BIOMASS PRODUCTION POTENTIAL, THEORETICAL ETHANOL YIELD, ENVIRONMENTAL SUSTAINABILITY OF MISCANTHUS x GIGANTEUS AND NITROGEN FERTILIZER EFFECT ON QUANTITY AND QUALITY IN FIVE LIGNOCELLULOSIC BIOMASS CROPS IN NORTH-CENTRAL US

By

Sichao Wang

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Crop and Soil Sciences – Master of Science

2017

ABSTRACT

BIOMASS PRODUCTION POTENTIAL, THEORETICAL ETHANOL YIELD, ENVIRONMENTAL SUSTAINABILITY OF MISCANTHUS x GIGANTEUS AND NITROGEN FERTILIZER EFFECT ON QUANTITY AND QUALITY IN FIVE LIGNOCELLULOSIC BIOMASS CROPS IN NORTH-CENTRAL US

By

Sichao Wang

Increasing concerns on climate change and energy security leads to growing emphasis has been placed on lignocellulosic ethanol industry in the U.S. Among many lignocellulosic feedstock crops, little information about long-term production and quality of giant miscanthus (Miscanthus × giganteus) is available in U.S. This study evaluated yield and quality parameters of giant miscanthus in southwest Michigan (KBS) and southcentral Wisconsin (ARL). An attributional life cycle assessment was also performed on giant miscanthus and switchgrass production phases by using empirical data. Nitrogen responses on yield and quality parameters were examined for five perennial bioenergy cropping systems: 1) switchgrass (Panicum virgatum L.); 2) giant miscanthus (Miscanthus × giganteus); 3) a native grass mixture (5 species); 4) an early successional field; and, 5) a restored prairie (18 species). The highest yield of miscanthus reached 22.81 ± 1.023 Mg ha^{-1} at KBS and 15.7 ± 0.898 Mg ha^{-1} at ARL. Giant miscanthus exhibited a positive yield response to nitrogen fertilization at both KBS and ARL and had the highest nitrogen fertilizer use efficiency among five cropping systems evaluated in this study. Compared to switchgrass cropping system, the giant miscanthus cropping system is more favorable in GHG emissions reduction when taking gasoline displacement credits into account. Due to higher yield, giant miscanthus had higher energy return on investment than switchgrass at both KBS and ARL.

ACKNOWLEGEMENTS

I would like to express heartfelt thanks to Dr. Kurt Thelen, for providing the opportunity to do this research project, for the guidance for my academic growth and your patience to answer my questions. I was so fortunate to be part of the Great Lakes Bioenergy Research Area 4.1 Team. Being part of this team exposes me to a diverse research environment. I would also like to thank my graduate committee members, Dr. Brian Teppen and Dr. Chris Saffron, for their guidance during my program, and inspirations from their classes.

Thank you to all the people I met along this rewarding journey- Andrew Adkins, Randy Laurenz, Bill Widdicombe, Lori Williams, Todd Martin. A big thanks to Pavani Tumbalam, you are the “mother” in the lab. Thank you for your help and company. Also, thank you to Monica Jean, for your friendship and exploring Asian food together.

Last but not least, thank you to my family and friends, for your endless love and supports during my life, especially my grandmother and my mother. My grandmother was the person who was always there for me. You are forever in my heart. My mother is the most optimistic person I have ever known. I could not have gotten through graduate school without you. I love you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

KBS	W.K. Kellogg Biological Station - Michigan State University
ARL	Arlington Research Station - University of Wisconsin-Madison
GHGs	Greenhouse gases
[Glc]	Glucose
[Xyl]	Xylose
[EtOH]	Theoretical ethanol concentration
DM	Dry matter
RFS	The Renewable Fuel Standard
EISA	Energy Independence and Security Act of 2007
LSD	Fisher`s protected least significant difference
AIC	Akaike information criterion
SOC	Soil organic carbon
GWP	Global warming potential
LCA	Life Cycle Analysis
ISO	International standard organization
LCI	Life cycle inventory
LCIA	Life cycle impact assessment
EP	Eutrophication potential
AP	Acidification potential
dLUC	Direct land use change
ΔSOC	Soil organic carbon change

EROI Energy return on invest

UAN Urea ammonium nitrate

TRACI Tool for Reduction and Assessment of Chemicals and Other Environmental Impacts

CHAPTER 1

BIOMASS PRODUCTION POTENTIAL AND THEORETICAL ETHANOL YIELD OF MISCANTHUS x GIGANTEUS

ABSTRACT

The dedicated bioenergy crop giant miscanthus (Miscanthus × giganteus) has a significant potential to provide stable feedstocks for the emerging lignocellulosic bioenergy industry. Field research was initiated in 2008 to evaluate giant miscanthus quantity and quality at two sites in the north-central US region (Michigan-KBS, 42˚23’47” N, 85˚22’26” W and Wisconsin-ARL, 43˚17’45” N, 89˚22’48” W). This study provides an evaluation of the giant miscanthus crop during a 5-year period within the crop’s production phase (2010-14). The result showed that giant miscanthus at KBS yielded from 9.21± 0.346 Mg ha^{-1} to 22.81 ± 1.023 Mg ha^{-1} with a yearly average of 16.84±5.65 Mg ha^{-1} yr^{-1}. At ARL, average dry matter yield (12.43±2.97 Mg ha^{-1} yr^{-1}) was lower than KBS with a range from 9.34 ± 1.322 Mg ha^{-1} to 15.7 ± 0.898 Mg ha^{-1}. Giant miscanthus is sensitive to water availability which led to low dry matter yield at both KBS and ARL (9.21± 0.346 Mg ha^{-1}, 9.34 ± 1.322 respectively) in the drought year of 2012. Viability during the establishment year and stand age play a critical role in the productivity of giant miscanthus. As the stand age increased, giant miscanthus showed a clear trend of increasing productivity. Fermentable sugar (glucose and xylose) levels of the giant miscanthus were analyzed from 2012-14 and did not vary significantly between KBS and ARL ([Glc]: 0.162 ± 0.0136 g g^{-1} yr^{-1} and 0.162 ± 0.0059 g g^{-1} yr^{-1}; and [Xyl]: 0.083 ± 0.0054 g g^{-1} yr^{-1} and 0.083 ± 0.0038 g g^{-1} yr^{-1} respectively). Theoretical gravitational ethanol yield also did not vary significantly between KBS and ARL (0.113 ± 0.0087 g g^{-1} yr^{-1}, 0.113 ± 0.0018 g g^{-1} yr^{-1}) respectively. KBS had the highest glucose level (0.183 ± 0.0075 g g^{-1}), xylose level (0.093± 0.0031 g g^{-1}) and theoretical gravitational ethanol yield (0.128± 0.0044 g g^{-1}) in the drought year

2012 relative to the other years. Theoretical ethanol yield on a land area basis was mainly driven by biomass quantity rather than biomass quality.

INTRODUCTION

Increasing concerns on global warming and energy security continue to drive interest in developing bioenergy technology despite recent declines in gasoline prices. Current data show that atmospheric concentrations of CO_2, one of the primary Green House Gases (GHGs), have increased by 40% since the 1800s and are now at their highest levels in at least 800,000 years (IPCC, 2013). One of the key competitive advantages of bioenergy relative to fossil fuels is the carbon neutrality of the former. Instead of releasing carbon that has been sequestered below ground for millions of years, renewable fuels recycle carbon that has been captured by the plant through photosynthesis.

