THE IMPACT OF LONG-TERM TILLAGE, CROP ROTATION AND N APPLICATION ON SOIL CARBON SEQUESTRATION

by

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Soil organic carbon (SOC) stock is controlled by many factors, but for given conditions it is the long term summation of the balance between inputs and outputs. Management practices will alter this balance by affecting the system's productivity and the speed of residue and soil organic matter decomposition. Given that annual changes in SOC are generally small, compared with the large and variable SOC background, long-term experiments, and soil samples taken at the start of experiments (archived samples) are necessary to determine SOC trends over time. Changes in SOC were analyzed for two long-term experiments; one an irrigated site at Mead, NE (1997) with continuous corn (CC) (*Zea mays* L.) and corn-soybean [*Glycine max* (L.) Merr.] (CS) rotation) and 3 nitrogen (N) application rates (0, 100 and 300 kg ha^{-1}); and the other a rainfed site at Concord, NE, (1985) with three tillage treatments (no-till, NT, disk, DK, and moldboard plow, MP), two crop sequences (CC and CS) and three N application rates (0, 80 and 160 kg ha^{-1}). Soil samples taken at early stages of the experiments were used as a benchmark to analyze carbon trends over time. Results showed that in spite of considerable differences, C inputs did not affect changes in SOC. Nitrogen fertilizer

application resulted in a 3% increase in SOC, but required 24 years to generate detectable differences in the surface 400 kg of soil m^{-2}. Over the last 12 years, MP lost 1.52 ±0.4 kg of C m^{-2} while NT lost 0.73 ±0.4 and DK lost 0.76 ±0.4 kg of C m^{-2} in the 1200 kg of soil m^{-2} profile. None of the evaluated treatments under the conditions of these experiments were able to sequester atmospheric C since all lost SOC over time. The use of archived samples made possible the determination of the rate of SOC change over time, and allowed an accounting for natural soil variability.

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INTRODUCTION

Climate change has become one of the most important issues of the first decade of the 21st century. Atmospheric enrichment of carbon dioxide (CO_2) from anthropogenic activity is considered to be the main cause of climate change. There is great interest in determining different ways to reduce CO_2 emissions to the atmosphere and/or ways to sequester CO_2 from the atmosphere. Soils are considered to be an important source and sink of atmospheric CO_2, and although soil only contain 1500 Gt of SOC compared to the 38,000 Gt of carbon (C) contained in the world's oceans, it is of particular interest because the annual flux of CO_2 from soil to the atmosphere is large and responds to anthropogenic modifications (Baker et al., 2007).

Soil C also has an important impact on the soil productivity by providing and holding nutrients and water, improving soil aeration through modification of soil bulk density, and reducing potential for soil erosion. Soil organic matter (SOM) is correlated to the organic C fraction of the soil (SOC), since organic C comprises 48% to 58% of the SOM mass (Collins et al., 1997). The importance of SOM is related to plant nutrition since it contributes to the cation exchange capacity and serves as a source of nutrients for plant growth. Soil organic matter has also a biological function as a primary energy source for microflora and microfaunal organisms, a physical function of promoting good soil structure and by lowering bulk density, and finally a physio-chemical function of increasing buffering and exchange capacity of soils (Stevenson, 1994).

The general hypothesis on SOC is that for a given location the SOC level at any point in time can be summarized as a balance between input and output of carbon. The equilibrium between the inputs and outputs is influenced by the soil type and by environmental variables such as temperature and precipitation (West and Six, 2007) which have direct and indirect effects on both, C inputs and outputs. Climate affects C fixation through plant growth and yield, and it mediates C decomposition rates by impacting the quantity and rate of C cycling. Therefore, the C balance hypothesis indicates that for a given location, by reducing C outputs or increasing the C returned to the soil, SOC stocks should increase (Paustian et al., 1997; Flessa et al., 2000; Kong et al., 2005; Puget and Lal, 2005).

Although many management practices affect several processes that control C balance, the two main factors, as suggested by Paustian et al. (1997), are (1) controlling the amount and composition of the plant residue returned to the soil, and (2) the degree of soil disturbance by tillage. The choice of management practices used in agricultural production has great impact on both of these areas. The cropping sequence (hereafter referred to as crop rotation) will directly impact the amount and quality of the residue returned to the soil. Nitrogen (N) fertilizer application rate will impact not only the amount of residue returned by increasing production but also the turnover rate of the residue and the SOM. Finally, tillage treatments will mainly affect the distribution of SOC in the profile and the degree of soil disturbance, reducing SOC stocks as tillage intensity increases.

Assessing and determining the effects of management practices on SOC will aid in the adoption of more sustainable and productive agricultural systems and will support the implementation of more accurate theories (models) on carbon sequestration for agricultural systems. In order to understand the effect of management practices on SOC, long-term studies are of great importance since the annual change is relatively small compared to the SOC background. (Baker et al., 2007; Angers and Eriksen-Hamel, 2008) Similarly, deep sampling (VandenBygaar and Angers, 2006; Gal et al., 2007) and reporting SOC stocks on a constant mass basis (Ellert and Bettany, 1995) are critical when comparing the effect of management practices on SOC. Finally, the use of archived samples provides valuable information on the initial SOC level, allowing the calculation of change over time, which reduces the impact of soil variability inherent in field experiments, and potentially unknown at the start of an experiment.

The objective of this study was to determine the effects of long-term tillage, crop rotation, and N rate on SOC. We hypothesize that

(i) as tillage intensity increases SOC will be decrease at the soil surface (0 to 25-30 cm.

(ii) as tillage intensity increases there will be more SOC below the 20-25 cm soil surface.

(iii) more SOC will be sequestered under continuous corn (CC) than corn-soybean rotation (CS).

(iv) increasing N fertilizer application will result in greater C sequestration

(v) higher residue input will result in higher SOC content regardless of the tillage treatment and

(vi) initial soil samples will allow proper understanding of management effects on SOC levels.

CHAPTER 1. Literature Review

Tillage, crop rotation and fertilizer application are common agricultural management practices that have unique effects on the soil carbon balance. Numerous studies have been conducted that address these effects, and are summarized in the following sections.

1.1 Critical Measurement Issues for SOC

There are three important issues that should be considered when determining tillage and soil depth influence on soil organic carbon. They are: sampling depth, bulk density variations, and the duration of the experiment.

The first issue in assessing SOC change over time is the depth of sampling. Many studies have focused only on the surface layer(s) of the soil as shown by Baker et al. (2007). VandenBygaar and Angers (2006) suggest that soil organic carbon might be redistributed with depth by tillage, and that the carbon redistributed by tillage to the lower soil profile would not be considered if only the top soil was sampled. Baker et al. (2007) established that differences in root distribution with depth, coupled with tillage induced alterations in the soil, could lead to differences in depth distribution of SOC. This difference cannot be properly interpreted when only the upper layer of the soil is sampled. Similarly, Gal et al., (2007) determined that sampling depth greatly affected the conclusions about SOC sequestration rates in NT vs. CT fields. The literature review of Angers and Eriksen-Hamel (2008) highlighted the importance of taking into account the whole soil profile when comparing SOC. In most cases, significant SOC differences were observed for no-till in the upper soil layer, however, important increases in SOC were also observed for the tillage treatment in the deeper soil layers. This leads to the conclusion that sampling the whole soil profile, and not just the upper layer of soil, is necessary to account for deep placement of carbon caused by tillage.

Another important factor in determining alterations in soil C content is the sampling procedure and the units in which the results are expressed. Physical changes often occur over time with a change in soil tillage practice. Bulk density often increases in the upper layer under no-till systems, with differences among tillage systems occurring over time. If soil C content and density are measured to the same depth for the comparison of SOC changes, then more soil mass would be present in no-till samples than in tilled samples (Ellert and Bettany, 1995; VandenBygaart and Angers, 2006). Thus SOC changes over time when tillage systems are involved could lead to confounding conclusions. Equivalent soil mass instead of fixed depth can be used to correct for differences in soil bulk density, thus allowing a more precise and unbiased comparison (Ellert and Bettany, 1995; Gifford and Roderick, 2003; Wuest, 2009).

The time factor also needs to be considered. Baker et al. (2007) suggested that multiyear experiments are necessary because annual changes in SOC are generally small compared to the SOC background. To effectively sequester carbon from the atmosphere we need to increase its mean residence time in the soil and this could only be properly analyzed through long term experiments. For example, Angers and Eriksen-Hamel (2008) found a weak but significant increase in SOC by NT over time in comparison with tillage, nevertheless, the time necessary to detect differences between treatments is highly variable among studies. Since SOC changes are relatively small compared to the SOC content, initial SOC measurement could aid in distinguishing effective changes due to management practices by documenting natural soil variability (Potter, 2006).

Unfortunately, rarely have adequate initial measurements been made in long-term studies, thus researchers have made the assumption that initial SOC contents were uniform across the study site, which is seldom true.

In conclusion, deep sampling is essential when comparing tillage effects on SOC, because large variations in the soil surface layer may be compensated for by small variations in deeper layers. Given the generally small variations in annual C stock compared to the SOC background, long-term experiments are necessary to measure differences between treatments, and initial soil sampling documents soil variability so that real C changes due to management are determined. Finally, expressing results on a constant mass basis allows a fair comparison when there are bulk density changes among the tillage treatments.

1.2 Tillage Influence on SOC

Tillage methods have a pronounced effect on the quantity and distribution of crop residue in the soil, but the long-term effect of tillage on SOC is not agreed on. Tillage practice has been established as one of the main causes of SOC loss by affecting SOM decomposition (Lal, 2004). Original SOM levels in soils have decreased by 40 to 50% under cultivation. Studies have shown that intensive tillage results in SOC loss, thereby increasing greenhouse gas emissions (e.g. Reicosky, 2003). Therefore, the use of less intensive tillage methods could reduce the SOC loss (Baker et al., 2007) or contribute to sequestration of C from the atmosphere. No-till (NT) and minimum tillage have been promoted as practices that will reduce the rate of soil CO_2 flux (loss) to the atmosphere and increase the rate of soil C sequestration as compared to conventional tillage (CT) (Baker et al., 2007; Christopher and Lal, 2007). Soil C sequestration in this study was defined as the capture and secure storage of C that would otherwise be emitted to or remain in the atmosphere.

Six et al. (2004) suggested that soil disturbance through tillage disrupts macroaggregates, reducing the physical protection of SOM and therefore increasing the rate of SOM decomposition. Tillage mixes the surface soil, disrupting aggregates and increasing exposure of SOM to microorganisms and the environment, thereby increasing the rate of SOM decomposition. To the contrary, under NT the percentage of large aggregates (Diaz-Zorita et al., 2002) and the volume of macro pores (Kay and VandenBygaart, 2002) increases. Six et al., (1999) determine that NT had a slower rate of macroaggregate turnover than CT. Consequent observations of Six et al. (2004) established that the slower the macroaggregates turnover, the higher the protection level of stable SOM.

In contrast, Lal and Kimble (1997) suggested that an effective strategy to sequester C was to place crop residue beneath the plow layer where it would not easily decompose. Angers and Eriksen-Hamel (2008) conducted a meta-analysis that indicated that at the 21 to 25 cm soil depth, which was the mean plowing depth for these experiments, SOC was greater under CT than NT in most studies analyzed. Even greater differences in SOC favored the tillage treatment just below the plow layer (>25 cm). Gal et al. (2007) showed carbon accumulation in the in the 30 to 50 cm soil depth although plowing was done to less than 25 cm depth. Similarly, Christopher et al. (2009) observed greater SOM accumulation in the 30 to 50 cm soil depth in 7 out of the 12 plots they analyzed. But the accumulation of C at or below the plowing depth under the tillage treatment is not consistent in every study (e.g. Angers and and Eriksen-Hamel, 2008;

Blanco-Canqui and Lal, 2008) and the increase of C in soil sampled under tillage depth did not always compensate for the reductions observed in the soil surface layers (Gal et al, 2007).

By incorporating crop residue into the soil through plowing, the decomposition rate is increased by placing residue into intimate contact with soil particles and microorganisms. However, Smith et al. (2007) under laboratory conditions determined a positive correlation between the initial substrate decomposition rate and C persistence in soil over time. Stemmer et al. (1999) determined that decomposition of residue was delayed when the residue was left on the surface. Moreover, Balesdent et al. (2000) affirmed that rapid incorporation of organic C within aggregates was observed under tillage, and was favored by intimate contact between crop residue and the soil. This suggests that incorporation of crop residue into the soil through tillage and the subsequent increase in microbial activity and decomposition of crop residue could increase the persistence of C in soil at deep soil layers.

The distribution of residues and therefore SOC is different under the different tillage practices since NT tends to accumulate SOC in the surface while more intensive tillage practices distribute the SOC with depth. The literature is not consistent as to which tillage practice manages to sequester more carbon when the whole soil profile (>60 cm) is analyzed.

1.3 Crop rotation influence on SOC

The most common rotations used in the western Corn Belt are continuous corn (CC) and corn-soybean (CS) rotation (Grassini et al., 2011). Since the content of SOC is related to the quantity and quality of carbon in crop residue (Follett, 2001; Lal, 2004), crop rotation affects C substrate availability and decomposition rates by: (1) controlling the quantity, (2) quality and (3) timing of C inputs via plant residue and roots (Huggins et al., 2007; Govaerts et al., 2009).

Given that plant residue is the main C input to the system, increases in crop residue production due to management practices should increase SOC or at least moderate the rate of SOC loss (Lal and Kimble, 1997). Lal et al. (1980, cited by Lal and Kimbel, 1997) confirmed this by finding a positive relationship between crop residues returned to the soil and the SOC level. Corn and soybean have different C productivity during their cycle. Verma et al. (2005) estimated that the net ecosystem productivity (e.i. amount of CO_2 fixed into organic compounds minus ecosystem respiration) of corn, under irrigation and rainfed, was substantially larger (ca. 4:1) than that for soybean. In comparison with soybean, corn is a higher-residue-producing crop (Buyanovsky and Wagner, 1986; Christopher and Lal, 2009) returning to the soil in the order of 1.5 to 1.8 times more C than soybeans (Paustian et al., 1997). Buyanovsky and Wagner (1986) found similar results when they compared soybean and corn in Missouri. Subsequently, Dobermann et al. (2005) in Nebraska observed higher (+11%) crop residue input in CC than in CS rotation that led to a significant build-up of SOM in the soil under the CC rotation while SOM under CS quantities decreased.

The amount of C stored in a soil is also a function of the rate of biomass decomposition and SOM turnover. The residue quality influences the rate of decomposition, altering the transition of C into the SOM fraction (Johnson et al., 2007). Russell et al. (2005) observed that the C:N ratio of crops residue determined the speed of decomposition. According to Zhang et al. (2008), the residue quality variables (N content, C:N ratio, and lignin) collectively accounted for more that 70% of the variation in the residue decomposition rate. They indicated that the combined effect of total N and C:N of residue had a large influence on the decomposition rate. Since soybean has residue with lower C:N ratio than corn (Broder and Wagner, 1988), it tends to have higher rate of residue decomposition than corn (Huggins et al., 2007). Consequently, differences in crop residue quality between soybeans and corn could result in faster decomposition rates for soybean; hence CC could positively impact SOC.

In addition, soybean removes more N in grain than is symbiotically fixed from the atmosphere. Salvagiotti et al. (2008) observed that on average soybean N fixation averaged 50% to 60% of its requirements. Thus, the higher yields of succeeding crops (Vanotti and Bundy, 1995) and the N credit commonly given in N recommendations (e.g. for corn in the USA) cannot be attributed to symbiotic N fixation (Salvagiotti et al., 2008). The N contribution of soybean to succeeding crops is the result of rapid degradation of crop residue releasing N when most needed by the succeeding crop (Vanotti and Bundy, 1995). Similarly, Dobermann et al. (2005) reported a rapid cycling of soybean residue through young OM fraction and argued that the N credit attributed to soybean was due to increased extraction of N from the soil reserves resulting in a decrease in SOC and N. These observations are in accordance with evaluations on the effects of rotation carried out by Studdert and Echeverria (2000) who concluded that SOC increased if soybean was not present in the rotation.

Roots also can make a larger contribution of C to SOC than above ground plant material. Differences in root mass, quality and rhizodeposition between corn and soybean could influence differences in SOC. Johnson et al. (2007) observed that corn root residue had a longer half-life than soybean roots and above ground plant parts. They affirmed that chemical recalcitrance not C:N appears to be the cause of the greater contribution of corn root C to SOC as compared to soybean. In addition, the amount of root biomass returned to the soil by corn is three times greater than by soybean (Buyanovsky and Wagner, 1986). In addition to root biomass C, net rhizodeposited C must also be considered when estimating the total contribution of roots to SOC. But the difficulty of measurement and the great range of values reported do not allow precise estimations as shown by Amos and Walters (2006) who observed values of net rhizodeposited C in the range of 5 to 62 %. Due to its large and extensive root system and its lower degradation rate, maize has the potential to sequester more carbon than soybean.

In summary, soybean produces less biomass and therefore, less crop residue is returned to the soil than by corn. In addition, the residue return by soybean has a faster degradation time due to a lower stover C:N ratio than corn. Soybean also has a negative effect on soil N, influencing crop production and C reserves. These characteristics are part of the reason for the hypothesis that more soil carbon will be sequestrated under continuous corn than when soybean is in rotation with corn.

1.4 Effect of N fertilizer application and SOC.

Nitrogen (N) supply is one of the most variable and critical factors determining crop yields (Carpenter-Boggs et al., 2000). Alvarez (2005) affirmed that crop yields and the quantity of residues produced and returned to the soil are usually greater in fertilized fields. Thus by increasing crop yield, residue inputs are increased as well, and fertilizer application can contribute to increase SOC (Paustian et al., 1997). Alvarez (2005) observed for a wide set of climates that carbon sequestration increased as more nitrogen was applied to the soil and determined that the N effect on SOC was positive in 79% of the cases analyzed. He concluded that N application increased the amount of residue and C returned to the soil and the SOC over time. Similarly, Halvorson et al. (1999) observed at Akron, CO a positive relation between increasing N rate and biomass production and that incremental biomass production was related to increasing SOC levels in soil. But there are several studies were there have been no relation between the residue input and SOC. Russell et al. (2005) in a 23-year experiment in Iowa found great yield increases with N application, but no correlation between the quantity of crop residue and SOC stocks across diverse rotations or sites when the whole soil profile was evaluated. Similar conclusions were obtained by Poirier et al. (2009) who determined increasing C inputs from crop residue with increasing N rates for corn (but not for soybeans) in Quebec, Canada. However, the increased crop residue did not influence SOC storage in the soil profile. These results lead to the conclusion that although N application increases crop and residue yields its effect on SOC is not consistent.

