

BIOFUEL CROPPING SYSTEM IMPACTS ON SOIL C, MICROBIAL COMMUNITIES
AND N₂O EMISSIONS

by

ANDREW R. MCGOWAN

B.S., Kansas State University, 2010

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2015

Abstract

Substitution of cellulosic biofuel in place of gasoline or diesel could reduce greenhouse gas (GHG) emissions from transportation. However, emissions of nitrous oxide (N₂O) and changes in soil organic carbon (SOC) could have a large impact on the GHG balance of cellulosic biofuel, thus there is a need to quantify these responses in cellulosic biofuel crops. The objectives of this study were to: (i) measure changes in yield, SOC and microbial communities in potential cellulosic biofuel cropping systems (ii) measure and characterize the temporal variation in N₂O emissions from these systems (iii) characterize the yield and N₂O response of switchgrass to N fertilizer and to estimate the costs of production. Sweet sorghum, photoperiod-sensitive sorghum, and miscanthus yielded the highest aboveground biomass (20-32 Mg ha⁻¹). The perennial grasses sequestered SOC over 4 yrs, while SOC stocks did not change in the annual crops. Root stocks were 4-8 times higher in the perennial crops, suggesting greater belowground C inputs. Arbuscular mycorrhizal fungi (AMF) abundance and aggregate mean weight diameter were higher in the perennials. No consistent significant differences were found in N₂O emissions between crops, though miscanthus tended to have the lowest emissions. Most N₂O was emitted during large events of short duration (1-3 days) that occurred after high rainfall events with high soil NO₃⁻. There was a weak relationship between IPCC Tier 1 N₂O estimates and measured emissions, and the IPCC method tended to underestimate emissions. The response of N₂O to N rate was nonlinear in 2 of 3 years. Fertilizer induced emission factor (EF) increased from 0.7% at 50 kg N ha⁻¹ to 2.6% at 150 kg N ha⁻¹. Switchgrass yields increased with N inputs up to 100-150 kg N ha⁻¹, but the critical N level for maximum yields decreased each year, suggesting N was being applied in excess at higher N rates. Yield-scaled costs of production were minimized at 100 kg N ha⁻¹ (\$70.91 Mg⁻¹). Together, these results show that crop selection

and fertilizer management can have large impacts on the productivity and soil GHG emissions
biofuel cropping systems.

BIOFUEL CROPPING SYSTEM IMPACTS ON SOIL C, MICROBIAL COMMUNITIES
AND N₂O EMISSIONS

by

ANDREW R. MCGOWAN

B.S., Kansas State University, 2010

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2015

Approved by:

Major Professor
Charles W. Rice

Copyright

ANDREW R. MCGOWAN

2015

Abstract

Substitution of cellulosic biofuel in place of gasoline or diesel could reduce greenhouse gas (GHG) emissions from transportation. However, emissions of nitrous oxide (N₂O) and changes in soil organic carbon (SOC) could have a large impact on the GHG balance of cellulosic biofuel, thus there is a need to quantify these responses in cellulosic biofuel crops. The objectives of this study were to: (i) measure changes in yield, SOC and microbial communities in potential cellulosic biofuel cropping systems (ii) measure and characterize the temporal variation in N₂O emissions from these systems (iii) characterize the yield and N₂O response of switchgrass to N fertilizer and to estimate the costs of production. Sweet sorghum, photoperiod-sensitive sorghum, and miscanthus yielded the highest aboveground biomass (20-32 Mg ha⁻¹). The perennial grasses sequestered SOC over 4 yrs, while SOC stocks did not change in the annual crops. Root stocks were 4-8 times higher in the perennial crops, suggesting greater belowground C inputs. Arbuscular mycorrhizal fungi (AMF) abundance and aggregate mean weight diameter were higher in the perennials. No consistent significant differences were found in N₂O emissions between crops, though miscanthus tended to have the lowest emissions. Most N₂O was emitted during large events of short duration (1-3 days) that occurred after high rainfall events with high soil NO₃⁻. There was a weak relationship between IPCC Tier 1 N₂O estimates and measured emissions, and the IPCC method tended to underestimate emissions. The response of N₂O to N rate was nonlinear in 2 of 3 years. Fertilizer induced emission factor (EF) increased from 0.7% at 50 kg N ha⁻¹ to 2.6% at 150 kg N ha⁻¹. Switchgrass yields increased with N inputs up to 100-150 kg N ha⁻¹, but the critical N level for maximum yields decreased each year, suggesting N was being applied in excess at higher N rates. Yield-scaled costs of production were minimized at 100 kg N ha⁻¹ (\$70.91 Mg⁻¹). Together, these results show that crop selection

and fertilizer management can have large impacts on the productivity and soil GHG emissions
biofuel cropping systems.

Table of Contents

List of Figures	xii
List of Tables	xvii
Acknowledgements	xix
Chapter 1 - Introduction.....	1
Biofuel and GHG Emissions.....	2
Nitrous Oxide Emissions	5
Soil Carbon Sequestration	7
Biofuel Crops	9
Study Objectives	11
References.....	12
Chapter 2 - Soil organic carbon, aggregates and microbial community structure in biofuel crops	22
Abstract.....	22
Introduction.....	23
Materials and Methods.....	26
Study Site	26
Root Stocks	27
Microbial Lipid Analysis	29
Water Stable Aggregates.....	30
Soil Organic Carbon Stocks.....	31
Statistical Analysis.....	32
Results.....	33

Aboveground Biomass	34
Root Density and Stocks	35
Microbial Lipids.....	36
Water-Stable Aggregate Distribution.....	38
Soil Organic Carbon Stocks	40
Discussion.....	41
Conclusions.....	46
References.....	47
Chapter 3 - Nitrous oxide emissions from biofuel cropping systems.....	70
Abstract.....	70
Introduction.....	71
Materials and Methods.....	74
Study Site	74
Nitrous Oxide Measurements	76
Ancillary Measurements	77
IPCC Tier 1 Estimates	78
Statistical Analysis.....	80
Results.....	81
Biomass and Grain Yield	81
Soil Nitrogen.....	82
Seasonal Emissions.....	83
Total Annual N ₂ O and Biomass-Scaled N ₂ O.....	84
IPCC Estimates	85

Discussion.....	86
Conclusions.....	91
References.....	92
Chapter 4 - Impact of nitrogen rate on switchgrass yield, production costs and N ₂ O emissions	106
Abstract.....	106
Introduction.....	107
Materials and Methods.....	111
Study Site	111
Nitrous Oxide Measurements	112
Ancillary Measurements	113
Cost Analysis	114
Statistical Analysis.....	114
Results.....	115
Soil Nitrogen.....	115
Seasonal N ₂ O Emissions.....	116
Total N ₂ O Emissions.....	117
Biomass Yield.....	118
Costs of Production.....	118
Discussion.....	119
Conclusions.....	123
References.....	125
Chapter 5 - Summary	143
Appendix A - Chapter 2.....	148

Appendix B - Chapter 3 154

List of Figures

- Figure 2.1. Root density by crop and depth taken in fall 2013. Error bars show 95% confidence intervals of the median. Sorghum is abbreviated with “S.” 54
- Figure 2.2. Total root stocks from 0-120 cm depth. Error bars represent 95% confidence interval of the median. Bars with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.” 55
- Figure 2.3. Effect of depth averaged over crop on PLFA concentration of total PLFA, gram positive bacteria, gram negative bacteria and fungi. Error bars represent 95% confidence intervals. Bars of the same group with different letters are significantly different ($p < 0.05$).
..... 56
- Figure 2.4. Effect of crop averaged over depth on total PLFA concentration. Error bars represent standard error. Bars with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.” 57
- Figure 2.5. Effect of crop averaged over depth on the 18:1 ω 6,9 PLFA biomarker (fungi) concentration. Error bars represent the 95% confidence interval of the median. Bars with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.” 58
- Figure 2.6. Effect of crop and depth on the 16:1 ω 5 NLFA AM biomarker (arbuscular mycorrhizae fungi) concentration. Error bars represent the 95% confidence interval of the median. Bars of the same depth with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.” 59
- Figure 2.7. Effect of crop and depth on the PLFA fungi:bacteria ratio. Error bars represent the 95% confidence interval of the median. Bars of the same depth with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.” 60

Figure 2.8. Water-stable macroaggregates (>250 μm) by crop at 0-5 cm. Error bars represent standard error. Bars of the same aggregate size with different letters are significantly different (p < 0.05). Sorghum is abbreviated with “S.” 61

Figure 2.9. The effect of crop on water-stable aggregate mean weight diameter averaged over depth. Error bars represent standard error. Bars with different letters are significantly different (p < 0.05). Model variance was allowed to vary between crops, resulting in a larger standard error in miscanthus than in big bluestem and switchgrass. This resulted in no significant difference between miscanthus and big bluestem, while switchgrass and big bluestem were significantly different. Sorghum is abbreviated with “S.” 62

Figure 2.10. The effect of water-stable aggregate size on SOC concentration in aggregates, averaged over crop and depth. Error bars represent standard error. Bars with different letters are significantly different (p < 0.05). 63

Figure 2.11. Effect of crop on the percent of bulk soil organic carbon contained within macroaggregates (>250 μm) averaged over depth. Error bars represent standard error. Bars with different letters are significantly different (p < 0.05). Sorghum is abbreviated with “S.” 64

Figure 3.1. Soil nitrate (a) and ammonium (b) by crop at 0 – 5 cm soil depth in 2011 – 2013. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum. Error bars indicate 95% confidence intervals..... 98

Figure 3.2. Mean daily N₂O flux by crop, percent water-filled pore space (%WFPS), daily precipitation and average daily temperature observed in 2011 – 2012. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum. Labels “urea” and “DAP” indicated date urea and the 2 applications of diammonium

phosphate were applied. “Corn” indicates the date that corn was planted and “sorghum” indicates the planting date of photoperiod sorghum and soybean. Rainfall data recorded at nearby meteorological station. 99

Figure 3.3. Mean daily N₂O flux by crop, percent water-filled pore space (%WFPS), daily precipitation and average daily temperature observed in 2012 – 2013. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum. Label “urea, corn” indicate date that urea was applied and corn was planted. “Sorghum” indicates date photoperiod-sensitive sorghum and soybean were planted. Rainfall data recorded at nearby meteorological station. 100

Figure 3.4. Mean daily N₂O flux by crop, percent water-filled pore space (%WFPS), daily precipitation and average daily temperature observed in 2013 – 2014. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum. “Corn”, “sorghum” and “soybean” indicate dates of corn, photoperiod sorghum and soybean planting, respectively. “MAP” and “urea” indicate dates of monoammonium phosphate and urea application. Rainfall data recorded at nearby meteorological station. 101

Figure 3.5. (a) Cumulative annual N₂O emissions in 2011–2013 by crop. (b) Percent of total annual N₂O emissions emitted by crop. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum. 102

Figure 3.6. Direct N₂O emissions estimated by IPCC Tier 1 method by crop using the default emission factor (1.0%) and uncertainty range (0.3% and 3.0%). Measured emissions are shown for comparison. Error bars represent 95% confidence intervals of the mean. 103

Figure 4.1. Figure 1. Soil nitrate and ammonium by N fertilizer rate from 2012-2014. Black lines show effect of sampling date averaged over time when interaction between date and N rate

was not significant. Error bars indicate 95% confidence intervals. 'a' and 'b' are nitrate and ammonium at 0-5 cm, respectively. 'c' and 'd' are nitrate and ammonium at 5-15 cm. 130

Figure 4.2. Mean daily N₂O flux by N fertilizer rate, percent water-filled pore space, daily precipitation, and average daily temperature from 2012-2013. Label "urea" indicates the date that urea was applied. Rainfall data recorded at nearby meteorological station. 131

Figure 4.3. Mean daily N₂O flux by N fertilizer rate, percent water-filled pore space, daily precipitation, and average daily temperature from 2013-2014. Label "urea" indicates the date that urea was applied. Rainfall data recorded at nearby meteorological station. 132

Figure 4.4. Mean daily N₂O flux by N fertilizer rate, percent water-filled pore space, daily precipitation, and average daily temperature in 2014 growing season. Label "urea" indicates the date that urea was applied. Rainfall data recorded at nearby meteorological station. .. 133

Figure 4.5. (a) Cumulative annual N₂O emissions for 2012-2014 by N fertilizer rate. (b) Percent of total annual N₂O emissions emitted by N fertilizer rate. 134

Figure 4.6. Total annual N₂O emissions in 2012-2014 over N fertilizer rate. Points and error bars represent the mean and standard error of emissions by N rate and year. Lines represent the predicted values of the best-fit model in each year..... 135

Figure 4.7. Effect of N fertilizer rate on switchgrass biomass yield (Mg dry matter ha⁻¹) in 2012-2013. Points and error bars represent the mean and standard error of emissions by N rate and year. Lines represent the predicted values of the best-fit model in each year..... 136

Figure 4.8. Quantity of N removed in switchgrass in 2012-2014. Points and error bars represent the mean and standard error of emissions by N rate and year. Lines represent the predicted values of the best-fit model in each year..... 137

Figure A.1. Sampling scheme root stock sampling in annual row crops (left) and switchgrass and big bluestem (right). c_1 , c_2 and c_3 indicate locations where soil cores were taken. A_1 , A_2 and A_3 indicate the areas assumed to be represented by c_1 , c_2 and c_3 , respectively. l_1 and l_2 represent the diameter of the grass plant and the length from the nearest plant, respectively.
..... 148

Figure A.2. Monthly average air temperatures at study site. 149

Figure B.1. Linear regression of IPCC Tier 1 N_2O estimates with 1% emission factor and measured N_2O emissions (blue line). Dashed line through the intercept with slope of 1 provided for reference. 154

List of Tables

Table 1.1. Summary of fuel categories under RFS2. GHG requirements are reductions in LCA GHG emissions relative to gasoline or diesel.	20
Table 1.2. Summary of biofuel pathways approved by the EPA (Renewable Fuel Standard, 2010).	21
Table 2.1. Annual aboveground biomass production.	65
Table 2.2. ANOVA results for Phospholipid fatty acid and water-stable aggregate data.	66
Table 2.3. ANOVA p-values for average annual change in soil organic carbon stocks by depth.	67
Table 2.4. Soil organic carbon stocks in 2009, 2014 and average annual change in soil organic carbon stocks (0-15 cm).	68
Table 2.5. Pearson correlation coefficients among soil organic carbon, root density, arbuscular mycorrhizae, saprophytic fungi, fungi:bacteria ratio and aggregate mean-weight diameter. Analyses were performed across all crop systems and depths (0-30 cm) unless stated otherwise.	69
Table 3.1. Annual aboveground biomass and grain yields	104
Table 3.2. Total annual N ₂ O emissions and biomass-scaled N ₂ O emissions.	105
Table 4.1. Mean emissions factor (EF) by N fertilizer rate.	138
Table 4.2. Best-fit models of switchgrass yield as influenced by N fertilizer rate, by year.	139
Table 4.3. Mean biomass N concentration by year and by N fertilizer rate.	140
Table 4.4. Prices and other parameter values used in cost estimation.	141
Table 4.5. Summary of 11 year costs of production of switchgrass at different N fertilizer rates.	142
Table A.1. Cultivars planted over the duration of the study.	150

Table A.2. Seeding rates and fertilizer rates over the duration of the study.....	151
Table A.3. Growing Season Precipitation and 30-year average at the study site. Measurements are from a meteorological station less than 1 km from the study site.....	152
Table A.4. ANOVA and contrast p-values for crop yield.	153
Table B.1. Average monthly temperature, April – October and October precipitation in Manhattan, KS. Left column shows 1981-2010 30 year averages.....	155

Acknowledgements

I thank my major professor, Dr. Charles Rice, for his guidance, support, and for exposing me to the many facets of science. I am also grateful to my committee members, Dr. Peter Tomlinson, Dr. Scott Staggenborg, and Dr. Donghai Wang for their guidance throughout my dissertation. I extend my gratitude to Dr. Mary Rezac, for giving me the opportunity to be a graduate fellow in the K-State Renewable Bioenergy IGERT.

I am grateful to Dr. Jeffery Williams for his help with the cost analysis. I also thank Dr. Kraig Roozeboom, Dr. Lucas Haag, Kyle Shroyer, and Jason Waite for their invaluable help managing the Long-Term Bioenergy Plots. I extend my thanks to Vernon Schaffer and the North Farm staff for their training and support.

I greatly appreciate the technical support and friendship of past and current members of the Soil Microbial Ecology Laboratory, especially Dr. Paul White, Dr. Maysoon Mikha, Dr. Miguel Arango, Dr. Habib Diop, Dr. Priscilla Mfombep, Leonardo Bastos, Stewart Watts, Edwin Akley, Noortje Notenbaert, Johanie Rivera-Zayas, Tiffany Carter, and Jerry Hsiao. I extend a big thanks to Meaghan Dustin, Elias Yishak, Breanna Leake, Brett Sattazahn, and James Inouye for their contributions to my research as summer REU participants. I also thank the many undergraduate technicians who put in countless hours collecting and analyzing data for this project.

Finally, I am grateful to my family for their patience, support, and encouragement during my studies, especially to my wife, Yan Wei, and to my parents, Bill and Joby.

Chapter 1 - Introduction

Global climate change is one of the most pressing issues of the 21st century. Between 1880 and 2012, global average surface temperature has increased by 0.85°C and the last 3 decades have each been warmer than any since 1850 (IPCC, 2014). This warming effect is mainly attributed to positive changes in radiative forcing from anthropogenic greenhouse gas (GHG) emissions, of which carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are the largest contributors (Myhre et al., 2013). Concentrations of atmospheric CO₂, CH₄, and N₂O have all increased substantially since pre-industrial (40%, 150%, 20%, respectively) and have reached levels not seen in at least 800,000 years (IPCC, 2014). The potential impacts of climate change are far-reaching and could include negative impacts on surface and groundwater availability, food security, human health, and economic growth (IPCC, 2014). Without mitigation efforts, warming by 2100 could rise 3.7-4.8°C above pre-industrial levels. Increases above 4°C will likely result in widespread impacts on threatened systems, species extinction, food security, tripping of tipping points, and limited potential for future adaptation (IPCC, 2014). Therefore, implementing strategies to mitigate GHG emissions is essential.

Taking steps to reduce global GHG emissions will require addressing emissions from the transportation sector. The transportation sector contributed 14% of anthropogenic GHG emissions in 2010 (7.0 Gt CO₂-eq), and made up 28% of U.S. GHG emissions (IPCC, 2014; USEPA, 2014). Since 1970, transportation sector emissions have more than doubled, increasing faster than any other energy-use sector. Road vehicles account for 80% of this increase (Sims et al., 2014). A few key measures to mitigate emissions from road vehicles include investing in infrastructure for public transport, walking, and cycling; lowering the energy intensity of vehicles by improving vehicle and engine performance; and reducing the carbon intensity of

fuels by utilizing natural gas, bio-methane or sustainable biofuels, electricity, and hydrogen (Sims et al., 2014). Of these solutions, biofuels are attractive because they can be implemented in the near- to mid-future, are more energy dense than other alternative fuels, and are compatible with the existing petroleum fuel infrastructure (Sims et al., 2014).

Biofuel and GHG Emissions

Biofuels are any organic material that is used directly as a solid, liquid or gaseous fuel to produce energy, usually through the combustion of the material (Bessou et al., 2011).

Transportation biofuels are frequently classified as 1st and 2nd generation biofuel. First generation biofuels only use a specific portion of the plant, usually one rich in sucrose, starch or oils. Starch and sucrose are fermented to produce ethanol while oils are converted into biodiesel through transesterification (Bessou et al., 2011). Second generation biofuels utilize lignocellulosic biomass to produce fuel. A number of possible pathways exist for converting lignocellulosic biomass into liquid fuels. Strategies to produce second generation biofuels can broadly be categorized by those that involve gasification to produce syngas, pyrolysis or liquifaction to produce bio-oils, or hydrolysis to produce aqueous sugars and lignin (Huber et al., 2006). These intermediary products can then be converted into a variety of liquid fuels. A common pathway that is being used in most commercial-scale biofuel facilities in the U.S. is hydrolysis of lignocellulosic biomass followed by fermentation to produce ethanol (Balan et al., 2013).

In order to promote energy independence, promote the development of clean, renewable fuels and reduce GHG emissions, Congress passed the Energy Independence and Security Act of 2007 (U.S. Congress, 2007). Title II, subtitle A of the EISA, which is commonly referred to as the Renewable Fuel Standard 2 (RFS2), establishes mandated volumes of biofuel to be blended

with motor fuels by oil companies. The mandated volumes are to increase from 34 billion liters per year in 2009 up to 136 billion liters per year in 2022. Even though biofuels are a promising alternative fuel source, substantial quantities of GHGs are emitted throughout the biofuel production cycle. To ensure that these emissions do not offset the mitigative capacity of biofuel production, RFS2 defines four categories of biofuel, each with its own mandated volume: renewable fuel, advanced biofuel, biomass-based diesel and cellulosic biofuel. These different fuel categories are distinguished by the feedstock used to produce the fuel as well as by reductions in GHG emissions from the production and use of the fuel, relative to GHG emissions from fossil fuel-based gasoline or diesel (Table 1.1-1.2). This mandate highlights the need to accurately estimate GHG emissions associated with the production and use of biofuels to ensure that they are reducing GHG emissions, relative to conventional fossil fuels. This stipulation of the EISA also creates the need for reliable methods of estimation.

Life Cycle Assessment (LCA) is an environmental accounting method designed for the evaluation of product chains and has been widely used to quantify GHG emissions from different biofuel systems (von Blottnitz and Curran, 2007; Bessou et al., 2011). Life Cycle Assessment of biofuels generally include GHG emissions from the production and transportation of the feedstock, conversion of the feedstock to biofuel, fuel transportation and distribution, and tailpipe emissions (USEPA, 2010). Accounting for emissions from these steps requires modeling, data inputs and system assumptions. Unfortunately, these often differ greatly, causing LCA results to be highly variable (Cherubini, 2010; Bessou et al., 2011).

Much of the difficulty in performing LCA of biofuel systems is due to the difficulty of applying the LCA method to the agricultural phase of biofuel production (Bessou et al., 2011). In a review of biofuel LCA studies, Adler et al. (2012) found the agricultural phase of production

contributed 46-68% of total GHG emissions. Because agricultural systems depend on environmental factors like climate and soils, agricultural data sets are time and space dependent, making it difficult to find high-quality data that is representative of a particular agricultural system (Bessou et al., 2011). Two components of agricultural systems that are especially important, but difficult to predict, are N₂O emissions from agricultural soils and changes in soil organic carbon (SOC) due to land use change.

Many studies have demonstrated the potentially large effect N₂O can have on the GHG balance of biofuel. Adler et al. (2007) conducted an LCA on 1st and 2nd generation biofuel produced from various crops and found that N₂O emissions represented the largest GHG source in each case. Kim and Dale (2009) conducted an LCA on soybean-based biodiesel and corn based ethanol in the US Corn Belt and found N₂O emissions to contribute 13-57% and 11-37% of the total GHG emissions for biodiesel and ethanol, respectively. Klemedtsson and Smith (2011) used field measurements of N₂O in a LCA of ethanol from wheat and found N₂O emissions to contribute 18-57% of GHG emissions. In a review of studies on GHG emissions from direct land-use change in bioenergy systems, Don et al. (2012) found that, on average, direct N₂O emissions contribute 27% of the GHG emissions of biofuels from food crops, with contributions ranging between 5-80%.

Fewer LCA studies have attempted to account for GHG emissions or uptake from changes in SOC stocks (Bessou et al., 2011; Goglio et al., 2015). Currently there is no consensus on how to account for SOC changes in LCA, including what time span over which to consider changes, how to account for the reversibility of changes, and what to use as a reference scenario (Bessou et al., 2011; Goglio et al., 2015). Studies that have included changes in SOC stocks have found potentially large impacts of biofuel GHG emissions. Wang et al. (2012) found that

accounting for changes in SOC reduced the GHG savings from using corn-based ethanol instead of gasoline 10%, but increased savings 13% for miscanthus-based cellulosic ethanol. Adler et al. (2015) found SOC changes due to corn stover residue removal with and without addition of high-lignin fermentation product caused GHG changes of +45.94 to -13.38 g CO₂-eq MJ⁻¹ in the LCA GHG emissions of ethanol, which are significant considering the LCA GHG emissions of gasoline were 93 CO₂-eq MJ⁻¹. These results highlight the large impact both N₂O and SOC can have on LCA GHG emissions of biofuel.

Nitrous Oxide Emissions

Nitrous oxide is a potent GHG with a global warming potential (GWP) 298 times that of CO₂, and has the third largest radiative forcing of anthropogenic GHGs (Myhre et al., 2013). Nitrous oxide emissions from agricultural soils account for 4.7% of total GHG emissions and 74.8% of total N₂O emissions in the U.S. (USEPA, 2014). Applications of synthetic fertilizer are an important source of N₂O, accounting for 22.2% of emissions from U.S. agricultural soils (USEPA, 2014).

The potentially large contribution of N₂O to GHG emissions and the uncertainty of N₂O emissions suggests that care should be taken in selecting the N₂O inputs into LCA models of biofuel. Currently, many studies rely on simple emission factors, such as the IPCC Tier 1 factor (Smeets et al., 2009; Don et al., 2012), which assumes that 1% of soil and fertilizer N is lost as N₂O (De Klein et al., 2006). The emissions factors are based on a large body of evidence showing N₂O emissions from soils increase with increasing N inputs (Bouwman et al., 2002; Stehfest and Bouwman, 2006). Various factors have been used in LCAs, and the selection of the N₂O emission factor can sometimes determine whether or not the GHG balance of biofuel is

higher or lower than that of conventional fossil-based fuels (Crutzen et al., 2008; Mosier et al., 2009).

Reports have criticized the use of emission factors as estimates of direct N₂O emissions (Smeets et al., 2009; Klemetsson and Smith, 2011; Reijnders and Huijbregts, 2011; Don et al., 2012). Emission factors such as the IPCC Tier 1 estimate ignore many factors that control N₂O emissions. Because many biofuel LCAs are performed for specific crops at regional scales or smaller, ignoring these factors could ignore critical differences in N₂O emissions that arise from variations in soil, climate and local management practices. For these reasons, some researchers promote the use of process-based models like DAYCENT or DNDC to estimate N₂O emissions (Adler et al., 2007; Kim and Dale, 2009; Smeets et al., 2009). These process-based models can provide N₂O estimates that account for differences in the various drivers behind N₂O production (Del Grosso et al., 2006; Li, 2000).

For many first-generation biofuel feedstocks, such as corn and soybean, there is a large body of data from field measurements of N₂O emissions which can be used as a basis of comparison in modeling approaches in LCA. Unfortunately, there are few field studies measuring N₂O from soils for 2nd generation biofuel feedstock production (Don et al., 2012). This makes it difficult to predict N₂O emissions from these crops and to know the accuracy of various N₂O models to predict N₂O emissions. More field studies measuring N₂O emissions from cellulosic biofuel crops are needed to understand biofuels potential to mitigate GHG emissions in the transportation sector.

An additional issue with the use of emission factors is that N₂O emissions may increase non-linearly with increasing N inputs. In a meta-analysis including 233 site-years of N rate studies, Shcherbak et al. (2014) found positive changes in EF with increasing N inputs in N-

fixing crops, upland grain crops, rice and perennial grass/forage crops. In another meta-analysis, Kim et al. (2013) found only 5 datasets where N₂O emissions increased linearly with increasing N inputs, while 16 datasets experienced exponential increases, and 2 datasets were best explained using a hyperbolic model. A non-linear response of N₂O emissions to N inputs could result in N₂O losses much larger than would be predicted by a single EF, especially if N inputs exceed plant requirements. It would also result in much higher GHG emissions in agricultural systems where N inputs exceed plant needs. These losses could be especially important in the context of biofuel production, where N inputs are necessary to maximize biomass production but resulting N₂O emissions could turn biofuels into a net source of GHG emissions (Crutzen et al., 2008; Smith et al., 2012). More field studies are needed to clarify the relationship between N inputs and N₂O emissions, especially in biofuel cropping systems.

Soil Carbon Sequestration

The global soil C pool contains 2500 Pg of C, which is more than the atmospheric and biotic pools combined (Lal, 2005). Soil C sequestration is one of the primary GHG mitigation strategies in the agricultural sector, contributing around 89% of the 5500-6000 MG CO₂-eq yr⁻¹ mitigation potential in agriculture (Smith et al., 2008). Management of biofuel cropping systems to increase SOC could substantially increase the mitigative capacity of biofuels. Depending on crop choice and management, SOC could increase, acting as a sink for atmospheric CO₂, or decrease, acting as a source of CO₂ emissions. Changes in SOC stocks result from an imbalance between organic C inputs into soil and outputs, primarily through microbial decomposition. Soil C sequestration can occur by increasing C inputs into the soil, increasing the residence time of SOC in the soil, or both (Jastrow et al., 2007). Increasing C inputs could be achieved through

management or crop selection that results in increased biomass production, particularly belowground biomass or increased crop residue retention.

Crop choice and management can also influence the residence time of SOC through changes in soil aggregate formation and stability. Carbon incorporated into soil aggregates, especially microaggregates, is protected from decomposition by physical impediment of decomposers and by creating O₂ and moisture conditions that limit decomposer activity (Six et al., 2002; Jastrow et al., 2007). Conditions that encourage the stabilization of macroaggregates (>250 µm diameter) increase the formation and stabilization of microaggregates within macroaggregates and the physical protection of SOC (Six et al., 2002). Macroaggregate formation and stabilization is facilitated by fine roots and hyphae of arbuscular mycorrhizal fungi (AMF), which enmesh soil particles (Jastrow et al., 2007). Selecting crops with high belowground biomass and high affinity for AMF, such as perennial grasses, could aid in the stabilization of aggregates and SOC.

Consideration of SOC inputs and stability is especially important in the context of cellulosic biofuel because these cropping systems require the removal of large quantities of aboveground biomass that would otherwise be returned to the soil. Many studies have observed negative effects of annual row crop residue removal on a variety of soil properties, including SOC and soil aggregates (Wilhelm et al., 2004; Hammerbeck et al., 2012; Osborne et al., 2014; Villamil et al., 2015). Such declines in SOC during crop production will negatively impact the GHG footprint of biofuel. Perennial grasses like switchgrass (*Panicum virgatum* L.) or miscanthus (*Miscanthus x giganteus*) are attractive alternatives to annual row crops because their higher belowground biomass could help maintain SOC while still producing high quantities of aboveground biomass. Given the numerous positive and negative impacts biofuel cropping

systems could have on SOC cycling, more field studies are needed to examine the long-term impacts of biofuel crops on SOC stocks and belowground C dynamics.

Biofuel Crops

There are a variety of bioenergy crops being considered for use in the Great Plains Region. In addition to conventional annual row crops like corn (*Zea mays* L.) and grain sorghum (*Sorghum bicolor* (L.) Moench), several annual dedicated bioenergy crops may be grown for cellulosic biofuel. Photoperiod-sensitive sorghums are sorghum cultivars that do not initiate reproductive flowering in most of the U.S. and can produce high amounts of lignocellulosic biomass. Photoperiod-sensitive sorghum commonly yields 20-30 Mg ha⁻¹ with yields reported as high as 35 Mg ha⁻¹ (Propheter et al., 2010; Maughan et al., 2012a). Sweet sorghum cultivars can produce quantities of cellulosic biomass similar to photoperiod sensitive cultivars. In addition, they accumulate high levels of fermentable carbohydrates (9-24% sugar), which can be directly converted in to ethanol (Regassa and Wortmann, 2014). In addition to high yields, advantages of photoperiod-sensitive and sweet sorghums include production practices similar to grain sorghum, which is already a commonly grown crop in Kansas, and is drought tolerant compared too many other biofuel crops (USDOE, 2011).

Several perennial crops are being considered as potential sources for cellulosic feedstock because of their low input requirements, low management needs, and ability to grow on marginal soils (Mitchell et al., 2008; USDOE, 2011). Switchgrass is a perennial warm-season grass that is native to the prairies of the U.S. Average yields in the U.S. are 8.7 Mg ha⁻¹ and 12.9 Mg ha⁻¹ for upland and lowland ecotypes, respectively (Wullschleger et al., 2010). Big bluestem (*Andropogon gerardii* Vitman) is another perennial warm-season grass native to the U.S. Biomass production of big bluestem ranges from 2-9 Mg ha⁻¹ (Hall et al., 1982; Barnhart, 1989;

Bartholomew et al., 1995; Tober et al., 2008). An additional advantage of both switchgrass and big bluestem is that well-established seed industries already exist. Miscanthus (*Miscanthus x giganteus*) is a sterile hybrid of a perennial warm-season grass originating in Japan. Sterility reduces concerns about miscanthus becoming an invasive species, but requires that stands are established by planting rhizomes, which makes establishment costs high compared to perennials like switchgrass and big bluestem (USDOE, 2011). Miscanthus has the potential to produce high amounts of biomass. Yields of 14-40 Mg ha⁻¹ are common from mature stands in the U.S but can yielded as high as 60 Mg ha⁻¹ (Heaton et al., 2008; Maughan et al., 2012b; Arundale et al., 2014).

Though the fertilizer requirements of perennial grass species are generally lower than annual crops, there is debate over the appropriate levels of fertilizer, particularly N, required for high yielding stands. Some studies have observed high yields in miscanthus receiving no N, and there have been reports of biological N in miscanthus systems (Heaton et al., 2008; Davis et al., 2010). However, reviews of miscanthus and switchgrass field trials have found that nitrogen fertilizer may be required to replenish removed nitrogen and maintain yields over the life of a stand (Miguez et al., 2008; Arundale et al., 2014). Switchgrass can also produce high yields without N inputs, but optimizing yields requires N additions (Mitchell et al., 2008). In a meta-analysis of switchgrass productivity, Wulschleger et al. (2010) found both upland and lowland switchgrass cultivars responded significantly to N additions with optimum rates at 100 kg N ha⁻¹. However, switchgrass response to N is highly variable and depends on soil conditions, climate, cultivar productivity and management (Mitchell et al., 2008). Many studies have observed yields in unfertilized stands to be similar to those receiving N inputs (Wulschleger et al., 2010; Jung et al., 2011; Kering et al., 2012; Sadeghpour et al., 2014; Wile et al., 2014). These findings

illustrate need for more studies to characterize switchgrass and miscanthus response to N across a range of conditions.

Study Objectives

Chapter 2: Changes in soil organic carbon, soil aggregates and microbial community structure in bioenergy crops.

The objectives of this study were to compare (i) biomass yield; (ii) carbon stocks; and long-term changes in SOC; and (iii) factors related to belowground C inputs and physical protection of SOC in annual and perennial bioenergy cropping systems.

Chapter 3: Nitrous oxide emissions from bioenergy cropping systems.

The objectives of this study were to: (i) measure N₂O emissions and yields of potential cellulosic biofuel cropping systems; and (ii) characterize the temporal variations in N₂O emissions in these cropping systems.

Chapter 4: Impact of nitrogen rate on switchgrass yield, production costs and N₂O emissions.

The objectives of this study were to: i) characterize the yield and N₂O response of switchgrass to N fertilizer; and ii) determine the costs of production of switchgrass across N fertilizer rates.

References

- Adler, P.R., S.J.D. Grosso, and W.J. Parton. 2007. Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems. *Ecol. Appl.* 17:675-691.
- Adler, P.R., J.G. Mitchell, G. Pourhashem, S. Spatari, S.J. Del Grosso, and W.J. Parton. 2015. Integrating biorefinery and farm biogeochemical cycles offsets fossil energy and mitigates soil carbon losses. *Ecol. Appl.* 25:1142-1156.
- Adler, P.R., S.J. Del Grosso, D. Inman, R.E. Jenkins, S. Spatari, and Y. Zhang. 2012. Chapter 12 - mitigation opportunities for life-cycle greenhouse gas emissions during feedstock production across heterogeneous landscapes. p. 203-219. *In* M.A.L.J.F.F. Follett (ed.) *Managing agricultural greenhouse gases*. Academic Press, San Diego.
- Arundale, R.A., F.G. Dohleman, E.A. Heaton, J.M. McGrath, T.B. Voigt, and S.P. Long. 2014. Yields of *Miscanthus x giganteus* and *Panicum virgatum* decline with stand age in the Midwestern USA. *GCB Bioenergy* 6:1-13.
- Balan, V., D. Chiaramonti, and S. Kumar. 2013. Review of US and EU initiatives toward development, demonstration, and commercialization of lignocellulosic biofuels. *Biofuels Bioproducts & Biorefining-Biofpr* 7:732-759.
- Barnhart, S.K. 1989. Warm-season grasses for hay and pasture. Rep. PM-569. Iowa State Univ., Ames, IA.
- Bartholomew, H.M., R.M. Sulc, R. Hendershot, and J. Cline. 1995. Perennial warm season grasses for Ohio. Rep. AGF-022-95. Ohio State Univ. Ext. Dept. of Hort. and Crop Sci., Columbus, OH.
- Bessou, C., F. Ferchaud, B. Gabrielle, and B. Mary. 2011. Biofuels, greenhouse gases and climate change. A review. *Agron. Sustain. Dev.* 31:1-79.

- Bouwman, A.F., L.J.M. Boumans, and N.H. Batjes. 2002. Emissions of N₂O and NO from fertilized fields: Summary of available measurement data. *Global Biogeochem. Cycles* 16:1058.
- Cherubini, F. 2010. GHG balances of bioenergy systems - overview of key steps in the production chain and methodological concerns. *Renew. Energy* 35:1565-1573.
- Crutzen, P.J., A.R. Mosier, K.A. Smith, and W. Winiwarter. 2008. N₂O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. *Atmos. Chem. Phys.* 8:389-395.
- Davis, S., W. Parton, F. Dohleman, C. Smith, S. Grosso, A. Kent, and E. DeLucia. 2010. Comparative biogeochemical cycles of bioenergy crops reveal nitrogen-fixation and low greenhouse gas emissions in a *Miscanthus × giganteus* agro-ecosystem. *Ecosys.* 13:144-156.
- De Klein, C., R. Novoa, S. Ogle, K. Smith, P. Rochette, T. Wirth, B. McConkey, A. Mosier, and K. Rypdal. 2006. N₂O emissions from managed soils, and CO₂ emissions from lime and urea application. *In* 2006 IPCC guidelines for national greenhouse gas inventories. IPCC.
- Del Grosso, S.J., W.J. Parton, A.R. Mosier, M.K. Walsh, D.S. Ojima, and P.E. Thornton. 2006. DAYCENT national-scale simulations of nitrous oxide emissions from cropped soils in the United States. *J. Environ. Qual.* 35:1451-1460.
- Don, A., B. Osborne, A. Hastings, U. Skiba, M.S. Carter, J. Drewer, H. Flessa, A. Freibauer, N. Hyvonen, M.B. Jones, G.J. Lanigan, U. Mander, A. Monti, S.N. Djomo, J. Valentine, K. Walter, W. Zegada-Lizarazu, and T. Zenone. 2012. Land-use change to bioenergy production in Europe: Implications for the greenhouse gas balance and soil carbon. *GCB Bioenergy* 4:372-391.