A stable and reliable biomass feedstock supply is paramount to establishing a foundation of bioenergy technologies on a large scale. Generating adequate biomass production necessitates the development of novel cropping systems for bioenergy feedstocks. The Renewable Fuel Standard (RFS) established in the Energy Independence and Security Act of 2007 (EISA) boosts the total volume requirement of renewable fuel to 36 billion gallons by 2022. Among the qualifying renewable fuels, the cellulosic biofuel volume requirement is set to be no less than 16 billion gallons and as an advanced biofuel it carries with it the requirement of reducing lifecycle Green House Gases (GHGs) emissions by 60% relative to a 2005 petroleum baseline. Meanwhile, the volume requirement of "conventional" biofuel, which is the ethanol derived from corn starch, is capped at 15 billion gallons. Clearly, the U.S has shifted the focus from corn ethanol based conventional biofuel to more environmentally favorable cellulosic biofuel. This high demand for cellulosic biofuel calls for active growth of dedicated energy crops. The U.S Billion-Ton Update shows dedicated energy crops have the potential of supplying 400 million –

799 million tons of biomass out of approximately 1.1-1.6 billion dry tons' biomass provided by all possible sources by 2030 (One Billion Ton Study Update, 2011).

Giant miscanthus is a promising dedicated energy crop mainly due to its high biomass productivity and relatively lower input requirements which lead to environmental sustainability. Miscanthus is a warm season perennial grass which has a C4 photosynthetic pathway and originates from East Asia. It was introduced as an ornamental plant in Europe and North America (ANDERSON 2010). The most commonly used species of the Miscanthus genus is Miscanthus × giganteus, which is also referred to as giant miscanthus. Giant miscanthus is a naturally occurring triploid hybrid between Miscanthus × sinensis (diploid, 2n=2x=38) and Miscanthus × sacchariflorus (tetraploid, 2n=4x=76). Due to having an odd-numbered ploidy level, giant miscanthus does not bear viable seed. This prevents the offsite spread of giant miscanthus and gives the plant a very low potential to be an invasive plant (Anderson et al., 2011).

As a C4 grass, giant miscanthus has a higher nutrient and water use efficiency than C3 plants. The C4 photosynthesis pathway concentrates CO_2 in the bundle sheath cells around the rubisco enzyme to an almost saturated level. The higher CO_2 concentration suppresses photorespiration, increasing the efficiency and rate of photosynthesis relative to the C3 photosynthesis pathway. This gives C4 plants a higher N use, and water use efficiency than C3 plants under atmospheric CO_2 concentrations and high temperature conditions (Brown 1978).

Relative to other similar dedicated C4 bioenergy plants such as switchgrass, giant miscanthus has been reported to have an advantage in leaf level photosynthesis rate, leaf nitrogen use efficiency and leaf water use efficiency. Dohleman et al., (2009) found that miscanthus had a 33% higher leaf-level photosynthesis rate, significantly higher leaf nitrogen use efficiency and higher leaf water use efficiency than switchgrass.

Due to different properties of the rubisco enzyme and the amount of pyruvate phosphate dikinase (PPDK), giant miscanthus has remarkable cold tolerance which is unique for C4 grasses. Even at temperatures as low as 6 ºC, giant miscanthus still maintains active leaf photosynthesis. Therefore, giant miscanthus gains more time to grow, which leads to more biomass accumulation (Oa, et al., 2008).

Giant miscanthus has a strong deep root system and rhizomes combined with a relatively low decomposition rate that creates a strong, long term C sink (Dondini, et al., 2009). This serves the purpose of reducing net GHG emissions.

Rhizomes of giant miscanthus normally are planted during the spring. Yields are minimal in the first growing season and gradually increase through year two. Generally, giant miscanthus hits a yield plateau at the third year of growth and then maintains productivity from 15 years to 20 years (Lewandowski, et al., 2000). Harvest of giant miscanthus biomass is best conducted after the first fall frost, when natural senescence is complete, which means nutrients have been remobilized back into the root system. Beale and Long (1997) reported that N, P, K concentration of above-ground dry matter of giant miscanthus after senescence declined by 83%, 82%, 63%, respectively. Giant miscanthus is very effective in remobilizing nutrients back to below ground biomass.

Biomass Yield

Giant miscanthus has been studied in Europe for a longer timeframe than in the U.S. One model study across Ireland (52˚39' N, 07˚50' W) shows the potential biomass yield of giant miscanthus ranges between 16 and 26 Mg/ha (J C Clifton-Brown, et al., 2000). Field trials in Illinois showed that biomass yield after giant miscanthus completely senesced reached 29.6±1.8 Mg ha^{-1} (Heaton 2008)

Existing Challenges

A current challenge of giant miscanthus biomass production is cost effective propagation and profitability for farmers during the establishment period. Rhizomes are not easily planted and most farms do not have the specialized equipment needed for planting. Another potential challenge with giant miscanthus production is related to the lack of genetic variability. Most root stock available within the US are hybrid clones or at best closely related. The lack of genetic variability within the population combined with the fact that the species is not native to the Americas, makes it vulnerable to attack from disease or insect pests with little genetic base for resistance mechanisms.

Objective of Study

In order to evaluate the viability of giant miscanthus as a suitable dedicated bioenergy crop in the U.S., productivity of giant miscanthus biomass must be examined on a regional basis. The objectives of this study were 1) evaluate the productivity of giant miscanthus in the North-Central US region; 2) analyze the harvested giant miscanthus biomass for glucose and pentose content; and 3) estimate theoretical ethanol yield on a gravimetric and land-area basis from the studied giant miscanthus biomass.

MATERIALS AND METHODS

Field locations and experimental design

This study was conducted at two locations: W.K. Kellogg Biological Station in Hickory Corners, Michigan (KBS, 42˚23’47” N, 85˚22’26” W) and the Arlington Agricultural Research Station in Arlington, Wisconsin (ARL, 43˚17’45” N, 89˚22’48” W). The dominant soil series at KBS are the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (Coarse-Loamy, Mixed, Semiactive, Mesic Typic Hapludalfs) series. These soils are well drained with moderate fertility, consisting of mainly alfisols which developed on uplands under forest vegetation (Crum, J.R., 1995). The dominant soil series at ARL is Plano silt loam (Fine-Silty, Mixed, Superactive, Mesic Typic Argiudolls). These soils are well drained with relatively high fertility, consisting of mainly mollisols and developed in loess or other silty material under prairie (UW Extension 2005).

Field Experiment Design

Experimental fields at both locations were established in 2008. The experimental design for this study was a randomized complete block design with 5 blocks at both locations (Figs. 1and 2). Each plot was 27 m wide × 43 m long (0.12 ha). The previous crop at KBS was alfalfa, while ARL had corn and alfalfa as the previous crops.

