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IMPACT OF CROP AND RESIDUE MANAGEMENT ON THE PHYSICAL AND CHEMICAL STABILIZATION OF SOIL ORGANIC MATTER AT FARM LEVEL

by

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Advisers: Rhae Drijber and Daniel Olk

This dissertation explores changes over time in soil organic matter (SOM) stabilization of two irrigated production fields: a continuous maize sequence that was converted from no-till to conservation deep tillage (Site 1), and a no-till maize-soybean rotation (Site 2). An integrated approach using humic acid extractions and density-based physical fractionation of SOM within aggregate size classes was developed to evaluate the changes in SOM stabilization (physical protection, organo-mineral associations and humification). At Site 1, loss of SOM in the surface layer was compensated for by increased SOM in deeper soil layers with no net change in C stocks. Whole field C-stocks in the 0-400 kg soil m^{-2} layer of Site 1 were 7.09 ± 0.29, 7.47 ± 0.36, 7.15 ± 0.37 and 7.18 ± 0.41 kg C m^{-2} in fall of 2005, 2006, 2007 and 2008, respectively. This outcome is encouraging, given the challenge of stabilizing aboveground residues into the mineral matrix without increasing tillage-induced C losses from native SOM and residues due to enhanced decomposition. At Site 2, there was an increase in C and N stocks in surface soil after the second maize year (2007). Whole field C stocks in the 0-400 kg soil m^{-2} layer of Site 2 were 6.42 ± 0.30, 6.18 ± 0.32, 6.81 ± 0.26 and 6.72 ± 0.29 kg C m^{-2} in fall of 2005, 2006, 2007 and 2008, respectively. The changes in soil ^{13}C signature and monosaccharide composition, together with changes in SOM fraction amount support a large input of soybean residues as key to promote stabilization of standing maize litter through physical protection and humification. Preferential SOM accumulation in the largest, most C-enriched aggregates was associated with less humified SOM fractions while similar accumulation rates of more humified fractions were found among aggregate classes. These findings support stabilization of crop residues as SOM under high yield maize and soybean management at field scale.

EVALUACION DEL MANEJO DEL CULTIVO EN CONDICIONES DE CAMPO SOBRE LA ESTABILIZACION FISICA Y QUIMICA DE LA MATERIA ORGANICA DEL SUELO

Ana Beatríz Wingeyer, Ph.D.

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Asesores: Rhae Drijber y Daniel Olk

Esta disertación evalúa la variación en el tiempo de la materia orgánica (MO) del suelo en dos sistemas de cultivo bajo riego: rotación de maíz continuo bajo siembra directa convertido a labranza conservacionista profunda (Sitio 1), y rotación maíz-soja bajo siembra directa (Sitio 2). Se desarrolló un método combinado de extracción de fracciones físicas (fracciones livianas-FL) y químicas (ácidos húmicos-AH) de la MO dentro de agregados estables para evaluar los diferentes mecanismos de estabilización. En el Sitio 1, la pérdida de MO en la superficie (desestabilización de agregados, mezcla de capas de suelo) fue compensada por su incremento en capas más profundas (mezcla de capas de suelo, encapsulación y humificación de los residuos) resultando en similares reservas de carbono (C) después de tres años del cambio en la labranza. Las reservas de C en la capa 0-400 kg suelo m^{-2} fueron 7.1 ± 0.3, 7.5 ± 0.4, 7.2 ± 0.4 y 7.2 ± 0.4 kg C m^{-2} en 2005, 2006, 2007 y 2008, respectivamente. En la rotación maíz-soja (Sitio 2), se registró un incremento de MO en las capas superficiales luego del segundo ciclo de maíz. Las reservas de C en la capa 0-400 kg suelo m^{-2} en el Sitio 2 fueron 6.4 ± 0.3, 6.2 ± 0.3, 6.8 ± 0.3 y 6.7 ± 0.3 kg C m^{-2} en 2005, 2006, 2007 and 2008, respectivamente. La importancia de los residuos de soja para la estabilización del rastrojo de maíz remanente fue demostrada mediante análisis de ^{13}C y perfil de monosacáridos del suelo, y cantidad de las distintas fracciones de la MO (FMO). Como segundo objetivo se evaluó la acumulación de las FMO por tamaño de agregados. En comparación a los agregados de 250-53 µm, los agregados >250 µm presentaron mayores tasas de acumulación de AH menos condensados así como de FL. Los resultados obtenidos respaldan el manejo para altos rendimientos del cultivo de maíz y soja como así también de los rastrojos para incrementar las reservad de MO del suelo.

To My Family, Ana and Raúl, Eve, Mina,
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Ad Astra per aspera

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CHAPTER 1. INTRODUCTION

1. Literature Review

The productivity and environmental buffering capacity of soils are directly and indirectly influenced by the amount and quality of soil organic matter (SOM) (Magdoff and Weil 2004). Cultivation of land for agricultural purposes has resulted in exposure of SOM to enhanced decomposition and erosion. Estimated losses of SOM due to cultivation range from 10 to 50 % of the original soil organic carbon (SOC) content (Davidson and Ackerman 1993, Paustian et al. 2000, Guo and Gifford 2002) which accounts for 50 to 60 Pg C worldwide (Paustian et al. 1997, Lal 2003). Using agricultural ecosystems as a sink for atmospheric CO_2 to mitigate the greenhouse effect has attracted attention from both scientific and political spheres. Changes in agricultural management practices that increase the amount of carbon (C) inputs and/or reduce the amount of C outputs can potentially accomplish the restoration or even enrichment of native SOC levels, (Ismail et al. 1994, Studdert et al. 1997, Follett et al. 1997, Studdert and Echeverria 2000).

Incorporation of atmospheric CO_2 into soil requires two intermediate processes: fixation of CO_2 by plants and incorporation of the plant tissues into SOM. Gross primary productivity of an ecosystem constitutes the total amount of C fixed through photosynthesis. Irrigated maize fields in the Midwest can fix 16 to 18 Mg C ha^{-1} $year^{-1}$ (gross primary productivity), approximately 80% more C than soybean crops (Verma et al. 2005). Given a fraction of the fixed C is returned to the atmosphere by plant respiration, the remaining C constitutes the net primary production of the ecosystem.

When respiration from both soil autotrophic and heterotropic organisms is considered, the remaining fixed C constitutes the net ecosystem productivity (NEP). This NEP represents the potentially available C for sequestration into SOM. Under agricultural systems only a portion of NEP will enter the soil system as C inputs (roots and litter), as a fraction will be harvested for off-field use. Given that belowground C (roots, root exudates) is already in the soil, it is the "effective" inclusion of aboveground biomass that will determine the magnitude of C inputs.

Effective incorporation of aboveground crop residue inputs: effect of tillage.

Tillage practices control the relative importance of direct physical incorporation as compared to movement of particulate, colloidal and/or soluble residue derived C into soil (Figure 1.1). Under no-till systems, residue-derived C moves mainly through colloidal and soluble forms, enhanced through soil faunal activity. The reduced soil-crop residue interface under no-till restricts the volume of soil in which residue-derived C moves (Coppens et al. 2006 a,b, Murage and Voroney 2008), which leads to stratification of SOM in the soil profile (Poirier et al. 2009) and also restricts the amount of crop residue that can be stabilized as SOM (Stemmer et al. 1999). Tillage practices facilitate the physical incorporation of residues and mixing of soil layers. Burial of the residues leads to an increase in the soil-residue interface and in the volume of soil for stabilization of colloidal and soluble residue-derived C (Angers et al. 1995, Wander et al. 1998, Murage and Voroney 2008), resulting in a more homogeneous C concentration throughout the entire tilled depth (Poirier et al. 2009).

Tillage practices also modify the timing and magnitude of C losses. At the same time residues are incorporated, tillage also stimulates the exposure of SOM to oxidation and decomposition and breakdown of soil aggregates (Six et al. 1999). These processes lead to a transient pulse of CO_2 efflux from soil (Hendrix et al. 1988). Nevertheless, no-till management systems may result in larger total C losses due to soil respiration compared to tilled soils (Hendrix et al. 1988). The aerobic yet moist conditions that can typify standing litter on a no-till soil surface may promote oxidation and decomposition of the residues compared to more anaerobic conditions deeper in the soil profile where aboveground residues are incorporated through tillage. Verma et al. (2005) reported that an increase in C losses by enhanced respiration under irrigated no-till systems offset the advantage of irrigation on gross primary productivity. The authors indicated that irrigated no-till continuous maize sequence was a C-neutral to slight C-source system, while irrigated no-till maize-soybean rotation was a moderate source of C.

Current management practices for increasing SOM content focus on increasing C inputs (increasing crop productivity, use of manure and cover crops) and reducing exposure of SOM to decomposition and oxidation (e.g. no-till). Selection of the optimal tillage management for promoting C sequestration under high yield maize systems constitutes a dilemma. Is it better to incorporate the high volume of residue C into soil while enhancing oxidation and decomposition of SOM, or to leave them on the soil surface potentially leading to greater C loss as soil respiration? Although soil residue cover has a beneficial effect on reducing wind and water erosion, the high volume of residues can make planting and crop emergence a challenge due to increased soil moisture and cooler soil conditions in the spring. Use of no-till limits the use of in-season split applications of N fertilizer (recommended best management practice) by knife injection, which better reduce N losses by volatilization compared to fertigation.

Based on anecdotal experiences (Reetz, 2000), a different approach to residue management was tested from 1999-2009 in experimental plots of the Ecological Intensification Project at the University of Nebraska-Lincoln. Fall conservation deep tillage together with N fertilizer was used to enhance the stabilization of crop residues into SOM deeper in the soil profile. Promising results were obtained under high yielding continuous maize systems after six years: soil C and N stocks in the 0-300 kg soil m^{-2} layer increased by 3.1 and 0.35 Mg ha^{-1}, respectively (Walters et al. 2009). Angers et al. (1995) suggested that environmental conditions following fall tillage may play an important role in favoring humification of crop residues without increasing native SOM losses.

Stabilization mechanisms of soil organic matter

Decomposition of crop residues is a necessary step for soil organic matter formation and stabilization as well as for recycling of organically bound nutrients (Guggenberger 2005). Decomposition is the overall process that leads to the transformation of organic materials into increasingly stable forms that can adsorb onto mineral surfaces or be incorporated into aggregates. Decomposition includes physical (fragmentation, dissolution and leaching), chemical (oxidation and condensation) and biological alteration (Berg and McClaugherty 2008) of residues. Microbially mediated reactions progressively derive energy and nutrients from organic residues while transforming them into various products (Gregorich 2000). Humification, formation of organo-mineral complexes, and incorporation into aggregates constitute distinct SOM stabilization mechanisms that reduce the bioavailability of SOM for microbial decomposition and lead to its accumulation in soil (Figure 1.2).

The process of humification is not wholly understood but it is characterized by the accumulation of products formed by both chemical and microbially mediated transformations of organic materials. Nitrogen enrichment and condensation of modified lignins, quinones and reducing sugars to form complex dark colored polymers constitute early theories of humus formation (Stevenson 1994). These transformations may lead to the formation of recalcitrant compounds not easily degradable by soil microorganisms. Accumulation of humic substances on decomposing residues may act as chemical barriers to the decomposing microorganisms or their enzymes, thus preventing further decomposition of readily available substrates (Mindermann 1968).

Organo-mineral associations are mainly the result of adsorption of organic compounds to clay surfaces (Sollins et al. 1996). Clays are mineral soil particles having a diameter less than 2 μm, with higher specific surface area than silt or sand particles and positive or negative charge. The adsorption of SOM by clay minerals can be due to several bonding mechanisms: van der Waals' forces, cation bridging, ligand exchange, hydrogen bonding, and adsorption by association with hydrous oxides (Sparks 2003). Van der Waals' forces result in weak adsorption but are important for the absorption of organic acids, long chain hydrocarbons, waxes and microbial cell walls. Bonding by polyvalent cation bridges is key for the adsorption of organic anions such as humic and fulvic acids to negatively charged clays. In this bonding type, the divalent Ca^{2+} and Mg^{2+} ions act as a bridge between the clay and the humic material.

Soil particles in soil do not stand in isolation, but they organize into aggregates. Soil aggregates can be considered as niches of different size, with varying resistance to mechanical breakdown and thus turnover. Microaggregates (<53 µm aggregates – silt and clay aggregates) are associated with more persistent SOM, small macroaggregates (53-250 µm) are associated with more transient SOM, and macroaggregates (>250 µm) are associated with more labile SOM (Gregorich et al. 1989, Besnard et al. 1996, Puget et al. 2000). This classification is operational, since the aggregate size classes are defined by the size of the sieves used, and larger aggregates contain varying proportions of smaller aggregates. A size classification of aggregates introduces a functional perspective by grouping the aggregates based on the size and volume of pores they contain or create with adjacent aggregates. Aggregation contributes to SOM stabilization by preventing the decomposition of newly incorporated C due to its occlusion into soil pores (Cambardella and Elliot 1994, Jastrow et al. 1996, Six et al. 1998, Gale et al. 2000, Kong et al. 2005), thus creating physical barriers between SOM and soil microorganisms.

Each one of these mechanisms; humification, association into organo-mineral complexes and incorporation into aggregates, theoretically defines a functional SOM pool (Kögel-Knabner et al. 2008a). In practice, it is not possible to isolate a uniquely functional SOM pool, because the protection mechanisms act together within the soil matrix (Kögel-Knabner et al. 2008b). This complexity leads to the stabilization mechanisms presented in Figure 1.2. Humification constitutes a continuum of organic compounds in different stages of decomposition and re-synthesis that present different degrees of condensation and recalcitrance resulting in different solubility, reactivity and degree of association with clay minerals. Moreover, decomposition and humification occur in a three-dimensional soil matrix that modifies aeration, water availability, and pore connectivity: 1) located within and between aggregates of different size, 2) located at different position inside soil profile, and 3) located at varying distances from C inputs. As a result, several niches for preservation of readily available substrates exist (Figure 1.2).

Methodological approaches for assessing soil organic matter stabilization mechanisms

Operationally defined SOM pools summarize the effects of several stabilization mechanisms acting together, and can be used as indicators of soil processes (Olk and Gregorich 2006). Comprehensive reviews exist for SOM fractionation techniques involving physical fractionations (Christensen 2001, Gregorich et al. 2006), chemical fractionations (Olk 2006, Paul et al. 2006) and physical and chemical fractionations (von Lutzow et al. 2007). A synthesis of the methodologies for SOM fractionation and the resulting SOM pools and characteristics is presented in Table 1.1 (Adapted from Olk and Gregorich 2006 and von Lutzow et al. 2007).

A combination of base extractions for humic acid fractions together with extractions for physically uncomplexed SOM fractions can be used to trace the progress of humification as crop residues are transformed into SOM. Physically uncomplexed SOM represents a transitory pool between litter and mineral associated SOM (Table 1.1). One form of uncomplexed SOM is recovered by floating material in a solution of density between 1.4 to 2 g cm^{-3} and is called the light fraction (LF). LF can be termed “free-“, “occluded-“ or “total-LF” depending on the degree of disruption of soil aggregates

(Christensen 2001). Free LF is extracted with a minimum dispersion of soil aggregates and represents a less decomposed fraction consisting mainly of plant litter, while occluded LF is released after dispersion of aggregates and is more decomposed (Golchin et al. 1994, Gregorich et al. 1997). The contribution of LF to the total soil C in the arable horizon is sensitive to the frequency and intensity of soil disruption (Gregorich et al. 1997, Six et al. 1998) and the quantity and quality of crop residue inputs (Legorreta-Padilla 2005).

Extraction of the mobile humic acid (MHA) and calcium-bound humic acid (CaHA) fractions can provide insight into more humified SOM fractions. Their extraction is based on solubility in alkali and cationic binding: they are distinguished by extraction before and after removal of soil calcium by HCl. The MHA constitutes a younger, less condensed and less aromatic material that is richer in N compared to the CaHA (Mahieu et al. 2002, Ve et al. 2004, Olk et al. 1996; Legorreta-Padilla 2005; Wingeyer and Walters 2008). The magnitude of seasonal change and long-term variation in the amount of MHA were related to the quality and quantity of residue inputs (Legorreta-Padilla 2005). Low inputs of N fertilizer and inclusion of soybean in the crop rotation significantly reduced the amount of MHA-C and -N, while high C inputs under continuous maize and adjusted N fertilizer doses favored the buildup of the MHA fraction (Legorreta-Padilla 2005). Measurements of ^{14}C activity of the MHA and CaHA fractions before and after soil incubation showed that the recently incorporated C in both fractions was preferentially mineralized (Wingeyer and Walters 2008).

Integrated approaches that use combined physical and chemical fractionations and account for the location of the SOM fractions within soil structure (e.g. within aggregate size classes) can provide valuable information regarding how crop and soil management practices modify the stabilization of SOM. Studies of SOM fractions within aggregate size classes are related to physical fractionation by density, particle size separations, or both (Besnard et al. 1996, Puget et al. 1995, 2000, John et al. 2005, Six et al. 1998, 2001, Yamashita et al. 2006, Helfrich et al. 2006).

Analytical tools for the study of soil organic matter stabilization mechanisms

In addition to using an integrated SOM fractionation scheme, several analytical techniques can aid in the interpretation of stabilization mechanisms and SOM dynamics: elemental composition analysis, stable isotope ($\delta^{13}C$) contents, optical properties of humic acid fractions, monosaccharide composition, and microscopy of free and occluded LF.

The C:N ratio can be used as indicator of the quality of C inputs and also as an indicator of the degree of decomposition or humification of the SOM fractions (Gregorich et al. 1997, Bending and Turner 1999, Johnson et al. 2007). The photosynthetic pathways of C3 and C4 plant species discriminate against the heavier ^{13}C isotope to different extents. This difference allows tracing the C incorporation from distinct labeled plant residues (e.g., maize and soybean) into SOM by measuring the ^{13}C enrichment, which is expressed as $\delta^{13}C‰$ (Balesdent et al. 1987). The $\delta^{13}C$ signature of soil aggregates after a change from C3 (native grass) to C4 (maize) species indicated a preferential incorporation of new C inputs from crop residues into macroaggregates (i.e. >250 µm) (Jastrow et al. 1996, Angers and Giroux 1996, John et al. 2005). Measurement of the δ^{13}C‰ of LF, MHA, and CaHA during the crop growing season showed preferential incorporation of new C into LF > MHA > CaHA (Legorreta-Padilla 2005).

Molar absorptivity at 465 nm (E4) and 665 nm (E6) and their ratio have been used as indicators of the molecular weight, degree of humification and chemical recalcitrance of humic materials to microbial decomposition (Chen et al. 1977, Zech et al. 1997, Olk et al. 1999, Mahieu et al. 2002, Ve et al. 2004). The positive correlation of E4 values with degree of humification (Olk et al. 1996, 1999, Mahieu et al. 2002) can track the incorporation of maize residues into humic fractions through lowering the E4 value. Ve et al. (2004) and Olk et al. (1996) indicated greater sensitivity of E4 values to residue management than the E4/E6 ratios.

Monosaccharide abundance in the soil profile can be used as an indicator of incorporation of C inputs and decomposition activity. Given the distinct monosaccharide compositions of plants and microorganism, changes in the total and relative amount of monosaccharides can be used as an indicator of the extent of residue decomposition (Murayama 1984). The morphology of free and occluded LF was used by Golchin et al. (1994) and Besnard et al. (1996) to reinforce the nature and extent of decomposition of these two fractions.

2. Research proposal and dissertation outline

Effective C sequestration from the atmosphere occurs when C inputs are stabilized into protected niches. Crop and crop residue management practices modify the quantity, quality, frequency, and placement of C inputs, as well as the timing and amount of C outputs. Thus, accumulation of SOC depends on the ability to increase the stability of highly labile C inputs, either by protecting them from decomposition and/or enhancing their chemical recalcitrance to decomposition via humification. To evaluate the impact of field-scale high production management strategies on SOM sequestration mechanisms over time provides a unique opportunity for advancing the understanding of SOM stabilization. It also constitutes a valuable precedent for more integrated assessment of management practices.

The studies presented in this dissertation were part of a larger study, the UNL Carbon Sequestration Program. Soil C stabilization mechanisms at two irrigated production-agriculture fields: the irrigated continuous maize sequence (Site 1) and the irrigated maize-soybean rotation (Site 2) were studied. Soil samples were collected during four years (2005-2008) from both fields for laboratory determination of changes in SOM stabilization mechanisms over time. Both fields were under no-till management from the beginning of the program in 2001 until 2005. In fall 2005, management at Site 1 was changed after the maize harvest: the no-till system was replaced by conservation deep tillage to incorporate maize residues together with N fertilizer. Site 2 was maintained under no-till management.

Objectives and hypotheses

The main objective of this dissertation was to contribute to the understanding of soil C stabilization mechanisms at two irrigated production-agriculture fields of the UNL Carbon Sequestration Program. To accomplish this objective, the following two sets of hypotheses were developed regarding the impact of ongoing crop and soil management strategies on SOM stabilization mechanisms:

A) Incorporation of maize residues plus N fertilizer with fall conservation deep tillage in Site 1 will enhance stabilization of maize residues as SOM compared to previous no-till management.

i. The change in management at Site 1 will expose recently protected C inputs in the upper soil layer to enhanced decomposition.

ii. The balance between enhanced respiration due to exposure of SOM by breakdown of aggregates and the protection of new C inputs by physical occlusion and humification will be negative in the upper soil layer, resulting in net C loss.

iii. The SOM content of deeper soil layers will increase over time:

- An initial increase in SOM in deeper soil layers due to mixing of soil layers.
- Subsequent increases in SOM of deeper soil layers will result from a positive balance between exposure of SOM by breakdown of aggregates and the protection of new C inputs by physical occlusion and humification.

B) Differences in the quantity and quality of maize and soybean crop residues will affect the dynamics of SOM stabilization mechanisms at Site 2.

i. The dynamics of stabilization mechanisms in the surface soil layer will reflect the differences in quantity and quality of aboveground maize and soybean residues placed on the soil surface.

ii. In deeper soil layers, the dynamics of physical and chemical stabilization mechanisms will reflect any differences in quantity and quality of root tissue between maize and soybean.

A second objective of this dissertation was to contribute to the understanding of SOM stabilization in relation to soil structure by exploring the accumulation of SOM fractions by aggregate size classes. Using an integrated SOM fractionation approach involving aggregate size fractionation and physical and chemical extractions of SOM, five operationally defined SOM fractions representing a range in the degree of association with the soil mineral matrix were studied for two aggregate size classes. The specific hypotheses were:

i. As aggregate size increases, the amount of SOM stabilized by physical protection will increase.

ii. As aggregate C concentration increases within an aggregate size class:

   - Physically protected SOM will exhibit a non-saturation behavior.
   - Organo-mineral associated SOM will exhibit a saturation behavior.

Outline

This dissertation explores different SOM stabilization mechanisms together with the concept of effective incorporation of aboveground crop residues for stabilization as SOM (Figure 1.1 and 1.2). An integrated approach using base extractions and density-based physical fractionation of SOM was developed to evaluate the hypothesized changes in SOM stabilization mechanisms with regard to soil structure (Figure 1.3). This approach was then used to investigate the magnitude of different stabilization mechanisms in relation to crop rotation and tillage management. Chapter 2 presents the impact of change in tillage management at Site 1 on SOM stabilization mechanisms at three soil depths. Chapter 3 presents a more detailed analysis of the change in tillage management at Site 1 and its effects on SOM stabilization mechanisms in relation to soil structure. Chapter 4 investigates the effects of varying the amount and composition of crop residue inputs at Site 2 on the relative significance of SOM stabilization mechanisms within the soil profile. Chapter 5 presents a more detailed exploration of this same topic now in relation to soil structure.

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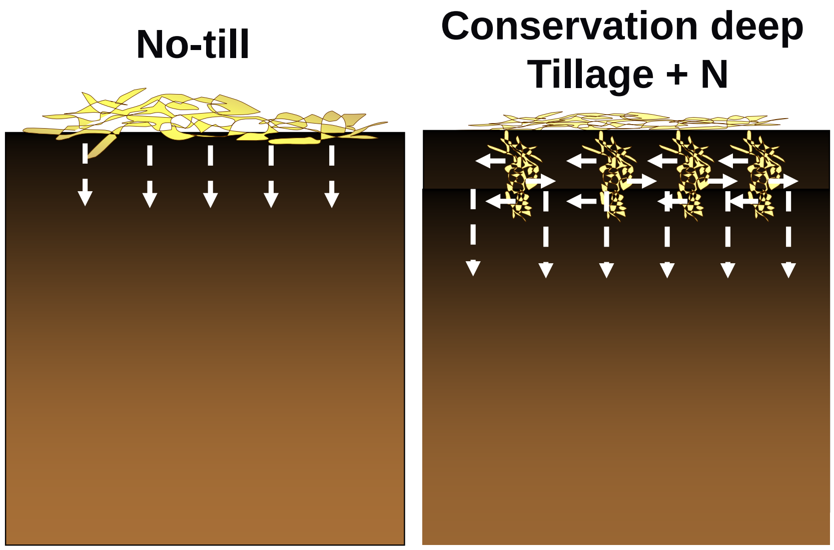

Decrease of C:N ratio	**Limited by N in residue - Soil**	**Facilitated**
Soil-residue interface	**Restricted to soil surface**	**Facilitated to deeper layers**
Movement of colloidal and soluble C into soil (arrows)	**Restricted to few first inches**	**Facilitated to deeper layers**

Figure 1. 1. Conceptual framework for the effect of tillage on the relative importance of direct physical incorporation as compared to movement of particulate, colloidal and/or soluble residue derived C into soil for SOM stabilization.

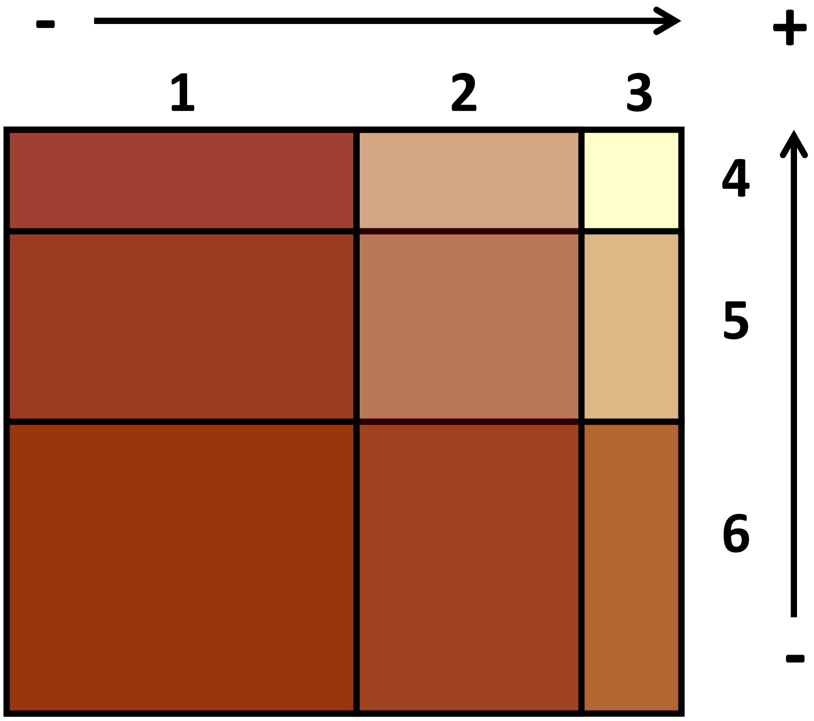

Figure 1. 2. Conceptual framework for the assessment of soil organic carbon (SOC) bioavailability for microbial decomposition in soil. Arrows indicate the direction of increase in bioavailability. Accessibility: 1 to 3, 1 being the less accessible material due to its location inside stable-small-pores and/or strongly associated to minerals, 2 is somewhat accessible material due to its location inside medium to large pores (tortuosity may limit the accessibility) and/or less stable organo-mineral association, and 3 is accessible material (not protected SOC or exposed SOC in the outside of aggregates or soil channels). Degradability: 4 to 6, 4 being the less decomposed plant residues (more degradable C source), 5 represents somewhat decomposed plant material (comprises a wide range of degradability, but the C compounds are more condensed than the original plant-microorganism material), and 6 constitutes the least degradable C due to the highest recalcitrance and condensation of C compounds. Thus the combination 1 and 6 represents the least bioavailable SOC, while the combination 3 and 4 represents the most bioavailable SOC for microbial decomposition.

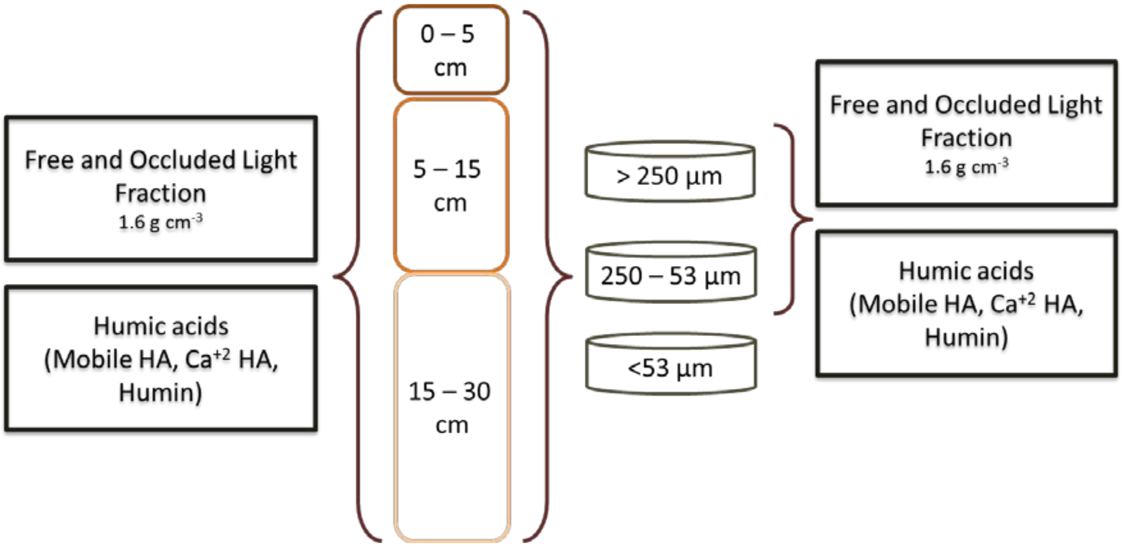

Figure 1. 3. Integrated SOM fractionation approach for assessing changes in SOM stabilization mechanisms with regard to soil structure.

Table 1. 1. Methodologies for the study of SOM stabilization mechanisms (adapted from Olk and Gregorich 2006 and von Lutzow et al. 2007).

Methodology	SOM pools	Characteristics of the fraction	Limitations in the use and interpretation
Physical fractionations			
Physical Uncomplexed (Gregorich et al. 2006, Christensen 2001)	Uncomplexed SOM – Light fraction SOM: Free Occluded (inside aggregates)	Relatively unaltered plant material with wide C:N ratio. Its a labile pool. Readily available source of energy. Does not usually represent labile soil organic N.	The material is fragmented by sample preparation. The quantity recovered depends on the amount of energy used (sonication) or the solution density.
Primary Particles (Christensen 2001)	Sand associated SOM Silt associated SOM Clay associated SOM	SOM is isolated into associated soil particle sizes: Clay, Silt and Sand. Chemical composition of SOM differs with the associated particle size: Clay size = microbial products, Silt size = plant-derived aromatics, Sandy size = plant polymers.	Achieve a complete disruption of aggregates, maintain the particles dispersed, and avoid the transfer of material among fractions are difficult. Deviations from spherical shape compromise the assumption for Stokes' Law.
Aggregates (Christensen 2001)	Intra-aggregate SOM Inter-aggregate SOM Micro-aggregate SOM Small-macroaggregate SOM Large-macroaggregate SOM	Aggregates represent an assemblage of mineral-associated and uncomplexed organic matter together with microorganisms and fine roots. Micro and Macro aggregates have different binding mechanisms and turnover. Elucidation of aggregate formation process (Six et al. 1998). Has been used to understand C dynamics and mineralization.	There is no standard methodology. The fractionation affects the biological properties to be measured. It is not clear its usefulness for study of N dynamics.

Chemical fractionations			
Acid extractions	Hydrolyzable N Non Hydrolizable N Non identifiable N	Strong acids are used to solubilize SOM compounds (HCl, H_2SO_4, HF). It extracts relatively younger C materials and N as amino acid forms (Paul et al. 2006)	Recovers only half of all organic N, and the extracted N forms can suffer modifications. It has little value for determining soil active N. It is not useful to distinguish C compounds by their decomposition susceptibility.
Base extractions (Stevenson 1994, Olk 2006)	Humic acid: Mobile Humic acid (MHA) Polyvalent bound humic acid (CaHA) Fulvic acid Humin	NaOH has been the most used base to solubilize SOM compounds. SOM might not have a regular chemical structure, but an irregular pattern of component molecules and chemical bonds that enhance the resistance of SOM to microbial degradation (MacCarthy 2001, Hayes 2006). MHA represents a young humic fraction and an active soil N pool. Proposed humification pathway MHA<CaHA<Humin (Legorreta-Padilla 2005)	It does not consider the spatial distribution of SOM in soil (no distinction between physically inaccessible from fully accessible materials). The material can be mixed between fractions, and cleaved into subunits or re-polymerized in new compounds. The material can be exposed to oxidation.

CHAPTER 2. FROM NO-TILL TO FALL CONSERVATION DEEP TILLAGE: MPACT ON FIELD SOIL C AND N STOCKS AND SOM FRACTIONS.

1. Abstract

Increasing the soil organic matter (SOM) content under agricultural settings represents a win-win situation. Efforts to date have focused on management practices that reduce exposure of SOM to decomposition via minimum tillage and promotion of soil aggregation. We assess the effect of an alternative approach, termed ‘conservation deep tillage’, on stabilizing SOM through fall incorporation of crop residues into the soil profile together with N fertilizer. Soil C and N stocks on an equal soil mass basis from the 0-30 cm depth were measured in the final no-till year and after one, two and three years of conservation deep tillage under irrigated continuous maize in eastern Nebraska. Two humic acid fractions (mobile humic acid (MHA) and calcium humates (CaHA)) and two uncomplexed organic matter fractions (free light fraction (fLF) and occluded LF) were extracted and analyzed for C and N content. No significant changes in soil C and N stocks in the 30 cm soil layer were observed after three years of tillage, either by sampling location or field weighted means adjusted for soil type abundance. Conservation deep tillage led to the redistribution of total soil-C and -N stocks and soil organic matter fractions in the soil profile. An increase in the soil−crop residue interface by conservation deep tillage favored the persistence of fLF deeper into the soil profile. Change from no-till to conservation deep tillage led to the reduction of C and N stocks as MHA and CaHA fractions after one year of tillage, suggesting enhanced decomposition and/or condensation. By the second and third years of conservation deep tillage, incorporation of crop residues into the soil profile together with fertilizer N promoted stabilization of crop residues into humus. These field-scale results are similar to those obtained in prior plot-scale experiments from the same region. The trends observed after three years of fall conservation deep tillage suggest that a positive balance of soil C and N accrual and losses may be achieved by enhancing the soil-residue interface in these soils.

Key words: humification, C sequestration, SOM stabilization

2. Introduction

The productivity and environmental buffering capacity of soils are directly and indirectly influenced by the amount and quality of soil organic matter (SOM) (Magdoff and Weil 2004). Agricultural production in the Great Plains over the last century led to a decline in SOM (Reeves 1997, Guo and Gifford 2002). The potential to restore SOM content in agricultural land at the expense of atmospheric CO_2 enrichment represents a win-win situation for soil quality and environmental improvement and for mitigation of greenhouse warming potential (Robertson and Swinton 2005). Crop management practices (i.e. planting date, population density, rotation, irrigation, fertilizers, and pest and weed control) that increase crop productivity also promote an increase in crop residues (i.e. C inputs) that can lead to increased SOM content (Studdert and Echeverría 2000, Halvorson et al. 2002, Allmaras et al. 2004, Huggins et al. 2007).

Irrigated maize fields in the Midwest can fix 16 to 18 Mg C ha^{-1} $year^{-1}$ (gross primary productivity) and thus have large potential for soil C sequestration (Verma et al.

2005). Estimations of the net ecosystem productivity (NEP) of maize-based systems indicated that despite enhanced gross primary productivity, irrigated no-till maize systems had larger respiration losses due to residue decomposition than did rainfed maize systems, which led to similar seasonal maize NEP (Verma et al. 2005). After accounting for grain harvest, net biome productivity of irrigated continuous maize during the first three years of the experiment was neutral, meaning no remaining C was available to build up SOM (Verma et al. 2005). This finding was confirmed by independent measurements of soil C stocks. Nevertheless, increased soil C and N stocks have been reported for high yielding irrigated continuous maize under intensive management (MM-Int) in deep Mollisols of the Midwest (Walters et al. 2009). Management of the high volume of maize residues following harvest at the Ecological Intensification Project was achieved with a modified moldboard plow that allowed for 30% residue cover after incorporation of residues along with 50 kg N ha^{-1}. The changes in soil C and N stocks in the MM-Int system after six years were +3.1 and +0.35 Mg ha^{-1} in the 0-30 cm depth, respectively (Walters et al. 2009). Effective incorporation of aboveground residues into the mineral matrix (i.e. as stable SOM) without increasing C losses from native SOM and residues due to enhanced decomposition constitutes the challenge to increasing SOM in managed ecosystems. Angers et al. (1995) suggested that environmental conditions following fall tillage may play an important role in favoring creation of SOM from crop residues, or “humification”, without increasing native SOM losses under plow tillage.

Decomposition of crop residues is a necessary step for soil organic matter formation and stabilization (Guggenberger 2005). Microbially mediated reactions progressively derive energy and nutrients from organic residues while transforming them into various products (Gregorich and Janzen 2000) that can react with mineral surfaces or be incorporated into aggregates (von Luetzow et al. 2006). Management of crop residue decomposition (i.e. by modifying residue placement, particle size, amount and composition) may shift the process toward formation of more stable SOM pools (Kogel-Knabner 2002). Yang and Jenssen (2002) proposed that the larger the initial reactivity of the crop residue is, (i.e. its potential mineralization), the faster its decomposition products will age, leading to greater persistence of the decomposition products in soil (Smith et al. 2007). Residues with reduced N content, and thus having a larger C/N ratio, have reduced C mineralization rates (Johnson et al. 2007). Mixing of high C:N ratio residues into soil reduces the availability of mineral N (Coppens et al. 2006, Wingeyer 2007) through immobilization into associated microbial biomass (Recous et al. 1995) suggesting N availability as a limiting factor during the initial stages of decomposition (Recous et al. 1995, Mary et al. 1996). Moderate additions of mineral N to the soil enhanced the reactivity of the high C/N ratio residues by relieving the N limitation (Recous et al. 1995). Management of high crop residue inputs for C sequestration will require synchronous N availability to increase residue reactivity (Yang and Jenssen 2002). Greater accessibility to soil N when residues are mixed with the soil compared to surface mulching may stimulate growth of microbial biomass and support greater transfer of residue derived C and N to more protected soil fractions (Stemmer et al. 1999) .

The relative importance of direct physical incorporation as compared to movement of particulate, colloidal and/or soluble residue derived C into soil is largely controlled by tillage practices (Stemmer et al. 1999, Coppens et al. 2006). Under no till systems, residue derived-C moves mainly through colloidal and soluble forms, enhanced through soil faunal activity. The reduced soil-crop residue interface under no-till restricts the volume of soil for movement of residue derived-C (Coppens et al. 2006, b, Murage and Voroney 2008) that leads to stratification of SOM in the soil profile (Blanco-Canqui and Lal 2008, Poirier et al. 2009). Although increases in more stable humified fractions in the surface layer of no-till soils have been reported (Murage and Voroney 2008), the preferential sequestration of labile SOM in surface layers of no-till soil (Olchin et al. 2008, Yoo and Wander 2008) suggests C saturation of the mineral surfaces (Hassink et al. 1997).

Tillage practices facilitate the physical incorporation of residues and mixing of soil layers. This leads to an increase in the soil-residue interface and in the volume of soil for stabilization of colloidal and soluble residue derived C (Angers et al. 1995, Wander et al. 1998, Stemmer et al. 1999, Rasse et al. 2006, Murage and Voroney 2008, Olchin et al. 2008) resulting in a more homogeneous C concentration throughout the entire tilled depth (Poirier et al. 2009). At the same time residues are incorporated, exposure of SOM to oxidation and decomposition and breakdown of aggregates are stimulated by tillage (Six et al. 1999). Incorporation of corn residues deeper into soil profile (i.e. mixing residues in the 0-30 cm soil layer) has been reported to increase the stabilization per unit C input of residue derived C compared to shallower incorporation (Olchin et al. 2008). Increased stabilization of the newly added C as humic fractions (Spaccini et al. 2000, Murage and Voroney 2008) and physically protected SOM (Wander and Bidart 2000, Wander and Yang 2000, Ding 2002) may explain the high retention of corn derived C with depth under tillage in all aggregate fractions reported by Olchin et al. (2008), and it may counterbalance the negative effects of tillage on potential C sequestration due to aggregate disruption (Six et al. 1999).

The primary objective of this study was to test the hypothesis that deep incorporation of maize residues plus fertilizer N using fall conservation deep tillage will expose more maize residues to physical and chemical stabilization mechanisms leading to increased SOM accumulation compared to previous no-till management. Although it was anticipated that this management will expose protected SOM in the upper soil layer to enhanced decomposition due to breakdown of aggregates, the SOM content of deeper soil layers should increase over time from both the mixing of soil layers and the enhanced protection of residue derived C into humic and physically protected SOM fractions.

3. Materials and Methods

Study site

This study was part of the UNL Carbon Sequestration Program (CSP) focused on studying C sequestration in agricultural systems (Verma et al. 2005). A detailed account of the CSP study can be found at http://csp.unl.edu/public/. The experiment was conducted on a large, production-scale, agricultural field (48.7 ha) equipped with center-pivot irrigation located at the University of Nebraska Agricultural Research and Development Center near Mead, NE (41º09’54.2’’N, 96º28’35.9’’W, 361 m). The soils are deep silty clay loams consisting of four soil series: Yutan (fine-silty, mixed, superactive, mesic Mollic Hapludalfs), Tomek (fine, smectitic, mesic Pachic Argialbolls), Filbert (fine, smectitic, mesic Vertic Argialbolls), and Filmore (fine, smectitic, mesic Vertic Argialbolls). Before initiation of the CSP study, six 20 m x 20 m intensive measurement zones (IMZs) were established for detailed process-level studies of soil C dynamics, crop growth and productivity, crop residue decomposition, and soil and plant gas exchange. The six spatial classes were delineated in the field using fuzzy-k-means clustering technique (Minasny and McBratney 2002) based on spatially dense information (4 m x 4 m) of elevation, soil type, electrical conductivity, soil organic matter content, digital aerial photographs and near infrared band of multispectral IKONOS satellite images. The spatial classes encompassed landscape level variation required to scale measurements to the entire field. Once the fuzzy environmental classes were delimited, the locations of the IMZs were randomly chosen to represent each of those fuzzy classes. This approach allowed quantification of the maximum natural variability of the measured variables within an agricultural field (Minasny and McBratney 2002).

Previous to 2001, the field was under a no tilled maize-soybean rotation for 10 years. Before initiation of the CSP study in 2001, the field was uniformly tilled by disking to homogenize the top 10 cm of soil and to incorporate P and K fertilizers as well as previously accumulated surface residues. The field has been under irrigated continuous maize since 2001. From 2001 to fall 2005, the site was under no-till. From fall 2005 to present the site has been under conservation deep tillage where N fertilizer (45 kg N ha^{-1}) as urea ammonium nitrate (UAN) was sprayed over crop residues in the fall after crop harvest and immediately incorporated using a modified moldboard plow to a final depth of 20 cm. Remaining soil cover after tillage has been greater than 30%, thus conforming to conservation tillage guidelines (CTIC 2011).

Crop management practices (i.e., plant populations, herbicide and pesticide applications, irrigation, fertilization) have been employed in accordance with standard best management practices prescribed for production-scale maize systems (Table 2.1). At physiological maturity, hand harvesting of six maize plants in each of 24 locations within the field was done to determine dry matter, harvest index and C and N concentrations in plant tissue (grain, cobs or podwalls, and vegetative biomass). Samples were dried at 70ºC, ground and analyzed for C and N using a Costech ECS 4010 elemental analyzer. Harvest index and tissue C and N mass fractions measured in the hand-harvested samples were used in combination with the whole-field grain yield estimate to calculate aboveground biomass and C and N inputs as crop residues (Table 2.1).