- Goglio, P., W.N. Smith, B.B. Grant, R.L. Desjardins, B.G. McConkey, C.A. Campbell, and T. Nemecek. 2015. Accounting for soil carbon changes in agricultural life cycle assessment (LCA): A review. *J. Clean. Prod.* 104:23-39.
- Hall, K.E., J.R. George, and R.R. Riedl. 1982. Herbage dry-matter yields of switchgrass, big bluestem, and indiangrass with N-fertilization. *Agron. J.* 74:47-51.
- Hammerbeck, A.L., S.J. Stetson, S.L. Osborne, T.E. Schumacher, and J.L. Pikul Jr. 2012. Corn residue removal impact on soil aggregates in a no-till corn/soybean rotation. *Soil Sci. Soc. Am. J.* 76:1390-1398.
- Heaton, E.A., F.G. Dohleman, and S.P. Long. 2008. Meeting US biofuel goals with less land: The potential of miscanthus. *Glob. Change Biol.* 14:2000-2014.
- Huber, G.W., S. Iborra, and A. Corma. 2006. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chem. Rev.* 106:4044-4098.
- IPCC. 2014. Climate change 2014: Synthesis report. p. 151. *In* R.K., Pachauri, and L.A. Meyer (eds.) Contribution of working groups I, II, and III to the fifth assessment report of the IPCC. IPCC, Geneva, Switzerland.
- Jastrow, J.D., J.E. Amonette, and V.L. Bailey. 2007. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. *Clim. Change* 80:5-23.
- Jung, J.Y., R. Lal, J.D. Jastrow, and D.D. Tyler. 2011. Nitrogenous fertilizer effects on soil structural properties under switchgrass. *Agric. Ecosys. Environ.* 141:215-220.
- Kering, M.K., T.J. Butler, J.T. Biermacher, and J.A. Guretzky. 2012. Biomass yield and nutrient removal rates of perennial grasses under nitrogen fertilization. *BioEnergy Res.* 5:61-70.

- Kim, D., G. Hernandez-Ramirez, and D. Giltrap. 2013. Linear and nonlinear dependency of direct nitrous oxide emissions on fertilizer nitrogen input: A meta-analysis. *Agric. Ecosys. Environ.* 168:53-65.
- Kim, S., and B.E. Dale. 2009. Regional variations in greenhouse gas emissions of biobased products in the United States-corn-based ethanol and soybean oil. *Int. J. Life Cycle Assess.* 14:540-546.
- Klemedtsson, A.K., and K.A. Smith. 2011. The significance of nitrous oxide emission due to cropping of grain for biofuel production: A Swedish perspective. *Biogeosciences* 8:3581-3591.
- Lal, R. 2005. World crop residues production and implications of its use as a biofuel. *Environ. Int.* 31:575-584.
- Li, C.S. 2000. Modeling trace gas emissions from agricultural ecosystems. *Nutr. Cycl. Agroecosys.* 58:259-276.
- Maughan, M., T. Voigt, A. Parrish, G. Bollero, W. Rooney, and D.K. Lee. 2012a. Forage and energy sorghum responses to nitrogen fertilization in central and southern Illinois. *Agron. J.* 104:1032-1040.
- Maughan, M., G. Bollero, D.K. Lee, R. Darmody, S. Bonos, L. Cortese, J. Murphy, R. Gaussoin, M. Sousek, D. Williams, L. Williams, F. Miguez, and T. Voigt. 2012b. *Miscanthus giganteus* productivity: The effects of management in different environments. *GCB Bioenergy* 4:253-265.
- Miguez, F.E., M.B. Villamil, S.P. Long, and G.A. Bollero. 2008. Meta-analysis of the effects of management factors on *miscanthus* × *giganteus* growth and biomass production. *Agricultural and Forest Meteorol.* 148:1280-1292.

- Mitchell, R., K.P. Vogel, and G. Sarath. 2008. Managing and enhancing switchgrass as a bioenergy feedstock. *Biofuels Bioproducts & Biorefining-Biofpr* 2:530-539.
- Mosier, A., P. Crutzen, K. Smith, and W. Winiwarter. 2009. Nitrous oxide's impact on net greenhouse gas savings from biofuels: Life-cycle analysis comparison. *Int. J. Biotech.* 11:60-74.
- Myhre, G., D. Shindell, F.-. Breon, w. Collins, J. Fuglestedt, J. Huang, D. Koch, J.-. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura, and H. Zhang. 2013. Anthropogenic and natural radiative forcing. p. 659. *In* T.F., Stocker, D. Qin, G.-. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P.M. ,Midgley (eds.) *The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change.* Cambridge University Press, Cambridge, U.K.
- Osborne, S.L., J.M.F. Johnson, V.L. Jin, A.L. Hammerbeck, G.E. Varvel, and T.E. Schumacher. 2014. The impact of corn residue removal on soil aggregates and particulate organic matter. *BioEnergy Res.* 7:559-567.
- Propheter, J.L., S.A. Staggenborg, X. Wu, and D. Wang. 2010. Performance of annual and perennial biofuel crops: Yield during the first two years. *Agron. J.* 102:806-814.
- Regassa, T.H., and C.S. Wortmann. 2014. Sweet sorghum as a bioenergy crop: Literature review. *Biomass Bioenerg.* 64:348-355.
- Reijnders, L., and M.A.J. Huijbregts. 2011. Nitrous oxide emissions from liquid biofuel production in life cycle assessment. *Curr. Opin. Environ. Sustain.* 3:432-437.
- Renewable Fuel Standard. 2010. Code of Federal Regulations Title 40, chapter I, subchapter C, part 80, subpart M.

- Sadeghpour, A., L.E. Gorlitsky, M. Hashemi, S.A. Weis, and S.J. Herbert. 2014. Response of switchgrass yield and quality to harvest season and nitrogen fertilizer. *Agron. J.* 106:290-296.
- Shcherbak, I., N. Millar, and G.P. Robertson. 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N₂O) emissions to fertilizer nitrogen. *Proc. Natl. Acad. Sci. U. S. A.* 111:9199-9204.
- Sims, R., R. Schaeffer, F. Creutzig, X. Cruz-Nunez, M. D'Agosto, D. Dimitriu, M.J. Figueroa Meza, L. Fulton, S. Kobayashi, O. Lah, A. McKinnon, P. Newman, M. Ouyang, J.J. Schauer, D. Sperling, and G. Tiwari. 2014. Transport. p. 599. *In* O., Edenhofer, R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlomer, C. von Stechow, T. Zwickel, and J.C. Minx (eds.) *Climate change 2014: Mitigation of climate change. Contribution of working group III to the fifth assessment report of the intergovernmental panel on climate change.* Cambridge University Press, Cambridge, UK.
- Six, J., R.T. Conant, E.A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant Soil* 241:155-176.
- Smeets, E.M.W., L.F. Bouwmanw, E. Stehfest, D.P. van Vuuren, and A. Posthuma. 2009. Contribution of N₂O to the greenhouse gas balance of first-generation biofuels. *Glob. Change Biol.* 15:1-23.
- Smith, K.A., A.R. Mosier, P.J. Crutzen, and W. Winiwarter. 2012. The role of N₂O derived from crop-based biofuels, and from agriculture in general, in earth's climate. *Phil. Trans. Royal Soc. B-Biol. Sci.* 367:1169-1174.

Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, O. Sirotenko, M. Howden, T. McAllister, G. Pan, V. Romanenkov, U. Schneider, S. Towprayoon, M. Wattenbach, and J. Smith. 2008. Greenhouse gas mitigation in agriculture. *Philos. Trans. R. Soc. B-Biol. Sci.* 363:789-813.

Stehfest, E., and L. Bouwman. 2006. N₂O and NO emission from agricultural fields and soils under natural vegetation: Summarizing available measurement data and modeling of global annual emissions. *Nutr. Cycl. Agroecosys.* 74:207-228.

Tober, D.W., W. Duckwitz, and M. Knudson. 2008. Big bluestem biomass trials in North Dakota, South Dakota, and Minnesota. USDA, NRSC Plant Materials Center, Bismark, ND.

U.S. Congress. 2007. Energy independence and security act of 2007. 201-210.

USDOE. 2011. U.S. billion-ton update: Biomass supply for a bioenergy and bioproducts industry. Rep. ORNL/TM-2011/224. Oak Ridge National Laboratory, Oak Ridge, TN.

USEPA. 2014. Inventory of U.S. greenhouse gas emissions and sinks: 1990 - 2012. Rep. EPA 430-R-14-003.

USEPA. 2010. Renewable fuel standard program (RFS2) regulatory impact analysis.

Villamil, M.B., J. Little, and E.D. Nafziger. 2015. Corn residue, tillage, and nitrogen rate effects on soil properties. *Soil Till. Res.* 151:61-66.

von Blottnitz, H., and M.A. Curran. 2007. A review of assessments conducted on bio-ethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. *J. Clean Prod.* 15:607-619.

Wang, M., J. Han, J.B. Dunn, H. Cai, and A. Elgowainy. 2012. Well-to-wheels energy use and greenhouse gas emissions of ethanol from corn, sugarcane and cellulosic biomass for US use. *Environ. Res. Ltts.* 7:045905.

- Wile, A., D.L. Burton, M. Sharifi, D. Lynch, M. Main, and Y.A. Papadopoulos. 2014. Effect of nitrogen fertilizer application rate on yield, methane and nitrous oxide emissions from switchgrass (*Panicum virgatum* L.) and reed canarygrass (*Phalaris arundinacea* L.). *Can. J. Soil Sci.* 94:129-137.
- Wilhelm, W.W., J.M.F. Johnson, J.L. Hatfield, W.B. Voorhees, and D.R. Linden. 2004. Crop and soil productivity response to corn residue removal: A literature review. *Agron. J.* 96:1-17.
- Wullschleger, S.D., E.B. Davis, M.E. Borsuk, C.A. Gunderson, and L.R. Lynd. 2010. Biomass production in switchgrass across the United States: Database description and determinants of yield. *Agron. J.* 102:1158-1168.

Table 1.1. Summary of fuel categories under RFS2. GHG requirements are reductions in LCA GHG emissions relative to gasoline or diesel.

Category	Feedstock	GHG Requirement	2022 Volume (L)
Renewable fuel†	Any renewable biomass	20%	136 billion
Advanced biofuel	Any renewable biomass except corn starch	50%	79 billion
Cellulosic biofuel‡	Cellulose, hemicellulose, lignin from renewable biomass	60%	61 billion
Biomass-based diesel	Any renewable biomass used to make biodiesel	50%	3.8 billion

† Advanced biofuel, cellulosic biofuel and biomass-based diesel are subclasses of renewable fuel and contribute to the renewable fuel mandated volume

‡ Cellulosic biofuel is a subclass of advanced biofuel

Table 1.2. Summary of biofuel pathways approved by the EPA (Renewable Fuel Standard, 2010).

Renewable fuel – 20% GHG Reduction
<ul style="list-style-type: none">• Biofuel from plants that existed or commenced construction before December 19, 2007• Ethanol or biobutanol from corn starch at new facilities using advanced efficiency technologies• Ethanol from grain sorghum at dry mill facilities using natural gas for process energy
Advanced biofuels – 50% GHG Reduction
<ul style="list-style-type: none">• Ethanol from sugarcane using fermentation• Naphtha and liquefied petroleum gas from camelina oil using hydroheating• Biodiesel and renewable diesel from soybean oil, annual covercrops, algal oil, biogenic waste oils/fats/greases, non-food grade corn oil or camelina oil produced by trans-esterification or hydroheating in facilities that co-process biomass and petroleum• Ethanol from grain sorghum at dry mill facilities using biogas for process energy
Biomass-based diesel – 50% GHG Reduction
<ul style="list-style-type: none">• Biodiesel or renewable diesel from soybean oil, annual covercrops, algal oil, biogenic waste oils/fats/greases, non-food grade corn oil or camelina oil produced by trans-esterification or hydroheating• Biodiesel from canola oil produced by trans-esterification using natural gas or biomass for process energy
Cellulosic biofuels (cellulosic ethanol or cellulosic diesel) – 60% GHG Reduction
<ul style="list-style-type: none">• Ethanol or biodiesel from crop residue, slash and tree residues, switchgrass, miscanthus, energy cane, <i>Arundo donax</i>, <i>Pennisetum purpureum</i>, yard waste, cellulosic components of either annual cover crops or yard waste, biogenic components of municipal solid waste• Renewable gasoline from feedstocks mentioned above from approved processes

Chapter 2 - Soil organic carbon, aggregates and microbial community structure in biofuel crops

Abstract

The substitution of cellulosic biofuel in place of conventional gasoline or diesel could reduce greenhouse gas (GHG) emissions from transportation. However, the effectiveness of cellulosic biofuel in reducing emissions depends on emissions during production, transportation and use of the biofuel. Changes in soil organic carbon (SOC) and soil quality during biofuel crop production could have a major impact on the GHG balance of biofuel and on maintaining soil quality. Thus there is a need for understanding the impacts of bioenergy crop on SOC and soil quality. This study measured aboveground biomass production, root stocks, soil microbial community structure, water-stable aggregate distribution, and SOC stocks in annual and perennial bioenergy crops. Sweet sorghum (*Sorghum bicolor* (L.) Moench), photoperiod sensitive sorghum, and miscanthus (*Miscanthus x giganteus*) yielded the highest aboveground biomass (20-32 Mg ha⁻¹). After 5 years miscanthus yields were similar to those of photoperiod sensitive and sweet sorghum. Root stocks were 3.7-7.8 times higher in perennials than in annual crops. Abundance of arbuscular mycorrhizal fungi was higher in the perennial grasses. Miscanthus and switchgrass had more macroaggregates (>250 µm) than annual crops. Significant differences in SOC stocks were detected to a 15 cm depth. Perennial grasses tended to have higher SOC stocks at the end of the study than the annual crops. As a result, the perennial grasses had sequestered SOC over 4 yrs, while SOC stocks had not changed in the annual crops. Thus some perennial bioenergy systems have the potential to produce high amounts of biomass while mitigating GHG emissions through C sequestration.

Introduction

The U.S. is the largest consumer of petroleum in the world. The majority of petroleum in the U.S. is consumed as gasoline and diesel in engines. In 2013 the U.S. consumed 734 billion liters of gasoline and diesel (EIA, 2014). Thus U.S. liquid fuel consumption is a major source of greenhouse gas (GHG) emissions. Of the 6,526 Tg of CO₂ equivalents emitted by the U.S. in 2012, 28% came from the transportation sector (USEPA, 2014). Consequently, action by the U.S. to minimize the impacts of climate change will require addressing GHG emissions from the transportation sector.

In order to promote energy independence, promote the development of clean, renewable fuels and reduce GHG emissions, Congress passed the Energy Independence and Security Act of 2007 (U.S. Congress, 2007). Title II, subtitle A of the EISA, which is commonly referred to as the Renewable Fuel Standard 2 (RFS2), mandates the production of 136 billion liters of biofuel by 2022. Of this amount, 61 billion liters must come from cellulosic biofuel.

Even though biofuels are a promising alternative fuel source substantial quantities of GHGs are emitted throughout the biofuel production cycle. To ensure that these emissions do not offset the mitigative capacity of biofuel production as mandated in the EISA, the act stipulates that cellulosic biofuel must have GHG emissions 60% below those of conventional fossil fuel (U.S. Congress, 2007). This mandate highlights the need to accurately estimate GHG emissions associated with the production and use of biofuels to ensure that they are actually reducing GHG emissions, relative to conventional fossil fuel.

Changes in soil organic carbon (SOC) during biofuel crop production could have a major impact on the GHG balance of biofuel. Depending on crop choice and management, SOC could increase, acting as a sink for atmospheric CO₂, or decrease, acting as a source of CO₂ emissions.

Changes in SOC stocks result from an imbalance between organic C inputs into soil and outputs, primarily through microbial decomposition. Carbon sequestration can occur by increasing C inputs into the soil, increasing the residence time of SOC in the soil, or both (Jastrow et al., 2007). Increasing C inputs could be achieved through management or crop selection that results in increased biomass production, particularly belowground biomass or increased crop residue retention.

Crop choice and management can also influence the residence time of SOC through changes in soil aggregate formation and stability. Carbon incorporated into soil aggregates, especially microaggregates, is protected from decomposition by physical impediment of decomposers and by creating O₂ and moisture conditions that limit decomposer activity (Six et al., 2002; Jastrow et al., 2007). Conditions that encourage the stabilization of macroaggregates (>250 µm diameter) increase the formation and stabilization of microaggregates within macroaggregates and the physical protection of SOC (Six et al., 2002). Macroaggregate formation and stabilization is facilitated by fine roots and hyphae of arbuscular mycorrhizal fungi (AMF), which enmesh soil particles (Jastrow et al., 2007). Selecting crops with high belowground biomass and high affinity for AMF, such as perennial grasses, could aid in the stabilization of aggregates and SOC.

Consideration of SOC inputs and stability is especially important in the context of cellulosic biofuel because these cropping systems require the removal of large quantities of aboveground biomass that would otherwise be returned to the soil. Many studies have observed negative effects of annual row crop residue removal on a variety of soil properties, including SOC and soil aggregates (Wilhelm et al., 2004; Hammerbeck et al., 2012; Osborne et al., 2014; Villamil et al., 2015). Such declines in SOC during crop production will negatively impact the

GHG footprint of biofuel. Perennial grasses like switchgrass (*Panicum virgatum* L.) or miscanthus (*Miscanthus x giganteus*) are attractive alternatives to annual row crops because their higher belowground biomass could help maintain SOC while still producing high quantities of biomass.

In addition to the ability to maintain SOC stocks, there are numerous characteristics desirable of crops grown for cellulosic biofuel production. A few additional desirable characteristics are high biomass production, low input costs, ease of management, and compatibility with current cropping systems. Consequently, there are a variety of bioenergy crops being considered for use in the Great Plains region. In addition to conventional annual row crops like corn (*Zea mays* L.) and grain sorghum (*Sorghum bicolor* (L.) Moench), several annual dedicated bioenergy crops may be grown for cellulosic biofuel. Photoperiod-sensitive sorghums are sorghum cultivars that do not initiate reproductive flowering in most of the U.S. and can produce high amounts of lignocellulosic biomass. Photoperiod-sensitive sorghum commonly yields 20-30 Mg ha⁻¹ with yields reported as high as 35 Mg ha⁻¹ (Propheter et al., 2010; Maughan et al., 2012a). Sweet sorghum cultivars can produce quantities of cellulosic biomass similar to photoperiod sensitive cultivars. In addition, they accumulate high levels of fermentable carbohydrates (9-24% sugar), which can be directly converted in to ethanol (Regassa and Wortmann, 2014).

Several perennial crops are being considered as potential sources for cellulosic feedstock because of their low input requirements, low management needs and ability to grow on marginal soils. Switchgrass is a perennial warm-season grass that is native to the prairies of the U.S. Average yields in the U.S. are 8.7 Mg ha⁻¹ and 12.9 Mg ha⁻¹ for upland and lowland ecotypes, respectively (Wullschleger et al., 2010). Big bluestem (*Andropogon gerardii* Vitman) is another

perennial warm-season grass native to the U.S. Biomass production of big bluestem ranges from 2-9 Mg ha⁻¹ (Tober et al., 2008; Bartholomew et al., 1995; Barnhart, 1989; Hall et al., 1982).

Miscanthus x giganteus is a sterile hybrid of a perennial warm-season grass originating in Japan. *Miscanthus* has the potential to produce high amounts of biomass. Yields of 14-40 Mg ha⁻¹ are common from mature stands in the U.S but can get as high as 60 Mg ha⁻¹ (Heaton et al., 2008; Maughan et al., 2012b; Arundale et al., 2014).

Side-by-side studies comparing both perennial and annual bioenergy crops are necessary to gain a better understanding of crop selection on relative yield potential and on soil carbon dynamics. Such information is necessary to better predict the impact of bioenergy cropping systems on the GHG balance of biofuel. The objectives of this study were to compare: (i) biomass yield; (ii) carbon stocks and long-term changes in SOC; and (iii) factors related to belowground C inputs and physical protection of SOC in annual and perennial bioenergy cropping systems.

Materials and Methods

Study Site

This experiment was conducted at the Kansas State University (KSU) Agronomy Research Farm in Manhattan, Kansas (39°11' N, 96°35' W). The soil types on the study site were Ivan, Kennebec, and Kahola silt loams (fine-silty, mixed, superactive, mesic Cumulic Hapludolls). Soils at the study site had an average SOC content of 14.5 g kg⁻¹ and a pH of 6.6 (Propheter et al., 2010). The experimental plots were previously planted in annual crops which were tilled intermittently. The last tillage event was in spring of 2005. Starting in 2007, corn, photoperiod-sensitive sorghum, sweet sorghum, grain sorghum, forage sorghum, switchgrass, miscanthus, and big bluestem were planted at the study site. Soybean [*Glycine max* (L.) Merr.]

was also planted as a part of corn-soybean and sorghum-soybean rotation. Continuous corn was also included in addition to the corn-soybean rotation. The study was arranged as a randomized complete block design with four replications. Details on plot establishment can be found in Prophet et al. (2010). All crops were under no-tillage management.

Cultivars, seeding rates, and fertilizer rates used over the course of the study are summarized in Appendix Table A.1-A.2. In 2007 and 2008 dual-purpose sorghum was included in the study. From 2009 onward, grain sorghum was planted instead of dual-purpose sorghum. For brevity, the dual-purpose/grain sorghum treatment will be referred to as grain sorghum. On 1 June 2011, 45.5 kg P ha⁻¹ of diammonium phosphate (DAP) was applied to all treatments. The evening after fertilizer application, heavy rains caused minor flooding on the plots. Soil tests showed that much of the DAP-N entered the soil of the plots, but the P had been washed off the plots. A second application of DAP was made at the same rate on 15 June.

Annual crops were harvested in September or October after reaching physiological maturity, or after the first killing frost if maturity was not reached. Perennial grasses (miscanthus, big bluestem and switchgrass) were harvested in November or early December after the plants became dormant. Annual crops were harvested by sampling the center two rows of each plot at a stubble height of 10 cm. The perennial grass plots were harvested using either a modified flail mower (1.2 by 10.7 m harvest area) or a sickle mower followed by hand raking (0.91 by 10.7 m harvest area). Yields are reported as total aboveground biomass and are reported on a dry-mass basis.

Root Stocks

Root stocks were measured in fall of 2013. Soil cores (8 cm dia.) were taken from each plot using a Giddings hydraulic probe (Giddings Machine Company, Windsor, CO, USA) to a

soil depth of 120 cm. Each core was separated into 8 depth increments: 0-5, 5-15, 15-30, 30-45, 45-60, 60-75, 75-90, 90-120 cm.

Samples of continuous corn, rotated corn, sweet sorghum, grain sorghum were taken during grain fill (22 Aug. for corn, 9 Sept. for sorghum). Miscanthus, switchgrass, big bluestem and photoperiod sensitive sorghum were sampled at the end of the growing season (25-26 Oct.). To account for spatial variability of root biomass in rows and interrows, three cores were taken from each plot in annual crops: one directly over a plant, one in the interrow (38 cm from plant row) and one in-between the first 2 cores (19 cm from the plant row). The pattern of spatial variability was much different in the perennial grass plots. In switchgrass and big bluestem, grasses were arranged in scattered clumps that were 20 to 70 cm apart. To account for this spatial variability, at each plot two soil cores were taken, one directly over a clump and one in-between clumps. In miscanthus, plant stands were thick and homogenous. Plant density was high enough that it was not possible to take a soil core between plants. Thus, only one core was taken in each plot directly over a plant.

After sampling, root biomass was separated from soil by washing the soil core increments in an automated root washer (Benjamin and Nielsen, 2004). Soil samples were inserted into stainless steel mesh cylinders with 300 μm openings. Cylinders were loaded onto the washer, which then rotated the samples under a high-pressure water spray. After root washing, the live roots were manually picked from organic debris and coarse soil fragments, rinsed and oven dried at 50°C.

Root density (g cm^{-3}) was calculated by dividing dry root mass by soil core segment volume. For plots in which multiple soil cores were taken, the root density of each core was

weighted by area (Appendix Fig. A.1). Total root stocks were calculated by summing the root densities from 0-120 cm.

Microbial Lipid Analysis

Soil samples for lipid analysis were taken on 19 Sept. 2013. Samples were divided into 3 depths: 0-5, 5-15, and 15-30 cm. Samples were frozen, lyophilized and ground with a mortar and pestle. Phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) were extracted from 5 g of soil using the method of Bligh and Dyer (1959) as modified by White and Ringelberg (1998). Soils were incubated in a 2:1:0.8 methanol:chloroform:phosphate buffer and PLFA and NLFA were isolated using silicic acid chromatography. The phospholipid and neutral lipids were then saponified using KOH and methylated to form fatty acid methyl esters (FAME). FAME were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a DB5-MS column (30m x 250 μm i.d. x 0.25 μm film thickness; Agilent Technologies, Santa Clara, California, USA). FAME peaks were identified by comparison with the bacterial acid methyl esters mix (BAME; Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania, USA). Tentative assignments of FAME peaks not present in the BAME mix were made by mass spectral interpretation. Peak concentration was quantified using the internal standard nonadecanoate and converted into nmol PLFA/NLFA g^{-1} dry soil. PLFA were classified into the following groups: gram positive bacteria (i15:0, a15:0, i16:0, i17:0, a17:0), gram negative bacteria (2-OH 10:0, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 16:1 ω 7c, cy17:0, cy19:0), arbuscular mycorrhizal fungi (AMF) (16:1 ω 5) and saprophytic fungi (18:2 ω 6,9c). NLFA were classified into arbuscular mycorrhizal fungi (16:1 ω 5) and saprophytic fungi (18:2 ω 6,9c). The fungal:bacterial ratio (F:B) was calculated by

dividing the sum of AMF and saprophytic fungi PLFA by the sum of gram positive and gram negative bacteria PLFA.

Water Stable Aggregates

Intact soil samples for water-stable aggregate analysis were taken using a spade on 8 April 2014. Soil was sieved through a 6mm sieve and air-dried prior to analysis. Two replicates of 50 g of air-dry soil were wet sieved through 20, 53, 250 and 2000 μm sieves with a wet sieving apparatus as described by Mikha and Rice (2004). Soil was placed in stacked 250 and 2000 μm sieves and slaked by submersion in water for 10 min. The sieves were then oscillated 4 cm lengths at 0.5 Hz for 10 min. Soil that passed through both sieves was passed through the 53 and 20 μm sieves. Soil remaining on all four sieves was collected, and one of each replicate was dried at 50°C or 105°C for 2 days. Soil dried at 105°C was weighed and used for sand correction. Aggregate subsamples dried at 50°C were ground with mortar and pestle and analyzed for aggregate associated C by dry combustion using a Carlo-Erba C and N analyzer (Thermo Finnegan Flash EA1112, Milan, Italy).

Sand correction was performed by adding a fivefold volume of 5 g L⁻¹ sodium hexametaphosphate to 1-5 g of intact aggregates. Aggregates were left overnight and then shaken at 350 rpm for 4 h. Dispersed sand was collected on a 53 μm sieve, dried at 105°C for 24 h and weighed for the sand correction. Sand collected on the sieve was dried at 105°C and weighed.

Water stable aggregate distribution was reported as the total weight of macroaggregates (>250 μm) and microaggregates (<250 μm), as well as the mean weight diameter (MWD) of treatment, which is calculated from the sum of the aggregate mass remaining on each sieve after

sieving, multiplied by the mean aperture of adjacent sieves. Aggregate associated C concentration was reported as a percentage of the bulk SOC.

Soil Organic Carbon Stocks

For determination of SOC stocks, soil cores (3-cm diameter) were taken to a depth of 120 cm using a Giddings hydraulic probe in 2009 and 2014. Each core was separated into 8 depths: 0-5, 5-15, 15-30, 30-45, 45-60, 60-75, 75-90, 90-120 cm. In Fall 2009, one core per plot was taken as described by Diop (2014). In 2014 samples were taken on 14 Mar. 2014, before planting (and thus occurred 4 growing seasons after the 2009 samples). Four cores per plot were taken in 2014 to decrease within-plot variability and increase precision of estimates. After sampling in 2009 and 2014, sample wet weight was recorded before taking a subsample to oven-dry at 105°C for determination of bulk density. The remaining sample was air-dried, sieved through a 6 mm sieve, and then ground with mortar and pestle to pass through a 500 µm sieve. All visible plant material was removed during the sieving process. To remove carbonates, all samples were treated with 0.1 mL 4 N phosphoric acid and allowed to dry. The acid treatment was repeated until there was no visible effervescence. Preliminary tests found no significant changes in SOC concentration occurred between samples with and without acid treatment (data not shown). Sample SOC concentration was determined by dry combustion using a Carlo-Erba C and N analyzer. Soil organic carbon stocks (Mg C ha⁻¹) were calculated using measured bulk density estimates. Differences in bulk density of soils between cropping systems and sampling dates required that SOC stocks be compared on a basis of equivalent soil mass. The equivalent soil mass for each soil layer was calculated using the method of Ellert and Bettany (1995). A simplified version of the Ellert and Bettany equation from Wendt et al. (2013) is shown below:

$$M_{OC(0-ref)} = M_{OC(0-ref)} = M_{OC(0-a)} + (M_{SOIL(0-ref)} - M_{SOIL(0-a)}) \times C_{OC(a-b)} \quad [2.1]$$

Where $M_{\text{soil}(0\text{-ref})}$ is the reference soil mass from the surface to the layer of interest, $M_{\text{OC}(0\text{-ref})}$ is the mass of SOC in the reference mass, $M_{\text{SOIL}(0\text{-a})}$ is the soil mass from the soil surface to the soil layer above which the reference mass is attained, $M_{\text{OC}(0\text{-a})}$ is the SOC mass in $M_{\text{SOIL}(0\text{-a})}$, $C_{\text{OC}(a\text{-b})}$ is the SOC concentration in soil layer below the $M_{\text{SOIL}(0\text{-a})}$ layer.

Once the equivalent soil mass SOC stocks were calculated, the annual change in SOC stock ($\text{Mg C ha}^{-1} \text{ yr}^{-1}$) was calculated from the difference between 2014 and 2009 samples.

Statistical Analysis

Differences in reported variables were evaluated by ANOVA using PROC GLIMMIX (SAS 9.4, SAS® Institute Inc., Cary, NC, USA). To avoid bias due to missing crop biomass yields in some years (all perennial grasses in 2012 and big bluestem in 2013), ANOVA of aboveground biomass was performed by fitting a cell means model where crop x year was a fixed effect and block was a random effect. A series of contrasts were then used to evaluate ‘partial’ crop x year interactions where years with missing data or crops with missing data were omitted from analysis such that subsets of the data which contained no missing crop x year combinations were analyzed. Multiple significant ‘partial’ interactions within each variable were taken as evidence for crop x year interactions.

Root density, PLFA, NLFA, water-stable aggregate distribution, aggregate MWD, aggregate carbon and SOC stocks were analyzed using two-way ANOVA, with crop, depth and crop x depth as fixed effects and block as a random effect. Tests for the conditional independence between soil depths were performed using the ‘COVTEST’ option in PROC GLIMMIX. In cases where soil depths were found to be correlated, depth was analyzed as a repeated variable with either unstructured, first-order ante-dependence or first-order

autoregressive structure. The covariance structure which minimized the Akaike information criterion (AIC) was used in the final model.

Total root stocks, SOC stocks and annual SOC change were analyzed using one-way ANOVA with crop as a fixed effect and block as a random effect. SOC stocks and annual rate of change at different depths were analyzed separately. All data were checked for normality and homogeneous variance. When the assumption of homogeneous variance was not met, model residual variance was allowed to vary using the 'GROUP' option in the 'RANDOM' statement of GLIMMIX. Non-normal data was logarithmically transformed and means converted back to their original scale for presentation. Mean separation was performed using Fisher's LSD. All statistical comparisons were made at the $\alpha = 0.05$ probability level.

Pearson correlation coefficients were estimated among SOC, macroaggregates, root density, AMF, and F:B using PROC CORR in SAS.

Results

Mean monthly air temperatures and growing season precipitation measured at a nearby meteorological station are summarized in Appendix Table A.3 and Fig. A.2 (Kansas State Univ., 2014). Precipitation in 2008 and 2009 was 15 and 4 cm above the 30 yr. average (72.7 cm). All other years were below the 30 yr. average. Years 2011, 2012, and 2013 were particularly dry, with precipitation 21, 39, and 18 cm below the 30 yr. average, respectively. Growing season temperatures were relatively cool in 2009, with July-Sept. monthly average temperatures 2-3.5°C below the 30 yr. average. The first half of the 2012 growing season was abnormally warm, with average temperatures 2.9°C above average in April-July. Average monthly temperature in July 2011 was 3.2°C above average.

Aboveground Biomass

The partial crop x year interaction was significant ($p < 0.0001$) for ANOVA of 2007-2011 biomass data that included all crops (Appendix Table A.4). The partial crop x year interaction was significant ($p < 0.0001$) for ANOVA of 2007-2011 and 2013 data that included all crops except big bluestem. The partial crop x year interaction was significant ($p < 0.0001$) for ANOVA of 2007-2013 data that included only annual crops.

There were several trends in aboveground biomass over the course of the study (Table 2.1). Sweet sorghum and photoperiod sensitive sorghum tended to be the highest yielding crops in most years of the study. An exception to this trend was 2009, when sweet sorghum and photoperiod sensitive sorghum biomass were both below 10 Mg ha^{-1} . The highest yield recorded during the study period was 2008 sweet sorghum (32 Mg ha^{-1}). The perennial grasses initially had the lowest biomass yields in 2007 and 2008. However, as the study progressed, switchgrass and miscanthus biomass increased. By 2011 and 2013, miscanthus yields reached 19.1 Mg ha^{-1} and were not significantly different from photoperiod sensitive sorghum and sweet sorghum. The highest biomass yield for switchgrass was 14.3 Mg ha^{-1} in 2013. Big bluestem did not show consistent yield trends over time and tended to have the lowest yields of all crops. Dual purpose sorghum yield in 2008 (labeled 'grain sorghum') and grain sorghum yield in 2009 were higher than 2010 - 2013 grain sorghum yields, which ranged $8.9\text{-}13.1 \text{ Mg ha}^{-1}$. Grain sorghum had the highest biomass of all crops in 2009 (20.9 Mg ha^{-1}). Rotated corn and continuous corn biomass trends were similar to each other. Corn yields were highest in 2007 and 2008. In 2009 corn yields dropped significantly and remained low until increasing slightly in 2013. Yield between continuous and rotated corn was often insignificant, except in 2010 and 2011, where rotated corn produced greater biomass.

Root Density and Stocks

The interaction of crop by depth was highly significant for root density ($p < 0.0001$). Miscanthus had significantly higher root density than all other crops to a depth of 30 cm (Fig. 2.1). At 0-5 cm switchgrass and big bluestem had significantly higher root biomass than rotated corn, grain sorghum and sweet sorghum. At both 5-15 cm and 15-30 cm switchgrass and big bluestem had significantly higher root density than all annual crops. From 30-90 cm, all three perennial grasses had significantly higher root density than the annual crops. At 30-90 cm, there were no significant differences between root densities of the perennial grasses, except at 75-90 cm, where switchgrass root density was higher than both big bluestem and miscanthus. There were few significant differences among the annual crops from 5-90 cm. At 90-120 cm switchgrass had the highest root density, but was not significantly higher than that of miscanthus. Miscanthus and big bluestem root density was not significantly higher than that of continuous and rotated corn at 90-120 cm.

Root distribution varied with depth among the crops. All sorghum cultivars had greater than 80% of root stocks in the top 30 cm. Corn had around 77% of roots in the top 30 cm. Big bluestem and switchgrass had higher root stocks deeper in the soil, with 70% and 57% of roots within the top 30 cm, respectively. Even though miscanthus had similar root densities to the other perennials below 30 cm, ~85% of miscanthus root stocks occurred in the surface 30 cm.

Significant differences were found between the total root stocks to a depth of 120 cm ($p < 0.0001$). Miscanthus had significantly higher root stocks than all other crops (Fig. 2.2). Big bluestem and switchgrass root stocks were significantly higher than those of all annual crops. Photoperiod sensitive sorghum had the highest root stocks among the annuals, but was only

significantly different from grain sorghum. No significant differences were found between sweet sorghum, grain sorghum, rotated corn and continuous corn.

Microbial Lipids

No significant interaction between crop and soil depth was found for total PLFA, gram positive bacteria, gram negative bacteria or fungal PLFA biomarkers and the fungal NLFA biomarker (Table 2.2). The effect of depth was significant for all of these biomarkers ($p < 0.05$). The concentration of all four PLFA biomarkers decreased with increasing soil depth (Fig. 2.3). Fungal NLFA followed the same trend as the fungal PLFA (data not shown).

Total PLFA differed significantly among crops. Total PLFA of miscanthus and switchgrass was higher than all other crops, but only miscanthus was significantly higher (Fig. 4). No other significant differences existed with the other crops. The effect of crop on the concentration of gram negative and gram positive bacteria biomarkers was not significant.

Significant differences in the 18:1 ω 6,9 fungal biomarker were found between crops. Miscanthus soils had a significantly higher concentration of fungal PLFA than all other crops, indicating an increased presence of saprophytic fungi in miscanthus (Fig. 2.5). Big bluestem, switchgrass and photoperiod sensitive sorghum had similar concentrations of the fungal biomarker and were not significantly different from each other. In big bluestem soils the fungal biomarker was significantly higher than in sweet sorghum, grain sorghum, rotated corn and continuous corn. The fungal biomarker in switchgrass was significantly higher than that of sweet sorghum. No significant differences existed in the fungal biomarker between the annual crops. There was a positive relationship between the fungal biomarker and root density ($r = 0.574$). Similar trends were seen in the 18:1 ω 6,9 NLFA concentration (data not shown).

Trends were similar between the 16:1 ω 5 arbuscular mycorrhizal (AM) PLFA and NLFA biomarkers. However, the 16:1 ω 5 PLFA biomarker exists in some bacteria, and the NLFA biomarker is considered a more reliable indicator for AMF (Frostegard et al., 2011; Ngosong et al., 2012) so the discussion will focus on the AM 16:1 ω 5 NLFA biomarker. The interaction between crop and depth was significant for the AM biomarker. At 0-5 cm, AM in miscanthus was significantly higher than that of all other crops, suggesting an increased abundance of AMF in miscanthus (Fig. 2.6). In switchgrass AM was higher than in rotated corn, photoperiod sensitive sorghum and sweet sorghum, but not significantly different from big bluestem, continuous corn or grain sorghum at 0-5 cm. Concentrations of AM biomarker were similar between soils of big bluestem, continuous corn, rotated corn and grain sorghum. Photoperiod sensitive sorghum and sweet sorghum had the lowest concentrations of the AM biomarker at 0-5 cm.

At 5-15 cm miscanthus and switchgrass soils had significantly higher concentrations of the AM biomarker than in grain sorghum, photoperiod sensitive sorghum and sweet sorghum. Miscanthus and switchgrass concentrations of the AM biomarker were not significantly different from those in big bluestem, continuous corn or rotated corn. In big bluestem, continuous corn and rotated corn, AM concentrations were significantly higher than in photoperiod sensitive sorghum and sweet sorghum but not significantly different from grain sorghum at 5-15 cm. Grain sorghum had significantly higher levels of the AM biomarker than photoperiod sensitive sorghum and sweet sorghum at 5-15 cm.

At 15-30 cm miscanthus, big bluestem, switchgrass and continuous corn had significantly greater concentrations of AM than in photoperiod sensitive sorghum and sweet sorghum. Concentrations of AM at 15-30 cm in rotated corn were significantly greater than that of sweet

sorghum. There were no significant differences between the concentrations of AM in soils of grain sorghum, photoperiod sensitive sorghum and sweet sorghum at 15-30 cm. Trends observed in AM may be related to root density, which were much higher in miscanthus at 0-5 cm and also tended to be higher in perennials down to 30 cm. There was a positive relationship between root density and AM ($r = 0.603$).

