

**CORN PERFORMANCE, NITROGEN USE EFFICIENCY,
PARTICULATE AND AGGREGATE ORGANIC MATTER
AFTER 25 YEARS OF DIFFERENT CROP MANAGEMENT**

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Masters of Science

(Soil Science and Agroecology)

at the

UNIVERSITY OF WISCONSIN-MADISON

2014

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ABSTRACT

Mineralization of nitrogen (N) from soil organic matter (SOM) is an important component of crop N supply, but we do not know which pools of SOM are the biggest contributors. Agricultural management choices have been shown to affect the buildup of SOM in macroaggregates (>250 μm), microaggregates (53 to 250 μm), occluded microaggregates within macroaggregates and particulate organic matter (POM). Our objectives were to measure the corn yield, N use efficiency, POM, and aggregate organic matter after 20 years of best management practices in three grain cropping systems and explore correlations between the dynamic soil properties measured and yield at 0 kg ha⁻¹ N (0N) and agronomically optimum N rate (AONR). The three systems were chisel-plowed continuous corn (*Zea mays* L.) (CC), strip-tilled corn-soybean [*Glycine max* (L.) Merr.] (CS), and an organically managed corn-soybean-winter wheat (*Triticum aestivum* L.) with green manure (CSW) at the Wisconsin Integrated Cropping Systems Trial (WICST) in southern Wisconsin, USA. In a drought year, the CS system out-yielded CC and CSW with a higher partial factor productivity and partial N balance, and response to N was so inconsistent that we could not determine AONR. In a second year where precipitation was not limiting, CS and CSW

both yielded greater than CC and had a greater partial factor productivity, but recovery efficiency was greater in CC due to very low yields when no N was applied in CC. In the second year, the AONR in CS was 99 kg N ha⁻¹, CSW was 113 kg N ha⁻¹, and CC was 153 kg N ha⁻¹. Although we found a positive effect on yield of reducing tillage in dry years, and that rotating corn with other crops both improves yields and reduces optimum N, the system effect on NUE was not consistent. We found higher POM concentration at 0 to 5 cm in CS, but no difference in POM-C and POM-N among the three systems. The CSW system had a significantly lower proportion of soil in occluded silt and clay at 0 to 5 cm, a lower proportion of soil N and carbon (C) in free silt and clay at 25 to 50 cm, and a greater proportion of soil C and N in free microaggregates at 25 to 50 cm, suggesting that the tillage for weed control in CSW increased the relative abundance of C and N not associated with macroaggregates in that system. The correlation analysis revealed significant positive correlations between free silt and clay and free microaggregate C and N and yield at 0N and AONR, and significant negative correlations between occluded microaggregate C and N. This suggests that there may be a tradeoff between promoting long-term storage of inaccessible C and maintaining SOM in a manner that renders it accessible to mineralization and crop uptake on an agronomic timescale. As perennials are known to increase both POM and aggregate C and N, the experiment was expanded to include the three WICST systems that incorporate perennial forages: three years of alfalfa (*Medicago sativa* L.) followed by corn (C3A), organically managed oats (*Avena sativa* L.)/alfalfa for 2 years followed by corn (C2A) and a rotationally grazed pasture seeded to a mixture of red clover, timothy (*Phleum pratense* L.), smooth brome grass (*Bromus inermis* L.) and orchardgrass

(*Dactylisglomerata* L.) (P). We found significantly lower concentrations of POM in CS and CSW, and significantly greater concentrations of POM-C in P and POM-N in P and C2A. The CSW system had a lower proportion of soil in macroaggregates and lower stocks of C and N within macroaggregates. It appears that in systems that are chisel plowed to produce corn every 1 to 3 years, high levels of biomass C inputs may support levels of POM-C and POM-N as well as soil aggregate C and N equivalent to the fully perennial P system. Potential future work might include measuring the potentially mineralizable N in aggregate fractions in order to further illuminate the source of soil N accessed by crops. A deeper exploration of how modest perennialization of annual agriculture affects the soil function and structure could also help inform managers concerned with the sustainability of their farming efforts.

ACKNOWLEDGMENTS

First and foremost thanks go to Matt Ruark, who has been accessible, supportive, and encouraging throughout this degree. At our first meeting, seeing my hesitation to make the big commitment, he told me that what would get me through was my own enthusiasm. That got me in the door, but I owe a lot to the rest of my support network as well.

I'm grateful to Bill Bland and Alfred Hartemink for serving on my committee, and answering and asking questions whenever I dropped in on them. To my last committee member, Erika Marin-Spiotta, I owe special thanks. Her seminar on soil organic matter opened my mind wide and provides the basis for much of this thesis, and she has continued to be a great help in developing my ideas and understanding.

Several other students and staff have been generous with their time: Mack Naber helped me do anything I needed to get done, Gregg Sanford dived into the weeds of my data repeatedly; Gary Oates ran many hundred samples with efficiency and good humor; Janet Hedtecke patiently shared her decades of experience at WICST and saved me from many errors; Peyton Smith made sure my macroaggregate isolator was functional after David Sloan meticulously put it together; James Tesmer always answered a call for help from the field; David Duncan and Cole Cook answered my statistical queries; Sabrina Ruis, Laura Smith, and Adam von Haden were willing to talk aggregates with me; the Agroecology program participants truly expanded my vision of what agriculture should be, and while many people helped me with field work I'm particularly grateful to the Jackson lab WICST crew, Mack Naber, Jaimie West, Nick Bero,

Julie Arriaga, Sarah Collier, Megan Chawner, Megan Wolf, Rita Argus, Elliot Morris, Ryan Curtin, and Adam Mittermaier. This work rests on a foundation provided by the late Dr. Josh Posner, whose vision for and dedication to WICST made it all possible.

Lastly, to my parents, my sisters, Dave, all my friends and family who've shared beans and pigs and bike rides and laughs with me, thank you. I especially treasure my parents' love and fantastic curiosity about what I'm up to, as well as their gentle and timely reminders to relax and appreciate that even a Masters student might not figure it all out.

Research

The distance between
my nose and the wall
is four feet three inches
according to this tape
plus the width of my thumb
which measures
a certain distance
nobody is certain of.

by David Allan Cates, 2012

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INTRODUCTION

This work investigates how a rich, productive soil can be changed over 25 years by varying agricultural management. The research takes place on the Plano silt loam, a mesic Typic Argiudolls, which covers over 172,000 ha in the U.S. Midwest. The Plano was formed on loess under tallgrass prairie between the end of the last ice age in the region, roughly 14,000 years ago, and cultivation approximately 150 years ago. Prairie vegetation contributes to the formation of a carbon-rich mollic epipedon due to the thick perennial root system (Jackson et al., 1996). As plants die, root biomass is preferentially stabilized as soil organic matter (SOM) rich in soil carbon (C) (Rasse et al., 2005; Mendez-Millan et al., 2010). Udolls formed under prairie like the Plano series make up much of the U.S. Corn Belt, especially in Iowa and Illinois, and the high organic matter content of these soils is vital to their productivity. The 25-year-old Wisconsin Integrated Cropping Systems Trial (WICST) on the Plano silt loam in southern Wisconsin represents an opportunity to investigate how these productive soils are affected by agriculture.

The replacement of prairie ecosystems with annual agriculture has had drastic effects on the soil organic carbon (SOC) content of U.S. Corn Belt Mollisols as well as other properties such as pH and bulk density. DeLuca and Zabinski's (2011) review comparing SOC in U.S. Midwest remnant prairies to cultivated adjacent lands determined that on average, in the top 22 cm, cultivated soils have only half the SOC of remnant prairies. Maintaining or increasing SOC is desirable to maintain soil fertility and to reduce the respiration of CO₂ from soil to the atmosphere, where it contributes to

global warming. Some literature suggests that no-till agriculture, in addition to decreasing erosion by maintaining surface residue instead of incorporating it, also increases SOC (e.g., Lal, 1984; Havlin et al., 1990; Six et al., 1999; Halvorson et al., 2002), but there is also evidence of no change in SOC in tilled systems, especially when SOC is measured deeper in the soil profile (Baker et al., 2007). For example, Bundy et al. (2011) found no change in SOC in the top 15 cm of soil after 50 years of moldboard plowing in continuous corn with high rates of N fertilization. The variation in SOC response to tillage may be due to the variation in mechanism of SOM storage. Organic compounds with more complex structures, such as lignins, waxes, phenols, aliphatics, or aromatics, may have slower initial decomposition rates (Marschner et al., 2008). However, simple proteins and saccharides may persist in the soil for as long or longer than more complex organic compounds, so physical protection, either through direct association with mineral surfaces or aggregation of soil particles is thought to have a significant effect on decomposition rates of SOM (Schmidt et al., 2011).

Aggregation in soil is a dynamic process whereby mineral particles are essentially glued together by organic residues (Fig. 1). Clay-size particles aggregate into domains, held together by inorganic binding agents as well as very small organic materials. These may congregate around a small piece of sticky organic material, forming a micro-aggregate, and the microaggregates in turn are held together by films of polysaccharides or the fungal byproduct glomalin, to form macroaggregates. The binding agents that hold particles together are proportional to the size of the particles being aggregated. Over time, any binding agent may break down as bacteria access and mineralize the material. Particles that are unbound may form new micro- or

macroaggregates. As proposed by Tisdall and Oades (1982) and corroborated by many other researchers, aggregate hierarchy states that smaller aggregates are more stable, turning over more slowly than larger aggregates, and microbial access to aggregates is limited, protecting aggregate associated SOC from decomposition (e.g., Reid and Goss, 1981; Angers and Giroux, 1996; Besnard et al., 1996; Jastrow, 1996; Six et al., 2004; Abiven et al., 2007; Oorts et al., 2007; Kong et al., 2007; Elmholt et al., 2008; Verchot et al., 2011; Six and Paustian, 2014). The particulate organic matter (POM), a large fraction of SOM that may form the nuclei of some aggregates, has been shown to be especially sensitive to changes in management, suggesting that it is an appropriate indicator of early changes in SOC due to management (Marriott and Wander, 2006b; Mirsky et al., 2008; Coulter et al., 2009).

There is a feedback between this aggregate turnover and agronomic performance. Kong et al. (2007) investigated the effects of management and source of nitrogen (N) on rate of aggregate-N turnover. They found that microaggregate-associated N and silt-and-clay-associated N had a shorter residence time in a conventionally managed system than an organically managed system, that fertilizer N was preferentially stored on silt and clay over aggregates, and that cover-crop- or manure-derived N cycled more slowly than fertilizer N. We cannot pinpoint exactly how much of the N turnover from aggregates is taken up by crops, although we know soil N mineralization is an important component of crop N supply. An ^{15}N tracer study performed by Stevens et al. (2005) suggests that 50% of crop N uptake is not accounted for by that year's fertilizer, but must be provided either by residual N fertilizer or N mineralization from SOM. Predictions of N mineralization from SOM are difficult because of the strong influence

of seasonal temperature and moisture on mineralization rates, but it may be possible to investigate the physical origin of N mineralized for crop growth.

The interaction between crop growth and SOM is extremely complex, and our investigation focuses primarily on the effects of tillage intensity and crop rotation. Tillage has been shown to influence the persistence of SOC in microaggregates within macroaggregates, as the disruption increases the turnover rate of macroaggregates such that microaggregates do not form or are bound by less persistent SOM (Six et al., 1998). Tillage has a varied effect on crop yield, depending on the soil type and growing season (Ismail et al., 1994; Malhi and Lemke, 2007; Andraski and Bundy, 2008). Increasing crop residue cover lowers the soil temperature, which may be harmful to yields in wetter years or on fine-textured soils, but beneficial to crops when moisture is limiting or on coarser soil. Crop rotation influences SOM through the type and quantity of residues introduced to the soil. Inclusion of perennial plants increases the growing season and total belowground bio-mass (DuPont et al., 2010). Rotation of corn with other crops has a consistently positive effect on yield for unknown reasons. The high C:N ratio of corn residue is thought to perhaps lead to immobilization of soil N to the detriment of crop growth, but when N is not limiting corn grown in rotation still out-yields corn grown following corn (e.g., Varvel and Peterson, 1990a; Stanger and Lauer, 2008; Gentry et al., 2013).

The complex nature of the relationships among SOM, rotation, tillage, and aggregates necessitates a holistic look at how management affects SOM, and how SOM may in turn affect crop performance. This study measured POM and aggregate organic matter after 25 years of cropping systems management at the Wisconsin Integrated

Cropping Systems Trial (WICST). The long history of consistent management allowed us to measure the effect of whole cropping systems on POM and aggregate organic matter, and the corn yield and N use efficiency (NUE) after SOM had been affected by this management. We explored the relationships further by analyzing correlations between the SOM measurements taken and corn yield. The dynamic nature of SOM demands that this type of study be replicated on many soil types, but as these prairie-derived soils are among the richest in the world, the potential benefits to understanding how SOC is stored and mineralized for plant growth here are enormous. The prairie-derived Mollisols at one time stored enough SOM to support abundant plant growth as well as increase SOC over many centuries, and by increasing our understanding of how SOC is stored in these soils they may be able to do so again.

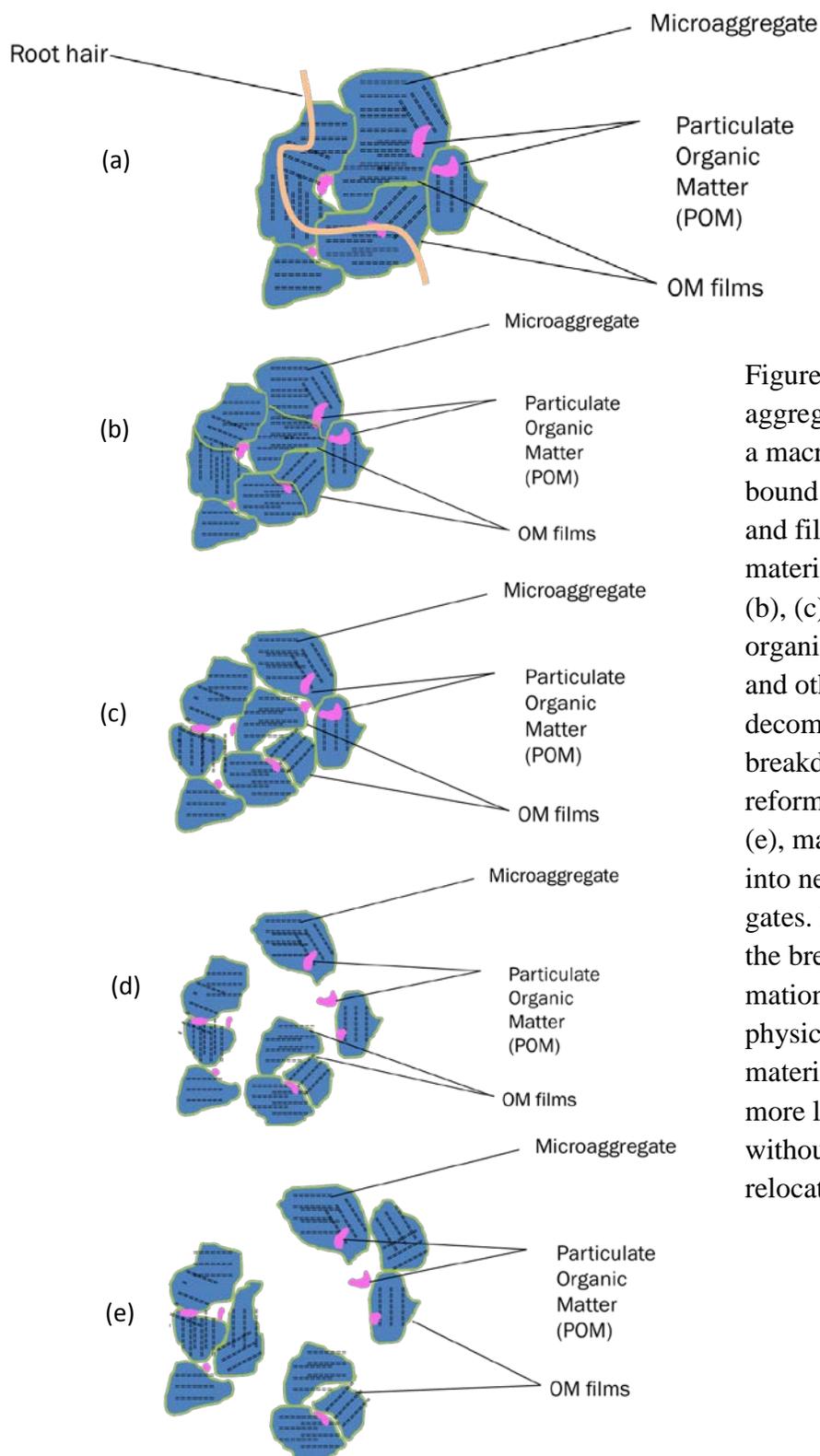


Figure 1. A schematic of aggregate dynamics. In (a) a macroaggregate is shown bound by root hair, POM and films of organic material (OM). Through (b), (c), and (d) some organic material is added and other material is decomposed, leading to breakdown and reformation of bonds. By (e), material has reformed into new macroaggregates. Note that although the breakdown and reformation is represented as physical movement of material, in truth this is more likely to happen without significant relocation of soil particles.

CHAPTER 1

Effects of Long-term Management on Corn Yield and Nitrogen Use Efficiency

ABSTRACT

While numerous studies have investigated the effects shifting from conventional to no-till agriculture and rotating corn with other crops, there is a paucity of data reflecting the cumulative effect of these management choices on yield after a long period of time. This study investigated yield and nitrogen use efficiency (NUE) metrics by performing nitrogen (N) rate trials within at the Wisconsin Integrated Cropping Systems Trial (WICST), where three grain systems varying in rotation and tillage intensity have been in place for over 20 years. The three systems were chisel-plowed continuous corn (*Zea mays* L.) (CC), strip-tilled corn-soybean (*Glycine max* L.) (CS) and an organically managed corn-soybean-winter wheat (*Triticum aestivum* L) with an oat (*Avena sativa* L.) cover crop inter-seeded with red clover (*Trifolium pretense* L.) (CSW). In a drought year, the CS system out-yielded CC and CSW with a higher partial factor productivity and partial N balance. In a second year where precipitation was not limiting, CS and CSW both yielded greater than CC and had a greater partial factor productivity, but recovery efficiency was greater in CC due to very low yields when no N was applied in CC. We found evidence that there is a positive effect on yield of reducing tillage in dry years and of rotating corn with other crops under varying conditions, but a mixed effect of management on NUE metrics.

INTRODUCTION

Nitrogen (N) fertilizer application to corn in the Midwest U.S. is a major contributor to buildup of N in the Gulf of Mexico (Alexander et al., 2008). Improving the N use efficiency (NUE) of crop production would have positive environmental and economic impacts, but it is a challenge to predict crop response to N. Variability in N response occurs due to edaphic and climatic conditions that cannot be controlled, but it also occurs in response to management decisions. Nitrogen is a critical input for high-yielding modern agriculture, but work remains to hone management practices to maximize efficiency.

Crop rotation and tillage change soil properties such as aggregation, bulk density, and soil organic carbon (SOC) (Jagadamma et al., 2007, 2008; Bhattacharyya et al., 2013; Tian et al., 2014), which in turn affects crop growth and access to nutrients. Corn grown in rotation with other crops commonly out-yields corn grown continuously (Posner et al., 2008; Stanger and Lauer, 2008). Gentry et al. (2013) identified N availability, corn residue accumulation, and weather as the primary causes of a continuous corn yield penalty. Annual inputs of cornstalks, a high C:N ratio residue, in the continuous corn system may lead to N immobilization during the growing season (Varvel and Peterson, 1990a; Kaboneka et al., 1997). Rotating other crops with corn generally decreases corn response to N fertilizer such that less N is needed to attain similar yields (Shrader et al., 1966; Jagadamma et al., 2008). Where alfalfa precedes corn, residual fixed N may supply enough N for high corn yields (Stanger and Lauer, 2008) but soybean, the most common crop in rotation with corn, contributes only marginal N to the following year's corn. It is clear that supply of N is not driving the yield increase in

rotation over continuous corn because, where N is not a limiting factor, continuous corn has decreased yields compared to corn-soybean rotations (Erickson, 2008). Minimizing tillage has the benefits of slowing erosion and conserving soil moisture and structure (Lal, 1984; Holland, 2004), but the effects of reducing tillage on yield have been mixed. When crop residues are left on the surface instead of tilled in, the soil is cooler in the spring, which may depress yields in wet years or on fine-textured soils, but improve yields in dry years or on well-drained soils (Ismail et al., 1994; Malhi and Lemke, 2007; Andraski and Bundy, 2008).

While yield response to management practices has been extensively studied, the long-term effects of crop rotation and tillage practices on NUE are not well documented. Rotation may alter the amount and rate of N mineralization from crop residue, the frequency of N fertilizer inputs, microbial N fixation and biomass, and total soil organic N (Pierce and Rice, 1988), and so the precise effect of rotation with a specific crop is difficult to extract. For example, Wortmann et al. (2011) studied corn in rotation with soybean and dry bean in Nebraska, and found that rotation with soybean resulted in equivalent recovery of N fertilizer in corn but an increase in corn yield per unit N applied, and that rotation with dry bean significantly decreased fertilizer N recovery and yield increase per unit N applied. More research is needed to explain how other preceding crops affect corn NUE. Reducing tillage may complicate the effect of rotation, as the yield gap between monoculture and rotation corn was greater in a no-till system than a system receiving moldboard plowing (Dick and Van Doren, 1985). No till systems leave more residue on the surface, and when other crops precede corn the C:N ratio of residue may be more favorable for decomposition and subsequent N miner-

alization (Gentry et al., 2013). However, reducing tillage has been shown to increase soil organic N (Pierce and Rice, 1988; Jagadamma et al., 2007) and microbial biomass N (Carter, 1986; Liang et al., 2012), indicating that decreasing disturbance increases the stable soil N pool which may lead to long-term NUE gains.

In their review of global NUE, Cassman et al. (2002) declare that research which illuminates the feedback between dynamic soil properties, crop management tactics, and NUE in realistic systems is sorely lacking. We aimed to address this gap by investigating corn response to N over two very different growing seasons in a long-term cropping systems experiment, the Wisconsin Integrated Cropping Systems Trial (WICST), where a range of best management practices have been in place since 1990. Our objectives were to investigate the effect of long-term management on response to N fertilizer, NUE metrics, and agronomically optimum nitrogen rate (AONR).

MATERIALS AND METHODS

The Wisconsin Integrated Cropping Systems Trial

The WICST is located at the University of Wisconsin Agricultural Research Station in Arlington, Wis. on a Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudolls). Prior to settlement in the mid-1800s, this area was likely tallgrass prairie. Wheat and feed for dairy herds was produced on the land until 1960. Between 1960 and the initiation of WICST, the primary crops grown were corn and alfalfa with dairy manure serving as major nutrient source. In 1989, corn was planted throughout the 9.71-ha trial in order to improve the uniformity of the land's history and take baseline

measurements. At that time, the average organic matter content (0 to 15 cm) was 47 g kg⁻¹ (based on loss on ignition), pH was 6.5 (1:1.3 soil/water), soil test P (Bray-1) was 108 mg kg⁻¹ and exchangeable K was 255 mg kg⁻¹ (Posner et al., 1995). The mean annual temperature at Arlington is 6.9°C and mean annual precipitation is 803 mm (1982-2012, National Climate Data Center).

