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Litter-Carbon Dynamics: The Importance of Decomposition, Accretion, and Sequestration in Understanding Ecosystem Carbon Cycling

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LITTER-CARBON DYNAMICS: THE IMPORTANCE OF DECOMPOSITION, ACCRETION, AND SEQUESTRATION IN UNDERSTANDING ECOSYSTEM CARBON CYCLING

by

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The atmospheric CO_2 concentration has been increasing since the industrial revolution. A proposed mitigation strategy is sequestering carbon (C) in terrestrial ecosystems, either in plant biomass or soil organic matter. The litter-C pool is the second largest C pool in agroecosystems post-harvest, and the amount of litter-C loss has been correlated with ecosystem respiration. Yet, the potential importance of the litter pool as one of the major C pools in a system is relatively unknown. We do, however, know that the size of the litter pool can be affected by increases or decreases in both litter-C production and decomposition, respectively, and is therefore a highly dynamic C pool. With the increase in productivity, and the decrease in litter burial and soil disturbance in agroecosystems, the propensity for substantial litter build up is likely and yet the magnitude and temporal dynamics of litter-C accretion is generally unknown. Therefore, in order to understand ecosystem carbon dynamics, and make accurate predictions of C sequestration, careful quantification of litter-C production, losses, and accretion is essential. In this dissertation, I detail my exploration of litter-C dynamics in maize-based agroecosystems. I first investigate the impact of management on the decomposition of one annual maize litter cohort and examine potential changes in litter tissue quality, decomposition rates, and the changes in this annual litter-C pool over three years of *in situ* decomposition (Ch.2). I then report changes in litter-C production and decomposition for four annual litter cohorts of both maize and soybean litter to examine litter-C accretion under different management regimes (Ch.3). Thirdly, I investigate the effect of inorganic nitrogen additions to litter and how this influences litter and soil organic matter decomposition with both field and laboratory incubation conditions (Ch. 4). Finally, I finish with a study about how the addition of charred plant material impacts litter and soil organic matter decomposition and whether it is an effective sequestration strategy in prairie ecosystems (Ch.5).

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Chapter 1

General Introduction and Outline

Fossil fuel burning and land clearing for agriculture has led to increased atmospheric CO_2 concentrations (Keeling, 1993; Keeling et al., 1989; Vitousek, 1992). The conversion of millions of acres of natural land to agricultural systems has resulted in massive losses of soil organic carbon (C), exacerbating the already increasing atmospheric CO_2 concentration. Presently, in the U.S alone, 340 million acres of the total land area is devoted to crop production, and, globally, agroecosystems comprise 34 % of the earth's terrestrial land area (Cassman et al., 2003; Lubowski et al., 2006). Over the last 60 years we have been able to increase carbon inputs in these systems through crop management techniques, such as irrigation and fertilization, while concurrently reducing soil-C losses to the atmosphere through conservation or no-till practices (Allmaras et al., 2000; Cassman et al., 2003; Lal et al., 1999). The combination of large land area, fertile soils, and increased productivity with irrigation and fertilization, as well as the potential for increasing soil carbon content, suggests that agroecosystems have large potential for ecosystem carbon sequestration (Alvarez, 2005; Follett, 2001; Sauerbeck, 2001).

Agroecosystems, like natural ecosystems, have two large pools of C post-harvest: 1) soil-C and 2) litter-C. The litter -C pool is divided between above and belowground biomass and is largely untouched in no-till systems. In large production-scale no-till fields in Nebraska, seed is harvested at the end of the growing season, but the remainder of the plant, including the seedless cob, stalks, leaves, as well as all below ground portions of the plant, remains in the field to decompose on the soil surface without being

incorporated into the soil matrix via tillage. While contemporary agricultural practices have been successful at increasing productivity in these systems, the effect of different management regimes on the decomposition of crop residues is relatively unknown (Kochsiek et al., 2009).

Litter decomposition and litter-C production are likely to change in response to management, such as irrigation, fertilization, and crop rotation for a number of reasons. Water limitation, an important aspect of microclimate, is one of the globally most significant factors controlling productivity (Leith, 1975) and decomposition (Aerts, 1997; Couteaux et al., 1995; Meentemeyer, 1978). Improved water availability via irrigation could have a direct impact on decomposition by creating a more hospitable abiotic environment for decomposers. In addition, fertilization is known to not only increase growth, but also to increase tissue quality (Berg and Tamm, 1991), by increasing N concentrations (Alberda, 1965; Meentemeyer, 1978; Melillo et al., 1982; Russell, 1988b; Taylor et al., 1989; Tian et al., 1992a; Witkamp, 1966) and the proportion of soluble carbon in plant residues (McClaugherty, 1983). Studies have also shown that the effects of inorganic-N addition to litter, such as in a fertilization event, have variable effects on litter decomposition rates. While some studies show that inorganic N addition to litter can increase litter decomposition rates (Carreiro et al., 2000; Green et al., 1995; Henriksen and Breland, 1999a; Hobbie, 2005; Hunt et al., 1988), others show no effect (Biederbeck et al., 1996; Carreiro et al., 2000; Hobbie, 2005; McClaugherty and Berg, 1987) or even a decrease in litter decomposition rates (Carreiro et al., 2000; Knorr et al., 2005). While fertigation (nutrient enrichment) has the potential to impact decomposition rates, it is more likely to impact litter-C production, as fertigation events commonly are

scheduled at times when the developing crop has the most need for N. Thus, the precise timing of nitrogen additions through fertigation alleviates the need for added N at key times in crop development and can lead to greater amounts of litter-C production. Also, crop rotation rather than constant cropping with a single crop can have impacts on the standing litter pool both through differences in litter-C production and decomposition patterns.

In order to attain long-term carbon sequestration, litter-C must be physically and chemically protected as soil organic matter carbon (SOM-C). Merely increasing litter-C inputs through enhanced productivity may not be enough to increase the sequestration of litter-C in the soil, if increases in productivity are offset by concurrent increases in litter and/or soil organic matter decomposition. Therefore, understanding the decomposition patterns and the ultimate fate of litter-C is necessary to determine how long an ecosystem can retain C. Yet, the potential contribution of the litter pool to SOM as one of the major C pools in a system is relatively unknown. Despite increased productivity, decreased litter burial and soil disturbance from no-till practices, and the propensity for substantial litter build up in most large-scale agroecosystems, the magnitude and temporal dynamics of litter C accretion remain poorly constrained. Verma et al. (2005) estimated that 65-75% of gross ecosystem primary production in intensively managed agricultural systems is emitted as ecosystem respiration, and others have found that field CO_2 fluxes are similar to litter-C inputs (Jacinthe et al., 2002a; Paul et al., 1999). Thus, plant litter may be a pool of carbon that dominates short-term carbon sequestration and, in the long-term, an important part of the overall carbon balance of agroecosystems.

The studies included in this thesis were part of a larger carbon sequestration study examining the potential to sequester C in agricultural systems, which includes the three main cropping systems typical in the Western US corn belt (Verma et al., 2005), namely irrigated continuous maize, irrigated maize-soybean rotation, and rainfed maize-soybean rotation. We used three production- scale agricultural fields at the University of Nebraska Agricultural Research and Development Center near Mead, NE. Each field was no-till, where the grain was harvested at the end of the growing season, but the remainder of the plant, including the seedless cob, stalks, leaves, as well as all of the below ground portions of the plant were left in the field to decompose without being incorporated into the soil matrix via tillage. Crop growth, soil moisture, soil carbon, soil and plant gas exchange, and productivity also were measured at regular intervals within each management regime. Thus, we could make detailed estimates of carbon cycling under different management strategies for production-scale agricultural systems.

Outline of dissertation

In this dissertation I detail my exploration of litter-C dynamics in maize-based agroecosystems. I first investigate the impact of management on the decomposition of one annual maize litter cohort and examine potential changes in litter tissue quality, decomposition rates, and the changes in this annual litter-C pool over three years of *in situ* decomposition (Chapter.2). I then report changes in litter-C production and decomposition for four annual litter cohorts of both maize and soybean litter to examine litter-C accretion under different cropping and management regimes (Chapter 3). In these chapters, I show that litter tissue quality, decomposition, and litter-C accretion were all impacted by management. Decomposition was highly variable, but rapid. Regardless

of management, there was approximately 20% litter-C remaining on average after three years of *in situ* decomposition. I argue that the litter-C pool is highly dynamic and much more responsive to changes in litter-C production than decomposition.

I also investigate the effect of inorganic nitrogen additions to litter and how this influences litter and soil organic matter decomposition with both field and laboratory incubation conditions (Chapter 4). I found no impact of inorganic N addition on litter decomposition in the laboratory or field, nor did I find an impact of inorganic N addition on the decomposition of soil organic matter. However, I did find that the addition of litter decreased the total amount of soil decomposed and could potentially lead to a net C gain in soils. Therefore, while the decomposition process is difficult to manipulate with inorganic N additions, at least at low levels of addition, more studies need to simultaneously monitor litter decomposition and soil organic matter decomposition to determine the ability of a system to sequester carbon.

Finally, I finish with a study about how the addition of charred plant material impacts litter and soil organic matter decomposition in two different prairie soils (Chapter 5). I show that charred additions to soil can lead to very small increases in litter and soil organic matter decomposition under ideal incubation conditions. However, I argue that because the effects were small under ideal conditions, charred material should not have a significant effect on ecosystem carbon cycling under natural variable environmental conditions found in the field.

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Chapter 2

Impacts of management on decomposition and the litter carbon balance in irrigated and rainfed no-till agricultural systems

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ABSTRACT- The litter carbon (C) pool of a single litter cohort in an agroecosystem is the difference between net primary productivity and decomposition and comprises 11-13% of the total C pool (litter and soil 0-15 cm depth) post-harvest. This litter-C pool is highly dynamic and up to 50% can be decomposed in the first 12 months of decomposition. Thus, understanding litter-C dynamics is key in understanding monthly and annual total ecosystem carbon dynamics. While the effects of management practices such as irrigation and fertilization on productivity are well understood, the effects on decomposition are less studied. While irrigation and fertilization increase productivity, this will only lead to increased litter-C residence time and litter-C pool accretion if these techniques do not also result in equivalent or greater increases in decomposition. Management could potentially have impacts on litter-C accretion by increasing litter inputs, changing plant-C allocation, plant tissue quality, or decomposition rates. We examined carbon loss of one annual cohort of maize litter using *in situ* nylon litterbags for three years in three no-till fields with differing management regimes: irrigated continuous maize with a pre-planting fertilization application and two fertigation events, irrigated maize-soybean rotation with the same fertilization regime as the irrigated continuous maize management regime, and rainfed maize-soybean rotation with a single pre-planting fertilization event. We addressed the effects of these different management

regimes on net primary productivity and litter inputs, litter nitrogen (N) concentrations and carbon quality measures, plant C allocation, decomposition rates and the potential changes in the overall litter-C balance. We found that irrigation/fertigation management increased litter inputs, led to changes in plant tissue quality, had no effect on carbon allocation, and increased decomposition rates. This balance of both greater litter inputs and outputs of C from the irrigated management regimes led to a similar litter-C balance for this litter cohort in the irrigated and rainfed management regimes after three years of decomposition. Our data clearly show that merely increasing litter-C inputs through irrigation/fertigation practices is not sufficient to increase litter-C residence time because decomposition rates also increase. Therefore, close monitoring of decomposition rates is essential for understanding litter-C pool dynamics.

Keywords: decomposition, carbon sequestration, litter pools, carbon loss, fertigation

1. INTRODUCTION

Atmospheric CO_2 concentration has been increasing since the industrial revolution (Hutchinson et al., 2007; Keeling, 1993). A proposed mitigation strategy is sequestering carbon in terrestrial ecosystems, either in plant biomass or soil organic matter. In the temperate northern hemisphere, several agricultural ecosystems have been identified as potential carbon sinks (Allmaras et al., 2000; Lal et al., 1999; Sauerbeck, 2001) .

Agroecosystems comprise 38 percent of the Earth's terrestrial land area and those devoted to grain production are generally situated on highly productive, fertile soils (Cassman et al., 2003). Large losses of soil carbon occurred with the conversion of natural land areas to agricultural systems due to plowing and soil disturbance (Matson et al., 1997). However, irrigation and fertilization have increased primary productivity and grain yield over the last 60 years, while alternative management practices, such as the implementation of conservation or no-till management, have decreased soil disturbance (Allmaras et al., 2000; Cassman et al., 2003; Lal et al., 1999). The combination of large land area, fertile soils, and increased productivity with irrigation and fertilization, and the potential for increasing soil carbon content suggests that agroecosystems have a large carbon sequestration potential (Alvarez, 2005; Follett, 2001; Sauerbeck, 2001).

A detailed budget of carbon inputs and losses are required in evaluating the carbon sequestration potential of agroecosystems. Yet, there is a paucity of system level studies investigating the effects of irrigation, fertilization, and progressive management strategies on the ecosystem-level carbon balance (Bernacchi et al., 2005; Halvorson et al., 2002; Verma et al., 2005). Such studies are needed because the same management

factors that increase primary productivity may also influence the fate of the litter stock by changing decomposition rates.

Carbon pools in agroecosystems include two major components: a soil organic matter pool, with a residence time of months to thousands of years, and a litter pool with a turnover of months to several years. The litter-C pool represents a short-retention time C pool that will either be respired back to the atmosphere via decomposer organisms or incorporated into stable soil organic matter-C (Hutchinson et al., 2007). In order to attain long-term carbon storage in temperate maize-based agroecosystems, C must be physically and chemically protected as humified soil organic carbon. Therefore, understanding the decomposition patterns of plant litter and the fate of this C is necessary to determine how long agricultural systems can retain carbon in increased litter pools and the amount of litter-C that is eventually incorporated into stable soil organic matter. It is possible that an increase in litter carbon inputs through management practices that increase crop yield may allow for short-term C sequestration if these management practices do not also lead to increased C losses through decomposition of litter and soil organic matter-C.

Litter decomposition is likely to change in response to irrigation and fertilization for a number of reasons. Water limitation, an important aspect of microclimate, is one of the globally most significant factors controlling productivity (Leith, 1975) and decomposition (Aerts, 1997; Couteaux et al., 1995; Meentemeyer, 1978). Better water availability could have a direct impact on decomposition by creating a more hospitable abiotic environment for decomposers, or indirectly by changing plant biomass allocation and/or tissue quality. Tissue quality refers to the decomposability of a substrate with

high tissue quality referring to substrates that are easy to decompose, such as substrates with high N or soluble concentrations. Low tissue quality would result from increased lignin or other complex structural components which leads to higher recalcitrance of litter (Berg et al., 1993; Russell, 1988a; Vasconcelos and Laurance, 2005). In addition, fertilization is known to not only increase growth, but also increase tissue quality (Berg and Tamm, 1991), by increasing N concentrations (Alberda, 1965; Meentemeyer, 1978; Melillo et al., 1982; Russell, 1988b; Taylor et al., 1989; Tian et al., 1992a; Witkamp, 1966) and soluble fractions (McClaugherty, 1983). Increases in tissue quality generally lead to increased rates of decomposition (Aerts and deCaluwe, 1997; Berg and Tamm, 1991; Sanchez, 2005). Studies have also shown that the effects of inorganic-N addition to litter, such as in a fertigation event, have variable effects on litter decomposition rates. While some studies show that inorganic N addition can increase litter decomposition rates (Carreiro et al., 2000; Green et al., 1995; Henriksen and Breland, 1999a; Hobbie, 2005; Hunt et al., 1988), others show no effect (Biederbeck et al., 1996; Carreiro et al., 2000; Hobbie, 2005; McClaugherty and Berg, 1987) or even a decrease in litter decomposition rates (Carreiro et al., 2000; Knorr et al., 2005). Further, changes in biomass partitioning between leaves, supportive structures, or belowground structures could have an impact on litter pool build-up.

Here we report changes in maize litter quality, decomposition and net litter pool changes for different management regimes. Our first objective was to investigate if management changes litter-C production. Second, we asked if management changes tissue quality of maize, either directly for each tissue type or through the allocation among tissues. Third, we asked if management changes litter decomposition rates, and if

these changes are caused directly by microclimate changes or indirectly through impacts on tissue quality. Fourth, we coupled litter-C production with litter decomposition to investigate the effects management has on the litter–C balance of a single litter cohort in irrigated and rainfed agroecosystems.

2. MATERIALS AND METHODS

2.1. Study sites

Our decomposition study was part of a larger carbon sequestration study examining the potential to sequester C in agricultural systems (Verma et al., 2005). We used three production- scale agricultural fields at the University of Nebraska Agricultural Research and Development Center near Mead, NE. Each field was no-till, where the grain was harvested at the end of the growing season, but the remainder of the plant including the seedless cob, stalks, leaves, as well as all of the below ground portions of the plant were left in the field to decompose without being incorporated into the soil matrix via tillage. All fields contained the same four related soil series: Yutan (fine-silty, mixed, superactive, mesic Mollic Hapludalf), Tomek (fine, smectic, mesic Pachic Argialboll), Filbert (fine, smectitic, mesic Vertic Argialboll), and Filmore (fine, smectitic, mesic Vertic Argialboll). Previous to this study, fields 1 and 2 had 10 years of no-till maize-soybean rotation while field 3 had a much more variable cropping history that included soybean, maize, oats and wheat grown in 2-4 ha plots with tillage. At the initiation of the study, the soil in all three fields was disk tilled in order to incorporate accumulated surface residues from previous management and incorporate P and K fertilizers. All three fields were approximately 65 ha and were within 1.6 km of each

other. Field 1 was continuous maize irrigated with a center pivot irrigation system. Field 2 was an annual maize-soybean rotation irrigated in the same way. Both of the irrigated fields received a pre-emergence fertilization application by coulter injection of 128 kg N/ha (28% urea ammonium nitrate) and two subsequent fertigation events coinciding with plant development (Table 1). Field 3 was a rainfed, annual maize-soybean rotation, relying solely on natural precipitation and received one pre-emergence fertilization application at the same rate and by the same method as the irrigated fields. These three management practices represent the three main cropping systems in the mid-western part of the US (Verma et al., 2005).

We conducted our decomposition study in six 20 m x 20 m intensive measurement zones (IMZs) within each management regime. Crop growth, soil moisture, soil carbon, soil and plant gas exchange, and productivity were also measured at regular intervals within each IMZ. Before the initiation of the study, IMZ locations were selected by using a fuzzy-k mean clustering technique which classified each management regime into six categories based on elevation, soil type, electrical conductivity, soil organic matter content, near infrared remotely-sensed imagery and digital aerial photographs (Minasny and McBratney, 2003). Once the management regime was separated into the six different fuzzy class environmental categories, the exact location of the IMZ was placed randomly within each category area for a total of six IMZs for each management regime. The purpose of classifying each site into six IMZs was to capture landscape-level spatial variability so that the measurements could be scaled up to the entire management site. This approach allowed us to quantify the natural variability within each management regime to gain an estimate of the maximum

variability of our measured variables within a biological/agricultural relevant field scale (Minasny and McBratney, 2003). Because our within-site replication showed little variation in litter-C loss among IMZs, we used individual IMZ measurements as replicates for each management regime and applied statistics and made conclusions about treatment differences on this basis (Cottenie and De Meester, 2003; Hurlbert, 1984; Hurlbert, 2004). Do note that each management regime is not replicated. However, replication of 65-ha fields was not possible and using small replicated plots would not represent realistic estimates of entire agricultural production fields, because the equipment and irrigation are designed for large agricultural production fields. Our approach, therefore, was to measure litter decomposition across the widest range of potential variability within each 65-ha management regime.

2.2. Field methods

In 2001, all three management regimes were planted with maize. At the end of the growing season in October of 2001, the aboveground portions of three plants, and the belowground portion of six plants, were harvested from each IMZ in each management regime. The aboveground portion of the plant was separated into cobs, leaves, and stalks and dried to constant weight at 75°C. Belowground portions of the plants were washed, dried to constant weight at 75°C, and separated into root stalks, coarse and fine roots. The root stalk was defined as the belowground portion of the stalk where the roots branch off. Coarse roots were defined as the large primary roots that branch directly off the root stalk, while fine roots were the portions of the root that branch off of the coarse roots and have no direct contact with the root stalk.

Twelve replicate litter bags per IMZ were prepared for leaves as well as stalks for a total of 24 litter bags per IMZ. Six replicate litter bags per IMZ were prepared for root stalks as well as cobs for each IMZ for a total of 12 litter bags per IMZ. There were a total of 144 bags for both leaves and stalks and 72 bags for root stalks as well as cobs in each management regime. Each litter bag was 20 cm x 20 cm with a mesh size of 1 mm and 5-10 g of plant tissue was packed per litter bag (Burgess et al., 2002). Leaf, stalk, and cob litter bags were placed on the soil surface while root stalk litter bags were buried at a 5-cm soil depth. Due to the mesh size of the litter bags, macrofaunal decomposers were excluded, thus making our decomposition rates conservative. From 0.15 to 0.25 g of coarse and fine roots were packed in mini-containers with a volume of 1.5 cm^3. Mini-containers are small polyethylene tubes with mesh closing either end (Eisenbeis et al., 1999). Once the mini-containers are packed with root biomass, they were placed in PVC bars with mini-container sized holes drilled in them hereafter referred to as "root bars" and buried horizontally at approximately 5-cm depth in each management regime (Paulus et al., 1999). Each root bar contained six mini-containers filled with coarse roots and six with fine roots for a total of 12 root samples per root bar. Three root bars were made for each IMZ in each management regime for a total of 216 mini-containers per management regime: 108 fine root samples and 108 coarse root samples. Two mesh sizes, 20 μm and 2 μm, were used to make mini-containers. However, we detected no difference in decay rate among these mesh sizes and so we report pooled results. It should be noted, however, that either of these mesh sizes will exclude soil macrofauna and therefore may underestimate root decomposition. In November 2001, the litterbags and root bars were placed in each management regime. For our statistical analyses, we treated each IMZ as

a replicate for management regime (n=6 per management regime) and averaged all sample replicates within each IMZ to determine the overall litter-C loss for each tissue type. Six harvests of litter bags were made after the initial placement in November 2001. One-sixth of the litter bags from each litter type in each IMZ was harvested every six months for three years, cleaned of any soil contamination and weighed to determine mass loss.

Above-ground and below-ground crop biomass as well as grain yield were determined by destructive harvest. Above-ground biomass was collected at physiological maturity by harvesting 12m of row in each IMZ. Below-ground root biomass was determined at the R1 stage of growth in the following manner. Within each IMZ, three replicate transects of four cores each were taken perpendicular to the row at 13cm increments to the center of the interrow space 38cm from the crop row. Root cores were taken to a depth of 0.6m and separated into 0.15m increments and washed to remove soil and gross organic residue material. After washing, roots were stained with congo-red to identify dead from live root material. Roots were then hand sorted, dried, and weighed. Root weight density of each core was integrated over distance to obtain an estimate of root mass at each soil depth. These replicated estimates were then extrapolated to obtain total root mass on a square-meter basis. All biomass samples were analyzed for C with a Costech 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, Ca). Grain yield was determined on a whole-field basis by weighing the amount of grain removed through combine harvesting and measuring grain percent moisture in each load. Grain yield was then adjusted to a standard moisture content of 15% (Verma et al., 2005).

2.3. Tissue quality analysis

Initial tissue C and N contents of harvested plant organs for each tissue type, location (IMZ) and sampling time were determined by grinding a portion of biomass from each sample in a Wiley mini-mill with a 40 mesh (2 mm) screen (Thomas Scientific, Swedesboro, NJ). Total C and N were analyzed with a Costech ECS 4010. In addition, ash content was determined by burning a sample at 475°C in a muffle furnace and used to correct mass loss data for ash content. We also estimated initial carbon quality with the Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, NY), which is a common technique used to determine forage digestibility (Goering and Van Soest, 1970; Van Soest et al., 1991). This technique uses a sequential extraction to determine the amount of soluble, hemicellulose, cellulose and lignin fractions within each sample. These classifications do not represent strictly identical chemical compounds, but rather groups of similar compounds with similar resistance to decomposition. The data for tissue fractions analysis are presented as the four fractions (soluble, hemicellulose, cellulose and lignin) totaling 100% of the plant tissue carbon quality. Therefore, any increase in one fraction leads to an equivalent decrease in the other fractions.

2.4. Statistical analysis

We used a type III general linear model multivariate analysis of variance (MANOVA) to determine initial tissue quality differences among management regimes and tissues types, with % N, % soluble, % hemicellulose, % cellulose and % lignin as dependent variables. We used Pillai's trace test statistic for the MANOVA because it is more robust to violations of assumptions, whereas Roy's largest root has the greatest power (Scheiner, 2001). Pillai's trace and Roy's largest root gave the same results. If significant, we subsequently analyzed each variable separately, using two-way general

linear model analysis of variance (ANOVA) with management regime and tissue type as independent factors. All data were natural log-transformed to improve normality and meet the assumptions of parametric statistical tests. Post-hoc comparisons were conducted using the least significant difference (LSD) test.

Litter mass loss data showed the same pattern as litter-C loss data (C was on average 35-45% of all of the mass samples) and we report only the litter-C loss data here. Litter-C loss refers to C lost from the nylon litter bag and does not assume a fate of this C, whether respired back to the atmosphere or incorporated into some fraction of soil organic-C. To assess the changes in C loss over time we used a three-way ANOVA with time, plant tissue type and management as independent factors. If significant, we subsequently analyzed each sampling time separately with a one-way ANOVA with carbon loss as the dependent variable and management regime as the independent variable for each tissue type in each management regime. Post-hoc comparisons were conducted using the LSD test. To determine the rate and time of C loss for each tissue type and each management regime we log transformed carbon loss remaining and regressed it against time using the equation:

$$\ln(\%\ \text{C loss}) = y - kt \qquad (1)$$

where y= intercept, k=exponential decay constant and t=time. Residence time was evaluated as 1/k (Olson, 1963). One-way ANOVA was used to determine significant differences in k among fields by pooling all tissue types in each management regime. All statistical analyses were performed with SPSS Inc., v. 15 for Windows.

3. RESULTS

3.1.Litter-carbon production

Irrigation greatly increased net primary production in these systems as grain C was 559 g C/m^2 in the irrigated continuous maize management regime, 549 g C/m^2 in the irrigated maize-soybean rotation management regime, and 372 g C/m^2 in the rainfed maize-soybean rotation management regime (Table 1, Fig.6). Not only was grain-C increased, but litter-C input was approximately 100 g C/m^2 higher in the irrigated than the rainfed management regime ($F_{2,17}$=10.51, P=0.002, Table 1, Fig. 6). These increases in litter-C production were not driven by one plant tissue type, rather the irrigated management regimes produced more litter-C in all tissue types compared to the rainfed plants. Soil moisture decreased in the rainfed management regime from week 5 after crop emergence to week 13 during the time in which water and nutrients are necessary to attain high grain yields (Fig. 1). The irrigated management regimes received irrigation throughout this time, as well as fertigation events at key times in crop development. The reduction in soil moisture and lack of N application during the growing season in the rainfed management regime reduced litter-C production and grain yield for all tissue types (Table 1).

3.2. Tissue quality

Tissue quality differed significantly among tissue types and crop management regimes and there was a significant interaction between management regime and tissue type (Table 2). All aspects of carbon quality and % N contributed to this overall tissue difference (Table 3). Fine roots, leaves, root stalks and cobs all had % N of

approximately 0.95-1.00%, while stalks had the least with 0.5% N. Cobs and stalks had significantly more % soluble than all the other tissue types while coarse and fine roots had the least. In contrast, below-ground structures such as fine roots had the highest % lignin and cobs had the least with 2% lignin. The rainfed management regime had significantly higher %N and % soluble than the irrigated management regimes, while the irrigated management regimes had significantly more cellulose, hemicellulose and lignin. The significant interaction between management regime and tissue type indicates that differences in initial litter quality did not change consistently with each tissue type in each management regime (Table 3). It is clear that both % N and the soluble fraction were significantly higher in the rainfed management regime than either of the irrigated management regimes for cobs, stalks, root stalks, and coarse roots (Fig. 2 and Fig. 3). Nitrogen concentration was approximately twice as high in the rainfed management regime for stalks and cobs than either of the irrigated management regimes while for root stalks and coarse roots the rainfed management regime was about 0.49% and 0.41% higher than the irrigated maize-soybean management regime, respectively (Fig. 2). The soluble fraction in the rainfed management regime was consistently 12-15% higher than either of the irrigated management regimes for stalks, cobs and root stalks and 18% higher in the rainfed management regime for coarse roots (Fig. 3). Therefore, it is clear that tissue quality is responsive to management as the rainfed management regime had enhanced tissue quality with higher %N and soluble C, which could have the potential to increase decomposition rates for this field. While the rainfed management regime saw increases in soluble carbon and % N in some tissue types, the overall plant allocation of C was not significantly different for the management regimes in any of the tissue types

except for roots ($F_{2,18}$=8.54, P=0.003, Fig. 4). Thus, plant biomass partitioning was highly conserved and not affected by management.

3.3. Litter-carbon loss

Percent litter-C remaining differed among time, tissue type, and management regime as well as in all the interactions of these three main factors (Table 4). In the first six months of decomposition, cobs and stalks had approximately 90-95% C remaining while other tissues had 70-80% C remaining (Fig. 5). Tissue type differences were not always consistent among management regimes and sampling times, and differences such as these were not maintained throughout the three years of decomposition. The rainfed management regime had less % litter-C remaining than the irrigated management regimes at the six-month harvest for all tissue types except cobs (Fig. 5). Yet by 12 months, all three management regimes had similar % litter-C remaining for all tissue types except stalks and fine roots. While there are some significant differences in carbon loss between management regimes for each tissue type within harvests, these differences rarely consistently persisted from harvest to harvest (Table 4, Fig. 5). However, we do see more litter-C loss during the summer than winter months for all tissue types and we see equivalent losses of carbon from both of the irrigated regimes whether the maize tissue was decomposing during a maize crop year with fertigation events or a soybean crop (Fig. 4, Fig. 5). By 36 months there was between 10-30 % litter-C remaining in all management regimes. Consequently, while we saw enhanced tissue quality in the rainfed management regime, it only had an effect on decomposition in the short-term and after three years of litter decomposition all management regimes had lost similar amounts of litter carbon.

3.4. Litter-carbon balance

Carbon loss rate, litter-C residence time and litter inputs were all affected by management. The rate of litter decomposition (k) was higher for the irrigated management regimes than the rainfed management regime ($F_{2,107}$=8.21, P<0.0001). There was significantly more initial litter in the irrigated management regimes than the rainfed management regime ($F_{2,107}$=11.7, P<0.0001) and the residence time of litter-C in the rainfed management regime was significantly longer than in the irrigated management regimes ($F_{2,107}$=11.88, P<0.0001, Table 5). However, even with significant differences in k and residence time, litter carbon in the rainfed management regime only had an increase in residence time of approximately one additional year compared to the irrigated management regimes (Table 5). However, even with this increase in C input as litter, all three management regimes had about 100 g C/m^2 of litter remaining after three years of decomposition (Fig. 6). Thus, while irrigation can increase the grain production and primary productivity in these systems, it also increased decomposition so that by the end of three years the litter-C remaining of one litter cohort in each of the systems was similar regardless of management (Fig. 6).

4. DISCUSSION

Management techniques, such as irrigation and fertilization that increase productivity can only increase litter-C residence time if they do not also affect decomposition processes either indirectly through changes in litter quality and allocation or directly by changing microclimate to enhance decomposition. To understand the

impact of management on the carbon balance of the litter pool both productivity and decomposition must be precisely monitored.

4.1. Litter-carbon production

Irrigation allowed for administering water to the crop at times of crop need and when water potentially became limiting. Because precipitation was less than predicted, the rainfed field experienced reduced yields compared to the irrigated fields.

4.2. Litter tissue quality and carbon allocation

The tissue type differences we found in initial litter quality (Fig. 2, Fig. 3) are not surprising as plant parts are well known to have different constituent elements depending upon whether the function of that plant part is structural, photosynthetic, or reproductive (Chapin, 1980). We found that belowground structures had higher lignin concentrations than aboveground structures (Fig. 3). Overall management differences showed that the rainfed management regime had a higher concentration of N and soluble C than the irrigated management regimes (Fig. 2 and Fig. 3).