The concentration of AM in miscanthus soils decreased significantly with depth. Concentration of AM at 0-5 cm was significantly greater than at 5-15 cm and 15-30 cm. No significant difference was found between AM concentration at 5-15 cm and 15-30 cm in miscanthus soils. No other significant differences were found in AM between soil depths in other crops.

The interaction between crop and depth was significant for the F:B ratio. At all three depths, miscanthus had a significantly higher F:B ratio than all annual crops (Fig. 2.7). Big bluestem tended to have a higher F:B ratio than most annuals at all 3 depths but the difference was not always significant. The F:B in both miscanthus and big bluestem remained relatively constant with decreasing depth, around 0.50 and 0.25 for miscanthus and big bluestem respectively. Switchgrass and the annuals did not show a consistent pattern between depths. Correlation between F:B and root density was not significant.

Water-Stable Aggregate Distribution

The crop x depth interaction was significant for water-stable macroaggregates (>250 μm) (Table 2.2). At 0-5 cm miscanthus and switchgrass soils had significantly greater amounts of macroaggregates than all other crops (Fig. 2.8). There were no other significant differences in macroaggregates between the other crops at 0-5 cm. No significant differences were found between crops in macroaggregates amounts at 5-15 cm (data not shown). In switchgrass and

miscanthus soils, macroaggregates were significantly lower at 5-15 cm than at 0-5 cm, while macroaggregates in soils of grain sorghum and sweet sorghum were significantly greater at 5-15 cm than at 0-5 cm.

The crop x depth interaction and the effect of depth on water-stable aggregate mean weight diameter were not significant. There was a significant effect of crop type on aggregate mean weight diameter. The mean weight diameter of aggregates in soils of miscanthus and switchgrass was significantly greater than that of all of the annual crops (Fig. 2.9). There was no significant difference between the mean weight diameter of aggregates in miscanthus and big bluestem soils. The mean weight diameter of aggregates in switchgrass was significantly greater than in big bluestem. Big bluestem soils had aggregates with a mean weight diameter significantly greater than in photoperiod sensitive sorghum, and continuous corn, but was not significantly different from sweet sorghum, grain sorghum or rotated corn. The aggregates with the lowest mean weight diameter occurred in soils of the annual crops, which were not significantly different from each other.

Aggregate size was positively correlated with root density, AM, fungi and F:B (Table 2.5) at 0-5 cm. Pearson correlation coefficient was 0.529 for roots and MWD, and was 0.464, 0.436, and 0.599 for MWD and AM, fungi and F:B, respectively.

To evaluate the effect of aggregate size on water-stable aggregate C concentration, ANOVA was performed on the C content of aggregates averaged over crops and depths. Aggregate size had a significant effect on SOC concentration ($p < 0.0001$). Carbon concentration decreased with aggregate size. Aggregates $>2000 \mu\text{m}$ had 26 g C kg^{-1} aggregate while aggregates $<53 \mu\text{m}$ had 8 g C kg^{-1} aggregate (Fig. 2.10). No significant differences were found in the concentration of aggregate C in the different aggregate size classes between crops.

The interaction of crop x depth and the main effect of depth on the percent of bulk soil organic carbon contained within macroaggregates (WSAC:SOC) were not significant. There was a significant effect of crop type on macroaggregate WSAC:SOC. Macroaggregate WSAC:SOC was significantly higher in miscanthus as compared to photoperiod sensitive sorghum, sweet sorghum, grain sorghum and continuous corn, but was not significantly different from big bluestem, switchgrass and rotated corn (Fig. 2.11). Switchgrass macroaggregate WSAC:SOC was significantly higher than that of photoperiod sensitive sorghum and continuous corn, but not significantly different from that of big bluestem, sweet sorghum, grain sorghum and rotated corn. Big bluestem and the annual crops tended to have the lowest macroaggregate WSAC:SOC, which were not significantly different from each other.

Soil Organic Carbon Stocks

The interaction between crop and year was significant for SOC stocks at 0-5 and 0-15 cm (Table 2.3). No significant effects were found in cumulative SOC or individual layers at depths below 15 cm. Miscanthus, big bluestem and switchgrass all had significantly higher SOC stocks at 0-15 cm in 2014 than in 2009. No significant differences were found in 0-15 cm SOC stocks of annual crops over time. In 2009 there were no significant differences in SOC stocks between crops. In 2014, miscanthus had significantly higher SOC than all annual crops. Big bluestem and switchgrass had higher SOC stocks than the annual crops, but were only significantly different from sweet sorghum.

Significant differences in the annual change in SOC stocks (Δ SOC) were found at 0-5 and 0-15 cm (Table 2.4). All depths below 15 cm were not significantly different for Δ SOC. At 0-15cm, Δ SOC in the perennial crops was positive and significantly different from 0. No annual crops exhibited Δ SOC significantly different from 0. The Δ SOC of miscanthus was significantly

higher than all other crops. Big bluestem Δ SOC was significantly higher than photoperiod sensitive sorghum, sweet sorghum, grain sorghum and continuous corn, but not switchgrass or rotated corn. Switchgrass and rotated corn Δ SOC was significantly higher than grain sorghum.

Root density and MWD were positively correlated with SOC concentration ($r = 0.549$, 0.474 , respectively). There was a weak positive relationship between AM and SOC concentration ($r = 0.265$) as well as fungi and SOC concentration ($r = 0.298$).

Discussion

Photoperiod-sensitive sorghum and sweet sorghum yields reported in this study were within the range reported in other studies (Tamang et al., 2011; Maughan et al., 2012a; Olson et al., 2012; Rocateli et al., 2012; Snider et al., 2012; Regassa and Wortmann, 2014). Switchgrass and miscanthus yields were initially low, but increased substantially over the course of the study. This is expected as perennial grasses often take several years before reaching maximum yield potential. There have been relatively few studies on the productivity of miscanthus in the U.S. Maughan et al. (2012b) reported maximum yields of 16.9-27.4 Mg ha⁻¹ at sites in Kentucky, Nebraska and New Jersey. Yields observed in New Jersey and Kentucky were similar to those observed in our study. Heaton et al. (2008) reported average miscanthus yields of 31.2-45.5 Mg ha⁻¹ over three years from sites in Illinois. The highest miscanthus annual yield reported was 60.8 Mg ha⁻¹. The yields reported by Heaton et al. (2008) are much higher than our study with differences likely due to precipitation and growing season temperatures.

There have been many studies reporting switchgrass biomass production. In a meta-analysis including 18 studies in the U.S., Wullschleger et al. (2010) reported the average yield of lowland ecotypes to be 12.9 Mg ha⁻¹, although the range varied widely depending on factors such as climate and fertilization. In some cases, yields above 28 Mg ha⁻¹ were reported in the

southern U.S. The 2011 and 2013 yields of switchgrass reported in this study were close to the average reported by Wullschelger et al. (2010). Big bluestem biomass was the lowest of all crops in the study, but within the range reported by other studies (Hall et al., 1982; Barnhart, 1989; Bartholomew et al., 1995; Tober et al., 2008).

The corn and sorghum root stock measurements in this study were within the observed range previously reported in other studies (Amos and Walters, 2006; Monti and Zatta, 2009; Schittenhelm and Schroetter, 2014). The estimated mean belowground biomass of corn in the reproductive stages from 8 studies included in a meta-analysis of Amos and Walters (2006) was found to be 2.1 Mg ha⁻¹, with a maximum of 3.24 Mg ha⁻¹. Most studies sampled to a 60-90 cm soil depth. The root stocks measured in our study are similar at 60-90 cm depths (3-4 Mg ha⁻¹). Schittenhelm et al. (2014) observed root dry weights in corn and sweet sorghum (3-4.3 Mg ha⁻¹; 3.8-4.4 Mg ha⁻¹ at 0 – 30 cm, respectively), which were slightly higher than corn and sorghum root stocks at equivalent depth in this study. Monti and Zatta (2009) measured root biomass of 2.1 Mg ha⁻¹ in fiber sorghum at 0-120 cm, which is 1-2.5 Mg ha⁻¹ lower than observed root stocks of sweet sorghum and photoperiod sensitive sorghum in this study.

The root biomass of switchgrass was within the range reported in other studies (Ma et al., 2001; Garten et al., 2010; Ontl et al., 2013; Wayman et al., 2014). Ma et al. (2001) observed root stocks between 13.3-22.9 Mg h⁻¹ at 0-3 m and Garten et al. (2010) observed root stocks of 16.2 Mg ha⁻¹ at 0-90 cm. Wayman et al. (2014) measured root stocks 9.1-13.3 Mg ha⁻¹ at 0-115 cm. Ontl et al. (2013) measured root stocks 4.3-6.4 Mg ha⁻¹, similar to switchgrass in our study at the same depth. In contrast, miscanthus root stocks measured in this study were greater than values reported elsewhere. Neukirchen et al. (1999) observed root stocks of 12.4 Mg ha⁻¹ in a 4-year stand of miscanthus. Monti and Zatta (2009) observed 4.2 Mg ha⁻¹ at 0-35 cm and Goetz et

al. (2015) reported root stocks 12.9-14.7 Mg ha⁻¹ down to 100 cm. It is unclear why root stocks of miscanthus were over twice as large as those reported in other studies. Aboveground production of miscanthus was lower in Goetz et al. (2015) than our study, but similar to that of Neukirchen et al. (1999). Sampling methods differed between studies as well, with some estimates derived from samples taken directly over plants, between plants, or both.

Similar to other studies, switchgrass tended to have more root stocks deeper in the soil than other crops, including miscanthus. Garten et al. (2010) found 69% of live roots to be in the top 30 cm of switchgrass. Monti and Zatta (2009) found that 90% of miscanthus root stocks were above 35 cm, while switchgrass only had 35%. Goetz et al. (2015) found 78% of miscanthus roots in the top 30 cm. Studies have found that 70-90% of corn roots are in the upper 15-30 cm (Aina and Fapohunda, 1986; Crozier and King, 1993; Osaki et al., 1995; Dwyer et al., 1996).

Few studies have examined the impacts of bioenergy crops on soil microbial communities. Jesus et al. (2015) and Liang et al. (2012) measured microbial communities in mixed prairie, switchgrass and corn. They found higher, but not consistently significant, amounts of total lipids in perennial grasses compared to continuous corn. Both studies observed elevated levels of AMF in perennial systems compared to corn, but only Liang et al. (2012) observed a significant response. Liang et al. (2012) observed a significant increase in gram negative bacteria under mixed prairie. No other significant differences in bacterial groups were observed in either study. These findings are similar to our study, where miscanthus and switchgrass showed higher microbial biomass than the annual crops and all perennial grasses showed evidence of elevated levels of saprophytic fungi and AMF compared to annuals, though this trend was only consistently significant in miscanthus.

Perennial grasses tended to have more macroaggregates and greater mean weight diameter than the annual crops. Both Tiemann et al. (2015) and Ontl et al. (2015) observed elevated macroaggregates in switchgrass compared to corn. However, Tiemann et al. (2015) observed no significant differences in macroaggregates of miscanthus and corn 4 yrs after miscanthus establishment. Dondini et al. (2009) found significantly more macroaggregates in miscanthus as compared to conventionally tilled cropland. O'Brien and Jastrow (2013) found macroaggregate levels of restored prairie at levels found in native prairie within 3 years after conversion to prairie vegetation. Mikha et al. (2010) observed significantly higher levels of macroaggregates under perennial grasses as compared to various conventional and no-tillage crop rotations.

The SOC increased in the perennial grasses but not in the annual crops. Root biomass is considered one of the most important factors predicting potential C sequestration, so perennial grasses tend to sequester higher rates of SOC (Lemus and Lal, 2005). Evidence of increased fungal biomass and greater WSA mean weight diameter in the perennial grasses suggest that mechanisms for physical protection of SOC may be contributing to the increase in SOC. Additionally, indicators of fungal biomass (AM, fungi, and F:B) were positively correlated with aggregate size, as was aggregate size and SOC. Increased AMF abundance has been found to increase soil the production of macroaggregates, which can contribute to the stabilization of SOC (Miller and Jastrow, 2000; Six et al., 2002; Jastrow et al., 2007; Wilson et al., 2009).

Many studies have reported elevated SOC under perennials such as miscanthus and switchgrass as compared to annual cropping systems (Lemus and Lal, 2005; Dondini et al., 2009; Monti et al., 2012; Cattaneo et al., 2014). In switchgrass, SOC sequestration rates of 0-1 Mg ha⁻¹ yr⁻¹ are common, though rates as high as 4 Mg ha⁻¹ yr⁻¹ have been observed. Most of the changes

in SOC tend to occur in the upper soil layers because of the greater impacts of climate, microbial biomass, and larger root biomass (Lemus and Lal, 2005). Miscanthus can also sequester high amounts of SOC. Studies using carbon stable isotope have estimated miscanthus to contribute between 0.3-3.2 Mg C ha⁻¹ yr⁻¹ to SOC pools, usually in the top 15 to 30 cm (Hansen et al., 2004; Dondini et al., 2009; Felten and Emmerling, 2012; Zimmermann et al., 2012; Poeplau and Don, 2014; Richter et al., 2015). Poeplau et al. (2014) estimated the mean SOC sequestration rate of miscanthus at 20 sites in Europe to be 1.46 Mg ha⁻¹ yr⁻¹, which is similar to the observed SOC sequestration rate in this study.

In contrast to the perennials, changes in SOC of annuals over the duration of the study ranged from slight increases to slight decreases. In this study annual crops were under no-tillage, but all standing biomass was removed to simulate harvest for bioenergy production. It is well established that high rates of residue removal can have a negative impact on SOC stocks, even in no-tillage systems (Wilhelm et al., 2004; Johnson et al., 2014; Osborne et al., 2014). Vilamil et al. (2015) observed decreases in in SOC after 8 years of residue removal in no-tillage corn at 3 locations in Illinois. Kenney et al. (2015) observed lower SOC levels after 2 years of residue removal in no-tillage corn, but observed no significant change in 2 locations under conventional tillage. Osborne et al. (2015) observed significant decreases in SOM in conventional and no-tillage corn after 11 years of residue removal. Johnson et al. (2014) estimated that roughly 5.7 Mg ha⁻¹ of stover would be needed to maintain SOC stocks. With all standing biomass removed from plots, the amount of stover remaining in this study was certainly below the threshold estimated by Johnson et al. (2014).

Conclusions

Our results show that sweet sorghum, photoperiod-sensitive sorghum, and miscanthus exhibit high yield potential, with all 3 crops producing approximately 20 Mg ha⁻¹ during the last 3 study years. However, both miscanthus and switchgrass took several years after establishment to reach biomass yields similar to the annual crops. Soils of the perennial cropping systems increased in SOC over 4 years, sequestering 0.86-1.97 Mg C ha⁻¹ yr⁻¹. The high C sequestration capacity of the perennial crops demonstrates their potential to mitigate GHG emissions while providing feedstock for biofuel. Even though all crops were under no-tillage management, the annual systems did not show significant changes in SOC over 4 years. However both positive (corn-soybean, photoperiod-sensitive sorghum) and negative (sweet sorghum, grain sorghum) trends in SOC stocks were observed in this study. If residue removal was continued for longer periods of time, there might be significant decreases in SOC in some of the annual crops. This study also represented an extreme case where all standing biomass was removed at harvest. In highly productive cropping systems like sweet sorghum and photoperiod-sensitive sorghum, leaving a small percentage of aboveground biomass on the field at harvest would result in large quantities of stover to protect soils from erosion and SOC loss, while still providing high volumes of feedstock for biofuel production.

This study found evidence of several mechanisms that may be driving the increases in SOC stocks of the perennial crops. Root stocks were 4-8 times higher in the perennial crops, suggesting greater belowground C inputs. Additionally, evidence of elevated AM fungi and increased aggregate size in the perennials suggests physical protection of SOC may be enhanced in these systems, especially in miscanthus. The increases in SOC and aggregate size in the perennials systems suggest that these systems have the potential to improve soil quality while

providing feedstock for biofuel production, which could be important in cases where these crops are utilized on marginal lands.

References

- Aina, P.O., and H.O. Fapohunda. 1986. Root distribution and water-uptake patterns of maize cultivars field-grown under differential irrigation. *Plant Soil* 94:257-265.
- Amos, B., and D.T. Walters. 2006. Maize root biomass and net rhizodeposited carbon: An analysis of the literature. *Soil Sci. Soc. Am. J.* 70:1489-1503.
- Arundale, R.A., F.G. Dohleman, E.A. Heaton, J.M. McGrath, T.B. Voigt, and S.P. Long. 2014. Yields of *miscanthus x giganteus* and *Panicum virgatum* decline with stand age in the midwestern USA. *GCB Bioenergy* 6:1-13.
- Barnhart, S.K. 1989. Warm-season grasses for hay and pasture. Rep. PM-569. Iowa State Univ., Ames, IA.
- Bartholomew, H.M., R.M. Sulc, R. Hendershot, and J. Cline. 1995. Perennial warm season grasses for Ohio. Rep. AGF-022-95. Ohio State Univ. Ext. Dept. of Hort. and Crop Sci., Columbus, OH.
- Benjamin, J.G., and D.C. Nielsen. 2004. A method to separate plant roots from soil and analyze root surface area. *Plant Soil* 267:225-234.
- Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.* 37:911-917.
- Cattaneo, F., L. Barbanti, P. Gioacchini, C. Ciavatta, and C. Marzadori. 2014. C-13 abundance shows effective soil carbon sequestration in miscanthus and giant reed compared to arable crops under mediterranean climate. *Biol. Fertility Soils* 50:1121-1128.
- Crozier, C.R., and L.D. King. 1993. Corn root dry-matter and nitrogen distribution as determined by sampling multiple soil cores around individual plants. *Commun. Soil Sci. Plant Anal.* 24:1127-1138.
- Diop, E.H.H.S. 2014. Biofuel feedstocks implications for sustainability and ecosystem services. Manhattan, Kan. Kansas State University, Manhattan, Kan.

- Dondini, M., K. Van Groenigen, I. Del Galdo, and M.B. Jones. 2009. Carbon sequestration under miscanthus: A study of C-13 distribution in soil aggregates. *GCB Bioenergy* 1:321-330.
- Dwyer, L.M., B.L. Ma, D.W. Stewart, H.N. Hayhoe, and D. Balchin. 1996. Root mass distribution under conventional and conservation tillage. *Can. J. Soil Sci.* 76:23-28.
- EIA. 2014. U.S. product supplied for crude oil and petroleum products.
- Ellert, B.H., and J.R. Bettany. 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Can. J. Soil Sci.* 75:529-538.
- Felten, D., and C. Emmerling. 2012. Accumulation of miscanthus-derived carbon in soils in relation to soil depth and duration of land use under commercial farming conditions. *J. Plant Nutr. Soil Sci.* 175:661-670.
- Frostegard, A., A. Tunlid, and E. Baath. 2011. Use and misuse of PLFA measurements in soils. *Soil Biol. Biochem.* 43:1621-1625.
- Garten, C.T., J.L. Smith, D.D. Tyler, J.E. Amonette, V.L. Bailey, D.J. Brice, H.F. Castro, R.L. Graham, C.A. Gunderson, R.C. Izaurralde, P.M. Jardine, J.D. Jastrow, M.K. Kerley, R. Matamala, M.A. Mayes, F.B. Metting, R.M. Miller, K.K. Moran, W.M. Post, R.D. Sands, C.W. Schadt, J.R. Phillips, A.M. Thomson, T. Vugteveen, T.O. West, and S.D. Wullschlegel. 2010. Intra-annual changes in biomass, carbon, and nitrogen dynamics at 4-year old switchgrass field trials in west Tennessee, USA. *Agric. Ecosyst. Environ.* 136:177-184.
- Hall, K.E., J.R. George, and R.R. Riedl. 1982. Herbage dry-matter yields of switchgrass, big bluestem, and indiangrass with N-fertilization. *Agron. J.* 74:47-51.
- Hammerbeck, A.L., S.J. Stetson, S.L. Osborne, T.E. Schumacher, and J.L. Pikul Jr. 2012. Corn residue removal impact on soil aggregates in a no-till corn/soybean rotation. *Soil Sci. Soc. Am. J.* 76:1390-1398.
- Hansen, E.M., B.T. Christensen, L.S. Jensen, and K. Kristensen. 2004. Carbon sequestration in soil beneath long-term miscanthus plantations as determined by ¹³C abundance. *Biomass Bioenergy* 26:97-105.

- Heaton, E.A., F.G. Dohleman, and S.P. Long. 2008. Meeting US biofuel goals with less land: The potential of miscanthus. *Glob. Change Biol.* 14:2000-2014.
- Jastrow, J.D., J.E. Amonette, and V.L. Bailey. 2007. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. *Clim. Change* 80:5-23.
- Jesus, E.d.C., C. Liang, J.F. Quensen, E. Susilawati, R.D. Jackson, T.C. Balser, and J.M. Tiedje. 2015. Influence of corn, switchgrass, and prairie cropping systems on soil microbial communities in the upper Midwest of the United States. *GCB Bioenergy*. DOI: 10.1111/gcbb.12289.
- Johnson, J.M.F., J.M. Novak, G.E. Varvel, D.E. Stott, S.L. Osborne, D.L. Karlen, J.A. Lamb, J. Baker, and P.R. Adler. 2014. Crop residue mass needed to maintain soil organic carbon levels: Can it be determined? *BioEnergy Res.* 7:481-490.
- Kansas State Univ. 2014. Kansas Mesonet. Weather Data Library. 2014.
- Lemus, R., and R. Lal. 2005. Bioenergy crops and carbon sequestration. *Crit. Rev. Plant Sci.* 24:1-21.
- Liang, C., E.d.C. Jesus, D.S. Duncan, R.D. Jackson, J.M. Tiedje, and T.C. Balser. 2012. Soil microbial communities under model biofuel cropping systems in southern Wisconsin, USA: Impact of crop species and soil properties. *Appl. Soil Ecol.* 54:24-31.
- Ma, Z., C.W. Wood, and D.I. Bransby. 2001. Impact of row spacing, nitrogen rate, and time on carbon partitioning of switchgrass. *Biomass Bioenergy* 20:413-419.
- Maughan, M., T. Voigt, A. Parrish, G. Bollero, W. Rooney, and D.K. Lee. 2012a. Forage and energy sorghum responses to nitrogen fertilization in central and southern Illinois. *Agron. J.* 104:1032-1040.
- Maughan, M., G. Bollero, D.K. Lee, R. Darmody, S. Bonos, L. Cortese, J. Murphy, R. Gaussoin, M. Sousek, D. Williams, L. Williams, F. Miguez, and T. Voigt. 2012b. *Miscanthus giganteus* productivity: The effects of management in different environments. *GCB Bioenergy* 4:253-265.
- Mikha, M.M., and C.W. Rice. 2004. Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. *Soil Sci. Soc. Am. J.* 68:809-816.
- Mikha, M.M., J.G. Benjamin, M.F. Vigil, and D.C. Nielson. 2010. Cropping intensity impacts on soil aggregation and carbon sequestration in the central Great Plains. *Soil Sci. Soc. Am. J.* 74:1712-1719.

- Miller, R.M., and J.D. Jastrow. 2000. Mycorrhizal fungi influence soil structure. p. 3-18. *In* Y. Kapulnik and D.D. Douds (eds.) *Arbuscular mycorrhizas: Physiology and function*. Dordrecht Boston: Kluwer Academic, Dordrecht; Boston.
- Monti, A., L. Barbanti, A. Zatta, and W. Zegada-Lizarazu. 2012. The contribution of switchgrass in reducing GHG emissions. *GCB Bioenergy* 4:420-434.
- Monti, A., and A. Zatta. 2009. Root distribution and soil moisture retrieval in perennial and annual energy crops in northern Italy. *Agric. Ecosyst. Environ.* 132:252-259.
- Neukirchen, D., M. Himken, J. Lammel, U. Czyionka-Krause, and H.W. Olf. 1999. Spatial and temporal distribution of the root system and root nutrient content of an established miscanthus crop. *Europ. J. Agron.* 11:301-309.
- Ngosong, C., E. Gabriel, and L. Ruess. 2012. Use of the signature fatty acid 16:1 ω 5 as a tool to determine the distribution of arbuscular mycorrhizal fungi in soil. *J. Lipids* 2012:236807-236807.
- O'Brien, S.L., and J.D. Jastrow. 2013. Physical and chemical protection in hierarchical soil aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands. *Soil Biol. Biochem.* 61:1-13.
- Olson, S.N., K. Ritter, W. Rooney, A. Kemanian, B.A. McCarl, Y. Zhang, S. Hall, D. Packer, and J. Mullet. 2012. High biomass yield energy sorghum: Developing a genetic model for C4 grass bioenergy crops. *Biofuels Bioprod. Biorefining* 6:640-655.
- Ontl, T.A., C.A. Cambardella, L.A. Schulte and R.K. Kolka. 2015. Factors influencing soil aggregation and particulate organic matter responses to bioenergy crops across a topographic gradient. *Geoderma* 255:1-11.
- Ontl, T.A., K.S. Hofmockel, C.A. Cambardella, L.A. Schulte, and R.K. Kolka. 2013. Topographic and soil influences on root productivity of three bioenergy cropping systems. *New Phytol.* 199:727-737.
- Osaki, M., T. Shinano, M. Matsumoto, J. Ushiki, M.M. Shinano, M. Urayama, and T. Tadano. 1995. Productivity of high-yielding crops. V. Root-growth and specific absorption rate of nitrogen. *Soil Sci. Plant Nutr.* 41:635-647.

- Osborne, S.L., J.M.F. Johnson, V.L. Jin, A.L. Hammerbeck, G.E. Varvel, and T.E. Schumacher. 2014. The impact of corn residue removal on soil aggregates and particulate organic matter. *BioEnergy Res.* 7:559-567.
- Poeplau, C., and A. Don. 2014. Soil carbon changes under miscanthus driven by C-4 accumulation and C-3 decomposition - toward a default sequestration function. *GCB Bioenergy* 6:327-338.
- Propheter, J.L., S.A. Staggenborg, X. Wu, and D. Wang. 2010. Performance of annual and perennial biofuel crops: Yield during the first two years. *Agron. J.* 102:806-814.
- Regassa, T.H., and C.S. Wortmann. 2014. Sweet sorghum as a bioenergy crop: Literature review. *Biomass Bioenerg.* 64:348-355.
- Richter, G.M., F. Agostini, M. Redmile-Gordon, R. White, and K.W.T. Goulding. 2015. Sequestration of C in soils under miscanthus can be marginal and is affected by genotype-specific root distribution. *Agric. Ecosyst. Environ.* 200:169-177.
- Rocateli, A.C., R.L. Raper, K.S. Balkcom, F.J. Arriaga, and D.I. Bransby. 2012. Biomass sorghum production and components under different irrigation/tillage systems for the southeastern US. *Ind. Crop. Prod.* 36:589-598.
- Schittenhelm, S. and, S. Schroetter. 2014. Comparison of drought tolerance of maize, sweet sorghum and sorghum-sudangrass hybrids. *J. Agron. Crop Sci.* 200:46-53.
- Six, J., R.T. Conant, E.A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant Soil* 241:155-176.
- Snider, J.L., R.L. Raper, and E.B. Schwab. 2012. The effect of row spacing and seeding rate on biomass production and plant stand characteristics of non-irrigated photoperiod-sensitive sorghum (sorghum bicolor (L.) moench). *Ind. Crop. Prod.* 37:527-535.
- Tamang, P.L., K.F. Bronson, A. Malapati, R. Schwartz, J. Johnson, and J. Moore-Kucera. 2011. Nitrogen requirements for ethanol production from sweet and photoperiod sensitive sorghums in the southern high plains. *Agron. J.* 103:431-440.

- Tiemann, L.K., and A.S. Grandy. 2015. Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *GCB Bioenergy* 7:161-174.
- Tober, D.W., W. Duckwitz, and M. Knudson. 2008. Big bluestem biomass trials in North Dakota, South Dakota, and Minnesota. USDA, NRSC Plant Materials Center, Bismark, ND.
- U.S. Congress. 2007. Energy independence and security act of 2007. 201-210.
- USEPA. 2014. Inventory of U.S. greenhouse gas emissions and sinks: 1990 - 2012. Rep. EPA 430-R-14-003.
- Villamil, M.B., J. Little, and E.D. Nafziger. 2015. Corn residue, tillage, and nitrogen rate effects on soil properties. *Soil Tillage Res.* 151:61-66.
- Wayman, S., R.D. Bowden, and R.B. Mitchell. 2014. Seasonal changes in shoot and root nitrogen distribution in switchgrass (*Panicum virgatum*). *BioEnergy Res.* 7:243-252.
- Wendt, J.W., and S. Hauser. 2013. An equivalent soil mass procedure for monitoring soil organic carbon in multiple soil layers. *Eur. J. Soil Sci.* 64:58-65.
- White, D.C., and D.B. Ringelberg. 1998. Signature lipid biomarker analysis. p. 255. *In* R.S. Burlage, R. Atlas, D. Stahl, G. Geesey and G. Sayler (eds.) *Techniques in microbial ecology*. Oxford Univ. Press, New York.
- Wilhelm, W.W., J.M.F. Johnson, J.L. Hatfield, W.B. Voorhees, and D.R. Linden. 2004. Crop and soil productivity response to corn residue removal: A literature review. *Agron. J.* 96:1-17.
- Wilson, G.W.T., C.W. Rice, M.C. Rillig, A. Springer, and D.C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments. *Ecology Ltrs.* 12:452-461.
- Wullschleger, S.D., E.B. Davis, M.E. Borsuk, C.A. Gunderson, and L.R. Lynd. 2010. Biomass production in switchgrass across the United States: Database description and determinants of yield. *Agron. J.* 102:1158-1168.

Zimmermann, J., J. Dauber, and M.B. Jones. 2012. Soil carbon sequestration during the establishment phase of *miscanthus x giganteus*: A regional-scale study on commercial farms using C-13 natural abundance. *GCB Bioenergy* 4:453-461.

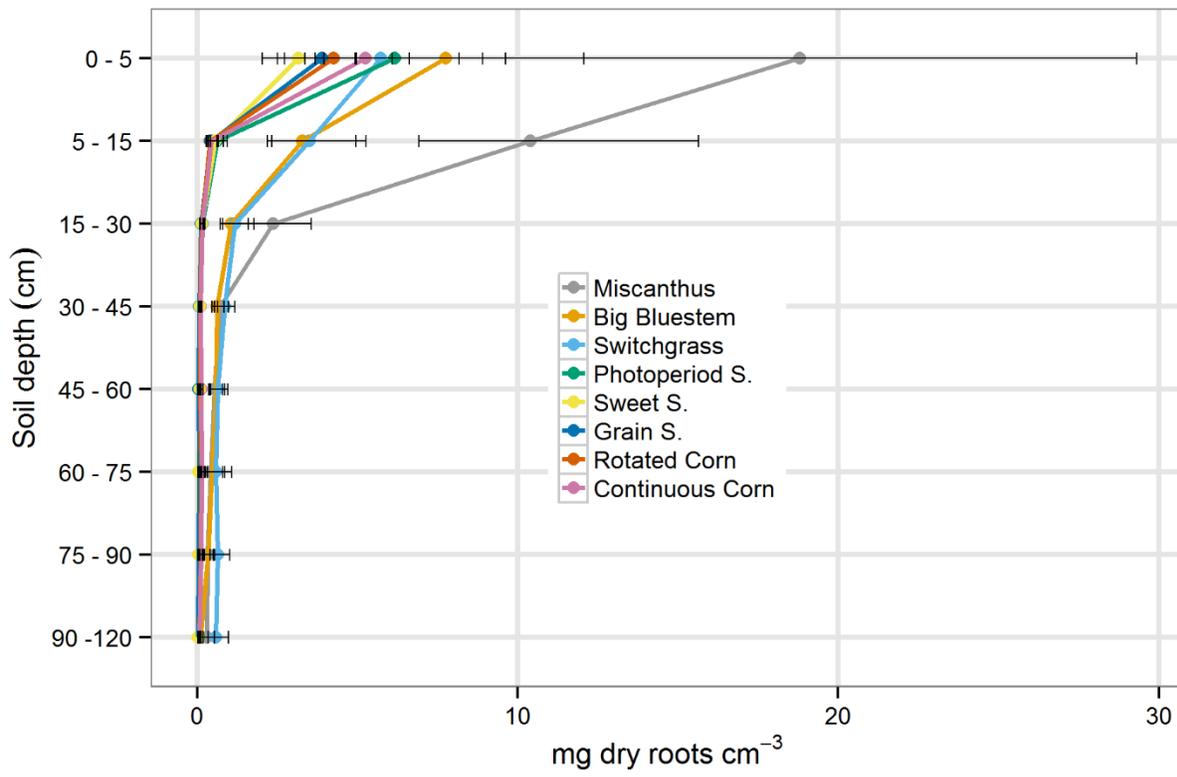


Figure 2.1. Root density by crop and depth taken in fall 2013. Error bars show 95% confidence intervals of the median. Sorghum is abbreviated with “S.”

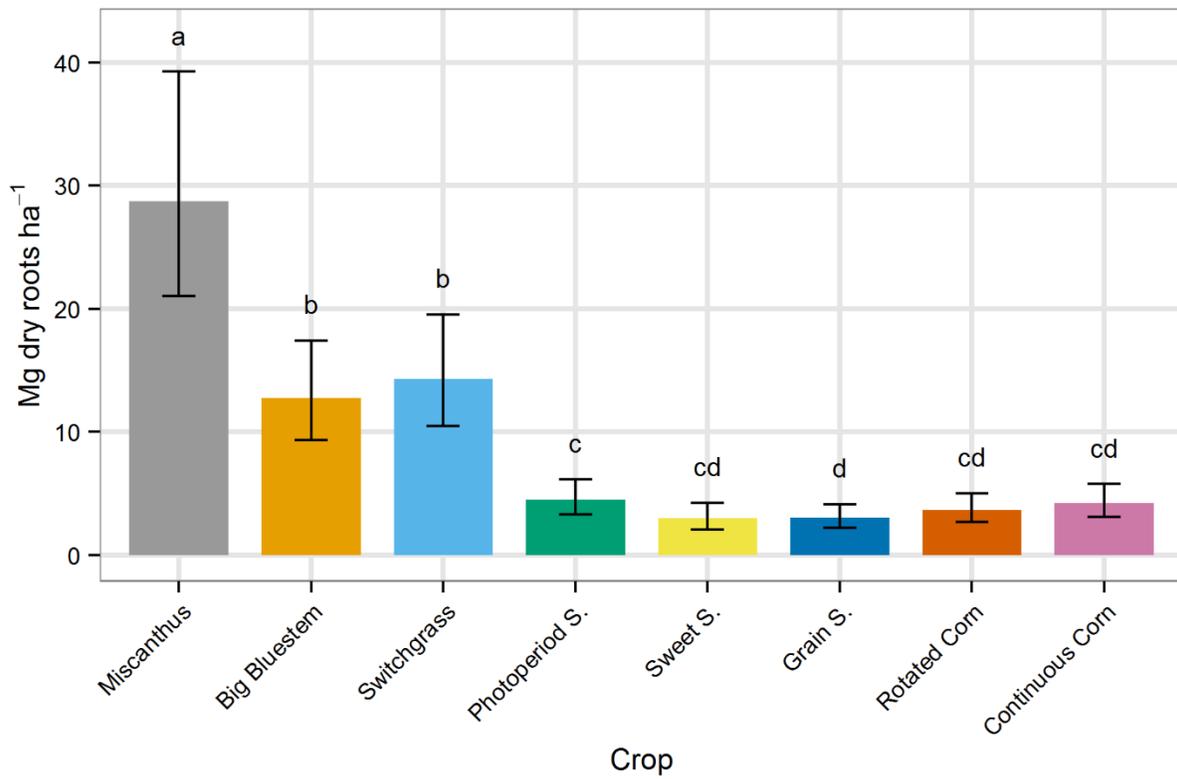


Figure 2.2. Total root stocks from 0-120 cm depth. Error bars represent 95% confidence interval of the median. Bars with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.”

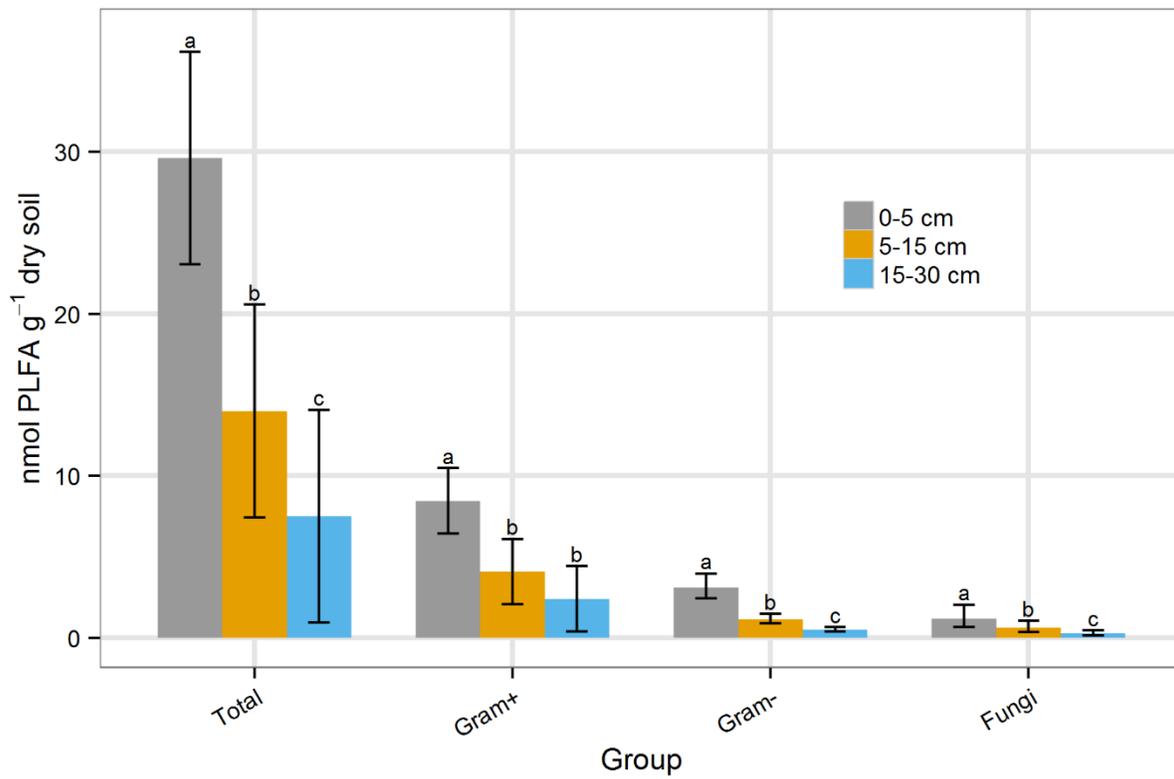


Figure 2.3. Effect of depth averaged over crop on PLFA concentration of total PLFA, gram positive bacteria, gram negative bacteria and fungi. Error bars represent 95% confidence intervals. Bars of the same group with different letters are significantly different ($p < 0.05$).

