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A comparison of cellulosic fuel yields and separated soil-surface CO2 fluxes in maize and prairie biofuel cropping systems

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A comparison of cellulosic fuel yields and separated soil-surface CO$_2$ fluxes in maize and

prairie biofuel cropping systems

by

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This [alcohol] was a substance produced by a tiny creature called yeast. Yeast organisms ate sugar and excreted alcohol. They killed themselves by destroying their environment.

-Kurt Vonnegut, *Breakfast of Champions*

DEDICATION

For my father, who shared his love of the outdoors; for my mother, who shared her love of science; for my brother, who helps me interpret the world; for Mikey, who is patient.

Lastly, for Fernando, who taught me to think like a scientist.

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CHAPTER 1: GENERAL INTRODUCTION

Introduction and Thesis Organization

Recently, there has been increasing demand from the public for agricultural systems to not only produce food, fuel, and fiber, but to do so without compromising common public goods such as air and water quality, game habitat, and aesthetically pleasing landscapes (Gulf of Mexico Watershed Nutrient Task Force 2008; Arbuckle et al. unpublished data). The latter provisions may be collectively referred to as ecosystem services. It has been suggested that strategic incorporation of perennial vegetation into agricultural landscapes can provide disproportional ecosystem services while maintaining agricultural productivity (Asbjornsen et al. 2013). The burgeoning cellulosic biofuel market, if carefully regulated, could provide an economic backdrop to support incorporation of perennial vegetation and its associated benefits. In order to address a lack of side-by-side comparisons of maize and perennial prairie biofuel feedstock production, the Comparison of Biofuel Systems (COBS) project was established at Iowa State University in 2008. COBS consists of 5 cropping systems: a 2-year rotation of maize and soybean grown for grain only, continuous maize grown for grain and stover both with and without a winter rye cover crop, and a diverse reconstructed tallgrass prairie grown for fall harvest of biomass both with and without spring nitrogen fertilization. One goal of the COBS project is to produce quantitative data in order to compare potential Midwestern biofuel cropping systems and evaluate their associated tradeoffs. The following four criteria were developed to help guide these comparisons (Thompson et al. 2010):

(1) Potential for biomass production, fossil-fuel replacement, and net energy returns

(2) Potential to reduce greenhouse gas emissions and to increase belowground carbon storage

(3) Potential to maintain soil quality and reduce water-quality impacts of nutrient exports

(4) Net economic return to biomass producers and the environment

This work aims to provide information pertaining to criteria #1 (potential for biomass production) and #2 (potential to reduce greenhouse gas emissions). Chapter 2, titled '*Comparing cellulosic ethanol yields of Midwestern maize- and reconstructed tallgrass prairie-systems managed for bioenergy*' examines the cellulosic production and harvests realized from the maize and prairie systems between 2009-2013, as well as the potential biochemical conversion rates of the harvested biomass. Chapter 3, titled '*Root-derived component of soil-surface CO_2 flux differs among continuous maize, unfertilized prairie, and N-fertilized prairie biofuel cropping systems*' is an in-depth two year study (2012-2013) on the two components of the largest output of carbon from the cropping systems' soil: the CO_2 flux from the soil surface.

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CHAPTER 2: COMPARING CELLULOSIC ETHANOL YIELDS OF MIDWESTERN MAIZE- AND RECONSTRUCTED TALLGRASS PRAIRIE-SYSTEMS MANAGED FOR BIOENERGY

Modified from a paper to be submitted to Bioenergy Research

Authors TBD

Abstract

We investigated potential use of prairie as a Midwestern cellulosic feedstock by conducting a 9 ha side-by-side comparison of maize- and prairie-based systems on fertile soils in Boone County Iowa, USA. Investigated systems were: maize-soybean rotation grown for grain only, continuous maize grown for grain and stover both with and without a winter rye cover crop, and a 31-species reconstructed prairie grown with and without spring nitrogen fertilization for fall-harvested biomass. We compared amounts of cellulosic biomass produced and harvested, ethanol conversion ratios of biomass as measured by both dietary and detergent methodology, as well as each method's estimated ethanol yields per unit land area. From 2009-2013, the highest producing system was N-fertilized prairie, averaging 10.4 Mg ha^{-1} yr^{-1} above-ground biomass with average harvest removals of 7.8 Mg ha^{-1} yr^{-1}. The unfertilized prairie produced 7.4 Mg ha^{-1} yr^{-1}, with average harvests of 5.3 Mg ha^{-1} yr^{-1}. Lowest cellulosic biomass harvests were realized from continuous maize systems, averaging 3.5 Mg ha^{-1} yr^{-1} when grown with, and 3.7 Mg ha^{-1} yr^{-1} when grown without a winter rye cover crop, respectively. Un-fertilized prairie biomass and maize stover had equivalent dietary conversion ratios at 330 g ethanol kg^{-1} dry biomass, but N-fertilized prairie was lower at 315. Detergent method over-prediction depended upon both growing environment and biomass source. Harvested biomass accounted for >90% of ethanol yield variation. Over four years prairie systems averaged 1287 L cellulosic ethanol ha^{-1} yr^{-1} more than maize systems, with fertilization increasing prairie ethanol production by 865 L ha^{-1} yr^{-1}. On both a biomass-per-unit-land area and ethanol-per-unit biomass basis, the reconstructed prairies met or surpassed cellulosic standards set by maize cellulosic systems, indicating prairies could function as an industrial cellulosic feedstock in the Midwest.

Introduction

In 2012 approximately 20,000 gallons of ethanol were produced in the United States via cellulosic biomass feedstocks, with a projection of more than 5 million gallons by 2014 (US EIA 2013). This 250 fold increase in feedstock demand will likely be met using a combination of agricultural waste and dedicated biomass crops (US DOE 2011). As the US has seen with grain ethanol, biofuel feedstock demand has the potential to significantly impact on-farm economics and therefore land-use decision making (Faber et al. 2012, Wright & Wimberly 2013), as well as the environment (Donner & Kucharik 2008). In order for the cellulosic biofuel industry to proceed in a sustainable manner, potential feedstocks must be carefully evaluated and policy proactively written. Given the documented environmental services provided by both restored and native prairies (Schilling & Jacobson 2010, Helmers et al. 2011, Fornara & Tillman 2012, Hirsh et al. 2013) prairie biomass could offer an environmentally sustainable source of cellulosic feedstock.

When evaluating a potential biofuel feedstock system one must consider the amount of ethanol the system will yield on a per unit land area basis. This is a function of two components: the quantity of biomass produced as well as the ethanol conversion ratio of the biomass (biochemical composition, or quality). In order to analyze each individual component's effect on ethanol yield, we considered biomass production and biomass quality separately.

Biomass Production

Currently, many estimates of Midwestern tallgrass prairie production are limited to low-input systems, often grown on degraded land unsuited for agricultural production. In the most recently updated Billion Ton Report, prairies were estimated to contribute 3.9 Mg ha^{-1} yr^{-1} for low input reconstructed prairie, and 5.6 Mg ha^{-1} yr^{-1} for managed native prairie (US DOE 2011). Studies performed on agricultural-grade land have found prairie yields ranging from 3.1-5.3 Mg ha^{-1} yr^{-1} (Brye et al. 2002, Camill et al. 2004). Some studies have shown prairies increase biomass production in response to N-fertilization (Camill et al. 2004, Jarchow & Liebman 2013), suggesting that the full production potential of prairie biomass

has not been fully investigated. Estimates of prairie productivity when managed explicitly for biomass production on agricultural land are needed to accurately estimate potential contributions of prairie biomass to US production goals. In the Midwest, where maize (*Zea mays*) stover is projected to be a major supplier of cellulosic ethanol (Graham et al. 2007, US DOE 2011), it is useful to evaluate how prairie systems perform when compared side-by-side to maize stover systems.

Biomass Quality

A reliable supply of feedstock across years is an important consideration for the cellulosic ethanol industry, but the supply should also be of a consistent quality. Both genotype and variation in environmental growing conditions have been shown to alter biomass biochemical composition (Sluiter et al. 2001, Jung & Lamb 2004). When considering potential biomass feedstocks, it is important to identify the range of quality that can be expected from that feedstock. Annuals and perennials exhibit varying growth patterns, and it is therefore possible that biochemical makeup will also vary. Since prairies are a heterogeneous mix of forbs and grasses with relative contributions that can change from year to year, it is unknown whether the range of prairie composition will be comparable to that of a monoculture of the annual grass maize. This comparison is particularly pertinent in Iowa, considering the first generation of cellulosic ethanol plants are being designed to accept maize stover (eg. DuPont 2013, POET 2013). If prairies exhibit comparable composition ranges, it is feasible that these industries could accept prairie biomass as feedstock with minimal process alterations.

Ethanol Yields

Owing to the finite availability of arable land, the amount of ethanol produced per unit land area is an important metric in evaluating biomass feedstocks. Due to lack of an established industry and thus large-scale conversion values, biochemical conversion potential of cellulosic biomass must be estimated using laboratory-scale methods. Ethanol is produced via biochemical conversion of both 5- and 6-carbon sugars. While the exact type of sugars will vary, 5-carbon sugars can be generally categorized as hemicelluloses, and 6-

carbon sugars as celluloses. Therefore, estimates of hemicellulose and cellulose contents will define the maximum amount of ethanol that can be produced from the biomass. There are two main methods available to analyze heterogeneous samples for hemicellulose and cellulose content: the detergent fiber and the dietary fiber method.

The detergent fiber method, also called the filter-bag method, was originally developed for estimating forage quality (Van Soest 1967, Grabber et al. 2009). While it provides an estimate of cellulose and hemicellulose contents, the method was not developed specifically for the biofuel industry and as such detergent results may not provide accurate predictions of bioenergy yields, nor accurately reflect true biochemical composition. However, due primarily to the analysis' simplicity, it has been widely used to predict ethanol yields from cellulosic biomass.

The second available method is the dietary fiber analysis, also referred to as the Uppsala method (Theander & Westerlund 1993). The National Renewable Energy Laboratory (NREL) has developed a protocol based on the Uppsala method that is specifically tailored to estimate biochemical conversion. The method uses several extractions, acid hydrolysis, and liquid chromatography to quantify neutral sugar polysaccharides (Sluiter et al. 2008). The individual sugar species can be grouped based on their 5- or 6-carbon structure, producing estimates of hemicellulose and cellulose contents. While the NREL method has been recognized as being both precise and accurate, it is often prohibitively expensive for large numbers of samples. Correlation between the two methods is inconsistent and depends upon the type, age, and pre-treatment of the biomass (Theander & Westerlund 1993, Jung & Lamb 2004, Dien et al. 2006, Wolfrum et al. 2009). It is therefore likely that correlation will differ depending on both feedstock characteristics and the environmental conditions under which they were grown. The detergent method may exaggerate or be unable to detect differences between biomass from different plant sources across years. For long-term projects such as the one presented here it would be advantageous to know how the method of analysis affects the calculated ethanol production potential of the different systems.

In order to investigate the impact of the chosen method on ethanol yield calculations, we calculated potential maximum ethanol yields for both the maize and prairies systems utilizing composition data from both dietary and detergent methods.

In summary, the goals of this study were to address the following three questions:

1. How does prairie cellulosic biomass production compare to maize stover production on prime Midwestern agricultural land?

2. Do maize stover and prairie biomass exhibit similar ranges of cellulosic ethanol feedstock quality across a range of environmental growing conditions?

3. How does the method of ethanol conversion estimation affect theoretical ethanol yields per unit land area?

Materials and Methods

Experimental site and design

The experimental site was located at Iowa State University's South Reynoldson Farm in Boone County, IA (41^o55 N 93^o45 W). The predominant soil types are Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls; USDA-NRCS 2014). Subsurface drainage of the plots was completed in spring of 2008, after which all cropping systems for this experiment were established. The site has been managed without tillage since establishment.

Five cropping systems were investigated in this study: a maize (*Zea mays*) and soybean (*Glycine max*) rotation (C2/S2) grown for grain only; continuous maize (CC) and continuous maize with a winter rye (*Secale cereale*) cover crop (CCW) grown for both grain and stover; a reconstructed tall grass prairie seeded with 31 species (P) and a prairie of the same seed mix spring fertilized with 84 kg nitrogen (N) ha^{-1} (PF), both grown for fall-harvested biomass. For the C2/S2 rotation, each crop phase of the rotation was present every year. Plots were 27 x 61 m, arranged as a randomized complete block design with four

replicates. A meteorological station located at the research site collected air temperature, solar radiation, wind speed, humidity, and precipitation measurements.

For a complete account of row crop management details, see Table 4 and Table 5 in Appendix A. All maize was a 104 day maturity hybrid, planted in 76 cm rows. All maize plots (C2, CC, and CCW) received 87-101 kg N ha^{-1} (32% UAN injected at 7.6 cm depth) at planting. Based on results from a late spring nitrate test (Blackmer 1997), all maize plots were side-dressed with 18-134 kg N ha^{-1} in the form of 32% UAN injected every other row. The large range is due to 2013, when two of the field replications showed very high levels of soil nitrate, and thus required very low post-emergence application of nitrogen fertilizer. Lime, phosphorus and potassium were applied based on soil tests to the maize plots as needed. All weeds and pests were adequately controlled. Maize grain was harvested after physiological maturity. After grain harvest, stover from the CC and CCW plots was shredded using a flail chopper, then windrowed. Approximately 1 kg of biomass was collected by hand from windrows of each maize plot (CC, CCW, C2) for moisture determination and subsequent biomass analysis. Collected biomass included cobs, stems, and leaves. The stover was then baled and weighed. 'Rymin' winter rye (*Secale cereale* L., cv. Rymin) was planted as a winter cover crop in CCW plots after stover removal. Rye was terminated the following spring using glyphosate. The maturity group for soybean was in the 2.2 - 2.6 range. Based on soil tests, lime, phosphorus and potassium were applied to all soybean plots as needed. Weeds and pests were adequately controlled with pesticides. Soybean grain was harvested after physiological maturity, and all soybean residue remained on the field.

The prairie seed mix contained 31 species Appendix A; Prairie Moon Nursery, Winona, MN). The seed mix was comprised of, by weight, 12% C_3 grasses, 56% C_4 grasses, 8% legumes, and 24% non-leguminous forbs. For management details please see Table 7 in Appendix A. The fertilized prairie (PF) received 84 kg N ha^{-1} (broadcast UAN 28% or 32%) in late March or early April. Both P and PF biomass was harvested after a killing frost, usually mid-October. The prairies were mowed at a height of 7-20 cm with all loose biomass being

removed, leaving only stubble. The fresh biomass was weighed, and subsamples from each plot were collected for moisture determination and biomass analysis.

