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Microbial Ecology, Nitrogen, and Nitrous Oxide Trends in Marginal Soils Used for Cellulosic Biofuel Production in Eastern Nebraska

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MICROBIAL ECOLOGY, NITROGEN, AND NITROUS OXIDE TRENDS IN MARGINAL SOILS USED FOR CELLULOSIC BIOFUEL PRODUCTION IN EASTERN NEBRASKA

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There is a growing demand for diverse biofuels in the United States. Potential feedstocks for cellulosic ethanol include corn (*Zea mays,* L.) stover and switchgrass (*Panicum virgatum,* L). Lands used to provide for corn production can provide some cellulosic feedstock through corn stover, but crop residue removal can have negative impacts on soil quality. Furthermore, arable land must supply both fuel and food. To meet both demands, lands considered marginal for row-crop production will likely be used to produce dedicated bioenergy crops such as switchgrass. Marginal lands are typically placed in conservation programs because they are prone to erosion and soil quality degradation, so it is imperative to understand the impacts biofuel production have on marginal soils. A long term, no-till, marginal site in eastern Nebraska was established to assess the impacts of management on soil quality. This study considers continuous corn, switchgrass, and soybean (*Glycine max* (L.) Merr.) as an interim crop, as well as nitrogen (N) and harvest practices in corn (residue retention/removal) and switchgrass plots (harvest in August or October). Soil microbial data, nitrous oxide (N_2O) emissions, crop N use efficiency, and soil N were measured to assess management impacts on soil, water, and air quality at this site. Abundances of soil microbial biomarkers and biomass

responded to all treatments, but crop type was the strongest determining factor. In one of two years of observation, there was no significant treatment effect on N_2O emissions. In the second year, N_2O emissions increased with N fertilization. Crop N recovery varied with all treatments and year. Relatively low levels of soil nitrate (NO_3^-) were found under switchgrass and soybean plots, compared to higher levels under corn plots. Switchgrass production on marginal soils appears less likely to result in N loss as N_2O or NO_3^- than corn. High microbial biomass, soil organic matter and low NO_3^- concentrations also suggest soil quality will be better retained under switchgrass than corn.

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Table of Contents

Abstract…….....ii

Acknowledgements…….iv

Chapter 1: Introduction…….……...1

References……..10

Figures……15

Chapter 2: Soil Microbial Communities in Corn and Switchgrass-based Biofuel Production Systems: Effects of residue and nitrogen management practices in a long-term, no-till study in Eastern Nebraska……16

Abstract……..16

Introduction……....17

Methods……..20

Results……....25

Discussion……..30

Conclusions……....34

References……..35

Tables and Figures…….39

Table of Contents Continued

Chapter 3: Nitrogen Dynamics of Corn, Switchgrass and Soybean Production Systems:

Nitrous oxide emissions, soil nitrogen, and crop nitrogen balances……..68

Abstract……..68

Introduction……...69

Methods…….74

Results……....82

Discussion……..84

Conclusions……89

References……..90

Tables and Figures…….97

Chapter 4: Summary and Conclusions…….112

References……...120

List of Tables

Chapter 2

Table 2.1. Pearson correlation matrix for soil physical and chemical properties and for abundances of microbial groups and total annual N_2O emissions…….39

Table 2.1a. Soil chemical properties in CC plots by N-rate at 0-5 cm……..40

Table 2.1b. Soil chemical properties in CC plots by N-rate at 5-10 cm……40

Table 2.1c. Soil chemical properties in CC plots by N-rate at 10-30 cm…….....41

Table 2.3a. Soil chemical properties in SG plots by N-rate at 0-5 cm……..42

Table 2.3b. Soil chemical properties in SG plots by N-rate at 5-10 cm……42

Table 2.3c. Soil chemical properties in SG plots by N-rate at 10-30 cm……..…43

Table 2.4a. Soil chemical properties in SB plots by N-rate at 0-5 cm. Treatments were last applied in 2009…….……...44

Table 2.4b. Soil chemical properties in SB plots by N-rate at 5-10 cm. Treatments were last applied in 2009……44

Table 2.4c. Soil chemical properties in SB plots by N-rate at 10-30 cm. Treatments were last applied in 2009……45

Table 2.5a. Pairwise squared Mahalanobis distances between for FAMEs between N-rate and harvest treatments at 0-5 cm in CC plots……46

Table 2.5b. Pairwise squared Mahalanobis distances between for FAMEs between N-rate and harvest treatments at 0-5 cm in SG plots……46

Table 2.5c : Pairwise squared Mahalanobis distances between for FAMEs between residual N-rate and harvest treatments in SB plots at 0-5 cm……47

Table 2.6a: Pairwise squared Mahalanobis distances between for FAMEs between N-rate and harvest treatments at 5-10 cm in CC plots……..48

Table 2.6b: Pairwise squared Mahalanobis distances between for FAMEs between N-rate and harvest treatments at 5-10 cm in SG plots……..48

Table 2.6c: Pairwise squared Mahalanobis distances between for FAMEs between residual N-rate and harvest treatments in SB plots at 5-10 cm……..49

Table 2.7a: Pairwise squared Mahalanobis distances between for FAMEs between crop types at 0-5 cm. Table corresponds with Figure 2.4……..…50

Table 2.7b: Pairwise squared Mahalanobis distances between for FAMEs between crop types at 5-10 cm. Table corresponds with Figure 2.4……...50

Table 2.7a. Pairwise squared Mahalanobis distances for FAMEs between N-rate at 0-5 cm. Table corresponds with Figure 2.5…….51

Table 2.7b. Pairwise squared Mahalanobis distances for FAMEs between N-rate at 5-10 cm. Table corresponds with Figure 2.5……..…..51

Table 2.8a. Pairwise squared Mahalanobis distances for FAMEs between crop and N rate at 0-5 cm. N treatments in SB plots refer to residual treatments. Table corresponds with Figure 2.6……...52

Table 2.8b. Pairwise squared Mahalanobis distances for FAMEs between crop and N-rate at 5-10 cm. N treatments in SB plots refer to management prior to 2010. Table corresponds with Figure 2.6…….53

Table 2.9a. Pairwise squared Mahalanobis distances for FAMEs between crop and harvest treatment at 0-5 cm. Harvest treatments in SB plots refer to management prior to 2010. Table corresponds with Figure 2.7…….54

Table 2.9b. Pairwise squared Mahalanobis distances for FAMEs between crop and harvest treatment at 5-10 cm. Harvest treatments in SB plots refer to management prior to 2010. Table corresponds with Figure 2.7…….54

Table 2.10a. Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 0-5 cm in CC plots……55

Table 2.10b. Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 5-10 cm in CC……..55

Table 2.11a. Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 0-5 cm in SG plots……56

Table 2.11b. Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 5-10 cm in SG plots…..56

Table 2.12a. Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 0-5 cm in SB plots. Treatments were last applied in 2009……57

Table 2.12b. Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 5-10 cm in SB plots. Treatments were last applied in 2009……57

Chapter 3

Table 3.1a. Average nitrogen inputs and removals in 2010, listed by crop……...97

Table 3.1b. Average nitrogen inputs and removals in 2011, listed by crop…….....97

Table 3.2a. Average cumulative N_2O emissions (kg N ha^{-1} yr^{-1}) by crop type and N-rate in 2010……...98

Table 3.2b. Average cumulative N_2O emissions (kg N ha^{-1} yr^{-1}) by crop type and N-rate in 2011……...98

Table 3.3a. Average values of soil properties from deep soil cores N-rate in CC plots…99

Table 3.3b. Average values of soil properties from deep soil cores N-rate in SG plots……..….100

Table 3.3c. Average values of soil properties from deep soil cores N-rate in SB plots, listed by N-rates formerly applied to the previous switchgrass crop……..…101

Table 3.4. Average SOM and POM from aggregate samples by crop and N-rate ..…...102

Table 3.5a. Daily N_2O flux data (g N ha^{-1} d^{-1}) from 2010 in soybean plots which received 120 kg N ha^{-1} fertilization in 2009……..….103

Table 3.5b. Daily N_2O flux data (g N ha^{-1} d^{-1}) from 2011 in soybean plots which received 120 kg N ha^{-1} fertilization in 2009……..…….103

Table 3.6. Extractable NO_3^- (kg N ha^{-1}) in the rooting zone and below in SB plots, listed by the fertilization applied to the previous crop in 2009……104

List of Figures

Chapter 1

Figure 1.1. Individual gas contribution to total greenhouse gas emissions in the United States (US EPA, 2012)……...15

Figure 1.2. Agricultural contributions to greenhouse gas production reported as CO_2 equivalents (US EPA, 2012)…….....15

Chapter 2

Figure 2.1. Plot plan of study site……..58

Figure 2.2. Canonical discriminant analysis of FAMEs by all treatments (A) and selected biomarkers (B) at 0-5 cm…….59

Figure 2.3. Canonical discriminant analysis of FAMEs by all treatments (A) and selected biomarkers (B) at 5-10 cm……60

Figure 2.4. Canonical discriminant analysis of FAMEs by crop type at (A,B) 0-5cm and (C,D) 5-10 cm……61

Figure 2.5. Canonical discriminant analysis of FAMEs by N-rate at (A,B) 0-5cm and (C,D) 5-10 cm……..62

Figure 2.6. Canonical discriminant analysis of FAMEs by crop and N-rate at (A,B) 0-5 cm and (C,D) 5-10 cm……..……...63

Figure 2.7. Canonical discriminant analysis of FAMEs by crop and harvest treatment at (A, B) 0-5 cm and (C, D) 5-10 cm…….64

Figure 2.8. Molar percentages of individual FAMEs by crop type at 0-5 cm……...65

Figure 2.9. Molar percentages of individual FAMEs by crop at 5-10 cm……66

Figure 2.11: Average microbial biomass (total FAMEs, nmol g^{-1}) of each crop type at 0-5 cm and 5-10 cm depth…….67

Chapter 3

Figure 3.1. Plot plan of study site……105

Figure 3.2. Precipitation (A), daily N_2O flux (B), and cumulative annual flux (C) from 120N CC and SG plots in 2010……...106

Figure 3.3. Precipitation (A), daily N_2O flux (B), and cumulative annual flux (C) from 120N CC and SG plots in 2011……...107

Figure 3.4. Total N in CC (A) and SG (B) plots by N-rate…….108

Figure 3.5. Extractable NO_3^- concentrations under CC (A) and SG (B) plots by N-rate……...109

Figure 3.6. Extractable NO_3^- (kg N ha^{-1}) under CC (A) and SG (B) plots by N-rate…..110

Figure 3.7: Volumetric water content under CC and SG plots. Depths are 1, 2, 3, and 4 ft (0.31, 0.61, 0.91, and 1.22 m)……..111

CHAPTER 1:

Introduction

*"**Biomass is the only renewable resource that can supplant petroleum-based liquid transportation fuels in the near term**." (US DOE, 2011)*

Biofuels can be made from almost anything. The United States Department of Energy (US DOE) lists "agricultural residues, forest resources, perennial grasses, woody energy crops, wastes (municipal solid waste, urban wood waste, and food waste), and algae" as potential feedstocks for biofuels (US DOE, 2011). This gamut of energy sources is part of what make biofuels so appealing. Biofuels can provide a local and diverse fuel source for transportation in the US while simultaneously reducing dependence on foreign fuels and reducing greenhouse gas emissions (GHGs) (US DOE, 2011). Rather than depending on one main source, regions in the US can be responsible for their own fuel needs. For example, cities could power their transit systems with wastes produced *in situ* and forested areas could use trees and waste products from timber production.

An America filled with biofuels seems inevitable. In 2007, the US Congress passed the Energy Independence and Security Act, and within this act set a Renewable Fuel Standard (RFS), which mandates that 36 billion gallons of biofuels will be blended into transportation fuel by 2022. Of this amount, 16 billion gallons are required to come from cellulosic biofuel (Congressional Research Service, 2011). Already it is estimated

that 90% of fuel consumed in the United Sates is blended with ethanol (Biofuels Hearing, 2011). Yet with growing demands and increasing production, biofuel research must also focus on the environmental impacts of biofuel production and consumption. After several years of research by scientists across the world, studies have found that biofuel production and consumption can have negative environmental impacts (Scharlemann and Laurance, 2008; Fargione et al., 2008). The potential negative impacts of biofuel production on the environment necessitate further study.

Beyond the over-arching need to understand what impacts biofuel production has on soil quality, it is especially important to monitor the development of production on marginal lands. As demand for biofuels increases, so will the demand for land to produce them on. Due to increasing demand for agronomic space, it is likely that biofuel production from crops like switchgrass will likely occur on marginal soils (Robertson et al., 2008; Campbell et al., 2008; Gopalakrishnan et al., 2009). Marginal soils are considered marginal for row crop production because they are highly erodible, too wet, low in nutrients, or otherwise unsuitable for conventional crop production (Stubbs, 2012). Much of the marginal land that will be used for switchgrass production is enrolled in or potentially eligible for the Conservation Reserve Program (CRP).

The high price of commodity crops in recent years may encourage landowners to remove land from CRP in favor of crop production. Removing land from CRP will likely result in reduced soil quality and increased soil erosion (Stubbs, 2012). This site discussed in this thesis was originally chosen for study partly because it is representative of land enrolled in CRP (Varvel et al., 2008).

The marginal site discussed in this thesis is a long-term cellulosic biofuel production site. In 1998, the site was planted in two cultivars of switchgrass and continuous corn.

Soil Ecology

Soil nutrient cycling is the foundation of soil quality, and nutrient cycling is largely dependent on the soil microbial community. Greenhouse gas emissions occur through microbial transformations of carbon (C) and nitrogen (N). Soil organic matter, which is critical to plant productivity, depends on microbial communities that break down plant matter. Agricultural management practices like tillage and residue management directly alter soil C and N and therefore change the composition of soil communities by altering the chemical and physical environment of the soil (Drijber et al., 2000; Spedding et al., 2004; Liebig et al., 2006). Furthermore, a changing global climate and the associated increases in atmospheric carbon dioxide (CO_2) will have impacts on soil community size and composition (Hu et al., 2001; Staddon et al., 2002; Drigo et al., 2010, Jin et al., 2010, Jin et al., 2011).

Human activities change soil quality, but soil microbes are the medium of change. It is therefore worthwhile to take note of how crop management impacts soil ecology. Chapter 2 of this thesis addresses the impacts of long-term cellulosic ethanol feedstock production on soil ecology. We focus on microbial biomass and on abundances of bacterial, actinomycete, and saprophytic and arbuscular mycorrhizal (AM) fungal groups to assess the impacts of crop, nitrogen, and harvest management practices on the soil

microbial community. Chapter 2 focuses on the structure of the microbial communities at this site.

Crop Nitrogen Use

Optimizing crop uptake of N is critical to ensuring limited loss of N. Excessive use of fertilizers has led to extensive nitrate (NO_3^-) pollution of ground and surface water in the U.S. (Wu and Babcock, 1999; EPA, 2000). Though producers have responded by adjusting the amounts and timing of fertilization to reduce NO_3^- loss through leaching and runoff, it is still possible to improve fertilization management techniques. In a given year, recovery of N fertilizer by corn plants can range from 14 to 65% (Meisinger et al., 1985), so even when producers manage fertilizer inputs carefully a substantial portion of fertilizer N could become susceptible to leaching (Dinnes et al., 2002). Much of this N is transformed by soil microbes and through microbial transformations can be incorporated into soil organic matter (SOM) or emitted as dinitrogen gas (N_2) or nitrous oxide gas (N_2O) (Robertson and Groffman, 2007). Maximizing crop N use efficiency is important because inefficient use of fertilizer inputs can lead to increased N_2O emissions and/or NO_3^- leaching.

Nitrous Oxide Emissions

Increasing concentrations of atmospheric greenhouse gases (GHGs) contribute to global climate change, and the greater the anthropogenic contribution of GHGs, the greater the potential change. The three GHGs of greatest interest are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). Several other GHGs exist in the

atmosphere (such as water vapor and hydrofluorocarbons, see Fig. 1). Most scientists focus on CO_2, CH_4, and N_2O because they are persistent, effective at trapping heat, and while each of these gases exist naturally, human activities have drastically increased the amount of these gases that reach the atmosphere (IPCC, 2007; US EPA, 2012). Figure 1.1 shows the relative amount of anthropogenic GHGs emitted into the atmosphere in the United States.

It is important to study the role soils play in climate change because soils can be both sources and sinks for each of the three major gases. The agriculture sector in the US produces approximately 6.3% of the country's GHG emissions, and accounts for 67.9% of total annual N_2O emissions (US EPA, 2012). The majority of agricultural GHG emissions are controlled by soil management (see Fig. 1.2). Due to the significant contribution agriculture makes to GHG emissions, understanding and implementing effective agricultural management practices are important for contributing to the mitigations of the US's total GHG emissions. Soil management's dominant role in agricultural GHG emission therefore warrants special attention.

Soils are the third largest storage pool for carbon (C), after fossil fuels and oceans. They have great potential as enormous CO_2 sinks—but also as enormous CO_2 sources. It is challenging to understand soil GHG fluxes because the soil system is infinitely complex, and because climate change can both directly and indirectly influence soil C and N cycles (Bardgett, 2011). However, management practices such as converting to reduced-till or no-till systems can increase the ability of soils to store organic C and offset N_2O and CO_2 emissions, and there have been several reviews that thoroughly

explore the potential of landscapes in the US to do so (see Grace et al., 2006; Jonhson et al., 2007a; Fissore et al., 2010; Cambardella et al., 2012).

Greenhouse gas emissions from soil is influenced by soil chemical, physical, and biological properties (Flessa et al., 1995; Lee et al., 2006), soil moisture (Flessa et al., 1995; US EPA, 2012), tillage (West and Marland, 2002; Lee et al., 2006; Johnson et al., 2007a; US EPA, 2012), fertilization and residue management (Halvorson et al., 2008; US EPA, 2012), transition from native ecosystems to agroecosystems (Fargione et al., 2008), and cropping systems (Johnson et al., 2007a). Soil properties and soil moisture are often beyond control in non-irrigated production systems, but tillage, fertilization and cropping system are all management practices that can be tailored to meet both agricultural and GHG management goals.

Plant-microbe relations also influence GHG emissions. For example, increased CO_2 concentrations can stimulate plant growth, which in turn can stimulate microbial activity by adding C to the soil through plant roots (Bardgett, 2011). Drake et al. (2011) demonstrated how complex plant-microbe relations can complicate estimations of GHG emissions. Drake and colleagues found that elevated CO_2 levels encouraged both plant growth and microbial activity in an N-limited pine forest in South Carolina. The breakdown of organic matter (caused by increased microbial activity) released N which further promoted plant growth. The result was greater C storage in the tree biomass, but not in the soil. In this particular study area, plant biomass rather than the soil acted as a CO_2 sink (Drake et al., 2011). To further complicate the situation, de Graaff et al. (2007) found that increased CO_2 concentrations encouraged rhizodeposition of N by wheat. This in turn increased the immobilization of N deposited by plant roots. While the CO_2

stimulated wheat biomass production, it eventually led to a decreased N availability that limited growth.

Research has shown that under increased atmospheric CO_2, fertilization increases N_2O emissions (Ineson et al., 1998; Kammann et al., 2008; Welzmiller et al., 2008), but the number of studies investigating N_2O emissions under increased CO_2 are limited (Dijkstra and Morgan, 2012). The impacts of management on N_2O emissions are better understood. Nitrous oxide emissions are influenced by soil water content (Blackmer and Bremmer, 1978; Linn and Doran, 1984; Anderson and Levine, 1987; Bateman and Baggs, 2005), organic matter (Jacinthe and Lal, 2003) temperature (Anderson and Levine, 1987), pH (Blackmer and Bremner, 1978) and N availability especially as NO_3^- (Anderson and Levine, 1987; Hefting et al., 2003; Halvorsen et al., 2008), all of which are influenced by management practices such as tillage (Six et al., 2004, Wagner-Riddle et al., 2007; Rochette, 2008).

Nitrous oxide emissions are discussed in Chapter 3. Emissions are investigated as part of a system N balance that also considers crop N use and soil total and inorganic N.

Soil Nitrogen

Fertilizer is not generally applied as NO_3-, but once fertilizer N is transformed into NO_3^- it can become susceptible to leaching. Groundwater contamination by NO_3^- is a concern in Nebraska, particularly in the western and central regions of the state (Kessavlou et al., 1996; Klocke et al., 1999). Spalding and Kitchen (1999) found that over a 15 year period as much as 600 lbs of NO_3^--N per acre (673 kg N ha^{-1}) accumulated

in the vadose zone under corn plots in Nebraska. As previously mentioned, excessive application of N fertilizer beyond crop nutrient requirements can be a major contributor to NO_3^- leaching. Proponents of switchgrass production for biofuels often point to reduced NO_3^- leaching potential of switchgrass compared to row-crops. Buffer strips vegetated with perennials are effective at NO_3^- removal (Groffman et al., 1992; Sanderson et al., 2001; Lee et al., 2003), thus limiting potential leaching and groundwater contamination.

Organic N is also important. Nitrogen stored in organic matter serves as a pool that feeds many processes of the N cycle. Nitrogen stored in organic matter can later be mineralized to provide nutrients for plants and microbes. This potential makes organic stores of N important to consider, because it can both fuel plant growth and N_2O production.

Interim Soybean Crop

The site used in this study was designed to compare corn and switchgrass production. In 1998, two cultivars of switchgrass, Trailblazer and Cave-in-Rock, were seeded into a field that was cropped in soybeans in 1997. The two varieties remained in place for nine years. In 2009, the Trailblazer switchgrass died out. In the spring of 2010, the Trailblazer plots were cleared of switchgrass using herbicide, and soybeans were planted in the plots as an interim crop for two years, following recommended best management practices for switchgrass cultivar replacement. Corn and remaining Cave-in-Rock switchgrass plots have fertilizer and harvest treatments applied to them. Former

Trailblazer plots did not have fertilizer or harvest treatments applied to them while cropped in soybeans. However, the effects of the former management practices were discernible in fall of 2011 in both the soil microbial community (discussed in Chapter 2) and soil N (discussed in Chapter 3).

Objectives

Several papers have reported results from this site, and these studies have focused on determining best management practices for switchgrass (Vogel et al., 2002), investigating the impacts of residue removal on corn yield (Varvel et al., 2008), and long-term management practice effects on soil organic C (Follett et al., 2012). Chapter 2 seeks to determine the impacts of long-term corn and switchgrass management practices on microbial community size, structure and functional composition. Chapter 3 addresses the impacts of these same management practices on crop N uptake efficiency, N_2O emissions, and soil organic and inorganic N.

References

Anderson, I.C. and Levine, J.S. (1987). Simultaneous field measurements of biogenic emissions of nitric oxide and nitrous oxide. *Journal of Geophysical Research*:Atmospheres 92: 965-976.

Bardgett, R.D. (2011). Plant-soil interactions in a changing world. F1000 Biology Reports 3(16): doi:10.3410/B3-16.

Bateman, E.J., and Baggs, E.M. (2005). Contributions of nitrification and denitrification N_2O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils* 41: 379–388.

Biofuels: Hearing before the Senate Committee on Energy and Natural Resources (Serial 112-187) 112th Cong. (2011).

Blackmer, A.M. and Bremner, J.M. (1978). Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil microorganisms. *Soil Biology and Biochemistry* 10(3): 187-191.

Cambardella, C.A., Johnson, J.M.F., Varvel, G.E. 2012. Soil carbon sequestration in central U.S. agroecosystems. In Liebig, M.A., Franzluebbers, A.J., and Follett (R.F.) (Eds.), *Managing Agricultural Greenhouse Gases: Coordinated Agricultural Research through GRACEnet to Address our Changing Climate* (41-58). Waltham, MA, USA: Academic Press.

Campbell, J. E., Lobell, D. B., Genova, R. C., Field, C. B. The global potential of bioenergy on abandoned agricultural lands. 2008. *Environmental Science and Technology* 42(15): 5791–5794.

Congressional Research Service. (2011). *Meeting the Renewable Fuel Standard Mandate for Cellulosic Biofuels: Questions and Answers* by Bracmort, K. Report for Congress, R41106.

Chan, A.S.K., Parkin, T.B. (2001). Methane oxidation and production activity in soils from natural and agricultural systems. *Journal of Environmental Quality* 30: 1896-1903.

Dijkstra, F.A., and Morgan, J.A. (2012). Elevated CO2 and warming effects on soil carbon sequestration and greenhouse gas exchange in agroecosystems: A review. In Liebig, M.A., Franzluebbers, A.J., and Follett (R.F.) (Eds.), *Managing Agricultural Greenhouse Gases: Coordinated Agricultural Research through GRACEnet to Address our Changing Climate* (467-486). Waltham, MA, USA: Academic Press.

Drake J.E., Gallet-Budynek, A., Hofmockel, K.S., Ernhardt, E.S., Billings, S.A., Jackson, R.B., Johnsen, K.S., Lichter, J., McCarthy, H.R., McCormack, M.L., Moore, D.J.P, Oren, R., Palmroth, S., Phillips, R.P., Pippen, J.S., Pritchard, S.G., Treseder, K.K., Schlesinger, W.H., DeLucia, E.H., Finzi, A.C. (2011). Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecology Letters* 14: 349-357.

Drigo, B., Pijl, A. S., Duyts, H., Kielak, A. M., Gamper, H. A., Houtekamer, M. J., Boschker, H.T.S., Bodelier, P.L.E., Whiteley, A.S., van Veen, J.A., Kowalchuk, G. A. (2010). Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *Proceedings of the National Academy of Science* 107(24): 10938-10942.

Fargione J., Hill, J., Tilman, D., Polasky, S., Hawthorne, P. (2008). Land clearing and the biofuel carbon debt. *Science* 319: 1235-1237.

Farrell A.E., Plevin, R.J., Turner, B.T., Jones, A.D., O'Hare, M., Kammen, D.M. (2006). Ethanol and contribute to energy and environmental goals. *Science* 311: 506-508.

Fissore, C., Espeleta, J., Nater, E. A., Hobbie, S. E., & Reich, P. B. (2009). Limited potential for terrestrial carbon sequestration to offset fossil-fuel emissions in the upper midwestern US. *Frontiers in Ecology and the Environment* 8(8): 409-413.

Flessa, H., Dörsch, P., Beese, F. (1995). Seasonal variation of N2O and CH4 fluxes in differently managed arable soils in southern Germany. *Journal of Geophysical Research* 100(D11) 23,115-23,124.

Follett, R.F., Vogel, K.P., Varvel, G.E., Mitchell, R.B., Kimble, J. (2012). Soil carbon sequestration by switchgrass and no-till maize grown for bioenergy. *Bioenergy Research* 4(4): 1-10. doi:10.1007/s12155-012-9198-y.

de Graaff, M., Six, J., van Kessel, C. (2007). Elevated CO2 increases nitrogen rhizodeposition and microbial immobilization of root-derived nitrogen. *New Phytologist* 173(4): 778-786.

Grace, P. R., Colunga-Garcia, M., Gage, S. H., Robertson, G. P., & Safir, G. R. (2006). The potential impact of agricultural management and climate change on soil organic carbon of the North Central Region of the United States. *Ecosystems* 9(5): 816-827.

Gopalakrishnan, G., Negri, M. C., Wang, M., Wu, M., Snyder, S. W., & Lafreniere, L. (2009). Biofuels, land, and water: a systems approach to sustainability. *Environmental science & technology*, *43*(15), 6094-6100.

Halvorson, A.D., Del Grosso, S.J., Reule, C.A. (2008). Nitrogen, tillage, and crop rotation effects on nitrous oxide emissions from irrigated cropping systems. *Journal of Environmental Quality* 37: 1337-1344.

Hefting, M.M., Bobbink, R., de Caluwe, H. (2003). Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones. *Journal of Environmental Quality* 32: 1194-1203.

Hernandez-Ramirez, G., Brouder, S.M., Smith, D.R., Van Scoyoc, G.E. (2009). Greenhouse gas fluxes in an Eastern Corn Belt soil: weather, nitrogen source, and rotation. *Journal of Environmental Quality* 38: 841-854.

IPCC. (2007). Climate Change 2007: Synthesis Report. Intergovernmental Panel on Climate Change Valencia, Spain.

Jacinthe, P.A., Lal, R. (2003). Nitrogen fertilization of wheat residue affecting nitrous oxide and methane emission from a central Ohio Luvisol. *Biology and Fertility of Soils* 37: 338-347.

Jin, V. L., & Evans, R. D. (2010). Microbial ^{13}C utilization patterns via stable isotope probing of phospholipid biomarkers in Mojave Desert soils exposed to ambient and elevated atmospheric CO_2. *Global Change Biology*, *16*(8), 2334-2344.

Jin, V. L., Schaeffer, S. M., Ziegler, S. E., & Evans, R. D. (2011). Soil water availability and microsite mediate fungal and bacterial phospholipid fatty acid biomarker abundances in Mojave Desert soils exposed to elevated atmospheric CO_2. *Journal of Geophysical Research: Biogeosciences (2005–2012), 116*(G2).

Johnson, J.M-F. Franzluebbers, A.J., Weyers, S.L., Reicosky, D.C. (2007). Agricultural opportunities to mitigate greenhouse gas emissions. *Environmental Pollution* 150(1): 107-124.

Kessavalou, A., Doran, J.W., Powers, W.L., Kettler, T.A., Qian, J.H. (1996). Bromide and Nitrogen-15 tracers of nitrate leaching under irrigated corn in Central Nebraska. *Journal of Environmental Quality* 25:1008-1014.

Klocke, N.L., Watts, D.G., Schneekloth, J.P., Davis, D.R., Todd, R.W., Parkhurst, A.M. (1999). Nitrate leaching in irrigated corn and soybean in a semi-arid climate. *Biological Systems Engineering: Papers and Publications* 42(6): 1621-1630.

Lee, J., Six, J., King, A.P., van Kessel, C., Rolston, D.E. (2006). Tillage and field scale controls on greenhouse gas emissions. *Journal of Environmental Quality* 35: 714 725.

Liebig, M., Carpenter-Boggs, L., Johnson, J. M. F., Wright, S., & Barbour, N. (2006). Cropping system effects on soil biological characteristics in the Great Plains. *Renewable Agriculture and Food Systems*, 21(1): 36-48.

Linn, D.M. and Doran, J.W. (1984). Effect of water-filled pore space on CO2 and N2O production in tilled and non-tilled soils. *Soil Science Society of America Journal* 48: 1267-1272.

Pacala, S. and Socolow, R. (2004). Stabilization Wedges: Solving the climate problem for the next 50 years with current technology. *Science* 305: 968-971.

Robertson, G.P., and Groffman, P.M. (2007). Nitrogen Transformations. In Paul, E.A. (Ed.) *Soil Microbiology, Ecology, and Biochemistry*, Third Edition. Academic Press, Burlington, MA, USA.

Robertson, P. G., Dale, V. H., Doering, O. C., Hamburg,S. P., Melillo, J. M., Wander, M. M., Parton, W. J., Adler, P. A., Barney, J. N., Cruse, R. M., Duke, C. S., Fearnside, P. M., Follett, R. F., Gibbs, H. K., Goldemberg, J., Mladenoff, D. J., Ojima, D., Palmer, M. W., Sharpley, A., Wallace, L., Weathers, K. C., Wiens, J. A., Wilhelm, W. W. (2008). Sustainable biofuels redux. *Science* 322: 49–50.

Rochette, P. (2008). No-till only increases N_2O emissions in poorly aerated soils. *Soil Tillage Research* 101: 97-100.