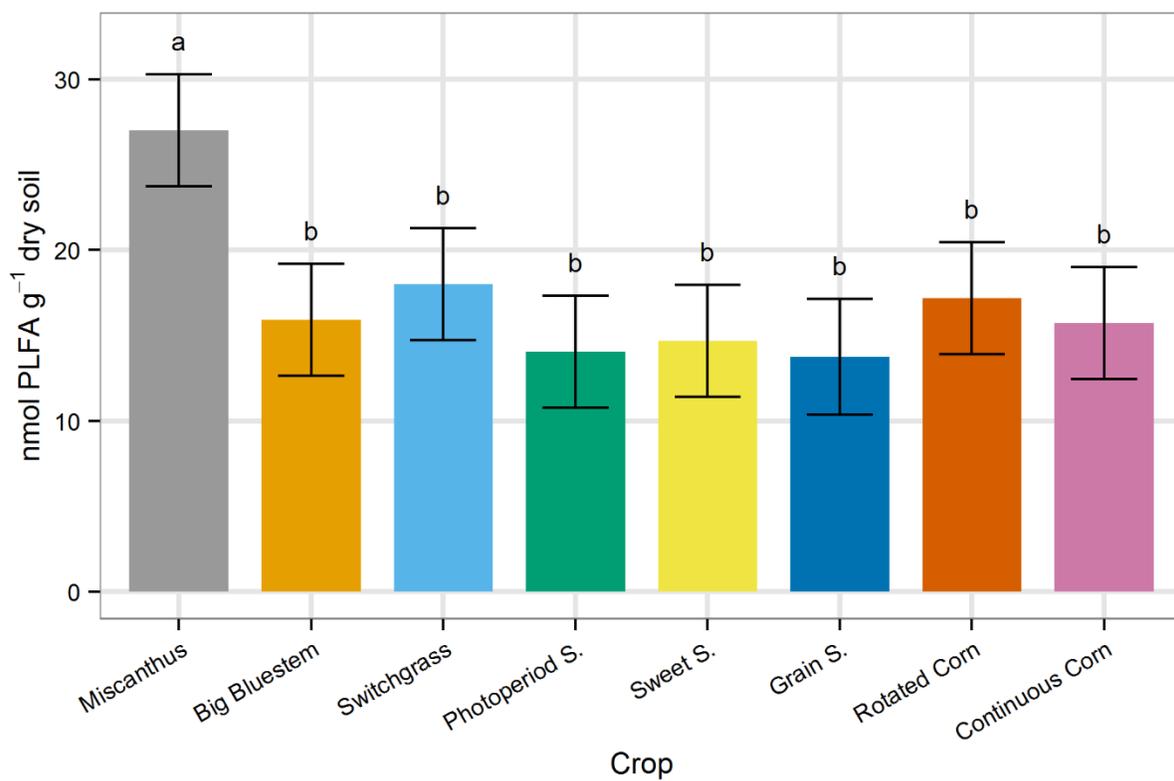


Figure 2.4. Effect of crop averaged over depth on total PLFA concentration. Error bars represent standard error. Bars with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.”

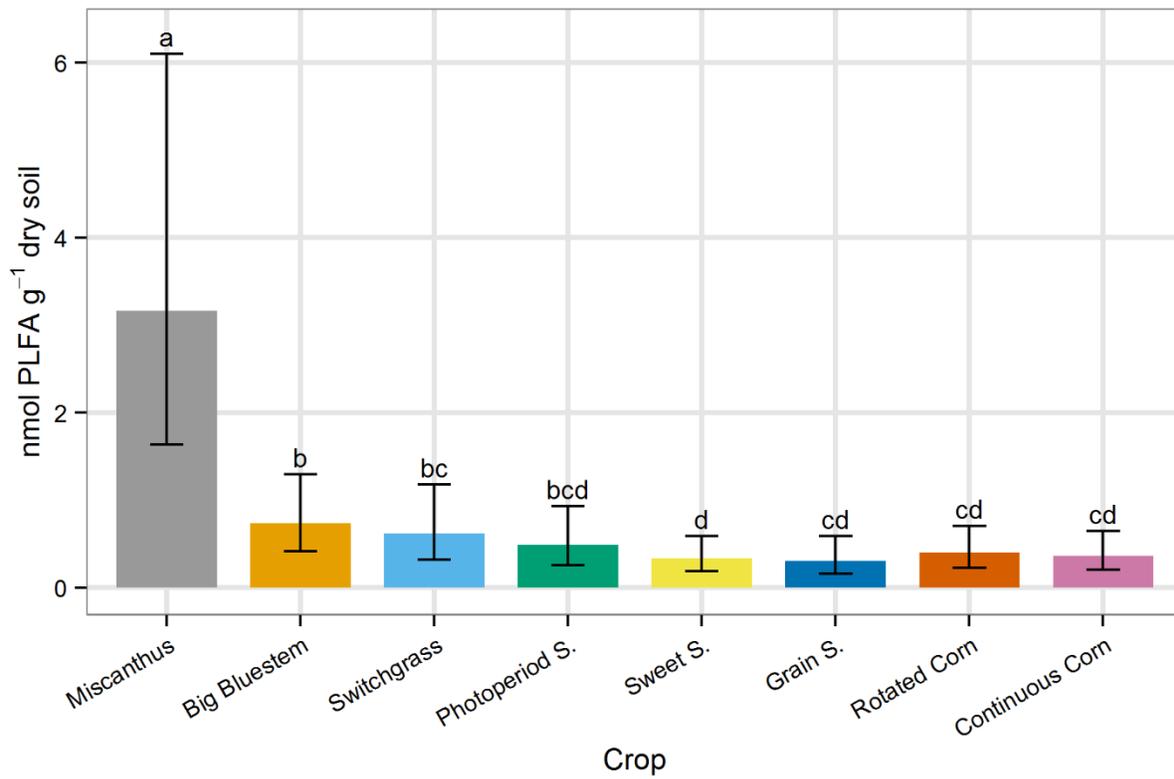


Figure 2.5. Effect of crop averaged over depth on the 18:1 ω 6,9 PLFA biomarker (fungi) concentration. Error bars represent the 95% confidence interval of the median. Bars with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.”

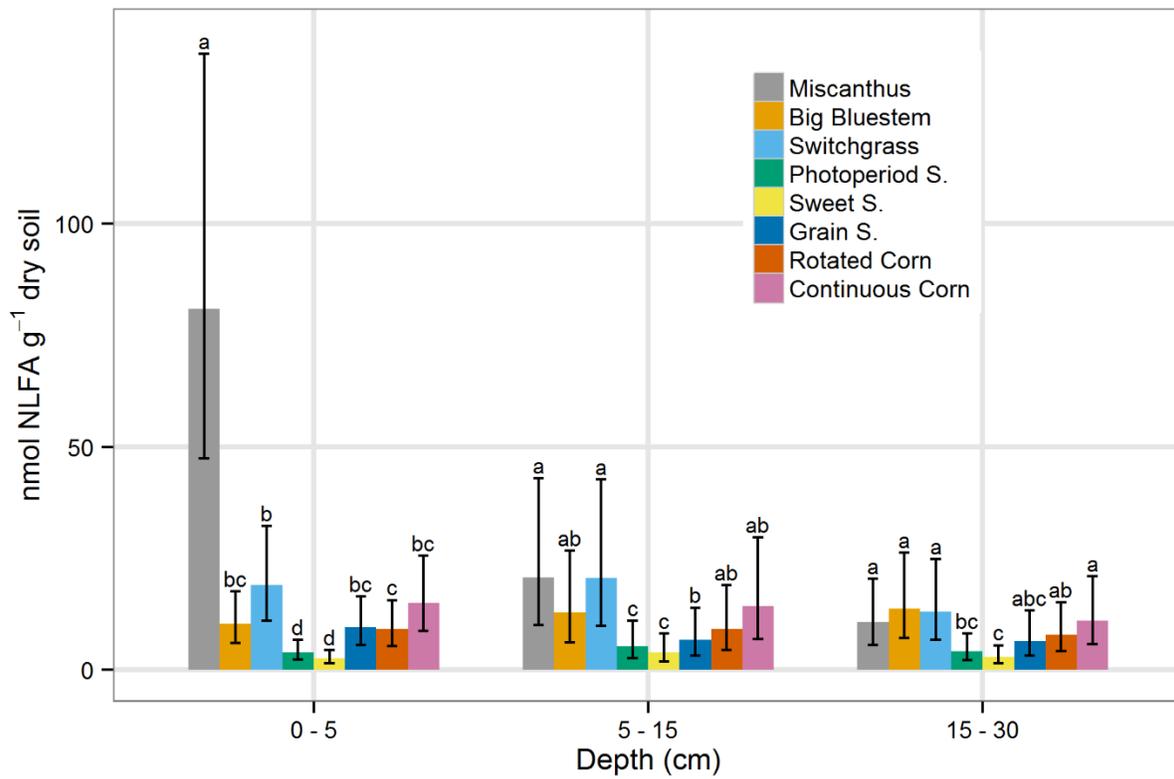


Figure 2.6. Effect of crop and depth on the 16:1 ω 5 NLFA AM biomarker (arbuscular mycorrhizae fungi) concentration. Error bars represent the 95% confidence interval of the median. Bars of the same depth with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.”

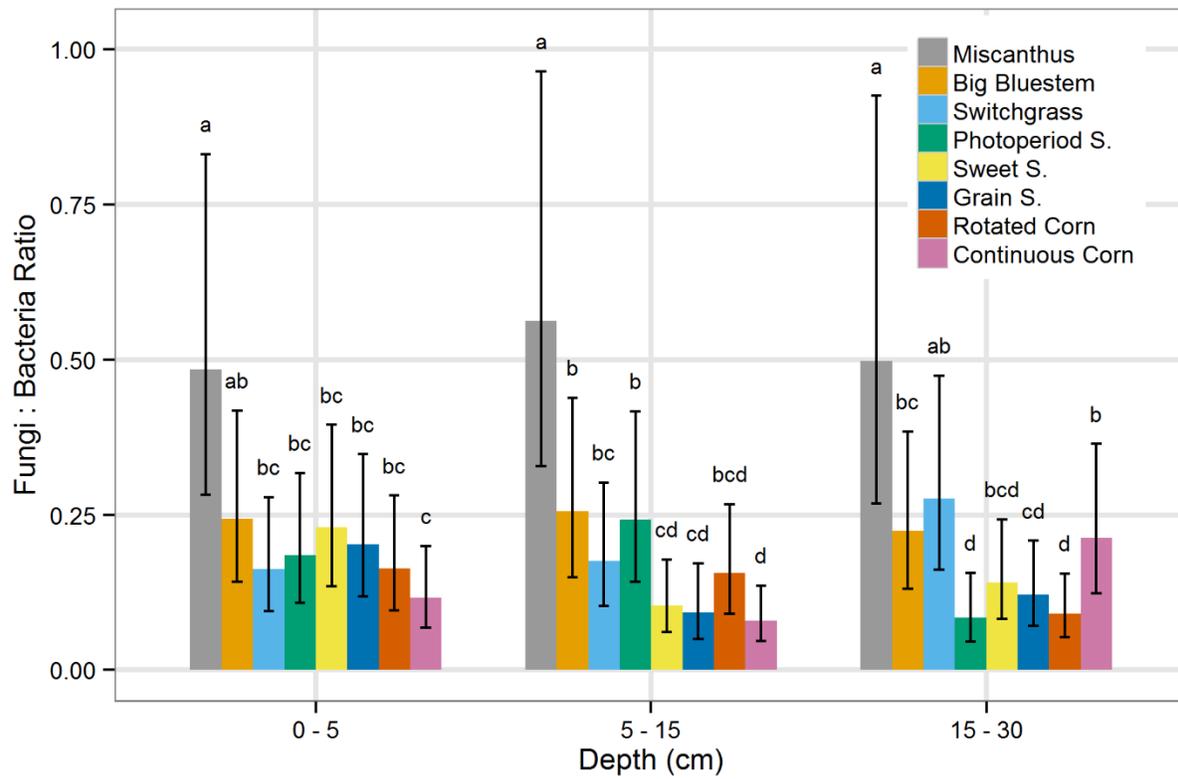


Figure 2.7. Effect of crop and depth on the PLFA fungi:bacteria ratio. Error bars represent the 95% confidence interval of the median. Bars of the same depth with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.”

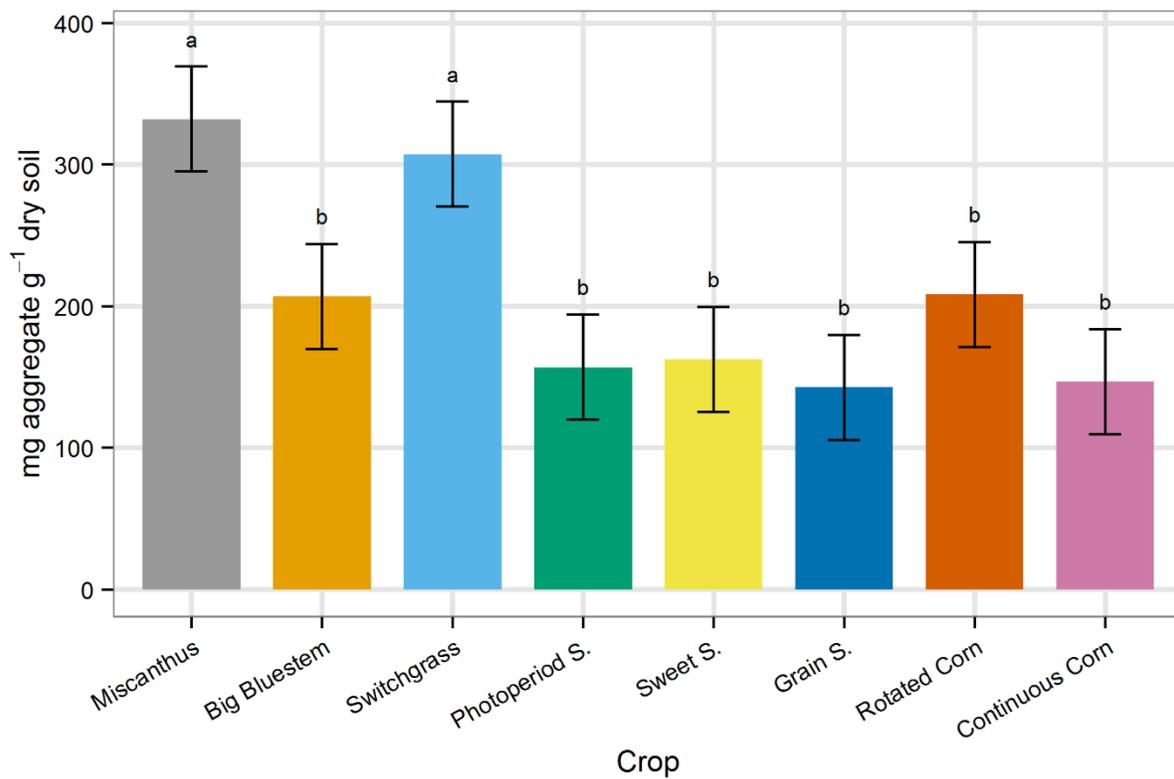


Figure 2.8. Water-stable macroaggregates (>250 μm) by crop at 0-5 cm. Error bars represent standard error. Bars of the same aggregate size with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.”

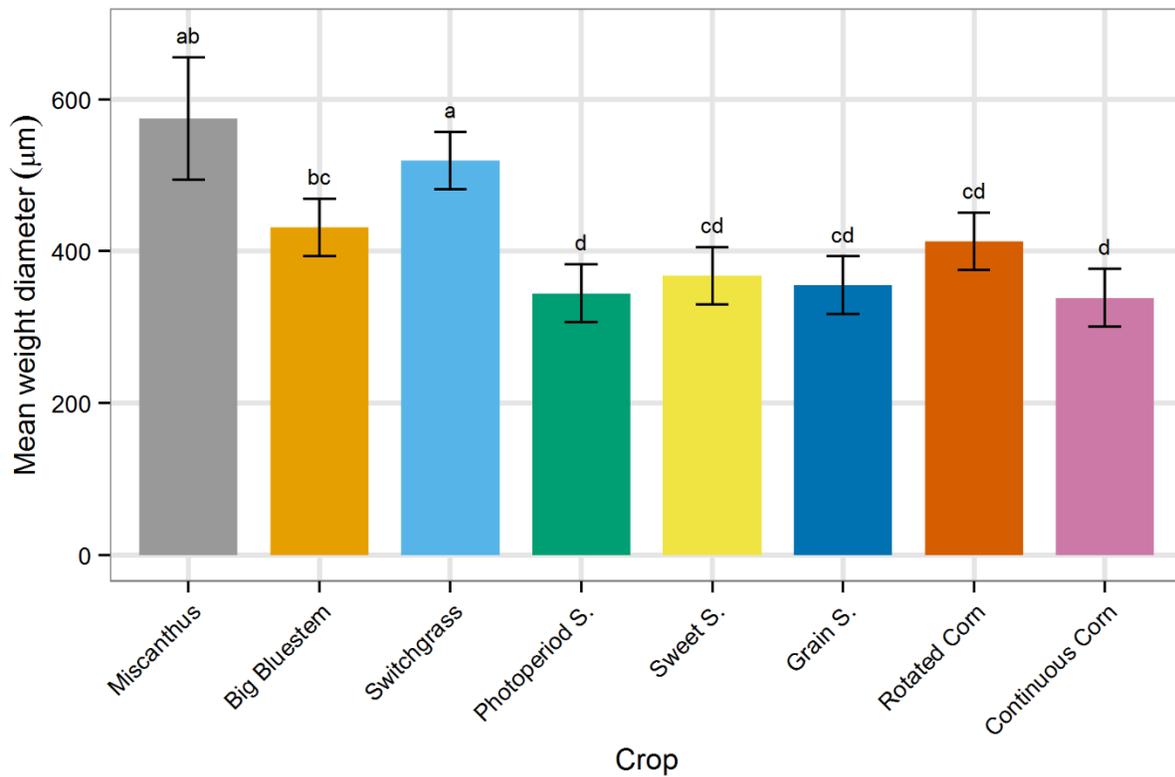


Figure 2.9. The effect of crop on water-stable aggregate mean weight diameter averaged over depth. Error bars represent standard error. Bars with different letters are significantly different ($p < 0.05$). Model variance was allowed to vary between crops, resulting in a larger standard error in miscanthus than in big bluestem and switchgrass. This resulted in no significant difference between miscanthus and big bluestem, while switchgrass and big bluestem were significantly different. Sorghum is abbreviated with “S.”

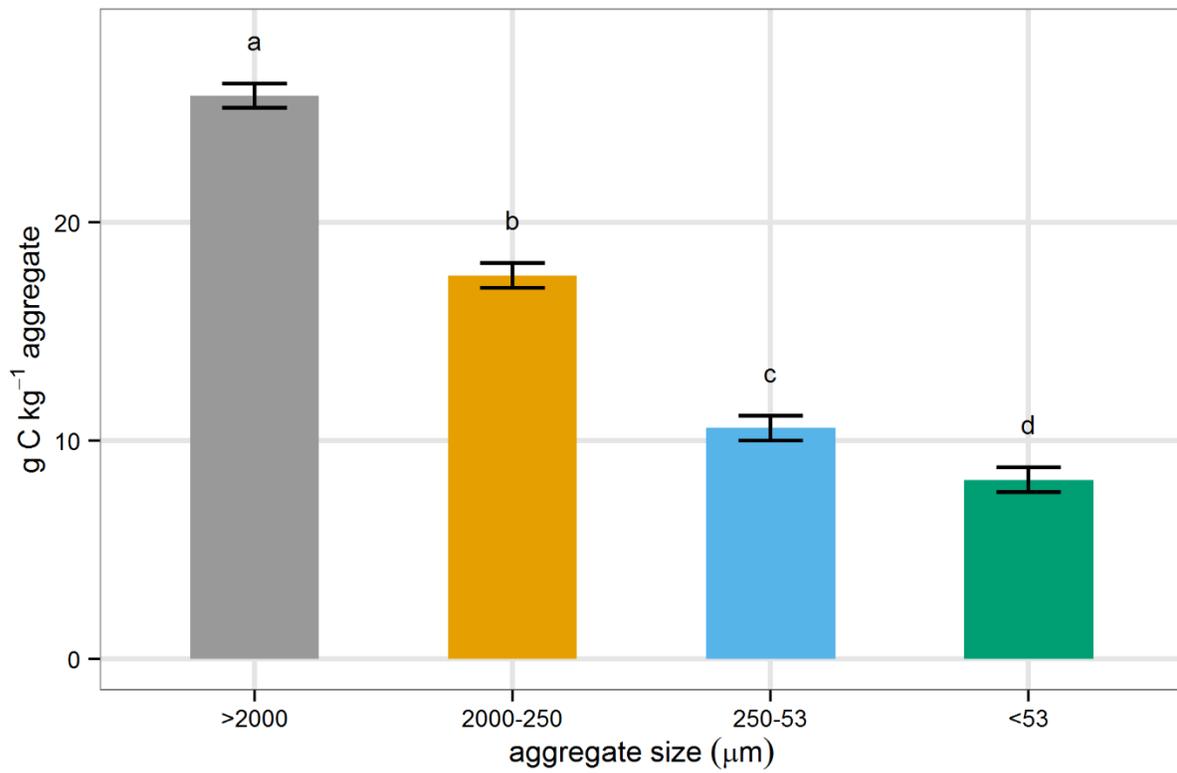


Figure 2.10. The effect of water-stable aggregate size on SOC concentration in aggregates, averaged over crop and depth. Error bars represent standard error. Bars with different letters are significantly different ($p < 0.05$).

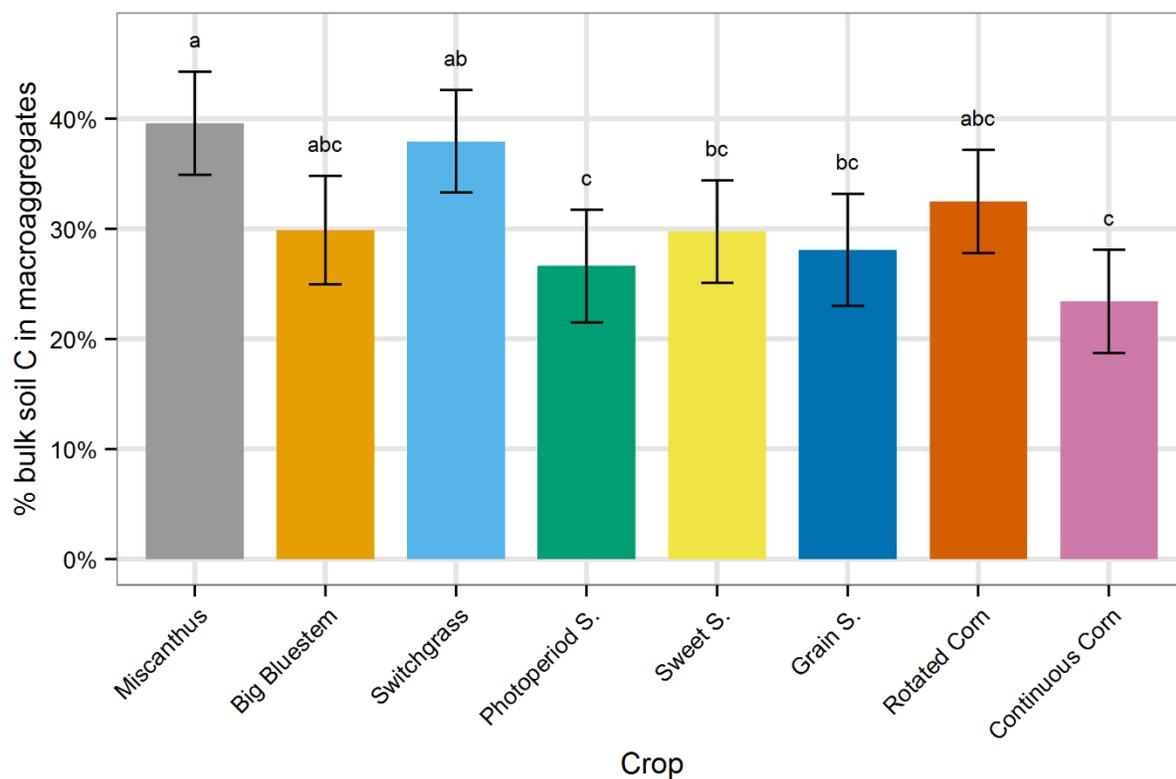


Figure 2.11. Effect of crop on the percent of bulk soil organic carbon contained within macroaggregates (>250 μm) averaged over depth. Error bars represent standard error. Bars with different letters are significantly different ($p < 0.05$). Sorghum is abbreviated with “S.”

Table 2.1. Annual aboveground biomass production.

Year	2007	2008	2009	2010	2011	2012	2013
Crop	Aboveground Biomass (Mg dry matter ha ⁻¹)						
Miscanthus	2.7 D†c‡	11.8 Db	12.1 Bb	9.7 BCb	19.1 Aa	.	19.1 ABa
Big Bluestem	3.8 Db	7.7 Cab	4.7 Db	3.8 Eb	7.9 Da	.	.
Switchgrass	3.6 Dd	7.2 Dcd	9.1 BCDbc	7.9 CDc	12.5 Bab	.	14.3 Ca
Photoperiod Sorghum	26.8 Aa	22.2 Bb	7.5 CDc	20.8 Ab	21.2 Ab	19.0 Ab	19.8 Ab
Sweet Sorghum	27.6 Ab	32.0 Aa	9.1 BCDD	20.1 Ac	19.4 Ac	18.7 Ac	19.8 Ac
Grain Sorghum§	13.7 Cb	19.0 BCa	20.9 Aa	8.9 BCc	13.1 BCb	12.7 Bbc	9.1 Dc
Rotated Corn	21.3 Ba	20.4 BCa	13.2 Bbc	12.1 Bc	11.3 Cdc	13.1 Bbc	16.3 BCb
Continuous Corn	20.6 Ba	17.3 Cab	11.1 BCcd	7.3 DEd	8.5 Dd	9.7Bd	14.7 Cbc

† Within column, means followed by same capital letter are not significantly different according to Fisher's LSD (0.05)

‡ Within row, means followed by same lower-case letter are not significantly different according to Fisher's LSD (0.05)

§ Dual purpose sorghum was planted in 2007 and 2008. From 2009 onward, dual purpose sorghum plots were planted with grain sorghum

Table 2.2. ANOVA results for Phospholipid fatty acid and water-stable aggregate data.

Variable	Group	Crop	Depth	Crop x Depth
		p-values		
PLFA	AMF	0.0150	<0.0001	0.0183
	Fungi	<0.0001	<0.0001	0.6403
	Gram -	0.0826	<0.0001	0.1660
	Gram +	0.6437	<0.0001	0.3375
	F:B	<0.0001	0.2757	0.0327
	Total	0.0133	<0.0001	0.0980
NLFA	AMF	<0.0001	0.0153	0.0032
	Fungi	0.0200	<0.0001	0.7765
WSA Distribution	>250 μ m	0.0580	0.1853	0.0004
	<250 μ m	0.0039	0.2966	0.0067
Mean Weight Diameter		0.0002	0.7394	0.1724
WSAC:SOC	>250 μ m	0.0332	0.4918	0.5567
	<250 μ m	<0.0001	<0.0001	0.0088

Table 2.3. ANOVA p-values for average annual change in soil organic carbon stocks by depth.

Depth	0-5	0-15	0-30	0-45	0-60	0-75	0-90
	p-value						
SOC change	<0.0001	0.0003	0.3968	0.5761	0.6626	0.7458	0.6430

Table 2.4. Soil organic carbon stocks in 2009, 2014 and average annual change in soil organic carbon stocks (0-15 cm).

Crops	SOC Stocks				Annual Change	
	2009		2014		Mg C ha ⁻¹ yr ⁻¹	
Miscanthus	25.31 B‡	(1.78) ¶	33.17 Aa†	(1.78)	1.97 a	(0.27)
Big Bluestem	26.72 B	(1.78)	30.97 Aab	(1.78)	1.06 b	(0.27)
Switchgrass	26.83 B	(1.94)	30.91 Aab	(1.78)	0.86 bc	(0.31)
Photoperiod Sorghum	27.51	(1.78)	28.12 bc	(1.78)	0.15 cd	(0.27)
Sweet Sorghum	27.90	(1.78)	27.08 c	(1.78)	-0.20 d	(0.27)
Grain Sorghum	28.06	(1.94)	27.62 bc	(1.78)	-0.26 d	(0.31)
Rotated Corn	27.68	(2.21)	28.66 bc	(1.78)	0.76 bc	(0.38)
Continuous Corn	28.91	(1.94)	28.53 bc	(1.78)	0.00 cd	(0.31)
ANOVA						
Source			p-value			
Crop			0.8480		0.0003	
Year			0.0046		-	
Crop x Year			0.0170		-	

† Within column, means with different lowercase letters are significantly different according to Fisher's LSD (0.05)

‡ Crop means with different uppercase letter are significantly different between years

¶ Standard error

Table 2.5. Pearson correlation coefficients among soil organic carbon, root density, arbuscular mycorrhizae, saprophytic fungi, fungi:bacteria ratio and aggregate mean-weight diameter. Analyses were performed across all crop systems and depths (0-30 cm) unless stated otherwise.

Variables	SOC	Roots	AM	Fungi	F:B
SOC (g kg ⁻¹)	-				
Roots (mg cm ⁻³)	0.549**	-			
AM (nmol g ⁻¹)	0.265*	0.603**	-		
Fungi (nmol g ⁻¹)	0.298*	0.574**	0.862**	-	
F:B	-0.141	0.179	0.320*	0.500*	-
MWD (µm)	0.474*	0.529*	0.464*	0.436*	0.559**

* Significant at 0.05 probability level

** Significant at 0.001 probability level

† Correlation at 0-5 cm depth only

Chapter 3 - Nitrous oxide emissions from biofuel cropping systems

Abstract

Burning of fossil fuels in the transportation sector accounts for 28% of U.S. greenhouse gas (GHG) emissions. Substitution of cellulosic biofuel in place of conventional gasoline or diesel could reduce GHG emissions from transportation. However, the effectiveness of cellulosic biofuel in reducing emissions depends on emissions during production, transportation and use of biofuel. Emissions of nitrous oxide (N_2O) can have a large impact on the GHG balance of cellulosic ethanol, thus quantifying N_2O emissions is needed from soils producing different cellulosic biofuel crops. The objectives of this study were: (i) to measure N_2O emissions and yields of potential cellulosic biofuel cropping systems, and (ii) to characterize the temporal variations in N_2O emissions in these cropping systems. Annual N_2O emissions were measured in corn/soybean and photoperiod-sensitive sorghum/soybean rotations as well as in switchgrass and miscanthus from 2011-2013 in Manhattan, KS using static chambers.

Photoperiod sensitive sorghum and miscanthus had the highest biomass, producing 19-21 Mg ha^{-1} . No consistent significant differences were found in N_2O emissions between crop types, though miscanthus tended to have the lowest emissions. Most N_2O was emitted during large events of short duration (1-3 days) that occurred after high rainfall events with high soil NO_3^- . In 2011 and 2012, a majority of N_2O was emitted during the growing season. However, in 2013, 30-50% of N_2O was emitted after September which was attributed to freeze-thaw cycles. There was a weak relationship between IPCC Tier 1 N_2O estimates and measured emissions, and the IPCC method tended to underestimate emissions.

Introduction

The transportation sector contributes 28% of total greenhouse gas (GHG) emissions in the U.S. (USEPA, 2014). A number of strategies are proposed to reduce GHG emissions in this sector, including the partial substitution of biofuels for fossil fuels. To encourage the implementation of biofuels in the U.S., Congress passed the Energy Independence and Security Act (EISA) in 2007 which includes a mandate for the production of 60 billion liters per year (16 billion gallons) of cellulosic ethanol by 2022 (U.S. Congress, 2007).

Potential feedstocks for cellulosic ethanol include agricultural residues such as corn or wheat stover as well as dedicated energy crops. Dedicated energy crops could include annual crops such as photoperiod-sensitive sorghum and perennial grasses such as switchgrass and miscanthus (USDOE, 2011). Photoperiod-sensitive sorghum commonly yields 20-30 Mg ha⁻¹ with yields reported as high as 35 Mg ha⁻¹ (Propheter et al., 2010; Maughan et al., 2012a). Perennial grass yields can also be high. Average switchgrass yields range from 9-13 Mg ha⁻¹ and miscanthus 30-60 Mg ha⁻¹ (Heaton et al., 2008; Wulschleger et al., 2010). The potentially high productivity of dedicated energy crops could make them an important feedstock for biofuel production.

Even though biofuels are a promising alternative fuel source substantial quantities of GHGs are emitted throughout the biofuel production cycle. To ensure that these emissions do not offset the mitigative capacity of biofuel production as mandated in the EISA, the act stipulates that cellulosic biofuels must have GHG emissions 60 % below those of conventional fossil fuels (U.S. Congress, 2007). This mandate highlights the need to accurately estimate GHG emissions associated with the production and use of biofuels to ensure that they are actually

reducing GHG emissions, relative to conventional fossil fuels. This stipulation of the EISA also creates the need for reliable methods of estimation.

Life Cycle Assessment (LCA) is an environmental accounting method designed for the evaluation of product chains and has been widely used to quantify GHG emissions from different biofuel systems (Bessou et al., 2011; von Blottnitz and Curran, 2007). Life Cycle Assessment of biofuels generally include GHG emissions from the production and transportation of the feedstock, conversion of the feedstock to biofuel, fuel transportation and distribution, and tailpipe emissions (USEPA, 2010). Accounting for emissions from these steps requires modeling, data inputs and system assumptions. Unfortunately, these often differ greatly, causing LCA results to be highly variable (Cherubini, 2010; Bessou et al., 2011).

Much of the difficulty in performing LCA of biofuel systems is due to the difficulty of applying the LCA method to the agricultural phase of biofuel production (Bessou et al., 2011). Because agricultural systems depend on environmental factors like climate and soils, agricultural data sets are time and space dependent, making it difficult to find high-quality data that is representative of a particular agricultural system (Bessou et al., 2011). One component of agricultural systems that can be especially important, but difficult to predict, is N₂O emissions from agricultural soils. Nitrous oxide emitted from soils during biofuel crop production could be a potentially large source of GHG emissions. Nitrous oxide has a global warming potential 298 times that of CO₂ (Myhre et al., 2013). Atmospheric levels of N₂O have increased nearly 20% since pre-industrial times, and it is the third largest radiative forcing agent (Hartmann et al., 2013). This increase is primarily from anthropogenic emissions, of which agricultural soils contribute over 50% (Robertson, 2004).

Because of the high global warming potential of N₂O, small quantities released during feedstock production can have a large impact on the GHG balance of ethanol. In a life cycle assessment of ethanol produced from corn stover, miscanthus and switchgrass, Wang et al. (2012) found N₂O emissions from soils account for 0, 14 and 32% of GHG emissions in cellulosic ethanol, respectively. Despite the potentially large contribution of N₂O to the GHG balance of cellulosic ethanol, there is much uncertainty regarding the quantities emitted from these crops (Wang et al., 2012).

The potentially large contribution of N₂O to GHG emissions and the uncertainty of N₂O emissions suggests that care should be taken in selecting the N₂O inputs into LCA models of biofuels. Currently, many studies rely on simple emission factors, such as the IPCC Tier 1 factor (Smeets et al., 2009; Don et al., 2012), which assumes that 1% of soil and fertilizer N is lost as N₂O (De Klein et al., 2006). Various factors have been used in LCAs, and oftentimes the selection of the N₂O emission factor can determine whether or not the GHG balance of biofuel is higher or lower than that of conventional fossil-based fuels (Crutzen et al., 2008; Mosier et al., 2009).

Reports have criticized the use of emission factors as estimates of direct N₂O emissions (Smeets et al., 2009; Klemetsson and Smith, 2011; Reijnders and Huijbregts, 2011; Don et al., 2012). Emission factors such as the IPCC Tier 1 estimate ignore many factors that control N₂O emissions. Because many biofuel LCAs are performed for specific crops at regional scales or smaller, ignoring these factors could ignore critical differences in N₂O emissions that arise from variations in soil, climate and local management practices. For these reasons, some researchers promote the use of process-based models like DAYCENT or DNDC to estimate N₂O emissions (Adler et al., 2007; Kim and Dale, 2009; Smeets et al., 2009). These process-based models can

provide N₂O estimates that account for differences in the various drivers behind N₂O production (Li, 2000; Del Grosso et al., 2006).

For many first-generation biofuel feedstocks, such as corn and soybean, there is a large body of data from field measurements of N₂O emissions which can be used as a basis of comparison in modeling approaches in LCA. Unfortunately, there are few field studies measuring N₂O from soils for 2nd generation biofuel feedstock production (Don et al., 2012). This makes it difficult to predict N₂O emissions from these crops and to know the accuracy of various N₂O models to predict N₂O emissions.

More field studies measuring N₂O emissions from cellulosic biofuel crops are needed to understand biofuels potential to mitigate GHG emissions in the transportation sector. Therefore, the objectives of this study were (i) to measure N₂O emissions and yields of potential cellulosic biofuel cropping systems and (ii) to characterize the temporal variations in N₂O emissions in these cropping systems.

Materials and Methods

Study Site

This experiment was conducted from 2011 to 2013 at the Kansas State University Agronomy Research Farm in Manhattan, Kansas (39°11' N, 96°35' W). The soil types on the study site were Ivan, Kennebec, and Kahola silt loams (fine-silty, mixed, superactive, mesic Cumulic Hapludolls). Soils at the study site had an average soil organic C content of 14.5 g kg⁻¹ and a pH of 6.6 (Propheter et al., 2010). In 2007, corn (*Zea mays* L.), photoperiod-sensitive sorghum (*Sorghum bicolor* (L.) Moench), switchgrass (*Panicum virgatum* L.) and miscanthus (*Miscanthus x giganteus*) were planted at the study site. Soybean [*Glycine max* (L.) Merr.] was also planted as part of a corn-soybean and sorghum-soybean rotations. The study was arranged

as a randomized complete block design with four replications. All crops were under no-tillage management. Additional details on crop establishment can be found in (Propheter et al., 2010). Cultivars used included Sorghum Partners '1990CA' photoperiod-sensitive sorghum, KSU Foundation 'KS3406RR' soybean and Dekalb 'DKC63-49' corn. Perennial grass species were 'Kanlow' switchgrass and Miscanthus.

Corn was planted on 2 May, 11 May, and 30 April in 2011, 2012, and 2013, respectively. Photoperiod-sensitive sorghum was planted on 6 June 2011 and 2012 and 24 May 2013. Soybean was planted on 6 June 2011 and 2012 and 26 May 2013. Corn grain and stover were harvested on 6 Sept., 13 Oct. and 30 Sept. in 2011, 2012 and 2013 respectively. Sorghum was harvested on 21, 13, 16 Oct. in 2011, 2012 and 2013, respectively. Soybean biomass was harvested after flowering (28 July and 21 Aug. 21 in 2012 and 2013, respectively). Soybean grain was harvested on 13 and 8 Oct. in 2012 and 2013, respectively. Soybean was not harvested in 2011. Switchgrass and miscanthus were harvested on 6 and 8 Nov. in 2011 and 2013, respectively.

On 12 May 2011, 78 kg N ha⁻¹ was applied as urea to corn, sorghum, miscanthus and switchgrass. On 1 June 2011, 49.5 kg P ha⁻¹ and 44.5 kg N ha⁻¹ were applied as diammonium phosphate (DAP) to all crops. On the evening of 2 June, heavy rains caused minor flooding. Based on soil tests much of the DAP-N entered the soil, but the P had runoff. A second DAP application was made at the same rate on 15 June. On 11 May 2012, 168 and 112 kg N ha⁻¹ was applied as urea to corn and sorghum, respectively. On 3 June 2013, 20 kg P ha⁻¹ and 10 kg N ha⁻¹ were applied to all crops as monoammonium phosphate (MAP). On 6 and 7 June 2013, 158 and 102 kg N ha⁻¹ was applied as urea to corn and sorghum, respectively, and 74 kg N ha⁻¹ was applied as urea to miscanthus and switchgrass. Grain yield and biomass yield were expressed on

a dry weight basis. To simulate crop harvest for bioenergy production, all plant biomass was removed from corn, sorghum, miscanthus and switchgrass each year.