Data Collection

Data were collected during the 2009-2013 growing seasons. Figure 1A presents cumulative daily precipitation for each year, along with 30 year means taken from a site 15 miles from this experiment. Stress degree days are calculated using the daily high air temperature. We assumed a base temperature of 30 degrees Celsius, meaning if the maximum air temperature is over 30 degrees Celsius, one stress-degree-day is accumulated for each degree over 30. We chose 30 degrees Celsius because that is the threshold above which maize development is negatively affected and the plant is likely to suffer from water stress (Tollenaar et al. 1979). Cumulative stress degree days are presented in Figure 1B along with 30-year means. This data set encompasses varied growing season environments, ranging from warm to cool and flooding to drought. In general the years are as follows (30-year mean annual precipitation is 846 mm): 2009 spring flooding (938 mm) with cooler temperatures; 2010 summer flooding (1443 mm) with average temperatures; 2011 average precipitation (805 mm) with warm temperatures; 2012 an extreme drought (566 mm) with equally extreme warm temperatures; 2013 spring flooding and late season drought (866 mm) with cool temperatures.

Biomass Analyses

All biomass samples were weighed, dried at 60 ° C for at least 48 hours, then ground to 2mm using a Wiley Mill. Samples were further ground to 1mm using an Udi Mill and stored in air tight containers at room temperature and humidity.

Compositional Analysis

For the detergent method, ground biomass samples were analyzed for cellulose, hemicellulose, lignin, and ash via ANKOM's sequential filter bag method using an ANKOM-200 Fiber Analyzer (ANKOM Technology, Macedon NY). The 2009-2011 seasons' biomass
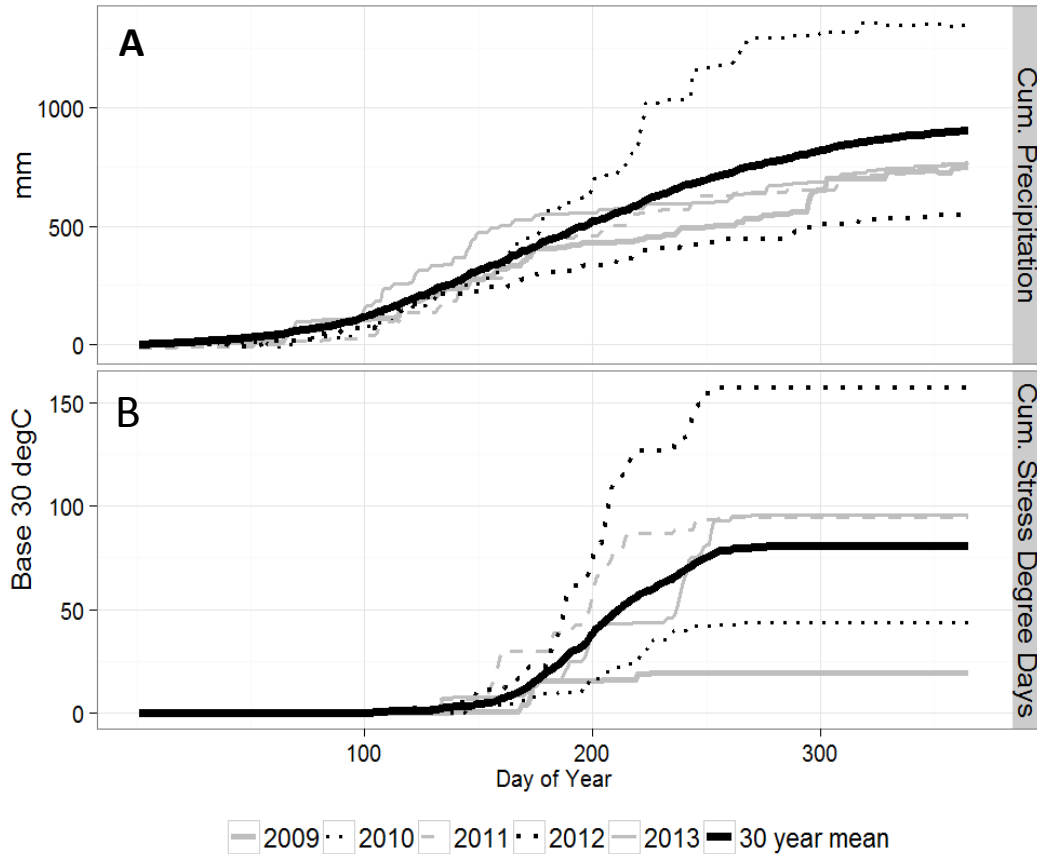
Figure 1. Weather summaries for 2009-2013.
A) Cumulative precipitation. Severe flooding occurred in 2010, and equally severe drought in 2012 B) Cumulative stress degree days at a base temperature of 30 degrees Celsius. 2009 was unusually cool, while 2012 was extremely warm

was analyzed in duplicate at a 1mm grind. In 2012, preliminary analyses indicated that a 1mm grind was too fine for the filter bags. Therefore the 2012 biomass was analyzed with a 2mm grind and run in triplicate through the ANKOM fiber analyzer. Following terminology recommended by Udén and colleagues (2005) hemicellulose was estimated as the difference between aNDF (amylase treated neutral detergent fiber) and ADF (acid detergent fiber), and cellulose as the difference between lignin(sa) and ADF.

In 2013, samples from 2009-2012 were analyzed using a modified procedure developed by NREL for determination of structural carbohydrates and lignin in biomass (Sluiter et al. 2008). The 1 mm (2009-2011 samples) or 2 mm (2012 samples) ground

biomass were used for these analyses. Our interest was to estimate the potential ethanol yield of structural carbohydrates – no analyses were performed to determine extractives, lignin or uronic acid. Briefly, samples were treated with 72% sulfuric acid, heated, diluted to 4% acid concentration, autoclaved, then analyzed for structural carbohydrates using High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPLC-PAD, Thermo Scientific Dionex). All analyses were done at the USDA Agricultural Research Service labs in Peoria, IL. Due to time and equipment constraints, only 2009 and 2012 samples were analyzed in duplicate using HPLC.

Theoretical Cellulosic Ethanol Conversion Ratio

The cellulose and hemicellulose fractions were converted to theoretical ethanol yields assuming anhydrous sugar-to-ethanol stoichiometric yields of 0.567 and 0.580 for hemicelluloses and celluloses, respectively (Dien 2010). Theoretical ethanol yields per unit biomass, or theoretical ethanol conversion ratios, were calculated as presented in Equations 1-3. We assumed a 100% recovery and 100% fermentation efficiencies for both cellulose and hemicellulose. Therefore, the results from Equations 1-3 represent maximum theoretical ethanol yields per unit biomass.

Eqn. 1 Calculation of hemicellulose-derived ethanol conversion ratio

$$\frac{g\ C5\ ethanol}{g\ biomass} = \left(\frac{g\ hemicellulose}{g\ dry\ biomass}\right) \times \left(\frac{0.567\ g\ ethanol}{g\ hemicellulose}\right)$$

Eqn. 2 Calculation of cellulose-derived ethanol conversion ratio

$$\frac{g\ C6\ ethanol}{g\ biomass} = \left(\frac{g\ cellulose}{g\ dry\ biomass}\right) \times \left(\frac{0.580\ g\ ethanol}{g\ cellulose}\right)$$

Eqn. 3 Calculation of maximum ethanol conversion ratio using results from Eqns. 1 and 2

$$\frac{g\ ethanol}{g\ biomass} = \frac{g\ C5\ ethanol}{g\ biomass} + \frac{g\ C6\ ethanol}{g\ biomass}$$

The dietary method's estimates will here-after be referred to as NREL estimates, and the detergent as ANKOM.

Theoretical Cellulosic Ethanol Yield Per Unit Land

The maximum ethanol conversion ratios (grams of ethanol per gram dry biomass, Eqn. 3) were multiplied by the amount of dry biomass harvested on a per hectare basis, yielding a theoretical amount of ethanol produced per hectare of land (Eqn. 4).

Eqn. 4 Maximum theoretical ethanol yield per unit land

$$\frac{L\ ethanol}{hectare} = \left(\frac{g\ ethanol}{kg\ biomass}\right) \times \left(\frac{kg\ _{harvested}\ cellulosic\ biomass}{hectare}\right) \times \left(\frac{1\ L\ ethanol}{789\ grams}\right)^{*}$$

   * Density of ethanol at 20 deg C

Statistical Analysis

The linear models used to statistically analyze dependent variables are presented in Appendix A. These models were analyzed using the MIXED procedure of SAS (Littell et al. 1996). The assumption of equal variances for each year was tested using the REPEATED statement. For the dependent variables 'biomass produced' and 'biomass removed', we found the mixed model that accommodated unequal variances provided the better fit based on Akaike's Information Criteria (AIC). For the models that included the 'measurement method' effect, we found the assumption of equal variances was satisfied for all dependent variables analyzed.

Pairwise comparisons were performed using the PDIFF and ESTIMATE statements. Unless otherwise specified differences were considered significant at $p < 0.05$.

Results and Discussion

Biomass Yields per unit Land

Maize and soybean yields were comparable to county averages during the study period, presented in Table 1 (USDA 2014).

Table 1. Maize and soybean grain yields (2009-2013)
Yearly Boone County IA averages are provided for comparison. No grain was harvested from prairie systems.

	2009	2010	2011	2012	2013
	----------------------------Mg ha^{-1}----------------------------				
County Average Maize/Soybean	(11.5 / 3.5)	(10.2 / 3.2)	(11.5 / 3.4)	(9.2 / 3.0)	(9.7 / 2.7)
CC	12.1	9.8	9.7	9.0	10.2
CCW	12.3	9.7	9.4	5.9	8.0
C2/S2	12.8	10.1	11.4	10.7	10.7
S2/C2	3.7	3.2	3.8	4.0	2.9
P	-	-	-	-	-
PF	-	-	-	-	-

CC=continuous maize, CCW=cont. maize with winter rye cover-crop, C2 = maize phase of maize-soybean rotation, S2 = soybean phase of maize-soybean rotation, P=un-fertilized prairie, PF=N-fertilized prairie

The amount of cellulosic biomass produced by each cropping system, along with statistical comparisons is presented in Table 2. These values are presented in Figure 2 along with standard errors of the means. Trendlines are presented for ease of viewing only. Note that the C2/S2 system's maize stover production per year is averaged over the 2 years of the rotation.

Table 2. Cellulosic biomass produced with statistical comparisons (2009-2013)
Uppercase letters indicate significant differences within a cropping system between years.
Lowercase letters indicate significant differences between cropping systems within a given year.
Soybean residue is not under consideration as a cellulosic feedstock and is therefore omitted.

	2009	2010	2011	2012	2013
	----------------------------Mg ha^{-1} yr^{-1}----------------------------				
CC	^{C}7.43^c	^{CD}6.51^c	^{D}5.25^c	^{A}9.90^a	^{B}8.43^b
CCW	^{BC}7.15^c	^{C}6.70^c	^{D}5.15^c	^{A}8.51^b	^{B}7.72^b
C2/S2	^{B}4.11^d	^{C}3.37^d	^{C}3.21^d	^{A}4.95^d	^{A}4.63^d
S2/C2	-	-	-	-	-
P	^{A}9.63^b	^{A}9.10^b	^{B}7.26^b	^{D}4.69^d	^{C}6.11^c
PF	^{A}13.48^a	^{B}11.86^a	^{C}9.74^a	^{D}7.61^c	^{C}9.36^a

CC=continuous maize, CCW=cont. maize with winter rye cover-crop, C2 = maize phase of maize-soybean rotation, S2 = soybean phase of maize-soybean rotation, P=unfertilized prairie, PF=N-fertilized prairie
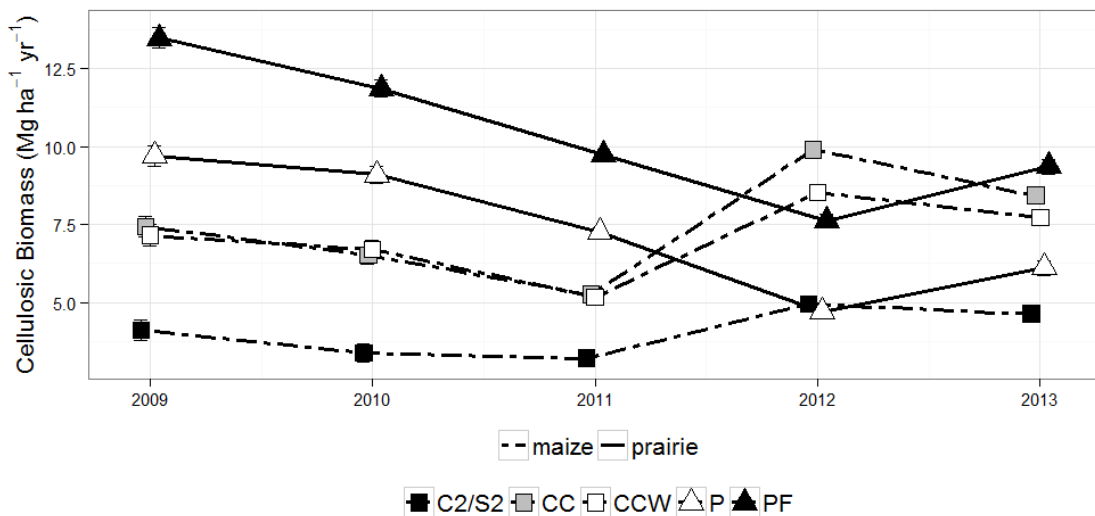
Figure 2. Aboveground cellulosic biomass produced (2009-2013)
The two prairies produced their maximum amount of cellulosic biomass in 2009, with PF and P producing 13.5 and 9.6 Mg ha^{-1}, respectively. All three maize systems exhibited maximum cellulosic production in 2012, with CC both producing 9.9 Mg ha^{-1}, CCW producing 8.5 Mg ha^{-1}, and the C2 phase of the C2/S2 system producing 5.0 Mg ha^{-1}.

There was a significant interaction between crop and year ($p < 0.001$), with the prairies having lowest biomass production in the drought years of 2012 and 2013, while all

maize systems had highest stover production in 2012 and 2013. Within the maize systems, in all years the CCW system was equal-to or lower-than the CC system in cellulosic biomass production per year. Within the prairie systems, the fertilized prairie (PF) produced significantly more biomass than the unfertilized (P) in every year of this study.