In addition, N applications over the required rate for optimum crop yield (economically optimum nitrogen rate) will result in higher N uptake and thus improve the stover quality by reducing the C:N ratio (Liang and MacKenzie, 1994). Increasing the N rate can in turn increase the rate of decomposition of the residue (Carreiro et al., 2000; Kochsiek et al., 2009) and therefore negatively impact SOC. But the application of N fertilization has also an effect on the rate of SOM decomposition. In a literature review by Fogg (1988), he concluded that N addition had no effect or a negative effect on SOM decomposition, therefore SOM was less decomposed with the application of N. Similarly, Carpenter-Boggs et al. (2000) observed that under their "zero N" treatment more N was mineralized from the SOM than when N was applied. They found a negative relationship between N fertilizer application and N mineralization and decomposition of SOM.

The effect of N supply on soil C is an integration of increasing grain and residue yields, and consequently greater crop residue returned to the soil; however, enhanced residue quality (lower C:N ratio) leading to more rapid initial residue decomposition, and a negative effect on OM decomposition. Therefore with increasing rates of N fertilizer application, we would expect increases in SOC.

1.5 Interactions between Tillage, Rotation and N Application.

Each of these practices previously discussed affect several processes which regulate both the input and outputs of C from the system as well as the micro-environmental conditions that regulate the SOC balance. Several studies (e.g. Varvel, 1994; Reicosky et al., 1995; Franzluebber, 2005) have studied the effects of more than one management factor on SOC. In general, the interactions between tillage and rotations are not significant (Gal et al., 2007; Franzluebbers, 2005; Puget and Lal, 2005; Dolan et al. 2006; Yang and Kay, 2001; Reicosky et al., 1995). The effect of tillage is the most profound and consistent effect, but it seems that the greater changes produced by tillage mask the effect of rotation (Reicosky et al., 1995). The interactions between the effects of tillage and N rates on SOC are not significant either (Franzluebbers, 2005; Dolan et al. 2006). Finally, when the interactions of effects of rotation (CC or CS) with N application on SOC were evaluated, results showed no significance after 8 years (Varvel, 1994) or an inconsistent interaction at best after 12 years (Russell et al., 2005).

Only a few studies reported significant interactions between rotations, N rate and/or tillage. Studdert and Echeverria, (2000) observed an increase in SOC with CC and

N application but a decrease in SOC whenever soybean was present. Moreover, Priori et al., (2009) established significant interactions between N application and tillage treatments that were evident only in the top 20 cm of soil. They observed that the lowest SOC content was under MP with the highest N rate while the highest SOC content was under NT with the highest N rate. They argued that the increased N rate under MP accelerated the speed of decomposition of the residue and the native SOM while under NT greater amount of residue was retained in the soil surface.

The literature supports that the effects of tillage are much greater than those produced by either rotation or N application. Any interactions, if present, could be observed in the soil surface (0-20 cm). While the influence of each of these management practices is fairly well understood, the net interaction effects will be dependent on site specific factors not totally understood.

1.6 Analyzing the effect of management practices on SOC change over time.

The effect of different practices on SOC can be evaluated in two ways: (1) Spatial comparison: by comparing the SOC storage among different management practices after a given time period or (2) Temporal comparison: by comparing initial to final measurements in order to determine the rate of SOC change over time under each management practice. As reported by Ellert et al. (2001), the first approach may not provide a true measurement of absolute C change, but it compares the SOC response to different practices. This method relies on the assumption that the initial SOC stocks under all management practices were equivalent. The temporal comparison allows the true measurement of SOC changes over time under each management practice and can account for initial differences in SOC but requires archived samples which are not always available.

Ellert et al., (2002) added a known amount of recalcitrant coal to the soil in order to assess the ability to detect a change due to management with the different methods. They observed that the added C was undetectable with spatial comparisons when the soil thickness exceeded 35 cm. When comparisons were made among samples taken over time in the same plot, they confidently detected small amounts (3.8 Mg ha^{-1}) of added carbon. VandenBygaar and Angers (2006) reported that differences in SOC may be due to pedologic or geomorphologic differences, and can mask changes in SOC due to treatment. This becomes of greater importance at depth where the impacts of treatments are less pronounced and soil variability increases. The lack of initial SOC determinations and the inherently large soil variability could mask small effects due to management practices.

CHAPTER 2. Material and Methods

First a brief description of the site and the history of the experiment will be presented, followed by a description of how data was obtained from the two long-term studies and how SOC stocks were calculated. The process includes calculating C and N inputs and outputs from crops, the soil sampling procedure, and adjusting archived soil samples in order to compare them with recent sampling.

2.1 Site Description and History

The data for this study were obtained from two long-term field experiments in eastern Nebraska; one situated near Concord and the other near Mead (Table 2.1). Conditions between sites varied, not only in terms of atmospheric and soil characteristics, but in management intensity and length of the experiment. Characterization and summary of experimental setup of each site are presented in Table 2.1.

The experiment at Concord was initiated in 1985 and established as a split-split plot design with four replications under rainfed conditions. Treatments consisted of a) tillage treatment as main plot with 3 levels: moldboard plow (MP) (followed by a disk), disk tillage (DK) and no-till (NT); b) crop rotation as subplot with 2 levels: continuous corn [*Zea mays* L.](CC) and corn-soybean [*Glycine max* (L) Merr.] rotation (CS); and c) N fertilizer rates as sub-subplots with 3 levels; 0 kg N ha^{-1} (0N), 80 kg N ha^{-1} (80N) and 160 kg N ha^{-1} (160N). These were a subset of treatments from a larger experiment which had 3 crop rotations and 5 N levels. There were 4 replications and two soil sampling dates (1997 and 2009). Samples were taken each spring prior to tillage, which also took place in spring just before planting. Nitrogen fertilization was only applied to corn as ammonium nitrate broadcasted usually before tillage took place. Corn seeding rates were initially planted at a rate of 44,500 seeds ha^{-1} but over time, the seeding rate has increased to 63,500 plants ha^{-1}. Phosphorus fertilizer was applied when necessary based on soil test using the University of Nebraska interpretations. Prior to the establishment of the experiment the field had been under continuous corn since approximately 1970 using mainly disk as the tillage method.

The experiment at Mead was initiated in 1997, designed as a split-plot with 3 replications under irrigation. Treatments established were crop rotation as the main plot with 2 levels: CC and CS; and N rate as subplot with 3 levels: 0 kg N ha^{-1} (0N), 100 kg ha^{-1} (100N) and 300 kg ha^{-1} (300N) applied only to corn. There were two soil sampling dates (1998 and 2009). Samples were taken in spring before fertilizer application and the tillage treatment, which consisted of a spring disk after N fertilization. Corn was planted at a rate of 79,000 seeds ha^{-1}. Prior to 1997 all experimental units were under a CS rotation since 1988.

2.1.1 Plant and grain harvest

a) Grain

Corn and soybean grain yields were quantified yearly for each treatment. At Concord, corn yield was estimated by harvesting 3 central rows with a MF 300 combine equipped with a 3 row corn head. Soybean yield was estimated by harvesting two rows with a 2 row combine. Yields were expressed on a dry matter basis for C balance calculations.

$$\mathbf{CGY = Gwt*(1\text{-}moist)*(10000/(rl*w))} \qquad \mathbf{(1)}$$

where CGY (kg ha^{-1}) is the corn grain yield on a per hectare basis, Gwt (kg) is the fresh grain weight from of 3 central rows harvested, moist is the grain moisture (as a decimal fraction), rl is the length (m) and w is the width (m) of the harvested rows, for corn it was 2.23 m. Soybean was calculated with the same procedure except w was 1.5 since only 2 rows were harvested. Corn grain N concentration was analyzed each year to determine the amount of N being removed from the system with grain harvest.

b) Stover

After combine harvest at Concord through the 2000 cropping season, an adjacent non-grain harvest row in each plot had the ears removed, and then the remaining plants were harvested using a specially designed stover harvester (Frerichs et al., 1993). After weighing the stover, a sub-sample was taken for moisture determination and N analysis. The remainder of the stover sample was spread back over the row from where it was harvested. Corn stover yield (CSY) (kg ha^{-1}) was calculated using equation 1, replacing Gwt by stover fresh weight (sfw) (kg) of the sampled row and setting w at 0.76 m.

At Mead and from 2001 on at Concord, the method used to estimate corn vegetative dry matter was slightly different. Before grain harvest, at the onset of physiological maturity PM (at least 1/2 or more of the ear at black layer (Ritchie and Hanway, 1982)) within the harvest row area, 6 plants were randomly selected when the plants had just reached physiological maturity. These six plants were cut at ground level and removed from the plot. Ears (w/o husk) were removed from the 6-plant sample for determination of grain and cob dry weight. After drying the whole ear at 70C, the ears were carefully shelled to obtain an accurate dry grain weight as well as cob weight. The 6-plant stover (stalk, leaf, and husk) samples were weighed and chopped then sub sampled for moisture and nitrogen concentration. Cob weight was added in later after shelling the 6-ear sample. A ratio (RatGS) was determined with equation 2.

RatGS = 6grainDW / (6stoverDW + 6cobDW) **(2)**

where 6grainDW is the grain dry (70°C) weight of 6-plants, 6stoverDW is the stover dry weight of 6-plants and 6cobDW is the cob dry weight of the 6-plants. The ratio estimated for the 6 plants were later used in order to estimate vegetative dry mass and N uptake for the whole plot (equation 3).

CSY = CGY / RatGS **(3)**

where CSY (kg ha^{-1}) is the corn stover yield.

Prior to 2001 the harvest index (HI=CGY/(CGY+CSY)) was 0.52 (0.01) at Concord compared to after 2001 where the average HI was 0.47 (0.01). This change was due to the timing of stover harvest. When stover harvest was taken at PM, more of the leaf material was still on the plant. Considering that eventually the stalks and leaves do drop to the ground the higher stover DM numbers are closer to the actual dry weight. In order to account for the reductions in stover calculations in Concord prior to 2001, given that stover was collected not at PM but after grain harvest, the average HI for every year in Concord prior to 2001 was adjusted to the average HI between 2001 and 2008. The pre-2001 dry matter numbers were adjusted for each year according to the following formula:

NHI = (0.47/0.52)*HIx **(4)**

where NHI is the new adjusted HI for a year X and HIx is the HI of a year X prior to 2001.

c) Assumptions

Different assumptions were made for each crop in order to quantify the above and belowground C and N inputs as well as outputs from the system (Table 2.2). For soybean, root to shoot ratio (R/S)(root biomass/(grain + aboveground biomass)) was assumed to be 0.14 and the harvest index to be 0.43 simulated using SoySim (Setiyono et al., 2010) a soybean growth model developed at the University of Nebraska. Stover and root C concentration were both assumed to contain 450 g C kg^{-1} of the dry mass, while N concentration was estimated at 11 g N kg^{-1} of soybean stover dry mass (leaf, stem and pod) (Lagorreta-Padilla, 2005) and 7.5 g N kg^{-1} of soybean root dry mass (Johnson et al., 2007), while soybean grain N content was assumed to be 60 g N kg^{-1} of grain. For corn the C content was assumed to be 437 g C kg^{-1} for stover and 343 g C kg^{-1} for root dry mass, while the N content was assumed to be 9 g N kg^{-1} (Johnson et al., 2007) for root dry mass. R/S ratio for maize (root biomass/(aboveground biomass - grain)) was considered to be 0.16 at physiological maturity (Amos and Walters, 2006).

2.2 Soil Sampling

In spring of 2009, each experimental unit was sampled at 6 depths (0-5, 5-15, 15-30, 30-60, 60-90, and 90-120 cm) and analyzed for total soil carbon (see below). Three soil cores for the 0-30 cm, divided in 0-5, 5-15 and 15-30 cm intervals were taken in each experimental unit. For the 0-5 cm sample, two additional 0-5 cm cores were taken with a hand probe and mixed with samples from the same depth interval for a composite sample. Additional replicate samples for the 30-120 cm, two per plot, were obtained with a hydraulic probe (21.5 mm diameter). The cores were divided into 3 depths (30-60 cm, 60-90 cm and 90-120 cm) and mixed for a composite sample at each depth. All samples (surface and deep) were taken ¼ of the way from the plated row. Visible root segments were handpicked from the samples. Samples were air dried to a constant weight first and then ground and passed through a 2mm sieve. Samples were prepared for C and N analysis with the same procedure followed on the surface 30 cm.

Each site was also sampled at the same depth intervals for bulk density. Samples were also taken ¼ of the way from the planted row. Bulk density in the first 30 cm was taken with a lubricated plastic sleeve mounted inside a hand probe. For every plot two sample cores, 21.5 mm in diameter were taken. Each soil core divided into three segments corresponding with depths used to determine both soil C and N, later dried at 105°C for 72 hrs and weighed. Multiple cores were taken for soil depths below 30 cm up to 120 cm, with a 21.5 mm diameter hydraulic probe and divided in three depths (30-60 cm, 60-90 cm and 90-120 cm).

Archived samples from Concord had been taken in 1997. Two soil cores (21.5 mm) were taken per plot in 30 cm increments up to 120 cm deep and mixed for composite samples at each depth interval. Samples were air dried and ground to pass a 2 mm sieve. Samples were used for N analysis and stored under dry conditions afterwards. Archived samples from Mead had been taken in 1998 with the same sampling protocol and preserved under the same conditions. Carbon and N determination for these archived samples were conducted in 2009 using the same protocol used for 2009 samples (see below). No bulk density measurements were taken at the time of sampling the archived samples in 1997.

2.3 Soil C and N Determination

From each depth interval a sub-sample of soil was fine-ground using a roller mill device. Twenty milligrams of fine-ground soil were then weighed for total C and N analysis using an elemental analyzer (ESC 4010, Costech Analytical Technologies Inc.

Valencia, CA). A standard soil (SS) was run with samples at 12-sample intervals to correct for daily machine drift. Samples were analyzed in the laboratory in 52 sample-runs with 4 being SS's in each run. The average of the 4 SS in each run was compared to the mean value of all the SS analyzed and the difference (in percentage) was used to obtain a correction factor for all the samples in the run. Based on the SS samples, the coefficient of variation (CV) of C analysis was within 1-2% range. Soil C and N values were adjusted for moisture content based on average carbon values of several SS samples oven dried at 105°C.

Soil organic carbon analysis can be confounded by mineral carbon compounds such as calcium carbonate. At Mead, soil samples showed no trace of carbonates for the two soil types present in the experiment. The acid (HCl) reaction test (Nelson and Sommers, 1996) was used on all samples from 1998 and 2009 at the 60-90 cm and 90-120 cm depth with no positive reaction. At Concord, samples (from 1997 and 2009) with a positive reaction to acid treatment were from the 60-90 cm and 90-120 cm depth layers. Samples which reacted positively (22 out of 432 samples, all corresponding to the 60-90 and 90-120 cm depths) were selected and carbonate content was determined. Phosphoric acid was used to eliminate carbonates from the sample (Follett et al., 1997) and the difference between treated and untreated samples determined the amount of inorganic carbon in the soil. Samples with and without phosphoric acid (1M PO_4H_3) treatment were analyzed for carbon using an elemental analyzer. Given that samples contained in general small (<0.4%) amounts of total carbon (they belonged to the deepest two soil layers) the normal machine drift (1 standard deviation =0.04% C) was relatively too big to allow the proper determination of carbonates that represented a small portion of the total carbon in the samples. These variations masked the presence of carbonates and, therefore, the presences of carbonates in these samples were ignored. However, three samples out of 144 (all belonging to the 60-90cm layer) were not considered in the estimation of bulk density of archived samples because carbonate levels were higher than the machine drift, therefore, the presence of carbonate was certain. Carbonate levels, whenever present, were assumed constant over a period of 25 years for the 60-90 cm and 90-120 cm depth layers.

2.4 Soil Bulk Density Estimation for Archived Samples

In order to report and compare carbon and nitrogen values on a constant mass basis, missing BD values for archived samples were estimated. Previous research has found that BD correlates strongly with SOC having been established as the main predictor of BD in cultivated and uncultivated soils (Adams, 1973; Harrison and Bocock, 1981; De Vos et al., 2005; Martin et al., 2009). Many pedotransfer functions (PTFs) have been developed to estimate soil BD (e.g. Martin et al., 2009), but as reported by De Vos et al., (2005) the predictive potential of PTFs is sometimes very limited. Harrison and Bocock (1981) recommended, that in order to obtain high accuracy and precision in BD estimations researchers should derive equations for their own research site rather than to rely on general equations. The relationships between BD and SOC in 2009 samples were used to estimate BD values for the archived samples based on their SOC content. Fitted equations are shown in Fig. 2.1 and 2.2.

De Vos et al., (2005) and Martin et al., (2009) compared the root mean square error (RMSE) of their proposed PTF's predictions to the standard deviation of direct BD measurements in the field.

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\hat{p}_i - p_i)}$$

were $\hat{p}_i$ and p_i are the estimated and observed bulk densities for the *i*th observation, respectively.

The standard deviations of direct measurements of BD on relatively homogeneous fields reported in previous studies are in the range of 0.09 Mg m^{-3} (Warrick and Nielsen, 1980) to 0.14 Mg m^{-3} (Alexander, 1980 cited by Martin et al., 2009). The RMSE values (which were equal to the SE in the regressions) obtained for the regressions in this study range from 0.042 Mg m^{-3} to 0.091 Mg m^{-3}, therefore, meeting the standard deviation of direct measurements of bulk density.

At Concord, for the first 30 cm depth layer, different equations were fitted (lowest-order best-fit polynomial) for each tillage treatment, since the different intensity of the tillage process affects BD and C distribution in the soil profile differently (Chen et al., 1998). Different relationships were established for 0-30 cm depth since tillage induced changes on BD are generally limited to the surface 30 cm (Chen et al., 1998, Martin et al., 2009). In the 30-90 cm soil interval, BD values for MP were significantly higher than for the other tillage treatments. A different curve for each tillage treatment was fitted and compared, but no statistical difference was observed among them, therefore, a single relationship was established for all treatments (Fig 2.1 and 2.2). Bulk density at the 90-120 cm depth layer was assumed constant over time so values obtained in 2009 were used in calculations for 1997.