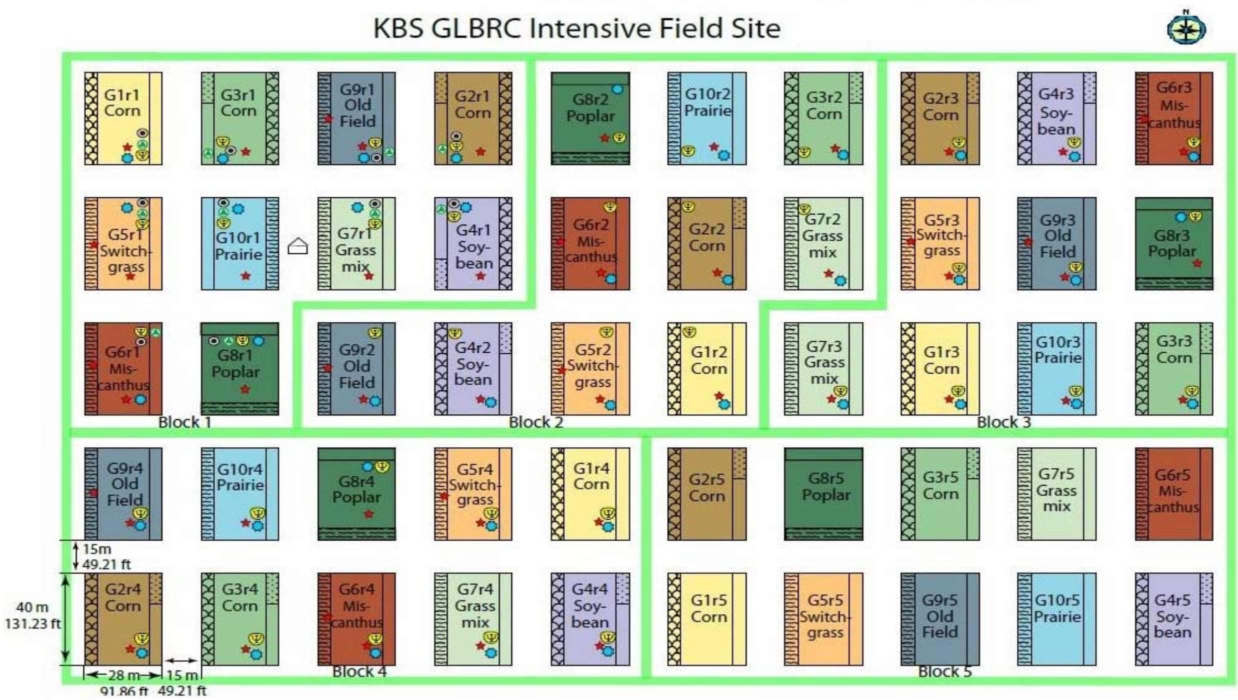

Figure 1 Schematic graph of GLBRC biofuel cropping system intensive field site at KBS.

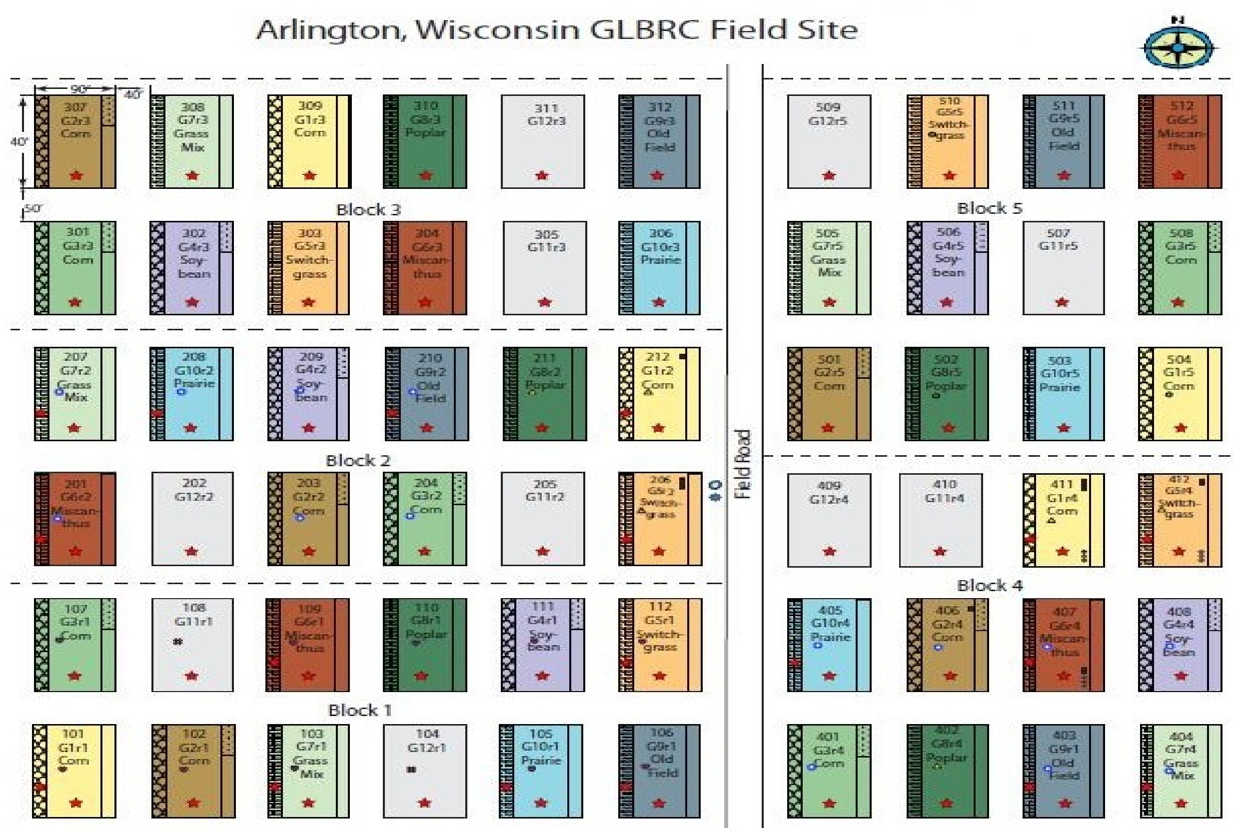

Figure 2 Schematic graph of GLBRC biofuel cropping system intensive field Site at ARL.

Establishment and agronomic management

In spring 2008, soil preparation was done at both locations by chisel plow and soil finisher. Giant miscanthus rhizomes, with one or two active growing points, were hand planted at a depth of 0.1m in late-May 2008. The rhizomes were planted on a 0.76m × 0.76m grid spacing. Agronomic practices such as the fertilizer and herbicide program were the best management practices recommended by Michigan State University (MSU) and University of Wisconsin (UW) agronomists and are detailed below.

Weed Control

During the establishment period of giant miscanthus, herbicides were applied to minimize weed competition. Quinclorac at 0.56 kg ha^{-1} was applied as post emergence weed control in mid-May 2009. 2,4-D amine herbicide at 2.24 kg ha^{-1} was applied as broadleaf weed control in mid-May 2010. Details are listed in Table 1.

Fertilization and Nutrient Management

No fertilizer was applied during establishment to reduce potential weed competition. Since 2010, 56 kg ha^{-1} applied as 28% N fertilizer was applied early to mid-May. No P and K fertilizers were applied followed recommendations from University of Wisconsin Extension and Michigan State University Soil and Plant Nutrient Laboratory based on annual fall soil sampling. Table 2 shows details of annual fertilization and nutrient management for each site.

Harvest and Biomass Yield

Giant miscanthus was harvested beginning in the fall of 2010 at KBS. Because of high mortality over winter at ARL, giant miscanthus were replanted in May 2010, resulting in postponing harvest until fall 2011. The giant miscanthus biomass harvest happened within two weeks after killing frost (-3.5˚C). At KBS, a John Deere (John Deere, Moline IL) 7350 self-propelled forage harvester equipped with a John Deere 676 Kemper cutting head was adopted for giant miscanthus biomass harvest. Plant material was chopped into a tare-weighed grain/forage truck which was reweighed to determine harvestable biomass. Cutting height of remaining plant stubble was 15.2 cm in all plots. Grab samples from each plot were placed in paper bags weighed for wet weight and placed in a drying oven at 60 °C until dry and reweighed to determine moisture content for each plot. A John Deere 7500 self-propelled forage harvester with a 600C series grass header was used at ARL. Plant material was chopped into a Miller (Art`s Way, Armstrong IA) Pro 8015 dump wagon. The dump wagon was equipped with load cells to determine bulk biomass weight. Moisture content was determined by weighing samples and placing in a drying oven at 60 °C until dry. Total dry matter yield was calculated for both locations following equation 1:

Dry Matter Yield (Mg ha^{-1}) = (1- Moisture Content in Percent) × Harvest yield   Equation 1

Theoretical Ethanol Yield Estimate

Fermentable Sugar Determination through Digestibility Platform (Enzymatic Hydrolysis)

After the harvested giant miscanthus biomass was dried, about 20-40 mg dry material was ball milled with 7/32 inch (5.56 mm) stainless steel balls (Salem Specialty Ball Co, Canton, CT) until the material became a fine powder (< 1mm). Then, a 1.5 mg subsample of biomass underwent 750 µL 0.25% (wt/vol) NaOH (62.5 mM) pretreatment solution in water bath at 90°C for 3 hours. Where necessary, reactions were neutralized with ~7.5ul 6N Hydrochloric acid. A solution containing 0.5 µL Accellerase 1000 (Genencor, Rochester, NY), 33.3ul 1 M citrate buffer (pH 4.5) plus 10ul 1% w/v sodium azide; 72nL C-Tec2 and 8nL H-tec2 enzymes were added to pretreated subsamples, then incubated for 20h in a rotisserie oven at 50°C. Next, racks were centrifuged and supernatants were transferred to 0.8 mL deep-well plates. Then, enzyme-based assay kits (Megazyme, Ireland) were used to determine glucose (Glc) and pentose (Xyl) content of samples was determined using enzyme-based assay kits. The assay kits for Glucose and Pentose were K-GLUC (Megazyme, Ireland) and K-XYLOSE (Megazyme) respectively (Santoro et al. 2010).

Theoretical ethanol yield was calculated based on the empirically derived fermentable [Glc] and Xyl levels using Equation 2:

$$([Glc] + [Xyl]) * 51.1\% * \text{metabolic yield} = \text{EtOH } (g\ g^{-1}) \qquad \text{Equation 2}$$

Where [Glc] is the glucose concentration of the biomass following pretreatment and enzymatic hydrolysis ($g\ g^{-1}$) and [Xyl] is the xylose concentration of the biomass following pretreatment and enzymatic hydrolysis ($g\ g^{-1}$). The mass conversion of fermentable sugars to ethanol is 51.1%, and metabolic yield equals to the ratio of ethanol to the consumed sugars in the fermentation process divided by 51.1% (Lau and Dale 2009).

Metabolic yield values for miscanthus (89.7%) were determined using a separate hydrolysis and fermentation (SHF) process and are derived from Jin et al., (2010). Total theoretical ethanol yield ($Mg\ ha^{-1}$) was calculated by multiplying theoretical ethanol yield from equation 2 with its corresponding dry matter yield.

Data analysis

Proc Mixed, SAS 9.4 (SAS Institute, Cary NC) was used to evaluate the effect of year and location on total giant miscanthus yield, biomass quality and theoretical ethanol yield. Different years and locations represent climatic and geological differences. Analysis of variance (ANOVA) was conducted. Year was treated as a random effect and location was considered as fixed for biomass yield. Since biomass quality data in this study were limited (3 years), both year and location were considered as fixed effects in statistics analysis for biomass quality data. The

Normality of residuals was checked with by examining their histogram relative to a normal probability plot. Homogeneity of variances was checked by looking at plot of residuals vs. predicted values and side-by-side boxplots. Levene`s test was also used to check homogeneity of variances if necessary. AIC value was the determinant of better model choice. Due to unequal variance as determined by Levene`s test, repeated measures by year was chosen as our statistical model. Multiple pair-wise comparisons among the means were conducted by using Fisher`s protected least significant difference (LSD) when fixed effect turned out to be significant (α=0.05).

RESULTS AND DISCUSSION

Climatology Data Summary

Daily air temperature and precipitation data during the study period (2008- 2014) were collected from weather stations near both field sites. The Arlington University Farm Station and, the Gull Lake Biological Station were the respective weather stations used for each site. After collecting data, all the data were summarized into monthly mean temperature and precipitation values across the growth cycle of giant miscanthus, and then compared with 30-year average climatology data.

At both locations, monthly average air temperatures did not vary significantly during the growing phase (Table 3 & 4). However, a higher monthly average temperature tendency is noticeable when compared to the 30-year average temperature. July was the hottest month during study years. Generally, the KBS monthly average air temperature was slightly higher than Arlington`s during the study period.

It is noteworthy that 2012 was the driest year during the study period and was also drier than the 30-year average at both locations, particularly during the growing season. At KBS, total precipitation during the growing phase in 2009, 2010, 2012 and 2014 was 0.4%, 4.8%, 42.7% and 10.9% respectively drier than the 30-year average. At Arlington, total precipitation during the growing phase in 2009, 2011 and 2012 was 13.4%, 22.9% and 38.8% respectively drier than 30-years average. With the exception of the drought year, June and July tended to be wetter than other months.

Table 1 Monthly mean temperatures (ºC) and precipitation (cm) during the study years (2008-14) compared to the 30-years means (1985-2014) at KBS. The 30-years averages were obtained from NOAA website.

Location	Cropping Phase	Mean Temperature (ºC)								
		Month	2008	2009	2010	2011	2012	2013	2014	30-yrs Avg.
KBS	Growth Phase	Apr	11.4	9.9	13.2	7.9	9.3	8.2	8.4	9.7
		May	14.9	15.7	16.3	16.3	16.4	17.4	14.9	15.9
		Jun	21.7	20.6	22.1	21.4	21.5	20.4	19.1	21.2
		Jul	23.1	20.2	23.8	24.8	25.9	23.0	18.1	23.0
		Aug	22.3	21.3	22.7	21.7	21.7	21.1	20.7	21.8
		Sep	18.9	17.4	18.1	16.9	16.0	18.2	16.7	17.3
	Fall Harvest	Oct	10.6	8.9	12.9	11.4	10.1	11.9	11.3	11.2
		Nov	3.7	7.3	5.5	7.2	3.9	3.8	0.9	4.8
Location	**Cropping Phase**	**Total Precipitation (cm)**								
		Month	2008	2009	2010	2011	2012	2013	2014	30-yrs Avg.
KBS	Growth Phase	Apr	6.3	14.1	7.3	13.3	10.7	21.4	6.7	9.4
		May	4.7	6.3	3.0	17.2	7.3	11.3	8.1	9.7
		Jun	15.0	15.3	20.8	5.7	4.0	10.8	14.7	10.0
		Jul	17.3	1.3	15.2	23.2	3.9	11.7	10.4	10.1
		Aug	2.1	19.9	1.8	9.8	4.9	13.6	7.4	10.5
		Sep	35.7	3.2	9.4	7.6	3.8	1.9	6.6	10.6
	Fall Harvest	Oct	9.0	12.2	4.5	9.0	14.2	5.5	9.4	9.5
		Nov	3.8	2.5	4.6	10.4	1.4	11.3	8.2	8.3

Table 2 Monthly mean temperatures (ºC) and precipitation (cm) during the study years (2008-14) compared to the 30-years means (1985-2014) at Arlington. The 30-years averages were obtained from NOAA website.