Figure 3 presents five-year averages and standard errors of the mean for each cropping system's biomass production and harvest.
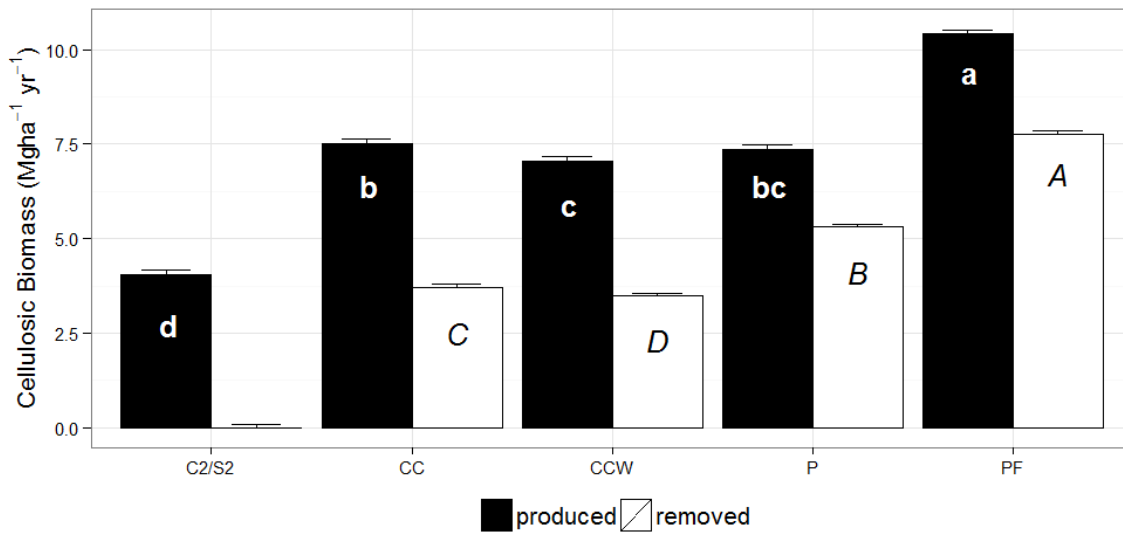
Figure 3. Mean yearly cellulosic biomass produced and removed averaged over 2009-2013 Lowercase letters indicate significant differences in produced biomass, capitalized letters indicate significant differences in harvested biomass amounts. C2/S2 is grown for grain only, and therefore has no cellulosic biomass removed.

Averaged over the 5-years, the prairie systems produced an estimated 2.7 Mg ha^{-1} yr^{-1} more cellulosic biomass than the maize systems ($p < 0.001$). The prairie showed a strong response to fertilization in all years, increasing production by an average of 3 Mg ha^{-1} yr^{-1} ($p < 0.001$).

On average, highest biomass harvests were realized from PF with 7.8 Mg ha^{-1} yr^{-1}, followed by P with 5.3, CC with 3.7, and finally CCW at 3.5 Mg ha^{-1} yr^{-1}

How does prairie cellulosic biomass production compare to maize stover production on prime agricultural land?

There are two considerations regarding the amount of biomass in a biofuel system – the amount of biomass produced, which represents a maximum, and the amount of biomass which is actually harvested, which depends on management. We will first discuss maximum cellulosic biomass production potentials of the maize and prairie systems.

Over a span of diverse weather years the maize systems produced an average of 6.2 Mg ha^{-1} yr^{-1}, while the P and PF produced an average of 7.4 and 10.4 Mg ha^{-1} yr^{-1}, respectively. The maize stover production values are consistent with other studies performed in Central Iowa (Hoskinson et al. 2007, Karlen et al. 2011). Both the P and PF values are substantially higher than the 1-6 and 6-8 Mg ha^{-1} yr^{-1} previously reported for unfertilized and fertilized prairies in the Midwest (Camil et al. 2004, Tilman et al. 2006, Jungers et al. 2013) as well as the 5.6 ha^{-1} yr^{-1} harvests assumed for 'managed prairie' in the US Billion Ton Update (Mitchell et al. 1996, US DOE 2011). In 2009 PF exceeded the 'breakeven' production rate of 13.4 Mg ha^{-1} yr^{-1}, which is the amount of biomass required (at $60 per Mg) to effectively compete economically with maize systems (James et al. 2010), although the cost of fertilization is not considered in those calculations.

In our study, the maize and prairie systems responded to drought years differently. In 2012, the most severe drought year, all maize systems (CC, CCW, C2) produced the lowest grain yields of this five year study, but produced the highest amount of cellulosic biomass. This is likely due to the timing of the drought – the maize experienced favorable growing conditions during vegetative growth, but water-limiting conditions during tasseling, pollination, and grain fill (Hay & Porter 2006). In 2012 the P and PF treatments' production dropped to only 48% and 56% of their maximum production, respectively. These results are consistent with the results from a study done by Tillman and Haddi (1992), which found a 47% decrease in unfertilized remnant prairie production during a drought in Minnesota in 1988. Drought also affected the efficacy of prairie fertilization, increasing prairie production by 2.9 Mg ha^{-1} in 2012 compared to the 3.9 Mg ha^{-1} increase observed in its most effective year, 2009. The early summer timing of the 2012 drought may have allowed for early-spring fertilization to be effective before the systems became water-limited.

The amount of cellulosic biomass produced versus harvested is not necessarily proportional, as harvesting regimes vary for different systems. For maize stover harvesting, it has been shown that soil erosion can remain 'tolerable' for removal rates up to 70% (Sheehan et al. 2004), but harvest rates should remain under 20% to maintain soil carbon (Wilheim et al. 2007). Our study utilized a rake and bale system, which has been shown to collect approximately 55% of the stover (Sokhansanj et al. 2012). During the five years of our study, we removed between 38-61% of the maize stover. Under this harvesting regime, in 4 of the 5 years the CC and CCW maize systems did not produce sufficient biomass to provide the 4.48 Mg ha^{-1} yr^{-1} (2 ton acre^{-1} yr^{-1}) desired by industry (eg. POET 2013, DuPont 2013).

As an ecosystem, prairies require periodic disturbance such as mowing, grazing, or fire in order to suppress growth of woody shrubs and trees (Anderson 1982, Collins & Barber 1985). Studies on perennial prairie grasses indicate nutrient cycling is altered not only by the amount of biomass removed, but also the timing of removal (Adler et al. 2006, Heaton et al. 2009, Wilson et al. 2014) In our study, we harvested biomass in the fall after a hard frost, usually late October. For a detailed study of the system's nutrient dynamics the reader is referred to Jarchow and collegues (2014) and Dietzel (in preparation). Due to variable mowing heights, the amount of above-ground material harvested from the prairie systems varied from 52 to nearly 100% removal. In all 5 years of this study, the PF system provided more than 4.48 Mg ha^{-1} (2 ton acre^{-1}) of harvested biomass, while the P provided more than 4.48 Mg ha^{-1} in 3 of the 5 years. This indicates that on a per area basis, prairies could provide cellulosic biomass in sufficient amounts to satisfy the requests of the Iowa cellulosic biofuel industry.

Ethanol Yields per unit Biomass

Ethanol yields per unit biomass as predicted via the NREL and ANKOM methods are presented in Figure 4 along with trendlines.
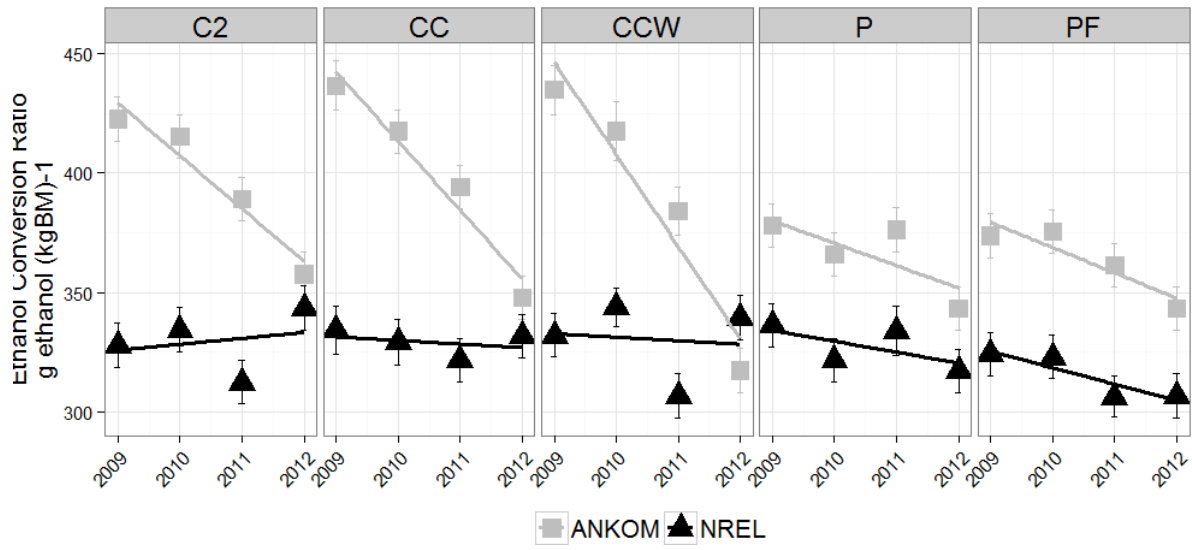
Figure 4. ANKOM and NREL ethanol conversion ratios for 2009-2012 cellulosic biomass
ANKOM predicted higher conversion values and suggested compositional changes in the maize
stover which were not reflected in the NREL method.

The ANKOM and NREL methods produced significantly different ethanol conversion ratios for every crop in every year ($p<0.0001$ to $p =0.02$), except for the 2012 C2 ($p=0.21$), CC ($p=0.15$) and CCW ($p=0.05$). In the prairie systems, there was no significant interaction between year and method ($p=0.29$), while in the maize systems there was ($p<0.0001$).

Over the 4 years, the ANKOM estimates were higher than NREL conversion ratios in the maize systems by 64 g ethanol (kg BM)$^{-1}$, while only 44 g ethanol (kg BM)$^{-1}$ in the prairie systems. It is generally recognized that the NREL method is the more accurate method (Theander and Westerlund 1993). Therefore, on average the ANKOM method over-predicted the maximum ethanol yield by an estimated 56 g ethanol (kg BM)$^{-1}$ ($p <0.001$), or by roughly 15%.

The biochemical constituents that contribute to the potential ethanol yield of biomass can be split into two categories: cellulose (6-carbon sugars) and hemicellulose (5-carbon sugars). Examining these two generalized components, as measured by both methods, provides insight into why ANKOM and NREL conversion rates differ. Cellulose and hemicellulose, as measured by the two methods, are shown in Figure 5.
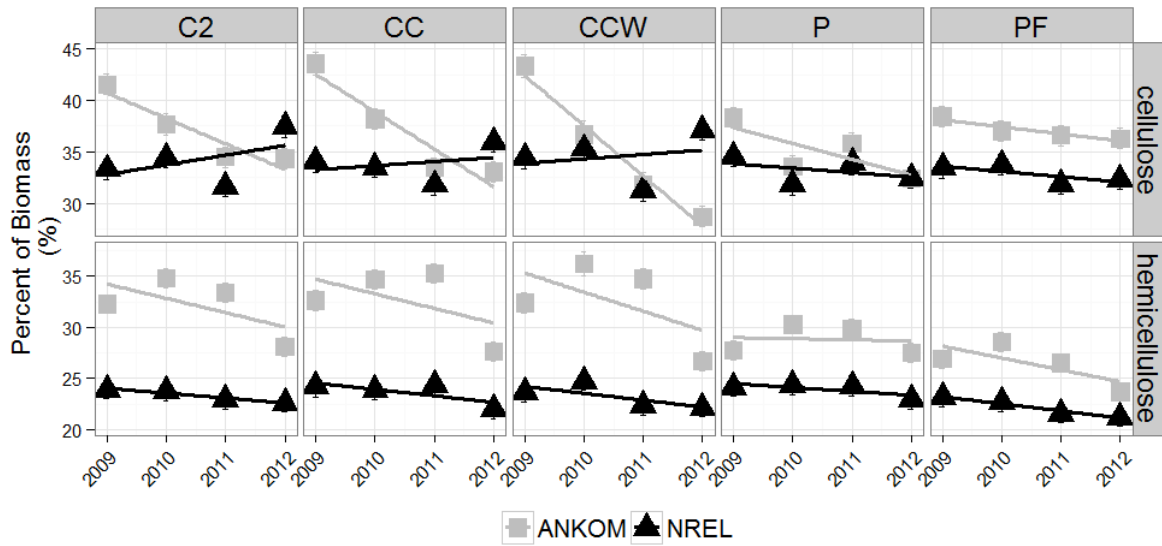
Figure 5. ANKOM and NREL estimates of generalized biomass components cellulose and hemicellulose. ANKOM cellulose measurements exhibit differential responses to both feedstocks and environments as compared to the NREL method, while ANKOM and NREL hemicellulose exhibit similar trends.

NREL and ANKOM hemicellulose estimates were significantly correlated ($p<0.001$), but the two methods' cellulose estimates were not. In general, the ANKOM predicted a larger range in both cellulose and hemicellulose than that predicted by NREL. The range of compositions for each system as predicted by the NREL method is presented in Table 3.

Table 3. NREL range of cellulose and hemicellulose estimates for maize and prairie systems

	NREL Cellulose	NREL Hemicellulose
	----------Percentage of Biomass (%)----------	
maize systems	32-37	22-24
prairie systems	32-35	21-24

Again assuming the NREL method produces more accurate results, the cellulose results from 2012 were the only instance in which a significant difference was observed between the maize and prairie systems. Over the 4 years, the maize systems averaged less

than 1% higher cellulose content than the prairies (p< 0.01), but 2.5% higher hemicellulose content (p <0.0001).

When averaged over the 4 years, the NREL conversion values of CC, CCW, C2, and P stover/biomass were not significantly different, predicting 330 g ethanol $(kgBM)^{-1}$. Fertilization significantly reduced the prairie biomass NREL conversion (p= 0.03), estimating 315 g ethanol $(kgBM)^{-1}$ from PF.

Do maize stover and prairie biomass exhibit similar ranges of cellulosic ethanol feedstock quality across a range of environmental growing conditions?

The ANKOM method works based on cell wall solubility under neutral and acidic conditions. Since plants vary in their exact cell wall composition and arrangement, they will exhibit differential responses to this method. The NREL dietary method, on the other hand, has been shown to be accurate in its estimations of cellulose and hemicellulose across feedstocks (Theander & Westerlund 1993). As has previously been found, the ANKOM method overestimated both the cellulose and hemicellulose contents as compared to the NREL method, with the amount of over-estimation depending on the plant source and year (Theander & Westerlund 1993, Dien et al. 2006). The decreasing ANKOM ethanol conversion ratio observed in the maize systems could be the result of either an increase in a water soluble component being removed in the neutral detergent step (Jung & Lamb 2004) or a change in either amount or configuration of an insoluble component. We observed a decreasing NDF value in the maize systems from 2009-2012, indicating an increase in one or more of the following water soluble cell wall components: starches, pectin, sugars, lipids, or easily digested proteins (Mertens 2002). The NREL samples were not subjected to water nor ethanol extraction before acid hydrolysis (Sluiter 2005) – the NREL values would therefore include these soluble fractions that were removed in the ANKOM procedure.