Scharlemann, J.P.W., Laurance, W.F. (2008). How green are biofuels? *Science* 319:43 44.

Searchinger T., Heimlich, R., Houghton, R.A., Dong, F., Elobeid, A., Fabiosa, J., Tokgoz, S., Hayes, D., Yu, T. (2008). Use of U.S. Croplands for biofuel increases greenhouse gases through emissions from land-use change. *Science* 319: 1238-1240.

Six, J., Ogle, S.M., F.J., Breidt, R.T., Moiser, A.R., Paustian, K. (2004). The potential to mitigate global warming with no-tillage is only realized when practiced in the long term. *Global Change Biology* 10: 155-160.

Spalding, R.F., and Kitchen, L.A. (1998). Nitrate in the intermediate vadose zone beneath irrigated cropland. *Ground Water Monitoring & Remediation* 8(2): 89-95.

Sperow, M., Eve, M., Paustian, K. (2003). Potential soil C sequestration on US agricultural soils. *Climatic Change* 57: 319-339.

Staddon, P. L., Fitter, A. H., & Graves, J. D. (1999). Effect of elevated atmospheric CO_2 on mycorrhizal colonization, external mycorrhizal hyphal production and phosphorus inflow in *Plantago lanceolata* and *Trifolium repens* in association with the arbuscular mycorrhizal fungus *Glomus mosseae*. *Global Change Biology*, *5*(3), 347-358.

Stubbs, M. (2012). *Conservation Reserve Program(CRP): Status and Issues*. Report for Congress, R42783. Congressional Research Service.

US DOE. (2010). Annual Energy Review. US Department of Energy, Energy Information Administration, Washington DC.

US DOE. (2011).Biomass Multi-Year Program Plan. US Department of Energy, Office of Energy Efficiency and Renewable Energy, Washington DC.

U.S. Environmental Protection Agency. (2000). Groundwater and Drinking Water Chapters. In *National Water Quality Inventory, 1998: Report to Congress*. EPA 816-R-00-013, August 2000.

U.S. Environmental Protection Agency. (2012). Inventory of US greenhouse gas emissions and sinks: 1990-2010. US EPA 430-R-12-001 United State Environmental Protection Agency, Office of Atmospheric Programs, Washington DC.

Varvel, G.E., Vogel, K.P., Mitchell, R.B., Follett, R.F., Kimble, J.M. (2008). Comparison of corn and switchgrass on marginal soils for bioenergy. *Biomass and Bioenergy* 32: 18-21.

Vogel, K.P., Brejda, J.J., Walters, D.T., Buxton, D.R. (2002). Switchgrass biomass production in the Midwest USA: Harvest and nitrogen management. *Agronomy Journal* 94: 413-420.

Wagner-Riddle, C., Furon, A., McLaughlin, N.L., Lee, I., Barbeau, J., Jayasundara, S.,Parkin, G., von Bertoldi, P., Warland, J. (2007). Intensive measurement of nitrous oxide emissions from a corn-soybean-wheat rotation under two contrasting management systems over 5 years. *Global Change Biology* 13:1722-1736.

West, T.O. and Marland, G. (2002). A synthesis of carbon sequestration, carbon emissions and net carbon flux in agriculture: comparing tillage practices in the United States. *Agriculture, Ecosystems and Environment* 91: 217-232.

Wu, J., and Babcock, B. A. (1999). Metamodeling potential nitrate water pollution in the central United States. *Journal of Environmental Quality 28*(6): 1916-1928.

Zhu, Y. and Fox, R.H. (2003). Corn-soybean rotation effects on nitrate leaching. *Agronomy Journal* 95: 1028-1033.

Figures

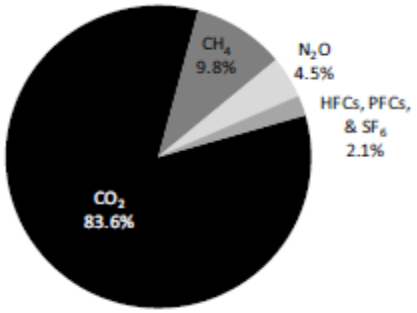

Figure 1.1: Individual gas contribution to total greenhouse gas emissions in the United States (US EPA, 2012).

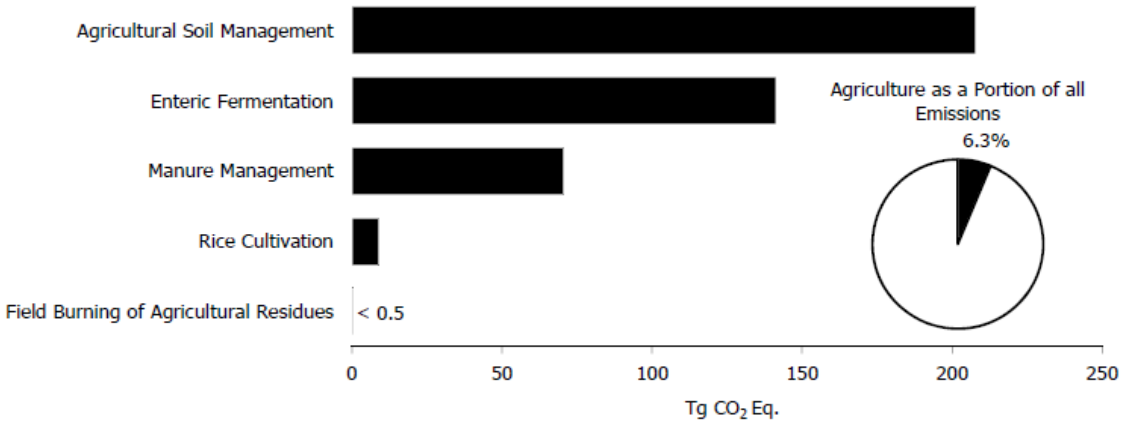

Figure 1.2: Agricultural contributions to greenhouse gas production reported as CO_2 equivalents (US EPA 2012).

CHAPTER 2:

Soil microbial communities under contrasting residue and N management in a long-term, corn and switchgrass-based biofuel production system in Eastern Nebraska.

Abstract

In recent years there has been increasing interest in alternative fuels in the United States, especially cellulosic fuels such as switchgrass (*Panicum virgatum,* L.) and corn (*Zea mays*, L.) stover. Due to already high demand for cropland, switchgrass production will likely occur on marginal lands. This makes understanding the impacts of switchgrass production on soil quality especially important. Despite the importance of monitoring soil quality as a result of management, there have been few investigations into the impacts of switchgrass production on soil microbial ecology. Fatty acid methyl ester (FAME) analyses were conducted on soils used for biofuel feedstock production in eastern Nebraska. Plots were cropped in continuous corn or switchgrass. Soybeans (*Glycine max* (L.) Merr.) were studied as an interim crop between successive switchgrass varieties. Corn and switchgrass received one of two harvest treatments, and each crop was fertilized at three different nitrogen (N) rates. EC_a (dS m^{-1}), pH, percent soil organic matter (SOM), total particulate organic matter (POM, mg g^{-1}), extractable inorganic N, and FAMEs were measured in bulk surface soils (0-5, 5-10 cm). The objective of this exploratory study was to determine how long-term management practices at this site have shaped the size, structure, and composition of the soil microbial community. There was a significant effect of depth on total microbial biomass, with the top 5 cm of soil having significantly higher biomass than the lower 5 cm. Biomass and community structure

were most strongly determined by crop type, but nitrogen and harvest treatments significantly influenced abundances of individual biomarkers. Switchgrass plots had higher total microbial biomass than corn or soybean plots. Biomarkers for saprophytic and arbuscular mycorrhizal (AM) fungi were significantly higher in switchgrass plots than corn or soybean plots. Nitrogen treatments significantly influenced the abundance of individual biomarkers, but did not impact overall biomass. Bacterial biomarker abundances were similar across crop types, but they composed a greater portion of the microbial community in corn plots where fungal abundances were lower. We predict that soils used for long-term switchgrass production will support larger microbial populations than soils used for row-crop production. However, we also expect that if soils under long-term switchgrass production are transitioned back into a row-crop system, even if tillage is avoided, there will be a loss of microbial biomass

Introduction

Switchgrass (*Panicum virgatum* L.) is a potential feedstock for cellulosic biofuels, and since the 1990s scientists have been investigating how to create varieties especially suited to biofuel production (Vogel, 1996). It is commonly predicted that if switchgrass becomes a major crop in the United States, it will primarily be produced on marginal lands due to its lower requirements for water and nutrients compared to many other agronomic species such as corn (Bouton, 2007; Jessup 2009; Hartman et al., 2011). In a 2011 review, Hartman et al. summarized that switchgrass production is unlikely to change soil quality or organic content compared to land in the Conservation Reserve

Program. Furthermore, switchgrass production would be preferable to row-crop production in terms of retaining soil quality. Studies have found overall soil quality improvement under switchgrass compared to row crops due to limited erosion and increased soil organic carbon (SOC) (Liebig et al., 2005; Liebig et al., 2008; Hartman et al., 2011; Follett et al., 2012).

While many studies have investigated switchgrass' influence on soil quality as a monoculture crop, very few have considered its impacts on soil microbial communities. Chaudhary and colleagues (2012) noted: "There is virtually no information on how microbial communities are affected by switchgrass". They used phospholipid fatty acid (PLFA) analysis to compare bulk soil and soil under switchgrass and jatropha (*Jatropha curcas* L.). Chaudhary et al. (2012) found that microbial biomass was significantly higher in rhizosphere soils under switchgrass than bulk soil, and that bacterial marker concentrations were higher in switchgrass than jatropha. Fungal groups were present in higher amounts in soils under jatropha compared to switchgrass.

Considerably more is known about soil microbial communities in corn, soybean, and grassland systems. Corn and soybean systems are typically dominated by bacteria (Vargas Gil et al., 2010), while grasslands have stronger fungal representation compared to row crops (West and Grant, 1987; Djajakirana et al., 1996; Stahl and Parkin, 1996; Stahl et al., 1999; Bailey et al., 2002). Corn and soybean systems may have lower total microbial biomass than rangeland systems (Lalande et al., 2005; McKinley et al. 2005), and soybeans in rotation or monoculture typically show reduced biomass compared to corn monocultures due to lower C inputs from residue (Meriles et al., 2009; Vargas Gil et al., 2010). Spedding et al. (2006) found that corn residue increased soil microbial C and

N, as well as total SOC. However, they did not find clear impacts of residue removal or addition on PLFA profiles (Spedding et al., 2006).

When cropland is restored to prairie, soil quality can improve over time. McKinley et al. (2005) studied several sites in Illinois ranging from cropland, recently restored prairie, prairie restored twenty years prior to the study, and virgin prairie. McKinley and colleagues found several improvements in soil quality in each of the prairie sites compared to the agricultural soil, with the virgin prairie site having the highest overall quality. The restored soils showed improvements, but the improvements in soil quality were linked to the time under restoration. Furthermore, microbial biomass followed the same trends as soil quality indicators, and stress indicators from PLFA markers and poly-β-hydroxybutyrate levels were negatively correlated with prairie age. McKinley and colleagues note that, while there is significant improvement in soil quality after prairie restoration, improvement takes time as even after 20 or more years under restoration, restoration sites significantly differed from virgin prairie. It should be noted that the sites used in this study were all tilled for decades prior to restoration, and some were previously strip-mined (McKinley et al., 2005).

Prior tillage may prove important in soil quality as marginal lands are either converted from CRP/non-use or row-crops to switchgrass. No-till or reduced tillage systems generally have higher total microbial biomass as well as higher fungal biomass and often higher bacterial biomass as well (Drijber et al., 2000; Helgason et al., 2007; Meriles et al., 2009; van Groenigen et al. 2010), although tillage effects may be less noticeable than other treatment effects such as crop type and crop rotation (Spedding et al., 2004; Ng et al., 2012).

In this study we investigate the long-term impacts of corn and switchgrass management on microbial ecology in a marginal soil in Eastern Nebraska. We also consider the short-term impacts of removing switchgrass and planting soybeans. We hypothesized that total microbial biomass and fungal biomass would be smaller in corn and soybean plots compared to switchgrass. We also predicted that there would be a significant shift in the microbial community in switchgrass plots that were transitioned into soybeans, and that N fertilization rate and residue management practice would significantly impact the size and structure of the microbial community.

Methods

Site Description

The site used in this study is also discussed in Varvel et al. (2008) and Follett et al. (2012). This rain-fed, no-till study site is located on the University of Nebraska Agricultural Research and Development Center near Ithaca, NE (latitude 41.15°, longitude -96.40°). The soils are considered marginal for row-crop development, and are classified as Yutan silty clay loams (fine-silty, mixed, superactive, mesic Mollic Hapludalfs) and Tomek silt loams (fine, smectitic, mesic Pachic Argiudolls). Prior to site establishment in 1998, the site was cropped in soybeans and sorghum (*Sorghum bicolor*) and was disk-tilled. Plots are arranged in a split-split plot, randomized complete block design (Fig. 2.1). Whole plots are divided into three subplots that are 30m long x 18.3m wide, and subplots are separated by 15m wide alley for equipment access.

Main treatments are continuous corn (CC) and two cultivars of switchgrass (SG), Trailblazer and Cave-in-Rock,. Corn has been planted continuously since 1999. In 1998,

both switchgrass cultivars were seeded into a field that was previously cropped in soybeans. Switchgrass crops were not fertilized in 1998, but afterwards received fertilizer treatments described below. In 2009, the Trailblazer cultivar died out and was planted in unfertilized soybeans in 2010 and 2011 as a transition crop to an improved bioenergy switchgrass cultivar. The interim unfertilized soybean (S) crop is evaluated in the present study. No-till management began in 1998 for switchgrass plots and in 1999 for corn plots.

N-fertilizer treatments are applied at the scale of sub-plot and harvest treatments to the scale of sub-sub-plot. In 1999, fertilizer rates were 0, 80, 180, or 240 kg N ha^{-1}. From 2000 on, N fertilizer rates were 0, 60, 120, or 180 kg N ha^{-1} (0N, 60N, 120N, and 180N). Prior to 2007, fertilizer was applied as NH_4NO_3 broadcasted with a bulk spreader on all plots. Beginning in 2007, fertilizer was applied to corn plots as subsurface banded urea and to switchgrass plots as surface broadcast sulfur-coated urea. 0N, 60N, and 120N rates were applied to switchgrass plots. In 2010 and 2011, soybean (formerly Trailblazer) plots did not receive fertilizer, but we refer to these plots according to their prior fertilizer treatments. Corn plots received 60N, 120N, and 180N treatments.

There are two harvest treatments in this study for each crop. In Cave-In-Rock and Trailblazer switchgrass, H1 indicates an August harvest, and H2 a post killing-frost harvest in October. In 2010 and 2011, soybeans did not receive any harvest treatment. In CC plots R^+ indicates residue retention after harvest and R^- indicates residue removal after harvest.

Soil Sampling and Analysis

Bulk soil and deep soil cores were sampled on 1 November, 2011. Deep soil cores were taken on taken at depths 0-5, 5-10, 10-30, 30-60, 60-90, and 90-120, and 120-150 cm, but we only discuss 0-5, 5-10, and 10-30 cm depths in this chapter (See Chapter Three for all deep core data). Soil tube diameter was 3.81 cm (1.5 in), and cores were separated according to the depths listed previously. Adjustments were made for moisture by drying a subset of samples at 105° C to determine depth-specific moisture content to calculate oven-dry bulk density (Mg m^{-3}). Bulk soil samples were taken using a flat-edged spade near the deep soil cores. Deep soil cores were used to analyze for EC_a, pH, NO_3^--N and NH_4^+-N, while bulk soil was used for SOM, POM, and FAMEs analyses.

Air-dried, ground and 2-mm sieved deep core soil samples were used for determination of soil chemical properties and nutrient concentrations. EC_a and pH were measured using a 1:1 soil/water slurry (Smith and Doran, 1996). For extractable inorganic nitrogen analyses, air-dried and 2mm sieved soil samples were weighed were mixed with of 2 M KCl in a 1:10 soil/KCl slurry and shaken for 30 min at 200 rpm. The resulting suspension was gravity filtered through Whatman #42 paper and analyzed colorimetrically by Cd-reduction for nitrate-nitrate. Ammonium concentrations were determined using the phenolate method. Both NO_3^- and NH_4^+ were analyzed colometrically with a Lachat QuikChem 8000 Continuum Series (Zellweger Analytics, Inc.) automated flow injection ion analyzer (Keeney and Nelson, 1982).

Bulk soil was used for SOM, POM, and FAMEs analysis. Soil samples used for SOM and POM analysis were passed through a 2-mm sieve and air-dried by weight-loss-on-ignition (Cambardella et al., 2000). Methods for FAMEs analysis is described below.

Quantification and Identification of FAMEs

Fresh soil sub-samples were stored in plastic bags and refrigerated at 5 °C within 12 hours of sampling. Sub-samples were passed through a 4-mm sieve and visible plant matter was removed, then frozen at -20°C within 48 hours of sampling.

FAMEs were quantified and identified by the methods detailed in Grigera et al. (2006). Approximately 10 g of frozen soil was hydrolyzed with freshly prepared 0.2 M potassium hydroxide in methanol, and the resulting fatty acids were partitioned into hexane (White et al. 1979). Saponification released ester-linked FAMEs, and then methyl-nonadeconoate (0.05 μg μL^{-1}) was added to the extract as an internal standard. FAMEs were separated by gas chromatography using helium as a carrier gas, with an Ultra 2HP (50 m, 0.2 mm i.d., 0.33 μm film thickness) capillary column. The gas chromatograph ran in a split mode (44:1) with a 0.75-min purge time (Grigera et al. 2006). FAMEs were quantified from peak areas and reported at nmol g^{-1} soil or as a molar percentage of total FAMEs.

FAMEs were designated as the total number of C atoms followed by a colon, the number of double bonds followed by the position of the double bond from the carboxyl end of the molecule and its *cis* or *trans* configurations in brackets. The prefixes *a* and *i* indicate *antieso* and *iso* branching, respectively. *Cy* indicates cyclopropane fatty acids,

br indicates an unknown branch position, and 10Me indicates a methyl branch of the 10th C atom from the carboxyl end of the molecule (Grigera et al. 2006). Forty FAMEs were detected, and 31 were completely identified while 9 were identified to carbon chain length. FAMEs that were not fully identified are followed by a question mark. For example, C16:1c5? is strongly suspected to be C16:1c5. FAMEs with only chain length identified are listed as unknown (unkC17), and some FAMEs have a known chain length but may or may not be cyclopropane fatty acids (such as *unkC15:1 or cyC15*). FAMEs with <0.05 nmol g^{-1} concentrations were not considered in any analysis. Bacterial makers included iC15:0, aC15:0, iC16:0, iC17:0, aC17:0, cyC17:0 (9,10) (Frostegård and Bååth, 1996). Actinomycetes were designated by 10MeC18:0 and i10MeC18:0 (Kroppenstedt, 1985). Saprophytic fungal markers were C18:1c9 and C18:2(9,12), though it should be noted that C18:1c9 can also occur in bacteria (Frostegård and Bååth, 1996, Stahl and Klug, 1996). C18:1c11 and C16:1c11 were designated as AM fungal markers (Olsson and Johansen, 2000, Drijber et al. 2000), but C18:1c11 can also derive from Gram-negative bacteria (Haack et al., 1994, White et al., 1996). Eukaryotic organisms were represented by C20:4 (White et al., 1996).

Statistical Analysis

A Pearson correlation analysis was used to assess linear relationships between the soil concentrations (nmol g^{-1}) of bacteria, actinomycete, saprophytic fungi, AMF, total FAMEs, and the measured soil properties (EC_a, pH, SOM, POM, NO_3^--N and NH_4^+-N).

A stepwise analysis was used to select FAMEs for canonical discriminant (CANDISC) analysis. The discriminant analyses used mole fraction (nmol%) of individual FAMEs, We included the 9 FAMEs that were not fully identified in the discriminant analyses. Initial CANDISC procedures considered all treatment combinations within each of the two depths. We conducted additional CANDISC analyses to more easily assess the effects of each treatment. CANDISC analyses were conducted considering 1) all treatments, 2) crop alone, 3) nitrogen rate alone, 4) crop and nitrogen rate, and 5) crop and harvest management. We also report Mahalanobis distances (MD) between treatments. Distances between treatments are considered significant at the p=0.05 level.

We also used analysis of variance (ANOVA, PROC GLIMMIX) using a completely randomized split-split plot design to evaluate the effect of treatment on concentration of individual biomarkers. Sidak grouping was used to separate means of soil chemical properties and microbial group abundances within crop type and by depth.

All statistical analyses were performed with the PC Version 9.3 of the Statistical Analyses System for Windows (SAS, Inc., Cary, NC) , and significance was determined at α=0.05 for all statistical analyses unless otherwise specified.

Results

Pearson correlation analysis showed strong relationships between organic matter and each microbial group (see Table 2.1). Both SOM and POM were positively correlated with bacteria [iC15:0, aC15:0, iC16:0, iC17:0, aC17:0, cyC17:0 (9,10)],

actinomycete (10MeC18:0 and i10MeC18:0), saprophytic fungi [C18:1c9 and C18:2c(9,12)], and AMF (C16:1c11 and C18:1c11) groups of biomarkers ($p < 0.001$). Relationships between microbial groups and EC_a were positive, and significantly influenced biomass of each group except bacteria. Bacteria and actinomycete groups had significant negative relationships with soil pH. Saprophytic fungi groups also had a negative relationship to pH, but it was not significant. AMF showed a non-significant positive relationship to pH. Nitrogen from NO_3^- had significant positive correlations with bacterial and actinomycete biomarkers, but a significantly negative correlation with AMF biomarkers. Nitrogen from NH_4^+ was significantly correlated with actinomycete biomarkers, but no other group.

There were no clear trends between treatment and EC_a under any crop type or soil depth (Tables 2.2a through 2.4c). R^+ plots of CC had significantly higher SOM and POM than R^- plots, but this effect was only significant from 0-5 cm. Increasing fertilization significantly decreased pH from 5-30 cm (Tables 2.2b and 2.2c). Nitrogen fertilization did not influence SOM or POM in CC plots. At 0-5 cm, NO_3^- concentrations were significantly influenced by residue removal, but not by N-rate. Nitrate concentration increased with fertilization, but the effect was not significant (Table 2.2a). However, both residue management and fertilization influenced NH_4^+ at that depth. There was no significant treatment effect on either NO_3^- or NH_4^+ from 5-10 cm, but at 10-30 cm both residue and N treatments significantly influenced NO_3^- concentrations.

In SG plots, pH was only significantly influenced by treatment from 0-5 cm, and pH decreased with increasing fertilization (Table 2.2a). Soil organic matter and POM were significantly influenced by both harvest and N treatments from 0-5 cm. Both

treatments also significantly influenced POM at 5-10 cm, but there were no other significant differences by depth in SOM or POM (Tables 2.3a through 2.3c). Ammonium concentrations increased significantly with fertilization at 0-5 cm, but there were no other significant treatment effects on NO_3^- or NH_4^+ at any depth.

Residual harvest and fertilization treatments did not significantly alter soil properties of SB plots below 5 cm (Tables 2.4b and 2.4b). At 0-5 cm, pH decreased significantly with increased level of former N fertilization (Table 2.4a). Nitrate and NH_4^+ concentrations were significantly higher in 120N plots than 0N or 60N plots, but there were no significant trends in SOM or POM by treatment.

Figures 2.2 to 2.7 show graphical representations of the first two discriminant functions of each CANDISC analysis. Tables 2.2 through 2.7 show the corresponding Mahalanobis distances for FAMEs between treatments. Figures 2.2 and 2.3 show the results of CANDISC analyses that considered all treatment combinations at 0-5 cm and 5-10 cm, respectively. Tables 2.2 and 2.3 likewise show MD for the respective analyses. Figures 2.4 through 2.7 (and corresponding Tables 2.4a through 2.7b) show results of CANDISC analyses that consider individual treatments. Analyses that do not account for all treatment combinations (Figures/Tables 2.4 through 2.7) were conducted to aid interpretation of the all-treatment analyses (Figures/Tables 2.2 and 2.3) and should be considered accordingly. When discussing the results of CANDISC analyses, we refer to the all-treatment analyses (Figures/Tables 2.2 and 2.3) unless otherwise specified.

Canonical discriminant analyses revealed strong separation of CC plots from both SG and SB, especially in the top 5 cm of soil (Figure 2.2). CANDISC identified 6

significant discriminant functions for both depths. From 0-5 cm, the first two discriminant functions accounted for 37.1 and 22.6% of variation, respectively (Figure 2.2). At 5-10 cm, the first two functions accounted for 30.8 and 24.9% of variation, respectively (Figure 2.3). CC treatments generally clustered together throughout CANDISC analyses, and the trend was especially clear when crop alone, or crop along with residue was considered (Figures 2.2, 2.4, and 2.7). When considering crop and N treatment without harvest, CC plots were less distinguishable from SG or SB plots (Figure 2.6). When all treatments were considered, CC plots clearly clustered apart from SG or SB plots at 0-5 cm (Figure 2.2), but not at 5-10 cm (Figure 2.3). However, at both depths MD were significant between CC treatments and SB treatments (Tables 2.8a and 2.8b). Mahalanobis distances between residue management treatments in CC plots were significant within N treatments at 0-5 cm (Table 2.5a), but not at 5-10 cm (Table 2.6a).

In every CANDISC analysis, bacteria and actinomycete biomarkers generally clustered in the same quadrants as CC treatments. Fungal biomarkers usually appeared in separate quadrants from bacteria and actinomycete markers. Arbuscular mycorrhizal fungal biomarkers often clustered in quadrants alone or with larger (C20) fatty acids. Eukaryotic makers tended to cluster in the same quadrants as SG plots, and AMF markers in particular clustered with SG plots. This clustering is most apparent in CANDISC analyses that did not consider all treatment combinations.

Nitrogen treatments had more subtle impacts on community structure than crop type. At 0-5 cm, the influence of N on CC distribution is unclear. SG and SB clustered together by N treatment, but 0N treatments clustered closer to 120N treatments than 60N.

At 5-10 cm, N rate appears to have a stronger influence as N treatments follow Discriminant Axis 2 (Figure 2.3a).

Figures 2.8 and 2.9 show the average molar percentages of individual FAMEs at 0-5 cm and 5-10 cm, respectively. Biomarker C16:0 made up a large portion of total biomass in all crops, as did saprophytic fungal and AM fungal biomarkers. The arbuscular mycorrhizal fungi biomarker C16:1c11 was the single largest contributor of microbial biomass in SG plots, accounting for 14 and 18% of total FAMEs at 0-5 and 5-10 cm, respectively. Bacteria and actinomycete biomarkers made up a larger portion of overall biomass in CC plots than SG or SB plots. The arbuscular mycorrhizal fungi marker C16:1c11 made up a far greater proportion of total biomass in SG plots than CC or SB plots.

In CC plots, bacterial and actinomycete biomarkers were not significantly influenced by treatments at either 0-5 or 5-10 cm (Tables 2.10a and 2.10b). Saprophytic fungi were more abundant in 60N and 120N plots than in 180N plots at 0-5cm, as were AMF at 5-10 cm (Tables 2.10a and 2.10b). Total biomass tended to be highest at 120N.

In SG plots, bacteria and actinomycete biomarkers were more abundant in H2 plots than H1 plots from 0-5 cm (Table 2.11a). At 5-10 cm, saprophytic fungal maker concentrations were higher in 0N plots compared to 60N or 120N plots (2.11b).

Soybean plots had no significant differences due to treatment at either depth. Saprophytic fungal markers were more abundant in H2 plots compared to H1 plots from 5-10 cm (Tables 2.12a and 2.12b), but this trend did not hold in 0N plots.

Discussion

Nitrogen and Residue Management in Corn Plots

One of the objectives of this study is to address the sustainability of residue removal in corn (Varvel et al., 2008). Residue removal did seem to influence community structure (see Figures 2.2, 2.3 and 2.7), and MD between R^+ and R^- plots were often significant within N-treatments (see Tables 2.2, 2.3 and 2.7), but the mechanisms behind the differences is unclear. Total microbial biomass tended to be higher in R- plots (see tables 2.8 and 2.9), and there were no clear trends between treatments with individual biomarkers. This is surprising, because previous studies at this site have shown reduced corn yields when residue is removed (Varvel et al., 2008), and Follett et al. (2012) found significantly higher soil C in R^+ plots compared to R^- plots. In 2011, grain yields were higher in R^+ plots than in R^- plots, and R^+ plots did have higher SOM and POM compared to R^- plots (see Chapter , but this did not translate to higher microbial biomass in CC plots as it did in SG plots.

Part of the lack of clarity may be due to the timing of sampling. Residue was removed from R^- plots on October 30, only two days before soil samples were taken. Soil sampling soon after residue removal likely did not provide enough time for soil microbial communities to respond to harvest treatment differences. However, further study is still needed to determine how residue removal influences the abundance of different microbial groups.

High Fungal Biomass under Switchgrass

The corn, soybean, and switchgrass communities differ in fungal biomass. Canonical discriminant analyses showed that bacterial biomarkers were key in separating CC plots from SB and SG plots, and also (especially at 5-10 cm) separating SB from SG plots. While bacterial communities are undoubtedly important, because fungal biomass makes up such a large portion of total biomass the differences in fungal communities are probably influencing the analyses much more strongly than bacterial communities. Thus, the separation between crops was driven largely by the amount of fungal biomass present. Similarly, this difference in fungal biomass separated SG and SB plots, especially from 5-10 cm where fungal and overall microbial biomass dropped.

Differences in fungal biomass across crop type likely are driven by the rooting structures of the different crops, and also by amount of plant litter remaining on the surface. The dense rooting structure of switchgrass along with the year-round cover directly increases microbial biomass by proving food sources to microbes through root exudates and aboveground litter, while at the same time altering the physical and chemical properties of the soil (Ma et al., 2000; Tolbert et al., 2002) which further influence microbial populations. Several studies have shown increases in soil organic carbon under switchgrass (Liebig et al., 2005; Liebig et al., 2008; Hartman et al., 2011; Follett et al., 2012) which is largely due to the extensive rooting structure of switchgrass (Zan et al., 2001; Ma et al., 2000; Frank et al., 2004; Liebig et al., 2008).

Furthermore, thatch layers in switchgrass plots were likely thicker than under corn or soybeans. We did not take any measurements on the litter layer, but due to the nature of the three crops we feel it is reasonable to assume that there is generally a thicker layer

of dead and decaying organic matter on switchgrass plots compared to corn or soybean plots. The higher litter density of switchgrass plots is likely contributing to higher C availability for microbes. The high level of C produced in the rooting zone, along with increased inputs from litter, in SG plots is likely driving high microbial biomass by providing a greater source of consumable C than is available under CC or SB plots.

The differences in total microbial biomass between crop types were driven largely by fungal abundance. The increased amount of biomass from switchgrass roots and litter provides a greater source of lignin and cellulose—accounting for the increased abundances of saprophytic fungi in SG plots. The high abundance of AMF in SG plots compared to CC or SB is likely due to the increased root biomass and the associated increased root exudates, as AMF require C inputs from their plant symbionts for survival (Powell and Klironomos, 2007).