Location	Cropping Phase	Mean Temperature (ºC)								
		Month	2008	2009	2010	2011	2012	2013	2014	30-yrs Avg.
ARL	Growth Phase	Apr	7	5.9	9.1	5.2	6.4	4.1	4.9	7.7
		May	11.6	13.3	14	12	15	13.3	12.6	13.7
		Jun	18.6	17.8	18.9	18.2	19.8	17.9	19.2	19.5
		Jul	20.9	17.2	21.8	22.7	24.3	20.4	18.1	21.5
		Aug	19.3	17.8	21.4	19.9	19.4	19.2	20.6	20.2
		Sep	16.3	15.6	14.3	13.6	14.3	15.5	15.4	15.9
	Fall Harvest	Oct	8.5	5.4	10	9.7	6.8	7.9	8.9	9.2
		Nov	1.3	3.4	2.1	2.1	1.2	-0.8	-2.2	1.6

Table 2 (cont`d)

Location	Cropping Phase	Month	2008	2009	2010	2011	2012	2013	2014	30-yrs Avg.
			Total Precipitation (cm)							
ARL		Apr	18.4	11	9.3	9	7.8	13.8	16.4	8.8
		May	8	9.1	10.5	6.1	7.5	15.3	7.1	9.4
		Jun	34.7	10.8	19.3	10.4	0.7	19.1	23.7	12.1
		Jul	12.4	5.9	23.6	6.3	10.1	7.6	4.8	10.1
	Growth Phase	Aug	4.2	8.2	11.9	3.7	7.3	4.5	9.4	9.5
		Sep	3.8	6	11.5	9.8	2.6	7.5	4.5	8.9
	Fall Harvest	Oct	6	11.7	4.3	4	10.1	3.9	7	6.1
		Nov	3.4	3.3	3.6	8.3	2.8	6.7	4.4	5.7

Table 3 Total growth phase (April - September) precipitation (cm) during study years (2008-14) compared and 30-years average (1985-2014).

	Total Precipitation (cm)							
Location	2008	2009	2010	2011	2012	2013	2014	30-yrs Avg.
KBS	81.1	60.1	57.5	76.8	34.6	70.8	53.8	60.3
ARL	81.5	50.9	86.2	45.3	36.0	67.9	66.0	58.8

Dry Matter Yield

For biomass yield, the effect of location (P<0.001) was statistically significant. Average DM yield (16.84±5.65 Mg ha^{-1} yr^{-1}) from year 2010 to year 2014 at KBS outyielded average DM yield (12.43±2.97 Mg ha^{-1} yr^{-1}) at ARL. Due to a statistically significant interaction between location and year (P= 0.0422), biomass yield at KBS and ARL are presented by year in Figure 3.

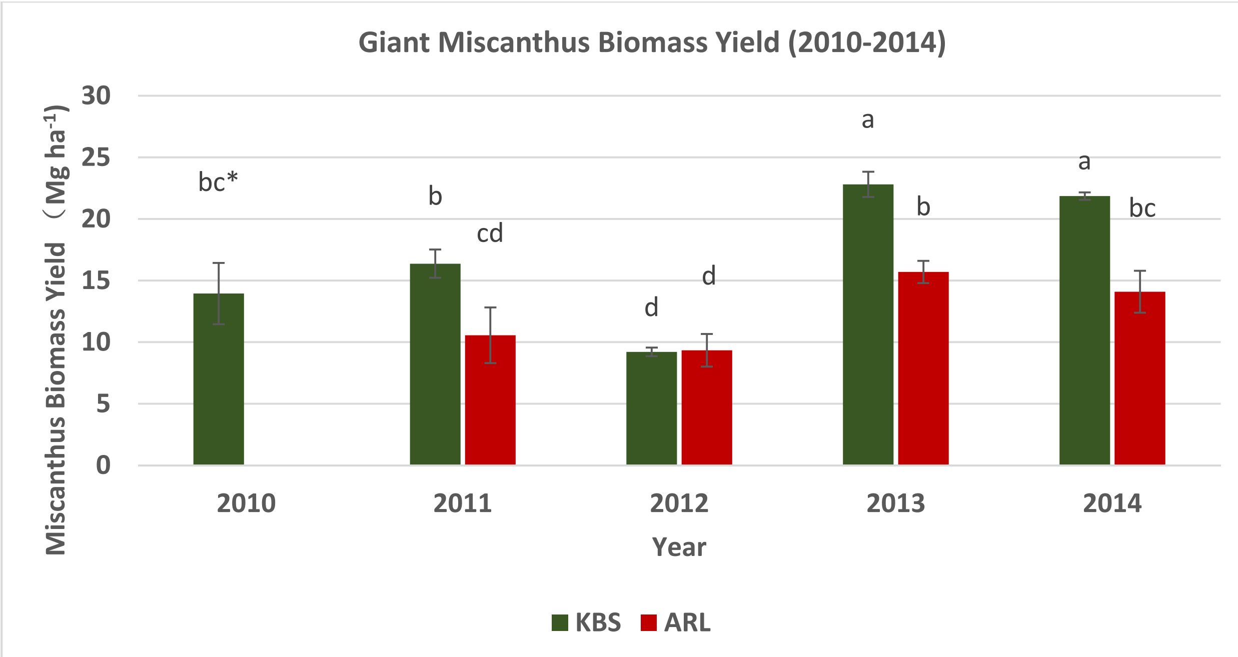

Figure 3 Giant miscanthus DM yield responses (2010-2014) at KBS and ARL. Error bars show one standard error from the giant miscanthus biomass yield mean. * Giant miscanthus DM yield with the same letter(s) are not statistically significant different (α=0.05).

Giant miscanthus DM yield ranged from 9.21± 0.346 Mg ha^{-1} to 22.81 ± 1.023 Mg ha^{-1} at KBS and giant miscanthus DM yield ranged from 9.34 ± 1.322 Mg ha^{-1} to 15.7 ± 0.898 Mg ha^{-1} at ARL, which fall into the ranges of giant miscanthus DM yield reported in studies conducted at similar latitudes (Heaton 2008; Christian, Riche, and Yates 2008; Larsen et al. 2014). Giant miscanthus DM yield in 2012 was the lowest across all study years. In 2012, DM yields at KBS (9.21± 0.346 Mg ha^{-1}) and Arlington (9.34 ± 1.322 Mg ha^{-1} yr^{-1}) were 45.3% and 24.8% lower than average DM yield (16.84 ± 2.526 Mg ha^{-1} yr^{-1} and 12.43 ± 1.487 Mg ha^{-1} yr^{-1} respectively) during the study years. Clearly, drought stress negatively impacted giant miscanthus DM yield, which agrees with other studies on giant miscanthus. (Emerson et al., 2014; Garlock et al., 2016; Lewandowski et al., 2000). Generally, higher DM yield within a given year was achieved at KBS with the exception of year 2012. The generally greater yields at KBS were not expected because of the generally less productive Alfisol soil at KBS relative to the more productive

Mollisol soil at Arlington. This can be attributed to lower precipitation in July at ARL with exception of year 2012. In the drought year of 2012, there was no significant difference between biomass yield at ARL and DM yield at KBS.

Winter kill during the first over-winter period of 2008-09 at ARL resulted in near complete loss of the giant miscanthus crop. Even though giant miscanthus has its unique cold tolerance among C4 plants, cold weather still could be lethal during establishment years (Beale, Bint, & Long, 1996; J. C. Clifton-Brown & Lewandowski, 2000; Naidu et al., 2003). After replanting in 2010, biomass at Arlington began to be harvested again in 2011. Even though biomass was harvested in 2011 at Arlington, technically miscanthus at Arlington was still in the phase of establishment at that time. The one-year older stands of giant miscanthus at KBS had a competitive advantage over Arlington in the early years of the study. Giant miscanthus stands in the study were relatively young, being successfully established in 2008 at KBS and 2010 at Arlington but the study did include production years of the stand which are generally considered to begin after the second year. Overall, stand age affects biomass productivity of giant miscanthus (Arundale et al., 2014). Nevertheless, research on long-term M. x giganteus stands of 15-20 years of age would be beneficial. Biomass yield tended to be lower at Arlington with the highest yield of 15.7 Mg ha^{-1} yr^{-1} in 2013. The highest biomass yield of 22.81 Mg ha^{-1} yr^{-1} at KBS also was achieved in 2013. Biomass yields in 2011, 2013 and 2014 were statistically significantly different between KBS and Arlington (P= 0.0081, P= 0.0015 and P=0.0006 respectively). However, there was no statistically significant difference in biomass yields between 2013 and 2014 within locations. Nonetheless, with the exception of the 2012 drought year, there was a clear trend of increasing biomass yield at both locations. Biomass yield for the first 2 years were significantly lower than the following years at both KBS and ARL.