The WICST is a randomized complete block trial, with six cropping systems represented in all phases with four replications. A staggered start was performed so that replication occurred in time as well as space (Posner et al., 1995). Plots are 155.5 by 18.3 m, or 0.3 ha, and commercial farm-scale equipment is used for all field work. Management has changed slightly over the years to reflect best management practices in the area. The six cropping systems were chosen to represent a spectrum of biological diversity and inputs: three grain and three forage systems. The three cash-grain systems used for this study are: (1) continuous corn (*Zea mays* L.), which receives fall chisel plowing (CC), (2) a 2-year rotation of corn-soybean [*Glycine max* (L.) Merr.], which is no-till during the soybean phase and strip-till during the corn phase (CS), and (3) a 3-year rotation of corn-soybean-winter wheat (*Triticum aestivum* L.) with an oat (*Avena sativa* L.) cover crop inter-seeded with red clover (*Trifolium pretense* L.), which is organically managed and receives tillage as well as cultivation as needed for weed control (CSW). Pelletized chicken manure (CPM) is applied in the spring prior to corn in CSW. Corn in CC and CS receives commercial fertilizer in the form of urea at planting at UW-Madison recommended rates based on presidedress nitrate testing (Laboski and Peters, 2012). The CC and CSW systems are all chisel plowed in the fall prior to corn planting and the CS system is strip tilled in the spring immediately prior to

corn planting. A chisel plow is also used prior to soybean planting in CSW, and soybeans in CS are planted using a no-till drill. Additional cultivation for weed control is used as needed in all phases of CSW while CC and CS weeds are chemically controlled.

Nitrogen Rate Trials

The N rate trials were designed as a randomized complete block split plot conducted in the corn phase of each of the three grain systems during the growing seasons of 2012 and 2013. The WICST systems were treated as whole plots, and N rate as a subplot treatment at levels of 0, 67, 106, 146, 185, or 224 kg N ha⁻¹. The hybrid Pioneer 35F40 was planted 26 April 2012 in the northwest corner of the WICST whole plots in CC, CS, and CSW. Each N-rate plot was 4.57 by 11.6 m, or 0.021 ha, encompassing six rows of corn. In 2013, six N-rate plots were established in the southwest corner of each corn plot of CC, CS, and CSW and treated with the same N rates. The hybrid FS53VT4 was planted 5 May 2013. In order not to overlap with the 2012 N-rate trial, 2013 N-rate plots in CC were only 8.53 m by 11.6 m, or 0.016 ha, still encompassing six corn rows. In CS and CSW, 2013 plots were again 0.021 ha. In both years, N was applied at planting in the form of urea with a stabilizing coating of N-methyl-2-pyrrolidone and N-(n-butyl) thiophosphorictriamide (Agrotain).

Soil samples were collected pre-plant (0 to 30 and 30 to 60 cm) and pre-plant on 13 June 2012 and 24 June 2013 (0 to 30 cm). Pre-plant soil samples were collected as a composite of six soil cores per plot collected across the whole plot. In-season samples were collected within 0 kg N ha⁻¹ (0N) split plots when corn reached 150 to 250 cm in height. Soil samples were air-dried, and ground to pass a 1-mm sieve.

In 2012, soil nitrate analysis was conducted in-house using a microplate colorimetric procedure (Doane and Horwath, 2003). In 2013, soil nitrate analysis was conducted by the University of Wisconsin Soil and Plant Analysis Laboratory using a microplate colorimetric procedure.

On 3 Oct. 2012 and 18 Oct. 2013, corn had reached full maturity and six corn plants were collected from each of the N rate plots. Corn ears were removed and air-dried, and grain was removed from ears and ground. Stalks and leaves were air-dried and ground. Subsamples weighing 5 to 6 mg were packed in 5 by 9 mm aluminum tins for combustion analysis. Subsamples of both corn and stalks and leaves were analyzed for total C and N on a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy).

Yield was determined from the center four rows of each six-row plot. Nitrogen uptake was determined by multiplying the %N in the plant by the total mass (conducted separately for grain and stalks plus leaves). Plots were harvested using a two-row combine on 17 Oct. 2012 and 23 Oct. 2013 and yields are reported at 15.5% moisture. Reported yields include corrections for grain harvested in subsampling. Nitrogen use efficiency was evaluated using partial factor productivity (PFP), agronomic efficiency (AE), recovery efficiency (RE), and a partial nutrient balance (PNB), according to the following equations:

$$\text{PFP} = \text{grain yield}/\text{N applied as fertilizer} \quad [1]$$

$$\text{AE} = (\text{grain yield} - \text{grain yield with no N applied})/\text{N applied as fertilizer} \quad [2]$$

$$\text{PNB} = \text{N in grain}/\text{N applied as fertilizer} \quad [3]$$

$$\text{RE} = (\text{total aboveground biomass N} - \text{total aboveground biomass N with no N applied}) / \text{N applied as fertilizer} \quad [4]$$

Note that each of these metrics includes N fertilizer application as the denominator, and so no NUE metrics were calculated for split plots receiving 0 kg N ha⁻¹.

Statistical Analysis

Analysis of variance was conducted to determine the effect of cropping system on grain yield, N uptake, RE, AE, PNB, and PFP using PROC MIXED in SAS 9.2 (SAS Institute Inc). Years were analyzed separately, system and N rate were treated as fixed effects, and block and block by system interactions were treated as random effects. The agronomic optimum N rate (AONR) was calculated separately for each system and year separately by fitting a linear plateau model using PROC NLIN in SAS 9.2. Alpha=0.10 was used to determine statistical significance unless otherwise noted.

RESULTS AND DISCUSSION

Growing Conditions

The 2012 growing season was characterized by early warming, lower than average rainfall, especially in the months of June and September, and higher than average temperatures (Fig. 1.5). Total precipitation between 1 March and 1 Nov. was 531 mm, significantly less than the 30-year average of 803 mm. According to the U.S. Drought Monitor, Arlington was abnormally dry the week of 26 June 2012, suffering from moderate drought the week of 3 July, severe drought the week of 10 July, and extreme drought 17 to 30 July, followed by severe drought through 15 Sept. 2012.

Growing conditions in 2013 were closer to typical conditions for the region, with a total of 765 mm precipitation between 1 March and 1 Nov. Only 3 to 23 Sept. were classified as moderate drought. The average daily temperatures in 2013 were lower than the 30-year average in April (5.25° in 2013, 7.45° 30-year average) and July (19.4° in 2013, 21.6° 30-year average), but otherwise within 1° of monthly averages (UW-Extension Arlington Agricultural Research Station weather data). Given these different growing conditions, 2012 and 2013 data were analyzed separately.

Corn Yield and Nitrogen Use Efficiency in 2012

Corn yields in 2012 were not significantly responsive to N rate but did differ by system with CS>CSW>CC when averaged across N rates (Table 1.2). Although linear, quadratic, linear plateau, and quadratic plateau models relating yield to N rate were fitted to the data, none were significant (data not shown). The interaction of N rate with system was significant in yield and yields in CSW were greater at 185 kg N ha⁻¹ than 0 kg N ha⁻¹ (Table 1.1). The CS system yields were significantly greater than CC at 0, 106, 146, and 224 kg N ha⁻¹, and greater than CSW at 106 and 146 kg N ha⁻¹ (Fig. 1.1a). Greater yields and N uptake at 0N in the CS system suggest that this system supplied more soil N to crops than the CC system (Fig. 1.1; Table 1.2). Our results are in agreement with a large body of work showing that rotation with other crops increases corn yields (Peterson and Varvel, 1989; Crookston et al., 1991). Although the difference in tillage and prior organic inputs confounds our results, it does not appear that increasing the length and diversity of rotation in CSW compared to CS has an effect on corn yields.

There was a significant effect of system and N rate on total N uptake, grain N uptake, and stalk and leaves N uptake (Table 1.2). Grain N uptake was by far the larger contributor to total N uptake, and corn in the CS system had greater total uptake and grain N uptake than CSW or CC. Although there was an overall effect of system on stalk and leaves N uptake, there were no significant differences between any two systems. In general, increasing N rate increased N uptake, but results were more variable for stalk and leaves N uptake than grain or total N uptake. There was a significant system by N rate interaction effect in all uptake measurements driven by the inconsistent pattern in response to N in each system (Fig. 1.1b-d).

Since 2012 was a drought year in Wisconsin, it is likely that nutrient uptake was limited by water stress in all three systems (Tanguilig et al., 1987). Rainfall in June was 7.37 mm, corresponding with the vegetative growth stage of corn (Fig. 1.5). July rainfall was concentrated in two major events, with potential for water stress in between, perhaps limiting corn as it moved into the reproductive stage. Water stress during tasseling has been found to impact grain yield more severely than water stress during any other growth stage (Çakir, 2004). The strip tillage employed in the corn phase of the CS system may have conserved moisture to attain the greater N uptake and yields observed. Decreased tillage increases surface residue coverage, which has been found to lower the soil temperature and increase soil moisture (Horton et al., 1996). Reduced tillage has been shown to be especially beneficial in warmer years with reduced precipitation (Wilhelm and Wortmann, 2004), such as 2012 in Wisconsin.

Preplant soil $\text{NO}_3\text{-N}$ (PPNT) concentrations were significantly affected by system and depth in 2012 (Fig. 1.3). The CSW system had significantly greater $\text{NO}_3\text{-N}$

levels than the other two systems at 0 to 30 cm. However, there was no difference among systems at 30 to 60 cm. Previous work (Varvel and Peterson, 1990b) suggests that CC has a smaller soil N pool than more complex rotations and is therefore more dependent on fertilizer N additions than more complex rotation systems. In our case, though we found larger PPNT in a more complex rotation; this did not lead to greater N uptake in CSW. However, none of the measured PPNT values were over the 56 kg ha⁻¹ threshold for N credits (Laboski and Peters, 2012) and so perhaps all values were too low to influence N uptake. Samples analyzed for presidedress NO₃-N (PSNT) showed no effect of system, and the average value of 14.6 mg NO₃-N kg⁻¹ warrants 35 lb ac⁻¹ (39.2 kg ha⁻¹) reduction in N sidedress application (Laboski and Peters, 2012). Since none of these systems receives sidedress N, this PSNT value serves only as an indicator of substantial soil reserves of NO₃-N in all three systems at the time of sampling. These reserves were likely protected from leaching by the minimal rainfall observed in early June 2012.

There was a significant effect of N rate on PFP and PNB in 2012, with smaller values signifying less efficient systems at higher N rates (Table 1.2). While corn in CS had significantly greater PFP and PNB, high yields and high N uptake at 0N in the CS system (Fig. 1.1a,c) lead to extremely low RE and AE. In comparison, a review of global NUE (Cassman et al., 2002) found that maize in North America typically has an RE value of 0.37. By comparing with 0N, RE indirectly addresses response to N fertilizer and so it is not surprising that all values were low in 2012, when N addition did not uniformly increase N uptake (uptake at 67 and 106 kg N ha⁻¹ were statistically equivalent to uptake at 0 kg N ha⁻¹). There were significant system by N rate interaction

effects in PFP, AE, PNB, and RE with widely scattered data again reflecting the poor growing conditions in 2012. The corn in CS had greater PFP than CSW or CC at 106 and 146 kg N ha⁻¹, indicating greater productivity per nutrient input (Fig. 1.4). Since PFP does not take into account yield at 0N, a high PFP may indicate greater yields due to high soil N. However since PPNT and PSNT were not elevated in CS, the high PFP may be more reflective of the CS system's increased capacity to access N in drought conditions.

Corn Yield and Nitrogen Use Efficiency in 2013

When averaged across all N rates, 2013 yield was greater in CS and CSW than CC and all three systems showed a significant yield response to N fertilizer. The interaction between N rate and system was not significant (Table 1.3; Fig. 1.2). There was no effect of system or N rate on total or grain N uptake, but stalk and leaves N uptake was increased with the addition of N fertilizer. The system by N rate interaction effect was not significant for total, grain or stalks, and leaves uptake (Table 1.3). There were no significant differences among systems in AE or PNB, but PFP in CS and CSW was significantly greater than in CC (Table 1.3; Fig. 1.4), and RE in CC was greater than in CS or CSW. The high uptake and yields at low N rates help to explain significantly higher PFP values in CS and CSW at 67 and 106 kg N ha⁻¹ (Fig. 1.4), as well as the very low RE values. Low RE may be misleading in situations like this where uptake at 0N is very high, but it serves as an indicator that the soil is supplying a significant portion of crop N. From the PNB, which does not take into account uptake at 0N, it appears that all three systems are removing the same amount of N from the system proportional to fertilizer applied. All NUE metrics generally decreased as N rates

increased, but it appears that applying fertilizer N up to 106 kg N ha^{-1} maintains AE, PNB and RE comparable to lower N rates. Lack of interaction effects in AE, PNB, and RE suggest that the three systems are similarly efficient in N use, while the significant interaction in PFP appears to be driven by differences in yields.

There was a significant effect of system, depth, and system by depth interaction in PPNT in 2013. At 0 to 30 cm, no differences were seen between systems, but at 30 to 60 and 60 to 90 cm, CC had greater concentrations of $\text{NO}_3\text{-N}$ than CS or CSW (Fig. 1.3). All values were much higher than 2012 PSNT or PPNT, evidence of residual soil $\text{NO}_3\text{-N}$ from the 2012 season, when low precipitation did not promote $\text{NO}_3\text{-N}$ leaching and in CC, low yields and low N uptake (Table 1.2) did not deplete the soil N. The total $\text{NO}_3\text{-N}$ in the profile of CC is sufficient for a 30 lb N ac^{-1} (33.6 kg ha^{-1}) credit according to the UW-Madison Extension nutrient application guidelines (Laboski and Peters, 2012). However, this increased soil $\text{NO}_3\text{-N}$ did not improve the yield of corn in the CC system nor lower the AONR, indicating that the $\text{NO}_3\text{-N}$ measured was not taken up by the crop. Since PPNT measurements were taken in April, $\text{NO}_3\text{-N}$ deep in the profile could have leached out before crops were able to use it. Pre-plant $\text{NO}_3\text{-N}$ measures are widely used and generally improve NUE, but the variability of soil nitrate and growing conditions limits their effectiveness as a prescriptive tool (Schröder et al., 2000; Haberle et al., 2004). Presidedress $\text{NO}_3\text{-N}$ values did not show any effect of system or depth, and average values of $39.4 \text{ mg NO}_3\text{-N kg}^{-1}$ in the upper 30 cm qualify for an N credit of 89.2 lb ac^{-1} (100 kg N ha^{-1}) (Laboski and Peters, 2012). No sidedress N was applied in any WICST system, so 100 kg N ha^{-1} credit indicates that N was likely overapplied at planting in all systems.

Linear plateau models relating yield to N rate were fit to each system and models were significant ($P \leq 0.005$) in all three systems. The yield plateau, or AONR (standard errors in parentheses), for CS was the lowest at 99.2 (29.8) kg N ha⁻¹, followed by 113.1 (19.9) kg N ha⁻¹ for CSW and 152.7 (29.1) kg N ha⁻¹ for CC (Fig. 1.2). Note that although the AONR is lower in CSW than CC, the slope of the linear plateau model is nearly identical (CSW slope = 0.0306, CC slope = 0.0308), so the gain in AONR in CSW is based on the larger yield at 0N in CSW. A large yield at 0N would suggest larger soil N supply in CSW and CS than CC, but the PPNT would suggest the opposite. Plants may be accessing organic N (Schimel and Bennett, 2004), or other factors, such as increased soil moisture in CS, may be affecting crop ability to utilize soil N. Using 106 kg N ha⁻¹ as an estimate of AONR for CS and CSW, and 146 kg N ha⁻¹ as an estimate of AONR for CC, it is possible to compare the systems' NUE values at their individual optimal N rates. The PFP in CS is 36% greater than CC and the PFP in CSW is 40% greater than CC (Fig. 1.4). The high PFP at the lower N rates in CS and CSW indicate a greater productivity for less N input in these systems than in the CC system.

In the organically managed CSW system, we saw a significant response to inorganic N in 2013 after 23 years of N supplied by legumes with the addition of pelletized chicken manure since 2009. Kong et al. (2007) suggested that applications of inorganic N in alternate years may increase PFP in organic systems where N is primarily applied as legumes or manure. These organic sources are more stable in the soil, forming a long-term, slow-release N pool that supports crop growth later in the season when inorganic N is no longer available (Omay et al., 1998; Kramer et al., 2002;

Kong et al., 2007). In 2013, the CSW system required inorganic N additions in order to reach a yield plateau, but part of the productivity of this system may be due to the slow-release soil organic N.

CONCLUSIONS

This experiment conducted in Southern Wisconsin confirms the continuous corn yield penalty seen in many other studies, in both dry and normal years, despite equal or greater soil $\text{NO}_3\text{-N}$. Although the effects of tillage and rotation are confounded in this systems study, the strong performance of strip-tilled corn following soy in a dry year, with yields greater than the organic rotation of corn, soy and wheat with cultivation for weed control, demonstrates that minimizing tillage can have agronomic benefits when plants are water stressed. However, the cultivation for weed control in the organically managed system did not depress performance when precipitation was sufficient in 2013, indicating that in many scenarios an organic system may equal or surpass the performance of continuous conventionally managed corn. Drought stress caused an inconsistent pattern of N uptake and NUE metrics in 2012, illustrating the complexity of the systems' response to N. In 2013 when growing conditions were more favorable, no one system showed a significant superiority in NUE across PFP, AE, PNB, and RE. Rotating other crops with corn appears to leads to higher yields than continuous corn, which boosted PFP, but RE and AE were more affected by crop performance at 0N.

Table 1.1. 2012 mean yield by system and N rate in WICST N rate plots.

N rate, kg ha ⁻¹	0	67	106	146	185	224
	Yield, Mg ha ⁻¹					
System†						
CC	7.49	7.73	7.84	7.5	7.77	7.71
CS	9.57	9.45	10.27	10.83	9.33	10.47
CSW	7.88 a‡	9.13 ab	8.22 ab	8.58 ab	9.47 a	8.98 ab

† CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover.

‡ Values followed by different lowercase letters are significantly different ($P \leq 0.10$) from other N rates within the same system.

Table 1.2. 2012 mean yield, N uptake, and N use efficiency values averaged across system and N rate in Wisconsin Integrated Cropping Systems Trial (WICST) N rate plots.

	Yield	Aboveground biomass N uptake	Grain N uptake	Stalk and leaves N uptake	PFPP†	AE	PNB	RE
	Mg ha ⁻¹	kg N ha ⁻¹	kg N ha ⁻¹	kg N ha ⁻¹	kg yield per kg N applied	kg yield increase per kg N applied	proportion of fertilizer N recovered in grain	proportion of fertilizer N recovered by crop
System ‡								
CC	7.67 c§	120 b	109 b	11.8	70.9 c	2.141	1.00 b	0.110
CS	9.99 a	138 a	127 a	11.7	91.6 a	3.46	1.14 a	0.028
CSW	8.71 b	118 b	109 b	8.67	81.4 b	9.00	1.03 b	0.231
N rate (kg ha ⁻¹)								
0	8.32	110 b	101 b	8.61 b	—	—	—	—
67	8.77	121 ab	111 ab	10.1 ab	146 a	7.53	1.85 a	0.183
106	8.78	120 ab	108 ab	11.2 ab	92.4 b	4.84	1.14 b	0.101
146	8.97	136 a	124 a	11.7 a	70.0 c	5.01	0.954 c	0.199
185	8.86	131 a	120 a	10.4 ab	53.7 d	3.28	0.728 d	0.126
224	9.05	136 a	124 a	12.3 a	45.3 e	3.67	0.618 d	0.130
ANOVA					<i>P</i> -value			
System (S)	0.001	0.0201	0.0107	0.0872	0.0004	0.3742	0.0309	0.1633
N Rate (N)	0.1238	0.0021	0.0033	0.0069	<0.0001	0.7104	<0.0001	0.1293
S x N	0.0136	0.0355	0.0472	0.0565	0.0248	0.0416	0.1097	0.0052

† PFP, partial factor productivity; AE, agronomic efficiency; PNB, partial nutrient balance; RE, recovery efficiency.

‡ CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover.

§ Values followed by a lowercase letter are significantly different ($P \leq 0.10$) from other systems or N rates.

Table 1.3. 2013 mean yield, N uptake, and N use efficiency values averaged across system and N rate in Wisconsin Integrated Cropping Systems Trial (WICST) N rate plots.

	Yield	Aboveground biomass N uptake	Grain N uptake	Stalk and leaves N uptake	PFP†	AE	PNB	RE
	Mg ha ⁻¹	kg N ha ⁻¹	kg N ha ⁻¹	kg N ha ⁻¹	kg yield per kg N applied	kg yield increase per kg N applied	proportion of fertilizer N recovered in grain	proportion of fertilizer N recovered by crop
System‡								
CC	8.17 b§	147	135	5.99	67.4 b	24.7	1.08	0.648 a
CS	10.4 a	123	115	6.76	88.4 a	26.9	0.958	0.072 b
CSW	11.0 a	136	126	7.04	91.4 a	21.1	1.03	0.100 b
N rate (kg ha ⁻¹)								
0	7.09 c	138	128	4.78 b	—	—	—	—
67	8.88 b	124	115	5.68 ab	132 a	26.6 ab	1.71 a	0.339 ab
106	10.4 a	154	143	6.87 ab	97.3 b	30.7 a	1.35 a	0.502 a
146	10.9 a	137	127	6.76 ab	74.8 c	26.1 ab	0.875 b	0.242 ab
185	10.9 a	134	124	7.71 a	59.0 d	20.7 bc	0.672 bc	0.178 ab
224	10.9 a	125	114	7.77 a	48.7 e	17.1 c	0.513 c	0.103 b
ANOVA					P-value			
System (S)	0.0060	0.4097	0.4539	0.1499	0.0095	0.8673	0.7291	0.0419
N Rate (N)	< 0.0001	0.4591	0.4503	0.8164	<0.0001	0.0035	<0.0001	0.0569
S x N	0.4110	0.8708	0.8969	0.5318	0.0012	0.1103	0.8647	0.2306

† PFP, partial factor productivity; AE, agronomic efficiency; PNB, partial nutrient balance, RE, recovery efficiency.

‡ CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover.

§ Values followed by a lowercase letter are significantly different ($P \leq 0.10$) from other systems or N rates.

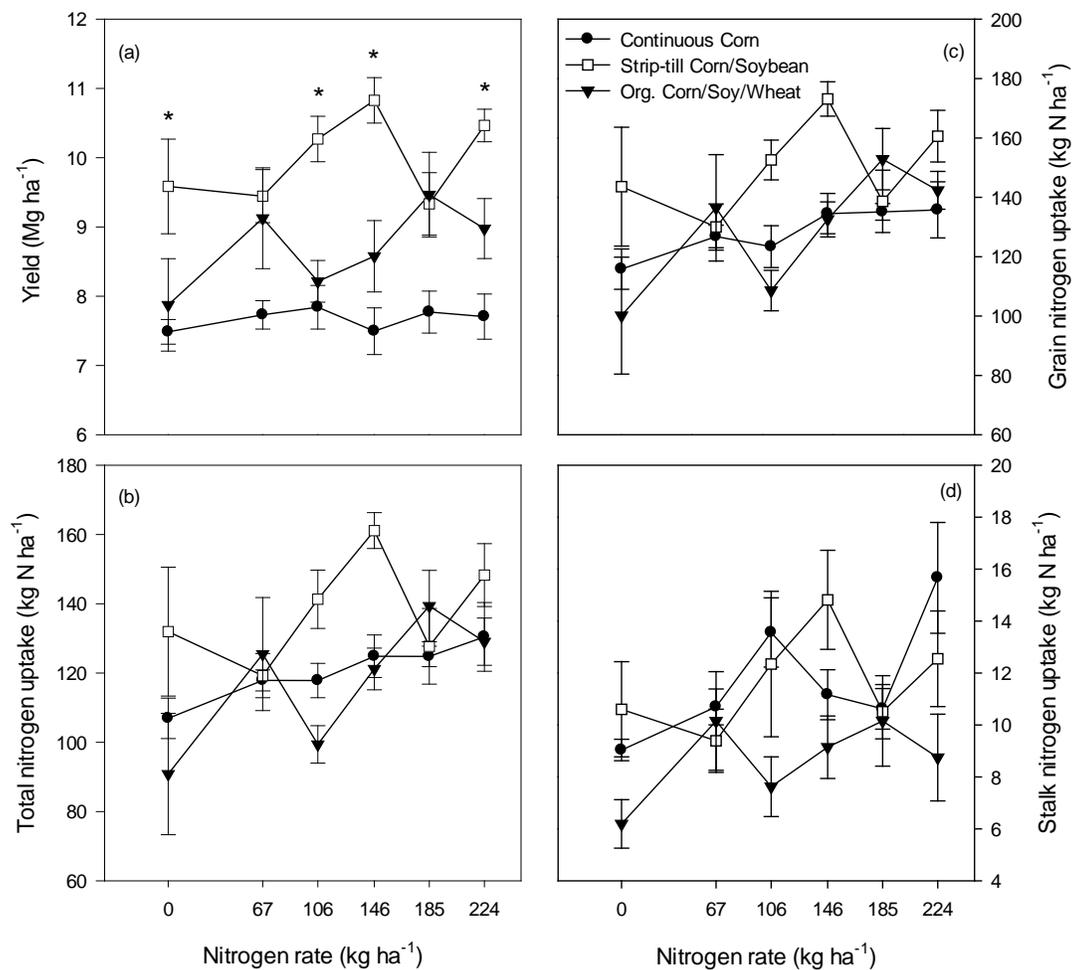


Figure 1.1. Interaction plots of N rate with system for yield and N uptake of corn in 2012 Wisconsin Integrated Cropping Systems Trial (WICST) N rate plots. (a) Grain yield. (b) Total N uptake. (c) Grain N uptake. (d) Silage N uptake. CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover. An asterisk indicates a significant difference ($P \leq 0.10$) among systems at each N rate.

