

THE CARBON FOOTPRINT OF BIOENERGY SORGHUM PRODUCTION IN  
CENTRAL TEXAS: PRODUCTION IMPLICATIONS ON GREENHOUSE GAS  
EMISSIONS, CARBON CYCLING, AND LIFE CYCLE ANALYSIS

A Dissertation

by

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Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2013

Major Subject: Soil Science

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## ABSTRACT

Enhanced interest in biofuel production has renewed interest in bioenergy crop production within the United States. Agriculture's role in biofuel production is critical because it has the potential to supply renewable energy while minimizing greenhouse gas (GHG) emissions. However, agronomic management practices influence direct and indirect GHG emissions, and both can have a significant impact on biofuel production efficiency. Our overall objective was to determine the carbon (C) footprint of bioenergy sorghum (*Sorghum bicolor L.*) production in central Texas. Specifically, we determined the impacts of crop rotation, nitrogen (N) fertilization, and residue return on direct and indirect GHG emissions, theoretical biofuel yield, C pools, and life cycle GHG emissions from bioenergy sorghum production in 2010 and 2011.

An experiment established in 2008 near College Station, TX to quantify the impacts of crop management practices on bioenergy sorghum yield and soil properties was utilized, and included two crop rotations (sorghum-sorghum or corn-sorghum), two fertilization levels (0 or 280 kg N ha<sup>-1</sup> annually), and two residue return rates (0 or 50% biomass residue returned) to assess management impacts on sorghum production, C cycling, and life cycle GHGs. Corn production was poor under moderate drought conditions, while bioenergy sorghum produced relatively large yields under both moderate and severe drought conditions. Nitrogen addition increased crop yields, and rotated sorghum had higher yield than monoculture sorghum. Fluxes of CO<sub>2</sub> and N<sub>2</sub>O were higher than those reported in literature and highest soil fluxes were frequently observed following precipitation events during the growing season. Residue return increased cumulative CO<sub>2</sub> emissions and N fertilization

increased N<sub>2</sub>O emissions. Residue return also increased soil microbial biomass-C, an important indicator of soil quality. Continuous sorghum significantly increased soil organic C (SOC) concentrations near the soil surface and at two depths below 30 cm. Analysis of change in SOC across time to estimate net CO<sub>2</sub> emissions to the atmosphere revealed bioenergy sorghum production accrued high amounts of SOC annually. Most treatments accrued more than 4 Mg C ha<sup>-1</sup> yr<sup>-1</sup> from 2008 to 2012, which indicated great potential for C sequestration and offsetting GHG emissions. Life cycle GHG emissions (as g CO<sub>2</sub>-eq MJ<sup>-1</sup>) were all negative due to high SOC increases each year and indicated all bioenergy sorghum production treatments sequestered atmospheric CO<sub>2</sub> per unit of theoretical energy provided. Despite its relatively low production efficiency, rotated sorghum with N addition and residue return was selected as the ideal bioenergy sorghum production scenario due to a number of sustainability factors. Bioenergy sorghum may offer great benefit as a high-yielding biofuel feedstock with minimal impacts to net GHG emissions.

## DEDICATION

This dissertation is dedicated to my wife and family. I am extremely grateful to my parents and everyone in my family for their unconditional support throughout my academic career and helping me get where I am today. Despite living a great distance apart, their patience, encouragement and love have always felt present. I would like to thank my wonderful wife, Jessica, who has contributed greatly to my sanity, success, well-being and has always been supportive of whatever adventure we embark on. I am also grateful to her for providing me with endless inspiration to succeed in the world and invaluable support throughout my graduate program.

## ACKNOWLEDGEMENTS

First and foremost, I would like to thank my co-advisors, Frank Hons and Terry Gentry, who provided me with a unique combination of mentoring, intellectual support, guidance, and encouragement that allowed my research to develop from ideas to a finished project. I am extremely grateful for the creative freedom and resources provided by my advisors, as they allowed me to explore many ideas and mature through strong learning experiences. I learned and experienced a tremendous amount through my time working with them, which I will carry with me throughout my career. I further thank them for the friendship we have developed over the years and the ability to talk about sports, gardening, and other things to keep me grounded outside the realm of research.

I would like to thank my other committee members, Jim Heilman and Tom Boutton, for the mentoring, support, encouragement, and resources that they provided me throughout my time as a doctoral student. For someone with little prior experience in soil physics and trace gas measurement, I could not have asked for a better advisor than someone as experienced and knowledgeable as Jim. His generosity with support and resources allowed my research to go far beyond my initial expectations. I am deeply grateful to Tom Boutton for providing encouraging and critical feedback, teaching the favorite course of my entire academic career, the opportunity to join him on an exciting research expedition, offering his guidance and friendship, and introducing me to the world of ecosystems research. Overall, I am honored to have such an amazing committee of distinguished scientists and am humbled to have their names associated with my research.

I am thankful to have been a part of the entire Soil & Crop Sciences community at Texas A&M while a Ph.D. student. I have appreciated the generosity and kindness of all students and faculty in the Soil & Crop Sciences Dept., you have made the department feel like family. I especially want to thank David Zuberer, Kevin McInnes, Hamid Shahandeh, Cristine Morgan, Tom Hallmark, Bill Rooney, and Tony Provin for their guidance, providing me with abundant resources, and offering generous levels of support. I am sincerely thankful to Katie, Yasu, Justin, Christina, Jim, Leo, Ben, Landon, Clark, Haly, Jason, and many other graduate students for offering assistance of all kinds over the years. I received a great deal of field support and data collaboration from Jason Wight and would like to thank him for his contributions to my research. I had the opportunity to work with many undergraduate students in the Soil Fertility and Nutrient Cycling Laboratory who made research fun and enjoyable and I thank them for all their contributions to my research. I would also like to thank Mitch Bender for his continued mentoring, advice, and encouragement from miles away and for shaping my early research career.

Finally, I would like to extend my gratitude for the following sources of funding: Texas A&M AgriLife Research, USDA-NIFA, Sun Grant Initiative, Tom Slick Graduate Research Fellowship, Graduate Research Mentoring Scholarship from the Department of Soil & Crop Sciences, and the Dudley and Angela Smith Graduate Travel Award.

# TABLE OF CONTENTS

	Page
ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vii
LIST OF FIGURES .....	ix
LIST OF TABLES.....	xiv
 CHAPTER	
I INTRODUCTION: THE MOTIVATION FOR RESEARCH .....	1
Biofuels.....	2
Greenhouse Gas Emissions.....	4
Carbon Cycling.....	10
Life Cycle Analysis.....	12
Summary.....	16
II GREENHOUSE GAS EMISSIONS ASSOCIATED WITH BIOENERGY SORGHUM PRODUCTION UNDER VARIOUS AGRONOMIC MANAGEMENT PRACTICES IN CENTRAL TEXAS.....	19
Introduction.....	19
Materials and Methods.....	26
Results.....	36
Discussion.....	77
Conclusion.....	98
III CARBON POOL DYNAMICS WITHIN BIOENERGY SORGHUM PRODUCTION SYSTEMS IN CENTRAL TEXAS .....	101
Introduction.....	101
Materials and Methods.....	104
Results.....	115
Discussion.....	144

CHAPTER	Page
Conclusion .....	163
IV LIFE CYCLE GREENHOUSE GAS EMISSIONS ASSOCIATED WITH BIOENERGY SORGHUM PRODUCTION UNDER VARIOUS AGRONOMIC MANAGEMENT PRACTICES IN CENTRAL TEXAS .....	167
Introduction.....	167
Materials and Methods.....	170
Results.....	183
Discussion.....	208
Conclusion .....	223
V CONCLUSION.....	226
Summary .....	226
Recommendations.....	231
REFERENCES .....	233



## LIST OF FIGURES

FIGURE		Page
1	Mean monthly air temperature and total monthly precipitation during the 2010 (a) and 2011 (b) sampling seasons at the research site near College Station, TX. Asterisks indicate irrigation in addition to rainfall total .....	39
2	Mean soil temperature and soil moisture content, reported as % water-filled pore space, at the field site during the 2010 (A) and 2011 (B) growing season. Mean (n=24) values are estimated from the entire field. Arrows indicate irrigation event .....	40
3	Mean bioenergy sorghum and corn yields in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).....	42
4	Mean theoretical ethanol yields in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Ethanol yield based on corn grain and sorghum biomass yield and NREL conversion estimates.....	44
5	Mean soil gas fluxes of CO <sub>2</sub> from 2010 growing seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Lines above sampling events denote significant (P < 0.05) difference in gas flux rate due to crop rotation (solid bar), N fertilization (dashed bar), or residue return (double bar), respectively. Asterisk denotes irrigation date.....	46
6	Mean soil gas fluxes of CO <sub>2</sub> from 2011 growing seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Lines above sampling events denote significant (P < 0.05) difference in gas flux rate due to crop rotation (solid bar), N fertilization (dashed bar), or residue return (double bar), respectively. Asterisk denotes irrigation date.....	47

7	Mean soil gas fluxes of N <sub>2</sub> O from 2010 growing seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Lines above sampling events denote significant (P < 0.05) difference in gas flux rate due to crop rotation (solid bar), N fertilization (dashed bar), or residue return (double bar), respectively. Asterisk denotes irrigation date.....	49
8	Mean soil gas fluxes of N <sub>2</sub> O from 2011 growing seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Lines above sampling events denote significant (P < 0.05) difference in gas flux rate due to crop rotation (solid bar), N fertilization (dashed bar), or residue return (double bar), respectively. Asterisk denotes irrigation date.....	53
9	Mean cumulative growing season CO <sub>2</sub> emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).....	56
10	Mean cumulative growing season N <sub>2</sub> O emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).....	60
11	Mean cumulative annual CO <sub>2</sub> emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).....	65
12	Mean cumulative annual N <sub>2</sub> O emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).....	68
13	Mean cumulative annual CO <sub>2</sub> -eq emissions from 2010 and 2011 from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).....	71
14	Mean cumulative annual CO <sub>2</sub> -eq emissions of the two-year average from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).....	73

FIGURE

Page

15	Mean yield-scaled GHG emissions from 2010 and 2011 from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Theoretical ethanol estimated from measured yield and NREL-provided conversion values.....	75
16	Mean yield-scaled GHG emissions of the two-year average from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Theoretical ethanol estimated from measured yield and NREL-provided conversion values.....	77
17	Mean soil gas fluxes of CO <sub>2</sub> from 2010 sampling seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Asterisk denotes irrigation date.....	82
18	Mean soil gas fluxes of CO <sub>2</sub> from 2011 sampling seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Asterisk denotes irrigation date.....	83
19	Mean soil gas fluxes of N <sub>2</sub> O from 2010 sampling seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Asterisk denotes irrigation date.....	87
20	Mean soil gas fluxes of N <sub>2</sub> O from 2011 sampling seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Asterisk denotes irrigation date.....	88
21	Mean monthly air temperature and total monthly precipitation during the 2010 (a) and 2011 (b) sampling seasons at the research site near College Station, TX. Asterisks indicate irrigation in addition to rainfall total .....	117
22	Mean corn grain yields in 2010 for treatment combinations of crop rotation (CS only), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).....	120
23	Mean corn stover or sorghum biomass dry yields in 2010 and sorghum biomass yields in 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).....	121

FIGURE

Page

24	Mean aerial biomass-C yields in 2010 and 2011 for treatment combinations of crop rotation (CS only), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).....	124
25	Mean cumulative soil respiration emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).....	128
26	Mean change in soil organic C (SOC) in the 0 – 5 cm soil depth since 2008 from combinations of crop rotation [continuous sorghum (SS) or corn sorghum rotation (CS)], N fertilization rate (- N or + N), and residue return rate [zero biomass (0%R) or 50% biomass return (50%R)] are reported with one standard error (n = 4).....	131
27	Mean change in SOC in 2010 and 2011 at soil depths of 5 – 15, 15 – 30, 30 – 60, and 60 – 90 cm since 2008 from combinations of crop rotation [continuous sorghum (SS) or corn-sorghum rotation (CS)], N fertilization rate (- N or + N), and residue return rate [zero biomass return (0%R) or 50% return (50%R)] are reported with one standard error (n = 4).....	132
28	Soil organic C (SOC) concentrations with depth under bioenergy sorghum production. Field soils were sampled at five depth intervals: 0-5, 5-15, 15-30, 30-60, and 60-90 cm and compared by crop rotation [continuous sorghum (SS) or corn-sorghum rotation (CS)] in 2008 and 2011. Horizontal bars indicate standard error of the mean (n = 4).....	135
29	Soil organic C (SOC) concentrations with depth under bioenergy sorghum production. Field soils were sampled at five depth intervals: 0-5, 5-15, 15-30, 30-60, and 60-90 cm and compared by N fertilization rate (+N or -N) (a) and residue return rate [zero biomass return (0%R) or 50% return (50%R)] (b) in 2011 to initial (2008) concentrations. Horizontal bars indicate standard error of the mean (n = 4).....	136
30	Mean soil microbial biomass C (SMBC) from 0 – 5 and 5 – 15 cm in 2008 and 2011 from combinations of crop rotation [continuous sorghum (SS) or corn-sorghum rotation (CS)], N fertilization rate (- N or + N), and residue return rate [zero biomass return (0%R) or 50% return (50%R)] are reported with one standard error (n = 4).....	138
31	Mean percentage of soil organic C (SOC) as soil microbial biomass C (SMBC) from 0 – 5 and 5 – 15 cm depths in 2008 and 2011 from combinations of crop rotation [continuous sorghum (SS) or corn-sorghum rotation (CS)], N fertilization rate (- N or + N), and residue return rate [zero biomass return (0%R) or 50% return (50%R)] are reported with one standard error (n = 4).....	143

FIGURE	Page
32 Mean monthly air temperature and total monthly precipitation during the 2010 (a) and 2011 (b) sampling years at the research site near College Station, TX. Asterisks indicate irrigation and rainfall events.....	185
33 Mean energy yields in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).....	187
34 Mean energy yields of the two-year average from treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).....	190
35 Mean cumulative N <sub>2</sub> O emissions in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4). Emissions of N <sub>2</sub> O reported as CO <sub>2</sub> -eq, assuming N <sub>2</sub> O GWP of 298 g CO <sub>2</sub> -eq per g N <sub>2</sub> O.....	192
36 Mean net changes in SOC from 2008 to 2012 from treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4). Means are the sum of net change in SOC from 5 sampling depths to 90 cm.....	197
37 Mean (n = 4) contributions of life cycle greenhouse gas emissions by activities in 2010 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R).....	199
38 Mean (n = 4) contributions of of life cycle greenhouse gas emissions by activities in 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R).....	201
39 Mean net well-to-wheel greenhouse gas emissions associated with bioenergy sorghum production in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).....	205
40 Mean net two-year average well-to-wheel net greenhouse gas emissions associated with bioenergy sorghum production from treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).....	207

## LIST OF TABLES

TABLE		Page
1	Field operations during two calendar years at the research site near College Station, TX .....	28
2	Mean (and standard error) soil temperature, mean volumetric water content, N fertilization rate, and previous harvest residue returned as biomass-C for each treatment in 2010 and 2011. Soil temperature and moisture averaged across growing season. Soil temperature reported from 31 May to 28 September in 2010 and 6 May to 28 August in 2011. Average volumetric water content reported from 1 June to 28 September in 2010 and 25 May to 28 August in 2011 .....	34
3	Means (and standard errors) of spring pre-plant soil properties. A summary of total carbon (TC), organic carbon (OC), total nitrogen (TN), extractable nitrate-nitrogen (NO <sub>3</sub> -N), extractable phosphorus (P), and extractable potassium (K) from 2010 and 2011 are presented .....	51
4	Means (and standard errors) of fall harvest biomass residue properties. A summary of total carbon (TC), total nitrogen (TN), total phosphorus (P), total potassium (K), and the carbon-to-nitrogen ratio (C:N) from 2010 and 2011 are presented .....	58
5	Summary of p-values from tests of difference between individual treatments and factorial analyses from 2010, 2011, and two-year average...	63
6	Summary of field operations since the initiation of the study in 2008 at the research site near College Station, TX.....	107
7	Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) on corn stover or sorghum biomass yield (Yield), C uptake, and C:N from 2010 and 2011. Note: Crop rotations (CS and SS) analyzed separately in 2010 due to bimodal distribution.....	122
8	Mean (and standard error) residue return rate (reported as C) applied and C:N of residue from harvest in 2009, 2011, and 2012 for each treatment (n = 4).....	125
9	Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) on cumulative soil respiration in 2010 and 2011.....	128

TABLE	Page
10 Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) by soil depth on change in SOC (since 2008) in 2010 and 2011 .....	130
11 Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) by soil depth on SMBC in 2011, change in SMBC (since 2008) and percentage of SOC as SMBC in 2008 and 2011 .....	139
12 Field operations during 2010 and 2011 calendar years at the research site near College Station, TX .....	173
13 Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) on energy yield, N <sub>2</sub> O Emissions, net annual change in SOC ( $\Delta$ SOC), and well-to-wheel life cycle greenhouse gas emissions (W2W LCGHG) .....	188
14 Net annual change in SOC ( $\text{Mg C ha}^{-1} \text{ yr}^{-1}$ ) for each experimental unit (n = 32) and each sampled depth and cumulative sum to 90 cm. Values are organized by treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R). Net change values were derived from regressions of mass of SOC across time from 2008 to 2012.....	194
15 Summary of mean (n = 4) contributions of life cycle greenhouse gas emissions by activities in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R).....	202

## CHAPTER I

### INTRODUCTION: THE MOTIVATION FOR RESEARCH

Innovative opportunities in biofuels and bioenergy are sprouting up nearly every day, often quite literally. Many societies around the globe have been tirelessly engaged in these efforts for decades (Fulton et al., 2004). A shift in interest toward alternative energy systems has largely been spurred worldwide by several national security and environmental issues stemming from dependence on petroleum. Biofuels were among several major carbon (C)-mitigation strategies identified by Pacala and Socolow (2004) to stabilize increasing atmospheric CO<sub>2</sub> concentrations. It is believed that the CO<sub>2</sub> released during biofuel combustion will be taken up in equal amounts via photosynthesis into the biomass of the plant feedstocks used to produce biofuels (St. Clair et al., 2008). However, without quantifying the net greenhouse-gas (GHG) emissions and other environmental impacts, developing and evaluating various C-mitigation strategies is not possible (Robertson and Grace, 2004).

Despite the large amount of progress made, researchers in this field are still considered pioneers in the realm of energy science. This sector of science is ripe for discovery, with more intensive research efforts than perhaps ever before. Several potential feedstocks and forms of biofuel have relatively recently been identified. Cropping systems devoted to producing feedstocks emit GHGs such as nitrous oxide (N<sub>2</sub>O), methane (CH<sub>4</sub>), and carbon dioxide (CO<sub>2</sub>) potentially negating some of the net GHG mitigation. Thus, without quantifying the net GHG emissions, developing and evaluating various bioenergy



strategies is not possible. The ‘carbon footprint’ of bioenergy feedstock production represents its sustainability based upon a number of parameters which take into account global warming potential or carbon intensity.

## **BIOFUELS**

High demand for renewable energy, including biofuels, is changing the landscape of agricultural and energy sectors globally. Large scale biofuel production within the U.S. has been justified through federal support in the form of renewable fuel standards, subsidies, and declared production goals (U.S. Congress, 2007). Reducing reliance on foreign oil and reducing GHG emissions relative to conventional gasoline are two primary assumptions behind the federal interest in increasing domestic biofuel production. Already, corn grain biofuel production capacity in the U.S. has risen from approximately 13 billion liters in 2004 to nearly 30 billion liters in 2008 (Renewable Fuels Association, 2008).

In 2005, a study by the U.S. Department of Energy explored the potential energy which could be provided from harvesting biofuel feedstocks from across the entire country (Perlack et al., 2005). The study estimated over one billion dry tons of biomass feedstocks could be harvested domestically for biofuels that could offset nearly 30% of the gasoline consumed as transportation fuel in the U.S. (Perlack et al., 2005). Shortly after the “billion ton study”, the U.S. Congress passed the 2007 Energy Independence and Security Act (EISA) which provided federal support for domestic biofuel production. The Renewable Fuel Standard was established by EISA and mandated the goal to produce over 136 billion liters of biofuels by the year 2022 (U.S. Congress, 2007). The total volume of biofuel is to be derived from three major sources; 42% (57 billion liters) from corn grain ethanol, 44% (61

billion liters) from cellulosic ethanol, and 14% (19 billion liters) from other advanced fuels (U.S. Congress, 2007). These production goals are highly ambitious, particularly considering cellulosic fuel conversion technologies were still not capable of large-scale implementation by 2013, but were established based on evidence provided by the “billion ton study”. However, the “billion ton study” developed by Perlack et al. (2005) did not thoroughly address the vast complexities associated with producing the targeted amount of feedstock nor the implications of producing and harvesting select biofuel feedstocks and potential issues associated with land-use change.

One issue with large-scale biofuel production is whether high biomass feedstock yields can be achieved with minimal impact to soil and environmental quality. The optimal balance between high agronomic productivity and sustainability must be determined for successful deployment of domestic biofuels. At the heart of the issue is concern regarding increased GHG emissions within the atmosphere and global climate change. Biofuels represent an opportunity to provide renewable energy with relatively low GHG emissions. However, intensification of agriculture may impact GHG emissions from agricultural lands and eradicate GWP benefits associated with biofuel production.

Corn-grain ethanol is the most widespread biofuel produced in the U.S. (Renewable Fuels Association, 2008). Technology used to produce corn grain ethanol has been available for a number of years and continues to advance (Liska et al., 2009; Wang et al., 2011). However, the 2007 EISA enhanced research efforts towards obtaining cellulosic biofuel conversion technology, representing a potentially vast source of biofuel feedstock throughout the U.S. (Tarkalson et al., 2009; U.S. Congress, 2007). Cellulosic ethanol is derived through the fermentation of cellulose and hemi-cellulose, generally following a pre-treatment process

(Mosier et al., 2005; Wang et al., 2011). Tremendous potential lies with cellulosic biofuel production due to the many types of feedstocks potentially available for fuel production, including, but not limited to crop residues, dedicated energy crops, woody plants and forest residues, and animal wastes (Perlack et al., 2005; Wang et al., 2011). In addition to the general abundance of these materials, research has found a number of cellulosic feedstocks to be economically-feasible (Wyman, 2003; Zhang, 2008). The “billion ton study” determined agricultural residues represent over half the total domestic cellulosic feedstock sources and the most plausible candidate for wide-scale cellulosic biofuel production (Perlack et al., 2005).

The specific agricultural residues receiving the greatest attention recently are corn stover and dedicated “energy” crops grown for biomass. Corn stover is the non-edible part of the corn plant left in the field after harvest (Lal, 2005). It is believed for every ton of corn grain harvested, one ton of dry stover is produced (Wang, 2008). Assuming the average weight of one bushel of corn is 25.4 kg, more than 332.7 million metric tons of corn stover was produced in 2007 in the United States (National Corn Growers Association, 2008). Corn stover and other potential cellulosic feedstocks, such as high-biomass sorghum require careful analysis to weigh the costs and benefits of harvesting this biomass from soil.

## **GREENHOUSE GAS EMISSIONS**

It is believed that anthropogenic increases in GHG emissions since the industrial era have caused increased radiative forcing of the Earth’s climate (Forster et al., 2007). Greenhouse gases are necessary to sustain life on Earth, as they allow some of the sun’s energy to be trapped and heat its surface. They can be defined as any gas, natural or

anthropogenic, which absorbs and emits infrared radiation within the Earth's atmosphere (IPCC, 1996). Greenhouse gases such as CO<sub>2</sub>, N<sub>2</sub>O, CH<sub>4</sub>, ozone, and water vapor may be produced naturally. Gases such as hydrofluorocarbons and perfluorocarbons do not occur naturally and are the result of anthropogenic activities (IPCC, 1996). Global warming potential (GWP), is the most frequently utilized measure of climate forcing potential and is a physical measure based upon an individual gas's radiative forcing potential and the longevity of the gas in the atmosphere (IPCC, 1996). The agriculture sector is a major contributor of GHG emissions (U.S. EPA, 2007) and three of the most studied greenhouse gases related to agricultural activities are CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub>.

### **Carbon Dioxide**

Conversion of native lands into production agriculture has manipulated the carbon cycle, reducing soil organic carbon (SOC) through decomposition and releasing it to the atmosphere as CO<sub>2</sub> (Anderson-Teixeira et al., 2009; David et al., 2009; Guo & Gifford, 2002; Schlesinger, 1986; West et al., 2004; Zinn et al., 2005). This phenomenon has recently garnered increasing attention due to the increased concern for global climate change. It is estimated more than 50 Gt of C have transitioned from the global soil C pool to the atmospheric C pool due to postindustrial era land conversion (Lal, 2004b). Tilling of native soils for crop production enhanced aerobic decomposition of SOC and resulted in reduction in SOC and increased CO<sub>2</sub> flux rates (Lal, 2004b).

These actions have shifted the balance between autotrophic fixation of C from the atmosphere to the biosphere and respiration of C by heterotrophic organisms back to the atmosphere. In relatively recent time, the concentration of CO<sub>2</sub> in the atmosphere has

increased from 295 ppm in the eighteenth century (Bolin et al., 1979) to more than 370 ppm in 2004 (Keeling and Whorf, 2005). It is believed that concentrations continue to rise and could near 500 ppm at the end of the century (IPCC, 1996).

The majority of all respiratory activity on earth occurs within the soil. The respiration of plant roots and decomposition of soil organic matter is known as soil respiration. Terrestrial soil respiration rates can vary based upon needs of the community of heterotrophic microbes responsible for decomposition. Organic materials with highly resistant forms of C do not decay as easily as labile forms. The C:N ratio must be narrow enough to supply adequate N to microbes for cell synthesis. Soil pH, moisture, aeration, and temperature are all factors that significantly affect soil respiration rates as well (Wolf & Wagner, 2005).

### **Nitrous Oxide**

The greenhouse gas  $N_2O$  has received significant attention recently due to its relatively high potency and increasing concentration within the atmosphere. Nitrous oxide is approximately 296 times as potent as  $CO_2$  when comparing radiative forcing potential over 100-year time horizon (Forster et al., 2007). The concentration of  $N_2O$  in the atmosphere is quite low compared to  $CO_2$ , approximately 319 ppbv, but is rising each year nearly 0.25% and has already risen 17% in the past 20 years alone (Forster et al., 2007). The primary source of anthropogenic  $N_2O$  in the world is agriculture (Forster et al., 2007). In the U.S., agricultural activities account for nearly 80% of man-made  $N_2O$  emissions (Mosier et al., 1998). Soil management activities, especially nitrogen (N) fertilization, constitutes the largest

fraction of the agricultural emissions, accounting for more than 70% of the total agriculturally-derived N<sub>2</sub>O emissions (Kim & Dale, 2008).

Nitrous oxide emissions from the soil may result from nitrification, denitrification, or even chemo-denitrification, although denitrification is often considered the major emission process from agricultural soils (Venterea & Rolston, 2000). Nitrous oxide production and emission vary due to many soil environmental parameters including moisture, temperature, oxygen availability, available C, available inorganic N (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>), and pH (Johnson et al., 2010; Mosier et al., 1998). These parameters have great effect on the key processes controlling soil N cycling, namely nitrification and denitrification.

Nitrification is the microbial oxidation process which converts ammonium and organic N to NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>. Nitrous oxide may be a byproduct released through this process. Nitrification may be carried out by archaea (Leininger et al., 2006) and chemoautotrophic or heterotrophic microbes, but aerobic chemoautotrophic bacteria are typically the dominant organisms. Major factors affecting nitrification include the quantity of ammonium, oxygen availability, soil pH, temperature, CO<sub>2</sub> availability, and soil moisture content (Mosier et al., 1998).

In contrast to nitrification, denitrification is the microbial reduction of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> to gaseous byproducts (N<sub>2</sub>, NO, and N<sub>2</sub>O) (Wallenstein et al., 2006). Denitrification is the major process which cycles N from the biosphere back to the atmosphere and is considered the major source of soil N<sub>2</sub>O emissions (Morkved et al., 2006; Wallenstein et al., 2006). Denitrification is commonly carried out by anaerobic bacteria and archaea with the ability to utilize NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> as a terminal electron acceptor under low oxygen conditions (Wallenstein et al., 2006). Major factors affecting denitrification include the quantity of

substrate ( $\text{NO}_3^-$ ), oxygen availability, soil pH, temperature, C availability, and soil moisture content (Wallenstein et al., 2006). Carbon content is a major factor; in fact there is generally a positive correlation between  $\text{N}_2\text{O}$  emissions and soil C content, especially more labile forms (Mosier et al., 1998).

Studies indicate significant variation in  $\text{N}_2\text{O}$  emissions coming from various cropping systems around the world. Cumulative annual  $\text{N}_2\text{O}$  emissions from ten different studies ranged from approximately 0.3 to 5.0 kg N ha<sup>-1</sup> (Dambreville et al., 2008; Drury et al., 2008; Gregorich et al., 2008; Zebarth et al., 2008; Tan et al., 2009; Archer & Halvorson, 2010; Halvorson et al., 2010; Johnson et al., 2010; Sistani et al., 2010; Venterea et al., 2010). Although variability existed between studies, major factors determining  $\text{N}_2\text{O}$  emissions included precipitation and temperature (Dambreville et al., 2008; Gregorich et al., 2008), fertilizer N input (Zebarth et al., 2008; Tan et al., 2009; Archer & Halvorson, 2010; Venterea et al., 2010), and crop rotation (Drury et al., 2008; Archer & Halvorson, 2010). The variability and contrasting conclusions among the studies speaks to the difficulty one may have in accurately assessing overall  $\text{N}_2\text{O}$  emissions from proposed bioenergy cropping systems. Several challenges exist which make quantifying cumulative  $\text{N}_2\text{O}$  emissions from biofuel cropping systems difficult. Clearly, further investigation is required to better understand  $\text{N}_2\text{O}$  emissions from cropping systems in Texas and elsewhere.

## **Methane**

Methane is another potent GHG commonly associated with agricultural activities. Methane has a GWP of 25 compared to  $\text{CO}_2$  over a 100-year time horizon (Forster et al., 2007). Although  $\text{CO}_2$  is responsible for over half the estimated anthropogenic radiative

forcing on earth, CH<sub>4</sub> accounts for approximately 20% (Lassey, 2007). Concentrations of CH<sub>4</sub> have been on the rise since the beginning of the eighteenth century, when concentrations were believed to have been near 700 ppb (Etheridge et al., 1998). Currently, the concentration of CH<sub>4</sub> in the atmosphere is above 1700 ppb and rising (Forster et al., 2007). It is estimated that approximately 30% of CH<sub>4</sub> emissions in the U.S. are attributed to agricultural activities (U.S. EPA, 2007). However, the majority of the agricultural CH<sub>4</sub> emissions come from enteric fermentation and manure management (>90%), with very small amounts, if any, attributed to traditional row-crop production (U.S. EPA, 2007).

Archaea produce the vast majority of agriculturally produced CH<sub>4</sub>. The major anaerobic methane-producers are known as methanogens (Whitman et al., 1992). Methanogens are obligate anaerobes and when redox potentials drop low enough, they reduce CO<sub>2</sub> into CH<sub>4</sub> to produce energy (Schimel and Holland, 2005). Obviously, soil moisture content (anaerobic or aerobic conditions) largely dictate methane production by methanogens, but other factors such as temperature and substrate availability can play a large role (Schimel and Holland, 2005).

Methane cycling may be more complex than CO<sub>2</sub> cycling in soils because soils may simultaneously produce or consume methane. When soils are not saturated, and do not exhibit anaerobic conditions, they support another group of bacteria known as methanotrophs (Lidstrom, 1992). These bacteria utilize CH<sub>4</sub> as their sole source of C and energy by oxidizing it into CO<sub>2</sub> and H<sub>2</sub>O. This sink for atmospheric CH<sub>4</sub> may annually account for approximately 25 Tg CH<sub>4</sub>-C. However, anthropogenic activities, such as tillage and fertilization, present opportunities to manipulate this soil sink (Schimel and Holland, 2005).



Greenhouse gas emissions from bioenergy cropping systems have the potential to undermine the thesis of producing biofuels to reduce atmospheric GHGs throughout the world. Thorough evaluation of GHG emissions from agricultural soils is necessary to determine which potential bioenergy cropping systems may be used in the reduction of net GHGs. The research proposed will provide a better understanding of GHG emissions from high-biomass sorghum cropping systems, contribute valuable field data to validate modeled data, and provide a foundation for understanding the environmental consequences of several management practices on a biofuel cropping system.

## **CARBON CYCLING**

The C cycle is critical for sustaining life on earth. The C cycle affects the Earth's climate by balancing the pools of C in the biosphere, lithosphere, hydrosphere, and atmosphere. Carbon cycling is also critical in cropping systems to sustain microbial communities and overall soil quality. The largest anthropogenically-produced greenhouse gas is CO<sub>2</sub> (Houghton, 2007), thus understanding its cycling will be critical in better understanding global climate change. Furthermore, if biofuel production efforts are at least partially driven by hopes of enhancing overall C sequestration, a thorough understanding of how C is cycled within a biofuel cropping system is required.

Carbon cycling within traditional agroecosystems is much smaller than global C cycling, but remains important due to the ability to manipulate it through human activities. The sizes of C pools in an agroecosystem pale in comparison to C pools of the oceans. The oceans contain approximately 38,000 Pg of C, whereas the atmosphere contains only about

780 Pg C and all the vegetation, organic residues, and soil C on earth account for about 2050 Pg C (Houghton, 2007).

Harvesting crop residues, such as from corn stover or biomass sorghum, for biofuel feedstock, may have a number of unintended consequences. Organic residues are derived of organic carbon and are a valuable source of potential soil nutrients and can improve soil quality (Doran, 2002). The largest pool of organic C on earth is in the soil (Lal, 2004b; Post et al., 1982). Soil organic carbon is a necessity in many biogeochemical cycles and soil organic matter can improve numerous soil qualities directly related to agriculture productivity (Lal, 2004b).

Harvesting agricultural residues means removing potential sources of SOM. Over time, harvesting agricultural residues and depleting potential SOM and SOC may not only reduce crop productivity, but could deteriorate soil structure, reduce water infiltration, increase runoff and losses of essential nutrients (Franzluebbers et al., 1995; Moebius-Clune et al., 2007; Powell and Hons, 1992; Sims and Kleinman, 2005). Retaining agricultural residues as sources of SOC has been shown to improve agronomic productivity in short and long-term studies (Anderson-Teixeira et al., 2009; Blanco-Canqui and Lal, 2007; Franzluebbers, 2002; Jastrow et al., 1998; Lal, 2009; Powell and Hons, 1992; Six et al., 2004).

Cellulosic feedstocks, such as high-biomass sorghum, are under intense examination for their potential exploitation as major feedstocks for future biofuels. Crop biomass or residue is not waste or trash, but is critical to SOC maintenance and accumulation, aids in retention of nutrients, and serves to prevent erosion. To better understand how the dynamic C pools change over time in a biofuel cropping system due to management activities, several

different pools must be examined. The primary C pools of interest may include biomass C, crop residue C, soil C (both inorganic and organic), soil microbial C, plant root C, C lost to leaching or erosion, and respired C (both plant and soil respiration).

## **LIFE CYCLE ANALYSIS**

The same federal energy bill that established a goal of 136 billion liters of biofuels to be produced in the United States by 2022 has stipulations on how these fuels may be produced. Of the 136 billion liters produced, 57 billion is to come from conventional corn-based ethanol, 61 billion from cellulosic ethanol, and 19 billion from advanced biofuels (U.S. Congress, 2007). Additionally, life cycle analyses (LCAs) of biofuels have been mandated under the 2007 Energy Independence and Security Act. The LCA requirement in the bill calls for a net reduction in life cycle GHGs based upon a 2005 petroleum fuel replacement standard. Corn-ethanol, cellulosic ethanol, and other advanced biofuels must reduce GHG emissions by at least 20%, 60%, and 50% below the 2005 petroleum baseline, respectively (U.S. Congress, 2007).

A life cycle analysis is a process used to quantify the environmental impacts of a product or process. A LCA is a valuable tool to compare the environmental performance between two or more products of interest (Kim & Dale, 2005). A full life cycle includes raw material acquisition through production, use, end-of-life treatment, recycling and final disposal, also called a cradle-to-grave assessment (Wang et al., 2007). A LCA is often used to compare a baseline product, a standard, versus an alternative product to determine potential benefits. Biofuel LCAs are commonly used to compare one or more alternative biofuel product with a traditional petroleum-derived fuel (e.g. gasoline).

Life cycle assessments of biofuels are becoming increasingly popular (Landis et al., 2007). Biofuels are commonly deemed an environmentally-friendly form of energy because they come from renewable plant life as opposed to petroleum (Landis et al., 2007). However, some believe these fuels are not as environmentally-friendly as they are perceived because large amounts of GHGs and energy are expelled to create the fuel. The objective of performing a LCA on bioenergy crop production systems may be to quantify the net energy gained/lost, the net GHG emissions produced/sequestered and overall environmental impacts. The U.S. Environmental Protection Agency has defined lifecycle greenhouse gases as:

“the aggregate quantity of greenhouse gas emissions (including direct emissions and significant indirect emissions such as significant emissions from land use changes), as determined by the Administrator, related to the full fuel lifecycle, including all stages of fuel and feedstock production and distribution, from feedstock generation or extraction through the distribution and delivery and use of the finished fuel to the ultimate consumer, where the mass values for all greenhouse gases are adjusted to account for their relative global warming potential.”

(U.S. Congress, 2007). Life cycle analyses of biofuels are vastly complex and remain highly controversial (Plieninger and Bens, 2006).

Quantifying net GHG fluxes is a critical LCA criterion because the presumed GHG mitigation provided by biofuels has been a major reason for increasing biofuel consumption. Growing crops, harvesting, transporting, and converting feedstocks into fuel also requires energy, much of it coming from fossil fuel sources. Overall environmental impacts of producing biofuels vary greatly. Factors include climate, soil properties, crop management systems, plant life cycle, yields, feedstock conversion efficiencies, nutrient demands and

cycling, fuel sources, byproduct credits, and transportation factors (Kim and Dale, 2005, Adler et al., 2007). All of these various factors have an impact on the overall net GHG emissions and other environmental impacts (Adler et al., 2007).

Models are frequently utilized in LCAs to help estimate or quantify various components within the production process. Models have been used to calculate emission factors, GHG emission fluxes, agricultural sector modeling, land use changes, fuel production processes, tailpipe emissions, and energy sector predictions (Wang, 2008). Most LCA models calculate GHG effects of fuels by quantifying the CO<sub>2</sub> equivalent emissions (Kammen et al., 2007). One model which incorporates several of these lifecycle components is the Greenhouse Gases, Regulated Emissions, and Energy use in Transportation (GREET) model. This model was developed at the Department of Energy's Argonne National Laboratory by Dr. Michael Wang in 1995 (Wang, 1999). This software began evaluating ethanol production in 1997, and has since been upgraded to include a variety of biofuels and production processes (Wang, 1999). The model is constantly being updated to continue studying energy and GHG emission impacts of biofuels as part of Argonne National Lab's efforts to evaluate the well-to-wheels energy and emission effects of a range of advanced vehicle technologies and transportation fuels (Wang, 2008).

The GREET model is popular for performing LCA in the U.S. (Wang et al., 2007; 2008). This model may calculate the consumption of total energy, emissions of GHGs, and emissions of the EPA's six criteria pollutants for a given vehicle and fuel system. The model includes more than 100 fuel production pathways, with diverse feedstocks such as crude oil, natural gas, coal, corn, soybean, sugarcane, and cellulosic biomass, to name a few. The model calculates a well-to-wheels assessment which implies a full lifecycle (cradle-to-grave)

analysis including feedstock growth and collection, feedstock transportation, fuel production, fuel transportation, and vehicle fuel usage information (Wang et al., 2007).

Several studies using the GREET model and other LCA models or data sources have noted specific factors that significantly influence the overall well-to-wheels results of many biofuels. Farm management factors significantly impact LCA results through variability in crop and biomass yields as well as energy and chemical inputs required for production (Kim & Dale, 2005; Kim & Dale, 2008; Wang, 2008). Nitrogen fertilizer sources, a component input of feedstock production, have a large impact on LCA results depending upon the fertilizer plant's energy source and whether it utilizes coal or natural gas as a feedstock source (Wang, 2008).

Nitrogen fertilizer efficiency also plays a large role in overall GHG emission results and remains a controversial factor. The conversion of some applied N fertilizer to  $N_2O$ , through nitrification and denitrification in the soil environment remains a factor of some speculation (Wang et al., 2003). The GREET model and the Intergovernmental Panel on Climate Change (IPCC) estimates conclude 1.325% of the N fertilizer applied will be denitrified to  $N_2O$  (Smith et al., 2007; Wang, 2008). However, Crutzen et al. (2008) contend that 3 to 5% of the N fertilizer applied will be lost as  $N_2O$  from the soil. One complication is in distinguishing between background and N fertilizer-induced  $N_2O$  emissions (Wang, 2008). Like other nutrients, N cycling is highly site specific and will vary greatly between studies.

Life cycle analyses are helpful tools for analyzing the overall environmental impacts of a production process. These tools appear to be gaining commonality in the biofuel industry (Wang, 1999; Wang, 2003; Kim & Dale, 2005; Farrell et al., 2006; Adler et al., 2007; Landis et al., 2007; Kim & Dale, 2008; St. Clair et al., 2008; Wang, 2008), likely due to their

requirement by the 2007 Energy Independence and Security Act. Life cycle analyses performed on agricultural products and processes present significant complexities due to numerous multifaceted variables such as nutrient cycling, plant life cycle, and other natural variables which cannot be directly controlled by human activities.

Validation of overall LCA conclusions may be close to impossible when factoring in global factors such as economics and land use change. Price changes of various commodities (fertilizer, grain, industrial machinery) around the globe occur regularly and have significant effects on overall production costs. Land use change is a result of a variety of economic, social, political, and scientific factors which may be unpredictable and rapidly changing (Kim et al., 2009; Searchinger et al., 2008). Nutrient cycling is highly site specific and modeling of these processes on a large scale may lead to high margins of error. Finally, potential climate change may impact several growth, nutrient cycling, and production cost parameters of an LCA, but has yet to be accurately modeled. Most of these complex variables are found in many global economic and environmental models which merely provide general guidance and must be used to derive results with great caution. However, direct field measurements, such as those provided by this research, may offer additional validation opportunities for LCA models and guide future model improvements.

## **SUMMARY**

Rapid changes occurring in renewable energy markets within the last five years have opened up numerous opportunities for the biofuel economy. The character of this industry may be determined largely as the result of governmental policy. Sound biofuel policy must consider the overall environmental impacts of these energy systems for them to succeed. The

LCA of biofuels has evolved as a valuable tool for evaluating numerous components of the system, making justified research investments easier and producing a rapid assessment of overall environmental sustainability of various biofuel production systems.

Cellulosic biofuels will likely be a major solution for future transportation fuel needs. However, the overall impacts of these biofuel systems have yet to be quantified. No commercial cellulosic biofuel production facilities currently exist, so large uncertainty surrounds the potential success for this industry and its potential future impacts. Several cellulosic feedstocks and potential cropping systems have only recently begun to receive attention. Significant research is ongoing and only minimal conclusive findings have been made available for scientific discussion and policy guidance. However, the research proposed herein will evaluate a potential superior biofuel production system and contribute to general agronomic and soil science through a better understanding of C cycling and GHG emissions from a biofuel cropping system in central Texas.

A field study established in 2008 to investigate the effects of crop rotation (sorghum-sorghum vs. corn-sorghum), N fertilization rate (0 vs. 280 kg urea-N ha<sup>-1</sup>), and residue return rate (0 vs. 50% biomass-residue returned to soil) on bioenergy sorghum yield and soil quality provided an opportunity to explore the impact of agronomic management practices on soil GHG emissions, C pool dynamics, and life cycle GHG emissions. Specifically, we wanted to evaluate the impact of these management effects on i) cumulative GHG emissions from the soil, ii) yield-scaled emissions, iii) changes in C pools including aerial biomass-C, cumulative soil respiration-C, soil organic C, soil microbial biomass-C and percentage soil organic C as soil microbial biomass, iv) theoretical energy yield, v) annual net change in SOC to 90 cm, vi) life cycle GHG emissions, and vii) determine whether any bioenergy



sorghum production scenarios examined meet GHG emission reductions required by 2007 EISA.

## CHAPTER II

# GREENHOUSE GAS EMISSIONS ASSOCIATED WITH BIOENERGY SORGHUM PRODUCTION UNDER VARIOUS AGRONOMIC MANAGEMENT PRACTICES IN CENTRAL TEXAS

### **INTRODUCTION**

The recent global push for biofuel production is transforming both agricultural and energy industries worldwide. The emphasis on producing biofuels has arisen largely due to concerns about energy security and sustainability of the petroleum industry and reliance on foreign petroleum-based fuels. The finite amount of extractable petroleum leads to market uncertainty and fluctuation. A lack of domestic production capacity in the United States to meet consumer demand has resulted in a reliance on foreign oil sources, which come with a series of political, economic, and national security issues (Asif and Muneer, 2007; Luque et al., 2008; Perlack et al., 2005). Finally, recent attention given to the impacts of greenhouse gases (GHGs), such as CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub>, on global climate change have illuminated biofuels as a potential energy source which may reduce GHG emissions compared to petroleum-based fuels.

The 2007 Energy Independence and Security Act provided a justified demand for biofuels in the U.S. with the Renewable Fuel Standard that requires the production of 36 billion gallons of biofuels annually by the year 2022. Of the total 36 billion gallons, 44% (16 billion gallons) is to be from cellulosic ethanol, 42% (15 billion gallons) from traditional corn ethanol, and 14% (5 billion gallons) from other advanced fuels. The U.S. Department of

Energy has determined that more than 1 billion dry tons of biomass could be “sustainably” harvested from forests and fields in the U.S. and converted to fuel, which could replace approximately 30% of the annual US petroleum transportation fuel demand (Perlack et al., 2005). This controversial study, termed the “billion ton study”, does not address the complex agronomic and conversion issues related to biomass-derived fuels.

Currently, corn grain-derived biofuels are the most common, but interests are shifting to a potentially larger source of biofuel feedstock: cellulosic biofuels (Tarkalson et al., 2009). Cellulosic biofuel is produced through the pretreatment, hydrolysis, fermentation, and purification of carbohydrate polymers of biomass materials (Mosier et al., 2005). Many existing cellulosic feedstocks, such as herbaceous crops, agricultural residues, woody plants, forest residues, animal manure and various waste products are excellent candidates for biofuel industries (Perlack et al., 2005) and represent an abundant, economically-feasible energy source (Wyman, 2003; Zhang, 2008). Agricultural residues remain the most abundant potential feedstock for production of cellulosic biofuel (Perlack et al., 2005). The “billion ton study” determined these residues represented over half of the total cellulosic biomass sources (Perlack et al., 2005).

Agricultural food production must increase at a rate of at least 1.3% each year until 2025 to feed the growing world population (Cassman et al., 2003). The world population is expected to reach 9 billion in less than 40 years and with it, demand for food, water, and natural resources are expected to climb (Godfray et al., 2010). Biofuel production must be performed in a way that minimizes impacts to food crop production and natural resources. Limiting biofuel production from existing food crops and preventing land used for traditional crop production to be converted to biofuel crop production is an important consideration

when selecting bioenergy crops. Bioenergy crops which can be grown with minimal inputs are ideal because of savings to valuable natural resources, such as water, in addition to increasing final profit margins for the fuel product.

Novel bioenergy crops and cropping systems are continuously being developed in an attempt to meet domestic biofuel production goals. How these cropping systems will impact GHG emissions and overall soil health is still not fully understood. Bioenergy sorghum (*Sorghum bicolor* L.) is one type of cellulosic bioenergy feedstock being examined for utilization in biofuel cropping systems. Bioenergy sorghum represents a crop which is not utilized as a current food source, can produce large yields (minimizing land use requirements), has suitable biomass chemical compositional properties for fuel conversion, and requires relatively fewer inputs than many other bioenergy crops (Buxton et al., 1999; Miller and Mcbee, 1993; Rocateli et al., 2012; Rooney et al., 2007; Zegada-Lizarazu et al., 2012). The warm temperatures and moderate rainfall (>750 mm) associated with climates in the southeastern and southcentral U.S. may be particularly well-suited for bioenergy sorghum production (Rocateli et al., 2012; Rooney et al., 2007). However, these warm, moist climates may also be particularly favorable for large GHG emissions from the soil (Almaraz et al., 2009; Castellano et al., 2010; Dobbie and Smith, 2003; Schimel and Holland, 2005; Xu and Luo, 2012). Based upon a recent literature review, the impact of bioenergy sorghum cropping systems on net GHG emissions has not been examined. Furthermore, this review indicates little data exists on GHG emissions from cropping systems of any kind in Texas.

The measured value of climate forcing potential by a radiative gas is commonly referred to as global warming potential (GWP), and is a function of a specific gas's radiative forcing potential and the lifespan of the gas within the atmosphere (IPCC, 1996). Three

particular GHGs which are commonly associated with agricultural activities include CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub>. Most row-cropping systems in central Texas and throughout the Midwest are not considered major sources of CH<sub>4</sub> emissions. Aerobic soil conditions found in most row-cropping fields in these regions inhibit the production of CH<sub>4</sub>, and in many cases are considered a net sink for this gas (Adviento-Borbe et al., 2007; Robertson et al., 2000; Schimel and Holland, 2005). Thus, greater emphasis on CO<sub>2</sub> and N<sub>2</sub>O emissions from these systems is critical to understanding the role of row-cropping systems as a potential net source of GHGs.

Historically, the transition of native lands into production agriculture has caused a significant reduction in soil organic C (SOC) (Anderson-Teixeira et al., 2009; David et al., 2009; Guo and Gifford, 2002; Schlesinger, 1986; West and Post, 2002; Zinn et al., 2005) and increased anthropogenic CO<sub>2</sub> emissions. Though the annual flux of C into the atmosphere is via soil respiration accounts for approximately 50 Pg C (Houghton, 2007; Wolf and Wagner, 2005), the net emissions to the atmosphere attributed to agricultural activities is small compared to other sources globally (Smith et al., 2007), largely due to net primary productivity (C uptake) regaining approximately 50 Pg C annually (Wolf and Wagner, 2005). The exposure of tilled soils to an oxygen-rich environment promotes the decomposition of previously sequestered organic C within the soil, releasing CO<sub>2</sub> via microbial decomposition, and reducing SOC concentrations. Lal (2004b) estimated that more than 50 Pg of C have been released from the terrestrial soil C pool (and not regained via net primary productivity) and deposited into the atmospheric pool as a result of land conversion during the postindustrial era. Despite its relatively small contribution to global C fluxes annually, agricultural soils are of profound interest to GHG researchers because of their ability to

potentially re-sequester large amounts of C previously lost to the atmosphere (Lal et al., 2007; Reicosky et al., 2000). Identifying agronomic management practices which minimize release of CO<sub>2</sub> and promote sequestration of C within the soil are imperative when evaluating bioenergy cropping systems to reduce net GHG emissions.

Agriculture accounts for more than half of the annual global anthropogenic emission of N<sub>2</sub>O (Galloway et al., 2003; Smith et al., 2007). The advent of the Haber-Bosch method of N fertilizer production and subsequent increased availability of commercial fertilizer dramatically increased the use of N fertilizer in agriculture and is believed to be a major factor in N<sub>2</sub>O emissions from agriculture (Davidson, 2009; Smith et al., 2007). Emissions of N<sub>2</sub>O from agricultural soils are typically a gaseous byproduct from the microbial-mediated processes of nitrification and denitrification (Bremner, 1997; Hutchinson and Davidson, 1993; Schimel and Holland, 2005; Smith and Conen, 2004). Nitrous oxide is an important greenhouse gas with 296 times the GWP of CO<sub>2</sub> over a 100-year time horizon (Forster et al., 2007). The current atmospheric concentration is fairly low, at 319 ppbv, but is estimated to be increasing at a rate of approximately 0.25%, annually (Forster et al., 2007). In the past 20 years alone, N<sub>2</sub>O production has increased by 17% globally (Forster et al., 2007). In addition to its radiative potency and role in the earth's natural radiation balance, N<sub>2</sub>O is of further concern because of its contribution to the photochemical destruction of atmospheric ozone (Duxbury, 1994; Rasmussen and Khalil, 1986; Schimel and Holland, 2005). Thus, finding suitable bioenergy cropping systems that minimize N<sub>2</sub>O emissions can reduce the impact of agriculture on the earth's natural radiation balance and the destruction of its ozone layer. Furthermore, few observations have been made to actually quantify flux rates of N<sub>2</sub>O from agricultural soils from regions in the southern U.S., such as Texas, reaffirming the

significance to obtain measured values to validate previously modeled estimates (Franzluebbbers, 2005; Johnson et al., 2007).

Agronomic management practices have a significant impact on C and nutrient cycling, microbial activity, and GHG emissions from soils. Cropping rotation and intensity can affect soil C and nutrient cycling (Dou et al., 2008; Franzluebbbers et al., 1994; West and Post, 2002; Wright and Hons, 2005), soil microbial activity (Acosta-Martinez et al., 2011; Dou et al., 2008; Franzluebbbers et al., 1994), and GHG emissions (Adviento-Borbe et al., 2010; Halvorson et al., 2008; Hernandez-Ramirez et al., 2009). Emissions of N<sub>2</sub>O from agricultural soils generally increase with increasing additions of N fertilizer (Adviento-Borbe et al., 2007; Forster et al., 2007; Gregorich et al., 2005; Johnson et al., 2005; Mosier et al., 2006; Pelster et al., 2011). Nitrogen fertilization is typically one of the largest factors in GHG emissions, particularly of N<sub>2</sub>O (Snyder et al., 2009), but crop residue return is a major contributor as well (Rochette et al., 2008). Similar to other management practices, residue management in a cropping system can have a significant effect on overall biogeochemical cycling and soil health (Kallenbach and Grandy, 2011; Malhi and Lemke, 2007; Powell and Hons, 1991; Rochette et al., 2008; Wilhelm et al., 2004). Each agronomic management practice may have a primary effect and may often have more complex interactive effects since biogeochemical cycling is connected through dynamic soil and microbial processes. Furthermore, site-specific factors such as soil properties, vegetation type, and climate further confound the complex effects of various management practices. Thus, the various agronomic management practices utilized to produce bioenergy crops can have a significant impact on GHG emissions from the soil, but further information is necessary to determine the potential effects of bioenergy sorghum production in central Texas.

Quantifying yield-scaled GHG emission values allows researchers to apply an efficiency value to a crop production system. This efficiency is based upon maximizing crop production while simultaneously minimizing GHG emissions. These calculations have been applied to agronomic studies recently as a better measure of GHG emissions relative to crop productivity (Grassini and Cassman, 2012; Johnson et al., 2012; Linqvist et al., 2012; Van Groenigen et al., 2010; Venterea et al., 2011). If GHG reduction practices fail to sustain or improve crop yields, any potential benefits accrued would provide little appeal to producers and lack practicability for most cropping systems. Furthermore, bioenergy cropping systems which minimize GHG emissions and contribute to global climate change mitigation must be identified, but not at the sacrifice of crop yield growth required to sustain growing global demands for food, fiber, and fuel (Grassini and Cassman, 2012).

If, through the production of bioenergy crops, net GHG emissions are increased from the soil via agronomic management practices, producers would be undermining one of the overall central goals to producing biofuels. Thus, the impact of various agronomic management practices on bioenergy crop production must be examined. The objective of this study was to determine: (i) the effect of crop rotation (corn-sorghum vs. sorghum-sorghum), N fertilization (0 vs. 280 kg urea-N ha<sup>-1</sup>), residue management (0 vs. 50% biomass sorghum residue returned), and their interactions on cumulative fluxes of CO<sub>2</sub>-C and N<sub>2</sub>O-N, (ii) the cumulative (GWP), expressed as CO<sub>2</sub>-equivalents, of each crop production practice considering both direct and indirect GHG emissions, and (iii) the ethanol yield-scaled GWP of each crop production system.



## MATERIALS AND METHODS

### Site Description and Experimental Design

The experimental site was located at the Texas Agrilife Research Farm, approximately 8 km southwest of College Station, Texas, USA (30° 32' 15"N, 96° 25' 37"W). The site is located within the Brazos River floodplain in south-central Texas. The soil used was a calcareous (pH 8.2) Weswood silty clay loam (100 g sand kg<sup>-1</sup>, 560 g silt kg<sup>-1</sup>, 340 g clay kg<sup>-1</sup>) and is classified by the USDA NRCS as a Fine-silty, mixed, superactive, thermic Udifluventic Haplustept. Climate at the site is classified as humid subtropical; the mean annual precipitation is 978 mm and the mean annual temperature is 20°C. Prior to the start of the bioenergy sorghum study in 2008, the field was in cotton (*Gossypium hirsutum* L.) in 2007, and rotated annually with corn (*Zea mays* L.) under conventional disk tillage.

This study was conducted within a larger study investigating the yield potential and agronomic responses of bioenergy sorghum (*Sorghum bicolor* L.) to various integrated management practices (Wight et al., 2012). The bioenergy sorghum cropping system study, established in 2008, utilized four row (1.02-m row centers) plots which measured 9.14 m long and 4.08 m wide. The larger study used a randomized complete block design with crop rotation, N fertilization rate, and biomass residue return rate as the three major factors. This study was limited to two levels of each of the three major factors and was replicated 3 times. The eight experimental treatments included every combination of; crop rotation (corn-sorghum [CS] or sorghum-sorghum [SS]), N fertilization (0 kg N ha<sup>-1</sup> [- N] or 280 kg N ha<sup>-1</sup> [+ N]), and biomass residue return (0% residue returned [0%R] or 50% residue returned [50%R]). The bioenergy sorghum variety, “4Ever Green”, used within the study was a photoperiod-sensitive, high-tonnage hybrid forage sorghum (Walter Moss Seed Co, Waco,

TX, U.S.A.). This particular variety was chosen because of its low water requirements, high biomass yield, and low lodging potential. When corn was rotated with sorghum, Dekalb DKC68-05 was the corn variety utilized in the corn-sorghum cropping sequence.

Bedded rows were prepared prior to planting with a rolling cultivator, applying pre-plant herbicide (Atrazine) on 17 March 2010, and planting to corn on 23 March 2010 and sorghum on 13 April 2010. In 2011, a pre-plant herbicide was applied on 2 March 2011, rolling cultivation occurred on 24 March 2011, and the entire field was planted to sorghum on 25 March 2011. A four-row inter-row cultivator was additionally used to control weeds on 22 May 2010 and 5 May 2011. Treatments receiving N fertilization were sidedressed with granular urea at 7.5 cm depth on 22 May 2010 and 5 May 2011; approximately the 4-leaf stage for sorghum and 6-leaf stage for corn. When corn was fertilized, it received a reduced rate of 168 kg N ha<sup>-1</sup>, based on local recommendation. Randomly selected 3.05 m segments of the middle two rows of corn were hand-harvested for grain and residue samples on 2 July 2010. Estimates of total corn biomass and grain yield were made on this date, however, the remaining non-sampled portion of the corn stand was not harvested/mulched onto the soil until the later sorghum harvesting date. The remaining corn biomass and biomass sorghum were harvested on 7 October 2010 and in 2011, sorghum was harvested on 1 September. A single-row silage harvester (cutting at approximately 20 cm height) with an attached weigh-bucket was utilized to harvest sorghum in 2010 and 2011. Biomass yield was estimated from the middle two rows of each plot and a random grab sample of chopped residue was captured for determining moisture and nutrient composition. The harvester mulched sorghum biomass into small pieces approximately 0.04 m by 0.04 m. After biomass yield weights were collected, the 50%R treatments received the biomass residue returned from the middle two

rows evenly distributed across the soil of the entire plot. This study was not interested in removing corn residue for biofuel conversion (only grain), so when corn was harvested in 2010, 100% of residue was returned to each plot, regardless of residue return treatment. After harvest each fall, residue was disked into the field and the plots were bedded into furrows. Disking and bedding occurred on 12 October 2010 and 5 September 2011. Furrow irrigation was used sparingly as needed on 31 May 2010 and 12 April, 9 May, 14 July, and 4 August in 2011. Eleven cm of irrigation water was applied in each event except 9 May 2011, when 9 cm was applied. A summary of these field operations can be found in Table 1.