Glucose content (g g^{-1})

Giant miscanthus biomass grown at KBS and ARL had a similar [Glc] (0.162 ± 0.0136 g g^{-1} yr^{-1} and 0.162 ± 0.0059 g g^{-1} yr^{-1} respectively). However, a significant difference due to the year effect (P=0.0071) was observed. There was also a statistically significant difference in [Glc] due to the two-way interaction between location and year (P=0.003).

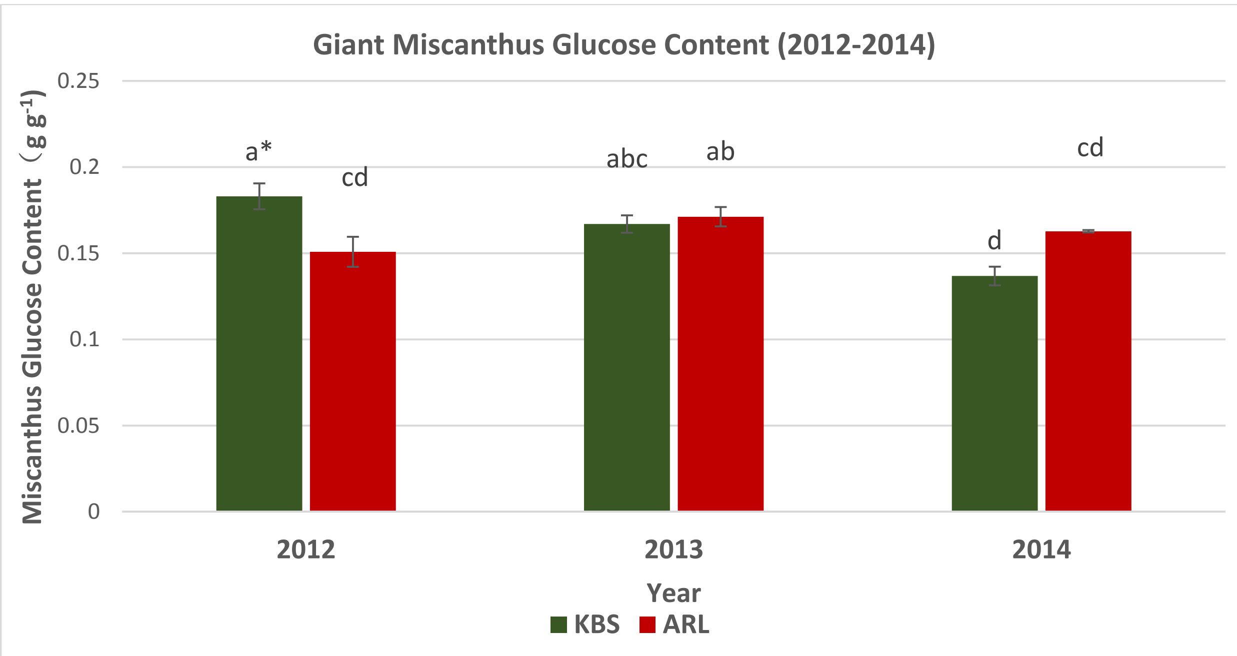

Figure 4 Glucose content of giant miscanthus in percentage (g g^{-1}) from digestibility test at KBS and ARL. Error bars show one standard error from the glucose content mean. * Glucose content means with the same letter(s) are not statistically significant different (α=0.05).

There were no significant differences between KBS and ARL in both 2013 and 2014, although giant miscanthus at ARL tended to have a numerically slightly higher [Glc] than KBS. However, the [Glc] of giant miscanthus (0.183±0.0075 g g-1) at KBS was statistically significantly higher than ARL (0.15082±0.0087 g g-1) in 2012. At KBS the drought year 2012 [Glc] (0.183±0.0075 g g-1) in giant miscanthus feedstock was significantly higher than year 2014 (0.137±0.0054 g g-1) , which is opposite with the findings of other studies(Chaves, et al., 2003; Garlock et al., 2016; Iraki, et al., 1989; Keyvan, 2010; Mostajeran,et al., 2009). In these studies, structural sugar content declined due to the increase of soluble sugars in response to drought stress. Even though year 2012 was a drought year at KBS, giant miscanthus experienced higher precipitation (14.2 cm) and lower temperature (10.1 °C) in October than the 30-year average (9.5 cm, 11.2 °C, respectively) before harvest. This late season precipitation at KBS may explain the lack of a decrease in giant miscanthus [Glc], which agrees with a study on giant miscanthus in Illinois (Emerson et al. 2014). However, a similar response was not observed at ARL.

Xylose content (g g^{-1})

Consistent with glucose content, a statistically significant difference due to year effect (P= 0.0007) was observed with regard to feedstock xylose (Xyl) levels. There was also a statistically significant difference in [Xyl] due to a two-way interaction between location and year (P=0.0011).

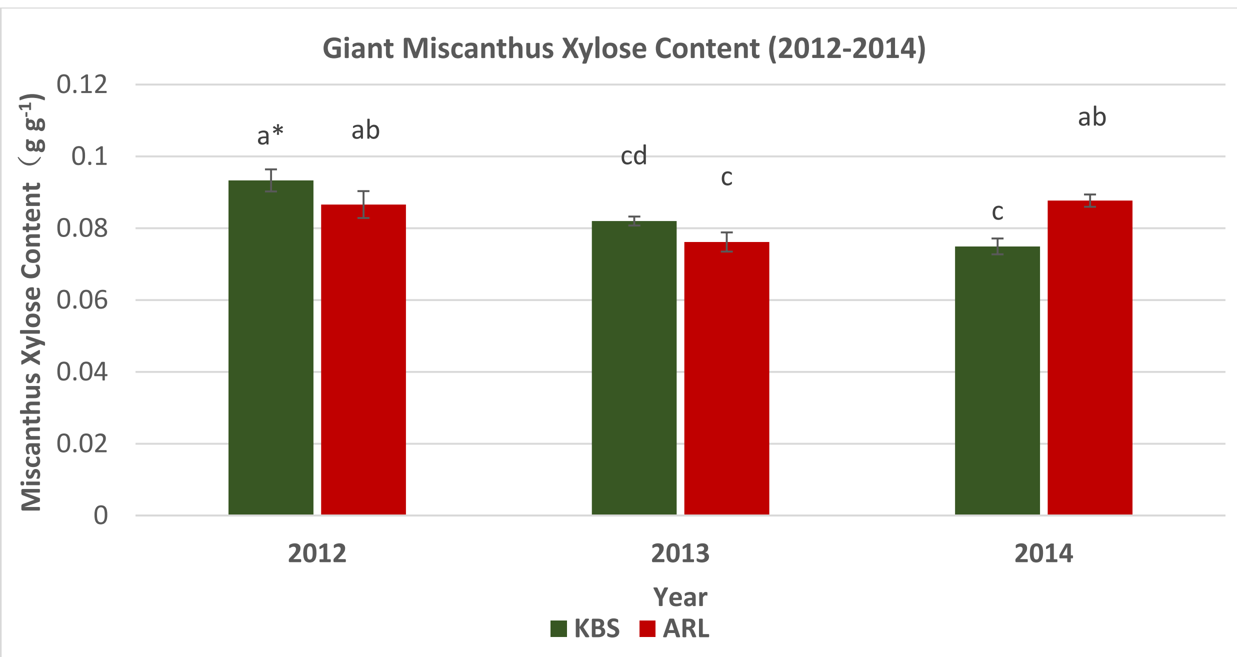

Figure 5 Xylose content of giant miscanthus (g g^{-1}) at KBS and Arlington. Error bars show one standard error from the xylose content mean. * Xylose content means with the same letter(s) are not statistically significant different (α=0.05).