The majority of the water solubles in stover are monomeric or short-chain sugars such as glucose (Chen et al. 2007). It is therefore possible that the observed increase in stover water solubles are sugars, which, due to the lack of 'sink' strength, were not mobilized from stover to grain during grain fill (Daynard et al. 1969, Hume & Campbell

1972). This is supported by the observation that from 2009 – 2012, low maize grain yields were correlated with higher amounts of water solubles in the stover, presented in Figure 6.
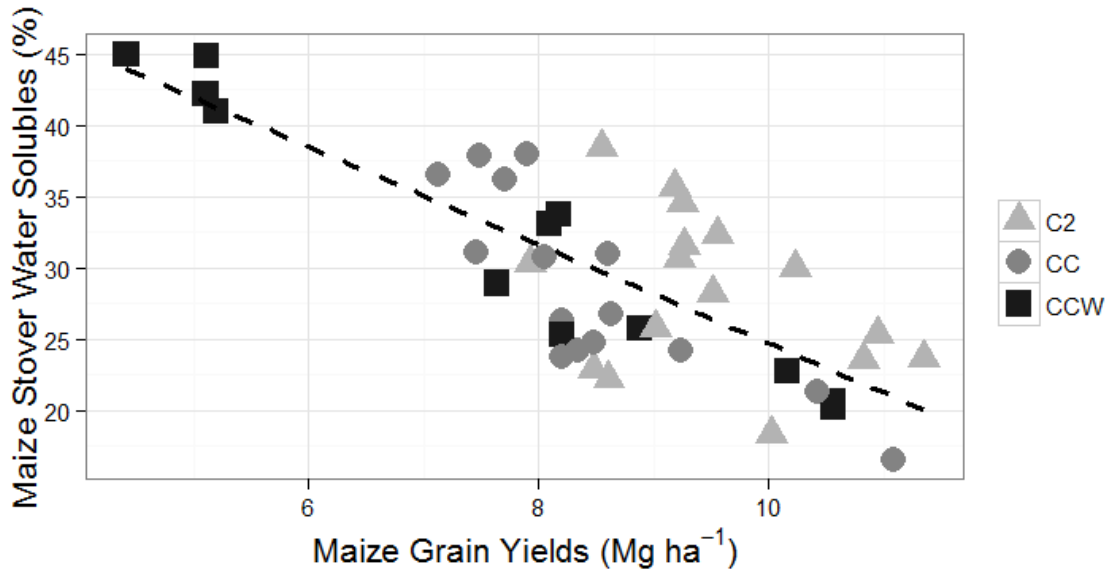
Figure 6. Lower maize grain yields are correlated with a higher percentage of water soluble components in the stover.

The ANKOM's amylase and neutral detergent step would remove these water soluble sugars and therefore preclude their inclusion in the theoretical ethanol yields. The NREL procedure directly measures glucose and would therefore include both soluble and structural polymeric sugars. These observations only add to the uncertainty associated with the ANKOM method's estimates of ethanol conversion.

Despite the disparate plant compositions of the systems, we found that the range in both cellulose and hemicellulose fractions as measured by the more accurate NREL method was similar in the prairie and maize systems.

Due to the observed differential response of the maize and prairie feedstocks to the ANKOM method, we do not advocate using a single 'correction factor' for the ANKOM value. We do note that the ANKOM consistently over-estimates ethanol yields on a per unit biomass regardless of feedstock, consistent with other studies (Theander & Westerlund 1993, Jung & Lamb 2004, Dien et al. 2006, Lorenz et al. 2009).

In some scenarios, it may be appropriate to assume a constant conversion factor from the NREL estimates of 330 g ethanol $(kgBM)^{-1}$ for maize stover and the C_4-grass dominated prairie, and 315 for the fertilized prairies, which contain a mixture of C_3- and C_4-grasses and forbs (Jarchow & Liebman 2013). These values are within the ranges reported for these types of feedstocks (Lee 2007), and are consistent with other studies that show lower conversion values for mixtures as compared to C_4 grasses (Jarchow et al. 2012).

Ethanol Yields per Unit Land

The maximum theoretical ethanol yields per unit land were calculated based on the ANKOM and NREL methods, as well as by assuming constant conversion ratios for the CC, CCW, and P (330 g ethanol $(kgBM)^{-1}$) , and PF (315 g ethanol $(kgBM)^{-1}$) systems. Ethanol yields were calculated using Eqn. 4, results are presented in Figure 7.
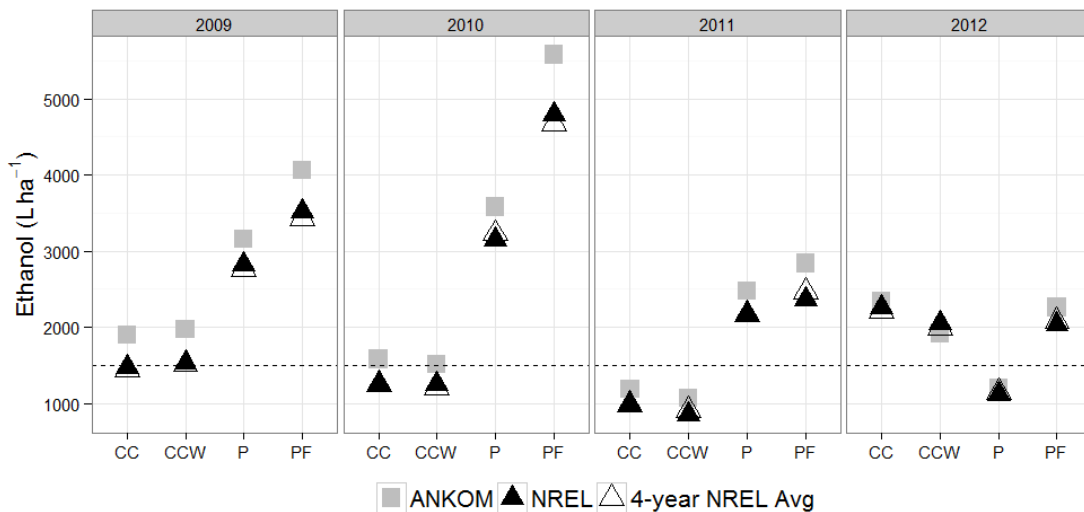
Figure 7. Effect of estimation method on calculated ethanol yields
ANKOM produces significantly higher estimates than NREL (p<.0001). NREL estimates were not significantly different from those predicted using 4-year NREL average conversion values for the maize (330), prairie(330), and fertilized prairie (315), respectively. Standard errors are not visible at this scale. As a reference, the dashed line represents the 1500 L ha^{-1} yr^{-1} yields expected by Iowa cellulosic fuel industries (eg. DuPont 2013, POET 2013).

The ANKOM and NREL methods' estimates were significantly different in all cases except for the CC, CCW, and P in 2012. Overall, the ANKOM method estimated a significantly higher ethanol yield than the NREL method (p<0.001) by an estimated 345 L ha^{-}

[1], with this estimate being larger in the prairie systems (448 L ha^{-1}) and lower in the maize systems (241 L ha^{-1}). In all cases, the NREL method's ethanol yields were not significantly different (p=0.67) from those calculated using 4-year NREL averages of 330, 330, and 315 g ethanol (kgBM)$^{-1}$ for the maize (CC and CCW), prairie (P), and fertilized prairie (PF) systems, respectively.

According to NREL estimates, when averaged across years the prairie systems averaged 1287 L cellulosic ethanol ha^{-1} yr^{-1} more than the maize systems, with fertilization increasing prairie ethanol production an average of 865 L ha^{-1} yr^{-1}.

Despite the significant differences between the NREL and ANKOM methods' results, the amount of biomass harvested accounted for 90% of the variability in ethanol yield per unit area.

How does the method of ethanol conversion estimation affect theoretical ethanol yields per unit land area?

Our results are consistent with other studies that have found that the amount of biomass is the more dominant variable dictating ethanol yields (Jarchow et al. 2012, Jungers et al. 2013). For example, at a constant biomass harvest of 3 Mg ha^{-1} yr^{-1}, if the maize stover conversion value were to drop from ~345 to 310 g ethanol (kg BM)$^{-1}$, which is the range of conversion ratios observed via the NREL method, the ethanol yield per unit area would drop ~100 L ha^{-1} yr^{-1}, or about 10%. Thus, while composition is an important consideration in biofeedstock evaluation, we found that a system's ethanol output is strongly dictated by its biomass production. In our study, 2009-2012 covered an impressively wide range of growing conditions, with all systems' 2013 biomass productions falling within the range of the previous four years'. This indicates that the 4-year average (2009-2012) NREL ethanol conversion ratios obtained for maize, prairie, and fertilized prairie systems could provide more accurate ethanol yield predictions as compared to using estimates obtained using yearly determined ANKOM values.

Conclusions

Prairie systems have the potential to produce significantly more biomass than previously reported, particularly those receiving modest amounts of N-fertilization in the spring. Our study indicates that the most productive management strategies of reconstructed prairie systems have not yet been exploited; with further research it is feasible that reconstructed prairies could be managed to optimize biomass production as well as other ecosystem services. Although the exact botanical make-up of prairies may vary, we found that as a biofuel feedstock the range in biochemical composition fell within the range expected from maize stover. We found that the method used to determine biochemical composition significantly affects the ethanol conversion ratio, but that the dominating variable in ethanol yield is the amount of harvestable biomass. If the interest of a study lies in comparing ethanol yields per unit land area, utilizing available ANKOM data or assuming constant conversion rates may be sufficient.

Acknowledgements

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Appendix A

Table 4. Detailed management of maize systems C2, CC, and CCW (2009-2013)

Year	CCW Cover crop termination	Variety *Seeding Rate*	Planting Date / Emergence/ Harvest	Cover Crop Planting*	N Fertilization C2/CC/CCW
2009	May 6	Agrigold 6325 VT3 (104-d) *82,670 sds ha^{-1}*	May 7/ May 19/ Oct 21	Nov 6	**Planting (all):** 84 kg N ha^{-1} 32% UAN **June 17:** 56/84/134 kg N ha^{-1} 32% UAN every other row
2010	May 5	Agrigold 6325 VT3 (104-d) *82,670 sds ha^{-1}*	May 6/ May 21/ Sept 29	Oct 4	**Planting (all):** 87 kg N ha^{-1} 32% UAN **June 17:** 18/36/82 kg N ha^{-1} 32% UAN every other row
2011	May 10	Agrigold 6325 VT3 (104-d) *82,670 sds ha^{-1}*	May 11/ May 21/ Oct 3	Oct 10	**Planting (all):** 87 kg N ha^{-1} 32% UAN **June 29:** 18/36/82 kg N ha^{-1} 32% UAN every other row
2012	April 18	Pioneer P0448XR (104-d) *80,200 sds ha^{-1}*	May 11/ May 18/ Sept 25	Oct 1	**Planting (all):** 87 kg N ha^{-1} 32% UAN **June 12:** 112/134/134 kg N ha^{-1} 32% UAN every other row
2013	May 7	Pioneer P0448XR (104-d) *80,200 sds ha^{-1}*	May 17/ May 24/ Oct 9	Oct 21	**Planting (all):** 90 kg N ha^{-1} 32% UAN **June 12:** 101 or 157/67 or 157/22 or 157 kg N ha^{-1} 32% UAN every other row

*222 seeds ha^{-1}, 19.1 cm rows

Table 5. Detailed soybean crop management (2009-2013)

	Variety *Seeding Rate*	Planting Date	Emergence	Harvest
2009	Pioneer 92M53 *399,260 sds ha^{-1}*	May 8	May 21	Oct 13
2010	Pioneer 92Y30 (2.3 maturity group) *399,500 sds ha^{-1}*	May 6	May 23	Oct 13
2011	Pioneer 92Y20 (2.2 maturity group) *399,500 sds ha^{-1}*	May 11	May 22	Sept 30
2012	Pioneer 92Y60-N343 (2.6 maturity group) *399,500 sds ha^{-1}*	May 11	May 19	Sept 25
2013	Pioneer 92Y60-N343 (2.6 maturity group) *399,500 sds ha^{-1}*	May 17	May 27	Oct 1

Table 6. Prairie seed mix species, functional group identity, and seeding rates
The same seed mix was used for the fertilized and unfertilized prairie

Latin Name	Common Name	Functional Group	Seeds m^{-2}	% by weight
Amorpha canescens	Lead plant	Legume	8	1.0
Andropogon gerardii	Big bluestem	C4 grass	99	20.0
Asclepias tuberosa	Butterfly milkweed	Forb	5	2.5
Aster novae-angliae	New England aster	Forb	16	0.5
Astragalus Canadensis	Canadian milk vetch	Legume	4	0.5
Baptisia leucantha	White wild indigo	Legume	2	2.0
Dalea purpurea	Purple prairie clover	Legume	19	2.5
Desmanthus illinoensis	Illinois bundleflower	Legume	3	1.5
Desmodium canadense	Showy ticktrefoil	Legume	4	1.5
Echinacea pallida	Purple prairie coneflower	Forb	8	3.0
Elymus Canadensis	Canada wildrye	C3 grass	31	12.0
Erungium yuccifolium	Rattlesnake master	Forb	6	1.5
helianthus laetiflorus	Showy sunflower	Forb	5	0.7
helianthus maximiliani	Maximillian's sunflower	Forb	5	0.7
Heliopsis helianthoides	Early sunflower	Forb	6	2.0
Lepedeza capitata	Round-headed bushclover	Legume	8	2.0
Liatris pycnostachya	Prairie blazing star	Forb	8	1.5
Monarda fistulosa	Wild bergamot	Forb	26	0.8
Panicum virgatum	Switchgrass	C4 grass	55	8.0
Pycnanthemum virginianum	Common mountain mint	Forb	33	0.3
Ratibida pinnata	Yellow coneflower	Forb	22	1.5
Rudbeckia hirta	Black-eyed susan	Forb	46	1.0
Schizachyrium scoparium	Little bluestem	C4 grass	59	8.0
Silphium integrifolium	Rosin weed	Forb	0.3	0.5
Silphium laciniaturn	Compass plant	Forb	0.5	1.5
Solidago rigida	Stiff goldenrod	Forb	20	1.0
Sorghastrum nutans	Indiangrass	C4 grass	119	20.0
Sporobolus heterolepis	Prairie dropseed	C4 grass	4	0.5
Tradescantia ohiensis	Ohio spiderwort	Forb	6	1.5
Vernonia fasciculate	Common ironweed	Forb	9	0.8
Veronicastrum virginicum	Culver's root	Forb	8	0.2

Table 7. Management summary for both unfertilized- (P) and fertilized-prairie (PF) systems (2009-2013)

	N Fertilization (PF only)	Harvest Date (P and PF)
2009	**April 17** 84 kg N ha^{-1} Broadcast ammonium nitrate	**Oct 19**
2010	**March 29** 84 kg N ha^{-1} Broadcast ammonium nitrate	**Oct 21**
2011	**April 11** 84 kg N ha^{-1} Broadcast 32% urea ammonium nitrate	**Oct 20**
2012	**March 28** 84 kg N ha^{-1} Broadcast 28% urea ammonium nitrate	**Oct 10**
2013	**April 26** 84 kg N ha^{-1} Broadcast 28% urea ammonium nitrate	**Oct 28**

Statistical model for dependent variables 'biomass produced' and 'biomass removed'

$$y_{ijk} = \mu + b_i + \alpha_j + \beta_k + \alpha\beta_{jk} + e_{ijk}$$

where

y_{ijk} = observation in the ith BLOCK receiving j^{th} level of factor CROP (α_j) and k^{th} level

of factor YEAR (β_k)

μ = overall mean

b_i = random effect due to the ith level of factor BLOCK (i=1,2,3,4) N(0,$\sigma_b{}^2$)

α_j = fixed effect due to the jth level of factor YEAR (k=2009, 2010, 2011, 2012, 2013)

β_k = fixed effect due to the kth level of factor CROP (j=maize of a maize-soybean

rotation, continuous maize, continuous maize with a winter rye cover crop,

prairie, N-fertilized prairie)

$\alpha\beta_{jk}$ = fixed interaction effect due to the jth level of factor YEAR and k^{th} level of

factor CROP

e_{ijk} = residual error effect assumed identically and independently distributed (i.i.d)

N(0, $\sigma_w{}^2$)

w_{ij} and e_{ijk} are assumed to be independent of one another

For dependent variables that were measured using multiple methods (ethanol
conversion rate, hemicellulose, cellulose) the following model was used.