At Mead, the lowest order best-fit polynomial equations were used to explain the relationship between soil organic carbon content and bulk density. A polynomial equation was fitted for the first 30 cm soil layer and a linear equation for the 30-60 cm and 60-90 cm deep samples grouped together. The bulk density values for the last foot depth were considered constant between the two sample dates since their carbon values did not correlate with bulk density values and, therefore, it was unreasonable to estimate values of BD based on carbon content of archive samples.

2.5 Analyzing SOC stocks and changes over time.

Calculations of SOC stocks were done for 0-50, 50-200, 200-400, 400-800 and 800-1200 kg of soil dry mass m^{-2} which approximately represents the 0-5, 5-15, 15-30, 30-60 and 60-90 cm depth intervals. Since archived samples were collected on 30 cm increments, SOC changes over time was analyzed on 400 kg of soil m^{-2} (approx.30 cm) intervals to a cumulative soil mass of 1200 kg m^{-2} (approx. 90 cm). The procedure used to transform and express values on a constant mass basis is presented in Appendix A. Archived samples (1997 at Concord, NE and 1998 at Mead, NE) were taken at 30 cm depth increments, therefore, analysis of the effect of management practices on SOC in the surface soil layers (0-50, 50-200 and 200-400 kg of soil m^{-2}) was done using spatial comparisons which assumes an equivalent initial SOC level.

Statistical Analysis

Data obtained for SOC and residue carbon and yield were analyzed statistically with PROC MIXED module in SAS (SAS Institute, 2009) with a split-split-plot in time design for Concord and a split-plot in time design for Mead. The ANOVA model tables for each site are presented in Table 2.3 and 2.4. Analysis of the 2009 samples, rates of SOC change, and average residue input was conducted as a simple split-plot in Mead and a split-split-plot in Concord for each soil depth interval separately. Orthogonal polynomial contrasts were also used to determine differences induced by N rates with and without interaction with tillage and rotation. Contrasts were also used to determine differences induced by N rate on residue C input. The probability level (p-value) at which the null hypothesis was rejected was set at $p<0.05$. LSMeans test were determined for comparisons among treatments and years whenever the ANOVA was significant.

Table 2.1 Experimental setup, site characteristics and soil present at each location.

	Concord, NE	**Mead, NE**
Physical location	42° 23'' N and 96° 59'' W	42° 23'' N and 96° 50'' W
Mean annual rainfall (mm)	670	737
Mean seasonal temperature (°C)	17.5	18.2
Initiated	1985	1997
Experimental design	Split-split-plot	Split-plot
Tillage	No-till, Disk and Plow	Disk
Crop rotation	Continuous Corn and Corn/Soybean	Continuous Corn and Corn/Soybean
N rates	0, 80 and 160 kg ha^{-1}	0, 100 and 300 kg ha^{-1}
Irrigation	Rainfed	Irrigated
Soil Sampled	1997 and 2009	1998 and 2009
Site history	Continuous corn-Disk (since approx. 1970)	Corn-soybean (since 1988)
	Soil series	**Family**
Concord, NE	Coleridge	fine-silty, mixed, superactive, mesic Cumulic Haplustolls
	Maskell	fine-loamy, mixed, superactive, mesic Cumulic Haplustolls
	Baltic	fine, smectitic, calcareous, mesic cumulic Vertic Endoaquolls
Mead, NE	Yutan	fine-silty, mixed, superactive, mesic Mollic Hapludalfs
	Tomek	fine, smectitic, mesic Pachic Argiudolls
	Filbert	fine, smectitic, mesic Vertic Argialbolls

Table 2.2 Summary of assumptions used to determine the above and belowground carbon (C) and (N) inputs and outputs from the system

	Corn	**Soybean**
Root/Shoot ratio	0.16	0.14
Stover C (g kg^{-1})	437	450
Stover N (g kg^{-1})	-	11
Root C (g kg^{-1})	343	450
Root N (g kg-1)	9.0	7.5
Grain N (g kg^{-1})	-	60
Harvest Index	-	0.43

Table 2.3 Analysis of variance (ANOVA) for SOC (g kg^{-1}) and SOC stocks (kg m^{-2}) over time.

Mead

Source		DF
Block (B)	(B-1)	2
Rotation (R)	(R-1)	1
Ea= B x R	(B-1)(R-1)	2
Nitrogen (N)	(N-1)	2
R x N	(R-1)(N-1)	2
Eb= N X B(R)	(N-1)(B-1)R	8
Time (t)	(t-1)	1
Ec= B x t	(B-1)(t-1)	2
R x t	(R-1)(t-1)	1
Ed= B x R x t	(B-1)(R-1)(T-1)	2
N x t	(N-1)(t-1)	2
R x N x t	(R-1)(N-1)(t-1)	2
Ee=t x B x N(R)	(t-1)(B-1)(N-1)R	8
Total	BRNt-1	35

Concord

Source		DF
Block (B)	(B-1)	3
Tillage (T)	(T-1)	2
Ea= B x T	(B-1)(T-1)	6
Rotation (R)	(R-1)	1
T x R	(T-1)(R-1)	2
Eb= R x B(T)	(R-1)(B-1)T	9
Nitrogen (N)	(N-1)	2
T x N	(T-1)(N-1)	4
N x R	(N-1)(R-1)	2
N*R*T	(N-1)(R-1)(T-1)	4
Ec= N x B(R x T)	(N-1)(B-1)RT	36
Time (t)	(t-1)	2
Ed= B x t	(B-1)(t-1)	6
T x t	(T-1)(t-1)	4
Ee= B x T x t	(B-1)(T-1)(t-1)	12
R x t	(R-1)(t-1)	2
T x R x t	(T-1)(R-1)(t-1)	4
Ef= B x R x t (T)	(B-1)(R-1)(t-1)T	18
N x t	(N-1)(t-1)	4
T x N x t	(T-1)(N-1)(t-1)	8
R x N x t	(R-1)(N-1)(t-1)	4
T x R x N x t	(T-1)(R-1)(N-1)(t-1)	8
Eg=B xNx t(T x R)	(B-1)(N-1)(t-1)T R	72
Total	BTRNt-1	215

Table 2.4 Analysis of variance (ANOVA) for rates of soil organic carbon (SOC) change, average C and N residue input, average yields, bulk density (BD) and SOC stocks in a given sampling date.

Mead

Source	DF	
rep (B)	(B-1)	2
rot (R)	(R-1)	1
Ea=rep*rot	(B-1)(R-1)	2
nrate (N)	(N-1)	2
rot*nrate	(N-1)(R-1)	2
Residual	(B-1)(N-1)R	8

Concord

Source	DF	
rep (B)	(B-1)	3
till (T)	(T-1)	2
Ea=rep*till	(B-1)(T-1)	6
rot (R)	(R-1)	1
till*rot	(T-1)(R-1)	2
Eb=rep*rot(till)	(B-1)(R-1)T	9
nrate (N)	(N-1)	2
till*nrate	(T-1)(N-1)	4
rot*nrate	(R-1)(N-1)	2
nrate*till*rot	(N-1)(T-1)(R-1)	4
Residual	(N-1)(B-1)RT	36

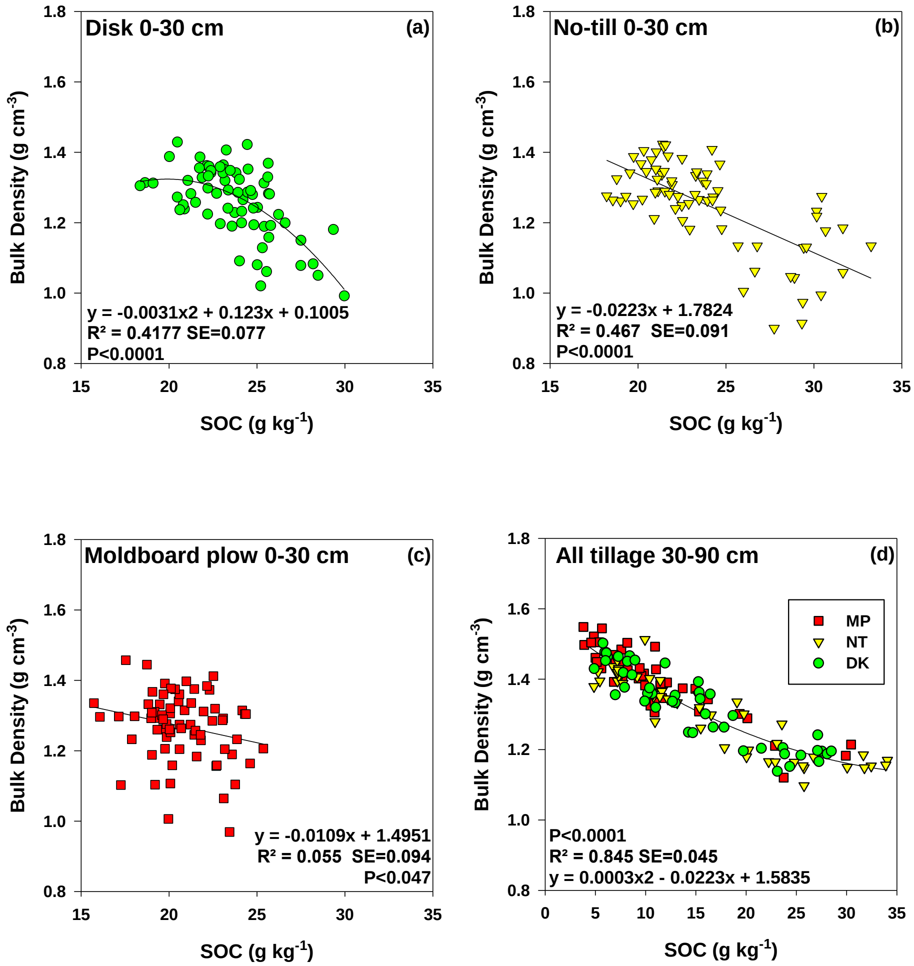

Figure 2.1 Bulk density-soil organic carbon (SOC) relations established for 2009 Concord samples under (a) disk tillage, (b) no-till and (c) plow tillage for surface 30 cm soil depth and for (d) the 30-90 cm soil depth (all tillage treatments).

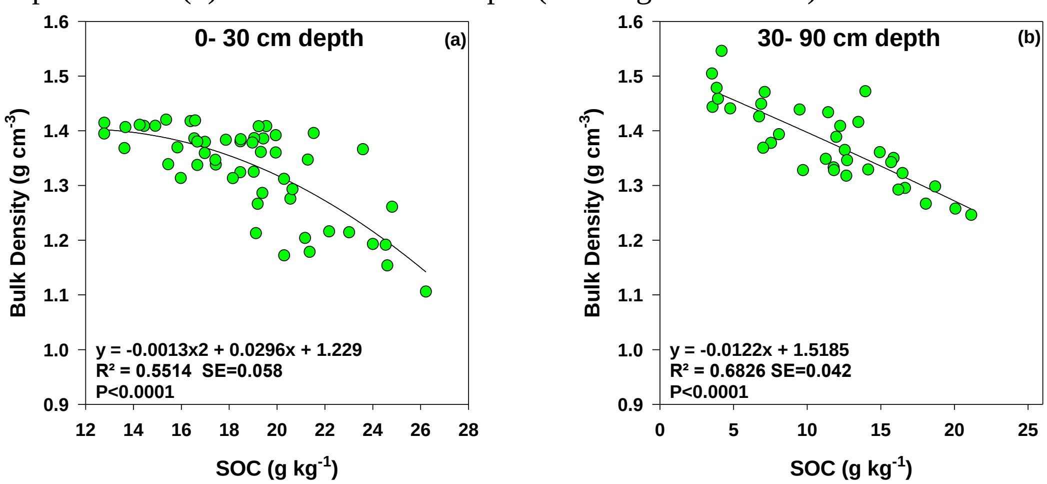

Figure 2.2 Bulk density-soil organic carbon (SOC) relations established for 2009 Mead samples for (a) surface soil depth (0-30cm) and (b) 30-90cm soil depth.

CHAPTER 3. Long-term study at Mead, NE

3.1 Results and Discussion

When analyzed by depth interval, BD was affected neither by rotation nor by N rate at the 0-5 and 5-15 cm intervals. A significant difference between rotations was present at the 15-30 cm interval (Table 3.1). This difference between rotations is thought to be related to the soils natural variability given that although not statistically significant, this difference between rotations was present in subsequent soil layers (30-60, 60-90 and 90-120 cm) and associated with lower SOC contents under CC than under CS. In addition, an isolated rotation by N interaction was present at the 60-90 cm depth interval. Given that BD was only slightly affected by treatments, analyzing the effects of management practices on SOC produces the same interpretation as when done on a concentration basis or an equivalent soil mass basis (Table 3.2). Mean SOC concentrations for each depth interval, sampling date, and statistical analysis are presented in Tables 3.2 and 3.3, but only values of SOC stocks will be discussed.

3.1.1 Effects of long-term rotation and N input on SOC sequestration: Spatial comparison.

Rotation (CC vs. CS) and N fertilizer rates (0, 100 and 300 kg ha^{-1}) had considerable impacts on grain yields as well as on C input from crop residue (stover + root). Yields between 1997 and 2008 for CC increased with increasing N rates averaging 7.4, 9.9 and 10.3 Mg ha^{-1} under the 0N, 100N and 300N treatment. During the same period for the CS rotation, corn yields were slightly higher in the 100N and 300N treatments than in the 0N treatments averaging 12.3, 12.2 and 12.0 Mg ha^{-1} respectively

(Table 3.4). Soybean yields averaged 3.6 Mg ha^{-1} following N applied to corn with 0N or 100N and 3.8 Mg ha^{-1} with 300N (Table 3.4).

Residue C inputs followed variations observed in grain yields. The average quantity of residue C input for CC increased with higher N rate applications (Fig. 3.1). The amount of C returning to the soil (stover + root) under CC averaged 3.3, 4.4 and 4.8 Mg ha^{-1} a year with 0N, 100N and 300N (Table 3.4). But for the CS rotation the average residue C had no response to increasing rate of N application. The average residue C inputs to the soil for the CS rotation (average of corn and soybean years) were 4.0, 4.3 and 4.1 Mg ha^{-1} yr^{-1} with 0N, 100N and 300N, respectively (Table 3.4).

Although differences in residue C inputs were evident between rotations at different N rates, SOC stocks were not impacted by either rotation or N fertilizer application at the 0-50, 50-200 or 200-400 kg of soil m^{-2} intervals. Our results are similar to those reported by Gal et al., (2007) who also reported no significant impact of crop rotation on SOC, even though they observed 30% more C residue returned under CC than under the CS rotation. In our study, there was 21% higher C inputs in the CS rotation at low N rates (0N) and 14% higher C inputs in CC system at high N rates (300N) but these differences in C inputs did not impact SOC stocks. Similarly, Russell et al. (2005) at Kanawha, IA determined no significant effect on SOC stocks with increasing N rates. They reported an increase in grain and residue yields for CC and CS with increasing N fertilizer rates but did not observe differences in the SOC stocks. High variability in SOC stocks observed throughout the field (see section 3.1.2) relative to the differences among treatments could have contributed to the lack of effect of residue C input on SOC. Not having initial samples for the surface soil layers (0-50, 50-200 and 200-400 kg of soil

m^{-2}) limited our capacity to differentiate SOC natural variability from management effects.

In our study, the differences in quantity of C input to the system by the CC system at different N rates were not reflected in a significant increase in the SOC. Soil C contents were not higher under CC-300N than under CC-0N although the amount of residue returned was 40% more. The lack of effect of different C inputs on SOC stocks could have been in part due to the assumption a fixed R:S ratio which might have underestimated the root biomass at low N rates. Amos and Walters (2006) reported a slight decrease in corn root biomass (-6.5%) when N was deficient but a 41% increase in the R:S. According to this, under low N availability the plant would reduce considerably more its aboveground biomass than its belowground biomass. Moreover, roots are an important factor in determining SOC levels (Paustian et al., 1997) since roots can contribute up to 1.5 times more C to SOC than the shoot, mainly attributed to a slow biodegradation of root-derived material (Balesdent and Balabane, 1996). In addition, the C input from rhizodeposition, which has a great contribution to SOC (Amos and Walters, 2006), is extremely difficult to estimate given the wide range of values reported. Therefore, the absence of initial SOC values for the surface soil layers to account for the inherent soil variability, together with the difficulty in the estimation of belowground C input could explain the lack of correlation between residue C inputs and SOC stocks.

3.1.2 Soil variability at depth.

When comparing SOC stocks, it has been argued that deep sampling is required to consider possible accumulation (and depletion) of SOC at depths, sometimes greater than the tillage depth (VandenBygaart and Angers, 2006). Therefore, the whole soil profile was considered (1200 kg of soil m^{-2}) observing slightly higher SOC stocks under CS than under CC. Values were 18.1 for CS and 14.5 kg C m^{-2} for CC (Fig. 3.2). In order to better understand the differences, SOC was separated at three different soil mass intervals. Although not statistically different, SOC stocks were greater under CS in the 0-400, 400-800 and 800-1200 kg of soil m^{-2} intervals (Fig. 3.3). Differences were greater in the two deepest soil mass intervals than in the surface 400 kg of soil m^{-2} (approx. 30 cm). Since rotation and N application were expected to influence SOC in the surface layer rather than at depth, great differences at depth were suspected to be caused by the soils' natural variability. However, results obtained solely from samples taken in 2009 did not allow us to determine if these differences were due to natural soil variability or due to treatment effects.

Experimental units (EU) with similar SOC content in the 400-1200 kg of soil m^{-2} interval were observed relatively close together in the field regardless of the rotation (Fig. 3.4). Overall, 66% of the plots under CC and 22% of the plots under CS were situated in areas related to low SOC stock (<6 kg m^{-2}), which would explains why SOC values were lower compared under CC. At depths greater than 30 cm the soils in the study had considerable differences in type of soil horizons. This would have affected the variation and content of SOC with increasing depths. Because samples were taken at fixed depths increments (30 cm), different soil horizons were sampled for each soil series at increasing depths (Fig. 3.5). For example, for a Yutan soil series, the horizon at the 30-60 cm depth consisted mainly of a Bt while for the same depth a sample taken from a Tomek series, which is also present at the study site consisted mainly of an AB horizon which would be expected to have higher SOC concentration. Great differences in SOC stocks between given soil horizons would mask changes due to management practices. Thus, the assumption of equivalent initial SOC stocks required for a spatial comparison may not be appropriate for deep samples. For a correct determination of the effect of management practices on SOC, initial measurements (1998) of each experimental unit sampled under the same protocol were used to account for the natural SOC variability.