Table 1. Field operations during two calendar years at the research site near College Station, TX.

Field operation	Year			
	2010		2011	
		<i>notes</i>		<i>notes</i>
Pre-plant herbicide†	17 Mar.		2 Mar.	
Soil sampling	5 Apr.		14 Mar.	
Pre-plant cultivation	17 Mar.		24 Mar.	
Planting	23 Mar.	<i>corn</i>		
	13 Apr.	<i>sorghum</i>	25 Mar.	
Fertilization	22 May		5 May	
Interrow cultivation	22 May		5 May	
Irrigation‡	31 May	<i>11 cm</i>	12 Apr.	<i>11 cm</i>
			9 May	<i>9 cm</i>
			14 July	<i>11 cm</i>
			4 Aug.	<i>11 cm</i>
Harvest	2 July	<i>corn</i>		
	7 Oct.	<i>sorghum</i>	1 Sept.	
Post-harvest disking and bedding	12 Oct.		5 Sept.	

† applied as Atrazine

‡ applied as furrow irrigation

## **Greenhouse Gas Flux Measurement**

Soil GHG ( $\text{CO}_2$ ,  $\text{N}_2\text{O}$ ,  $\text{CH}_4$ ) fluxes were directly measured using a static, vented chamber and a field photoacoustic gas analyzer. Specifically, the measurements were made by integrating a Li-Cor 20-cm survey chamber (model 8100-103, Li-Cor Inc., Lincoln, NE) with an INNOVA 1412 Photoacoustic gas analyzer (Innova AirTech Instruments A/S, Denmark). The flux chamber was selected for its portability, adaptability, and ability to minimize pressure disequilibrium between the chamber headspace and ambient atmosphere (Xu et al., 2006). A PVC flux chamber soil-collar was installed to a depth of approximately 12 cm near the middle of each of the 24 sampled plots. The collars were placed on top of beds on level surfaces which were equidistant from the crop and injected fertilizer. Height measurements from the soil surface to the top of each collar were measured at four quadrants inside the collar and averaged to estimate height for periodic headspace volume calculation. To minimize disturbance effects, soil-collars were installed no less than 24 hours prior to the initial gas sampling event and remained in place through the entire growing and fallow seasons (Parkin and Venterea, 2010; Tiedje et al., 1989). Collars were only removed briefly in spring and fall for field operations.

Greenhouse gas fluxes were measured by placing the Li-Cor 20-cm chamber atop the PVC collars for a deployment time of 20 minutes. The analyzer was programmed to collect a measurement every 2 minutes during deployment. The gas analyzer was set-up with a 5-second sampling integration time and auto-flushing for the corresponding tube length (typically 2 m). All tubing, fittings, and connection components were made of polytetrafluoroethylene (PTFE) or stainless steel. The gas analyzer was lab calibrated at least every two months with a 2-point calibration of a zero gas and mixed gas standard. The

primary gas standards (CO<sub>2</sub> at 450 ppm, CH<sub>4</sub> at 5 ppm, and N<sub>2</sub>O at 2 ppm) and zero gas (UHP N<sub>2</sub>) were mixed to at least 99.99% accuracy (Airgas Specialty Gases, LaPorte, TX). The gas analyzer was routinely checked prior to field sampling with gas standards to ensure calibration stability.

Preliminary analysis of trace gas data determined the majority of fluxes exhibited a nonlinear increase in concentration over time within the chamber headspace. Thus, nonlinear fluxes of GHGs were calculated by fitting a nonlinear regression of trace gas concentration across deployment time (Wagner et al., 1997) and on a few occasions where linear fluxes were observed, a linear method was utilized. While the nonlinear quadratic regression method selected may not be the unconditional best, its simplicity of use and significant improvement over linear methods (given our nonlinear data) (Livingston et al., 2006; Venterea et al., 2009) coupled with high temporal resolution and the large number of data points collected for each flux calculation with our analyzer warranted its use for this study. Due to relatively high flux rates, CO<sub>2</sub> flux calculations only utilized 12 minutes of sampling data (6 individual data points) to minimize increased ‘chamber effect’ observed in the full 20 minute deployment time. Relatively lower flux rates of N<sub>2</sub>O warranted calculating fluxes from at least the first 14 minutes of sampling (7 data points), but often utilized the full 20-minute deployment time which allowed concentrations to increase to detectable limits while the chamber was closed. Detectable fluxes of CH<sub>4</sub> were infrequent and were eliminated from analysis and will not be discussed further in this study. Any regression with a coefficient of determination ( $r^2$ ) less than 0.8 was rejected and a net flux of zero was assumed. Calculation of trace gases fluxes were based on GRACEnet protocols (Parkin and Venterea, 2010) and were calculated as:

$$F_1 = \frac{V \cdot \frac{\Delta C}{\Delta t}}{A_s} \quad [1]$$

where  $F_1$  is the volumetric gas flux in  $\mu\text{L}$  ( $\text{CO}_2$  or  $\text{N}_2\text{O}$ )  $\text{m}^{-2} \text{min}^{-1}$ ,  $\frac{\Delta C}{\Delta t}$  is the change in trace gas concentration over time in ppm (v) ( $\text{CO}_2$  or  $\text{N}_2\text{O}$ )  $\text{min}^{-1}$ ,  $V$  is the calculated chamber volume (L), and  $A_s$  is the surface area ( $\text{m}^2$ ). Further calculations were made as:

$$F_2 = C \cdot \frac{F_1 \cdot p}{R \cdot T} \quad [2]$$

where  $F_2$  is the trace gas flux in  $\mu\text{mol}$  ( $\text{CO}_2$  or  $\text{N}_2\text{O}$ )  $\text{m}^{-2} \text{min}^{-1}$ ,  $F_1$  is the volumetric gas flux in  $\mu\text{L}$  from [1],  $p$  is pressure (atm),  $R$  is the gas law constant ( $0.08206 \text{ L atm mol}^{-1} \text{ K}^{-1}$ ),  $T$  is temperature ( $^\circ\text{K}$ ), and  $C$  is a conversion coefficient ( $0.63648$  for  $\text{N}_2\text{O}$  to  $\text{N}_2\text{O-N}$  and  $0.2727$  for  $\text{CO}_2$  to  $\text{CO}_2\text{-C}$ ).

Due to deployment time and the ability to only measure a single plot at a time, the 24 total plots (8 cropping system treatments x 3 replicates) were divided into two consecutive days of sampling. Sampling order was randomized to reduce bias due to temperatures increasing from morning hours. Gas sampling was normally performed between 9:00 and 15:00. Specific days within a week selected for a sampling event were determined based on weather forecasts. Sampling events only occurred on consecutive days with stable weather patterns (e.g. no precipitation, no weather fronts, etc.).

Soil gas measurements were initiated on 25 May 2010, after N fertilization. Trace gas flux measurements were performed approximately weekly through the growing season and at a reduced frequency during the fallow period. Precipitation events and technical difficulties inhibited the uniform sampling frequency initially planned for 2010 and 2011. Cumulative growing-season GHG emissions were calculated from 12 sampling events in both 2010 and

2011. Cumulative annual emissions of GHG fluxes were calculated from 18 sampling events in 2010 and 20 sampling events in 2011. Measurements for year 1 (2010) occurred from 27 May 2010 through 3 March 2011 and for year 2 (2011) from 6 May 2011 to 1 March 2012.

### **Environmental and Soil Properties**

Several environmental variables were monitored throughout the study to supplement trace gas flux measurements. Air temperature, relative humidity, wind speed, solar radiation, and rainfall were measured with a weather station, approximately 400 m from the field plots, every half hour. Soil temperature was measured hourly by type T thermocouples at 10 cm depth near gas sampling collars within each plot. A time domain reflectometry (TDR) system was utilized to measure soil moisture every 6 hours. The TDR array consisted of sensors near gas flux collars, using a TDR100, and five SDMX50 multiplexers (Campbell Scientific, Inc., Logan, UT). The volumetric water content was determined to a depth of 15 cm by analyzing TDR wave forms with PC-TDR (Campbell Scientific, Inc., Logan, UT) and Topp's equation (Topp et al., 1980). Soil temperature and moisture data from monitoring systems were collected within the field with a CR1000 data logger (Campbell Scientific, Inc., Logan, UT). Environmental variables were paired with each trace gas flux measurement based on time stamps nearest to flux measurement time.

Three, (4-cm) soil cores were composited from each plot in the spring on 5 April 2010 and 14 March 2011, prior to each growing season for analysis. The soil cores collected from within each plot and separated into depths of 0 - 5, 5 - 15, 15 - 30, 30 - 60, and 60 - 100 cm for composite sampling. Soil and plant tissue samples were analyzed by methods described by Wight et al. (2012). Briefly, soil samples were oven dried at 60°C and ground to

pass a 1.75 mm sieve. Soil pH was determined with a 1:2 soil to water ratio suspension. Inorganic soil nitrate-N was determined from a KCl extraction and spectrophotometric analysis methods (Keeney and Nelson, 1982). Other soil nutrients, such as P, K, Ca, and Mg were extracted with Mehlich III methodology (Mehlich, 1984) and analyzed by inductively coupled spectroscopy (ICP). A subsample from each composite soil sample was finely ground and used to determine total C, organic C, and total N via combustion analysis methods (McGeehan and Naylor, 1988; Schulte and Hopkins, 1996; Storer, 1984). Plant samples were collected during harvest annually for composition analysis. Following oven-drying and grinding to pass a 1 mm sieve, a subset of samples were finely ground for elemental analysis of C and N by combustion methods (McGeehan and Naylor, 1988). Total P and K concentration in plant tissue was determined with a nitric acid digestion and ICP analysis (Havlin and Soltanpour, 1980). A summary of N and C applied as urea fertilizer and biomass residue, respectively, is presented in Table 2. Additional site and soil characteristics at the initiation of the larger study, in 2008, were reported by Wight et al. (2012). Soil and biomass residue properties from 2010 and 2011 are presented in Tables 3 and 4, respectively.



Table 2. Mean (and standard error) soil temperature, mean volumetric water content, N fertilization rate, and previous harvest residue returned as biomass-C for each treatment in 2010 and 2011. Soil temperature and moisture averaged across growing season. Soil temperature reported from 31 May to 28 September in 2010 and 6 May to 28 August in 2011. Average volumetric water content reported from 1 June to 28 September in 2010 and 25 May to 28 August in 2011.

Treatment	2010				2011			
	Soil Temp <sup>†</sup> (°C)	$\theta^{\ddagger}$ (m <sup>3</sup> m <sup>-3</sup> )	N applied (kg N ha <sup>-1</sup> )	C applied (kg C ha <sup>-1</sup> )	Soil Temp <sup>†</sup> (°C)	$\theta^{\ddagger}$ (m <sup>3</sup> m <sup>-3</sup> )	N applied (kg N ha <sup>-1</sup> )	C applied (kg C ha <sup>-1</sup> )
<b>SS, - N, 50%R</b>	29.4 (0.2)	0.09 (0.02)	0	2738 (241)	29.5 (1.4)	0.12 (0.02)	0	1895 (248)
<b>SS, - N, 0%R</b>	29.3 (0.1)	0.15 (0.00)	0	0	30.4 (0.0)	0.12 (0.02)	0	0
<b>SS, + N, 50%R</b>	28.2 (0.3)	0.15 (0.02)	280	3994 (468)	29.4 (0.6)	0.13 (0.03)	280	2935 (689)
<b>SS, + N, 0%R</b>	27.8 (0.1)	0.15 (0.02)	280	0	30.3 (0.3)	0.11 (0.01)	280	0
<b>CS, - N, 50%R</b>	31.4 (0.2)	0.13 (0.01)	0	4932 (350)	30.4 (0.2)	0.09 (0.01)	0	987 (16) <sup>§</sup>
<b>CS, - N, 0%R</b>	31.6 (0.0)	0.16 (0.01)	0	0	30.1 (0.4)	0.08 (0.00)	0	1043 (41) <sup>§</sup>
<b>CS, + N, 50%R</b>	30.8 (0.0)	0.15 (0.01)	168 <sup>*</sup>	4969 (55)	29.5 (0.1)	0.09 (0.02)	280	1133 (79) <sup>§</sup>
<b>CS, + N, 0%R</b>	30.5 (0.2)	0.15 (0.03)	168 <sup>*</sup>	0	29.5 (0.1)	0.09 (0.03)	280	1116 (50) <sup>§</sup>

<sup>†</sup> Mean soil temperature (n = 3) at 10 cm depth

<sup>‡</sup> Mean volumetric water content (n = 3) from 0 - 15 cm depth

<sup>\*</sup> N fertilization applied at reduced rate when corn is grown

<sup>§</sup> All corn biomass returned

## Data Analysis

Cumulative CO<sub>2</sub>-C and N<sub>2</sub>O-N emissions were estimated by linearly interpolating between sampling events and integrating the underlying area (Gilbert, 1987). Cumulative emissions were calculated for both the growing season and the entire year. Annual nitrogen emission factors (*NEF*) were calculated as:

$$NEF = \frac{(N_2O_{+N}) - (N_2O_{-N})}{N_m} \cdot 100 \quad [3]$$

where  $N_2O_{+N}$  is the mass of N<sub>2</sub>O-N lost from fertilized treatments in kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>,  $N_2O_{-N}$  is the mass of N<sub>2</sub>O-N lost from unfertilized treatments in kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>, and  $N_m$  is the mass of N fertilizer applied to the fertilized treatments in kg urea-N ha<sup>-1</sup>. Cumulative annual emissions of CO<sub>2</sub>-C and N<sub>2</sub>O-N were converted to CO<sub>2</sub>-equivalents (CO<sub>2</sub>-eq), assuming the global warming potential of N<sub>2</sub>O is 298 (Forster et al., 2007) and summed for estimation of kg CO<sub>2</sub>-eq ha<sup>-1</sup> for each plot.

Near infrared reflectance (NIR) spectroscopy analysis was performed on bioenergy sorghum biomass tissues in 2011 to estimate chemical composition of sorghum residues. Theoretical ethanol yields from each bioenergy sorghum plot was estimated by combining yield data, plant tissue data, and conversion estimates provided by the National Renewable Energy Laboratory's (NREL) 'Theoretical Ethanol Yield Calculator' ([http://www1.eere.energy.gov/biomass/ethanol\\_yield\\_calculator.html](http://www1.eere.energy.gov/biomass/ethanol_yield_calculator.html)). Ethanol yields from harvested corn grain were estimated from NREL's straight-forward conversion value of 0.519 L kg<sup>-1</sup> dry feedstock. Yields of sorghum biomass and corn grain were obtained from annual harvests for each plot, converted to kg dry feedstock ha<sup>-1</sup> and multiplied by the NREL's estimated ethanol conversion value to obtain an estimate of L ethanol ha<sup>-1</sup>. Cumulative annual GHG emissions (kg CO<sub>2</sub>-eq ha<sup>-1</sup>) from each plot were divided by the

estimated amount of ethanol produced (L ethanol ha<sup>-1</sup>) from each plot for an overall yield-scaled GHG emission value reported as kg CO<sub>2</sub>-eq L<sup>-1</sup> ethanol.

A mixed ANOVA in SAS (Version 9.2) was used to identify differences ( $P < 0.05$ ) in cumulative CO<sub>2</sub>-C, N<sub>2</sub>O-N, CO<sub>2</sub>-eq, and theoretical yield-scaled GHG emissions between the eight individual treatments. Following overall treatment significance, means separation was performed using Fisher's LSD ( $\alpha = 0.05$ ). Next, the effects of crop rotation, N fertilization, and residue return and their interactions on trace gas fluxes, cumulative CO<sub>2</sub>-C, N<sub>2</sub>O-N, CO<sub>2</sub>-eq, and theoretical yield-scaled GHG emissions were tested using a mixed ANOVA in SAS using PROC mixed procedures. Crop rotation, N fertilization, and residue return were considered fixed effects, while replicate (block) was considered a random effect. Treatment significance was determined using a mixed ANOVA where treatment was a fixed effect and replicate (block) was considered a random effect. Thus, while treatment differences may not have existed between each of the production scenarios, the impact of any of the three primary factors could be elucidated. Year was determined to be a significant ( $P = 0.003$ ) effect. Thus, all analyses were performed separately in 2010 and 2011.

## **RESULTS**

### **Site Climate and Environmental Conditions**

The climatic conditions in 2010 and 2011 provided a unique opportunity to explore GHG fluxes under drought conditions in central Texas (Figs. 1 and 2). The 2010 growing season was considered a relatively moderate drought, while 2011 was the most severe drought on record (Nielsen-Gammon, 2011). Air temperatures observed in 2010 were slightly above the 30-year average, while air temperatures recorded during the 2011 growing

season were well above the 30-year average (OSC, 2012). Mean temperature during the 2010 measurement period (1 May 2010 – 30 April 2011) was 21.8 °C, while that during the 2011 measurement period (1 May 2010 – 30 April 2011) was 22.6 °C. During the 2010 growing season, mean monthly soil temperatures were 29.5, 29.8, and 32.1 during June, July, and August, respectively. Corresponding temperatures during the 2011 growing season were generally higher, measuring 30.9, 31.0, and 31.7, respectively. The daily sinusoidal pattern of soil temperature in 2011 had considerably greater daily fluctuation compared to observations from 2010 (Fig. 2); soil thermal properties were likely altered by very low soil moisture conditions throughout the 2011 growing season.

The average (30-year average) annual precipitation total at the study location is 1009 mm (OSC, 2012). During the 2010 sampling year (05/27/2010 – 05/01/2011), 317 mm of total precipitation was measured at the site with an additional 220 mm of irrigation applied (Fig. 2a). Even with irrigation, the research location had only half the normal cumulative precipitation in 2010, providing little soil moisture reserves for the 2011 season. During the 2011 sampling year (05/01/2011 – 05/01/2012), 1021 mm of precipitation was measured at the site with an additional 310 mm of irrigation applied. Of the 1021 mm of cumulative precipitation in 2011, 80% of it fell after fall harvest and 45% of it fell in February and March alone (Figs. 1 and 2). Thus, outside of irrigation, very little water was available during the growing season in 2010. In 2010, 143 mm of cumulative precipitation was measured during the growing season compared to 200 mm during the 2011 season. Despite greater precipitation during the 2011 growing season, the drought effects were heightened for several reasons (Fig. 2). Little precipitation occurred from late fall 2010 through planting in spring 2011. This period of time normally sees the highest precipitation accumulation for the entire

year, and without it, the soil profile was depleted of soil moisture prior to the start of the growing season. Additionally, abnormally high air temperatures combined with high winds on many days throughout the 2011 growing season elevated evapotranspiration (ET) levels across the study area. Irrigation was used to replenish some soil moisture to the profile prior to planting in spring 2011, to help germinate the crop, and to minimize drought stress effects early in the growing season. Much of the precipitation during the 2011 sampling period occurred during the fallow season. In February 2012, a daily rainfall record was set when 159 mm of precipitation occurred within 24 hours. Additionally, a weak tornado accompanied the storm and blew debris into the study area and damaged some environmental monitoring equipment. Another 24-hour precipitation record was set on 20 March 2012 when over 60 mm of precipitation occurred. March 2012 set a monthly cumulative rainfall record; 169 mm of precipitation was deposited over the course of the month. The abnormally heavy precipitation which occurred in February and March 2012 contributed greatly to replenishing soil moisture throughout the profile and lifting drought conditions in the area for the first time since October 2010.

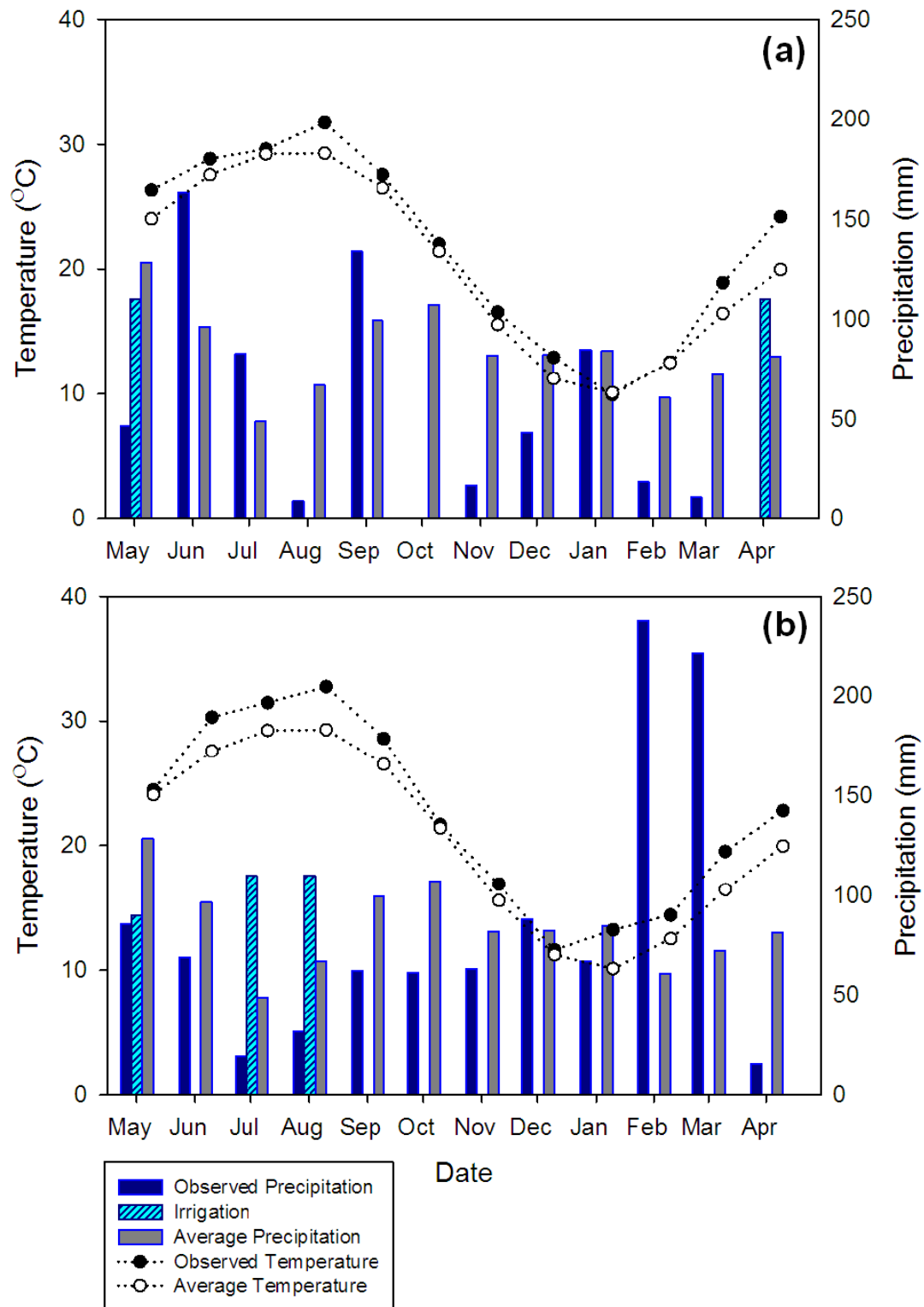


Figure 1. Mean monthly air temperature and total monthly precipitation during the 2010 (a) and 2011 (b) sampling seasons at the research site near College Station, TX. Asterisks indicate irrigation in addition to rainfall total.

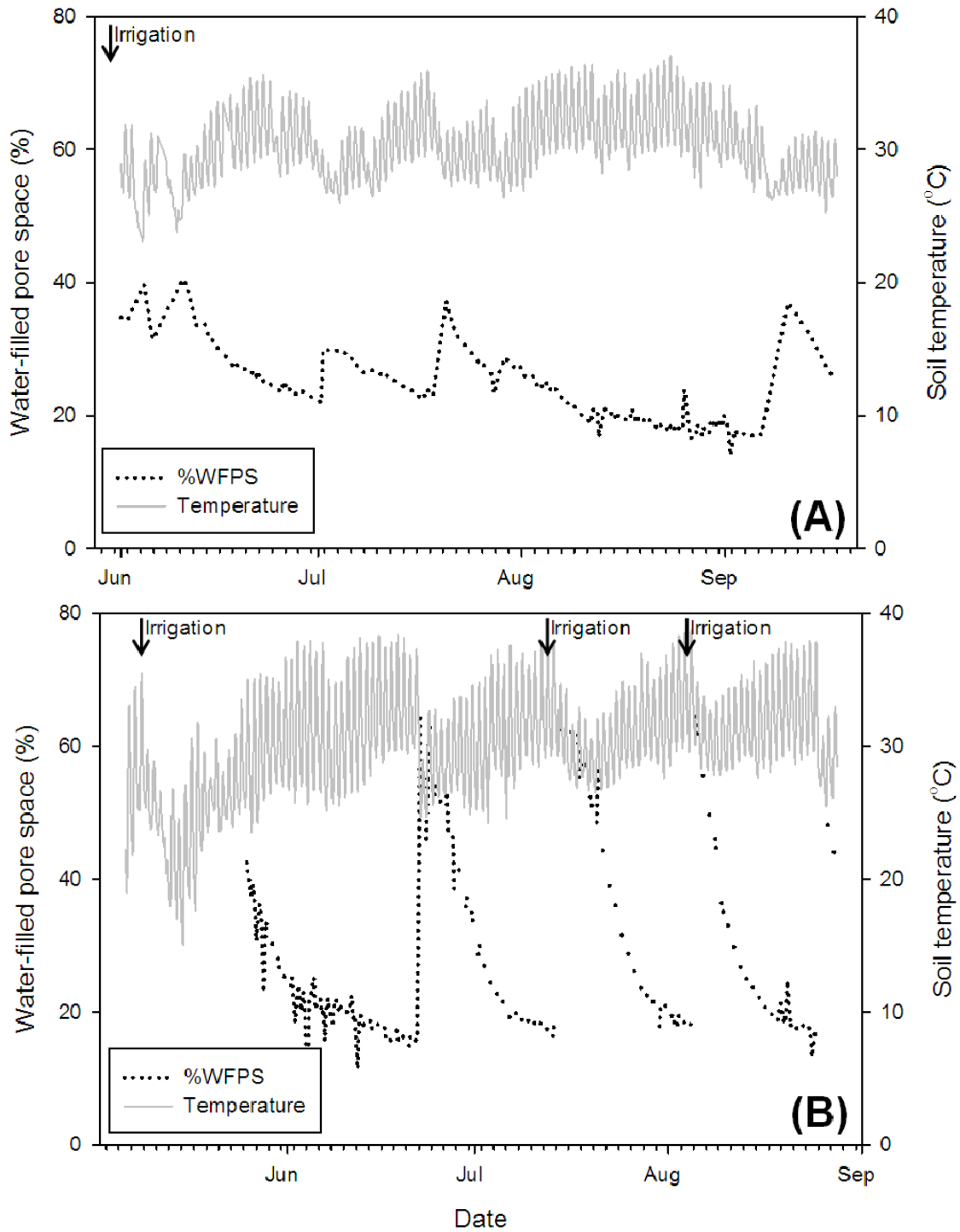


Figure 2. Mean soil temperature and soil moisture content, reported as % water-filled pore space, at the field site during the 2010 (A) and 2011 (B) growing season. Mean (n=24) values are estimated from the entire field. Arrows indicate irrigation event.

## **Crop and Theoretical Ethanol Yield**

All crop rotation sequences were observed across both years of analysis, but because of study design, corn was only grown in 2010. Nitrogen fertilization significantly ( $P = <0.001$ ) increased yield in 2010 and 2011 (Fig. 3). In addition, fertilization significantly ( $P = <0.001$ ) increased plant biomass-N concentration compared to unfertilized treatments in 2011, but not in 2010. The effect of crop rotation on bioenergy sorghum could only be assessed in 2011 because CS rotation was in the corn sequence in 2010. Overall, CS treatment yields in 2010 were lower due to the presence of corn in rotation compared to the much larger aerial biomass yield provided by sorghum in the SS treatments. In 2011, when the entire study was in the bioenergy sorghum sequence, crop rotation significantly ( $P = <0.001$ ) affected total biomass yield; CS produced 28% more biomass yield than SS. Additionally, crop rotation had a significant ( $P = <0.001$ ) impact on plant composition; CS significantly increased the C and N concentrations of biomass compared to SS treatments. Residue return had no significant effect on total biomass yield (when 50%R was not subtracted from total yield) in 2010. However, 50%R produced significantly ( $P = <0.001$ ) more biomass yield than 0%R in 2011. Residue return had no impact on plant biomass-C or N concentration in either 2010 or 2011.



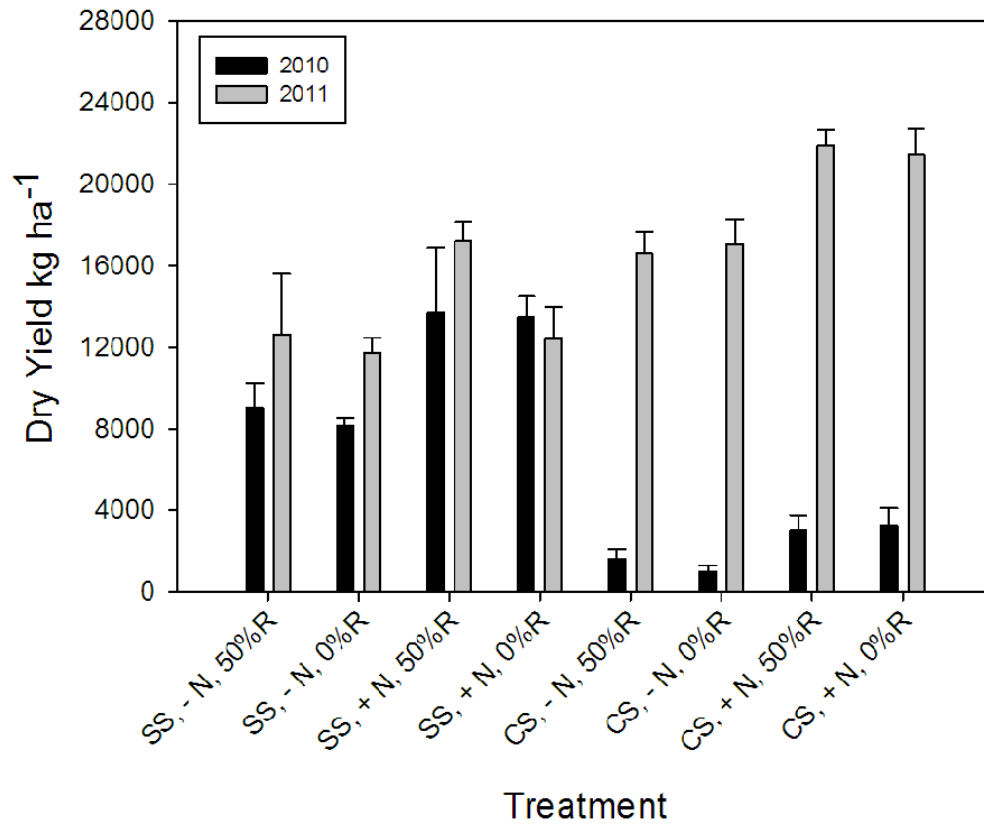


Figure 3. Mean bioenergy sorghum and corn yields in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).

The NREL estimate of theoretical ethanol yield was obtained from our NIR-determined plant tissue composition and total biomass yield produced. These values are theoretical estimates and are used solely for treatment comparison in this study. Theoretical ethanol yields in this study were based on C5 and C6 polymer content within the feedstock. Starch content can significantly increase theoretical ethanol yield per unit of feedstock (Stefaniak et al., 2012) but was not included in this study. Bioenergy sorghum estimated conversion values ranged from 0.32 to 0.34 L ethanol kg<sup>-1</sup> dry feedstock. These theoretical ethanol conversion yields were similar to those found by Stefaniak et al. (2012) in biomass sorghum. Only corn grain was harvested for simulated biofuel production, and no corn residues were removed in this study. The NREL standard estimated conversion value for corn was 0.52 L ethanol kg<sup>-1</sup> dry grain. In 2010, theoretical ethanol production ranged from only 615 L ethanol ha<sup>-1</sup> in [CS, - N, 0%R] to more than 4600 L ethanol ha<sup>-1</sup> in [SS, + N, 0%R] (Fig. 4). The large variation was due to the vast difference in yield between corn and bioenergy sorghum. Theoretical ethanol production in 2011 was based on bioenergy sorghum yield across the entire study and estimates ranged from approximately 1700 L ethanol ha<sup>-1</sup> in [SS, - N, 50%R] to more than 7000 L ethanol ha<sup>-1</sup> in [CS, + N, 0%R]. One discernible result of 50%R treatments was a large reduction in utilizable yield due to half the total dry yield being returned to the soil as an amendment; thus less total biomass available for ethanol production. Wide variation in theoretical ethanol yield from CS treatments in 2010 to 2011 can be attributed to a poor corn grain yield in 2010 compared to high bioenergy sorghum yield in 2011.

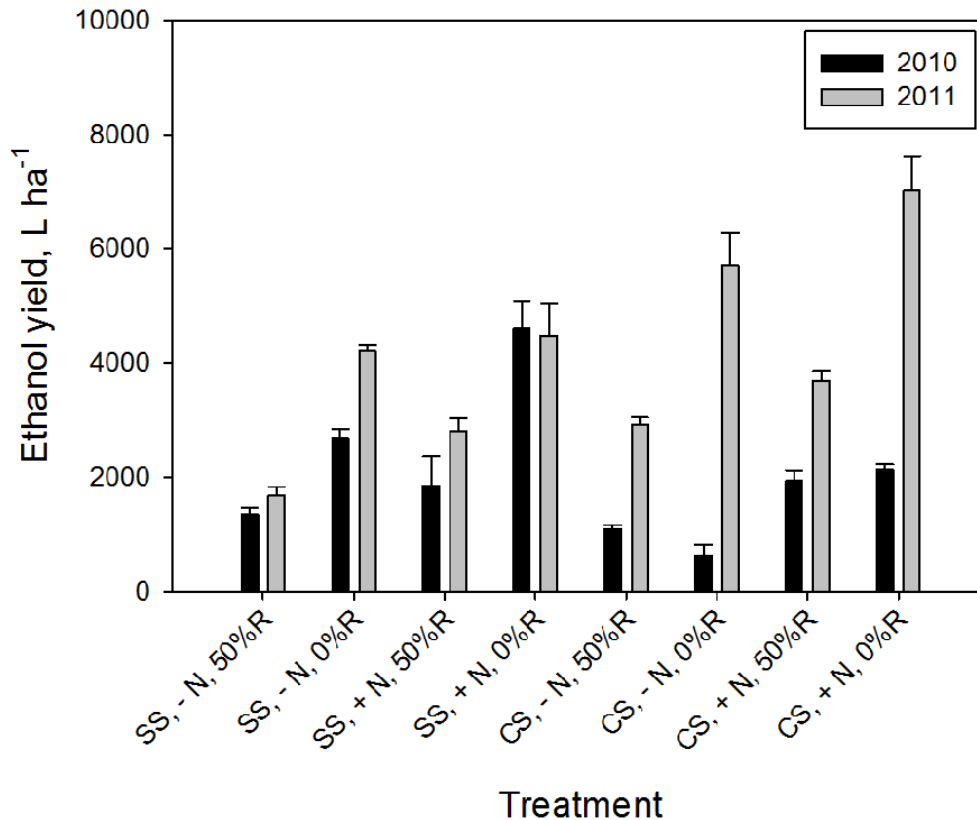


Figure 4. Mean theoretical ethanol yields in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Ethanol yield based on corn grain and sorghum biomass yield and NREL conversion estimates.

### Growing Season Greenhouse Gas Fluxes

Following N fertilization, fluxes of CO<sub>2</sub> were generally high when the soil moisture was also relatively high. Peak fluxes were generally observed after irrigation/precipitation events. As the soil dried, the fluxes decreased until a precipitation or irrigation event increased soil moisture once again (Figs. 2, 5 and 6). Early in 2010, crop rotation and residue return significantly affected CO<sub>2</sub> fluxes. Residue return significantly increased CO<sub>2</sub> fluxes on several sampling events until early August. It is likely that the additional organic carbon supplied by returning residue to the soil increased microbial decomposition and subsequent heterotrophic respiration. Interestingly, for the majority of the growing season, crop rotation

did not significantly affect emissions of CO<sub>2</sub>, despite corn harvest and senescence occurring in early July 2010. We expected the photosynthetically-active sorghum crop to have higher CO<sub>2</sub> loss for a longer period of time than the senesced corn crop. However, the senescence of the corn may have initiated additional belowground decomposition as the labile portions of the senesced roots were degraded by soil microbes over time. However, late in the season, crop rotation emerged as a significant effect driving CO<sub>2</sub> emissions, where higher emissions were found in the SS treatment compared to the CS rotation (Fig. 5). Labile portions of senesced corn crop biomass may have decomposed during the mid-growing season and decomposition, overall, slowed as soil moisture decreased throughout August. Precipitation in late August and early September likely increased microbial activity and photosynthetic activity in the sorghum crop. As labile portions of SOC were diminished in the late part of the growing season in the CS rotation, and as the SS treatment continued to be photosynthetically-active, belowground C allocation and decomposition may have lowered CO<sub>2</sub> fluxes in the CS rotation while increasing fluxes in the SS treatment.

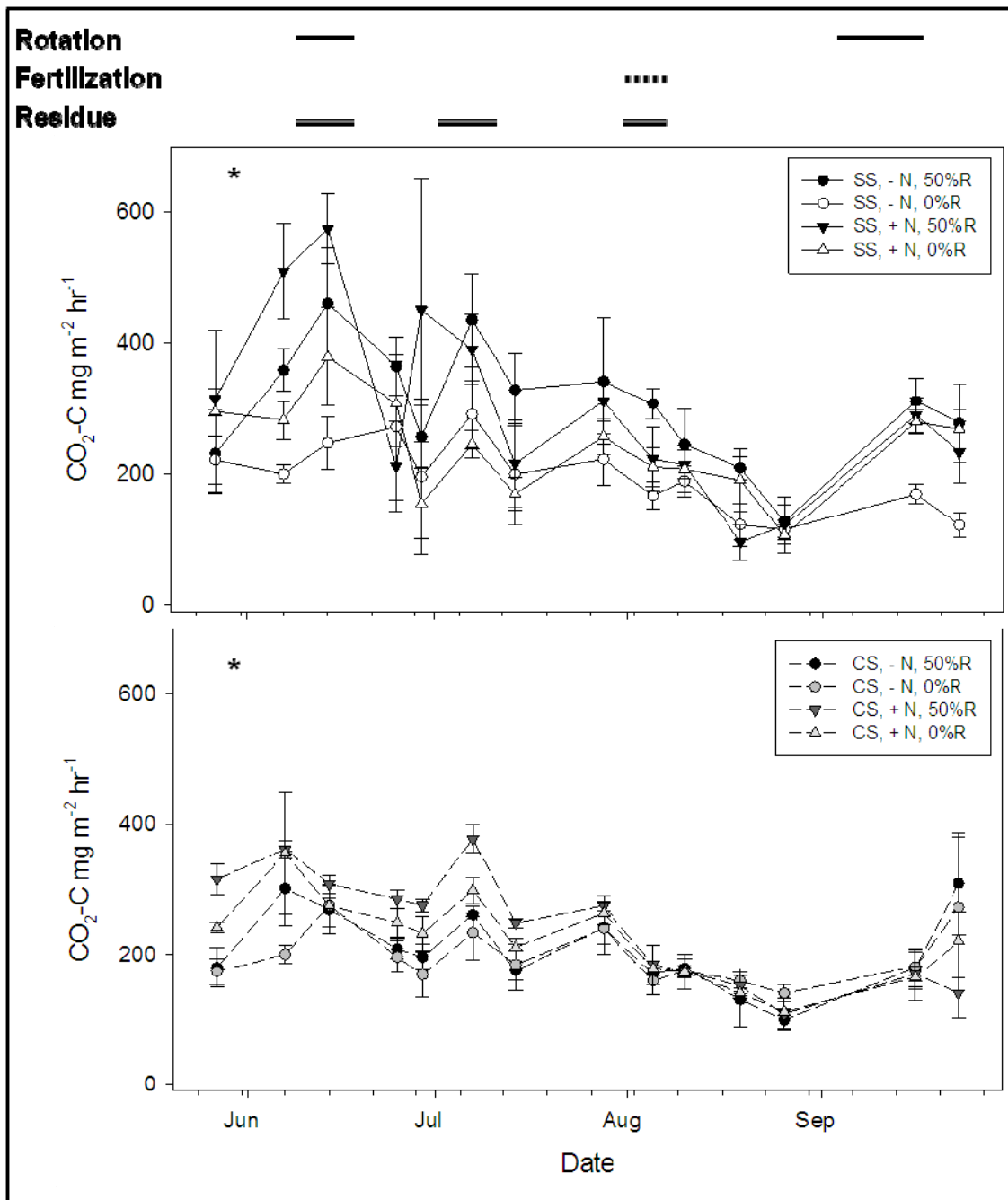


Figure 5. Mean soil gas fluxes of CO<sub>2</sub> from 2010 growing seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Lines above sampling events denote significant (P < 0.05) difference in gas flux rate due to crop rotation (solid bar), N fertilization (dashed bar), or residue return (double bar), respectively. Asterisk denotes irrigation date.

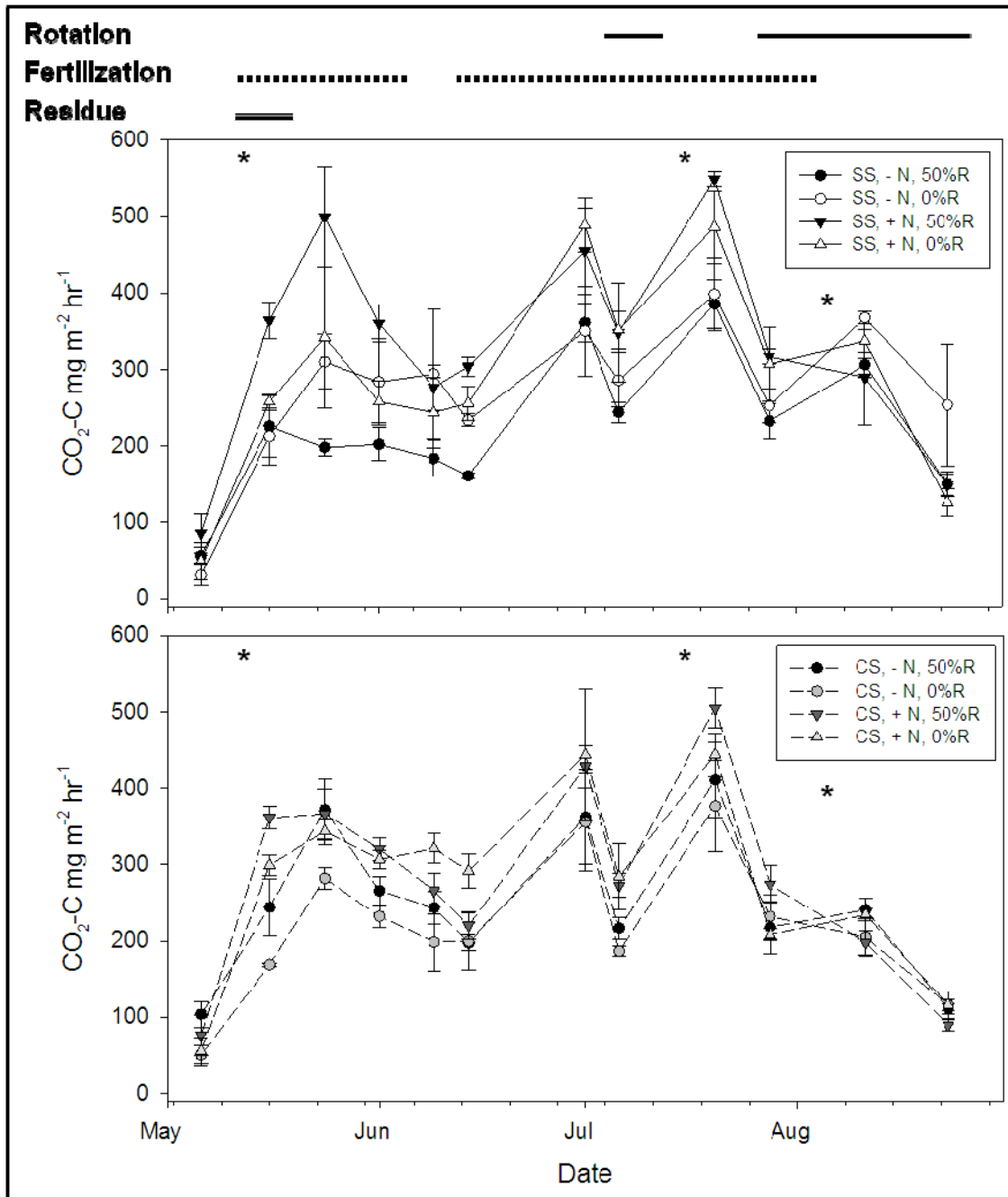


Figure 6. Mean soil gas fluxes of CO<sub>2</sub> from 2011 growing seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Lines above sampling events denote significant (P < 0.05) difference in gas flux rate due to crop rotation (solid bar), N fertilization (dashed bar), or residue return (double bar), respectively. Asterisk denotes irrigation date.

Nitrous oxide fluxes in 2010 were sporadic in the bioenergy cropping system (Fig. 7). As expected, fluxes of N<sub>2</sub>O were higher following N fertilization and fertilization was implicated as a significant driver of N<sub>2</sub>O loss. By late June, soil moisture was significantly depleted (Fig. 2a) and associated N<sub>2</sub>O flux rates were low (Fig. 7). Shortly following precipitation events throughout the year, flux rates again increased, but never to the highest rates observed initially after fertilization. Similarly, after the initial peak early in the season, N fertilization was no longer found as a significant factor driving N<sub>2</sub>O fluxes. Beginning in mid-July, crop rotation became significantly associated with N<sub>2</sub>O flux. It is possible that the senesced corn crop was not providing a labile source of organic C to soil microbes in the CS rotation late in the growing season, whereas, sorghum may have continued to provide a source of labile organic C because of photosynthate production throughout the growing season. It may be possible that less labile organic C (via active plant root exudates) reduced microbial activity and potential gaseous N loss later in the season in the CS rotation relative to the SS rotation.

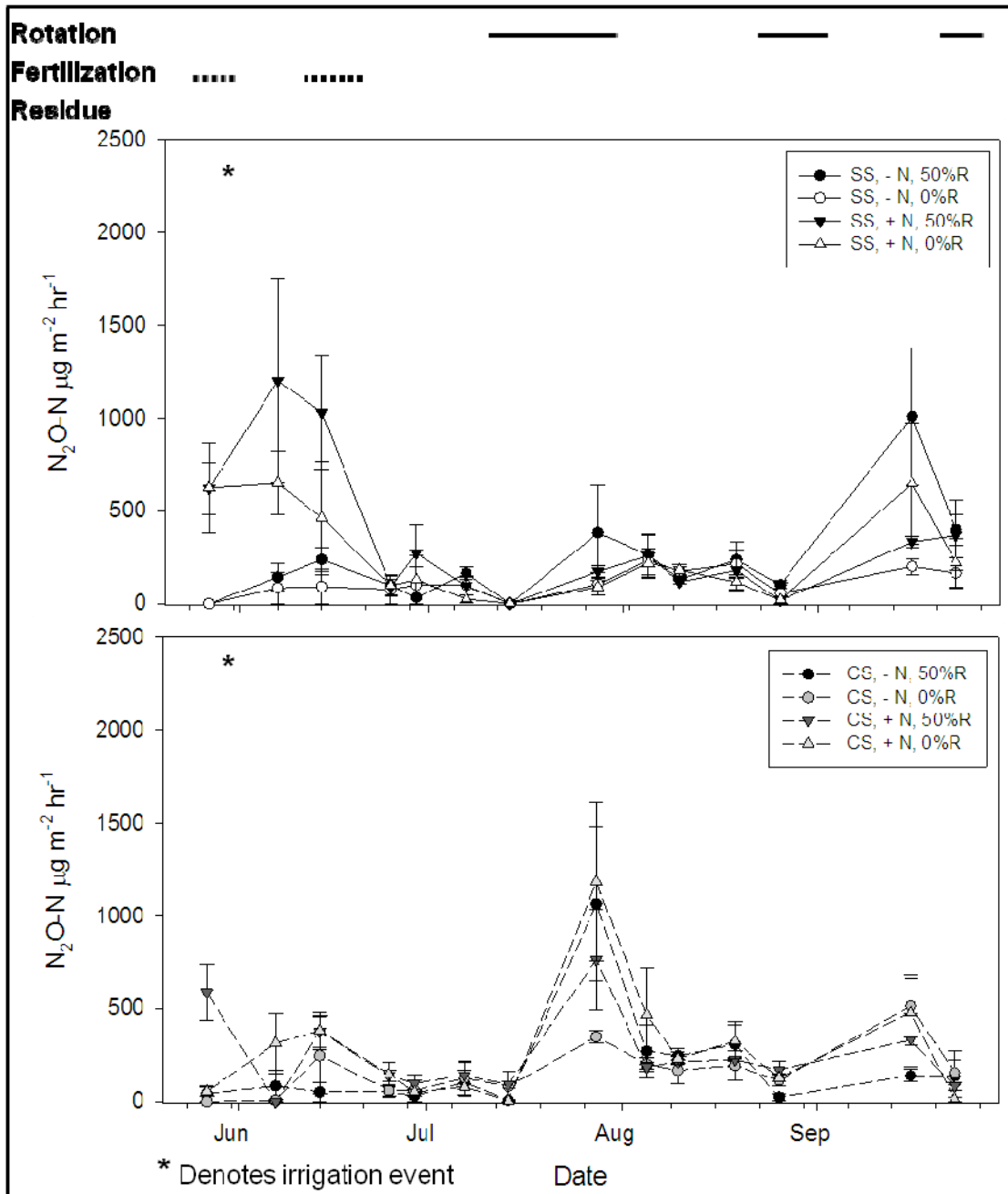


Figure 7. Mean soil gas fluxes of  $N_2O$  from 2010 growing seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error ( $n = 3$ ). Lines above sampling events denote significant ( $P < 0.05$ ) difference in gas flux rate due to crop rotation (solid bar), N fertilization (dashed bar), or residue return (double bar), respectively. Asterisk denotes irrigation date.



Fluxes of CO<sub>2</sub> were low immediately after fertilization in 2011 (Fig. 6). Low soil moisture content (Fig. 2b) may have limited soil microbial activity and may have been a large factor in why fluxes were low initially. However, following irrigation, fluxes increased in mid-May and hit a peak in late May. Nitrogen fertilization was a significant factor causing elevated CO<sub>2</sub> emissions throughout much of the growing season (Fig. 6). Increased CO<sub>2</sub> flux due to N fertilization was likely related to multiple biophysical factors. Increased N availability in the fertilized plots increased primary productivity and probably subsequent belowground C allocation in both root exudates and increased root respiration. Elevated CO<sub>2</sub> fluxes also tended to coincide with increased soil moisture conditions (Fig 2b). When soil moisture dropped in mid-June, N fertilization no longer was a significant factor (Figs. 2 and 6). Similarly, late in the season, as soils dried, N fertilization was no longer a significant factor (Figs. 2 and 6). However, N availability within the soil environment may have also diminished by late in the season, causing direct effects of fertilization to no longer be observed. Residue return was found to be significantly related to CO<sub>2</sub> flux on only one sampling event early in the season in mid-May (Fig. 6). Residue remaining in the soil from previous harvests may have continued to decompose early in the season, with more labile fractions continuing to be available for soil microbes initially, but may have diminished in availability by late May. Interestingly, despite the entire field being planted to sorghum in 2011, crop rotation became a significant factor that increased CO<sub>2</sub> flux late in the season (Fig. 6). Crop rotation was a significant factor beginning in early July and again in late July through harvest. It was surprising that the CS rotation had significantly lower CO<sub>2</sub> flux compared to SS late in the season, despite the fact the CS rotation had higher biomass yield (primary productivity) than the SS rotation (Fig. 3). One potential explanation may be related

to the belowground organic C concentrations in SS treatments. The SS treatments had relatively higher SOC, compared to CS (Table 3), possibly from greater annual root C inputs from biomass sorghum. Increased SOC in the SS rotation may have provided a larger available C pool for microbial decomposition that was evident later in the year.

Table 3. Means (and standard errors) of spring pre-plant soil properties. A summary of total carbon (TC), organic carbon (OC), total nitrogen (TN), extractable nitrate-nitrogen (NO<sub>3</sub>-N), extractable phosphorus (P), and extractable potassium (K) from 2010 and 2011 are presented.

Treatment	<i>Soil properties</i>					
	<b>2010</b>					
	TC	OC	TN	NO <sub>3</sub> -N	P	K
	g kg <sup>-1</sup>		mg kg <sup>-1</sup>			
<b>SS, - N, 50%R</b>	19.6 (0.3)	11.6 (0.3)	1236 (116)	3.5 (0.2)	23.8 (0.8)	287.0 (19.6)
<b>SS, - N, 0%R</b>	18.9 (0.2)	10.8 (0.2)	1205 (95)	4.0 (0.4)	15.9 (3.2)	201.6 (22.1)
<b>SS, + N, 50%R</b>	20.8 (0.4)	15.1 (2.2)	1328 (112)	2.8 (0.3)	22.0 (1.7)	313.8 (28.3)
<b>SS, + N, 0%R</b>	19.3 (0.2)	11.0 (0.1)	1112 (19)	4.4 (0.5)	17.8 (2.1)	224.4 (28.7)
<b>CS, - N, 50%R</b>	16.4 (1.0)	8.9 (0.8)	1082 (67)	2.5 (0.3)	23.3 (1.9)	195.8 (13.5)
<b>CS, - N, 0%R</b>	17.0 (0.8)	8.9 (0.5)	1033 (16)	3.6 (0.2)	23.2 (0.1)	201.0 (14.1)
<b>CS, + N, 50%R</b>	18.1 (0.9)	10.2 (0.8)	1009 (74)	3.3 (0.3)	27.3 (0.6)	218.8 (16.5)
<b>CS, + N, 0%R</b>	17.1 (0.5)	9.2 (0.8)	979 (178)	4.7 (0.2)	23.5 (0.8)	201.0 (7.9)
	<b>2011</b>					
	TC	OC	TN	NO <sub>3</sub> -N	P	K
	g kg <sup>-1</sup>		mg kg <sup>-1</sup>			
<b>SS, - N, 50%R</b>	19.5 (0.6)	11.0 (1.2)	1305 (32)	3.7 (0.3)	24.8 (0.2)	224.8 (13.3)
<b>SS, - N, 0%R</b>	18.9 (0.1)	11.0 (0.3)	1298 (10)	5.9 (0.4)	24.1 (0.3)	213.2 (5.2)
<b>SS, + N, 50%R</b>	20.5 (0.4)	13.4 (0.4)	1392 (8)	2.6 (0.8)	22.3 (0.7)	237.7 (18.9)
<b>SS, + N, 0%R</b>	19.2 (0.2)	12.1 (0.6)	1318 (23)	6.2 (0.9)	22.3 (0.4)	191.5 (3.8)
<b>CS, - N, 50%R</b>	16.9 (0.7)	9.5 (0.7)	1040 (38)	7.4 (1.5)	26.6 (1.0)	193.0 (15.4)
<b>CS, - N, 0%R</b>	16.7 (0.1)	9.2 (0.1)	1101 (12)	7.8 (1.1)	24.1 (0.7)	194.1 (15.7)
<b>CS, + N, 50%R</b>	17.0 (0.3)	9.9 (0.2)	1087 (61)	7.9 (0.4)	25.6 (1.6)	196.7 (21.3)
<b>CS, + N, 0%R</b>	17.8 (0.4)	10.4 (0.8)	1168 (32)	8.2 (1.1)	22.6 (0.9)	190.1 (5.1)

Fluxes of N<sub>2</sub>O during the 2011 growing season were a bit more sporadic than those in 2010 and driven largely by soil moisture conditions (Fig. 8). Fewer detectable fluxes of N<sub>2</sub>O occurred during the 2011 growing season compared to the 2010 growing season (Figs. 7 and 8). The initial gas sampling event following fertilization on 6 May 2011 showed no measurable N<sub>2</sub>O flux. However, following irrigation, subsequent sampling events yielded measurable fluxes. The highest measured flux rate of the study (2,182 µg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup>) was measured in late May in a [SS, +N, 50%R] treatment replicate. Nitrogen fertilization significantly increased fluxes of N<sub>2</sub>O, early in the growing season, but did not persist beyond mid-June (Fig. 8). Crop rotation significantly influenced N<sub>2</sub>O flux at several points throughout the growing season, but impacts were not consistent with a single rotation treatment (Fig. 8). The rotation effect generally coincided with relatively high flux events, and was not likely detected during lower flux events throughout the growing season because of lack of measurable loss. The rotation effect was also not consistent with a single crop rotation treatment throughout the growing season; the SS treatment had significantly higher N<sub>2</sub>O loss near the beginning and at the end of the study, but CS treatments had higher fluxes near the middle of the growing season (Fig. 8). This observation may be partially related to the inherent variability experienced with N<sub>2</sub>O fluxes during the 2011 growing season. Finally, residue return significantly increased N<sub>2</sub>O flux late in the growing season (Fig. 8). The C:N ratio of the biomass residue returned may have narrowed significantly by late in the season, allowing for some additional N mineralization and nitrification.

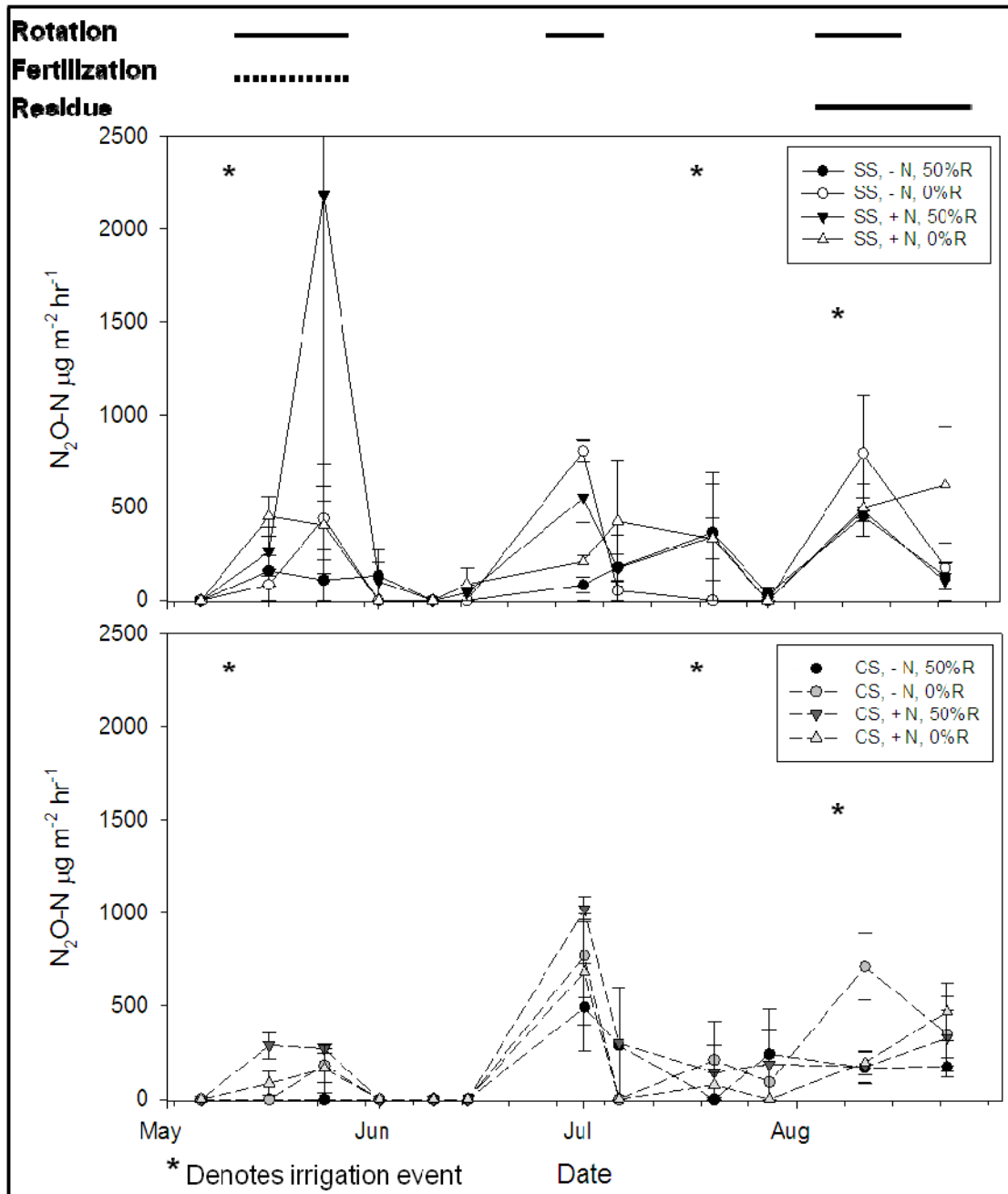


Figure 8. Mean soil gas fluxes of  $N_2O$  from 2011 growing seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error ( $n = 3$ ). Lines above sampling events denote significant ( $P < 0.05$ ) difference in gas flux rate due to crop rotation (solid bar), N fertilization (dashed bar), or residue return (double bar), respectively. Asterisk denotes irrigation date.