Statistical model for dependent variables involving 'method of measurement'

$$y_{ijk} = \mu + b_i + \alpha_j + b\alpha_{ij} + \beta_k + b\beta_{ik} + \alpha\beta_{jk} + \gamma_l$$
$$+ b\gamma_{il} + \alpha\gamma_{jl} + \beta\gamma_{kl} + \alpha\beta\gamma_{ijl} + b\alpha\beta_{ijk} + e_{ijkl}$$

where

y_{ijk} = observation in the ith BLOCK receiving j^{th} level of factor CROP (α_j) and k^{th} level of factor YEAR (β_k)

μ = overall mean

b_i = random effect due to the ith level of factor BLOCK (i=1,2,3,4) N(0,σ_b^2)

α_j = fixed effect due to the jth level of factor YEAR (j=2009, 2010, 2011, 2012)

$b\alpha_{ij}$= random error associated with BLOCK by YEAR interaction (i.i.d) N(0, σ_w^2)

β_k = fixed effect due to the kth level of factor CROP (k=maize of a maize-soybean rotation, continuous maize, continuous maize with a winter rye cover crop, prairie, N-fertilized prairie)

$b\beta_{ik}$= random error associated with BLOCK by CROP interaction (i.i.d) N(0, σ_w^2)

$\alpha\beta_{jk}$ = fixed interaction effect due to the jth level of factor YEAR and k^{th} level of factor CROP

γ_l= fixed effect due to the lth level of factor METHOD OF MEASUREMENT (l=Ankom, NREL)

$b\gamma_{il}$= random error associated with BLOCK by METHOD OF MEASUREMENT interaction (i.i.d) N(0, σ_w^2)

$\alpha\gamma_{jl}$= fixed interaction effect due to the jth level of factor YEAR and l^{th} level of factor METHOD OF MEASUREMENT

$\boldsymbol{\beta\gamma_{kl}}$= fixed interaction effect due to the kth level of factor CROP and l^{th} level of factor METHOD OF MEASUREMENT

$\boldsymbol{\alpha\beta\gamma_{ijl}}$= fixed 3-way interaction effect due to the jth level of factor YEAR and k^{th} level of factor CROP and l^{th} level of factor METHOD OF MEASUREMENT

$\boldsymbol{b\alpha\beta_{ijk}}$= random error associated with BLOCK by YEAR by CROP interaction (i.i.d) $N(0, \sigma_w^2)$

$\boldsymbol{e_{ijkl}}$ = residual error effect assumed identically and independently distributed (i.i.d) $N(0, \sigma_w^2)$

$\boldsymbol{b\alpha_{ij}}$ and $\boldsymbol{b\beta_{ik}}$ and $\boldsymbol{b\gamma_{il}}$ and $\boldsymbol{b\alpha\beta_{ijk}}$ and $\boldsymbol{e_{ijk}}$ are assumed to be independent of one another

For the dependent variable 'ethanol yield per unit land' this model was modified to include 3 levels of 'method of measurement' (ANKOM, NREL, 4-year NREL constants).

CHAPTER 3: ROOT-DERIVED COMPONENT OF SOIL-SURFACE CO$_2$ FLUX DIFFERS AMONG CONTINUOUS MAIZE, UNFERTILIZED PRAIRIE, AND N-FERTILIZED PRAIRIE GROWN FOR BIOFUEL

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Abstract

A major hurdle in closing carbon budgets is the separation of the soil-surface CO$_2$ flux into its respective components. We used a shading method over two seasons to separate the CO$_2$ resulting from oxidation of soil organic matter and CO$_2$ derived from live-root activity. We compared separation values in an unfertilized prairie and prairie receiving spring nitrogen fertilization, both perennial systems, and an annual system of continuous maize. Contrary to our hypothesis, the total growing-season root CO$_2$ flux was not proportional to the end-of-season root biomass of the cropping systems: the unfertilized prairie contained nearly twice the root biomass of the fertilized prairie, but their total root CO$_2$ fluxes were not significantly different from one another in either year. Root growth, rather than total root biomass, may be the more dominant factor dictating total root respiration. This may be particularly apparent in perennial systems where total root biomass contains roots of heterogeneous ages. We found that the total growing-season flux of both root- and organic matter-derived CO$_2$ was higher in the perennial systems compared to the maize system. However, on a percentage basis, the prairies' soil-surface CO$_2$ flux from May-September averaged 29% root-derived while from mid-June through September the maize averaged 22% root-derived. The percentage of the total CO$_2$ flux that was root-derived in a given system varied from year to year, indicating there is no set relationship for a given cropping system.

Introduction

The soil contains a major portion of the globe's readily exchangeable carbon stores, second only to the ocean, and twice that which is present in the atmosphere (Eswaran et al. 1993). Therefore, not only does agriculturally driven release of soil carbon (C) degrade the

production potential of the land, but it also contributes to already rising atmospheric CO_2 levels. It would therefore seem prudent for US biofuel production, which strives to reduce the release of fossil CO_2 (US House 2007), to rely on cropping systems that will maintain or improve the carbon content of the soil. Currently, there is great uncertainty associated with soil-C sustainability of harvesting agricultural residues such as maize stover for biofuel feedstock (Mann et al. 2002, Laird & Chang 2013). Long term studies have identified perennial systems such as prairie as being soil-carbon builders (Matmala et al. 2008, Anderson-Teixeira et al. 2009), although the exact mechanisms are not well understood (Brye et al. 2002, Rasse et al. 2005, Fornara & Tilman 2012). New fuel standards set by the Environmental Protection Agency require feedstocks to meet stringent lifecycle carbon requirements (US House 2009, Environmental Protection Agency 2013). In order to better inform these policy requirements, researchers must gain an increased understanding of candidate systems' effects on soil C.

Predicting a cropping system's effect on long-term carbon storage is difficult. Direct measurements are subject to large errors due to spatial variability of soil C, may require 10+ years before exposing significant changes, and give no insight into mechanisms for C storage. Approaches that quantify C inputs, outputs, and their subsequent balance could provide an indication of how systems build or deplete carbon reserves over time (Cahill et al. 2009). The largest output of carbon from non-calcareous soils is C lost via microbial decomposition of soil organic matter (SOM; Buyanovsky et al. 1987, Cahill et al. 2009). This C output, which can be considered fossil C (Schimel 1995), materializes as CO_2 diffusing from the soil surface (R_{SOM}). In vegetated soils, below-ground portions of plants also produce CO_2 (R_{ROOT}), directly via respiration, and indirectly by exuding carbon compounds that are immediately oxidized by microbes living in the rhizosphere. The substrate for R_{ROOT} is C that was recently fixed from the atmosphere via photosynthesis; R_{ROOT} is therefore simply recycling C back to the atmosphere. This recycled C (R_{ROOT}) mixes with the SOM-derived fossil C (R_{SOM}), resulting in a carbon flux from the soil surface (R_{TOT}) that is difficult to interpret as a carbon output. In order to more accurately estimate carbon balances, as well as predict future climate impacts on SOM, each component must be considered

separately (Cannell & Thornley 2000, Pendall et al. 2004, Trumbore 2006, Kucharik et al. 2006).

There are several methods available for separating R_{TOT}, and choice of method should reflect the goals of the study (Hanson et al. 2000, Kuzyakov 2006, Hopkins et al. 2013). The goal of this study was to quantify seasonal differences in fluxes from both prairie and maize systems while working within a larger cropping system experiment; we required a method that could be applied and removed weekly and was non-destructive to the sampling environment. The shading method can be applied and removed easily throughout the season, is non-destructive to above-ground biomass, and is non-invasive to the soil structure (Kuzyakov 2006). It is therefore well-suited to seasonal studies on collaborative sites. Assuming an ideal situation where soil temperature remains unaffected, shading of vegetation affects R_{ROOT} by disrupting photosynthate production, and therefore substrate for root respiration and exudation, while R_{SOM} remains unchanged. By comparing R_{TOT} under various levels of shading and natural conditions, one can deduce the R_{ROOT} contribution as the portion of R_{TOT} that is responsive to light. Shading has been successfully utilized to separate CO_2 emissions in grasslands (Craine et al. 1999, Wan & Luo 2003). Grasslands are spatially heterogeneous, containing many species of plants with different rooting structures. It is therefore likely that the shading method works as well, if not better, in spatially regular systems such as annual maize.

To our knowledge the relationship between R_{ROOT} and below-ground biomass (BGB) has not been explored on a field scale. Some field studies have shown a linear relationship between R_{TOT} and BGB, indicating there may be a simple relationship between R_{ROOT} and root biomass (Kucera & Kirkham 1971, Koerber et al. 2010). Conversely, root metabolism relies on photosynthate produced by the aboveground portion of the plant, which may mean that R_{ROOT} is dependent on the amount of above-ground biomass (ABG) of the system. Zhang and colleagues found R_{TOT} showed better correlation to AGB than BGB (Zhang et al. 2009). Norman and colleagues demonstrated that the incorporation of leaf area index (LAI) into an empirical model predicting R_{TOT} improved the fit of the model (Norman et al. 1992).

Additionally, some studies have suggested that the gross primary productivity of the system, which would include both ABG and BGB, is the main driver of R_{ROOT} (Zhang et al. 2009, Hopkins et al. 2013).

We hypothesized that once R_{TOT} is separated, the R_{ROOT} component would be proportional to the BGB of the system. From 2008-2011, the perennial prairie systems at this site showed an average root production 7 times greater than the annual maize systems (Jarchow et al. 2014). We therefore hypothesized that in the perennial prairie systems a larger portion of the season-cumulative R_{TOT} would be derived from roots. The high root biomass and longer growing seasons of the prairies might also increase the 'priming effect', stimulating more SOM decomposition (R_{SOM}), thus diluting the relative increase in R_{ROOT} (Kuzyakov 2002, Cheng et al. 2014). Additionally, annual maize systems exhibit higher peak growth rates than perennial prairies (Dietzel, in preparation). High growth rates may correspond to a spike in R_{ROOT} that may counteract the effects of the annual maize's smaller root biomass and shorter growing season.

In this study, we tested the hypotheses that the season-cumulative R_{ROOT} would be linearly related to the end-of-season root biomass of the system, and that the perennial systems' growing-season cumulative R_{TOT} would have a higher proportion R_{ROOT} compared to the annual maize. We tested our hypothesis by using the shading method to separate the soil-surface CO_2 flux (R_{TOT}) of an unfertilized prairie, an N-fertilized prairie, and a continuous maize system into SOM- and root-derived components (R_{SOM} and R_{ROOT}, respectively) for two growing seasons (2012-2013).

Materials and Methods

Experimental site, design, and general management

We conducted a field experiment from May 2012 to October 2013 at Iowa State University's South Reynoldson Farm in Boone County IA (41°55 N 93°45 W). For a complete site description and history, please see Jarchow et al. (2014). The predominant soil types are Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls; USDA-NRCS 2014).

Subsurface drainage of the plots was completed in spring of 2008 after which all cropping systems were established. The site has been managed without tillage since establishment. A micro-meteorological station located at the research site provided air temperature, solar radiation, wind speed, humidity, and precipitation measurements.

Five potential biofuel cropping systems were arranged in a randomized complete block design with four replicates. Plots were 27 x 61 m. Only three of the five cropping systems were investigated in this experiment: continuous maize (*Zea mays* L.) of a 104 day maturity hybrid (CC), 31 species-seeded reconstructed tall grass prairie (P), and a spring nitrogen (N) fertilized prairie of the same seed mix (PF). The prairie seed mix contained, by weight, 12% C_3 grasses, 56% C_4 grasses, 8% legumes, and 24% non-leguminous forbs (Prairie Moon Nursery, Winona, MN). A detailed plant list can be found in Jarchow & Liebman (2013), and a detailed management description can be found in Jarchow et al. (2014).

At planting, the CC received 87 (May 11 2012) and 90 (May 17 2013) kg ha^{-1} (32% UAN injected at 7.6 cm depth). Based on a late spring soil nitrate test, the CC plots were side-dressed with 112 (June 12 2012) and 67 or 157 kg (June 28 2013) N ha^{-1} in the form of 32% UAN injected every other row. Lime, phosphorus and potassium were applied based on soil tests to the maize plots as needed. All pests and weeds were adequately controlled using chemicals. Grain was harvested from CC plots after physiological maturity (Sept 25 2012 and Oct 9 2013, respectively), followed by shredding and windrowing of the remaining stover. Approximately 50% of the stover was baled and removed from the site. Maize grain yields at this site were 9.0 and 10.2 Mg ha^{-1} in 2012 and 2013, respectively, on par with the county averages of 9.2 Mg ha^{-1} in 2012 and 9.7 Mg ha^{-1} in 2013 (USDA 2013, 2014).

On March 28 2012 and April 26 2013 PF received 84 kg N ha^{-1} (28% UAN broadcast). Prairie biomass (both P and PF) was harvested on October 10 2012 and October 28 2013, after a killing frost. The prairies were mowed to 3-10 cm, with all loose biomass being removed, leaving only the stubble. In 2012 and 2013, the prairies (P) produced 4.7 and 6.1 Mg ha^{-1} of aboveground biomass, while the fertilized prairies (PF) produced 7.6 and 9.4 Mg ha^{-1}, respectively.

Treatments

We used a split-plot design with cropping system (CC, P, or PF) as the main plot and shade (0%, 50%, 90% reduction in light) as a subplot. Three steel frames 1 m x 1 m x 1 m in size were installed every season in each main plot. Subplots were located at least 3 m from the edge of the main plots. In CC each shade subplot encompassed two planted rows, one alley row receiving N-side dressing, and one alley with tractor wheel traffic. Light reduction was achieved using shade cloth of varying mesh sizes (Greenhouse Megastore). Shade cloth covered the top and three sides of the frame, leaving the north face open to facilitate air flow. Shades were attached to the frames at dusk, remained in place for 2-3 full days, after which they were removed. At the beginning of the season, shade treatment was randomly assigned to sub plots, restricted by main plot. Shade treatments were rotated for subsequent rounds of measurements using a William's Latin Square design in time, ensuring that every subplot received the same sequence of shade treatments. This was done to avoid a subplot randomly receiving 90% shade over multiple sampling periods, as chronic shading could permanently affect plant growth. Shade treatments were applied every 1-2 weeks from May through September of both 2012 and 2013.