3.1.3 Changes in SOC over time: Temporal comparison.

When SOC was evaluated over the last 11 years, crop rotation and N rates did not have a significant effect at any of the analyzed soil intervals (Table 3.5). Quantities of SOC at the 0-400 kg of soil m^{-2} interval decreased under all of the treatments evaluated. Stocks of SOC in the top 400 kg of soil m^{-2} decreased from 7.46 to 7.15 kg of C m^{-2} between 1998 and 2009, which represented a change of -0.31 ±0.03 kg of C m^{-2} (Table 3.6). At the same site, Lagorreta-Padilla (2005) reported a 2% decrease in total soil carbon in the first 15 cm under CS after the first 6 years of the study (1997-2003). Although he did not adjust values for BD measurements and considered only the first 15 cm, this rate of loss was in the same range as with the 4% observed after 11 years in the surface 400 kg of soil m^{-2} (approx. 30 cm) of our study at Mead. Our results clearly show that for the given conditions of this study (climatic conditions, soil type, tillage system and irrigation) none of the evaluated treatments were able to sequester atmospheric C or even achieve a balance between inputs and outputs. Levels of SOC were clearly lower after 11 years of cultivation and future research will be necessary to determine if and when the system achive a balance or steady state.

Soil C content in the 400-800 and 800-1200 kg of soil m^{-2} intervals did not change after 11 years. Differences in SOC observed between crop rotations in 2009 were present in 1998 (Fig 3.6). Changes over the length of the study averaged over rotation and N rate were -0.10 ±0.09 and -0.09 ±0.11 kg C m^{-2} in the 400-800 and 800-1200 kg of soil m^{-2} intervals. This is equivalent to an average yearly change rate of 8.8 and 8.1 g C m^{-2} yr^{-1} for each mentioned soil mass interval (Table 3.6). Given the error associated to these estimations, SOC stock at the 400-800 and 800-1200 kg of soil m^{-2} intervals were small and less than the precision of our ability to measure.

When SOC changes over time were considered for the whole 1200 kg of soil m^{-2}, rotation and N fertilization had no effect on SOC stocks (Table 3.5). Apparent differences observed in the 2009 samples between rotations were also present in the 1998 samples (Fig. 3.6). Under CS, SOC changed from 18.93 to 18.26 kg of C m^{-2} between 1998 and 2009 and under CC from 14.83 kg to 14.50 kg of C m^{-2} between 1998 and 2009. Archived samples showed that the rate of SOC depletion under CS was double than that of CC (Fig.3.6), but this difference was not statistically significant. These results emphasize the importance of having archived samples in order to determine the true effect of management practices on SOC over time (Potter, 2006). Averaged over rotation and N fertilizer rate, stocks of SOC in 1200 kg of soil m^{-2} (approx. 0-90 cm) decreased by -0.5 ±0.22 kg m^{-2} from 16.85 to 16.30 kg m^{-2} between 1998 and 2009 (Table 3.6), however these changes were not significant ($P > 0.142$). Even though the amount of SOC in the 1200 kg of soil m^{-2} interval between 1998 and 2009 were not statistically significant, trends show that C is being lost from the soil rather than sequestered from the atmosphere. Small differences in SOC stocks over time observed in the surface 400 kg of soil m^{-2} were not observed when the 1200 kg of soil m^{-2} mass was considered.

These results are interesting because they support the idea that to understand the effect of management practices on SOC, long-term studies need to be analyzed since changes are relatively small compared to the great SOC background. For example, in the 0-400 kg of soil m^{-2} profile, observed changes were close to 28 g of C m^{-2} yr^{-1} while the content of SOC was more than 7.46 kg m^{-2}, which is just a 0.37 percent annual difference (Table 3.6). Observations over long periods of time would be required to determine if there have been effective changes in the SOC content at this soil mass layer.

These results also highlight the importance of having initial soil samples in order to accurately determine the effect of different treatments. This is especially important in deep samples (>30cm) where management practices have less impact and there is a greater natural soil variability (Potter, 2006). Similarly, if we had based our conclusions on samples obtained in 2009 our results would have been somewhat different. Varvel (2006) on a study in a nearby location also pointed out the importance of more than one sampling date when assessing SOC trend over time. Although he analyzed surface samples (0-30 cm), the effect of changes in management practices on SOC during the course of the experiment could have only been observed by having more than one sampling date. In our study management practices remain relatively constant during the last 11 years, thus having initial and final sampling times allowed the determination of the effects of management practices on SOC changes. VandeBygaart and Angers (2006) and Ellert et al. (2002) suggested that future studies, in order to overcome soil variability, should require intensive initial sampling.

Most importantly, based on the results from this study, neither CC nor CS at the evaluated N rates, were an effective management practice to sequester atmospheric C.

Although C inputs varied from 3.3 to 4.7 Mg ha^{-1} yr^{-1}, this difference did not translate in to a significant difference in the SOC. It seems that the amount of returned residue under these management practices and for the given conditions (weather, soil, tillage irrigation, SOC levels, etc) are not enough to compensate for the losses from decomposition of residue and SOM.

3.2 Conclusions

Differences in C input between CC and CS at different N rates did not impact SOC. The absence of initial soil samples for the surface soil layers (0-50, 50-200 and 200-400 kg m^{-2}) in order to account for the inherent SOC variability, or deficiencies in estimating belowground C input could explain, in part, the lack of response of the SOC to residue C input. West and Six (2007) observed that the average duration of studies which had detected differences in SOC due to changes in rotation intensity was 20 ± 6 years. Probably more time is required to observe differences between the treatments given that changes are relatively small compared to the SOC background and variability.

Under these given climatic and soil condition of the site, none of the evaluated management practices were sufficient to stop the depletion of C from the soil. The loss of SOC was limited to the surface 400 kg of soil m^{-2} (approx. 0-30 cm) and the rate of SOC loss for this part of the soil profile was 0.310 ± 0.03 kg C m^{-2} after 11 years. When 1200 kg of soil m^{-2} (approx. 90 cm) were considered, no differences could be detected after 11 years.

Finally, in order to interpret SOC change in the whole soil profile, initial soil samples were necessary to account for soils natural variability, especially when sampling exceeded 30 cm. Given the great SOC variability at depth and the relatively small impact of management practices, having initial measurements allowed the determination of the true rate of change in SOC due to management.

Table 3.1. Bulk density (Mg m^{-3}) values from 2009 samples at different soil depth intervals and ANOVA performed at given depth intervals at Mead, NE.

Rotation	**N rate**	**0-5 cm**	**5-15 cm**	**15-30 cm**	**30-60 cm**	**60-90 cm**	**90-120 cm**
	kg ha^{-1}	**Mg m^{-3}**					
CC	**0**	1.230	1.373	1.380	1.383	1.453	1.467
	100	1.210	1.390	1.363	1.387	1.507	1.450
	300	1.217	1.387	1.393	1.337	1.400	1.473
CS	**0**	1.223	1.357	1.357	1.320	1.390	1.460
	100	1.220	1.363	1.373	1.317	1.357	1.493
	300	1.283	1.373	1.347	1.347	1.357	1.437
CC		1.219	1.383	1.379	1.369	1.453	1.463
CS		1.242	1.364	1.359	1.328	1.368	1.463
	0	1.227	1.365	1.368	1.352	1.422	1.463
	100	1.215	1.377	1.368	1.352	1.432	1.472
	300	1.250	1.380	1.370	1.342	1.378	1.455
Average BD		1.231	1.374	1.369	1.348	1.411	1.463
Source	**Den DF**	Prob > F					
rot	**2**	0.250	0.535	0.027	0.518	0.128	1.000
nrate	**8**	0.874	0.715	0.997	0.796	0.005	0.843
rot*nrate	**8**	0.855	0.935	0.592	0.082	0.005	0.402

Table 3.2 Soil organic carbon (SOC) concentration (g kg^{-1}) for 1998 and 2009 samples at different depths at Mead, NE. ANOVA for SOC concentration at given soil depth.

		1998				2009					
Rotation	**N rate**	**0-30 cm**	**30-60 cm**	**60-90 cm**	**90-120 cm**	**0-5 cm**	**5-15 cm**	**15-30 cm**	**30-60 cm**	**60-90 cm**	**90-120 cm**
	kg ha^{-1}		---g kg^{-1}---								
CC	**0**	17.34	12.22	7.16	5.11	21.26	18.41	15.94	11.67	7.10	5.08
	100	17.67	11.31	7.36	4.41	21.27	17.20	14.99	11.48	7.23	4.46
	300	18.73	13.31	8.11	5.06	22.45	18.02	16.63	11.55	7.01	4.47
CS	**0**	20.57	17.46	12.48	10.08	22.25	19.68	18.57	16.96	11.61	9.84
	100	18.52	15.75	11.26	6.47	21.66	17.02	16.72	15.14	10.87	6.35
	300	19.43	15.53	11.78	8.01	21.81	17.72	16.91	14.99	11.26	8.05
CC		17.91	12.28	7.54	4.86	21.66	17.88	15.85	11.56	7.11	4.67
CS		19.51	16.25	11.84	8.19	21.91	18.14	17.40	15.69	11.25	8.08
	0	18.96	14.84	9.82	7.59	21.75	19.05	17.26	14.32	9.35	7.46
	100	18.09	13.53	9.31	5.44	21.47	17.11	15.85	13.31	9.05	5.41
	300	19.08	14.42	9.94	6.54	22.13	17.87	16.77	13.27	9.13	6.26

Table 3.3 ANOVA for bulk density and soil organic carbon (SOC) concentration (g kg^{-1}) at different soil depths as affected by treatments and ANOVA for SOC stocks (kg m^{-2}), SOC rate of change over time at different soil masses and residue carbon (C) input as affected by treatments at Mead, NE (2009).

		Rot (R)	**Nitrogen (N)**	**R*N**
		-------------------------Prob > F-------------------------		
Bulk Density (Mg m^{-3})	0-5 cm	0.250	0.874	0.855
	5-15 cm	0.535	0.715	0.935
	15-30 cm	0.027	0.997	0.592
	30-60 cm	0.518	0.796	0.082
	60-90 cm	0.128	0.005	0.005
	90-120 cm	1.000	0.843	0.402
SOC (g kg^{-1})	0-5 cm	0.785	0.851	0.781
	5-15 cm	0.731	0.321	0.774
	15-30 cm	0.319	0.454	0.571
	30-60 cm	0.389	0.517	0.614
	60-90 cm	0.261	0.916	0.839
	90-120 cm	0.283	0.267	0.496
	0-30 cm	0.300	0.441	0.655
SOC (kg m^{-2})	0-50 kg m^{-2}	0.814	0.837	0.776
	50-200 kg m^{-2}	0.716	0.321	0.781
	200-400 kg m^{-2}	0.335	0.444	0.559
	400-800 kg m^{-2}	0.377	0.518	0.587
	800-1200 kg m^{-2}	0.264	0.890	0.691
	0-200 kg m^{-2}	0.320	0.493	0.638
SOC rate of change (kg m^{-2} yr^{-1})	0-400 kg m^{-2}	0.227	0.146	0.338
	400-800 kg m^{-2}	0.849	0.439	0.338
	800-1200 kg m^{-2}	0.859	0.597	0.408
	0-1200 kg m^{-2}	0.792	0.238	0.348
Residue C input (kg ha^{-1})		0.666	<0.001	0.001

Table 3.4 Average residue carbon (C) input, average residue nitrogen (N) input and average grain yield from 1997 to 2008 as a function of N fertilizer rates at Mead, NE.

Rotation[€]	**Crop**	**Grain Yield^**			**Residue C input**[‡]			**Residue N input**		
		0N*	**100N**	**300N**	**0N**	**100N**	**300N**	**0N**	**100N**	**300N**
		-------------------kg ha^{-1}-------------------								
CC	**Corn**	7379a	9902b	10339b	3328aA¶	4381b	4775cA	67a	95b	120c
CS		-	-	-	4039aB	4251a	4142aB	92a	104ab	106b
	Corn	12027a	12318b	12252b	5373a	5799a	5488a	122a	147b	148b
	Soybean	3641a	3638a	3764a	2706a	2703a	2797a	62a	62a	64a

‡ Residue = aboveground + root biomass. Corn stover and grain quantified annually. Assumptions= root/shoot ratio for Corn=0.16 and soybean=0.14; corn stover C content=43.7% and root C content=34.3%, corn root N content=0.9%.; soybean residue based on an average HI=0.43 and average C content of combined leaf, stem, pod and root C of 45%. Content of N in soybean stover (leaf, stem and pod) assumed = 1.1%, root N assumed = 0.75%

*Nitrogen fertilizer rate, 0, 100 and 300 kg of N ha^{-1} applied to corn

^Grain yield averaged from 1997-2008

€ CC, continuous corn; CS, corn-soybean rotation

¶Values within a row (N rate) followed by different lower case letter differ significantly among N treatments (P=0.05). Values of residue C input within a column (Rotation) followed by different upper case letter differ significantly between rotations (P=0.05).

Table 3.5 ANOVA for soil organic carbon (SOC) stocks (kg m^{-2}) and SOC concentration (g kg^{-1}) in different soil mass intervals as affected by treatments and time at Mead, NE (1998-2009).

		Rot (R)	Nitrogen (N)	R*N	time (t)	R*t	N*t	R*N*t
		---------------	---------------	---------------	Prob > F	---------------	---------------	---------------
SOC (kg m^{-2})	0-400 kg m^{-2}	0.288	0.453	0.487	0.013	0.219	0.140	0.333
	400-800 kg m^{-2}	0.366	0.509	0.494	0.254	0.851	0.442	0.340
	800-1200 kg m^{-2}	0.224	0.835	0.700	0.331	0.859	0.603	0.412
	0-800 kg m^{-2}	0.343	0.467	0.463	0.079	0.737	0.238	0.632
	0-1200 kg m^{-2}	0.292	0.539	0.522	0.142	0.793	0.241	0.348
SOC (g kg^{-1})	0-30 cm	0.284	0.469	0.456	0.016	0.922	0.409	0.292
	30-60 cm	0.381	0.506	0.483	0.181	0.889	0.341	0.304
	60-90 cm	0.227	0.847	0.728	0.275	0.889	0.680	0.553
	90-120 cm	0.277	0.283	0.524	0.603	0.858	0.835	0.550

Table 3.6 Soil organic carbon (SOC) stocks in different soil mass intervals (averaged over rotation and nitrogen rate) in 1998 and in 2009 at Mead, NE.

	SOC (± Standard error)			
	0-1200 kg of soil m^{-2}	**0-400 kg of soil m^{-2}**	**400-800 kg of soil m^{-2}**	**800-1200 kg of soil m^{-2}**
	---------------	--------------- kg m^{-2} ---------------		---------------
1998	16.85	7.46	5.57	3.81
2009	16.35	7.15*	5.47	3.72
Difference	-0.50 ±0.22	-0.31 ±0.03	-0.10 ±0.09	-0.09 ±0.11
	---------------	--------------- g m^{-2} yr^{-1} ---------------		---------------
Annual C rate of change	-45.5	-28.2	-8.8	-8.1

*Significant difference between years at α=0.05

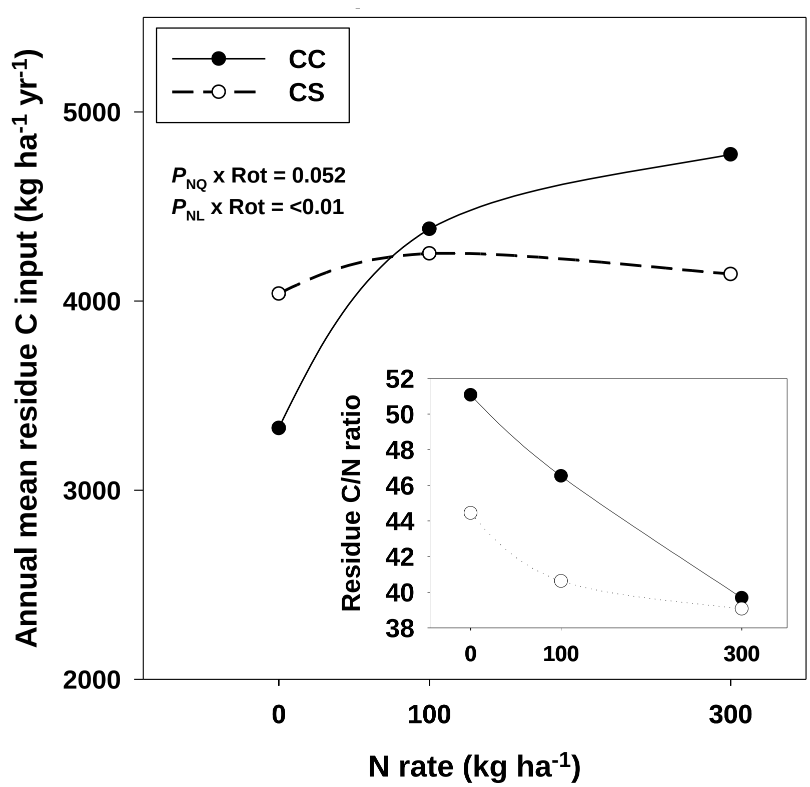

Figure 3.1 Interactive effect of N fertilization rate (0, 100 and 300 kg N ha^{-1}) and rotation (continuous corn (CC) and corn-soybean (CS) rotation on mean annual residue carbon (C) input between 1997 and 2008 at Mead, NE. Insert shows average C:N ratio of CC and CS residue (shoot + root) at increasing N rate application (P_{NL} = linear effect of N rate; P_{NQ} = quadratic effect of N rate; Rot = rotation effect).