In spring 2012, an application of glyphosate was mistakenly sprayed on the switchgrass and miscanthus treatments. The grasses were promptly mowed to prevent glyphosate uptake into the plant roots and rhizomes. Within weeks of mowing, the grass re-emerged and stand density appeared unaffected by the glyphosate application. However, information on miscanthus and switchgrass were omitted from the study in 2012.

Nitrous Oxide Measurements

Fluxes of N₂O were measured from April 2011 until March 2014 using static, vented, polyvinyl chloride (PVC) chambers (7.5 cm high x 20 cm diameter), as described in Hutchinson and Mosier (1981). PVC anchors (20 cm high x 20 cm diameter) were placed randomly within the perennial grasses and placed 3 cm from rows in corn and sorghum, such that the chamber did not interfere with plant growth but covered soil in both the row and interrow. Anchors were driven approximately 15 cm into the soil. Samples were collected 1-2 times per week during the growing season and once every 2-4 weeks for the rest of the year. During the winter months, on sampling dates where the soil was determined to be frozen, it was assumed there was no N₂O flux. On measurement days, chambers were installed on anchors and 30 mL gas samples removed from each chamber after 0, 15, and 30 minutes and injected into pre-evacuated 12 mL Labco Exetainers (Labco Limited, United Kingdom) with Labco grey butyl rubber septa. Gas samples were transported back to the laboratory and N₂O concentrations were determined by gas chromatography (GC) using a Shimadzu Model 14A GC (Shimadzu Corporation, Japan) equipped with a ⁶³Ni electron capture detector and a stainless steel column (0.318-cm dia. by 74.5 cm long) with Poropak Q (80-100 mesh). The GC was calibrated daily using analytical-

grade standards containing 0.2, 3.5, and 15.3 parts per million N₂O. The concentration of N₂O in each sample was converted to μg N₂O-N m⁻² using equation 3.1:

$$X = \frac{CPVM}{ART} \quad [3.1]$$

where X is μg N₂O-N m⁻², C was the volumetric concentration of N₂O (μL N₂O L⁻¹), P was the atmospheric pressure at 304.8 m (0.965 atm), V was chamber volume (L), M was the mass of N in N₂O (28 μg N μmol⁻¹ N₂O), A was the chamber surface area (m²), R was the Universal Gas Constant (0.08206 atm μL μmol⁻¹ K⁻¹), and T was air temperature (K) during sampling. Fluxes of N₂O were calculated from linear regression of the converted N₂O values over time. Total annual flux was estimated using linear interpolation between sampling points and calculation of the area under the curve using equation 3.2:

$$\text{Cumulative } N_2O \text{ (g N ha}^{-1}\text{)} = \sum_i^n \frac{(F_i + F_{i+1})}{2} (t_{i+1} - t_i) \quad [3.2]$$

where F_i and F_{i+1} were the N₂O-N fluxes (g ha⁻¹ day⁻¹) at sampling points i and $i+1$; t_i and t_{i+1} were the sampling dates (Julian date) at sampling points i and $i+1$; and n was the number of sampling points taken in a given year. Biomass-scaled fluxes were calculated by dividing the total annual flux by the dry biomass yield of each crop.

Ancillary Measurements

On each gas sampling date, volumetric soil water content and soil temperature were measured. Volumetric soil water content was measured at 0-5 cm soil depth using Stevens Hydra Probe II soil sensor (Stevens Water Monitoring Systems, Inc.). Soil water content was converted to percent water-filled pore space using soil bulk density values. Soil temperature was measured at a soil depth of 5 cm. Soil samples were taken at 0-5 and 5-15 cm soil depths 6-8 times during the growing season for determination of NO₃⁻-N and NH₄⁺-N. Soil NO₃⁻-N and NH₄⁺-N were determined using a continuous flow analyzer (Alpkem Corp., Bulletins A303-S021

and A303-S170, Clackamas, OR) after extraction with 1 M KCl (soil:solution ratio 1:5). Daily precipitation, air temperature and 5 cm soil temperature were collected at a meteorological station less than 1 km from the study site (Kansas State Univ., 2014). Number of days that experienced freezing soil conditions were estimated using weather station soil temperature data. Days in which the 5 cm maximum daily soil temperature did not exceed 0°C were assumed to have frozen soil. On several days with freezing conditions, soil temperatures were measured at the experimental site and found to correspond well to soil temperature from the weather station.

IPCC Tier 1 Estimates

Direct emissions of N₂O due to crop cultivation were estimated using the Tier 1 methodology outlined by the IPCC (De Klein et al., 2006). Direct emissions of N₂O were estimated on a per hectare basis using equation 3.3:

$$N_2O_{Direct} - N = (F_{SN} + F_{ON} + F_{CR} + F_{SOM}) \times EF_1 \quad [3.3]$$

where $N_2O_{Direct} - N$ was the annual emission of N₂O (kg N₂O-N yr⁻¹), F_{SN} was the amount of synthetic fertilizer N applied to soils (kg N yr⁻¹ ha⁻¹), F_{ON} was the annual amount of animal manure, compost or other organic N additions applied, F_{CR} was the amount of N in above and belowground crop residues returned to soils (kg N yr⁻¹ ha⁻¹), F_{SOM} was the annual amount of N in mineral soils that is mineralized during loss of soil organic matter as the result of changes from land management (kg N yr⁻¹ ha⁻¹) and EF_1 was the emission factor for N₂O emissions from N inputs (kg N₂O-N kg⁻¹ N input). The IPCC value assigned to EF_1 is 0.01 with an uncertainty range of 0.003-0.03. Because no organic amendments were added in this study $F_{ON} = 0$.

F_{CR} was estimated using equation 3.4:

$$F_{CR} = (Area - Area_{burnt} \times C_f) \times Frac_{Renew} \times [AG_{DM} \times N_{AG} \times (1 - Frac_{Remove}) + (AG_{DM} \times 1000 + Crop) \times R_{BG-BIO} \times N_{BG}] \quad [3.4]$$

where $Area$ was the total annual area harvested of the crop ($ha\ yr^{-1}$), $Areaburnt$ was the annual area of crop area burnt ($ha\ yr^{-1}$), $Frac_{Renew}$ was the fraction of total area that was renewed annually ($1/X$, where X was the number of years the crop was grown before replanting), AG_{DM} was the annual aboveground dry matter production ($kg\ biomass$), N_{AG} was the N content of above-ground residues for a given crop ($kg\ N\ kg^{-1}\ biomass$), $Frac_{Remove}$ was the fraction of above-ground residues of crop T removed annually ($kg\ N\ kg^{-1}\ crop-N$), R_{BC-BIO} was the ratio of below-ground residues to total above-ground biomass ($kg\ root\ biomass\ kg^{-1}\ above\ ground\ biomass$) and N_{BG} was the N content of below-ground residues for a given crop. Because no burning of crops or crop residues took place in this study and the calculation was done on a per hectare basis, $(Area - Areaburnt * C_f) = 1$. $Frac_{Renew}$ was 1 for all annual crops and 10 for switchgrass and miscanthus. This assumes that producers would harvest switchgrass and miscanthus for 10 yrs before replanting. The total harvested stover (or dry biomass at flowering for soybean) for each crop was used for AG_{DM} . For soybean, $Frac_{Remove} = 0$, since all stover was left on the field. $Frac_{Remove}$ was estimated for other the crops by estimating remaining residue using measurements made after harvest in 2011 and 2012 using a 72 x 60 cm quadrat and adding this to the harvested stover to get total biomass. Harvested stover was divided by total biomass to get $Frac_{Remove}$. The measured grain yield was used for $Crop$ in soybean and corn. The default N_{AG} , N_{BG} and R_{BG-BIO} values provided by the IPCC for maize, soybean, sorghum and perennial grasses were used for corn, soybean, photoperiod-sensitive sorghum, switchgrass and miscanthus, respectively. Because soybean yield and biomass were not measured in 2011, soybean yields from a nearby field were used to estimate $Crop$ for 2011 soybean, and equation 3.5 from De Klein et al. (2006) used to estimate AG_{DM} .

$$AG_{DM(soy)} = \left(\frac{Crop}{1000} \right) \times 0.93 + 1.35 \quad [3.5]$$

F_{SOM} was estimated from equation 3.6:

$$F_{SOM} = \Delta C_{Mineral,LU} \times \frac{1}{R} \times 1000 \quad [3.6]$$

where $\Delta C_{Mineral,LU}$ was the average annual loss of SOC for a given crop and R was the C:N ratio of the organic matter. Unpublished values of long-term SOM changes and SOM C:N from the study site were used for $\Delta C_{Mineral,LU}$ and R , respectively, such that F_{SOM} was 22 and 60 kg N ha⁻¹ yr⁻¹ for corn/soybean and photoperiod-sensitive sorghum/soybean rotations, respectively.

Switchgrass and miscanthus gained SOC over the course of the study, which is not accounted for in Tier 1 methodology.

IPCC estimates were calculated separately for each replicate in each crop, thus reflecting the variation of N inputs from grain and stover production. This allowed for statistical comparison of IPCC estimates to measured emissions.

Statistical Analysis

Differences in biomass yield, stover yield, grain yield, total annual N₂O emissions, and biomass-scaled N₂O emissions were evaluated by ANOVA using PROC GLIMMIX (SAS 9.3, SAS® Institute Inc., Cary, NC, USA). ANOVA for these variables was conducted for each year, with crop type as a fixed effect and block as a random effect. Soil nitrate and ammonium were analyzed by year using crop, sampling date and crop x sampling date as fixed effects and block as a random effect. Sampling date was modeled as a repeated variable using the first-order antedependence structure when significant covariance between sampling dates was detected. All data were checked for normality and homogeneity of variance. When the assumption of homogeneous variance was not met, model residual variance was allowed to vary using the ‘GROUP’ option in the ‘RANDOM’ statement of GLIMMIX. Non-normal data was logarithmically transformed and means converted back to their original scale for presentation.

Mean separation was performed using Fisher's LSD. Paired t-tests were used to compare IPCC estimates to measured N₂O emissions using the 't.test' function in R 3.2 (R Core Team, Vienna, Austria). Goodness-of-fit was evaluated between measured annual N₂O emissions and IPCC Tier 1 estimates (1% emission factor) using the 'lm' function in R. All statistical comparisons were made at the $\alpha = 0.05$ probability level.

Results

Growing season precipitation (Apr-Oct) in 2011, 2012 2013 was 520, 338, and 550 mm, respectively (Appendix Table B.1). All 3 years were relatively dry compared to the 30 yr average rainfall (727 mm).

Biomass and Grain Yield

In all three study years there was a significant effect of crop on biomass (non-grain). Photoperiod-sensitive sorghum produced the greatest biomass for all three years of the study (Table 3.1). Miscanthus biomass was not statistically different from photoperiod-sensitive sorghum in 2011 and 2013. In 2011, switchgrass biomass was less than photoperiod-sensitive sorghum and miscanthus, but greater than corn. In 2012, corn biomass was significantly less than photoperiod-sensitive sorghum but greater than biomass of both soybean rotations. In 2013 there were no significant differences in biomass between photoperiod-sensitive sorghum, miscanthus, and switchgrass. The 2013 corn biomass was less than that of photoperiod-sensitive sorghum, miscanthus and switchgrass but greater than the stover yield of both soybean rotations. There were no significant differences in biomass between soybean rotations in both 2012 and 2013. In both 2012 and 2013, corn grain yields were significantly greater than grain yields of both soybean rotations. There was no difference between the grain yields of the soybean rotations in both 2012 and 2013.

Soil Nitrogen

There was a significant interaction of crop and sampling date on soil NO_3^- for all 3 yrs at the 0-5 and 5-15 cm depths. The crop by sampling date interaction was significant for soil NH_4^+ during all 3 yrs at the 0-5 cm depth, but only in 2011 at the 5-15 cm depth. In 2012 and 2013 there was no significant effect of crop type on NH_4^+ . Only the sampling date had a significant effect in 2012 and 2013.

Soil NO_3^- at 0-5 cm increased rapidly after fertilizer application, with the highest concentrations typically occurring between June and July (Fig. 3.1). Corn and photoperiod-sensitive sorghum had higher soil NO_3^- than the other crops, though the difference was not always significant for photoperiod-sensitive sorghum. Soil NO_3^- gradually decreased towards background levels as the growing season progressed. In 2011 and 2013, soil NO_3^- in miscanthus minimally increased after fertilizer application and remained low throughout the growing season. In 2012, a slight but significant increase in soil NO_3^- occurred in corn between August and September before dropping to background levels. Soil NO_3^- concentrations at the 5-15 cm depth were of much lower magnitude but followed similar trends to 0-5 cm depth (data not shown).

Soil NH_4^+ at 0-5 cm was similar to, but less pronounced than soil NO_3^- (Fig. 3.4). Soil NH_4^+ concentrations were highest in the weeks following fertilizer application and then decreased quickly to background levels by July (2012, 2013) or August (2011). Soil NH_4^+ after fertilizer application tended to be highest in corn and photoperiod-sensitive sorghum, but differences from the other crops were often not significant. Soil NH_4^+ at 5-15 cm showed few consistent trends over the growing season for all 3 yrs and concentrations were generally $< 5 \mu\text{g NH}_4^+\text{N g}^{-1}$ (data not shown).

Seasonal Emissions

The daily N₂O emissions varied greatly over the study period, with the highest fluxes typically occurring in early summer following N fertilizer application and rainfall events (Figs. 3.2-3.4). The highest N₂O fluxes occurred in late May and early June of 2011 during which approximately 180 mm of precipitation fell within a 2-wk period, resulting in minor flooding and extremely wet soil conditions. Corn, photoperiod-sensitive sorghum, switchgrass and miscanthus all had high emissions during this period which contributed 60-80% of the total N₂O emissions that occurred in 2011. In 2012, the highest fluxes were observed in corn and photoperiod-sensitive sorghum on 16 and 22 June, following rainfall events of 36 and 42 mm, respectively. In 2013, high emissions occurred on 31 May in both soybean rotations and in photoperiod-sensitive sorghum after 27 mm of rainfall but before fertilizer application. Large fluxes occurred in all crops in late June after two rainfall events totaling 27 mm each.

Few N₂O fluxes were observed after September in both 2011 and 2012. In both years, the late autumn and winter months were characterized by relatively cool, dry conditions in which %WFPS rarely rose above 50%. More than 80% (78-88%) of the total annual N₂O emissions occurred in all crops by September 2011 and 86-90% by September 2012 in soybean and corn (Fig. 3.5). A notable exception in 2012 was photoperiod-sensitive sorghum which had two events in February and March of 2013 which contributed approximately 40% to the total annual emissions that year. By September 2013, 87% of total annual emissions had occurred in photoperiod-sensitive sorghum, but only 48-68% had occurred in the soybean, corn, miscanthus and switchgrass. There were large precipitation events in autumn of 2013, followed by N₂O fluxes which contributed to the total emissions for soybean and switchgrass. Low fluxes occurred

in early 2014 in soybean, miscanthus and switchgrass that may have been due in part to prolonged periods of moist soil following soil thawing.

Total Annual N₂O and Biomass-Scaled N₂O

There was a significant effect of crop on total annual N₂O emissions in all three years (Table 3.2). In 2011 the highest annual N₂O emissions were in photoperiod-sensitive sorghum, followed by corn, switchgrass and miscanthus. Of these crops, only miscanthus had emissions significantly lower than photoperiod-sensitive sorghum. Emissions of N₂O in 2011 from both soybean rotations were lower but not significantly different from corn, switchgrass and miscanthus. Emissions from soybean in 2011 were significantly lower than emissions from photoperiod-sensitive sorghum. In 2012 the highest N₂O emissions occurred in photoperiod-sensitive sorghum and corn. Differences between photoperiod-sensitive sorghum and corn in 2012 were not significant. Both photoperiod-sensitive sorghum and corn had significantly higher emissions than soybean in 2012. In 2013, soybean in rotation with corn had the highest emissions, followed by soybean in rotation with photoperiod-sensitive sorghum and switchgrass, but differences between these three crops were not significantly different. Soybean in rotation with corn had significantly higher emissions than corn, photoperiod-sensitive sorghum and miscanthus in 2013. Emissions of N₂O in 2013 from soybean in rotation with photoperiod-sensitive sorghum and switchgrass were significantly higher than emissions from corn and miscanthus, but were not significantly different than emissions in photoperiod-sensitive sorghum. In 2013, N₂O emissions in miscanthus were the lowest, but were not significantly different from emissions in photoperiod-sensitive sorghum and corn. Differences in annual N₂O emissions of the soybean rotations did not vary significantly in 2011, 2012 or 2013.

Study year 2013 was the only year in which there was a significant effect of crop on biomass-scaled N₂O emissions. In 2013, both soybean rotations had biomass-scaled N₂O emissions significantly higher than all other crops. The 2013 biomass-scaled emissions of switchgrass were higher than corn, photoperiod-sensitive sorghum and miscanthus, but were not significantly different from corn and photoperiod-sensitive sorghum. Miscanthus had the lowest yield-scaled emissions, but these were not significantly different from corn and photoperiod-sensitive sorghum.

IPCC Estimates

The IPCC Tier 1 method using the default emissions factor of 1% yielded N₂O estimates that were often similar to measured field emissions (Fig. 3.6). Large variability between plots in the field measurements resulted in a large uncertainty range that often contained the IPCC estimates. Thirty-eight percent of the IPCC Tier 1 estimated emissions were significantly different from those measured in the field. In 2011 measured emissions in photoperiod-sensitive sorghum were higher than the IPCC 1% estimate, but in 2012 measured emissions were lower. Measured emissions from corn in 2013 were less than the IPCC 1% estimate. Measured emissions were lower than the IPCC 1% estimate for soybean in rotation with sorghum in 2012, while in 2013 measured emissions were higher. Measured emissions from corn in 2013 were greater than the IPCC 1% estimate. Soybean in rotation with corn in 2013 was the only instance where measured N₂O emissions fell outside the IPCC tier 1 uncertainty range (0.3-3% emission factor) estimates. However, in this instance, the measured emissions were not significantly different from the IPCC upper estimate.

The IPCC Tier 1 estimates were weakly correlated with measured N₂O emissions ($r = 0.36$). Even though only 53% of measured emissions were greater than IPCC estimates, the

discrepancy between measured and predicted emissions tended to be greater in instances where the measured emissions were higher than the IPCC estimates, indicating the model slightly underestimated emissions.

Discussion

Photoperiod-sensitive sorghum yields reported in this study were within the range reported in other studies (Propheter et al., 2010; Tamang et al., 2011; Maughan et al., 2012a). Few studies have examined the productivity of miscanthus in the U.S. Maughan et al. (2012b) observed maximum yields of 16.9-27.4 Mg ha⁻¹ in Kentucky, Nebraska and New Jersey. New Jersey and Kentucky yields were similar to those observed in our study. Heaton et al. (2008) reported miscanthus yields of 31.2-45.5 Mg ha⁻¹ over three years in Illinois. The yields reported by Heaton et al. (2008) are higher than in our study with differences likely due to growing season temperatures and precipitation. The biomass production of switchgrass has been reported in many studies. In a meta-analysis including 18 studies in the U.S., Wullschelger et al. (2010) reported the average yield of lowland ecotypes to be 12.9 Mg ha⁻¹, although the range varied widely depending on factors such as climate and fertilization. The average yield reported by Wullschelger et al. (2010) was close to those observed in this study.

High N₂O fluxes occurred at the beginning of each growing season, when soil NO₃⁻ was high from recent fertilizer application, and high rainfall events. In 2011, 60-80% of total N₂O emissions occurred during the first 10 weeks of the growing season. In 2012, 40-55% of total N₂O emissions had been emitted during the first 10 wks. In 2013, 40-50% of total N₂O emissions had been emitted by week 10 in all crops except photoperiod-sensitive sorghum which had over 70%. These findings emphasize the importance of implementing N management

strategies to reduce N availability early in the growing season, when the potential for high losses is present.

In late fall and early spring of 2012 and 2013 there were several small fluxes that contributed to the total annual emissions. There may be several factors contributing to the late- and post-growing season fluxes. In 2013, several small N₂O fluxes occurred in Oct-Nov which did not occur in 2011 or 2012, likely due to large precipitation events that resulted in several days where WFPS was above 60%. The late- and post-growing season fluxes in 2013 could be related to freeze-thaw events. Winter temperatures in 2013-2014 were substantially lower than in 2011-2012 and 2012-2013 resulting in 40 more days of frozen soil. Freeze-thaw events often result in large emissions of N₂O (Risk et al., 2013). Large fluxes after thawing events were thought to be due to the release of accumulated N₂O under the impermeable layer of frozen soil. This mechanism is unlikely to play a significant role in eastern Kansas as soils are rarely frozen continuously for an extended period of time (>2 weeks). In a review of freeze-thaw impacts on N₂O emissions Risk et al. (2013) presented evidence for the production of N₂O at the onset of a thawing event. Increased C and N substrate availability from microbe turnover and aggregate disintegration during soil freezing, as well as increased microbial activity at the onset of thawing were proposed as possible mechanisms driving these emissions. It seems possible that the low N₂O emissions measured in Jan and Feb 2014 may have been produced by this mechanism since they occurred after recent thaws when soil conditions were moist (55-65% WFPS). It is harder to explain the reason behind the post-growing season fluxes that occurred in photoperiod-sensitive sorghum in 2012-2013. These fluxes did not occur after thawing events. Soil conditions in photoperiod-sensitive sorghum were very wet (~60% WFPS), but were similar to the other crops.

Annual N₂O emissions can vary greatly between sites due to different soil types and climate as well as within sites between years due to varying precipitation patterns and management. Smith et al. (2013) observed fluxes ranging 0.6-1.4 kg N₂O-N ha⁻¹ in unfertilized miscanthus in Illinois. These fluxes are lower than this study observed in 2011 (2.87 kg N₂O-N ha⁻¹) when fertilized with 167 kg N ha⁻¹ but similar to fluxes in 2013 (0.84 kg N₂O-N ha⁻¹) fertilized with 84 kg N ha⁻¹. Behnke et al. (2012) measured fluxes that ranged from 0.35 to 2.91 kg N₂O-N ha⁻¹ from miscanthus receiving 0 to 120 kg N ha⁻¹. The fluxes observed in this study in miscanthus are within the range of those in Behnke et al. (2012) at 60 and 120 kg N ha⁻¹.

Smith et al. (2013) observed N₂O fluxes ranging 0.8-1.4 kg N₂O-N ha⁻¹ over 3 years from switchgrass receiving 56 kg N ha⁻¹. Schmer et al. (2012) measured annual fluxes of 0.09 and 0.29 kg N₂O-N ha⁻¹ from switchgrass in North Dakota receiving 0 and 67 kg N ha⁻¹, respectively. Our study measured higher N₂O emissions from switchgrass, 3.77 and 2.58 kg N₂O-N ha⁻¹ in 2011 and 2013, respectively. The discrepancy could be due in part to the N rates and soil moisture conditions.

There are few published studies that report N₂O emissions from photoperiod-sensitive sorghum. Storlien et al. (2014) observed annual N₂O emissions of approximately 7 and 10 kg N₂O-N ha⁻¹ from photoperiod-sensitive sorghum receiving 0 and 280 kg N ha⁻¹, respectively, when averaged over rotation type and crop residue removal rate. These emissions are much higher than N₂O emissions measured in our study, which ranged 1.68-5.15 kg N₂O-N ha⁻¹. The higher emissions reported by Storlien et al. (2014) may be related to differences in rainfall distribution between studies. Additional studies measuring N₂O emissions from photoperiod-sensitive sorghum are needed.

The emissions from corn and soybean in our study fall within the range reported by other researchers. Hoben et al. (2011) reported annual N₂O emissions ranging from 1-4 kg N₂O-N ha⁻¹ from corn receiving 180 kg N ha⁻¹. Sistani et al. (2011) reported annual emissions of 3.31 and 1.70 kg N₂O-N ha⁻¹ in corn receiving 168 kg N ha⁻¹. Halvorson et al. (2010) reported fluxes of 0.865 and 0.805 kg N ha⁻¹ from corn receiving 246 and 202 kg N ha⁻¹, respectively. The lower fluxes reported by Halvorson et al. (2010) could be due in part to the much drier climate of the study site.

Smith et al. (2013) observed an annual flux of 2.2 kg N₂O-N ha⁻¹ from soybean in a corn-corn-soybean rotation. Parkin and Kasper (2006) reported a wider range of emissions from soybean in a corn-soybean rotation in Iowa, measuring fluxes of 2.17 and 6.96 kg N₂O-N ha⁻¹ in 2003 and 2004, respectively. The magnitude of the N₂O emissions in our study are within the range reported by Smith et al. (2013) and Parkin and Kasper (2006). However, in both studies, the N₂O emissions from soybean were well below those of corn planted in the same year. This is in contrast to 2013 of our study, where the N₂O emissions from soybean were higher than all other crops, including corn and photoperiod-sensitive sorghum. This was an unexpected result because soybean received around 100 and 160 kg ha⁻¹ less N than did photoperiod-sensitive sorghum and corn, respectively.

One possible explanation for high N₂O emissions from soybean in 2013 could be the presence of residual NO₃⁻ in the subsoil. Hot, dry conditions during the 2012 growing season resulted in the lowest corn grain yield recorded in all 3 study years, and could have resulted in a buildup and carryover of residual NO₃⁻ in the subsoil into the 2013 soybean rotation. Iqbal et al. (2015) found that soybeans planted a year after N fertilized corn subjected to drought had flux

rates 35-70% higher than soybeans following non-fertilized corn subjected to drought. This carryover effect was not observed in years that did not follow a drought.

Both soybean systems had more late- and post-growing season N₂O emissions than corn and photoperiod-sensitive sorghum in 2013, which may be due to increased N availability from plant residue and nodule decomposition. Several studies have noted observed N mineralization after soybean flowering and harvest, which can result in increased N₂O emissions (Uchida and Akiyama, 2013). Uchida and Akiyama (2013) found mean post-harvest N₂O emissions from soybeans were 0.62 kg N₂O-N ha⁻¹ and a ranged from 0.001-5.01 kg N₂O-N ha⁻¹. They also noted that high post-harvest emissions occurred only when conditions were favorable for N₂O production. The results from our study support the findings of Uchida and Akiyama (2013). Emissions after Sept. 1 from soybean in 2011 and 2012 were lower in than those in 2013, when soil conditions were wetter and thus favorable for N₂O production.

Few studies have done side-by-side comparisons of the N₂O emissions of perennial and annual bioenergy cropping systems in the U.S. In this study, there were not consistent differences in the annual N₂O emissions between crops. This differs from the findings of Smith et al. (2013) who found emissions from in a corn-corn-soybean rotation to be higher than those of miscanthus and switchgrass. This discrepancy could be due in part to the lower fertilizer rates applied to miscanthus and switchgrass in Smith et al. (2013). Some studies have observed high yields in miscanthus receiving no N (Heaton et al., 2008). However, reviews of miscanthus and switchgrass field trials have found N fertilizer may be required to replenish removed N and maintain stand yields (Miguez et al., 2008; Wullschleger et al., 2010; Arundale et al., 2014). Wullschleger et al. (2010) found that although high switchgrass yields could sometimes be achieved without fertilization, both upland and lowland ecotypes responded to N fertilizer and

appeared to reach optimum yields around 100 kg N ha⁻¹. Nitrogen rates for these crops need to be carefully chosen to minimize negative environmental effects.

The IPCC Tier 1 method of estimating direct N₂O emissions produced estimates that were not statistically different from annual emissions in 10 out of 16 crop-years (Fig. 3.6). However, this may be in large part due to the high variability in the field measurements, more than due to the accuracy of the IPCC estimates. There were several instances where there was a 1-2 kg N₂O-N ha⁻¹ discrepancy between measured and IPCC estimates that were not significantly different due to high variability in the measured annual emissions. The correlation coefficient from the regression of IPCC predictions and measured emissions was 0.36, indicating a weak relationship between measured and predicted emissions. Since the IPCC Tier 1 method accounts only for N inputs into soil on a large scale, it would not be able to capture variability due to changing soil conditions and may in part explain the weak relationship between measured and IPCC estimated emissions in this study. The discrepancy between measured and predicted emissions tended to be greatest in instances where the measured emissions were higher than the IPCC estimates, indicating a tendency for the model to underestimate emissions.

Conclusions

A large portion (40-80%) of annual N₂O emissions were released in the first 10 weeks of the growing season. This finding emphasizes the importance of implementing N management strategies to reduce N availability early in the growing season, when the potential for high losses is present. In this study, fertilizer timing was the same for all crops in a given growing season. If the timing of N application to each crop had been made when N demands were high, observed N₂O emissions may have been lower. Future studies should examine the impact of fertilizer timing on N₂O emissions in these systems. In 2013, post-growing season emissions contributed

up to 40% of annual N₂O in some crops, which may be attributable to mineralization of soybean roots and nodules as well as freeze-thaw cycles. These findings demonstrate that it is important to measure N₂O emissions for the entire year, even in climates that do not experience prolonged freezing conditions.

No consistent significant differences were observed in annual N₂O emissions between photoperiod-sensitive sorghum, corn, switchgrass and miscanthus, which may be due to variable N rates and weather between study years. However, in both 2011 and 2013 miscanthus tended to have lower emissions than the other cellulosic biofuel crops. More measurements of N₂O emissions need to be made in these cropping systems to determine if there are differences in N₂O emissions between these systems. Application of N to miscanthus in this study likely resulted in elevated N₂O emissions. There is debate over the appropriate N rate for miscanthus, which could have a large impact on N₂O emissions. Future studies examining yield and N₂O response of miscanthus to N inputs are needed. This study found a weak correlation between measured N₂O emissions and IPCC Tier 1 estimates, likely due to high variability of measured emissions. The discrepancy between measured and predicted emissions tended to be greatest in instances where the measured emissions were higher than the IPCC estimates, indicating a tendency for the model to underestimate emissions. Future research should utilize process-based models to try to improve the accuracy of predictions of N₂O emissions in these cropping systems.

References

- Adler, P.R., S.J.D. Grosso, and W.J. Parton. 2007. Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems. *Ecol. Appl.* 17:675-691.
- Arundale, R.A., F.G. Dohleman, E.A. Heaton, J.M. McGrath, T.B. Voigt, and S.P. Long. 2014. Yields of *miscanthus x giganteus* and *panicum virgatum* decline with stand age in the midwestern USA. *GCB Bioenergy* 6:1-13.

- Behnke, G.D., M.B. David, and T.B. Voigt. 2012. Greenhouse gas emissions, nitrate leaching, and biomass yields from production of *miscanthus x giganteus* in Illinois, USA. *BioEnergy Res.* 5:801-813.
- Bessou, C., F. Ferchaud, B. Gabrielle, and B. Mary. 2011. Biofuels, greenhouse gases and climate change. A review. *Agron. Sustain. Dev.* 31:1-79.
- Cherubini, F. 2010. GHG balances of bioenergy systems - overview of key steps in the production chain and methodological concerns. *Renew. Energy* 35:1565-1573.
- Crutzen, P.J., A.R. Mosier, K.A. Smith, and W. Winiwarter. 2008. N₂O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. *Atmos. Chem. Phys.* 8:389-395.
- De Klein, C., R. Novoa, S. Ogle, K. Smith, P. Rochette, T. Wirth, B. McConkey, A. Mosier, and K. Rypdal. 2006. N₂O emissions from managed soils, and CO₂ emissions from lime and urea application. *In* N₂O emissions from managed soils, and CO₂ emissions from lime and urea application. 2006 IPCC guidelines for national greenhouse gas inventories. IPCC.
- Del Grosso, S.J., W.J. Parton, A.R. Mosier, M.K. Walsh, D.S. Ojima, and P.E. Thornton. 2006. DAYCENT national-scale simulations of nitrous oxide emissions from cropped soils in the United States. *J. Environ. Qual.* 35:1451-1460.
- Don, A., B. Osborne, A. Hastings, U. Skiba, M.S. Carter, J. Drewer, H. Flessa, A. Freibauer, N. Hyvonen, M.B. Jones, G.J. Lanigan, U. Mander, A. Monti, S.N. Djomo, J. Valentine, K. Walter, W. Zegada-Lizarazu, and T. Zenone. 2012. Land-use change to bioenergy production in Europe: Implications for the greenhouse gas balance and soil carbon. *GCB Bioenergy* 4:372-391.
- Halvorson, A.D., S.J. Del Grosso, and F. Alluvione. 2010. Nitrogen source effects on nitrous oxide emissions from irrigated no-till corn. *J. Environ. Qual.* 39:1554-1562.
- Hartmann, D.L., A.M.G. Klein Tank, M. Rusticucci, L.V. Alexander, S. Bronnimann, Y. Charabi, F.J. Dentener, E.J. Dlugokencky, D.R. Easterling, A. Kaplan, B.J. Soden, P.W. Thorne, M. Wild, and P.M. Zhai. 2013. Observations: Atmosphere and surface. p. 159. *In* T.F. Stocker, D. Qin, G.-.

- Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P.M. Midgley (eds.)
The physical science basis. Contribution of working group I to the fifth assessment report of the
intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK.
- Heaton, E.A., F.G. Dohleman, and S.P. Long. 2008. Meeting U.S. biofuel goals with less land: The
potential of miscanthus. *Glob. Change Biol.* 14:2000-2014.
- Hoben, J.P., R.J. Gehl, N. Millar, P.R. Grace, and G.P. Robertson. 2011. Nonlinear nitrous oxide (N₂O)
response to nitrogen fertilizer in on-farm corn crops of the U.S. Midwest. *Glob. Change Biol.*
17:1140-1152.
- Hutchinson, G.L., and A.R. Mosier. 1981. Improved soil cover method for field measurement of nitrous
oxide fluxes. *Soil Sci. Soc. Am. J.* 45:311-316.
- Iqbal, J., D.C. Mitchell, D.W. Barker, F. Miguez, J.E. Sawyer, J. Pantoja, and M.J. Castellano. 2015.
Does nitrogen fertilizer application rate to corn affect nitrous oxide emissions from the rotated
soybean crop? *J. Environ. Qual.* 44:711-719.
- Kansas State Univ. 2014. Kansas Mesonet. Weather Data Library. 2014.
- Kim, S., and B.E. Dale. 2009. Regional variations in greenhouse gas emissions of biobased products in
the United States-corn-based ethanol and soybean oil. *Int. J. Life Cycle Assess.* 14:540-546.
- Klemedtsson, A.K., and K.A. Smith. 2011. The significance of nitrous oxide emission due to cropping of
grain for biofuel production: A Swedish perspective. *Biogeosciences* 8:3581-3591.
- Li, C.S. 2000. Modeling trace gas emissions from agricultural ecosystems. *Nutr. Cycling Agroecosyst.*
58:259-276.
- Maughan, M., T. Voigt, A. Parrish, G. Bollero, W. Rooney, and D.K. Lee. 2012a. Forage and energy
sorghum responses to nitrogen fertilization in central and southern Illinois. *Agron. J.* 104:1032-1040.
- Maughan, M., G. Bollero, D.K. Lee, R. Darmody, S. Bonos, L. Cortese, J. Murphy, R. Gaussoin, M.
Sousek, D. Williams, L. Williams, F. Miguez, and T. Voigt. 2012b. *Miscanthus giganteus*
productivity: The effects of management in different environments. *GCB Bioenergy* 4:253-265.

- Miguez, F.E., M.B. Villamil, S.P. Long, and G.A. Bollero. 2008. Meta-analysis of the effects of management factors on *miscanthus* × *giganteus* growth and biomass production. *Agric. For. Meteorol.* 148:1280-1292.
- Mosier A., P. Crutzen, K. Smith, and W. Winiwarter. 2009. Nitrous oxide's impact on net greenhouse gas savings from biofuels: Life-cycle analysis comparison. *Int. J. Biotechnol.* 11:60-74.
- Myhre G., D. Shindell, F. Breon, W. Collins, J. Fuglestedt, J. Huang, D. Koch, J. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura, and H. Zhang. 2013. Anthropogenic and natural radiative forcing. p. 659. *In* T.F. Stocker, D. Qin, G. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P.M. Midgley (eds.) *The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change.* Cambridge University Press, Cambridge, U.K.
- Parkin ,T.B., and T.C. Kaspar. 2006. Nitrous oxide emissions from corn–soybean systems in the Midwest. *J. Environ. Qual.* 35:1496-1506.
- Propheter, J.L., S.A. Staggenborg, X. Wu, and D. Wang. 2010. Performance of annual and perennial biofuel crops: Yield during the first two years. *Agron. J.* 102:806-814.
- Reijnders, L. and M.A.J. Huijbregts. 2011. Nitrous oxide emissions from liquid biofuel production in life cycle assessment. *Curr. Opin. Environ. Sustain.* 3:432-437.
- Risk, N., D. Snider, and C. Wagner-Riddle. 2013. Mechanisms leading to enhanced soil nitrous oxide fluxes induced by freeze–thaw cycles. *Can. J. Soil Sci.* 93:401-414.
- Robertson, G.P. 2004. Abatement of nitrous oxide, methane and other non-CO₂ greenhouse gases: The need for a systems approach. p. 493-506. *In* C.B. Field and M.R. Raupach (eds.) *The global carbon cycle.* Island Press, Washington, DC.
- Schmer, M.R., M.A. Liebig, J.R. Hendrickson, D.L. Tanaka, and R.L. Phillips. 2012. Growing season greenhouse gas flux from switchgrass in the northern Great Plains. *Biomass Bioenerg.* 45:315-319.

- Sistani, K.R., M. Jean-Baptiste, N. Lovanh, and K.L. Cook. 2011. Atmospheric emissions of nitrous oxide, methane, and carbon dioxide from different nitrogen fertilizers. *J. Environ. Qual.* 40:1797-1805.
- Smeets, E.M.W., L.F. Bouwmanw, E. Stehfest, D.P. van Vuuren, and A. Posthuma. 2009. Contribution of N₂O to the greenhouse gas balance of first-generation biofuels. *Glob. Change Biol.* 15:1-23.
- Smith, C.M., M.B. David, C.A. Mitchell, M.D. Masters, K.J. Anderson-Teixeira, C.J. Bernacchi, and E.H. DeLucia. 2013. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. *J. Environ. Qual.* 42:219-228.
- Storlien, J.O., F.M. Hons, J.P. Wight, and J.L. Heilman. 2014. Carbon dioxide and nitrous oxide emissions impacted by bioenergy sorghum management. *Soil Sci. Soc. Am. J.* 78:1694-1706.
- Tamang, P.L., K.F. Bronson, A. Malapati, R. Schwartz, J. Johnson, and J. Moore-Kucera. 2011. Nitrogen requirements for ethanol production from sweet and photoperiod sensitive sorghums in the southern high plains. *Agron. J.* 103:431-440.
- U.S. Congress. 2007. Energy independence and security act of 2007. 201-210.
- Uchida, Y., and H. Akiyama. 2013. Mitigation of postharvest nitrous oxide emissions from soybean ecosystems: A review. *Soil Sci. Plant Nutr.* 59:477-487.
- USDOE. 2011. U.S. billion-ton update: Biomass supply for a bioenergy and bioproducts industry. Rep. ORNL/TM-2011/224. Oak Ridge National Laboratory, Oak Ridge, TN.
- USEPA. 2014. Inventory of U.S. greenhouse gas emissions and sinks: 1990 - 2012. Rep. EPA 430-R-14-003.
- USEPA. 2010. Renewable fuel standard program (RFS2) regulatory impact analysis.
- von Blottnitz, H., and M.A. Curran. 2007. A review of assessments conducted on bio-ethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. *J. Clean Prod.* 15:607-619.