It is possible that the increased N and soluble C concentrations in the rainfed management regime were due to lack of irrigation and differences in fertilization regime (Table 1; Fig.1). In the rainfed management regime, fertilizer was applied at the beginning of the season and the fertilization rate was calculated to maximize maize grain yield based on average annual precipitation (Table 1). It has been shown that maize can take up about 71% of total N uptake before the period of maximum crop growth rate (Greef et al., 1999). Under conditions in which crops receive optimal amounts of water via precipitation or irrigation, the large amounts of N taken up initially would be diluted as more biomass accumulates during the period of maximum growth rate (Plenet and

Lemaire, 2000). Because soil moisture was significantly less in the rainfed management regime than the irrigated regimes at essential times in maize development, maize grain and biomass yield were less than predicted and therefore the plants were fertilized in excess and N dilution did not occur (Fig. 1). In the rainfed management regime, N that was not incorporated into the seed, because of reduced grain yield, remained in the structural tissue types (Fig. 2). The plants at the rainfed site had so much N in their tissues, that % N in grain was significantly higher than at the irrigated site (data not shown). The remobilization of N from structural tissue types to fill the grain was not enough to diminish N stocks in these tissue types to levels similar to the irrigated sites (Ta and Weiland, 1992). For the irrigated management regimes, because fertigation events were synchronized with plant need for water and nitrogen due to weather and phenology, they reduced the likelihood of fertilizing in excess.

4.3. Litter-carbon loss

Short-term decomposition patterns showed that in the rainfed management regime, structural tissues with significantly more % N decomposed more rapidly than in the irrigated management regimes in the first six months of decomposition (Fig. 5). Many decomposition studies have shown strong positive correlations between N content and decomposition rate, at least in the initial stages of decomposition (Lupwayi et al., 2004; Melillo et al., 1982; Taylor et al., 1989; Tian et al., 1992a; Tian et al., 1992b; Witkamp, 1966). Also, the soluble fraction of decomposing tissue is the portion that is most rapidly decomposed because it is comprised of carbohydrates and simple sugars that can either be leached out of the litter as dissolved organic carbon or can be easily assimilated by the microbial community (Christensen, 1985; Reinertsen et al., 1984;

Schreiber and Mc Dowell, 1985). Reinertsen et al. (1984) postulated that the controls on the early stages of decomposition are largely dependent upon the soluble C and other C that may not necessarily be soluble but is easily decomposed. The early pattern of increased decomposition in the rainfed field disappeared after six months suggesting that it was driven by the increase in % N and the soluble fractions that could be leached or rapidly consumed by decomposers and was therefore merely an ephemeral trend. Therefore, the tissue quality changes only had minor impact on the decomposition process and did not influence litter C pools except in the very short term.

Long-term decomposition patterns showed that maize litter in the irrigated management regimes decomposed more rapidly than in the rainfed management regime during three years of decomposition (Table 5). The indirect effects of enhanced tissue quality on decomposition in the rainfed field did not affect the overall rate of decomposition in the long term. The result of increased decomposition rate with increased water availability is in agreement with many other studies (Austin, 2002; Austin and Vitousek, 2000; Schomberg et al., 1994; Stott et al., 1986). The fertigation events at stages V-6 and V-12 in the irrigated management regimes, not only added water, but they also added a source of soluble N to the litter pool that microbes could utilize to enhance litter decomposition. Inorganic N addition to litter has been shown to have variable effects on decomposition rates (Henriksen and Breland, 1999b; Hobbie, 2005; Jacinthe et al., 2002b; Knorr et al., 2005). While we cannot definitively exclude the potential effects of added inorganic N through fertigation, we did not see differences in litter-C losses between the irrigated continuous maize regime where our litter bags could have been exposed to added N and those in the irrigated maize-soybean rotation where no

N was added during soybean years (Fig. 5, Fig. 6). After three years of decomposition, 80-90% of the initial fixed carbon was lost and all three fields had similar amounts of litter-C remaining regardless of management regime (approximately 100 g C/m^2; Fig. 6).

4.4. Litter-carbon balance

Management had significant impacts on litter-C inputs as well as litter-C decomposition (Table 4 and Table 5). The irrigated management regimes had approximately 80-100 g C/m^2 more litter input than the rainfed management regime due to increased net primary productivity by irrigation, and yet the apparent decomposition rate (k) over the three years was significantly faster in both of the irrigated management regimes than the rainfed management regime when all the tissue types were pooled for each management regime (Table 5). In the irrigated management regimes, irrigation/fertigation increased litter-C inputs by increasing productivity but also increased litter-C losses through decomposition, therefore these effects canceled each other out and the overall C balance of this litter cohort was similar regardless of management. We took precise measurements of decomposition for one litter cohort and found that by the end of three years of decomposition each field had approximately 100 g C m^2 litter remaining. So while the carbon balance of this litter cohort was similar by the end of the experiment, the overall litter dynamics of each system would be influenced by multiple annual cohorts of litter from each annual crop. However, our measurements showed that an increase in productivity due to irrigation/fertigation management was met with a similar increase in decomposition for agroecosystems common in Nebraska.

The importance of litter pools in carbon dynamics in agroecosystems should not be underestimated as it contributes to ecosystem respiration (Kucharik and Twine, 2007).

Verma et al. (2005) estimated that 65-75% of gross ecosystem primary production is emitted as ecosystem respiration. Jacinthe et al. (2002) found a positive relationship between litter-C input and annual CO_2 flux, suggesting that litter dynamics had a major effect on the overall carbon dynamics of the system. Annual net ecosystem production (NEP) is the balance between plant CO_2 uptake minus plant/rhizosphere respiration, litter decomposition, and also the balance between soil organic matter decomposition and formation. Soil organic matter decomposition and formation are long-term, slow processes that probably contribute little to NEP on an annual basis. It is clear that during the growing season NEP is mostly driven by the balance between plant uptake minus plant and rhizosphere respiration. However, our data demonstrate that after harvest, the litter pool comprises about 11-13% of the total field-C pool (litter and soil 0-15 cm depth) and as much as 50% of this litter-C can be lost in the first 12 months of decomposition. The highly dynamic nature of this pool suggests that it could be key in understanding ecosystem carbon dynamics. Thus, in order to determine the ability of these ecosystems to sequester C, it will be necessary to quantify the ultimate fate of this pool, whether it is respired back to the atmosphere or stored as stable soil organic matter.

5. CONCLUSIONS

This study provides clear evidence that management can have an impact on litter quality, litter inputs and litter losses through decomposition. Maize in the rainfed management regime with one pre-emergence fertilizer application had greater %N and soluble fractions, but reduced grain productivity compared to the irrigated management regimes with a pre-emergence fertilizer application and two fertigation events. Irrigation and fertigation allowed for more precise calculation of plant need for N at key times

during the season and allowed for higher plant productivity, greater N use efficiency, and less build-up of plant tissue N. The increased tissue quality (% N) in the rainfed management regime only produced increased decomposition rates in the first six months of decomposition whereas the irrigated management regimes saw faster decomposition over a three-year period. The irrigated management regimes not only led to greater litter-C inputs but also greater decomposition rates. The most important result of this study shows that the combination of greater inputs and outputs of litter-C led to a similar litter pool C balance after three years of decomposition. This result indicates the highly responsive nature of the litter-C pool to changes in management. Yet, our data also exemplify that while the litter pool is dynamic, many changes are transient as increased inputs of litter-C due to management can be met with equivalent or even greater increases in decomposition rates. This study demonstrates that precise measurements of both productivity and decomposition are crucial to understanding the overall litter-C balance of a system.

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Fig. 1. Soil volumetric water content at 25 cm soil depth for the 2001 growing season. Arrows denote fertigation events, critical stages in crop development, and crop harvest. Fertigation events coincided with periods of greatest plant need for N.

Fig. 2. Maize litter percent nitrogen (A) and percent of total plant nitrogen in each tissue type (B) in the three fields. Given are the means +/- 1 S.E. (n=6) Different letters denote $P<0.05$ of a LSD posthoc comparison of a one-way ANOVA (see Table 3).

Fig. 3. Maize litter carbon quality i.e. percent soluble, hemicellulose, cellulose and lignin in each tissue type for each field. Different letters denote $P<0.05$ of a LSD posthoc (n=6) in a one-way ANOVA (see Table 3).

Fig. 4. Percent of litter-C comprised by each tissue type in each management regime. One-way ANOVA was performed and the different letters denote significant differences at $P<0.05$ level. In each management regime the mass of cobs, stalks, leaves, and roots, as well as the % C of each of these tissue types were quantified in each IMZ. This allowed us to determine the amount of carbon in the litter pool for each tissue type in each management regime.

Fig. 5. Percent maize litter-C remaining over 36 months of *in situ* decomposition. Significant differences represent differences between each management regime in each harvest for each tissue type. The six IMZs in each field were used as replicates and significant differences were set at the 0.05 level. The rainfed management regime had

significantly greater C loss than the irrigated management regimes in the first six months for all tissue types except stalks and cobs.

Fig. 6. Litter-C loss (g/m^2) for all maize tissue types over 36 months of decomposition. Grain carbon is harvested at the end of the season and so is only represented in the initial harvest. In each management regime, the mass of cobs, stalks, leaves, and roots, as well as the % C of each of these tissue types were quantified in each IMZ at each harvest. We summed the litter-C for all tissue types in each IMZ at each harvest to determine the amount of carbon remaining in the litter pool over time. The six IMZs in each management regime were used as replicates and significant differences were set at the 0.05 level. Letters denote significant differences for total litter-C ($g\ C/m^2$) among management regimes within each harvest.

Table 1. Fertilization regime (A) and plant-C production (B) for each management regime.

A. Management regime	Applied N	kg N/ha
Irrigated Continuous Maize	Pre-emergence	128
	V-6 Fertigation	33
	V-12 Fertigation	35
	Total	196
Irrigated Maize-Soybean Rotation	Pre-emergence	128
	V-6 Fertigation	34
	V-12 Fertigation	34
	Total	196
Rainfed Maize-Soybean Rotation	Pre-emergence	128
	Total	128

Table 1 continued.

B. Management regime	Litter Tissue Type					
Irrigated Continuous Maize	Cob	Leaves	Stalk	Roots	Grain	Total
Biomass (g/m^2)	129±4.0	302±12.0	730±41.9	181±0.0	1227±10.5	2569
%C	43.02±0.2	41.58±0.3	43.25±0.3	44.03±0.4	45.54±0.06	
g C/m^2	55±1.9a	126±5.5a	316±20.3a	80±0.7a	559±4.7a	1136
Irrigated Maize-Soybean Rotation	Cob	Leaves	Stalk	Roots	Grain	Total
Biomass (g/m^2)	128±4.2	274±15.7	674±32.3	203±0.0	1202±13.1	2481
%C	43.42±0.1	41.60±0.5	42.99±0.2	44.25±0.4	45.69±0.05	
g C/m^2	56±1.9a	114±6.8a	289±13.0ab	90±0.7b	549±6.0a	1098
Rainfed Maize-Soybean Rotation	Cob	Leaves	Stalk	Roots	Grain	Total
Biomass (g/m^2)	105±7.2	226±8.4	574±22.3	169±0.0	817±24.2	1891
%C	43.17±0.2	41.89±0.2	44.27±0.8	43.53±0.3	45.52±0.1	
g C/m^2	46±3.3b	95±3.7b	254±10.5b	74±0.4c	372±10.6b	841
Statistics						
F	5.70	8.07	4.22	128.39	195.15	
P	0.014	0.004	0.035	0.000	0.000	

Table 2. Initial maize litter tissue quality at harvest: multivariate analysis of tissue quality with management regime and tissue type as independent factors and %N, % soluble, % hemicellulose, % cellulose and % lignin as dependent factors. Shown are the Pillai's trace value, *F*, and *P* of the Pillai's trace multivariate test statistic. All data were LN transformed to improve normality.

Treatment (*d.f.*)	Tissue quality		
	Pillai's value	*F*	*P*
Tissue type (*5, 102*)	2.35	15.11	<0.0001
Management (*2, 102*)	0.65	7.90	<0.0001
Management * Tissue type	1.47	3.54	<0.0001

Table 3. Maize litter quality. Shown are the F and P value of GLM analyses of respectively, % nitrogen, % soluble, % hemicellulose, % cellulose, and % lignin as the dependent factor with management regime and tissue type as the independent factors. Data were LN transformed to improve normality.

Treatment (*d.f.*)	% Nitrogen		% Soluble		% Hemicellulose		% Cellulose		% Lignin	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Tissue type (*5 ,107*)	21.74	0.000	34.61	0.000	55.45	0.000	24.55	0.000	119.37	0.000
Management (*2, 107*)	17.92	0.000	46.26	0.000	10.25	0.000	34.83	0.000	30.13	0.000
Management * Tissue type	3.86	0.001	2.95	0.010	5.91	0.000	3.59	0.000	2.50	0.011

Table 4. Percent carbon remaining of maize litter. Shown are the *F* and *P* values for a three way univariate general linear model with time, management regime, and plant tissue type as independent factors. Significant differences were set at the 0.05 level.

Source	*d.f.*	*F*	*P*
Time	*5*	931.27	0.000
Tissue type	*5*	134.74	0.000
Management	*2*	9.03	0.000
Management *Tissue type	*10*	10.13	0.000
Time*Tissue type	*25*	5.71	0.000
Time*Management	*10*	12.44	0.000
Time*Management * Tissue type	*50*	2.43	0.000

Table 5. Maize decomposition rate constants (k) ± 1 S.E. of carbon loss and residence time for each tissue type in each management regime. k was determined as the slope of the regression of log (carbon remaining) against time. Mean residence time is defined as $1/k$ (Olson 1963). The r^2 values represent the fit for each individual tissue type in each management regime. To determine the overall decay rate, tissue types were pooled for each field.

Tissue type	**$-k$** ($year^{-1}$)	**r^2**	**Residence time** (year)
Irrigated continuous maize			
Cob	0.21±0.020	0.73	4.96
Stalk	0.22±0.014	0.86	4.75
Leaf	0.33±0.014	0.93	3.09
Root stalk	0.24±0.017	0.82	4.30
Coarse roots	0.25±0.014	0.89	4.03
Fine roots	0.32±0.028	0.77	3.37
Irrigated maize-soybean rotation			
Cob	0.26±0.017	0.86	3.84
Stalk	0.21±0.015	0.83	4.96
Leaf	0.35±0.019	0.89	2.86
Root stalk	0.36±0.025	0.83	2.90
Coarse roots	0.30±0.021	0.83	3.55
Fine roots	0.28±0.023	0.79	3.74
Rainfed maize-soybean rotation			
Cob	0.20±0.020	0.71	5.11
Stalk	0.16±0.010	0.88	6.29
Leaf	0.35±0.018	0.91	2.86
Root stalk	0.18±0.018	0.72	5.89
Coarse roots	0.20±0.016	0.82	5.04
Fine roots	0.21±0.018	0.77	5.16
Overall (all tissue types combined)			
Irrigated continuous maize	0.26±0.009	0.75	3.84
Irrigated maize-soybean rotation	0.29±0.010	0.77	3.45
Rainfed maize-soybean rotation	0.22±0.010	0.67	4.54

Fig. 1.

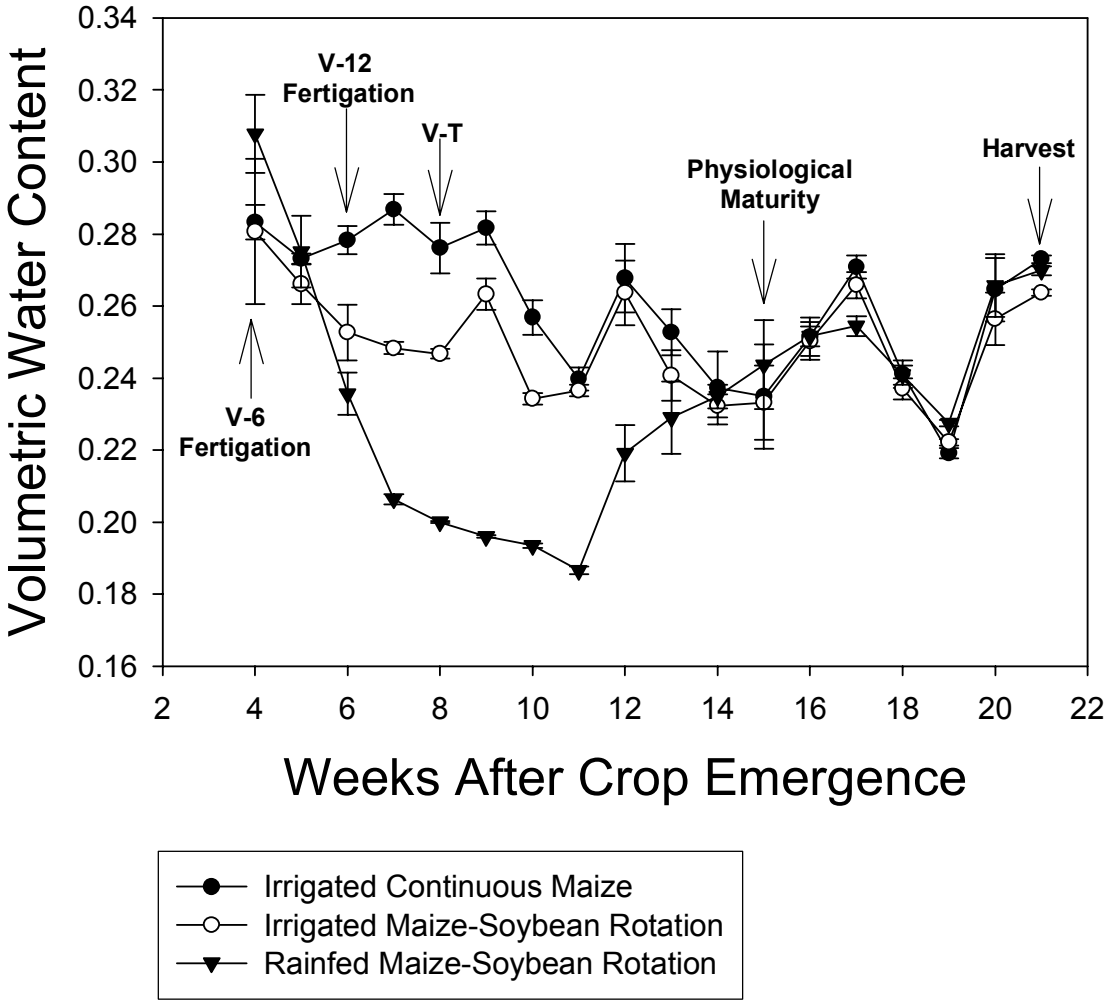

Volumetric Water Content
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
2
4
6
8
10
12
14
16
18
20
22
Weeks After Crop Emergence
V-12 Fertigation
V-T
Physiological Maturity
Harvest
V-6 Fertigation
Irrigated Continuous Maize
Irrigated Maize-Soybean Rotation
Rainfed Maize-Soybean Rotation

Fig. 2.

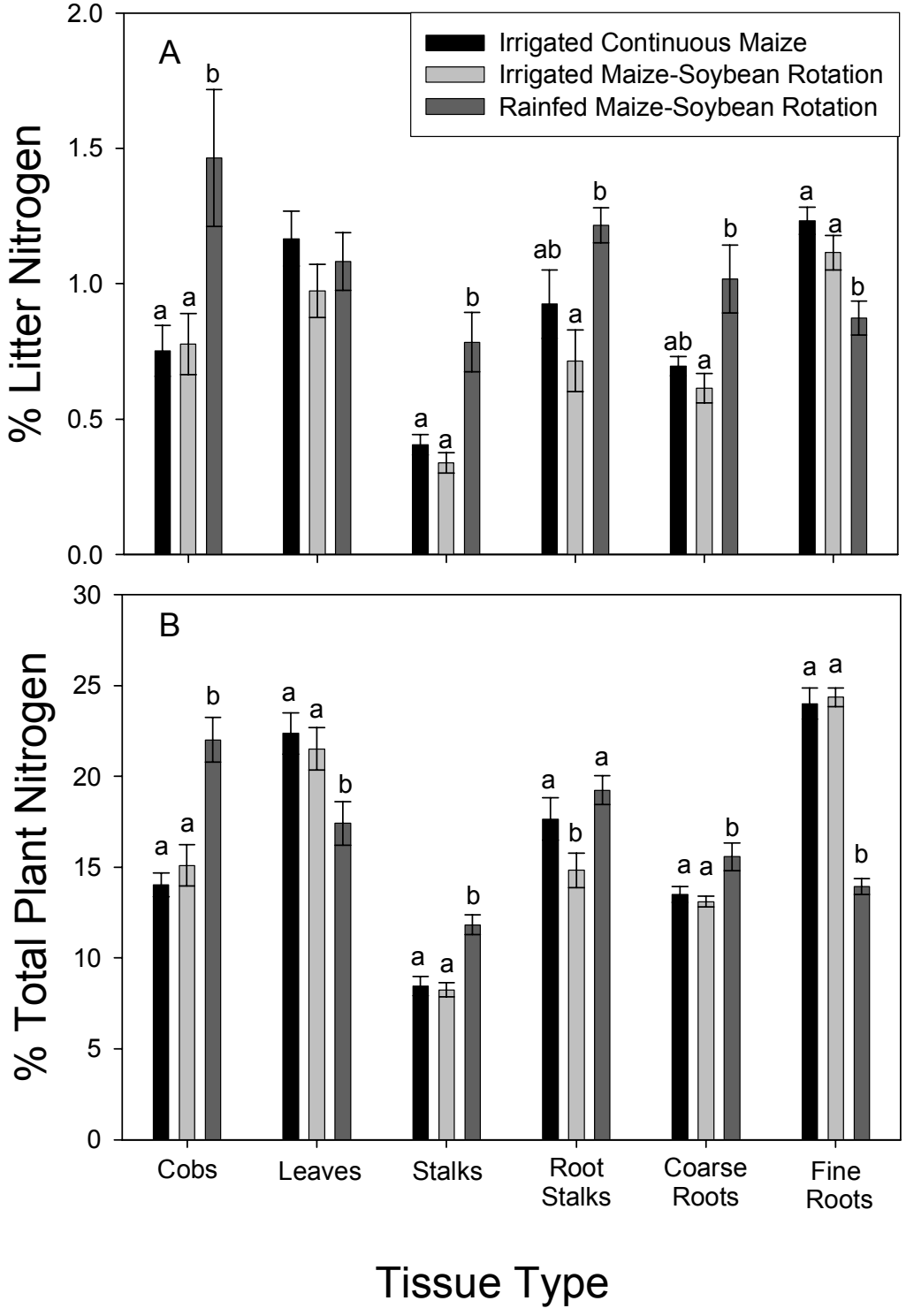

A
B
Irrigated Continuous Maize
Irrigated Maize-Soybean Rotation
Rainfed Maize-Soybean Rotation
% Litter Nitrogen
% Total Plant Nitrogen
Cobs
Leaves
Stalks
Root Stalks
Coarse Roots
Fine Roots
Tissue Type

Fig. 3.

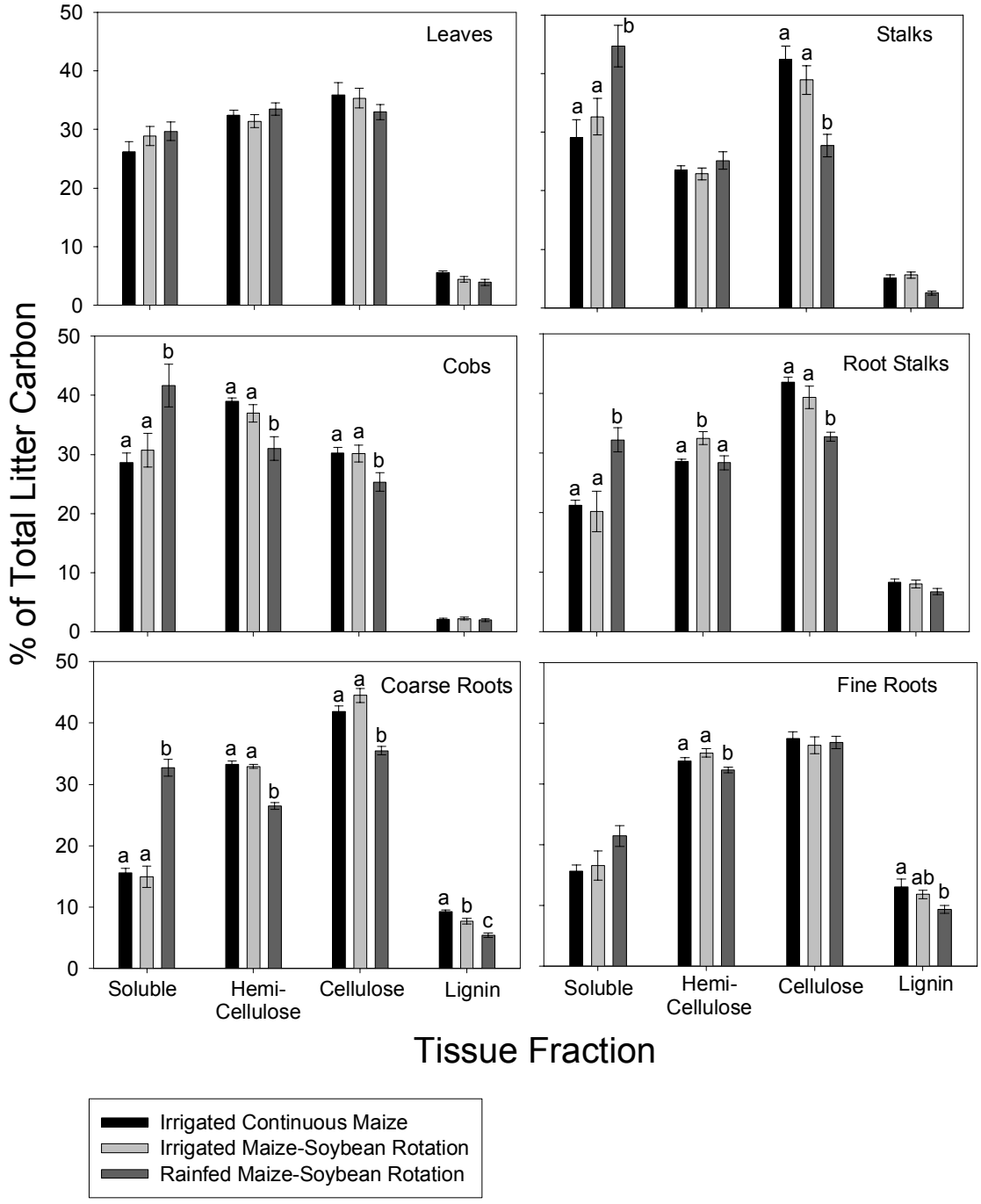

Leaves
Stalks
Cobs
Root Stalks
Coarse Roots
Fine Roots
% of Total Litter Carbon
Soluble
Hemi-Cellulose
Cellulose
Lignin
Tissue Fraction
Irrigated Continuous Maize
Irrigated Maize-Soybean Rotation
Rainfed Maize-Soybean Rotation

Fig. 4.

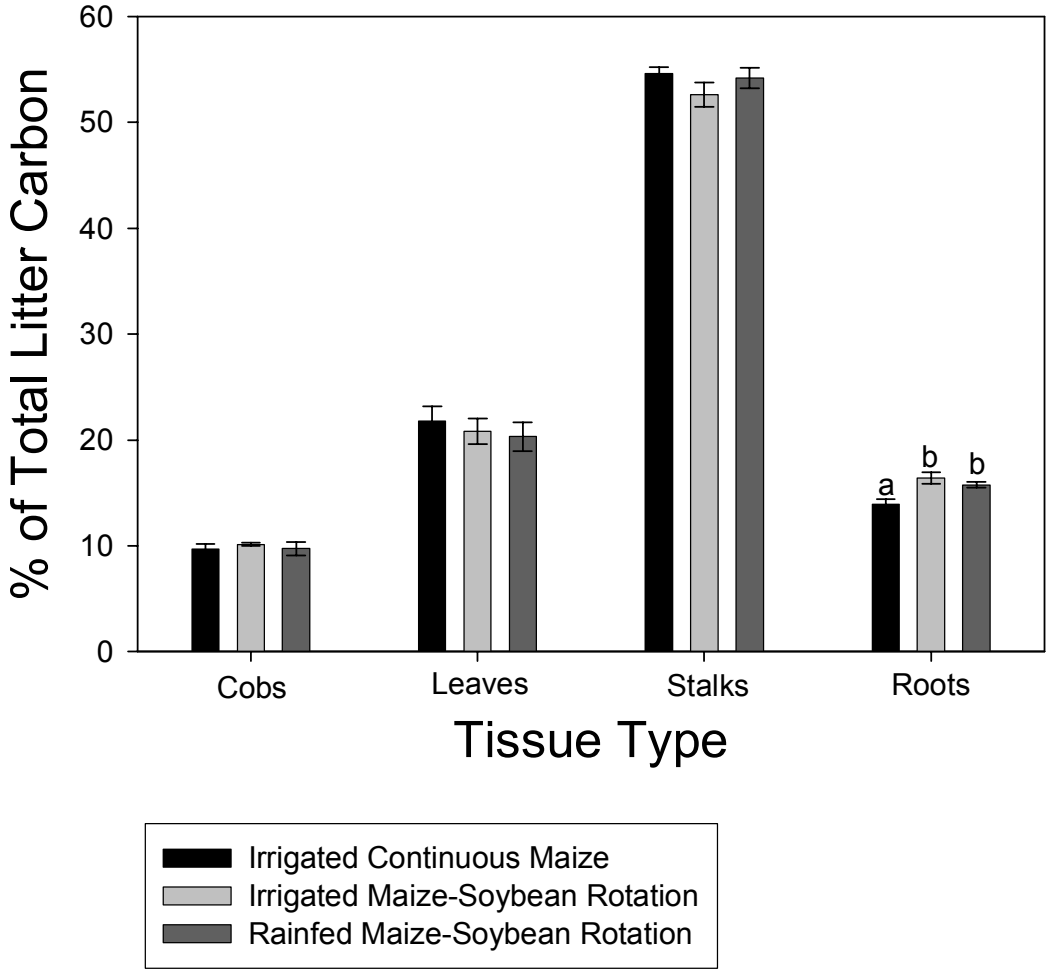

% of Total Litter Carbon
60
50
40
30
20
10
0
Cobs
Leaves
Stalks
Roots
a
b
b
Tissue Type
Irrigated Continuous Maize
Irrigated Maize-Soybean Rotation
Rainfed Maize-Soybean Rotation

Fig. 5.