Measurements

Due to equipment and time restrictions, from May – August 2012 shading and all flux-associated measurements were staggered between the four blocks. Two randomly chosen blocks received shading on Day 1, while the other two received shading the following day (Day 2). Flux, photosynthesis, and moisture measurements were then made on the first group of blocks on Day 4, while the second group was measured on Day 5. These consecutive day measurements were treated as replicates, barring significant weather changes from one measurement day to the next (such as rain). In September 2012 and for the entire 2013 season, shading was applied to all 4 blocks and all measurements were made on the 3rd day of shading.

Photosynthetically Active Radiation

Photosynthetically active radiation (PAR) was measured above and below the ceiling shade cloth at each sampling (AccuPAR LP-80, Decagon Devices Inc., Pullman WA). Results were analyzed using the *summary* and *sd* functions of R (R Core Team 2013).

Photosynthesis

Leaf level photosynthesis was measured using a portable infrared gas analyzer (LI-COR 6400, LI-COR, Inc., Lincoln, NE) with a leaf chamber attachment. In 2012 photosynthesis measurements were made between 1000 and 1300, done concurrently with the soil flux measurements. In 2013, photosynthesis measurements were made between 0900 and 1130 one day before soil flux measurements.

In CC, four maize plants were randomly selected from within each shaded plot, and photosynthesis was measured at the shaded PAR value on the last fully expanded green leaf until tasseling, and thereafter on the ear leaf. Shaded prairie plots contained a diverse plant community that shifted throughout the season due to each plant's different growing season. In 2012, for each sampling we chose one species that appeared in every shade plot and measured 3 separate leaves from the same species under each shade regime. In 2013 we instead chose 3 plants at each sampling that we felt best represented the community of the individual shade plot on that day and measured on the last fully expanded leaf of those plants.

The effect of shading on photosynthesis was analyzed using the MIXED procedure of SAS (SAS release 9.3; SAS Institute, Cary, NC, USA). Year, date, crop, shade, and their 2, 3, and 4-way interactions were fixed variables and block was random. The effect of shade was analyzed for each crop on each date, and effects were considered significant at $p < 0.05$.

Soil Surface CO_2 flux

For the 2012 season PVC collars 9 cm in diameter were installed in the prairies in May, and in the maize after N side-dressing in mid-June; all collars were removed in

October. In 2013 prairie collars were again installed in May, but the maize collars were installed one week after emergence, were removed for side-dressing, and were then re-installed. All collars were inserted to a depth of ~3 cm, with approximately 2 cm remaining above the soil surface. In the prairie the 4 collars were randomly placed in each of the three shade sub-plots, ensuring the collar was free from live vegetation. Maize collars were carefully placed to account for spatial variability (Rochette et al. 1991, Daigh 2013) – 2 collars were placed in alleys between planting rows, with one being in an N-fertilized alley. The remaining 2 collars were placed between maize plants in a planted row. We waited at least 1 week after collar installation before taking CO_2 flux measurements.

Soil surface CO_2 flux measurements were taken using a portable infrared gas analyzer (LI-COR 6400, LI-COR, Inc., Lincoln, NE) with a soil respiration chamber (LI-COR 6400-09), coupled with soil temperature at 7 cm depth within 30 cm of the collar (LI-COR thermocouple attachment). Volumetric water content of the soil was measured within 2 hours of CO_2 measurements at a depth of 5 cm (Field Scout TDR 100, Spectrum Technologies, Aurora, IL). CO_2 flux measurements were conducted by placing the soil chamber over the PVC collar, reducing the CO_2 concentration below the ambient air concentration, and measuring the CO_2 concentration change over a 10-30 second interval. The instrument plotted CO_2 concentration against time, and reported the slope of the line at the ambient air concentration as the soil CO_2 flux (LI-COR Biosciences 2012). All measurements were conducted in the morning between 0800 and 1200. When the site received more than 25 mm rain we waited at least 36 hours before measuring soil fluxes.

From July 7-9 2012 we performed semi-continuous soil surface CO_2 flux measurements. Shading was applied to 2 blocks on the evening of July 6, and soil CO_2, soil temperature, and soil moisture measurements were made as described above at approximately 0600, 1000, and 1800 on July 7 and 8, then at 1000 on July 9. Results were analyzed using the MIXED procedure of SAS with block as random, and all other effects and their interactions as fixed. Comparisons were made using the PDIFF statement, and unless otherwise specified differences were considered significant at $p<0.05$.

Outliers were assessed as described in Appendix B, and resulted in the removal of approximately 1% of data. Temperature correction is also described in detail in Appendix B. Briefly, when sampling periods warranted correction, fluxes were adjusted using fitted Q_{10} relationships.

Results

Weather

Cumulative daily precipitation and stress degree days are presented in Figure 8 with 30-year means from a site 15 miles from this experiment. Stress degree days are calculated using the daily high air temperature. We assumed a base temperature of 30 degrees Celsius, meaning if the maximum air temperature is over 30 degrees Celsius, one stress-degree-day is accumulated for each degree over 30. We chose 30 degrees Celsius because that is the threshold above which maize development is negatively affected and the plant is likely to suffer from water stress (Tollenaar t al. 1979).
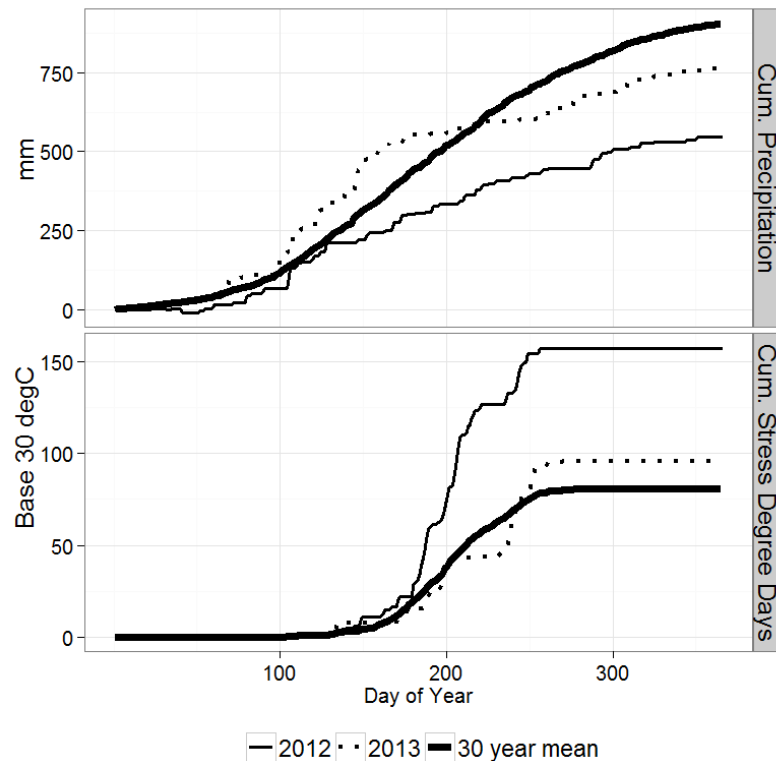

Figure 8. 2012-2013 precipitation and temperature summaries.
 2012 was extremely dry and hot. 2013 experienced spring flooding with late season drought.

Photosynthetically Active Radiation (PAR)

Over the 2012-2013 seasons, the 50% shade treatment resulted in an average of 54%(sd±3%) reduction in PAR, while the 90% treatment achieved an average of 87%(sd±2%) reduction.

Photosynthesis

In both prairie systems, a reduction in light resulted in a significant reduction in leaf level photosynthesis on all dates. In all but the last sampling date (Sept 24 2013) shading significantly reduced photosynthesis in the maize systems. Figure 9 presents sample data from each cropping system taken on various dates.
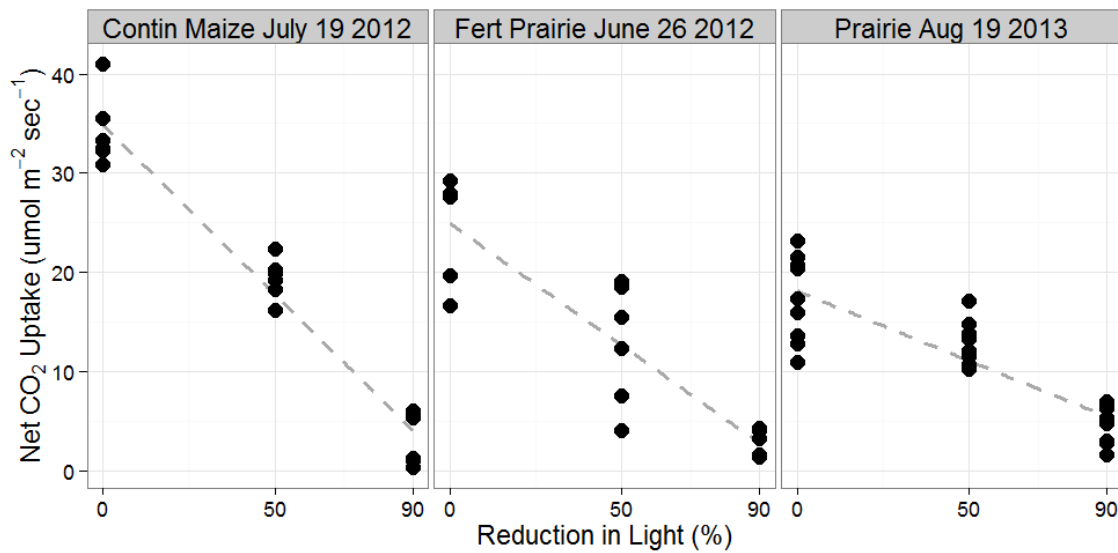

Figure 9. Decreasing available light corresponded with decreasing leaf level photosynthesis in all cropping systems

Soil Temperature and Moisture

We corrected for changes in soil temperature as described in Appendix B. The following table summarizes instances that received temperature correction.

Table 8. Summary of dates in which fluxes were adjusted to account for effect of shade on soil temperature

	P	PF	CC
2012	June 1 June 13	June 1 June 13	-
2013	June 3 June 13 June 29 July 27 Aug 1	June 13 June 18	June 13

We observed no consistent effect of shading on 5cm soil moisture, nor did we see any relationship between CO_2 flux and soil moisture.

Soil Respiration

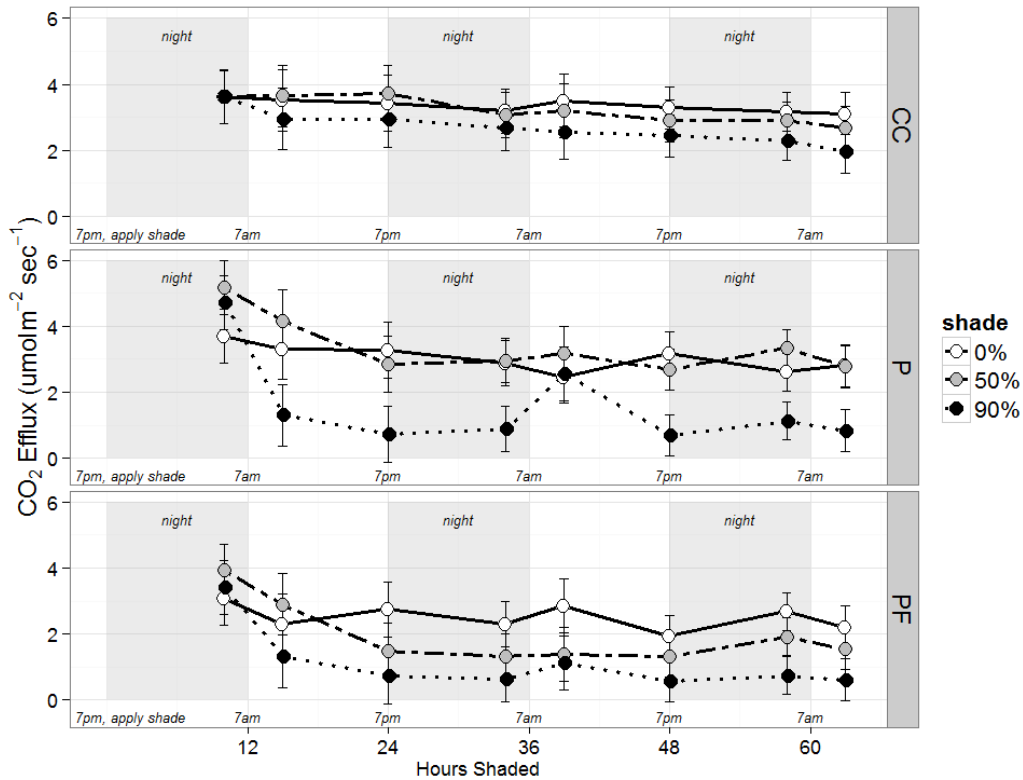The results from semi-continuous flux measurements taken July 8-10 2012 are presented in Figure 10.

Figure 10. Results from 2012 semi-continuous CO_2 flux measurements

In all three crops, the flux values after 24 hours of shading were not significantly different from those after 60.

Reduction in ambient light did not always result in a reduced surface soil CO_2 flux. The relative effect of shading (as calculated by Eqn. 7 in Appendix B at each date is presented in Figure 11.
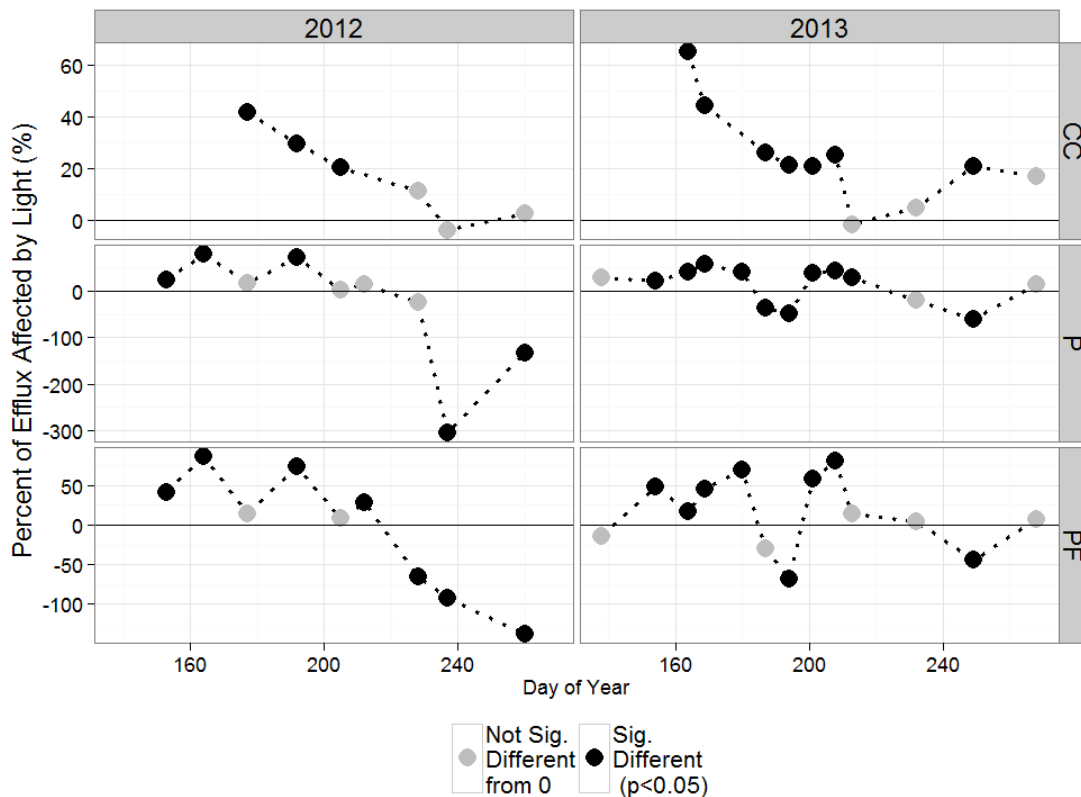
Figure 11. The effect of shading versus day of year.
Positive values represent a decrease in CO_2 flux in response to increased shading. Higher absolute values indicate a larger difference between 90% shade and non-shade fluxes. Note the different y-axis scales for each cropping system.

