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Ana B. Wingeyer
University of Nebraska-Lincoln, anitawingeyer@yahoo.com.ar

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THE EFFECT OF RESIDUE C:N RATIO ON THE TURNOVER OF N AND C IN VARIOUS SOIL ORGANIC MATTER FRACTIONS

by

Ana B. Wingeyer

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THE EFFECT OF RESIDUE C:N RATIO ON THE TURNOVER OF N AND C IN VARIOUS SOIL ORGANIC MATTER FRACTIONS

Ana B. Wingeyer, M. S.

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Adviser: Daniel T. Walters

Identifying soil organic matter (SOM) fractions that contribute to soil indigenous nitrogen (N) supply and understanding their turnover under different management constitute necessary tools toward an efficient N use. The objectives of this study were: i) trace the endpoint of carbon (C) flux from residue inputs into SOM; and ii) asses the role of the light fraction (LF), mobile humic acid (MHA) fraction and calcium humate (CaHA) fraction as N sources for heterotrophic decomposition of fresh plant residues with contrasting C:N ratio. A long-term aerobic soil incubation was carried out on ^{15}N-labeled soil samples from Lincoln and Mead, NE. Pre-incubation three residue treatments were assigned: MAIZE stover; SOYBEAN leaves, and NO-RESIDUE added. Pre- and post-incubation LF, MHA and CaHA were extracted. The soil was periodically leached, and the leachate was analyzed for N and ^{15}Natom%. SOM fractions were analyzed for %C, %N, δ^{13}C‰ and ^{15}Natom%. Cumulative mineralized N was ~60% higher at Mead. MAIZE addition resulted in N immobilization at Mead (until t=90d) and Lincoln (until t=300d), while SOYBEAN increased N mineralization by 42% at

Lincoln and 23% at Mead. Post-incubation, CaHA mass was reduced by 16 and 11% at Lincoln and Mead, respectively, and MHA and LF mass varied among treatments , with a significant increase of both fractions at Lincoln and no differences at Mead. All SOM fractions had a significant loss of ^{15}Natom% and ^{15}N mass across treatments. The relationship between ^{15}N mass loss and change in N mass indicated CaHA as a N donor fraction with a preferentiall loss of recently added materials. The turnover of MHA reflected a wider range of situations. A N donor under N mineralization at Mead, and N and C storage under N immobilization at Lincoln. The LF %C4-C increased under MAIZE, supporting LF as the primary pool for residues into SOM. This study indicates that the C and N flux from Residue > LF > MHA > CaHA, can be modified to CaHA > MB > MHA > LF under high N demand for decomposition of C, where both humic fractions constitute N sources to LF and residue decomposition.

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Table of Contents

Acknowledgements i

Table of Contents ii

List of Tables iii

List of Figures iv

Introduction 1

Materials and Methods 10

Results

1. Repeatability of MHA and CaHA fractions' mass yield, C, N, δ13C and 15N atom% composition 18
2. Soil N mineralization 19
3. Pre and post- incubation properties in WS and SOM fractions 23
4. Source of N under different residue additions 32

Discussion 37

Conclusions 42

References 44

List of Tables

Table 1. Average annual fertilizer N input and residue C and N input at the Lincoln and Mead, NE sites. Averages for the 1999 - 2004 period at Lincoln, and 1997 - 2004 at Mead. 52

Table 2. Means and standard errors of mass yield, %N, ^{15}N atom%, %C and δ13C‰ in MHA and CaHA fractions from 6 soil samples. The value for each plot represents three independent extractions. The probability value (p-value) corresponds to the repeatability among extractions. 52

Table 3. Means and standard errors of %N, ^{15}N atom%, %C and δ^{13}C‰ analysis performed on the same extracted MHA and CaHA samples. Three sub-samples were analyzed for the same soil sample and extraction time. The probability value (p-value) corresponds to the repeatability among determinations. 53

Table 4. Average ^{15}N atom % values in the initial (t = 0 d) and final (t = 300 d) leachate, and in pre- and post-incubation LF, MHA and CaHA fractions. 53

Table 5. Probability values for SOM fractions and WS properties with probability values. ns: p > 0.05. 54

Table 6. Pre- and post-incubation change in mass and C and N composition of whole soil (WS) and SOM fractions (LF, MHA and CaHA). Means by site, residue treatment and rotation. WS* Pre-incubation values for no-residue treatment. 56

Table 7. N turnover statistic for SOM fractions. Means and (se) by site, residue addition and rotation. 59

List of Figures

Figure 1. Means and standard errors of cumulative mineralized N and ^{15}N (mg kg soil^{-1}) after 300 days of aerobic soil incubation at Lincoln and Mead by residue amended. 60

Figure 2. Change in ^{15}N atom% in the MHA, CaHA and LF SOM fractions at Lincoln and Mead. Means and SE by residue amended and rotation. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize. 61

Figure 3. Change in LF, MHA and CaHA fraction mass (%) after incubation at Lincoln and Mead as affected by rotation and residue treatment. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize. Error bars are standard errors. 62

Figure 4. Change in %C4-C (%) in the MHA, CaHA and LF SOM fractions at Lincoln and Mead. Means and SE by residue amended and rotation. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize. 63

Figure 5. Change in C4-C mass (%) in the MHA, CaHA and LF SOM fractions at Lincoln and Mead. Means and SE by residue amended and rotation. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize. 64

Figure 6. Change in ^{15}N mass in the MHA, CaHA and LF SOM fractions at Lincoln and Mead. Means and SE by residue amended and rotation. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize. 65

INTRODUCTION

Among all crop production systems, nitrogen (N) constitutes the most critical plant nutrient in terms of amount required and potential impact on environmental quality. To produce profitable yields without increasing N losses requires a synchrony between crop N demand and N supply from all sources throughout the growing season. To achieve this synchrony requires reliable prediction of N supply from indigenous sources so that the amount and timing of N fertilizer applications can be utilized most efficiently by the crop and the negative effect of N on the environment can be reduced. Likewise, thorough knowledge of how agricultural management practices affect the size and dynamics of the indigenous soil N pool over both the short- and long-term is necessary to develop crop and soil management systems that maintain or even increase the soil indigenous N supply. Such knowledge is critical to development of sustainable agricultural systems.

The indigenous N supply of the soil represents the ability of a soil to provide N for a crop. Soil N will ordinarily cycle continuously between organic and inorganic forms through the processes of mineralization-immobilization turnover (MIT) (Jansson and Persson, 1982). The measurement of net N mineralization, the difference between gross N mineralization and immobilization, has been used to represent the size of the soil indigenous N supply pool often termed potentially mineralizable N (Jansson, 1963). The kinetics of the many biological and physical processes governing net soil N mineralization can be assessed by

simple functional approaches, where the parameters for modeling are obtained from laboratory soil incubation studies by fitting cumulative N mineralization to the time of incubation. When soil samples are incubated under constant temperature and moisture, the amount of cumulative mineralized N results in a comparable measurement of the soil N mineralization capacity, the quality of available substrates for mineralization and their interaction with soil matrix (Wang et al., 2003; Benbi and Richter, 2002).

In a posterior analysis of N mineralization, Wang et al. (2004) proposed that the dynamics of indigenous soil N supply could be related to specific soil organic matter (SOM) pools. They defined the total mineralized N as composed by N from the “active N pool” responsible of the N mineralization flush in the first weeks and the “slow N pool” responsible for the long-term capacity of N supply. As SOM represents a continuum of organic compounds in several stages of decomposition and re-synthesis, it is possible that N availability will vary with the degree of physical and chemical stabilization of these pools (Nguyen et al., 2004a; Baldock and Skjemstad, 2000; Paul, 1984). Thus, the study of SOM pools with different degree of decomposition and physical-chemical protection along with the soil N supply will contribute to the understanding of the indigenous soil N supply capacity and dynamic (Paul et al., 2003).

The stabilization of SOM is determined by its accessibility to microbes, which is characterized by the degree of chemical recalcitrance and the accessibility to microbes within the soil matrix (Krull et al., 2003). The chemical recalcitrance of SOM depends on the original composition of the organic material

and the chemical transformation processes that it undergoes over time. Solid state ^{13}C-NMR has been used to asses the SOM composition and expected speed of turnover. These include a fast degrading pool (months to a few years) mainly composed by carbohydrates identified in the O-alkyl region; an intermediate degrading pool (10 – 100 yr) composed by aliphatic and aromatic compounds identified in the alkyl region; and the recalcitrant pool (> 1000 yr) mainly composed of charcoal, a highly condensed and aromatic SOM fraction identified in aryl C region (Gleixner et al., 2001; Skjemstad et al., 2001, 1996). Despite the variation in SOM turnover expected by its composition, the actual rate at which the organic fractions are decomposed depends also in the degree of protection by the soil matrix (Baldock and Skjemstad, 2000), as well as temperature, aeration and water content that influence microbial activity.

The protection mechanisms of SOM by soil minerals include the absorption and chemical binding of organic compounds onto soil mineral surfaces and the physical occlusion into aggregates (Krull et al., 2003; Six et al., 2002; Baldock and Skjemstad, 2000; Sollins et al., 1996). The chemical nature of soil minerals, the presence of multivalent cations, and the presence of reactive mineral surfaces determine the capability of SOM chemical protection (Baldock and Skjemstad, 2000). The most common protection mechanism of SOM against microbial decomposition is the sorption of negatively-charged organic groups to clay minerals through polyvalent cation bridging (Sollins et al., 1996). Soil layer silicates and sesquioxides with reactive surfaces have the capacity to adsorb and protect SOM with the concomitant increase in content and specific surface area

of these minerals resulting in an increase of the soil protective capacity (Oades, 1989). The physical occlusion of SOM is possible through aggregation. As aggregates are being formed and destroyed continually, the protection of SOM will be greater where aggregate stability is high and turnover is low (Krull et al., 2003). The incorporation of new organic materials seems to occur preferentially into macro aggregates and then they are progressively distributed to smaller aggregates (Puget et al., 2000). However as macro aggregates are more easily disturbed than smaller aggregates, there is a faster turnover of occluded SOM resident in them (Skjemstad et al., 1996). Soil organic matter is then a heterogeneous mixture of compounds ranging in size, complexity, turnover rate and location in the soil matrix.

The fractionation of SOM into relevant pools with different turnover rates is based on its composition and degree of physico-chemical stabilization. These extraction procedures attempt to isolate fractions that respond to land management and use and that represent a significant proportion of SOM (Olk and Gregorich, 2006). Physical fractionations segregate SOM pools as a function of their association to soil minerals and position within the architecture of soil aggregates. These fractionation procedures are based on disturbance of soil aggregates and subsequent separation by sedimentation or sieving into particulated or aggregated size fractions, or by specific density into light fraction (LF) and heavy fractions (Gregorich et al., 2006; Cambardella and Elliot, 1992; Christensen, 1992; Janzen et al., 1992). Chemical fractionations are based on the different solubility in water, alkalis and acids associated with the type of

chemical binding between SOM and minerals and within SOM (Olk and Gregorich, 2006).

Physically extracted SOM has been reported as a rapid response fraction to crop management and amount and quality of residue additions (Cookson et al., 2005; Lagorreta-Padilla, 2005; Christensen and Olsen, 1998; Feller and Beare, 1997; Boone, 1994; Cambardella and Elliot, 1994; Christensen, 1992). One physically extracted SOM fraction, the LF pool, is a mix of plant residues at several stages of degradation along with microbial biomass and constitutes a rich carbon (C) source with a rapid turnover time in the soil since it generally lacks physical protection (Legorreta-Padilla, 2005; Christensen, 1992). This fraction can be conceptualized as the first step in C stabilization as crop residues transform to SOM. Legorreta-Padilla (2005) measured the δ^{13}C‰ of LF during crop growing season and found significant changes in LF composition from planting to harvest, which was evidence of the labile behavior of the fraction and the role of its C and N composition in its turnover. The contribution of LF to the indigenous soil N supply will depend on the inner composition of the fraction, its C:N ratio, and the soil conditions that promote the decomposition of the fraction (Gregorich and Janzen, 1996). Wang et al. (2004) reported that N mineralized from the “active N pool” was positively associated to the initial dissolved organic N representing readily mineralizable organic N, while the slow pool was linked to the quality of SOM - LF.

The chemical extraction of humic acid fractions before and after an acid wash isolates two pools of different composition and chemical stabilization: the

mobile humic acid (MHA) fraction and the polyvalent cation bound humic acid (CaHA) fraction. Nitrogen concentration in MHA ranges from 4.0 to 5.5%, which is approximately twice the N content found in the CaHA fraction, while C concentration varies among 48 to 53% in MHA and 54 to 57% in CaHA (Legorreta-Padilla, 2005; Nguyen et al., 2004a, b; Mahieu et al., 2002a; Olk et al., 1996). The mean residence time of C as determined by ^{14}C was found to be on the order of 10 - 100 years in aerated soils for MHA and 250 - 1000 years for CaHA (Legorreta-Padilla, 2005). Chemical characterization of both these fractions by NMR has showed that the MHA fraction contains a lesser amount of aromatic-C and a lower molecular weight, representing an earlier stage in SOM stabilization compared to the more condensed CaHA (Mahieu et al., 2002a, b). Optical density analysis at 465 nm to determine the concentration of humified materials and its degree of condensation showed that MHA is composed of less humified materials than CaHA (Nguyen et al., 2004a). There are then two SOM stabilization mechanisms acting together on humic acids: biochemical recalcitrance and cation binding to minerals. They appear to drive the humification sequence from the younger, less condensed and N rich MHA to the older and more humified CaHA.

