

2-1-2014

# A long-term N fertilizer gradient has little effect on soil organic matter in a high-intensity maize production system.

Kimberly H. Brown  
*Iowa State University*

Elizabeth M. Bach  
*Iowa State University*

Rhae A. Drijber  
*University of Nebraska - Lincoln*

Kirsten S. Hofmockel  
*Iowa State University, khof@iastate.edu*

Elizabeth S. Jeske  
*University of Nebraska - Lincoln*

Follow this and additional works at: [http://lib.dr.iastate.edu/agron\\_pubs](http://lib.dr.iastate.edu/agron_pubs)



*next page for additional authors*

Part of the [Agriculture Commons](#), and the [Ecology and Evolutionary Biology Commons](#)

The complete bibliographic information for this item can be found at [http://lib.dr.iastate.edu/agron\\_pubs/202](http://lib.dr.iastate.edu/agron_pubs/202). For information on how to cite this item, please visit <http://lib.dr.iastate.edu/howtocite.html>.

---

This Article is brought to you for free and open access by the Agronomy at Iowa State University Digital Repository. It has been accepted for inclusion in Agronomy Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact [digirep@iastate.edu](mailto:digirep@iastate.edu).

---

# A long-term N fertilizer gradient has little effect on soil organic matter in a high-intensity maize production system.

## Abstract

Global maize production alters an enormous soil organic C (SOC) stock, ultimately affecting greenhouse gas concentrations and the capacity of agroecosystems to buffer climate variability. Inorganic N fertilizer is perhaps the most important factor affecting SOC within maize-based systems due to its effects on crop residue production and SOC mineralization. Using a continuous maize cropping system with a 13 year N fertilizer gradient (0–269 kg N ha<sup>-1</sup> yr<sup>-1</sup>) that created a large range in crop residue inputs (3.60–9.94 Mg dry matter ha<sup>-1</sup> yr<sup>-1</sup>), we provide the first agronomic assessment of long-term N fertilizer effects on SOC with direct reference to N rates that are empirically determined to be insufficient, optimum, and excessive. Across the N fertilizer gradient, SOC in physico-chemically protected pools was not affected by N fertilizer rate or residue inputs. However, unprotected particulate organic matter (POM) fractions increased with residue inputs. Although N fertilizer was negatively linearly correlated with POM C/N ratios, the slope of this relationship decreased from the least decomposed POM pools (coarse POM) to the most decomposed POM pools (fine intra-aggregate POM). Moreover, C/N ratios of protected pools did not vary across N rates, suggesting little effect of N fertilizer on soil organic matter (SOM) after decomposition of POM. Comparing a N rate within 4% of agronomic optimum (208 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and an excessive N rate (269 kg N ha<sup>-1</sup> yr<sup>-1</sup>), there were no differences between SOC amount, SOM C/N ratios, or microbial biomass and composition. These data suggest that excessive N fertilizer had little effect on SOM and they complement agronomic assessments of environmental N losses, that demonstrate N<sub>2</sub>O and NO<sub>3</sub> emissions exponentially increase when agronomic optimum N is surpassed.

## Disciplines

Agriculture | Ecology and Evolutionary Biology

## Comments

This is an article from Brown KH, Bach EA, Drijber RA, Hofmockel K, Jeske ES, Sawyer JE, Castellano MJ. 2014. A long-term N fertilizer gradient has little effect on soil organic matter in a high-intensity maize production system. *Global Change Biology* doi: [10.1111/gcb.12519](https://doi.org/10.1111/gcb.12519). Posted with permission.

## Authors

Kimberly H. Brown, Elizabeth M. Bach, Rhae A. Drijber, Kirsten S. Hofmockel, Elizabeth S. Jeske, John E. Sawyer, and Michael J. Castellano

# **A long-term nitrogen fertilizer gradient has little effect on soil organic matter in a high-intensity maize production system**

Running Head: Long-term nitrogen fertilizer

Kimberly H. Brown<sup>1</sup>, Elizabeth Bach<sup>2</sup>, Rhae A. Drijber<sup>3</sup>, Kirsten Hofmockel<sup>2</sup>, Elizabeth S. Jeske<sup>3</sup>, John E. Sawyer<sup>1</sup>, Michael J. Castellano<sup>1\*</sup>

<sup>1</sup>Department of Agronomy, Iowa State University, Ames, IA 50011

<sup>2</sup>Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011

<sup>3</sup>Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583

\*corresponding author: [castellanomichaelj@gmail.com](mailto:castellanomichaelj@gmail.com); 515-294-3963

## **Abstract**

Global maize production alters an enormous soil organic C (SOC) stock, ultimately affecting greenhouse gas concentrations and the capacity of agroecosystems to buffer climate variability. Inorganic N fertilizer is perhaps the most important factor affecting SOC within maize-based systems due to its effects on crop residue production and SOC mineralization. Using a continuous maize cropping system with a 13-year N fertilizer gradient (0-269 kg N ha<sup>-1</sup>y<sup>-1</sup>) that created a large range in crop residue inputs (3.60-9.94 Mg dry matter ha<sup>-1</sup>y<sup>-1</sup>), we provide the first agronomic assessment of long-term N fertilizer effects on SOC with direct reference to N rates that are empirically determined to be insufficient, optimum, and excessive. Across the N fertilizer gradient, SOC in physico-chemically protected pools was not affected by N fertilizer rate or residue inputs. However, unprotected particulate organic matter (POM) fractions increased with residue inputs. Although N fertilizer was negatively linearly correlated with POM C/N ratios, the slope of this relationship decreased from the least decomposed POM pools (coarse POM) to the most decomposed POM pools (fine intra-aggregate POM). Moreover, C/N ratios of protected pools did not vary across N rates, suggesting little effect of N fertilizer on soil organic matter (SOM) after decomposition of POM. Comparing a N rate within 4% of agronomic optimum (208 kg N ha<sup>-1</sup>y<sup>-1</sup>) and an excessive N rate (269 kg N ha<sup>-1</sup>y<sup>-1</sup>), there were no differences between SOC amount, SOM C/N ratios, or microbial biomass and composition. These data suggest excessive N fertilizer had little effect on SOM and they complement agronomic assessments of environmental N losses that demonstrate N<sub>2</sub>O and NO<sub>3</sub> emissions exponentially increase when agronomic optimum N is surpassed.

## Introduction

Maize-based cropping systems cover over 39 million ha in the US (USDA-ERS, 2012) and represent an enormous stock of actively managed soil organic carbon (SOC). However, the status of SOC balances in maize systems is uncertain. Positive, negative, and neutral balances are reported (Khan *et al.*, 2007; Adviento-Borbe *et al.*, 2007; David *et al.*, 2009; Senthilkumar *et al.*, 2009). Perhaps the greatest factor affecting SOC balances in maize-based cropping systems is nitrogen (N) fertilizer application (Cassman, 1999). Nitrogen fertilizer is applied to ~95% of the Midwest US maize crop at an average rate of 148 kg N ha<sup>-1</sup> y<sup>-1</sup> (USDA-ERS, 2012). Nitrogen fertilizer can increase SOC by increasing crop residue (organic matter) inputs to the soil or decrease SOC by increasing C mineralization (Russell *et al.*, 2009). Although the net effect of inorganic N fertilizer on SOC is highly debated (Robertson *et al.* 2013), inorganic N fertilizer typically has a positive effect on SOC when not applied in large excess beyond crop demand (Alvarez, 2005; Russell *et al.* 2009).

In the absence of inorganic N fertilizer, a positive effect of organic matter (OM) inputs (manure or crop residues) on SOC is frequently observed and represented in process-based models with linear first-order kinetics so that SOC increases in constant proportion to OM inputs (e.g., Paustian *et al.*, 1997). These models suggest an infinite potential for SOC accumulation. However, in some long-term experiments with high initial SOC, broad ranges of OM inputs do not produce differences in SOC storage across treatments or time (Reicosky *et al.*, 2002; Soon, 1998; Huggins & Fuchs, 1997; Huggins *et al.*, 1998). These results led to the concept that soils have a finite capacity to store SOC, termed 'C saturation' (Six *et al.*, 2002).

Two models have been developed to describe C saturation dynamics (Stewart *et al.* 2007). A one-pool whole-soil C saturation model suggests soils have a finite C storage capacity and the efficiency of C storage decreases with increasing input. Alternatively, a two-pool model suggests soils contain two distinct C pools: A stable (protected) pool that saturates and a labile (unprotected) pool that does not saturate. The stable pool includes SOC fractions that are physico-chemically protected from mineralization through mineral-association and micro-aggregation (Jastrow *et al.*, 1996; Sollins *et al.*, 1996; von Lützow *et al.*, 2008). The labile pool includes SOC that is not mineral-associated or occluded within micro-aggregates and thus has no physical limit on accumulation. However, the labile pool is easily mineralized and typically a small proportion of total SOC in annual cropping systems. In the two-pool model, the transfer efficiency of decomposition products from labile to stable pools decreases in proportion to amount of C in the stable pool; OM inputs that are not transferred to the stable pool accumulate in the labile pool or are mineralized (Stewart *et al.*, 2007; Gulde *et al.*, 2008; Castellano *et al.*, 2012).

Similar to the two-pool C saturation model which suggests the amount of C in the stable pool affects partitioning of new OM inputs among stable and labile pools, inorganic N fertilizer can also affect the stabilization and mineralization of new OM inputs. Inorganic N fertilizer can enhance microbial C use efficiency which can elevate the conversion rate of plant litter-C to

mineral-associated C thus increasing SOC independent from the effect of N fertilizer on the quantity of crop residue inputs (Kirkby *et al.*, 2013). In contrast, N fertilizer can increase C mineralization (Salinas-Garcia *et al.*, 1997) and decrease aggregate-protected C by increasing aggregate turnover rate (Fonte *et al.*, 2009; Chivenge *et al.*, 2011; Plaza *et al.* 2013). Importantly, soil microbes play a critical role in both these processes and N fertilizer can alter microbial biomass and microbial community composition (Liebig *et al.*, 2002; Liang *et al.*, 2010; Ramirez *et al.*, 2012).

