ENHANCING SOIL CARBON SEQUESTRATION WITH PLANT RESIDUE QUALITY AND SOIL MANAGEMENT

by

PAUL MARK WHITE, JR.

B.S., University of Arkansas, 1999
M.S., University of Arkansas, 2002

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Abstract

Atmospheric concentrations of the greenhouse gases (GHG) carbon dioxide (CO_2), nitrous oxide, and methane have been increasing since the Industrial Revolution. An expanding human population, increased fossil fuel use, extensive ecosystem disturbance, and intensive production agriculture have contributed to this increase. Storing carbon (C) in soil in natural and agricultural ecosystems has the potential to offset a portion of the future atmospheric increases in CO_2 levels. Laboratory and field studies were conducted to evaluate basic mechanisms of C sequestration. The research reported here focuses on identifying strategies to reduce C loss from soil by (1) slowing plant residue decomposition rates, or (2) increasing soil fungal dominance and physical protection of soil C. Grain sorghum (*Sorghum bicolor*) hybrids were used in a laboratory experiment to determine the effect of varied amounts of lignin on plant residue C mineralization. The different levels of lignin in the hybrids was not strongly correlated with plant residue C mineralization. Another laboratory experiment investigated larger differences in lignin content between crop plants. Plant residue exhibiting the natural mutation referred to as brown midrib (bmr) also had lowered total lignin and different lignin chemistry. The bmr plants decomposed faster than the normal isolines, and the addition of nitrogen lowered overall mineralization. Nitrogen additions also significantly impacted the microbial community by lowering total phospholipid fatty acids (PLFA) and shifting fungal energy storage physiology. A field experiment was conducted to measure the soil microbial response to adding grain sorghum residue in both tillage (CT) and no-tillage (NT) agricultural ecosystems. The residue mineralized similarly in both systems, but the NT microbial population was stimulated significantly greater than CT. The fungal PLFA in NT 0-5 cm was higher than NT 5-15 cm, CT 0-5 cm, or CT 5-15 cm. A significantly greater amount of plant residue C was found in soil macroaggregates, as

compared to microaggregates, at the conclusion of the experiment, regardless of tillage. More N was found in NT macroaggregates than in CT macroaggregates. The experiment identified two mechanisms for increased C storage in NT soils, as compared to CT soils.

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Approved by:

Major Professor
Charles W. Rice

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CHAPTER 1 - General Introduction

Atmospheric concentrations of the greenhouse gases (GHG) carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) have been increasing at an exponential rate since the Industrial Revolution in the 19th century (IPCC, 2001). In addition to natural causes, an expanding human population, increased fossil fuel use, more extensive ecosystem disturbance, and more intensive production agriculture have contributed to this increase (Vitousek, 1994; IPCC, 2001). Storing carbon (C) in soil in natural and agricultural ecosystems has the potential to offset a portion of the future atmospheric increases in CO_2 levels until alternative energy and other GHG mitigation strategies become available (Paustian et al., 1998; Caldeira et al., 2004). The overall objective of this research was to determine if components of the plant-soil system can be manipulated to increase the amount of C stored in soil. This introduction provides an overview of (1) the global C cycle; (2) GHG and global warming; (3) mitigation options and strategies for C sequestration; (4) plant residue quality effects on C sequestration; (5) soil aggregate effects on C sequestration; (6) general hypotheses; and (7) descriptions of chapters 2 through 4.

Global C Cycle

The C cycle is an important biogeochemical cycle due to the central role of C in the atmospheric, marine, and terrestrial environments. About 10^8 Pg of C exists on Earth, most of which is buried in sedimentary rocks (8×10^7 Pg or 80%) as organic compounds or carbonates. Buried coal, petroleum, and organic sediments are estimated to range from 4,000 to 7,000 Pg total C. (Schimel et al., 1994; Falkowski et al., 2000; Lal, 2004). Of the remaining unburied C,

only about 5 x 10^4 Pg (or 0.05% of the total) is located in the active surface pools of the atmosphere, the oceans, and the terrestrial environment, containing approximately 750; 38,000; and 3000 Pg total C, respectively. The terrestrial C pool is subdivided into soil inorganic C, soil organic C, and vegetation with global masses of 950, 1550, and 560 Pg C, respectively (Lal, 2004). The major fluxes of C between the atmosphere and the terrestrial environment are driven by photosynthesis and respiration and constitute about 120 Pg C y^{-1}. The major flux between the atmosphere and oceans involves about 92 and 91 Pg C y^{-1} absorbed and desorbed from seawater, respectively (Sposito, 1989; Schlesinger, 1997). The global C cycle is completed by including the 0.02 to 0.05 Pg of carbonaceous materials that are recycled as CO_2 to the atmosphere through hydrothermal and volcanic activity resulting from subduction of the ocean floor over time (Schlesinger, 1997).

Greenhouse Gases and Global Warming

Energy radiating from the Sun is mostly absorbed by the Earth's surface water, causing evaporation and driving the hydrologic cycle; 30% of the incoming radiation is reflected into space by the surface and the atmosphere (CAST, 2004). The surface of the Earth cools by radiating heat upwards into the atmosphere where it is absorbed by atmospheric gases that reemit it back to the Earth’s surface. Thus, increasing the concentrations of gases in the atmosphere that absorb the radiation would lead to an increase in the temperature of the atmosphere and of surface temperature of the Earth. The term "greenhouse effect" was coined in 1896 by Svante Arrhenius to describe the effects of CO_2 on the temperature of the atmosphere.

In addition to CO_2, water vapor (H_2O), ozone (O_3), CH_4, and N_2O are also important GHG in the atmosphere. Other greenhouse gases, such as chlorofluorocarbons (CFCs) and sulfur hexafluoride are also contributors to the greenhouse effect but are less of a concern due to the

elimination of CFC production under the Montreal Protocol of 1987 and the presence of extremely low concentrations of sulfur hexafluoride (CAST, 2005). While the GHG are present in the atmosphere at trace levels, usually in the μmol GHG mol air^{-1} range or lower, their concentrations fluctuate highly over time.

Ice core data from Antarctica have indicated fluctuating atmospheric levels of CO_2 and CH_4 during four distinct "ice age" events in the past 240,000 y with levels from 180 to 290 μmol CO_2 mol air^{-1} and 325 to 710 nmol CH_4 mol air^{-1} (Petit et al., 1999). Present day levels of 370 μmol CO_2 mol air^{-1} and 1500 CH_4 nmol mol air^{-1} are greater than at any other time in this ice record. In fact, for about the last 800 y, levels of CO_2 and CH_4 were stable at about 280 μmol CO_2 mol air^{-1} and 740 nmol mol air^{-1} until about the middle of the 19th century, the beginning of the Industrial Revolution (Hirsch et al., 1988; CAST, 2004). Corresponding with the increases in GHG is the observed average surface temperature increase of 0.2°C since the late 19th century with most of the increases occurring between 1910 to 1945 and 1980 to 2000. In addition 15% of the snow cover and mountain glacier ice has disappeared since the 1950s and the 1960s, along with a 40% decline in Arctic sea-ice (IPCC, 2001). Melting ice sheets could impact coastal areas worldwide by raising sea level up to 70 cm (Alley et al., 2005).

There are many sources of GHG, but burning fossil fuels, industrial processes, deforestation, and agriculture are the major contributors (Cunningham and Saigo, 1999). Agriculture is responsible for 38 and 22% of the annual N_2O and CH_4 emissions, respectively, but act as a slight sink for CO_2 (CAST, 2004). In terms of CO_2, the burning of fossil fuels and the production of cement account for 6.3 Pg CO_2 y^{-1}, but this level is buffered by retention in the oceans and in the terrestrial environment (Table 1.1) (CAST, 2004). Other anthropogenic sources of CO_2 include land disturbance such as tillage agriculture and deforestation. Sources of CH_4

include natural sources such as wetlands and termites and also anthropogenic sources such as natural gas leakage, coal mining, rice paddies, enteric fermentation, animal wastes, and landfills. Sources of N_2O include natural ecosystems as well as industrial combustion and manufacturing, biomass burning, and agriculture practices (CAST, 2004). In terms of global warming potential, CH_4 and N_2O are 62 and 275 times more potent, respectively, than CO_2 in a 20 y time span. In a 100 y time span, CH_4 and N_2O are 23 and 296 times more potent, respectively, than CO_2, the difference being the long-term stability of CH_4 and N_2O in the atmosphere (IPCC, 2001). Thus, these GHG represent a threat to humans and the environment.

Political action on the threat of increasing GHG emissions began in the 1990s. In 1992 the United Nations adopted the Framework Convention on Climate Change (UNFCCC) in Rio de Janeiro. The convention proposed to stabilize GHG concentrations at levels which would prevent anthropogenic interference with climate. In 1995 the Intergovernmental Panel on Climate Change (IPCC) issued a report authored by almost 80 scientists concluding that (1) world climate had changed significantly in the past 100 y; (2) evidence suggests anthropogenic causes of climate change; and (3) climate change models predict temperature increases of 1°C to 3.5°C by the year 2100 (Cunningham and Saigo, 1999). Using the computer model MiniCam 98.3, the IPCC predicted that a "business-as-usual" scenario could result in an increase in predicted 2005 C emissions from less than 8 to nearly 19 Pg C y^{-1} by 2095 (CAST, 2000). To combat global warming, the UNFCCC met again in 1997 in Kyoto, Japan, and drafted a protocol that would place limits on GHG emissions, either by decreasing the rate at which GHG are emitted or by increasing the rate at which they are removed from the atmosphere. Not all countries ratified the Kyoto Protocol (e.g., United States and Australia), which went into effect on February 16, 2005. Nevertheless, political recognition of global climate change was achieved,

as even U.S. President George W. Bush acknowledged that the Earth's temperature "has risen by 0.6°C over the past 100 years" and that "the National Academy of Sciences indicates that the increase is due in large part to human activity" (Bush, 2001).

Global warming will not affect all areas of the planet equally. Tropical latitudes will probably not get much warmer than they are today, but the mid-latitude and high-latitude regions are likely to experience more extreme change (Cunningham and Saigo, 1999). Giorgi and Francisco (2000a,b) compared five Atmosphere-Ocean General Circulation Models to predict global temperature and precipitation changes as a result of increases in CO_2. They found that, for temperature, (1) no areas were predicted to cool; (2) the Northern Hemisphere areas of Alaska (U.S.), Greenland, Northern Europe, and Siberia would warm to 40% more than the global average of 2 to 7°C; (3) precipitation decreases were predicted for Central America, East Africa, parts of Central Asia and the Mediterranean, and Australia; and (4) precipitation would increase for the Northern Hemisphere areas and Antarctica.

Mitigation of GHG and C Sequestration

Strategies aimed at reducing GHG are very diverse and include natural and engineered solutions. An extensive review of C management options was compiled by Caldeira et al. (2004). They introduce the concept of reducing sources or increasing sinks, but were careful to point out that no single option available now will stabilize the effects of increased GHG. They sorted mitigation options based on a time scale to achieve significant GHG reductions and the potential to reduce the C emitted to the energy consumed ratio (C/E). The C storage in agricultural soils ranked as a rapidly deployable technology and was a major contributor in reducing C/E (Table 1.2). In a 1998 workshop, the U.S. Department of Energy's Pacific Northwest and Oak Ridge National Laboratories and the Council for Agricultural Science and Technology (CAST)

addressed questions related to C sequestration (Rosenberg et al., 1999). These questions included the feasibility of C sequestration in agricultural soils and whether methods could be developed to increase both the quantities and residence time of C sequestered in soils. In 2000 the Consortium for Agriculture Soils Mitigation of Greenhouse Gases (CASMGS) received funding from the US Government to study agriculture's role in reducing GHG (website: www.casmgs.colostate.edu). One of the tasks of CASMGS was to gain a better understanding of the effects of plant residue quality on soil C storage and the biophysical processes related to soil C sequestration.

Soil C sequestration is defined as the process of transforming C in the air (CO_2) into stored soil C. For more information, visit the K-State Soil Carbon Center at: http://soilcarboncenter.k-state.edu. The transformations and storage of this C in soil is a function of biotic, chemical, and physical controls and is related to plant residue quality or biochemistry, and its accessibility to organisms (Figure 1.1). Plant productivity and soil microbial activity are two biological processes governing soil C dynamics (Rice and Angle, 2004). Plant C is transported belowground and enters the soil food web through (1) arbuscular mycorrhizal (AM) fungi, (2) root exudates, (3) root material - especially fine roots, or (4) bacterial autotrophic CO_2 fixation. A portion of the aboveground plant biomass also enters the soil food web by incorporation through tillage or leaching. In soil, plant derived C acts as a growth substrate for bacteria, actinomycetes, and fungi to reproduce or maintain cells. The C is recycled within the soil food web into higher trophic levels by a complex consortium of organisms including microbes, nematodes, protozoa, collembola, mites, oligochaetes, and insects (Bottomley, 2005). In agricultural ecosystems, about 70-80% of the original plant C is mineralized to CO_2 and returned to the atmosphere through respiration in one year. However, some of the decomposition

products and microbial secondary metabolites may be converted into stable soil humus, which could have residence times up to centuries long (Stevenson, 1994; Six and Jastrow, 2002).

Plant Residue Quality Effects on C Sequestration

Plant residues differ in their decomposability due to their inherent different chemical characteristics. Plants contain non-structural components such as soluble sugars and proteins, and structural components such as cellulose, hemicellulose, and lignin (Johnson and Barbour, 2002). Quantities of each of these components differ between plants and within plant organs. These components decompose in the soil at different rates, generally being more difficult to decompose in the following order: soluble materials < hemicellulose < cellulose < lignin (Wolf and Wagner, 2004). Increasing the amount of lignin in plant residue may have a positive impact on soil C storage (Melillo et al., 1982). This could represent an opportunity for biotechnology to breed or create a plant less susceptible to microbial decay. Additionally, lignin biosynthesis has been a major target of genetic manipulation (Pillonel et al., 1991; Halpin et al., 1994; Baucher et al., 1999). Conceptually, this development may be a means to slow decomposition of the plant residue (Fig. 1.2). This chould lead to decreased CO_2 release and increased soil C storage, but not necessarily increased soil organic matter (SOM). Factors affecting the biological activity, such as water, temperature, and oxygen status, also will influence soil C cycling.

Soil Aggregate Effects on C Sequestration

The biological, chemical, and physical interactions affecting soil resource heterogeneity and soil architecture should also be considered important for soil C cycling. Physical and chemical protection provided by soil aggregates and iron oxides play a role in decomposition and mineralization kinetics (Six et al., 2002). Soil aggregate dynamics are increasingly becoming an

important topic in soil science research, especially in regards to soil C sequestration (Mikha et al., 2006; Jastrow and Six, 2006; Vadakattu et al., 2006; Veenstra et al., 2006). Soil macroaggregates are composed of particulate organic matter and microaggregates, which are primarily composed of chemically protected organic matter (Six and Jastrow, 2006). Macroaggregates contain a greater concentration of C than microaggregates (Mikha and Rice, 2004). The mean resonance time is highly dependent on management, with macro- and microaggregate C times of between 1.3 to 140 and 7 to 691 y, respectively, with averages of 42 and 209, respectively (Six and Jastrow, 2002).

Macroaggregates will outnumber free, non-macroaggregate associated-microaggregates in natural ecosystems, and the reverse is true in disturbed ecosystems, as studies of tillage or AM fungi suppression have shown (Mikha and Rice, 2004; Wilson and Rice, unpublished data). Arbuscular mycorrhizal and saprophytic fungi abundances are often correlated with macroaggregate abundance (Frey et al., 1999; Six et al., 2006; Wilson and Rice, unpublished data). Jastrow and Miller (2000) hypothesized that AM fungal hyphae acts as a "web" to enclose macroaggregates and Wright (2005) proposed that glycoproteins, such as glomalin, produced by AM fungi act as an adhesive to bind soil aggregates together. Thus, soil management strategies aimed at increasing or maintaining soil fungi could result in greater C sequestration. In addition to a role in macroaggregate formation, fungi produce secondary metabolites from the plant residue C that may be more resistant to further decomposition by other soil microbes (Martin and Haider, 1971; Linhares and Martin, 1978; Linhares and Martin, 1979) and become chemically bound to clay minerals within microaggregates (Simpson et al., 2004). Management enhancing soil fungal dominance could result in greater soil aggregation and soil C retention and less CO_2 release (Fig. 1.3).

General Hypotheses

H_1: Increasing levels of lignin in plant residue slow its decomposition by soil microbes, delaying its conversion to SOM and eventually CO_2 (Fig. 1.2).

H_2: Management strategies that benefit soil fungi will result in a greater proportion of macroaggregates and soil C, with less plant residue C released as CO_2 (Fig. 1.3).

Summary of Chapters 2-4

Chapter 2 - Potential for Enhanced Soil C Sequestration Using Lodging-Resistant Grain Sorghum Hybrids

Lignin has been shown to be more resistant to microbial decomposition than other plant residue components. Grain sorghum hybrids were chosen from around Kansas based on different degrees of lodging resistance, due presumably due to different lignin contents. The lignin content of each hybrid was evaluated, and the residue C mineralization kinetics were determined by conducting a laboratory incubation experiment.

H_1: Sorghum plants with higher amounts of lignin will decompose slower in soil than plants with lower amounts of lignin.

Chapter 3 - Soil Biological Properties Following Additions Of Bmr Mutant Grain Sorghum

The potential may exist for biotechnology to play a role in creating a plant with characteristics well suited to C sequestration. Grain sorghum hybrids that were either normal or natural brown midrib (bmr) mutants were used to study residue decomposition and changes in microbial communities. In a laboratory experiment, the plant residue was added to soil and the C mineralization kinetics were monitored for a duration of 194 d. Following the incubation

phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) from the soil were extracted in order to characterize the microbial community. Soil and plant residue natural abundance $\delta^{13}C$ signatures were used to estimate the plant residue mineralization extent.

H_1: The bmr mutation will result in lower lignin levels and altered lignin chemistry as compared to normal plants.

H_2: The bmr mutation will result in greater residue C mineralization as compared to the normal plants.

H_3: Adding lignin-modified plant residue to soil will not change the composition of the soil microbial community. The null hypothesis is that the lignin-modified plant residue will alter the soil microbial community.

Chapter 4 - Microbial Ecology and Carbon and Nitrogen Flux during Plant Residue Decomposition

No-till (NT) soils contain more C and have greater soil aggregation than tilled (CT) soils, possibly due to increased fungal dominance. The purpose of this experiment was to determine if soils with a higher fungal population would lead to greater retention of plant residue C and N. We added ^{13}C- and ^{15}N-labeled grain sorghum residue to NT and CT continuous sorghum plots and measured residue C mineralization, soil inorganic N, PLFA and NLFA, and incorporation of residue ^{13}C and ^{15}N into soil aggregates over a 100 d time span.

H_1: NT has a greater fungal dominated microbial community as compared to CT, leading to reduced residue C mineralization and greater C and N retention.

H_2: The different soil aggregate size classes will exhibit different amounts of both ^{13}C and ^{15}N indicating recent residue C and N retention.

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Table 1.1. Sources and sinks of the greenhouse gases CO_2, CH_4, and N_2O. Data from CAST (2005) and IPCC (2001).

Trace Gas		Amount
CO_2	**Sources**	**Pg C y^{-1}**
	Emission from fossil fuels and cement	6.3 ±0.4
	Sinks	
	Ocean-atmosphere flux	1.7 ±0.5
	Land-atmosphere flux	1.4±0.7
	Net atmospheric increase	3.2±0.1
N_2O	**Sources**	**Tg N_2O-N y^{-1}**
	Industrial	
	Combustion	0.9
	Manufacturing	0.3
	Biomass burning	0.6
	Natural ecosystems	
	Oceanic	3.0
	Terrestrial	6.0
	Agriculture	
	Synthetic fertilizer	0.9
	Grazing animals	0.6
	Biological N_2 fixation	0.1
	Crop residues	0.4
	Cultivated peat soils	0.1
	Animal waste handling	2.1
	Nitrate leaching	1.6
	Human sewage	0.2
	N deposition on ag land	0.3
	(Total Agriculture)	6.3
	Sinks	
	Photolysis/other reactions in atmos.	12.3
	Net atmospheric increase	3.9
CH_4	**Sources**	**Tg CH_4 y^{-1}**
	Natural	
	Wetland	100-200
	Termites	10-50
	Oceans	5-20
	Freshwater	1-25
	CH_4 hydrate	0-5
	Anthropogenic sources	
	Coal mining, natural gas, petrol	70-120
	Rice paddies	9-25
	Enteric fermentation	65-100
	Animal wastes	10-30
	Domestic sewage treatment	25
	Landfills	20-70
	Biomass burning	20-80
	Sinks	
	Atmospheric removal	450-600
	Oxidation in soils	15-45
	Net atmospheric increase	28-37

Table 1.2. Examples of greenhouse gas mitigation strategies based on deployment time and effectiveness at reducing C units emitted per unit of energy consumed. Adapted from Caldeira et al (2004).

	Rapidly deployable[a]	Not rapidly deployable[b]
Minor Contributor*	Expanded use of natural gas	Photovoltaics
	Hydropower	Forest management/fire suppression
	Wind without energy storage	Ocean fertilization
Major Contributor*	**Carbon storage in agricultural soils (no-till, cover crops)**	Biomass to transportation fuel
	Improved vehicle efficiency	Next generation nuclear fission
	Reforestation/land restoration	Cessation of net deforestation
	Stratospheric sulfate aerosol geoengineering	Reduced population growth
		Wind with energy storage

[a]significant fraction of technology's potential achieved in < 2 decades.
[b]unlikely to achieve significant fraction of technology's potential in < 2 decades.
*Minor contributors capable of <0.2 Pg C y^{-1}; major contributors cable of >0.2 Pg C y^{-1}

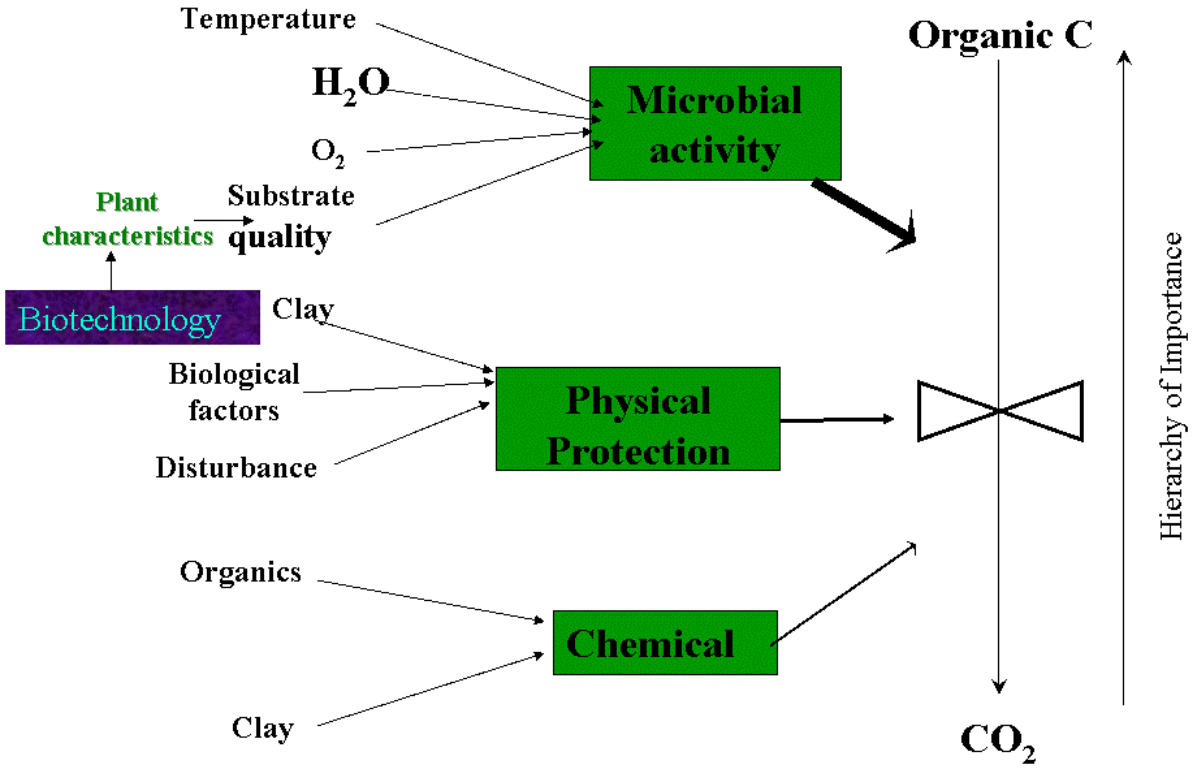
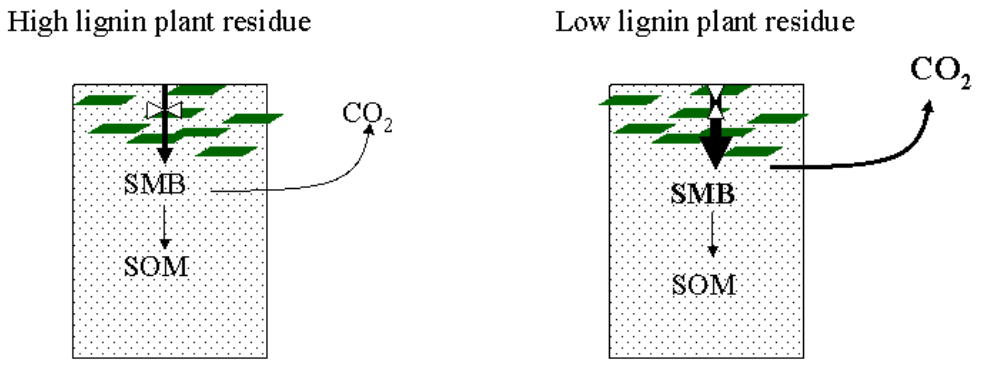


Figure 1.1. Detail of the conceptual biological, physical, and chemical controls on the conservation of soil C. From Rice and Angle, 2004. Used with permission.



SMB = soil microbial biomass
SOM = soil organic matter
Overall, less plant residue C is lost from the system as CO_2. The plant residue decomposes slower. In this model, the SOM pool does not increase.

Figure 1.2. Increasing soil C storage may be accomplished by altering the quality of applied plant residues. The same amount of plant residue C is added to the soil, but the plant residue C has a longer residence time because it is decomposed more slowly by the soil microorganisms.

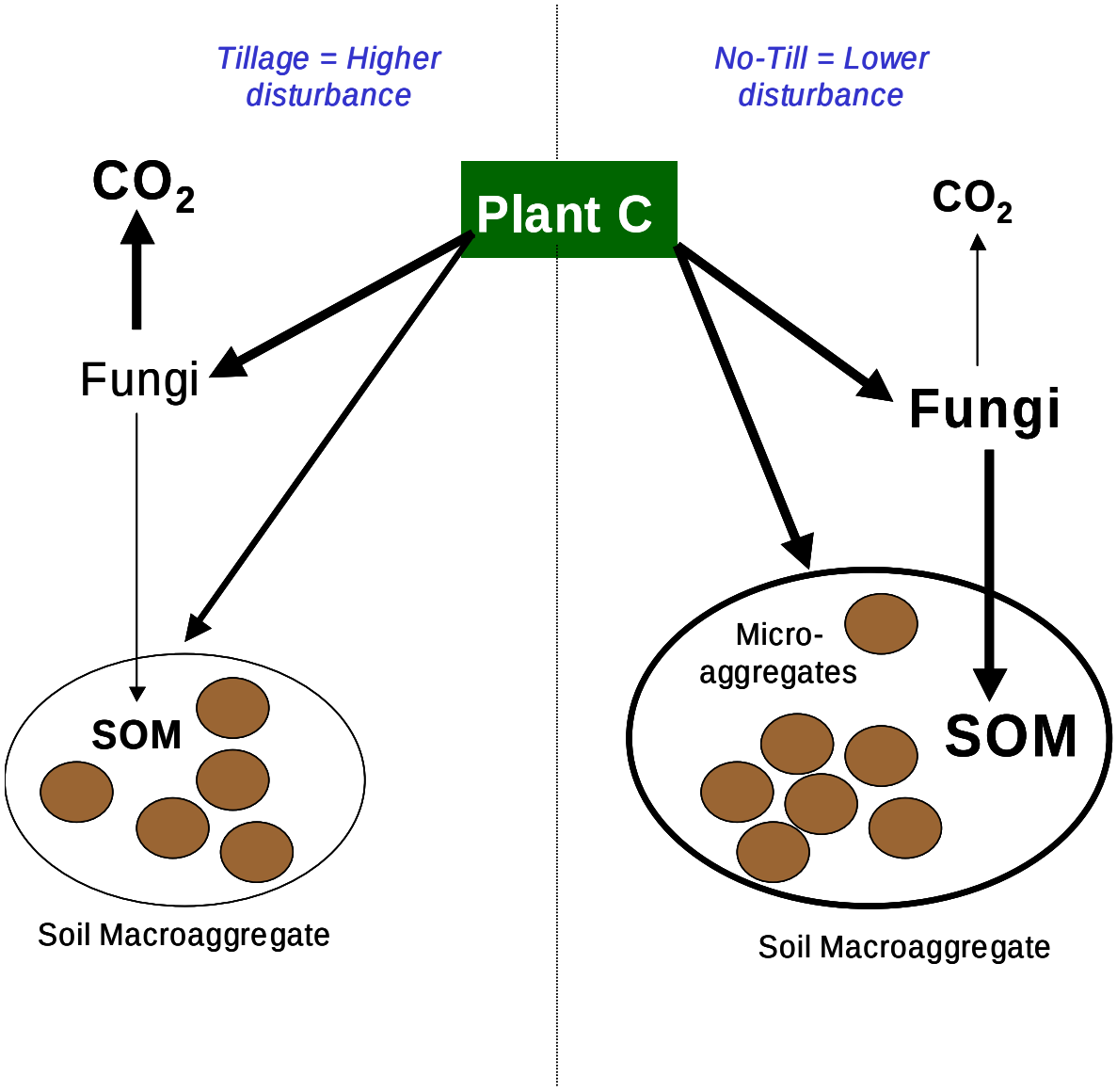


Figure 1.3. Increasing soil C may be accomplished by selecting management strategies that stimulate fungal communities and increase soil aggregation. The same amount of plant residue C is added to the soil, but fungal byproducts of decay, coupled with greater physical protection in macroaggregates, increase SOM. No-till soils also contain a higher proportion of macroaggregates, as compared to tilled soils.

CHAPTER 2 - Potential for Enhanced Soil C Sequestration Using Lodging-Resistant Grain Sorghum Hybrids

ABSTRACT

Carbon (C) sequestration may be a viable technology to assist in reducing increased atmospheric levels of CO_2. Lignin is generally more resistant to microbial decomposition, and choosing plants with increased lignin levels may lead to increased soil C sequestration. Four grain sorghum (*Sorghum bicolor*) hybrids grown in Kansas were selected for the study based on historical lodging resistance. Samples were taken from Riley, Reno, Labette, Harvey and Chase counties. Plant residue chemistry was determined on the four hybrids to assess lignin content. Grain sorghum residue from Riley County was selected for the mineralization study. Ground shoots and roots were incubated in soil, with and without added nitrogen (N), for 228 d to assess the effects of increased lignin content on C mineralization. No differences were detected between the hybrids for rapid, intermediate, or slow phases of mineralization. Nitrogen additions resulted in significantly lower intermediate and slow phases of mineralization. Mycogen 1506, which exhibited a high lignin content, resulted in the highest amount of C mineralization. The results indicate that small changes in lignin content did not alter C mineralization. However, plant residue architecture may influence C mineralization to some extent.

INTRODUCTION

Nineteenth and twentieth century fossil fuel use coupled with forest clearing have increased atmospheric CO_2 levels from 260 to 370 mmol mol^{-1} air (IPPC, 2001). Increasing soil C storage in agricultural systems can effectively reduce atmospheric CO_2 levels, and has the potential to sequester 20 to 30 Pg C over the next 50 to 100 years (Paustian et al., 1998). The increase in soil C can be achieved either by increasing inputs or decreasing outputs. Carbon sequestration is defined as the process of transforming C in the air (CO_2) into stored soil C. Plant productivity and soil microbial activity are two biological processes governing soil C dynamics (Rice and Angle, 2004).

