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CHARACTERIZATION OF SOIL CARBON STABILIZATION IN LONG-TERM ROW-CROPPED AGRO-ECOSYSTEMS

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ABSTRACT OF DISSERTATION

Soraya Patricia Alvarado-Ochoa

The Graduate School

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CHARACTERIZATION OF SOIL CARBON STABILIZATION IN LONG-TERM ROW-CROPPED AGRO-ECOSYSTEMS

ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By
Soraya Patricia Alvarado-Ochoa

Lexington, Kentucky

Director: Dr. John Grove, Associate Professor

Lexington, Kentucky

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Soil organic matter (SOM) is a dynamic soil property, sensitive and responsive to many factors. The possibility of increasing soil carbon (C) sequestration by changing land use and management practices has been of great interest recently due to concerns with global changes in the atmospheric carbon dioxide (CO_2) balance. Nonetheless, as a result of the complex dynamics of SOM, there is still the need for SOM characterization procedures capable of monitoring SOM stabilization, taking into account all the factors involved.

This study characterized SOM stabilization as affected by management practices in three long-term field experiments, considering physical, chemical and biological components. The field experiments are located near Lexington, Kentucky, on a Maury silt loam (fine, mixed, mesic Typic Paleudalfs). The first experiment evaluates tillage and nitrogen (N) rate effects. The second experiment studies manure and N rate effects. The third experiment evaluates the five corn components of three crop rotations [continuous (monoculture) corn, corn-wheat/double crop soybean, and hay-hay-corn-corn-corn]. Soil organic matter content, stability, and composition, for physically separated fractions, were assessed using $\delta^{13}C$ natural abundance and diffuse reflectance Fourier transformed infrared (DRIFT) spectroscopy. In addition, management effects on microbial biomass and microbial function as indicated by phenol oxidase enzyme activity were evaluated.

The results indicate that management practices affect SOM content, stability, and composition, and these effects differ by the soil aggregate fraction. No-tillage (NT), N fertilization, manure application and increased corn in crop rotations enhanced SOM levels. However, the effect of NT was observed mainly at the soil surface. Soil organic matter storage was determined by the aggregate size distribution. The proportion of recently deposited C was generally positively related to aggregate size, especially for the first and third experiments. Most of the recently deposited C was stabilized in microaggregates within macroaggregates, across the management treatments and field experiments. In addition, this fraction consistently exhibited low to medium SOM reactivity. These results suggest that SOM stabilization, as influenced by management practices, required achieving a specific composition and location within the soil matrix. This implies that soil C forms and aggregate size and stability are closely interrelated.

Keywords: Carbon stabilization, No-tillage systems, SOM composition, $\delta^{13}C$ natural abundance, DRIFT spectroscopy.

Soraya Patricia Alvarado-Ochoa

March, 2010

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By

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March, 2010

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This dissertation is dedicated to Thomas and Daniel

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Chapter 1

Introduction

Soils play a key role in the global C cycle, being both a source and a sink for atmospheric CO_2. Soil C is lost as CO_2 after SOM mineralization, while C is gained by the soil through plant growth that fixes atmospheric CO_2 via photosynthesis. Soil organic matter is a fundamental but transient component of the soil that impacts many chemical, physical, and biological properties (Gregorich et al., 1994). Greater than two-thirds of the organic C stored in terrestrial ecosystems is contained in SOM (Schlesinger, 1997), but intense cultivation on a global scale has contributed significantly to CO_2 release to the atmosphere (Cole et al., 1997). Changes in the type and intensity of land management have been linked to the potential for agricultural soils to regain some of this lost C (DeGryze et al., 2004; Lal, 2004), and soil/crop management modifications could play an important role in the mitigation of climate change.

Soil management practices can modify SOM levels by affecting organic matter inputs and turnover. Studies have shown that increasing C inputs (with additions of manure and fertilizer, inclusion of legumes and cover crops, and reduction in fallow frequency) can cause gradual SOM accumulation (Kuo et al., 1997; Rasmussen et al., 1980). In addition, tillage is another soil management practice affecting SOM levels. No-tillage usually exhibits increased SOM and aggregation compared to plow tillage (Blevins et al., 1977; Blevins et al., 1983; Lamb et al., 1985; Havlin et al., 1990; Paustian et al., 1992; Smith et al., 1998; Diaz-Zorita and Grove, 2002; Amado et al., 2006). This has been explained by reduced aggregate turnover in NT, leading to formation of stable microaggregates that also stabilize and sequester C for a longer period (Six et al., 2000a). Nonetheless, the extent of SOM changes due to NT management varies among soils and according to other management practices being used.

Increasing the C sequestration potential of agricultural soils requires a careful understanding of the mechanisms controlling soil C stabilization. Soil C stabilization has been explained by one (or all) of the following mechanisms, depending on soil type, environment and management (Sollins et al., 1996): (1) organo-mineral interactions, which are strongly related to the type and amount of clay; (2) inaccessibility, which is determined by soil structural characteristics, such as aggregate size and stability; and (3) biochemical stabilization via formation of recalcitrant SOM compounds.

No SOM fractionation procedure has proved to be most suitable for distinguishing SOM pools based on all the possible factors involved in SOM stabilization. Physical fractionations capture the effects of spatial arrangement on SOM dynamics without considering the chemical agents of SOM stabilization, while chemical fractionations are used for advanced chemical characterization and ignore any spatial arrangement. In this context, SOM researchers have been called upon to develop integrated fractionation procedures that include physical, chemical, and/or biological components; and to identify the most suitable extraction procedure for a specific research situation, i.e., the specific land use, crop type, crop management, soil type, and nutrient or compound in question (Olk and Gregorich, 2006).

The soil biological component is inherent to SOM stabilization, because it represents an active portion of the SOM (Smith and Paul, 1990). Furthermore, soil microbial community function, as indicated by extracellular enzyme activity, has been used as a sensitive indicator of the degree of C cycling associated with different management practices (Bandick and Dick, 1999). In this context, phenol oxidase is an important lignolytic extracellular enzyme involved in organic matter decomposition and humification, and has been reported to be responsive to N amendments (Carriero et al., 2000; Davies et al., 2001; Matocha et al., 2004). Nevertheless, little is known about the response of phenol oxidase enzyme activity to soil management practices in agro-ecosystems.

Accordingly, we studied three long-term field experiments to characterize SOM stabilization in row-cropped agro-ecosystems, considering the physical, chemical and biological components of that stabilization in no-till and moldboard plow soils under different N fertilization, manure application and crop rotation systems. The methodological approach used in this study recognizes: (i) that long-term agricultural field experiments have been established to study the effect of constant management practices and are especially appropriate for SOM studies, because SOM contents change relatively slowly and these changes may be small when compared to initial values or when evaluated within the context of the soil's spatial variation in SOM (Powlson, 1996);

and (ii) that soil aggregation and organic matter accumulation are interrelated, i.e., organic matter is basic to the aggregation process and organic matter sequestered within aggregates is protected against degradation (Carter and Stewart, 1996). Consequently, SOM fractions were obtained by aggregate separation.

The specific research objectives were:

- To quantify the long-term effects of tillage, N fertilization, manure application and crop rotation on the SOM storage capacity of both whole soil and physically separated fractions.
- To characterize soil C stabilization as affected by management practices for physically fractionated SOM pools using $\delta^{13}C$ natural abundance.
- To determine if microbial community function, as indicated by phenol oxidase enzyme activity, could be a sensitive indicator of differences in carbon stabilization due to different management practices.
- To assess the effect of management practices on the SOM composition of physically separated fractions.

Chapter 2

Soil Organic Carbon Storage in Long-term Row-cropped Soils Under Different Management Practices

Introduction

Organic carbon storage in agricultural soils provides well-known beneficial effects on nutrient dynamics and soil structure, and is also a potential sink for atmospheric carbon dioxide (Smith et al., 2007). The soil organic carbon (SOC) storage capacity, which is not necessarily characterized by the native soil organic C level, is controlled by factors such as soil type, climate, soil landscape processes, and crop and soil management practices (VandenBygaart et al., 2002). The native soil organic C level decreases drastically with cultivation (Bauer and Black, 1981), and this decline continues when cropping systems involving fallow are used rather than continuous cropping (Unger, 1982). On the other hand, the native soil organic C level can be increased by selecting the appropriate crop and soil management practices (Ismail et al., 1994).

Soil management practices influence soil organic C storage and ultimately, determine the relative importance of different mechanisms of soil organic C stabilization. Conservation tillage, where shoot residues are left on the soil surface and root residues are left in the undisturbed soil, can generally increase surface soil organic C (Blevins et al., 1977; Blevins et al., 1983; Lamb et al., 1985; Havlin et al., 1990; Paustian et al., 1992; Smith et al., 1998; Diaz-Zorita and Grove, 2002; Amado et al., 2006). Blevins et al. (1983) reported that OC was approximately twice as great with NT as with moldboard plowing (MP) in the soil surface (0-5 cm depth increment) after 10 years of continuous corn. Paustian et al. (1992) estimated that NT resulted in an average soil C increase of 285 g m^{-2} with respect to conventional tillage (CT). Smith et al. (1998) found that the average increase in soil organic C, when changing from CT to NT, was $0.73 \pm 0.39\%$ yr^{-1}, and that soil organic C may reach a new equilibrium in approximately 50 to 100 yr. The Intergovernmental Panel on Climate Change (IPCC, 2000) suggests using a multiplication factor of 1.1 for change from CT to NT, essentially corresponding to a 10% increase in soil organic C. However, the extent of soil organic C change with NT is closely related to soil depth, type of soil, climate, and other management practices being used (Gregorich et al., 1996; VandenBygaart et al., 2002). A global data analysis of soil organic C sequestration rates emphasized the importance of crop rotation with changing tillage practices, pointing out that a transition from CT to NT can sequester 57 ± 14 g C m^{-2} yr^{-1}, excluding wheat-fallow systems where soil organic C accumulation may not result (West and Post, 2002).

Frequently, a synergistic effect between reduced tillage practices and increased cropping intensity on soil organic C accumulation has been reported. According to Amado et al. (2006), NT performance is improved by high inputs of biomass C and N, via biologic fixation and mineral N fertilization, and accomplished in more intensive and diversified cropping systems. They reported that under an intensive cropping system, NT had 0.69 Mg ha^{-1} yr^{-1} more C addition than CT. The intensive cropping system had 1.84 Mg ha^{-1} yr^{-1} more C addition than the double-cropping system, both under NT.

Soil management practices which influence yield and the proportion of crop residues returned to the soil are likely to influence soil organic C levels (Rasmussen et al., 1980; Cole et al., 1993; Kuo et al., 1997). Therefore, application of manure, nitrogen (N), phosphorus (P), potassium (K), and sulphur (S) increases soil organic C by increasing crop yields (Juma et al., 1993). In addition, the application of these sources can alter rates of soil organic matter (SOM) formation and the release of plant nutrients from litter through their effects on the composition and function of microbial communities (Solberg et al., 1997). Juma et al. (1993) reported that application of manure increased SOM even more than fertilizer, which is explained by the fact that in addition to the nutrient value of manure, it represents an additional source of organic C.

The beneficial effects of manure application on SOM will depend on the type of manure applied. Bremer et al. (1994) observed that long-term application of low rates of animal manure resulted in a nearly two-fold increase in the light fraction C in a wheat-fallow system. Paustian et al. (1992) reported that organic amendments with high-lignin content (farmyard manure) resulted in greater C accumulations per unit C input than low-lignin amendments (straw, green manure). They suggested that the residue-quality differences impact the direct stabilization of lignin degradation products in the slow organic matter pool as opposed to the more complete metabolism of nonlignin residue fractions by the soil microbial biomass. Soil management impacts can be additive. It has also been reported that manure application, in combination with NT, further improved soil aggregation and aggregate-protected C and N (Mikha and Rice, 2004).

Crop rotation is another management practice influencing soil organic C storage, because it enables manipulation of the amount and mechanism of C return to the soil, and the characteristics and temporal distribution of crop residues. The total amount of photosynthate deposited below ground, the composition of this photosynthate (Blum et al., 1991), and the resistance of this material to complete mineralization varies considerably between plant species (Kay, 1990). Davenport and Thomas (1988) showed that the total amount of rhizodeposition with bromegrass (*Bromus inermis* Leyss) can be twice as high as that deposited by corn. Additionally, C originating from bromegrass has been reported to be more persistent in the soil (Davenport et al., 1988). Accordingly, it has been suggested that soil organic C loss can be reduced by including pastures in the rotation or by increasing the amount of crop residues returned to the soil (Cole et al., 1993). It has been observed that inclusion of legumes and cover crops (Kuo et al., 1997), and a reduction in fallow frequency (Rasmussen et al., 1980), both linearly increase soil organic C levels.

Larson et al. (1972) suggested that the type of residue applied to a soil only weakly relates to soil organic C levels and that the overriding effect of various cover crop species on soil organic C was due to the magnitude of the cover crop C inputs. However, according to Six et al. (2002a), additional C inputs will not always be stabilized as soil organic C, and this has been observed in long-term field experiments where soils with a high C content exhibited little or no increase in soil C content with 2 to 3 fold increases in C input (Campbell et al., 1995; Wilts et al., 2004).

The soil organic C can be separated into fractions that are either (1) physical-chemically stabilized through aggregation or intimate association with silt and clay particles, (2) biochemically stabilized through the formation of recalcitrant compounds, or (3) physically uncomplexed, which includes light and particulate organic matter (POM) not occluded within microaggregates (Six et al., 2002a). Each fraction will have different functional roles in SOM dynamics and nutrient cycling, and may respond differently to various soil and crop management practices. Despite this conceptual model, there is still not a full understanding of the effects of crop and soil management practices on these SOM fractions.

Several methods have been developed to isolate SOM fractions and quantify their organic C content in order to evaluate turnover times and relationships between the organic C distribution among fractions and soil management (Hassink et al., 1997; Sohi et al., 2001; Six et al., 2002b). Chemical fractionation techniques cannot consider soil organic C spatial arrangement and produce purely organic fractions that are used to elucidate molecular-level interactions, but are not consistently related to the dynamics of SOM turnover (Oades and Ladd, 1977).

On the other hand, physical fractionation techniques such as particle size fractionation, density fractionation, and aggregate fractionation emphasize the role of soil minerals in SOM stabilization and turnover. Physical fractionation is considered less chemically destructive than chemical fractionation, and its results are likely related to the structure and function of SOM in-situ (Christensen, 1992). These techniques have been applied to determine the association of SOM with primary particles and to quantify the amount of POM between and within soil aggregates (Beare et al., 1994; Six et al., 1998). Macroaggregation is sensitive to changes in land use and cultivation practices; while microaggregation is less sensitive (Tisdall and Oades, 1982; Franzluebbers and Arshad, 1997) and the dynamics of aggregate formation seem to be closely linked with SOM storage in soils (Golchin et al., 1997). Microaggregation within macroaggregates has been called the ideal indicator of C sequestration potential in sustainable agroecosystems (Kong et al., 2005); whereas the light POM fraction, which plays a crucial role in the formation of aggregates (Six et al., 2001), has been reported to be the most management sensitive fraction, because its size is a function of C input and the specific decomposition of the various C components (Bremer et al., 1994; Franzluebbers and Stuedemann, 2002).

The hierarchical model proposed by Tisdall and Oades (1982) indicates that stable microaggregates (<250 μm) are bound together to form stable macroaggregates (>250 μm) with organic compounds of different origin as intermicroaggregate binding agents (Elliot, 1986; Golchin et al., 1994). Tisdall and Oades (1982) and Hassink (1997) proposed that organic matter addition to soils results first in the formation of SOM associations with clay and silt particles and with microaggregates and that macroaggregate formation begins only if the SOM binding capacity of clay and silt fractions is saturated. The distribution of recently incorporated litter C has not been found to be homogeneous in soil aggregates. Puget et al. (2000) found that recently added maize residue C particularly enriched the particulate organic matter located in macroaggregates. Sohi et al. (2005) reported that inter- and intra-aggregate organic matter occupy contrasting positions in the decomposition sequence, and are likely to display reactivities sufficiently distinct so as to operate as discrete pools in most recent SOM models.

Summarizing the findings above, there is still considerable uncertainty about the effects of different soil management practices on soil organic C storage among soil physical organic matter fractions. Accordingly, the main objective of the present study was to evaluate the effect of tillage, N fertilization, and crop rotation on soil organic C and N storage in long-term row-cropped soils under the hypotheses that every agro-ecosystem possesses different soil organic C and N storage potentials, directly related to management practices, and that the C and N cycles are not independent. The specific objectives were: i) to assess soil organic C and N storage in whole soils ii) to evaluate soil organic C and N storage in water-stable aggregates of different size [>2000 µm: large macroaggregates (LM), 2000-250 µm: small macroaggregates (SM), 250-53 µm: microaggregates, and <53 µm: silt and clay], iii) to determine the SOC and N storage in intra-macroaggregate fractions [free light <1.6 g cm^{-3} (free), occluded light <1.6 g cm^{-3} (occluded), >250 µm within small macroaggregates (>250 μm_{SM}), >250 µm within large macroaggregates (>250 μm_{LM}), 250-53 µm within small macroaggregates (250-53 μm_{SM}), 250-53 µm within large macroaggregates (250-53 μm_{LM}), <53 µm within small macroaggregates (<53 μm_{SM}) and <53 µm within large macroaggregates (<53 μm_{LM})].

Material and Methods

Field Experiments

Three long-term field experiments located at the University of Kentucky's Agricultural Experimental Station Farm (Spindletop), near Lexington, Kentucky (38^o 07'N, 84^o 29'W), were investigated. Average annual temperature is 13 ^{o}C and average annual precipitation is 113 cm. The soil series is a well-drained Maury silt loam (fine, mixed, mesic Typic Paleudalfs) developed in the residuum of phosphatic Ordovician limestone with smectite-vermiculite, mica, and kaolinite as the predominant clay mineralogy (Karathanasis, 1991). All three experimental sites had been in bluegrass (*Poa pratensis,* L.) since 1920 (Blevins et al., 1977).

Field Experiment 1-Tillage by N rate.

The long-term effects of tillage and fertilizer N rate treatments have been evaluated in this experiment since 1983. Tillage treatments include MP, disk tillage, chisel plowing, and NT, and are main plots, laid out in four randomized blocks. Crop residues are left intact in the NT treatment, and the only soil perturbation is a narrow slit where seed are sown during planting. Moldboard plowing is performed to a depth of 20 to 25 cm in late April of each year before corn (*Zea mays* L.) planting. Nitrogen treatments are four N (NH_4NO_3) rates (0, 75, 150, and 225 kg ha^{-1}), and these are subplot within main plots. Nitrogen is applied annually within two weeks of corn establishment. Corn is grown every year, and a winter annual cereal cover crop is established after the corn harvest. Plots that were sampled for this study included the 0 and 150 kg N ha^{-1} subplot treatments for both MP and NT systems, giving a total of 16 sampled plots.

Field Experiment 2-Manure by N rate.

The long-term effects of crop/manure and fertilizer N rate treatments have been tested in this experiment since 1992. Crop/manure treatments consist of six manure timing-application treatments (spring each year, fall each year, spring and fall in corn years, spring and fall in soybean years, spring and fall each year, and unmanured), and two crops (soybean and corn), which are rotated annually. Crop/manure treatments are main plots, laid out in three randomized blocks. Nitrogen rate subplot treatments are three

N (NH_4NO_3) rates (0, 84, and 168 kg ha^{-1}). Nitrogen is applied annually 3-4 weeks after planting. Soil samples were taken from unmanured and spring-of–each-year-manured main plots at each of two N rates (0 and 168 kg ha^{-1}), giving a total of 12 sampled plots.

Field Experiment 3-Corn in crop rotations.

The long-term effects of several continuous NT crop rotations on corn yield have been evaluated in this experiment since 1983. Crop rotation treatments consist of continuous corn (CCCCC), corn-wheat/double crop soybean (w/sc), and hay-hay-corn-corn-corn (hhccc), which are main plots, laid out in four randomized blocks. Nitrogen rate subplot treatments were begun in 1994 and are six N (NH_4NO_3) rates (0, 45, 90, 135, 180, and 225 kg ha^{-1}), which are the subplot treatments. Nitrogen is applied annually 1-3 weeks after planting. Soil samples were taken from the five corn components of the crop rotation treatment plots, i.e. continuous corn (CCCCC), corn in w/sc (w/sC), first year of corn in hhccc (hhCcc), second year of corn in hhccc (hhcCc), and third year of corn in hhccc (hhccC), at one N rate (180 kg ha^{-1}), giving a total of 20 sampled plots.

Soil Sampling and Handling

The three long-term field experiments were sampled twice in 2006. Two depth increments (0-10 and 10-20 cm) were sampled during spring (late April); and three depth increments (0-10, 0-5, and 5-10 cm) were sampled during fall (late November). Additionally, soil samples were taken from the sod soil adjancent to each long-term field experiment as a control soil during the spring sampling.

There were two sampling sites per plot or sod site. At each site, three core samples were taken at each depth, 25 cm from the corn row using a cylindrical core sampler with a diameter of 5.4 cm. The first core sample was used for assessing bulk density and antecedent moisture content. The second core sample was composited across sampling sites, and used for aggregate stability analysis. Once the aggregate stability analysis was completed, the sample was crushed to pass a 2 mm sieve for further physical and chemical characterization. The third core sample was composited across sampling sites and used for water-stable aggregate fractionation, and for some biological assays that are described in Chapter 4.

Characterization of the Study Soils

General soil analysis.

Bulk density was determined by the core method (Blake and Hartge, 1986), in which the mass is determined after drying to constant weight at 105 ^{o}C, and the volume is that of the sample as taken in the field. Antecedent soil moisture content was assessed from the weight change with drying. The rest of the physical and chemical analyses were performed on air dried soil crushed to pass a 2 mm sieve. Soil pH was determined in a water suspension (soil:solution ratio of 1:2.5). Soil texture was assessed using the pipette method (Sheldrick and Wang, 1993). Bioavailable P, K, Ca, Mg, Zn, and Mn were determined by inductively coupled plasma spectroscopy (ICP) on a Mehlich III extract, which contains 0.2 N acetic acid, 0.25 N NH_4NO_3, 0.015 N NH_4F, 0.013 N HNO_3, and 0.001 N EDTA.

Total soil C and N assessment.

Total C and total N contents were determined by dry combustion (Nelson and Sommers, 1982) of air-dry 2-mm-sieved whole soil samples from each depth.

Aggregate stability analysis.

The size distribution of aggregates was assessed by two methods. The first method was dry sieving of previously drop shattered and air-dried soil (Perfect et al., 2002). Briefly, the fresh core soil samples were placed in paper bags and dropped from heights ranging from 1.5 to 2.0 m onto a tray to induce fragmentation of moist soil with a mean specific energy input of 0.017 J g^{-1}. The fragmented soil was air-dried for at least two weeks before sieving. The sieving was done with a Fritsch Analysette 3 vibratory sieve shaker for 30 s using a frequency of approximately 50 Hz and oscillation amplitude of 2 mm. The nest of sieves consisted of 5 aperture sizes: 16, 8, 4, 2, and 1 mm. The masses collected on each sieve were normalized with respect to total mass, and summed to give the normalized cumulative mass of fragments. The second method was wet sieving (Yoder, 1936), which was performed using nested 12.5 cm diameter sieves of 3 aperture sizes: 5, 2, and 0.5 mm and a Yoder apparatus containing 4 L of deionized water. The top sieve of the stack contained about 50 g of previously misted dry-sieved aggregates (2 to 8 mm diameter). When the apparatus was activated, the motor moved the stack through a vertical distance of 2 cm, completing 33 cycles min^{-1}. After 10 min, the motor was stopped and the sieve stack removed from the tank. Material retained on each sieve was gently washed into tared specimen cups and dried overnight at 105°C. Dry weights were recorded. The dry masses of primary particles (sand and gravel) in intact aggregates, from each size class, were determined by shaking and dispersing the aggregates in a sodium hexametaphosphate solution. Total and size class weights were corrected for gravel/sand recovered on each sieve size class. As a measure of soil structural stability, the mean weight diameter (MWD) was calculated according to the following equation,

$$MWD = \sum_{i=1}^{n} x_i W_i$$

where i= 1, 2, 3,….,n and corresponds to each fraction collected, including the one that passed the finest sieve; x_i is the mean diameter of each size fraction (i.e., mean intersieve size) and W_i is the weight proportion of each aggregate size fraction.

Water-stable Aggregate Fractionation

Water-stable aggregates were obtained as described by Elliot (1986), and Angers and Mehuy (1993), which is a method based upon the slaking property of soils. Fresh field soil samples were gently passed through an 8 mm sieve and air dried at room temperature. A 25 g sub-sample was wet sieved by hand through a series of three sieves to obtain 4 size fractions: (1) >2000 µm, (2) 2000-250 µm, (3) 250-53 µm, and (4) <53 µm. Soils were submersed in water on the largest screen for 5 min before sieving started, and slaking was allowed to occur as aggregates were directly immersed in water without pre-wetting. Soils were sieved under water by gently moving the sieve 3 cm vertically 50 times over a period of 2 min through water contained in a shallow pan. Material remaining on the sieve was transferred to a plastic container and dried at 60°C in a forced-air oven. Soil passing a particular sieve and remaining in the shallow pan was then transferred to the next finer sieve and the process repeated. Material smaller than 53 µm (silt and clay particle size fraction) was allowed to settle, the water siphoned off, and oven dried. Two replicate sievings were completed on each field sample. A sub-sample of each 60^{o}C-dried soil aggregate fraction was dried at 105^{o}C for final soil aggregate moisture corrections. A correction for the presence of the dry mass of primary particles and of sand (>53 μm) in intact aggregates was accomplished by shaking and dispersing 3-4 g of 60^{o}C-dried soil aggregates in 20 ml of 5% (w/v) solution of sodium hexametaphosphate overnight in a reciprocal shaker set at a low speed (180 osc min^{-1}). Then, the dispersed particles were resieved, washed using ionized water, and dried at 105 ^{o}C. The mass of coarse primary particles was subtracted from the previously obtained mass of stable aggregates. Sub-samples of the 60^{o}C-dried aggregates were ground using an agate mortar and pestle and their organic C and total N contents determined.

Isolation of Intra-aggregate Fractions from Large and Small Macroaggregates

A selected water-stable aggregate fraction from each field experiment was subject to further density and wet sieving fractionation, which is summarized in Figure 2.1, to obtain the free, occluded, >250 μm_{SM}, >250 μm_{LM}, 250-53 μm_{SM}, 250-53 μm_{LM}, <53 μm_{SM} and <53 μm_{LM} fractions. The method was modified after Skjemstand et al. (1990), Golchin et al. (1994), Six et al. (1998), and Sohi et al. (2001). Briefly, a 5 g sub-sample of water-stable small macroaggregates (Field Experiments 1 and 2) or water-stable large macroaggregates (Field Experiment 3) was suspended in 15 ml of 1.6 g cm^{-3} sodium polytungstate (SPT) in a 50 ml centrifuge tube. The suspended sample was mixed by slowly reciprocal shaking by hand (15 strokes) to bring the whole sample in suspension. The material left on the cap and sides of the centrifuge tube were washed into suspension with 5 ml of SPT. The sample was then put under vacuum for 10 min to evacuate air entrapped within aggregates. After a 30 min equilibration period, sedimentation of heavy particles was accelerated by centrifuging at 2000 g for 30 min. The floating material, the free light fraction, was aspirated and filtered using a pre-weighed Whatman Gf/A glass fiber filter. Then, this fraction was rinsed thoroughly with deionized water to remove SPT and dried at 60 ^{o}C for 12 hours. The filtrate from each sample was returned to the respective centrifuge tube. The occluded light fraction was released by re-suspending the contents of the centrifuge tube (i.e., the soil residue and replaced SPT) and reciprocally shaking with 25 glass beads (4 mm diameter) for 8 min. After 30 min of equilibration, the occluded light fraction was recovered by centrifugation using the same procedure described for the free light fraction. The remaining (heavy) fraction was re-suspended in 20 ml of water and subjected to wet sieving using two sieves with 250 μm and 53 μm apertures. Heavy fractions were washed into specimen cups, oven dried at 60 ^{o}C for 24 hours, and weighed. The recovered dried fractions were ground using a mortar and their organic C and N contents determined.

Statistical Analysis

SAS (1999) was used for all statistical analysis. Management effects on soil physical and chemical properties, soil aggregate-size distributions, and organic C and total N concentrations of SOM fractions were evaluated by analysis of variance (ANOVA) for each soil depth increment at each sampling time. The chosen model was based on the experiment design for each field experiment, and significant differences among means were determined by LSD means separation (Saxton, 1998). The probability level used to define statistical significance is specified in each related table or graph. In addition, the effects of aggregate size and type of intra-aggregate fraction on organic C and total N concentrations were analyzed using ANOVA for a completely randomized design, which was performed separately for each field treatment. Finally, a repeated measures procedure was used to evaluate seasonal variation in total C, total N, and the C/N ratio for the 0-10 cm soil depth increment.

Results and Discussion

Characterization of the Study Soils

General soil analysis-Field Experiment 1.

Selected soil physical and chemical properties from the spring sampling of Field Experiment 1 are presented in Table 2.1. For the 0-10 cm soil depth increment, all soil properties but Mn were significantly affected by tillage, N rate, or their interaction. Tillage significantly affected silt, clay, and extractable Ca and Zn. Higher silt, extractable Ca and Zn, and lower clay were observed for the NT system. These results are supported by the stratification of nutrients reported under NT (Dick, 1983; Selles et al., 1997; Diaz-Zorita and Grove, 2002). Additionally, higher clay content under MP is explained by the process of inversion plowing, which could bring clay particles from deeper horizons, and by the increased macroaggregate turnover under MP (Six et al., 2000a). The N rate effect was significant for bulk density and extractable Mg, giving higher bulk density and extractable Mg when N was not applied. These unexpected results can be explained by the impact of N fertilization on SOM levels. Nitrogen fertilization is responsible for higher crop yields and consequently greater soil C inputs and nutrient removal. Both tillage and N rate main effects were significant for soil pH (Table 2.1). Higher soil pH was observed in the NT system, while, as expected, lower pH was observed with N fertilization. The interaction of tillage by N rate was significant for gravimetric water, sand, and bioavailable P and K. The highest value for all of these soil properties (except bioavailable K) was associated with N fertilized NT system. Extractable K exhibited higher values in the NT soil, especially when unfertilized. These results are not fully consistent with previous findings. Blevins et al. (1983) reported no significant effect of tillage on bulk density in the 0-15 cm soil depth increment, but lower soil pH and exchangeable Ca for NT, after 10 years of continuous NT or MP corn. On the other hand, the soil in the surrounding sod exhibited the lowest pH and extractable Ca. The sod soil's extractable P, K, Mg, Zn and Mg, gravimetric water content, bulk density, and sand, silt, and clay contents, were similar to those observed for the NT soils.

In the 10-20 cm soil depth increment, tillage, N rate, and their interaction did not significantly influence gravimetric water content and bulk density. In contrast, the tillage effect was significant for sand, extractable Ca, Zn, and Mn concentrations. Lower extractable Ca, Zn, and Mn, and higher sand, were observed for the NT system. Nitrogen significantly affected silt and extractable P. Lower silt and higher extractable P were observed without N fertilization. Tillage and N rate main effects were observed for extractable Mg and clay. Lower Mg and clay were associated with the NT system, while higher Mg and clay were observed without N fertilization. The interaction of tillage by N rate was significant for soil pH and extractable K. Lower soil pH was observed with N fertilization but only under the NT system. Extractable K exhibited higher values in unfertilized soils, with the highest value found in the NT system.

At the deeper depth, the sod sample exhibited more acidity than most of the tillage by N rate treatments (Table 2.1). Extractable Ca was consistent with soil pH. Extractable K was similar to that observed for the 150N rate treatment; while extractable P and Mg were more similar to the 0N rate treatment. The sod's gravimetric water content was above that observed in the cropped soils, and bulk density was within the range observed among the tillage by N rate treatments. Sand, silt, clay, and extractable Zn and Mn for the sod were more similar to the NT soils.

The 0-10 cm soil depth increment was studied in more detail by taking samples from the 0-5 and 5-10 cm soil depths during the fall sampling. Table 2.2 summarizes some soil physical and chemical properties from this extra sampling. For the 0-5 cm soil depth increment, all soil properties except for soil pH, and extractable Ca and Mn were significantly affected by tillage, N rate, or their interaction. Tillage significantly affected extractable K and Zn. Higher K and Zn were observed in the NT soils. The N rate effect was significant for extractable Mg, which was greater when N was not applied. Both tillage and N rate main effects significantly influenced bulk density and sand content. Higher bulk density and lower sand content were associated with the MP system, while lower bulk density and higher sand content were observed with N fertilization. The interaction of tillage and N rate was significant for gravimetric water, silt and clay content, and for extractable P. The highest value for all of these soil properties, except clay content, was associated with the N fertilized NT soil. Clay contents were highest for unfertilized MP soil.

In the 5-10 cm soil depth increment, all soil properties but soil pH and extractable Ca were significantly affected by tillage, N rate, or their interaction. Tillage significantly influenced silt content and extractable Zn, with greater values for both variables in NT soils. Nitrogen rate significantly influenced bulk density and sand content. Higher bulk density and lower sand contents were observed without N fertilization. Tillage and N rate main effects were observed for clay content and extractable K and Mg. Higher extractable K and lower extractable Mg and clay content were found in NT soil. Lower extractable K and Mg, and clay contents were observed N fertilized soil. The interaction of tillage and N rate was significant for gravimetric water content and extractable P and Mn. The highest extractable P and gravimetric water content was associated with N fertilized NT soil. The highest extractable Mn was observed with MP soils, regardless of N rate, but the unfertilized NT soil exhibited the lowest level of extractable Mn.

General soil analysis-Field Experiment 2.

The soil physical and chemical properties from the spring sampling of Field Experiment 2 are presented in Table 2.3. In the 0-10 cm soil depth increment, bulk density, sand, silt, clay and extractable Mn were not significantly affected by manure, N rate, or their interaction. Manure use significantly influenced gravimetric water content, with higher values when manure was used. Nitrogen use significantly affected extractable Mg with higher values found when N fertilizer was not applied. Manure and N rate main effects were observed for soil pH and extractable P, K, Ca and Zn. Higher soil pH and extractable P, K, Ca and Zn values were associated with manure application, while, as expected, lower soil pH and extractable P, K, Ca and Zn values were observed with N fertilization. The surrounding sod exhibited the lowest soil pH and extractable K, Ca, Mg and Zn values. The sod soil’s extractable P level was comparable to the unmanured soils, but gravimetric water content was closer to that of manured soils. Bulk density, sand, silt and clay contents, and extractable Mn levels in sod soil were within the range exhibited by the study treatments.

In the 10-20 cm soil depth increment, manure, N rate, and their interaction did not significantly affect gravimetric water content, bulk density, sand content, soil pH or extractable P, Ca, Zn and Mn. However, manure and N rate significantly influenced extractable K. Higher extractable K was associated with manure application, and when N was not applied. The interaction of manure and N rate was significant for silt and clay contents and for extractable Mg. Silt and clay contents were higher when N was not applied, and with manure application. At this deeper depth, the sod soil exhibited soil pH, gravimetric water content, bulk density, sand, silt, and clay contents, and extractable P, Ca, Zn and Mn values similar to those observed in unmanured soil. Extractable K and Mg were lower than those observed in any soil in the study.

Some of the soil physical and chemical properties from the fall sampling (0-5 cm and 5-10 cm soil depth increments) of Field Experiment 2 are summarized in Table 2.4. In the 0-5 cm soil depth increment, all soil properties except for bulk density, gravimetric water, and extractable Mn were significantly affected by manure, N rate, or their interaction. Manure application significantly and positively affected extractable P and Zn. The greater N rate was significantly associated with higher sand content values. Manure and N rate main effects were observed for soil pH and extractable K, Ca and Mg. Higher soil pH and extractable K, Ca and Mg were associated with manure application, while lower soil pH and extractable K, Ca and Mg were observed with N fertilization. The interaction of manure and N rate was significant for silt and clay contents. Lower silt content was found with N fertilization and manure application, but there was no difference in silt content due to N rate when manure was not applied. Clay contents were higher when N and manure were not applied.

At the 5-10 cm soil depth increment, sand, silt and clay content and extractable Mg and Mn were not significantly affected by manure, N rate, or their interaction. In contrast, manure application significantly impacted bulk density, gravimetric water content, and extractable P and Zn. Lower bulk density and a higher gravimetric water and extractable P and Zn content were observed after manure application. Both manure and N rate main effects were observed for extractable K, with higher values subsequent to manure application and lower values on N fertilized soil. The interaction of manure and N rate was significant for soil pH and extractable Ca. Lower soil pH values were found on N fertilized soil only when manure was applied, while higher extractable Ca levels were associated with manured but unfertilized soil.

General soil analysis-Field Experiment 3.

Table 2.5 summarizes some of the soil physical and chemical properties from the spring sampling of Field Experiment 3. In the 0-10 cm soil depth increment, all soil properties, except for the gravimetric water and extractable Mn content, were not significantly affected by crop rotation. Higher gravimetric water contents were associated with increased corn in the crop rotation (hhccC and CCCCC). The highest extractable Mn was observed for the hhCcc treatment, while the lowest extractable Mn was associated with hhccC and CCCCC treatments. The nearby sod sample exhibited a greater soil pH, content of sand, and extractable P, Ca and Mg and a lower bulk density and clay content than the crop rotation treatments. Sod soil content of silt and extractable K and Zn were similar that observed in the CCCCC treatment. In the 10-20 cm soil depth increment, none of the soil properties were significantly affected by crop rotation. The sod soil exhibited higher extractable P and lower bulk density and clay contents than the crop rotation treatments. Gravimetric water content, soil pH, sand and silt content and extractable Ca, Mg, Zn and Mn were similar to those observed among the crop rotation treatments. Extractable K was similar to that observed in the CCCCC treatment.

Some of the soil physical and chemical properties found with the fall sampling (0-5 and 5-10 cm soil depth increments) of Field Experiment 3 are presented in Table 2.6. In the 0-5 cm soil depth increment, except for bulk density, soil pH and extractable K and Mn, soil properties were not significantly affected by crop rotation. The lowest bulk density and extractable Mn, but the highest extractable K, were observed for the hhCcc treatment. The highest soil pH was associated with the w/sC treatment, while the lowest soil pH was related to the CCCCC and hhcCc treatments. In the 5-10 cm soil depth increment, only bulk density, soil pH, sand and silt content, and extractable K and Ca were significantly affected by crop rotation. The lowest bulk density and highest sand content were observed in the hhCcc treatment. The lowest soil pH and extractable Ca were found in the CCCCC treatment, while the highest extractable K was observed in the CCCCC and hhCcc treatments.

Total soil C and N assessment-Field Experiment 1.

Table 2.7 presents total C and N concentrations in the whole soil from the spring sampling of Field Experiment 1. In the 0-10 cm soil depth increment, total C and N were significantly affected by the interaction of tillage and N rate. Greater total C and N were found with NT, which is consistent with previous findings (Blevins et al., 1977; Blevins et al. 1983; Denef et al., 2004). Tillage promotes loss of soil organic C via macroaggregate disruption and exposure of SOM to microbial decomposition (Six et al., 2000a). Tillage also increases aeration, which enhances C mineralization and CO_2 fluxes to the atmosphere (Elliott, 1986). In addition, N application enhanced total C and N in NT, but not in MP (Table 2.7). Total C and N were 1.4 times higher in NT than MP in unfertilized soil, but 1.7 times higher at the 150 kg N ha^{-1} rate. Similar results have been reported (Blevins et al., 1977; Blevins et al., 1983; Allmaras et al., 2004), and they illustrate the importance of soil N in the soil organic C sequestration process (Campbell and Zentner, 1993). On the other hand, the C/N ratio was significantly affected only by tillage, where the NT soils exhibited greater values, independent of N rate. The nearby sod soil exhibited slightly higher total C and N contents, relative to those observed for the N fertilized NT soil, the C/N ratio was lower than any observed among the treatments; but was closest to those found for the MP soils. In the 10-20 cm soil depth increment, total C and N were lower than those observed in the 0-10 cm soil depth increment in NT but not MP soils, which reinforces the concept of stratification in NT systems (Lamb et al., 1985; Diaz-Zorita and Grove, 2002). In addition, total C and N in the 10-20 cm soil depth increment were still significantly affected by N rate. Higher total C and N were observed with N fertilization, regardless of tillage. In contrast, the C/N ratio was significantly affected by both tillage and N rate. The sod sample exhibited total C, total N, and C/N ratio values not very different from those observed for the tillage by N rate treatments.

Total C and N levels in the whole soil from the fall sampling of Field Experiment 1 are presented in Table 2.8. For both the 0-5 cm and 5-10 cm soil depth increments, total C and N were significantly affected by tillage and N rate. Higher levels of total C and N were observed for the NT system, especially with N fertilization. These results are not fully consistent with what was reported by Six et al. (1999), who did not observe any significant difference in aggregate soil C in the 5-20 cm layer due to tillage. On the other hand, the C/N ratio for the 0-5 cm soil depth increment was significantly affected by tillage and N rate, exhibiting higher values in the N fertilized NT soil. In contrast, the C/N ratio for the 5-10 cm soil depth increment was significantly affected only by N rate, with higher values for N fertilized soil. In addition, the total C and N for the 5-10 cm soil depth increment were lower than those observed in the 0-5 cm soil depth increment of the NT soil, which is consistent with the stratification observed in the spring sampling (Table 2.7) and with the results of Kern and Johnson (1993) and West and Post (2002). The latter concluded that a change from CT to NT causes sequestration of the greatest amount of C in the top 7-8 cm of soil, a lesser amount in the 8-15 cm depth, and no significant amount below 15 cm.

Looking at seasonal variation in the 0-10 cm soil depth increment, it was found that total C and N were significantly affected by the interaction of season and tillage. Higher total C was found in the spring sampling (Tables 2.7, 2.8), but only for the NT system. Higher total N was also associated with spring sampling of the NT system, while lower total N was found with spring sampling of the MP system. The C/N ratio exhibited a significant seasonal effect, with higher values in the spring sampling. The overall trend observed from these results is consistent with those found by Andreux et al. (1990) who stated that C concentrations reach a first maximum in spring, followed by a decrease during summer and a second, lower maximum, in fall. Additionally, Leinweber et al. (1994) reported decreases in soil C between June and August, decreases in soil N between August and September, and the lowest C/N ratio between July and August.

Total soil C and N assessment-Field Experiment 2.

Table 2.9 gives total C and N concentrations for the whole soil from the spring sampling of Field Experiment 2. In the 0-10 cm soil depth increment, total C and N were significantly affected by manure application, which enhanced total C and N. This has been found in other studies (Roder et al., 1988). On the other hand, the C/N ratio exhibited a manure by N rate interaction. Slightly higher C/N values were observed with N fertilization of the unmanured soil, but lower C/N values were observed with N fertilization of the manured soil. The nearby sod exhibited total C and N values similar to those observed for the manured soils. In the 10-20 cm soil depth increment, total C, total N, and the C/N ratio were lower than those observed for the 0-10 cm soil depth increment, stratified similarly as reported for Field Experiment 1. In addition, only total C in the 10-20 cm soil depth increment was significantly affected by manure. Higher total C was observed with manure application. At this depth, the sod exhibited total C, total N, and C/N ratio values within the range observed for the manure by N rate treatments measured in the study.

Total C and N values for the whole soil from the fall sampling of Field Experiment 2 are presented in Table 2.10. For the 0-5 cm soil depth increment, total C and N were significantly affected only by manure, and higher total C and N were observed with manure application. On the other hand, the C/N ratio was significant affected by the manure by N rate interaction. Higher values were observed with N fertilization, especially with manure application. In the 5-10 cm soil depth increment, total C and N were significantly and positively affected by manure application. On the other hand, the C/N ratio was not significantly affected by either manure or N rate. The total C and N for the 5-10 cm soil depth increment were lower than those observed for the 0-5 cm soil depth increment, especially for unmanured soils, which suggests that C and N from manure application have been moved into the 5-10 cm soil layer. This was not observed for the deeper soil depth increment (10-20 cm) sampled in the spring.

The analysis of seasonal variation for total C and N in the 0-10 cm soil depth increment revealed that both parameters were significantly affected by the interaction of season, manure, and N rate. In contrast, the C/N ratio was significantly affected only by season exhibiting higher C/N ratios in the spring. Total C (Figure 2.2) was lower in fall-sampled unmanured soils with slight differences due to N rate. In contrast, total C was lower in spring-sampled manured soils, especially with N fertilization. Total N (Figure 2.3) was greater with fall sampling of manured soil, especially when no N was applied. There was no significant difference in total N due to season or N rate when manure was not applied.

Total soil C and N assessment-Field Experiment 3.

Table 2.11 presents total C and total N values for the whole soil from the spring sampling of Field Experiment 3. Total C, total N and the C/N ratio in both the 0-10 and 10-20 cm soil depth increments were not significantly affected by crop rotation. Nevertheless, lower total C, total N, and C/N ratios were observed for the 10-20 cm soil layer. This result further exemplifies the C and N stratification observed for the NT in Field Experiments 1 and 2. In addition, the sod sample exhibited higher total C and total N values, and similar C/N ratios, in comparison to samples taken in the crop rotation treatments.

In contrast to spring samples, the fall samples (Table 2.12) revealed crop rotation effects. In the 0-10 cm soil depth increment, total C and the C/N ratio were significantly affected by crop rotation, exhibiting the lowest total C in the hhcCc rotation and the highest C/N ratio in the w/sC rotation. In the 0-5 cm soil depth increment, total C, total N, and the C/N ratio were all significantly affected by crop rotation. The highest total C and N were associated with the CCCCC and hhCcc treatments, while the highest C/N ratio was observed for the w/sC treatment, followed by the CCCCC rotation. In the 5-10 cm soil depth increment, total C and N were also significantly affected by crop rotation, exhibiting trends similar to those observed in the 0-5 cm soil depth increment. In contrast, the C/N ratio in this deeper soil layer was not significantly affected by crop rotation. Total C, total N, and the C/N ratio were generally lower in the deeper soil layer.

The analysis of seasonal variation in the 0-10 cm soil depth increment revealed that total C was not significantly affected by season in this experiment. The opposite was true for total N and the C/N ratio. Total N was higher in the fall season and the C/N ratio was lower. Nevertheless, fall sampling of the 0-10 cm soil depth increment exhibited a significant crop rotation effect on total C that was not detected with spring sampling.

Aggregate stability analysis.

Mean weight diameter was calculated for each described aggregate stability method (wet and dry) and for the water-stable aggregate fractionation, which was also based on the wet sieving technique, but with smaller size fractions. Mean weight diameter values for Field Experiment 1 are presented in Table 2.13. In the 0-10 cm soil depth increment, MWD by the dry method was not significantly influenced by the tillage by N rate treatments. In contrast, MWD by both wet methods exhibited significant tillage and N rate effects. Higher MWD was associated with the NT system and lower MWD was observed with N fertilization. These results are consistent with those reported by Wander and Bollero (1999). Similarly, Angers et al. (1993a, b) reported that the MWD of water-stable aggregates in NT soils was 40% greater than that of MP soils, which was attributed to reduced aggregate turnover in the NT systems that leads to formation of stable microaggregates (Six et al., 2000a). In the 10-20 cm soil depth increment, the dry aggregate MWD was significantly affected by N rate, with lower MWD values for N fertilized soil. Further, MWD by the wet method exhibited significant differences for tillage and N rate, with the same trends as observed in the 0-10 cm soil depth increment. For the nearby sod, MWD (0-10 cm depth increment) by both wet methods was similar to that found in the unfertilized NT soil. In contrast, sod MWD by the dry method was much higher than that associated with any of the tillage by N rate treatments, suggesting that the dry method was more sensitive to a dense root system at sampling. In the 10-20 cm sod soil, MWD by the dry method was similar to that found in the N fertilized soils, while MWD by the wet method was most similar to that found in the unfertilized soils.