Together, these data suggest OM inputs, C saturation status, and N fertilizer rate interact to affect SOC storage and the partitioning of SOC among labile and stable pools. However, the relative importance of these factors and the balance between positive and negative effects of N fertilizer on SOC stabilization is unknown, particularly with direct reference to agronomic optimum N inputs as empirically determined from long-term yield response to N fertilizer (*i.e.*, N maximizes yield and crop residue production, but does not exceed crop demand). Our objectives were to evaluate C saturation concepts as well as the net effect of N fertilizer rate on SOC storage through its effects on crop residue inputs and SOC storage in physico-chemically protected and unprotected pools. To accomplish these objectives we used a continuous maize cropping system that received one of five N fertilizer rates (0-269 kg N ha<sup>-1</sup>) annually for 13 years while all other nutrients and pH were managed for optimum production. The site was well suited to address our objectives because it included three N rates that were insufficient to maximize crop production, one rate within 6 kg N ha<sup>-1</sup> y<sup>-1</sup> (4%) of agronomic optimum N rate, and one rate that provided a large excess of inorganic N fertilizer (61 kg N ha<sup>-1</sup> y<sup>-1</sup>) beyond agronomic optimum.

## **Materials and methods**

### *Site Description and Experimental Design*

The study site was established in 1999 on the Iowa State University Research Farm (42°0'38" N, 93°47'19" W). Soil classification was Typic Hapludoll, Clarion soil series. Research plots were maintained in continuous-maize with fall chisel plow to ~30 cm and spring disk/field cultivated tillage. Plots contained six maize rows in a 4.5 m by 15.25 m area with an average density of ~76,000 plants ha<sup>-1</sup> over the duration of the experiment; however, commensurate with agronomic advances, plant density has increased over time with the release of new maize hybrids to a present density of ~85,000 plants ha<sup>-1</sup>. Five N application rates were applied in a randomized complete block design with four replications (N = 20). Experimental blocks were designed to account for landscape variations that affected soil organic matter. Nitrogen rates were maintained in the same plot location each year. Nitrogen was applied annually either pre-plant in the spring or mid-May within two weeks after planting. Application rates included 0, 67, 135, 202, 269 kg N ha<sup>-1</sup> y<sup>-1</sup> and were applied as pre-plant broadcast urea or urea-ammonium nitrate solution (32% UAN) sidedressed in subsurface bands (1.5 m spacing). After grain harvest, crop residue was tilled into the soil by fall chisel plowing with secondary disk and field cultivation for seedbed preparation, creating a C input gradient across treatments. This tillage management strategy is commonly practiced in continuous maize production systems in the U.S. Corn Belt. All nutrients

other than N were maintained for optimum maize production (soil pH, P and K levels were tested; lime and fertilizer were applied if needed). The agronomic optimum N rate (AONR) was estimated as the join point of a fitted quadratic-plateau regression model (Anderson and Nelson, 1975) of the mean across years yield data (1999-2011). Prior to 1999 when the N fertilizer rate experiment was initiated, the site was managed in various crops such as alfalfa (*Medicago sativa* L.), oat (*Avena sativa* L.), wheat (*Triticum aestivum* L.), maize, and soybean [*Glycine max* (L.) Merr.] from the late 1980s to the early 1990s when it was converted to a continuous maize system. Exact years of the change in cropping system are unknown.

### *Crop Residue Inputs*

Crop residue inputs were estimated from the average annual grain yield (1999-2011) per N rate treatment by assuming a 0.5 harvest index, which evidence suggests is accurate and does not vary with N rate (Russell *et al.*, 2009; Lorenz *et al.*, 2010). Belowground residue was not included in our estimations of residue inputs because belowground production and root:shoot ratio of maize grown across an identical long-term N gradient (0-269 kg N ha<sup>-1</sup>) in Iowa did not vary with N rate (Russell *et al.*, 2009). Also, the range of root:shoot ratios across the N fertilizer gradient in that study was narrow: 0.11-0.12. Because root:shoot ratios did not vary with N rate in previous work, including an estimate of belowground production in our results would not alter any relationships. Moreover, belowground production in maize is notoriously difficult to estimate; in a literature review, Amos and Walters (2006) found that net rhizodeposited root C ranged from 5-62% of standing root biomass at crop senescence.

In addition to the fact that aboveground production accounts for ~90% of total dry matter production, it is also likely that aboveground production is a large contributor to SOC at our site due to intensive fall tillage that mixes the aboveground crop residues into the top 30 cm of soil shortly after harvest (i.e., senescence). Although some recent studies suggest root inputs are the dominant contributor to SOC (e.g., Katterer *et al.* 2011), intimate association between roots and the soil matrix has been identified as a potential cause; intensive tillage at our site would promote intimate association between aboveground residues and the soil matrix.

### *Soil Sampling and General Soil Property Analyses*

In July of 2011, ten soil cores (2.5 diameter x 30 cm depth) from each plot were sampled. We selected a depth of 30 cm because it represents the plow layer, which is annually mixed in this study's tilled systems. Soil samples were only taken within the three center rows of the six row plots to avoid border rows and any possible N or crop residue movement across plots. Three cores were used to determine bulk density. After bulk density measurement, all ten cores were passed through an 8 mm sieve and air-dried. Subsequently, the soil was homogenized and a portion was ground for quantification of plant-available phosphorus (Bray-P1) and pH (1:1 soil: water ratio). Bray-P1 analysis was conducted at the Iowa State University Soil and Plant Analysis Laboratory. The portion of soil not ground was used for textural analysis with the method of Kettler *et al.* (2001). The remaining soil from the homogenized cores was used for fractionation procedures detailed below.

### *Fractionation Procedure*

The fractionation procedure (supplementary materials) follows Gulde *et al.* (2008), with a slight modification in the light fraction (LF) removal. Instead of removing the LF after wet-sieving, the LF was removed during wet-sieving. This procedure isolated water-stable aggregates by wet-sieving, and then further fractionated large and small macroaggregates to isolate POM and silt and clay fractions within these aggregates.

### *Water-stable Aggregate Isolation and Light Fraction Removal*

Isolated water-stable aggregate fractions included: large macroaggregates (LM), small macroaggregates (SM), free microaggregates (Free-m), and free silt and clay (Free-SC). Diameters of these fractions are  $> 2000 \mu\text{m}$ ,  $250\text{-}2000 \mu\text{m}$ ,  $53\text{-}250 \mu\text{m}$ , and  $< 53 \mu\text{m}$ , respectively. Briefly, about 100 g of composite air-dry soil was slaked for five minutes prior to wet-sieving. After slaking, the soil was agitated over a  $2000 \mu\text{m}$  sieve by re-submerging the sieve into water 50 times over two minutes. The LF, or any floating residue, was removed at this step with a net, and any soil remaining on the sieve was washed into a drying pan and dried at  $65^\circ\text{C}$  overnight. The procedure was repeated by agitating the remaining soil over a  $250 \mu\text{m}$  and a  $53 \mu\text{m}$  sieve by the same procedure. Return in soil mass after the wet-sieving process averaged  $99.22\%$  ( $\pm 0.63$ ) and ranged from  $99.12\text{-}100.49\%$  of total soil. All aggregate fractions were corrected for sand and LF.

### *Large and Small Macroaggregate Fractionation*

We isolated microaggregates within macroaggregates (i.e., inter-microaggregates (inter-m)), with the protocol outlined by Six *et al.* (2000). Briefly, 10-15 g of macroaggregates were slaked for 15-20 min before being exposed to minor abrasion mechanical shaking in running water with 50 glass beads. This was done over a  $250 \mu\text{m}$  sieve to collect coarse particulate organic matter (CPOM) that was within macroaggregates. All soil  $< 250 \mu\text{m}$  ran through the sieve and was collected in a receptacle equipped with a  $53\mu\text{m}$  sieve. The wet-sieving procedure, as mentioned above, was done on the soil collected on this sieve. This procedure separated the inter-microaggregates from the silt and clay that was within macroaggregates, but not within microaggregates (i.e., inter-silt and clay (inter-SC)). All fractions isolated with this procedure were oven-dried at  $60^\circ\text{C}$ .

### *Inter-microaggregate Fractionation*

To disperse inter-microaggregates, which consist of fine intra-aggregate particulate organic matter (fiPOM) encrusted with silt and clay (i.e., intra-silt and clay (intra-SC)), samples were shaken in 0.5 % sodium hexametaphosphate ( $(\text{NaPO}_3)_6$ ) at a 1:3 ratio (soil:liquid; v,v) for 12 h. Dispersed samples were washed through a  $53 \mu\text{m}$  sieve to separate fiPOM from intra-SC. All fractions isolated with this procedure were oven-dried at  $60^\circ\text{C}$ .

### *Density Flotation*

Sodium polytungstate ( $\text{Na}_6[\text{H}_2\text{W}_{12}\text{O}_{40}]$ ) at a density of  $2.3 \text{ g cm}^{-3}$  was used to remove sand contamination from the CPOM (sand  $> 250 \mu\text{m}$ ) and fiPOM ( $53 \mu\text{m} < \text{sand} < 250 \mu\text{m}$ ) fractions. A density flotation at  $1.85 \text{ g cm}^{-3}$  was also used to remove fPOM from the Inter-m fraction before dispersion. Briefly, samples were added to sodium polytungstate at the desired density with a 1:3 ratio (soil:liquid; v,v). Suspended soil within the solution was gently stirred once to ensure light material trapped below dense material was released and left to settle overnight. After settling overnight, floating material was aspirated from the surface by vacuum filtration using a  $20 \mu\text{m}$  nylon mesh filter. Any remaining sodium polytungstate was washed off with deionized water 7-10 times before oven-drying at  $60 \text{ }^\circ\text{C}$ .

### *Carbon and Nitrogen Measurement and Interpretation*

Carbon and N content were determined with a dry combustion elemental analyzer for the whole soil, water-stable aggregates, free-SC, CPOM, fPOM, fiPOM, Inter-SC, and Intra-SC. We interpreted C concentrations for each fraction as a percent of whole soil C ( $\text{g fraction C kg}^{-1}$  whole soil). When evaluating C saturation dynamics, we also interpreted C concentrations within individual free-SC, inter-SC, and intra-SC fractions ( $\text{g fraction C g}^{-1}$  fraction) because silt and clay, unlike aggregates, do not have the ability to increase in proportion of the whole soil on timescales relevant to management. Instead, silt and clay have a relatively finite capacity to stabilize C (Hassink & Whitmore, 1997).