Plant residue decomposition is a function of substrate quality, soil temperature, moisture, nutrients, and clay content. Typical plant components include soluble sugars, hemicellulose, cellulose, and lignin, which vary in proportions between plant species, within plant species, and within plant organs (Johnson and Barbour, 2002). Decomposition rates of plant components generally increase with increasing water solubility (Broder and Wagner, 1988). Decomposition is often described kinetically as a first order process, and is usually divided into separate fractions based on rate constant (k) values (Ladd and Martin, 1984). Understanding the decomposition rates of different plant components is important in terms of residence time of plant C and to which pools of soil C the components of plant C reside. Of these fractions, lignin is most resistant to decomposition, thus increasing the lignin concentration of the plant may have a positive impact on soil C storage. Hybrids of grain sorghum (*Sorghum bicolor*) grown in Kansas vary in resistance to lodging (Kansas Performance Tests, 2000-2002), possibly due to differences

in lignin levels in the stalk. The differences in lignin content may have a positive impact on soil C storage, by increasing the turnover time of the residue C upon addition to soil.

The objectives of the experiment were to (1) collect and quantify the soluble, hemicellulose, cellulose, and lignin content of four grain sorghum hybrids, and (2) determine if differences in plant components correlate with C mineralization. Our hypothesis was that lignin content would differ between high lodging and low lodging hybrids, and that the hybrids with increased lignin would decompose slower once added to soil, resulting in lower C mineralization.

MATERIALS AND METHODS

Grain sorghum collection

Grain sorghum root and shoot material was collected post-harvest from the 2002 Kansas State University Variety Performance test plots located in Chase, Harvey, Labette, Reno, and Riley counties. Plant material from grain sorghum hybrids Mycogen 1506, OK11xTX2741, TX3042x2737, and TX2752xTX430 was collected from each location. Four replications of each plant hybrid at Chase, Labette, Reno, and Riley counties, and three replications from Harvey County were collected. Historically, Mycogen 1506 and OK11xTX 2741 exhibit a higher resistance to lodging, and TX3042xTX2737 and TX2752xTX430 exhibit a lower resistance to lodging (M. Tuinstra, personal communication). Leaves were removed from the shoots and the roots collected were primarily large roots (> 1 mm in diameter) from 0-10 cm depth below the plants. The plant samples were dried at 60°C for 1 wk before being ground with a Wiley mill to pass a 1-mm screen. Plant residue chemical composition was performed according to the method of Goering and Van Soest (1970), using an Ankom™ technologies system. Total C and N were

determined on ball-milled samples by dry combustion on a Flash EA1112 (Thermo Electron Corp.) (Table 2.1). The remaining residue was stored at 25°C in sealed containers until the start of the incubations.

Soil characteristics

Smolan silt loam soil (Fine, montmorillonitic, mesic, Pachic, Arguistolls) was collected from a depth of 5 to 15 cm on 14 February 2003 from the Kansas State University (KSU) North Agricultural Farm in Manhattan, KS, sieved through a 1-mm sieve, air-dried, and stored at 4°C until use. The field had been previously cropped to a *Bromus* sp. for hay production for 50 y (C. Owensby, personal communication). A subsample was sent to the KSU Soil Testing Laboratory (Manhattan, KS) for chemical and physical analysis. The soil contained 260, 520, and 220 g kg^{-1} sand, silt, and clay, respectively, as determined by the hydrometer method (Gee and Bauder, 1986). Total C and TN were determined with the same procedures used with the plant residue and the soil contained 16.01 g TC kg^{-1} soil and 1.4 g TN kg^{-1} soil. The 1:1 soil:water pH of the soil was 7.7. A portion of the soil was extracted with 1 M KCl and the inorganic N content in the extract was determined by colorimetric analysis with values of 3 and 5 mg NH_4-N and NO_3-N kg^{-1} soil, respectively.

Soil-plant residue incubations

The soil was brought to a gravimetric water content of 0.15 g H_2O g^{-1} dry soil. Twenty-five g dry weight equivalent soil was weighed into 125-mL Erlenmeyer flask "microcosms" that were covered with parafilm to allow gas exchange and were placed in a 25°C incubator in the dark for 7 d. After the 7-d preincubation, the soil was brought to a gravimetric water content of 0.23 g H_2O g^{-1} dry soil, with either 2 mL of distilled water or 2 mL of a $(NH_4)_2SO_4$ solution to

supply 35 mg N kg^{-1} dry soil. Inorganic N was added to half of the microcosms to minimize the impacts of N limitation on C mineralization. The final soil water content corresponded to approximately -0.03 MPa. Ground (< 1 mm) plant residue was added at a rate of 15 mg residue g^{-1} dry soil, the flask contents were thoroughly mixed, and the microcosms placed into 1-L mason jars and capped with airtight lids that were fitted with rubber septa. About 50 mL of distilled water was placed into the bottom of each jar to prevent soil dehydration. Controls without plant residue (with and without N) were also included to measure the background soil C mineralized. The jars were incubated in the dark at 25°C for 228 d. Carbon mineralization was measured frequently during the 228 d incubation by sampling the headspace gas and measuring the CO_2 concentration with a Shimadzu GC-8A gas chromatograph (Colombia, MD) with a Porapak Q 80/100 mesh column (Alltech Part #2701PC). The column and injector/detector temperatures were 65 and 100°C, respectively, and the carrier gas was He. The jars were opened to purge excess CO_2 and replenish O_2 after each sampling. Cumulative CO_2 collected was natural log transformed and used to compute the % residue C mineralization during the experiment.

Experimental design

The experiment design in the laboratory was an complete factorial with five plant residue levels (4 hybrids + no-plant control) and two N levels (0 and 35 mg N kg^{-1} soil) and four replications. The experiment was set up in a randomized complete block design. Data were analyzed using PROC GLM and means were separated by t-tests; plant residue chemical composition data were compared with C mineralization data over time using PROC CORR in SAS version 9.0 (SAS Institute, Cary, N.C.). The significance level of $p<0.05$ was used to identify differences between means.

RESULTS

Plant residue chemical composition

The plant residue was harvested as soon as possible after grain harvest, which was 0, 4, 6, 5, and 9 d for Riley, Reno, Labette, Harvey, and Chase counties, respectively. The grain sorghum in Reno County had a high amount of lodging several weeks before grain harvest. Soluble material constituted about 40% of the total dry plant residue weight, followed by cellulose and hemicellulose with about 20% each, and lignin and ash made up the difference with about 5-8 and 2-8%, respectively (Fig. 2.1-2.5). When analyzed statistically there were numerous 2-way interactions including county*root/shoot and hybrid*root/shoot (data not shown). The Riley county material was used in the subsequent laboratory incubations because 1) the material was collected the same day as harvest and subjected to the least post-harvest decomposition; 2) the forage fiber analysis variability was low (Fig. 2.6); and 3) the lignin content was significantly different between hybrids (Fig 2.7).

For the Riley County samples, the main effect of plant organ was significant for soluble material, hemicellulose, and ash content. Roots contained significantly greater soluble material than the shoots, with values of 509 and 459 g soluble material kg^{-1} plant residue, respectively (Fig. 2.6). Shoots contained significantly greater amounts of hemicellulose and cellulose than roots with values of 232 and 217 g hemicellulose kg^{-1} plant residue, and 258 and 214 g cellulose kg^{-1} plant residue. The roots contained more ash than the shoots with values of 36 and 15 g ash kg^{-1} plant residue, respectively. The 2-way hybrid*plant organ interaction was significant for lignin. Mycogen 1506 roots had higher lignin content than any other hybrid plant organ combination, with a value of 77 g lignin kg^{-1} plant residue (Fig. 2.7). Mycogen 1506 shoot lignin content was 52 g lignin kg^{-1} plant residue. Hybrid TX2752xTX430 root lignin content was

greater than shoot lignin content, with values of 53 and 42 g lignin kg^{-1} plant residue. Hybrid TX3042xTX2737 roots and shoots contained 56 and 51 g lignin kg^{-1} plant residue and hybrid OK11xTX2741 roots and shoots each contained 53 g lignin kg^{-1} plant residue.

Plant residue mineralization kinetics

Total C mineralization was monitored for 228 d for each of the plant residue+soil and no plant residue controls (Fig. 2.8, 2.9). Carbon mineralization from soils receiving hybrid TX2752xTX430 showed that generally, N increased total C mineralized during the incubation, and shoots mineralized less than roots (Fig. 2.8). However, N reduced the C mineralized from soils receiving additions of hybrid TX3042xTX2737, and shoots mineralized more than roots (Fig. 2.8). Mycogen 1506 shoots mineralized more than roots, but not when N was added (Fig. 2.8). Similar to hybrid TX3042xTX2737, C mineralization from soils receiving hybrid OK11xTX2741 was lower upon additions of N (Fig. 2.8). In summary, C mineralization following plant residue additions and N were generally different between hybrids. Nitrogen did not largely affect C mineralization from the no-plant controls (Fig. 2.9).

The data was natural log transformed, and regression lines were fit to the data to maximize r^2 values (>0.92) by minimizing the sum of squares of the differences between predicted and measured data. The data best fit a two-pool first order reaction model, with a rapid and a slow phase, and an intermediate transition phase. Nitrogen did not affect the rapid, intermediate, and slow kinetics, in terms of duration, with values of 7, 76, and 145 d, respectively (data not shown). Carbon mineralization kinetics were not different between hybrids, with a mean rapid, intermediate, and slow phase mineralization rate of 0.1030, 0.0218, and 0.0064 d^{-1} (Table 2.2). Adding N significantly decreased the rate of C mineralization in the intermediate and slow phases by 51 and 40%, respectively, but had no affect on the rapid phase

(Table 2.2). Total plant residue mineralized during the experiment was estimated from the natural log transformed data. The hybrid and N rate main effects were significant (Table 2.3). Mycogen 1506 was mineralized more than TX3042xTX2737 or OK11xTX2741, but not more than TX2752xTX430. Nitrogen reduced C mineralization of all hybrids from 36 to 25% (Table 2.3).

DISCUSSION

Values reported for soluble material, hemicellulose, cellulose, and lignin are similar to those reported elsewhere (Wang et al., 2004; Chapter 3). County, hybrid, and plant organ all contributed variability, which was different for each plant component, as demonstrated by the numerous 2-way interactions. The variability was likely due to genetics and environmental conditions at each site. The differences between sites could partially be attributed to lodging, and the advanced decomposition rate of lodged material, as compared to upright. For example, the higher lignin concentrations observed at Reno County could be an artifact of the removal of other, more labile compounds in the residue prior to collection (Kononova, 1966). The similarity between the hybrid chemistries, when compared to other plants (e.g. *Quercus* sp.), could partially explain the similar plant residue mineralization kinetics. Within the Riley County dataset, the differences seen in plant residue chemistry, especially in lignin content, should have been adequate to test the hypothesis of the experiment, whether or not small changes in lignin content affect C mineralization.

The total C mineralization data for the no-plant control samples was subtracted from the plant residue + soil samples and the remaining C evolved was assumed to be derived solely from plant residue. This would assume no soil organic matter priming effect, which may not be correct (Chapter 3; Kuzyakov, 2006; Kuzyakov and Bol, 2006). Thus, the C mineralization data is best

described as an estimate. Mineralization followed first order patterns similar to those seen in experiments by others (Gilmour and Gilmour, 1985; Broder and Wagner, 1988; Hopkins et al., 2001; Wang et al., 2004) and similar to those used in many C mineralization models including Century and RothC. No differences were detected between hybrids in the rapid, intermediate, or slow phases of decomposition (Table 2.2). However, nitrogen lowered the kinetic rate during the intermediate and slow phases. This could be related to plant lignin content, as high N levels were shown to lower the production of ligninase by basidiomycetes *Phanerochaete chrysosporium* and *Trametes versicolor* (Keyser et al., 1978). The total C mineralized from the hybrids was different, as Mycogen 1506 mineralized to a greater extent than did TX3042xTX2737 or OK11xTX2741 (Table 2.3). This could be related to the C/N ratio of the Mycogen 1506 residue. Reid (1979) found that a higher C/N ratio resulted in higher lignin decomposition, and Mycogen 1506 exhibited high lignin levels (Fig. 2.7) and the highest C/N ratio (Table 2.1). Reid (1979) found that glucose additions lowered extracellular N levels in cultures, resulting in greater mineralization of lignin. However, the addition of N should have lowered the mineralization of Mycogen 1506, and this was not seen as the 2-way interaction was not significant ($p=0.6021$). One possible explanation is the complex chemistry of the plant residue along with the soil organic matter muted the effect. Another explanation is the differences in lignin content alters the physical barrier for decomposition by microorganisms. Hénault et al. (2006) found that plants with genetic mutation in lignin biosynthesis pathways decomposed more rapidly, due to more readily available plant polysaccharides.

CONCLUSIONS

Grain sorghum hybrids were shown to have different lignin contents, however, environmental parameters contributed significant variability. Overall the hybrids decomposed in

soil at rates consistent with literature. However, measured differences in Riley county grain sorghum lignin content were not consistent with residue C mineralization. Mycogen 1506, which exhibited high lignin content, mineralized greater than two other hybrids with lower lignin contents. Nitrogen significantly reduced C mineralization during the intermediate and slow phases of decomposition, possibly by suppressing lignin degradation enzymes. Future research may look at plants with larger differences in lignin, and on the suppression of lignolytic enzymes using N.

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Table 2.1 Total C and N contents of plant residue

Hybrid	TC	TN	C/N
	----- g kg^{-1} -----		
TX2752xTX430	367 a	7.73 a	48 c
TX3042xTX2737	391 a	5.38 b	73 b
Mycogen 1506	406 a	4.04 b	100 a
OK11xTX2741	400 a	7.64 a	52 c

*Means with the same letter in a column are not different ($p<0.05$).

Table 2.2. Carbon mineralization kinetics after additions of plant residue to soil.

Sorghum hybrid	Rapid	Intermediate	Slow
	---------- k (d^{-1}) ----------		
TX2752xTX430	0.1069	0.0218	0.0066
TX3042xTX2737	0.1005	0.0199	0.0061
Mycogen 1506	0.1020	0.0253	0.0066
OK11xTX2741	0.1025	0.0201	0.0063

N rate	Rapid	Intermediate	Slow
---mg N kg^{-1} soil---	---------- k (d^{-1}) ----------		
0	0.1039	0.0293 a*	0.0094 a
35	0.102	0.0143 b	0.0056 b

*Means in a column followed by the same letter are not different ($p<0.05$).

Table 2.3. The extent of plant residue mineralized. The hybrid and N rate main effects were significant while the 2-way interaction was not.

Sorghum hybrid	Mineralized
	-------%-------
TX2752xTX430	31.4 ab*
TX3042xTX2737	28.7 b
Mycogen 1506	33.3 a
OK11xTX2741	29.6 b

N rate	Mineralized
	-------%-------
0	36.3 x
35	24.7 y

*Means in a column followed by the same letter are not different ($p<0.05$).

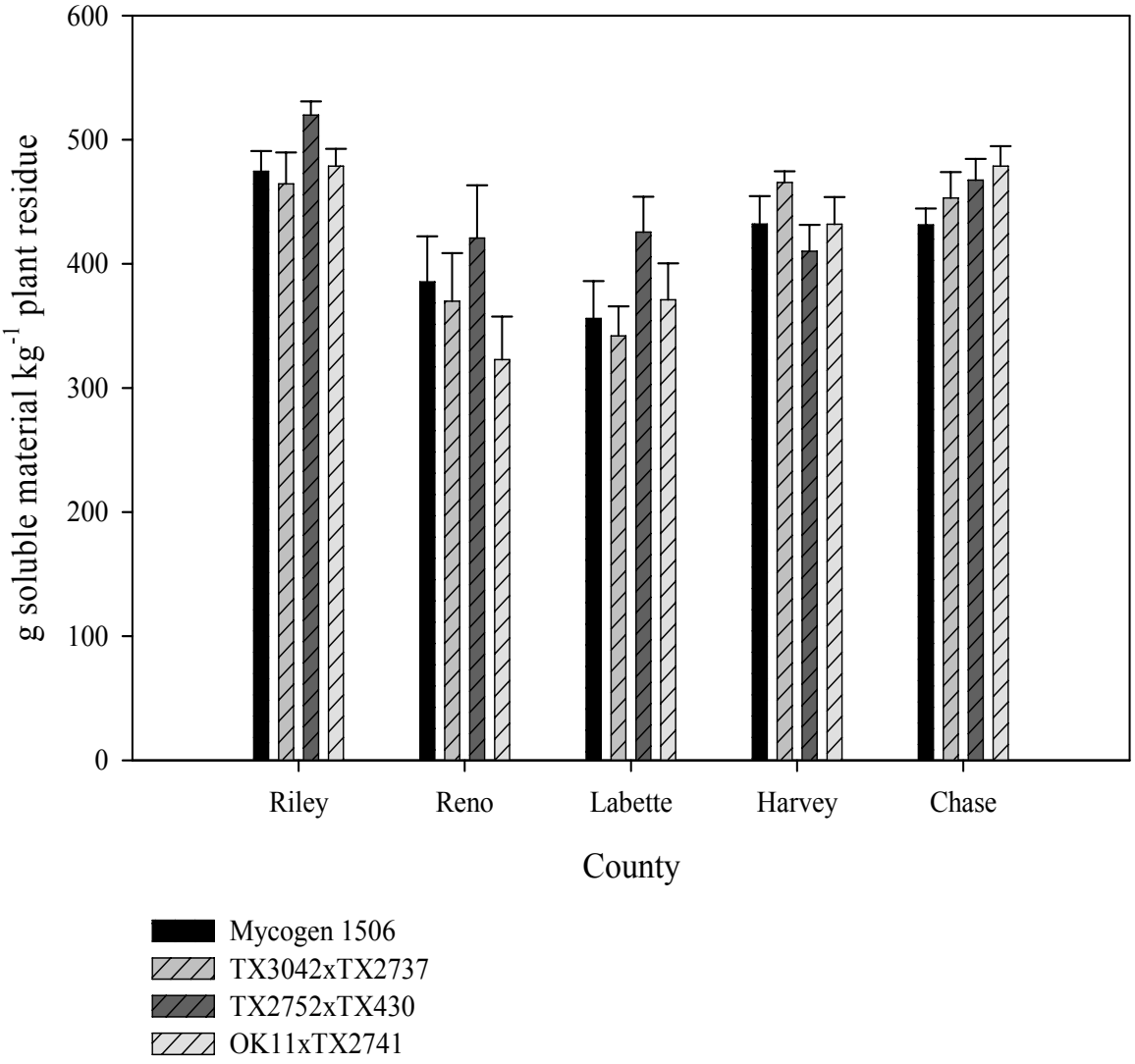

Figure 2.1. Soluble material measured in grain sorghum hybrids for five counties in 2002. Bars are means ± one standard error.

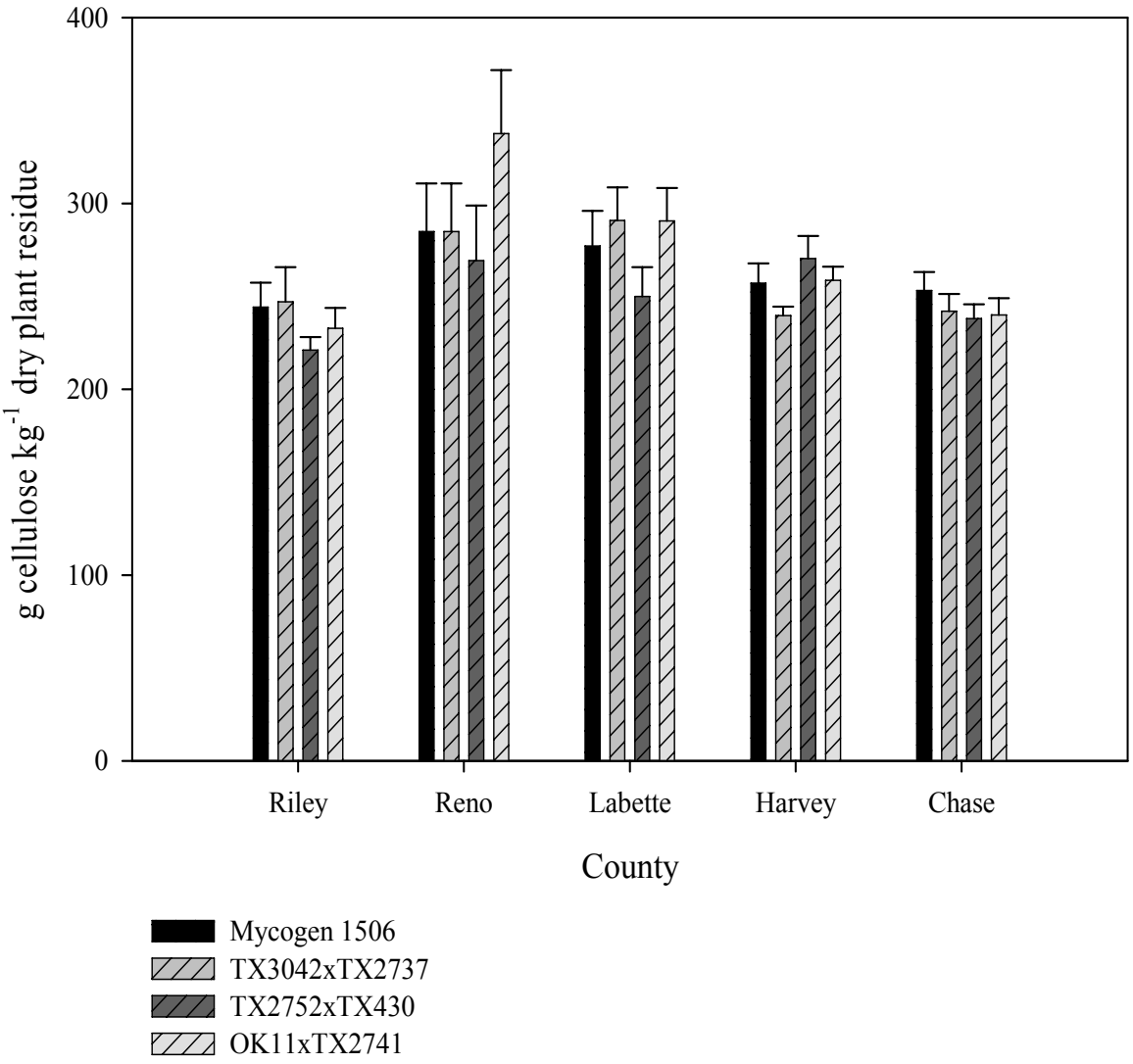

Figure 2.2. Cellulose measured in grain sorghum hybrids for five counties in 2002. Bars are means ± one standard error.

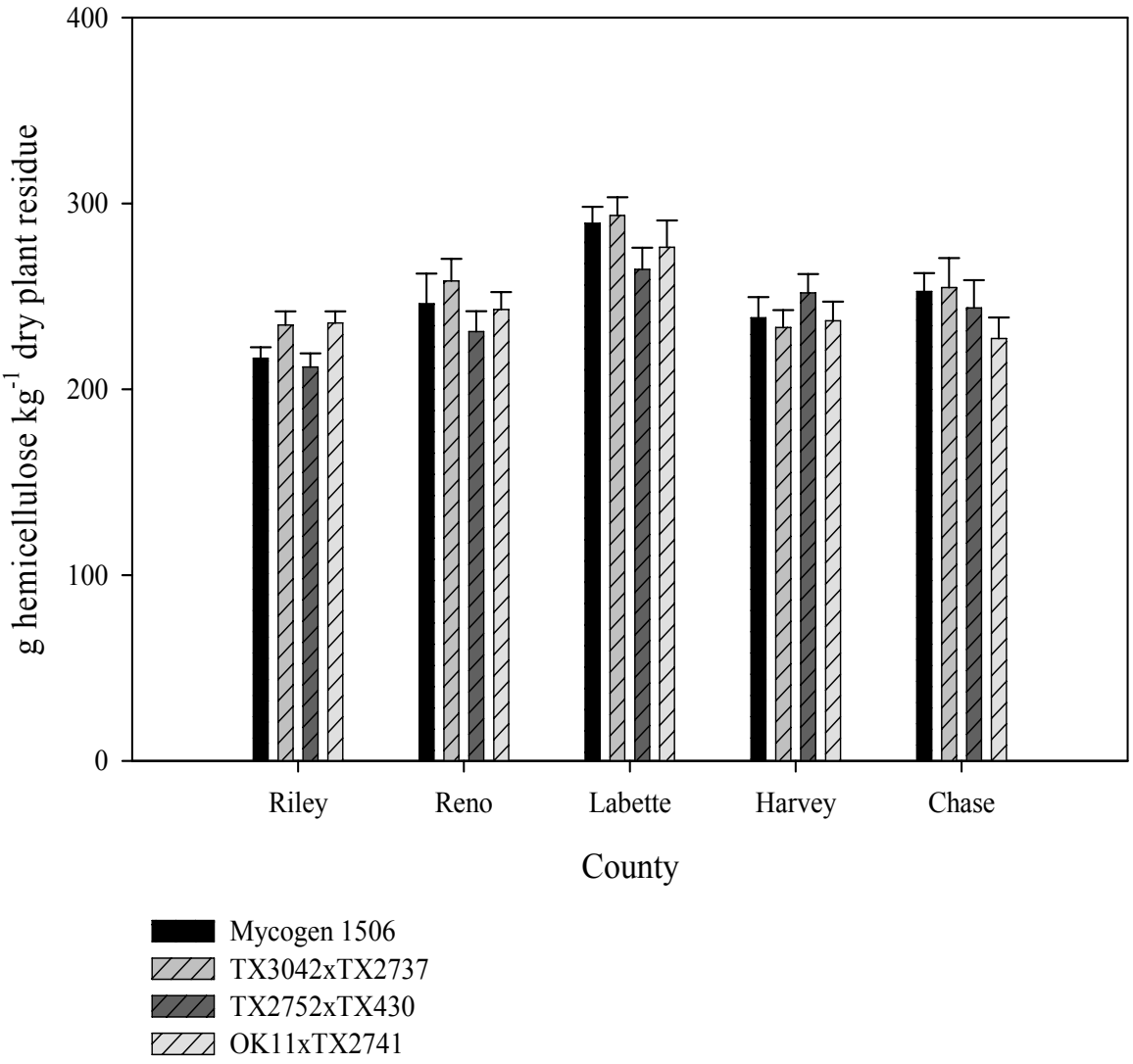

Figure 2.3. Hemicellulose measured in grain sorghum hybrids for five counties in 2002. Bars are means ± one standard error.

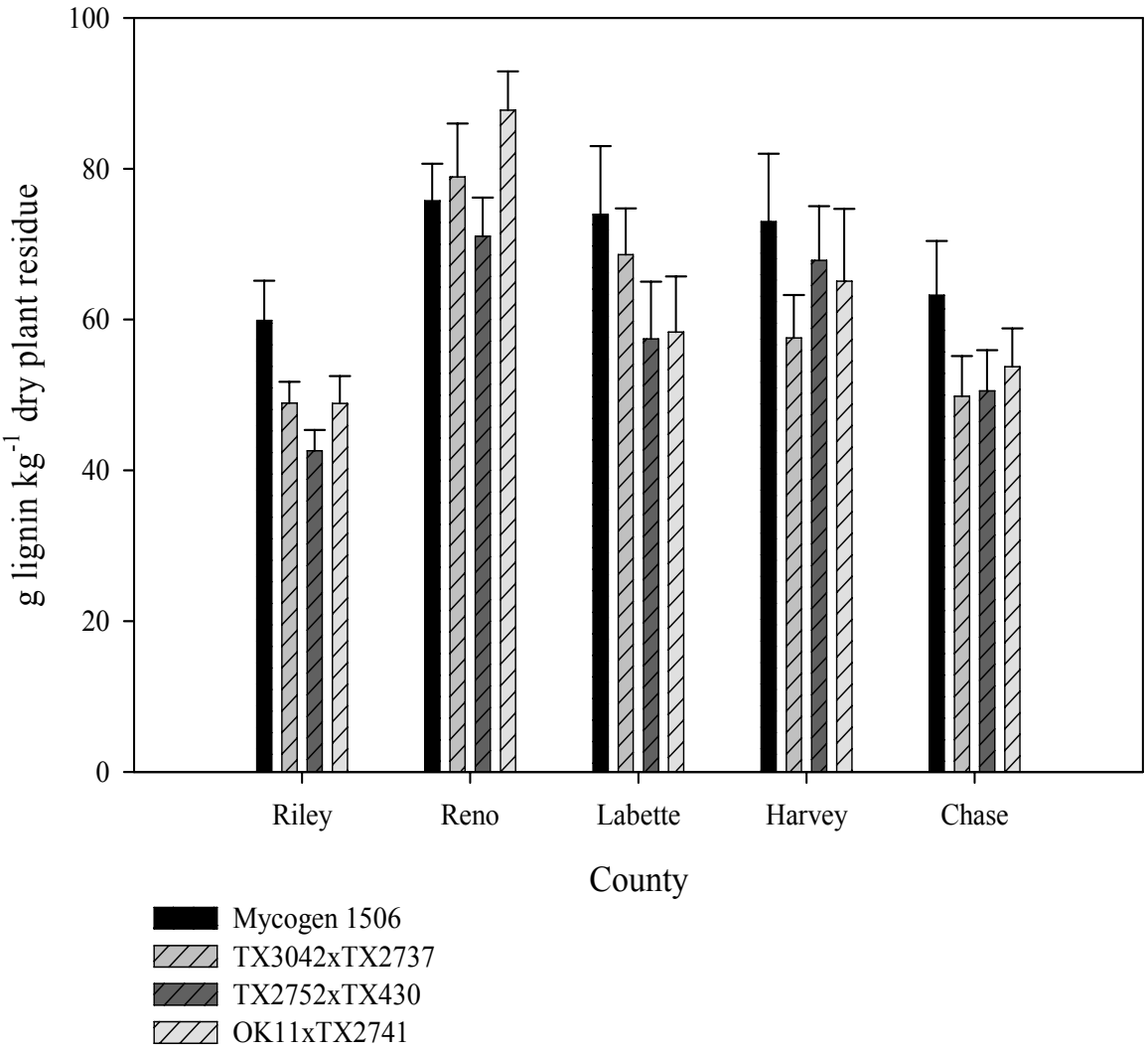

Figure 2.4. Lignin measured in grain sorghum hybrids for five counties in 2002. Bars are means ± one standard error.