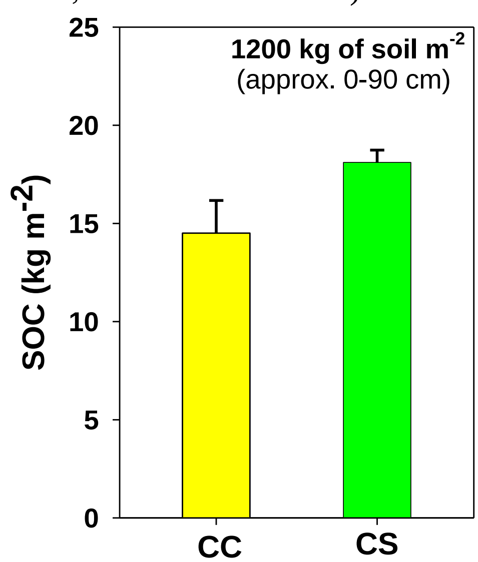

Figure 3.2 Soil organic carbon (SOC) stocks under continuous corn (CC) and corn-soybean (CS) rotation at 0-1200 kg of soil m^{-2} at Mead, NE (2009). Different letters mark significant difference at $p<0.05$.

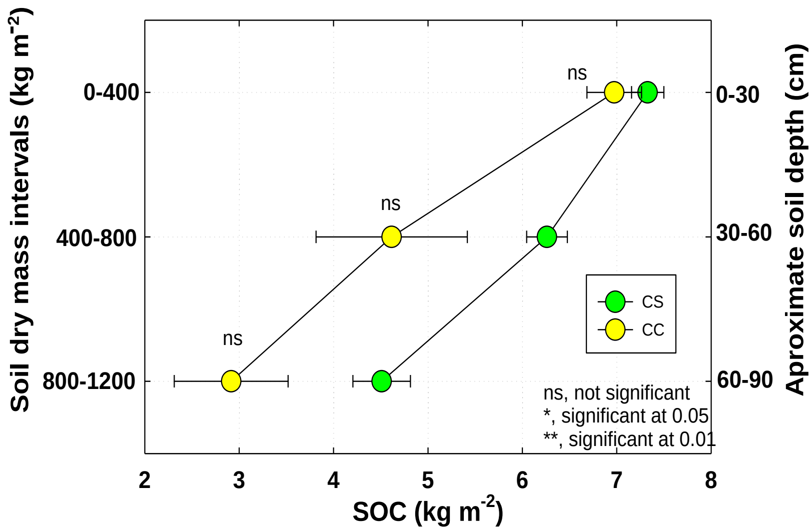

Figure 3.3 Soil organic carbon stocks in 2009 under continuous corn (CC) and corn-soybean (CS) rotation (averaged over nitrogen rates) at different soil mass intervals ± 1 standard error at Mead, NE.

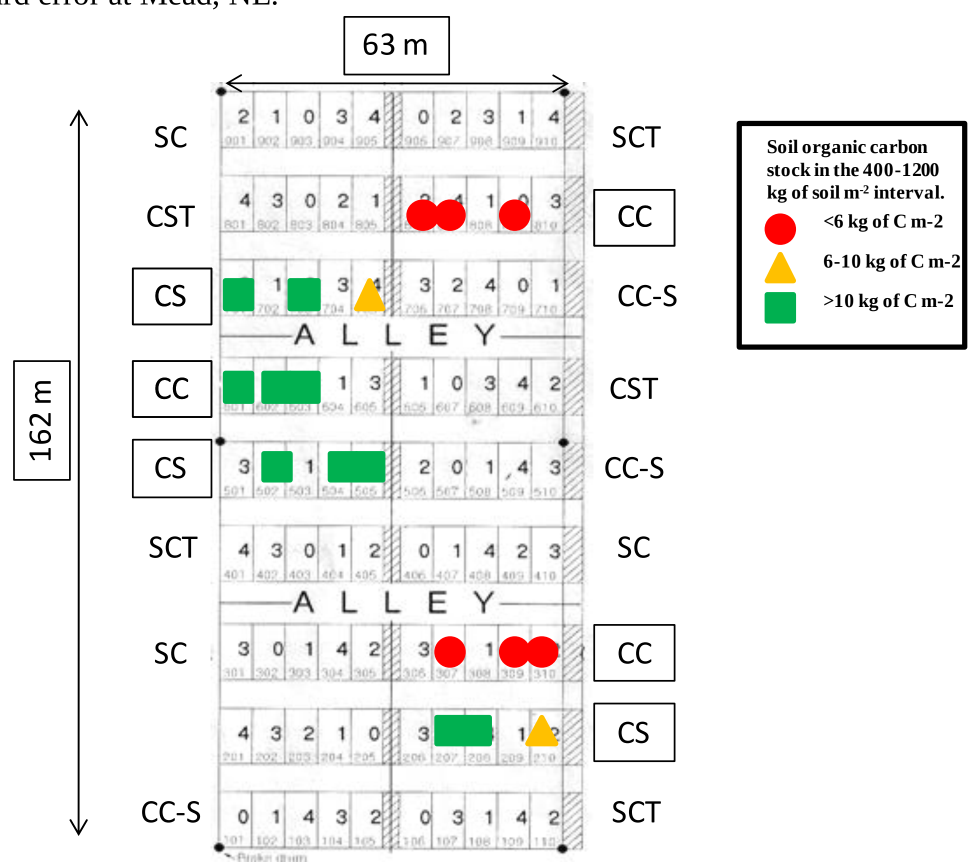

Figure 3.4 Complete experiment design (6 x 5 Split-plot with 3 replications) and distribution of sampled experimental units in the field under continuous corn (CC) and corn soybean (CS). Colored circles denote the quantity of soil organic carbon in the 400-1200 kg of soil m^{-2} interval (approx 30-90 cm).

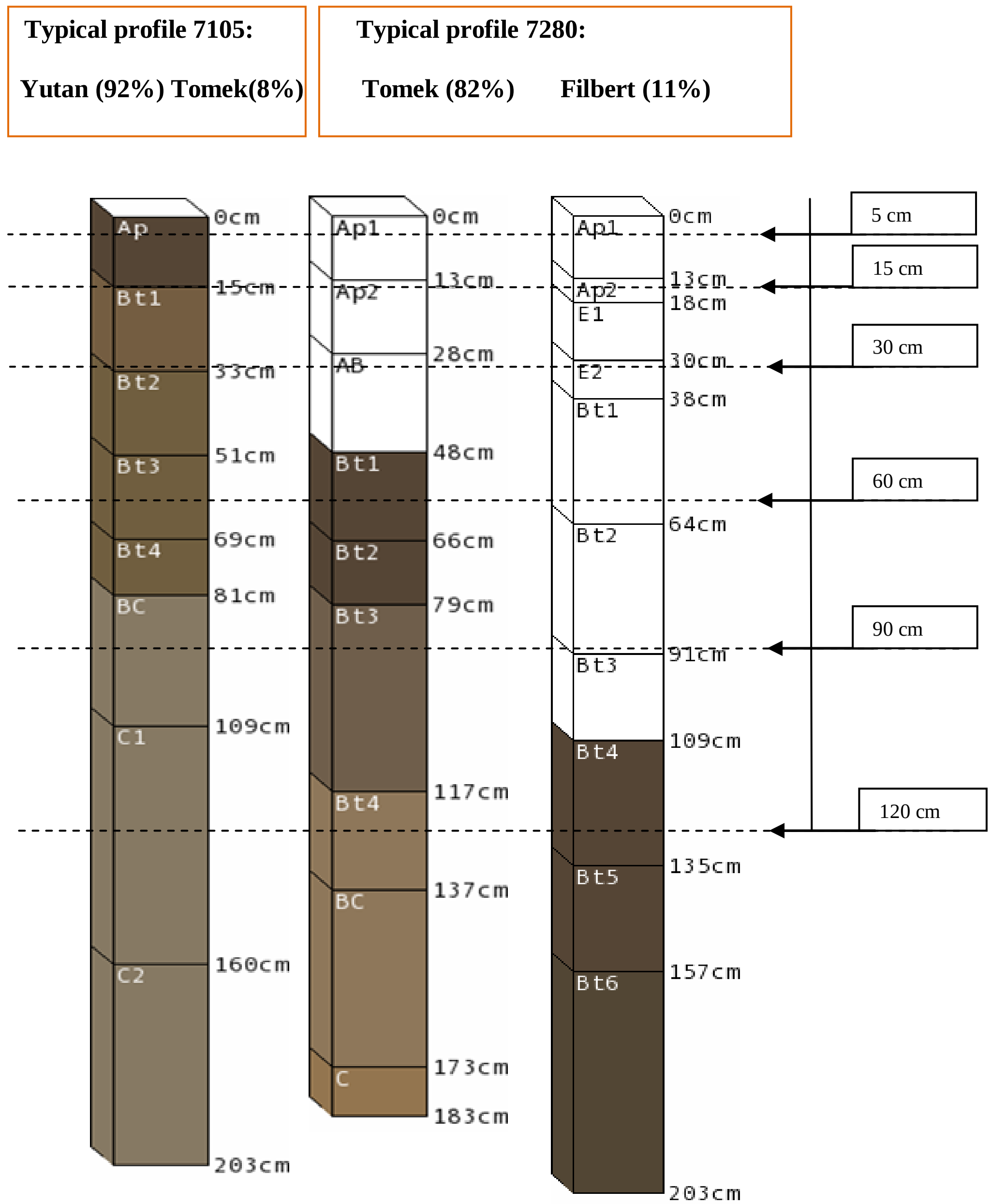

Figure 3.5 Typical profiles and soil series present in the study area (Natural Resource Conservation Service, NRCS-USD, 2010). Soil horizon types and depth distribution related to sampling depth.

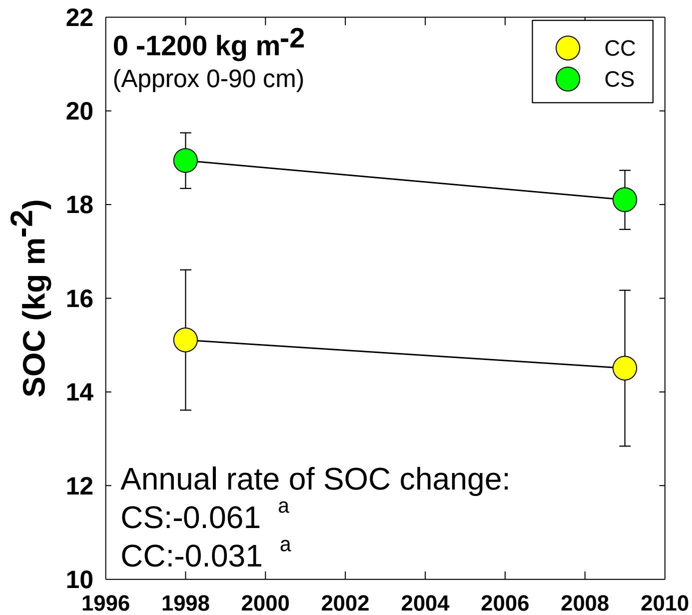

Figure 3.6 Soil organic carbon (SOC) levels and annual rates of SOC change between 1998 and 2009 in 1200kg m^{-2} (approx. 0-90cm) of soil dry mass ± 1 standard error at Mead, NE. Annual rates of SOC (kg m^{-2} yr^{-1}) between years are presented for continuous corn (CC) and Corn-soybean (CS) rotation. Annual rates lettered differently mark significant differences at $p<0.05$.

CHAPTER 4. Long-term study at Concord, NE

4.1 Results and Discussion

4.1.1 Bulk density and SOC variability.

Data on soil BD required to express SOC on a constant mass basis are presented in Table 4.1. At each depth interval BD was tested for significant effect by the different treatments (Table 4.2). Analysis of variance indicated that rotation and tillage had no effect on BD in the 0-30 cm. Samples were collected in spring before tillage allowing the longest time for soil to restore to its pre-tillage condition which could partially explain the lack of difference between tillage treatments. Previous studies (e.g. Angers et al., 1997, Blanco-Canqui and Lal, 2008) have similarly reported no effect on BD of tillage in the surface 30 cm. Higher N rates decreased BD in the 5-15 cm depth interval (Table 4.2) which we speculate may have been due to increased root biomass production in this depth interval.

In general, since BD was not severely affected by treatments in the surface 30 cm, SOC analyses on a concentration (g kg^{-1}) or constant mass basis (kg m^{-2}) yield roughly the same results (Table 4.2). The exceptions were a rotation by N fertilizer interaction in the 0-5 cm and a main N fertilizer effect in the 15-30 cm interval were significant when SOC concentration was analyzed but not significant after adjusting values to a constant mass basis (Table 4.2). The reason for this difference was that the increases in SOC concentration (Table 4.3) were compensated by a slight decrease in BD (Table 4.1).

Spatial variability confounded results for samples below 30 cm. In the 30-60, 60-90 and 90-120 cm depths, MP had consistently higher BD than either DK or NT.

Differences in BD were likely due to natural soil spatial variability since the increase in BD was also associated with a decrease in SOC content and plots with similar SOC stock in the 400-1200 kg of soil m^{-2} (approx. 30-90 cm) intervals were closely situated in the field. Overall, 80% of the plots under MP were situated in areas with low SOC stocks at depth while only 58% and 54% of the plots under DK and NT were situated in similar areas (Fig. 4.1). Differences in soil horizons between soil series present in the study site could explain this variability. Since the sampling was conducted at constant depth increments, we could be comparing contrasting soil horizons with inherently different SOC depending on the soil series considered. For example, in the 30-60 cm interval we are comparing SOC stocks in a A1 horizon, from a Coleridge soil against a Bw horizon from a Baltic soil which would be expected to have different SOC stocks (Fig. 4.2). Therefore, since the assumption of equivalent starting SOC stocks under every treatment may not be valid for subsurface samples (>30 cm), only changes in SOC levels over the last 12 years of the study using archived samples as a benchmark will be discussed below the 400 kg of soil m^{-2} (approx. 30 cm).

4.1.2 Grain yield and Residue C input

Average corn grain yield between 1985 and 2008 for CC increased with increasing N applications while yields were not affected by tillage system (Table 4.4). Corn yields increased from 4.4 to 5.9 Mg ha^{-1} yr^{-1} in the CC system and from 5.7 to 6.5 Mg ha^{-1} yr^{-1} in the CS system with increasing N application (averaged over tillage systems). Soybean yields were relatively constant with varying N rates showing little to no response of soybean yields to residual N applied from previous corn crop.

Estimates of residue C produced by each rotation were obtained based on soybean yields and measured above-ground biomass in corn. Analysis of variances for the average residue C input (Table 4.2) showed that the amount of C returned by CC was overall 7% higher under NT than under DK or MP while tillage had no effect on C input in the CS rotation. The mean amount of C input to the soil (stover + roots) by each rotation from 1985 to 2009 averaged over tillage systems is presented in Fig. 4.3. Residue C input from CC increased 30% from 0N to 160N (Fig. 4.3) but since increased C input from corn in response to higher N rates in the CS rotation was relatively low (6% under DK and 13% under NT and MP between 0N and 160N) and no increase was observed by soybeans (Tables 4.4), when the CS system was considered, averaged C input was relatively constant with increasing N rates (Fig. 4.3). The amount of residue C returned to the soil was equivalent for both rotations (average between soybean and corn years for the CS rotation) at 0N, but CC returned 23% more C than CS with 160N.

4.1.3 Effect of rotation on SOC

When considering the surface soil layers, results show that rotation had no impact on SOC at the 0-50, 50-200 or 200-400 kg of soil m^{-2} intervals (Table 4.2) despite the differences in estimated residue C inputs. These results are in agreement with the results from Gal et al., (2007) who also observed 30% more C residue returned under the CC system, but no difference in SOC. Similarly, Poirier et al. (2009) found no differences in SOC stock in the 60 cm profile between corn receiving 0 kg N ha^{-1} and 160 kg N ha^{-1}, even when C input where 60% higher in the fertilized treatment. Moreover, Varvel and Wilhelm (2008) in a study in Nebraska under irrigation observed similar trends of C input to the ones in our study between CC and CS in a relatively equivalent range of N fertilizer application, but reported no difference in SOC between CC and CS.

When comparing SOC stocks to archived samples in order to analyze change over time, rotation did not impact SOC trends in the surface 400 kg of soil m^{-2} over the last 12 years. Results show that after 12 years no differences were observed between CC and CS. This is reasonable since we did not observe differences in the spatial comparison which evaluated the impact of rotation after conducting the experiment for 24 years (assuming an equivalent SOC starting level).

4.1.4 The effect of tillage on the SOC.

The distribution of SOC in the soil profile under each tillage treatment is shown in Fig 4.4. In the surface 50 kg of soil m^{-2} (approx 0-5 cm) SOC stocks were higher under NT and decrease as tillage intensity increases. Since residue is incorporated into the soil profile with tillage, slightly less SOC was left at surface under DK, but significantly less under MP. The increase in SOC in the surface layer under NT is attributed to the accumulation of crop residue and the lack of mechanical disturbance which generates the conditions for a higher level of aggregation and therefore, physical protection of the SOC (Six et al., 2000).

It has been widely documented that tillage redistributes SOC in the surface soil layer while under NT SOC is accumulated in the surface (e.g. Angers et al., 1997, Gal et al., 2007, Blanco-Canqui and Lal, 2008, Christopher et al., 2009). Results in this study show that there was a redistribution of SOC in the first 400 kg of soil m^{-2} (approx. 30 cm) profile under DK as compared to NT. While under NT SOC stocks were greater (+10%) in the top 50 kg m^{-2} than under DK, they were slightly lower in the 50-200 kg m^{-2} (-5.2%)

and 200-400 kg m^{-2} (-3.7%) soil intervals (Fig. 4.4). But SOC stocks under MP were consistently lower in 0-50, 50-200 and 200-400 kg of soil m^{-2} intervals than either under DK or NT (Fig. 4.4). When the 0-400 kg of soil m^{-2} interval is considered, no differences in SOC stocks were observed between NT and DK, but 13% less SOC was observed under MP (Fig. 4.5). Soil disturbance generated by MP in the surface 400 kg of soil m^{-2} could have increased the rate of SOC loss relative to DK or NT. Conventional tillage is known to disrupt aggregates, thus reducing the physical protection (Six et al., 2004), and exposing previously inaccessible SOC to microbial degradation (Stevenson, 1994).