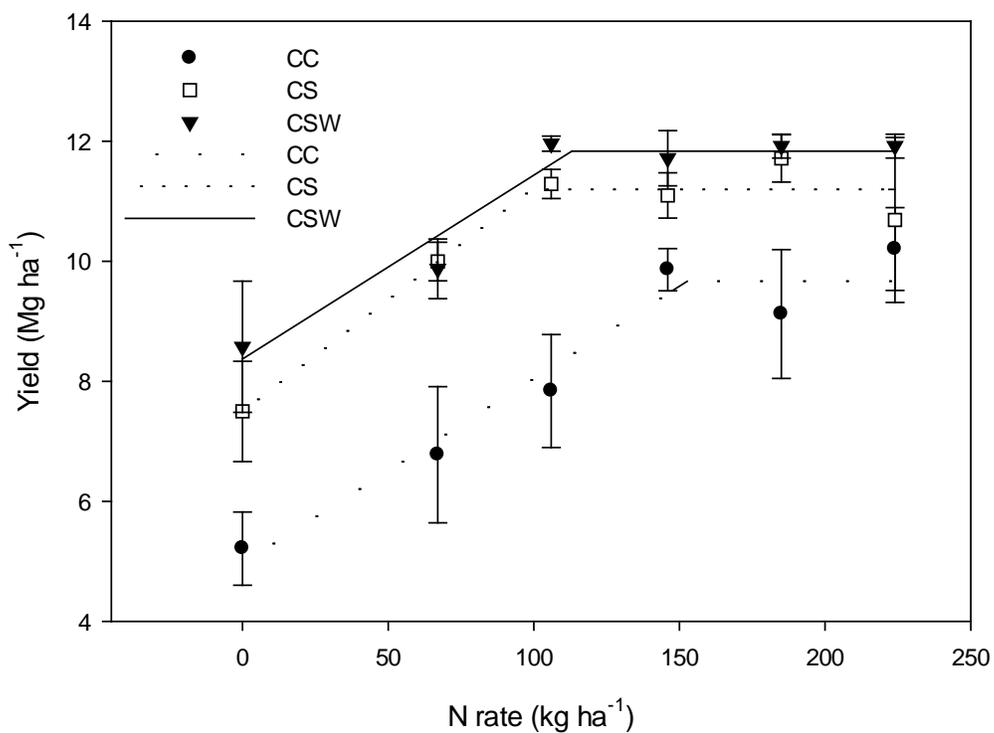


Figure 1.2. Corn yield in 2013 at Wisconsin Integrated Cropping Systems Trial (WICST) N rate plots. Lines represent yields predicted by linear plateau models for each system. CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover. All models are significant at $P \leq 0.0005$.

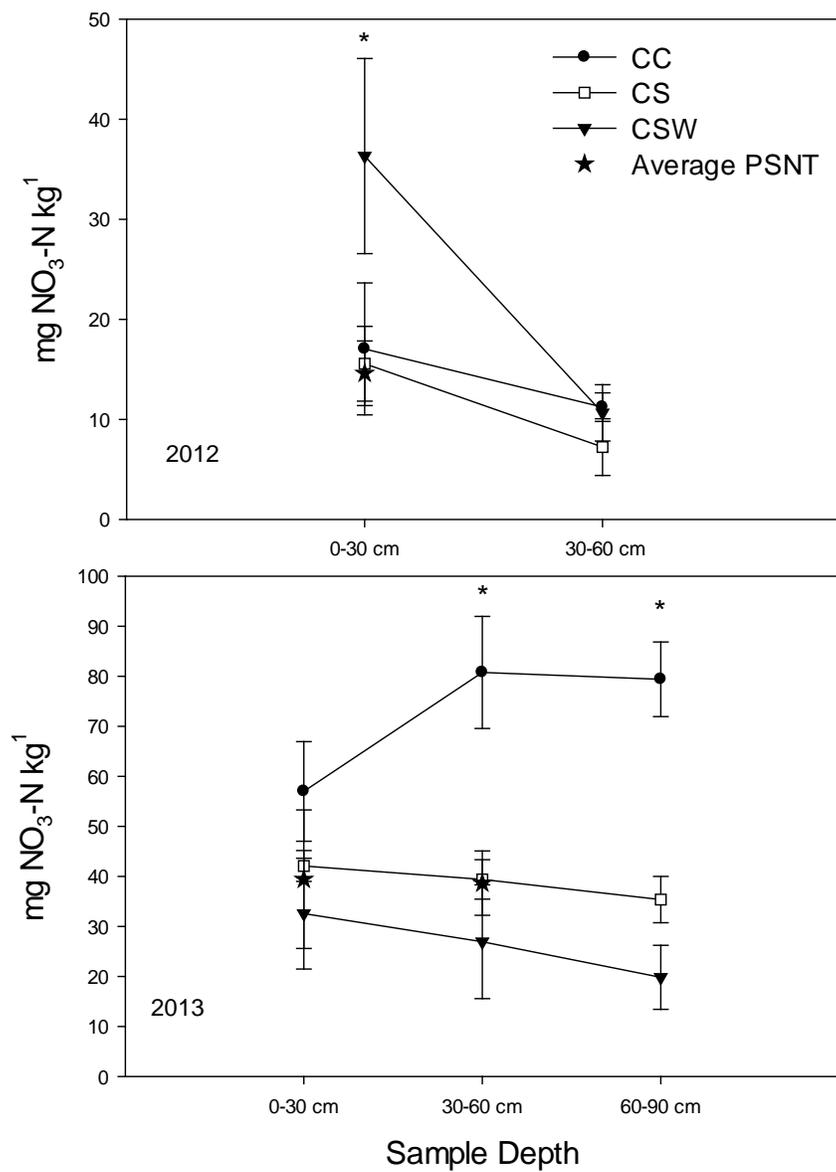


Figure 1.3. Preplant NO₃-N (PPNT) concentrations by system and depth and residue nitrate (PSNT) concentrations in unfertilized plots averaged across systems at a given depth. An asterisk indicates a significant difference ($P \leq 0.10$) among systems at each depth.

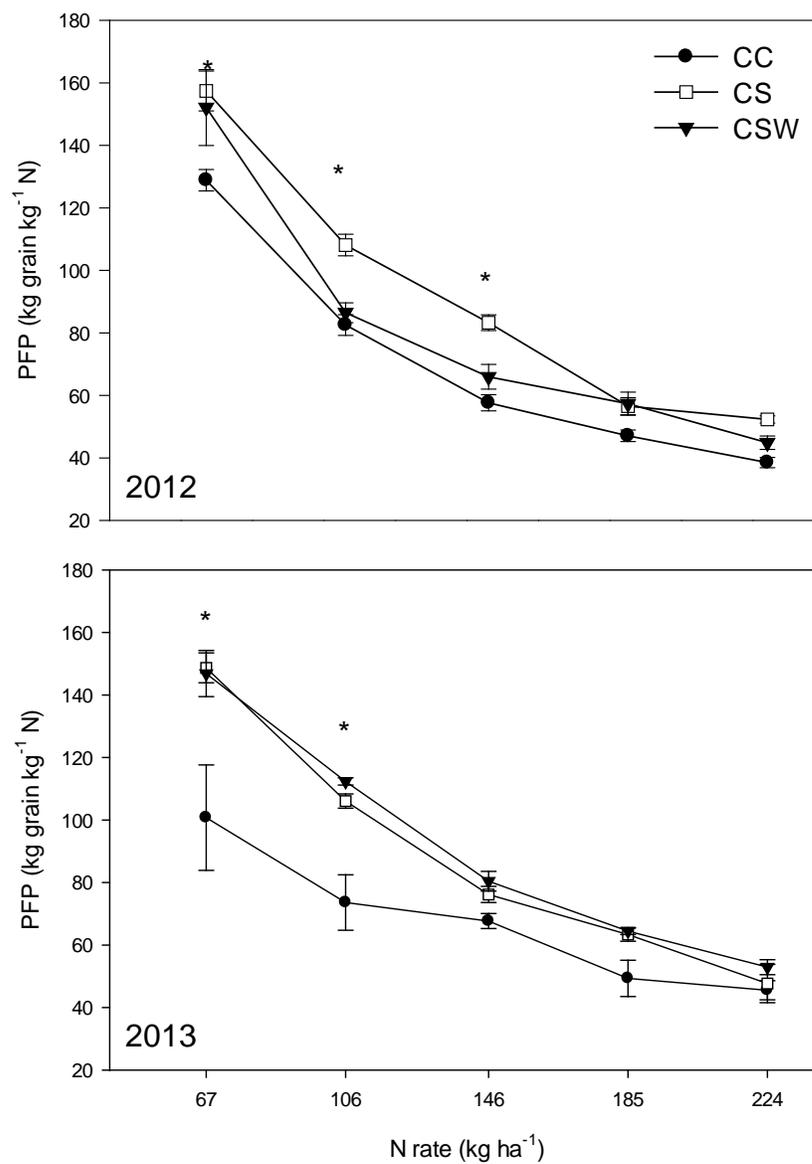


Figure 1.4. Partial factor productivity (PFP) in Wisconsin Integrated Cropping Systems Trial (WICST) N rate plots. CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover. An asterisk indicates a significant difference ($P \leq 0.10$) among systems at each N rate.

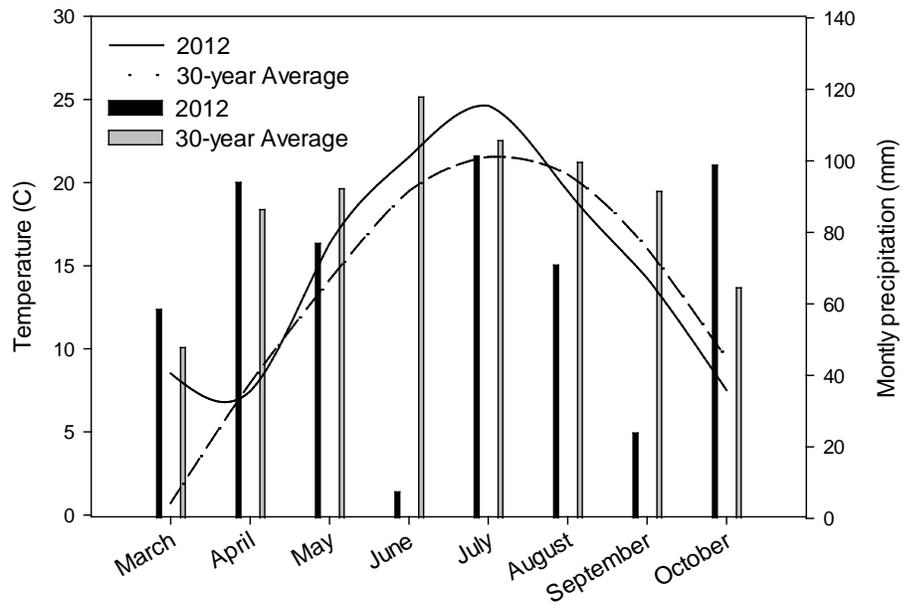


Figure 1.5. Average monthly temperatures and total monthly precipitation during the growing season at Arlington Agricultural Research Station, 2012 and 1981 to 2010. Source: UW Extension Ag Weather.

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CHAPTER 2

Investigating the Effects of Long-term Management on Particulate and Aggregate Organic Matter and Their Correlations with Corn Yield

ABSTRACT

Mineralization of nitrogen (N) from soil organic matter (SOM) is an important component of crop N supply, but we do not know which pools of SOM are the biggest contributors. This study measured particulate organic matter (POM) and aggregate organic matter after 20 years in three grain cropping systems and explored correlations between the SOM fractions measured and yield at 0 kg ha⁻¹ N (0N) or agronomically optimum N rate (AONR). The three systems were chisel-plowed continuous corn (*Zea mays* L.) (CC), strip-tilled corn-soybean (*Glycine max* L.) (CS), and organically managed corn-soybean-winter wheat (*Triticum aestivum* L) with an oat (*Avena sativa* L.) cover crop inter-seeded with red clover (*Trifolium pretense* L.) (CSW) at the Wisconsin Integrated Cropping Systems Trial (WICST) in southern Wisconsin, USA. When linear plateau models were fit to N rate trial yields, the AONR was 152 kg N ha⁻¹ in CC, 99.2 kg N ha⁻¹ in CS, and 113 kg N ha⁻¹ in CSW. We found higher POM concentration at 0 to 5 cm in CS, but no difference in POM-C and POM-N. The CSW system had a significantly lower proportion of soil in occluded silt and clay in CSW at 0 to 5 cm, a lower proportion of soil N and C in free silt and clay at 25 to 50 cm, and a greater proportion of soil C and N in free microaggregates at 25 to 50 cm, suggesting that the tillage for weed control in CSW decreased macroaggregate formation. Correlation analysis revealed significant positive correlations between free silt and clay and

free microaggregate C and N and yield at 0N and AONR, and significant negative correlations between occluded microaggregate C and N. This suggests that there may be a tradeoff between promoting long-term storage of inaccessible C and maintaining SOM in a manner that renders it accessible to mineralization and crop uptake on an agronomic timescale.

INTRODUCTION

Soil organic matter (SOM) is a heterogeneous mixture of organic compounds derived from soil fauna and vegetation. It is an important component of soil structure and fertility due to its contribution to cation exchange capacity and soil aggregation (Brady and Weil, 2010). Over time, SOM is decomposed by bacteria and fungi. Decomposition may mineralize nutrients quickly, or material may persist in the soil for years, decades, or centuries. Controls on decomposition include temperature, moisture, microbial community, chemical composition of organic material, and physical protection (Schmidt et al., 2011). Macroaggregates (generally defined as larger than 250 μm) are held together by the temporary binding agents such as roots, fungal hyphae and polysaccharides, while microaggregates (generally defined as 53 to 250 μm) are bound by more persistent organic materials such as glomalin (Tisdall and Oades, 1982; Wright and Upadhyaya, 1998; Abiven et al., 2007). When soil organic matter (SOM) is exposed due to natural or tillage-induced aggregate turnover, physically protected nitrogen (N) may be mineralized into plant-available nitrate or ammonia. Elliot (1986) measured an increase in N mineralized from crushed macroaggregates compared to intact macroaggregates, indicating that when aggregates break down, organic material is

released and accessed by microbes. While aggregate turnover time estimates vary widely among studies and ecosystems, there is some evidence that turnover occurs throughout the growing season (Plante et al., 2002; Kong et al., 2007), and mineralized SOM from these aggregates may be the source of N for during corn grain fill when fertilizer N is not present (Bender et al., 2013).

Aggregate fractionation has illuminated patterns of aggregate stability and carbon (C) sequestration under varying management. Based on ^{13}C dating of fractions in a variety of soil types, SOM associated with the mineral fraction contains the oldest and most stable C, followed by microaggregates and then macroaggregates (John et al., 2005; Marín-Spiotta et al., 2008; Gunina and Kuzyakov, 2014). The decrease in SOC often observed in conventionally plowed compared to no-till agriculture has been shown to be largely due to lower levels of C found in microaggregates occluded within macroaggregates (Six et al., 1999, 2000a; Deneff et al., 2004, 2007; Chung et al., 2008; Álvaro-Fuentes et al., 2009). Occluded microaggregates form within macroaggregates due to slow decomposition and redistribution of material within the macroaggregates, but the faster turnover time of macroaggregates under conventional tillage does not allow this to take place (Six et al., 1999; Kristiansen et al., 2006). The physical protection of SOM afforded by aggregates helps to explain persistence of very old soil C that is not mineral-associated better than older models relating decomposition primarily to chemical composition of SOM, which appears to be correlated with only the initial decomposition rate of plant residues (Marschner et al., 2008; Schmidt et al., 2011). Other controls on decomposition of SOM include temperature, moisture, microbial community, and nutrient availability, all of which may vary naturally, seasonally, or

anthropogenically (Craine et al., 2007; Marschner et al., 2008; Schmidt et al., 2011; Hobbie et al., 2012). Roots contribute more to SOM than aboveground biomass, especially in production of exudates (Rasse et al., 2005). Crop rotation may have a direct effect on soil aggregation due to the varying nature of root exudates from different crops. For example, perennial ryegrass roots were shown to increase aggregate stability while corn roots decreased aggregate stability despite larger corn root weight (Reid and Goss, 1981).

Particulate organic matter (POM), the large ($>53 \mu\text{m}$) chemically labile portion of organic matter has been shown to be more sensitive to changes in management than more broad measurements of SOM. A study comparing continuous corn to corn-soybean under various N rates found a greater increase of POM-C and POM-N than SOC and total nitrogen (TN) in continuous corn compared to corn-soybean rotation (Coulter et al., 2009). Increasing N rate did not affect TN or SOC, but it did increase POM-N, POM C:N ratio, and ratio of POM-N to TN, perhaps because higher rates of synthetic N increased plant N, and this plant N was incorporated more quickly into POM than the total soil N pool (Coulter et al., 2009). Compared to native sod, cultivation has been shown to deplete POM-C and N, but not mineral-associated C and N (Cambardella and Elliott, 1992), and moldboard plowing has been shown to reduce POM-N by 60% and POM-C by 66% compared to no-till wheat (Martín-Lammerding et al., 2013). Given this sensitivity to rotation, fertilization and tillage and the relatively simple lab analysis to extract POM, Marriott and Wander (2006a) have suggested that POM may be an early indicator of soil changes due to organic management.

Since SOM is spatially and compositionally heterogeneous, POM and soil aggregates have proven useful indicators for gauging the ability of an agroecosystem to protect and store SOM. It is desirable to increase protection of SOM in order to maintain soil fertility and protect C from respiration as CO₂, and it is also desirable to maintain a system in which N is steadily mineralized from SOM for crop growth. Thus far, our ability to predict soil N mineralization over the course of a growing season is mediocre (Schomberg et al., 2009), but it may be that incorporating information about dynamic soil properties could improve models. Improved predictions of soil N supply could reduce reliance on synthetic fertilizer N, which has negative environmental impacts due to vulnerability to losses from leaching, volatilization, and denitrification (Cassman et al., 2002; Donner, 2007). Our study leverages 23 years of the Wisconsin Integrated Cropping Systems trial (WICST) to (1) investigate how POM and aggregate C and N are affected by grain management systems that vary in crop rotation diversity and tillage intensity, and (2) explore the correlations between these dynamic soil properties and corn yield. We hypothesize based on past work that organic management will increase POM and that increasing tillage intensity will decrease POM and aggregate C and N, and that the fast turnover of macroaggregates will cause them to be most closely related to corn yield.

MATERIALS AND METHODS

The Wisconsin Integrated Cropping Systems Trial (WICST)

The study took place within the WICST, which was established at the University of Wisconsin Agricultural Research Station in Arlington, Wis. in 1990, on the Plano silt

loam (fine-silty, mixed, superactive, mesic Typic Argiudolls). Prior to settlement in the mid-1800s, this area was likely tallgrass prairie. Wheat and feed for dairy herds was produced on the land until 1960. Between 1960 and the initiation of WICST, the primary crops grown were corn and alfalfa with dairy manure serving as major nutrient source. In 1989, corn was planted throughout the 9.71-ha trial in order to improve the uniformity of the land's history and take baseline measurements. At that time, the average organic matter content (0 to 15 cm) was 47 g kg^{-1} (based on loss on ignition), pH was 6.5 (1:1.3 soil/water), soil test P (Bray-1) was 108 mg kg^{-1} , and exchangeable K was 255 mg kg^{-1} (Posner et al., 1995). The mean annual temperature at Arlington is 6.9°C and mean annual precipitation is 898 mm. (1981-2010, National Climate Data Center).

The WICST is a randomized complete block trial, with six cropping systems represented in all phases with four replications. A staggered start was performed so that replication occurred in time as well as space. Plots are 155.5 by 18.3 m, or 0.3 ha, and commercial farm-scale equipment is used for all field work. The trial was designed to test the feasibility of sustainable agricultural practices in the most realistic conditions over time, and so management has changed slightly to reflect best management practices over time. The three cash-grain systems, representing a gradient of high to low inputs and low to high diversity, are: (1) continuous corn (*Zea mays* L.) (CC), (2) 2 year corn-soybean [*Glycine max* (L.) Merr.] rotation that is no-till during the soybean phase and strip-till during the corn phase (CS), and (3) a 3-year organically managed rotation of corn-soybean-winter wheat (*Triticum aestivum* L.) with an oat (*Avena sativa* L.) cover crop inter-seeded with red clover (*Trifolium pretense* L.) (CSW). Pelletized

chicken manure (CPM) is applied to the corn and wheat phases of CSW. Corn in CC and CS receives commercial fertilizer in the form of urea at planting at UW-Madison recommended rates based on presidedress nitrate testing (Laboski and Peters, 2012). The CC and CSW systems are all chisel plowed in the fall prior to corn planting and the CS system is strip tilled in the spring immediately prior to corn planting. A chisel plow is also used prior to soybean planting in CSW, and soybeans in CS are planted using a no-till drill. Additional cultivation for weed control is used as needed in all phases of CSW while CC and CS weeds are chemically controlled.

Particulate Organic Matter (POM)

Soils were sampled for POM in April 2013. Sampling was conducted using a hydraulic tractor-mounted probe (2.4 cm diam.) at 0 to 5, 5 to 25 and 25 to 50 cm depths. Eight samples were taken from each whole WICST plot which was to be planted to corn in 2013 and composited into one sample per plot. Samples were dried at 100°C and ground to pass a 1-mm sieve. The POM analysis was conducted to reflect updates to the method proposed by Marriott and Wander (2006a). Ten-gram soil samples were placed in 30-mL plastic bottles with 20 mL sodium hexametaphosphate (SHMP, 100 g per 2 L solution). Soils soaked 17 to 21 hours. Bottles were capped with 53- μ m mesh and a top with a 1-cm hole. This allowed smaller material to escape while material >53 μ m, POM, was retained in bottles. Fifteen 30-mL bottles at a time were placed into a 26 by 17 by 7.5 cm Ziploc storage container with 700 mL SHMP. The whole container was placed on a reciprocal shaker and shaken at low speed for 1 hour. After 1 hour, the SHMP was poured off and replaced with 700 mL deionized water. The container was shaken on low speed for 10 minutes and then water was removed and

replaced with 700 mL fresh deionized water. Rinsing and shaking with deionized water was repeated 12 times so that samples were shaken for a total of 180 minutes. Finally, POM samples from each 30-mL bottle were emptied into a pre-weighed square of 53- μ m mesh. Samples were rinsed with deionized water until water ran clear. Mesh squares containing samples were dried at 50 to 60°C for a minimum of 24 hours, then weighed and stored in plastic Whirl-pak bags. Particulate organic matter concentration was determined by dividing the mass of POM by the mass of soil analyzed (10.0 g).