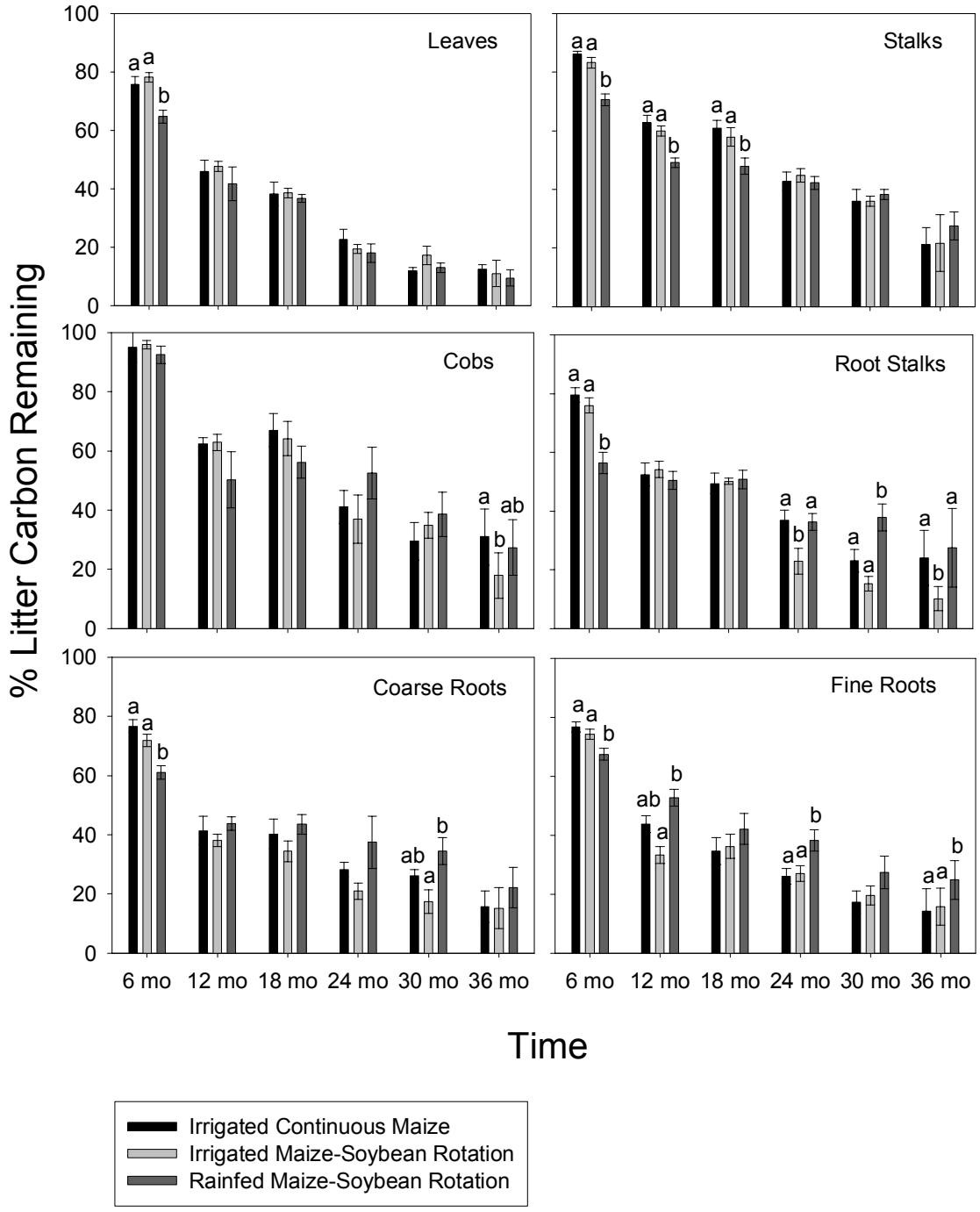
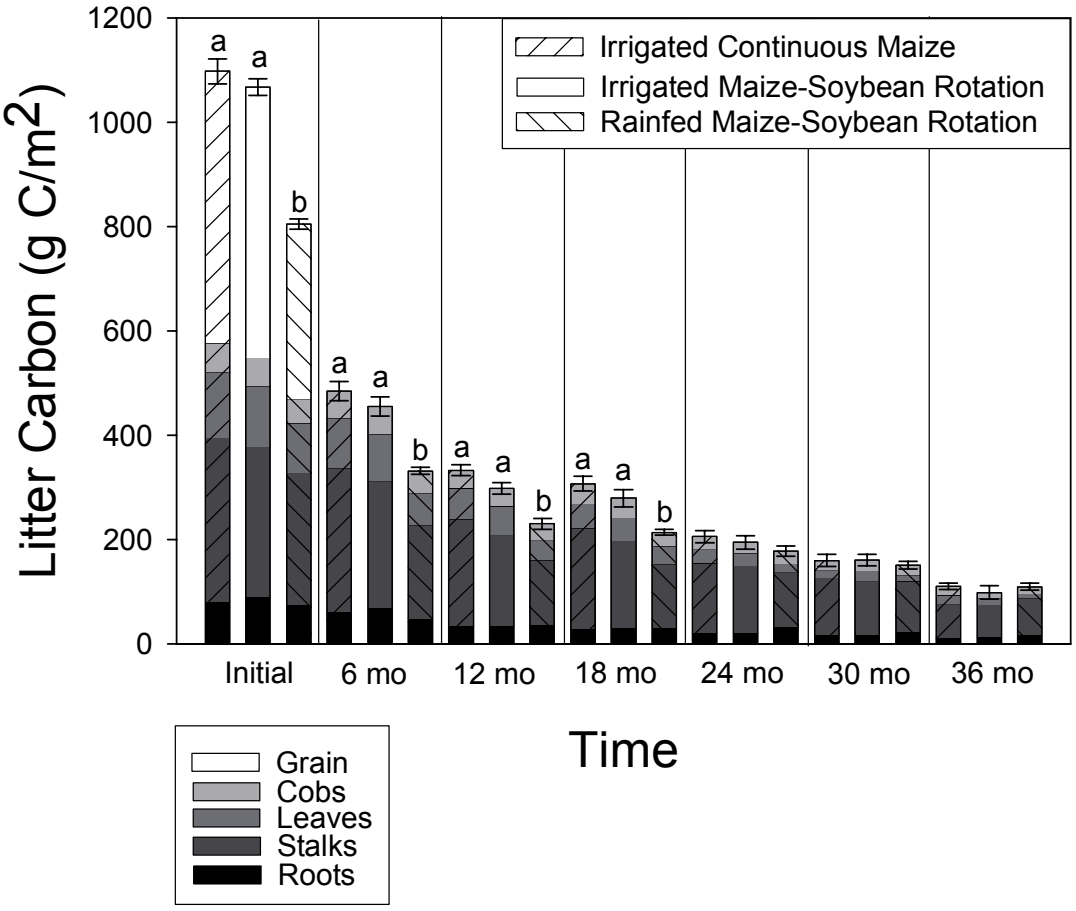

Leaves
Stalks
Cobs
Root Stalks
Coarse Roots
Fine Roots
% Litter Carbon Remaining
0
20
40
60
80
100
6 mo
12 mo
18 mo
24 mo
30 mo
36 mo
Time
Irrigated Continuous Maize
Irrigated Maize-Soybean Rotation
Rainfed Maize-Soybean Rotation

Fig.6.


Litter Carbon (g C/m2)
1200
1000
800
600
400
200
0
Irrigated Continuous Maize
Irrigated Maize-Soybean Rotation
Rainfed Maize-Soybean Rotation
a
a
b
a
a
b
a
a
b
a
a
b
Initial
6 mo
12 mo
18 mo
24 mo
30 mo
36 mo
Time
Grain
Cobs
Leaves
Stalks
Roots

Chapter 3

Litter-C production and decomposition effects on litter-C accretion in three no-till management regimes

Amy E. Kochsiek, Johannes M.H. Knops, Chad Brassil, Daniel T. Walters, and Timothy J. Arkebauer

ABSTRACT-Post-harvest, the litter carbon (C) pool of maize-based no-till agricultural systems is the second largest C-pool after soil-C. Therefore, understanding the dynamics of the litter-C pool and the controls on its decomposition is important in determining the overall C dynamics of the system and its potential of to sequester C. The size of the litter-C pool can be impacted by both litter-C production and decomposition. In order to understand litter-C accretion (litter-C production minus decomposition), we investigated litter-C production and *in situ* decomposition of maize and soybean litter using four annual litter cohorts (2001-2004) in three no-till management regimes: irrigated continuous maize, irrigated maize-soybean rotation, and rainfed maize-soybean rotation. We found that litter-C production was impacted by management and crop type, with the irrigated management regimes producing between 20%-30% more litter-C than the rainfed management regime and maize producing approximately twice as much litter-C as soybean. Irrigation also reduced annual variation in litter-C production for maize crops. Decomposition was highly variable, but overall, after three years of decomposition, only 20% litter-C remained on average. Litter-C accretion was impacted by management, as the irrigated continuous maize management regime had 15 and 35% more litter-C after ten years of management than either the irrigated maize-soybean rotation or the rainfed maize-soybean rotation, respectively. The litter-C pool proved to be much more responsive to changes in litter-C production than decomposition and was

driven by the most recent litter-C inputs. Our data clearly show that the litter-C pool is highly dynamic, with as much as a 60% increase in the litter-C pool within one year. Due to the potential for large amounts of litter-C buildup in systems such as these, understanding litter-C dynamics is key for determining C fluxes and for quantification of the carbon sequestration potential of agroecosystems.

INTRODUCTION

Predicting the ability of an ecosystem to sequester carbon (C) is becoming increasingly important due to the increase in atmospheric CO_2 caused by fossil fuel combustion (Hutchinson et al., 2007; Keeling, 1993). Agroecosystems comprise 38 percent of the Earth's terrestrial land area, and those systems devoted to grain production are generally situated on highly productive, fertile soils (Cassman et al., 2003). Large losses of soil carbon occurred with the conversion of natural land areas to agricultural systems due to plowing and soil disturbance, and within the highly productive temperate US agroecosystems, there has been on average a 50% reduction in soil carbon over the last century due to agriculture practices (Matson et al., 1997; Paul et al., 1997). However, irrigation and fertilization have increased primary productivity and grain yield over the last 60 years, while alternative management practices, such as the implementation of conservation or no-till management, have decreased soil disturbance (Allmaras et al., 2000; Cassman et al., 2003; Lal et al., 1999). The combination of large land area, fertile soils, increased productivity with irrigation and fertilization, and reduced C losses associated with recent management practices enhances the potential for increasing soil carbon content and suggests that agroecosystems have a large potential for carbon sequestration (Alvarez, 2005; Follett, 2001; Sauerbeck, 2001).

In agroecosystems, as in most terrestrial ecosystems, the carbon balance at the earth's surface is the difference between productivity and decomposition (Austin, 2002), and carbon can be stored in transient pools of carbon, such as the litter pool, or in more stable long-term pools, such as the soil-C pool. Soil-C represents the long-term C storage pool with a residence time estimated between months to thousands of years. The litter-C pool represents a short-term C pool with a turnover time of months to several years and a C pool that either will be respired back to the atmosphere via decomposer organisms or incorporated into stable soil organic matter-C (Hutchinson et al., 2007). In order to attain long-term carbon storage in temperate maize-based agroecosystems, C must be physically and chemically protected as humified soil organic carbon. Therefore, understanding the decomposition patterns of plant litter and the fate of litter C is necessary in order to determine how long agricultural systems can retain carbon in increased litter pools and the amount of litter-C that is eventually incorporated into stable soil organic matter. In addition, an increase in litter carbon inputs through management practices that increase crop yield also may allow for short-term C sequestration if these management practices do not also lead to increased C losses through decomposition of litter and soil organic matter-C. Verma et al. (2005) estimated that 65-75% of gross ecosystem primary production in intensively managed agricultural systems is emitted as ecosystem respiration, and others have found the field CO_2 fluxes are similar to litter-C inputs (Jacinthe et al., 2002; Paul et al., 1999). Thus, plant litter may also be an important pool of carbon that dominates short-term carbon sequestration and in the long-term an important part of the overall carbon balance of agroecosystems.

In general, the importance of the litter pool as one of the major C pools in terrestrial systems is relatively unknown. We do, however, know that the size of the litter pool can be affected by increases or decreases in both productivity and decomposition, respectively, and is therefore a highly dynamic C pool. With the increase in productivity, and the decrease in litter burial and soil disturbance, the propensity for substantial litter build up in agroecoystems seems likely, and yet the magnitude and temporal dynamics of litter C accretion is generally unknown.

In large-scale, no-till production fields in Nebraska, seed is harvested at the end of the growing season, but the remainder of the plant including the seedless cob, stalks, leaves, as well as all below ground portions of the plant are left in the field to decompose without being incorporated into the soil matrix via tillage. Although productivity has been increased in these systems, the effect of different management regimes on the decomposition of crop residues is relatively unknown (Kochsiek et al., 2009). For example, irrigation increases productivity, but it has also been shown to affect decomposition patterns (Aerts, 1997; Couteaux et al., 1995; Kochsiek et al., 2009; Leith, 1975; Meentemeyer, 1978). The availability of water could have a direct impact on decomposition by improving the abiotic environment for decomposers and indirect impacts by either enhancing or worsening plant tissue quality. Also, crop rotation rather than constant cropping with a single crop can have impacts on the standing litter pool both through differences in productivity and decomposition patterns.

Litter decomposition is likely to change in response to fertilization for a number of reasons. Fertilization is known to not only increase growth, but also increase tissue quality (Berg and Tamm, 1991), by increasing N concentrations (Alberda, 1965;

Meentemeyer, 1978; Melillo et al., 1982; Russell, 1988; Taylor et al., 1989; Tian et al., 1992; Witkamp, 1966) and soluble fractions (McClaugherty, 1983). Studies also have shown that the effects of inorganic-N addition to litter, such as in a fertigation event, have variable effects on litter decomposition rates. While some studies show that inorganic N addition to litter can increase litter decomposition rates (Carreiro et al., 2000; Green et al., 1995; Henriksen and Breland, 1999; Hobbie, 2005; Hunt et al., 1988), others show no effect (Biederbeck et al., 1996; Carreiro et al., 2000; Hobbie, 2005; McClaugherty and Berg, 1987) or even a decrease in litter decomposition rates (Carreiro et al., 2000; Knorr et al., 2005). While fertigation has the potential to impact decomposition rates, it is more likely to impact litter-C production because fertigation events are scheduled at times when the developing crop has the most need for nitrogen. Thus, the precise timing of nitrogen additions through fertigation alleviates need for added N at key times in crop development and can lead to greater amounts of litter-C production.

Here we report changes in litter-C production and decomposition for four annual litter cohorts, each of which decomposed *in situ* for three years in three no-till management regimes that represent the major cropping systems in the western USA corn belt. Our first objective was to investigate how annual variability and different field management changes litter-C production. Second, we asked if there were significant annual variation and management impacts on litter decomposition rates. Third, we generated site-specific decomposition models using maximum likelihood analysis to characterize the decomposition processes. Fourth, we coupled decomposition and litter-C production to investigate the effects of management on the litter–C balance and litter-C accretion over ten year of management. In total, this allows us to evaluate both how

important the litter pool is in the overall carbon budget of these agroecosystems and how sensitive the litter pool is to management changes.

MATERIALS AND METHODS

Study sites

This decomposition study was part of a larger carbon sequestration project to examine the potential to sequester C in agricultural systems (Verma et al., 2005). We used three production- scale agricultural fields at the University of Nebraska Agricultural Research and Development Center near Mead, NE. Each field was no-till, where the grain was harvested at the end of the growing season, but the remainder of the plant including the seedless cob, stalks, leaves, as well as all of the below ground portions of the plant were left in the field to decompose without being incorporated into the soil matrix via tillage. All fields contained the same four related soil series: Yutan (fine-silty, mixed, superactive, mesic Mollic Hapludalf), Tomek (fine, smectic, mesic Pachic Argialboll), Filbert (fine, smectitic, mesic Vertic Argialboll), and Filmore (fine, smectitic, mesic Vertic Argialboll)(Verma et al., 2005). Prior to this study, fields 1 and 2 were split in two and had 10 years of no-till alternating maize-soybean rotation, while field 3 had a much more variable cropping history that included soybean, maize, oats and wheat grown in 2-4 ha plots with tillage. At the initiation of the study, the soil in all three fields was disk tilled in order to incorporate accumulated surface residues from previous management and incorporate phosphorus (P) and potassium (K) fertilizers. All three fields were approximately 65 ha and were within 1.6 km of each other. Field 1 was continuous maize, irrigated with a center pivot irrigation system. Field 2 was an annual

maize-soybean rotation irrigated in the same way. Both of the irrigated fields received a pre-emergence fertilization application by coulter injection of 128 kg N/ha (28% urea ammonium nitrate) and two subsequent fertigation events coinciding with plant development (Table 1). Field 3 was a rainfed, annual maize-soybean rotation, relying solely on natural precipitation, and received one pre-emergence fertilization application at the same rate and by the same method as the irrigated fields. These three management practices represent the three main cropping systems in the mid-western part of the US (Verma et al., 2005).

We conducted our decomposition study in six 20 m x 20 m intensive measurement zones (IMZs) within each management regime. Crop growth, soil moisture, soil carbon, soil and plant gas exchange, and productivity also were measured at regular intervals within each IMZ. Before the initiation of the study, IMZ locations were selected by using a fuzzy-k mean clustering technique, which classified each management regime into six categories based on elevation, soil type, electrical conductivity, soil organic matter content, near infrared remotely-sensed imagery and digital aerial photographs (Dobermann and Ping, 2004; Minasny and McBratney, 2003). Once the management regime was separated into the six different fuzzy class environmental categories, the exact location of the IMZ was placed randomly within each category area for a total of six IMZs for each management regime. The purpose of classifying each site into six IMZs was to capture landscape-level spatial variability so that the measurements could be scaled up to the entire management site. This approach allowed us to quantify the natural variability within each management regime to gain an estimate of the maximum variability of our measured variables within a

biological/agricultural relevant field scale (Minasny and McBratney, 2003). There was within-site variation in productivity, with an average coefficient of variation (COV) of 9% within each field and year. Soil-C varied by approximately 26%, and litter-C lost was, in general, the most variable measurement, with an average COV of 38% for each tissue type, field, and year. However, these factors are not highly correlated with one another. We used individual IMZ measurements as replicates for each management regime and applied statistics and made conclusions about treatment differences on this basis (Cottenie and De Meester, 2003; Hurlbert, 1984; Hurlbert, 2004). Note that each management regime is not replicated. However, replication of 65-ha fields was not possible, and using small replicated plots would not represent realistic estimates of entire agricultural production fields, because the equipment and irrigation are designed for large agricultural production fields. Our approach, therefore, was to measure litter decomposition and remaining litter pools and to maximize the potential variability within each 65-ha management regime.

Field methods

There were four annual litter cohorts from 2001 to 2004. The fertilization and irrigation regimen for each management regime in each litter production year (2001-2004) is shown in Table 1. Each year, at the end of the growing season (October), above and belowground biomass was sampled next to each IMZ in each management regime. In 2001 and 2003, all three management regimes were planted with maize. In 2002 and 2004, the irrigated maize-soybean rotation and the rainfed maize-soybean rotations were planted with soybean. In the years that the management regimes were planted with maize,

the aboveground portions of three plants, and the belowground portion of six plants, were harvested from each IMZ in each management regime. The aboveground portion of the plant was separated into cobs, leaves, and stalks and dried to constant weight at 75°C. Belowground portions of the plants were washed, dried to constant weight at 75°C, and separated into root stalks, coarse and fine roots. The root stalk was defined as the belowground portion of the stalk where the roots branch off. Coarse roots were defined as the large primary roots that branch directly off the root stalk, while fine roots were the portions of the root that branch off of the coarse roots and have no direct contact with the root stalk. In soybean years, leaf litter traps were created to collect senesced leaves, and then the above and belowground biomass was harvested from twelve plants adjacent to each IMZ. The aboveground portion of the plants was separated into pod walls, leaves, and stalks and dried to constant weight at 75°C. Belowground portions of the plants were washed, dried to constant weight at 75°C, and separated into coarse and fine roots. Soybean biomass does not have a definable root stalk, and so this tissue type is not included in soybean litter cohorts. All other tissue types were defined in the same manner as in maize years.

For each annual litter cohort, twelve replicate litter bags per IMZ were prepared for leaves, as well as stalks, for a total of 24 litter bags per IMZ. Six replicate litter bags per IMZ were prepared for root stalks, as well as cobs, for each IMZ for a total of 12 litter bags per IMZ. There were a total of 144 bags for both leaves and stalks and 72 bags for root stalks, as well as cobs, in each management regime for each annual litter cohort. Each litter bag was 20 cm x 20 cm with a mesh size of 1 mm, and 5-10 g of plant tissue were packed per litter bag (Burgess et al., 2002). Leaf, stalk, and cob litter bags were

placed on the soil surface, while root stalk litter bags were buried at a 5-cm soil depth. From 0.15 to 0.25 g of coarse and fine roots were packed in mini-containers with a volume of 1.5 cm^3. Mini-containers are small polyethylene tubes with mesh closing either end (Eisenbeis et al., 1999). Once the mini-containers are packed with root biomass, they were placed in PVC bars with mini-container sized holes drilled in them, hereafter referred to as "root bars", and buried horizontally at approximately 5-cm depth in each management regime (Paulus et al., 1999). Each root bar contained six mini-containers filled with coarse roots and six with fine roots for a total of 12 root samples per root bar. Three root bars were made for each IMZ in each management regime for a total of 216 mini-containers per management regime in each annual litter cohort: 108 fine root samples and 108 coarse root samples. Two mesh sizes, 20 μm and 2 μm, were used to make mini-containers. However, we detected no difference in decay rate among these mesh sizes, and so we report pooled results. It should be noted, however, that either of these mesh sizes will exclude soil macrofauna and therefore may underestimate root decomposition. In November of each year, the litterbags and root bars were placed in each management regime. For our statistical analyses, we treated each IMZ as a replicate for management regime (n=6 per management regime) and averaged all sample replicates within each IMZ to determine the overall litter-C loss for each tissue type. Six harvests of litter bags were made after the initial placement in November of each year (Figure 1). One-sixth of the litter bags from each litter type in each IMZ were harvested every six months for three years, cleaned of any soil contamination, and weighed to determine mass loss.

Above-ground and below-ground crop biomass, as well as grain yield, were determined by destructive harvest. Above-ground biomass was collected at physiological maturity by harvesting 12m of row in each IMZ. Below-ground root biomass was determined at the R1 stage of growth in the following manner. Within each IMZ, three replicate transects of four cores each were taken perpendicular to the row at 13 cm increments to the center of the inter-row space 38 cm from the crop row. Root cores were taken to a depth of 0.6 m and separated into 0.15m increments and washed to remove soil and gross organic residue material. After washing, roots were stained with congo-red to identify dead from live root material. Roots were then hand sorted, dried, and weighed. Root weight density of each core was integrated over distance to obtain an estimate of root mass at each soil depth. These replicated estimates were then extrapolated to obtain total root mass on a square-meter basis. All biomass samples were analyzed for C with a Costech 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, Ca). Grain yield was determined on a whole-field basis by weighing the amount of grain removed through combine harvesting and measuring grain percent moisture in each load. Grain yield was then adjusted to a standard moisture content of 15% (Verma et al., 2005).

Tissue quality analysis

Initial tissue C and N contents of harvested plant organs for each tissue type, location (IMZ) and sampling time were determined by grinding a portion of biomass from each sample in a Wiley mini-mill with a 40 mesh (2 mm) screen (Thomas Scientific, Swedesboro, NJ). Total C and N were analyzed with a Costech ECS 4010. In

addition, ash content was determined by burning a sample at 475°C in a muffle furnace and used to correct mass loss data for ash content. We also estimated initial carbon quality with the Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, NY), which is a common technique used to determine forage digestibility (Goering and Van Soest, 1970; Van Soest et al., 1991). This technique uses a sequential extraction to determine the amount of soluble, hemicellulose, cellulose and lignin fractions within each sample. These classifications do not represent strictly identical chemical compounds, but rather groups of similar compounds with similar resistance to decomposition. The data for tissue fractions analysis are presented as the four fractions (soluble, hemicellulose, cellulose and lignin) totaling 100% of the plant tissue carbon quality. Therefore, any increase in one fraction leads to an equivalent decrease in the other fractions.

Statistical Analyses

The effect of year and management regime on the initial amount of litter produced for each tissue type for each litter cohort was determined using a two-way analysis of variance (ANOVA) with year and management regime as the main factors.

We determined differences in %C loss for each tissue type in each management regime for all four annual litter cohorts. For each tissue type, we determined the main effects of year and management regime with a two-way ANOVA. If either year or management regime proved significant, we determined differences between either year and/or management regime using separate one-way ANOVAs. All analyses included harvest time as a covariate.

We fit decomposition models using maximum likelihood analysis to determine the decomposition rates for each tissue type in each management regime for the four annual litter cohorts for each six month decomposition period using Mathematica v.7. Because decomposition tends to be rapid during the first year and then slow over time we created a model with separate decomposition rates for each winter and summer decomposition period. Thus, we had three winter decomposition parameters and three summer decomposition parameters.

$$y = e^{-w1t} \; ; \; t \leq 0.5; \text{ 0-6 months (winter)}$$

$$y = e^{-0.5w1} \, e^{-s1\,(t-0.5)} \; ; \; 0.5 < t \geq 1.0; \text{ 6-12 months (summer)}$$

$$y = e^{-0.5(s1+w1)} \, e^{-w2(1-1.0)}; \; 1.0 < t \geq 1.5; \text{ 12-18 months (winter)}$$

$$y = e^{-0.5(w2+s1+w1)} \, e^{-s2(t-1.5)} \; ; \; 1.5 < t \geq 2.0; \text{ 18-24 months (summer)}$$

$$y = e^{-0.5(s2+w2+s1+w1)} \, e^{-w3(t-2.0)} \; ; \; 2.0 < t \geq 2.5; \text{ 24-30 months (winter)}$$

$$y = e^{-0.5(w3+s2+w2+s1+w1)} \, e^{-s3(t-2.5)} \; ; \; 2.5 < t \geq 3.0; \text{ 30-36 months (summer)}$$

Where w1= the first winter decomposition rate from 0-6 months; s1= the first summer decomposition rate from 6-12 months of decomposition; w2= winter decomposition rate for 12-18 months of decomposition; s2= summer decomposition rate for 18-24 months of decomposition; w3= winter decomposition rate for 24-30 months of decomposition; s3= summer decomposition rate for 30-36 months of decomposition. We also fit two other less complex decomposition models. Since our decomposition data showed that in the first six months of decomposition, which is also the first winter period, there was more rapid decomposition than in the later winter periods, we fit a model with a separate decomposition rate for the first winter period of decomposition (w1). We then

used a common winter decomposition rate for the two other winter periods (w) and a common decomposition rate for the summer periods (s). The third and most simplistic model had common decomposition rates for all the winter decomposition periods (w) and all summer decomposition periods (s).

We then fit our three decomposition models using maximum likelihood analysis (Bolker, 2008; Hilborn and Mangel, 1997). Percent C loss was characterized best by a beta distribution where all values fall between 0 and 1 and a defined mean and shape parameter (Evans et al., 2000). The beta distribution can appear to be normal, but as the values get closer to 0 or 1 the distribution becomes more skewed. Thus, we used the beta distribution to parameterize our decomposition models. The normal distribution also was used, but beta distribution produced better model fits in all cases. We then added tissue type, field, and year incrementally to each of the three models to test the fit of adding each category to the previous simpler model. We compared the fit of each model using Akaike Information Criterion (AIC), which takes into account not only the model fit but also penalizes the addition of parameters that make the model unnecessarily complex (Burnham and Anderson, 2002; Sakamoto et al., 1986).

We then used the decomposition parameters generated from our best fit model to predict % C loss over time. We used litter-C production and decomposition parameters to determine how much of a litter cohort remained at any period of time. For years after 2004, litter-C production was not monitored directly, so we used the grain harvest data and the proportion of each litter type in previous years to determine the litter-C production for each litter type. For 2009-2010, we used the mean litter-C production for each tissue type. We also used the mean % C remaining for each tissue type to predict

decomposition in years after 2004. By summing the remaining fraction of litter cohorts for any period of time, we could determine the amount of litter C accretion in each management regime. We then increased productivity and decomposition rates by 10% to determine potential effects on litter-C accretion in each of the three management regimes.

RESULTS

During the growing seasons when litter was produced (2001-2004), air and soil temperatures were similar for all years and management regimes (Figure 2a, 2b). In each year, from July-September, the rainfed management regime had reduced soil moisture compared to the irrigated management regime (Figure 2c). Generally, the irrigated maize-soybean rotation had the highest soil moisture compared to the other management regimes throughout the growing season.

Productivity was highly variable between crop type, management regime, and year (Table 2; Figure 1 &2). In 2001 and 2003, when all three management regimes were cropped with maize, the irrigated management regimes were significantly more productive than the rainfed management regime (Figure 2). Irrigation tended to decrease variability in maize production, as the irrigated continuous maize and the irrigated maize-soybean management regimes had COVs of 12% and 9%, respectively while the rainfed site had 16%. Also, maize was always approximately two fold more productive than soybean. Irrigation increased litter-C production for soybean, but this effect was only significant in 2002 (Figure 2). Irrigation also did not lead to reduced variability for soybean production as it did with maize. However, it should be noted that in 2004 soybean was planted late due rainy conditions, so a short season hybrid was used, which

produced less litter-C. Therefore, there was increased variability in soybean production in both the irrigated (COV=31.7) and rainfed (COV=23.8) management regimes. Generally, there was a decrease in litter-C production over the four years that were monitored.

Decomposition rates were also highly variable, with significant annual variation (COV=40) and management impacts (COV=41.4) (Table 3&4; Figure 4). We investigated the impact of tissue quality and environmental measures, such as VWC and soil temperature at 10 cm depth, on decomposition rates, and, while they varied among years and management regimes, there was no significant correlation between any of these variables and decomposition rates (Appendix). Generally, the belowground tissue types were more responsive to irrigation than the aboveground tissue types, because they tended to decompose slower in the rainfed management regimes than in the irrigated management regimes, regardless of crop type (Table 3&4). Soybean tissue types also decomposed significantly faster than their maize counterparts for all tissue types ($p=0.000$), except for stalks ($f_{1,70}=0.207$; $p=0.650$). Regardless of crop type or management regime, there was on average 20% of the litter-C remaining after 3 years of *in situ* decomposition, and it varied between 2 and 40% depending on tissue type.

For both maize and soybean, the model with the best fit included the three factors (year, tissue type and field) with the six separate decomposition parameters that characterized decomposition in each six month period (w1, w2, w3, s1, s2, s3)(Table 5). While decomposition had significant management and annual variation effects (Table 3 & 4), the model fit points to the factors that explain the data better than others. For example, including tissue type (t) with any of the three decomposition models had a

lower log likelihood and AIC value and thus fit the data better than including either field (f) or year (y). Also, generally including more decomposition parameters increased model fit, where the common winter (w) and summer (s) decomposition parameters were a poorer fit than including a separate decomposition parameter for the first winter period (w1) and then common decomposition parameters for the remaining winter periods (w) and all summer periods (s) (Table 5).

By combining litter-C production and decomposition, we determined the amount litter-C after ten years of management. The irrigated continuous maize regime had approximately 15% and 35% more litter-C than the irrigated maize-soybean rotation and the rainfed maize-soybean rotation, respectively (Figure 5A). Increasing the decomposition rates by 10% had small impacts on litter-C accretion, and only increased litter-C by 5% on average (Figure 5B). Litter-C was decreased more in the maize-soybean rotations than the continuous maize system by 2.5-3.5%. Increasing litter-C production, however, was directly related to the amount of standing litter-C, as each management regime increased its standing litter-C pool by 10% (Figure 5C). We also calculated litter-C accretion in the spring and fall (after harvest) after 10 years of management (Figure 4). From spring to post harvest, there is a dramatic increase in the litter pool of each management regime, with the biggest increases seen in the maize-soybean rotations, with increases in the standing litter-C pool of 55 % and 60%, respectively (Figure 4). The large increases seen in the maize-soybean rotations are due to the differences in litter-C production, with the maize crop being approximately twice as productive as the soybean. When decomposition was decreased by 10%, the difference between the spring and post-harvest litter pools, while still dramatic, was

lessened by only 2% in the irrigated continuous maize regime, but was 10-12% less in the maize-soybean rotations. Because decomposition rates decreased, this led to more litter-C remaining in the spring and therefore less of a difference between spring and the post-harvest standing litter pools.

DISCUSSION

Irrigation and fertigation allowed the administering of water and nitrogen to the crop at key times in crop development and/or when water became limiting for plant growth. Because precipitation was less than predicted in some years, the rainfed field experienced reduced yields compared to the irrigated fields. When the irrigated management regimes were cropped with maize, they tended to have a less variation in litter-C production compared with the rainfed regimes, because the crops always had sufficient water and fertilizer inputs. 2004 was a particularly bad year for litter-C production in all of the sites due to a late freeze that damaged the corn plants in the irrigated continuous maize management regime. In the irrigated and rainfed maize-soybean rotations, soybean planting was delayed because of large amounts of rain, and thus a short season hybrid was used.

While there were significant management effects and annual variation in litter-C decomposition, all tissue types decomposed rapidly, and after three years of decomposition 80% of the litter-C was lost. Litter-C loss was highly variable among tissue types, management, and years, and it was not significantly correlated with environmental variation, such as soil temperature or moisture or initial tissue quality (Appendix; Kochsiek et al., 2009). This is contrary to studies in natural systems, where

decomposition has been shown to be impacted by both the environment and plant tissue quality (Aerts, 1997; Aerts et al., 2003; Alberda, 1965; Swift, 1979). Maize tissue generally has about 50% lower lignin concentrations than natural C_4 grasses (Pastor et al., 1987; Wedin et al., 1995) and this may favor its rapid decomposition Instead of tissue quality or environmental variation leading to decomposition differences, the observed differences in litter-C loss between tissue types seemed to be more related to plant tissue structure than tissue quality. For example, cob tissue is a large dense structure which takes time for microbial colonization and is more resistant to fragmentation than other tissue types (Foley and Vander Hooven, 1981). Thus, cob tissue had the slowest decomposition rates. While we did not formally quantify litter structure, there is at least a qualitative relationship between litter-C loss and litter structure.

In the first six months of decomposition, which was a winter period,between 20-30% litter-C was lost. Our winter C losses for leaf and stalks (~21% lost) are in agreement with other studies of corn decomposition in Southwestern Quebec (~ 20%) (Burgess et al., 2002), southeastern Ontario(Gregorich and Ellert, 1994) and were slightly slower than the 25% loss seen in Missouri (Ghidey and Alberts, 1993). The significant amount of litter-C loss during this time points to the potential importance of physical processes such as freeze/thaw dynamics, precipitation interception, and litter fragmentation in the decomposition process. Other studies also show that some portion of the decomposer community is active at cold temperatures (Stott et al., 1986). Thus, those studies that ignore winter decomposition patterns and only investigate decomposition during the summer months are potentially missing critical decomposition processes.