Soil organic C associated with CPOM and fPOM were considered unprotected or labile since they are located inside macroaggregates, which are destroyed upon relatively minor soil disruption. If not incorporated into microaggregates, these POM fractions lack long-term physical protection (Six *et al.*, 2000). Soil organic C associated with free-m, free-SC, fiPOM, Inter-SC, and Intra-SC were considered protected or stable pools. We quantified the amount of unprotected C by summing C from CPOM and fPOM located within large and small macroaggregates. The remaining C within the macroaggregates, along with the C from free-m and free-SC, represented the protected pool. For each macroaggregate class, the fiPOM:CPOM ratio was calculated as a proxy for aggregate turnover (Six *et al.*, 2000).

In addition to physical fractionations, we also measured potentially mineralizable C during laboratory incubations (Robertson and Paul 1999). For these analyses, 10 g of soil that was used for physical fractionation procedures (air dried, 8 mm sieved) was brought to 60% water holding capacity and incubated in the dark for 30 d at  $22 \text{ }^\circ\text{C}$ . Water content was maintained daily and  $\text{CO}_2$  headspace accumulation was measured with an infrared gas analyzer at days 1, 2, 3, 5, 7, 10, 19, 22, 25 and 30. Between each measurement the headspace was flushed. Cumulative  $\text{CO}_2$  flux was calculated with linear interpolation and numerical integration.

### *Microbial Sampling and Analysis*

Fatty Acid Methyl Ester (FAME) analysis was used to determine total soil microbial biomass and community composition (Grigera *et al.*, 2007a). In 2012, soil samples were taken from the field site in a similar fashion to those sampled for physical fractionation except that samples

were passed through a 4mm sieve upon returning to the laboratory and then immediately frozen. Briefly, 10 ml of 0.2 M potassium hydroxide (KOH):methanol (MeOH) (1:1, v/v) was added to 5g soil samples and placed into a water bath at 37 °C for 1 hour, shaking occasionally. Extracted fatty acid methyl esters were dissolved (after being dried under N<sub>2</sub> gas) in hexane (C<sub>6</sub>H<sub>14</sub>) containing the fatty acid internal standard C19:0 before inserting 50 µl aliquot to the conical gas chromatograph vial. Biomarkers for bacteria included, iC14:0, iC15:0, aC15:0, C15:0, iC16:0, i10MeC17:0, C17:1, iC17:0, aC17:0, C17:1c9, C17:0, 10MeC19:0, cyC19(9,10), and cyC19(11,12). Biomarkers for fungi differentiated between saprophytic fungi and arbuscular mycorrhizal fungi (AMF). The biomarkers C18:2c9 and C16:1c11 were used to represent saprophytic fungi and AMF, respectively. Total microbial biomass was estimated by summing nmol g<sup>-1</sup> FAMES representing bacteria, AMF, and saprophytic fungi.

### *Statistical Analyses*

All statistical tests were performed with SAS 9.2 (SAS Institute, Cary, NC). Large and small macroaggregates contained three types of POM (CPOM, fPOM, fiPOM), and although there are documented distinctive characteristics among POM fractions (i.e. CPOM vs. fiPOM; Six et al., 2000), it is not clear if the originating aggregate size class adds distinctiveness (i.e. large macroaggregate CPOM vs small macroaggregate CPOM). Large and small macroaggregates were also fractionated into two types of silt and clay fractions, and similarly, it is not clear if there is distinctiveness between these fractions (i.e. Inter- vs. Intra-SC) or among originating aggregates (i.e. Large macroaggregate Inter-SC vs. Small macroaggregate Inter-SC). We tested for differences between POM and SC between large and small macroaggregates with an ANOVA model that included the fraction and the originating aggregate as fixed categorical factors. In both cases, statistical results showed distinctiveness was present between fractions (i.e., CPOM vs. fPOM vs. fiPOM and Inter vs. Intra SC), but the originating aggregate did not matter. Therefore, data from large and small macroaggregate classes were combined (N=40).

Relationships between the dependent variables (C and N concentrations, C:N ratios and microbial biomass) and independent variables (N fertilizer rate and crop residue input) were analyzed with linear and nonlinear regression models. The best fit equation (linear vs. nonlinear) was based on the lowest sum of squares error. To test for linear relationships as well as significant differences among regression coefficients or slopes the SAS REG procedure was used. To test for nonlinear exponential relationships, the SAS NLIN procedure was used. The SAS NLIN procedure was also used to fit a quadratic model to the relationship among yield and N fertilizer rate as is the standard procedure in soil fertility literature (e.g., Cassman and Plant, 1992). The R values were calculated for nonlinear relationships with the following equation:  $R = \sqrt{1 - SSE/SST}$ , where SSE is the sum of squares error and the SST is the sum of squares of the corrected total (Kvalseth, 1985). It is important to note that all regression analyses were computed with all data points (N = 20 plots); however, figures display only the means and standard errors from each of the five N rates for visual clarity.

## **Results**

### *General soil properties*

Bulk density and soil texture did not significantly differ among N applications (Table 1). Plant available (Bray) P (mean  $\pm$  standard error) averaged 34 ppm ( $\pm 4.5$ ) and all plots were above the agronomic optimum P soil test level (0-30cm) for maize production in Iowa (Sawyer *et al.*, 2002). Soil pH averaged 6.03 ( $\pm 0.12$ ) and all plots were within the agronomic optimum for the Clarion soil series in Iowa (Sawyer *et al.*, 2002).

### *Crop Residue Input*

Average annual aboveground crop residue production ranged from 3.60-9.94 Mg dry matter ha<sup>-1</sup> y<sup>-1</sup>, and significantly increased with N application (Fig. 1). The relationship between crop residue production and N rate was fit to a quadratic model (Table 1 and Figure 1). Based on yield data from 1999-2011, the agronomic optimum N rate (AONR) during those years was 208 kg N ha<sup>-1</sup> with a corresponding residue production of 9.77 Mg dry matter ha<sup>-1</sup>.

### *Soil Carbon and Nitrogen Fractions*

Total SOC ranged from 16.22-24.49 g C kg<sup>-1</sup> soil among individual plots and did not increase with N rate or crop residue input (Table and Figure 1). The C:N ratios for the whole soil, protected organic matter within macroaggregates, free-m, free-SC, inter-SC, and intra-SC did not differ among N rates (Figure 2). Means and standard errors for these C:N ratios were 11.9 ( $\pm 0.2$ ), 13.2 ( $\pm 1.1$ ), 12.7 ( $\pm 0.1$ ), 11.1 ( $\pm 0.1$ ), 10.9 ( $\pm 0.1$ ), and 11.4 ( $\pm 0.1$ ), respectively. Particulate organic matter C:N ratios ranged from 25.2-14.7, 28.4-14.4, and 16.8-13.9 for CPOM, fPOM, and fiPOM, respectively (Figure 3). All POM fraction C:N ratios significantly decreased with N application, but the slopes of these relationships significantly differed. The regression coefficient for CPOM was greater than both fPOM and fiPOM, but regression coefficients did not differ between fPOM and fiPOM (Figure 3). In addition, average POM C:N ratios decreased in the following order: CPOM > fPOM, > fiPOM. Because the effect of N application rate on C:N ratios was limited to the POM fractions, we focus all further analyses on relationships with crop residue input rather than N application rate.

The proportions of water-stable aggregate classes did not differ across crop residue input levels. Aggregate class means were 2.14 ( $\pm 0.38$ ), 28.67 ( $\pm 0.96$ ), and 22.89 ( $\pm 0.93$ ) g 100 g<sup>-1</sup> soil for LM, SM, and free-m, respectively. The mass of free-SC averaged 8.20 ( $\pm 0.29$ ) g 100 g<sup>-1</sup> soil and also did not differ across crop residue inputs (Table 2).

The protected SOC pool, on a whole soil basis (mass of fraction in whole soil\*fraction C concentration), did not significantly differ for any fraction across crop residue inputs. Protected classes averaged 0.49 ( $\pm 0.11$ ), 5.13 ( $\pm 0.33$ ), 4.48 ( $\pm 0.24$ ), and 1.78 ( $\pm 0.06$ ) g C kg<sup>-1</sup> whole soil for LM, SM, free-m, and free-SC, respectively. Note that CPOM, and fPOM are not included in protected fraction calculations, and also that all fractions are corrected for sand and LF (although inclusion of these relatively small amounts of C did not affect our results). In addition, average protected SOC stored in each fraction decreased in the following order: SM > free-m > free-SC > LM (Figure 3).

In contrast to the protected SOC pool, the unprotected SOC pool (sum of CPOM and fPOM) increased with crop residue input (Figure 4). Among individual plots, unprotected C ranged from 0.2111-0.5360 g C kg<sup>-1</sup> whole soil. After fractionating the soil into 12 fractions and summing the C from each, the calculated value for whole soil C was very similar to the actual value of whole soil C. There was a difference of 0.0123 g C kg<sup>-1</sup> soil with standard errors of 0.5085 and 0.4903 for actual and calculated values, respectively.

Although there was no effect of crop residue input on the fiPOM:CPOM ratio for SM, the fiPOM:CPOM ratio for LM did increase with crop residue input (Figure 5). Among individual plots, LM fiPOM:CPOM ratio ranged from 0.1451-1.2516.

There were no significant differences in C concentrations within free-SC fractions across crop residue inputs, and averaged 21.72 g C kg<sup>-1</sup> free-SC ( $\pm 0.2104$ ). Carbon concentration of silt and clay fractions held within macroaggregates (Inter- and Intra-SC), however, increased with crop residue input (Figure 6). Among individual plots, C concentrations for inter- and Intra-SC ranged from 22.06-28.28 and 15.46-31.98 g C kg<sup>-1</sup> SC, respectively.

Although we did find small increases in unprotected SOC with crop residue inputs, there was no relationship between potentially mineralizable SOC and crop residue input or N fertilizer rate. As crop residue inputs increased from the lowest to highest N fertilizer rate (Figure 1), 30 d potentially mineralizable C was: 283, 295, 334, 297, and 332 mg C kg soil<sup>-1</sup>. Normalizing these data to total SOC (mg potentially mineralizable C kg<sup>-1</sup> total SOC) had no effect.

#### *Microbial Biomass and Community Composition*

Lipid abundance, among all plots, ranged from 29.65-50.50, 6.75-10.52, and 20.05-34.89 nmol g<sup>-1</sup> soil for bacteria, AMF, and saprophytic fungi, respectively (Figure 7). Bacteria increased, but AMF decreased with crop residue input. Saprophytic fungi did not change across crop residue input rates. The increase in bacterial populations was not enough to compensate for the decrease in AMF populations, however, and total microbial biomass decreased exponentially with crop residue input. Total microbial biomass ranged from 53.16-76.14 nmol g<sup>-1</sup> soil. Although the decrease in total microbial biomass with crop residue input was best fit by an exponential decay model, this relationship was driven by the four data points from plots receiving zero N application; if these data were removed from the analysis, there was no relationship (linear or exponential) between crop residue inputs or N fertilizer and total microbial biomass. In contrast, within bacteria and AMF, removal of data points from plots receiving zero N application from the analysis did not eliminate the significant linear relationships between AMF biomass and crop residue inputs or bacterial biomass and crop residue inputs.