In maize-belt soils, Legorreta-Padilla (2005) studied the effect of crop management (rotation, tillage, nutrient input) on N and C content of total soil, MHA, CaHA and LF pools. He found that C and N mineralization rates were different between MHA and CaHA, with a loss in MHA C and N as result of crop rotation with soybean, no N fertilizer inputs and limited crop productivity. The

MHA loss was attributed also to the hypothesized CaHA precursor function of MHA as N is released from MHA and then Ca^{+2} complexed to CaHA. The author characterized the MHA pool as an easily depleted substrate under N limiting situations or under high crop N demand, and concluded that depletion of MHA-N may occur in the short term if N inputs do not compensate the demand of the cropping system. For almost all the studied situations, the depletion of total soil N and the N of MHA pool fit an exponential decay model suggesting that N demand and availability controls the utilization of these pools as substrate for microbial activity (Legorreta-Padilla, 2005).

In the same study, the $\delta^{13}C$‰ signatures of MHA showed the effect of residue quantity and quality on its formation and persistence into the soil. Short-term (five months) $\delta^{13}C$‰ measurements during the growing season displayed rapid change in the $\delta^{13}C$‰ of both LF and MHA fractions. Since LF is a rich C source bearing plant characteristics, it is most likely a precursor to more humified materials. However, the decomposition rate of LF will be related to its own N content, C:N ratio, and possibly to the readily mineralizable N content of MHA. Studies with ^{15}N labeled soil in these sites showed that ^{15}N enrichment decreased in the order LF > MHA > CaHA implying that the flux of N and degree of stabilization follows this same sequence.

Nguyen et al. (2004b) analyzed the contribution of MHA and CaHA extracted from seven soils on their N mineralization potential, by addition of these two fractions to two different soils and incubating them under anaerobic conditions for six weeks. They reported that cumulative N mineralization from

MHA amended soils was greater than from CaHA amended soils, indicating MHA as a more labile humic fraction with a greater contribution to indigenous soil N supply as confirmed by the chemical characterization of these fractions (Legorreta-Padilla, 2005; Nguyen et al., 2004a, b; Mahieu et al., 2002a). After addition of MHA to a soil rich in exchangeable calcium, the N supply was reduced compared to that added to a low exchangeable calcium soil (Nguyen et al., 2004b). Similarly, Legorreta-Padilla (2005), observed that when soil chemical conditions allowed the stabilization of humic acids as CaHA, the MHA pool was quantitatively reduced as CaHA increased over a period of several months. This evidence suggests that mineralization of N from young N-rich humic acid compounds (MHA) can be replaced by divalent cations (i.e. Ca^{+2}) to form chemically stabilized humates such as CaHA (Olk, 2006; Legorreta-Padilla, 2005; Nguyen et al., 2004a, b; Mahieu et al., 2002a; Baldock and Skjemstad, 2000 and Olk et al., 1995).

Agricultural practices influence the decomposition rates of SOM due to physical soil disturbance, erosion processes, and residue inputs with a greater impact on the less stabilized fractions (Besnard et al., 1996; Paustian et al., 1995). The amount and quality (C:N ratio) of added residues will also influence the size and supply of the indigenous soil N pool (Paustian et al., 1997; Janssen, 1996). Studies conducted at these Lincoln and Mead, NE sites have demonstrated the impact of crop rotation (through residue quality) and fertilization management (by residue quantity) on the C and N dynamics in LF, MHA and CaHA pools (Legorreta Padilla, 2005). At Lincoln, intensive nutrient

(NPK) management practices for continuous maize along with high plant populations have resulted in a build up of soil C and N. In contrast the maize-soybean rotation has resulted in a net loss of soil N and C. In this study, the soil under continuous maize (CC) rotation and intensive management accumulated 3100 kg C ha^{-1} and 340 kg N ha^{-1} after six years (Adviento-Borbe et al., 2007). The net effect of maize-soybean (CS and SC) rotation under recommended and intensive nutrient management was an increase in the growing season soil N supply, which led to a net loss of both soil C and N reserves (Adviento-Borbe et al., 2007). Conversely, in the CC rotation under both intensive and conventional management, there was an increase in SOM that has resulted in an increase in indigenous soil N supply over time and less dependence on fertilizer N input over time (Walters et al., 2004). A study conducted by Legorreta-Padilla (2005) in these sites showed that crop rotation had a significant impact on MHA formation and persistence in soil, primarily due to differences in residue quality and C inputs. Over the long term, the MHA content declined in the CS rotation, and increased under CC management which implies that a direct link exists between MHA and indigenous soil N supply. Moreover the increase in the crop productivity under high nutrient inputs resulted in stabilization of MHA and CaHA fractions (Legorreta-Padilla, 2005).

With the aim of understanding the role of chemically and physically extracted SOM fractions on C and N flux, a laboratory experiment was designed to accomplish the following objectives: i) trace the endpoint of C flux from residue inputs into SOM fractions; ii) asses the role of the LF, MHA and CaHA as

sources of N during the heterotrophic decomposition of fresh plant residue in both net immobilizing and net mineralizing environments via manipulation of the C:N of residue addition.

The hypothesis for this experiment were: i) C flux from residue inputs into SOM follows the sequence LF > MHA > CaHA; ii) MHA is an important source of N during the heterotrophic decomposition of fresh plant residue in an immobilizing environment (high C:N residue addition); iii) high C:N ratio residue decomposition (net immobilizing environment) will result in the transfer of MHA-N to the microbial biomass (MB) during the decomposition of the energy rich LF and formation of CaHA; iv) low C:N ratio residue decomposition (net mineralizing environment) will result in flux of residue N and C to MHA.

MATERIALS AND METHODS

A long-term soil incubation was carried out on ^{15}N labeled soil samples to evaluate the effect of fresh plant residue additions of different C:N ratio and $\delta^{13}C$‰ on the fate of C and N among soil organic matter fractions.

Soil material

The soils used for this experiment were collected from two long-term maize experiments located at Lincoln, NE (42º 12'N - 96º 35'W - Fine-silty, mixed, mesic Cumulic Hapludoll) and at Mead, NE (42º 23' N – 96º 50' W - Fine-montmorillonitic, mesic Typic Argiudoll). The amounts of C and N inputs from residue and N from fertilizer are presented in Table 1. In May 2003, ^{15}N labeled ammonium - nitrate fertilizer ($^{15}NH_4{}^{15}NO_3$) was applied to field micro plots to

maize (CC and CS rotations with 200 kg N ha^{-1} ≈ 10% AE ^{15}N) or soybean (SC rotation with 10 kg N ha^{-1} ≈ 99% AE ^{15}N). The number of replications were four at Lincoln (n = 12) and three at Mead (n = 9). After harvest, the crop ^{15}N labeled residues were incorporated by plowing (Lincoln) or disking (Mead). Prior to planting in the spring of 2004, the ^{15}N micro plots were sampled at 0 - 20 cm depth in Lincoln and 0 - 15 cm depth in Mead, which represented the historical tillage and sampling depths. The samples were gently handled to pass through a 2 mm sieve, bigger residue particles were removed and soil was stored in closed plastic bags at -1 ºC.

Amended Crop Residue Treatments

Prior to packing soil samples in incubation units, three levels of finely ground unlabeled crop residue were established. These included a.) control (no residue addition), b.) soybean leaf residue (C:N ratio = 12), and c.) maize stover residue (C:N ratio = 75). Both maize and soybean residues were added at a rate to deliver 23.7 mg N kg $soil^{-1}$. This rate of N application as residue resulted in 290 mg C kg $soil^{-1}$ added as soybean leaf residue and 1770 mg C kg $soil^{-1}$ added as maize stover residue.

Incubation specifications

After mixing crop residue treatments with soil, the soil was aerobically incubated in plastic incubation units of 115 ml capacity with a 0.2 μm pore size cellulose nitrate membrane and a 115 ml receiver sealed to the filter unit (Nalgene® 121-0020). Each filter unit (experimental unit) was filled with field moist soil (60 g dry soil basis) and packed to a 1.1 Mg m^{-3} soil bulk density. Soil

packing was carried out by successive filling and compaction to achieve a uniform soil density. A previous soil packing trial was performed using soil samples with moisture contents in the same range that the experimental soil samples until it resulted in a density range of 1.09 to 1.13 Mg m^{-3}, and the packed soil exhibited an internal uniform soil density. The incubation units were incubated during 43 weeks (301 d) in a growth chamber at constant air temperature (25 ºC) and soil moisture (60 ± 4.2% water filled pore space (WFPS): θ_v/ε). Incubation vessels were sealed and aerated with a forced stream of humidified CO_2 free air at a rate of 10 ml min^{-1}. The θ_v of these soils was determined in a previous determination after equilibrium under 0.33 bar and WFPS averaged 60 ± 2.1% WFPS. The tare of each experimental unit was individually recorded before starting the soil filling. For each leaching process, the weight of each units was monitored to unplug them from the vacuum line when soil reached 60% WFPS. The humidified CO_2 free air circulation was achieved by bubbling air into a flask containing 4N NaOH, followed by a flask containing water and a flask without water (to trap excess water).

Net N mineralization

A periodic leaching was performed by addition of 100 ml 0.01M $CaCl_2$ followed by 25 ml of minus N Stanford and Smith (1972) nutrient solution. Leachings were performed at 0, 1, 2, 4, 7, 10, 13, 16, 19, 24, 28, 34 and 43 weeks from the inception of incubation. The leached solution was first collected on the unit reservoir and then transferred via vacuum trap to disposable plastic bottles with known tare. After the leaching was completed the bottles were

weighed to estimate the leachate volume. A 5 ml aliquot was then preserved for N analysis (ammonium and nitrate) on a LACHAT® 8000. After the last leaching (301 d) soil was quickly removed from the incubation units and air dried. To determine the ^{15}N atom% content of the leachate 30 to 60 mL of the leached solution were placed into 125 mL disposable plastic cups, containing 0.3 g MgO heavy powder, 0.4 g Devarda's alloy, and two glass beads. A 10 mm diameter Whatman GF/D filter disk treated with 10 μl of 2.5M $KHSO_4$ was then suspended above the solution on a stainless wire and the cup lied was tightly closed. The cup was gently shaken to allow mixing but without wetting the filter disk and then placed on a bench for diffusion occur during six days (Brooks et al., 1989). After that time, the filter paper was removed and desiccated for 24 h before packing the filter disk into a tin cup for ^{15}N atom% analysis. A separate incubation of unlabeled soil from the same sites and treatments was used to obtain the reference ^{15}N atom% enrichment on the leachate.

Soil indigenous N pool

The N mineralization dynamic was evaluated by fitting a first order kinetic model to the cumulative mineralized N (*N*) (Eq. 1):

$$N = N_0 \times \left(1 - e^{kt}\right) \qquad [1]$$

where N_0 represents the size of the soil indigenous N pool, t is the time from the beginning of the mineralization, and k is the rate of mineralization. The parameters N_0 and k were estimated using the routines included in the software SigmaPlot 9.0® (SYSTAT, 2004).

Humic acid fractionation

The humic acid extraction employed in this study is based on the sequential removal of NaOH soluble mobile humic acid (MHA) until the fraction is exhaustively removed, followed by acid washes to ensure the removal of cations, and the exhaustive extraction of calcium humate (CaHA). The large number of steps and time that this procedure takes, the need to preserve SOM against oxidation, and the fact that there are no clear boundaries between the extracted humic acids along with the dependence of CaHA yield on the effectiveness of the acid wash requires extreme care during the extraction procedure.

A small study was conducted whereby repeated humic acid extractions and analysis were performed in triplicate on six different soil samples used in the study to test repeatability of extraction mass and consistence in the analyses of %N, ^{15}N atom%, %C and $\delta^{13}C$‰ in separately extracted samples. In addition, one of the replicates of CaHA and MHA from each of these plots was subdivided into three sub-samples and analyzed for %N, ^{15}N atom%, %C and $\delta^{13}C$‰ to test the repeatability of these analyses on a single sample.

Humic acid fractionation into MHA and CaHA was performed on soil prior to incubation and addition of residue treatments and after 301 d of incubation. The humic acid extraction procedure followed that prescribed by Olk et al. (1995) with some modification. The first humic fraction, MHA, was obtained by two consecutive extractions with a 10:1 ratio of 0.25M NaOH:dry soil. Ultra pure N_2 gas was bubbled into the suspension for 5 min to remove oxygen that might oxidize humic acids. Samples were shaken at 150 RPM in a reciprocating

mechanical shaker for 15 min every 2 h for 24 h. After this regimen, the samples were centrifuged for 20 minutes at 13,000 g. The supernatant was acidified with 2N HCl to pH = 2 to precipitate MHA. After the second MHA extraction, the soil pellet was subject to two acid washes using an 8:1 ratio 0.25M HCl: dry soil followed by a water wash. The second humic fraction, CaHA, was then extracted and precipitated following the same steps outlined for the MHA fraction. After the final CaHA extraction, the remaining soil was acid and water washed and dried at 40 ºC. The humic acid precipitates were cleaned to reduce clay contamination by dissolution with 0.28M KOH and 0.4M KCl under an N_2 environment, shaking for 10 minutes and then centrifuged at 13,000 g. The supernatant was then re-precipitated. The precipitates were then de-ashed by shaking 24 h with a solution of HF-HCl, centrifuged, then packed in cellulose dialysis membrane, and dialyzed under constant stirring for three and a half days with solution changes from 0.01M HCl to 0.001M HCl and finally deionized water during the first, second and third days of dialysis, respectively. Dialysis solutions were changed twice daily. After dialysis the content of each membrane was washed into glass bottles and freeze-dried. The dried humic acid fractions were then weighed, homogenized in a high speed ball mill, and transferred to 4 mL amber glass vials for storage. The dried soil remaining after the last extraction (humin) was weighed, ground with mortar and pestle, and stored in glass vials to await analysis.