Fluxes of CO<sub>2</sub> in 2010 and 2011 generally coincided with precipitation or irrigation events (Figs. 5 and 6). Both CO<sub>2</sub> and N<sub>2</sub>O fluxes increased shortly after fertilization in 2010. Fluxes of CO<sub>2</sub> and N<sub>2</sub>O were generally higher from N fertilized plots, but the fertilization effect was only significant (P = 0.006) for the N<sub>2</sub>O fluxes. Following corn harvest (2 July 2010), some of the higher CO<sub>2</sub> fluxes were observed in the SS crop sequence and reached more than 500 mg CO<sub>2</sub>-C m<sup>-2</sup> hr<sup>-1</sup>. However, in the CS rotation, variation between treatments was less and fluxes during the same period only reached approximately 300 mg CO<sub>2</sub>-C m<sup>-2</sup> hr<sup>-1</sup>. Fluxes of both CO<sub>2</sub> and N<sub>2</sub>O then decreased, but periodically spiked during the remainder of the season in response to precipitation events. In late July 2010, fluxes of N<sub>2</sub>O peaked following a precipitation event (Fig. 7). Fluxes from the CS rotation were generally greater than those from the SS sequence during this event, but results were not significant. It is possible that decomposing residues from the senesced corn plants may have increased levels of available soil N and led to greater losses of N<sub>2</sub>O. Approximately one month later, near the end of August, a significant difference in N<sub>2</sub>O flux was attributed to crop rotation, with greater loss occurring from the CS rotation. Decomposing corn residue potentially supplied more available soil N, similar to the event observed in late July. Although N fertilization was not determined to be a significant effect on N<sub>2</sub>O loss on the second sampling event on 7 June 2010, we noted that only half the plots were able to be sampled on this date because a pop-up thunderstorm caused a cancellation of further sampling, preventing measurement of all plots.

## **Cumulative Growing Season Greenhouse Gas Emissions**

Cumulative emissions of CO<sub>2</sub> from the 2010 growing season were affected by agronomic management practices employed within the various treatments (Fig. 9). CS treatments were in the corn sequence of the rotation in 2010, causing fundamental differences in crop physiology and impacts to the soil environment. Corn was harvested early in July in 2010, so the crop in the CS rotation was no longer photosynthetically active beyond that date and was senescing near mid-June. Therefore, CO<sub>2</sub> fluxes after late June were driven largely by microbial respiration in the CS rotation with little root respiration contribution. We cannot eliminate all plant respiration as a source of CO<sub>2</sub> flux because some weeds were present in the plots and were photosynthetically-active for the entire growing season. Factorial analysis revealed that, across the entire growing season, the CS rotation significantly decreased ( $P = 0.004$ ) cumulative CO<sub>2</sub> emissions by 20% compared to the SS rotation. Harvesting all biomass residue (0%R) reduced ( $P = 0.014$ ) cumulative emissions of CO<sub>2</sub> by 16% compared to 50%R. The SS treatments receiving 50% residue return had the highest cumulative growing season CO<sub>2</sub> emissions during 2010 ( $\sim 8500$  kg CO<sub>2</sub>-C ha<sup>-1</sup>) and all CS treatments were significantly less ( $\sim 5500$  kg CO<sub>2</sub>-C ha<sup>-1</sup>). An interactive effect ( $P = 0.026$ ) between rotation and residue return showed that regardless of N fertilization, the SS, 50%R treatments had 35% higher cumulative CO<sub>2</sub> emissions than the CS, 50%R treatments in 2010.

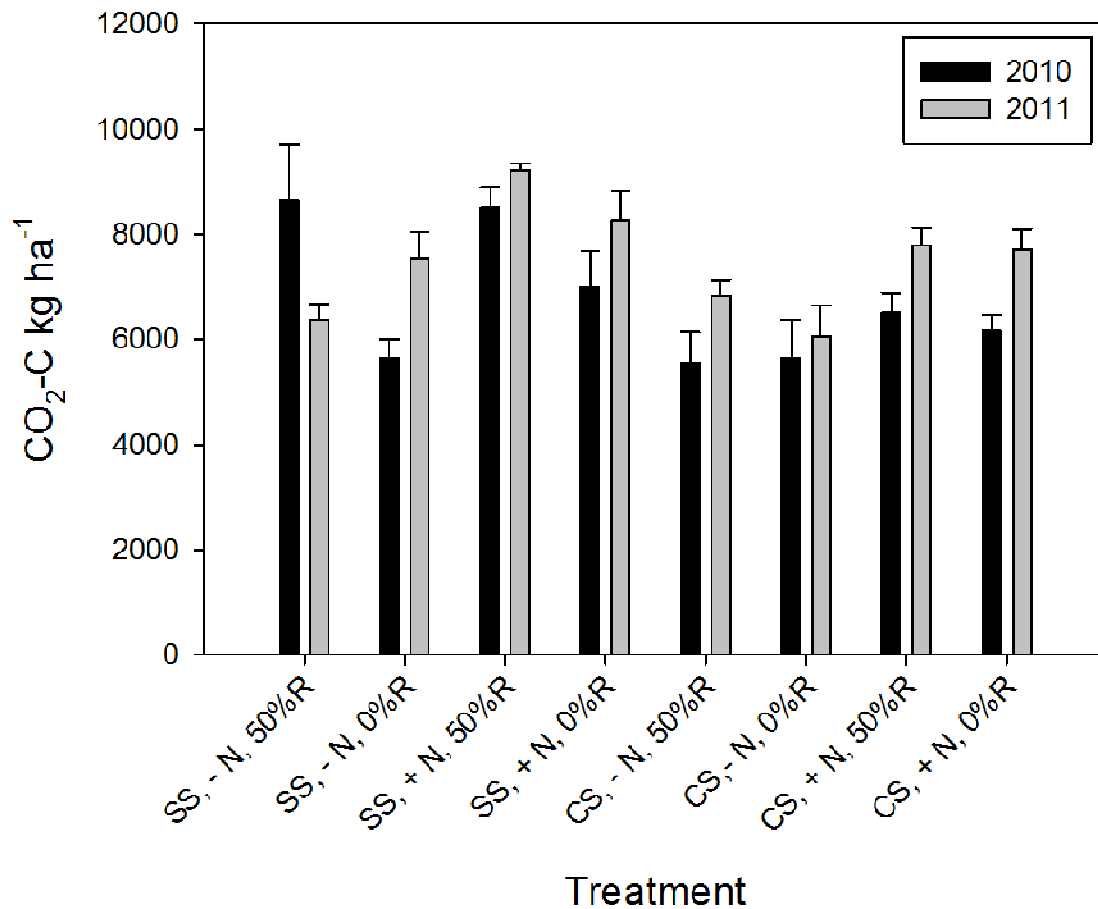


Figure 9. Mean cumulative growing season CO<sub>2</sub> emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).

Overall, greater differences were observed in cumulative growing season CO<sub>2</sub> emissions among treatments in 2011 compared to 2010. This was a surprising observation because both cropping sequence treatments were in the sorghum phase of the crop rotation during 2011. While the cumulative CO<sub>2</sub> emissions among the treatments ranged from approximately 6000 to 9000 kg CO<sub>2</sub>-C ha<sup>-1</sup>, relatively similar to the 2010 range, greater means separation was determined between treatments in 2011. Agronomic management practices significantly affected cumulative CO<sub>2</sub> emissions from the 2011 growing season. For the second year, crop rotation was a significant factor. The CS rotation reduced (P = 0.018)

cumulative CO<sub>2</sub> emissions by 10% compared to SS treatments. This observation was surprising because the CS treatments yielded higher sorghum biomass than the SS treatments in 2011 (Fig. 3). Nitrogen fertilization also had a highly significant ( $P = <0.001$ ) effect on cumulative CO<sub>2</sub> emissions, where - N treatments reduced CO<sub>2</sub> emissions by 19% compared to plots receiving N fertilization. Plots receiving N fertilization had significantly higher ( $P = <0.001$ ) biomass yield than unfertilized treatments, and may have contributed greater CO<sub>2</sub> emissions via root respiration. Another potential factor affecting cumulative CO<sub>2</sub> loss may be related to the high C:N of the biomass residue, which may have limited microbial decomposition/respiration in the - N soil environments. The C:N of bioenergy sorghum (average of SS and CS) in 2009 was 55, bioenergy sorghum in 2010 (SS only) was 70, and corn in 2010 (CS only) was 50 (Table 4). The [SS, + N, 50%R] treatment exhibited some of the highest cumulative emissions of CO<sub>2</sub> in both 2010 and 2011. Interestingly, this treatment did not correspond to one of the highest biomass yielding treatments among those investigated. The SS rotation had less biomass yield, but greater CO<sub>2</sub> emissions in 2011 compared to the CS rotation implying possible greater microbial respiration in the SS rotation and corresponding with soil organic carbon and microbial biomass results which found higher soil organic carbon and soil microbial biomass-C (higher potential microbial activity) in the SS rotation compared to the CS rotation (Chapter III).



Table 4. Means (and standard errors) of fall harvest biomass residue properties. A summary of total carbon (TC), total nitrogen (TN), total phosphorus (P), total potassium (K), and the carbon-to-nitrogen ratio (C:N) from 2010 and 2011 are presented.

<i>Biomass residue properties</i>					
<b>2010†</b>					
	C	N	P	K	C:N
Treatment	kg ha <sup>-1</sup>				
<b>SS, - N, 50%R</b>	3790 (497)	48.1 (6.6)	9.0 (1.6)	151.7 (12.5)	79
<b>SS, - N, 0%R</b>	3443 (188)	48.2 (2.6)	8.1 (1.2)	138.6 (9.2)	71
<b>SS, + N, 50%R</b>	5870 (1378)	98.2 (21.8)	9.8 (2.6)	202.9 (47.0)	60
<b>SS, + N, 0%R</b>	5761 (383)	82.7 (7.8)	14.3 (3.3)	235.4 (39.0)	70
<b>CS, - N, 50%R</b>	987 (16)	22.7 (1.7)	5.3 (1.4)	23.4 (1.7)	43
<b>CS, - N, 0%R</b>	1043 (41)	20.3 (1.3)	3.7 (2.0)	28.0 (2.0)	51
<b>CS, + N, 50%R</b>	1133 (79)	21.2 (1.4)	1.5 (0.1)	34.6 (1.7)	53
<b>CS, + N, 0%R</b>	1116 (50)	23.6 (1.8)	1.5 (0.1)	30.8 (1.7)	47

<b>2011</b>					
	C	N	P	K	C:N
Treatment	kg ha <sup>-1</sup>				
<b>SS, - N, 50%R</b>	5067 (1175)	70.0 (21.9)	11.5 (1.7)	220.8 (45.7)	72
<b>SS, - N, 0%R</b>	4715 (290)	81.5 (8.2)	12.3 (1.0)	214.1 (26.8)	58
<b>SS, + N, 50%R</b>	6901 (351)	127.7 (10.1)	12.5 (1.3)	292.5 (15.0)	54
<b>SS, + N, 0%R</b>	4985 (622)	85.8 (10.9)	9.3 (2.2)	225.2 (38.8)	58
<b>CS, - N, 50%R</b>	6765 (420)	103.9 (5.8)	11.2 (1.6)	284.1 (26.2)	65
<b>CS, - N, 0%R</b>	6982 (536)	102.5 (9.8)	11.1 (0.7)	285.4 (16.4)	68
<b>CS, + N, 50%R</b>	8998 (328)	182.1 (9.1)	14.7 (0.8)	385.0 (34.8)	49
<b>CS, + N, 0%R</b>	8737 (554)	182.2 (19.8)	15.0 (1.9)	338.0 (31.5)	48

† CS was corn biomass residue

In 2010, cumulative growing season N<sub>2</sub>O emissions varied greatly among treatments (Fig 6b). Cumulative N<sub>2</sub>O emissions ranged from approximately 3 to 10 kg N<sub>2</sub>O-N ha<sup>-1</sup>. Nitrogen fertilization significantly (P = 0.002) increased cumulative emissions of N<sub>2</sub>O by 61% compared to unfertilized treatments. A rotation by residue interaction (P = 0.012) was observed in which, regardless of N fertilization, SS, 50%R treatments had 65% greater

cumulative N<sub>2</sub>O emissions than SS, 0%R treatments. The addition of residue appeared to increase cumulative N<sub>2</sub>O emissions in the SS sequence, but not in the CS rotation. The larger mass of residue (with high C:N) applied to the CS treatments from the 2009 harvest may have caused net N immobilization within the soil and limited N availability for gaseous loss processes. In addition to potential immobilization, the higher microbial biomass in the SS, 50%R treatments (Chapter III) may have enhanced nitrification/denitrification which released gaseous N from those treatments. The largest variation between treatments occurred within the SS sequence. A difference of nearly 7 kg N<sub>2</sub>O-N ha<sup>-1</sup> was observed between [SS, - N, 0%R] and [SS, + N, 50%R]. During the 2010 growing season, [SS, - N, 0%R] and [CS, - N, 50%R] exhibited some of the lowest emissions of cumulative CO<sub>2</sub> and N<sub>2</sub>O, while [SS, - N, 50%R] and [SS, + N, 50%R] had some of the highest cumulative GHG emissions. Larger variation was associated with cumulative N<sub>2</sub>O emissions than cumulative CO<sub>2</sub> emissions.

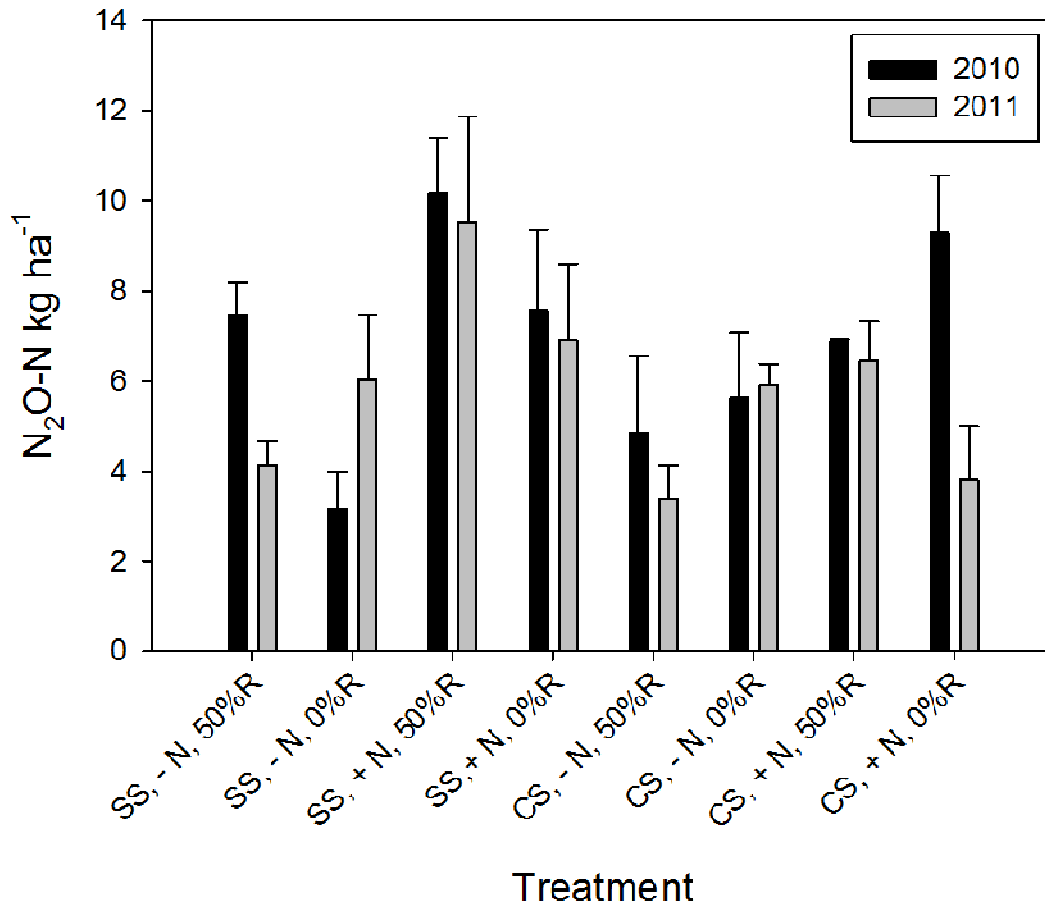


Figure 10. Mean cumulative growing season N<sub>2</sub>O emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).

The severe drought during the 2011 growing season provided an interesting analysis of cumulative N<sub>2</sub>O emissions from cropping system treatments that were highly water-limited and highly stressed. As a result, we observed highly sporadic fluxes of N<sub>2</sub>O which led to large variation within cumulative emission estimates (Fig. 10). There was only a slight difference (P = 0.081), at the P<0.10 level of significance, in cumulative growing season N<sub>2</sub>O emissions among any of the treatments in 2011. The mean cumulative N<sub>2</sub>O emissions among the treatments ranged from 3.5 to 9.5 kg N<sub>2</sub>O-N ha<sup>-1</sup>, relatively similar to the range observed in 2010, but with less means separation. A significant (P = 0.020) interaction

between N fertilization and residue return was observed and regardless of rotation, + N, 50%R increased N<sub>2</sub>O emissions by 113% compared to – N, 50%R. The high C:N of biomass residue (and potential lack of decomposition during the fallow period due to drought conditions) may have created net immobilization conditions in the soil environment and the addition of N via urea likely enhanced mineralization and provided labile N for gaseous N loss processes. Due to the high variation within the 2011 cumulative N<sub>2</sub>O data, it might be reasonable to consider effects which were slightly outside the strict P = 0.05 significance level. The effect of N fertilization on cumulative N<sub>2</sub>O emissions during the 2011 growing season fell just outside the default significance level (P = 0.069). Treatments with N fertilization had 37% higher N<sub>2</sub>O loss than – N treatments. Rotation was significant at P = 0.078, with SS treatments showing 36% higher N<sub>2</sub>O emission than CS treatments.

### **Cumulative Annual Greenhouse Gas Emissions**

While the most active period of trace gas emissions is typically the growing season, the fallow season can contribute a significant portion of annual emissions. In central Texas, fallow season conditions are generally characterized by relatively mild temperatures (commonly above freezing) and increased precipitation, relative to the growing season. Although fallow season GHG fluxes are likely lower than those from the growing season (lower temperatures and lack of crop growth), these mild, wet conditions may be conducive to sustaining significant GHG fluxes. Thus, complete analysis of GHG emissions from cropping systems in central Texas necessitates accounting for both growing season and fallow season emissions.

Cumulative annual analysis of CO<sub>2</sub> emissions revealed less difference among treatments than for growing season emissions, with no differences between any of the 8 individual treatments being found at the 0.05 level of significance (Table 5). The moderate drought during the summer of 2010 and the continuing lack of moisture in the fall of 2010 through the winter of 2011 may have contributed to a lack of sustained GHG fluxes through the 2010 fallow. The lack of soil moisture probably limited organic matter decomposition. Overall, the treatments were similar to one another, with only a slight difference ( $P = 0.065$ ) in annual CO<sub>2</sub> emissions from any of the cropping treatments analyzed in 2010. The SS sequence contained the only possible difference (assuming  $P = 0.065$  is significant), where [SS, - N, 0%R] showed significantly less CO<sub>2</sub> loss than the other three SS treatments (Fig. 11). Sorghum-sorghum treatment combinations usually lost more CO<sub>2</sub> during 2010, but the treatment receiving neither N fertilization nor residue return generally had the lowest emissions. Residue return had a significant ( $P = 0.047$ ) impact on CO<sub>2</sub> losses during 2010 (Table 5), with 50%R increasing losses of CO<sub>2</sub> 13% compared to treatments receiving no residue return. The effect of residue return on CO<sub>2</sub> emissions was anticipated, since the greater amount of organic C applied to the soil in these treatments led to greater microbial biomass (Chapter III) and potentially greater CO<sub>2</sub> fluxes via microbial decomposition. A rotation by residue interaction ( $P = 0.024$ ) was also observed in CO<sub>2</sub> emissions in 2010. Regardless of N fertilization, SS treatments with 50%R had 22% higher losses of CO<sub>2</sub> than CS, 50%R. This may be partially explained by the difference in mass of residue applied to the 50%R plots from the 2009 harvest; the CS rotation had higher biomass sorghum yield in 2009 and subsequently more residue returned.

Table 5. Summary of p-values from tests of difference between individual treatments and factorial analyses from 2010, 2011, and two-year average.

Statistic or effect	CO <sub>2</sub> -C		N <sub>2</sub> O-N		CO <sub>2</sub> -eq	Yield-scaled GHG
	GS	YR	GS	YR		
			<i>p-value</i>			
			<u>2010</u>			
Individual differences	<b>0.009</b>	0.065	<b>0.022</b>	0.058	<b>0.009</b>	< <b>0.001</b>
<i>Factorial</i>						
Rotation	<b>0.004</b>	0.236	0.624	0.410	0.174	<b>0.001</b>
N-Fert.	0.137	0.303	<b>0.002</b>	<b>0.013</b>	<b>0.018</b>	<b>0.002</b>
Residue	<b>0.014</b>	<b>0.047</b>	0.303	0.312	<b>0.043</b>	<b>0.023</b>
Rotation*N-Fert.	0.884	0.329	0.691	0.822	0.407	<b>0.007</b>
Rotation*Residue	<b>0.026</b>	<b>0.024</b>	<b>0.012</b>	<b>0.014</b>	<b>0.003</b>	< <b>0.001</b>
N-Fert.*Residue	0.527	0.251	0.361	0.426	0.188	0.205
Rotation*N-Fert.*Residue	0.266	0.250	0.993	0.966	0.405	0.207
			<u>2011</u>			
Individual differences	<b>0.001</b>	<b>0.010</b>	0.081	0.223	<b>0.013</b>	< <b>0.001</b>
<i>Factorial</i>						
Rotation	<b>0.018</b>	0.589	0.078	0.491	0.321	< <b>0.001</b>
N-Fert.	< <b>0.001</b>	<b>0.013</b>	0.070	<b>0.027</b>	<b>0.001</b>	0.277
Residue	0.575	<b>0.020</b>	0.820	0.309	0.396	< <b>0.001</b>
Rotation*N-Fert.	0.409	0.820	0.172	0.304	0.278	0.848
Rotation*Residue	0.352	0.101	0.868	0.758	0.153	0.168
N-Fert.*Residue	0.232	0.431	<b>0.020</b>	0.273	0.132	0.197
Rotation*N-Fert.*Residue	<b>0.025</b>	<b>0.005</b>	0.859	0.397	0.152	0.462

Table 5 continued

Statistic or effect	CO <sub>2</sub> -C		N <sub>2</sub> O-N		CO <sub>2</sub> -eq	Yield-scaled GHG
	GS	YR	GS	YR		
			<i>p-value</i>			
			<u>2-yr. avg.</u>			
Individual differences	<b>0.001</b>	<b>0.020</b>	<b>0.015</b>	<b>0.048</b>	<b>0.003</b>	<b>&lt;0.001</b>
<i>Factorial</i>						
Rotation	<b>&lt;0.001</b>	0.197	0.107	0.289	<i>0.084</i>	<b>0.031</b>
N-Fert.	<b>&lt;0.001</b>	<b>0.015</b>	<b>0.002</b>	<b>0.002</b>	<b>&lt;0.001</b>	<b>0.002</b>
Residue	<b>0.020</b>	<b>0.004</b>	0.383	0.927	<b>0.031</b>	<b>0.009</b>
Rotation*N-Fert.	0.737	0.354	0.207	0.354	0.166	<b>0.001</b>
Rotation*Residue	0.149	0.424	<i>0.053</i>	0.124	<i>0.075</i>	<b>&lt;0.001</b>
N-Fert.*Residue	0.884	0.696	0.229	0.761	0.979	0.144
Rotation*N-Fert.*Residue	0.685	0.220	0.893	0.503	0.738	<i>0.082</i>

Treatment effects included crop rotation (Rotation), N fertilization (N-Fert.), and residue return (Residue)

Values in bold significant at 0.05 level.

Values in italics significant at 0.10 level.

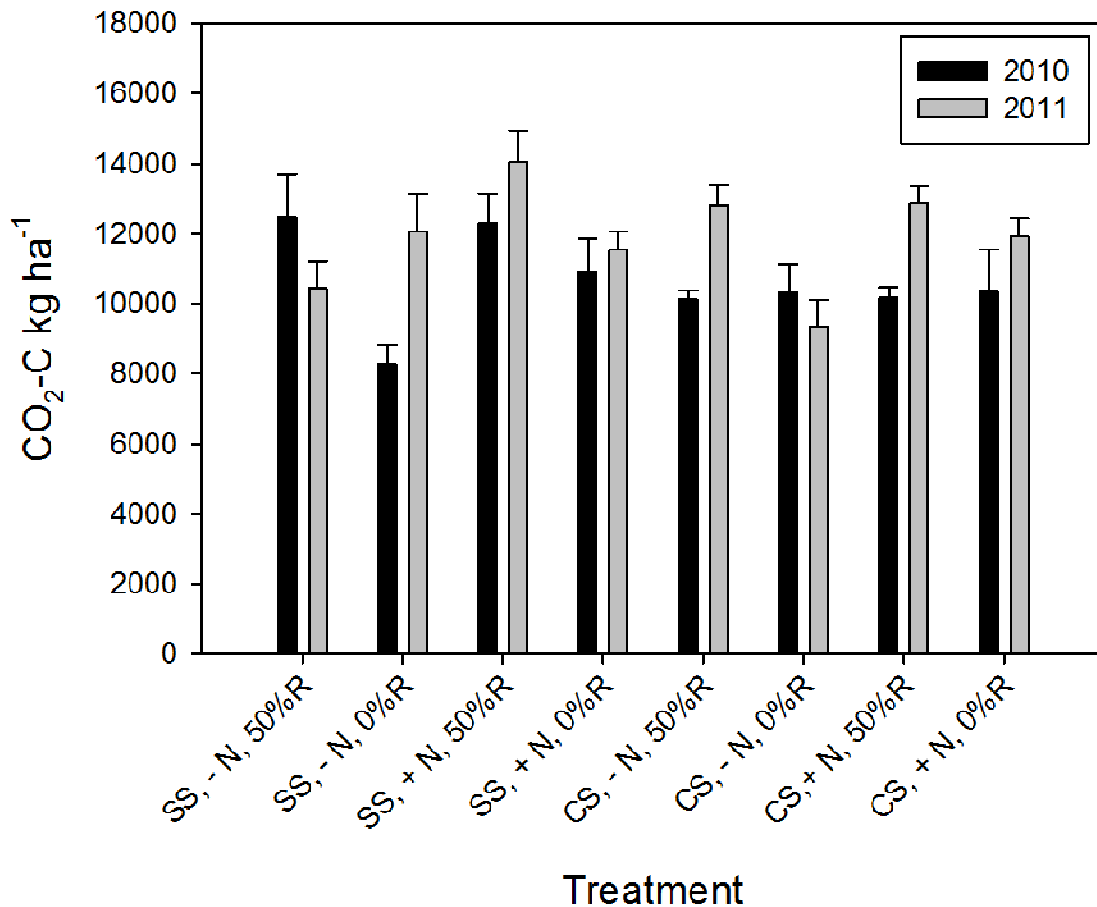


Figure 11. Mean cumulative annual CO<sub>2</sub> emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).

The entire field was planted to sorghum in 2011 and it was anticipated that less difference in annual CO<sub>2</sub> loss between treatments would be observed in 2011 than in 2010. However, there were significant differences (P = 0.010) (Table 5) which were due to a number of factors, including differences in yield/plant productivity within each cropping system, amounts and composition of residue returned to the soil, or potential differences in soil physical properties between the treatments. The [SS, + N, 50%R] treatment continued to have some of the highest cumulative CO<sub>2</sub> emissions in 2011 (approximately 14,000 kg CO<sub>2</sub>-



C ha<sup>-1</sup>), while some of the lowest emissions of CO<sub>2</sub> were observed in the [CS, - N, 50%R] treatment (approximately 9,000 kg CO<sub>2</sub>-C ha<sup>-1</sup>) (Fig. 11). Nitrogen fertilization had a significant (P = 0.013) effect on annual CO<sub>2</sub> emissions in 2011 (Table 5). Fertilized plots lost nearly 13% more CO<sub>2</sub> than unfertilized plots, likely due to greater soil N availability and less immobilization, and greater soil organic matter (SOM) decomposition. Residue return also significantly (P = 0.020) influenced cumulative annual CO<sub>2</sub> emissions in 2011, where 50%R had approximately 12% higher CO<sub>2</sub> emissions than 0%R (Fig. 11). The lower amount of organic matter available for decomposition in 0%R treatments likely contributed to lower cumulative CO<sub>2</sub> emissions. A three-way rotation by N fertilization by residue return effect (P = 0.005) was observed for cumulative annual CO<sub>2</sub> emissions in 2011 as well.

Cumulative annual emissions of N<sub>2</sub>O in 2010 were not different between the eight individual treatments at the 0.05 level of significance, but were at a slightly higher, 0.058 level of significance (Table 5). The [SS, + N, 50%R] treatment had the highest cumulative annual N<sub>2</sub>O emission, nearly 13 kg N<sub>2</sub>O-N ha<sup>-1</sup>, while the [SS, - N, 0%R] treatment had the lowest with only 4 kg N<sub>2</sub>O-N ha<sup>-1</sup> (Fig. 12) While the + N treatments were not significantly different from each other, the SS, - N treatments were different from each other. The [SS, - N, 50%R] treatment resulted in significantly larger cumulative annual N<sub>2</sub>O loss compared with [SS, - N, 0%R], possibly due to returned biomass supplying greater organic N which provided additional N over time for mineralization or additional organic C which may have contributed to increased denitrification. Soil moisture, however, likely limited potential denitrification. Nitrogen fertilization was found to be a major controlling factor (P = 0.013), affecting cumulative annual N<sub>2</sub>O emissions in 2010 (Table 5). Fertilized treatments had 53% higher cumulative annual N<sub>2</sub>O emission than treatments that were unfertilized (Fig. 12). A

rotation by residue return interaction ( $p = 0.014$ ) was also observed in 2010, where, regardless of N fertilization, the SS, 50%R treatments had 66% higher cumulative  $N_2O$  emissions than the CS, 50% treatments. The CS treatments receiving 50%R were supplied with a larger mass of sorghum biomass returned to the soil from the 2009 harvest. However, the summation of OC returned from 2008 and 2009 was relatively similar between the SS and CS treatments (12,384 and 11,668 kg C ha<sup>-1</sup>, respectively) because biomass sorghum yield gains by CS treatments in 2009 were somewhat negated by lower total OC applied from corn in 2008. However, the greater amount of biomass from 2009 may have caused net soil N immobilization for a longer period of time than SS treatments, resulting in a pool of slowly mineralizing organic N over time. The slow-release of organic N (via mineralization or organic residues and SOM) may have improved availability of N throughout the growing season, when crop needs were highest (Alvarez et al., 2011). Release of N slowly throughout the growing season can increase NUE and reduce cumulative  $N_2O$  emissions (Sistani et al., 2010; Van Groenigen et al., 2010; Venterea et al., 2011). Another potential reason for greater  $N_2O$  emissions in the SS, 50%R treatments may be related to the longer lifespan of the bioenergy sorghum relative to corn during the 2010 growing season. The bioenergy sorghum was photosynthetically-active longer and likely continued to supply soil microbes with labile C (via root exudates) longer than the corn crop which was no longer photosynthetically-active by late June 2010. This enhanced microbial activity within SS treatments may have enhanced residue degradation, mineralization, and nitrification or denitrification processes. The NEF from both SS and CS were generally higher in 2010 than in 2011. The SS treatments had an average NEF of 1.7%, while the CS rotation had an NEF of 2.5%. The higher NEF in the CS rotation may be due to corn uptake of N being less efficient than with

bioenergy sorghum and because bioenergy sorghum was actively extracting N for a longer period of time.

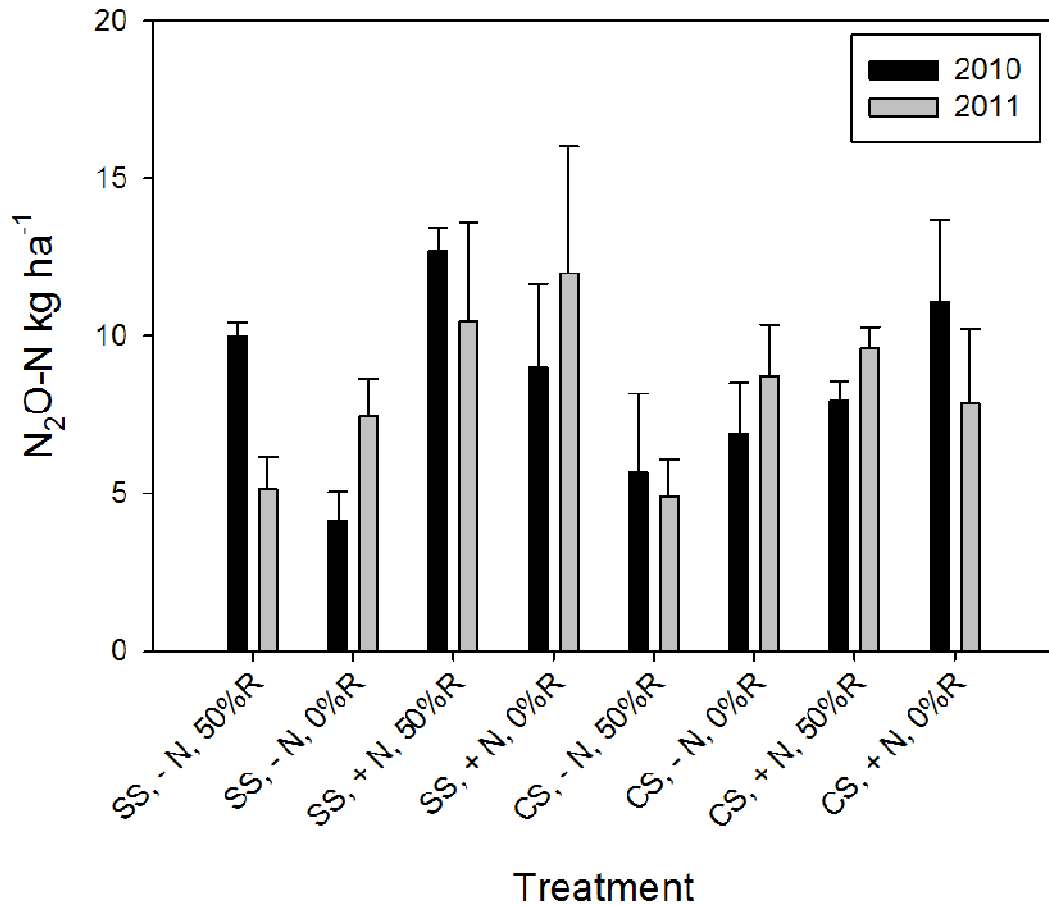


Figure 12. Mean cumulative annual N<sub>2</sub>O emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).

There was no significant difference ( $P = 0.223$ ) in cumulative annual N<sub>2</sub>O emissions from any of the treatments in 2011 (Table 5). The high variability in the 2011 N<sub>2</sub>O data may have limited detection of significant differences between treatments. Based on mean estimates, regardless of residue return rate, the SS, + N treatments had the highest average annual emission, losing over 10 kg N<sub>2</sub>O-N ha<sup>-1</sup> in 2011 (Fig. 12). In contrast, regardless of

crop sequence, the - N, 50%R treatments had relatively low cumulative N<sub>2</sub>O emissions with approximately 5 kg N<sub>2</sub>O-N ha<sup>-1</sup> lost in 2011. The only variable which significantly affected (P = 0.027) cumulative annual N<sub>2</sub>O emissions in 2011 was N fertilization. Fertilization results from 2011 were consistent with 2010 findings; fertilized treatments had 53% higher annual cumulative N<sub>2</sub>O emissions than unfertilized treatments (Fig. 12). The NEF from SS and CS in 2011 were 1.6% and -0.3%, respectively. The observed negative NEF was peculiar; review of the data revealed that the highly sporadic N<sub>2</sub>O fluxes measured during the 2011 drought made small fluxes of N<sub>2</sub>O from unfertilized plots play a larger role in NEF when fertilized plots had no detectable fluxes during the same sampling event. We believe that the variable and relatively low N<sub>2</sub>O fluxes associated with extreme drought conditions explain much of the negative NEF observed in CS treatments in 2011.

### **Cumulative CO<sub>2</sub>-equivalent Emissions**

To understand the net impact of bioenergy sorghum production under various production scenarios on overall global warming potential, CO<sub>2</sub> and N<sub>2</sub>O were converted to CO<sub>2</sub>-equivalents. This conversion particularly emphasizes the importance of N<sub>2</sub>O, which may have relatively low cumulative emissions, but high global warming potential (Smith et al., 2007). Analyses were performed to evaluate the effect of crop production scenarios on cumulative CO<sub>2</sub>-eq emissions in 2010, 2011, and an overall 2-year average.

The global warming potential (GWP) from the eight crop production scenarios differed significantly (P= 0.009) from one another in 2010 (Table 5). Similar to observations of cumulative CO<sub>2</sub> and N<sub>2</sub>O emissions in 2010, the SS, 50%R treatments were among the highest in cumulative emissions, while the lowest emissions were associated with [SS, - N,

0%R] (Fig. 13). Cumulative CO<sub>2</sub>-eq emissions ranged from approximately 10,000 kg to 18,000 kg CO<sub>2</sub>-eq ha<sup>-1</sup>. All of the + N treatments had relatively high cumulative CO<sub>2</sub>-eq emissions and [SS, - N, 50%R] had relatively high emission despite no fertilization. Factors which significantly influenced cumulative CO<sub>2</sub>-eq emissions in 2010 appeared similar to factors that influenced either CO<sub>2</sub> or N<sub>2</sub>O emissions or both. Nitrogen fertilization significantly (P = 0.018) increased cumulative CO<sub>2</sub>-eq emissions by 17% in 2010 (Table 5 and Fig. 13). Returning residue also significantly (P = 0.043) increased cumulative CO<sub>2</sub>-eq emissions by 14% (Table 5 and Fig. 13). The rotation by residue interaction observed for both CO<sub>2</sub> and N<sub>2</sub>O emissions was again found to be significant (P = 0.003) for cumulative CO<sub>2</sub>-eq emissions. Regardless of N fertilization, a 28% reduction in cumulative CO<sub>2</sub>-eq emissions was observed from the SS, 0%R treatments compared to the SS, 50%R treatments (Table 5 and Fig. 13), probably due to the large amount of residue returned, which likely stimulated microbial activity.

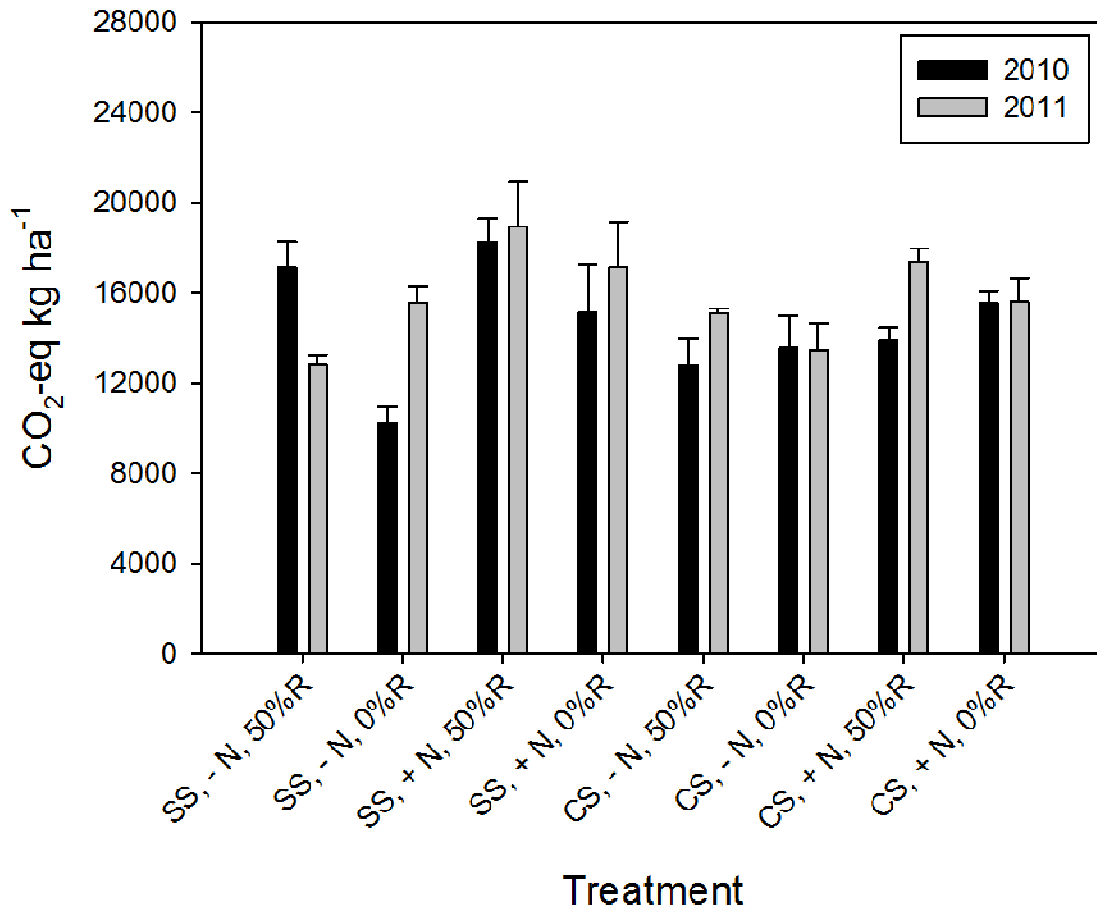


Figure 13. Mean cumulative annual CO<sub>2</sub>-eq emissions from 2010 and 2011 from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).

Despite no overall significant treatment differences for cumulative N<sub>2</sub>O emissions in 2011, cumulative CO<sub>2</sub>-eq emissions were significantly different (P = 0.013) (Table 5). In general, cumulative annual CO<sub>2</sub>-eq emissions were higher in 2011 compared to 2010. The highest cumulative emission in 2011 reached nearly 19,000 kg CO<sub>2</sub>-eq ha<sup>-1</sup> while the lowest was approximately 13,000 kg CO<sub>2</sub>-eq ha<sup>-1</sup> (Fig. 13). Although not significantly larger than all other treatments, the + N, 50%R treatments from both crop rotations were among the highest in cumulative CO<sub>2</sub>-eq emissions. In contrast, [SS, - N, 50%R] and [CS, - N, 0%R] were among the treatments with the lowest cumulative CO<sub>2</sub>-eq emissions in 2011. Similar to

observations found in both cumulative CO<sub>2</sub> and N<sub>2</sub>O emissions, N fertilization significantly (P = 0.001) affected the GWP from crop production scenarios in 2011 (Table 5). Treatments receiving N fertilizer had 21% higher cumulative CO<sub>2</sub>-eq emissions than unfertilized treatments (Fig. 13).

To assess the impact of agronomic practices on cumulative CO<sub>2</sub>-eq emissions across both years, the average for each plot was calculated and analyzed. Across both years, significant differences (P = 0.003) in cumulative CO<sub>2</sub>-eq were observed among the eight bioenergy sorghum production scenarios (Table 5). One treatment, [SS, + N, 50%R], had significantly higher GWP than any other treatment, averaging over 18,500 kg CO<sub>2</sub>-eq ha<sup>-1</sup> annually (Fig. 14). This treatment was consistently one of the highest emitters of both CO<sub>2</sub> and N<sub>2</sub>O. In contrast, [SS, - N, 0%R] had relatively low GWP, accounting for less than 13,000 kg CO<sub>2</sub>-eq ha<sup>-1</sup> each year. In general, + N treatments had higher average annual cumulative CO<sub>2</sub>-eq than - N treatments, and among the fertilized treatments, the SS treatments were higher than the CS treatments. In contrast, SS and CS plots receiving neither N fertilizer nor any residue returned generally had lower cumulative CO<sub>2</sub>-eq emissions across 2010 and 2011 (Fig. 14). Nitrogen fertilization significantly (P = <0.001) increased cumulative CO<sub>2</sub>-eq emissions (Table 5); fertilized treatments had 19% higher cumulative CO<sub>2</sub>-eq emissions annually compared to unfertilized treatments. Residue return also significantly (P = 0.031) increased the average annual cumulative CO<sub>2</sub>-eq emissions (Table 5); 50%R treatments lost 9% more average annual cumulative CO<sub>2</sub>-eq than 0%R treatments (Fig. 14). These findings suggest that N fertilization increased N availability for gaseous-N loss processes and residue return supplied greater organic C which likely enhanced microbial activity or organic N that could also influence soil N processes.

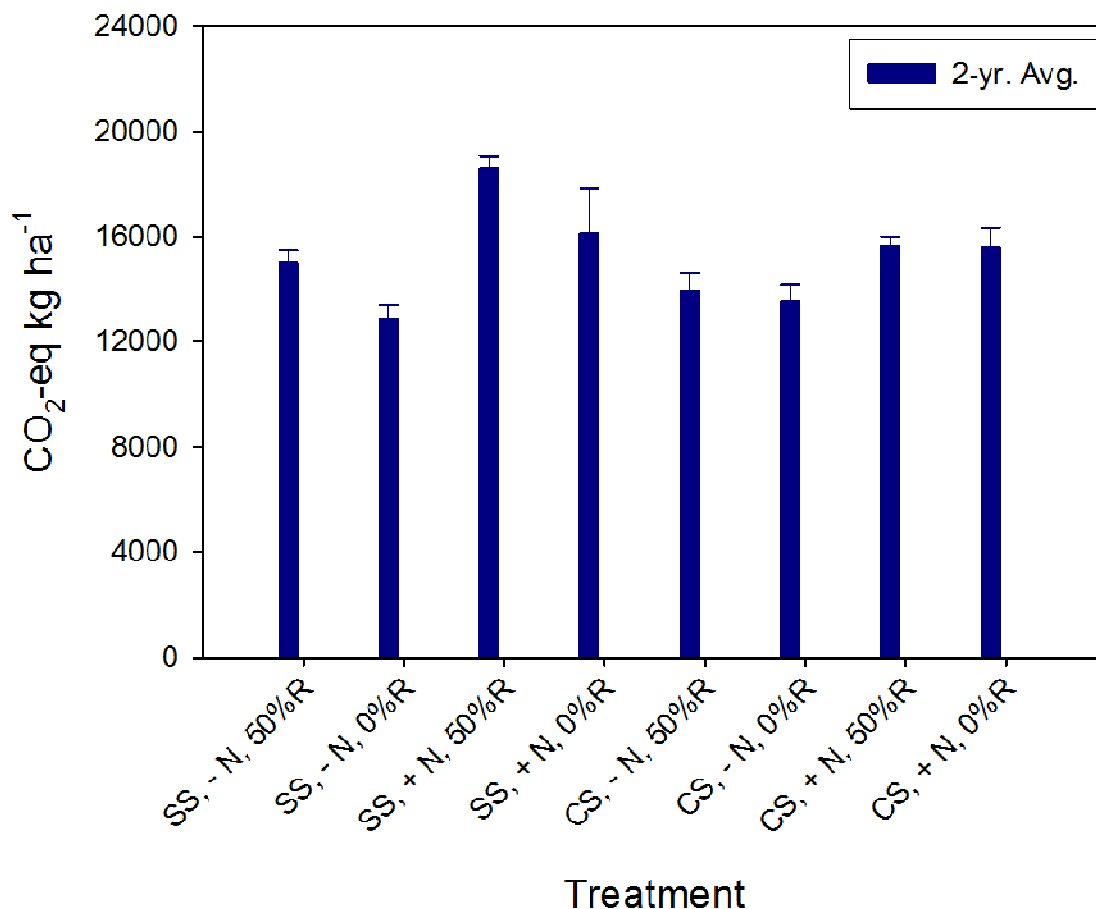


Figure 14. Mean cumulative annual CO<sub>2</sub>-eq emissions of the two-year average from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).

### Yield-scaled Greenhouse Gas Emissions

Determining GHG emissions on a yield-scaled basis greatly increased the number and significance of differences compared to other characterizations (Table 5). The eight bioenergy sorghum production treatments examined were different ( $P = <0.001$ ) from one another in 2010 based upon the yield-scaled GHG emissions. The [CS, - N, 0%R] treatment was the least efficient bioenergy production scenario, losing over 25 kg CO<sub>2</sub>-eq for each potential liter of ethanol produced (Fig. 15). Both the SS treatments with 0%R exhibited the most efficient yield-scaled production in 2010, losing less than 4 kg CO<sub>2</sub>-eq for each liter of



ethanol. Crop rotation also significantly ( $P = 0.001$ ) impacted the efficiency of bioenergy sorghum production in 2010. SS reduced  $\text{CO}_2\text{-eq}$  emissions by 36% for each theoretical liter of ethanol produced (Fig. 15). Nitrogen fertilization significantly ( $P = 0.002$ ) improved the yield-scaled GHG emissions by 43% compared to unfertilized treatments, largely through increasing yield. Residue return, in contrast, significantly ( $P = 0.007$ ) decreased yield-scaled GHG production efficiency. A 10% reduction in yield-scaled GHG efficiency associated with 50%R treatments was attributed to biomass being returned to plots as an organic amendment, and subsequently reducing the overall yield available for biofuel production (Fig. 15). It is interesting to note that only a 10%, not a 50%, reduction was observed with 50%R. A significant ( $P = 0.022$ ) interaction between crop rotation and nitrogen fertilization was observed in 2010 because, regardless of residue return rate, CS, + N treatments had 61% increased efficiency compared to CS, - N treatments. An additional interaction ( $P = <0.001$ ) was observed between crop rotation and residue return, where regardless of N fertilization rate, the SS, 0%R treatments significantly increased production efficiency by more than 70% compared to SS, 50%R treatments (Table 5 and Fig. 15). The rotation by residue interaction in 2010 was likely due to corn being grown in the CS rotation where only corn grain was considered for total ethanol production yield, as opposed to SS rotations where residue return resulted in a significant portion of biomass yield being returned to the soil.

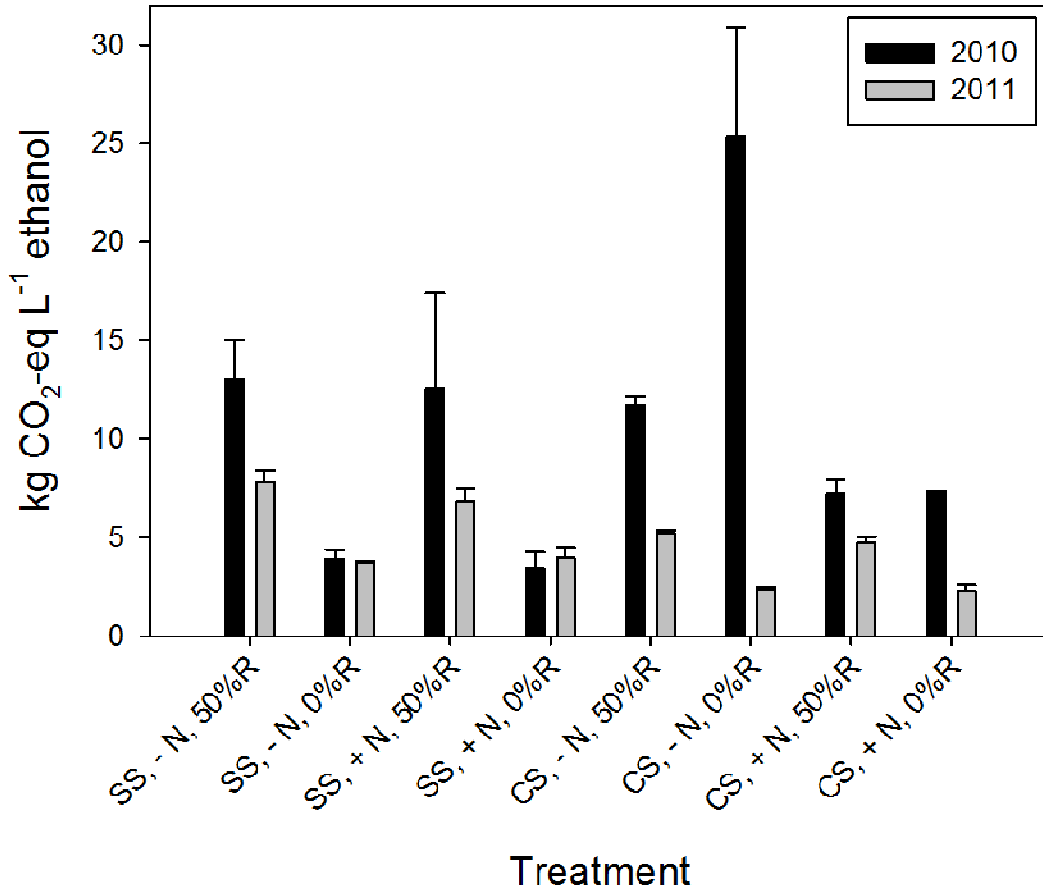


Figure 15. Mean yield-scaled GHG emissions from 2010 and 2011 from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Theoretical ethanol estimated from measured yield and NREL-provided conversion values.

In 2011, the entire study was in the sorghum sequence of the crop rotation and significant ( $P = <0.001$ ) differences between production scenarios were again found, but fewer factors were significant (Table 5). Both SS, 50%R treatments were the least efficient at producing biofuel while minimizing GHG emissions in 2011 and emitted over 6 kg CO<sub>2</sub>-eq per liter of fuel (Fig. 15). Regardless of N fertilization, the CS, 0%R treatments were the most efficient at sustainably producing biofuel, losing just over 2 kg CO<sub>2</sub>-eq per liter of fuel. Yield-scaled GHG emissions were significantly ( $P = <0.001$ ) impacted by crop rotation in 2011. The SS sequence reduced efficiency by 34% compared to the CS rotation (Fig. 15).

The CS rotation had significantly ( $P = <0.001$ ) higher sorghum biomass yield, accounting for a portion of the increased overall efficiency in 2011. Similar to our findings in 2010, the 50%R treatments were significantly ( $P = <0.001$ ) less efficient at producing biofuel while minimizing GHG emissions. Treatments returning residue to the soil were 50% less efficient than treatments where all yield was used for fuel conversion.

Across two years of study, the average yield-scaled GHG emissions from the bioenergy sorghum production treatments were significantly ( $P = 0.001$ ) different (Table 5). The various treatments generally fell into two groups of more or less efficient biofuel production scenarios. Both the SS, 50%R treatments and the [CS, - N, 0%R] treatment were among the poorest in terms of overall sustainable biofuel production efficiency, while both SS, 0%R treatments were among the best performers (Fig. 16). The two-year average for the most efficient treatments ranged from 3 to 6 kg CO<sub>2</sub>-eq per liter of fuel whereas the worst performers lost 8 to 14 kg CO<sub>2</sub>-eq per liter of fuel. The SS crop sequence significantly ( $P = 0.031$ ) improved yield-scaled GHG emissions, curbing emissions by 17% (Fig. 16). Nitrogen fertilization significantly ( $P = 0.002$ ) increased the average biofuel production efficiency. Fertilized treatments increased efficiency by nearly 34% over unfertilized. As anticipated, residue return significantly ( $P = <0.001$ ) impacted efficiency due to differences in biomass available for fuel conversion. Complete biomass removal decreased yield-scaled GHG emissions by 24% compared to treatments returning half of the residue (Fig. 16). A crop rotation by N fertilization rate interaction ( $P = 0.001$ ) was observed and regardless of residue return rate, CS, + N was greater than 50% more efficient than CS, - N (largely attributed to yield reductions observed in - N). An additional interaction ( $P = <0.001$ ) between crop

rotation and residue return rate was found, in which regardless of N fertilization rate, SS, 0%R had nearly 63% lower yield-scaled GHG emissions than SS, 50%R (Fig.9b).

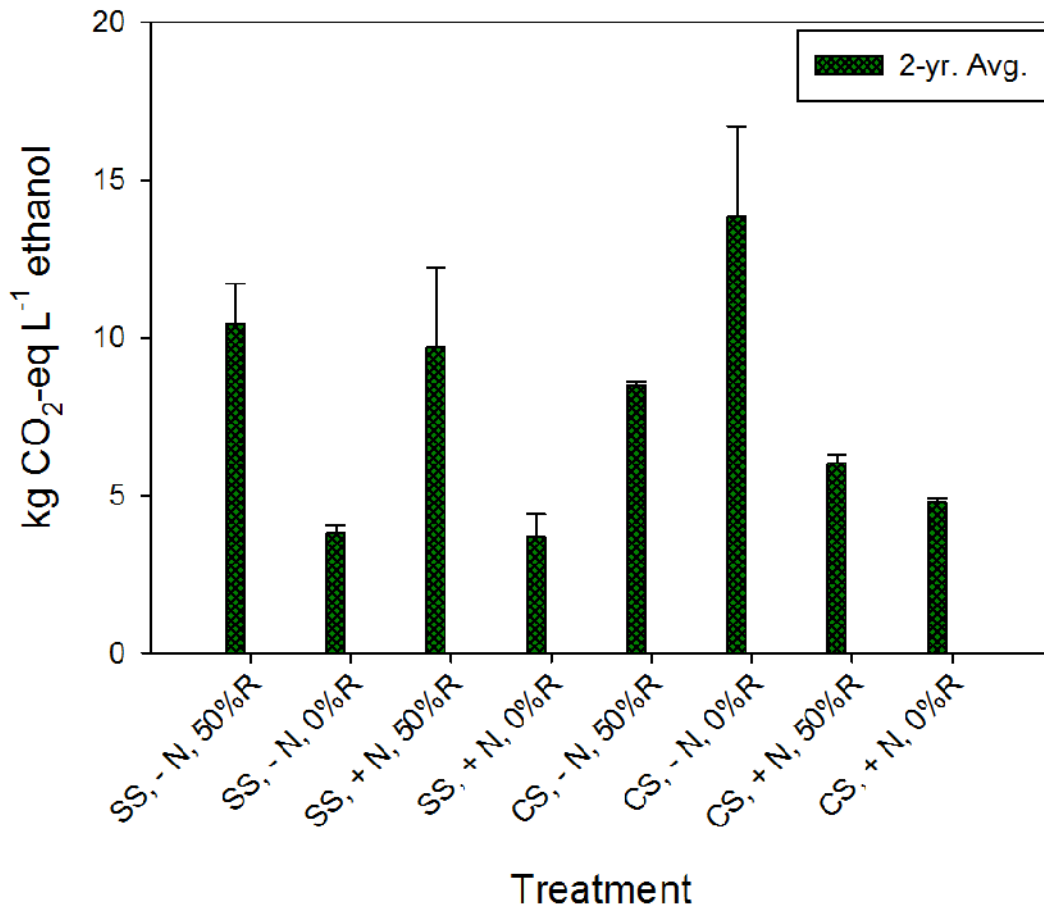


Figure 16. Mean yield-scaled GHG emissions of the two-year average from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Theoretical ethanol estimated from measured yield and NREL-provided conversion values.