#### **Discussion**

Despite 13 years of N fertilizer application and aboveground crop residue inputs that ranged from 3.60-9.94 Mg dry matter ha<sup>-1</sup>y<sup>-1</sup>, we found no differences in total SOC or physico-chemically protected SOC fractions. These results suggest that either: *i*) N fertilization concurrently increased crop residue inputs and SOC mineralization (Russell *et al.*, 2009) or *ii*)

soils were effectively C-saturated given the current management and climate (Six *et al.*, 2002; Stewart *et al.*, 2007). We cannot say with certainty that soils were effectively C saturated because there was no sampling at the initiation of the experiment. However, our data are consistent with the two-pool C saturation model that posits unprotected SOC fractions increase linearly with organic matter inputs when protected fractions are saturated (Stewart *et al.*, 2007). Furthermore, data from our soil fractionation procedures and potentially mineralizable C assay suggest that N fertilizer has relatively little effect on SOC.

### *Soil Organic Carbon Storage*

Protected SOC fractions did not differ across the range of crop residue inputs (Figures 3 and 4) and differences in the amount of C represented by each fraction in the protected pool were associated with differences in their mass proportion of the whole soil rather than differences in C concentrations within each fraction (Table 2). Although C concentrations of Inter-SC and Intra-SC significantly increased with crop residue inputs (Figure 6), these increases were extremely small when scaled to a whole soil basis (<1% of total SOC). Together, these data suggest protected fractions cannot accumulate more SOC under current management practices. Thus, we expect that further increases in C inputs to these soils could only increase unprotected SOC fractions. Nevertheless, unprotected fractions were relatively minor contributors to total SOC (1.93-3.35%), which is one reason why we observed no differences in total SOC (Figure 2). High variability in SOC stocks may have also contributed to the lack of observed significant differences in total SOC stocks (Kravchenko & Robertson 2011). However, potentially mineralizable C, which responds more quickly to changes in management than total SOC, also exhibited no differences across treatments.

The lack of SOC data from experiment initiation in 1999 precludes determination of whether SOC stocks were increasing, decreasing or in equilibrium. However, Russell *et al.* (2006) reported 0-15 cm SOC stocks at a site 100 km to the north and on the same soil series. The site was managed in continuous maize with treatments receiving 0, 90, 180 or 270 kg N ha<sup>-1</sup> y<sup>-1</sup> for 47 years. Although the authors found no difference in SOC stocks across N fertilizer rates in 2002, measurements of change in SOC stocks from 1990-2002 determined that SOC stocks at the zero N fertilizer rate were not changing while SOC stocks at the 180 kg N ha<sup>-1</sup> y<sup>-1</sup> fertilizer rate were increasing by ~0.6 Mg C ha<sup>-1</sup> y<sup>-1</sup>. These data are not directly comparable to our study because we sampled from 0-30 cm. However, soil sampling at our site from 0-15 cm in 2009 produced similar data: Russell *et al.* (2006) reported a range of SOC stocks from 42.4-51.3 Mg C ha<sup>-1</sup> while we observed a range of 37.2-50.0 Mg C ha<sup>-1</sup> (Castellano and Sawyer unpub. data). Although the range of SOC data at our site was large, it was partially accounted for by the blocking factor in our experimental design: compared to the range of SOC across all blocks (12.8 Mg C ha<sup>-1</sup>), the range of SOC within blocks was 3.2-7.8 Mg C ha<sup>-1</sup>. Because agronomic management in Russell *et al.* (2006) and our study were nearly identical, we speculate that treatments in our study with crop residue inputs greater than Russell *et al.* (2006 & 2009) may have neutral or increasing SOC stocks.

### *Nitrogen Fertilizer Rate Effect*

The C:N ratios of protected and unprotected fractions indicated little effect of N fertilizer rate on SOC (Figure 2). Due to the flexible stoichiometry of plants, maize residue C:N ratios decrease with increasing N application. Across the identical N fertilization gradient studied by Russell *et al.* (2009), maize residue C:N ratios decreased from ~80 to ~30.

Our data were consistent with this pattern; the C:N ratio of POM, which is dominated by recognizable plant residues, decreased with N fertilizer rate. However, the effect of N fertilizer rate on POM C:N ratios decreased from the least decomposed POM fraction to the most decomposed POM fraction so that the range of C:N ratios across N application rates narrowed with each step of decomposition from CPOM to fPOM and fiPOM. The slope of the relationship between N fertilizer input and POM fraction C:N ratios significantly decreased from CPOM to fPOM by 64%, and from fPOM to fiPOM by 52% (see Figure 2 caption).

Across the N fertilizer rates, the range of C:N ratios spanned 4.8 units for CPOM, 1.7 units for fPOM, and 0.5 units for fiPOM. Moreover, there was no effect of N fertilizer rate on C:N ratios of protected fractions or total SOC. At the highest N fertilizer rate, which provided 61 kg N ha<sup>-1</sup> y<sup>-1</sup> in excess of the AONR, SOM C:N ratios in protected and unprotected fractions were similar to those in the treatment that was fertilized within 4% (6 kg N ha<sup>-1</sup> y<sup>-1</sup>) of the AONR.

In general, these results suggest that the effect of N fertilizer on SOC is largely limited to the least decomposed POM fraction (CPOM), which accounts for <5% of total SOC. Although the relationships between N fertilizer, fPOM and fiPOM were statistically significant, it is questionable whether ranges in C:N ratios of 1.7 and 0.5 are ecologically relevant given the large variability in C:N ratios within and among plants and the relative importance of various environmental factors controlling microbial physiology. Moreover, in the absence of N fertilizer application (0 kg N ha<sup>-1</sup> y<sup>-1</sup> treatment), C:N ratios decreased approximately 2.3 units as CPOM decomposed to fPOM and 2.9 units as fPOM decomposed to fiPOM. These decreases in POM C:N ratios, as decomposition proceeds from fPOM to fiPOM (within the 0 kg N ha<sup>-1</sup> y<sup>-1</sup> treatment), are greater than the decrease in C:N ratios within fPOM and fiPOM fractions across the N fertilizer gradient. Thus, as POM is decomposed beyond CPOM, the C:N ratios of fPOM and fiPOM appear to be more affected by the level of decomposition than by N fertilizer rate or initial residue C:N ratio because the decrease in C:N ratio from one POM fraction to the next within N fertilizer rates was greater than the decrease in fiPOM C:N ratio across the N fertilizer rate gradient. As organic matter inputs decomposed from CPOM to fPOM, and fiPOM, and subsequently stabilized in silt-clay adsorbed pools, the C:N ratio of these pools progressively narrowed towards the C:N ratio of microbial biomass. This pattern supports two key aspects of physico-chemically-based models of SOC dynamics: *i*) POM is increasingly processed by microbes as it is degraded from CPOM to fPOM and fiPOM; and *ii*) microbes decompose POM and the products of this process are transferred to silt/clay adsorbed SOM pools (Grandy & Neff 2008; Plaza *et al.* 2013).

### *Microbial Biomass and Community Composition*

Similar to SOC concentrations and C:N ratios in physico-chemically protected fractions, the rate of N fertilizer application had little effect on microbial biomass: microbial biomass was 15-16% lower in N-fertilized plots regardless of the N fertilizer rate. This is consistent with a meta-analysis of 82 published studies where soil microbial biomass declined under N fertilization (Treseder, 2008). In contrast to total microbial biomass, bacterial biomass linearly increased across the crop residue input gradient while saprophytic fungi were unresponsive. It is difficult to speculate whether this pattern in abundance was affected by crop residue or N inputs (because N inputs produced the increase in crop residue). However, the biomass of bacteria was virtually identical among plots that received very different N rates (202 & 269 kg N ha<sup>-1</sup> y<sup>-1</sup>) but similar residue inputs (9.59 & 9.94 Mg dry matter ha<sup>-1</sup> y<sup>-1</sup>) suggesting residue inputs and the associated increase in unprotected carbon may have led to the increase in bacterial biomass. The lack of differences in protected SOM fractions (particularly between 0 and 269 kg N ha<sup>-1</sup> y<sup>-1</sup> fertilizer rates where microbial biomass and composition were very different) are consistent with the recent finding that microbial residue source and biochemistry does not have a large impact SOC storage (Throckmorton *et al.*, 2012).

Nitrogen fertilization is known to reduce AMF biomass in soil (Bradley, 2006, Jeske, 2012), which is consistent with our study where AMF linearly declined across the crop residue/N input gradient. Because AMF are obligate plant symbionts, their behavior is largely dependent on plant C allocation (Fitter, 2006) and residue inputs would impact AMF *indirectly* through habitat and nutrient modification. Thus, it is difficult to speculate whether the decline in extramatrical AMF biomass along the crop residue/nitrogen input gradient stems from plant resource allocation or fungal response to increased N availability.

### *Aggregate Dynamics*

Soil aggregate fractions remained relatively unchanged with crop residue and N fertilizer inputs. This was unexpected given the large decline in AMF biomass, a known contributor to aggregate formation (Strickland & Rousk, 2010), with N fertilizer addition. Because the AMF lipid biomarker is confined to the 'active portion' of the fungus, it may not truly represent the totality of AMF biomass in the soil that is important to aggregation (Grigera *et al.*, 2007b). Fungal abundance, however, is only one of many factors that affect soil aggregation (Wilson *et al.*, 2009). Crop residue inputs are also known to benefit aggregation and a potential positive effect on aggregation caused by the 176% increase in crop residue inputs across the N fertilizer gradient may have been sufficient to negate any negative effect of the 69% decrease in AMF.