There is no baseline to compare too, but is likely that fungal populations increased at the whole site after conversion to no-till in 1998 (Drijber et al., 2000; Helgason et al., 2007; Meriles et al., 2009; van Groenigen et al. 2010). It is therefore reasonable to assume that, while fungal abundances in CC plots were lower than fungal abundances in SB or SG plots, that they have increased since the study began. This further supports the argument that roots are driving the fungal dynamics at this site: if tillage was the largest factor in of fungal biomass, CC plots would have abundances of fungi closer to that of SG plots.

Legacy of Switchgrass Management under Soybeans

Soybean plots had similar community structure to SG plots (see Figures 2.2 and 2.3). At 5-10 cm, SB plots began to cluster between SG and CC plots, suggesting that the community is beginning to shift under the soybean cropping system. However, the legacy of not only Trailblazer switchgrass but the N and harvest management practices is still discernible after two years. Despite the difference in overall and fungal biomass between SG and SB plots (Figures 2.10 and 2.11), the community structure of SB plots is still more similar to SG plots than to CC plots. We hypothesize that this legacy effect is largely related to switchgrass roots which remained in the soil. Johnson et al. (2007) estimated the half-life of recalcitrant portions of switchgrass roots is 1450 days. This estimation was based on a laboratory study and is difficult to compare to field conditions, but it is not unreasonable to assume that the slowly decaying roots of Trailblazer switchgrass are still influencing the soil microbial community. Furthermore, when sieving samples for FAMEs analysis, we observed (but did not quantify) switchgrass roots in soils from SB plots.

It is impossible to know for certain if the difference between fungal biomass in SG and SB plots is due solely to the transition from a switchgrass cultivar to soybeans, but given the similarities in community composition between SG and SB plots, we suspect this is the case. Future studies at this site can compare the changes which occur after the SB plots are re-seeding with a new switchgrass cultivar to address this uncertainty.

Conclusions

Total microbial biomass was significantly affected by depth and crop type, but not by N or harvest treatments. Community structure was influenced by all treatments, but crop type was the strongest factor. Differences in community structure between crops were largely driven by fungal abundance, and arbuscular mycorrhizal fungi were especially abundant in switchgrass plots. Soybean plots, despite not receiving any N fertilizer or harvest treatment for two years prior to sampling, showed a strong legacy effect from their former Trailblazer switchgrass management regimes. It is still unclear exactly how the microbial community changed when switchgrass is replaced by a row crop as no samples were taken prior to the soybean plot. It also remains unclear what impacts residue removal in corn has on microbial communities. Switchgrass plots had higher total microbial and fungal biomass than row crop plots, but future studies should investigate the differences between microbial communities under monoculture switchgrass and mixed grasslands.

References

Bailey, V.L., Smith, J.L., Bolton, H.J. (2002). Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. *Soil Biology & Biochemistry* 34: 997 1007.

Bouton J. (2007). The economic benefits of forage improvement in the United States. *Euphytica* 154 (3): 263-270.

Cambardella, C. A., Gajda, A. M., Doran, J. W., Wienhold, B. J., & Kettler, T. A. (2001).Estimation of particulate and total organic matter by weight loss-on-ignition. In Lal, R., Kimble, J.M., Follett, R.F., Steward, B.A., *Assessment methods for soil carbon* (349-359). Boca Raton, FL, USA: Lewis Publishers.

Chaudhary, D.R., Saxena, J., Lorenz, N., Dick, L.K., Dick, R.P. (2012). Microbial profiles of rhizosphere and bulk soil microbial communities of biofuel crops switchgrass (*Panicum virgatum* L.) and jatropha (*Jatropha curcas* L.). *Applied and Environmental Soil Science* 2012: Article ID 906864, 6 pages, 2012. doi:10.1155/2012/906864.

Dalal, R. C., and Mayer, R. J. (1986). Long term trends in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. II. Total organic carbon and its rate of loss from the soil profile. *Soil Research*, *24*(2), 281-292.

Djajakirana, G., Joergensen, R.G., Meyer, B. (1996). Ergosterol and microbial biomass relationship in soil. *Biology and Fertility of Soils* 22: 299–304.

Drijber, R.A., Doran, J.W., Parkhurst, A.M., Lyon, D.J. (2000). Changes in soil microbial community structure with tillage under long-term wheat-fallow management. *Soil Biology and Biochemistry* 32: 1419–143.

Haack, S.K., H. Garchow, D.A. Odelson, L.J. Forney, M.J. Klug. (1994). Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. *Applied and Environmental Microbiology* 60(7): 2483-2493.

Follett, R.F., Vogel, K.P., Varvel, G.E., Mitchell, R.B., Kimble, J. (2012). Soil carbon sequestration by switchgrass and no-till maize grown for bioenergy. *Bioenergy Research* 4(4): 1-10. doi:10.1007/s12155-012-9198-y.

Frank, A.B., Berdahl, J.D., Hanson, J.D., Liebig, M.A., Johnson, H.A. (2004). Biomass and carbon partitioning in switchgrass. *Crop Science* 44:1391–1396.

Frostegård, A. and Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* 2 (12): 59-65.

Grigera, M.S., Drijber, R.A., Eskridge, K.M., Wienhold, B.J. (2006). Soil microbial biomass relationships with organic matter fractions in a Nebraska corn field mapped using apparent electrical conductivity. *Soil Science Society of America Journal* 70: 1480-1488.

Hartman J.C., Nippert, J.B., Orozco, R.A., Springer, C.J. (2011). Potential ecological impacts of switchgrass (*Panicum virgatum* L.) biofuel cultivation in the Central Great Plains, USA. *Biomass and Bioenergy* 35: 3415-3421.

Helgason, B.L., Walley, F.L., Germida, J.J. (2007). Fungal and bacterial abundance in long-term no-till and intensive-till soils of the Northern Great Plains. *SoilScience Society of America Journal* 73(1): 120-127.

Lalande, R., Gagnon, B., Chapman, R.A., Barnett, G.M. (2005). Soil microbial populations, activity, and community structure in continuous corn or forage systems under organic or inorganic fertilization in eastern Canada. *Canadian Journal of Soil Science* 85(1): 27-38.

Liebig, M.A., Johnson, H.A., Hanson, J.D., Frank, A.B. (2005). Soil carbon under switchgrass stands and cultivated cropland. *Biomass Bioenergy* 28: 347–354.

Liebig, M.A., Schmer, M.R., Vogel, K.P., Mitchell, R.B. (2008). Soil carbon storage by switchgrass grown for bioenergy. Bioenergy Research 1: 215-222. doi:10.1007/s12155-008-9019-5.

Keeney, D.R., and Nelson, D.W. (1982). Nitrogen-inorganic forms. In Page, A.L., Miller, R.H., and Keeney, D.R. (Eds). *Methods of Soil Analaysis*. Part 2. 2nd ed. Agronomy Monograph No. 9. (643-698). Madison, WI, USA, Agronomy Society of America and Soil Science Society of America.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. Society for Applied Bacteriology Technical Series 20: 173-199.

Jessup, JW. 2009. Development and status of dedicated energy crops in the United States. *In Vitro Cell Developmental Biology-Plant* 45: 282-290.

Ma, Z., Wood, C.W., Bransby, D.I. (2000). Impacts of soil management on root characteristics of switchgrass. *Biomass and Bioenergy* 18: 105-112.

McKinley, V.L., Peacock, A.D., White, D.C. (2005). Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. *Soil Biology and Biochemistry* 37: 1946-1958.

Meriles, J.M., Vargas Gil, S., Conforto, C., Figoni, G., Lovera, E., March, G.J., Guzma´n, C.A. (2009). Soil microbial communities under different soybean cropping systems: Characterization of microbial population dynamics, soil microbial activity, microbial biomass, and fatty acid profiles. *Soil Tillage and Research* 103: 271-281.

Ng, J.P., Hollister, E.B., González-Chávez, M.d.C.A., Hons, F.M., Zuberer, D.A., Aitkenhead-Peterson, J.A., Loeppert, R., Gentry, T.J. (2012). Impacts of cropping systems and long-term tillage on soil microbial population levels and community composition in dryland agricultural setting. *International Scholarly Research Network Ecology* 2012: 1-11, doi doi:10.5402/2012/487370.

Olsson, P.A. and Johansen, A. (2000). Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. *Mycological Research* 104(4): 429-434.

Powell, J., Klironomos, J. 2007. The ecology of plant-microbial mutualisms. In Paul, E.A. (Ed.) *Soil Microbiology, Ecology, and Biochemistry*. Third Edition (259-281). Burlington, MA, USA: Academic Press.

Smith, J.L., and Doran, J.W. (1996). Measurement and use of pH and electrical conductivity for soil quality analysis. In Doran, J.W. and Jones, A.J. (Eds.) *Methods for assessing soil quality* (169-186). Madison, WI, USA: Agronomy Society of America, Crop Science Society of America, Soil Science Society of America.

Spedding, T.A., Hamel, C., Mehuys, G.R., Madramootoo, C.A. (2004). Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. *Soil Biology and Biochemistry* 36: 499-512.

Stahl, P.D. and Klug, M.J. (1996). Characterization and differentiation of filamentous fungi based on fatty acid composition. *Applied and Environmental Microbiology* 62(11):4136-4146.

Stahl, P.D. and Parkin, T.B. (1996). Relationship of soil ergosterol concentration and fungal biomass. *Soil Biology & Biochemistry* 28: 847–855.

Stahl, P.D., T.B. Parkin, M. Christensen. (1999). Fungal presence in paired cultivated and uncultivated soils in central Iowa, USA. *Biology and Fertility of Soils* 29: 92-97.

Tolbert, V. R., Todd Jr, D. E., Mann, L. K., Jawdy, C. M., Mays, D. A., Malik, R., Bandaranayake, W., Houston, A. Tyler, D., Pettry, D. E. (2002). Changes in soil quality and below-ground carbon storage with conversion of traditional agricultural crop lands to bioenergy crop production. *Environmental Pollution* 116: S97-S106.

Van Groenigen, K., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bodé, S., Forristal, D., Jones, M.B. (2010). Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. *Soil Biology and Biochemistry* 42: 48-55.

Vargas Gil, S., Meriles, J., Conforto, C., M. Basanta, Radl, V., Hagn, A.. Schloter, M., March, G.J. (2011). Response of soil microbial communities to different management practices in surface soils of a soybean agroecosystems in Argentina. *European Journal of Soil Biology* 47: 55-50.

Varvel, G.E., Vogel, K.P., Mitchell, R.B., Follett, R.F., Kimble, J.M. (2008). Comparison of corn and switchgrass on marginal soils for bioenergy. *Biomass and Bioenergy* 32: 18-21.

Vogel K.P. (1996). Energy production from forages (or American agriculture-back to the future*). Journal of Soil and Water Conservation* (51) 137–9.

Vogel, K.P., Brejda, J.J., Walters, D.T., Buxton, D.R. (2002). Switchgrass biomass production in the Midwest USA: Harvest and nitrogen management. *Agronomy Journal* 94: 413-420.

West, A.W., Grant, W.D. (1987). Use of ergosterol, diaminopimelic acid and glucosamine contents of soils to monitor changes in microbial populations. *Soil Biology & Biochemistry* 19: 607–612.

White, D.C., Stair, J.O., Ridgelberg, D.B. (1996). Quantitative comparisons of *in situ* microbial biodiversity by signature biomarker analysis. *Journal of Industrial Microbiology* 17(34):185-196.

Zan, C.S., Fyles, J.W., Girouard, P., Samson, R.A. (2001) Carbon sequestration in perennial bioenergy, annual corn and uncultivated systems in southern Quebec. *Agriculture, ecosystems & environment*, *86*(2): 135-144.

Table 2.1: Pearson correlation matrix for soil physical and chemical properties and for abundances of microbial groups.

*p≤0.05, **p ≤ 0.01, ***p≤0.0001

	EC	pH	SOM	POM	NO_3^--N	NH_4^+-N	Bact	Actino	Sapro	AMF	TF
EC	1										
pH	0.26*	1									
SOM	-0.07	-0.34***	1								
POM	0.07	-0.28**	0.95***	1							
NO_3^--N	0.16	-0.54***	0.11	0.15	1						
NH_4^+-N	0.25**	-0.39***	0.17	0.22*	0.34***	1					
Bact	0.11	-0.33**	0.59***	0.59***	0.24***	0.16	1				
Actino	0.20*	-0.27**	0.44***	0.44***	0.20*	0.28**	0.84***	1			
Sapro	0.29**	-0.02	0.43***	0.51***	-0.07	0.07	0.68***	0.52***	1		
AMF	0.25**	0.09	0.32**	0.35**	-0.20*	0.25	0.53***	0.47***	0.74***	1	
TF	0.22*	-0.15	0.53***	0.57***	-0.19	0.10	0.88***	0.73***	0.89***	0.84***	1

POM= 0.05-2 mm; NO_3^--N = extractable N; NH_4^+-N = extractable N; Bact= bacterial markers iC15:0, aC15:0, iC16:0, iC17:0, aC17:0, cyC17:0 (9,10); Actino= actinomycete markers 10MeC18:0 and i10MeC18:0; Sapro= saprophytic fungal markers C18:1c9 and C18:2(9,12); AMF= Arbuscular mycorrhizal markers C16:1c11 and C18:1c11. TF = Total FAMEs in nmol g^{-1} soil.

Table 2.2a: Soil chemical properties in CC plots by N-rate at 0-5 cm.

CC	Depth (cm)	EC	pH	SOM (%)	POM (0.05-2 mm)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
60N R^+	0-5	0.23 ± 0.02	5.94 ± 0.15	53.67 ± 5.69	20.12 ± 3.58	2.92 ± 1.22	3.99 ± 0.80
60N R^-	0-5	0.22 ± 0.05	6.01 ± 0.34	46.96 ± 4.08	13.82 ± 2.60	1.43 ± 0.17	2.78 ± 0.44
120N R^+	0-5	0.24 ± 0.02	5.89 ± 0.38	48.90 ± 3.91	16.41 ± 3.91	4.36 ± 2.39	5.32 ± 1.86
120N R^-	0-5	0.24 ± 0.03	5.61 ± 0.30	38.65 ± 2.53	9.46 ± 2.53	3.11 ± 1.06	5.05 ± 1.20
180N R^+	0-5	0.21 ± 0.03	5.35 ± 0.20	47.54 ± 4.15	14.74 ± 4.15	7.69 ± 2.83	5.91 ± 1.00
180N R^-	0-5	0.18 ± 0.03	5.56 ± 0.09	40.08 ± 6.44	9.01 ± 6.44	4.62 ± 0.73	5.05 ± 1.29

Table 2.2b: Soil chemical properties in CC plots by N-rate at 5-10 cm.

CC	Depth (cm)	EC	pH	SOM (%)	POM (0.05-2mm)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
60N R^+	5-10	0.21 ± 0.03	6.05 ± 0.14	39.19 ± 3.27	6.39 ± 1.24	1.30 ± 0.96	2.99 ± 0.58
60N R^-	5-10	0.20 ± 0.03	6.20 ± 0.28	40.15 ± 2.47	6.19 ± 1.83	0.66 ± 0.28	2.91 ± 0.53
120N R^+	5-10	0.23 ± 0.03	6.30 ± 0.24	34.74 ± 1.02	3.99 ± 0.45	2.60 ± 1.84	3.71 ± 0.96
120N R^-	5-10	0.20 ± 0.03	6.04 ± 0.32	35.07 ± 0.96	4.06 ± 0.37	1.24 ± 0.62	4.22 ± 0.89
180N R^+	5-10	0.21 ± 0.03	5.46 ± 0.32	37.91 ± 3.85	5.28 ± 1.15	5.24 ± 3.93	4.25 ± 0.74
180N R^-	5-10	0.20 ± 0.02	5.93 ± 0.25	34.96 ± 3.71	3.40 ± 0.60	2.80 ± 0.91	4.98 ± 1.36

Table 2.2c: Soil chemical properties in CC plots by N-rate at 10-30 cm.

CC	Depth (cm)	EC	pH	SOM (%)	POM (0.05-2mm)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
60N R^+	10-30	0.18 ± 0.03	6.27 ± 0.19	34.36 ± 2.04	2.63 ± 0.60	0.07 ± 0.08	1.81 ± 0.44
60N R^-	10-30	0.19 ± 0.04	6.49 ± 0.22	35.62 ± 1.85	2.62 ± 0.63	0.28 ± 0.27	2.09 ± 0.49
120N R^+	10-30	0.27 ± 0.07	6.75 ± 0.24	31.02 ± 2.47	2.25 ± 0.10	2.55 ± 2.35	2.54 ± 0.71
120N R^-	10-30	0.25 ± 0.06	6.66 ± 0.13	32.20 ± 0.86	2.38 ± 0.22	0.34 ± 0.13	2.78 ± 0.68
180N R^+	10-30	0.27 ± 0.06	5.86 ± 0.26	33.54 ± 3.72	2.16 ± 0.26	4.21 ± 1.93	3.14 ± 0.68
180N R^-	10-30	0.23 ± 0.01	6.16 ± 0.18	33.80 ± 3.42	2.40 ± 0.41	5.07 ±1.87	3.20 ± 0.90

Table 2.3a: Soil chemical properties in SG plots by N-rate at 0-5 cm.

SG	Depth (cm)	EC	pH	SOM (%)	POM (0.05-2mm)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
0N H1	0-5	0.25 ± 0.02	6.03 ± 0.08	48.09 ± 3.99	15.31 ± 4.23	0.05 ± 0.04	4.08 ± 0.79
0N H2	0-5	0.30 ± 0.05	5.80 ± 0.29	50.50 ± 0.54	17.47 ± 3.06	0.00 ± 0.00	4.64 ± 0.98
60N H1	0-5	0.22 ± 0.02	5.78 ± 0.16	56.03 ± 1.67	20.21 ± 1.60	0.04 ± 0.02	4.44 ± 0.79
60N H2	0-5	0.27 ± 0.04	5.83 ± 0.19	58.24 ± 3.63	21.75 ± 1.82	0.27 ± 0.16	4.90 ± 0.11
120N H1	0-5	0.19 ± 0.02	5.47 ± 0.09	83.48 ± 11.52	40.65 ± 9.04	0.04 ± 0.04	5.90 ± 0.58
120N H2	0-5	0.22 ± 0.04	5.26 ± 0.20	59.19 ± 2.51	20.68 ± 1.29	6.42 ± 4.49	6.26 ± 1.24

Table 2.3b: Soil chemical properties in SG plots by N-rate at 5-10 cm.

SG	Depth (cm)	EC	pH	SOM (%)	POM (0.05-2mm)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
0N H1	5-10	0.27 ± 0.04	6.09 ± 0.13	37.26 ± 0.90	5.88 ± 0.80	0.00 ± 0.00	4.53 ± 1.29
0N H2	5-10	0.23 ± 0.03	6.02 ± 0.10	39.27 ± 1.48	7.19 ± 0.86	0.00 ± 0.00	4.05 ± 0.82
60N H1	5-10	0.31 ± 0.06	6.30 ± 0.20	40.76 ± 3.15	7.66 ± 1.57	0.00 ± 0.00	3.53 ± 0.27
60N H2	5-10	0.23± 0.01	6.07 ± 0.22	41.85 ± 4.25	8.41 ± 1.66	0.00 ± 0.00	3.95 ± 0.32
120N H1	5-10	0.17 ± 0.02	5.78 ± 0.08	49.63 ± 3.27	12.15 ± 0.78	0.00 ± 0.00	4.92 ± 0.64
120N H2	5-10	0.23 ± 0.04	5.70 ± 0.20	41.37 ± 1.72	6.25 ± 0.27	2.24 ± 1.58	4.40 ± 1.09

Table 2.3c: Soil chemical properties in SG plots by N-rate at 10-30 cm.

SG	Depth (cm)	EC	pH	SOM (%)	POM (0.05-2mm)	NO_3^-N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
0N H1	10-30	0.19 ± 0.02	6.39 ± 0.19	34.31 ± 1.91	3.20 ± 0.18	0.00 ± 0.00	2.82 ± 0.52
0N H2	10-30	0.20 ± 0.02	6.28 ± 0.06	35.15 ± 1.89	3.69 ± 0.24	0.00 ± 0.00	2.70 ± 0.50
60N H1	10-30	0.22 ± 0.03	6.45 ± 0.12	35.29 ± 2.05	4.05 ± 0.70	0.00 ± 0.00	1.83 ± 0.68
60N H2	10-30	0.19 ± 0.01	6.55 ± 0.15	34.96 ± 2.58	4.37 ± 0.04	0.00 ± 0.00	2.27 ± 0.29
120N H1	10-30	0.16 ± 0.02	6.33 ± 0.08	41.69 ± 2.53	6.57 ± 1.14	0.00 ± 0.00	3.05 ± 0.86
120N H2	10-30	0.16 ± 0.03	6.23 ± 0.12	37.40 ± 1.47	4.71 ± 0.31	0.23 ± 0.23	2.69 ± 0.80

Table 2.4a: Soil chemical properties in SB plots by N-rate at 0-5 cm. Treatments were last applied in 2009.

SB	Depth (cm)	EC	pH	SOM (%)	POM (0.05-2mm)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
0N H1	0-5	0.23 ± 0.03	6.13 ± 0.03	48.70 ± 6.31	14.26 ± 3.39	2.76 ± 0.72	3.93 ± 0.95
0N H2	0-5	0.24 ± 0.03	6.02 ± 0.03	50.33 ± 4.20	15.43 ± 1.62	0.71 ± 0.05	4.19 ± 0.16
60N H1	0-5	0.26 ± 0.01	5.60 ± 0.05	56.09 ± 3.09	19.82 ± 1.69	7.51 ± 3.19	5.50 ± 0.28
60N H2	0-5	0.21 ± 0.03	5.70 ± 0.07	56.02 ± 2.28	19.56 ± 1.17	5.43 ± 1.97	4.39 ± 0.72
120N H1	0-5	0.24 ± 0.03	5.35 ± 0.15	53.86 ± 2.83	18.06 ± 2.87	6.68 ± 1.18	6.10 ± 0.73
120N H2	0-5	0.27 ± 0.03	5.35 ± 0.20	61.57 ± 5.34	23.14 ± 4.37	7.47 ± 4.13	5.83 ± 0.60

Table 2.4b: Soil chemical properties in SB plots by N-rate at 5-10 cm. Treatments were last applied in 2009.

SB	Depth (cm)	EC	pH	SOM (%)	POM (0.05-2mm)	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
0N H1	5-10	0.25 ± 0.04	6.12 ± 0.16	37.86 ± 2.57	5.63 ± 0.88	1.00 ± 0.56	5.61 ±1.37
0N H2	5-10	0.25 ± 0.03	6.05 ± 0.07	40.93 ± 3.94	7.34 ± 1.57	0.20 ± 0.18	3.47 ± 0.93
60N H1	5-10	0.18± 0.06	6.00 ± 0.10	40.37 ± 0.46	6.98 ± 0.09	1.65 ± 1.21	4.24 ± 1.13
60N H2	5-10	0.17 ± 0.01	5.89 ± 0.13	40.70 ± 1.78	6.79 ± 1.14	0.84 ± 0.34	5.18 ± 1.30
120N H1	5-10	0.21 ± 0.02	5.87 ± 0.28	36.72 ± 1.68	4.54 ± 0.16	1.60 ± 0.62	4.36 ± 0.46
120N H2	5-10	0.22 ± 0.04	5.66 ± 0.19	41.72 ± 4.75	7.13 ± 2.59	2.18 ± 1.15	4.49 ± 0.57

Table 2.4c: Soil chemical properties in SB plots by N-rate at 10-30 cm. Treatments were last applied in 2009.

SB	Depth (cm)	EC	pH	SOM (%)	POM (0.05-2mm)	NO_3^-N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
0N H1	10-30	0.19 ± 0.02	6.38 ± 0.04	34.48 ± 2.20	3.11 ± 0.38	0.16 ± 0.14	3.39 ± 0.88
0N H2	10-30	0.21 ± 0.03	6.26 ± 0.08	35.77 ± 2.62	3.67 ± 0.47	0.05 ± 0.05	2.37 ± 0.65
60N H1	10-30	0.18 ± 0.02	6.25 ± 0.10	35.43 ± 0.43	3.17 ± 0.25	0.32 ± 0.19	2.39 ± 0.50
60N H2	10-30	0.16 ± 0.00	6.30 ± 0.08	34.95 ± 1.68	3.21 ± 0.39	0.20 ± 0.10	2.62 ± 0.48
120N H1	10-30	0.18 ± 0.02	6.40 ± 0.10	34.89 ± 2.43	2.46 ± 0.12	0.42 ± 0.20	3.40 ± 0.16
120N H2	10-30	0.20 ± 0.04	6.27 ± 0.17	36.56 ± 2.02	3.09 ± 0.66	0.67 ± 0.36	2.99 ± 0.53

Table 2.5a : Pairwise squared Mahalanobis distances between for FAMEs between N-rate and harvest treatments at 0-5 cm in CC plots.

From Crop	CC 60N R^+	CC 60N R^-	CC 120N R^+	CC 120N R^-	CC 180N R^+	CC 180N R^-
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)						
CC 60N R^+	0 (1)					
CC 60N R^-	32 (0.034)	0 (1)				
CC 120N R^+	11 (0.7366)	34 (0.0256)	0 (1)			
CC 120N R^-	62 (0.0005)	68 (0.0003)	35 (0.0214)	0 (1)		
CC 180N R^+	116 (<.0001)	124 (<.0001)	85) (0.0002	39 (0.0273)	0 (1)	
CC 180N R^-	137 (<.0001)	103 (<.0001)	106 (<.0001)	69 (0.0002	51 (0.0056)	0 (1)

Table 2.5b : Pairwise squared Mahalanobis distances between for FAMEs between N-rate and harvest treatments at 0-5 cm in SG plots.

From Crop	SG 0N H1	SG 0N H2	SG 60N H1	SG 60N H2	SG 120N H1	SG 120N H2
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)						
SG 0N H1	0 (1)					
SG 0N H2	22 (0.3584)	0 (1)				
SG 60N H1	98 (0.0001)	98 (0.0001)	0 (1)			
SG 60N H2	131 (<.0001)	111 (<.0001)	19 (0.4805)	0 (1)		
SG 120N H1	148 (<.0001)	141 (<.0001)	255 (<.0001)	324 (<.0001)	0 (1)	
SG 120N H2	83 (0.0025)	75 (0.0047)	39 (0.128)	36 (0.1779)	186 (<.0001)	0 (1)

Table 2.5c : Pairwise squared Mahalanobis distances between for FAMEs between residual N-rate and harvest treatments in SB plots at 0-5cm.

From Crop	SB 0N H1	SB 0N H2	SB 60N H1	BS 60N H2	SB 120N H1	SB 120N H2
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)						
SB 0N H1	0 (1)					
SB 0N H2	21 (0.3958)	0 (1)				
SB 60N H1	42 (0.0362)	54 (0.0096)	0 (1)			
SB 60N H2	51 (0.013)	40 (0.047)	26 (0.2257)	0 (1)		
SB 120N H1	86 (0.0004)	70 (0.0017)	53 (0.0105)	40 (0.0441)	0 (1)	
SB 120N H2	143 (<.0001)	100 (0.0001)	109 (<.0001)	61 (0.0042)	29 (0.1559)	0 (1)

Table 2.6a: Pairwise squared Mahalanobis distances between for FAMEs between N-rate and harvest treatments at 5-10 cm in CC plots.

From Crop	CC 60N R^+	CC 60N R^-	CC 120N R^+	CC 120N R^-	CC 180N R^+	CC 180N R^-
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)						
CC 60N R^+	0 (1)					
CC 60N R^-	15 (0.4934)	0 (1)				
CC 120N R^+	42 (0.0126)	31 (0.0254)	0 (1)			
CC 120N R^-	19 (0.3137)	18 (0.2193)	15 (0.3424)	0 (1)		
CC 180N R^+	39 (0.0185)	38 (0.0089)	11 (0.6158)	21 (0.1357)	0 (1)	
CC 180N R^-	84 (<.0001)	78 (<.0001)	53 (0.0001)	59 (0.0005)	27 (0.0489)	0 (1)

Table 2.6b: Pairwise squared Mahalanobis distances between for FAMEs between N-rate and harvest treatments at 5-10 cm in SG plots.

From Crop	SG 0N H1	SG 0N H2	SG 60N H1	SG 60N H2	SG 120N H1	SG 120N H2
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)						
SG 0N H1	0 (1)					
SG 0N H2	58 (0.0151)	0 (1)				
SG 60N H1	53 (0.0064)	169 (<.0001)	0 (1)			
SG 60N H2	46 (0.0157)	128 (<.0001)	23 (0.2583)	0 (1)		
SG 120N H1	53 (0.0064)	155 (<.0001)	41 (0.0273)	47 (0.0135)	0 (1)	
SG 120N H2	51 (0.0082)	166 (<.0001)	22 (0.3096)	27 (0.1628)	14 (0.6902)	0 (1)

Table 2.6c: Pairwise squared Mahalanobis distances between for FAMEs between residual N-rate and harvest treatments in SB plots at 5-10 cm.

From Crop	SB 0N H1	SB 0N H2	SB 60N H1	SB 60N H2	SB 120N H1	SB 120N H2
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)						
SB 0N H1	0 (1)					
SB 0N H2	40 (0.0882)	0 (1)				
SB 60N H1	46 (0.0453)	89 (0.0002)	0 (1)			
SB 60N H2	43 (0.064)	61 (0.0027)	31 (0.0961)	0 (1)		
SB 120N H1	82 (0.0016)	86 (0.0002)	54 (0.0057)	90 (0.0002)	0 (1)	
SB 120N H2	38 (0.1057)	52 (0.0078)	28 (0.1475)	47 (0.0136)	18 (0.4377)	0 (1)

Table 2.7a: Pairwise squared Mahalanobis distances between for FAMEs between crop types at 0-5 cm. Table corresponds with Figure 2.4.

From Crop	CC	SG	SB
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)			
CC	0 (1)		
SG	73 (<.0001)	0 (1)	
SB	80 (<.0001)	11 (<.0001)	0 (1)

Table 2.7b: Pairwise squared Mahalanobis distances between for FAMEs between crop types at 5-10 cm. Table corresponds with Figure 2.4.

From Crop	CC	SG	SB
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)			
CC	0 (1)		
SG	38 (<.0001)	0 (1)	
SB	14 (<.0001)	19 (<.0001)	0 (1)

Table 2.8a. Pairwise squared Mahalanobis distances for FAMEs between N-rate at 0-5 cm. Table corresponds with Figure 2.5.

From N-Rate	0N	60N	120N	180N
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)				
0N	0 (1)			
60N	34 (<.0001)	0 (1)		
120N	41 (<.0001)	19 (<.0001)	0 (1)	
180N	84 (<.0001)	61 (<.0001)	42 (<.0001)	0 (1)

Table 2.8b. Pairwise squared Mahalanobis distances for FAMEs between N-rate at 5-10 cm. Table corresponds with Figure 2.5.

From N	0N	60N	120N	180N
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)				
0N	0 (1)			
60N	15 (<.0001)	0 (1)		
120N	17 (<.0001)	0 (0.6261)	0 (1)	
180N	34 (<.0001)	11 (<.0001)	9 (<.0001)	0 (1)

Table 2.8a. Pairwise squared Mahalanobis distances for FAMEs between crop and N-rate at 0-5 cm. N treatments in SB plots refer to residual treatments. Table corresponds with Figure 2.6.