When compared to archived soil samples, 12 years of treatment showed a decrease in SOC stocks under all of the tillage treatments in first 400 kg of soil m^{-2} (Table 4.7 and Fig 4.6). The implementation of NT did not result in C sequestration but rather a decrease in the rate of SOC loss compared to MP. Although the depletion of SOC from the 0-400 kg of soil m^{-2} interval was not statistically different among treatments (Table 4.7), the rate of change was clearly greater in MP (Table 4.8). This suggests that12 years were not enough to generate measurable changes in SOC between NT, DK and MP. Results are in accordance with several studies (e.g. Ellert and Bettany, 1995, VandenBygaart and Angers, 2006, Baker et al., 2007) which affirm that given the great SOC background in the whole soil profile, and the small annual changes, long-term studies are vital in order to determine differences in the effect of management practices.

In the soil layer immediately below the plow layer (400-800 kg m^{-2}), when SOC stocks were evaluated over time it was evident that in the period between soil samplings (1997-2009), the SOC stocks had decreased considerably under MP while remaining practically unchanged under DK or NT (Fig. 4.6). There was no difference between SOC stocks in 1997 and 2009 under NT and DK (Table 4.7). The annual rate of SOC loss in the 400-800 kg of soil m^{-2} interval under MP was -42.4 g C m^{-2} yr^{-1} while under NT and DK the rate of SOC change was +1.5 g C m^{-2} yr^{-1} and -6.9 g C m^{-2} yr^{-1}, respectively (Table 4.8). Given the error associated to the estimations, SOC stocks under NT and DK were considered unaffected my tillage at this soil mass interval. The depth of soil disturbance is greater in MP (25 cm) than in DK (15 cm) altering soil to a greater depth. Therefore, the increased soil disturbance with MP could have produced a sudden increase in soil aeration (as well as changes in soil temperature and moisture) at greater depths compared to NT or less invasive tillage as DK. Exposing SOM at depth to more oxidative environments would speed decomposition (Stevenson, 1994; Halvorson et al., 2002), and could be the cause of SOC depletion at the 400-800 kg of soil m^{-2} interval under MP. As expected, in the 800-1200 kg of soil m^{-2} interval (approx. 60-90 cm), SOC was unaffected by management practices and remained invariable under all of the evaluated treatments (Fig. 4.6).

Finally, when considering soil C changes in the whole 1200 kg of soil m^{-2} (approx. 90 cm depth) there were no differences among tillage treatments in 1997 but were close to becoming significant ($P < 0.052$) in 2009. Soil C stocks decreased by 0.74, 0.76 and 1.52 kg C m^{-2} under NT, DK and MP over the last 12 years of the experiment (Table 4.8). Assuming a constant rate of change of the SOC stocks over the last 12 years, MP doubled the rate of SOC change under NT or DK being -126.2, 61.2 and 63.3 g C m^{-2} yr^{-1} (Fig. 4.7). Despite these observed differences between treatments, when C changes for each treatment were analyzed over time, the differences were not statistically significant (Table 4.7 and 4.8).

Greater SOC stocks under NT and DK as compared to MP were observed in the surface 400 kg of soil m^{-2} after 24 years. Temporal comparisons using archive samples showed that although not declared significant, more SOC was being lost under MP than under either NT or DK. This suggest that probably more than 12 years are required, given the conditions of this experiment, to detect differences among the evaluated tillage systems in the surface 400 kg of soil m^{-2} (approx. 30 cm). In the 400-800 kg of soil m^{-2}, SOC stocks were observed to decrease under MP after only 12 years while remaining invariable under NT and DK. Tillage did not impact SOC stocks in the 800-12000 kg of soil m^{-2} interval. Having archived soil samples allowed the determination of the true rate of change. By comparing current soil samples to archived soil samples it was possible to determine that although more carbon was found for NT and DK than for MP, all of the tillage treatments had lost SOC over time. Archived samples also aided in accounting for the soils natural variability, especially when samples exceeded the surface soil interval (>400 kg of soil m^{-2}).

4.1.5 Effect of N fertilizer application rate.

When considering the surface 50 kg of soil m^{-2} (approx. 0-5 cm), the increase in SOC stocks with increasing N application over all of the tillage systems (Fig. 4.8) does not seem to be related to the amount of returned residue. Even though the C input under both rotations was relatively constant between 80N and 160N, SOC stocks continued to increase up to 160N. Given that increased C inputs can only build SOM if there is available N for humification processes (Dobermann et al., 2005; Moran et al., 2005), results suggest that SOC levels in the studied system, might have been limited more by N than by C inputs. Similarly, Jogadamma et al. (2007) observed no increase in aboveground residue yield with N rates higher that 150 kg ha^{-1}, but after adjusting their values to a constant 1200 Mg of soil ha^{-1} (approx. 10 cm), SOC stocks showed a constant increase up to 300 kg ha^{-1}. Moreover, Varvel and Wilhelm (2008) in an irrigated study in Nebraska, observed little to no increase in aboveground residue yield from CC and CS with N rates greater than 100 kg ha^{-1}, but reported that SOC increased up to 200 kg of N ha^{-1}.

Interaction between tillage treatment and N application was observed in the 50-200 kg of soil m^{-2} interval (Table 4.2). Increasing N fertilizer rates under DK and MP caused an increase in SOC stocks, but had a contrary effect under NT. Under N, SOC stocks decreased with higher N rates (Fig. 4.8). Possibly, the decomposition of residue by microbes and transformation to stable SOM under low N rates could have been limited by N availability. Moran et al. (2005) found that the addition of N accelerated residue decomposition transforming C residue into stable SOM. In our study, the presence of C from incorporated residue and N from fertilizer (also incorporated) under DK and MP could have led to a faster decomposition of the residue and stabilization into SOM in the 50-200 kg of soil m^{-2} interval under higher N rates. This is further supported by Balesdent et al. (2000) who established that rapid incorporation of organic C within aggregates was observed under tillage, and was favored by intimate contact between crop residue and the soil. Lower SOM content under MP than under DK was likely related to the increased disturbance of soil with MP and the fact that residue is mostly clustered at the plow depth under MP (Staricka et al., 1991) and not equally mixed with soil. Most of the residue under NT remains in the surface, and part of N applied on the surface will leach to deeper soil layers. When N reaches the 50-200kg of soil m^{-2} interval (approx. 5-15 cm depth) the presence of easily available N could have stimulated the mineralization of SOM. In the absence of residue C, microbes could have used SOM as a C source, therefore reducing the levels of SOC under NT.

When the 0-400 kg of soil m^{-2} interval (approx. 0-30 cm) was considered, the previously discussed interaction was not evident (Table 4.2). Nitrogen fertilizer application had a positive effect on the SOC level under all of the tillage systems (Fig. 4.5) resulting in a 3% increase in SOC stocks (averaged over tillage systems) between 0N and 160N after 24 years. When a temporal comparison was conducted using 1997 samples, N fertilizer had no impact on SOC change over the last 12 years in the 0-400, 400-800 or 800-1200 kg of soil m^{-2} soil horizons. Since the effect of N fertilization was only 3% after 24 years it is not surprising that no differences were detected after only 12 years of treatment.

Although interesting, increasing N application rates explained only 2.5% of the variability in the data while tillage explains almost 39% of the variability in the surface 400 kg of soil m^{-2}. The difference in the magnitude of the impact of the different management practices and the time required for these effects to be observed, seem to support the idea that the greater changes produced by tillage mask the effect of N application or crop rotation as suggested by Reicosky et al. (1995). Moreover, given the lack of initial values for SOC in the surface 0-5, 50-200 and 200-400 kg m^{-2} intervals, results should be cautiously analyzing and interpreted to avoid erroneous conclusions.

4.2 Conclusions

Results from 24-years experiment showed no difference in SOC stocks under either CC or CS despite up to 23% greater amount of C returned to the soil by the CC rotation. After 24 years, increasing SOC stocks in the surface 400 kg of soil m^{-2} with increasing N application under all of the tillage systems suggest that N was a stronger limitation to SOC sequestration than C input in this study. Interactions between N rate and tillage systems in the sub-surface soil interval (50-200 kg of soil m^{-2}) were not evident when the 0-400 kg of soil m^{-2} interval was considered or when changes were considered over time. More than 12 yrs were required to observe a positive effect of N fertilizer application on SOC stocks in the surface 400 kg of soil m^{-2} (approx. 30 cm).

Tillage affected not only distribution of SOC in the profile but also SOC stocks after 24 years. Higher SOC stocks in the surface soil intervals (50kg of soil m^{-2}) under NT were compensated for by greater SOC stocks at deeper soil intervals (200-400 kg of soil m^{-2}) under DK, but MP had consistently less SOC in the surface 400 kg of soil m^{-2}. Changes in SOC over the last 12 years were not significant between tillage systems in the surface 400 kg of soil m^{-2}, although the rates of SOC loss were noticeably greater for MP. After 24 years soils under DK and NT contained 13% more SOC than under MP in the surface 400 kg of soil m^{-2} (approx. 30 cm). Moreover, MP was the only treatment that produced SOC losses in the 400-800 kg of soil m^{-2} interval.

Finally, results from this study further emphasize the importance of having archived samples when determining the effect of management practices on SOC, especially in sites with great soil variability or at depth where the impact of management practices is less pronounced. With the aid of archived samples it was possible to determine differences between soils natural variability and the impact of management practices at depth greater than 30 cm. Future studies should focus on carefully determining soil variability prior to the establishment of the experiment in order to increase their capacity of detecting management effects on SOC stocks in the future. But most importantly, archived samples allowed us to determine that under the soil and climatic conditions of this experiment none of the tillage treatments, rotations, and N rates evaluated were able to increase SOC stocks. In fact, SOC was lost under every treatment combinations evaluated over time.

Table 4.1 Bulk density (BD) values and ANOVA of BD at different soil depth intervals at Concord, NE in 2009.

Tillage System	Rotation	N rate	2009 0-5 cm	5-15 cm	15-30 cm	30-60 cm	60-90 cm	90-120 cm
		kg ha^{-1}	---Mg m^{-3}---					
NT			1.10	1.36	1.27	1.27	1.33	1.41
DK			1.13	1.35	1.28	1.29	1.37	1.46
MP			1.18	1.33	1.29	1.36	1.45	1.58
	CC		1.15	1.34	1.29	1.32	1.39	1.50
	CS		1.13	1.35	1.27	1.30	1.37	1.47
		0	1.17	1.36	1.29	1.31	1.37	1.48
		80	1.15	1.35	1.28	1.31	1.39	1.48
		160	1.10	1.32	1.27	1.30	1.38	1.49
Averaged over treatments			1.14	1.35	1.28	1.31	1.38	1.48
		Den DF	---Prob > F---					
till		**6**	0.166	0.273	0.477	0.025	0.026	0.029
rot		**9**	0.551	0.330	0.285	0.340	0.689	0.394
till*rot		**9**	0.066	0.500	0.919	0.741	0.373	0.472
nrate		**36**	0.085	0.033	0.553	0.580	0.326	0.981
till*nrate		**36**	0.265	0.597	0.239	0.945	0.479	0.844
rot*nrate		**36**	0.091	0.977	0.660	0.439	0.110	0.162
till*rot*nrate		**36**	0.311	0.310	0.693	0.513	0.051	0.869

Table 4.2 ANOVA for bulk density (BD) and soil organic carbon (SOC) concentration (g kg^{-1}) at different soil depths as affected by treatments and ANOVA for SOC stocks (kg m^{-2}) , SOC rate of change over time at different soil messes as affected by treatments and average residue carbon (C) input at Concord, NE (2009).

		till (T)	rot (R)	t*r	nitrogen (N)	T*N	R*N	T*R*N
		------------------------------------Prob > F------------------------------						
SOC (g kg^{-1})	0-5 cm	0.005	0.881	0.252	<0.001	0.386	0.024	0.739
	5-15 cm	0.208	0.614	0.537	0.306	0.006	0.872	0.455
	15-30 cm	0.022	0.097	0.357	0.040	0.533	0.713	0.116
	30-60 cm	0.086	0.121	0.494	0.637	0.568	0.415	0.274
	60-90 cm	0.126	0.371	0.546	0.929	0.351	0.430	0.333
	90-120 cm	0.137	0.521	0.583	0.790	0.456	0.567	0.562
	0-30 cm	0.009	0.137	0.350	0.003	0.098	0.393	0.303
SOC (kg m^{-2})	0-50 kg m^{-2}	0.006	0.910	0.199	<0.001	0.347	0.062	0.723
	50-200 kg m^{-2}	0.167	0.528	0.508	0.257	0.006	0.813	0.488
	200-400 kg m^{-2}	0.022	0.111	0.510	0.208	0.767	0.818	0.192
	400-800 kg m^{-2}	0.095	0.124	0.507	0.675	0.482	0.318	0.349
	800-1200 kg m^{-2}	0.138	0.344	0.605	0.980	0.357	0.473	0.389
	0-400 kg m^{-2}	0.006	0.129	0.478	0.035	0.229	0.597	0.398
SOC rate of change (kg m^{-2} yr^{-1})	0-400 kg m^{-2}	0.128	0.722	0.152	0.488	0.161	0.456	0.784
	400-800 kg m^{-2}	0.007	0.764	0.073	0.451	0.707	0.249	0.270
	800-1200 kg m^{-2}	0.885	0.876	0.753	0.655	0.518	0.236	0.213
	0-800 kg m^{-2}	0.020	0.985	0.278	0.749	0.628	0.212	0.488
	0-1200 kg m^{-2}	0.076	0.915	0.293	0.728	0.604	0.161	0.357
Residue C input (kg ha^{-1})		0.112	<0.001	<0.001	<0.001	0.869	<0.001	0.549

Table 4.3 Soil organic carbon (SOC) concentration (g kg^{-1}) at different soil depths in 1997 and 2009 at Concord, NE**.**

			1997				2009						
Tillage System	**Rotation**	**N rate**	**0-30 cm**	**30-60 cm**	**60-90 cm**	**90-120 cm**	**0-5 cm**	**5-15 cm**	**15-30 cm**	**0-30 cm**	**30-60 cm**	**60-90 cm**	**90-120 cm**
		kg ha^{-1}	----------	----------	----------	----------	----------	----------	g kg^{-1}	----------	----------	----------	----------
NT	**CC**	**0**	24.77	13.87	13.92	9.97	26.81	22.39	21.85	22.86	20.13	13.92	10.30
		80	24.35	13.58	13.63	10.02	28.50	21.72	20.64	22.31	19.16	13.63	10.00
		160	24.21	12.33	13.13	9.76	29.73	20.76	21.70	22.73	19.85	13.13	9.47
	CS	**0**	24.67	12.52	12.66	8.16	28.68	22.27	21.22	22.81	19.85	12.66	8.77
		80	25.46	13.52	12.09	9.45	27.94	22.18	21.47	22.78	19.68	12.09	10.03
		160	24.72	13.30	13.39	9.13	29.34	21.85	21.69	23.02	19.90	13.39	9.99
DK	**CC**	**0**	24.28	10.81	10.76	8.08	24.13	22.45	20.76	21.89	16.19	10.76	8.54
		80	24.24	10.86	10.77	7.98	26.57	23.58	21.40	22.99	16.02	10.77	7.97
		160	25.27	11.31	11.04	8.33	28.16	24.21	23.30	24.41	16.75	11.04	8.79
	CS	**0**	25.93	14.04	14.71	9.81	24.27	22.63	22.32	22.75	21.34	14.71	9.65
		80	25.56	10.79	10.55	6.97	25.50	22.67	22.83	23.23	18.15	10.55	7.34
		160	25.13	13.40	12.64	9.06	25.48	23.75	23.38	23.85	19.92	12.64	8.37
MP	**CC**	**0**	22.73	6.19	6.02	3.92	19.93	20.42	17.77	19.01	10.91	6.02	3.25
		80	22.19	6.39	6.63	2.92	20.81	20.92	19.56	20.22	12.10	6.63	3.38
		160	23.92	5.78	4.77	2.52	21.61	21.46	18.96	20.24	9.86	4.77	2.71
	CS	**0**	23.51	7.95	7.28	3.73	21.18	21.15	21.41	21.29	14.96	7.28	3.92
		80	23.15	11.35	10.60	9.69	21.50	21.46	20.09	20.78	15.53	10.60	9.27
		160	24.50	10.56	11.07	5.51	21.71	21.62	21.94	21.80	17.63	11.07	7.73
NT			24.70	13.19	13.14	9.42	28.50	21.86	21.43	22.75	19.76	13.14	9.76
DK			25.07	11.87	11.75	8.37	25.69	23.22	22.33	23.19	18.06	11.75	8.44
MP			23.33	8.04	7.73	4.71	21.12	21.17	19.95	20.56	13.50	7.73	5.04
	CC		24.00	10.12	10.08	7.05	25.14	21.99	20.66	21.85	15.66	10.08	7.16
	CS		24.74	11.94	11.67	7.95	25.07	22.18	21.82	22.48	18.55	11.67	8.34
		0	24.31	10.90	10.89	7.28	24.17	21.89	20.89	21.77	17.23	10.89	7.41
		80	24.16	11.08	10.71	7.84	25.14	22.09	21.00	22.05	16.77	10.71	8.00
		160	24.62	11.11	11.01	7.39	26.01	22.28	21.83	22.67	17.32	11.01	7.84

Table 4.4 Average residue carbon (C) input, average residue nitrogen (N) input and average grain yield from 1985 to 2008 as a function of N fertilizer rates, under different tillage systems at Concord.

Tillage[ρ]	Rotation[€]	Crop	Grain Yield^			Residue C input[‡]			Residue N input		
			0N*	80N	160N	0N	80N	160N	0N	80N	160N
			---kg ha^{-1}---								
MP	**CC**	**corn**	4489a	5504b	5565b	2452a¶	3021bA	3106bA	41a	60b	70c
	CS		-	-	-	2439a	2755bB	2702bB	47a	58b	61c
		corn	5733a	6592b	6478b	2897a	3408b	3334b	49a	68b	75c
		soybean	2665a	2830b	2785ab	1980a	2102a	2069a	45a	48a	47a
DK	**CC**	**corn**	4421a	5645b	5821b	2415a	2982bA	3119bA	41a	55b	65c
	CS		-	-	-	2464a	2535aB	2623aB	48a	52b	58c
		corn	6029a	6198ab	6515b	2970a	3138a	3239a	51a	60b	70c
		soybean	2635a	2599a	2701a	1958a	1931a	2007a	45a	44a	46a
NT	**CC**	**corn**	4510a	5553b	5944b	2577aA	3177bA	3383cA	44a	60b	74c
	CS		-	-	-	2371aB	2570bB	2570bB	46a	53b	56c
		corn	5763a	6217b	6408b	2857a	3215b	3197b	49a	62b	68c
		soybean	2537a	2591a	2615a	1885a	1925a	1943a	43a	44a	45a

‡ Residue = stover + root. Corn stover and grain quantified annually. Assumptions= root/shoot ratio for Corn=0.16 and soybean=0.14; corn stover C content=43.7% and root C content=34.3%, corn root N content=0.9%.; soybean residue based on an average HI=0.43 and average C content of combined leaf, stalk pod and root C of 45%. Content of N in soybean stover (leaf, stem and pod) assumed = 1.1%, root N assumed = 0.75%

*Nitrogen fertilizer rate, 0, 80 and 160 kg of N ha-1 applied to corn

^Grain yield averaged from 1985-2008

€ CC, continuous corn; CS, corn-soybean rotation

ρ MP, moldboard plow; DK, disk tillage; NT, no-till

¶Values within a row (N rate) followed by different lower case letter differ significantly among N treatments (P=0.05). Values of residue C input within tillage system and column (Rotation) followed by different upper case letter differ significantly between rotations (P=0.05).