Light fraction extraction

The light fraction (LF) extraction was performed on both the pre-incubation and post-incubation soil as follows. Each air dried soil sample was placed in a 70

mL plastic centrifuge tube and suspended in sodium polytungstate solution (SPT) of 1.6 g cm^{-3} density in an 8:1 SPT:soil weight ratio. The tubes were then shaken at 300 RPM in a reciprocating mechanical shaker for 15 min and then allowed to stand 30 min before centrifugation at 2000 RPM for one hour. The supernatant was carefully transferred to a disposable plastic filtration unit with a cellulose nitrate filter of 0.45 μm pore size to separate the light fraction of the SPT. To speed the filtration, the units were connected to a vacuum pump at 0.3 bar. The material collected on the filter was washed with 350 ml deionized water, transferred to a 50 ml porcelain crucible and dried at 40 ºC. After dried, the LF was cooled in a desiccator, weighed, and homogenized using a high-speed ball mill and stored in 4 mL amber glass vials prior to analysis.

Ash content, C, N, δ^{13}C‰ and ^{15}N determination.

Ash content in SOM fractions (with the exception of Humin) were determined by weighing 3 to 10 mg into a previously tared silver cup. The silver cups were placed on ceramic trays and burned at 500 ^{o}C for 4 h. All SOM comparisons (mass and composition) are reported in an ash free basis.

Whole soil (WS), LF, MHA, CaHA and Humin fractions, and maize and soybean residues were analyzed for C and N concentrations in an automated Costech Analytical Incorporated Elemental Combustion System. The ^{15}N atom% analysis was doing on an Europa Scientific INTEGRA Isotope Ratio Mass Spectrometer and δ^{13}C‰ was determined on a Thermo Finnigan Delta-S Isotope Ratio Mass Spectrometer. To obtain the reference ^{15}N atom% the same SOM

fractionation procedures were applied to a separate incubation experiment of unlabeled soil samples from the same sites and treatments.

Carbon and Nitrogen fluxes

At the end of 301 d of incubation, the change in C and N content of WS and SOM fractions was analyzed as the change in content and mass of C, C-4 derived C (C4-C), N and ^{15}N in pre and post-incubation soil. The proportion of C4-C was calculated using the $\delta^{13}C$‰ of the sample (Balesdent and Mariotti, 1996) as (Eq. 2):

$$\%\ C4-C = \frac{\delta^{13}C_{sample} - \delta^{13}C_{C3veg}}{\delta^{13}C_{C4veg} - \delta^{13}C_{C3veg}} \times 100 \qquad [2]$$

where sample refers to the sample in question, and veg refers to vegetation. The $\delta^{13}C$‰ signature was set -27‰ for C3 vegetation and -12‰ for C4 vegetation.

The ^{15}N atom% excess in each sample was calculated as indicated in Eq. 3:

$$^{15}N\ atom\ \%\ excess = A_S - A_R \qquad [3]$$

where A_S is the ^{15}N atom% in the sample and A_R is the ^{15}N atom% in the reference sample. The mass of ^{15}N present in the samples (^{15}N mass) was calculated as in Eq. 4 (Powlson and Barraclough, 1993):

$$^{15}N\ mass = TN \times {}^{15}N\ atom\ \%\ excess \times C.F. \qquad [4]$$

where TN is the total N in the sample (g kg^{-1}), and C.F. is a correction factor for the true mass of ^{15}N in each sample (Eq. 5):

$$C.F. = \frac{14 \times \left(1 - {}^{15}N\ atom\%\ in\ the\ sample/100\right) + 15 \times \left(1 - {}^{15}N\ atom\%\ in\ the\ sample/100\right)}{14} \qquad [5]$$

Statistical analysis

The incubation experimental design is a randomized complete block split-plot design with field rotation as the main factor with three levels of rotation: CC, CS and SC, and with four replications at Lincoln and three replications at Mead. The residue addition treatment is the subplot factor with three levels: Control (no residue), maize stover, and soybean leaf.

Prior to incubation all soil fractions and their properties were analyzed as a Randomized Complete Block (RCBD). Post-incubation soil parameters and cumulative mineralized N were analyzed as a split-plot RCBD. The post- vs pre-incubation measurements were analyzed as split-split-plot RCBD. The reproducibility of humic acid extractions and δ^{13}C‰ and ^{15}N analyses of these fractions were analyzed as repeated measurements. The statistical analyses were performed using the routines included in SAS 8.0 (SAS Institute, 2001) and SigmaStat 3.1® (SYSTAT, 2004).

RESULTS

1. Repeatability of MHA and CaHA fractions' mass yield, C, N, δ^{13}C and ^{15}N atom% composition.

The mean and standard error of CaHA and MHA extractions mass and the analysis for %N, %C, ^{15}N atom%, and δ^{13}C in each are displayed in Table 2. There were no significant differences in extracted mass or N and C analysis among the repeated measures. These results indicate that the procedures used were sufficiently robust to insure low error in extractability. Also the fact that N

and C analyses were similar across replicates within a sample suggests that the constitution of the extracted masses was unique and repeatable.

The repeatability of the Costech analyses of %C and %N as well as mass spectrophotometer determination of both ^{15}N atom% and $\delta^{13}C$ are shown in Table 3. Readings and the sample preparation for analysis were done on repeated analysis of the same extracted sample. There were no significant differences in the determination of these properties performed on the same extracted sample for MHA or CaHA.

2. Soil N mineralization

a. Cumulative mineralized N

The total cumulative mineralized N was affected by residue amendment and rotation, with Mead site yielding more than twice the mineralized N than at Lincoln (Figure 1). The difference between sites could be related to the history of N and C management, since the total N inputs from fertilizer and residues in the years prior to extraction of these samples from the field to soil sampling were similar at both sites but the amount of C returned with the residues was higher at Lincoln site. During the 300 days of incubation there was an evident effect of the C:N ratio of the residues added on all the rotation soils at both sites. When soil was amended with maize stover (C:N ratio = 75) we observed a net N immobilization environment that lasted until the no residue treatment accumulated about 15 mg mineralized N kg soil^{-1}. Thus maize induced N immobilization until the end of incubation at Lincoln, t = 300 d, and until the day t

= 90 at Mead. Where soybean leaf had been amended (C:N ratio = 12) there was an increase in net N mineralization over the non-residue treatment that accounted for a total increase in cumulative mineralized N of 42% and 23% above the no-residue treatment at Lincoln and Mead, respectively.

b. Estimate of indigenous soil N pool size, N_0

The size of the indigenous soil N supply was estimated from the non-amended treatments. At Mead, the rate of cumulative N mineralization declined overtime but at t = 169 d, all the Mead soils showed an increase in the rate of net N mineralization. For the Lincoln treatments a single first order exponential function was fitted, while at Mead a first order exponential function was fitted with the cumulative mineralized N until t = 169 d. The estimated N_0 values were 13.7, 18.5 and 18.2 mg N kg soil^{-1} for CC, SC and CS at Lincoln and 48.7, 30.8 and 43.7 mg N kg soil^{-1} at Mead, respectively. At Lincoln, the size of N_0 was larger under rotations compared to CC, while at Mead site, the size of N_0 was significantly higher under CC and CS than SC. The difference between sites could be related to N management. At Mead, soybean crops did not receive any N from fertilizer during the growing season, while the maize crop was fertilized with 300 kg N ha^{-1}. This N rate was far in excess of crop needs. In essence, there was a significant pool of inorganic N available to aid in the decomposition and humification of maize residues at the Mead site. The mass of MHA at Mead was 50% greater than at Lincoln under the CC rotation and 83% greater following maize in the CS rotation at Lincoln. In contrast, MHA mass was nearly equivalent at Mead and Lincoln following soybean in the SC rotation (see Table 6). As MHA

is a young humic acid fraction with high N content (< 5%), it may then constitute a significant source of indigenous N supply.

c. Cumulative mineralized ^{15}N

The total cumulative mineralized ^{15}N was similar between the non-amended and soybean amended treatments at each site. In contrast, the maize-amended treatment showed the effects of net N immobilization (Figure 1). The similarity between mineralized ^{15}N between the non-residue and soybean amended treatments quantifies the net mineralization of added N in the soybean leaf amended treatment. The proportion of cumulative mineralized ^{15}N to cumulative mineralized N was higher at Mead than Lincoln (0.39% vs 0.30%, 0.33% vs 0.28%, and 0.33% vs 0.25%, for none, maize, and soybean, respectively) which can be associated with more enriched materials.

d. ^{15}N atom% of mineralized N and SOM fractions

The ^{15}N atom% of leached inorganic N declined over time following an exponential decay function (data not shown). The ^{15}N atom% values at the initial leaching were similar to those of the pre-incubation LF at both sites (Table 4). At the end of incubation, the ^{15}N atom% in the leachate was similar to that of post-incubation LF for no-residue and soybean amended treatments at Lincoln, and smaller than LF for all the treatments at Mead and maize amended treatment at Lincoln.

As the ^{15}N label of LF was the highest among SOM fractions the contribution of that N to the indigenous N supply is evident. At Lincoln that label coincided with the signature of first and last leaching with the pre and post-

incubation LF determination, which suggest LF is the main source of indigenous N supply contributing to net N mineralization. At Mead, the lower ^{15}N atom% values of mineralized N at the end of incubation compared to the LF, suggest that other N sources, along with LF, are being used as N supply toward the end of incubation. The ^{15}N values suggest a mixture of LF-N and MHA-N or LF-N and residue-N. Other evidence supporting the role of LF is the change in ^{15}N atom% of the LF compared to MHA and CaHA.

The ^{15}N atom% label of LF was the highest among the pre-incubation SOM fractions (Table 4). All treatments experienced a significant loss in ^{15}N atom% post-incubation for all the SOM fractions (Figure 2). At Lincoln, the change in LF ^{15}N atom% post-incubation averaged -24% and at Mead -21%, but the loss in label was largest under SC rotation at Mead (-29.6%). The pre-incubation ^{15}N atom% label of MHA and CaHA was higher at Mead (0.465 and 0.412, respectively) compared to Lincoln (0.450 and 0.402 for MHA and CaHA, respectively). The pre-incubation ^{15}N label of both humic fractions was higher under the SC rotation at both sites, which can be associated with higher N inputs under for CS and CC and thus dilution of ^{15}N. After 300 d of incubation, there was a significant reduction in the ^{15}N atom% of both fractions at both sites. At Mead, the ^{15}N atom% change of both fractions was smaller under the maize amended treatment than either the soybean or no-residue treatments. Also, the post-incubation differences in ^{15}N atom% among rotations for both fractions were similar to the pre-incubation ones. Initially, the MHA fraction was 13% richer in ^{15}N label than CaHA but experienced a reduction in ^{15}N label of more than twice

that of the more humified CaHA. This ^{15}N label loss indicates that both fractions contribute to the soil indigenous N supply, but that MHA plays a more active role than CaHA.

3. Pre and post- incubation properties in WS and SOM fractions

The post-incubation analysis of variance for the WS and SOM fractions properties is presented in Table 5.

a. Change in SOM fractions' mass

Light Fraction (LF): Following 300 d of incubation there was an increase in the extracted LF mass at Lincoln. This increase was higher under SC rotation (+36%) with only 5 and 10% increase under CC and CS rotations, respectively. At the Mead site there were no significant changes in LF mass post-incubation (Figure 3).

Mobile Humic Acid (MHA): The post-incubation MHA mass significantly increased by 6% (soybean), 10% (maize), and 17% (no-residue) at Lincoln compared to the pre-incubation MHA mass (Figure 3). At Mead site there was a greater effect of rotation and amended residue that resulted in no mass differences in MHA between pre- post-incubation. With the addition of residues to the Mead soil, the post incubation MHA mass was 12% smaller than under no residue addition. Between rotations, under CC there was an increase of MHA mass post-incubation, while for CS and SC there was a net loss in MHA mass.

Calcium Humate (CaHA): The post-incubation CaHA fraction mass was reduced at both sites by more than 1000 mg kg soil^{-1}. The possibility of soil

acidification during incubation as the cause of CaHA loss was ruled out upon measurement of only a -0.2 unit pH change after 300 d of incubation. At Lincoln the CaHA mass loss was affected by rotation, with -21% CaHA mass under CC compared to -14% CaHA mass for CS or SC (Figure 3).