Aggregate MWD values by the different methods, for Field Experiment 2, are presented in Table 2.14. In the 0-10 cm soil depth increment, MWD by the dry method was positively and significantly affected by manure application. These results are explained by the fact that manure application generally increases soil organo-mineral interactions due to manure's high concentration of polysaccharides, which favors macroaggregate formation and stability (Juma et al., 1993; Petersen et al., 1999; Werner, 1997). In contrast, MWD by one wet method (larger size fractions) exhibited a significant manure by N rate interaction, with greater MWD for the unfertilized soil especially without manure application. Aggregate MWD by the other wet method (smaller size fractions) did not exhibit any significant differences due to the treatments. These results emphasize the importance of both method and chosen size fraction in aggregate stability analysis and its interpretations.

In the 10-20 cm soil depth increment (Table 2.14), MWD by the dry method exhibited no significant differences due to treatments. Nevertheless, MWD by the large-size wet method did give a significantly negative response to N rate. For the surface (0-10 cm) sod, MWD by both wet methods were most similar to those found in the unfertilized, unmanured soil. In contrast, surface sod MWD by the dry method was much higher than that associated with any of the manure by N rate treatments. In the subsurface (10-20 cm) sod, MWD by the dry method was lower than that observed for the manure by N rate treatments. Aggregate MWD by the larger-size wet method was similar to that found in the unfertilized soils.

Aggregate MWD values for Field Experiment 3 are presented in Table 2.15. The dry and large size wet methods were not able to detect any significant rotation treatment effect on MWD for either 0-10 cm or 10-20 cm soil depth increments. However, the small size wet method exhibited a significant crop rotation effect in the 0-10 cm soil depth increment, where the lowest MWD values were associated with the hhCcc and w/sC rotations. The highest MWD was found in the CCCCC, hhccC and hhcCc rotations. Those results support the findings of Power (1990), who reported that short 2-year corn and soybean rotations often reduce aggregation, relative to well-fertilized continuous corn, presumably because soybean returns less crop residue. Kong et al. (2005) emphasized that long-term management practices that augment residue-C return to the soil will result in increased aggregate stability. On the other hand, these results contrast with reports by other investigators where crop rotations that included legumes and/or grasses were generally beneficial to aggregate stability (Kay, 1990; van Bavel and Schaller, 1950). The surface (0-10 cm) and subsurface (10-20 cm) sod soil exhibited MWD values, by all the methods, which were generally greater than those found in the crop rotation treatments. The only exception was the dry aggregate MWD for the 10-20 cm sod soil, which was lower than that found in the cropped soils.

Water-stable Aggregate Fractionation

Water-stable aggregate weight distribution.

The large management effect on the weight distribution among water-stable aggregate size classes across the three field experiments was very evident in Field Experiment 1 (Figure 2.2). No-tillage gave significantly higher large macroaggregate (>2000 μm) fraction values, which is consistent with Denef et al. (2004) who proposed a reduced macroaggregate turnover as a mechanism promoting C sequestration in temperate NT soils dominated by 2:1 clay mineralogy. On the other hand, the tillage by N rate interaction effect was significant for the rest of the water-stable aggregate size classes. The N fertilized NT soil exhibited the highest small macroaggregate (2000-250 μm) fraction, while the MP soil, especially the N fertilized MP soil, exhibited significantly greater microaggregate (250-53 μm) and the silt plus clay (<53 μm) fractions, consistent with the observations of Mikha and Rice (2004) and John et al. (2005).

The NT soils were dominated (~80%) by equal proportions of large and small macroaggregates, with a slightly higher proportion of small macroaggregates in the N fertilized NT soil. These fractions were followed by the microaggregate fraction (~10-15%) and then by the silt plus clay fraction (~5%). In contrast, the MP soils were dominated (~90%) by equal proportions of microaggregates and small macroaggregates, with a slightly higher proportion of microaggregates in the N fertilized MP soil. These fractions were followed by equal proportions of large macroaggregates and silt plus clay in the unfertilized MP soil. The silt plus clay size fraction was greater than the large macroaggregate fraction in the N fertilized MP soil. The sod soil behaved similarly to the N fertilized NT soil treatment. Analogous to these results, Six et al. (2000b) reported that microaggregates were the most abundant fraction in tilled soils, and the 250-1000 μm fraction was important in NT soils.

The management treatments had no impact on the weight distribution among the water-stable aggregate fractions in Field Experiment 2 (Figure 2.3). However, slightly higher amounts of large and small macroaggregates were observed with manure application. Mikha and Rice (2004) reported a significant increase in macroaggregation after manure application. On the other hand, there were significant differences among the aggregate fractions, within each management treatment. The dominant fraction (~60%) was the small macroaggregates, followed by the large macroaggegates (~20%), the microaggregates (~15%), and the silt plus clay (~5%). In contrast, the sod sample presented equally large amounts of large and small macroaggregates (~43% each), followed by microaggregates (~10%), and silt plus clay (~4%).

The management (rotation) effect in Field Experiment 3 significantly affected the weight distribution of all but the small macroaggregate size fraction (Figure 2.4). The smallest large macroaggregate fraction was observed in the hhCcc rotation, followed by the w/sC rotation. The highest relative weight of microaggregates and clay plus silt was found in the hhCcc rotation, followed by the w/sC rotation. These results are not consistent with a report by Tisdall and Oades (1982), who pointed out that the water-stability of microaggregates depends on soil characteristics, rather than management practices. In addition, differences among the weight proportions for aggregate fractions within each rotation treatment indicated that the CCCCC, hhccC, and hhcCc rotation treatments have equal amounts (~43%) of small and large macroaggregates, followed by the microaggregate (~10%) and silt plus clay (~4%) fractions. On the other hand, the hhCcc and w/sC treatments were dominated (around 50%) by small macroaggregates, followed by the large macroaggegates (~30%), microaggregates (~15%), and the silt plus clay (~5%). The sod soil exhibited an aggregate distribution similar to that observed in the CCCCC, hhccC, and hhcCc treatments.

Organic C and total N in wate-stable aggregate fractions-Field Experiment 1.

Table 2.16 gives the organic C and total N associated with each of the four water-stable aggregate size fractions from Field Experiment 1. Management significantly affected organic C, total N, and the C/N ratio in every aggregate size fraction. In the >2000 μm fraction, organic C, total N, and the C/N ratio were significantly and positively affected by N fertilization. For the 2000-250 μm fraction, organic C and total N were significantly affected by the tillage by N rate interaction. Nitrogen fertilization favored greater organic C and total N, but only in the NT system. On the other hand, the C/N ratio in this size fraction was affected only by tillage, exhibiting higher values in the NT soil. In the 250-53 μm and the <53 μm fractions, organic C and total N were significantly affected by tillage, with higher values found in the NT system. The C/N ratio of the 250-53 μm fraction was also significantly affected by tillage, and it was significantly affected by the tillage by N interaction in the <53 μm fraction.

Additionally, size fractions within a given management treatment exhibited significantly different values for organic C, total N, and C/N ratio. The lowest organic C, total N, and C/N ratio values were associated with the smallest aggregate size class (<53 μm) in all management treatments. The highest organic C, total N, and C/N ratio values were not consistently found in any of the other aggregate size classes.

These results do not completely support the concept of aggregate hierarchy (Tisdall and Oades, 1982; Six et al., 2000b). Increases in C concentration with increasing aggregate class size would be expected, because larger aggregates are composed of smaller aggregates plus organic binding agents (Elliot, 1986). However, the lowest C/N ratio was associated with the silt plus clay fraction, suggesting that this fraction contains more humified organic material and tends to be more enriched in N than C as compared to rest of the separated fractions. The sod sample exhibited organic C, total N, and C/N ratio values within the range observed for the tillage by N rate treatments, for each aggregate size class. The only exception was the sod soil's greater organic C in the 250-53 μm fraction, relative to those observed in the tillage by N rate treatments. In addition, the significant effect of aggregate size class on organic C, total N, and C/N ratios in the sod sample was similar to that observed in the tillage by N rate treatments.

The distribution of organic C and total N among the water-stable aggregate fractions for the whole soil is presented in Figure 2.5 for Field Experiment 1. Tillage affected significantly the amount of organic C and total N in large macroaggregates, while tillage by N rate interaction significantly affected the distribution of organic C and total N among the rest of aggregate size fractions. No-tillage favored higher organic C and total N in the two larger aggregate size fractions, and N fertilization enhanced the tillage effect on organic C and total N in these size fractions only for the NT system. On the other hand, the MP treatment resulted in higher organic C and total N in the two smaller aggregate size fractions, and N fertilization enhanced the tillage effect only in the 250-53 μm size fraction. The large and small macroaggregates contained the majority of organic C and total N in the NT soils, while small macroaggregates and microaggregates contained the majority of organic C and total N in MP soils. In short, the overall trend observed for the mass distribution of organic C and total N among water-stable aggregates in the whole soil resembled the trend observed for the mass distribution of water-stable aggregates (Figure 2.2). These results were not consistent with what was reported by Denef et al. (2004). They found for three soils differing in clay mineralogy [a

2:1 clay dominated soil, a soil with mixed clay mineralogy (2:1, 1:1 and oxide clays), and a soil dominated by 1:1 clay minerals and oxides], that more than 90% of the total difference in soil organic C between NT and MP soils was explained by the difference in microaggregate C.

Organic C and total N in water-stable aggregate fractions-Field Experiment 2.

Table 2.17 presents organic C and total N concentrations for each of the four water-stable aggregate fractions for Field Experiment 2. Management significantly affected organic C and total N concentrations in all of the aggregate fractions but the smallest (<53 μm). In contrast, management significantly affected the C/N ratio only in the <53 μm fraction. In the >2000 μm and 2000-250 μm fractions, organic C and total N were significantly and positively affected by manure. In the 250-53 μm fraction, organic C and total N were significantly affected by both manure and N rate. Higher values were observed with manure application, and lower values were observed with N fertilization. In the <53 μm fraction, the C/N ratio was significantly affected by the tillage by N rate interaction, exhibiting higher values in the unfertilized soil, but only after manure application.

In addition, the size fractions within a given management treatment exhibited significantly different values for organic C, total N, and C/N ratio. The lowest organic C and total N was associated with the smallest aggregate size class (<53 μm); while organic C and total N concentrations were similar among the rest of the aggregate size classes, for all management treatments. Because animal manure provides an additional input of biologically processed material (Christensen, 1992), enrichment of the clay fraction in total C with manure application was expected. However, this was not observed in this study. The lowest C/N ratio was associated with the smallest aggregate size class, but the highest C/N ratio was not associated with any specific aggregate size fraction, regardless of management treatment. The sod sample exhibited slightly lower organic C and total N concentrations, and slightly higher C/N ratios, as compared to those observed among the study treatments, regardless of aggregate size fraction. In addition, the impact of aggregate size fraction on organic C, total N and the C/N ratio in the sod soil was similar to that observed among the manure by N rate treatments.

The distribution of organic C and total N among the water-stable aggregate fractions for the whole soil is presented in Figure 2.6 for Field Experiment 2. Management did not significantly affect the amount of organic C and total N found in any of the aggregate size classes. Nevertheless, a trend for slightly higher organic C and total N was observed with manure application, especially in the two larger aggregate size fractions. On the other hand, small macroaggregates concentrated the majority of organic C and total N, across all management treatments, followed by large macroaggregates, microaggregates, and the silt plus clay fraction. As was observed in Field Experiment 1, the overall trend in the mass distribution of organic C and total N among water-stable aggregates for the whole soil resembled the observed trend in the mass distribution of water-stable aggregates (Figure 2.3).

Organic C and total N in water-stable aggregate fractions-Field Experiment 3.

Table 2.18 presents organic C and total N concentrations found in each of the four water-stable aggregate size fractions from the rotation treatments in Field Experiment 3. In the >2000 μm and 250-53 μm fractions, organic C and total N were significantly affected by crop rotation with the highest concetrations associated with the hhccC and the CCCCC treatments. In the 2000-250 μm fraction, only total N was significantly affected by crop rotation with the highest value found in the hhccC and CCCCC crop rotations. In the <53 μm fraction, total N and the C/N ratio were affected significantly by crop rotation with the highest values found in the hhccC and hhcCc rotations, respectively. These results suggest that long-term management practices that raise the return of residue-C to the soil will result in increased aggregate soil organic C levels and long-term C sequestration (Kuo et al., 1997). In contrast, C quality would explain the low level of aggregate soil organic C found in the w/sC rotation. Martens (2000) reported that soil humic acids decrease under soybean residues, which are low in phenolic acids compared to corn residues.

On the other hand, aggregate size fractions (within a rotation treatment) exhibited significant differences in organic C, total N, and the C/N ratio. The lowest organic C, total N, and C/N ratios were associated with the smallest size class (<53 μm) in all crop rotations. The highest organic C, total N, and C/N ratios were not consistently associated with any aggregate size class across all crop rotations. The sod soil exhibited C/N ratios range similar to those observed in the same aggregate size class for the crop rotation treatments. Organic C and total N were greater in the sod aggregates than in comparable aggregate sizes from the crop rotation treatments, and especially for the 250-53 μm fraction. Similar trends were observed for the sod surrounding Field Experiment 1. In addition, aggregate size fraction significantly impacted organic C, total N, and the C/N ratio in the sod soil, following the same trend as that observed among aggregate sizes in the crop rotation treatments.

The mass distributions for organic C and total N among the water-stable aggregates for the whole soil are presented in Figure 2.7. Crop rotation did not significantly affect the distribution of organic C and total N in any but the large macroaggregate size fraction. The highest organic C and total N portions were associated with the hhccC and CCCCC crop rotations. The large and small macroaggregate fractions concentrated the majority of organic C and total N in all the crop rotation treatments. Lower organic C and total N fractions were associated with the large macroaggregate fractions, compared to the small macroaggregates fraction, in the hhCcc and w/sC crop rotations. The organic C and total N fractions for both large and small macroaggregates were similar across the CCCCC, hhccC, hhcCc crop rotations. The sod soil exhibited organic C and total N distributions, among aggregate fractions, similar to that observed for the hhccC crop rotation. In agreement with Field Experiments 1 and 2, the mass distribution of organic C and total N among water-stable aggregates for the whole soil resembled that observed for the mass distribution of the water-stable aggregates themselves (Figure 2.4).

Isolation of Intra-aggregate Fractions from Large and Small Macroaggregates

Intra-aggregate particle size distribution.

Table 2.19 presents the distribution of intra-aggregate particle sizes of Field Experiment 1. Treatment effects were significant on all but the free and occluded fractions. The tillage by N rate interaction effect was significant for the coarser fractions (>250 μm_{SM} and 250-53 μm_{SM}). A greater value for the >250 μm_{SM} fraction was associated with the MP soils, especially with N fertilization. Greater values for the 250-

53 μm_{SM} fraction were found with the NT system, especially with N fertilization. The N rate significantly affected the finest fraction (<53 μm_{SM}) exhibiting lower values with N fertilization. On the other hand, there were significant differences in the mass distribution among the intra-aggregate fractions for a given management treatment. The <53 μm_{SM} fraction dominated all the tillage by N rate treatments and the nearby sod. The 250-53 μm_{SM} fraction was the second largest fraction in the NT soils and for the sod sample; while the >250 μm_{SM} was the second largest fraction in the MP soils. The free and the occluded fractions were the least important intra-small macroaggregate fractions in the sod and tillage by N rate treatments.

Table 2.20 gives the intra-small macroaggregate mass distribution found in Field Experiment 2. Management effects were not significant in any of the fractions. Conversely, significant differences in the mass distribution of intra-small macroaggregate fractions within each management treatment were observed. The <53 μm_{SM} fraction dominated small macroaggregates in Field Experiment 2, including the nearby sod, followed by the 250-53 μm_{SM}, >250 μm_{SM}, free, and occluded fractions.

The mass distribution of particulate materials within the large macroaggregates found in Field Experiment 3 is presented in Table 2.21. Crop rotation significantly affected only the free fraction. The highest free fraction was associated with the hhccC and w/sC rotations. Significant differences in the mass distribution fractions, for a given crop rotation treatment, were found. The <53 μm_{LM} fraction dominated, followed by the 250-53 μm_{LM}, the >250 μm_{LM}, free, and occluded fractions in both sod and all crop rotation treatments except for w/sC, for which the >250 μm_{LM} fraction was the second most abundant mass fraction.

Organic C and total N in intra-macroaggregate fractions-Field Experiment 1.

Table 2.22 presents organic C and total N concentrations (on both intra and inter aggregate basis) associated with each of the five intra-small macroaggregate fractions in Field Experiment 1. Organic C and total N and the C/N ratio of all separated fractions were significantly affected by management, except for the C/N ratio of the occluded fraction. However, there was no consistency in the management effect (tillage, N rate, or tillage by N rate interaction) that significantly affected these concentrations /ratio. These results indicate that the light fraction is not necessarily the most sensitive to management practices, as stated by Bremer et al. (1994) and Franzluebbers and Stuedemann (2002). This could be explained because in this study the light fraction was not isolated from the whole soil, and also that the fractionation efficiency of light fractions and their SOM content are sensitive to the density of fractionation liquids (Ladd and Amado, 1980).

On the other hand, the particular intra-aggregate fraction, within a given management treatment, significantly affected organic C, total N, and C/N ratio. The highest organic C and total N were observed in the 250-53 μm_{SM} fraction, in all of the management treatments, while the lowest organic C and total N were associated with the occluded fraction. These results are similar to those reported by Hamblin (1977) and Cameron and Poster (1979), who reported significant accumulations of SOM in silt and sand size separates. Degryze et al. (2004) and Kong et al. (2005) also reported that most of the soil organic C accumulation due to additional C inputs was preferentially stored in microaggregates-within-small-macroaggregates. In addition, the C/N ratios decreased in the order: free>occluded>250 μm_{SM}>250-53 μm_{SM}>53 μm_{SM}, indicating an increasing degree of degradation and humification of the organic matter, in this order, for all of the management treatments. These results are consistent with John et al. (2005) and Christensen (1992), who pointed out that the free or noncomplexed SOM consists of decomposing plant and animal residues with a relatively high C/N ratio; while the heavy fraction, which includes the organomineral complexed SOM, exhibits a lower C/N ratio. The sod sample exhibited values for organic C, total N, and C/N ratio that fell within the range observed for the NT system.

The distribution of organic C and total N among the intra-macroaggregate fractions is shown in (Figure 2.8). The management effect on organic C and total N was no longer significant in the free and occluded fractions, due to their small proportion. The highest organic C and total N was associated with the <53 μm_{SM} fraction, followed by the 250-53 μm_{SM}, the >250 μm_{SM}, the free, and the occluded fractions, for all of the tillage by N rate treatments. These results are in agreement with those of John et al. (2005). They reported that most of the soil organic C is associated with the heavy mineral fraction. For the sod sample, the highest organic C and total N were observed for the <53 μm_{SM} and the 250-53 μm_{SM} fractions, followed by the >250 μm_{SM}, the free and the occluded fractions. These results were consistent with trends observed among the intra-macroaggregate size fractions (Table 2.19).

Organic C and total N in intra-macroaggregate fractions-Field Experiment 2.

Table 2.23 presents organic C and total N concentrations (on both intra-small macroaggregate and small macroaggregate fraction basis) for each of the five intra-small macroaggregate fractions of Field Experiment 2. Organic C and total N in intra-small macroaggregate fractions exhibited significant management effects. For the free and the occluded fractions, the interaction of manure by N rate was significant for organic C, total N and the C/N ratio. The exception was total N in the occluded fraction, which did not exhibit any significant management effect. For the >250 μm_{SM} fraction, the main effect of manure was significant for organic C and total N. For the 250-53 μm_{SM} fraction, the interaction of manure by N rate was significant for organic C and total N. In contrast, the <53 μm_{SM} fraction was not significantly affected by management. In addition, the type of intra-small macroaggregate fraction, within a management treatment, significantly affected organic C, total N, and the C/N ratio. The highest organic C and total N was observed for the 250-53 μm_{SM} fraction in all of the management treatments and for the sod sample; while the lowest organic C and total N was associated with the occluded fraction. The C/N ratio exhibited the highest value in the free fraction, followed by the occluded, the >250 μm_{SM}, the 250-53 μm_{SM}, and the <53 μm_{SM} fractions, for all of the management treatments and for the sod sample. These results are consistent with those observed in Field Experiment 1 and reflect the dominant influence of plant litter on light fractions.

The distribution of organic C and total N among the intra-macroaggregate fractions within the small macroaggregate fraction exhibited less management influence. Organic C in the free and occluded fractions was not significantly affected by management, nor was total N in the free, occluded, and the <53 μm_{SM} fractions. In addition, the highest organic C and total N levels were found in the <53 μm_{SM} fraction, followed by the 250-53 μm_{SM}, the >250 μm_{SM}, the free and the occluded fractions, for all of the manure by N rate treatments and for the sod sample. These results were driven by the weight (mass) intra-small macroaggregate distribution (Table 2.20).

On a whole soil basis, the distribution of organic C and total N among the intra-small macroaggregate fractions (Figure 2.9) was significantly affected by management only for the >250 μm_{SM} fraction. Higher organic C and total N were observed with manure application. On a whole soil basis, the highest organic C and total N concentrations were observed for the <53 μm_{SM} fraction, followed by the 250-53 μm_{SM}, the >250 μm_{SM}, the free and the occluded fractions, in all manure by N rate treatments and for the sod sample. Those results were consistent with what was observed for the distribution of organic C and total N among the intra-small macroaggregate fractions, but expressed on the whole small macroaggregate basis.

Organic C and total N in intra-large macroaggregate fractions-Field Experiment 3.

Table 2.24 presents organic C and total N (on both a large intra-large macroaggregate and a large macroaggregate fraction basis) for each of the five intra-large macroaggregate fractions of samples taken in Field Experiment 3. Organic C and total N in all intra-large macroaggregate fractions but the >250 μm_{LM} fraction were significantly affected by crop rotation. The C/N ratio was significantly affected by crop rotation only in the free and the 250-53 μm_{LM} fractions. Organic C, total N, and the C/N ratio of the sod sample were in a range similar to that observed for the crop rotation treatments, for each intra-large macroaggregate fraction. In addition, the particular intra-large macroaggregate fraction, within a management treatment, significantly affected organic C, total N, and the C/N ratio. The highest organic C and total N concentrations were found in the 250-53 μm_{LM} fraction in all of the management treatments and also in the sod sample. The lowest organic C and total N concentrations were associated with the occluded fraction. The expected enrichment of the silt fraction in SOM derived from decomposition of plant residues (Christensen, 1992) was not observed in this soil. The C/N ratio exhibited the highest value in the free fraction, followed by the occluded, the >250 μm_{LM}, the 250-53 μm_{LM}, and the <53 μm_{LM} fractions for the sod sample and for all crop rotations but the CCCCC. Continuous corn exhibited the lowest C/N ratio in the <53 μm_{LM} fraction of all the crop rotations.

The distribution of organic C and total N among the intra-large macroaggregate fractions (large macroaggregate fraction basis) was influenced by rotation only for organic C in the >250 μm$_{LM}$ fraction, where the highest organic C was associated with the hhCcc crop rotation. In addition, the highest organic C and total N distribution, among the intra-aggregate fractions within a crop rotation, were observed for the <53 μm$_{LM}$ fraction, followed by the 250-53 μm$_{LM}$, the >250 μm$_{LM}$, the free and the occluded fractions, across all crop rotation treatments and the sod sample. These results were consistent with the trend in mass distribution among the intra-large macroaggregate fractions (Table 2.20) and with higher organic C concentrations observed in the 250-53 μm$_{LM}$ fraction, as compared to the >250 μm$_{LM}$ fraction.

The distribution of organic C and total N among intra-large macroaggregate fractions, on a whole soil basis (Figure 2.10) showed that organic C was significantly affected by crop rotation only in the >250 μm$_{LM}$ fraction, while total N was significantly affected by crop rotation in both the >250 μm$_{LM}$ and <53 μm$_{LM}$ fractions. The highest organic C and total N concentrations (whole soil basis) were observed in the hhccC crop rotation, followed by CCCCC rotation. Additionally, the highest whole soil organic C and total N concentrations, among the intra-large macroaggregate fractions within a crop rotation, were observed for the <53 μm$_{LM}$ fraction, followed by the 250-53 μm$_{LM}$, the >250 μm$_{LM}$, the free, and the occluded fractions, for all of the crop rotations and for the sod sample. Those results were consistent with what was observed for the mass distribution of organic C and total N among the intra-large macroaggregate fractions within the large macroaggregate fraction.

Summary of C and N stocks in the soil physical fractions

The distributions of C and N stocks in the soil physical fractions for Field Experiments 1, 2 and 3 are presented in Tables 2.25, 2.26, and 2.27, respectively. Across all three field experiments, the sum of organic C and total N from water-stable aggregate fractions closely mached the organic C and total N of the whole soil, suggesting that the loss of C as dissolved organic C (DOC) during the wet sieving procedure was negligible, and that most of the C in these soils was organic in nature and was physically protected. There was a high (98-100%) recovery index in this fractionation procedure (data not shown).

On the other hand, the sum of C stocks from the intra-macroaggregate fractions, for every field experiment, was lower than that observed for the respective macroaggregate fraction. The sum of N stocks closely matched that observed for the respective macroaggregate fraction. The recovery for this fractionation procedure was also high (96-100%), but the greater loss of C suggested that DOC was relatively more important C form within macroaggregates and/or that the SPT solution was able to complex part of the DOC pool. In addition, the small size of the recovered light fractions could have decreased the accuracy of organic C determination.

Conclusions

Soil physical and chemical properties are significantly affected by management practices, and the effects are specific for each soil depth in each agro-ecosystem evaluated. Most of the tillage, manure, and crop rotation effects are reflected in the soil surface layer, showing that NT, manure application, and increased corn in the crop rotation sequence clearly enhanced soil gravimetric water content, while N application and crop rotation have a major impact on soil pH and bulk density and manure application enhanced the overall soil nutrient status. Additionally, soil K content deserves special attention, because it responded significantly to all management practices, across all three field experiments especially in the soil surface layer.

Aggregate stability analysis by the wet sieving method was more effective in detecting management effects than the dry method. Aggregate stability, as indicated by MWD, was decreased by N application, but enhanced by NT, manure application, and increased corn in the crop rotation. Across all three field experiments, the water-stable aggregate size distribution was mainly influenced by tillage, as MP especially destroyed large macroaggregates, and NT favored small-macroaggregation. The increased use of corn in the crop rotation enhanced the size of the large-macroaggregate fraction.

Total C and N storage capacity of whole soils was affected by tillage, N and manure application, and by crop rotation. No-tillage increased surface soil total C and N compared to MP, and N application enhanced the effect of NT on total C and N storage. In the soil subsurface (10-20 cm soil depth increment), tillage no longer affected total soil C storage, but N application still favored higher levels of total C, independent of the tillage system. Animal manure application enhanced surface and subsurface total soil C, and only surface soil total N. Continuous corn, and hhCcc enhanced surface soil total C and N. In addition, stratification of total C and N were associated with NT across all three field experiments, exhibiting their highest concentrations at the soil surface. On the other hand, total C and N concentrations in whole surface soils varied seasonally in NT soils, with the highest values found in spring. When manure was applied to a NT system, the highest values for total C and N were during fall. Crop rotation effects on total C and N storage were more evident during fall than spring.

Soil organic C and total N in water-stable aggregates were affected by management practices. However, not all the management effects were consistently reflected at every water-stable aggregate size. No-till and N application enhanced soil organic C and total N across all water-stable aggregate fractions, while manure application enhanced soil organic C and total N in all but the smallest water-stable aggregate fraction. Increasing corn in the crop rotation enhances soil organic C and total N in large macroaggregates and microaggregates, and only total N in the rest of the water-stable fractions.

The concept of aggregate hierarchy was not observed among the three biggest water-stable aggregate size classes, across the three field experiments. However, the decreasing C/N ratios with decreasing aggregate size suggest that soil organic C in the silt and clay fraction is older and less labile than soil organic C in the rest of water-stable aggregates. In addition, the storage capacity of the water-stable aggregates for soil organic C and total N was greatly determined by the management practices that controlled their aggregate size distribution. Increased soil organic C and total N storage was closely associated with small- macroaggregates and microaggregates with MP, with large macroaggregates in the NT corn crop rotations, and with large- and small-macroaggregates under NT and manure application.

Management practices affected the mass distribution, and the soil organic C and total N concentrations of the intra-macroaggregate fractions. Tillage and N rate affected the mass distribution among the coarse intra-macroaggregates. Crop rotation affected the free fraction. Nevertheless, silt and clay were the dominant fraction in the studied water-stable macroaggregate fractions, across the three field experiments. There was no consistency in the type of management effect on soil organic C and total N concentrations associated with each intra-macroaggregate fraction, across the three field experiments. The most and least soil organic C or total N enriched intra-macroaggregate fractions were the microaggregate (250-53 μm_{SM} and 250-53 μm_{LM}) and occluded fractions, respectively. However, the contribution of soil organic C and total N from the intra-macroaggregate fractions to their respective macroaggregate fraction, or to whole soil, was consistent with their respective mass distributions. In addition, an increasing degree of degradation and humification of the SOM, taken as free<occluded<250 μm$_{SM}$<250-53 μm$_{SM}$<53 μm$_{SM}$ was suggested by the C/N ratios observed.

Physical and chemical characterization of the nearby sod soil, across all three field experiments, showed that row-cropping has decreased total C and N contents in the whole soil, especially when MP tillage has been adopted. Also, the major differences in physical and chemical properties between sod soils and soils from the row-cropped systems are related to soil pH. In terms of aggregate stability, both NT with N application and continuous corn were the management treatments resulting in soil aggregate stabilities similar to that of the nearby sod, suggesting an important role of soil pH on aggregation. Finally, the distribution of organic C and total N among water-stable aggregate fractions, for the whole soil under sod, followed the same trend as that observed for the row-cropped soils. In contrast, the distribution of organic C and total N among the intra-macroaggregate fractions, for the respective whole macroaggregate fraction, exhibited higher microaggregation.

Tables

Table 2.1. Soil properties from the spring sampling (0-10 cm and 10-20 cm soil depth increment) in Field Experiment 1-tillage by N rate.

Tillage	N Rate	pH_{Water}	Mehlich III extractable (mg/kg):						Θ_g	D_b	Sand	Silt	Clay
	(kg ha^{-1})		P	K	Ca	Mg	Zn	Mn	(%)	(g cm^{-3})		(%)	
								0-10 cm					
NT	0	6.66†	242a‡	488a‡	2421§	143¶	5.5#	108ns	28.9b‡	1.18¶	12.8b††	71.4§	15.9§
	150	6.42	255a	383b	2568	98	5.0	110	30.2a	1.13	14.2a	71.0	14.8
MP	0	6.27	215b	290c	2045	140	1.9	114	25.8c	1.18	11.3b	67.9	20.7
	150	5.96	196b	229d	1882	95	3.6	115	25.7c	1.13	10.2c	69.3	20.5
Sod		5.67	232	350	1603	183	3.7	92	31.7	1.14	14.8	72.1	13.1
								10-20 cm					
NT	0	6.46a††	200¶	412a††	2139#	120†	1.5#	75§	25.2ns	1.31ns	11.6§	68.6¶	19.8†
	150	5.90b	176	249bc	1799	74	1.4	65	25.9	1.28	10.8	70.8	18.4
MP	0	6.31a	211	256b	2186	148	1.9	114	25.2	1.30	10.3	67.4	22.3
	150	6.34a	188	172c	2163	102	2.2	120	24.4	1.26	10.3	69.7	20.1
Sod		6.01	201	216	1832	141	1.5	68	26.0	1.29	10.8	71.2	18.0

† Main effect of tillage and N rate statistically significant ($P<0.05$)
‡ Interaction effect of tillage by N rate statistically significant ($P<0.10$)
§ Main effect of tillage statistically significant ($P<0.05$)
¶ Main effect of N rate statistically significant ($P<0.05$)
# Main effect of tillage statistically significant ($P<0.10$)
†† Interaction effect of tillage by N rate statistically significant ($P<0.05$)
ns = Not statistically significant ($P>0.10$)
Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column
NT (no-tillage); MP (moldboard plow); Θ_g (gravimetric water content); D_b (bulk density)

Table 2.2. Soil properties from the fall sampling (0-5 cm and 5-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

Tillage	N Rate	pH_{Water}	Mehlich III extractable (mg/kg):						Θ_g	D_b	Sand	Silt	Clay
	(kg ha^{-1})		P	K	Ca	Mg	Zn	Mn	(%)	(g cm^{-3})		(%)	
								0-5 cm					
NT	0	6.16ns	220 b†	421‡	1729ns	123§	8.9‡	108ns	32.1b†	1.35¶	12.8¶	71.8a#	15.4c†
	150	5.94	272a	429	2147	89	10.6	108	37.3 b	1.19	14.1	70.4 a	15.5 c
MP	0	6.22	212bc	284	2016	136	1.9	105	26.5 c	1.47	9.5	67.6 b	22.9 a
	150	5.91	199c	316	1940	96	1.7	236	28.0 c	1.35	10.6	67.9 b	21.5 b
								5-10 cm					
NT	0	6.19ns	196b†	359¶	1926ns	118¶	2.9‡	62c†	26.7b#	1.5§	10.2§	70.5‡	19.3¶
	150	6.25	235a	277	2262	70	2.4	79b	28.7a	1.3	10.7	70.4	18.8
MP	0	6.33	204b	270	2141	140	1.9	104a	26.4b	1.5	9.6	67.3	23.1
	150	6.29	190b	220	2236	102	1.6	99a	27.0ab	1.4	10.4	68.3	21.4

† Interaction effect of tillage by N rate statistically significant ($P<0.05$)

‡ Main effect of tillage statistically significant ($P<0.05$)

§ Main effect of N rate statistically significant ($P<0.05$)

¶ Main effect of tillage and N rate statistically significant ($P<0.05$)

Interaction effect of tillage by N rate statistically significant ($P>0.10$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

NT (no-tillage); MP (moldboard plow); Θ_g (gravimetric water content); D_b (bulk density)

Table 2.3. Soil properties from the spring sampling (0-10 cm and 10-20 cm soil depth increment) in Field Experiment 2-manure by N rate.

Manure	N Rate	pH_{Water}	Mehlich III extractable (mg/kg):						Θ_g	D_b	Sand	Silt	Clay
	(kg ha^{-1})		P	K	Ca	Mg	Zn	Mn	(%)	(g cm^{-3})		(%)	
								0-10 cm					
Unmanured	0	6.26†	146†	318†	1751†	288‡	7.4†	145ns	26.1§	1.24ns	11.2ns	74.6ns	14.2ns
	168	5.66	122	253	1407	231	6.5	141	25.8	1.27	11.3	74.1	14.5
Manured	0	6.53	321	601	2158	327	16.8	154	29.7	1.16	11.3	74.2	14.5
	168	5.87	305	515	1741	236	14.9	123	28.3	1.21	12.9	71.8	15.2
Sod		5.48	146	140	1167	134	1.9	138	29.3	1.20	13.7	73.3	13.0
								10-20 cm					
Unmanured	0	5.77ns	107ns	132†	1333ab#	181b¶	1.5ns	90ns	24.9ns	1.29ns	9.3ns	73.6ab¶	17.1a#
	168	5.79	93	88	1333ab	202ab	1.5	86	25.5	1.31	8.5	73.9ab	17.6a
Manured	0	6.18	184	339	1700a	234a	5.3	100	26.0	1.29	10.9	74.4a	17.8b
	168	5.86	166	281	1493b	196ab	3.2	97	26.3	1.26	10.6	71.8b	17.6a
Sod		5.71	115	63	1285	78	1.0	88	25.9	1.29	11.0	72.0	17.0

† Main effect of manure and N rate statistically significant ($P<0.05$)

‡ Main effect of N rate statistically significant ($P<0.05$)

§ Main effect of manure statistically significant ($P<0.05$)

¶ Interaction effect of manure by N rate statistically significant ($P<0.05$)

Interaction effect of manure by N rate statistically significant ($P<0.10$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

Θg (gravimetric water content); Db (bulk density)

Table 2.4. Soil properties from the fall sampling (0-5 cm and 5-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

Manure	N Rate (kg ha^{-1})	pH$_{Water}$	Mehlich III extractable (mg/kg): P	K	Ca	Mg	Zn	Mn	Θ_g (%)	D_b (g cm^{-3})	Sand	Silt (%)	Clay
								0-5 cm					
Unmanured	0	6.30‡	179†	321‡	1755‡	286‡	7.5§	135ns	31.8ns	1.32ns	10.9¶	73.9ab#	15.2a††
	168	5.79	163	291	1507	225	6.4	125	30.5	1.31	12.1	73.8ab	14.1b
Manured	0	6.63	331	602	2389	343	16.9	113	35.0	1.27	10.8	75.6a	13.6ab
	168	6.23	324	520	2077	271	16.8	103	34.9	1.25	13.1	72.9b	14.0ab
								5-10 cm					
Unmanured	0	6.17ab#	152†	157‡	1609b#	253ns	6.9§	95ns	27.2§	1.37§	9.6ns	75.2ns	15.3ns
	168	6.18ab	133	114	1661ab	241	4.7	83	26.9	1.39	9.9	73.1	17.0
Manured	0	6.62a	302	493	2154a	315	11.9	93	30.5	1.33	9.6	75.2	15.3
	168	6.20b	278	356	1986ab	265	10.9	80	30.4	1.25	12	73.2	14.8

† Main effect of manure statistically significant (P<0.10)

‡ Main effect of manure and N rate statistically significant (P<0.05)

§ Main effect of manure statistically significant (P<0.0001)

¶ Main effect of N rate statistically significant (P<0.05)

Interaction effect of manure by N rate statistically significant (P<0.10)

†† Interaction effect of manure by N rate statistically significant (P<0.05)

ns = Not statistically significant (P>0.10)

Different letters indicate significant differences (P<0.05, Fisher's LSD) within a column

Θ_g (gravimetric water content); D_b (bulk density)

Table 2.5. Soil properties from the spring sampling (0-10 cm and 10-20 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

Crop Rotation	pH_{Water}	Mehlich III extractable (mg/kg):						Θ_g	D_b	Sand	Silt	Clay
		P	K	Ca	Mg	Zn	Mn	(%)	(g cm^{-3})		(%)	
							0-10 cm					
CCCCC	6.06ns	47.8ns	282ns	1600ns	171ns	4.5ns	104b†	27.47ab†	1.22ns	15.3ns	70.8ns	13.9ns
hhccC	6.31	53.0	234	1993	203	4.8	99b	29.2 a	1.16	16.5	68.9	14.6
hhcCc	6.69	45.5	213	1966	206	5.0	114ab	27.4 b	1.19	14.8	70.6	14.7
hhCcc	6.50	43.0	209	2071	193	4.4	135a	26.5 b	1.20	14.9	69.5	15.6
w/sC	6.31	44.5	170	1857	195	7.1	117ab	27.4 b	1.21	15.5	70.4	14.2
Sod	6.91	80.6	254	2379	242	4.1	98	24.2	1.07	17.2	70.6	12.1
							10-20 cm					
CCCCC	5.88ns	33.5ns	146ns	1435ns	99ns	1.8ns	85ns	25.1ns	1.31ns	13.8ns	70.0ns	16.7ns
hhccC	6.17	40.0	83	1765	106	2.2	87	24.7	1.31	15.0	67.7	17.4
hhcCc	6.44	42.3	92	1677	121	3.9	79	24.8	1.31	13.8	70.0	16.2
hhCcc	6.31	40.5	84	1675	107	4.3	93	25.0	1.31	13.6	69.0	17.4
w/sC	6.09	36.5	88	1506	99	2.2	80	24.2	1.31	14.5	69.2	16.3
Sod	6.26	61.0	150	1619	128	2.1	92	23.0	1.24	16.6	70.3	13.1

† Effect of crop rotation statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

Θ_g (gravimetric water content); D_b (bulk density)

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation); hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation)

hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/doble crop soybean crop rotation)

Table 2.6. Soil properties from the fall sampling (0-5 cm and 5-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

Crop Rotation	pH_{Water}	Mehlich III extractable (mg/kg):						Θ_g	D_b	Sand	Silt	Clay
		P	K	Ca	Mg	Zn	Mn	(%)	(g cm^{-3})		(%)	
							0-5 cm					
CCCCC	6.17b†	49.5ns	390b‡	1634ns	179ns	4.1ns	90c†	30.6ns	1.17a†	14.7ns	72.6ns	12.8ns
hhccC	6.65ab	48.8	373b	1895	212	5.0	108bc	31.3	1.08a	14.3	72.2	13.6
hhcCc	6.49b	44.8	350b	1728	197	3.9	120ab	29.2	1.14a	15.4	70.6	14.0
hhCcc	6.58ab	51.0	548a	1823	214	4.6	89c	30.7	0.98b	15.9	70.4	13.7
w/sC	7.12a	56.0	416ab	2142	239	4.2	144a	31.7	1.15a	17.0	70.8	12.2
							5-10 cm					
CCCCC	6.20b‡	41.3ns	255a‡	1573b†	165ns	3.4ns	76 ns	27.5ns	1.30a†	13.3c†	72.5a†	14.2ns
hhccC	6.76a	38.8	169ab	1878a	173	4.1	85	27.3	1.26a	13.6c	70.1ab	16.3
hhcCc	6.61ab	35.5	141b	1857a	155	3.4	89	26.9	1.24ab	14.2bc	69.5ab	16.3
hhCcc	6.59ab	38.0	247a	1782ab	187	4.1	73	28.0	1.15b	15.8a	68.3b	16.0
w/sC	7.03a	41.3	190ab	2033a	198	3.4	88	26.6	1.33a	15.2ab	70.7ab	14.1

† Effect of crop rotation statistically significant ($P<0.05$)

‡ Effect of crop rotation statistically significant ($P<0.10$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

Θ_g (gravimetric water content); D_b (bulk density)

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation); hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation)
hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/doble crop soybean crop rotation)

Table 2.7. Total C and N concentration of the whole soil from the spring sampling (0-10 cm and 10-20 cm soil depth increments) in Field Experiment 1-tillage by N rate.

Tillage	N Rate	0-10 cm			10-20 cm		
	(kg ha^{-1})	Total C	Total N	C/N	Total C	Total N	C/N
		(%)			(%)		
NT	0	2.16b†	0.20b†	10.79‡	1.58§	0.16§	10.23a¶
	150	2.73a	0.25a	10.92	1.65	0.17	10.02a
MP	0	1.52c	0.15c	10.16	1.46	0.15	10.09a
	150	1.58c	0.16c	10.19	1.69	0.16	10.52a
Sod		2.85	0.28	10.05	1.61	0.16	9.85

† Interaction of tillage by N rate statistically significant ($P<0.05$)

‡ Only main effect of tillage statistically significant ($P<0.05$)

§ Only main effect of N rate statistically significant ($P<0.05$)

¶ Interaction of tillage by N rate statistically significant ($P<0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

NT (no-tillage); MP (moldboard plow)

Table 2.8. Total C and N concentrations of the whole soil from the fall sampling (0-10 cm, 0-5 cm and 5-10 cm soil depth increments) in Field Experiment 1-tillage by N rate.

Tillage	N Rate	0-10 cm			0-5 cm			5-10 cm		
	(kg ha^{-1})	Total C	Total N	C/N	Total C	Total N	C/N	Total C	Total N	C/N
		(%)			(%)			(%)		
NT	0	1.85b†	0.19‡	9.64b†	2.16b†	0.22b†	9.99‡	1.71‡	0.18‡	9.38§
	150	2.34a	0.23	10.19a	2.88a	0.28a	10.40	1.94	0.20	9.62
MP	0	1.46d	0.16	9.32b	1.45d	0.16d	9.39	1.46	0.16	9.27
	150	1.65c	0.18	9.38b	1.75c	0.18c	9.77	1.63	0.17	9.42

† Interaction of tillage by N rate statistically significant ($P<0.05$)

‡ Only main effect of tillage and N rate statistically significant ($P<0.0001$)

§ Only main effect of N rate statistically significant ($P<0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

NT (no-tillage); MP (moldboard plow)

Table 2.9. Total C and N concentrations of the whole soil from the spring sampling (0-10 cm and 10-20 cm soil depth increments) in Field Experiment 2-manure by N rate.

Manure	N Rate	0-10 cm			10-20 cm		
	(kg ha^{-1})	Total C	Total N	C/N	Total C	Total N	C/N
		(%)			(%)		
Unmanured	0	2.09†	0.20‡	10.51ab§	1.47‡	0.15ns	9.92ns
	168	2.05	0.19	10.69ab	1.40	0.14	9.95
Manured	0	2.60	0.24	10.72a	1.66	0.16	10.35
	168	2.40	0.24	10.04b	1.58	0.15	10.50
Sod		2.34	0.23	10.35	1.45	0.15	9.87

† Only main effect of manure statistically significant ($P<0.001$)

‡ Only main effect of manure statistically significant ($P<0.10$)

§ Interaction effect of manure by N rate statistically significant ($P<0.10$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

Table 2.10. Total C and N concentrations of the whole soil from the fall sampling (0-10 cm, 0-5 cm and 5-10 cm soil depth increments) in Field Experiment 2-manure by N rate.

Manure	N Rate	0-10 cm			0-5 cm			5-10 cm		
	(kg ha^{-1})	Total C	Total N	C/N	Total C	Total N	C/N	Total C	Total N	C/N
		(%)			(%)			(%)		
Unmanured	0	2.00b†	0.21c‡	9.82ns	2.33§	0.22§	10.42c‡	1.70§	0.18§	9.35ns
	168	1.93b	0.20c	9.76	2.36	0.23	10.60b	1.67	0.18	9.28
Manured	0	2.49a	0.25b	9.77	2.85	0.29	9.70d	2.23	0.23	9.24
	168	2.71a	0.28a	9.70	3.10	0.31	10.66a	2.29	0.24	9.39

† Interaction of manure by N rate statistically significant ($P<0.10$)

‡ Interaction of manure by N rate statistically significant ($P<0.01$)

§ Only main effect of manure statistically significant ($P<0.001$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

Table 2.11. Total C and N concentrations of the whole soil from the spring sampling (0-10 and 10-20 cm soil depth increments) in Field Experiment 3-corn in crop rotations.

Crop Rotation	0-10 cm			10-20 cm		
	Total C	Total N	C/N	Total C	Total N	C/N
	(%)			(%)		
CCCCC	2.15ns	0.22ns	10.02ns	1.32ns	0.14ns	9.68ns
hhccC	2.17	0.22	10.01	1.33	0.14	9.88
hhcCc	1.96	0.19	10.38	1.25	0.13	9.62
hhCcc	2.12	0.20	10.53	1.33	0.14	9.60
w/sC	2.00	0.20	10.36	1.23	0.12	9.97
Sod	2.60	0.25	10.51	1.49	0.15	9.86

ns = Not statistically significant (P>0.10)

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation)

hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation); hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation)

w/sC (corn in corn-wheat/double crop soybean crop rotation)

Table 2.12. Total C and N concentrations of the whole soil from the fall sampling (0-10 cm, 0-5 cm and 5-10 cm soil depth increments) in Field Experiment 3-corn in crop rotations.

Crop Rotation	0-10 cm			0-5 cm			5-10 cm		
	Total C	Total N	C/N	Total C	Total N	C/N	Total C	Total N	C/N
	(%)			(%)			(%)		
CCCCC	2.20a†	0.23ns	9.58b‡	2.66a†	0.26ab‡	10.07ab‡	1.97a‡	0.21a‡	9.35ns
hhccC	2.14ab	0.22	9.61ab	2.39ab	0.25b	9.85bc	1.61c	0.18c	9.04
hhcCc	1.86b	0.20	9.22b	2.28b	0.24b	9.56c	1.58c	0.18c	8.77
hhCcc	2.24a	0.24	9.58b	2.61a	0.27a	9.52c	1.8ab	0.20ab	9.02
w/sC	2.17a	0.22	10.05a	2.51ab	0.24b	10.42a	1.71bc	0.19bc	9.11

† Crop rotation effect statistically significant (P<0.10)

‡ Crop rotation effect statistically significant (P<0.05)

ns = Not statistically significant (P>0.10)

Different letters indicate significant differences (P<0.05, Fisher's LSD) within a column

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation)

hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation); hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation)

w/sC (corn in corn-wheat/double crop soybean crop rotation)

Table 2.13. Mean weight diameter from the spring sampling (0-10 cm and 10-20 cm soil depth increments) in Field Experiment 1-tillage by N rate.