Aggregate Fractionation

Soil samples for aggregate fractionation were taken prior to fall tillage in late October 2012. Six cores (5.2 cm diam.) were taken using a hydraulic tractor-mounted probe from each CC, CS, and CSW plot that would be planted in corn in 2013. Cores were kept refrigerated until analysis. Cores were separated into 0 to 5, 5 to 25 and 25 to 50 cm depth segments and then composited into a single sample for each plot. Each sample was sieved to 4 mm; clumps were gently broken up and large organic material was removed by hand.

The fractionation procedure followed that outlined by Six et al. (2002). A 5.0-g subsample was weighed immediately and dried at 50 to 60°C to determine the moisture content of the soil at the start of the procedure. An 80.0 g sample of moist soil was weighed for the aggregate fractionation. The sample was placed on a 250- μ m sieve inside a plastic basin with the water level 2 cm above the top of the sieve. After 5 minutes of soaking, soil was sieved for 2 minutes by moving the sieve up and down 50 times (approximately 3 cm amplitude). Fine particles on the bottom and sides of the sieve were rinsed into the basin and material on top of the 250- μ m sieve, the

macroaggregates, were backwashed into a pre-weighed aluminum pan. All water and particles remaining were poured onto a 53- μm sieve in a plastic basin. This material was sieved for 2 minutes by moving the sieve up and down 50 times at approximately 3 cm amplitude. The bottom and sides of the 53- μm sieve were rinsed into the basin, and material on top of the sieve, the microaggregates, was backwashed into a pre-weighed aluminum pan. Material and water remaining in the basin was considered free silt and clay, and was rinsed into pre-weighed aluminum pans. All material was dried at 50 to 60°C for a minimum of 24 hours.

A subsample of 15.0 g oven-dried macroaggregates was weighed and placed in a beaker with approximately 50 mL water for 20 minutes. After soaking, macroaggregates were placed in the microaggregate isolator (built by David Sloan, UW-Madison Dept. of Soil Science, based on specifications from Johan Six; e.g., Six et al., 2002) with 30 metal beads on a piece of 250- μm mesh on a reciprocal shaker. Soil and beads were shaken under running deionized water for 3 to 5 minutes, until all macroaggregates were destroyed and water ran clear through the 250- μm mesh through the tube below. Material remaining on top of the 250- μm mesh was poured into a 2000- μm sieve, which caught the metal beads, over a 53- μm sieve, which caught remaining sand and coarse particulate organic matter (cPOM), which was larger than 250 μm in diameter. The cPOM was backwashed into a pre-weighed aluminum drying pan. Material that flowed through the microaggregate isolator's 250 μm mesh was tubed to a 53- μm sieve in a plastic basin. Material in this basin was sieved 50 times for 2 minutes at approximately 3-cm amplitude. The sides and bottom of the 53- μm sieve was rinsed into the basin, and material on top of the 53- μm sieve was backwashed into a pre-

weighed aluminum pan. Material from the top of the 53- μm sieve was classified as occluded microaggregates. Material that passed through the 53- μm sieve was considered occluded silt and clay particles, and was rinsed into a pre-weighed aluminum drying pan. All material was dried at 50 to 60°C for a minimum of 24 hours and then weighed and stored in plastic Whirl-pak bags.

Carbon and Nitrogen Analysis

A subsample of approximately 1 g of each whole soil, aggregate fraction, and POM sample was ground in a 2-mL micro-centrifuge tube with a ball bearing on a shaker to homogenize samples for C and N analysis. Subsamples weighing 8 to 10 mg were packed into 5-by-9 mm tin capsules. Carbon and N were determined by dry combustion using a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). Since inorganic C is negligible in these soils ($<0.05 \text{ g kg}^{-1}$; Paul et al., 2001), organic C was assumed to be the same as total C. The POM-N was determined by multiplying the concentration of N in POM by the concentration of POM in the bulk soil. The POM-N:TN was determined by dividing the POM-N, g kg^{-1} soil, by the total N, g kg^{-1} soil. The POM-C and POM-C:SOC were calculated in the same manner. Aggregate C and N are reported as a proportion, the concentration of C or N in the aggregate fraction divided by the total C and N found by summing all aggregate fractions. Since we did not measure bulk density at these depths, all C and N are reported on a concentration basis.

Based on summing aggregate fractions C and N, we found sum soil C to be 6.2% less than the whole soil C on average and sum soil N 0.13% less than whole soil N on average. Summing total C and N from aggregate fractions was less variable than

total C and N as measured from whole soil samples, perhaps due to the small sample (5.0 g) representing each large plot and the inherent field variability of SOC. Given the seasonal variation we observed in whole soil samples, we present the aggregate fraction data as a proportion of summed aggregate fractions. Recovery from the wet sieving process averaged 100%, and recovery from the macroaggregate isolation process averaged 99.6%, so we are confident that all material is represented in the summation of fraction C and N.

Nitrogen Rate Trials

The nitrogen (N) rate trials were designed as a randomized complete block split plot conducted in the corn phase of each of the three grain systems during the growing seasons of 2013. The WICST systems were treated as whole plots, and N rate as a subplot treatment at levels of 0, 67, 106, 146, 185, or 224 kg N ha⁻¹. The hybrid FS53VT4 was planted 5 May 2013 in six N-rate plots in the southwest corner of each corn plot of CC, CS, and CSW. In order not to overlap with previous N-rate trials, 2013 N-rate plots in CC were 8.53 m by 11.6 m, or 0.016 ha, but in CS and CSW, plots were 4.57 by 11.6 m, or 0.021 ha. Fertilizer N was applied at planting in the form of urea with a stabilizing coating of N-methyl-2-pyrrolidone and N-(n-butyl) thiophosphorictriamide (Agrotain). The center four rows of each six-row plot were used to determine yield. Plots were harvested using a two-row combine on 23 Oct. 2013 and yields were normalized to 15.5% moisture. The agronomic optimum N rate (AONR) was determined for each system separately by fitting a linear plateau model using PROC NLIN in SAS 9.2. For CC, the AONR (standard error in parentheses) was 152.7 (29.1), for CS AONR was 99.2 (29.8) and for CSW AONR was 113.1 (19.9) kg N ha⁻¹ (Fig.

1.2). Yields are reported for the N rate closest to AONR in each plot, which was 146 kg N ha⁻¹ for CC and 106 kg N ha⁻¹ for CS and CSW.

Statistical Analysis

Soil data, including POM, POM-C, POM-N, SOC, TN, proportion of C and N in POM and all aggregate fractions, and C:N ratio of whole soil and POM, were analyzed using PROC MIXED in SAS 9.2 (SAS Institute Inc) to develop ANOVA statistics. Depth and system were treated as fixed effects while block and block by system interactions were treated as random effects. Where assumptions of equal variance appeared to be violated, the transformations $\arcsin(x)$, $\arcsin(\sqrt{x})$, $x/1-x$, and $\log(x/(1-x))$, were modeled and assessed in residual plots. Where more than one transformation improved the fit of the data by visual examination, the model with the lowest AIC value was selected. For the proportion of soil found in microaggregates, the transformation $[x/1-x]$ was used; for the proportion of soil found in cPOM, the transformation $[\arcsin(\sqrt{x})]$ was used; for the proportion of soil C found in cPOM, the transformation $[\arcsin(\sqrt{x})]$ was used; for the proportion of soil C found in occluded silt and clay, the transformation $[\log(x/(1-x))]$ was used; for the proportion of soil C found in microaggregates, the transformation $[\arcsin(\sqrt{x})]$ was used; for the proportion of soil N found in microaggregates, the transformation $[\log(x/(1-x))]$ was used; for the proportion of soil N found in occluded silt and clay, the transformation $[\arcsin(x)]$ was used; for the proportion of soil N found in cPOM the transformation $[\log(x/(1-x))]$ was used. While statistics are reported for transformed data, actual values are shown in figures. Aggregate C:N ratios were analyzed using Friedman's two-way non-parametric ANOVA in PROC ANOVA in SAS 9.2. Correlations between soil data and yield data

were explored using the pairs function in R, and significance of these correlations determined using the cor.test function. Outliers were assessed using the Grubb's test for outliers and points were removed if they fell further than two standard deviations from the mean. Paired-t tests comparing spring and fall soil sampling were performed using the t.test function in R.

RESULTS AND DISCUSSION

Particulate Organic Matter

There was a significant effect of depth on all POM measurements, with values of all measurements following the pattern 0 to 5 > 5 to 25 > 25 to 50 cm (Table 2.1). The CS rotation had a significantly greater concentration of POM than the other systems at 0 to 5 cm, and this trend continued at 5 to 25 cm, but not at a significant level (Fig. 2.1). There were no significant differences among systems in SOC, TN, POM-C, POM-N, or proportion of SOC and TN found in POM. The three grain systems differ in rotation, tillage regime, and organic management. The POM was not influenced by tillage in the comparison between reduced tillage and no-till systems (Sequiera et al., 2011). Our data do not show a consistent effect of tillage except in the 0 to 5 cm depth, where the minimal tillage in CS allowed greater accumulation of POM. However, the CS system receives strip tillage in years when corn is grown, which may disturb soil structure enough to interrupt POM accumulation. Besnard et al. (1996) found less POM in CC than in a forested ecosystem after 35 years of corn, but effects were not apparent after 7 years, so it may be that the WICST systems have not been in place long enough to observe differences in POM. In the CSW system, there was cultivation twice in fall

of 2012 to establish and then incorporate the oats/clover cover crop, and over the 2013 corn growing season a rotary hoe was used twice and a cultivator once for weed control, which may account for lower values in POM concentration, POM-C and POM-N.

Contrary to hypotheses, measures of POM, SOC, and TN in the organically managed CSW system were not significantly different from either of the two conventionally managed grain systems. Researchers using the same method for fractionating POM have found that organic management increases POM and lowers POM C:N ratio (Marriott and Wander, 2006b; Ugarte and Wander, 2012; Maltas et al., 2013). The difference in POM quality is attributed to the use of legumes and manure as an N source. Manure has been shown to have a long-term beneficial effect on soil aggregation, which in turn protects POM (Jenkinson, 1991; Aoyama et al., 1999; Abiven et al., 2007). The organic corn in the CSW system at WICST received pelletized chicken manure (CPM) at approximately 4.48 kg ha^{-1} in 2013 and Hafez (1974) found that due to higher fiber content, dairy and swine manure has a greater effect on soil physical properties than chicken manure. We are not aware of any study that tests the effect of manure type on POM specifically, but less fibrous manure combined with low overall plant inputs and frequent tillage in the CSW system could explain why no increase in POM was found with organic management at WICST. The CPM application in CSW began in 2007; prior to that, N was supplied solely by a red clover/oat green manure. The green manure was found to be insufficient for corn nutrient needs based on low yields (G.R. Sanford, personal communication, 2014). After 7 years of CPM additions, total biomass C inputs in the CSW system are approximately $3319 \text{ kg C ha}^{-1}$, much less than CS with $5460 \text{ kg C ha}^{-1}\text{yr}^{-1}$ or CC with $6502 \text{ kg C ha}^{-1}\text{yr}^{-1}$ (average of 2012 and

2013 data based on the approach of Bolinder et al. (2007). The CSW corn yielded less on average than CS or CC between 1989 and 2009 (Sanford et al., 2012) and the wheat and soybean in rotation create less biomass in a growing season than corn. Greater diversity in rotation is, in this case, reducing plant inputs to the system, which may be reducing POM accrual since POM is primarily fresh plant inputs.

There was no significant difference among systems in the C:N ratio of POM or whole soil in our study. The POM C:N ratio reflects the C:N ratio of the inputs as well as the degree of decomposition of the material (Aoyama et al., 1999; Christensen, 2001). The average POM C:N ratio (16.7) was significantly greater than whole soil C:N ratio (11.5), confirming that POM is more similar to inputs of fresh plant material than whole soil. The most recent inputs to the grain systems were corn residue in CC, soybean residue in CS, and oat biomass in CSW. In fall 2012, the C:N ratio of oat biomass was 31:1 and the C:N ratio of corn stover was 70:1. Soybean residue C:N was not measured in the fall of 2012, but estimates from the literature range from 10.6 to 14.5:1 (Wong et al., 2001; Toma and Hatano, 2007). Marriott and Wander (2006b), also found that the C:N ratio of POM, fractionated by size as in our work, did not differ between organic systems with diverse crop rotations, and less diverse conventional systems. However, Marriott and Wander (2006b) found that POM that was fractionated by density into free and aggregate-occluded POM did show higher C:N ratios in conventional systems than organic systems. It is more difficult to detect differences among systems when measuring size-fractionated POM (as conducted in our study) because it includes more decomposed and relatively inert material than density-fractionated POM and therefore the signature of the most recent inputs is muted; however size fractionation

is more efficient (Gregorich et al., 1996; Marriott and Wander, 2006b). It is also possible that differences in C:N ratio were not detected here because POM recovered in April had already undergone significant microbial processing. Since the C:N ratio of biomass inputs was much higher in CC but the C:N ratio of soil and POM was not different among systems, it appears that microbial decomposers accessed and respired much more C in the CC system than decomposers in CS or CSW over the winter months.

Aggregate Fractions

There was a significant effect of depth on all aggregate fractions across all three systems, with higher proportions of free silt and clay found at the 25 to 50 cm depth and more macroaggregates and microaggregates found at 0 to 5 and 5 to 25 cm (Table 2.2; Fig. 2.2). There was more cPOM and occluded microaggregates found at 0 to 5 cm than either lower depth, and more occluded silt and clay found at 0 to 5 and 5 to 25 cm than 25 to 50 cm. The decrease in aggregation at depth is likely due to the greater presence of roots and associated biological activity in the upper depths (Jackson et al., 1996; Tufekcioglu et al., 1998) as roots and other organic material are the binding agents of soil aggregates (Tisdall and Oades, 1982; Schmidt et al., 2011).

Only fractions isolated from within macroaggregates were affected by system. Previous work indicates that chisel plowing, as in CC and CSW, disrupts aggregate formation (Beare et al., 1994; Six et al., 1998, 1999; Oorts et al., 2007; Tian et al., 2014) but other research suggests that plowed systems may bind aggregates using more fresh, labile C inputs and bacterial byproducts rather than fungal hyphae and glomalin (Bossuyt et al., 2002; Simpson et al., 2004; Elmholt et al., 2008). There was some

evidence that reducing tillage increased soil aggregation, as the CS system contained more occluded microaggregates than CC or CSW, and more occluded silt and clay than CSW. No differences were observed in the cPOM fraction across depths, as this fraction was extremely small and quite variable (averaged across all systems and depths, 15 g cPOM kg⁻¹ soil, with a standard error of 4.4 g cPOM kg⁻¹ soil). There was an interaction between system and depth on microaggregates, silt and clay, and cPOM, indicating that the soil profiles under these three cropping systems differ significantly in the way the aggregate distributions change with depth. For example, CSW had the greatest quantity of free silt and clay at 0 to 5 cm but CS has larger quantities of free silt and clay at 5 to 25 and 25 to 50 cm (not significant). This may be an effect of regular shallow cultivation for weed control in CSW, which by disrupting the formation of aggregates at the surface would increase the proportion of unassociated silt and clay. The CS system had the greatest quantity of cPOM at 0 to 5 cm, likely due to the larger quantity of crop residue left behind by the minimal tillage regime, and at 25 to 50 cm the CS system had the smallest quantity of cPOM, but neither trend was significant. Within free microaggregates, CSW>CS>CC at all three depths, but only significant at 25 to 50 cm (Fig. 2.1). The increased proportion of soil of free microaggregates in the CSW system may also be a result of cultivation preventing the formation of macroaggregates, as has been seen on a loess-derived soil in France (Oorts et al., 2007) and a Michigan Hapludalf (Grandy and Robertson, 2007).

Aggregate storage of C and N was significantly affected by depth in all cases except proportion of N in silt and clay and proportion of C or N in occluded silt and clay (Table 2.2; Fig. 2.3 and 2.4). These effects may be due to a change in C and N

concentration of the soil fraction, or a change in the proportion of soil found in that fraction. For example, the proportion of soil C and N found in macroaggregates follows the pattern established by the proportion of total soil found in macroaggregates, 5 to 25 = 0 to 5 > 25 to 50 cm, indicating that this effect is largely based on an increased mass of macroaggregates above 25 cm and not an increased concentration of C and N within those macroaggregates. The lack of effect of depth on most silt and clay C and N may be because SOM associated with silt and clay is bound through organo-mineral associations to a limited quantity of binding sites (Kleber et al., 2007), so C and N on silt and clay are more affected by mineralogy and overall quantity of SOM than management. Since the proportion of total soil weight found in silt and clay increases with depth, it follows that there is an increase in silt and clay C at 25 to 50 over 0 to 5 cm (Table 2.1; Fig. 2.2). The proportion of soil N associated with silt and clay does not increase with depth, and we can conclude that the SOM bound to silt and clay at depth is depleted in N compared to the SOM bound to silt and clay in the upper soil. There is a concurrent non-significant increase in C:N ratio of the silt and clay at 25 to 50 cm (Fig. 2.5). In occluded silt and clay, there is no significant effect of depth on proportion of C and N, but the overall proportion of soil found in occluded silt and clay is greater at 25 to 50 cm, so SOM bound to occluded silt and clay is less enriched in C and N at 25 to 50 cm than the upper two depths.

The effect of depth on C (0 to 5 = 25 to 50 > 5 to 25 cm) and N (0 to 5 > 25 to 50 > 5 to 25 cm) in microaggregates appears to be more driven by the stability of C and N concentration in microaggregates than the proportion of microaggregates in the soil. Although significantly more soil is found in microaggregates at 0 to 5 and 5 to 25 cm

than 25 to 50 cm, the proportion of soil C and N found in microaggregates is significantly greater at 25 to 50 cm than 5 to 25 cm (Table 2.1; Fig. 2.2). Since microaggregates are more stable than macroaggregates or cPOM (Denef et al., 2001; Gunina and Kuzyakov, 2014), they maintain similar concentrations of C and N at depth, and while less protected C and N concentrations decrease, their stable C and N concentration makes up a larger proportion of total soil C and N. The large proportions of soil C and N in microaggregates at 25 to 50 cm were found primarily in the CSW system, which had significantly greater proportions of C and N in microaggregates than CC or CS at 25 to 50 cm (Fig. 2.2, 2.3).

There was no significant effect among systems on C:N ratio of any fraction (Table 2.2). There was a significant effect of depth on the C:N ratios of macroaggregates ($P=0.0781$), occluded microaggregates ($P=0.0199$), and occluded silt and clay ($P=0.0269$). Overall, cPOM had a greater C:N ratio than all other fractions (Fig. 2.5). Many studies have found that the C:N ratio of SOM decreases with depth (Post and Mann, 1990), reflecting reduced plant inputs and increased proportion of soil C and N in microbial biomass, but the cPOM C:N suggests that decomposition is relatively consistent at 0 to 50 cm in these systems.

Correlations

Particulate organic matter was shown to be unrelated to agronomic performance in this study (Table 2.3). The POM concentration, POM-C and POM-N were not correlated to yield at 0N or AONR. While these exploratory analyses would not indicate a causal relationship, the lack of correlation suggests that although POM has been shown to vary in response to management, it is not the most promising diagnostic tool for

predicting crop yield. The lack of correlation between POM concentration or C and N enrichment and yield may be due to the minimal variation in POM among systems measured in this study. While we would expect that the slight increase in POM concentration in CS (Fig. 2.1) would support increased yields due to increased availability of labile material, perhaps the difference in concentration was too small to be relevant. Macroaggregates made up a similar portion of soil mass in each system, so POM experienced a similar degree of physical protection, and the quality of POM, as measured by C and N concentration and C:N ratio, was not different among systems. These similarities in POM quality and physical accessibility should lead to similar mineralization rates and subsequently similar plant-available N. This is supported by our measurements of total N and $\text{NO}_3\text{-N}$, which did not differ among systems (Fig. 1.3).

Within aggregate fractions, more significant correlations to yield were found at 5 to 25 and 25 to 50 cm than at 0 to 5 cm. This may be due to the distribution of corn roots in the profile. For example, Hilfiker and Lowery (1988) found that the maximum rooting density of corn occurred between 10 and 40 cm, and Dwyer et al. (1996) found an exponential relationship between rooting density and depth, suggesting that the greatest concentrations of roots are found near the surface. In addition, since cores from the lower depths in this study contained greater volumes of soil and roots, perhaps they are therefore more strongly correlated to agronomic performance.

Carbon and N associated with silt and clay were strongly positively correlated with yield at both AONR and 0N at 5 to 25 cm (Table 2.3). This may indicate that SOM associated with free silt and clay contributes significantly to crop performance. Aggregate hierarchy theory predicts that SOM associated with silt and clay particles is highly

processed and strongly bound with the mineral soil (Tisdall and Oades, 1982; Gunina and Kuzyakov, 2014). However, the mean residence time of mineral-associated C in a forest soil did not differ from that of the light fraction C in some of Crow et al.'s (2007) incubations of forest soils. Recent work has shown that some of the mineral-associated SOM is bioavailable, and the large bacterial population associated with silt and clay may facilitate its decomposition (Kleber et al., 2007; Kögel-Knabner et al., 2008). The zonal model of organo-mineral associations presented in Kleber et al. (2007) suggests that there may be several layers of organic material on mineral surfaces, and that turnover of material in the outermost or "kinetic" layer may be rapid depending on exchange kinetics and other soil solution factors. Since the silt and clay in this fraction is not bound into aggregates, SOM which becomes unbound to the mineral surfaces is immediately accessible to microbes for mineralization. Furthermore, Kong et al.'s (2007) ^{15}N tracer study showed that synthetic fertilizer N was retained more by association with silt and clay particles than aggregates, and that additions of synthetic fertilizer N lead to faster turnover of N on the silt and clay fraction. The positive correlation between silt and clay C and N and yield suggests this process benefits the corn crop. Although we assume that N mineralization is more important for crop yields than C mineralization, it is interesting to note that silt and clay C is as strongly correlated to yield as silt and clay N at AONR, and more strongly correlated at 0N. A strong relationship between soil C and yield was also found by Culman et al. (2013), who determined that soil C mineralization at planting to be a better predictor of grain yield, total biomass and total N than pre-plant $\text{NO}_3\text{-N}$. There were very strong

correlations between C and N in all fractions (data not shown), for example, silt and clay C and silt and clay N at 5 to 25 cm, $r = 0.95$, $P < 0.0001$.

Yield at AONR and 0N were negatively correlated with the proportion of soil in occluded microaggregates at 0 to 5 cm, and occluded microaggregate C and N at 5 to 25 cm. Yield at AONR was also negatively correlated with occluded microaggregate N at 0 to 5 cm. This is likely due to the inaccessibility of C and N associated with microaggregates within macroaggregates. Many studies have shown the stability of occluded microaggregates, especially in systems with reduced tillage, and suggested that protecting C in occluded microaggregates is desirable for soil health and C sequestration (Six et al., 2000a; Denef et al., 2007; Lal, 2013). However, if a soil stores a large proportion of SOM in occluded microaggregates, less mineralization of plant-available nutrients will occur than in a system that stores more SOM in physically accessible areas of the soil matrix. In soils like the Plano silt loam, there may be a tradeoff between promoting long-term storage of inaccessible C and maintaining SOM in a manner that renders it accessible to mineralization and subsequent crop uptake on an agronomic timescale.

At 25 to 50 cm, there was a significant positive correlation between free microaggregate weight, C and N, and yield at AONR (Table 2.3). This may reflect the importance of these stable aggregates as a source of slowly releasing nutrients at depth. The positive relationship with microaggregates may also reflect the water holding capacity of the soil. Small pores in microaggregates favor water retention more than macroaggregates. Zhuang et al. (2008) showed that the presence of SOM in pores in microaggregates increased water retention in microaggregates, and so where we found enrichment

in microaggregate-C and N, for example in CSW at 25 to 50 cm, this may have positively impacted crop growth.