Although SOC levels in the total soil and aggregate fractions did not differ with crop residue inputs, the increase in LM fiPOM:CPOM ratio with increasing crop residue input (Figure 5) suggests aggregation improves very slightly with residue input. The fiPOM:CPOM ratio is a proxy for aggregate turnover rate; high fiPOM:CPOM ratios indicate macroaggregate turnover rates are slower and there is more opportunity for fiPOM stabilization within microaggregates (Six *et al.*, 2000). Over time, this process may slowly increase SOC levels by increasing the proportion of LM and free-m. In our results, however, an increase in the fiPOM:CPOM ratio is

not accompanied with an increase in LM or free-m likely because LM represented a very small proportion of the total soil (1.43-3.31 g 100 g<sup>-1</sup> soil) and the treatments were only in place for 13 years at the time of sampling. It is possible that slower LM turnover may eventually increase microaggregates and fiPOM occluded therein, but the timescale to such an increase is likely irrelevant to management.

Soil organic C concentrations in the Free-SC fraction did not differ among crop residue input levels, suggesting this fraction is effectively C-saturated. In contrast, SOC concentrations in the SC fraction that were trapped inside aggregates (Inter-SC and Intra-SC) increased with increasing crop residue input levels (Figure 6). These data demonstrate that the ‘effective soil C stabilization capacity’ or ‘effective C saturation’ (*sensu* Stewart *et al.*, 2007) of SC particles within aggregates is higher than free SC particles (Liao *et al.* 2006; Plaza *et al.* 2013). This is consistent with the concept that a change in management could increase the point at which protected SOC fractions become saturated (i.e., the point of effective soil C saturation).

#### *Managing N fertilizer inputs and SOC*

Although many reports examine the effect of N fertilization on SOC in agronomic systems, they do not empirically determine whether experimental N rates were agronomically insufficient, optimum or excessive (Russell *et al.*, 2005, 2009; Alvarez, 2005; David *et al.*, 2009). This information is critical because crop residue input levels are the source of SOC: if work is limited to a gradient of N rates that are agronomically insufficient, one might expect a positive effect of N fertilizer on SOC due to the positive effect of N fertilizer on crop residue inputs. In contrast, if work is limited to a gradient of N rates that are agronomically excessive, there is greater likelihood of a negative effect of N fertilizer on SOC because in this scenario N fertilizer does not increase crop residue inputs. However, the AONR is highly variable and can only be determined *post hoc* from an experiment that includes a broad N fertilizer gradient (Sawyer *et al.*, 2006; Robertson & Vitousek 2009). We report the first agronomic assessment of N fertilizer effects on SOM that is directly based on empirically determined, long-term optimized N rates.

After 13 years of an N fertilizer rate that provided 61 kg N ha<sup>-1</sup> y<sup>-1</sup> above the AONR, we observed no negative effects of this excess N on SOC pools or microbial communities (compared to the rate near the AONR). Although POM, Inter-SC, and Intra-SC organic C and N pools increased with N fertilizer rate, the increase in these pools across N fertilizer rates (~40-80 kg N ha<sup>-1</sup>) accounted for a small amount of total SOC/N that was far less than the cumulative amount of excess N fertilizer application (793 kg N ha<sup>-1</sup>). Moreover, there were no differences in the size of these pools between the AONR and excessive N rate. Given that we could not detect the excess N in SOM pools, it likely that significant portions were lost to the atmosphere and subsoil leaching. Accordingly, these data complement agronomic assessments of environmental N losses that demonstrate nitrous oxide and nitrate losses exponentially increase when the AONR is surpassed (Lawlor *et al.*, 2008; Van Groenigen *et al.*, 2010).

The lack of detectable differences in SOC despite 13 years of crop residue inputs that ranged from 3.60-9.94 Mg dry matter ha<sup>-1</sup>y<sup>-1</sup> has important implications for the modeling and

management of SOC stocks. These data underscore recent suggestions that ecosystem process models should incorporate the C saturation concept (Stewart *et al.*, 2007). Although it is generally accepted that N fertilizer and manure applications cannot result in net SOC sequestration due to the C emissions associated with the production of those inputs (Schlesinger 2000), our results raise an important question: In the absence of N fertilizer additions, can increases in organic matter inputs increase SOC storage in intensively managed Midwest US continuous maize systems? Our results suggest that meaningful SOC increases in intensively managed continuous maize systems may be difficult to achieve at current levels of crop residue production.

### **Acknowledgements**

This work was funded by the Iowa Department of Agriculture and Land Stewardship, the Leopold Center for Sustainable Agriculture, and United States Department of Agriculture National Institute of Food and Agriculture.

### **Literature Cited**

- Adviento-Borbe M a. a., Haddix ML, Binder DL, Walters DT, Dobermann a. (2007) Soil greenhouse gas fluxes and global warming potential in four high-yielding maize systems. *Global Change Biology*, **13**, 1972–1988.
- Alvarez R (2005) A review of nitrogen fertilizer and conservation tillage effects on soil organic carbon storage. *Soil Use and Management*, **21**, 38–52.
- Amos B, Walters DT (2006) Maize Root Biomass and Net Rhizodeposited Carbon. *Soil Science Society of America Journal*, **70**, 1489.
- Anderson, RL, Nelson, LA (1975) A family of models involving intersecting straight lines and concomitant experimental designs useful in evaluating response to fertilizer nutrients. *Biometry*, **31**, 301-308.
- Bradley, K., Drijber, R.A., Knops, J., 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. *SBB* 38, 1583-1595.
- Cassman KG (1999) Ecological intensification of cereal production systems: yield potential, soil quality, and precision agriculture. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 5952–9.
- Cassman KG, Plant RE (1992) A model to predict crop response to applied fertilizer nutrients in heterogeneous fields. *Fertilizer Research*, **31**, 151-163.

- Castellano MJ, Kaye JP, Lin H, Schmidt JP (2012) Linking Carbon Saturation Concepts to Nitrogen Saturation and Retention. *Ecosystems*, **15**, 175-187.
- Chivenge P, Vanlauwe B, Gentile R, Six J (2011) Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. *Soil Biology and Biochemistry*, **43**, 657–666.
- Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soils: Is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, **85**, 235-252.
- David MB, McIsaac GF, Darmody RG, Omonode R a (2009) Long-term changes in mollisol organic carbon and nitrogen. *Journal of environmental quality*, **38**, 200–211.
- Fitter, A. H. 2006. What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. *New Phytologist* 172:3-6.
- Fonte SJ, Yeboah E, Ofori P, Quansah GW, Vanlauwe B, Six J (2009) Fertilizer and Residue Quality Effects on Organic Matter Stabilization in Soil Aggregates. *Soil Science Society of America Journal*, **73**, 961.
- Gentile R, Vanlauwe B, Six J (2011) Litter quality impacts short- but not long-term soil carbon dynamics in soil aggregate fractions. *Ecological applications : a publication of the Ecological Society of America*, **21**, 695–703.
- Grandy a S, Neff JC (2008) Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. *The Science of the total environment*, **404**, 297–307.
- Grigera SM, Drijber RA, Wienhold BJ (2007a) Increased abundance of arbuscular mycorrhizal fungi in soil coincides with the reproductive stages of maize. *Soil Biology and Biochemistry*, **39**, 1401–1409.
- Grigera SM, Drijber RA, Shores-Morrow RH, Wienhold BJ (2007b) Distribution of the arbuscular mycorrhizal biomarker C16:1cis11 among neutral, glyco and phospholipids extracted from the soil during reproductive growth of corn. *Soil Biology and Biochemistry*, **39**, 1589-1596.
- Gulde S, Chung H, Amelung W, Chang C, Six J (2008) Soil Carbon Saturation Controls Labile and Stable Carbon Pool Dynamics. *Soil Science Society of America Journal*, **72**, 605.
- Hassink J, Whitmore AP (1997) A Model of the Physical Protection of Organic Matter in Soils. *Soil Science Society of America Journal*, **61**, 131–139.
- Huggins DR, Fuchs DJ (1997) Long term nitrogen management effects on corn yield and soil carbon of an aquic haplustoll in Minnesota. In: Soil organic matter in temperate agroecosystems. Eds. Elliot TE, Paul EA, Paustian K, Cole CV. CRC Press.

- Huggins D, Clapp C, Allmaras R, Lamb J, Layese M (1998) Carbon Dynamics in Corn-Soybean Sequences as Estimated from Natural Carbon-13 Abundance. **62**, 195–203.
- Jastrow JD, Boutton TW, Miller RM (1996) Carbon Dynamics of Aggregate-Associated Organic Matter Estimated by Carbon-13 Natural Abundance. *Soil Science Society of America Journal*, **60**, 801–807.
- Jeske ES (2012) Soil microbial community response to maize grown for high yield in rotation with soybeans. PhD dissertation, University of Nebraska-Lincoln.
- Katterer T, Bolinder MA, Andren O, Kitchmann H, Menichetti L (2011) Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. *Agriculture, Ecosystems & Environment*, **141**, 184-192.
- Kettler TA, Doran JW, Gilbert TL (2001) Simplified Method for Soil Particle-Size Determination to Accompany Soil-Quality Analyses. *Soil Science Society of America Journal*, **65**, 849–852.
- Khan S a, Mulvaney RL, Ellsworth TR, Boast CW (2007) The myth of nitrogen fertilization for soil carbon sequestration. *Journal of environmental quality*, **36**, 1821–32.
- Kirkby CA, Richardson AE, Wade LJ, Batten GD, Blanchard C, Kirkegaard J a. (2013) Carbon-nutrient stoichiometry to increase soil carbon sequestration. *Soil Biology and Biochemistry*, **60**, 77–86.
- Kravchenko AN, Robertson GP (2011) Whole-profile soil organic carbon stocks: the danger of assuming too much from analysis of too little. *Soil Science Society of America Journal*, **75**, 235-240.
- Kvalseth TO. 1985. Cautionary Note About  $R^2$ . *The American Statistician*. 39:279-285.
- Lawlor PA, Helmers MJ, Baker JL, Melvin SW, Lemke DW (2008) Nitrogen application rate effect on nitrate-nitrogen concentration and loss in subsurface drainage for a corn-soybean rotation. *Transactions of the ASABE*, **51**, 83–94.
- Leff JW, Wieder WR, Taylor PG, Townsend AR, Nemergut DR, Grandy a. S, Cleveland CC (2012) Experimental litterfall manipulation drives large and rapid changes in soil carbon cycling in a wet tropical forest. *Global Change Biology*, **18**, 2969–2979.
- Liao JD, Boutton TW, Jastrow JD (2006) Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. *Soil Biology and Biochemistry* **38**, 3184–3196.
- Liang B, Yang X, He X, Zhou J (2010) Effects of 17-year fertilization on soil microbial biomass C and N and soluble organic C and N in loessial soil during maize growth. *Biology and Fertility of Soils*, **47**, 121–128.