Wang, M., J. Han, J.B. Dunn, H. Cai, and A. Elgowainy. 2012. Well-to-wheels energy use and greenhouse gas emissions of ethanol from corn, sugarcane and cellulosic biomass for U.S. use. *Environ. Res. Ltts* 7:045905.

Wullschleger, S.D., E.B. Davis, M.E. Borsuk, C.A. Gunderson, and L.R. Lynd. 2010. Biomass production in switchgrass across the United States: Database description and determinants of yield. *Agron. J.* 102:1158-1168.

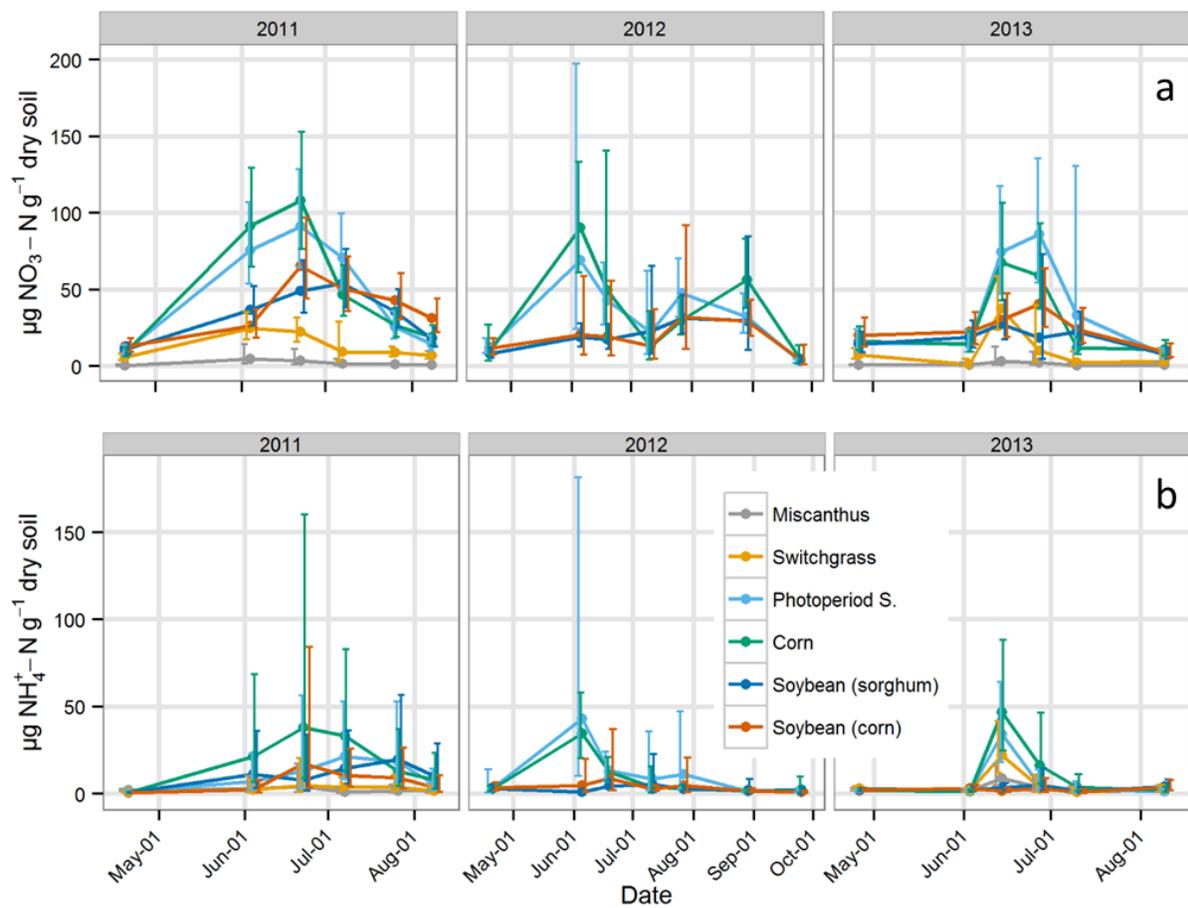


Figure 3.1. Soil nitrate (a) and ammonium (b) by crop at 0 – 5 cm soil depth in 2011 – 2013. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum. Error bars indicate 95% confidence intervals.

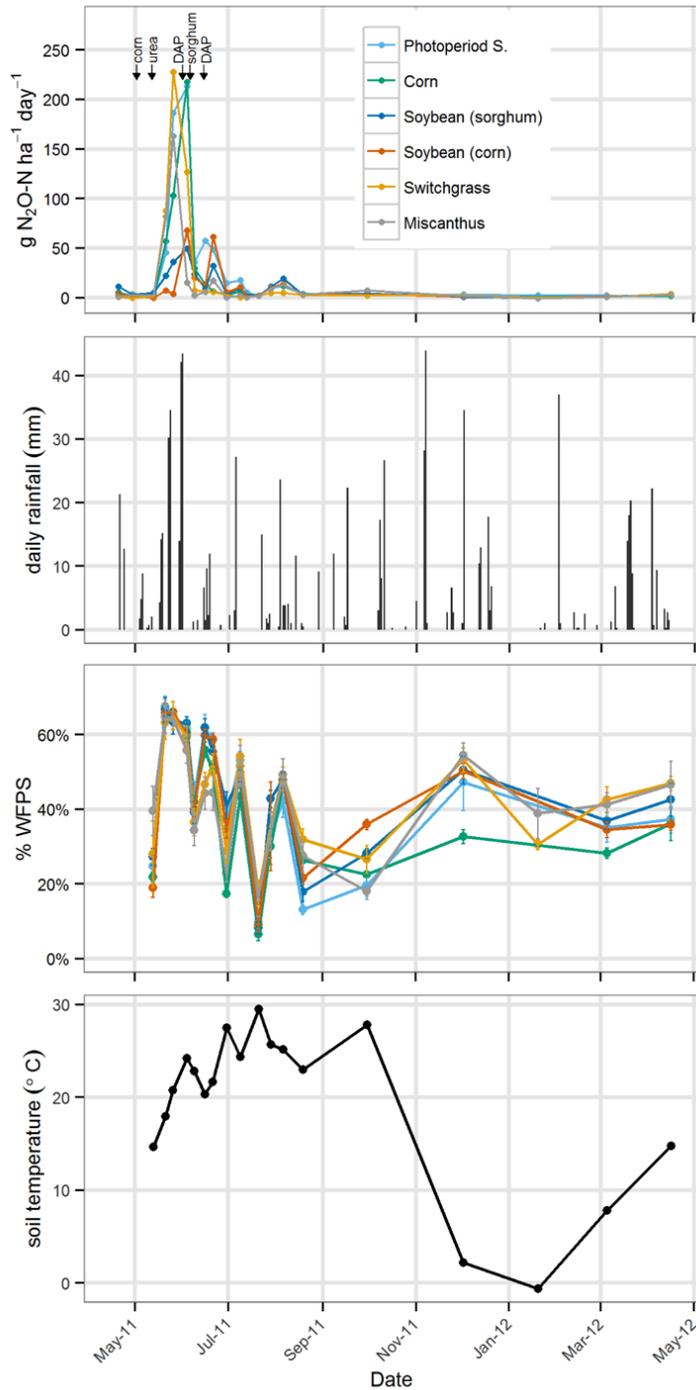


Figure 3.2. Mean daily N_2O flux by crop, percent water-filled pore space (% WFPS), daily precipitation and average daily temperature observed in 2011 – 2012. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum. Labels “urea” and “DAP” indicated date urea and the 2 applications of diammonium phosphate were applied. “Corn” indicates the date that corn was planted and “sorghum” indicates the planting date of photoperiod sorghum and soybean. Rainfall data recorded at nearby meteorological station.

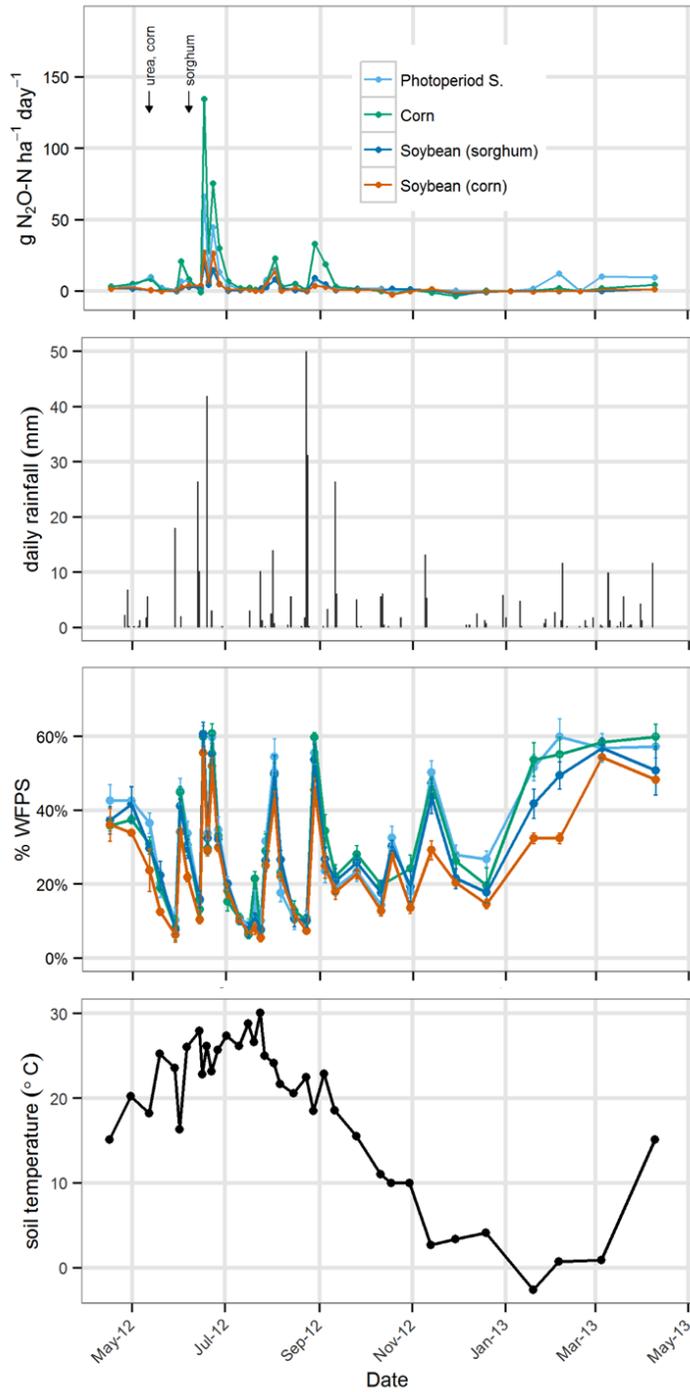


Figure 3.3. Mean daily N_2O flux by crop, percent water-filled pore space (%WFPS), daily precipitation and average daily temperature observed in 2012 – 2013. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum. Label “urea, corn” indicate date that urea was applied and corn was planted. “Sorghum” indicates date photoperiod-sensitive sorghum and soybean were planted. Rainfall data recorded at nearby meteorological station.

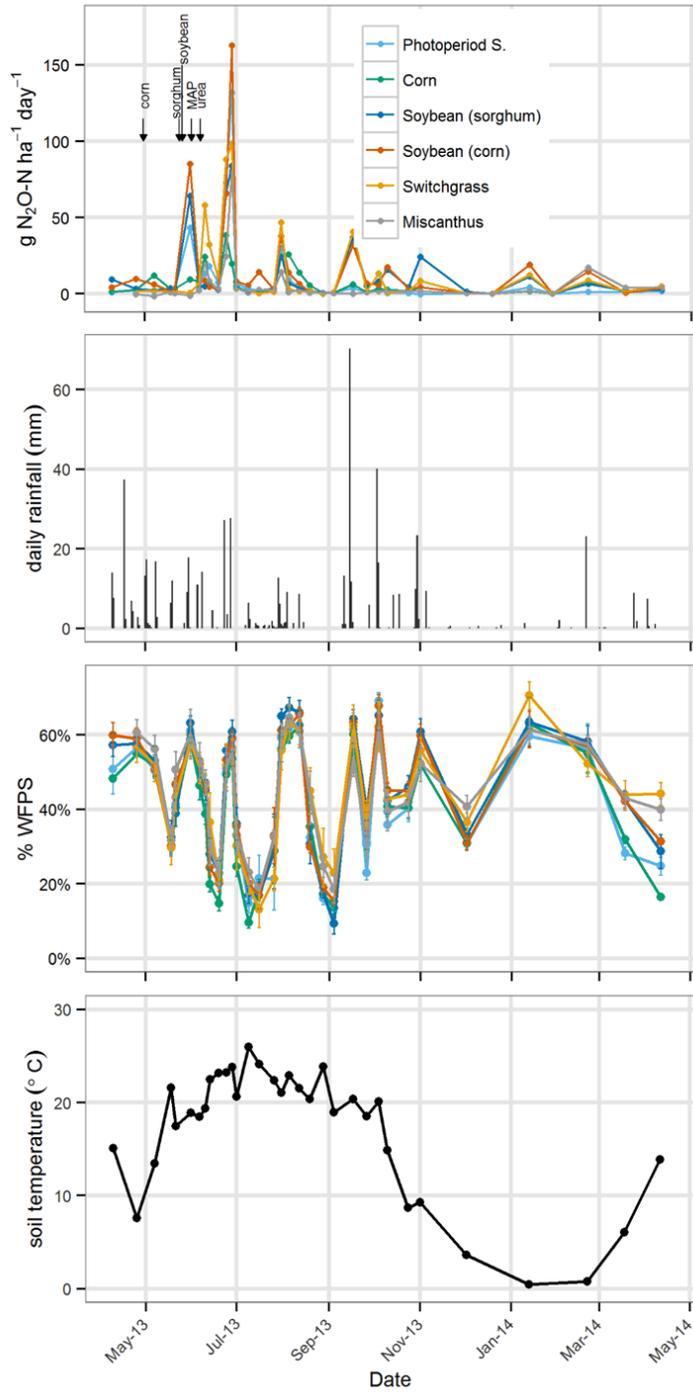


Figure 3.4. Mean daily N_2O flux by crop, percent water-filled pore space (%WFPS), daily precipitation and average daily temperature observed in 2013 – 2014. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum. “Corn”, “sorghum” and “soybean” indicate dates of corn, photoperiod sorghum and soybean planting, respectively. “MAP” and “urea” indicate dates of monoammonium phosphate and urea application. Rainfall data recorded at nearby meteorological station.

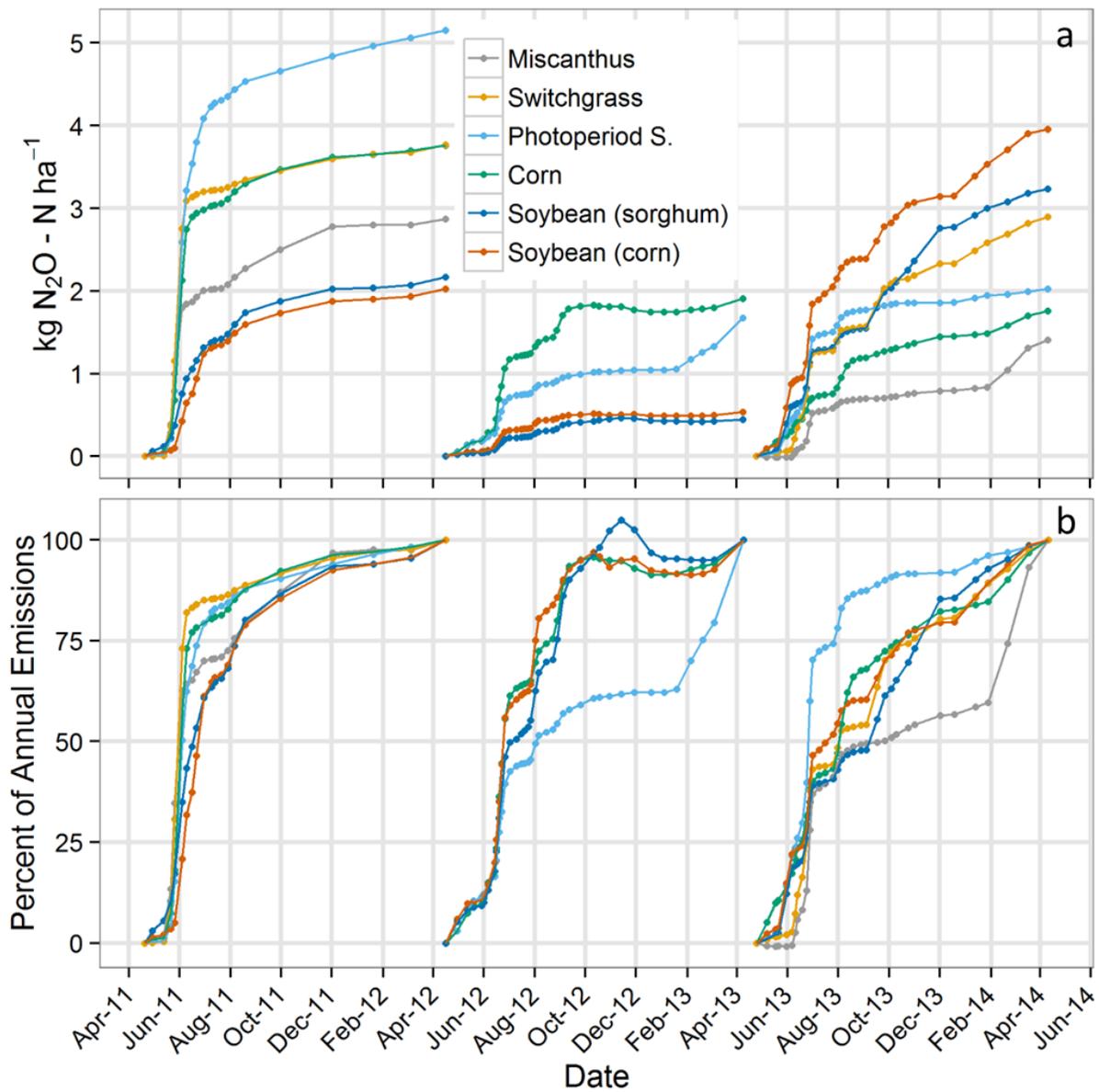


Figure 3.5. (a) Cumulative annual N₂O emissions in 2011–2013 by crop. (b) Percent of total annual N₂O emissions emitted by crop. Soybean (corn) is soybean in rotation with corn and soybean (sorghum) is soybean in rotation with sorghum.

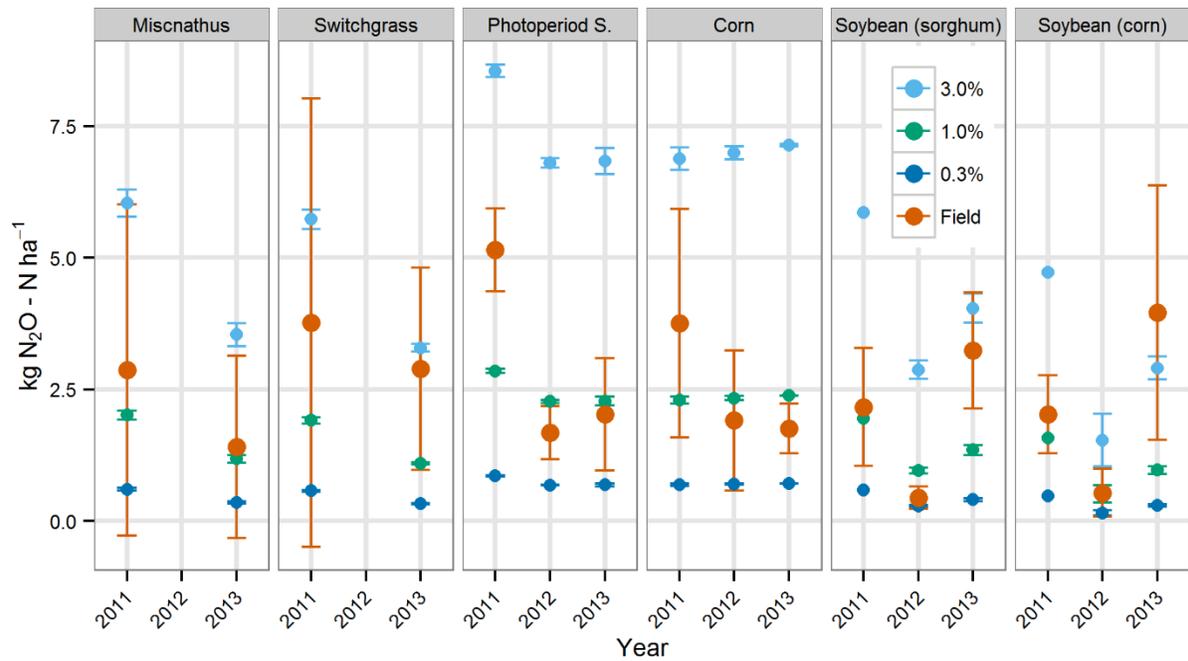


Figure 3.6. Direct N₂O emissions estimated by IPCC Tier 1 method by crop using the default emission factor (1.0%) and uncertainty range (0.3% and 3.0%). Measured emissions are shown for comparison. Error bars represent 95% confidence intervals of the mean.

Table 3.1. Annual aboveground biomass and grain yields

Crop	Biomass (non-grain)			Grain		
	2011	2012	2013	2011	2012	2013
	————— Mg dry matter ha ⁻¹ —————					
Corn	7.0 c†	10.0 b	9.4 b	4.2	3.1 a	7.0 a
Sorghum	21.2 a	19.0 a	19.8 a			
Soy - corn‡	.	2.7 c	6.4 c	.	2.3 b	2.9 b
Soy - sorghum¶	.	3.4 c	6.4 c	.	2.4 b	2.9 b
Switchgrass	12.5 b	.	14.3 a			
Miscanthus	19.1 a	.	19.1 a			

· Missing value

† Within column, means followed by same letter are not significantly different according to Fisher's LSD (0.05)

‡ Soybean in rotation with corn

¶ Soybean in rotation with sorghum

Table 3.2. Total annual N₂O emissions and biomass-scaled N₂O emissions.

Crop	Annual Emissions						Biomass-Scaled Emissions					
	2011		2012		2013		2011		2012		2013	
	kg N ₂ O-N ha ⁻¹ yr ⁻¹						g N ₂ O-N Mg ⁻¹					
Corn	3.76 ab †	(0.68) ‡	1.91 a	(0.42)	1.76 cd	(0.15)	343a	(54)	141 a	(26)	91 bc	(10)
Sorghum	5.15 a	(0.25)	1.68 a	(0.16)	2.02 bcd	(0.32)	243a	(14)	89 a	(11)	102 bc	(22)
Soy (corn) §	2.03 b	(0.23)	0.53 b	(0.14)	3.96 a	(0.75)	.	.	101 a	(29)	384 a	(81)
Soy (sorghum) ¶	2.17 b	(0.35)	0.44 b	(0.07)	3.25 ab	(0.34)	.	.	77 a	(12)	326 a	(46)
Switchgrass	3.77 ab	(1.34)	.	.	2.89 abc	(0.67)	320 a	(98)	.	.	189 b	(55)
Miscanthus	2.87 b	(0.99)	.	.	1.40 d	(0.33)	141 a	(33)	.	.	48 c	(38)

· Missing value

† Within column, means followed by same letter are not significantly different according to Fisher's LSD (0.05)

‡ Standard error of the mean

§ Soybean in rotation with corn

¶ Soybean in rotation with sorghum

Chapter 4 - Impact of nitrogen rate on switchgrass yield, production costs and N₂O emissions

Abstract

Switchgrass (*Panicum virgatum* L.) has been promoted as a potential feedstock for cellulosic biofuel in the United States. Switchgrass is known to respond to N fertilizer, but optimal rates remain unclear. Given the potential non-linear response of nitrous oxide (N₂O) emissions to N inputs, N additions to switchgrass above optimal levels could have large impacts on the greenhouse gas balance of switchgrass-based biofuel. Additionally, N additions are likely to have a large impact on the switchgrass production costs. Yield, N₂O emissions, N removal, and costs of production were measured in switchgrass receiving 0-200 kg N ha⁻¹ in Manhattan, KS from 2012-2014. Response of N₂O to N rate varied each year, showing an exponential, linear and sigmoidal response to N₂O emissions in 2012, 2013 and 2014, respectively. Fertilizer induced emission factor (EF) increased from 0.7% at 50 kg N ha⁻¹ to 2.6% at 150 kg N ha⁻¹, demonstrating a non-linear increase in N₂O emissions. Switchgrass yields increased with N inputs up to 100-150 kg N ha⁻¹, but the critical N level for maximum yields decreased each year, suggesting N was being applied in excess at higher N rates. Removal of N at harvest increased linearly with increasing N rate. Yield-scaled costs of production were minimized at 100 kg N ha⁻¹ (\$70.91 Mg⁻¹). Harvest costs, land rent and N fertilizer made up the majority of production costs. These results demonstrate N inputs are necessary to increase switchgrass productivity, but rates exceeding optimal levels could result in excessive N₂O emissions and increased costs for producers.

Introduction

Nitrous oxide (N_2O) is a greenhouse gas (GHG) with a global warming potential (GWP) 298 times that of CO_2 and has the third largest radiative forcing of anthropogenic GHGs (Myhre et al., 2013). The global average concentration of N_2O was 324 ppb in 2011, nearly 20% higher than the concentration in 1750 (Hartmann et al., 2013). Nitrous oxide emissions from agricultural soils account for 4.7% of total GHG emissions and 74.8% of total N_2O emissions in the U.S. (USEPA, 2014). Applications of synthetic fertilizer are an important source of N_2O , accounting for 22% of emissions from U.S. agricultural soils (USEPA, 2014).

There is a large body of evidence showing N_2O emissions from soils increase with increasing N inputs, including synthetic fertilizer (Bouwman et al., 2002; Stehfest and Bouwman, 2006; Kim et al., 2013; Shcherbak et al., 2014). To account for the effect of N fertilizer on N_2O in GHG inventories, the IPCC Tier 1 method uses an emission factor (EF) of 1%, which assumes that 1% of fertilizer N is lost as direct emissions of N_2O (De Klein et al., 2006). However, there is a growing body of evidence that suggests N_2O emissions increase non-linearly with increasing N inputs. In a meta-analysis including 233 site-years of N rate studies, Shcherbak et al. (2014) found positive changes in EF with increasing N inputs in N-fixing crops, upland grain crops, rice and perennial grass/forage crops. In another meta-analysis, Kim et al. (2013) found only 5 datasets where N_2O emissions increased linearly with increasing N inputs, while 16 datasets experienced exponential increases, and 2 datasets were best explained using a hyperbolic model.

Kim et al. (2013) hypothesized that if enough N inputs are added to soil, a 3-phase N_2O response may be observed that includes a linear increase at low N rates where there is high competition for N by plants and denitrifiers, an exponential increase once available N exceeds

plant requirements, and a hyperbolic response once N additions have exceeded the ability of microbes to utilize N for N₂O production, causing N₂O production to plateau. The linear, exponential and hyperbolic response are shown in Equations 4.1, 4.2 and 4.3 respectively:

$$y = ax + b \quad [4.1]$$

$$y = ae^{(bx)} \quad [4.2]$$

$$y = y_0 + ax(b + x)^{-1} \quad [4.3]$$

where x is the N input (kg N ha⁻¹) and y is the total annual N₂O emissions (kg N₂O-N ha⁻¹). This 3-phase response is similar to non-Michaelis-Menten kinetics, which follows a sigmoidal response of enzyme reaction rate over changing substrate concentration (Kim et al., 2013). This response can be explained by the Hill Equation (Weiss, 1997; Goutelle et al., 2008) which can be expressed as:

$$v = \frac{V_{max}*[S]^n}{(K_{0.5})^n + [S]^n} \quad [4.4]$$

where v is the reaction rate, V_{max} is the maximum reaction rate, $[S]$ is substrate concentration, $K_{0.5}$ is the half-maximal activity constant, and n is the Hill coefficient, which provides a measure of cooperativity of substrate binding to enzymes. The equation can be applied to N₂O emissions by substituting N rate for $[S]$, annual N₂O emissions for v , the level at which N₂O emissions plateau for V_{max} , and one-half the amount of N needed to reach maximum N₂O emissions for $K_{0.5}$.

A non-linear response of N₂O emissions to N inputs could result in N₂O losses much larger than would be predicted by a single EF, especially if N inputs exceed plant requirements. It would also result in much higher GHG emissions in agricultural systems were N inputs exceed plant needs. These losses could be especially important in the context of bioenergy production, where N inputs are necessary to maximize biomass production but resulting N₂O emissions

could turn biofuels into a net source of GHG emissions (Crutzen et al., 2008; Smith et al., 2012). Many studies have demonstrated the potentially large effect N₂O can have on the GHG balance of biofuel. Adler et al. (2007) conducted an LCA on 1st and 2nd generation biofuel produced from various crops and found that N₂O emissions represented the largest GHG source in each case. Kim and Dale (2009) conducted an LCA on soybean-based biodiesel and corn based ethanol in the US Corn Belt and found N₂O emissions to contribute 13-57% and 11-37% of the total GHG emissions for biodiesel and ethanol, respectively. Klemetsson and Smith (2011) used field measurements of N₂O in a LCA of ethanol from wheat and found N₂O emissions to contribute 18-57% of GHG emissions. In a review of studies on GHG emissions from direct land-use change in bioenergy systems, Don et al. (2012) found that, on average, direct N₂O emissions contribute 27% of the GHG emissions of biofuels from food crops, with contributions ranging between 5-80%. Because biofuels are promoted as one strategy to reduce GHG emissions in the transportation sector, it is essential to quantify the N response and resulting N₂O emissions from bioenergy crops to ensure they are managed in a way that maximizes productivity while minimizing GHG emissions.

Switchgrass (*Panicum virgatum* L.) has been promoted as a potential feedstock for cellulosic biofuel in the U.S. Switchgrass is a perennial grass native to North America. It has a number of desirable characteristics giving it advantages over both first-generation and other cellulosic feedstock sources: no annual establishment costs, low inputs of fertilizer and pesticides, a well-established seed industry, the ability to grow on and improve the quality of marginal soils and the potential to produce high amounts of biomass (Mitchell et al., 2008; USDOE, 2011). Average yields of switchgrass grown in the U.S. are 8.7 Mg ha⁻¹ and 12.9 Mg ha⁻¹ for upland and lowland ecotypes, respectively, but yields over 30 Mg ha⁻¹ have been reported

(Wullschleger et al., 2010). Switchgrass can produce high yields without N inputs, but optimizing yields requires N additions (Mitchell et al., 2008). In a meta-analysis of switchgrass productivity, Wullschleger et al. (2010) found both upland and lowland switchgrass cultivars responded significantly to N additions and found the optimum rate to be approximately 100 kg N ha⁻¹. However, switchgrass response to N is highly variable and depends on soil conditions, climate, cultivar productivity and management (Mitchell et al., 2008). Many studies have observed yields in unfertilized stands to be similar to those receiving N inputs (Wullschleger et al., 2010; Jung et al., 2011; Kering et al., 2012; Sadeghpour et al., 2014; Wile et al., 2014). These findings illustrate need for more studies to characterize switchgrass response to N across a range of conditions.

Few studies have examined explicitly the impact of N rate on the costs of production of switchgrass. However, costs of production have been found to vary nearly 200% depending on assumed switchgrass yields (Duffy and Nanhou, 2002). Estimates of switchgrass production costs vary substantially, ranging \$44-\$149 Mg⁻¹ (Hallam et al., 2001; Duffy and Nanhou, 2002; Khanna et al., 2008; Vadas et al., 2008; Mooney et al., 2009; Perrin et al., 2012; Haque et al., 2013). Much of the variation may be accounted for in assumed switchgrass yield, N inputs required, and assumptions regarding land cost.

Given the potential importance of N input levels on switchgrass yields, costs of production and N₂O emissions, studies that examine the relationship between these factors are needed in order to develop switchgrass systems that can mitigate GHG emissions while remaining economically viable. The objectives of this study were to: i) characterize the yield and N₂O response of switchgrass to N fertilizer; and ii) determine the costs of production of switchgrass across N fertilizer rates.

Materials and Methods

Study Site

This experiment was conducted from 2012 to 2014 at the Kansas State University Agronomy Research Farm in Manhattan, Kansas (39°11' N, 96°35' W). The soil type was a Smolan silt loam (fine, smectic, mesic Pachic Argiustoll). Total soil C content at 0-5 cm and 5-15 cm was 13.9 and 10.9 g C kg⁻¹, while total N content was 1.31 and 1.07 g N kg⁻¹ at 0-5 and 5-15 cm respectively. Bulk density and pH at 0-15 cm were 1.43 g cm⁻¹ and 5.9 respectively. Average annual precipitation and temperature at the study site are 82.4 mm and 12.7°C. Annual precipitation was 47.8, 61.7 and 66.8 mm in 2012, 2013 and 2014, respectively.

In 2008, Cave-in-Rock switchgrass was seeded with a grass drill at a depth of 0.6 to 1.3 cm after disk and field cultivation. Seeding rate was 4 kg pure live seed ha⁻¹ with 20.3 cm row spacing. No fertilizers or inputs were applied from establishment through 2010. Switchgrass was burned in spring annually through 2012. Prior to switchgrass establishment, the study site was planted with winter wheat. Starting in 2012, N fertilizer treatments were assigned to 3.0 x 6.1 m plots arranged as a randomized complete block design with 4 replicates. Nitrogen treatments were 0, 50, 100, 150 and 200 kg N ha⁻¹ (0N, 50N, 100N, 150N and 200N, respectively) applied as urea. Urea was applied on 18 May, 25 May, and 28 April in 2012, 2013 and 2014, respectively. Switchgrass aboveground biomass was harvested from 0.91 x 1.83 m quadrats using a sickle-bar mower on 16, 14, and 12-13 Nov. in 2012, 2013, and 2014, respectively. Remaining biomass was cut to a stubble height of 6 cm and removed from the plots. Biomass subsamples were taken for evaluation of gravimetric moisture and total C and N content. Biomass C and N content was determined using a using a Carlo-Erba C and N analyzer

(Thermo Finnegan Flash EA1112, Milan, Italy). All biomass yields are reported on a dry matter basis.

Nitrous Oxide Measurements

Fluxes of N₂O were measured from May 2012 through Oct. 2014 using static, vented, polyvinyl chloride (PVC) chambers (7.5 cm high x 20 cm diameter), as described in (Hutchinson and Mosier, 1981). PVC anchors (20 cm high x 20 cm diameter) were placed randomly within the plots. Anchors were driven approximately 15 cm into the soil. Switchgrass was allowed to grow within the anchors, until shoots were too large to fold into the chamber during sampling, at which point grass was clipped to a height that permitted chamber attachment. This usually occurred around mid-June to July. Samples were collected 1-2 times per week during the growing season and once every 2-4 weeks for the rest of the year. During the winter months, on sampling dates where the soil was determined to be frozen, it was assumed there was no N₂O flux. On measurement days, chambers were installed on anchors and 30 mL gas samples removed from each chamber after 0, 15, and 30 min. and injected into pre-evacuated 12 mL Labco Exetainers (Labco Limited, United Kingdom) with Labco grey butyl rubber septa. Gas samples were transported back to the laboratory and N₂O concentrations were determined by gas chromatography using a Shimadzu Model 14A GC (Shimadzu Corporation, Japan) equipped with a ⁶³Ni electron capture detector and a stainless steel column (0.318-cm dia. by 74.5 cm long) with Poropak Q (80-100 mesh). The GC was calibrated daily using analytical-grade standards containing 0.2, 3.5, and 15.3 parts per million N₂O. The concentration of N₂O in each sample was converted to µg N₂O-N m⁻² using equation 4.5:

$$X = \frac{CPVM}{ART} \quad [4.5]$$

where X is $\mu\text{g N}_2\text{O-N m}^{-2}$, C was the volumetric concentration of N_2O ($\mu\text{L N}_2\text{O L}^{-1}$), P was the atmospheric pressure at 304.8 m (0.965 atm), V was the chamber volume (L), M was the mass of N in N_2O ($28 \mu\text{g N } \mu\text{mol}^{-1} \text{N}_2\text{O}$), A was the chamber surface area (m^2), R was the Universal Gas Constant ($0.08206 \text{ atm } \mu\text{L } \mu\text{mol}^{-1} \text{ K}^{-1}$), and T was the chamber headspace temperature (K) when the sample was taken. Fluxes of N_2O were calculated from linear regression of the converted N_2O values over time. Total annual flux was estimated using linear interpolation between sampling points and calculation of the area under the curve using equation 4.6:

$$\text{Cumulative N}_2\text{O (g N ha}^{-1}\text{)} = \sum_i^n \frac{(F_i + F_{i+1})}{2} (t_{i+1} - t_i) \quad [4.6]$$

where F_i and F_{i+1} were the $\text{N}_2\text{O-N}$ fluxes ($\text{g ha}^{-1} \text{ day}^{-1}$) at sampling points i and $i+1$; t_i and t_{i+1} were the sampling dates (Julian date) at sampling points i and $i+1$; and n was the number of sampling points taken in a given year. Fluxes after switchgrass harvest were minimal in 2012 and 2013, so no samples were collected after harvest in 2014. Fertilizer induced emission factors (EF) were estimated by dividing the difference between total annual emissions at a given N rate and emissions at 0N by the rate of N applied.

Ancillary Measurements

On each gas sampling date, volumetric soil water content and soil temperature were measured. Volumetric soil water content was measured at 0-5 cm soil depth using Stevens Hydra Probe II soil sensor (Stevens Water Monitoring Systems, Inc.). Soil water content was converted to percent water-filled pore space (WFPS) using soil bulk density values. Soil temperature was measured at a soil depth of 5 cm. Soil samples were taken at 0-5 and 5-15 cm soil depths 5-6 times during the growing season for determination of soil NO_3^- -N and NH_4^+ -N. Soil NO_3^- -N and NH_4^+ -N were determined using a continuous flow analyzer (Alpkem Corp., Bulletins A303-S021 and A303-S170, Clackamas, OR) after extraction with 1 M KCl

(soil:solution ratio 1:5). Daily precipitation and air temperature were collected at a meteorological station less than 100 m from the study site (Kansas State Univ., 2014).

Cost Analysis

The costs of production were estimated for switchgrass at different levels of N fertilization using the 3 yr average yields at each N level measured in this study. Costs of inputs, field operations, harvest, and land rental rate are displayed in Table 4.4. Field operations, harvest costs and land rent are based on values reported for brome hay management in Eastern Kansas (Ibendahl et al., 2015). Herbicide rates and prices for control of broadleaf weeds during switchgrass establishment are based on values recommended for weed control in native grasses by Thompson et al. (2015). Cost of urea was based on the March 2013 average U.S. farm prices reported by the National Agricultural Statistics Service (USDA NASS, 2015). Switchgrass stands were assumed to have a life of 11 years. It was assumed switchgrass was not harvested in year 1 due to low yields that are typical immediately after establishment. Total costs of production and yield-scaled production costs are presented as net present value (NPV) at switchgrass stand establishment, assuming a nominal discount rate of 6% and 3% inflation.