Tillage	N Rate	0-10 cm			10-20 cm	
	(kg ha^{-1})	MWD$_d$	MWD$_w$	MWD$_{we}$	MWD$_d$	MWD$_w$
				(mm)		
No-tillage	0	5.82ns	4.64†	2.47†	17.81‡	4.16†
	150	6.46	4.07	2.30	13.45	2.42
Moldboard Plow	0	5.89	2.14	0.94	17.69	3.10
	150	4.78	1.26	0.59	13.49	1.89
Sod		12.61	5.29	2.57	13.02	3.34

† Main effect of tillage and N rate statistically significant ($P<0.05$)
‡ Main effect of N rate statistically significant ($P<0.05$)
ns = Not statistically significant ($P>0.10$)
Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column
MWDd = Mean weight diameter based on the dry sieving method (16-1 mm size fractions)
MWDw = Mean weight diameter based on the wet sieving method (5-0.5 mm size fractions)
MWDwe = Mean weight diameter based on the wet sieving method (2-0.053 mm size fractions)

Table 2.14. Mean weight diameter from the spring sampling (0-10 cm and 10-20 cm soil depth increments) in Field Experiment 2-manure by N rate.

Manure	N Rate	0-10 cm			10-20 cm	
	(kg ha^{-1})	MWD$_d$	MWD$_w$	MWD$_{we}$	MWD$_d$	MWD$_w$
				(mm)		
Unmanured	0	4.78†	4.72a‡	1.60ns	14.29ns	3.59a§
	168	3.97	2.26b	1.75	13.67	1.69b
Manured	0	6.65	3.72ab	1.88	10.67	3.57a
	168	8.12	3.04b	1.87	14.66	1.73b
Sod		13.61	5.02	2.61	10.40	4.06

† Main effect of manure statistically significant ($P<0.10$)
‡ Interaction effect of manure by N rate ($P<0.10$)
§ Main effect of N rate statistically significant ($P<0.05$)
ns = Not statistically significant ($P>0.10$)
Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column
MWD$_d$ = Mean weight diameter based on the dry sieving method (16-1 mm size fractions)
MWD$_w$ = Mean weight diameter based on the wet sieving method (5-0.5 mm size fractions)
MWD$_{we}$ = Mean weight diameter based on the wet sieving method (2-0.053 mm size fractions)

Table 2.15. Mean weight diameter from the spring sampling (0-10 cm and 10-20 cm soil depth increments) in Field Experiment 3-corn in crop rotations.

Crop Rotation	0-10 cm			10-20 cm	
	MWD_d	MWD_w	MWD_{we}	MWD_d	MWD_w
			(mm)		
CCCCC	8.86ns	4.00ns	2.54a†	10.30ns	2.16ns
hhccC	7.03	4.09	2.48a	10.05	3.10
hhcCc	8.09	4.09	2.42a	10.45	2.49
hhCcc	7.21	3.22	1.88b	10.54	2.73
w/sC	6.64	3.44	2.11ab	10.71	2.06
Sod	9.35	4.85	2.67	7.28	2.96

† Main effect of crop rotation statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column for each treatment effect

MWD_d = Mean weight diameter based on the dry sieving method (16-1 mm size fractions)

MWD_w = Mean weight diameter based on the wet sieving method (5-0.5 mm size fractions)

MWD_{we} = Mean weight diameter based on the wet sieving method (2-0.053 mm size fractions)

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation)

hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation); hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation)

w/sC (corn in corn-wheat/double crop soybean crop rotation)

Table 2.16. Organic C and total N associated with water-stable aggregate fractions for the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

Property	Aggregate size class	No-tillage 0 kg N ha^{-1}	No-tillage 150 kg N ha^{-1}	Moldboard Plow 0 kg N ha^{-1}	Moldboard Plow 150 kg N ha^{-1}	Sod
C/N	>2000 µm	10.67†AB	11.36A	10.65A	11.73A	10.47A
	2000-250 µm	10.99‡A	11.13AB	10.19B	10.31B	10.59A
	250-53 µm	10.88‡A	10.87B	10.07B	10.06B	10.45A
	<53 µm	10.16a§B	10.25aC	9.62bC	9.19bC	9.40B
N (g N/100 g soil fraction)	>2000 µm	0.21†B	0.26AB	0.18A	0.28A	0.28B
	2000-250 µm	0.24b§A	0.27aA	0.18cA	0.19 cB	0.31AB
	250-53 µm	0.24‡A	0.25B	0.18A	0.18 B	0.34A
	<53 µm	0.13‡C	0.14C	0.12B	0.13 C	0.15C
C (g C/100 g soil fraction)	>2000 µm	1.94†B	2.51AB	1.68A	2.80 A	2.48B
	2000-250 µm	2.24b§A	2.61aA	1.57cA	1.72 cB	2.84AB
	250-53 µm	2.23‡A	2.29B	1.52A	1.56 B	3.03A
	<53 µm	1.16‡C	1.20C	0.97B	0.98 C	1.18C

† Only main effect of N rate statistically significant ($P<0.05$)

‡ Only main effect of tillage statistically significant ($P<0.05$)

§ Interaction of tillage by N rate statistically significant ($P<0.05$)

Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different ($P>0.05$, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different ($P>0.05$, Fisher's LSD)

Table 2.17. Organic C and total N associated with water-stable aggregate fractions for the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

Property	Aggregate	Unmanured		Manured		Sod
	size class	0 kg N ha^{-1}	168 kg N ha^{-1}	0 kg N ha^{-1}	168 kg N ha^{-1}	
C/N	>2000 µm	10.63 nsA	10.98A	10.81A	10.76A	11.03AB
	2000-250 µm	10.49nsA	10.66AB	10.71A	10.49B	11.04AB
	250-53 µm	10.52nsA	10.45BC	10.72A	10.38B	11.23A
	<53 µm	9.99b†B	9.95bC	10.41aA	9.94bC	10.33B
N (g N/100 g soil fraction)	>2000 µm	0.24‡A	0.26A	0.30A	0.28A	0.19A
	2000-250 µm	0.25‡A	0.24A	0.30A	0.28A	0.23A
	250-53 µm	0.26§A	0.27A	0.29A	0.27A	0.20A
	<53 µm	0.12nsB	0.12B	0.14B	0.14B	0.11B
C (g C/100 g soil fraction)	>2000 µm	2.19‡A	2.41A	2.74A	2.58A	1.82A
	2000-250 µm	2.23‡A	2.19A	2.72A	2.54A	2.14A
	250-53 µm	2.30§A	2.03A	2.62A	2.41A	1.97A
	<53 µm	1.07nsB	0.98B	1.22B	1.15B	1.01B

† Interaction of manure by N rate statistically significant (P<0.10)

‡ Only main effect of manure statistically significant (P<0.05)

§ Main effects of manure and N rate statistically significant (P<0.05)

ns = Not statistically significant (P>0.10)

Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different (P>0.05, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD)

Table 2.18. Organic C and total N associated with water-stable aggregate fractions for the spring sampling (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

Property	Aggregate size class	Crop Rotation					Sod
		CCCCC	hhccC	hhcCc	hhCcc	w/sC	
C/N	>2000 µm	11.06nsA	10.85A	10.95A	10.98A	11.30A	10.80A
	2000-250 µm	10.81nsA	10.67A	10.81A	10.87AB	10.98AB	10.65A
	250-53 µm	10.84nsA	10.64A	10.78A	10.58B	10.75B	10.67A
	<53 µm	10.24 a†B	9.72bB	10.36aB	9.78bC	10.01abC	9.95B
N (g N/100 g fraction)	>2000 µm	0.22 ab†A	0.23aA	0.20bA	0.22abA	0.20bA	0.28B
	2000-250 µm	0.23ab†A	0.24aA	0.21bA	0.21bA	0.21bA	0.29B
	250-53 µm	0.23a†A	0.23aA	0.20abA	0.19bB	0.20abA	0.32A
	<53 µm	0.11 ab†B	0.12abB	0.10bB	0.10bC	0.10bB	0.15C
C (g C/100 g fraction)	>2000 µm	2.07 ab†A	2.18aAB	1.86cA	2.03abcA	1.96bcA	2.55B
	2000-250 µm	2.15 nsA	2.23A	1.91A	1.98A	2.02A	2.65B
	250-53 µm	2.08 a†A	2.06aB	1.84abA	1.69bB	1.86abA	2.95A
	<53 µm	0.97 nsB	0.97C	0.89B	0.88C	0.89B	1.29C

† Crop rotation effect statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different ($P>0.05$, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different ($P>0.05$, Fisher's LSD)

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation); hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation)

hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/double crop soybean crop rotation)

Table 2.19. Mass distribution of light particles and aggregates separated from the water-stable small macroaggregate (SM) fraction from different management treatments and for the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

Soil Fraction	No-tillage		Moldboard Plow		Sod
	0 kg N ha^{-1}	150 kg N ha^{-1}	0 kg N ha^{-1}	150 kg N ha^{-1}	
	(%)				
Free	0.24nsC	0.33D	0.20C	0.24D	0.37D
Occluded	0.04nsC	0.06D	0.06C	0.09D	0.08D
>250 μm_{SM}	14.04 b†B	13.11bC	15.68abB	20.18bB	13.95C
250-53 μm_{SM}	14.46 b†B	16.75aB	10.34cB	9.88cC	23.29B
<53 μm_{SM}	71.21§A	69.75A	73.72A	69.61A	62.32A

† Interaction of tillage by N rate statistically significant (P<0.05)

‡ Interaction of tillage by N rate statistically significant (P<0.10)

§ Main effect of N rate statistically significant (P<0.10)

ns = Not statistically significant (P>0.10)

Mean values followed by the same lowercase letter within a size fraction and between management treatments are not significantly different (P>0.05, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between size fractions are not significantly different (P>0.05, Fisher's LSD)

Table 2.20. Mass distribution of light particles and aggregates separated from the water-stable small macroaggregate (SM) fraction from different management treatments and for the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

Soil Fraction	Unmanured		Manured		Sod
	0 kg N ha^{-1}	168 kg N ha^{-1}	0 kg N ha^{-1}	168 kg N ha^{-1}	
	(%)				
Free	0.14nsD	0.24D	0.22D	0.19D	0.23C
Occluded	0.04nsD	0.03D	0.06D	0.04D	0.03C
>250 μm_{SM}	9.99nsC	9.72C	10.81C	11.13C	12.27B
250-53 μm_{SM}	17.51nsB	16.26B	18.02B	19.14B	16.48B
<53 μm_{SM}	72.32nsA	73.76A	70.88A	69.49A	70.99A

ns = Non statistically significant differences (P>0.10) between management treatments and within a size fraction

Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD)

Table 2.21. Mass distribution of light particles and aggregates separated from the water-stable large macroaggregate (LM) fraction from different management treatments and for the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

Soil Fraction	Crop Rotation					Sod
	CCCCC	hhccC	hhcCc	hhCcc	w/sC	
			(%)			
Free	0.13b†C	0.31aC	0.11bC	0.15bC	0.31bD	0.20D
Occluded	0.07nsC	0.04C	0.04C	0.08C	0.06D	0.07D
>250 μm_{LM}	13.55nsB	17.18B	15.37B	17.25B	17.12B	12.39C
250-53 μm_{LM}	14.21nsB	13.91B	12.46B	11.70B	12.03C	19.32B
<53 μm_{LM}	72.04nsA	68.57A	72.02A	70.82A	70.48A	68.02A

† Crop rotation effect statistically significant (P<0.05)

ns = Non statistically significant differences (P>0.10) between management treatments and within an fraction size

Mean values followed by the same lowercase letter within a size fraction and between management treatments are not significantly different (P>0.05, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between size fractions are not significantly different (P>0.05, Fisher's LSD)

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation);
hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation); hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation);
w/sC (corn in corn-wheat/double crop soybean crop rotation)

Table 2.22. Organic C and total N associated with intra-small macroaggregate fractions for the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

Property	Intra-small macroaggregate fraction	No tillage		Moldboard Plow		Sod
		0 kg N ha^{-1}	150 kg N ha^{-1}	0 kg N ha^{-1}	150 kg N ha^{-1}	
N (g/100 g ISM)	Free	0.15†B	0.17C	0.09C	0.17B	0.21B
	Occluded	0.04b‡C	0.07aD	0.04bcD	0.03cD	0.05C
	>250 μm_{SM}	0.15b‡B	0.19aBC	0.09cC	0.10CDc	0.22B
	250-53 μm_{SM}	0.36b‡A	0.45aA	0.26Ad	0.30cA	0.46A
	<53 μm_{SM}	0.19§B	0.20B	0.16B	0.16BC	0.20B
C (g/100 g ISM)	Free	2.29a‡B	2.10abB	1.42bB	2.18abB	2.89B
	Occluded	0.57¶D	0.73D	0.35E	0.37D	0.53D
	>250 μm_{SM}	1.59†C	2.06B	0.79D	0.86C	2.43B
	250-53 μm_{SM}	3.46b‡A	4.31aA	2.48dA	2.83cA	4.10A
	<53 μm_{SM}	1.38§C	1.52C	1.12C	1.14C	1.58C
C/N$_{ISM}$	Free	18.14†A	15.03A	19.60A	17.17A	16.14A
	Occluded	15.34nsB	12.41B	11.33B	12.28B	11.29C
	>250 μm_{SM}	12.66¶BC	12.72B	10.57BC	10.48B	13.00B
	250-53 μm_{SM}	11.36¶CD	11.33C	10.98B	11.02B	10.29CD
	<53 μm_{SM}	8.79¶D	8.91D	8.24C	8.26B	9.02D
N (g/100 g SM)	Free	$3.7x10^{-4}$ nsD	$5.4x10^{-4}$D	$2.5x10^{-4}$D	$2.8x10^{-4}$D	$7.2x10^{-4}$C
	Occluded	$2.0x10^{-5}$nsD	$3.0x10^{-5}$D	$3.0x10^{-5}$D	$4.0x10^{-5}$D	$4.0x10^{-5}$C
	>250 μm_{SM}	0.02§C	0.03C	0.01C	$1.9x10^{-2}$C	0.03B
	250-53 μm_{SM}	0.05b‡B	0.08aB	0.03cB	0.03cB	0.11A
	<53 μm_{SM}	0.13¶A	0.14A	0.01A	0.11A	0.13A
C (g/100 g SM)	Free	$5.3x10^{-3}$nsD	$7.0x10^{-3}$D	$3.8x10^{-3}$D	$7.0x10^{-3}$D	0.01C
	Occluded	$2.3x10^{-4}$nsD	$3.2x10^{-4}$D	$2.8x10^{-4}$D	$3.8x10^{-4}$D	$4.3x10^{-4}$C
	>250 μm_{SM}	0.22§C	0.27C	0.12C	0.18C	0.35B
	250-53 μm_{SM}	0.50b‡B	0.73aB	0.26cB	0.28cB	0.96A
	<53 μm_{SM}	0.98¶A	1.06A	0.82A	0.80A	0.98A

† Only main effect of N rate statistically significant ($P<0.05$)

‡ Interaction of tillage by N rate statistically significant ($P<0.05$)

§ Main effects of tillage and N rate statistically significant ($P<0.05$)

¶ Only main effect of tillage statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Mean values followed by the same lowercase letter within a size fraction and between management treatments are not significantly different ($P>0.05$, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between size fractions are not significantly different ($P>0.05$, Fisher's LSD)

ISM (intra-small macroaggregate fraction basis; SM (small macroaggregate fraction basis)

Table 2.23. Organic C and total N concentrations associated with intra-small macroaggregate fractions for the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

Property	Intra-small macroaggregate fraction	Unmanured		Manured		Sod
		0 kg N ha^{-1}	168 kg N ha^{-1}	0 kg N ha^{-1}	168 kg N ha^{-1}	
N (g N/100 g ISM)	Free	0.09b†D	0.08bD	0.08bD	0.10aD	0.11C
	Occluded	0.03nsE	0.03E	0.04E	0.03E	0.04D
	>250 μm_{SM}	0.11‡C	0.12C	0.14C	0.13C	0.13C
	250-53 μm_{SM}	0.35d†A	0.36cA	0.44aA	0.40bA	0.37A
	<53 μm_{SM}	0.18nsB	0.18B	0.20B	0.20B	0.18B
C (g C/100 g ISM)	Free	1.45b†B	0.81cC	1.54bB	2.09aB	2.15B
	Occluded	0.39b†D	0.32cD	0.64aC	0.33cD	0.51D
	>250 μm_{SM}	1.14‡C	1.17B	1.60B	1.42C	1.49C
	250-53 μm_{SM}	3.26c†A	3.43cA	4.29aA	3.80bA	3.54A
	<53 μm_{SM}	1.35nsBC	1.34B	1.59B	1.50C	1.27C
C/N$_{ISM}$	Free	19.52c†A	11.72dB	21.63bA	23.59aA	22.26A
	Occluded	13.88b†B	13.30bA	17.76aB	12.27cB	16.86B
	>250 μm_{SM}	11.83nsC	11.58B	13.25C	12.54B	13.11C
	250-53 μm_{SM}	10.94D	11.10B	11.42D	11.06C	11.08D
	<53 μm_{SM}	8.57nsE	8.62C	9.21E	8.81D	8.41E
N (g N/100 g SM)	Free	1.0x10^{-4}nsC	2.0x10^{-4}D	2.0x10^{-4}D	2.0x10^{-4}D	3.0x10^{-4}C
	Occluded	1.0x10^{-5}nsC	1.0x10^{-5}D	2.0x10^{-5}D	2.0x10^{-5}D	1.0x10^{-5}C
	>250 μm_{SM}	0.01‡C	0.01C	0.02C	0.02C	0.02C
	250-53 μm_{SM}	0.06‡B	0.06B	0.08B	0.08B	0.06B
	<53 μm_{SM}	0.13nsA	0.13A	0.14A	0.14A	0.12A
C (g C/100 g SM)	Free	1.8x10^{-3}nsD	2.4x10^{-3}C	3.9x10^{-3}D	3.1x10^{-3}D	4.9x10^{-3}C
	Occluded	1.8x10^{-4}nsD	8.0x10^{-5}C	2.0x10^{-4}D	2.1x10^{-4}D	1.5x10^{-4}C
	>250 μm_{SM}	0.11‡C	0.11C	0.17C	0.02C	0.18C
	250-53 μm_{SM}	0.57‡B	0.56B	0.77B	0.73B	0.59B
	<53 μm_{SM}	0.98b†A	0.99bA	1.12aA	1.04bA	0.89A

† Interaction of manure by N rate statistically significant ($P<0.05$)

‡ Only main effect of manure statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Mean values followed by the same lowercase letter within a size fraction and between management treatments are not significantly different ($P>0.05$, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between size fractions are not significantly different ($P>0.05$, Fisher's LSD)

ISM (intra-small macroaggregate fraction basis; SM (small macroaggregate fraction basis)

Table 2.24. Organic C and total N associated with intra-large macroaggregates fractions for the spring sampling (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

Property	Intra-large macroaggregate fraction	Crop Rotation CCCCC	hhccC	hhcCc	hhCcc	w/sC	Sod
N (g N/100 g ILM)	Free	0.11b†C	0.12bC	0.10bC	0.10bD	0.17aB	0.16C
	Occluded	0.04b‡D	0.04bD	0.04bD	0.05aE	0.04bD	0.07D
	>250 μm$_{LM}$	0.13nsC	0.12C	0.10C	0.13C	0.11C	0.15C
	250-53 μm$_{LM}$	0.45c† A	0.49abA	0.43cA	0.50aA	0.46bcA	0.50A
	<53 μm$_{LM}$	0.19b† B	0.20aB	0.17cB	0.19bB	0.18bcB	0.21B
C (g C/100 g ILM)	Free	1.20b†B	1.94abB	1.17bB	1.12bB	2.77aB	2.11B
	Occluded	0.37b†C	0.36bC	0.38bD	0.48aC	0.37bD	0.64D
	>250 μm$_{LM}$	1.30nsB	1.08BC	0.90C	1.34B	0.95CD	1.60C
	250-53 μm$_{LM}$	4.19bc†A	4.49abA	3.98cA	4.82aA	4.52abA	4.75A
	<53 μm$_{LM}$	1.27bc†B	1.39aB	1.17cB	1.29bB	1.27bcC	1.53C
C/N$_{ILM}$	Free	12.07b†A	16.16abA	14.08abA	13.27bA	18.33abA	15.65A
	Occluded	11.21nsA	10.14B	10.86B	10.75B	10.47BC	11.02B
	>250 μm$_{LM}$	11.13nsA	10.51B	9.92B	11.53B	10.54BC	12.07B
	250-53 μm$_{LM}$	10.97bc†A	10.60cB	10.88bcB	11.17abB	11.46aB	11.03B
	<53 μm$_{LM}$	7.93nsB	8.00B	8.08C	8.08C	8.17C	8.38C
N (g N/100 g LM)	Free	1.5x10^{-4}nsD	3.6x10^{-4}D	1.7x10^{-4}D	1.7x10^{-4}D	4.2x10^{-4}D	3.4x10^{-4}D
	Occluded	3.0x10^{-5}nsD	2.0x10^{-5}D	2.0x10^{-5}D	3.0x10^{-5}D	3.0x10^{-5}D	5.0x10^{-5}D
	>250 μm$_{LM}$	0.02Cns	0.02C	0.020C	0.02C	0.02C	0.02C
	250-53 μm$_{LM}$	0.06nsB	0.07B	0.05B	0.06B	0.06B	0.10B
	<53 μm$_{LM}$	0.13nsA	0.14A	0.12A	0.13A	0.13A	0.14A
C (g C/100 g LM)	Free	1.9x10^{-3}nsD	3.9x10^{-3}D	2.6x10^{-3}D	2.3x10^{-3}D	5.6x10^{-3}D	4.5x10^{-3}D
	Occluded	2.6x10^{-4}nsD	1.4x10^{-4}D	1.5x10^{-4}D	2.8x10^{-4}D	2.5x10^{-4}D	4.6x10^{-4}D
	>250 μm$_{LM}$	0.16ab‡C	0.18abC	0.13bC	0.22aC	0.16abC	0.19C
	250-53 μm$_{LM}$	0.60nsB	0.62B	0.49B	0.57B	0.54B	0.92B
	<53 μm$_{LM}$	0.91nsA	0.95A	0.84A	0.91A	0.90A	1.03A

† Crop rotation effect statistically significant (P<0.05)

‡ Crop rotation effect statistically significant (P<0.10)

Different letters indicate significant differences (P<0.05, Fisher's LSD) within a column

Mean values followed by the same lowercase letter within a size fraction and between management treatments are not significantly different (P>0.05, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between size fractions are not significantly different (P>0.05, Fisher's LSD)

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation); hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation); hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/double crop soybean crop rotation);

ILM (intra-small macroaggregate fraction basis; LM (large macroaggregate fraction basis)

Table 2.25. Summary for organic C and total N distribution among water-stable and intra-small macroaggregate fractions for the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

	Organic Carbon (g C/100 g soil)					Total Nitrogen (g N/100 g soil)				
	No-tillage		Moldboard Plow		Sod	No-tillage		Moldboard Plow		Sod
	0 kg N ha^{-1}	150 kg N ha^{-1}	0 kg N ha^{-1}	150 kg N ha^{-1}		0 kg N ha^{-1}	150 kg N ha^{-1}	0 kg N ha^{-1}	150 kg N ha^{-1}	
Whole soil	*2.16*	*2.73*	*1.52*	*1.58*	*2.85*	*0.20*	*0.25*	*0.15*	*0.16*	*0.28*
Water-stable aggregate fractions										
>2000 μm	0.85	0.95	0.17	0.06	1.09	0.09	0.10	0.02	0.01	0.12
† 2000-250 μm	**0.85**	**1.25**	**0.60**	**0.64**	**1.41**	**0.09**	**0.13**	**0.07**	**0.07**	**0.16**
250-53 μm	0.30	0.25	0.63	0.78	0.15	0.03	0.03	0.07	0.09	0.02
<53 μm	0.05	0.03	0.10	0.10	0.02	0.01	4.0×10^{-3}	0.01	0.01	2.0×10^{-3}
Sum	*2.05*	*2.48*	*1.50*	*1.58*	*2.67*	*0.22*	*0.26*	*0.17*	*0.18*	*0.30*
Intra-small macroaggregate fractions										
Free	2.0×10^{-3}	3.3×10^{-3}	1.4×10^{-3}	1.6×10^{-3}	5.1×10^{-3}	1.4×10^{-4}	2.5×10^{-4}	9.0×10^{-5}	1.1×10^{-4}	3.6×10^{-4}
Occluded	9.0×10^{-5}	1.5×10^{-4}	1.1×10^{-4}	1.5×10^{-4}	2.2×10^{-4}	1.0×10^{-5}	2.0×10^{-5}	1.0×10^{-5}	2.0×10^{-5}	2.0×10^{-5}
>250 μm$_{SM}$	9.0×10^{-3}	0.13	0.04	0.07	0.17	8.0×10^{-3}	0.01	0.01	0.01	0.02
250-53 μm$_{SM}$	0.19	0.35	0.10	0.10	0.48	0.02	0.04	0.01	0.01	0.05
<53 μm$_{SM}$	0.37	0.52	0.32	0.30	0.49	0.05	0.07	0.04	0.04	0.06
† Sum	**0.58**	**1.00**	**0.46**	**0.47**	**1.15**	**0.08**	**0.12**	**0.06**	**0.06**	**0.13**

† Small-macroaggregate fraction in bold for comparison with sum of intra-small macroaggregate fractions, also in bold

Table 2.26. Summary for organic C and total N distribution among water-stable and intra-small macroaggregate fractions for the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

	Organic Carbon (g C/100 g soil)					Total Nitrogen (g N/100 g soil)				
	Unmanured		Manured		Sod	Unmanured		Manured		Sod
	0 kg N ha^{-1}	168 kg N ha^{-1}	0 kg N ha^{-1}	168 kg N ha^{-1}		0 kg N ha^{-1}	168 kg N ha^{-1}	0 kg N ha^{-1}	168 kg N ha^{-1}	
Whole soil	*2.09*	*2.05*	*2.60*	*2.40*	*2.34*	*0.20*	*0.19*	*0.24*	*0.24*	*0.23*
Water-stable aggregate fractions										
>2000 μm	0.44	0.53	0.71	0.68	0.81	0.05	0.06	0.08	0.08	0.09
† 2000-250 μm	**1.37**	**1.24**	**1.55**	**1.45**	**0.95**	**0.15**	**0.14**	**0.17**	**0.16**	**0.10**
250-53 μm	0.34	0.32	0.34	0.31	0.17	0.03	0.04	0.04	0.04	0.02
<53 μm	0.04	0.04	0.05	0.04	0.03	0.01	$5.0x10^{-3}$	$5.0x10^{-3}$	$5.0x10^{-3}$	$3.0x10^{-3}$
Sum	*2.18*	*2.14*	*2.65*	*2.48*	*1.96*	*0.24*	*0.23*	*0.29*	*0.28*	*0.21*
Intra-small macroaggregate fractions										
Free	$1.1x10^{-3}$	$1.3x10^{-3}$	$2.2x10^{-3}$	$1.8x10^{-3}$	$2.2x10^{-3}$	$7.0x10^{-5}$	$1.0x10^{-4}$	$1.0x10^{-4}$	$9.0x10^{-5}$	$1.1x10^{-4}$
Occluded	$1.1x10^{-4}$	$5.0x10^{-5}$	$1.3x10^{-4}$	$1.2x10^{-4}$	$7.0x10^{-5}$	$1.0x10^{-5}$	$1.0x10^{-5}$	$1.0x10^{-5}$	$1.0x10^{-5}$	$1.0x10^{-5}$
>250 μm$_{SM}$	0.07	0.06	0.10	0.09	0.08	0.01	0.01	0.01	0.01	0.01
250-53 μm$_{SM}$	0.35	0.32	0.44	0.42	0.27	0.04	0.03	0.05	0.04	0.03
<53 μm$_{SM}$	0.60	0.56	0.64	0.60	0.39	0.08	0.07	0.08	0.08	0.05
† Sum	**1.02**	**0.94**	**1.18**	**1.11**	**0.74**	**0.13**	**0.11**	**0.14**	**0.13**	**0.09**

† Small-macroaggregate fraction in bold for comparison with sum of intra-small macroaggregate fractions, also in bold

Table 2.27. Summary for organic C and total N distribution among water-stable and intra-large macroaggregate fractions for the spring sampling (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

	Organic Carbon (g C/100 g soil)						Total Nitrogen (g N/100 g soil)					
	CCCCC	hhccC	hhcCc	hhCcc	w/sC	Sod	CCCCC	hhccC	hhcCc	hhCcc	w/sC	Sod
Whole soil	*2.15*	*2.17*	*1.96*	*2.12*	*2.00*	*2.60*	*0.22*	*0.22*	*0.19*	*0.20*	*0.20*	*0.25*
Water-stable aggregate fractions												
† >2000 µm	**0.94**	**0.95**	**0.79**	**0.60**	**0.68**	**1.25**	**0.10**	**0.10**	**0.08**	**0.06**	**0.07**	**0.14**
2000-250 µm	0.90	0.94	0.84	0.92	0.96	1.08	0.10	0.10	0.09	0.10	0.10	0.12
250-53 µm	0.21	0.22	0.20	0.32	0.25	0.24	0.02	0.02	0.02	0.04	0.03	0.03
<53 µm	0.03	0.03	0.03	0.04	0.03	0.03	$3\text{x}10^{-3}$	$4\text{x}10^{-3}$	$3\text{x}10^{-3}$	$5.0\text{x}10^{-3}$	$4\text{x}10^{-3}$	$4.0\text{x}10^{-3}$
Sum	*2.08*	*2.14*	*1.86*	*1.88*	*1.92*	*2.60*	*0.22*	*0.23*	*0.20*	*0.20*	*0.20*	*0.29*
Intra-large macroaggregate fractions												
Free	$8.9\text{x}10^{-4}$	$1.6\text{x}10^{-3}$	$1.1\text{x}10^{-3}$	$8.2\text{x}10^{-4}$	$1.9\text{x}10^{-3}$	$2.0\text{x}10^{-3}$	$7.0\text{x}10^{-5}$	$1.5\text{x}10^{-4}$	$8.0\text{x}10^{-5}$	$6.0\text{x}10^{-5}$	$1.4\text{x}10^{-4}$	$1.6\text{x}10^{-4}$
Occluded	$1.3\text{x}10^{-4}$	$6.0\text{x}10^{-5}$	$6.0\text{x}10^{-5}$	$9.0\text{x}10^{-5}$	$8.0\text{x}10^{-5}$	$2.2\text{x}10^{-4}$	$1.0\text{x}10^{-5}$	$1.0\text{x}10^{-5}$	$1.0\text{x}10^{-5}$	$1.0\text{x}10^{-5}$	$1.0\text{x}10^{-5}$	$2.0\text{x}10^{-5}$
>250 µm$_{LM}$	0.07	0.08	0.06	0.06	0.06	0.09	0.01	0.01	0.01	0.01	0.01	0.01
250-53 µm$_{LM}$	0.27	0.28	0.21	0.17	0.19	0.45	0.03	0.03	0.02	0.02	0.02	0.05
<53 µm$_{LM}$	0.41	0.42	0.36	0.27	0.31	0.50	0.06	0.06	0.05	0.04	0.04	0.07
† Sum	**0.76**	**0.78**	**0.63**	**0.50**	**0.56**	**1.04**	**0.10**	**0.10**	**0.08**	**0.06**	**0.07**	**0.13**

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation); hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation)
hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/double crop soybean crop rotation)
† Large-macroaggregate fraction in bold for comparison with sum of intra-large macroaggregate fractions, also in bold

Figures

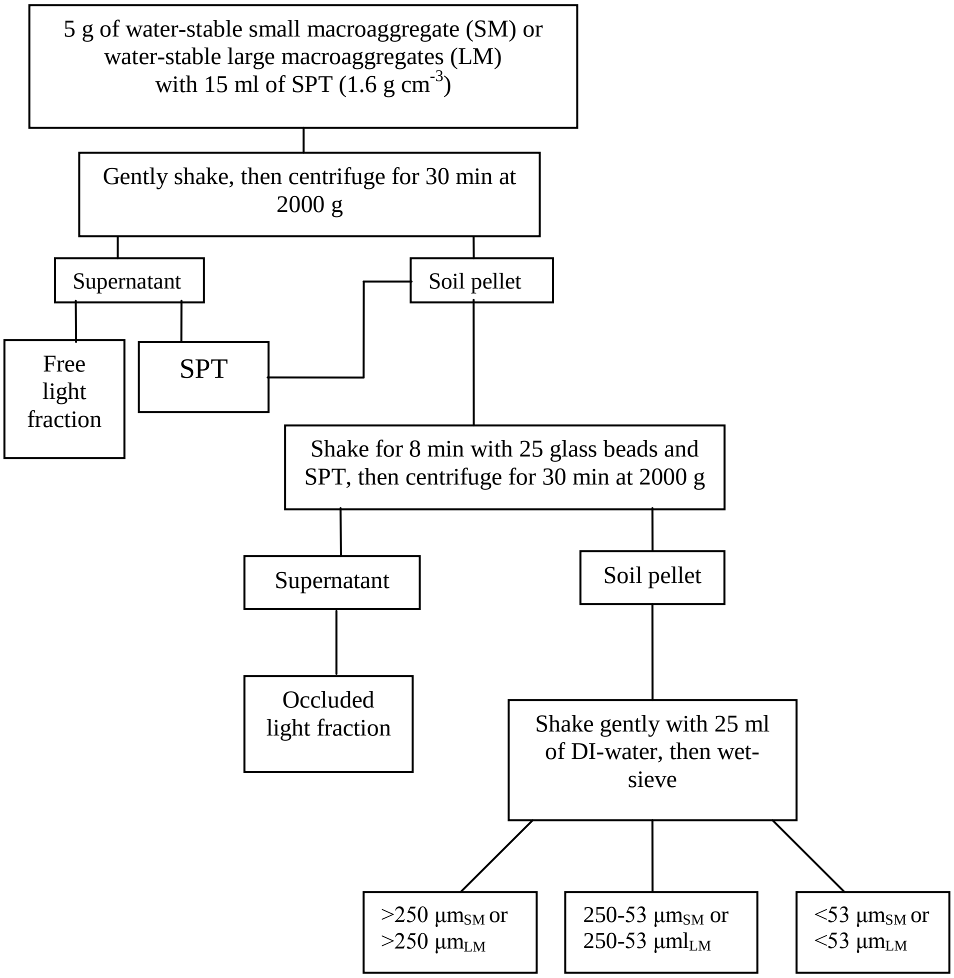

Figure 2.1. Scheme for isolation of intra-macroaggregate fractions from water-stable macroaggregates using sodium polytungstate solution (SPT) as heavy liquid. Modified after Skjemstand (1990), Golchin et al. (1994), Six et al. (1998), and Sohi et al. (2001).

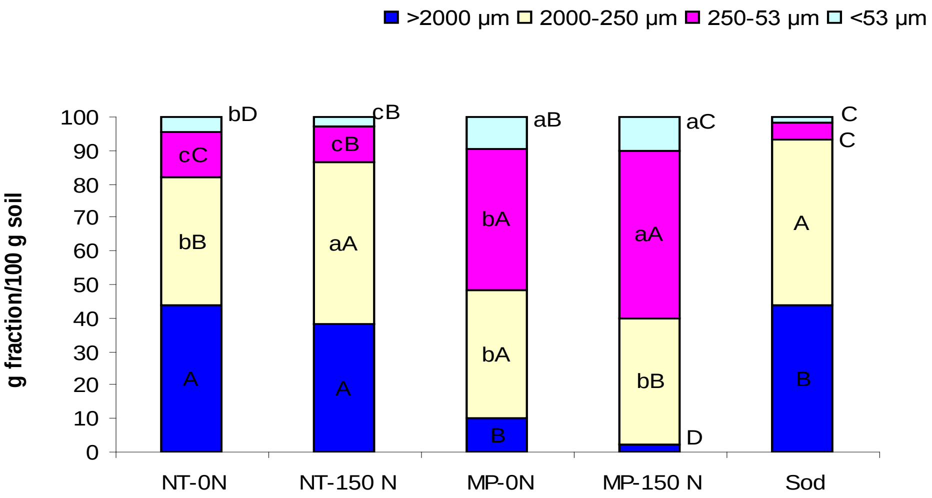

Figure 2.2. Weight distribution among water-stable aggregate size classes for the different management treatments and the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate. Mean values followed by the same lowercase letter within an aggregate size class and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate size classes are not significantly different (P>0.05, Fisher's LSD).

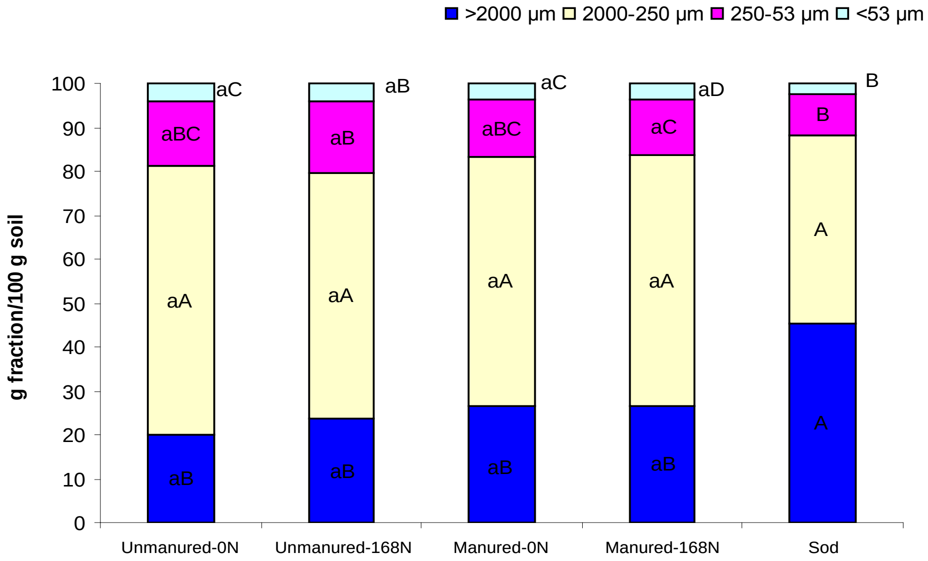

Figure 2.3. Weight distribution among water-stable aggregate size classes for the different management treatments and the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate. Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD).

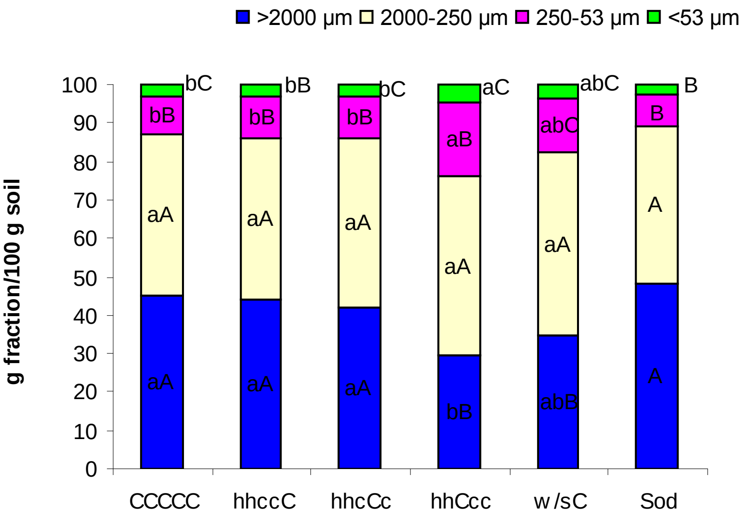

Figure 2.2. Weight distribution among water-stable aggregate size classes for the different management treatments and the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations. Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD).

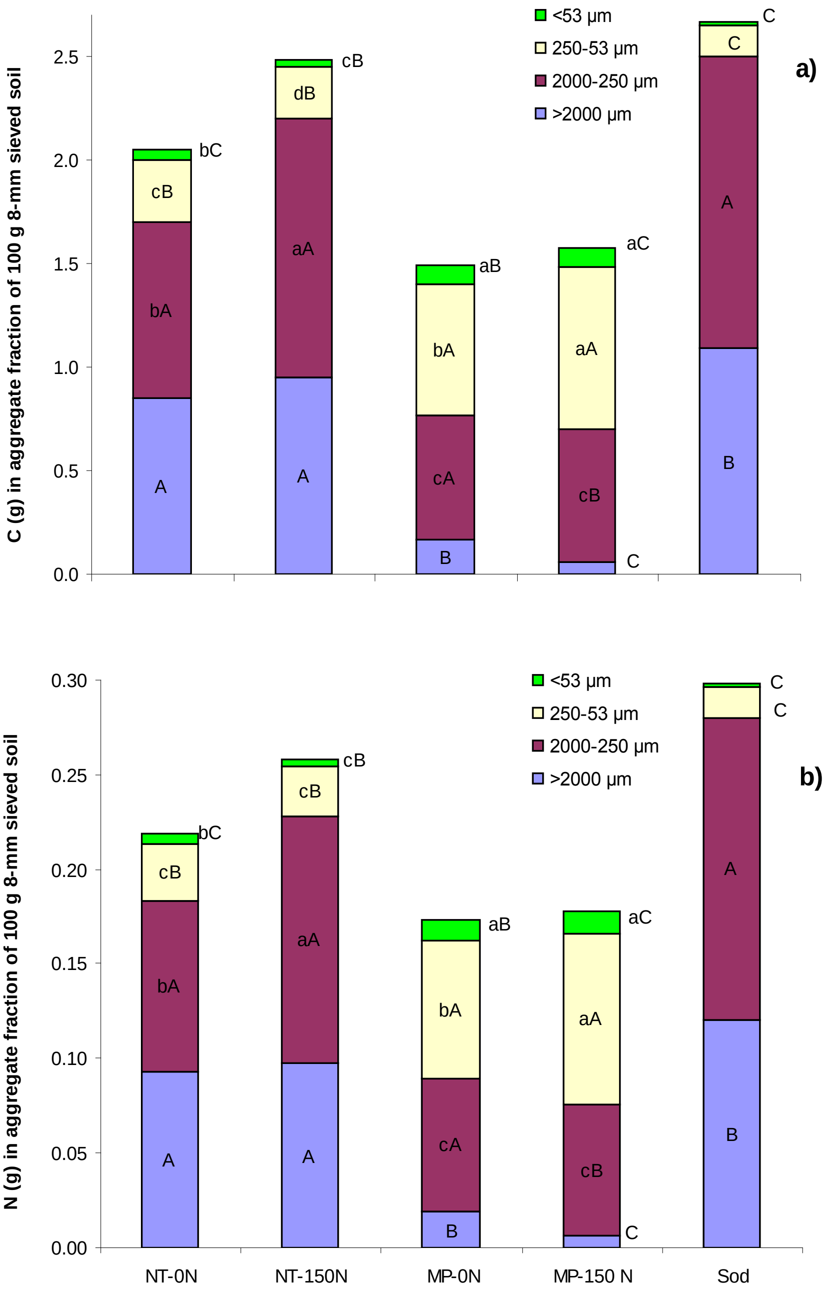

Figure 2.3. Distribution of organic C (a) and total N (b) among water-stable aggregates in 100 g of whole soil for the different management treatments and the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate. Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD).

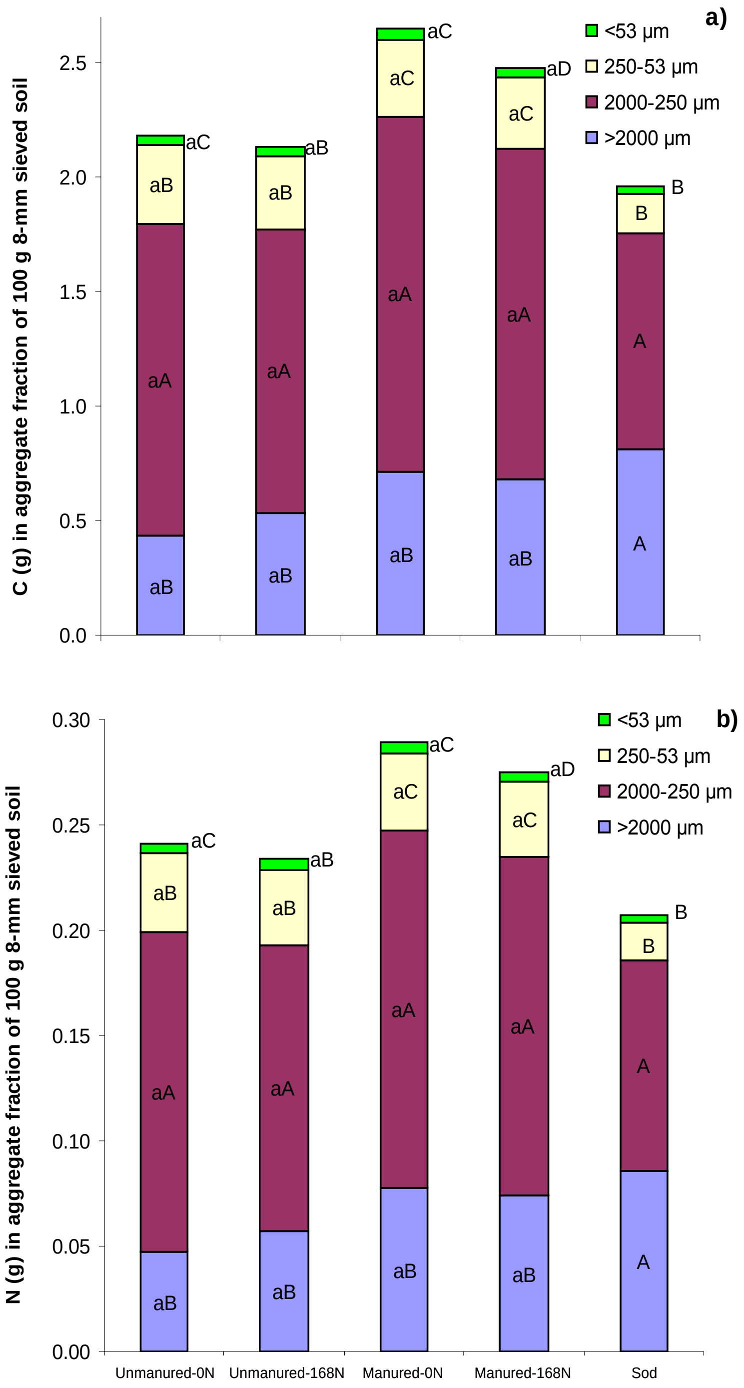

Figure 2.4. Distribution of organic C (a) and total N (b) among water-stable aggregates in 100 g of whole soil for the different management treatments and the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate. Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD).