## DISCUSSION

Bioenergy crops are produced with the intent of providing renewable biofuel feedstock, while simultaneously minimizing net GHG emissions during production. While most yield-scaled emissions studies have attempted to identify management practices which

minimize GHG emissions while meeting growing worldwide demands for food (Grassini and Cassman, 2012; Johnson et al., 2012; Linqvist et al., 2012; Van Groenigen et al., 2010; Venterea et al., 2011), this study employed the technique to evaluate biofuel production scenarios to potentially meet the growing demand for domestic biofuels. The aim of this study was to analyze the effects of three primary agronomic management practices under eight unique production scenarios in order to identify suitable practices which can maximize biofuel production potential while minimizing impacts of GHG emissions. Crop rotation, N fertilization, and residue return are all important agronomic management practices that potentially impact nutrient cycling and GHG emissions, and that may either beneficially or detrimentally influence sustainable biofuel production.

### **Growing Season vs. Annual Analysis**

Analysis of cumulative GHG emissions was performed for the sum of both on a growing season and annual basis. Most of the agronomic management practices found to significantly affect GHG emissions during the growing season remained dominant through the entire year. However, some differences were found based on the accounting technique, which illustrates the necessity to perform full year analysis when striving to identify cropping system treatments which minimize overall GHG emissions. One particularly interesting discrepancy between each summation was the identification of crop rotation as a significant effect on cumulative growing season emissions of CO<sub>2</sub> in 2010 and 2011, but not on cumulative annual emissions of CO<sub>2</sub> either year. This may be due to inherent differences in yield and the crop itself which directly impact CO<sub>2</sub> emissions via plant respiration. Particularly in 2010, when the CS rotation was in the corn sequence, we hypothesized that

the distinctly different crops would cause variation in CO<sub>2</sub> flux rates. In 2011, when all plots were planted to sorghum, differences in crop yield between the SS and CS rotation may have also accounted for differences in cumulative growing season CO<sub>2</sub> emissions. The lack of a crop rotation effect in the full year analysis suggested that treatments with lower fluxes in the growing season may have exhibited increased soil respiration during fallow. Subtraction of cumulative growing season CO<sub>2</sub> emissions from cumulative annual CO<sub>2</sub> emissions from each plot provided an estimate of emissions from the fallow season. Analysis revealed that CS rotation treatments had higher CO<sub>2</sub> emissions than SS treatments during the fallow season, but differences attributable to crop rotation were not significant in 2010 ( $P = 0.123$ ) or 2011 ( $P = 0.237$ ) (data not shown). It is possible that the recalcitrant nature of some belowground biomass from crop roots required time for microbial decomposition (partially explaining a delay in CO<sub>2</sub> evolution). Residue return did not significantly impact growing season CO<sub>2</sub> emissions in 2011, but did impact annual emissions (Figs. 9 and 11). The extreme drought during the 2010 fallow season and 2011 growing season may have limited microbial activity and decomposition of plant residues in the soil. Residue decomposition was likely enhanced during the 2011 fallow season due to increased precipitation, and may explain the significance of annual CO<sub>2</sub> emissions caused by residue return. Finally, the interaction of N fertilization by residue return on N<sub>2</sub>O emissions was significant for N<sub>2</sub>O emissions during the 2011 growing season, but was not a factor in 2011 annual N<sub>2</sub>O emissions (Table 5 and Figs. 10 and 12). This result may be partially due to the distinctly different amounts and type of residue returned to the soil by 50%R from the 2010 harvest. All corn residue was returned to the soil and was a much smaller amount of biomass returned compared to sorghum residue from the 2010 harvest. Furthermore, potential differences in chemical composition between

corn and sorghum biomass residue (Table 4) may have impacted the decomposition rate and interacted with N fertilization in 2011 to cause differences in gaseous N loss.

### **Carbon Dioxide Emissions**

Overall, trace gas emissions from our study were higher than those observed by many other studies across North America albeit little research has been performed in the southern U.S. (Franzluebbers, 2005) and little data exists on GHG emissions from bioenergy sorghum. Emissions of CO<sub>2</sub> in this study do not necessarily represent those going directly to the atmosphere because measurements taken at the soil surface represent soil respiration (microbial and root respiration) and do not account for CO<sub>2</sub> that may have been taken up by plants before exiting the plant canopy. Cumulative annual CO<sub>2</sub> emissions in this study averaged between 10,600 and 11,900 kg CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup> in 2010 and 2011, respectively. These values are on the higher side of the range of cumulative CO<sub>2</sub> emissions (2,300 to 10,500 kg CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>) reported from other cropping systems (Adviento-Borbe et al., 2007; Alvarez et al., 1995; Heller et al., 2010; Hernandez-Ramirez et al., 2009; Ussiri and Lal, 2009), but do not appear unrealistic considering the humid subtropical climate associated with the study location. Furthermore, bioenergy sorghum remains in a vegetative stage until day length is shorter than approximately 12 hours (Tamang et al., 2011), giving bioenergy sorghum a longer life cycle than most conventional agricultural crops. Cumulative CO<sub>2</sub> emissions in 2011 were significantly (P=0.016) larger than emissions from 2010 (Fig. 11). The higher temperatures throughout 2011 may partially explain this observation in addition to a mild, wet fallow season.

The highest CO<sub>2</sub> fluxes (Figs. 17 and 18) were generally observed during the growing season, particularly after fertilization and after precipitation/irrigation events. Increased soil respiration following N fertilization has been frequently observed in other cropping systems (Alluvione et al., 2010; Fernandes Cruvinel et al., 2011; Ussiri and Lal, 2009). We suspect the physical disturbance of soil via knifing of urea to 10-cm depth, increased plant growth, as well as enhanced soil organic N mineralization via additional N availability may have been contributing factors. Enhanced soil respiration following irrigation/precipitation events have also been observed in a number of studies (Adviento-Borbe et al., 2007; Adviento-Borbe et al., 2010; Hernandez-Ramirez et al., 2009) and was observed particularly during the growing season in our study (Figs. 17 and 18). Wetting and drying cycles during the growing season likely enhanced SOM mineralization and re-wetting provided sufficient moisture to increase heterotrophic microbial activity, enhancing decomposition (Wolf and Wagner, 2005; Xu and Luo, 2012).



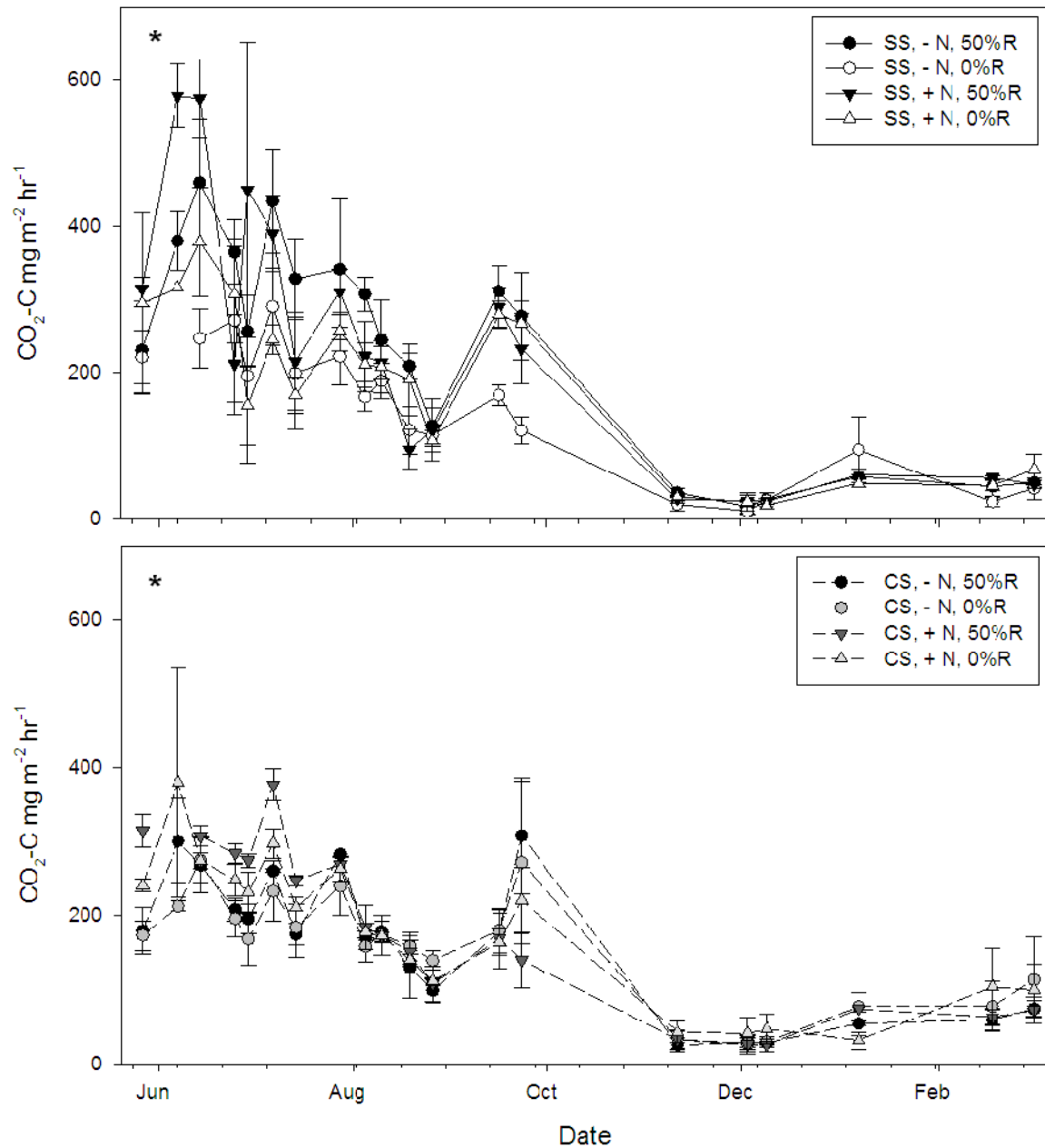


Figure 17. Mean soil gas fluxes of CO<sub>2</sub> from 2010 sampling seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Asterisk denotes irrigation date.

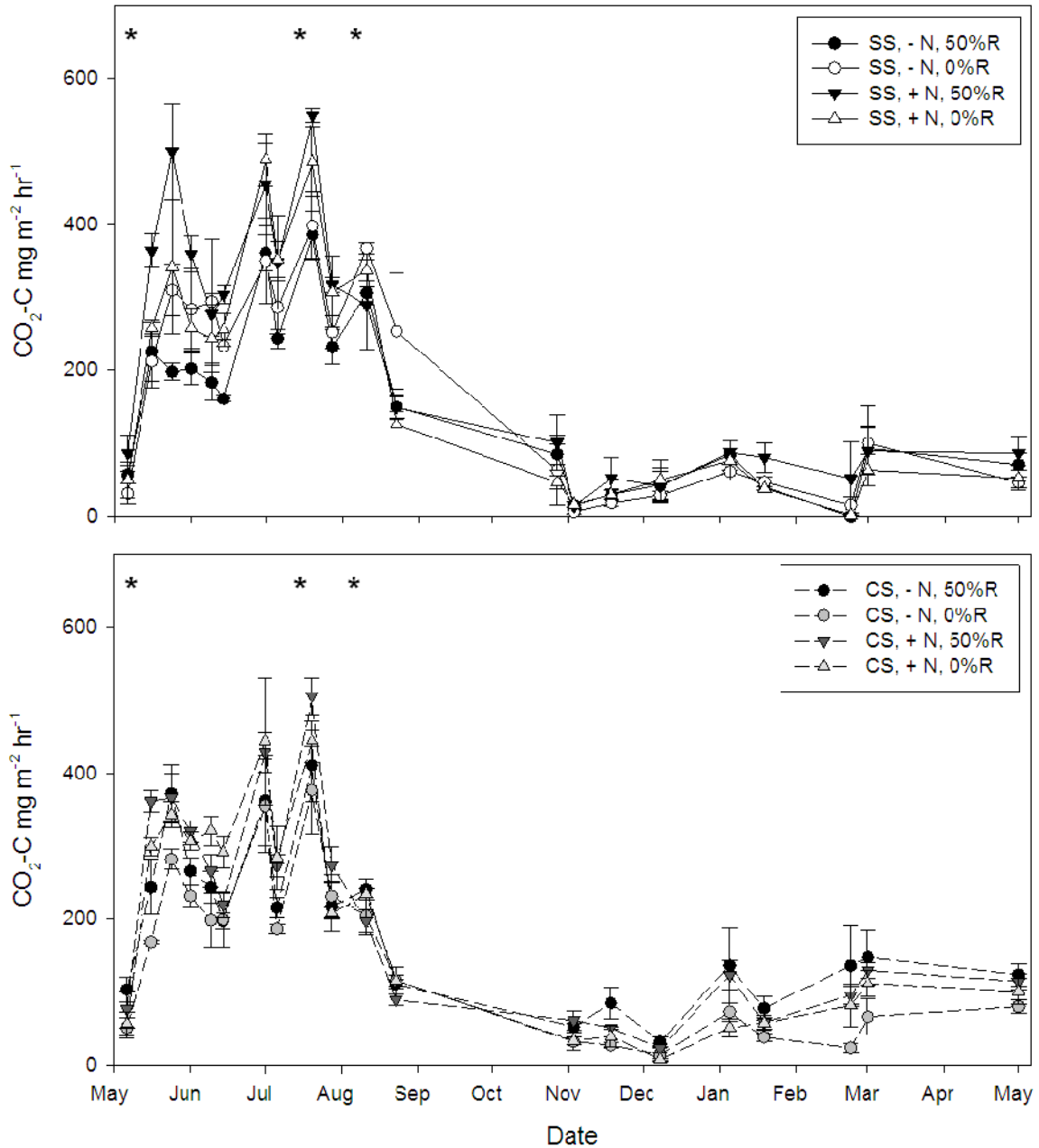


Figure 18. Mean soil gas fluxes of CO<sub>2</sub> from 2011 sampling seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Asterisk denotes irrigation date.

Climatic factors at our location probably also contributed to higher than average cumulative CO<sub>2</sub> emissions. Numerous studies cite air (Adviento-Borbe et al., 2007; Adviento-Borbe et al., 2010; Almaraz et al., 2009; Ussiri and Lal, 2009) and soil (Adviento-

Borbe et al., 2007; Adviento-Borbe et al., 2010; Allaire et al., 2012; Hernandez-Ramirez et al., 2009; Liebig et al., 2010; Ussiri and Lal, 2009) temperature as drivers of soil respiration rates. Microbial activity increases with increasing temperature which directly influences soil respiration. Soil and air temperatures throughout the growing season frequently exceeded 30°C (Figs. 1 and 2), and mean annual temperatures in 2010 and 2011 were 21.8 and 22.6°C, respectively. These observations are higher than the temperatures reported in many other studies of soil respiration (Adviento-Borbe et al., 2007; Adviento-Borbe et al., 2010; Allaire et al., 2012; Almaraz et al., 2009; Hernandez-Ramirez et al., 2009; Liebig et al., 2010; Ussiri and Lal, 2009) and were generally sustained for a longer period of time. Lower flux rates during the fallow season were likely related to the lack of autotrophic respiration as well as lower temperatures which reduced both autotrophic and heterotrophic microbial activity. Mean monthly temperatures during the fallow months rarely exceeded 20°C (Fig. 1).

Cumulative respiration differences between treatments were likely due to distinct differences in both autotrophic (plant root) and heterotrophic (soil microbe) respiration. Similar to Adviento-Borbe et al. (2010), we hypothesized that treatments exhibiting higher cumulative CO<sub>2</sub> would correspond to higher crop yield, due to increased photosynthetic activity. However, the highest yielding treatments did not directly correspond to those treatments with the highest respiration values, suggesting that autotrophic, or plant root, respiration was not the primary cause of differences in CO<sub>2</sub> emissions among treatments (Adviento-Borbe et al., 2010). However, during the 2010 growing season, CO<sub>2</sub> flux rates in SS remained highly variable within treatments until harvest, unlike CS rotations with corn that had lower fluxes late in the summer characterized by relatively low treatment variation (Fig. 5). Thus, while no distinct association between autotrophic, or root, respiration and

cumulative annual respiration values was observed, we suggest that respiration flux rates from individual sampling events throughout the growing season are a better indication of treatment differences attributable to autotrophic respiration.

### **Nitrous Oxide Emissions**

Nitrous oxide may be the more important GHG measured in our study. Unlike CO<sub>2</sub>, no portion of this soil gas flux will be taken up by the vegetation through photosynthesis. Nitrous oxide also represents a monetary loss in terms of potential N leaving the cropping system, rather than remaining as a valuable nutrient to enhance plant growth. Nitrous oxide also has almost 300 times the GWP of CO<sub>2</sub> (Smith et al., 2007). Thus, N<sub>2</sub>O emission remain a critical factor when evaluating bioenergy crop production.

Average annual cumulative emission of N<sub>2</sub>O observed across both years of this study was approximately 8 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>. In 2010, the annual range between treatments was 4 to 13 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> and in 2011 it was 5 to 12 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> (Fig. 12). Similar to CO<sub>2</sub>, measured N<sub>2</sub>O values were relatively high compared to several studies in the U.S., but fall within the range of cumulative N<sub>2</sub>O emissions observed by several studies within North America [0.6 – 15.4 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>] (Adviento-Borbe et al., 2007; Duxbury and McConnaughey, 1986; Hernandez-Ramirez et al., 2009; Johnson et al., 2012; Parkin and Kaspar, 2006; Venterea et al., 2011) and globally [0.3 – 40 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>] (Denmead et al., 2010; Rozas et al., 2001; Stehfest and Bouwman, 2006; Weier, 1998).

In general, substantial variation within the N<sub>2</sub>O flux measurements was observed. Growing season fluxes were particularly variable, ranging from no detectable flux to a maximum of approximately 2,100 mg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup> (Figs. 7 and 8), while the maximum

fallow season flux only reached 60 mg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup> (Figs. 19 and 20). Pelster et al. (2011) suggested high variation in N<sub>2</sub>O fluxes in some cropping systems may be attributable to localized soil microenvironments which may favor nitrification/denitrification based upon prevailing environmental conditions and available organic C. High variation may partially explain some of the lack of detectable differences from residue return or crop rotation in this study. Other studies have found similar flux differences early in the year with no detectable differences in annual cumulative emissions (Johnson et al., 2012). Furthermore, inter-annual variation may have been partially responsible for residue return interactions observed in 2010 and 2011, making it difficult to understand the magnitude of a possible management effect. A number of studies have found significant inter-annual variation in N<sub>2</sub>O emissions (Adviento-Borbe et al., 2007; Chantigny et al., 2010; Pelster et al., 2011), illustrating the difficulty in interpreting whether treatment effects are truly caused by a management effect or are simply inter-annual variation and environment variability.

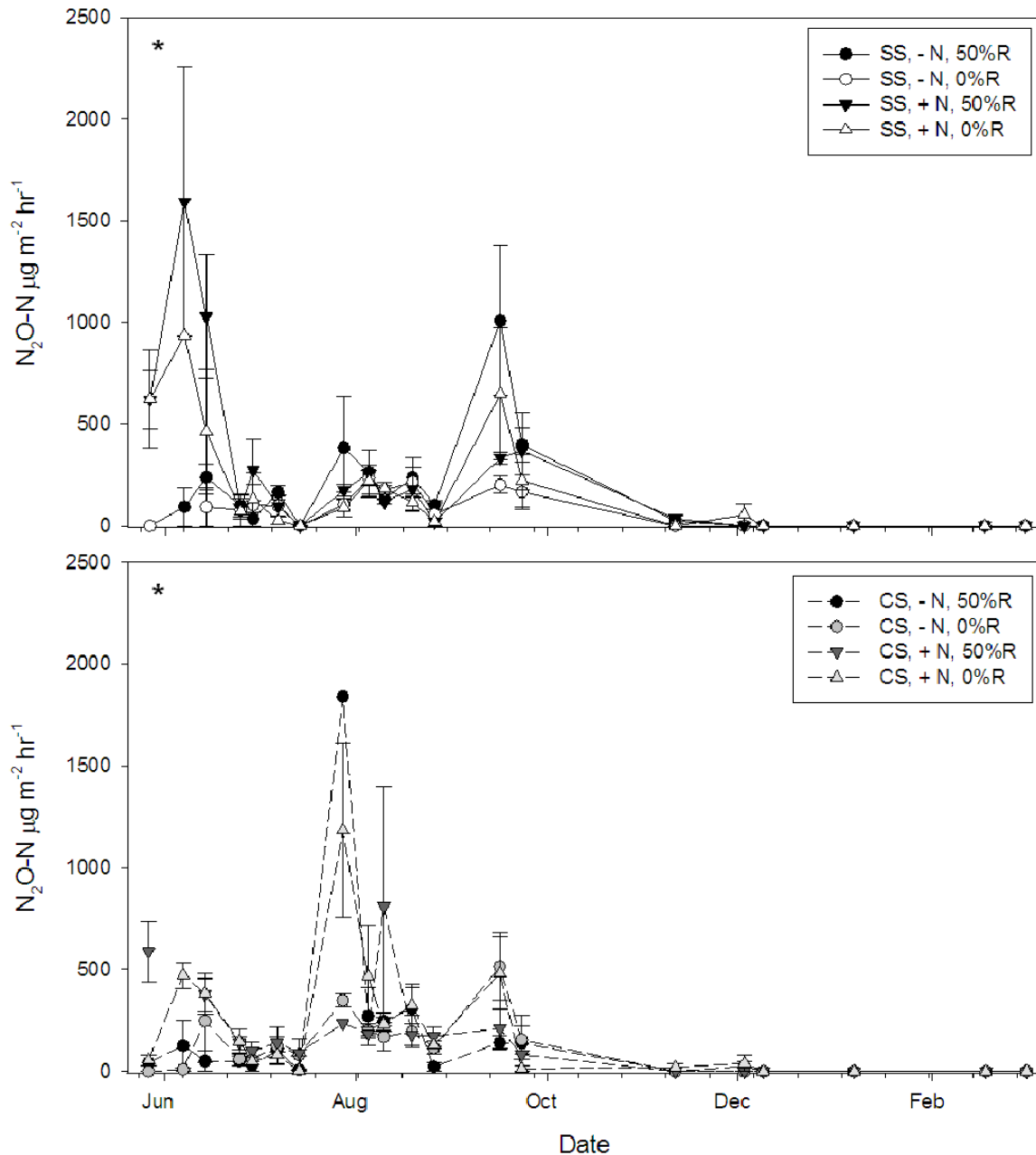


Figure 19. Mean soil gas fluxes of N<sub>2</sub>O from 2010 sampling seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Asterisk denotes irrigation date.

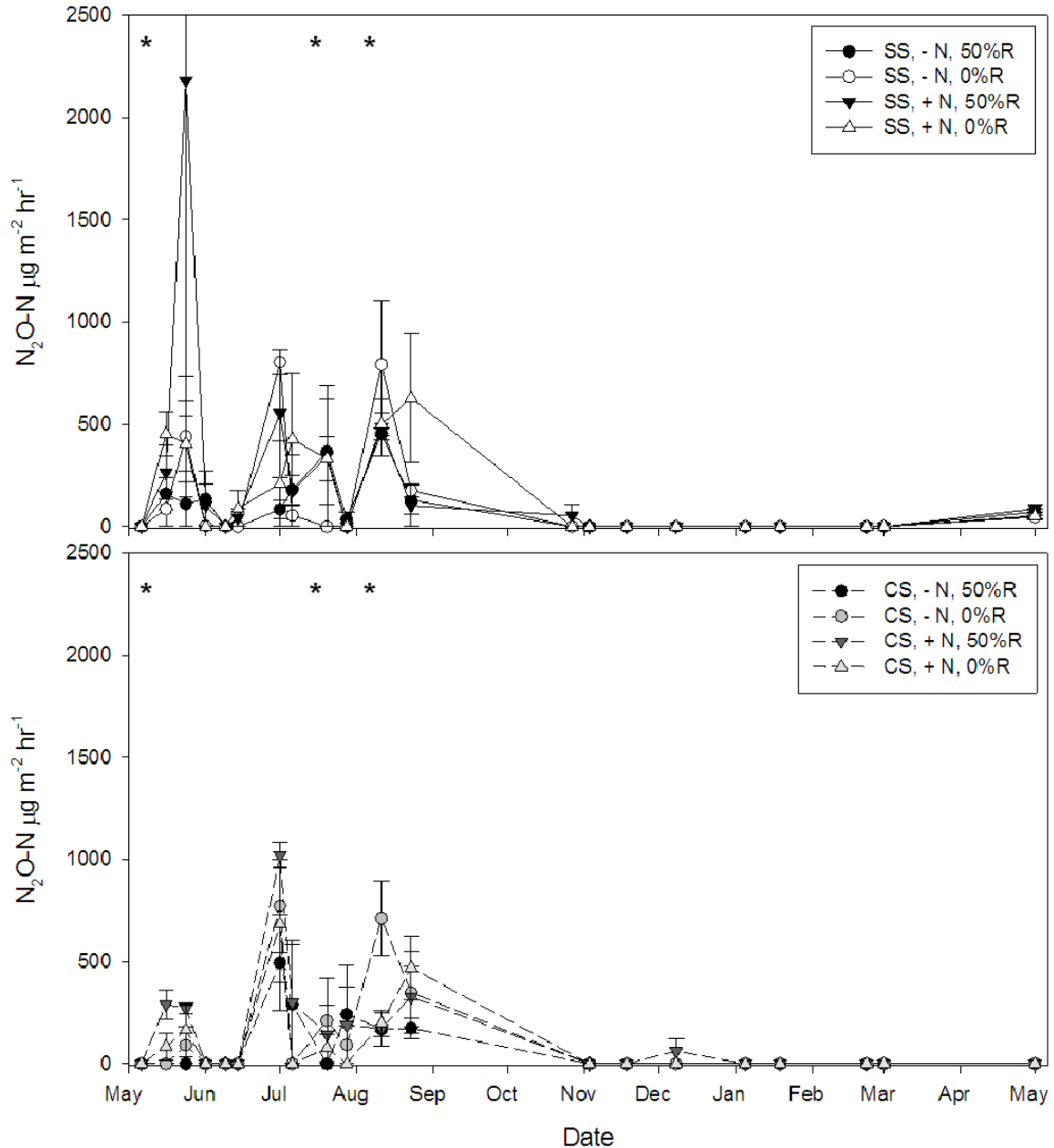


Figure 20. Mean soil gas fluxes of N<sub>2</sub>O from 2011 sampling seasons for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3). Asterisk denotes irrigation date.

The highest N<sub>2</sub>O fluxes in SS were observed early in the growing season (Figs. 6 and 8) when temperatures were warm, soil moisture was relatively high, and crops were likely providing belowground C inputs from photosynthesis. In contrast, the highest fluxes of N<sub>2</sub>O

observed in CS treatments occurred later in the summer in 2010 and 2011, following irrigation/precipitation events and when temperatures were very warm (Figs. 6 and 8). This finding is particularly interesting because while corn grown in CS rotations in 2010 may explain part of the observation, sorghum was grown in both SS and CS in 2011 and the similar pattern of relatively higher fluxes existed later in the season rather than during the early portion for both. Cumulative growing season N<sub>2</sub>O emissions were influenced by a rotation by residue interaction in 2010, perhaps due to lower N addition to CS in 2010 compared to SS (Table 2), but no rotational effect or interaction was observed in 2011. The soil environment responded differently to residue return between crop rotations in 2010. It is possible that the higher amount of residue returned to the soil in CS, 50%R treatments in 2009 immobilized soil N until late in the season, when the C:N may have narrowed, possibly resulting in increased available soil N and subsequent higher N<sub>2</sub>O emissions. Despite no rotational effects on cumulative N<sub>2</sub>O emissions (Table 5), differences in individual gas flux rates from the sampling events late in the growing season showed a rotational effect (Figs. 7 and 8). The effect may have been sufficiently short-lived in 2011 and not strong enough to impact either growing season or annual cumulative N<sub>2</sub>O emissions.

Higher N<sub>2</sub>O emissions were generally observed under conditions where N availability, temperature, and soil moisture were high. The effect of N fertilization on cumulative N<sub>2</sub>O emissions was very strong, and was significant in 2010 and 2011 for both growing season and annual cumulative emissions (Table 5). Increased N availability from fertilization often increases N<sub>2</sub>O emissions and has been widely observed (Fernandes Cruvinel et al., 2011; Halvorson et al., 2008; Hernandez-Ramirez et al., 2009; Sistani et al., 2010; Snyder et al., 2009; Venterea et al., 2011). However, initial gas sampling each year



occurred less than a week after fertilization, yet large increases in flux rates were frequently not observed for more than one to two weeks after the start of our gas sampling campaign. This lag in N<sub>2</sub>O flux may be due to low soil moisture initially which may have limited both autotrophic and heterotrophic microbial activity. Several studies have shown that when soil moisture is increased via irrigation/precipitation following fertilization, N<sub>2</sub>O fluxes increased (Almaraz et al., 2009; Barton et al., 2008; Fernandes Cruvinel et al., 2011; Pelster et al., 2011; Smith et al., 1998). Thus, we theorize that dry soil conditions at the start of each sampling campaign were not conducive for large N<sub>2</sub>O fluxes to be observed following fertilization. Warm soil temperatures observed throughout both growing seasons in central Texas (Fig. 2) have been shown to promote greater soil N<sub>2</sub>O fluxes (Adviento-Borbe et al., 2010; Barton et al., 2008; Rochette et al., 2010), and would indicate potential elevated microbial activity. Throughout the growing season, N<sub>2</sub>O fluxes generally increased following irrigation/precipitation events (Figs. 7 and 8) and were congruent with other similar research (Adviento-Borbe et al., 2010; Almaraz et al., 2009; Denmead et al., 2010; Halvorson et al., 2008; Venterea et al., 2011). Soil drying and re-wetting cycles have also been reported to promote N<sub>2</sub>O emissions from soil (Almaraz et al., 2009; Fernandes Cruvinel et al., 2011; Pelster et al., 2011; Venterea et al., 2011). Thus, soil moisture can have a profound impact on N<sub>2</sub>O emissions. The soils in this study became very dry during the droughty conditions experienced in 2010 and 2011, and water films surrounding soil particles likely became discontinuous and limited substrate supply to microbes (Schimel and Holland, 2005) performing nitrification/denitrification. Simultaneously, limited transport of mineral N, possibly allowed greater quantities to concentrate in the soil over the dry periods and became available for nitrification/denitrification upon wetting of the soil (Pelster et al., 2011).

Drying and re-wetting cycles frequently experienced across the growing season in this study likely enhanced soil organic and N mineralization, minimized transport of soil  $\text{NO}_3^-$  during dry periods, and then increased substrate availability upon wetting (Aguilera et al., 2013). Additionally, furrow irrigation utilized in this study may have been more conducive to  $\text{N}_2\text{O}$  emissions than other methods of irrigation. Aguilera et al. (2013) found furrow irrigation to contribute to higher  $\text{N}_2\text{O}$  emissions compared to several other types of irrigation utilized in Mediterranean climates.

Emissions of  $\text{N}_2\text{O}$  from cropped soils are most often a byproduct of nitrification or denitrification (Schimel and Holland, 2005). Nitrification is the likely source of the majority of  $\text{N}_2\text{O}$  measured from this study. Denitrification prevails under anaerobic conditions, commonly associated with excessively wet soils, although very high  $\text{CO}_2$  concentrations in microsites within the soil may be conducive to denitrification on a small scale (Barton et al., 2008). When %WFPS in soil reaches more than 80%, we anticipate denitrification to be the dominant  $\text{N}_2\text{O}$ -producing process, while lower soil moisture levels would be more conducive to nitrification (Schimel and Holland, 2005). The soil at our location contained relatively high clay (35%) and silt (55%) content and field roads often became impassable following precipitation events. Subsequently, it was not possible to reach the field to measure soil gas fluxes until soils were well below saturation. Since %WFPS in our study only peaked near a maximum of 40% in 2010 and 65% in 2011 (Fig. 2), we believe soil conditions were generally more conducive to nitrification being the source of  $\text{N}_2\text{O}$ . This conclusion also aligns with a number of studies which report nitrification as the dominant mechanism producing  $\text{N}_2\text{O}$ , due to relatively low %WFPS (Adviento-Borbe et al., 2007; Bremner, 1997; Pelster et al., 2011).

Nitrogen emission factors (NEFs) were estimated annually for each crop rotation in addition to the two-year average. The Intergovernmental Panel on Climate Change (IPCC) considers this as the percentage of added N fertilizer lost to the atmosphere as  $\text{N}_2\text{O-N}$ , and is calculated by subtracting the mass of  $\text{N}_2\text{O-N}$  lost from an unfertilized treatment from the mass of  $\text{N}_2\text{O-N}$  lost from a similar treatment which was fertilized, dividing by the mass of fertilizer applied and multiplying by 100 (Smith et al., 2007). The IPCC utilizes a default approximation of 1.25% of applied fertilizer N being lost annually as  $\text{N}_2\text{O-N}$  (Smith et al., 2007). Despite the constant value utilized to estimate NEF by the IPCC, other research has found a wide range of NEFs ranging from 0.02 to 3.42% (Adviento-Borbe et al., 2010; Aguilera et al., 2013; Alluvione et al., 2010; Barton et al., 2008; Fernandes Cruvinel et al., 2011; Halvorson et al., 2008; Linqvist et al., 2012; Sistani et al., 2010; Venterea et al., 2011). Emission factors have been found to significantly vary due to N source, N application rate, type of crop, and climate regime, among a myriad of other factors. The NEFs from this study varied between years, particularly in the CS cropping rotation. In 2010, estimated NEFs for the SS and CS treatments were 1.74 and 2.50%, respectively. The SS and CS sequences had NEFs of 1.63 and -0.30%, respectively, in 2011. The severe drought in 2011 likely contributed to the negative NEF estimated in the CS rotation. The CS estimate seemed atypical, but as noted before, the sporadic fluxes of  $\text{N}_2\text{O}$  during the 2011 growing season created high variation in the data set. It is likely a small amount of detectable fluxes were found in the CS unfertilized treatments while no detectable fluxes were found in fertilized treatments from the similar time period, resulting in the negative value. The SS rotation had a two-year average NEF of 1.68%, while the CS rotation had an average of 1.10%, both of

which were similar to the IPCC estimate of 1.25%. Averaged across crop sequence and years, the overall NEF for the entire study was 1.39%.

### **Cumulative CO<sub>2</sub>-equivalent Emissions**

Emissions of CO<sub>2</sub> and N<sub>2</sub>O were integrated with their GWPs to estimate GWP intensity from the soil surface of each plot. We acknowledge that this methodology likely resulted in an overestimation of GWP because at least a portion of the emitted CO<sub>2</sub> was taken up by the crops during the growing season via photosynthesis and never reached the atmosphere. Emissions of CO<sub>2</sub> from the soil surface are not equal to net CO<sub>2</sub> emissions to the atmosphere (Mosier et al., 2006) and as such, changes in SOC are commonly utilized as an estimate of net CO<sub>2</sub> exchange to the atmosphere from a cropping systems (Robertson et al., 2000). However, this study was concerned with the consequences of various agronomic management practices on the exchange of gas at the soil surface rather than the net exchange to the atmosphere.

As anticipated, the general trend of cumulative CO<sub>2</sub>-eq emissions closely followed that of cumulative CO<sub>2</sub>-C emissions. In 2010, a slightly greater difference in CO<sub>2</sub>-eq emissions between treatments was observed compared to cumulative CO<sub>2</sub>-C emissions that year (Table 5 and Figs. 11 and 13). In contrast, less difference was seen between the treatments in 2011 (Table 5 and Fig. 13). This difference may be explained by lower N<sub>2</sub>O emissions in 2011 that resulted in less change between cumulative CO<sub>2</sub>-C and CO<sub>2</sub>-eq emissions. Across both years, fertilized plots generally exhibited greater CO<sub>2</sub>-eq emissions than unfertilized plots and this effect was more evident in the SS sequence than the CS rotation (Fig. 14).

The contribution of N<sub>2</sub>O to total cumulative CO<sub>2</sub>-eq emissions varied with treatment. Generally, N<sub>2</sub>O emissions from N fertilized contributed more to total CO<sub>2</sub>-eq than did unfertilized treatments. In 2010, the contribution of N<sub>2</sub>O to CO<sub>2</sub>-eq emissions ranged from 18.7% in [SS, - N, 0%R] to 33.3% in [CS, + N, 0%R]. Increased CO<sub>2</sub>-eq attributable to N<sub>2</sub>O was slightly lower in 2011 and ranged from 15.1% in [CS, - N, 50%R] to 30.8% in [SS, + N, 0%R]. The highest percentage each year was observed in fertilized treatments with no residue return, which may suggest residue return temporarily immobilized available soil N, resulting in less overall N<sub>2</sub>O loss. Across years and treatments, N<sub>2</sub>O contributed approximately 25% to CO<sub>2</sub>-eq emissions and is congruent with our hypothesis that N<sub>2</sub>O would contribute a smaller, yet significant portion, of the total GWP intensity from soil gas fluxes.

### **Yield-scaled Greenhouse Gas Emissions**

This study is the first to our knowledge that analyzed yield-scaled soil gas fluxes in terms of theoretical ethanol yield from any crop, particularly bioenergy sorghum. Grain yield has traditionally been the parameter used to measure overall efficiency (Grassini and Cassman, 2012; Johnson et al., 2012; Linnquist et al., 2012; Van Groenigen et al., 2010; Venterea et al., 2011). Theoretical ethanol yield from bioenergy sorghum biomass and corn grain was the yield parameter used in the current study due to the focus on bioenergy crop production. In addition, other studies have focused on either N<sub>2</sub>O, N<sub>2</sub>O plus CH<sub>4</sub>, or near full life cycle GHG accounting (Grassini and Cassman, 2012; Johnson et al., 2012; Linnquist et al., 2012; Van Groenigen et al., 2010; Venterea et al., 2011), not CO<sub>2</sub> plus N<sub>2</sub>O emissions from the soil surface, such as in our study. The purpose of our study was to determine how

various agronomic management practices affected CO<sub>2</sub> and N<sub>2</sub>O emissions from the soil surface. This type of comparison may be the first of its kind, and making direct numerical comparisons with other yield-scaled GHG studies' values was not possible. However, the impact of management practices on overall yield-scaled GHG emissions is documented and was used for comparison in this study.

Crop rotation and residue return were the dominant factors controlling yield-scaled GHG emissions in 2010 and 2011 (Table 5 and Fig. 15). The impact of crop rotation had an opposite effect in 2010 and 2011; SS was significantly more efficient in 2010, while it was less efficient in 2011. SS had significantly greater ethanol yield in 2010 and SS had lower yield in 2011 compared to CS treatments. Thus, crop rotation was vital to yield-scaled GHG emissions due to its impact on ethanol yield in 2010 and 2011. Similarly, yield-scaled GHG emissions were consistently affected by residue return in both 2010 and 2011 (Table 5 and Fig. 15). Treatments which retained half the residue on the field as an organic amendment had significantly less theoretical ethanol yield, and subsequently had reduced yield-scaled GHG efficiency. While the residue amendment addition may likely improve soil health, its negative impact on production efficiency from a yield-scaled GHG flux perspective will likely inhibit its adoption.

Across both years of study, all three primary factors significantly impacted production efficiency. While crop rotation results varied by year, the SS sequence had the strongest effect across years by consistently producing relatively high ethanol yields, and had the lowest two-year average yield-scaled GHG emissions (Figs. 4 and 16). The yield reduction found in 50%R treatments, attributed to returning biomass to soil (direct reduction in biofuel feedstock), across years caused a decrease in efficiency or elevated yield-scaled

GHG emissions. Interestingly, N fertilization improved efficiency by providing greater ethanol yield across years. While N fertilization increased N<sub>2</sub>O emissions and cumulative CO<sub>2</sub>-eq (Figs. 12 and 14), the benefit from providing greater ethanol yield was more dominant. Residue return did not have the same effect on yield-scaled GHG emissions in each cropping sequence; returning half the residue had a larger impact in the SS sequence, where bioenergy sorghum is grown annually, compared to CS, where corn is grown every other year (Table 5 and Fig. 16). In the same way, N fertilization had a different effect on yield-scaled GHG emissions in SS than CS; fertilized CS treatments were much more efficient than unfertilized while SS treatments were relatively similar, regardless of fertilization.

Agronomic practices employed to produce a given crop may not only impact GHG emissions, but also total yield. Thus, due to the dynamic changes from one crop system to another, yield-scaled GHG emission values are helpful in identifying those systems with maximum efficiency. This study examined the effect of crop rotation, N fertilization, and residue return on system efficiency. In addition to these, other practices such as type of crop (Linquist et al., 2012), water management method (Grassini and Cassman, 2012), organic and conventional cropping systems (Johnson et al., 2012), and tillage practices (Venterea et al., 2011) have been examined for yield-scaled GHG efficiency.

Similar to other studies (Grassini and Cassman, 2012; Linquist et al., 2012; Van Groenigen et al., 2010), we found N fertilization to be a significant factor for yield-scaled GHG emissions. Nitrogen fertilization has often been identified as a critical component of cropping system efficiency and meta-analyses have frequently revealed that moderate N application rates typically have the greatest yield-scaled GHG emission efficiency (Linquist

et al., 2012; Van Groenigen et al., 2010). Linnquist et al. (2012) found that low to medium rates of N fertilization on rice, maize, and wheat generally produced a constant efficiency value, while high rates caused much higher GWP and lowered efficiency. Cellulosic energy crops are often grown to maximize biomass yield which can require relatively high N rates. The current study determined that a relatively high N application rate (280 kg N ha<sup>-1</sup>) increased cumulative CO<sub>2</sub>-eq emissions from the soil, but yield gains from fertilization outweighed GHG minimization benefits provided by unfertilized plots. Nitrogen use efficiency (NUE) of cropping systems has been noted on several occasions as a critical component of balancing GHG emissions and sustained yields (Grassini and Cassman, 2012; Linnquist et al., 2012; Van Groenigen et al., 2010). While this study was unable to examine the effects of variable N application rates on yield-scaled GHG emissions, it did reveal outcomes from two rates and may lay the basis for future research.

Theoretical ethanol yield was a prevailing factor in determining overall cropping system efficiency. Like many studies, boosts in yield from management practices were central to efficiency because all GHG emissions were eventually compared relative to total yield achieved (Grassini and Cassman, 2012; Johnson et al., 2012; Linnquist et al., 2012; Van Groenigen et al., 2010). This study demonstrated that returning high amounts of biomass (potential biofuel feedstock) back to the soil or producing a poor biomass yield immediately decreased efficiency via reduction in theoretical ethanol yield. Similarly, yield reductions caused by organic compared to conventional cropping systems (Johnson et al., 2012) or dryland compared to irrigated cropping systems (Grassini and Cassman, 2012) increased yield-scaled GHG emissions and reduced cropping system efficiency. Thus, in order to improve overall efficiency of bioenergy cropping systems, additional research to improve



crop yields or conversion methods is important for enhancing biofuel yield and boosting efficiency.

## **CONCLUSION**

Sources of sustainable bioenergy feedstock that minimize GHG emissions while sustaining high yield are critical components of future domestic energy sources and policy. Largely due to difficulties in direct quantification, estimates of GHG emissions from bioenergy cropping systems are often obtained by estimated emission factors or models. These tools are helpful in providing estimates to guide conservation and mitigation efforts throughout the world. However, many of these models can benefit from additional direct measurement to help calibrate them to new cropping systems and regions of interest. Little information is available on GHG emissions from bioenergy sorghum cropping systems. Moreover, central Texas and much of the southern U.S. remain as areas with very little data available to help guide estimates of trace gas emissions from agricultural cropping systems. This study provided insight on the impact of agronomic management practices on soil GHG fluxes in bioenergy sorghum cropping systems in central Texas.

Trace gas emissions in this study were generally higher than reported in the literature and were variable across treatments. The highest fluxes of CO<sub>2</sub> and N<sub>2</sub>O were observed during the growing season and frequently followed N fertilization and/or precipitation or irrigation events. Severe drought during the 2011 growing season caused N<sub>2</sub>O fluxes to be sporadic and few detectable fluxes were obtained when the soil was extremely dry. Inter-annual variability in soil gas fluxes and the corresponding influential drivers of loss

processes made it difficult to elucidate clear patterns from one growing season to another. Additional longer-term research is required.

This study found that cumulative growing season GHG emissions were not equal to those of cumulative annual emissions. Our analysis revealed larger variation between individual treatments at the end of each growing season, and greater similarity in annual emissions. Retaining half of the bioenergy sorghum biomass on the field as an organic amendment consistently increased CO<sub>2</sub> emissions, and addition of N fertilizer consistently increased N<sub>2</sub>O emissions each year of the study. The NEF for the entire study was 1.39%, similar to the IPCC estimate of 1.25%. The [SS, + N, 50%R] treatment consistently exhibited some of the highest losses of CO<sub>2</sub> and N<sub>2</sub>O each year and had the highest cumulative CO<sub>2</sub>-eq emissions across both years. Regardless of crop sequence, unfertilized treatments with 100% biomass removal generally showed the lowest cumulative CO<sub>2</sub>-eq emissions across years. Nitrogen fertilization generally resulted in higher cumulative CO<sub>2</sub>-eq emissions.

Yield-scaled GHG emissions were utilized to measure efficiency by identifying those treatments that minimized soil GHG emissions while still producing high theoretical ethanol yields. In 2010 and 2011, there was a general segregation into two or more groups of treatments which performed well and those that did not. Surprisingly, despite the severe drought in 2011, most treatments performed relatively well based on yield-scaled GHG emissions, and may have been attributable to the lower GHG emissions and relatively high biomass yield across all treatments from the drought-resistant sorghum and increased irrigation. Across both years of study, both SS treatments without residue return and the [CS, + N, 0%R] treatment were the most efficient for supplying theoretical ethanol while simultaneously minimizing soil GHG emissions. While the treatments harvesting all residue

for ethanol production performed the best in this study, we acknowledge that further research is needed into the implications of removing nearly 100% of above-ground biomass across several years. Complete biomass removal will inherently mine the soil of valuable organic C and nutrients over time and may not sustain current yield levels. N fertilization was also identified as an important factor influencing yield-scaled GHG emissions. As others have noted, optimization of cropping system NUE may be one of the principal attributes for improved efficiency. Future work with bioenergy sorghum should determine N application rates that result in optimum yield-scaled GHG emission efficiency. Finally, while this study highlights the impact of various bioenergy sorghum production scenarios on soil GHG fluxes, future work should focus on other sustainability factors such as C cycling and life cycle analyses.

## CHAPTER III

### CARBON POOL DYNAMICS WITHIN BIOENERGY SORGHUM PRODUCTION SYSTEMS IN CENTRAL TEXAS

#### **INTRODUCTION**

The production and removal of organic carbon (C)-containing residues, such as corn stover or bioenergy sorghum, for biofuel feedstock, can have significant consequences on C cycling within agroecosystems. When bioenergy feedstocks are harvested, care must be taken to minimize negative impacts on soil C stocks, nutrient retention, greenhouse (GHG) emissions, soil erosion, crop yield, global warming potential, and overall soil quality (Gelfand et al., 2011; Graham et al., 2007; Lal, 2009). Under proper management conditions, biomass residues may provide a biofuel feedstock while sustaining soil and environmental quality (Graham et al., 2007; Johnson et al., 2006; Karlen et al., 2011; Lal, 2009) and reducing net GHG emissions compared to traditional fossil fuel energy sources (Adler et al., 2007).

Several important characteristics of soil fertility and soil quality are attributed to soil organic matter (SOM) and soil organic C (SOC) (Doran, 2002). More than half of SOM is SOC (approximately 58%) (Schulte and Hopkins, 1996) and it represents the largest reservoir of organic C in terrestrial ecosystems (Lal, 2004b; Post et al., 1982). Availability of SOC is a critical factor in numerous biogeochemical cycles vital for soil fertility (Lal, 2004b). Soil organic matter has beneficial impacts on soil physical, chemical, and biological properties, resulting in a direct relationship with agriculture productivity (Lal, 2004b).

Removal of agricultural residues translates into removal of potential SOM. Removal of crop residues over time will likely reduce SOM and SOC, which may not only reduce crop productivity, but may deteriorate soil structure, reduce water infiltration, and increase runoff and losses of essential nutrients (Blanco-Canqui and Lal, 2007; Franzluebbers et al., 1995; Lal, 2004b; Lal, 2009; Moebius-Clune et al., 2008; Powell and Hons, 1991). Short-term impacts of retaining SOC can be observed in enhanced crop yields and in the long-term impacts on overall agronomic productivity (Lal, 2009). Blanco-Canqui and Lal (2007) showed that the magnitude of SOC depletion associated with corn stover removal was directly proportional to the reduction in amount of residue-C input. In a study modeling C fluxes under various biofuel crops, the rate of C lost increased significantly with the percentage of residue removed in all models evaluated (Anderson-Teixeira et al., 2009). Additionally, crop residues serve as important sources of C and nutrients for soil biota, which control many nutrient cycles, further enhancing the importance of C retention for soil fertility (Franzluebbers, 2002). The biological availability of various nutrients is also essential for microbial conversion of biomass C into stable-C pools such as humus, which can enhance C sequestration (Jastrow et al., 1998, Six et al., 2004).

Soil aggregation is an important parameter of overall soil quality and increased C sequestration. Soil aggregation plays significant roles in limiting soil erosion and protecting organic matter (Jastrow et al., 1998). Changes in soil aggregation have direct impacts on how well organic matter is physically protected and chemically stabilized, resulting in variability of organic matter turnover rates (Jastrow et al., 1998; Six et al., 2004). Maintaining or increasing soil aggregation is important to C cycling and sequestration as well as overall soil quality (Jastrow et al., 1998; Six et al., 2004). Harvesting agricultural residues, such as from

corn or biomass sorghum, can reduce soil aggregation and increase SOM decomposition rates (Malhi and Lemke, 2007; Moebius-Clune et al., 2008; Singh and Malhi, 2006), thus causing negative effects on soil quality and shifting greater amounts of C back into the atmosphere.

Agronomic management practices utilized in production of bioenergy crops can have a significant impact on C cycling. Crop rotations and increased cropping intensity can influence several soil C pools (Dou et al., 2008; Franzluebbers and Follett, 2005; Franzluebbers et al., 1995; Omonode et al., 2007; Wright and Hons, 2005), total biomass yield (Wight et al., 2012), improve soil fertility by increasing SOM (Wright and Hons, 2005), enhance SMBC and soil aggregation (Acosta-Martinez et al., 2011; Six et al., 2006), and alter soil respiration rates (Adviento-Borbe et al., 2010; Omonode et al., 2007). Beyond obvious biomass yield increases, N fertilization can have several impacts on C cycling, including the potential to increase SOC (Allmaras et al., 2004; Christopher and Lal, 2007; Lemke et al., 2010; Poirier et al., 2009); increase SOC retention time (Moran et al., 2005; Paustian et al., 1995); influence soil respiration rates (Alluvione et al., 2010; Dick, 1992; Heller et al., 2010; Van Vleck and King, 2011) or in some instances enhance microbial activity and cause priming of SOM mineralization which can reduce SOC (Kuzyakov et al., 2000). Residue management can influence total crop biomass C (Wight et al., 2012), active pools of SOC (Franzluebbers et al., 1995; Kallenbach and Grandy, 2011), and soil C respiration rates (Kuzyakov et al., 2000). Due to complex C cycling dynamics, any of these management impacts may have diverging results on any one C pool over a given time period. Furthermore, little data is available on the impact of crop rotation, N fertilization, and residue return on C pool dynamics within bioenergy sorghum cropping systems.

The impact of bioenergy sorghum production systems on plant, soil, microbial, and gaseous C pools is not well understood. The aim of this study was to provide additional knowledge related to C cycling in bioenergy sorghum production scenarios in central Texas. To better understand the effects of crop rotation, N fertilization, and residue return on C cycling dynamics, we measured i) biomass C yield, ii) SOC, iii) SMBC, and iv) soil respiration C over time.

## **MATERIALS AND METHODS**

### **Site Description and Experimental Design**

The experimental site was located at the Texas A&M Agrilife Research Farm, approximately 8 km southwest of College Station, Texas, USA (30° 32' 15"N, 96° 25' 37"W). The site is located within the Brazos River floodplain in south-central Texas. The soil used was a Weswood silty clay loam (100 g sand kg<sup>-1</sup>, 560 g silt kg<sup>-1</sup>, 340 g clay kg<sup>-1</sup>) and is classified by the USDA NRCS as a Fine-silty, mixed, superactive, thermic Udifluventic Haplustept. Climate at the site is classified as humid subtropical; the mean annual precipitation is 978 mm and the mean annual temperature is 20°C. Prior to the start of the bioenergy sorghum study in 2008, the field was in cotton (*Gossypium hirsutum* L.) in 2007, and rotated annually with corn (*Zea mays* L.) under conventional disk tillage.

This study was conducted within a larger study investigating the yield potential and agronomic responses of bioenergy sorghum (*Sorghum bicolor* L.) to various integrated management practices (Wight et al., 2012). The bioenergy sorghum cropping system, established in 2008, was planted into four row (1.02-m row centers) plots which measured 9.14 m long and 4.08 m wide. The larger study used a randomized complete block design

with crop rotation, N fertilization rate, and biomass residue return rate as the three major factors. This study was limited to two levels of each of the three major factors and was replicated 4 times (3 times for CO<sub>2</sub>-C measurements). The eight experimental treatments included every combination of: crop rotation (corn-sorghum [CS] or sorghum-sorghum [SS]), N fertilization (0 kg N ha<sup>-1</sup> [- N] or 280 kg N ha<sup>-1</sup> [+ N]), and biomass residue return (0% residue returned [0%R] or 50% residue returned [50%R]). The bioenergy sorghum variety, “4Ever Green”, used within the study was a photoperiod-sensitive, high-tonnage hybrid forage sorghum (Walter Moss Seed Co, Waco, TX, U.S.A.). This particular variety was chosen because of its low water requirements, high biomass yield, and low lodging potential. When corn was rotated with sorghum, Dekalb DKC68-05 was the corn variety utilized in the corn-sorghum cropping sequence.

A summary of field management operations in 2008 and 2009 can be found in Wight et al. (2012) and Table 6. The field management operations in 2010 and 2011 can be found in summary Table 6 or as described here. The field beds were prepared for planting with a rolling cultivator and pre-plant herbicide on 17 March 2010 and planted to corn on 23 March 2010 and sorghum on 13 April 2010. In 2011, a pre-plant herbicide was applied on 2 March 2011, cultivation occurred on 24 March 2011, and the entire field was planted to sorghum on 25 March 2011. A four-row cultivator was used to control weeds on 22 May 2010 and 5 May 2011. Treatments receiving N fertilization were sidedressed with granular urea at 7.5 cm depth on 22 May 2010 and 5 May 2011; approximately the 4-leaf stage for sorghum and 6-leaf stage for corn. When corn was fertilized, it received a reduced rate of 168 kg N ha<sup>-1</sup>, based on local recommendation. Randomly selected 3.05 m segments of the middle two rows of corn were hand-harvested for grain and stover samples on 2 July 2010. Estimates of total



corn biomass and grain yield were made on this date, however, the remaining non-sampled portion of the corn stand was not harvested/mulched onto the soil until the later sorghum harvesting date. The remaining corn biomass and biomass sorghum were harvested on 7 October 2010 and in 2011, sorghum was harvested on 1 September. A single-row silage harvester with an attached weigh-bucket was utilized to harvest sorghum in 2010 and 2011. Biomass yield was estimated from the entire length of the middle two rows of each plot and a random grab sample of chopped residue was captured for determining moisture and nutrient composition. The harvester mulched sorghum biomass into small pieces approximately 0.04 m by 0.04 m. After biomass yield weights were recorded, the 50%R treatments received the biomass from the middle two rows evenly distributed across the area of the entire plot. This study was not interested in removing corn residue for biofuel conversion (only grain), so when corn was harvested in 2010, 100% of residue was returned to each plot, regardless of residue return treatment. After harvest each fall, residue was disked into the field and the plots were bedded. Disking and bedding occurred on 12 October 2010 and 5 September 2011. Furrow irrigation was used sparingly as needed on 31 May 2010 and 12 April, 9 May, 14 July, and 4 August in 2011. Eleven cm of irrigation water was applied in each event except 9 May 2011, when 9 cm was applied. A summary of these field operations can be found in Table 6.

Table 6. Summary of field operations since the initiation of the study in 2008 at the research site near College Station, TX.

Field operation	Year							
	2008		2009		2010		2011	
		<i>notes</i>		<i>notes</i>		<i>notes</i>		<i>notes</i>
Pre-plant herbicide†	3 Mar.		9 Mar.		17 Mar.		2 Mar.	
Soil sampling	24 Mar.		6 Apr.		5 Apr.		14 Mar.	
Pre-plant cultivation	25 Mar.		6 Apr.		17 Mar.		24 Mar.	
Planting	26 Mar.	<i>corn</i>	7 Apr.	<i>sorghum</i>	23 Mar.	<i>corn</i>	25 Mar.	<i>sorghum</i>
	26 Mar.	<i>sorghum</i>			13 Apr.	<i>sorghum</i>		
Fertilization	1 May		20 June		22 May		5 May	
Interrow cultivation	24 Apr.		5 June		22 May		5 May	
Irrigation‡	10 June	<i>9 cm</i>	16 July	<i>6 cm</i>	31 May	<i>11 cm</i>	12 Apr.	<i>11 cm</i>
	10 July	<i>9 cm</i>	24 Aug.	<i>6 cm</i>			9 May	<i>9 cm</i>
							14 July	<i>11 cm</i>
Harvest							4 Aug.	<i>11 cm</i>
	5 Aug.	<i>corn</i>	5 Nov.	<i>sorghum</i>	2 July	<i>corn</i>	1 Sept.	<i>sorghum</i>
Post-harvest disking and bedding	14 Oct.	<i>sorghum</i>			7 Oct.	<i>sorghum</i>		
	15 Oct.		6 Nov.		12 Oct.		5 Sept.	

† applied as Atrazine

‡ applied as furrow irrigation

## **Soil Respiration Measurement**

Soil respiration ( $\text{CO}_2\text{-C}$ ) fluxes were directly measured using a static, vented chamber and a field photoacoustic gas analyzer. Specifically, the measurements were made by integrating a Li-Cor 20-cm survey chamber (model 8100-103, Li-Cor Inc., Lincoln, NE) with an INNOVA 1412 Photoacoustic gas analyzer (Innova AirTech Instruments A/S, Denmark). The flux chamber was selected for its portability, adaptability, and ability to minimize pressure disequilibrium between the chamber headspace and ambient atmosphere (Xu et al., 2006). A PVC flux chamber soil-collar was installed to a depth of approximately 12 cm near the middle of each of the 24 sampled plots. The collars were placed on top of beds on level surfaces which were equidistant from the crop and injected fertilizer. Height measurements from the soil surface to the top of each collar were measured at four quadrants inside the collar and averaged to estimate height for periodic headspace volume calculation. To minimize disturbance effects, soil-collars were installed no less than 24 hours prior to a gas sampling event and remained in place through the entire growing and fallow seasons (Parkin and Venterea, 2010; Tiedje et al., 1989). Collars were only removed briefly in spring and fall for field operations.

Soil respiration fluxes were measured by placing the Li-Cor 20-cm chamber atop the PVC collars for a deployment time of 20 minutes. The analyzer was programmed to collect a measurement every 2 minutes during deployment. The gas analyzer was programmed with a 5-second sampling integration time and auto-flushing for the corresponding tube length (typically 2 m). All tubing, fittings, and connection components were made of polytetrafluoroethylene (PTFE) or stainless steel. The gas analyzer was lab calibrated every two months with a 2-point calibration of a zero gas and mixed gas standard. The primary gas

standard (CO<sub>2</sub> at 450 ppm) and zero gas (UHP N<sub>2</sub>) were mixed to at least 99.99% accuracy (Airgas Specialty Gases, LaPorte, TX). The gas analyzer was routinely checked prior to field sampling with gas standards to ensure calibration stability.