- Liebig MA, Varvel GE, Doran JW, Wienhold BJ (2002) Crop Sequence and Nitrogen Fertilization Effects on Soil Properties in the Western Corn Belt. **601**, 596–601.
- Lorenz AJ, Gustafson TJ, Coors JG, Leon N De (2010) Breeding Maize for a Bioeconomy: A Literature Survey Examining Harvest Index and Stover Yield and Their Relationship to Grain Yield. *Crop Science*, **50**, 1.
- Paustian K, Collins HP, Paul EA (1997) Management controls on soil carbon. In: Cole CV (ed) Soil organic matter in temperate agroecosystems: long-term experiments in North America. CRC Press, New York, pp 15-49.
- Plaza C, Courtier-Murias D, Fernández JM, Polo A, Simpson AJ (2013) Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: A central role for microbes and microbial by-products in C sequestration. *Soil Biology and Biochemistry* **57**, 124–134.
- Ramirez KS, Craine JM, Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, **18**, 1918–1927.
- Reicosky DC, Evans SD, Cambardella CA et al. (2002) Continuous corn with moldboard tillage: Residue and fertility effects on soil carbon. *Journal of Soil and Water Conservation*. **57**, 277-284.
- Robertson GP, Paul EA (1999) Decomposition and soil organic matter dynamics. In: Sala O, Jackson R, Mooney H, Howarth R, editors. *Methods in Ecosystem Science*. Springer-Verlag, p 104-116.
- Robertson GP, Vitousek PM (2009) Nitrogen in agriculture: Balancing the cost of an essential resource. *Annual Review of Environment and Resources*, **34**, 97-125.
- Roberston GP, Bruulsema TW, Gehl R, Kanter D, Mauzerall D, Rotz A, Williams C (2013) Climate-nitrogen interactions in agriculture. Biogeochemistry, special issue climate-nitrogen interactions, *Biogeochemistry*
- Russell AE, Cambardella CA, Laird DA, Jaynes DB, Meek DW (2009) Nitrogen fertilizer effects on soil carbon balances in midwestern U.S. agricultural systems. *Ecological applications*, **19**, 1102–13.
- Russell A, Parkin TB, Mallarino A (2006) Impact of Nitrogen Fertilization and Cropping System on Carbon Sequestration in Midwestern Mollisols. *Soil Science Society of America Journal*, **69**, 413.
- Salinas-Garcia J, Hons FM, Matocha JE (1997) Long-Term Effects of Tillage and Fertilization on Soil Organic Matter Dynamics. *Soil Science Society of America Journal*, **61**, 152–159.

- Sawyer JE, Mallarino SP, Killorn R, Barnhart SK. 2002. General guide for crop nutrient recommendations in Iowa. Publ. Pm-1688 (Rev.). Iowa State. Univ. Extension.
- Sawyer J, Nafziger E, Randall G, Bundy L, Rehm G, Joern B (2006) *Concepts and Rationale for Regional Nitrogen Rate Guidelines for Corn*. Ames, IA, Iowa State University Extension.
- Schlesinger WH (2000) Carbon sequestration in soils: some cautions amidst optimism. *Agriculture Ecosystems Environment*, **82**, 121-127.
- Senthilkumar S, Basso B, Kravchenko a. N, Robertson GP (2009) Contemporary Evidence of Soil Carbon Loss in the U.S. Corn Belt. *Soil Science Society of America Journal*, **73**, 2078.
- Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter : Implications for C-saturation of soils. *Plant and Soil*, **241**, 155–176.
- Six J, Elliott ET, Paustian K (2000) Soil macroaggregate turnover and microaggregate formation : a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry*, **32**, 2099–2103.
- Sollins P, Homann P, Caldwell BA (1996) Stabilization and destabilization of soil organic matter : mechanisms and controls. *Geoderma*, **74**, 65–105.
- Soon YK (1998) Crop residue and fertilizer management effects on some biological and chemical properties of a Dark Grey Solod. *Canadian Journal of Soil Science*, **78**, 707-713.
- Stewart CE, Paustian K, Conant RT, Plante AF, Six J (2007) Soil carbon saturation: concept, evidence and evaluation. *Biogeochemistry*, **86**, 19–31.
- Strickland MS, Rousk J (2010) Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, **42**, 1385–1395.
- Throckmorton HM, Bird J a, Dane L, Firestone MK, Horwath WR (2012) The source of microbial C has little impact on soil organic matter stabilisation in forest ecosystems. *Ecology letters*, **15**, 1257–65.
- Treseder K (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters* **11**, 1111-1120.
- van Groenigen JW, Velthof GL, Oenema O, Van Groenigen KJ, Van Kessel C (2010) Towards an agronomic assessment of N<sub>2</sub>O emissions: a case study for arable crops. *European Journal of Soil Science*, **61**, 903–913.
- von Lützow M, Kögel-Knabner I, Ludwig B, *et al.* (2008) Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. *Journal of Plant Nutrition and Soil Science*, **171**, 111–124.

USDA – ERS. 2013. Fertilizer use and price. 2012 . Percentage of corn acreage receiving nitrogen fertilizer, selected States. National Agricultural Statistics Service, Economic Research Service, U.S. Department of Agriculture, Washington, D.C., USA.  
[www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx#26720](http://www.ers.usda.gov/data-products/fertilizer-use-and-price.aspx#26720)

Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecology letters*, **12**, 452–61.

Table 1. Spatial means and standard errors of general soil properties sampled in 2011 and temporal mean and standard error of grain yield (1999-2011).

Nitrogen application kg ha <sup>-1</sup> y <sup>-1</sup>	Grain Yield Mg ha <sup>-1</sup> y <sup>-1</sup>	Total Organic Carbon g C kg soil <sup>-1</sup>	Total Nitrogen g N kg soil <sup>-1</sup>	Whole Soil C:N	Bulk Density g cm <sup>-3</sup>	Texture - Sand g 100 g soil <sup>-1</sup>	Texture - Silt g 100 g soil <sup>-1</sup>	Texture - Clay g 100 g soil <sup>-1</sup>
0	3.60 ±0.24	17.97 ±0.72	1.53 ±0.04	11.76 ±0.22	1.07 ±0.04	38.58 ±1.65	35.92 ±0.52	25.50 ±1.18
67	6.96 ±0.04	19.10 ±1.77	1.62 ±0.12	11.79 ±0.46	1.08 ±0.04	36.15 ±2.21	37.37 ±1.61	26.48 ±0.79
135	9.01 ±0.13	19.36 ±0.89	1.65 ±0.11	11.77 ±0.32	1.00 ±0.04	37.47 ±1.36	36.42 ±1.16	26.11 ±1.27
202	9.59 ±0.09	20.53 ±1.37	1.64 ±0.10	12.52 ±0.37	1.06 ±0.02	36.37 ±3.14	37.83 ±2.07	25.80 ±1.14
269	9.94 ±0.11	19.80 ±1.03	1.67 ±0.08	11.87 ±0.30	1.13 ±0.03	37.28 ±2.25	36.14 ±0.84	26.58 ±1.52

Table 2. Means and standard error for the total mass proportion aggregate classes and free silt and clay of the whole soil. There no significant differences among N rates for proportions of any aggregate class or free silt and clay.

Nitrogen application kg ha <sup>-1</sup> y <sup>-1</sup>	Large Macros g 100 g <sup>-1</sup> soil	Small Macros g 100 g <sup>-1</sup> soil	Free-micros g 100 g <sup>-1</sup> soil	Free-SC g 100 g <sup>-1</sup> soil
0	1.43 ±0.14	28.16 ±1.34	22.40 ±0.73	8.95 ±0.59
67	1.19 ±0.19	31.32 ±2.09	21.23 ±3.84	6.95 ±0.56
135	2.34 ±0.65	29.57 ±3.22	22.36 ±2.50	7.91 ±0.57
202	2.46 ±1.23	28.39 ±2.27	24.79 ±1.63	8.04 ±0.29
269	3.31 ±1.30	25.90 ±1.54	23.68 ±0.87	9.17 ±0.70

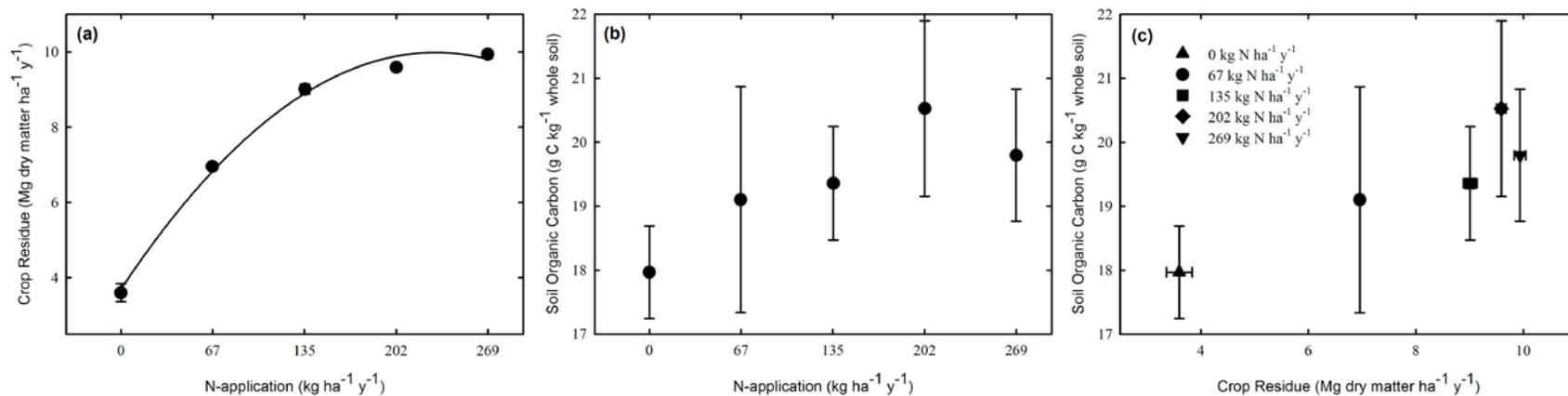
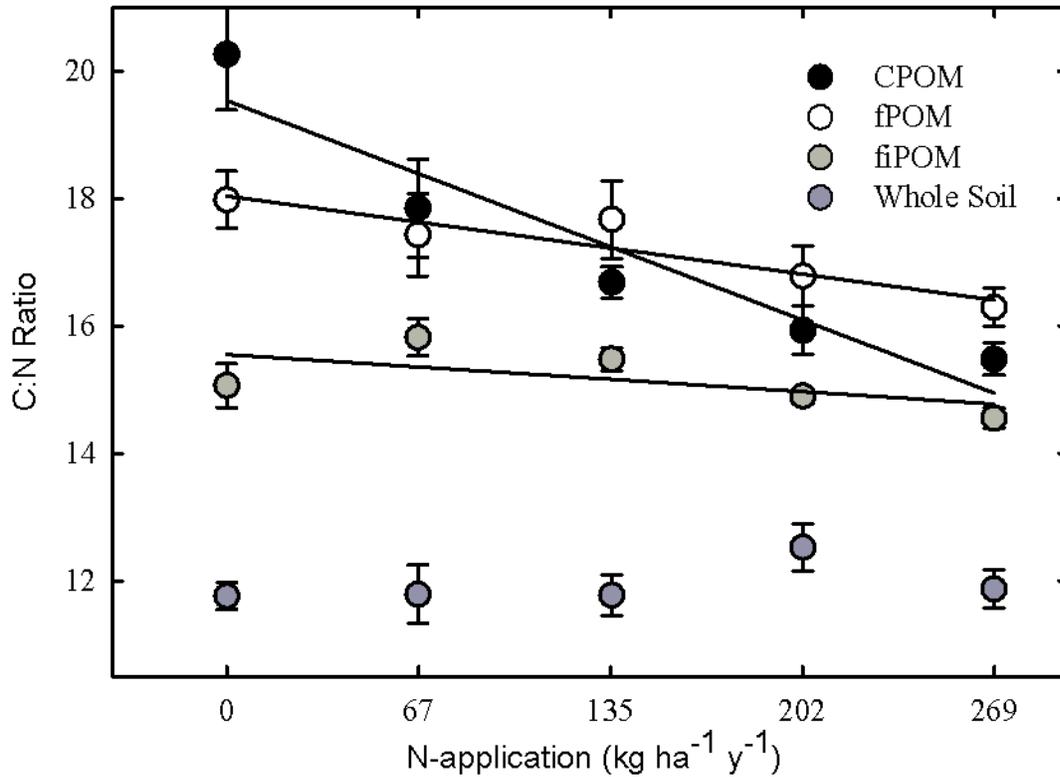