There were no statistically significant differences between KBS and ARL in both 2012 and 2013. In these two years, ARL had numerically slightly lower [Xyl] than KBS. However, [Xyl] of ARL giant miscanthus (0.088±0.0017 g g^{-1}) was statistically significant higher than KBS in 2014 (0.075±0.0022 g g^{-1}). From May to September, ARL experienced higher precipitation and lower temperature, which may have led to higher [Xyl] (Emerson et al. 2014). At KBS the giant miscanthus [Xyl] was significantly higher in 2012 (0.093±0.0030 g g^{-1}) than the other two study years. Across years, [Xyl] in the giant miscanthus feedstock tended to decrease at KBS. Unlike KBS, giant miscanthus [Xyl] at ARL stayed relatively consistent across the study years with the exception of year 2013 (0.082±0.0027 g g^{-1}) which was significantly lower than year 2012 (0.087±0.0037 g g^{-1}) and year 2014 (0.088±0.0017 g g^{-1}).

Theoretical gravitational ethanol yield (g g^{-1})

Per equation 2 theoretical ethanol yield is mathematically directly correlated with biomass [Glc] and [Xyl]. KBS and ARL giant miscanthus biomass had similar theoretical ethanol yield (0.113 ± 0.0086 g g^{-1} yr^{-1} and 0.113 ± 0.0018 g g^{-1} yr^{-1} respectively). Consistent with giant miscanthus glucose and xylose levels, a significant difference in theoretical ethanol yield due to year effect (P=0.01) was observed. Additionally, an interaction between location and year was observed (P=0.0002).

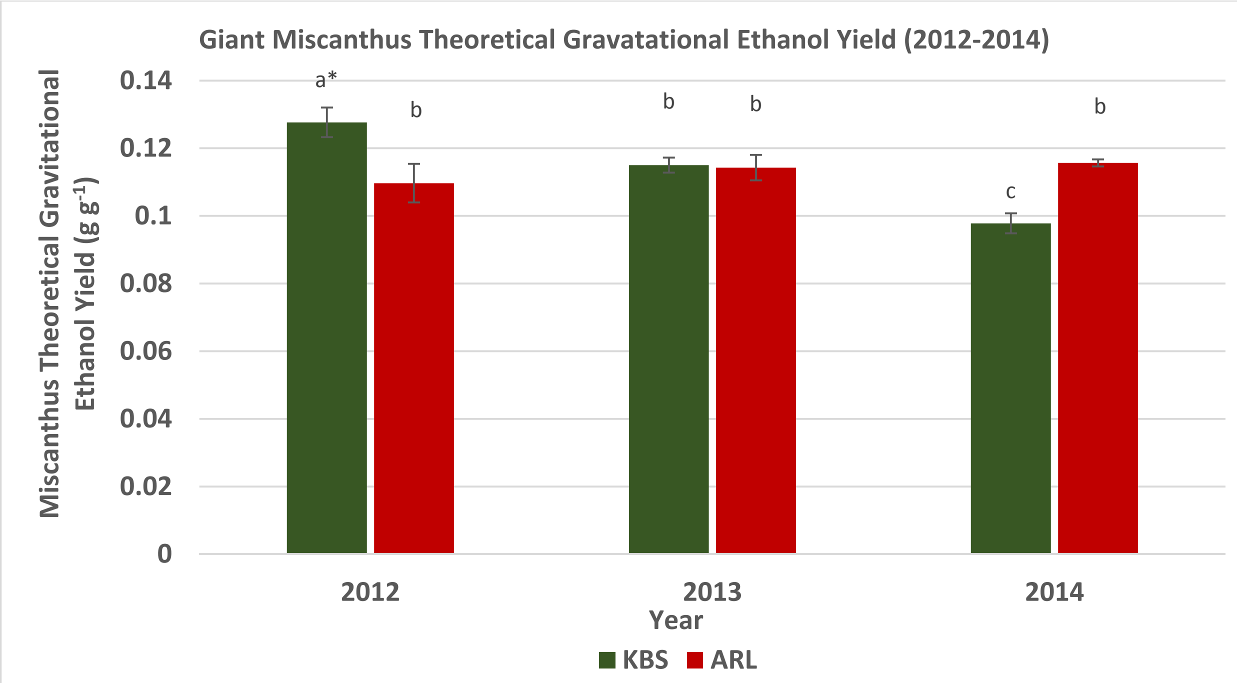

Figure 6 Theoretical gravitational ethanol yield (g g^{-1}) of giant miscanthus at KBS and ARL. Error bars show one standard error from the theoretical gravitational ethanol yield mean. * Means with the same letter(s) are not statistically significant different (α=0.05).

There was no statistically significant difference in theoretical gravitational ethanol yield (g g^{-1}) of the giant miscanthus feedstock between KBS (0.115±0.0022 g g^{-1}) and ARL (0.114±0.0038 g g^{-1}) in 2013. Theoretical gravitational ethanol yield (g g^{-1}) was highest in 2012 (0.128±0.0044 g g^{-1}) at KBS relative to other years. Across years, theoretical gravitational ethanol yield tended to decrease at KBS but remained steady at ARL.

Theoretical ethanol yield on a land area basis (Mg ha^{-1})

Similar to the other giant miscanthus crop quality parameters, a statistically significant interaction between location and year (P=0.0352) was detected. Therefore, multiple pair-wise comparisons were conducted.