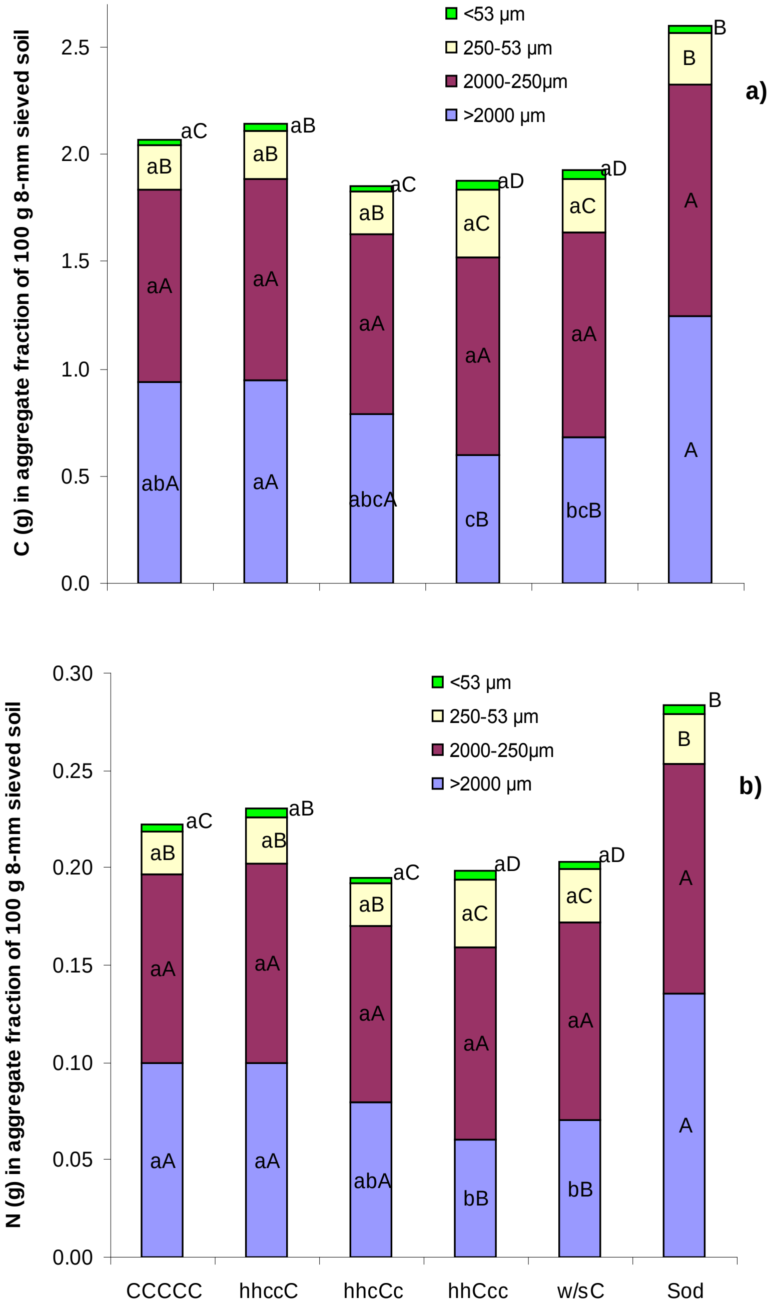

Figure 2.5. Distribution of organic C (a) and total N (b) among water-stable aggregates in 100 g of whole soil for the different crop rotation treatments and the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations. Mean values followed by the same lowercase letter within an aggregate size class and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD).

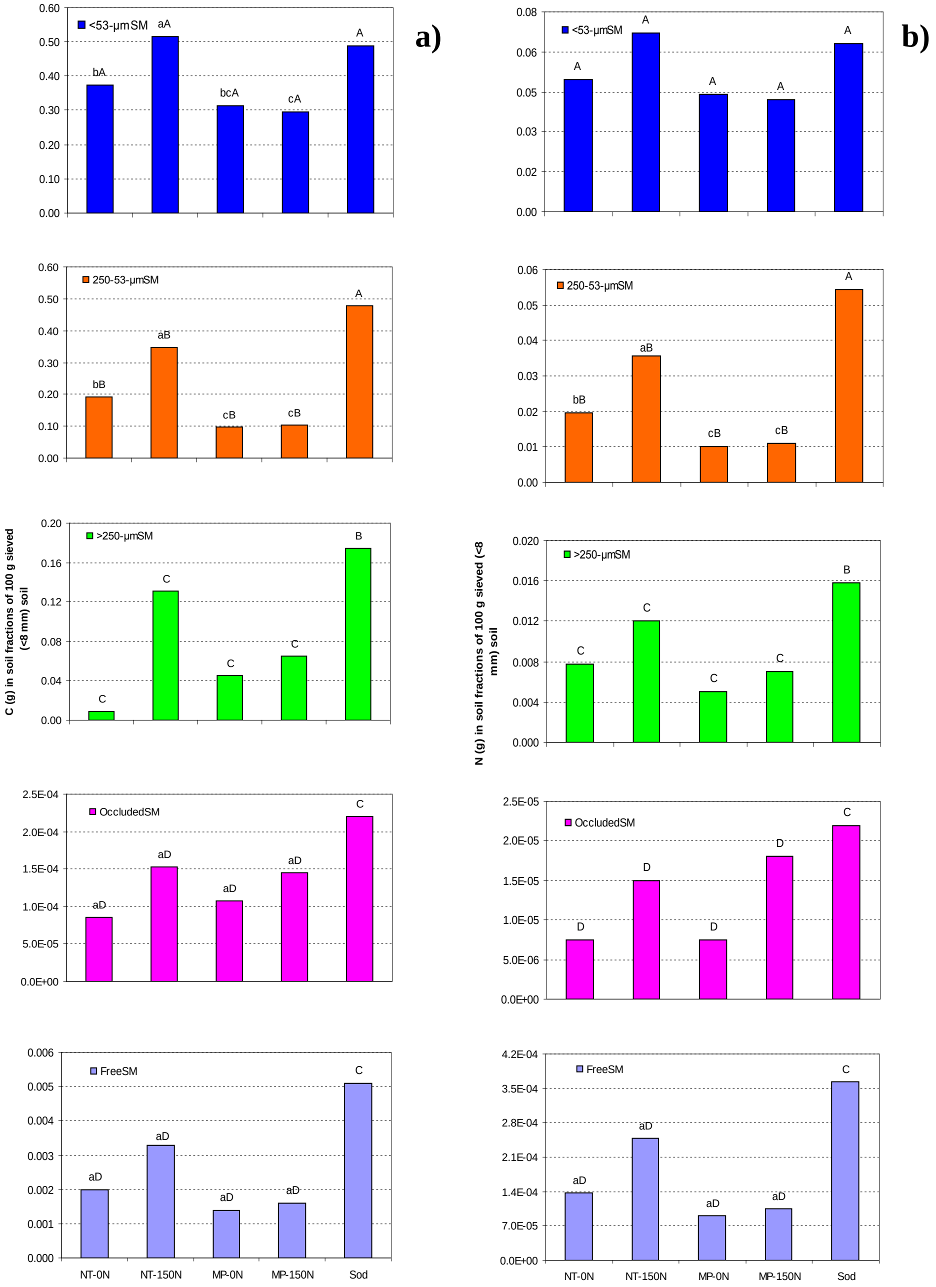

Figure 2.6. Distribution of organic C (a) and total N (b) among intra-small macroaggregate fractions in 100 g of whole soil for the different management treatments and the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate. Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD).

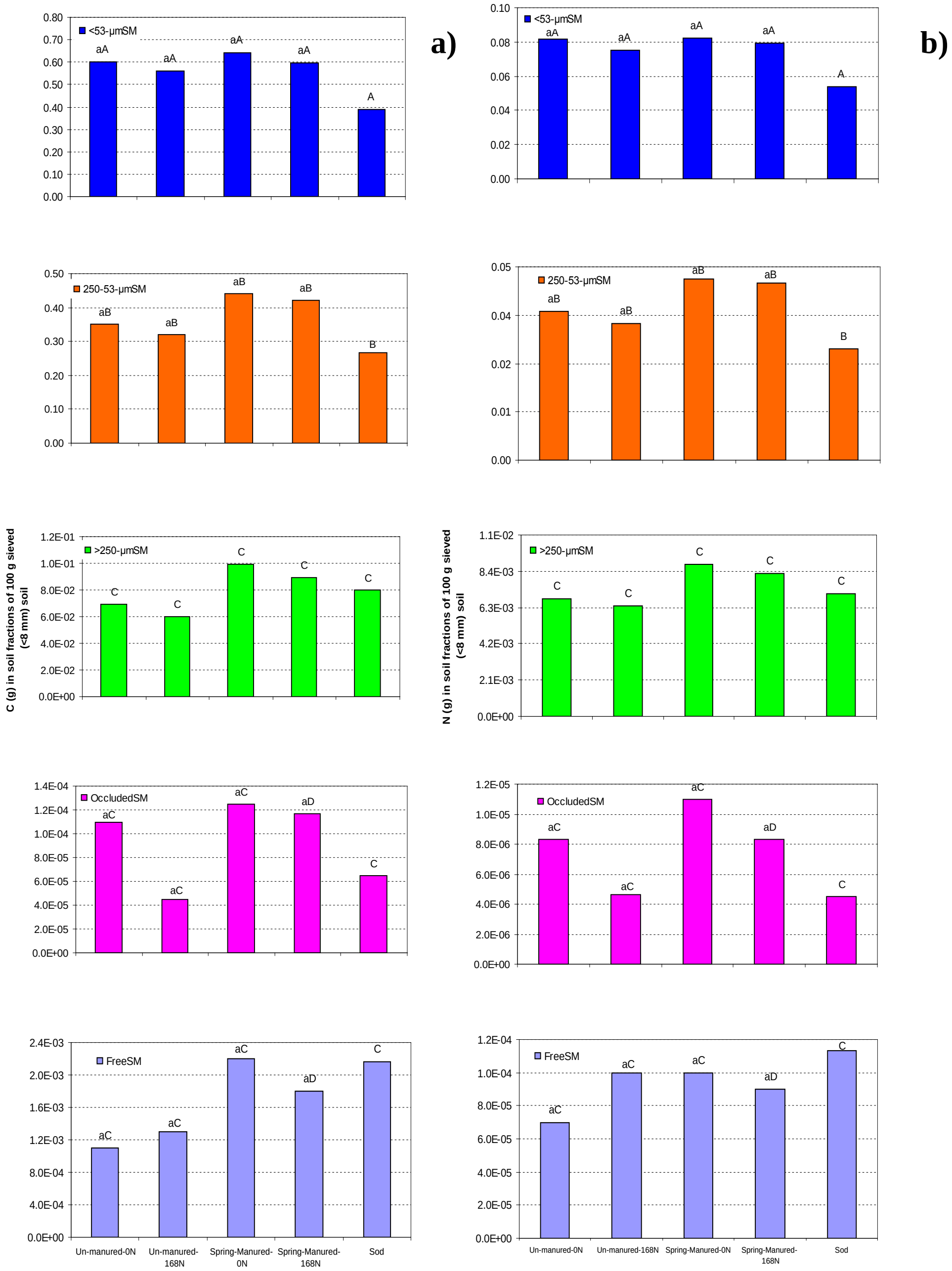

Figure 2.7. Distribution of organic C (a) and total N (b) among intra-small macroaggregate fractions in 100 g of whole soil for the different management treatments and the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate. Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD).

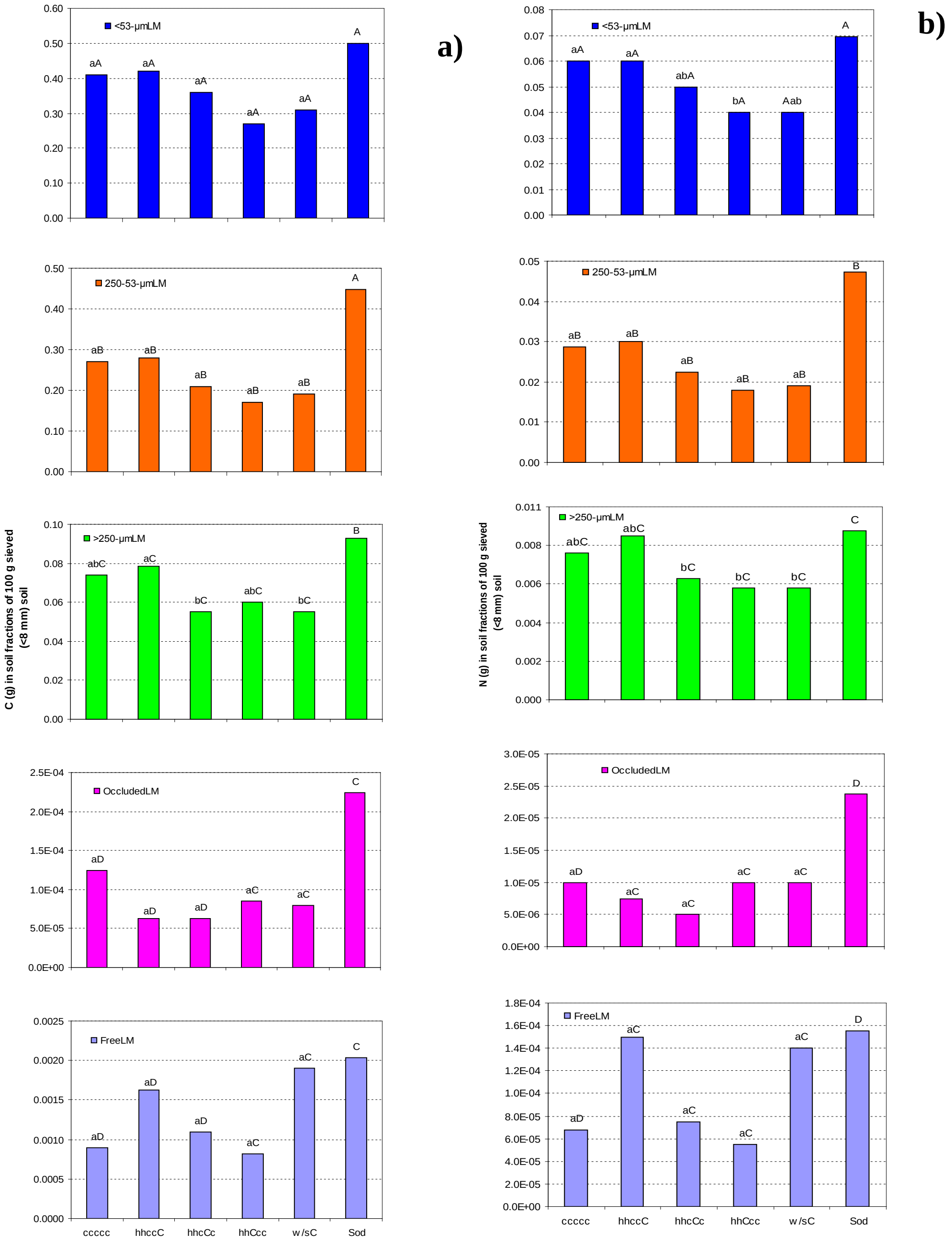

Figure 2.8. Distribution of organic C (a) and total N (b) among intra-small macroaggregate fractions in 100 g of whole soil for the different management treatments and the nearby sod for the spring sampling (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations. Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different (P>0.05, Fisher's LSD).

Chapter 3

Stability of Soil Organic Carbon Pools in Aggregate Fractions of Long-term Row-cropped Soils under Different Management Practices

Introduction

Soil organic matter (SOM) is dynamic in nature. The amount of SOM can be increased or decreased by diverse environmental factors and human activities. However, even when the amount of organic matter in a given soil is at equilibrium, SOM is still part of a cycle where decomposition generates new inputs into and through organic matter pools of various qualities and replaced materials that are either transferred to other pools or mineralized (Paul, 1984). Consequently, the response of soil organic C in agricultural soils to management practices should not only be assessed in terms of total C stocks but also with respect to soil organic C stability and turnover to understand the functioning of these ecosystems.

The dynamic behavior or turnover of SOM is often quantified as mean residence time (MRT), which is defined as the average time required to completely renew the content of the pool at steady state. Specifically, the turnover of SOM-C has been estimated by techniques such as simple first-order modeling, ^{13}C natural abundance, and ^{14}C dating. The first two techniques have been used in medium-term observations or experiments (5-50 yr) because the relative recent inputs and C pools dominate estimates by these techniques. Radiocarbon dating techniques have been used to estimated turnover dominated by the oldest and most recalcitrant C pools (200-40,000 yr) (Six and Jastrow, 2002).

The ^{13}C natural abundance technique uses natural differences in $\delta^{13}C$ values between plants with the C_3 and C_4 pathways of photosynthesis. Atmospheric CO_2 has a $\delta^{13}C$ value of approximately -8‰ (Levin et al., 1987). During photosynthesis, plants with the C_3 pathway discriminate against atmospheric $^{13}CO_2$ to a greater extent than C_4 plants (O'Leary, 1988). The C_3 plants have $\delta^{13}C$ values ranging from -32 to -20‰; while C_4 plants have $\delta^{13}C$ values ranging from -17 to -9‰. Thus, C_3 and C_4 plants have distinct stable C isotope ratios and differ from each other by approximately 14‰ on average (Smith and Epstein, 1971). Although there are small isotopic differences between different parts of the same plant and between specific biochemical fractions within plants, the C isotopic signature of the whole plant is largely preserved as dead plant tissue decomposes and enters the SOM pool (Melillo et al., 1989). As a result, SOM inherits the isotopic composition of plant communities. This natural isotopic “label” of SOM enables reconstruction of the prior history of plant communities (Dzurec et al., 1985) and also permits estimation of SOM dynamics without any type of disturbance. The use of the ^{13}C natural abundance technique in SOM dynamics studies is obviously limited to situations where there has been a change in vegetation. The rate of loss of the C derived from the original vegetation and the incorporation of C derived from the new vegetation are inferred from the resulting change in the ^{13}C natural abundance signature of the soil. The turnover of C derived from the original vegetation is calculated by using the first order decay model (Balesdent and Mariotti, 1996).

Soil organic matter turnover is controlled by biological processes such as: primary production (specifically, the rate of organic matter transfer below ground), soil microbial activity (specifically, the rates of SOM transformation and decay), climate (especially temperature and precipitation), type of vegetation, parent material, topography, time, and disturbance or management practices (Six and Jastrow, 2002).

Soil management practices influence SOM turnover through direct effects on inputs and outputs and through indirect effects on the factors controlling these fluxes (Six and Jastrow, 2002). It has been reported that the MRT of whole soil C is significantly longer under NT than under CT practices (Six et al., 1998; Clapp et al., 2000; Balesdent et al., 1990). In addition, Gregorich et al. (1996) reported significant soil organic C turnover as influenced by long-term N fertilization of continuous corn. Also, there is evidence that crop selection and sequence can have an effect on soil organic C stability. Cambardella and Elliot (1992) found that wheat derived particulate organic matter (POM) turned over faster than native grass derived POM. They reported a half-life of grass derived POM to be about 13 years.

Total SOM consists of many different pools that turn over at different rates. van Venn and Paul (1981) reported that surface soils have a very stable or recalcitrant organic C pool that has a mean age of several thousand years; while Balesdent et al. (1988) reported a labile SOM pool with a half-life of 10 to 15 years, which is extinguished after

30 to 40 years of cultivation. Parton et al. (1987), on the other hand, pointed out that surface soils have a slow or intermediate organic C pool with a turnover time of 20 to 50 years, and a very labile, active organic C pool that has a mean age of 1 to 5 years. Consequently, considering only the MRT of whole-soil C, which is a function of the turnover rates of its constituent pools, could mislead. Soils with similar MRT of whole soil C can have very different distributions of organic matter among pools with fast, slow, and intermediate turnover rates (Davidson et al., 2000). Simulation models are used to describe SOM dynamics accounting for variations in turnover rates for different SOM pools (Parton et al., 1987).

For a given set of biotic and abiotic conditions the turnover of different SOM pools depends on the quality and biochemical recalcitrance of organic matter and its accessibility to decomposers (Six and Jastrow, 2002). Readily decomposable materials can become chemically protected from decomposition by association with clay minerals and by sorption to humic colloids (Christensen, 1992), consequently, mineralogy and quantity of clay play a major role in SOM protection and MRT values. Christensen (1992) and Feller and Beare (1997) reported that MRT values for primary organo-mineral associations generally increase with decreasing particle size. In addition, relatively labile materials may become physically protected by incorporation into soil aggregates or by deposition in micropores inaccessible even to bacteria. As a result, slower turnover of organic matter in microaggregates compared to macroaggregates, has been reported (Angers and Giroux, 1996; Six et al., 1999).

In this context, physically separated SOM fractions may be meaningful fractions for evaluation of SOM stabilization. Few studies have used stable isotopes to trace the turnover of C in different physically separated SOM fractions (Angers et al., 1997; Monreal et al., 1997; Puget et al., 2000; Denef et al., 2001; Frey et al., 2003; John et al., 2005). Their findings showed that stable aggregates appear to be involved in the storage and stabilization of C in silty soils (Angers et al., 1997). Monreal et al. (1997) reported that the mean turnover time of soil organic C was 14 yr in macroaggregates, 61 yr in microaggregates and 275 yr in the <50 μm fraction for a long-term experiment with continuous maize cultivation on a Gleysol. John et al. (2005) reported, for a long-term experiment with continuous maize on a Luvisol, that the mean turnover time of soil organic C was 37 yr in macroaggregates and 86 yr in the <53 μm fraction. For density fractions, they reported the following order: free POM (22 yr) <dense occluded POM (49 yr) <mineral-SOM (63 yr) <light occluded POM (83 yr). Nonetheless, a heterogeneous distribution of recently incorporated litter C has been reported in soil aggregates. According to Puget et al. (2000), the particulate organic matter located in macroaggregates is particularly enriched with maize residue C.

There is still considerable uncertainty about how the physical SOM fractions are affected by management practices and how these fractions differ in soil organic C stability. Accordingly, this study may provide more insight into the effect of tillage, N fertilization, and manure application on SOM dynamics by assessing the stability and turnover of physically fractionated SOM pools. The specific objectives were: i) to analyze the stability of physically fractionated SOM fractions using $\delta^{13}C$ in formation, ii) to determine the apparent turnover time or mean age of soil organic C in physically fractionated SOM fractions, and iii) to characterize $\delta^{15}N$ in physically fractionated SOM fractions.

Material and Methods

Field Experiments

Samples were taken from the same treatments and field experiments described in Chaper 2, with the exception of Field Experiment 3, where all components of the crop rotation treatment plots were sampled, i.e. continuous corn (CCCCC), corn in w/sc (w/sC), first year of corn in hhccc (hhCcc), second year of corn in hhccc (hhcCc), third year of corn in hhccc (hhccC), first year of hay in hhccc (Hhccc), second year of hay in hhccc (hHccc), and wheat/soybeans in w/sc (W/Sc) at one N rate (180 kg ha^{-1}).

Soil Sampling and Handling

The three long-term field experiments were sampled twice in 2006 (late April and late November) as indicated in Chaper 2. However, only the soil surface samples (0-10 cm soil depth increment) coming from the spring sampling were used for this study with the exception of Field Experiment 3 that had samples coming from the fall sampling for the Hhccc, hHccc, w/Sc crop rotation treatment plots. The physical and chemical properties of these soils are discussed in detail in Chapter 2 and summarized in Tables 3.1, 3.2, and 3.3.

Elemental and Isotopic Analysis

Each water-stable aggregate fraction and intra-macroaggregate fraction isolated as previously described in Chaper 2 was subject to isotopic analysis ($\delta^{13}C$ and $\delta^{15}N$), which was conducted at the University of Kentucky's Environmental Research Training Laboratory (ERTL). First, samples were weighed to approximately 20 mg in 5 x 9 mm silver capsules (Costech Analytical code 041067) using a Sartorius microbalance. Each sample was treated repeatedly with 50 µL H_2SO_4 (to remove carbonate) until no reaction was observed. Samples were analyzed via continuous flow using a Costech 4010 elemental analyzer, interfaced with a Thermo Finnigan Conflo III to a Thermo Finnigan Delta Plus XP isotopic ratio mass spectrometer (IRMS). Isotopic compositions were standardized using IAEA-N1 (NIST RM 8547) for $\delta^{15}N$ and U.S.G.S. 24 (NIST RM 8541) for $\delta^{13}C$. The $\delta^{13}C$ and $\delta^{15}N$ values were calculated from the measured C isotope ratios ($^{13}C/^{12}C$) and ($^{15}N/^{14}N$), respectively, of the sample (R_1) and standard (R_2) gas:

$$\delta^{13}C \ \ or \ \delta^{15}N\left(‰\right) = \frac{(R_1 - R_2)}{R_2} \times 10^3$$

Using mean δ^{13}C values, we applied the natural abundance method to estimate the proportion of corn-derived C (C_4-derived C) under the different management treatments according to Angers and Giroux (1996):

$$f = \frac{(\delta_s - \delta_m)}{(\delta_c - \delta_m)}$$

where *f* = proportion of corn-derived C, $\delta_s = \delta^{13}$C value of sample (isolated soil aggregate fractions), $\delta_m = \delta^{13}$C value of the corresponding fraction from the sod soil, and $\delta_c = \delta^{13}$C value of corn root residues, for which a typical value of -12.3 ‰ was assumed.

The quantity of C_4-derived C (C_1) and C_3-derived C (C_0) of aggregate fractions can be calculated as

$$C_1 = C \times f \ \text{ and } \ C_0 = C \times (1 - f)$$

where C is the total C concentration of the aggregate fraction (Zach et al., 2006).

Calculation of the apparent turnover time or mean age of organic carbon

The apparent turnover time T of soil organic C fractions was calculated by using the first-order decay model (Balesdent and Mariotti, 1996):

$$T = 1/k = -(t - t_0)/\ln(C_t / C_{t0})$$

where k is the rate constant of the first-order decay equation, t gives the time of sampling (year), t_0 the time of vegetation change (year), C_t the proportion of remaining C labeled from C_3-derived C in the soil at the time of sampling (%) and C_{t0} the percentage of C labeled from C_3 plants at t_0 (%). For the three field experiments, t was 2006; t_0 was 1983 for Field Experiments 1 and 3 and 1992 for Field Experiment 2, C_{t0} was 100%; and C_t was calculated as 100%-(*f* x 100%).

Nonetheless, John et al. (2005) pointed out that the calculated apparent turnover times of organic C in aggregate fractions does not describe C turnover within aggregates because the dynamics of organic matter in aggregate fractions is strongly interrelated with the life-time of aggregates and C may have resided in other fractions before entering aggregates. Consequently, they use the term “mean age” instead of “apparent turnover time” for organic C in soil aggregate fractions.

Statistical analysis

SAS (1999) was used for all statistical analysis. Management effects on $\delta^{13}C$, $\delta^{15}N$, C_3-derived C, C_4-derived C, and mean age of SOM fractions were evaluated by ANOVA. The chosen model was based on the experimental design for each field experiment, and significant differences among means were determined by LSD means separation (Saxton, 1998). The probability level used to define statistical significance is specified in each related table or graph. In addition, the effects of aggregate size and type of intra-macroaggregate fraction on $\delta^{13}C$, δ^{15} N, C_3-derived C, C_4-derived C, and mean age were analyzed using ANOVA for a completely randomized design, which was performed separately for each field treatment.

Results and Discussion

Delta ^{13}C and δ^{15} N values of SOM in water-stable aggregate fractions-Field Experiment 1

The δ^{13}C and δ^{15}N values of SOM in water-stable aggregate fractions of Field Experiment 1 are presented in Table 3.4. Delta ^{13}C in large macroaggregates and small macroaggregates was not significantly affected by management practices. In contrast, δ^{13}C in microaggregates was significantly affected by tillage showing δ^{13}C enrichment under NT, which is consistent with Clapp et al. (2000) and Six et al. (2002a) and suggests the formation of stable microaggregates under NT that stabilize and sequestered C in the long term. Delta ^{13}C in the silt and clay fraction was significantly affected by the interaction of tillage and N rate showing that NT promotes δ^{13}C enrichment especially when N was not applied. As expected the most negative δ^{13}C value was associated with the sod soil for every water-stable aggregate fraction because *Poa pratensis* is a grass with the C_3 pathway and discriminates against atmospheric $^{13}CO_2$ to a greater extent than C_4 plants. In addition, the analysis of aggregate size effect on δ^{13}C values showed increasing δ^{13}C enrichment of soil organic C with increasing aggregate size for every tillage by N rate treatment; while this trend was reversed for the sod soil sample. These results suggest that the <53 μm fraction is the least enriched in recently deposited C, which is consistent with other findings (Angers and Giroux, 1996; John et al., 2005).

On the other hand, δ^{15}N in every water-stable aggregate fraction was significantly affected by management practices. The main effect of N fertilization was observed on δ^{15}N in large macroaggregates, the tillage effect was observed on δ^{15}N in microaggregates, and both tillage and N fertilization effects were observed on δ^{15}N in small macroaggregates. Nitrogen fertilization and NT reduced δ^{15}N in these aggregate fractions. In contrast, the interaction effect of tillage by N rate was observed for the <53 μm fraction showing δ^{15}N reduction with N fertilization only under MP. Delta ^{15}N in every water-stable fraction of the sod soil was slightly lower than that observed for the tillage by N rate treatments, suggesting that C_3 plants were discriminating against ^{15}N more than C_4 plants. In addition, increasing δ^{15}N enrichment of SOM with decreasing aggregate size was observed in the sod soil and every tillage by N rate treatment except for NT without N fertilization. These results suggest accumulation of more processed

SOM, probably of microbial origin (Tiessen et al., 1984) in smaller aggregate size classes. Nonetheless, Angers et al. (1997) and Aita et al. (1997) found a greater proportion of $\delta^{15}N$, than $\delta^{13}C$, in the fraction <50 μm and ascribed this to higher solubility of the $\delta^{15}N$ source, as compared to the source of $\delta^{13}C$.

Delta ^{13}C and δ^{15} N values of SOM in intra-macroaggregate fractions-Field Experiment 1

The $\delta^{13}C$ values of SOM in every intra-aggregate fraction but the "free" from samples taken in Field Experiment 1 were significantly affected by management practices (Table 3.4). Delta ^{13}C in the occluded fraction was significantly affected by the interaction of tillage and N rate, showing $\delta^{13}C$ enrichment under NT only when N was not applied. Delta ^{13}C in the >250 μm_{SM} fraction showed the main effects of tillage and N fertilization; while $\delta^{13}C$ in the 250-53 μm_{SM} and <53 μm_{SM} fractions showed only the main effects of tillage. Delta ^{13}C enrichment was observed under NT. Also, $\delta^{13}C$ in the free, and especially in the occluded, fractions for all the tillage by N rate treatments was as negative as that found for the sod soil sample; while $\delta^{13}C$ in the remaining fractions was more negative for the sod soil than for the tillage by N rate treatments. In addition, the order of increasing $\delta^{13}C$ enrichment was occluded<free<(<53 μm_{SM})<(>250 μm_{SM})<(250-53 μm_{SM}), indicating distinct differences in the stability of these soil organic C pools. The 250-53 μm_{SM} fraction accumulated most of the recently deposited C, and this fraction exhibited the highest soil organic C content among the rest of the intra-macroaggregate fractions (reported in Chapter 2). Angers et al. (1997) reported the 50-250 μm stable aggregate fraction as the one involved in the storage and stabilization of C for a silty soil in a field experiment using $^{13}C^{15}N$-labelled wheat straw.

Delta ^{15}N values in every intra-macroaggregate fraction but the "free" were significantly affected by tillage and N rate. Depletion in $\delta^{15}N$ was associated with NT and N fertilization. Also, $\delta^{15}N$ in intra-macroggregate fractions of the sod soil was lower that that observed in the tillage by N rate treatments, especially in the heavy fractions. The highest $\delta^{15}N$ enrichment of soil organic C was associated with the <53 μm_{SM} fraction for every tillage by N rate treatment and the sod soil suggesting that this fraction is the more stable soil N pool (Christensen, 1992).

Delta ^{13}C and δ^{15} N values of SOM in water-stable aggregate fractions-Field Experiment 2

The δ^{13}C and δ^{15}N values of SOM in water-stable aggregate fractions of Field Experiment 2 are presented in Table 3.5. Significant management effects on δ^{13}C were observed only for the microaggregate fraction. Depletion in δ^{13}C was found after N fertilization especially when manure was applied. The manure isotopic composition could not be claimed as a factor for these results because the unmanured-0N and the manured-0N treatments exhibited similar δ^{13}C values. Nonetheless, the depletion in δ^{13}C could be explained by the slower decay of ^{13}C depleted lignin in the early steps of C decay following manure and N application (Balesdent and Mariotti, 1996). As expected, the sod soil exhibited greater δ^{13}C depletion compared to the manure by N rate treatments. In addition, aggregate size did not have an effect on δ^{13}C values for any manure by N rate treatment. Nonetheless, the highest depletion in δ^{13}C was observed in the microaggregate fraction for the sod soil suggesting once again that this fraction accumulates most of the recently deposited C.

On the other hand, δ^{15}N in every water-stable aggregate fraction was significantly affected by management practices. The main effects of manure and N rate were observed for δ^{15}N in large macroaggregates and the silt and clay fraction. Enrichment in δ^{15}N was found with manure application, while depletion in δ^{15}N was observed with N fertilization. The interaction of manure by N rate for δ^{15}N was observed in small macroaggregates and microaggregates. The δ^{15}N in every water-stable fraction of the sod soil was also lower than that observed in the manure by N rate treatments, similar to what was observed in Field Experiment 1. In addition, increasing δ^{15}N enrichment of soil organic C with decreasing aggregate size was observed, especially when manure was not applied. This was not found in the presence of manure. The remaining treatments, and the sod soil, did not show any aggregate size effect on δ^{15}N. These results suggested that δ^{15}N enriched manure accumulates first in the biggest aggregate size class.

Delta ^{13}C and δ^{15} N values of SOM in intra-macroaggregate fractions-Field Experiment 2

The δ^{13}C values of SOM in the free and occluded fractions were the only ones significantly affected by management treatments in Field Experiment 2 (Table 3.5). Delta

^{13}C in the free fraction was significantly affected by N fertilization showing enrichment in δ^{13}C after N fertilization. Delta ^{13}C in the occluded fraction exhibited the manure by N rate interaction, showing δ^{13}C enrichment after N fertilization only when manure was not applied. The δ^{13}C values in the soil sod fractions were also more negative than those observed for the manure by N rate treatments; the difference was greater for microaggregates and for the silt and clay fraction. In addition, the increasing order of δ^{13}C enrichment; occluded<free<(>250 μm_{SM})<(<53 μm_{SM})<(250-53 μm_{SM}); for all the manure by N rate treatments and the sod soil, indicated that most of the recently deposited C accumulation is found in the 250-53 μm_{SM} fraction. These results support the findings from Field Experiment 1, but the second most enriched δ^{13}C pool is not consistent between the two experiments.

Delta ^{15}N values in every intra-macroaggregate fraction but the “free” were significantly affected by management. The N rate effect was significant for δ^{15}N in the occluded fraction; while the effect of manure and N rate were observed for δ^{15}N in the >250 μm_{SM} and the <53 μm_{SM} fractions, exhibiting enrichment in δ^{15}N with manure application, and depletion in δ^{15}N with N fertilization. The interaction of manure by N rate was observed on δ^{15}N in the 250-53 μm_{SM} fraction showing depletion in δ^{15}N with N fertilization only when manure had been applied and suggesting that manure stimulates N fertilizer use. The δ^{15}N in intra-macroggregate fractions of the sod soil was also in the range found among the manure by N rate treatments, indicative of similar N fractionation between C_3 and C_4 plants. In addition, the highest δ^{15}N enrichment was associated with the <53 μm_{SM} fraction for the unmanured treatments and the sod soil; while the effect of the type of the intra-macroaggregate fraction on δ^{15}N was less evident in the manured treatments, especially with N fertilization.

Delta ^{13}C and δ^{15} N values of SOM in water-stable aggregate fractions-Field Experiment 3

The δ^{13}C and δ^{15}N values of SOM in water-stable aggregate fractions of Field Experiment 3 are presented in Table 3.6. Delta ^{13}C in every water-stable aggregate fraction was significantly affected by crop rotation. The highest δ^{13}C enrichment was observed in continuous corn, while the lowest δ^{13}C enrichment was found in the hHccc crop rotation. As was expected, increased $\delta^{13}C$ enrichment was associated with the increase of corn in the hhccc crop rotation. The w/sC crop rotation exhibited $\delta^{13}C$ values similar to those observed for the hhccC treatment. The sod soil also showed more negative $\delta^{13}C$ values in every water-stable aggregate fraction when compared to the crop rotation treatments. In addition, the aggregate size effect on $\delta^{13}C$ values was not consistent through all the crop rotation treatments. Increasing $\delta^{13}C$ enrichment of SOM with increasing aggregate size was observed for continuous corn and for the Hhccc treatment; while the most $\delta^{13}C$ enriched fraction was the fraction <53 μm for the hhccC, hhcCc, hhCcc, w/sC treatments and the sod soil. Delta ^{13}C in the hHccc and the W/Sc was not significantly affected by aggregate size. These results suggest that C stabilization in different aggregate size fractions is closely related to quality and composition of C inputs.

On the other hand, $\delta^{15}N$ in every water-stable aggregate fraction but the fraction <53 μm was significantly affected by crop rotation. The highest $\delta^{15}N$ depletion was observed in the hHccc treatment for all aggregate fractions; while the highest enrichment in $\delta^{15}N$ was observed in the CCCCC and w/sC treatments for the large macroaggregate fraction, in the CCCCC and W/Sc treatments for the small macroaggregate fraction, and in the W/Sc treatment for the microaggregate fraction. The smallest water-stable aggregate fraction of the sod soil showed consistently lower $\delta^{15}N$ values than those observed for the crop rotation treatments, while the rest of the water-stable aggregate fractions of the sod and crop rotations showed $\delta^{15}N$ values in a similar range with the exception of the continuous corn treatment. These results reinforce what was observed in Field Experiments 1 and 2 in terms of differences in discrimination of ^{15}N between C_3 and C_4 plants. In addition, increasing $\delta^{15}N$ enrichment of soil organic C with decreasing aggregate size was observed for every crop rotation treatment; while the $\delta^{15}N$ in the sod soil showed no significant difference due to aggregate size, implying that N metabolic activity is less intensive in larger water-stable aggregates under the studied crop rotations.

Delta ^{13}C and δ^{15} N values of SOM in intra-macroaggregate fractions-Field Experiment 3

The $\delta^{13}C$ values of SOM in every intra-aggregate fraction but the free one of Field Experiment 3 were significantly affected by crop rotation (Table 3.6). As expected, the

CCCCC treatment showed the highest $\delta^{13}C$ enrichment in every intra-macroaggregate fraction. Increasing $\delta^{13}C$ enrichment was also observed with increased corn in the hhccc crop rotation, for every intra-macroaggregate fraction. Delta ^{13}C in fractions of the w/sC treatment was not significant different from that observed for the hhccC treatment. Every intra-macroaggregate fraction from the sod soil also presented more negative $\delta^{13}C$ value compared to crop rotation treatments. In addition, the effect of the type of intra-macroaggregate fraction on $\delta^{13}C$ was not consistent throughout the crop rotation treatments. The increasing $\delta^{13}C$ enrichment as free<occluded<(<53 μm_{LM}, >250 μm_{LM})<(250-53 μm_{LM}) was observed only for the CCCCC treatment; while there was no effect of type of intra-macroaggregate fraction on $\delta^{13}C$ for the w/sC treatment. On the other hand, increasing corn in the hhccc crop rotation diversified the effect of type of fraction on $\delta^{13}C$. Higher $\delta^{13}C$ enrichment for the three heavy fractions (<53 μm_{LM}, 250-53 μm_{LM}, and >250 μm_{LM}) compared to the light fractions (free and occluded) was observed for the hhCcc treatment, higher $\delta^{13}C$ enrichment for the smaller heavy fractions (<53 μm_{LM}, 250-53 μm_{LM}) was observed for the hhcCc treatment, and higher $\delta^{13}C$ enrichment for the free fraction was observed for the hhccC treatment. The sod soil showed increasing $\delta^{13}C$ enrichment as free<occluded<(>250 μm_{LM})<(250-53 μm_{LM}, <53 μm_{LM}) suggesting that recently deposited C accumulation is mainly taking place in the light fraction and the coarser heavy fraction.

Delta ^{15}N was significantly affected by crop rotation only in the two smallest intra-macroaggregate fractions. Delta ^{15}N enrichment was observed in both fractions for the CCCCC treatment. The $\delta^{15}N$ in every fraction for the sod soil regarding the crop rotation treatments followed the same trend observed for the water-stable aggregate fractions. In addition, the effect of fraction type on $\delta^{15}N$ values showed $\delta^{15}N$ depletion in the free fraction and $\delta^{15}N$ enrichment in the <53 μm_{LM} fraction for every crop rotation treatment and the sod soil.

Corn-derived C in water-stable aggregate fractions-Field Experiment 1

The percentage of corn derived C in water-stable aggregates of Field Experiment 1 is presented in Figure 3.1, and the respective quantity of C_4- and C_3-derived C is summarized in Table 3.7. The proportions of corn derived C in large macroaggregates and small macroaggregates were not significantly affected by management practices. In contrast, the proportion of corn derived C in microaggregates was significantly affected by tillage, showing higher values associated with NT, and the proportion of corn derived C in the fraction <53 μm was significantly affected by the interaction of tillage by N rate showing a higher proportion of corn derived C under NT, especially when N had not been applied. In addition, the increasing proportion of corn derived C was associated with increasing aggregate size for every tillage by N rate treatment. The enrichment of larger aggregates with recently added C has been widely reported (Puget et al., 1995; Angers and Giroux, 1996; Angers et al., 1997; Puget et al., 2000, John et al., 2005). Angers and Giroux (1996) reported a decreasing percentage of corn derived C with decreasing aggregate size: 20.5% (>2000 μm), 18.2% (1000-2000 μm), 11.6% (500-1000 μm), 8.2% (250-500 μm), 3.4% (50-250 μm) and 0.3% (<50 μm) from a soil under 15 yr of continuous corn. John et al. (2005) found that 24% of corn derived C in the <53 μm fraction steadily increased with increasing aggregate size to 47% in the >1000 μm fraction.

Corn-derived C in water-stable aggregate fractions-Field Experiment 2

The proportion of corn derived C in water-stable aggregates of Field Experiment 2 is presented in Figure 3.2, and the respective quantity of C_4- and C_3-derived C is summarized in Table 3.8. The proportion of corn derived C was not significantly affected by management practices in large macroaggregate, small macroaggregate, and silt and clay fractions. In contrast, the interaction effect of manure by N rate was significant for the proportion of corn derived C in microaggregates showing higher values after manure application only when N was not applied. The highest proportion of corn derived C was also observed in the microaggregate fraction, followed by the large and small macroaggregate fractions, and then by the silt and clay fraction for all the manure by N rate treatments but the Manured-168N one. This treatment showed a lower proportion of corn derived C in the fraction <53 μm compared to the remaining fractions.

Corn derived C in water-stable aggregate fractions-Field Experiment 3

The proportion of corn derived C in water-stable aggregates from Field Experiment 3 are presented in Figure 3.3, and the quantities of C_4- and C_3-derived C are summarized in Table 3.9. The proportion of corn derived C in every aggregate size class was significantly affected by crop rotation. However, the crop rotation effect was more evident in larger aggregate size classes. As expected, the highest and the lowest proportion of corn-derived C were associated with the CCCCC and the hHccc treatments, respectively, for every aggregate size class. The second highest proportion of corn derived C was related to the W/Sc and the Hhccc treatments for the three highest aggregate size classes. Also, the increase of corn in the hhccc crop rotation nicely corresponded to the increasing proportion of corn derived C as hhCcc<hhcCc<hhccC, which was observed for the large and small macroaggregate fractions, but not for smaller aggregate size classes. On the other hand, the trend of increasing proportion of corn derived C with increasing aggregate size was nicely observed for the CCCCC treatment and was supported by similar results for the crop rotation treatments, except for hhCcc, which showed the highest proportion of corn derived C in the microaggregate fraction.

Corn derived C in intra-macroaggregate fractions-Field Experiment 1

The proportion of C derived from corn in intra-macroaggregates from Field Experiment 1 is presented in Figure 3.4. The proportion of corn derived C, in every intra-macroaggregate fraction was significantly affected by management treatments, except for the free fraction. Tillage and N rate effects were significant for the occluded and the >250 μm_{SM} fractions; while tillage effect was observed for the 250-53 μm_{SM} and the <53 μm_{SM} fractions. The proportion of corn derived C was higher under NT, compared to MP, across all of the aggregate fractions, but the increase in the proportion of corn derived C due to N fertilization was observed mainly under MP in the occluded and the >250 μm_{SM} fractions. On the other hand, the highest proportion of corn derived C was associated with the 250-53 μm_{SM} fraction for all of the tillage by N rate treatments, except for the MP-150N one, which showed the same proportion of corn derived C in every intra-macroaggregate fraction. These results were consistent with Degryze et al. (2004) and Kong et al. (2005). They suggested that the microaggregates (250-53 μm) within small macroaggregates are an ideal indicator of C sequestration potential in sustainable agroecosystems.

Corn derived C in intra-macroaggregate fractions-Field Experiment 2

The proportion of C derived from corn in intra-macroaggregates from Field Experiment 2 is presented in Figure 3.5. Only the proportions of corn derived C in free and occluded fractions were significantly affected by management treatments. Nitrogen fertilization significantly affected the proportion of corn derived C in the free fraction, which exhibited higher values after N fertilization. Manure and N rate effects significantly affected the proportion of corn derived C in the occluded fraction, exhibiting higher values after N fertilization, especially in the unmanured system. In addition, the highest proportion of corn derived C was associated with the free, the 250-53 μm_{SM} and the <53 μm_{SM} fractions from the N fertilized treatments (Unmanured-168N and Manured-168N); while the highest proportion of corn derived C was associated with the 250-53 μm_{SM} and the <53 μm_{SM} fractions only for the un-N-fertilized treatments (Unmanured-0N and Manured-0N).

Corn-derived C in intra-macroaggregate fractions-Field Experiment 3

The proportion of C derived from corn in intra-macroaggregates from Field Experiment 3 is presented in Figure 3.6. The proportion of corn derived C, in every intra-macroaggregate fraction except the free fraction, was significantly affected by crop rotation. As expected, the highest proportion of corn derived C was associated with the ccccc treatment for every aggregate fraction. Also, the increase of corn in the hhccc crop rotation corresponded to the increasing proportion of corn-derived C as hhCcc<hhcCc<hhccC in the 250-53 μm_{LM} and the <53 μm_{LM} fractions. However, this trend was not observed in the other aggregate fractions. In addition, the proportion of corn derived C in every aggregate fraction for the w/sC treatment was in the upper level of the range observed for the hhccc treatments. Moreover, the effect of aggregate type on the proportion of corn derived C was not consistent across the crop rotation treatments. The highest proportion of corn-derived C was observed in the 250-53 μm_{LM} fraction for the CCCCC and the hhcCc treatment, in the free fraction for the hhccC treatment, and in the >250 μm_{LM} fraction for the hhCcc treatment. In contrast, equal proportion of corn derived C was observed across all of the aggregate types for the w/sC treatment.

Mean age of soil organic C in aggregate fractions-Field Experiment 1

Soil organic C mean age in water-stable aggregates and intra-macroaggregates of Field Experiment 1 is presented in Table 3.7. Soil organic C mean age in every water-stable aggregate fraction except for the large macroaggregate one was affected significantly by management practices. Tillage significantly affected soil organic C mean age in the small macroaggregate and the microaggregate fractions; while tillage and N rate effects significantly affected soil organic C mean age in the <53 μm fraction. Longer soil organic C mean age was was found with MP, compared to NT, and with N fertilization. Those results are not consistent with Gregorich et al. (1996), who reported that soil organic C turnover was significantly influenced by long-term N fertilization under no-tilled continuous corn. In addition, the effect of aggregate size on the mean age of soil organic C was significant across all of the tillage by N rate treatments. The mean age of soil organic C increased with decreasing aggregate size, indicating that organic matter associated with macroaggregates was younger and more readily mineralized than that associated with microaggregates and the <53 μm fraction (Elliot, 1986; Martin et al., 1990; Christensen, 1992; Feller and Beare, 1997; John et al., 2005). Nonetheless, the mean age of soil organic C calculated in this study does not necessarily represent soil organic C turnover in aggregates, because aggregate turnover also affects soil organic C storage in the aggregates.

On the other hand, mean age of soil organic C in intra-macroaggregate fractions was significantly affected by tillage only when those were heavy fractions. Longer mean age of soil organic C was found under MP than under NT. The effect of the type of aggregate fraction significantly affected mean age of soil organic C in every tillage by N rate treatment except for the MP-150N treatment. Longer mean age of soil organic C was observed for the light (free and occluded) fractions, compared to the heavy ones. Unlike our results, Martel and Paul (1974) and Trumbore et al. (1996) found that low density SOM from whole soils turns over faster than high density, mineral associated SOM. Gregorich et al. (1997) also reported that most of the free light fraction organic matter was corn derived in fertilized corn soils. Nonetheless, John et al. (2005) found a longer mean soil organic C age in light occluded fractions compared to free POM and mineral-SOM fractions. In addition, longer mean soil organic C age in the free light fraction found in this study may be explained by the likely presence of un-decomposed C_3-plant tissue in this fraction, coming from the cover crop also used in this field experiment.