From Crop	CC 60N	CC 120N	CC 180N	SG 0N	SG 60N	SG 120N	SB 0N	SB 60N	SB 120N
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)									
CC 60N	0 (1)								
CC 120N	20 (0.0042)	0 (1)							
CC 180N	48 (<.0001)	35 (<.0001)	0 (1)						
SG 0N	92 (<.0001)	117 (<.0001)	112 (<.0001)	0 (1)					
SG 60N	105 (<.0001)	129 (<.0001)	107 (<.0001)	58 (<.0001)	0 (1)				
SG 120N	116 (<.0001)	118 (<.0001)	100 (<.0001)	119 (<.0001)	47 (0.0001)	0 (1)			
SB 0N	103 (<.0001)	115 (<.0001)	109 (<.0001)	57 (<.0001)	43 (0.0002)	85 (<.0001)	0 (1)		
SB 60N	133 (<.0001)	132 (<.0001)	107 (<.0001)	117 (<.0001)	36 (0.0005)	26 (0.0105)	56 (<.0001)	0 (1)	
SB 120N	110 (<.0001)	110 (<.0001)	82 (<.0001)	115 (<.0001)	60 (<.0001)	16 (0.1195)	75 (<.0001)	33 (0.0011)	0 (1)

Table 2.8b. Pairwise squared Mahalanobis distances for FAMEs between crop and N-rate at 5-10 cm. N treatments in SB plots refer to management prior to 2010. Table corresponds with Figure 2.6.

From Crop	CC 60N	CC 120N	CC 180N	SG 0N	SG 60N	SG N3	SB 0N	SB 60N	SB 120N
	Squared Mahalanobis Distance/(Probability > Mahalanobis distance)								
CC 60N	0 (1)								
CC 120N	10 (0.1403)	0 (1)							
CC 180N	27 (0.0002)	26 (0.0002)	0 (1)						
SG 0N	78 (<.0001)	100 (<.0001)	126 (<.0001)	0 (1)					
SG 60N	45 (<.0001)	48 (<.0001)	74 (<.0001)	56 (<.0001)	0 (1)				
SG N3	42 (<.0001)	49 (<.0001)	75 (<.0001)	70 (<.0001)	24 (0.0032)	0 (1)			
SB 0N	36 (0.0001)	38 (<.0001)	67 (<.0001)	47 (<.0001)	45 (<.0001)	74 (<.0001)	0 (1)		
SB 60N	37 (<.0001)	26 (0.0006)	58 (<.0001)	87 (<.0001)	42 (<.0001)	67 (<.0001)	26 (0.0033)	0 (1)	
SB 120N	39 (<.0001)	29 (0.0002)	35 (<.0001)	117 (<.0001)	46 (<.0001)	39 (<.0001)	67 (<.0001)	48 (<.0001)	0 (1)

Table 2.9a: Pairwise squared Mahalanobis distances for FAMEs between crop and harvest treatment at 0-5 cm. In CC plots, H1 = residue retained and H2 = residue removed. In SG plots, H1= August harvest and H2 = October harvest. Harvest treatments in SB plots refer to management prior to 2010. Table corresponds with Figure 2.7.

From Crop	CC R^+	CC R^-	SG H1	SG H2	SB H1	SB H2
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)						
CC R^+	0 (1)					
CC R^-	10 (0.0008)	0 (1)				
SG H1	91 (<.0001)	99 (<.0001)	0 (1)			
SG H2	69 (<.0001)	86 (<.0001)	14 (0.0007)	0 (1)		
SB H1	83 (<.0001)	108 (<.0001)	23 (<.0001)	9 (0.0199)	0 (1)	
SB H2	74 (<.0001)	101 (<.0001)	33 (<.0001)	13 (0.0014)	5 (0.1852)	0 (1)

Table 2.9b: Pairwise squared Mahalanobis distances for FAMEs between crop and harvest treatment at 5-10 cm. In CC plots, H1 = residue retained and H2 = residue removed. In SG plots, H1= August harvest and H2 = October harvest. Harvest treatments in SB plots refer to management prior to 2010. Table corresponds with Figure 2.7.

From Crop	CC R^+	CC R^-	SG H1	SG H2	SB H1	SB H2
Squared Mahalanobis Distance/(Probability > Mahalanobis distance)						
CC R^+	0 (1)					
CC R^-	3 (0.1558)	0 (1)				
SG H1	39 (<.0001)	45 (<.0001)	0 (1)			
SG H2	26 (<.0001)	32 (<.0001)	4 (0.0918)	0 (1)		
SB H1	13 (<.0001)	20 (<.0001)	31 (<.0001)	18 (<.0001)	0 (1)	
SB H2	7 (0.0017)	12 (<.0001)	26 (<.0001)	14 (<.0001)	3 (0.1833)	0 (1)

Table 2.10a: Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 0-5cm in CC plots. Total biomass = sum of all biomarker concentrations.

CC	Bacteria	Actinomycete	Saprophytic Fungi	Arbuscular Mycorrhizal Fungi	Total Biomass
60N R+	28.68 ± 5.10	8.11 ± 1.15	33.03 ± 6.04	24.23 ± 3.42	174.75 ± 29.00
60NN R-	35.03 ± 6.19	7.57 ± 0.57	42.97 ± 9.99	36.25 ± 8.60	229.06 ± 45.14
120N R+	35.98 ± 3.27	9.72 ± 0.36	40.55 ± 4.75	28.12 ± 5.59	213.09 ± 20.09
120N R-	42.40 ± 8.03	8.92 ± 0.36	54.21 ± 14.83	27.05 ± 4.74	247.68 ± 51.61
180N R+	31.23 ± 4.44	8.54 ± 0.76	26.55 ± 2.98	15.60 ± 2.81	164.88 ± 24.59
180N R-	32.43 ± 7.49	8.35 ± 1.08	24.70 ± 6.57	17.62 ± 3.07	170.13 ± 40.54

Table 2.10b: Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 5-10 cm in CC. Total biomass = sum of all biomarker concentrations.

CC	Bacteria	Actinomycete	Saprophytic Fungi	Arbuscular Mycorrhizal Fungi	Total Biomass
60N R+	17.93 ± 3.49	7.01 ± 1.00	16.55 ± 0.59	16.52 ± 2.36	105.45 ± 16.21
60NN R-	23.33 ± 2.60	7.02 ± 0.24	21.82 ± 3.62	26.44 ± 3.15	150.76 ± 19.11
120N R+	23.33 ± 4.42	7.32 ± 0.87	19.26 ± 2.75	24.15 ± 2.62	138.42 ± 19.48
120N R-	18.60 ± 1.01	6.54 ± 0.47	15.30 ± 1.99	16.59 ± 2.39	104.53 ± 8.27
180N R+	18.83 ± 1.44	7.19 ± 1.36	14.07 ± 0.98	17.48 ± 2.84	108.01 ± 8.52
180N R-	25.13 ± 5.49	7.73 ± 0.72	20.65 ± 7.57	16.84 ± 2.22	134.78 ± 29.29

Table 2.11a: Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 0-5 cm in SG plots. H1= August harvest, H2= October harvest. Total biomass = sum of all biomarker concentrations.

SG	Bacteria	Actinomycete	Saprophytic Fungi	Arbuscular Mycorrhizal Fungi	Total Biomass
0N H1	41.83 ± 7.85	11.46 ± 1.59	103.45 ± 2.11	91.07 ± 15.49	386.26 ± 70.81
0N H2	41.63 ± 5.69	11.31 ± 0.86	75.05 ± 14.39	66.99 ± 13.31	318.11 ± 48.64
60N H1	39.70 ± 6.16	9.14 ± 1.13	56.20 ± 8.10	80.58 ± 23.06	317.63 ± 50.34
60N H2	54.60 ± 4.10	13.64 ± 1.94	57.67 ± 3.92	69.21 ± 8.02	350.85 ± 24.36
120N H1	41.27 ± 4.18	9.02 ± 1.34	61.92 ± 3.91	50.79 ± 14.77	293.91 ± 9.18
120N H2	80.70 ± 4.25	17.38 ± 3.07	84.21 ± 3.61	84.80 ± 1.73	484.77 ± 13.37

Table 2.11b: Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 5-10 cm in SG plots. H1= August harvest, H2= October harvest. Total biomass = sum of all biomarker concentrations.

SG	Bacteria	Actinomycete	Saprophytic Fungi	Arbuscular Mycorrhizal Fungi	Total Biomass
0N H1	26.60 ± 0.93	8.13 ± 0.34	68.91 ± 4.51	53.67 ± 5.68	249.36 ± 11.35
0N H2	22.70 ± 6.29	6.85 ± 2.20	56.28 ± 11.39	26.60 ± 5.59	181.50 ± 44.53
60N H1	29.50 ± 6.46	8.39 ± 1.54	36.02 ± 2.98	71.16 ± 6.04	254.82 ± 42.79
60N H2	27.30 ± 4.33	8.33 ± 1.48	35.97 ± 7.10	63.24 ± 11.17	232.36 ± 6.72
120N H1	25.40 ± 2.62	7.44 ± 0.97	31.35 ± 3.04	53.63 ± 12.58	209.13 ± 24.12
120N H2	35.73 ± 6.45	9.77 ± 1.97	39.66 ± 3.40	61.69 ± 7.58	264.48 ± 28.13

Table 2.12a: Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 0-5 cm in SB plots. Total biomass = sum of all biomarker concentrations. Treatments were last applied in 2009.

SB	Bacteria	Actinomycete	Saprophytic Fungi	Arbuscular Mycorrhizal Fungi	Total Biomass
0N H1	41.07 ± 1.68	10.72 ± 0.65	46.41 ± 9.84	40.68 ± 5.56	250.84 ± 21.60
0N H2	39.17 ± 2.60	10.17 ± 0.70	41.72 ± 7.26	38.09 ± 3.69	231.72 ± 22.30
60N H1	49.07 ± 3.41	12.27 ± 0.47	47.81 ± 3.58	37.94 ± 2.46	276.82 ± 10.97
60N H2	47.60 ± 4.30	11.46 ± 0.94	43.45± 4.59	42.78 ± 4.05	270.11 ± 23.11
120N H1	44.97 ± 6.95	12.04 ± 1.22	40.05 ± 6.29	30.44 ± 3.51	242.62 ± 32.79
120N H2	44.63 ± 4.77	12.04 ± 1.38	39.27 ± 5.50	29.18 ± 5.08	239.76 ± 25.75

Table 2.12b: Average nmol g^{-1} soil concentrations for bacterial, actinomycete, saprophytic fungi and AMF biomarkers by treatment at 5-10 cm in SB plots. Total biomass = sum of all biomarker concentrations. Treatments were last applied in 2009.

SB	Bacteria	Actinomycete	Saprophytic Fungi	Arbuscular Mycorrhizal Fungi	Total Biomass
0N H1	26.87 ± 2.10	7.84 ± 0.51	29.16 ± 7.15	26.87 ± 3.17	166.02 ± 10.83
0N H2	26.65 ± 1.67	8.38 ± 0.56	41.69 ± 9.14	26.33 ± 3.03	173.87 ± 10.25
60N H1	27.33 ± 0.44	9.57 ± 2.03	21.41 ± 2.54	26.75 ± 0.70	161.70 ± 5.14
60N H2	31.87 ± 1.94	9.61 ± 0.74	24.83 ± 2.29	30.59 ± 2.86	183.96 ± 7.73
120N H1	21.37 ± 3.02	7.67 ± 0.49	16.47 ± 2.19	24.24 ± 2.30	128.43 ± 15.33
120N H2	25.77 ± 2.12	9.03 ± 0.48	25.35 ± 5.53	29.99 ± 4.33	161.09 ± 7.55

Figure 2.1: Plot plan of study site. Plots labeled Trailblazer were the Trailblazer variety of switchgrass through 2009, and in 2010 and 2011 were cropped in soybeans. N-rates indicate kg N ha^{-1} applied each year. H1 in switchgrass and soybean plots indicates a pre-killing frost harvest in August, while H2 is a post-killing freeze harvest (while cropped in soybeans, there was no harvest treatment practiced). In corn plots, R^+ designates residue retained after harvest, and R^- designates maximum residue removal after harvest.

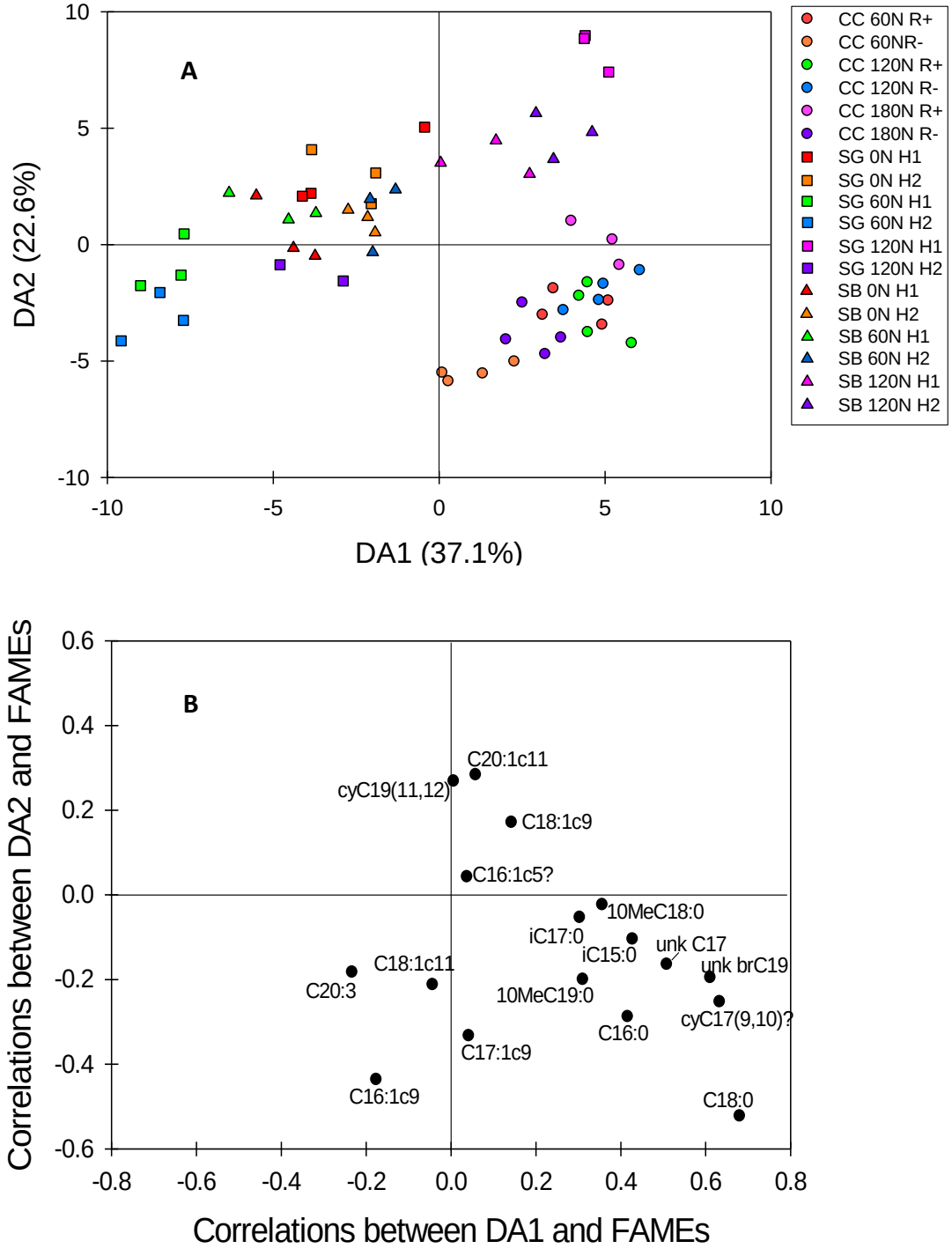

Figure 2.2: Canonical discriminant analysis of FAMEs by all treatments (A) and selected biomarkers (B) at 0-5 cm. In CC, H1= residue retained and H2= residue removed. In SG and SB H1= August harvest and H2= October harvest. N and harvest treatments in SB are residual. DA= Discriminant axis.

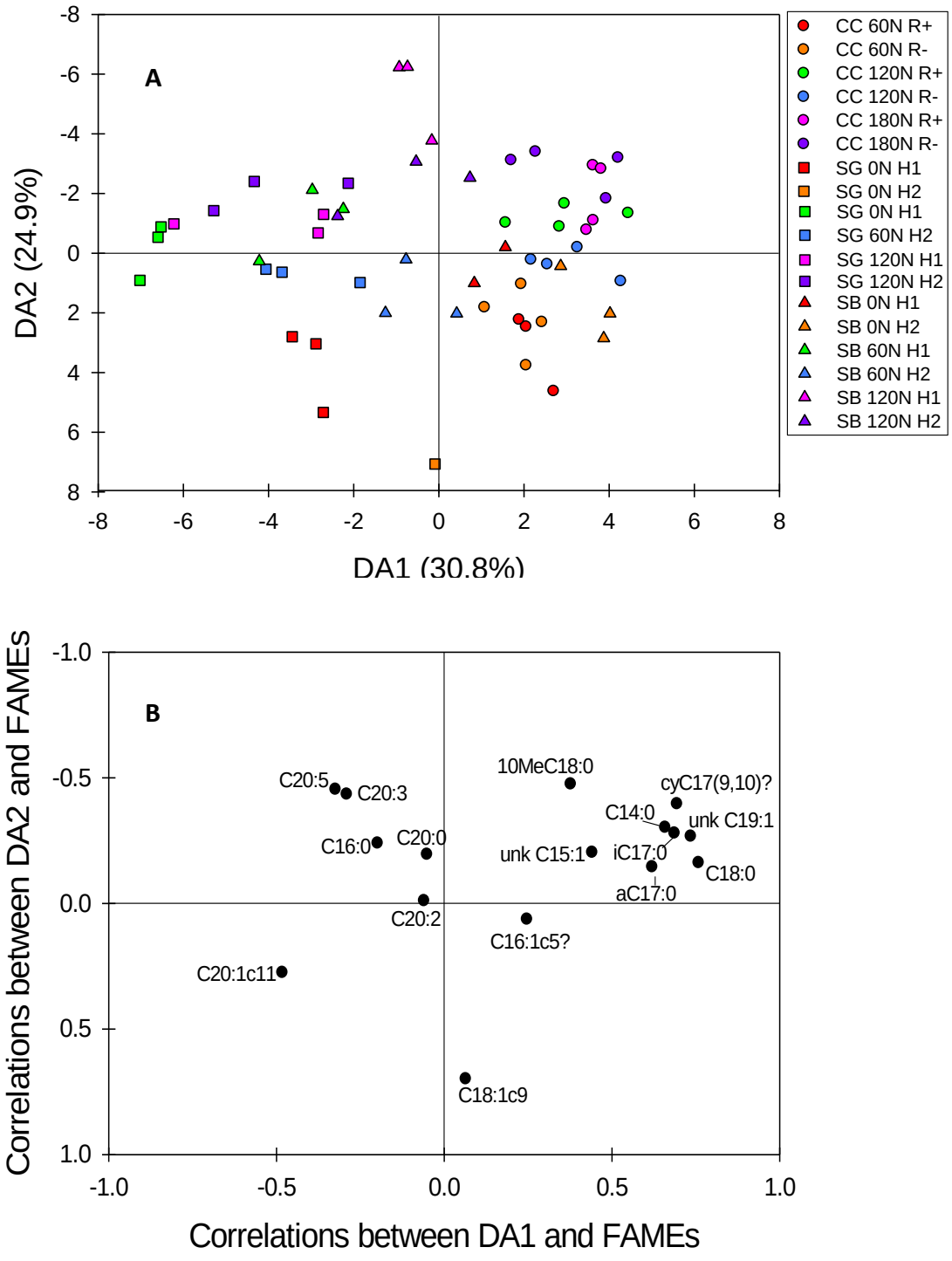

Figure 2.3: Canonical discriminant analysis of FAMEs by all treatments (A) and selected biomarkers (B) 5-10 cm. In CC, H1= residue retained and H2= residue removed. In SG and SB H1= August harvest and H2= October harvest. N and harvest treatments in SB are residual. DA= Discriminant axis.

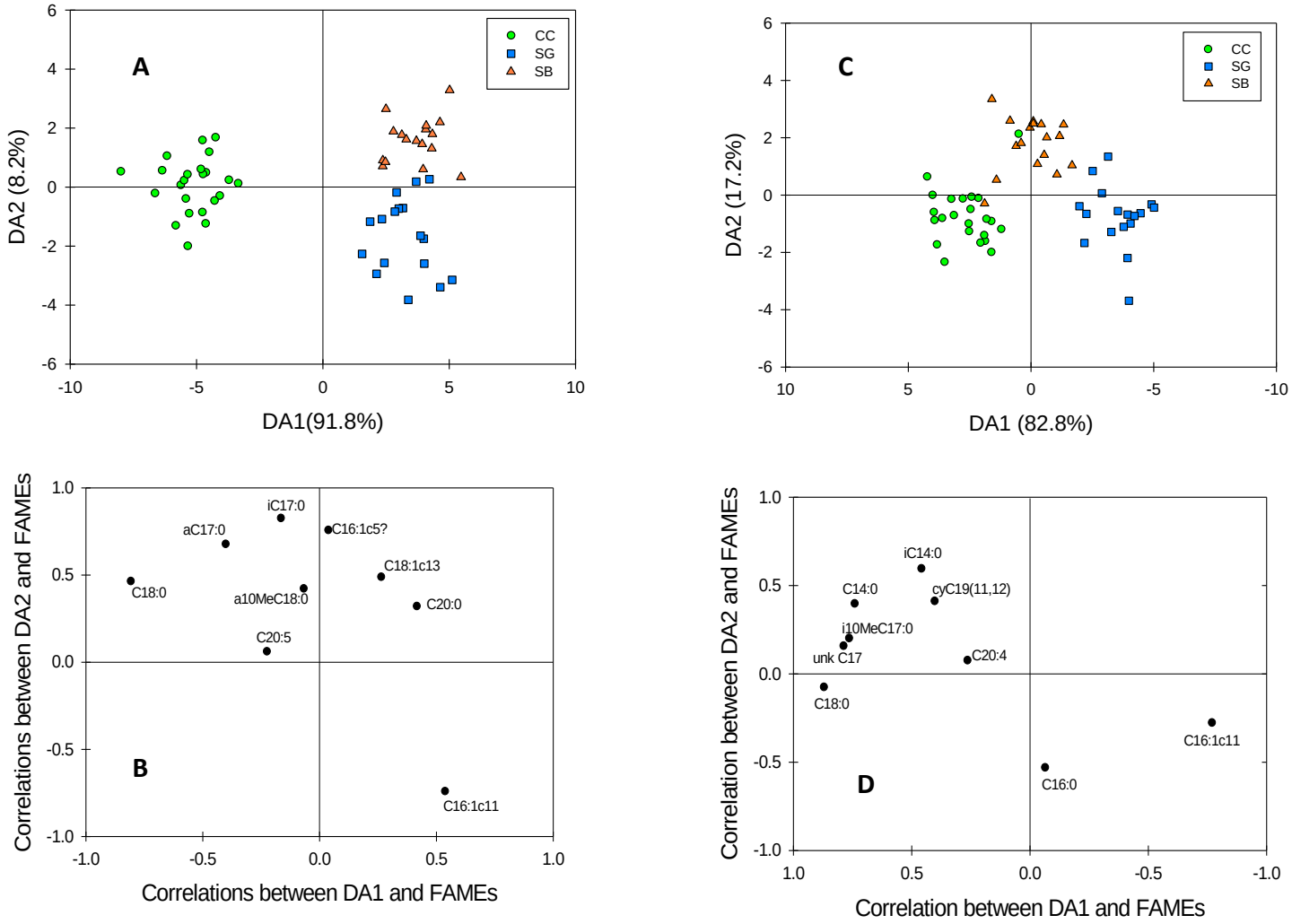

Figure 2.4: Canonical discriminant analysis of FAMEs by crop type at (A,B) 0-5 cm and (C,D) 5-10 cm. DA= Discriminant Axis

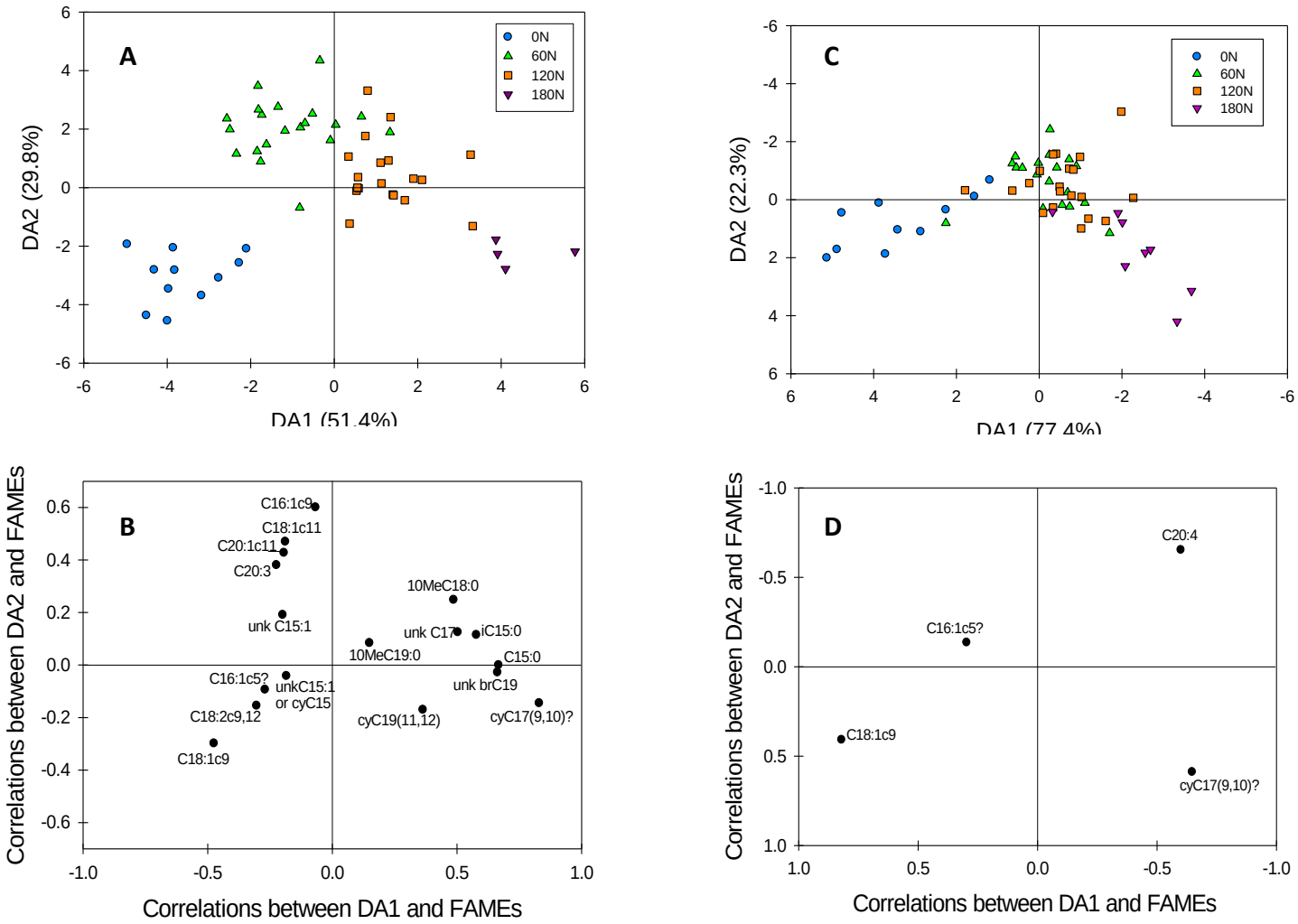

Figure 2.5: Canonical discriminant analysis of FAMEs by N-rate at (A,B) 0-5 cm and (C,D) 5-10 cm. DA= Discriminant axis.

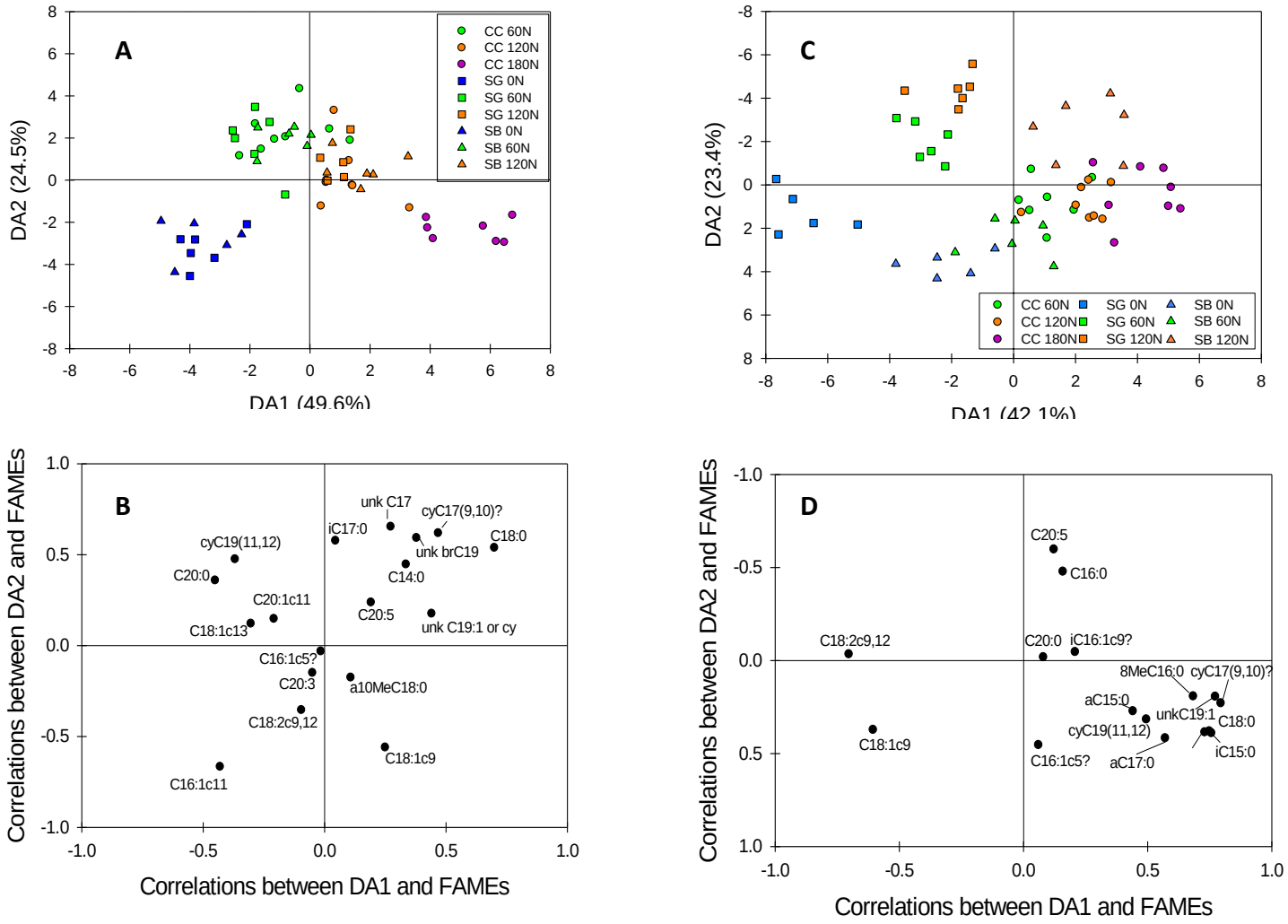

Figure 2.6: Canonical discriminant analysis of FAMEs by crop and N-rate at (A,B) 0-5 cm and (C,D) 5-10 cm. DA= Discriminant axis.