Preliminary analysis of trace gas data determined the majority of fluxes exhibited a nonlinear increase in concentration over time within the chamber headspace. Thus, nonlinear fluxes of GHGs were calculated by fitting a nonlinear regression of trace gas concentration across deployment time (Wagner et al., 1997) and on a few occasions where linear fluxes were observed, a linear method was utilized. While the nonlinear quadratic regression method selected may not be the unconditional best, its simplicity of use and significant improvement over linear methods (given our nonlinear data) (Livingston et al., 2006; Venterea et al., 2009) coupled with high temporal resolution and the large number of data points collected for each flux calculation with our analyzer warranted its use for this study. Due to relatively high flux rates, CO<sub>2</sub> flux calculations only utilized 12 minutes of sampling data (6 individual data points) to minimize potential ‘chamber effect’ observed in the full 20-minute deployment time. Any regression with a coefficient of determination ( $r^2$ ) less than 0.8 was rejected and a net flux of zero was assumed. Less than 1% (7 of 985) of soil respiration values had to be rejected due to poor coefficients of determination. Calculation of trace gases fluxes were based on GRACEnet protocols (Parkin and Venterea, 2010) and were calculated as:

$$F_1 = \frac{V \cdot \frac{\Delta C}{\Delta t}}{A_s} \quad [4]$$

where  $F_1$  is the volumetric gas flux in  $\mu\text{L} (\text{CO}_2) \text{ m}^{-2} \text{ min}^{-1}$ ,  $\frac{\Delta C}{\Delta t}$  is the change in trace gas concentration over time ppm (v)  $(\text{CO}_2) \text{ min}^{-1}$ ,  $V$  is the calculated chamber volume (L), and  $A_s$  is the surface area ( $\text{m}^2$ ). Further calculations were made as:

$$F_2 = C \cdot \frac{F_1 \cdot p}{R \cdot T} \quad [5]$$

where  $F_2$  is the trace gas flux in  $\mu\text{mol} (\text{CO}_2) \text{ m}^{-2} \text{ min}^{-1}$ ,  $p$  is pressure (atm),  $R$  is the gas law constant ( $0.08206 \text{ L atm mol}^{-1} \text{ K}^{-1}$ ),  $T$  is temperature ( $^{\circ}\text{K}$ ), and  $C$  is a conversion coefficient ( $0.2727$  for  $\text{CO}_2$  to  $\text{CO}_2\text{-C}$ ).

Due to required deployment time and the ability to only measure a single plot at a time, the 24 total plots (8 cropping system treatments x 3 replicates) were divided into two consecutive days of sampling. Sampling order was randomized for each sampling event to reduce bias due to temperatures increasing from morning hours. Gas sampling was normally performed between 9:00 and 15:00. Specific days selected for a sampling event were determined based on weather forecasts. Sampling events only occurred on consecutive days with stable weather patterns (e.g. no precipitation, no weather fronts, etc.).

Soil gas measurements were initiated on 25 May 2010, after N fertilization. Trace gas flux measurements were performed approximately weekly through the growing season and at a reduced frequency during the fallow period. Precipitation events and technical difficulties inhibited uniform sampling frequency planned throughout 2010 and 2011. Cumulative growing-season GHG emissions were calculated from 12 sampling events in both 2010 and 2011. Cumulative annual emissions of GHG fluxes were calculated from 18 sampling events in 2010 and 20 sampling events in 2011. Measurements for “sampling year 1” (2010) occurred from 27 May 2010 through 3 March 2011 and for “sampling year 2” (2011) from 6 May 2011 to 1 March 2012.

## **Environmental and Soil Properties**

Several environmental variables were monitored throughout the study to supplement trace gas flux measurements. Air temperature, relative humidity, wind speed, solar radiation, and rainfall were measured with a weather station, approximately 400 m from the field plots, every half hour. Soil temperature was measured hourly by type T thermocouples at 10-cm depth near gas sampling collars within each plot. A time domain reflectometry (TDR) system was utilized to measure soil moisture every 6 hours. The TDR array consisted of sensors near gas flux collars, using a TDR100, and five SDMX50 multiplexers (Campbell Scientific, Inc., Logan, UT). The volumetric water content was determined to a depth of 15 cm by analyzing TDR wave form with PC-TDR (Campbell Scientific, Inc., Logan, UT) and performing Topp's equation (Topp et al., 1980). Soil temperature and moisture data from monitoring systems were collected within the field with a CR1000 data logger (Campbell Scientific, Inc., Logan, UT). Environmental variables were paired with each trace gas flux measurement based on time stamps nearest to flux measurement time.

## **Soil Organic Carbon Determination**

Three, (4-cm) soil cores were composited from each plot in the spring on 24 March 2008, 6 April 2009, 5 April 2010, and 14 March 2011, prior to each growing season. The soil cores collected from within each plot were composited into depths of 0 - 5, 5 - 15, 15 - 30, 30 - 60, and 60 - 100 cm. Samples were oven-dried (60°C) for 7 d, weighed to determine bulk density, coarse ground with a flail grinder, and sieved to pass 1.75 mm. A subsample of each composite sample was finely ground with a puck-and-ring grinder and analyzed for organic and inorganic C via combustion analysis (McGeehan and Naylor, 1988; Schulte and

Hopkins, 1996; Storer, 1984) with an Elementar Americas Inc., VarioMAX CN analyzer (Mt. Laurel, NJ). Separation of inorganic and organic C was achieved via differential heating. A flow rate of 2 L O<sub>2</sub> min<sup>-1</sup> was used within the furnace and temperatures were set to 650°C and 900°C for organic and total C, respectively (Rabenhorst, 1988; Wang and Anderson, 1998).

### **Plant Carbon Determination**

Bioenergy sorghum was harvested manually in August 2008 to ~10-cm stubble height. In November 2009, October 2010, and September 2011, bioenergy sorghum was harvested with a mechanical forage harvester. Yield determination was made by harvesting the inner two rows of each (four row) plot. Random grab samples from each row were collected for biomass tissue analysis. Residue return treatments with 50% biomass return applied each of the middle two rows back across the entire plot following weight determination, while 100% biomass removal (0%R) returned no aboveground biomass. Moisture content of aboveground biomass was estimated from a 600 g subsample from 5 randomly sampled plants which were mulched with a commercial chipper/shredder. Weights of subsamples were collected before and following oven-drying at 60°C for 7 d to determine moisture content. Oven-dried plant tissue samples were coarse ground to pass a 1 mm sieve, then fine ground to pass a 0.5 mm sieve using a puck-and-ring grinder in preparation for elemental C determination via combustion analysis (McGeehan and Naylor, 1988) with an Elementar Americas Inc., VarioMAX CN analyzer (Mt. Laurel, NJ). Total C accumulation in aboveground biomass was estimated by multiplying total dry weight by elemental C concentration.

## **Soil Microbial Biomass Carbon**

Initial soil samples were taken 24 March 2008, prior to the beginning of the study. Three (4-cm diameter) composited soil cores were collected at 0 – 5 and 5 – 15 cm depths from each experimental unit. Samples were oven-dried at 60 °C for 7 d, coarse ground using a flail grinder, sieved to pass through 1.75 mm mesh, and stored in a non-climate-controlled storage facility at air temperature.

Soil samples collected on 14 March 2011 were sampled and scheduled to be processed in the same manner as those in 2008. However, technical issues caused flail-ground soil samples to be over-ground (relative to 2008 coarse ground samples) to a very fine consistency where no small aggregates remained, such as observed in the 2008 samples (and normal coarse ground samples). Measurements of SMBC from these samples appeared to have been modified during processing and results will not be discussed further.

Due to technical issues with spring 2011 soil samples, an additional soil sampling event occurred during the 2011 fallow season to collect soil samples better suited for SMBC analysis. On 18 November 2011, six composited (2.5 cm diameter) soil cores were collected at two depths (0 - 5 and 5 - 15 cm) from each experimental unit. Soil samples were allowed to remain at field moisture content, sieved to 4 mm, and stored at 4°C for no more than 3 months before extraction. A 10 g subsample was used to determine gravimetric moisture content of each soil sample.

## **Chloroform Fumigation Extraction Methods**

Soil microbial biomass C was determined by combustion following fumigation-extraction methods proposed by Vance et al. (1987) with slight modifications. For each depth



in each plot, two 10 g subsamples were placed into 50-mL glass beakers and brought to 50% water-holding capacity (based upon prior gravimetric moisture measurement) with de-ionized water. Soils were incubated at 25°C in 1 L air-tight glass jars with approximately 10 mL of water in the bottom to maintain humidity for 5 days. Each soil subsample was then placed in a vacuum desiccator lined with wet paper towels to maintain humidity. One desiccator containing a set of subsamples was fumigated with ethanol-free chloroform (stabilized with amylene), while the other containing subsamples used as controls remained non-fumigated. Both desiccators were sealed and covered with a black trash bag to protect from light and incubated at room temperature for 24 h. Following vacuum removal of chloroform, soils were transferred from beakers into 50-mL centrifuge tubes and extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> at a 4:1 ratio of extract to soil. Each tube was sealed and shaken for 1 h at 200 rpm on a reciprocating shaker. Samples were subsequently centrifuged for 5 minutes at 2500 rpm and filtered through 2.5 µm filter paper (Whatman 42). Filtrates were stored frozen (-20°C) until analyzed for total dissolved C with a Shimadzu TOC-VCSH (Shimadzu Corporation, Kyoto, Japan) analyzer. Soil microbial biomass-C (*SMBC*) was calculated from Paul et al. (1999) as:

$$SMBC = \frac{EC_f - EC_c}{k_{ec}} \quad [6]$$

where  $EC_f$  is extractable C in the fumigated sample (mg C L<sup>-1</sup> extractant),  $EC_c$  is extractable C in the control sample (mg C L<sup>-1</sup> extractant), and  $k_{ec} = 0.45$  (Wu et al., 1990).

### **Data Analysis**

Cumulative soil respiration (CO<sub>2</sub>) emissions were estimated by linearly interpolating between sampling events and integrating the underlying area from each sampling year

(Gilbert, 1987). The effects of crop rotation, N fertilization, and residue return and their interactions on crop yield, biomass C content, C:N ratio of biomass tissue, cumulative soil respiration, change in SOC by depth, SMBC and percentage of SOC attributed to SMBC were tested using a mixed ANOVA in SAS using PROC mixed procedures. Crop rotation, N fertilization, and residue return were considered fixed effects, while replicate (block) was considered a random effect. When year or soil depth was determined to be a significant, all analyses were performed separately by year and/or depth.

## **RESULTS**

### **Environment**

The climatic conditions in 2010 and 2011 provided a unique opportunity to explore C pool dynamics under drought conditions in central Texas (Fig. 21). The 2010 growing season was considered a relatively moderate drought, while 2011 was the most severe drought on record (Nielsen-Gammon, 2011). Average air temperatures observed in 2010 were slightly above the 30-year average, while air temperatures recorded during the 2011 growing season were well above the 30-year average (OSC, 2012). Mean monthly air temperatures between May and September (approximate growing season) in 2010 averaged 1.49°C above normal, while the same time period in 2011 averaged 2.17°C above normal (Fig. 21).

The average (30-year average) annual precipitation total at the study location is 1009 mm (OSC, 2012). During the 2010 soil respiration sampling year (05/27/2010 – 05/01/2011), 317 mm of total precipitation was measured at the site with an additional 220 mm of irrigation applied (Table 6 and Fig. 21). Even with irrigation, the research location had only half the normal cumulative precipitation in the 2010 period, providing little soil moisture

reserves for the 2011 season. During the 2011 sampling year (05/01/2011 – 05/01/2012), 1021 mm of precipitation was measured at the site with an additional 310 mm of irrigation applied (Table 6 and Fig. 21). Of the 1021 mm of cumulative precipitation in 2011, 80% of it fell after fall 2011 biomass harvest and 45% of it fell in February and March alone (Fig. 21). Thus, outside of irrigation, very little water was available during the growing season in 2011. In 2010, 143 mm of cumulative precipitation was measured during the growing season compared to 200 mm during the 2011 season. Despite greater precipitation during the 2011 growing season, the drought effects were heightened for several reasons. Little precipitation occurred from late fall 2010 through planting in spring 2011. This period of time normally sees the highest precipitation accumulation for the entire year, and without it, the soil profile was depleted of soil moisture prior to the start of the growing season. Additionally, abnormally high air temperatures, combined with high winds on many days throughout the 2011 growing season, elevated evapotranspiration (ET) levels across the study area. Irrigation was used to replenish some profile soil moisture prior to planting in spring 2011, to help germinate the crop, and to minimize drought stress effects early in the growing season. Much of the precipitation during the 2011 sampling period occurred during the fallow season. In February and March 2012, daily rainfall records were set on different rainfall events (OSC, 2012). March 2012 set a monthly cumulative rainfall record; 169 mm of precipitation was deposited over the course of the month (OSC, 2012).

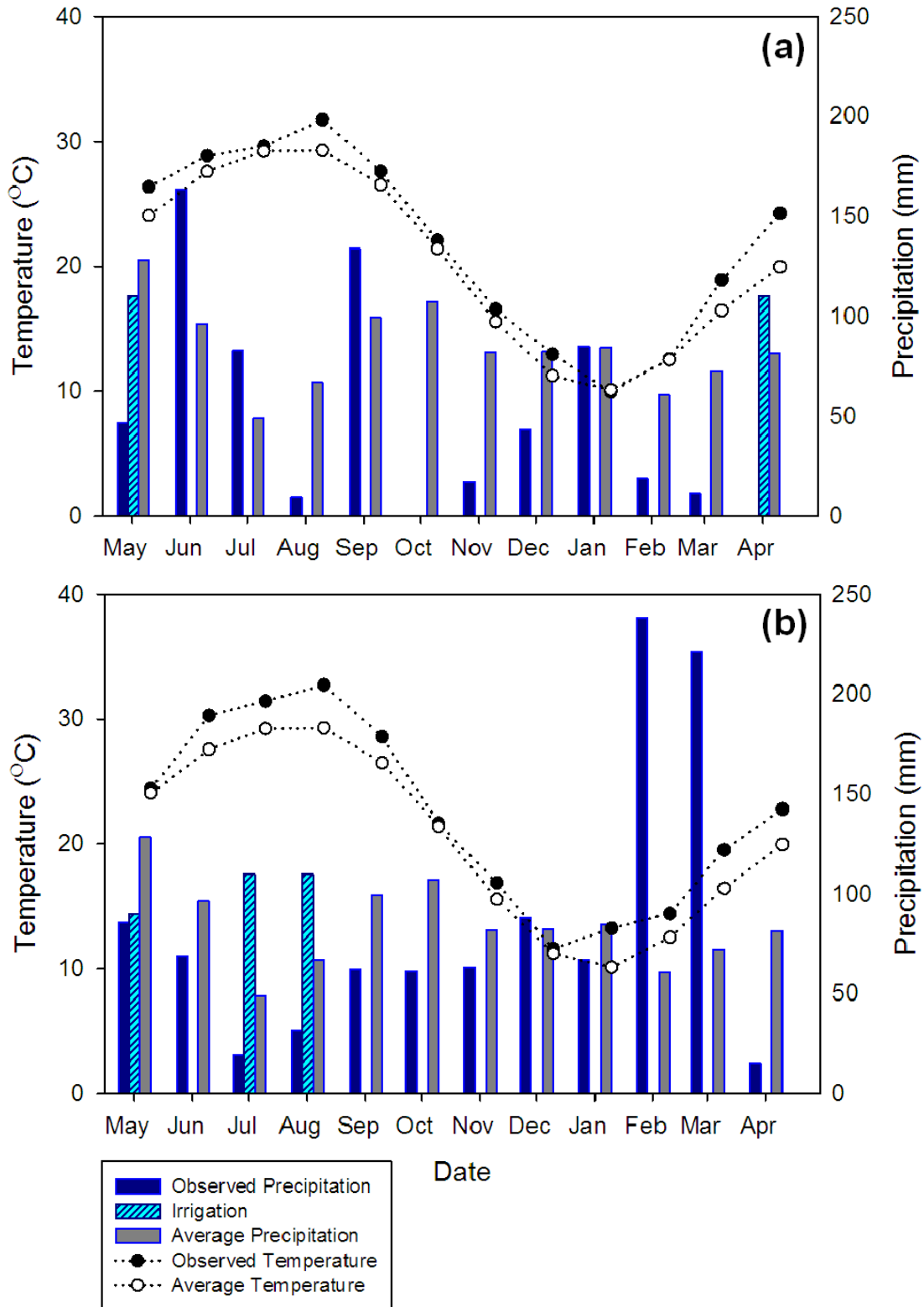


Figure 21. Mean monthly air temperature and total monthly precipitation during the 2010 (a) and 2011 (b) sampling seasons at the research site near College Station, TX. Asterisks indicate irrigation in addition to rainfall total.

## Plant Biomass Carbon

Corn began to senesce in June 2010 and grain harvest was completed at the beginning of July (Table 6). After 3 m-sections of the inner two rows were harvested for biomass yield and plant tissue analysis, the remaining aboveground biomass remained in place until tillage following sorghum harvest (Table 6). Corn harvest was exceptionally poor in 2010. Corn grain yields ranged between 0.2 to 0.9 Mg ha<sup>-1</sup> and aboveground biomass from each of the CS treatments ranged from 2.4 to 2.8 Mg ha<sup>-1</sup> (Figs. 22 and 23). Late planting combined with drought stress (Table 6 and Fig. 21) most likely contributed to the poor yield. Sorghum biomass harvest was completed at the beginning of October in 2010 and the beginning of September in 2011 (Table 6). Drought stress and increased plant lodging caused earlier sorghum harvest in 2011 than in 2010. Sorghum biomass yields in 2010 ranged from 8 to 14 Mg ha<sup>-1</sup> and from 12 to 22 Mg ha<sup>-1</sup> in 2011 (Fig. 23).

Yield and crop C content were highly related, but may have differed slightly based upon plant tissue composition. In this study, we focused on the effects of bioenergy sorghum production practices on C pools and were interested in the fate of total C in each pool examined. Thus, crop C contents in 2010 and 2011 were analyzed separately from biomass yield. Largely due to different crops grown in 2010 in each of the crop sequences (corn vs. sorghum), crop rotation significantly affected yield ( $P = <0.001$ ) and C content ( $P = <0.001$ ) (data not shown). The yield and C content data from 2010 contained a bimodal distribution, so each crop rotation was further analyzed separately for impacts from N fertilization and/or residue return.

Agronomic management practices affected corn grain and corn stover yields in 2010. Despite very poor yields overall, impacts from management practices were still observed in

corn grain and stover yields (Figs. 22 and 23). Nitrogen fertilization significantly ( $P = <0.001$ ) increased corn grain yield in 2010, - N treatments had 78% less harvestable corn grain than + N treatments (Fig. 22). Residue return had no impact ( $P = 0.417$ ) on corn grain yield in 2010 and means were nearly identical (Fig. 22). Biomass yield (corn stover) from CS treatments in 2010 was lower than usual, likely due to late planting date combined with moderate drought stress (Fig. 21 and Table 6). Nitrogen fertilization had a slight ( $P = 0.087$ ) effect on CS stover or biomass yield in 2010 (Table 7). Surprisingly, N fertilization had less of an effect on corn yield than it did in grain yield, only increasing stover yield by 10% in 2010 (Fig. 23). We anticipated a better response to N fertilization, but the late planting date and moderate drought may have reduced the potential for the corn to take full advantage of additional N. Residue return had no effect ( $P = 0.806$ ) on corn stover yield in 2010 (Table 7), and means from both return rates were nearly identical. Nitrogen fertilization had a stronger impact ( $P = 0.063$ ) on biomass C content of corn stover in 2010 than on total yield, but still only increased C assimilated by 11% (Fig. 24). Residue return had no effect ( $P = 0.721$ ) on corn stover C uptake in 2010 (Table 7).

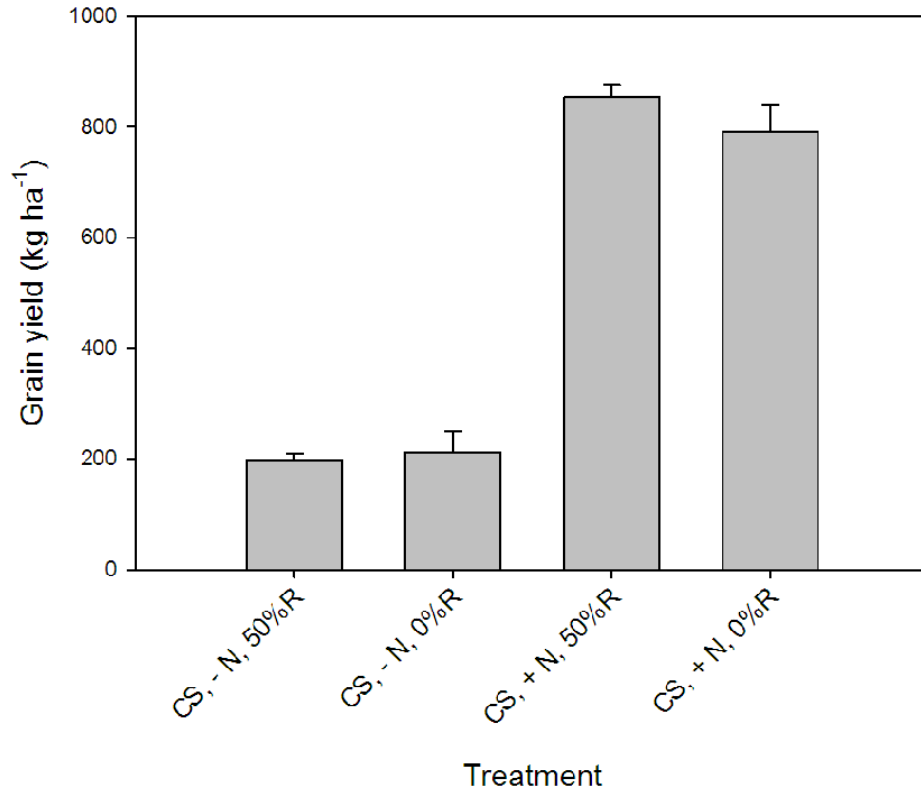


Figure 22. Mean corn grain yields in 2010 for treatment combinations of crop rotation (CS only), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).

The SS sequence showed a significant ( $P = 0.022$ ) increase in biomass yield due to N fertilization in 2010 (Table 7). Addition of N fertilizer increased total sorghum biomass dry yield by more than 58%. Returning half the residue (50%R) back to the field in 2009 generally improved SS yields in 2010, but the effect was not significant ( $P = 0.767$ ). The total dry biomass yields for SS in 2010 ranged from 8,100 to 13,600 kg ha<sup>-1</sup> (Fig. 23). Similar to yield, SS crop C content was significantly ( $P = 0.018$ ) affected by N fertilization in 2010 (Table 7). Plant C contents of SS treatments were increased nearly 61% by N fertilization in 2010 (Fig. 24). As anticipated, N appeared to be a limiting-nutrient in 2010 and N fertilizer was critical to improvement of both total yield and C content (Figs. 23 and 24). The crop C contents of SS ranged from 3,400 to 5,800 kg ha<sup>-1</sup> in 2010 (Fig. 24).

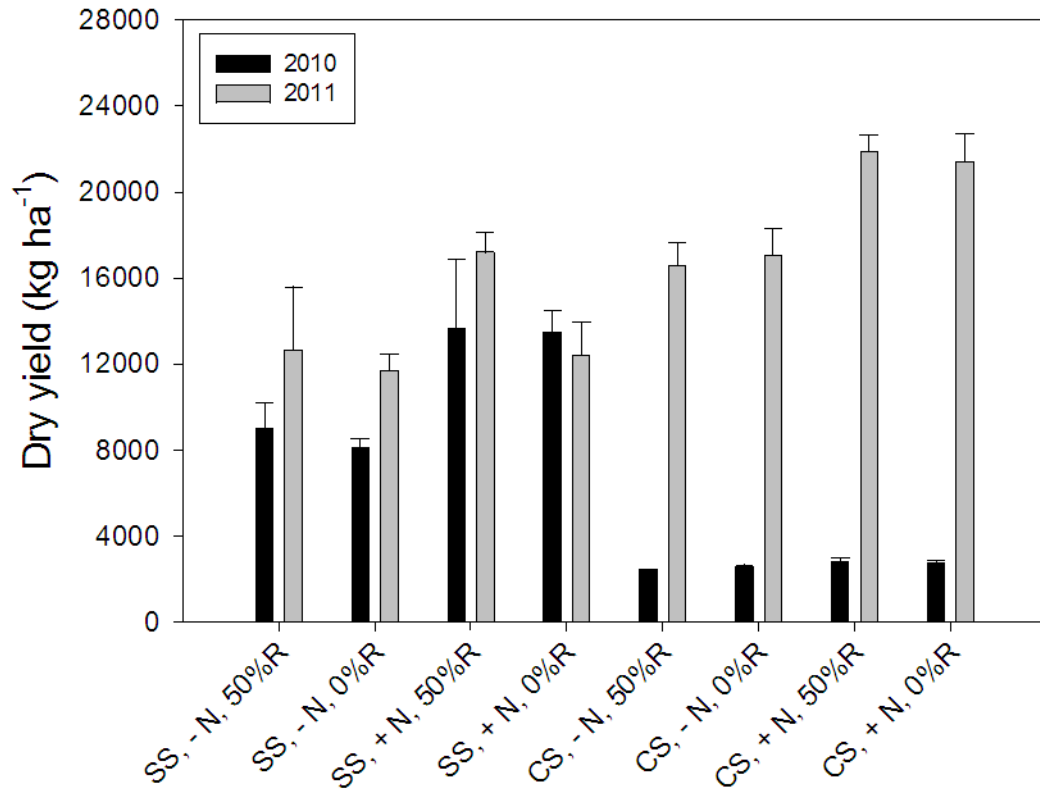


Figure 23. Mean corn stover or sorghum biomass dry yields in 2010 and sorghum biomass yields in 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (-N or +N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).



Table 7. Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) on corn stover or sorghum biomass yield (Yield), C uptake, and C:N from 2010 and 2011. Note: Crop rotations (CS and SS) analyzed separately in 2010 due to bimodal distribution.

Effect	Yield		C Uptake		C:N	Yield	C Uptake	C:N
	<i>p-value</i>							
			<u>2010</u>		<u>2011</u>			
	<i>CS<sup>†</sup></i>	<i>SS</i>	<i>CS<sup>†</sup></i>	<i>SS</i>				
Rotation	n/a	n/a	n/a	n/a	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.220
N-Fert.	<i>0.087</i>	<b>0.022</b>	<i>0.063</i>	<b>0.018</b>	0.194	<b>0.002</b>	<b>0.002</b>	<b>&lt;0.001</b>
Residue	0.806	<i>0.767</i>	0.721	<i>0.772</i>	0.660	0.182	0.186	0.415
Rotation*N-Fert.	n/a	n/a	n/a	n/a	<b>0.027</b>	0.305	0.277	0.324
Rotation*Residue	n/a	n/a	n/a	n/a	0.915	0.187	0.202	0.213
N-Fert.*Residue	0.521	0.850	0.501	0.880	0.644	0.263	0.240	0.226
Rotation*N-Fert.*Residue	n/a	n/a	n/a	n/a	<b>0.008</b>	0.493	0.527	<i>0.056</i>

<sup>†</sup>CS 'Yield' and 'C Uptake' were analyzed on corn stover in 2010.

Values in bold significant at 0.05 level.

Values in italics significant at 0.10 level.

In 2011, each crop sequence was in sorghum, allowing for yield comparison between the same crops. Despite each crop sequence being planted to sorghum in 2011, the strongest observed management effect was crop rotation ( $P = <0.001$ ) (Table 7). The CS treatments increased total biomass yield by 43% in 2011 compared to SS treatments (Fig. 23). The strong response to rotated sorghum vs. continuous sorghum may have been due to several factors including potentially stronger allelopathic effects (Nimbal et al., 1996; Weston and Duke, 2003) in SS treatments, differences in nutrient removal rates in each crop rotation (Wight et al., 2012), or other factors within each crop sequence. Yield was significantly ( $P = 0.002$ ) increased by N fertilization in 2011 (Table 7). Treatments receiving N had 26% more biomass yield than treatments that did not receive N fertilizer in 2011 (Fig. 23). Returning residue slightly improved harvested sorghum yield in 2011, although the effect was not significant ( $P = 0.182$ ) (Table 7). The CS treatments significantly ( $P = <0.001$ ) increased crop C assimilated compared to SS treatments in 2011, accruing 45% more C (Table 7 and Fig. 24). Plant C assimilated was significantly ( $P = 0.002$ ) improved with the addition of N fertilizer in 2011, with + N having 26% higher total plant C content (Table 7 and Fig. 24).

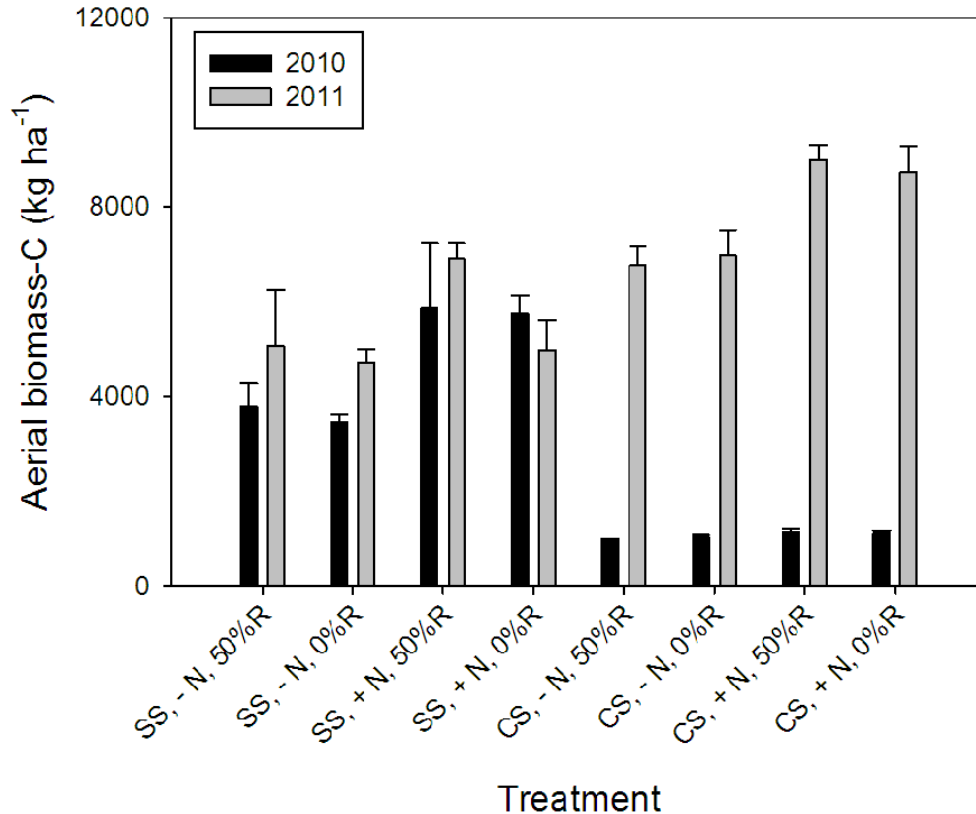


Figure 24. Mean aerial biomass-C yields in 2010 and 2011 for treatment combinations of crop rotation (CS only), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).

Crop management practices had no impact on the C:N ratio in 2009 and the ratios varied from 51 to 59 across all treatments harvested (Table 8). The C:N for each crop species in 2010 was significantly ( $P = <0.001$ ) different (Tables 7 and 8). It was anticipated that corn and sorghum would have different C:N ratios due to fundamental differences between the two crops. Within each crop rotation in 2010, sorghum had a C:N of 70 and corn had a C:N of 49 (Table 8). In 2011, when each crop rotation treatment was in the sorghum sequence, rotation did not affect C:N ( $P = 0.220$ ) (Table 7 and 8). However, N fertilization significantly ( $P = <0.001$ ) influenced the C:N of sorghum biomass (Table 7 and 8). The C:N of - N treatments was 62, while the C:N of + N treatments was 58 in 2011 (Table 8). The C:N ratio across all treatments in 2011 ranged from 44 to 79 in 2011.

Table 8. Mean (and standard error) residue return rate (reported as C) applied and C:N of residue from harvest in 2009, 2011, and 2012 for each treatment (n = 4).

Treatment	C applied (kg ha <sup>-1</sup> )			C:N		
	<u>2009</u>	<u>2010†</u>	<u>2011</u>	<u>2009</u>	<u>2010‡</u>	<u>2011</u>
SS, - N, 50%R	2738 (241)	1895 (248)	2534 (588)	59 (9)	79 (1)	77 (6)
SS, - N, 0%R	0	0	0	55 (5)	72 (3)	59 (6)
SS, + N, 50%R	3994 (468)	2935 (689)	3450 (176)	56 (4)	60 (5)	55 (4)
SS, + N, 0%R	0	0	0	51 (4)	71 (6)	58 (4)
CS, - N, 50%R	4932 (350)	987 (16)	3383 (210)	56 (5)	44 (4)	65 (3)
CS, - N, 0%R	0	1043 (41)	0	56 (5)	52 (3)	69 (5)
CS, + N, 50%R	4969 (55)	1133 (79)	4499 (164)	53 (6)	54 (1)	50 (3)
CS, + N, 0%R	0	1116 (50)	0	54 (5)	48 (3)	49 (3)

†100% of corn residue returned in CS treatments

‡ Represents corn residue in CS treatment

## Cumulative Annual Greenhouse Gas Emissions

Cumulative soil respiration emissions among treatments in 2010 were similar (Table 9 and Fig. 25). A moderate drought during the summer of 2010 and the continued lack of moisture in the fall of 2010 through the winter of 2011 (OSC, 2012) may have limited organic matter decomposition. A test of means found only marginal difference ( $P = 0.065$ ) in cumulative soil respiration-C from any of the cropping treatments analyzed in 2010 (data not shown). Among the SS sequence treatments, [SS, - N, 0%R] showed significantly less  $\text{CO}_2$  loss than the other three SS treatments (Fig. 25). Although not significant ( $P = 0.236$ ), SS treatment combinations usually lost more  $\text{CO}_2$  during 2010 than CS treatments, but the SS treatment receiving neither N fertilization nor residue return generally had the lowest emissions (Table 9). Residue return had a significant ( $P = 0.047$ ) impact on soil respiration during 2010 (Table 9), with 50%R increasing soil respiration-C by 13% compared to treatments receiving no residue return (Fig. 25). The effect of residue return on soil respiration was anticipated, since the greater amount of organic C applied to the soil in these treatments should potentially result in greater microbial activity and potentially greater  $\text{CO}_2$  fluxes via microbial decomposition. A rotation by residue interaction ( $P = 0.024$ ) was also observed in  $\text{CO}_2$  emissions in 2010 (Table 9). Regardless of N fertilization, SS treatments with 50%R had 22% higher losses of  $\text{CO}_2$  than CS, 50%R (Fig. 25). This may be partially explained by consistently high rates of C applied to SS treatments compared to CS (Table 8), potentially sustaining relatively higher microbial activity and labile pools of C which turnover quickly.

The entire field was planted to sorghum in 2011 and we anticipated that less difference in soil respiration-C loss between treatments would be observed in 2011 than in

2010. However, differences did occur, possibly due to a number of factors, including differences in yield/plant productivity within each cropping system (Fig. 23), amounts and composition of residue returned to the soil (Table 9), or potential differences in soil physical properties between the treatments. The [SS, + N, 50%R] treatment continued to have some of the highest cumulative soil respiration emissions in 2011 (approximately 14,000 kg CO<sub>2</sub>-C ha<sup>-1</sup>), while some of the lowest emissions of CO<sub>2</sub> were observed in the [CS, - N, 50%R] treatment (approximately 9,000 kg CO<sub>2</sub>-C ha<sup>-1</sup>) (Fig. 25). The cumulative soil respiration emissions observed in [SS, + N, 50%R] treatments were more than twice the total biomass C from 2010 (Figs. 24 and 25). While this seemed extreme, we reiterate these emissions were direct soil emissions which represent the sum of heterotrophic and autotrophic soil respiration and may explain how the amount of C lost as CO<sub>2</sub> seems particularly high relative to plant biomass-C pool. Nitrogen fertilization had a significant ( $P = 0.013$ ) effect on soil respiration-C in 2011 (Table 9). Nitrogen fertilized plots lost nearly 13% more CO<sub>2</sub> than unfertilized plots (Fig. 25), potentially due to greater soil N availability and less immobilization, greater soil organic matter (SOM) decomposition, and enhanced plant productivity (and root respiration) with N addition. Residue return also significantly ( $P = 0.020$ ) influenced soil respiration in 2011 (Table 9), where 50%R had approximately 12% higher CO<sub>2</sub> emissions than 0%R (Fig. 25). The lower amount of organic matter available for decomposition in 0%R treatments likely contributed to lower soil respiration via microbial decomposition. A three-way rotation by N fertilization by residue return effect ( $P = 0.005$ ) was observed for soil respiration-C in 2011 as well (Table 9).

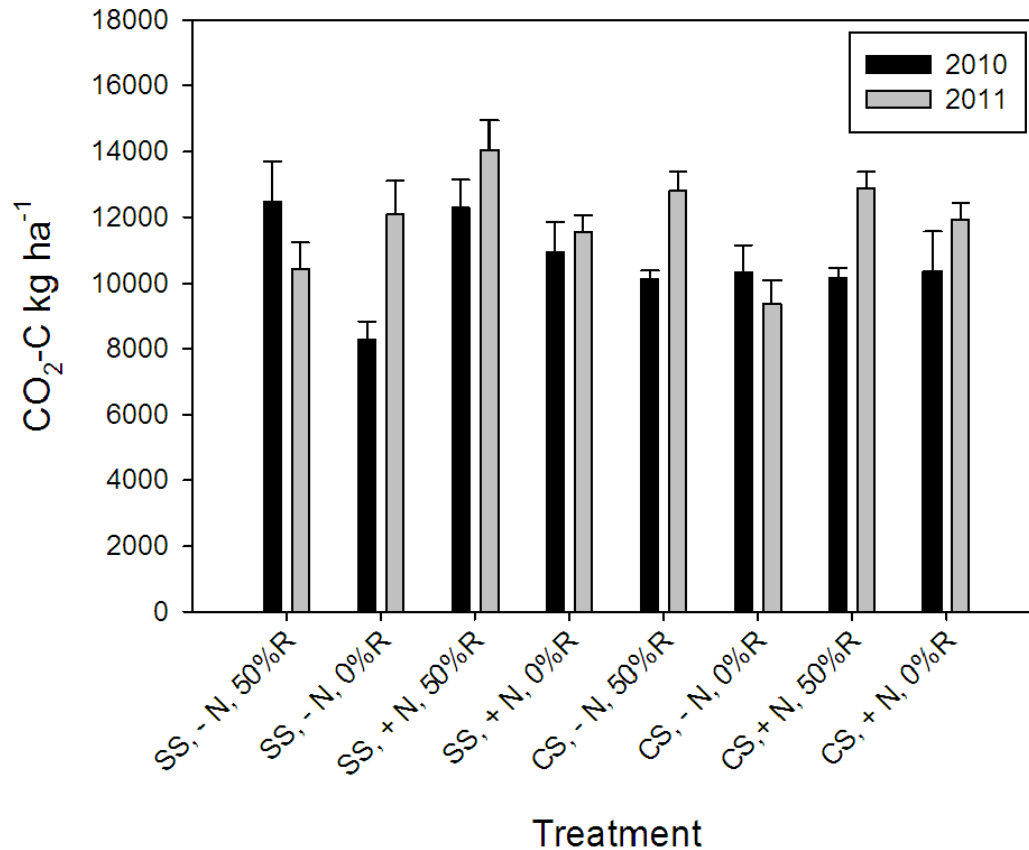


Figure 25. Mean cumulative soil respiration emissions from combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 3).

Table 9. Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) on cumulative soil respiration in 2010 and 2011.

Effect	Cumulative soil respiration	
	<i>p-value</i>	
	<u>2010</u>	<u>2011</u>
Rotation	0.236	0.589
N-Fert.	0.303	<b>0.013</b>
Residue	<b>0.047</b>	<b>0.020</b>
Rotation*N-Fert.	0.329	0.820
Rotation*Residue	<b>0.024</b>	0.101
N-Fert.*Residue	0.251	0.431
Rotation*N-Fert.*Residue	0.250	<b>0.005</b>

Values in bold significant at 0.05 level.

Values in italics significant at 0.10 level.

## Soil Organic Carbon

Several management practices affected SOC change at given depths in 2010. Most noticeable effects were observed in the surface 0 to 5-cm depth (Fig. 26). The SS rotation significantly ( $P = <0.001$ ) increased SOC at 0 – 5 cm compared to CS treatments (Table 10). The SS rotation generally increased in SOC over time more than CS treatments (Fig. 26). Similarly, residue return had a strong effect ( $P = <0.001$ ), where 50%R had significantly more SOC at the surface than 0%R (Fig. 26). Direct annual addition of C in biomass after each harvest was most likely responsible for higher SOC in the surface soil of 50%R treatments. A slight N fertilization effect ( $P = 0.073$ ) was observed; + N increased SOC in the surface compared to – N (Table 10 and Fig. 26). A significant N fertilization by residue return interaction ( $P = 0.016$ ) was observed in surface soil where regardless of crop rotation, + N, 50%R increased SOC relative to + N, 0%R (Table 10 and Fig. 26). This may have been related to differences observed in yield and subsequent amount of residue return applied to the soil; +N had more total biomass yield, which translated to greater quantities of residue to return than 0%R treatments.



Table 10. Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) by soil depth on change in SOC (since 2008) in 2010 and 2011.

Effect	Depth (cm)				
	0 - 5	5 - 15	15 - 30	30 - 60	60 - 90
	<i>p-value</i>				
	<u>2010</u>				
Rotation	<b>&lt;0.001</b>	0.711	0.790	<b>0.017</b>	<i>0.062</i>
N-Fert.	<i>0.073</i>	0.886	0.461	<i>0.056</i>	<i>0.079</i>
Residue	<b>&lt;0.001</b>	0.109	0.633	0.709	0.238
Rotation*N-Fert.	0.765	0.546	0.918	0.411	0.956
Rotation*Residue	0.351	0.908	0.106	<i>0.079</i>	0.716
N-Fert.*Residue	<b>0.016</b>	0.393	0.480	0.658	0.427
Rotation*N-Fert.*Residue	0.397	0.759	0.433	0.579	0.168
	<u>2011</u>				
Rotation	<b>0.010</b>	0.851	0.238	<b>0.027</b>	<b>0.010</b>
N-Fert.	0.453	0.237	0.354	0.209	0.569
Residue	0.119	0.216	0.153	0.430	0.625
Rotation*N-Fert.	0.144	0.657	0.102	0.368	0.875
Rotation*Residue	0.432	0.820	<b>0.047</b>	0.995	0.305
N-Fert.*Residue	0.502	0.719	0.367	0.177	0.529
Rotation*N-Fert.*Residue	0.476	0.782	0.176	0.402	0.208

Values in bold significant at 0.05 level.

Values in italics significant at 0.10 level.

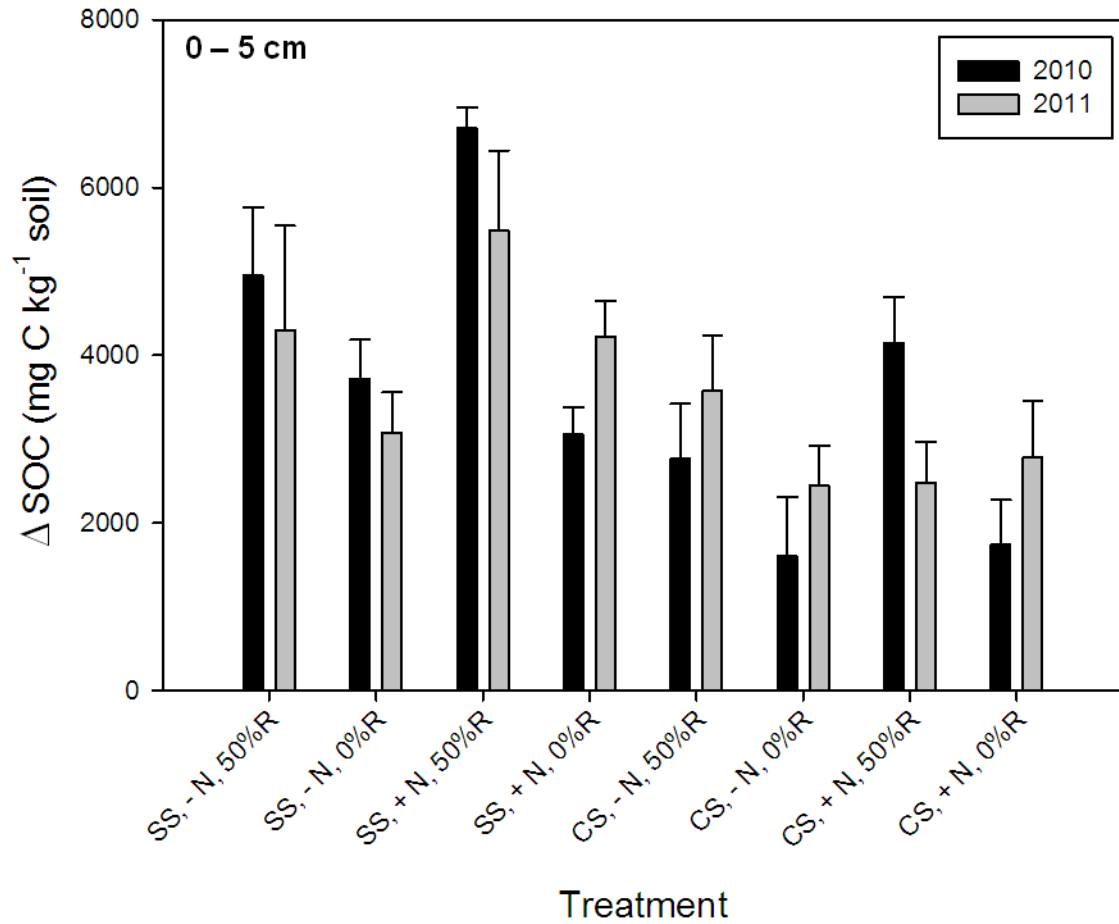


Figure 26. Mean change in soil organic C (SOC) in the 0 – 5 cm soil depth since 2008 from combinations of crop rotation [continuous sorghum (SS) or corn sorghum rotation (CS)], N fertilization rate (- N or + N), and residue return rate [zero biomass (0%R) or 50% biomass return (50%R)] are reported with one standard error (n = 4).

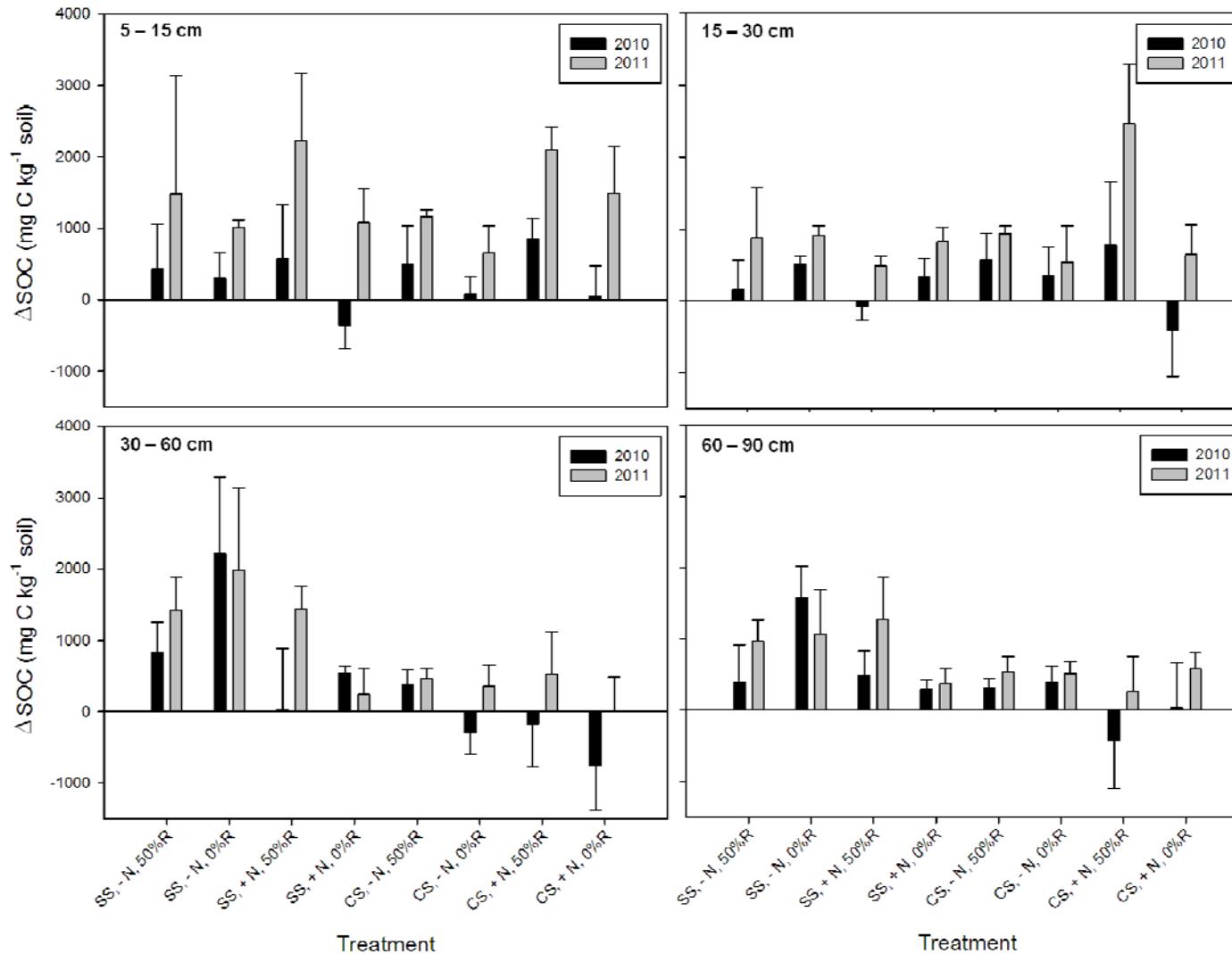


Figure 27. Mean change in SOC in 2010 and 2011 at soil depths of 5 – 15, 15 – 30, 30 – 60, and 60 – 90 cm since 2008 from combinations of crop rotation [continuous sorghum (SS) or corn-sorghum rotation (CS)], N fertilization rate (- N or + N), and residue return rate [zero biomass return (0%R) or 50% return (50%R)] are reported with one standard error (n = 4).

Below the surface, management practices also had some impact on SOC concentration. From 5 – 15 cm, the only potential management effect ( $P = 0.109$ ) in 2010 came from residue return; 50%R increased SOC over 0%R (Table 10 and Fig. 27). This may be related to carry-over of the residue effect at the surface, and light disk tillage may have failed to fully incorporate the biomass C returned beyond the full 15 cm desired. Another potential weak effect ( $P = 0.106$ ) in 2010 was detected at 15 – 30 cm, where a rotation by residue return interaction caused CS, 50%R to have higher SOC than CS, 0%R (Table 10 and Fig. 27). At 30 – 60 cm within the soil in 2010, a significant ( $P = 0.017$ ) crop rotation effect was found; SS soil at this depth exhibited significantly more SOC than CS treatments (Table 10 and Fig. 27). This may have been related to the relatively more consistent accrual of SOC over time from SS treatments compared to CS. Nitrogen fertilization also slightly affected ( $P = 0.056$ ) SOC from 30 – 60 cm; - N had more SOC than + N (Table 10 and Fig. 27). The addition of N fertilizer may have enhanced mineralization of SOM at depth in the soil profile, or decreased organic C deposited at depth (via plant roots). A weak rotation by residue return interaction ( $P = 0.079$ ) was found from 30 – 60 cm; SS, 0%R had greater SOC than CS, 0%R (Table 10 and Fig. 27). Harvesting all biomass may have had more of an impact on SOC in the SS treatments rather than the CS treatments because of relatively less SOC addition each year in the CS treatments. At the deepest sampling depth, 60 – 90 cm, two slight effects on SOC were detected from the experimental factors in 2010. The first observed potential effect at that depth was for crop rotation ( $P = 0.062$ ), where SOC in the SS treatments increased compared to CS (Table 10 and Fig. 27). This may have been related to the continued trend of slightly higher SOC accrual over time in the SS treatments relative to CS. The other management impact with a slight statistical effect ( $P = 0.079$ ) was N fertilization; - N had

more SOC than + N (Table 10 and Fig. 27). This may have been related to either additional SOM mineralization from the addition of N and subsequent reduction in SOC, or increased root-derived C from unfertilized treatments.

In 2011, fewer detectable effects from management practices were found on the change in SOC from the initiation of the study (2008). Consistent with the strongest SOC changes detected in the 2010 analysis, most of the detectable differences in SOC were attributable to crop rotation (Table 10 and Fig. 28). The monoculture sorghum significantly increased SOC at the surface ( $P = 0.010$ ) and at 30 – 60 cm of depth ( $P = 0.027$ ) compared to the CS rotation (Table 10 and Figs. 26 and 27). The trend continued down to 60 – 90 cm, where a slight ( $P = 0.099$ ) rotational effect was observed and SS still had higher SOC than CS (Table 10 and Figs. 27 and 28). Despite being marginally significant, the trends of SOC change below 30 cm were consistent and increased in both 2010 and 2011 (Fig. 27). In 2010, SOC in SS had increased by 908 and 693 mg C kg<sup>-1</sup> soil since 2008 at both 30 – 60 and 60 – 90 cm, respectively (Fig. 27). In 2011, the trend of increasing SOC continued as SS increased SOC by 1271 and 921 mg C kg<sup>-1</sup> soil since 2008 at 30 – 60 and 60 – 90 cm, respectively (Fig. 27). Thus, despite weaker significance levels at the lower depths, the trend of consistently increasing SOC in SS treatments since 2008 prevailed. Two management interaction effects on SOC were found at 15 – 30 cm in 2011. First, a weak ( $P = 0.102$ ) rotation by N fertilization interaction was observed; CS, + N had more SOC than SS, + N (Table 10 and Fig. 27). This result was rather surprising, considering CS produced less biomass in 2010 while in the corn phase of the corn-sorghum rotation. The SS sequence with N addition may have provided greater amounts of labile C belowground in 2010, which could have acted as a priming effect, enhancing mineralization temporarily. The stronger

effect ( $P = 0.047$ ) observed at 15 – 30 was a rotation by residue return interaction; CS, 50%R had significantly more SOC accumulation since 2008 than CS, 0%R (Table 10 and Fig. 27). The impact of returning residue may be more significant in the CS rotation because of slightly higher recalcitrant (higher C:N) nature of the SS residue in 2011 (Table 8), which likely reduced decomposition rate and potentially enhanced longer term C accrual. While SOC generally increased across most plots since 2008, N fertilization and residue return showed no significant influence over change in SOC (Table 10 and Fig. 29).

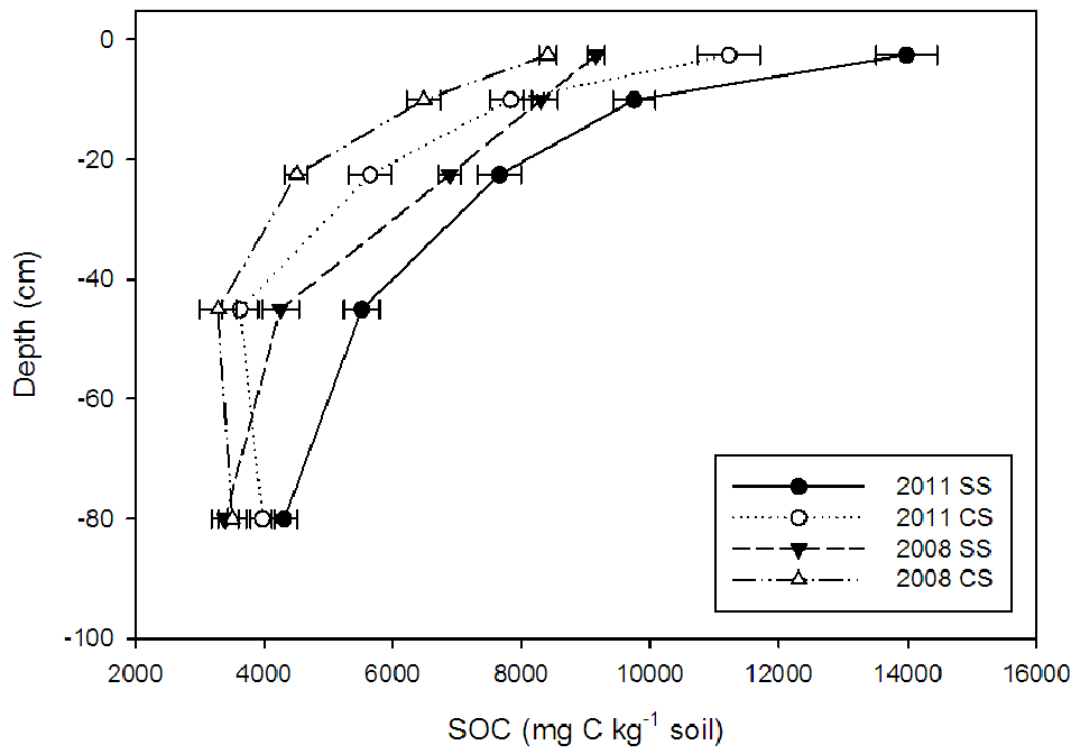


Figure 28. Soil organic C (SOC) concentrations with depth under bioenergy sorghum production. Field soils were sampled at five depth intervals: 0-5, 5-15, 15-30, 30-60, and 60-90 cm and compared by crop rotation [continuous sorghum (SS) or corn-sorghum rotation (CS)] in 2008 and 2011. Horizontal bars indicate standard error of the mean ( $n = 4$ ).

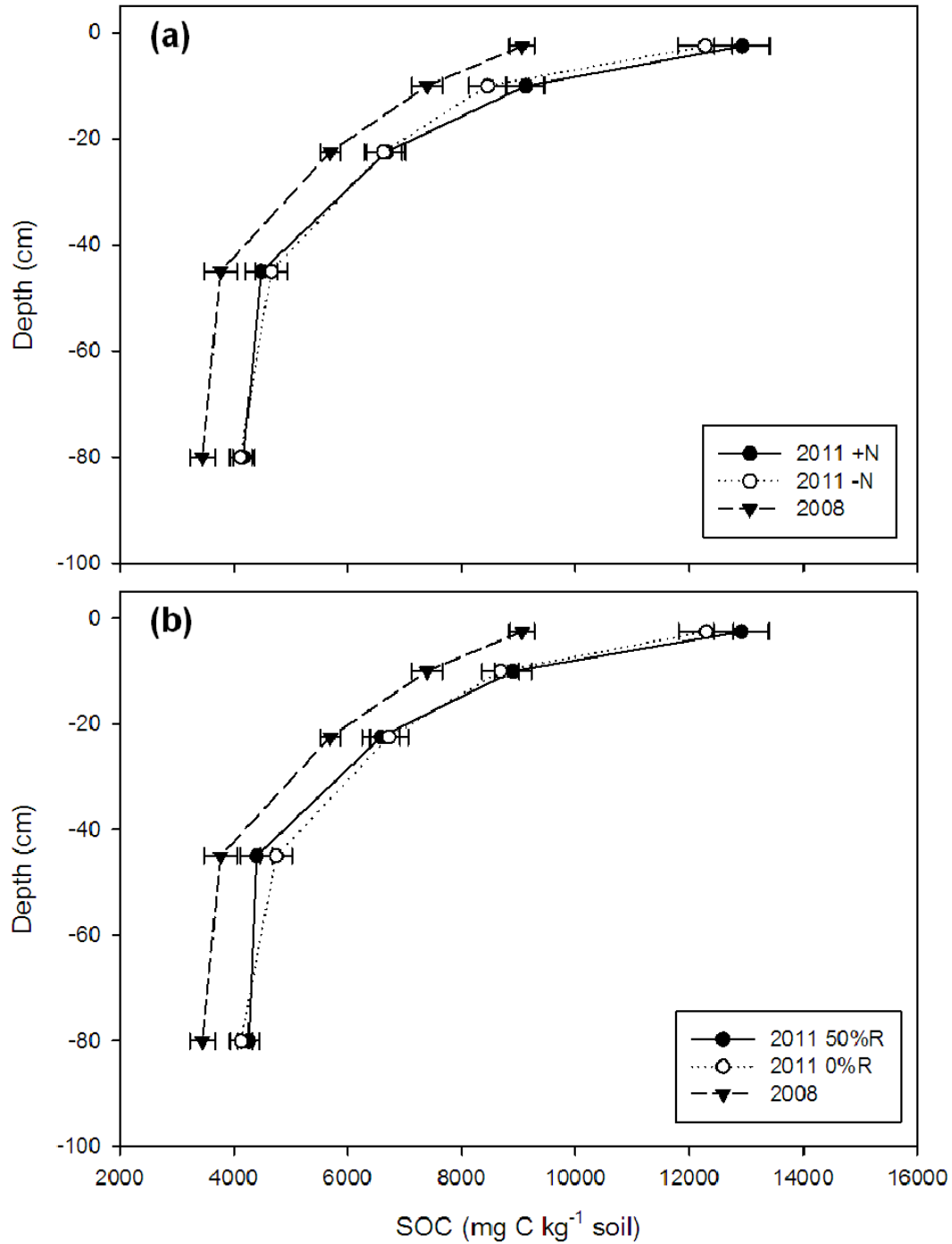


Figure 29. Soil organic C (SOC) concentrations with depth under bioenergy sorghum production. Field soils were sampled at five depth intervals: 0-5, 5-15, 15-30, 30-60, and 60-90 cm and compared by N fertilization rate (+N or -N) (a) and residue return rate [zero biomass return (0%R) or 50% return (50%R)] (b) in 2011 to initial (2008) concentrations. Horizontal bars indicate standard error of the mean (n = 4).