At both sites the pre-incubation mass of LF and humic acids was similar, with around 760 mg LF kg soil^{-1} and 10900 mg humic materials kg soil^{-1}, but the proportion MHA:CaHA was different with a higher MHA proportion at Mead. This fact might be related to the difference in N_0 between both sites and may help to explain the observed SOM pool changes to the addition of residues with different quality. The time that the sites were under N management is different, Mead has longer period of high N fertilizations compared to Lincoln, and smaller mass of returned residues. However, the N content of Mead maize residues was higher than that at the Lincoln site. This may promote a more rapid decomposition and humification of the residues at Mead and the formation of more N-rich humic acids as MHA compared to Lincoln. Despite the gain in post-incubation MHA mass there was a net reduction in the amount of humic materials at the end of incubation for all treatments. Because of the removal of net mineralization product during incubation, humic materials were used by microorganisms as a source of C and N pointing out that these are active N pools that exhibit short term change.

b. Compositional analysis of SOM fractions and whole soil (%C, %N, %C4-C, and ^{15}N atom%)

Whole soil (WS): The pre-incubation WS C content (1.6% and 1.9% at Lincoln and Mead, respectively) was reduced after incubation in all treatments. For both sites, the addition of maize residues resulted in a higher loss of C than addition of soybean residues or none. The δ^{13}C signature of whole soil indicated a pre-incubation derived C4-C content of 75% and 80% at Lincoln and Mead, respectively. At both sites, the %C4-C was higher under CC and lower for the SC rotation. At Mead, the change in the pre to post-incubation %C4-C was insignificant for no-residue treatment and less than 3% for both maize and soybean amended residue treatments.

The pre-incubation %N was 0.13 and 0.18 at Lincoln and Mead, respectively. After incubation both sites showed a net N loss, which was higher under the soybean amended treatment. At Mead, the change in %N after incubation was higher under CC rotation. The ^{15}N atom% label of WS was significantly reduced after incubation at both sites (0.415 to 0.408, and 0.423 to 0.417, for pre to post-incubation change at Lincoln and Mead, respectively). At Mead there was a bigger change in ^{15}N atom% (-2%) under SC rotation than CS or CC (-1.6 and -1.1%, respectively).

The observed N loss agreed with the pattern in cumulative mineralized N observed: larger WS-N loss and N mineralization under amended soybean, and smaller under amended maize. The N immobilization under amended maize and

the larger WS C and C4-C losses suggest that a considerably greater proportion of the C4 residue added was lost due to respiration.

Light Fraction (LF): This SOM fraction exhibited the largest differences in composition between sites. The pre-incubation C content of LF was similar among rotations at each site and averaged 56.7 and 49.3% at Lincoln and Mead, respectively (Table 6). Except for the maize-amended treatment at Mead, all the treatments showed a significant reduction in the post-incubation LF %C. The pre-incubation %C4-C in the LF at Lincoln was 57, 40 and 37% for CC, CS and SC rotations. Post-incubation, the %C4-C was higher under maize amended (and similar to the pre-incubation value) than the soybean amended or no residue treatment, and the values ranged between 32 to 52%. At Mead, the pre-incubation %C4-C of LF was significantly different between rotations: 73, 56 and 41% for CC, CS and SC rotations. Post incubation the %C4-C among amended residue treatments was similar to Lincoln, with higher %C4-C under maize amended residue treatment than under the soybean amended and no-residue treatments. The magnitude in the post- incubation %C4-C change at Mead was highly dependent on the crop rotation with no change under CC, loss under CS, and slight gain under SC (Figure 4). The pre- incubation %N of the LF was 2.36 and 2.76% at Lincoln and Mead, respectively, with a significantly higher %N under CC and SC rotations. For both sites there was no significant effect of the residue treatment on the post-incubation change of %N, but there was a significant rotation effect on this change in the LF (Table 6). At Lincoln the %N in LF under CS increased but decreased in the CC and SC rotations, At Mead an

increase in %N of LF was observed under the CC and CS rotations and a loss was observed under SC.

The larger magnitude in the post-incubation ^{15}N atom% loss compared to the %N change implies that LF pool is an active N pool as there is a significant loss of ^{15}N with less of a change in the overall mass of LF-N (Figure 2, Table 6). Moreover the similarity between ^{15}N label of LF and that of the leachate at the beginning and end of incubation suggests that the LF-N was the principal source of readily mineralizable N. The effect of previous crop (by rotation) and the C4 vs C3 residue addition on the post-incubation LF %C4-C, along with the contrasting environments of net immobilization at Lincoln and net mineralization at Mead highlights the fast turnover of C in this fraction.

Mobile Humic Acid (MHA): The pre-incubation C content for both sites averaged 52%. After incubation, there was a gain in C content of about 2% for MHA (Table 6). At Lincoln, the %C change in MHA was also influenced by residue addition, and rotation. The addition of maize or soybean residues resulted in higher post- incubation %C than no-residue addition. Under the Lincoln SC rotation there was a larger MHA-C content than under CC or CS. The pre-incubation %N was similar for MHA at both sites (5.7 and 5.8% for Lincoln and Mead, respectively). After incubation there was a significant reduction in %N for all treatments and sites. The change in %N to the pre-incubation N content was -6% at Lincoln and -4% at Mead (Table 6).

Calcium Humate (CaHA): The pre-incubation C content for both sites was 54% which was increased after incubation to 55%. The pre-incubation %N was slightly

higher for CaHA at Mead (2.9 and 3.2 at Lincoln and Mead). After incubation there was a significant reduction in %N for all treatments and sites. The change in %N was -6% at Lincoln, and -4% at Mead of the pre-incubation content (Table 6).

At both sites, the pre-incubation %C4-C was affected by previous crop in the rotation in MHA ($p < 0.05$) and CaHA ($p < 0.1$), with a sequence of %C4-C CC > CS > SC. Post- incubation MHA %C4-C resulted in no change under CC, and increase under CS and SC rotations at Lincoln (Figure 4). At Mead there was also no change for the %C4-C in MHA under CC, small increase in SC and higher increase for CS in the %C4-C post incubation. The addition of maize residues increased the %C4-C of MHA more than the addition of soybean residues The higher and more uniform pre- incubation %C4-C across rotations and sites for CaHA compared to MHA, and the small changes in the %C4 signature could indicate a more strong degree in biochemical stabilization of C into CaHA (Figure 4).

Humin: This fraction of SOM was the remaining SOM after the removal of humic materials. The C:N ratio was around 10 at both sites and the %C and %N were very small (< 1% C and < 0.1% N) due to the high proportion of soil minerals in the fraction. Despite the smallest C and N contents, this fraction represented about half of the total mass of WS, total C and total N. After incubation, there was a loss in humin %C and %N at Lincoln under amended soybean and no-residue (%C: from 0.72% to 0.66% and 0.67% and %N from 0.066% to 0.063% for no- and soybean residue, respectively), with almost no change for amended maize

residue (0.72% to 0.69%C). At Mead there was a uniform reduction in the post incubation %C among residue treatments (0.83% to 0.79%) after incubation, and a %N loss under no- residue (0.086% N pre-incubation to 0.082, 0.087 and 0.087%N for no-, soybean and maize residues). The pre- incubation %C4-C of humin (>70%) was higher under CC rotations in both sites. After incubation there was small change (< 1%) in the %C4-C and there was the same trend among rotations than pre-incubation for both sites. The differences observed in %C4-C between residues at Lincoln were < 1%. The ^{15}N atom% label of Humin was lower than that of humic fractions and LF, but it indicated an enrichment of this fraction. The differences in ^{15}N label among rotations were insignificant, as well as those between pre- and post-incubation. This suggests that adsorbed N in this fraction is relatively inert biologically.

c. Change in C, N, ^{15}N and C4 mass

LF: The C mass of LF showed minor changes after incubation and displayed the same trends in change as observed in fraction mass. At Lincoln there was a significant increase in the LF-C mass under SC rotation at the post-incubation time across all residue treatments (+24%) (Table 6). The pre-incubation LF C4-C mass was higher under CC rotation at both sites and lower at the SC rotation, indicating a direct effect of immediate preceding crop on the origin of LF. After incubation there was a significant increase in C4 mass under the maize amended treatment at Lincoln that was more pronounced under the SC rotation. At Mead there was also a significant increase in C4 mass of LF under SC rotation, with a larger change for the maize amended treatment (Figure 5).

The pre-incubation N mass of LF was similar across rotations for both sites and represented 1.3% of WS-N. After 300d of incubation, there was no change in the N mass of LF at Mead, but an increase in the N mass under SC and CS rotations at Lincoln (Table 6). The observed increase in N mass was higher for the no-residue treatment (+21%) compared to the maize (+7%) and soybean (+11%) residue treatments. The pre- incubation ^{15}N mass of LF was higher at Mead site (0.188 and 0.106 mg ^{15}N kg soil^{-1} at Lincoln and Mead, respectively), and there was no statistical differences among rotations at either site. Post incubation, there was a larger ^{15}N mass loss in all treatments (Figure 6). For both sites, the ^{15}N mass loss under both maize and soybean amended treatments was larger under SC rotation, while the no-residue treatment had larger ^{15}N mass loss in CC rotation. The larger loss of ^{15}N in this fraction under SC can be associated to the higher ^{15}N atom% label and a lower C:N ratio that promoted the decomposition of the LF. In addition, the larger loss in ^{15}N mass observed under amended soybean suggests that the shift in N mineralization could be due to an increase in the degree of LF decomposition when a low C:N ratio material is the source. The greater increase in LF-C mass, C4 mass and N mass under SC maize amended treatment, suggests that the more available N of this rotation contributed to the fixation of the C present in the maize residue.

Mobile Humic Acid (MHA): The pre-incubation C mass of total humic materials was similar between sites (5834 and 5774 mg C kg soil^{-1}). At Lincoln, the pre-incubation C mass of MHA was similar between rotations, but was 36% lower in SC compared to CC and CS rotations at Mead (Table 6). At Lincoln there was an

increase in the post incubation C mass of MHA for all treatments (23%, 14% and 10% increase under amended maize, no-residue, and soybean treatments, respectively). At Mead, all the rotations with the no-residue treatment had an increase in MHA C mass compared to the pre-incubation mass. Also, the CC rotation at Mead had an increase in the post incubation MHA C mass across al residues compared to C and SC. As observed with the C mass, the pre-incubation N mass of MHA was similar among rotations at Lincoln, and was lower in SC compared to CC and CS rotations at Mead (-30%). At Mead, the CS rotation had a larger MHA N loss across rotations compared to the loss observed in SC, while CC showed an increase in MHA N mass. Among residue treatments, the maize and soybean amended treatments had larger MHA N loss than no residue added (Table 6).

Calcium Humate (CaHA): The pre-incubation C mass of CaHA was similar among rotations at each site. The post-incubation C mass of CaHA was reduced in all treatments and sites (Table 6). At Lincoln, there was a 12% lost in CaHA C mass under CS and SC rotations, while CC had a larger reduction of CaHA C mass (-20%). At Mead the loss of C mass in CaHA were smaller in magnitude than at Lincoln (3%, 12% and 7% loss under maize, no-residue and soybean amended treatments). The pre-incubation N mass of CaHA was similar among rotations at each site. Post-incubation N mass of MHA was not influenced by residue treatment. The post-incubation N mass of CaHA was reduced compared to the pre-incubation N mass for all rotations and treatments at both sites. At

Lincoln there was a trend of higher CaHA N mass loss under CC, while at Mead the higher CaHA N loss was under no residue addition.

The pre-incubation ^{15}N mass of each humic fraction was similar among rotations at each site but the mass of label was significantly greater in MHA than CaHA (0.241 and 0.166 mg ^{15}N kg soil^{-1} at Lincoln, and 0.404 and 0.200 mg ^{15}N kg soil^{-1} at Mead, for MHA and CaHA, respectively). The post-incubation ^{15}N mass of both fractions was reduced for all treatments and sites, indicating mineralization of the pre-incubation N in the most recently immobilized N fraction (Figure 6). The loss of MHA ^{15}N mass was higher at Mead compared to Lincoln, and for both sites the ^{15}N mass loss was larger under the soybean amended treatment (25% and 32% at Lincoln and Mead, respectively) than was observed under the no residue or maize amended treatments (21% and 13% at Lincoln, and 25% and 24% at Mead). The ^{15}N mass loss of CaHA was higher at Lincoln (-35%) than Mead. At Lincoln there were no differences among residue treatments and small differences between rotations in the CaHA ^{15}N mass loss. At Mead there was a higher CaHA ^{15}N mass loss under no residue addition (35%) compared to the soybean amended (27%) or maize amended (17%) treatments.

4. Source of N under different residue additions

The significant reduction of both %N and ^{15}N atom% of MHA and CaHA after 300 d of incubation indicates that both fractions, when exposed to a N mining environment (removal of net mineralization product), will release part of their N and contribute to the soil indigenous N pool. The relationship between pre

and post-incubation mass of SOM fractions, and its C and N composition is showed in Table 6. In Table 6, the net difference of post – pre-incubation mass (SOM fraction, C or N mass) is called transformed mass. A negative number means that there was a loss of mass at the end of incubation compared to pre-incubation. The C and N composition of that transformed mass (%C and %N in transformed mass) was calculated by dividing the transformed C or N mass by the corresponding transformed SOM fraction mass and multiplying by 100. A positive % means that the fraction showed a net unidirectional flux of fraction and nutrient mass (e.g. a net loss in CaHA mass along with a net loss in C mass in the same fraction) while negative % means that the net change in fraction mass was opposite to the net change in C or N mass in that fraction (e.g. a net gain in MHA mass along a net loss in N mass in the same fraction). The % C and N values for transformed mass that were too far from the original composition are not shown. These extreme values, which predominate among MHA and LF, indicate the possible occurrence of several recycling processes into the fraction with a net change in fraction mass too small or too big compared to the net change in nutrient.