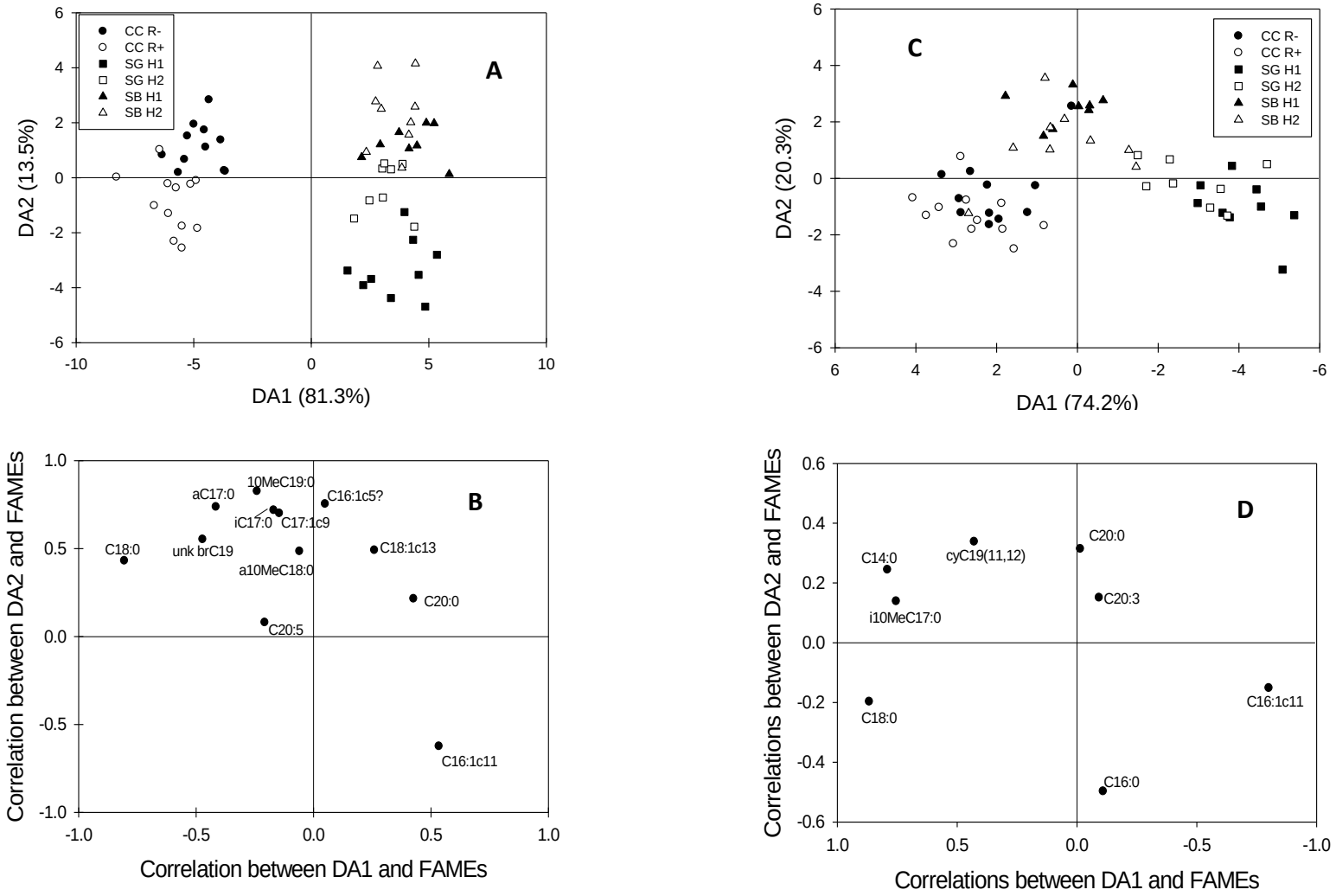

Figure 2.7: Canonical discriminant analysis of FAMEs by crop and harvest treatment at (A, B) 0-5 cm and (C, D) 5-10 cm. CC R^+ = residue retained, CC R^- = residue removed. SG and SB H1 = August harvest and H2= October Harvest. DA= Discriminant axis.

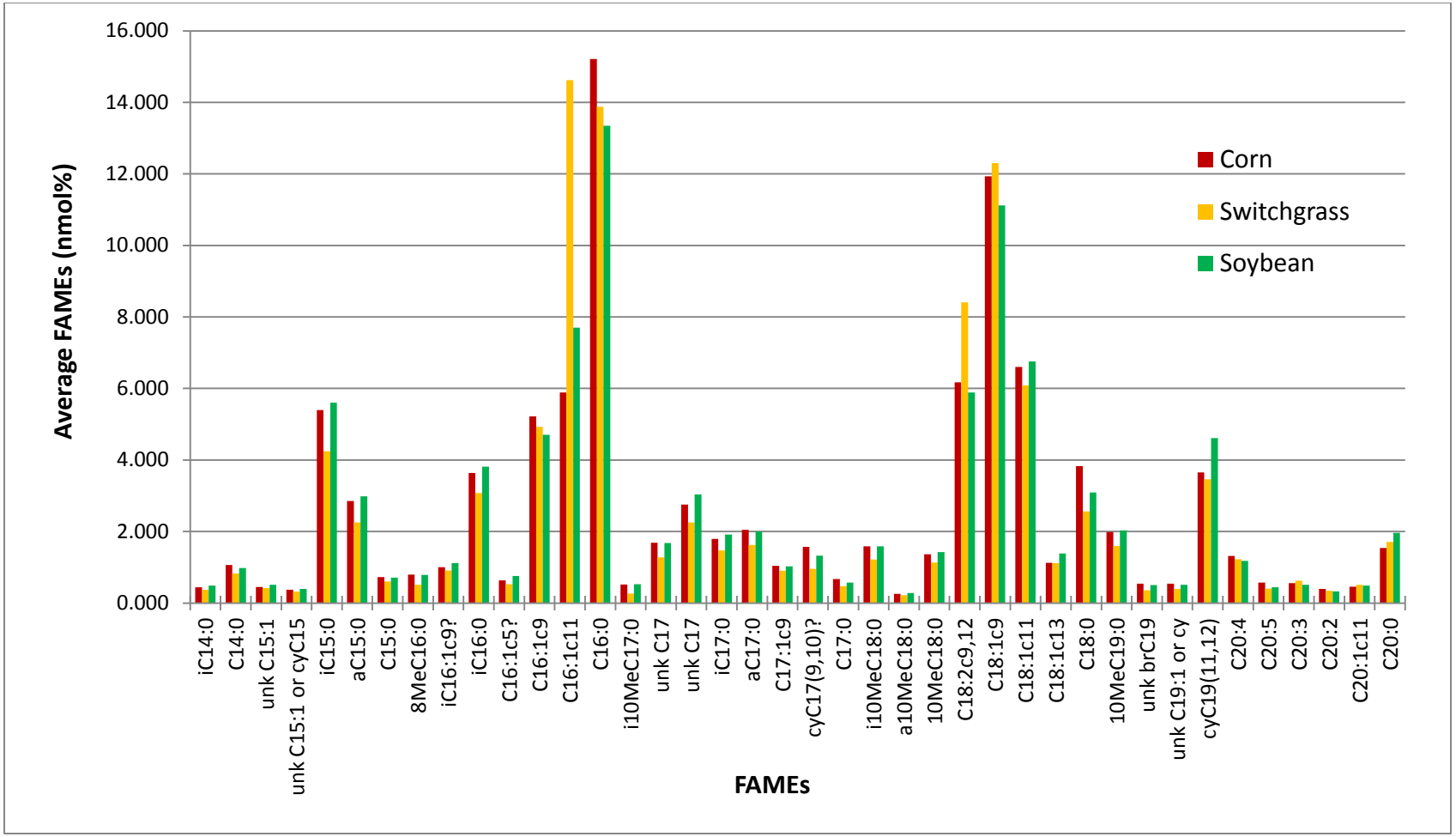

Figure 2.8: Molar percentages of individual FAMEs by crop type at 0-5 cm.

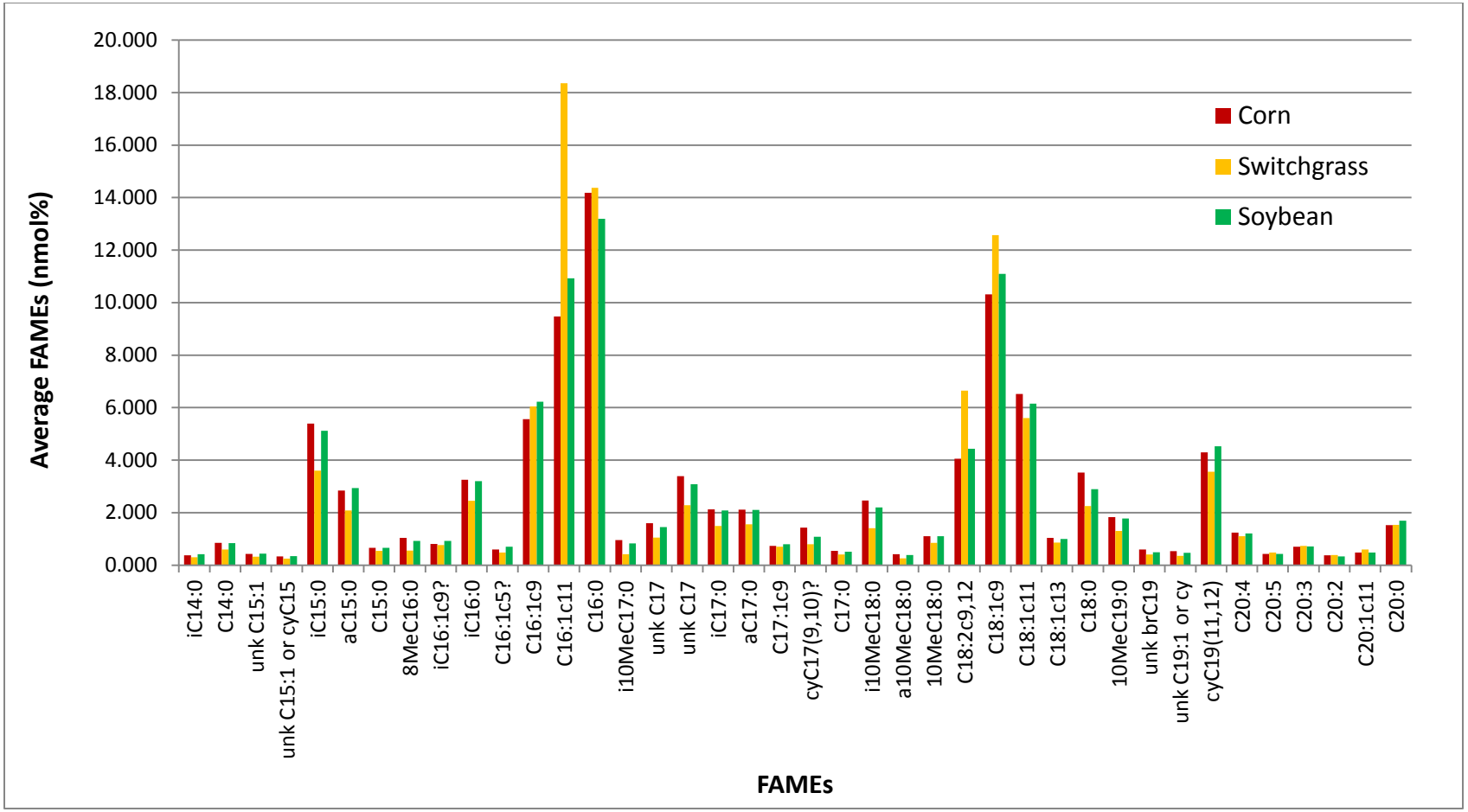

Figure 2.9 Molar percentages of individual FAMEs by crop at 5-10 cm.

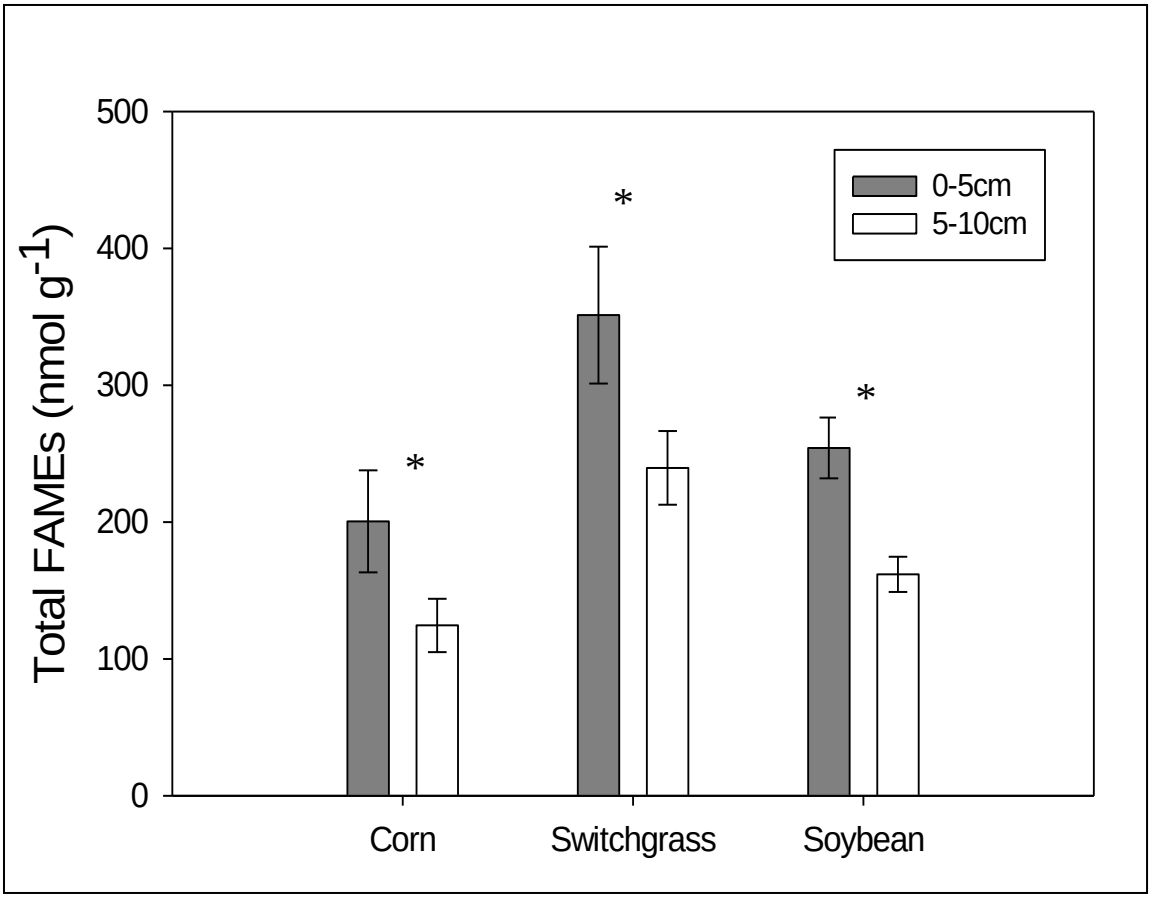

Figure 2.11: Average microbial biomass (total FAMEs, nmol g^{-1}) of each crop type at 0-5 cm and 5-10 cm depth.

* depth means within crop type differ significantly (p = 0.001).

Chapter 3:

Nitrogen in Corn, Switchgrass and Soybean Production Systems: Crop nitrogen balances, nitrous oxide emissions, and soil nitrogen

Abstract

As biofuel industries advance, demand will grow for cellulosic feedstocks from current annual cropping systems such as corn (*Zea mays,* L.) residue, or as dedicated feedstocks like perennial grasses such as switchgrass (*Panicum virgatum,* L.) The objectives of this study were to investigate the potential impacts of nutrient management on potential N losses as N_2O and NO_3^- for two bioenergy crops: continuous no-tillage corn (CC) and long-term Cave-in-Rock switchgrass (SG), grown on a marginal soil. The effects of nutrient inputs on potential N losses from previously fertilized switchgrass were also evaluated for an interim soybean (SB) crop used between successive switchgrass cultivars. CC plots were fertilized at 0, 60, 120, or 180 kg N ha^{-1} yr^{-1}. SG plots were fertilized at 0, 60, or 120 kg N $ha^{-1}yr^{-1}$. SB plots were not fertilized during the study period and were evaluated based on N-rates previously added to the former switchgrass crop (0, 60, or 120 kg N ha^{-1} yr^{-1}). Nitrogen balances were estimated for each crop and N-rate in 2010 and 2011, including soil N_2O emissions (except N4 in corn). Soil data from 2011 were analyzed for total and extractable inorganic N. In CC plots, only the highest N-rate (180 kg N ha^{-1}) provided enough N to replace N removed in aboveground biomass. In SG plots, N2 and N3 fertilization rates were in excess of N removed in aboveground biomass. In 2010, neither crop nor N treatment affected soil N_2O emissions. In 2011, N_2O emissions were greatest in 120 kg N ha^{-1} CC and SB plots.

SB 120 kg N ha^{-1} plots had the highest NO_3^- concentrations in the top 5 cm, consistent with the measured high N_2O emissions. Below 5 cm, 180 kg N ha^{-1} CC plots had the highest NO_3^- concentrations. Management impacts on potential N_2O losses at this site are unclear, but the higher residual soil nitrate concentrations suggest a potential for NO_3^- losses in corn plots fertilized above 120 kg N ha^{-1}.

Introduction

Maintaining Soil Quality in a Growing Industry

There is a growing demand for diverse biofuels in the United States (US DOE, 2011). Potential feedstocks for cellulosic ethanol include corn stover and switchgrass. Lands used to provide corn grain can provide some cellulosic feedstock through corn stover, but there is reason to suspect that crop residue removal will have negative impacts on soil quality (Karlen et al., 1994; Wilhelm et al., 2002). Furthermore, arable land must supply both fuel and food. To meet both demands, lands considered marginal for row-crop production will likely be used to produce dedicated non-food bioenergy crops (Robertson et al., 2008; Campbell et al., 2008; Gopalakrishnan et al., 2009). Unless there are strong economic incentives to maintain cash crops, marginal lands are typically placed in conservation programs, such as the Conservation Reserve Program (CRP), because they are prone to erosion and soil quality degradation (Stubbs, 2012). Because bioenergy crops can be grown on land used for conservation programs, it is imperative to understand the impacts biofuel production have on marginal soils to ensure they are not degraded further through erosion or soil C losses.

As the cellulosic biofuel production industry continues to develop, switchgrass may also be a viable alternative for farmers who are unable to enroll land in CRP. Multiple studies have shown that continuous switchgrass leads to increasing soil organic C (Ma et al., 2000; Liebig et al., 2005; Liebig et al. 2008; Follett et al., 2012), though few studies have investigated soil N dynamics under switchgrass production. Behnke et al. (2012) investigated GHG emissions from *Miscanthus x giganteus* used for biofuel production. Behnke and colleagues (2012) found N fertilization increased N_2O emissions in one of two years, but did not influence CO_2 emissions. There was also an increased amount of NO_3^- leaching from *M. x gianteus* plots with increasing fertilization (Behnke et al., 2012).

Greenhouse Gas Emissions

Managing soil nitrous oxide (N_2O) emission is of great interest in modern cropping systems. Agricultural soils were estimated to produce approximately 68% of the N_2O emitted in the United States in 2010, making them the single largest source of N_2O in the country (US EPA 2012). Furthermore, 92% of agricultural N_2O emissions are likely a result of soil and nutrient management practices (US EPA 2012). Any plan to mitigate greenhouse gas emissions must therefore include an evaluation of methods to reduce N_2O emissions from soils. However, controlling N_2O production is difficult because different management practices can have confounding impacts on emission rates, and because emissions must be managed alongside crop production and soil quality.

Because many factors controlling production are site specific, local reduction of N_2O emissions from agricultural soils can only be achieved through fine-tuning of

management practices. Due to the complicated and variable nature of emissions which depend on soil and environmental conditions, some management practices can either increase or decrease total output and the necessary actions by producers will depend on largely on local climate and local soils. A review of the literature suggests that N_2O emissions are influenced by pH (Blackmer and Bremner, 1978) soil water content (Blackmer and Bremmer, 1978; Linn and Doran, 1984; Anderson and Levine, 1987; Bateman and Baggs, 2005), organic matter content (Jacinthe and Lal, 2003), temperature (Anderson and Levine, 1987), and nitrogen availability especially as nitrate (NO_3^-) (Anderson and Levine, 1987; Hefting et al., 2003; Halvorsen et al., 2008). Soil management practices such as tillage can influence N_2O emissions by changing soil temperature and aeration (Six et al., 2004, Wagner-Riddle et al., 2007; Rochette, 2008).

However, an overriding factor appears to be that increased nitrogen fertilization leads to an increase in N_2O emissions (Snyder et al., 2009; Millar et al., 2010a; Cavigelli and Parkin, 2012). The Intergovernmental Panel on Climate Change (IPCC) assumes a linear relationship between N application and soil N_2O emissions in predicting greenhouse gas trends (IPCC, 2007), but there is increasing evidence of a rising exponential relationship (Zebarth et al., 2008; Snyder et al., 2009; Ma et al., 2010; Millar et al., 2010a; Hoben et al, 2011; Cavigelli and Parkin, 2012). The link between agricultural N_2O flux and fertilization suggest that carefully planning the timing, amount, and application method of fertilizer may be the most effective way to control N_2O losses from agriculture (Cavigelli and Parkin, 2012; Robertson et al., 2012).

Soil Fertility, Crop Nitrogen Use and Potential for Nitrate Leaching

Nitrogen is essential to plant health. One factor controlling native soil N availability is microbial decomposition of organic N in soil organic matter (SOM). This decomposition also generates N that is available for plant uptake (N mineralization). Studies have shown intimate and complex interactions between plant roots and the soil microbial community (Wardle et al., 2002; Bardgett and Wardle, 2003; Wardle et al., 2004). When N is applied as fertilizer to agricultural soils, it can be taken up by plants or microbes, or it can be removed from the system by N_2O emission or NO_3^- leaching if the fertilizer N supply exceeds plant and microbial demand. Excessive use of fertilizers has lead to extensive NO_3^- pollution of ground and surface water in the U.S. (Wu and Babcock, 1999; U.S. EPA, 2000), so it is important to closely monitor N use of crops. Balancing N supply with the appropriate time and amount of crop demand will help control N_2O emissions and NO_3^- leaching (Millar et al., 2010b; Cavigelli and Parkin, 2012)

Soil N also directly influences the activity and ecology of soil microbes (Lalande et al., 2005; Wallenstein et al., 2006), which in turn control organic matter decomposition (Albrecht, 1938; Franzluebers, 2002). Furthermore, N has a notable, if not fully-understood, role in the sequestration of C in the soil (Cambardella et al., 2012). Nitrogen fertilization has been shown to increase soil carbon in some systems (Gregorich et al., 1997; Halvorson et al., 1999;Liebig et al., 2002; Varvel et al., 2006; Varvel and Wilhelm, 2008), but not at others (Halvorson et al., 2002; Dolan et al., 2006; Khan et al., 2007; Russell et al., 2009). At the same site used in this study, Follett et al. (2012) found no

effect of N fertilization rate on C sequestration in CC plots, but fertilized switchgrass plots had higher C sequestration than non-fertilized switchgrass plots.

Yet addition of N is not simply important for its use to maximize crop productivity or indirect control of soil carbon. Nitrogen as N_2O or NO_3^- is an environmental pollutant, and can contribute to global climate change as N_2O (IPCC, 2007; discussed above) and groundwater contamination as NO_3^- (EPA, 2000). Nitrate leaching has been shown to be a problem in Nebraska (Kessavalou et al.,1996; Spalding and Kitchen, 1999).

In a review, Dinnes et al. (2002) noted that in any given year, corn plants can obtain a significant portion of their N from mineralized SOM, even when using fertilizer additions. One study estimated that first year recoveries of fertilizer N by corn ranged from 14 to 65% (Meisinger et al., 1985), suggesting that a considerable portion of fertilizer N could be susceptible to leaching (Dinnes et al., 2002). Accumulation of NO_3^- over time can be concerning as limited transformation is expected to occur, and leaching will result in future contamination. Spalding and Kitchen (1999) found that over a 15 year period as much as 600 lbs of N per acre (673 kg N ha^{-1}) accumulated in the vadose zone under corn plots in Nebraska.

Because of lower crop N requirements, different cropping methods and plant characteristics, it may be reasonable to assume that the potential for NO_3^- leaching in switchgrass production systems is less than under corn systems. Switchgrass has a much more extensive rooting system than corn, allowing switchgrass more opportunity to capture NO_3^- as it moves through the soil profile. We consider the rooting zone to extend to 30 cm in depth for this study, but switchgrass roots have been found to extend 330 cm

below the surface (Ma et al., 2000). Furthermore, studies of buffer strips have shown that perennials with high root densities are effective at NO_3^- removal (Groffman et al., 1992; Sanderson et al., 2001; Lee et al., 2003).

In this chapter, we focus on evaluating crop N-balances, N_2O emissions, and soil N data. Our objectives are to determine if there are best management practices that can minimize losses of N from the cropping system as N_2O and/or NO_3^-.

Methods

Site Description

The site used in this study is also discussed in Varvel et al. (2008) and Follett et al. (2012). This rain-fed, no-till study site is located on the University of Nebraska Agricultural Research and Development Center near Ithaca, NE (latitude 41.15°, longitude -96.40°). The soils are considered marginal for row-crop development, and are classified as Yutan silty clay loams (fine-silty, mixed, superactive, mesic Mollic Hapludalfs) and Tomek silt loams (fine, smectitic, mesic Pachic Argiudolls). Prior to site establishment in 1998, the site was cropped in soybeans and sorghum (*Sorghum bicolor*) and was disk-tilled. Plots are arranged in a split-split plot, randomized complete block design (Fig. 3.1). Whole plots are divided into three subplots that are 30m long x 18.3m wide, and subplots are separated by 15m wide alley for equipment access.

Main treatments are continuous corn (CC) and two cultivars of switchgrass (SG), Trailblazer and Cave-in-Rock. Corn has been planted continuously since 1999. In 1998, both switchgrass cultivars were seeded into a field that was previously cropped in soybeans. Switchgrass crops were not fertilized in 1998, but afterwards received fertilizer

treatments described below. In 2009, the Trailblazer cultivar died out and was planted in unfertilized soybeans in 2010 and 2011 as a transition crop to an improved bioenergy switchgrass cultivar. The interim unfertilized soybean (SB) crop is evaluated in the present study. No-till management began in 1998 for switchgrass plots and in 1999 for corn plots.

N-fertilizer treatments are applied at the scale of sub-plot and harvest treatments to the scale of sub-sub-plot. In 1999, fertilizer rates were 0, 80, 180, or 240 kg N ha^{-1}. From 2000 on, N fertilizer rates were 0, 60, 120, or 180 kg N ha^{-1} (0N, 60N, 120N, and 180N). Prior to 2007, fertilizer was applied as NH_4NO_3 broadcasted with a bulk spreader on all plots. Beginning in 2007, fertilizer was applied to corn plots as subsurface banded urea and to switchgrass plots as surface broadcast sulfur-coated urea. 0N, 60N, and 120N rates were applied to switchgrass plots. In 2010 and 2011, soybean (formerly Trailblazer) plots did not receive fertilizer, but we refer to these plots according to their prior fertilizer treatments. Corn plots received 60N, 120N, and 180N treatments.

There are two harvest treatments in this study for each crop. In Cave-In-Rock and Trailblazer switchgrass, H1 indicates an August harvest, and H2 a post killing-frost harvest in October. In 2010 and 2011, soybeans did not receive any harvest treatment. In CC plots R^+ indicates residue retention after harvest and R^- indicates residue removal after harvest. In this chapter, we only consider the two most likely biofuel management scenarios for each crop: H2 in switchgrass, which is the accepted best management practice (Vogel et al., 2002;Varvel et al., 2008); and R^- in corn, where both grain and residue are assumed to be feedstock for biofuel production. Greenhouse gas data was

only collected in these plots. Grain and biomass harvest data, as well as soil data were collected in all plots.

Crop Nitrogen Balance

We calculated a N-balance for aboveground inputs and removals of N (kg N ha^{-1} y^{-1}) using the following equation based on Legg and Meisinger (1982):

$$N_{net} = [Deposition + Fertilization + Fixation] – [Grain + Biomass + N_2O],$$

where N_{net} = net nitrogen in excess or in deficit for the production system; Deposition = wet + dry atmospheric N inputs; Fertilization = rate of commercial N applied; Fixation = N_2 fixation from symbionts; Grain = Corn and soy grain; Biomass = Corn residue harvested (removed); and N_2O = measured soil N_2O emissions.

Residue N removals were calculated using biomass yield data and N content of dry residue. Cave-in-Rock switchgrass balances considered biomass harvested, atmospheric deposition, and fertilizer inputs. We assumed there was negligible N_2 fixation in CC and SG plots. Approximately 6.5 kg N ha^{-1} per year of atmospheric deposition were used in the calculations, based on data from the National Atmospheric Deposition Program (2006).

Soybean plot removals were also used in calculating aboveground biomass N removal (grain only) and N_2O emissions data. Soybean stover residue was not sampled or analyzed for N content. We estimated N inputs from soybeans based on a review of N fixation rates by Salvagiotti et al. (2008), and also included atmospheric deposition. We assumed soybean N_2 fixation supplied 125 kg N ha^{-1} per year in all soybean plots based on the Salvgiotti et al. (2012) review. Soybeans received no N or harvest management.

Corn grain yields were determined from hand-harvested sub-samples at physiological maturity. Sub-samples were collected by sampling 15 feet (4.57 m) of a representative row in the center of each plot. Total yield and biomass production were calculated by scaling up from the sub-sample: Rows are 0.76 m wide, and we sub-sample 4.57 m of length. Grain and biomass yield data reported are based on dry mass. Biomass was calculated by removing corn stalks from the 15-foot sub-sample after grain harvest with flail forage harvesters (Varvel et al., 2008). Nitrogen content was determined using dry combustion (Follett et al., 2012).

Switchgrass biomass yields were also determined from sub-sampling. Swaths of 1.8 m wide by the length of the plot were cut and weighed using a flail forage harvester. Cutting height was set to 10 cm. Yields were determined for the 1.8 m strip and applied to the full plot area. After yields were determined, the remainder of the plots were harvested and biomass was removed but not weighed. Biomass was weighed on site, and subsamples were hand-collected from each subplot. The subsamples were dried at 50 °C for 48 hours in a forced-air oven and re-weighed to determine dry matter content. Nitrogen content was determined using dry matter content (Follett et al., 2012). Near infra-red spectroscopy using calibration techniques developed by NREL (Golden, CO).

Soybean grain yields were combine-estimated. We did not estimate biomass from soybean crops because all residue remained in the plots.

Greenhouse Gas Collection and Analysis

Gas samples for N_2O were collected using fixed-chambers positioned in each plot from May 24, 2010 through December 20, 2011 using standard sampling protocols from USDA-Agricultural Research Service's Greenhouse Gas Reduction through Agricultural Carbon Enhancement Network (GRACEnet; Parkin and Venterea 2010).The site was sampled weekly during the growing season and monthly during the non-growing season, weather and ground conditions permitting. Time intervals between sampling events was often unpredictable due to inclement field conditions, especially during winter months.