Partitioning

In order to compare relative contributions of root- and SOM-derived respiration, we calculated fluxes relative to the maximum observed that year. The results are presented in Figure 12.

Figure 12. Cumulative growing season fluxes relative to maximum of that year, separated into root- and SOM-derived components.
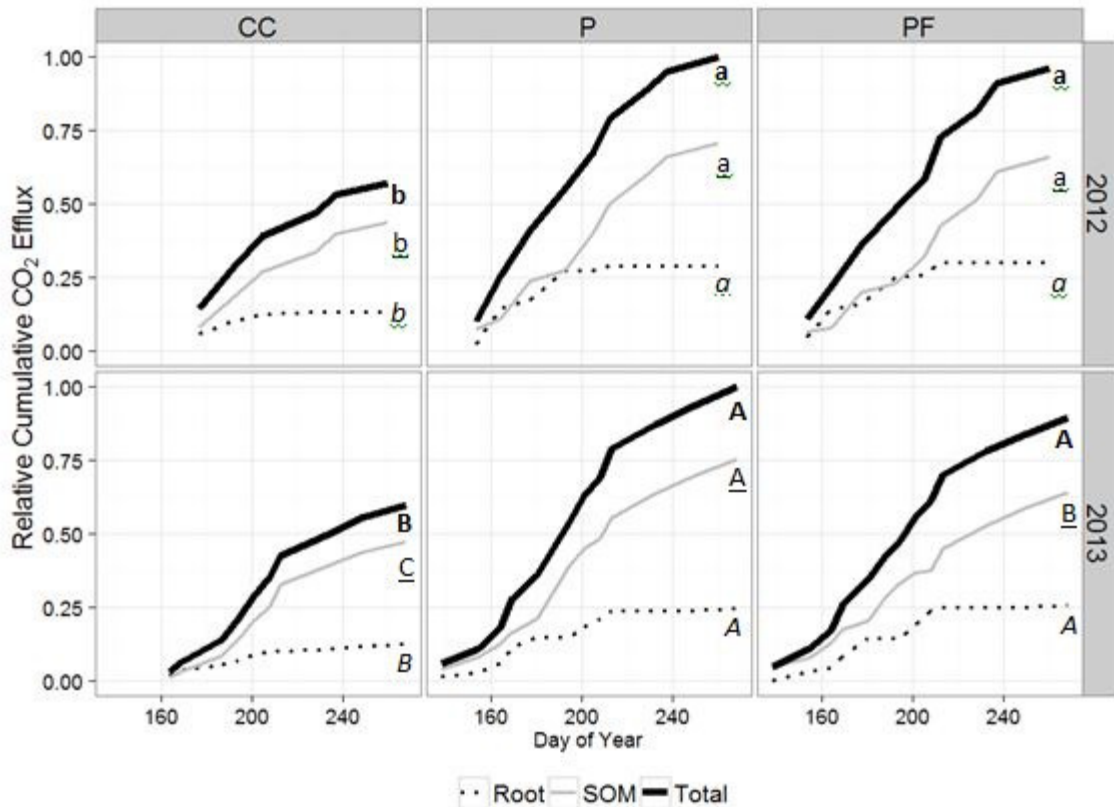Bold letters indicate within year differences between a crop's relative 'total' CO_2 emissions, underlined letters indicate differences between 'SOM-derived', and italicized letters indicate between relative 'Root-derived'. Lowercase letters are reserved for 2012, uppercase for 2013.

From May-September of both 2012 and 2013, P and PF exhibited the same relative R_{TOT}. In 2012, P and PF released the same amount of R_{SOM} over their growing season, but in 2013 P released significantly more R_{SOM} than PF ($p<0.05$). In both years, the relative R_{ROOT} fluxes were the same from P and PF. The CC has a shorter growing season as compared to the prairies, and from June-September of both years it released significantly less R_{SOM}, R_{ROOT}, and R_{TOT} compared to both P and PF ($p<0.0001$).

Each season's total root flux can be expressed as a percentage of that season's total combined (root- and SOM-derived) CO_2 flux – statistical comparisons are presented in Table 9.

Table 9. Statistical differences between the percent root contribution.
Uppercase letters indicate differences between cropping systems within a year, lowercase letters indicate differences within a cropping system between 2012 and 2013.

	2012			2013		
	CC	P	PF	CC	P	PF
Percent Root-derived (%)	[B]24[a]	[A]31[a]	[A]30[a]	[C]19[b]	[B]24[b]	[A]28[a]

CC=continuous corn, P=unfertilized prairie, PF=N-fertilized prairie

It is important to note that these values represent the percentage of the total CO_2 flux within a cropping system's growing season – the prairies having a longer growing season as compared to the maize. A significantly higher percentage of the prairie systems' (P and PF) growing season flux was root-derived as compared to CC's, averaging 7% higher over the 2 years ($p<0.0001$). In 2012, the $\%R_{ROOT}$ in P and PF were statistically equivalent, while in 2013 the $\%R_{ROOT}$ in PF was significantly higher than in P ($p<0.05$). The CC and P systems' $\%R_{ROOT}$ was significantly different in 2012 compared to 2013.

Discussion

Semi-continuous Measurements

Our semi-continuous measurements confirmed that in early July of 2012, the effects of shading were manifested in soil surface CO_2 emissions within 60 hours. Our data indicate that in both the maize and prairie systems, CO_2 flux responded to a reduction in light within 24 hours of shade application, consistent with other studies finding photosynthate transport in herbaceous plants varies from hours to days (Meharg & Killham 1988, Warembourg & Estelrich 2001, Davidson & Holbrook 2009, Kuzyakov & Gavrichkova 2010, Gomez-Casanovas et al. 2012).

Effect of Shading on Soil-Surface CO_2 Flux

Instances in which shading increased soil surface CO_2 fluxes were unexpected. To our knowledge, no studies have reported this phenomenon. It was only observed in the perennial prairie systems, and the magnitude of increase was most pronounced in the unfertilized prairie as compared to the fertilized prairie. In 2012 and 2013, the end of

season root biomass (to a depth of 1 meter) of P was 1.6 and 1.8 times larger than PF (Dietzel, unpublished data). While the total root biomass of P and PF differ, the types of roots are also different as P has been shown to have a higher percentage of grasses as compared to PF (Jarchow & Liebman 2013). Thus the response may be related to the amount of root biomass or be specific to the amount of grass root biomass. We hypothesize that it has to do in part with the complex dynamics associated with root turnover in perennial systems. Additionally, because a greater amount of both prairie systems' roots extend to greater depths than the maize system (Jarchow et al. 2014), deep soil moisture and diffusion may play a more dominant role in these systems' CO_2 emissions. The consistency of the results indicates there are biophysical reasons for this occurrence, and more research is needed to identify variables that may help to explain our observations.

Relative Root Contribution to Soil-Surface CO_2

As we expected, a higher proportion of the growing season CO_2 flux in the perennial systems was root-derived compared to the annual maize system (Table 9). Roots contributed an average of 31% and 26% of the growing season CO_2 emissions in the prairie systems in 2012 and 2013, as compared to 24% and 19% in the continuous maize. The prairie values are modest estimates for two reasons. Firstly, we assumed there was no root contribution when shading increased CO_2 fluxes - it is unlikely the root-derived flux at these times was actually zero. Secondly, there was already green biomass present in May when we began our measurements. Even with these caveats, our data indicate that within each system's respective growing season, the prairies had a higher root contribution than the annual maize system. This signifies the higher amount of respiration from the root biomass was more prominent than the increase in R_{SOM} from the roots' rhizosphere priming. We emphasize that these are growing season percentages– approximately May-September for the prairies and June-September for maize. If we had measured the maize system beginning in May, the maize's root contribution would be diluted by the SOM-derived flux of unplanted soil, resulting in a lower proportion of root-derived CO_2.

The observed differences in %R_{ROOT} within a system from year to year (Table 9), as well as the varying seasonal patterns (Figure 11) indicate that the R_{ROOT} to R_{TOT} ratio is not fixed for a given system. Each component responds differently to environmental conditions, likely on a daily or even hourly timescale. Thus, assuming there is a universal percentage that applies to, for example, prairies, will likely lead to inaccurate conclusions in carbon budgets. Utilizing biophysical-based models that have been validated using field experiments such as the one presented here may be the only way to produce accurate separation estimates.

Relationship of Root CO$_2$ to Total Root Biomass

Contrary to our hypothesis, the cumulative amount of root-derived respiration was not proportional to end-of-year root biomass. The unfertilized prairie contains nearly twice the root biomass compared to the fertilized prairie, yet the two systems produced comparable amounts of root-derived CO_2 in both years, Table 10.

Table 10. Total relative root CO_2 flux compared to end-of-season root biomass (Dietzel, unpublished data)

	2012		2013	
	Relative Root-derived	Root biomass Mg ha^{-1}	Relative Root-derived	Root biomass Mg ha^{-1}
Prairie	0.25	11.1	0.24	10.2
Fertilized Prairie	0.25	6.8	0.24	5.8
Contin. Corn	0.11	1.5	0.11	2.0

To help explain this we looked to mathematical models of respiration. Many models conceptually group root respiration into two categories: growth and maintenance respiration (Amthor 1984, Thornley & Johnson 1990, Hay & Porter 2006). The growth component is thought to be directly related to photosynthesis, while maintenance only indirectly (Hay & Porter 2006). Therefore, shading for short periods of time may only affect the growth component of root respiration. Thornley and Johnson (1990) mathematically

represent maintenance respiration as a function of the total root biomass and temperature, and growth respiration as a more complex function that includes growth rate as well as nitrogen content of roots. If our shade estimates of root-derived respiration reflect growth respiration only, it is conceivable that while the P and PF have different total root biomasses and therefore different maintenance respirations, their root growth respirations may be similar. Data from Dietzel (in preparation) indicate that in both 2010 and 2011 the unfertilized prairie, when compared to the fertilized prairie, had higher peak root growth rates but lower root nitrogen content. Since the growth component of root respiration is thought to depend on both growth rate and nitrogen content, these observations, in conjunction with Table 10, suggest P and PF could have similar root growth respirations.

The ratio of maintenance to growth respiration is complex, and changes depending on several factors (eg. plant growth stage, nutrient status, root structure). Assuming shading is only affecting the growth component of root respiration Table 9 shows that this component can contribute up to 31% of the soil's total CO_2 flux. These estimates would not include root maintenance respiration, and would therefore represent minimum values, but it is not clear how large an underestimation these values may be. Using data from Daigh (2013) and Jarchow et al. (2014) we compared May-September cumulative CO_2 emissions from the prairie, unfertilized prairie, and continuous corn to the total end-of-season root biomass of the system. The results, presented in Figure 13, demonstrate that in 2011 the total root biomass of the prairie was twice that of the fertilized prairie, however their total CO_2 emissions were similar.

If root maintenance respiration were a significant source of CO_2, season total CO_2 fluxes should reflect the differences in root biomass observed between the two prairies. This suggests root maintenance respiration plays a small role in soil-surface CO_2 fluxes. These observations, in conjunction with our data, indicate that growth respiration of roots is possibly the dominant driving force in root respiration of the perennial systems.
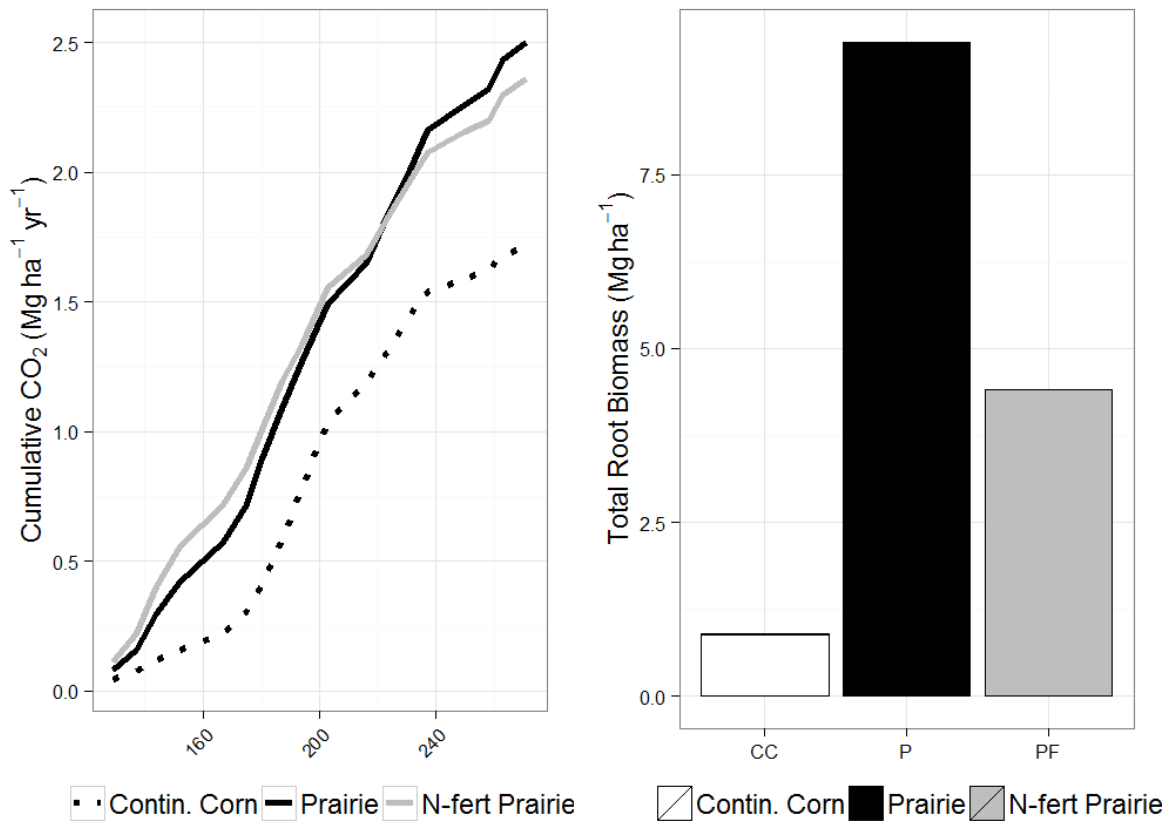
Figure 13. Comparing 2011 cumulative CO_2 emissions (Daigh 2013) and total root biomass (Jarchow et al. 2014).