Soil sampling

Annual soil sampling of the top 30 cm of soil was conducted in the fall of 2005, 2006, 2007 and 2008. Sampling was done in each of the six IMZs after crop harvest and before fall tillage. Within each IMZ, six separate soil cores (4 cm diameter by 30 cm depth) were collected along an east–west transect between previous crop rows and inter-row center (approximately 0.16 m away from row). The field moist cores were split into three soil depths: 0-5, 5-15 and 15-30 cm, and then gently broken apart along natural planes of weakness. The samples were combined into one composite sample for each year (4) by IMZ (6) by depth (3) combination (n=72), air dried to constant weight, sieved to 8 mm and stored in boxes for further fractionations and analyses.

At the same time soil core samples were taken in the fall for C and N analyses and SOM fractionation, five smaller soil cores (2.15 cm diameter by 30 cm depth) were taken in each transect of each IMZ every year using a lubricated plastic sleeve mounted inside a soil hand probe to determine bulk density. These cores were also split into 0-5, 5-15 and 15-30 cm depths, dried at 105 ºC for 24 h and weighed. Soil bulk density (BD, Mg m-3) for each core by depth was calculated as:

$$BD = \frac{Soil_m}{Soil_v}$$

where Soil_m is the oven dried soil mass(g), and Soil_v is the volume of soil of each depth increment.

Extraction of SOM fractions

Two humic acid fractions were sequentially extracted to assess the degree of humification of SOM in the 0-5, 5-15 and 15-30 cm soil depths of all years. The mobile humic acid (MHA) fraction represents a young humic material, less associated with soil minerals, and the calcium-bound humic acid (CaHA) fraction constitutes an older, more condensed humic material associated with soil minerals (Campbell et al. 1967, Olk et al. 1995). The extraction was adapted from Olk et al. (1995). Briefly, the MHA was extracted with 0.25 M NaOH under an N_2 atmosphere. The solubilized material (MHA + fulvic acids) was acidified with HCl to precipitate the MHA. The extraction was performed twice on each sample to remove the majority of the MHA. The soil remaining after extraction of the MHA was decalcified with 0.25 M HCl. The CaHA was then extracted in similar way as the MHA with 0.25 M NaOH under an N_2 atmosphere. The solubilized material was acidified to precipitate the CaHA. Both the MHA and CaHA were de-ashed with a 0.5% HFl and 0.5% HCl solution for 24 hours, and de-salted by dialysis for three days in weak HCl and then water solutions. After dialysis the material was freeze dried, weighed, finely ground and stored in amber glass vials. The remaining soil containing the unextracted humin was oven-dried at 50ºC until constant weight, finely ground with mortar and pestle, and stored in glass scintillation vials.

A sequential density fractionation, adapted from Golchin et al. (1994) and Besnard et al. (1996), was used to determine the incorporation of residue-derived organic matter into two loosely physically protected fractions, the free light fraction (fLF) and light fraction occluded inside aggregates (oLF) for the 0-5, 5-15 and 15-30 cm soil depths from 2005 and 2008. Briefly, a 10 g soil sample was placed in a 60 mL centrifuge tube and 40 mL of sodium polytungstate (NaPT) of 1.6 g cm^{-3} density was added. The tube was capped and inverted gently by hand five times to avoid disruption of aggregates. Soil particles adhering to the lid and tube walls were washed into suspension using more NaPT to a final volume of 50 mL. The suspension was allowed to stand for 30 min before centrifuging at 2000 rpm for 1 hour. The supernatant with floating particles (free LF <1.6 g cm^{-3}) was poured onto a nitro-cellulose filter (0.20 μm), and filtered under vacuum. Ten (5 mm) glass beads and 40 mL of NaPT solution (1.6 g cm^{-3}) were added to the soil remaining in the centrifuge and the tubes were horizontally shaken for 16 hours at 60 oscillations per minute. After shaking, the particles adhering to the lid and tube walls were washed into suspension using more NaPT to a final volume of 50 mL. The tubes were allowed to stand for 30 min before centrifuging at 2000 rpm for one hour. The supernatant with floating particles (o-LF with a density <1.6 g cm^{-3}) was poured onto a nitro-cellulose filter (0.20 μm), and filtered under vacuum. The NaPT was removed from the f-LF and o-LF by washing with 350 mL distilled water. The LF on the filter was backwashed into a weighed porcelain crucible and dried at 50°C until constant weight. The extraction was performed at least twice for each sample to ensure enough material for analyses. Duplicates were combined, finely ground in a ball mill and stored in amber glass vials for further analysis. The NaPT was recycled according to Six et al. (1999b) to avoid cross contamination of C between samples.

Analytical methods

- C and N analysis

A sub-sample of whole soil was finely ground using a roller mill to pass a 100 mesh sieve. Any recognizable plant material and/or $CaCO_3$ concretions (> 1 mm) were removed before grinding. Twenty milligrams of the ground soil and 2 mg of humic materials were analyzed for C and N concentration using an elemental analyzer (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA).

To better account for the variation in effective sampling depth and soil mass due to changes in soil bulk density over time, the cumulative mass approach described by Gifford and Roderick (2003) was used to compare SOC and N changes over time. Estimates of whole soil SOC (g C m^{-2}) and N (g N m^{-2}) were calculated for each of the three soil depth intervals based on the measured bulk density corresponding to the time of sampling, and the C and N concentrations. Three reference soil masses were used to evaluate SOC and N changes over time (Equation 4, Gifford and Roderick 2003) (See detailed explanation in Appendix 1): (i) the top 60 kg dry soil m^{-2} (approximately 0–5 cm depth), (ii) the top 200 kg dry soil m^{-2} (approximately 0–15 cm depth) and (iii) the top 400 kg dry soil m^{-2} (approximately 0–30 cm depth).

- Absorbance

Light absorption at 465 (A4) and 665 nm (A6) was measured in duplicate for humic acids fractions from 2005 and 2008 (all depths) with a Spectronic® 20 Genesys™ spectrophotometer. Approximately, 1.5 mg and 3 mg of C as CaHA and MHA, respectively, were dissolved in 10 mL 0.05N $NaHCO_3$ (Chen et al. 1977). Molar absorptivity (E) for each wavelength was calculated as: $E=A/c*l$, where c is the C concentration of the solution (g C L^{-1}) and l is the length of the cuvette (1 cm). The E4/E6 ratio was calculated by dividing the absorbance of a sample at 465 nm by that at 665 nm. Molar absorptivity at 465 nm (E4) and the E4/E6 ratio have been used as an indicator of the molecular weight, degree of humification and chemical recalcitrance of the humic material to microbial decomposition (Chen et al. 1977, Zech et al. 1997, Olk et al. 1999, Ve et al. 2004, Mahieu et al. 2002).

Statistical analysis

The equality of the means among years was tested using repeated measurements (RM) ANOVA to account for the nature of the field sampling procedures and the natural variability of each individual IMZ. Comparisons of C and N mass among years for each soil layer (i.e. equal soil mass basis) for whole soil and humic fractions were done using a one-way RM ANOVA (Table 2.2). Two-way RM ANOVA (soil layer and year) was used for comparison of whole soil C:N ratio, all SOM fractions and for the light absorption data (Table 2.2). The C and N mass of the free and occluded LF for each soil layer were analyzed with a paired t-test, because only two years (2005 and 2008) were analyzed. The probability values (p-values) among year comparisons of C and N mass are shown in

Table 2.3. Post-hoc separation of means was done using Fisher LSD on significant variables ($p<0.05$). Data were analyzed using SigmaPlot® 11.0 software (Systat Software, Inc. 2008).

4. Results

Crop residue C and N inputs

The annual C and N inputs from aboveground crop residues averaged 4500 and 75 kg ha^{-1}, respectively (Table 2.1). Under no-till management, from 2001 to 2005 crop residues accumulated on the soil surface. The decomposition of these residues measured over a three year period (Kochsiek et al. 2009) contributed an estimated additional input in fall 2005 of 4970 kg C ha^{-1} and 140 kg N ha^{-1}, respectively. In the following years, the fall tillage events incorporated only the organic C and N inputs from the crop residues of that year together with the added fertilizer N (Table 2.1). The C:N ratio of the crop residue inputs (crop residues + litter) incorporated with tillage was between 45 and 60. Addition of N fertilizer to the residues prior to tillage reduced the C:N ratio of the inputs to 37, 31 and 40 in 2005, 2006 and 2007, respectively.

C and N stocks on an equal soil mass basis

Bulk densities of the 0-5 and 5-15 cm soil depths a year after the first tillage event were reduced compared to prior no-till (Figure 2.1). The higher bulk density under no-till (fall 2005 sampling) resulted in an effective sampling depth for 400 kg soil m^{-2} of 29.2 cm, while after tillage the reduction in bulk density increased the effective sampling depth to 31.7, 30.5, and 30.4 cm for fall 2006, 2007 and 2008, respectively. Given the changes in soil bulk density following tillage and thus the soil mass at fixed depths, using the cumulative mass approach proposed by Gifford and Roderick (2003) for the calculation of soil C and N stocks better accounted for the differences in effective sampling depth across years. The equal soil mass layers were set as 60, 200 and 400 kg soil m^{-2}, which approximated the 5, 15 and 30 cm depths, respectively. Both soil C and N stocks followed a similar trend among years (Figure 2.2). From fall 2005 to fall 2008, soil C and N stocks were reduced by 23% in the first layer (60 kg soil m^{-2}), with the greatest reduction after the first tillage (17% decline for both stocks compared to 2005 levels). A significant increase of C (+22%) and N (+16%) stocks after the first tillage was observed in the 60-200 kg soil m^{-2} layer. By fall 2008, the difference in C and N stocks in the 60 to 200 kg soil m^{-2} layer compared to 2005 were +7% and +4% respectively (Figure 2.2). The slight increases in the soil C and N stocks detected in the 200 to 400 kg soil m^{-2} layer (3 and 4%, respectively) were not significant. When the three soil layers were summed, soil C and N stocks after three years of tillage were similar to those under prior no-till (Figure 2.2, Table 2.3). Soil C and N stocks across IMZs ranged from 4.36 to 8.18 kg C m^{-2} and from 0.44 to 0.72 kg N m^{-2} in the 0-400 kg soil m^{-2} soil layer. Using proportional area weighting of the IMZs, we determined whole field C and N stocks in the 0-400 kg soil m^{-2} soil layer. Soil C stocks were 7.09 ±0.29, 7.47±0.36, 7.15±0.37 and 7.18±0.41 kg C m^{-2}, in fall 2005, 2006, 2007 and 2008 respectively.

C and N stocks in SOM fractions

Before tillage, the two humic acid fractions plus humin comprised 74.1, 75.8, and 84.3% of soil C stocks in the 0-60, 60-200 and 200-400 kg soil m^{-2} layers, respectively.

By 2008, these fractions represented 79.0, 79.2 and 85.2 % of soil C in the same soil layers. Similar trends were observed for the proportion of soil N. The three fractions together accounted for 62.5, 60.5 and 65.3% of soil N stocks in 2005, and 65.1, 65.2 and 68.8% of soil N in 2008 for the 0-60, 60-200 and 200-400 kg soil m^{-2} layers, respectively. In 2005, C and N in free plus occluded LF accounted for 5.6, 2.3, and 1.6% of soil C stocks and 3.4, 1.2, and 0.7 % of soil N stocks for the 0-60, 60-200 and 200-400 kg soil m^{-2} layers, respectively. In 2008, fLF and oLF together represented 4.5, 4.1, and 3.2% of soil C stocks and 2.5, 2.1, and 1.5% of soil N stocks for the same soil layers, demonstrating a shift in C and N stocks down the profile.

Stratification of SOM fractions with soil depth was larger in 2005 than in 2008 for all fractions (Figure 2.8). The degree of stratification in 2005 was in the order fLF > MHA > oLF > CaHA. In 2005, for the 0-400 kg soil m^{-2} layer approximately 70-85% of the C mass in the fLF, MHA and oLF was found in the uppermost 200 kg soil m^{-2} layer. By 2008, all the fractions showed more even distribution of C in the profile with 35-45% of the 0-400 kg soil m^{-2} layer C mass stored in the 200-400 kg soil m^{-2} layer (Figure 2.8).

The reduction in MHA-C and -N in the uppermost layer was mirrored by an increase in the second layer from 2005 to 2006, with minor changes in the following years (Figure 2.3, Table 2.3). For the 200-400 kg soil m^{-2} layer, MHA-C and -N increased by 2008 (Figure 2.3). No significant changes in MHA-C and -N were determined when pooling all soil layers together (Figure 2.3, Table 2.3). Changes in CaHA-C and -N in the first soil layer had similar trends to those of the MHA fraction, but of smaller magnitude. The CaHA-N was reduced by 20% in the 60 kg soil m^{-2} layer after the first year, and the CaHA-C increased by 15% in 2008 in the 60-200 kg soil m^{-2} (Figure 2.4, Table 2.3). The

C and N trends in the pooled 0-400 kg soil m^{-2} layer followed the trend in the 200-400 kg soil m^{-2} soil layer. The CaHA-C and -N showed a reduction after the first year, and a subsequent increase by 2008 (Figure 2.4). The reduction in CaHA-C was significant in 2006 and 2007 for the 0-400 kg soil m^{-2} layer, however, when pooling all soil layers together the CaHA-C and -N were similar for 2005 and 2008 (Figure 2.4, Table 2.3). Changes in humin-C and -N stocks in all three layers were similar to those of the MHA (Figure 2.5), namely small reduction of C and N stocks in the 0-60 kg soil m^{-2} layer and increase in the C and N stocks in the 60-200 and 200-400 kg soil m^{-2} layer (Figure 2.6, Table 2.3). For the 0-400 kg soil m^{-2} layer, humin-C and -N stocks increased over time by 4 and 5%, respectively (Table 2.3).

The small reduction in fLF-C and –N stocks in the 0-60 kg m^{-2} soil layer was offset by large increases in the 60-200 and 200-400 kg soil m^{-2} layers (Figure 2.7, Table 2.3). Free LF-C and -N in the 0-60 kg m^{-2} soil layer were reduced by 30% in 2008 compared to 2005. In the 60-200 and 200-400 kg soil m^{-2} layers, however, the incorporation of crop residues increased the fLF-C and -N by 172% and 149%, and by 318% and 285%, respectively (Figure 2.6, Table 2.3). The total fLF-C and -N in 2005 in the 0-400 kg soil m^{-2} layer was approximately twice that of 2005.Changes in oLF-C and -N from 2005 to 2008 were of large negative magnitudes in the first soil layer, accompanied by smaller increases in both the 60-200 and 200-400 kg soil m^{-2} layers (Figure 2.7). Similar to the MHA-C and -N, no significant changes in the oLF-C and -N were determined when pooling all soil layers together (Figure 2.7, Table 2.3).

SOM characteristics (Light absorption, C:N ratio)

The E4/E6 ratio of the MHA ranged from 6.8 to 10.3, and for the CaHA from 4.3 to 7.7. The E4/E6 ratio for CaHA did not show any significant response to management or depth in the soil profile (Table 2.4). For MHA, the E4/E6 ratio increased in 2008 compared to 2005 across all depths. Light absorption at 465 nm (E4) ranged from 2.5 to 8.8, and from 3.8 to 9.8 (g C $L^{-1})^{-1}$ for MHA and CaHA, respectively. Contrary to E4/E6, the E4 values of both MHA and CaHA fractions varied with soil depth and were sensitive to management (Table 2.4). In 2005, E4 values of both fractions increased with depth, while no significant changes in E4 values were determined among depths in 2008. A significant reduction of E4 values in 2008 compared to 2005 was found for the 5-15 and 15-30 cm soil depths for MHA and for the 15-30 cm soil depth for CaHA. In 2008, increased E4 values were measured for CaHA in the first soil layer compared to 2005.

The C: N ratios of WS and SOM fractions showed in general gradual trends with depth in 2005 that changed to more uniform C:N ratios across depths in 2008 (Table 2.5). With the exception of fLF and the humin, SOM fractions and WS showed a depth by year interaction effect on C:N ratio (oLF and MHA $p<0.05$, WS and CaHA $p<0.06$) (Table 2.5). Across depths and years, WS had the most uniform C:N ratio with an overall average of 10.5 ± 0.1 (Table 2.5). Free LF was the only fraction that showed a significant difference across years in its C:N ratio, with an increase of two units.

5. Discussion

Current management practices for increasing SOM content focus on increasing C inputs (increasing crop productivity, use of manure and cover crops) and reducing exposure of SOM to decomposition and oxidation (e.g. no-till). In this study we evaluate a different approach, enhancing the stabilization of crop residues into SOM by fall incorporation of maize residues into the soil profile together with N fertilizer using conservation deep tillage. This approach, based on anecdotal experiences (Reetz, 2000), was tested in experimental plots in the Ecological Intensification Program at the University of Nebraska-Lincoln. Successful results were obtained under high yielding continuous maize systems after 6 years: soil C and N stocks increased by 3.1 and 0.35 Mg ha^{-1}, respectively (Walters et al. 2009). In our study, fall conservation deep tillage with N fertilizer was evaluated at field scale for continuous maize that was under no-till management until 2005. After three years of implementing this management, we found that stabilization of maize residue inputs can offset increased C losses commonly associated with tillage. In addition to monitoring soil C and N stocks, the analysis of SOM fractions allowed us to better understand how this management enhanced incorporation and stabilization of maize residues compared to no-till management.

Soil C and N stocks were unchanged after three years of incorporation of maize residues along with N fertilizer in the 0-400 kg soil m^{-2} layer. The increase in the total amount of C and N in the 400 kg soil m^{-2} layer in fall 2006 was due to an increase in the C and N stocks in the 0-200 kg soil m^{-2} layer and reflects the additional inputs of 0.5 kg C and 0.01 kg N m^{-2} litter which were incorporated along with 0.47 kg C and 0.01 kg N m^{-2} as crop residue in 2005. There were also no changes in C stocks during the four years prior to the initiation of this experiment in 2005 (Verma et al. 2005). Lack of differences in soil C stocks on an equal soil mass basis between no-tillage and plow tillage was previously reported for paired experiments (Puget and Lal 2005, Puget et al. 2005, Angers and Eriksen-Hamel 2008, Poirier et al. 2009, Christopher et al. 2009) and for successional experiments where uncultivated or no-tilled soils had been converted into tillage (VanderBygaart and Kay 2004, Grandy and Robertson 2006).

The lack of differences in C and N stocks among tillage systems when using equal soil mass comparisons for sampling depths of 30 cm or more has been attributed to changes in the distribution of C within the soil profile (Angers and Eriksen-Hamel 2008). While no-till practices enhance accumulation of C and N close to the soil surface, plow tillage homogenizes C and N concentrations throughout the plowed depth and increases C and N concentrations below the tillage depth (Gal et al. 2007, Poirier et al. 2009, Christopher et al. 2009, Angers and Eriksen-Hamel 2008). Similarly, in the present study the distribution of C and N stocks in the soil profile was affected by tillage. Under no-till, 21% and 45% of soil C and N stocks in the 0-400 kg soil m^{-2} layer were in the 0-60 and 200-400 kg soil m^{-2} layers, respectively. After three years of tillage, the proportions of C and N stocks changed to 16% and 47% for the 0-60 and 200-400 kg soil m^{-2} layers, respectively. Despite a nominal plow depth of 20 to 25 cm (8-10 inches), the most significant changes in C and N stocks were in the 0-60 and 60-200 kg soil m^{-2} layer (0-5 and 5-15 cm approximately). In a meta-analysis of soil profile C distribution by tillage, Angers and Eriksen-Hamel (2008) determined that placement of crop residues at the bottom of the plow layer with full inversion tillage (i.e. moldboard plow) increased the soil C concentration at the bottom (21-25 cm depth) and below (31-35 cm) the depth of plow. It can be inferred that the modified moldboard plow used in this study to generate 30% residue cover resulted in more mixing of the residues and litter with the whole plow layer compared to burial of the residues at the bottom of the plow layer. Different explanations have been offered regarding the lack of significant changes in total soil C and N stocks after changes in management: a large C and N background (Grandy and Robertson 2006), similar magnitudes of accrual and losses, and limited sampling depth (Angers and Eriksen-Hamel 2008).

Increasing the soil-residue interface for stabilization of residue derived C and N as physically protected SOM fractions

Results from our study are consistent with previous studies indicating that tillage promotes the incorporation of residue derived C into SOM fractions deeper in the profile (Gregorich et al. 1995, Angers et al. 1995, Wander et al. 1998, Murage and Voroney 2008). All SOM fractions showed some degree of incorporation of crop residues and litter inputs with depth, either by net gain in fraction C and N stocks or changes in the properties of the fractions. Given its labile nature, the persistence of significant amounts of fLF with depth suggests either reduced decomposition deeper in the profile due to lower temperatures (Anger et al. 1995), or an oxygen limitation caused by excessive soil moisture at depth brought on by irrigation, and/or displacement by CO_2. Occluded LF represents more persistent SOM that is protected by encapsulation into stable aggregates (Golchin et al. 1994, Helfrich et al. 2006). Occluded LF may also include the fungal hyphae that bind together aggregates and is released during the breakdown of aggregates. The lack of changes with tillage in oLF-C and -N stocks in the 0-400 kg soil layer is contrary to the expected decline in aggregate stability due to tillage. Six et al. (1999) hypothesized that tillage will promote faster turnover of aggregates and result in both the loss of the protected oLF especially in macroaggregates, and reduced stability and stabilization capacity of macroaggregates. However, when aggregate turnover is slowed under no-till, stabilization of new oLF is limited (Yoo and Wander 2008). Plante and McGill (2002) found that tillage promoted the incorporation of crop residues into aggregates of all sizes during a laboratory experiment. The significant changes we observed in the C/N ratio of the oLF indicated a more decomposed fraction in 2008 compared to 2005, which may be indicative of processes beyond mere mixing of the soil layers. This is also supported by the change in the physical appearance on the oLF fraction (Figure 2.9). The visual inspection under dissecting microscope indicated more fragmented and less recognizable material in 2008 compared to 2005. Thus, in addition to physical redistribution of oLF in the soil profile, our results support loss of existing oLF and accrual of new oLF into aggregates. Six et al. (1999) hypothesized that oLF released by tillage is counterbalanced by gains of more decomposed oLF in new or repacked aggregates.

Increasing the soil-residue interface for stabilization of residue derived C and N as humus SOM fractions

The distributions of the MHA and CaHA fractions in the soil profile and their chemical compositions were distinct and affected by placement of the aboveground maize residue. Similar to Ve et al. (2004) and Olk et al. (1996), we found greater sensitivity of E4 values to management than the E4/E6 ratios. The positive correlation of E4 values with degree of humification (Olk et al. 1996, 1999, Mahieu et al. 2002) led us to use this as an indicator for the incorporation of maize residues into humic fractions, namely by lowering the E4 value. Under no-till, larger MHA-C and -N stocks together with wider C/N ratios and lower E4 values in the surface soil layer compared to deeper layers suggests this young MHA fraction preferentially forms and accumulates close to decomposing residues. A possible explanation is that as MHA diffuses down into soil it may encounter less C-saturated clays and cations (i.e. Ca^{+2}) allowing adsorption to clays as CaHA. Thus, the stratified distribution of MHA with soil depth may respond to two factors: the location of decomposing residues and the availability of sites for stabilization as CaHA. Carbon and N stocks of the more stable CaHA fraction were less stratified with depth after five years of continuous maize under no-till. Similar to MHA, E4 values were lowest closest to the soil surface. The preferential accumulation of less humified CaHA closest to the surface suggests that the formation of older and more humified fractions under no-till is also restricted by crop residue placement. Murage and Voroney (2008) evaluated the influence of tillage on the incorporation of corn residues into humified fractions using a $\delta^{13}C$ tracer. After 11 years of continuous maize, both no-till and conventional tillage systems showed incorporation of maize residues into all humic fractions, but with a distinctive depth distribution (Murage and Voroney, 2008). Physical incorporation of the residues by tillage increased the retention of maize-derived C in labile and recalcitrant humic fractions throughout the plow depth (20 cm), compared to only the first 5 cm under no-till management (Murage and Voroney 2008). After three years of conservation deep tillage, we found C and N stocks of the MHA and CaHA fractions were less stratified, and incorporation of humified crop residues into both MHA and CaHA fractions occurred at all depths.

The loss of C and N stocks as MHA and CaHA after one year of tillage was accompanied by a larger increase in the humin-C and -N stocks. By fall 2006, the MHA and CaHA fractions showed reductions of 24 g C m^{-2} and 82 g C m^{-2} , and 2.4 g N m^{-2} and 3.1 g N m^{-2}, respectively, while the humin fraction increased by 175 g C m^{-2} and 18 g N m^{-2} and the soil C and N stocks increased by 283 g C m^{-2} and 15 g N m^{-2}, respectively. The changes introduced by conservation deep tillage and incorporation of residues may have increased the humification of both fractions or may have resulted in the enhanced decomposition of MHA and CaHA fractions during the first year. The recovery of the C and N stocks as MHA and CaHA fractions by 2008 compared to 2006 together with no changes in humin-C and -N stocks are indicative of enhanced humification of maize residues. The changes in the chemical compositions of the MHA and CaHA fractions showing less condensed materials in 2008 compared to 2005 indicating a net stabilization of crop residue inputs into these humified fractions.

Stabilization of residue derived-C and -N into SOM fractions: speculation on the role of humic acid N.

Yang and Jenssen (2002) proposed that the larger the initial reactivity (i.e. its potential mineralization) of the crop residues the faster its decomposition products will age, leading to greater persistence of the decomposition products in soil (Smith et al. 2007). The main hypothesis of the present study is that under high-yielding continuous maize, increasing crop residue activity by increasing the soil-residue interface and adding fertilizer N will increase the stabilization and persistence of C and N from the residues. This hypothesis implies that management of high crop residue inputs for C sequestration will require synchronous N availability to increase residue reactivity. Decomposition of crop residues with wide C:N ratio may immobilize not only mineral N into the microbial biomass but also N from SOM fractions (Jansson 1958). Field and laboratory studies involving ^{15}N tracers have demonstrated that both the MHA and CaHA fractions constitute important sources of N for residue decomposition and further stabilization into SOM fractions (Legorreta-Padilla 2005, Wingeyer 2007). Legorreta-Padilla (2005) indicated that the seasonal dynamics of MHA and CaHA fractions were affected by crop rotation and use of N fertilizer. Under continuous maize, the MHA fraction was preferentially mined for N during winter and replenished during the cropping season, while the CaHA did not appeared to be affected by the N immobilization conditions (Legorreta-Padilla 2005). Ve et al. (2004) measured N mineralization after additions of MHA and CaHA to rice soils. The authors reported greater N mineralization from the younger less humified MHA fraction, suggesting the MHA as a more labile source of N. After a long term laboratory incubation of ^{15}N-labeled soil, the most recently incorporated N into the CaHA fraction was also a N source for the decomposition of wide C:N ratio maize residues (Wingeyer 2007). Under such conditions ^{14}C analysis indicated that the most labile sub-fraction of the CaHA fraction was not bound to soil cations and was co-extracted with the more labile MHA fraction (Wingeyer and Walters 2008). These results indicate that both MHA and CaHA may constitute available N fractions for decomposing residues. In this current study, in fall 2005 about 1000 g C m^{-2} and 20 g N m^{-2} from plant origin were incorporated into the soil together with 5 g N m^{-2} as N fertilizer through conservation deep tillage. The following fall, both MHA and CaHA fractions had reduced C and N stocks, but the total mass of humus was larger due to increased humin-C and -N stocks, suggesting humification of the crop residues and litter. In subsequent tillage operations, the amounts of C and N inputs were smaller than in

2005 since no litter was allowed to accumulate on the surface, but similar N fertilizer additions were made before tillage. Based on findings from previous studies, it can be speculated that the loss in MHA and CaHA fractions after the first year constituted a new N supply for decomposition of residues and litter after the first tillage operation.

6. Conclusions

Stabilization of aboveground residues into the mineral matrix without increasing C losses from native SOM and residue due to enhanced decomposition constitutes a challenge when attempting to maximize soil C sequestration. In this study we evaluated the hypothesis that under high-yielding continuous maize, increasing crop residue activity by increasing the soil-residue interface and adding fertilizer N can increase the stabilization and persistence of C and N from the residues. After one, two and three years of change from no-till to conservation deep tillage of an irrigated continuous maize field, redistribution of C and N stocks with no net overall change in amount was determined for the 0-400 kg soil m^{-2} layer. Three years may constitute a short time interval to measure net accrual or loss of C and N stocks at field level, based on data available from an experimental site. Nevertheless, no net loss in soil C and N stocks together with the changes in C and N stocks of specific SOM fractions and their depth distribution indicate novel management of crop residues may compensate for the normally detrimental effect of tillage on SOM content.

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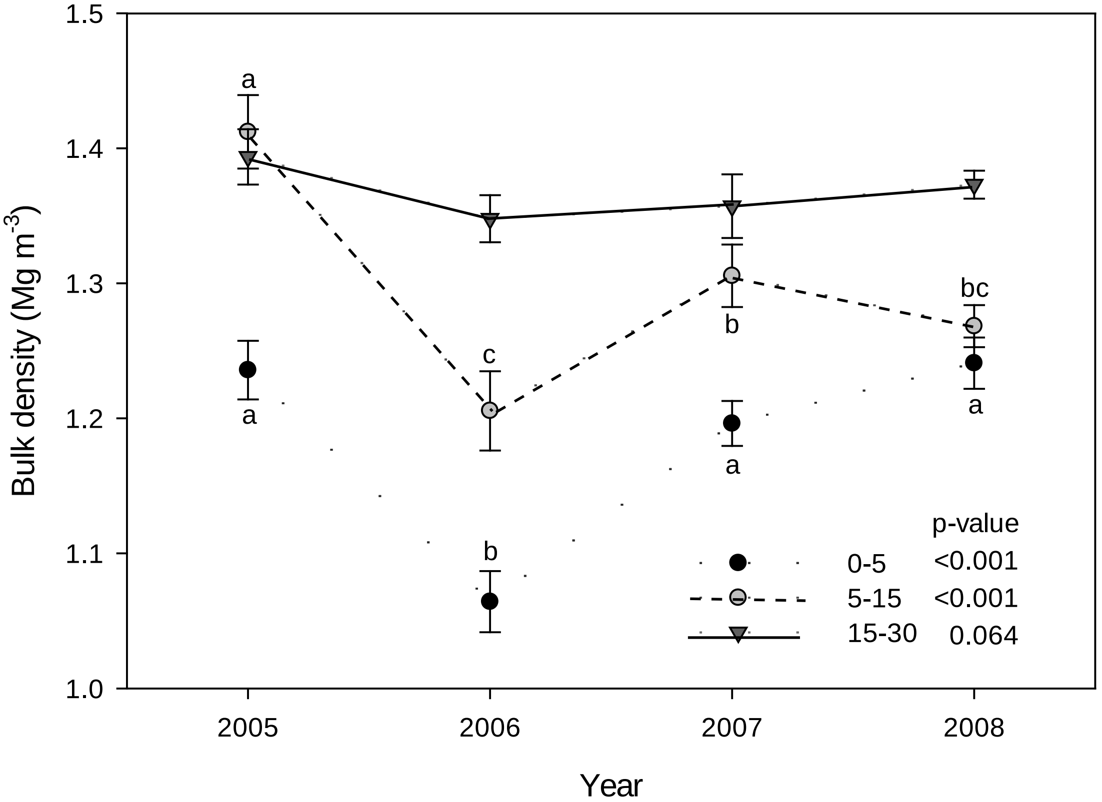


Figure 2. 1. Changes in soil bulk density by depth and year. Values followed by similar small letters do not differ significantly (α=0.05) between years. P-values for each soil layer are for among year comparisons from repeated measurement ANOVA.

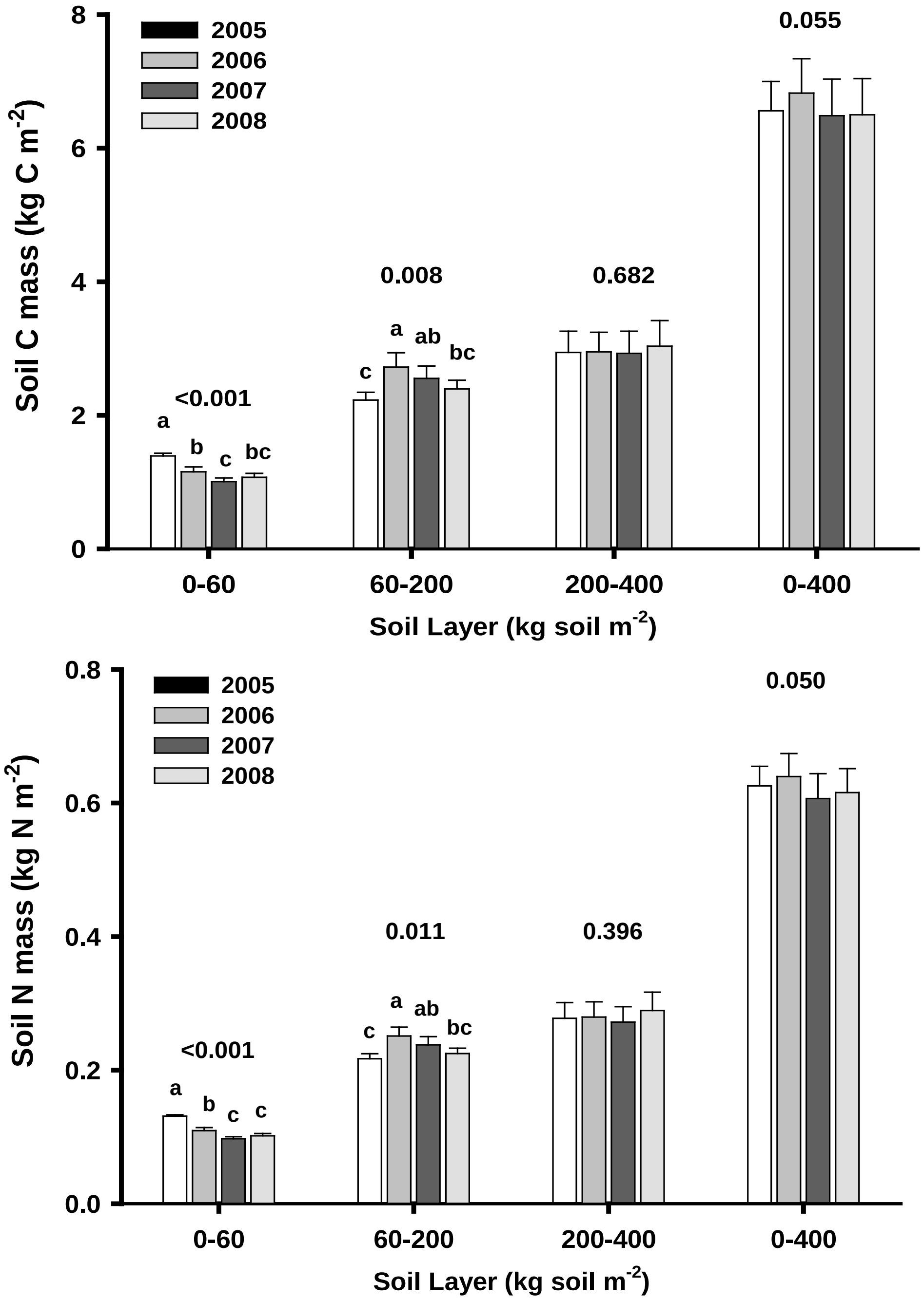


Figure 2. 2. Soil C and N mass per soil mass layer and year. Values followed by similar small letters do not differ significantly (α=0.05) between years P-values for each soil layer is for among year comparisons from repeated measurement ANOVA.

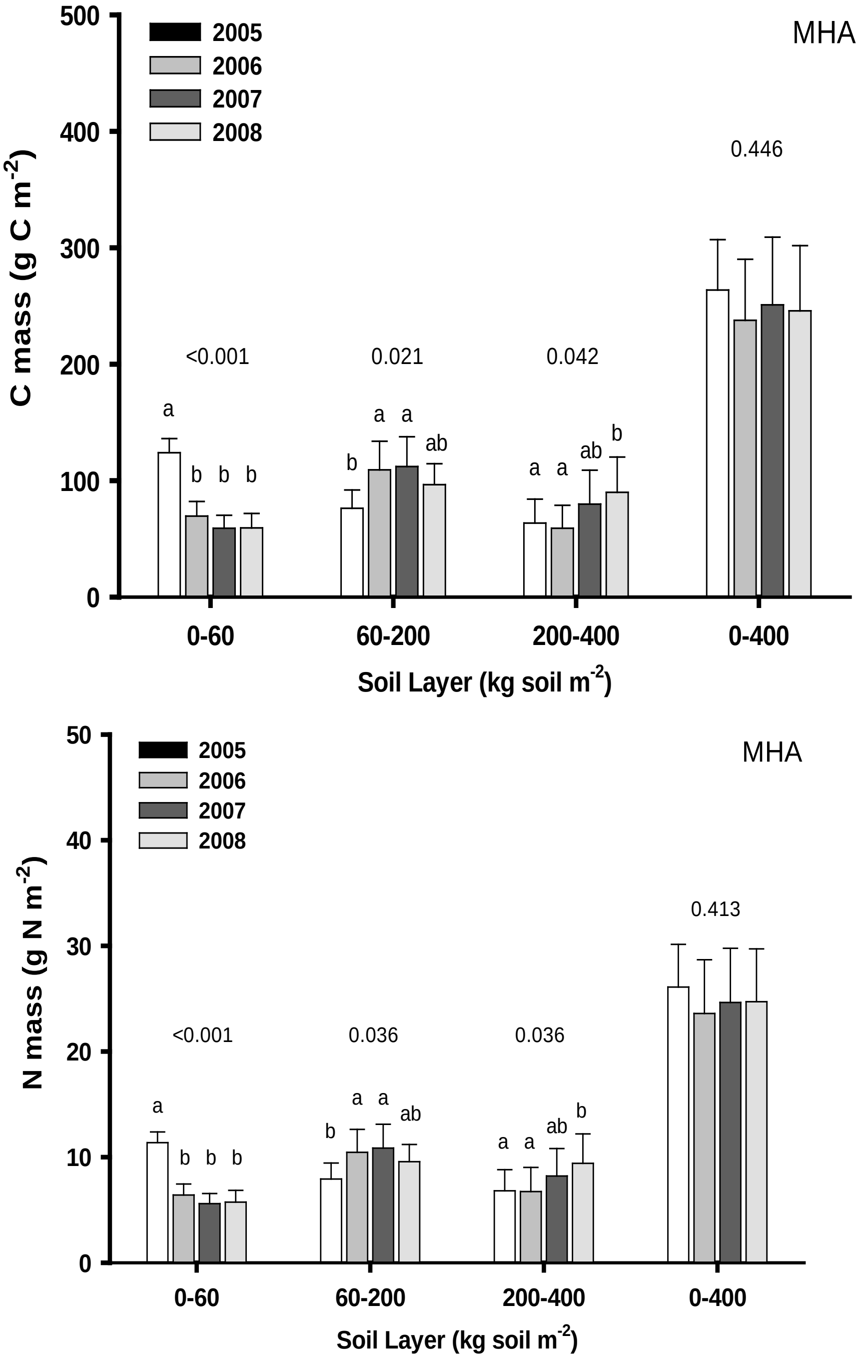


Figure 2. 3. Carbon and N mass in MHA fraction per soil mass layer. Values followed by similar small letters do not differ significantly (α=0.05) between years. P-values for each soil layer are for among year comparisons from repeated measurement ANOVA.