The fixed chambers were removed and re-installed periodically to allow field equipment to pass through the plots for fertilization and harvest, but otherwise remained stationary throughout the year. Chamber anchor depths were measured after each installation. Soil area measured was 1706.9 cm^2. Chambers were placed in R^- treatments of CC and H2 treatments of SG and SB. Although no measurements were taken in 180N CC plots, N_2O emissions were measured from a 0N subplot nested within the 60N treatment for corn. This resulted in N_2O measurements from all cropping systems at three identical N rates, 0, 60, 120 kg N ha^{-1} (Figure 3.1). Daily flux estimates as well as cumulative flux estimates were calculated for each year.

Immediately prior to sampling, chamber lids were attached to anchors with 4 large clips to seal the chamber. Samples were taken by injecting a needle attached to a 30

mL syringe into a sampling port located on the top of a given chamber lid, and 25 mL samples were immediately transferred to a vacuum-evacuated vial at 0, 10, 20, and 30 minute intervals. Corn and soybean plants were removed from the anchor area after sprouting. Switchgrass plants were clipped and kept as low to the ground as possible prior to each sampling (Sainju et al., 2012).

Gas samples were analyzed using a Varian 450 GC Greenhouse Gas Analyzer (Bruker Daltronics). The GC was fully automated with an electron capture detector (ECD) for analysis of N_2O concentrations, and fluxes were calculated as linear changes in concentration over time, then converted to an areal basis (kg N ha^{-1}) (Hutchinson and Moiser, 1981). Flux calculations were corrected for theoretical flux underestimation using the methods described by Venterea et al. (2010). Daily fluxes were reported after accounting for chamber area, soil temperature at 0-15 cm, and volumetric water content (m^3 m^{-3}), which were determined at the time of each sampling. Volumetric water content was determined at 0-15 cm using a time-domain reflectrometry (TDR) hand-held device (Gardner et al., 2000). Measurements were taken using a Fieldscout 300 Soil Moisture Meter (Spectrum Technologies, Inc., Plainfield, IL, USA).

Precipitation data is presented along with daily flux estimates. Daily precipitation data was retrieved from a metrological station located near the site, provided by the High Plains Regional Climate Center in Lincoln, NE.

Soil Properties and Nitrogen Data

Deep soil cores (0-150 cm; 3.81 cm DIA) were taken in all plots of the site on November 1, 2011. All soil samples were taken with a hydraulic soil corer at depths 0-5, 5-10, 10-30, 30-60, 60-90, and 90-120, and 120-150 cm. For each soil depth increment, electrical conductivity (EC_a; dS m^{-1}), pH, extractable inorganic N (mg/kg), total N (g/kg), total C (g/kg), and bulk density (Mg/m^3) were calculated using the deep soil cores. Bulk density was determined using the core method (Blake and Hartge, 1986). Soil tube diameter was 3.81 cm (1.5 in), and cores were separated according to the depths listed previously. Adjustments were made for moisture by drying a subset of samples at 105° C to determine depth-specific moisture content to calculate oven-dry bulk density (Mg m^{-3}). In addition to deep cores, larger surface soil samples were taken using a flat-edged spade at 0-5, 5-10, and 10-30 cm near the deep cores. Soil organic matter (SOM) and particulate organic matter (POM) content were determined on these samples only.

Air-dried, ground and 2-mm sieved soil samples were used for determination of soil chemical properties and nutrient concentrations. EC_a and pH were measured using a 1:1 soil/water slurry (Smith and Doran, 1996). For extractable inorganic nitrogen analyses, air-dried and 2 mm sieved soil samples were weighed were mixed with of 2 M KCl in a 1:10 soil/KCl slurry and shaken for 30 min at 200 rpm. The resulting suspension was gravity filtered through Whatman #42 paper and analyzed colorimetrically by Cd-reduction for nitrite-nitrate. Ammonium concentrations were determined using the phenolate method. Both NO_3^- and NH_4^+ were analyzed colorimetrically with a Lachat QuikChem 8000 Continuum Series (Zellweger Analytics, Inc.) automated flow injection ion analyzer (Keeney and Nelson, 1982).

Total N and TC were determined by dry combustion using a FlashEA 1112 Series NC Soil Analyzer (Thermo Electron Corportaion) (Mikha et al., 2005). SOM and POM estimates were determined by weight-loss-on-ignition (Cambardella et al., 2000).

Samples used for SOM and POM analysis were passed through a 2-mm sieve and air-dried by weight-loss-on-ignition (Cambardella et al., 2000). *In-situ* soil moisture data were collected from two CC and two SG plots at the field site. Matric potential (centibar) was measured by Watermark Monitors (Model 900 M, Irrometer Company, Inc.) at 1, 2, 3, and 4 ft (0.30, 0.61, 0.91, and 1.22 m). Matric potential was converted to volumetric water content ($m^3\ m^{-3}$) using the Soil Water Characteristcs Hydraulic Properties Calculator (v6.02.75) in Saxton et al., (2006). Soil water data was recorded hourly. We report averaged hourly data from October 11 (the last day data was available).

Statistics

Cumulative annual N_2O fluxes were used to determine management effects on differences in emissions. All statistical analyses were performed with the PC Version 9.2 of the Statistical Analyses System for Windows using PROC GLIMMIX (SAS, Inc., Cary, NC). Nitrous oxide data were analyzed with N treatment nested within crop type. Soil data were analyzed at each soil depth to test the split-split plot treatment of N nested within crop type. Fisher's protected LSD test was used to identify significant differences in pair-wise treatment mean comparisons within each crop type. Significance was determined at $\alpha = 0.10$ for N_2O statistical analyses and at $\alpha = 0.05$ for soil statistical analyses.

Results

Crop Nitrogen Balance

In 2010 and 2011, nitrogen balance calculations indicated that residue removal lead to net N removals for CC plots in 0N, 60N, and 120N treatments. At the highest CC application rate (180N) more N was added than was removed as corn biomass and grain. Switchgrass plots that received N fertilization had net N additions according to the N balances. In 2010, 35% of the fertilizer N added to 60N SG plots was removed as biomass at harvest, and 53% was removed in 2011. In 2010, 46% of N added as fertilizer in 120N SG plots was removed as aboveground biomass, and in 2011 68% was removed. (Tables 3.1a and 3.1b).

Nitrous Oxide Emissions

In 2010 there were no observable effects of fertilizer application or crop on measured emissions of N_2O. In CC and SB plots, non-fertilized plots tended to have lower emissions fertilized plots. In SG plots, emissions appeared highest in the 120N treatment, though no treatments were significant (Table 3.2a). In 2011, the N_2O emissions in 120N treatments were higher than 0N or 60N in both CC and SB. There was no significant N effect in SG plots, although N_2O emissions in 0N appeared somewhat lower than 60N or 120N treatments (Table 3.2b). Cumulative annual emissions in both years ranged from 0.54 to 3.93 kg N ha^{-1} yr^{-1}. In fertilized CC and SG plots, these emissions accounted for 2.5 to 3% of N applied as fertilizer in 2010 and 2011.

There was a time delay between major precipitation events and daily flux response (Figures 3.2 and 3.3). In 2011, the highest emissions occurred in 120N SB plots, and most of this flux was measured during a single flux event (Table 3.5b).

Soil Total N and Extractable Inorganic N

Concentrations of total N were highest in the top 5 cm of soil in all plots (Tables 3.3a to 3.3c). N-fertilizer application rate did not correlate to total N under CC plots. Nonetheless, total N in under 180N was highest in surface soils (0-30 cm) but lowest in soils below 30 cm compared to 60N and 120N treatments (Figure 3.4a). In SG plots, total N was highest under 120N at all depths (Figure 3.4b). In SB plots, 120N plots had the lowest total N from 0-10 cm, and had the highest total N below 30 cm (Table 3.3c). At 0-5 cm, SG plots had higher total N than CC or SB plots at all N-rates, but below 5 cm total N did not notably vary by crop type.

As expected, extractable inorganic N was directly related to N fertilization rate. Below 5 cm, 180N CC plots consistently had much higher residual inorganic N concentrations than any other treatment. Below 90 cm, 120N and 180N plots of CC had similar concentrations of NO_3^- (Figure 3.5a). Based on the samples collected, very little if any NO_3^- accumulation below 90 cm was observed in any of the SG or SB plots (Figure 3.5b; Tables 3.3b and 3.3c). At 0-5cm, 120N SB plots had the highest concentrations of NO_3^- of any treatment combination (Table 3.3c). For 60N CC plots, virtually all NO_3^- was found in the top 30 cm, though in 120N and 180N plots there was more NO_3^- below

the rooting zone (Table 3.6). In all of the SG and SB plots, the vast majority of NO_3^- exists in the top 30 cm of soil regardless of N treatment (Figure 3.6b and Table 3.6).

Soil water data was only collected from CC and SG plots. Volumetric water content increased with depth, and below 2 ft (0.61 m) SG plots had slightly higher water content than CC plots (Figure 3.7). In order to accommodate harvesting equipment, data loggers had to be removed in October. Weather stations recorded 1.43 cm of precipitation between October 11 and November 1 (when soil samples were taken).

CC plots had the lowest SOM and POM of the three crop types from 0-10 cm (Table 3.4). SG plots generally had higher SOM and POM than SB plots from 0-10 cm. In SG and SB plots, SOM and POM increased with N-rate, but N-rate did not influence SOM or POM in CC plots. Soil organic matter did not notably differ between crop types or N-rate below 10 cm. From 10-30 cm, SG plots had higher soil POM concentrations than either CC or SB plots.

Discussion

Highest Net N-Losses in Corn

In both years, more N was removed from the system than was added in R- corn plots at 0N, 60N, and 120N. However, grain yields were not higher in 180N plots than 120N plots in either year. N-balance and yield compare with estimates published by Varvel et al. (2008). N removal estimates from grain indicate more N from grain was removed in 120N plots than 180N plots (Tables 3.1a and 3.1b). Most of the extra N

added in 180N compared to 120N plots was not taken up by the plant in aboveground biomass. In 60N and 120N plots, the N removed in excess of fertilizer supply was likely supplied by mineralization (Meisinger et al., 1985, Dinnes et al., 2002). The N balance data show that 120 kg N ha^{-1} alone is insufficient to meet crop N demands, but the corn crops are unable to uptake the full 180 kg N ha^{-1}.

Total N in the soil was not related to fertilizer application rate, although it appeared highest in CC 180N plots than any other crop or N treatment combination. Elevated soil NO_3^- concentrations in CC 180N, however, indicated a greater risk for N leaching under the highest application rate relative to all other management treatments. Not only were NO_3^- concentrations high throughout the profile, but high concentrations (>25 mg kg^{-1}) were observed below the rooting zone (Figure 3.6a). The 180N CC plots may also have produced high N_2O emissions not sampled because of field conditions. As previously mentioned, there is very likely an exponential relationship between N application and N_2O emissions (Zebarth et al., 2008; Snyder et al., 2009; Ma et al., 2010; Millar et al., 2010a; Hoben et al, 2011; Cavigelli and Parkin, 2012). Though a relationship between N-rate and N_2O emissions could not be established because too few N levels were tested in this field study, total emissions doubled from 60N to 120N in CC in 2011. Given the elevated soil NO3-levels at the highest N rate, it is likely that a greater percentage of fertilizer N in 180N plots is being lost as NO_3^- and/or N_2O.

Similar to 180N plots, 120N plots of corn also showed large NO_3^- concentrations in soils below the rooting zone. While the N-balance showed that more N was removed as aboveground biomass than was added as fertilizer in both years, the soil data show that N is still being lost as NO_3^- from 120N CC plots (Figure 3.5a). Higher soil

concentrations of NO_3^-, suggest greater potential of groundwater contamination by NO_3^- leaching. Loss of N to NO_3^- leaching may be a larger concern at this site than N_2O emissions in CC plots fertilized at 120N. In 2011, N3 CC plots had much higher emissions than 0N or 60N plots (Table 3.2b). Cumulative emissions even at the 120N rate were within the lower range of annual N_2O emissions in a review of corn and soybean systems in the Eastern and Central U.S. (0.8 to 19.3 kg N ha^{-1}) (Cavigelli and Parkin, 2012).

Residual Nitrate Low under Switchgrass

Crop N-balances suggest that more N is added as fertilizer than is removed as aboveground biomass in SG plots, but this N may be largely tied up in root biomass. Soil N data showed higher TN in SG plots than CC or S plots, especially in the top 5 cm. Also, there was little NO_3^- in the soil under SG plots, especially below 10 cm. The higher concentration of TN and low concentrations of NO_3^- show that while more N is added as fertilizer than is removed, the extra N is stored as soil organic N and probably stored in root biomass as well. These data support Vogel et al.'s (2002) findings that 120 kg N ha^{-1} is an optimal rate of fertilization for switchgrass, and support the hypothesis that NO_3^- leaching potential under switchgrass will be less than that of row crops for a given N fertilizer rate. In 2010, the highest emissions were measured in 120N SG plots, but this was not significant due to high standard error. Further investigation is needed to determine the timing and magnitude of N_2O emissions from soils under switchgrass production.

Nitrous oxide Emissions Highest under Interim Soybean

In 2011, the highest N_2O emissions occurred in SB plots. 120N plots of CC and SB plots were comparable (Table 3.2b) and both were far higher than any other crop-N treatment combination. In CC plots, N_2O emission is directly related to fertilizer N application rate, but SB plots were not fertilized after spring of 2009. High levels of NO_3^- were measured in the top 5 cm of soil in SB 120N, and it is probable that this NO_3^- is the result of N_2 fixation and subsequent nitrification from the previous year. Considerable levels of nitrate have been found under soils following N_2 fixation by legumes (Peoples et al., 1995), but N-fixers do not always readily utilize available NO_3^- (Herridge and Bergersen, 1988). Nitrate sparing may have contributed to high NO_3^- levels in the top 5 cm of soil. However, N_2 fixation in soybean crops peak between the R3 and R5 growth stages (Zapata et al., 1987). Furthermore, peak emissions in 2011 from 120N plots were measured in March, before soybeans were planted, while NO_3^- data were collected in November. Any NO_3^- left in March from NO_3^- sparing would have been produced by the soybean crop in the fall of 2010. However, 0N and 60N plots of soybeans had the lowest emissions at the site. If NO_3^- sparing alone could explain the high emissions from SB plots, 0N and 60N SB plots should also have shown high soil emissions of N_2O.

When sampling both deep cores and bulk soil, switchgrass roots were frequently observed in the soil samples. Johnson et al. (2007) estimated the half-life of rapidly decomposing portion of switchgrass roots is 22 days, while the half-life of recalcitrant portions of roots is 1450 days. This estimation was based on a laboratory study and is difficult to compare to field conditions, but it is reasonable to assume that after two years

there is some decomposition of switchgrass roots. Switchgrass rhizomes may exist for many years in the soil (Hartnett, 1989), so it is possible that roots remained viable in the soil and began to decompose in the spring of 2011. Switchgrass aboveground biomass has been shown increase with fertilization (Vogel et al., 2002; Varvel et al., 2008), and while belowground biomass was not measured it is reasonable to assume that the same trend applies. By that assumption, 120N plots of Trailblazer likely had higher root biomass than 0N or 60N plots, leaving more root biomass even after the Trailblazer was removed and soybeans were planted. It is possible that NO_3^- from the previous year (2010) remained in the soil and when the thawed in March, the combination of available N and C from switchgrass roots provided the fuel for high N_2O production.

It is also possible that similar early-season emissions occurred in 2010, but because sampling did not occur until May it is impossible to know. Root biomass probably played a role in 120N SB plots having higher emissions than 0N or 60N plots.. Yield data has shown that aboveground biomass in switchgrass roots increased rapidly at this site (Vogel et al., 2002; Varvel et al., 2008), so it is reasonable to assume that belowground biomass followed similar trends. It is also worth noting that N_2O emissions from fertilized corn and switchgrass plots did not account for more than about 3% of N from fertilization. The IPCC estimates that approximately 0.3-3% of applied fertilizer N is later lost as N_2O-N (IPCC, 2006), so the emissions from corn and switchgrass plots in both 2010 and 2011 fell within the IPCC's predicted range.

Conclusions

Crop N balances showed that in corn plots receiving less than 120 kg N ha^{-1} yr^{-1} more N was removed as biomass than was applied as fertilizer when residue was removed. In switchgrass plots, more N was removed as biomass than was added as fertilizer.

We found that N_2O emissions from a marginal soil in eastern Nebraska were variable by year and N application rate. There was no significant treatment effect in 2010 on emissions, but switchgrass plots receiving 120 kg N ha^{-1} had higher emissions than other treatments. In 2011, corn plots receiving 120 kg N ha^{-1} and soybean plots which received the same amount of N in 2009 had the highest emissions. We suspect that decomposition of switchgrass roots from previous years, combined with NO_3^- from soybean N sparing, is responsible for the high N_2O emissions in soybean plots. Corn plots receiving 120 and 180 kg N ha^{-1} showed high concentrations of NO_3^- below the rooting zone (>15 mg kg^{-1}), raising cause for concern about groundwater contamination from fertilization at this site. We are likely missing the largest N_2O emissions at this site because 180 kg N ha^{-1} corn plots were not sampled. Low concentrations of NO_3^- beneath switchgrass plots suggest a low risk of NO_3^- leaching.

References

Anderson, I.C. and Levine, J.S. (1987). Simultaneous field measurements of biogenic emissions of nitric oxide and nitrous oxide. *Journal of Geophysical Research*: Atmospheres 92: 965-976.

Bardgett, R. D., and Wardle, D. A. (2003). Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* 84(9): 2258-2268.

Bateman, E.J., and Baggs, E.M. (2005). Contributions of nitrification and denitrification N_2O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils* 41: 379–388.

Blackmer, A.M. and Bremner, J.M. (1978). Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil microorganisms. *Soil Biology and Biochemistry* 10(3): 187-191.

Blake, G. R., & Hartge, K. H. (1986). Bulk density. In Klute, A. (Ed.) *Methods of Soil Analysis: Part 1—Physical and Mineralogical Methods* (363-375). American Society of Agronomy, Inc: Madison, WI, USA.

Burke, I. C., Yonker, C. M., Parton, W. J., Cole, C. V., Schimel, D. S., & Flach, K.(1989). Texture, climate, and cultivation effects on soil organic matter content in US grassland soils. *Soil Science Society of America Journal*, *53*(3), 800-805.

Campbell, J. E., Lobell, D. B., Genova, R. C., Field, C. B. The global potential of bioenergy on abandoned agricultural lands. 2008. *Environmental Science and Technology* 42(15): 5791–5794.

Cavigelli, M.A., and Parkin, T.B. (2012). Cropland management contributions to greenhouse gas flux: Central and Eastern U.S. In Liebig, M.A., Franzluebbers, A.J., and Follett (R.F.) (Eds.), *Managing Agricultural Greenhouse Gases: Coordinated Agricultural Research through GRACEnet to Address our Changing Climate* (129-165). Academic Press: Waltham, MA, USA.

Dolan, M.S., Clapp, C.E., Allmaras, R.R., Baker, J.M., Molina, J.A.E. (2006). Soil organic carbon and nitrogen in a Minnesota soil as related to tillage, residue and nitrogen management. *Soil Tillage Research* 89: 221-231.

Follett, R.F., Vogel, K.P., Varvel, G.E., Mitchell, R.B., Kimble, J. (2012). Soil carbon sequestration by switchgrass and no-till maize grown for bioenergy. *Bioenergy Research* 4(4): 1-10. doi:10.1007/s12155-012-9198-y.

Gardner, C.M.K., Robinson, D., Blyth, K. & Cooper, J.D. (2001). Soil water content. In Smith, K.A., and Mullins, C.E. (Eds.) *Soil and environmental analysis: physical methods* (1–64). Marcel Dekker Inc., New York, NY.

Gregorich, E.G., Ellert, B.H., Drury, C.F., Liang, B.C. (1996). Fertilization effects on soil organic matter turnover and corn residue C storage. *Soil Science Society of America Journal* 60: 472-476.

Groffman P.M., Gold, A.J. Simmons, R.C. (1992). Nitrate dynamics in riparian forest: microbial studies. *Journal of Environmental Quality* 21: 666–671.

Halvorson, A.D., Reule, C.A., Follett, R.F. (1999). Nitrogen fertilizer effects on soil carbon and nitrogen in a dryland cropping system. *Soil Science Society of America* 63: 912-917.

Halvorson, A.D., Wienhold, B.J., Black, A.L. (2002). Tillage, nitrogen, and cropping system effects on soil carbon sequestration. *Soil Science Society of America Journal* 66: 906-912.

Halvorson, A.D., Del Grosso, S.J., Reule, C.A. (2008). Nitrogen, tillage, and crop rotation effects on nitrous oxide emissions from irrigated cropping systems. *Journal of Environmental Quality* 37: 1337-1344.

Hartnett, D. C. (1989). Density-and growth stage-dependent responses to defoliation in two rhizomatous grasses. *Oecologia*, *80*(3), 414-420.

Hatfield, J.L., and Cambardella, C.A. (2001). Nutrient management in cropping systems. *In* J. McFarland and M. Sanderson (Eds.) *Integrated management of land application of animal waste*. American Society of Agricultural Engineering. St. Joseph, MI, USA.

Hefting, M.M., Bobbink, R., de Caluwe, H. (2003). Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones. *Journal of Environmental Quality* 32: 1194-1203.

Herridge DF, Bergersen FJ (1988) Symbiotic nitrogen fixation. In. Wilson, J.R. (Ed.) *Advances in Nitrogen Cycling in Agricultural Ecosystems* (46-65). CAB International. Wallingford, UK.

Hoben, J.P., Gehl, R.J., Millar, N., Grace, P.R., Robertson, G.P. (2011). Nonlinear nitrous oxide (N_2O) response to nitrogen fertilizer in on-farm corn crops of the US Midwest. *Global Change Biology* 17:1140-1152.

Hutchinson, G.L., and Moiser, A.R. (1981). Improved soil cover method for field measurements of nitrous oxide fluxes. *Soil Science Society of America Journal* 45:311-316.

Kessavalou, A., Doran, J.W., Powers, W.L., Kettler, T.A., Qian, J.H. (1996) Bromide and Nitrogen-15 tracers of nitrate leaching under irrigated corn in central Nebraska. *Journal of Environmental Quality* 25:1008-1014.

Intergovernmental Panel on Climate Change. (2007). Contribution of Working Group Ito the Fourth Assessment Report of the Intergovnernmental Panel on Climate Change, 2007. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jacinthe, P.-A., and Dick, W.A. (1997). Soil management and nitrous oxide emissions from cultivated fields in southern Ohio. *Soil Tillage Research* 41:221–235.

Jacinthe, P.A., Lal, R. (2003). Nitrogen fertilization of wheat residue affecting nitrous oxide and methane emission from a central Ohio Luvisol. *Biology and Fertility of Soils* 37: 338-347

Karlen, D. L., Wollenhaupt, N. C., Erbach, D. C., Berry, E. C., Swan, J. B., Eash, N. S., Jordahl, J. L. (1994). Crop residue effects on soil quality following 10-years of no-till corn. *Soil and Tillage Research* 31(2):149-167.

Keeney, D.R., and Nelson, D.W. (1982). Nitrogen-Inorganic forms. In Page, A.L., Miller, R.H., Keeney, D.R. (Eds.) *Methods of Soil Analysis: Part 2*. American Society of Agronomy, Inc., Madison, WI, USA.

Kessavalou, A., Doran, J.W., Powers, W.L., Kettler, T.A., Qian, J.H. (1996). Bromide and Nitrogen-15 tracers of nitrate leaching under irrigated corn in Central Nebraska. *Journal of Environmental Quality* 25:1008-1014.

Khan, S.A., Mulvaney, R.L., Ellsworth, T.R., Boast, C.W. (2007). The myth of nitrogen fertilization for soil carbon sequestration. *Journal of Environmental Quality* 36: 1821-1832.

Lee, K. H., Isenhart, T. M., Schultz, R. C. (2003). Sediment and nutrient removal in an established multi-species riparian buffer. *Journal of Soil and Water Conservation*, *58*(1): 1-8.

Leibig, M.A., Varvel, G.E., Doran, J.W., Wienhold, B.J. (2002). Crop sequence and nitrogen fertilization effects on soil properties in the western Corn Belt. Soil Science Society of America Journal 66: 596-601.

Leibig, M.A., Johnson, H.A., Hanson, J.D., Frank, A.B. (2005). Soil carbon under switchgrass stands and cultivated cropland. *Biomass and Bioenergy* 28: 347-354.

Liebig, M.A., Schmer, M.R., Vogel, K.P., Mitchell, R.B. (2008). Soil carbon storage by switchgrass grown for bioenergy. *Bioenergy Research* 1:215-222.

Linn, D.M. and Doran, J.W. (1984). Effect of water-filled pore space on CO_2 and N_2O production in tilled and non-tilled soils. *Soil Science Society of America Journal* 48: 1267-1272.

Ma, Z., Wood, C.W., Bransby, D.I. (2000). Impacts of management on root characteristics of switchgrass. *Biomass and Bioenergy* 18: 105-112.

Ma, Z., Wood, C.W., Bransby, D.I. (2000). Soil management impacts on soil carbon sequestration by switchgrass. *Biomass and Bioenergy* 18: 469-477.

Ma, B.L., Wu, T.Y., Tremblay, N., Deen, W., Morrison, M.J., McLaughlin, N.B., Gregorich, E.G., Steward, G. (2010). Nitrous oxide fluxes from corn fields: on-farm assessment of the amount and timing of nitrogen fertilizer. *Global Change Biology* 16:156-170.

Meisinger, J.J., Bandel, V.A., Stanford, G., Legg, J.O. (1985). Nitrogen utilization of corn under minimal tillage and moldboard plow tillage: I. Four year results using labled fertilizer on an Atlantic Coastal Plain soil. *Agronomy Journal* 77:602-611.

Mikha, M.M., Vigil, M.F., Liebig, M.A., Bowman, R.A., McConkey, B., Deibert, E.J., Pikul, J.L. Jr. (2006). Cropping system influences on soil chemical properties and soil quality in the Great Plains. *Renewable Agriculture and Food Systems* 21(1): 26-35.

Millar, N., Robertson, G.P., Grace, P.R., Gehl, R.J., Hoben, J.P. (2010a). Nitrogen fertilizer management for nitrous oxide (N_2O) mitigation in intensive corn (Maize) production: an emissions reduction protocol for US Midwest agriculture. Mitigation and Adaption Strategies for Global Change 15(2): 185-204.

Millar, N., Robertson, G.P., Grace, P.R., Gehl, R.J., Hoben, J.P. (2010b). Nitrogen fertilizer rate management as a nitrous oxide mitigation in intensive corn (maize) production: an emissions reduction protocol for US Midwest agriculture. *Mitigation and Adaptation Strategies for Global Change* 15(2): 185-204.

National Atmospheric Deposition Program. (2006). Total Inorganic N deposition, 2006. Retrieved from <http://nadp.sws.uiuc.edu/amon/>

Parkin, T.B., and Kaspar, T.C. (2006). Nitrous oxide emissions from corn–soybean systems in the Midwest. *Journal of Environmental Quality* 35:1496–1506.

Peoples M.B., Herridge D.F., Ladha J.K. (1995). Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant and Soil* 174:3-28.

Rochette, P. (2008). No-till only increases N_2O emissions in poorly aerated soils. *Soil Tillage Research* 101: 97-100.

Russell, A.E., Cambardella, C.A., Laird, D.A., Jaynes, D.B., Meek, D.W. (2009). Nitrogen fertilizer effects on soil carbon balances in Midwestern U.S. agricultural systems. *Ecological Applications* 19: 1102-1113.

Salvagiotti, F., Cassman, K.G., Specht, J.E., Walters, D.T., Weiss, A., Dobermann, A. (2008). Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research* 108(1): 1-13. doi:10.1016/j.fcr.2008.03.001.

Sainju, U.M., Stevens, W.B., Ceasar-TonThat, T., Liebig, M.A. (2012). Soil greenhouse gas emissions affected by irrigation, tillage, crop rotation, and nitrogen fertilization. *Journal of Environmental Quality* 41(6): 1774-1786.

Saxton KE, Willey PH. 2006. The SPAW model for agricultural field and pond hydrologic simulation. In VP Singh and DK Frevert (eds.) *Watershed Models* (401-435). CRC Press: Boca Raton, FL, USA.

Six, J., Ogle, S.M., F.J., Breidt, R.T., Moiser, A.R., Paustian, K. (2004). The potential to mitigate global warming with no-tillage is only realized when practiced in the long term. *Global Change Biology* 10: 155-160.

Smith, J.L., and Doran, J.W. (1996). Measurement and use of pH and electrical conductivity for soil quality analysis. In Doran, J.W. and Jones, A.J. (Eds.) *Methods for assessing soil quality* (169-186). Madison, WI, USA: Agronomy Society of America, Crop Science Society of America, Soil Science Society of America.

Snyder, C.S., Bruulsema, T.W., Jensen, T.L., Fixen, P.E. (2009). Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agriculture, Ecosystems and Environment* 133: 247-266.

Spalding, R. F., and Kitchen, L. A. (1988). Nitrate in the intermediate vadose zone beneath irrigated cropland. *Ground Water Monitoring & Remediation* 8(2): 89-95.

Streeter, J.G., 1985. Nitrate inhibition of legume nodule growth and activity. II. Short term studies with high nitrate supply. *Plant Physiology* 77: 325–328.

Streeter, J.G., 1988. Inhibition of legume nodule formation and N_2 fixation by nitrate. CRC Crit. Rev. *Plant Science* 7: 1–23.

Stubbs, M. (2012). *Conservation Reserve Program(CRP): Status and Issues*. Report for Congress, R42783. Congressional Research Service.

Tisdall, J.M., and J.M. Oades. (1982). Organic matter and water-stable aggregates in soils. *Journal of Soil Science* 33:141-163.

U.S. Environmental Protection Agency. (2000). Groundwater and Drinking Water Chapters. In *National Water Quality Inventory, 1998: Report to Congress*. EPA 816-R-00-013, August 2000.

U.S. Environmental Protection Agency. (2012). Inventory of U.S. greenhouse gas emissions and sinks: 1990-2010. U.S. Environmental Protection Agency, Officeof Atmospheric Programs, EPA 430-R-11-005, April 2011. Retrieved from <www.epa.gov/climatechange/ghgemissions/usinventoryreport.html>

Varvel, G.E. (2006). Soil organic carbon changes in diversified rotations of the western Corn Belt. *Soil Science Society of America Journal* 70: 426-433.

Varvel, G.E., and Wilhelm, W.W. (2003). Soybean nitrogen contribution to corn and sorghum in western Corn Belt rotations. *Agronomy Journal* 95(5): 1220-1225.

Varvel, G.E., and Wilhelm, W.W. (2008). Soil carbon levels in irrigated western Corn Belt rotations. *Agronomy Journal* 100: 1180-1184.

Varvel, G.E., Vogel, K.P., Mitchell, R.B., Follett, R.F., Kimble, J.M. (2008). Comparison of corn and switchgrass on marginal soils for bioenergy. *Biomass and Bioenergy* 32: 18-21.

Varvel, G.E., and Wilhelm, W.W. (2011). No-tillage increases soil profile carbon and nitrogen under long-term rainfed cropping systems. *Soil Tillage Research* 114: 28-36.