Mean age of soil organic C in aggregate fractions-Field Experiment 2

The mean ages of soil organic C in water-stable aggregates and intra-macroaggregates from Field Experiment 2 are presented in Table 3.8. The mean soil organic C age for every water-stable aggregate fraction was unaffected by management treatments. Nevertheless, the effect of aggregate size on the mean age of soil organic C was significant across all manure by N rate treatments. The longest mean soil organic C age was found in the silt and clay fraction from every manure by N rate treatment, which is consistent with what was observed for Field Experiment 1.

On the other hand, mean soil organic C age only in the light intra-macroaggregate fractions, was significantly affected by treatments. The mean age of soil organic C in the free fraction was significantly affected by N fertilization exhibiting lower values with N fertilization. The mean soil organic C age for the occluded fraction was significantly affected by the manure by N rate interaction. Shorter mean soil organic C age was found with N fertilization, especially in unmanured soils. In addition, the effect of the type of aggregate fraction significantly affected the mean soil organic C age in every manure by N rate treatment. The longest mean age of soil organic C was observed for the occluded fraction across all of the manure by N rate treatments except for Manured-0N, which had the longest mean age of soil organic C associated with the free fraction. These results were consistent with those observed in Field Experiment 1. The longest mean age of soil organic C for the light fractions in this experiment may be also explained by the likely presence of un-decomposed C_3-plant residues coming from the cover crop used in this experiment.

Mean age of soil organic C in aggregate fractions-Field Experiment 3

The mean ages of soil organic C in water-stable aggregates and intra-macroaggregates from Field Experiment 3 are presented in Table 3.9. The mean soil organic C age in every water-stable aggregate fraction was significantly affected by crop rotation. The longest mean soil organic C age was associated with the hHccc treatments for every aggregate size fraction except for the large macroaggregate fraction, which also had the longest mean soil organic C age associated with the hhCcc treatment. In contrast, the shortest mean soil organic C age was associated with the CCCCC treatment, for every aggregate size fraction, and the mean soil organic C age in the w/SC treatment was as large as that observed in the continuous corn treatment. These results are in line with the findings of Studdert and Echeverria (2000). Additionally, the effect of aggregate size on the mean soil organic C age was significant across all crop rotation treatments. The largest mean soil organic C age was found in the <53 μm fraction for all crop rotation treatments except for the hhCcc treatment, which exhibited the largest mean soil organic C age in the large macroaggregate and <53 μm fractions.

On the other hand, mean soil organic C age in intra-macroaggregate fractions was affected significantly by crop rotation only when those fractions were heavy. The shortest mean soil organic C age was observed for the CCCCC treatment in every heavy intra-macroaggregate fraction; while the highest mean soil organic C age was associated with the hhCcc treatment, for the two smallest aggregate size fractions, and with the hhcCc and w/sC treatments for the >250 μm_{LM} fraction. The effect of aggregate fraction significantly affected the mean soil organic C age in every crop rotation treatment except for hhCcc. Higher mean soil organic C age was observed for the light fractions, as compared to the heavy ones. These results are consistent with the results from Field Experiments 1 and 2. The likely presence of un-decomposed C_3-plant tissue in the light fraction coming from the hay, wheat, or soybeans would contribute to the longer mean soil organic C age observed for light fractions in the hhccc and w/sc crop rotations; while high ^{13}C-depleted lignin content (Balesdent and Mariotti, 1996) in corn residues could have contributed to the larger mean soil organic C age in light fractions from the continuous corn treatment.

Conclusions

The soil organic C stability based on $\delta^{13}C$ of physically fractionated SOM pools was sensitive to the type of management practice. For Field Experiment 1, $\delta^{13}C$ in smaller water-stable aggregate size classes was most affected by tillage and N fertilization. No-tillage was associated with $\delta^{13}C$ enrichment; while N fertilization was related to $\delta^{13}C$ depletion. In addition, increasing $\delta^{13}C$ enrichment with increasing size fraction indicated that most of the recently deposited C was accumulated in water-stable macroaggregates and that the most stable soil organic C was associated with the silt and clay fraction, which was consistent with the calculated mean age of soil organic C for these fractions. On the other hand, intra-macroaggregate fractions were also sensitive to the type of management. Tillage and N fertilization affected the occluded and the >250 μm_{SM} fractions; while tillage mainly affected the two smallest fractions. The greatest accumulation of corn-derived C within small macroaggregates was associated with the 250-53 μm_{SM} fraction, which was consistently the youngest intra-macroaggregate fraction.

For Field Experiment 2, $\delta^{13}C$ in the water-stable microaggregate fraction was the only fraction sensitive to manure and N fertilization. The greatest $\delta^{13}C$ enrichment was observed after manure application without N fertilization. Microaggregates had the highest accumulation of corn derived C, and consequently they exhibited the lowest mean soil organic C age among water-stable aggregate size classes. In contrast, the silt and clay fraction was the oldest, more stable, fraction. Additionally, among the intra-macroaggregate fractions, the light fractions were the only fractions sensitive to management. The free fraction responded to N fertilization, while the occluded fraction responded to the interaction of manure and N fertilization. Nitrogen fertilization was responsible for greater accumulation of recently deposited C in the free fraction; while N fertilization without manure was associated with the highest recently deposited C accumulation in the occluded fraction. Nonetheless, the greatest $\delta^{13}C$ enrichment among intra-macroaggregate fractions was observed for the 250-53 μm_{SM} fraction followed by the <53 μm_{SM} fraction, which was consistent with the calculated mean soil organic C age for these fractions.

For Field Experiment 3, $\delta^{13}C$ in every water-stable aggregate fraction was sensitive to crop rotation. Delta ^{13}C, and consequently the proportion of corn derived C, increased steadily in every water-stable aggregate fraction with years of corn cropping. However, the proportion of corn derived C in the w/sC crop rotation, in every fraction, was close to that observed for continuous corn, indicating that C quality and composition are key factors in C stabilization. In addition, the proportion of corn derived C decreased with decreasing aggregate size and consequently older and more stable soil organic C was observed in the <53 μm fraction for most of the crop rotations. On the other hand, $\delta^{13}C$ in every intra-macroagggregate fraction but the "free" was sensitive to crop rotation. Increased corn in the rotation was reflected by the increasing $\delta^{13}C$ enrichment, by the increasing proportion of corn derived C, and consequently by the decreasing mean soil organic C age in every intra-macroaggregate fraction. However, the longest mean soil organic C age was not associated with the same crop rotation, across the intra-macroaggregate fractions. Also, the distribution of corn derived C across intra-macroaggregate fractions was not consistent for every crop rotation. The greatest corn derived C accumulation in the 250-53 μm_{LM} fraction was only observed for the continuous corn and hhcCc rotations.

On the other hand, ^{15}N natural abundance was also sensitive to management treatments across the three field experiments. For water-stable aggregate fractions, tillage affected $\delta^{15}N$ mainly in the smaller aggregate size classes, N fertilization and manure application affected $\delta^{15}N$ in every water-stable fraction, and crop rotation affected $\delta^{15}N$ in every fraction but the <53 μm fraction. No-tillage and N fertilization were associated with $\delta^{15}N$ depletion; while manure application, continuous corn and the w/sC rotation were related to $\delta^{15}N$ enrichment. Also, increasing $\delta^{15}N$ enrichment with decreasing aggregate size indicated the accumulation of more processed SOM in the clay and silt fraction. For intra-macroaggregate fractions, tillage, N fertilization, and manure application affected $\delta^{15}N$ in every fraction except for the "free". In contrast, crop rotation affected $\delta^{15}N$ only in microaggregates and the <53 μm fractions. The highest $\delta^{15}N$ enrichment was associated with the silt and clay fraction.

In short, physical fractionation of soil organic C and the use of ^{13}C and ^{15}N natural abundance showed that SOM associated with different aggregate fractions had different ages and dynamics and responded specifically to differences in management. Nonetheless, these results suggested that not only physical protection, but also quality and composition of C inputs, deserve special attention when C stabilization in aggregate fractions is studied. Consequently, the mean age of organic C calculated for these physically separated fractions may also be expressing differences in chemical structure and recalcitrance.

Tables

Table 3.1. Physical and chemical properties from the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

Tillage	N Rate	pH_{Water}	Θ_g	D_b	Sand	Silt	Clay	MWD	Total C	Total N	C/N
	(kg ha^{-1})		(%)	(g cm^{-3})		(%)		(mm)	(g /100 g soil)		
NT	0	6.66†	28.9b‡	1.18¶	12.8b††	71.4§	15.9§	2.47 †	2.16 b††	0.20 b††	10.79 a§
	150	6.42	30.2a	1.13	14.2a	71	14.8	2.3	2.73 a	0.25 a	10.92 a
MP	0	6.27	25.8c	1.18	11.3b	67.9	20.7	0.94	1.52 c	0.15 c	10.16 b
	150	5.96	25.7c	1.13	10.2c	69.3	20.5	0.59	1.58 c	0.16 c	10.19 b
Sod		5.67	31.7	1.14	14.8	72.1	13.1	2.57	2.85	0.28	10.05

† Main effect of tillage and N rate statistically significant ($P<0.05$)

‡ Interaction effect of tillage by N rate statistically significant ($P<0.10$)

§ Main effect of tillage statistically significant ($P<0.05$)

†† Interaction effect of tillage by N rate statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

NT (no-tillage); MP (moldboard plow); Θ_g (gravimetric water content); D_b (bulk density);

MWD (mean weight diameter on the wet sieving method using 2-0.053 mm size fractions)

Table 3.2. Physical and chemical properties from the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

Manure	N Rate	pH_{Water}	Θ_g	D_b	Sand	Silt	Clay	MWD	Total C	Total N	C/N
	(kg ha^{-1})		(%)	(g cm^{-3})		(%)		(mm)	(g /100 g soil)		
Unmanured	0	6.26†	26.1§	1.24ns	11.2ns	74.6ns	14.2ns	1.60ns	2.09§	0.20§	10.51ab‡
	168	5.66	25.8	1.27	11.3	74.1	14.5	1.75	2.05	0.19	10.69a
Manured	0	6.53	29.7	1.16	11.3	74.2	14.5	1.88	2.60	0.24	10.72ab
	168	5.87	28.3	1.21	12.9	71.8	15.2	1.87	2.40	0.24	10.04b
Sod		5.48	29.3	1.2	13.7	73.3	13	2.61	2.34	0.23	10.35

† Main effect of manure and N rate statistically significant (P<0.05)

‡Interaction effect of manure by N rate statistically significant (P<0.05)

§ Main effect of manure statistically significant (P<0.05)

ns = Not statistically significant (P<0.10)

Different letters indicate significant differences (P<0.05, Fisher's LSD) within a column

Θ_g (gravimetric water content); D_b (bulk density); MWD (mean weight diameter on the wet sieving method using 2-0.053 mm size fractions)

Table 3.3. Physical and chemical properties from the spring and the fall samplings (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

Crop Rotation	pH_{Water}	Θ_g	D_b	Sand	Silt	Clay	MWD	Total C	Total N	C/N
		(%)	(g cm^{-3})		(%)		(mm)	(g /100 g soil)		
CCCCC	6.17ns	29.0bc†	1.24a†	14.6ns	71.9ns	13.6ns	2.54a†	2.20 ab†	0.23 bc†	9.58 bc†
hhccC	6.89	29.3bc	1.17ab	14.1	71.4	14.6	2.48ab	2.14 bc	0.22 bcd	9.61 bc
hhcCc	6.60	28.0c	1.20ab	14.5	69.9	15.6	2.42ab	1.86 c	0.20 d	9.22 c
hhCcc	6.58	29.3bc	1.06c	16.4	68.8	14.8	1.88cd	2.24 ab	0.24 abc	9.58 bc
w/sC	7.06	29.1bc	1.24a	15.3	71.2	13.5	2.11bc	2.17 abc	0.22 cd	10.05 a
W/Sc	6.45	30.7ab	1.16ab	14.4	71.9	13.8	1.47e	2.13 bc	0.22 cd	9.65 ab
Hhccc	6.43	30.6ab	1.21ab	14.4	71.1	14.5	1.55de	2.32 ab	0.25 ab	9.32 bc
hHccc	6.68	31.7a	1.15b	14.1	72.2	13.7	1.35e	2.48 a	0.26 a	9.64 ab
Sod	6.91	24.2	1.07	17.2	70.6	12.1	2.67	2.60	0.25	10.51

† Effect of crop rotation statistically significant ($P<0.05$)

ns = Not statistically significant ($P<0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

Θ_g (gravimetric water content); D_b (bulk density); MWD (mean weight diameter on the wet sieving method using 2-0.053 mm size fractions);

CCCCC (Continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation); hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation);

hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/doble crop soybean crop rotation);

W/Sc (wheat/doble crop soybean in corn-wheat/double crop soybean crop rotation); Hhccc (first year of hay in hay-hay-corn-corn-corn crop rotation);

hHccc (second year of hay in hay-hay-corn-corn-corn crop rotation)

Table 3.4. $\delta^{13}C$ and $\delta^{15}N$ of water-stable aggregates and intra-small macroaggregates from the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

	$\delta^{13}C$					$\delta^{15}N$				
	No tillage		Moldboard plow		Sod	No tillage		Moldboard plow		Sod
	0 kg N ha^{-1}	150 kg N ha^{-1}	0 kg N ha^{-1}	150 kg N ha^{-1}		0 kg N ha^{-1}	150 kg N ha^{-1}	0 kg N ha^{-1}	150 kg N ha^{-1}	
Water-stable aggregate fractions										
>2000 µm	-21.66nsA	-21.61A	-21.99A	-21.59A	-24.72B	5.22A§	4.86B	5.45B	4.83B	4.65AB
2000-250 µm	-21.76nsA	-21.54A	-22.04A	-22.03AB	-24.88B	5.02A¶	4.76B	5.65AB	5.53A	4.53B
250-53 µm	-21.76A‡	-21.95AB	-22.20AB	-22.40B	-24.61B	5.15A‡	5.31A	5.94A	5.91A	4.81A
<53 µm	-21.88a†A	-22.28bB	-22.41bB	-22.53bB	-23.85A	5.01c†A	5.02cAB	6.07aA	5.84bA	4.84A
Intra-small macroaggregate fractions										
Free	-22.64nsC	-23.06BC	-22.80B	-20.19A	-23.77A	2.00nsC	5.04AB	3.60D	4.32B	2.45B
Occluded	-24.70a†D	-25.48abC	-26.90cC	-25.50bB	-25.18A	5.00B¶	3.52B	8.43A	6.29AB	5.25A
>250 µm$_{SM}$	-20.80B¶	-19.93A	-22.80B	-22.20A	-24.29A	5.03B¶	3.94AB	6.66BC	6.16AB	3.61AB
250-53 µm$_{SM}$	-19.10A‡	-19.13A	-20.09A	-19.94A	-23.55A	4.98B¶	4.41AB	5.93C	5.23AB	4.12AB
<53 µm$_{SM}$	-21.68BC‡	-21.46AB	-22.76B	-22.73AB	-23.60A	6.81A¶	6.40A	7.51B	7.42A	5.69A

† Interaction of tillage by N rate statistically significant ($P<0.05$)

‡ Only main effect of tillage statistically significant ($P<0.05$)

§ Only main effect of N rate statistically significant ($P<0.05$)

¶ Main effects of tillage and N rate statistically significant ($P<0.05$)

ns = Not statistically significant ($P<0.10$)

Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different ($P<0.05$, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different ($P<0.05$, Fisher's LSD)

Table 3.5. $\delta^{13}C$ and $\delta^{15}N$ of water-stable aggregates and intra-small macroaggregates from the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

	$\delta^{13}C$					$\delta^{15}N$				
	Unmanured		Manured		Sod	Unmanured		Manured		Sod
	0 kg N ha^{-1}	168 kg N ha^{-1}	0 kg N ha^{-1}	168 kg N ha^{-1}		0 kg N ha^{-1}	168 kg N ha^{-1}	0 kg N ha^{-1}	168 kg N ha^{-1}	
Water-stable aggregate fractions										
>2000 µm	-22.82nsA	-22.56A	-22.62A	-22.93A	-24.93AB	5.75B‡	5.47A	8.04AB	7.29A	4.56A
2000-250 µm	-23.04nsA	-22.72A	-22.80A	-23.00A	-25.11AB	5.94c†AB	5.71cA	7.92aAB	7.20bA	4.04A
250-53 µm	-22.91A§	-22.72A	-22.60A	-23.09A	-25.25B	6.12c†AB	5.89cA	8.08aA	7.30bA	4.28A
<53 µm	-22.92nsA	-22.86A	-22.73A	-23.00A	-24.54A	6.30A‡	5.92A	7.76B	7.28A	3.93A
Intra-small macroaggregate fractions										
Free	-25.99D¶	-23.87C	-26.33D	-22.08AB	-27.21D	3.88nsC	3.96BC	4.48C	8.36A	3.39B
Occluded	-26.58c†E	-25.98aD	-26.25bD	-26.13bC	-27.43D	4.48BC¶	3.35C	5.17B	4.43A	4.35AB
>250 µm$_{SM}$	-24.37nsC	-23.81C	-24.03C	-24.12BC	-25.53C	5.48A‡	4.57B	7.35A	6.42A	4.02AB
250-53 µm$_{SM}$	-21.32nsA	-21.04A	-20.85A	-21.33A	-23.65A	4.66c†B	4.31cB	7.14aA	6.33bA	3.53B
<53 µm$_{SM}$	-21.74nsB	-21.69B	-21.52B	-21.79AB	-24.43B	5.81A‡	5.55A	7.27A	6.87A	5.42A

† Interaction of manure by N rate statistically significant ($P<0.05$)

‡ Main effects of manure and N rate statistically significant ($P<0.05$)

§ Interaction of manure by N rate statistically significant ($P<0.10$)

¶ Only main effect of N rate statistically significant ($P<0.05$)

ns = Not statistically significant ($P<0.10$)

Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different ($P<0.05$, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different ($P<0.05$, Fisher's LSD)

Table 3.6. $\delta^{13}C$ and $\delta^{15}N$ of water-stable aggregates and intra-large macroaggregates from the spring and the fall samplings (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

	CCCCC	hhccC	hhcCc	hhCcc	w/sC	W/Sc	Hhccc	hHccc	Sod
					$\delta^{13}C$				
Water-stable aggregate fractions									
>2000 µm	-20.02a†A	-22.78bcAB	-23.24cdB	-23.75dC	-22.87bcAB	-22.24bcA	-22.44bcA	-23.66dA	-26.58B
2000-250 µm	-20.34a†AB	-22.79cdAB	-23.16cdeB	-23.35deB	-22.85cdAB	-22.23bA	-22.68bcAB	-23.71eA	-26.45B
250-53 µm	-20.62a†BC	-23.06cB	-23.15cB	-23.27cB	-23.08cB	-22.36bcA	-22.86bcB	-23.80dA	-26.54B
<53 µm	-20.85a†C	-22.60bA	-22.78bA	-22.80bA	-22.70bA	-22.57bA	-22.95bB	-23.87cA	-25.51A
Intra-large macroaggregate fractions									
Free	-23.42nsD	-19.69A	-23.96B	-24.95B	-22.25A				-26.49C
Occluded	-22.27a†C	-24.42bC	-24.06bB	-25.88cB	-23.74bA				-26.24C
>250 μm_{LM}	-19.60a†B	-21.21abAB	-23.35cB	-22.45bcA	-22.91bcA				-25.77B
250-53 μm_{LM}	-17.59a†A	-21.01bAB	-22.04cA	-22.58cA	-21.20bA				-25.18A
<53 μm_{LM}	-19.94a†B	-22.24bBC	-22.55bcA	-22.74cA	-22.32bA				-24.86A
					$\delta^{15}N$				
Water-stable aggregate fractions									
>2000 µm	4.03a‡B	3.45abC	3.70abC	3.56abB	3.92aB	3.74abC	3.20bC	3.09bC	3.70A
2000-250 µm	4.11ab‡B	3.67bcBC	3.90abcBC	3.89abcB	4.20abAB	4.29aBC	3.68bcB	3.40cBC	3.76A
250-53 µm	4.21abc†AB	3.92cdAB	4.12bcdAB	4.48abA	4.23abcAB	4.74aAB	3.85cdB	3.62dB	3.75A
<53 µm	4.49nsA	4.19A	4.37A	4.81A	4.61A	5.04A	4.27A	4.20A	3.85A
Intra-large macroaggregate fractions									
Free	2.08nsC	2.12C	1.56C	2.02C	1.13C				1.20C
Occluded	4.59nsB	3.33B	4.42AB	4.14B	3.53B				3.41B
>250 μm_{LM}	4.58nsB	4.58A	5.17AB	4.12B	5.15A				4.00AB
250-53 μm_{LM}	4.00a†B	3.28bcB	3.75abB	2.90cBC	3.68abB				3.43B
<53 μm_{LM}	6.15a†A	5.48cA	6.08abA	5.80bA	6.07abA				5.21A

† Crop rotation effect statistically significant ($P<0.05$)

‡ Crop rotation effect statistically significant ($P<0.10$)

ns = Not statistically significant ($P<0.10$)

Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different ($P<0.05$, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different ($P<0.05$, Fisher's LSD)

CCCCC (Continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation); hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation); hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/doble crop soybean crop rotation); W/Sc (wheat/doble crop soybean in corn-wheat/double crop soybean crop rotation); Hhccc (first year of hay in hay-hay-corn-corn-corn crop rotation); hHccc (second year of hay in hay-hay-corn-corn-corn crop rotation)

Table 3.7. C$_4$- and C$_3$-derived C and mean apparent SOC mean age of water-stable aggregates and intra-small macroaggregates from the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

	No-tillage		Moldboard plow	
	0 kg N ha^{-1}	150 kg N ha^{-1}	0 kg N ha^{-1}	150 kg N ha^{-1}
	C$_4$ Carbon (g/100 g soil fraction)			
Water-stable aggregate fractions				
>2000 μm	0.48 b§A	0.63aAB	0.37bA	0.68aA
2000-250 μm	0.56 A‡	0.69A	0.35A	0.39B
250-53 μm	0.52 A¶	0.50B	0.30B	0.28C
<53 μm	0.20 B‡	0.16C	0.12C	0.11D
Intra-small macroaggregate fractions				
Free	0.42 a#BC	0.16aC	0.21aB	0.90aA
Occluded	0.11 nsC	0.11C	0.02C	0.06B
>250 μm$_{SM}$	0.56 b§B	0.85aB	0.15cB	0.20cB
250-53 μm$_{SM}$	1.50 A‡	1.86A	0.86A	1.02A
<53 μm$_{SM}$	0.31 b#BC	0.37aBC	0.15cB	0.15cB
	C$_3$ Carbon (g/100 g soil fraction)			
Water-stable aggregate fractions				
>2000 μm	1.46 B†	1.88A	1.31A	2.13A
2000-250 μm	1.68 A‡	1.91A	1.21A	1.33B
250-53 μm	1.71 A¶	1.79A	1.22A	1.28B
<53 μm	0.96 C¶	1.04B	0.85B	0.87B
Intra-small macroaggregate fractions				
Free	1.88 a¶A	1.95AB	1.22B	1.28B
Occluded	0.46 b§C	0.63aC	0.33bE	0.31b
>250 μm$_{SM}$	1.04 B‡	1.24BC	0.64D	0.66C
250-53 μm$_{SM}$	1.97 b§A	2.46aA	1.62dA	1.80cA
<53 μm$_{SM}$	1.08 B‡	1.16C	0.97C	0.99B
	Mean Age (yr)			
Water-stable aggregate fractions				
>2000 μm	82.3 nsB	80.9B	93.7B	84.3C
2000-250 μm	82.7 B¶	75.1B	90.3B	90.6B
250-53 μm	87.7 B¶	96.9B	106.3B	118.4B
<53 μm	125.7 A‡	161.0A	173.3A	191.3A
Intra-small macroaggregate fractions				
Free	269.0 nsA	1168A	150.3B	251.8A
Occluded	107.8 nsAB	154.9B	742.8A	154.0A
>250 μm$_{SM}$	56.1 B¶	44.7B	117.7B	96.8A
250-53 μm$_{SM}$	40.9 B¶	41.3B	54.5B	51.4A
<53 μm$_{SM}$	93.6 B¶	86.8B	167.4B	164.9A

† Only main effect of N rate statistically significant ($P<0.05$)

‡ Main effects of tillage and N rate statistically significant ($P<0.05$)

§ Interaction of tillage by N rate statistically significant ($P<0.05$)

¶ Only main effect of tillage statistically significant ($P<0.05$)

Interaction of tillage by N rate statistically significant ($P<0.10$)

ns = Not statistically significant ($P<0.10$)

Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different ($P<0.05$, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different ($P<0.05$, Fisher's LSD)

Table 3.8. C_4- and C_3-derived C and mean apparent SOC mean age of water-stable aggregates and intra-small macroaggregates from the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

	Unmanured		Manured	
	0 kg N ha^{-1}	168 kg N ha^{-1}	0 kg N ha^{-1}	168 kg N ha^{-1}
	C_4 Carbon (g/100 g soil fraction)			
Water-stable aggregate fractions				
>2000 μm	0.37 nsA	0.47A	0.50A	0.40A
2000-250 μm	0.36 b§A	0.41abA	0.49aA	0.41Aab
250-53 μm	0.42 A¶	0.40A	0.54A	0.41A
<53 μm	0.14 nsB	0.14B	0.18B	0.14B
Intra-small macroaggregate fractions				
Free	0.12 b#C	0.18Cb	0.10Db	0.73ABa
Occluded	0.02 nsD	0.03D	0.05D	0.03C
>250 μm$_{SM}$	0.15 nsC	0.21C	0.25C	0.22C
250-53 μm$_{SM}$	0.77 b#A	0.89Ab	1.18Aa	0.89Ab
<53 μm$_{SM}$	0.32 B†	0.33B	0.41B	0.35BC
	C_3 Carbon (g/100 g soil fraction)			
Water-stable aggregate fractions				
>2000 μm	1.82 nsA	1.95A	2.24A	2.18A
2000-250 μm	1.87†A	1.78AB	2.23A	2.13A
250-53 μm	1.89‡A	1.63B	2.09A	2.01A
<53 μm	0.92 nsB	0.85C	1.04B	1.01B
Intra-small macroaggregate fractions				
Free	1.33 a#B	0.62Cb	1.44 Ba	1.37Ba
Occluded	0.37 b#D	0.29Dc	0.59 Da	0.30Cc
>250 μm$_{SM}$	0.99 C†	0.96B	1.35 BC	1.20B
250-53 μm$_{SM}$	2.50 A†	2.54A	3.11A	2.91A
<53 μm$_{SM}$	1.03 nsC	1.02B	1.18C	1.16B
	Mean Age (yr)			
Water-stable aggregate fractions				
>2000 μm	76.9 ABns	70.0B	69.3CB	85.1B
2000-250 μm	83.1 ABns	67.8B	70.5B	82.6B
250-53 μm	70.6 Bns	64.6B	61.3C	81.5B
<53 μm	98.6 Ans	94.8A	88.2A	109.7A
Intra-small macroaggregate fractions				
Free	160.8B¶	53.7C	204.1A	40.4C
Occluded	234.4a#A	135.5cA	166.7bB	152.4bcA
>250 μm$_{SM}$	101.0Cns	80.7B	91.8C	90.4B
250-53 μm$_{SM}$	52.3Cns	46.8C	43.6D	56.6C
<53 μm$_{SM}$	51.6Cns	50.6C	47.5D	53.1C

† Only main effect of manure statistically significant ($P<0.05$)

‡ Main effects of manure and N rate statistically significant ($P<0.05$)

§ Interaction of manure by N rate statistically significant ($P<0.10$)

¶ Only main effect of Nrate statistically significant ($P<0.05$)

Interaction of manure by N rate statistically significant ($P<0.05$)

ns = Not statistically significant ($P<0.10$)

Mean values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different ($P<0.05$, Fisher's LSD)

Mean values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different ($P<0.05$, Fisher's LSD)

Table 3.9. C_4- and C_3-derived C and mean apparent SOC mean age of water-stable aggregates and intra-macroaggregates from the spring and the fall samplings (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

	CCCCC	hhccC	hhcCc	hhCcc	w/sC	W/Sc	Hhccc	hHccc
	C_4 Carbon (g/100 g soil fraction)							
Water-stable aggregate fractions								
>2000 μm	0.95a†	0.58bcd	0.43de	0.40eA	0.51de	0.73bA	0.70bc	0.54cde
2000-250μm	0.93a†	0.58bc	0.44dA	0.43dA	0.52bcd	0.63bB	0.61bA	0.45cd
250-53 μm	0.87a†	0.50bcd	0.43dA	0.39dB	0.46cd	0.59cbB	0.58bB	0.48bcd
<53 μm	0.34a†B	0.21bcC	0.18cdB	0.18dC	0.19bcd	0.22bC	0.21bcd	0.14eB
Intra-macroaggregate								
Free	0.27nsC	1.19A	0.23B	0.12CD	1.22AB			
Occluded	0.11a†	0.05bcB	0.07bC	0.02cD	0.07abC			
>250 $μm_{LM}$	0.65a†B	0.40abB	0.19bB	0.37abB	0.23bB			
250-53$μm_{LM}$	2.53a†	1.57bA	1.08cA	1.10cA	1.05bA			
<53 $μm_{LM}$	0.52a†B	0.33bB	0.25dB	0.25cdB	0.29bcB			
	C_3 Carbon (g/100 g soil fraction)							
Water-stable aggregate fractions								
>2000 μm	1.12	1.60bA	1.43bc	1.63bA	1.45bcA	1.67bA	1.70abA	2.12aA
2000-250μm	1.22	1.65ab	1.47bA	1.55bA	1.51bA	1.47bc	1.69abA	1.89aA
250-53 μm	1.21	1.56bc	1.41cd	1.30dB	1.41cdA	1.37cd	1.68bcA	1.94aA
<53 μm	0.63	0.76bC	0.71bc	0.70bcC	0.70bcB	0.76bC	0.86aB	0.96aB
Intra-macroaggregate fractions								
Free	0.93	0.75C	0.94B	1.00B	1.56B			
Occluded	0.26	0.31bD	0.32bD	0.46aC	0.30bD			
>250 $μm_{LM}$	0.66	0.68C	0.71C	0.97B	0.73C			
250-53$μm_{LM}$	1.66	2.92bA	2.90bA	3.72aA	3.01bA			
<53 $μm_{LM}$	0.74	1.06aB	0.93bB	1.03aB	0.98abC			
	Mean Age (yr)							
Water-stable aggregate fractions								
>2000 μm	37.5	75.4bc	87.4ab	106.1aA	78.8bcB	64.5cB	70.3bcB	101.1aB
2000-250μm	40.7	77.6bc	87.9bc	94.3abA	81.0bcd	65.2dB	76.7cdB	107.1aB
250-53 μm	43.1	83.2bB	87.1bB	89.0bB	84.1bB	66.5cB	78.6bcB	108.0aB
<53 μm	53.3	93.3bA	103.1b	102.2bA	98.0bA	91.8bA	110.9bA	174.9aA
Intra-macroaggregate fractions								
Free	89.3	43.6C	111.3A	1184A	1153A			
Occluded	68.5	147.9A	136.2A	1248A	118.6B			
>250 $μm_{LM}$	37.2	54.0bc	107.1a	83.5abA	93.3aB			
250-53$μm_{LM}$	24.9	54.6cC	73.8ab	90.7aA	58.1bcB			
<53 $μm_{LM}$	43.2	86.0bB	97.9ab	105.5aA	89.5abB			

† Crop rotation effect statistically significant ($P<0.05$)
ns = Not statistically significant ($P<0.10$)
Values followed by the same lowercase letter within an aggregate size and between management treatments are not significantly different ($P<0.05$, Fisher's LSD)
Values followed by the same uppercase letter within a management treatment and between aggregate sizes are not significantly different ($P<0.05$, Fisher's LSD)
CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation);
hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation); hhCcc (first year of corn in hay- hay-corn-corn-corn crop);
w/sC (corn in corn-wheat/doble crop soybean crop rotation); W/Sc (wheat/doble crop soybean in corn-wheat/doble crop soybean);
Hhccc (first year of hay in hay-hay-corn-corn-corn crop rotation); hHccc (second year of hay in hay-hay-corn-corn-corn crop)

Figures

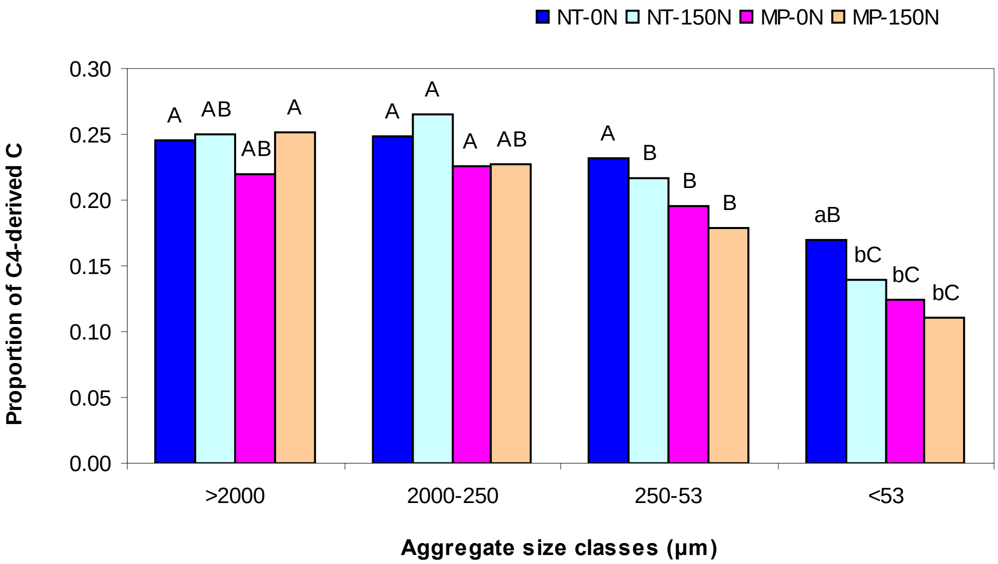

Figure 3.1. Proportion of C derived from corn (C4) of water-stable aggregates from the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate. Mean values followed by the same lowercase letter within a size fraction and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between size fractions are not significantly different (P>0.05, Fisher's LSD).

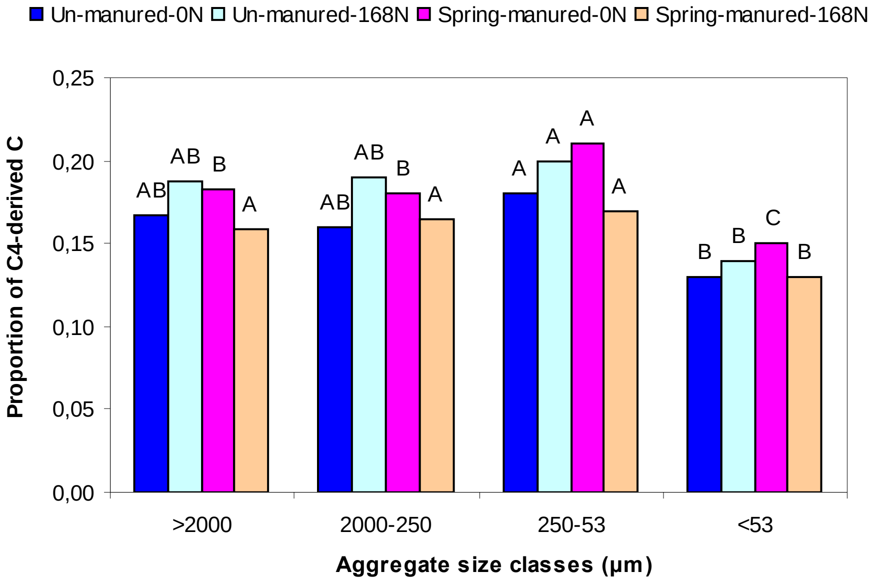

Figure 3.2. Proportion of C derived from corn (C_4) of water-stable aggregates from the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate. Mean values followed by the same uppercase letter within a management treatment and between size fractions are not significantly different (P>0.05, Fisher's LSD).

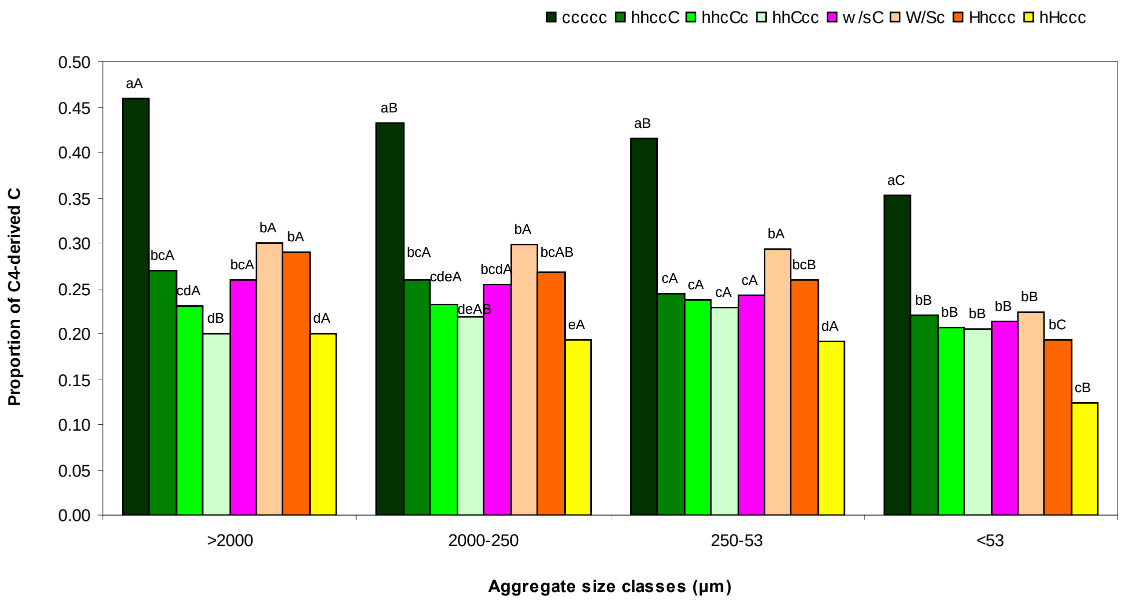

Figure 3.3. Proportion of C derived from corn (C_4) of water-stable aggregates from the spring and the fall samplings (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations. Mean values followed by the same lowercase letter within a size fraction and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between size fractions are not significantly different (P>0.05, Fisher's LSD).

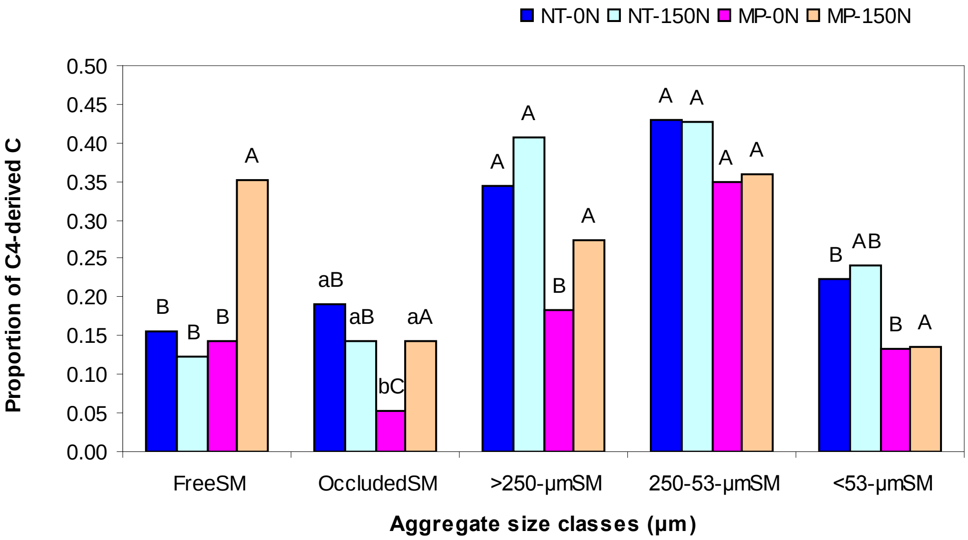

Figure 3.4. Proportion of C derived from corn (C_4) of intra-macroaggregates from the spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate. Mean values followed by the same lowercase letter within an aggregate type and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate types are not significantly different (P>0.05, Fisher's LSD).

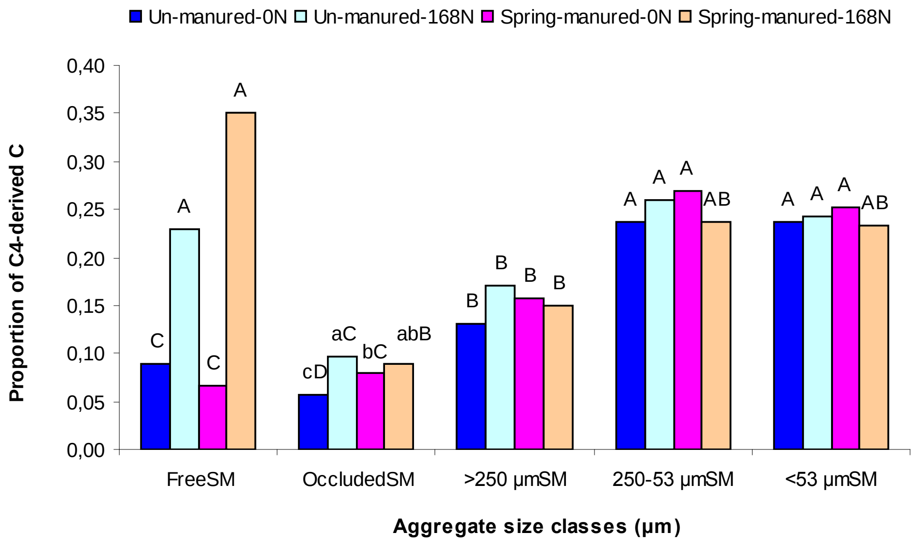

Figure 3.5. Proportion of C derived from corn (C4) of intra-macroaggregates from the spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate. Mean values followed by the same lowercase letter within an aggregate type and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate types are not significantly different (P>0.05, Fisher's LSD).

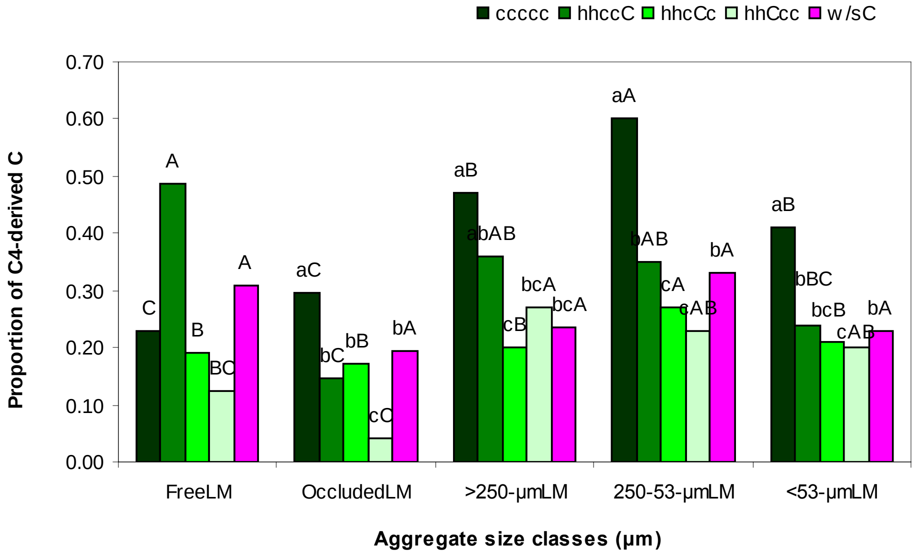

Figure 3.6 Proportion of C derived from corn (C_4) of intra-macroaggregates from the spring sampling (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations. Mean values followed by the same lowercase letter within an aggregate type and between management treatments are not significantly different (P>0.05, Fisher's LSD). Mean values followed by the same uppercase letter within a management treatment and between aggregate types are not significantly different (P>0.05, Fisher's LSD).

Chapter 4

Soil Microbial Biomass and Phenol Oxidase Enzyme Activity Response to Management Practices under Long-term Row-cropped Soils

Introduction

Soil microbial biomass is an active fraction of organic matter, which represents a reservoir of nutrients and participates in nutrient cycling (Smith and Paul, 1990). Nutrient fluxes through microbial biomass are of at least one order of magnitude faster than in the remaining organic matter (Dalal, 1998), leading to the suggestion that variations in the microbial biomass content induced by management practices could be used as an early indicator of changes in SOM level and soil quality (Powlson et al., 1987; Saffigna et al., 1989; Zelles, 1999). Microbial community function, as indicated by extracellular enzyme activity, has also been used as an indicator of C cycling sensitive to management practices (Bandick and Dick, 1999). Moreover, microbial activity and the unprotected soil C pool are closely related because the rate of decomposition of this pool is, by definition, independent of the level of soil chemical and physical protection, but is related to soil moisture, temperature, intrinsic biodegradability and N availability as principal controls on microbial activity (Bremer et al., 1994). This puts in perspective the fact that soil C stabilization in agroecosystems cannot be fully understood without considering its biological component, especially when the dynamics of microbially derived organic matter and their relationship with soil properties and management practices still need to be investigated further.

Soil management and fertilization practices affect the amount of soil microbial biomass and microbial community composition, as well as microbially derived organic matter, because these agricultural practices are closely related to C inputs and to changes in soil physical and chemical properties. Tillage intensity affects the magnitude of organic C depletion (Angers et al., 1993a; Cambardella and Elliot, 1993). As a result, the reduction of microbial biomass content (Doran, 1980; Angers et al., 1993a, 1993c; Feng et al., 2003) and microbial activity (van de Weft and Verstraete, 1987; Bergstrom et al., 1998) have been observed. In addition, shifts in the structure of the soil microbial community are linked with changes in soil microbial biomass content (Bardgett et al., 1999). Tillage leads to the development of soil microbial communities dominated by aerobic microorganisms, typically bacteria, whereas under conservation practices, plant residues left at or near the soil surface encourage fungal growth (Pankhurst et al., 2002; Spedding et al., 2004). In addition, more active microbial populations, as indicated by higher extracellular enzyme activity, have been associated with the accumulation of soil organic C in NT systems (Doran, 1980; Dick, 1984; Matocha et al., 2004).

Research has shown that soil microbial biomass and activity respond to crop management practices such as cover crop (Kirchner et al., 1993; Mullen et al., 1998), crop type (Fyles et al., 1988; Kaiser and Heinemeyer, 1993), and crop rotation (Insam et al., 1989; Dick, 1992; Acosta-Martinez et al., 2003; Adeboye et al., 2006). Kirchner et al. (1993) reported that cover crops enhance microbial biomass C in a conventionally-tilled continuous corn system. Mullen et al. (1998) observed that cover crops also significantly increased several enzyme activities, relative to no cover, in NT corn. Studying the influence of macroclimate on soil microbial biomass, Insam et al. (1989) found that the ratio of soil microbial biomass to organic C in soils under monocultures was significantly lower than that in soils under crop rotations. In addition, Acosta-Martinez et al. (2003) reported that soils under crop rotation show increased soil enzyme activity when compared with soils under continuous monocropping. Nonetheless, Balota et al. (2003) found that tillage reduction had a much bigger effect on microbial biomass, particularly in the 0-5 cm soil depth increment, than did crop rotation. However, they also found that inclusion of maize in the rotation resulted in more than twice as much biomass input as soybean or cotton in a Brazilian Oxisol. In addition, Adeboye et al. (2006) stated that crop rotation affects distinct sub-components of microbial biomass, even though the gross microbial biomass seems unaffected. To date there is still the need for a closer investigation of microbial biomass, by soil type and crop rotation.