## **Soil Microbial Biomass-Carbon**

Soil microbial biomass-C (SMBC) was measured in 2008 to provide a baseline for future analyses. Ideally, analyses would have revealed no detectable impacts from the three primary factors (crop rotation, N fertilization, or residue return) in 2008, prior to the start of the field experiment. However, an apparent crop rotation effect was identified. In 2008, analyses determined SS to have significantly greater SMBC than CS treatments from 0 – 5 (P = <0.001) and 5 – 15 cm (P = 0.001) (Fig. 10). The spatial layout of the experimental design in the field coincided with natural soil textural differences within the field. SS treatments were located in a portion of the field which generally contains higher clay content and lower silt content than the CS treatments. Thus, we do not believe an effect was truly present at the beginning of the study, rather natural spatial variation attributed to soil textural differences correlated with SS treatments in the field experiment. To remedy this issue, differences in SMBC attributed to bioenergy sorghum management practices in 2011 were analyzed on a net difference (2011 minus 2008 SMBC) basis in addition to the normal analysis to remove potential bias in crop rotation treatments (Fig. 30 and Table 11). Years were analyzed separately due to a significant effect on SMBC (P = <0.001) and SOC as SMBC (P = <0.05) at both depths examined (Figs. 30 and 31).



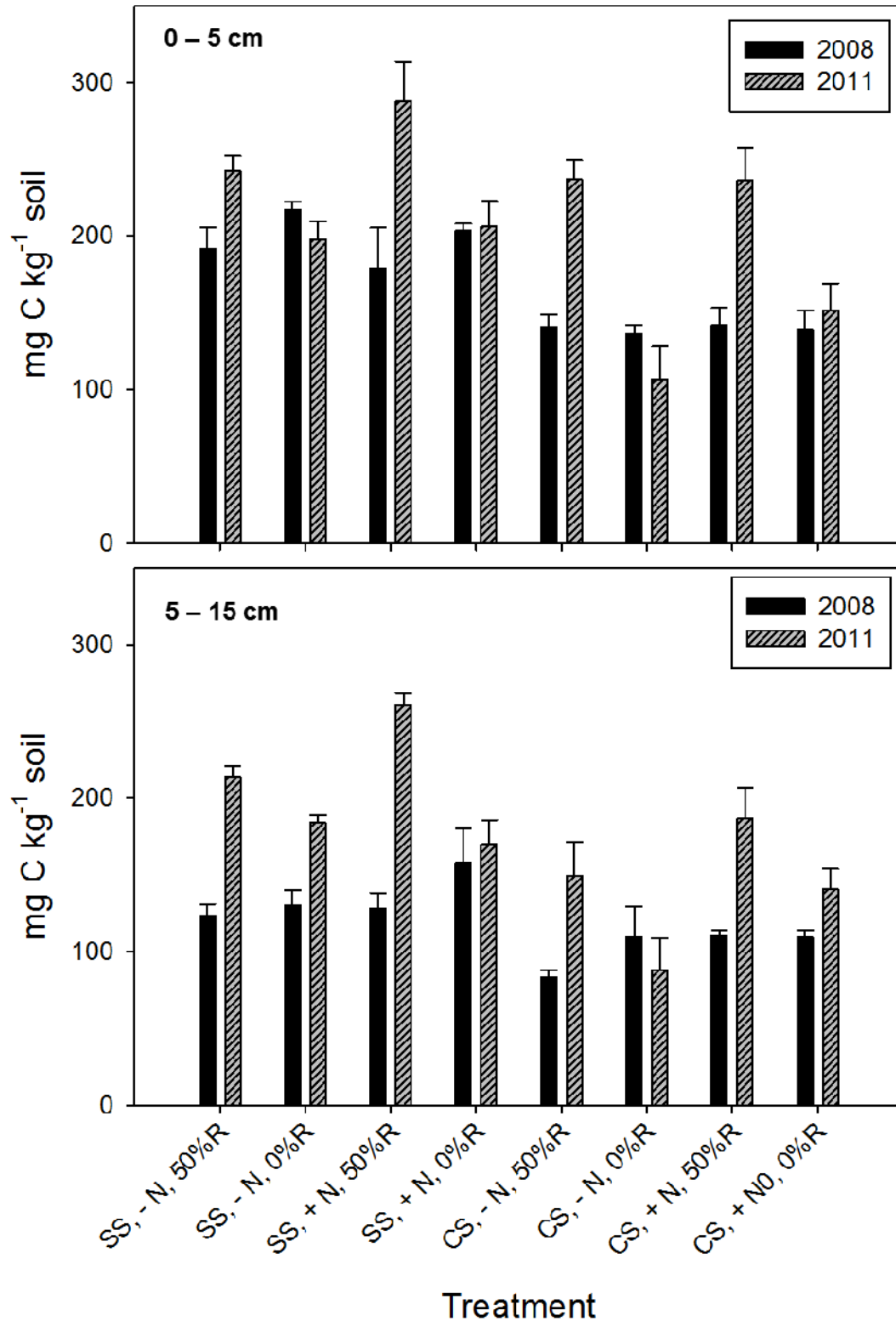


Figure 30. Mean soil microbial biomass C (SMBC) from 0 – 5 and 5 – 15 cm in 2008 and 2011 from combinations of crop rotation [continuous sorghum (SS) or corn-sorghum rotation (CS)], N fertilization rate (- N or + N), and residue return rate [zero biomass return (0%R) or 50% return (50%R)] are reported with one standard error (n = 4).

Table 11. Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) by soil depth on SMBC in 2011, change in SMBC (since 2008) and percentage of SOC as SMBC in 2008 and 2011.

Effect	SMBC <sup>†</sup>		Δ SMBC		% SOC as SMBC		% SOC as SMBC	
	0 - 5 cm	5 - 15 cm	0 - 5 cm	5 - 15 cm	0 - 5 cm	5 - 15 cm	0 - 5 cm	5 - 15 cm
	<i>p-value</i>							
	<u>2011</u>		<u>(2011 - 2008)</u>		<u>2008</u>		<u>2011</u>	
Rotation	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.597	<b>0.023</b>	<b>0.004</b>	0.904	0.849	<b>0.029</b>
N-Fert.	<i>0.067</i>	<b>0.010</b>	<i>0.055</i>	0.264	0.364	<i>0.094</i>	0.374	0.175
Residue	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.643	0.759	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Rotation*N-Fert.	0.862	0.214	0.526	0.285	0.441	0.395	0.425	0.145
Rotation*Residue	<i>0.094</i>	0.782	0.607	0.662	0.149	0.519	<i>0.070</i>	0.365
N-Fert.*Residue	0.865	0.302	0.887	0.456	0.743	0.399	0.791	0.644
Rotation*N-Fert.*Residue	0.120	<i>0.095</i>	0.189	0.033	0.810	0.127	0.320	0.265

<sup>†</sup> Unmodified SMBC concentrations from 2011

Values in bold significant at 0.05 level.

Values in italics significant at 0.10 level.

In 2011, each of the three primary factors impacted the unmodified SMBC concentrations at both 0 – 5 and 5 – 15 cm depths (Fig. 30 and Table 11). As anticipated, depth impacted the distribution of SMBC, near-surface contained significantly ( $P = <0.001$ ) more SMBC than the subsurface depth (Fig. 30). At 0 – 5 cm, 50%R significantly ( $P = <0.001$ ) increased SMBC compared to 0%R (Fig. 30 and Table 11). The addition of biomass C back to the soil likely created an environment where microbial activity was less limited by organic C sources. Crop rotation significantly ( $P = <0.001$ ) also affected SMBC at 0 – 5 cm; SS had more SMBC than CS (Table 11 and Fig. 30). This result may have been a carryover effect from the 2008 observations or the SS rotation may have supplied relatively greater SOC annually, which may have enhanced microbial activity, compared to CS treatments. A marginal ( $P = 0.067$ ) N fertilization effect was found at the surface, where +N treatments had higher SMBC compared to – N (Table 11 and Fig. 30). The same trends observed at the surface in 2011 were consistent with trends observed in the subsurface (5 – 15 cm), with even higher significance (Table 11). Residue return and crop rotation each had highly significant ( $P = <0.001$ ) impact on SMBC in the subsurface; SS increased SMBC compared to CS and 50%R increase SMBC compared to 0%R (Table 11 and Fig. 30). The causes for each of the effects were probably similar to those observed at the surface, including potential 2008 carryover effect from the rotational treatments. Nitrogen fertilization was a stronger ( $P = 0.010$ ) influence on SMBC in the subsurface than at the surface; + N treatments continued to have higher SMBC than – N (Table 11 and Fig. 30). The consistency in impacts of the management practices from the surface to the subsurface soil may have been caused by light disk tillage which mixed the surface layers of the soil.

Due to the crop rotational effect observed at the start of the study on SMBC data, analysis was performed on the difference of 2008 SMBC subtracted from 2011 SMBC. The difference estimates potentially removed bias previously observed due to soil textural differences coinciding with the crop rotation treatments in the experimental layout. Fewer differences were found for the difference values than for the unmodified 2011 SMBC values (Table 11). Residue return had a highly significant ( $P = <0.001$ ) impact on change values for SMBC at both 0 – 5 and 5 – 15 cm depths (Table 11). Crop rotation influenced the vertical distribution of SMBC. Continuous sorghum significantly ( $P = 0.023$ ) increased SMBC in the subsurface (5 – 15 cm), but not directly at the surface (0 – 5 cm) (Table 11). This effect was somewhat surprising because in 2011, CS was in the sorghum sequence of the rotation and had higher yields than SS. However, since SS consistently added more C to the SOC pool each year, compared to CS (Table 8), it may help explain increases in SMBC in the subsurface. Nitrogen fertilization had a marginal effect ( $P = 0.055$ ) on SMBC in surface soil; + N treatments increased SMBC compared to – N (Table 11).

Soil microbial biomass-C represents a small, but important portion of the total SOC pool. The proportion of SOC which was attributable to SMBC was determined at the initiation of the study and again in 2011 from 0 – 5 cm and 5 – 15 cm. The effect of crop rotation, N fertilization, and residue return on percentage of SOC as SMBC was determined each year at each sampled depth (Table 11). Differences were found and none of the observed effects were similar between 2008 and 2011 (Fig. 31 and Table 11). There was a difference in the vertical distribution of SOC as SMBC. The fraction of SOC as SMBC was significantly ( $P = 0.015$ ) greater from 0 – 5 cm (1.86%) than from 5 – 15 cm (1.64%) (Fig. 31 and Table 11). At the initiation of the study, the contribution of SMBC to the total SOC

pool was higher ( $P = 0.004$ ) in SS treatments than CS (Table 11 and Fig. 31). This observed effect was likely due to the inherent spatial soil textural variation in the field which aligned with location of rotational treatments; higher percentage clay found in most SS treatments than CS. The only other potential effect was a marginal ( $P = 0.094$ ) N fertilization effect (Table 11); + N caused a greater percentage of SOC to be attributable to SMBC than – N (Fig. 31). Such a marginal effect observed at the initiation of the study may have been simply attributable to inherent spatial variation which favored + N treatments, though there was no detectable textural correlation with + N and – N treatments similar to SS and CS treatments at the beginning of the study. A difference in the vertical distribution of SOC as SMBC was observed from the initiation of the study to 2011. The amount of SOC as SMBC was significantly ( $P = 0.042$ ) higher from 0 – 5 cm in 2008 compared to 2011 (Fig. 31). In contrast, the proportion of SOC as SMBC was significantly ( $P = 0.002$ ) lower from 5 – 15 cm in 2008 compared to 2011 (Fig. 31).

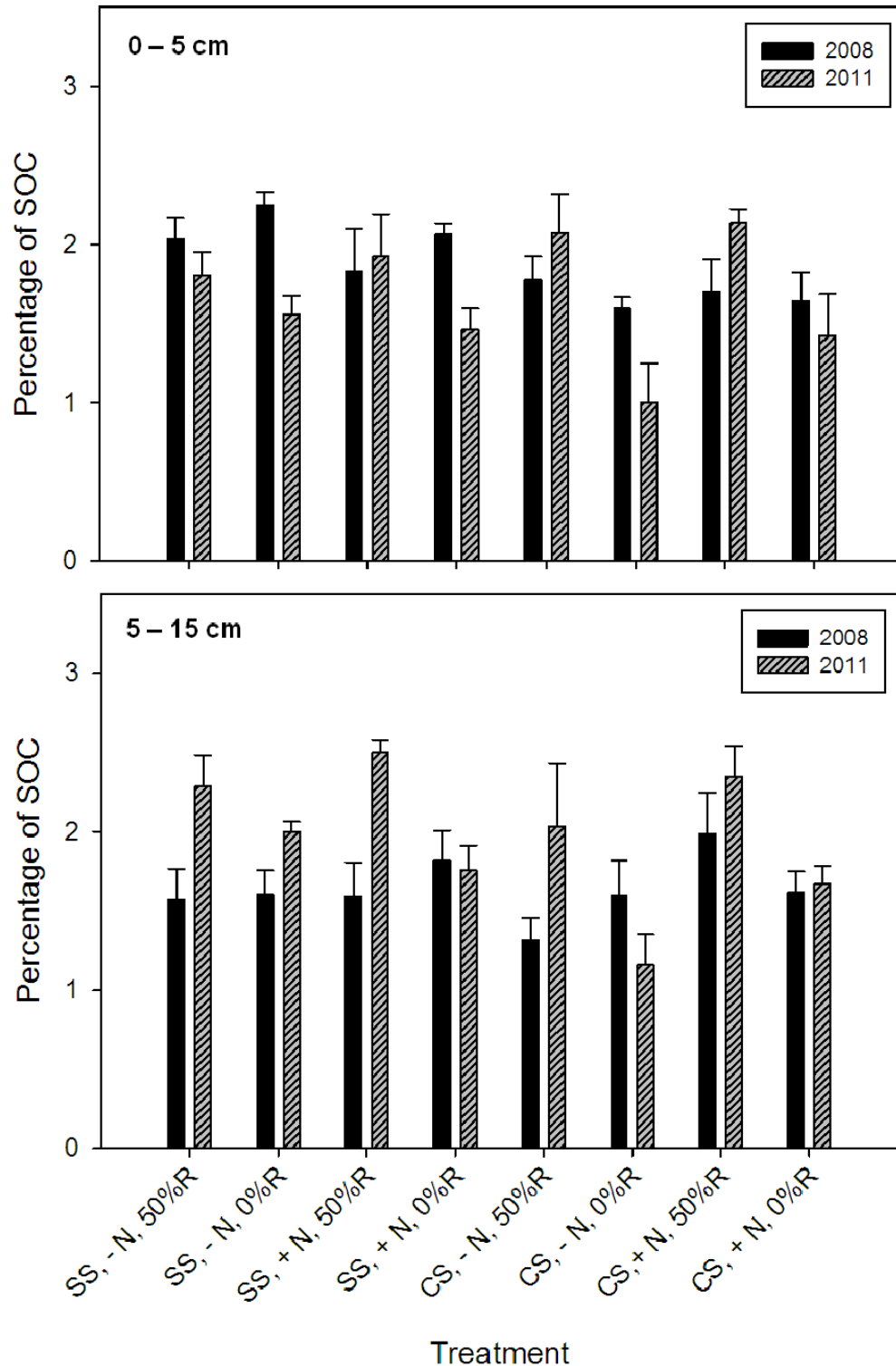


Figure 31. Mean percentage of soil organic C (SOC) as soil microbial biomass C (SMBC) from 0 – 5 and 5 – 15 cm depths in 2008 and 2011 from combinations of crop rotation [continuous sorghum (SS) or corn-sorghum rotation (CS)], N fertilization rate (- N or + N), and residue return rate [zero biomass return (0%R) or 50% return (50%R)] are reported with one standard error (n = 4).

After four full growing seasons, the management practices had modified the percentage of SOC as SMBC. The surface soil had a significantly ( $P = 0.005$ ) smaller fraction (1.67%) of SOC as SMBC than from the subsurface (1.97%) (Fig. 31). The percentage of SMBC comprising total SOC in 2011 was affected by two of the primary agronomic management practices examined. Returning half of the biomass back to the soil (50%R) significantly ( $P = <0.001$ ) increased the fraction of SOC attributed to SMBC from 0 – 5 and 5 – 15 cm (Table 11). This result aligned with trends in SMBC which found residue return increased the overall size of the SMBC pool at both examined depths. Crop rotation significantly ( $P = 0.029$ ) influenced the proportion of SMBC as SOC from 5 – 15 cm; SS increased SMBC as SOC compared to CS (Table 11 and Fig. 31). This effect may have been attributed to a greater annual increase in SOC in SS (Table 10 and Fig. 28), which could enhance overall microbial activity. Finally, SOC as SMBC at the surface was weakly ( $P = 0.070$ ) influenced by a crop rotation by residue interaction; SMBC in CS, 50%R was a greater portion of SOC than CS, 0%R (Table 11 and Fig. 31). Similar to an observation in SOC, the impact of residue return in CS treatments may be enhanced due to relatively less consistent organic C addition for the entirety of the study.

## **DISCUSSION**

### **Plant Carbon Pool**

Sorghum yields in 2010 were generally lower than those observed in 2011 (Fig. 23). Sorghum yields for different treatments in 2010 ranged from 8 to 14 Mg ha<sup>-1</sup>, and from 12 to 22 Mg ha<sup>-1</sup> in 2011. While yields from 2010 were lower than anticipated, the yields from 2011 were similar to those observed by several studies utilizing bioenergy sorghum [13.1 –

30.1 Mg ha<sup>-1</sup>] (Goff, et al., 2010; Ra et al., 2012; Rocateli et al., 2012; Tamang et al., 2011; Wight et al., 2012). Lower yields in 2010 may have been due to a later planting date coupled with a moderate drought and less irrigation (Table 6 and Fig. 21).

The plant C pool (yield and C assimilated) was significantly influenced by agronomic management practices in 2010 and 2011, but did not share the same results between years. In 2010, cropping sequence significantly increased biomass yield and C assimilated, as SS had much higher biomass yield and C assimilation than CS, largely due to CS being in the corn phase of the rotation. However, in 2011, when both crop sequence treatments were in sorghum, CS treatments had significantly larger total biomass yield and C content. This aligns with previous work which found significantly higher sorghum biomass yields following corn (Wight et al., 2012). These findings illustrated tradeoffs associated with continuously cropped versus rotated sorghum. While corn was in production, total biomass yields and C assimilated were decreased, yet the following year, the yields rebounded and exceeded those in the SS sequence.

As anticipated, N fertilization significantly improved biomass yield and crop C assimilated over unfertilized treatments in both 2010 and 2011. Nitrogen fertilization increased yield and C uptake in years 2008 and 2009 of the study at our location (Wight et al., 2012). Additional N was shown to improve yield and total C content of both sorghum and corn, illustrating the need for N fertilization to sustain high yields across years. This result agrees with results from long-term modeling of bioenergy sorghum production in the U.S.; N addition was required to sustain high yield over time (Meki et al., 2013).

Residue return had no detectable impact on biomass yield or crop C assimilated in 2010 or 2011. Reported effects of residue removal in sorghum cropping systems have varied.



Harvesting half the aboveground sorghum biomass reduced yield and overall soil quality after three cropping seasons (Powell and Hons, 1991). A recent study found that returning 25% of aboveground sorghum biomass from the first harvest of the season improved yields of the second harvest and was believed to have been related to increased soil moisture retention with the return (Wight et al., 2012). Results from modeled bioenergy cropping systems in the southern U.S. determined that 75% of aboveground biomass yields could be sustainably harvested at a rate of more than 15 Mg ha<sup>-1</sup> for 50 years under no-till management (Meki et al., 2013). Similar to previous work (Wight et al., 2012), our study found no impact on sorghum yields from 0% or 50% residue return rates. This study cannot determine potential yield improvements attributed to returning 25% of aboveground residue to the soil, as others have. However, it may take a longer period of time to detect the impacts of residue return, as the soil used may contain residual nutrients sufficient to satisfy relatively high yield productivity in the short term. Biomass residue returned represents a valuable pool of C and nutrients which aides in sustaining long-term soil health (Lal, 2009) and harvesting all biomass can mine the soil of these vital nutrients (Meki et al., 2013).

### **Soil Respiration Carbon**

Cumulative soil respiration measurements were higher than those observed by many other studies across North America albeit little research has been performed in the southern U.S. (Franzluebbers and Follett, 2005) and no published data was available from bioenergy sorghum cropping systems. Emissions of CO<sub>2</sub> in this study do not necessarily represent those going directly to the atmospheric C pool because measurements taken at the soil surface represent soil respiration (microbial and root respiration) and do not account for CO<sub>2</sub> that

may have been taken up by plants before exiting the plant canopy. Cumulative soil respiration emissions in this study averaged 10,600 and 11,900 kg CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup> in 2010 and 2011, respectively. These values are on the higher side of the range of cumulative respiration emissions (2,300 to 10,500 kg CO<sub>2</sub>-C ha<sup>-1</sup> yr<sup>-1</sup>) reported from other cropping systems (Adviento-Borbe, Haddix, et al., 2007; Alvarez et al., 1995; Heller et al., 2010; Hernandez-Ramirez et al., 2009; Ussiri and Lal, 2009), but do not appear unrealistic considering the humid subtropical climate and the long lifecycle of bioenergy sorghum. Cumulative soil respiration emissions in 2011 were significantly (P=0.016) larger than emissions from 2010 (Fig. 25). The higher temperatures throughout 2011 may partially explain this observation in addition to a following mild, wet fallow season.

Climatic factors at our location probably also contributed to higher than average cumulative soil respiration emissions. Numerous studies cite air (Adviento-Borbe et al., 2007; Adviento-Borbe et al., 2010; Almaraz et al., 2009; Ussiri and Lal, 2009) and soil (Adviento-Borbe et al., 2007; Adviento-Borbe et al., 2010; Allaire et al., 2012; Hernandez-Ramirez et al., 2009; Liebigh et al., 2010; Ussiri and Lal, 2009) temperature as drivers of soil respiration rates. A study of corn-soybean cropping systems in Iowa determined daily root-derived respiration emissions were influenced primarily by environmental conditions, particularly temperature (Van Vleck and King, 2011). Microbial activity increases with increasing temperature which directly influences soil respiration. Air temperatures throughout the growing season frequently exceeded 30°C (data not shown), and mean annual temperatures in 2010 and 2011 were 21.8 and 22.6°C, respectively (Fig. 21). These observations are higher than the temperatures reported in many other studies of soil respiration (Adviento-Borbe et al., 2007; Adviento-Borbe et al., 2010; Allaire et al., 2012;

Almaraz et al., 2009; Hernandez-Ramirez et al., 2009; Liebig et al., 2010; Ussiri and Lal, 2009) and were generally sustained for a longer period of time. Lower soil respiration rates observed during the fallow season were likely related to the lack of autotrophic respiration, reduced microbial activity due to less active rhizodeposition, and lower temperatures which reduced both autotrophic and heterotrophic microbial activity. Mean monthly temperatures during the fallow months rarely exceeded 20°C (Fig. 21).

Cumulative respiration differences between treatments were likely due to distinct differences in both autotrophic (plant root) and heterotrophic (soil microbe) respiration. Similar to Adviento-Borbe et al. (2010), we hypothesized that treatments exhibiting higher cumulative soil respiration would correspond to higher crop yield, due to increased photosynthetic activity. However, the highest yielding treatments did not directly correspond to those treatments with the highest respiration values (Figs. 23 and 25), particularly in 2011, suggesting that autotrophic, or plant root, respiration was not the primary cause of differences in cumulative soil respiration emissions among treatments (Adviento-Borbe et al., 2010). While the amount of autotrophic respiration sorghum roots contributed to cumulative soil respiration is unknown, recent research on corn root-derived respiration found less than 10% of total soil respiration during the growing season was attributed to corn root respiration (Van Vleck and King, 2011). This research supports our observations that crop rotation had limited impact on cumulative soil respiration (Table 9). We anticipated that corn and sorghum would have different cumulative soil respiration quantities in 2010, particularly because of the relatively long life cycle of sorghum compared to corn. However, rotation did not affect soil respiration, rather residue return influenced cumulative soil respiration in 2010 and 2011 (Table 9). The 50%R treatments significantly increased SMBC (Table 11), which

subsequently may have increased cumulative soil respiration (heterotrophic microbial respiration). Our result supports the aforementioned study which found the contribution of microbial respiration to overall soil respiration to be much larger than crop root-derived respiration (Van Vleck and King, 2011).

Nitrogen fertilization increased cumulative soil respiration in 2011, but not 2010. Increased soil respiration following N fertilization has been frequently observed in other cropping systems (Adviento-Borbe et al., 2010; Alluvione et al., 2010; Ussiri and Lal, 2009). Enhanced SOM and SOC mineralization (heterotrophic respiration) has been attributed to N fertilization (Khan et al., 2007; Kuzyakov et al., 2000). Nitrogen addition and the form of N fertilizer applied can have a significant influence on cumulative root-derived soil respiration emissions as well (Van Vleck and King, 2011). Furthermore, the addition of N increased crop yields (primary productivity) (Figs. 22 and 23 and Table 7) and likely autotrophic respiration and belowground C allocation. We also suspect the physical disturbance of soil via knifing of urea to 10-cm depth, increased plant growth, as well as enhanced soil organic N mineralization via additional N availability, may have been contributing factors.

### **Soil Organic Carbon Pool**

The SOC pool in 2010 exhibited more differences due to management practices than in 2011. Each of the three primary factors (crop rotation, N fertilization, and residue return) impacted SOC at one or more depths in 2010. The surface soil was impacted by all three management practices. Any treatment which had SS, + N, or 50%R had higher SOC at the surface 0 – 5 cm compared CS, - N, or 0%R treatments, respectively, since 2008.

Residue return significantly increased SOC at 0 – 5 and 5 – 15 cm depths. In 2009, all rotation treatments were in the sorghum sequence, and all biomass returned was sorghum residue. When sorghum was grown, residue returned was a greater mass than returning 100% of corn residue (Table 8). The relatively large amount of sorghum residue disked into the soil from the 2009 harvest likely caused an increase in SOC in the top 15 cm of soil. The increase in SOC near the surface of the soil due to residue return is in agreement with long-term model estimates of SOC change from similar management practices (Meki et al., 2013). However, this effect was not observed in 2011, as residue return had no detectable effect on SOC at any of the depths examined. Corn residues were not harvested/removed in the study, and when CS was in the corn sequence, 100% of corn residues were returned to the soil. The impact of returning all corn residue (despite contributing a relatively smaller mass of C compared to sorghum), may have negated a residue return impact from 50%R from the 2010 harvest (impact on 2011 SOC) in CS treatments. Thus, applying 100% of corn residue back to the plot, regardless of residue return rate, may have negated the direct effect of residue return in 2011. The residue return effect may not have been strong enough to prevail over two years. However, long-term modeling suggests returning sorghum residue will increase SOC near the surface (Meki et al., 2013).

Nitrogen fertilization significantly impacted SOC in 2010, but had no detectable impacts in 2011. Interestingly, N fertilization did not have the same impact on SOC at the surface as it did on SOC at deeper soil depths. At the surface (0 – 5 cm), N fertilization significantly increased SOC, and the increase may have been related to increased primary productivity. Nitrogen fertilization increased biomass yield and C assimilation, which often translates to additional crop residues returned to the soil (Lemke et al., 2010). Larger biomass

yield may have caused larger plant crowns to be left near the surface following harvest in + N treatments compared to – N treatments. Additionally, N fertilization may have also stimulated greater root activity near the surface in + N treatments compared to – N treatments. The greater mass of root-C or greater density of roots may have directly contributed to increases in SOC at the surface (Lemke et al., 2010). Below 30 cm, N fertilization significantly decreased SOC. This result aligns with some long-term studies of change in SOC over time with the application of N fertilizers to various corn cropping systems (Khan et al., 2007). One hypothesis for this observation was that the addition of N increased N availability in + N plots and if NO<sub>3</sub>-N formed, it mobilized upon irrigation/rainfall event and increased N concentrations below 30 cm via leaching. If additional N was more available below 30 cm, it may have acted to prime decomposition of SOM/SOC (Khan et al., 2007), although microbial activity would be relatively low at this depth in the profile (Babujia et al., 2010).

The impact of crop rotation on SOC was the strongest effect observed and significantly influenced SOC at multiple depths in both 2010 and 2011. While the influence of other management practices on SOC was only observed in 2010, crop rotation consistently impacted SOC from 0 – 5, 30 – 60 cm, and 60 – 90 cm in both 2010 and 2011. These results suggest the impact of crop rotation on SOC is strong enough to supersede inter-annual variation in the cropping system. The SS rotation consistently increased SOC from 0 – 5 cm, 30 – 60 cm, and 60 – 90 cm in 2010 and 2011.

Several mechanisms may be causing an increase in SOC across years. One hypothesis, in relation to increased SOC near the surface, may be related to the continuous sorghum treatments and subsequent high C:N of sorghum biomass tissue (Table 8).

Monoculture corn showed the greatest increase in SOC above 30 cm over time compared to rotated corn cropping systems (Halvorson and Schlegel, 2012; Varvel and Wilhelm, 2010). Larger increase in SOC over time of continuously cropped corn was attributed to the relatively higher corn tissue C:N in this system (Halvorson and Schlegel, 2012). The SS treatments had significantly higher C:N than corn in 2010 and relatively higher C:N than CS sorghum in 2011 (Table 8). Thus, any aboveground residue incorporated into the soil may have been more recalcitrant in the SS treatments opposed to the CS treatments.

Increased SOC at the soil surface could be related to consistent addition of organic C to the surface. Bioenergy sorghum is a photo-period sensitive C<sub>4</sub> plant with high aboveground productivity (Rocateli et al., 2012; Rooney et al., 2007), large leaves and a tall, dense canopy. Throughout the growing season, as the crop grew in height, lower leaves would periodically senesce from the plant and fall onto the soil, and the plant would continue growth upward and with new leaves being produced within the upper canopy. While the leaves themselves did not appear to be a large mass of organic C, the cumulative mass across the entire growing season may have been substantial enough to increase SOC near the surface of the soil, particularly considering the higher C:N of sorghum plant tissue (Table 8).

Another theory was that the SS sequence provided a more shaded canopy over the soil surface throughout much of the middle and late portions of the growing season in 2010 compared to the corn canopy in CS treatments. The CS treatments were in the corn sequence in 2010, and due to wide row spacing (102-cm row centers) (Wight et al., 2012), the corn canopy did not completely shade the soil within plots. Without shading from the crop canopy, the soil surface may have received greater solar radiation and surface warming than the shaded canopy provided by sorghum. Higher soil temperature can enhance heterotrophic

decomposition of organic C and soil respiration (Davidson et al., 2006; Van Vleck and King, 2011). Temperature increase near the surface may have stimulated heterotrophic respiration and subsequent SOC decomposition and caused a reduction in SOC near the surface in CS treatments compared to SS treatments. However, this theory is not supported by the soil respiration data (Fig. 25 and Table 9), because cumulative CO<sub>2</sub> emissions from CS treatments were relatively less than those from SS treatments in 2010. One explanation may be that a greater portion of respired C in CS treatments was attributable to decomposition in the soil compared to SS treatments where root respiration may have been a larger contributor to soil respiration. This would support the hypothesis that enhanced soil temperature due to lack of shading increased decomposition in CS treatments compared to SS treatments.

Perhaps the most surprising observation related to increased SOC concentration was the significant increase observed consistently below 30 cm. In most traditional agricultural cropping systems, significant increases in SOC at subsurface depths usually takes much longer than 2 to 3 years to become evident (Anderson-Teixeira et al., 2009; Varvel and Wilhelm, 2010) and little long-term data is available on SOC change below 30 cm (Meki et al., 2013; Varvel and Wilhelm, 2010). The impact of SS on SOC at relatively deep depths in the soil appeared strong, because SS increased SOC below 30 cm in 2010 and 2011, albeit the significance from 60 – 90 cm was not particularly strong each year; ( $P = 0.062$ ) and ( $P = 0.099$ ) for 2010 and 2011, respectively (Table 10). However, as noted in the Results section, the trend of increasing SOC in SS treatments was very consistent and SS treatments in 2011 had accumulated more SOC than SS treatments in 2010 relative to the start of the study. While changes in SOC near the soil surface due to management have frequently been observed in row cropping systems, subsurface changes have less frequently been documented



(Varvel and Wilhelm, 2010). We do not suspect a significant quantity of organic C leached into the subsurface depths due to the relatively fine soil texture (silty clay loam) of the soil used in this study (Wight et al., 2012), nor would it have been solely relegated to SS treatments. We hypothesized the source of increased SOC below 30 cm likely was associated with sorghum root-derived C.

The sorghum crop likely had an extensive root system that was distributed deep within the soil. It is plausible that a crop which produced high aboveground biomass also produced large belowground biomass. The developments of aboveground and belowground biomass are correlated and ratios can be modified with time, water and nutrient availability, and additional factors (Zegada-Lizarazu et al., 2012). Unfortunately, few studies were available on sorghum root-to-shoot ratios to estimate the potential mass of belowground C, based upon known aboveground C quantities. The few studies available were limited to greenhouse study (Calderon et al., 2012) and another growing a sweet sorghum variety (Zegada-Lizarazu et al., 2012). Zegada-Lizarazu et al. (2012) reported sweet sorghum under drought stressed conditions had a root-to-shoot ratio of approximately 0.12 111 days after sowing. In contrast, Calderon et al. (2012) found a much higher root-to-shoot ratio after 76 days in a greenhouse experiment testing the effects of a mycorrhizal inoculants on C allocation; 0.59 to 0.74 for inoculated and uninoculated sorghum varieties, respectively. For reference, root-to-shoot ratio of corn has been well documented and varies across management systems, but is assumed to be approximately 0.16 at full maturity (Amos and Walters, 2006). Further evidence in variation of belowground C allocation by C<sub>4</sub> plants was provided in a study of C<sub>4</sub> prairie grasses and maize (a C<sub>4</sub> plant) in Iowa which determined a broad range in belowground C allocation between C<sub>4</sub> plant species (Jarchow and Liebman,

2012). The Iowa study found maize had relatively small amounts of C allocated belowground, while a much larger portion was allocated aboveground. In contrast, the C<sub>4</sub> prairie grasses had similar total biomass C, yet a large portion of it was allocated belowground rather than aboveground (Jarchow and Liebman, 2012). Thus, without further information, the suggestion that significant quantities of SOC from the sorghum root system were deposited belowground at depths beyond 30 cm cannot be documented, but also cannot be ruled out.

Furthermore, the moderate drought in 2010 and severe drought in 2011 may have enhanced root proliferation in a search for additional water resources. As a drought resistant crop, sorghum has multiple mechanisms to improve water use efficiency (WUE) under water-stressed conditions (Wright and Smith, 1983; Zegada-Lizarazu et al., 2012). For example, in response to drought conditions, grain sorghum will allocate greater amounts of C belowground as a mechanism to improve WUE (Wright and Smith, 1983). A study of drought effects on maize and sweet sorghum root systems determined the root system of irrigated and drought-stressed maize were nearly the same 111 days after sowing, but drought-stressed sorghum was 1.7 times larger than the control (Zegada-Lizarazu et al., 2012). The study also determined drought-stressed sorghum had significantly higher root-to-shoot ratio after 82 days than irrigated sorghum.

Similar to the potential root scavenging for water resources, sorghum roots may have also produced an extensive root system in order to scavenge for nutrients. While not significant, the SS, - N treatments had higher SOC change than SS, + N treatments from 30 – 60 and 60 – 90 cm in both 2010 and 2011. Unfertilized C<sub>4</sub> prairie grasses had more total root biomass than those fertilized, and more than 20 times more biomass than corn in an Iowa

bioenergy cropping system (Jarchow and Liebman, 2012). This was parallel to results by Amos and Walters (2006) which found N or P deficiency caused increased root-to-shoot ratio in corn crops from several studies. These results support the hypothesis that sorghum plants without adequate nutrient and water resources may allocate large amounts of C belowground to scavenge for additional resources.

### **Soil Microbial Biomass Carbon**

The portion of the SOC pool which was of particular interest for this study was the SMBC pool. This pool is a vital one for sustaining long-term soil quality and soil health and is an important indicator for changes in soil quality (Powlson et al., 1987). The microbial biomass C pool is highly responsive to changes to soil and environmental conditions and will frequently display changes before the SOC pool (Powlson et al., 1987). This particularly dynamic pool of SOC is frequently monitored to predict changes in soil quality induced via agronomic management practices (Franzluebbers et al., 1994).

Concentrations of SMBC may vary over time, depending upon prevailing environmental and soil conditions (Franzluebbers et al., 1995; Liebig et al., 2006; Salinas Garcia et al., 1997; Wardle, 1992). Estimates of SMBC in this study were made from different months which may have caused some inherent bias. The initial samples of the study taken in 2008 were collected under pre-plant conditions in March, while 2011 samples were collected under post-harvest conditions in late November. However, the late fall samples occurred nearly 3 months after fall harvest and tillage. Thus, while not ideal, the SMBC measurements were made from samples collected in the fallow season, which is generally

considered a more ideal sampling period due to weather and soil condition stability (Liebig et al., 2006).

Concentrations of SMBC observed in 2008 ranged from 120 to 170 mg C kg<sup>-1</sup> soil at the surface (0 - 5 cm) and subsurface (5 - 15 cm), respectively. Concentrations of SMBC increased in 2011 and ranged between 170 to 210 mg C kg<sup>-1</sup> soil. These concentrations fall within the range most frequently observed in agricultural soils, based upon meta-analysis of more than 400 studies (Kallenbach and Grandy, 2011). The range in SMBC concentrations was lower than previous studies near our research location (Franzluebbers et al., 1995), but differences in methodologies or soils can lead to differences in absolute concentrations observed (Haney et al., 2001; Wardle and Parkinson, 1991). Additionally, we observed SMBC decreased with depth. This differed from a previous study near the research location (Dou et al., 2008), but agreed with another study near our research location (Franzluebbers et al., 1994) and several other agricultural studies (Babujia et al., 2010; Feng et al., 2003; Wardle, 1992).

The analysis of impacts of crop rotation, N fertilization, and/or residue return on SMBC from 0 – 5 and 5 – 15 cm focused on the net change from 2008 to 2011. The management practice with the strongest impact was residue return (Fig. 30 and Table 11). Treatments receiving 50% of harvested biomass returned to the soil contained significantly more SMBC at both sampling depths. Organic C availability, agronomic management practices related to C inputs, and dry matter application rate are known to play major roles in SMBC concentration in soils (Franzluebbers et al., 1994; Kallenbach and Grandy, 2011; Wardle, 1992). Concentrations of SMBC in fine texture soils generally show greater response to organic amendments, such as crop residues, due to their SOM stabilization properties

(Franzluebbers et al., 1996; Six et al., 2006; Wardle, 1992). Thus, addition of relatively large quantities of biomass sorghum to the silty clay loam soil at our study location likely had a strong influence over SMBC concentrations. Furthermore, the 50%R treatments may have provided additional beneficial aspects related to soil moisture retention, which can improve SMBC resilience against effects from drought (Hueso et al., 2011).

Nitrogen fertilization had a small effect on SMBC concentration in near surface soil. Treatments which received N fertilization had a larger increase in SMBC than treatments which did not receive additional N. Some studies have found N addition may decrease SMBC (Khan et al., 2007). Others have shown slight increase in SMBC (Franzluebbers et al., 1994; Franzluebbers et al., 1995; Majumder and Kuzyakov, 2010). The impact of N fertilization was not as strong as residue return and other studies note the relatively small influence of N fertilization compared to other management practices (Franzluebbers et al., 1994; Franzluebbers et al., 1995).

Despite both crop sequences being in sorghum in 2011, SMBC from 5 – 15 cm was significantly higher in SS than CS treatments. This result contrasts with some other studies that found higher SMBC in rotated rather than continuous cropping systems (Acosta-Martinez et al., 2011; Franzluebbers et al., 1995; Six et al., 2006). While not significant, SOC in the 5 – 15 cm depth was higher in SS than CS treatments (Table 10 and Fig. 28). Soils with relatively higher SOC are frequently associated with higher SMBC (Fierer et al., 2009; Kallenbach and Grandy, 2011; Wardle, 1992). At most depths, SOC was generally higher in SS treatments and may help explain higher SMBC concentrations.

Similar to the role that SMBC can play as an indicator of changes to soil quality, SMBC as a fraction of SOC has been noted as a valuable indicator of changes to the SOM

pool (Powlson et al., 1987). Increases in the fraction of SOC as SMBC are believed to indicate an increasing pool of SOM or improved soil quality (Powlson et al., 1987). Microbial biomass normally makes up a small fraction of total SOC, and in agricultural soils frequently accounts for less than 3% of SOC (Wardle, 1992). These results are similar to those of our study where values ranged from 1.0 to 2.5% SOC as SMBC. At the initiation of the study, the portion of SOC as SMBC decreased with depth, but the trend reversed by 2011 and the subsurface contained a greater percentage of SOC as SMBC. The initial observation is similar to other research near our location studying the impact of cropping intensity and tillage regimes in wheat cropping systems (Franzluebbbers et al., 1994). Perhaps the cotton crop prior to the initiation of the current experiment (rotated annually with corn) (Wight et al., 2012) had different soil C allocation properties than the bioenergy sorghum in the current study.

At the beginning of the study, SS had a higher portion of SOC as SMBC than CS treatments from 0 – 5 cm, but no differences were found below 5 cm. This observation may have been related to the previously noted soil textural variability coinciding with SS treatments. Franzluebbbers et al. (1996) found the portion of SOC as SMBC increased with increasing clay content in a number of soils, which helps explain the difference observed at the initiation of this study. In 2011, crop rotation did not have an impact on SOC as SMBC at the surface, but SOC as SMBC represented a larger percentage from 5 – 15 cm in SS than CS treatments. Others studies have found increased SOC as SMBC with greater cropping diversity and intensity (Franzluebbbers al., 1994; Franzluebbbers et al., 1995), but none evaluated bioenergy sorghum. The increased SOC attributed to SS compared to CS treatments may indicate greater C inputs into those treatments, which may have contributed

to a greater fraction of SOC attributed to SMBC. Greater C inputs were associated with increased cropping intensity which increased the portion of SOC as SMBC in a study near our location (Franzluebbers et al., 1994), and coincides with the observations of increased SOC as SMBC attributed to 50%R treatments from 0 – 5 and 5 – 15 cm (Fig. 31). We found increasing C inputs via residue return significantly increased SMBC and subsequently, the portion of SOC as SMBC. While we did not observe a consistent trend of residue return on SOC, these active biological C pool indicators may indicate changes to SOC dynamics which ultimately may be observed in long-term total SOC.

## **Review**

The impact of crop rotation on SOC was the most dominant of the three management practices examined. Crop rotation consistently influenced SOC at multiple depths in the soil in both 2010 and 2011, while other management practices did not consistently affect SOC in both years. The SS treatments increased SOC in near surface soil and below 30 cm. One of the most surprising results in this study was the ability of SS treatments to significantly increase SOC below 30 cm. The implications of these findings may be large and certainly warrant further investigation. Deposition of C deep in the soil profile, where microbial activity is relatively low, can provide greater SOC longevity and enhance C sequestration potential. Photoperiod sensitive biomass (bioenergy) sorghum is a relatively new crop and much of the research to date has focused on aboveground production. Little data exists on belowground C dynamics within bioenergy sorghum crop production scenarios. We speculate it may be possible that a crop that frequently produces more than 15 Mg ha<sup>-1</sup> of aboveground biomass may also produce a relatively smaller, but significant amount of

biomass belowground. Changes in SOC below 30 cm in SS treatments, where sorghum was grown each year, indicate increasing SOC, likely due to root-derived C deposits. Further research must be focused on the source of increased SOC below 30 cm, as the results from this study can only be speculative.

Continuous sorghum treatments increased both soil C pools examined (SOC and SMBC), but reduced yield and plant C assimilated, but had no effect on cumulative soil respiration. Thus, some of the benefits achieved by SS treatments in regard to soil C sequestration may not be practical if yields are suppressed over the long-term. Soil C sequestration potential must be balanced with high yielding cropping systems, and SS did not provide higher sorghum yields than CS. However, when compared to the corn sequence in CS treatments under drought conditions, SS outperformed CS by a wide margin. If soil moisture has been depleted throughout the profile and drought potential is high, SS may provide a cellulosic biofuel crop with relatively high yield, particularly compared to corn. Further research is needed to quantify the impact of corn on C cycling within CS treatments under more optimal conditions (uninhibited by late planting and adequate moisture throughout the growing season). The corn yield data from 2010 was particularly poor and may not give an accurate representation of how the crop could perform on an average yearly basis. Further research is required to understand the impact of corn in the CS treatments under more optimal growing conditions.

While SS treatments offered a number of benefits including increased SOC at various depths, SMBC from 5 – 15 cm, and increased yield relative to corn, there were some issues associated with monoculture cropping not addressed in this study. Continuous sorghum cropping may increase disease and pest stresses which could significantly reduce yields and



likely impact other C pools (Moore et al., 2009). Cultivating the same crop for many years may essentially mine the soil of nutrients if the crop consistently extracts the same nutrients from the soil year after year (Powell and Hons, 1992). Crop rotation has been recommended in sorghum cropping systems to minimize threat from disease (Rocateli et al., 2012).

Residue return had a major impact on SMBC and soil respiration C by increasing the microbial biomass as well as cumulative respiration in 2010 and 2011. These results were similar to other research which determined crop (autotrophic) respiration contributed a relatively small amount to cumulative respiration values (Van Vleck and King, 2011). The microbial C pool was influenced largely by residue return rate, with 50%R significantly increasing SMBC from 0 – 5 cm and 5 – 15 cm. The greater amount of available C likely allowed microbes to proliferate more in the 50%R treatments. The strong impact of residue return on SMBC, but not SOC, after three years is fairly common because SMBC represents a pool of C that is more sensitive to impacts from agronomic management practices (Acosta-Martinez et al., 2011).

Increased SMBC attributable to residue management practices has potential implications beyond SMBC itself. Greater microbial biomass and subsequent microbially-derived binding agents may translate to greater aggregate formation (Degens 1997, Jastrow, Miller, et al., 1998). SMBC and microbial activity may have played a particularly large role in aggregate formation at our location due to the fine-textured soils (Degens, 1997; Six et al., 2004). The irrigation and drought-induced wetting and drying cycles may have enhanced aggregate stabilization across 2010 and 2011 in our study (Denef et al., 2001). With enhanced aggregation, these soils could protect C for a longer period of time and enhance C sequestration potential (Six et al., 2006). Residue return significantly increased cumulative

respiration in 2011, however, the additional CO<sub>2</sub> lost through soil respiration may be partially offset in the long-term if greater SMBC promotes greater aggregation and C sequestration over time. Residue return also increased the fraction of SOC as SMBC which may indicate an increasing pool of SOM (Powlson et al., 1987). Building SOM concentrations can provide a multitude of benefits and remains one of the most valuable indicators of overall soil health (Ehmke, 2013; Lal, 2009).

Besides improving microbial activity, increasing aggregation, and SOM, residue return has benefits related to soil fertility and soil physical properties. While not examined in this study, returned crop biomass is also a source of nutrients which were applied back to the field. While this study focused on impacts to C cycling, other nutrient cycling pools may have been significantly impacted by residue return. Continuous harvest of all aboveground biomass in bioenergy sorghum production systems may provide high yields initially, but as the soil is mined of nutrients, long-term yields may diminish (Meki et al., 2013; Powell and Hons, 1992). Furthermore, retaining residue at the surface may aid in retaining moisture and lower soil surface temperatures which could further slow decomposition of surface residues as well as belowground C (Stott et al., 1986). Thus, while residue return was not a major impact factor on all C pools in this study, impacts of residue management may take longer to be detected and could have significant consequences to C dynamics.

## **CONCLUSIONS**

Few efforts have been made to assess the impact of agronomic management practices on C dynamics within bioenergy sorghum production systems. A very negative outcome of biofuel production would be to address near-term problems (over-reliance on imported fossil

fuels), while creating or aggravating longer-term problems (reduction in the productive capacity of soils and environmental degradation). To help avoid this outcome, we determined the net effects of bioenergy sorghum management practices on cumulative soil respiration emissions, SOC, and C fixation in plant and microbial biomass.

Studied management practices had major impacts on C cycling within bioenergy sorghum cropping systems. Different management practices differentially impacted each C pool. The climatic conditions that occurred across the two years of study were somewhat atypical, and environmental factors may have influenced C dynamics to some degree. Previous research found that improvement in some C quality parameters can be achieved despite drought conditions which may lower overall crop yields (Acosta-Martinez et al., 2011). Our study concurred, and in spite of moderate drought in 2010 and a severe drought in 2011 that may have limited maximum yield potential, increases in several C pools were observed.

Nitrogen addition increased corn and sorghum yield, plant C assimilated, SMBC near the surface and cumulative soil emissions in 2011. The aboveground C pools were generally more impacted by N fertilization than belowground C pools. Nitrogen fertilization was imperative to sustaining high yield each year, which will be vital to the feasibility and sustainability of any biofuel cropping system. However, N fertilization also increased soil respiration, and if it increased net GWP (including other gases such as  $N_2O$ ), it may offset some long-term SOC sequestration benefits.

Residue return primarily influenced belowground C pools across 2010 and 2011. Based upon SOC concentration change since 2008, returning half the biomass residue to the soil as an organic amendment significantly increased SOC at 0 – 5 and 5 – 15 cm in 2010,

but had no detectable effect in 2011. Residue return had a highly significant effect on SMBC concentrations at 0 – 5 and 5 – 15 cm, likely due to enhanced carbon availability. Returning residue also increased the % SOC as SMBC, indicating a potentially increasing pool of SOM, and subsequently increased soil quality. Returning half the biomass to the soil significantly increased cumulative soil respiration values in 2010 and 2011. The relatively large mass of organic C applied to the soil from 50%R treatments did not consistently impact SOC. We believe that since this study utilized conventional disc tillage and saw significantly higher respiration-C emissions from 50%R treatments across years and had no consistent impact to SOC concentration, most of the residue-C applied was quickly decomposed and lost as CO<sub>2</sub>.

Crop rotation had the strongest impact across all C pools. Rotation influenced both above and belowground C pools. Crop rotation affected aboveground C pools in 2010 and 2011, but had contrasting results; increasing SS treatments increased yield and C assimilated in 2010, but decreasing them in 2011 compared to CS treatments. Continuous sorghum treatments significantly increased SOC pools at various depths in 2010 and 2011, specifically deeper soil depths that are generally less frequently impacted by management practices in the short term. The ability of SS treatments to significantly increase SOC below 30 cm compared to CS treatments may have large ramifications on C sequestration potential and further investigation is necessary.

Each management practice examined in this study influenced at least one of the four C pools examined in 2010 and 2011, and resulted in a better understanding of how crop management practices may influence C dynamics in a bioenergy sorghum cropping system. However, to better evaluate the sustainability of the system, further research is needed to

evaluate the efficiency of biofuel yield production in terms of all net inputs utilized weighed against potential C sequestration benefits and net GWP.

## CHAPTER IV

# LIFE CYCLE GREENHOUSE GAS EMISSIONS ASSOCIATED WITH BIOENERGY SORGHUM PRODUCTION UNDER VARIOUS AGRONOMIC MANAGEMENT PRACTICES IN CENTRAL TEXAS

### **INTRODUCTION**

Biofuels present both challenges and opportunities to meet sustainable energy demands within the United States. There is high demand for transportation fuels throughout the United States, as evidenced by increasing prices and increased energy consumption in the transportation sector, continuing at least in the short term (U.S. Energy Information Administration, 2013). Along with the demand is a growing need to identify renewable energy sources for the transportation sector. While total transportation consumption may eventually decrease slightly (by 2040) due to increased vehicle efficiency standards, biofuel consumption is projected to increase to nearly 6% of total U.S. liquid fuel consumption by 2040 (U.S. Energy Information Administration, 2013). It is anticipated that these renewable fuels can supplement traditional foreign petroleum fuel sources with sources of domestic energy, as well as mitigate greenhouse gas emissions in an effort to minimize impacts to climate change.

The 2007 Energy Independence and Security Act (EISA) established a mandate to produce over 136 billion liters of biofuels by the year 2022 (U.S. Congress, 2007). Of that amount, more than half of the fuel must come from advanced biofuels, or fuels which are different from traditional corn grain ethanol (U.S. Congress, 2007). These aggressive

renewable energy mandates demonstrate that the U.S. federal government is committed to domestic biofuel production as a viable transportation fuel supplement for the foreseeable future. Concerns associated with greenhouse gas (GHG) emissions by the energy sector and impacts on global climate change led to the inclusion of mandated life cycle GHG emission reduction by advanced biofuels in the 2007 EISA. Specifically, cellulosic biofuel must meet a 60% reduction in life cycle GHGs relative to gasoline (U.S. Congress, 2007). Thus, success of advanced biofuels must balance high production output with minimal GHG emissions. Biofuel production scenarios which maximize efficiency by producing high yield with minimal GHG emissions must be identified to achieve mandated renewable fuel goals.

Life cycle analyses (LCA) are tools utilized to estimate the environmental impact of a product or process from its production through its entire life cycle, and are being used to determine whether biofuel production scenarios meet or exceed reductions in GHGs compared to gasoline (Hsu, 2012; Swana et al., 2011; Wang et al., 2012). However, bioenergy crop production is highly complex due to the vast number of environmental, management, and production output scenarios. Biomass production remains a major portion of total life cycle GHG emissions from the biofuel production process (Adler et al., 2007; Liska and Cassman, 2008). Even LCAs on common U.S. crops, such as corn, have wide variations in crop production GHGs (Liska and Cassman, 2008). Thus, relatively new bioenergy crops, such as biomass sorghum, require study to determine the impact of management practices on life cycle GHGs. Furthermore, due to the vast complexity associated with producing bioenergy crops, it is necessary to provide as much measured data as possible to enhance estimation and guide future life cycle accounting efforts.

Agronomic management practices directly impact soil C cycling in crop production systems. These production systems could be net sources or net sinks of atmospheric C, depending upon their ability to lose C (typically as CO<sub>2</sub>) or sequester C [accrual of soil organic carbon (SOC)], respectively. However, few studies investigating LCA impacts of bioenergy crops consider changes to SOC attributed to production management practices (Adler et al., 2007; Anderson-Teixeira et al., 2009; Gollany et al., 2011; Hill et al., 2006, Liska et al., 2009; Plevin, 2009; Searchinger et al., 2008). Recent research indicates a number of bioenergy cropping systems have accrued significant amounts of SOC belowground during production (Clay et al., 2012; Follett et al., 2012) and illustrate the need for additional information to determine the impact of bioenergy crop production on SOC sequestration when investigating LCA GHG emissions.

Bioenergy sorghum is a relatively new agronomic crop which has been developed for its potential use as a biomass feedstock in the production of advanced biofuels. Research on the management practices utilized in the production of bioenergy sorghum has focused on maximizing yield for maximum biofuel production potential. However, little data is available on the capacity of bioenergy sorghum production systems to efficiently maximize yield while minimizing life cycle GHG emissions. Therefore, the objectives of this study were to quantify both direct and indirect GHG emissions associated with the production of bioenergy sorghum in central Texas. This data was then used to determine how measured crop production emissions and sequestered C (via SOC accrual) impacted the well-to-wheel (W2W) lifecycle GHG analysis of ethanol production. Furthermore, we determined which production scenario provided the greatest overall efficiency and determined if any were able to satisfy the 60% life cycle GHG reduction mandated by the 2007 EISA. The aim of this



research was to identify various sinks and sources of life cycle GHGs from bioenergy sorghum production. This study identified sustainable agronomic management practices that should reduce, both direct and indirect, GHG emissions associated with bioenergy sorghum production in the southern U.S.

## **MATERIALS AND METHODS**

### **Site Description and Experimental Design**

The experimental site is located at the Texas A&M Agrilife Research Farm, approximately 8 km southwest of College Station, Texas, USA (30° 32' 15"N, 96° 25' 37"W), and is situated within the Brazos River floodplain of south-central Texas. The soil used was a Weswood silty clay loam (100 g sand kg<sup>-1</sup>, 560 g silt kg<sup>-1</sup>, 340 g clay kg<sup>-1</sup>) and is classified by the USDA NRCS as a Fine-silty, mixed, superactive, thermic Udifluventic Haplustept. Climate at the site is classified as humid subtropical; the mean annual precipitation is 978 mm and the mean annual temperature is 20°C. Prior to the start of the bioenergy sorghum study in 2008, the field was in cotton (*Gossypium hirsutum* L.) in 2007, and rotated annually with corn (*Zea mays* L.) under conventional disk tillage.

This study was conducted within a larger study investigating the yield potential and agronomic responses of bioenergy sorghum [*Sorghum bicolor* L. (Moench.)] to various integrated management practices (Wight et al., 2012). The bioenergy sorghum cropping system, established in 2008, utilized four-row (1.02-m row center) plots which measured 9.14 m long and 4.08 m wide. The larger study used a randomized complete block design with crop rotation, N fertilization rate, and biomass residue return rate as the three major factors. The study reported herein was limited to two levels of each of the three major factors

and was replicated 4 times (3 times for CO<sub>2</sub>-C measurements). The eight experimental treatments included every combination of: crop rotation (corn-sorghum [CS] or sorghum-sorghum [SS]), N fertilization (0 kg N ha<sup>-1</sup> [- N] or 280 kg N ha<sup>-1</sup> [+ N]), and biomass residue return (0% residue returned [0%R] or 50% residue returned [50%R]). The bioenergy sorghum variety, “4Ever Green”, used was a photoperiod-sensitive, high-tonnage hybrid forage sorghum (Walter Moss Seed Co, Waco, TX, U.S.A.). This particular variety was chosen because of its low water requirement, high biomass yield, and low lodging potential. When corn was rotated with sorghum, Dekalb DKC68-05 was the corn variety utilized in the corn-sorghum cropping sequence.

A summary of field management operations in 2008 and 2009 can be found in Wight et al. (2012) and Table 12. The field management operations in 2010 and 2011 can be found in summary Table 12 or as described here. The field beds were prepared for planting with a rolling cultivator and pre-plant herbicide on 17 March 2010 and planted to corn on 23 March 2010 and sorghum on 13 April 2010. In 2011, a pre-plant herbicide was applied on 2 March 2011, cultivation occurred on 24 March 2011, and the entire field was planted to sorghum on 25 March 2011. A four-row cultivator was used to control weeds on 22 May 2010 and 5 May 2011. Treatments receiving N fertilization were sidedressed with granular urea at 7.5 cm depth on 22 May 2010 and 5 May 2011, and approximated the 4-leaf stage for sorghum and 6-leaf stage for corn. When corn was fertilized, it received a reduced rate of 168 kg N ha<sup>-1</sup>, based on local recommendation. Randomly selected 3.05 m segments of the middle two rows of corn were hand-harvested for grain and stover samples on 2 July 2010. Estimates of total corn biomass and grain yield were made on this date, however, the remaining non-sampled portion of the corn stand was not harvested/mulched onto the soil until the later

sorghum harvesting date. The remaining corn biomass and biomass sorghum were harvested on 7 October 2010 and in 2011, sorghum was harvested on 1 September. A single-row silage harvester with an attached weigh-bucket was utilized to harvest sorghum in 2010 and 2011. Biomass yield was estimated from the entire length of the middle two rows of each plot and a random grab sample of chopped residue was captured for determining moisture and nutrient composition. The harvester mulched sorghum biomass into approximately 0.04 m by 0.04 m pieces. After biomass yield weights were recorded, the 50%R treatments received the biomass from the middle two rows evenly distributed across the area of the entire plot. This study was not interested in removing corn residue for biofuel conversion (only grain), so when corn was harvested in 2010, 100% of residue was returned to each plot, regardless of residue return treatment. After harvest each fall, residue was disked and the plots were bedded. Disking and bedding occurred on 12 October 2010 and 5 September 2011. Furrow irrigation was used sparingly as needed on 31 May 2010 and 12 April, 9 May, 14 July, and 4 August in 2011. Eleven cm of irrigation water was applied in each event except 9 May 2011, when 9 cm was applied. A summary of these field operations can be found in Table 12.