Venterea, R.T. (2010). Simplified method for quantifying theoretical underestimation of chamber-based trace gas fluxes. *Journal of Environmental Quality* 39: 126-135.

Vogel, K.P., Brejda, J.J., Walters, D.T., Buxton, D.R. (2002). Switchgrass biomass production in the Midwest USA: Harvest and nitrogen management. *Agronomy Journal* 94: 413-420.

Wagner-Riddle, C., Furon, A., McLaughlin, N.L., Lee, I., Barbeau, J., Jayasundara, S.,Parkin, G., von Bertoldi, P., Warland, J. (2007). Intensive measurement of nitrous oxide emissions from a corn-soybean-wheat rotation under two contrasting management systems over 5 years. *Global Change Biology* 13:1722-1736.

Wallenstein, M.D., McNulty, S., Fernandez, I.J., Boggs, J., Schlesinger, W.H. (2006). Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management* 222: 459-468.

Wardle DA. (2002). In *Communities and Ecosystems: Linking the Aboveground and Belowground Components*. Princeton University Press: Princeton, New Jersey, USA.

Wardle, D.A., Bardgett, R.D., Klironomos , J.N., Setälä,H., van der Putten, W.H., Wall, D.H. (2004). Ecological linkages between aboveground and belowground biota. *Science* 304: 1634–1637.

Weier, K.L., Doran, J.W. , Power, J.F., Walters, D.T. (1993). Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal* 57(1): 66-72.

Wilhelm, W. W., Johnson, J. M. F., Hatfield, J. L., Voorhees, W. B., & Linden, D. R. (2004). Crop and soil productivity response to corn residue removal. *Agronomy Journal* 96(1), 1-17.

Wu, J., and Babcock, B. A. (1999). Metamodeling potential nitrate water pollution in the central United States. *Journal of Environmental Quality 28*(6): 1916-1928.

Zapata, F., Danso, S.K.A., Hardarson, G., Fried, M. (1987). Time course of nitrogen fixation in field-grown soybean using nitrogen-15 methodology. *Agronomy Journal* 79: 172–176.

Zebarth, B.J., Rochette, P., Burton, D.L. (2008). N_2O emissions from spring barley production as influenced by fertilizer nitrogen rate. *Canadian Journal of Soil Science* 88: 197-205.

Tables and Figures

Table 3.1a: Average nitrogen inputs and removals in 2010, listed by crop. R- = Residue removed. H2 = October Harvest.

	Inputs (kg N ha^{-1})			**Removals (kg N ha^{-1})**			**Net N**
	Atmospheric Deposition	Fertilization	N_2 Fixation	Grain	Biomass	N_2O	
CC							
0N R$^-$	6.5	0	0	24	21	1.4	**-40**
60N R$^-$	6.5	60	0	64	14	2	**-14**
120N R$^-$	6.5	120	0	103	28	2	**-7**
180N R$^-$	6.5	180	0	88	32	-	**66**
SG							
0N H2	6.5	0	0	-	11	1.2	**-6**
60N H2	6.5	60	0	-	32	1.5	**33**
120N H2	6.5	120	0	-	81	3.4	**42**
SB							
0N	6.5	-	125	151	-	1.3	**-20**
60N	6.5	-	125	159	-	1.3	**-28**
120N	6.5	-	125	154	-	1.4	**-23**

Table 3.1b: Average nitrogen inputs and removals in 2011, listed by crop. R- = Residue removed. H2 = October Harvest.

	Inputs (kg N ha^{-1})			**Removals (kg N ha^{-1})**			**Net N**
	Atmospheric Deposition	Fertilization	N_2 Fixation	Grain	Biomass	N_2O	
CC							
0N R$^-$	6.5	0	0	45	36	1.4	**-40**
60N R$^-$	6.5	60	0	70	24	1.9	**-29**
120N R$^-$	6.5	120	0	121	41	3.9	**-40**
180N R$^-$	6.5	180	0	86	46	-	**54**
SG							
0N H2	6.5	0	0	-	6	1.2	**-1**
60N H2	6.5	60	0	-	21	2.5	**43**
120N H2	6.5	120	0	-	55	2.3	**69**
SB							
0N	6.5	-	125	113	-	0.7	**18**
60N	6.5	-	125	135	-	1	**-3.5**
120N	6.5	-	125	114	-	5.2	**17**

Table 3.2a: Average cumulative N_2O emissions (kg N ha^{-1} yr^{-1}) by crop type and N-rate in 2010, followed by standard error. Numbers with different letter headings are significantly different ($p \leq 0.05$) within crop type.

Crop and N-rate	N_2O (kg N ha^{-1})
CC 0N	1.36 ± 0.9[a]
CC 60N	1.97 ± 0.5[a]
CC 120N	1.86 ± 0.5[a]
SG 0N	1.21 ± 0.3 [a]
SG 60N	1.50 ± 1.2[a]
SG 120N	3.41 ± 3.3[a]
SB 0N	1.26 ± 0.8[a]
SB 60N	1.31 ± 0.4[a]
SB 120N	1.74 ± 1.1[a]

Table 3.2b: Average cumulative N_2O emissions (kg N ha^{-1} yr^{-1}) by crop type and N-rate in 2011, followed by standard error. Numbers with different letter headings are significantly different ($p \leq 0.05$) within crop type.

Crop and N-rate	N_2O (kg N ha^{-1})
CC 0N	1.19 ± 0.9 [a]
CC 60N	1.60 ± 2.1[a]
CC 120N	3.58 ± 0.7[b]
SG 0N	0.92 ± 1.2[a]
SG 60N	1.90 ± 1.3[a]
SG 120N	1.80 ± 0.9[a]
SB 0N	0.54 ± 0.4[a]
SB 60N	0.74 ± 0.5[a]
SB 120N	3.93 ± 0.8[b]

Table 3.3a: Average values of soil properties from deep soil cores N-rate in CC plots. BDL indicates value is below detectable limits

	Depth (cm)	EC	pH	NO_3^- (mg N/kg)	NH_4^+ (mg/kg)	TN (g/kg)	TC (g/kg)	Bulk Density (g cm^{-3})
CC 60N	0-5	0.22 ± 0.03	5.97 ± 0.25	2.18 ± 0.93	3.39 ± 0.71	1.45 ± 0.17	16.43 ± 2.40	1.55 ± 0.17
	5-10	0.20 ± 0.03	6.13 ± 0.21	0.98 ± 0.68	2.95 ± 0.52	1.29 ± 0.14	14.33 ± 1.97	1.68 ± 0.09
	10-30	0.19 ± 0.03	6.38 ± 0.20	0.17 ± 0.19	1.95 ± 0.44	1.17 ± 0.17	13.62 ± 2.47	1.38 ± 0.05
	30-60	0.20 ± 0.07	6.79 ± 0.40	BDL	1.98 ± 0.58	1.08 ± 0.27	13.47 ± 2.93	1.37 ± 0.04
	60-90	0.18 ± 0.04	6.83 ± 0.55	BDL	2.20 ± 0.48	0.76 ± 0.23	9.48 ± 2.37	1.48 ± 0.09
	90-120	0.17 ± 0.02	6.89 ± 0.55	BDL	2.07 ± 0.54	0.53 ± 0.16	5.40 ± 1.61	1.55 ± 0.04
	120-150	0.16± 0.02	7.07 ± 0.57	0.04 ± 0.06	1.82 ± 0.58	0.40 ± 0.10	3.41 ± 0.84	1.65 ± 0.06
CC 120N	0-5	0.24 ± 0.03	5.75 ± 0.33	3.74 ± 1.76	5.18 ± 1.45	1.57 ± 0.07	17.38 ± 0.92	1.46 ± 0.09
	5-10	0.21 ± 0.03	6.17 ± 0.27	1.92 ± 1.34	3.96 ± 0.87	1.24 ± 0.05	13.33 ± 0.70	1.64 ± 0.04
	10-30	0.26 ±0.06	6.70 ± 0.18	0.42 ± 0.19	2.66 ± 0.65	1.02 ± 0.14	11.30 ± 2.06	1.43 ± 0.01
	30-60	0.30 ± 0.09	6.97 ± 0.36	0.30 ± 0.34	2.43 ± 0.80	0.99 ± 0.31	11.34 ± 4.01	1.35 ± 0.03
	60-90	0.32 ± 0.10	7.14 ± 0.54	0.92 ± 1.03	2.44 ± 0.46	0.79 ± 0.22	11.00 ± 2.46	1.39 ± 0.04
	90-120	0.30 ± 0.11	7.25 ± 0.58	0.67 ± 0.71	2.08 ± 0.56	0.66 ± 0.21	9.24 ± 2.36	1.41 ± 0.05
	120-150	0.27 ± 0.10	7.34 ± 0.57	1.00 ± 1.05	1.75 ± 0.50	0.45 ± 0.12	4.78 ± 1.33	1.49 ± 0.11
CC 180N	0-5	0.20 ± 0.02	5.45 ± 0.16	6.15 ± 2.14	5.48 ± 1.10	1.48 ± 0.13	16.76 ± 1.79	1.50 ± 0.15
	5-10	0.20 ± 0.03	5.69 ± 0.30	4.02 ± 2.75	4.62 ± 1.04	1.22 ± 0.13	13.56 ± 1.98	1.41 ± 0.14
	10-30	0.25 ± 0.04	6.01 ± 0.23	4.26 ± 1.84	3.17 ± 0.74	1.13 ± 0.21	12.76 ± 3.01	1.40 ± 0.05
	30-60	0.29 ±0.08	6.59 ± 0.38	1.76 ± 0.66	2.45 ± 0.67	0.90 ± 0.24	10.67 ± 3.24	1.37 ± 0.04
	60-90	0.25 ± 0.06	6.88 ± 0.48	0.85 ± 0.61	2.99 ± 0.60	0.66 ± 0.17	8.61 ± 2.20	1.40 ± 0.04
	90-120	0.26 ± 0.07	6.93 ± 0.43	1.28 ± 0.92	2.75 ± 0.48	0.51 ± 0.08	5.91 ± 1.38	1.36 ± 0.08
	120-150	0.21 ± 0.05	7.19 ± 0.49	1.19 ± 0.79	2.31 ± 0.36	0.36 ± 0.07	3.46 ± 0.92	1.51 ± 0.07

Table 3.3b: Average values of soil properties from deep soil cores N-rate in SG plots. BDL indicates value is below detectable limits.

	Depth (cm)	EC	pH	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	TN (g/kg)	TC (g/kg)	Bulk Density (g cm^{-3})
SG 0N	0-5	0.28 ± 0.04	5.45 ± 0.20	0.02 ± 0.03	4.36 ± 0.81	1.55 ± 0.09	19.80 ± 1.50	1.46 ± 0.06
	5-10	0.25 ± 0.03	6.06 ± 0.11	BDL	4.29 ± 0.98	1.21 ± 0.08	14.16 ± 0.93	1.62 ± 0.02
	10-30	0.20 ± 0.02	6.34 ± 0.13	BDL	2.76 ± 0.46	0.94 ± 0.13	10.55 ± 1.76	1.38 ± 0.02
	30-60	0.21 ± 0.03	6.58 ± 0.05	BDL	2.67 ± 0.76	0.68 ± 0.14	6.92 ± 2.21	1.39 ± 0.05
	60-90	0.21 ± 0.03	6.81 ± 0.07	0.23 ± 0.32	2.82 ± 0.78	0.53 ± 0.11	5.13 ± 1.80	1.38 ± 0.02
	90-120	0.26 ± 0.07	7.09 ± 0.34	BDL	1.85 ± 0.49	0.49 ± 0.12	6.10 ± 1.68	1.35 ± 0.03
	120-150	0.29 ± 0.08	7.32 ± 0.32	BDL	1.81 ± 0.26	0.45 ± 0.09	5.73 ± 1.26	1.40 ± 0.05
SG 60N	0-5	0.25 ± 0.03	5.81 ± 0.16	0.16 ± 0.12	4.67 ± 0.53	1.87 ± 0.09	23.32 ± 1.15	1.42 ± 0.02
	5-10	0.27 ± 0.05	6.18 ± 0.20	BDL	3.74 ± 0.29	1.30 ± 0.12	14.51 ± 1.14	1.62 ± 0.05
	10-30	0.20 ± 0.02	6.50 ± 0.12	BDL	2.05 ± 0.49	0.91 ± 0.11	10.16 ± 1.55	1.40 ± 0.03
	30-60	0.18 ± 0.01	6.69 ± 0.09	BDL	2.60 ± 0.45	0.65 ± 0.12	6.55 ± 1.73	1.39 ± 0.02
	60-90	0.18 ± 0.01	6.81 ± 0.12	BDL	2.54 ± 0.70	0.48 ± 0.08	4.08 ± 1.03	1.37 ± 0.04
	90-120	0.25 ± 0.07	7.01 ± 0.19	BDL	2.49 ± 0.64	0.41 ± 0.04	3.17 ± 0.57	1.37 ± 0.05
	120-150	0.22 ± 0.06	7.20 ± 0.26	BDL	2.30 ± 0.64	0.32 ± 0.05	2.97 ± 1.41	1.49 ± 0.07
SG 120N	0-5	0.21 ± 0.03	5.37 ± 0.15	3.23 ± 3.48	6.08 ± 0.87	2.13 ± 0.19	24.90 ± 2.29	1.31 ± 0.09
	5-10	0.20 ± 0.03	5.74 ± 0.14	1.12 ± 1.22	4.66 ± 0.82	1.46 ± 0.14	16.41 ± 1.70	1.47 ± 0.08
	10-30	0.16 ± 0.02	6.28 ± 0.10	0.11 ± 0.16	2.87 ± 0.75	1.22± 0.17	14.30 ± 2.46	1.32 ± 0.06
	30-60	0.15 ± 0.01	6.46 ± 0.15	BDL	2.53 ± 0.84	1.14 ± 0.28	13.27 ± 3.88	1.28 ± 0.03
	60-90	0.22 ± 0.08	6.71 ± 0.39	0.02 ± 0.03	3.00 ± 0.52	0.83 ± 0.22	10.16 ± 2.77	1.30 ± 0.05
	90-120	0.20 ± 0.06	6.98 ± 0.49	BDL	2.47 ± 0.40	0.75 ± 0.23	10.41 ± 2.55	1.43 ± 0.13
	120-150	0.18 ± 0.07	7.03 ± 0.57	BDL	2.68 ± 0.84	0.66 ± 0.21	8.10 ± 2.33	1.49 ± 0.11

Table 3.3c: Average values of soil properties from deep soil cores N-rate in SB plots, *listed by N-rates formerly applied to the previous switchgrass crop. BDL indicates value is below detectable limits

	Depth (cm)	EC	pH	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	TN (g/kg)	TC (g/kg)	Bulk Density (g cm^{-3})
SB 0N*	0-5	0.24 ± 0.02	6.08 ± 0.04	1.74 ± 0.79	4.06 ± 0.61	1.68 ± 0.14	19.81 ± 1.87	1.54 ± 0.05
	5-10	0.25 ± 0.04	6.09 ± 0.11	0.60 ± 0.45	4.54 ± 1.25	1.30 ± 0.11	14.43 ± 1.54	1.60 ± 0.03
	10-30	0.20 ± 0.02	6.32 ± 0.07	0.11 ± 0.10	2.88 ± 0.76	1.10 ± 0.20	12.59 ± 2.79	1.67 ± 0.43
	30-60	0.19 ± 0.02	6.45 ± 0.18	BDL	2.86 ± 1.04	0.93 ± 0.36	10.34 ± 5.02	1.39 ± 0.05
	60-90	0.26 ± 0.09	6.85 ± 0.49	0.02 ± 0.02	2.91 ± 0.84	0.71 ± 0.27	8.70 ± 3.38	1.35 ± 0.06
	90-120	0.26 ± 0.08	7.00 ± 0.51	BDL	2.40 ± 0.41	0.52 ± 0.16	7.17 ± 2.18	1.39 ± 0.06
	120-150	0.32 ± 0.07	7.25 ± 0.48	0.04 ± 0.05	2.46 ± 0.48	0.41 ± 0.06	4.90 ± 1.37	1.44 ± 0.11
SB 60N*	0-5	0.24 ± 0.03	5.65 ± 0.06	6.47 ± 2.46	4.94 ± 0.60	1.90 ± 0.10	21.81 ± 1.12	1.38 ± 0.08
	5-10	0.18 ± 0.01	5.95 ± 0.11	1.24 ± 0.84	4.71 ± 1.13	1.37 ± 0.08	15.31 ± 0.88	1.52 ± 0.05
	10-30	0.17 ± 0.02	6.28 ± 0.08	0.26 ± 0.14	2.50 ± 0.44	1.20 ± 0.08	13.76 ± 1.08	1.39 ± 0.03
	30-60	0.15 ± 0.01	6.55 ± 0.06	0.03 ± 0.03	2.22 ± 0.36	0.97 ± 0.19	11.17 ± 2.59	1.36 ± 0.04
	60-90	0.18 ± 0.02	6.65 ± 0.13	0.07 ± 0.10	2.42 ± 0.27	0.73 ± 0.16	8.12 ± 2.32	1.31 ± 0.06
	90-120	0.24 ± 0.08	6.92 ± 0.32	BDL	2.87 ± 0.59	0.71 ± 0.14	8.72 ± 1.21	1.29 ± 0.06
	120-150	0.22 ± 0.07	7.14 ± 0.34	BDL	2.62 ± 0.71	0.53 ± 0.09	6.21 ± 0.93	1.37 ± 0.07
SB 120N*	0-5	0.26 ± 0.03	5.24 ± 0.13	7.94 ± 2.02	6.25 ± 0.48	2.05 ± 0.16	23.03 ± 2.26	1.34 ± 0.07
	5-10	0.21 ± 0.02	5.74 ± 0.22	1.93 ± 0.81	4.42 ± 0.47	1.33 ± 0.12	14.20 ± 1.26	1.56 ± 0.07
	10-30	0.20 ± 0.03	6.32 ± 0.13	0.55 ± 0.27	3.25 ± 0.30	1.09 ± 0.11	11.87 ± 1.27	1.44 ± 0.03
	30-60	0.18 ± 0.02	6.54 ± 0.08	BDL	3.09 ± 0.37	0.73 ± 0.10	7.53 ± 1.41	1.46 ± 0.03
	60-90	0.17 ± 0.01	6.58 ± 0.09	0.01 ± 0.01	3.29 ± 0.46	0.52 ± 0.05	4.42 ± 0.61	1.49 ± 0.03
	90-120	0.20 ± 0.01	6.65 ± 0.14	0.16 ± 0.10	3.58 ± 0.59	0.44 ± 0.04	3.19 ± 0.47	1.47 ± 0.04
	120-150	0.18 ± 0.02	6.84 ± 0.08	0.22 ± 0.26	3.04 ± 0.69	0.35 ± 0.06	2.23 ± 0.41	1.50 ± 0.05

Table 3.4: Average SOM and POM from aggregate samples by crop and N-rate.

	Depth (cm)	SOM (%)	POM (mg kg^{-1} 0.05-2mm)
CC 60N	0-5	41.8 ± 6.98	10.5 ± 2.98
	5-10	37.2 ± 4.56	4.7 ± 0.98
	10-30	34.1 ± 3.79	2.2 ± 0.12
CC 120N	0-5	40.8 ± 0.46	10.5 ± 1.27
	5-10	35.1 ± 1.18	4.1 ± 0.44
	10-30	31.8 ± 0.92	2.3 ± 0.24
CC 180N	0-5	42.1 ± 7.36	10.7 ± 3.74
	5-10	35.5 ± 4.48	3.5 ± 0.72
	10-30	34.4 ± 4.09	2.5 ± 0.49
SG 0N	0-5	48.2 ± 2.03	18.2 ± 2.64
	5-10	37.3 ± 1.16	7.3 ± 0.82
	10-30	33.7 ± 1.86	4.0 ± 0.19
SG 60N	0-5	57.1 ± 3.39	19.4 ± 0.68
	5-10	43.2 ± 4.40	9.6 ± 1.67
	10-30	35.5 ± 2.70	4.7 ± 0.37
SG 120N	0-5	59.2 ± 2.51	20.7 ± 1.29
	5-10	41.4 ± 1.72	6.2 ± 0.27
	10-30	37.4 ± 1.47	4.7 ± 0.31
SB 0N	0-5	50.3 ± 4.20	15.4 ± 1.62
	5-10	40.9 ± 3.94	7.3 ± 1.57
	10-30	35.8 ± 2.62	3.7 ± 0.47
SB 60N	0-5	50.0 ± 3.69	17.3 ± 1.91
	5-10	37.4 ± 2.05	5.5 ± 0.44
	10-30	32.5 ± 0.81	3.0 ± 0.26
SB 120N	0-5	57.5 ± 6.32	20.7 ± 5.59
	5-10	37.8 ± 2.79	4.9 ± 1.24
	10-30	35.1 ± 1.58	2.4 ± 0.11

Table 3.5a: Daily N_2O flux data (g N ha^{-1} d^{-1}) from 2010 in soybean plots which received 120 kg N ha^{-1} fertilization in 2009.

Date	N_2O (g N ha^{-1} d^{-1})
5/24/2010	2.38
6/16/2010	17.03
6/24/2010	6.44
6/29/2010	7.08
7/9/2010	2.02
7/15/2010	4.71
7/22/2010	2.63
7/28/2010	28.57
8/4/2010	25.89
8/10/2010	2.97
8/19/2010	7.77
8/25/2010	9.79
9/10/2010	3.49
9/22/2010	3.47

Table 3.5b: Daily N_2O flux data (g N ha^{-1} d^{-1}) from 2011 in soybean plots which received 120 kg N ha^{-1} fertilization in 2009.

Date	N_2O (g N ha^{-1} d^{-1})
3/3/2011	41.90
6/22/2011	2.83
7/14/2011	1.40
7/27/2011	2.25
8/10/2011	1.06
8/23/2011	1.72
9/21/2011	0.47
10/6/2011	0.30
11/16/2011	0.00
12/20/2011	0.30

Table 3.6: Extractable NO_3^- in the rooting zone and below in SB plots, listed by the fertilization applied to the previous crop in 2009.

N-Rate	Depth (cm)	N-NO_3^- (kg N ha^{-1})
0 kg N ha-1	0-30	1.06
	30-150	0
60 kg N ha^{-1}	0-30	5.37
	30-150	0.86
120 kg N ha^{-1}	0-30	10.03
	30-150	0.68

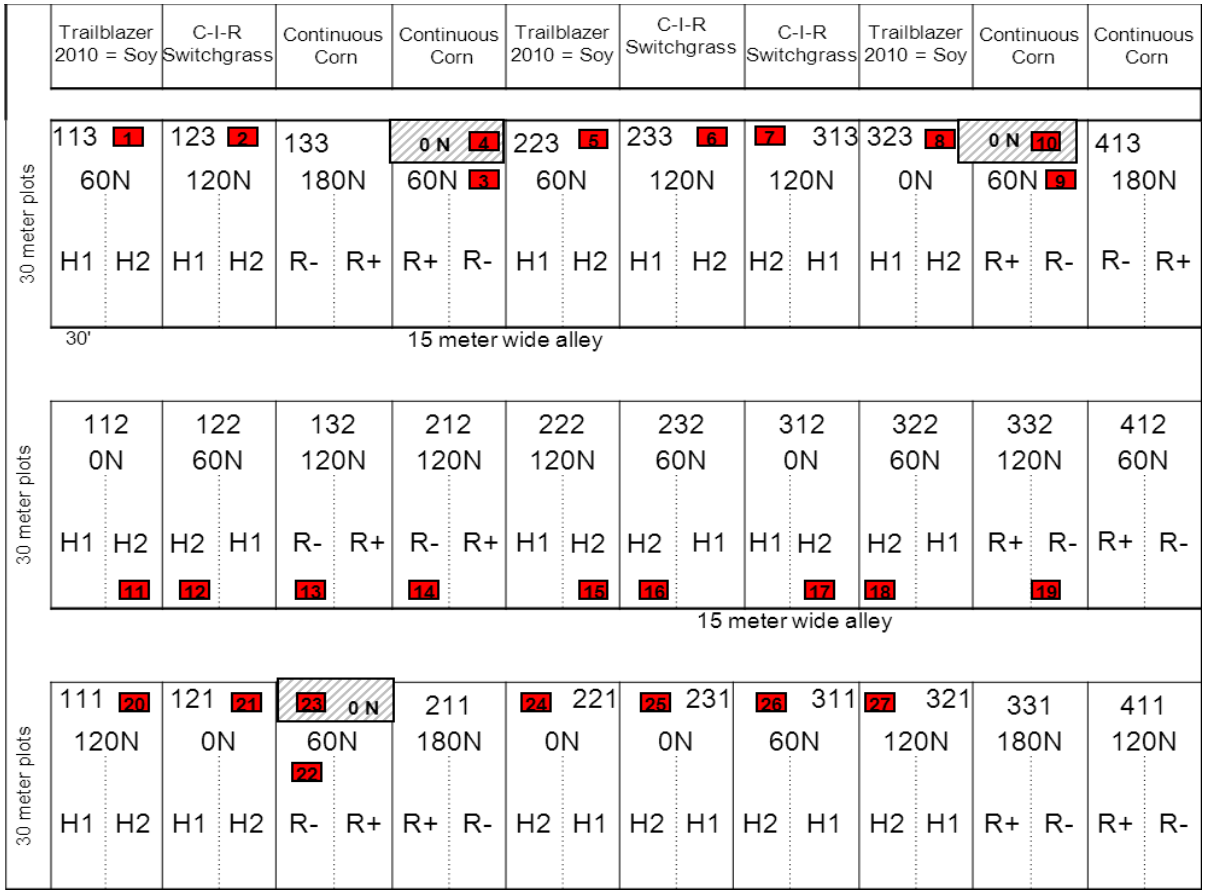

Figure 3.1: Plot plan of study site. Crop type is the main plot portion (main plots = columns). N-rate is applied at the sub-plot level, and harvest treatment is applied at the sub-sub-plot level (H1= Aug harvest, H2= Oct harvest; R+ = residue retained, R- = residue removed).

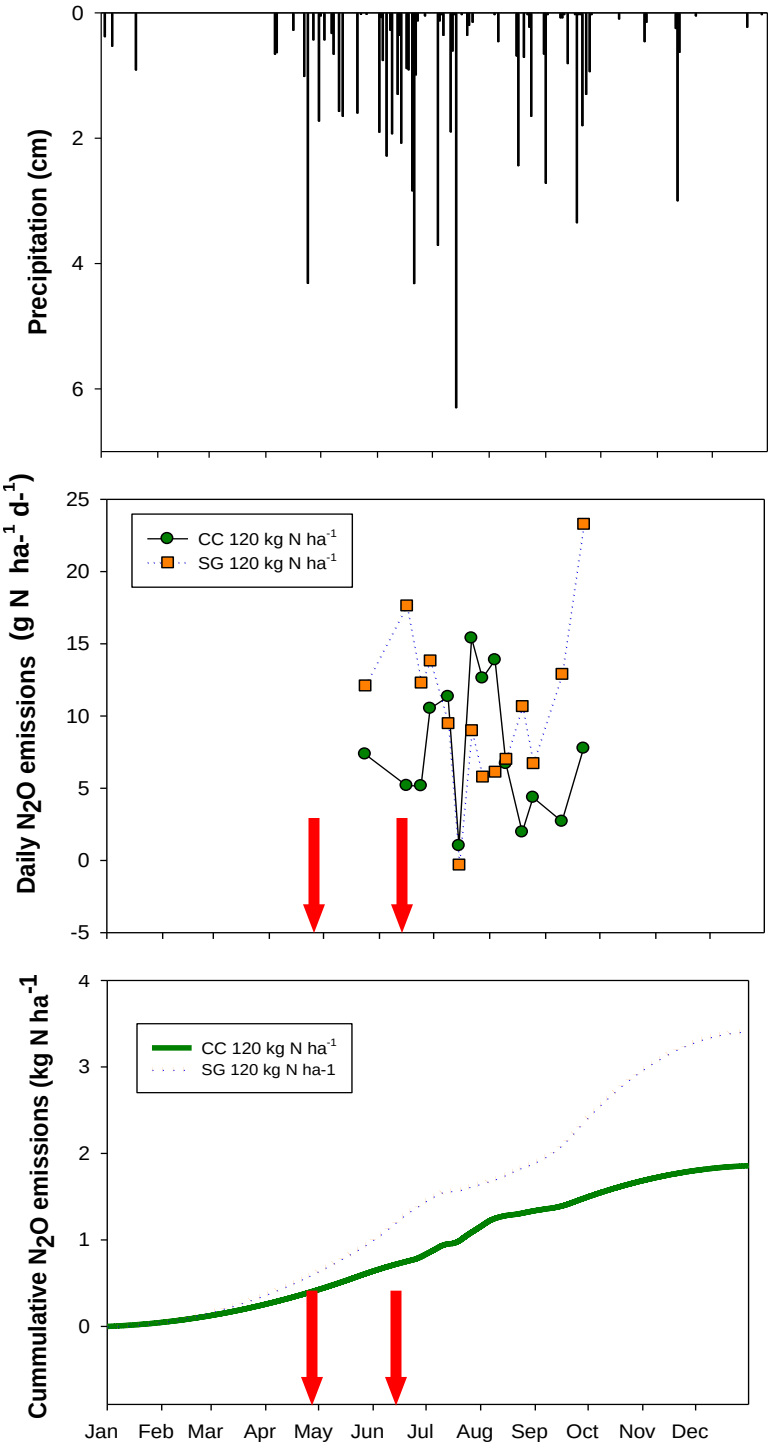

Figure 3.2: Precipitation (A), daily N_2O flux (B), and cumulative annual flux (C) from 120N CC and SG plots in 2010. Red arrows denote fertilization dates (April 29 for SG, June 16 for CC).