Table 4.5 ANOVA for soil organic carbon (SOC) content in different soil masses and SOC concentration at different soil depths as affected by treatments and time at Concord, NE (1997-2009).

		till (T)	rot (R)	t*r	nitrogen (N)	T*N	R*N	T*R*N	time (t)	T*t	R*t	T*R*t	N*t	T*N*t	R*N*t	T*R*N*t
		--Prob > F--														
SOC (kg m^{-2})	**0-400kg m^{-2}**	**0.018**	0.132	0.709	0.144	0.662	0.807	0.602	**0.008**	0.128	0.722	0.153	0.488	0.161	0.456	0.784
	400-800kg m^{-2}	0.154	0.131	0.590	0.903	0.527	0.610	0.484	0.204	**0.007**	0.762	0.071	0.446	0.708	0.244	0.267
	800-1200kg m^{-2}	0.163	0.338	0.624	0.961	0.387	0.630	0.469	0.929	0.881	0.875	0.755	0.653	0.522	0.242	0.207
	0-1200kg m^{-2}	0.090	0.183	0.596	0.855	0.476	0.742	0.475	0.060	0.076	0.915	0.293	0.727	0.603	0.160	0.357
SOC (g kg^{-1})	**0-30cm**	**0.038**	0.116	0.733	0.115	0.515	0.678	0.613	**0.003**	**0.033**	0.691	0.141	0.260	0.091	0.384	0.919
	30-60cm	0.145	0.119	0.588	0.856	0.601	0.731	0.419	0.126	**0.011**	0.737	0.096	0.592	0.690	0.346	0.455
	60-90cm	0.151	0.325	0.577	0.969	0.398	0.550	0.431	0.688	0.765	0.407	0.444	0.623	0.452	0.347	0.405
	90-120cm	0.159	0.565	0.603	0.784	0.389	0.539	0.371	0.342	0.752	0.347	0.242	0.752	0.555	0.782	0.454

Table 4.6 Soil organic carbon (SOC) stocks and annual rate of change in multiple soil mass intervals (averaged over rotation and nitrogen rate) in 1997 and in 2009 at Concord.

	Soil Organic Carbon (± Standard error)											
	0-1200kg of soil m^{-2} (approx. 0-90 cm)			0-400 kg of soil m^{-2} (approx. 0-30 cm)			400-800 kg of soil m^{-2} (approx. 30-60 cm)			800-1200 kg of soil m^{-2} (approx. 60-90 cm)		
	No-till	**Disk**	**Plow**	**No-till**	**Disk**	**Plow**	**No-till**	**Disk**	**Plow**	**No-till**	**Disk**	**Plow**
	---kg m^{-2}---											
1997	22.44	21.55	18.07	9.77	9.87	9.11	7.62	7.11	5.82	5.04	4.57	3.14
2009	21.70	20.79	16.55	8.98*	9.18*	8.12*	7.64	7.03	5.31*	5.08	4.58	3.12
difference	-0.73 ±0.4	-0.76 ±0.4	-1.52 ±0.4	-0.79 ±0.2	-0.69 ±0.2	-0.99 ±0.2	0.02 ±0.2	-0.08 ±0.2	-0.51 ±0.2	0.04 ±0.1	0.01 ±0.1	-0.02 ±0.1
	---g of C m^{-2} yr^{-1}---											
Annual SOC change rate	-61.7	-63.3	-126.7	-66.2	-57.4	-82.1	1.5	-7.0	-42.5	1.7	0.6	-1.8

*Significant difference between years at α=0.05

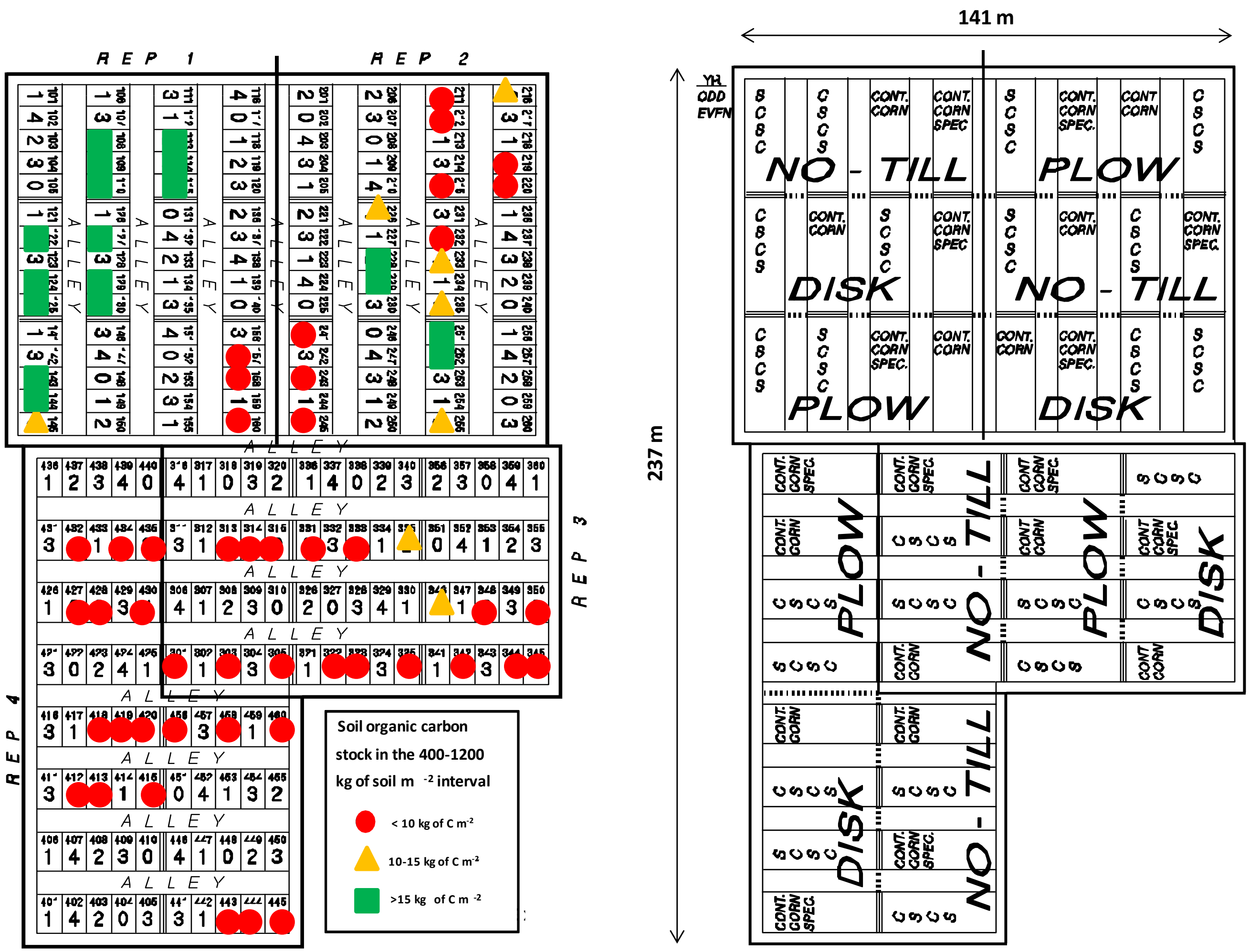

Figure 4.1 Complete experiment design (3 x 4 x 5 Split-split-plot with 4 repetitions) and distribution of sampled experimental units in the field under No-till, Disk and Plow. Colored circles denote the quantity of soil organic carbon in the 400-1200 kg of soil m^{-2} interval (approx 30-90 cm).

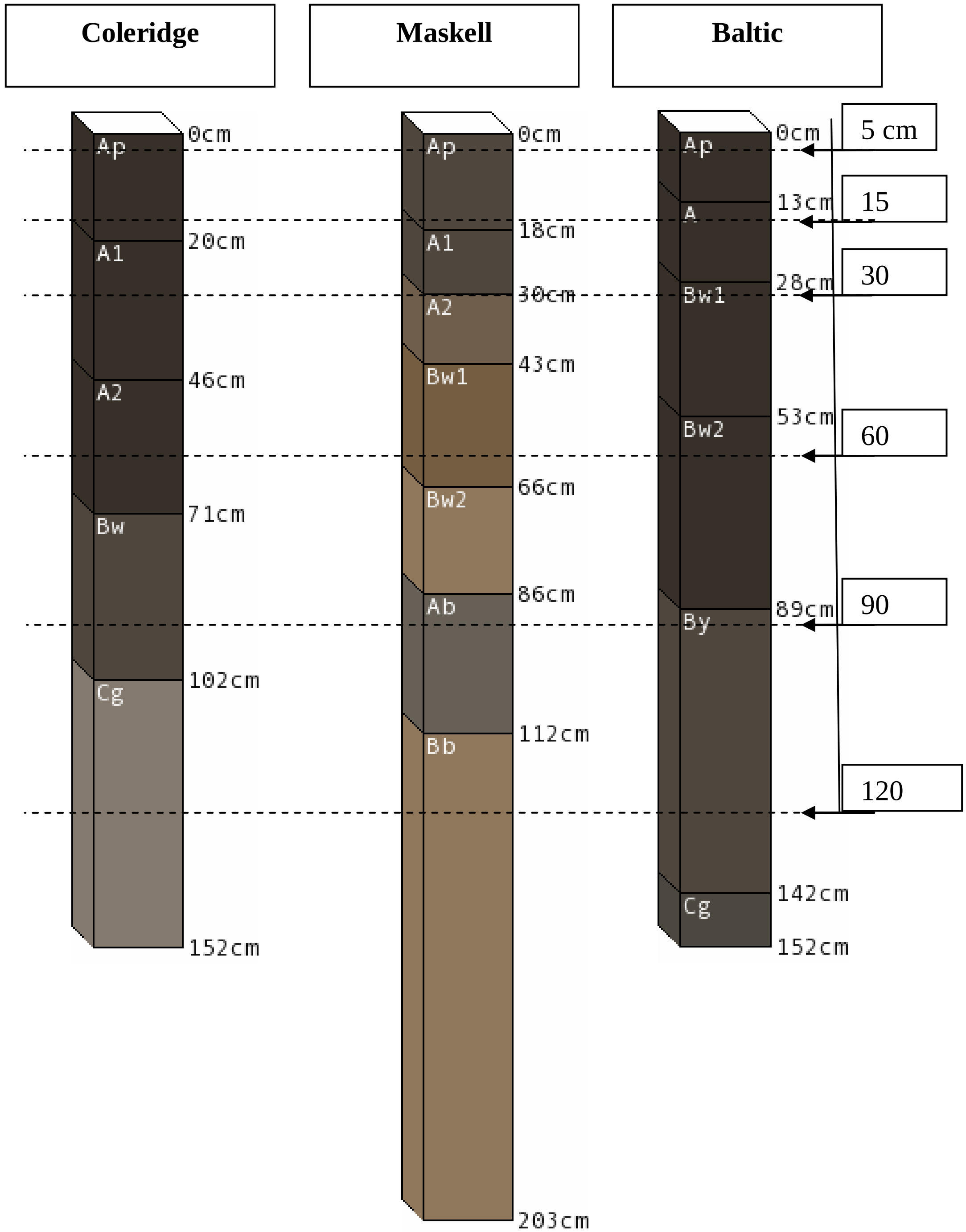

Figure 4.2 Typical profiles and mayor soil series present in the study area (Natural Resource Conservation Service, NRCS, 2010). Soil horizon types and depth distribution related to sampling depth.

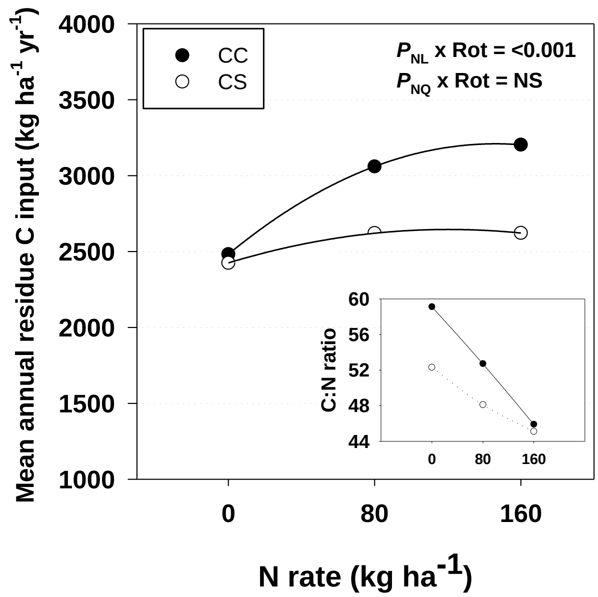

Figure 4.3 Interactive effect of mean annual residue carbon input between 1985 and 2008 in Concord averaged over tillage for each rotation (continuous corn, CC; corn-soybean, CS) and N rate application (0, 80, 160 kg N ha^{-1}). Insert shows C:N ratio for CS and CC residue (stover + root) at increasing N rate application (NS = not significant; P_{NL} = linear effect of N rate; P_{NQ} = quadratic effect of N rate; Rot = rotation effect). N applied to corn only.

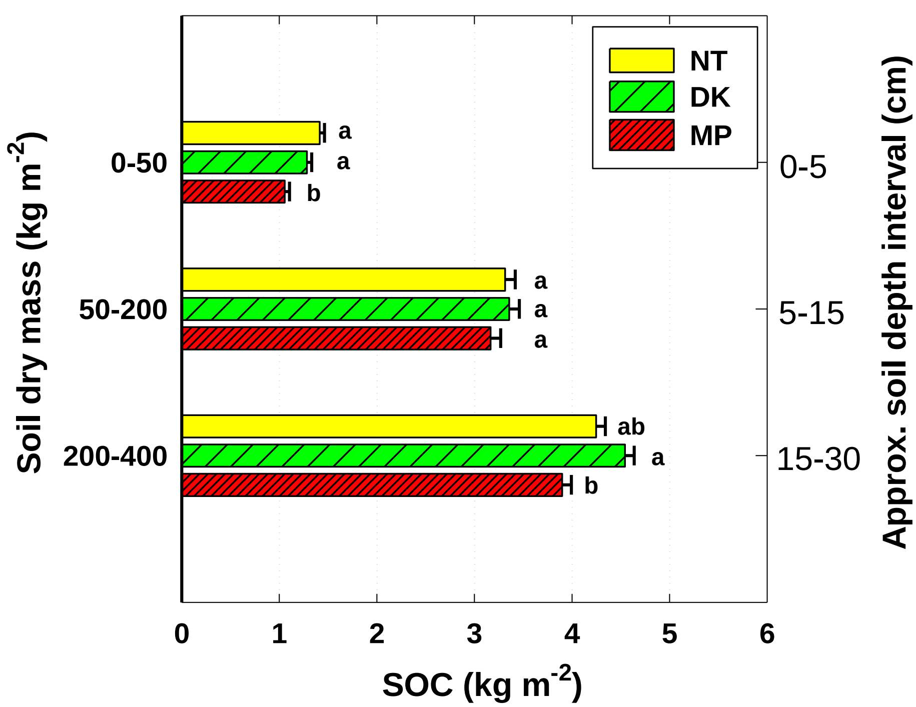

Figure 4.4 Soil organic carbon (SOC) under no-till (NT), disk tillage (DK) and moldboard plow (MP) at successive soil dry mass intervals. ± 1 standard error. Tillage treatments at each soil interval lettered differently mark significant differences at p<0.05.

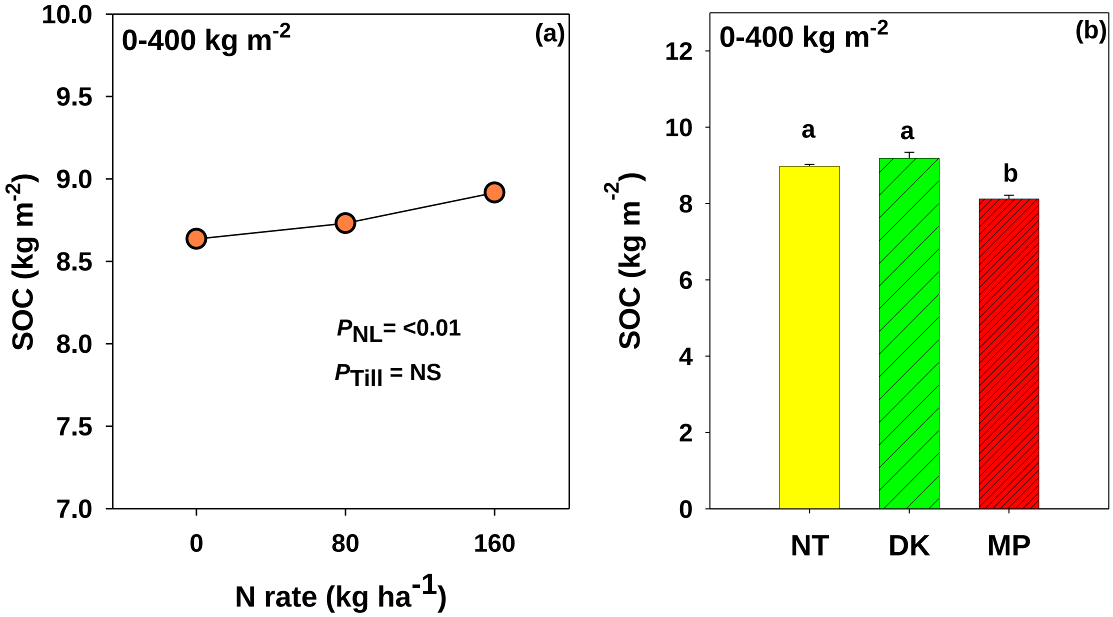

Figure 4.5 Effect of (a) nitrogen (N) fertilizer rate (0, 80 and 160 kg N ha^{-1}), and (b) tillage (no-till, NT; disk tillage, DK; and moldboard plow, MP) on soil organic carbon (SOC) measured in the 0-400 kg of soil m^{-2} averaged over the remaining management treatments at Concord, NE in 2009. (NS = not significant; P_{NL} = linear effect of N rate; Till = tillage treatment effect). N applied to corn only.