Percent C-loss during the first summer period for maize surface litter (cobs, leaves, and stalks) was 27%. This is higher than the 21% C-loss reported for summer decomposition rates in Southwestern Quebec (Burgess et al., 2002), but lower than the 35% lost reported in Quebec by (Rochette et al., 1999) and also lower than rates from Missouri (Broder and Wagner, 1988). After two years of decomposition, for surface litter we lost 73% litter-C which is within 1-3% of what was reported for similar tissue types over the same decomposition interval in Southwestern Quebec (Burgess et al., 2002) and surprisingly very close to rates of litter that was buried at 10 cm soil depth in North Platte, Nebraska (Tarkalson et al., 2008). Thus, it is clear that there is some consistency (±10 %) in decomposition rates over large geographic areas. However, it should also be noted that the litter bag mesh size used in this study excluded mesofaunal decomposers such as earthworms, resulting in conservative rates of decomposition.

Because our decomposition data were collected at six-month intervals, which were summer and winter seasons, there were distinct differences in decomposition rates for each period. Fitting exponential decay functions to these data did not accurately capture the seasonal dynamics in decomposition, and thus we fit decomposition models to the data that were tailored to incorporate seasonality. By doing this, we are able to make more precise estimates of litter-C remaining at each six month interval for the entire three years of decomposition for each annual litter cohort. This also allowed us to make within-year estimates of the change in the size of the litter-C pool from spring to post harvest.

Litter-C accretion was higher in the irrigated continuous maize regime than in either of the maize-soybean rotations. Because maize produces much more litter-C

annually, continuous maize regime had annual inputs around 5000 kg C/ha, whereas in a soybean year, productivity dropped to between 2000-3000 kg C/ha. Soybean also tended to decompose significantly faster than maize for all tissue types except for stalks. Thus, for the maize-soybean rotations, the combination of reduced litter-C production in soybean years plus the increased decomposition rates seen with soybean, led to decreased litter-C accretion rates. When we increased decomposition and litter-C production in these management regimes, it became clear that litter-C inputs have more of an impact on litter-C accretion than does decomposition. Increasing decomposition rates by 10% only lead to and average of a 5% increase in litter-C accretion over 10 years (Figure 5). When looking at the contribution of each annual litter cohort to the entire amount of litter-C accumulated over ten years, it is clearly driven by litter-C production and decomposition in the most recent 3-4 litter cohorts and after about 4 years of decomposition, very little remains in any litter cohort regardless of management regime. So even with the large observed differences in decomposition rates with different management, as well as significant annual variation in litter-C lost, litter decomposition is so rapid in these systems that this variation has little impact on litter-C accretion. Litter-C accretion is more driven by changes in litter-C production than by decomposition. The litter-C pool in intensively managed systems, such as these, tends to be dynamic and ephemeral, with large inputs and rapid losses of C. We clearly show that, even within one year, the litter-C pool can change by as much as 65%.

The importance of litter pools in carbon dynamics in agroecosystems should not be underestimated as it contributes to ecosystem respiration (Kucharik and Twine, 2007). Verma et al. (2005) estimated that 65-75% of gross ecosystem primary production is

emitted as ecosystem respiration. Jacinthe et al. (2002) found a positive relationship between litter-C input and annual CO_2 flux, suggesting that litter dynamics had a major effect on the overall carbon dynamics of the system. Annual net ecosystem production (NEP) is the balance between plant CO_2 uptake minus plant/rhizosphere respiration, litter decomposition, and also the balance between soil organic matter decomposition and formation. Soil organic matter decomposition and formation are long-term slow processes that probably contribute little to NEP on an annual basis. It is clear that during the growing season, NEP is mostly driven by the balance between plant uptake minus plant and rhizosphere respiration. However, our data demonstrate that after harvest, the litter pool comprises about 20-23% of the total field-C pool (litter and soil 0-15 cm depth) and as much as 80% of this litter-C can be lost in three years of decomposition. The highly dynamic nature of this pool suggests that it could be key in understanding ecosystem carbon dynamics. Thus, in order to determine the ability of these ecosystems to sequester C, it will be necessary to quantify the ultimate fate of this pool, whether it is respired back to the atmosphere or stored as stable soil organic matter.

Conclusions

This study shows that litter-C accretion is sensitive to changes in management, with the irrigated continuous maize rotation having significantly greater litter-C pool after 10 years of management than either the irrigated or the rainfed maize-soybean rotations. The differences among the litter-C pools can be related to higher litter-C production associated with annual inputs of maize, which produced approximately two fold more litter-C annually than soybean. Irrigation also reduced the variation in litter-C production for maize crops, allowing for consistently large inputs of litter-C. While

decomposition was variable, it tended to be rapid, with between 2-40% litter-C remaining after three years of in situ decomposition depending on tissue type. The most important result from this study is that the litter pool is a highly dynamic and ephemeral C pool that can change as much as 60% within one year. Also, post-harvest it is the second largest C pool in these systems after soil-C. This study demonstrates that precise measurements of both productivity and decomposition are crucial to understanding the overall litter-C balance of a system and that the litter can be a substantial short-term C pool in highly managed systems, such as these. Thus, understanding C cycling through this pool will help to determine entire ecosystem C gains and losses and how long a system will retain C in short-term pools such as the litter-C pool.

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Table 1. Management in each site for the four annual litter cohorts.

Site		**2001**	**2002**	**2003**	**2004**
		Irrigated Continuous Maize			
Crop		Maize	Maize	Maize	Maize
Pre-emergence fertilizer	Kg/ha	127.86 (N); 85.12(S)	134.4	133.5	159.04
V-6 fertigation	Kg/ha	33.04	44.80	45.47	33.6
V-12 fertigation	Kg/ha	34.72	45.36	45.02	33.6
Annual Irrigation	cm	33.60	28.68	37.84	22.81
Harvest	Mg/ha	13.51	12.97	12.12	12.12
		Irrigated Maize-Soybean Rotation			
Crop		Maize	Soybean	Maize	Soybean
Pre-emergence fertilizer	Kg/ha	127.86 (N); 85.12(S)		111.89	
V-6 fertigation	Kg/ha	33.6		28.89	
V-12 fertigation	Kg/ha	34.27		27.55	
Annual Irrigation (cm)	cm	32.97	20.96	34.80	15.88
Harvest (Mg/ha)	Mg/ha	13.41	3.99	14.00	3.36
		Rainfed Maize-Soybean Rotation			
Crop		Maize	Soybean	Maize	Soybean
Pre-emergence fertilizer	Kg/ha	127.68	None	89.82	None
Harvest	Mg/ha	8.72	3.32	7.72	3.14

Table 2. Changes in litter production for the four annual litter cohorts. Two-way ANOVA were used to determine the main effects of year and management regime on litter production for each tissue type. Significant differences were determined where $P<0.05$ in a LSD post-hoc comparison.

	Productivity		
	Maize		
Cob	*DF*	*F*	*P*
year	3, 40	3.21	0.033
management regime	2, 40	19.06	0.000
year*management regime	2, 40	4.44	0.018
Leaf			
year	3, 40	6.11	0.002
management regime	2, 40	18.05	0.000
year*management regime	3, 40	2.65	0.083
Stalk			
year	3, 40	9.47	0.000
management regime	2, 40	29.52	0.000
year*management regime	3, 40	3.71	0.033
Roots			
year	3, 40	229.04	0.000
management regime	2, 40	149.73	0.000
year*management regime	3, 40	34.90	0.000
	Soybean		
Pods	*DF*	*F*	*P*
year	1, 40	19.07	0.000
management regime	1, 40	9.77	0.005
year*management regime	1, 40	1.72	0.204
Leaf			
year	1, 40	63.67	0.000
management regime	1, 40	0.17	0.687
year*management regime	1, 40	0.03	0.857
Stalk			
year	1, 40	18.24	0.000
management regime	1, 40	30.39	0.000
year*management regime	1, 40	12.20	0.002
Roots			
year	1, 40	453.99	0.000
management regime	1, 40	0.711	0.409
year*management regime	1, 40	0.248	0.624

Table 3. Maize percent carbon loss for each tissue type in each litter cohort. For each tissue type, we determined the main effects of year and management regime with a two-way ANOVA. We then determined potential management and annual differences for each tissue type using one-way ANOVA. All analyses included harvest time as a covariate. Displayed are the *f* and *p* values for the two-way and both one-way ANOVAs. Significant differences were determined where P<0.05 in a LSD post-hoc comparison. Letters denote significant differences among managements regimes or years. If the letters are to the right of the mean, they represent annual differences. If the letters are to the left of the mean, they represent annual differences.

				Stalks				
	df		*f*		*p*			
Year	3, 565		45.17		0.000			
Field	2, 565		3.87		0.021			
Year*field	2, 565		2.84		0.059			
							Management Differences	
			Means± S.E.					
Management	Irrigated Continuous Maize		Irrigated Maize-Soybean Rotation		Rainfed Maize-Soybean Rotation		*f*	*p*
2001	a52.55±1.20a		51.30±1.08a		46.10±1.34b		8.20	0.000
2002	b56.14±1.25							
2003	c46.99±1.26		47.59±1.08		46.79±1.36		0.04	0.960
2004	d36.40±1.27							
Annual	*f*	*p*	*f*	*p*	*f*	*p*		
Differences	47.112	0.000	5.932	0.016	0.13	0.719		
				Leaves				
	df		*f*		*p*			
Year	3, 558		21.47		0.000			
Field	2, 558		6.38		0.002			
Year*field	2, 558		2.88		0.057			
							Management Differences	
			Means± S.E.					
Management	Irrigated Continuous Maize		Irrigated Maize-Soybean Rotation		Rainfed Maize-Soybean Rotation		*f*	*p*

2001	a27.50±1.40a		35.04±1.27b		29.73±1.39a		9.632	0.000
2002	b39.77±1.40							
2003	c32.45±1.38		33.69±1.28		32.99±1.39		0.348	0.706
2004	a25.16±1.40							
Annual	*f*	*p*	*f*	*p*	*f*	*p*		
Differences	21.402	0.000	0.559	0.456	2.734	0.100		

Cobs

	df	*f*	*p*
Year	3, 276	9.30	0.000
Field	2, 276	3.00	0.051
Year*field	2, 276	1.97	0.141

	Means± S.E.			**Management Differences**	
Management	Irrigated Continuous Maize	Irrigated Maize-Soybean Rotation	Rainfed Maize-Soybean Rotation	*f*	*p*
2001	b53.96±2.10	51.72±2.10	52.51±2.82	0.189	0.828
2002	ab50.14±2.07				
2003	a47.07±2.04 a	44.88±2.10a	37.71±2.82b	4.404	0.015
2004	a46.48±2.04				
Annual	*f*	*p*	*f*	*p*	*f*	*p*		
Differences	2.746	.045	5.293	.024	13.791	.000		

Root Stalks

	df	*f*	*p*
Year	3, 270	16.03	0.000
Field	2, 270	1.74	0.178
Year*field	2, 270	0.17	0.844

	Means± S.E.			**Management Differences**	
Management	Irrigated Continuous Maize	Irrigated Maize-Soybean Rotation	Rainfed Maize-Soybean Rotation	*f*	*p*
2001	a43.47±1.93	37.74±2.01	41.24±2.03	1.912	0.153
2002	b26.06±1.90				
2003	a43.04±1.90	39.23±2.03	42.54±2.00	0.429	0.653
2004	c37.71±1.90				
Annual	*f*	*p*	*f*	*p*	*f*	*p*		
Differences	18.151	.000	0.272	0.604	0.207	0.651		

Coarse Roots

	df	*f*	*p*
Year	3, 845	79.57	0.000
Field	2, 845	40.10	0.000
Year*field	2, 845	18.71	0.000

	Means± S.E.			**Management**

					Management Differences	
Management	Irrigated Continuous Maize		Irrigated Maize-Soybean Rotation	Rainfed Maize-Soybean Rotation	*f*	*p*
2001	38.04±1.31a		33.32±1.28b	39.22±1.13a	7.413	0.001
2002	19.36±1.28b					
2003	36.01±1.31a		37.86±1.26a	52.53±1.14b	45.517	0.000
2004	43.72±1.30c					
Annual Differences	*f* 66.369	*p* 0.000	*f* 6.438	*p* 0.012	*f* 68.445	*p* 0.000

Fine Roots

	df	*f*	*p*
Year	3, 830	38.91	0.000
Field	2, 830	23.06	0.000
Year*field	2, 830	0.73	0.483

Means ± S.E.

					Management Differences	
Management	Irrigated Continuous Maize		Irrigated Maize-Soybean Rotation	Rainfed Maize-Soybean Rotation	*f*	*p*
2001	a35.54±1.35a		34.42±1.36a	42.11±1.26b	11.918	0.000
2002	b23.92±1.30					
2003	c38.94±1.30a		40.67±1.33a	48.06±1.29b	11.172	0.000
2004	c39.92±1.30					
Annual Differences	*f* 31.825	*p* 0.000	*f* 10.826	*p* 0.001	*f* 10.917	*p* 0.001

Table 4. Soybean percent carbon loss for each tissue type in each litter cohort. For each tissue type, we determined the main effects of year and management regime with a two-way ANOVA. We then determined potential management and annual differences for each tissue type using one-way ANOVA. All analyses included harvest time as a covariate. Displayed are the *f* and *p* values for the two-way and both one-way ANOVAs. Significant differences were determined where P<0.05 in a LSD post-hoc comparison.

			Stalks			
	df		*f*		*p*	
Year	1, 279		209.50		0.000	
Field	1, 279		1.53		0.217	
Year* Field	1, 279		8.40		0.004	
	Means± 1 S.E.				**Management Differences**	
Management Year	Irrigated Maize-Soybean Rotation		Rainfed Maize-Soybean Rotation		*f*	*p*
2002	57.36±1.40		55.86±1.24		1.45	0.230
2004	34.37±1.42		40.51±1.20		7.96	0.005
Stats	*f*	*p*	*f*	*p*		
Annual Differences	132.12	0.000	78.95	0.000		
			Leaves			
	df		*f*		*p*	
Year	1, 273		44.75		0.000	
Field	1, 273		10.63		0.001	
Year* Field	1, 273		8.06		0.005	
	Means± 1 S.E.				**Management Differences**	
Management	Irrigated Maize-Soybean Rotation		Rainfed Maize-Soybean Rotation		*f*	*p*
Year						
2002	29.17±1.65		19.62±1.57		21.35	.000
2004	13.61±1.67		13.36±1.64		0.067	0.796
Stats	*f*	*p*	*f*	*p*		
Annual Differences	43.99	0.000	7.63	0.007		
			Pods			
	df		*f*		*p*	

Year	1, 135	6.58	0.011
Field	1, 135	0.01	0.914
Year* Field	1, 135	5.59	0.020

	Means± 1 S.E.		**Management Differences**	
Management	Irrigated Maize-Soybean Rotation	Rainfed Maize-Soybean Rotation	*f*	*p*
Year				
2002	21.20±2.32	15.31±1.82	1.99	0.163
2004	21.54±2.35	25.60±1.85	4.64	0.035
Stats	*f* *p*	*f* *p*		
Annual Differences	0.01 0.918	15.76 0.000		

Coarse Roots

	df	*f*	*p*
Year	1, 392	5.94	0.015
Field	1, 392	43.99	0.000
Year* Field	1, 392	6.36	0.012

	Means± 1 S.E.		**Management Differences**	
Management	Irrigated Maize-Soybean Rotation	Rainfed Maize-Soybean Rotation	*f*	*p*
Year				
2002	46.32±1.04	59.11±1.32	56.59	0.000
2004	46.37±1.04	53.24±1.21	7.55	0.007
Stats	*f* *p*	*f* *p*		
Annual Differences	0.001 0.973	10.762 0.001		

Fine Roots

	df	*f*	*p*
Year	1, 384	57.67	0.000
Field	1, 384	16.91	0.000
Year* Field	1, 384	1.84	0.175

	Means± 1 S.E.		**Management Differences**	
Management	Irrigated Maize-Soybean Rotation	Rainfed Maize-Soybean Rotation	*f*	*p*
Year				
2002	55.22±1.56	61.66±1.56	3.97	0.048
2004	41.56±1.56	52.18±1.40	25.15	0.000
Stats	*f* *p*	*f* *p*		
Annual Differences	38.32 0.000	20.44 0.000		

Table 5. Maize and soybean decomposition models. Shown is the fit for each model tested for both crop types. There were three possible factors to include: year (y), tissue type (t) and/or management regime (f). Each model tested is represented by the parameters included in the model. For example, the most complex model denoted (w1,w2,w3,s1,s2,s3) had separate decomposition parameters for each six month period of decomposition. Also included are the log likelihood values, total number of parameters, AIC value and the difference between the model tested and the model with the best fit (Δi). The model with the best fit has the lowest log likelihood and AIC values.

Maize

Log likelihood	Total Parameters	Factors	Model	AIC	Δi
-3456	241	y*t*f	w1,w2,w3,s1,s2,s3	-6429	0
-3156	121	y*t*f	w1, s, w	-6071	358
-3148	121	y*t	w1,w2,w3,s1,s2,s3	-6054	375
-2961	81	y*t*f	w, s	-5759	670
-2934	61	y*t	w1, s, w	-5747	682
-2782	41	y*t	w,s	-5482	947
-2682	91	t*f	w1,w2,w3,s1,s2,s3	-5182	1248
-2567	46	t*f	w1, s, w	-5041	1388
-2482	31	t	w1,w2,w3,s1,s2,s3	-4902	1527
-2471	31	t*f	w, s	-4879	1550
-2403	16	t	w1, s, w	-4773	1656
-2318	11	t	w, s	-4614	1815
-2220	25	y*f	w1, s, w	-4390	2039
-2206	25	y	w1,w2,w3,s1,s2,s3	-4363	2066
-2167	13	y	w1, s, w	-4308	2121
-2156	17	y*f	w, s	-4278	2151
-2135	19	f	w1,w2,w3,s1,s2,s3	-4232	2198
-2120	10	f	w1, s, w	-4221	2208
-2110	9	y	w, s	-4202	2227
-2079	7	f	w, s	-4143	2286

Soybean

Log likelihood	Total Parameters	Factors	Model	AIC	Δi
-1640	97	y*t*f	w1,w2,w3,s1,s2,s3	-3087	0
-1563	49	y*t*f	w1, s, w	-3028	59
-1523	49	y*t	w, s	-2948	-139

-1506	33	y*t*f	w, s	-2947	-140
-1473	25	y*t	w1, s, w	-2896	-191
-1426	17	y*t	w, s	-2819	-268
-1353	61	t*f	w1,w2,w3,s1,s2,s3	-2584	-503
-1296	25	t*f	w1, s, w	-2543	-544
-1295	31	t	w1,w2,w3,s1,s2,s3	-2528	-559
-1256	13	t	w1, s, w	-2486	-601
-1255	17	t*f	w, s	-2477	-610
-1219	9	t	w, s	-2419	-668
-562	13	y*f	w1, s, w	-1099	-1988
-556	7	y	w1, s, w	-1099	-1988
-562	13	y	w1,w2,w3,s1,s2,s3	-1098	-1989
-571	25	y*f	w1,w2,w3,s1,s2,s3	-1092	-1995
-542	9	y*f	w, s	-1065	-2022
-536	5	y	w, s	-1062	-2025
-521	13	f	w1,w2,w3,s1,s2,s3	-1016	-2071
-513	7	f	w1, s, w	-1013	-2074
-495	5	f	w, s	-979	-2108

Figure 1. Sampling regime for the four annual litter cohorts. Each cohort remained in the field for three years and was sampled at six month intervals.

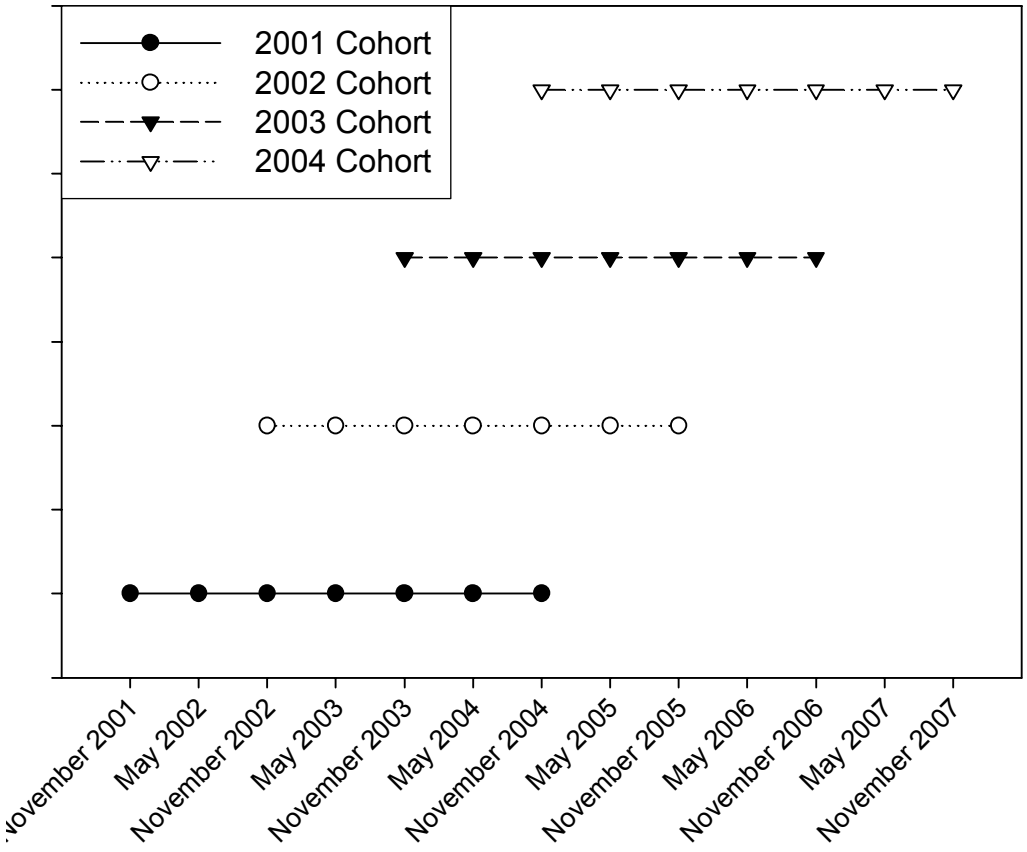


Figure 2. Environmental measurements for each month from 2001-2004. Shown are the mean of all four years±1 S.E. Air temperature was measured at the soil surface, while soil temperature and soil moisture (volumetric water content) were measured at 10 cm soil depth.

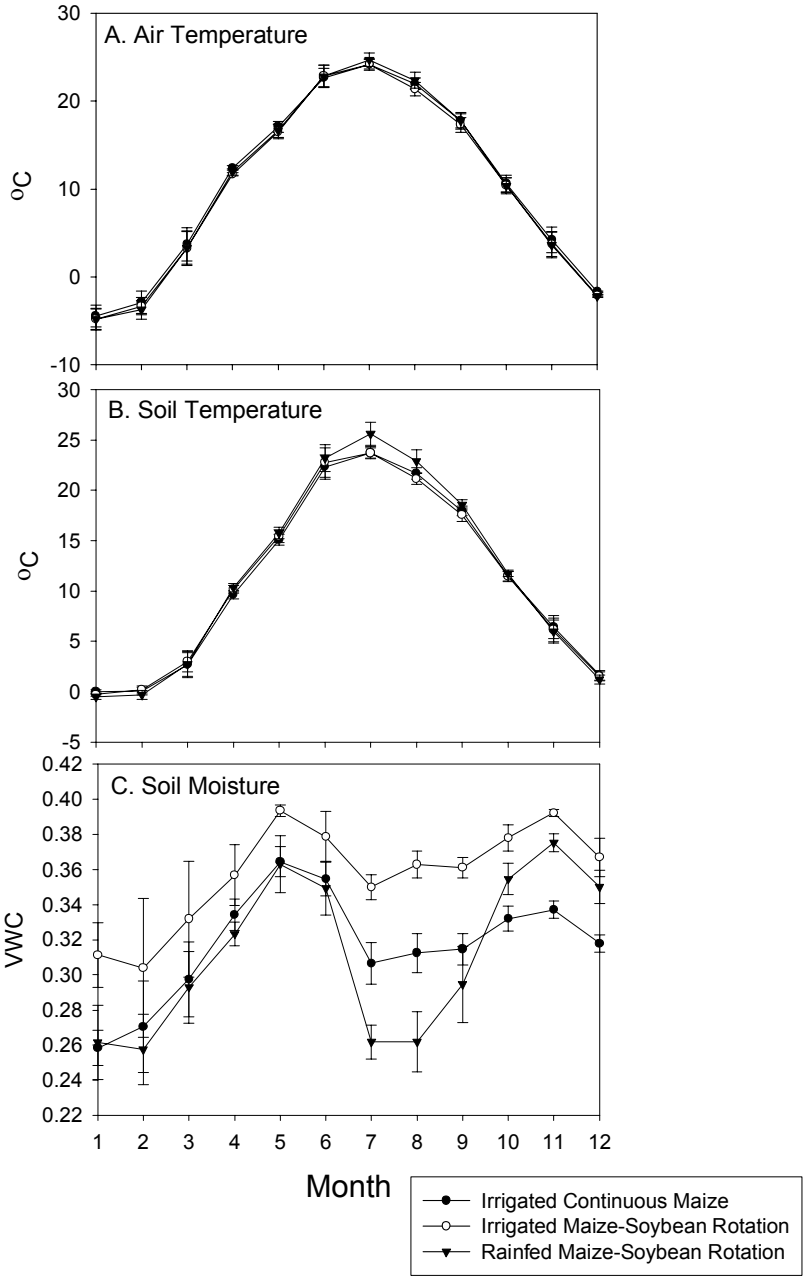


Figure 3. Total litter-C production in each management regime and litter-C production for each tissue type from 2001-2004. Letters denote significant annual differences for each litter type in each management regime and were determined with one-way ANOVA where $P<0.05$ in a LSD post-hoc comparison.

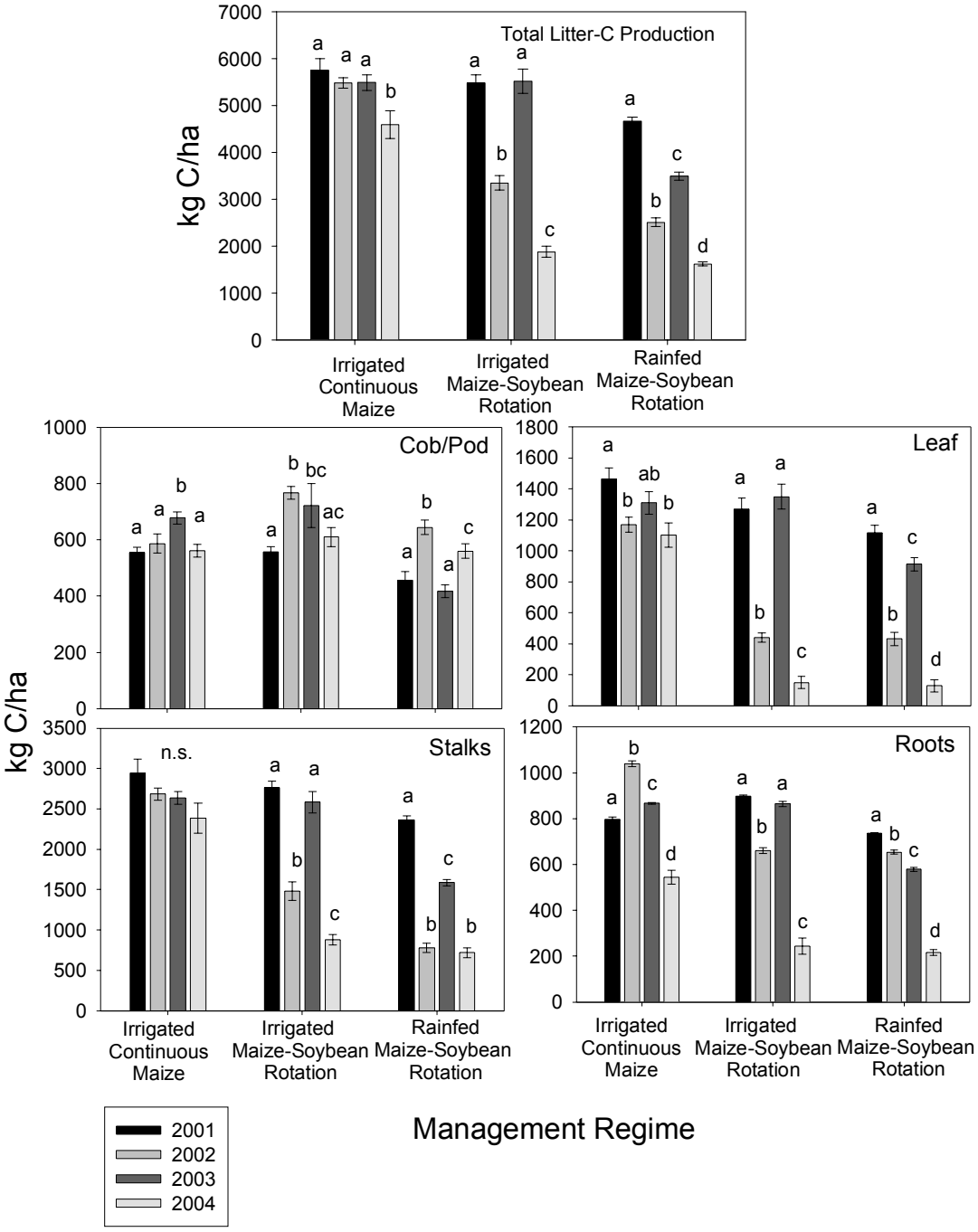


Figure 4. Litter-carbon loss in each management regime in each year. Shown are the mean±1 S.E. for each harvest. Soybean litter cohorts are denoted with a dashed line.