Animal manures are typically applied to supply crop N, P, and K need. However, the impact of manures on the soil goes further than the application of those nutrients. Animal manures have been shown to maintain or increase the amount of readily available C, resulting in a more diverse and dynamic microbial system as compared to inorganically fertilized soil (Dormaar et al., 1988; Insam et al., 1989; Ritz et al., 1997; Peacock et al., 2001). Wonprasaid (2003) reported that biologically active SOM, including microbial biomass C, microbial biomass N, mineralizable C and mineralizable N, was affected positively and significantly by manure application, whether the manure was applied to cultivated soil or a long-term sod.

Nitrogen amendments have been shown to affect microbial activity, as indicated by changes in enzyme activity in prairie soils (Ajwa et al., 1999), in litter and forest soils (Fog, 1988; Carriero et al., 2000; Saiya-Cork et al., 2002; DeForest et al., 2004), and in agro-ecosystems (Matocha et al., 2004). In addition, N fertilization is associated with changes in soil microbial community composition. Wallenstein et al. (2003) found a decreasing fungal to bacterial biomass ratio with increasing N input in forest systems. It has also been reported that N fertilization suppresses the activity of lignin-degrading fungi (Berg, 1986; Fog, 1988), hence decreasing the soil's capacity to degrade lignin and other polyphenols (Carreiro et al., 2000). DeForest et al. (2004) reported that N addition reduced microbial biomass by 18%, relative to the unfertilized control, and broadly suppressed all microbial groups, not just the activity and abundance of lignin degrading fungi in hardwood forests soils, which is consistent with the findings of Peacock et al. (2001), who reported a decrease in the typical Gram-negative bacteria PLFA (phospholipid fatty acid) biomarkers after N application to a Fragiudalf under NT silage corn.

Phenol oxidase is an important lignolytic extracellular enzyme involved in organic matter decomposition and may be one of the enzymes most responsive to N amendments. Although many fungi and bacteria produce oxidative enzymes that modify lignin to varying degrees, white rot fungi in the *Basidiomycota* and xylariaceous *Ascomycota* are primarily involved with the production of phenol oxidase and all the other enzymes that are essential for completely degrading lignin (Kirk and Farrell, 1987; Dick and Webster, 1995; Hammel, 1997). Carriero et al. (2000) found that N-induced attenuations of litter decomposition rates were closely linked to changes in phenol oxidase activity. They also reported that the effect of N application on litter decomposition depended on the initial lignin concentration of the decomposing material. These findings were consistent with Fog (1988), who speculated that N amendment may retard the decomposition of lignified litter by repressing the production of lignin degrading enzymes. Later, Saika-Cork et al. (2002) studied N deposition effects, not only on litter, but also on SOM in forest soils (considering that litter and SOM have some intrinsic differences). Soil organic matter has a lower C/N ratio than does litter and is largely humified, and bacterial activity may be more important than fungal activity in SOM degradation. Saika-Cork et al. (2002) reported that phenol oxidase activity decreased in soil, but increased in litter, suggesting that N inhibition of recalcitrant organic matter decomposition was not exclusive to lignin degrading basidiomycetes. Waldrop et al. (2004) also reported an ecosystem-specific response in phenol oxidase activity to N application to forest soils. In forest ecosystems with highly lignified litter (C/N = 133), the highest level of N deposition tended to reduce phenol oxidase activity. Conversely, in forest ecosystems with litter low in lignin (C/N = 80), N deposition tended to increase phenol oxidase activity. In contrast to temperate forest soils, phenol oxidase enzyme activity did not respond to long-term N deposition in semiarid grassland (Stursova et al., 2006). In an agricultural soil, Matocha et al. (2004) observed that phenol oxidase activity was 1.7 times greater under NT, compared to MP, and that N fertilization under NT had a negative impact on phenol oxidase activity. Further research is warranted to elucidate the potential use of phenol oxidase enzyme activity as an indicator of humification, especially in agroecosystems.

Evaluating the effect of management practices on soil biological properties becomes challenging when seasonal effects and spatial variation in the field are considered. Seasonal trends in microbial C and N are not well understood. Soil microbial biomass C and soil microbial biomass N have been shown to peak in summer months (Bardgett et al., 1999). Microbial biomass C is often closely related to organic matter input (He et al., 1997) and decomposition of organic material throughout the season is thought to result from successional populations of soil organisms. Seasonal crop growth may influence soil microbial dynamics by altering the temporal and spatial distribution of organic inputs due to rhizodeposition, crop roots and residues (Franzluebbers et al., 1995), soil moisture and nutrient conditions. Bossio et al. (1988) found that, for a given soil, microbial community changes over time were of greater magnitude than changes associated with management practices such as manure inputs, cover crops, and mineral fertilizers. Also, Spedding et al. (2004) reported that the distribution of soil microbial communities as indicated by phopholipid-linked fatty acid (PLFA) profiles also showed better distinction between sampling time and depth than between tillage treatments (NT, reduced tillage, and CT). Nevertheless, Feng et al. (2003) found a significant impact of tillage practices on soil microbial communities in winter fallow (February) and when influence of plant growth was minimal (May), but not in October. They concluded that changes in the microbial community during the growing season may be primarily determined by crop growth changes due to soil conditions and environmental variables such as moisture and temperature. In contrast, Patra et al. (1990) found no clear evidence for seasonal changes in biomass C and N within continuous wheat and grass fields.

Accordingly, the main objective of this study was to evaluate the effect of tillage, N fertilization, manure application, and crop rotation on biological properties of long-term row-cropped soils. The specific objectives were: i) to assess the management effect on soil microbial biomass and phenol oxidase enzyme activity, ii) to characterize the seasonal variation in these biological properties, and iii) to determine how biomass and phenol oxidase are related to extractable forms of C and N (water and potassium sulfate extracts, and amino sugar-N), and to other soil chemical and physical properties.

Material and Methods

Field Experiments

The treatments and field experiments used in this chapter were the same as those described in Chaper 2.

Soil Sampling and Handling

The three long-term field experiments were sampled twice in 2006, as indicated in Chapter 2. Two depth increments (0-10 and 10-20 cm) were sampled during spring (late April); and three depth increments (0-10, 0-5, and 5-10 cm) were sampled during fall (late November). Composited moist samples were stored at 4 ^{o}C until analyzed. The physical and chemical properties of these soils are discussed in detail in Chapter 2.

Characterization of Water Extractable C and N

Water soluble extracts were used in the characterization of soil solution C and N. These extracts were obtained by shaking 10 g of 2 mm sieved moist field soil with 20 mL of deionized water on a reciprocal shaker for 1 hour at 180 strokes per min. Samples were centrifuged at 8,000 rpm for 10 min and filtered through a 0.45 μm cellulose nitrate membrane filter (Whatman code 7184-004) (Zhang et al., 2006; Matocha et al., 2004). The filtrate was used to evaluate total Kjeldahl N, nitrate (NO_3^-), ammonium (NH_4^+), nitrite (NO_2^-), diphenolic content, and the specific ultraviolet absorbance at 272 nm ($SUVA_{272}$). Dissolved organic N was obtained by subtracting NO_3^-, NH_4^+ and NO_2^- from total Kjeldahl N.

Organic C Analysis.

The organic C of soil filtrate was measured by combustion/non-dispersive infrared gas analysis using a 2.5 mL sub-sample, which was acidified prior to the analysis by adding one drop of 3M sulfuric acid (H_2SO_4). Acidifying the samples drives off inorganic C, leaving only organic C.

Total Kjeldahl N Analysis.

Total Kjeldahl N was measured by sample digestion and colorimetric measurement of ammonium. Briefly, a 5 mL sub-sample of the soil filtrate was digested using 2.44 mL of 13.25N H_2SO_4 and selenized boiling chips. Once the digestion was completed, the sample was analyzed for ammonium.

Ammonium Analysis.

Ammonium was measured by a colorimetric-microplate method based on a modified Berthelot reaction. Indophenol is the product of the reaction between ammonia and phenol, hypochlorite, and sodium nitroprusside resulting in an intense blue color. Absorbance of the solution is read at 630 nm on a microplate reader, and ammonium concentrations of the samples are interpolated from a curve of standards (Ngo et al., 1982; Weatherburn, 1967).

Nitrate and Nitrite Analysis.

Nitrate and nitrite were measured using a colorimetric microplate technique, which is based on the reaction of nitrite with sulfanilamide and Greiss reagent under acidic conditions. A highly colored azo dye is formed and its concentration is measured at 542 nm (Dorich and Nelson, 1984). For nitrate analysis, prior reduction of nitrate to nitrite with copperized Cd metal is required (Mulvaney, 1996; Wood et al., 1967).

Diphenolic Analysis.

The diphenolic content was measured colorimetrically based on the o-diphenol reaction with nitrite to produce a bright red (λ_{max} 500 nm) chromophore in alkali (Waite and Tanzer, 1981). Briefly, a 2.4 mL subsample of each water soluble extract reacted with 0.3 mL of 0.5M HCl, 0.3 mL of 1.45M $NaNO_2$ and 0.41M $NaMoO_4.2H_2O$, and 0.3 mL of 1M NaOH solutions. Absorbance at 500 nm was measured and the concentration of diphenolics in the sample is calculated from a calibration curve that is based on L-B-3, 4-Dihydroxyphenylalanine (L-DOPA) standards.

Specific Ultraviolet Absorbance.

The aromatic C content of water soluble extracts was determined by measuring the specific ultraviolet (UV) absorbance at 272 nm after Traina et al. (1990) and Weishaar el al. (2003). The wavelength of 272 nm represents the λ max for the aromatic structures that are thought to be common to most humic acids (overlapping $\pi \rightarrow * \pi$ transitions for phenolic arenes, benzoic acids, polyenes, and polycyclic aromatic hydrocarbons with ring numbers of two or more. Each water soluble extract was analyzed using deionized water in the reference cell from 200-400 nm on a UV-VIS spectrophotometer. The $SUVA_{272}$ was estimated dividing the UV absorbance measure at 272 nm by the DOC concentration and the cell length (1 cm), and reported in the units of liter per milligrams C per meter ($L\ mg^{-1}\ m^{-1}$).

Phenol Oxidase Enzyme Activity

Phenol oxidase enzyme activity (EC 1.10.3.2) was measured spectrophotometrically after Matocha et al. (2004). This method is based on the oxidation of L-DOPA by phenol oxidase enzymes to a red compound, 2-carboxy-2, 3-dihydroindole-5, 6-quinone (DOPAchrome). DOPAchrome production is linear over short incubation times (<5 min), so initial formation of DOPAchrome is followed by measuring absorbance at 490 nm from 0 to 3 min to assess phenol oxidase enzyme activity (Pind et al., 1994). Briefly, 0.2 g of 2 mm sieved field moist soil sample was mixed with 3 mL of 0.05M 2-(N-Morpholino) ethane-sulfonic acid (MES) solution at pH 6.0 for 10 min in stirred batch reactors contained in a water-jacketed flask attached to a VWR circulating water bath controlled at 24 ^{o}C. Three mL of freshly prepared 0.01 M L-DOPA solution were added to soil suspension and stirred for exactly 3 min. Suspension samples were filtrated through 0.2 μm membrane paper (Fisher code 09-719-2A). The absorbance of the filtrate was measured at 490 nm, and it was converted to concentration using Beer's Law and a molar extinction coefficient of 3.7×10^4. Activity was expressed as nmoles DOPAchrome $mg^{-1}\ min^{-1}$. Blanks consisting of MES and L-DOPA solutions were included in each run. In addition, the abiotic component of this reaction was assessed by running sterile soil from each sample. The difference in phenol oxidase activity between fresh and sterile samples represented the net phenol oxidase activity.

Soil Sterilization.

Soil sterilization was accomplished by moist heat. The <2 mm field moist soil sample was incubated for 48 hours at room temperature to stimulate microbial growth. The soil was autoclaved at 0.10 MPa and 121 ^{o}C for 1 hour. After the initial autoclaving, the soil sample was incubated an additional 48 hours at room temperature and autoclaved a second time for 1 hour to eliminate any microorganisms that were not destroyed in the initial autoclaving (Wolf and Skipper, 1994). The sterile soil samples were stored at 4 ^{o}C until analyzed.

Microbial Biomass Analysis

Microbial biomass C and N were assessed on field moist samples by direct chloroform extraction. The method was modified after Gregorich et al. (1990), Mueller et al. (1992), Widmer et al. (1989), and Needelman et al. (2001). Briefly, 4 g of moist soil were sieved through a 4 mm sieve and extracted with 20 mL of 0.5 M K_2SO_4 solution. The soil and the extractant were shaken on a horizontal shaker at 180 osc min^{-1} for one hour. After shaking, the soil suspension was centrifuged at 10,000 rpm for 10 min and filtered using a 2.7 µm glass fiber filter (Fisher code APFD04700). The filtrate was collected and stored at 4 ^{o}C until analyzed. A blank filtrate, extractant alone, was run for each batch of samples to determine background levels of C and N. The tube with the remaining soil was weighed to assess the interstitial solution and 10 µL of chloroform ($CHCl_3$) were added. The tube was vortexed and incubated at room temperature (20 ^{o}C) for 5 days. After incubation, the tubes were evacuated using vacuum for 1 hour and extracted with 20 mL of 0.5 M K_2SO_4 solution using the same procedure described for the first extraction. Blanks consisting of tubes with and without extractant were analyzed to control for the efficiency of the evacuation. The soil filtrates were analyzed for organic C, total Kjeldahl N, NH_4^+ and NO_3^- using the techniques previously described. The amount of soluble C and N found after fumigation represents biomass C and biomass N, respectively, so these were calculated from the extractable C and N found in the second soil filtrate as follows:

$$\text{Biomass C} = \text{Organic C}$$

$$\text{Biomass N} = [(\text{total Kjeldahl N}) - (NH_4^+) - (NO_3^-)]$$

Amino sugar-N Analysis

Amino sugar-N was quantified by a diffusion method after Khan et al. (2001) and Mulvaney and Khan (2001). Briefly, 100 mg of air dried soil sieved through a 2 mm sieve were weighed directly into disposable 5 mL beakers. The beakers were fitted into polystyrene holders and placed into small diffusion jars containing 4 mL of 0.06N H_2SO_4 trapping solution. One mL of 2M NaOH was pipetted onto the soil in each beaker. The jars were quickly capped, swirled to mix the solutions and placed in a digital oven at 55 ^oC for 5 hours to liberate (NH_4^+ amino sugar)-N as gaseous NH_3. The trapping solutions were analyzed for NH_4^+ using the colorimetric-microplate method.

Statistical analysis

SAS (1999) was used for all statistical analysis. Management effects on all of the measured variables were evaluated by ANOVA. The chosen model was based on the experimental design for each field experiment, and significant differences among means were determined by LSD means separation (Saxton, 1998). The probability level used to define statistical significance is specified in each related table or graph. In addition, correlation and multiple linear regression analyses were performed among the set of variables and some of the soil physical and chemical properties presented in Chapter 2. Finally, a repeated measures procedure was used to evaluate seasonal variation in these variables.

Results and Discussion

Characterization of extractable C and N

Water extractable C and N.

The water extractable forms of C and N for Field Experiment 1 are presented in Table 4.1. Higher water extractable C due to N fertilization was expected, based on previous research (Freeman et al., 2001, Matocha et al., 2004, Sinsabaugh et al., 2004), but was not observed in our study. Only nitrates were significantly affected by the interaction between tillage and N rate in both the spring and the fall samplings. Organic N in the fall sampling was significantly affected by N fertilization. A higher level of nitrates was observed under NT, compared to MP, and the trend was reinforced by N fertilization. The opposite trend was observed for organic N. In addition, the diphenolic content and the $SUVA_{272}$ data for both sampling times showed that tillage significantly affected the level of aromatic C content in water extracts. No-tillage was related with lower levels of aromatic C in the water extract, suggesting higher humification associated with NT compared to MP. Finally, seasonal variation was observed for every evaluated variable but organic C. Higher levels were associated with fall, as compared to spring for all the variables, except for nitrates, which exhibited highest concentrations in spring.

The water extractable C and N forms of Field Experiment 2 are summarized in Table 4.2. Organic C and nitrates were sensitive to the interaction of manure and N fertilization, but only in spring. Manure application increased organic C and nitrates, especially without N fertilization. Organic N was affected by the interaction of manure and N rate at both sampling times, exhibiting slightly higher levels after manure and N application. Manure has been shown to significantly increase soluble organic C in soil (Gregorich et al., 1998; Liang et al., 1998), which supported these results. Additionally, the diphenolic content and the $SUVA_{272}$ exhibited significant responses to management treatments in the spring. Lower diphenolic content was observed with manure application; which is consistent with the $SUVA_{272}$ trend, suggesting a decrease in aromatic C content in soil solution with manure use, and especially when N is not applied. Only nitrates, diphenolics, and $SUVA_{272}$ exhibited significant seasonal variation. The fall sampling was generally lower in nitrates, but higher in $SUVA_{272}$ and diphenolics, as compared to the spring sampling.

The water extractable forms of C and N for Field Experiment 3 are presented in Table 4.3. Only nitrates and nitrites were significantly affected by crop rotation, and only nitrates were consistently affected at both sampling times. The highest level of nitrate was observed for the w/sC treatment, in both the spring and fall samplings. In addition, diphenolic content and $SUVA_{272}$ exhibited significant crop rotation effect. Consistently, the lowest aromatic C content in the water extract was observed with the w/sC treatment. The highest aromatic C content was associated with the hhcCc treatment. Significant seasonal variation was observed for all the measured variables but organic C and ammonium. Higher levels were associated with the fall sampling, as compared to the spring sampling, for all the variables except for nitrate.

Potassium Sulfate Extractable C and N.

For Field Experiment 1, the inorganic and organic forms of C and N extracted with K_2SO_4 were significantly affected by tillage and N fertilization (Table 4.4). At both sampling times, higher extractable organic C, organic N and ammonium at the soil surface and sub-surface were associated with NT, especially after N fertilization. In contrast, a higher level of nitrate was found in N fertilized NT soil, at the 10-20 cm depth in the spring and at all depths in the fall. In addition, depth stratification of every extractable form of C and N was observed under NT. Seasonal differences were observed only for extractable organic N and ammonium, with higher levels in fall, compared to spring, across all tillage by N rate treatments.

For Field Experiment 2, the inorganic and organic forms of C and N extracted with K_2SO_4 were significantly affected by manure and N fertilization, but the trends were not consistent, across either soil depth increments or sampling times (Table 4.5). Nitrogen fertilization significantly affected organic C at both sampling depths in the spring. Manure application significantly affected organic N at 10-20 cm, and nitrate at 0-10 cm, in the spring. The interaction of manure and N fertilization significantly affected organic C and organic N at 0-5 cm in the fall sampling, and ammonium at 10-20 cm in the spring and at 0-5 and 0-10 cm in the fall. In addition, stratification of every extractable C and N form was observed in this experiment, and only extractable organic

C and nitrate exhibited seasonal differences. Higher levels of organic C, but lower levels of nitrate, were found in spring, compared to fall, across the manure by N rate treatments.

For Field Experiment 3, only organic C and nitrate exhibited a significant crop rotation effect (Table 4.6). Organic C was significantly affected by crop rotation only in fall with highest concentrations in the continuous corn treatment. In contrast, nitrate exhibited a significant crop rotation effect in the spring with the greatest concentrations found in the w/sC treatment. Also, the stratification of every C and N form was evident in both the spring and the fall samplings. Significant seasonal variation was observed only for organic forms of C and N, which exhibited higher levels in fall, compared to spring, across all the crop rotation treatments. There was an interaction between season and crop rotation on nitrates. All crop rotation treatments but w/sC, exhibited higher nitrate levels in fall than in spring.

Phenol Oxidase Response to Management and Seasonal Effects

Phenol oxidase activity was sensitive to the interaction of tillage and N fertilization only at the soil surface (0-10 cm soil depth increment) in the spring sampling (Table 4.7). Unlike Matocha et al. (2004), there was higher enzyme activity after N fertilization under NT. There was not a difference in enzyme activity between N rates under MP. These results may be explained by differences in residue lignin levels or microbial community composition between the two experiments (Carriero et al., 2000; Waldrop et al., 2004). Management effects on phenol oxidase activity were not observed at any soil depth increment in the fall sampling. Stratification of the enzyme activity was observed under NT, with higher levels in the 0-5 cm soil depth increment, as compared with the deeper one. These results are consistent with higher biomass found in the surface soil depth increment. In addition, there was significant seasonal variation in phenol oxidase activity, with greater concentrations observed in fall than spring, across all the tillage by N rate treatments. Phenol oxidase activity was positively related to extractable nitrate and negatively related with soil pH. Multiple linear regression analysis found that 53% of variation in phenol oxidase enzyme activity was explained by soil pH, gravimetric water content, and K_2SO_4 extractable nitrate [Phenol oxidase = 52.48 – 7.87 pH + 0.83 gravimetric water + 3.75 $(NO_3^-)_{K2SO4}$]. These results are not consistent with phenol oxidase behavior reported for peat and wetland environments where enzyme activity was reduced by low pH values and low oxygen supply (Pind et al., 1994; McLatchey and Reddy, 1988), implying that phenol oxidase activity may be site specific or regulated by other, unevaluated, factors. The positive relationship between phenol oxidase activity and extractable nitrate also contrasts with previous findings in forest and mineral soils (Saiya-Cork et al., 2002; Matocha et al., 2004), which indicates that N supply is not responsible for phenol oxidase activity suppression in this soil. Finally, phenol oxidase was not significantly correlated with the amount, or aromatic content, of water extractable organic C, which is consistent with the findings of DeForest et al. (2005) for forest soils. However, tillage did have an effect on the aromatic content of water extractable organic C, with the highest level found NT soils, independent of the level of N fertilization. These results indicated that neither phenol oxidase activity nor N fertilization is linked with less humic water extractable organic C.

For Field Experiment 2, N fertilization was also associated with higher phenol oxidase activity in the 0-5 cm soil depth increment in the fall sampling (Table 4.8), which is consistent with the trend observed for Field Experiment 1, suggesting that the growing season could have modified the microbial community composition and/or enriched the soil with more labile C substrate than that associated with manure application. On the other hand, N fertilization was associated with lower phenol oxidase activity in spring, at the 0-10 cm soil depth increment, which is consistent with the trend observed by Matocha et al. (2004). Phenol oxidase activity was positively related to soil pH and extractable ammonium; not related to the aromatic C content of water extractable organic C, and negatively related to the amount of extractable organic C and extractable organic N (Table 4.11). These results suggest that N fertilization is most likely not the only factor determining phenol oxidase suppression in this soil, and that the difference in microbial community composition after manure application could also have played an important role. The multiple regression analysis found that 51% of the variation in phenol oxidase activity was explained by soil pH, C to N ratio, K, gravimetric water content, and potassium sulfate extractable ammonium [Phenol oxidase = $-2.64 + 6.27\ \mathrm{pH} - 4.94\ \mathrm{C/N} - 0.03\ \mathrm{K} + 2.02\ \theta_g + 14.1\ (\mathrm{NH_4^+})_{\mathrm{K2SO4}}$]. Phenol oxidase activity in the 10-20 cm depth increment was significantly affected by manure application. Higher enzyme activity was observed with manure application, implying that less lignified forms of C favoring activity of this enzyme were readily located at this depth, which also explains the lack of stratification observed for phenol oxidase activity, even though this experiment is under NT. In addition, there was no significant seasonal variation in phenol oxidase activity observed in this experiment.

In Field Experiment 3, phenol oxidase activity in the 0-10 cm soil depth increment was significantly affected by crop rotation, but only in the fall sampling (Table 4.9). However, there was no seasonal variation in phenol oxidase activity, across all the crop rotation treatments. These results imply an impact of C inputs, crop residues, on soil microbes. The highest phenol oxidase enzyme activity was associated with the hhcCc and the CCCCC treatments, which is not consistent with Acosta-Martinez et al. (2003), who reported higher soil enzyme activity under crop rotation than under continuous monocropping. Similar to phenol oxidase enzyme activity, aromatic C content exhibited highest concnetrations under the hhcCc and CCCCC treatments. These results suggest that high phenol oxidase activity is not associated with less humic water extractable C. In addition, phenol oxidase enzyme activity exhibited a significant negative correlation with water extractable ammonium, and also with potassium sulfate extractable C and N. Multiple linear regression found that 60% of the variation in phenol oxidase enzyme activity was explained by soil pH, biomass C, biomass N, water extractable ammonium, and potassium sulfate extractable organic N [Phenol oxidase = 67.95 - 5.39 pH – 2.74 $(NH_4^+)_{H2O}$ + 0.51 Biomass N -0.18 Biomass C – 1.93 $(SON)_{K2SO4}$]. There was stratification in phenol oxidase activity. Higher enzyme activity was associated with the surface soil depth increment.

Biomass Response to Management and Seasonal Effects

For Field Experiment 1, biomass C was sensitive to tillage and N fertilization at the soil surface (Table 4.7). Higher biomass C was observed under NT especially after N fertilization, suggesting that C and N substrate are more readily available to microorganisms under NT, which is consistent with higher soil organic C and extractable forms of C and N observed for NT. As presented in Table 4.10, biomass C and biomass N were significantly correlated with potassium sulfate-extractable organic C and N. These results are supported by the findings of Feng et al. (2003), who reported that microbial biomass C content under NT was at least 60% greater than under CT for a Paleudult under continuous cotton. Nonetheless, Alvarez et al. (1995b) found that microbial biomass was not affected by tillage on an Argiudoll under moldboard plowing and reduced tillage treatments. The stratification of biomass C was observed for both samplings, with the highest accumulation of biomass C at the soil surface. These results are explained by the fact that soils under NT have greater crop residues and higher SOM contents in the surface layer, which results in increased infiltration and higher water holding capacity at the surface. Thus, NT soils are not only high in available substrate, but are also wetter, cooler and fluctuate less in moisture and temperature (Doran, 1980). These conditions stimulate the growth and activity of soil microorganisms. Unlike biomass C, biomass N was significantly affected by tillage and N fertilization at the soil surface and sub-surface for both sampling times. No-tillage favored higher biomass N in the surface soil, and N fertilization enhanced biomass N, especially under NT. Biomass N decreases with increasing depth under NT, which is consistent with the stratification observed for other soil properties. In addition, unlike biomass C, biomass N exhibited a significant seasonal variation in this experiment with higher values in fall, which is consistent with the higher levels of extractable organic N found in fall. These results suggest the occurrence of a shift in soil microbial population, in favor of species with elevated N content, and could be attributed to inputs of mineralizable N (Ross et al., 1995), either from plant residues or N fertilizer. Similar with these results, Spedding et al. (2004) found little seasonal change in microbial biomass C, compared to microbial biomass N, as a result of post-emergence mineral N fertilization on a corn cropped loamy sand soil in southwestern Quebec.

For Field Experiment 2, biomass was significantly affected by manure regardless of N fertilization (Table 4.8). Biomass C at the soil surface was affected by manure both sampling times, while biomass N was affected by manure only in fall. As expected, higher levels of biomass C and N were associated with manure application. These results are supported by previous findings (Dormaar et al., 1988; Peacock et al., 2001; Wonprasaid, 2003) and are explained by the fact that manure is a good source of bioavailable C for microorganisms, stimulating production of microbial biomass and microbial products. In addition, stratification of both biomass C and N was observed in both samplings. Extractable forms of C and N were significantly correlated with biomass C (Table 4.12), suggesting the abundance of readily available C and N at the soil surface for biomass development. These results are consistent with Peacock et al. (2001), who found nearly twice as much microbial biomass in the 0-5 cm soil depth increment as at 5-10 and 10-15 cm. Finally, seasonal variation was significant only for biomass C, exhibiting higher levels in spring than in fall, which is consistent with the spring manure applications.

In Field Experiment 3, biomass was not significantly affected by crop rotation in any soil depth increment or at any sampling time (Table 4.9). Lack of differences in biomass in this experiment may reflect both, the long-term stability of the substrate and similar N immobilization in crop residues across the rotation treatments. These results are consistent with Alvarez et al. (1995a), who reported no crop effects on microbial C in a wheat-double crop soybean rotation on an Argiudoll. Adeboye et al. (2006) pointed out that crop rotation does not necessarily influence gross soil microbial biomass, but may affect physiologically distinct subcomponents of the microbial biomass. This thesis is supported by the significant crop rotation effect on potassium sulfate extractable organic C, on amino sugar-N, and on phenol oxidase enzyme activity observed in this study. Microbial biomass C exhibited a significant correlation with water extractable organic C and potassium sulfate extractable organic C (Table 4.12). In addition, microbial biomass C and water extractable organic C exhibited a significant correlation with both soil pH and total soil organic C, which suggests that soil pH and total soil organic C influence the flux of soil microbial biomass and the amount of water extractable C in these soils. Multiple linear regression found that 51.2% of the variation in biomass C was explained by soil pH, aggregate stability (MWDw) and phenol oxidase activity (biomass C = 16.75 + 7.43 pH + 5.29 MWDw – 0.22 phenol oxidase activity) suggesting that aggregate stability protects and improves habitat for microbiota (Alvarez et al., 1995c). On the other hand, biomass C and biomass N exhibited higher concentrations at the soil surface, both sampling times. This stratification pattern was expected because this experiment was entirely under NT. In addition, only biomass C exhibited significant seasonal variation, with the highest level in fall. Fall samples were taken after corn harvest, so the soil microbial community at this time may be dominated by the organisms responding to corn death and residue availability.

Amino Sugar-N Response to Management and Seasonal Effects

Amino sugar-N analysis is considered a chemical extraction of microbially derived organic matter (Six et al., 2006). It has also been proposed to be the specific fraction of soil organic N that affects crop responsiveness to N fertilization (Mulvaney et al., 2001). These results support these statements, as amino sugar-N exhibited a significant positive correlation with soil microbial biomass and was sensitive to management practices, across the three field experiments. The effects of tillage and N rate on amino sugar-N were significant in every soil depth increment tested, in both spring and fall samplings (Table 4.7). Higher levels of amino sugar-N were observed for NT, as compared to MP, and N fertilization enhanced amino sugar-N level only under NT, and especially at the soil surface. In addition, the NT soil surface exhibited the highest amino sugar-N level, which is consistent with the stratification observed for biomass and phenol oxidase enzyme activity in this tillage system. Finally, there was no seasonal variation in amino sugar-N across the tillage by N rate treatments.

A significant effect of manure application on amino sugar-N was detected in the fall sampling (Table 4.8). Manure application increased amino sugar-N. Nitrogen fertilization increased amino sugar-N after manure application, but decreased amino sugar-N without manure application. The highest amino sugar-N level was associated with the soil surface in both spring and fall samplings. In addition, the interaction effect of manure by season was significant. The trend for greater amino sugar-N level with manure application was enhanced in fall, as compared to spring.

The effect of crop rotation on amino-sugar N was evident at the soil surface (0-5 cm soil depth increment) and only for the fall sampling (Table 4.9), suggesting that the growing season had an impact on microbially derived organic matter. The highest amino sugar-N level was associated with the hhCcc treatment followed by the CCCCC one. As expected for a NT system, the soil surface exhibited the highest amino sugar-N levels in both spring and fall samplings. In addition, amino sugar-N did not exhibit significant seasonal variation across the crop rotation treatments.

Conclusions

Phenol oxidase activity was sensitive to management practices, but it was not a good indicator of humification in these agricultural soils, due to the great agro-ecosystem-specific response. Tillage, N fertilization, and crop rotation affected phenol oxidase enzyme activity at the soil surface (0-10 cm soil depth increment); while manure affected phenol oxidase enzyme activity in the sub-surface (10-20 cm soil depth increment). No-tillage was associated with higher enzyme activity than MP, but only when N was applied. Manure application and the CCCCC and hhcCc crop rotation treatments were associated with higher enzyme activity, compared to the remaining treatments. In addition, the significant seasonal variation in phenol oxidase activity was not observed consistently across the three field experiments. Field Experiment 1 exhibited greater enzyme activity in fall, while Field Experiment 2 and 3 exhibited fairly constant enzyme activity across the two seasons. Accordingly, phenol oxidase enzyme activity in these agricultural soils appeared to be driven not only by N inputs but also by the SOM biochemistry (degree of recalcitrance), some soil physical and chemical properties such as pH and gravimetric water content, and, most likely, microbial community composition. Further research is needed to evaluate the weight of each of these factors and the physiological response of microbial communities to N fertilization.

The amount of soil microbial biomass was an effective indicator of management effects, except for crop rotation. Biomass was affected by tillage, N fertilization, and manure application, especially at the soil surface. No-tillage favored higher biomass C and biomass N than did MP, while N fertilization enhanced the effect of NT. Manure application enhanced biomass independently of N fertilization. In addition, NT caused stratified biomass, with the highest level at the soil surface in all three field experiments. Nonetheless, seasonal variation in biomass was not consistent across field experiments, responding not only to management effects but likely also to changes in soil conditions that occur over the growing season. As a result, fall was associated with the highest biomass N in the tillage by N rate treatments, and the highest biomass C in the crop rotation treatments. Spring was associated with the highest biomass C for the manure by N rate treatments.

The water and potassium sulfate extractable forms of C and N were sensitive to management treatments, across the three field experiments, suggesting their utility as soil quality indicators that respond to changes in the rhizosphere caused by management practices. For the water extract, organic N was sensitive to tillage and N fertilization effects, while organic C was more sensitive to manure application. Among the inorganic forms of N, only nitrates were consistently affected by management treatments across the three field experiments. In addition, the level of aromatic C content in the water extracts was sensitive to management practices across the three field experiments and was consistently detected by the two techniques used. No-tillage and manure application resulted in less aromatic C content in water extracts, while N fertilization enhanced the NT effect and diminished the manure effect. Finally, the water extractable forms of C and N were most abundant in fall, compared to spring. It is important to point out that even though organic C level did not vary with season across the crop rotation and the tillage by N rate treatments, the level of aromatic C did, indicating the importance of evaluating seasonal variation in both quantity and quality of C.

The less labile SOM, represented by the potassium sulfate extract (compared to the water extract) exhibited slightly different impacts due to management treatments. No-till favored higher levels of extractable C and N especially after N fertilization. The manure effect was not consistent across extracted C and N materials and sampling times. Continuous corn was associated with the highest level of extractable organic C in fall, but the w/sC treatment caused the highest level of nitrate in spring. In addition, the NT soil surface exhibited the highest levels of extractable C and N, across the three field experiments. Extractable forms of C and N exhibited seasonal variation. Nonetheless, the trend was not consistent across field experiments or across extractable C and N compounds.

Amino sugar-N was sensitive to management practices. No-tillage and manure application increased amino sugar-N, and N fertilization enhanced the effect of both NT and manure application. The hhCcc and the CCCCC crop rotation treatments were associated with the highest amino sugar-N level. In addition, amino sugar-N exhibited stratification under NT, positive correlation with biomass across field experiments, and seasonal stability across management treatments, except in the manure by N rate experiment. Accordingly, amino sugar-N was a good indicator of microbially derived organic matter and the claim that it could estimate potential mineralization of soil organic N and improve N fertilizer recommendations was supported.

Tables

Table 4.1. Water extractable C and N for the soil surface (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

Treatment	Organic C	Organic N	NO_3^-	NO_2^-	NH_4^+	Diphenolics	$SUVA_{272}$
	(µg C/g dry soil)					mg/g dry soil	l mg^{-1} m^{-1}
	Spring Sampling						
NT-0N	33.98ns	6.77ns	42.25b†	0.13ns	0.05ns	3.87‡	3.26§
NT-150N	40.61	6.74	69.41a	0.11	0.04	2.32	1.98
MP-0N	33.05	8.12	17.26c	0.13	0.04	12.68	8.35
MP-150N	43.09	9.63	20.34c	0.13	0.04	8.24	4.92
	Fall Sampling						
NT-0N	39.74ns	13.16¶	22.18§	0.17ns	0.20ns	14.21§	8.43§
NT-150N	40.74	9.51	30.71	0.14	0.13	6.31	4.28
MP-0N	49.70	19.07	8.32	0.27	0.23	29.45	13.34
MP-150N	53.72	16.97	16.82	0.20	0.22	19.30	9.02

† Interaction effect of tillage by N rate statistically significant ($P<0.10$)

‡ Main effect of tillage statistically significant ($P<0.05$)

§ Main effect of tillage and N rate statistically significant ($P<0.05$)

¶ Main effect of N rate statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

NT-0N (no-tillage and 0 kg N ha^{-1}); NT-150N (no-tillage and 150 kg N ha^{-1}); MP-0N (moldboard plow and 0 kg N ha^{-1}); MP-150N (moldboard plow and 150 kg N ha^{-1}); $SUVA_{272}$ (specific ultraviolet absorbance at 272 nm)

Table 4.2. Water extractable C and N for the soil surface (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

Treatment	Organic C	Organic N	NO_3^-	NO_2^-	NH_4^+	Diphenolics	$SUVA_{272}$
	(µg C/g dry soil)					mg/g dry soil	l mg^{-1} m^{-1}
	Spring Sampling						
Unmanured-0N	22.86a†	5.71ab‡	33.43c‡	0.11ns	0.10ns	9.16§	8.97a†
Unmanured-168N	23.97a	5.35ab	29.30c	0.07	0.19	8.03	7.54ab
Manured-0N	29.74a	5.55b	67.74a	0.09	2.65	5.68	5.24b
Manured-168N	28.07a	6.31a	50.20b	0.1	4.31	7.56	7.54ab
	Fall Sampling						
Unmanured-0N	26.92ns	7.41a†	25.91ns	0.12ns	0.04ns	14.14ns	11.39ns
Unmanured-168N	24.68	5.18a	21.73	0.09	0.04	9.87	9.20
Manured-0N	24.66	5.90a	31.48	0.12	0.08	9.85	9.61
Manured-168N	29.94	7.00a	35.61	0.12	3.45	11.36	9.82

† Interaction effect of manure by N rate statistically significant ($P<0.10$)

‡ Interaction effect of manure by N rate statistically significant ($P<0.05$)

§ Main effect of manure statistically significant ($P<0.10$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

unmanured-0N (unmanured and 0 kg N ha^{-1}); unmanured-168N (unmanured and 168 kg N ha^{-1});
manured-0N (manured and 0 kg N ha^{-1}); manured-168N (manured and 168 kg N ha^{-1});
$SUVA_{272}$ (specific ultraviolet absorbance at 272 nm)

Table 4.3. Water extractable C and N for the soil surface (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

Treatment	Organic C	Organic N	NO_3^-	NO_2^-	NH_4^+	Diphenolics	$SUVA_{272}$
	(µg C/g dry soil)					mg/g dry soil	$l\ mg^{-1}\ m^{-1}$
	Spring Sampling						
CCCCC	21.20ns	4.09ns	59.35bc†	0.07a†	0.11ns	3.41a†	4.49ab†
hhccC	21.55	3.54	71.95ab	0.04b	0.36	1.75b	2.48bc
hhcCc	23.23	4.29	52.69c	0.08a	0.86	4.31a	4.96a
hhCcc	20.88	3.33	50.91c	0.07a	0.36	3.13ab	4.12abc
w/sC	20.57	3.27	74.53a	0.04b	0.03	1.62b	2.24c
	Fall Sampling						
CCCCC	22.34ns	7.00ns	30.5ab‡	0.11ns	3.77ns	8.64ns	9.98ns
hhccC	26.94	7.04	28.24ab	0.13	0.05	7.84	7.85
hhcCc	20.08	5.75	23.06b	0.09	0.92	7.75	9.79
hhCcc	22.29	5.74	36.63a	0.13	0.71	4.83	5.83
w/sC	25.23	7.20	23.42b	0.14	0.05	8.08	9.97

† Crop rotation effect statistically significant ($P<0.05$)

‡ Crop rotation effect statistically significant ($P<0.10$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

CCCCC (continuous corn); hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation);

hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation);

hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/double crop soybean crop rotation);

$SUVA_{272}$ (specific ultraviolet absorbance at 272 nm)

Table 4.4. Potassium sulfate extractable C and N in Field Experiment 1-tillage by N rate.

Treatment	Spring Sampling		Fall Sampling		
	0-10 cm	10-20 cm	0-5 cm	5-10 cm	0-10 cm
	Organic C (μg C/g dry soil)				
NT-0N	65.20b§	46.10†	52.33bc¶	47.01b¶	45.87b¶
NT-150N	94.05a	57.08	102.16a	98.89a	97.0a
MP-0N	43.94c	37.09	43.54c	41.64b	42.76b
MP-150N	53.95bc	45.72	55.40b	53.01b	57.57b
	Organic N (μg N/g dry soil)				
NT-0N	7.55‡	4.06‡	9.02b¶	7.40†	8.79b¶
NT-150N	9.12	5.17	15.53a	13.67	14.60a
MP-0N	2.07	1.50	4.79c	6.14	4.96b
MP-150N	3.62	2.79	8.39b	7.48	6.46b
	NO_3^- (μg N/g dry soil)				
NT-0N	0.09ns	0.11b§	3.15†	1.90#	1.98bc¶
NT-150N	0.45	0.40a	7.18	4.38	6.03a
MP-0N	0.28	0.06ab	1.29	2.09	1.75c
MP-150N	0.02	0.01ab	4.23	3.15	3.22b
	NH_4^+ (μg N/g dry soil)				
NT-0N	1.11†	0.74a¶	1.27‡	0.82ns	1.32‡
NT-150N	0.82	0.34bc	1.36	0.71	1.10
MP-0N	0.46	0.37c	0.63	1.04	0.61
MP-150N	0.40	0.46b	1.09	0.89	0.68

† Main effect of tillage and N rate statistically significant ($P<0.05$)

‡ Main effect of tillage statistically significant ($P<0.05$)

§ Interaction effect of tillage by N rate statistically significant ($P<0.10$)

¶ Interaction effect of tillage by N rate statistically significant ($P<0.05$)

Main effect of N rate statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

NT-0N (no-tillage and 0 kg N ha^{-1}); NT-150N (no-tillage and 150 kg N ha^{-1});

MP-0N (moldboard plow and 0 kg N ha^{-1}); MP-150N (moldboard plow and 150 kg N ha^{-1})

Table 4.5. Potassium sulfate extractable C and N in Field Experiment 2-manure by N rate.

Treatment	Spring Sampling		Fall Sampling		
	0-10 cm	10-20 cm	0-5 cm	5-10 cm	0-10 cm
	Organic C (μg C/g dry soil)				
Unmanured-0N	63.46‡	59.09‡	63.28b§	58.35ns	55.96ns
Unmanured-168N	73.50	62.22	79.50a	56.03	62.04
Manured-0N	65.64	56.00	69.49ab	56.63	57.44
Manured-168N	73.81	61.63	64.71b	60.49	61.44
	Organic N (μg N/g dry soil)				
Unmanured-0N	28.43ns	31.86†	23.61b§	22.43‡	23.60ns
Unmanured-168N	33.08	31.93	38.69a	25.41	31.83
Manured-0N	30.36	26.14	30.45ab	23.01	29.73
Manured-168N	30.5	27.58	28.38b	30.08	29.27
	NO_3^- (μg N/g dry soil)				
Unmanured-0N	0.01†	0.06ns	0.62ns	0.44ns	0.71†
Unmanured-168N	0.01	0.50	2.10	1.20	0.18
Manured-0N	0.49	0.25	0.77	1.04	1.50
Manured-168N	0.16	0.25	0.81	0.97	0.78
	NH_4^+ (μg N/g dry soil)				
Unmanured-0N	0.81ns	0.73a§	1.25a¶	1.03ns	0.78ab§
Unmanured-168N	0.54	0.48b	2.18a	0.59	0.89ab
Manured-0N	0.91	0.28ab	1.51a	0.76	1.39a
Manured-168N	0.55	0.41ab	0.52a	0.55	0.45b

† Main effect manure statistically significant ($P<0.05$)
‡ Main effect of N rate statistically significant ($P<0.05$)
§ Interaction effect of manure by N rate statistically significant ($P<0.05$)
¶ Interaction effect of manure by N rate statistically significant ($P<0.10$)
ns = Not statistically significant ($P>0.10$)
Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column
Unmanured-0N (unmanured and 0 kg N ha^{-1}); unmanured-168N (unmanured and 168 kg N ha^{-1}); Manured-0N (manured and 0 kg N ha^{-1}); manured-168N (manured and 168 kg N ha^{-1})

Table 4.6. Potassium sulfate extractable C and N in Field Experiment 3-corn in crop rotations.

Treatment	Spring Sampling		Fall Sampling		
	0-10 cm	10-20 cm	0-5 cm	5-10 cm	0-10 cm
	Organic C (μg C / g dry soil)				
CCCCC	79.45ns	61.79ns	96.89ns	81.13a†	91.73a†
hhccC	73.46	58.16	90.34	63.51b	84.04ab
hhcCc	69.02	56.31	80.23	61.94b	68.14c
hhCcc	73.15	56.31	83.12	71.09ab	76.98bc
w/sC	68.92	58.00	101.83	71.64ab	83.56ab
	Organic N (μg N / g dry soil)				
CCCCC	4.60ns	0.53ns	9.26ns	7.85ns	8.58ns
hhccC	1.42	0.49	9.47	7.37	7.78
hhcCc	1.48	0.60	7.36	3.98	8.15
hhCcc	2.04	1.31	6.84	6.32	7.73
w/sC	2.94	1.18	9.85	7.27	9.73
	NO_3^- (μg N / g dry soil)				
CCCCC	2.47a†	2.49ns	7.84ns	10.06ns	7.96ns
hhccC	3.61a	2.51	7.90	4.46	6.24
hhcCc	2.68a	1.81	6.21	1.59	5.41
hhCcc	2.54a	2.29	9.00	6.59	9.25
w/sC	20.09b	4.26	8.61	3.31	4.77
	NH_4^+ (μg N / g dry soil)				
CCCCC	1.56ns	0.51ns	1.03ns	0.94ns	1.8ns
hhccC	1.10	0.66	1.14	2.94	1.37
hhcCc	1.06	0.59	0.93	0.63	4.23
hhCcc	1.14	0.86	1.50	0.44	0.60
w/sC	1.71	0.56	1.70	1.74	0.72

† Crop rotation effect statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation);

hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation);

hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation);

w/sC (corn in corn-wheat/double crop soybean crop rotation)

Table 4.7. Phenol oxidase enzyme activity, biomass, and amino sugar-N in Field Experiment 1-tillage by N rate.

Treatment	Spring Sampling		Fall Sampling		
	0-10 cm	10-20 cm	0-5 cm	5-10 cm	0-10 cm
	Phenol Oxidase Enzyme Activity (nmoles min^{-1} g dry soil^{-1})				
NT-0N	23.2b†	26.0ns	34.6ns	31.4ns	31.6ns
NT-150N	29.8a	26.5	37.6	32.0	31.5
MP-0N	26.7ab	30.9	30.1	34.1	35.3
MP-150N	26.4ab	25.5	36.1	35.1	31.8
	Biomass C (μg C / g dry soil)				
NT-0N	82.5‡	46.8ns	63.1b§	43.1b§	47.4¶
NT-150N	105.1	46.1	177.4a	112.3a	127.9
MP-0N	52.5	35.8	35.7b	34.3b	34.1
MP-150N	46.1	42.6	52.4b	57.0b	50.4
	Biomass N (μg N / g dry soil)				
NT-0N	16.9‡	9.1a†	27.3¶	17.1§	19.6b§
NT-150N	19.4	4.5ab	36.9	24.9	30.6a
MP-0N	3.8	3.5b	12.4	13.7	12.9c
MP-150N	5.0	5.9ab	18.8	17.0	17.5b
	Amino Sugar-N (μg NH_4-N / g dry soil)				
NT-0N	164.4b§	134.1a†	187.2b§	162.7¶	171.2b§
NT-150N	205.4a	140.2a	247.2a	182.2	209.3a
MP-0N	129.8c	100.9b	123.1d	139.5	138.7c
MP-150N	134.6c	140.8a	157.3c	155.1	149.9c

† Interaction effect of tillage by N rate statistically significant ($P<0.10$)

‡ Main effect of tillage statistically significant ($P<0.05$)

§ Interaction effect of tillage by N rate statistically significant ($P<0.05$)

¶ Main effect of tillage and N rate statistically significant ($P<0.05$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

NT-0N (no-tillage and 0 kg N ha^{-1}); NT-150N (no-tillage and 150 kg N ha^{-1});

MP-0N (moldboard Plow and 0 kg N ha^{-1}); MP-150N (moldboard Plow and 150 kg N ha^{-1})

Table 4.8. Phenol oxidase enzyme activity, biomass and amino sugar-N in Field Experiment 2-manure by N rate.