Figure 1. Crop residue as a function of nitrogen fertilizer application  $P < 0.0001$ ;  $R = 0.99$ ;  $y = 0.0545x - 0.0001x^{2+3.6902}$  (a); note yield is equivalent to residue based on the harvest index assumption of 0.5. Whole soil carbon concentration was not affected by N fertilizer application rate (b) or crop residue input (c). All error bars represent standard errors (N=4). Symbol shape indicates nitrogen fertilizer rate.

1



2

3

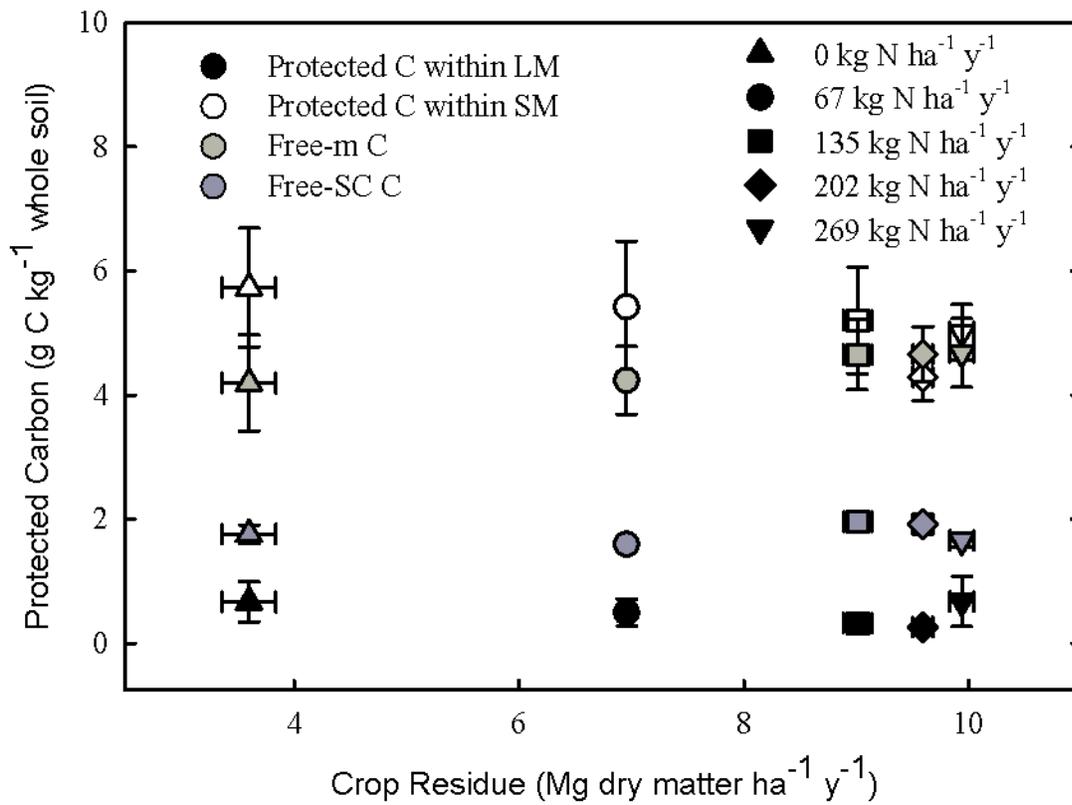
4 Figure 2. C:N ratios for POM fractions within small plus large macroaggregates. Significant linear  
5 relationships were found with nitrogen application rate and CPOM ( $P < 0.0001$ ,  $R = 0.71$ ), fPOM ( $P = 0.0079$ ,  
6  $R = 0.45$ ), and fiPOM ( $P = 0.0220$ ,  $R = 0.36$ ). Regression equations for CPOM, fPOM, and fiPOM were:  $y =$   
7  $-0.0171x + 19.54$ ,  $y = -0.0061x + 18.04$ , and  $y = -0.0029x + 15.55$ , respectively and the regression slope  
8 coefficients significantly differed between CPOM and fPOM ( $P = 0.0027$ ) and between CPOM and fiPOM  
9 ( $P < 0.0001$ ). Error bars represent standard errors.

10

11

12

13



14

15 Figure 3. There was no significant effect of crop residue on the amount of protected C fractions. Values  
 16 represent protected carbon within macroaggregates (the sum of C from inter-m, inter-SC, and intra-SC),  
 17 protected carbon within microaggregates (the sum of free-m and intra-SC), and free protected C (free-  
 18 SC). Error bars represent standard errors.

19

20

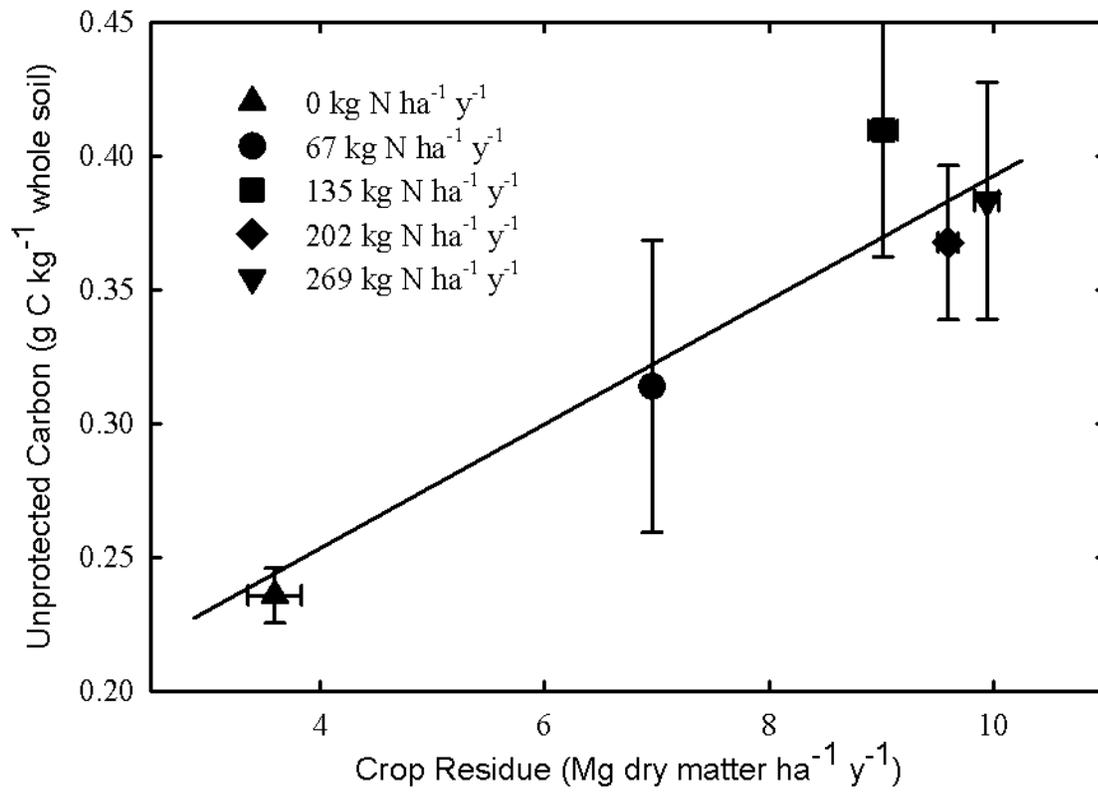
21

22

23

24

25



27

28 Figure 4. Unprotected carbon (sum of CPOM and fPOM) was positively correlated with crop residue  
 29 input. Error bars represent standard errors;  $P=0.0063$ ;  $R=0.59$ ;  $y=0.0232x+0.1604$ .