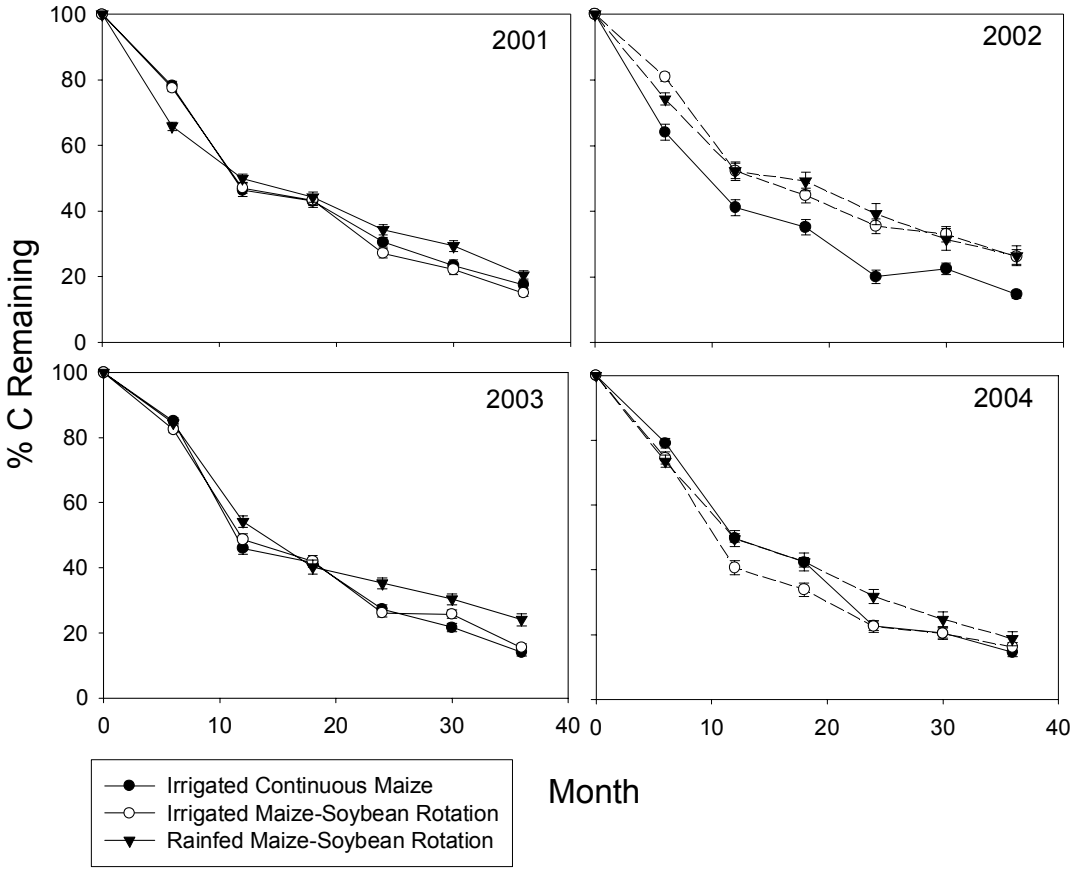
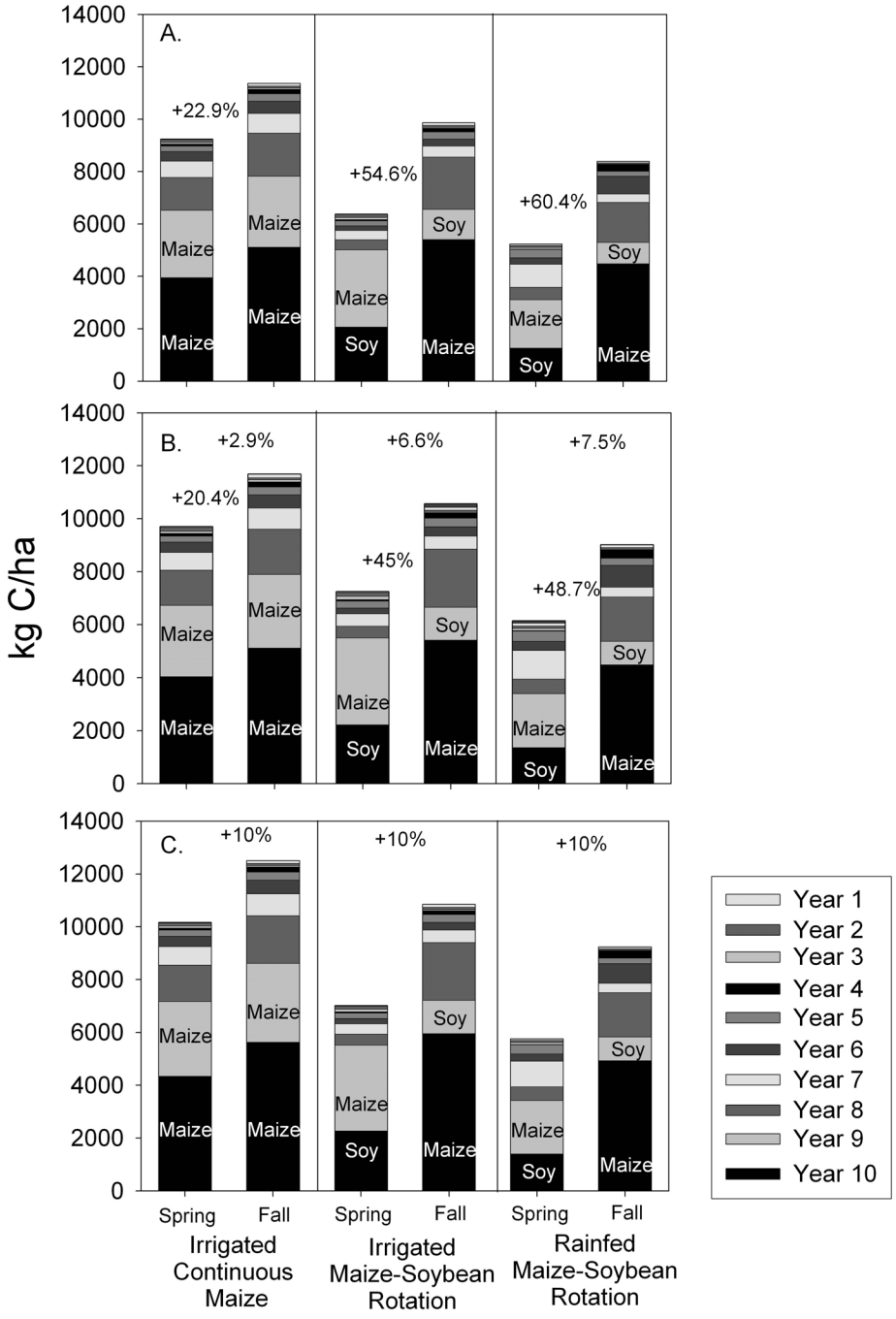


Figure 5. Litter carbon accretion in each management regime over ten years. A) litter carbon accretion with known productivity and decomposition parameters B) litter carbon accretion with a 10% decrease in decomposition rates C) litter carbon accretion with a 10% increase in productivity.



Appendix.

Environmental Factors: Monthly mean ± 1 S.E. Air temperature at the soil surface, Ts soil temperature at 10 cm soil depth, Volumetric water content at 10 cm soil depth (VWC). S.E. for VWC not shown because it was always ≤0.001.

Year	Period	Site 1: Irrigated Continuous Maize			Site 2: Irrigated Maize-Soybean Rotation			Site 3: Rainfed Maize-Soybean Rotation		
		Ta	Ts	VWC	Ta	Ts	VWC	Ta	Ts	VWC
2001	Growing Season (May-Sept)									
	May	17.6±0.45	-	0.36	-	-	-	-	-	-
	June	22.7±0.25	25.1±0.13	0.35	24.2±0.27	25.8±0.13	0.34	23.9±0.31	25.7±0.18	0.35
	July	24.6±0.15	24.5±0.05	0.34	24.9±0.17	24.1±0.05	0.37	25.8±0.19	27.3±0.07	0.27
	August	22.3±0.16	22.3±0.08	0.33	22.4±0.18	22.1±0.08	0.36	23.6±0.22	24.9±0.11	0.29
	September	16.9±0.20	17.6±0.10	0.33	17.0±0.22	17.4±0.10	0.37	17.1±0.24	18.6±0.12	0.35
2001-2002	Winter (Oct-Feb)	3.1±0.29	4.9±0.07	0.31	2.5±0.30	4.6±0.07	0.37	0.1±0.29	4.56±0.07	0.33
2002	Pre-planting (March-April)	6.3±0.34	5.1±0.09	0.33	5.7±0.33	5.3±0.10	0.36	5.8±0.32	5.2±0.10	0.31
	Growing Season (May-Sept)									
	May	16.2±0.30	14.5±0.12	0.39	15.9±0.30	14.4±0.13	0.40	16.0±0.31	14.6±0.13	0.37
	June	25.6±0.27	23.6±0.08	0.37	25.7±0.27	24.7±0.12	0.38	25.9±0.27	25.0±0.11	0.31
	July	24.8±0.18	23.9±0.05	0.30	25.3±0.21	24.9±0.08	0.34	25.6±0.26	27.4±0.09	0.23
	August	22.0±0.17	21.8±0.06	0.32	21.8±0.17	21.4±0.06	0.38	22.1±0.19	23.3±0.08	0.30
	September	18.7±0.25	18.6±0.11	0.29	18.6±0.27	18.7±0.09	0.35	18.7±0.28	19.9±0.09	0.25
2002-2003	Winter (Oct-Feb)	-0.4±0.27	2.85±0.05	0.30	-0.7±0.28	3.0±0.06	0.33	-0.9±0.28	2.8±0.06	0.32
2003	Pre-planting (March-April)	8.0±0.36	5.6±0.12	0.30	7.5±0.35	6.6±0.15	0.30	7.8±0.41	6.2±0.14	0.29
	Growing Season (May-Sept)									
	May	16.0±0.27	14.6±0.09	0.36	15.6±0.26	15.3±0.13	0.40	15.8±0.28	16.3±0.14	0.39
	June	21.5±0.27	20.0±0.12	0.37	20.9±0.26	20.6±0.12	0.41	20.9±0.26	21.9±0.14	0.39

Year	Period	Site 1: Irrigated Continuous Maize			Site 2: Irrigated Maize-Soybean Rotation			Site 3: Rainfed Maize-Soybean Rotation		
		Ta	Ts	VWC	Ta	Ts	VWC	Ta	Ts	VWC
	July	25.1±0.23	24.5±0.07	0.28	23.8±0.20	22.9±0.06	0.33	24.9±0.22	25.2±0.06	0.27
	August	23.4±0.19	22.5±0.04	0.28	22.2±0.16	21.3±0.04	0.36	23.9±0.22	23.7±0.04	0.24
	September	15.7±0.28	16.7±0.09	0.31	15.1±0.25	15.8±0.10	0.37	15.9±0.29	17.6±0.12	0.31
2003-2004	Winter (Oct-Feb)	0.6±0.27	3.7±0.04	0.30	0.29±0.26	3.4±0.05	0.36	0.12±0.27	3.4±0.05	0.33
2004	Pre-planting (March-April)	9.8±0.33	7.7±0.10	0.33	9.2±0.32	7.8±0.12	0.38	9.4±0.33	8.1±0.11	0.33
	Growing Season (May-Sept)									
	May	18.6±0.31	16.1±0.11	0.35	18.1±0.31	16.6±0.13	0.39	18.1±0.31	16.4±0.12	0.33
	June	21.0±0.26	20.6±0.08	0.33	20.8±0.26	19.9±0.09	0.39	20.7±0.25	20.1±0.09	0.35
	July	22.3±0.19	21.9±0.07	0.31	22.9±0.24	22.8±0.10	0.35	22.6±0.23	22.6±0.09	0.28
	August	20.3±0.20	20.0±0.07	0.32	19.1±0.19	19.7±0.08	0.35	19.9±0.23	19.6±0.08	0.22
	September	19.8±0.25	18.8±0.06	0.32	18.6±0.25	18.4±0.05	0.36	19.5±0.28	18.1±0.06	0.27
2004-2005	Winter (Oct-Feb)	1.6±0.27	4.4±0.05	0.32	1.13±0.28	4.7±0.05	0.34	1.0±0.27	4.0±0.06	0.32
2005	Pre-planting (March-April)	8.6±0.30	7.5±0.08	0.33	8.1±0.30	7.7±0.09	0.36	8.1±0.30	7.5±0.09	0.34
	Growing Season (May-Sept)									
	May	17.3±0.30	15.5±0.13	0.34	17.1±0.31	16.9±0.16	0.38	17.0±0.30	17.2±0.16	0.35
	June	24.5±0.26	22.5±0.11	0.32	24.0±0.23	23.6±0.12	0.33	24.2±0.24	24.2±0.14	0.30
	July	24.4±0.20	23.6±0.05	0.31	23.9±0.19	23.6±0.06	0.29	25.1±0.23	25.0±0.09	0.25
	August	22.4±0.18	21.9±0.04	0.31	21.8±0.18	21.8±0.05	0.30	22.2±0.20	23.1±0.05	0.24
	September	20.4±0.27	19.6±0.07	0.29	20.0±0.27	19.6±0.09	0.30	19.9±0.27	20.7±0.09	0.26
2005-2006	Winter (Oct-Feb)	2.6±0.28	4.4±0.08	0.25	1.8±0.31	4.4±0.07	0.32	1.4±0.30	4.1±0.08	0.32
2006	Pre-planting (March-April)	8.7±0.26	9.0±0.10	0.26	8.1±0.28	8.1±0.09	0.35	8.0±0.27	7.8±0.09	0.33
	Growing Season (May-Sept)									
	May	19.0±0.28	18.8±0.17	0.29	18.5±0.30	16.6±0.12	0.36	18.9±0.30	17.1±0.14	0.35
	June	24.6±0.29	25.7±0.10	0.19	24.6±0.34	22.5±0.08	0.31	25.2±0.34	23.9±0.09	0.32
	July	24.2±0.17	23.4±0.05	0.21	24.7±0.22	23.6±0.04	0.30	25.0±0.30	23.4±0.07	0.26
	August	22.5±0.15	22.7±0.06	0.25	21.3±0.14	22.0±0.05	0.35	25.7±0.24	24.2±0.05	0.33

Year	Period	Site 1: Irrigated Continuous Maize			Site 2: Irrigated Maize-Soybean Rotation			Site 3: Rainfed Maize-Soybean Rotation		
		Ta	Ts	VWC	Ta	Ts	VWC	Ta	Ts	VWC
	September	16.3±0.21	16.7±0.08	0.28	15.3±0.22	16.5±0.05	0.37	22.1±0.16	22.3±0.05	0.36
2006-2007	Winter (Oct-Feb)	0.4±0.28	3.1±0.09	0.22	-0.2±0.29	3.5±0.07	0.30	-0.3±0.30	2.6±0.08	0.28
2007	Pre-planting (March-April)	9.1±0.33	8.6±0.20	0.36	8.7±0.34	7.4±0.17	0.33	8.7±0.34	7.7±0.19	0.36
	Growing Season (May-Sept)									
	May	19.7±0.22	19.7±0.10	0.38	19.2±0.22	18.6±0.10	0.37	19.4±0.22	18.6±0.08	0.37
	June	22.9±0.21	22.9±0.10	0.33	22.5±0.21	22.7±0.10	0.33	23.0±0.22	23.6±0.12	0.34
	July	24.0±0.14	23.5±0.04	0.30	23.4±0.15	23.0±0.05	0.32	25.0±0.19	24.7±0.06	0.24
	August	24.3±0.15	23.8±0.05	0.37	23.7±0.14	23.4±0.05	0.37	24.7±0.17	24.1±0.05	0.33
	September	18.7±0.24	18.6±0.10	0.33	18.4±0.25	18.6±0.09	0.33	19.0±0.28	19.3±0.09	0.31

Appendix Figure 1. Litter tissue type nitrogen content. Letters denote significant field differences for each tissue type in each field for the four annual litter cohorts. Significant differences were determined with one-way ANOVA where $P<0.05$ in a LSD post-hoc comparison.

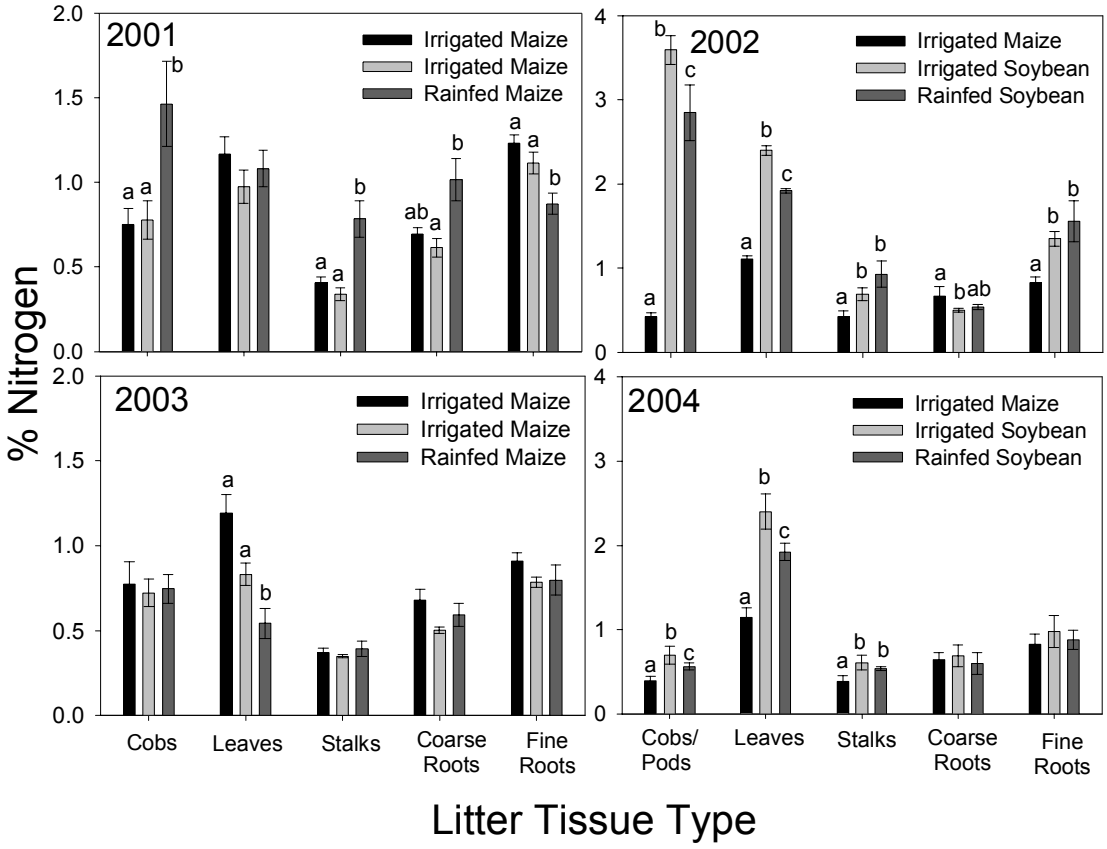


Figure 2. Maize litter carbon quality i.e. percent soluble, hemicellulose, cellulose and lignin in each tissue type for each annual litter cohort. All tissue types were pooled to determine mean carbon quality for the entire field.

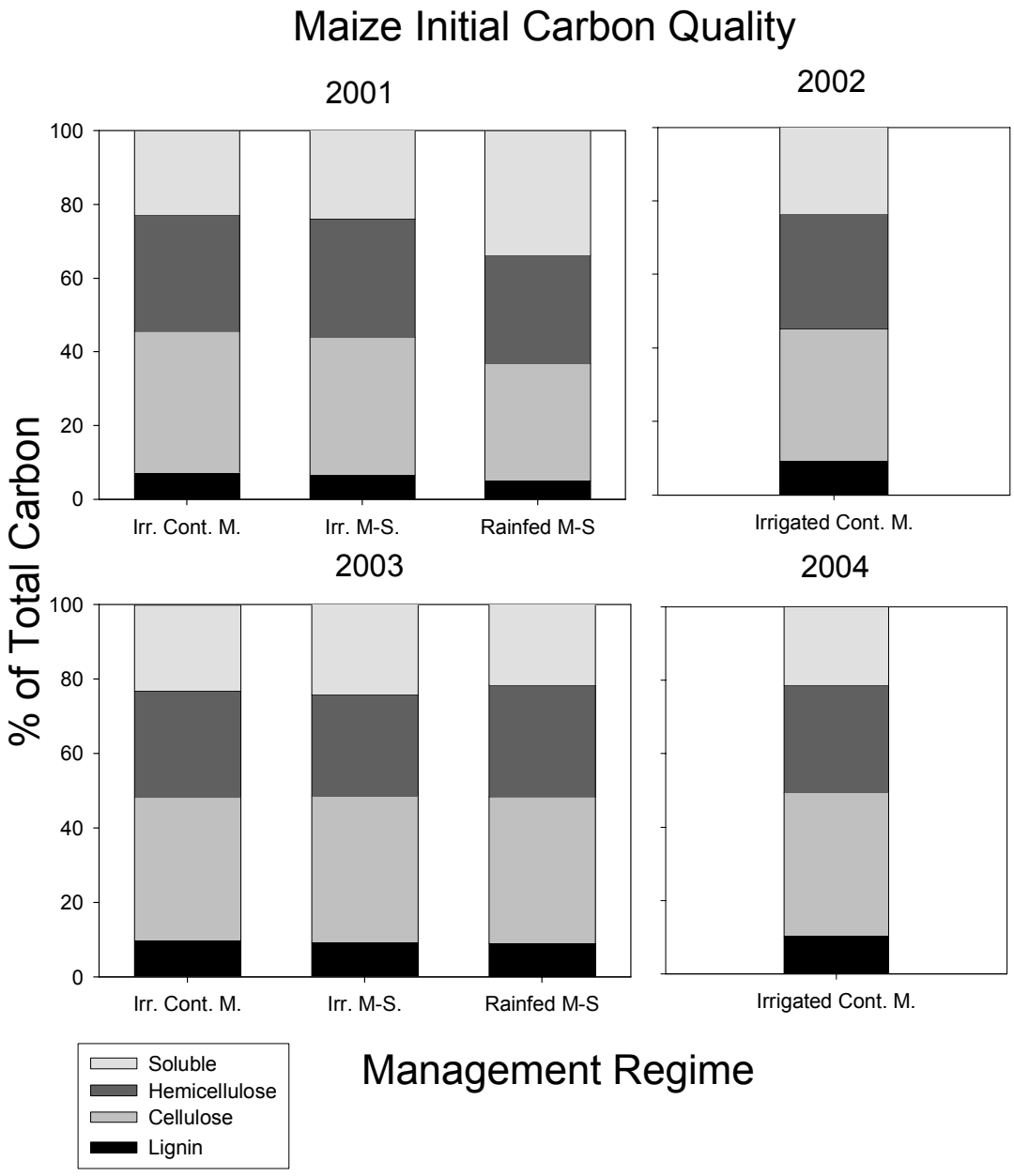
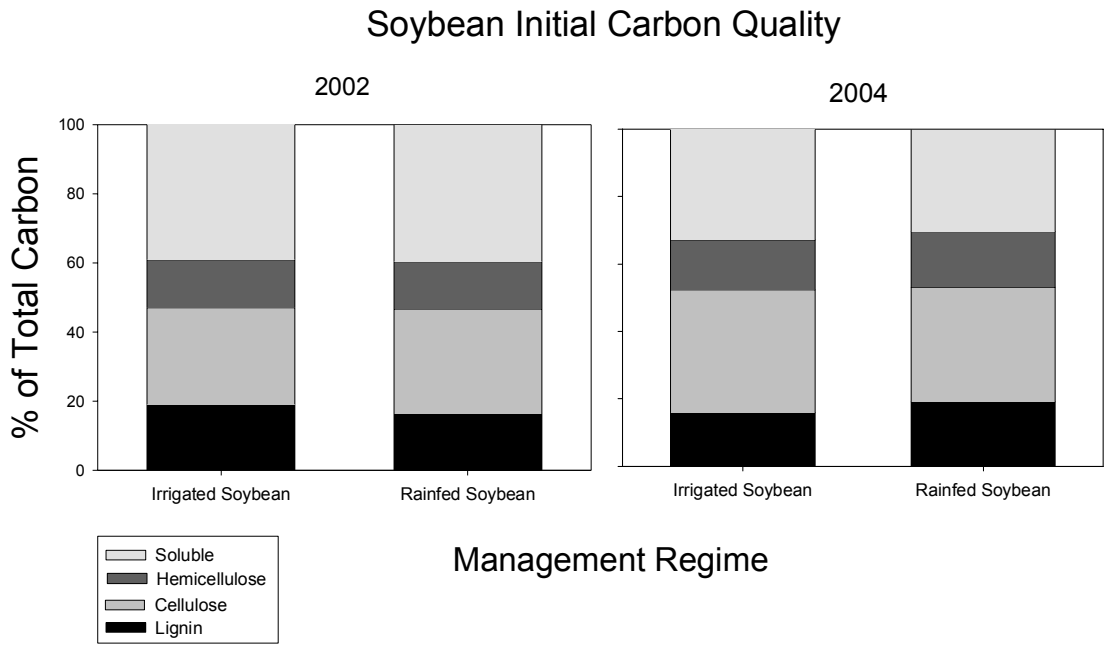


Figure 3. Soybean litter carbon quality i.e. percent soluble, hemicellulose, cellulose and lignin in each tissue type for each annual litter cohort. All tissue types were pooled to determine mean carbon quality for the entire field.



Chapter 4

Inorganic-N addition effects on litter and soil organic matter decomposition

Amy E. Kochsiek and Johannes M.H. Knops

ABSTRACT- Most work that addresses the effects of inorganic nitrogen (N) addition on carbon (C) cycling has been conducted in forested and other natural ecosystems. This body of research on the relationship of inorganic nitrogen and carbon in natural systems generally has concluded that decomposers are primarily limited by the availability of carbon (energy). However, this relationship may not translate to agroecosystems, because large inputs of monospecific litter with high C/N ratio and low lignin content potentially causes nitrogen limitation to have a stronger impact on decomposers. Therefore, we hypothesized that: 1) the microbial community accesses N from the soil organic matter (SOM)-N pool, in order to metabolize litter-C for energy; and 2) the addition of an easily usable N source such as an inorganic-N fertilizer, will reduce the need for SOM-N and lead to faster litter decomposition and decreased SOM decomposition by the soil microbial community. Further, while the C/N ratio of litter can be used as a predictor of litter decomposition rates, we have very little knowledge of how the C/N ratio of litter could affect SOM-C decomposition. Thus, we set up a 3 x 2 factorial laboratory incubation experiment with soil and no litter addition, soil and maize leaf litter (C/N~40), and soil and maize stalk litter (C/N~102) with two levels of NH_4NO_3 fertilization (0 g/m^2 and 5 g/m^2). The soil used in the experimental incubations had been consistently cropped with wheat for 30+ years, allowing us to differentiate between litter (C4 $\delta^{13}C$ signature) and SOM-C (C3 $\delta^{13}C$ signature) decomposition. We incubated these

samples in the dark for 120 days at 25°C and measured CO_2 evolved and $\delta^{13}C$ signature of evolved CO_2 over time. We also monitored decomposition of fertilized (5 g N/m^2 urea ammonium nitrate) and unfertilized leaf and stalk tissue placed at the surface and buried at 10 cm soil depth for one year. We found no impact of inorganic N addition on litter decomposition in the laboratory or field, nor did we find an impact of inorganic N addition on the decomposition of soil organic matter. However, we did find that the addition of litter decreased the total amount of soil decomposed and could potentially lead to a net C gain in soils. Therefore, while the decomposition process is difficult to manipulate with inorganic N additions, at least at this low level of addition, more studies need to simultaneously monitor litter decomposition and soil organic matter decomposition to determine the ability of a system to sequester C.

Introduction

Human activities drive many of the environmental changes that we see today. For example, industrial nitrogen (N) fixation for the production of N fertilizers and increased atmospheric N deposition has led to increased N availability in most ecosystems, while fossil fuel burning and land clearance has led to increased atmospheric CO_2 (Keeling, 1993; Keeling et al., 1989; Vitousek, 1992). The conversion of millions of acres of natural land to agricultural systems has resulted in massive losses of soil carbon (C), exacerbating the already increasing atmospheric CO_2 concentration. Presently, in the U.S alone, 340 million acres of the total land area are devoted to crop production and globally, agroecosystems comprise 34 % of the earth's terrestrial land area (Cassman et al., 2003; Lubowski et al., 2006). Over the last 60 years, carbon inputs to these systems have been increased through crop management techniques, such as irrigation and fertilization while concurrently reducing soil-C losses to the atmosphere through conservation or no-till practices (Allmaras et al., 2000; Cassman et al., 2003; Lal et al., 1999). While most of the original soil-C lost with the initial conversion has yet to be regained, these practices have prevented further loss of soil-C. The combination of large land area, fertile soils, and increased productivity with irrigation and fertilization, as well as the potential for increasing soil carbon content suggests that agroecosystems are probable sites for carbon sequestration (Alvarez, 2005; Follett, 2001; Sauerbeck, 2001).

Agroecosystems, like natural ecosystems, have two large pools of C post-harvest: 1) soil-C and 2) litter-C. The litter-C pool is divided between above and belowground litter, and is largely untouched in no-till systems. Post-harvest the litter pool can represent 20-23% of ecosystem C and is a highly dynamic ephemeral pool of C (Chapter

3). While successful techniques for managing agricultural systems for increased crop productivity and yield are well known, management of the decomposition process in order to increase sequestration of litter-C into soil organic matter carbon (SOM-C) has received less attention. Furthermore, since most ecosystems are experiencing increased N availability because of increased atmospheric N deposition (Reay et al., 2008), questions regarding the relationship between litter-C decomposition, sequestration of litter-C as SOM-C, and increased N availability also must be addressed.

Most work examining the effects of inorganic nitrogen (N) addition on litter and soil organic matter decomposition has been done in forested and other natural ecosystems (Fog, 1988; Hobbie, 2005; Knorr et al., 2005; Pregitzer et al., 2004; Waldrop et al., 2004; Xu et al., 2004). While some studies show that inorganic N addition can increase litter decomposition rates (Carreiro et al., 2000; Green et al., 1995; Henriksen and Breland, 1999; Hobbie, 2005; Hunt et al., 1988), others show no effect (Biederbeck et al., 1996; Carreiro et al., 2000; Hobbie, 2005; McClaugherty and Berg, 1987) or even a decrease in litter decomposition rates (Carreiro et al., 2000; Knorr et al., 2005). A meta-analysis of N fertilization effects on litter decomposition by Knorr et al (2005) showed that the differential impact of N addition on litter decay could be explained by litter tissue quality, fertilization rate, and length of the experiment. They found that N addition stimulated decomposition of litter with low lignin concentrations and inhibited the decomposition of high lignin litters. This finding is supported by other studies in hardwood forests, which showed an increase in cellulase activity and a concurrent decrease in lignolytic enzyme activity with N addition (Carreiro et al., 2000; Gallo et al., 2004). Maize-based agroecosystems are characterized by high exogenous inorganic N inputs from fertilizer

and large inputs of monospecific litter with high C/N ratio and low lignin content. Litter inputs, such as these, create a nitrogen limiting environment for decomposers, as compared with previous work on the relationship of inorganic nitrogen and carbon in natural systems where decomposers are primarily limited by carbon (energy). This shift from carbon to nutrient limitation may cause nitrogen availability to exert more control over decomposers in agroecosystems than carbon availability. Nutrient limitation of the decomposers in agroecosystems makes the impact of inorganic nitrogen additions potentially important for carbon dynamics in these systems. How inorganic N can affect the decomposition and the stabilization of litter-C in stable SOM is a vital question in the determination of sinks and sources of C. Accurate predictions of C sink or source strength of ecosystems are necessary to predict future changes in the atmospheric CO_2 concentration.

In order to attain long-term carbon sequestration, litter-C must be physically and chemically protected as SOM-C. Therefore, understanding the decomposition patterns and the ultimate fate of litter-C is necessary in order to determine how long an ecosystem can retain C. The C balance of any ecosystem is the difference between C inputs (primary productivity) and C losses (decomposition of litter-C and SOM-C). Studies have shown that an increase in available N due to increased N deposition leads to short-term increases in plant productivity and litter inputs (Bassin et al., 2007; Clark et al., 2007; Knops et al., 2007). However, merely increasing litter-C inputs through enhanced productivity may not be enough to increase litter-C sequestration, if increases in productivity are offset by concurrent increases in decomposition. While the effects of inorganic-N additions on decomposition are often inconsistent (Carreiro et al., 2000;

Green et al., 1995; Henriksen and Breland, 1999; Hunt et al., 1988; Knorr et al., 2005), even less is known about how inorganic N additions affect SOM decomposition or the stabilization of litter-C as SOM-C. In addition, there are interactions between litter decomposition and SOM decomposition as the application of litter can prime the microbial breakdown of stable SOM (Fontaine et al., 2004; Kuzyakov et al., 2000). These "priming effects" could lead to no net C gain or even net C loss if increased litter C inputs lead to increased SOM-C decomposition. Further, while we know C/N ratio of litter can be used as a predictor of litter decomposition rates (Aerts, 1997), we have very little knowledge of how C/N ratio of litter could affect litter-C stabilization as SOM-C and SOM decomposition. As such, we investigated the impact of inorganic N addition on the decomposition of litter with different C/N ratios *in situ* and in a laboratory incubation experiment. Further, in the laboratory incubation experiment we also examined the decomposition of SOM, and the stabilization of litter-C in SOM with the addition of inorganic N addition to litter with different C/N ratios.

Because maize litter and soil have C/N ratios of 40 and 10, respectively, because microbes need both C and N, we hypothesized that the microbial community accesses N from SOM-N pool, in order to utilize litter-C for energy. The addition of an easily usable N source such as an inorganic-N fertilizer will supplement microbial demand for N thereby reducing the need for SOM-N. The result is faster litter decomposition and increased stabilization of litter-C in SOM by increasing soil microbial biomass. The soil used in this study had been consistently cropped with wheat for 30+ years. By using this soil we could differentiate between microbial decomposition of litter (C4 $\delta^{13}C$ signature)

and SOM-C (C3 $\delta^{13}C$ signature) while simultaneously monitoring total litter and soil organic matter pools.

Materials and Methods

Laboratory methods

Soil was sampled at the High Plains Agricultural Laboratory in Sidney, Nebraska in a site consistently cropped with wheat for over 30 years. The soil type at this site is categorized as Pachic Haplustoll with a soil texture of 25 % clay, 35 % silt and 40 % sand (Lyon et al., 1997). Ten random soil samples were taken at 0-10 cm depth in plots that had received tillage. Soil was brought back to the lab and stored at 4ºC until use.

Maize litter was harvested from Mead, Nebraska in a no-till irrigated continuous maize field at the end of the growing season just before harvest. Litter was separated into leaf and stalk material, dried to a constant mass at 70ºC, and ground in a Wiley mini-mill with a 40 mesh (2 mm) screen (Thomas Scientific, Swedesboro, NJ). After grinding, leaf and stalk litter was analyzed for total C and N in a Costech 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA.). We also estimated initial carbon quality with the Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, NY), which is a common technique used to determine forage digestibility (Goering and Van Soest, 1970; Van Soest et al., 1991). This technique uses a sequential extraction to determine the amount of soluble, hemicellulose, cellulose and lignin fractions within each sample. These classifications do not represent strictly identical chemical compounds, but rather groups of similar compounds with similar resistance to decomposition. The data for tissue fractions analysis are presented as the four fractions (soluble, hemicellulose,

cellulose and lignin) totaling 100% of the plant tissue carbon quality. Therefore, any increase in one fraction leads to an equivalent decrease in the other fractions. Maize leaf (C/N~40) and stalk litter (C/N~102) were used because they have similar tissue fractions, but significantly different initial C/N (Table 1).