Overall, the mix of positive and negative correlations between yield and soil measurements reflect the fact that SOM accumulation and protection is not driven by the same variables as corn performance. Agronomic performance is primarily related to nutrient and water availability, as well as favorable temperatures. Since these factors, especially soil moisture in microclimates, may influence microbial activity, and agronomic performance determines quality and quantity of plant biomass inputs to soil, the agronomic system affects the SOM. Particulate organic matter accrual and C and N enrichment are driven by biomass inputs (Fig. 3.2), physical disturbance of soil structure, and microbial access to the POM material. While increasing inputs and microbial activity may be beneficial to both POM accrual and corn growth, the effect of disturbance is opposite. Crops may benefit from disturbance of the soil structure in order to prepare a seedbed and control weeds, but disturbance will likely lead to disruption of aggregates and increased exposure of POM to microbial decomposition.

Although past studies have found aggregate stability, along with SOC, to be a top determinant of corn grain yield (Shukla et al., 2004; Blanco-Canqui et al., 2006), our work contradicts these relationships. Macroaggregates were negatively correlated with yields at all depths and although the relationship was not significant this suggests that increasing the proportion of soil which is aggregate is not beneficial to corn yields. Spring sampled SOC and TN were also significantly negatively correlated with yield. Although SOC has been positively correlated with yield in a variety of previous studies (e.g., Aref and Wander, 1997; Jagadamma et al., 2008; Culman et al., 2013), the high

temporal and spatial variation of SOC and TN (Cambardella et al., 1994) make them unsuitable for yield prediction across the landscape. Our exploratory correlations are not robust enough to build predictive models of yield from the dynamic properties measured, but they suggest that further research might explore SOM outside of macroaggregates as a diagnostic of crop performance.

Fall and spring sampling had a significant effect on SOC and TN, as evidenced by the opposite signs on correlations with yield in many cases. For example, spring SOC at 5 to 25 cm was significantly negatively correlated to yield at AONR at -0.57, while fall SOC at 5 to 25 cm was non-significantly positively correlated to yield at AONR at 0.22 (Table 2.3). The correlation between spring and fall sampled SOC was 0.34 (not significant) at 0 to 5 cm, 0.71 ($P=0.031$) at 5 to 25 cm, and 0.53 ($P=0.088$) at 25 to 50 cm, indicating that seasonal sampling variation diminished with depth. Correlation coefficients for spring and fall sampled TN are also more positive with depth, but none were significant: at 0 to 5 cm, $r=0.099$, at 5 to 25 cm, $r=0.29$, at 25 to 50 cm, $r=0.48$. Paired t tests of the two sampling dates revealed a significant difference of 0.46 g C kg⁻¹ ($P=0.005$) soil and 0.029 g N kg⁻¹ ($P=0.007$) soil, with greater values found in the fall. It may be that C and N were exposed to decomposition when tillage was performed after fall soil sampling, and that some mineralization occurred over the winter months. The harvest of annual plants leads to a flush of bioavailable C and N, and Martín-Lammerting et al. (2013) found significantly higher values of SOC, TN, POM-C, and POM-N in July after wheat harvest compared to other sampling points in the same growing season. Seasonal variation of C and N is not often discussed in the literature as it is assumed to be considerably less than spatial variation (Cambardella et al., 1994),

but the timing of sampling appears to have a significant effect on data and should be taken into consideration.

CONCLUSIONS

We explored correlations between POM and aggregate-associated C and N and yield at AONR or 0N, and found that POM is not a good predictor of yield. Silt and clay associated C and N were significantly positively correlated to yields, suggesting that SOM released from un-aggregated soil may be an important source of mineralized N. Systems varying in rotation and tillage had a significant effect on POM concentration only at 0 to 5 cm, but aggregate distribution and C and N enrichment varied down to 50 cm. The interaction between system and depth on aggregate measurements suggests that the cropping systems distribute and store C and N differently throughout the soil profile although significant differences were found among systems primarily at 25 to 50 cm, where CSW was enriched in microaggregate-C and N and depleted in silt and clay-C and N. Our work suggests that in a temperate highly aggregated Mollisol, tillage does have an effect on aggregate distribution and C and N enrichment, and these properties in turn affect crop yields.

Table 2.1. Treatment means and ANOVA p-values for particulate organic matter (POM) and whole soil C and N in three grain cropping systems at the Wisconsin Integrated Cropping Systems Trial (WICST). Lowercase letters indicate a significant difference across systems among depths, $P < 0.10$.

Treatment	POM	POM-N	POM-C	POM C:N	POM-C: SOC	POM-N: TN	SOC	TN	Whole C:N
	g kg ⁻¹						g kg ⁻¹		
System†									
CC	0.498	0.159	2.445	16.0	0.110	0.078	18.7	1.78	12.0
CS	0.582	0.149	2.317	16.5	0.121	0.081	15.6	1.58	11.4
CSW	0.465	0.136	2.044	17.2	0.113	0.077	16.1	1.07	11.2
Depth									
0 to 5 cm	0.649 a	0.305 a	4.889 a	18.7 a	0.208 a	0.138 a	24.1 a	2.25 a	12.5 a
5 to 25 cm	0.497 b	0.106 b	1.523 b	16.7 b	0.087 b	0.060 b	17.9 b	1.76 b	11.8 a
25 to 50 cm	0.399 c	0.0335 c	0.394 c	14.4 c	0.050 c	0.037 c	8.46 c	0.951 c	10.4 b
ANOVA					<i>P</i> -value				
System (S)	0.0038	0.3808	0.2442	0.3751	0.2929	0.6426	0.1187	0.1780	0.5017
Depth (D)	<0.0001	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	0.0054
S x D	0.0585	0.3287	0.2347	0.1030	0.1116	0.1792	0.0923	0.7435	0.2716

† CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover.

Table 2.2. Results of one-way ANOVA analyses for effect of cropping system and depth on aggregate distribution and proportion of soil C and N found in aggregates. Under treatment effects, values are presented in order from greatest to smallest and significance is based on $\alpha=0.10$. Interaction effects are presented in Figures 1-3.

Source of variation	Soil fraction					
	Macroaggregates		Microaggregates		Silt and clay	
	Proportion of total soil dry weight found in fraction					
	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects
System (S)	0.1893		0.5355		0.4700	
Depth (D)	≤ 0.0001	b=a>c†	≤ 0.0001	a=b>c	0.0004	c>b>a
S x D	0.0823		0.0923		0.0145	
	Proportion of total soil N found in fraction					
	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects
System (S)	0.4663		0.0029	CC=CSW>CS ‡	0.3586	
Depth (D)	0.0015	b>a>c	0.0015	a>c>b	0.1751	
S x D	0.7768		0.0064		0.0198	
	Proportion of total soil C found in fraction					
	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects
System (S)	0.5463		0.0047	CSW>CC>CS	0.0782	CS>CSW
Depth (D)	0.0031	b=a>c	0.0016	a=c>b	0.0748	c>a
S x D	0.6732		0.0025		0.0097	

† a, 0 to 5 cm; b, 5 to 25 cm; c, 25 to 50 cm.

‡ CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseem clover.

Table 2.2. (continued) Results of one-way ANOVA analyses for effect of cropping system and depth on aggregate distribution and proportion of soil C and N found in aggregates. Under treatment effects, values are presented in order from greatest to smallest and significance is based on $\alpha=0.10$. Interaction effects are presented in Figures 2.1 to 2.3.

Source of variation	Soil fraction					
	Occluded microaggregates		Occluded silt and clay		Coarse particulate organic matter	
	Proportion of total soil dry weight found in fraction					
	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects
System (S)	0.0969	CC>CS=CSW‡	0.0777	CS>CSW	0.7290	
Depth (D)	≤0.0001	a>b>c†	0.0020	a=b>c	0.0044	a>c=b
S x D	0.1939		0.0556		0.0315	
	Proportion of total soil N found in fraction					
	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects
System (S)	0.1824		0.4331		0.9059	
Depth (D)	0.0005	b>a>c	0.4140		0.0028	a>b>c
S x D	0.7932		0.6331		0.0653	
	Proportion of total soil C found in fraction					
	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects	<i>P</i> -value	Treatment effects
System (S)	0.1871		0.5212		0.7154	
Depth (D)	0.0003	b>a>c	0.4786		0.0025	a>b>c
S x D	0.5841		0.7493		0.1676	

† a, 0 to 5 cm; b, 5 to 25 cm; c, 25 to 50 cm.

‡ CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseem clover.

Table 2.3. Results of correlations between aggregate and particulate organic matter (POM) concentration and C and N and yield at the agronomically optimum nitrogen rate (AONR) and 0 kg N ha⁻¹ (0N).

	Grain Yield at ANOR	Grain Yield at 0N		Grain Yield at ANOR	Grain Yield at 0N		Grain Yield at ANOR	Grain Yield at 0N	Grain Yield at ANOR	Grain Yield at 0N	
Whole Soil <i>g C or N kg⁻¹ soil</i>			C in fractions <i>proportion SOC in fraction</i>			N in fractions <i>proportion TN in fraction</i>			Soil Fraction <i>proportion of whole soil mass</i>		
Depth	<i>r value</i>			<i>r value</i>			<i>r value</i>			<i>r value</i>	
0-5 cm‡											
SOC, spring	-0.25	-0.26	POM-C	-0.20	-0.15	POM-N	-0.22	-0.13	POM	0.22	0.078
SOC, fall	0.13	0.28	Macro-C	-0.17	0.078	Macro-N	-0.30	-0.061	Macro	-0.40	-0.41
TN, spring	-0.21	-0.068	Micro-C	-0.13	0.28	Micro-N	-0.10	0.29	Micro	0.21	0.23
TN, fall	-0.049	-0.098	S&C-C	0.23	0.17	S&C-N	0.23	0.17	S&C	0.39	0.39
			OMicro-C	-0.47	-0.11	OMicro-N	-0.52†	-0.16	OMicro	-0.58†	-0.57†
			OS&C-C	0.04	-0.16	OS&C-N	0.14	-0.069	OS&C	0.0092	-0.042
			cPOM-C	-0.29	0.12	cPOM-N	-0.32		cPOM	-0.14	-0.083
5-25 cm											
SOC, spring	-0.57†	-0.23	POM-C	-0.099	-0.12	POM-N	-0.089	-0.11	POM	-0.12	0.10
SOC, fall	0.22	0.39	Macro-C	0.024	0.15	Macro-N	0.089	0.11	Macro	-0.22	-0.089
TN, spring	-0.52†	-0.62**	Micro-C	0.36	0.20	Micro-N	0.26	0.056	Micro	0.34	0.18
TN, fall	0.25	0.44	S&C-C	0.74**	0.65*	S&C-N	0.70*	0.57†	S&C	0.16	0.054
			OMicro-C	-0.64*	-0.36	OMicro-N	-0.78**	-0.52†	OMicro	-0.15	0.028
			OS&C-C	-0.17	-0.25	OS&C-N	-0.1	-0.17	OS&C	-0.36	-0.34
			cPOM-C	0.47	0.035	cPOM-N	0.55†	0.12	cPOM	0.39	0.12

† Significant at the 0.10 probability level.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

‡SOC, soil organic carbon; TN, total nitrogen; POM, particulate organic matter; Macro, macroaggregates; Micro, microaggregates; S&C, silt and clay; OMicro, occluded microaggregates; OS&C, occluded silt and clay; cPOM, coarse particulate organic matter.

Table 2.3. (continued). Results of correlations between aggregate and particulate organic matter (POM) concentration and C and N and yield at the agronomically optimum nitrogen rate (AONR) and 0 kg N ha⁻¹ (0N).

	Grain yield at ANOR	Grain yield at 0N		Grain yield at ANOR	Grain yield at 0N		Grain yield at ANOR	Grain yield at 0N		Grain yield at ANOR	Grain yield at 0N
Whole soil			C in fractions			N in fractions			Soil fraction		
g C or N kg ⁻¹ soil			proportion SOC in fraction			proportion TN in fraction			proportion of whole soil mass		
Depth	<i>r value</i>		<i>r value</i>			<i>r value</i>			<i>r value</i>		
25 to 50 cm‡											
SOC, spring	-0.40	-0.38	POM-C	-0.25	0.11	POM-N	-0.37	-0.12	POM	-0.19	-0.037
SOC, fall	-0.43	-0.24	Macro-C	-0.15	-0.021	Macro-N	-0.20	0.15	Macro	-0.24	-0.087
TN, spring	-0.40	-0.51	Micro-C	0.59*	0.36	Micro-N	0.59*	0.35	Micro	0.57†	0.46
TN, fall	-0.38	-0.29	S&C-C	-0.32	-0.24	S&C-N	-0.30	-0.21	S&C	-0.29	-0.33
			OMicro-C	-0.068	0.083	OMicro-N	-0.10	0.099	OMicro	-0.33	-0.038
			OS&C-C	0.16	0.12	OS&C-N	0.12	-0.0092	OS&C	-0.085	-0.054
			cPOM-C	-0.20	-0.44	cPOM-N	-0.24	-0.47	cPOM	-0.11	-0.29

† Significant at the 0.10 probability level.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

‡SOC, soil organic carbon; TN, total nitrogen; POM, particulate organic matter; Macro, macroaggregates; Micro, microaggregates; S&C, silt and clay; OMicro, occluded microaggregates; OS&C, occluded silt and clay; CPOM, coarse particulate organic matter.

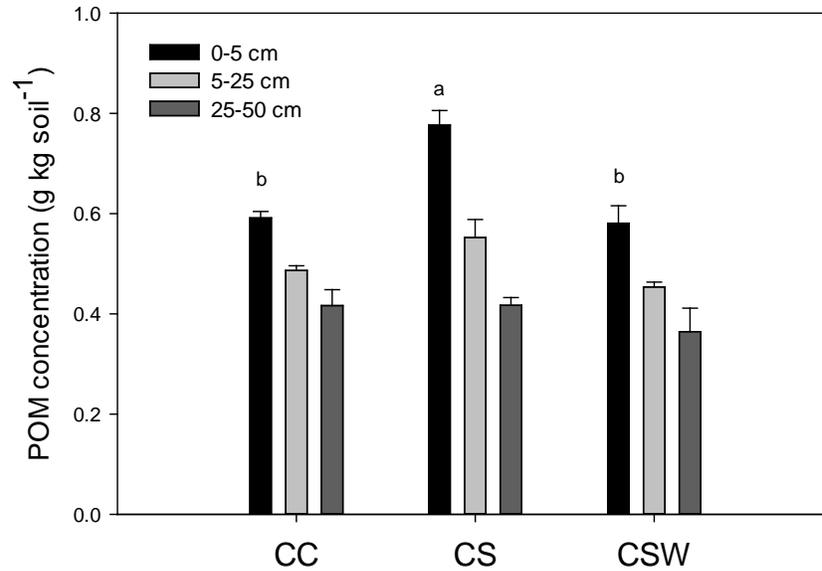


Figure 2.1. Concentration of particulate organic matter (POM) by depth in three grain cropping systems at the Wisconsin Integrated Cropping Systems Trial (WICST). Error bars represent standard error and lowercase letters denote significant differences among systems at a given depth: CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover.

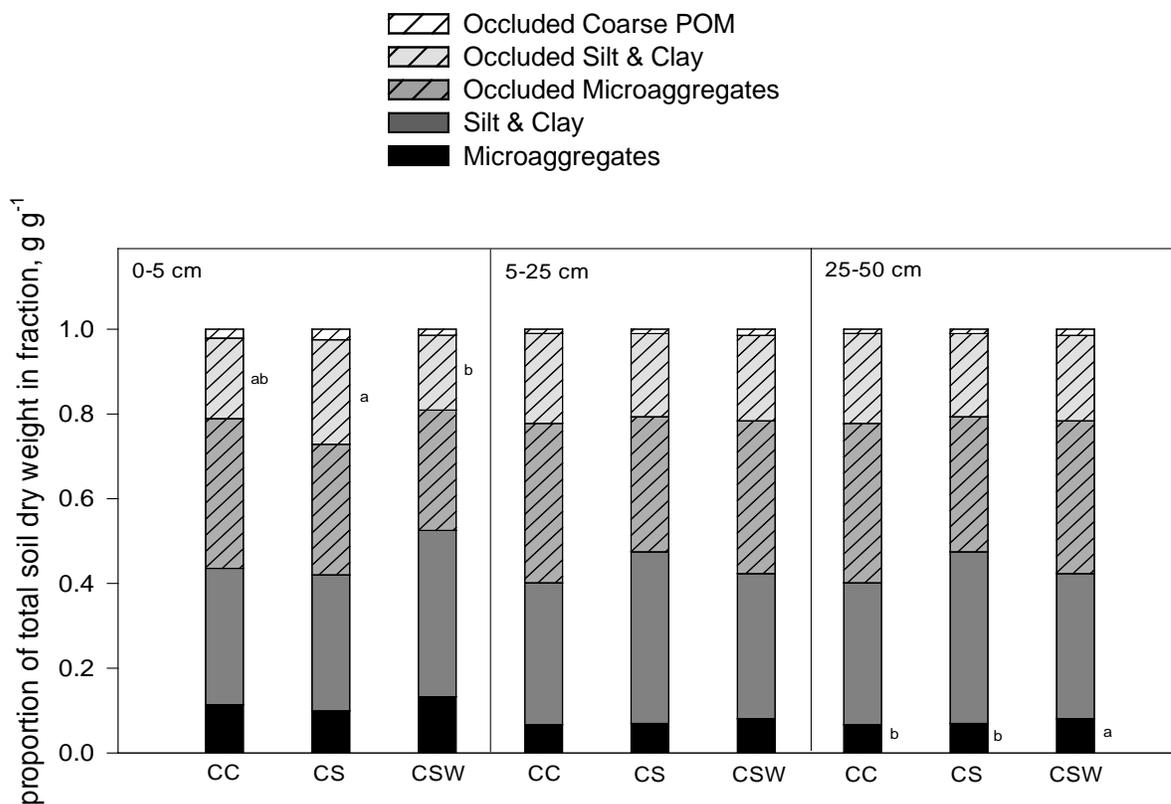


Figure 2.2. Proportion of soil mass found in each soil fraction in each cropping system at depths 0 to 5, 5 to 25, and 25 to 50 cm. Bars representing fractions isolated from within macroaggregates include diagonal lines. Lowercase letters represent significant differences among systems at a given depth. CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseem clover.

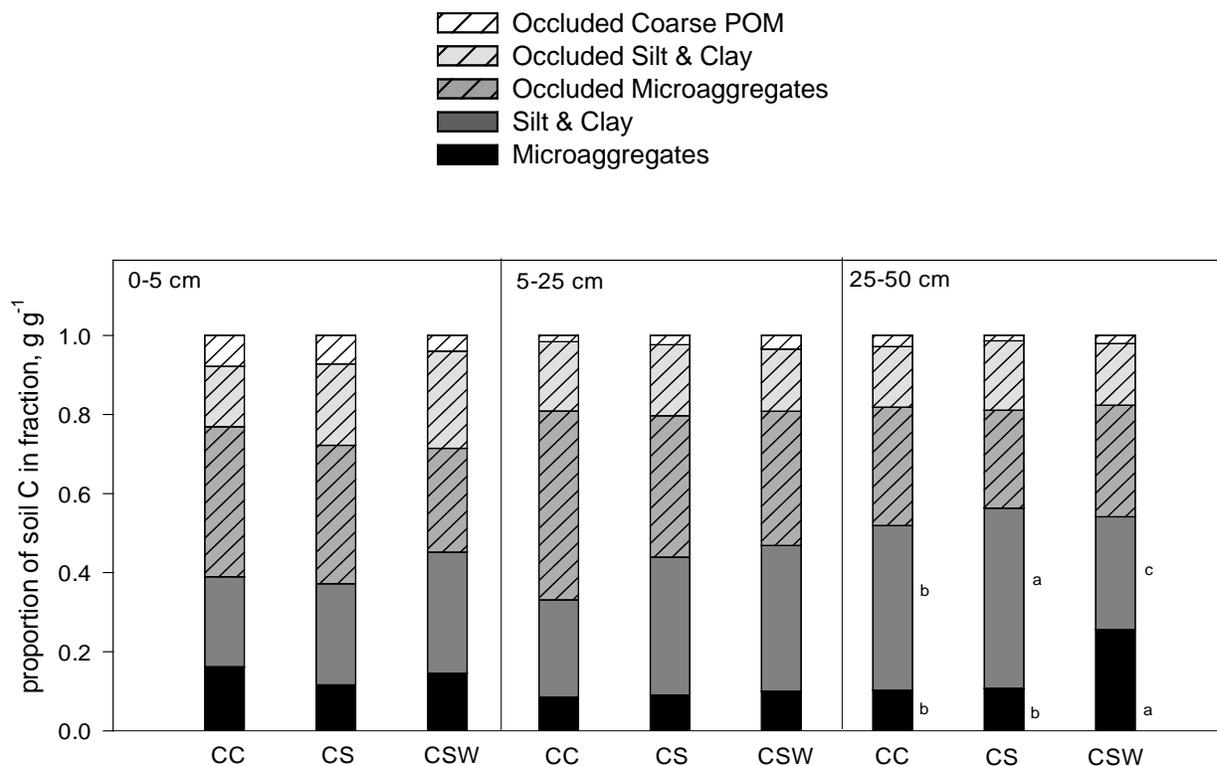


Figure 2.3. Proportion of soil C found in each soil fraction in each cropping system at depths 0 to 5, 5 to 25, and 25 to 50 cm. Bars representing fractions isolated from within macroaggregates include diagonal lines. Lowercase letters represent significant differences among systems at a given depth. CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseemclover.

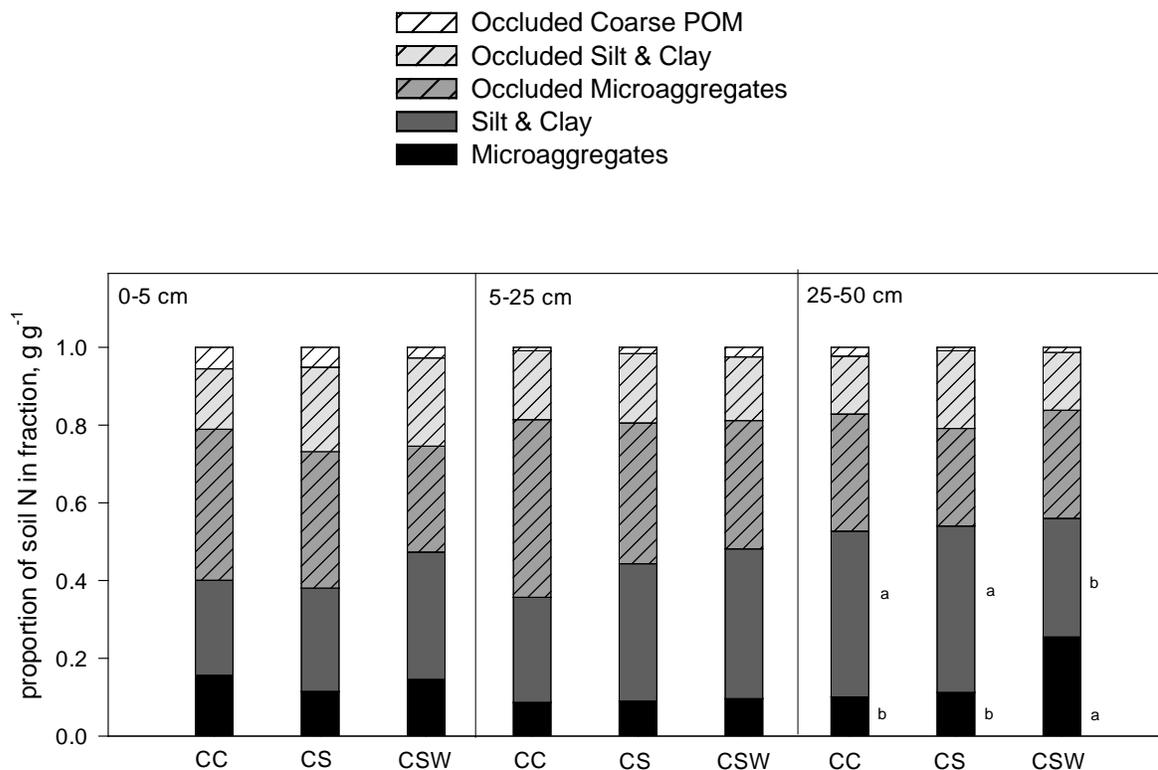


Figure 2.4. Proportion of soil N found in each soil fraction in each cropping system at depths 0 to 5, 5 to 25, and 25 to 50 cm. Bars representing fractions isolated from within macroaggregates include diagonal lines. Lowercase letters represent significant differences among systems at a given depth. CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseem clover.