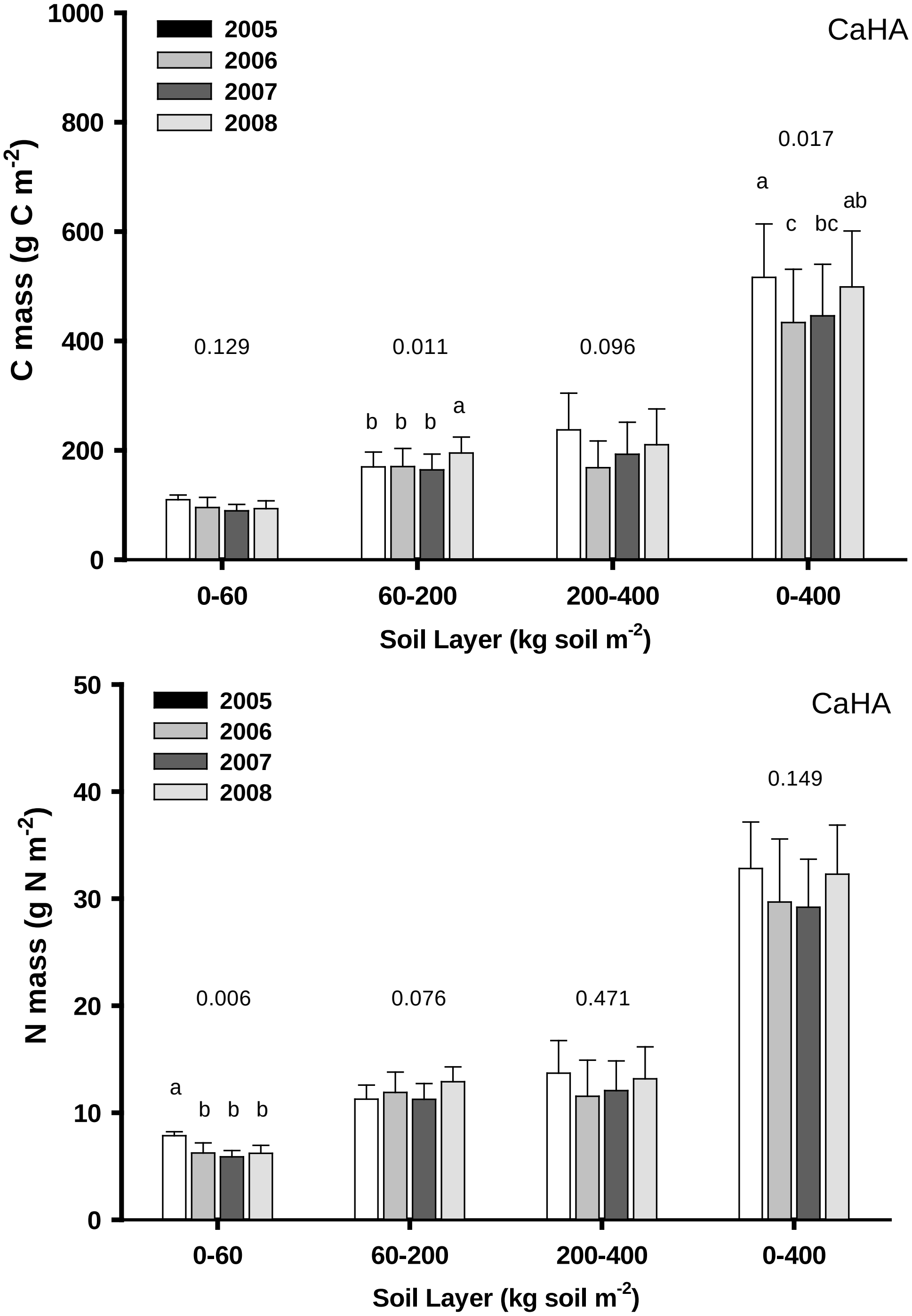


Figure 2. 4. Carbon and N mass in CaHA fraction per soil mass layer. Values followed by similar small letters do not differ significantly (α=0.05) between years. P-values for each soil layer is for among year comparisons from repeated measurement ANOVA

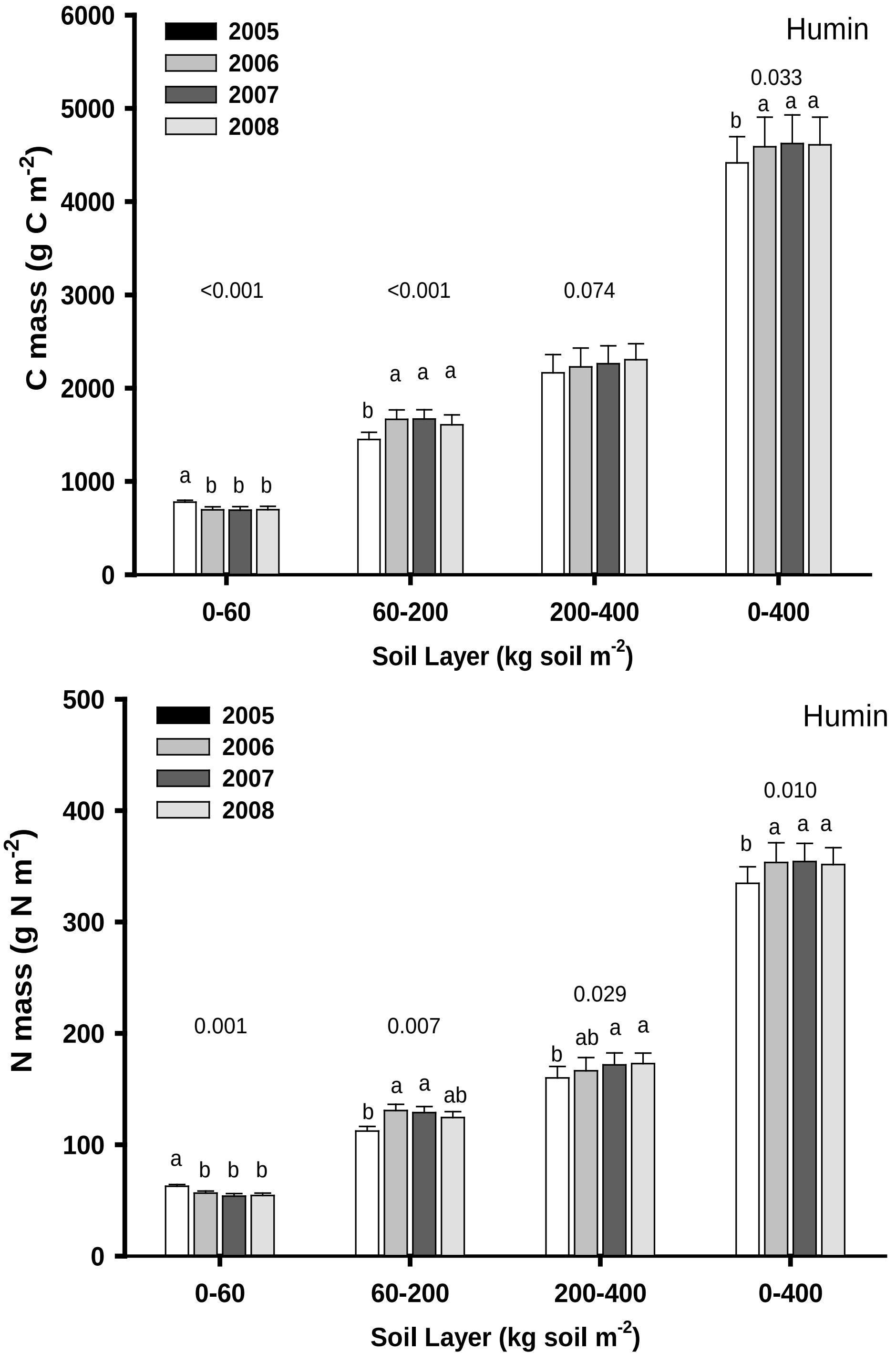
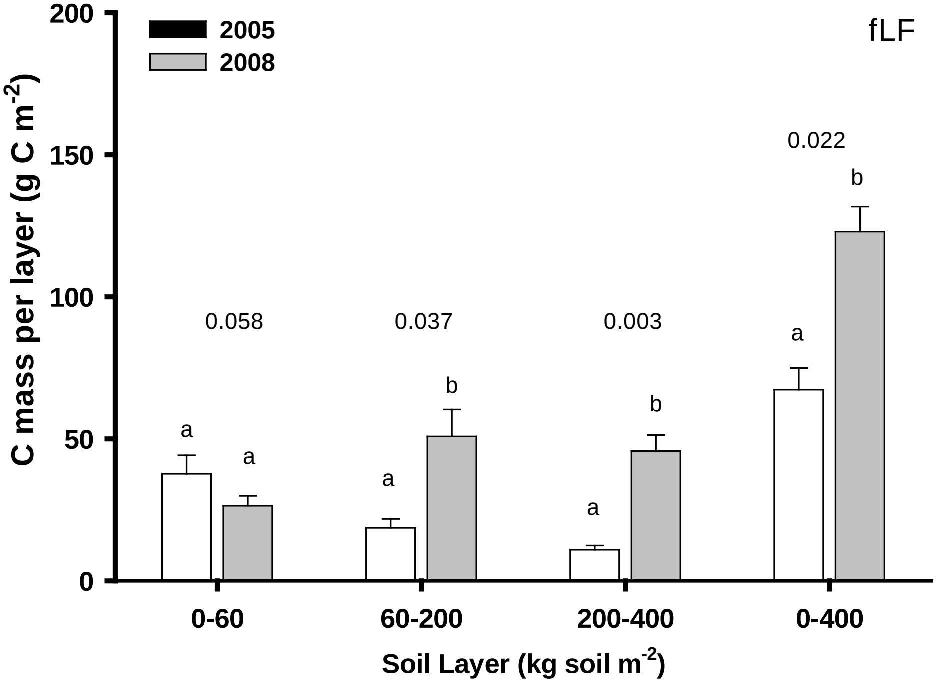


Figure 2. 5. C and N mass in humin fraction per soil mass layer. Values followed by similar small letters do not differ significantly (α=0.05) between years. P-values for each soil layer is for among year comparisons from repeated measurement ANOVA.



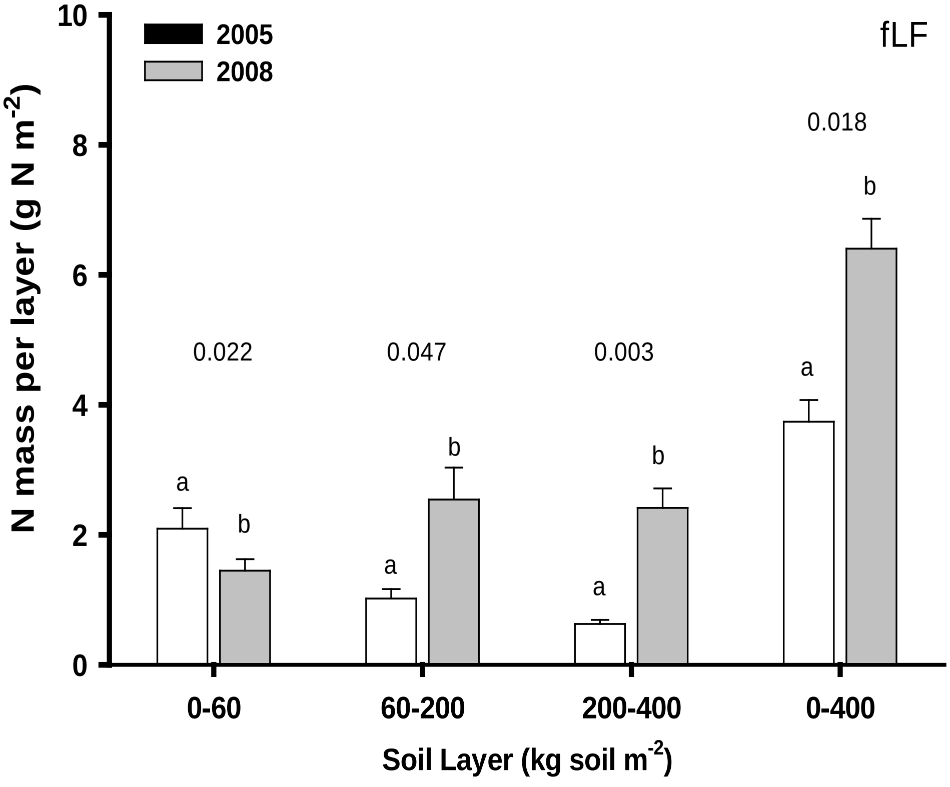


Figure 2. 6. C and N mass in free LF per soil mass layer. Values followed by similar small letters do not differ significantly (α=0.05) between years. P-values for paired-t test with 5 df.

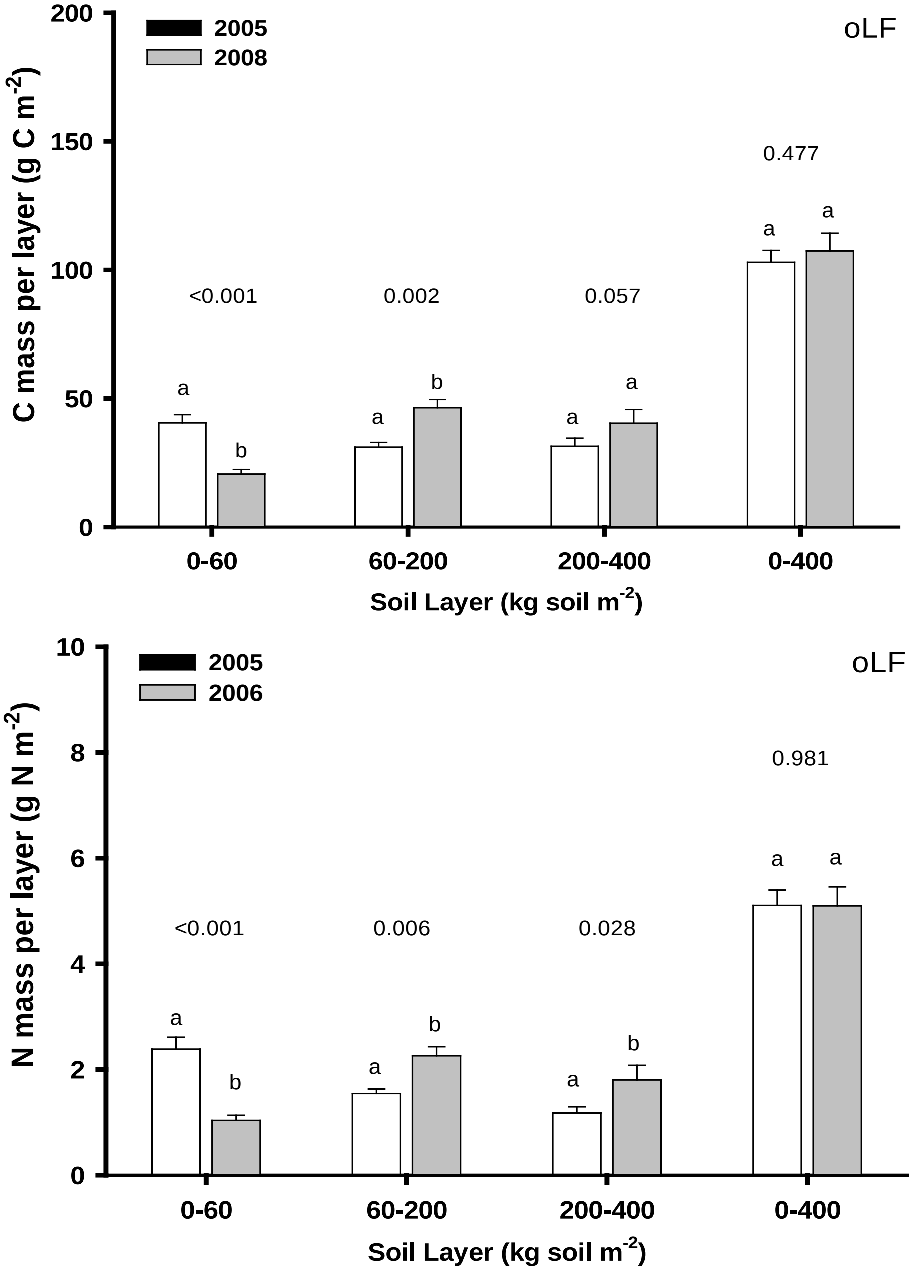


Figure 2. 7. C and N mass in occluded LF per soil mass layer. Values followed by similar small letters do not differ significantly (α=0.05) between years. P-values for paired-t test with 5 df.

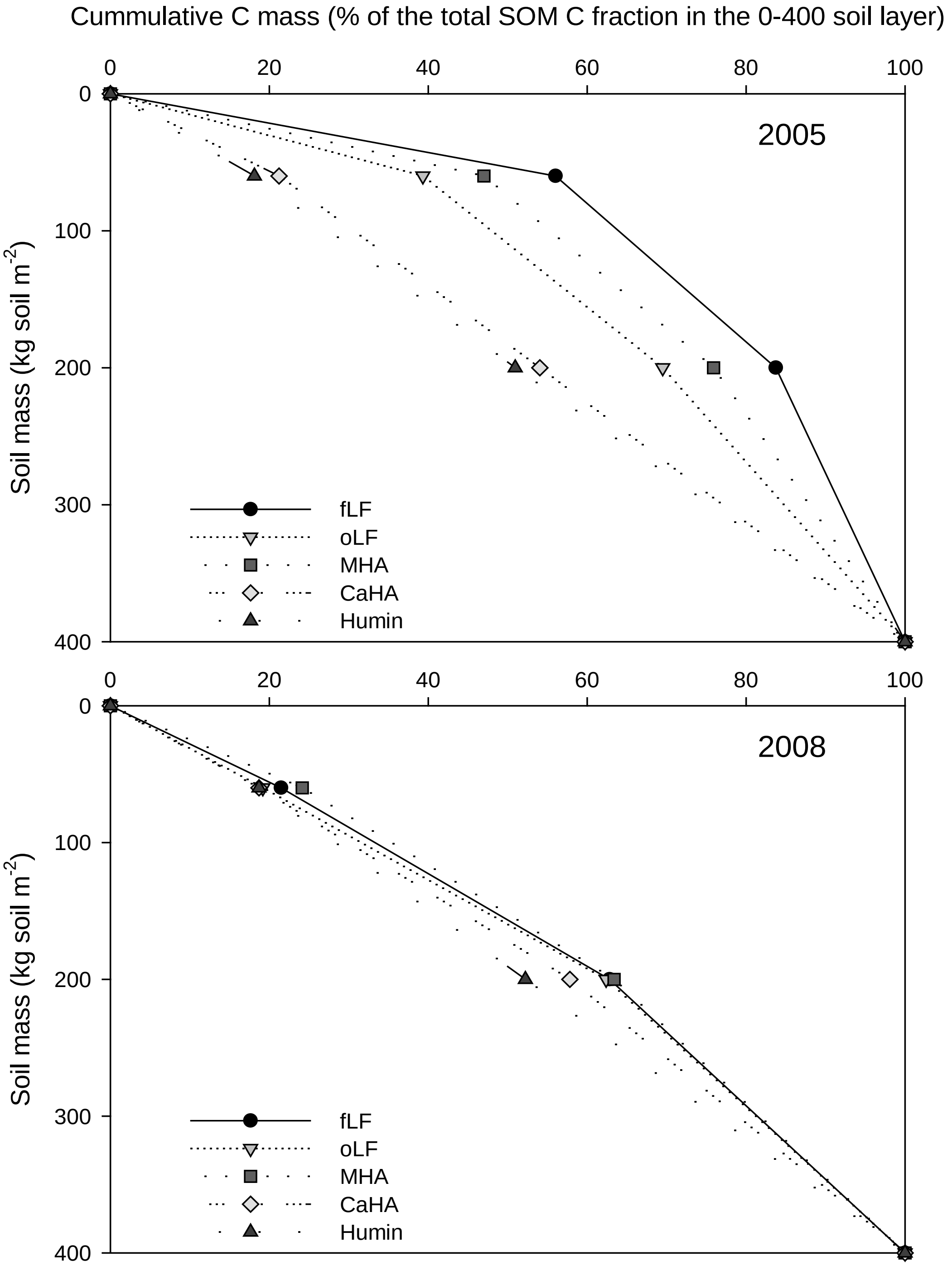


Figure 2. 8. Proportion of fraction C per soil layer

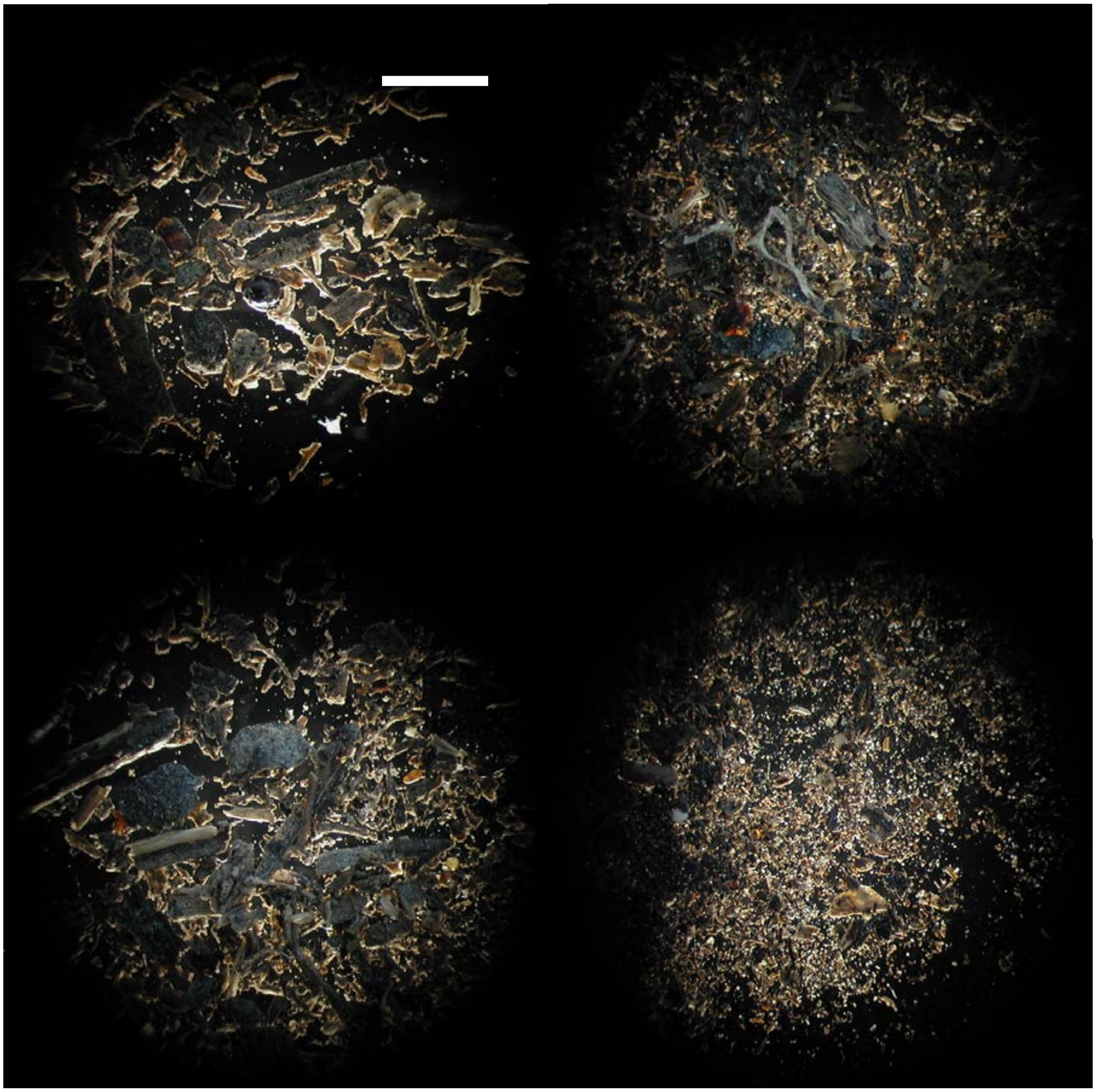


Figure 2. 9. Free (left) and occluded (right) LF in 2005 (top) and 2008 (bottom). White bars represents 2 mm.

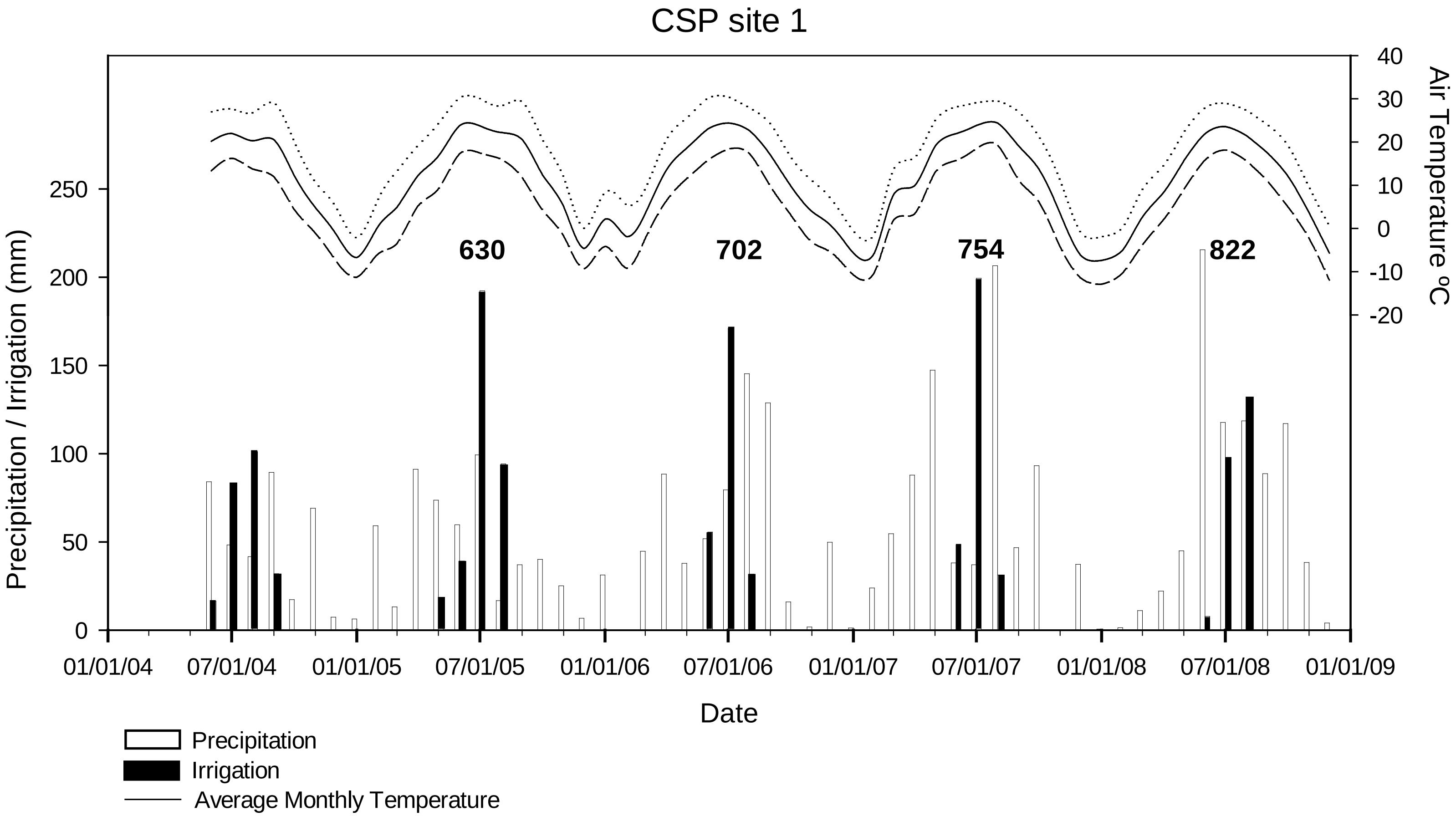


Figure 2. 10. Monthly average air temperatures (1 m) and monthly total precipitation and irrigation.

Table 2. 1. Crop management and grain yield (adjusted to 15.5% moisture) for the 2001–2008 period.

Year	Hybrid	Plant density (pl. ha^{-1})	Planting date	Harvest date	Sampling date	Tillage date	N to crop (kg N ha^{-1})	N to residues (kg N ha^{-1})	Yield (Mg ha^{-1})	Residue C* (kg C ha^{-1})	Residue N* (kg N ha^{-1})
2001	Pioneer 33P67	82000	5/10	10/18		-	163	-	14.5	5236	88
2002	Pioneer 33P67	81000	5/9	11/4		-	225	-	13.6	4697	74
2003	Pioneer 33B51	77000	5/15	10/27		-	269	-	13.1	4738	85
2004	Pioneer 33B51	81000	5/3	10/13		-	226	-	13.1	4116	64
2005	DeKalb 63-75	82000	5/4	10/12	10/20	10/26	246	45	12.8	4720**	77**
2006	Pioneer 33B53	82000	5/4	10/4	10/20	11/1	210	48	12.9	4011	80
2007	Pioneer 31N30	79000	5/1	11/6	11/8	11/13	181	45	15.3	4960	78
2008	Pioneer 31N30	81000	4/29	11/10	11/20	12/4	196	45	12.0		

*C and N inputs from above ground crop residues (leaf, stalks, cobs, husks). The number shown does not account for C and N inputs from previous litter, root inputs, or root exudates.

**With the tillage on fall 2005, crop residues and litter from previous years (2001 to 2004) were incorporated. Estimated additional C and N inputs are 5 and 0.14 Mg ha^{-1}, respectively (after Kochsiek et al. 2010).

Table 2. 2. ANOVA Table for one way and two way repeated measurements (RM) models.

One Way RM[1] (4 years)		Two way RM[2] (2 years)		Two way RM[3] (4 years)	
Source	df	Source	df	Source	Df
Between IMZs	5	Between IMZs	5	Between IMZs	5
Between Years	3	Between Depths	2	Between Depths	2
Residual	15	Ea=IMZ*Depth	10	Ea=IMZ*Depth	10
Total	23	Between Years	1	Between Years	3
		Eb=IMZ*Year	5	Eb=IMZ*Year	15
		Depth*Year	2	Depth*Year	6
		Residual	10	Residual	30
		Total	35	Total	71

[1]: Used for C and N mass per soil layer as WS, MHA, CaHA, and humin

[2]: Used for C/N ratio of LF data and for light absorption data on MHA and CaHA fractions.

[3]: Used for C/N ratio of WS, MHA, CaHA, and humin fractions.

Table 2. 3. Annual soil and SOM fractions' C and N stocks.

Soil layer (kg m^{-2})		0-60	0-200	200-400	0-400	0-60	0-200	200-400	0-400
	Year	------------------ kg C m^{-2} ---------------				------------------ kg N m^{-2} ---------------			
Soil	2005	1.392a	2.228c	2.940	6.559	0.131a	0.217c	0.278	0.626
	2006	1.153b	2.739a	2.950	6.842	0.109b	0.253a	0.279	0.641
	2007	1.008c	2.551ab	2.926	6.484	0.097c	0.238ab	0.272	0.607
	2008	1.070bc	2.393bc	3.035	6.499	0.102c	0.225bc	0.289	0.616
	p-value (year)	<0.001	0.008	0.682	0.055	<0.001	0.011	0.396	0.050
		------------------ g C m^{-2} ---------------				------------------ g N m^{-2} ---------------			
fLF	2005	37.7	18.7b	10.9b	67.3b	2.1a	1.0b	0.6b	3.7b
	2008	26.4	50.8a	45.7a	123.0a	1.4b	2.5a	2.4a	6.4a
	p-value (year)	0.058	0.037	0.003	0.022	0.022	0.047	0.003	0.018
oLF	2005	40.5a	31.1b	31.4	103.0	2.4a	1.5b	1.2b	5.1
	2008	20.6b	46.4a	40.4	107.4	1.0b	2.3a	1.8a	5.1
	p-value (year)	<0.001	0.002	0.057	0.477	<0.001	0.006	0.028	0.981
MHA	2005	123.9a	76.2b	63.5b	263.6	11.4a	7.9b	6.8b	26.1
	2006	69.5b	111.0a	59.0b	239.4	6.4b	10.6a	6.7b	23.7
	2007	59.1b	112.1a	79.8ab	251.0	5.6b	10.8a	8.2ab	24.6
	2008	59.4b	96.4ab	90.0a	245.8	5.7b	9.6ab	9.4a	24.7
	p-value (year)	<0.001	0.021	0.042	0.446	<0.001	0.036	0.036	0.413
CaHA	2005	109.5	169.4b	237.2	516.1a	7.8a	11.3	13.7	32.8
	2006	95.2	170.8b	168.2	434.2c	6.2b	11.9	11.5	29.7
	2007	89.2	164.1b	192.7	445.9bc	5.9b	11.2	12.1	29.2
	2008	93.3	195.1a	210.2	498.6ab	6.2b	12.9	13.2	32.3
	p-value (year)	0.129	0.011	0.096	0.017	0.006	0.076	0.471	0.149
Humin	2005	801a	1449b	2165	4414b	63a	112b	160b	335b
	2006	696b	1665a	2228	4589a	56b	131a	166ab	353a
	2007	691b	1669a	2262	4622a	54b	129a	172a	354a
	2008	696b	1607a	2305	4608a	54b	124a	173a	351a
	p-value (year)	<0.001	<0.001	0.074	0.033	0.001	0.007	0.029	0.010

Means followed by similar letter do not differ significantly (α=0.05) among years for each soil layer. P-value for one-way RM ANOVA (Soil, MHA and CaHA) and paired t-test (fLF and oLF).

Table 2. 4. Molar absorptivity at 465 nm (E6) and E4/E6 ratio of mobile humic acid (MHA) and calcium humate (CaHA) extracted from all soil depths in 2005 and 2008 (n=6).

	E4/E6		$E4^1$	
Depth	2005	2008	2005	2008
MHA			------------ $(g\ C\ L^{-1})^{-1}$	-------------
0-5	8.07	8.60	4.39 a A	4.41 a A
5-15	8.77	9.07	4.85 a AB	4.31 b A
15-30	8.24	9.01	5.61 a B	3.99 b A
Average	8.36 a	8.89 b	4.95	4.24
p-value depth	0.157		0.614	
p-value year	0.014		<0.001	
p-value depth*year	0.292		0.035	
CaHA			------------ $(g\ C\ L^{-1})^{-1}$	------------
0-5	5.09	5.14	5.98 a A	6.76 b A
5-15	5.27	5.23	7.21 a B	6.94 a A
15-30	5.28	5.71	7.84 a B	6.47 b A
Average	5.21	5.36	7.01	6.72
p-value depth	0.273		0.046	
p-value year	0.258		0.116	
p-value depth*year	0.115		<0.001	

[1]: for the interactions, values followed by similar small letters do not differ significantly (α=0.05) between years, and values followed by similar cap letters do not differ significantly (α=0.05) between depths. p-value is the (P>F).

Table 2. 5. C:N ratio of whole soil (WS), free and occluded LF (fLF, oLF), mobile humic acid (MHA) fraction, calcium humate (CaHA) fraction, and humin in 2005 and 2008 from all soil depths (n=6)

	WS		fLF		oLF		MHA		CaHA		Humin	
Depth	2005	2008	2005	2008	2005	2008	2005	2008	2005	2008	2005	2008
	---------- C:N ratio ----------											
0-5	10.6	10.5	17.8	18.2	17.1 a A	20.0 b A	10.9 a A	10.2 b A	13.9	14.7	12.8	12.8
5-15	10.2	10.6	18.1	20.2	20.2 a B	20.5 a A	9.4 a B	10.0 a A	14.7	14.8	12.9	12.8
15-30	10.4	10.3	17.2	18.9	27.0 a C	22.9 b B	8.9 a B	9.0 a B	15.7	14.5	13.4	13.3
Average (year)	10.4	10.5	17.7 a	19.1 b	21.4	21.1	9.7	9.7	14.8	14.7	13.0	13.0
p-value depth	0.784		0.047		<0.001		0.001		0.496		0.085	
p-value year	0.602		0.031		0.367		0.989		0.636		0.642	
p-value depth*year	0.057		0.163		<0.001		0.019		0.059		0.899	

[1]: for the interactions, values followed by similar small letters do not differ significantly (α=0.05) between years, and values followed by similar cap letters do not differ significantly (α=0.05) among depths.

CHAPTER 3. AGGREGATE STABILIZATION OF C AND N POOLS AFTER CONVERSION FROM NO TILL TO FALL CONSERVATION DEEP TILLAGE

1. Abstract

The amounts and composition of soil organic matter (SOM) fractions in aggregate size classes were assessed in three soil depths using a combined fractionation approach involving light fraction (LF) separation and humic acid (HA) fractionation from slake-resistant aggregates (>250 µm, 250-53 µm and <53 µm). Soil samples from 0-5, 5-15 and 15-30 cm depth from an irrigated continuous maize field were collected under no-till and after 1, 2, and 3 years of fall incorporation of crop residues and N fertilizer with a modified moldboard plow. The change in management resulted in the redistribution of aggregates (and their SOM) between the 0-5 and 5-15 cm depths. After three years of tillage, the proportion of >250 µm aggregates decreased in the 0-5 (-36%) and 5-15 (-15%) cm soil depths, with a concomitant increase in mid-size and small-size aggregates. In the 15-30 cm depth, the reduction of mid-size aggregates was accompanied by an increase in small-size aggregates and no change in the largest aggregates. From 2005 to 2008, aggregate-C and -N contents in SOM fractions of the 0-5 cm depth decreased from 7 to 35%, while increases of 9 to 120% were observed for the 5-15 cm depth and of 2 to 110% for the 15-30 cm depth. Both monosaccharide abundance, microbial to plant monosaccharide ratio and LF distribution with soil depth indicated preferential protection of plant-derived C in larger aggregates as well as a shift to more homogenous distribution of residue inputs across all depths in 2008 compared to 2005. From 2005 to 2008, a reduction in the light absorption at 465 nm of HA fractions in the 5-15 and 15-30 cm depths of the largest and mid-size aggregates indicated humification of new C inputs with change in management. The trends observed after three years of the fall conservation deep tillage indicates loss of C and N in the 0-5 cm depth was counterbalanced by preferential stabilization of new C and N inputs with soil depth by both physical protection and humification into >250 μm aggregates and largely humification in mid-size and small-size aggregates.

Key words: Carbohydrates, Mobile humic acid, Calcium humic acid, free light fraction, occluded light fraction.

2. Introduction

Soil aggregates are structural units consisting of soil minerals and soil organic matter (SOM) having different size, resistance to mechanical breakdown, and thus turnover (Tisdall and Oades 1982, Gregorich et al. 1989). Stabilization of SOM is achieved by humification, organo-mineral associations and by physical protection due to occlusion into intraagregate light fraction (oLF) and free light fraction in interaggregate pore space (fLF). The relative importance of organo-mineral association and physical occlusion for soil carbon (C) and nitrogen (N) stabilization is dependent on the size of stable aggregates (Puget et al. 2000, Yamashita et al. 2006) and may influence the degree of humification of aggregate-associated SOM. In general, increasing aggregate size is associated with greater intraagregate pore space (McCarthy et al. 2008), greater content of occluded non-mineral associated SOM (Golchin et al. 1994, Six et al. 2002), and a greater loss of C if the aggregate is disrupted (Gregorich et al. 1989). Thus, the mechanisms of SOM stabilization of individual aggregates depend on aggregate size.

Studies using the $\delta^{13}C$ signature of soil aggregates after a change from C3 (native grass) to C4 (maize) species indicated preferential incorporation of new C inputs from crop residues inside macroaggregates (i.e. >250 µm) (Jastrow et al. 1996, Angers and Giroux 1996, John et al. 2005), where the main mechanism of SOM stabilization is occlusion. Nevertheless, the faster recycling/turnover of larger aggregates (Six et al. 1999) together with their increased susceptibility to disruption may constrain their capacity to protect labile crop residue inputs. Microaggregates (i.e. <53 µm) represent very stable niches for SOM protection due to slower aggregate turnover (Six et al. 2000). Given that the main mechanism of SOM stabilization in microaggregates is the formation of organo-mineral complexes, the current C saturation theory indicates that stabilization of new C inputs by microaggregates will be restricted to their unsaturated mineral surfaces (Hassink et al. 1997, Stewart et al. 2007). Mid-size aggregates (i.e. 53-250 µm) have greater stability against mechanical breakdown than do larger aggregates, where SOM can be stabilized by either occlusion or formation of organo-mineral complexes. Mid-size aggregates have been suggested as the preferential niche for stabilization of newly formed SOM from decomposing crop residues (Angers et al. 1997, Six et al. 1999).

Tillage can alter the incorporation of crop residues into aggregates by modifying both the soil-residue interface and turnover rates. At the same time residues are incorporated, tillage exposes SOM to oxidation and decomposition leading to breakdown of aggregates and altered aggregate class size distribution in the soil profile (Jastrow et al.

1996, Angers and Giroux 1996, John et al. 2005). Loss of labile, physically protected SOM inside mid- and large-size aggregates is considered the main mechanism of C loss caused by aggregate breakdown during tillage (John et al. 2005). Nonetheless, deep incorporation of crop residues can increase aggregation and C retention by aggregates deeper in the soil profile (Olchin et al. 2008), and thereby counterbalance the negative effects of tillage on potential C sequestration due to aggregate disruption at shallower soil depths. In the previous chapter, increased stabilization of newly added C as humic fractions and physically protected SOM at depth was reported after conversion from no-till to fall conservation deep tillage under continuous high yielding maize (Chapter 2). Analysis of whole soil and SOM fractions suggests both mechanisms of SOM stabilization were important in deeper soil layers to compensate for SOM losses nearer the surface (Chapter 2). Although some insights regarding the role of aggregates on SOM stabilization were gained in the previous chapter, a more detailed account of the stabilization mechanisms of each aggregate size is needed to assess their function.

This chapter focuses on the SOM protection mechanisms in different aggregate sizes of an irrigated, high yielding continuous maize field that was under no-till management and converted to conservation deep tillage. Aggregate C and N composition and stocks are presented for >250, 250-53 and <53 µm aggregate size fractions under no-till and after 1, 2, and 3 years of fall incorporation of crop residues and N fertilizer with a modified moldboard plow. The objectives of this study were: i) to evaluate the impact of management change on the SOM protection capacity of >250, 250-53 and <53 µm aggregates; ii) to evaluate the impact of management change on the protection mechanisms of >250, 250-53 and <53 µm aggregates; and iii) to test whether mid-size aggregates (250-53 µm) are the preferential niche for stabilization of new C inputs under conservation deep tillage.

3. Materials and Methods

Study site

This study was part of the UNL Carbon Sequestration Program (CSP) focused on examining the potential to sequester C in agricultural systems (Verma et al. 2005). The experiment was conducted in a large production-scale, agricultural field (48.7 ha) equipped with center-pivot irrigation at the University of Nebraska Agricultural Research and Development Center near Mead, NE (41º09’54.2’’N, 96º28’35.9’’W, 361 m). The soils are deep silty clay loams consisting of four soil series: Yutan (fine-silty, mixed, superactive, mesic Mollic Hapludalfs), Tomek (fine, smectitic, mesic Pachic Argialbolls), Filbert (fine, smectitic, mesic Vertic Argialbolls), and Filmore (fine, smectitic, mesic Vertic Argialbolls). Previous to 2001, the field was under no till maize-soybean rotation for 10 years. Before initiation of the CSP study in 2001, the field was uniformly tilled by disking to homogenize the top 10 cm of soil and to incorporate P and K fertilizers as well as previously accumulated surface residues. The field has been under irrigated continuous maize since 2001. From 2001 to fall 2005, the site was under no-till. From fall 2005 to present the site has been under conservation deep tillage where N fertilizer (45 kg N ha^{-1}) as urea ammonium nitrate (UAN) was sprayed over crop residues in the fall after crop harvest and immediately incorporated using a modified moldboard plow to a final depth of 20 cm. Remaining soil cover after tillage has been greater than 30%, thus conforming to conservation tillage guidelines (CTIC 2011). Crop management practices (i.e., plant populations, herbicide and pesticide applications, irrigation, fertilization) have been employed in accordance with standard best management practices prescribed for production scale maize systems (see Chapter 2).

Soil sampling

Annual soil sampling of the top 0.3 m of soil was conducted in the fall of 2005, 2006, 2007 and 2008. Sampling was done in each of the six intensive management zones (IMZ, see Chapter 2 for details) after crop harvest and before fall tillage. Within each IMZ, six separate soil cores (4 cm diameter by 30 cm depth) were collected along a transect in east–west direction between previous crop rows and inter-row center (approximately 0.16 m away from row). The field moist cores were split into three soil depths: 0-5, 5-15 and 15-30 cm, and then gently broken apart along natural planes of weakness. The samples were combined into one composite sample for each year (4) by IMZ (6) by depth (3), then air-dried to constant weight, sieved through 8mm and stored in boxes for further fractionations and analyses.

In addition to the soil core samples, five smaller soil cores (2.15 cm diameter by 30 cm depth) were taken every year to determine bulk density in each transect of each IMZ, using a lubricated plastic sleeve mounted inside a soil hand probe. These cores were also split into 0-5, 5-15 and 15-30 cm depths, dried at 105 ºC for 24 h and weighed. Soil bulk density (BD, Mg m^{-3}) for each depth increment of each core was calculated as:

$$BD = \frac{Soil_m}{Soil_v}$$

where $Soil_m$ is the oven dried soil mass (g), and $Soil_v$ is the volume of soil of each depth increment.

Extraction of SOM fractions

In order to assess the different mechanisms for protecting SOM, physical and chemical techniques for extracting SOM were combined (Figure 3.1). The soil samples were first separated into aggregate classes, and then two humic acid fractions and two light fractions were extracted from each aggregate class. A wet-sieving procedure, which allowed for slaking to occur, was used to separate water-stable aggregates from the soil. The soil was separated into three aggregate size fractions: >250, 250-53 and <53 μm. One hundred grams of the air-dried soil was put on the top of a 250 μm sieve and quickly immersed in distilled water inside a plastic pail. After ten minutes of immersion the sieve was moved up and down (3 cm) 60 times during a period of two minutes without breaking the water surface. After sieving the sieve was set aside to drain and the contents in the plastic pail were poured on top of the 53μm sieve inside a second plastic pail. The sieve was moved up and down (3 cm) 60 times during a period of two minutes without breaking the water surface. After the second sieving the sieve was set aside to drain and the <53 μm soil fraction was transferred to 2L flasks and brought to volume together with 2 ml of a saturated calcium chloride solution. After overnight sedimentation of the suspension, most of the water was removed by siphoning and the sediments were backwashed into weighed metal boats and dried at 50ºC until constant weight. The aggregates in the sieves were backwashed into porcelain crucibles with distilled water and dried at 50ºC until constant weight. Each combination of soil depth, IMZ and year was extracted at least three times to insure enough aggregate material for subsequent fractionations.

Two humic acid fractions were sequentially extracted from the >250 and 250-53 µm aggregates for all soil depths and years. These extractions were not performed in the <53 µm size class due to insufficient material. The mobile humic acid (MHA) fraction represents young humic material that is less associated with soil minerals and the Ca-bound humic acid fraction constitutes an older, more condensed humic material associated with soil minerals (Campbell et al. 1967, Olk et al. 1995). The extraction was adapted from Olk et al. (1995). Briefly, the MHA was extracted from soil with 0.25 M NaOH under N_2 atmosphere. The solubilized material (MHA + fulvic acids) was acidified with HCl to precipitate the MHA fraction. The extraction was performed twice on each sample to remove the majority of MHA. The soil remaining after the MHA extraction was decalcified through washes with 0.25 M HCl. The CaHA was then extracted in a similar way to MHA with 0.25 M NaOH under N_2 atmosphere. The solubilized material was acidified to precipitate the CaHA fraction. Both, MHA and CaHA, were de-ashed with a 0.5% HFl and 0.5% HCl solution for 24 hours, and de-salted by dialysis for three days in weak HCl and then deionized (DI) water solutions. After dialysis the material was freeze-dried, weighed, finely ground and stored in amber glass vials. The remaining soil containing the unextracted humin was oven-dried at 50ºC until constant weight, finely ground with mortar and pestle and stored in glass scintillation vials.