Table 12. Field operations during 2010 and 2011 calendar years at the research site near College Station, TX.

Field operation	Year			
	2010		2011	
		<i>notes</i>		<i>notes</i>
Pre-plant herbicide†	17 Mar.		2 Mar.	
Soil sampling	5 Apr.		14 Mar.	
Pre-plant cultivation	17 Mar.		24 Mar.	
Planting	23 Mar.	<i>corn</i>		
	13 Apr.	<i>sorghum</i>	25 Mar.	<i>sorghum</i>
Fertilization	22 May		5 May	
Inter-row cultivation	22 May		5 May	
Irrigation‡	31 May	<i>11 cm</i>	12 Apr.	<i>11 cm</i>
			9 May	<i>9 cm</i>
			14 July	<i>11 cm</i>
			4 Aug.	<i>11 cm</i>
Harvest	2 July	<i>corn</i>		
	7 Oct.	<i>sorghum</i>	1 Sept.	<i>sorghum</i>
Post-harvest disking and bedding	12 Oct.		5 Sept.	

† applied as Atrazine

‡ applied as furrow irrigation

### Feedstock and Energy Yield

Bioenergy sorghum was harvested by techniques and on dates previously discussed. Yield determination was made by harvesting the inner two rows of each (four-row) plot. Random grab samples were collected for analysis and half the biomass residue was returned to 50%R treatments. Moisture content of aboveground biomass was estimated from a 600 g subsample from 5 randomly sampled plants which were mulched with a commercial chipper/shredder. Weights of subsamples were collected before and following oven-drying at 60°C for 7 d to determine moisture content. Oven-dried plant tissue samples were coarsely ground in a large Wiley mill to pass a 1 mm sieve, then finely ground to pass a 0.5 mm sieve using a puck-and-ring grinder in preparation for elemental C determination via combustion

analysis (McGeehan and Naylor, 1988) with an Elementar Americas Inc., VarioMAX CN analyzer (Mt. Laurel, NJ). Total C accumulation in aboveground biomass was estimated by multiplying total dry weight by elemental C concentration.

Based upon measured yield values and bioenergy feedstock conversion estimates, theoretical energy yield was calculated on a per hectare basis. Ethanol yields from harvested corn grain (CS in 2010) were estimated from the National Renewable Energy Laboratory's straight-forward conversion value of 519 L Mg<sup>-1</sup> dry feedstock (NREL, 2012). Ethanol yields from harvested bioenergy (photoperiod-sensitive) sorghum were estimated from the 452 L Mg<sup>-1</sup> conversion value provided by Stefaniak et al. (2012), assuming conversion of both sugar and starch to ethanol. For further verification, we compared published values (Stefaniak et al., 2012) with several plant tissue composition samples analyzed with NIR from our own study. The values aligned well and we concluded the published conversion estimate of 452 L ethanol Mg<sup>-1</sup> biomass feedstock would be suitable for the study. Yields of sorghum biomass and corn grain were obtained from annual harvests for each plot, converted to Mg dry feedstock ha<sup>-1</sup> and multiplied by ethanol conversion value to obtain an estimate of L ethanol ha<sup>-1</sup>. Finally, L ethanol ha<sup>-1</sup> was multiplied by ethanol energy value (21.2 MJ L<sup>-1</sup>) and converted to final energy yield units of GJ ha<sup>-1</sup>.

### **Nitrous Oxide Emissions**

Nitrous oxide (N<sub>2</sub>O-N) fluxes were directly measured using a static, vented chamber and a field photoacoustic gas analyzer. Specifically, the measurements were made by integrating a Li-Cor 20-cm survey chamber (model 8100-103, Li-Cor Inc., Lincoln, NE) with an INNOVA 1412 Photoacoustic gas analyzer (Innova AirTech Instruments A/S, Denmark).

The flux chamber was selected for its portability, adaptability, and ability to minimize pressure disequilibrium between the chamber headspace and ambient atmosphere (Xu et al., 2006). A PVC flux chamber collar was installed to a depth of approximately 12 cm near the middle of each of the 24 sampled plots. The collars were placed on top of beds on level surfaces which were equidistant from the crop and injected fertilizer. Height measurements from the soil surface to the top of each collar were measured at four quadrants inside the collar and averaged to estimate height for periodic headspace volume calculation. To minimize disturbance effects, soil-collars were installed no less than 24 hours prior to a gas sampling event and remained in place through the entire growing and fallow seasons (Parkin and Venterea, 2010; Tiedje et al., 1989). Collars were only removed briefly in spring and fall for field operations.

Nitrous oxide fluxes were measured by placing the Li-Cor 20-cm chamber atop the PVC collars for a deployment time of 20 minutes. The analyzer was programmed to collect a measurement every 2 minutes during deployment. The gas analyzer was programmed with a 5-second sampling integration time and auto-flushing for the corresponding tube length (typically 2 m). All tubing, fittings, and connection components were made of polytetrafluoroethylene (PTFE) or stainless steel. The gas analyzer was lab calibrated every two months with a 2-point calibration of a zero gas and mixed gas standard. The primary gas standard ( $\text{N}_2\text{O}$  at 2 ppm) and zero gas (UHP  $\text{N}_2$ ) were mixed to at least 99.99% accuracy (Airgas Specialty Gases, LaPorte, TX). The gas analyzer was routinely checked prior to field sampling with gas standards to ensure calibration stability.

Preliminary analysis of trace gas data determined the majority of fluxes exhibited a nonlinear increase in concentration over time within the chamber headspace. Thus, nonlinear

fluxes of GHGs were calculated by fitting a nonlinear regression of trace gas concentration across deployment time (Wagner et al., 1997) and on a few occasions where linear fluxes were observed, a linear method was utilized. While the nonlinear quadratic regression method selected may not be the unconditional best, its simplicity of use and significant improvement over linear methods (given our nonlinear data) (Livingston et al., 2006, Venterea et al., 2009) coupled with high temporal resolution and the large number of data points collected for each flux calculation with our analyzer warranted its use for this study. Fluxes of N<sub>2</sub>O were calculated from the the first 14 minutes of sampling (7 data points) to minimize ‘chamber effect’, but often utilized the full 20-minute deployment time which allowed concentrations to increase to detectable limits while the chamber was closed. Any regression with a coefficient of determination (r<sup>2</sup>) less than 0.8 was rejected and a net flux of zero was assumed. Calculation of trace gases fluxes were based on GRACEnet protocols (Parkin and Venterea, 2010) and were calculated as:

$$F_1 = \frac{V \cdot \frac{\Delta C}{\Delta t}}{A_s} \quad [7]$$

where  $F_1$  is the volumetric gas flux in  $\mu\text{L (N}_2\text{O) m}^{-2} \text{ min}^{-1}$ ,  $\frac{\Delta C}{\Delta t}$  is the change in trace gas concentration over time ppm (v) (N<sub>2</sub>O) min<sup>-1</sup>,  $V$  is the calculated chamber volume (L), and  $A_s$  is the surface area (m<sup>2</sup>). Further calculations were made as:

$$F_2 = C \cdot \frac{F_1 \cdot p}{R \cdot T} \quad [8]$$

where  $F_2$  is the trace gas flux in  $\mu\text{mol (N}_2\text{O) m}^{-2} \text{ min}^{-1}$ ,  $p$  is pressure (atm),  $R$  is the gas law constant (0.08206 L atm mol<sup>-1</sup> K<sup>-1</sup>),  $T$  is temperature (°K), and  $C$  is a conversion coefficient (0.63648 for N<sub>2</sub>O to N<sub>2</sub>O-N).

Due to required deployment time and the ability to only measure a single plot at a time, the 24 total plots (8 cropping system treatments x 3 replicates) were divided into two consecutive days of sampling. Sampling order was randomized for each sampling event to reduce bias due to temperatures increasing from morning hours. Gas sampling was normally performed between 9:00 and 15:00. Specific days selected for a sampling event were determined based on weather forecasts. Sampling events only occurred on consecutive days with stable weather patterns (e.g. no precipitation, no weather fronts, etc.).

Soil gas measurements were initiated on 25 May 2010, after N fertilization, and were performed approximately weekly through the growing season and at a reduced frequency during the fallow period. Precipitation events and technical difficulties inhibited uniform sampling frequency planned throughout 2010 and 2011. Cumulative growing-season GHG emissions were calculated from 12 sampling events in both 2010 and 2011. Cumulative annual emissions of GHG fluxes were calculated from 18 sampling events in 2010 and 20 sampling events in 2011. Measurements for “sampling year 1” (2010) occurred from 27 May 2010 through 3 March 2011 and for “sampling year 2” (2011) from 6 May 2011 to 1 March 2012.

### **Environmental and Soil Properties**

Several environmental variables were monitored throughout the study to supplement trace gas flux measurements. Air temperature, relative humidity, wind speed, solar radiation, and rainfall were measured with a weather station, approximately 400 m from the field plots, every half hour. Soil temperature was measured hourly by type T thermocouples at 10-cm depth near gas sampling collars within each plot. A time domain reflectometry (TDR) system



was utilized to measure soil moisture every 6 hours. The TDR array consisted of sensors near gas flux collars, using a TDR100 and five SDMX50 multiplexers (Campbell Scientific, Inc., Logan, UT). The volumetric water content was determined to a depth of 15 cm by analyzing TDR wave form with PC-TDR (Campbell Scientific, Inc., Logan, UT) and performing Topp's equation (Topp et al., 1980). Soil temperature and moisture data from monitoring systems were collected within the field with a CR1000 data logger (Campbell Scientific, Inc., Logan, UT). Environmental variables were paired with each trace gas flux measurement based on time stamps nearest to flux measurement time.

### **Soil Organic Carbon Determination**

Three, (4-cm) soil cores were composited from each plot in the spring on 24 March 2008, 6 April 2009, 5 April 2010, 14 March 2011, and 7 March 2012, prior to each growing season. The soil cores were collected from within each plot and composited into depths of 0 - 5, 5 - 15, 15 - 30, 30 - 60, and 60 - 90 cm. Samples were oven-dried (60°C) for 7 d, weighed to determine bulk density, coarsely ground with a flail grinder, and sieved to pass 1.75 mm. A subsample of each composite sample was finely ground with a puck-and-ring grinder and analyzed for organic and inorganic C via combustion analysis (McGeehan and Naylor, 1988; Schulte and Hopkins, 1996; Storer, 1984) with an Elementar Americas Inc., VarioMAX CN analyzer (Mt. Laurel, NJ). Separation of inorganic and organic C was achieved via differential heating. A flow rate of 2 L O<sub>2</sub> min<sup>-1</sup> was used within the furnace and temperatures were set to 650°C and 900°C for organic and total C, respectively (Rabenhorst, 1988; Wang and Anderson, 1998).

Soil bulk density was annually estimated from the three 4-cm composited soil cores collected for chemical analysis from each plot (Blake and Hartge, 1986). No difference in soil bulk density was observed between treatments and years, so an average soil bulk density (1.45, 1.45, 1.42, 1.43, 1.38 g cm<sup>-3</sup> for 0 – 5, 5 – 15, 15 – 30, 30 – 60, and 60 – 90 cm soil depths, respectively) was used for each depth for all treatments and years to calculate mass of SOC. Mass of SOC (Mg C ha<sup>-1</sup>) was calculated from measured C concentration, bulk density, and volume of soil from each sampled depth in one hectare.

### **Soil Carbon Life Cycle Greenhouse Gas Emissions**

Since direct measurement of CO<sub>2</sub> emissions from the soil surface does not necessarily represent net emissions to the atmosphere (a portion of soil CO<sub>2</sub> will be taken up by plants via photosynthesis), net annual change in SOC was converted to CO<sub>2</sub>-eq using similar methods as suggested by Robertson et al. (2000). The composited soil samples taken at multiple depths within each plot were used to estimate change in SOC at each depth. A regression was performed on change in mass of SOC across time (utilizing all years available from 2008 to 2012). Individual regressions were performed for every experimental unit (n = 32) at every depth (5 depths to 90 cm). The slopes (annual net change in SOC) from each depth within each experimental unit were summed to provide a net annual change value (kg C ha<sup>-1</sup>) for the entire 90-cm sampling depth. Conversion of net C change per plot to CO<sub>2</sub>-eq was achieved by multiplying by 3.667 (44 kg CO<sub>2</sub>/12 kg C) for integration into final life cycle GHG accounting.

## Indirect Life Cycle Greenhouse Gas Emissions

Indirect GHG emissions from agronomic management practices were estimated for the LCA. Life cycle greenhouse gases for each management practice were estimated based upon their global warming potential (GWP) and reported as CO<sub>2</sub>-eq. Indirect emissions associated with production of N fertilizer (urea), diesel fuel, and herbicide (atrazine) inputs were calculated from the GREET model estimates (GREET1\_2012\_rev2) (Argonne National Laboratory, 2012). Diesel fuel consumption was estimated in L ha<sup>-1</sup> from each type of management practice utilized and corresponding diesel consumption rate (Frisby, 1993). GREET-derived GWP conversion estimates associated with diesel fuel, urea fertilizer, and atrazine production were 3406.7 g CO<sub>2</sub>-eq L<sup>-1</sup>, 3507.5 g CO<sub>2</sub>-eq kg<sup>-1</sup>, and 17.1 g CO<sub>2</sub>-eq g<sup>-1</sup>, respectively (Argonne National Laboratory, 2012). We utilized the 51.6 g CO<sub>2</sub>-eq m<sup>-3</sup> irrigation water GWP conversion estimate suggested by Lal (2004a).

Following edge-of-field GWP estimate calculation, further analysis included using GREET model estimates to complete a well-to-wheel analysis. The GREET model did not include a cellulosic fuel production pathway for bioenergy sorghum at the time of the study (Argonne National Laboratory, 2012). However, it did provide fuel production pathways for a number of cellulosic crops such as corn stover, switchgrass, and miscanthus for cellulosic ethanol production (Wang et al., 2012). Regardless of cellulosic feedstock type, GREET assumed an ethanol conversion value of 375 L Mg<sup>-1</sup> of dry cellulosic feedstock (Wang et al., 2012), largely due to limited conversion data availability. For the current study, we utilized more recent research on chemical composition of photoperiod-sensitive sorghum to provide an estimate of theoretical cellulosic ethanol conversion (452 L Mg<sup>-1</sup>) (Stefaniak et al., 2012). Three well-to-wheel life cycle GHG components were estimated through extrapolating

REET-derived estimates on three popular cellulosic ethanol production pathways. Estimates of GWP were provided for ethanol production, transportation and distribution, and combustion in g CO<sub>2</sub>-eq MJ<sup>-1</sup> from corn stover, switchgrass, and miscanthus ethanol (Wang et al., 2012). Global warming potential associated with ethanol production ranged from 10 to 11 g CO<sub>2</sub>-eq MJ<sup>-1</sup> (Wang et al., 2012), and 11 g CO<sub>2</sub>-eq MJ<sup>-1</sup> was selected for use in this study. Transportation and distribution accounted for 2 to 3 g CO<sub>2</sub>-eq MJ<sup>-1</sup> in cellulosic ethanol pathways (Wang et al., 2012), 3 g CO<sub>2</sub>-eq MJ<sup>-1</sup> was utilized for this study. Life cycle GHG emissions associated with cellulosic ethanol combustion remained constant at 1 g CO<sub>2</sub>-eq MJ<sup>-1</sup> among the three cellulosic ethanol production pathways (Wang et al., 2012), and the same value was utilized for estimates in this study. Thus, estimates of GWP associated with ethanol production, transportation and distribution, and combustion were calculated by multiplying energy yield (MJ ha<sup>-1</sup>) from each plot by the respective REET-derived life cycle GHG value. Indirect emissions associated with land use change remain highly controversial and sound estimates are highly difficult to quantify (Kim et al., 2009) and will not be explored in this study.

### **Well-to-Wheel Analysis**

A well-to-wheel (W2W) analysis was made to determine if the bioenergy sorghum production scenarios met the 60% LCA GHG reduction goals established by the 2007 EISA. The W2W analysis combined measured management production GWP values with default estimates provided by the REET model. First, field production GWP was calculated by subtracting net annual SOC change values from measured cumulative N<sub>2</sub>O emissions and indirect emissions from management inputs. The resulting value provided a GWP reported in

Mg CO<sub>2</sub>-eq ha<sup>-1</sup> corresponding to the net C sequestration (negative) or net C loss (positive) associated with agronomic production of bioenergy sorghum at the field-scale, or ‘edge-of-field GWP’. Next, indirect life cycle GHGs associated with ethanol production, transportation and distribution, and combustion from each plot were subtracted from its ‘edge-of-field’ GWP. This value represented the sum of all W2W life cycle GHG emissions associated with theoretical cellulosic biofuel production in the study. Finally, similar to yield-scaled GHG emissions, a GWP efficiency value was estimated per unit of theoretical energy produced (g CO<sub>2</sub>-eq MJ<sup>-1</sup>). The W2W life cycle GHGs (kg CO<sub>2</sub>-eq ha<sup>-1</sup>) were divided by the energy yield (MJ ha<sup>-1</sup>) and converted to g CO<sub>2</sub>-eq MJ<sup>-1</sup> as a measure of GWP efficiency and W2W life cycle GHG emissions.

### Data Analysis

Cumulative N<sub>2</sub>O emissions were estimated by linearly interpolating between sampling events and integrating the underlying area from each sampling year (Gilbert, 1987). The regression function of Microsoft Excel’s Analysis ToolPak was utilized to calculate the regression of mass of SOC across time (utilizing all years available, from 2008 to 2012) (Microsoft, 2007). Cumulative emissions were calculated for both the growing season and the entire year. Annual nitrogen emission factors (*NEF*) were calculated as:

$$NEF = \frac{(N_2O_{+N}) - (N_2O_{-N})}{N_m} \cdot 100 \quad [9]$$

where  $N_2O_{+N}$  is the mass of N<sub>2</sub>O-N lost from fertilized treatments in kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>,  $N_2O_{-N}$  is the mass of N<sub>2</sub>O-N lost from unfertilized treatments in kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>, and  $N_m$  is the mass of N fertilizer applied to the fertilized treatments in kg urea-N ha<sup>-1</sup>. Cumulative annual emissions of N<sub>2</sub>O-N were converted to CO<sub>2</sub>-equivalents (CO<sub>2</sub>-eq),

assuming the global warming potential of N<sub>2</sub>O is 298 (Forster et al., 2007) for estimation of kg CO<sub>2</sub>-eq ha<sup>-1</sup> for each plot. The effects of crop rotation, N fertilization, and residue return and their interactions on energy yield, cumulative N<sub>2</sub>O emissions, net annual change in SOC, and well-to-wheel life cycle GHG emissions were tested using a mixed ANOVA in SAS using PROC mixed procedures. Crop rotation, N fertilization, and residue return were considered fixed effects, while replicate (block) was considered a random effect. When year or soil depth was determined to be significant, all analyses were performed separately by year and/or depth.

## **RESULTS**

### **Environment**

The climatic conditions in 2010 and 2011 provided a unique opportunity to explore life cycle GHG emissions under drought conditions in central Texas (Fig. 32). The 2010 growing season was considered a relatively moderate drought, while 2011 was the most severe drought on record (Nielsen-Gammon, 2011). Average air temperatures observed in 2010 were slightly above the 30-year average, while air temperatures recorded during the 2011 growing season were well above the 30-year average (OSC, 2012). Mean monthly air temperatures between May and September (approximate growing season) in 2010 averaged 1.49°C above normal, while the same time period in 2011 averaged 2.17°C above normal (Fig. 32).

The 30-year average annual precipitation total at the study location is 1009 mm (OSC, 2012). During the 2010 N<sub>2</sub>O sampling season (05/27/2010 – 05/01/2011), 317 mm of total precipitation was measured at the site with an additional 220 mm of irrigation applied

(Table 12 and Fig. 32). Even with irrigation, the research location had only half the normal cumulative precipitation in the 2010 period, providing little soil moisture reserves for the 2011 season. During the 2011 sampling year (05/01/2011– 05/01/2012), 1021 mm of precipitation was measured at the site with an additional 310 mm of irrigation applied (Table 12 and Fig. 32). Of the 1021 mm of cumulative precipitation in 2011, 80% of it fell after fall 2011 biomass harvest and 45% of it fell in February and March alone (Fig. 32). Thus, outside of irrigation, very little water was available during the growing season in 2011. In 2010, 143 mm of cumulative precipitation was measured during the growing season compared to 200 mm during the 2011 season. Despite greater precipitation during the 2011 growing season, the drought effects were heightened for several reasons. Little precipitation occurred from late fall 2010 through planting in spring 2011. This period of time normally sees the highest precipitation accumulation for the entire year, and without it, the soil profile was depleted of soil moisture prior to the start of the growing season. Additionally, abnormally high air temperatures, combined with high winds on many days throughout the 2011 growing season elevated evapotranspiration (ET) levels across the study area. Irrigation was used to replenish some profile soil moisture prior to planting in spring 2011, to help germinate the crop, and to minimize drought stress effects early in the growing season. Much of the precipitation during the 2011 sampling period occurred during the fallow season. In February 2012, a daily rainfall record was set when 159 mm of precipitation occurred within 24 hours (OSC, 2012). Another 24-hour precipitation record was set on 20 March 2012 when over 60 mm of precipitation occurred. March 2012 set a monthly cumulative rainfall record, when 169 mm of precipitation was deposited over the course of the month (OSC, 2012). The abnormally heavy precipitation which occurred in February and March 2012 contributed greatly to

replenishing soil moisture throughout the profile and lifting drought conditions in the area for the first time since October 2010.

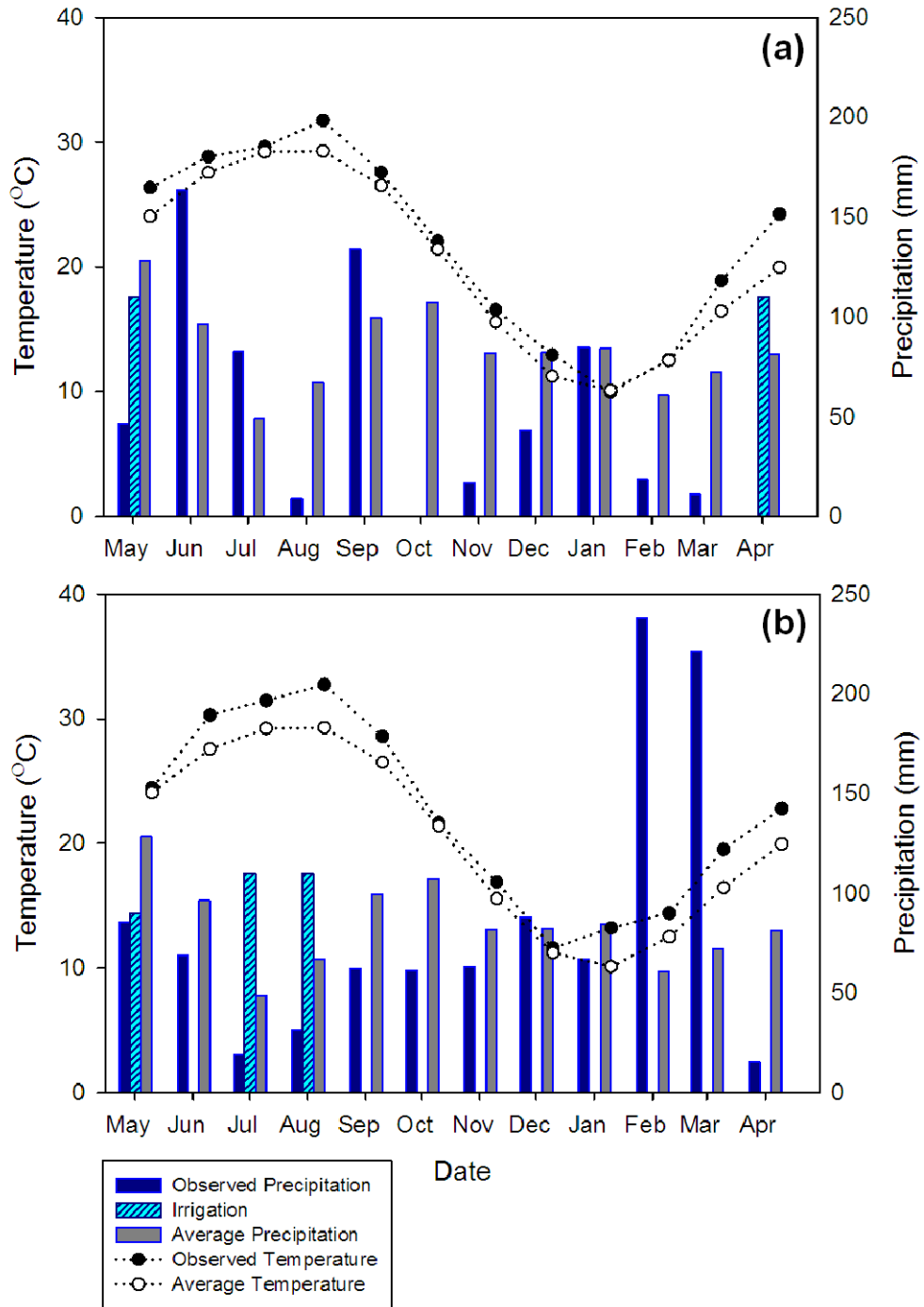


Figure 32. Mean monthly air temperature and total monthly precipitation during the 2010 (a) and 2011 (b) sampling years at the research site near College Station, TX. Asterisks indicate irrigation and rainfall events.



## Energy Yield

The bioenergy feedstock (sorghum biomass and corn grain) dry yields in 2010 were bimodal, likely due to distinctly different crops harvested with different dry yields. However, when the bioenergy feedstock yields were converted to theoretical energy yields ( $\text{GJ ha}^{-1}$ ), the data distribution was normalized. Corn grain in CS treatments provided significantly ( $P < 0.001$ ) less energy yield, 61% less  $\text{GJ ha}^{-1}$  than SS treatments. In 2010, N fertilization significantly ( $P < 0.001$ ) increased energy yield of treatments by more than 70% compared to unfertilized treatments (Fig. 33 and Table 13). Treatments with 50% residue returned exhibited significantly ( $P < 0.001$ ) less energy yield, as was anticipated, because of 50% less utilizable yield. Removing 100% of biomass for biofuel production (0%R) increased energy yield by 50% in 2010 compared to plots with half of the residue returned (50%R) (Fig. 33). All corn residues were returned to the field (only grain harvested) regardless of treatment residue return rate, while half of the biomass yield from 50%R treatments was returned to the soil as an organic amendment. A rotation by residue return rate interaction ( $P < 0.001$ ) was observed in 2010, with SS treatments with 100% biomass removal providing 90% higher energy yield than treatments returning half of the harvested residue (Fig. 33).

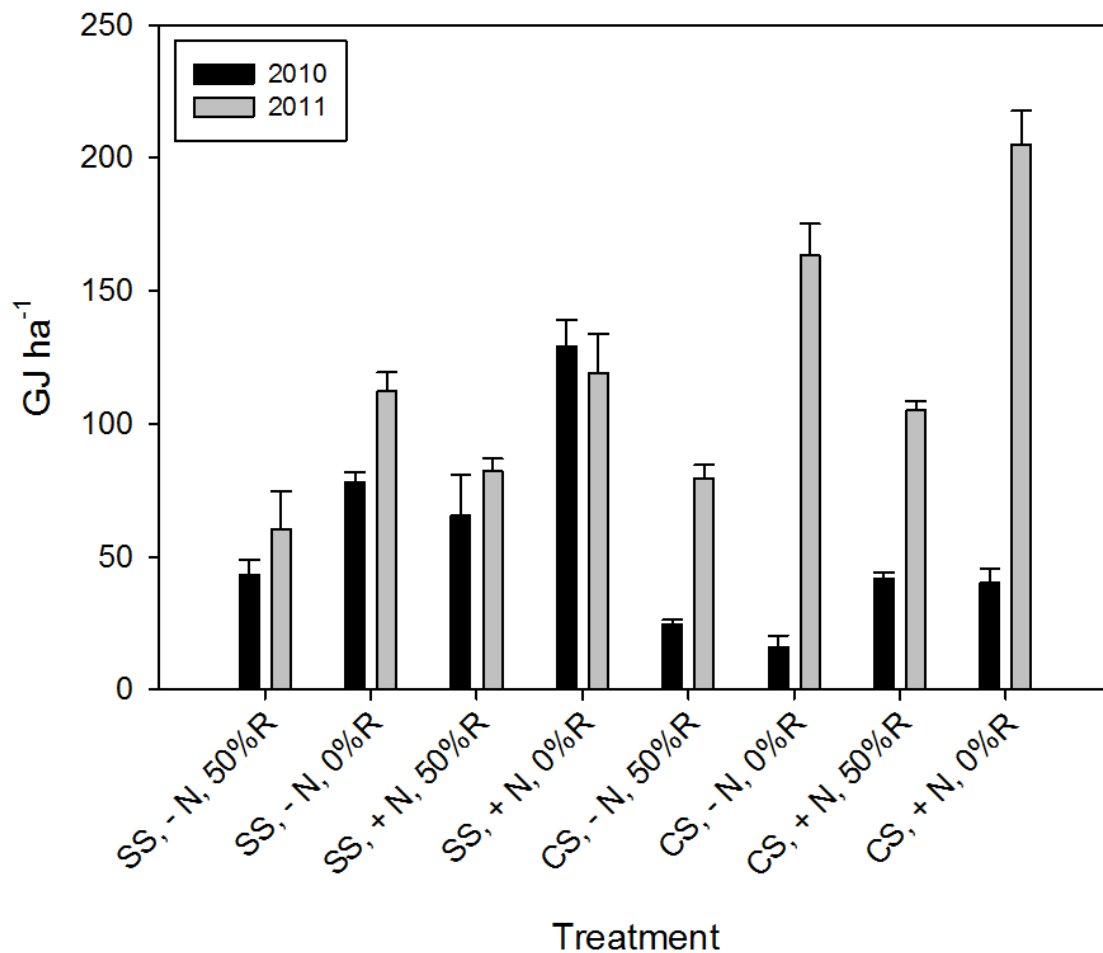


Figure 33. Mean energy yields in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).

Table 13. Summary of p-values testing the effects of crop rotation (Rotation), N fertilization (N-Fert.), and residue return rate (Residue) on energy yield, N<sub>2</sub>O Emissions, net annual change in SOC ( $\Delta$  SOC), and well-to-wheel life cycle greenhouse gas emissions (W2W LCGHG).

Effect	Energy Yield			N <sub>2</sub> O Emissions		$\Delta$ SOC	W2W LCGHG		
	<u>2010</u>	<u>2011</u>	<u>2-yr. avg.</u>	<u>2010</u>	<u>2011</u>		<u>2010</u>	<u>2011</u>	<u>2-yr. avg.</u>
					<i>p-value</i>				
Rotation	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.707	0.410	0.510	<i>0.064</i>	0.299	<b>0.004</b>	0.865
N-Fert.	<b>&lt;0.001</b>	<b>0.003</b>	<b>&lt;0.001</b>	<b>0.013</b>	0.751	0.584	<b>0.011</b>	<i>0.069</i>	<b>0.015</b>
Residue	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.312	0.154	0.872	0.608	<i>0.084</i>	0.361
Rotation*N-Fert.	0.127	0.194	0.877	0.822	0.308	0.862	0.354	0.470	0.632
Rotation*Residue	<b>&lt;0.001</b>	<b>0.003</b>	0.752	<b>0.014</b>	0.529	0.593	0.271	0.247	0.243
N-Fert.*Residue	<i>0.094</i>	0.966	0.335	0.426	0.431	0.563	0.653	0.128	0.424
Rotation*N-Fert.*Residue	0.295	0.285	0.806	0.966	<b>0.042</b>	0.503	0.766	0.849	0.869

Values in bold significant at 0.05 level.

Values in italics significant at 0.10 level.

In 2011, the entire field was planted to bioenergy sorghum and energy yields were normally distributed. Despite each crop sequence being planted to sorghum in 2011, one of the strongest observed management effects was crop rotation ( $P < 0.001$ ) (Table 13). The CS treatments increased total energy yield by 48% in 2011 compared to SS treatments (Fig. 33). The strong response of rotated sorghum vs. continuous sorghum was primarily associated with greater yields that may have been due to several factors including potentially stronger allelopathic effects (Nimbal et al., 1996; Weston and Duke, 2003) in SS treatments, differences in nutrient removal rates in each crop rotation (Wight et al., 2012), or other factors within each crop sequence. Energy yield was significantly ( $P = 0.003$ ) increased by N fertilization in 2011 (Table 13). Treatments receiving N provided 23% more GJ of energy per hectare than treatments that did not receive N fertilizer in 2011 (Fig. 33). As anticipated, residue return significantly ( $P < 0.001$ ) impacted energy yield in 2011, with 100% biomass removal (0%R) increasing energy yield by 83% compared to 50%R treatments. The impact of residue return on total biomass yield (assuming 100% residue removal) was also evaluated in 2011 (data not shown) for reference. However, we determined returning residue only slightly improved total harvested sorghum yield, and the effect was not significant ( $P = 0.179$ ).

To evaluate the impact of agronomic practices on potential energy yield across both years, the average for each plot was calculated and analyzed. Across both years, N fertilization significantly ( $P < 0.001$ ) increased energy yield (Table 13). Treatments receiving N fertilization provided 36% greater average energy yield across 2010 and 2011 compared to unfertilized treatments (Fig. 34). Removing all biomass for fuel production significantly ( $P < 0.001$ ) increased energy yield across years, with 0%R treatments resulting in 72% greater

theoretical ethanol yield than treatments returning half the residue (Fig. 34). Across both years, + N, 0%R treatments generally provided the greatest potential energy yield, averaging more than 120 GJ ha<sup>-1</sup> annually (Fig. 34). In contrast, - N, 50%R treatments provided the lowest energy yield across 2010 and 2011, averaging about 50 GJ ha<sup>-1</sup> annually (Fig. 34).

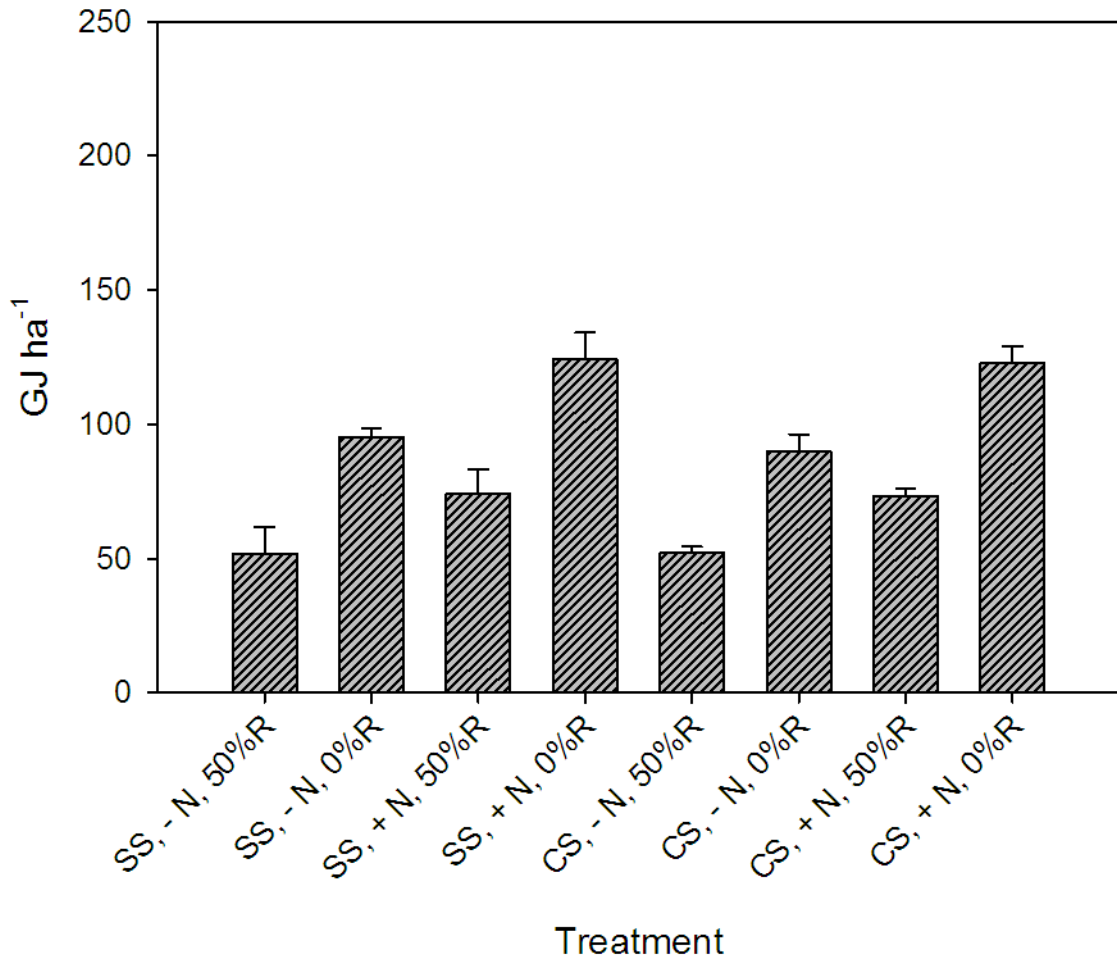


Figure 34. Mean energy yields of the two-year average from treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).

### Cumulative Nitrous Oxide Emissions

All cumulative emissions of nitrous oxide were analyzed following conversion to CO<sub>2</sub>-eq and are reported as such. The [SS, + N, 50%R] treatment had the highest cumulative

annual N<sub>2</sub>O emission, nearly 6,000 kg CO<sub>2</sub>-eq ha<sup>-1</sup>, while the [SS, - N, 0%R] treatment had the lowest with less than 2,000 kg CO<sub>2</sub>-eq ha<sup>-1</sup> (Fig. 35). Nitrogen fertilization was found to be a major controlling factor (P = 0.013), affecting cumulative N<sub>2</sub>O emissions in 2010 (Table 13). Fertilized treatments had 53% higher cumulative N<sub>2</sub>O emission than treatments that were unfertilized (Fig. 35). A rotation by residue return interaction (P = 0.014) was also observed in 2010, where, regardless of N fertilization, the SS, 50%R treatments had 66% higher cumulative N<sub>2</sub>O emissions than the CS, 50%R treatments (Fig. 35). The CS treatments receiving 50%R were supplied with a larger mass of sorghum biomass returned to the soil from the 2009 harvest. However, the summation of organic C returned from 2008 and 2009 was relatively similar between the SS and CS treatments (12,384 and 11,668 kg C ha<sup>-1</sup>, respectively) because biomass sorghum yield gains by CS treatments in 2009 were somewhat negated by lower total organic C applied from corn in 2008. While 50%R treatments had slightly higher N<sub>2</sub>O emissions than 0%R treatments in 2010, the effect was not significant (P = 0.312) (Fig. 35 and Table 13). Likewise, SS treatments had slightly higher N<sub>2</sub>O emissions than CS treatments in 2010, but the impact of crop rotation was not significant (P = 0.410) (Fig. 35 and Table 13). The nitrogen emission factors (NEF) from both SS and CS were generally higher in 2010 than in 2011. The SS treatments had an average NEF of 1.7%, while the CS rotation had an NEF of 2.5%. The higher NEF in the CS rotation may be due to corn uptake of N being less efficient than with bioenergy sorghum and because bioenergy sorghum was actively extracting N for a longer period of time.

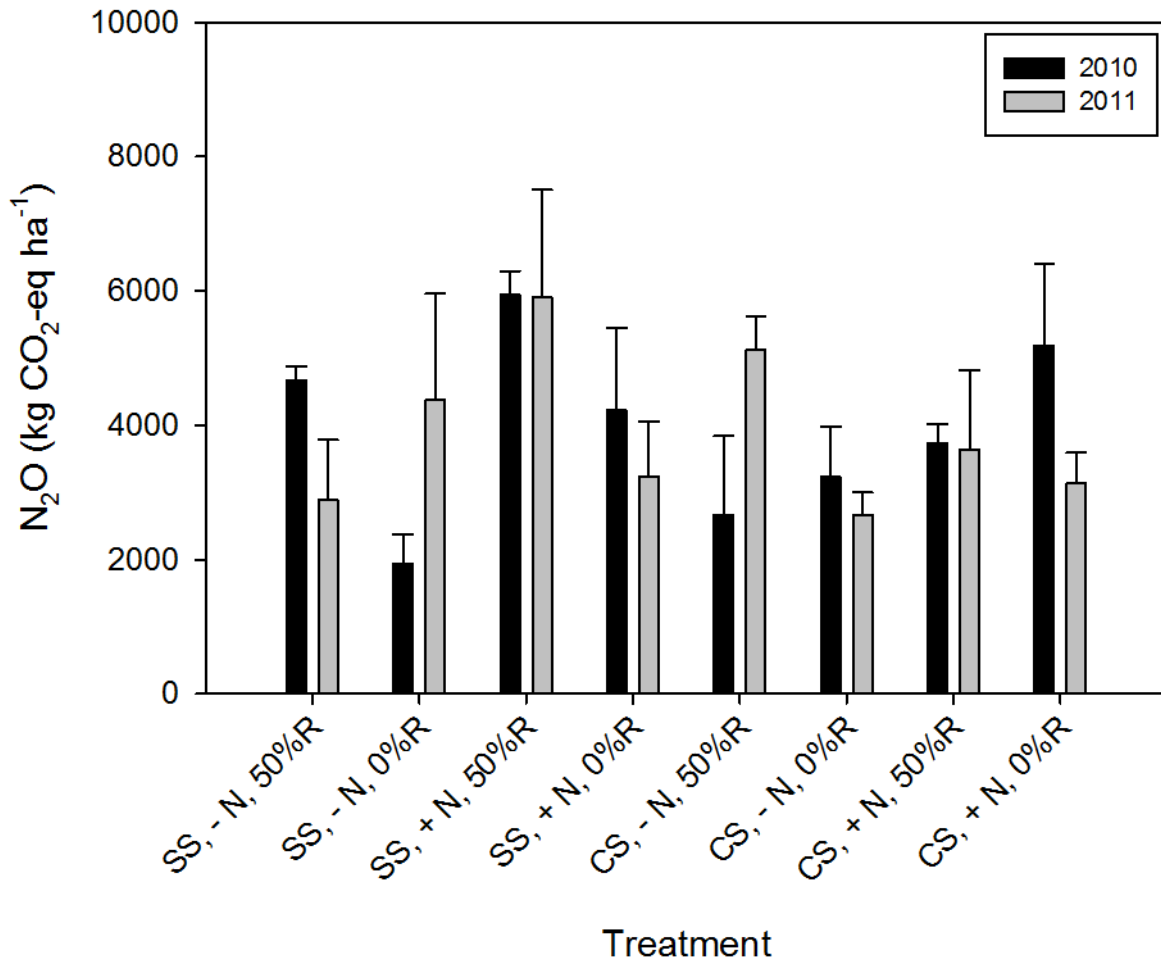


Figure 35. Mean cumulative N<sub>2</sub>O emissions in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4). Emissions of N<sub>2</sub>O reported as CO<sub>2</sub>-eq, assuming N<sub>2</sub>O GWP of 298 g CO<sub>2</sub>-eq per g N<sub>2</sub>O.

Cumulative N<sub>2</sub>O emissions in 2011 were not significantly impacted by crop rotation, N fertilization, or residue return. The high variability in the 2011 N<sub>2</sub>O data may have limited detection of significant differences between treatments. Based on mean estimates, [SS, + N, 50%R] and [CS, - N, 0%R] had the highest and lowest cumulative N<sub>2</sub>O emissions, respectively (Fig. 35). Cumulative N<sub>2</sub>O emissions ranged from 2,670 to 5,910 kg CO<sub>2</sub>-eq ha<sup>-1</sup> among treatments in 2011 (Fig. 35). There was a significant (P = 0.042) three-way interaction by the primary factors on cumulative annual N<sub>2</sub>O emissions in 2011 (Table 13).

Based upon means separation, [SS, + N, 50%R] emitted approximately double the cumulative N<sub>2</sub>O emissions than [SS, - N, 50%R] in 2011 (Fig. 35). The NEF from SS and CS in 2011 were 1.6% and -0.3%, respectively. The observed negative NEF was peculiar; review of the data revealed that the highly sporadic N<sub>2</sub>O fluxes measured during the 2011 drought made small fluxes of N<sub>2</sub>O from unfertilized plots play a larger role in NEF when fertilized plots had no detectable fluxes during the same sampling event. We believe that the variable and relatively low N<sub>2</sub>O fluxes associated with extreme drought conditions explain much of the negative NEF observed in CS treatments in 2011.

### **Soil Organic Carbon**

Regressions were performed on mass of SOC with time from 2008 (initiation of study) to 2012 in each plot at each of the five sampling depths. Of the 160 total regressions, approximately one-third (52) were significant at the 0.10 level of significance and 18% (28) were significant at the 0.05 level of significance (Table 14). The SS treatments had 34 significant regressions compared to 18 in the CS treatments. Treatments receiving N fertilization had 29 significant regressions compared to 23 significant regressions for unfertilized plots. Significant regressions among residue return treatments were almost evenly split between 27 significant regressions in 50%R treatments and 25 significant regressions in 0%R treatments (Table 14).



Table 14. Net annual change in SOC ( $\text{Mg C ha}^{-1} \text{ yr}^{-1}$ ) for each experimental unit ( $n = 32$ ) and each sampled depth and cumulative sum to 90 cm. Values are organized by treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R). Net change values were derived from regressions of mass of SOC across time from 2008 to 2012.

<b>Treatment</b>	<b>SS, - N, 50%R</b>				<b>SS, - N, 0%R</b>			
Rep	1	2	3	4	1	2	3	4
	<i>Mg C ha<sup>-1</sup> yr<sup>-1</sup></i>							
0 - 5 cm	1.37**	0.30	0.71	1.10**	0.58	0.85	-0.10	0.64
5 - 15 cm	1.07*	0.20	0.59	2.09**	1.10*	0.04	0.80	1.30*
15 - 30 cm	1.47	-0.83	-0.02	0.50	1.10**	1.62*	0.21	1.20*
30 - 60 cm	2.68*	1.48	-1.90	7.25**	-2.33	1.80**	1.71	8.09**
60 - 90 cm	3.82**	2.97	4.30	0.98	0.18	0.39	4.13*	5.25**
<b>Cumulative sum</b>	10.41	4.12	3.68	11.91	0.63	4.71	6.75	16.51

<b>Treatment</b>	<b>SS, + N, 50%R</b>				<b>SS, + N, 0%R</b>			
Rep	1	2	3	4	1	2	3	4
	<i>Mg C ha<sup>-1</sup> yr<sup>-1</sup></i>							
0 - 5 cm	1.12*	1.39**	1.10	1.43*	0.18	0.28	1.59**	1.02**
5 - 15 cm	0.63	0.94*	1.05*	0.97	0.41	0.23	0.59*	1.71**
15 - 30 cm	0.28	0.81**	-0.17	1.94	1.03	1.76*	0.40	1.51**
30 - 60 cm	2.73	1.94*	2.84*	0.13	1.09	0.70	3.26	1.56**
60 - 90 cm	4.26*	3.53**	0.14	-0.36	-0.64	5.34	2.76**	-0.62
<b>Cumulative sum</b>	9.02	8.61	4.97	4.11	2.08	8.32	8.60	5.18

Table 14 continued

<b>Treatment</b>	<b>CS, - N, 50%R</b>				<b>CS, - N, 0%R</b>			
Rep	1	2	3	4	1	2	3	4
	<i>Mg C ha<sup>-1</sup> yr<sup>-1</sup></i>							
0 - 5 cm	0.88**	0.40	0.97**	0.47	0.49	0.80	0.26	0.14
5 - 15 cm	0.99**	0.34	2.12	-0.11	0.55	1.02	-0.20	-0.19
15 - 30 cm	2.03	-0.03	0.23	1.04*	-0.29	1.04	1.79**	-0.18
30 - 60 cm	0.54	1.81	1.02	-0.12	4.13	-0.33	3.32	3.48
60 - 90 cm	0.61	0.27	2.30	3.71*	-1.14	-1.95	0.53	3.20*
<b>Cumulative sum</b>	5.05	2.80	6.65	4.99	3.73	0.59	5.70	6.45
<b>Treatment</b>	<b>CS, + N, 50%R</b>				<b>CS, + N, 0%R</b>			
Rep	1	2	3	4	1	2	3	4
	<i>Mg C ha<sup>-1</sup> yr<sup>-1</sup></i>							
0 - 5 cm	0.66	0.61	0.55	1.30**	0.90	1.02*	-0.05	1.12**
5 - 15 cm	0.81	0.04	1.27**	0.45	1.08	0.17	0.83	1.37**
15 - 30 cm	1.64*	1.63	0.74	1.45	-0.55	2.23	-0.84	2.45
30 - 60 cm	1.05	-3.69*	-0.59	0.53	0.66	-0.49	0.87	3.67*
60 - 90 cm	-4.35	3.32*	2.49	0.77	2.22	0.24	0.98**	3.34**
<b>Cumulative sum</b>	-0.20	1.91	4.47	4.49	4.31	3.18	1.78	11.96

\*\* Significant at the 0.05 level.

\* Significant at the 0.10 level.

While a number of individual slopes within a given rep and depth may have been negative, indicating net loss of C, most were positive, indicating net C accrual (Table 14). When summed to estimate net C change to 90 cm, every plot indicated net C accrual with the exception of a single plot. The single plot indicating a net loss of C over time appears to have been driven by a single regression performed at the 60 - 90 cm depth (Table 14). There were generally more significant regressions near the surface and at the bottom sampling depth, with slightly fewer found in the two depths from 15 - 60 cm. The range of change in SOC across all individual reps ranged from -0.20 to 16.51 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (Table 14). The values were highly variable and many were unusually high. Some particular outliers, such as 16.51 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, were difficult to justify removal from the data set because they contained several significant regressions, some significant at the 0.05 level (Table 14). Thus they were included for this study, but future research will re-evaluate select outliers to better determine validity, possibly by re-analyzing select samples.

The impact of crop rotation, N fertilization, and residue return was evaluated on the net change in soil C from 2008 to 2012. Means from every treatment combination were positive, indicating all bioenergy sorghum cropping systems were a net sink for atmospheric C. The net SOC change values ranged from 7.53 Mg C ha<sup>-1</sup> yr<sup>-1</sup> for [SS, - N, 50%R] to 2.67 Mg C ha<sup>-1</sup> yr<sup>-1</sup> for [CS,+ N, 50%R] (Fig. 36). The SOC data were variable and the coefficient of variation across all treatments was 0.66. The only management practice to have a detectable impact on SOC change from 2008 to 2012 was crop rotation (Table 13). The SS treatments significantly (P = 0.064) increased soil C accrual by 62% compared to CS treatments (Table 13). Despite adding significantly more biomass C back to the soil, 50%R treatments were not different from 0%R treatments in net SOC change across 5 years of

investigation. Similarly, N fertilization had no effect on net C change, despite fertilized plots producing significantly greater amounts of aboveground biomass C.

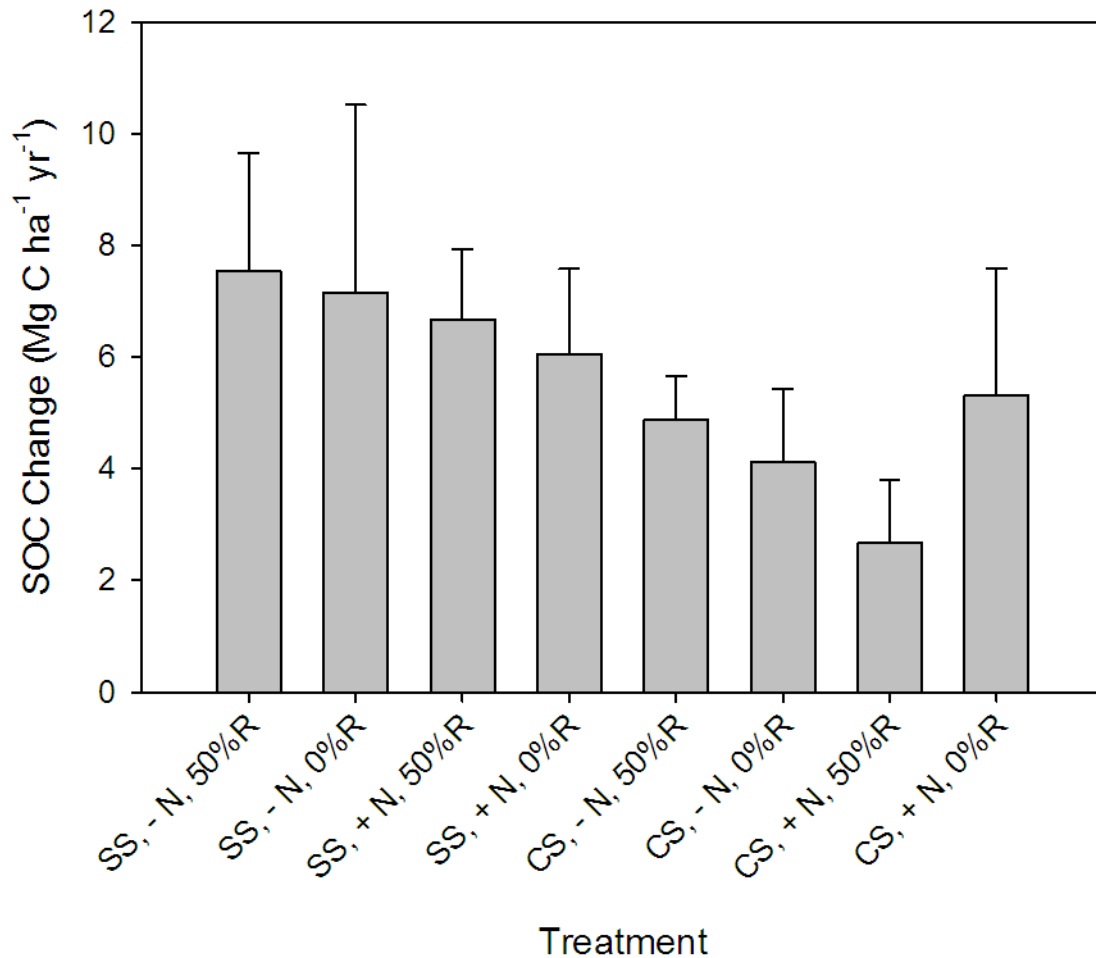


Figure 36. Mean net changes in SOC from 2008 to 2012 from treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4). Means are the sum of net change in SOC from 5 sampling depths to 90 cm.

### Life Cycle Greenhouse Gas Emissions

Accounting for ‘edge of field’ life cycle GHG emissions (LCGHG) included direct measurement of N<sub>2</sub>O emissions and several inputs required to produce bioenergy feedstock. Inputs at the field level included urea fertilizer, diesel fuel, and herbicide production

emissions as well as emissions associated with irrigation. The ‘field to wheel’ LCGHGs were associated with inputs required to transport, produce, and utilize the ethanol as a transportation fuel. These emissions were estimated through extrapolating GREET-derived values for cellulosic ethanol production (Wang et al., 2012) and were directly tied to energy yield ( $\text{MJ ha}^{-1}$ ) through a multiplier. Thus, greater yields translated to greater ‘field to wheel’ LCGHGs. According to GREET model developers, this was a conservative approach because the improved conversion estimate utilized in this study for bioenergy sorghum ( $452 \text{ L Mg}^{-1}$ ) would produce a decrease in ‘well-to-wheel’ energy consumption (J. Dunn, personal communication, 2013) compared to default cellulosic ethanol conversion estimates ( $375 \text{ L Mg}^{-1}$ ) utilized by the GREET model (Wang et al., 2012).

The summation of all LCGHGs associated with cellulosic ethanol production via bioenergy sorghum cropping systems revealed which inputs and processes were of greatest influence. Nitrous oxide emissions were by far the largest contributor to overall LCGHGs, frequently accounting for much more than half of the total emissions (Table 15 and Fig 28). In 2010, when bioenergy sorghum was grown in the SS treatments, LCGHGs attributable to cumulative  $\text{N}_2\text{O}$  emissions ranged from 57 to 84% (Table 15 and Fig 28). Nitrous oxide emissions were a larger portion of LCGHG in CS treatments in 2010 (corn sequence of rotation) than SS treatments, ranging from 73 to 88% of total  $\text{CO}_2\text{-eq}$  emissions (Table 15 and Fig 28). The other major LCGHGs in 2010 were attributable to ethanol and urea fertilizer production (Table 15 and Fig 28). Ethanol production accounted for more than 11% of LCGHGs across all treatments. Similarly, urea fertilizer production accounted for more than 11% of LCGHGs across all plots receiving N fertilization.

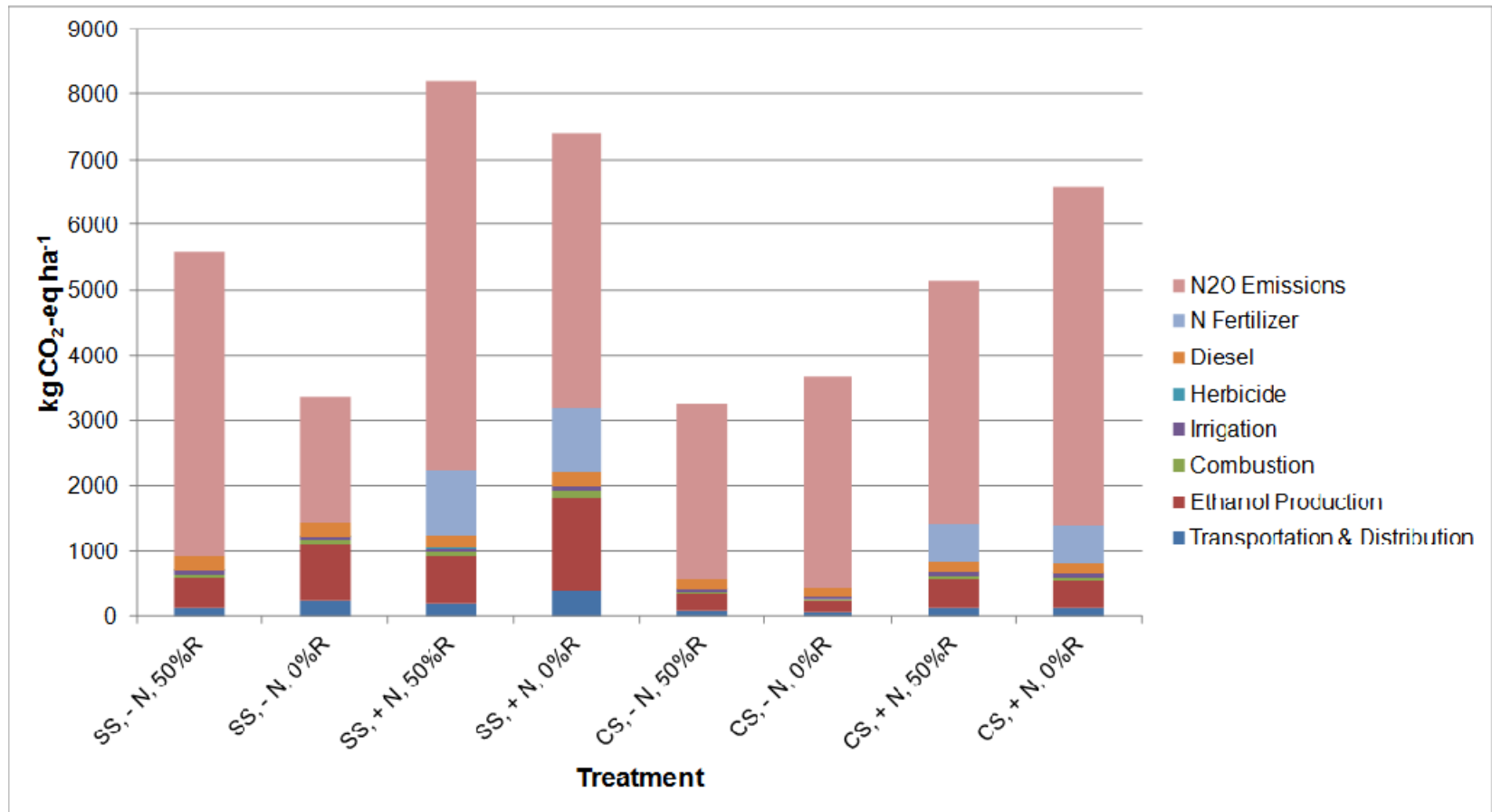


Figure 37. Mean (n = 4) contributions of life cycle greenhouse gas emissions by activities in 2010 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R).