Statistical Analysis

Differences in EF, biomass N concentrations and soil NO_3^- and NH_4^+ were evaluated by ANOVA using PROC GLIMMIX (SAS 9.3, SAS® Institute Inc., Cary, NC, USA). Soil NO_3^- and NH_4^+ were analyzed separately by each year and depth using N rate, sampling date and the interaction between N rate and sampling date as fixed effects and block as a random effect. When significant covariance between sampling dates was detected, sampling date was modeled as a repeated variable using the covariance structure providing the best model fit based on the Akaike Information Criterion (AIC). Biomass N and EF were analyzed using year, N rate and

year x N rate as fixed effects and block as a random effect. All data were checked for normality and homogeneity of variance. When the assumption of homogeneous variance was not met, model residual variance was allowed to vary using the ‘GROUP’ option in the ‘RANDOM’ statement of GLIMMIX. Non-normal data was logarithmically transformed and means converted back to their original scale for presentation. Mean separation was performed using Fisher’s LSD.

Total annual N₂O, switchgrass biomass yield and biomass N removal were analyzed using linear and non-linear least square regression using ‘lm’ and ‘nls’ in R 3.2 (R Core Team, Vienna, Austria). The relationship between N rate and total N₂O emissions was evaluated by fitting linear (Eq. 4.1), exponential (Eq. 4.2) and sigmoidal (Eq. 4.4) models to the N₂O data each year. Linear and linear-plateau models were fit to each year of biomass yield data. For biomass yield and total N₂O, the model providing the best fit each year was selected on the basis of AIC.

Results

Soil Nitrogen

There was a significant interaction of N rate and sampling date on soil NO₃⁻ and NH₄⁺ for all 3 yrs at the 0-5 cm depth. At 0-5 cm NO₃⁻ and NH₄⁺ increased rapidly after fertilizer application, with the highest measured levels (20-40 kg N ha⁻¹) occurring between mid-May and mid-June (Fig. 4.1). Soils receiving higher N rates had more NO₃⁻ and NH₄⁺ in the month after fertilizer application, though these differences were not always significantly different from the concentrations of adjacent N rates. As the growing season progressed, concentrations decreased towards background levels. Minimal differences were found between N treatments after July,

except in 2012, where soil NO_3^- and NH_4^+ remained significantly above background levels until August.

At 5-15 cm, variation in soil NO_3^- was much less over the growing season, typically varying by no more than 6 kg N ha^{-1} . Only NO_3^- in 2013 showed a significant interaction of N rate and sampling date at 5-15 cm. The effect of sampling date on NO_3^- and NH_4^+ was significant for all 3 yrs at the 5-15cm depth. In 2013, 5-15 cm soil NO_3^- was higher in soils receiving higher N rates following fertilizer application and dropped to background levels as the growing season proceeded. In 2013 and 2014, NO_3^- increased after fertilizer application and reached maximum levels in mid-June, which was 2-4 weeks later than the maximum NO_3^- measured at 0-5 cm. Temporal variation in 2012 NO_3^- at 5-15 cm was similar to 0-5 cm, with the highest levels occurring after fertilizer application and decreasing over time. Temporal variation in soil NH_4^+ at 5-15 cm was low ($< 3 \text{ kg N ha}^{-1}$) and did not follow a consistent pattern between years. At 5-15 cm, the effect of N rate on NO_3^- and NH_4^+ was significant in all years except NH_4^+ in 2014. Soil NO_3^- and NH_4^+ increased with increasing N rate, but only differences between the highest N rates were significantly different from the unfertilized control (data not shown).

Seasonal N₂O Emissions

Daily N_2O emissions varied greatly over the study period, with the highest fluxes occurring in late May and early June following N fertilizer application and rainfall events (Figs. 4.2-4.4). In 2012 large fluxes reaching 259 and $382 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$ were observed on 16 and 22 June following rainfall events of 37 and 42 mm, respectively. In 2013, the largest fluxes were observed on 31 May and 6 June (88 and $197 \text{ g N}_2\text{O-N ha}^{-1} \text{ d}^{-1}$) after rainfall totaling 27 and 11 mm, respectively. The largest fluxes throughout the study were observed between 22 May and

11 June 2014. Over 208 mm of rain fell during this period, resulting in wet soil conditions and fluxes up to 470 g N₂O-N ha⁻¹ d⁻¹. In all 3 yrs, the largest fluxes usually occurred in soils receiving high rates of N fertilizer (100N-200N) and occurred when soil WFPS was 55-65%. Fluxes in the unfertilized control were much lower (< 15 g N₂O-N ha⁻¹ d⁻¹). Fluxes also occurred in July and Aug after rainfall events, but these tended to be lower than fluxes observed in May and June. Few fluxes were observed after Sept., even when soil WFPS rose above 60%. This is likely attributable to low available NO₃⁻ late in the growing season.

Total N₂O Emissions

In all 3 yrs, the majority of total annual N₂O emissions occurred in May and June (Fig. 4.5). In 2012 between 80-90% of total annual N₂O was emitted by 1 July from soils receiving N fertilizer. Soils receiving N fertilizer had emitted 40-70% of total N₂O by 1 July 2013 and 72-95% by 1 July 2014. In the unfertilized control, total annual N₂O of the control increased throughout the year, with only 34-45% occurring by 1 July. This is likely due to the absence of large N₂O fluxes caused by N fertilizer inputs.

Total annual N₂O increased with increasing N fertilizer rate, but the relationship between N rate and total N₂O varied each year (Fig. 4.6). The exponential curve best described the N₂O response to fertilizer in 2012. In 2013 the linear model best described the N₂O response, while the sigmoidal model best described the 2014 response. All 3 best-fit models were significant (p < 0.05). Annual emissions in 2014 were higher than in other years, reaching a maximum of 6.67 kg N₂O-N ha⁻¹ at 150N. Maximum annual emissions in 2012 and 2013 were 3.85 and 2.24 kg N₂O-N ha⁻¹, respectively.

Emission factors (EF) varied significantly between N fertilizer rates, but not between years (Table 4.1). The EF at 150N was significantly higher than the EF at 50N, but not

significantly different from the EF at 100N. Emission factors at 150N and 200N were both significantly different from the IPCC Tier 1 EF of 1%.

Biomass Yield

Switchgrass biomass production responded positively to low rates of N fertilizer but plateaued at higher N rates (Fig. 4.7). In all 3 yrs, the linear-plateau model best described switchgrass yield response. The critical level of N at which maximum yield was attained was similar in 2012 and 2013 (161 and 156 kg N ha⁻¹) but lower in 2014 (79 kg N ha⁻¹) (Table 4.2). Switchgrass yield increased in each year of the study, but yields plateaued at similar levels in 2013 and 2014 (9.6 and 10.2 Mg ha⁻¹). Maximum yield was lower in 2012 (6.1 Mg ha⁻¹).

Biomass N concentration varied significantly over different N rates and between years. Biomass N was significantly higher at 150N and 200N than at lower N rates when averaged over all years. There were no other significant differences between N rates. Biomass N in 2014 was significantly lower than biomass N in 2012 and 2013 when averaged over N rate. Biomass N in 2012 did not differ significantly from 2013 biomass N. The quantity of N removed in harvested biomass (N removed), increased linearly with increasing N rate in all 3 yrs (Fig. 4.8). Removal of N increased all 3 years at a similar rate (0.19-0.21 kg N removed kg⁻¹ N applied). Removed N in 2012 was 10-14 kg ha⁻¹ lower than N removed in 2013 and 2014.

Costs of Production

The cost of production per hectare increased with increasing N rate (Table 4.5). Production costs were \$3,110.90 ha⁻¹ at 0N and increased to \$7,086.69 ha⁻¹ at 200N. Most of the increase in cost between 0N and 200N was from urea and higher baling costs during harvest. At 0N, harvest costs and land charge were the largest costs (40% and 50% of unadjusted total). At 200N, major costs were urea (35%), harvest costs (36%) and land rent (22%). The yield scaled

cost of production was highest at 0N (\$94.40 Mg⁻¹). Production costs decreased with increasing N rate to \$70.91 Mg⁻¹ at 100N, then increased with additional N inputs. This cost trend is explained by high switchgrass yield gains with N additions up to 100N. Above 100N additional N inputs resulted in minimal yield gains.

Discussion

Switchgrass yield response to N fertilizer tends to be positive, but highly variable (Mitchell et al., 2008; Wullschleger et al., 2010). Wullschleger et al. (2010) found the optimum N rate for switchgrass was approximately 100 kg N ha⁻¹, but noted that in many studies unfertilized switchgrass performed as well as fertilized stands. These findings are in agreement with the yield response of switchgrass in our study, which responded positively to fertilizer rates but reached optimum levels between 79-161 kg N ha⁻¹. Wullschleger et al. (2010) reported an average yield for upland switchgrass ecotypes to be 8.7 Mg ha⁻¹, which was similar to yields of treatments above 100 kg N ha⁻¹ in this study.

Switchgrass yields increased and the critical N rate at which maximum yields were achieved decreased over the study. Annual precipitation was substantially lower in 2012 than in 2013-2014 (48, 62, 67 cm, respectively) and 41% below the 30-yr average (82 cm), which may partially explain the lower yields. However, an 80 kg N ha⁻¹ decrease in the critical N rate as well as observations of increased pre-fertilization green-up and growth of fertilized plots in 2013 and 2014, suggest that there may have been a carryover effect of fertilizer between years. Soil N levels did not show signs of residual N at 0-15 cm, but it is possible N was accumulating deeper in the soil profile. It is likely that much of the carryover was from N stored in switchgrass roots. Studies have observed increases in switchgrass root biomass N concentrations with increasing N rate, and root N stocks as high as 315 kg N ha⁻¹ (Heggenstaller et al., 2009; Garten et al., 2011).

Many researchers have observed decreases in aboveground biomass N accompanied by increases in root or rhizome N of warm-season grasses at the end of the growing season, suggesting translocation of N to belowground biomass occurs during senescence (McKendrick et al., 1975; Clark, 1977; Dell et al., 2005; Lemus et al., 2008a). This N may be retained in perennial grasses for extended periods of time. Dell et al. (2005) observed the retention of ^{15}N taken up by native prairie grass 5 yrs after N addition, and Lemus et al. (2008a) observed significant yield response to N additions 3 yrs after application. McKendrick et al. (1975) estimated that 18% of annual N requirements in big bluestem and indiangrass were supplied from belowground N. Drops in aboveground N concentrations of 20-60% during senescence are commonly reported in switchgrass, suggesting that translocation of N to roots may be substantial (Lemus et al., 2008a; Yang et al., 2009; Kering et al., 2012). Availability of N translocated to belowground biomass in subsequent growing seasons could explain why the critical N rate of switchgrass decreased throughout the study.

Both switchgrass biomass N concentration and N removal increased with increasing N fertilizer. Several studies have observed increases in switchgrass N concentration at higher N rates (Lemus et al., 2008b; Heggenstaller et al., 2009; Garten et al., 2011). Increases in N concentration at higher N rates may explain why N removal increased linearly, even at rates beyond which a positive yield response was observed. Other studies have also observed linear increases in N removal with increasing yield (Jung and Lal, 2011). Removal of N accounted for 20-50% of fertilizer N applied each year. Removal at 0N was 7-17 kg N ha⁻¹. These results, as well as the positive yield response to N, demonstrate that N inputs would be necessary to maintain long-term productivity in switchgrass.

Few other studies have measured N₂O emissions from soils in switchgrass. Schmer et al. (2012) observed fluxes of 0.14-0.46 kg N₂O-N ha⁻¹ in switchgrass receiving 0 and 67 kg N ha⁻¹ and Smith et al. (2013) measured emissions of 0.80-1.4 kg N₂O-N ha⁻¹ in switchgrass receiving 56 kg N ha⁻¹. Emissions in both studies fall within the range of our study at similar N rates. Wile et al. (2014) and Nikiema et al. (2011) reported maximum emissions of 0.93 and 0.64 kg N₂O-N ha⁻¹ from switchgrass receiving 120 and 112 kg N ha⁻¹, respectively. The fluxes reported by Wile et al. (2014) and Nikiema et al. (2011) are lower than the fluxes observed at similar N rates in this study, which may be attributable to differences in climate or different soil textures.

The relationship between N₂O emissions and N rate differed in each year of this study and increased nonlinearly in 2 out of 3 years. In two meta-analyses of N₂O response to N rate, 71 datasets were best explained by exponential models, 35 by linear models, and 13 with hyperbolic models (Kim et al., 2013; Shcherbak et al., 2014). Kim et al. (2013) hypothesized that if enough N inputs are added to soil, a 3-phase N₂O response may be observed that includes a linear increase at low N rates where there is high competition for N by plants and denitrifiers, an exponential increase once available N exceeds plant requirements, and a hyperbolic response once N additions have exceeded the ability of microbes to utilize N for N₂O production and N₂O production reaches a plateau. This response can be modeled using the sigmoidal response of the Hill Equation (Kim et al., 2013), which best explained 2014 N₂O emissions in this study. The decrease in critical N rate of switchgrass yield in 2014 suggests that the N demand of switchgrass was lower than in previous years. This may result in more available N for denitrifying bacteria, which could explain the higher N₂O emissions and plateau effect observed at high N rates in 2014. These results suggest that N fertilizer application beyond switchgrass needs could

result in large losses of N₂O, with negative impacts on the GHG balance of switchgrass-based biofuel.

One effect of nonlinear N₂O responses is that one EF cannot be applied across levels of N inputs (Shcherbak et al., 2014). This study observed an increase in EF with increasing fertilizer rate to levels over 2.5 higher than the IPCC 1% EF. These findings are in agreement with Shcherbak et al. (2014), who found that the IPCC 1% EF under predicts N₂O emissions at high N rates.

The majority of N₂O emissions occurred within 2 months of fertilizer application. At N rates above 100 kg N ha⁻¹, over 70% of N₂O was emitted by July 1. Differences in precipitation during this period of time partially explain differences observed between the maximum N₂O emissions, which occurred at 150N all 3 yrs. Annual fluxes were highest in 2014, when 27.4 cm of precipitation fell within 2 months of fertilizer application. Only 10.5 and 13.3 cm of precipitation fell in the 2 months after fertilizer application in 2012 and 2013, and maximum fluxes were 34% and 58% lower than in 2014, respectively. These findings emphasize the importance of implementing N management strategies to reduce N availability early in the switchgrass growing season, especially at high rates of N application.

Yield-scaled costs of production were minimized at 100N. High yield gains with N additions at rates up to 100 kg N ha⁻¹ decreased yield-scaled production costs. At N rates above 100N, yield gains from additional N did not offset increases in fertilizer cost. These results demonstrate the importance of N inputs in increasing the economic efficiency of switchgrass production, but that excessive N can drive up costs. Estimates of cost of production in the literature vary greatly because of assumptions behind the estimates, which can make comparisons between studies difficult. Duffy et al. (2002) found that land rental cost and yield

had particularly large impact on switchgrass costs of production. However, many studies reported production costs within the range reported in this study. Duffy et al. (2002) estimated cost of production to be \$65-74 Mg⁻¹ at switchgrass yields of 9.0 Mg ha⁻¹, while estimates by Haque et al. (2013) and Perrin et al. (2012) were \$81-\$84 Mg⁻¹ and \$70.70 Mg⁻¹, respectively. Khanna et al. (2008) estimated cost without land rent to be \$56.93 Mg⁻¹, which is similar to our study when land rent is excluded from the analysis.

Conclusions

Switchgrass yield responded positively to N fertilizer application at fertilizer rates below 100-150 kg N ha⁻¹. The critical N rate required to reach maximum yield decreased by 82 kg N ha⁻¹ over 3 yrs, possibly due to carryover of N translocated to switchgrass roots at the end of each growing season. The relationship between N₂O emissions and N rate differed in each year of this study and increased nonlinearly in 2 out of 3 years. In 2014 N₂O emissions were the highest in the study, and followed a sigmoidal response that suggests N availability had exceeded the ability of microbes to utilize N for N₂O production. The yield and N₂O responses suggest that at N rates above 100 kg ha⁻¹, N was exceeding plant needs, especially in the 3rd year of the study. Based on these findings, N rates above 100 kg ha⁻¹ in switchgrass should be discouraged due to minimal yield gains, increased costs, and increased N₂O emissions. If this study was continued, it is possible the critical N rate would continue to decrease. Nitrogen response studies that span the entire life of the switchgrass stand are needed in order to determine how N carryover impacts optimum N rate over longer time spans.

Emission factors (EF) for fertilizer induced N₂O emissions increased from 0.7% to 2.6% with increasing N, further demonstrating a non-linear response of N₂O to N inputs. This differs from the IPCC Tier 1 EF, which is 1% regardless of fertilizer rates. These findings suggest that

N₂O emissions would be under predicted by the IPCC Tier 1 model at higher N rates, which could lead to the underestimation of GHG emissions from biofuel if used in life cycle assessments. Future life cycle assessments of biofuel need to use methodology that will account for the nonlinear response of N₂O to N inputs, such as implementing process-based models or N rate dependent EFs.

In all 3 study years, most N₂O emissions (40-95%) occurred early in the growing season, when available soil nitrogen and rainfall were both high. Delaying fertilizer application to later in the growing season or splitting fertilizer application could help reduce emissions by providing N when uptake by switchgrass will be more rapid. Future studies should examine the impact of fertilizer application timing on switchgrass yield and N₂O emissions.

Total costs of production in switchgrass increased by nearly \$4,000 ha⁻¹ when 200 kg N ha⁻¹ was applied, mainly due to increased N fertilizer and harvest costs. Costs of production per unit biomass were minimized at 100 kg N ha⁻¹, which was approximately \$23 Mg⁻¹ lower than when no N was applied. Costs increased with N applications above 100 kg ha⁻¹. These results demonstrate the importance of N inputs in maximizing the economic potential of switchgrass, but also that N inputs in excess can result in increased costs and higher GHG emissions. Together, these results show that fertilizer management can have large impacts on the productivity, soil GHG emissions, and production costs of switchgrass.

References

- Adler, P.R., S.J.D. Grosso, and W.J. Parton. 2007. Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems. *Ecol. Applications* 17:675-691.
- Bouwman, A.F., L.J.M. Boumans, and N.H. Batjes. 2002. Emissions of N₂O and NO from fertilized fields: Summary of available measurement data. *Global Biogeochem. Cycles* 16:1058.
- Clark, F. 1977. Internal cycling of N-15 in shortgrass prairie. *Ecology* 58:1322-1333.
- Crutzen, P.J., A.R. Mosier, K.A. Smith, and W. Winiwarter. 2008. N₂O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. *Atmos. Chem. Phys.* 8:389-395.
- De Klein, C., R. Novoa, S. Ogle, K. Smith, P. Rochette, T. Wirth, B. McConkey, A. Mosier, and K. Rypdal. 2006. N₂O emissions from managed soils, and CO₂ emissions from lime and urea application. *In* N₂O emissions from managed soils, and CO₂ emissions from lime and urea application. 2006 IPCC guidelines for national greenhouse gas inventories. IPCC.
- Dell, C., M. Williams, and C. Rice. 2005. Partitioning of nitrogen over five growing seasons in tallgrass prairie. *Ecology* 86:1280-1287.
- Don, A., B. Osborne, A. Hastings, U. Skiba, M.S. Carter, J. Drewer, H. Flessa, A. Freibauer, N. Hyvonen, M.B. Jones, G.J. Lanigan, U. Mander, A. Monti, S.N. Djomo, J. Valentine, K. Walter, W. Zegada-Lizarazu, and T. Zenone. 2012. Land-use change to bioenergy production in Europe: Implications for the greenhouse gas balance and soil carbon. *GCB Bioenergy* 4:372-391.
- Duffy, M.D., and V.Y. Nanhou. 2002. Costs of producing switchgrass for biomass in southern Iowa. ASHS Press, Alexandria, USA.
- Garten, C.T., Jr., D.J. Brice, H.F. Castro, R.L. Graham, M.A. Mayes, J.R. Phillips, W.M. Post III, C.W. Schadt, S.D. Wullschleger, D.D. Tyler, P.M. Jardine, J.D. Jastrow, R. Matamala, R.M. Miller, K.K. Moran, T.W. Vugteveen, R.C. Izaurralde, A.M. Thomson, T.O. West, J.E. Amonette, V.L. Bailey,

- F.B. Metting, and J.L. Smith. 2011. Response of "alamo" switchgrass tissue chemistry and biomass to nitrogen fertilization in west Tennessee, USA. *Agric. Ecosys. Environ.* 140:289-297.
- Goutelle, S., M. Maurin, F. Rougier, X. Barbaut, L. Bourguignon, M. Ducher, and P. Maire. 2008. The Hill equation: A review of its capabilities in pharmacological modelling. *Fundam. Clin. Pharmacol.* 22:633-648.
- Hallam, A., I.C. Anderson, and D.R. Buxton. 2001. Comparative economic analysis of perennial, annual, and intercrops for biomass production. *Biomass Bioenergy* 21:407-424.
- Haque, M., J.T. Biermacher, M.K. Kering, and J.A. Guretzky. 2013. Economics of alternative fertilizer supply systems for switchgrass produced in phosphorus-deficient soils for bioenergy feedstock. *BioEnergy Res.* 6:351-357.
- Hartmann, D.L., A.M.G. Klein Tank, M. Rusticucci, L.V. Alexander, S. Bronnimann, Y. Charabi, F.J. Dentener, E.J. Dlugokencky, D.R. Easterling, A. Kaplan, B.J. Soden, P.W. Thorne, M. Wild, and P.M. Zhai. 2013. Observations: Atmosphere and surface. p. 159. *In* T.F. Stocker, D. Qin, G.-. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.) *The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change.* Cambridge University Press, Cambridge, UK.
- Heggenstaller, A.H., K.J. Moore, M. Liebman, and R.P. Anex. 2009. Nitrogen influences biomass and nutrient partitioning by perennial, warm-season grasses. *Agron. J.* 101:1363-1371.
- Hutchinson, G.L., and A.R. Mosier. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Sci. Soc. Am. J.* 45:311-316.
- Ibendahl, G., D.M. O'Brien, D. Shoup, and S. Duncan. 2015. Brome hay cost-return budget in central and eastern Kansas. Rep. MF2143. Kansas State Univ.
- Jung, J.Y., and R. Lal. 2011. Impacts of nitrogen fertilization on biomass production of switchgrass (*Panicum virgatum* L.) and changes in soil organic carbon in Ohio. *Geoderma* 166:145-152.
- Jung, J.Y., R. Lal, J.D. Jastrow, and D.D. Tyler. 2011. Nitrogenous fertilizer effects on soil structural properties under switchgrass. *Agric. Ecosyst. Environ.* 141:215-220.

- Kansas State Univ. 2014. Kansas Mesonet. Weather Data Library. 2014.
- Kering, M.K., T.J. Butler, J.T. Biermacher, and J.A. Guretzky. 2012. Biomass yield and nutrient removal rates of perennial grasses under nitrogen fertilization. *BioEnergy Res.* 5:61-70.
- Khanna, M., B. Dhungana, and J. Clifton-Brown. 2008. Costs of producing miscanthus and switchgrass for bioenergy in Illinois. *Biomass Bioenergy* 32:482-493.
- Kim, D., G. Hernandez-Ramirez, and D. Giltrap. 2013. Linear and nonlinear dependency of direct nitrous oxide emissions on fertilizer nitrogen input: A meta-analysis. *Agric. Ecosys. Environ.* 168:53-65.
- Kim, S., and B.E. Dale. 2009. Regional variations in greenhouse gas emissions of biobased products in the United States-corn-based ethanol and soybean oil. *Int. J. Life Cycle Assess.* 14:540-546.
- Klemedtsson, A.K., and K.A. Smith. 2011. The significance of nitrous oxide emission due to cropping of grain for biofuel production: A Swedish perspective. *Biogeosci.* 8:3581-3591.
- Lemus, R., D.J. Parrish, and O. Abaye. 2008a. Nitrogen-use dynamics in switchgrass grown for biomass. *BioEnergy Res.* 1:153-162.
- Lemus, R., E.C. Brummer, C.L. Burras, K.J. Moore, M.F. Barker, and N.E. Molstad. 2008b. Effects of nitrogen fertilization on biomass yield and quality in large fields of established switchgrass in southern Iowa, USA. *Biomass Bioenergy* 32:1187-1194.
- McKendrick, J.D., C.E. Owensby, and R.M. Hyde. 1975. Big bluestem and indian grass vegetative reproduction and annual reserve carbohydrate and nitrogen cycles. *Agro-Ecosyst.* 2:75-93.
- Mitchell, R., K.P. Vogel, and G. Sarath. 2008. Managing and enhancing switchgrass as a bioenergy feedstock. *Biofuels Bioproducts Biorefining* 2:530-539.
- Mooney, D.F., R.K. Roberts, B.C. English, D.D. Tyler, and J.A. Larson. 2009. Yield and breakeven price of 'alamo' switchgrass for biofuels in Tennessee. *Agron. J.* 101:1234-1242.
- Myhre, G., D. Shindell, F. Breon, W. Collins, J. Fuglestedt, J. Huang, D. Koch, J. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura, and H. Zhang. 2013. Anthropogenic and natural radiative forcing. p. 659. *In* T.F. Stocker, D. Qin, G. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.) *The physical science basis.*

- Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, U.K.
- Nikiema, P., D.E. Rothstein, D.H. Min, and C.J. Kapp. 2011. Nitrogen fertilization of switchgrass increases biomass yield and improves net greenhouse gas balance in northern Michigan, USA. *Biomass Bioenerg.* 35:4356-4367.
- Perrin, R., J. Sesmero, K. Wamisho, and D. Bacha. 2012. Biomass supply schedules for Great Plains delivery points. *Biomass Bioenerg.* 37:213-220.
- Sadeghpour, A., L.E. Gorkitsky, M. Hashemi, S.A. Weis, and S.J. Herbert. 2014. Response of switchgrass yield and quality to harvest season and nitrogen fertilizer. *Agron. J.* 106:290-296.
- Schmer, M.R., M.A. Liebig, J.R. Hendrickson, D.L. Tanaka, and R.L. Phillips. 2012. Growing season greenhouse gas flux from switchgrass in the northern Great Plains. *Biomass Bioenerg.* 45:315-319.
- Shcherbak, I., N. Millar, and G.P. Robertson. 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N₂O) emissions to fertilizer nitrogen. *Proc. Natl. Acad. Sci. U. S. A.* 111:9199-9204.
- Smith, C.M., M.B. David, C.A. Mitchell, M.D. Masters, K.J. Anderson-Teixeira, C.J. Bernacchi, and E.H. DeLucia. 2013. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. *J. Environ. Qual.* 42:219-228.
- Smith, K.A., A.R. Mosier, P.J. Crutzen, and W. Winiwarter. 2012. The role of N₂O derived from crop-based biofuels, and from agriculture in general, in earth's climate. *Philosophical Transactions of the Royal Society B-Biological Sci.* 367:1169-1174.
- Stehfest, E., and L. Bouwman. 2006. N₂O and NO emission from agricultural fields and soils under natural vegetation: Summarizing available measurement data and modeling of global annual emissions. *Nutr. Cycl. Agroecosys.* 74:207-228.
- Thompson, C.R., D.E. Peterson, W.H. Fick, P.W. Stahlman, and J.W. Slocombe. 2015. Chemical weed control for field crops, pastures, rangeland, and noncropland. Rep. SRP1117. Kansas State Univ.
- USDA NASS. 2015. Average U.S. farm prices of selected fertilizers. *Agricultural Prices.*

- USDOE. 2011. U.S. billion-ton update: Biomass supply for a bioenergy and bioproducts industry. Rep. ORNL/TM-2011/224. Oak Ridge National Laboratory, Oak Ridge, TN.
- USEPA. 2014. Inventory of U.S. greenhouse gas emissions and sinks: 1990 - 2012. Rep. EPA 430-R-14-003.
- Vadas, P.A., K.H. Barnett, and D.J. Undersander. 2008. Economics and energy of ethanol production from alfalfa, corn, and switchgrass in the upper Midwest, USA. *BioEnergy Res.* 1:44-55.
- Weiss, J. 1997. The Hill equation revisited: Uses and misuses. *FASEB J.* 11:835-841.
- Wile, A., D.L. Burton, M. Sharifi, D. Lynch, M. Main, and Y.A. Papadopoulos. 2014. Effect of nitrogen fertilizer application rate on yield, methane and nitrous oxide emissions from switchgrass (*Panicum virgatum* L.) and reed canarygrass (*Phalaris arundinacea* L.). *Can. J. Soil Sci.* 94:129-137.
- Wullschleger, S.D., E.B. Davis, M.E. Borsuk, C.A. Gunderson, and L.R. Lynd. 2010. Biomass production in switchgrass across the United States: Database description and determinants of yield. *Agron. J.* 102:1158-1168.
- Yang, J., E. Worley, M. Wang, B. Lahner, D.E. Salt, M. Saha, and M. Udvardi. 2009. Natural variation for nutrient use and remobilization efficiencies in switchgrass. *BioEnergy Res.* 2:257-266.

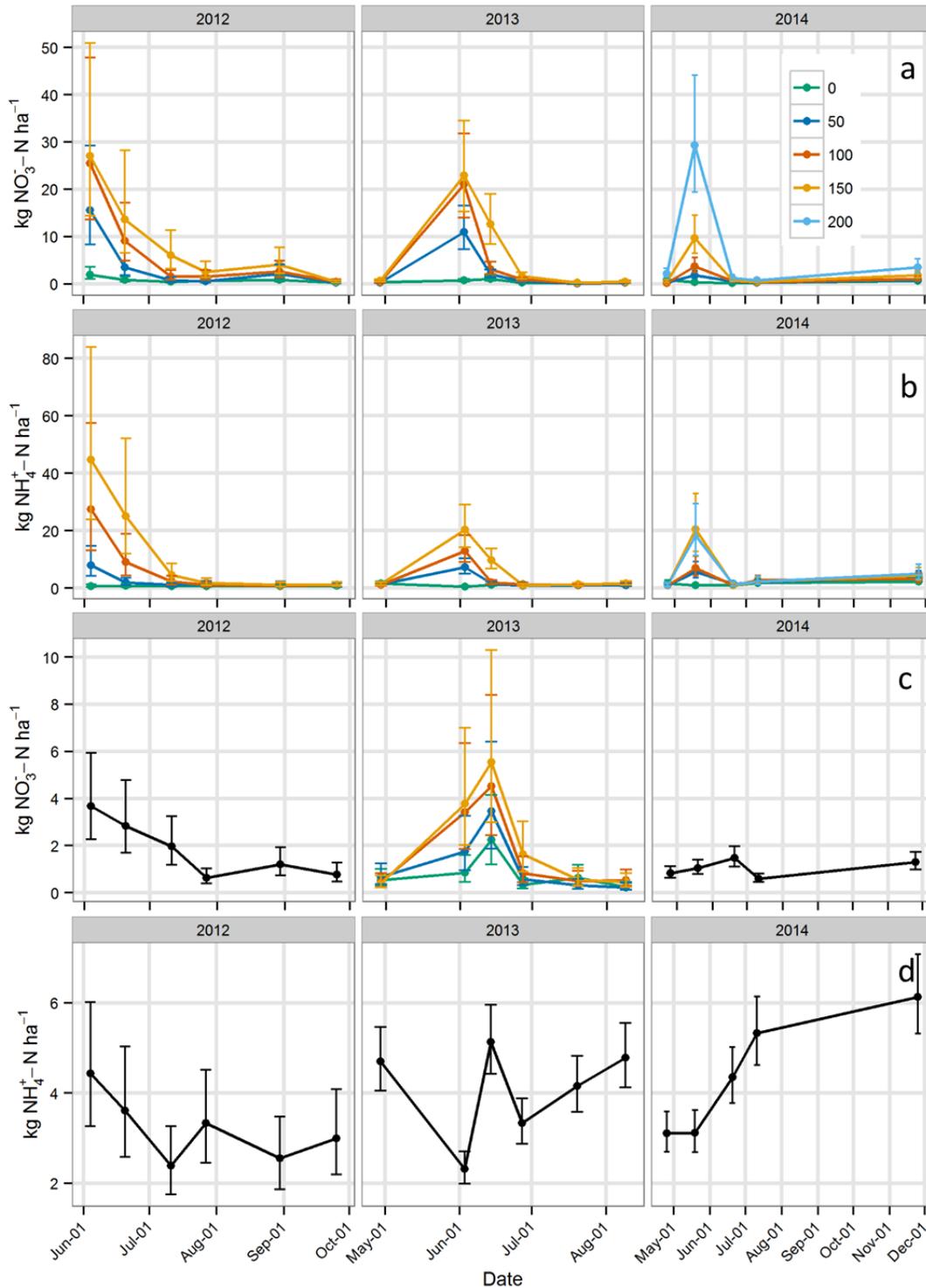


Figure 4.1. Figure 1. Soil nitrate and ammonium by N fertilizer rate from 2012-2014. Black lines show effect of sampling date averaged over time when interaction between date and N rate was not significant. Error bars indicate 95% confidence intervals. 'a' and 'b' are nitrate and ammonium at 0-5 cm, respectively. 'c' and 'd' are nitrate and ammonium at 5-15 cm.

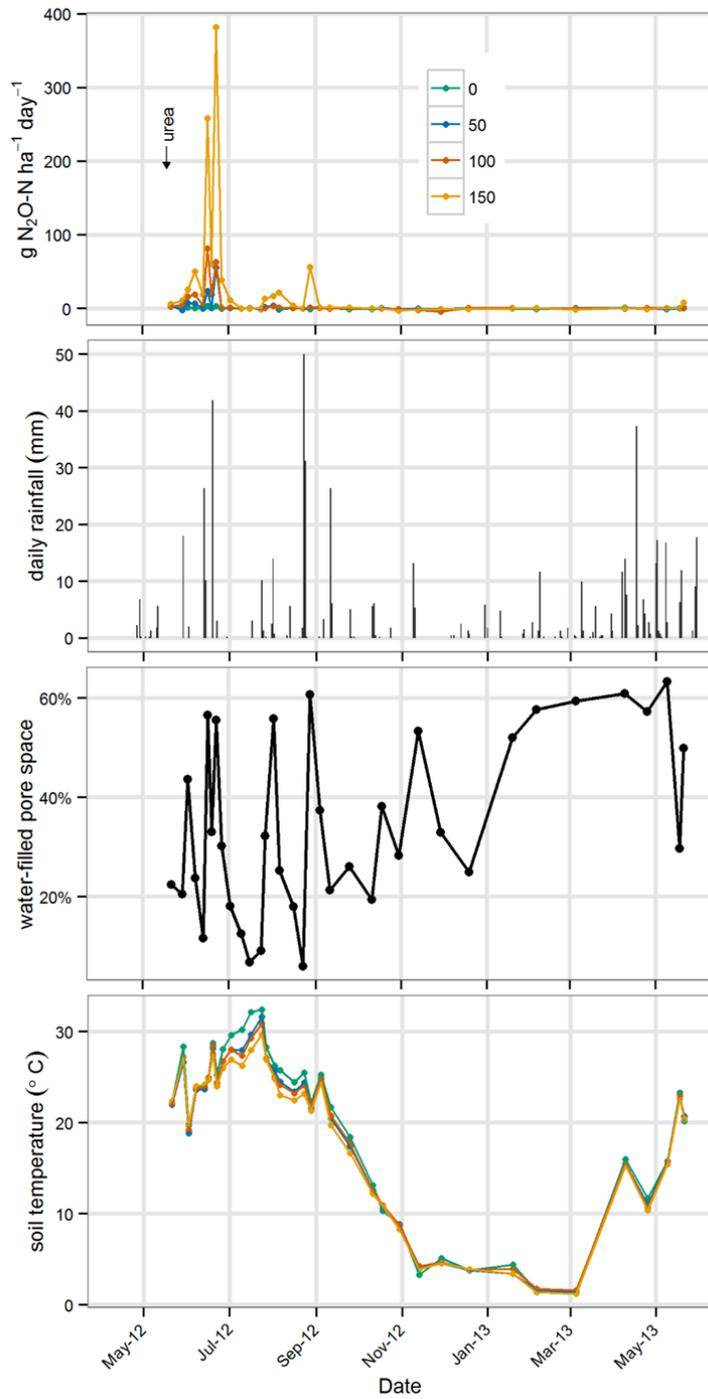


Figure 4.2. Mean daily N_2O flux by N fertilizer rate, percent water-filled pore space, daily precipitation, and average daily temperature from 2012-2013. Label “urea” indicates the date that urea was applied. Rainfall data recorded at nearby meteorological station.

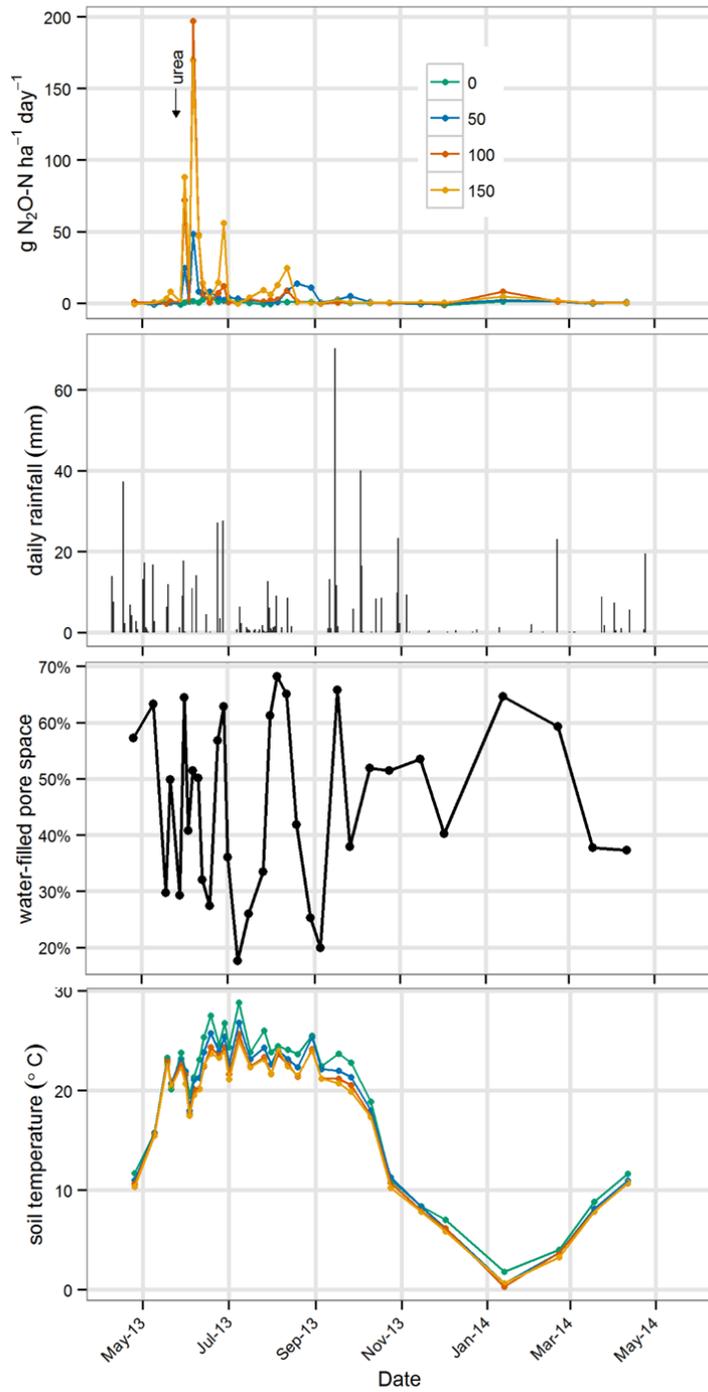


Figure 4.3. Mean daily N_2O flux by N fertilizer rate, percent water-filled pore space, daily precipitation, and average daily temperature from 2013-2014. Label “urea” indicates the date that urea was applied. Rainfall data recorded at nearby meteorological station.