A sequential density fractionation, adapted from Golchin et al. (1994) and Besnard et al. (1996), was used to extract two loosely physically protected fractions, the free light fraction (fLF) and the light fraction occluded inside aggregates (oLF) in the >250 and 250-53 μm aggregates for all soil depths and years. Briefly, 10 g aggregates were placed in duplicate in a 60 mL centrifuge tube and 40 mL of sodium polytungstate (NaPT) of 1.6 g cm^{-3} density was added. The tube was capped with a plastic lid and inverted gently by hand five times to avoid disruption of aggregates. The particles adhering to the lid and tube walls were washed into suspension using more NaPT to a final volume of 50 mL. The suspension was allowed to stand for 30 min before centrifuging at 2000 rpm, for 1 hour. The supernatant with floating particles (free LF <1.6 g cm^{-3}) was poured onto a nitro-cellulose filter unit (0.20 μm), and filtered under vacuum. Ten (5 mm) glass beads and 40 mL of NaPT solution (1.6 g cm^{-3}) was added to the soil remaining in the centrifuge and the tubes were shaken horizontally for 16 hours at 60 oscillations per minute. After shaking, the particles adhering to the lid and tube walls were washed into suspension using more NaPT to make a final volume of 50 mL. The tubes were allowed to stand for 30 min before centrifuging at 2000 rpm for 1 hour. The supernatant with floating particles (oLF with a density <1.6g cm^{-3}) was poured onto a nitro-cellulose filter unit (0.20 μm), and filtered under vacuum. The NaPT was removed from the fLF and oLF by submerging the material at least four times in 80 mL deionized (DI) water and removing the leachate with a vacuum filtration system while adding more DI water using a squirt bottle to keep the LF material in suspension (Crow et al. 2007). The LF on the filter was backwashed into a weighed porcelain crucible and dried at 50°C until constant weight. The extraction was performed at least twice in each sample to ensure enough material for analyses. Duplicates were combined, finely ground in a ball mill and stored in amber glass vials for further analysis. The NaPT was recycled according to Six et al. (1999b) to avoid cross contamination of C between samples.

Analytical methods

- C and N analysis

A sub-sample of each aggregate size was finely ground to pass a 100 mesh sieve using a roller mill. Any recognizable plant material and/or $CaCO_3$ concretions (> 1 mm) were removed before grinding. Twenty milligrams of the ground aggregates and humin, 2 mg of humic materials, and 5 mg of light fraction were weighed for C and N concentration using an elemental analyzer (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA).

To better account for the variation in effective sampling depth and soil mass due to changes in soil bulk density over time, the cumulative mass approach described by Gifford and Roderick (2003) was used to compare C and N changes over time for each aggregate size. Carbon (g C m^{-2}) and N (g N m^{-2}) stock of each aggregate size were estimated for each of the three soil depth intervals based on the measured bulk density at the time of sampling, the C and N concentrations of the aggregates and the abundance of the aggregate class. Three reference soil masses were used to evaluate aggregate C and N changes over time (Equation 4, Gifford and Roderick, 2003): (i) the top 60 kg dry soil m^{-2} (approximately 0–5 cm depth), (ii) the top 200 kg dry soil m^{-2} (approximately 0–15 cm depth) and (iii) the top 400 kg dry soil m^{-2} (approximately 0–30 cm depth).

- Absorbance

Light absorption at 465 (A4) and 665 nm (A6) was measured in duplicate for humic acids fractions from 2005 and 2008 (all depths) with a Spectronic® 20 Genesys™ spectrophotometer. Approximately, 1.5 mg and 3 mg of C as CaHA and MHA, respectively, were dissolved in 10 mL 0.05N $NaHCO_3$ (Chen et al. 1977). Molar absorptivity (E) for each wavelength was calculated as: E=A/c*l, where c is the C concentration of the solution (g C L^{-1}) and l is the length of the cuvette (1 cm). The E4/E6 ratio was calculated by dividing the absorbance of a sample at 465nm by that at 665nm. Molar absorptivity at 465 nm (E4) and E4/E6 ratio has been used as an indicator of the molecular weight, degree of humification and chemical recalcitrance of the humic material to microbial decomposition (Chen et al. 1977, Zech et al. 1997, Olk et al. 1999, Ve et al. 2004, Mahieu et al. 2002).

- Monosaccharides

The monosaccharide composition of all soil aggregates classes in 2005 and 2008 was determined for all depths following the weak acid extraction of the optimized procedure by Martens and Loeffelmann (2002). Briefly, 100 mg soil was treated with 6M H_2SO_4 for 30 min in culture tubes, then diluted with 4 mL DI water and autoclaved at 121ºC for 30 min. The hydrolysates were diluted with DI to 10 mL, their pH values were adjusted to 5 with NaOH, and they were centrifuged at 2500 rpm. The monosacharides in the supernatant (fucose, arabinose, rhamnose, galactose, glucose, xylose, and mannose) were separated on a Dionex DX-500 (Dionex Corp., Sunnyvale, CA) anion chromatograph with a CarboPac PA10 column. The ratio of microbial to plant monosaccharides was approximated as (galactose + mannose) / (xylose + arabinose) (GM/XA) (Oades, 1984).

Statistical analysis

Equality of the means among years was tested with repeated measurements (RM) ANOVA to account for the nature of the field sampling procedures and the natural variability among individual IMZs. The comparisons of C and N stocks among years for each aggregate size and for the SOM C and N stocks in each aggregate class were done using a one-way RM ANOVA (year) (Table 3.1). Fully crossed three-way RM ANOVA (soil layer, aggregate size and year) was used to evaluate properties of aggregate (C and N concentrations, C:N ratio, monosaccharide composition and plant to microbial monosaccharide ratio, mass fraction) and of SOM fractions (C and N contents in aggregates as SOM fractions, C:N ratio, and E4 and E4/E6 ratio of humic acids) (Table 3.1). When the three-way interaction was significant, the differences among years were analyzed for each combination of aggregate size and depth. Post-hoc separation of means was done with Fisher LSD procedure if overall ANOVA indicate significant differences ($p<0.05$). Data were analyzed using PROC MIXED procedures in SAS 9.3 (SAS Institute 2009), with IMZ as the random effect and year as the repeated effect. Linear regression analysis of aggregate C content as SOM fractions as a function of aggregate C concentration were performed with SigmaPlot® 11.0 software (Systat Software, Inc. 2008).

4. Results

Aggregate size distribution

About 50 percent of the soil mass was recovered as >250 µm aggregates in all depths in 2005 after wet sieving fractionation (Table 3.2). Given the significance of the three-way interaction ($p<0.001$), the changes in the proportion of each aggregate class among years were analyzed for each combination of aggregate size and depth. After three years of tillage, the proportion of the largest aggregates decreased in the 0-5 cm (-36%) and 5-15 (-15%) cm soil depths, with no changes in the 15-30 cm depth (+2%) (Table 3.2). From 2005 to 2008, the proportions of both mid-size (250-53 µm) and small-size (<53 µm) aggregates increased in the 0-5 cm depth (+23% and +72% respectively), perhaps as a consequence of either breakdown of larger aggregates or mixing of soil layers, while in the 5-15 cm soil depth only the proportion of the smallest aggregates increased (+41%). In the 15- 30 cm soil depth, the proportion of mid-size aggregates decreased in 2008 by 15% compared to 2005, which was accompanied by a slight numeric increase in smaller aggregates (+26%, $p>0.05$).

C and N concentrations in aggregates

Carbon and N concentrations of the aggregates decreased with decreasing aggregate size at each soil depth (Tables 3.4 and 3.5). In 2005, aggregate C and N concentrations were especially stratified in the uppermost depths. Given the significance of the three-way interaction ($p<0.001$) the changes in C and N concentration among years were analyzed for each combination of aggregate size and soil depth. After three years of tillage, aggregate C concentration decreased in the largest and mid-size aggregates in the

0-5 cm depth by 15% and 14%, respectively, and increased in both aggregate classes in the 5-15 cm depth by 22% and 13%, respectively. Similarly, compared to 2005, aggregate N concentration in 2008 decreased in the largest and mid-size aggregates in the 0-5 cm depth by 13% and increased in both aggregate classes in the 5-15 cm depth by 17% and 13%, respectively. Small-size aggregates showed similar trends as the largest aggregates at both depths, although levels of significance were much less ($p>0.05$ for C concentration, and $p<0.05$ for N concentration in both depths). For the 15-30 cm depth, C and N concentrations for each aggregate class were similar among years (Tables 3.4 and 3.5). As a result of these trends, aggregate C and N concentrations in 2008 were similar between the 0-5 and 5-15 cm depths for mid-size and small-size aggregates, while the largest aggregates had more C and N content in the 0-5 cm depth than in lower depths.

The C:N ratio of aggregates narrowed with decreasing aggregate size in all depths, indicating the presence of less decomposed litter or root derived C in the larger aggregates and older, more degraded materials in the <53µm aggregates (Table 3.6). Similar to aggregate C and N concentrations, the three-way interaction was significant for the C:N ratio. The C:N ratio narrowed from 2005 to 2008 in all aggregate classes of the 0-5 cm depth and in the mid-size class of the 15-30 cm depth after tillage, while the CN ratio of the >250 aggregates increased with time in the 5-15 cm depth.

C and N stocks

Before tillage in 2005, >250 µm aggregates accounted for 62%, 54% and 54% of the C stocks in the 0-60, 60-200, and 200-400 kg soil m^{-2} layers, respectively (Table 3.3). In 2008, after three years of conservation deep tillage, >250 µm aggregates accounted for

43%, 49% and 54% of the C and N stocks in the same layers. The significant reduction over three years in cumulative C and N stocks of the >250 µm aggregates in the 0-60 kg soil m^{-2} layer was masked when expanded over the 0-400 kg soil m^{-2} layer (Table 3.2). Cumulative C and N stocks for the 250-53 µm aggregates did not vary significantly ($P>0.13$) among years (Table 3.2). The C and N stocks in the <53 µm aggregates increased from 2005 to 2008 by 59 and 67%, respectively, for the 0-60 kg soil m^{-2} layer, 49 and 50%, respectively, for the 0-200 kg soil m^{-2} layer, and 39 and 41% in the 0-400 kg soil m^{-2} layer (Table 3.3).

C and N content in SOM fractions of >250 and 250-53 µm aggregate classes

The fractionation of the largest and mid-size aggregates into fLF, oLF, MHA, CaHA and humin fractions recovered 82-96% of the C in large aggregates and 71-86% of the C in the mid-size aggregates (Table 3.4). These fractions together accounted for 66-76% and 57-73% of the N content in large- and mid-size aggregates, respectively (Table 3.5). The distributions of aggregate C and N for all aggregate size classes and years ranged from 56-71% and 47-59% as humin, 9-14% and 6-9% as CaHA, 2-10% and 2-10% as MHA, 1-6% and 0.3-3.8% as oLF, and 0.3-10 and 0.1-5.5% as fLF, respectively (Tables 3.4 and 3.5).

Changes over time in aggregate C and N content of SOM fractions were affected by depth and aggregate size (Tables 3.4 and 3.5). In general, the largest aggregates contained more C and N in extracted SOM fractions than did the mid-size aggregates (Tables 3.4 and 3.5). Trends over time and among aggregate size classes within each depth for each SOM fraction largely mirrored those for whole aggregates, as described above. Aggregate C and N contents as oLF and MHA showed a significant three-way interaction, while aggregate C and N content as fLF, CaHA and humin showed a significant depth by size interaction. From 2005 to 2008, aggregate C and N content in each SOM fractions of the 0-5 cm depth decreased by 7 to 35% ($p<0.05$), while increases of 9 to 120% ($p<0.05$) were observed for the 5-15 cm depth and of 2 to 110% for the 15-30 cm depth ($p<0.1$ for fLF and $p<0.05$ for all other fractions) (Tables 3.4 and 3.5). For aggregate C and N contents as oLF and MHA, the trends in the lower two depths were also affected by aggregate size, where the largest aggregates showed greater increases from 2005 to 2008 compared to mid-size aggregates. Depth by size interactions were associated with a larger difference in aggregate C and N content as SOM fraction between >250 and 250-53 aggregate classes in the 0-5 cm depth and smaller differences in the 15-30 cm depth (Table 3.3 and 3.4).

The aggregate C content extracted in SOM fractions as a function of aggregate C concentration (Figure 3.4) can be used to interpret the changes in aggregate C concentration among depths and aggregate classes (Table 3.4). As aggregate C increased, the masses of all SOM fractions also increased, but the slopes and y axis intercepts differed among SOM fractions, aggregate size and soil depth (Table 10). Within each depth, slopes in the largest aggregate size generally decreased in the order humin > CaHA > MHA > fLF > oLF, with the exception of MHA>CaHA in the 0-5 cm depth, while for the mid-size aggregates the two LF fractions were reversed (Table 3.10). The slope of the humin-C relationship was similar among depths for each aggregate class. The other SOM fractions had different slopes among depths within each aggregate class. Compared to the 0-5 cm layer, the slopes of the CaHA-C were larger in the 5-15 and 15-

30 depths for the >250 µm aggregates, and larger only in the 5-15 cm depth for the mid-size aggregates. The slope of the MHA-C and oLF-C decreased clearly with depth for each aggregate size, while that of the fLF-C decreased regularly with depth only for the >250 µm aggregates in the 15-30 depth. Across depths, the slopes of the MHA-C, oLF-C and fLF-C were greater for the >250 µm compared to 250-53 µm aggregates. The aggregate C contents as oLF and fLF were in general poorly associated with aggregate C content, with all coefficients of determination less than 0.50 (Table 3.10).

Large differences among the SOM fractions in their y axis intercepts reflected different ranges of aggregate C contents in which each SOM fraction accumulated C (Table 3.10). The large intercept of the humin fraction resulted from its significant C accumulation even at low aggregate C contents. The CaHA intercept was less negative than the MHA intercept for the entire (0-30 cm) profile only because a large difference in the 0-5 cm depth outweighed smaller differences of the opposite magnitude in the lower depths. In most cases the oLF and fLF had modest to negligible intercepts, probably due to their weak associations with aggregate C content. The y axis intercept did not change regularly with soil depth for any SOM fraction. Regarding aggregate size classes, the humin had clearly greater intercepts for the >250 µm aggregates than for the 250-53 µm aggregates, and in most cases the CaHA also had less negative intercepts for the >250 µm aggregates. Intercepts of the less tightly bound SOM fractions, fLF, oFL, and MHA, showed opposite trends.

Degree of humification of SOM fractions

Averaged across depths, years, and aggregate size classes, the C:N ratio of the SOM fractions increased in the order MHA (10.0) > humin (12.4) >CaHA (15.8)> fLF (19.8) = oLF (20.1) (Table 3.6). Similar to their C and N contents, the C:N ratio of the SOM fractions was simultaneously affected by depth, aggregate size and tillage management. In general, in all years the C:N ratio of the fLF ($p<0.05$), oLF ($p<0.05$), and CaHA fractions ($p>0.05$) was narrower in the 0-5 cm depth than in the 5-15 and 15-30 cm depths, while it narrowed with increasing soil depth for the MHA fraction ($p<0.05$) and was not affected by depth for the humin fraction ($p>0.05$). The C:N ratio of the fLF, oLF, and CaHA was significantly wider for mid-size aggregates than for the largest aggregates at all depths, while the C:N ratio of the MHA fraction was significantly narrower for mid-size aggregates compared to the largest aggregates at all depths. In the 0-5 cm depth, the magnitude of the changes in C:N ratio of SOM fractions among years was larger for the mid-size aggregates than for the >250 µm aggregates for oLF, MHA and humin, while for CaHA and fLF the changes in C:N ratio of the largest aggregates was greater than for mid-size aggregates. In the 5-15 and 15-30 cm depth, the C:N ratio of fLF, oLF and CaHA varied more in mid-size aggregates than in the largest aggregates while for MHA and humin, the C:N ratio of largest aggregates varied more than the midsize aggregates. From 2005 to 2008, the C:N ratio of the fLF, oLF and CaHA increased in the 0-5 cm depth and narrowed in the 15-30 cm depth, while at the 5-15 cm depth the C:N ratio declined from 2005 to 2007 before increasing in 2008 (Table 3.9). The trends among years in the C:N ratio of the humin fraction for the 5-15 cm depth were similar to those of the CaHA fraction. By contrast, from 2005 to 2008 the C:N ratio of the MHA narrowed in the 0-5 cm depth, and increased in the 5-15 and 15-30 cm depths.

Light absorption of the MHA fraction indicated a less humified pool in all depths and aggregates sizes compared to the CaHA fraction (Table 3.7). The E4/E6 ratio was larger (i.e. less humified) in 2008 compared to 2005 for the MHA fraction across depths and aggregate sizes ($p<0.05$), and for the CaHA fraction in the 15-30 cm depth (Table 7). Molar absorptivity at 465 nm (E4) of both the MHA and CaHA was more sensitive than was the E4/E6 ratio, as evidenced by significant interactions between depth, time and aggregate size. In general, E4 values of both fractions increased with depth ($p>0.05$), suggesting a greater degree of humification, and was larger in mid-size aggregates compared to larger aggregates for all three depths. For the MHA fraction, the depth by size ($p<0.05$), size by time ($p<0.05$) interactions of the E4 values were further analyzed by aggregate size. For the 250-53 μm aggregates, E4 values were similar among depths and years ($p>0.05$, Table 3.7). The E4 values of the MHA in >250 μm aggregates, were smaller in the 5-15 and 15-30 cm depth in 2008 compared to 2005, with no change in the 0-5 cm depth between years. The significant depth by size and depth by time interactions for E4 of the CaHA fraction were also further analyzed by depth (Table 3.7). The differences in E4 values between the >250 and 250-53 μm aggregate classes decreased with depth, similarly to MHA (Table 3.7). From 2005 to 2008, the E4 values of the CaHA fraction increased in the 0-5 cm depth ($p<0.05$), decreased slightly in the 5-15 cm depth ($p>0.05$) and decreased yet more in the 15-30 cm depth ($p<0.05$) (Table 3.7).

Monosaccharide composition of aggregate classes

The monosaccharide concentrations of aggregates decreased with aggregate size (Table 3.8). The depth by time interaction across monosaccharides followed the trend of C concentration and indicated a change from stratified monosaccharide concentration in 2005, with larger monosaccharide concentrations in the 0-5 cm depth, to a more homogenous distribution of monosaccharide concentration across all depths in 2008. The ratio GM/XA across all replicates ranged from 0.64 to 0.99. Given the significant depth by time and size by time interactions, the data were further analyzed by year. In 2005, the GM/XA ratio had a significant depth by size interaction ($p=0.039$); the >250 μm aggregates had a greater proportion of plant-derived monosaccharides than did the 250-53 and <53 μm aggregates in the 0-5 cm soil layer, while the GM/XA ratio did not differ significantly among aggregate classes at lower depths. In 2008, the >250 μm aggregates had a greater proportion of plant-derived monosaccharides than did the 250-53 and <53 μm aggregates at all depths ($p < 0.001$) and aggregates in the 0-5 and 5-15 cm depths had greater proportions of plant-derived monosaccharides than those in the 15-30 cm depth ($p = 0.001$). Compared to 2005, the largest aggregates had a significant increase in the proportion of plant-derived monosaccharides at all depths, while the smallest aggregates had an increase in the proportion of microbial-derived monosaccharides in 2008. Principal component analysis of carbohydrate concentrations found that nearly all carbohydrates followed similar trends across depths, aggregate sizes, and years (data not shown).

5. Discussion

Stabilization of SOM within the soil matrix constitutes a crucial step for C sequestration. Integrated fractionation approaches are required to study the complexity of SOM stabilization within soil structure. We use the distributions of C and N contents in aggregates, their extracted SOM fractions, and the characteristics of the SOM fractions as influenced by a change in tillage management to test the hypothesis that incorporation of maize residue and fertilizer N with fall conservation deep tillage can increase the stabilization and persistence of C and N from crop residues (Chapter 2).

Mechanisms of C and N stabilization by aggregate classes

The main mechanism of SOM stabilization of individual aggregates may depend on the size of the aggregate (Six et al. 2002). Increasing aggregate size is associated with a greater content of occluded non-mineral associated SOM (Golchin et al. 1994, Six et al. 1999). The proportion of aggregate C extracted as fLF was several-fold greater in the largest aggregates than in the 250-53 µm aggregates, namely by 9, 6 and 4 times for the 0-5, 5-15 and 15-30 cm depths, respectively in 2005 and 13, 14, and 6 times for the same depths in 2008. Corresponding ratios for the oLF were 2.3, 1.9, and 1.4 times for 2005 and 2 times for 2008 across depths, respectively. Similar to Chapter 5, the reduction in the amount of LF and increased difference in LF C:N ratio between aggregate classes with increasing depth under no-till may indicate that C inputs as a driving force for SOM stabilization inside aggregates is diluted as the distance to the soil-residue interface increases. The larger differences between aggregates for the fLF may be associated with unwanted movement of the lighter material into suspension during wet sieving fractionation of aggregates (see discussion in Chapter 5). Preliminary extractions of free and occluded LF from the <53 µm aggregates (not shown) did not yield enough fraction to be detectable (less than 0.5 mg fraction in 10 g aggregates). Consequently, the mechanism for SOM stabilization in the <53 µm aggregates was attributed to organo-mineral associations.

Nonetheless, the greater content of occluded non-mineral associated SOM with increased aggregate size does not completely account for the increased C content of larger aggregates. The largest aggregates had greater proportions of aggregate C as MHA, similar proportions as humin, and smaller proportions as CaHA, compared to mid-size aggregates (Figure 3.4). Under no-tillage in 2005, the amount of MHA-C that was extracted from the largest aggregates was 1.4, 1.5 and 1.1 times greater than the MHA-C from the mid-size aggregates in the 0-5, 5-15 and 15-30 cm depths, respectively. The corresponding values for the CaHA were 0.9, 1.2 and 1.0 respectively, and for the humin they were 0.96, 1.1, and 0.96. These indicate comparable adsorption rates of CaHA and humin among aggregate size classes but a preferential stabilization of MHA in the largest aggregates in the upper two depths, similar to the results presented in Chapter 5. Regardless of aggregate size, the onset of each stabilization mechanism seems to be regulated by the C concentration of the aggregate: the onset of accumulation increased in the order humin < CaHA < MHA. The later onset in MHA accumulation compared to CaHA, together with the preferential accumulation of MHA above the accumulation threshold is perhaps indicative of a competitive stabilization process between these two humic fractions where the threshold is governed by the capacity for formation of new organo-mineral associations. Our results agree with the increased capacity for SOM stabilization by physical protection with increasing aggregate size as proposed by Six et al. (2002) and Gulde et al. (2008). Nonetheless, the increased proportion of MHA-C and lack of difference in the proportion of humin-C in the largest aggregates compared to the mid-size aggregates suggests that the increased capacity to stabilize SOM by the largest aggregates is due to both physical occlusion and humification mechanisms (Figure 3.4).

Tillage effects on C and N stabilization by aggregate classes.

- Aggregate size distribution

The change from no-till to conservation deep tillage influenced the aggregate size distribution by mixing soil layers and through the breakdown and repacking of aggregates (Angers and Giroux 1996, John et al. 2005). One year after the change in tillage management, the increased mass of the largest aggregates in the 5-15 cm and 15-30 cm depths accounted for about 60% more than their loss in the 0-5 cm depth. This net gain in largest aggregates after one year of conservation deep tillage indicates both mixing of the soil layers and repacking of aggregates. This increase in aggregation may be associated with the volume of maize residues and litter incorporated during the 2005 fall tillage operation. Similarly, Kong et al. (2005) found a positive linear relationship between aggregation and C inputs. Nonetheless, by 2008, the mass of largest aggregates decreased in the 0-5 (-34%) and 5-15 cm (-14%) depths compared to 2005, which was not compensated by the increase in the 15-30 cm depth. The significant reduction in the mass of larger aggregates with tillage is in agreement with previous studies (Yamashita et al. 2006, Six et al. 1999). The net reduction in mass of the largest aggregates indicates that after three years of deep incorporation of crop residues into the soil profile, the detrimental effect of tillage on aggregate stability is larger than any positive effect on aggregation due to increased C inputs.

- Monosaccharides

The change from no-till to conservation deep tillage was hypothesized to increase the stabilization and persistence of C and N from the residues. In Chapter 2, the increase in the soil−crop residue interface by conservation deep tillage together with addition of N fertilizer favored the persistence of fLF deeper into the soil profile and promoted stabilization of crop residues into humus. In this study, the overall increase in the monosaccharide abundance (plant and microbial derived) with depth in 2008 is indicative of increased C inputs at those depths brought about by the change in tillage management. The changes in xylose and arabinose were used as indicators of plant derived C, while changes in mannose and galactose were used as indicators of microbial derived C (Oades, 1984). By 2008, stocks of the plant derived monosaccharides xylose and arabinose in the <53µm aggregates increased by 82 and 71% in the 0-60 kg soil m^{-2} layer, 79 and 63% in the 60-200 kg soil m^{-2} layer and 52 and 29% in the 200-400 kg soil m^{-2} layer. Although arabinose may originate from the microbial biomass, the increase in both xylose and arabinose indicates stabilization of decomposing plant residues in soil (Murayama, 1984). Our findings also agree with the greater transfer of residue derived C to finer soil fractions when residues are mixed with the soil compared to surface mulching reported by Stemmer et al. (1999). The increase in the proportion of microbial derived monosaccharides in the small-size aggregates was explained by an even larger increase in the stocks of mannose and galactose at all depths by 2008. Given the negligible recovery of fLF and oLF from the smallest aggregates, stabilization of maize residues largely as organo-mineral complexes of microbial origin contributed to the increase in C stocks of the <53 µm aggregate class.

Monosaccharide stocks in mid- and large size aggregates also indicated stabilization of residue C in these aggregate classes. By 2008, C stocks in the largest aggregates decreased in the 0-60 and 60-200 soil layers with no changes in the 200-400 kg soil m^{-2} layer. Although stocks of xylose in the largest aggregates decreased by 43% in the 0-60 kg soil m^{-2} layer, increases in the 60-200 kg soil m^{-2} layer (+61%) and the 200-400 kg soil m^{-2} layer (+54%) led to an overall net gain of this monosaccharide (+27%) indicating new stabilization of maize derived C by the largest aggregates over and above mixing of the soil layers. Concentrations of arabinose, mannose and galactose were also increased in the largest aggregates at the lower two depths in 2008 compared to 2005. Nonetheless, stocks of arabinose, mannose, and galactose in the largest aggregates in the 0-400 kg soil m^{-2} layer did not differ in 2008 compared to 2005, indicating that the increased stabilization of maize residues with depth in the largest aggregates only compensated for the reduction in the surface layer over the four year period.

Interestingly, although there were no significant changes in C stocks of the mid-size aggregates by 2008, there were changes in monosaccharide composition. Reduced xylose (-16%) stocks in the 0-60 kg soil m^{-2} layer, increased xylose (+82%), arabinose (+42%), mannose (+46%) and galactose (+49%) stocks in the 60-200 kg soil m^{-2} layer, and the reduction in all four monosaccharides (-12 to -25%) in the 200-400 kg soil m^{-2} layer, resulted in a net gain in xylose, arabinose, and galactose stocks in mid-size aggregates for the 0-400 kg soil m^{-2} layer. Thus, stabilization of maize derived C was also favored in this aggregate size class. However, the changes over time in the proportion of aggregates indicate that the stabilization of new C inputs in mid-size aggregates is limited compared to the largest aggregates.

- Occluded Light fraction

The results presented in this study demonstrate that conservation deep tillage with the addition of N fertilizer increases mid- and large size aggregate turnover and favor the stabilization of more humified SOM as oLF into both aggregate classes. Loss of occluded SOM inside mid- and large-size aggregates is considered the main mechanism of C loss caused by aggregate breakdown during tillage (John et al. 2005). The lack of changes with tillage in oLF-C and -N stocks in the 0-400 kg m^{-2} soil layer (Chapter 2); however, was contrary to this expectation. In this study, both aggregate classes in the 5-15 and the largest aggregates in the 15-30 cm depths exhibited a gradual increase over time in C and N contents of oLF compared to 2005. These trends agree with the increased abundance of plant and microbial derived monosaccharides discussed previously. The gradual increase in the C:N ratio of the oLF inside water stable aggregates from 2005 to 2008 in the 0-5 cm depth may be the result of mixing of 0-5 and 5-15 cm layers. The decrease from 2005 to 2008 in the C:N ratio in the 5-15 and 15-30 cm depths was accompanied by an increase in aggregate C as oLF (Table 4) greater than what expected by mixing of the layers (net gain in C as oLF) and was accompanied by an even larger increase in aggregate N mass as oLF (Table 3.5). Thus not only is redistribution of oLF in the soil profile occurring, but more humified SOM is being stabilized as oLF into the mid- and large size aggregates in deeper soil layers.

The increased stabilization of oLF (greater oLF-C and –N stocks in the 0-400 kg soil layer m^{-2}) is indicative of repacking of aggregates. Despite the fact that tillage induces the breakdown of aggregates, reduced aggregate turnover under no-till can constrain stabilization of new oLF (Yoo and Wander 2008) in all aggregate fractions compared to tillage (Plante and McGill 2002). The GM/XA monosaccharide ratio indicated preferential enrichment of plant-derived carbohydrates in large compared to mid-size aggregates in the 5-15 cm depth, and only in the largest aggregates in the 15-30 cm depth, suggesting limited stabilization of new crop residue into midsize aggregates. The similar trends in aggregate C and N content as oLF suggest that conversion to conservation deep tillage with addition of N fertilizer promotes stabilization of maize derived SOM as oLF perhaps by increasing aggregate turnover (e.g. breakdown and repacking of aggregates).

- Humic acids and humin fraction

Conversion from no-till to conservation deep tillage also impacted the amount and distribution of MHA, CaHA and humin fractions. After three years of tillage, the C and N contents of each humic fraction decreased within both aggregate classes in the 0-5 cm layer and increased in both aggregate size classes in the lower two depths, which agrees with the results presented in Chapter 2. In 2008, the amount of MHA-C that was extracted from the largest aggregates was 1.7, 1.7 and 1.5 times that of the MHA-C extracted from mid-size aggregates for the 0-5, 5-15 and 15-30 cm depths, respectively. Corresponding values for the CaHA were 0.82, 0.84 and 0.85, and for humin they were 0.91, 0.96, and 0.94. These results demonstrate preferential stabilization of MHA in the largest aggregates, and suggest a shift in either the preferential adsorption of CaHA and humin materials to mid-size aggregates or preferential loss of CaHA and humin materials from the largest aggregates. When accounting for changes in soil bulk density and aggregate size distribution, the increase in humified C with depth in 2008 compared to 2006 was consistent with whole soil trends presented in Chapter 2 and with previous studies (Murage and Voroney, 2008). The increases in C and N stocks from 2006 to 2008 as CaHA in the 60-200 kg soil m^{-2} layer (+24 g C m^{-2} and +1 g N m^{-2}), and as MHA (+31 g C m^{-2} and +2.7 g N m^{-2}) and CaHA (+42 g C m^{-2} and +1.6 g N m^{-2}) in the 200-400 kg soil m^{-2} layer reported in Chapter 2 were attributed to newly humified maize residues. In this study, the loss in C stocks for the 0-400 kg soil m^{-2} layer from 2006 to 2008 as MHA and CaHA from the >250 µm aggregates (-11 g MHA-C m^{-2} and -42 g CaHA-C m^{-2}) was more than compensated by the increase in C stocks in 250-53 µm aggregates (+22 g MHA-C m^{-2} and +58 g CaHA-C m^{-2}).

The overall increase in MHA and CaHA stocks indicate that conversion to conservation deep tillage with addition of N fertilizer promotes stabilization of maize derived SOM as MHA and CaHA. As in Chapter 2, we determined greater sensitivity of E4 values to management than the E4/E6 ratios. The larger E4 values of both MHA and CaHA of mid-size aggregates compared to the MHA and CaHA of the largest aggregates indicates either more humified material is stabilized as aggregate size is reduced or slower aggregate turnover of mid-size aggregates compared to larger aggregates restricts the incorporation of newly humified material into mid-size aggregates. The similar E4 values over time for MHA in the 250-53 µm aggregates suggests that the increased mass of MHA in 250-53 µm aggregates of the 5-15 cm depth may be the result of mixing of the 0-5 and 5-15 cm depths. The E4 values of MHA in the >250 µm aggregates of the 5-15 and 15-30 cm depths indicates a less humified fraction in 2008 compared to 2005, while no changes were determined between years in the 0-5 cm depth. From 2005 to

2008, the largest aggregates in the 0-5 cm depth had 44% less MHA-C, and 48% and 44% more MHA-C in the 5-15 and 15-30 cm depths. From 2005 to 2008, E4 of MHA from the largest aggregates increased by 1%, 16% and 39% in the 0-5, 5-15 and 15-30 cm depths, respectively. The increase in MHA-C content with depth in the larger aggregates after three years of conservation deep tillage together with the lower E4 values can be partially attributed to mixing of soil depths due to tillage (nominal tillage depth was 25 cm). Nonetheless, the overall reduction in E4 values of MHA in both aggregate size classes for the 5-15 and 15-30 cm depths together with the increase in C stocks as MHA in the 200-400 kg soil m^{-2} layer indicates that maize residues deeper in the soil profile were also stabilized as humified MHA. The increase in CaHA-C in both aggregate classes for the 5-15 cm depth and lack of changes in aggregate CaHA-C in the 15-30 cm depth after three years of conservation deep tillage were also associated with a reduction in E4 values. As discussed previously, C stocks as CaHA significantly increased from 2006 to 2008. Thus, similar to the MHA fraction, tillage-induced mixing of soil layers and stabilization of new residue inputs into humified SOM largely explains the change in amount and distribution of CaHA in the soil profile.

The differences in aggregate C content as SOM fractions indicate larger aggregates as a preferential site for stabilization of newly humified C. The mid- and large size aggregates did not differ greatly in their rates of increase of CaHA-C or humin-C with increasing aggregate-C, indicating comparable adsorption rates and possibly adsorption mechanisms across both aggregate size classes, at least over the ranges of aggregate-C where both fractions gain mass. The increase in the slope of CaHA with depth (Table 3.10) may indicate larger availability of binding sites for stabilization of this fraction in lower depths. As previously presented, from 2005 to 2008 the aggregate C concentration in the 0-5 cm depth decreased for both large and mid-size aggregates, while in the 5-15 cm depth it increased for both aggregate classes and in the 15-30 cm depth it increased only for the largest aggregates ($p>0.05$). As aggregate C decreased in the 0-5 cm depth, MHA appeared less labile in the mid-size aggregates compared to larger aggregates. Nonetheless the increases in aggregate-C concentration of the 5-15 cm and 15-30 cm depths were accompanied by a yet larger increase in the MHA in the largest aggregates. These results along with the light absorption data and onset point of SOM accumulation with increasing aggregate C concentration, support larger water-stable aggregates as a preferential site for stabilization of newly humified maize residues, especially in the more loosely bound SOM fractions of the MHA, fLF, and oLF.

6. Conclusion

The change from no-till to fall conservation deep tillage had several effects on the capacity and mechanisms of SOM stabilization of aggregates. The different sets of variables; overall aggregate C and N composition, C and N composition of SOM fractions in each aggregate size class, and humic acid light absorption and aggregate associated monosaccharides, complemented each other in the interpretation of the SOM protection capacity and protection mechanisms of >250 µm, 250-53 µm and <53 µm aggregates. The change in tillage management resulted in the redistribution of aggregates (and their SOM) between the 0-5 and 5-15 cm depths, along with a breakdown of larger aggregates and repacking of aggregates as evidenced by the more uniform aggregate C content as SOM fractions in 2008 compared to 2005 and the more uniform light absorption of MHA and CaHA. Incorporation of new C inputs as uncomplexed and humified SOM fractions was measured in the 5-15 and 15-30 cm depths as an increase in C and N stocks as fLF, oLF, MHA and CaHA fractions, and monosaccharide stocks. The increase in monosaccharide stocks were accompanied by increase in xylose and arabinose, plant derived monosaccharides. The largest aggregates in the lower depths of the soil profile constituted a preferential site for stabilization of new C and N inputs by physical occlusion, while both >250 µm and 250-53 µm aggregates stabilized newly humified maize residues.

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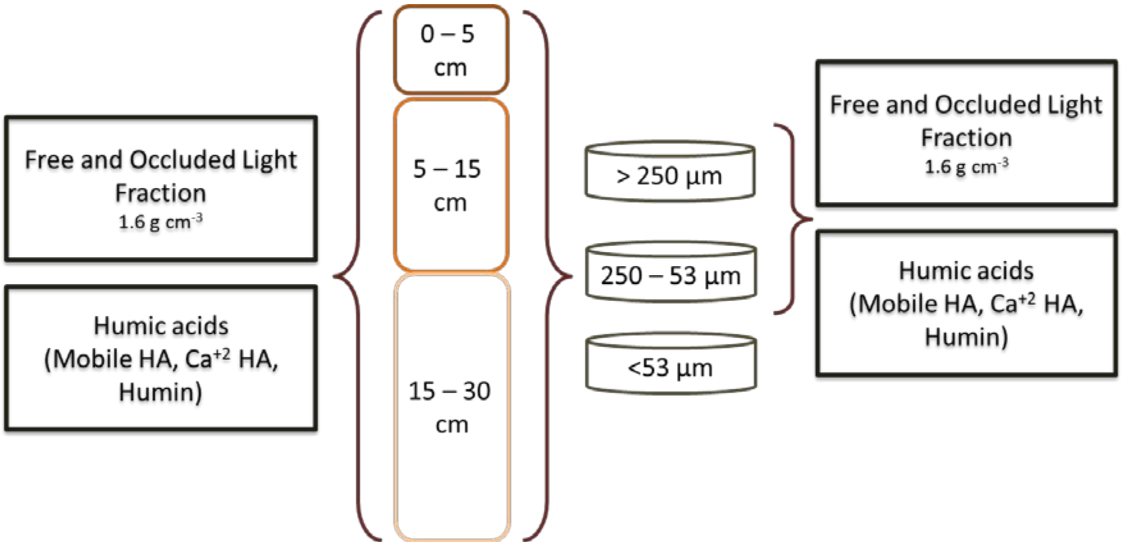

Figure 3. 1. Procedure for extraction of soil organic matter fractions

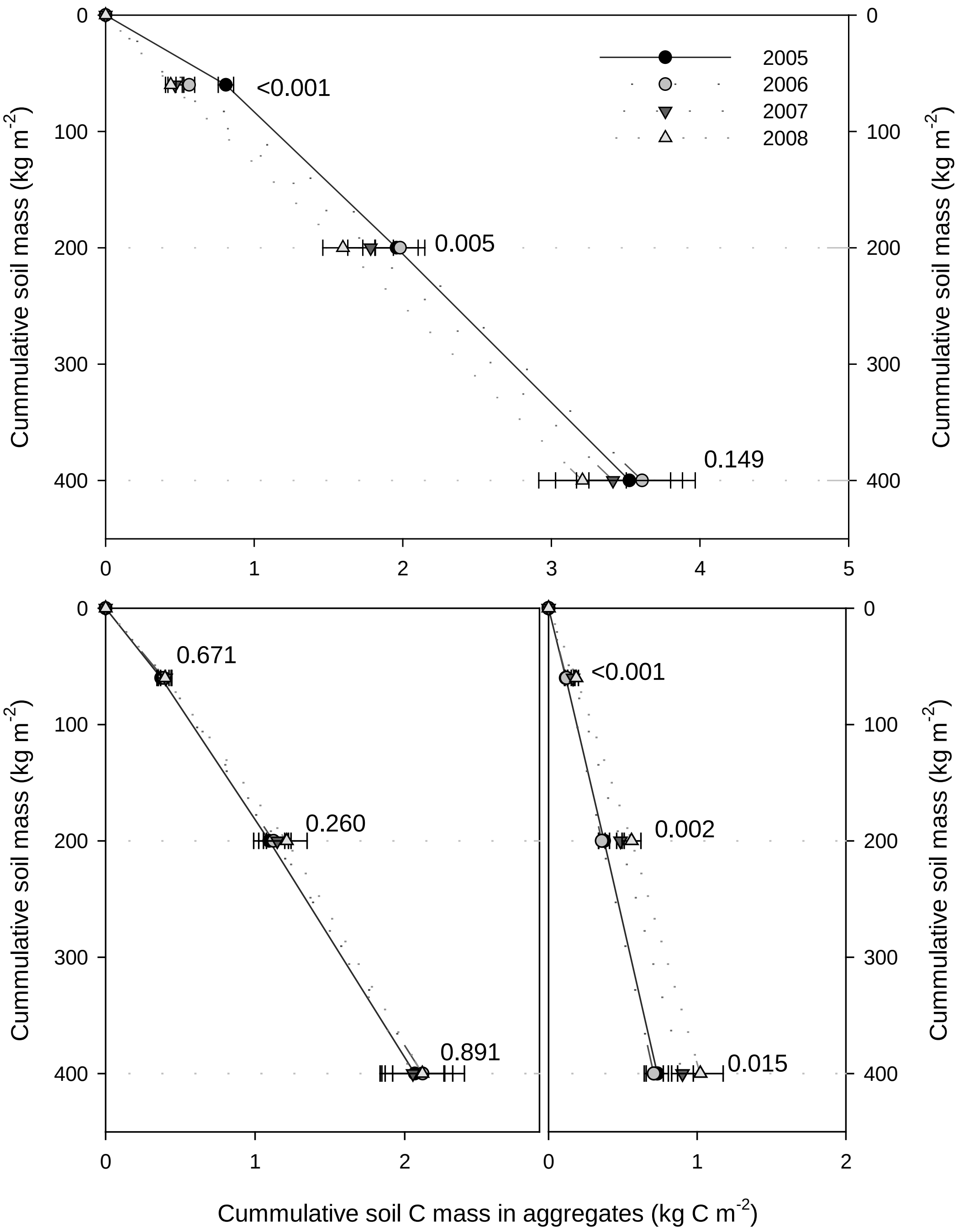

Figure 3. 2. Aggregate C stocks on an equivalent soil mass basis. Probability value (p>F) for comparison between years at each cumulative layer. Top: >250 µm aggregates, bottom left: 250-53 µm aggregates, bottom right: <53 µm aggregates.

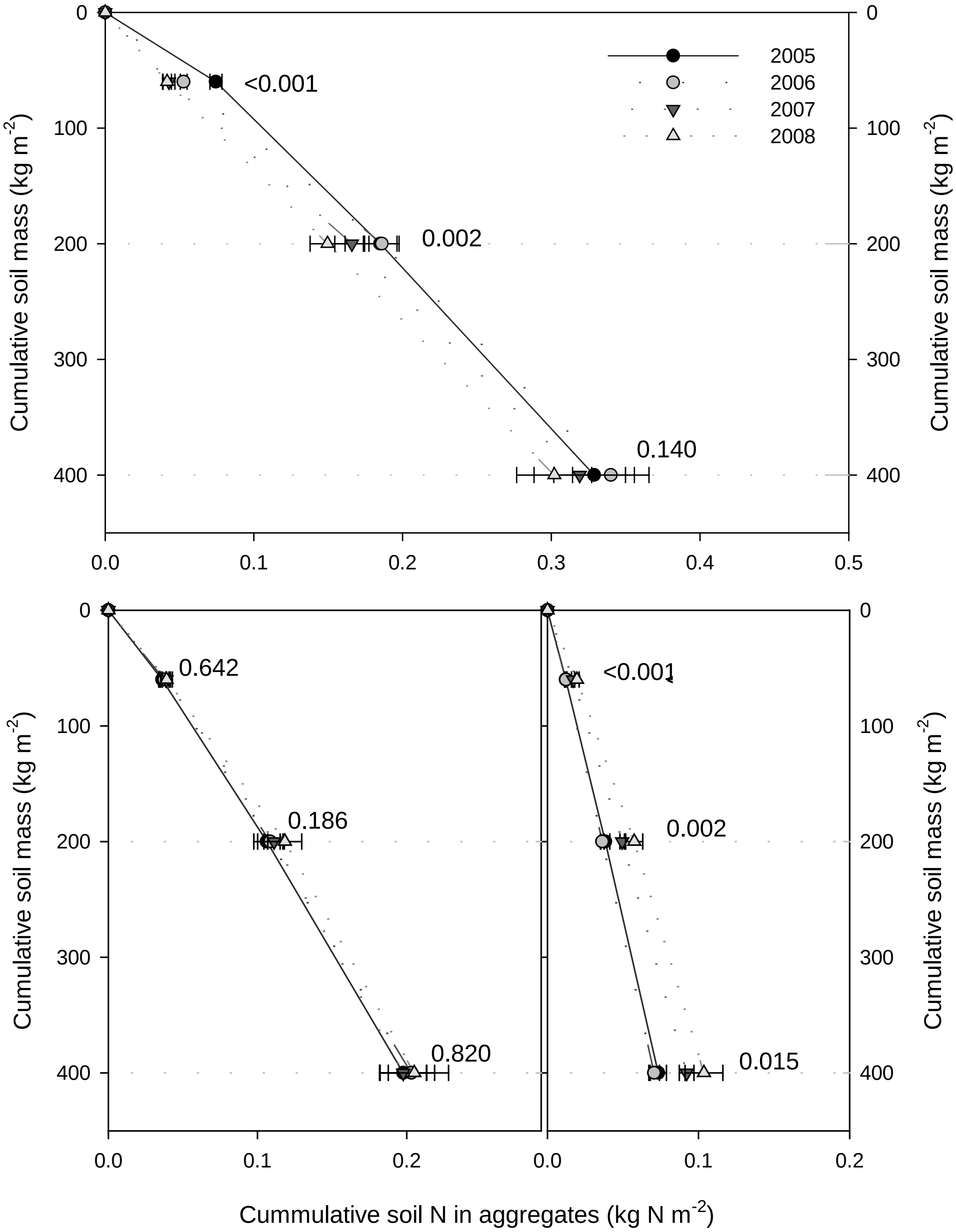

Figure 3. 3. Aggregate N stocks on an equivalent soil mass basis. Probability value (p>F) for comparison between years at each cumulative layer. Top: >250 µm aggregates, bottom left: 250-53 µm aggregates, bottom right: <53 µm aggregates.