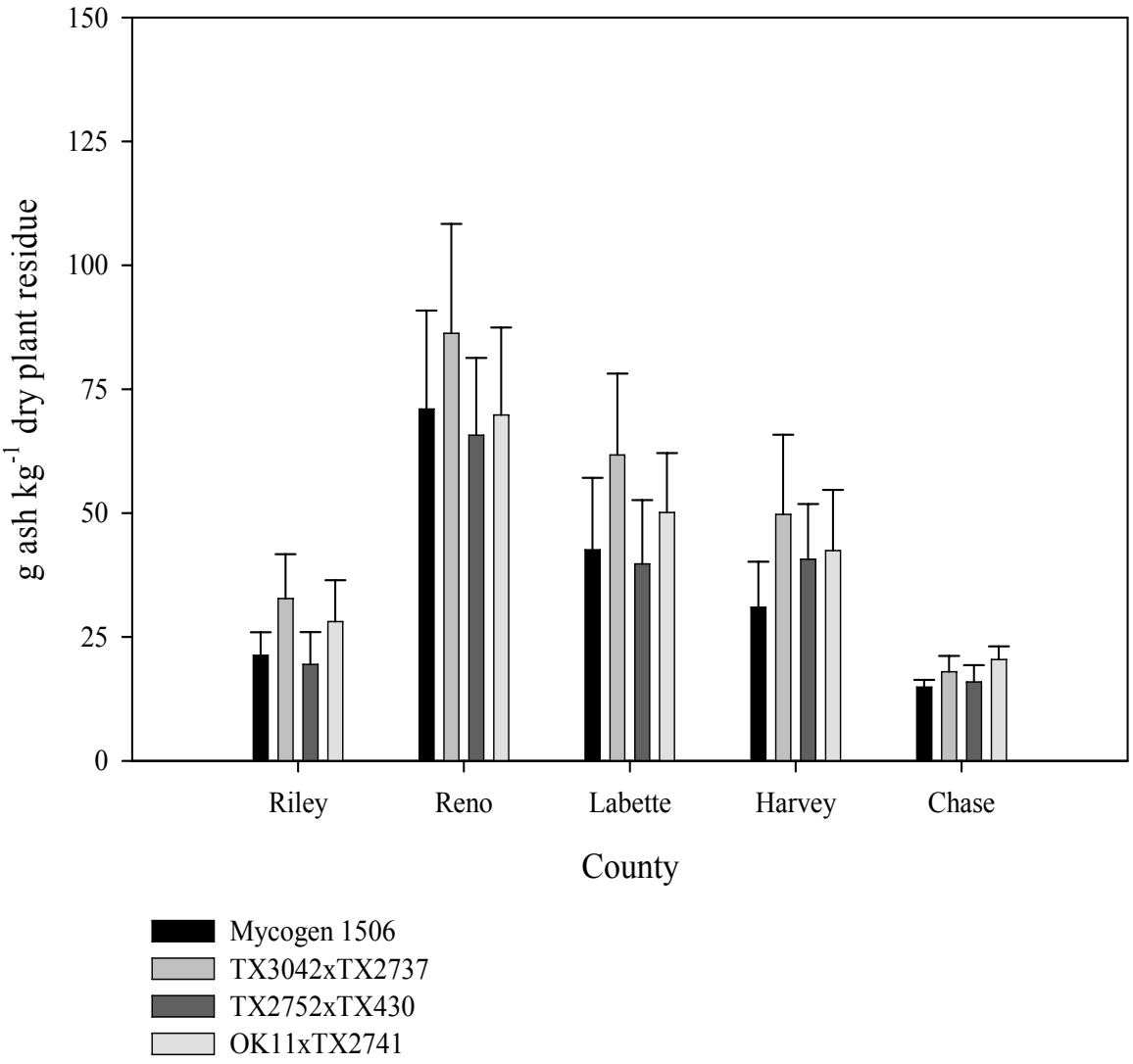

Figure 2.5. Ash content in grain sorghum hybrids for five counties in 2002. Bars are means ± one standard error.

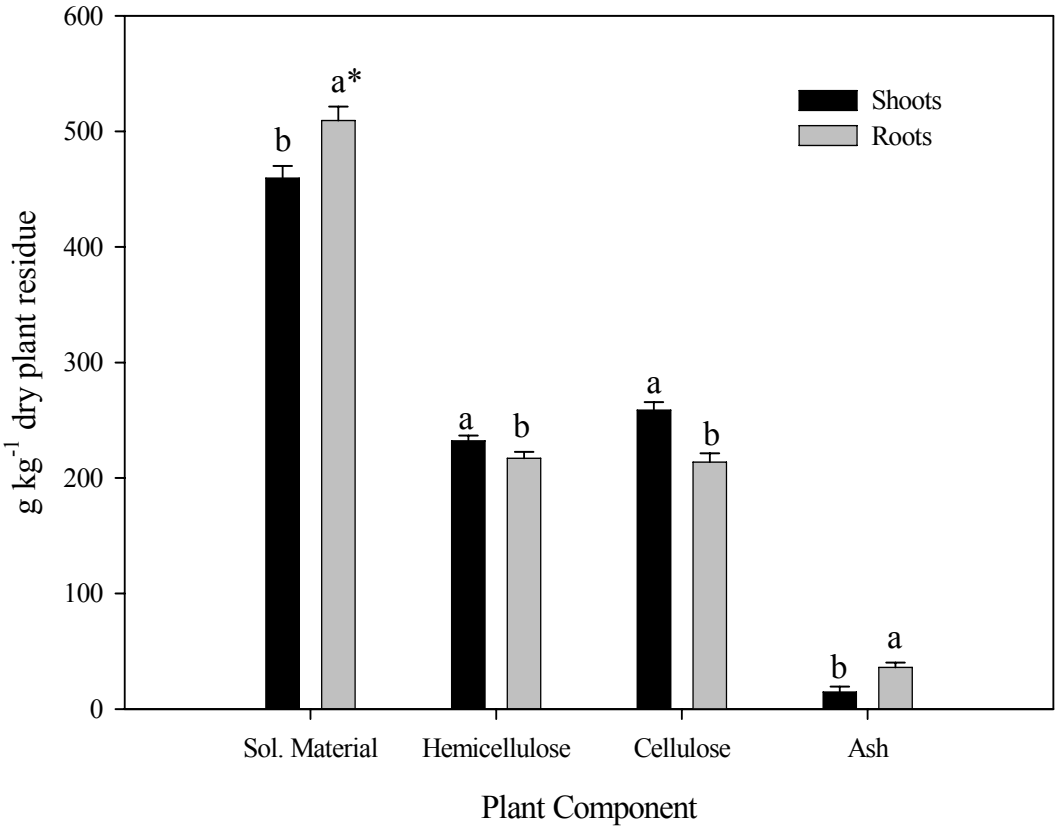

Figure 2.6. Riley County soluble material, cellulose, hemicellulose, and ash content combined over four grain sorghum hybrids. The plant organ main effect was significant. Bars are means ± 1 standard error. Bars within plant components with the same letter are not different (p>0.05).

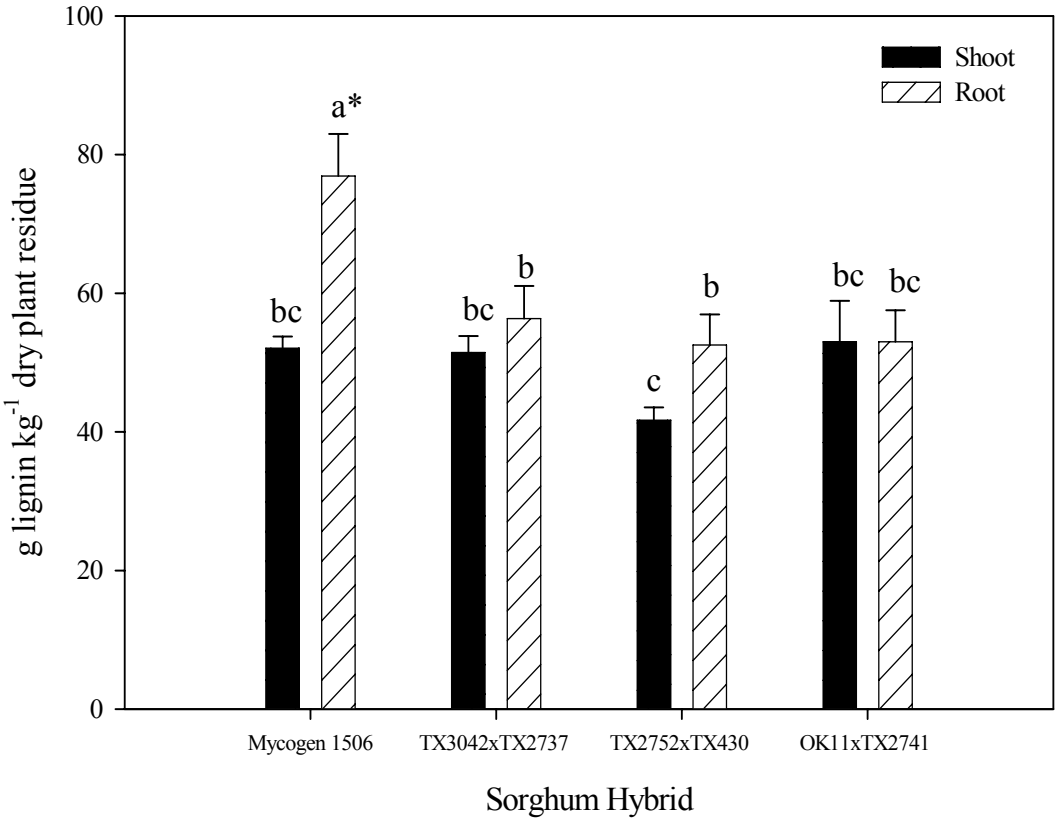

Figure 2.7. Lignin content measured in Riley County grain sorghum hybrids. The two-way hybrid*plant organ interaction was significant. Bars are means ± 1 standard error. Bars with the same letter are not different ($p<0.05$).

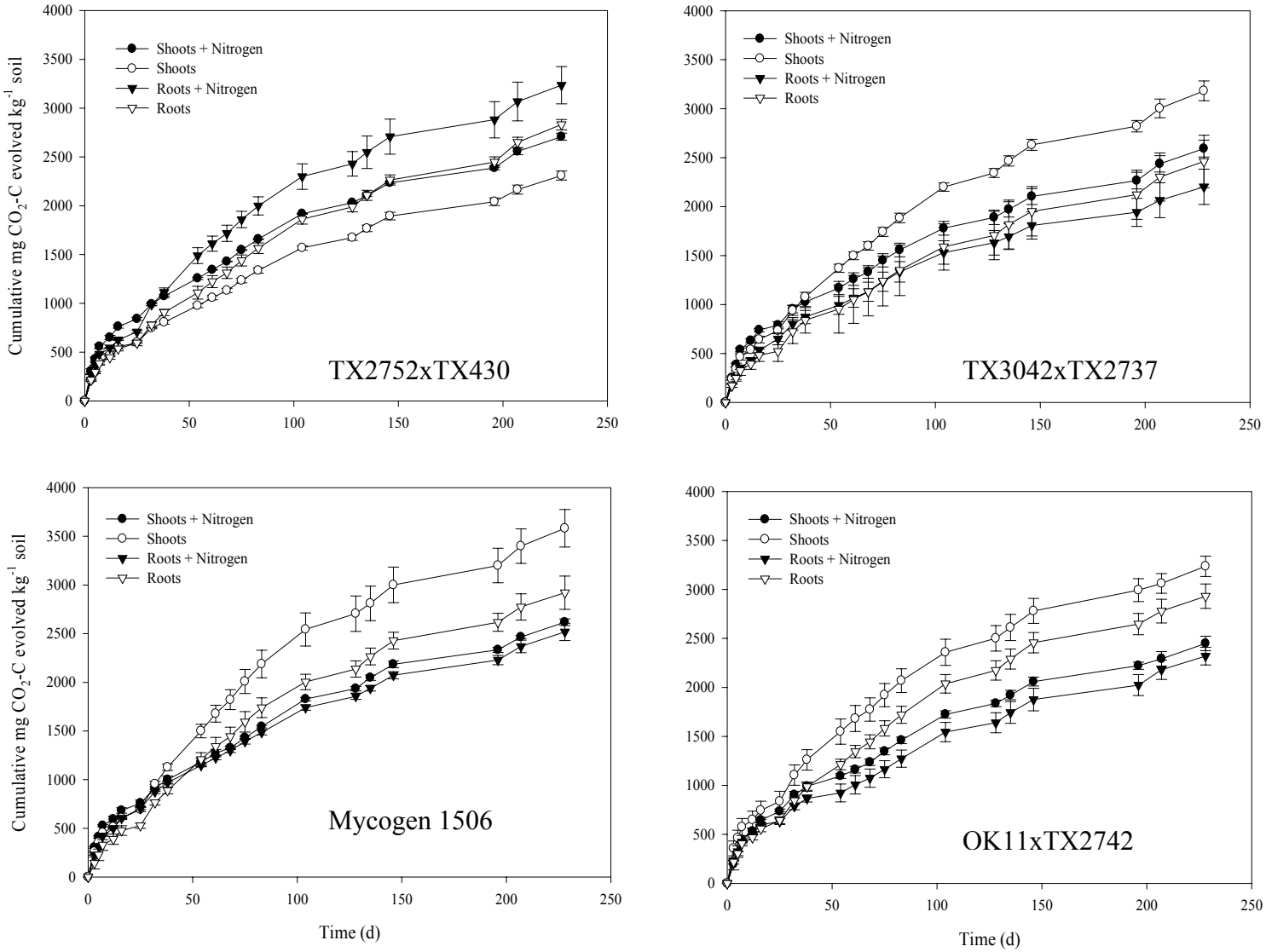

Figure 2.8. Cumulative CO_2 evolved from the four sorghum hybrids during the 228 d experiment. Values are means ± 1 standard error.

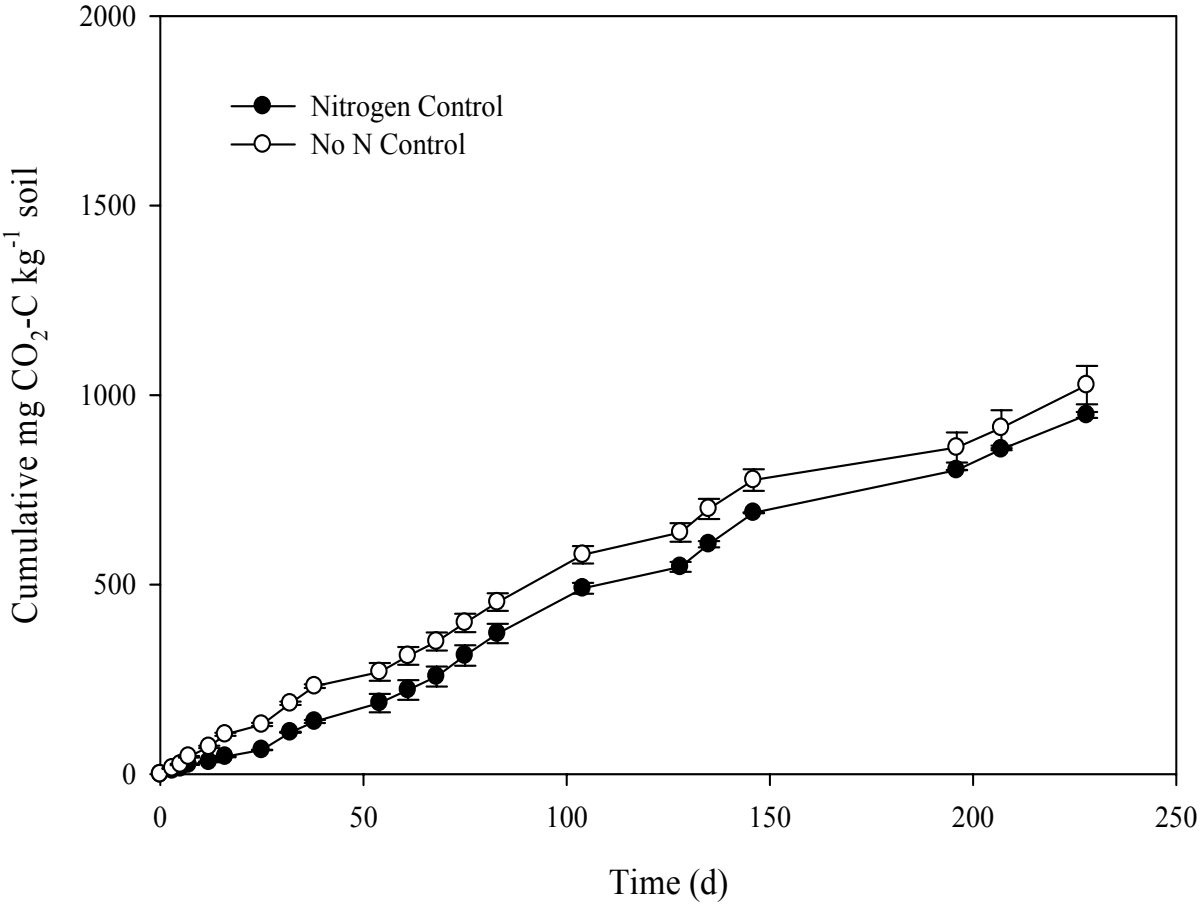

Figure 2.9. Cumulative CO_2 evolved from the control, no-plant samples during the 228 d experiment. Values are means ± 1 standard error.

CHAPTER 3 - Soil Biological Properties Following Additions of bmr Mutant Grain Sorghum

ABSTRACT

Carbon sequestration may be a viable technology to reduce increases in greenhouse gas emissions until cleaner fuel technology is available. Crop plants with increased lignin levels may lead to increased soil C sequestration. Grain sorghum exhibiting lower lignin due to the naturally occurring brown midrib mutation (bmr) may allow an assessment of the potential of biotechnology to affect soil C sequestration by manipulating plant lignin concentrations. A 194-d laboratory microcosm experiment was conducted to investigate the mineralization of grain sorghum (*Sorghum bicolor*) brown midrib mutants (bmr) and their normal isolines. Cross-polarization ^{13}C-nuclear magnetic resonance of the residue agreed with chemical analysis that the bmr residue contained altered lignin and less lignin per mass weight. Ground bmr or normal grain sorghum residue was added to soil, with or without an inorganic N amendment. Initial C mineralization from microcosms receiving bmr residue was higher than from microcosms receiving normal residue, but the differences were not maintained through the 194-d experiment. Total residue C mineralization was not different between bmr or normal isolines, and accounted for only 26% of the originally added residue C. Greater variability was observed between sorghum lines than between genotypes. The addition of N to soil resulted in increased soil C mineralization. With added N, microcosm C mineralization was most strongly correlated with hemicellulose content. With no added N, however, microcosm C mineralization was most strongly correlated with the lignin/N ratio. The soil microbial community, as assessed by

phospholipid and neutral-lipid fatty acid analysis, was not affected by plant residue genotype, but the addition of N resulted in significant changes to the soil microbial community, most notably changes to the soil fungi. Results indicate that potential does exist to modify plant residue chemistry to increase soil C sequestration, but soil fertility and microbial community dynamics are important considerations.

INTRODUCTION

Over the past century, carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) have increased rapidly as a result of human activity (IPCC, 2001), and these gases are considered the major contributors to potential global warming (CAST, 2004). The largest flux of C into and out of the atmosphere is with the plant-soil system. A better understanding of the biological mechanisms and controls on belowground C flux and retention could lead to knowledge that would enhance soil C sequestration (Rice et al., 2004). In agro-ecosystems, a substantial proportion of above- and belowground plant residue remains in the field following harvest. Decomposition of this residue and the incorporation of residue C into soil C pools is the primary input of C into soil, whereas residue and fertilizer additions constitute N inputs.

Lignin, and other aromatic structures such as fungal byproducts, decay more slowly than other plant components (Kononova, 1966; Martin and Haider, 1971; Linhares and Martin, 1978; Linhares and Martin, 1979). Provided that total dry matter is not reduced, increasing the proportion of plant dry matter allocated to lignin may have a positive impact on soil C storage. Additionally, increased lignin content in residues may provide fungi with a competitive advantage over other soil microorganisms and protect residue C from decomposition within stable soil aggregates (Rice et al., 2004; Six et al., 2004). However, lignin is not generally desirable in plants due to its low utilization efficiency in the paper production and animal feed industries (Ralph et al., 1998).

The naturally occurring mutation, known as brown midrib (bmr), results in plants with lower levels of lignin. These bmr mutants may be utilized more efficiently as a silage (Marita et al., 2003). Genes involved in lignin down-regulation include cinnamyl alcohol dehydrogenase (CAD), caffeic acid O-methyltransferase (COMT), and cinnamoyl CoA-reductase (CCR) (Marita

et al., 1999; Hopkins et al., 2001; Marita et al., 2003). These genes are responsible for modifying the side groups present on the 4, 5, and 6 C of the aromatic ring structure of the phenylpropanoid lignin precursor. Cinnamyl alcohol dehydrogenase is involved in the conversion of p-coumaraldehyde, coniferaldehyde, and sinapaldehyde into p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, respectively, which are subsequently polymerized into lignin (Croteau et al., 2000; Ralph et al., 2001). Down regulation of CAD leads to plants with similar amounts of lignin but altered chemistry due to increased aldehyde abundance (Ralph et al., 2003). Caffeic acid O-methyltransferase catalyses the conversion of caffeic acid to ferulic acid (Crouteau et al., 2000) and COMT down regulation results in altered lignin chemistry due to a decrease in syringyl phenylpropanoid units and formation of 5-hydroxy-guaiacyl units (Atanassova et al., 1995). Decreased CCR expression results in reductions in total lignin and altered lignin (Piquemal et al., 1998; Hopkins et al., 2001) Decreasing the production of lignin precursors by selecting for mutants with lowered lignin gene expression might result in a plant tissue more readily mineralized by soil microorganisms. Modified lignin may afford less physical protection to the plant residue from microbial decay (Hénault et al., 2006). More readily accessible polysaccharide may result in faster decomposition of lignin modified plants (Webster et al., 2005). More rapid mineralization is not desirable in the context of C sequestration, but studying mineralization of lignin-modified residue may generate information about the importance of lignin in soil C cycling. To date, little is known about the effects of down-regulation of lignin biosynthesis in grain sorghum on soil C content or on the soil microbial community. Hopkins et al. (2001) studied the mineralization of non-bmr and bmr tobacco (*Nicotiana tabacum* L.), and found that bmr mutants mineralized faster than non-bmr isolines. However, the tobacco was

specifically targeted for genetic mutation, and results obtained with naturally occurring mutations may differ.

Thus, the objectives of the research reported here were to (1) determine if the naturally occurring bmr mutant grain sorghum has altered lignin content and chemistry; (2) determine if the altered lignin changes the mineralization kinetics of C after additions of normal and bmr grain sorghum residue to soil; and (3) determine the effects of bmr mutation on residue C mineralization, soil C levels, and microbial community structure. We hypothesized that bmr residue will have altered lignin content and/or chemistry and that will result in (1) more rapid or complete residue C mineralization and (2) lower retention in soil of residue C.

MATERIALS AND METHODS

Plant materials and residue chemical analyses

The sorghum test was planted at the KSU East Central Experiment Field near Ottawa, KS, on 29 May 2003. The field was fertilized with 100 kg N ha^{-1} before planting and weeds were controlled using 1.14 kg S-metolachlor ha^{-1} and 0.91 kg Atrazine (1,3,5-Triazine-2,4-diamine) ha^{-1}. The sorghum hybrids were evaluated in single row plots (0.75 m x 6.1 m) with four replications using a randomized complete block design at a population of 170,000 plants ha^{-1}. Whole plant samples for laboratory analyses were harvested at the end of the season when grains had dried to at least 0.15 g H_2O g^{-1} grain (20 September 2003).

Three field replications of four genetic lines were used for the laboratory experiment. Each line tested included the normal isoline and the naturally occurring bmr mutant. The grain was removed, and the remaining stover was chopped, dried, and ground to pass a 1-mm screen. The plant residue was analyzed for total C (TC) and N (TN) by dry combustion on a Flash EA

1112 (ThermoElectron, Waltham, MA) and analyzed for $\delta^{13}C$ signature using a Europa Anca SL - 20-20 isotope ratio mass spectrometer system (Sercon, Crewe, UK). All $\delta^{13}C$ data reported here is referenced to the Vienna Pee Dee Belemnite standard (1.12372% ^{13}C or 0‰) (Table 3.1). The grain sorghum residue was analyzed according to the forage fiber methodology of Goering and Van Soest (1971) for total soluble matter, cellulose, hemicellulose, lignin, and ash content (Table 3.1). A portion of the grain sorghum residue samples (1 replicate of each line) was analyzed using cross-polarization magic angle spinning ^{13}C-nuclear magnetic resonance (CPMAS-^{13}C-NMR). The analysis was conducted to measure changes in plant chemistry between the normal line and the corresponding bmr mutant for that line. The analyses were completed with a Varian Unity 200 spectrometer (Varian Inc, Palo Alto, CA) operating at a ^{13}C frequency of 50.3 MHz. A measured mass of finely ground sample (300 to 400 mg) was packed into a 7-mm cylindrical zirconia rotor with Kel-F end-caps and spun at 5000 ±100 Hz. A 1-ms contact time and a 5-s recycle delay (>7 times the longest T1H value in the samples) were used for all samples, and between 2,500 and 10,000 transients were collected. Total signal intensities and the proportions found within given chemical shift regions were defined by integration. Chemical shifts were externally referenced to the methyl resonance of hexamethylbenzene at 17.36 ppm. The proportion of NMR observable C in each sample was assessed using glycine as an external standard and comparing the amount of the sample signal intensity per unit of sample C to that of the glycine after correcting for variations in spin lattice relaxation in the rotating frame (T1ρH), according to Smernik and Oades (2000a, b). Differences in the distribution of NMR signal intensity across the 300 to 50 ppm chemical shift range were used to determine specific changes in plant residue chemistry (Table 3.2).

Incubations

Smolan silt loam soil (Fine, montmorillonitic, mesic, Pachic, Arguistolls) was collected from a depth of 5 to 15 cm on 14 February 2003 from the Kansas State University (KSU) North Agricultural Farm in Manhattan, KS, sieved through a 1-mm sieve, air-dried, and stored at 4°C until use. The field had been previously cropped to a C_3 plant (*Bromus* sp.) for hay production for 50 y. A subsample was sent to the KSU Soil Testing Laboratory for chemical and physical analysis. The soil contained 260, 520, and 220 g sand, silt, and clay, respectively, kg^{-1} soil as determined by the hydrometer method (Gee and Bauder, 1986). Total C, TN, and $\delta\ ^{13}C$ were determined with the same procedures used with the plant residue; the soil contained 16.01 g TC kg^{-1} soil and 1.4 g TN kg^{-1} soil, and had a $\delta^{13}C$ signature of -20.32‰. The 1:1 soil:water pH of the soil was 7.7. A portion of the soil was extracted with 1 M KCl and the inorganic N content in the extract was measured using a colorimetric analysis with values of 3 and 5 mg NH_4-N and NO_3-N kg^{-1} soil, respectively. A 5-cm diameter x 15-cm deep soil core was removed from the same location on 11 November 2003, sieved through a 2-mm sieve, and added to the previously collected soil as an inoculant. The ratio of dried to fresh soil was about 20:1. The entire soil mixture was immediately brought to a gravimetric water content of 0.15 g H_2O g^{-1} dry soil. Twenty-five g dry weight equivalent soil was weighed into 125-ml Erlenmeyer flask "microcosms" that were covered with parafilm to allow gas exchange and were placed in a 25°C incubator in the dark for 25 d. The pre-experimental incubation was conducted to allow the soil microbial population to reestablish in the soil that had previously been allowed to air dry.

After the 25-d preincubation, the soil was brought to a gravimetric water content of 0.23 g H_2O g^{-1} dry soil, with either 2 ml of distilled water or 2 ml of a $(NH_4)_2SO_4$ solution to supply 35 mg N kg^{-1} dry soil. Inorganic N was added to half of the microcosms to minimize the impacts

of N limitation on C mineralization. The final soil water content corresponded to approximately -0.03 MPa. Plant residue was added at a rate of 15 mg residue g^{-1} dry soil, the flask contents were thoroughly mixed, and the microcosms were placed into 1 l mason jars and capped with airtight lids that were fitted with rubber septa. About 50 ml of distilled water was placed into each jar to prevent soil dehydration. No-plant-residue controls, with and without N, were also included to measure the background soil C mineralized. The jars were incubated in the dark at 25°C.

Carbon mineralization kinetics

Carbon mineralization was measured frequently during the 194 d incubation by sampling the headspace gas and analyzing the CO_2 concentration with a Shimadzu GC-8A gas chromatograph (Colombia, MD) with a Porapak Q 80/100 mesh column (Alltech Part #2701PC). The jars were opened to purge collected CO_2 after each headspace sampling and replenish O_2. The CO_2 headspace data was use to estimate C mineralization kinetics but not used quantitatively due to losses of microcosm C during the periodic purging of CO_2 from the jars and error introduced by high laboratory CO_2 levels (>550 μmol mol^{-1} air).

Evaluation of the priming effect

To measure plant residue mineralization, the C mineralized from the soil organic matter (SOM) as a result of adding plant residue needs to be quantified and subtracted from the total C mineralization measured. The percentage of microcosm C attributed to the plant residue (%X) following the 194 d experiment was calculated according to Gregorich et al. (1995):

$$\%X = (\delta - \delta_b)/(\delta_{gsr} - \delta_b) \times 100$$

where $\delta = \delta^{13}C$ value of whole soil after the 194 d experiment, δ_b = -20.32‰, the $\delta^{13}C$ value of pre-experiment soil, and δ_{gsr} = the $\delta^{13}C$ value of the pre-experiment grain sorghum residue.

Soil microbial community analysis

Changes to the soil microbial community were assessed at 194 d by measuring the soil phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) content. The PLFA are indicators of viable microbial biomass (Kennedy and Gewin, 1997) whereas the NLFA are indicators of soil fungal cell composition (Harwood and Russell, 1984). After measuring headspace CO_2 on day 194, 10 g of the moist soil was immediately frozen at -20°C and lyophilized. The total lipids were extracted from the lyophilized soil following the methods of White and Ringelberg (1998). The total lipid extract was then separated into PLFA and NLFA using silicic acid chromatography; the fatty acids were then cleaved from the glycerol backbone by KOH saponification; and the harvested fatty acids were then methylated to form fatty acid methyl esters (FAME) (Allison and Miller, 2005). The resulting FAME were analyzed with a Hewlett Packard 6890 GC with a 5871 Mass Selection Detector (MSD) (Palo Alto, CA). The gas chromatograph analyzed a 1 µl splitless injection where the inlet temperature was 230˚C, the gas chromatograph to MSD interface was 280˚C, helium was the carrier gas, and the column used was an Agilent Ultra-2 (Cross-linked 5% PH ME) 25-m length x 0.2-mm ID x 33-µM film thickness (Palo Alto, CA). The temperature program was 80˚C for 1 min, ramp 20˚C min^{-1} to 155˚C, ramp 5˚C min^{-1} to 270˚C, and hold for 5 min (33 min total run time). Peaks were identified using commercially available standards and the Wiley 138K mass spectral database. Sample peaks were quantitated based on comparison of the abundance with the internal standard nonadecanoic acid methyl ester. The abundance data was converted to mole % before statistical analysis. The nomenclature used to describe the identified fatty acids (FA) is as follows (Bossio

and Scow, 1998): total number of C atoms:number of double bonds, the position of the double bonds, ***cis*** or ***trans*** isomers identified by **c** or **t**. Prefixes of **a**, **i**, and **Me** indicated anteiso-branching, iso-branching, and methylation, respectively. For example, the molecule a10Me18:1ω6c would be 18 C FA with a CH_3 branch 2 C from the tail end of the FA, a double bond in the ***cis*** configuration at the 6 position from the tail, and another CH_3 branch at the 10 C from the head of the FA.

Experimental design

The grain sorghum residue obtained from the field was a complete block design with three replicates. There were nine residue levels: Line 2 (normal and bmr), Line 12 (normal and bmr), Line 26 (normal and bmr), Line 28 (normal and bmr), and no-plant residue control. There were two N levels: 0 or 35 mg N kg^{-1} soil. The experiment design in the laboratory was a randomized complete block design using the field replicates as laboratory replicates. Data was analyzed using PROC GLM and means were separated by t-tests; plant residue chemical composition data was compared with C mineralization data over time using PROC CORR in SAS version 9.0 (SAS Institute, Cary, N.C.). The significance level of $p<0.05$ was used to identify differences between means.

RESULTS

Plant residue chemistry

Sorghum line (2, 12, 26, and 28) effects and genotype (normal vs. bmr) effects were important throughout the experiment. Total C contents differed slightly between lines and genotypes, with a range from 404 to 423 mg C g^{-1} residue (Table 3.1). Total N differed more widely, with values of 4.33 to 9.43 mg N g^{-1} residue, which resulted in a wide range of C/N

ratios from 54 to 90. The $\delta^{13}C$ signature of the plant residue ranged from -11.18 to -11.66‰, which is in the range for C_4 plants (Bender, 1970). Soluble matter consisted of about 400 mg g^{-1} plant residue. Cellulose and hemicellulose constituted most of the remaining plant residue with values ranging from 239 to 297 and 165 to 216 mg g^{-1} plant residue, respectively. The main effect of genotype was significant for plant residue lignin content differences, as the normal lines had more lignin than the bmr mutants with mean values of 79 and 55 mg g^{-1} plant residue, respectively. Ash content was about 72 mg g^{-1} plant residue.