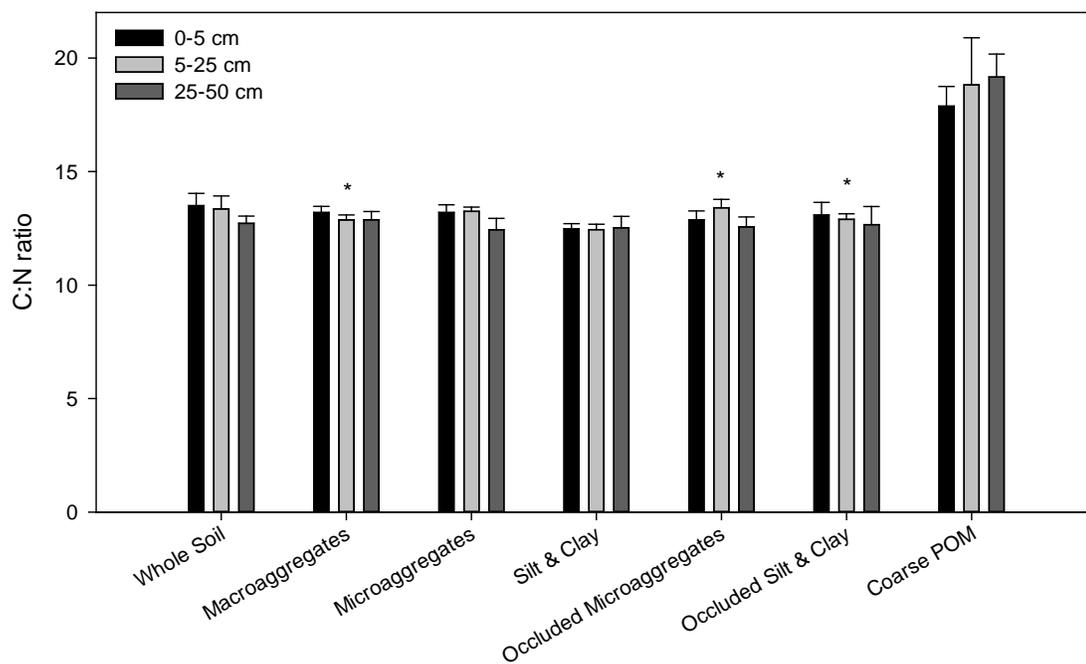


Figure 2.5. Carbon to nitrogen ratio of aggregate fractions and whole soil. An asterisk above a set of three bars indicates that the C:N ratio of that aggregate fraction differs significantly among depths, $P < 0.10$. The C:N ratio of the coarse particulate organic matter (POM) is greater than other fractions at all depths.

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CHAPTER 3

Long-term Tillage, Rotation and Perennialization Effects on Particulate and Aggregate Organic Matter

ABSTRACT

Perennialization and reducing tillage have shown increased soil organic carbon (SOC) in both protected aggregate and particulate organic matter (POM). This study investigates how 20 years of management varying in crop rotation, tillage intensity, and organic management have affected POM and aggregate C and N. The six systems from the Wisconsin Integrated Cropping Systems Trial (WICST) were: chisel plowed continuous corn (*Zea mays* L.) (CC), strip-tilled corn-soybean (*Glycine max* L.) (CS), organically managed corn-soybean-winter wheat (*Triticum aestivum* L.) with an oat (*Avena sativa* L.) cover crop inter-seeded with red clover (*Trifolium pretense* L.) (CSW), 3 years of alfalfa (*Medicago sativa* L.) followed by corn (C3A), organically managed oat/alfalfa for 2 years followed by corn (C2A) and a rotationally grazed pasture seeded to a mixture of red clover, timothy (*Phleum pretense* L.), smooth brome grass (*Bromus inermis* L.) and orchardgrass (*Dactylis glomerata* L.) (P). We found significantly lower concentrations of POM in CS and CSW, and significantly greater concentrations of POM-C in P and POM-N in P and C2A. The CSW system had a lower proportion of soil in macroaggregates and lower stocks of C and N within macroaggregates. Our results indicate that the regular cultivation for weed control in CSW is likely disrupting formation of aggregates and storage of C and N therein. However, in systems that were chisel plowed every 1 to 3 years, high levels of biomass

C inputs may support high levels of POM-C and POM-N as well as soil aggregation equivalent to the P system.

INTRODUCTION

Soil aggregate dynamics have proven to be a useful, measurable tool for investigating carbon (C) storage in natural and agroecosystems (Six and Paustian, 2014). Aggregate hierarchy theory as proposed by Tisdall and Oades (1982) maintains that stability of aggregates increases as size decreases, due to the nature of the soil organic matter (SOM) acting as aggregate binding agents. Physical fractionation and ^{14}C abundance have revealed that organo-mineral associations with silt and clay are the most stable, followed by binding into microaggregates (53 to 250 μm) by persistent binding agents such as glomalin, and finally binding into macroaggregates (<250 μm) by more temporary organic compounds such as polysaccharides, fungal hyphae and roots (Tisdall and Oades, 1982; Jastrow, 1996; Deneff et al., 2001; Six et al., 2004; Kleber et al., 2007; Gunina and Kuzyakov, 2014). Although more complex organic molecules such as aromatics and lignin have a lower initial decomposition rate, physical protection plays a critical role in stabilizing even simple bioavailable C for decades or even centuries (Marschner et al., 2008; Schmidt et al., 2011). However, this apparent stabilization is vulnerable to disturbance due to land management practices. Tillage has been shown to especially affect occluded microaggregates, which form within macroaggregates, and the loss of C associated with these occluded microaggregates has been shown to account for the reduced soil organic carbon (SOC) often seen in conventionally tilled farmland compared to no-till (Six et al., 1999; Deneff et al., 2004, 2007). Soil

texture also has an effect on the degree of aggregation (Tiemann and Grandy, 2014) as there must be sufficient free silt and clay particles available for aggregation to occur (Jastrow, 1996).

Soil under perennial cropping systems has been shown to store more C than annual crops, as the increased root presence and associated microbial activity provides SOM around which aggregates are formed, and lack of tillage maintains even chemically labile C in protected microsites (Culman et al., 2010; DeLuca and Zabinski, 2011). Jastrow showed that as prairie restoration progressed, the change in vegetation and increasing biomass C inputs lead to increased SOC and aggregation compared to an adjacent cornfield (1996). Perennials' year-round root system encourages greater fungal abundance in the microbial community, and the important role of fungal hyphae in aggregation is shown by the concurrent increase in aggregation and fungal amino sugars in a no-till trial compared to conventional tillage (Simpson et al., 2004), and a decline in aggregation after application of fungicide in an incubation experiment (Bossuyt et al., 2001). The no-till conversion of perennial grassland to annual wheat halved root production and significantly reduced microbial biomass C in the top 40 cm of soil (DuPont et al., 2010), so changes in SOC upon shifts to annual crop production are due to crop physiology as well as disturbance.

The extent and mechanism of soil C storage may be dynamic when a system is undergoing drastic changes in management, which makes it difficult to predict whether SOC is at equilibrium at the time of measurement. Long-term studies show that SOC declined quite rapidly when prairies were converted to agriculture in the U.S. Corn Belt, but a stable C pool persists (Balesdent et al., 1988; Huggins et al., 1998; DeLuca and

Zabinski, 2011). By separating the large-size, labile particulate organic matter (POM), researchers have been better able to discern early shifts in C and N due to management regime. The POM-C has been shown to be easily decomposed (Mirsky et al., 2008) and reflective of inputs (Marriott and Wander, 2006b). The POM-C and POM-N were increased in continuous corn compared to corn-soybean rotation (Coulter et al., 2009), and decreased in cultivated soils compared to native sod more drastically than SOC or total nitrogen (TN) (Cambardella and Elliott, 1992). Rotations with livestock, prairie and manure treatment have also increased POM-C compared to cornfields (Hernandez-Ramirez et al., 2009; Maughan et al., 2009). The sensitivity to management shown in these studies suggests that POM is a biologically labile but physically protected pool of SOM, so when management disrupts soil structure, POM-C and POM-N are affected before SOC or TN.

More diverse rotations and minimal tillage are often seen as effective at sequestering SOC by the farming community, but trials have shown both positive and negative responses of SOC to no-till regime and increasing rotational diversity. Many field studies have shown that reducing tillage increases storage of SOC (Lal, 1984; Havlin et al., 1990; Six et al., 1999; Halvorson et al., 2002), but there is also evidence that tillage regime does not change total SOC in some conditions, or may redistribute SOC lower in the soil profile (Baker et al., 2007). For example, Bundy et al. (2011) found no change in SOC in the top 15 cm of soil after 50 years of moldboard plowing in continuous corn with high rates of N fertilization. The variation in SOC response to tillage may be due to the variation in mechanism of SOM storage. Tillage has been shown to influence the persistence of SOC in microaggregates within macroaggregates,

as the disruption increases the turnover rate of macroaggregates such that microaggregates do not form or are bound by less persistent SOM (Six et al., 2000b), while C associated with silt and clay is more stable despite short-term management shifts (Beare et al., 1994; Chivenge et al., 2007). The effect of rotation on SOC has been less well studied, although it appears that a more diverse blend of organic inputs supports a more diverse microbial system (Liang et al., 2012). When tillage and rotation treatments were studied jointly, no-till increased macroaggregate C in a continuous barley system but not in a barley-fallow system, and only at 0 to 5 cm (Álvaro-Fuentes et al., 2009). These interactions have not been broadly studied.

A wide range of management strategies are practiced by farmers. In Wisconsin, over one million hectares of forage and over half a million hectares of hay were grown in 2012, primarily for the state's dairy livestock (Wisconsin Agricultural Statistics Service, 2013), and management intensive grazing is practiced on 22% of Wisconsin dairy farms and 42% of Wisconsin beef farms to supplement feed during the growing season (Paine and Gildersleeve, 2011a, b). The inclusion of forage perennials in a cropping system may have important effects on storage of POM and aggregate organic matter, even if they are part of a rotation which also includes annuals. In this work, we measure aggregate organic matter and POM in the Wisconsin Integrated Cropping Systems Trial (WICST), which was designed to test the feasibility of various sustainable agricultural practices within a holistic systems trial. The six management systems combine tillage, crop rotation, and fertility inputs in a manner intended to reflect realistic conditions. Previous work (Chapter 2) focused on the three annual grain systems in WICST and found that aggregate fractions C and N varied more drastically

than POM C and N, but it remains to be seen whether these metrics can discern the degrees of perennialization in the other three WICST systems, which are designed as forage rotations exemplary of Wisconsin's dairy industry. In this study, our objectives were to investigate whether aggregate and POM C and N are appropriate metrics for evaluating C and N storage across a diverse range of cropping systems. Based on past work, we hypothesized that POM would be a sensitive indicator of tillage and organic management and that systems with less tillage and greater perennialization would show greater aggregate stability and enrichment in C and N.

MATERIALS AND METHODS

The Wisconsin Integrated Cropping Systems Trial (WICST)

The study took place within the WICST, which was established at the University of Wisconsin Agricultural Research Station in Arlington, Wis. in 1990. The soil is Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudolls). Prior to settlement in the mid-1800s, this area was likely tallgrass prairie. Wheat and feed for dairy herds was produced on the land until 1960. Between 1960 and the initiation of the WICST, the primary crops grown were corn and alfalfa with dairy manure serving as major nutrient source. In 1989, corn was planted throughout the 9.71-ha trial in order to improve the uniformity of the land's history and take baseline measurements. At that time, the average organic matter content (0 to 5 cm) was 47 g kg^{-1} (based on loss on ignition), pH was 6.5 (1:1.3 soil/water), soil test P (Bray-1) was 108 mg kg^{-1} , and exchangeable K was 255 mg kg^{-1} (Posner et al., 1995). The mean annual temperature at

Arlington is 6.9°C and mean annual precipitation is 898 mm (1981-2010, National Climate Data Center).

The WICST is a randomized complete block trial, with six cropping systems represented in all phases with four replications. A staggered start was performed so that replication occurred in time as well as space. Plots are 15.5 by 18.3 m, or 0.3 ha, and commercial farm-scale equipment is used for all field work. Management has changed slightly over the years to reflect best management practices in the area. The WICST was designed with input from farmers, researchers, and extension agents, and applies six sets of management practices as whole systems (Posner et al., 1995). The weakness of this trial is that no one practice can be tested by itself (i.e., tillage intensity across a range of rotations), but the strength of WICST is that each system represents an on-farm context along a gradient of low to high input systems. The six cropping systems were chosen to represent a spectrum of biological diversity in two overarching categories: three cash-grain and three forage systems. The three cash-grain systems are: (1) continuous corn (*Zea mays* L.) (CC), (2) corn-soybean [*Glycine max* (L.) Merr.] (CS), and (3) an organically managed corn-soybean-winter wheat (*Triticum aestivum* L.) with an oat (*Avena sativa* L.) cover crop inter-seeded with red clover (*Trifolium pretense* L.) (CSW). The three forage systems are: (1) 3 years of alfalfa (*Medicago sativa* L.) followed by corn system (C3A), (2) an organically managed oat/alfalfa for 2 years followed by corn (C2A) and (3) a rotationally grazed pasture seeded to a mixture of red clover, timothy (*Phleum pretense* L.), smooth brome grass (*Bromus inermis* L.) and orchardgrass (*Dactylis glomerata* L.) (P). Slurry manure is applied in the fall prior to corn planting and the first year alfalfa seeding in C3A and C2A and pelletized chicken

manure is applied to corn and wheat in CSW. Corn in CC, CS and C3A receives commercial fertilizer in the form of urea at planting at UW-Madison recommended rates (Laboski and Peters, 2012). The CC, CSW, C3A, and C2A systems are all chisel plowed in the fall prior to corn planting and the CS system is strip tilled in the spring immediately prior to corn planting. A chisel plow is also used prior to soybean planting in CSW and alfalfa seeding in C3A and C2A. Soybeans in CS are planted using a no-till drill.

Particulate Organic Matter (POM)

Soils were sampled to 50 cm for POM in April 2013 using a hydraulic tractor-mounted probe of 2.4 cm diam. In the three grain systems, samples were split into 0 to 5 and 5 to 25 cm sections, analyzed for POM separately, and the 0 to 25 cm data presented here is a weighted average of 0 to 5 and 5 to 25 cm. In the three forage systems, samples were split into 0 to 25 and 25 to 50 cm. Six cores per plot were composited into a single sample. Samples were dried at 100°C and ground to pass a 1-mm sieve. To extract POM, samples were analyzed according to an updated version of the method in Marriott and Wander (2006a; Ugarte, personal communication, 2013). Ten-gram soil samples were placed in 30-mL plastic bottles with 20 mL sodium hexametaphosphate (SHMP) (100 g per 2 L solution). Soils soaked 17 to 21 hours. Bottles were capped with a square of 53- μ m mesh and a top with a 1-cm hole drilled through it, allowing small material to escape while POM, the material $> 53 \mu$ m, was retained in the bottle. Fifteen 30-mL bottles at a time were placed into a 26 by 17 by 7.5 cm Ziploc storage container with 700 mL SHMP. The whole container was placed on a reciprocal shaker and shaken at low speed for 1 hour. After 1 hour, the SHMP was

poured off and replaced with 700-mL deionized water. The container was shaken on low speed for 10 minutes and then water was removed and replaced with 700-mL fresh deionized water. This was repeated for a total of 12 rinses. Finally, each 30-mL bottle was emptied into a pre-weighed square of 53- μ m mesh. Samples were rinsed with deionized water until water ran clear. Mesh squares containing samples were dried at 50 to 60°C. The POM concentration was calculated as the mass of POM divided by the mass of dry soil analyzed, in this case, 10.0 g. Dry samples of POM were weighed and scraped into plastic Whirl-pak bags for storage.

Aggregate Fractionation

Soil samples for aggregate fractionation were taken November 2013 using a hydraulic probe on the back of a tractor. Nine cores (5.2 cm diam.) were taken to a depth of 25 cm in all six systems. Samples were taken from the plots that would be planted in corn in 2014 in CC, CS, CSW, C2A, and C3A, and from the rotationally grazed pasture. Cores were kept refrigerated until analysis. Three cores were dried and weighed to determine bulk density. For aggregate fractionation, soil from the remaining six cores was composited into a single sample for each plot. Each sample was sieved to 4 mm; clumps were gently broken up and large organic material was removed by hand.

A 5.0-g subsample was weighed and dried at 50 to 60°C immediately to determine the moisture content of the soil at the start of the procedure. A 100.0-g sample was weighed for the aggregate fractionation. The sample was placed on top of a 250- μ m sieve inside a plastic basin with the water level 2 cm above the top of the sieve. After 5 minutes of soaking, soil was sieved for 2 minutes by moving the sieve up and down 50 times (approx. 3-cm amplitude). Fine particles on the bottom and sides of the sieve were

rinsed into the basin and fine particles and material on top of the 250- μm sieve, the macroaggregates, were backwashed into a pre-weighed aluminum pan. All water and particles remaining in the plastic basin were poured onto a 53- μm sieve. This material was sieved for 2 minutes by moving the sieve up and down 50 times at approximately 3-cm amplitude. The bottom and sides of the 53- μm sieve were rinsed into the basin, and material on top of the sieve, the microaggregates, was backwashed into a pre-weighed aluminum pan. Material and water remaining in the basin was considered free silt and clay and was rinsed into pre-weighed aluminum pans. All material was dried at 50 to 60°C for a minimum of 24 hours.

A subsample of 20.0 g oven-dried macroaggregates was weighed and placed in a beaker with approximately 50 mL water for 20 minutes. After soaking, macroaggregates were placed in the microaggregate isolator (built by David Sloan, UW-Madison Department of Soil Science, based on specifications from Johan Six; e.g., Six et al., 2002a) with 30 metal beads on a piece of 250- μm mesh on a reciprocal shaker. Soil and beads were shaken on low with running deionized water for 3 to 5 minutes, until all macroaggregates were destroyed and water ran clear through the 250- μm mesh and tube below. Material remaining on top of the 250- μm mesh was placed on top of a 2000- μm sieve nested over a 53- μm sieve. The 2000- μm sieve caught the metal beads and the 53- μm sieve caught remaining sand and coarse particulate organic matter (cPOM), which was larger than 250- μm in diam. The cPOM was backwashed into a pre-weighed aluminum drying pan. Material and water that flowed through the 250- μm mesh in the device were tubed to a 53- μm sieve in a plastic basin. Material in this basin was sieved 50 times for 2 minutes at approximately 3-cm amplitude. The sides and bottom of the

53- μm sieve was rinsed into the basin, and material on top of the 53- μm sieve was backwashed into a pre-weighed aluminum pan. Material from the top of the 53- μm sieve was classified as occluded microaggregates. Material that passed through the 53- μm sieve was considered occluded silt and clay, and was rinsed into a pre-weighed aluminum drying pan. All material was dried at 50 to 60°C for a minimum of 24 hours. All fractions were weighed after removing from the oven and cooling to room temperature. Weights were recorded and fractions were stored in plastic Whirl-pak bags until C and N analysis.

Carbon and Nitrogen Analysis

A subsample of each fraction, whole soil, and POM was ground in a 2-mL micro-centrifuge tube with a ball bearing on a paint shaker. Subsamples weighing eight to 10 mg were packed into 5 by 9 mm tin capsules. Carbon and N were determined by dry combustion using a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). Since inorganic C is negligible in these soils, ($<0.05 \text{ g kg}^{-1}$; Paul et al., 2001), organic C was assumed to be the same as total C. Aggregate C and N concentration were determined by multiplying the concentration of C or N in an aggregate fraction by the concentration of C or N in the whole soil. These concentrations were converted to mass C or N per unit area using bulk density measurements taken at the same time. The POM-N was determined by multiplying the concentration of N in POM by the overall concentration of POM in the bulk soil. The proportion of soil N found in POM was determined by dividing the POM-N, g kg^{-1} soil, by the total N, g kg^{-1} soil. The POM-C and proportion of soil C found in POM were calculated in the same manner.

Statistical Analysis

All soil data, including bulk density, POM, POM-C, POM-N, SOC, TN, proportion of C and N in POM, and all aggregate fractions, and C:N ratio of whole soil, POM, and aggregate fractions, were analyzed with ANOVA using PROC MIXED in SAS 9.2 (SAS Institute Inc). Depth (for POM data) and system were treated as fixed effects while block and block-by-system interactions were treated as random effects. Where assumptions of equal variance appeared to be violated, the transformations $\arcsin(x)$, $\arcsin(\sqrt{x})$, $x/1-x$, and $\log(x/(1-x))$, were modeled and assessed in residual plots. Where more than one transformation improved the fit of the data by visual examination, the model with the lowest AIC value was selected. While statistics are reported for transformed data, actual values are shown in figures. For the proportion of soil N found in POM, the transformation $[\log(x/(1-x))]$ was used; for the proportion of soil C found in POM the transformation $[\log(x/(1-x))]$ was used. Linear regression models between POM measurements and biomass estimates were created in SigmaPlot 12.0 (Systat Software, San Jose, CA). Unless otherwise specified, all significance was determined at $\alpha=0.10$.

RESULTS AND DISCUSSION

Soil Organic Carbon, Total Nitrogen, and Particulate Organic Matter

There was a significant effect of system on POM concentration, POM-N, POM-C, and the proportion of soil C in POM (Table 3.1). Significant differences in whole SOC and TN were also measured among the six systems. The P system had significantly greater SOC than CSW, and significantly greater TN than CS and CSW

(Table 3.1). In the P and C2A systems, POM-C made up a greater proportion of the whole soil C than CC. The SOC, POM concentration, and POM-C were not significantly different among P, C2A, and CC, but POM-C was increased enough in P and C2A over CC to significantly affect the ratio of POM-C:SOC. No effect of system was seen on C:N ratio of POM or whole soil, but larger C:N ratios in POM than whole soil reflect the plant origin of POM material. As decomposition occurs, C is respired by microbes while N is retained in the soil, so a lower C:N ratio reflects a greater degree of decomposition in the whole soil than the POM. The whole soil C:N ratio range from 10.2 to 11.7 is closer to the reported range of microbial biomass C:N ratio, 9:1 to 12:1 (Cleveland and Liptzin, 2007), suggesting that the whole soil C and N may be largely microbial biomass. There was a significant effect of depth on all measurements, with greater values observed in 0 to 25 cm than 25 to 50 cm.

There was a significant interaction between system and depth in POM-N and POM-C (Table 3.1; Fig. 3.1). At 0 to 25 cm, the rotationally grazed pasture had significantly greater POM-C than all other systems, and significantly greater POM-N than all systems except C2A. The CSW system has significantly lower POM-C and POM-N than C2A, C3A, and P at 0 to 25 cm. At 25 to 50 cm, all systems had statistically similar concentrations of POM-C and POM-N. There is less root biomass at lower depths (Jackson et al., 1996; Tufekcioglu et al., 1998), which leads to lesser accumulation of POM and whole soil C and N at the lower depths. The significantly lower POM-C:SOC and POM-N:TN ratios at the 25 to 50 cm relative to 0 to 25 cm illustrates that POM is not a large component of SOM at this depth. Since it occurred at such small concentrations at 25 to 50 cm even in perennial systems, POM may not be

an appropriate measure for comparing systems at lower depths. Wander et al. (2007) also found no differences between conventional and organic farming systems when POM-C and POM-N were very low.