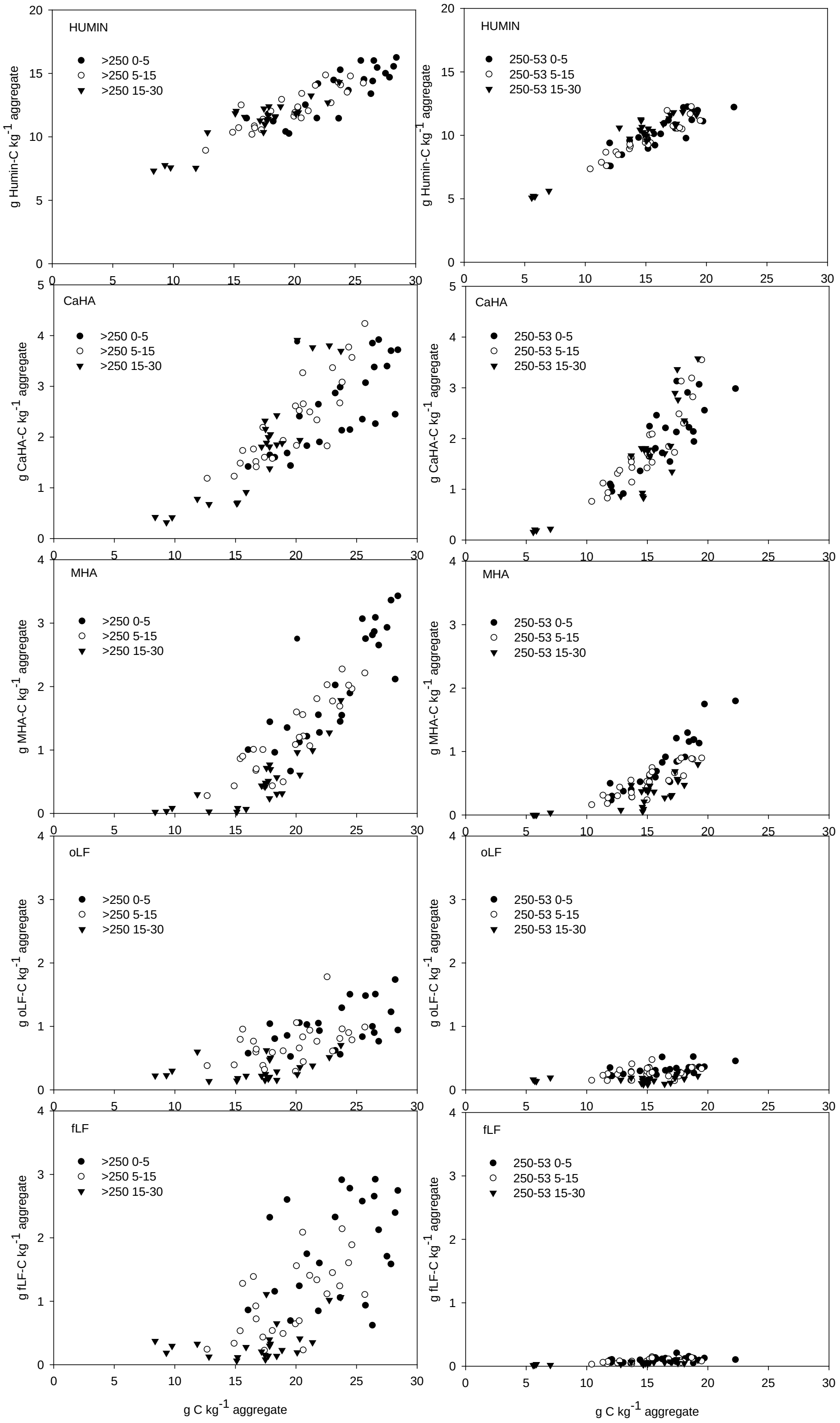

Figure 3. 4. Carbon content as soil organic matter fractions in relation to aggregate C content, size and soil depth.

Table 3. 1. ANOVA source of variation and degree of freedom (df) for one-way and three-way repeated measurements (RM) models.

One-Way RM[1]		Three-way RM[2]		Three-way RM[3]	
Source	df	Source	Df	Source	Df
Between IMZs	5	Between IMZs	5	Between IMZs	5
Between Years	3	Between Years (Y)	3	Between Years (Y)	3
Residual	15	Ea=IMZ*Y	15	Ea=IMZ*Y	15
Total	23	Between Depths (D)	2	Between Depths (D)	2
		Eb=IMZ*D	10	Eb=IMZ*D	10
		D*Y	6	D*Y	6
		Ec=IMZ*D*Y	30	Ec=IMZ*D*Y	30
		Between Size (S)	2	Between Size (S)	1
		Ed=IMZ*S	10	Ed=IMZ*S	5
		S*Y	6	S*Y	3
		Ee=IMZ*S*Y	30	Ee=IMZ*S*Y	15
		S*D	4	S*D	2
		Ef=IMZ*S*D	20	Ef=IMZ*S*D	10
		S*D*Y	12	S*D*Y	6
		Residual	60	Residual	30
		Total	215	Total	143

[1]: Used for Aggregate C and N stocks per soil layer
[2]: Used for Aggregates C and N concentration and C:N ratio
[3]: Used for SOM fractions C and N concentration and C:N ratio.

Table 3. 2. Aggregate size distribution per soil layer.

Depth (cm)	0-5			5-15			15-30		
Fraction (μm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
	---------- kg aggregate kg soil^{-1} ----------								
2005	0.512^{a}	0.339^{b}	0.148^{c}	0.475^{ab}	0.368	0.157^{ab}	0.467	0.364	0.170
2006	0.422^{b}	0.415^{a}	0.163^{c}	0.515^{a}	0.345	0.140^{b}	0.479	0.363	0.158
2007	0.350^{c}	0.434^{a}	0.216^{b}	0.462^{ab}	0.355	0.184^{ab}	0.470	0.331	0.199
2008	0.329^{c}	0.416^{a}	0.255^{a}	0.405^{b}	0.375	0.221^{a}	0.476	0.310	0.214
LSD	0.052	0.048	0.038	0.073	0.045	0.036	0.066	0.039	0.043
p-value(year)	<0.001	0.050	<0.001	0.040	0.515	<0.001	0.977	0.025	0.572

Means in the same column followed by same letter are not significantly different. p-value: probability (P > F) for differences among years by each depth by aggregate size combination. LSD: Fisher's least significant difference for comparisons among years.

Table 3. 3. Aggregate C and N stocks per soil layer.

Soil layer (kg m^{-2})	0-60			0-200			0-400		
Fraction (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
C stock	-- kg C m^{-2} --								
2005	0.810	0.372	0.117	1.958	1.094	0.374	3.526	2.068	0.732
2006	0.563	0.389	0.121	1.982	1.123	0.359	3.610	2.120	0.708
2007	0.468	0.404	0.165	1.784	1.148	0.484	3.415	2.055	0.902
2008	0.439	0.397	0.186	1.597	1.211	0.558	3.209	2.117	1.022
LSD	0.068	0.057	0.019	0.212	0.123	0.079	0.364	0.224	0.176
p-value (year)	<0.001	0.671	<0.001	0.005	0.260	0.002	0.149	0.891	0.015
N stock	-- kg N m^{-2} --								
2005	0.074	0.036	0.012	0.185	0.106	0.038	0.329	0.198	0.073
2006	0.053	0.038	0.012	0.186	0.109	0.036	0.340	0.203	0.071
2007	0.043	0.039	0.017	0.166	0.111	0.050	0.319	0.198	0.092
2008	0.042	0.039	0.020	0.150	0.118	0.058	0.302	0.205	0.104
LSD	0.006	0.005	0.002	0.019	0.012	0.008	0.033	0.021	0.017
p-value (year)	<0.001	0.642	<0.001	0.002	0.186	0.002	0.140	0.820	0.015

p-value: probability (P > F) for differences among years by each depth by aggregate size combination.
LSD: Fisher’s least significant difference for among years comparisons.

Table 3. 4. Aggregate C concentration and C content as SOM fractions.

Depth (cm)	0-5			5-15			15-30		
Size (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
Aggregate	-- g C kg aggregate^{-1} --								
2005	26.4	18.3	13.2	17.1	13.9	11.7	16.2	14.0	10.9
2006	22.1	15.9	12.8	20.5	15.4	12.1	16.7	14.1	11.3
2007	22.4	15.5	12.9	20.4	15.1	12.6	17.0	14.0	11.2
2008	22.5	15.7	12.4	20.9	15.7	12.2	17.3	14.5	10.9
LSD	2.6	1.0	0.8	1.7	0.5	0.7	1.4	0.9	0.7
p-value	0.009	<0.001	0.152	<0.001	<0.001	0.083	0.397	0.548	0.409
Free LF	-- g fLF-C kg aggregate^{-1} --								
2005	1.63	0.12		0.37	0.05		0.18	0.04	
2006	2.21	0.11		1.20	0.10		0.26	0.06	
2007	1.69	0.10		1.30	0.11		0.46	0.06	
2008	1.53	0.08		1.11	0.06		0.39	0.05	
LSD	1.02	0.04		0.53	0.03		0.29	0.02	
p-value	0.320	0.202		0.003	0.155		0.032	0.277	
Occluded LF	-- g oLF-C kg aggregate^{-1} --								
2005	1.47	0.45		0.37	0.16		0.20	0.12	
2006	1.00	0.28		0.76	0.30		0.29	0.15	
2007	0.80	0.27		0.77	0.27		0.34	0.16	
2008	0.99	0.31		0.82	0.30		0.42	0.18	
LSD	0.39	0.06		0.18	0.05		0.15	0.05	
p-value	0.008	<0.001		<0.001	0.009		0.019	0.221	

Depth (cm)	0-5			5-15			15-30		
Size (μm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
MHA	---------- g MHA-C kg aggregate^{-1} ----------								
2005	2.60	1.25		0.74	0.40		0.37	0.28	
2006	1.97	0.69		1.28	0.47		0.43	0.25	
2007	1.72	0.67		1.35	0.58		0.48	0.32	
2008	1.81	0.76		1.41	0.62		0.66	0.37	
LSD	0.49	0.18		0.35	0.09		0.19	0.07	
p-value	0.015	<0.001		0.009	<0.001		0.017	0.010	
CaHA	---------- g CaHA-C kg aggregate^{-1} ----------								
2005	2.82	2.17		1.91	1.32		1.86	1.61	
2006	2.12	1.66		2.23	1.77		1.77	1.45	
2007	2.66	1.95		2.70	1.97		1.86	1.55	
2008	2.51	2.13		2.50	2.23		1.77	1.75	
LSD	0.33	0.34		0.35	0.29		0.26	0.27	
p-value	0.003	0.025		0.002	<0.001		0.767	0.168	
Humin	---------- g Humin-C kg aggregate^{-1} ----------								
2005	14.92	10.81		11.04	7.94		10.71	9.64	
2006	13.97	10.68		13.06	9.77		11.48	9.76	
2007	12.93	9.88		11.79	9.70		11.04	9.94	
2008	12.62	9.64		12.71	9.94		11.29	10.05	
LSD	1.619	0.634		0.795	2.478		0.796	0.445	
p-value	0.032	0.002		<0.001	0.309		0.235	0.253	

p-value: probability (P > F) for differences among years by each depth by aggregate size combination. LSD: Fisher's least significant difference for among years comparisons.

Table 3. 5. Aggregate N concentration and N content as SOM fractions.

Depth (cm)	0-5			5-15			15-30		
Size (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
Aggregate	---------- g N kg aggregate^{-1} ----------								
2005	2.425	1.777	1.367	1.652	1.353	1.192	1.500	1.298	1.062
2006	2.112	1.537	1.305	1.885	1.485	1.228	1.588	1.330	1.110
2007	2.050	1.498	1.317	1.907	1.465	1.283	1.602	1.327	1.120
2008	2.132	1.550	1.292	1.940	1.528	1.242	1.635	1.392	1.088
LSD	0.218	0.103	0.075	0.129	0.049	0.067	0.125	0.074	0.061
p-value	0.008	<0.001	0.182	<0.001	<0.001	0.070	0.171	0.096	0.225
Free LF	---------- g fLF-N kg aggregate^{-1} ----------								
2005	0.100	0.007		0.019	0.002		0.009	0.002	
2006	0.116	0.006		0.060	0.005		0.014	0.003	
2007	0.096	0.005		0.074	0.005		0.024	0.003	
2008	0.081	0.004		0.058	0.003		0.020	0.002	
LSD	0.049	0.001		0.026	0.002		0.011	0.001	
p-value	0.506	0.030		0.003	0.012		0.042	0.082	
Occluded LF	---------- g oLF-N kg aggregate^{-1} ----------								
2005	0.092	0.025		0.021	0.007		0.009	0.005	
2006	0.058	0.014		0.042	0.014		0.015	0.006	
2007	0.047	0.013		0.044	0.013		0.017	0.007	
2008	0.056	0.016		0.046	0.015		0.022	0.008	
LSD	0.021	0.004		0.011	0.003		0.007	0.002	
p-value	0.002	<0.001		<0.001	<0.001		0.013	0.004	

Depth (cm)	0-5			5-15			15-30		
Size (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
MHA	g MHA-N kg aggregate^{-1}								
2005	0.2318	0.1198		0.0736	0.0413		0.0380	0.0298	
2006	0.1723	0.0683		0.1161	0.0476		0.0448	0.0273	
2007	0.1535	0.0665		0.1237	0.0598		0.0471	0.0352	
2008	0.1646	0.0743		0.1269	0.0609		0.0641	0.0394	
LSD	0.038	0.016		0.029	0.009		0.016	0.007	
p-value	0.002	<0.001		0.005	<0.001		0.021	0.013	
CaHA	g CaHA-N kg aggregate^{-1}								
2005	0.192	0.140		0.121	0.080		0.102	0.084	
2006	0.142	0.105		0.145	0.111		0.102	0.083	
2007	0.170	0.121		0.170	0.122		0.105	0.090	
2008	0.164	0.130		0.159	0.133		0.108	0.099	
LSD	0.021	0.021		0.019	0.014		0.015	0.014	
p-value	0.001	0.018		<0.001	<0.001		0.755	0.098	
Humin	g Humin-N kg aggregate^{-1}								
2005	1.219	0.915		0.909	0.637		0.842	0.747	
2006	1.111	0.883		1.047	0.821		0.909	0.755	
2007	1.031	0.820		0.971	0.812		0.872	0.777	
2008	1.010	0.784		0.995	0.799		0.871	0.789	
LSD	0.111	0.053		0.063	0.196		0.055	0.033	
p-value	0.005	<0.001		0.003	0.187		0.119	0.051	

p-value: probability (P > F) for differences among years by each depth by aggregate size combination. LSD: Fisher's least significant difference for among years comparisons.

Table 3. 6. Aggregate and SOM fractions C:N ratio.

Depth (cm)	0-5			5-15			15-30		
Size (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
Aggregate									
2005	10.86	10.27	9.63	10.32	10.23	9.73	10.65	10.57	10.00
2006	10.63	10.30	9.75	10.57	10.30	9.81	10.40	10.37	9.98
2007	10.84	10.32	9.71	10.64	10.24	9.77	10.43	10.30	9.71
2008	10.53	10.06	9.49	10.73	10.22	9.72	10.48	10.24	9.78
LSD	0.220	0.179	0.155	0.233	0.159	0.113	0.266	0.163	0.249
p-value	0.024	0.026	0.024	0.008	0.700	0.330	0.244	0.003	0.086
Free LF									
2005	16.44	18.07		19.13	21.24		20.49	24.15	
2006	18.91	19.25		19.59	21.12		18.57	23.27	
2007	17.66	19.42		17.39	19.49		18.64	20.78	
2008	18.70	19.77		19.25	21.77		20.08	22.92	
LSD	1.739	1.824		1.488	1.928		1.935	1.956	
p-value	0.031	0.260		0.028	0.112		0.112	0.015	
Occluded LF									
2005	16.01	18.02		17.84	22.80		21.41	27.67	
2006	17.24	19.71		17.98	21.58		19.31	25.33	
2007	17.14	20.37		17.59	20.75		19.85	23.56	
2008	17.51	19.87		17.92	20.41		19.13	23.08	
LSD	0.803	0.887		0.913	0.948		1.280	1.495	
p-value	0.006	<0.001		0.809	<0.001		0.007	<0.001	

Depth (cm)	0-5			5-15			15-30		
Size (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
MHA									
2005	11.17	10.37		9.92	9.45		9.07	8.93	
2006	11.22	9.92		10.79	9.65		9.12	8.70	
2007	11.05	9.93		10.80	9.66		9.35	8.78	
2008	10.92	10.11		10.87	10.03		9.62	9.16	
LSD	0.450	0.190		0.562	0.257		0.503	0.266	
p-value	0.509	<0.001		0.007	0.002		0.122	0.030	
CaHA									
2005	14.46	15.35		15.51	16.14		16.75	17.44	
2006	14.79	15.54		15.05	15.54		16.05	16.26	
2007	15.32	15.86		15.57	15.79		16.34	15.98	
2008	14.99	16.04		15.33	16.40		15.46	16.33	
LSD	0.605	0.609		0.500	0.451		0.922	0.887	
p-value	0.052	0.111		0.153	0.005		0.057	0.016	
Humin									
2005	12.24	11.81		12.11	12.33		12.66	12.79	
2006	12.52	12.05		12.46	11.85		12.56	12.77	
2007	12.51	12.02		12.14	11.91		12.62	12.63	
2008	12.46	12.62		12.77	12.42		12.89	12.62	
LSD	0.373	0.512		0.313	0.280		0.296	0.299	
p-value	0.379	0.355		0.001	<0.001		0.133	0.501	

p-value: probability (P > F) for differences among years by each depth by aggregate size combination. LSD: Fisher's least significant difference for among years comparisons.

Table 3. 7. Molar absorptivity at 465 nm (E4) and E4/E6 ratio of mobile humic acid (MHA) and calcium humic acid (CaHA) of >250 and 250-53 µm aggregates from all soil depths in 2005 and 2008 (n=6).

Depth (cm)	0-5			5-15			15-30		
Size (µm)	>250	250-53	Average	>250	250-53	Average	>250	250-53	Average
MHA					---------------------------- E4/E6 ----------------------------				
2005	8.36	8.61	8.49	9.19	9.29	9.24	8.17	8.28	8.23
2008	8.90	9.00	8.95	8.91	9.24	9.07	8.73	8.74	8.73
Average	8.63	8.80	8.72	9.05	9.26	9.15	8.45	8.51	8.48
CaHA									
2005	4.79	4.74	4.77	4.93	5.05	4.99	5.02	5.02	5.02a
2008	4.91	4.83	4.87	4.88	4.90	4.89	5.31	5.42	5.36b
Average	4.85	4.79		4.91	4.98		5.16	5.22	
MHA					------------------------------ E4 (g C L^{-1})$^{-1}$ -------------------------*				
2005	4.31aA	4.64a	4.43	4.80aAB	5.04a	4.92	6.36aB	6.04a	6.20
2008	4.34aA	5.06a	4.65	4.13bA	4.65a	4.39	4.58bA	5.32a	4.95
Average	4.33	4.83A		4.47A	4.84A		5.47	5.68A	
CaHA									
2005	6.01	7.28	6.64a	7.36	8.03	7.69a	7.84	8.40	8.12a
2008	6.66	7.79	7.23b	7.01	7.72	7.36a	7.10	7.67	7.39b
Average	6.33A	7.53B		7.18A	7.87B		7.47A	8.03B	

If size by time interaction was not significant (Table 2.9) then average columns are used for the main effect comparison. Otherwise, means in a column followed by same lower case letter (between years) are not significantly different. MHA: Means in a row within each aggregate size (depth comparison) followed by the same upper case letter do not differ significantly. CaHA: Means in a row within each depth (size comparison) followed by the same upper case letter do not differ significantly.

Table 3. 8. Carbohydrates in aggregates in 2005 and 2008 from all soil depths (n=6).

Depth (cm)	0-5			5-15			15-30		
Size (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
Fucose	-------------------------------- (µg carbohydrate g aggregate^{-1}) ------------------------------------								
2005	131	96	72	82	57	52	85	71	51
2008	118	80	68	114	89	64	108	75	52
Arabinose									
2005	1186	940	741	911	697	594	950	838	607
2008	1157	821	737	1111	972	687	1061	815	623
Rhamnose									
2005	416	351	278	284	224	176	294	262	171
2008	368	281	212	358	301	202	344	254	193
Galactose									
2005	923	711	512	660	489	410	693	589	413
2008	838	598	530	840	715	499	784	606	443
Glucose									
2005	1849	1418	965	1160	880	709	1183	1038	713
2008	1700	1131	889	1657	1313	809	1424	1044	710
Xylose									
2005	516	295	137	248	138	96	218	177	95
2008	455	202	145	470	246	122	330	175	115
Mannose									
2005	611	528	372	477	371	291	571	512	318
2008	579	429	394	580	530	362	592	448	348
Total	-- (µmol g^{-1}) --								
2005	34	26	18	23	17	14	24	21	14
2008	31	21	18	30	25	16	28	20	15
Ratio[1]	----------------------- (Galactose + Mannose) / (Xylose + Arabinose) ---------------------								
2005	0.74	0.83	0.84	0.82	0.86	0.85	0.90	0.90	0.89
2008	0.73	0.84	0.88	0.75	0.85	0.89	0.83	0.90	0.90

[1]: Ratio of microbial to plant derived carbohydrates (Oades, 1984)

Table 3. 9. Three-way repeated measurement ANOVA for a) aggregate C concentration and C content as SOM fractions, b) aggregate N concentration and N content as SOM fractions, c) aggregate and SOM fractions C:N ratio, d) monosaccharide concentration in aggregates, e) light absorption of humic acids.

Source	Aggregates	fLF	oLF	MHA	CaHA	Humin
a)			p > F			
Depth	0.018	<0.001	0.000	0.002	0.120	0.034
Size	<0.001	<0.001	<0.001	0.002	0.004	<0.001
Time	0.912	0.079	0.303	0.404	0.019	0.086
D*S	<0.001	<0.001	0.001	0.002	0.003	0.005
D*T	<0.001	0.098	<0.001	<0.001	<0.001	0.011
S*T	0.407	0.093	0.643	0.432	0.032	0.427
D*S*T	<0.001	0.117	0.001	0.004	0.791	0.716
b)			p > F			
Depth	0.0056	<0.0001	<0.0001	0.0014	0.0153	0.0029
Size	<0.0001	<0.0001	<0.0001	0.0016	0.0011	<0.0001
Time	0.6707	0.0928	0.3954	0.2629	0.0181	0.0695
D*S	<0.0001	<0.0001	0.0003	0.0016	0.0003	0.009
D*T	<0.0001	0.0519	<0.0001	<0.0001	<0.0001	0.0065
S*T	0.3076	0.1292	0.7261	0.3904	0.1238	0.2773
D*S*T	<0.0001	0.0765	0.0008	0.0056	0.7489	0.6139
c)			p > F			
Depth	0.883	<0.001	<0.001	0.135	0.301	0.109
Size	<0.001	0.001	<0.001	0.013	0.001	0.089
Time	0.151	0.059	0.018	0.109	0.202	0.014
D*S	0.009	0.001	0.010	0.125	0.015	0.035
D*T	0.002	0.017	<0.001	0.045	0.022	0.054
S*T	0.052	0.779	0.024	0.162	0.003	0.189
D*S*T	0.014	0.098	0.000	0.033	0.578	0.006

	Fucose	Arabinose	Rhamnose	Galactose	Glucose	Xylose	Mannose	Total	Ratio
d)				p > F					
Depth (D)	0.016	0.099	0.071	0.124	0.039	0.004	0.512	0.065	<0.001
Size (S)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Time (T)	0.352	0.303	0.596	0.314	0.327	0.367	0.535	0.334	0.649
D*S	0.214	0.009	0.688	0.105	0.002	<0.001	0.560	0.006	0.006
D*T	0.001	0.002	<0.001	0.002	<0.001	0.009	0.011	0.001	0.061
S*T	0.943	0.905	0.847	0.852	0.517	0.415	0.547	0.836	0.033
D*S*T	0.164	0.103	0.805	0.096	0.017	0.032	0.155	0.066	0.479

e)	df	df (error)	MHA		CaHA	
			E4	E4/E6	E4	E4/E6
Depth (D)	2	10	0.458	0.788	0.091	0.324
Size (S)	1	5	0.003	0.074	<0.001	0.692
Time (T)	1	5	0.018	0.033	0.095	0.038
D*S	2	10	0.016	0.699	0.004	0.252
D*T	2	10	0.052	0.141	0.003	0.020
S*T	1	5	0.009	0.591	0.879	0.988
D*S*T	2	10	0.231	0.321	0.907	0.284

Table 3. 10. Linear regression parameters for the composition of aggregate C as SOM fractions as a function of aggregate C concentration presented in Figure 3.4.

Depth (cm)		0-30			0-5			5-15			15-30		
Aggregate class	SOM fraction	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept	R^2	Slope	Intercept
>250 µm	Humin	0.83	0.401a	4.297a	0.68	0.402A	4.220X	0.83	0.417A	3.930X	0.82	0.418A	4.114X
	CaHA	0.69	0.175	-1.275a	0.61	0.164A	-1.292X	0.81	0.229B	-2.178X	0.76	0.246B	-2.307X
	MHA	0.79	0.175	-2.259	0.75	0.189A	-2.378X	0.78	0.160A	-1.961X	0.63	0.089B	-1.006Y
	oLF	0.44	0.058	-0.471a	0.17	0.043A	0.057X	0.28	0.038A	-0.060X	0.08ns	0.015B	0.067X
	fLF	0.45	0.124	-1.404	0.12 ns	0.080AB	-0.04XY	0.41	0.116A	-1.268X	0.14	0.032B	-0.190Y
250-53 µm	Humin	0.84	0.471b	2.797b	0.74	0.447A	2.947X	0.86	0.476A	2.569X	0.92	0.524A	2.433X
	CaHA	0.73	0.213	-1.436a	0.67	0.206A	-1.382X	0.83	0.268B	-2.203X	0.69	0.200A	-1.243X
	MHA	0.59	0.090	-0.815	0.84	0.146A	-1.544X	0.79	0.084B	-0.748Y	0.57	0.043C	-0.305Z
	oLF	0.15	0.013	0.059b	0.13	0.014A	0.092X	0.08ns	0.010AB	0.103X	0 ns	0.002A	0.125X
	fLF	0.31	0.007	-0.022	0.06 ns	0.004A	0.035X	0.35	0.007A	-0.035X	0.51	0.004A	-0.011X

R^2= adjusted R^2. If R^2 is followed by ns, then the regression model was not significant (p-value regression >0.05), otherwise regression was significant. n=24 for 0-5, 5-15 and 15-30 cm depths, n=72 for 0-30 cm depth. Parameters followed by similar upper case letter (A B C for Slope, X Y Z for intercept) within a row are not significantly different among depths. Parameters for the same fraction within a column followed by similar lower case letters are not significantly different among aggregate size.

CHAPTER 4. ANNUAL CHANGES IN SOIL C AND N STOCKS UNDER NO-TILL MAIZE-SOYBEAN ROTATION

1. Abstract

Carbon (C) and nitrogen (N) inputs from above ground crop residues are crucial for maintaining soil C and N stocks. A maize-soybean rotation has annual contrasts in N fertilization regime and also in the biomass and composition of crop residues after harvest. Annual changes in soil C and N stocks in production fields summarize the balance between inputs and losses associated with the crop and its interactions with crop management and the environment. This study monitors an irrigated no-till production field in eastern Nebraska over four years for annual changes in (1) soil C and N stocks (equivalent soil mass basis), (2) two light fraction pools (free and occluded), (3) soil carbohydrates, and (4) the soil δ^{13}C signature. Soil samples from 0-5, 5-15 and 15-30 cm depth were collected for C and N determination in fall 2005 (maize), 2006 (soybean), 2007 (maize) and 2008 (soybean). Soil C and N stocks increased in the surface layer in 2007. This followed a large input of soybean residue of 3.9 Mg C ha^{-1} and 0.2 Mg N ha^{-1} in 2006, more than twice the biomass of any other year. Compared to 2005 and 2006, significant increases in soil C and N stocks were measured in fall 2007 after the maize harvest in the 0-60 (+25% and 21%, respectively) and 60-200 kg soil m^{-2} layers (+8% and +6%, respectively), but not in the 200-400 kg soil m^{-2} layer. Compared to 2005 and 2006, both LF pools in 2007 increased by 2-3 fold and carbohydrate concentrations increased by an average of 30% in the surface 0-5 cm. The ^{13}C signature indicated that the increase in C stocks in 2007 was derived from both C3 and C4 sources. Our results support the hypothesis that the high volume of highly decomposable 2006 soybean residue contributed to the decomposition and humification of previous (2005) maize residues during the 2007 maize cropping season, resulting in enhanced soil C and N stocks.

Key words: soil monosaccharides, free and occluded light fraction, δ^{13}C, equal soil mass basis.

2. Introduction

Maize-soybean rotation under irrigated no-till management represents a challenging scenario for stabilization and accumulation of soil carbon (C) and nitrogen (N). Use of no-till has been proposed as a key practice to achieve C sequestration by a reduction in soil organic matter (SOM) decomposition (Lal et al. 2003). However, the soil-residue interface necessary to effectively sequester C and N inputs is limited to the soil surface, resulting in stratification of C and N in the soil profile. Stabilization of crop residue inputs into SOM requires both physical incorporation into the soil matrix and decomposition-humification of the plant material into more stable forms (Krull et al. 2003). Under no-till systems, soil C and N enrichment of the surface layer can saturate the niches for stabilization of C and N as clay-associated complexes, resulting in the preferential accumulation of C and N inputs into less stable SOM pools (i.e. light fraction) (Six et al. 2002, Gulde et al. 2008, Yoo and Wander 2008).

In addition, maize and soybean crops have contrasting N fertilization and residue amounts/composition at harvest. Soil organic matter has a C:N ratio around 10, while maize residues and soybean residues have a C:N ratio above 55 and below 30, respectively. Thus incorporation of maize C into SOM will rely on the availability of soil N or other N sources for decomposition compared to incorporation of soybean C into SOM (Stemmer et al. 1999). During the soybean cropping season, the combination of (i) high mass and high C:N ratio of the maize residues placed on the soil surface, (ii) absence of N fertilization, and (iii) irrigation will promote crop residue loss in the surface layer primarily through increased C respiration (Coppens et al. 2006). After the soybean year, reduced mass of crop residues together with a lower C:N ratio and the addition of N fertilizer to the next maize crop may stimulate decomposition of remaining maize residues and build up of SOM. It is hypothesized that differences in quantity and quality of crop residues together with N management can impact the stabilization and storage of soil C and N under maize-soybean rotation.

Annual estimation of soil C and N stocks summarizes accrual and loss processes, while the study of SOM pools that respond quickly to management provide better insight into the underlying processes. Light fractions that are either free (fLF) or occluded inside aggregates (oLF) represent different stages of humification and play different roles in SOM turnover (Golchin et al. 1997). Free LF is a less decomposed fraction that consists mainly of plant litter, while oLF has undergone more decomposition (Golchin et al. 1994). The contribution of LF to total soil C in the plow layer is sensitive to the quantity and quality of crop residue inputs and is dynamic, peaking soon after residue incorporation and then declining until the next residue incorporation (Legorreta-Padilla 2005, Yoo and Wander 2008). Some of the newly formed LF can become physically protected inside new aggregates, which allows it to gradually age, i.e. humify. Therefore physical protection of new C inputs as oLF may constitute a crucial step to retard decomposition and favor humification. Moreover, Six et al. (1999) hypothesized that, in cases of high relative C abundance such as in the surface soil layer of no-till systems, stabilization of new C and N inputs occurs through protection of uncomplexed SOM fractions by aggregates.

Carbohydrates are the most common type of plant biomolecule and with the exception of cellulose are readily decomposed by soil microorganisms. Therefore monosaccharide abundances in the soil profile can be used as an indicator of incorporation of C inputs and decomposer activity. The relative proportions of individual monosaccharides differ between plants and microorganisms, so changes in the total and relative amounts of monosaccharides can be used as an indicator of the extent of residue decomposition (Murayama 1984).

This study quantified annual soil C and N stocks in the 0-30 cm depth of an irrigated maize-soybean rotation for four years. The objectives of this study were to evaluate the effects of aboveground crop residues on the amount, time and depth of total soil C and N stocks and two physically protected SOM pools.

3. Materials and Methods

Study site

This study was part of the Carbon Sequestration Program (CSP) focused on studying C sequestration in agricultural systems (Verma et al. 2005). A detailed account of the CSP study can be found at http://csp.unl.edu/public/. The experiment was conducted on a large, production-scale, agricultural field (52.4 ha) equipped with center-pivot irrigation located at the University of Nebraska Agricultural Research and Development Center near Mead, NE (41° 9' 53.5"N, 96° 28' 12.3"W, 362 m). The soils are deep silty clay loams consisting of four soil series: Yutan (fine-silty, mixed, superactive, mesic Mollic Hapludalfs), Tomek (fine, smectitic, mesic Pachic Argialbolls), Filbert (fine, smectitic, mesic Vertic Argialbolls), and Filmore (fine, smectitic, mesic Vertic Argialbolls). Before initiation of the CSP study, six 20 m x 20 m intensive measurement zones (IMZs) were established for detailed process-level studies of soil C dynamics, crop growth and productivity, crop residue decomposition, and soil and plant gas exchange. Six spatial classes were delineated in the field using fuzzy-k-means clustering technique (Minasny and McBratney 2002) based on spatially dense information (4 m x 4 m) of elevation, soil type, electrical conductivity, soil organic matter content, digital aerial photographs and near infrared band of multispectral IKONOS satellite images. The spatial classes encompassed landscape level variation required to scale measurements to the entire field. Once the fuzzy environmental classes were delimited, the locations of the IMZs were randomly chosen to represent each of those fuzzy classes. This approach allowed quantifying the maximum natural variability of the measured variables within an agricultural field (Minasny and McBratney 2002).

Previous to 2001, the field was under no till maize-soybean rotation for 10 years. Before initiation of the CSP study in 2001, the field was uniformly tilled by disking to homogenize the top 10 cm of soil and to incorporate P and K fertilizers and previously accumulated surface residues. The field has been under an irrigated no-till maize-soybean rotation since 2001. Crop management practices (i.e., plant populations, herbicide and pesticide applications, irrigation, fertilization) have been employed in accordance with standard best management practices prescribed for production-scale maize-soybean systems. No fertilizer N was applied during the soybean years, while in the maize years on average 200 kg N ha^{-1} was split-applied, with three-quarters applied at pre-plant and the remainder fertigated and split equally between the six- and twelve-leaf growth stages.

Table 4.1 summarizes major crop management information for the 2001–2008 period. At physiological maturity, six maize plants or a 1-m row for soybeans were harvested by hand in 24 locations within the field to determine dry matter, harvest index and C and N concentrations in plant tissue (grain, cobs or podwalls, and vegetative biomass). Samples were dried at 70ºC, ground and analyzed for C and N using a Costech ECS 4010 elemental analyzer. Harvest index and tissue C and N mass fractions measured in the hand-harvested samples were used in combination with the combine whole-field grain yield estimate to calculate aboveground biomass and C and N inputs as crop residues (Table 4.1).

Soil sampling

Annual soil sampling of the top 30 cm of soil was conducted in the fall of 2005, 2006, 2007 and 2008. Sampling was done in each of the six IMZs after crop harvest. Within each IMZ, six separate soil cores (4 cm diameter by 30 cm depth) were collected along an east–west transect between previous crop rows and inter-row center (approximately 16 cm away from row). The field-moist cores were split into three soil depths: 0-5, 5-15 and 15-30 cm, and then gently broken apart along natural planes of weakness. The samples were combined into one composite sample for each year (n=4) and depth (n=3) and field rep (IMZ, n=6) combination (n=72), then air-dried to constant weight, sieved to 8 mm and stored in boxes for further fractionations and analyses. The six IMZ samples served as replicates for statistical analyses.

In addition to the soil core samples, five smaller soil cores (2.15 cm diameter by 30 cm depth) were taken every year to determine bulk density in each transect of each IMZ, using a lubricated plastic sleeve mounted inside a soil hand probe. These cores were also split into 0-5, 5-15 and 15-30 cm depths, dried at 105 ºC for 24 h and weighed. Soil bulk density (BD, Mg m^{-3}) for each depth increment of each core was calculated as (1):

$$BD = \frac{Soil_m}{Soil_v} \tag{1}$$

where $Soil_m$ is the oven-dried soil mass (g), and $Soil_v$ is the volume of soil of each depth increment.

Extraction of free and occluded Light Fraction

A sequential density fractionation, adapted from Golchin et al. (1994) and Besnard et al. (1996), was used to determine the incorporation of crop residue-derived material into the fLF and the oLF for the 0-5, 5-15 and 15-30 cm soil depth layers of each year. Extraction was performed by duplicates. Briefly, a 10 g soil sample was placed in a 60 mL centrifuge tube, and 40 mL of sodium polytungstate (NaPT) of 1.6 g cm^{-3} density was added. The tube was capped with a plastic lid and inverted gently by hand five times, taking care to avoid disruption of soil aggregates. The particles which adhered to the lid and tube walls were washed into suspension using more NaPT to a final volume of 50 mL. The suspension was allowed to stand for 30 min before centrifuging at 2000 rpm for 1 hour. The supernatant with floating particles (free LF <1.6 g cm^{-3}) was poured onto a nitro-cellulose filter (0.20 μm), and filtered under vacuum. Ten (5 mm) glass beads and 40 mL of NaPT solution (1.6 g cm^{-3}) was added to the soil remaining in the centrifuge and the tubes were shaken horizontally for 16 hours at 60 oscillations per minute. After shaking, the particles which adhered to the lid and tube walls were washed into suspension using more NaPT to a final volume of 50 mL. The tubes were allowed to stand for 30 min before centrifuging at 2000 rpm for 1 hour. The supernatant with floating particles (oLF with a density <1.6 g cm^{-3}) was poured onto a nitro-cellulose filter (0.20 μm), and filtered under vacuum. The NaPT was removed from the fLF and oLF by submerging the material at least four times with deionized (DI) water and removing the leachate with a vacuum filtration system while adding more DI water using a squirt bottle to keep the LF material in motion (Crow et al. 2007). The LF on the filter was backwashed into a weighed porcelain crucible and dried at 50°C until constant weight. Duplicates were combined, finely ground in a ball mill, and stored in amber glass vials until further analysis. The NaPT was recycled according to Six et al. (1999) to avoid cross contamination of C between samples.

Analytical methods

- C and N analysis

A sub-sample of whole soil was finely ground to pass a 100 mesh sieve using a roller mill. Any recognizable plant material and/or $CaCO_3$ concretions (> 1 mm) were removed before grinding. Twenty mg of the ground soil and 6 mg of LF materials were analyzed for C and N concentration using an elemental analyzer (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA).

To better account for the variation in effective sampling depth and soil mass due to changes in soil bulk density over time, the cumulative mass approach described by Gifford and Roderick (2003) was used to calculate soil C and N changes over time. Estimates of whole soil C (g C m^{-2}) and N (g N m^{-2}) were calculated for each of the three soil depth intervals based on the measured bulk density at the time of sampling and the C and N concentration. Three reference soil masses were used to evaluate SOC and N changes over time (Equation 4 in Gifford and Roderick, 2003) (See Appendix 1): (i) the top 60 kg dry soil m^{-2} (approximately 0-5 cm depth), (ii) the top 200 kg dry soil m^{-2} (approximately 0-15 cm depth) and (iii) the top 400 kg dry soil m^{-2} (approximately 0–30 cm depth).

- Carbohydrates

The soil carbohydrate composition was determined for all depths and years following the weak acid extraction of the optimized procedure by Martens and Loeffelmann (2002). Briefly, 100 mg soil was treated with 6M H_2SO_4 for 30 min in culture tubes, then diluted with 4 mL DI water and autoclaved at 121ºC for 30 min. The hydrolysates were diluted with DI to 10 mL, their pH values were adjusted to 5 with NaOH, and they were centrifuged at 2500 rpm. The monosacharides in the supernatant (fucose, arabinose, rhamnose, galactose, glucose, xylose, and mannose) were separated on a Dionex DX-500 (Dionex Corp., Sunnyvale, CA) anion chromatograph with a CarboPac PA10 column. The ratio of plant to microbial carbohydrates was approximated as xylose / mannose (Murayama, 1984).

- δ^{13}C analysis

Soil samples from all depths and years were analyzed for δ^{13}C on a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility at the University of California, Davis. The sample δ^{13}C (Eq. 2) represents the relative difference in the ^{13}C to ^{12}C ratio of the sample compared to the Vienna Pee Dee Belemnite standard (*std*), in per mil:

$$\delta^{13}C_{sample} = \frac{\left({}^{13}C/{}^{12}C\right)_{sample} - \left({}^{13}C/{}^{12}C\right)_{std}}{\left({}^{13}C/{}^{12}C\right)_{std}} * 1000 \qquad (2)$$

During photosynthesis, discrimination against the ^{13}C isotope leads to depletion of this isotope in plant tissues compared to the standard. The different photosynthetic pathways of maize (C4 pathway) and soybean (C3 pathway) result in different extents of depletion in δ^{13}C. The δ^{13}C‰ signature was set at -27‰ for soybean residues and -12‰ for maize residues. Balesdent and Mariotti (1997) indicated that the proportion of maize-derived C (%C4-C) can be calculated using the δ^{13}C‰ of the sample as (Eq. 3):

$$\%\ C4 - C = \frac{\delta^{13}C_{sample} - \delta^{13}C_{C3veg}}{\delta^{13}C_{C4veg} - \delta^{13}C_{C3veg}} \times 100 \qquad (3)$$

The %C4-C and %C3-C (estimated as %C3-C = 100 – (%C4-C)) was used to calculate the mass of C4- and C3-derived C in each soil depth, and by accounting for bulk density changes in C4-C and C3-C stocks on an equivalent mass basis.

Statistical analysis

The equality of the means among years was tested using repeated measurements (RM) ANOVA to account for the nature of the field sampling procedures and the natural variability of each individual IMZ. The C and N stocks and the C and N contents of the two LF fractions for each soil layer were compared with one-way RM ANOVA in SAS (SAS Institute, 2009). PROC MIXED routine procedure was used with IMZ as random effect and year as a repeated factor (Loughin 2006) (Table 4.2). A planned contrast for C and N stocks was maize years (2005 and 2007) versus soybean years (2006 and 2008), which was estimated using the CONTRAST statement. Two-way RM ANOVA (soil layer and year) was used to evaluate soil BD, soil carbohydrate concentrations, soil δ^{13}C signature and %C4-C, and the C:N ratio for whole soil and the two LF fractions (Table 4.2). Post-hoc separation of means for significant variables ($p<0.05$) was done using Fisher LSD.