Treatment	Spring Sampling		Fall Sampling		
	0-10 cm	10-20 cm	0-5 cm	5-10 cm	0-10 cm
	Phenol Enzyme Oxidase Activity (nmoles min^{-1} g dry soil^{-1})				
Unmanured-0N	37.9‡	30.5†	37.5‡	38.8ns	41.3ns
Unmanured-168N	32.2	25.9	42.8	33.0	40.2
Manured-0N	39.1	32.7	39.6	43.7	36.5
Manured-168N	33.3	34.4	45.5	40.1	38.7
	Biomass C (µg C / g dry soil)				
Unmanured-0N	77.8†	58.1ns	78.4ns	53.8†	58.9†
Unmanured-168N	88.1	60.0	92.0	56.2	63.7
Manured-0N	104.7	62.9	96.7	76.2	78.0
Manured-168N	104.1	63.5	95.8	76.4	74.7
	Biomass N (µg N / g dry soil)				
Unmanured-0N	13.9ns	8.7ns	24.4†	17.8†	19.2ns
Unmanured-168N	16.9	8.4	25.4	16.8	17.7
Manured-0N	26.9	9.9	33.2	24.4	25.0
Manured-168N	19.6	9.4	27.9	21.0	21.6
	Amino Sugar-N (µg NH_4-N / g dry soil)				
Unmanured-0N	213.0ns	163.5ns	191.2c¶	148.9†	161.4b§
Unmanured-168N	219.8	142.6	178.2c	142.2	156.6b
Manured-0N	257.9	167.6	237.1b	189.4	210.4a
Manured-168N	202.1	156.5	257.5a	187.1	234.2a

† Mean effect of manure statistically significant ($P<0.05$)

‡ Main effect of N rate statistically significant ($P<0.10$)

§ Interaction effect of manure by N rate statistically significant ($P<0.10$)

¶ Interaction effect of manure by N rate statistically significant ($P<0.05$)

ns = Not statistically significant ($P<0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

Unmanured-0N (unmanured and 0 kg N ha^{-1}); unmanured-168N (unmanured and 168 kg N ha^{-1}); Manured-0N (manured and 0 kg N ha^{-1}); manured-168N (manured and 168 kg N ha^{-1})

Table 4.9. Phenol oxidase enzyme activity, biomass and amino sugar-N in Field Experiment 3-corn in crop rotations.

Treatment	Spring Sampling		Fall Sampling		
	0-10 cm	10-20 cm	0-5 cm	5-10 cm	0-10 cm
	Phenol Oxidase Enzyme Activity (nmoles min^{-1} g dry soil^{-1})				
CCCCC	31.1ns	25.9ns	31.6ns	36.9ns	33.0ab‡
hhccC	29.2	20.8	29.7	31.2	27.35b
hhcCc	25.7	19.5	29.7	37.2	38.19a
hhCcc	27.3	23.0	26.4	27.0	26.93b
w/sC	27.0	23.1	32.9	33.6	29.76b
	Biomass C (μg C/g dry soil)				
CCCCC	79.7ns	47.7ns	129.7ns	87.0ns	103.4ns
hhccC	78.0	47.9	133.8	74.0	107.6
hhcCc	78.4	44.1	110.1	70.2	80.5
hhCcc	76.5	45.8	117.2	79.7	111.7
w/sC	76.1	46.3	162.7	93.8	98.0
	Biomass N (μg N/g dry soil)				
CCCCC	35.3ns	15.1ns	34.6ns	24.3ns	29.9ns
hhccC	24.3	12.8	34.6	24.2	27.8
hhcCc	26.3	14.2	28.1	19.6	23.7
hhCcc	23.4	15.4	30.6	22.4	27.3
w/sC	30.9	25.9	40.2	23.7	26.4
	Amino Sugar-N (μg NH$_4$-N/g dry soil)				
CCCCC	241.6ns	166.0ns	237.1ab†	200.5ns	209.7ns
hhccC	234.4	152.3	220.3b	170.2	220.3
hhcCc	234.4	163.5	221.7b	175.8	197.5
hhCcc	216.2	153.4	250.7a	185.8	211.9
w/sC	205.9	148.3	222.5b	178.5	222.1

† Crop rotation effect statistically significant ($P<0.05$)

‡ Crop rotation effect statistically significant ($P<0.10$)

ns = Not statistically significant ($P>0.10$)

Different letters indicate significant differences ($P<0.05$, Fisher's LSD) within a column

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation);
hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation);
hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation);
w/sC (corn in corn-wheat/double crop soybean crop rotation)

Table 4.10. Pearson correlation coefficients among soil properties from spring sampling (0-10 cm soil depth increment) in Field Experiment 1-tillage by N rate.

	SOC_T	N_T	pH	θ_g	MWDw	AmiSug	SOC_W	SON_W	$(NO_3^-)_W$	$(NH_4^+)_W$	SOC_{PS}	SON_{PS}	$(NO_3^-)_{PS}$	$(NH_4^+)_{PS}$	PheOxi	Diphe	$SUVA_{272}$	BioC	BioN
SOC_T	1.000																		
N_T	0.997	1.000																	
pH	0.507	0.485	1.000																
θ_g	0.728	0.743	0.371	1.000															
MWDw	0.777	0.758	0.755	0.652	1.000														
AmiSug	0.751	0.736	0.485	0.247	0.589	1.000													
SOC_W	0.133	0.138	-0.389	0.202	-0.071	-0.050	1.000												
SON_W	-0.242	-0.227	-0.445	0.070	-0.308	-0.413	0.790	1.000											
$(NO_3^-)_W$	0.784	0.788	0.199	0.724	0.601	0.554	0.079	-0.301	1.000										
$(NH_4^+)_W$	-0.208	-0.176	-0.481	-0.375	-0.457	-0.076	0.060	0.084	-0.151	1.000									
SOC_{PS}	0.798	0.789	0.242	0.641	0.509	0.585	0.269	-0.183	0.864	-0.165	1.000								
SON_{PS}	0.681	0.695	0.541	0.808	0.629	0.300	0.058	-0.079	0.599	-0.374	0.682	1.000							
$(NO_3^-)_{PS}$	0.170	0.188	-0.192	0.281	-0.051	-0.020	0.003	-0.003	0.475	-0.044	0.301	0.150	1.000						
$(NH_4^+)_{PS}$	0.616	0.624	0.793	0.676	0.807	0.336	-0.118	-0.201	0.409	-0.395	0.360	0.801	0.002	1.000					
PheOxi	0.229	0.271	-0.466	0.357	-0.068	-0.158	0.240	0.131	0.407	0.119	0.221	0.175	0.561	-0.075	1.000				
Diphe	-0.679	-0.665	-0.381	-0.482	-0.601	-0.502	0.060	0.526	-0.710	0.157	-0.694	-0.481	0.122	-0.438	-0.012	1.000			
$SUVA_{272}$	-0.670	-0.662	-0.200	-0.560	-0.533	-0.395	-0.283	0.227	-0.727	0.098	-0.756	-0.503	0.060	-0.399	-0.141	0.930	1.000		
BioC	0.610	0.585	0.494	0.462	0.614	0.404	0.018	-0.148	0.464	-0.370	0.667	0.720	-0.076	0.456	-0.042	-0.447	-0.403	1.000	
BioN	0.834	0.817	0.591	0.573	0.745	0.596	0.099	-0.170	0.560	-0.097	0.702	0.688	0.020	0.669	-0.081	0.546	-0.554	0.729	1.000

SOC_T (total soil organic C); N_T (total soil N); pH (soil pH in water); MWD_W (mean weight diameter from the wet sieving method); AmiSug (amino sugar-N); SOC_w (water-extractable organic C);
SON_w (Water-extractable organic N); $NO_3^-{}_w$ (Water-extractable nitrate); $NH_4^+{}_w$ (water-extractable ammonium); SOC_{PS} (potassium sulfate-extractable organic C); SON_{PS} (potassium sulfate-extractable organic N);
$NO_3^-{}_{PS}$ (potassium sulfate-extractable nitrate); $NH_4^+{}_{PS}$ (potassium sulfate-extractable ammonium); PheOxi (Phenol oxidase enzyme activity); Diphe (diphenolic content of water extract);
$SUVA_{272}$ (specific ultra-violet absorbance at 272 nm of water extract); Bio C (biomass C); BioN (biomass N)

Table 4.11. Pearson correlation coefficients among soil properties from spring sampling (0-10 cm soil depth increment) in Field Experiment 2-manure by N rate.

	SOC_T	N_T	pH	θ_g	MWDw	AmiSug	SOC_W	SON_W	$(NO_3^-)_W$	$(NH_4^+)_W$	SOC_{PS}	SON_{PS}	$(NO_3^-)_{PS}$	$(NH_4^+)_{PS}$	PheOxi	Diphe	$SUVA_{272}$	BioC	BioN
SOC_T	1.000																		
N_T	0.950	1.000																	
pH	0.421	0.269	1.000																
θ_g	0.872	0.883	0.313	1.000															
MWDw	0.248	0.197	0.601	0.028	1.000														
AmiSug	0.389	0.328	0.316	0.561	-0.012	1.000													
SOC_W	0.751	0.723	0.362	0.706	-0.098	0.532	1.000												
SON_W	0.227	0.139	-0.033	0.242	-0.162	0.177	0.505	1.000											
$(NO_3^-)_W$	0.717	0.655	0.567	0.710	0.110	0.291	0.639	0.085	1.000										
$(NH_4^+)_W$	0.117	0.231	0.188	0.099	-0.02	0.119	0.454	-0.098	0.382	1.000									
SOC_{PS}	-0.205	-0.015	-0.570	0.009	-0.699	0.156	0.266	0.096	-0.120	0.512	1.000								
SON_{PS}	-0.170	-0.049	-0.205	-0.035	-0.289	0.443	0.230	-0.219	-0.005	0.630	0.775	1.000							
$(NO_3^-)_{PS}$	0.385	0.226	0.473	0.286	-0.040	0.069	0.395	-0.060	0.698	0.401	-0.113	-0.023	1.000						
$(NH_4^+)_{PS}$	-0.036	-0.237	0.632	-0.191	0.224	-0.278	-0.122	-0.170	0.303	-0.034	-0.582	-0.465	0.653	1.000					
PheOxi	0.107	0.001	0.663	0.117	0.334	-0.056	-0.140	-0.211	0.236	0.193	-0.689	-0.608	0.231	0.675	1.000				
Diphe	-0.054	-0.094	-0.075	-0.178	0.190	0.205	0.064	0.597	-0.488	-0.329	-0.127	-0.157	-0.574	-0.313	-0.172	1.000			
$SUVA_{272}$	-0.187	-0.175	-0.189	-0.301	0.302	0.022	-0.218	0.409	-0.608	-0.367	-0.198	-0.221	-0.683	-0.333	-0.136	0.931	1.000		
BioC	0.726	0.814	0.087	0.836	-0.274	0.482	0.794	0.182	0.601	0.359	0.449	0.326	0.263	-0.319	-0.215	-0.229	-0.392	1.000	
BioN	0.654	0.642	0.433	0.657	-0.052	0.228	0.525	-0.100	0.563	-0.019	-0.096	-0.136	0.248	0.164	0.365	-0.301	-0.458	0.688	1.000

SOC_T (total soil organic C); N_T (total soil N); pH (soil pH in water); MWD_W (mean weight diameter from the wet sieving method); AmiSug (amino sugar-N); SOC_w (water-extractable organic C);

SON_w (Water-extractable organic N); $NO_3^-{}_w$ (Water-extractable nitrate); $NH_4^+{}_w$ (water-extractable ammonium); SOC_{PS} (potassium sulfate-extractable organic C); SON_{PS} (potassium sulfate-extractable organic N);

$NO_3^-{}_{PS}$ (potassium sulfate-extractable nitrate); $NH_4^+{}_{PS}$ (potassium sulfate-extractable ammonium); PheOxi (Phenol oxidase enzyme activity); Diphe (diphenolic content of water extract);

$SUVA_{272}$ (specific ultra-violet absorbance at 272 nm of water extract); Bio C (biomass C); BioN (biomass N)

Table 4.12. Pearson correlation coefficients among soil properties from spring sampling (0-10 cm soil depth increment) in Field Experiment 3-corn in crop rotations.

	SOC_T	N_T	pH	θ_g	MWDw	AmiSug	SOC_W	SON_W	$(NO_3^-)_W$	$(NH_4^+)_W$	SOC_{PS}	SON_{PS}	$(NO_3^-)_{PS}$	$(NH_4^+)_{PS}$	PheOxi	Diphe	$SUVA_{272}$	BioC	BioN
SOC_T	1.000																		
N_T	0.863	1.000																	
pH	-0.063	-0.297	1.000																
θ_g	0.285	0.327	-0.210	1.000															
MWDw	0.494	0.422	0.017	0.190	1.000														
AmiSug	0.297	0.362	-0.134	0.101	0.184	1.000													
SOC_W	0.323	0.089	0.615	0.095	0.246	-0.198	1.000												
SON_W	0.249	0.094	0.096	-0.101	0.357	0.534	0.200	1.000											
$(NO_3^-)_W$	0.044	0.074	-0.001	0.361	-0.054	-0.207	0.292	-0.212	1.000										
$(NH_4^+)_W$	-0.061	-0.123	0.050	-0.024	-0.197	0.592	-0.162	0.212	-0.258	1.000									
SOC_{PS}	0.400	0.429	-0.222	-0.138	0.407	0.176	-0.194	0.326	-0.332	-0.064	1.000								
SON_{PS}	0.393	0.405	-0.201	-0.001	0.159	0.060	-0.137	0.141	-0.134	-0.030	0.547	1.000							
$(NO_3^-)_{PS}$	-0.272	-0.225	-0.040	-0.289	-0.205	-0.254	-0.202	-0.053	0.530	-0.187	-0.067	0.003	1.000						
$(NH_4^+)_{PS}$	0.311	0.423	-0.183	-0.223	0.163	-0.044	-0.066	0.051	0.079	-0.172	0.380	0.801	0.314	1.000					
PheOxi	-0.186	-0.076	-0.291	0.138	-0.053	-0.360	-0.018	-0.327	0.250	-0.486	-0.394	-0.327	-0.040	-0.273	1.000				
Diphe	0.083	-0.046	0.118	-0.303	0.020	0.403	0.113	0.327	-0.402	0.120	0.113	0.327	-0.402	0.120	-0.292	1.000			
$SUVA_{272}$	0.021	-0.053	-0.030	-0.327	-0.031	0.419	0.194	0.359	-0.388	0.100	0.190	0.359	-0.388	0.100	-0.229	0.961	1.000		
BioC	0.351	0.154	0.453	-0.121	0.600	0.074	0.476	0.247	0.050	-0.119	0.443	0.132	-0.155	0.065	-0.342	-0.003	-0.078	1.000	
BioN	0.179	0.143	-0.057	0.004	0.142	-0.141	0.194	0.070	0.238	-0.282	0.035	0.707	0.063	0.617	0.161	0.195	0.158	0.115	1.000

SOC_T (total soil organic C); N_T (total soil N); pH (soil pH in water); MWD_W (mean weight diameter from the wet sieving method); AmiSug (amino sugar-N); SOC_w (water-extractable organic C);

SON_w (Water-extractable organic N); $NO_3^-{}_w$ (Water-extractable nitrate); $NH_4^+{}_w$ (water-extractable ammonium); SOC_{PS} (potassium sulfate-extractable organic C); SON_{PS} (potassium sulfate-extractable organic N);

$NO_3^-{}_{PS}$ (potassium sulfate-extractable nitrate); $NH_4^+{}_{PS}$ (potassium sulfate-extractable ammonium); PheOxi (Phenol oxidase enzyme activity); Diphe (diphenolic content of water extract);

$SUVA_{272}$ (specific ultra-violet absorbance at 272 nm of water extract); Bio C (biomass C); BioN (biomass N)

Chapter 5

Composition of Soil Organic Carbon Pools within Aggregate Fractions in Long-term Row-cropped Soils under Different Management Practices

Introduction

The content and composition of SOM depend on many factors, such as type of soil, vegetation, land use, soil management practices, and climatic conditions. Management practices change SOM via the annual input of organic matter and the rate at which it decays (Jenkinson, 1988; West and Post, 2002). These changes in SOM are not only quantitative, but also qualitative in terms of composition (Murray and Quirk, 1990). Furthermore, differences in SOM composition of agricultural soils have been found where changes in SOM concentrations due to management practices were difficult to detect (Ellerbrock et al., 1999). The term SOM composition defines the spatial arrangement, at the molecular scale, of functional groups such as carboxylic and hydroxylic groups, as well as the C frame. These functional groups are responsible for the chemical reactivity and sorptivity of SOM (e.g. hydrophobicity or cation exchange capacity) (Capriel et al., 1995; Gressel et al. 1995). Unlike changes in SOM content as a function of management practices, changes in SOM composition are still poorly understood. A major reason for this is the lack of reliable analytical methods to quantitatively measure such changes, as well as the complexity of the soil-plant system. To further broaden our understanding of SOM dynamics, more information is needed on the chemical composition of meaningful SOM fractions.

The effects of soil amendments and fertilizer application on SOM composition have been of considerable interest. Wander and Traina (1996) investigated the effects of organic and conventional management practices on the SOM composition of physically and chemically isolated SOM fractions. They found that humic acid in the manure-amended organic rotation was more reactive than that isolated from crop-residue-amended soils. They also reported a change in reactivity associated with season for the fulvic acid and light fractions. Ellerbrock et al. (1997) studied different fertilized long-term experiments on a sandy soil and found that the content of the carboxyl- and hydroxyl-groups in SOM pyrophosphate extracts was higher in plots fertilized with cattle manure than in those that received straw plus mineral N. They also suggested that differences in crop yields might be explained by variations in the hydrophilic character of the SOM. Later, Ellerbrock et al. (1999) extended their study to soils with higher clay content, and reported that the type of amendment influenced SOM composition in a similar way across soil textural classes. However, the differences between fertilizer treatments were relatively lower in soils with higher clay content. These findings were supported by those of Capriel (1997), who investigated the effect of long-term management on two soils with contrasting textures and found that the amount of aliphatic C-H units and, implicitly, the hydrophobicity of SOM, were influenced by the type of crop rotation and fertilization. They reported that the decrease in organic C due to management was accompanied by a decrease in hydrophobicity, microbial biomass, and soil aggregate stability. In addition, Francioso et al. (2000) reported that SOM extracted from plots amended with cattle manure exhibited a structural composition characterized by aliphatic and aromatic groups that are more resistant to microbiological decomposition, promoting their accumulation over time. Furthermore, Francioso et al. (2005) found that structural changes in the humic fraction were related to the type of amendment. Crop residues and cow manure slurries caused a decrease in oxygenated functional groups and aromatic C-H, in contrast to cattle manure amendment, which produced an increase in aliphatic and carboxylate groups with both aromatic and aliphatic structures.

Tillage is another management practice that causes changes in SOM composition. Carbohydrates and amino sugars of microbial origin are often significantly higher under NT than CT (Arshad et al., 1990; Ball et al., 1996; Beare et al., 1997). Cambardella and Elliot (1994) hypothesized that those materials are sequestered as inter-microaggregate SOM within macroaggregates where they serve as binding agents for aggregate formation, which was supported by the findings of Simpson et al. (2004). Ding et al. (2002) characterized SOM fractions from a Kandiudult under long-term tillage management and reported that humic acid from CT was less aliphatic and more aromatic than humic acid from conservation tillage, as determined by solid-state ^{13}C NMR. In addition, based on reactive/recalcitrant peak ratio comparisons using DRIFT, they found a more reactive humic acid under conservation tillage than under CT. It has also been reported that the degree of humification, which has been associated with the concentration of semiquinone-type free radicals determined by electron spin resonance (ESR), can decrease in NT systems, especially with high rates of crop residue addition (Bayer et al., 2000). Nonetheless, Bayer et al. (2002) found that the degree of SOM humification depended not only on tillage but also on the size of the SOM fraction. They reported that the degree of humification decreased in both the 53-20 μm and 20-2 μm fractions under NT. Humification in the <2 μm fraction was unaffected by tillage, a fraction that normally exhibits the lowest degree of humification.

Most of the studies characterizing the effect of management on SOM composition have been done on chemically separated SOM fractions. However, chemical fractionation procedures are criticized for being operationally defined, potentially altering SOM composition, and for disregarding the spatial functionality of C stabilization (Stevenson et al., 1989). On the other hand, physically separated SOM fractions are isolated according to aggregate size and/or density. Physical separation methods are based on the premise that the association of the primary soil particles and their spatial arrangement play a key role in SOM function (Gregorich et al., 2006). Chemical characterization indicates that physical fractions isolated by size are not identical with those separated by density. Materials separated using variations of a particular fractionation method (i.e., different sizes or different densities) have different chemical or biological properties.

Distinct differences in composition have been observed between physically separated SOM fractions. Compared with the whole soil or the fine or heavy fractions, light fraction and POM (e.g., >53 μm) have higher O-alkyl, lower carbonyl, and higher O-aryl C contents. The last is explained by the presence of higher amounts of lignin in the partially decomposed plant residues dominating these fractions (Golchin et al., 1994; Skjemstad et al., 1999). In addition, Gregorich et al. (1996a) reported that the light fraction contained greater amounts of carbohydrates and aliphatic compounds compared to POM. On the other hand, Sohi et al. (2001) reported differences in SOM composition between free and intra-aggregate fractions. They found greater proportions of aliphatic hydrocarbon, carboxylic anions, and aromatic C in intra-aggregate fractions than in free ones, suggesting that the intra-aggregate fraction comprises more decomposed and transformed organic matter relative to the free fraction. Furthermore, Ellerbrock and

Gerke (2004) found, for the surface of an arable soil, that the content of the carboxylic groups in hot-water-soluble organic matter taken from soil aggregate coatings was less than that in the interiors, suggesting greater hydrophobic behavior for aggregate coatings compared to aggregate interiors. These differences in SOM composition, associated with the location of organic matter within soil aggregates, are nicely illustrated by Kinyangi et al. (2006). They conducted a nanoscale study of unaltered soil microaggregates and found that organic matter in exterior regions was highly processed, containing more carboxyl-C relative to aromatic-C. Organic matter in interior regions was more complex and contained significant amounts of aromatic and aliphatic C. These results indicate that more research is needed to understand the spatial interrelationships between soil C forms and aggregate stability.

Physically separated SOM fractions have been useful in evaluating SOM stabilization, because they are associated with differing C dynamics (C turnover) (Christensen, 1987; Gregorich et al., 1989), and biochemical characteristics (Catrouxx and Schinitzer, 1987; Angers and Mehuys, 1990). Kong et al. (2005) reported that most of the accumulation of soil organic C due to additional C input was preferentially sequestered in microaggregates within macroaggregates. These results are supported by the findings discussed in Chapter 1 and 2 of this dissertation. In brief, across management treatments and for all three field experiments, microaggregates within macroaggregates (250-53 μm_{SM} or 250-53 μm_{LM}) was the fraction with the highest total C content (g C/100 g fraction) and the highest content of C_4 derived C (recently deposited SOM). Nonetheless, little is known about the SOM composition of this important fraction.

Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy is considered the most sensitive routine infrared technique (Griffiths, 1983), and has been applied extensively to characterize the functional group composition of heterogeneous materials. According to Painter et al. (1985) and Niemeyer et al. (1992), DRIFT offers several advantages over transmission IR spectroscopy: i) a simpler sample preparation procedure is required; ii) interferences due to water absorption are reduced; and iii) resolution of the spectra is improved. Nonetheless, direct quantitative analysis through application of Beer’s law is not possible with DRIFT spectroscopy (Griffiths and Haseth, 1975). The relative heights or intensities of selected peaks have been used for comparative analysis of DRIFT spectra (Griffiths and Fuller, 1982; Niemeyer et al., 1992; Ding et al. 2002).

Niemeyer et al. (1992) characterized humic acids, compost, and peat by DRIFT. They found that the relative enrichment and depletion of specific functional groups during humification could be assessed through the use of peak ratios within complex organic and organo-mineral spectra. Using a similar approach, Inbar et al. (1989) used DRIFT peak ratios to semiquantitatively assess the maturation of composted cattle manure. Their results showed that as the manure was composted, its aliphatic peak constituents were relatively decreased and the proportion of aromatic functional groups increased. Wander and Traina (1996) reported that reactive/recalcitrant (O/R) peak ratios of the humic acid fraction best reflected overall SOM availability; while the O/R peak ratios of fulvic acid, light, and litter fractions reflected the cultural or temporal farming system impacts on SOM lability. However, they could not assess management effects on SOM composition from spectra of the fine clay and sand size fractions due to mineral contaminants. Therefore, there is a need to clarify the relationship between the lability of the different SOM fractions and their functional significance in mineral soils.

The objective of this study was to investigate the impact of management practices on SOM composition of physically separated fractions using DRIFT spectroscopy. The specific objectives were: i) to evaluate the effects of tillage, N fertilization, manure application, and crop rotation on SOM reactivity of intra-macroaggregate fractions as indicated by O/R peak ratios; and ii) to assess the impact of the type of intra-macroaggregate fraction on SOM reactivity.

Material and Methods

Field Experiments

Samples were taken from the same treatments and field experiments described in Chaper 2.

Soil Sampling and Handling

The three long-term field experiments were sampled twice in 2006 (late April and late November) as indicated in Chaper 2. However, only the soil surface samples (0-10 cm soil depth increment) coming from the spring sampling were used in this study. The physical and chemical properties, the aggregate size distribution, and the contents of organic C and total N associated with these aggregate fractions are described in detail in Chapter 2; while the soil organic C stability, based on $\delta^{13}C$ for the same fractions, is discussed in Chapter 3.

Spectroscopic Analysis

Each intra-macroaggregate fraction isolated as described in Chaper 2 was subject to DRIFT analysis. Infrared spectra were recorded with a Nicolet 6700 Fourier Transform Infrared Spectrometer, equipped with a Nicolet X700 XT-KBr beam splitter, a liquid N_2 cooled detector, and a Thermo Fisher Smart Collector Diffuse Reflectance kit. The intra-macroaggregate fractions were powdered with a mortar and pestle, and diluted at 3.0% on potassium bromide (KBr) to reduce scatter light intensity. Some samples of the light and occluded fractions were diluted to a lower concentration (ranging from 0.7 to 3.0%) using as much sample as could be recovered from the membrane filter. A blade was used to smooth the sample surface, and the spectra were acquired with a minimum of 200 scans over the range from 4000 cm^{-1} to 400 cm^{-1} (mid-infrared region), with a resolution of 2 cm^{-1}. Potassium bromide background spectra were collected for every 5 samples.

Because the heavy fractions (>250 μm_{SM}, >250 μm_{LM}, 250-53 μm_{SM}, 250-53 μm_{LM}, <53 μm_{SM}, and <53 μm_{LM}) have an important mineral component, all spectra were corrected by mathematical subtraction, to eliminate bands corresponding to the mineral phases. Ashes were obtained by combustion at 430 ^{o}C for 8 hours (Ellerbrock and Gerke, 2004), and spectra of the SOM fraction minus ash were obtained using Thermo

Electron's OMNIC^{TM} Spectroscopy Software. The absorption bands in spectra of both ash and SOM fraction were compared, especially in the regions relevant for bands of mineral components (which maintained themselves during heating). Figure 5.1 presents DRIFT spectra of the SOM fraction and corresponding ash for one of the field treatments. The same pattern was observed consistently for the rest of the samples. The ash spectrum exhibited lower absorption intensity at 3300-3279 cm^{-1}, 2930-2924 cm^{-1}, 1660-1650 cm^{-1}, and 1600-1585 cm^{-1} resulting from organic matter lost by heating, and small changes at 3500-2800 cm^{-1} resulting from water lost. There were no changes in the absorption bands for Si-O-Si (1100-1000 cm^{-1}) or a shift in the absorption maximum. These results are supported by the findings of Ellerbrock and Gerke (2004). In addition, the 2930/2850 peak ratio in spectra of both SOM fraction and SOM fraction minus ash was calculated to evaluate possible variation introduced by the subtraction technique. The results showed consistency in this peak ratio, for both spectra, and across all the samples. Thus the 430^{o}C ashing temperature and the subtraction technique used in this study are justified.

All the absorption bands of the DRIFT spectra were assigned to functional groups (Table 5.1), since the mineral component was already corrected. The presence of absorption bands representing the labile (O containing) and recalcitrant (C and H and/or N) functional groups was not entirely consistent across all spectra. Consequently, only absorption bands common across management treatments, within each intra-macroaggregate fraction, were used to identify management effects on SOM reactivity. Common absorption bands among management treatments and intra-macroaggregate fractions were used to identify the effect of the intra-macroaggregate fraction type on SOM reactivity. In this study absorption bands representing the labile functional groups were equated with SOM reactivity because they are present in all major SOM functional groups (carboxyl, phenolic, hydroxyl, alcohol, and carbonyl) and can then be associated with SOM binding characteristics and solubility (Wander and Traina, 1996).

Data Analysis

The single-sample approach (Baes and Bloom, 1989; Inbar et al., 1989; Schnitzer and Schuppili, 1989; Niemeyer et al., 1992) was used in this study to evaluate the effects of management and type of intra-macroaggregate fraction on SOM composition due to the large number of samples However, every spectrum was obtained from a sample composited across all field replications of each intra-macroaggregate fraction. Consequently, no statistical analysis was involved.

Results and Discussion

Spectra and Peak Ratios of Field Experiment 1-Tillage by N Rate

The DRIFT spectra of the organic matter fractions from each management treatment in Field Experiment 1 are presented in Figures 5.2 to 5.5. Tillage and N fertilization treatments affected the SOM composition of each intra-macroaggregate fraction (Tables 5.2 and 5.3). However, major differences among management treatments were observed for the free and the <53 μm_{SM} fractions. Minor differences were associated with the 250-53 μm_{SM} fraction.

For the free fraction, the 1509 cm^{-1} peak was present and the 1630 cm^{-1} peak absent, for N-fertilized treatments across both tillage systems, suggesting that N application promoted amide formation (Francioso et al., 2000). On the other hand, only NT soils exhibited the 1280 cm^{-1} peak, indicating the presence of organic matter with potentially higher hydrophilic behavior (solubility and acidity) (Niemeyer et al., 1992). However, the degree of humification was modified under NT with N fertilization, because the NT-0N treatment was the only one without the 2850 cm^{-1} peak suggestive of a higher degree of humification (Niemeyer et al., 1992). The overall reactivity of this fraction, as indicated by O/R ratio, was affected more by N fertilization than tillage. Higher reactivity was associated with N fertilization, across tillage treatments, but especially under MP.

In the occluded fraction, the 1660 cm^{-1} peak was only observed for N-fertilized treatments, in both tillage systems. The 1600 cm^{-1} band was only observed in NT soils, suggesting that, as for the free fraction, the amide groups are favored by N fertilization while the carboxyl groups are enhanced by NT. The O/R ratios revealed that SOM reactivity was increased by N fertilization under NT, while the reverse was true under MP.

With the <53 μm_{SM} fraction, the 1600 cm^{-1} and the 1550 cm^{-1} peaks were absent with N fertilization of NT soils, suggesting a decrease in aromatic moieties. On the other hand, the 1280 cm^{-1} peak was observed with N fertilization of MP soils, which implies the presence of hydrophilic groups. These results are supported by the O/R ratio, indicating higher SOM reactivity was associated with unfertilized MP soils than with N fertilized MP soils. In contrast, N rate had no impact on reactivity in NT soils.

For the 250-53 µm$_{SM}$ fraction, the 1550 cm^{-1} peak was the only one exhibiting an impact ffrom the management treatments. This peak was present in N fertilized NT soils, and the reverse was observed with MP soils, suggesting differences in the type of ortho-substituted aromatic rings among management treatments (Bellamy, 1975). The O/R ratio for this fraction revealed that N fertilization did not affect SOM reactivity under NT, but decreased reactivity under MP.

With the >250 µm$_{SM}$ fraction, the 1509 cm^{-1} peak was present only under NT indicating the presence of aromatic moieties derived from lignin (Francioso and Montecchio, 2007). Like the free fraction, the 2850 cm^{-1} peak was observed only with N fertilization of NT soils, suggesting lower humification (Niemeyer et al., 1992). Nonetheless, the O/R ratio indicated that SOM reactivity in this fraction was the same for both fertilized and unfertilized NT soils, which was consistent across the three heavy fractions. In contrast, SOM reactivity was higher with, than without, N fertilization of MP soils.

The type of intra-macroaggregate fraction affected SOM composition across management treatments. The occluded fraction exhibited the highest abundance of absorption bands, followed by the free fraction, which was the only one without the 1660 cm^{-1} peak. The <53 µm$_{SM}$ and 250-53 µm$_{SM}$ fractions did not have the 1630 cm^{-1}, 1509 cm^{-1}, 1460 cm^{-1}, and 1420 cm^{-1} peaks compared to the rest of the fractions, indicating a lack of aromatic components. In addition, the 250-53 µm$_{SM}$ and the >250 µm$_{SM}$ fractions did not exhibit the 1280 cm^{-1} peak, suggesting less hydrophilic behavior (low solubility). The >250 µm$_{SM}$ fraction was the only fraction without the 1550 cm^{-1} peak. The reactivity of SOM, expressed as the (1400+1084)/2930 ratio (Table 5.4) was also affected by the type of intra-macroaggregate fraction, even though there was no consistent trend due to the management treatments. The <53 µm$_{SM}$ fraction possessed the lowest reactivity in NT soils, and the highest reactivity in unfertilized MP soil. The >250 µm$_{SM}$ fraction exhibited lowest reactivity in N fertilized MP soils. The light fractions were associated with the highest reactivity across all management treatments except for the unfertilized MP soils. These results indicate that MP soil, especially without N fertilization, causes otherwise stable fractions such as the <53 µm$_{SM}$ fraction to be more reactive. In addition, there was no clear relationship among O/R ratio, C/N ratio and the content of recently deposited C, because the <53 μm_{SM} fraction exhibited the lowest C/N ratio while the 250-53 μm_{SM} fraction possessed the highest amount of recently deposited C, across all management treatments. These results are consistent with those of Wander and Traina (1996), who did not find a clear link between lability, O/R ratios, and other measures of biological availability. However, the medium to low reactivity associated with the 250-53 μm_{SM} fraction, and its low hydrophilic behavior implying resistance to microbial decomposition (Francioso et al., 2002), could explain why recently deposited C concentrations were highest in this fraction.

Spectra and Peak Ratios of Field Experiment 2-Manure by N Rate

Figures 5.6 to 5.9 illustrate the DRIFT spectra of organic matter fractions for each management treatment in Field Experiment 2. Unlike other studies (Wander and Traina, 1996; Ellerbrock et al., 1999), which had reported higher reactivity and/or carboxylic acid group content of SOM associated with manure-amended management treatments, these results indicated that the effect of manure application on SOM reactivity depended on N fertilization. The difference in results might be due to the fact that previous studies analyzed chemically separated SOM fractions and that the source of manure was not the same as that used here.

The manure and/or N rate effect on SOM composition was unique to every intra-macroaggregate fraction (Tables 5.5 and 5.6). The presence or absence of absorption bands in the free fraction was affected mainly by manure application. The 1550 cm^{-1} peak was observed only in the manured treatments, while the 1600 cm^{1} peak was observed only in the unmanured treatments, suggesting that manure application was associated with decreased carboxyl groups and the formation of amide groups. However, the O/R ratio for this fraction revealed lower reactivity associated with N fertilization of the manured soils, while slightly higher reactivity was associated with N fertilization of the unmanured soils.

The occluded fraction from manured unfertilized soils exhibited 1660 cm^{-1}, 1550 cm^{-1}, 1420 cm^{-1}, and 1280 cm^{-1} peaks, which suggests an increase in aromatic, amide, carbonyl, and carboxylic groups, respectively. However, the O/R ratio indicated lower reactivity for unfertilized manured soils. On the other hand, N-fertilized and manured soils exhibited an absence of 2850 cm^{-1}, 1660 cm^{-1}, 1460 cm^{-1}, and 1190 cm^{-1} peaks, implying an increase in aliphatic moieties, especially polysaccharides (Inbar et al., 1989; Ellerbrock and Gerke, 2004). This is characteristic of the initial stages of decomposition (Inbar et al., 1989). Nonetheless, SOM reactivity was the same for N-fertilized manured and unmanured soils as indicated by the O/R ratio.

The <53 μm_{SM} fraction exhibited differences only for N-fertilized, manured soils. The loss of the 1280 cm^{-1} peak and the presence of the 1600 cm^{-1} peak suggested an abundance of aromatic moieties. These observations were consistent with the O/R ratio. Higher reactivity was associated with N-fertilized manured soils. No difference in reactivity due to N rate was observed for the unmanured soils.

The 250-53 μm_{SM} fraction revealed no difference among management treatments in terms of the presence of functional groups. However, the O/R ratio showed that N fertilization increases SOM reactivity in both manured and unmanured soils, but manure application did not have any effect on reactivity in this fraction.

The >250 μm_{SM} fraction of unfertilized manured soils exhibited peaks at 2850 cm^{-1}, 1509 cm^{-1}, and 1460 cm^{-1}, suggesting an increase in aromatic and aliphatic groups. The O/R ratio also evidenced a decrease in reactivity in these soils. In contrast, N-fertilized manured soils exhibited a band at 1509 cm^{-1}, implying the presence of aromatic C=C vibrations derived from lignin (Francioso and Montecchio, 2007). However, the reactivity, as indicated by the O/R ratio, was similar for unfertilized manured and unmanured soils.

The different intra-macroaggregate fractions affected SOM composition across the manure by N rate treatments. The free and occluded fractions exhibited the highest abundance of absorption bands among all the fractions. As in Field Experiment 1, the 250-53 μm_{SM} and the >250 μm_{SM} fractions did exhibit a peak at 1280 cm^{-1}, suggesting low hydrophilic behavior (Niemeyer et al., 1992). The >250 μm_{SM} fraction was also the only fraction without a peak at 1550 cm^{-1}, implying differences in aromatic composition for this fraction. In addition, the occluded, <53 μm_{SM}, and 250-53 μm_{SM} fractions did not exhibit a 1509 cm^{-1} peak, suggesting a decrease in aromatic moieties (Francioso and Montecchio, 2007). The <53 μm_{SM} and 250-53 μm_{SM} fractions did not show any peak at 1460 cm^{-1}, suggesting a decrease in aliphatic structures, as well (Bellamy, 1975).

Soil organic matter reactivity was different for the different intra-macroaggregate fractions, as indicated by the 1400/2930 ratio (Table 5.7). The highest reactivity was associated with the occluded fraction in every manure by N rate treatment, except for the unfertilized-manured treatment. There was not a specific fraction associated with the lowest reactivity across all the management treatments. As in Field Experiment 1, there was no relationship between the O/R ratio and the C/N ratio and/or the recently deposited C content in each fraction. The lowest C/N ratio and the highest recently deposited C content were consistently associated with the <53 μm_{SM} and the 250-53 μm_{SM} fractions, respectively, across the manure by N rate treatments. However, the medium to low O/R ratio and low hydrophilic behavior associated with the 250-53 μm_{SM} fraction may be explained by the highest accumulation of C input in this fraction.

Spectra and Peak Ratios of Field Experiment 3-Corn in Crop Rotations

The DRIFT spectra of organic matter fractions for each management treatment in Field Experiment 3 are illustrated in Figures 5.10 to 5.14. Crop rotation affected the SOM composition of every aggregate fraction (Tables 5.8 and 5.9). In the free fraction, the same functional groups were observed for the hhccC and the hhCcc treatments. In contrast, the spectra for the w/sC treatment did not exhibit the 2850 cm^{-1} peak, suggesting fewer hydrophobic groups (Capriel et al., 1995; Ellerbrock and Gerke, 2004), spectra from the CCCCC treatment was absent the 1420 cm^{-1} peak evidencing fewer aromatic moieties, and spectra from the hhcCc treatment was absent the 1550 cm^{-1}, 1460 cm^{-1}, and 1420 cm^{-1} peaks, suggesting decreased aliphatic and aromatic functional groups. Despite differences in the absence/presence of absorption bands, the O/R ratio revealed that the w/sC, CCCCC, and hhCcc treatments had greater reactivity than that observed in the hhcCc and hhccC treatments.

With the occluded fraction, the only difference among treatments was in the 1550 cm^{-1} peak, which was present only in the CCCCC and hhCcc treatments, suggesting that differences in SOM composition of this fraction due to the crop rotation treatments was related to ortho-substituted aromatics (Bellamy, 1975). However, similar to what was observed for the light fraction, the highest SOM reactivity (expressed as O/R ratio) was associated with the w/sC, CCCCC, and hhCcc treatments.

For the <53 μm$_{LM}$ fraction, the w/sC treatment was the only one without the 1280 cm^{-1} peak, evidencing absence of CO_2H groups (Niemeyer et al., 1992). In contrast, the hhcCc and hhCcc treatments were the only treatments with the 1600 cm^{-1} peak, suggesting the presence not only of COO^- groups, but also of differences in aromaticity in comparison to the rest of management treatments. Nonetheless, the SOM reactivity was slightly higher for the w/sC, CCCCC, and hhCcc treatments, compared to the rest of the rotation treatments.

In the 250-53 μm$_{LM}$ fraction, major differences with respect to the presence or absence of absorption bands due to to the rotation treatments were not observed. Spectra from the hhcCc and hhCcc treatments were the only ones without the 1280 cm^{-1} peak, evidencing low hydrophilic behavior, and this has been consistently observed in this fraction across the three field experiments. Nonetheless, the highest SOM reactivity was still found in the w/sC, CCCCC, and hhCcc treatments.

With the >250 μm$_{LM}$ fraction, the CCCCC treatment was enriched in aliphatic groups because this spectra exhibited the 2850 cm^{-1} peak. The spectra from the hhccC treatment was the only one exhibiting the 1509 cm^{-1} peak, suggesting the presence of aromatic C=C vibrations derived from lignin (Francioso and Montecchio, 2007). Spectra from the w/sC and hhcCc treatments did not exhibit the 1660 cm^{-1} and 1600 cm^{-1} peaks, but did exhibit the 1630 cm^{-1} peak, implying enrichment in aromatic functional groups. The SOM reactivity for this fraction, as indicated by the O/R ratio, exhibited a different pattern of response to the treatments, compared to the rest of the fractions. The highest reactivity was associated with the CCCCC and hhccC treatments, followed by the w/sC and hhCcc treatments, and finally by the hhcCc treatment, which is more consistent with the findings of Wander and Traina (1996), who reported higher O/R ratios in the spectra of fulvic and humic acids isolated from plots producing corn than in spectra isolated from plots producing soybeans.

The individual intra-macroaggregate fraction affected SOM composition across the crop rotation treatments. The occluded and free fractions exhibited the highest abundance of peaks, relative to the rest of the fractions, which was consistent with what was observed in Field Experiments 1 and 2. In addition, the occluded fraction was the only fraction without the peak at 1190 cm^{-1}; while the >250 μm$_{LM}$ fraction was the only fraction exhibiting the 1630 cm^{-1} peak and absent the 1280 cm^{-1} peak. These results suggest the absence of polysaccharides in the occluded fraction and the presence of more aromatic compounds in the >250 μm_{LM} fraction. Unlike the light fractions, the <53 μm_{LM}, 250-53 μm_{LM}, and >250 μm_{LM} fractions did not exhibit the 1460 cm^{-1} peak, and the <53 μm_{LM} fraction was the only one without a peak at 1509 cm^{-1}, indicating fewer aromatic groups derived from lignin (Francioso and Montecchio, 2007). In contrast to Field Experiments 1 and 2, the 1280 cm^{-1} peak was no longer a distinct feature of spectra from the 250-53 μm_{LM} fraction. On the other hand, SOM reactivity, as indicated by the (1400+1084)/2930 ratio (Table 5.10) did not exhibit a consistent trend across the rotation treatments. Nonetheless, the highest reactivity was associated with the occluded fraction in the CCCCC, hhcCc, and w/sC treatments, while the highest reactivity was in the free fraction of the hhCcc treatment, and in the >250 μm_{LM} fraction of the hhccC treatment. The lowest reactive fraction was usually found in the <53 μm_{LM} or >250 μm_{LM} fractions. Unlike Field Experiments 1 and 2, this experiment exhibited the expected relationship between O/R and C/N in some treatments. Low C/N ratio and low reactivity were associated with the <53 μm_{LM} fraction, considered the most recalcitrant fraction. High C/N and high reactivity were related to the free light fraction, considered the most labile pool (Ding et al., 2002). In addition, the low to medium reactivity of the 250-53 μm_{LM} fraction is probably related to its high content of recently deposited C as in Field Experiments 1 and 2.

Spectra and Peak Ratios across Field Experiments

Visually, the DRIFT spectra across the three experiments exhibited similar patterns, especially within the same intra-macroaggregate fraction. However, only the 1400 cm^{-1} and 2930 cm^{-1} peaks were consistently observed in the spectra for every management treatment across the three field experiments. Table 5.11 summarizes the 1400/2930 ratio for N-fertilized NT soils. Differences in SOM reactivity were specific to every type of intra-macroaggregate fraction. The free and the >250 $\mu m_{SM \text{ or } LM}$ fractions exhibited the highest SOM reactivity in Field Experiment 1. The occluded fraction exhibited the highest SOM reactivity in Field Experiment 2. The <53 $\mu m_{SM \text{ or } LM}$ fraction exhibited higher SOM reactivity in Field Experiments 1 and 2, and in the CCCCC treatment in Field Experiment 3. The 250-53 $\mu m_{SM\ or\ LM}$ fraction was the only fraction exhibiting similar SOM reactivity across the field experiments. Slightly higher reactivity was observed in the hhcCc treatment, compared with the rest of the treatments. These results suggest a common impact from continuous corn, across field experiments, for the 250-53 $\mu m_{SM\ or\ LM}$ fraction.

Conclusions

The use of single composite samples for DRIFT analysis does not allow us to draw statistically supported conclusions regarding the effect of management treatments and intra-macroaggregate fraction on SOM composition. Nonetheless, DRIFT spectra and the O/R ratio indicate that management practices affect SOM composition, and that this effect is not the same across all the intra-macroaggregate fractions.

Tillage affected the SOM reactivity of every intra-macroaggregate fraction except for the free one, which was only affected by N fertilization. However, there was a strong interaction between tillage and N fertilization. Soil organic matter reactivity of the heavy fractions was less affected by N fertilization under NT than under MP. The effect of N fertilization on SOM reactivity was specific to each fraction under MP. Manure application also affected SOM reactivity and that effect was closely linked to N fertilization. Nonetheless, the interaction of manure by N fertilization on SOM reactivity was specific to each intra-macroaggregate fraction. Finally, crop rotation also affected SOM reactivity. Higher SOM reactivity was associated with CCCCC, w/sC and hhCcc, compared to hhcCc and hhccC, for every intra-aggregate fraction except for the >250 μm_{LM} fraction.

The type of intra-macroaggregate fraction affected SOM reactivity across management treatments, and this effect seemed to be experiment specific. In the tillage by N rate and the crop rotation experiments, the light fraction was associated with higher SOM reactivity than the heavy fractions, especially the <53 $\mu m_{SM\ or\ LM}$ fraction. In contrast, the manure by N rate experiment revealed that the light fraction could exhibit both high and low SOM reactivity, depending on its physical location within the aggregate. Heavy fractions had moderate SOM reactivity. Nonetheless, the consistent low to medium SOM reactivity associated with the 250-53 $\mu m_{SM\ or\ LM}$ fraction, across field experiments, might be explained by its high level of recently deposited C content. In addition, this fraction exhibited the most consistent peak assignment across management treatments and field experiments, suggesting there is potential to use this fraction to monitor changes in SOM reactivity.