Soil was homogenized, sieved through a 2 mm mesh, and larger organic fragments such as root and litter were removed by hand. The experimental soil was amended with two factors, litter and N addition, with six experimental treatments: 1) No litter (soil alone), 2) No litter with N addition, 3) Leaf litter 4) Leaf litter + N addition 5) Stalk litter 6) Stalk litter + N addition. Each experimental unit (1 specimen cup) received 40 g of soil. Each treatment was replicated eight times for a total of 48 samples. All treatments with litter additions received 0.2805±0.0002 g C which was equivalent to 375 g C/m^2 annual aboveground productivity of leaves and stems combined. Ground litter was mixed with the soil to facilitate more rapid decomposition and treatment effects due to limited incubation time. Each N addition treatment received 3.7 mg $NH_4^+NO_3^-$ per 40 g soil which is equivalent to a fertilization rate of 5 g N/ m^2. Each experimental unit was set to a bulk-density of 1 g/cm^3 and 60% water-filled pore space and maintained throughout the experiment. All experimental units were incubated in the dark for 120 days at 25ºC. Incubation time of 120 days at 25ºC was equivalent to approximately two thermal years and was chosen so as to allow enough time for adequate decomposition of litter.

Each experimental unit remained open to the atmosphere during the incubation except during sampling periods to avoid O_2 limitation. CO_2 emissions were sampled (n= 6 per treatment) on days 1, 5, 10, 15, 20, 35, 50, 75, 90, and 120 days. During sampling,

the experimental units were enclosed in jars, and CO_2 was cleared from the each jar by pumping CO_2 free air through the jar. Twenty-four hours after clearing the jars and sealing the experimental units in the incubation jars, headspace was sampled and the CO_2 concentration measured on a Shimadzu gas chromatograph-17A (version 3) with an electron capture detector and a Porapak Q column. $\delta^{13}C$ of the headspace samples was also taken on days 5, 15, 35, 75 and 120 by sampling 12 ml of headspace gas and transferring it to an evacuated exetainer (LABCO, UK) and analyzed at the UC Davis Stable Isotope Facility using a SerCon Cryoprep TGII trace gas concentration system interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

In all treatment combinations we decomposed C_4 plant material on C_3 soil. Because C_3 and C_4 plants differ in discrimination of $^{13}C/^{12}C$, the soil carbon will have a more negative $\delta^{13}C$ than the plant material (Table 1). By using the differentiation between the two signatures we could determine the amount of CO_2 respired carbon that had originated from soil organic matter and from litter decomposition.

At the end of the 120 day experiment, soil was physically fractionated (n= 8 per treatment) into four aggregate size classes: >2000μm, 250-2000μm, 53-250μm, and <53μm. Each sample was immersed in room temperature water for five minutes on the largest sieve. The sieve was then moved up and down three cm for two minutes, poured into the next smaller sieve, and repeated (Denef and Six, 2005; Elliott, 1986). Each size class was separated, dried to a constant mass at 70ºC, weighed, and analyzed for total C and N, organic C, and $\delta^{13}C$ (n=8 for each fraction in each treatment; n=192 total). Total C and N as well as organic C were measured at the Ecosystem Analysis Laboratory in Lincoln,

NE on a Costech 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA.). Organic C for each fraction was determined using a 1 M H_3PO_4 digest to remove soil inorganic C and then organic C was determined on a Costech 4010 elemental analyzer. Typically, HCL is used to remove soil inorganic C, but this interferes with C analysis on the Costech so we modified the method to use H_3PO_4. $\delta^{13}C$ was determined at the UC Davis Stable Isotope Facility with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Field Methods

In the fall of 2006, stalk and leaf litter was collected from maize plants in an irrigated

agricultural field at the University of Nebraska Agricultural Research and Development Center near Mead, NE. Six mature plants were harvested by hand near areas designated as intensive measurement zones (IMZs) just before grain harvest in October of 2006. The IMZ's were designated sampling areas within this field. Before the initiation of the study, IMZ locations were selected by using a fuzzy-k mean clustering technique, which classified this field into six categories based on elevation, soil type, soil electrical conductivity, soil organic matter content, near infrared remotely-sensed imagery and digital aerial photographs (Dobermann and Ping, 2004; Minasny and McBratney, 2003). Once the field was separated into the six different fuzzy class environmental categories, the exact location of the IMZ was placed randomly within each category area for a total of six IMZs for the field. The purpose of classifying this field into six IMZs was to capture landscape-level spatial variability so that the measurements could be scaled up to

the entire field. This approach allowed us to quantify the natural variability within the field to gain an estimate of the maximum variability of our measured variables within a biological/agricultural relevant field scale (Minasny and McBratney, 2003). The aboveground portion of the each plant sampled was separated into leaves and stalks and dried to constant weight at 75°C. A subsample of the dried litter was ground and analyzed for total C and N content on a Costech 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA.). A 32% solution of urea ammonium nitrate was applied to half of the dried stalk and leaf litter at a rate of 5 g N/m^2 yr. Sixteen replicate litter bags were prepared for both fertilized and unfertilized stalk and leaf litter for a total of 64 litter bags per IMZ. Each litter bag was 20 cm x 20 cm with a mesh size of 1 mm and 5-10 g of plant tissue was packed per litter bag (Burgess et al., 2002). These 64 litter bags per IMZ were placed near the IMZ locations within the irrigated continuous maize field where litter was originally harvested for a total 384 litter bags in the field. Half of the litter bags for fertilized and unfertilized leaf and stalk litter were placed on the soil surface (n=8), while the other half were buried at 10 cm soil depth (n= 8). We then harvested half of the litter bags for each depth, litter type, and fertilization at six and twelve month intervals (n= 4 per type at each harvest, n=24 for the entire field for each type and each harvest). After the bags were harvested they were dried to a constant weight at 75°C, weighed, ground in a Wiley mini-mill with a 40 mesh (2 mm) screen (Thomas Scientific, Swedesboro, NJ), and analyzed for total C/N on a Costech 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA.). After total C/N analysis, ash content was determined by burning a sample at 475°C in a muffle furnace and used to correct mass loss data for ash content.

Statistical Analysis

All statistics were performed using SPSS v.17. Cumulative respiration rates were determined by fitting linear regressions to the first 10 days of respiration rates and subsequently to 3 parameter exponential decay functions for days 10-120. We then used these fitted lines to determine the amount of CO_2-C respired for every day of the experiment. These amounts were then summed for the 120-day experiment to determine cumulative amounts of CO_2-C respired. These cumulative differences in total (litter and SOM), SOM, and litter CO_2-C respired were determined using two-way ANOVA with addition (no addition, leaf, or stalk litter) and nitrogen (0 or 5 g N/m^2) as the main effects. The effects of day, addition and nitrogen on CO_2-C respired for each sampling date over the entire 120 day experiment were tested using repeated measures ANOVA. To determine the amount of litter and SOM decomposition we used the $\delta^{13}C$ of the CO_2-C respired (n=6 per treatment per sampling day) and assumed that there was preferential litter decomposition. Thus, any deviation of the $\delta^{13}C$ signature from the litter signature was attributed to soil organic matter decomposition. We could then calculate the percent of the CO_2-C respired in each sample that originated from soil organic matter decomposition. By multiplying the total CO_2-C respired on each sampling day by the percent of litter and soil respiration determined from the $\delta^{13}C$ measurements, we could determine the amount of CO_2-C respired from soil and litter, respectively.

To determine the effect of inorganic nitrogen addition on total SOM and litter respiration rates, we subtracted the unfertilized treatments from the fertilized treatment at each sampling day (n= 6 per treatment). Non-overlapping 95% confidence intervals in a repeated measures analysis of variance (ANOVA) with day and treatment as the main

effects were used to determine significant differences from zero. If the assumption of sphericity was violated in any of the repeated measures ANOVAs performed, we used the greenhouse-geisser correction. This test modification applied a correction factor to the degrees of freedom making the F-ratio more conservative. This correction never changed the overall significance of the test.

Differences in the percent of total mass, amount of organic-C and $\delta^{13}C$ for each soil fraction were analyzed using one way ANOVAs with treatment as the main effect. LN transformation was used to improve normality for the impact of nitrogen additions on soil organic-C in each fraction. Within each soil fraction, we used a two-way ANOVA with addition and nitrogen as the main effects to determine significant differences in both amount of organic-C and $\delta^{13}C$.

Litter decomposition *in situ* was determined after six and twelve months of decomposition. The effect of harvest, litter placement (depth), tissue type (stalks or leaves) and fertilization (0 or 5 g N/m^2) were determined using a four-way ANOVA. When harvest proved highly significant we analyzed both harvests separately with three-way ANOVA with litter placement, tissue type, and fertilization as the main effects.

We also calculated the amount of litter C and N remaining by determining the percent litter –C remaining after 12 months of field decomposition and the C/N ratio of the litter to determine litter-N remaining. We also used the total cumulative amount of soil decomposed over the 120 day incubation and the C/N ratio of the soil to determine that amount of C and N lost.

Results

Incubation Litter Decomposition

Overall, the addition of litter increased the total cumulative CO_2-C respired but nitrogen additions had no significant effect on total CO_2-C respired (Figure 1A; Table 2). In fact, when litter respiration is separated from total respiration, nitrogen addition has a significant negative effect on stalk litter decomposition and a slight trend for decreased decomposition of litter in the leaf treatment (Figure 1C). Between days 5-10 rates of CO_2-C respired were highest for all treatments (Figure 2A). All litter addition treatments regardless of C/N ratio or nitrogen addition were higher than the soil treatments for the first 75 days of the experiment. By 90 days, all treatments were respiring at the same rate. We found that litter addition, regardless of C/N ratio, caused a significant increase in CO_2-C respired (Figure 1A), and that by the end of the incubation experiment the high C/N ratio stalk litter decomposed more than the low C/N leaf litter, regardless of nitrogen addition (Figure 1C; Table 2C). Nitrogen addition significantly increased CO_2-C respired for the stalk treatments early in the experiment, but then after day 20 there was a trend for decreased CO_2-C respired in the nitrogen addition treatment versus the unfertilized treatment (Figure 3B). Over the course of the 120 day experiment, there were only a few instances where the difference between the fertilized and unfertilized leaf and soil treatments were significantly different than zero.

In the field, we saw that the low C/N ratio leaf litter decomposed significantly faster than the high C/N ratio stalk litter and that litter burial increased decomposition anywhere between 5-30% (Figure 4; Table 4). Yet, nitrogen had no effect on litter decomposition in the field at any harvest time, litter type, or litter placement.

Soil organic matter decomposition

We found that the addition of litter, whether stalk or leaf, decreased the total amount of soil decomposition, but that nitrogen had no significant effect on SOM decomposition for any treatment (Figure 1B; Table 2B & 3B). Nitrogen addition had no significant effect on the amount of SOM decomposition for any addition treatment. For high C/N ratio stalk tissue, fertilization only significantly increased SOM decomposition on day 5 and day 35, but this was not enough to lead to cumulative increases in soil decomposition (Figure 3C; Table3B). When testing the main effect of day on the impact of nitrogen addition on SOM decomposition, the assumption of sphericity was violated in the repeated-measures ANOVA. Thus, we used the greenhouse-geisser correction, which applied a correction factor to the degrees of freedom making the F-ratio more conservative. This correction did not change the overall significance of the test. The significant addition differences were driven by the differences between the stalk and soil treatments in the first 35 days as fertilization increased soil decomposition in the stalk treatment and decreased soil decomposition in the soil only treatment.

When we calculated the litter C and N remaining at the end of the 120 day experiment using the SOM-C and SOM-N lost, we saw that there is potential for increasing C content in the SOM pool for all of the litter treatments except for the stalk treatment which was C neutral (Figure 6). SOM-N decreased in all treatments regardless of litter addition or C/N ratio of litter additions.

Soil Fractionation

There were no differences between the percent of each sample in each size class regardless of litter or nitrogen additions (Table 6). We presented the data in this way, because each treatment had a different total mass. Each experimental unit originally received 40 g of soil, and the litter additions were scaled by %C. Therefore, the total mass of each treatment depended upon the type of litter added.

Generally, the amount of organic-C in each fraction was variable and was more affected by litter additions than by nitrogen additions (Table 5 & 6). We did see significantly lower $\delta^{13}C$ values for all of the litter treatments compared to the soil treatments for all fractions, but there was no difference between the high and low C/N ratio litter. There was also a small trend for increased organic-C in the litter additions as compared to the no litter treatment, but this was not consistent across soil aggregate size classes (Table 5 & 6). Furthermore, there was no effect of nitrogen addition on the $\delta^{13}C$ values or organic-C incorporated into each fraction. In the 250-2000μm fraction the leaf +N, stalk, and stalk+N treatments had significantly more organic-C than the other treatments, but this pattern did not hold for the other fractions. Within each litter treatment, nitrogen only significantly increased organic-C in the 250-2000μm fraction for leaf litter (Figure 4). Nitrogen significantly decreased organic-C in the 50-250μm fraction in both the soil and leaf treatments. Overall, nitrogen had very little effect on incorporation of organic-C in any of the litter types or the soil fractions.

Discussion

Litter Decomposition

Inorganic-N addition to litter has highly variable effects on litter decomposition rates (Knorr et al., 2005) with some studies showing that inorganic N addition can increase litter decomposition rates (Carreiro et al., 2000; Green et al., 1995; Henriksen and Breland, 1999; Hobbie, 2005; Hunt et al., 1988) while others others show no effect (Biederbeck et al., 1996; Carreiro et al., 2000; Hobbie, 2005; McClaugherty and Berg, 1987) or even a decrease in litter decomposition rates (Carreiro et al., 2000; Knorr et al., 2005). We found that, at least in the short-term (1-10 days), nitrogen additions increased litter decomposition in all of the litter addition treatments (Figure 1A). The high C/N ratio stalk litter saw a more sustained increase in litter decomposition in the fertilized treatment for the first 20 days. Fertilization had a slight negative impact on decomposition in the soil only treatment in the first 20 days, but after that seemed to have no effect (Figure 1B). Since the addition of nitrogen was a single pulse addition at the beginning of the experiment, it is not surprising that we see fairly short-term, ephemeral effects of the added N. We also saw that the lower C/N ratio leaf tissue decomposed at a higher rate than the higher C/N ratio stalk litter for the first 15 days. After that period of time, stalk litter then decomposed at a higher rate until day 90. This result might be due to both the lower C/N ratio of the leaf tissue and the fact that the leaf tissue had more easily usable portions, which were rapidly broken down by the soil microbial community, leaving the more recalcitrant portions that were harder to decompose. This would directly lead to the lower respiration rates we saw after day 15. Due to the fact that the sieved soil was taken from a 4ºC cold room, and the litter applications were applied to the

chilled soil, we did not start the first CO_2 measurements for five days to allow for the soil to equilibrate. Thus, we may have missed some portion of CO_2-C respired and therefore, our estimates of litter decomposition are conservative. Generally, we saw no effect of N addition in the total (litter + soil) amount of decomposition, and N addition decreased litter decomposition in the high C/N ratio stalk litter (Figure 1). Nor did we see evidence for N addition effects on decomposition in situ (Figure 4; Table 4). This is directly contrary to our prediction that increased inorganic N would alleviate N limitation of the soil decomposer community leading to increased litter-C decomposition.

Stalk litter decomposed significantly faster than leaf litter in the laboratory incubation, which was the opposite of the field decomposition study. These contradictory results may occur because the litter for the laboratory incubation was ground and incorporated into the soil, while in the field, the natural structure of the litter was maintained, and whole tissue was placed in litter bags and put into the field. The litter was ground in the laboratory incubation so as to increase the availability of the litter in order to see potential treatment effects over the 120-day period. These contrasting results point to two possible conclusions. Firstly, in the field, where the litter was not ground, stalk tissue does not fragment as easily as leaf tissue and maintains its shape for much longer periods of time. This may make microbial colonization of intact stalk litter more difficult as compared to leaf litter, which fragments more easily in the field, leading to decreased stalk decomposition rates (Burgess et al., 2002). Stalk litter tends to have a tough outer sheath around the stalk, whereas the interior, where the main nutrient and water transport take place, was much more porous and spongy. After one year of decomposition was complete, we would still find the outer portion of the stalk remaining

in the bag, but the inner portion was completely degraded (personal observation). In the incubation experiment, by grinding the stalk litter and incorporating it into the soil, we were allowing for direct decomposer access to the more easily usable portions of the stalk litter that could be degraded rapidly and that in the field would have been protected by the tough outer tissue. Our initial tissue quality analysis also showed that stalk litter has higher soluble and lower hemicellulose concentrations than leaf litter which could lead to increased decomposition when ground (Table 1). Our results are in concordance with another recent incubation study, where stalk and leaf maize litter were ground. They also showed that stalk litter had more sugar concentration and less hemicellulose which lead to more % C remaining in leaf litter than stalk litter (Johnson et al., 2007). The disruption of litter structure by grinding and the differences in quality may be why we saw stalk litter decomposing more rapidly than leaf litter in this incubation experiment, and leads us to conclude that litter structure and other measures of litter quality such as soluble and hemicellulose fractions may govern decomposition patterns more than C/N ratio.

During the first six months of decomposition during the winter period, surface applied leaf litter lost approximately 25% of its carbon and belowground it lost 50%. Stalk litter saw less of a difference between surface and buried litter, but still lost 18% and 20% respectively. Other studies have found similar winter decomposition rates, and due to the low temperatures during this time, point to the importance of physical processes on decomposition at these times. Physical processes, such as fragmentation of the litter due to interception of precipitation, compressive forces for buried litter from overlaid soil, and freeze-thaw dynamics can all lead to break down of litter (Burgess et

al., 2002; Ghidey and Alberts, 1993; Gregorich and Ellert, 1994; Parker, 1962). So while microbial activity is, of course, essential for the decomposition process, and decomposition has been shown to occur at temperatures around 0ºC (Stott et al., 1986) early stages of field decomposition, particularly during the winter months, can be driven by the fragility of litter structure.

Soil organic matter decomposition

Overall, we saw that nitrogen additions had very little effect on SOM decomposition, except for a trend in increased soil decomposition in the fertilized stalk treatment. Our study is in agreement with a study in a rice system that also found no difference in soil-C respired in their fertilized vs. unfertilized addition treatments at any time throughout their experiment (Moran et al. 2005).

While it seems that the addition of inorganic-N additions did not impact SOM decomposition, we did find that the addition of litter decreased SOM decomposition. This is suggestive of a shift in the soil microbial community composition and/or functioning with the addition of litter from a community that is degrading soil to one that is now primarily degrading litter and reducing the total amount of soil being decomposed. Studies have shown that the presence and quality of litter additions can impact decomposer diversity as well as enzyme diversity (Bending et al., 2002; Dilly et al., 2004; Dilly and Munch, 2004; McMahon et al., 2005). While we did not directly measure soil microbial community composition or functioning, this study does show that the application of litter can decrease the amount of soil decomposition in a system. This pattern may be ecosystem specific and only occur in maize systems where there are large

inputs of litter that has very low lignin content. Relatively easy litter to degrade could stimulate a suite of decomposers that create enzymes that specialize in cleaving bonds commonly found in plant litter, which are not as complex or difficult to break as those in SOM. . Thus litter in natural systems with higher lignin content might also stimulate decomposers that can break more difficult humic bonds, like those found in SOM, and in turn may not result in decreased SOM decomposition. It is clear from our work that the stimulation of a certain suite of microbes through the addition of litter can have a direct impact on SOM decomposition.

We did find evidence for the potential to increase soil C but not soil N, when we calculated the litter C and N remaining at the end of the 120 day experiment with the soil C and N lost (Figure 6). This is dependent upon the amount of litter C remaining in the treatments, and since we found that the stalk litter decomposed more than the leaf litter, this resulted in more leaf litter C remaining and a higher net C gain in soil with leaf additions. At least under these ideal incubation conditions, our data do show the potential for C sequestration in soils through a combination of decreased soil decomposition and input of the recalcitrant portion of litter.

Soil Fractionation

Generally, we found that nitrogen additions had very little effect on litter-C storage in any treatment. There was a small, but insignificant trend for increased organic-C in the fertilized soil treatment in all fractions except for the 53-250μm fraction. The fertilized leaf treatment saw increases in organic C in the large macroaggregate (>2000μm) and a significant increase in the small macroaggregate fraction (250-2000μm), but then

decreases in both the microaggregate (53-250μm) and mineral associated (<53μm) fractions. The relatively high C/N ratio stalk tissue had no significant changes in organic-C storage in any fraction with a trend for decreased organic-C with fertilization in the large macroaggregate fraction (>2000μm). The small and generally insignificant trends that we saw for fertilization increasing macroaggregate formation could be due to the initial stimulation of the soil microbial community as we saw higher respiration rates for all litter treatments in the first 10 days. These macroaggregates are bound by microbial polysaccharides or easily decomposable substrates in the early stages of decomposition and therefore tend to be unstable and transient (Blanco-Canqui and Lal, 2004). Microaggregate fractions (53-250μm and <53μm), on the other hand, represent fractions in which long-term stabilization of carbon occurs. They tend to be formed by more recalcitrant compounds, forming organo-mineral complexes, which are highly stable. Thus, for the leaf tissue, where we saw fertilization leading to an increase in macroaggregate formation but a decrease in microaggregate formation, long-term C sequestration does not occur, because it is the microaggregate fraction that is more stable. Because maize litter has relatively low amounts of lignin, maize based systems may not have the high amounts of recalcitrant compounds to form nucleation sites for microaggregate formation. We did not see a significant amount of carbon sequestration in any treatment regardless of litter addition or litter C/N ratio.

Conclusions

Overall, it is clear that manipulation of litter and soil organic matter decomposition with inorganic nitrogen additions is difficult at fairly low fertilization rates. We also saw no evidence for increased organic-C stabilization in soil due to

inorganic nitrogen additions. However, we did see a significant effect of litter addition, which decreased the amount of SOM decomposition. This could be due to shift in the decomposer community to microorganisms that specialize on litter and thus decrease the total amount of soil C lost. We found a positive net balance of C in this incubation study with the amount of soil decomposed and litter remaining for all the litter treatment except for stalk litter. This suggests that increasing C content in soils is possible.

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Figure 1. Cumulative CO_2-C respired over the 120 day experiment. A) Total (litter + soil) CO_2-C respired over the 120 day experiment, B) cumulative soil respiration, and C) cumulative litter respiration. To calculate the cumulative amount of CO_2-C respired for litter and SOM, amounts were calculated using the rate of CO_2-C respired and the $\delta^{13}C$ signature of each sample. We assumed preferential decomposition of litte at each time period r. Thus, any difference in the $\delta^{13}C$ signature of the CO_2-C respired at the litter signature was attributed to soil decomposition. We then used this difference between the CO_2-C respired and the litter signature to determine the mean percent of CO_2-C respired of each sample that was derived from soil organic matter decomposition. Shown are the mean±1 S.E. for each treatment. Different letters denote significant differences among treatments where $P<0.05$ of a LSD posthoc (n=6) in a one-way ANOVA.

Figure 2. Rate of CO_2-C respired over the 120 day experiment for A) total (soil+litter) B) soil and C) litter at each sampling day. Shown are the mean±1 S.E. for each treatment at each sampling date (n=6 per treatment at each sampling day).

Figure 3. The effect on N on respiration rate. We subtracted the unfertilized treatment from the fertilized treatment to determine the change in respiration rate for A) total (litter + soil) B) soil and C) litter at each sampling day. Open symbols show means that are significantly different from zero while closed symbols denote non-significant differences. Significant differences were determined with non-overlapping 95% confidence intervals in a repeated measures ANOVA.

Figure 4. Percent carbon loss of litter *in situ*. Shown are the mean±1 S.E. for litter at the surface and 10 cm soil depth for the A) first 6 months which is from November to May, B) 6-12 months which represents decomposition from May to November, and C) the total amount of %C loss over the entire one year period. 6-12 months of decomposition was determined by subtracting the % C loss at 6 months from the total % C loss for the entire year. Letters denote treatment differences and were determined with a one-way ANOVA where $P<0.05$ of a LSD posthoc comparison.

Figure 5. Amount of soil C and N lost and the amount of litter C and N remaining that will be incorporated into SOM. Shown are the mean±1 S.E. for litter C and N remaining and soil C and N lost. The amount of C and N lost from soil was calculated from the soil C and N lost in the laboratory incubation experiment while the amount of litter C and N remaining of litter decomposing *in situ* after twelve months of decomposition. Letters denote treatment differences and were determined with a one-way ANOVA where $P<0.05$ of a LSD posthoc comparison.

Table 1. Quality and $\delta^{13}C$ of soil and litter additions. Shown are the mean ± 1 S.E. N=3 for $\Delta^{13}C$ means and n=6 for all other quality measurements.

	% soluble	% hemi-cellulose	% cellulose	% lignin	C	N	C/N	$\Delta^{13}C$
Stalk	30.8±1.2	24.3±0.4	38.6±0.9	6.22±0.3	44.1±0.1	0.43±0.03	102.6	-11.8±0.01
Leaf	28.7±0.7	31.0±0.4	33.9±0.7	6.40±0.3	41.9±0.1	1.01±0.42	41.4	-12.8±0.13
Soil	-	-	-	-	2.1±0.02	0.20±0.001	10.5	-22.1±0.63

Table 2. The effect of litter addition and nitrogen on A) cumulative CO_2-C respired, B) cumulative CO_2-C respired from SOM and C) cumulative CO_2-C respired from litter. Shown are the *d.f.*, *f*, and *p*-values from a two-way ANOVA with addition and nitrogen as the main effects. Significant differences were determined where $P<0.05$ in a LSD post-hoc comparison.

A. Cumulative CO_2-C respired			
Main effect	*d.f.*	*f*	*p*
Addition	2, 30	1082.88	0.000
Nitrogen	1, 30	0.22	0.645
Addition* Nitrogen	2, 30	5.22	0.011
B. Cumulative CO_2-C respired from SOM			
Main effect	*d.f.*	*f*	*p*
Addition	2, 30	513.05	0.000
Nitrogen	1, 30	1.78	0.192
Addition*Nitrogen	2, 30	5.13	0.012
C. Cumulative CO_2-C respired from Litter			
Main effect	*d.f.*	*f*	*p*
Addition	1, 20	254.95	0.000
Nitrogen	1, 20	6.52	0.019
Addition*Nitrogen	1, 20	2.70	0.116

Table 3. The effect of litter addition and nitrogen on A) CO_2-C respired, and B) amount of CO_2-C respired from SOM. Shown are the *d.f.*, *f*, and *p*-values from a repeated-measures ANOVA with day, addition, and nitrogen as the main effects. Significant differences were determined where $P<0.05$ in a LSD post-hoc comparison. Portions of the table labeled "only litter additions" are repeated-measures ANOVA with day, addition, and nitrogen as the main effects only with the soil treatment excluded from the analysis.

A. CO_2-C respired			
Main effect	*d.f.*	*f*	*p*
Day	9, 45	3927.01	0.000
Addition	2, 10	1295.52	0.000
Nitrogen	1,5	1.68	0.252
Day*Addition	18, 90	320.92	0.000
Day*Nitrogen	9, 45	11.66	0.000
Addition*Nitrogen	2, 10	27.87	0.000
Day*Addition*Nitrogen	18, 90	14.91	0.000
Only litter additions			
Main effect			
Day	9, 45	2990.77	0.000
Addition	1, 5	0.395	0.557
Nitrogen	1, 5	10.00	0.025
Day*Addition	9, 45	173.79	0.000
Day*Nitrogen	9, 45	18.22	0.000
Addition*Nitrogen	1, 5	26.23	0.004
Day*Addition*Nitrogen	9, 45	14.83	0.000
B. Amount of CO_2-C respired derived from SOM			
Main effect	*d.f.*	*f*	*p*
Day	4, 20	1942.96	0.000
Addition	2, 10	287.84	0.000
Nitrogen	1, 5	0.24	0.644
Day*Addition	8, 40	37.21	0.000
Day*Nitrogen	4, 20	0.525	0.719
Addition*Nitrogen	2, 10	5.528	0.024
Day*Addition*Nitrogen	8, 40	4.12	0.001
Only litter additions			
Main effect			
Day	4, 20	6530.98	0.000
Addition	1, 5	21.11	0.006
Nitrogen	1, 5	5.67	0.063

Day*Addition	4, 20	8.35	0.000
Day*Nitrogen	4, 20	1.84	0.161
Addition*Nitrogen	1, 5	5.62	0.064
Day*Addition*Nitrogen	4, 20	5.18	0.058

Table 4. The effect of depth and fertilization on decomposition *in situ*. Shown are the *d.f.*, *f*, and *p*-values from three separate ANOVAs. A) a four-way ANOVA with harvest, depth, tissue type and nitrogen as the main effects with the data from both the 6 and 12-month harvests included. Because harvest was highly significant in the overall analysis we separated the data by harvest and ran three-way ANOVAs for B) the 6-month harvest and C) the 12-month harvest. In all analyses shown, significant differences were determined where $P<0.05$ in a LSD post-hoc comparison. Summer decomposition (6-12 months) was determined by subtracting % C loss at 6 months from % C loss at 12 months.

A. Overall (All Harvests Included)		
Main effect (*d.f.*)	*f*	*p*
Harvest (*1, 363*)	2458.04	**0.000**
Depth (*1, 363*)	279.20	**0.000**
Tissue (*1, 363*)	353.91	**0.000**
Fertilization (*1, 363*)	0.268	0.605
Harvest*Depth (*1, 363*)	4.08	**0.044**
Harvest*Tissue (*1, 363*)	2.24	0.135
Harvest*Fertilization (*1, 363*)	0.13	0.723
Depth*Tissue (*1, 363*)	1.18	0.278
Depth*Fertilization (*1, 363*)	0.15	0.700
Tissue*Fertilization (*1, 363*)	7.27	**0.007**
Harvest*Depth*Tissue (*1, 363*)	64.11	**0.000**
Harvest*Depth*Fertilization (*1, 363*)	0.03	0.870
Harvest*Tissue*Fertilization (*1, 363*)	2.06	0.152
Depth*Tissue*Fertilization (*1, 363*)	0.629	0.428
Harvest*Depth*Tissue*Fertilization (*1, 363*)	1.81	0.179
B. Winter Decomposition (0-6 months)		
Main effect (*d.f.*)	*f*	*p*
Depth (*1, 179*)	153.29	**0.000**
Tissue (*1, 179*)	213.01	**0.000**
Fertilization (*1, 179*)	0.02	0.891
Depth*Tissue (*1, 179*)	58.76	**0.000**
Depth*Fertilization (*1, 179*)	0.35	0.852
Tissue*Fertilization (*1, 179*)	1.13	0.289
Depth*Tissue*Fertilization (*1, 179*)	0.22	0.642
C. Summer Decomposition (6-12 months)		
Main effect (*d.f.*)	*f*	*p*

Depth (*1, 186*)	3.24	**0.074**
Tissue (*1, 186*)	1.58	0.210
Fertilization (*1, 186*)	0.08	0.782
Depth*Tissue (*1, 186*)	64.56	**0.000**
Depth*Fertilization (*1, 186)*	0.31	0.579
Tissue*Fertilization (*1, 186*)	1.35	0.247
Depth*Tissue*Fertilization (*1, 186*)	1.93	0.167
D. Total Decomposition (1 year)		
Main effect (*d.f.)*	*f*	*p*
Depth (*1, 184*)	137.18	**0.000**
Tissue (*1, 184*)	161.30	**0.000**
Fertilization (*1, 184*)	0.30	0.586
Depth*Tissue (*1, 184*)	18.73	**0.000**
Depth*Fertilization (*1, 184*)	0.12	0.731
Tissue*Fertilization (*1, 184*)	6.67	**0.011**
Depth*Tissue*Fertilization (*1, 184*)	1.79	0.183

Table 5. Soil physical fractionation after 120-day incubation. The mean±1 S.E. are shown for % total mass, mg organic-C/g soil, and $\delta^{13}C$ for each soil fraction in each treatment. There were no treatment differences in the % of total mass of each fraction in each treatment ($F_{5,167}$=0.008; p=1.0) so only the mean for each fraction is shown. Different letters denote significant differences among treatments in each soil fraction where P<0.05 of a LSD posthoc (n=8) in a one-way ANOVA

Soil fraction (µm)	**>2000**	**250-2000**	**53-250**	**<53**
		% of total mass		
Mean	6.28±0.44	39.74±0.74	25.24±0.69	28.77±0.66
		mg Organic-C/g soil		
Soil	17.60±1.10	19.58±1.29ab	16.89±0.78a	17.68±0.54
Soil + N	20.63±0.19	21.96±0.43a	12.84±0.34b	19.88±0.81
Leaf	20.13±1.21	19.15±1.23b	18.34±1.06a	19.40±1.01
Leaf + N	21.80±0.74	23.69±0.57c	15.87±0.66a	18.03±0.41
Stalk	22.18±0.24	23.00±0.61c	17.25±0.75a	20.62±1.15
Stalk + N	19.97±1.16	22.70±0.55c	16.41±0.64a	20.00±1.14
$F_{5, 48}$	3.43	4.85	6.49	1.71
P	0.11	**0.001**	**0.000**	0.153
		$\delta^{13}C$		
Soil	-21.99±0.11a	-22.19±0.04a	-22.70±0.09a	-21.17±0.12a
Soil + N	-22.00±0.05a	-22.37±0.07a	-22.90±0.07a	-21.23±0.11a
Leaf	-21.19±0.09b	-21.08±0.09bc	-21.61±0.05b	-20.47±0.05b
Leaf + N	-21.01±0.13bc	-21.12±0.03b	-21.54±0.04bc	-20.61±0.16b
Stalk	-20.87±0.10c	-20.93±0.06c	-21.38±0.05c	-20.52±0.19b
Stalk + N	-20.86±0.04c	-21.06±0.08bc	-21.48±0.08bc	-20.62±0.19b
$F_{4, 42}$	32.58	101.32	110.68	5.43
P	**0.000**	**0.000**	**0.000**	**0.001**

Table 6. The effect of litter addition and nitrogen on A) organic carbon in each fraction (mg organic-C/g soil) and B) $\delta^{13}C$ of each soil fraction. Shown are the *d.f.*, *f*, and *p*-values from a two-way ANOVA with addition and nitrogen as the main effects. Significant differences were determined where P<0.05 in a LSD post-hoc comparison.