Chemical data obtained from NMR spectroscopy revealed that the most abundant component was O-alkyl C, but there was no trend between genotypes, and values were >530 mg C g^{-1} TC. Di-O-alkyl C content was between 120 and 142 mg C g^{-1} TC (Table 3.2). Line 28 exhibited greater N-alkyl + Methoxyl C content than did the other lines with mean values of about 102 and 53 mg C g^{-1} TC, respectively, for Line 28 and the mean of Lines 12, 2, and 26. Alkyl C values ranged from 36 to 80 mg C g^{-1} TC, amide-carboxyl C ranged from 42 to 55 mg C g^{-1} TC, and ketone C accounted for <5 mg C g^{-1} TC. In terms of possible lignin components, aromatic C values ranged from 74 to 98 mg C g^{-1} TC whereas phenolic C values ranged from 23 to 36 mg C g^{-1} TC. A detailed inspection of the 165 to 110 ppm aryl/O-aryl region revealed that there were some consistent differences between normal and bmr mutants (Figure 3.1, Table 3.3). Data for all lines was combined to allow for an analysis of genotype effects on the aryl regions, and significant differences were found in the 141 to 132 ppm, 158 to 152 ppm, and 162 to 158 ppm chemical shift regions in which lignin subunits resonate (Table 3.3).

Carbon mineralization

Carbon mineralization after plant residue additions followed a first order model, with rapid and slow fractions, with an intermediate transition phase (Figures 3.2-3.5). The no-plant-

residue control samples exhibited a constant C mineralization after a short initial period characterized by more rapid C mineralization, most likely associated with the liquid additions of water or $(NH_4)_2SO_4$ and subsequent mixing (Figure 3.6). For determining mineralization kinetics, data for the no-plant-residue control samples was subtracted from the plant residue+soil samples. The subsequent data was natural log transformed, and regression lines were fit to each of the three phases to maximize r^2 values ($\geq$0.92) by minimizing the sum of squares of the differences between predicted and measured data. The N and sorghum line main effects were significant for several phases and the time of the phase (Table 3.4). The addition of N increased the rapid fraction mineralization kinetics (k) from 0.0582 to 0.0642 d^{-1} (Table 3.4). Nitrogen also increased the duration of the rapid phase, but, decreased the duration of the intermediate transition phase, and increased the duration of slow phase, as compared to the no N treatment. The different sorghum lines differed in their slow k value, with values from 0.0017 to 0.0029 d^{-1} (Table 3.4). The genotype x line interaction was significant as there was some variability between the normal and bmr-mutant plant residue, but the differences seemed to be present only in the initial stages of the experiment and, by the slow phase, there were no differences in mineralization kinetics (Table 3.5). For lines 12, 2, and 26, the bmr mutant had a higher rapid k than did the normal isoline. In line 28, the values for rapid k were not different between normal and bmr mutant. Nitrogen reduced and increased the intermediate transition phase k value for line 2 and line 28, respectively, and reduced the slow k for lines 2 and 26. (Table 3.5). No difference was detected in the intermediate transition phase time between the normal or bmr mutant-plant residue with no N added, but in the + N treatment, the values were higher for the normal, as compared to the bmr mutant-plant residue (Table 3.5).

Correlation between plant residue chemistry and C mineralization

For the +N treatment, the soluble matter was positively correlated with microcosm C mineralization until about d 18, and hemicellulose content was most strongly negatively correlated with microcosm C mineralization until d 74 and again during the last 60 d of incubation (Table 3.6). Lignin content was only significantly correlated with C mineralization when expressed as a ratio (lignin to soluble matter, lignin to hemicellulose), which could be an additive artifact of the forage fiber analysis, which subtracts mass loss for each successive determination of soluble matter (neutral detergent fiber remaining), cellulose (acid detergent fiber remaining), and lignin (acid digestible lignin), with the balance going to hemicellulose. For the no-N-added treatment, the ratio of lignin to residue total N content (lignin/N) was the plant parameter most strongly correlated to C mineralization for the first 88 d of the incubation (Table 3.7). As the lignin/N ratio increased, C mineralization decreased. The residue total N and lignin correlated with C mineralization only until d 39 and d 16, respectively. Therefore, the lignin/N ratio may be a good predictor of C mineralization potential in soil ecosystems with no extraneous N inputs. The plant residue C/N ratio was correlated with C mineralization, but not for as long a duration as the correlation was observed for the lignin/N ratio. Cellulose content was positively correlated with C mineralization from d 5 to d 25, indicating a possible C source for decomposing microorganisms. In contrast to the +N treatments, it was only toward the end of the incubation (116 d) that the soluble matter content correlated positively with C mineralization. Ash content also was positively correlated with C mineralization during the final 2/3 of the incubation.

Carbon mass balance

A C mass balance was constructed to estimate the losses of C from the soil and added residue as a function of sorghum line and genotype (Table 3.8). The proportion of residue C in the microcosms at 194 d was calculated from ^{13}C isotope data and ranged from 0.194 to 0.285 mg C mg^{-1} added C (Table 3.8). The variability was mostly found within the sorghum lines and not between genotypes; residue C in lines 28 and 2 was significantly higher than in line 12, and the residue C line 26 was not different than in lines 28, 2, or 12, with means of 0.195, 0.253, 0.217, and 0.232 mg residue C g^{-1} microcosm C, respectively, for lines 12, 2, 26, and 28. The amount of residue C that was mineralized from the microcosms varied between 0.99 and 2.24 mg C g^{-1} soil, which represented 0.171 and 0.392 mg mineralized residue C mg^{-1} residue C added. Values for amounts of soil C mineralized were similar to amounts of residue C mineralized, and ranged from 0.79 to 1.68 mg C g^{-1} soil.

The N main effect was important for several aspects of the C balance, however no 2 way or 3-way interactions between N and line and/or genotype were significant (p=0.4965). The +N treatment resulted in significantly less total microcosm C at 194 d, compared with the no-N treatment, with values of 18.30 and 18.97 mg C g^{-1} soil, respectively, with a concomitant increase in calculated CO_2-C losses, with values of 3.81 and 2.53 mg CO_2-C respired g^{-1} soil, respectively. The values are lower than the CO_2-C measured during the incubation (Figures 3.2-3.5), and could be due to high background laboratory CO_2 levels (generally >550 μmol mol^{-1}) that would have replaced the jar CO_2 after each measurement. The proportion of residue C remaining in +N and no N treatments was not different (p=0.231), indicating that the additional loss of C from the microcosms receiving N had to come from soil C pools, which could indicate a stimulatory effect on SOM or changes in the microbial biomass C pool. Respiration data

indicate that CO_2-C losses derived from no-residue control samples was about 1.41 and 1.10 mg C g^{-1} soil for the +N and no-N treatments, respectively (Figure 3.6). Calculated values for soil C mineralization indicated significant differences of 1.82 and 1.16 mg C g^{-1} soil for the +N and no-N treatments, respectively, so there is some evidence for a stimulatory effect on soil C mineralization on the order of about 0.41 and 0.06 mg C g^{-1} soil for +N and no-N treatments, respectively.

Soil Microbial Community

The resolved FA were placed in groupings for common, Gm + bacteria, Gm- bacteria, actinomycetes, and fungi. Nitrogen significantly affected the extractable PLFA and NLFA (Table 3.9). The +N treatment reduced the total amount of PLFA from 29.2 to 24.1 nmol PLFA g^{-1} soil. The relationship of Bailey et al. (2002), where 1 nmol PLFA corresponds to 2.4 μg K_2SO_4-extractable microbial biomass C (MBC), indicates that these PLFA values correspond to about 70 and 58 μg MBC g^{-1} soil, respectively. The response of individual PLFA to the +N treatment was variable. The mole % of the common PLFA 16:0 increased from 15.7 to 18.0 with the addition of N. The +N treatment both increased and reduced the amount PLFA associated with Gm + bacteria, with mole % of i15:0 decreasing from 17.4 to 13.4, and i16:0 increasing from 0.8 to 1.1, possibly indicating shifts in the Gm^+ population. Concentrations of PLFA 16:1ω7c and 18:1ω7c, associated with Gm- bacteria, increased from 5.4 to 6.2 mole % and decreased from 5.8 to 4.7 mole %, respectively. The PLFA associated with fungi were less variable; the +N treatment increased the relative abundance of 18:2ω6,9c and 18:1ω9c from 1.9 and 5.4 mole % to 5.4 and 6.3 mole %, respectively. The PLFA associated with actinomycetes also changed with additions of N, with 10Me16:0 and 10Me18:0 increasing from 15.7 and 2.7 mole % to 18.0 and 3.3 mole %, respectively.

The total amount of NLFA, associated only with fungi, was not affected by the +N treatment, with about 18.8 nmol NLFA g^{-1} soil. The NLFA 18:2ω6,9c increased from 17.1 to 30.0 mole % with the addition of N, with a concomitant decrease in 18:1ω9c from 24.8 to 16.6 mole %. The NLFA 16:1ω5c was also reduced from 6.4 to 4.7 mole % as a result of the +N treatment. There was a considerable presence of the common FA 16:0 in the NLFA, but it was not affected by the +N treatment, with a mean value of 20.9 mole%.

DISCUSSION

Total C concentrations in the plant residue were different between lines but not genotypes, and were similar to those of agricultural crops such as wheat (Wang et al., 2004) and tobacco (Hopkins et al., 2001) (Table 3.1). Total N levels in the plant residue were low, and resulted in a wide range of C/N ratios. The plant residue was obtained after grain fill, when a majority of the plant N was translocated to the grain (Martin et al., 1967). This was one of the reasons N addition was included as a treatment. Soluble matter, cellulose, and hemicellulose values were similar to those reported for corn, but with less lignin than reported for soybean (*Glycine max*) or wheat (Broder and Wagner, 1988). Our first hypothesis was partly correct inasmuch as there was a significant decrease in lignin content for the bmr mutant, as compared to normal-plant residue. The bmr mutant also contained higher levels of soluble material, higher levels of cellulose, and lower levels of hemicellulose, as compared to the normal-plant residue (Table 3.1).

Chemical data obtained from NMR spectroscopy showed that, for the most part, the proportions of major components of the plant residue were O-alkyl C, Di-O-alkyl C, N-methoxy+carbonyl, alkyl C, and aromatics; total amounts did not differ significantly between lines or genotypes, and had values similar to those reported for a range of plant materials,

including wheat, sugarcane (*Saccharum officinarum*), and buffel grass (*Cenchrus ciliaris*) (Wang et al., 2004) (Table 3.2). But, there were consistent differences between the normal and bmr mutants within the aromatic region in the 160 to 110 ppm chemical shift region; the integrated areas between 141 to 132 ppm, 158 to 152 ppm, and 162 to 158 ppm were significantly greater for the normal residue than for the bmr mutant residue (Table 3.3). Carbon atoms resonating in these regions all are present in the 3, 4, or 5 positions on the aromatic ring portion of the syringyl, guaiacyl, or p-hydroxyphenyl lignin aromatic precursor structure (Wilson, 1981; Kögel-Knabner, 2002), and could result in different lignin precursors in the bmr mutants than in the normal isolines. Atanassova et al. (1995) found a decrease in syringyl units and an increase in 5-hydroxy guaiacyl units in bmr tobacco plants. In addition, syringyl precursors represent the last step in precursor biosynthesis (Croteau et al., 2000), and reducing their abundance may alter final lignin chemistry. Hopkins et al. (2001) found that similar changes in lignin chemistry resulted in increased C mineralization once the bmr tobacco residue was added to soil.

Microcosm C mineralization followed first order patterns similar to those seen in experiments by others (Gilmour and Gilmour, 1985; Broder and Wagner, 1988; Hopkins et al., 2001; Wang et al., 2004) and similar to those used in many C mineralization models including Century and RothC. Kinetic values for rapid, intermediate, and slow phases were similar to those reported (Paul and Clark 1989; Wolf and Wagner, 2004). There were several significant 2-way interactions associated with C mineralization. For sorghum lines 12, 2, and 26 the initial rate of C mineralization was highest for the bmr mutants; this was expected because they contain less lignin. However, the trend was not seen in the intermediate and slow phases (Table 3.5). As the cellulose and hemicellulose is decomposed, lignin concentrations in the remaining residue should increase (Kononova, 1966; Sjöberg et al., 2004), but the differences between lines and genotypes

should become smaller due to low levels of lignin decomposition by fungi. However, the overall differences between the normal and bmr mutant residue were not large enough to affect C mineralization. The CO_2-C collected in the headspace was not used quantitatively to calculate residue mineralization rates because the total CO_2 emitted could contain some soil C that has been mineralized (Kuzyakov and Bol, 2006; Kuzyakov, 2006).

The C mass balance revealed that only about half of the microcosm C mineralized was derived from the added plant residue (Table 3.8). Although it is generally accepted that about 67% of plant residue applied to soil is mineralized within a year (Ladd and Martin, 1984; Stevenson, 1994), only about 26% was accounted for in this experiment. The ^{13}C data indicated that about 72% was still present in the microcosms at 194 d. Possible explanations could include the short duration of the experiment or that an important portion of residue C was in the soil microbial biomass. By converting PLFA data into microbial biomass C (MBC) there is about 0.066 mg MBC g^{-1} soil in the microcosm samples receiving residue versus about 0.036 mg MBC g^{-1} soil in the no-residue control samples, with a difference of 0.030 mg MBC g^{-1} soil that presumably originated as residue C. That is a relatively small pool, compared with the residue C added originally or remaining in the microcosms at 194 d. Therefore, one explanation is that the plant residue was simply partly decomposed or unaltered. In an experiment in which rice (Oryza sativa L.) residue was added to soil, Moran et al. (2005) found between 19 to 26% of added residue-C was unaltered and about 10% existed as particulate organic matter after 90 d.

The effect of added N on C mineralization was interesting. Mineral N typically increases crop residue mineralization (Moran et al., 2005). But, Fog (1988) found that differences in residue C quality and in available N could increase, decrease, or have no effect on C mineralization. Moreover, increased N can affect residue C cycling by increasing or decreasing

the activities of residue breakdown enzymes β-glucosidase, phenol oxidase, and peroxidase (Waldrop et al., 2004). The effects of adding N were also seen within tobacco bmr mutants, as N additions increased C mineralization from CCR mutants, but did not affect those with COMT or CAD mutations (Hopkins et al., 2006). Gallo et al. (2004) found that the microbial ecology (assessed using PLFA) was significantly affected by N additions. Waldrop and Firestone (2004) concluded that the microbial activity and C cycling effects due to increased N deposition were ecosystem specific, possibly related to differences in the fungal communities in each ecosystem. In this study, the N treatment resulted in more soil C mineralization.

The bmr grain sorghum residue did not affect the soil microbial community present in the microcosms at 194 d differently than the normal isolines (data not shown). Similar to the C mineralization, however, adding N significantly changed total PLFA and several key PLFA and NLFA, some of those related to soil fungi (Table 3.9). The reduced total PLFA found in the microcosms receiving N could be related to the lowered total C content found at 194 d, because MBC and soil organic C or particulate organic C were shown to be correlated (Franzluebbers et al., 1999; Moore et al., 2000). In the N treatments, the increased microcosm C mineralization could be a result of the increased abundance of soil fungi; soil fungi are capable of decomposing more complex substrates, such as lignin, than are bacteria, through the production of extracellular enzymes such as lignin peroxidases (Field et al., 1992). The microbial community was assayed at the end of the experiment, and microbial dynamics within the rapid and intermediate stages of C mineralization could have provided important information, such as which microorganism groups were predominant at different stages of residue decomposition. For example, Ziegler et al. (2005) found two phases of microbial activity after amending a soil with U-^{13}C-glucose and tracking the ^{13}C into PLFA over 48 h; Gm+ bacteria dominated early

incorporation of the ^{13}C from glucose, but actinomycetes played a role in recycling the ^{13}C. In this experiment, rapidly growing bacteria could have immobilized the added N and the ambient soil mineral N, making the remaining soil environment N limited. This N system should favor k-strategists, such as actinomycetes and fungi, which could partly explain the significant increases observed for PLFA associated with actinomycetes and fungi. The shift in the fungal NLFA from 18:1ω9c to 18:2ω6,9c could represent a physiological change in the fungal population as an effect of the + N treatment. Changes in the activity of fungal enzymes related to lignin and humus decomposition (lignin peroxidase, phenol oxidase, glucose oxidase, and glyoxal oxidase, e.g.) have been observed upon addition of N in forest ecosystems (Gallo et al., 2004; Waldrop et al., 2004; Sinsabaugh et al., 2005), but, more research is needed to determine what changes in NLFA within soil fungal communities signal.

CONCLUSIONS

Differences were detected between total lignin content of normal and bmr mutant plant residue samples, as measured by standard chemical methods, and in lignin chemistry, using NMR spectroscopy. The bmr mutants had lower overall lignin, as compared to the normal isolines. The NMR data showed that the bmr mutants had lower amounts of many aromatic structures found in the aryl region (162-114 ppm), but the differences were not consistent with any one specific structure. However, the differences were not large enough to result in increased soil C retention of normal residue, compared with bmr mutant residue. Evaluation of the bmr mutation as a means of predicting altered lignin levels to increase soil C sequestration rates remains to be determined. The differences between lines suggests that plant genetics may offer opportunities to change microbial communities and biogeochemical cycling. The lignin/N ratio was the strongest predictor of C mineralization when no external N was added; but its

importance was greatly reduced in the +N treatment. The addition of the bmr mutant plant residue did not affect soil microbial ecology. But the addition of N to the microcosms stimulated soil fungal populations, resulting in an increase in SOM mineralization. Additional research is needed to determine if greater differences in lignin content can alter residue mineralization rates and additions of N need to be evaluated as to their affects on soil C dynamics.

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Table 3.1. Properties of each line of grain sorghum residue as determined by chemical analysis

Line	TC	TN	C/N	$\delta\ ^{13}C_{VPDB}$	Soluble matter	Cellu-lose	Hemi-cellulose	Lignin	Ash
	--- mg g^{-1} residue---			--- ‰ ---	---------- mg g^{-1} residue ----------				
Line 12	406 c	4.57 b	89.8 a	-11.53	443 a	239 c	191	62.4	64.2 bc
Line 2	411 ab	8.13 a	53.8 c	-11.63	379 c	283 a	203	57.7	77.0 ab
Line 26	419 a	6.48 ab	72.6 ab	-11.39	416 ab	254 b	186	81.2	60.1 c
Line 28	409 bc	8.05 a	55.2 bc	-11.59	389 bc	272 a	189	67.3	85.1 a
Normal	412	5.80 B	76.3 A	-11.51	391 B	253 B	202 A	78 A	75.5
bmr mutant	412	7.82 A	59.4 B	-11.55	422 A	271 A	184 B	55 B	67.7
Statistical analyses (P-values associated with the main and interaction effects)									
Genotype (G)	0.9834	0.0248	0.0110	0.6074	0.0094	0.0015	0.0155	0.0025	0.1012
Line (L)	0.0084	0.0228	0.0016	0.2657	0.0027	<0.0001	0.3873	0.0735	0.0048
G*L	0.1809	0.5200	0.1847	0.1420	0.1979	0.1683	0.1013	0.3057	0.4143

*Means with the same uppercase (genotype main effect) and lowercase (line main effect) are not different at the $p<0.05$ level. Data for lines is averaged over genotype (normal or bmr) and genotype data is averaged over times.

Table 3.2. Chemical properties of each line of grain sorghum residue as determined by NMR spectroscopy.

Line	Alkyl	N-Alkyl +Methoxyl	O-Alkyl	Di-O-Alkyl	Aromatic	Phenolic	Amide/Carboxyl	Ketone
	---------- mg C g^{-1} TC ----------							
Normal 12	36	49	586	142	98	36	50	3
bmr12	45	52	619	141	74	23	46	0
Normal 2	52	57	589	135	83	32	51	0
bmr2	44	50	591	139	84	33	54	4
Normal 26	46	57	609	138	79	30	42	0
bmr 26	55	53	591	134	84	27	55	2
Normal 28	80	107	538	120	74	29	49	2
bmr 28	70	96	550	123	74	27	54	5

Table 3.3. Chemical properties of the aryl region (162-114 ppm) of the NMR spectrum. Resonance identification data is from Kogel-Knabner (2002) and Wilson (1987).

	114 - 123	123 - 132	132 - 141*	141 - 152	152 - 158*	158 - 162*
	ppm					
Compounds found in this resonance region	C2, C5, C6, in guaiacyl, C3,C5 in *p*-hydroxy-phenyl subunits	C1, C2, C6 in *p*-hydroxy-phenyl subunits, and Cα, Cβ in Ring-CH=CH-CHOH	C1 in guaiacyl, syringyl, and C4 in syringyl	C3, C4 in guaiacyl, C3 in syringyl	C3, C5 in syringyl; C4 *p*-hydroxy-phenyl	C4 in *p*-hydroxy-phenyl
	mg C g^{-1} TC					
Normal 12	27	22	18	20	12	5
bmr 12	24	19	14	18	6	3
Normal 2	25	21	15	18	10	5
bmr 2	24	21	14	19	9	4
Normal 26	25	19	15	19	9	4
bmr 28	26	22	15	20	7	4
Normal 28	24	20	16	18	10	5
bmr 28	21	19	13	18	8	4

*Indicate significant differences between normal and bmr groups. The values for each line were pooled to permit statistical analysis.

Table 3.4. Microcosm C mineralization kinetics during the experiment: main effects.

Sorghum	Rapid Fraction		Intermediate Fraction		Slow Fraction	
	k	Time	k	Time	k	Time
	-- d^{-1}--	-- d --	-- d^{-1}--	-- d --	-- d^{-1}--	-- d --
Line 12	0.0599	6.33	0.0088	30.3	0.0029 a	143
Line 2	0.0651	6.33	0.0093	30.2	0.0022 bc	144
Line 26	0.0619	6.50	0.0087	31.9	0.0026 ab	142
Line 28	0.0579	6.50	0.0072	43.4	0.0017 c	131
Nitrogen						
- Nitrogen	0.0582 b	6.00 b	0.0084	53.75 a	0.0034	118.8 b
+ Nitrogen	0.0642 a	6.83 a	0.0086	14.17 b	0.0014	161.4 a
Statistical analyses (P-values associated with the main and interaction effects)						
Genotype (G)	0.0003	0.1519	0.3228	0.6509	0.1110	0.8742
Line (L)	0.2240	0.9090	0.0792	0.1690	0.0027	0.1674
Nitrogen (N)	0.0214	0.0009	0.7510	<0.0001	<0.0001	<0.0001
G*L	0.0089	0.1681	0.0161	0.2468	0.4491	0.3372
G*N	0.8511	0.1519	0.0190	0.6261	0.2240	0.6861
L*N	0.1527	0.4522	0.0007	0.1506	0.0009	0.1452
G*L*N	0.9975	0.0766	0.9472	0.2661	0.6631	0.4495

* Means followed by the same letter in a column are not different (p>0.05).

Table 3.5. Microcosm C mineralization kinetics during the experiment: 2 way interactions.

	Rapid Fraction		Intermediate Fraction		Slow Fraction	
G*L Interaction	k	Time	k	Time	k	Time
	---d^{-1}---	---d---	---d^{-1}---	---d---	---d^{-1}---	---d---
Normal 12	0.0510 c*	6.33	0.0086 a	22.5	0.0025	148
bmr 12	0.0689 ab	6.33	0.0090 a	38.2	0.0034	139
Normal 2	0.0575 c	6.00	0.0095 a	34.5	0.0021	141
bmr 2	0.0727 a	6.67	0.0091 a	25.8	0.0024	148
Normal 26	0.0553 c	6.67	0.0080 a	34.8	0.0026	138
bmr 26	0.0685 ab	6.33	0.0095 a	29.0	0.0025	147
Normal 28	0.0607 bc	6.00	0.0091 a	39.7	0.0016	136
bmr 28	0.0551 c	7.00	0.0054 b	47.2	0.0019	126
L*N Interaction						
Line 12, -N	0.0572	5.67	0.0085 ab	50.5	0.0045 a	121
Line 12, +N	0.0627	7.00	0.0091 ab	10.2	0.0014 b	166
Line 2, -N	0.0668	6.00	0.0105 a	43.8	0.0032 a	129
Line 2, +N	0.0634	6.67	0.0081 b	16.5	0.0013 b	160
Line 26, -N	0.0566	6.00	0.0097 ab	48.5	0.0038 a	125
Line 26, +N	0.0673	7.00	0.0078 b	15.3	0.0013 b	160
Line 28, -N	0.0522	6.33	0.0051 c	72.2	0.0020 b	101
Line 28, +N	0.0636	6.67	0.0094 ab	14.7	0.0015 b	161
G*N Interaction						
Normal, - N	0.0533	5.67	0.0080 ab	51.5	0.0031	120
bmr, - N	0.0631	6.33	0.0088 ab	56.0	0.0037	118
Normal, + N	0.0589	6.83	0.0096 a	14.3	0.0013	161
bmr, + N	0.0696	6.83	0.0076 b	14.1	0.0014	162
Statistical analyses (P-values associated with the main and interaction effects)						
Genotype (G)	0.0003	0.1519	0.3228	0.6509	0.1110	0.8742
Line (L)	0.2240	0.9090	0.0792	0.1690	0.0027	0.1674
Nitrogen (N)	0.0214	0.0009	0.7510	<0.0001	<0.0001	<0.0001
G*L	0.0089	0.1681	0.0161	0.2468	0.4491	0.3372
G*N	0.8511	0.1519	0.0190	0.6261	0.2240	0.6861
L*N	0.1527	0.4522	0.0007	0.1506	0.0009	0.1452
G*L*N	0.9975	0.0766	0.9472	0.2661	0.6631	0.4495

* Means followed by the same letter in a column are not different (p>0.05).

Table 3.6. Correlation coeffiecients (r) between plant residue variables and cumulative C mineralized under +N treatment.

	3	5	7	12	16	25	32	39	53	60	69	74	88	102	116	123	132	146	156	179	194
Residue Variable	---------- days----------																				
Total C	0.09	0.07	0.07	-0.08	-0.10	-0.09	-0.05	-0.05	-0.05	-0.62	-0.08	-0.08	-0.12	-0.13	-0.15	-0.15	-0.16	-0.17	-0.18	-0.16	-0.16
Total N	0.09	0.23	0.24	0.17	0.15	0.15	0.16	0.17	0.19	0.18	0.17	0.16	0.13	0.13	0.12	0.12	0.13	0.14	0.13	0.15	0.14
C/N Ratio	-0.14	-0.26	-0.26	-0.18	-0.17	-0.18	-0.19	-0.20	-0.21	-0.20	-0.19	-0.19	-0.17	-0.17	-0.16	-0.16	-0.17	-0.18	-0.18	-0.20	-0.19
Soluble	**0.59**	**0.52**	**0.54**	**0.46**	**0.42**	0.37	0.36	0.37	0.37	0.37	0.37	0.36	0.36	0.36	0.34	0.34	0.34	0.35	0.34	0.34	0.36
Cellulose	-0.09	0.05	0.10	-0.06	-0.08	-0.05	-0.03	-0.03	-0.02	-0.01	-0.02	-0.02	-0.03	-0.03	-0.03	-0.03	-0.04	-0.03	-0.03	-0.03	-0.03
Hemicellulose	**-0.48**	**-0.51**	**-0.52**	**-0.52**	**-0.48**	**-0.45**	**-0.46**	**-0.45**	**-0.44**	**-0.44**	**-0.43**	**-0.43**	-0.41	-0.41	-0.41	-0.41	**-0.43**	**-0.46**	**-0.45**	**-0.48**	**-0.49**
Lignin	-0.27	-0.32	-0.39	-0.15	-0.12	-0.11	-0.11	-0.14	-0.16	-0.17	-0.18	-0.18	-0.16	-0.16	-0.16	-0.16	-0.13	-0.13	-0.13	-0.10	-0.11
Ash	**-0.50**	**-0.44**	**-0.45**	-0.33	-0.29	-0.26	-0.26	-0.26	-0.26	-0.26	-0.25	-0.25	-0.24	-0.24	-0.22	-0.22	-0.21	-0.21	-0.21	-0.20	-0.22
Lignin/TN	-0.36	**-0.46**	**-0.50**	-0.29	-0.27	-0.27	-0.29	-0.30	-0.32	-0.32	-0.32	-0.32	-0.30	-0.30	-0.29	-0.29	-0.29	-0.29	-0.29	-0.29	-0.29
Lignin/Cellulose	-0.24	-0.31	-0.39	-0.15	-0.12	-0.11	-0.12	-0.15	-0.17	-0.17	-0.18	-0.18	-0.17	-0.17	-0.17	-0.17	-0.14	-0.14	-0.14	-0.12	-0.13
Lignin/Soluble	**-0.41**	**-0.44**	**-0.51**	**-0.25**	**-0.22**	-0.19	-0.19	-0.21	-0.23	-0.24	-0.25	-0.24	-0.22	-0.23	-0.22	-0.22	-0.20	-0.19	-0.19	-0.17	-0.19
Lignin/Hemicellulose	-0.03	**-0.06**	**-0.13**	0.06	0.06	0.07	0.06	0.04	0.01	0.01	-0.01	-0.01	0.00	-0.01	0.00	0.00	0.03	0.04	0.04	0.07	0.06

Bolded r values are significant at P>0.05

Table 3.7. Correlation coeffiecients (r) between plant residue variables and cumulative C mineralized under - N treatment.

	3	5	7	12	16	25	32	39	53	60	69	74	88	102	116	123	132	146	156	179	194
Residue Variable	---------- days ----------																				
Total C	0.29	0.21	0.19	0.17	0.16	0.28	0.32	0.36	0.36	0.34	0.32	0.31	0.28	0.24	0.22	0.21	0.19	0.18	0.16	0.18	0.19
Total N	**0.44**	**0.49**	**0.58**	**0.64**	**0.63**	**0.57**	**0.50**	**0.45**	0.40	0.36	0.28	0.24	0.15	0.11	0.06	0.04	0.03	0.00	-0.02	-0.05	-0.05
C/N Ratio	**-0.45**	**-0.52**	**-0.58**	**-0.63**	**-0.63**	**-0.58**	**-0.54**	**-0.50**	**-0.48**	**-0.45**	-0.38	-0.33	-0.23	-0.17	-0.13	-0.11	-0.09	-0.06	-0.02	0.01	0.01
Soluble	0.29	0.15	0.10	0.16	0.14	0.13	0.20	0.22	0.24	0.26	0.30	0.32	0.36	0.39	**0.41**	**0.42**	**0.42**	**0.43**	**0.45**	**0.46**	**0.46**
Cellulose	0.31	**0.42**	**0.50**	**0.49**	**0.49**	**0.43**	0.34	0.32	0.30	0.27	0.21	0.18	0.12	0.08	0.04	0.02	0.01	0.00	-0.03	-0.07	-0.06
Hemicellulose	-0.29	-0.24	-0.25	-0.35	-0.34	-0.29	-0.32	-0.30	-0.30	-0.30	-0.29	-0.28	-0.24	-0.22	-0.22	-0.22	-0.20	-0.19	-0.19	-0.17	-0.17
Lignin	**-0.47**	**-0.44**	**-0.44**	**-0.45**	**-0.43**	-0.36	-0.30	-0.30	-0.27	-0.26	-0.26	-0.26	-0.28	-0.28	-0.27	-0.27	-0.28	-0.29	-0.29	-0.28	-0.29
Ash	-0.23	-0.16	-0.10	-0.13	-0.11	-0.18	-0.28	-0.30	-0.36	-0.38	**-0.43**	**-0.46**	**-0.51**	**-0.52**	**-0.54**	**-0.55**	**-0.55**	**-0.55**	**-0.56**	**-0.58**	**-0.58**
Lignin/TN	**-0.63**	**-0.65**	**-0.67**	**-0.71**	**-0.71**	**-0.64**	**-0.59**	**-0.57**	**-0.56**	**-0.54**	**-0.50**	**-0.46**	**-0.40**	-0.36	-0.32	-0.30	-0.29	-0.27	-0.25	-0.22	-0.23
Lignin/Cellulose	**-0.48**	**-0.47**	**-0.48**	**-0.49**	**-0.48**	-0.40	-0.34	-0.34	-0.31	-0.30	-0.29	-0.28	-0.29	-0.28	-0.26	-0.25	-0.26	-0.26	-0.26	-0.24	-0.25
Lignin/Soluble	**-0.50**	**-0.45**	**-0.44**	**-0.47**	**-0.45**	-0.37	-0.33	-0.34	-0.30	-0.29	-0.31	-0.31	-0.34	-0.35	-0.35	-0.35	-0.35	-0.37	-0.38	-0.37	-0.38
Lignin/Hemicellulose	-0.29	-0.26	-0.25	-0.22	-0.20	-0.16	-0.10	-0.11	-0.07	-0.06	-0.06	-0.07	-0.11	-0.11	-0.11	-0.10	-0.11	-0.13	-0.13	-0.13	-0.14

Bolded r values are significant at $P<0.05$

Table 3.8. Carbon balance for grain sorghum lines and genotypes.