The greater concentrations of POM-C and POM-N in P and C2A may have been due to the inclusion of perennials in rotation. The P system has been in perennial cover for over 20 years and POM has been shown to be sensitive to plant species composition (Erfanzadeh et al., 2014). Annual crops were measured to have 43% less root biomass than perennial grassland 5 years after establishment (DuPont et al., 2010) and roots have been shown to be preferentially preserved in SOM over shoot biomass (Rasse et al., 2005; Mendez-Millan et al., 2010). Linear regressions between both above and belowground biomass C and POM-C and POM-N were significant at $P < 0.0001$, but no regression with POM concentration was significant (Fig. 3.2). The linear regressions between belowground biomass C and POM-C and POM-N had slopes 2.9 times that of the regression between aboveground biomass C and POM-C and POM-N, suggesting that belowground biomass C has a much stronger influence on POM enrichment in C and N. However, inclusion of perennials did not uniformly increase POM-C and POM-N, as C3A, which rotates 3 years of alfalfa with 1 year of corn, did not significantly differ from CC in POM-N, or CC and CS in POM-C. The minimal tillage in CS likely protected POM to some extent. While it is surprising that continuous plowed annual corn is equivalent in POM, POM-C, and POM-N to the primarily perennial C3A, this may be due to larger overall biomass-C in CC, where approximately $3800 \text{ kg C ha}^{-1}$ aboveground biomass is tilled in each fall. The annual tillage disturbance may be offset in CC by this large biomass input. Corn in the C3A system yields comparably to CC but

is only grown once every 3 years. In alfalfa years, total biomass C in C3A is 4114 kg C ha⁻¹ in the first year (including manure application), 3676 kg C ha⁻¹ in the second year, and 10306 kg C ha⁻¹ the third year, reflecting the time it takes to establish a vigorous stand. Although the biomass inputs in C3A are comparable to CC when averaged across the rotation, perhaps the low C:N ratio of alfalfa (approximately 15.1 according to (Bruulsema and Christie, 1987) leads to increased decomposition and less storage in POM in C3A. Corn residue in CC was measured to have a C:N ratio of 57:1 in 2013 (data not shown).

Aggregate Fractions

Significant differences were seen among systems in concentration of macro-aggregates, free silt and clay, and occluded microaggregates (Table 3.2; Fig. 3.3). The CSW system contained significantly less macroaggregates and occluded micro-aggregates and significantly more free silt and clay than C3A, C2A, and P. The CC system did not differ significantly from any system, and the CS system contained significantly less free silt and clay and more occluded microaggregates than CSW. Generally, these results indicate a significant decrease in soil aggregation, as measured by proportion of soil in micro- or macroaggregates, in the CSW system. Tillage for weed control in CSW consisted of one pass with a tine weeder and two passes with a field cultivator during the 2013 growing season, while CC was last cultivated during 2013 seedbed preparation and no other system experienced tillage in 2013. Tillage has been shown to negatively impact aggregate formation and stability on a loess-derived soil in France (Oorts et al., 2007) and a Michigan Hapludalf (Grandy and Robertson, 2007). Strip tillage on the WICST Argiudolls did not increase aggregate formation

relative to conventional plowing, as we found no significant differences in any aggregate fraction between CC, which is tilled at planting and after harvest annually, and CS, which is strip tilled before corn planting and not tilled in the soybean phase. There was also no significant difference in the macroaggregate concentration among CC and the three forage systems, when we expected that increased perennialization of the forage systems might increase aggregation due to increased root presence. Roots have been shown to hold macroaggregates together (Tisdall and Oades, 1982; Six et al., 2004), and this was likely occurring to some extent, as evidenced by the larger values in macroaggregate content in forage systems. It appears that large biomass inputs in CC are sufficient to maintain soil aggregation and SOM at the same level as corn/alfalfa rotations despite annual tillage.

Models were very similar when C and N were analyzed on a concentration and stock basis, while the aggregate proportion of SOC and TN illuminated fewer differences among systems (Table 3.2; Fig. 3.4 to 3.6). Bulk density differed significantly among the six systems at $P = 0.035$, with CS and CSW having a significantly greater bulk density (1.38 and 1.36 g cm⁻³) than the P system (1.23 g cm⁻³). The C in macroaggregates was greater on a stock and concentration basis in C3A and P than CSW, and N in macroaggregates was greater on a stock and concentration basis in C3A and P than CSW and CC (Fig. 3.5 and 3.6). However, only P had a greater proportion of TN and SOC in macroaggregates than CSW (Fig. 3.4). The occluded microaggregates in P had greater concentration and stock of C and N than CSW, and the occluded silt and clay in CS, C3A, and P had a greater concentration and stock of C and N than CSW. The proportion of C and N associated with free silt and clay was greater in CSW than P. It

has been shown that silt and clay associated C and N are not sensitive to tillage due to the strength of the association with mineral particles (Huggins et al., 1998; Kleber et al., 2007), so CSW, with its additional tillage, is more dependent on silt and clay for storage of SOM.

While comparing P, C3A, and CSW, the difference between highly perennialized and seldom tilled cropping systems and a tilled annual crop rotation is quite apparent: aggregates were more enriched in C and N in perennialized systems. But there were no significant differences in aggregate C and N among CC, CS, and C2A systems, which all include annual crops with disturbance every 1 to 3 years. It may be that soil C in these systems is not yet at equilibrium. Since the initiation of WICST, SOC was lost from all six systems, except in the top 15 cm of the P system (Sanford et al., 2012). The high initial SOC of this prairie-derived Mollisol was supplemented by manure applications, which has long-term stabilizing effects on aggregates for many years prior to the initiation of the WICST trial (Posner et al., 1995; Abiven et al., 2007). There is still prairie-derived C present at Arlington, according to Paul et al. (2001), who estimated the age of SOC to be 485 ± 50 years at 0 to 20 cm and 2620 ± 55 years at 25 to 50 cm, while the non-hydrolyzable C was 2840 ± 50 and 7797 ± 65 . All six systems continue to decompose that historical C and the rate of loss is only slightly affected by the varying management practices in place. However, Tiemann and Grandy's (2014) work on the same Plano silt loam with a similar management history in Wisconsin found evidence of increasing aggregate stability and SOC protection after 5 years of perennial bio-energy crops, especially a native grass mix. According to the aggregate fractionation we performed, the soil under the P system is no more strongly aggregated than the CS or

CC annual systems. The P system does have greater SOC than CS or CC and due to higher enrichment in C and N in the occluded microaggregates and occluded silt and clay fractions, concurring with Tiemann and Grandy's (2014) evidence for C accrual primarily in stable microaggregates. It appears that 20 years of perennialization in the WICST have not significantly affected soil aggregation but some protected microenvironments may be slowing the loss of historical SOC.

The cPOM had an increased C:N ratio compared to all other fractions, reflecting its plant origins, but C:N ratios did not differ among systems in any aggregate fraction (Fig. 3.7). Greater concentrations and stocks of C and N were found in cPOM in C2A than CS, and greater concentrations of C were found in cPOM in P than CS (Fig. 3.4 to 3.6). The cPOM is the material that persists in the microaggregate isolator after macroaggregates have been destroyed, and consists of large, relatively fresh material which has been physically protected with macro-aggregates (Six et al., 2002), so it is reasonable that 2 years' root presence of alfalfa in C2A contributed more cPOM than the annual rotation with significant tillage of CSW, although it is surprising that the C3A and P systems were not equally enriched in cPOM. Since there was no difference among systems in the weight of cPOM isolated from macroaggregates, the differences in cPOM-C and cPOM-N must be driven by the high enrichment in C and N of the cPOM sampled. The cPOM isolated with aggregate fractionation is larger ($>250 \mu\text{m}$) than the POM separated by the Marriott and Wander method ($>53 \mu\text{m}$) and is only macroaggregate protected material, so we do not expect results to be equivalent to the Marriott and Wander POM results. In addition, note that aggregate fractionation samples were taken in plots to be planted to corn in 2014, while POM samples were

taken in plots to be planted to corn in 2013. Although WICST is blocked to account for field variability, we should not compare cPOM and POM in this study to each other. The Marriott and Wander POM was more sensitive to the management gradient at WICST, which is consistent with expectations for this method. As the most variable aggregate fraction, partly due to the very small amount of cPOM present in soil, the cPOM is not as suited to discern differences between management regimes.

CONCLUSIONS

This study explored more subtle effects of management on POM and aggregate C and N than have been found in past studies. At 0 to 25 cm, POM-C and POM-N were increased in a perennial pasture and the proportion of silt and clay associated C and N were increased in an organically managed annual rotation that was tilled for weed control. The C3A and P systems were both enriched in macroaggregate C and N stock and concentration, as well as occluded microaggregate and silt and clay C and N. Coarse POM-N was depleted in a strip-till corn-soybean system, and increased where organically managed corn followed 2 years of alfalfa. Beyond these significant effects, systems that were more moderate in tillage or included only some perennials in rotation were not distinguishable in aggregate fraction C and N and POM. It appears that perennialization alone does not increase aggregate or POM C and N, especially on this Mollisol where all systems are still storing and decomposing SOC from ancient prairies and manure applications from the early 20th century. The first signs of differentiation due to management were found in the fractions isolated from within macroaggregates,

concurring with previous work that protection of SOM in aggregates is a critical component of C and N storage. The effect of cropping systems on other aggregate fractions' C and N and POM may not become apparent unless systems have been in place much longer.

Table 3.1. Treatment means and ANOVA p-values for particulate organic matter (POM) and whole soil C and N in six cropping systems at the Wisconsin Integrated Cropping Systems Trial (WICST).

Treatment	SOC	TN	POM	POM-N	POM-C	POM C:N	POM-C: SOC	POM-N: TN	Whole C:N
	g kg ⁻¹ soil								
System†									
CC	15.9 ab‡	1.55 ab	0.462 ab	0.0986	1.42	14.8	0.074 b	0.056	11.7
CS	12.8 ab	1.33 b	0.507 b	0.0869	1.28	15.5	0.082 ab	0.057	11.2
CSW	12.1 b	1.30 b	0.430 b	0.0889	1.26	16.9	0.087 ab	0.058	10.5
C3A	15.8 ab	1.74 ab	0.511 a	0.135	1.83	15.5	0.142 ab	0.084	10.2
C2A	16.2 ab	1.76 ab	0.489 ab	0.149	2.01	14.5	0.109 a	0.076	10.5
P	19.0 a	1.94 a	0.506 a	0.173	2.57	16.8	0.115 a	0.077	11.0
Depth									
0 to 25 cm	20.6 a	2.07 a	0.551 a	0.203 a	2.97 a	16.9 a	0.152 a	0.099 a	11.5 a
25 to 50 cm	10.0 b	1.13 b	0.417 b	0.0409 b	0.491 b	14.4 b	0.053 b	0.037 b	10.2 b
ANOVA	<i>P</i> -value								
System	0.0811	0.0191	0.0260	<0.0001	<0.0001	0.1148	0.0217	0.1502	0.3901
Depth	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	0.0021
System x depth	0.1180	0.5940	0.4621	0.0002	0.0005	0.2352	0.4300	0.2704	0.0915

† CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover; C3A, corn following three years of alfalfa; C2A, organic corn following 2 years of alfalfa; P, rotationally grazed mixed pasture.

‡ Lowercase letters denote significant differences among systems across depths, or between depths across systems, at $P < 0.10$.

Table 3.2. Results of two-way ANOVA analyses for effect of cropping system on aggregate distribution and proportion, stock, and concentration of C and N found in aggregates.

Measurement	Soil fraction					
	Macro [†]	Micro	S&C	OMicro	OS&C	cPOM
	<i>P</i> -value					
Proportion of total soil dry weight	0.0045	0.6115	0.0004	0.0084	0.1844	0.1785
Proportion of total soil N	0.2175	0.6713	0.0744	0.0716	0.579	0.0964
Proportion of total soil C	0.2186	0.6707	0.0437	0.0383	0.4308	0.1301
N in fraction, Mg ha ⁻¹	0.0055	0.2576	0.8802	0.0037	0.0309	0.0483
C in fraction, Mg ha ⁻¹	0.0109	0.5824	0.7482	0.0070	0.0192	0.0775
N in fraction, g kg ⁻¹ soil	0.0048	0.1016	0.8823	0.0069	0.0124	0.0390
C in fraction, g kg ⁻¹ soil	0.0144	0.3063	0.7721	0.0120	0.0060	0.0538

[†]Macro, macroaggregates; micro, microaggregates; S&C, silt and clay; OMicro, occluded microaggregates; O S&C, occluded silt and clay; cPOM, coarse particulate organic matter.

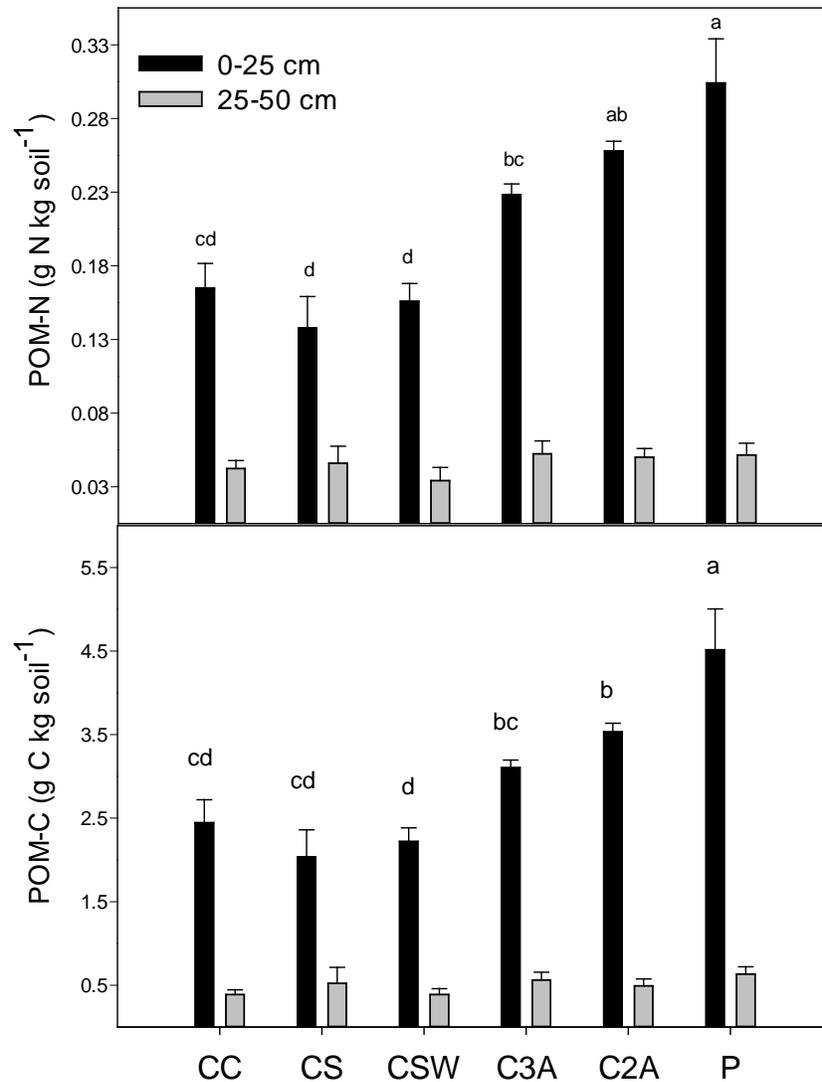


Figure 3.1. Concentrations of particulate organic matter (POM) N and C across six Wisconsin Integrated Cropping Systems Trial (WICST) cropping systems at two soil depths. Bars represent standard error and lowercase letters denote differences among systems at a given depth, $p < 0.10$. CC, continuous corn; CS, corn/soybean; CSW, organic corn/soybean/winter wheat and oats/berseem clover; C3A, corn following 3 years of alfalfa; C2A, organic corn following 2 years of alfalfa; P, rotationally grazed mixed pasture.

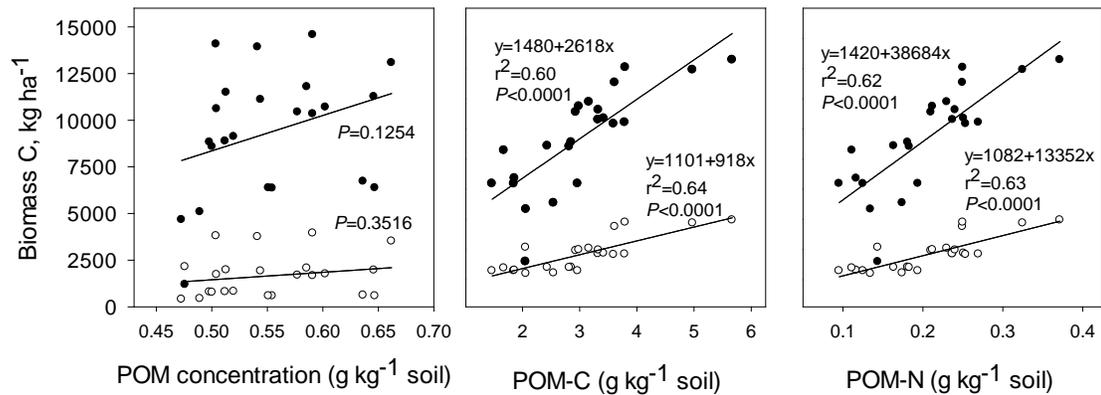


Figure 3.2. Linear regression analysis of biomass C estimates (Sanford et al., 2012) and 2013 particulate organic matter (POM) (a), POM-C (b), and POM-N (c) across six cropping systems. Filled circles represent belowground biomass and open circles represent aboveground biomass. Lines represent linear regression of above and belowground biomass C and POM measurements.

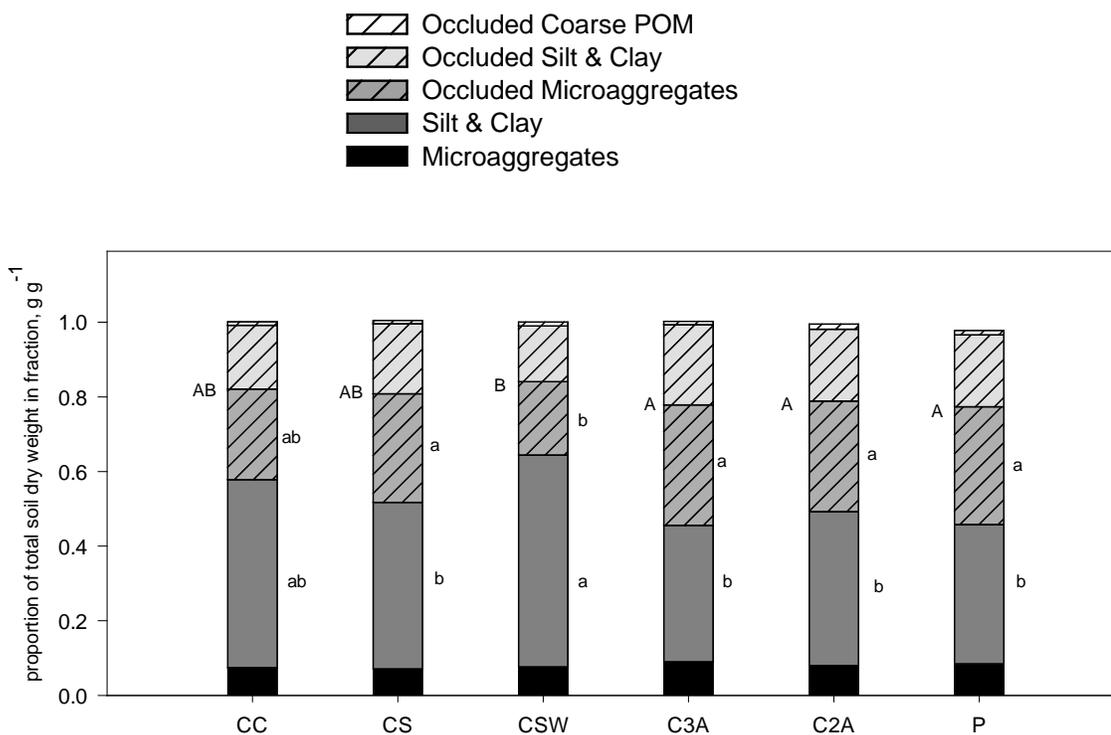


Figure 3.3. Proportion of soil mass found in each soil fraction in six Wisconsin Integrated Cropping Systems Trial (WICST) cropping systems at 0 to 25 cm. Bars representing fractions isolated from within macroaggregates include diagonal lines. Uppercase letters to the left of bars represent significant differences among systems in macroaggregates. Lowercase letters to the right of bars represent significant differences among systems. CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseem clover; C3A, corn following 3 years of alfalfa; C2A, organic corn following 2 years of alfalfa; P, rotationally grazed mixed pasture.

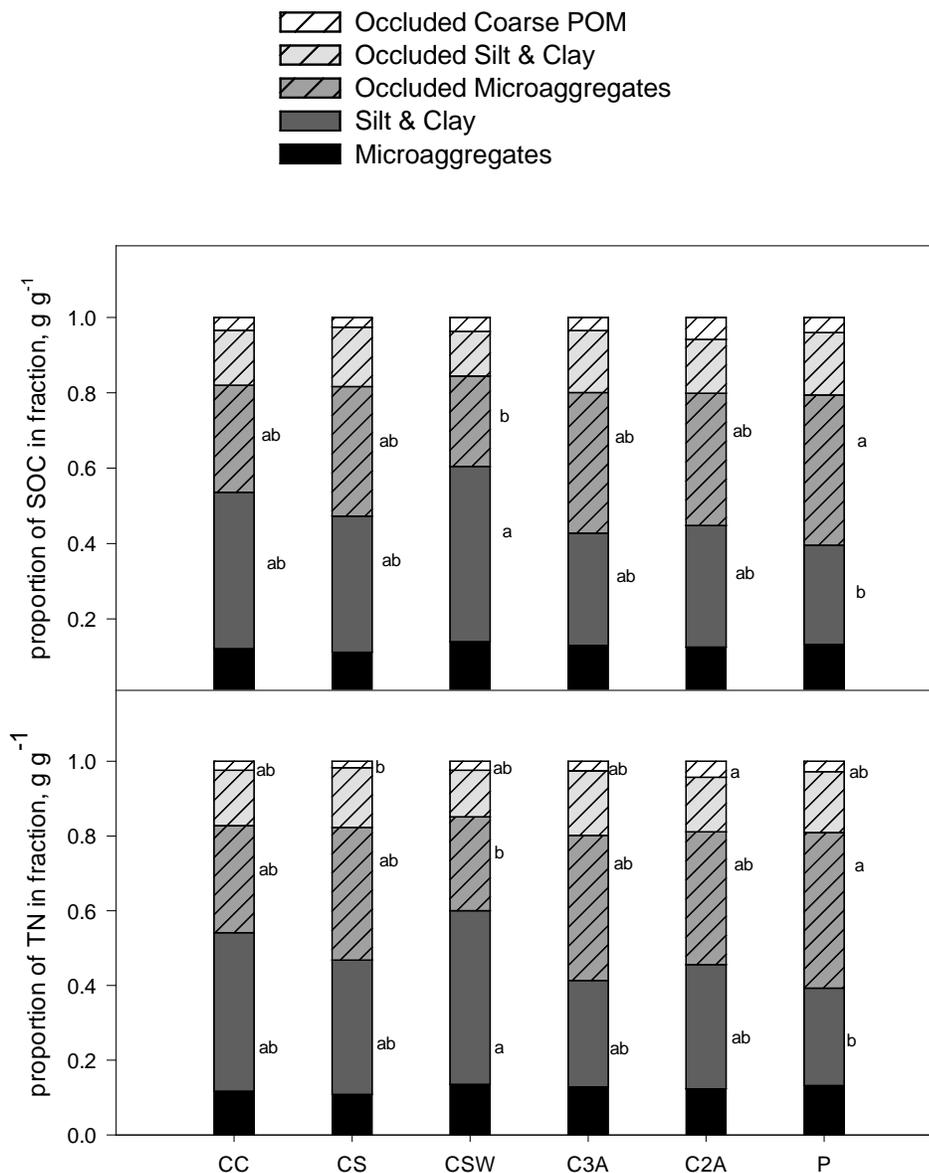


Figure 3.4. Proportion of soil carbon and nitrogen found in each soil fraction in six Wisconsin Integrated Cropping Systems Trial (WICST) cropping systems at 0 to 25 cm. Bars representing fractions isolated from within macroaggregates include diagonal lines. Lowercase letters to the right of bars represent significant differences among systems. CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseem clover; C3A, corn following 3 years of alfalfa; C2A, organic corn following 2 years of alfalfa; P, rotationally grazed mixed pasture.