Finally, it is evident from this study that analyzing water-stable macroaggregates without considering the intra-aggregate fractions could result in a simplistic description of SOM composition. The SOM composition of intra-aggregate fractions differs substantially. Also, these results show that long-term management practices can change the SOM composition of physically separated fractions, so management effects on SOM should be assessed both quantitatively and functionally.

Table 5.1. DRIFT spectra peak assignments for SOM samples.

Wavenumber (cm^{-1})	DRIFT Band Assignment
3340-3279	Hydroxyl (alcohols, phenol, water molecules), amide N-H[†, §, #]
2930-2924	CH_2 asymmetric stretch[†], CH_3, CH group of alkyls[#]
2850	CH_2 symmetric stretch[†]
1660-1650	C=O, C=O-H bonded, amide H[†, ¶]
1630-1608	C=C aromatic[†, #]
1600-1585	C=C aromatic, asymmetric COO^- stretch[‡, ¶]
1550	Aromatic ring, amide[†]
1509	Aromatic ring, amide[†]
1460-1457	CH_3 Asymmetric, CH bend[†, §]
1420	Aromatic ring stretching[†]
1400-1370	Symmetric COO^- stretch[†, ‡, ¶, #]
1280-1240	CO, COOH, COC, phenol OH[†, §]
1190-1115	C-C or C-OH stretch of aliphatic[†, ‡, ¶]
1084-1050	C-O-C eter, CO aliphatic alcohol[†, §, ¶, #]

† Wander and Traina (1996)

‡ Niemeyer et al. (1992)

§ Francioso et al. (2001)

¶ Francioso et al. (2005)

Ellerbrock et al. (1999)

Table 5.2. Absorption bands from DRIFT spectra in Field Experiment 1-tillage by N rate.

Fraction	Peak Reference Range	Tillage by N Rate Treatments			
		NT-150N	NT-0N	MP-150N	MP-0N
		Wavenumber (cm^{-1})			
Free	3300-3279	3331	3350	3332	3352
	2930-2924	2926	2929	2926	2924
	2850	2856		2856	2854
	1630-1608		1622		1628
	1600-1585	1589	1595	1589	1579
	1550		1576		
	1509	1514		1516	
	1400-1370	1389	1377	1389	1377
	1280-1240	1271	1273		
	1190-1115	1107	1109	1097	1103
	1084-1050	1074	1076	1074	
Occluded	3300-3279	3302	3311	3300	3305
	2930-2924	2926	2924	2924	2926
	2850	2856	2854	2854	2854
	1660-1650	1664		1655	
	1630-1608		1624		
	1600-1585	1593	1591		
	1550	1556	1578	1562	1578
	1509	1516	1516	1514	
	1460-1457			1456	
	1420	1417	1414	1417	
	1400-1370	1381	1379	1381	1379
	1280-1240	1261	1265	1254	1259
	1190-1115	1153	1155	1149	1157
	1084-1050	1093	1074	1095	1072
<53 μm_{SM}	3300-3279	3288	3280	3275	3286
	2930-2924	2926	2926	2926	2926
	2850	2852	2852	2852	2852
	1660-1650	1657	1655	1655	1655
	1600-1585		1591		
	1550		1560	1554	1558
	1400-1370	1414	1410	1410	1410
	1280-1240			1232	
	1190-1115	1161	1161	1163	1161
	1084-1050	1095	1097	1099	1095
250-53 μm_{SM}	3300-3279	3304	3298	3298	3305
	2930-2924	2924	2924	2924	2924
	2850	2852	2852	2852	2852
	1660-1650	1655	1655	1655	1657
	1600-1585	1589	1593	1595	1591
	1550	1560			1558
	1420	1410	1412	1414	1408
	1190-1115	1165	1159	1161	1163
	1084-1050	1097	1099	1095	1095
>250 μm_{SM}	3300-3279	3228	3234	3188	3226
	2930-2924	2929	2933	2929	2929
	2850	2852			
	1660-1650	1659	1657	1657	
	1630-1608		1632		1628
	1600-1585	1589	1589	1593	
	1509	1514	1518		
	1460-1457	1464	1462	1462	1466
	1400-1370	1402	1404	1402	1400
	1084-1050	1097	1095	1095	1095

NT-0N (no-tillage and 0 kg N ha^{-1}); NT-150N (no-tillage and 150 kg N ha^{-1});
MP-0N (moldboard plow and 0 kg N ha^{-1}); MP-150N (moldboard plow and 150 kg N ha^{-1})

Table 5.3. DRIFT absorption ratios suitable to compare among management treatments within an intra-macroaggregate fraction in Field Experiment 1-tillage by N rate.

Fraction	Wavenumber ratio†	Tillage by N Rate Treatments			
		NT-150N	NT-0N	MP-150N	MP-0N
Free	$\frac{1400 + 1190 + 1600}{2930}$	3.28	1.39	3.68	1.39
Occluded	$\frac{1400 + 1280 + 1084}{2930 + 2850 + 1550}$	1.00	0.81	0.64	0.99
<53 μm$_{SM}$	$\frac{1660 + 1400 + 1190 + 1084}{2930 + 2850}$	1.49	1.51	1.55	2.40
250-53 μm$_{SM}$	$\frac{1660 + 1400 + 1190 + 1084}{2930 + 2850}$	1.71	1.70	1.61	1.76
>250 μm$_{SM}$	$\frac{1400 + 1084}{2930 + 1460}$	0.92	0.91	0.81	0.73

† Wavenumber values are approximate and exact peak location varies between spectra
NT-0N (no-tillage and 0 kg N ha^{-1}); NT-150N (no-tillage and 150 kg N ha^{-1})
MP-0N (moldboard Plow and 0 kg N ha^{-1}; MP-150N (moldboard plow and 150 kg N ha^{-1})

Table 5.4. DRIFT absorption ratio suitable to compare among intra-macroaggregate fractions within a management treatment in Field Experiment 1-tillage by N rate.

Fraction	Tillage by N Rate Treatments			
	NT-150N	NT-0N	MP-150N	MP-0N
	(1400+1084)/2930†			
Free	1.89	2.06	1.83	1.75
Occluded	1.96	1.51	1.07	2.10
<53 μm$_{SM}$	1.24	1.33	1.53	2.97
250-53 μm$_{SM}$	1.64	1.55	1.43	1.74
>250 μm$_{SM}$	1.49	1.46	1.12	0.74

† Wavenumber values are approximate and exact peak location varies between spectra
NT-0N (no-tillage and 0 kg N ha^{-1}); NT-150N (no-tillage and 150 kg N ha^{-1});
MP-0N (moldboard plow and 0 kg N ha^{-1}; MP-150N (moldboard plow and 150 kg N ha^{-1})

Table 5.5. Absorption bands from DRIFT spectra in Field Experiment 2-manure by N rate.

Fraction	Peak Reference Range	Manure by N Rate Treatments			
		Manured-0N	Manured-168N	Unmanured-0N	Unmanured-168N
		Wavenumber (cm^{-1})			
Free	3300-3279	3311	3313	3302	3304
	2930-2924	2922	2922	2924	2926
	2850	2852	2852	2854	2854
	1660-1650	1655	1657	1653	1655
	1600-1585			1597	1591
	1550	1541	1537		
	1509	1514	1510	1516	1514
	1460-1457	1454	1448	1456	1454
	1420	1419	1431	1417	1417
	1400-1370	1369	1379	1377	1379
	1280-1240	1267	1271	1263	1265
	1190-1115	1157	1155	1157	1157
	1084-1050	1072	1078	1080	1078
Occluded	3300-3279	3280	3278	3278	3278
	2930-2924	2926	2931	2926	2926
	2850	2854		2854	2856
	1660-1650	1664			1664
	1600-1585	1601	1595	1589	1593
	1550	1551			
	1460-1457	1456		1446	1456
	1420	1421			
	1400-1370	1390	1389	1396	1387
	1280-1240	1271	1290		1286
	1190-1115				1105
	1084-1050	1074		1072	
<53 µm$_{SM}$	3300-3279	3298	3292	3302	3300
	2930-2924	2926	2926	2926	2926
	2850	2852	2852	2852	2852
	1660-1650	1655	1657	1655	1657
	1600-1585		1591		
	1550	1554	1551	1556	1552
	1400-1370	1412	1410	1414	1408
	1280-1240	1257			
	1190-1115	1163	1161	1161	1165
	1084-1050	1099	1097	1103	1095
250-53 µm$_{SM}$	3300-3279	3302	3302	3304	3304
	2930-2924	2926	2924	2924	2924
	2850	2852	2852	2852	2852
	1660-1650	1657	1659	1655	1657
	1600-1585	1593	1581	1589	1587
	1550	1558	1552	1558	1554
	1400-1370	1408	1406	1412	1410
	1190-1115	1163	1163	1163	1161
	1084-1050	1097	1099	1101	1097
>250 µm$_{SM}$	3300-3279	3226	3203	3226	3226
	2930-2924	2931	2931	2941	2931
	2850	2854			
	1660-1650	1655	1662	1651	1653
	1600-1585	1593	1583	1591	1595
	1509	1512	1516		
	1460-1457	1466	1466		1464
	1400-1370	1402	1402	1406	1406
	1190-1115	1165	1165	1161	1161
	1084-1050	1097	1097	1099	1099

Manured-0N (manured and 0 kg N ha^{-1}); manured-168N (manured and 168 kg N ha^{-1}); unmanured-0N (unmanured and 0 kg N ha^{-1}); unmanured-168N (unmanured and 168 kg N ha^{-1})

Table 5.6. DRIFT absorption ratios suitable to compare among management treatments within an intra-macroaggregate fraction in Field Experiment 2-manure by N rate.

Fraction		Manure by N rate treatments			
	Wavenumber ratio[†]	Manured		Unmanured	
		0 kg N ha^{-1}	168 kg N ha^{-1}	0 kg N ha^{-1}	168 kg N ha^{-1}
Free	$\frac{1660 + 1400 + 1280 + 1190 + 1084}{2930 + 2850 + 1509 + 1460 + 1420}$	0.85	0.80	0.80	0.82
Occluded	$\frac{1400 + 1600}{2930}$	1.99	1.02	3.42	1.02
<53 μm_{SM}	$\frac{1660 + 1400 + 1190 + 1084}{2930 + 2850}$	2.07	2.29	2.25	2.25
250-53 μm_{SM}	$\frac{1660 + 1600 + 1400 + 1190 + 1084}{2930 + 2850 + 1550}$	1.49	0.94	1.49	0.94
>250 μm_{SM}	$\frac{1660 + 1600 + 1400 + 1190 + 1084}{2930}$	3.26	2.36	3.50	2.35

† Wavenumber values are approximate and exact peak location varies between spectra

Table 5.7. DRIFT absorption ratio suitable to compare among intra-macroaggregate fractions within a management treatment in Field Experiment 2-manure by N rate.

Fraction	Manure by N rate treatments			
	Manured-0N	Manured-168N	Unmanured-0N	Unmanured-168N
		(1400/2930)		
Free	0.95	0.99	0.85	0.95
Occluded	0.96	1.60	1.11	1.36
<53 μm_{SM}	0.76	1.00	0.97	1.06
250-53 μm_{SM}	0.99	1.02	0.97	1.00
>250 μm_{SM}	0.83	1.00	1.07	0.96

† Wavenumber values are approximate and exact peak location varies between spectra
manured-0N (manured and 0 kg N ha^{-1}); manured-168N (manured and 168 kg N ha^{-1});
unmanured-0N (unmanured and 0 kg N ha^{-1}); unmanured-168N (unmanured and 168 kg N ha^{-1})

Table 5.8. Absorption bands from DRIFT spectra in Field Experiment 3-corn in crop rotations.

Fraction	Peak Reference Range	Management Treatments				
		CCCCC	hhcCc	w/sC	hhccC	hhCcc
		Wavenumber (cm^{-1})				
Free	3300-3279	3305	3304	3327	3305	3304
	2930-2924	2924	2924	2926	2922	2922
	2850	2854	2854		2852	2852
	1660-1650	1660	1655	1659	1657	1659
	1600-1585	1603	1605	1603	1605	1599
	1550	1551		1549	1545	1551
	1509	1516	1516	1512	1514	1514
	1460-1457	1454		1452	1454	1452
	1420			1421	1421	1421
	1400-1370	1379	1389	1379	1379	1379
	1280-1240	1271	1269	1265	1265	1267
	1190-1115	1159		1157	1157	1171
	1084-1050	1072	1086	1076	1078	1099
Occluded	3300-3279	3304	3304	3304	3288	3290
	2930-2924	2922	2924	2926	2922	2926
	2850	2852	2854	2856	2852	2856
	1660-1650	1660	1660	1660	1660	1664
	1600-1585	1603	1599	1589	1597	1599
	1550	1549				1547
	1509	1512	1516	1514	1514	1514
	1460-1457	1454	1456	1452	1448	1460
	1420	1425	1425	1421	1419	1423
	1400-1370	1379	1377	1379	1375	1373
	1280-1240	1257	1254	1252	1269	1261
	1084-1050	1095	1092	1097	1074	1090
<53 μm_{LM}	3300-3279	3280	3288	3275	3277	3300
	2930-2924	2926	2926	2924	2927	2929
	2850	2852	2852	2852	2852	2854
	1660-1650	1659	1655	1657	1655	1655
	1600-1585		1591			1591
	1550	1552	1554	1552	1552	1552
	1400-1370	1408	1410	1410	1410	1414
	1280-1240	1279	1265		1277	1271
	1190-1115	1161	1161	1161	1161	1165
	1084-1050	1101	1103	1103	1103	1101
250-53 μm_{LM}	3300-3279	3280	3290	3294	3288	3290
	2930-2924	2930	2926	2927	2926	2924
	2850	2850	2852	2854	2852	2852
	1660-1650	1650	1657	1657	1657	1657
	1600-1585	1580	1589	1589	1593	1589
	1550	1550	1552	1556	1554	1558
	1509	1510	1518	1516	1518	1516
	1400-1370	1420	1412	1414	1414	1416
	1280-1240	1270		1265	1271	
	1190-1115	1160	1161	1163	1163	1163
	1084-1050	1100	1101	1099	1099	1103
>250 μm_{LM}	3300-3279	3197	3201	3226	3213	3244
	2930-2924	2933	2933	2933	2945	2941
	2850	2854				
	1660-1650	1660			1659	1653
	1630-1608		1632	1628		1641
	1600-1585	1585			1593	1591
	1509				1514	
	1400-1370	1406	1406	1404	1406	1404
	1190-1115	1163	1161	1161	1161	1163
	1084-1050	1097	1099	1101	1105	1099

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation);
hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation);
hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation);
w/sC (corn in corn-wheat/doble crop soybean crop rotation)

Table 5.9. DRIFT absorption ratios suitable to compare among management treatments within an intra-macroaggregate fraction in Field Experiment 3-corn in crop rotations.

Fraction	Wavenumber ratio†	Crop Rotation Treatments				
		CCCCC	hhcCc	w/sC	hhccC	hhCcc
Free	$\frac{1660 + 1600 + 1400 + 1280 + 1084}{2930 + 1509}$	2.31	1.58	2.49	1.57	2.33
Occluded	$\frac{1660 + 1600 + 1400 + 1280 + 1084}{2930 + 2850 + 1509 + 1460 + 1420}$	1.04	0.69	1.05	0.69	1.04
<53 μm_{LM}	$\frac{1660 + 1400 + 1190 + 1084}{2930 + 2850 + 1550}$	1.11	1.04	1.11	1.02	1.14
250-53 μm_{LM}	$\frac{1660 + 1600 + 1400 + 1190 + 1084}{2930 + 2850 + 1550}$	1.44	0.94	1.46	0.94	1.55
>250 μm_{LM}	$\frac{1400 + 1084}{2930}$	1.69	1.05	1.30	1.66	1.34

† Wavenumber values are approximate and exact peak location varies between spectra

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation);

hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation);

hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/double crop soybean crop rotation)

Table 5.10. DRIFT absorption ratio suitable to compare among intra-macroaggregate fractions within a management treatment in Field Experiment 3-corn in crop rotations.

Fraction	Crop rotation treatments				
	CCCCC	hhcCc	w/sC	hhccC	hhCcc
	(1400+1084)/(2930)†				
Free	1.54	1.77	1.57	1.52	1.70
Occluded	2.13	1.86	1.99	1.60	1.64
<53 μm_{LM}	1.52	1.38	1.55	1.33	1.57
53-250 μm_{LM}	1.61	1.60	1.81	1.56	1.62
>250 μm_{LM}	1.69	1.05	1.30	1.66	1.34

† Wavenumber values are approximate and exact peak location varies between spectra

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation);

hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation);

hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/double crop soybean crop rotation)

Table 5.11. DRIFT absorption ratio suitable to compare among NT soils under N fertilization in the three field experiments.

Fraction	Experiment 1	Experiment 2	Experiment 3				
	NT-150N	Unmanured-168N	CCCCC	hhcCc	w/sC	hhccC	hhCcc
			(1400/2930)†				
Free	1.28	0.95	0.99	1.11	1.09	1.02	1.12
Occluded	1.11	1.36	1.05	0.84	1.02	1.10	0.91
<53 μm$_{LM}$	1.07	1.06	1.08	0.88	1.01	0.89	0.97
53-250 μm$_{LM}$	0.99	1.00	1.02	1.07	1.03	1.04	1.02
>250 μm$_{LM}$	1.14	0.96	1.03	0.71	0.91	0.90	0.82

† Wavenumber values are approximate and exact peak location varies between spectra

NT-150N (no-tillage and 150 kg N ha^{-1}); Unmanured-168N (unmanured and 168 kg N ha^{-1})

CCCCC (continuous corn); hhccC (third year of corn in hay-hay-corn-corn-corn crop rotation);

hhcCc (second year of corn in hay- hay-corn-corn-corn crop rotation);

hhCcc (first year of corn in hay- hay-corn-corn-corn crop rotation); w/sC (corn in corn-wheat/double crop soybean crop rotation)

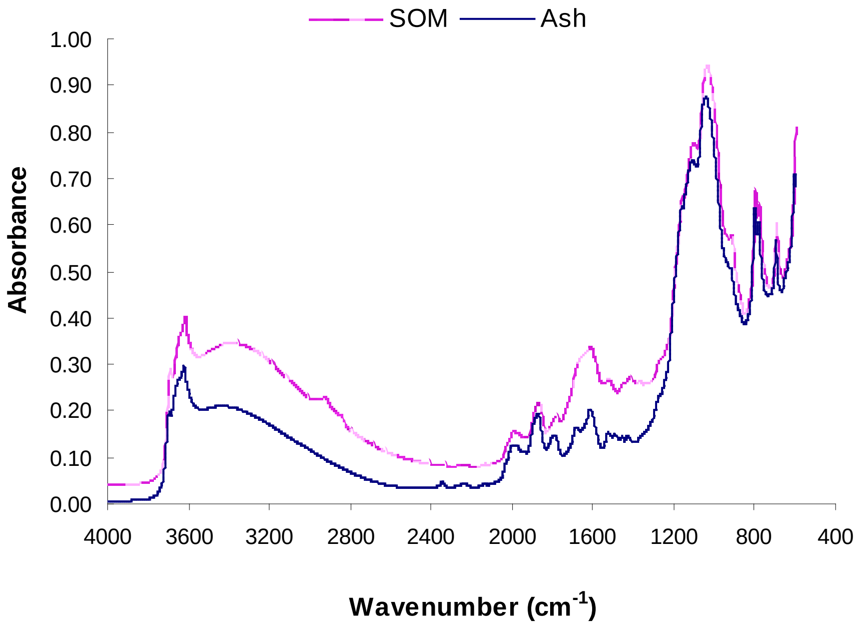

Figure 5.1. DRIFT spectra of SOM and its ashes remaining after 8 hours at 430^{o}C.

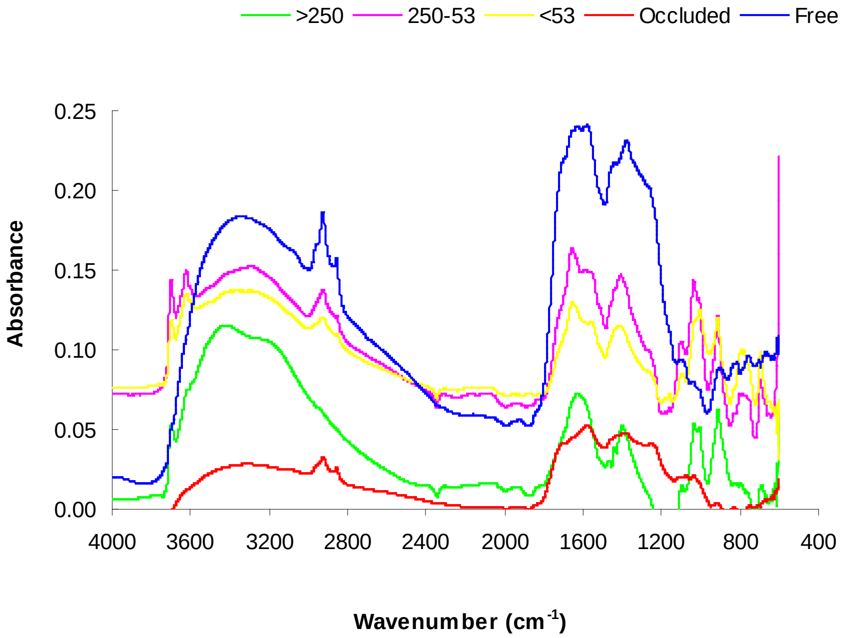

Figure 5.2. DRIFT spectra of intra-macroaggregate fractions for the moldboard plow and 0 kg N ha^{-1} treatment in Field Experiment 1-tillage by N rate.

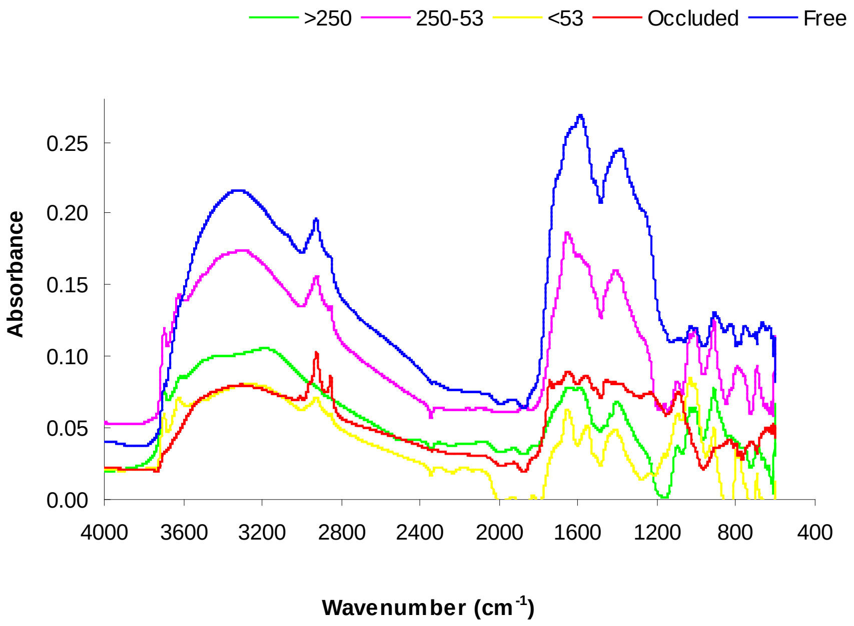

Figure 5.3. DRIFT spectra of intra-macroaggregate fractions for the moldboard plow and 150 kg N ha^{-1} treatment in Field Experiment 1-tillage by N rate.

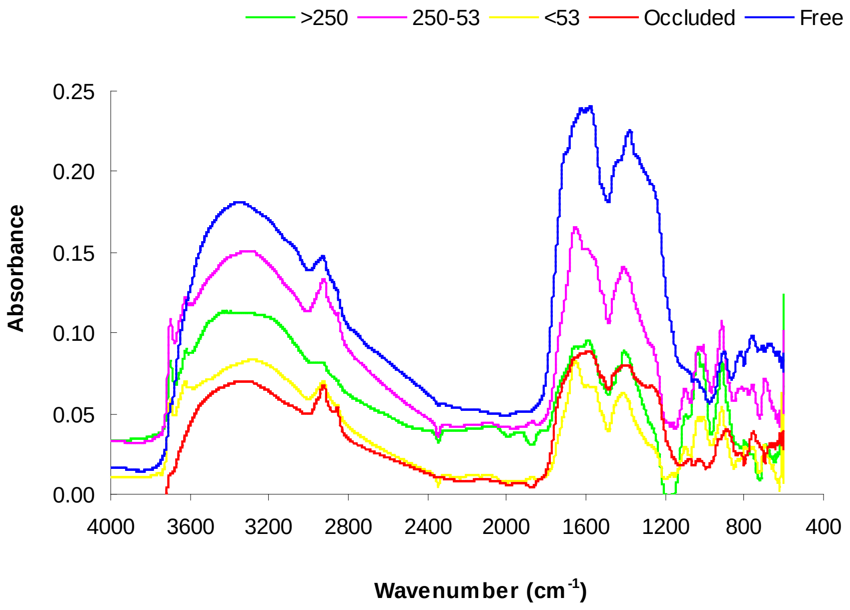

Figure 5.4. DRIFT spectra of intra-macroaggregate fractions for the no-tillage and 0 kg N ha^{-1} treatment in Field Experiment 1-tillage by N rate.

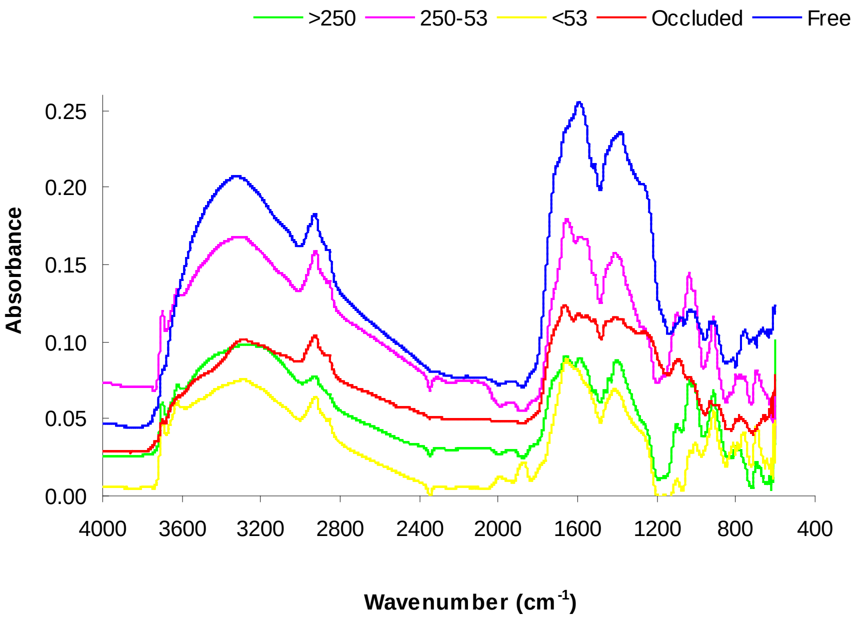

Figure 5.5. DRIFT spectra of intra-macroaggregate fractions for the no-tillage and 150 kg N ha^{-1} treatment in Field Experiment 1-tillage by N rate.

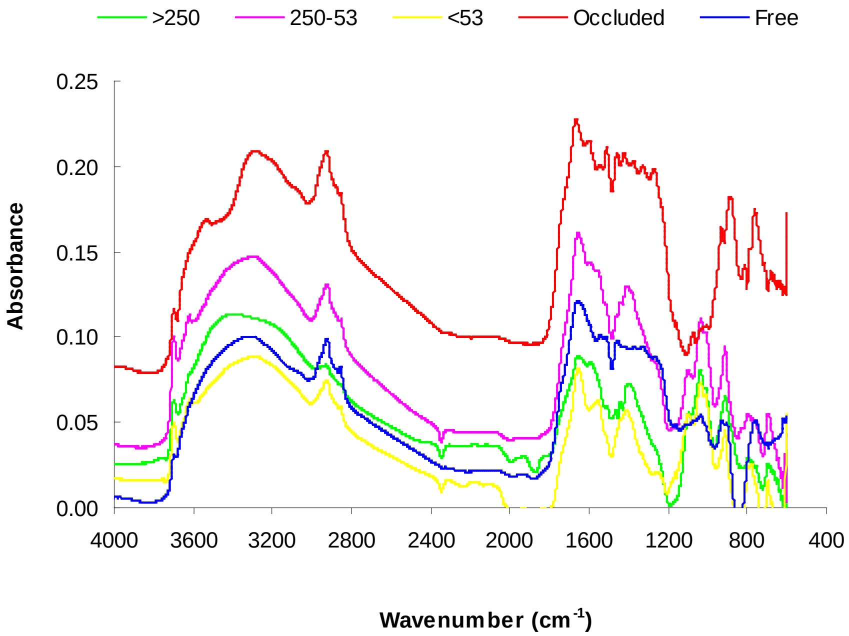

Figure 5.6. DRIFT spectra of intra-macroaggregate fractions for the manured and 0 kg N ha^{-1} treatment in Field Experiment 2-manure by N rate.

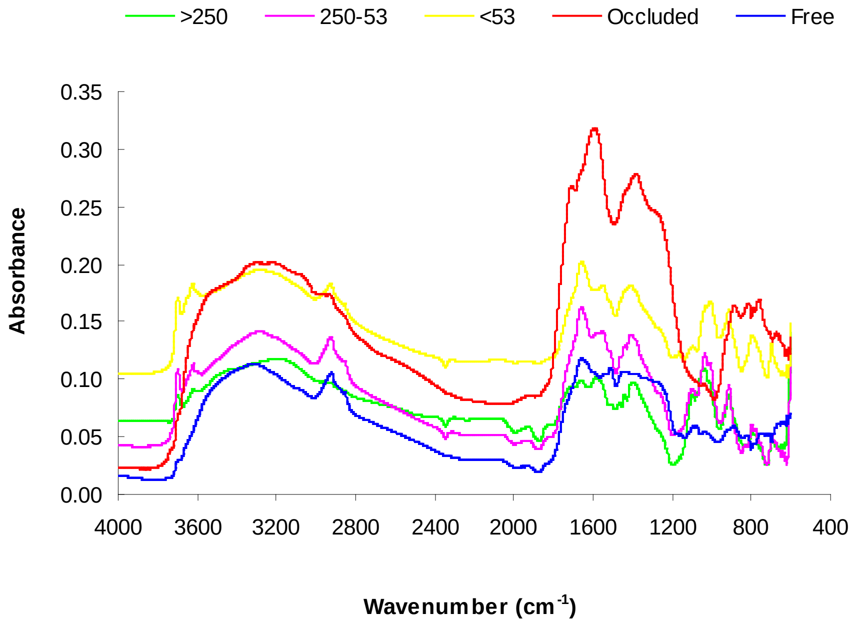

Figure 5.7. DRIFT spectra of intra-macroaggregate fractions for the manured and 168 kg N ha^{-1} treatment in Field Experiment 2-manure by N rate.

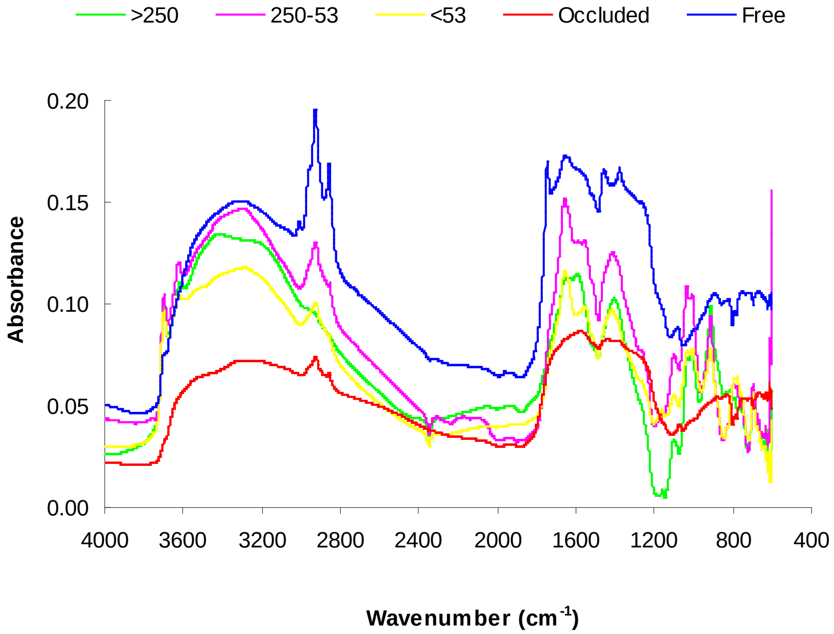

Figure 5.8. DRIFT spectra of intra-macroaggregate fractions for the unmanured and 0 kg N ha^{-1} treatment in Field Experiment 2-manure by N rate.

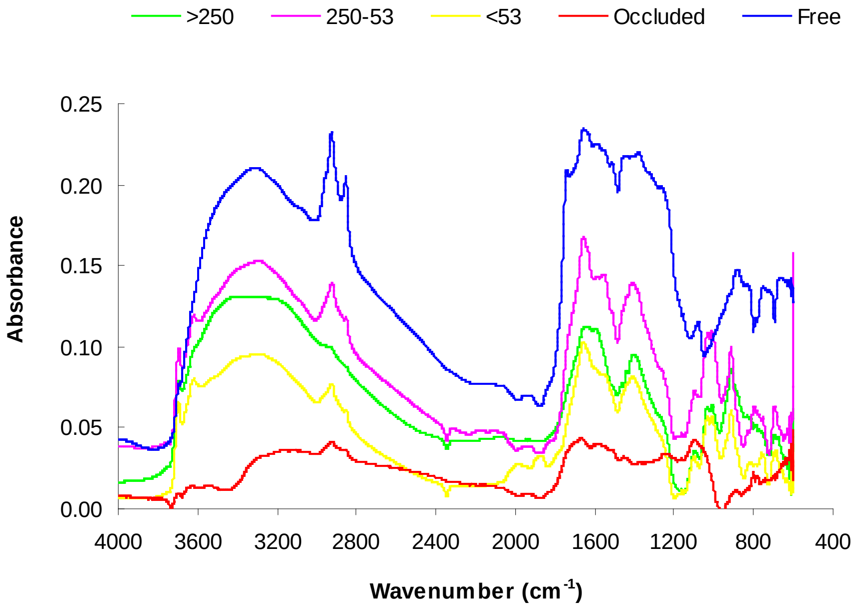

Figure 5.9. DRIFT spectra of intra-macroaggregate fractions for the unmanured and 168 kg N ha^{-1} treatment in Field Experiment 2-manure by N rate.

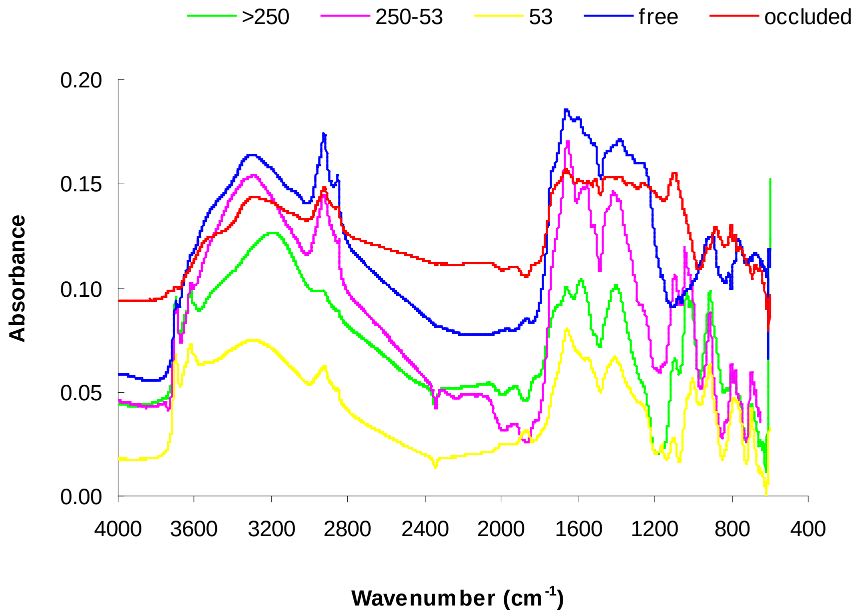

Figure 5.10. DRIFT spectra of intra-macroaggregate fractions for the continuous corn crop rotation in Field Experiment 3-corn in crop rotations.

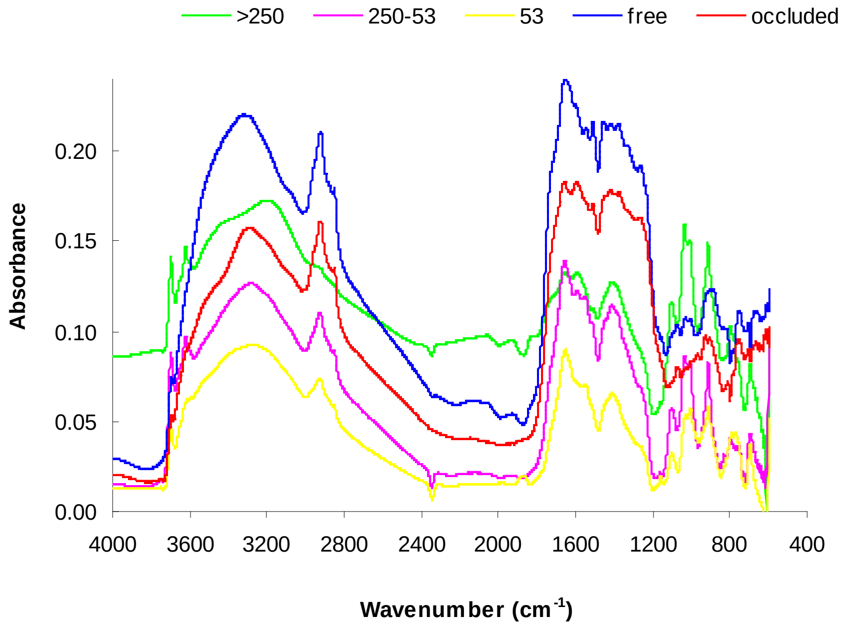

Figure 5.11. DRIFT spectra of intra-macroaggregate fractions for the third year of corn in the hay-hay-corn-corn-corn crop rotation in Field Experiment 3-corn in crop rotations.

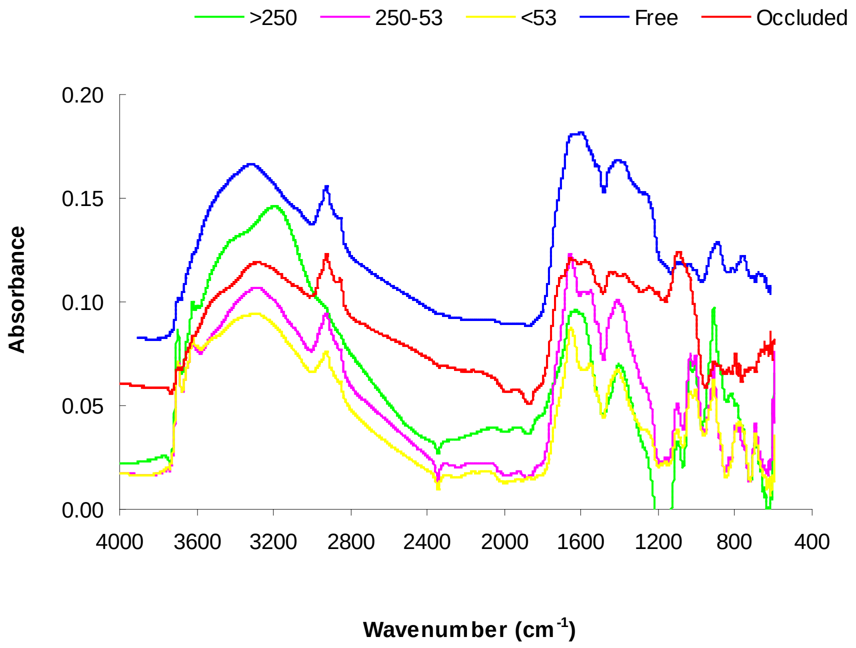

Figure 5.12. DRIFT spectra of intra-macroaggregate fractions for the second year of corn in the hay-hay-corn-corn-corn crop rotation in Field Experiment 3-corn in crop rotations.

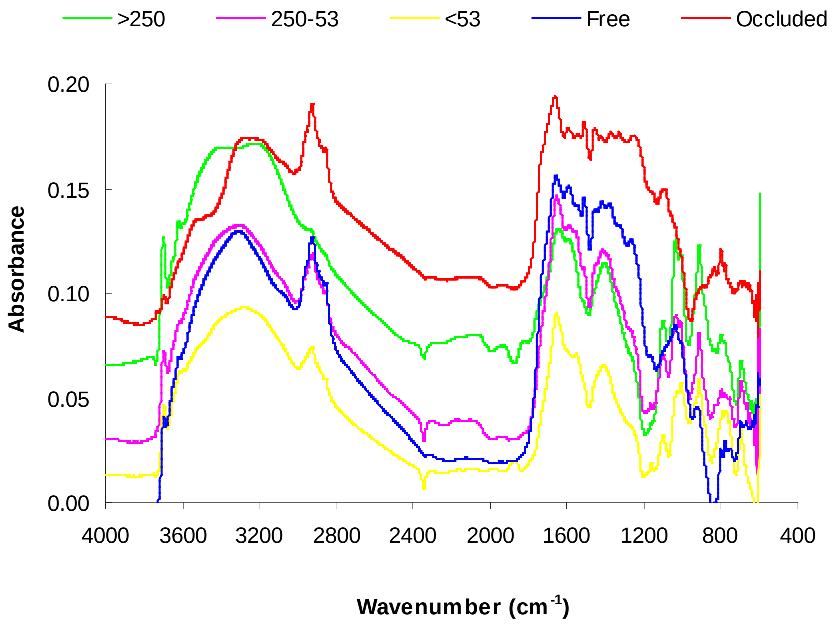

Figure 5.13. DRIFT spectra of intra-macroaggregate fractions for the first year of corn in the hay-hay-corn-corn-corn crop rotation in Field Experiment 3-corn in crop rotations.

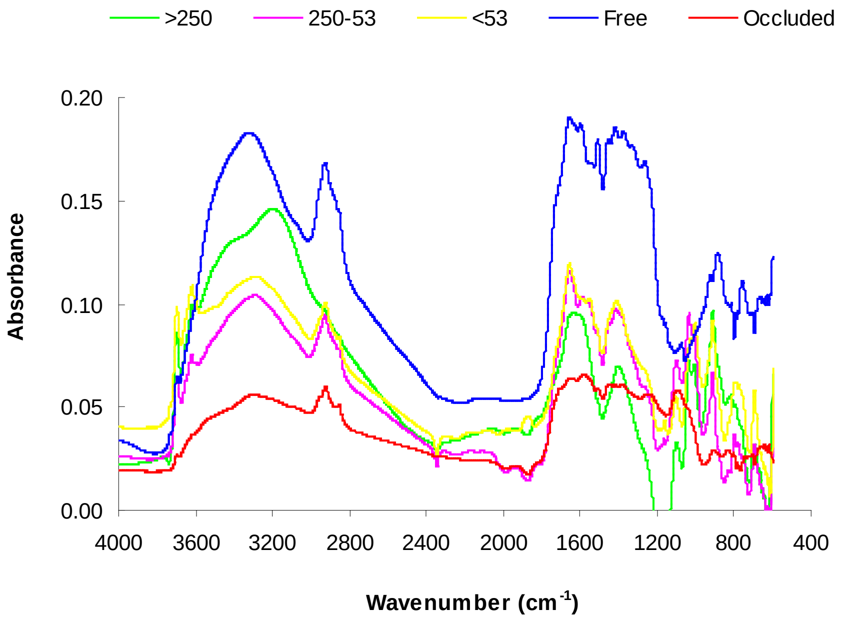

Figure 5.14. DRIFT spectra of intra-macroaggregate fractions for the corn in corn-wheat/doble crop soybean crop rotation in Field Experiment 3-corn in crop rotations.

Chapter 6

Conclusions

The original driving force for this work was an evaluation of the concept of soil C saturation, i.e., the maximal capacity of a soil to stabilize C (Six et al., 2002). However, after the initial results associated with SOM storage in these long-term field experiments were found, it was realized that an understanding of the reasons behind the different observed soil organic C concentrations in the several aggregate size fractions, and the functions of these "pools", first required more detailed characterization of the quantity, composition and stability of physically separated SOM fractions. As a result, that characterization became the main objective of this work and what follows are the overall results.

Management practices did affect SOM concentrations. However, these influences were not of equal concern to all parts of the soil system, because they differed with soil depth increment, and among the physically separated fractions. Overall, NT soil management was associated with greater surface SOM than was MP soil management. Nitrogen fertilization, manure application and the increased use of corn in the crop rotation also enhanced SOM accumulation. In addition, POM (>53 μm) was more enriched in SOM than was the <53 μm fraction, but the SOM storage capacity associated with each fraction was greatly impacted by management practices, because these practices impact their aggregate size distribution. Finally, the micro-aggregate-within-macroaggregates fraction (250-53 μm_{SM} or 250-53 μm_{LM}) was the most SOM enriched fraction among the intra-macroaggregate fractions, even though it did not respond consistently, across field experiments, to management practices.

Soil organic matter stabilization in physically separated fractions, as indicated by $\delta^{13}C$ natural abundance, was strongly affected by management. No-tillage, the corn-wheat-doublecrop soybean crop rotation and increased years of corn with the progression of time in the hay-hay-corn-corn-corn crop rotation enhanced the proportion of recently deposited C, and this was positively related to aggregate size, especially in Field Experiments 1 and 3. These results provide further evidence that persistence of SOM is closely related to aggregate size, and persistence is reduced with greater aggregate size. It is worth pointing out that most of the C recently deposited within macroaggregates was consistently associated with the 250-53 μm_{SM} or 250-53 μm_{LM} fraction, across field experiments, and only tillage and crop rotation treatments significantly affected this fraction.

The biological component involved in SOM stabilization is as important as the physical or chemical components, especially at the soil surface (0 to 5 cm soil depth increment). Soil microbial biomass concentrations and microbial biomass function, as indicated by phenol oxidase enzyme activity, were significantly affected by management practices. However, phenol oxidase enzyme activity was sensitive not only to N inputs but also to C inputs, and also to some physical and chemical properties such as soil pH and gravimetric water content. The potential use of this enzyme as a biological indicator of humification is questionable because of the agro-ecosystem specific response observed in this work. Further research is needed to evaluate the role that microbial community composition had on both phenol oxidase enzyme activity and general C stabilization in these agro-ecosystems.

Soil organic matter composition, as indicated by O/R ratios from the DRIFT spectra for physically separated fractions, was influenced by management treatments and this influence was not the same for all the intra-macroaggregate fractions evaluated. Overall, SOM reactivity responded to the interaction of either tillage or manure application with N fertilization in Field Experiments 1 and 2, respectively. Higher SOM reactivity was associated with continuous corn, corn-wheat-doublecrop soybean and the first year of corn in the hay-hay-corn-corn-corn rotations in Field Experiment 3. On the other hand, across management treatments, neither the lowest or highest degree of SOM reactivity was associated with any specific intra-macroaggregate fraction. However, the low to medium SOM reactivity was consistently associated with the 250-53 μm_{SM} or 250-53 μm_{LM} fraction, which also exhibited the most consistent peak assignment across both management treatments and field experiments. These results suggest that a more realistic approach to assessment of management effects on SOM should evaluate SOM content and composition, while considering the spatial arrangement of SOM.

In short, across these three field experiments, most of the recently deposited C has been stabilized in macroaggregates and, more specifically, in microaggregates within macroaggregates. This fraction consistently exhibited the highest total C concentration, the lowest mean age and a low to medium reactivity. These results indicated that SOM stabilization requires specificity in SOM composition and location within the soil matrix.

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