A. Organic C (mg OrganicC/gsoil)			
		>2000μm	
Main effect	*d.f.*	*f*	*p*
Addition	2, 42	3.13	0.054
Nitrogen	1, 42	1.33	0.256
Addition* Nitrogen	2, 42	4.77	0.014
		250-2000μm	
Main effect	*d.f.*	*f*	*p*
Addition	2, 42	3.10	0.056
Nitrogen	1, 42	10.00	0.003
Addition* Nitrogen	2, 42	4.02	0.025
		53-250μm	
Main effect	*d.f.*	*f*	*p*
Addition	2, 42	5.51	0.008
Nitrogen	1, 42	16.66	0.000
Addition* Nitrogen	2, 42	2.40	0.103
		<53μm	
Main effect	*d.f.*	*f*	*p*
Addition	2, 42	2.05	0.142
Nitrogen	1, 42	0.01	0.925
Addition* Nitrogen	2, 42	2.23	0.120
B. $\delta^{13}C$			
		>2000μm	
Main effect	*d.f.*	*f*	*p*
Addition	2, 42	80.53	0.000
Nitrogen	1, 42	0.58	0.452
Addition* Nitrogen	2, 42	0.63	0.539
		250-2000μm	
Main effect	*d.f.*	*f*	*p*
Addition	2, 42	250.03	0.000
Nitrogen	1, 42	5.25	0.027
Addition* Nitrogen	2, 42	0.64	0.531
		53-250μm	
Main effect	*d.f.*	*f*	*p*
Addition	2, 42	273.49	0.000
Nitrogen	1, 42	2.29	0.138
Addition* Nitrogen	2, 42	2.06	0.140
		<53μm	

Main effect	*d.f.*	*f*	*p*
Addition	2, 42	13.17	0.000
Nitrogen	1, 42	0.74	0.396
Addition* Nitrogen	2, 42	0.04	0.965

Figure 1.

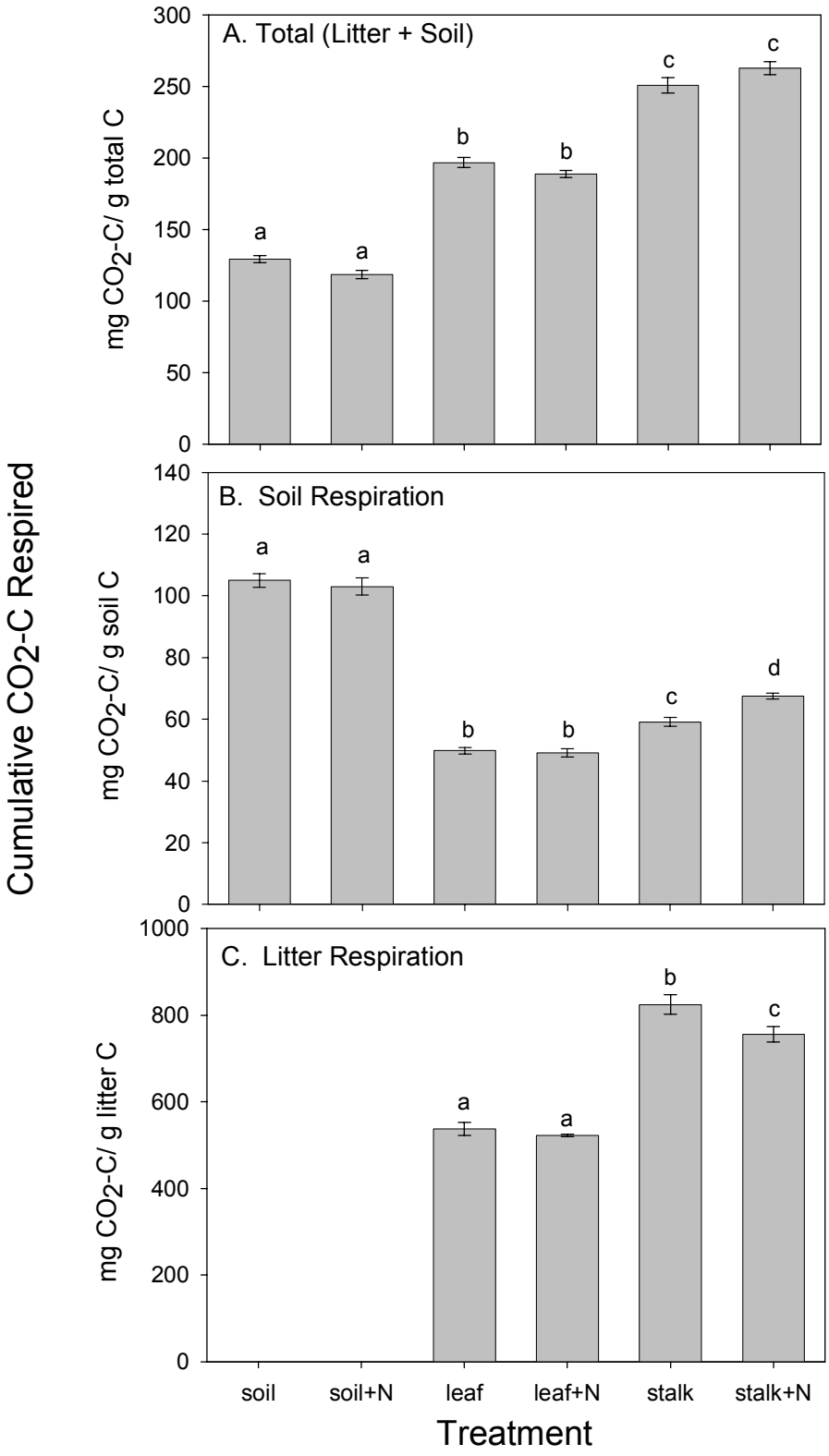

Cumulative CO2-C Respired
A. Total (Litter + Soil)
mg CO2-C/ g total C
300
250
200
150
100
50
0
a
a
b
b
c
c
B. Soil Respiration
mg CO2-C/ g soil C
140
120
100
80
60
40
20
0
a
a
b
b
c
d
C. Litter Respiration
mg CO2-C/ g litter C
1000
800
600
400
200
0
a
a
b
c
soil
soil+N
leaf
leaf+N
stalk
stalk+N
Treatment

Figure 2.

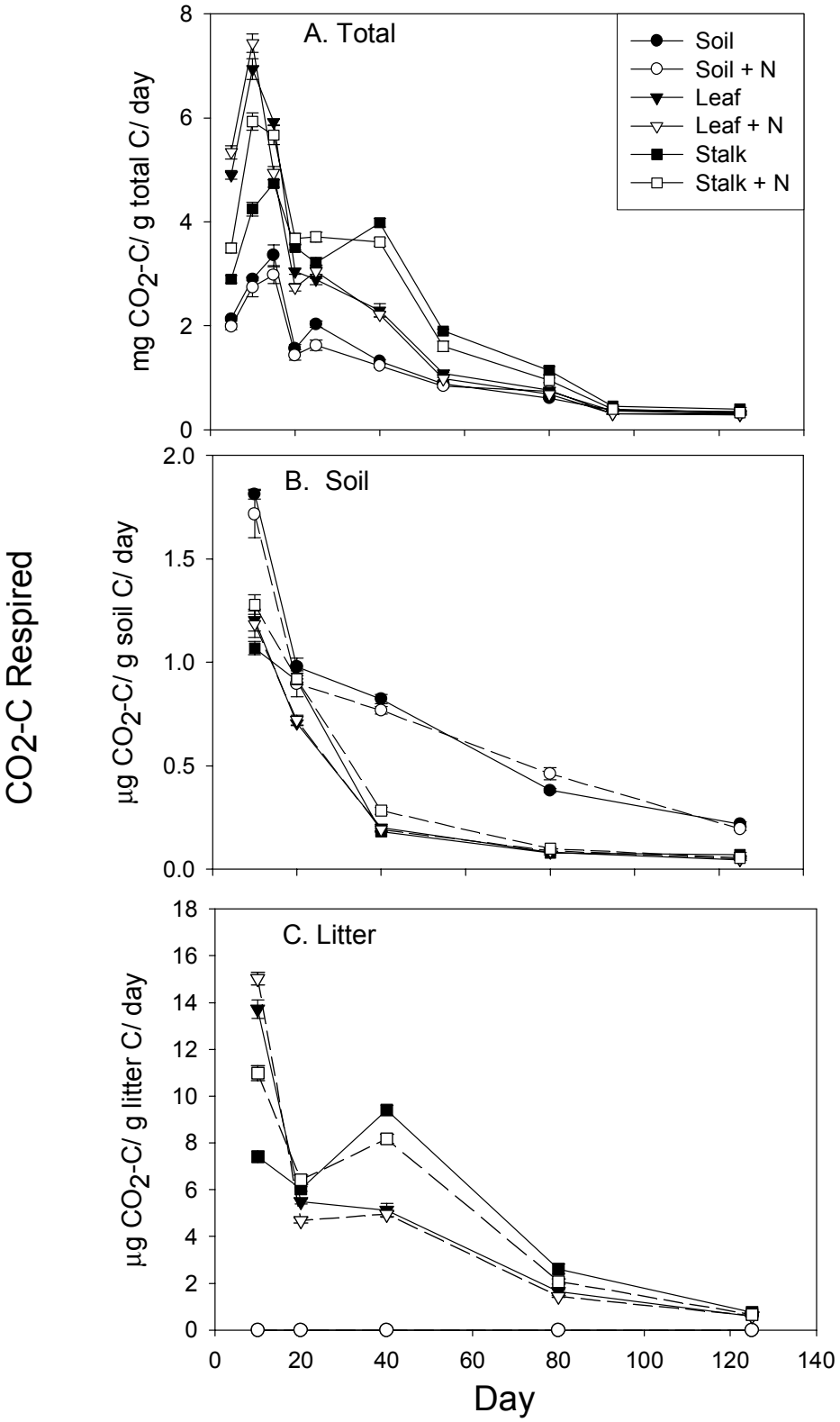

A. Total
Soil
Soil + N
Leaf
Leaf + N
Stalk
Stalk + N
mg CO2-C/ g total C/ day
B. Soil
µg CO2-C/ g soil C/ day
C. Litter
µg CO2-C/ g litter C/ day
CO2-C Respired
Day
0
20
40
60
80
100
120
140

Figure 3.

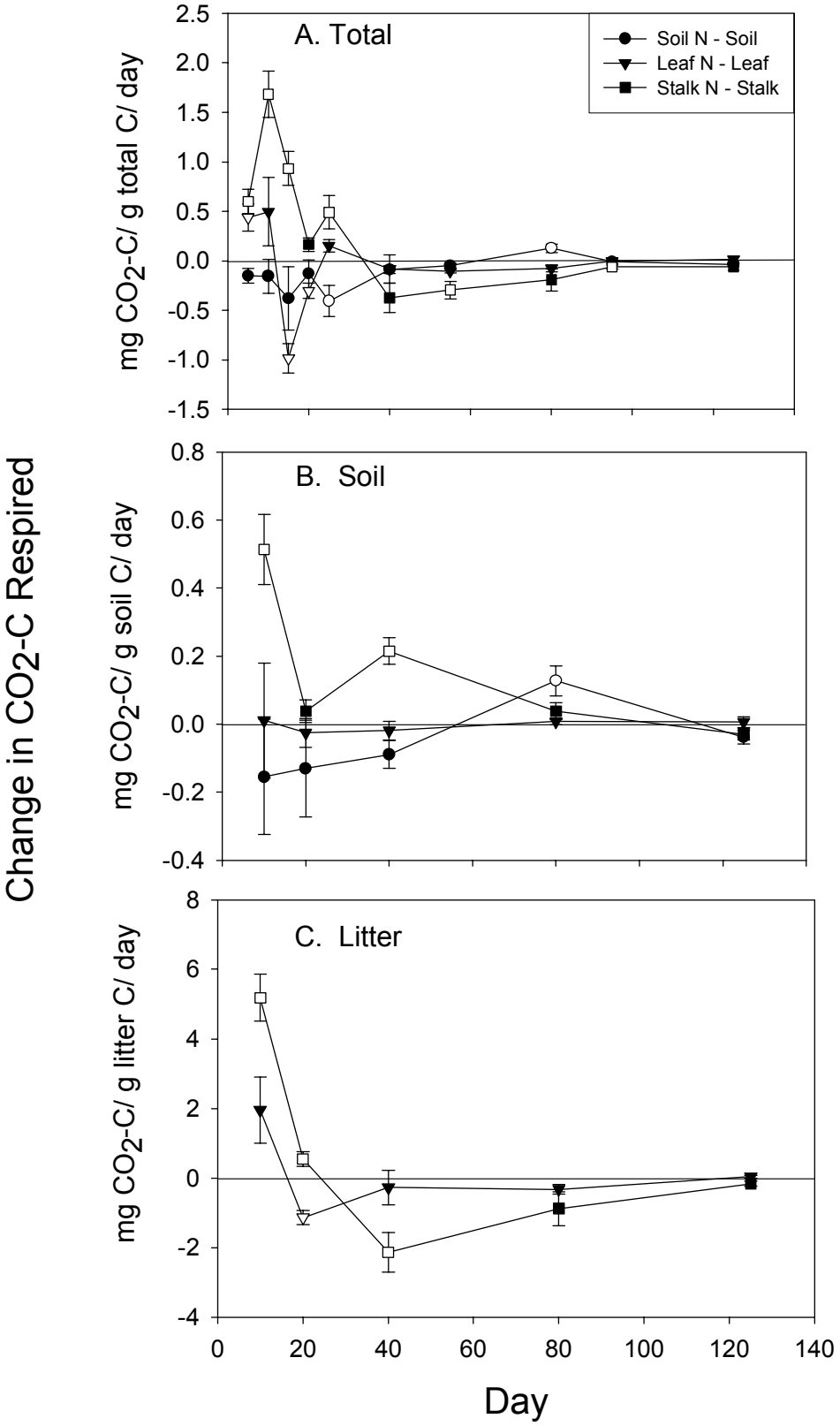

Change in CO2-C Respired
A. Total
Soil N - Soil
Leaf N - Leaf
Stalk N - Stalk
mg CO2-C/ g total C/ day
B. Soil
mg CO2-C/ g soil C/ day
C. Litter
mg CO2-C/ g litter C/ day
Day

Figure 4.

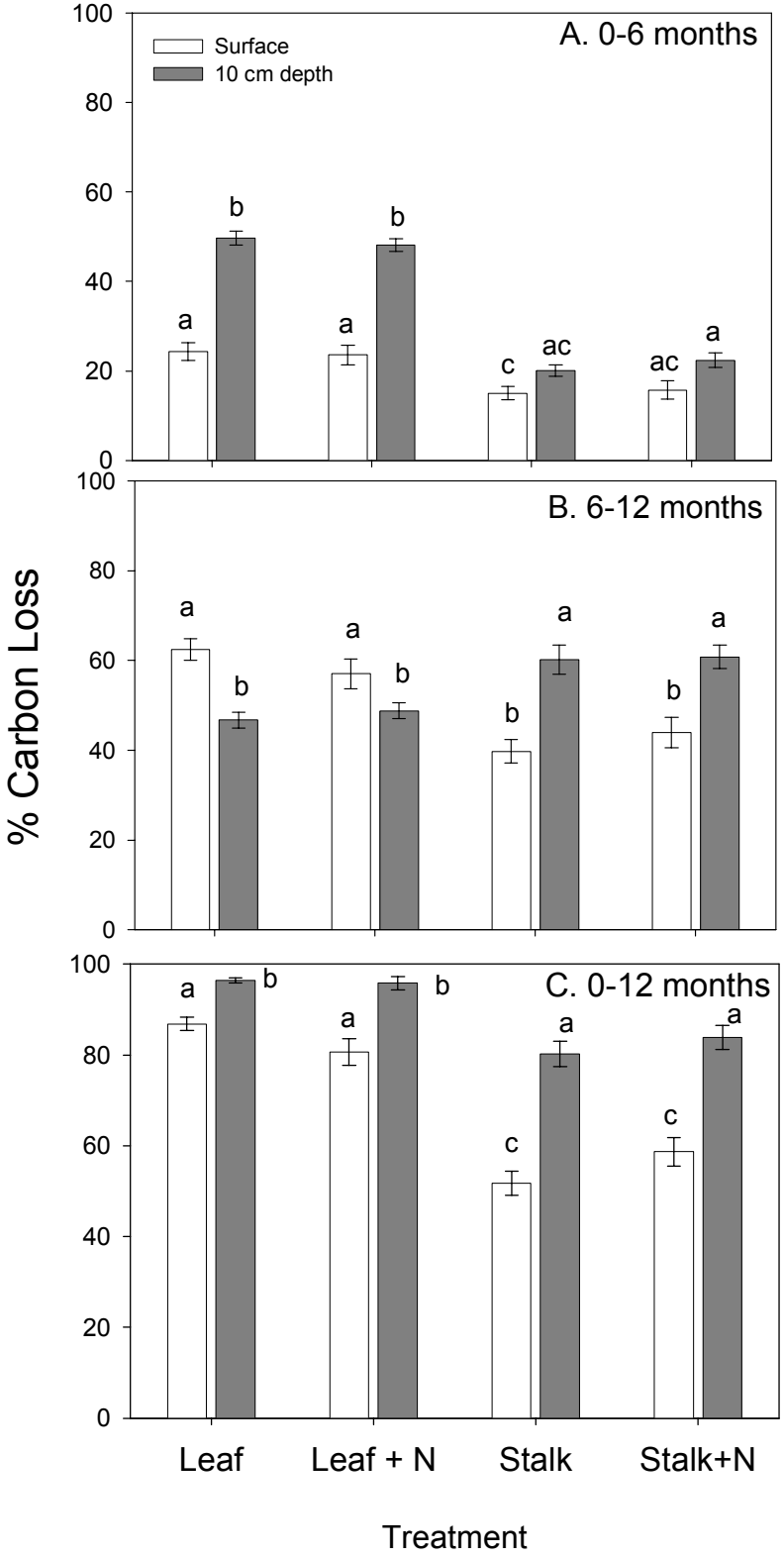
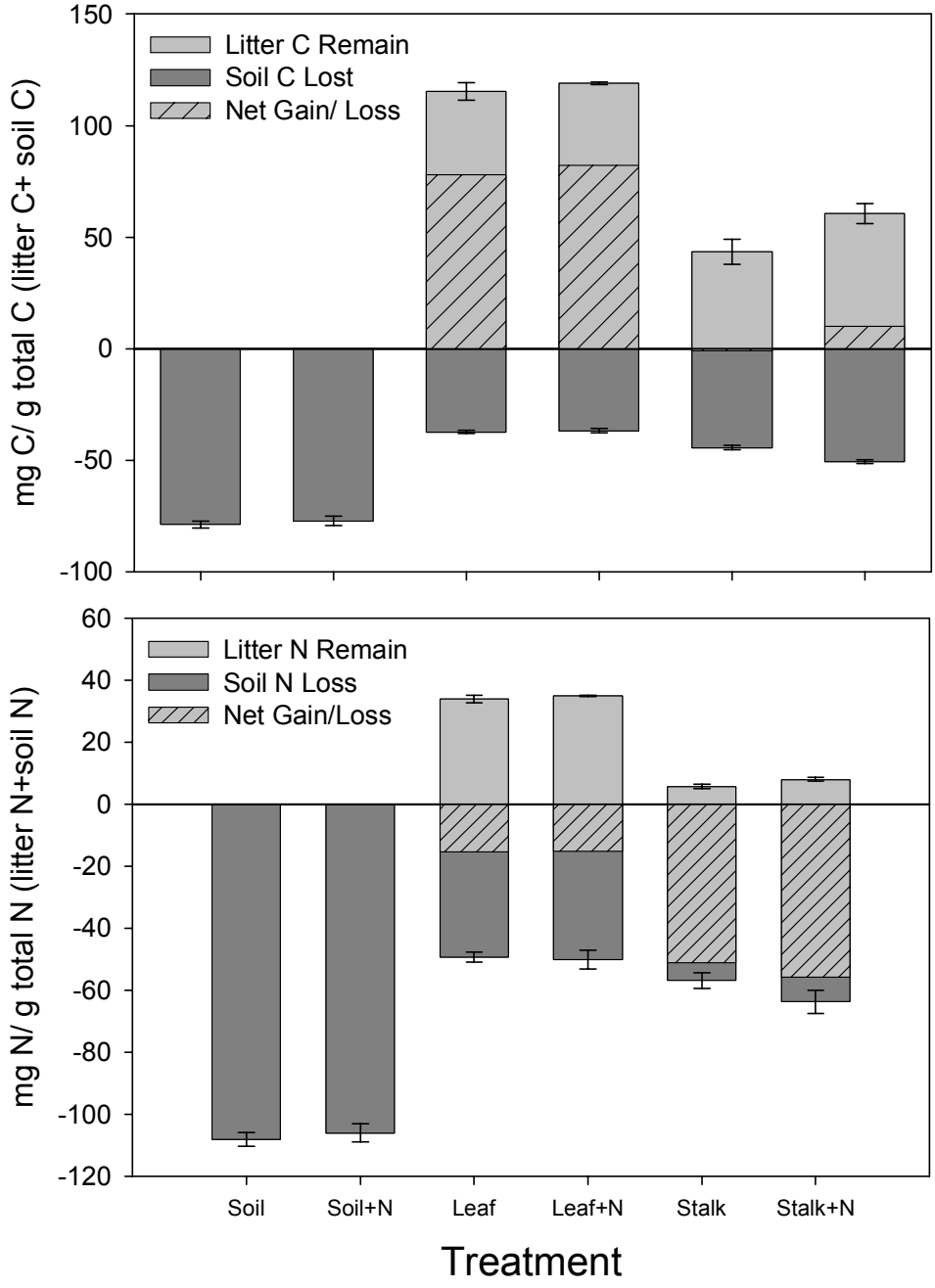

A. 0-6 months
Surface
10 cm depth
B. 6-12 months
C. 0-12 months
% Carbon Loss
Leaf
Leaf + N
Stalk
Stalk+N
Treatment

Figure 5.


Litter C Remain
Soil C Lost
Net Gain/ Loss
mg C/ g total C (litter C+ soil C)
150
100
50
0
-50
-100
Litter N Remain
Soil N Loss
Net Gain/Loss
mg N/ g total N (litter N+soil N)
60
40
20
0
-20
-40
-60
-80
-100
-120
Soil
Soil+N
Leaf
Leaf+N
Stalk
Stalk+N
Treatment

Chapter 5

The effects of biochar and charcoal additions on decomposition in two prairie soils

Amy E. Kochsiek and Johannes M.H. Knops

ABSTRACT-Fire has played a major role in creating and maintaining prairie ecosystems, but there are few studies examining the role of charred material in carbon and nitrogen cycling. While black carbon (C) is thought to be relatively inert and a viable compound to be used in C sequestration, recent evidence from a forest system suggests that charcoal additions can prime the decomposition of soil organic matter, leading to a net loss of C. Because prairie systems are dominated by grasses with fewer woody trees and shrubs, we tested the effects of both charred grass (biochar) and charred woody material (charcoal) on decomposition. We tested three main questions: i) does charcoal/biochar increase the decomposition of soil organic matter, ii) does charcoal/biochar increase the decomposition of litter, iii) does charcoal/biochar addition impact nitrogen cycling. We tested these questions in prairie soils from two locations (Nebraska and Minnesota), incubated them in the dark at 25°C for 120 days, and monitored CO_2 flux. At the end of the experiment, we measured extractable NH_4 and NO_3. Our results show that charcoal and biochar additions have soil and substrate-specific impacts. Biochar and charcoal additions led to small increases soil organic matter decomposition in the Nebraska soil but not in the Minnesota soil. Charcoal additions also increased litter decomposition slightly in the Nebraska soil, by approximately 7%. Nitrogen dynamics were highly variable between soils and between treatments within

soils, but there was no evidence that black-C additions changed N cycling. This demonstrates that charred materials can lead to small increases in litter and soil organic matter decomposition under ideal incubation conditions, but overall they do not significantly impact carbon or nitrogen cycling in prairie systems.

Introduction

Fire historically has played a major role in creating and maintaining prairie ecosystems, but there are few studies examining the role of charred material in carbon and nitrogen cycling (Shindo, 1991). Charred material could potentially be an important carbon pool in these soils, as it was recently reported that pyrogenic C in North American prairie soils can be equivalent to 4-18% of soil organic matter-C (SOM-C) (Glaser et al., 2003). During a fire event, much of the above ground biomass gets converted to CO_2, and the remaining charred material on the soil surface is eventually incorporated into the soil environment. In order for charred material to represent a major C sink in these systems, two requirements should be met: charred material must be resistant to decomposition, and it must not lead to the enhanced decomposition of litter or SOM-C.

Studies have shown that charred C is resistant to decomposition (Shindo, 1991; Liang et al., 2008). The stability of charred C in soils has been shown to depend upon the temperature at which it was created (Baldock et al., 2002) and/or the extent to which it becomes physically protected in soils (Glaser et al., 2000; Brodowski et al., 2005; Brodowski et al., 2006). Yet, while many studies have demonstrated charred C recalcitrance, recent evidence from a forest ecosystem suggests that charcoal additions prime the decomposition of SOM-C by adsorbing organic C and enhancing microbial

growth, leading to a net loss of carbon from this ecosystem (Pietikainen et al., 2000; Wardle et al., 2008).

The addition of black C has also been shown to increase cation exchange capacity and impact nitrogen availability (Lehmann et al., 2003; Berglund et al., 2004; Liang et al., 2006). Lehmann et al. (2003) demonstrated that addition of charcoal C reduced leaching of fertilizer N, and Berglund et al (2004) showed increased nitrification rates with the addition of activated C to a pine forest. It is also possible that the addition of high C/N ratio charcoal could lead to immobilization of N due to microbial demand (Lehmann et al., 2005). Thus, the effect of charcoal C additions on the decomposition process could be mediated by changes in nutrient cycling.

Most studies regarding the formation of charcoal and its potential effects on biogeochemical cycling and carbon sequestration have been in forested systems, where the majority of the source material is woody biomass. However, in prairie systems the dominant plant species are grasses with fewer woody trees and shrubs. Thus, it becomes important to test the effects of black carbon from different source materials: biochar with grass biomass as the source and charcoal produced from woody plant species. Because of this, we created charcoal from locally dominant trees and biochar from dominant grasses. We tested the effects of charcoal and biochar on decomposition by adding indigenous litter only, biochar only, and charcoal only and then combinations of 50 % litter and 50 % biochar and 50 % litter and 50 % charcoal to two different prairie soils. We then incubated these soils for 120 days and monitored CO_2 flux. At the end of the experiment, we measured extractable NO_3 and NH_4 in each treatment. We addressed three main questions: i) does charcoal/biochar increase soil organic matter-C decomposition, ii)

does charcoal/biochar increase the decomposition of litter, iii) does charcoal/biochar addition impact nitrogen cycling.

Materials and Methods

Grass, tree and soil samples were collected at Cedar Creek Ecosystem Science Reserve, MN (Latitude 45.40, Longitude -93.21) and Arapahoe Prairie, NE (Lat 41.48, Longitude -101.85). Cedar Creek Ecosystem Science Reserve lies on a glacial outwash sandplain. Soil series Sartell and Zimmerman, which are both sandy Entisols, dominate this area and are typically low fertility, high permeability soils (Grigal et al., 1974). Arapahoe prairie soil is comprised mainly of Valentine fine sand, which is a mixed mesic Typic Ustipsamments. The Valentine series is formed from eolian sands and are very deep, excessively drained soils (S.C.S., 1966; Yost et al., 1977). At Cedar Creek Ecosystem Science Reserve and Arapahoe prairie, soil was collected from 0-10 cm depth in five random locations so as to collect representative samples for each location. Soil properties are shown in table 1. Neither site has histories of fire in the sampling sites for 50+ years. For both sites, soil was brought back to the lab, sieved to 2 mm, homogenized, and stored at 4°C until use.

At Cedar Creek Ecosystem Science Reserve, *Schizachyrium scoparium* (Little Bluestem) was collected for the litter and biochar treatments and *Quercus macrocarpa* (Bur Oak) for the charcoal treatment. At Arapahoe Prairie, *Panicum virgatum* (Switchgrass) was collected for the litter and biochar treatments and *Juniperus virginiana* (Red Cedar) was collected for the charcoal treatment. These species were chosen because they represent the dominant grass and woody species at each location. All litter was collected randomly within each location from 5-10 individual plants so as to

incorporate potential variability within each site. Only senesced biomass and dead branches were collected for each plant species. Litter was then air dried to a constant mass at 70ºC.

Biochar and charcoal were produced by burning biomass in an oxygen-limited environment at 350ºC for 3.5 hours in a muffle furnace. Baldock and Smernick (2002) demonstrated that charcoal produced at temperatures greater than 200ºC had mineralization rates less than 2%. Therefore, we assumed that the charcoal/biochar in this experiment is not contributing to any increase in mineralization in any treatments where it was added. Any increased CO_2 respired was attributed to decomposition of SOM or litter. There were six treatments for each soil: 1) soil 2) litter 3) charcoal 4) biochar 5) charcoal/litter 6) biochar/litter with six replications for each treatment (n=72). Soil, litter, biochar, and charcoal for each soil were analyzed for total % C and % N with a Costech ECS 4010. We scaled the treatment additions based on the percent C of each biomass addition. All experimental units receive 0.168 g C in 50 g soil. We based this amount on productivity at Cedar Creek Natural History area of 500g biomass m^{-2}. For treatments where charcoal and litter or biochar and litter were added together, we added half the amount of material for each biomass type so that the C addition remained at 0.168 g C. All experimental units were packed to 1.0 g/cm^3 bulk density, maintained at 60% water-filled pore space, and incubated in the dark at 25ºC. On days 0, 5, 10, 15, 20, 35, 50, 70, 90, and 120 CO_2 measurements were taken. This amount of time is approximately equivalent to 1.5 thermal years of field decomposition. On sampling days, samples were placed in airtight mason jars, and CO_2 free air was pumped through each jar. Jars were then sealed for 24 hours, and gas samples were taken after that time. CO_2

samples were analyzed with a gas chromatograph (Shimadzu GC-17A). At the end of the experiment, extractable NH_4 and NO_3 were measured in each chamber and analyzed with a digital colorimeter (Bran-Luebbe AutoAnalyzer 3).