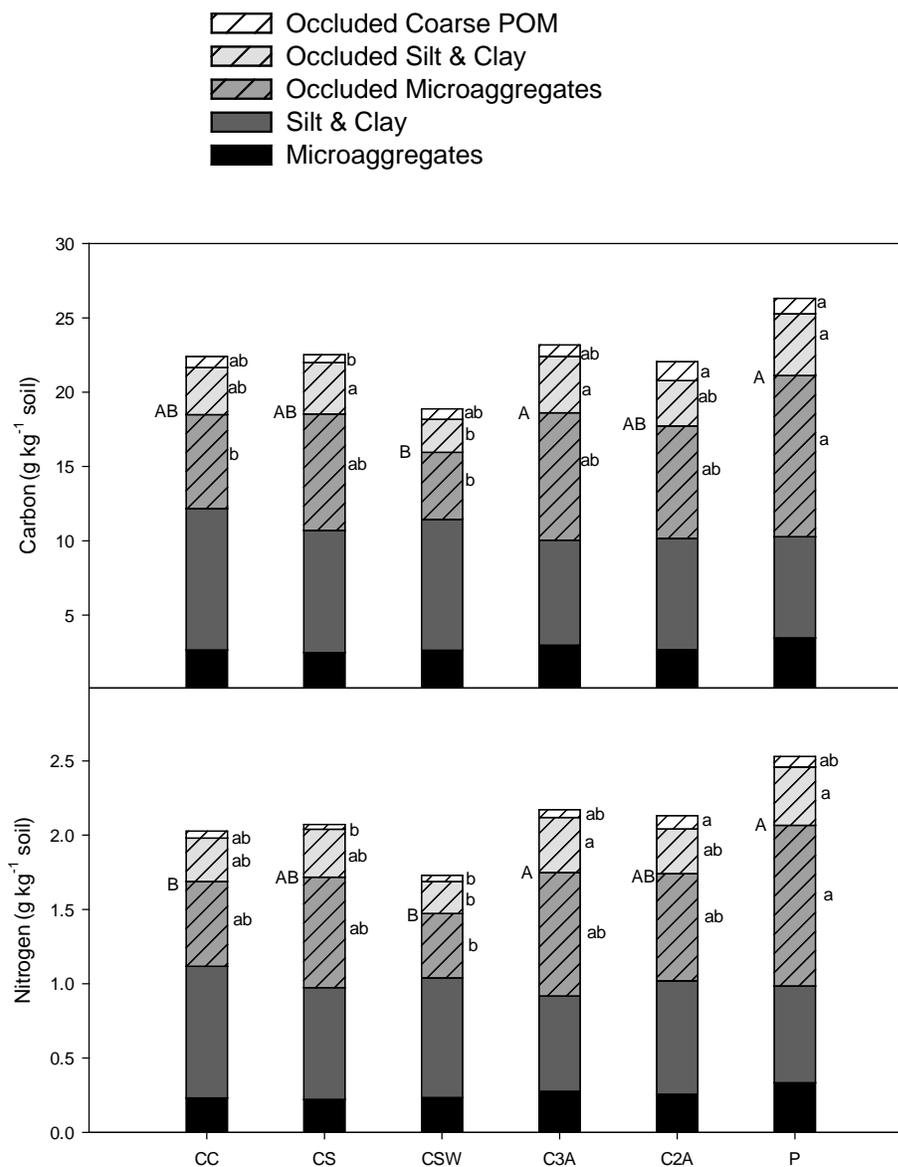


Figure 3.5. Concentration of carbon and nitrogen in each soil fraction in six Wisconsin Integrated Cropping Systems Trial (WICST) cropping systems at 0 to 25 cm. Bars representing fractions isolated from within macroaggregates include diagonal lines. Uppercase letters to the left of bars represent significant differences in macroaggregate C or N concentration among systems, lowercase letters to the right of bars represent significant differences among systems of all other fractions. CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseem clover; C3A, corn following 3 years of alfalfa; C2A, organic corn following 2 years of alfalfa; P, rotationally grazed mixed pasture.

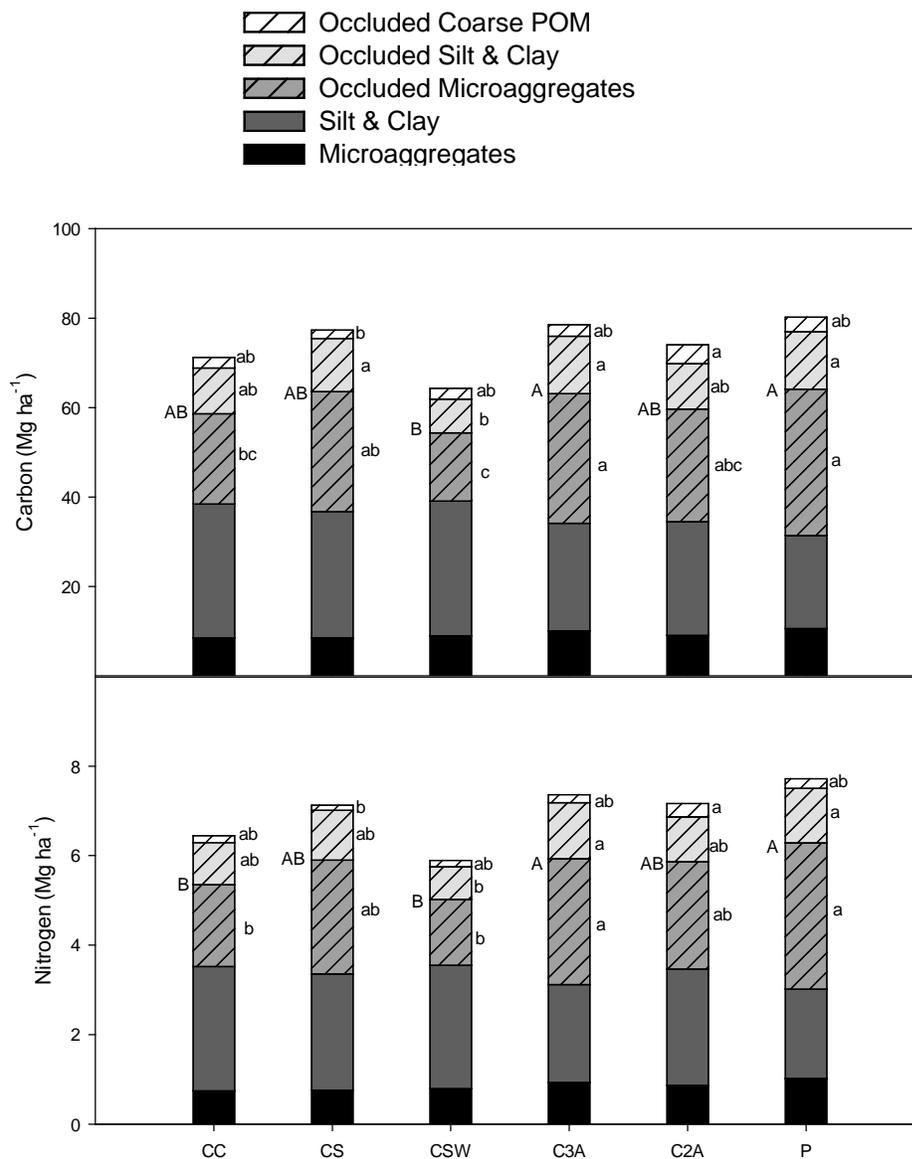


Figure 3.6. Stock of carbon and nitrogen in each soil fraction in six Wisconsin Integrated Cropping Systems Trial (WICST) cropping systems at 0 to 25 cm. Bars representing fractions isolated from within macroaggregates include diagonal lines. Uppercase letters to the left of bars represent significant differences in macroaggregate C or N stock among systems, lowercase letters to the right of bars represent significant differences among systems in C or N stock of all other fractions. CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseem clover; C3A, corn following 3 years of alfalfa; C2A, organic corn following 2 years of alfalfa; P, rotationally grazed mixed pasture.

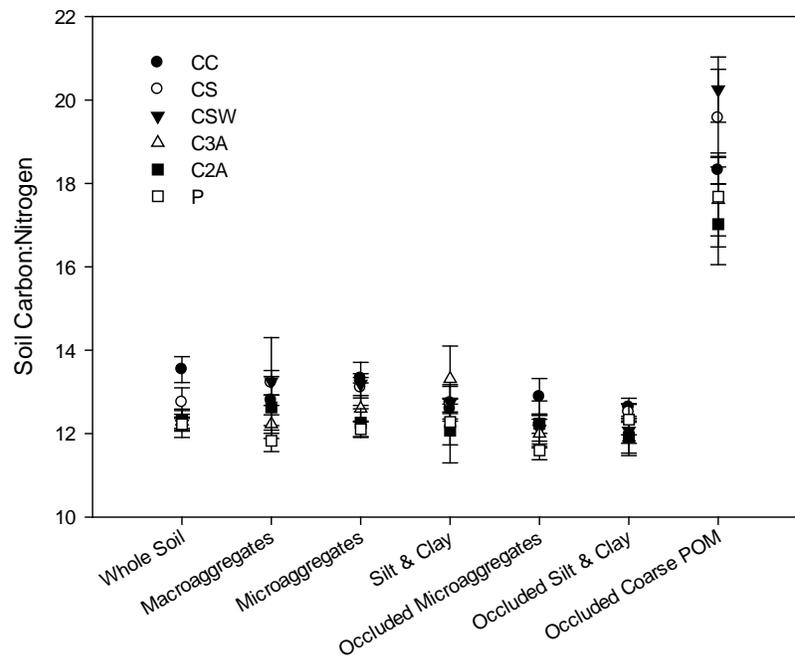


Figure 3.7. Carbon to nitrogen ratio of whole soil and aggregate fractions in the Wisconsin Integrated Cropping Systems Trial (WICST). Error bars represent standard error of the mean ($n=4$). The overall ANOVA shows a significant effect of systems ($P=0.0349$) but no differences were observed among systems based on least squares means comparisons. The C:N ratio of occluded coarse particulate organic matter (POM) is significantly greater than all other fractions, $P<0.0001$. CC, continuous corn; CS, strip-till corn/soybean; CSW, organically managed corn/soybean/winter wheat and oats-berseem clover; C3A, corn following 3 years of alfalfa; C2A, organic corn following 2 years of alfalfa; P, rotationally grazed mixed pasture.

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CONCLUSIONS

These results are specific to a highly productive Mollisol in a temperate climate. Aggregation and therefore physical protection of SOM have been shown to vary according to soil mineralogy (Denef et al., 2004; Kögel-Knabner et al., 2008). Specifically, soils with greater proportions of 2:1 minerals have been shown to disaggregate with loss of SOM more than soils dominated by 1:1 clays (Denef et al., 2004). The 2:1 mineral montmorillonite was the most abundant mineral in similar loess-derived soils in central WI (Fanning and Jackson, 1965), and so the loss of SOC upon cultivation may have had a more drastic impact on aggregation on Plano silt loam than in other environments. However, the tension that emerges in this work between long-term soil C storage to mitigate global warming, and predictable mineralization of SOM to provide high-yielding crops with nutrients may be universal.

This study was undertaken to better understand the interactions between management of a prime agricultural soil and the SOM storage of that soil, with an eye towards better management of N in U.S. Midwest cornfields. We found that while particulate and aggregate organic matter under a perennial pasture was greater than that under corn cropping systems varying in tillage and rotation, systems that included 2 or 3 years of perennial alfalfa in rotation with corn could not be distinguished from chisel plowed continuous corn by these SOM measurements. The organic management practiced in the corn/soy/wheat system had the lowest values of POM and aggregate organic matter, suggesting that contrary to expectations that organic management increases soil tilth, in this case low biomass inputs and regular cultivation for weed control lead to a decline in these SOM measurements. Our results suggest that none of the annual

cropping systems are creating conditions for soil aggregation to physically protect SOM, and this is reflected in the overall loss of SOC in all except the pasture system of WICST found by Sanford et al. (2012). The SOC being lost by the system is still prairie-derived, according to the radiocarbon age of nearly 500 years for surface SOC reported in Paul et al. (2001). It appears that although the variations in management practiced at WICST look drastic from an agronomic perspective, the protection of SOM on the Plano silt loam was most fundamentally affected by the shift from native vegetation to annual agriculture. The pasture comes closest to mimicking native vegetation and in conjunction shows an increase in aggregation and SOC compared to the other cropping systems.

In exploring which SOM measurements were best correlated to corn yield, we found that positive relationships between yield and C and N associated with free silt and clay, and negative relationships between yield and C and N associated with occluded microaggregates. Past research has focused on the role of occluded microaggregates in increasing SOC in no-till systems relative to tilled systems, suggesting that this fraction may be diagnostic of a cropping system's ability to sequester C (Six and Paustian, 2014). Aggregation is beneficial in building SOC because aggregates consist of the sum of the SOC of the parts plus the SOM in the organic binding agents of the aggregate itself (Tisdall and Oades, 1982). Increasing SOM stock may, in the long term, increase nutrient availability to plants, but our results suggest that the greater the proportion of this SOM stored in occluded microaggregates, the lower the yield of a given year's crop. There may be a tradeoff between long-term C storage and a ready supply of mineralizable SOM for crop growth. Achieving both vegetative growth and SOC

accrual was possible under native prairie vegetation, but that plant community may have had lower nutrient demands, a different microbial community which enhanced plant nutrient acquisition, or additional nutrient input from grazing animals' waste.

APPENDIX 1: Particulate Organic Matter Data from 2012

Particulate organic matter (POM) was extracted from samples taken in both 2012 and 2013; however, due to methodological changes only 2013 data are reported in the previous chapters. Based on the evidence that follows, it was decided not to include POM 2012 data in the analyses for this thesis.

In 2012, soil sampling was done in April using hand probes. In CC, CS, and CSW, soil samples (2.4 cm diam.) were taken at 0- to 5-, 5- to 25- and 25- to 50-cm depths. Eight samples were taken from each whole WICST plot that was to be planted to corn in 2012 and composited into one sample per plot. In C3A, C2A, and P, samples were taken and 0- to 25- and 25- to 50- cm depth. Six cores were taken from each whole plot that was to be planted to corn, and the rotationally grazed pasture. Samples were dried at 100°C and ground to pass a 1-mm sieve.

Samples were analyzed for POM following the procedure outlined in Marriot and Wander (2006). Ten-gram soil samples were placed in 30-mL plastic bottles with 20 mL sodium hexametaphosphate (SHMP) (100 g per 2 L solution). Soils soaked overnight, at least 12 hours. The next day, bottles were capped with a square of 53- μ m mesh and a top with a 1-cm hole drilled through it. Each 30-mL bottle was placed in a 250-mL centrifuge bottle and another 130 mL of SHMP was added to each 250-mL bottle before it was capped. The 250-mL bottles were placed upright on a reciprocal shaker and shaken on high for 1 hour. Then the SHMP was poured out of each bottle, replaced with 150 mL water and the bottles shaken again for 20 minutes. This rinse was repeated two more times with fresh water each time followed by a 20-minute shake, for a total shaking time of 120 minutes. Material larger than 53 μ m was thus trapped in the

bottle while material smaller than 53 μm was removed. After the final shake, POM was rinsed onto 53- μm mesh and rinsed with tap water until the water was clear. The mesh was rinsed into a pre-weighed aluminum pan and dried at 50 to 60°C for a minimum of 24 hours.

In 2013, the method was changed after a visit to the Wander lab at University of Illinois at Urbana-Champaign confirmed that Ugarte and Wander had substantially changed the protocol for greater efficiency. These updates have not been documented in any peer-review literature, but Ugarte (personal communication, 2013) claimed that adjustments to the POM extractions had not resulted in significantly different results. The new method is described in Chapter 2 and Chapter 3 of this document, but the most meaningful differences are the use of a single large container with all 30-mL bottles placed horizontally instead of individual upright 250-mL centrifuge bottles, and an increase in shaking time.

To test whether POM data collected in 2012 were significantly different than POM data collected in 2013, year was included in the PROC MIXED analysis as a fixed effect. Analyses were performed separately for POM at three depths in the three grain systems, and POM at 0 to 25 cm in all six WICST cropping systems. In both cases, the effect of year was significant in POM concentration, with 2012 exhibiting significantly lower concentrations of POM, and year did not significantly affect POM-N or POM-C. This indicates that overall, the 2012 procedure recovered more material, but the quality of that material was the same.

Paired t-tests were also performed in R Commander, and in the three grain systems all variables tested were significantly different in 2012 than 2013 with

$P < 0.0001$ except for TN, which did not differ significantly between years. In paired t-tests of all six systems between years, POM concentration, SOC, POM C:N and whole C:N were all significantly different at the $\alpha = 0.0001$ level, and TN was significantly different at the 0.10 level. These pairs were made up of samples taken from the same system and block of the WICST trials, but given that all sampling was done in the plots that were to be planted in corn, the physical plots differed between 2012 and 2013. This may have driven some of the variation we observed, but it is not possible to discern how much without further testing of the samples.

Based on these two statistical analyses, we elected to present only the 2013 data. The updates to the method of POM extraction appear to result in a more complete dispersion of soil, leading to collection of less material greater than 53 μm . We are confident that POM extracted by the 2013 method is comparable to other published POM data based on the same method, for example Marriott and Wander (Marriott and Wander, 2006b), Culman et al. (Culman et al., 2012), and Peralta and Wander (Peralta and Wander, 2008); however our POM data should not be compared to POM data based on a separation by density (as discussed in Chapters 2 and 3).

APPENDIX 2: SAS Code

Models for yield by N rate, Chapter 1.

```

OPTIONS LS=80 PS=60;
DATA A;
INPUT Nrate grainyld;
cards;
;

```

Title 'Regression models_Linear, Quadratic, Linear Plateau, Quadratic Plateau';

```

data b; set a;
N = Nrate;
N2 = N*N;

```

```
DATA REG; SET b;
```

```

PROC REG;
*LINEAR REGRESSION;
MODEL GRAINYLD=N;

```

```

PROC REG;
*QUADRATIC REGRESSION;
MODEL GRAINYLD=N N2;
OUTPUT OUT=X RESIDUAL=R;

```

```

PROC NLIN;
PARMS B0=120 B1=0.44 KNOT=100;
AGEPLUS=MAX (N - KNOT, 0);
MODEL GRAINYLD = B0 + B1*N - B1*AGEPLUS;
OUTPUT OUT=B PREDICTED=YP RESIDUAL=RR;
Proc Plot;
plot grainyld*n yp*n=*' /overlay;
plot rr*n;
run;

```

*LINEAR PLATEAU;

```

PROC NLIN;
PARMS A=120 B=0.44 C=-.0015;
N0=-.5*B/C; *ESTIMATE JOIN POINT;
DB=-.5/C; *DERIV OF NO WRT B;
DC=-.5*B/C**2; *DERIV OF NO WRT C;

```

*QUADRATIC PLATEAU;

```

IF N<N0 THEN DO; *QUADRATIC PART OF MODEL;
MODEL GRAINYLD=A+B*N+C*N*N;

```

```

DER.A=1;
DER.B=N;
DER.C=N*N;
END;

```

```

ELSE DO;          *PLATEAU PART OF MODEL;
MODEL GRAINYLD=A+B*N0+C*N0*N0;
DER.A=1;
DER.B=N0+B*DB+ 2*C*N0*DB;
DER.C= B*DC+N0*N0+2*C*N0*DC;
END;

IF _OBS_=1 & _ITER_>0 THEN DO; *PRINT OUT OBS;
PLATEAU=A+B*N0+C*N0*N0;

PUT N0=PLATEAU=;
end;

OUTPUT OUT=b predicted=grainyldp residual=gyresid;
proc plot;
plot GRAINYLD*N grainyldp*N='*' / overlay vpos=35;
plot gyresid*n;
run;

QUIT;

```

ANOVA for yield and NUE metrics, Chapter 1.

```
options ls=80 ps=50 nodate nonumber formdlim=" " formchar="|----|+|----|=|<>*";
```

```

proc import datafile="C:\Users\Ruark_GraStu\Dropbox\Anna's Data\harvest 13.txt"
    out=soil
    dbms=dlm
    replace;
    delimiter='09'x;
    datarow=2;
    getnames=Y;

proc print data=soil;

proc mixed data=soil method=type3;
class Nrate blk system;
model RE = Nrate system Nrate*system / outp=resids;
* model asstcpom_c = system depth depth*system / residual outp=resids;
* model asmacro = system depth depth*system / residual outp=resids;
* model odmacro = system depth depth*system / residual outp=resids;
* model lodmacro = system depth depth*system / residual outp=resids;
lsmeans Nrate|system / pdiff adj=TUKEY;
random blk ;

proc print data=resids;

proc plot data=resids;
plot resid*pred / vref=0 hpos=60 vpos=20;

proc univariate data=resids normal plot;
var resid;

```

```

proc sort;
  by system blk;

proc means noprint;
  by system blk;
  var RE;
  *   var asstcpom_c;
  *   var asmacro;
  *   var odmacro;
  *   var lodmacro;
  output out=REmean mean=mac;

proc print data=REmean;

proc mixed data=REmean method=type3;
  class system blk;
  model mac = system / residual outp=residout;
  random blk;

proc plot data=residout;
  plot resid*pred / vref=0 hpos=60 vpos=20;

proc univariate data=residout normal plot;
  var resid;

run;
ods pdf close;

```

ANOVA for soils data from three WICST grain systems, Chapter 2.

```

options ls=80 ps=50 nodate nonumber formdlim=" " formchar="|----|+|----+=|-\^<>*";

proc import datafile="D:\My Docs\field 12\fract data update 7.19.txt"
  out=soil
  dbms=dlm
  replace;
  delimiter='09'x;
  datarow=2;
  getnames=Y;

proc print data=soil;

proc mixed data=soil method=type3;
  class blk depth system;
  model macro = system depth depth*system / outp=resids;
  *   model asstmacro = system depth depth*system / residual outp=resids;
  *   model asmacro = system depth depth*system / residual outp=resids;
  *   model odmacro = system depth depth*system / residual outp=resids;
  *   model lodmacro = system depth depth*system / residual outp=resids;
  lsmeans depth|system / pdiff adj=TUKEY;
  random blk blk*system;

```



```

class plot blk system;
model BD = system / outp=resids;
* model asstcpom_c = system depth depth*system / residual outp=resids;
* model asmacro = system depth depth*system / residual outp=resids;
* model odmacro = system depth depth*system / residual outp=resids;
* model lodmacro = system depth depth*system / residual outp=resids;
lsmeans system / pdiff adj=TUKEY;
random blk ;

proc print data=resids;

proc plot data=resids;
plot resid*pred / vref=0 hpos=60 vpos=20;

proc univariate data=resids normal plot;
var resid;

proc sort;
by system blk;

proc means noprint;
by system blk;
var BD;
* var asstcpom_c;
* var asmacro;
* var odmacro;
* var lodmacro;
output out=BDmean mean=mac;

proc print data=BDmean;

proc mixed data=BDmean method=type3;
class system blk;
model mac = system / residual outp=residout;
random blk;

proc plot data=residout;
plot resid*pred / vref=0 hpos=60 vpos=20;

proc univariate data=residout normal plot;
var resid;

run;
ods pdf close;

```

Non-parametric tests for C:N ratios, Chapters 2 and 3.

```

proc import datafile="D:\My Docs\field 12\fract cn ratio.txt"

out=soil
dbms=dlm
replace;

```

```
delimiter='09'x;  
    datarow=2;  
getnames=Y;  
  
proc print data=soil;  
  
proc sort;  
by depth sys;  
  
proc rank;  
  
    var whole;  
    ranks Rwhole;  
run;  
  
proc print;  
    title2 'CN Ratio';  
run;  
proc means ;  
by depth sys;  
var cpom;  
var macro ;  
var micro;  
var mmicro;  
var osc;  
var sc;  
var whole;  
output out=means mean=mac;  
proc print data =means;  
proc anova;  
    class depth sys;  
    model Rwhole = depth sys;  
    title2 'Friedman"s Two-way Nonparametric ANOVA';  
run;
```