30

31

32

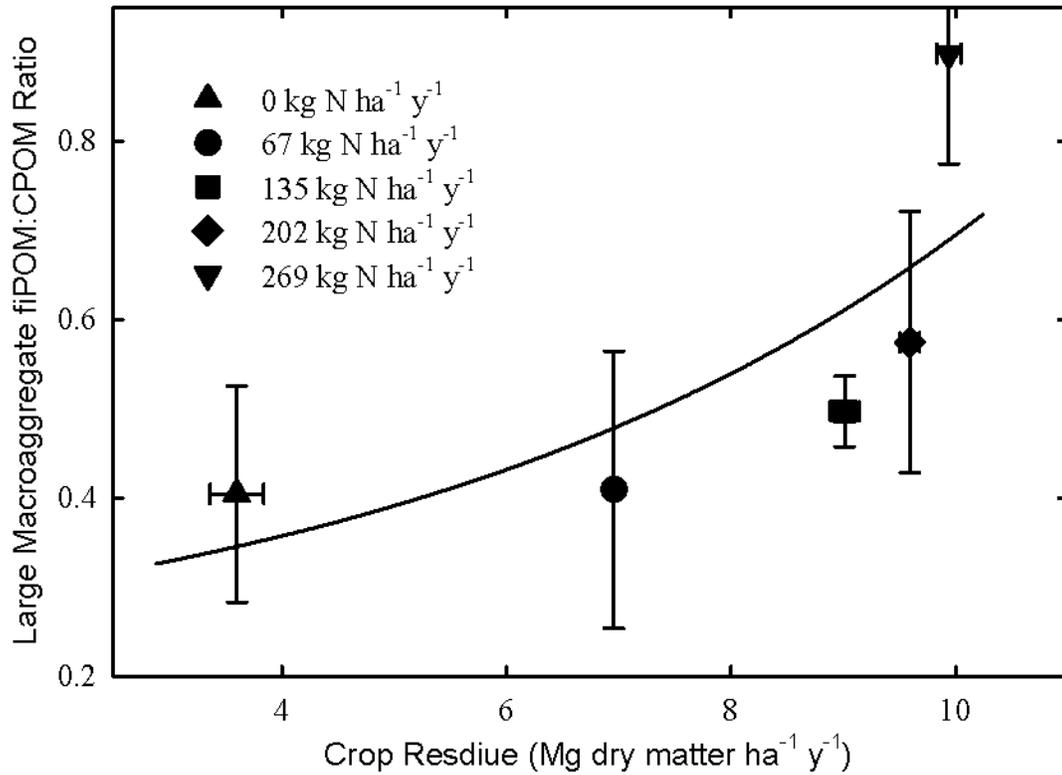
33

34

35

36

37



39

40 Figure 5. The ratio of fiPOM:CPOM in large macroaggregates increased with crop residue input. Error  
 41 bars represent standard errors;  $P=0.0540$ ;  $R=0.44$ ;  $y=0.1901+\log 1.202^x$ .

42

43

44

45

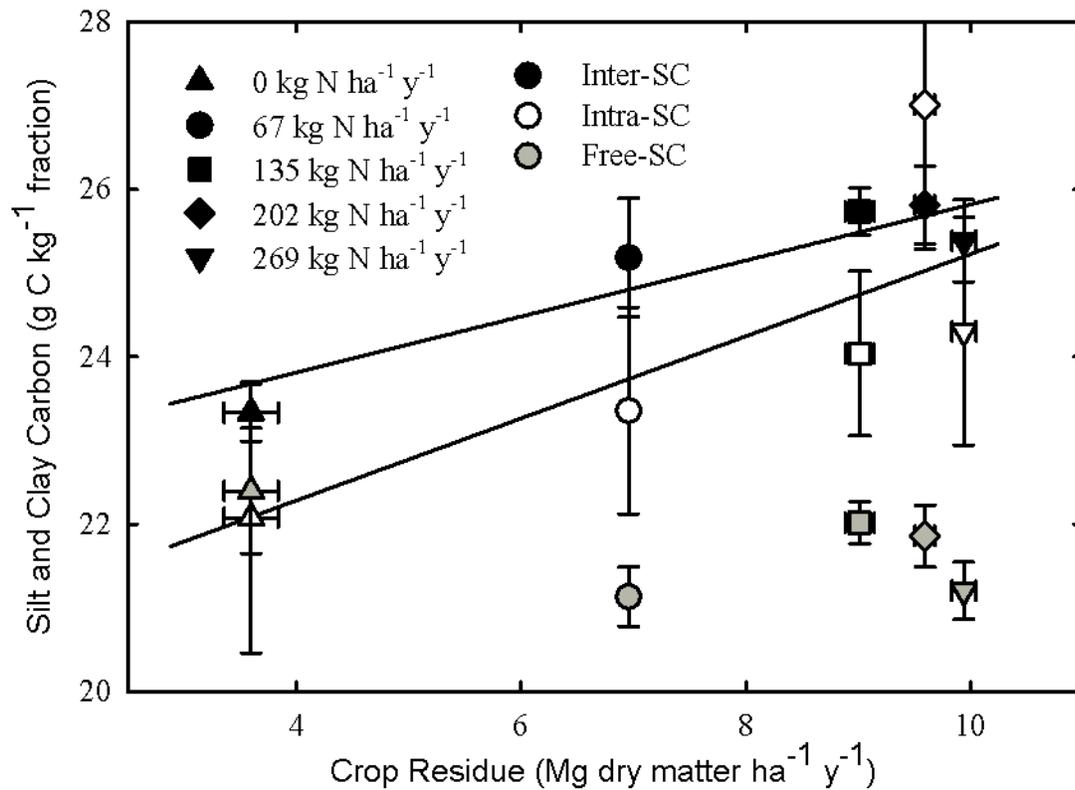
46

47

48

49

50



51

52

53 Figure 6. Carbon concentration of free silt and clay (free-SC) fractions as well as silt and clay fractions  
 54 trapped within aggregates (Inter- and Intra-SC). There were significant relationships between crop  
 55 residue and Inter-SC ( $P=0.0009$ ,  $R=0.51$ ) and slightly significant relationships between crop residue and  
 56 Intra-SC ( $P=0.0745$ ,  $R=0.29$ ). Regression equations for Inter-SC and Intra-SC were:  $y=0.3346x+22.47$  and  
 57  $y=0.4909x+20.32$ , respectively. Free-SC was not related to crop residue input. Error bars represent  
 58 standard errors.

59

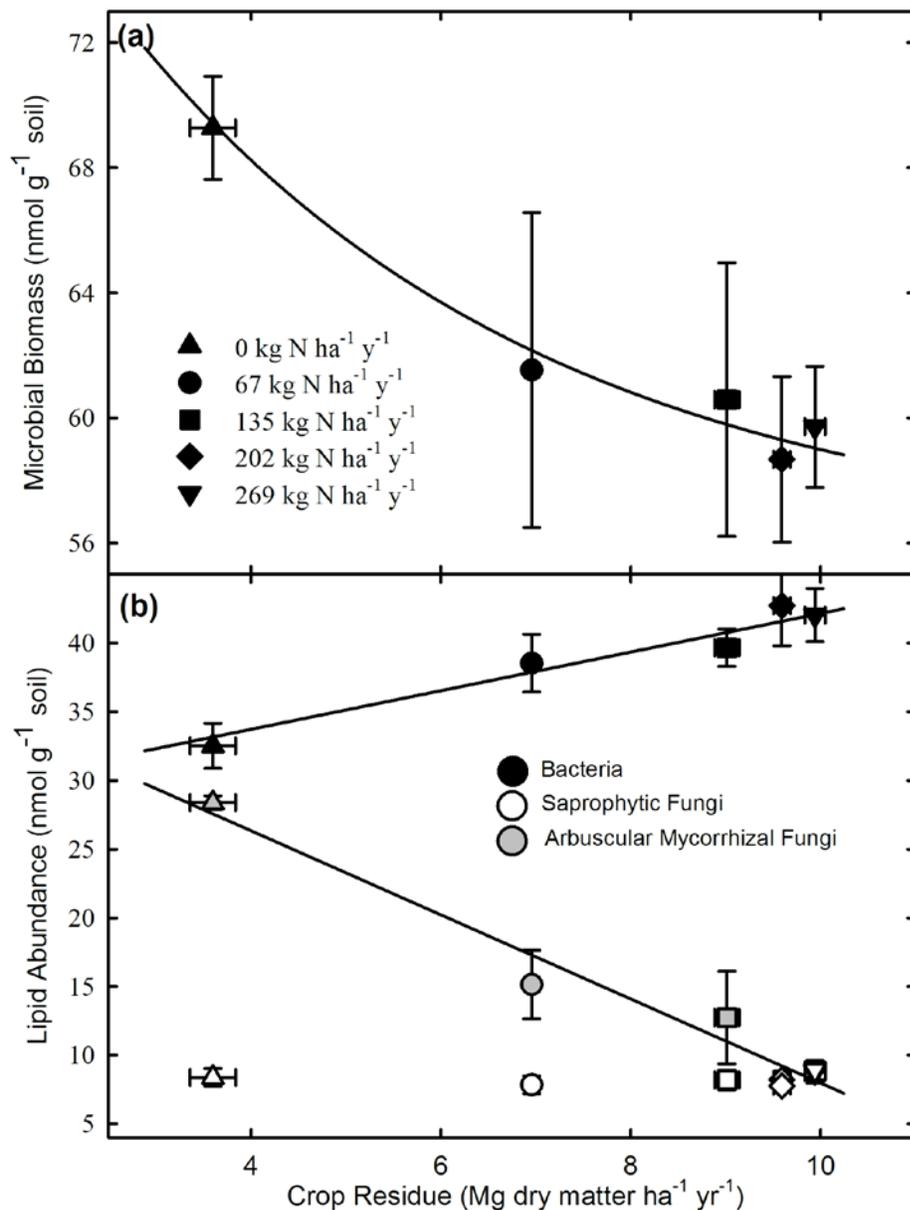
60

61

62

63

64



65

66 Figure 7 (a) Microbial biomass (sum of lipid abundance of bacteria, saprophytic fungi, and arbuscular  
 67 mycorrhizal fungi) decreased with crop residue input ( $P=0.0368$ ;  $R=0.57$ ); regression equation:  
 68  $y=55.81+30.85^{(-0.2272x)}$ . (b) Lipid abundance from fatty acid methyl ester analysis for bacteria and fungi.  
 69 Bacteria, saprophytic fungi and arbuscular mycorrhizal fungi are displayed. Crop residue was positively  
 70 correlated with bacteria ( $P=0.0018$ ,  $R=0.65$ ) lipid abundance and negatively correlated with AMF  
 71 ( $P<0.0001$ ,  $R=-0.90$ ) lipid abundance. Regression equations for bacteria and AMF were:  $y=1.41x+28.10$ ,  
 72  $y=-0.6873x+29.44$ , and  $y=-3.06x+38.60$ , respectively.