Statistical Analysis

All statistics were performed using SPSS v.17. We used a two-way univariate general linear model (GLM), with soil and addition as the independent factors, to determine differences in the cumulative CO_2-C respired after 120 days of decomposition. When soil proved significant, we used one way univariate GLM for each soil, with addition as the independent factor. Differences in the expected and observed 50:50 mix treatments also were tested using one-way univariate GLM. For all analyses, the expected values for the 50:50 mixed treatments were calculated using the sum of half of the observed CO_2-C respired for the litter only and biochar only treatments. The charcoal and litter 50:50 mixed treatment were calculated in the same manner but with half the observed CO_2-C respired for the litter only and the charcoal only treatments.

Differences in the rate of CO_2-C respired for the sampling days were determined using repeated-measures ANOVA, with addition as the main effect. Rate was calculated using CO_2 concentration measurements from the gas chromatograph and the amount of time the samples were incubated (n=6 per treatment). We subtracted the mean soil flux at each time period from each of the addition treatments to determine "relative" rates for each treatment at each sampling time. For the repeated measures ANOVA, we used the ten sampling periods with six replicates for each treatment within each sampling period (n=72 for each sampling period).

Total N lost was estimated by calculating the percent of total C lost as CO_2 –C after 120 days and then multiplying that amount by the total amount of N present in each treatment at the beginning of the experiment. Extractable NH_4 and NO_3 were also measured in each chamber at the end of the experiment. Thus, we could use the estimated total N lost compared to the extractable N to determine the contribution of N from the microbial community. If total extractable N (NH_4-N + NO_3-N) was greater than total N, the excess N was attributed to microbial mineralization. Conversely, if extractable N (NH_4-N + NO_3-N) was less than total N, it was attributed to microbial immobilization. We used a type III GLM multivariate analysis of variance (MANOVA) to test for overall impacts of soil and addition on N. For the MANOVA we used Pillai's trace test statistic to determine significant differences, because it is more robust to violations of assumptions, whereas Roy's largest root has the greatest power (Scheiner, 2001). Pillai's trace and Roy's largest root gave the same results, except for soil*addition for the charred 50:50 mix where Roy's largest root had an F=3.732 and p=0.019. If soil was significant, we used type III GLM MANOVA for each soil separately to determine differences in N due to addition. Then we used type III univariate GLM to determine which factor, NH_4-N, NO_3-N or microbe-N, contributed to the overall difference. All N data were LN transformed to improve normality.

Results

Charcoal and biochar impacts on soil organic matter decomposition

Soil, addition, as well as the soil*addition interaction all had significant impacts on the amount of cumulative CO_2 –C respired (Table 2A; Figure 1). However, there was no evidence for increased decomposition of SOM-C in the biochar and charcoal addition treatments in the Minnesota soil, whereas there was significantly greater decomposition in the biochar and charcoal addition treatments than soil in the Nebraska soil (Table 2B-D; Figure 1). The biochar and charcoal treatments in the Nebraska soil had increased cumulative CO_2-C loss of 14.7% and 16.9%, respectively. We examined the rate of CO_2-C respired for each treatment that led to the cumulative differences in decomposition (Figure 2). In a repeated-measures ANOVA on the rate of CO_2-C respired, soil, treatment, and the soil by treatment interaction were all highly significant (Table 3A). In the Minnesota soil, CO_2-C respired in the biochar and charcoal addition treatments were not significantly higher than soil for any day measured, whereas in the Nebraska soil they were significantly higher on day 5. While there were no other significant differences between soil and the biochar and charcoal treatments for the Nebraska soil, there was a general trend for slightly higher CO_2-C respired in the biochar and charcoal treatments than soil for the first 50 days of the experiment. These slightly higher rates led to the aforementioned trend of higher decomposition in the Nebraska soil for the biochar and charcoal treatments compared to the soil treatment.

Charcoal and biochar impacts on litter decomposition

In the Minnesota soil, we found no significant increase in decomposition of litter with the addition of charcoal and biochar, as there were no significant differences in the cumulative expected 50:50 mix of biochar and litter and charcoal and litter versus the

cumulative observed measurements (Table 2C-D; Figure 1). The rate of CO_2-C respired from the observed 50:50 mix of biochar and litter was not significantly different from the expected values for either soil on any sampling day (Table 2B-C). For the Nebraska soil, there was a slight, but statistically insignificant, increase of 5% greater cumulative observed measurements than the cumulative expected values for the 50:50 mix of biochar and litter. There was a significant increase in decomposition with the charcoal treatment, where the observed 50:50 mix of charcoal and litter was 7% greater than expected. The significant differences in the expected 50:50 mix of charcoal and litter compared to observed measurements in the Nebraska soil were driven only by greater respiration rates in the observed treatments at day 1 (927 mg CO_2-C/g soil C/day greater) and day 35 (1505 mg CO_2-C/g soil C/day greater). By 120 days, all treatments, with the exception of the litter treatments, were not significantly greater than soil flux, regardless of soil.

Charcoal and biochar impact on nitrogen cycling

N dynamics were highly variable and showed large differences between soils and between treatments within each soil (Figure 3). The two-way MANOVA of all measured treatments showed highly significant differences for soil, addition, and the soil*addition interaction (Table 4). At the initiation of the study, however, the Minnesota soil had 69% more N than the Nebraska soil (Table 1). Also, the addition treatments from the Minnesota soil had more N than the comparable addition treatments from the Nebraska soil (Table 1).

For all treatments in both the Minnesota and Nebraska soils, there was microbial immobilization of N, and available NH_4-N and NO_3-N was much higher in the Minnesota

soil than in the Nebraska soil (Figure 3). The differences between addition treatments within each soil were driven by NH_4-N, NO_3-N, and microbe-N and were not solely the result of just one of these N forms measured. However, there was no difference between the expected and observed charred 50:50 mixes (Table 4).

Discussion

Charcoal and biochar impacts on soil organic matter decomposition

Our results show that there are potential soil and/or charred substrate differences in charcoal and biochar additions, and yet these difference only lead to small increases in CO_2-C respired under ideal temperature and moisture conditions. In the Minnesota soil we saw no significant increases in SOM decomposition with charcoal and biochar additions, whereas the Nebraska soil had small increases. We are assuming that any increased CO_2-C respired from the biochar or charcoal treatments are due to SOM decomposition. The limited decomposition of biochar and charcoal has been noted in many experiments and points to the addition of these substrates as an effective tool for C-sequestration (Shindo, 1991; Baldock et al., 2002; Liang et al., 2008). The biochar and charcoal used in this experiment were created in the laboratory by burning biomass at 350ºC for 3.5 hours in a muffle furnace. Baldock and Smernik (2002) found that charcoal produced at temperatures above 200ºC had C mineralization rates of less than 2%. They also demonstrated using diffuse reflectance infrared Fourier transform (DRIFT) spectra, that there was loss of carbohydrate and lignin structures, with accompanying increases in aromatic and oxygenated aromatic ring structures, making materials charred at these

temperatures highly recalcitrant. Studies have also shown evidence of physical protection of biochar and charcoal in soil, which could also lead to reduced mineralization (Glaser et al., 2000; Brodowski et al., 2005; Brodowski et al., 2006). However, in these soils, physical protection is unlikely due to the very low clay content in both soils.

As a result of the experimental design, we are unable to tease apart the source of the increased CO_2 respired, whether it originates from soil organic matter or charcoal/biochar additions. We also are unable to attribute the soil differences that we see in the biochar and charcoal addition treatments to soil or substrate differences. And yet, other studies have shown that charred C is not completely inert, and thus, the oxidation of these substances may depend upon environmental effects, such as mean annual temperature or the availability of oxygen in sediments where charred C was incorporated (Gelinas et al., 2001; Cheng et al., 2006; Cheng et al., 2008). It has been suggested that in well-aerated soils, black C could be degraded on the order of 10s-100s of years (Bird et al., 1999). Further, certain types of microorganisms, such as some saprophytic fungi or those that create extracellular oxidative enzymes, do have the capacity to degrade black C (Fakoussa et al., 1999; Hockaday et al., 2006). If charcoal and biochar are not entirely inert, and decomposition is possible, this could lead to the small increases in CO_2-C respired without increasing litter or soil-C decomposition (Bird et al., 1999; Cheng et al., 2006; Cheng et al., 2008). The increases with biochar and charcoal additions seen in the Nebraska soil could be a product of the direct decomposition of these additions and not attributed to increases in soil organic matter decomposition. It is also possible that there was incomplete combustion of the substrates

from the Nebraska soil, which could explain the increased decomposition in the charcoal and biochar treatments compared to soil alone. All of the charred substrates were produced under the same conditions, thus incomplete combustion of both grass and tree litter from the Nebraska soil compared to the Minnesota soil seems unlikely.

Charcoal and Biochar impacts on litter decomposition

The addition of biochar and charcoal did not lead to increased litter decomposition in the Minnesota soil but did lead to small increases in the Nebraska soil. The Nebraska soil had greater CO_2-C respired from the observed 50:50 mix of charcoal and litter compared with the expected values. While small increases in decomposition due to the addition of charred substrates are possible (Wardle et al., 2008), other studies suggest that the availability of easily usable organic-C could prime the decomposition of charcoal, as seen with lignin decomposition (Willmann et al., 1997a; Willmann et al., 1997b; Hamer et al., 2004). Thus, it is possible that soluble substances in plant litter could have primed the decomposition of charcoal and biochar, which would explain the significantly higher observed 50:50 mix of charcoal and litter than expected in the Nebraska soil.

Charcoal and biochar impacts on nitrogen cycling

The large soil differences in the N dynamics are not surprising, as the Minnesota soil had more N in both the soil and in each of the addition treatments that then Nebraska soil. Our results suggest that the microbes in both the Minnesota and Nebraska soils were severely N limited and therefore immobilized N in all treatments (Figure 4). There are no

clear patterns in the N dynamics with the addition of charred products in either of the soils. Some studies have found increased nitrification rates with the addition of black-C, while others suggest that the addition of black-C could lead to immobilization of N (Berglund et al., 2004; Lehmann et al., 2005). In these two prairie soils, we see no evidence to support either of these processes. No differences in N dynamics were observed between the 50:50 mixes of charred and litter material, which suggests that there were no substantial changes in N cycling due to black-C additions in either soil.

Conclusions

Overall, it is clear that for mesic-prairie ecosystems, such as the ones studied here, black-C additions are highly recalcitrant. The Minnesota soil saw no increases in decomposition due to charred substrate addition, whereas the Nebraska soil had small increases in decomposition. While our work cannot definitively point to increased soil organic matter, litter, or charred substrate decomposition, it is clear that any increases in decomposition evident in this experiment were small and that the addition of charred material will not lead to drastic increases in carbon loss or changes in nitrogen dynamics. This study was conducted under optimal temperature and moisture conditions, and the small increases in decomposition seen under ideal conditions could prove to be negligible under field conditions. Thus, the addition of black-C in prairie systems would be an effective carbon sequestration strategy.

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Figure 1. Cumulative CO_2-C respired per gram of soil C over the 120 day experiment. We calculated the expected flux for the 50:50 mixtures based on the sum of½the flux in the litter only treatment and½the flux from either the biochar or charcoal only treatments. Standard errors for each bar represent the error around total flux. Different letters denote $P<0.05$ of a LSD posthoc comparison of a one-way ANOVA.

Figure 2. Relative rate of CO_2-C flux over time corrected for soil flux. Rate was calculated using CO_2 concentration measurements from the gas chromatograph and the amount of time the samples were incubated (n=6 per treatment). We subtracted the mean soil flux at each time period from each of the addition treatments to determine "relative" rates for each treatment at each sampling time. Open symbols denote significant differences in CO_2-C flux of each treatment versus soil flux, whereas closed symbols are not significantly different than soil flux. Error bars show standard error around the mean relative flux for each treatment. Significant differences were determined using non-overlapping 95% confidence intervals in a repeated-measures ANOVA with ten sampling periods. The repeated measures ANOVA showed significant soil ($f_{1, 75}$= 2790.20 p=0.000), addition ($f_{7,75}$=210.96 p=0.000) and soil* addition interaction ($f_{7,75}$=27.88 p=0.000).

Figure 3. Total Nitrogen after 120 days. We calculated the expected flux for the 50:50 mixtures based on the sum of½the flux in the litter only treatment and½the flux from either the biochar or charcoal only treatments. Total N lost was estimated by the %C lost as CO2 after 120 days multiplied by the amount of N in each chamber (soil N+ addition N). Thus, we could use the estimated total N lost compared to the total extractable N

(NH_4-N + NO_3-N) measured at the end of the experiment, to determine the contribution of N from the microbial community. If total extractable N was greater than total N lost, the excess N was attributed to microbial mineralization. Conversely, if extractable N was less than total N lost, it was attributed to microbial immobilization. Treatments with microbial mineralization are denoted with the hatched microbe-N boxes, while treatments with microbial immobilization are the open microbe-N boxes.

Table 1. Mean percent C and N ± standard error of soil and additions in each soil. Cedar Creek Ecosystem Reserve, MN soil data was referenced from (Grigal et al., 1974). Arapahoe Prairie, NE soil data was referenced from (S.C.S., 1966; Yost et al., 1977).

			Cedar Creek Ecosystem Reserve, MN						
	Species	%C	%N	C/N	pH	CEC (mg/100g)	Sand	Silt %	Clay
Soil	-	1.5 ± 0.07	0.10 ± 0.00	14.9 ± 0.4	5.8	2.1	93	3	4
Litter	*S. scoparium*	45.2 ± 0.05	0.57 ± 0.01	78.8 ± 0.9					
Biochar	*S. scoparium*	68.0 ± 0.22	1.55± 0.02	44.0 ± 0.5					
Charcoal	*Q. macrocarpa*	70.1 ± 0.88	0.63 ± 0.03	112.1 ± 4.6					
			Arapahoe Prairie, NE						
	Species	%C	%N	C/N	pH	CEC (mg/100g)	Sand	Silt %	Clay
Soil	-	0.7 ± 0.05	0.07 ± 0.01	10.8 ± 0.1	7.2	4.6	92.3	3.6	4.1
Litter	*P. virgatum*	42.0± 0.03	0.41 ± 0.01	102.7 ± 1.7					
Biochar	*P. virgatum*	49.5 ± 0.97	0.96 ± 0.01	51.5 ± 0.7					
Charcoal	*J. virginiana*	59.4 ± 0.57	0.62 ± 0.01	95.1 ± 0.8					

Table 2. Cumulative CO_2-C lost after 120 day incubation. Two-way analysis of variance was performed for each treatment combination presented (A-D). If soil was significant for the two-way analysis, data were split by soil and one way analysis of variance was performed for each soil separately.

A. All measured addition treatments (excluding expected)			
	df	*f*	*p*
Soil	1, 72	1810.82	**0.000**
Addition	5, 72	258.07	**0.000**
Soil*Addition	5, 72	34.30	**0.000**
Split by Soil:			
Cedar Creek Ecosystem Reserve, MN	5, 36	177.10	**0.000**
Arapahoe Prairie, NE	5, 36	140.67	**0.000**
B. Biochar and Charcoal Expected vs. Observed			
	df	*f*	*p*
Soil	1, 48	1986.73	**0.000**
Addition	3, 48	5.19	**0.004**
Soil* Addition	3, 48	1.78	0.167
C. Biochar Expected vs. Observed			
	df	*f*	*p*
Soil	1, 24	952.98	**0.000**
Addition	1, 24	5.39	**0.031**
Soil* Addition	1, 24	1.04	0.321
D. Charcoal Expected vs. Observed			
	df	*f*	*p*
Soil	1, 24	1034.08	**0.000**
Addition	1, 24	9.93	**0.005**
Soil* Addition	1, 24	2.71	0.115

Table 3. Rate of CO_2-C respired over 120 days. Repeated measures ANOVA was used to determine differences in the rate of CO_2-C respired with time and addition as the main effects. The Greenhouse-Geiser correction was used to correct for sphericity. This correction reduced the degrees of freedom to make the F-value more conservative. Presented are both the standard df and the Greenhouse-Geiser adjusted df denoted as adjusted df. There were no cases where the correction changed the significance of a test. Given are the f and p values of each test. Because soil was significant in the overall test (A), we subsequently tested each soil individually (B-C). There were 10 sampling days (Day) and six replicates for each treatment (Addition) with each sampling period (n=72 for each sampling day). We calculated the expected flux for the 50:50 mixtures based on the sum of½the flux in the litter only treatment and½the flux from either the biochar or charcoal only treatments.

A. Both Soils

All Measured Treatments

	df	*Adjusted df*	*f*	*p*
Day	9, 45	3.14, 15.59	359.94	0.000
Soil	1, 5	1.00, 5.00	1794.82	0.000
Addition	5, 25	1.90, 9.48	403.73	0.000
Day*Soil	9, 45	2.75, 13.73	38.57	0.000
Day*Addition	45, 225	4.21, 21.20	11.50	0.000
Soil*Addition	5, 25	2.17, 10.85	70.76	0.000
Day*Soil*Addition	45, 225	4.19, 20.95	3.12	0.000

B. Ecosystem Science Reserve, MN

All Measured Treatments

	df	*Adjusted df*	*f*	*p*
Day	9, 45	3.06, 15.30	404.52	0.000
Addition	5, 25	2.16, 10.78	251.48	0.000
Day*Addition	45, 225	3.70, 18.48	10.49	0.000

Biochar and Charcoal (Expected and Observed)

	df	*Adjusted df*	*f*	*p*
Day	9, 45	3.04, 15.21	366.41	0.000
Addition	3, 15	1.90, 9.47	2.23	0.162
Day*Addition	27, 135	3.91, 19.53	3.81	0.020

C. Arapahoe Prairie, NE

All Measured Treatments

	df	*Adjusted df*	*f*	*p*
Day	9, 45	3.11, 15.55	193.75	0.000
Addition	5, 25	2.02, 10.10	262.17	0.000
Day*Addition	45, 225	4.13, 20.64	6.65	0.001

Biochar and Charcoal (Expected and Observed)

	df	*Adjusted df*	*f*	*p*
Day	9, 45	2.87, 14.34	189.64	0.000
Addition	3, 15	1.93, 9.65	13.28	0.002
Day*Addition	27, 135	3.94, 19.70	3.29	0.033

Biochar (Expected and Observed)

	df	*Adjusted df*	*f*	*p*
Day	9, 45	2.95, 14.77	172.69	0.000
Addition	1, 5	1.00, 5.00	34.77	0.002
Day*Addition	9, 45	2.93, 14.66	2.76	0.081

Charcoal (Expected and Observed)

	df	*Adjusted df*	*f*	*p*
Day	9, 45	3.18, 15.88	108.97	0.000
Addition	1, 5	1.00, 5.00	20.95	0.006
Day*Addition	9, 45	3.11, 15.53	5.98	0.006

Table 4. Total nitrogen at the end of 120 day incubation: multivariate analysis of all observed treatments and the 50:50 mix of all charred treatments (biochar and charcoal expected and observed). NH_4-N , NO_3-N, and microbe-N were the dependent variables, whereas soil and addition were the independent factors. Given are the F and P value of the Pillai's trace. Pillai's trace and Roy's largest root gave the same results, except for soil*addition for the charred 50:50 mix where Roy's largest root had an F=3.732 and p=0.019. We used Pillai's trace, because it is more robust to violations of assumptions, whereas Roy's largest root has the greatest power (Scheiner, 2001). NH_4-N , NO_3-N, and microbe-N were all Ln transformed to improve normality.

	All Measured Treatments (Observed Only)	
Fixed factor (*df*)	f	p
Soil (3, 50)	3744.04	0.000
Addition (15, 156)	10.62	0.000
Soil*Addition (15, 156)	5.23	0.000
Split by Soil:		
Ecosystem Science Reserve, MN		
Addition (15, 90)	7.86	0.000
Arapahoe Prairie, NE		
Addition (15, 66)	5.39	0.000
	All Charred 50:50 Mix (Expected and Observed)	
Fixed factor (*df*)	f	p
Soil (3, 35)	2873.75	0.000
Addition (9, 111)	1.67	0.106
Soil*Addition (9, 111)	1.58	0.131

Figure 1.

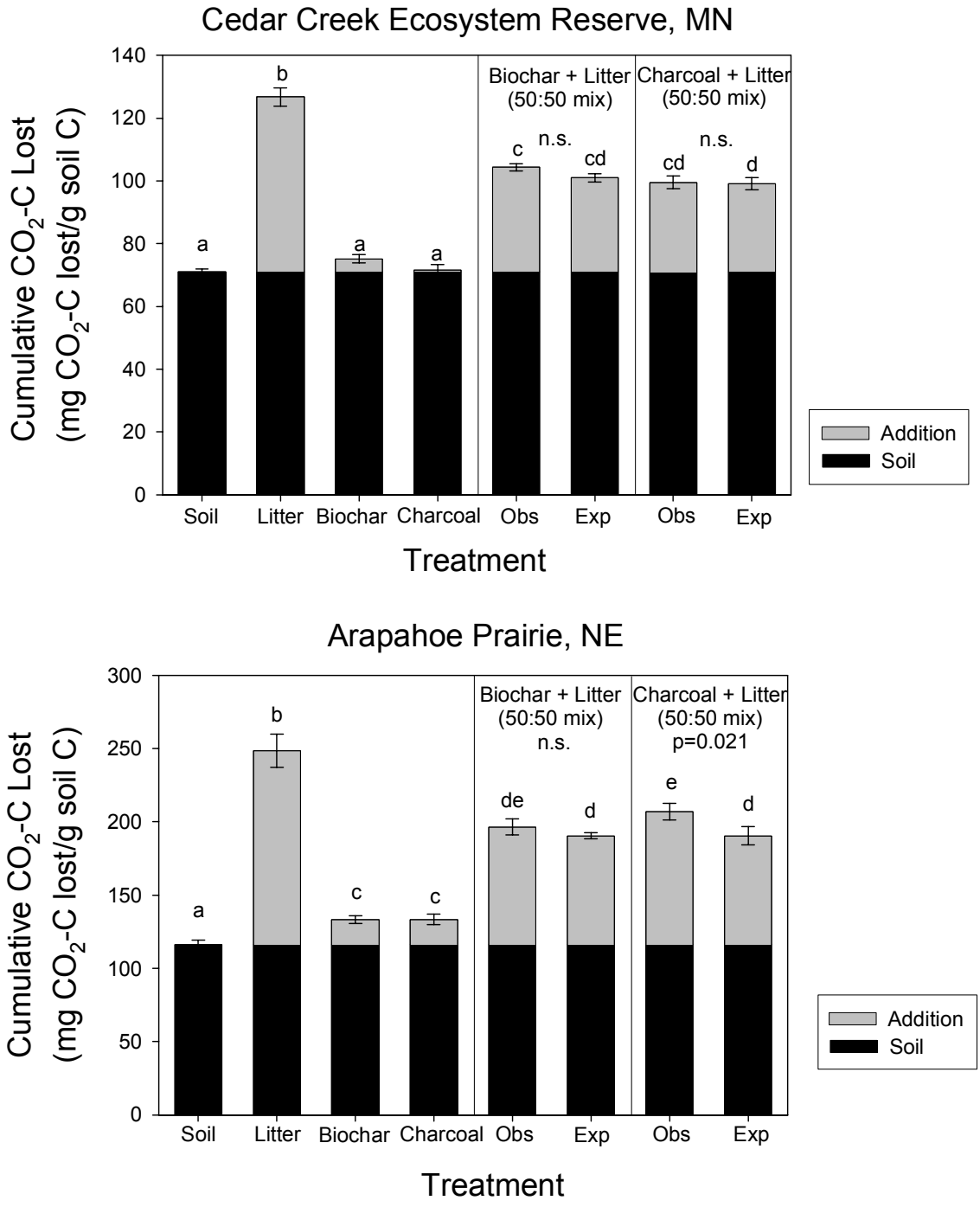

Cedar Creek Ecosystem Reserve, MN
Cumulative CO_2-C Lost (mg CO_2-C lost/g soil C)
Biochar + Litter (50:50 mix)
Charcoal + Litter (50:50 mix)
n.s.
n.s.
a
b
a
a
c
cd
cd
d
Soil
Litter
Biochar
Charcoal
Obs
Exp
Obs
Exp
Treatment
Addition
Soil
Arapahoe Prairie, NE
Cumulative CO_2-C Lost (mg CO_2-C lost/g soil C)
Biochar + Litter (50:50 mix) n.s.
Charcoal + Litter (50:50 mix) p=0.021
a
b
c
c
de
d
e
d
Soil
Litter
Biochar
Charcoal
Obs
Exp
Obs
Exp
Treatment
Addition
Soil

Figure 2.

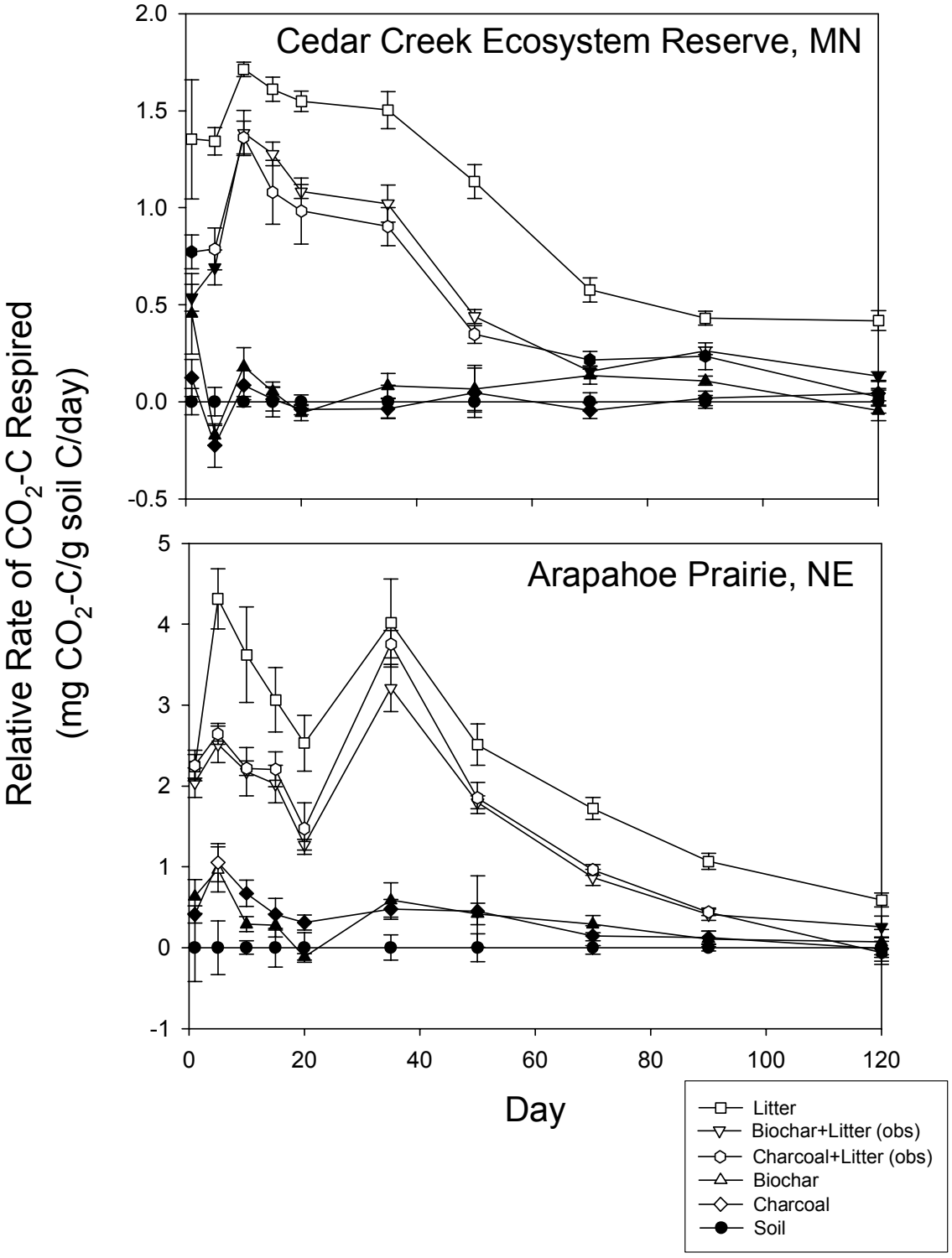
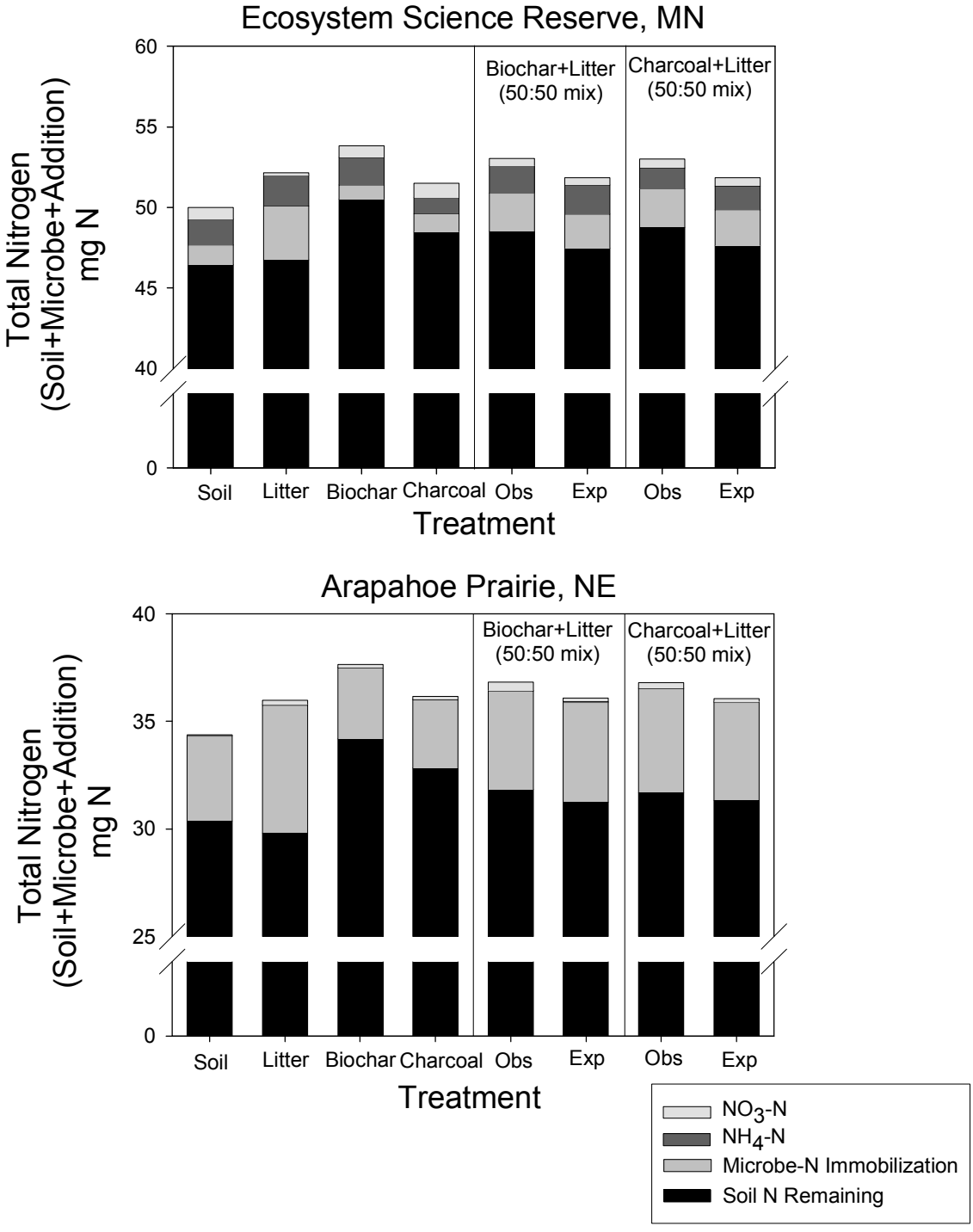

Cedar Creek Ecosystem Reserve, MN
Arapahoe Prairie, NE
Relative Rate of CO_2-C Respired
(mg CO_2-C/g soil C/day)
Day
Litter
Biochar+Litter (obs)
Charcoal+Litter (obs)
Biochar
Charcoal
Soil

Figure 3.


Ecosystem Science Reserve, MN
Total Nitrogen (Soil+Microbe+Addition) mg N
60
55
50
45
40
0
Biochar+Litter (50:50 mix)
Charcoal+Litter (50:50 mix)
Soil
Litter
Biochar
Charcoal
Obs
Exp
Obs
Exp
Treatment
Arapahoe Prairie, NE
Total Nitrogen (Soil+Microbe+Addition) mg N
40
35
30
25
0
Biochar+Litter (50:50 mix)
Charcoal+Litter (50:50 mix)
Soil
Litter
Biochar
Charcoal
Obs
Exp
Obs
Exp
Treatment
NO3-N
NH4-N
Microbe-N Immobilization
Soil N Remaining