The transformed CaHA mass was characterized as having greater %N and lower %C than the original material, which suggests a preferential release of a less stabilized or condensed humic substances extracted in the CaHA fraction. There is likely a range in both the fraction of C with long or short MRT of carbon and so there are fractions within CaHA that are more labile than the whole. The release of a more labile CaHA sub-fraction is expected to increase the proportion

of the more condensed ones in that fraction, increasing the %C of that fraction which was observed. Under the extended mineralization conditions of this experiment, the release of more labile CaHA materials could be responsible for the net gain of MHA mass. However the net loss in CaHA fraction, C and N mass was greater than the net gain in C and N by MHA, and for some treatments there was also a net loss in MHA fraction, C and N mass accompanying CaHA loss. It seems logical that the transformed CaHA material underwent further transformations by soil microorganisms before becoming part of MHA or being lost as mineralization products (e.g. CO_2). So to, MHA materials are also being modified by microorganisms to release the C and N in them. The %C in the transformed MHA mass varied widely, and was in general higher than the %C of the CaHA transformed mass. This suggests that MHA had a more active role in the cycling of C.

The N composition of transformed CaHA was higher than the original material, denoting a preferential loss of enriched N materials. The loss in ^{15}N atom% and mass of CaHA implied that this recently labeled N loss was stored in recently stabilized N compounds which are readily available for mineralization. The N composition of the transformed MHA mass varied widely between sites, residue addition, and rotations, indicating a higher N turnover in that fraction related to the N demand for C mineralization of added residue. At Lincoln, the increase in MHA mass was characterized by an increase in N mass under maize amended treatment, and no change in MHA N mass under soybean amended. This suggests that the immobilization environment under maize amended

resulted in N mineralization from MHA and CaHA to decompose the residue (as evidenced by the loss in ^{15}N mass of this fraction). The prevalent conditions of immobilization until the end of incubation suggest that the maize residue is still the energy source for microbial biomass at the Lincoln site. Thus, the released N-rich CaHA substances once have lost part of its N will probably become part of the MHA pool increasing its mass but with a lower %N. The net amount of LF-N involved in the transformed mass was considerably smaller than that of CaHA and MHA.

To assess the relative activity and importance of sources to the soil's indigenous N pool, an N turnover statistic relating the net change (%) in ^{15}N mass to the net change (%) in N mass for each fraction was computed. Table 7 shows the mean values of the N turnover statistic by amended residue and rotation along with the standard errors.

As the change in ^{15}N mass for all fractions was a net loss, a positive turnover statistic means a net loss in N mass by that fraction and a negative number will indicate a net gain in N mass in the fraction. The magnitude for the statistic can be 0 (meaning the net change in N mass is far larger than the net change in ^{15}N mass), 1 (the net change in N mass and ^{15}N mass are similar), or > 1 (the net change in ^{15}N mass is larger than that of N mass).

The turnover statistic for CaHA was > 1 among rotations and residue amended treatments for both sites. This fact points out that CaHA fraction is not labeled uniformly, and that the ^{15}N is in the more reactive compounds within the fraction. The CaHA fraction, therefore, donates N with higher ^{15}N label than the

bulk material. The analysis of transformed mass, implies that the higher %N observed in the CaHA mass loss is due to a preferential release of N-rich compound within CaHA fraction. All this suggests that the CaHA pool is composed of materials with different degree of stabilization, and that the new added materials (^{15}N labeled) are N-rich compounds that could be mineralized to donate N. At Mead there was a larger turnover statistic value of CaHA under CC among residue treatments, suggesting that the higher N inputs in this rotation have resulted in a larger pool of N-rich labile-CaHA.

The turnover statistic for MHA varied widely among treatments and sites, indicating a more dynamic N pool compared to CaHA. The negative values for the statistic indicates a net increase in N mass but a depletion in ^{15}N mass. This suggests MHA is an N donor pool for residue and LF decomposition as well as a storage pool for the humified compounds released from CaHA pool (as suggested by the %N of the transformed MHA mass). The positive values of the turnover at Mead indicated a flow of N out of MHA pool for the CS and SC rotations under all residue treatments. The higher %N in the transformed MHA mass for this treatments compared to the original material could indicate, as for the CaHA pool, that a preferential loss of the more labile and N-rich compound are being mineralized in this fraction.

The turnover for the LF pool also varied widely among treatments, showing a higher magnitude than the turnover of MHA. The positive values for all the rotations under soybean amended treatment, and for CC and SC with no

residue addition at Mead could constitute a hint about the nature of N pools contributing to the higher N mineralization observed under these treatments.

DISCUSSION

Soil N mineralization

The difference between the Lincoln and Mead sites in the cumulative mineralized N reflects two distinctive environments: a moderate to high soil indigenous N supply at Lincoln, where between 1.1 to 1.4% of the total whole soil N was mineralized in the no-residue treatment, and a very high soil indigenous N supply at Mead, where the total cumulative mineralized N accounted for 2.2 to 2.8% of total WS-N. The consequence of adding the same rate of fresh crop residues of contrasting quality to soils from both sites was a higher net N mineralization capacity with soybean residue amendment at Mead (2.6 to 3.4% of total WS-N was mineralized) and a net immobilization of the added residue N under the maize amended treatment at Lincoln (0.1 to 0.3% of total WS-N was mineralized). Thus a broad range of different environments for soil indigenous N supply expression was achieved because of different labile N storage in diverse SOM pools at each site (Figure 1). The similar amounts of mineralized ^{15}N among no-residue and soybean amended residue treatments suggest that the increase in N mineralization was achieved due to mineralization of non-labeled N sources (as the soybean residue).

Soil ^{15}N mineralization and SOM fractions

The soils used in this study had been amended with ^{15}N fertilizer in a field study approximately 1 year prior to sampling for this study. We found the majority of the recently immobilized ^{15}N mass resident in the LF, MHA and CaHA fractions. The decline in the ^{15}N atom% of the inorganic N in leachate overtime suggests that a large proportion of recently immobilized ^{15}N was stored in relatively labile forms N and released early during incubation. The similarity in ^{15}N atom% of initial leaching to the pre-incubation LF ^{15}N atom% suggests that LF decomposition is an initial easily mineralizable source of N. The LF, composed of a mixture of partially decomposed plant materials, constitutes a readily available N and C source (Bending and Turner, 1999). The observed change in the magnitude of its ^{15}N atom% and $\delta^{13}C$ signature upon application of C4 and C3 residues also identifies it as a primary energy and N pool for heterotrophic activity. Wang et al. (2004) determined that the long-term potential of N mineralization is mainly affected by the capacity to replenish bio-available substrate. They related this capacity to the quality of LF - its N content - and the amount of N mineralized during the first 4 weeks of incubation as indicators of the degree of substrate replenishment. The addition of soybean residues with a lower C:N ratio than LF (C:N = 12) constituted a substituting N source for mineralization, since it will be more easily decomposed than LF (Johnson et al., 2007). This substitution was observed as a drop in the ^{15}N atom% of the mineralized N from the initial 4 leachings (49 d) compared to the no-residue treatment at both sites. After that, the ^{15}N atom% of leachate was similar for the

no-residue and soybean amended treatments, suggesting that the initial substitution by soybean residues was over.

During the entire 300 d incubation, the ^{15}N label of the leachate decayed toward a constant value at the end of incubation. This final value indicated a ^{15}N enrichment of the leachate compared to the ^{15}N atom% of the humic acid pools, but similar (Lincoln) or lower (Mead) than the ^{15}N atom% of the LF. This fact can have multiple interpretations: a) LF is still the source of N mineralization at the end of incubation, b) there is a shared source of the mineralized N between LF and humic materials during incubation, c) one source of mineralized N is some labile fraction of humic materials which is enriched in ^{15}N (more recent) compared to the bulk material.

Considering the total N mass of LF at the beginning of incubation (≈ 20 mg N kg soil^{-1}), it is unlikely that LF constitutes the only source for N mineralization. Moreover, the replenishment of this pool at the end of incubation indicates that a dynamic turnover exists among SOM pools with replenishment of the LF from new sources of N and C. Along with the partially decomposed plant materials, the extracted LF can contain microbial constituents. Nichols and Wright (2006) found that approximately 9 to 12% of the LF pool was composed of fungal proteins with a C:N ratio of 15. Thus it is possible that the replenishment of LF pool observed at the end of incubation in the amended as well as no-residue amended treatments is associated with an increase in the contribution of microbial compounds into this fraction, some of which may initiate from labile humic material.

The soils used in this experiment were labeled with ^{15}N fertilizer in spring 2002 and the crop residues after harvest in fall 2002 were returned to the soil and incorporated. The ^{15}N atom% observed in the following spring in the SOM pools studied is indicative of N that was recently incorporated. The decline in ^{15}N atom% and ^{15}N mass observed in all SOM fractions over the course of incubation indicated preferential loss of newly immobilized N.

The analysis of transformed mass showed that the CaHA mass that was lost during incubation was characterized as having a greater %N and lower %C than the total CaHA pool, which suggests a preferential release of the less stabilized and condensed humic materials within this fraction. The turnover statistic of CaHA showed a net unidirectional and nearly proportional flux of N and ^{15}N out of this fraction, which suggests that a significant pool of labile N exists in the CaHA fraction that cannot be extracted in the lab without acidification (decalcification) of humate before alkali extraction. It is apparent, however, that this pool is accessible to microbial activity. In this study, the labile CaHA fraction constituted approximately 16% at Lincoln and 10% at Mead of the total CaHA pool.

It is presumed that the materials released from CaHA are initially utilized by the microbial biomass during residue and LF decomposition and humification. The byproducts then become part of the MHA pool. Indeed we observed a gain in MHA mass over the course of this long-term incubation especially under a net N immobilizing environment. The difference between transformed CaHA mass and its composition to the transformed MHA mass and its composition means

that any material gained by MHA from CaHA suffered more transformations and loss C and N during its transformation. The broad variation in transformed N mass of MHA between sites, rotations and residues, indicated a higher N turnover (or response to the N demand) in MHA than CaHA and is related to N demand for mineralization. This suggests that MHA is a primary N source that undergoes rapid turnover relative to CaHA. Also the loss in ^{15}N mass observed in the MHA fraction confirmed that the degree of ^{15}N atom% reduction was due to both, ^{15}N dilution from massive N deposition to this pool as well as flow of ^{15}N mass out of the fraction. The analysis of the turnover statistic between the net change in ^{15}N and N mass also varied widely but identified MHA as an active soil indigenous N pool, as N donor and N acceptor. The transformed C4 mass of CaHA indicated a loss of C4 mass in CaHA for all treatments at both sites, with a higher loss at Lincoln site. The transformed mass of C4 for MHA revealed a net gain at Lincoln, for all residue treatments and rotations, suggesting that part of the C lost by CaHA was recycled into MHA, and another portion was utilized for microbial respiration. At Mead, the transformed C4 mass of MHA showed a significant net gain under the no-residue treatment in CC and CS, which coincided with a net C4 mass loss of CaHA. The addition of maize residues or soybean residues resulted in a loss of C4 mass in both humic fractions at Mead, which can be attributed, in the case of the maize amended treatment, to a N demand for decomposition of high C:N ratio residue, thus promoting the mineralization of more labile humic materials richer in N.

The %C4-C of post incubation MHA and CaHA had very small changes compared to the changes observed for LF, which suggest that the C from the added residues was incorporated mainly into the less stabilized LF as well as lost by respiration.

CONCLUSION

The experimental conditions of this study resulted in a high N demanding environment. The 7500 cumulative degree days of incubation represented about 2.5 years of cumulative field degree days in the sample sites. Periodic leaching of the samples during incubation accounted for a cumulative water input equivalent to ~800 mm which was only 2/3 of site precipitation. The addition of high C:N ratio maize residue resulted in an N flux from humic acids to aid decomposition of the residue, while addition of low C:N ratio soybean residues promoted net N mineralization and loss of N via leaching. Interpretation of these results should consider the differences between field and laboratory incubation as there were no periodic residue or root C and N inputs in our laboratory incubation as would occur in the field. This may have promoted SOM pools as C and N microbial substrates to a greater extent in the laboratory incubation. Nevertheless, the intense immobilizing environment created in the laboratory helped to identify labile C and N SOM pools.

The fractionation of SOM into pools of increasing mean C residence time and therefore more diverse protection mechanisms allowed us to quantitatively isolate pools of different size and contribution to short-term soil N supply. We

learned that new material incorporated into CaHA (a fraction with ~500-1200 yr MRT for C) was labile enough to be mineralized and that the whole CaHA pool MRT was not especially a clear indicator of the turnover of the most recently incorporated material in CaHA. Analysis of the gain and loss of ^{15}N in CaHA and MHA over the course of the incubation suggests that N loss from CaHA was eventually incorporated into MHA, a pool with much higher turnover rate and unprotected by chemical recalcitrance.

Under the conditions of this experiment the more recalcitrant humic acid pool, CaHA, was capable of donating N to decomposers. Apparently the most recent C and N added to CaHA pool was less protected than the bulk material and so more easily mineralized. The MHA pool acted as both a source and sink of C and N compounds. At the site with higher net N mineralization (Mead) there was a loss of MHA mass during decomposition of added residues whereas at Lincoln (lower net N mineralization) there was a gain in MHA mass over the course of the incubation. It seems that growth in MHA N storage occurs under greater immobilization pressure (e.g. Lincoln).

The LF pool also acted as a source and sink of C and N, but the post-incubation materials were unlike the pre-incubation LF. Post-incubation the extracted LF mass was larger than at pre-incubation, and this increase in ash-corrected LF mass was correlated with the ash content of the fraction. As decomposition imparts smaller particle size and greater reactivity with clay, we interpret this as an indication that the extracted material was different in nature, with a more decomposed state in the LF at post- incubation.