4. Results

C and N inputs

The amount and quality of the residue inputs varied between maize and soybean crops. The annual C and N inputs from aboveground maize crop residues averaged 4800 ± 90 kg ha^{-1} and 77 ± 4 kg ha^{-1}, respectively (Table 4.1). The C and N harvest index for maize averaged 0.54 ± 0.004 and 0.69 ± 0.01, respectively. Annual C and N residue inputs from soybean varied greatly among seasons, ranging between 1700 to 3900 kg C ha^{-1} and 41 to 188 kg N ha^{-1}, respectively. Nonetheless soybean yields were steady, averaging 4056 ± 137 kg ha^{-1}. The harvest index for soybean ranged between 0.34 to 0.51 for C and 0.54 to 0.85 for N. The lowest C and N harvest indices were in 2006, which resulted in an unusually large amount of C and N residues that were incorporated following the 2006 harvest. The largest C and N harvest indices were in 2004, resulting in the smallest amounts of soybean C and N residue inputs after harvest. The average C:N ratio of aboveground residues was 62 ± 3 for maize and 31 ± 5 for soybean.

C and N stocks on equal soil mass basis

Bulk densities of the 0-5, 5-15 and 15-30 cm soil depths across years are shown in Table 4.3. The soil depth by year interaction was significant ($p=0.002$). In general, BD increased with depth for each year. In 2008, decreased BD in deeper layers probably resulted from the deep ripping tillage operation of the previous fall. In the surface layer, BD tended to be smaller in soybean years. Given the variation in soil bulk density between years and depths, the cumulative mass approach as proposed by Gifford and Roderick (2003) was used to account for differences in effective sampling depth for the calculation of soil C and N stocks. The equal soil mass layers were set as 60, 200 and 400 kg soil m^{-2}, which approximated the 5, 15 and 30 cm depths, respectively. Both soil C and N stocks followed similar trends, with significant differences among some years for the 0-60 and 60-200 kg soil m^{-2} layers that resulted in significant differences in the 0-400 kg m^{-2} layer (Table 4.4, Figure 4.1). The planned comparisons of crop rotation by C and N stocks (maize versus soybean) were not significant ($p>0.05$) (Table 4.4). Hence the soil C and N stocks did not change in response to the annual rotation of maize and soybean. The most significant difference among years was an increase in C and N stocks in 2007, compared to 2005 and 2006, and followed the large biomass of soybean residues produced after the 2006 harvest. Carbon and N stocks increased by 25% and 21%, respectively, in the 0-60 kg soil m^{-2} layer, and by 8% and 6%, respectively, in the 60-200 kg soil m^{-2} layer (Figure 4.1). In 2008, the C and N stocks showed a modest numeric decrease compared to 2007 for the 0-400 kg soil m^{-2} layer, because of a decrease in the uppermost soil layer. Carbon and N stocks in 2008 were only 13% and 11% greater than those in 2005 and 2006 for the 0-60 kg soil m^{-2}, but were similar to 2007 for the 60-200 kg soil m^{-2} and 200-400 kg soil m^{-2} layers (Figure 4.1, Table 4.4).

C and N content as free and occluded LF

Soil C and N contents as free and occluded LF were greater near the surface and decreased with depth (Table 4.5). In fact, both LF fractions were more stratified with soil depth than were whole soil C and N (Table 4.4). Free LF accounted for 6.2, 1.6, and 0.8% of soil C and 3.9, 0.8, and 0.3 % of soil N for the 0-60, 60-200 and 200-400 kg soil m^{-2} layers, respectively. Occluded LF accounted for 4.6, 1.8, and 1.0% of soil C and 3.0, 0.8, and 0.4 % of soil N for the 0-60, 60-200 and 200-400 kg soil m^{-2} layers, respectively. Free LF-C and -N contents in the first soil layer were more than double in 2007 compared to 2005 and 2006 (Table 4.5), and decreased by similar amounts in 2008. In this same surface layer, oLF-C and -N contents increased by 50% in 2007 and decreased by 27% in 2008 compared to 2005 and 2006. Occluded LF-C and -N contents in the second layer decreased in 2008 by 32% and 25%, respectively, compared to 2007.

The soil C:N ratio was slightly greater in the 0-5 cm depth than in the lower two depths and showed minor variation among years (Table 4.6). The C:N ratios of free and occluded LF were similar between fractions and increased with depth (Table 4.6). Both fractions showed a significant depth by year interaction, due to the larger soybean residue inputs in 2007. Significant differences among years for each fraction are presented by soil depth. The largest changes in the C:N ratio of the fLF were that in the 0-5 cm layer it increased between 2005 and 2007 from 16 to 20, while in the 15-30 cm depth it decreased between 2006 and 2008 from 24 to 20. At the 5-15 cm depth the C:N ratio of the fLF did not change consistently among years. The C:N ratio of the oLF decreased by 1.5 from 2005 to 2006 for both the 5-15 and 15-30 cm depths. Despite the similarity in C:N ratios of the fLF and oLF, their appearances differed. Both fractions were composed of small particulates, but the oLF was darker (dark brown to black) than the fLF. Using a dissecting microscope, fragments of plants and insects were observed in the fLF. By contrast, the oLF was a non-identifiable mass of dark particulates together with fungal hyphae.

Carbohydrates

Monosaccharide abundance decreased in the order glucose, arabinose, galactose, mannose, xylose, rhamnose, and fucose (Table 4.7). All carbohydrates showed a sharp decrease in concentration from the 0-5 cm depth to the lower two depths. A significant depth by year interaction for individual monosaccharides was mainly related to changes in the carbohydrate content in the 0-5 cm layer. All carbohydrates were found in greater concentration in 2007 compared to 2005 and 2006 for the 0-5 cm depth. In 2008, glucose and mannose content decreased to similar values as in 2005 and 2006, but arabinose, rhamnose, galactose, and xylose were still more abundant than in 2005 and 2006 (Table 4.7). The plant to microbial carbohydrate ratio decreased with depth (i.e. becomes more microbial derived) and increased slightly (p-value=0.066) from 2005 and 2006 to 2007 and 2008, i.e. it became more plant derived C (Table 4.7).

^{13}C analysis and maize-derived C

The soil δ^{13}C signature ranged from -14.9 to -16.8 ‰ indicating a predominance of C4-derived C (Table 4.8). There was a significant depth by year interaction for the C signature (Table 4.4). Percent C4-C increased with soil depth in all years. In 2008, the proportion of soil C that was C4-C derived was lower than in the previous years for the 0-5 cm and 5-15 cm depths, while for the 15-30 cm depth it was larger than in 2005 – 2007. The 2007 values indicated smaller proportions of C4-C in the 0-5 and 5-15 depths, consistent with the large biomass of soybean residue following the 2006 harvest. The changes in C stocks as C4- and C3 derived C are shown in Figure 4.2. Both the C4 and C3 stocks increased in 2007 for the 0-60 and 60-200 kg soil m^{-2} layer, and they decreased slightly in 2008. Despite the significant changes in C signature in the 5-15 cm depth, no significant changes among years were determined for the C4 and C3 stocks of the comparable 200-400 kg soil m^{-2} layer (Figure 4.2).

5. Discussion and Conclusion

Responses of soil C and N stocks to crop management over time carry obvious significance to several current issues, including climate change, soil quality and productivity. Their measurement under field conditions can also depict the modifying influences of crop rotation and year-specific variations. In this study, soil C and N stocks in a production field are reported 5 to 8 years after the initiation of irrigated no-till management. The soil C and N stocks and SOM fractions remained stratified under the ongoing no-tillage, with preferential accumulation near the soil surface. The proportion of soil C, fLF-C and oLF-C stocks in the 0-60 kg soil m^{-2} layer accounted for 24%, 62%, and 51% of their respective stocks in the 0-400 kg soil m^{-2} layer. For the 0-200 kg soil m^{-2} layer, the proportion of soil C, fLF-C and oLF-C stocks accounted for 57%, 85%, and 79% of their respective stocks in the 0-400 kg soil m^{-2} layer. Similar results were reported previously under no-till management for total soil C and LF-C (Poirier et al. 2009, Murage et al. 2007). Retention of crop residues aboveground with no tillage imposes a reduced soil-residue interface for effective incorporation of C inputs into SOM pools. The larger stratification of the LF pool compared to total soil C suggests not only a more restricted incorporation of particulate C inputs than soluble-colloidal forms under no till management, but also indicates aboveground crop residues as the origin of this labile fraction (Legorreta-Padilla 2005, Murage et al. 2007, Angers et al. 1995). In the following sections, annual changes of soil C and N stocks and SOM fractions are discussed in relation to the crop rotation and the quality and quantity of crop residues.

Influence of quantity and quality of crop residues on soil C and N stocks: Hypothesis versus field observations

Differences in management and productivity between maize and soybean crops resulted in contrasting conditions for C and N stabilization into SOM pools (Studdert and Echeverría, 2000). In our study, best management practices for maize include supplementary N from fertilizers while soybean did not receive additional N. Moreover, maize returned twice as much C as above ground residue than did soybean, but with an average C:N ratio above 60 compared to a C:N ratio of 30 or less for soybean residues. The differences in quantity and quality of crop residues together with N management were hypothesized to impact the stabilization and storage of soil C and N in this irrigated maize-soybean rotation. Placement of large masses of maize residues with a wide C:N ratio on the soil surface after harvest will lead to N immobilization during the following soybean year (Coppens et al. 2006), particularly at the soil surface. Since no additional N is applied as fertilizer, the small amount of available N in surface soil will likely limit maize C and N stabilization into SOM during the soybean year (Stemmer et al. 1999) and residue-C will largely be lost through enhanced respiration. In contrast, the narrower C:N ratio of soybean residues will promote N mineralization during the maize year. In addition, the application of N fertilizer through fertigation will provide an extra N source for decomposition of both soybean and maize residues and their transformation into SOM.

As expected, maize residues had greater mass and wider C:N ratios than did soybean residues. At the same time, the amount and quality of the soybean residues left on the soil surface prior to maize were highly different: the 2004 soybean residues (C:N ratio = 41) returned 57% less C and 78% less N into the soil than did the 2006 soybean residues (C:N ratio= 21). Nevertheless there were common changes in soil properties in both soybean years (2006 and 2008): 1) C and N stocks of the surface soil decreased slightly ($p>0.05$) during both years, and 2) the soil ^{13}C signature of surface soil also indicated a consistent reduction in C4-derived C during both soybean years (Figure 4.2). Several other soil properties differed between the 2005 and 2007 maize years, and may reflect the much greater return of soybean residues in 2006 compared to 2004: 1) the 0.6 kg C m^{-2} increase in soil C stocks in the 0-400 kg soil m^{-2} layer after the 2007 maize crop compared to 2005 and 2006, 2) the fact that 56% of that increase occurred in the 0-60 kg soil m^{-2} layer, 3) the increase in soil C included both C4- (+0.35 kg C4-C m^{-2} soil) and

C3- derived C (+0.25 kg C3-C m^{-2} soil), 4) the reduced contribution of the increased C stocks in both LF pools to the overall increase, and 5) the increase in the plant derived monosacharides in 2007. The increase in soil C stocks following the 2007 maize season is comparable to increases in soil C stocks with manure application (Kong et al. 2005) and was not previously observed in this field (Verma et al. 2005). The absence of this response following the 2005 maize season might be due to the low amount of soybean residue incorporated into the soil after 2004 soybean harvest. Consequently this dataset is consistent with, but does not prove the hypothesis, that soil conditions during maize are more conducive for soil C and N sequestration than those during soybean due to promotion of maize and soybean residue humification. However, this dataset does demonstrate the sensitivity of these measurements to annual variations in soybean C and N inputs and their effects on labile SOM fractions.

Trends in C and N stocks of soil and SOM fractions indicate that differences in the quantity and quality of soybean crop residues may play an important role in the stabilization of maize and soybean residues into SOM pools. The increase in C4-derived C in the surface layer during the 2007 maize season can result from either decomposition of previous maize residues (i.e. remaining 2005 residues) or rhizodeposition and decomposition of surface maize roots from the current year. The increase in the C3-derived C after the 2007 maize year indicates decomposition and incorporation into soil of the 2006 soybean residues. Estimated C inputs from the 2006 soybean were 0.39 kg C m^{-2}. Based on the 2005 maize residue C inputs and the estimated C litter decomposition rate from the first three years of this experiment (Kochsiek et al. 2009), the standing maize residue C input from maize in 2005 was estimated as 0.25 kg C m^{-2}. Assuming the increase in C4- and C3-derived C in the first layer was associated only with litter decomposition (and not C inputs from root decomposition of the 2007 maize crop), 36% of the 2006 soybean C residue and 80% of the remaining 2005 maize C residue remained in the soil at the end of 2007.

Nature of the increase in soil C and N stocks

Light fraction-C is considered more sensitive to changes in crop management than total soil C (Gregorich et al. 1995, Six et al. 1998). Free LF constitutes particulate organic matter that is not associated with the soil matrix and is composed mainly of plant-like material, while oLF is more decomposed SOM protected inside aggregates (Golchin et al. 1994, Gregorich et al. 1997). The 2-3 fold increase in free and occluded LF pools that occurred in the surface layer between 2006 and 2007 and the simultaneous increase in the fLF C:N ratio indicate that during the 2007 maize season standing crop residues were at least partially decomposed and their remaining materials were incorporated into SOM fractions. Nonetheless, the increase in free and occluded LF pools accounted for only 0.17 kg C m^{-2}, which indicates that 72% of the increase in soil C was related to stabilization of crop residues as humified forms. Further study of the aggregate-associated SOM confirmed significant increases in humified SOM fractions in this layer (Chapter 5).

Soil monosaccharide abundance and composition can be used as an indicator of the amount of C inputs and their extent of decomposition (Murayama 1984). In our study, individual monosaccharides were 1.5 to 3 times more abundant in the 0-5 cm soil layer compared to 5-15 and 15-30 cm depths. The stratification in the abundance of monosaccharides in the 0-30 cm soil depth indicates that the contribution of the aboveground crop residue inputs is limited to the first several centimeters of soil. The reduction in the xylose to mannose ratio from 1.05 to 0.51 from the 0-5 to the 15-30 cm depth indicates preferential retention of plant-derived C at the surface. These indicators of plant derived C are consistent with the increase in soil C and LF in surface soil and support the concept of limited volume for movement and stabilization of residue derived C into SOM under no-till (Chapter 2). The increase in total carbohydrates and in the xylose to mannose ratio at the 0-5 cm depth from 2005 and 2006 to 2007 support enhanced stabilization of crop residues through increased soil C and N stocks, C4-and C3-derived C, and fLF and oLF fractions.

An interesting feature of the data presented in this study is the lag in time and depth where changes in soil C and N occurred. The increase in C and N stocks in the 0-5 cm depth observed in 2007 seems to have moved down the profile in 2008 to the 5-15 cm depth. C stocks decreased in the surface layer from 2007 to 2008, which was accompanied by a loss of both LF pools. The reduction in the LF-C stocks on the surface layer doubled that of soil C, suggesting part of the reduction in the LF-C mass may have been the result of transformation of LF into humified fractions. Soil monosacharides were less abundant in the surface layer in 2008 compared to 2007, but were still larger than in 2005 and 2006, and showed a slight increase in the 5-15 and 15-30 cm depths. The xylose to mannose ratio also increased with depth in 2008, suggesting movement of new C inputs from aboveground crop residues down into the soil profile.

Stratified distribution of soil C and N stocks under no-till management with preferential accumulation near the soil surface supports the impact of above ground crop residues to the top several centimeters of soil. The distribution and composition of LF and monosaccharides also confirms a restricted volume for stabilization of C & N inputs from aboveground crop residues. The increased C and N stocks after the second maize year (2007) were attributed mostly to storage of C and N inputs in surface soil. Less than 30% of the increase was associated with LF fractions. The increase from 2006 to 2007 of both C4- and C3-derived C stocks in surface soil together with the reduced contribution from fLF and oLF indicates humification of both 2006 soybean and 2005 maize residues during the 2007 maize year. The fact that these trends were not apparent in the first maize year (2005) might be related to the reduced C and N soybean residue in 2004. Both the quantity and quality of C inputs can be important determinants of the amount of C residue that is stabilized as SOM (Studdert and Echeverría 2000, Kong et al. 2005). The significant contribution of 2006 soybean-derived C to soil C stocks in the 0-60 kg soil m^{-2} layer during the following (2007) maize cropping season can be attributed to both quantity and quality of those soybean residues. These data further support the hypothesis that the high volume of readily decomposable soybean residue from 2006 contributed to the decomposition and humification of previous (2005) maize residues during the 2007 maize cropping season.

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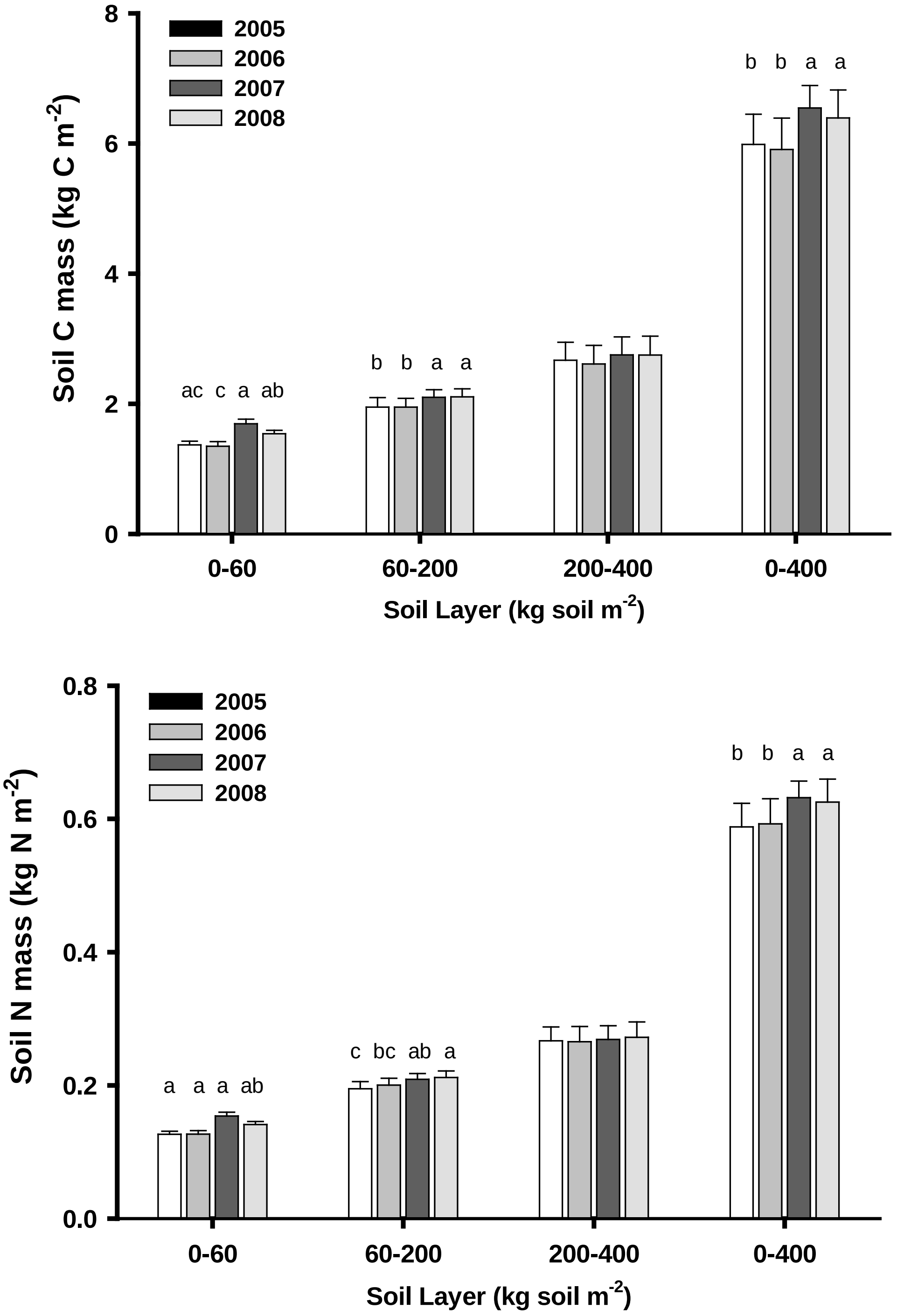

Figure 4. 1. Soil C and N stock (means and se) per soil mass layer and year. Means followed by the same lower case letters within a layer do not differ significantly (α=0.05) between years.

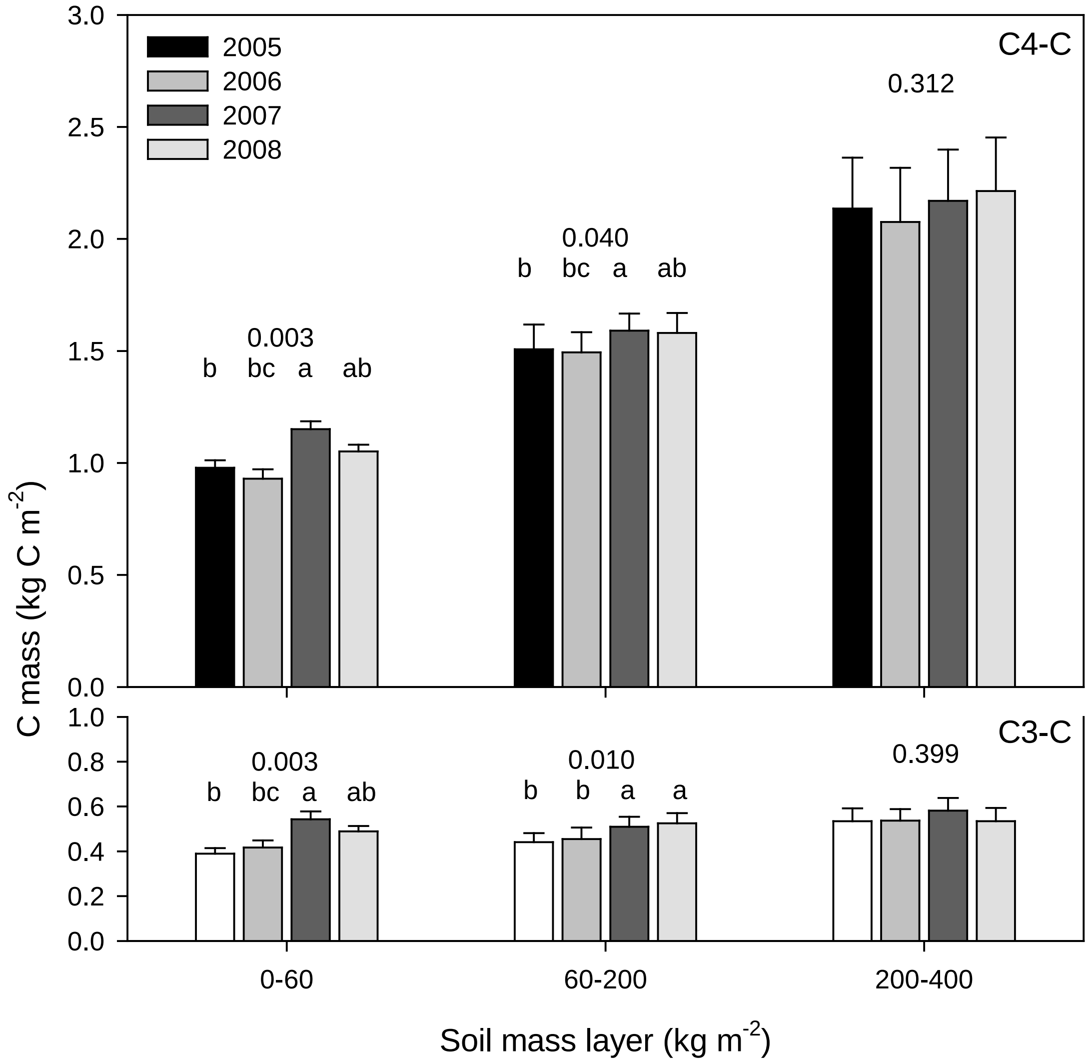

Figure 4. 2. C stocks as C4 and C3 derived C. Means followed by the same lower case letters within a layer do not differ significantly (α=0.05) between years.

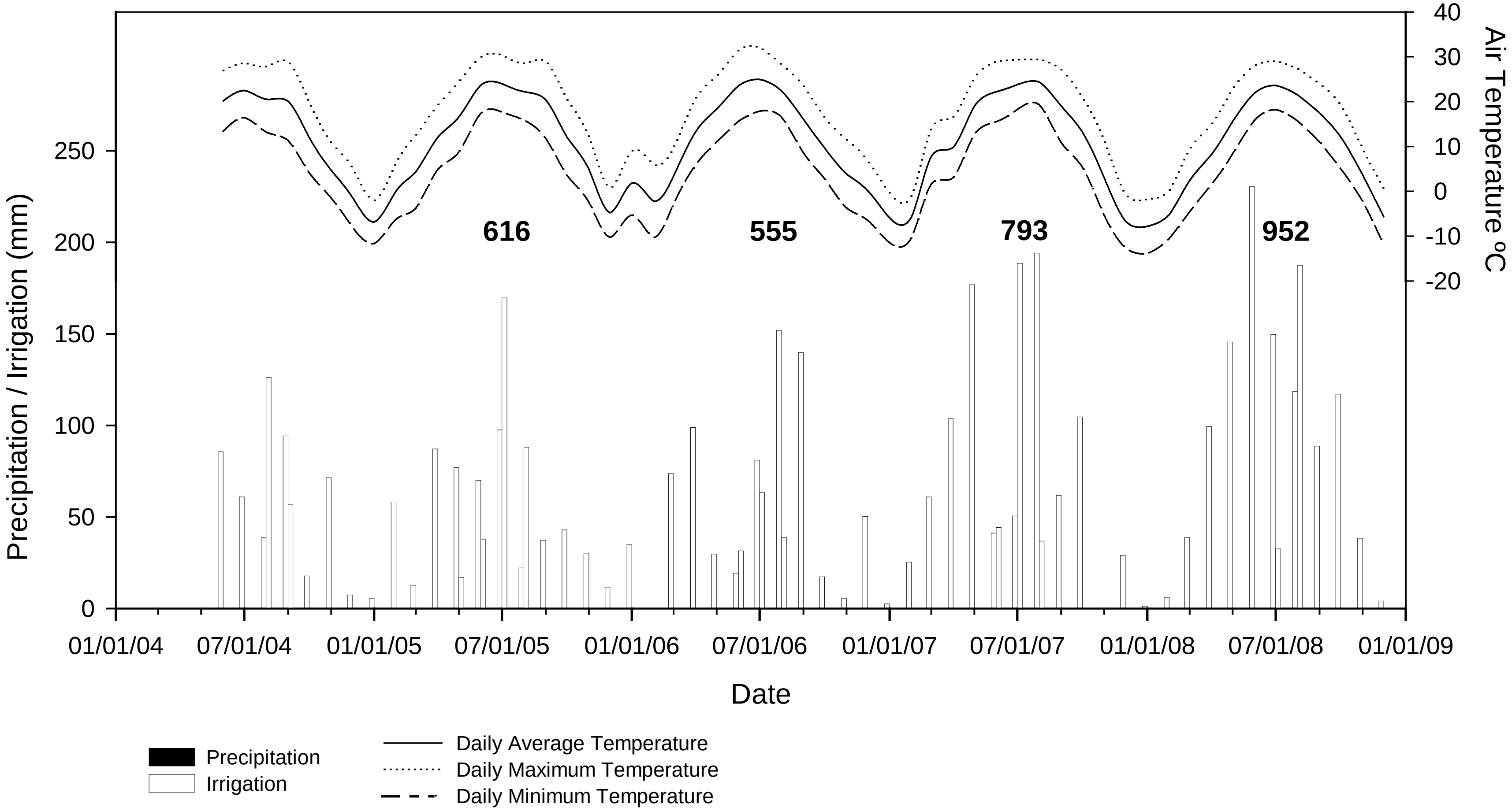

Figure 4. 3. Monthly average air temperatures (1 m) and monthly total precipitation and irrigation.

Table 4. 1. Crop management and grain yield for 2001–2008.

Year	Hybrid	Plant density (pl ha^{-1})[3]	Planting date	Harvest date	Sampling date	N to crop (kg N ha^{-1})	Irrigation cm	Yield[1] (Mg ha^{-1})	Residue C* (kg C ha^{-1})	Residue N* (kg N ha^{-1})
2001	Pioneer 33P67	81,000	5/11	10/22		196	32.97	13.4	4752	82
2002	Asgrow 2703	371,000	5/20	10/7		-	20.96	3.99	2736	84
2003	Pioneer 33B51	78,000	5/14	10/23		169	34.80	14.0	4826	85
2004	Pioneer 93B09	371,000	6/2	10/18		-	15.88	3.36	1683	41
2005	Pioneer 33B51	83,000	5/2	10/17	10/20	271	30.23	13.2	4515	68
2006	Pioneer 93M11	371,000	5/12	10/3	10/20	-	12.20	4.36	3879	188
2007	Pioneer 31N28	79,000	5/1	11/5	11/8[2]	184	26.01	13.2	4925	74
2008	Pioneer 93M11	370,000	5/14	10/9	11/20	-	22.00	4.16		

*Given the no-till management these inputs remain on soil surface.

[1] Maize grain yield: adjusted to 15.5% moisture content; Soybean grain yield: adjusted to 13% moisture content.

[2] After sampling, deep ripping tillage was performed on the east half of the field, affecting IMZs 4, 5, and 6.

[3] Plant density at sowing.

Table 4. 2. ANOVA table for one-way and two-way repeated measurements (RM) models.

One Way RM[1]

Source	Df
Between IMZs	5
Between Years	3
Residual	15
Total	23

Two way RM[2]

Source	df
Between IMZs	5
Between Depths	2
Ea=IMZ*Depth	10
Between Years	3
Eb=IMZ*Year	15
Depth*Year	6
Residual	30
Total	71

[1]: Used for C and N stocks as whole soil and SOM fractions.

[2]: Used for soil BD, and CN ratio.

IMZ: Intensive management zone (field reps). E= error term.

Table 4. 3. Mean annual soil bulk density (se) by soil layer (n=6)

Soil layer (cm)		0-5	5-15	15-30
Year	Crop	-------------------------------------	kg C m^{-2}	-------------------------------------
2005	Maize	1.20 (0.02) b A	1.43 (0.02) a B	1.48 (0.03) a C
2006	Soybean	1.18 (0.02) b A	1.46 (0.03) ab B	1.48 (0.03) a B
2007	Maize	1.26 (0.04) a A	1.49 (0.02) a B	1.49 (0.03) a B
2008	Soybean	1.23 (0.02) ab A	1.42 (0.02) b B	1.46 (0.03) b C

Means followed by the same lower case letter do not differ significantly (α=0.05) among years for each soil layer. Means followed by the same upper case letters do not differ significantly (α=0.05) among depths for each year. p-value (depth) < 0.001, p-value (year) = 0.048, p-value (depth*year) = 0.002.

Table 4. 4. Mean annual soil C and N stocks (se) for equivalent soil mass layers (n=6)

Soil layer (kg m^{-2})		0-60	60-200	200-400	0-400
Soil C			------------------ kg C m^{-2} --------------		
2005	Maize	1.367 (0.057) bc	1.949 (0.146) a	2.669 (0.276)	5.985 (0.464) a
2006	Soybean	1.347 (0.071) c	1.948 (0.135) a	2.613 (0.285)	5.907 (0.483) a
2007	Maize	1.629 (0.069) a	2.100 (0.115) b	2.751 (0.276)	6.545 (0.345) b
2008	Soybean	1.540 (0.053) ab	2.105 (0.124) b	2.748 (0.291)	6.393 (0.428) b
p-value (year)		*0.001*	*0.007*	*0.482*	*0.005*
p-value (maize versus soy)		*0.153*	*0.801*	*0.554*	*0.303*
Soil N			------------------ kg N m^{-2} ---------------		
2005	Maize	0.126 (0.005) a	0.195 (0.011) c	0.267 (0.021)	0.588 (0.035) a
2006	Soybean	0.127 (0.005) a	0.200 (0.010) bc	0.265 (0.023)	0.593 (0.038) a
2007	Maize	0.154 (0.006) b	0.209 (0.009) ab	0.269 (0.021)	0.632 (0.025) b
2008	Soybean	0.141 (0.005) ab	0.212 (0.010) a	0.272 (0.023)	0.625 (0.034) b
p-value (year)		0.003	0.003	0.896	0.008
p-value (maize versus soy)		*0.193*	*0.309*	*0.894*	*0.778*

Means followed by the same lower case letter do not differ significantly (α=0.05) among years for each soil layer. p-value(years) (p>F) for one-way RM ANOVA, p-value (maize vs soybean) (p>F) for contrast (df error, 15).

Table 4. 5. Mean (se) soil C and N content as free and occluded light fractions (fLF and oLF). (n=6)

Soil layer (cm)		0-5	5-15	15-30	0-5	5-15	15-30
Year	Crop	g C kg soil^{-1}			g N kg soil^{-1}		
fLF							
2005	Maize	1.285 (0.250) a	0.159 (0.025)	0.102 (0.026)	0.080 (0.016) a	0.008 (0.002)	0.004 (0.001)
2006	Soybean	1.011 (0.080) a	0.267 (0.074)	0.053 (0.013)	0.062 (0.005) a	0.013 (0.003)	0.002 (0.000)
2007	Maize	3.235 (0.619) b	0.246 (0.082)	0.151 (0.072)	0.163 (0.035) b	0.013 (0.005)	0.006 (0.002)
2008	Soybean	0.840 (0.149) a	0.197 (0.046)	0.097 (0.034)	0.053 (0.009) a	0.010 (0.002)	0.005 (0.001)
p-value (year)		***<0.001***	*0.284*	*0.615*	***0.001***	*0.241*	*0.204*
oLF							
2005	Maize	1.057 (0.111) a	0.286 (0.025) a	0.149 (0.012)	0.066 (0.007) b	0.013 (0.001) a	0.006 (0.001) a
2006	Soybean	1.069 (0.097) a	0.253 (0.017) a	0.115 (0.013)	0.065 (0.006) bc	0.013 (0.001) a	0.005 (0.001) b
2007	Maize	1.526 (0.203) a	0.257 (0.037) a	0.130 (0.018)	0.094 (0.013) a	0.012 (0.002) a	0.005 (0.001) b
2008	Soybean	0.796 (0.087) b	0.174 (0.012) b	0.116 (0.014)	0.047 (0.005) c	0.008 (0.001) b	0.005 (0.001) b
p-value (year)		***<0.001***	***0.002***	*0.066*	***<0.001***	***0.024***	***0.048***

Means followed by the same lower case letter do not differ significantly (α=0.05) among years for each soil layer. P-value (p>F) for one-way RM ANOVA.

Table 4.6. Mean (se) C:N ratios of whole soil and free and occluded light fractions (fLF, oLF) (n=6)

Soil layer (cm)		0-5	5-15	15-30	Depth Average
			Soil		
2005	Maize	10.8 (0.1)	9.9 (0.2)	9.8 (0.4)	*10.2 ab*
2006	Soybean	10.6 (0.2)	9.7 (0.2)	9.7 (0.4)	*10.0 b*
2007	Maize	11.0 (0.1)	10.0 (0.2)	10.1 (0.4)	*10.4 a*
2008	Soybean	10.9 (0.1)	9.9 (0.2)	10.0 (0.4)	*10.3 a*
Average		*10.8 A*	*9.9 B*	*9.9 B*	
			fLF		
2005	Maize	16.0 (0.2) a A	19.7 (1.3) a B	24.4 (2.5) a C	*20.0 (1.2)*
2006	Soybean	16.2 (0.3) a A	19.8 (1.2) a B	24.9 (1.1) a C	*20.3 (1.0)*
2007	Maize	19.9 (1.1) b A	20.8 (1.8) a A	22.7 (1.9) ab A	*21.1 (0.9)*
2008	Soybean	15.8 (0.4) a A	20.9 (0.9) a B	20.0 (0.7) b B	*19.0 (0.7)*
Average		*17.0 (0.5)*	*20.3 (0.6)*	*23.1 (0.9)*	
			oLF		
2005	Maize	16.1 (0.2) a A	21.8 (0.5) a B	24.3 (0.4) a C	20.7 (0.9)
2006	Soybean	16.6 (0.2) a A	20.3 (0.3) b B	22.9 (0.7) b C	19.9 (0.7)
2007	Maize	16.2 (0.2) a A	21.4 (0.6) a B	25.4 (0.9) a C	21.0 (1.0)
2008	Soybean	16.8 (0.3) a A	21.2 (0.5) a B	24.9 (1.0) a C	21.0 (0.9)
Average		16.4 (0.1)	21.2 (0.2)	24.4 (0.4)	

ANOVA	Soil	fLF	oLF
		--------p-value-------	
Depth	0.004	<0.001	<0.001
Year	0.046	0.477	0.056
*Depth*Year*	0.698	0.038	0.018

Means followed by the same lower case letters do not differ significantly (α=0.05) between years. Means followed by the same upper case letters do not differ significantly (α=0.05) between depths.

Table 4. 7. Mean (se) whole soil carbohydrate concentrations and xylose:mannose ratio.

Carbohydrate	Year	2005 (maize)	2006 (soybean)	2007 (maize)	2008 (soybean)
	Depth	------------------- ug kg soil $^{-1}$ --------------------			
Fucose	0-5	116 (7)	114 (7)	145 (17)	117 (11)
	5-15	57 (3)	58 (3)	59 (3)	57 (2)
	15-30	59 (6)	55 (6)	57 (6)	57 (6)
Arabinose	0-5	1239 (57) a B	1234 (61) a B	1483 (52) a A	1455 (60) a A
	5-15	894 (76) b AB	874 (64) b B	919 (58) b AB	1037 (69) b A
	15-30	921 (89) b	889 (103) b	967 (105) b	1027 (129) b
Rhamnose	0-5	363 (30) a B	385 (31) a B	467 (25) a A	481 (27) a A
	5-15	200 (21) b AB	201 (21) b B	212 (20) b AB	265 (18) b A
	15-30	202 (23) b	190 (28) b	222 (27) b	239 (30) b
Galactose	0-5	1073 (63) a B	1060 (66) a B	1296 (43) a A	1249 (65) a A
	5-15	658 (66) b	653 (57) b	689 (52) b	763 (57) b
	15-30	665 (72) b	642 (81) b	693 (81) b	765 (102) b
Glucose	0-5	1755 (68) a B	1779 (81) a B	2211 (107) a A	1953 (79) a B
	5-15	948 (61) b	948 (60) b	987 (48) b	1039 (66) b
	15-30	999 (97) b	934 (98) b	1002 (107) b	1028 (108) b
Xylose	0-5	681 (39) a C	645 (38) a C	917 (56) a A	799 (50) a B
	5-15	284 (21) b	266 (20) b	295 (16) b	330 (18) b
	15-30	270 (25) b	240 (25) b	262 (27) b	287 (23) b
Mannose	0-5	842 (24) a B	796 (30) a B	986 (38) a A	870 (36) a B
	5-15	582 (42) b	557 (35) b	560 (35) b	639 (37) b
	15-30	629 (54) b	583 (68) b	627 (64) b	636 (69) b
		------------------- umol kg soil $^{-1}$ --------------------			
Total	0-5	36.1 (1.5) a C	35.7 (1.7) a C	44.7 (1.7) a A	41.3 (1.7) a B
	5-15	21.6 (1.7) b	21.2 (1.5) b	22.2 (1.3) b	24.6 (1.5) b
	15-30	22.2 (2.1) b	21.0 (2.4) b	22.8 (2.4) b	24.0 (2.8) b
		--------------- Xylose / Mannose -----------			
Ratio*	0-5	0.97 (0.05)	0.97 (0.04)	1.11 (0.05)	1.11 (0.09)
	5-15	0.59 (0.02)	0.57 (0.02)	0.64 (0.03)	0.63 (0.05)
	15-30	0.52 (0.02)	0.53 (0.06)	0.51 (0.02)	0.56 (0.04)

Means followed by the same lower case letters do not differ significantly (α=0.05) between years. Means followed by the same upper case letters do not differ significantly (α=0.05) between depths. *Plant to microbial carbohydrates.

Anova for monosaccharide abundance and composition.

Anova	*Fuc*	Ara	Rha	Gal	Glu	Xyl	Man	Tot	Ratio
Depth	*0.003*	0.003	<0.001	<0.001	<0.001	0.001	0.005	<0.001	<0.001
Year	*0.174*	0.003	0.012	0.015	0.058	0.009	0.029	0.012	0.066
*D*Year*	*0.324*	*0.046*	*0.038*	0.017	0.030	0.008	0.004	0.005	0.144

Table 4. 8. Mean (se) soil δ^{13}C signature (‰) and percent carbon derived from maize C (% C4-C) by depth and year (n=6).

Soil layer (cm)		0-5	5-15	15-30
			δ13C (‰)	
2005	Maize	-16.26 (0.12) a A	-15.38 (0.13) a B	-15.04 (0.19) ab B
2006	Soybean	-16.64 (0.14) b A	-15.46 (0.19) ab B	-15.18 (0.24) ab B
2007	Maize	-16.80 (0.13) b A	-15.53 (0.17) ab B	-15.21 (0.22) a B
2008	Soybean	-16.75 (0.09) b A	-15.71 (0.17) b B	-14.94 (0.16) b C
			%C4-C	
2005	Maize	71.6 (0.8) a A	77.5 (0.8) a B	79.8 (1.2) ab B
2006	Soybean	69.1 (0.9) b A	77.0 (1.2) ab B	78.8 (1.6) ab B
2007	Maize	68.0 (0.8) b A	76.5 (1.1) ab B	78.6 (1.4) a B
2008	Soybean	68.3 (0.6) b A	75.3 (1.2) b B	80.4 (1.1) b C

Anova	δ13C (‰) and %C4-C
Depth	<0.001
Year	0.010
*Depth*Year*	0.017

Means followed by the same lower case letters do not differ significantly (α=0.05) between years. Means followed by the same upper case letters do not differ significantly (α=0.05) between depths.

CHAPTER 5. AGGREGATE-ASSOCIATED SOIL ORGANIC MATTER FRACTIONS UNDER NO-TILL MAIZE-SOYBEAN ROTATION.

1. Abstract

Soil aggregates constitute structural units of different sizes, resistance to mechanical breakdown, and thus turnover for stabilization of carbon (C) and nitrogen (N) inputs. The soil organic matter (SOM) amount and composition of aggregate size classes with respect to the standing crop and depth were assessed using a combined fractionation approach involving density separation and humic acid (HA) fractionation from slake-resistant aggregates (>250, 250-53 and <53 µm). Soil samples from the 0-5, 5-15 and 15-30 cm depths of an irrigated no-till maize‒soybean field were collected in fall 2005, 2006, 2007 and 2008. Carbon and N stocks decreased in the order >250 µm (60%), 250-53 µm (30%), <53 µm (10%), which was a function primarily of increased C and N contents and abundance of >250 µm aggregates. Stratification of C and N with soil depth was found for all aggregate classes and SOM pools. Free (fLF) and occluded (oLF) light fraction contributed significantly to aggregate C and N content only in the 0-5 cm depth (2 to 5% of aggregate C and N contents). The proportion of aggregate C and N recovered as mobile HA, calcium bound HA, and humin increased linearly with increasing aggregate C and N content for all depths. No clear relationship was found between fLF and oLF amount and aggregate C and N content. The hypothesized enhanced humification of aboveground crop residues during the 2007 maize year was corroborated by the increase in both MHA and CaHA fractions from larger aggregates in the 0-5 cm

depth. Thus, the formation and abundance of MHA and CaHA appears to be related to stability of slake-resistant aggregates >250 µm.

Key words: humic acids, soil structure, residue quality, humification.

2. Introduction

Soil aggregates constitute structural units of soil minerals and soil organic matter (SOM) of different sizes, resistance to mechanical breakdown, and thus turnover (Tisdall and Oades 1982, Gregorich et al. 1989). Stabilization of SOM is achieved by humification, organo-mineral associations and physical protection due to occlusion into intraagregate light fraction (oLF) and free light fraction in interaggregate pore space (fLF). The relative importance of organo-mineral association and physical occlusion for soil carbon (C) and nitrogen (N) stabilization is dependent on the size of stable aggregates (Puget et al. 2000, Yamashita et al. 2006) and may influence the degree of humification of aggregate associated SOM. As the aggregate size increases, the intra-aggregate pore volume increases, allowing for occlusion of SOM inside the aggregate (McCarthy et al. 2008). Thus smaller aggregates protect C mostly by organo-mineral associations while in larger aggregates both processes provide important contributions to stabilization of C inputs as SOM (Jastrow et al. 1996, Yamashita et al. 2006), resulting in distinct qualitative and quantitative SOM protection capacities by aggregate classes.