Conclusion

We found that within the system's growing season, the CO_2 derived from roots constitutes a higher percentage of the soil-surface CO_2 flux in the perennial prairie versus annual maize system. Although the prairies had higher end-of-season root biomass, season-total root CO_2 fluxes were not proportional to end-of-the-year root biomass of any system. The unfertilized prairie has approximately twice the root biomass of the fertilized prairie, but within years the season cumulative root flux of each system was statistically equivalent. The shading method may only capture growth respiration of roots, but our results suggest the growth component of root respiration contributes a substantial amount to the soil-surface CO_2 flux. As such, in addition to root mass, the growth rate and nitrogen content of roots may be an important factor in modelling.

Acknowledgements

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Appendix B

Soil CO_2 flux outliers were assessed as follows: for each crop at each date, a linear model was fit using the *lm* function in R. Carbon dioxide flux was the dependent variable, with block, shade, soil temperature, and soil moisture as fixed effects. Fluxes with residuals greater than 4 were removed from the dataset. Approximately 1% of all data was removed as a result. This outlier-free data set was used for all subsequent calculations.

Correction for Shading Effects on Soil Temperature & Moisture

Shading has the potential to alter the micro-environment of the affected area. Our interest was in isolating the effect of PAR on R_{TOT}. Soil temperature and moisture have been identified as possible drivers of soil CO_2 production (Mielnick and Dugas 2000; Skopp, Jawson, and Doran 1990), and shading-induced changes in these variables could be confounded with changes in PAR. We evaluated the effect of shading on soil temperature for each crop on each date. We began by analyzing CO_2 flux versus 7cm soil temperature. As an example, data from June 24 2012 in the prairie (P) system is presented in Figure 14.
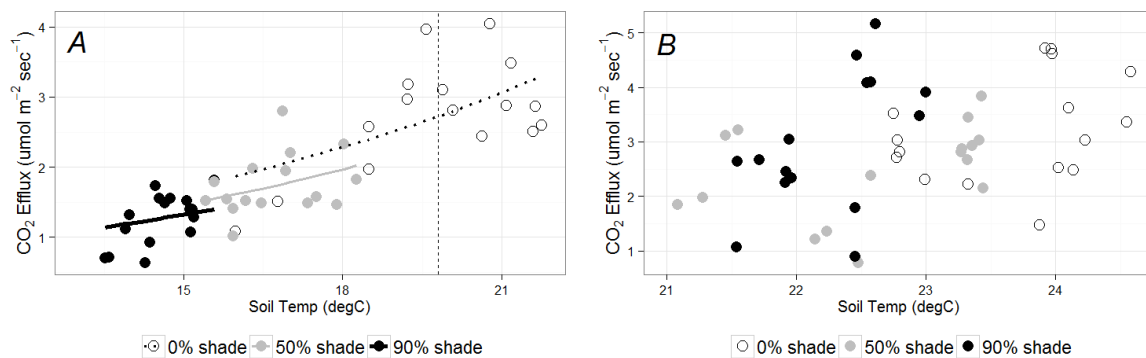

Figure 14. CO_2 flux grouped by shade versus 7cm soil temperature in P
A) June 24 2012, fluxes were adjusted to correct for effect of shading on soil temperature B) July 29 2012, no adjustments were made to fluxes

We fit an exponential function (Eqn. 5) using the non-linear least squares function in R , allowing the α parameter, the equivalent of the intercept, to vary according to shade treatment.

Eqn. 5 Exponential function fit to describe CO_2 as a function of soil temperature under each shade

$$R = \alpha \cdot e^{\beta T}$$

where

R = CO_2 flux in μmol m^{-2} sec^{-1}

T =temperature in Celsius

α = fitted parameter, unique for each shade treatment

β= fitted parameter, unique for each crop at each date, can be used to calculate a

$\quad$ Q_{10} value (Eqn. 6)

Eqn. 6 Calculating Q_{10} from fitted parameters

$$Q_{10} = e^{10\beta}$$

A Q_{10} < 1 indicates increasing soil temperature correlates with decreasing soil CO_2 flux. A Q_{10} of 1 indicates there is no relationship. We would expect a Q_{10} > 1, which indicates that with increasing soil temp, there is also in increase in soil CO_2 flux.

If the Q_{10} value was less than or equal to 1, we assumed no temperature correction was warranted for that crop on that date. If the Q_{10} was greater than 1, we used each shade's unique fitted equation (Eqn. 6) to predict that shade treatment's flux at the 0% shade treatment's mean soil temperature. An example of Figure 14A fluxes before and after temperature adjustment is presented in Table 11.

Table 11. Temperature adjustment increased flux values on June 24 2012 in the prairie system

	90% Shade	50% Shade	Full Sun
	------------------ μmol CO_2 m^{-2} sec^{-1} --------------------		
Un-adjusted	1.26	1.74	2.75
Temp adjusted	2.12	2.35	2.72

Partitioning of Soil Respiration

All partitioning was done after data was corrected for soil temperature using the methodology outlined above. At each date, each crop's CO_2 flux was plotted versus the percent reduction in ambient light (% shade). A regression line was fit to the data using the *lme* function in R. The same lme function was used to predict the flux at 0% shade (full sun), extrapolate a flux at 100% shade, and analyze the results statistically. For each crop at each date, we quantified the effect of shading on CO_2 as follows:

Eqn. 7 Calculating the magnitude and sign of CO_2 response to shading

$$\% \, \boldsymbol{Change} = \frac{CO2_{full\,sun} - CO2_{100\%\,shade}}{CO2_{full\,sun}} \times 100$$

Eqn. 7 will produce a negative value if the predicted flux under 100% shade is higher than the flux under full sun (eg. Figure 15B).

Figure 15 demonstrates partitioning. When shading resulted in a decrease in CO_2 emissions (a positive % change), the difference between the predicted flux at 0% shade and the extrapolated flux under 100% shade was assigned to root-derived (R_{ROOT}) respiration while the extrapolated CO_2 flux under 100% shade was assigned to SOM-derived (R_{SOM}), seen in Figure 15A. The flux at 0% shading always represented R_{TOT}. Instances in which shading *increased* CO_2 fluxes were assumed to have no root-derived contribution; in these cases R_{SOM} is equal to R_{TOT}, Figure 15B. Ten out of the sixty separations demonstrated a significant increase in CO_2 flux in response to shading.
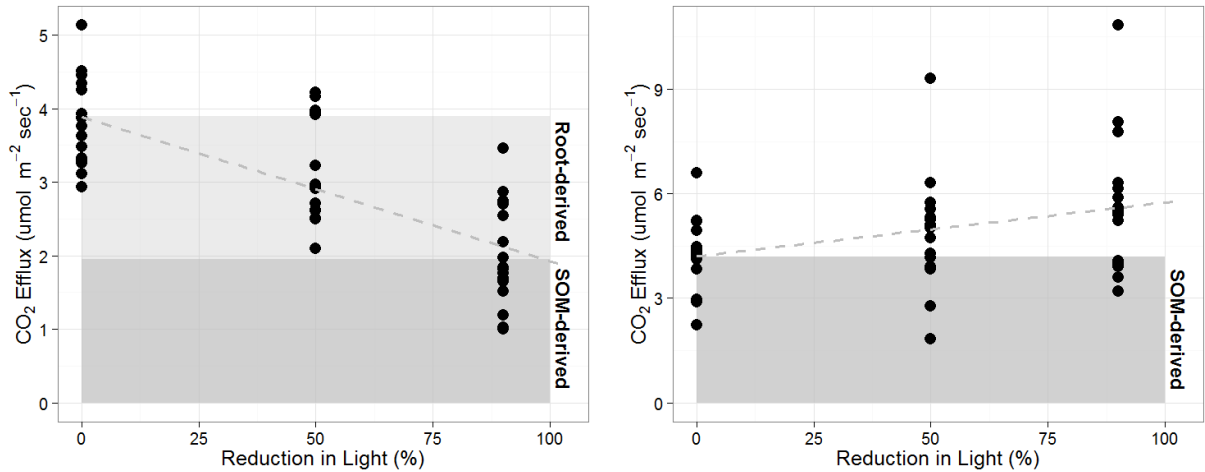
Figure 15. Total surface CO2 flux was partitioned depending on the flux's response to shading. A) Flux decreased in response to shading, resulting in a root contribution. B) Instances in which flux increased in response to shading were assumed to have no root-derived contribution.

Cumulative Values

Cumulative sums are affected by missing data. There were two instances in which we had missing data from a block (P block 4 on September 6 2013, and PF block 4 on August 1 2013). In these instances, we took the mean fluxes of the other 3 blocks and used these dummy values for that sampling period.

The total R_{ROOT} and R_{SOM} as percent of each season's cumulative R_{TOT} was calculated using Eqn. 8.

Eqn. 8 Calculating seasonal R_{ROOT} proportion of R_{TOT}

$$\left(\%_{Root}\right)_{y,c,b} = \frac{\sum_{t=1}^{t=N}(flux_{Root})_{y,c,b,t}}{\sum_{t=1}^{t=N}(flux_{Total})_{y,c,b,t}}$$

where

$\left(\%_{Root}\right)_{y,c,b}$= Percentage of the cumulative total CO_2 flux that is root-derived in

year ***y*** from crop ***c*** in block ***b*** from sampling period ***t=1*** through ***t=N(end of***

season)

$(flux_{Root})_{y,c,b,t}$ = Root-derived CO_2 flux in year y from crop c in block b at sampling

period t

$(flux_{Total})_{y,c,b,t}$ = Total CO_2 flux in year y from crop c in block b at sampling period t

The total R_{SOM} proportion was calculated in a like manner.

Season totals are sensitive to the number of times a system is sampled in a year. We sampled more often in 2013 compared to 2012, therefore raw season fluxes will appear higher in 2013. In order to compare systems between years, we scaled each component's seasonal flux by dividing by the largest season total (root plus SOM) flux observed within a year. For example, in 2012 the largest total flux was observed in the unfertilized prairie at 19.2, therefore all root, SOM, and total fluxes in 2012 were scaled to a value between 0 and 1 by dividing by 19.2. In 2013, the largest total flux was again in the unfertilized prairie at 46.9, so all 2013 fluxes were scaled relative to this maximum by dividing by 46.9.

End-of-season proportions and relative cumulative fluxes were compared using the MIXED procedure of SAS, with year, crop, and their interaction as fixed effects and block as random. Comparisons were made using PDIFF and ESTIMATE statements.

CHAPTER 4: GENERAL CONCLUSIONS

General Discussion

Both fuel production and soil sustainability are important metrics in evaluating potential biofuel cropping systems. In this study, the prairie systems met or exceeded cellulosic biomass production standards desired by Iowa bioenergy industries. When subjected to both flooding and extreme drought, the fertilized prairie produced sufficient biomass to provide the 4.48 Mg ha^{-1} of cellulosic biomass requested by industry, while in certain weather conditions both the maize and unfertilized prairie systems did not. The maximum amount of ethanol that could be produced from a unit biomass of maize stover was statistically equivalent to the amount from the prairie biomass. Fertilization reduced the prairie's ethanol conversion rate by approximately 5%. These are maximum ethanol yields, and realized ethanol yields will depend on the effectiveness of pretreatments, which may depend more heavily on biomass structure rather than composition. Overall, when comparing maize stover and prairie (fertilized or not) biomass, the system's ethanol yields depend heavily on the amount of harvested biomass, and only secondarily on the biomass composition.

Long term soil sustainability is a serious concern for biofuel systems. The soil-surface CO_2 flux is the largest output in the COBS systems' soil, and whether a system is assigned as a carbon source or sink is highly dependent on separating this output into root- and SOM-derived components (Dietzel, unpublished data). This study separated the CO_2 emissions for 2012-2013 seasons - due to the significant differences observed between years, as well as the dynamic nature of the prairie systems' roots, separation values found in this study should not be applied to previous years' carbon balances. Never-the-less, long term carbon statuses of the systems may be estimated using models, and data from this study may be useful in calibrating and informing these models. Our study, along with past years' data, indicates that the growth rate of the roots, rather than total root biomass, may be the more dominant factor contributing to the root-derived component. Therefore, when modeling

the total CO_2 flux emanating from the soil surface, root respiration resulting from root growth is an important component.

Recommendations for Future Research

COBS' forbs response to nitrogen fertilization

An experiment performed by Jarchow and Liebman (2012) found that both C3 and C4 prairie grasses' biomass production respond favorably to nitrogen fertilization, and without a significant change in composition. Legumes demonstrate no response to fertilization, but also exhibit no significant change in composition. This same study found the cell wall content of C3 grasses to be lower than either legumes or C4, and that a mixture containing one-third C3 grasses exhibited cell wall contents similar to that of the C3 monoculture. (Jarchow et al. 2012) Non-leguminous forbs were not included in the aforementioned study, yet they can contribute up to one-third of the COBS fertilized prairies' plant cover (Jarchow & Liebman 2013); it is therefore not clear whether the decrease in the COBS fertilized prairie biomass quality is due solely to an increased amount of C3 grasses, or if forbs are also contributing a lower quality biomass. Preliminary analysis of data taken in August of 2013 indicates the dominant forbs in the COBS fertilized prairie were *Ratibida pinnata*, *Monarda fistulosa*, and *Helianthus laetiflorus* (unpublished data). A small experiment designed similarly to Jarchow and Liebman's experiment (2012) could help to quantify these individual species' responses to nitrogen fertilization. Whole plots would be forb species, and subplots would be 0 or 84 kg N ha^{-1} applied in the spring (as is done in the COBS experiment). In contrast to grasses and legumes, forbs' leaf to stem ratio may change significantly in response to fertilization. The researcher should therefore separate each plots' biomass harvests into leaf and stem tissue, thus allowing the quantification of both the amount and composition of both leaf and stem biomass. The researcher could then tease out the effects of nitrogen fertilization on each component. This information would be useful in designing prairie seed mixes specifically engineered for biofuel production.

Depth of soil CO$_2$ production

The instances in which shading increased soil-surface CO$_2$ emissions call for additional investigation. Temperature and photosynthate are assumed to be major drivers in CO$_2$ production, yet under shading both of these factors are reduced. Understanding the depth at which CO$_2$ is being produced could provide insight as to why certain environmental conditions led to this increase in soil-surface CO$_2$ fluxes in response to shading. One could insert collars to a 2, 15, and 30cm depth under each shade plot and measure soil-surface CO$_2$ fluxes, as well soil temperature and moisture at these depths. In this way one could see where the CO$_2$ is being produced, and under what environmental conditions (temperature, moisture, and light availability). Coupled with knowledge of rooting depths provided by previous studies (Jarchow et al. 2014) this CO$_2$-by-depth information could strengthen, and perhaps help to quantify, the relationship between photosynthesis and root-derived respiration throughout the season.

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