Thus, the flux of C and N does not necessarily followed the sequence LF > MHA > CaHA > Humin for the conditions of this lengthy incubation time. Our findings indicate that there is a mineralization of C and N from CaHA through microbial biomass to MHA where we propose the most labile, newly incorporated materials of CaHA are released. The MHA apparently undergoes much greater turnover of N and C than CaHA. The N mineralized from these humic materials has contributed to the decomposition of LF and plant residues as evidenced by the change in the C and N composition and particle size of the LF.

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Table 1. Average annual fertilizer N input and residue C and N input at the Lincoln and Mead, NE sites. Averages for the 1999 - 2004 period at Lincoln, and 1997 - 2004 at Mead.

Site	Rotation	N and C returned in residue		Fertilizer N
		kg N $ha^{-1}yr^{-1}$	kg C $ha^{-1}yr^{-1}$	kg N $ha^{-1}yr^{-1}$
Lincoln	**CC**	112 ± 8	5914 ± 164	284 ± 19
	CS	106 ± 9	4997 ± 879	171 ± 32
	SC	100 ± 9	4890 ± 704	175 ± 35
Mead	**CC**	131 ± 28	4845 ± 836	272 ± 27
	CS	117 ± 26	3146 ± 654	127 ± 60
	SC	154 ± 31	4620 ± 1166	150 ± 67

Table 2. Means and standard errors of mass yield, %N, ^{15}N atom%, %C and δ13C‰ in MHA and CaHA fractions from 6 soil samples. The value for each plot represents three independent extractions. The probability value (p-value) corresponds to the repeatability among extractions.

Material	Plot	n	Mass yield mg kg^{-1} soil	% N	^{15}N Atom %	% C	δ^{13}C ‰
CaHA	1	3	9063 ± 92	2.52 ± 0.02	0.4078 ± 0.0005	54.55 ± 0.56	-15.54 ± 0.03
	2	3	7170 ± 87	2.53 ± 0.03	0.3941 ± 0.0005	53.97 ± 0.50	-15.40 ± 0.04
	3	3	7615 ± 74	2.48 ± 0.05	0.3849 ± 0.0002	53.61 ± 0.94	-15.84 ± 0.03
	4	3	8472 ± 98	2.54 ± 0.05	0.4010 ± 0.0002	54.80 ± 0.76	-15.39 ± 0.02
	5	3	6287 ± 51	2.41 ± 0.02	0.3864 ± 0.0001	51.72 ± 0.22	-15.32 ± 0.02
	6	3	7244 ± 100	2.63 ± 0.01	0.4063 ± 0.0015	54.12 ± 0.31	-15.73 ± 0.05
p-value		18	0.37	0.35	0.46	0.29	0.94
MHA	1	3	3025 ± 63	4.66 ± 0.03	0.4732 ± 0.0008	49.27 ± 0.13	-17.16 ± 0.02
	2	3	1621 ± 15	4.97 ± 0.02	0.4458 ± 0.0010	47.73 ± 0.05	-16.70 ± 0.05
	3	3	1599 ± 23	6.02 ± 0.03	0.4353 ± 0.0005	48.18 ± 0.06	-16.39 ± 0.04
	4	3	2751 ± 33	4.83 ± 0.00	0.4565 ± 0.0003	47.82 ± 0.12	-17.02 ± 0.03
	5	3	1848 ± 32	5.35 ± 0.03	0.4309 ± 0.0002	48.24 ± 0.08	-15.87 ± 0.07
	6	3	1958 ± 9	4.97 ± 0.03	0.4649 ± 0.0042	47.23 ± 0.03	-17.34 ± 0.01

p-value	18	0.65	0.88	0.38	0.09	0.96

Table 3. Means and standard errors of %N, ^{15}N atom%, %C and δ^{13}C‰ analysis performed on the same extracted MHA and CaHA samples. Three sub-samples were analyzed for the same soil sample and extraction time. The probability value (p-value) corresponds to the repeatability among determinations.

Material	Sample	n	% N		^{15}N Atom %		% C		δ^{13}C	
CaHA	2	3	2.56	± 0.02	0.3951	± 0.0001	54.59	± 0.21	-15.39	± 0.03
	4	3	2.59	± 0.01	0.4011	± 0.0002	55.51	± 0.17	-15.43	± 0.04
	6	3	2.63	± 0.01	0.4040	± 0.0001	53.55	± 0.13	-15.83	± 0.05
p-value		9	0.1		0.55		0.16		0.52	
MHA	2	3	4.66	± 0.01	0.4744	± 0.0001	49.07	± 0.08	-17.14	± 0.04
	4	3	6.00	± 0.01	0.4355	± 0.0001	48.07	± 0.02	-16.43	± 0.03
	6	3	5.36	± 0.02	0.4307	± 0.0000	48.16	± 0.09	-15.82	± 0.02
p-value		9	0.62		0.69		0.95		0.56	

Table 4. Average ^{15}N atom % values in the initial (t = 0 d) and final (t = 300 d) leachate, and in pre- and post-incubation LF, MHA and CaHA fractions.

Site	Time	Treatment	Leaching	LF	MHA	CaHA
Lincoln	**Initial**		0.6741	0.6737	0.4499	0.4022
	Final	**None**	0.5135	0.5052	0.4313	0.3958
		Soy	0.4979	0.4951	0.4291	0.3954
		Maize	0.4577	0.5118	0.4322	0.3968
Mead	**Initial**		0.7644	0.7522	0.4650	0.4122
	Final	**None**	0.5492	0.5866	0.4412	0.4021
		Soy	0.5370	0.6054	0.4410	0.4040
		Maize	0.5304	0.6048	0.4495	0.4078

Table 5. Probability values for SOM fractions and WS properties with probability values. ns: p > 0.05.

		Lincoln							Mead						
		Rot	Res	Rot* Res	Time	Rot* Time	Res* Time	Rot* Res* Time	Rot	Res	Rot* Res	Time	Rot* Time	Res* Time	Rot* Res* Time
WS	%C	ns	0.010	ns	0.008	0.020	0.012	ns	ns	0.010	ns	0.001	ns	ns	ns
	%N	ns	0.007	ns	0.001	ns	0.007	ns	ns	0.015	ns	0.001	0.006	ns	ns
	%C4-C	0.003	ns	ns	ns	ns	ns	ns	0.004	0.025	ns	0.006	ns	ns	ns
	^{15}N atom%	ns	ns	ns	0.001	ns	ns	ns	ns	ns	ns	0.001	0.009	ns	ns
	C mass	ns	0.001	ns	0.001	0.020	0.020	ns	ns	0.001	ns	0.001	ns	0.040	ns
	N mass	ns	0.001	ns	0.001	ns	0.010	ns	0.051	0.003	ns	0.001	0.005	ns	ns
	C4-C mass	ns	0.001	ns	0.001	0.001	0.040	ns	0.023	0.001	ns	0.001	0.015	0.026	ns
	^{15}N mass	ns	0.016	ns	0.009	ns	ns	ns	ns	ns	ns	0.015	ns	ns	ns
LF	%C	ns	ns	ns	0.001	ns	ns	ns	ns	0.002	ns	0.003	ns	ns	ns
	%N	ns	ns	ns	ns	0.005	ns	ns	ns	ns	ns	0.048	0.022	ns	ns
	%C4-C	ns	0.026	ns	ns	ns	ns	ns	0.014	ns	ns	ns	0.003	ns	ns
	^{15}N atom%	ns	ns	ns	0.001	ns	ns	ns	ns	ns	ns	0.001	0.031	ns	ns
	fraction mass	ns	ns	ns	0.005	0.012	ns	ns	ns	ns	ns	ns	ns	ns	ns
	C mass	ns	ns	ns	ns	0.008	ns	ns	ns	ns	ns	ns	ns	ns	ns
	N mass	ns	0.048	ns	ns	0.008	ns	ns	ns	ns	ns	ns	ns	ns	ns
	C4-C mass	0.049	0.017	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.060	ns	ns
	^{15}N mass	ns	ns	0.020	0.001	ns	ns	ns	ns	ns	ns	ns	0.001	ns	ns

Table 5. (Continued)

		Lincoln							Mead						
		Rot	Res	Rot* Res	Time	Rot* Time	Res* Time	Rot* Res* Time	Rot	Res	Rot* Res	Time	Rot* Time	Res* Time	Rot* Res* Time
MHA	%C	ns	0.006	ns	0.001	0.027	ns	ns	ns	ns	ns	0.001	ns	ns	ns
	%N	ns	ns	ns	0.001	ns	ns	ns	ns	ns	ns	0.001	ns	ns	ns
	%C4-C	0.001	ns	ns	ns	ns	ns	ns	0.001	0.021	ns	0.011	ns	ns	ns
	^{15}N atom%	ns	ns	ns	0.001	ns	ns	ns	0.025	0.003	ns	0.001	ns	ns	ns
	Fraction mass	ns	ns	ns	0.001	ns	ns	ns	0.001	0.009	ns	ns	ns	ns	ns
	C mass	ns	ns	ns	0.001	ns	ns	ns	0.001	0.006	ns	ns	ns	ns	ns
	N mass	ns	ns	ns	ns	ns	ns	ns	0.001	0.002	ns	0.005	0.004	ns	ns
	C4-C mass	ns	ns	ns	0.001	ns	ns	ns	0.001	0.006	ns	ns	ns	ns	ns
	^{15}N mass	ns	0.053	ns	0.001	ns	ns	ns	ns	ns	ns	0.001	ns	ns	ns
CaHA	%C	ns	ns	ns	0.001	ns	ns	ns	ns	ns	ns	0.001	ns	ns	ns
	%N	ns	ns	ns	0.001	ns	ns	ns	ns	ns	ns	0.001	ns	ns	ns
	%C4-C	ns	0.043	ns	0.020	ns	ns	ns	ns	0.029	ns	0.023	ns	ns	ns
	^{15}N atom%	ns	ns	ns	0.001	ns	ns	ns	0.029	0.003	ns	0.001	0.002	ns	ns
	Fraction mass	ns	ns	ns	0.001	0.025	ns	ns	ns	ns	ns	0.001	ns	ns	ns
	C mass	ns	ns	ns	0.001	0.029	ns	ns	ns	ns	ns	0.004	ns	ns	ns
	N mass	ns	ns	ns	0.001	ns	ns	ns	ns	ns	ns	0.001	ns	ns	ns
	C4-C mass	ns	ns	ns	0.001	0.014	ns	ns	ns	ns	ns	0.006	ns	ns	ns
	^{15}N mass	ns	ns	ns	0.001	ns	ns	ns	ns	0.001	ns	0.001	ns	ns	ns
Humin	%C	ns	ns	ns	0.001	0.033	ns	ns	ns	ns	ns	0.007	ns	ns	ns
	%N	ns	0.002	ns	0.001	0.034	ns	ns	ns	0.046	ns	ns	ns	ns	ns
	%C4-C	0.016	0.040	ns	0.051	ns	ns	ns	0.011	ns	0.007	ns	ns	ns	ns
	^{15}N atom%	0.004	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.012	ns	ns	ns

Table 6. Pre- and post-incubation change in mass and C and N composition of whole soil (WS) and SOM fractions (LF, MHA and CaHA). Means by site, residue treatment and rotation. WS* Pre-incubation values for no-residue treatment.

Lincoln		**Pre incubation**			**Post – incubation**								
					Maize			**No-residue**			**Soybean**		
		CC	**CS**	**SC**	**CC**	**CS**	**SC**	**CC**	**CS**	**SC**	**CC**	**CS**	**SC**
WS *	C mass	16075	15735	16208	16795	15262	16330	15768	14873	15545	15396	14328	15242
	Transformed C mass				-1050	-2243	-1647	-307	-862	-662	-969	-1697	-1256
	N mass	1309	1274	1326	1324	1267	1332	1284	1239	1307	1294	1240	1298
	Transformed N mass				-10	-30	-17	-25	-35	-19	-39	-57	-51
MHA	Fraction mass	2748	2469	2641	3326	2760	3146	2834	2865	2883	2991	2610	2722
	Transformed mass				578	291	505	87	396	242	243	141	81
	C mass	1440	1276	1382	1813	1481	1741	1510	1529	1566	1633	1391	1479
	Transformed C mass				373	204	360	70	253	184	193	115	98
	%C	52.4	51.7	52.2	54.3	53.6	55.3	53.2	53.2	54.1	54.3	53.3	54.3
	%C transformed mass				64.6	70.2	71.3	81.2	63.8	76.2	79.5	81.4	
	N mass	156	141	143	176	149	162	150	153	147	157	140	142
	Transformed N mass				20	8	20	-7	12	4	1	-1	-1
	%N	5.9	5.7	5.4	5.5	5.4	5.2	5.5	5.3	5.1	5.5	5.4	5.2
	%N transformed mass				3.5	2.7	3.9		3.0	1.8	0.5		
CaHA	Fraction mass	8633	7678	8543	6414	6596	7425	6804	6546	7281	7112	6848	7370
	Transformed mass				-2218	-1083	-1118	-1829	-1132	-1262	-1520	-831	-1173
	C mass	4611	4172	4622	3500	3681	4081	3668	3606	4011	3829	3776	4087
	Transformed C mass				-1110	-491	-541	-943	-565	-611	-781	-396	-535
	%C	53.6	54.3	54.0	54.9	55.8	54.8	54.0	55.1	55.0	54.0	55.1	55.4
	%C transformed mass				50.1	45.3	48.4	51.6	49.9	48.5	51.4	47.7	45.6
	N mass	237	223	246	170	179	201	180	178	201	186	184	202
	Transformed N mass				-66	-43	-45	-56	-44	-45	-50	-39	-44
	%N	2.8	2.9	2.9	2.7	2.7	2.7	2.7	2.7	2.8	2.6	2.7	2.7