No-till systems alter the incorporation of crop residues into soil by limiting the soil-residue interface to the soil surface, which leads to stratification of C and N in the soil profile (Puget et al. 2005, Angers and Ericksen-Hamel 2008). An observed consequence is the preferential accumulation of C and N inputs as inter- (free) and intra-aggregate (occluded) SOM pools in the surface soil (Chapters 2, 3 and 4). Gulde et al. (2008) proposed that as soil C and N increases, the C protection capacity of organo-mineral sites in the soil is saturated, such that further C and N inputs that are stabilized will be into uncomplexed, physically protected, niches. In the previous chapter, we analyzed the effect of irrigated maize-soybean rotation under no till on soil C and N stocks. A significant increase in soil C stocks was observed after a maize year (2007) and ^{13}C analysis indicated increases in both C3 (soybean) and C4 (maize) C sources (Chapter 4). Analysis of physically protected SOM fractions (fLF and oLF) indicated that they accounted for <30% of the increase in C and N stocks, while most of the loss in soil C and N stocks observed after the following crop could be attributed to loss of these uncomplexed SOM pools (Chapter 4). Thus other mechanisms were responsible for the increased stabilization of maize-and soybean-derived C and N in surface soil. Assessment of SOM within soil aggregates and with regard to the size and location of the aggregates in the soil profile may provide significant insights into the stabilization mechanisms of C and N inputs under no-till systems.

In this chapter we report on the results of a combined fractionation approach in which two density fractions and three humic materials were extracted from three size classes of water-stable aggregates (>250, 250-53 and <53 µm) after two maize (2005, 2007) and two soybean (2006, 2008) cropping seasons. Our objectives were to: 1) evaluate for each aggregate size class it its SOM protection capacity in relation to the type and amount of crop residues (maize and soybean), and the location of the aggregates in the soil profile, 2) quantify and compare SOM composition of aggregate size classes in relation to location in the soil profile and the type and amount of crop residues, 3) explore the stabilization mechanisms responsible for the increase in soil C and N stocks after the 2007 maize season and 4) further analyze the hypothesis presented in Chapter 4 that the increase in in soil C and N stocks was associated with enhanced humification of previous (2005) maize residues during the 2007 maize cropping season.

3. Materials and Methods

Study site

This study was part of the UNL Carbon Sequestration Program (CSP) focused on examining the potential to sequester C in agricultural systems (Verma et al. 2005). We used a large, production-scale, agricultural field (52.4 ha) equipped with center-pivot irrigation located at the University of Nebraska Agricultural Research and Development Center near Mead, NE (41° 9' 53.5"N, 96° 28' 12.3"W, 362 m). The soils are deep silty clay loams consisting of four soil series: Yutan (fine-silty, mixed, superactive, mesic Mollic Hapludalfs), Tomek (fine, smectitic, mesic Pachic Argialbolls), Filbert (fine, smectitic, mesic Vertic Argialbolls), and Filmore (fine, smectitic, mesic Vertic Argialbolls). Previous to 2001, the field was under no till maize-soybean rotation for 10 years. Before initiation of the CSP study in 2001, the field was uniformly tilled by disking to homogenize the top 10 cm of soil and to incorporate P and K fertilizers and previously accumulated surface residues. The field has been under an irrigated no-till maize-soybean rotation since 2001. Crop management practices (i.e., plant populations, herbicide and pesticide applications, irrigation, fertilization) have been employed in accordance with standard best management practices prescribed for production scale maize-soybean systems (see Chapter 4).

Soil sampling

Annual soil sampling of the top 0.3 m of soil was conducted in the fall of 2005, 2006, 2007 and 2008. Sampling was done in each of the six IMZs (intensive management zones, see Chapter 4 for details) after crop harvest and before fall tillage. Within each IMZ, six separate soil cores (4 cm diameter by 30 cm depth) were collected along a transect in east–west direction between previous crop rows and inter-row center (approximately 16 cm away from row). The field-moist cores were split into three soil depths: 0-5, 5-15 and 15-30 cm, and then gently broken apart along natural planes of weakness. The samples were combined into one composite sample for each year (4) by IMZ (6) by depth (3) combination, then air-dried to constant weight, sieved through 8mm and stored in boxes for further fractionations and analyses.

In addition to the soil core samples, five smaller soil cores (2.15 cm diameter by 30 cm depth) were taken every year to determine bulk density in each transect of each IMZ, using a lubricated plastic sleeve mounted inside a soil hand probe. These cores were also split into 0-5, 5-15 and 15-30 cm depths, dried at 105 ºC for 24 h and weighed. Soil bulk density (BD, Mg m^{-3}) for each depth increment of each core was calculated as:

$$BD = \frac{Soil_m}{Soil_v}$$

where $Soil_m$ is the oven dried soil mass(g), and $Soil_v$ is the volume of soil of each depth increment.

Extraction of SOM fractions

In order to assess the different protection mechanisms, physical and chemical SOM extraction techniques were combined similar to Chapter 3 (Figure 5.1). The soil samples were first separated into aggregate classes, and then humic acid fractions and light fractions were extracted from each aggregate class. A wet-sieving procedure, which allowed for slaking to occur, was used to separate water-stable aggregates from the soil. The soil was separated into three aggregate size fractions: >250, 53-250 and <53 µm. One hundred grams of the air-dried soil was put on the 250 µm sieve and quickly immersed in distilled water inside a plastic pail. After ten minutes of immersion the sieve was moved up and down (3 cm) 60 times during a period of two minutes without breaking the water. After sieving, the sieve was set aside to drain and the contents in the plastic pail were poured on top of the 53µm sieve inside a second plastic pail. The sieve was moved up and down (3 cm) 60 times during a period of two minutes without breaking the water. After the second sieving the sieve was set aside to drain and the <53 µm soil fraction was transferred to 2 L flasks and brought to volume together with 2 ml of a saturated calcium chloride solution. After overnight sedimentation of the suspension, most of the water was removed by siphoning and the sediments were backwashed into weighed metal boats and dried at 50ºC until constant weight. The aggregates in the sieves were backwashed into porcelain crucibles with distilled water and dried until constant weight at 50ºC. Each combination of soil depth, IMZ and year was extracted at least three times to insure enough aggregate material for subsequent fractionations.

Two humic acid fractions were sequentially extracted in the >250 and 250-53 µm aggregates for all soil depths and years. These extractions were not performed on the <53 µm size class due to insufficient material. The mobile humic acid (MHA) fraction represents young humic material that is less associated with soil minerals, and the Ca-bound humic acid fraction constitutes an older, more condensed humic material associated with soil minerals (Campbell et al. 1967, Olk et al. 1995). The extraction was adapted from Olk et al. (1995). Briefly, the MHA was extracted from soil with 0.25 M NaOH under N_2 atmosphere. The solubilized material (MHA + fulvic acids) was acidified with HCl to precipitate the MHA fraction. The extraction was performed twice on each sample to remove the majority of the MHA. The soil remaining after the MHA extraction was decalcified through washes with 0.25 M HCl. The CaHA was then extracted in a similar way to MHA with 0.25 M NaOH under N_2 atmosphere. The solubilized material was acidified to precipitate the CaHA. Both MHA and CaHA were de-ashed with a 0.5% HFl and 0.5% HCl solution for 24 hours, and de-salted by dialysis for three days in weak HCl and then water solutions. After dialysis the material was freeze-dried, weighed, finely ground and stored in amber glass vials. The remaining soil containing the unextracted humin was oven-dried at 50ºC until constant weight, finely ground with mortar and pestle and stored in glass scintillation vials.

A sequential density fractionation, adapted from Golchin et al. (1994) and Besnard et al. (1996), was used to extract two loosely physically protected fractions, the free light fraction (fLF) and light fraction occluded inside aggregates (oLF) in the >250 and 250-53 µm aggregates for all soil depths and years Briefly, 10 g aggregates were placed in duplicate in a 60 mL centrifuge tube and 40 mL of sodium polytungstate (NaPT) of 1.6 g cm^{-3} density was added. The tube was capped with a plastic lid and inverted gently by hand five times to avoid disruption of aggregates. The particles which adhered to the lid and tube walls were washed into suspension using more NaPT to a final volume of 50 mL. The suspension was allowed to stand for 30 min before centrifuging at 2000 rpm, for 1 hour. The supernatant with floating particles (free LF <1.6 g cm^{-3}) was poured into a nitro-cellulose filter unit (0.20 μm), and filtered under vacuum. Ten (5-mm) glass beads and 40 mL of NaPT solution (1.6 g cm^{-3}) were added to the aggregates remaining in the centrifuge tubes, and the tubes were shaken horizontally for 16 hours at 60 oscillations per minute. After shaking, the particles which adhered to the lid and tube walls were washed into suspension using more NaPT to a final volume of 50 mL. The tubes were allowed to stand for 30 min before centrifuging at 2000 rpm for 1 hour. The supernatant with floating particles (oLF with a density <1.6g cm^{-3}) was poured into a nitro-cellulose filter unit (0.20 μm), and filtered under vacuum. The NaPT was removed from the fLF and oLF by submerging the material at least four times in 80 mL deionized (DI) water and removing the leachate with a vacuum filtration system while adding more DI water using a squirt bottle to keep the LF material in suspension (Crow et al. 2007). The LF on the filter was backwashed into a weighed porcelain crucible and dried at 50°C until constant weight, duplicates were combined, finely ground in a ball mill and stored in amber glass vials for further analysis. The NaPT was recycled according to Six et al. (1999b) to avoid cross contamination of C between samples.

Analytical methods

- C and N analysis

A sub-sample of each aggregate size was finely ground to pass a 100 mesh sieve using a roller mill. Any recognizable plant material and/or $CaCO_3$ concretions (> 1 mm) were removed before grinding. Twenty milligrams of the ground aggregates and humin, 2 mg of humic materials, and 5 mg of light fraction were weighed for C and N concentration using an elemental analyzer (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA). The C:N ratio of the aggregates and SOM fractions can be used an index of humification, where lower C/N ratios are related to higher degree of humification (Martins et al. 2011).

To better account for the variation in effective sampling depth and soil mass due to changes in soil bulk density over time, the cumulative mass approach described by Gifford and Roderick (2003) was used to compare C and N changes over time for each aggregate size. Carbon (g C m^{-2}) and N (g N m^{-2}) contents of each aggregate size were estimated for each of the three soil depth intervals based on the measured bulk density at the time of sampling, the C and N concentration of the aggregates and the abundance of the aggregate class. Three reference soil masses were used to evaluate aggregate C and N changes over time (Equation 4, Gifford and Roderick 2003): (i) the top 60 kg dry soil m^{-2} (approximately 0–5 cm depth), (ii) the top 200 kg dry soil m^{-2} (approximately 0–15 cm depth) and (iii) the top 400 kg dry soil m^{-2} (approximately 0–30 cm depth).

Statistical analysis

Equality of the means among years was tested with repeated measurements (RM) ANOVA to account for the nature of the field sampling procedures and the natural variability among individual IMZ. The comparisons of C and N stocks among years for each aggregate size and for the SOM fractions of each aggregate class were done using a one-way RM ANOVA (year) (Table 5.1). Fully crossed three-way RM ANOVA (soil layer, aggregate size and year) was used for the comparisons of aggregate's C and N concentration and SOM composition (C and N contents in aggregates of SOM pools), C:N ratio and mass fraction of aggregates (Table 5.1). When the three-way interaction was significant, the differences among years were analyzed for each combination of aggregate size and depth. Post-hoc separation of means was done with Fisher LSD procedure if overall ANOVA indicate significant differences ($p<0.05$). Planned contrasts were used to assess the role of previous crop (maize versus soybean) on the aggregate C and N composition using the CONTRAST statement. Data were analyzed using PROC MIXED procedures in SAS 9.3 (SAS Institute 2009), with IMZ as the subjects and year as the repeated effect.

4. Results

Aggregate size class distribution

After wet aggregate size fractionation, 48-61 % of the soil mass was recovered as >250 µm aggregates, while midsize and smaller aggregates comprised 30-40% and 9-15% of the soil mass, respectively (Table 5.2). Both depth by size and size by year interactions were significant ($p<0.05$). The reduction in the proportion of largest aggregates with depth ($p>0.05$) was accompanied by increases in the mid-size ($p>0.05$) and small-size aggregates ($p<0.05$). The proportion of larger aggregates was greater during maize years than soybean years. Consequently, the proportions of mid-size and small-size aggregates were larger in soybean years than in maize years.

Aggregate C and N stocks

Carbon and N stocks on a cumulative basis and also for each soil depth are shown in Figure 5.2 and 5.3 and Table 5.3. The contribution of each aggregate class to soil C and N stocks changed with depth. Aggregates > 250 µm accounted for a similar percentage of C and N stocks in each layer: 64%, 59% and 50% in the 0-60, 60-200, and 200-400 kg soil m^{-2}, respectively (Table 5.3). Mid-size aggregates and small aggregates contributed increasingly to C and N stocks with increasing depth, accounting for 29%, 32% and 40% of the C and N stocks as mid-size aggregates and 7%, 9% and 10% as small size aggregates in the 0-60, 60-200, and 200-400 kg soil m^{-2} layers, respectively. Trends among years were similar for C and N stocks. The C and N stocks for the aggregates in the 0-60 and 200-400 kg soil m^{-2} layer were affected by the standing crop. From 2005 to 2006 and from 2007 to 2008 (maize to soybean), C and N stocks decreased in the largest aggregates and increased in the mid-size and smallest aggregates. From 2006 to 2007 (soybean to maize), C and N stocks increased in the larger aggregates, with a slight decrease in the mid-size and smallest size aggregates.

C and N concentrations in aggregate size classes

Carbon and N concentrations of the aggregates decreased with decreasing aggregate size within each depth (Tables 5.4 and 5.5, and Figure 5.4). Aggregate C and N concentrations were stratified, with larger concentrations near the surface. The degree of reduction in C and N concentrations with depth varied by aggregate size: C concentration of larger aggregates decreased by 43% from the 0-5 depth to the 5-15 cm depth, while the C concentration of mid-size aggregates and small-size aggregates decreased by 28% and

13%, respectively. From the 5-15 to 15-30 cm depths, the percent decrease in C concentration were smaller, being 7%, 3% and 4% for the >250, 250-53 and <53 µm sizes, respectively. Given the significance of the three-way interaction (Table 5.7), the changes in the C and N concentration among years were analyzed for each combination of aggregate size and depth. Differences in C concentration among years were significant only for the >250 µm aggregates in the 0-5 cm depth, where 2007 had the largest content. Similarly, differences in the N concentration of aggregate size classes were significant only for larger aggregates at the 0-5 depth with a maximum value in 2007. The significant difference among years in N concentration of larger aggregates in the 15-30 cm depth was caused by the small N concentration in 2007 (Table 5.4 and 5.5). During 2005, all aggregates in the 0-5 cm depth had numerically lower C and N concentrations than in other years.

The aggregate C:N ratio narrowed with decreasing aggregate size in all depths (Table 5.6) ($p<0.05$). Similar to C and N concentrations, the three-way interaction term was significant (Table 5.7). After a maize year, C:N ratios tended to be narrower in the smallest aggregate size class (all depths) and the mid-size class (5-15 and 15-30 depths only) compared to the soybean years.

C and N contents in SOM fractions of >250 µm and 250-53 µm aggregate classes

The fractionation of the largest and mid-size aggregates into free and occluded LF and the MHA, CaHA and humin fractions recovered 80-93% of the C in large aggregates and 77-87% of C in the mid-size aggregates (Table 5.4). These fractions together accounted for 63-79% and 61-73% of the N content of large and mid-size aggregates, respectively (Table 5.5). The distribution of aggregate-C and -N ranged from 61-78% and 53-61% as humin, 6-8% and 4-7% as CaHA, 2-11% and 3-9% as MHA, 1-4% and 0.3-2.6% as oLF, and 0.2-5.3% and 0.1-3.8% as fLF, respectively (Table 5.4 and 5.5).

Changes over time in aggregate C and N contents of SOM fractions were affected by depth and aggregate size. In general, the largest aggregates contained more C and N extracted as SOM than did the mid-size aggregates, and the decrease in the C and N contents of aggregates with soil depth was matched by a reduction in extracted C and N. Aggregate C contents extracted as fLF and MHA showed a significant three way interaction, while the aggregate C contents of oLF, CaHA and humin showed at least two significant interactions (depth by size and depth by year) (Table 5.7). Similar results were obtained for aggregate N content: fLF, MHA and CaHA had three-way interactions, while oLF and humin showed two significant interactions (depth by size and depth by year) (Table 5.7). The significant three-way interactions were related to the increase in the C and N contents of larger aggregates in the 0-5 cm depth in 2007 (Table 5.4 and 5.5). The significant interactions between depth and size (all fractions) were related to a larger reduction in the C and N content with depth in the >250 µm aggregates compared to the 250-53 µm aggregates. Depth by time interactions of oLF-C and -N were associated with cyclical C and N contents in the 0-5 and 15-30 cm depths. During soybean years, C and N contents as oLF were smaller than in maize years.

Using the C:N ratio as a negative index of the degree of SOM humification, the degree of humification increased for all depths in the order: (larger than 250 µm) < (250-53 µm) < (smaller than 53 µm), indicating the presence of less decomposed litter or root derived C in the larger aggregates and older more degraded materials in the <53 µm aggregates (Table 5.6). The C:N ratio of the SOM fractions narrowed from LF > CaHA > Humin > MHA (Table 5.5). The C:N ratio of the MHA fraction decreased with depth (similar to aggregate C:N ratio), while the C:N ratio of CaHA, fLF and oLF increased with depth (Table 5.5). The C:N ratio of the CaHA was similar among aggregate classes for each depth. The C:N ratio of humin was wider for the >250 µm aggregates than for the 250-53 µm aggregates in the 0-5 and 5-15 cm depths, but similar in the 15-30 cm depth. The C:N ratio of the aggregates showed an analogous trend. For the MHA fraction, the C:N ratio was larger in the >250 µm aggregates than in the 250-53 µm aggregates in all depths. Given the significant three-way interaction, years were compared for each combination of aggregate size and depth (Table 5.5). Among all years the C:N ratio of the MHA in the 0-5 cm depth was widest in 2007 for both aggregate classes and in general the C:N ratio of the MHA fraction was more sensitive to year than were the C:N ratios of the other fractions. The C:N ratio of fLF and oLF were influenced by aggregate class, year and depth. In general the 250-53 aggregates had wider C:N ratio than the larger aggregates, with larger differences in the 15-30 cm soil depth than in the 0-5 cm depth.

The composition of aggregate C as a function of aggregate C content varied with soil depth, while aggregate size had a small effect on aggregate composition (Figure 5.5). Within each depth, C contents of the humin, CaHA and MHA fractions in the >250 µm and 250-53 um size classes increased with aggregate C content (Table 5.8). The slope of the humin-C relationship was similar between aggregate classes. For aggregate-C contents at or below 10 g kg^{-1}, humin C accounted for >90% of the aggregate C and the remaining C was mostly extracted as CaHA-C. Between 10 and 20 g C kg^{-1} aggregate, C contents as MHA and CaHA increased linearly, with similar slopes in the >250 aggregate class. In that range, increase in aggregate C content was reflected by a larger increase in the CaHA pool than in the MHA pool in the 250-53 µm aggregate class (Table 5.8). Above 20 g C kg^{-1} aggregate, the increase in C content as MHA doubled that of the CaHA. However, aggregate C content > 20 g C kg^{-1} aggregate was achieved only by >250 µm aggregates in the 0-5 cm depth. At these high aggregate C levels, greater deviation from the best fit lines appeared in the humin, CaHA, and MHA fractions. Increases in C contents of the two LF fractions were not correlated with aggregate C content, especially at C levels less than 20 g C kg^{-1} aggregate.

5. Discussion

In the present study the concept of aggregate capacity to sequester C and N under no-till irrigated maize-soybean is evaluated. We studied the distribution of C and N in three aggregate size classes and their extracted SOM fractions as influenced by soil depth. We provide evidence that the high volume of low C:N ratio soybean residues in 2006 contributed to the decomposition and humification of previous (2005) maize residues during the 2007 maize cropping season (Chapter 4). First discussed is the aggregate C protection capacity with soil depth and aggregate size, and second the aggregate associated SOM fractions in relation to aggregate size and changes in soil C and N stocks.

Aggregate capacity to protect C and N: relationship to aggregate size and soil depth

- Soil depth

Carbon and N contents in all aggregate size classes decreased below the uppermost 5 cm soil depth. This stratification of aggregate C and N content under no-till management is in agreement with previous studies (Beare et al. 1994). The larger C and N contents in aggregates of the 0-5 cm depth compared to deeper layers points out the significant contribution of aboveground residue C and N inputs to SOM formation. Kong et al. (2005) found a positive linear relationship between aggregate C content and the amount of C inputs. In our study, the smaller C and N contents of all soil aggregates in the 5-15 and 15-30 cm depths indicate the contribution of C and N inputs from decomposing aboveground residues and belowground roots is significantly reduced in these layers compared to the 0-5 cm depth. In addition, the proportion of larger aggregates decreased with soil depth, similar to soil C content. Our results are consistent with the observations of Kong et al. (2005) who reported increased proportions of larger aggregates with larger C contents as a consequence of increased C inputs. Thus, the reduced soil-residue interface under no-till constitutes (i) a direct limitation to the protection of new C and N inputs by limiting the volume of soil for stabilization of C and N inputs, and (ii) an indirect restriction on C and N protective capacity of aggregates below the 5 cm soil depth by constraining aggregation.

Six et al. (2002) hypothesized that as the C and N content of soil approaches its potential capacity for sequestering C and N, the soil has decreased capacity to further sequester SOM. Given the larger C and N contents of all aggregates in the 0-5 cm soil depth, limited potential for further C and N sequestration in this layer can be expected. Approximately 56% of the increase in soil C and N stocks after the 2007 maize crop, as discussed in Chapter 4, occurred in the 0-60 kg soil m^{-2} layer (approximately 0-5 cm depth) and appeared to result from the decomposition and humification of both 2005 maize and 2006 soybean residues during the 2007 cropping season. In this study, after the maize 2007 season C and N contents increased in all aggregate size classes of the 0-5 cm depth but were significant only in the >250 μm aggregates. Thus, despite the larger soil C and N content, the capacity of the surface soil layer to protect more C and N inputs seems to have not reached its capacity.

- Aggregate Size

The C content of the smallest aggregates per unit aggregate mass were on average 50%, 76% and 79% of the C content in the larger aggregates for the 0-5, 5-15, and 15-30 cm depths, respectively. The C content of the mid-size aggregates was on average 67%, 86% and 89% of the C content in the larger aggregates for the same depths. The observed increase in the aggregate C and N contents with increasing aggregate size are in agreement with previous studies (Jastrow et al. 1996, Besnard et al. 1996, Puget et al. 2000, Yamashita et al. 2006) and has been used to indicate the capacity of aggregates to sequester C.

Assuming total soil C and N contents of these soils were in equilibrium with C and N inputs, the role of aggregate size in the aggregate protection capacity of C and N can be assessed by comparing the aggregate C and N concentrations to soil C and N concentrations. Similar slopes (i.e. 1) for aggregate C and N concentrations and soil C and N concentrations for all aggregate classes in the 5-15 and 15-30 cm depths indicates uniform C adsorption across aggregate size classes across these depths (Figure 5.4). In addition, the linear relationship for both C and N content was also found in the 0-5 cm depth for only the largest aggregates (R^2=0.94 for C and N, Figure 5.4), while the C and N contents of mid-size and small size aggregates seemed to reach a plateau (Figure 5.4). These relationships imply a larger capacity to protect C and N for >250 aggregates than for the smaller aggregate size classes but do not identify the responsible protection mechanisms. The finding that aggregate C concentration responded similarly across size classes to total soil C and N in the 5-30 cm depth but not in the 0-5 cm depth may be due either to: 1) saturation of the C protective capacity of 250-53 μm aggregates (physical occlusion and organo-mineral associations) and <53 μm aggregates (organo-mineral associations) in the 0-5 cm depth, 2) preferential protection of new C and N inputs by aggregation into larger units (physical occlusion), or 3) an operational shift in the distribution of uncomplexed SOM during wet sieving and 'slaking' of aggregates (release of light fraction from aggregates not stable in water that is recovered on the >250 μm sieve). The first two explanations are discussed further below.

If there was a finite capacity to sequester C that differs among the aggregate size classes (Gulde et al. 2008, Six et al. 2002), the slight increase from 2006 to 2007 of the C concentrations of the mid-size and small size aggregates (+3% and +0.3% respectively) in the 0-5 cm depth and the corresponding larger increase (+16%) for the >250 μm aggregates could be attributed to the <53 and 250-53 μm aggregate classes nearing their C saturation capacity, which is not the case for the >250 μm aggregates. This limited capacity, or C saturation level, is related to the protective capacity of each stabilization mechanism: interaction of organic materials with soil minerals (organo-mineral associations), and physical occlusion of organic material from decomposers and/or their enzymes. The <53 μm aggregates fraction is regarded as mostly clay- and silt-size aggregates in which SOM is stabilized by organo-mineral associations (Puget et al. 2000). Thus, the asymptotic relationship of the C and N concentration of the <53 μm aggregates to soil C and N concentration might suggest the saturation of sites for organo-mineral associations. In the 250-53 μm aggregates, both physical occlusion and organo-mineral associations stabilize SOM (Cambardella and Elliot, 1993). Assuming a mineral particle density of 2.65 g mL^{-1}, aggregate density of 1.8 g mL^{-1} (Chepil 1950, Bisal and Hinman 1972), and an oLF density ranging from 0.9 g mL^{-1} (oil and waxes) to 1.6 g mL^{-1} (density of the NaPT solution used), less than 5% (i.e. between 2.6 to 4.5%) of total intra-aggregate pore space was occupied by occluded SOM. McCarthy et al. (2008) studied the SOM filled porosity using ultra small X-ray scattering. They used soil aggregates extracted from contrasting soil management scenarios to evaluate the change in SOM filled porosity before and after removal of labile physically protected SOM. They found that pores <10 nm were completely filled by stable SOM, while physical protection of SOM occurred in pores larger than 10 nm. They determined that soil pores >1 μm could physically protect SOM, but the proportion of SOM protection (SOM-filled pore volume) dropped significantly. To conclude that an estimated mean intra-aggregate pore space occupied by occluded SOM of 5% challenges the validity of the saturation concept and would also require knowledge of the pore size distribution in this soil.

The second possible interpretation of the increased aggregate C and N content in the 0-5 cm depth after the 2007 maize crop, is that all aggregates may have similar saturation of their SOM protective capacity at this depth, but stabilization of new, labile C and N inputs promotes aggregation only in the >250 μm aggregates, perhaps by increasing mean aggregate diameter. This may be visualized as combinations of different aggregate sizes that nucleate around C inputs or that are clustered together by growing fungal hyphae, or both (Jastrow et al. 1996). From 2006 to 2007, we observed an increase (+8%) in the mass fraction of the >250 aggregates, at the expense of both mid-size (-7%) and small-size (-10%) aggregates. Puget et al. (2000) using ^{13}C to trace the incorporation of maize-derived C into particle size fractions of stable and unstable macro aggregates, speculated that the incorporation of young organic matter from plant debris is the responsible binding mechanism between microaggregates. Using the ^{13}C signature of soil aggregates after a change from C3 (grass) to C4 (maize) species, Jastrow et al. (1996), Angers and Giroux (1996) and John et al. (2005) reported 1) a preferential incorporation of maize C inputs into aggregates >250 μm in the short term (four years), and 2) slow incorporation of maize residues into smaller aggregates with time. Thus, it is plausible that the "saturated" behavior of 250-53 μm aggregates in this study instead reflects a slower rate of aggregation.

Aggregate protection mechanisms

As described previously, the C:N ratio indicated increasing degree of humification with decreasing aggregate size (Table 5.5). These results are consistent with the findings of Chapter 3 and with the increasing proportions of fLF and oLF pools with increasing aggregate size. In Chapter 3, the ratio of microbial to plant carbohydrates was found to increase as aggregate size decreased in all depths under continuous maize no-till management; microbial residues are likely to be more common in humified SOM, which has been degraded by microorganisms for a considerable time, than in younger, more plant-like materials. The proportions of fLF-C and oLF-C were 10 times and two times larger, respectively, for the C content of the >250 µm aggregates than for the C content of the 250-53 µm aggregates. Insignificant amounts of fLF and oLF were recovered from the <53 µm aggregates during the preliminary assessment of the physical density fractionation (see Appendix). In addition, visual inspection of these fractions under a dissecting microscope is in agreement with the observations of Golchin et al. (1994) regarding their predominance of plant-derived material.

The increasing proportion of oLF-C with aggregate size is in agreement with the known increase in intra-aggregate pore space as aggregates increase in size. On the other hand, the increased oLF in both aggregate classes in the 0-5 cm depth during maize years may be associated with increased aggregation. The 10-fold increase in free LF-C from the 250-53 um aggregates to the >250 um aggregates should be cautiously interpreted. During wet sieving, slaking of non-stable aggregates releases plant debris that was trapped inside aggregates of several sizes. Buoyancy of those oLF materials may have shifted their recovery to the larger aggregates regardless of the debris size. Thus release of oLF during slaking and the resulting shift in size recovery due to buoyancy in the wet sieving separation may explain the differences in fLF and oLF between the aggregates and the whole soil extraction, and the lower C:N ratios of fLF in the 0-5 cm depth in our study.

Preferential accumulation of C as MHA compared to CaHA in the largest and most C-enriched aggregates of the 0-5 cm depth suggests that significant proportions of humified crop residues associated with those aggregates were not stabilized by Ca^{+2} and or Mg^{+2}, perhaps due to inadequate access to these divalent cations. Furthermore, the preferential accumulation of MHA together with the steady increase in the humin C with increasing aggregate C in larger aggregates of 0-5 cm depth but not in the CaHA (Figure 5.5) suggests CaHA stabilization in larger aggregates may be constrained. At the same time, however, the increase in C content of the largest aggregates in the 0-5 cm depth after 2007 maize was accompanied by an increase in MHA-C content (+34%) and CaHA-C content (+20%) and no changes in humin-C.

The increase in humified C in the aggregates in 2007 compared to 2006 is consistent with the whole soil trends presented in Chapter 4. The increases in C stocks of the 0-60 kg soil m^{-2} layer from 2006 to 2007 were followed by an increase in physically protected C (Chapter 4). However, the physical fractions explained only <30% of the total soil C increase. An increase in humification of the aboveground residues was hypothesized to have contributed to the increase in soil C stocks. In this study, the increase in the MHA and CaHA fractions in the 0-5 cm depth of larger aggregates in 2007 compared to 2006 support the hypothesis that the increased C stocks of 2007 in the surface soil were associated with increased humification. The results also indicate a link between humification of crop residues and aggregate formation and stabilization.

6. Conclusion

A combined fractionation approach involving density separation and humic acid fractionation from slake-resistant aggregates allowed the evaluation of the composition and C-protective capacity of aggregate size classes with soil depth. Aggregate C and N content and SOM composition were affected by the both aggregate size and depth. Carbon and N stocks were stratified with soil depth across all aggregate size classes, as were the fLF and oLF pools within each aggregate class. These results corroborate the hypothesis that the reduced soil-residue interface constitutes a limitation for stabilization of new C and N inputs into all aggregate size niches. The proportions of aggregate C and N content extracted in humified fractions increased linearly with aggregate C and N content across all depths, while aggregate C and N content were not clearly related to fLF and oLF. The hypothesized enhanced humification of aboveground crop residues during the 2007 maize year was corroborated by the increase in both the MHA and CaHA fractions of larger aggregates in the 0-5 cm depth. The accumulation of MHA and CaHA, seems to be interrelated with the stability of slake- resistant aggregates >250 µm and the physical stabilization of new C and N inputs as oLF in the 0-5 cm surface soil.

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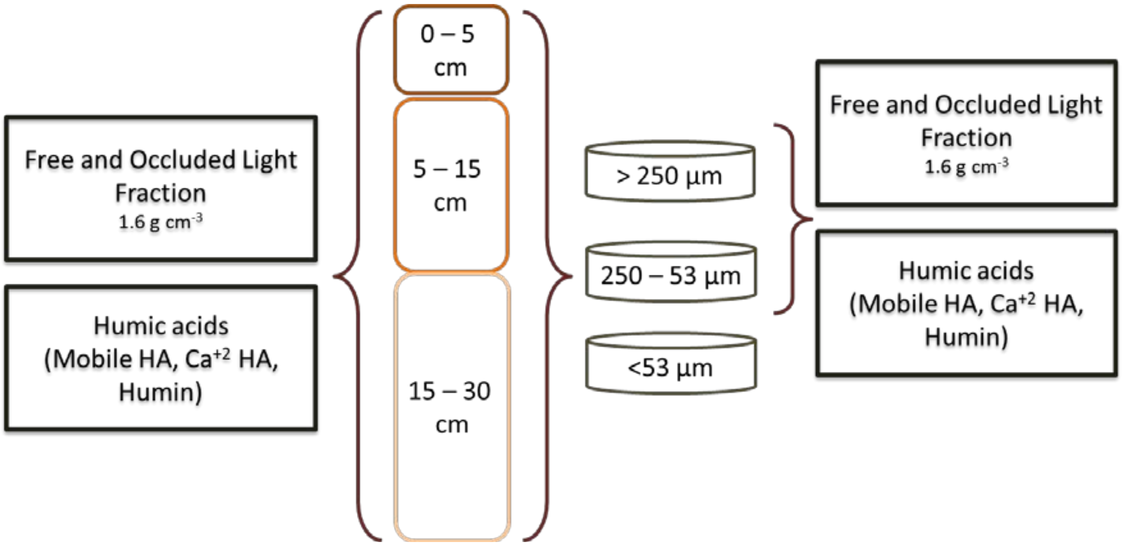

Figure 5. 1. Procedure for extraction of soil organic matter fractions

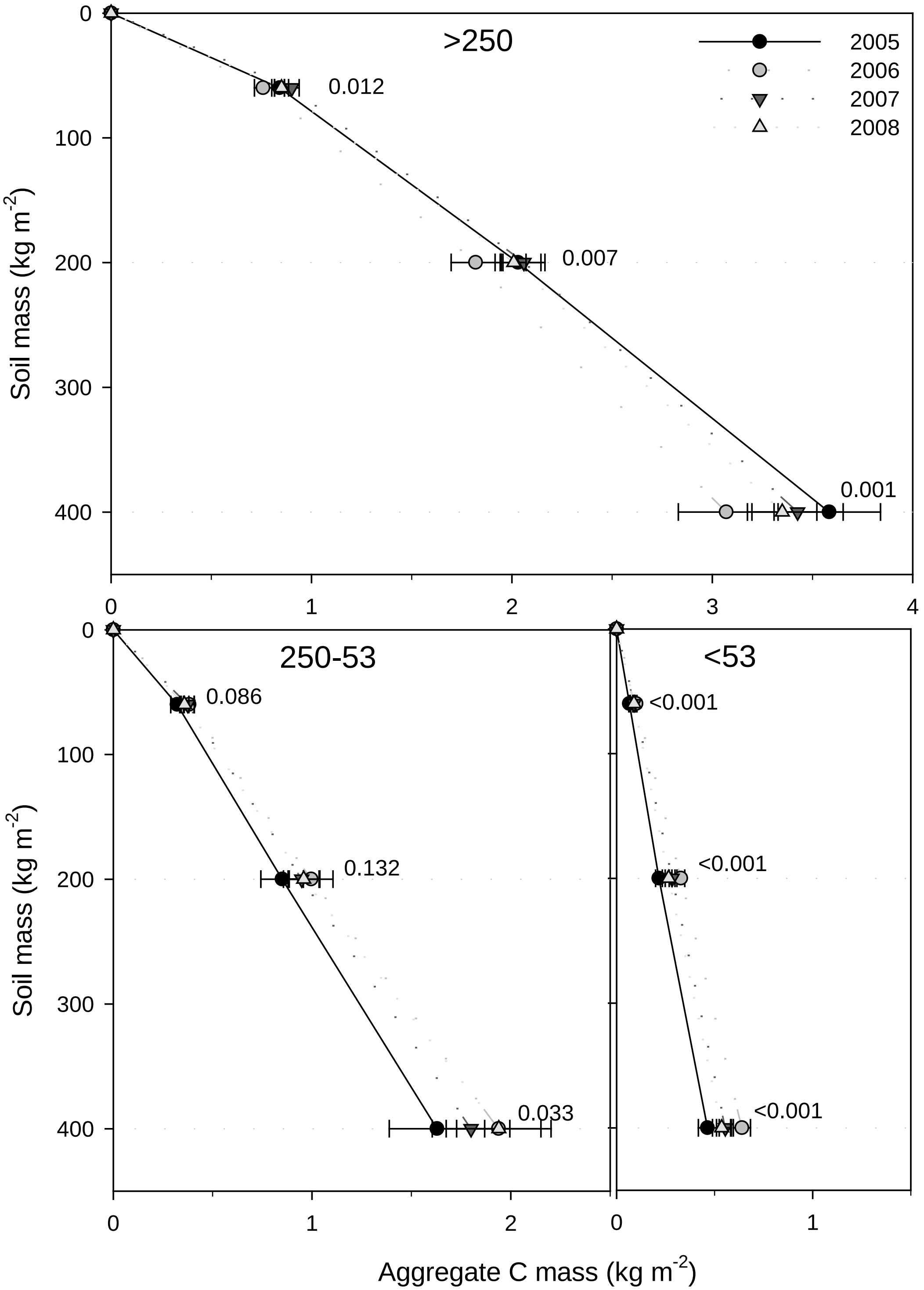

Figure 5. 2. Aggregate C stocks on an equivalent soil mass basis. Probability value (p>F) for comparison between years at each cumulative layer.

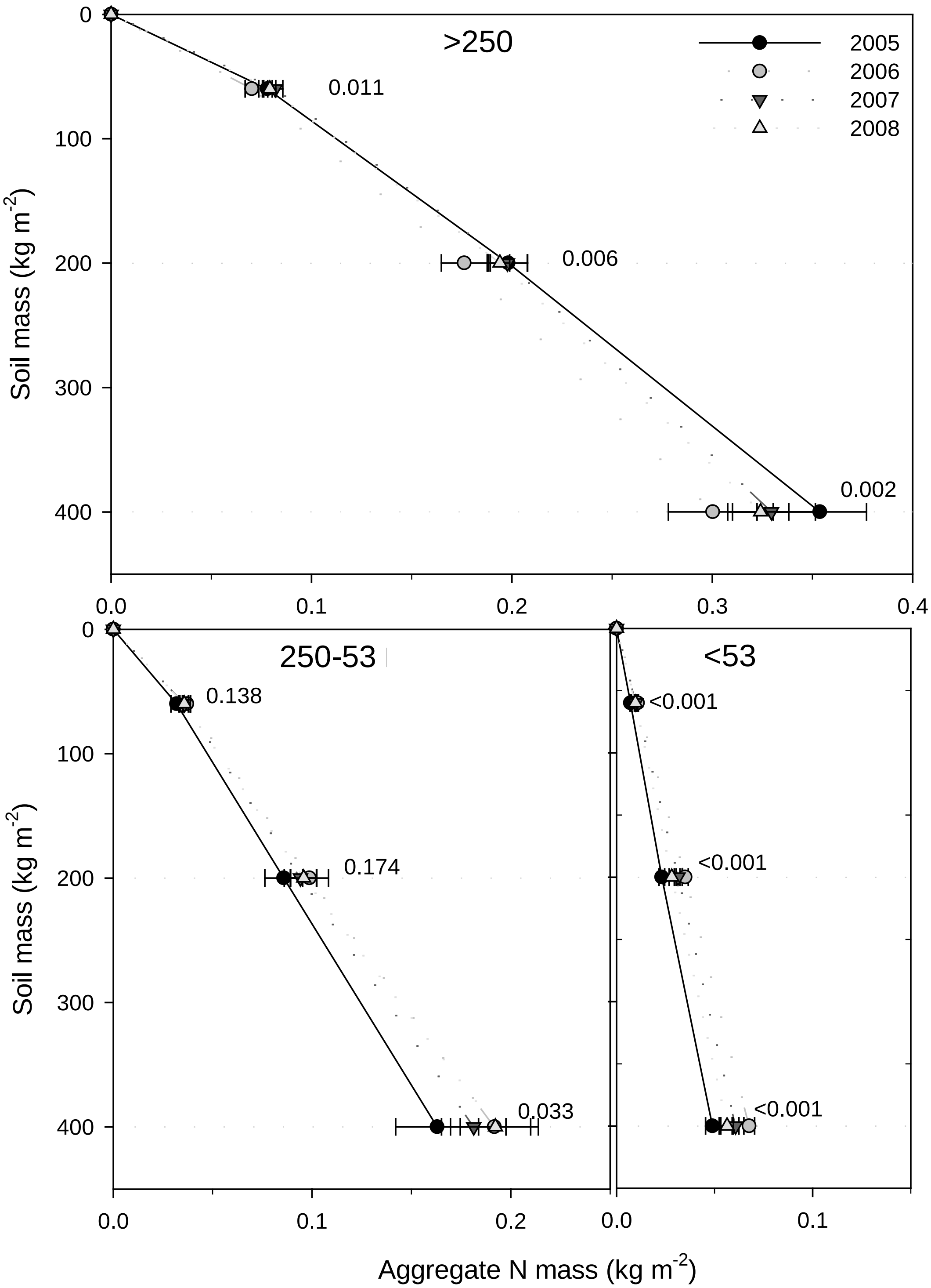

Figure 5. 3. Aggregate N stocks on an equivalent soil mass basis. Probability value (p>F) for comparison between years at each cumulative layer.

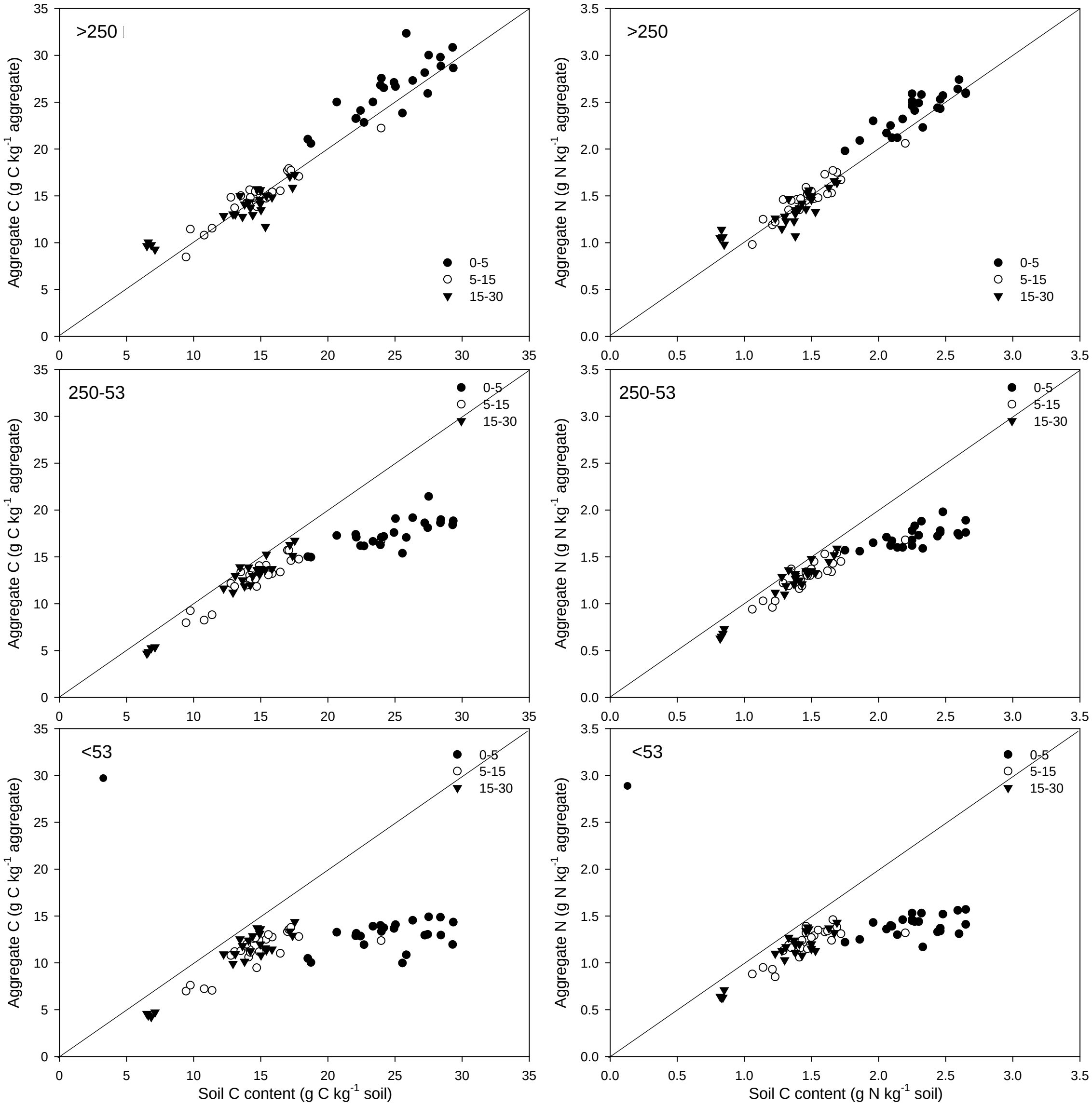

Figure 5. 4. Aggregate C and N concentration in relation to soil C and N concentration.