Sorghum Line	Initial soil C[1]	Residue C added[1]	Total micro-cosm C at T=0 d[2]	Total micro-cosm C at T=194 d[1]	Proportion of residue C in total micro-cosm C at T=194d[3]	Residue C mineralized[4]	Soil C mineralized[5]	Proportion of residue C mineralized[6]
	----------mg g^{-1} soil----------				mg mg^{-1}	----------mg g^{-1} soil----------		mg mg^{-1}
Line 12	16.01		21.73	17.86 b	0.194 b	2.23 a	1.64	0.391 a
Normal	16.01	5.705 c	21.71	17.88	0.194	2.23	1.60	0.392
bmr	16.01	5.733 bc	21.74	17.83	0.195	2.24	1.68	0.390
Line 2	16.01		21.83	19.29 a	0.231 a	1.36 b	1.21	0.234 b
Normal	16.01	5.805 bc	21.82	19.83	0.239	0.99	0.79	0.171
bmr	16.01	5.834 ab	21.84	18.75	0.221	1.71	1.43	0.292
Line 26	16.01		21.89	18.56 ab	0.216 ab	1.88 ab	1.51	0.321 ab
Normal	16.01	5.829 ab	21.83	18.76	0.209	2.02	1.46	0.345
bmr	16.01	5.936 a	21.95	18.36	0.224	1.75	1.55	0.295
Line 28	16.01		21.76	18.83 a	0.232 a	1.41 b	1.61	0.244 b
Normal	16.01	5.826 b	21.84	19.05	0.245	1.16	1.63	0.199
bmr	16.01	5.702 c	21.71	18.62	0.218	1.66	1.60	0.290
N Main Effect								
+ Nitrogen	n.a.[7]	n.a.	n.a.	0.190 a	0.224	1.7724	1.8224 a	0.2917
- Nitrogen	n.a.	n.a.	n.a.	0.183 b	0.212	1.6877	1.1614 b	0.3061
Statistical analyses (P-values associated with the main and interaction effects)								
Genotype (G)	nt[8]	0.6507	nt	0.2986	0.4691	0.2026	0.5013	0.2202
Line (L)	nt	<0.0001	nt	0.0073	0.0422	0.0033	0.5639	0.0042
Nitrogen (N)	nt	nt	nt	0.0189	0.2310	0.7379	0.0198	0.7414
G*L	nt	0.0056	nt	0.2894	0.4115	0.2338	0.8575	0.2115
G*N	nt	nt	nt	0.4965	0.5213	0.5880	0.6027	0.5881
L*N	nt	nt	nt	0.7558	0.6410	0.8853	0.5092	0.8542
G*L*N	nt	nt	nt	0.9184	0.8977	0.8849	0.9758	0.8895

[1]Measured; 2 Calculated by adding initial soil C and residue C added; [3]Using equation from Gregorich et al., 2005; [4]Calculated as the difference between residue C added and proportion of residue C remaining in total microcosm C. [5]Calculated as the difference between Total cumulative CO_2 evolved and residue C mineralized. [6]Calculated by finding of quotient of residue C mineralized and residue C added; [7]n.a. = not applicable; [8]nt = not tested.

Table 3.9. Phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) content in soil at the end of the 194 d experiment.

Fatty Acid	Grouping	PLFA		NLFA	
		No N	N Added	No N	N Added
		mean mole %			
14:0	Common	1.3	1.4	2.1 a	1.8 b
15:0	Common	0.6	0.6	0.6 a	0.2 b
16:0	Common	15.7 b	18.0 a	20.2	21.5
10Me17:0	Common	2.6	2.5	0.0	0.0
18:0	Common	3.1	3.2	4.4 b	5.4 a
20:0	Common	0.2	0.1	1.6	1.7
i15:0	Gram (+)	17.4 a*	13.4 b	4.0 a	2.7 b
a15:0	Gram (+)	8.1	7.1	3.0	2.7
i16:0	Gram (+)	0.8 b	1.1 a	0.0	0.0
a17:0	Gram (+)	11.3	11.3	2.5	2.3
i17:0	Gram (+)	3.9	3.8	1.6	1.1
10Me16:0	Gram (+), Actinomycetes	4.5 a	2.1 b	6.6 a	4.4 b
10Me18:0	Actinomycetes	2.7 b	3.3 a	0.0	0.1
16:1ω7c	Gram (-)	5.4 b	6.2 a	1.7	1.9
16:1ω5c	Gram (-), fungi	4.2	4.2	6.4 a	4.7 b
18:1ω7c	Gram (-)	5.8 a	4.7 b	3.3	2.8
cy19:0	Gram (-)	5.0	5.2	0.1	0.0
18:2ω6,9c	Fungi	1.9 b	5.4 a	17.1 b	30.3 a
18:1ω9c	Fungi, plants	5.4 b	6.3 a	24.8 a	16.6 b
Mole Sum		29.2 a	24.1 b	19.2	18.3

* Letters in the same row for a given PLFA or NLFA indicate significant differences ($p<0.05$).

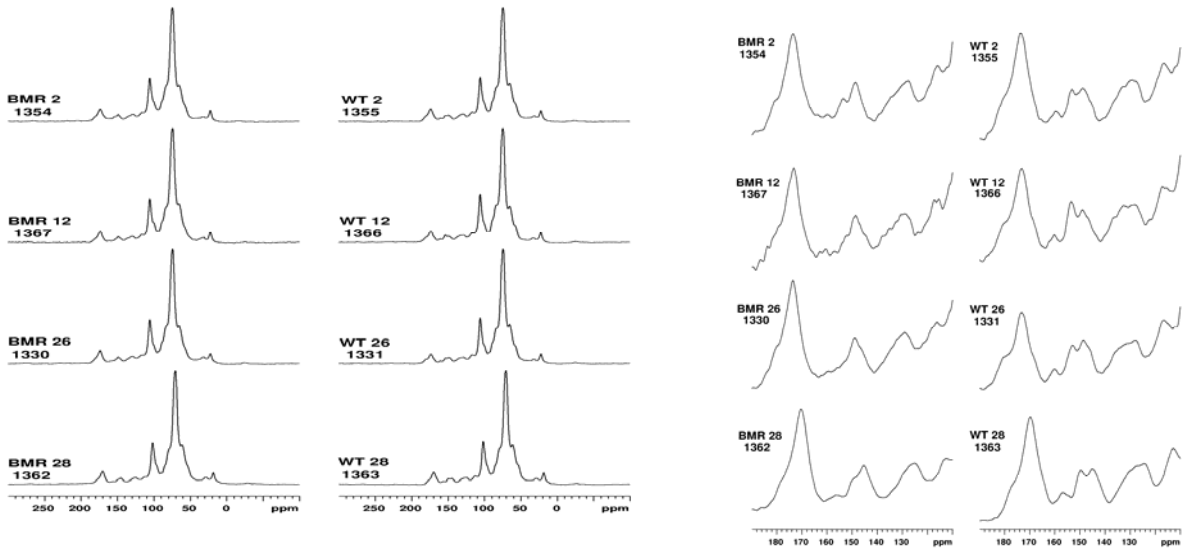

Figure 3.1. (a) CP-MAS ^{13}C NMR Spectra of stover from normal (wt) and bmr lines used in the laboratory incubation experiment. Differences between normal (wt) and bmr for each line are not clearly evident. (b) Detailed aryl region of CP-MAS ^{13}C- NMR spectra of the normal (wt) and bmr grain sorghum residue. Note the differences in the shape of the two resonances between 140 and 150 ppm and the width of the resonance near 130 ppm double peak reductions in bmr as compared to wt.

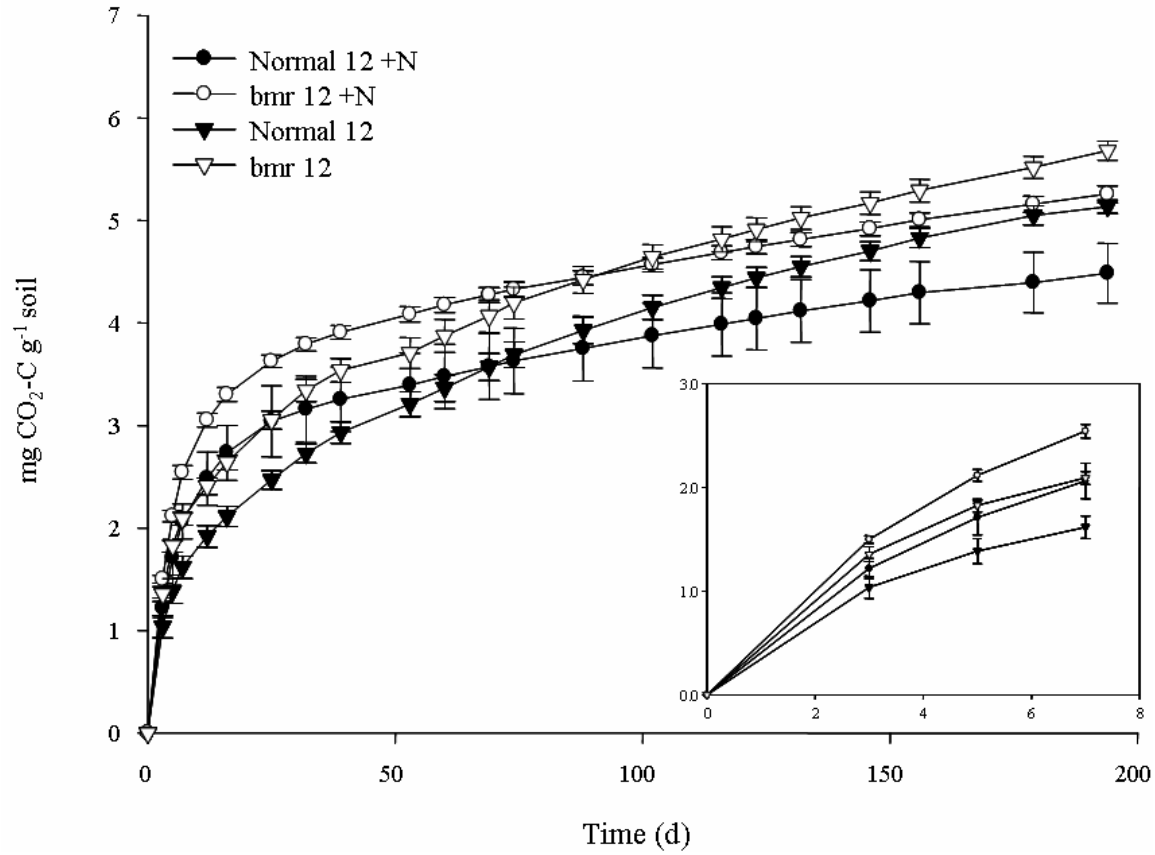

Figure 3.2. Cumulative microcosm C mineralization for sorghum line 12 during the 194 d experiment. Values are means (n=3) and bars are ± 1 standard error of the mean. Inset has same axis legends as main graph.

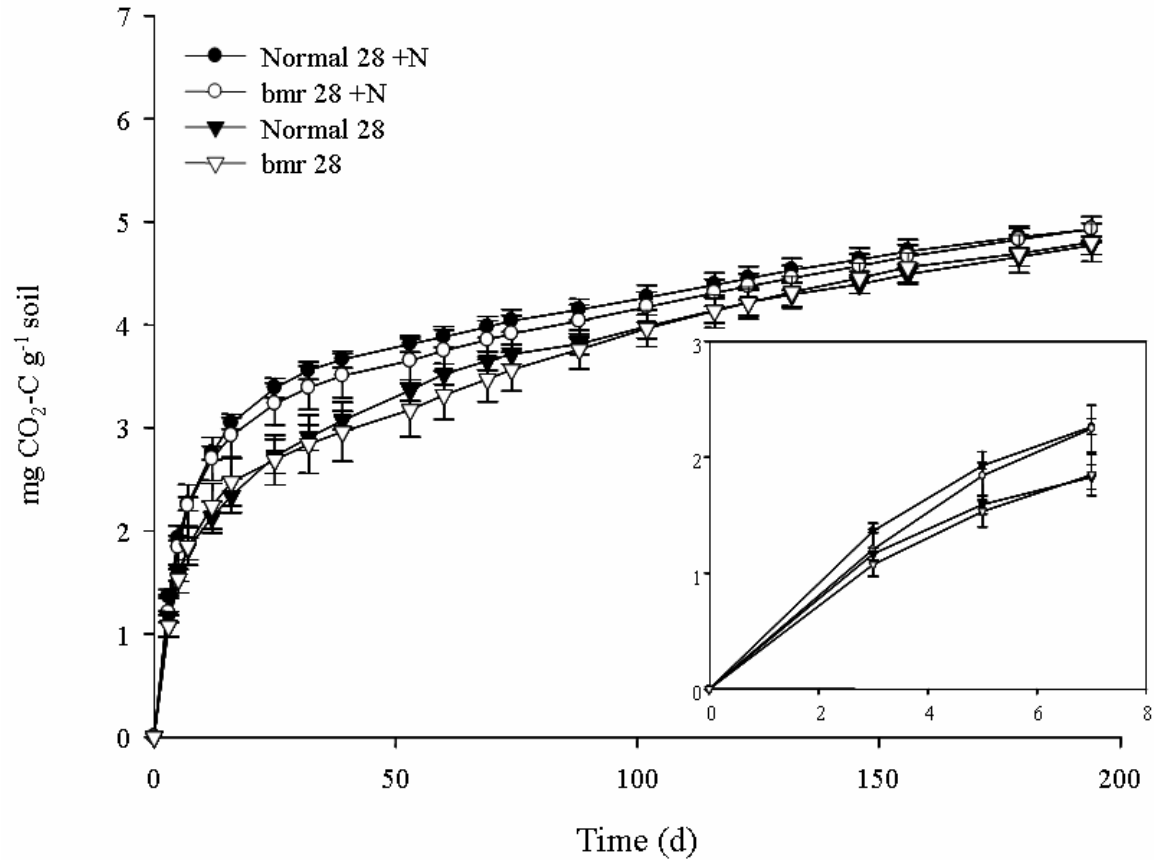

Figure 3.3. Cumulative microcosm C mineralization for sorghum line 28 during the 194 d experiment. Values are means (n=3) and bars are ± 1 standard error of the mean. Inset has same axis legends as main graph.

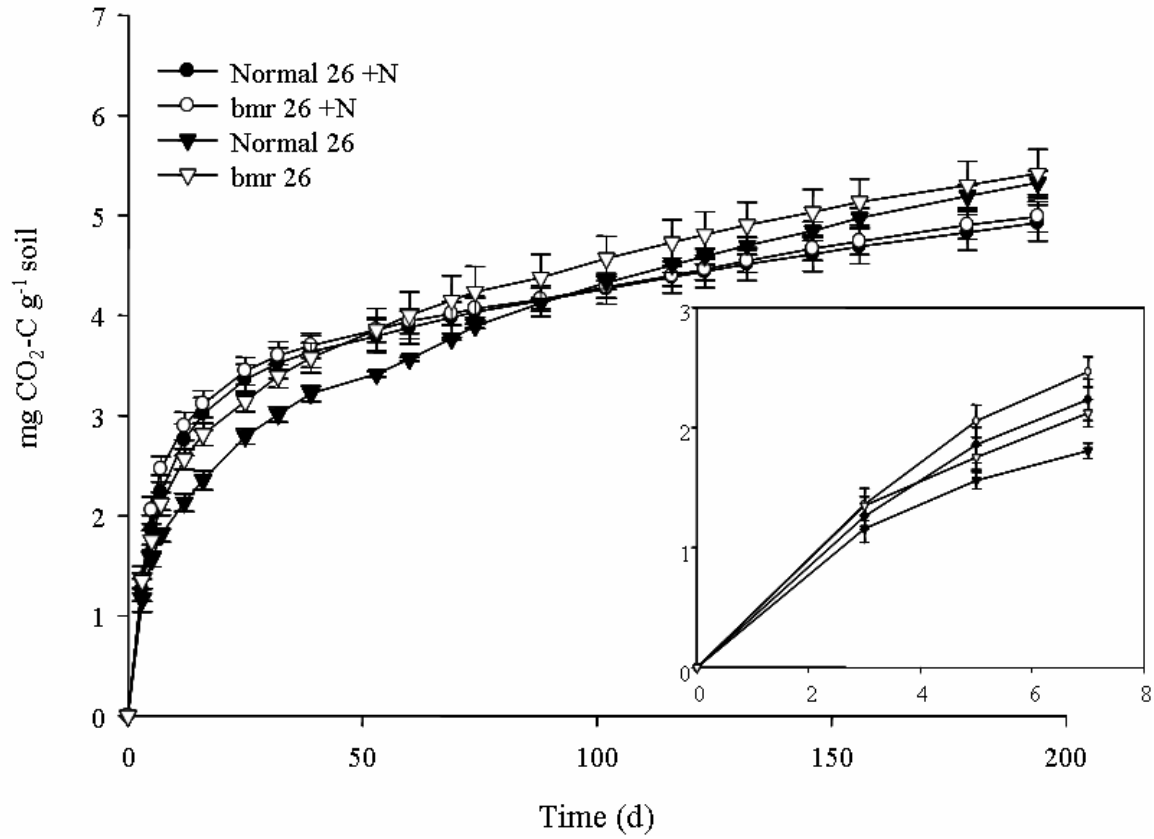

Figure 3.4. Cumulative microcosm C mineralization for sorghum line 26 during the 194 d experiment. Values are means (n=3) and bars are ± 1 standard error of the mean. Inset has same axis legends as main graph.

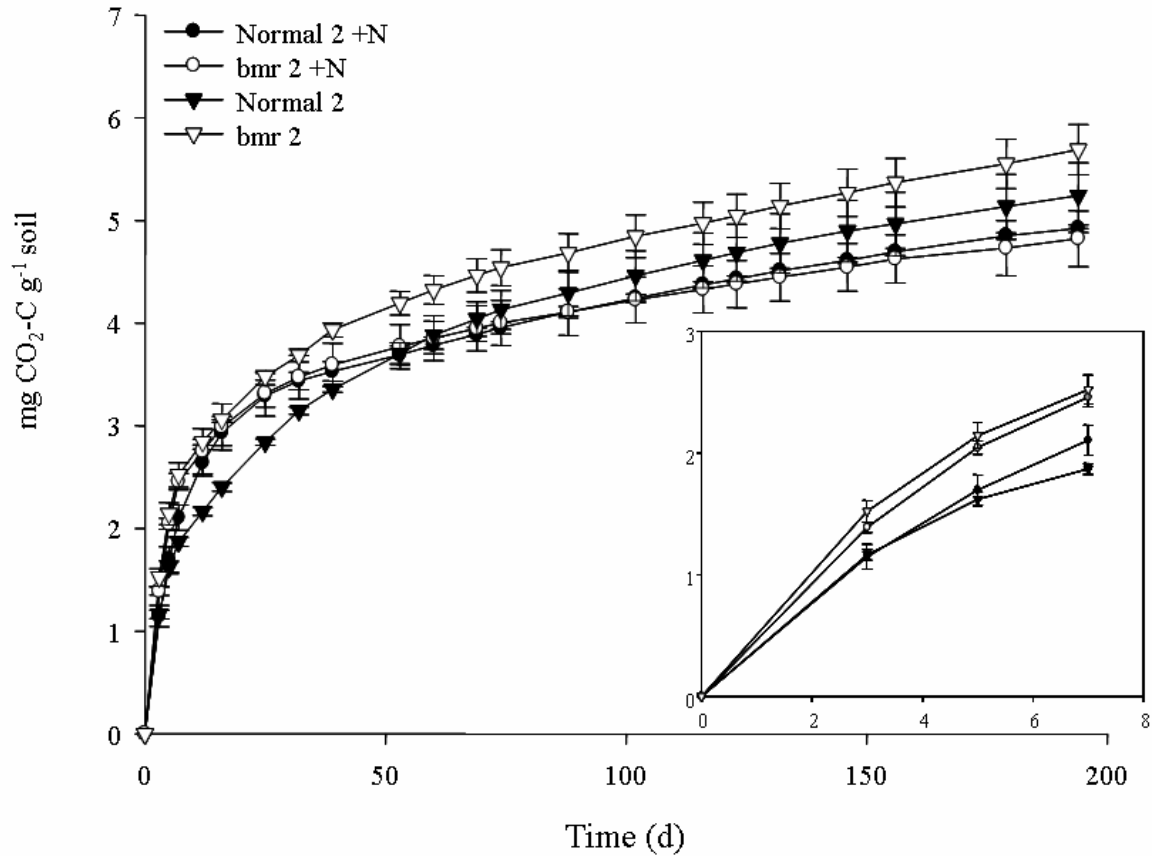

Figure 3.5. Cumulative microcosm C mineralization for sorghum line 2 during the 194 d experiment. Values are means (n=3) and bars are ± 1 standard error of the mean. Inset has same axis legends as main graph.

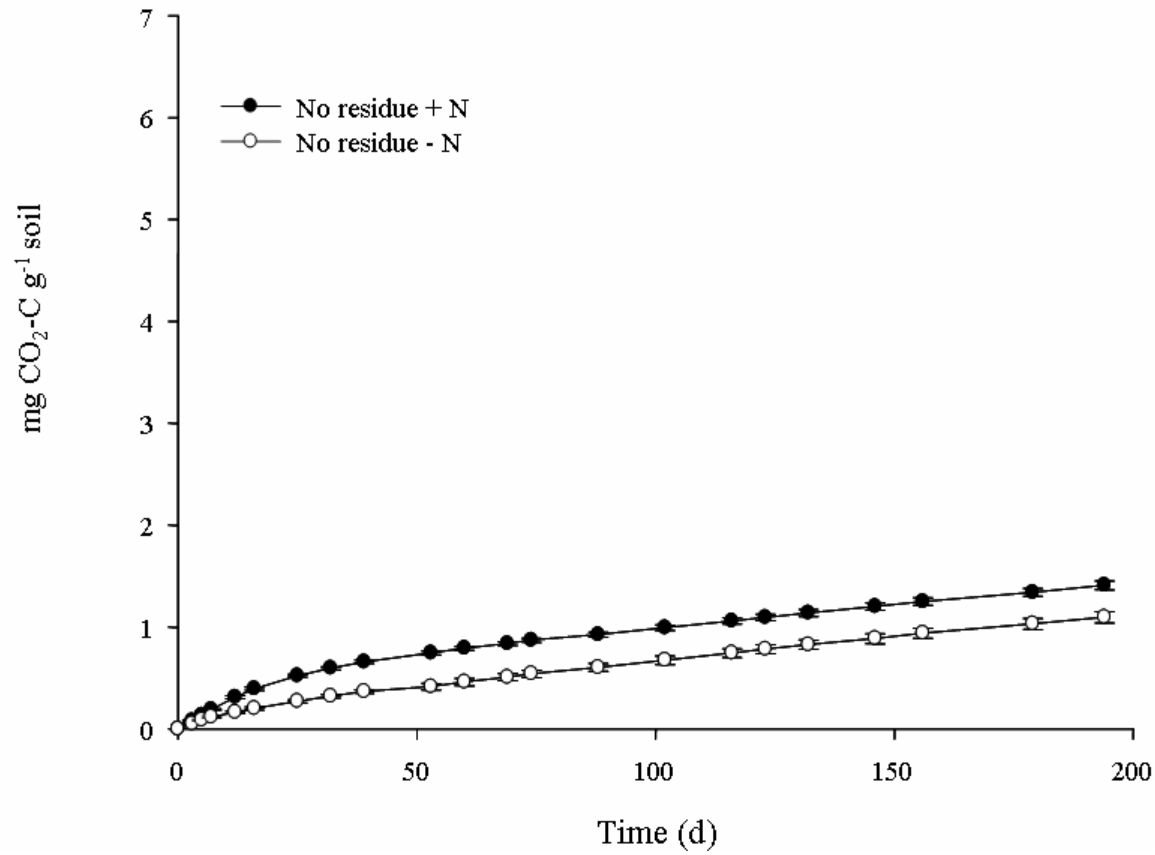

Figure 3.6. Cumulative microcosm C mineralization for no residue added controls. Values are means (n=3) and bars are ± 1 standard error of the mean. Inset has same axis legends as main graph. Inset has same axis legends as main graph.

CHAPTER 4 - Microbial Ecology and Carbon and Nitrogen Flux during Plant Residue Decomposition

ABSTRACT

One goal of soil C sequestration is to increase the mass of C stored in agricultural soils. Reducing soil disturbance, e.g. no-tillage, increases soil fungi and results in higher C sequestration rates. However, the specific mechanisms associated with short-term plant residue C and N retention are less clear. The objective of the experiment was to apply a ^{13}C- and ^{15}N-enriched grain sorghum (*Sorghum bicolor*) residue to no-tillage (NT) and tillage (CT) soils, and measure the ^{13}C and ^{15}N mineralization and redistribution, along with soil microbial ecology, during a growing season. The plant residue mineralized rapidly in both tillage systems, as indicated by ^{13}C and ^{15}N data. Mass balance calculations indicated that approximately 70% of the added ^{13}C was mineralized to CO_2 by 40 d. Inorganic N levels were highly variable due to field fertilization rates and rapid plant residue decomposition. Total, Gm+ and Gm- bacteria, and fungal phospholipid fatty acids (PLFA) were higher in NT 0-5 cm during the most active period of residue mineralization as compared to the CT 0-5 or CT 5-15 cm depth. No changes were observed in the NT 5-15 cm depth. The NT 0-5 cm depth PLFA displayed a higher evenness for 6 out of 7 time points sampled. The >1000 μm aggregate size class retained the most ^{13}C, regardless of tillage. The NT >1000 μm aggregates retained more ^{15}N at the end of the experiment than other aggregates size classes. The results indicate higher biological activity associated with NT soils, as compared to CT, and increased retention of plant residue C and N in

macroaggregates. Both higher total and fungal microbial biomass, and increased plant residue C and N retention in macroaggregates indicate that multiple mechanisms in NT systems are responsible for increased C and N levels.

INTRODUCTION

Storing or sequestering C in soil in natural and agricultural ecosystems has the potential to offset a portion of the future atmospheric increases in CO_2 until alternative energy and other GHG mitigation strategies become available (Caldeira et al., 2004; Paustian et al., 1998). However, the complex biological, chemical, and physical interactions resulting in C sequestration need to be evaluated to determine strategies to alter or boost the ability to sequester C. Carbon sequestration is defined as the process of transforming C in the air (CO_2) into stored soil organic C (SOC) primarily through plant photosynthesis and residue deposition.

Plant roots exude C compounds during the growing season but the majority of plant residue C deposition occurs after harvest, as residues are incorporated (CT) or left on the soil surface (NT). The transformations and storage of the plant residue C in soil is a function of biological, chemical, and physical factors and is heavily influenced by tillage practice. Mikha and Rice (2004) found significantly higher SOC levels in NT, as compared to CT under continuous corn. McVay et al. (2006) found greater amounts of SOC in 0-5 cm of NT soils throughout Kansas, as compared to CT. Higher levels of SOC in NT may be due to an increased amount of residue C deposited in the top 0-5 cm, as compared to CT, where the residue C is commonly incorporated to depths of 15 to 20 cm. Fabrizzi (Ph.D. dissertation) found increased SOC in 0-5 cm for NT, as compared to CT, in spite of similar grain yields and residue input. The increased SOC content in NT soils may also be related to increased soil aggregation, as soil aggregates provide chemical and physical protection against plant residue decomposition and alter C mineralization kinetics (Six et al., 2002). Soil macroaggregates are composed of particulate organic matter and microaggregates, which are primarily composed of chemically

protected organic matter (Six and Jastrow, 2002). Thus macroaggregates will generally contain a greater concentration of C than microaggregates (Mikha and Rice, 2004).

Soil biology is an important component of C sequestration and soil aggregation, as abundance of arbuscular mycorrhizal and saprophytic fungi are often correlated with the mass of macroaggregates (Six et al., 2006). Jastrow and Miller (2000) hypothesized that AM fungal hyphae act as a "web" to encase macroaggregates and Wright (2005) proposed that glycoproteins, such as glomalin, produced by AM fungi act as an adhesive to bind soil aggregates. Metabolic products from saprophytic fungi, such as melanins, chitosan, and quinones, may be more resistant to further decomposition by other soil microbes (Linhares and Martin, 1979; Linhares and Martin, 1978; Martin and Haider, 1971) and become chemically bound to clay minerals within microaggregates (Simpson et al., 2004). Thus, soil management strategies aimed at increasing or maintaining soil fungi, such as NT, could result in greater C sequestration (Rice and Angle, 2004).

The objective of the experiment was to apply ^{13}C- and ^{15}N-enriched plant residue to a NT and CT continuous grain sorghum field to evaluate (1) residue decomposition and mineralization; (2) incorporation of ^{13}C and ^{15}N into soil aggregates; and (3) microbial ecology. It is known that the NT continuous sorghum has greater SOC (McVay et al., 2006), higher abundance of fungal hyphae (Frey et al., 1999), and greater abundance of macroaggregates Fabrizzi (Ph.D. dissertation) as compared to the CT system. Therefore, the central goal of this experiment was to gather information during a short-term experiment to enhance our understanding of differences in residue processing, C and N retention, and microbial ecology between NT and CT agricultural systems.

MATERIALS AND METHODS

Production of ^{13}C and ^{15}N-enriched grain sorghum residue

Grain sorghum (Cv. Mycogen 1506) seed was planted in 1 L mason jars that were filled with sand and the moisture content brought to 0.15 g H_2O g^{-1} sand. The jars were placed in the laboratory at ambient temperature (20-25ºC) to germinate seeds. After radical emergence, seedlings were thinned to one seedling jar^{-1} and the jars were placed in the growth chamber. The temperature in the growth chamber was set to 25°C during light periods (16 hr d^{-1}) and 15°C for dark periods (8 hr d^{-1}). Jars were weighed twice daily and kept at 0.15 g H_2O g^{-1} soil by additions of 0.5X modified Hoagland's solution containing, in mM L^{-1}: $K^{15}NO_3$ (10 atom% ^{15}N), 5.0; Ca-Acetate, 2.5; $MgSO_4*7H_2O$, 1.0; KH_2PO_4, 0.5; and in mg L^{-1} Fe as $FeSO_4$ in H_2SO_4 (final pH = 6), 2.24; Cl as KCl, 1.77; B as H_3BO_3, 0.27, Mn as $MnSO_4*H_2O$, 0.27, Zn as $ZnSO_4*7H_2O$, 0.13, Cu as $CuSO_4*5H_2O$, 0.03, and Mo as $(NH_4)_6Mo_7O_{24}*4H_2O$, 0.01, (modified from Kirkham et al., 1969).

At about 2, 3, 4, and 5 wk after seedling emergence, the jars containing the grain sorghum plants were placed in an air-tight Plexiglas box and sealed. Approximately 1 L of 99.9 atom% $^{13}CO_2$ (Cambridge Isotope Laboratory, Andover, MA) was then injected into the box through a septum installed in the side. A second septum on the opposite side of the box was removed to allow the internal pressure to remain constant during the $^{13}CO_2$ injection. The injection increased the internal CO_2 concentration to about 10 times the ambient growth chamber CO_2 level, to 7000-8000 μmol CO_2 mol^{-1} air. Rubber septa were placed at various places around the box and a 50-mL syringe was used at alternate sides and heights of the box to mix the internal air again after the $^{13}CO_2$ injection. Each hour the box atmosphere was remixed and 1-mL aliquots of the box atmosphere were withdrawn and analyzed for CO_2 concentration using a

Shimadzu GC-8A gas chromatograph with a Porapak Q 80/100 mesh column (Alltech Part #2701PC). Once the internal CO_2 had been reduced to below ambient levels, the plants were removed from the box. The entire procedure was complete in 4 to 6 hours. The internal Plexiglas box temperature was monitored during the ^{13}C labeling and generally was between 0 and 5°C warmer than the growth chamber.