Table 6. (Continued)

Lincoln		Pre incubation			Post – incubation								
					Maize			No-residue			Soybean		
		CC	CS	SC	CC	CS	SC	CC	CS	SC	CC	CS	SC
CaHA	%N transformed mass				3.0	4.0	4.0	3.1	3.9	3.5	3.3	4.7	3.7
LF	Fraction mass	1028	703	576	1053	738	766	1000	816	885	1018	711	714
	Transformed mass				26	35	189	-28	113	308	-10	8	138
	C mass	600	401	323	560	382	398	550	431	446	551	364	362
	Transformed C mass				-40	-19	75	-50	30	123	-49	-37	39
	%C	57.5	56.7	56.0	52.6	51.4	51.8	53.5	52.5	50.5	53.1	51.0	50.4
	%C transformed mass						39.8		26.3	39.8			28.6
	N mass	24	15	15	21	17	16	23	18	20	22	16	18
	Transformed N mass				-3	2	1	-1	3	6	-2	1	3
	%N	2.4	2.1	2.5	2.1	2.4	2.1	2.4	2.3	2.3	2.3	2.3	2.5
	%N transformed mass						0.5	3.1	3.1	1.8			2.2
Mead													
WS *	C mass	20173	19630	17630	18300	18636	17175	17362	18186	15984	18163	17572	16448
	Transformed C mass				-3643	-2764	-2225	-2811	-1444	-1646	-2300	-2348	-1472
	N mass	1863	1774	1655	1743	1720	1658	1664	1662	1620	1743	1647	1595
	Transformed N mass				-142	-76	-18	-199	-111	-35	-143	-150	-82
MHA	Fraction mass	3968	4439	2657	3966	3885	2433	4436	4482	2746	4067	4005	2465
	Transformed mass				-3	-554	-224	468	43	89	99	-434	-192
	C mass	2056	2313	1367	2105	2064	1296	2388	2400	1437	2185	2138	1309
	Transformed C mass				50	-249	-71	333	88	70	130	-175	-57
	%C	51.7	52.0	51.3	53.1	53.0	53.0	53.9	53.6	52.1	53.6	53.4	52.6
	%C transformed mass					44.9	31.7	71.1		78.5		40.3	29.9
	N mass	215	251	162	209	208	144	237	236	159	218	214	145
	Transformed N mass				-5	-43	-19	22	-16	-3	3	-38	-18

Table 6. (Continued)

Mead		Pre incubation			Post – incubation								
					Maize			No-residue			Soybean		
		CC	CS	SC	CC	CS	SC	CC	CS	SC	CC	CS	SC
MHA	%N	5.4	5.7	6.3	5.3	5.4	6.0	5.4	5.3	5.9	5.4	5.4	6.0
	%N transformed mass					7.8	8.3	4.7			2.9	8.7	9.2
CaHA	Fraction mass	6831	8706	6235	6421	8178	5742	5914	7211	5226	6714	7677	5250
	Transformed mass				-410	-528	-493	-917	-1495	-1009	-117	-1030	-985
	C mass	3630	4674	3283	3431	4432	3070	3235	3970	2835	3630	4151	2844
	Transformed C mass				-199	-242	-214	-395	-704	-449	0	-522	-440
	%C	53.0	53.7	52.2	53.4	54.2	53.3	54.5	55.0	54.0	54.1	54.0	53.6
	%C transformed mass				48.4	45.8	43.4	43.1	47.1	44.5	0.3	50.7	44.7
	N mass	220	243	206	199	220	181	181	198	166	204	209	168
	Transformed N mass				-22	-23	-24	-40	-45	-40	-16	-34	-38
	%N	3.3	2.8	3.4	3.1	2.7	3.3	3.1	2.8	3.3	3.1	2.7	3.3
	%N transformed mass				5.3	4.4	4.9	4.3	3.0	3.9		3.3	3.8
LF	Fraction mass	942	799	781	782	732	920	825	792	834	838	759	747
	Transformed mass				-160	-66	140	-118	-7	53	-105	-39	-34
	C mass	460	394	387	387	355	458	376	359	386	386	354	353
	Transformed C mass				-72	-40	71	-84	-35	0	-74	-40	-34
	%C	49.2	49.1	49.7	50.2	48.4	50.1	45.4	45.4	46.3	46.3	46.6	47.8
	%C transformed mass				45.0	59.8	51.1	71.3			70.6		
	N mass	26	21	22	25	22	26	24	21	24	25	19	20
	Transformed N mass				-1	2	3	-2	0	2	-1	-1	-2
	%N	2.7	2.6	2.9	3.1	3.0	2.8	2.8	2.6	2.9	3.0	2.6	2.7
	%N transformed mass				0.8		2.4	1.8		4.1	0.8	3.5	5.5

Table 7. N turnover statistic for SOM fractions. Means and (se) by site, residue addition and rotation.

		Lincoln						Mead					
Residue	**Rotation**	**MHA**		**CaHA**		**LF**		**MHA**		**CaHA**		**LF**	
maize	**CC**	-1.76	(0.98)	1.41	(0.22)	1.45	(1.97)	2.11	(0.81)	2.11	(1.64)	-0.64	(2.23)
	CS	-1.95	(2.04)	1.73	(0.31)	-2.01	(1.52)	1.49	(0.80)	1.19	(0.12)	-0.28	(2.09)
	SC	-0.61	(0.79)	1.76	(0.25)	-3.75	(2.53)	1.80	(0.60)	1.12	(1.05)	-1.57	(0.28)
none	**CC**	1.72	(1.51)	1.84	(0.65)	2.06	(2.05)	-1.12	(1.53)	2.15	(0.95)	3.57	(0.25)
	CS	-2.35	(1.08)	1.90	(0.43)	-1.26	(0.79)	4.05	(0.08)	1.64	(0.10)	-1.75	(2.02)
	SC	-1.86	(1.74)	1.69	(0.44)	-0.98	(1.14)	1.84	(1.49)	1.37	(1.53)	1.96	(4.12)
soybean	**CC**	1.78	(2.47)	2.26	(0.86)	2.65	(2.12)	-1.45	(1.10)	3.18	(1.84)	2.41	(1.34)
	CS	1.45	(2.26)	2.08	(0.58)	-1.62	(2.54)	2.82	(1.07)	1.56	(0.23)	3.16	(0.80)
	SC	-1.34	(1.89)	1.80	(0.27)	-2.69	(2.34)	2.30	(1.27)	1.12	(0.94)	4.70	(0.73)

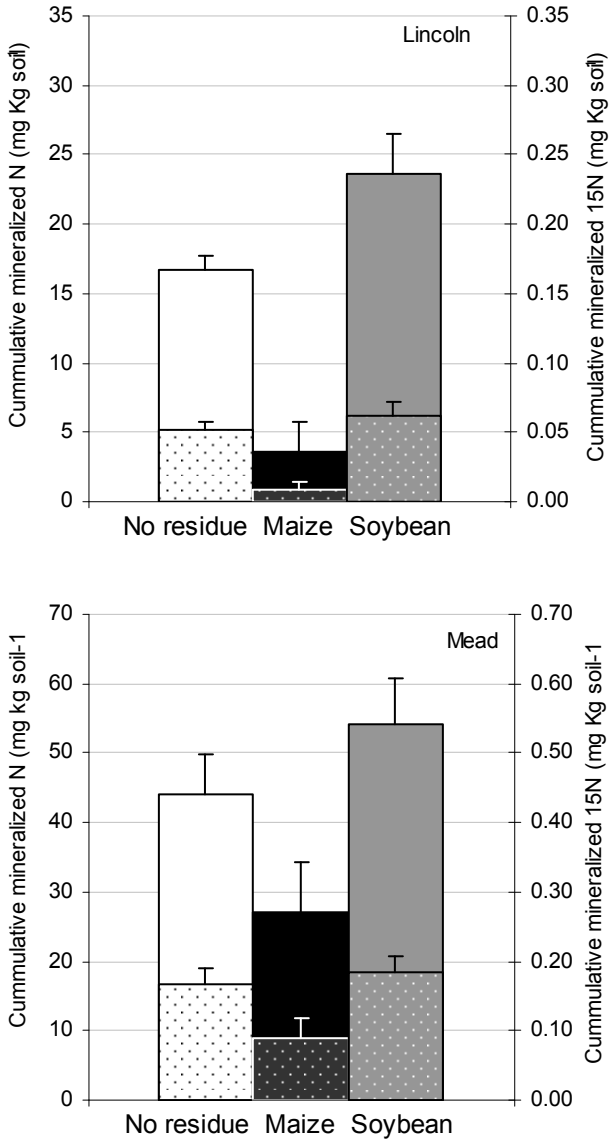

Figure 1. Means and standard errors of cumulative mineralized N and ^{15}N (mg kg soil^{-1}) after 300 days of aerobic soil incubation at Lincoln and Mead by residue amended.

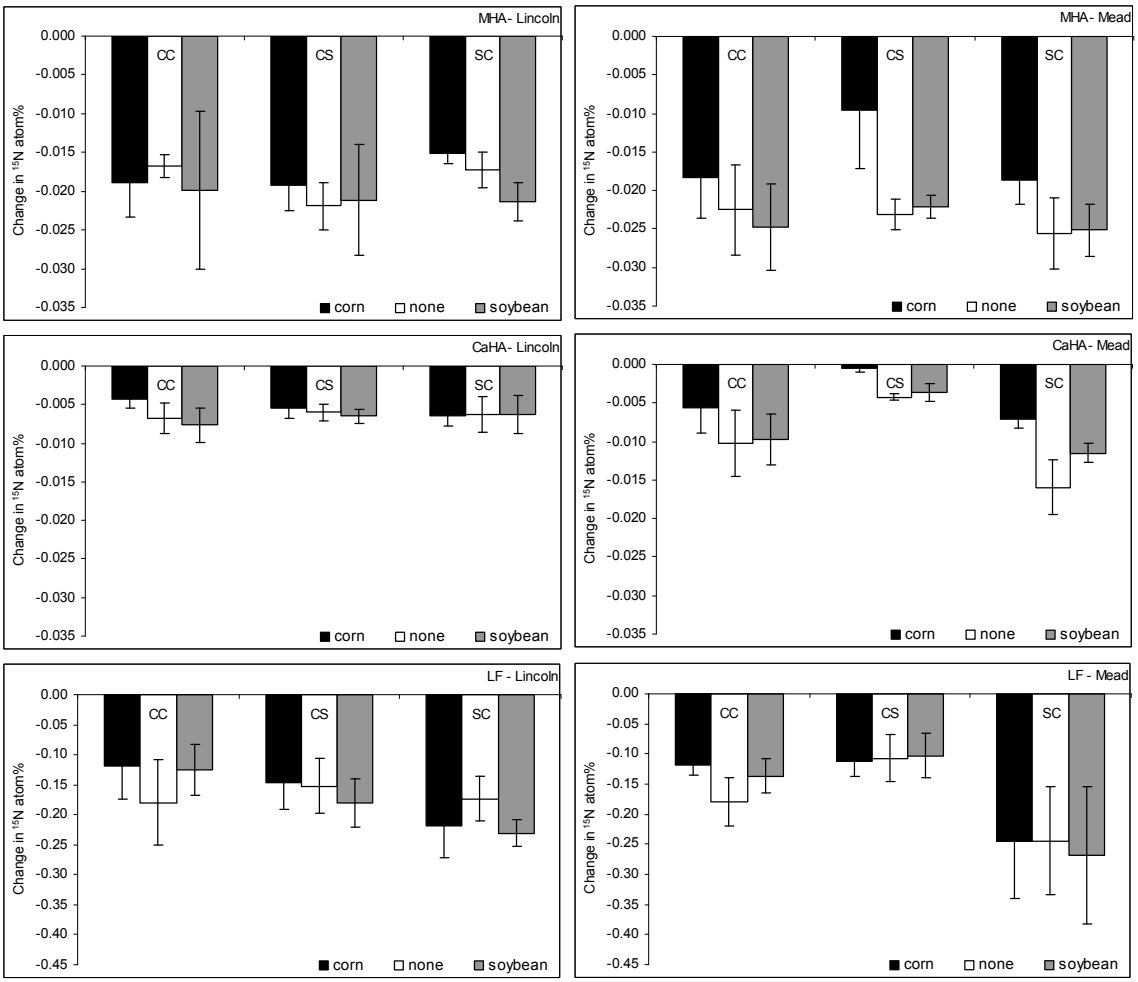

Figure 2. Change in ^{15}N atom% in the MHA, CaHA and LF SOM fractions at Lincoln and Mead. Means and SE by residue amended and rotation. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize.