Overall, LCGHGs were generally higher in 2011 compared to 2010. For many treatments, yields were higher in 2011 than in 2010, thus emissions directly correlated with energy yield were higher. Nitrous oxide emissions remained the largest contributor to overall LCGHGs in 2011, ranging from 41 to 76% of total emissions (Table 15 and Fig. 38). Across all treatments, ethanol production and transportation and distribution accounted for approximately 20% and 6% of LCGHGs, respectively. Approximately 14% of LCGHGs were attributable to urea fertilizer production in treatments receiving N fertilization. In 2010 and 2011, [SS, + N, 50%R] and [CS, + N, 0%R] treatments consistently had some of the highest LCGHGs, possibly due to high N<sub>2</sub>O emissions, relatively high yields (subsequent LCGHGs tied to yield) and relatively high CO<sub>2</sub>-eq emissions associated with the high urea-fertilization rate (Figs. 37 and 38).

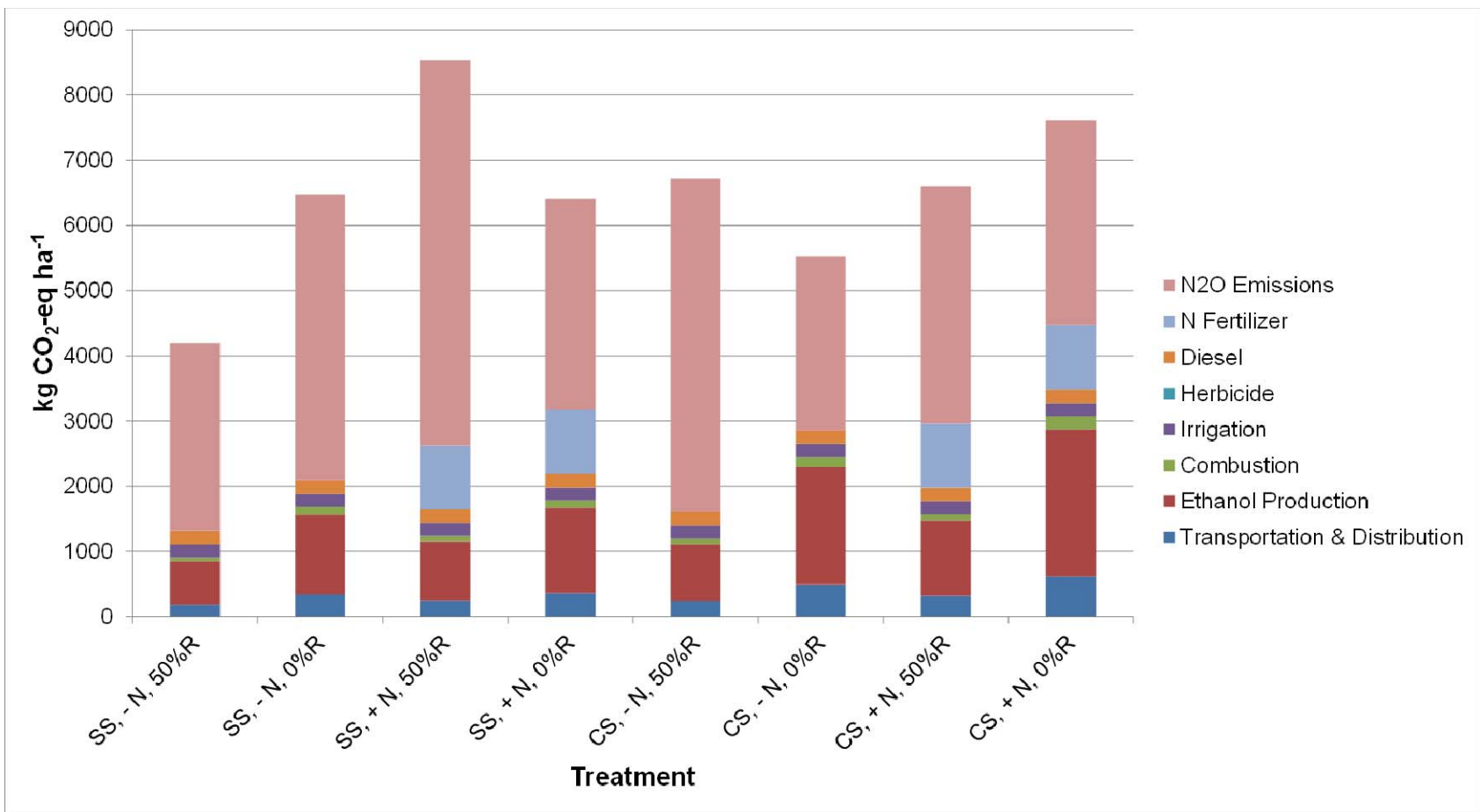


Figure 38. Mean (n = 4) contributions of of life cycle greenhouse gas emissions by activities in 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R).



Table 15. Summary of mean (n = 4) contributions of life cycle greenhouse gas emissions by activities in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R).

<b>Treatment</b>	<b>SS, - N, 50%R</b>	<b>SS, - N, 0%R</b>	<b>SS, + N, 50%R</b>	<b>SS, + N, 0%R</b>
	<i>kg CO<sub>2</sub>-eq ha<sup>-1</sup></i>			
	<u>2010</u>			
Transportation & Distribution	130	233	197	387
Ethanol Production	476	856	721	1420
Combustion	43	78	66	129
Irrigation	52	52	52	52
Herbicide Production	5	5	5	5
Diesel Consumption	209	209	209	209
Urea Fertilization Production	0	0	982	982
N <sub>2</sub> O Emissions	4672	1940	5949	4219
	<u>2011</u>			
Transportation & Distribution	182	337	247	357
Ethanol Production	666	1234	907	1308
Combustion	61	112	82	119
Irrigation	197	197	197	197
Herbicide Production	0	0	0	0
Diesel Consumption	210	210	210	210
Urea Fertilization Production	0	0	982	982
N <sub>2</sub> O Emissions	2885	4378	5910	3239

Table 15 continued

<b>Treatment</b>	<b>CS, - N, 50%R</b>	<b>CS, - N, 0%R</b>	<b>CS, + N, 50%R</b>	<b>CS, + N, 0%R</b>
	<i>kg CO<sub>2</sub>-eq ha<sup>-1</sup></i>			
	<u>2010</u>			
Transportation & Distribution	74	48	125	120
Ethanol Production	270	177	457	441
Combustion	25	16	42	40
Irrigation	52	52	52	52
Herbicide Production	5	5	5	5
Diesel Consumption	145	145	145	145
Urea Fertilization Production	0	0	589	589
N <sub>2</sub> O Emissions	2664	3228	3726	5191
	<u>2011</u>			
Transportation & Distribution	239	490	315	616
Ethanol Production	875	1798	1154	2257
Combustion	80	163	105	205
Irrigation	197	197	197	197
Herbicide Production	0	0	0	0
Diesel Consumption	210	210	210	210
Urea Fertilization Production	0	0	982	982
N <sub>2</sub> O Emissions	5115	2670	3639	3142

## Well-to-Wheel Life Cycle Greenhouse Gas Emissions

The effect of crop rotation, N fertilization, and residue return on well-to-wheel life cycle GHG emissions (W2W LCGHG) were evaluated in 2010, 2011, and across both years. Specifically, following subtraction of direct and indirect LCGHGs from net change in SOC, the final W2W value provided a value of efficiency (the mass of CO<sub>2</sub>-eq produced/sequestered per unit of cellulosic energy provided). The W2W LCGHGs from 2010 and 2011 were highly variable, largely due to inherent variability associated with a number of the values associated with final computation (eg. N<sub>2</sub>O emissions and net SOC change). Across all treatments, the coefficient of variation was 96% and 108% in 2010 and 2011, respectively. While a number of individual plots had positive values, indicating a net source of CO<sub>2</sub>-eq MJ<sup>-1</sup> ethanol, all treatment means were negative, indicating C sequestration per MJ energy. Therefore, all W2W LCGHG values reported represent g CO<sub>2</sub>-eq sequestered as SOC, unless otherwise noted.

Bioenergy sorghum cropping systems were generally more efficient in 2010 compared to 2011 (Fig. 39). This was likely due to lower energy yields produced overall in 2010 (corn grain ethanol in CS treatments) in addition to slightly lower LCGHGs and constant net SOC change values. Well-to-wheel LCGHGs ranged from -622 to -105 g CO<sub>2</sub>-eq MJ<sup>-1</sup> for [CS, - N, 50%R] and [SS, + N, 0%R] treatments, respectively, in 2010 (Fig. 39). The only management practice with a detectable impact on W2W LCGHGs in 2010 was N fertilization (P = 0.011) (Table 13). Treatments receiving urea fertilizer in 2010 were 61% less efficient than unfertilized treatments (Fig. 39). This may have been due to higher N<sub>2</sub>O emissions associated with N fertilized treatments as well as a relatively large LCGHG cost associated with urea fertilizer production. Sorghum rotated with corn generally provided

greater W2W efficiency (Fig. 39), but the overall effect was not significant ( $P = 0.299$ ). Residue return had no effect ( $P = 0.608$ ) on W2W efficiency in 2010. Since corn was grown in CS treatments in 2010, the effect of residue return was only directly noticeable on energy yields from SS treatments.

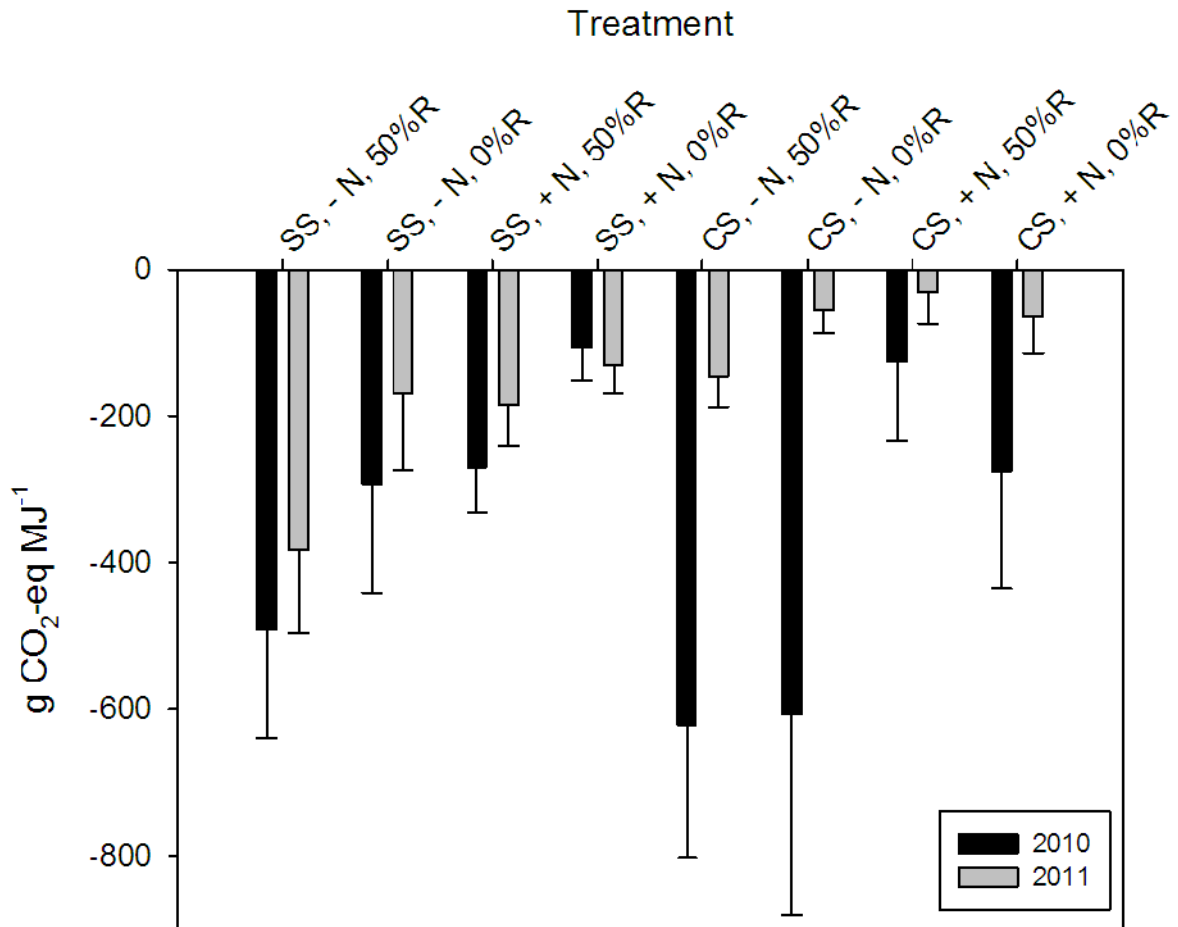


Figure 39. Mean net well-to-wheel greenhouse gas emissions associated with bioenergy sorghum production in 2010 and 2011 for treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error ( $n = 4$ ).

The W2W LCGHG efficiency of most sorghum production treatments in 2011 was lower than in 2010. All crop rotation treatments were in the sorghum sequence of the rotation

in 2011 and energy yield averaged across all treatments was more than double the energy yield from 2010. Well-to-wheel LCGHGs in 2011 ranged from -383 to -31 g CO<sub>2</sub>-eq MJ<sup>-1</sup> for [SS, - N, 50%R] and [CS, + N, 50%R] treatments, respectively. Each of the primary agronomic management practices examined had some effect on W2W LCGHGs in 2011. Crop rotation had the strongest impact (P = 0.004) on W2W LCGHGs in 2011, with SS treatments being 66% more efficient than CS treatments (Fig. 39). This observation could be partially explained by CS treatments having higher energy yield in 2011 than SS treatments. Nitrogen fertilization had a marginal effect (P = 0.069) on W2W LCGHGs in 2011, as - N treatments were 45% more efficient than + N treatments (Fig. 39). Treatments receiving N fertilizer generally had higher yield, but higher N<sub>2</sub>O emissions and indirect GHG emissions. Residue return had the weakest of the three significant effects (P = 0.084), but was still significant at the 0.10 level of significance in a highly variable data set. Treatments removing all biomass for biofuel feedstock had 43% lower W2W LCGHG efficiency compared to treatments returning half the biomass residue (Fig. 39). Treatments returning half the residue had approximately half the energy yield of those treatments with complete biomass removal, which partially explained improved W2W LCGHG efficiency.

Across two years of study, the impact of crop rotation, N fertilization, and residue return on the average W2W LCGHG was examined. The average W2W LCGHGs across both years ranged from -437 to -78 g CO<sub>2</sub>-eq MJ<sup>-1</sup> for [SS, - N, 50%R] and [CS, + N, 50%R] treatments, respectively (Fig. 40). Despite being averaged across years, the overall variation of the data set was still relatively high and the coefficient of variation across all treatments was 89%. Nitrogen fertilization was the only management practice to have a significant (P = 0.015) impact on the two-year averaged W2W LCGHGs. Treatments not receiving any N

fertilizer were 57% more efficient (at sequestering CO<sub>2</sub>-eq) than treatments receiving N fertilizer (Fig. 40). Lower yield and N<sub>2</sub>O emissions as well as lower indirect GHG emissions associated with unfertilized treatments may partially explain the high efficiency value associated with – N treatments. Treatments returning half the residue back to the soil were generally more efficient than 0%R treatments (Fig. 40), although the effect was not significant (P = 0.361). Crop rotation had no effect (P = 0.865) on two-year average W2W LCGHGs and the means were nearly identical, -241 and -254 g CO<sub>2</sub>-eq MJ<sup>-1</sup> for CS and SS treatments, respectively (Fig. 40).

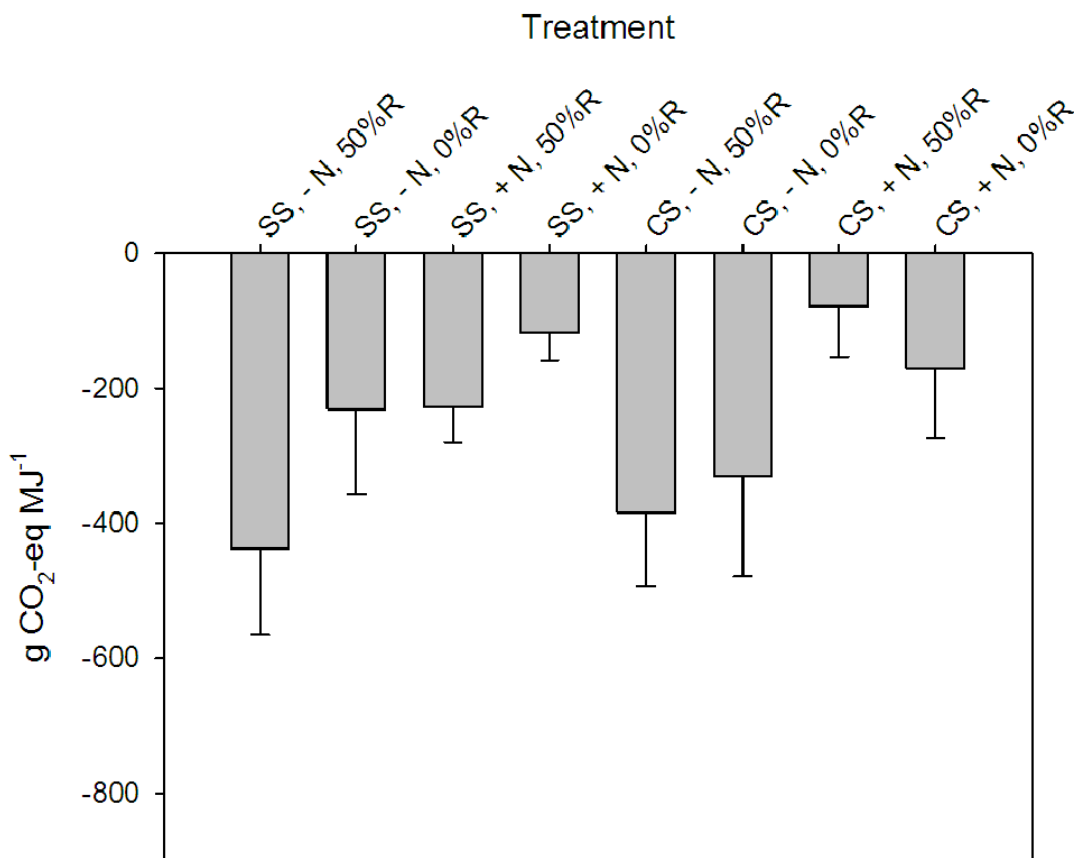


Figure 40. Mean net two-year average well-to-wheel net greenhouse gas emissions associated with bioenergy sorghum production from treatment combinations of crop rotation (SS or CS), N fertilization rate (- N or + N), and residue return rate (0%R or 50%R) are reported with one standard error (n = 4).

## **DISCUSSION**

### **Energy Yield**

Bioenergy sorghum is potentially an ideal biofuel crop because of its ability to consistently produce high yield. Based upon conversion estimates (Stefaniak et al., 2012), multiple studies have documented photo-period sensitive (bioenergy) sorghum can provide yields in excess of 250 GJ ha<sup>-1</sup> (Maughan et al., 2012; Ra et al., 2012; Rocateli et al., 2012). The highest energy yields observed in this study were observed for the [CS, + N, 0%R] treatment in 2011 and were just over 200 GJ ha<sup>-1</sup> (Fig. 33). Sorghum yields from 2010 and 2011 were potentially lower than anticipated due to moderate and severe drought conditions, respectively (Nielsen-Gammon, 2011), as well as a later planting date in 2010 (Table 12 and Fig. 32). Rocateli et al. (2012) observed that when bioenergy sorghum was stressed by excessive water stress and disease in 2009, it still produced an energy yield of more than 140 GJ ha<sup>-1</sup> within 20 weeks after planting. Averaged across years and N application rates, an experiment on photo-period sensitive sorghum near Lubbock, TX reported an energy yield of only 32 GJ ha<sup>-1</sup>. However, differences in energy yield calculation are likely due to their method that utilized neutral detergent fiber composition analysis and other published conversion estimates (Tamang et al., 2011). When reported dry matter yields of Tamang et al. (2011) were converted to energy yield based on conversion estimates of Stefaniak et al. (2012), the average energy yield across both years and all N application rates was approximately 145 GJ ha<sup>-1</sup>, which is comparable to energy yields observed in our study (Fig. 33).

As anticipated, N fertilization significantly improved energy yield over unfertilized treatments in both 2010 and 2011. Nitrogen fertilization increased yield in years 2008 and

2009 of the study at our location (Wight et al., 2012). Additional N was shown to improve yield of both sorghum and corn, illustrating the need for N fertilization to sustain high yields across years. This result agrees with results from long-term modeling of bioenergy sorghum production in the U.S., where N addition was required to sustain high yield over time (Meki et al., 2013). Other studies have demonstrated the importance of N addition for sustaining energy yield (Maughan et al., 2012; Tamang et al., 2011). Some studies did not observe a yield response to added N in the initial year of study, but in west Texas, energy yield responded quadratically to N application rate by the second year of study (Tamang et al., 2011).

Returning a portion of harvested sorghum biomass to the soil as an organic amendment had obvious implications on energy yield in the form of a direct reduction in total feedstock yield for fuel production, but other factors related to residue return may also have had an impact on energy yield. Reported effects of residue removal in sorghum cropping systems have varied. Harvesting half the aboveground sorghum biomass reduced yield and overall soil quality after three cropping seasons (Powell and Hons, 1991). A recent study found that returning 25% of aboveground sorghum biomass from the first harvest of the season improved yields of the second harvest and was believed to have been related to increased soil moisture retention with the return (Wight et al., 2012). When high bioenergy sorghum yields from Alabama were utilized in a recent crop modeling study, it was determined that 50% biomass removal rates still provided approximately 137 GJ ha<sup>-1</sup> in energy yield (Meki et al., 2013). Initial yields in the Alabama study were much higher than those observed in our study and even many treatments with complete biomass removal from our study were not able to provide as great an energy yield as treatments with 50% removal



rates from the study in Alabama (Fig. 33) (Meki et al., 2013). Biomass residue returned represents a valuable pool of C and nutrients which aides in sustaining long-term soil health (Hoskinson et al., 2007; Lal, 2009) and harvesting all biomass can mine the soil of these vital nutrients (Meki et al., 2013). Some research suggests 75% biomass removal rates may be sustainable in no-till cropping systems (Meki et al., 2013). However, if compaction is of concern long-term, then removal rates of no more than 50% were suggested (Meki et al., 2013). Thus, if 50%R treatments in this study are utilized for future biofuel production, we would expect these treatments to provide 50 – 100 GJ ha<sup>-1</sup> of future energy yield (Fig. 34). However, we also anticipate higher energy yields may be achieved under non-drought growing conditions.

### **Nitrous Oxide Emissions**

Direct N<sub>2</sub>O emissions from the various bioenergy sorghum crop production scenarios were quantified for inclusion into LCAs. Emissions of N<sub>2</sub>O during crop production are frequently cited as a major component of LCAs for bioenergy cropping systems (Kim and Dale, 2005; Kim et al., 2009, Landis et al., 2007; Liska and Cassman, 2008, Wang et al., 2012; Wang et al., 2011) and a major source of GWP from crop production (Stehfest and Bouwman, 2006). Nitrous oxide also represents a monetary loss in terms of potential N leaving the cropping system, rather than remaining as a valuable nutrient to enhance plant growth. Nitrous oxide also has almost 300 times the GWP of CO<sub>2</sub> (Smith et al., 2007). Thus, cumulative N<sub>2</sub>O emissions remain a critical factor when evaluating net GWP and life cycle GHGs from bioenergy crop production.

Average annual cumulative emission of N<sub>2</sub>O observed across both years of this study was approximately 3,746 kg CO<sub>2</sub>-eq ha<sup>-1</sup> yr<sup>-1</sup>. In 2010, the annual range between treatments was 1,871 to 6,085 kg CO<sub>2</sub>-eq ha<sup>-1</sup> yr<sup>-1</sup> and in 2011 it was 2,342 to 5,617 kg CO<sub>2</sub>-eq ha<sup>-1</sup> yr<sup>-1</sup> (Fig. 35). Similar to CO<sub>2</sub>, measured N<sub>2</sub>O values were relatively high compared to several studies in the U.S., but fall within the range of cumulative N<sub>2</sub>O emissions observed by several studies within North America [280 – 7,212 kg CO<sub>2</sub>-eq ha<sup>-1</sup> yr<sup>-1</sup>] (Adviento-Borbe et al., 2007; Duxbury and McConnaughey, 1986; Hernandez-Ramirez et al., 2009; Johnson et al., 2012; Parkin and Kaspar, 2006; Venterea et al., 2011) and globally [140 – 18,729 kg CO<sub>2</sub>-eq ha<sup>-1</sup> yr<sup>-1</sup>] (Denmead et al., 2010; Rozas et al., 2001; Stehfest and Bouwman, 2006; Weier, 1998).

While N<sub>2</sub>O emissions measured in this study were generally higher than some observed elsewhere, several factors may have contributed to elevated emissions. Warm soil temperatures observed throughout both growing seasons in central Texas (Fig. 32) have been shown to promote greater soil N<sub>2</sub>O fluxes (Adviento-Borbe et al., 2010; Barton et al., 2008; Rochette et al., 2010), and may indicate potential elevated microbial activity. Additionally, soil drying and re-wetting cycles have also been reported to promote N<sub>2</sub>O emissions from soil (Almaraz et al., 2009; Fernandes Cruvinel et al., 2011; Pelster et al., 2011; Venterea et al., 2011). The soils in our study became very dry during the droughty conditions experienced in 2010 and 2011 and were re-wetted through brief precipitation or irrigation events. Drying and re-wetting cycles frequently experienced across the growing season in this study likely enhanced soil organic N mineralization and nitrification, minimized transport of soil NO<sub>3</sub><sup>-</sup> during dry periods, and then increased substrate availability upon wetting (Aguilera et al., 2013).

Substantial variation within N<sub>2</sub>O measurements was observed in both 2010 and 2011. Measured fluxes were particularly variable, ranging from no detectable flux to a maximum of approximately 2,100 µg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup>. Pelster et al. (2011) suggested high variation in N<sub>2</sub>O fluxes in some cropping systems may be attributable to localized soil microenvironments which may favor nitrification/denitrification based upon prevailing environmental conditions and available organic C. High variation may partially explain some of the lack of detectable differences from residue return or crop rotation in this study. Other studies have found similar wide flux variation with no detectable differences in annual cumulative emissions (Johnson et al., 2012).

While N<sub>2</sub>O emissions were influenced by some management practices in 2010, little effect was observed in 2011. Cumulative N<sub>2</sub>O emissions were influenced by a rotation by residue interaction in 2010, potentially related to lower N addition rate to CS in 2010 compared to SS (lower N application rate when corn was grown), but no rotational effect or interaction was observed in 2011. The soil environment responded differently to residue return between crop rotations in 2010. It is possible that the higher amount of residue returned to the soil in CS, 50%R treatments in 2009 immobilized soil N until late in the season, when the C:N may have narrowed, possibly resulting in increased available soil N and subsequent higher N<sub>2</sub>O emissions. Nitrogen fertilization increased cumulative N<sub>2</sub>O emissions in 2010. Increased N availability from fertilization often increases N<sub>2</sub>O emissions and has been widely observed (Fernandes Cruvinel et al., 2011; Halvorson et al., 2008; Hernandez-Ramirez et al., 2009; Sistani et al., 2010; Snyder et al., 2009; Venterea et al., 2011). Thus, a common trade-off for increased bioenergy crop yield via N fertilization is

subsequently higher N<sub>2</sub>O emissions and GWP. It is important for future research to identify an optimal balance between high sorghum yields and relatively low N<sub>2</sub>O emissions.

Nitrogen emission factors (NEFs) were estimated annually for each crop rotation in addition to the two-year average. The IPCC utilizes a default approximation of 1.25% of applied fertilizer N being lost annually as N<sub>2</sub>O-N (Smith et al., 2007). Despite the constant value utilized to estimate NEF by the IPCC, other research has found a wide range of NEFs ranging from 0.02 to 3.42% (Adviento-Borbe et al., 2010; Aguilera et al., 2013; Alluvione et al., 2010; Barton et al., 2008; Fernandes Cruvinel et al., 2011; Halvorson et al., 2008; Linqvist et al., 2012; Sistani et al., 2010; Venterea et al., 2011). Emission factors have been found to significantly vary due to N source, N application rate, type of crop, and climate regime, among a myriad of other factors. The NEFs from this study varied between years, particularly in the CS cropping rotation. In 2010, estimated NEFs for the SS and CS treatments were 1.74 and 2.50%, respectively. The SS and CS sequences had NEFs of 1.63 and -0.30%, respectively, in 2011. The severe drought in 2011 likely contributed to the negative NEF estimated in the CS rotation. The CS estimate seemed atypical, but as noted before, the sporadic fluxes of N<sub>2</sub>O during 2011 created high variation in the data set. Small detectable fluxes were found in CS unfertilized treatments while no detectable fluxes were found in fertilized treatments from the same time period, resulting in the negative value. The SS rotation had a two-year average NEF of 1.68%, while the CS rotation had an average of 1.10%, both of which were similar to the IPCC estimate of 1.25%. Averaged across crop sequence and years, the overall NEF for the entire study was 1.39%.

Life cycle analysis models and methodologies utilize different NEFs to estimate the amount of N lost to N<sub>2</sub>O due to fertilization. As Plevin (2009) noted, two popular models, the

BESS and GREET models utilize slightly different NEFs that can impact total life cycle GHG results. The BESS model uses a fairly straight-forward assumption that 1.8% of applied fertilizer will be lost as N<sub>2</sub>O (Plevin, 2009). The original GREET model utilizes a slightly more complex estimate of 1.325% which was based on the IPCC estimates (Wang et al., 2012) but also accounted for additional N contained in crop residue which may end-up in the soil and had an effective NEF of 1.75% (Plevin, 2009). However, GREET model developers realized the great uncertainty associated with N<sub>2</sub>O emissions attributable to N fertilizer application, and based on additional research data, recently changed the NEF to 1.525% to take into account direct and indirect (eg. assuming some NO<sub>3</sub><sup>-</sup> leaching may occur and a portion may eventually become N<sub>2</sub>O) emissions (Wang et al., 2012). These recent NEFs compare favorably to the NEFs observed in this study which were generally slightly higher than 1.25%. If we used current versions of the BESS or GREET models, we would estimate that 2,425 or 2,000 kg CO<sub>2</sub>-eq ha<sup>-1</sup> yr<sup>-1</sup> (5.18 or 4.27 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>), respectively, would be attributable to bioenergy sorghum treatments which received urea-N fertilizer.

### **Soil Organic Carbon**

Bioenergy sorghum is a crop that may have tremendous C sequestration potential, particularly when compared with other row crops. Little information, however, is available on the impact of photo-period sensitive sorghum on belowground C accrual. Bioenergy sorghum is a relatively new crop and much of the research to date has largely focused on management impacts on aboveground C (yield) (Goff et al., 2010; Maughan et al., 2012; Ra et al., 2012; Rocateli et al., 2012; Rooney et al., 2007; Stefaniak et al., 2012; Tamang et al., 2011; Wight et al., 2012). Thus, comparison of SOC accrual rates may be limited to other

bioenergy crops. Annual changes to SOC were measured at 5 depths to 90 cm, which is considered a fairly deep sampling depth. Many other studies investigating SOC change attributable to crop management practices limit SOC examination to less than 50 cm (Al-Kaisi et al., 2005; Allmaras et al., 2004; Baker et al., 2007; Blanco-Canqui and Lal, 2008; Blanco-Canqui and Lal, 2009; Clay et al., 2012; Ussiri and Lal, 2009; West and Marland, 2002), and relatively few studies sample to depths close to 1 m (Collins et al., 2010; Follett et al., 2012; Liebigh et al., 2008; Syswerda et al., 2011; Varvel and Wilhelm, 2011). Furthermore, many LCA models and C modeling tools used to quantify management effects on C cycling in bioenergy cropping systems rely on published data with soil sampling depths less than half a meter (Follett et al., 2012). This study provided highly important research on changes in SOC to a depth of nearly 1 m for a relatively new bioenergy crop.

Cropping systems are frequently considered net sinks of CO<sub>2</sub> emissions because of their ability to assimilate atmospheric CO<sub>2</sub> into biomass-C (Kim and Dale, 2005). However, several studies cite serious issues related to not properly accounting for SOC changes in LCAs for determining GWP associated with bioenergy crop production (Clay et al., 2012; Follett et al., 2012). Clay et al. (2012) reported that the impact of agronomic management practices on SOC changes in bioenergy cropping systems is frequently overlooked in many LCAs and when included, it is frequently underestimated (Follett et al., 2012).

The average annual SOC accrual rates in our study ranged from approximately 2.5 to 7.5 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (Fig. 36). Carbon accrual rates of approximately 6 Mg C ha<sup>-1</sup> yr<sup>-1</sup> observed in this study translated to GWP savings of more than 20 Mg CO<sub>2</sub>-eq ha<sup>-1</sup> yr<sup>-1</sup>. This is considerably higher than C accrual rates observed in a number of other studies from bioenergy cropping systems ranging from maize to switchgrass to perennial grasses (Clay et

al., 2012; Collins et al., 2010, Follett et al., 2012; Hill et al., 2006; Liebig et al., 2008; Varvel and Wilhelm, 2011). However, several of these studies limited SOC change observations to surface depths (Clay et al., 2012; Hill et al., 2006) or had different crops with lower biomass yields compared to bioenergy sorghum (Collins et al., 2010; Follett et al., 2012; Liebig et al., 2008; Varvel and Wilhelm, 2011). Furthermore, SOC accrual rates depend upon factors such as initial quantity of soil C, mineralization rates, tillage regime, biomass- and root-C inputs supplied by a given crop, soil properties, and environmental conditions (Clay et al., 2012; Follett et al., 2012), so relatively high accrual rates observed in this study may be very different than others observed throughout the U.S.

Switchgrass is a bioenergy crop which has shown promise in providing significant aboveground yields while simultaneously offering SOC sequestration (Collins et al., 2010; Liebig et al., 2008). A study in the upper Midwest determined that switchgrass could provide yields of approximately 2.5 Mg C ha<sup>-1</sup> yr<sup>-1</sup> while accruing SOC at rates between 1.1 and 2.9 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (Liebig et al., 2008). This reported study determined that switchgrass cropping systems were able to significantly increase SOC in the surface 30 cm as well as to a depth of 120 cm. Similar to our study (Table 14), these authors observed highly variable SOC accrual rates which ranged from 0.6 to 4.3 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (Liebig et al., 2008). Another SOC accrual study by Collins et al. (2010) in the Pacific Northwest determined that switchgrass was able to produce nearly 4 Mg C ha<sup>-1</sup> m<sup>-1</sup> from root biomass-C only a few years after establishment. However, after 5 cropping seasons, SOC changes were limited to the top 15 cm, where SOC increased at a rate of approximately 1.2 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, and no detectable increases in SOC were found from 15 to 100 cm depths (Collins et al., 2010). Further analysis utilizing isotopic

measurement techniques found switchgrass-derived SOC accrued at a rate of approximately  $1 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ .

A long-term study in Nebraska examined the impact of N fertilization rate and harvest management in switchgrass and maize cropping systems on the net SOC change to a depth of 150 cm (Follett et al., 2012). Switchgrass and corn grain yields provided up to 5.1 and  $2.6 \text{ Mg C ha}^{-1}$ , annually (Follett et al., 2012). Nearly all the treatments in the maize and switchgrass cropping systems were found to significantly increase SOC to most depths examined. The SOC accrual rates ranged up to 2 and  $2.6 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  in switchgrass and maize, respectively (Follett et al., 2012). Similar to the previously mentioned studies and our own findings (Table 14), they observed considerable variability in the SOC data (Follett et al., 2012).

Thus, after five cropping seasons, bioenergy sorghum in our study accrued a very large mass of SOC compared to other reported bioenergy crops. There may be a number of reasons why SOC accrual was observed at high rates in our study. Factors that may have contributed to these findings were lower initial SOC concentrations and higher yield in our study, differences in crops produced and the long growing season may have contributed to the higher C increases observed in our study.

Based upon the average annual SOC accrual rate, and assuming measured increases in SOC were attributable to bioenergy sorghum, a large portion of the total plant biomass may have been belowground biomass-C. An estimate was simply derived by adding the average annual mass of aboveground C to the average annual mass of SOC accrual. In the two years examined, approximately 50 to 60% of the total plant biomass was belowground in SS treatments and 25 to 40% of plant biomass was belowground in CS treatments. These are



particularly high rates. Follett et al. (2012) found SOC accrual rates in switchgrass to be approximately 40% of aboveground harvestable C. However, the SOC accrual rates were derived from all 5 years of our study (since 2008), but were evaluated only on drought-stressed yields from 2010 and 2011, which may explain why accrual rates seem particularly large compared to aboveground sorghum biomass-C.

Of particular importance in this study was our findings that a significant portion of the SOC accrued annually occurred below 30 cm (Table 14). Soil C increases below 30 cm are very important because they represent a pool of C less likely to quickly turnover because of its depth below normal tillage zones (Follett et al., 2012). It is believed that nearly half of the SOC sequestered in many no-till maize cropping systems throughout the corn belt may occur below 30 cm, potentially representing a large C sequestration benefit currently overlooked in many LCAs (Follett et al., 2012). Several others have observed significant increases in SOC below 30 cm (Follett et al., 2012; Liebig et al., 2008; Varvel and Wilhelm, 2011). However, a number of those studies were long-term and increases below 30 cm in many cropping systems may not be observed within the short time period (5 years) utilized in this study (Follett et al., 2012; Varvel and Wilhelm, 2011). Thus, the SOC accrual observed at relatively deep depths in our study may have particularly significant implications on GWP of bioenergy sorghum cropping systems.

### **Life Cycle Greenhouse Gas Emissions**

The crop production phase of biofuel production in our study was the largest component of life cycle GHG emissions from direct and indirect inputs (excluding indirect SOC change emissions) (Figs. 37 and 38). This finding was similar to many life cycle studies

which cite crop production as a major source of total life cycle GHGs (Fazio and Monti, 2011; Kim and Dale, 2005; Kim et al., 2009; Landis et al., 2007; Liska and Cassman, 2008; Liska et al., 2009; Plevin, 2009; Searchinger et al., 2008; Wang et al., 2012; Wang et al., 2011). Specifically, Liska et al. (2009) found that the crop production phase accounted for 37 – 65% of life cycle GHG emissions, and ethanol production at the biorefinery accounted for the other major portion. Emissions associated with activities following crop production (transportation and distribution, ethanol production, and combustion) were a significant, but smaller, portion of total life cycle GHGs in this study and were directly tied to overall energy yield. Ethanol production has frequently been noted as a major life cycle GHG activity in most analyses (Liska and Cassman, 2008; Liska et al., 2009; Wang et al., 2012; Wang et al., 2007; Wang et al., 2011) and was the largest factor in one study (Hill et al., 2006). However different accounting techniques and categories generally cause significance from a specific activity to vary from one study to another.

Direct emissions of N<sub>2</sub>O from soil were the largest life cycle GHGs measured in our study (Figs. 37 and 38) and N fertilization significantly influenced W2W life cycle GHGs across both years. Treatments receiving N fertilizer generally had higher N<sub>2</sub>O emissions, greater indirect emissions from N fertilizer production, and higher yield, which increased life cycle GHGs directly related to energy yield. We experienced particularly high N<sub>2</sub>O emissions compared to other studies potentially due to a number of factors previously discussed. However, similar to our study, many LCAs have found both direct and indirect CO<sub>2</sub>-eq emissions associated with soil N<sub>2</sub>O emissions and N fertilizer production (indirect) emissions to be a significant component of total life cycle accounting (Kim et al., 2009; Landis et al., 2007; Liska and Cassman, 2008; Liska et al., 2009; Wang et al., 2012; Wang et

al., 2011), but none determined direct soil N<sub>2</sub>O emissions as having the largest life cycle GHG emissions.

Despite our calculated NEFs being relatively similar to other studies which utilized the GREET or BESS models (Landis et al., 2007; Liska and Cassman, 2008; Liska et al., 2009; Wang et al., 2012; Wang et al., 2007; Wang et al., 2011), CO<sub>2</sub>-eq emissions attributable to N<sub>2</sub>O were much higher than those studies. This may be partly explained by the method of calculation and relatively high N<sub>2</sub>O emissions from treatments not receiving urea fertilizer (Fig. 35). Since the difference in cumulative N<sub>2</sub>O between fertilized and unfertilized plots was used to calculate NEF, unfertilized treatments which had relatively high N<sub>2</sub>O emissions may have caused the NEF to be relatively low while the total mass of CO<sub>2</sub>-eq (from N<sub>2</sub>O) lost may still have been high. Thus, the NEFs from this study are comparable to those found elsewhere, yet the GWP associated with direct N<sub>2</sub>O emissions was considered high.

Life cycle GHG emissions from activities following crop production were directly tied to total energy yield (Figs. 37 and 38 and Table 15). While better estimates of these activities may be possible, we believe the estimates are fair based on data presented by Wang et al. (2012) and may be conservative estimates due to the relatively high fuel conversion rate associated with bioenergy sorghum compared to default cellulosic conversion values (Stefaniak et al., 2012; Wang et al., 2012). One dilemma associated with this estimation method was that high yielding treatments were at a disadvantage with these particular life cycle GHGs compared to relatively low yielding treatments, and cropping feasibility (economically) was not considered. Future research efforts will be made to verify these values.

The largest factor driving total life cycle GHG emissions from this study was net SOC change (Figs. 36 and 39). The SOC accrual found in our bioenergy sorghum cropping systems had a tremendous impact on overall GWP. Very few life cycle analyses account for soil C change, and those that do (Clay et al., 2012; Kim et al., 2009; Wang et al., 2012) determined C sequestration to have only a small impact on net life cycle GHG efficiency. The much smaller reported impact of net SOC change on life cycle GHGs could be attributed to SOC being measured only near the soil surface (top 15 cm) (Clay et al., 2012). A recent study on this issue cited the lack of adequate SOC change data in bioenergy crop life cycle accounting as a potentially large source of net benefit underestimation (in GWP) (Follett et al., 2012). This study provided a robust method of accounting for net SOC change across 5 years of study and determined SOC had highly significant results on overall life cycle GHG efficiency.

In some studies, net change in SOC over time (accounting for potential C sequestration) and direct emissions of N<sub>2</sub>O from bioenergy cropping systems were either omitted or controlled with a conservative estimate, due to great uncertainty generally associated with such factors (Fazio and Monti, 2011). It is well documented (Fazio and Monti, 2011) and observed in this study that SOC and N<sub>2</sub>O data may be highly variable. However, this represents a particularly beneficial aspect of this study because we were able to directly quantify both of these highly variable factors and provide greater confidence to estimates of life cycle GHGs associated with bioenergy sorghum production. Furthermore, the significance of each of these life cycle GHG activities necessitates accounting when analyzing a new feedstock source which has yet to be thoroughly investigated, such as bioenergy sorghum.

Our findings indicate bioenergy sorghum may be a unique feedstock source providing C sequestration and GWP savings not observed in other biofuel life cycle analyses (De Oliveira et al., 2005; Fazio and Monti, 2011; Hill et al., 2006; Hsu, 2012; Liska and Cassman, 2008; Liska et al., 2009; Plevin, 2009; Searchinger et al., 2008; Wang et al., 2012; Wang et al., 2007; Wang et al., 2011). Life cycle GHG emissions associated with gasoline production in 2005 were approximately 96 g CO<sub>2</sub>-eq emitted MJ<sup>-1</sup> (net source of C) (Hill et al., 2006; Liska and Cassman, 2008; Wang et al., 2011). This value was the baseline comparison established by the 2007 EISA (U.S. Congress, 2007) by which cellulosic biofuels must meet a 60% relative reduction. Averaged across 2010 and 2011, bioenergy sorghum production treatments examined in this study ranged from -437 to -78 g CO<sub>2</sub>-eq MJ<sup>-1</sup> ethanol or 78 to 437 g CO<sub>2</sub>-eq sequestered MJ<sup>-1</sup> ethanol (net sink of C) (Fig. 40). The [SS, - N, 50%R] treatments provided the greatest total life cycle GHG savings, sequestering over 400 g CO<sub>2</sub>-eq from the atmosphere per MJ ethanol produced (Fig. 40). Thus, bioenergy sorghum cropping systems met and exceeded the life cycle GHG emission reduction established by federal regulation. Additionally, these findings surpassed the emission reductions (relative to gasoline) compared to a number of other bioenergy crop studies reporting similar values, ranging from 22 to 84 g CO<sub>2</sub>-eq emitted MJ<sup>-1</sup> (all were a net source of C) (De Oliveira et al., 2005; Hill et al., 2006; Liska and Cassman, 2008; Liska et al., 2009; Wang et al., 2012; Wang et al., 2007).

Results found in this study were the first of its kind due to the cropping system and methods utilized to quantify net life cycle GHG emissions. Most other studies use large data sets of agronomic statistics or models such as GREET to determine life cycle greenhouse gases. These have great benefit because they may be more broadly applicable to a large

number of cropping systems within a given region or a given production practice. Our study is highly applicable to bioenergy sorghum cropping systems throughout the southern U.S. but is relatively specific because of many GWP estimates based on direct field measurements or through measured yield. Several of the most significant parameters, such as N<sub>2</sub>O emissions and net SOC change, were highly variable within our own field, suggesting difficulty in up-scaling with great confidence throughout a given region. However, this study provided a highly valuable reference for LCAs of bioenergy sorghum cropping systems for the future. Additionally, numerous studies cite the need for more field-measured data to make better estimates of various feedstock production activities associated with biofuel production (Clay et al., 2012; Follett et al., 2012; Kim et al., 2009; Liska and Cassman, 2008; Liska et al., 2009), which this study accomplished. The effect of accurately quantifying life cycle GHGs for any given biofuel production activity was presented by Liska et al. (2009), who upon providing updated data on biorefinery efficiency, observed major differences in total life cycle GHG emission efficiency compared with default (unmodified) model estimates. Thus, we believe measured data from this study provided better estimates of life cycle GHGs for bioenergy sorghum crop production than some model estimated data.

## **CONCLUSIONS**

Despite producing energy yields under drought conditions and having relatively high N<sub>2</sub>O emissions, all bioenergy sorghum production scenarios in this study met and exceeded life cycle GHG reduction goals established by the 2007 EISA. Regardless of crop rotation, unfertilized treatments returning half the residue were among the best performing treatments in this study and sequestered nearly 400 g CO<sub>2</sub>-eq MJ<sup>-1</sup> ethanol. This was the first study of its

kind to determine life cycle GHG emissions from bioenergy sorghum production and utilize a robust method to quantify net SOC change to 90 cm. All bioenergy sorghum treatments examined in this study annually sequestered large amounts of C during the first 5 years of cropping. As demonstrated by Follett et al. (2012), bioenergy crops can potentially sequester large amounts of C deep below ground where it will not turnover quickly. Our research suggested bioenergy sorghum can sequester significant amounts of SOC deep within the soil for at least the first 5 years of cropping. Large SOC sequestration benefits translated to large gains in life cycle GHG emission efficiency and production scenarios were a sink for atmospheric C per MJ ethanol.

Contrary to goals of finding renewable fuels which can feasibly be produced in a manner that minimizes environmental impacts, in some situations, W2W efficiency was greater under treatments with relatively low yield (Figs. 34 and 40). This effect was partly due to some indirect GHG emissions directly correlated with yield, but largely due to the final computation unit (MJ ethanol). If there was low energy yield for a particular treatment, then the final quotient may be higher simply because the denominator (energy yield) used to derive the efficiency value was low. In some situations, the relatively high W2W efficiency value may be unsuccessful because the cropping system may never be economically viable. Well-to-wheel efficiency values may be obstructive toward overall biofuel production goals if they purvey select treatments with low energy yield as having greatest benefit. Care must be given to interpretation of these values because they represent important GWP efficiency values associated with bioenergy crop production, but may not necessarily identify truly viable cropping systems.

While the findings of this study are not free from uncertainty, they did provide a straight-forward approach to life cycle GHG emissions from bioenergy sorghum production in central Texas during drought conditions. The variability in data from our study may be similar to variability in data utilized in other life cycle analyses with similar uncertainty. These results are not meant to provide an estimate of life cycle GHGs for all bioenergy sorghum production systems; rather they provide the first values used for life cycle accounting towards determining compliance with the 2007 EISA (U.S. Congress, 2007). Based on the measured parameters, we determined all bioenergy sorghum production scenarios examined in this study exceeded the life cycle GHG emission reductions mandated by the 2007 EISA. Due in large part to the substantial reductions in GWP attributable to SOC accrual to 90 cm, all treatments in the study were net sinks of atmospheric CO<sub>2</sub> per MJ ethanol produced. Future work is needed to verify the source of SOC sequestration due to its substantial implications on overall life cycle GHG efficiency. Additionally, we hope to utilize the newest version of the GREET model to incorporate field-scale measured values and simulate well-to-wheel life cycle GHG emissions from various GREET-derived biofuel production parameters.



## CHAPTER V

### CONCLUSION

#### **SUMMARY**

This study provided valuable information on GHG emissions from crop production systems in central Texas. High emissions of GHGs were observed despite moderate and severe drought conditions in 2010 and 2011, respectively. Trace gas emissions generally were highest following precipitation events and when soil temperatures were warm throughout the growing season. The largest portion of annual cumulative emissions was lost during the growing season, but a significant amount of emissions were lost during the fallow season as well. Results derived from analysis of cumulative growing season and annual emissions were different. This study illustrated the importance of full annual GHG accounting when determining cumulative GHG emissions from crop production. Fallow season emissions can have a significant impact on overall conclusions, particularly in climates where soils do not freeze during winter months.

Cumulative GHG emissions were significantly impacted by agronomic management practices in 2010 and 2011. Returning half the biomass residue to the soil (50%R) significantly increased cumulative CO<sub>2</sub> emissions in 2010 and 2011. Nitrogen addition significantly increased N<sub>2</sub>O emissions in 2010 and 2011. Crop rotation had no measurable impact on cumulative annual GHG emissions in 2010 and 2011. The N emission factors (NEFs) observed in this study were generally above the IPCC estimate of 1.25%. A negative NEF resulted from the 2011 data, likely due to sporadic fluxes throughout the growing

season and high variability. The [SS, + N, 50%R] treatments had the highest cumulative emissions of GHGs across 2010 and 2011.

Yield-scaled GHG emissions were largely driven by factors directly related to yield itself. Since treatments with 50% residue return had half the potential ethanol yield of treatments with 100% biomass removal, 50%R treatments had significantly higher yield-scaled GHG emissions. Averaged across both years of study, N fertilization and sorghum monoculture had higher yield, and subsequently decreased yield-scaled GHG emissions. The average yield-scaled GHG emissions of bioenergy sorghum production treatments from 2010 and 2011 were divided into two groups of similarly yield-scaled GHG performance; four better performing treatments and four poorer performing treatments. Considering multiple sustainability factors, the [CS, + N, 50%R] treatments were selected as the ideal bioenergy sorghum production scenario for achieving greatest yield-scaled GHG emissions savings.

Each of the agronomic management practices influenced one or more C pools in this study in at least 2010 or 2011. Crop rotation had the most consistent impact on multiple C pools in 2010 and 2011. Continuous sorghum increased aerial biomass-C and SOC at 0 – 5, 30 – 60, and 60 – 90 cm in 2010. In 2011, SS treatments had lower aerial biomass-C than CS, increased SMBC at 5 – 15 cm, and increased SOC at the same depths observed in 2010. Nitrogen addition increased aerial biomass-C and SOC at 0 – 5 cm, but decreased SOC at two depths below 30 cm in 2010. In addition to increasing aerial biomass-C, N fertilization increased cumulative soil respiration and SMBC at 0 – 5 cm in 2011. Residue return increased cumulative respiration-C and SOC in the top 15 cm in 2010. In 2011, 50%R treatments continued to increase soil respiration and significantly increased SMBC at both depths in the top 15 cm.

Despite a relatively large mass of organic-C returned to the soil by 50%R, we did not observe a consistent increase in SOC across years with this treatment. This field study utilized conventional disc tillage and we observed that returning residue consistently increased cumulative respiration-C across years. We believe this suggests that the biomass-C returned to the soil in 50%R treatments decomposed relatively quickly each year and was lost as CO<sub>2</sub> rather than remaining in the soil as SOC.

Investigation of C cycling in bioenergy sorghum crop production revealed trade-offs between providing high feedstock yield and sustainability. Returning half the residue as an organic amendment (50%R) caused increased CO<sub>2</sub> emissions and provided lower total harvestable yield, but it significantly increased SMBC, a pool of C sensitive to change and that may indicate enhanced soil quality. Nitrogen addition improved feedstock yield across years and increased SMBC from 0 – 5 cm, but increased CO<sub>2</sub> emissions in 2011. The monoculture sorghum (SS) treatments provided higher yield than corn and consistently increased SOC concentrations (including at two depths below 30 cm), but it did not increase yields compared to rotated sorghum and could increase risk from pest and disease pressure. Thus, ideal agronomic management practices for biomass feedstocks must be identified to achieve an optimal balance between producing high yield while ensuring long-term sustainability.

Theoretical ethanol yield estimated for bioenergy sorghum production scenarios remained high despite moderate and severe drought conditions through 2010 and 2011. Each of the management practices examined significantly influenced ethanol yields in both years. The addition of N fertilizer and biomass residue had differential effects on energy yield, with + N treatments increasing energy yield, and 50%R treatments decreasing energy yield. We

anticipate it will be critical to utilize N fertilizer to sustain future sorghum-derived energy yields. Biomass residue return decreased energy yield through direct decrease in feedstock, but may offer future benefits through sustaining nutrients and improving soil quality. Crop rotation had diverging effects by year; when corn was grown in CS treatments, it provided lower energy yield than SS, but when sorghum was grown CS produced higher yield. Across years, SS and CS produced approximately the same energy yield. Considering the beneficial impacts associated with crop rotation, we suggest utilizing crop rotation to produce similar energy yield while reducing risks associated with pest pressure.

We determined net change in SOC to 90 cm was one of the most promising aspects of bioenergy sorghum production for biofuel feedstock due to its potential ability to sequester large amounts of SOC, annually. Continuous sorghum increased the annual SOC accrual rate compared to CS treatments after 5 years of study. While the greatest C sequestration potential may be associated with the monoculture sorghum cropping system, rotated sorghum also offered potential C sequestration benefits, sequestering over 4 Mg C ha<sup>-1</sup> yr<sup>-1</sup> to 90 cm. Depending on what is deemed the highest priority, greatest C sequestration may be achieved through SS treatments, while CS treatments offer more promise in overall sustainability. While maximum C sequestration potential may not be achieved compared to SS treatments, overall cropping system sustainability is deemed a highly valuable aspect of the rotated bioenergy feedstock production. We believe benefits associated with crop rotation (eg. similar energy yield over multiple years, feedstock diversity, and reduced pest pressure) justify utilizing a CS treatment for bioenergy sorghum biofuels production.

Following the summation of all measured and estimated W2W LCGHGs from bioenergy sorghum ethanol production, they were subtracted from the net SOC change (to 90

cm) to provide an estimated LCGHG value for comparison with the 2007 EISA. Overall, direct emissions of N<sub>2</sub>O from the soil were the production activity with the greatest LCGHG emissions. This was likely due to higher than usual N<sub>2</sub>O emissions associated with this study, possibly from the warm climate and frequent wetting-drying cycles throughout the growing season. Nitrogen fertilization was the management practice which had a consistent effect on W2W LCGHGs across both years of study, with + N treatments resulting in significantly more W2W LCGHG emissions (lower efficiency) than – N treatments. While this suggests unfertilized treatments have the greatest ability to minimize production GWP, it does not necessarily represent the most feasible bioenergy crop production scenario. Nitrogen fertilization was required to provide high yield, a key to sustaining high energy yield and providing greater economic incentive for producer implementation (and subsequent long-term feasibility).

All bioenergy sorghum production scenarios examined in this study exceeded the LCGHG reduction goals mandated by the 2007 EISA. The most efficient bioenergy sorghum production scenario examined was the [SS, - N, 50%R] treatment and the least efficient was the [CS, + N, 50%R] treatment. Despite being the least efficient cropping system in terms of LCGHG emissions, [CS, + N, 50%R] may offer the greatest potential as a bioenergy sorghum production scenario due to the benefits associated with rotation, N fertilization, and residue return discussed in this document. To improve W2W LCGHG efficiency, reducing N application and residue return rates, may reduce both direct and indirect N<sub>2</sub>O emissions as well as enhance energy yield, respectively. Adjusting these rates could provide greater W2W LCGHG efficiency for the [CS, + N, 50%R] treatments, making it comparable to other treatments in this study while simultaneously having greater long-term sustainability. Carbon

sequestration associated with the large net increase in SOC to 90 cm over 5 years was the largest factor causing all treatments examined to have C sequestered for each MJ of cellulosic ethanol produced. Sequestering C for each unit of energy has generally not been observed in other published bioenergy crop life cycle analyses. This study determined that bioenergy sorghum has great potential as a future cellulosic feedstock due to its high energy yield potential and ability to significantly increase SOC. We believe many studies overlook the potential LCGHG savings associated with bioenergy crop production and/or the potential increase in SOC below 15 cm (a common sampling depth). This study determined that accounting for potential increases in SOC to 90 cm had significant impact on overall W2W LCGHG emissions and had major implications for bioenergy cropping system sustainability.

## **RECOMMENDATIONS**

Producing high yielding bioenergy feedstocks while minimizing life cycle GHG emissions is a major goal of domestic biofuel production. We determined bioenergy sorghum provided high yields despite drought conditions in 2010 and 2011. Poor energy yields were produced by corn under drought conditions. Bioenergy sorghum may be a bioenergy crop particularly well-suited for biomass feedstock for biofuel production under drought conditions. This characteristic could have significant implications related to potential global climate change. Future models predict Texas' climate will be warmer and drier in the future, suggesting bioenergy sorghum may be a particularly good bioenergy crop candidate for biofuel production within Texas and across similar climates in the southern U.S. While soil GHG emissions were high, net C sequestration from SOC change over time minimized net GHG emissions from bioenergy sorghum production. All bioenergy sorghum production

scenarios analyzed met the life cycle GHG emissions reductions mandated by the 2007 EISA. The optimal treatment in the short-term for minimizing GHG emissions per MJ of cellulosic ethanol was [SS, - N, 50%R]. However, we believe the most promising production scenario for long-term sustainability is [CS, + N, 50%R]. The CS rotation can provide significantly higher sorghum yields and reduce disease and pest pressure associated with monoculture cropping. Nitrogen fertilization will be required to sustain long-term yields over time. Returning biomass residue increased soil microbial biomass, a biological indicator of increased soil quality, and past research determined that removing 100% of biomass removed can mine the soil of carbon and nutrients over time. The [CS, + N, 50%R] treatment did not provide as much W2W life cycle GHG savings as other treatments examined, however, the efficiency might be improved by reducing N and residue return rates. I recommend exploring a lower rate of N fertilization and residue return to improve W2W life cycle GHG efficiency for [CS, + N, 50%R].

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