At 65 d after radical emergence, the aboveground portions of the plants was removed, chopped with scissors, and the 4-6 mm diameter fraction retained, subsequently lyophilized and stored at -20°C in a water-tight container. A subsample of the residue was ground in a mortar and pestle and analyzed for total C (TC) and N (TN) by dry combustion on a Thermo Electron Flash EA1112 (Waltham, MA) and analyzed for $\delta^{13}C$ and atom% ^{15}N using a Sercon GSL preparation unit fitted to a Europa 20-20 isotope ratio mass spectrometer (Sercon, Chesire, UK) (Table 4.1). The ^{13}C data is reported in δ notation where appropriate and standardized against the Vienna Pee Dee Belemnite (VPDB) standard (^{13}C:^{12}C = 0.012372 or 0‰). Another subsample was ground to pass 1-mm screen and analyzed according to the forage fiber methodology of Goering and Van Soest (1971) for total soluble matter, cellulose, hemicellulose, lignin, and ash (Table 4.1).

Field conditions and microcosm installation

The field site was located at Ashland Bottoms near Manhattan, KS (39º 07' N, 96º 36' W). The field was a Muir silt loam (Fine silty, mixed, mesic, Pachic Haplustolls) consisting of 86, 697, and 217 g kg^{-1} of sand, silt, and clay, respectively, by the hydrometer method (Gee and Bauder, 1986). The field had been cropped to continuous grain sorghum for 17 y and was either under NT or CT management. Conventional tillage consisted of fall chisel plow and spring cultivation and resulted in a lower bulk density as compared to the NT, with values of 1.36 and 1.40 g cm^{-3}, respectively. The field annually received 100 kg N ha^{-1} and 10 kg P ha^{-1} prior to

planting. The soil was sampled before planting at depths of 0-5 and 5-15 cm and sent to the Kansas State University Soil Testing Laboratory (Manhattan, KS) for chemical analysis (Table 4.1).

On 26 June 2004, 5 cm diameter x 17 cm deep beveled PVC "microcosms" were driven into the ground between the rows of grain sorghum to a depth of 15 cm. A total of eight plots were used for the experiment, four NT and four CT. There were 2 sets of 7 microcosms in each plot: set 1 consisted of applying the ^{13}C- and ^{15}N-enriched grain sorghum residue (R+) and set 2 were controls that did not receive grain sorghum residue (R-). The microcosms were carefully removed to minimize disturbance to the surrounding soil. For the NT microcosms, any surface residue was removed and 2.1 g of the ^{13}C and ^{15}N-enriched grain sorghum residue was placed on the soil surface, the original residue was returned on top of the enriched residue, and squares of 1-mm nylon mesh were duct taped to the top and the bottom of the PVC microcosm. The soil in the CT microcosms was removed and placed into a Ziploc® bag where it was thoroughly mixed with 2.1 g of the ^{13}C and ^{15}N-enriched grain sorghum residue. The soil was then placed back into the microcosm, and sealed with 2 squares of the 1-mm nylon mesh material using duct tape. The NT and CT microcosms not receiving residue were treated similarly except they did not receive any labeled residue. All microcosms were then returned to the original location from which they were extracted.

Plant residue mineralization and C and N dynamics

At 0, 3, 16, 25, 40, 68, and 159 d one R+ and one R- microcosm was removed from each plot (2 microcosms x 4 plots x 2 tillage = 16 microcosms). The soil was separated into 0-5 and 5-15 cm portions by extracting it from the bottom of the microcosm. The soil sample was

immediately sieved through a 4-mm mesh screen and stored according to the analysis specifications.

The ^{13}C loss over time was estimated by using the whole microcosm $\delta^{13}C$ values for 0, 3, 16, 25, 40, 68, and 159 d. The data was fitted to the following equation:

$$f = y0 + a*e^{-kt}$$

where f is the microcosm $^{13}C_{VPDB}$ value at time = t; y0 is the vertical asymptote representing the amount of plant residue-derived ^{13}C not mineralized, a is the size of ^{13}C pool mineralized, and k is the kinetic value for ^{13}C mineralization (d^{-1}). This model is incorporating only 1 pool of decomposing C in the residue, while most equations modeling plant residue mineralization incorporate 2 or more pools (e.g. rapid, slow, recalcitrant) (Gilmour and Gilmour, 1985; Wang et al., 2004). The actual plant residue C mineralization during the experiment was calculated according to the equation by Gregorich et al. (1995):

$$\%X = (\delta_{R+} - \delta_{R-})/(\delta_{gsr} - \delta_{R-}) \times 100$$

where δ_{R+} = $\delta^{13}C$ value of R+ microcosms soil at 159 d, δ_{R-} = the $\delta^{13}C$ value of the R- microcosms at 159 d, and δ_{gsr} = the $\delta^{13}C$ value of the original grain sorghum residue.

Inorganic N as NH_4-N and NO_3-N was extracted from a fresh subsample by adding 1 M KCl and shaking on an orbital shaker at 300 rpm for 1 h. Inorganic N was measured in the extracts using colorimetric analysis (Apkem RFA). The air-dried soil was analyzed for ^{15}N, ^{13}C, TC and TN as described above for the grain sorghum residue.

Soil water stable aggregates were separated by wet sieving through 20, 53, 250 and 1000-μm sieves with a Yoder-type apparatus as described by Mikha and Rice (2004). Air-dried soil samples were placed over stacked 250 and 1000 μm sieves and submersed in water for 10 min (slaking phase) and then subjected to 10 min of 4 cm length oscillations at a frequency of 0.5 Hz. The soil remaining on the sieves was collected and allowed to settle and air dry. The soil that passed both sieves was filtered through the 53 and 20 μm sieves, collected, and allowed to air dry. The soil from each fraction was ground with mortar and pestle and analyzed for ^{15}N and ^{13}C as described previously. Aggregate ^{15}N and ^{13}C data from the 0-5 cm, NT and CT, R+ treatments for 68 and 159 d was combined with aggregate TC and TN data from Fabrizzi et al. (200_) to estimate plant residue C and N storage in the different aggregate size classes. The %X was found for each data point as described above. The mole difference was calculated as:

$$\text{Mole Difference} = \frac{\text{Moles of C, N from plant residue}}{\text{Moles of C, N at t=0 in soil aggregate}}$$

The mole difference should provide an estimate of stabilized C and N in the different aggregate size classes, as the values to calculate %X were taken from the end of the experiment, when the % ^{13}C from the residue was most constant.

Soil Microbial Community Dynamics

The total lipids were extracted from the frozen, lyophilized soil using a modification of the Bligh and Dyer (1959) extraction (White and Ringelberg, 1998). The PLFA were then separated from the total lipid extract using silicic acid chromatography; the fatty acids were then cleaved from the glycerol backbone using KOH saponification; and the harvested fatty acids

were then methylated to form fatty acid methyl esters (FAME) (Allison and Miller, 2005; White and Ringelberg, 1998). The resulting FAMEs were analyzed using a Hewlett Packard 6890 GC with a 5871 Mass Selection Detector (MSD) (Allison and Miller, 2005). The GC analyzed a 1 μL splitless injection where the inlet temperature was 230˚C, the GC to MSD interface was 280˚C, helium was the carrier gas and the column used was an Agilent Ultra-2 (Cross linked 5% PH ME) 25 m length x 0.2 mm ID x 33 μM film thickness. The temperature program was 80˚C for 1 min, ramp 20˚C min-1 to 155˚C, ramp 5˚C min-1 to 270˚C, and hold for 5 min (33 min total run time). Peaks were identified using commercially available standards and the Wiley 138K mass spectral database. Sample peaks were quantitated based on comparison of the abundance with the internal standard nonadecanoic acid methyl ester.

The nomenclature used to describe the identified fatty acids (FA) is as follows (Bossio and Scow, 1998): total number of C atoms:number of double bonds, the position of the double bonds, ***cis*** or ***trans*** isomers identified by **c** or **t**. Prefixes of **a**, **i**, and **Me** indicated anteiso-branching, iso-branching, and methylation, respectively. For example, the molecule a10Me18:1w6c would be 18 C FA with a CH_3 branch 2 C from the tail end of the FA, a double bond in the ***cis*** configuration at the 6 position from the tail, and another CH_3 branch at the 10 C from the head of the FA. Fatty acids were grouped into Gm+ bacteria (i15:0, a15:0, 10Me16:0, i17:0, and a17:0), Gm- bacteria (18:1ω7c and cyclic 19:0), actinomycetes (10Me18:0 and 10Me17:0), and fungi (18:2ω6,9c and 18:1ω9c) (McKinley et al., 2005). The Simpson-Yule diversity index ($E = 1/\Sigma pi^2$) was used to calculate evenness (E) on the PLFA as an estimate of their diversity (Carney and Matson, 2005).

Experimental Design

The Ashland field site experimental design is a randomized block with four blocks containing three tillage treatments and four crop rotations. Only the continuous grain sorghum rotation under CT or NT was used for this experiment. Thus, there were four blocks of each tillage regime (NT and CT) and 7 R+ and 7 R- cores $plot^{-1}$. Data were subjected to an ANOVA using Proc Mixed in SAS version 9.0 (SAS Institute, Cary, N.C.). Means were separated by t-tests with a significance level of $p<0.05$. For the PLFA data, time was included as a split plot in the 3-way model (time, tillage, residue). The slice procedure in Proc Mixed was used to determine which time periods contained statistically different data, and another ANOVA was performed for each time period to examine 2-way differences (tillage, residue). Proc Corr was used for data correlations. Nonlinear regression analysis was carried out using SigmaPlot version 8.0 (SPSS, Inc., IL, USA).

RESULTS

Soil total C and N dynamics

Soil TC and TN values were combined across depths and tillage treatment to allow a statistical test of the effects of adding plant residue on TC and TN. Depth and tillage were not tested due to their significant differences at T=0 d (Table 4.1). The addition of plant residue did not change TC and TN levels during the experiment (Table 4.2), with mean values of 14.34 and 1.29 g kg^{-1} soil of TC and TN, respectively. Soil TC and TN were highly (0.973) and significantly ($p<0.0001$) correlated (data not shown).

Plant residue C mineralization

The highest rates of ^{13}C mineralization occurred during the first 25 d of the experiment (Fig. 4.1, 4.2). When the data was analyzed as a 2-compartment kinetic model the slow phase values were not significantly greater than 0 (data not shown) thus the 1 compartment model best fit the data. This was probably because of the low amount of lignin and hemicellulose in the immature plant residue (Table 4.1). The $\delta^{13}C$ values for the NT 5-15 cm, R+ treatment indicated that low amounts of the plant residue ^{13}C applied to the surface of the soil leached below 5 cm (Fig. 4.1). Data for the R- treatments were within the range for soils cropped to C_4 plants (Bender, 1970) (Fig. 4.3). Plant residue C mineralization for the R+ treatments was 0.711, 0.669, and 0.657 g residue C mineralized g^{-1} residue C added for NT 0-5 cm, CT 0-5 cm, and CT 5-15 cm, respectively (Table 4.3). These values correspond to about 70% of the added residue C being mineralized during the 159 d experiment (Table 4.3).

Soil inorganic N

Soil inorganic N levels varied throughout the experiment during the majority of the ^{13}C mineralization (Fig. A.1-A.4). There was a significant tillage*residue*depth interaction at 3 and 68 d for soil NH_4-N (Table A.1). At 3 d, the 0-5 cm depth of NT, R+ treatment had higher NH_4-N than any other treatment combinations, which were not different from each other. However, by 68 d, higher amounts of NH_4-N were found in the NT, R- treatment at the 0-5 cm depth than in the NT, R+ treatment at either depths, which was not different than the CT, R+ treatment at either depths, indicating that a high amount of NH_4-N mineralized from the residue was either immobilized in the soil microbial biomass, nitrified, or denitrified. Depth had a significant impact on soil NH_4-N levels at 16 d, with 0-5 cm depth having greater NH_4-N than 5-15 cm

depth. However there was high variability, especially within the CT, R+ treatments (Fig. A.3, A.4).

The tillage*depth interaction was significant at 3, 40, and 159 d for soil NO_3-N levels (Table A.1). At 3 and 40 d, the NT 0-5 cm had more NO_3-N than the NT 5-15 cm, but was not different than the CT at either depth. This can partially be explained by the placement of the grain sorghum residue, with the NT 5-15 cm treatment never receiving additional residue as a source of NH_4-N. The lower NO_3-N levels in the NT 5-15 may also indicate that leaching of NO_3 has not occurred by the 40 d mark. By 159 d, however, NT 0-5 cm had more NO_3-N than NT 5-15, CT 0-5, or CT 5-15 cm (Fig. A.1-A.4). This could indicate higher SOM levels in the NT 0-5 as compared to NT 5-15, CT 0-5, and CT 5-15 resulting in a greater N mineralization and subsequent nitrification rate. The residue*depth interaction was significant at 40 d, with R+ at 0-5 cm depths having greater NO_3-N levels than the other treatment combinations (Table A.1).

Soil microbial community dynamics

Data for PLFA was analyzed separately for each sample time by depth (0-5 or 5-15 cm) because differences in PLFA abundance were expected for the two depths (Table 4.4). A significant tillage*residue interaction was detected in the different PLFA groups at varied times, indicating that the response to residue additions was greater in NT than in CT treatments (Table 4.4). At the 0-5 cm depth in the NT, R+ treatment, total PLFA displayed a large increase that peaked at 25 d (Fig. 4.4), and then declined, opposite to the residue ^{13}C mineralization rates (Fig. 4.1, 4.2). The Gm+ and Gm- bacteria and fungi PLFA increased in response to added residue, similarly to the total PLFA (Fig. A.5-A.7). Actinomycetes responded marginally as compared to the other microbial populations (Fig. A.8). The 0-5 cm CT, R+ treatment responded, but not in terms of large PLFA increases, as did the NT, R+ treatment. At the 5-15 cm depth, an

increase in total, Gm+ and Gm- bacterial, and fungal PLFA was observed but to a lower extent as than with the 0-5 cm depth. Actinomycetes varied and differed little, on a gravimetric basis, as compared to 0-5 cm. Fungal PLFA was low in NT, as compared to CT, at the 5-15 cm depth. The addition of residue stimulated microbial growth in general, but the effect was reduced as the substrate C was depleted. No-tillage had a significantly higher amount of PLFA in the 0-5 cm depth in response to the residue addition, as compared to the CT.

Evenness was calculated as an indicator of PLFA diversity during the experiment. The residue main effect was significant for the 0-5 cm depth, as adding residue increased evenness from 6.45 to 7.49. There was a significant tillage main effect on evenness at the 5-15 cm depth, where CT had a higher evenness than NT, with values of 6.35 and 5.24, respectively. The time*tillage 2-way interaction was also significant in the 0-5 cm depth, as NT generally had a higher evenness value than CT, except for at 40 d, coinciding with the decrease in total, fungi, Gm+ and Gm- bacterial PLFA (Fig. A.9).

Incorporation of ^{13}C and ^{15}N into soil aggregates

Soil aggregates from CT and NT 0-5, R+ treatment were tested to determine if certain aggregate size classes retained more of the added plant residue ^{13}C or ^{15}N than other size classes. The 5-15 cm depth was not tested for ^{13}C and ^{15}N incorporation as it was assumed that CT 0-5 and 5-15 would be similar and NT 5-15 acquired very little ^{13}C (Fig. 4.1). The mole amount of plant residue ^{13}C and ^{15}N in the aggregates was calculated using data from T=68 d and T=159 d when the ^{13}C mineralization was not significantly greater than 0 and inorganic N had stabilized. This mole amount was normalized against the moles of C and N present in the aggregates before the plant residue additions (Fabrizzi et al., 200_). There was a significant tillage*aggregate size class interaction for aggregate N (Table 4.5). The NT >1000 μm aggregate size class had

significantly more plant residue derived N than any other tillage*aggregate combination (Table 4.5). The aggregate size class main effect was significant for C, as the >1000 μm size class had more plant residue derived C than any other size class (Table 4.5).

DISCUSSION

No-tillage soil contained more total soil C than CT, but only in the 0-5 cm depth. Differences in the soil C and soil N between tillage and soil depth are common in Kansas agro-ecosystems where NT 0-5 contains significantly more soil C than NT 5-15, CT 0-5, or CT 5-15 (Fabrizzi, Ph.D. dissertation; McVay et al., 2006; Mikha and Rice, 2004). The greater amounts of C present in the NT 0-5 cm depth is partially related to the increased plant residue deposition in that zone, as compared to CT, where the residue is incorporated. However, the NT 5-15 depth had the same amount of soil C as the CT, at either depth. Sá et al. (2001) found that 22 y of NT resulted in higher SOC levels in the top 20 cm of a Brazilian Oxisol, as compared to CT, but that 20-40 cm the CT contained higher SOC, as compared to the NT. However, on a mass basis, 22 y of NT and CT resulted in a 18.9 and -0.13 Mg SOC ha^{-1} change as compared to native forest (Sá et al., 2001). Thus, the higher soil C in the surface NT depth can not be fully explained as a redistribution of residue C. As soil C and soil N are highly correlated, similar accumulations in NT are seen here, and in other studies (McVay et al., 2006; Mikha and Rice, 2004; Sá et al., 2001).

Plant ^{13}C mineralization displayed first order kinetics similar to those seen in other experiments (Hopkins et al., 2001; Gilmour and Gilmour, 1985; Wang et al., 2004; White et al. 200_). However, a one-pool model fit the data better than a two-pool model, indicating the

residue was low in recalcitrant material. Mass balance calculations indicated that 71, 67, and 66% of the applied residue C was mineralized during the experiment for NT 0-5 cm, CT 0-5 cm, and CT 5-15 cm, respectively (Table 4.3). This value is similar to the 67% reported to mineralize from crop residues annually (Ladd and Martin, 1984; Stevenson, 1994). The plant residue had a C:N ratio of 32.1, but initial soil inorganic N levels, especially NO_3-N, would have promoted rapid residue decomposition. The positive values for y_0, however, indicate that a portion of the residue ^{13}C was retained in the soil and thus the residue ^{13}C and ^{15}N (see aggregate data) was an adequate tracer for the length of the experiment. The rapid residue mineralization could suggest low C and N sequestration, but it could also mean a greater rate of residue C and N incorporated in the microbial biomass and subsequent transformation into stable soil organic matter (Moran et al., 2005). In addition to nutrient status, temperature and moisture can have significant effects on decomposition rates. Temperature and moisture status were optimal for aerobic chemoheterotrophic activity, with air temperatures during the first 68 d ranging from 20-25°C, and soil moisture throughout the experiment averaging 0.24 g H_2O g^{-1} soil.

Inorganic N values were used as a supplemental measure of plant residue mineralization, but the high background of NO_3-N seen in the R- treatments confounded the analysis. High amounts of NH_4-N were seen in the 0-5 cm NT, R+ treatment, which persisted until about 40 d (Fig. A.1), similar to the ^{13}C levels (Fig. 4.1), again indicating rapid plant residue mineralization. The high amount of inorganic N present in the soil throughout the experiment could also explain the rapid residue mineralization, as the system would not have been N limited. Fog (1988) summarized over 60 papers and concluded that N has a positive effect on decomposition of organic materials with a low C:N ratio, which would apply to this experiment. Inorganic N has been shown to increase, decrease, or not alter C mineralization from a variety of soils, and most

likely is linked to factors such as residue quality (e.g., C:N ratio, lignin:N ratio, % lignin), soil pH, and the microbial community (Gallo et al., 2004; Sinsabaugh et al., 2005; Waldrop et al., 2004; Chapter 3).

The PLFA are indicators of viable microbial biomass (Kennedy and Gewin, 1997), and thus increases in PLFA abundance should represent the portions of the microbial community decomposing the plant residue. The microbial community structure was significantly affected by the addition of plant residue (Table 4.4), and the effect was different for NT and CT. At the 0-5 cm depth, NT responded much greater to the addition of plant residue, as compared to CT. The 0-5 cm NT, R+ received 3X the residue as the 0-5 cm CT, R+ treatment, and this is reflected in the total PLFA, where NT had 1.5, 2.3, 2.9, and 1.5 times the total PLFA, as compared to CT (Fig. 4.4). Similar increases were seen for the Gm+ and Gm- bacterial, and fungal PLFA (Fig. A.5-A.7), indicating that a large proportion of the soil microbial community was active in the decomposition process, and the overall greater microbial biomass in the NT 0-5 cm reflects the addition of larger amounts of substrate. A higher amount of actively metabolizing fungi could result in the buildup of recalcitrant metabolites, such as melanins, chitosan, and quinones (Linhares and Martin, 1979; Linhares and Martin, 1978; Martin and Haider, 1971), and this could lead to a greater amount of C sequestration through formation of recalcitrant byproducts or increased soil aggregation due to fungal activity (Six et al., 2006).

In the 5-15 cm depth, the CT soil responded to plant residue additions, following similar trends as the 0-5 cm NT and CT soils where residue was added, but to a lower extent, indicating that the rate of substrate addition was related to the degree of microbial growth. However, in the NT 5-15 cm soil, very low levels of fungal PLFA were observed. This could be due to several reasons. Fungi are known to store a large amount of metabolized C in neutral lipid fatty acids

(Allison and Miller, 2005), especially during periods where most energy acquired is used for maintenance and not new growth. The low levels of fungal PLFA could have just been an artifact of not adding any new C to the 5-15 cm depth in NT. However, where plant residue C was available, fungal PLFA were enhanced.

Soil PLFA evenness revealed that generally NT soils were more diverse than CT soils in the 0-5 cm depths, but the opposite was true at the 5-15 cm depth. This could be related to substrate availability, as soil %C was shown to be associated with changes in the soil microbial community (Carney and Madsen, 2005). In this study a higher amount of soil C and an increased rate of plant residue addition was associated with increased PLFA and often increased PLFA evenness.

Simpson et al. (2004) concluded that fungal-derived C was stabilized in NT soils, primarily by improving soil structural stability and channeling C into microaggregates within macroaggregates. A model proposed by Six et al. (2004) outlined macroaggregate formation as the complexation of particulate organic matter (POM) and microaggregates, encased in a biological film, with a relatively short (25-40 y) turnover rate. In this experiment plant residue C was rapidly mineralized and accompanied by a pulse of microbial activity, with high amounts of fungi present. Plant residue-derived ^{13}C and ^{15}N was observed in every size aggregate, however, significantly more was present in macroaggregates. Given that this NT soil had significantly greater >1000 μm aggregates, as compared to CT, a greater amount of plant residue C is expected to be present in NT as compared to CT. The higher levels of plant residue derived ^{13}C and ^{15}N in NT at the end of the 159 d experiment could indicate that POM, originating from plant residue, is accumulating in macroaggregates (Six et al., 2004). The delayed mineralization due to this physical protection could be a mechanism that results in greater C and N

sequestration. The presence of ^{13}C and ^{15}N in microaggregates could indicate microbial processing of plant residue into low-molecular weight compounds and subsequent chemical stabilization on clay mineral surfaces or iron oxides.

CONCLUSIONS

Plant residue decomposition proceeded rapidly as about 70% of the residue C mineralized in the 159 d experiment. However, significant amounts of plant residue ^{13}C and ^{15}N were present in the soil and in all soil aggregates at 159 d, as compared to soil in microcosms not receiving plant residue. Residue decomposition was accompanied by a pulse in microbial PLFA, with increases in Gm+ and Gm- bacterial, and fungal PLFA in the 0-5 cm NT, R+, and 0-5 and 5-15 cm CT, R+ treatments. Significantly greater amounts of residue ^{13}C and ^{15}N were found within macroaggregates, and could represent POM or microbially-derived organic matter. Overall, the greater amount of microbial biomass present in the 0-5 cm NT soil was presumably due to higher TC content related to surface application of residues. With a higher microbial biomass, a greater amount of soil fungi are present, which could lead to increased soil C sequestration through production of recalcitrant by-products and through increased soil aggregate formation.

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Table 4.1. Properties of the grain sorghum residue and Ashland field soil.

	Total C	Total N	C:N	$\delta^{13}C$	^{15}N	Particle Size	Soluble Material	Cellulose	Hemi-cellulose	Lignin	Ash
	----g kg^{-1} residue----			0%	atom %	mm	---------- g kg^{-1} residue----------				
Grain Sorghum Residue	443	13.8	32.1	533.4	9.25	4-6	491	206	284	18.3	none

							Mehlich 3 Extractable					
Soil Tillage	Depth	Total C	Total N	$\delta^{13}C_{VPBD}$	$^{15}N_{air}$	pH	P	Ca	K	Mg	Na	SO_4-S
	--- cm ---	----g kg^{-1} soil----		‰	atom %	1:1 s:w	---------- mg kg^{-1} soil ----------					
NT	0-5	18.98	1.74	-14.83	-1.75	5.0	158	1768	262	251	3.7	8.3
NT	5-15	14.20	1.31	-15.13	3.61	5.8	41	2201	173	291	5.8	6.5
CT	0-5	12.04	1.09	-18.10	-0.98	5.4	128	1844	317	281	3.6	7.5
CT	5-15	12.04	1.09	-15.32	-0.19	5.7	52	2209	193	308	6	7.6

Table 4.2. Soil total C and total N levels during the field experiment.

	Time (d)						
	0	3	16	25	40	68	159
Total Soil C	---------- g kg^{-1} soil ----------						
R+	14.32	15.57	14.38	14.91	14.14	13.61	14.86
R-	14.32	18.53	14.60	13.27	12.86	12.63	13.30
p-value	nt†	0.2747	0.8984	0.3474	0.3773	0.4259	0.3263
Total Soil N							
R+	1.30	1.37	1.29	1.28	1.34	1.23	1.37
R-	1.30	1.58	1.32	1.20	1.20	1.11	1.20
p-value	nt*	0.2219	0.8101	0.5427	0.2334	0.1846	0.1354
C:N Ratio							
R+	11.02	11.36	11.15	11.65	10.55	11.07	10.85
R-	11.02	11.73	11.06	11.06	10.72	11.38	11.08

†Not testable - prior to residue additions

Table 4.3. Mineralization of ^{13}C derived from added plant residue during the experiment.

Treatment	Initial Soil C[1]	Residue C Added	Total Micro-cosm C at T=0 d[2]	Total Micro-cosm C at T=159 d[1]	% of residue C in total micro-cosm C[3]	Mass of residue C in total micro-cosm C[4]	Proportion of mineralized residue C[5]
	----------g microcosm^{-1} ----------				--%--	g microcosm^{-1}	g g^{-1}
NT 0-5 cm R+	1.923	0.990	2.913	2.938	0.097	0.286	0.711
NT 5-15 cm R+	2.878	0.000	2.878	3.486	0.002	0.008	n.a
CT 0-5 cm R+	1.220	0.330	1.550	1.844	0.059	0.109	0.669
CT 5-15 cm R+	2.441	0.660	3.101	3.608	0.063	0.226	0.657
							p=0.6838

[1]Measured.

[2]Calculated by adding initial soil C and residue C added.

[3]Using equation from Gregorich et al., 2005.

[4]Calculated as the product of the total microcosm C at 159 d and the percent of residue C in total microcosm C.

[5]Calculated using the equation: (Residue C added - Mass of residue C in total microcosm C)/Residue C added.

Table 4.4. P-values for PLFA statistical tests: 2-way interactions.

		Gm^+		Gm-		Actinomycetes		Fungi		Total	
Time	Effect	0-5	5-15	0-5	5-15	0-5	5-15	0-5	5-15	0-5	5-15
		---------- cm ----------									
0*	Tillage (T)	0.5667	0.0662	0.2783	**0.0231**	0.6127	0.1014	0.2977	0.3805	0.3907	**0.0301**
3	Tillage (T)	0.4423	0.0551	0.1653	0.0845	0.2510	0.0952	0.0645	0.0550	**<0.0001**	0.0720
	Residue (R)	**0.0056**	**0.0476**	0.0705	**0.0382**	**0.0290**	**0.0284**	**0.0001**	0.0648	**<0.0001**	**0.0374**
	T*R	0.4994	0.7109	0.4385	0.6079	0.4335	0.9265	0.1240	0.3307	**<0.0001**	0.5165
16	Tillage (T)	**0.0100**	**0.0085**	0.0003	**0.0018**	0.7105	**0.0319**	0.0015	0.0051	0.5500	**0.0010**
	Residue (R)	0.1082	0.1876	0.0015	**0.0016**	**<0.0001**	0.0723	0.0007	0.0042	0.5960	**0.0038**
	T*R	0.1882	0.6607	**0.0314**	0.0654	0.8980	0.5697	**0.0010**	**0.0075**	0.7220	0.1032
25	Tillage (T)	0.0027	**0.0293**	0.0141	0.0047	**0.0254**	**0.0102**	0.0066	**0.0172**	0.0021	**0.0012**
	Residue (R)	0.0048	**0.0322**	0.0026	0.0032	0.6995	**0.0400**	0.0005	**0.0065**	0.0020	**0.0038**
	T*R	**0.0288**	0.2410	**0.0087**	**0.0420**	0.6007	0.3589	**0.0020**	0.1307	**0.0090**	0.0825
40	Tillage (T)	**0.0002**	**0.0284**	**0.0106**	**0.0021**	**0.0447**	0.0632	**0.0012**	0.2097	**0.0071**	**0.0024**
	Residue (R)	**0.0048**	0.0570	**0.0146**	**0.0014**	0.3447	0.1089	**0.0008**	**0.0802**	**0.0022**	**0.0086**
	T*R	0.1796	0.1550	0.1136	0.0943	0.9272	0.4515	0.0597	0.1214	0.3548	0.1460
68	Tillage (T)	**0.0361**	**<0.0001**	**0.0340**	**<0.0001**	0.1468	0.2845	0.1202	0.0002	0.0755	**<0.0001**
	Residue (R)	0.6225	**0.0224**	0.3408	0.3575	0.3140	0.5317	0.6095	0.7472	0.9794	0.3159
	T*R	0.1546	0.1906	0.3932	0.4979	0.1643	0.2229	0.4364	**0.0144**	0.2338	0.6112
159	Tillage (T)	**0.0403**	0.1777	**0.0126**	0.2633	**0.0136**	0.6171	**0.0005**	0.1061	0.0083	0.1144
	Residue (R)	0.8076	0.9173	0.1441	0.1814	0.6376	0.8748	0.3108	0.8161	0.0088	0.5117
	T*R	0.3468	0.4630	0.1666	0.9239	0.6821	0.5664	0.2556	0.7189	**0.0047**	0.6358

Table 4.5. Mole difference of C and N levels in soil aggregates for the NT and CT 0-5 cm depths.

2 way interaction		Aggregate N		Aggregate C	
Tillage	Aggregate Size	Mean	Std Err	Mean	Std Err
		---------- mole difference ----------			
	--- μm ---				
CT	>1000	**0.0228 b**	**0.0092**	0.1078	0.0474
	250-1000	**0.0206 b**	**0.0053**	0.0702	0.0182
	53-250	**0.0053 b**	**0.0040**	0.0460	0.0057
	20-53	**0.0029 b**	**0.0039**	0.0388	0.0032
NT	>1000	**0.1411 a***	**0.0281**	0.2250	0.1074
	250-1000	**0.0229 b**	**0.0022**	0.0820	0.0156
	53-250	**0.0043 b**	**0.0020**	0.0310	0.0059
	20-53	**0.0043 b**	**0.0016**	0.0384	0.0115
Aggregate Main Effect	>1000	0.0819	0.0345	**0.1664 a**	**0.0587**
	250-1000	0.0217	0.0032	**0.0761 b**	**0.0113**
	53-250	0.0047	0.0025	**0.0385 b**	**0.0047**
	20-53	0.0037	0.0021	**0.0386 b**	**0.0055**

P-values

Effect	Aggregate N	Aggregate C
Tillage (T)	0.0667	0.3271
Aggregate (A)	0.0047	**0.0133**
T*A	**0.0272**	0.3588

*Means in a column followed by the same letter are not different ($p<0.05$).