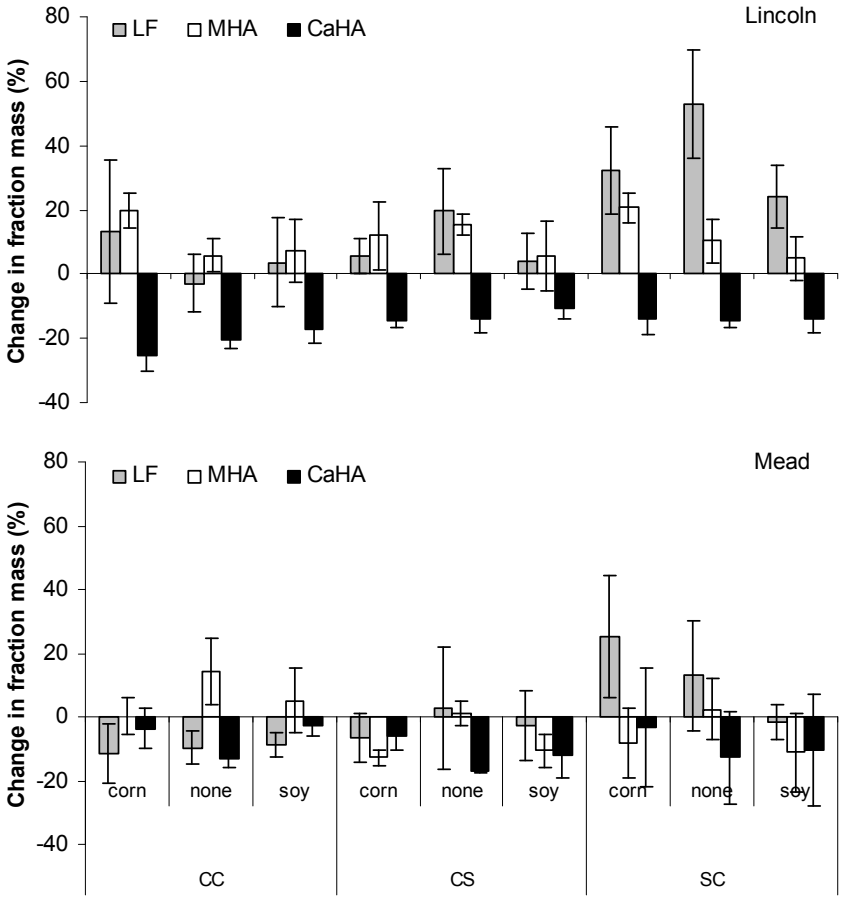

Figure 3. Change in LF, MHA and CaHA fraction mass (%) after incubation at Lincoln and Mead as affected by rotation and residue treatment. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize. Error bars are standard errors

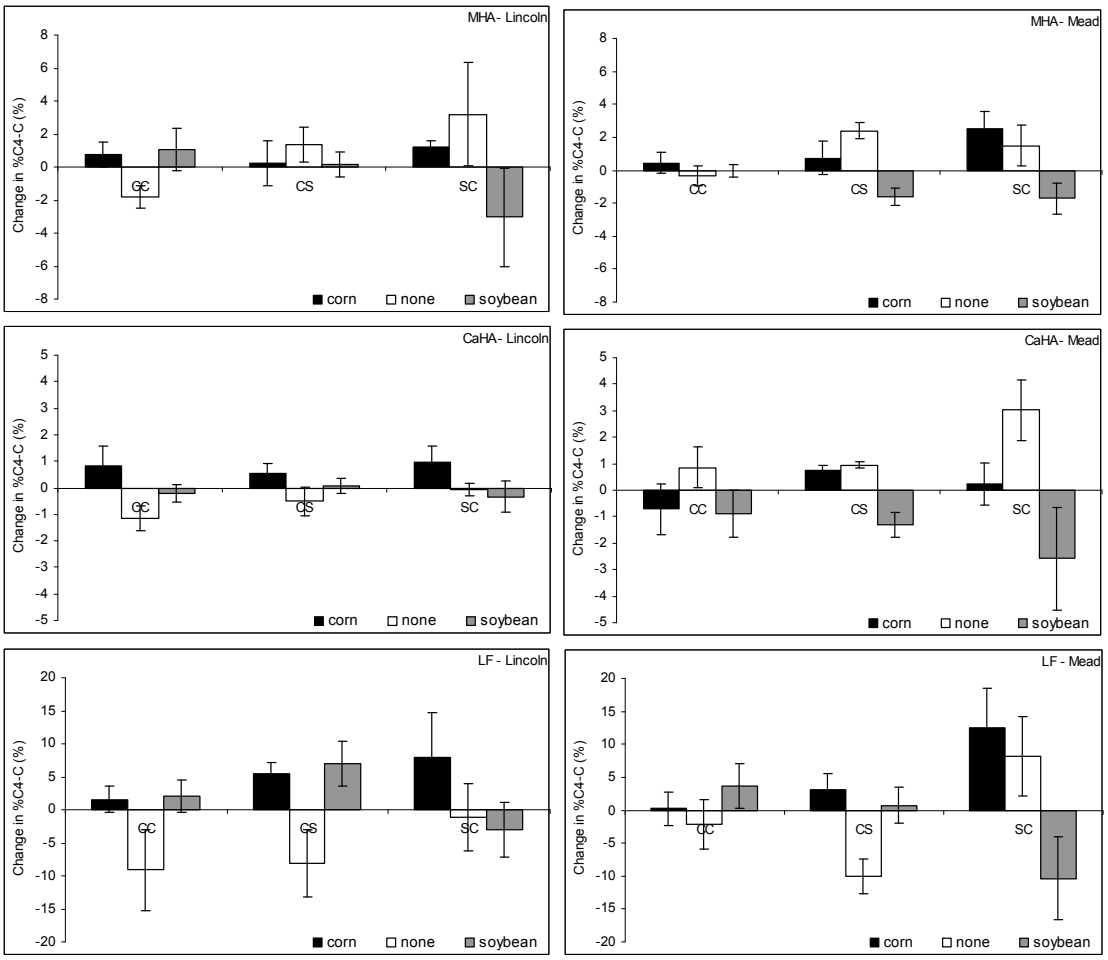

Figure 4. Change in %C4-C (%) in the MHA, CaHA and LF SOM fractions at Lincoln and Mead. Means and SE by residue amended and rotation. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize.

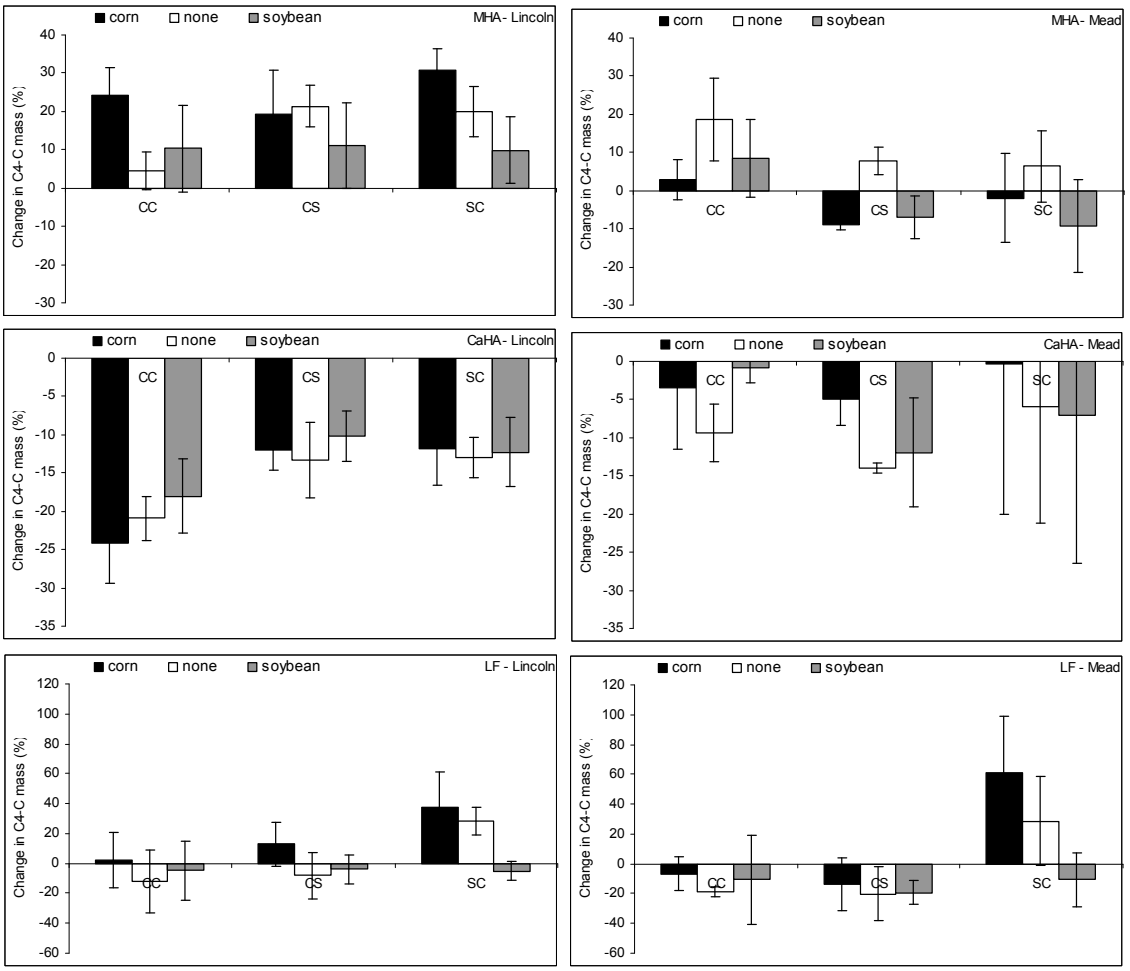

Figure 5. Change in C4-C mass (%) in the MHA, CaHA and LF SOM fractions at Lincoln and Mead. Means and SE by residue amended and rotation. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize.

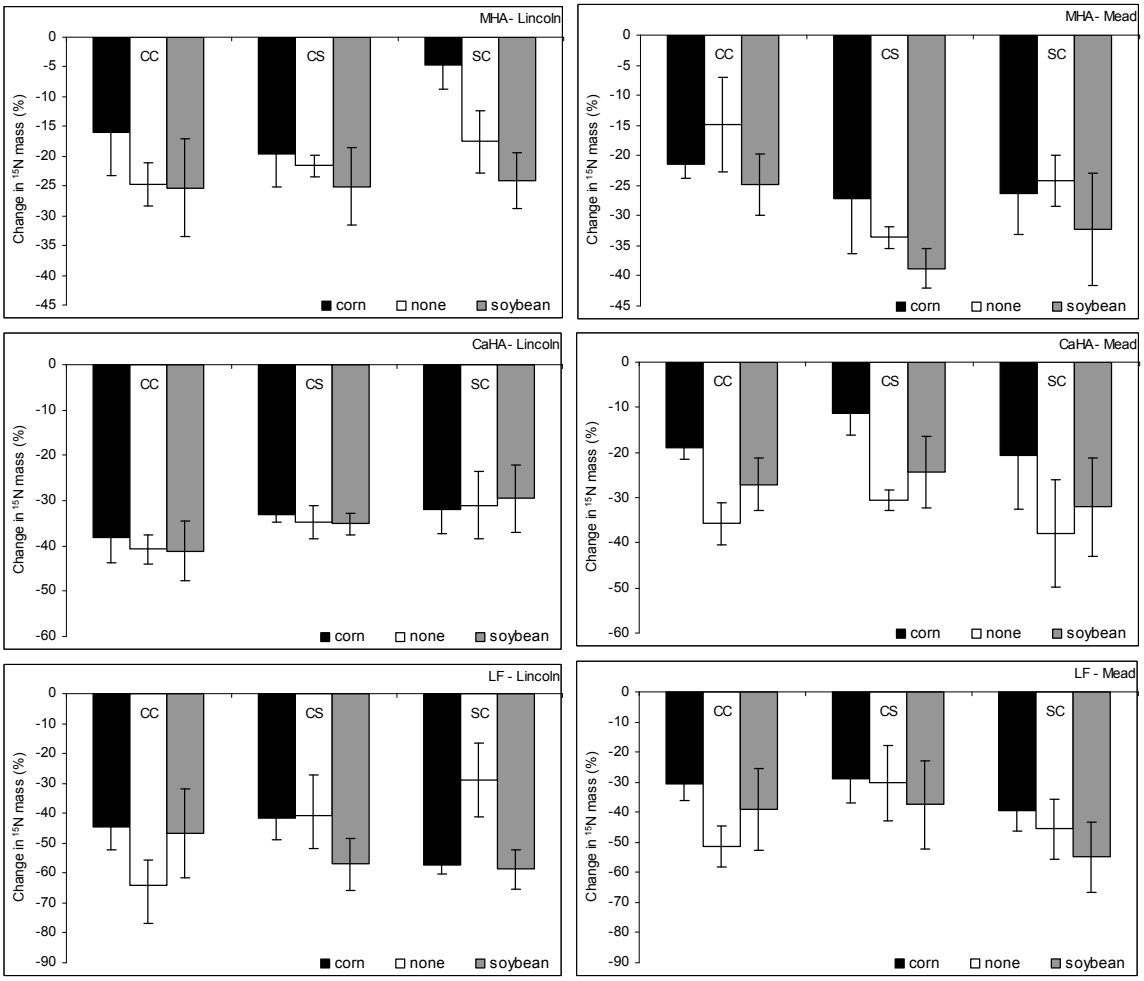

Figure 6. Change in ^{15}N mass in the MHA, CaHA and LF SOM fractions at Lincoln and Mead. Means and SE by residue amended and rotation. CC: continuous maize, CS: maize following soybean, and SC: soybean following maize.