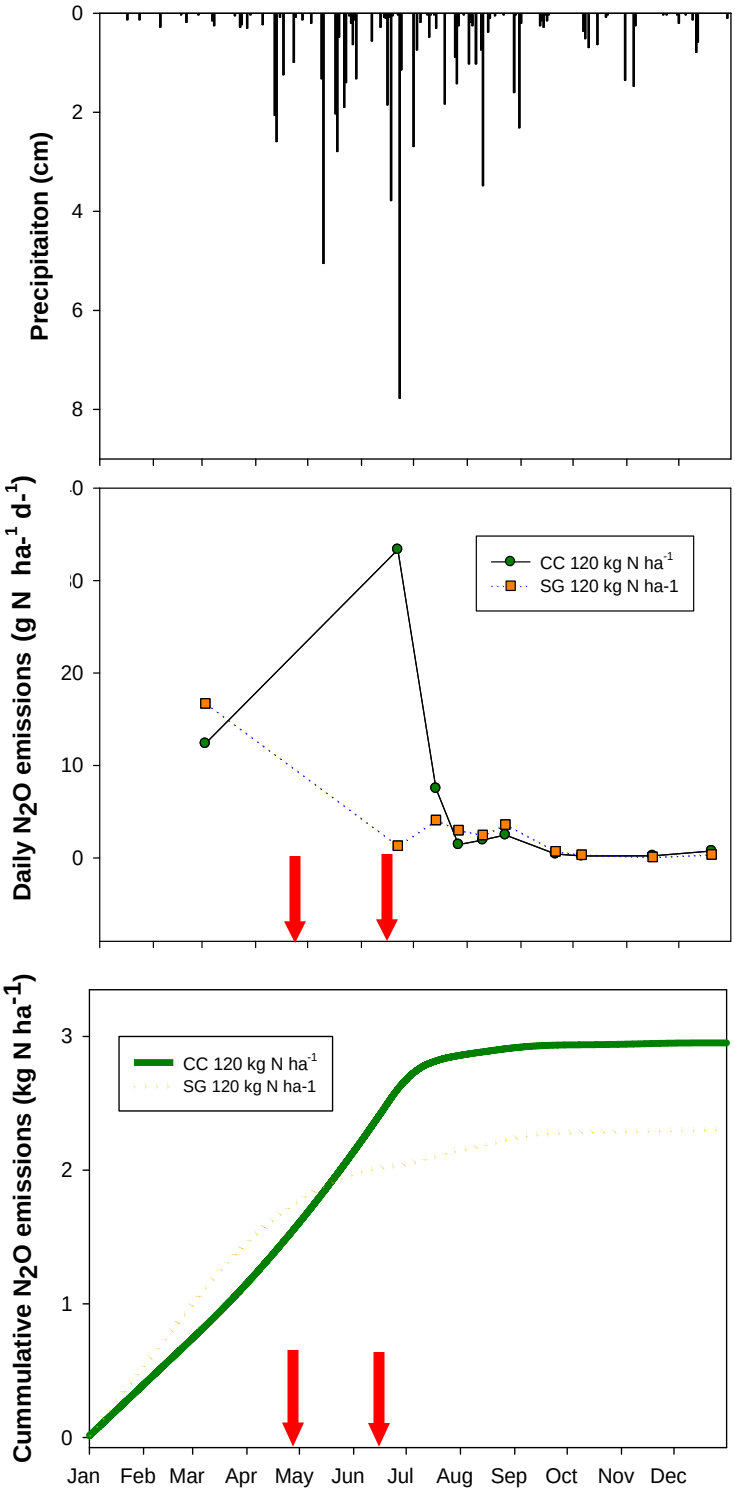

Figure 3.3: Precipitation (A), daily N_2O flux (B), and cumulative annual flux (C) from 120N CC and SG plots in 2011. Red arrows denote fertilization dates (April 29 for SG, June 14 for CC).

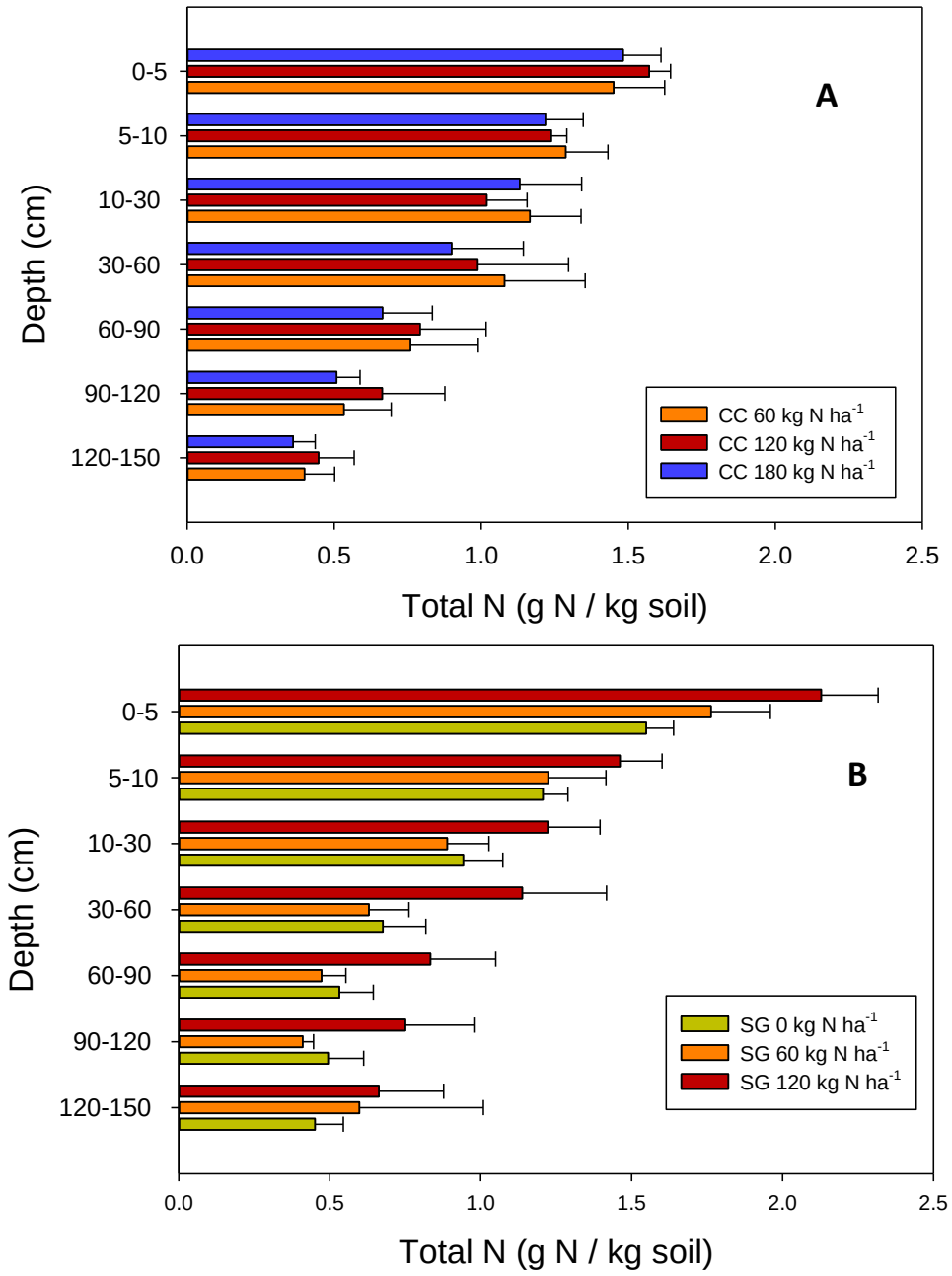

Figure 3.4: Total N in CC (A) and SG (B) plots by N-rate.

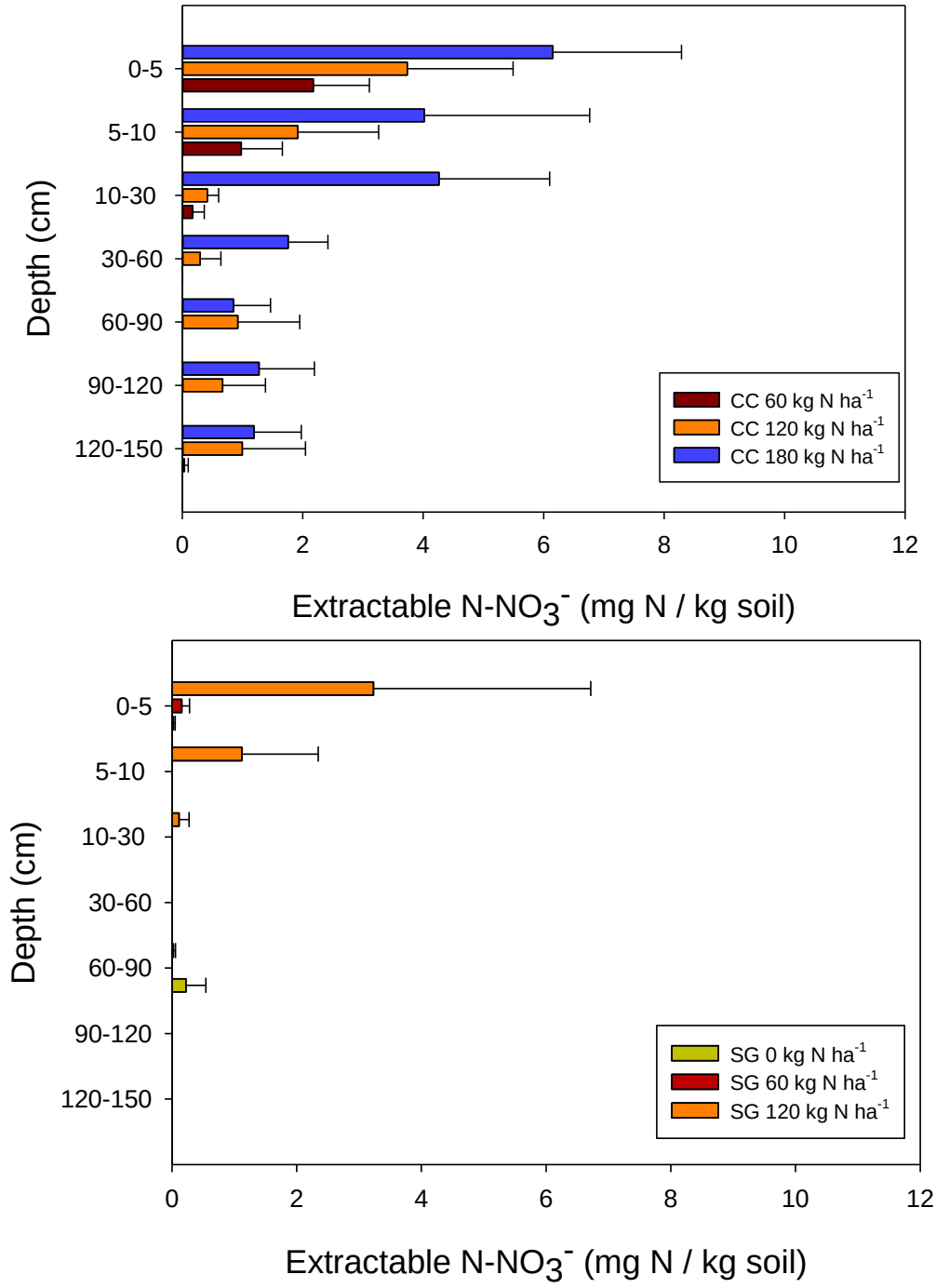

Figure 3.5: Extractable NO_3^- concentrations under CC (A) and SG (B) plots by N-rate.

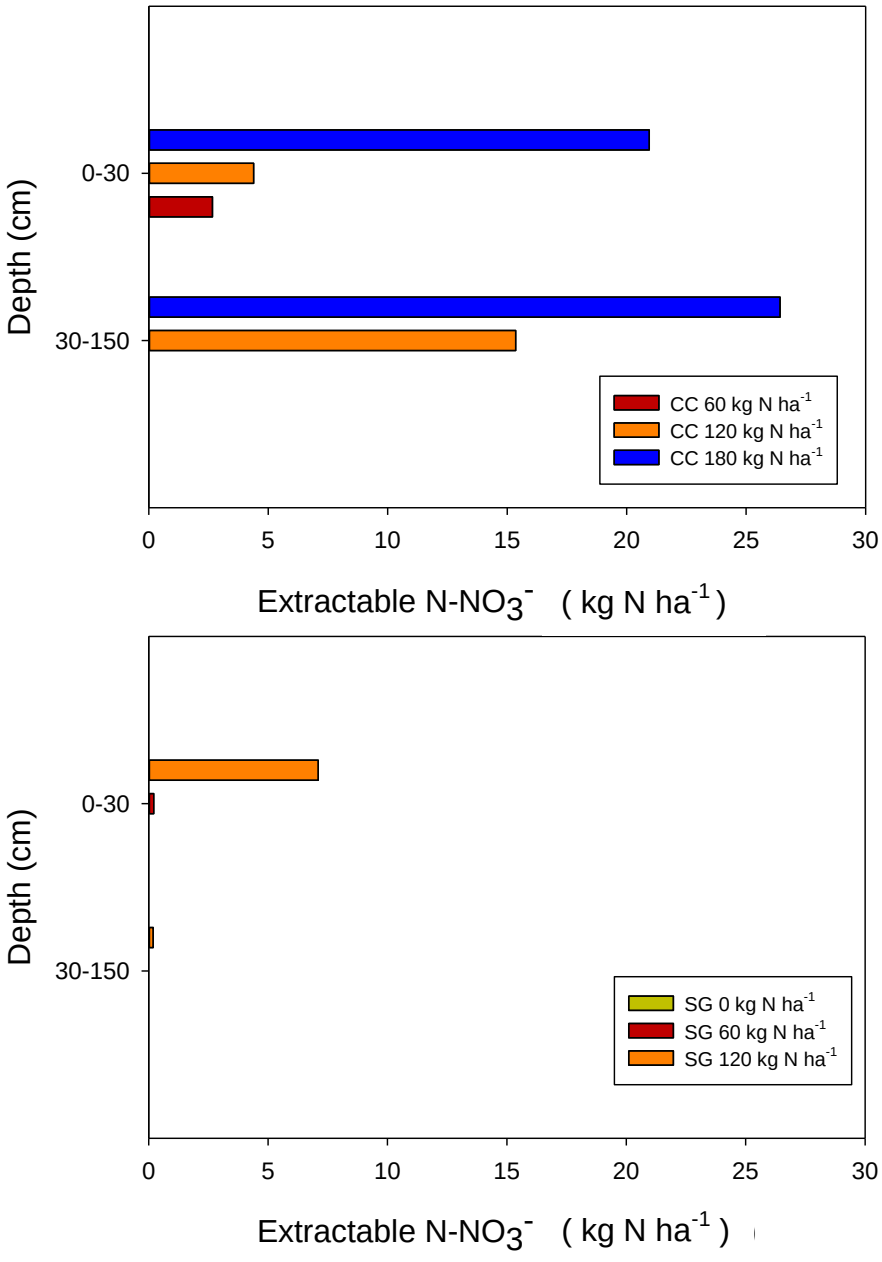

Figure 3.6: Extractable NO_3^- (kg N ha^{-1}) under CC (A) and SG (B) plots by N-rate

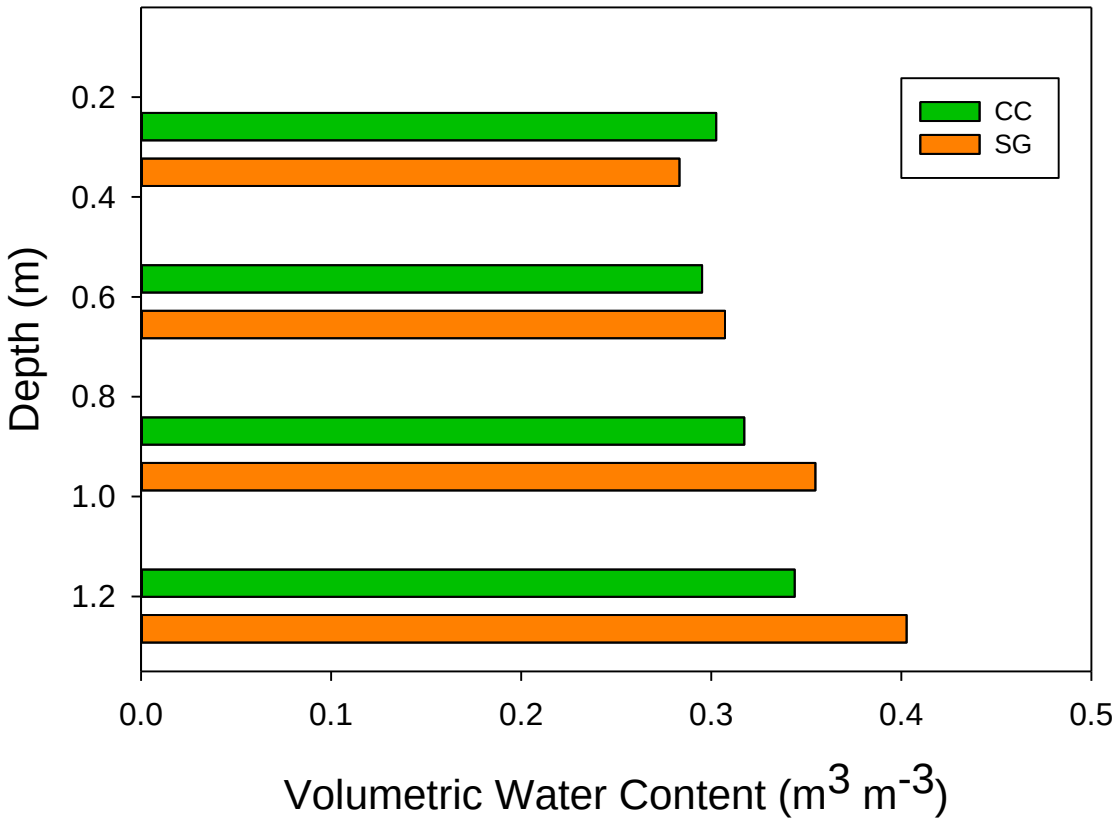

Figure 3.7: Volumetric water content under CC and SG plots. Depths are 1, 2, 3, and 4 ft (0.31, 0.61, 0.91, and 1.22 m).

Chapter 4:

Summary and Conclusions

Previous studies conducted at this site have shown management practices impacted crop yield over multiple years (Varvel et al., 2008) and soil organic carbon (SOC; Follett et al., 2012). The long-term management practices have also notably influenced soil microbial communities and soil nitrogen (N). The impacts of crop management on biomass-N removal and N_2O production varied by year, but N fertilization generally increased biomass and total emissions. This thesis focused on the potential impacts of corn (*Zea mays,* L.) and switchgrass (*Panicum virgatum,* L.) management practices on soil microbial ecology, crop N use, N_2O emissions, and soil N. This chapter summarizes the findings of chapters 2 and 3.

Residue Removal from Corn

Previous studies have shown that residue removal negatively impacts corn grain yield in Nebraska (Doran et al., 1984; Wilhelm et al., 1986; Varvel et al., 2008; Follett et al., 2012) and the influence of residue removal can last for years, even when removal is discontinued (Maskina et al., 1993). Follett et al. (2012) found that soils under corn had increases in SOC regardless of harvest treatment. In our study, harvest did not significantly influence total N or inorganic N, nor did it have a clear impact on microbial communities (but see Chapter 2 for a full discussion). However, residue removal did reduce soil organic matter (SOM) and particulate organic matter (POM) in corn plots.

Long term residue removal does not seem to impact larger pools of C and N at this site, but it does influence yield and more labile C pools.

Varvel et al. (2008) cautioned that residue removal should be considered carefully at a given site due to decreased yield potential. Our data show further cause to reconsider harvesting residue from corn plots on marginal soils. Residue removal increases potential for soil erosion, a special concern on marginal soils, and reduces SOM and POM. Producers may increase income by selling residue, but the difference in loss of income through reduced yields over time may negate any immediate economic benefit of residue removal. At this site, only about 50% of total residue available was removed each year. It may be possible that a lower removal rate may offer benefits without sacrificing grain yield or soil quality. Further research is needed to determine under what circumstances residue removal is appropriate on marginal soils in Nebraska.

Fertilization and Nitrate Leaching Potential from Corn

Increasing fertilization of corn at this site led to increased NO_3^- leaching potential and decreased soil microbial abundances and POM. At fertilizer application rates of 60 and 120 kg N ha^{-1} yr^{-1}, crop N balances suggested that corn crops relied on soil N mineralization to a degree. However, fertilizer additions in excess of 120 kg N ha^{-1} yr^{-1} did not result in increased amounts of N removed as aboveground biomass. In 2010, 120 kg N ha^{-1} was removed as aboveground biomass from 180N plots, and in 2011 132 kg N ha^{-1} was removed as aboveground biomass from 180N plots. Varvel et al. (2008) and Follett et al. (2012) reported that the total biomass yields from 120 and 180 kg N ha^{-1}

corn plots did not differ, and this was the case in 2010 and 2011 (biomass yield data not reported; see Chapter 3). Due to the time span covered by Vogel et al. (2008), Follett et al. (2012) and this study spanning from 1998-2007, and 2010-2011, it is clear that 180 kg N ha^{-1} is not beneficial for corn grain or residue biomass yields. The additional cost of applying 180 versus 120 kg N ha^{-1} is was not returned through increased biomass yield.

There are other reasons to consider the 180 kg N ha^{-1} fertilization disadvantageous. In corn plots, increasing N fertilization decreased microbial abundances, and 180 kg N ha^{-1} had the lowest abundances. There were also negatives correlations between N-rate and bacterial, actinomycete, and arbuscular mycorrhizal fungi (AMF) biomarkers, as well as with pH, and pH was lower in 180N plots. The NH_4^+ available in excess of crop N demand in 180 kg N ha^{-1} corn plots is likely lowering pH (Brady and Weil, 2004), and therefore influencing microbial abundance and activity (Wallenstein et al., 2006; Voroney et al., 2007). In this study, bacteria, and actinomycete had a negative correlation with pH across all cropping systems. Under continuous corn, total soil microbial biomass tended to decrease with increasing fertilization rate. Increasing fertilization also rapidly increased the potential for NO_3^- leaching to groundwater. At both 120 and 180 kg N ha^{-1}, there was more plant-available NO_3^- below the rooting zone than in the rooting zone. Though 120 kg N ha^{-1} is the accepted "best" N-rate at this site from a production standpoint (Varvel et al., 2008), corn plants are unable to capture all of the N added as fertilizer and it is being lost below the rooting zone before plants can utilize it. Plots receiving 180 kg N ha^{-1} have the highest NO_3^- concentrations at every depth at this site except 0-5 cm (discussed below). These plots also had more plant-available NO_3^- below the rooting zone than in it.

Some of the excess N in 180 kg N ha^{-1} plots is almost certainly being lost as N_2O. Plots receiving 120 kg N ha^{-1} had higher fluxes at this site than plots receiving less N fertilizer. We did not measure emissions from 180 kg N ha^{-1} plots, but there is an exponential relationship between N application and N_2O emissions (Zebarth et al., 2008; Snyder et al., 2009; Ma et al., 2010; Millar et al., 2010a; Hoben et al, 2011; Cavigelli and Parkin, 2012), so we hypothesize that there are even greater losses from 180 kg N ha^{-1} plots than from 120 kg N ha^{-1} plots.

There are several disadvantages to fertilizing corn above 120 kg N ha^{-1} at this site, and no discernible advantages. Increasing N fertilization to 180 kg N ha^{-1} decreased microbial biomass and POM while increasing NO_3^- leaching out of the root zone and likely increasing N_2O soil emissions, but offers no increase in biomass yield.

High Microbial Biomass and Low Nitrate Leaching from Switchgrass

Microbial biomass under switchgrass plots was higher than under corn or soybean plots. Both bacteria and fungal biomarkers were highest under switchgrass, but fungal abundance, particularly AMF markers, was two to three times more abundant under switchgrass. Soil organic matter, POM, total N, and total C were also highest under switchgrass plots in the top 30 cm of soil. The combination of these data show that soil quality under switchgrass plots is higher than under corn plots at this site.

Furthermore, though crop N balances showed fertilizer N was added in excess of biomass removal, soil total N and NO_3^- data showed that this N is not leaving the profile as NO_3^- and N_2O emissions are comparable to or less than emissions from corn plots.

Since this N is not being lost as N_2O or NO_3^-, and because total N was highest in switchgrass plots, we hypothesize that this fertilizer N not being removed as biomass exists in belowground biomass and in SOM. The corresponding increase in soil C pools under switchgrass plots along with increasing fertilization supports this hypothesis. Nitrogen fertilization has been shown to increase SOC in some systems (Paustian et al. 1997; Liebig et al., 2002; Varvel and Wilhelm, 2008), soil C does not necessarily follow N fertilization (Russell et al., 2005; Russell et al., 2009). At this site, N fertilization increased total C, SOM and POM in switchgrass plots but had no effect on total C or SOM and decreased POM in corn plots. Follett et al. (2012) found increases over time in SOC under corn plots at this site, but similarly did not find differences due to N fertilization.

Varvel et al. (2008) found that potential ethanol yield was equivalent in corn and switchgrass plots. There was no economic analysis conducted in this study, but assuming similar prices for feedstocks there is a strong incentive to choose switchgrass over corn production on marginal soils like the one used in this study. Fertilizer inputs were the same for corn and switchgrass, but because it is a perennial producers do not need to purchase switchgrass seed every year as they do for corn. Furthermore, there are several soil quality and environmental benefits to choosing switchgrass over corn for biofuel feedstock production. Producers will need to consider economic costs and benefits of the two crops, but from a soil quality perspective data from this site show greater benefits from switchgrass production than from continuous corn.

Soybean as an Interim Crop between Switchgrass Cultivars

Soybean plots had several soil quality traits that were clearly influenced by the previous switchgrass crop. The Trailblazer crop lasted from 1998 until 2009. Soil microbial data from soybean plots was more similar to switchgrass than corn. However, canonical discriminant analyses showed that soybean plots appeared to be changing and shifting away from the Trailblazer legacy. Extractable NO_3^- from soybean plots also mirrored switchgrass data. Below 5 cm, soybean plots had very low NO_3^- concentrations, like switchgrass. We attribute this NO_3^- trend to the former Trailblazer because NO_3^- leaching under corn-soybean rotations has been found to exceed leaching under continuous corn (Klocke et al., 1999; Zhu and Fox, 2003). Soil organic matter, POM, total N, and total C under soybean plots tended to be higher than in corn plots.

Follett et al. (2012) found no significant differences in SOC between Cave-In-Rock and Trailblazer cultivars. Even after two years under soybean management, the former Trailblazer plots were still very similar to Cave-In-Rock plots in many ways. There were notable differences in microbial communities between the two crop types. We hypothesize that this is due to the gradual decomposition of switchgrass roots and loss of above and belowground biomass.

Root decomposition is also a probable cause of high emissions from soybean plots in 2011. In 2011, the highest N_2O emissions occurred in soybean plots which received 120 kg N ha^{-1} in 2009. In the same year, soybean plots that received 0 or 60 kg N ha^{-1} in 2009 had the lowest N_2O emissions. Because aboveground biomass increased with increasing N fertilization in switchgrass plots, it is reasonable to assume that

belowground biomass also increased with fertilization. Trailblazer switchgrass plots that received 120 kg N ha^{-1} probably had higher root biomass than plots receiving 0 or 60 kg N ha^{-1}, and thus left more roots in the soil. The higher root biomass would translate to more C and N available for decomposition, and thus higher potential emissions. The majority of the cumulative emissions from soybean plots were measured during a single sample period in March. No samples were taken before May in 2010, so it is possible that similar emissions occurred in 2010 but were not measured.

Recommendations for Future Studies

There is limited knowledge about the influence of monoculture perennial grasses on microbial communities (Chaundhary et al., 2012). We look forward to further study of soil microbes at this site, and in particular are interested in how soybean plots will change when the new switchgrass cultivar is planted into them. While study at biofuel production sites is very useful, there are no studies to our knowledge that compare monoculture grass systems to native grasslands. We cannot completely understand how biofuel production impacts microbial communities unless we compare soils under production to soils under native vegetation.

Due to a lack of strong conclusions about corn versus switchgrass influence of N_2O emissions, it would be advantageous to continue study of greenhouse gas emissions at this site. The 180 kg N ha^{-1} treatment in corn was not sampled for any emissions because it could not directly be compared to switchgrass, but we suspect there are higher emissions from those plots than in the other treatments. After 2010, greenhouse gas

sampling protocol changed to increase sampling frequency and expand the number of months sampled (Jin, personal communication).

References

Brady, N.C., and Weil, R.R. (2004). *Elements of the nature and properties of soils.* Second Edition (266-315). Pearson Education Inc., Upper Saddle River, New Jersey, USA.

Chaudhary, D.R., Saxena, J., Lorenz, N., Dick, L.K., Dick, R.P. (2012). Microbial profiles of rhizosphere and bulk soil microbial communities of biofuel crops switchgrass (*Panicum virgatum* L.) and jatropha (*Jatropha curcas* L.). *Applied and Environmental Soil Science* 2012: Article ID 906864, 6 pages, 2012. doi:10.1155/2012/906864.

Doran, J.W., Wilhelm, W.W., Power, J.F. (1984). Crop residue removal and soil productivity with no-till corn, sorghum, and soybean. *Soil Science Society of America Journal* 48: 640-645.

Follett, R.F., Vogel, K.P., Varvel, G.E., Mitchell, R.B., Kimble, J. (2012). Soil carbon sequestration by switchgrass and no-till maize grown for bioenergy. *Bioenergy Research* 4(4): 1-10. doi:10.1007/s12155-012-9198-y.

de Klein C, Novoa RSA, Ogle S, Smith KA, Rochette P, Wirth TC, McConkey BG, Mosier AR, Rypdal K, Walsh M, Williams SA (2006) N2O emissions from managed soils, and CO2 emissions from lime and urea application. In *2006 IPC Guidelines for National Greenhouse Gas Inventories*. Eggleston S, Buendia L, Miwa K, Ngara T, Tanabe K (eds). Institute for Global Environmental Strategies, Japan, p. 11.1-11.54.

Klocke, N.L., Watts, D.G., Schneekloth, J.P., Davison, D.R., Todd, R.W. (1999). Nitrate leaching in irrigated corn and soybean in a semi-arid climate. *Biological Systems Engineering: Papers and Publications* 42(6): 1621-1630.

Leibig, M.A., Varvel, G.E., Doran, J.W., Wienhold, B.J. (2002). Crop sequence and nitrogen fertilization effects on soil properties in the western Corn Belt *Soil Science Society of America Journal* 66: 596-601.

Maskina, M.S., Power, J.F., Doran, J.W., Wilhelm, W.W. (1993). Residual effects of no-till crop residues on corn yield and nitrogen uptake. *Soil Science Society of America Journal* 57: 1555-1560.

Paustian, K., H.P. Collins, and E.A. Paul. (1997). Management controls on soil carbon. In Paul, E.A. (Ed.) Soil organic matter in temperate agroecosystems: Longer-term experiments in North America. 15-49. CRC Press: Boca Raton, FL, USA.

Russell, A.E., Laird, D.A., Parkin, T.B., Mallarino, A.P. (2005). Impact of nitrogen fertilization and cropping system on carbon sequestration in Midwestern Mollisols. *Soil Science Society of America Journal* 69: 413-422.

Russell, A.E., Cambardella, C.A., Laird, D.A., Jaynes, D.B., Meek, D.W. (2009). Nitrogen fertilizer effects on soil carbon balances in Midwestern U.S. agricultural systems. *Ecological Applications* 19: 1102-1113.

Spalding, R. F., and Kitchen, L. A. (1988). Nitrate in the intermediate vadose zone beneath irrigated cropland. *Ground Water Monitoring & Remediation* 8(2): 89-95.

Stein, L.Y., and Yung, Y.L. (2003). Production, isotopic composition, and atmospheric fate of biologically produced nitrous oxide. *Annual Review of Earth Planet Science* 31: 329-356. doi: 10.1146/annurev.earth.31.110502.080901.

Varvel, G.E., Vogel, K.P., Mitchell, R.B., Follett, R.F., Kimble, J.M. (2008). Comparison of corn and switchgrass on marginal soils for bioenergy. *Biomass and Bioenergy* 32: 18-21.

Voroney, R.P. (2007). The soil habitat. In Paul, E.A. (Ed.) *Soil Microbiology, Ecology, and Biochemistry*. Third Edition (25-49). Academic Press: Burlington, MA, USA.

Wallenstein, M.D., McNulty, S., Fernandez, I.J., Boggs, J., Schlesinger, W.H. (2006). Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management* 222: 459-468.

Wilhelm,W.W., J.W. Doran, and J.F. Power. (1986). Corn and soybean yield response to crop residue management under no-tillage production systems. *Agronomy Journal* 78: 184-189.

Zhu, Y., and Fox, R.H. (2003). Corn-soybean rotation effects on nitrate leaching. *Agronomy Journal* 95: 1028-1033.