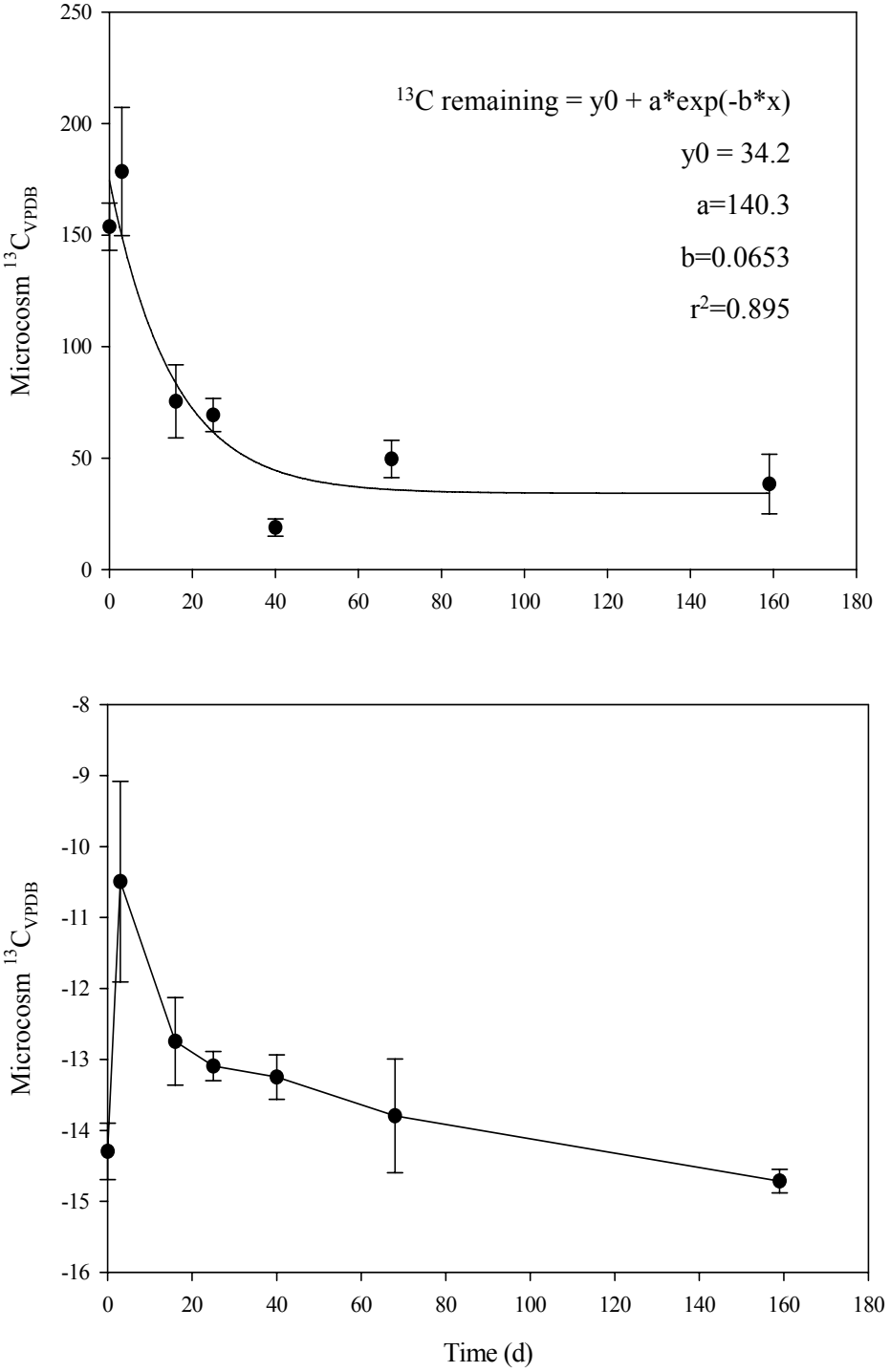

Figure 4.1. Plant residue ^{13}C mineralization in 0-5 cm NT, R+ treatment (A) and 5-15 cm NT, R+ treatment (B) during the 159 d experiment. Values are means and bars are ± 1 standard error. Note that very little ^{13}C was found in the 5-15 cm depth, indicating low leaching in this short term experiment. Inset data is for a one-pool kinetic model.

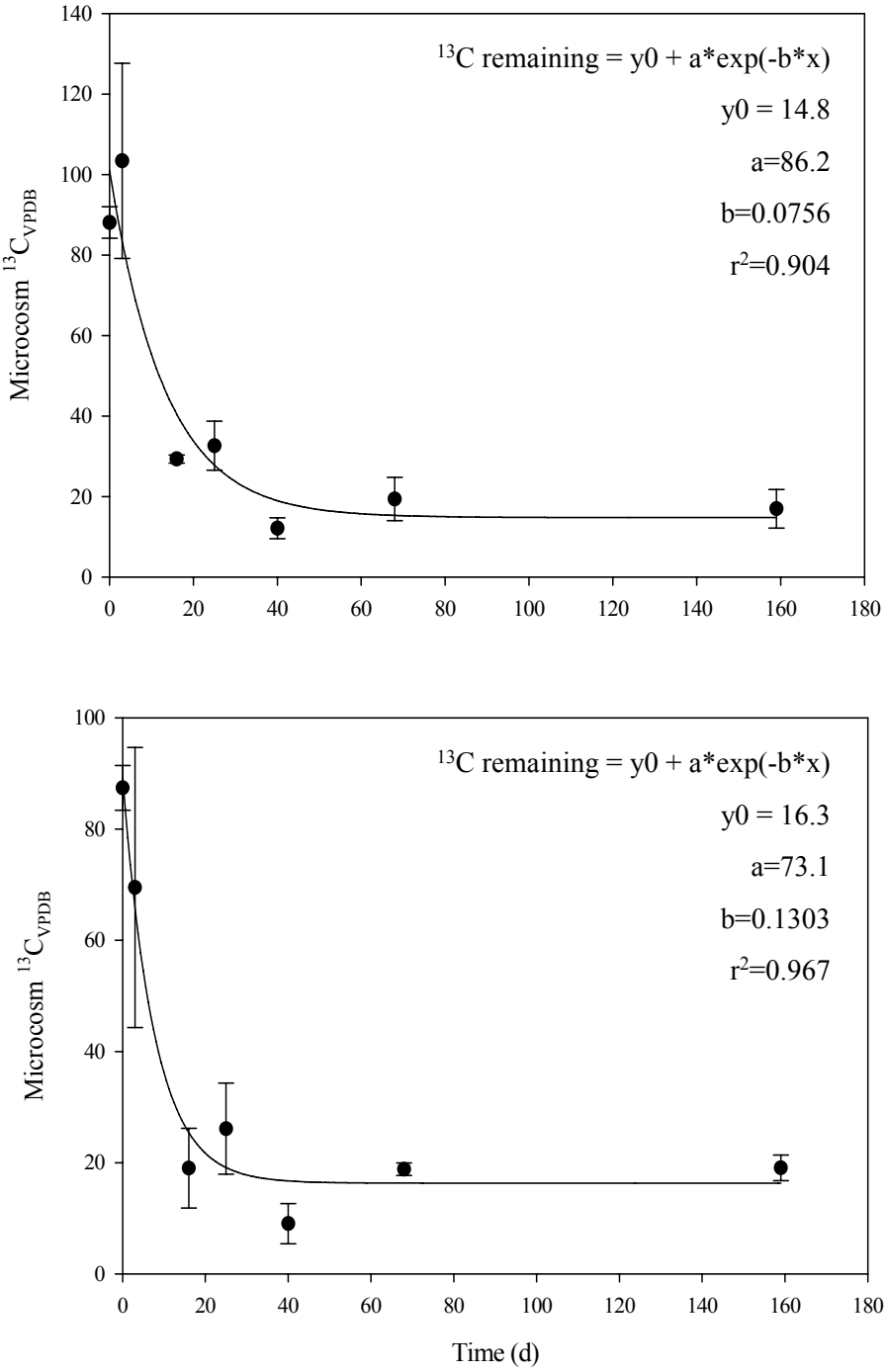

Figure 4.2. Plant residue ^{13}C mineralization in 0-5 cm CT, R+ treatment (A) and 5-15 cm CT, R+ treatment (B) during the 159 d experiment. Values are means and bars are ± 1 standard error. Inset data is for a one-pool kinetic model.

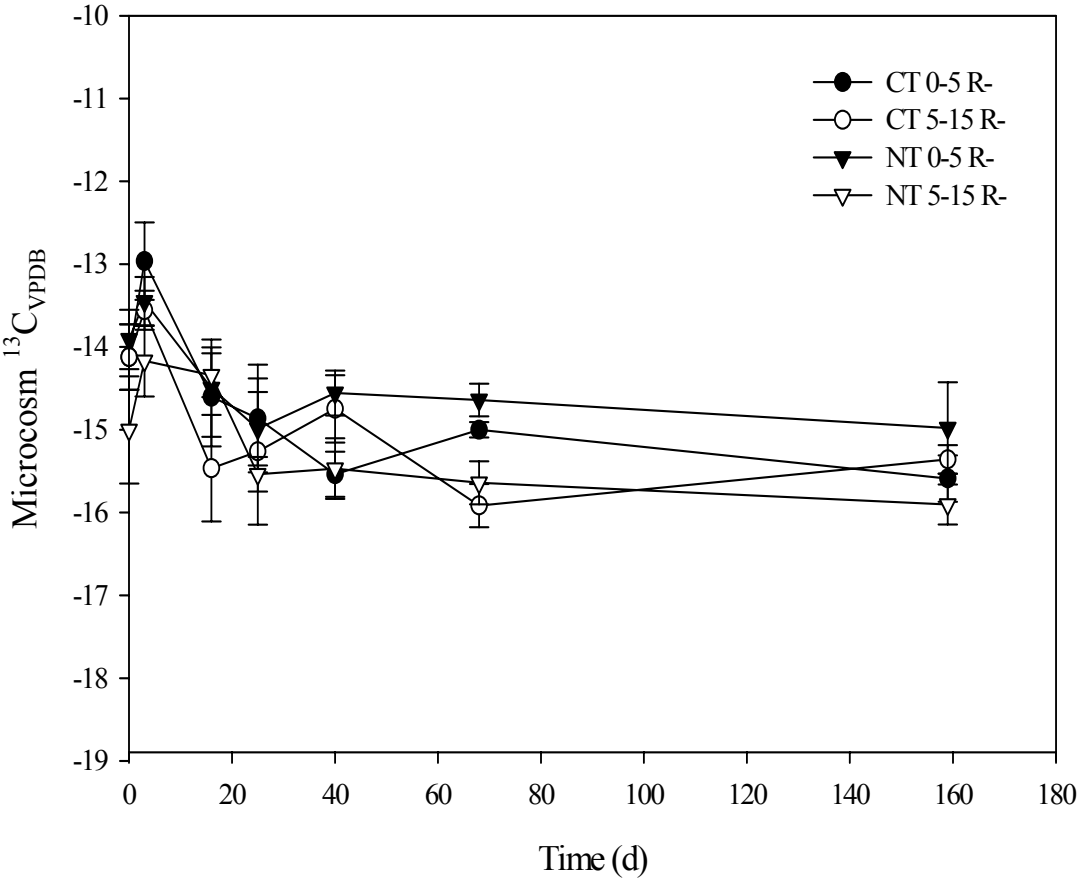

Figure 4.3. ^{13}C data collected from NT and CT microcosms not receiving any of the ^{13}C and ^{15}N-enriched plant residue. Values are means and bars are ± 1 standard error.

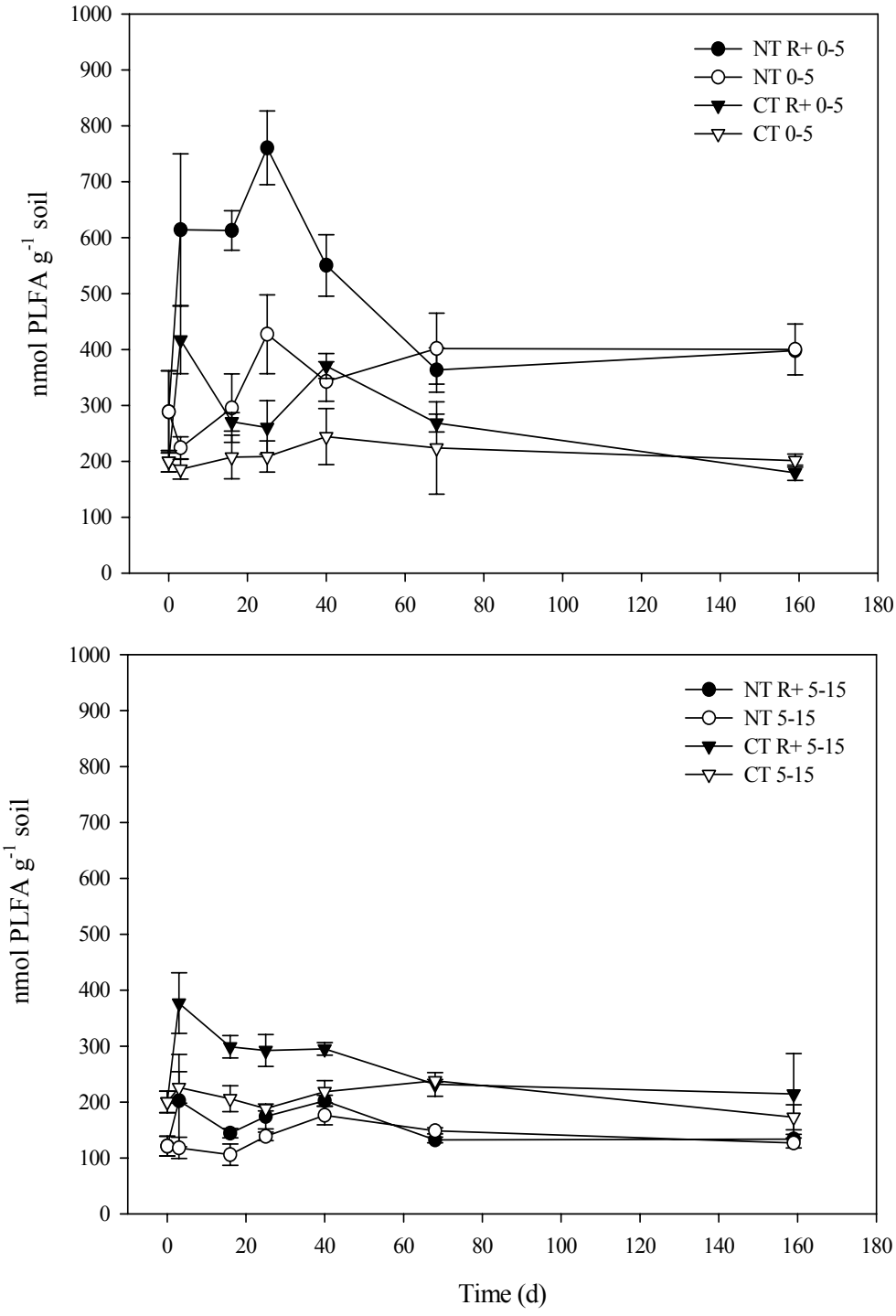

Figure 4.4. Soil total PLFA during the 159 d experiment for the 0-5 depth (A) and the 5-15 cm depth (B). Values are means ± 1 standard error.

CHAPTER 5 - Summary

Levels of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) have increased exponentially over the past 150 y, primarily due to human activities (IPCC, 2001). The increased levels of greenhouse gases (GHG) in the atmosphere have the potential to alter global temperature and precipitation patterns (CAST, 2004). Soil contains the largest terrestrial pool of C, and soil C sequestration is a rapidly deployable technology for reducing levels of atmospheric CO_2 and N_2O (Caldeira et al., 2004; Rice, 2006). The controls on soil C storage include physical, chemical, and biological processes. Understanding the impact of soil and plant management on soil C sequestration may allow for further enhancements of soil C storage capacity. From a mass balance perspective, increases to soil C can be made by increasing inputs or reducing outputs. The research reported here focused on identifying strategies to reduce C outputs, or losses, from soil by (1) slowing plant residue decomposition rates, or (2) increasing soil fungal dominance and physical protection of soil C.

Plant residue added to the soil at the end of harvest contains soluble materials, such as sugars and proteins, cellulose, hemicellulose, lignin, and ash. Of these, lignin is the most resistant to microbial decomposition. Increasing the proportion of lignin in crop residues may increase C sequestration rates by (1) slowing the conversion of plant residue C into soil C or CO_2, and (2) acting as chemical intermediates to soil organic matter (SOM) formation. Grain sorghum (*Sorghum bicolor*) varieties grown in Kansas contained different levels of lignin which contribute to differential lodging characteristics. The lignin content of shoots and roots varied from about 40-80 g lignin kg^{-1} plant residue. The differences observed in lignin content were not correlated with mineralization of plant residue C into CO_2 by the soil microorganisms. The variation in lignin levels were relatively low in this study, when compared to other plant

materials (Wang et al., 2004), and possibly do not constitute a large enough variation of plant residue C to result in measurable differences in mineralization rates. Rapid, intermediate, and slow phases of C mineralization were similar for each hybrid. Adding N reduced overall residue C mineralization, and reduced the intermediate and slow kinetic rates of mineralization. The overall residue C mineralized was possibly connected with the residue C/N ratio and lignin content. Reid (1979) concluded that a high C/N ratio resulted in greater lignin decomposition. Mycogen 1506 exhibited high lignin content, the highest C/N ratio, and the highest % mineralization.

Natural plant mutations, such as brown midrib mutation, result in the suppression of one or more of the lignin biosynthesis enzymes, such as cinnamyl alcohol dehydrogenase, caffeic acid O-methyl transferase, cinnamoyl CoA reductase (Marita et al., 2003). Reducing the expression of these enzymes has been shown to lead to altered lignin chemistry (Attanassova et al., 1995; Ralph et al., 1996) that results in more rapid mineralization for the bmr mutants, as compared to their normal isolines (Hopkins et al., 2001; Webster et al., 2005). We found normal grain sorghum plant residue to have higher amounts of lignin, as compared to its bmr counterpart. Additionally, the bmr plant residue had lower aromatic resonance, suggesting altered lignin chemistry. The combined effects of lowered lignin content and altered lignin chemistry allowed for a more detailed study of the effect of lignin on C mineralization.

The bmr residue resulted in higher initial C mineralization rates, as compared to the normal isolines, for three out of the four hybrids evaluated. However, little difference was observed at other times. Similarly, Webster et al. (2005) concluded that initial higher rates of decomposition of bmr tobacco (*Nicotiana tabacum* L.) were due to the higher availability of polysaccharides, as compared to unaltered tobacco. Generally, lignin content limited C

mineralization, but only when no external N was added. When external N was added, hemicellulose content limited C mineralization. Lignin provides a physical obstacle for microorganisms by preventing access to more labile substrates, such as cellulose (Hénault et al., 2006). Hemicellulose is a term used to describe polysaccharides that cross-link and bond to cellulose microfibrils (Carpita and McCann, 2000), possibly offering a similar physical barrier. As N is known to limit lignin degradative enzyme production (Fog, 1988), the switch from lignin to hemicellulose control of C mineralization may be the result of metabolic changes in the soil decomposer community. White rot fungi (*Phanerochaete chrysosporium*) were shown to only produce ligninases when grown in a low N medium (Keyser et al., 1978). The researchers described the production of ligninase as a metabolic shift in fungi induced by exhausting available N, not by the presence of lignin (Keyser et al., 1978). Indeed, Reid (1979) found glucose additions used to lower extracellular N content would increase lignin mineralization, and vice versa. Whatever the cause, there is evidence to support the importance of plant residue architecture to plant residue C mineralization. A possibility is the location of specific substrates within plant residue and the available N at the microbe-plant residue interface affects decomposition.

The addition of N altered the soil microbial phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) profiles. The added N reduced total soil PLFA, but had variable effects on Gm+ and Gm- bacteria, fungi, and actinomycetes, when assessed at the conclusion of the experiment. The NLFA total abundance was not affected by N, however the specific abundances of the fungal NLFA 18:2ω6,9c and 18:1ω9c were switched as a result of adding N, representing a possible change in energy storage. The PLFA and NLFA profiles associated with Gm+ and Gm- bacteria, fungi, and actinomycetes did not change as a result of the changes in plant residue

chemistry. However, changes during the most rapid phase of decomposition are probable, and shifts in microbial lipid profiles have been observed for a variety of substrates, including bmr tobacco (Hénault et al., 2006), wood (Waldrop et al., 2004), and glucose (Ziegler et al., 2005). The importance of these ephemeral shifts in microbial community structure, in terms of C sequestration, are not known. However, the formation of microbial byproducts of decomposition can play important roles in SOM formation and soil aggregate formation. Saprophytic fungi produce a variety of recalcitrant aromatic molecules, such as quinones, which may condense into humus-like materials (Martin and Haider, 1971). Glycoproteins and polysaccharides formed by arbuscular mycorrhizal fungi can bind soil particles together to form aggregates (Wright, 2005). The importance of saprophytic and arbuscular mycorrhizal fungi to soil C storage and soil aggregation is well documented (Frey et al., 1999; Jastrow and Miller, 2000; Mikha and Rice, 2004; Wright, 2005; Six et al., 2006). However, microbial community dynamics following plant residue additions in different tillage systems have received less attention. A field experiment was conducted to measure the soil microbial response to adding grain sorghum residue in both tillage (CT) and no-tillage (NT) agricultural ecosystems. The ^{13}C- and ^{15}N-enriched residue decomposed rapidly and to about the same extent in both systems. The total microbial PLFA increased rapidly and then declined in conjunction with plant residue C depletion. The total PLFA pulse was significantly greater in the NT 0-5 cm, as compared to the NT 5-15 cm, CT 0-5 cm, or 5-15 cm. A similar pulse was observed for Gm+ and Gm- bacteria, and fungi; however, the actinomycetes did not respond to residue additions. The placement of the plant residue in the NT soil (on the surface), as compared to the CT soil (mixed to 15 cm) concentrated available substrates. Residue placement could also explain the higher soil microbial biomass C and N found in surface NT soils, as compared to CT soils (Frey et al., 1999), as higher substrate levels

would support larger microbial populations (Mikha and Rice, 2004). Surface applied residue could also be colonized by aerial hyphae penetrating the soil, providing a niche for fungi. The higher amount of fungi, and therefore recalcitrant fungal byproducts, could indicate one possible long-term mechanism for C accumulation in NT soils, as compared to CT soils. However, the importance of fungi extends beyond their ability to produce recalcitrant C compounds.

Fungal hyphae, especially arbuscular mycorrhizal hyphae, are intricately involved with the formation of soil macroaggregates (Six et al., 2002). Macroaggregates directly provide physical protection for plant residue C and N (Simpson et al., 2004). In this experiment, we found significantly greater amounts of plant residue C and N present in the macroaggregates as compared to the microaggregates, after only 159 d. The high value for both ^{13}C and ^{15}N within the macroaggregates provides evidence for physical protection of plant residue. The larger proportion of macroaggregates, as compared to microaggregates, in undisturbed soils is a possible secondary mechanism of long-term C accumulation in NT soils.

More research is needed to extrapolate the complex mechanisms involved in plant residue decomposition. Recent evidence has indicated that plant architecture influences decomposition, as well as lignin chemistry or the addition of N (Webster et al., 2005; Hénault et al., 2006). Future research could include observational studies to examine the plant residue and soil microbial interface. An interesting study would investigate microbial gene expression of degradative enzymes (e.g., cellulase, lignin peroxidase) coupled with fluorescent and light microscopy to correlate microbial growth to the substrate source. A plasmid could be constructed that contains the degradative enzyme along with a fluorescent tag such as green reporter protein. The experiment could be strengthened by selectively labeling plant residue cellulose or lignin with ^{13}C (Crawford and Crawford, 1976) and measuring the $^{13}CO_2$ evolution to confirm substrate

utilization. Results could indicate that plant architectural considerations may be as important as differences in plant residue chemistry between similar plants.

Soil architecture and aggregation are important components of soil quality and C sequestration. Macroaggregates are coated with a relatively water insoluble consortium of glycoproteins, earthworm casts, root exudates, and hyphae (Wright, 2005). Macroaggregate turnover plays a critical role in many soil processes relating to C and N sequestration. De Gryze et al. (2006) used rare earth metals to model soil aggregate turnover and found macroaggregate turnover is <40 d. However, this experiments, and others, omitted plants from the experimental design. Field studies including plants are needed to more accurately model macroaggregate stability. Aggregate stability could be determined by added a water soluble, traceable material, such as bromine, and determining its location inside and outside of macroaggregates after a specified time period.

Microbial genetics may also be used to enhance soil C sequestration research. The growing use of functional genomic microarrays (Gentry et al., 2006) may provide a third tier of information, next to nutrient transformations and microbial community analysis, to further facilitate the understanding of the terrestrial C and N cycles. In summary, soil C sequestration is important for agriculture and the environment, as enhancing soil C storage increases soil quality and productivity.

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Appendix A - Appendix

Table A.1. P-values of ANOVA for soil NH_4-N and NO_3-N levels during the field experiment.

	Time						
	0	3	16	25	40	68	159
NH_4-N Effect	----------days----------						
Tillage (T)	0.1387	0.9699	0.9957	0.1984	0.8949	0.8508	0.3608
Residue (R)	no test	0.1477	0.1719	0.1485	0.1110	0.4975	0.3929
T*R	no test	0.2486	0.6864	0.2582	0.9539	0.1224	0.9472
Depth (D)	0.0167	**<0.0001**	**0.0143**	0.0941	0.0994	0.2445	0.5883
T*D	0.0167	**0.0005**	0.3329	0.1359	0.8105	0.0512	**0.0381**
R*D	no test	0.0600	0.0690	0.0625	0.0861	0.3197	0.3336
T*R*D	no test	**0.0028**	0.8102	0.1094	0.7509	**0.0405**	0.3777
NO_3-N Effect							
Tillage (T)	0.1646	0.7299	0.8991	0.8549	0.5493	0.4366	0.1314
Residue (R)	no test	0.5744	0.7227	0.4428	0.1950	0.3268	0.4364
T*R	no test	0.5170	0.1017	0.1555	0.4045	0.2857	0.9819
Depth (D)	0.0221	**0.0087**	0.2448	**0.0169**	**0.0005**	0.8300	**0.0090**
T*D	0.0221	**0.0213**	0.3272	0.3466	**0.0118**	0.0963	**0.0444**
R*D	no test	0.1718	0.4898	0.4230	**0.0056**	0.8248	0.2626
T*R*D	no test	0.4550	0.2987	0.9267	0.3753	0.7173	0.6301

Table A.2. P-values for statistical tests of PLFA data: 3-way interactions.

	PLFA Groupings - Test of 3 factor model									
	0-5 cm					5-15 cm				
Effect	Gm+	Gm-	Fungi	Actino.	Total	Gm+	Gm-	Fungi	Actino.	Total
Time (M)	0.0058	0.0004	0.0006	0.0069	0.0014	0.0086	0.0163	0.0023	0.0130	0.0127
Tillage (T)	<0.0001	0.0174	<0.0001	0.0036	0.0100	0.0001	<0.0001	0.0002	0.0253	0.0035
M*T	0.0608	0.0263	0.0625	0.9138	0.0112	0.8229	0.5816	0.0540	0.8706	0.4236
Residue (R)	0.0002	0.0019	<0.0001	0.1333	0.0006	0.0263	0.0003	0.0082	0.0583	<0.0001
M*R	0.0044	0.0201	<0.0001	0.2042	0.0007	0.0384	0.0100	0.0111	0.1374	0.0118
T*R	0.0703	0.0276	0.0046	0.7897	0.0425	0.2868	0.0712	0.0350	0.4471	0.0492
M*T*R	0.4695	0.2045	0.0086	0.7685	0.1247	0.6786	0.4522	0.7386	0.9393	0.9419
Sliced by Time - Indicates where differences are located						Sliced by Time - Indicates where differences are located				
0	0.8676	0.4434	0.4555	0.8114	0.4843	0.0940	0.0766	0.9521	0.2199	0.1026
3	**<0.0001**	**0.0103**	**<0.0001**	0.0556	**<0.0001**	**0.0001**	**0.0001**	**<0.0001**	**0.0196**	**<0.0001**
16	**0.0087**	**0.0001**	**<0.0001**	**0.0104**	**<0.0001**	**0.0067**	**<0.0001**	**0.0030**	**0.0118**	**<0.0001**
25	**<0.0001**	**<0.0001**	**<0.0001**	0.1221	**<0.0001**	**0.0079**	**<0.0001**	**0.0027**	**0.0140**	**0.0007**
40	**0.0003**	**<0.0001**	**<0.0001**	0.4148	**0.0013**	**0.0144**	**0.0001**	**0.0466**	**0.0164**	**0.0131**
68	0.1627	0.1360	0.2443	0.2570	0.1241	**0.0245**	**0.0472**	0.3706	0.1164	**0.0282**
159	0.1033	**0.0296**	**0.0319**	0.1744	**0.0417**	0.5102	**0.0358**	0.4605	0.7594	0.2722

Table A.3. P-values for PLFA evenness calculations

Effect	0-5 cm	5-15 cm
Time (M)	0.0017	0.7479
Tillage (T)	0.2872	0.0119
M*T	0.0352	0.2358
Residue (R)	0.0003	0.1030
M*R	0.3320	0.4157
T*R	0.4451	0.2850
M*T*R	0.5455	0.1205

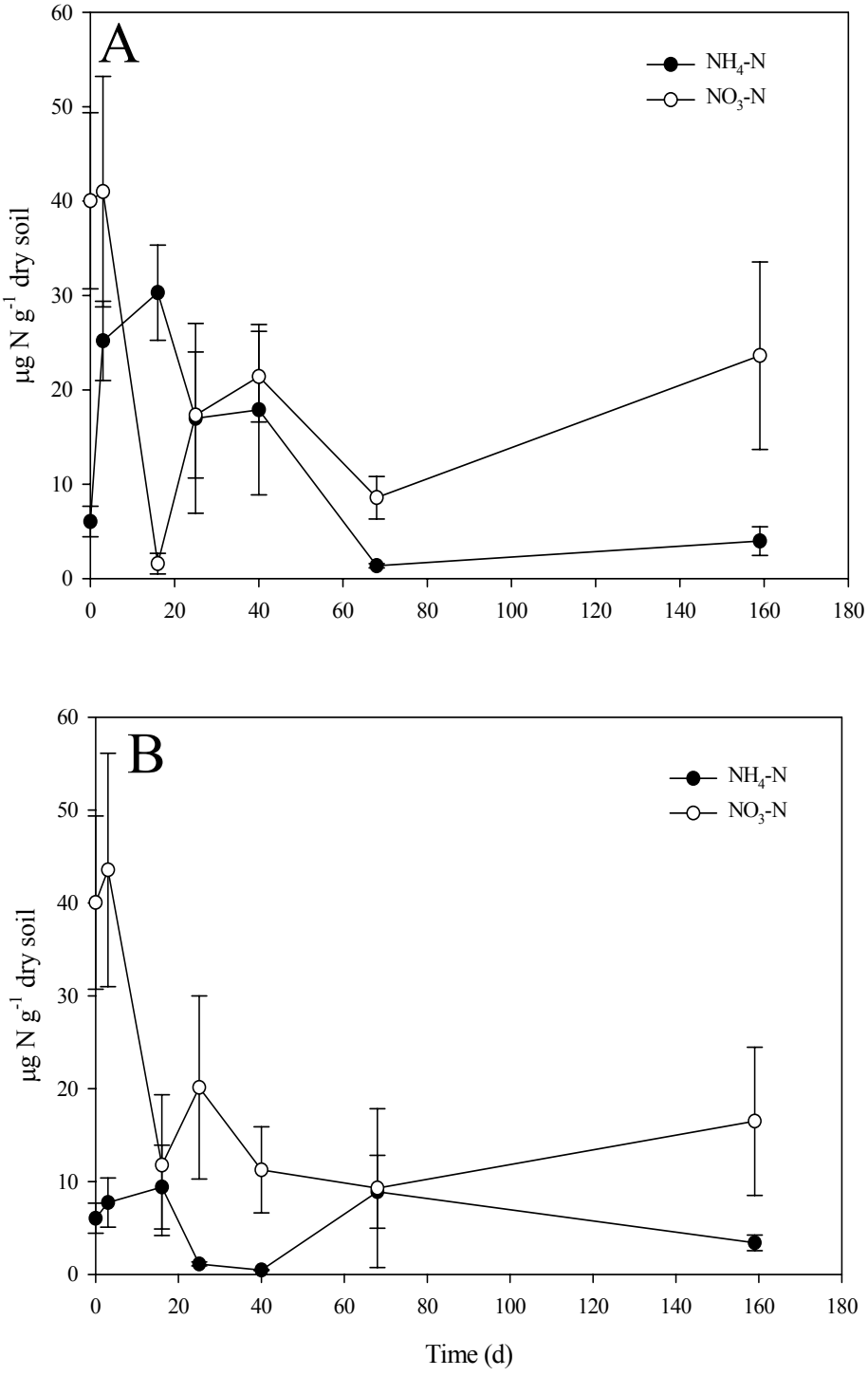

Figure A.1. Soil inorganic N levels for NT 0-5 cm R+ treatment (A) and NT 0-5 cm R- treatment (B) during the 159 d experiment. Values are means and bars are ± 1 standard error.