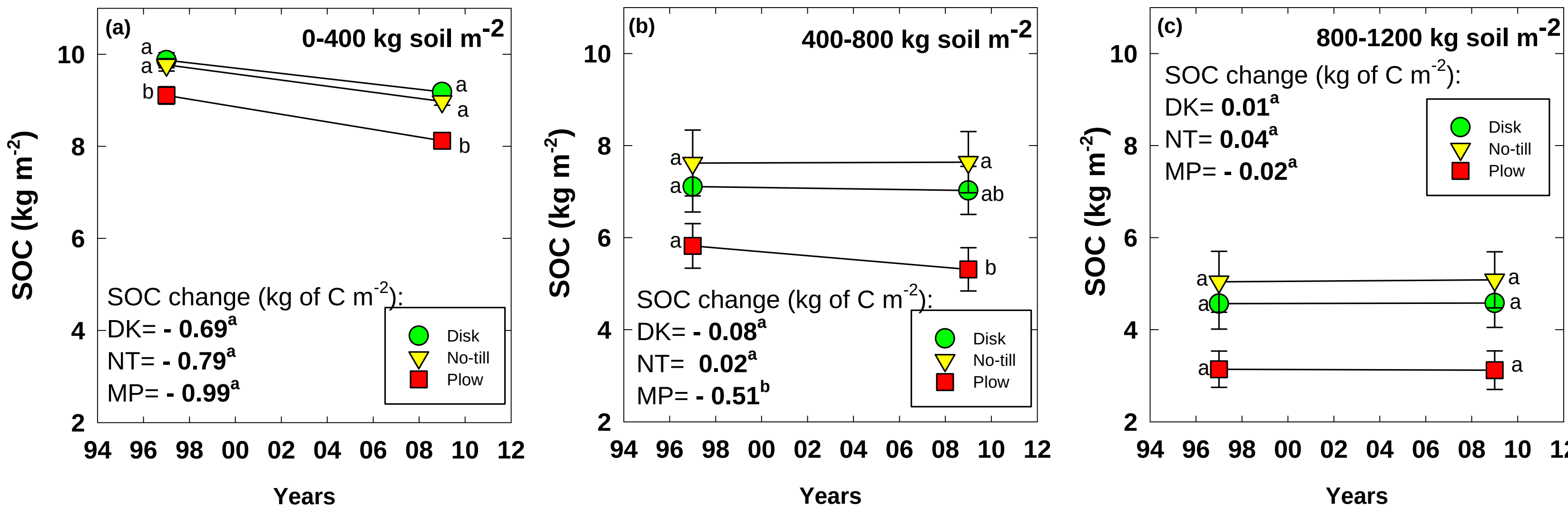

Figure 4.6 Soil organic carbon (SOC) trends between 1997 and 2009 ± 1 standard error at Concord for 3 soil mass intervals;(a) 0-400kg m^{-2} (approx. 0-30cm), (b) 400-800kg m^{-2} (approx. 30-60cm) and (c) 800-1200kg m^{-2} (approx. 60-90cm) of soil dry mass under disk (DK), not-till (NT) and moldboard plow (MP). Points lettered differently in a given year mark significant differences between tillage treatments at $p<0.05$. Differences in SOC stocks (kg m^{-2}) between 1997 and 2009 are presented for each tillage system. Changes lettered differently mark significant differences at $p<0.05$.

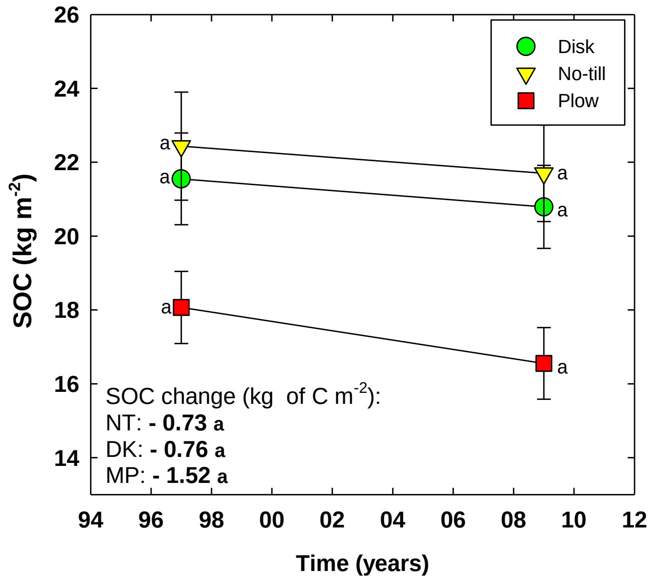

Figure 4.7 Soil organic carbon (SOC) stocks and annual rates of change in 1200 kg m^{-2} (approx. 90cm) of soil dry mass at Concord between 1997 and 2009 under no-till (NT), disk tillage (DK) and moldboard plow (MP) ± 1 standard error. Points lettered differently in a given year mark significant differences at p<0.05. SOC change (kg m^{-2}) between sampling dates are presented for each tillage treatment. Annual rates lettered differently mark significant differences at p<0.05.

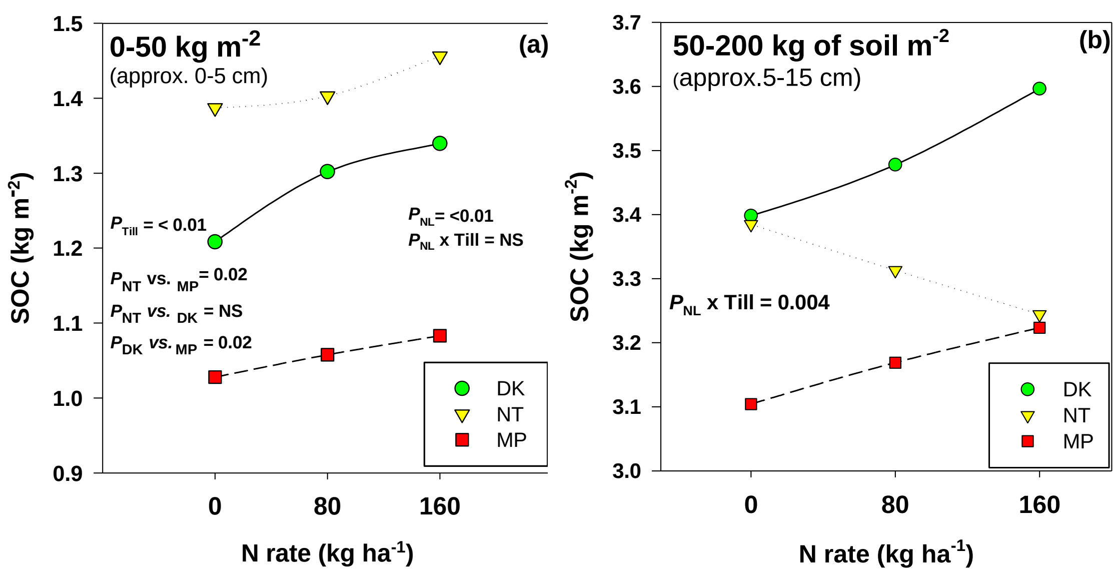

Figure 4.8 Effect of N fertilizer (0, 80 and 160 kg N ha^{-1}) and tillage treatment (no-till, NT; disk tillage, DK; moldboard plow, MP) on soil organic carbon (SOC), averaged over rotation measured in the (a) 0-50 kg of soil m^{-2} and (b) 50-200 kg of soil m^{-2} intervals. (NS = not significant; P_{NL} = linear effect of N rate; Till = tillage treatment effect). N applied to corn only.

CHAPTER 5. General Conclusions

The evaluation of the effects of management practices on SOC in two long-term studies led to somewhat similar conclusions, even though the conditions of the systems studied were relatively different. I presented six hypotheses in the introduction (p. 3) and will conclude by commenting on each.

The first and second hypotheses were that as tillage intensity increases SOC will be reduced from the soil surface (0-25 cm) but increased below 25 cm. As tillage intensity increased there was a redistribution of SOC in the profile as hypothesized, but it occurred only between NT and DK since under MP SOC stock decreased even below the plow layer. Increased SOC stock in the surface 50 kg m^{-2} under NT was compensated by greater SOC stocks in the 50-200 and 200-400 kg m^{-2} interval under DK, but SOC stocks under MP were consistently lower in the surface 400 kg m^{-2}. More than 12 years were required to produce detectable differences among tillage systems in the surface 400 kg of soil m^{-2}. After 24 years, SOC stock under NT and DK were 13% higher than under MP. Moreover, MP was the only tillage system which affected SOC stocks in the 400-800 kg of soil m^{-2} interval since most SOC changes under NT (at Concord) and DK (at Concord and Mead) where evident in the surface 400 kg of soil m^{-2} (Table 5.1).

The third hypothesis was that more SOC will be sequestered under continuous corn (CC) than corn-soybean rotation (CS). This hypothesis was rejected since the effect of rotation was not evident at either site.

The forth hypothesis was that increasing N fertilizer application would result in greater C sequestration. This hypothesis proved to be true, since the application of N fertilizer had a slightly positive effect in the surface 400 kg of soil m^{-2} in one of the sites evaluated, but required 24 years of treatment in order to be detected.

The fifth hypothesis was that higher residue input would result in higher SOC content regardless of the tillage treatment. In both studies, differences in estimated C input from crop production did not pproduce differences in SOC stocks. Better estimations of root biomass and rhizodeposition are necessary to properly address this issue in the future. More detailed surface initial samples could help future studies by increasing the precision to detect SOC changes.

The sixth hypothesis established that initial soil samples would allow proper understanding of management effects on SOC levels. Through the use of archived samples it was possible to determine differences between soils natural variability and the impact of management practices at depth greater than 30 cm. These results are in accordance with our hypothesis, since the results the interpretation of the 2009 soil samples would have been different without initial measurements. Future studies should focus on carefully determining soil variability prior to the establishment of the experiment in order to increase their capacity of detecting management effects on SOC stocks in the future.

Finally, in both studies, none of the evaluated treatments were able to sequester CO_2 from the atmosphere since SOC was lost under very treatment. Greater losses in Concord could be due to higher initial SOC stock (Senthilkumar et al., 2009) or due to lower productivity than the site at Mead (Paustian et al., 1997), but further research should be conducted to address this issue. These results clearly show that SOC in these systems and for the given conditions has not reached an equilibrium and therefore, SOC stocks could continue to decrease if management practices remain the same.

Table 5.1 Soil organic carbon (SOC) change between sampling dates for different soil mass intervals ± 1 standard error under different tillage treatments at Mead, NE and archived Concord, NE.

	Concord, NE (1997-2009)			**Mead, NE (1998-2009)**
	MP	**NT**	**DK**	**DK**
	kg m^{-2}			
0-400 kg of soil m^{-2}	-0.99 ±0.2*	-0.79 ±0.2*	-0.69 ±0.2*	-0.31 ±0.03*
400-800 kg of soil m^{-2}	-0.51 ±0.2*	0.02 ±0.2	-0.08 ±0.2	-0.10 ±0.09
800-1200 kg of soil m^{-2}	-0.02 ±0.1	0.04 ±0.1	0.01 ±0.1	-0.09 ±0.11

*Significant difference between years at α=0.05

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Appendix A. Procedure used to transform and express values on a constant mass basis.

Soil organic carbon values expressed on a constant mass basis (Ellert and Bethany, 1995):

(1) $\mathbf{M_{carbon} = conc. * BD * D * 10000\ m^2\ ha^{-1} * 0.001\ Mg\ kg^{-1}}$

M_{carbon} = carbon mass per unit area (Mg ha^{-1})
conc. = carbon concentration (%)
BD = soil bulk density (Mg m^{-3})
D = soil depth (m)

(2) $\mathbf{M_{soil} = BD * D * 10000\ m^2\ ha^{-1}}$

M_{soil} = mass of soil per unit area (Mg ha^{-1})

(3) $\mathbf{M_{carbon\ equiv.} = (M_{carbon\ surf} + ((M_{carbon\ add} / M_{soil\ add})*(M_{soil\ equiv.} - M_{soil\ surf})))}$

$M_{soil\ equiv.}$ = equivalent soil mass (Mg ha^{-1})
$M_{carbon\ equiv.}$ = carbon in equivalent soil mass (Mg ha^{-1})
$M_{soil\ surf}$ = soil mass of surface layer (Mg ha^{-1})
$M_{carbon\ surf}$ = carbon in surface soil layer (Mg ha^{-1})
$M_{soil\ add}$ = soil mass in added soil layer (Mg ha^{-1})
$M_{carbon\ add}$ = carbon in soil layer added (Mg ha^{-1})

If the thickness needed to obtain the desired soil mass equivalent is required:

(4) $\mathbf{T_{add} = ((M_{soil\ equiv.} - M_{soil\ surf}) * 0.0001\ ha\ m^{-2)} / BD_{subsurface}}$

T_{add} = additional thickness of subsurface layer required to attain the equivalent soil mass (m) $BD_{subsurface}$ = bulk density of subsurface layer (Mg m^{-3})

Appendix B. Statistical model and SAS code used in the analysis.

1-Statistical model and SAS code used to analyze the effect of management practices on SOC concentration and stocks over time at different soil depths or soil mass intervals at Concord, NE. (till = tillage system; rot = rotation; nrate = nitrogen rate; time = year of sample).

```
proc mixed method=type3;
class block till rot nrate time;
model carbon=block
             till
             rot
             till*rot
             nrate
             till*nrate
             rot*nrate
             nrate*till*rot
             time
             till* time
             rot* time
             till*rot* time
             nrate* time
             till*nrate* time
             rot*nrate* time
             till*rot*nrate* time;
random block*till
       rot*block(till)
       nrate*block(rot*till)
       block* time
       block*till* time
       block*rot* time (till);
run;
```

2- Statistical model and SAS code used to analyze the effect of management practices on (1) soil organic carbon (SOC) concentration at different soil depths in a given sampling date, (2) SOC stocks at different soil mass intervals in a given sampling date, (3) average residue carbon input between 1985-2008 (4) rate of SOC change between sampling dates and (5) soil bulk density at different soil depths at Concord, NE. (till = tillage system; rot = rotation; nrate = nitrogen rate).

```
;
proc mixed method=type3;
class block till rot nrate;
model carbon=block
             till
             rot
             till*rot
             nrate
             till*nrate
             rot*nrate
             nrate*rot*till;
random block*till
       rot*block(till);
```

```
lsmeans block till rot nrate till*rot*nrate/pdiff;
run;
```

3-Orthogonal polynomial contrast used to determine response curves induced by nitrogen (N) rates on soil organic carbon with and without interaction with tillage systems at different soil mass intervals and response curves induced by N rates on residue carbon input of each rotation at Concord, NE. (NT= no-till; D= Disk; P= moldboard plow; nrate = nitrogen rate; till =tillage system; CS= corn-soybean; CC= continuous corn).

```
contrast 'nrate linear' nrate -1 0 1;
contrast 'nrate quadratic' nrate 1 -2 1;
contrast 'CS vs CC linear' rot*nrate -1 0 1 1 0 -1;
contrast 'CS vs CC quadratic' rot*nrate 1 -2 1 -1 2 -1;
contrast 'D vs NT linear' till*nrate -1 0 1 1 0 -1 0 0 0;
contrast 'D vs P linear' till*nrate -1 0 1 0 0 0 1 0 -1;
contrast 'nt vs P linear' till*nrate 0 0 0 -1 0 1 1 0 -1;
contrast 'D vs NT quadratic' till*nrate 1 -2 1 -1 2 -1 0 0 0;
contrast 'D vs P quadratic' till*nrate 1 -2 1 0 0 0 -1 2 -1;
contrast 'nt vs P quadratic' till*nrate 0 0 0 1 -2 1 -1 2 -1;
```

4-Statistical model and SAS code used to analyze the effect of management practices on SOC concentration and stocks over time at different soil depths or soil mass intervals at Mead, NE. (rep= repetition; rot = rotation; nrate = nitrogen rate; time = year of sample).

```
;
proc mixed method=type3;
class rep rot nrate time;
model carbon=rep
             rot
             nrate
             rot*nrate
             time
             rot*time
             nrate*time
             rot*nrate*time;
random rep*rot
       nrate*rep(rot)
       rep*time
       rep*rot*time;
       lsmeans time rot nrate rot*time/pdiff;
run;
```

5- Statistical model and SAS code used to analyze the effect of management practices on (1) soil organic carbon (SOC) concentration at different soil depths in a given sampling date, (2) SOC stocks at different soil mass intervals in a given sampling date, (3) average residue carbon input between 1997-2008 (4) rate of SOC change between sampling dates and (5) soil bulk density at different soil depths at Mead, NE. (rot = rotation; nrate = nitrogen rate; rep=repetition).

```
;
proc mixed method=type3;
class rep rot nrate;
model carbon=rep
             rot
             nrate
             rot*nrate;
random rep*rot;
lsmeans rot nrate rot*nrate/pdiff;
run;
```

6-Orthogonal polynomial contrast used to determine response curves induced by N rates on residue carbon input of each rotation at Mead, NE. (CS= corn-soybean; CC= continuous corn; nrate = nitrogen rate).

```
contrast 'nrate linear' nrate -0.617213 -0.154303 0.771516;
contrast 'nrate quadratic' nrate 0.534522 -0.801784 0.267262;
contrast 'CB vs CC linear' rot*nrate -0.617213 -0.154303 0.771516
0.617213 0.154303 -0.771516;
contrast 'CB vs CC quadratic' rot*nrate 0.534522 -0.801784 0.267262 -
0.534522 0.801784 -0.267262;
```