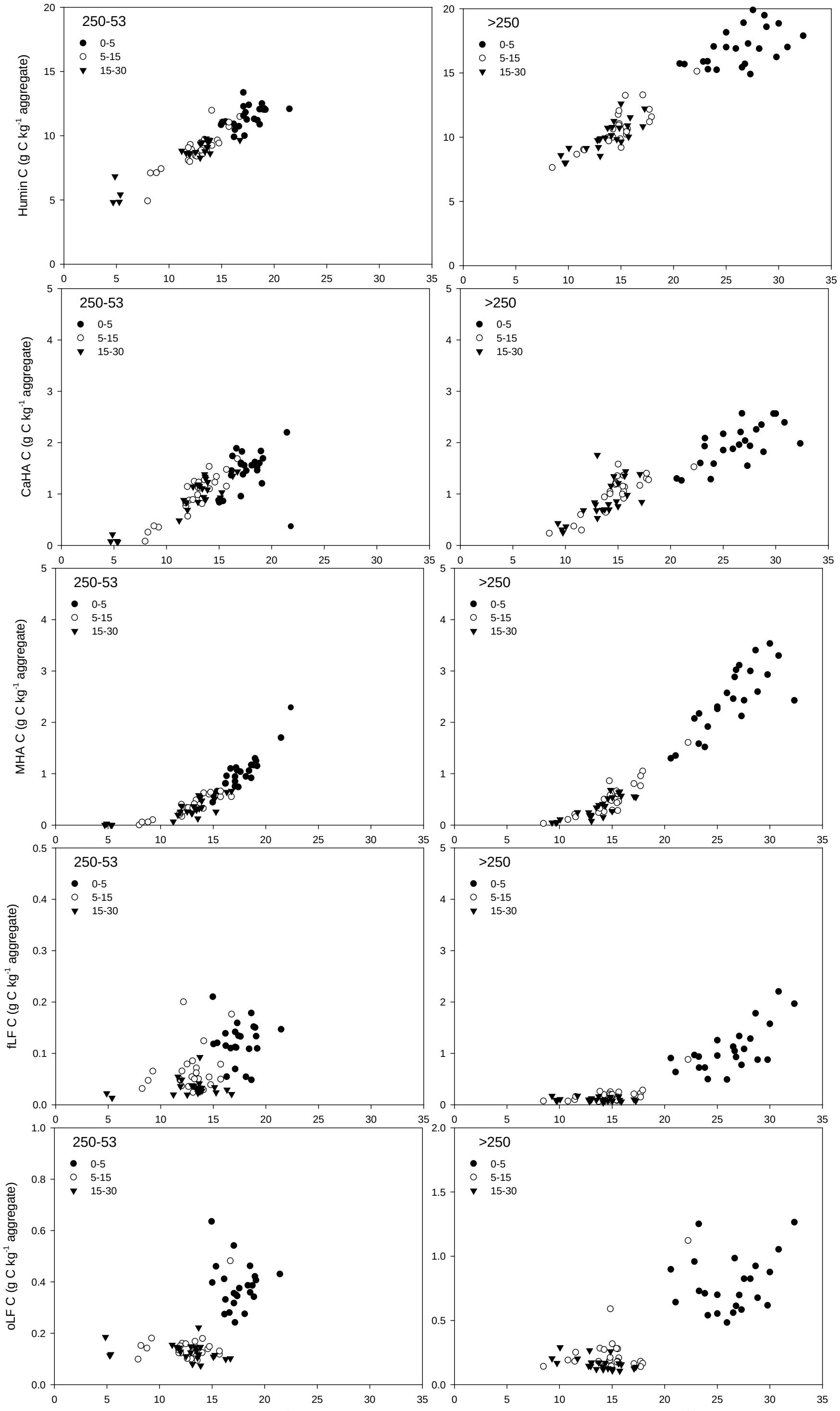

Figure 5. 5. Carbon content of soil organic matter fractions in relation to aggregate C content, size and soil depth. Table 5.8 presents the regression analyses.

Table 5. 1. ANOVA source of variation and degree of freedom (df) for one-way and three-way repeated measurements (RM) models.

One-Way RM[1]		Three-way RM[2]		Three-way RM[3]	
Source	df	Source	Df	Source	Df
Between IMZs	5	Between IMZs	5	Between IMZs	5
Between Years	3	Between Years (Y)	3	Between Years (Y)	3
Residual	15	Ea=IMZ*Y	15	Ea=IMZ*Y	15
Total	23	Between Depths (D)	2	Between Depths (D)	2
		Eb=IMZ*D	10	Eb=IMZ*D	10
		D*Y	6	D*Y	6
		Ec=IMZ*D*Y	30	Ec=IMZ*D*Y	30
		Between Size (S)	2	Between Size (S)	1
		Ed=IMZ*S	10	Ed=IMZ*S	5
		S*Y	6	S*Y	3
		Ee=IMZ*S*Y	30	Ee=IMZ*S*Y	15
		S*D	4	S*D	2
		Ef=IMZ*S*D	20	Ef=IMZ*S*D	10
		S*D*Y	12	S*D*Y	6
		Residual	60	Residual	30
		Total	215	Total	143

[1]: Used for Aggregate C and N stocks per soil layer

[2]: Used for C and N concentration, C/N ratio of Aggregates

[3]: Used for C and N concentration, C/N ratio of fLF, oLF, MHA, CaHA, and humin fractions.

Table 5. 2. Aggregate size distribution in each soil layer.

Fraction (µm)	>250		250-53		<53	
Depth						
0-5	----------------------------------		kg aggregate kg soil^{-1}		--------------------------------	
2005	0.60	A	0.32	A	0.09	A
2006	0.51		0.37		0.13	
2007	0.54		0.35		0.12	
2008	0.55		0.34		0.12	
5-15						
2005	0.61	A	0.30	A	0.10	A
2006	0.51		0.34		0.15	
2007	0.55		0.32		0.13	
2008	0.56		0.33		0.11	
15-30						
2005	0.56	B	0.32	B	0.12	A
2006	0.45		0.40		0.15	
2007	0.51		0.36		0.13	
2008	0.48		0.39		0.13	
Depth average						
2005	0.588 a		0.310 c		0.102 a	
2006	0.490 c		0.368 a		0.142 b	
2007	0.534 b		0.341 bc		0.125 ab	
2008	0.528 bc		0.355 ab		0.118 ab	

Means in the same column for each average followed by the same lower case letter are not significantly different. Means in the same column across depths followed by the same upper case letter are not significantly different.

Anova for aggregate size class distribution

Source	mf
Depth	1.000
Size	<0.001
Time	1.000
D*S	0.0142
D*T	1.000
S*T	<0.001
D*S*T	0.292

Table 5. 3. Aggregate C and N stocks in each soil layer.

Soil layer (kg m^{-2})	0-60			60-200			200-400		
Fraction (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
C stock					--- kg C m^{-2} ---				
2005	0.841a	0.322a	0.066a	1.190	0.528	0.150a	1.553a	0.779b	0.247a
2006	0.759b	0.382b	0.100c	1.061	0.614	0.228c	1.250b	0.943a	0.311b
2007	0.956a	0.367b	0.090b	1.163	0.573	0.194b	1.366b	0.852ab	0.271a
2008	0.851a	0.357ab	0.089b	1.158	0.600	0.176ab	1.340b	0.982a	0.270a
LSD	*0.079*	*0.050*	*0.009*	*0.111*	*0.104*	*0.027*	*0.145*	*0.134*	*0.036*
p-value (year)	*0.017*	*0.049*	*<0.001*	*0.117*	*0.340*	*<0.001*	*0.004*	*0.024*	*0.012*
N stock					--- kg N m^{-2} ---				
2005	0.078ab	0.032	0.007c	0.120	0.054	0.016c	0.156a	0.077b	0.026c
2006	0.070b	0.037	0.011a	0.106	0.062	0.024a	0.124b	0.093a	0.033a
2007	0.087a	0.036	0.010ab	0.116	0.058	0.022a	0.132b	0.087ab	0.029ab
2008	0.079ab	0.036	0.010b	0.115	0.060	0.019b	0.130b	0.097a	0.028bc
LSD	*0.007*	*0.005*	*0.001*	*0.011*	*0.010*	*0.003*	*0.016*	*0.013*	*0.004*
p-value (year)	*0.018*	*0.064*	*<0.001*	*0.077*	*0.396*	*<0.001*	*0.004*	*0.024*	*0.007*

Means in the same column followed by same letter are not significantly different. p-value: probability (P > F) for differences among years by each depth by aggregate size combination. LSD: Fisher’s least significant difference for comparisons among years.

Table 5. 4. Aggregate C concentration and C content as SOM fractions.

Depth (cm)	0-5			5-15			15-30		
Size (μm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
Aggregate	g C kg aggregate^{-1}								
2005	23.782a	16.937	12.540	14.135	12.485	11.133	13.817	12.110	10.518
2006	25.313ab	17.480	13.128	14.843	12.655	11.223	13.700	11.773	10.753
2007	29.428c	17.975	13.163	14.733	12.347	10.905	13.038	12.045	10.755
2008	26.093b	17.503	12.853	14.795	12.647	11.467	13.775	12.638	11.135
Free LF	g fLF-C kg aggregate^{-1}								
2005	0.975b	0.143		0.175	0.082		0.116	0.036	
2006	1.073b	0.135		0.166	0.068		0.089	0.029	
2007	1.505a	0.109		0.150	0.051		0.127	0.035	
2008	0.747c	0.110		0.119	0.038		0.076	0.023	
Occluded LF	g oLF-C kg aggregate^{-1}								
2005	0.898a	0.440a		0.238	0.132		0.196	0.157	
2006	0.807a	0.360b		0.188	0.131		0.131	0.101	
2007	0.882a	0.411ab		0.213	0.141		0.172	0.117	
2008	0.592b	0.333b		0.232	0.137		0.133	0.116	
MHA	g MHA-C kg aggregate^{-1}								
2005	2.190a	0.823		0.486	0.323		0.357	0.294	
2006	2.242a	1.022		0.522	0.340		0.349	0.324	
2007	3.011b	1.004		0.452	0.343		0.326	0.300	
2008	2.196a	0.997		0.481	0.361		0.304	0.298	

Depth (cm)	0-5			5-15			15-30		
Size (μm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
CaHA	---------- g CaHA-C kg aggregate^{-1} ----------								
2005	1.793ab	1.366		1.033	0.794		0.838	0.835	
2006	1.952a	1.574		1.086	0.980		0.927	0.945	
2007	2.351c	1.484		1.033	0.878		0.982	0.836	
2008	1.680b	1.538		0.954	1.123		0.728	0.997	
Humin	---------- g Humin-C kg aggregate^{-1} ----------								
2005	16.367b	11.552		10.587ab	8.644		10.760a	9.267a	
2006	17.582a	11.523		10.580ab	9.058		9.667b	8.847b	
2007	17.200ab	11.746		10.007a	8.728		9.725b	8.786ab	
2008	16.355b	11.282		11.057b	9.171		10.079ab	8.493b	

Means in the same column followed by same letter are not significantly different.

Table 5.5. Aggregate N concentration and N content as SOM fractions.

Depth (cm)	0-5			5-15			15-30		
Size (μm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
Aggregate	g N kg aggregate^{-1}								
2005	2.200c	1.688	1.355	1.425	1.283	1.177	1.382a	1.202	1.095
2006	2.340bc	1.697	1.412	1.477	1.282	1.190	1.358a	1.165	1.115
2007	2.653a	1.723	1.440	1.480	1.265	1.207	1.253b	1.232	1.143
2008	2.428b	1.748	1.387	1.468	1.272	1.207	1.340ab	1.240	1.150
Free LF	g fLF-N kg aggregate^{-1}								
2005	0.058b	0.008		0.008	0.004		0.006	0.002	
2006	0.067b	0.008		0.007	0.003		0.004	0.001	
2007	0.097a	0.006		0.007	0.003		0.005	0.001	
2008	0.047c	0.006		0.006	0.002		0.004	0.001	
Occluded LF	g oLF-N kg aggregate^{-1}								
2005	0.057a	0.023		0.011	0.005		0.008	0.006	
2006	0.051a	0.019		0.010	0.006		0.006	0.004	
2007	0.055a	0.022		0.011	0.006		0.007	0.004	
2008	0.037b	0.018		0.012	0.006		0.006	0.004	
MHA	g MHA-N kg aggregate^{-1}								
2005	0.190a	0.080		0.050	0.035		0.038	0.033	
2006	0.187a	0.094		0.052	0.036		0.037	0.034	
2007	0.240b	0.091		0.046	0.036		0.035	0.033	
2008	0.195a	0.095		0.051	0.040		0.034	0.033	

Depth (cm)	0-5			5-15			15-30		
Size (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
CaHA	-- g CaHA-N kg aggregate^{-1} --								
2005	0.135bc	0.102		0.070	0.053a		0.055	0.054	
2006	0.145b	0.116		0.074	0.064ab		0.059	0.059	
2007	0.177a	0.110		0.070	0.060ab		0.064	0.053	
2008	0.128c	0.113		0.065	0.072b		0.048	0.062	
Humin	-- g Humin-N kg aggregate^{-1} --								
2005	1.305	0.968ab		0.854	0.720		0.849a	0.732	
2006	1.308	0.986ab		0.840	0.758		0.761b	0.687	
2007	1.358	1.045a		0.800	0.726		0.745b	0.726	
2008	1.281	0.976b		0.855	0.765		0.796ab	0.674	

Means in the same column followed by same letter are not significantly different.

Table 5. 6. Aggregate and SOM fractions C:N ratio.

Depth (cm)	0-5			5-15			15-30		
Size (μm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
Aggregate									
2005	10.8	10.0b	9.2	9.8	9.7	9.4a	10.0a	9.9a	9.4
2006	10.8	10.3a	9.3	10.0	9.8	9.4a	10.0a	9.9a	9.4
2007	11.0	10.4a	9.1	9.9	9.7	9.0b	10.4b	9.6b	9.2
2008	10.7	10.0b	9.2	10.1	9.9	9.4a	10.2ab	10.0a	9.4
Free LF									
2005	16.9	18.2		23.4a	21.6		21.2a	22.4a	
2006	15.9	17.9		21.7ab	20.8		20.5a	24.8a	
2007	15.5	17.3		21.0ab	20.3		24.1b	27.8b	
2008	15.9	18.9		19.0b	23.2		21.2a	30.4b	
Occluded LF									
2005	15.9	19.0		21.3	26.0		24.6	27.7	
2006	15.9	18.9		19.7	23.9		23.1	27.9	
2007	16.1	18.9		18.7	23.4		23.2	28.1	
2008	15.9	18.9		19.3	24.2		22.7	29.2	
MHA									
2005	11.5b	10.3a		9.5	9.1a		9.3ab	8.8	
2006	12.0b	10.8bc		10.0	9.4ab		9.4ab	9.3	
2007	12.6a	11.1c		9.8	9.7b		9.7b	9.2	
2008	11.3c	10.5ab		9.5	9.0a		8.9a	9.1	

Depth (cm)	0-5			5-15			15-30		
Size (µm)	>250	250-53	<53	>250	250-53	<53	>250	250-53	<53
CaHA									
2005	13.2	13.3		14.4	14.5		14.8	14.7	
2006	13.4	13.5		14.6	14.9		15.2	15.1	
2007	13.3	13.5		14.4	14.4		14.9	14.9	
2008	13.1	13.4		14.5	15.4		14.7	15.6	
Humin									
2005	12.5	11.9		12.4	11.9		12.6	12.6	
2006	13.4	11.7		12.6	11.9		12.7	12.1	
2007	12.7	11.4		12.5	12.0		13.1	12.1	
2008	12.8	11.6		12.9	11.9		12.6	12.5	

Means in the same column followed by same letter are not significantly different.

Table 5. 7. ANOVA for a) aggregate C concentration and C content as SOM fractions, b) aggregate N concentration and N content as SOM fractions, and c) aggregate and SOM fractions C:N ratio.

a)

Source	Aggregates	fLF	oLF	MHA	CaHA	Humin
	-- p > F --					
Depth	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Size	<0.001	<0.001	<0.001	<0.001	0.012	<0.001
Year	0.152	0.032	0.013	0.138	0.114	0.825
D*S	<0.001	<0.001	**<0.001**	<0.001	**<0.001**	**<0.001**
D*T	0.004	0.004	**0.014**	0.002	**0.038**	**0.004**
S*T	0.029	0.017	0.240	<0.001	**<0.001**	0.396
D*S*T	**<0.001**	**<0.001**	0.169	**<0.001**	0.091	0.248

b)

Source	Aggregates	fLF	oLF	MHA	CaHA	Humin
	-- p > F --					
Depth	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Size	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
Time	0.087	0.012	0.017	0.411	0.039	0.741
D*S	<0.001	<0.001	**<0.001**	<0.001	<0.001	**<0.001**
D*T	0.013	0.001	**0.007**	0.031	0.005	0.054
S*T	0.364	0.007	0.231	0.001	<0.001	0.084
D*S*T	**<0.001**	**<0.001**	0.148	**<0.001**	**0.003**	0.658

c)

Source	Aggregates	fLF	oLF	MHA	CaHA	Humin
	-- p > F --					
Depth	0.174	<0.001	<0.001	<0.001	**0.001**	0.159
Size	<0.001	0.026	<0.001	<0.001	0.288	0.004
Time	0.424	0.366	**0.019**	0.001	0.349	0.679
D*S	<0.001	**0.003**	**0.013**	<0.001	0.717	**0.024**
D*T	0.006	**0.004**	0.388	0.269	0.734	0.080
S*T	<0.001	**<0.001**	0.236	0.181	0.071	0.074
D*S*T	**0.007**	0.218	0.195	**0.041**	0.932	0.116

Table 5. 8. Linear regression parameters for the composition of aggregate C as SOM fractions as a function of aggregate C concentration presented in Figure 5.5.

Soil layer (cm)		0-30			0-5			5-15			15-30		
Aggregate class	SOM fraction	Adj. R^2	Slope	Intercept	Adj. R^2	Slope	Intercept	Adj. R^2	Slope	Intercept	Adj. R^2	Slope	Intercept
>250 µm	Humin	0.897	0.514*	3.181	0.254	0.254*	10.224	0.585	0.479*	3.559	0.639	0.436*	4.135
	CaHA	0.803	0.087*	-0.299	0.465	0.090*	-0.400	0.693	0.134*	-0.932	0.433	0.118*	-0.738
	MHA	0.928	0.160*	-1.822	0.641	0.170*	-2.031	0.681	0.101*	-0.990	0.689	0.079*	-0.739
	oLF	0.800	0.074*	-0.894	0.000	0 ns	0.578	0.000	0 ns	0.220	0.235	-0.012*	0.335
	fLF	0.678	0.043*	-0.381	0.437	0.095*	-1.413	0.184	0.015*	-0.062	0.019	0 ns	0.156
250-53 µm	Humin	0.844	0.489*	2.813	0.192	0.261*	6.911	0.734	0.577*	1.620	0.857	0.450*	3.257
	CaHA	0.785	0.119*	-0.560	0.369	0.142*	-0.988	0.790	0.163*	-1.095	0.825	0.112*	-0.454
	MHA	0.785	0.096*	-0.813	0.737	0.158*	-1.803	0.825	0.081*	-0.671	0.638	0.048*	-0.274
	oLF	0.365	0.025*	-0.131	0.000	0 ns	0.520	0.000	0ns	0.148	0.028	0 ns	0.162
	fLF	0.292	0.009*	-0.054	0.000	0 ns	0.143	0.000	0 ns	0.044	0.000	0 ns	0.020

Adj R^2= adjusted R^2. ns= no significant (p-value regression >0.05). *= significant (p-value regression <0.05). n=24 for 0-5, 5-15 and 15-30 cm soil layers, n=72 for 0-30 cm soil layer.

CHAPTER 6. SYNTHESIS

Uniqueness of the data presented in this dissertation:

This dissertation explores the changes over four years in the stabilization of soil organic matter (SOM) in three soil depths of two agricultural production fields: Site 1, an irrigated continuous maize field that was converted from no-till to conservation deep tillage, and Site 2, an irrigated maize-soybean rotation under no-till. An integrated approach using base extractions and a density-based physical fractionation of SOM was developed to evaluate the hypothesized changes in SOM stabilization mechanisms (physical protection, organo-mineral associations and humification) within soil aggregates in relation to crop rotation and tillage management. The main findings and significance of this work are integrated in this section together with new concepts and hypotheses.

Hypotheses tested

The main objective of this dissertation was to contribute to the understanding of soil C stabilization mechanisms at the two irrigated production-agriculture fields of the UNL Carbon Sequestration Program. In the Introductory chapter, two sets of hypotheses were presented regarding the impact of ongoing crop and soil management scenarios on SOM stabilization (page 12).

Chapters 2 and 3 tested the first set of hypotheses regarding the impact of increasing soil-crop residue interface on stabilization of SOM by replacing no-tillage with incorporation of maize residues plus nitrogen (N) fertilizer in the fall through conservation deep tillage (Site 1).

Over time, this change in management resulted in:

a) Mixing of soil layers resulting in less stratified carbon (C) and N stocks and SOM fractions with soil depth.
b) Breakdown of largest aggregates resulting in less stratified C and N concentrations with aggregate size.
c) Repackaging of aggregates.
d) Changes in aggregate size distribution.
e) Increased physical protection of crop residues.
f) Stabilization of plant-derived C and N.
g) Humification of crop residues.

After three years of conservation deep tillage, C and N stocks in the uppermost soil layer decreased because of mixing with lower depths and a negative balance between enhanced respiration due to aggregate disruption and exposure of SOM with conservation deep tillage and protection of new C inputs by physical occlusion and humification. In deeper soil layers, the increase in C and N stocks was favored by the mixing of soil layers and a positive balance between exposure of SOM in disrupted aggregates and the protection of new C inputs by physical occlusion and humification.

Chapters 4 and 5 tested the second set of hypotheses on whether the quantity and quality of maize and soybean crop residues influences SOM stabilization under no-till management (Site 2).

No-till management resulted in:

a) Stratification of C and N stocks, SOM fractions, and monosaccharides with soil depth

b) Stratification of aggregate size distribution with soil depth
c) Stratification of C, N, SOM fractions, and monosaccharides with aggregate size

The alternation in successive years in both quantity and quality of crop residues resulted in:

a) Slight reductions in soil C and N stocks after the soybean years.
b) Increase in C and N stocks derived from soybean residues in surface soil during the maize year (2007) after a large addition of soybean residue in 2006.
c) Increase in all SOM fractions and monosaccharides in the 0-5 cm depth in 2007 accompanying the increase in soil C and N stocks.

The mechanisms for SOM stabilization in the surface soil layer were hypothesized to reflect the differences in quantity and quality of aboveground maize and soybean residues placed on the soil surface, but this was not the case. In Chapter 4, the high biomass of highly decomposable soybean residue from 2006 was hypothesized to contribute to the decomposition and humification of previous (2005) maize residues during the 2007 maize cropping season, resulting in enhanced soil C and N stocks. In Chapter 5, the increased mass of both humic acid fractions in the largest aggregates (>250 µm) in the 0-5 cm depth indicated enhanced humification of aboveground crop residues during the 2007 maize season. The mechanisms for physical and chemical stabilization of SOM were similar with depth and between crops.

Significant findings regarding field management effects on C and N stocks

As presented in Chapters 2 and 4, the reduced soil-crop residue interface under no-till restricted the volume of soil for stabilization of residue derived SOM leading to stratification of SOM in the soil profile. Three years after conversion of no-till to conservation deep tillage, soil C stocks were similar to the previous no-till condition. Using proportional area weighting of the IMZs, whole field C stocks in the 0-400 kg soil m^{-2} layer of Site 1 were 7.09 ± 0.29, 7.47 ± 0.36, 7.15 ± 0.37 and 7.18 ± 0.41 kg C m^{-2}, in fall 2005, 2006, 2007 and 2008 respectively. This outcome is encouraging, given the challenge of stabilizing aboveground residues into the mineral matrix without increasing C losses from native SOM and residue due to enhanced decomposition Three years may constitute a short time to measure net accrual or net loss of C and N stocks at field level, based on data available from the Ecological Intensification Project (Walters et al. 2009). Nevertheless, the changes in C and N stocks of humified SOM fractions and their distribution with depth indicate that novel management of crop residues may compensate for the normally detrimental effect of tillage on SOM content.

In Chapter 4, the magnitude of increase in surface soil C and N stocks after the second maize year (2007) at Site 2 constituted a new scenario for the study of SOM stabilization. Using proportional area weighting of the IMZs, whole field C stocks in the 0-400 kg soil m^{-2} layer of Site 2 were 6.42 ± 0.30, 6.18 ± 0.32, 6.81 ± 0.26 and 6.72 ± 0.29 kg C m^{-2}, in fall 2005, 2006, 2007 and 2008 respectively. The lack of increase in C stocks in the 0-200 kg soil m^{-2} layer after the first year of maize (2005) was attributed to the low return of C and N in soybean residues in 2004. The measured changes in ^{13}C signature, monosaccharide composition and SOM fraction masses indicated that a large return of soybean residues can promote the stabilization of standing maize litter through physical protection and humification. Thus, management practices that result in increased return of soybean residues may contribute to the stabilization of crop residues into SOM.

Significant findings on SOM stabilization mechanisms

A second objective of this dissertation was to study the stabilization of SOM within aggregates to evaluate the concept of a finite capacity to sequester C by aggregates and thus soil (i.e., C saturation). Previous studies indicated that the main mechanism of SOM stabilization in individual aggregates may depend on aggregate size, where the enhanced C concentration associated with increasing aggregate size was caused by preferential stabilization of occluded non-mineral associated SOM (Gulde et al. 2008, Stewart et al. 2007). In this dissertation, two physically protected SOM fractions, free and occluded light fraction (fLF and oLF), and three humified fractions, mobile humic acid (MHA) calcium-bound humic acid (CaHA) and humin, were extracted from >250 µm and 250-53 µm aggregates. The proportion of aggregate C extracted as fLF was 4-14 times greater in the largest aggregates than in the 250-53 µm aggregates across depths and 1.5 to 2 times greater for oLF across depths, respectively. Nonetheless, the greater content of occluded non-mineral associated SOM with increased aggregate size did not completely account for the increased C content of larger aggregates. At both field sites, increased aggregate C and N contents were correlated in each depth and for each aggregate size class with a linear increase in the proportions of aggregate C and N contents extracted in the three humified fractions, while aggregate C and N contents were not clearly related to fLF and oLF. Comparison of the slopes between aggregate C as each SOM fraction and aggregate C concentration indicates the preferential accumulation of each SOM fraction by aggregate size class, while comparison of the intercept of the relationship on the y axis indicates whether the onset of stabilization of each SOM fraction is regulated by the C concentration of the aggregate size. Similar results were obtained from both field sites: a) aggregate size class had little effect on the slopes of the humin and CaHA fractions, indicating comparable adsorption rates, while MHA was preferentially stabilized in the largest aggregates at C levels above 15 g C kg^{-1} aggregate; b) as aggregate C concentration increased from low to high values, C accumulated in humified SOM fractions followed the order humin, then CaHA, and finally MHA, indicating a different aggregate-C concentration threshold or onset of stabilization for each SOM fraction. The deferred start in MHA accumulation compared to CaHA, together with the preferential accumulation of MHA above 15 g C kg^{-1} aggregate, is perhaps indicative of a competitive stabilization process between these two humic fractions, where the onset of MHA stabilization may be triggered by decreasing availability of soil Ca^{+2} for CaHA stabilization. The preferential accumulation of MHA in the largest, most C-enriched aggregates and lack of difference in the accumulation rate of humin between the largest aggregates and the mid-size aggregates in those soil layers and years with greater C content suggests that the increased capacity to stabilize SOM within the largest aggregates is due to both increased humification (MHA) and also maintained rates of organo-mineral associations (humin), in addition to the previously noted increase of physical occlusion of LF.

Unanswered questions and new questions

Given the low recovery of the <53µm aggregate size class, only C and N analysis and monosaccharide abundance and composition were carried out. Preliminary extraction of fLF and oLF fractions from <53 µm aggregates yielded negligible amounts. Thus stabilization of SOM by this aggregate class was regarded as organo-mineral association. The nature of this organo-mineral associated SOM remains unknown. Based on the relationships of MHA, CaHA and humin accumulation with aggregate C for the largest and mid-size aggregates and the low C concentrations of <53 µm aggregates (<13.2 g C kg^{-1} aggregate), SOM in the small-size aggregates can be hypothesized to be stabilized mainly as humin.

The consistently thresholds for specific aggregate-C concentrations that marked the onset of SOM stabilization into the CaHA and MHA in both aggregate size classes seem to indicate a competitive stabilization process among these two humified fractions. It is hypothesized that the threshold for MHA stabilization may be triggered by diminishing soil Ca^{+2} availability that would occur after substantial stabilization of the CaHA fraction.

The mechanisms for SOM stabilization in the surface soil layer of Site 2 were hypothesized to reflect differences in quantity and quality of aboveground maize and soybean residues placed on the soil surface, but this was not the case. The increase in C and N stocks in the 0-200 kg soil m^{-2} layer in the second maize year (2007) was related to the larger C and N biomass return of previous soybean (2006), while the lower C and N stocks of the first maize year (2005) was hypothesized to be related to the low return of C and N in soybean residues in 2004. Is it possible to manage maize-soybean rotations to enhance soil C and N stocks? The measured changes in soil ^{13}C signature and monosaccharide composition, and amount of SOM fractions indicated that a large return of soybean residues may have promoted the stabilization of standing maize litter through physical protection and humification, perhaps by constituting a source of readily available N for microbial activity.

Conclusions

Some general conclusions can be stated regarding the impact of crop and residue management on stabilization of SOM at the two irrigated fields of the UNL Carbon Sequestration Program:

- Under high yielding continuous maize systems, the use of fall conservation deep tillage together with addition of fertilizer N to the residues can increase the stabilization of maize residues into SOM and compensate for the detrimental effect of tillage on SOM content.
- The increasing stabilization of crop residue into SOM under irrigated no-till maize-soybean rotation may be achieved with increased return of soybean residues.
- The linear increase of all SOM fractions with increasing aggregate C concentration of >250 µm and 250-53 µm aggregates did not support saturation behavior of any of the SOM fractions studied.

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APPENDIX A. ESTIMATION OF FIELD C AND N STOCKS

Introduction of tillage on no-till systems modifies soil bulk density. To better account for the variation in effective sampling depth and soil mass due to changes in soil bulk density over time, the cumulative mass approach described by Gifford and Roderick (2003) was used to compare carbon (C) and nitrogen (N) stocks over time. This appendix constitutes a detailed explanation of the steps and calculations used in this dissertation to express the C and N concentration as C and N stocks.

1. Soil sampling, soil handling and analysis

Annual soil sampling of the top 0.3 m of soil was conducted in the fall of 2005, 2006, 2007 and 2008. Sampling was done in each of the 6 IMZs after crop harvest and before fall tillage. Within each IMZ, six separate soil cores (40 mm diameter by 300 mm depth) were collected using a hydraulic probe along an east–west transect between previous crop rows and inter-row center (approximately 0.16 m away from row) (Figure A1.1).

The field moist cores were split into three soil depths: 0-0.05, 0.05-0.15 and 0.15-0.30 m, and then gently broken apart along natural planes of weakness. The samples were air dried to constant weight, combined into one composite sample for each Year(n=4)*IMZ(n=6)*depth(n=3), sieved to 8 mm and stored in boxes for further fractionations and analysis. A sub-sample of whole soil was finely ground to pass a 100 mesh sieve using a roller mill. Any recognizable plant material and/or $CaCO_3$ concretions (> 1 mm) were removed before grinding. Ground soil was kept in labeled and closed glass vials until analysis. Twenty milligrams of the ground soil per sample was analyzed for C and N concentration using an elemental analyzer (ECS 4010, Costech Analytical Technologies Inc., Valencia, CA). For QA/QC purposes and comparisons with C and N analyses from previous years, a standard soil (Soil Standard A) was packed (and run) every 11 soil samples. A week prior to weighing samples for C and N analysis, soil samples were brought to the lab and the vials caps were loosened to allow all the samples and the Soil Standard A to equilibrate their moisture with the controlled humidity (~30% rh) in the lab. At each weighing period a complete batch was prepared (approximately 50 samples).

2. Bulk density sampling and estimation

In addition to the soil core samples, five smaller soil cores (2.15 cm diameter by 30 cm depth) were taken in each transect of each IMZ every year (Figure A1.1). A lubricated plastic sleeve mounted inside a soil hand probe was used to collect the cores to determine bulk density. After taking the sample, the sleeve was removed from the sampler and plastic caps were placed on both ends. In the lab, the soil cores were measured for total soil length and then carefully removed from inside the sleeves by pushing from the bottom. The cores were split into 0-5, 5-15 and 15-30 cm depths, dried at 105 ºC for 24 h and weighed. Soil bulk density (BD, Mg m-3) for each core*depth was calculated as:

$$BD = \frac{Soil_m}{Soil_v}$$

where $Soil_m$ is the oven dried soil mass(g), and $Soil_v$ is the volume of soil of each depth increment (cm^3). The five subsamples BD were then averaged and that was the BD of each Depth*IMZ*Year combination.

3. Choosing reference soil layers

Reference soil masses were used to estimate C and N stocks. In order to determine the reference soil mass, we used the BD to calculate for each cumulative sampled depth the cumulative soil mass. The reference soil mass for each cumulative sample depth was set to be a whole amount close to the minimum soil mass at a given year for that layer. The three reference soil layers used were: (i) the top 60 kg dry soil m^{-2} (approximately 0–0.05 m depth), (ii) the top 200 kg dry soil m^{-2} (approximately 0–0.15 m depth) and (iii) the top 400 kg dry soil m^{-2} (approximately 0–0.30 m depth).

4. Calculating C and N stocks

The cumulative mass approach described by Gifford and Roderick (2003) was followed. A spreadsheet (Figure A1.2) was prepared with the sample identification, BD and % C and N. Estimates of Soil mass (kg m^{-2}) C (kg C m^{-2}) and N (kg N m^{-2}) stocks were first calculated for each of the three soil depth intervals based on the measured bulk density at the time of sampling, and the C and N concentration (Figure A1.2):

$\text{Soil mass}_{(0\text{-}0.05\text{m})} = \text{BD}_{(0\text{-}0.05)} * 1000 * 0.05 \text{ m} * 1 \text{ m}^2$

$\text{C stock}_{(0\text{-}0.05\text{m})} = \text{Soil mass}_{(0\text{-}0.05)} * \%\text{C}/100$

$\text{N stock}_{(0\text{-}0.05\text{m})} = \text{Soil mass}_{(0\text{-}0.05)} * \%\text{N}/100$

After each soil depth layer C and N mass was calculated, Equation 4 from Gifford and Roderick (2003) was used to calculate the C and N stocks in the reference layer. See Figure 2 for the detailed equations in columns Q, R and S. The equations contain a logical operator to decide which equation should be used based on the comparison of the soil mass in the depth layer with the reference layer.

5. Whole-field C and N stocks estimates

Given that only one (composite) measurement of C and N stock per IMZ was realized, estimates of the mean $\bar{x}$ and standard error $SE_{\bar{x}}$ annual C and N stock for the whole field were obtained similar to Verma et al. (2005):

$$\bar{x} = \sum_{i=1}^{m} w_i \, \bar{x}_i$$

$$w_i = \frac{area\ of\ \ fuzyy\ class\ k}{total\ area} \quad , \quad \sum w_i = 1$$

$$SE_{\bar{x}} = \sqrt{\frac{1}{m}\sum_{i=1}^{m} w_i \, (x_i - \bar{x})^2}$$

where w_i is the weight associated to an IMZ, $\bar{x}_i$ is the IMZ mean, and *m* is the number of IMZs per field. Weights w_i were assigned to each IMZ based on the proportional area of the fuzzy class represented, i.e., the sum of all weights per field is 1. Weights used in this dissertation (Table A1.1) differ from those used by Verma et al. (2005). The estimated weights in 2001 by fuzzy-k means clustering based on soil and landscape attributes were revised based on the soil C results from the grid soil sampling in 2005 (Dan Walters and Achim Dobermann).

6. Notes

We used 8 mm sieve opening since the soil organic matter fractionation involved aggregate size classes. Before sieving, large loose plant residues were removed. During sieving, clods larger than 8 mm were hand broken following natural planes of weakness.

Plastic sleeves used for bulk density sampling were lubricated with WD-40 spray oil to facilitate the sampling and extraction of the sample.

References

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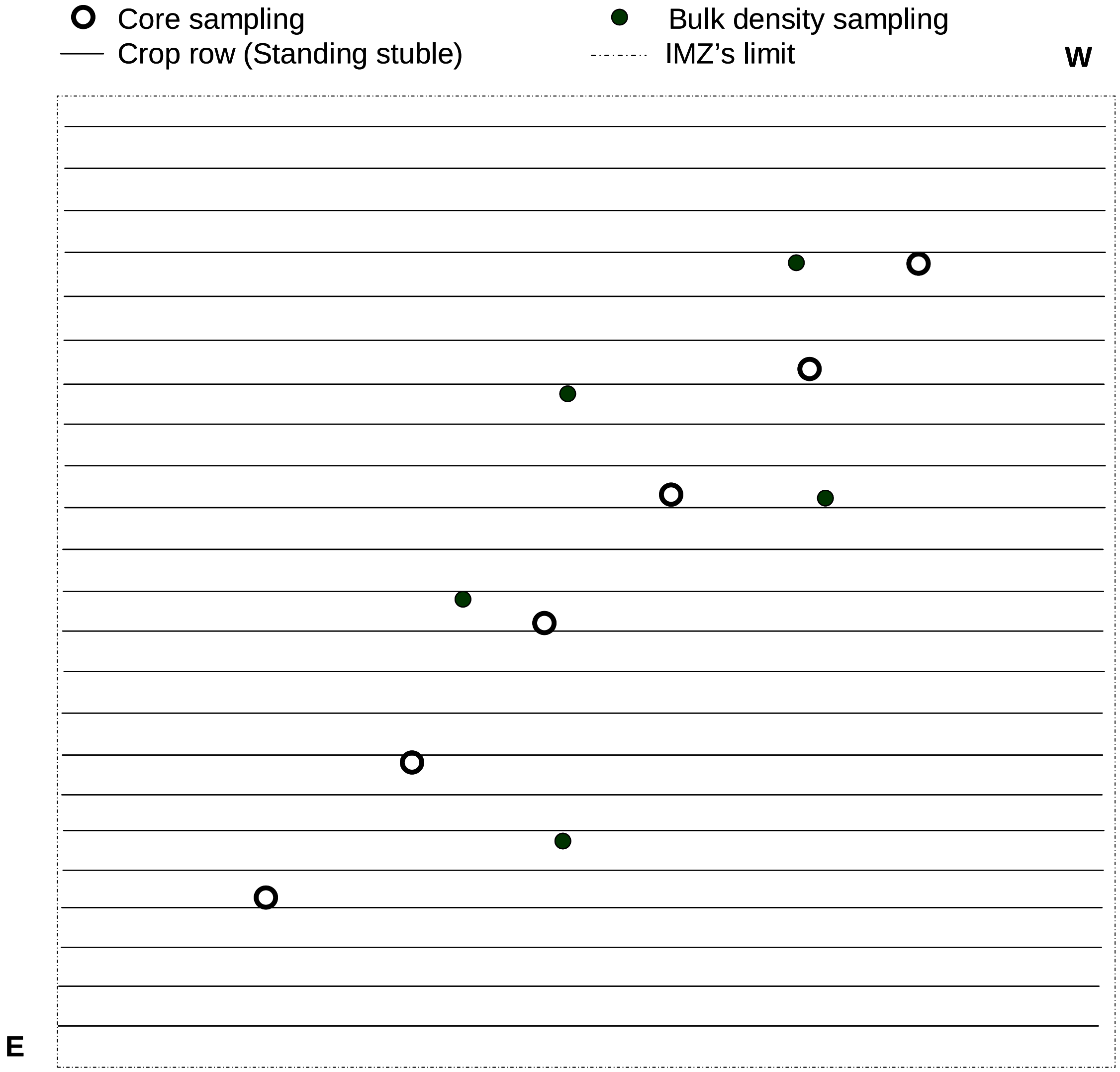

Figure A1. 1. Sampling layout of each individual Intensive Management Zone (IMZ)

col	A	B	C	D	E	F	G	H	I	J
row	Sample ID	BD 0-5 (Mg m^{-3})	BD 5-10 (Mg m^{-3})	BD 15-30 (Mg m^{-3})	% C 0-5	% C 5-15	% C 15-30	% N 0-5	% N 5-15	% N 15-30
1	2005 IMZ1	1.28	1.52	1.48	2.038	1.389	0.670	0.207	0.143	0.081
2	2005 IMZ2	1.20	1.34	1.36	2.316	1.758	1.723	0.214	0.169	0.170
3	2005 IMZ3	1.20	1.36	1.34	2.476	1.878	1.827	0.222	0.168	0.149

col	A	K	L	M	N	O	P
row	Sample ID	Soil mass 0-5 (kg m^{-2})	Soil mass 5-15 (kg m^{-2})	Soil mass 15-30 (kg m^{-2})	C mass 0-5 (kg C m^{-2})	C mass 5-15 (kg C m^{-2})	C mass 15-30 (kg C m^{-2})
1	2005 IMZ1	63.944	151.888	221.709	1.303	2.110	1.485
2	2005 IMZ2	=B2*0.05*1000	=C2*0.1*1000	=D2*0.15*1000	=K1*E1/100	=L1*F1/100	=M1*G1/100
3	2005 IMZ3						

col	A	Q	R	S	T	U	V
row	Sample ID	C mass 0-60 (kg C m^{-2})	C mass 60-200 (kg C m^{-2})	C mass 200-400 (kg C m^{-2})	C mass 0-60 (kg C m^{-2})	C mass 0-200 (kg C m^{-2})	C mass 0-400 (kg C m^{-2})
1	2005 IMZ1	1.223	1.970	1.454	1.223	3.193	4.647
2	2005 IMZ2	=IF($K1>60,N1*60/$K1,N1+((60-$K1)*O1/$L1))	=IF(($K1+$L1)<200,(N1+O1+P1*(200-$K1-$L1)/$M1)-Q1,(N1+O1*(200-$K1)/$L1)-Q1)	=IF(($K1+$L1+$M1)<400,(N1+O1+P1+P1*(400-$K1-$L1-$M1)/$M1)-Q1-R1,(N1+O1+P1*(400-$K1-$L1)/$M1)-Q1-R1)	=Q2	=T2+R2	=U2+S2
3	2005 IMZ3						

Figure A1.2. Example of spreadsheet for calculation of C and N stocks. Columns Q, R and S represent Equation 4 in Gifford and Roderick (2003). For N stock calculation, corresponding columns were omitted, but equations are similar to columns N to V.

Table A1. 1. Weights of soil-landscape classes established by fuzzy-k-means clustering in 2001 and corrected by soil C concentrations in 2008.

IMZ	1	2	3	4	5	6
	------------------------------Spatial weight------------------------------					
Site 1	0.0252	0.2912	0.3075	0.1186	0.1186	0.1389
Site 2	0.1090	0.0451	0.1382	0.1382	0.4311	0.1382