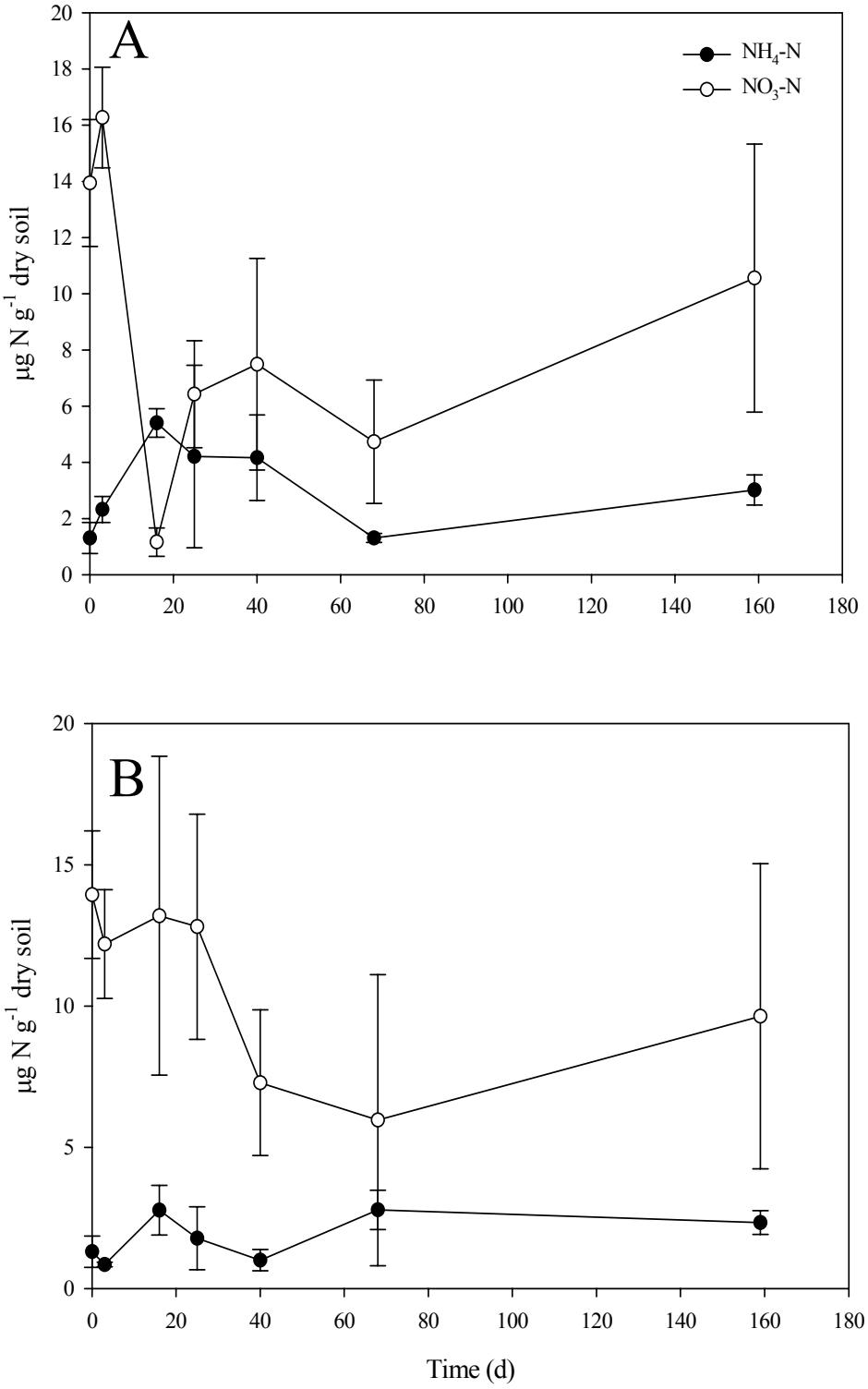

Figure A.2. Soil inorganic N levels for NT 5-15 cm R+ treatment (A) and NT 5-15 cm R- treatment (B) during the 159 d experiment. Values are means and bars are ± 1 standard error.

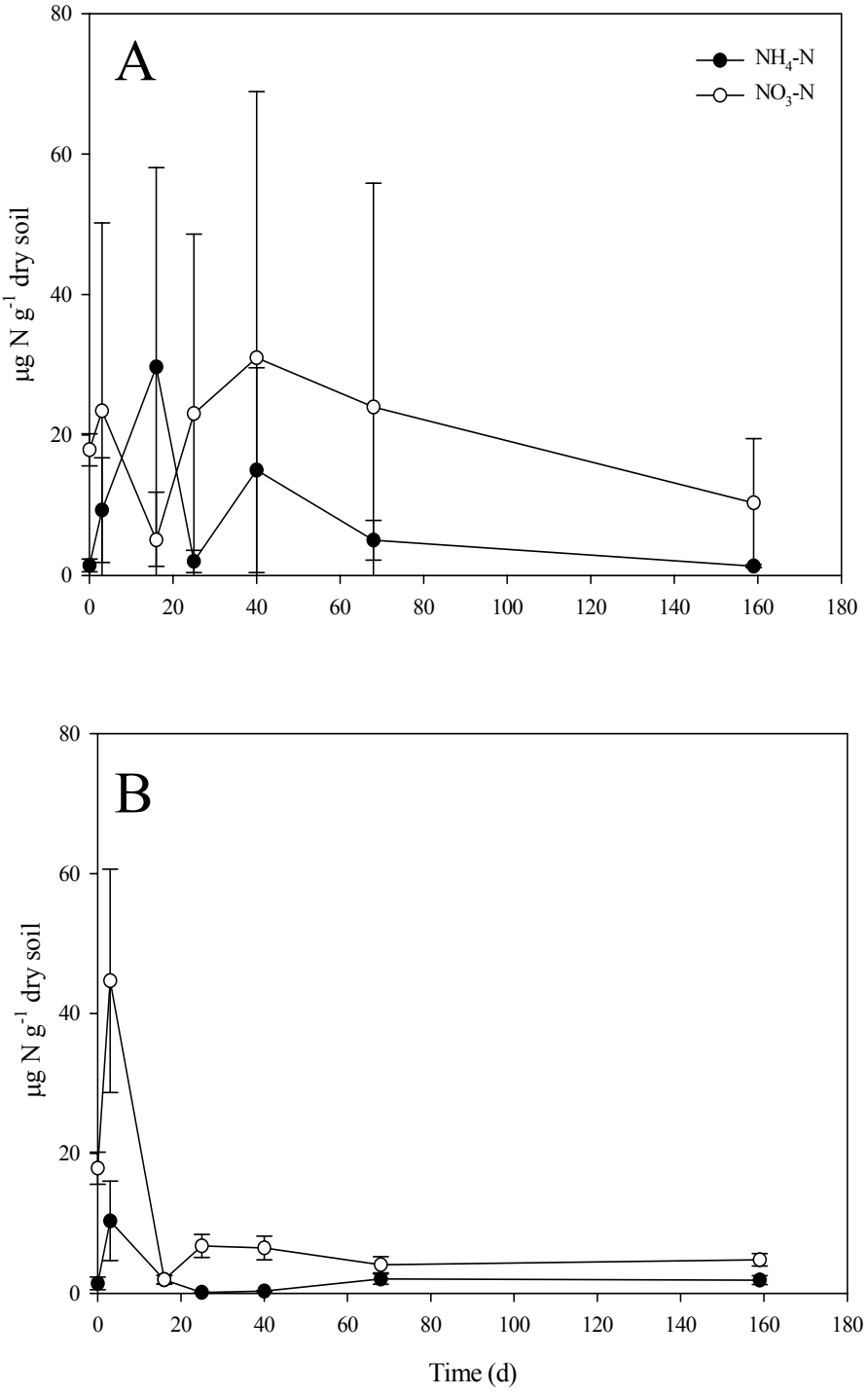

Figure A.3. Soil inorganic N levels for CT 0-5 cm R+ treatment (A) and CT 0-5 cm R- treatment (B) during the 159 d experiment. Values are means and bars are ± 1 standard error.

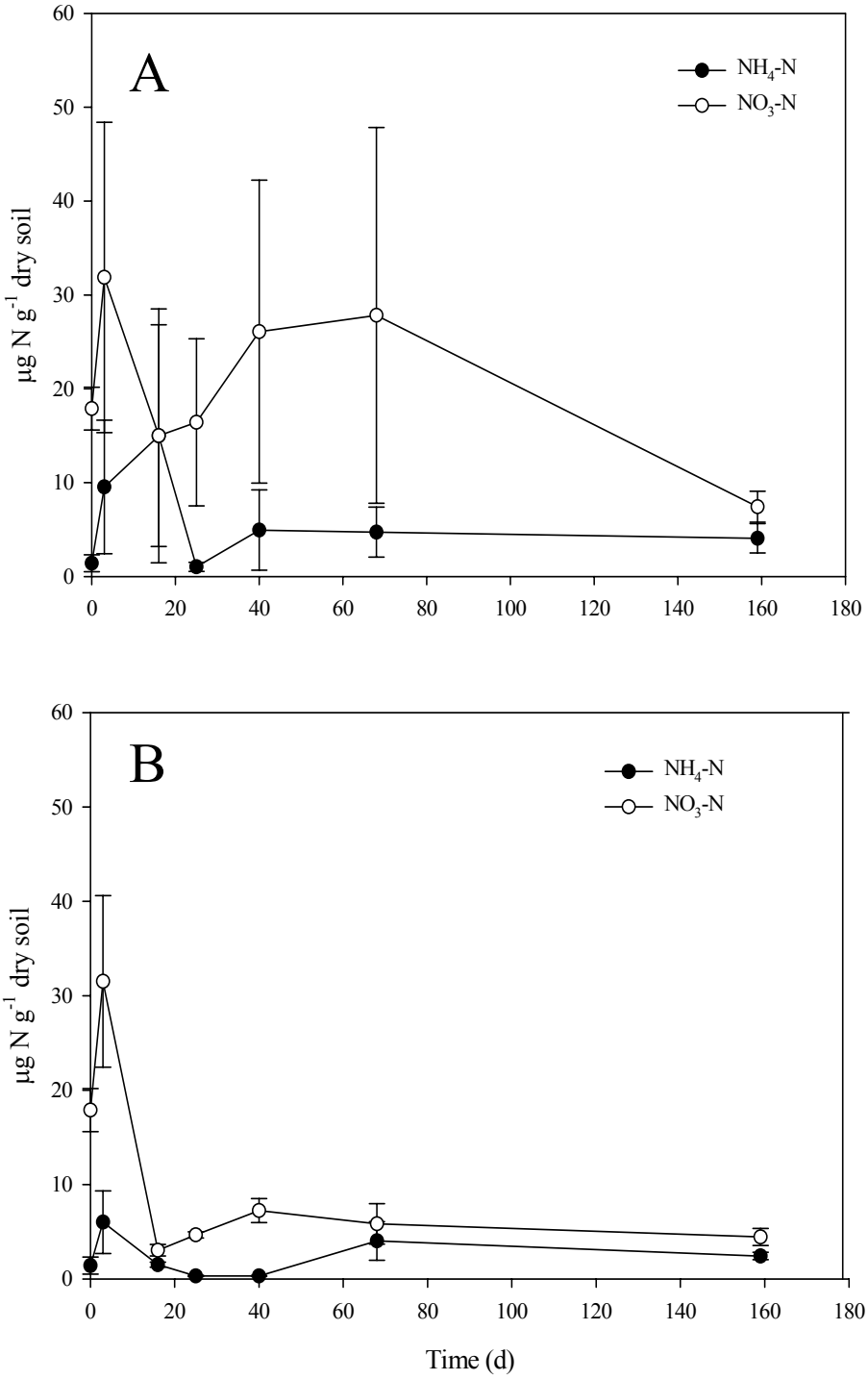

Figure A.4. Soil inorganic N levels for CT 5-15 cm R+ treatment (A) and CT 5-15 cm R- treatment (B) during the 159 d experiment. Values are means and bars are ± 1 standard error.

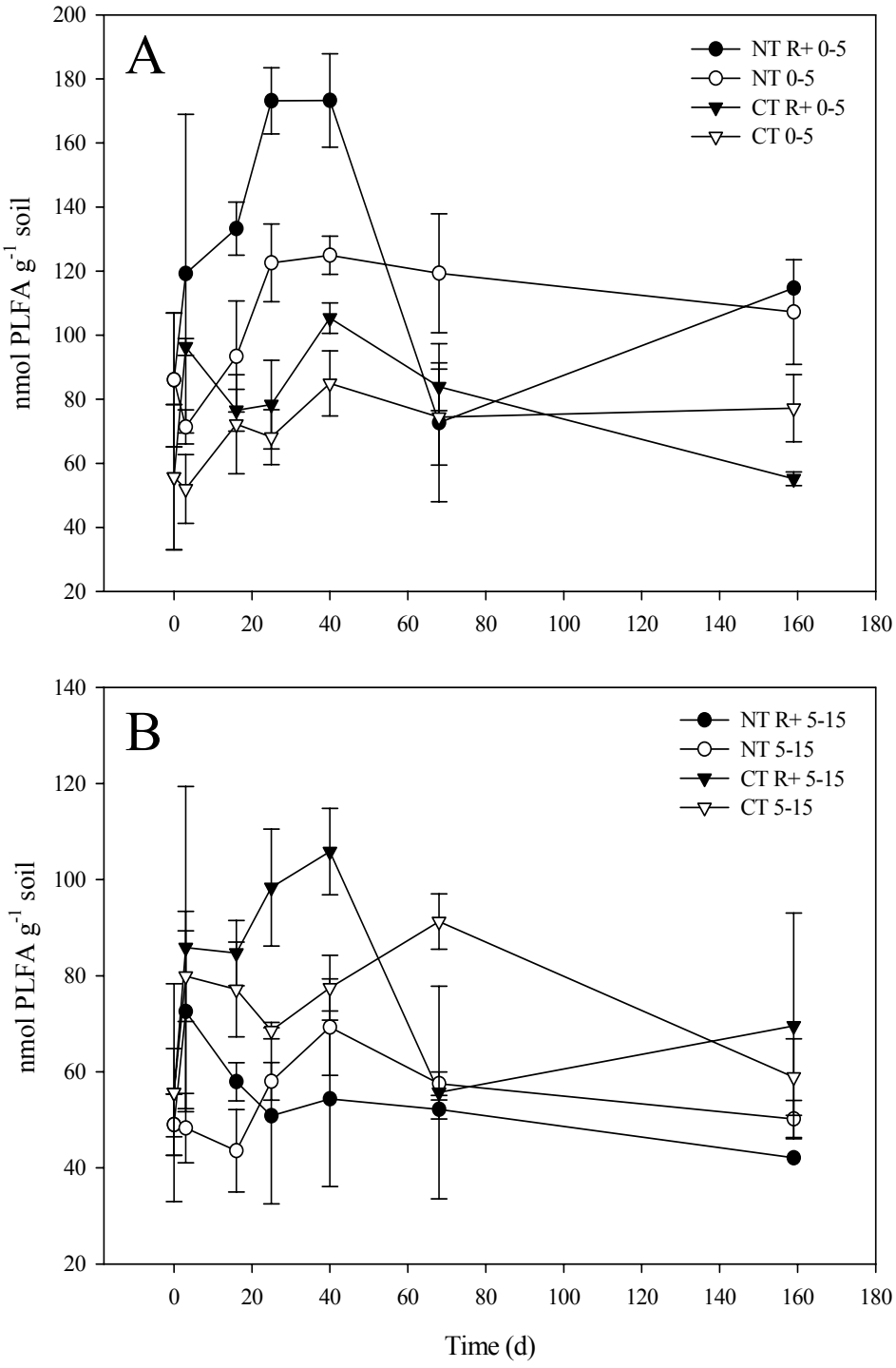

Figure A.5. Soil PLFA representing Gm^+ bacteria during the 159 d experiment for the 0-5 depth (A) and the 5-15 cm depth (B). Values are means ± 1 standard error

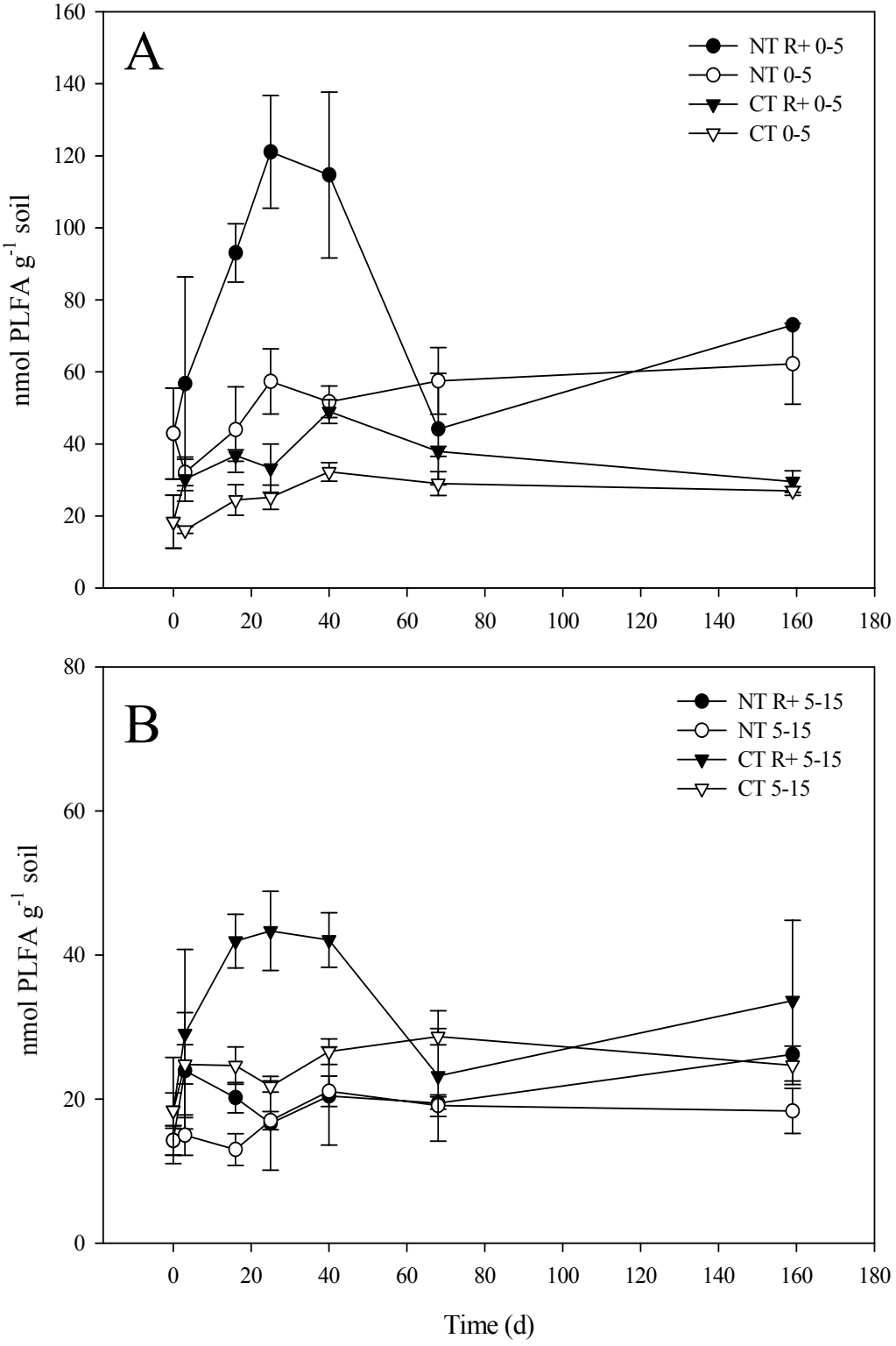

Figure A.6. Soil PLFA representing Gm^- bacteria during the 159 d experiment for the 0-5 depth (A) and the 5-15 cm depth (B). Values are means ± 1 standard error.

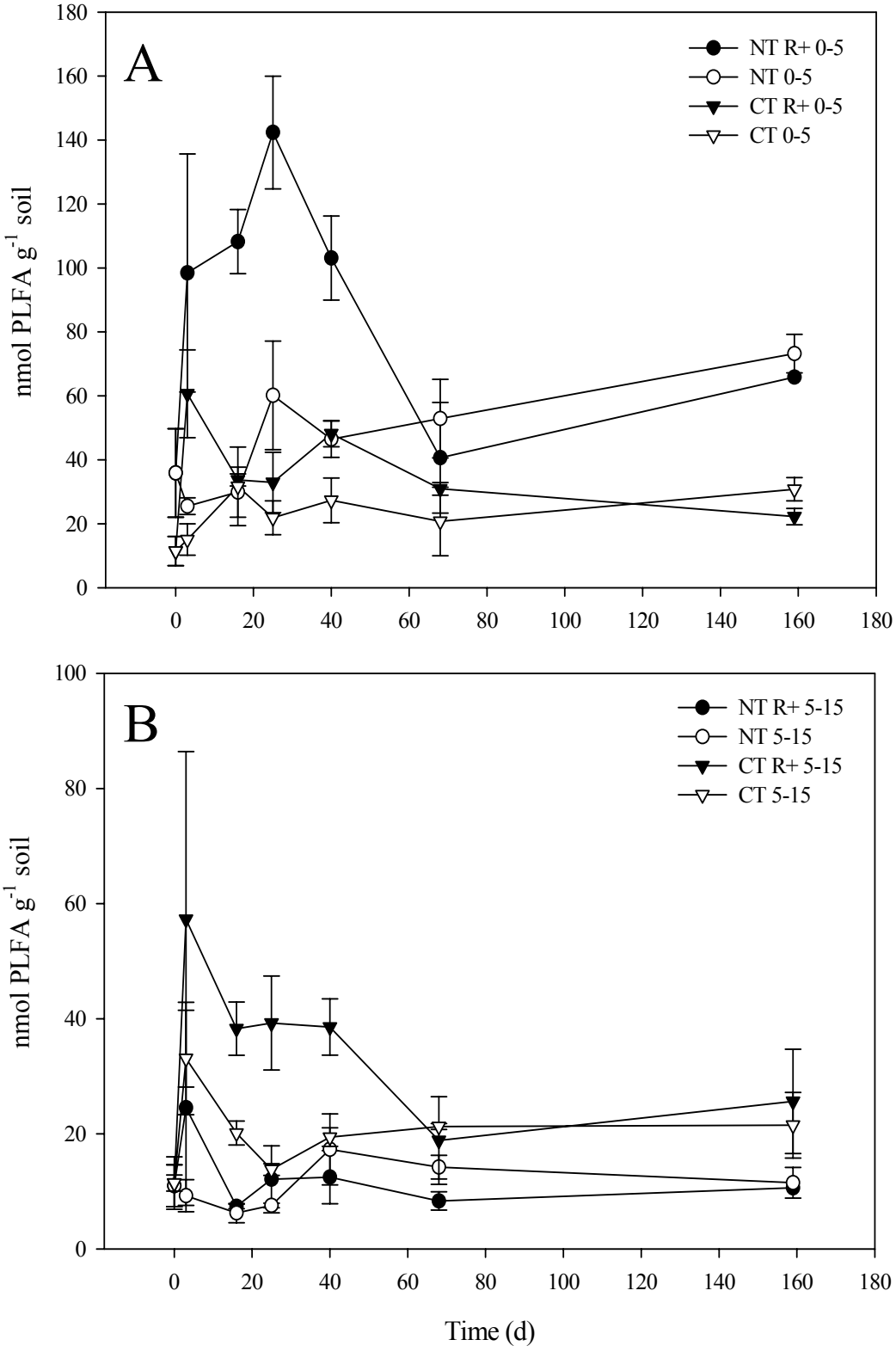

Figure A.7. Soil PLFA representing fungi during the 159 d experiment for the 0-5 depth (A) and the 5-15 cm depth (B). Values are means ± 1 standard error.

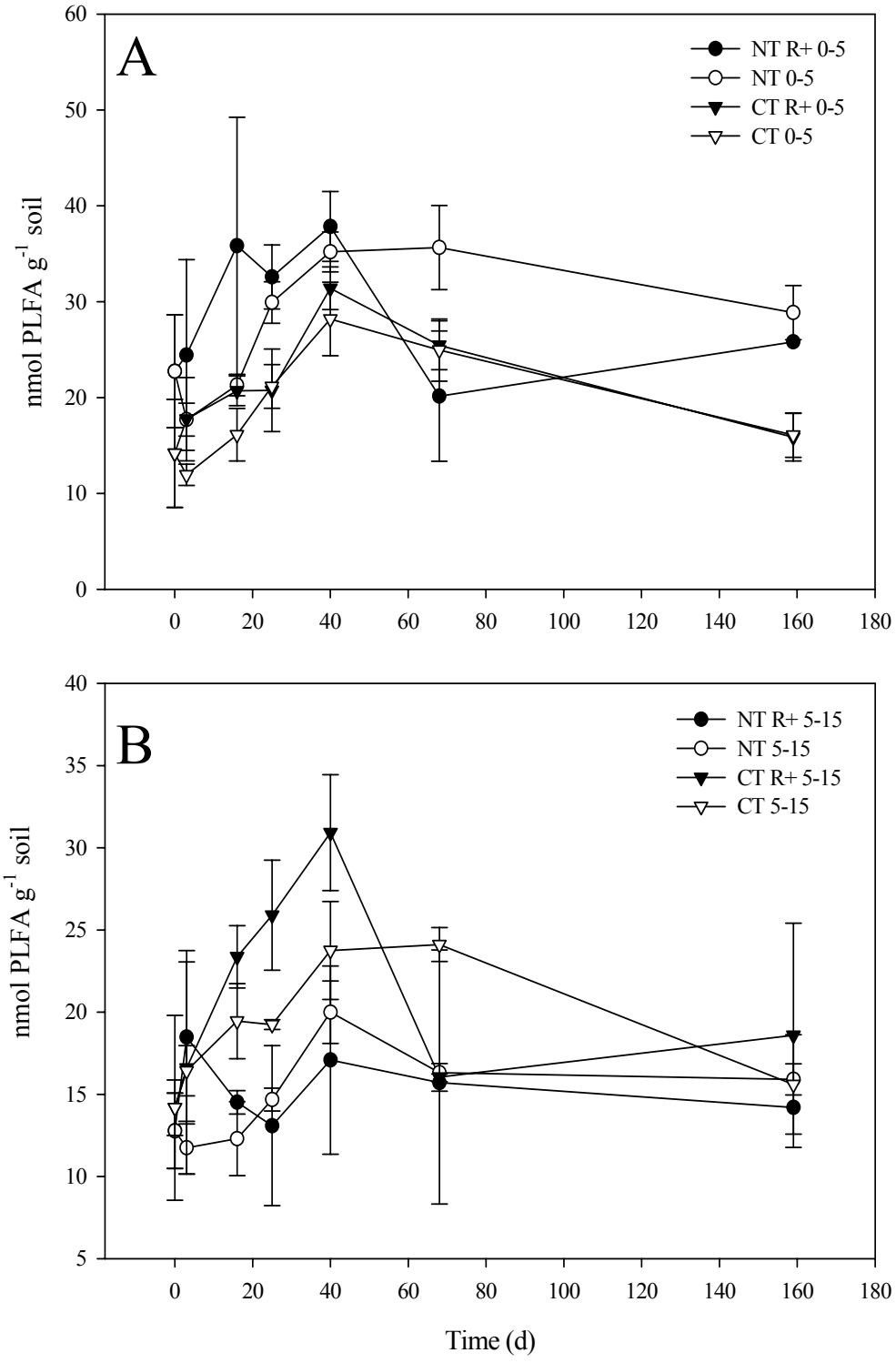

Figure A.8. Soil PLFA representing actinomycetes during the 159 d experiment for the 0-5 depth (A) and the 5-15 cm depth (B). Values are means ± 1 standard error.

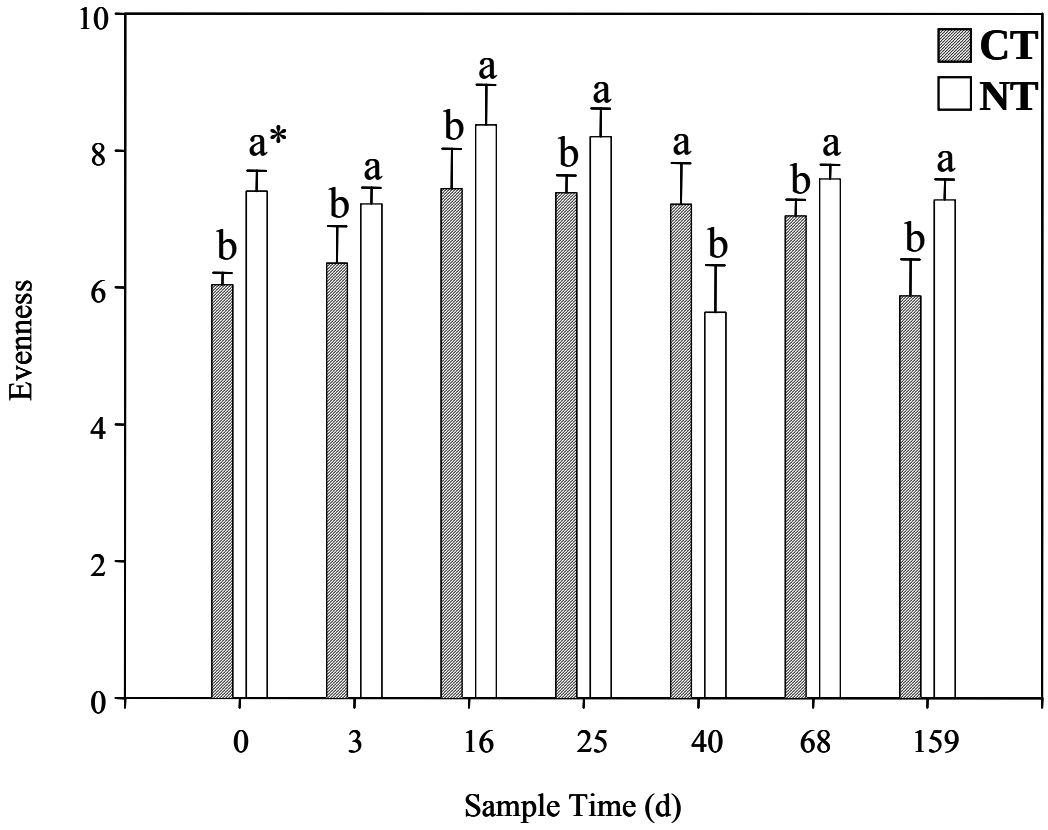

Figure A.9. Soil PLFA evenness for 0-5 cm during the 159 d experiment. The time*tillage interaction was significant. Values are means ± 1 standard error. *Bars for each time with the same letter are not different.