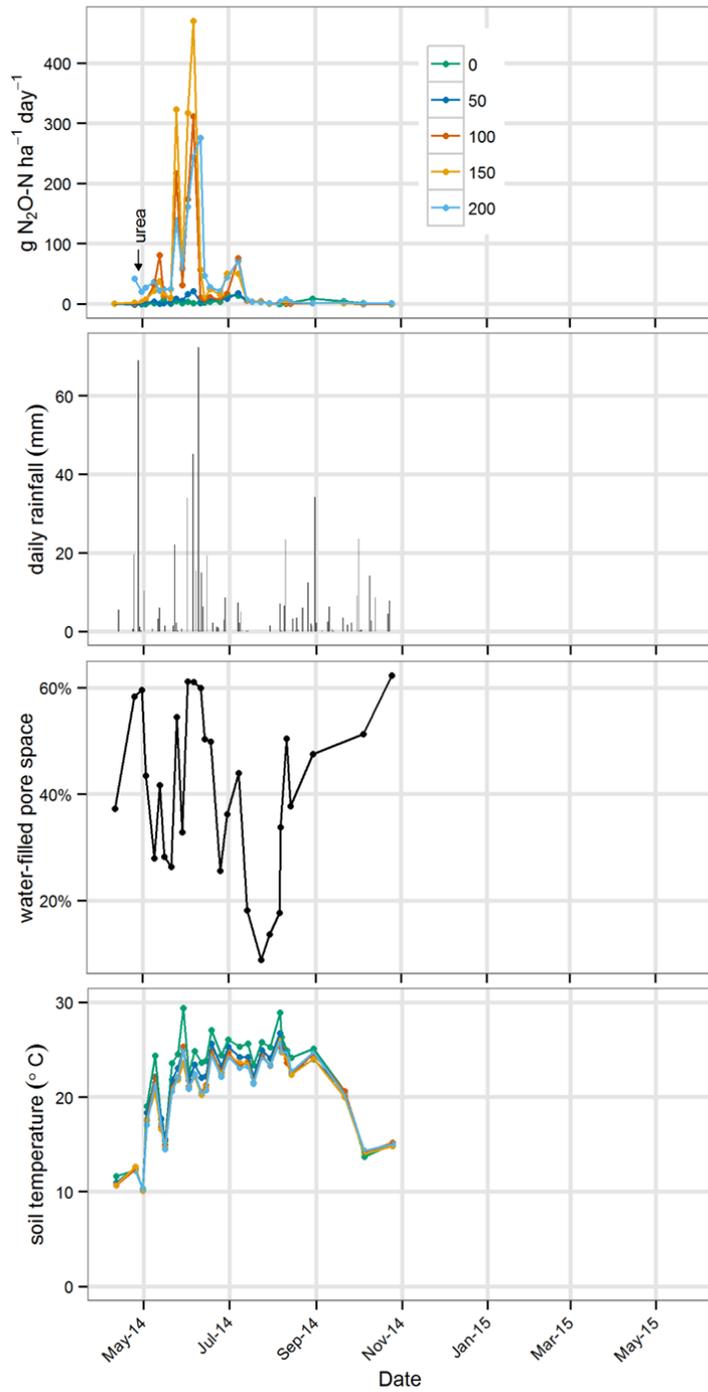


Figure 4.4. Mean daily N_2O flux by N fertilizer rate, percent water-filled pore space, daily precipitation, and average daily temperature in 2014 growing season. Label “urea” indicates the date that urea was applied. Rainfall data recorded at nearby meteorological station.

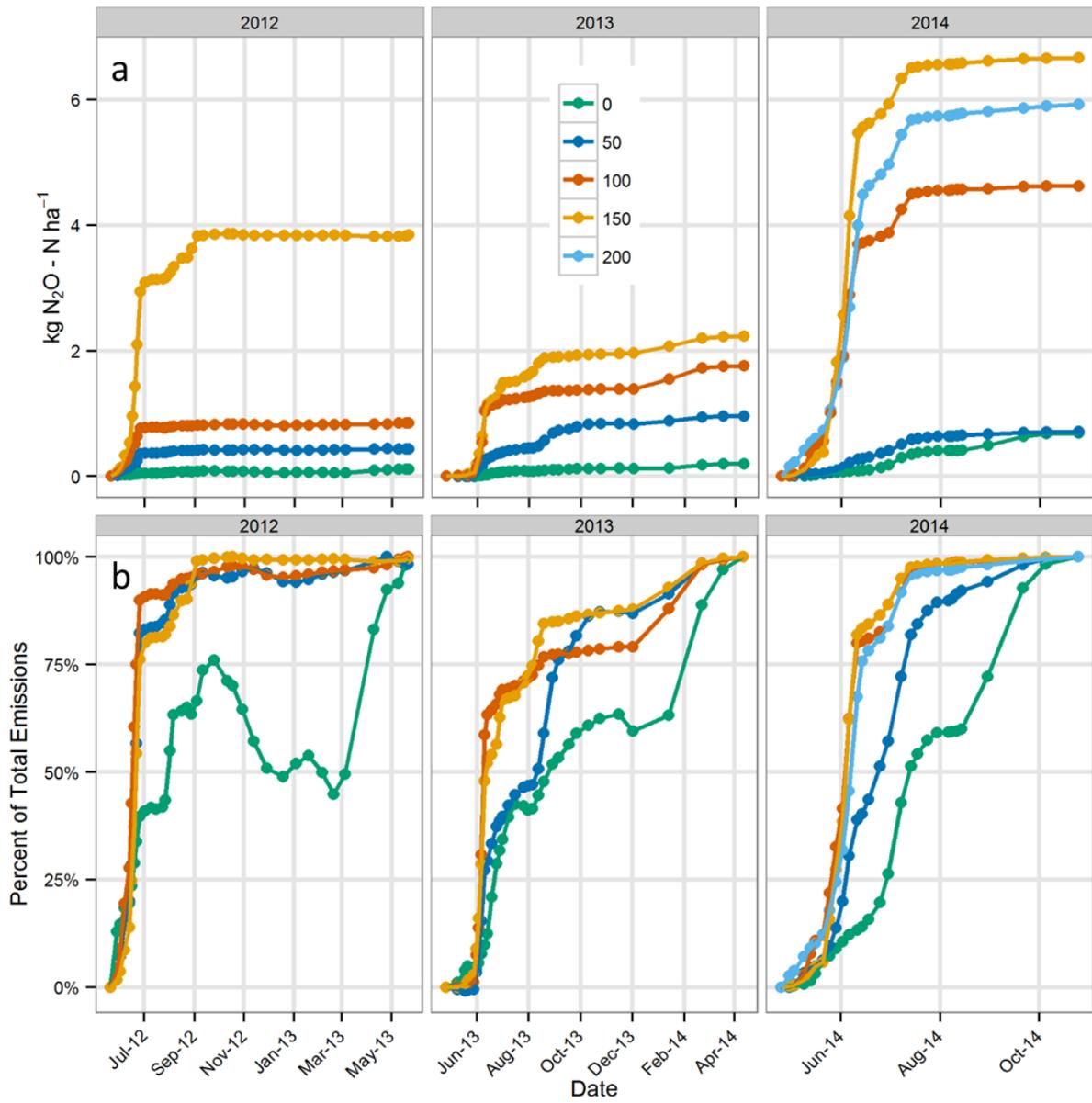


Figure 4.5. (a) Cumulative annual N_2O emissions for 2012-2014 by N fertilizer rate. (b) Percent of total annual N_2O emissions emitted by N fertilizer rate.

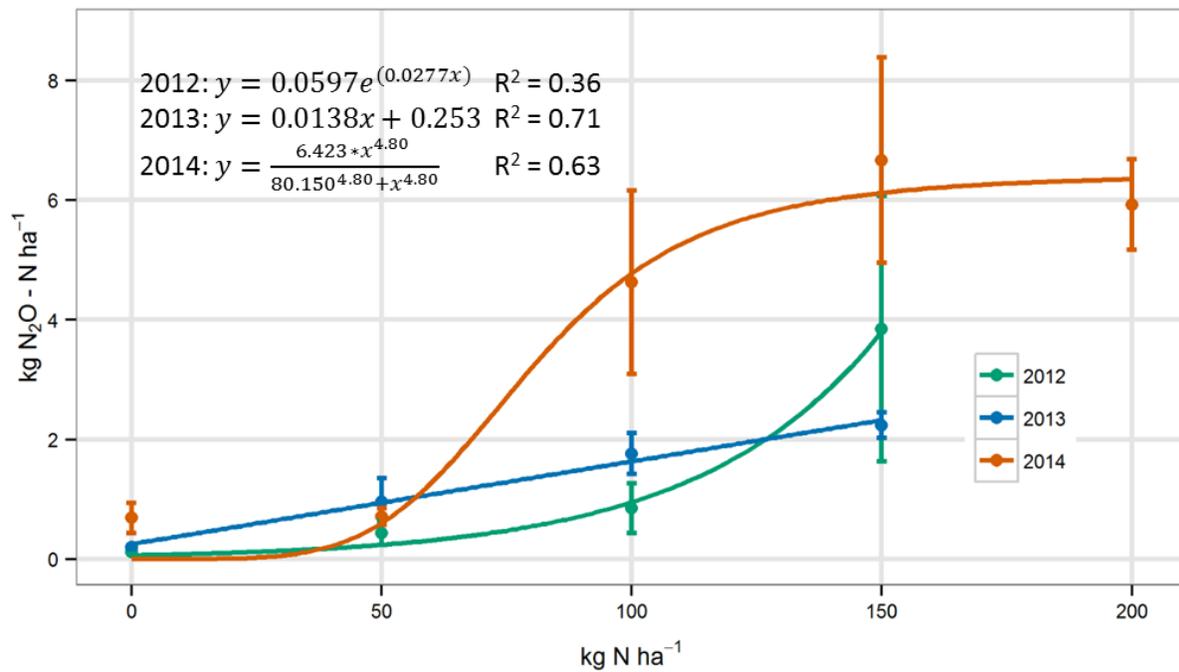


Figure 4.6. Total annual N₂O emissions in 2012-2014 over N fertilizer rate. Points and error bars represent the mean and standard error of emissions by N rate and year. Lines represent the predicted values of the best-fit model in each year.

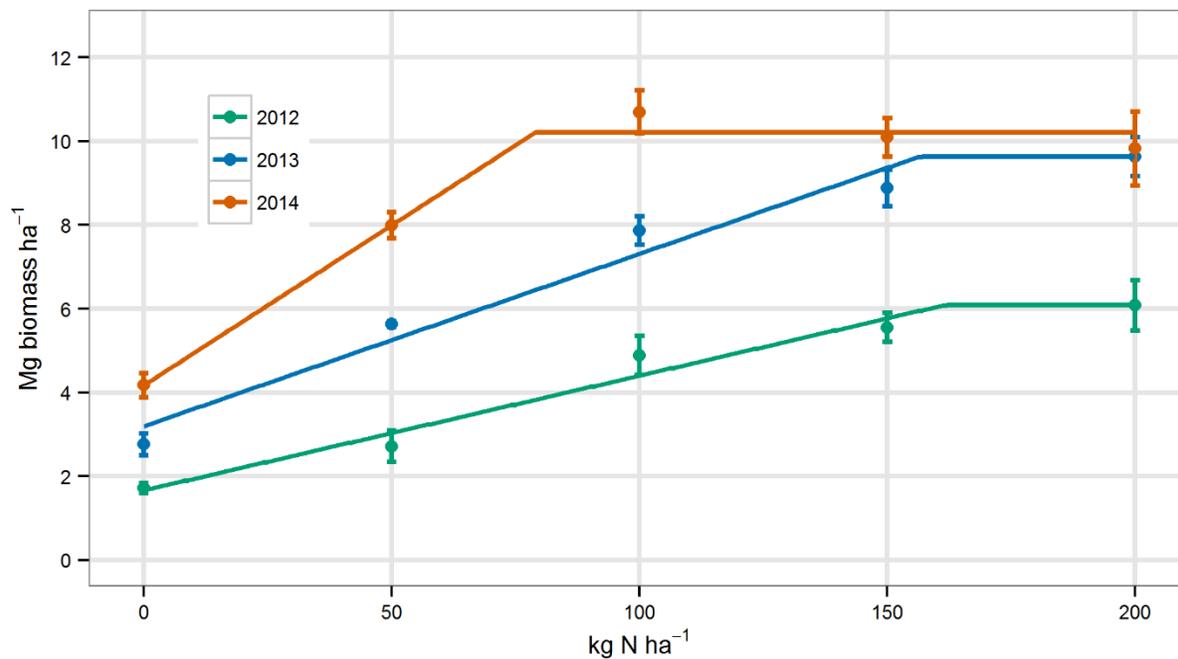


Figure 4.7. Effect of N fertilizer rate on switchgrass biomass yield (Mg dry matter ha⁻¹) in 2012-2013. Points and error bars represent the mean and standard error of emissions by N rate and year. Lines represent the predicted values of the best-fit model in each year.

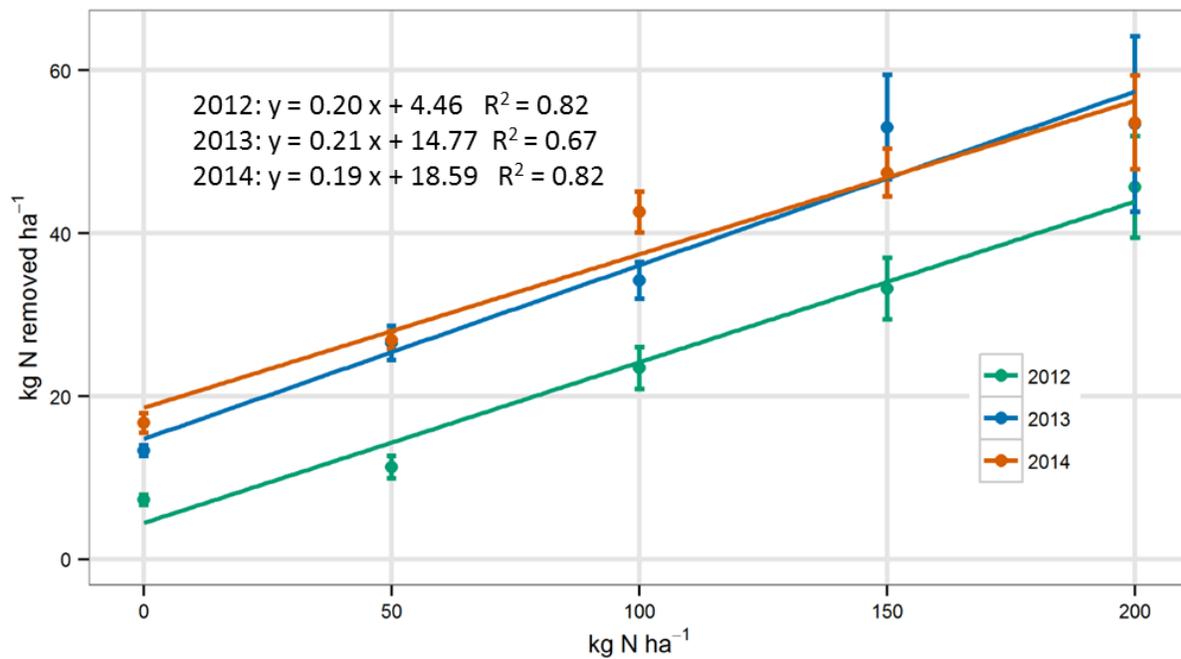


Figure 4.8. Quantity of N removed in switchgrass in 2012-2014. Points and error bars represent the mean and standard error of emissions by N rate and year. Lines represent the predicted values of the best-fit model in each year.

Table 4.1. Mean emissions factor (EF) by N fertilizer rate.

N rate (kg N ha ⁻¹)	EF (%)
50	0.7 b†
100	2.1 ab
150	2.6 a‡
200	2.6‡§
ANOVA	
Effect	p-value
N rate	0.03147
Year	0.11761
N rate x Year	0.07603

† Means followed by the same letter are not significantly different ($p < 0.05$)

‡ Significantly different from IPCC EF (1%) based on student's t-test

§ 2014 data only

Table 4.2. Best-fit models of switchgrass yield as influenced by N fertilizer rate by year.

Year	Model	Critical Level (x_0)	R^2
2012	$y = \begin{cases} 0.03x + 1.7, & x < x_0 \\ 0.03x_0 + 1.7, & x \geq x_0 \end{cases}$	161.3	0.82
2013	$y = \begin{cases} 0.04x + 3.2, & x < x_0 \\ 0.04x_0 + 3.2, & x \geq x_0 \end{cases}$	156.3	0.92
2014	$y = \begin{cases} 0.08x + 4.2, & x < x_0 \\ 0.08x_0 + 4.2, & x \geq x_0 \end{cases}$	78.9	0.86

Table 4.3. Mean biomass N concentration by year and by N fertilizer rate.

Year	g N kg ⁻¹ biomass
2012	5.32 a
2013	5.08 a
2014	4.32 b
N rate (kg N ha ⁻¹)	
0	4.39 b
50	4.09 b
100	4.37 b
150	5.55 a
200	6.13 a
ANOVA	
Effect	p-value
Year	0.0001
N rate	0.0009
Year x N rate	0.052

Table 4.4. Prices and other parameter values used in cost estimation.

Item	Price	Years
Seed, \$ kg ⁻¹	22.05	1
Urea, \$ kg ⁻¹ N	1.42	2-11
Ally XP, \$ g ⁻¹	0.35	1
Weathermax, \$ L ⁻¹	9.41	1
Banvel 4, \$ L ⁻¹	20.20	2
2, 4-D Amine, \$ L ⁻¹	4.23	2
Disk	32.47	1
Cultivate	29.53	1
Planting (Drill), \$ L ⁻¹	49.10	1
Fertilizer application, \$ ha ⁻¹	15.72	2-11
Herbicide application, \$ ha ⁻¹	13.74	1, 2
Swathing and conditioning, \$ ha ⁻¹	39.31	1-11
Sideraking, \$ ha ⁻¹	11.86	1-11
Baling, \$/ 0.68 Mg bale	18.83	1-11
Land rental rate, \$ ha ⁻¹	163.09	1-11
Interest on operating costs, %	6.5	1-11

Table 4.5. Summary of 11 year costs of production of switchgrass at different N fertilizer rates.

Category	Unit	Nitrogen rate (kg N ha ⁻¹)				
		0	50	100	150	200
Switchgrass yield†	Mg ha ⁻¹ yr ⁻¹	3.29	5.55	7.97	8.32	8.71
10 yr total yield†	Mg ha ⁻¹	32.95	55.59	79.66	83.21	87.14
Urea	\$ ha ⁻¹	0	709.31	1,418.63	2,127.94	2,837.25
Other input costs	\$ ha ⁻¹	140.09	140.09	140.09	140.09	140.09
Harvest costs	\$ ha ⁻¹	1,423.74	2,050.34	2,716.39	2,814.67	2,923.11
Other field operations	\$ ha ⁻¹	142.58	299.74	299.74	299.74	299.74
Interest	\$ ha ⁻¹	55.46	103.98	148.68	174.93	201.51
Land rent	\$ ha ⁻¹	1,793.98	1,793.98	1,793.98	1,793.98	1,793.98
Total Cost, NPV‡	\$ ha ⁻¹	3,110.90	4,431.80	5,648.56	6,363.02	7,086.69
Total Cost, NPV	\$ Mg ⁻¹	94.40	79.72	70.91	76.47	81.33

† Yields reported on dry matter basis

‡ Total costs presented as net present value at stand establishment

Chapter 5 - Summary

Global climate change is being driven by increases in atmospheric greenhouse gas (GHG) emissions. Minimizing the negative impacts of climate change requires strategies that can reduce GHG emissions while still meeting human needs. Biofuels are one strategy to reduce GHG emissions in the transportation sector. To ensure their effectiveness in mitigating emissions, measurements of GHG emissions during the production and use of biofuel are required. Emissions of nitrous oxide (N₂O) and changes in soil organic carbon (SOC) during biofuel feedstock production could have a large impact on the GHG footprint of biofuel, but there is uncertainty regarding SOC and N₂O responses in biofuel cropping systems.

Our results show that sweet sorghum, photoperiod-sensitive sorghum, and miscanthus exhibited the highest yield potential, with all 3 crops producing approximately 20 Mg ha⁻¹ during the last 3 study years. Both miscanthus and switchgrass took several years after establishment to reach biomass yields similar to the annual crops.

Soils of the perennial cropping systems increased in SOC over 4 years, sequestering 0.86-1.97 Mg C ha⁻¹ yr⁻¹. The high C sequestration capacity of the perennial crops demonstrates their potential to mitigate GHG emissions while providing feedstock for biofuel. The high C sequestration capacity of the perennial crops, demonstrates their potential to mitigate GHG emissions while providing feedstock for biofuel. Even though all crops were under no-tillage management, the annual systems did not show significant changes in SOC over 4 years. However both positive (corn-soybean, photoperiod-sensitive sorghum) and negative (sweet sorghum, grain sorghum) trends in SOC stocks were observed in this study. If residue removal was continued for longer periods of time, there might be significant decreases in SOC in some of the annual crops. This study also represented an extreme case where all standing biomass was

removed at harvest. In highly productive crops like sweet sorghum and photoperiod-sensitive sorghum, leaving a small percentage of aboveground biomass on the field at harvest would result in large quantities of stover to protect soils from erosion and SOC loss, while still providing high volumes of feedstock for biofuel production.

This study found evidence for several mechanisms that may be driving the increases in SOC stocks of the perennial crops. Root stocks were 4-8 times higher in the perennial crops, suggesting greater belowground C inputs. Additionally, evidence of elevated AM fungi and increased aggregate size in the perennials suggests physical protection of SOC may be enhanced in these systems, especially in miscanthus. The increases in SOC and aggregate size in the perennials systems suggest that these systems have the potential to improve soil quality while providing feedstock for biofuel production, which could be important in cases where these crops are utilized on marginal lands.

In both Chapter. 3 and Chapter. 4, a large portion (40-90%) of annual N₂O emissions were released in the first 8-10 weeks of the growing season. This finding emphasizes the importance of implementing N management strategies to reduce N availability early in the growing season, when the potential for high losses is present. Future studies should examine the impact of different strategies to reduce N₂O emissions in these systems, such as the use of split-applications or slow-release fertilizers. In 2013, post-growing season emissions contributed up to 40% of annual N₂O in some crops, which may be attributable to mineralization of soybean roots and nodules as well as freeze-thaw cycles. These findings demonstrate that it is important to measure N₂O emissions for the entire year, even in climates that do not experience prolonged freezing conditions.

No consistent significant differences were observed in annual N₂O emissions between photoperiod-sensitive sorghum, corn, switchgrass and miscanthus, which may be due to variable N rates and weather between study years. More measurements of N₂O emissions need to be made in these cropping systems to determine if there are long-term differences in N₂O emissions. Application of N to miscanthus in this study likely increased N₂O emissions relative to other crops. There is debate over the appropriate N rate for miscanthus, which could have a large impact on N₂O emissions. Future studies examining yield and N₂O response of miscanthus to N inputs are needed. This study found a weak correlation between measured N₂O emissions and IPCC Tier 1 estimates, likely due to high variability of measured emissions. The discrepancy between measured and predicted emissions tended to be greatest in instances where the measured emissions were higher than the IPCC estimates, indicating a tendency for the model to underestimate emissions.

Switchgrass yield responded positively to N fertilizer application. Yields increased by 50-80 kg ha⁻¹ for each kg N applied until a yield plateau was reached. The critical N rate required to reach maximum yield decreased by 82 kg N ha⁻¹ over 3 yrs, likely due to carryover of N translocated to switchgrass roots at the end of each growing season. The relationship between N₂O emissions and N rate differed in each year of this study and increased nonlinearly in 2 out of 3 years. In 2014 N₂O emissions were the highest in the study, and followed a sigmoidal response that suggests N availability had exceeded the ability of microbes to utilize N for N₂O production. The yield and N₂O responses suggest that at N rates above 100 kg ha⁻¹, N was exceeding plant needs, especially in the 3rd year of the study. Based on these findings, N rates above 100 kg ha⁻¹ in switchgrass should be discouraged due to minimal yield gains, increased costs, and increased N₂O emissions. If this study was continued, it is possible the critical N rate

would continue to decrease. Nitrogen response studies that span the entire life of the switchgrass stand are needed in order to determine how N carryover impacts optimum N rate over longer time spans.

Emission factors (EF) for fertilizer induced N₂O emissions increased from 0.7% to 2.6% with increasing N, further demonstrating a non-linear response of N₂O to N inputs. This differs from the IPCC Tier 1 EF, which is 1% across all fertilizer rates. These findings suggest that N₂O emissions would be under predicted by the IPCC Tier 1 model at higher N rates, which could lead to the underestimation of GHG emissions from biofuel if used in life cycle assessments. Future life cycle assessments of biofuel need to use methodology that will account for the nonlinear response of N₂O to N inputs, such as implementing process-based models or N rate dependent EFs.

Total costs of production in switchgrass increased by nearly \$4,000 ha⁻¹ when 200 kg N ha⁻¹ was applied, mainly due to increased N fertilizer and harvest costs. Costs of production per unit biomass were minimized at 100 kg N ha⁻¹, which was approximately \$23 Mg⁻¹ lower than when no N was applied. Costs increased with N applications above 100 kg ha⁻¹. These results demonstrate the importance of N inputs in maximizing the economic potential of switchgrass, but also that N inputs in excess can result in increased costs and higher GHG emissions.

Together, these results show that crop selection and fertilizer management can have large impacts on both the productivity and soil GHG emissions biofuel cropping systems. Sustainable biofuel production will depend on the selection of crops that are highly productive, but release minimal GHG emissions during cultivation. Based on the findings of this study, sweet sorghum, photoperiod-sensitive sorghum, and miscanthus appear to be the best biofuel crops for producers because of their high biomass production. However, careful management of sweet sorghum and

photoperiod-sensitive sorghum residues will be necessary to prevent the loss of SOC. In contrast, miscanthus had relatively low N₂O emissions when receiving 168 and 84 kg N ha⁻¹ and high C sequestration potential, making it an ideal crop for biofuel production.

Appendix A - Chapter 2

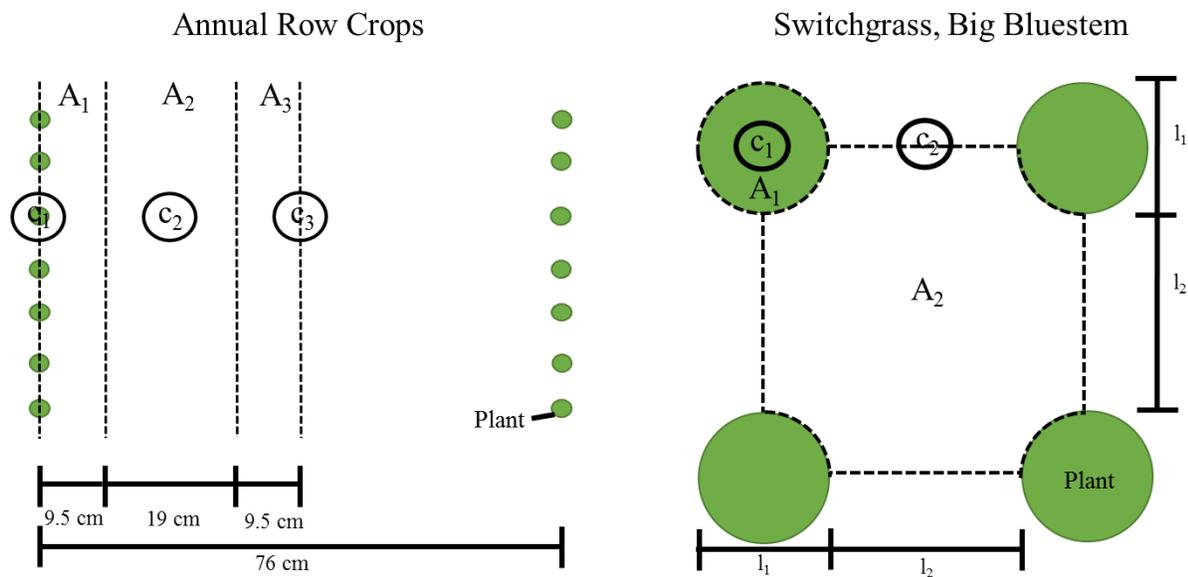


Figure A.1. Sampling scheme root stock sampling in annual row crops (left) and switchgrass and big bluestem (right). c_1 , c_2 and c_3 indicate locations where soil cores were taken. A_1 , A_2 and A_3 indicate the areas assumed to be represented by c_1 , c_2 and c_3 , respectively. l_1 and l_2 represent the diameter of the grass plant and the length from the nearest plant, respectively.

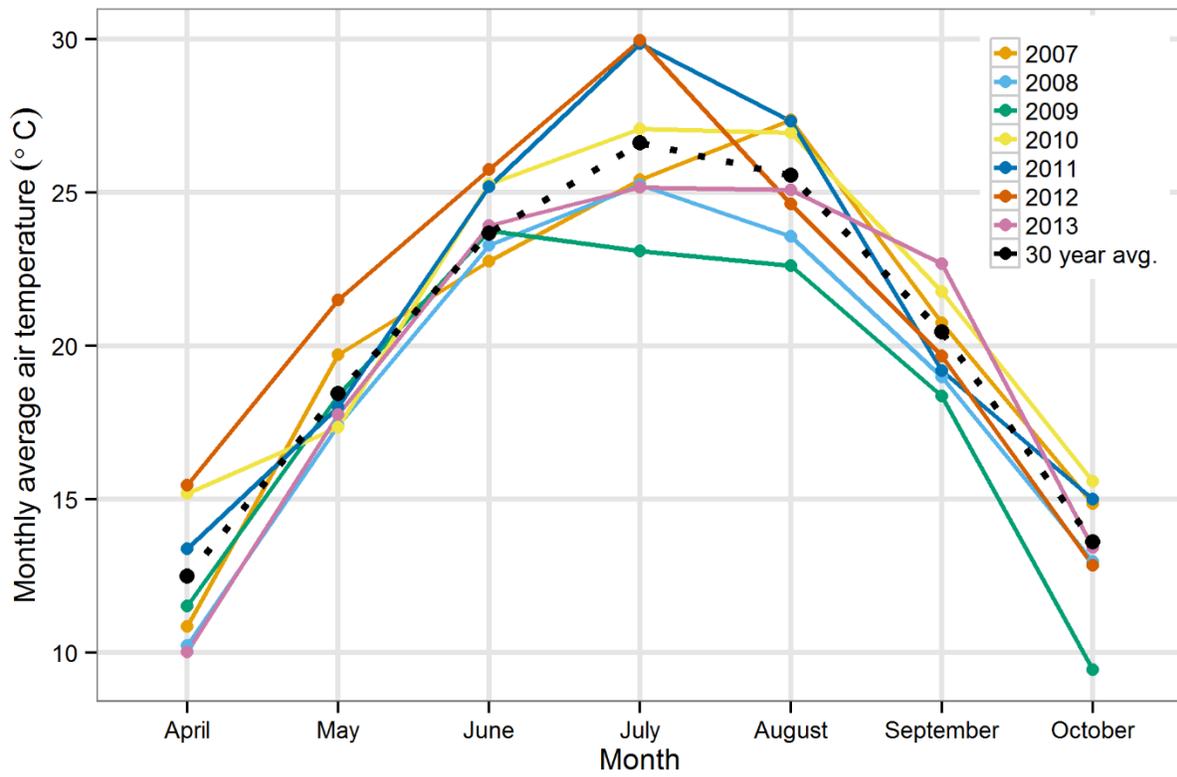


Figure A.2. Monthly average air temperatures at study site.

Table A.1. Cultivars planted over the duration of the study.

Crops	Year							
	2007	2008	2009	2010	2011	2012	2013	
Corn	Pioneer 33K40	Pioneer 33K44	Pioneer 33T57		DeKalb DKC63-49			
Photoperiod sorghum	Sorghum Partners 1990CA							
Sweet sorghum	Mississippi State M81E							
Dual Purpose/ Grain sorghum	Land-O-Lakes DKS59-09 dual purpose sorghum		Pioneer 84G62 grain sorghum					
Soybeans	KSU Foundation KS3406RR							
Miscanthus	Miscanthus x giganteus	Regrowth†						
Switchgrass	Kanlow	Regrowth						
Big bluestem	Kaw	Regrowth						

† Regrowth indicates regrowth from established perennial grasses and that no planting occurred.

Table A.2. Seeding rates and fertilizer rates over the duration of the study.

Crops	Year						
	2007	2008	2009	2010	2011	2012	2013
	Seed rate (seeds ha ⁻¹)						
Corn	68,000	68,000	28,000	30,000	28,000	28,000	28,000
Photoperiod sorghum	143,000	191,000	64,000	65,000	70,000	70,000	71,000
Sweet sorghum	143,000	191,000	64,000	50,000	70,000	70,000	71,000
Grain/Dual Purpose sorghum	143,000	191,000	64,000	65,000	70,000	70,000	71,000
Soybeans	250,000	296,000	140,000	140,000	140,000	140,000	140,000
Miscanthus	6,148†	-	-	-	-	-	-
Switchgrass	4.0‡	-	-	-	-	-	-
Big bluestem	6.3‡	-	-	-	-	-	-
	Fertilizer rate (kg ha ⁻¹)						
Corn	180 N 0 P 0 K	168 N 66 P 279 K	179 N 0 P 0 K	179 N 0 P 0 K	167 N 99 P 0 K	168 N 0 P 0 K	168 N 20 P 0 K
Photoperiod sorghum	180 N 0 P 0 K	168 N 66 P 279 K	179 N 0 P 0 K	179 N 0 P 0 K	167 N 99 P 0 K	112 N 0 P 0 K	112 N 20 P 0 K
Sweet sorghum	180 N 0 P 0 K	168 N 66 P 279 K	179 N 0 P 0 K	179 N 0 P 0 K	167 N 99 P 0 K	112 N 0 P 0 K	112 N 20 P 0 K
Grain/Dual Purpose sorghum	180 N 0 P 0 K	168 N 66 P 279 K	179 N 0 P 0 K	179 N 0 P 0 K	167 N 99 P 0 K	112 N 0 P 0 K	112 N 20 P 0 K
Soybeans	0 N 0 P 0 K	0 N 66 P 279 K	0 N 0 P 0 K	0 N 0 P 0 K	89 N 99 P 0 K	0 N 0 P 0 K	10 N 20 P 0 K
Miscanthus	§	45 N 66 P 279 K	112 N 0 P 0 K	112 N 0 P 0 K	167 N 99 P 0 K	84 N 0 P 0 K	84 N 20 P 0 K
Switchgrass	0 N 0 P 0 K	45 N 66 P 279 K	56 N 0 P 0 K	56 N 0 P 0 K	167 N 99 P 0 K	84 N 0 P 0 K	84 N 20 P 0 K
Big bluestem	0 N 0 P 0 K	45 N 66 P 279 K	56 N 0 P 0 K	56 N 0 P 0 K	167 N 99 P 0 K	84 N 0 P 0 K	84 N 20 P 0 K

† live plants ha-1

‡ kg live seed ha-1

§ 10.5 g Miracle Grow (24-8-16) applied to each miscanthus plant

Table A.3. Growing Season Precipitation and 30-year average at the study site. Measurements are from a meteorological station less than 1 km from the study site.

2007	2008	2009	2010	2011	2012	2013	30 yr avg
Growing season precipitation (cm)							
66.8	88.2	76.7	61.0	52.0	33.8	55.0	72.7

Table A.4. ANOVA and contrast p-values for crop yield.

Effect	crops included	Partial Interaction Contrasts			p-value
		years included	NDF†	DDF‡	
crop x year	all	2007-2011	27	153	<0.0001
crop x year	all but big bluestem	2007-2011, 2013	30	153	<0.0001
crop x year	all annuals	2007-2013	24	153	<0.0001
ANOVA					
crop x year¶	all	all	51	153	<0.0001

† Numerator degrees of freedom

‡ Denominator degrees of freedom

¶ To avoid model bias from missing crop x year combinations, ANOVA was performed using a cell-means model that included only the interaction of crop by year

Appendix B - Chapter 3

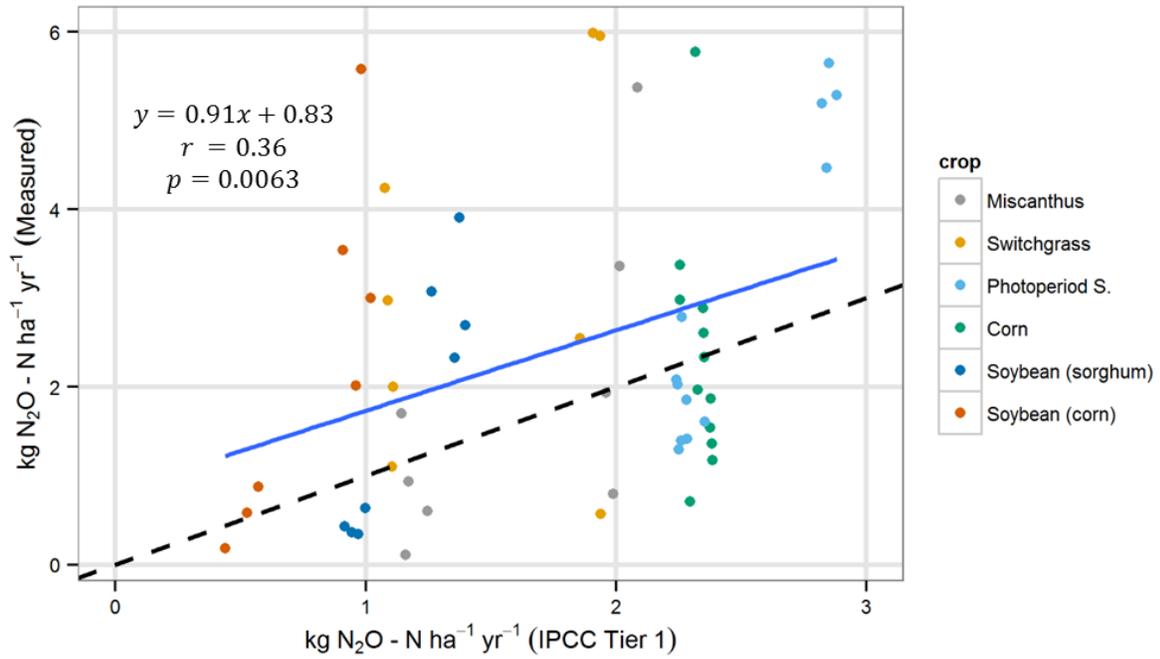


Figure B.1. Linear regression of IPCC Tier 1 N₂O estimates with 1% emission factor and measured N₂O emissions (blue line). Dashed line through the intercept with slope of 1 provided for reference.

Table B.1. Average monthly temperature, April – October and October precipitation in Manhattan, KS. Left column shows 1981-2010 30 year averages.

	Month	Year			
		2011-2012	2012-2013	2013-2014	30 yr. avg
Average Monthly Air Temperature (°C)	April	13.4	15.5	10.0	12.5
	May	18.0	21.5	17.8	18.4
	June	25.2	25.7	23.9	23.7
	July	29.9	29.9	25.2	26.6
	August	27.3	24.6	25.1	25.6
	September	19.2	19.7	22.7	20.4
	October	15.0	12.8	13.4	13.6
	November	6.9	8.8	5.6	6.2
	December	2.0	1.7	-2.0	-0.4
	January	2.1	0.7	-2.6	-1.6
	February	3.6	1.2	-2.9	1.1
	March	14.8	4.3	5.2	6.4
Precipitation (mm)	April – October	520	338	550	727
	November – March	292	78	50	97