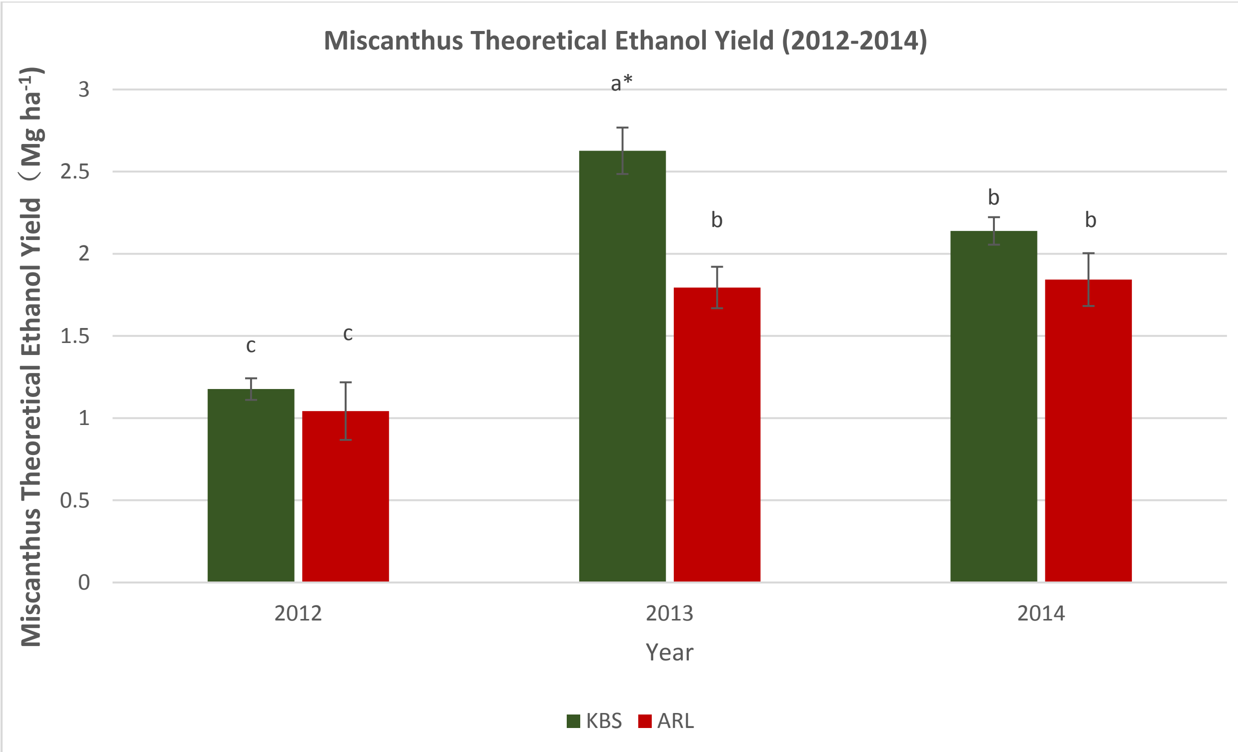

Figure 7 Theoretical ethanol yield on land area basis (Mg ha^{-1}) of giant miscanthus at KBS and ARL. Error bars show one standard error from the theoretical ethanol yield mean. * Means with the same letter(s) are not significantly different (α=0.05).

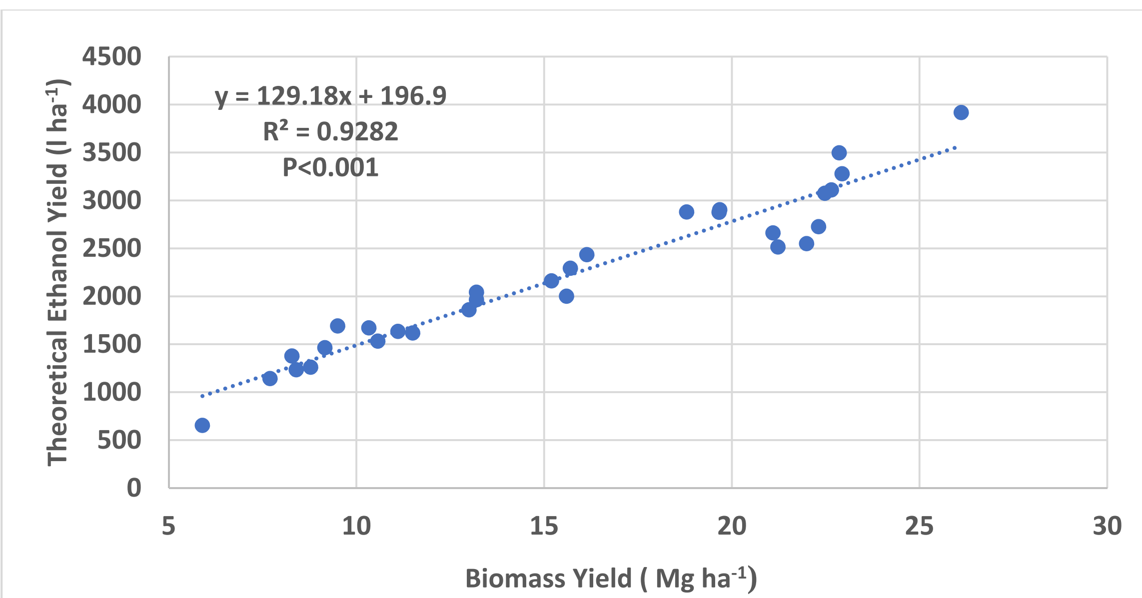

Figure 8 Correlation of giant miscanthus theoretical ethanol yield on land area basis (l ha^{-1}) with giant miscanthus biomass yield (Mg ha^{-1}) (α=0.05) at KBS and ARL during the study years from 2012-14 (observation number n=30).

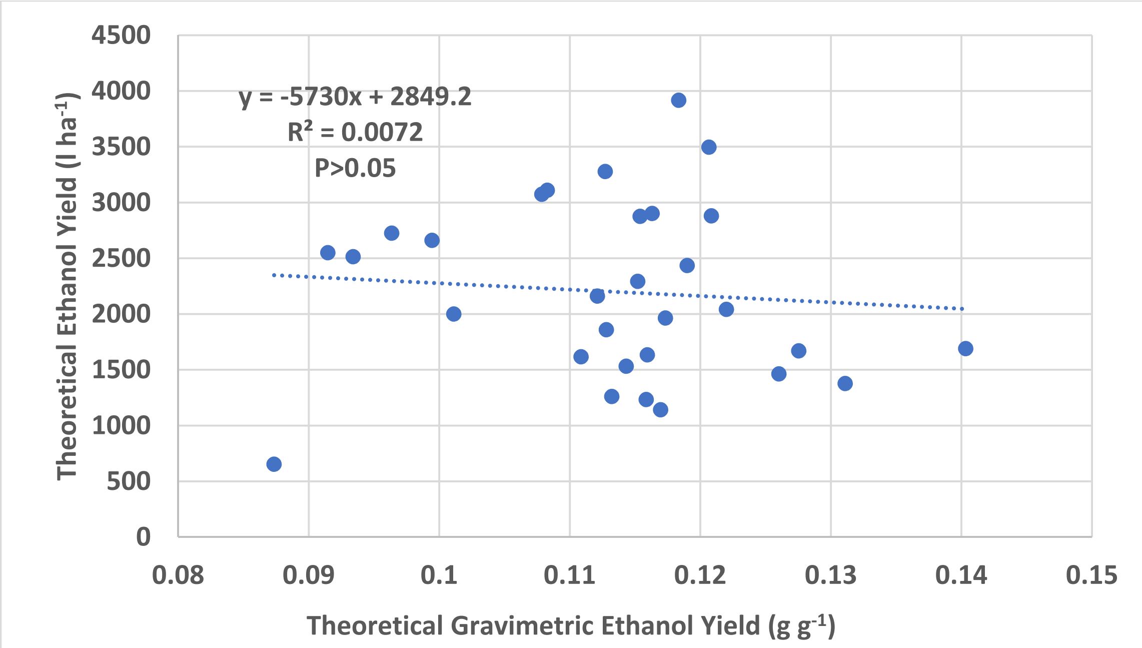

Figure 9 Correlation of giant miscanthus theoretical ethanol yield on land area basis (l ha^{-1}) and giant miscanthus theoretical gravitational ethanol yield (g g^{-1}) (α=0.05) at KBS and ARL during the study years from 2012-14 (observation number n=30).

In 2012, both KBS (1.177±0.0658 Mg ha^{-1}) and ARL (1.043±0.1751 Mg ha^{-1}) had significantly lower giant miscanthus theoretical ethanol yield on a land area basis within years and locations. However, there was no statistically significant difference in theoretical ethanol yield (Mg ha^{-1}) between KBS and ARL in 2012. The highest giant miscanthus theoretical ethanol yield was observed at KBS (2.627±0.1413 Mg ha^{-1}) in 2013. In Figure 8, and Figure 9, giant miscanthus theoretical ethanol yield on a land area basis was correlated with biomass yield (R^2=0.9282) and not theoretical ethanol yield on gravitational basis (R^2=0.0072), which indicates biomass quantity instead of quality plays a more significant role in theoretical ethanol yield on a land area basis. Jungers (2013) et al. also reported that ethanol yield on a land area basis was driven by biomass quantity rather than quality. Consistent with Sanford et al (2016), we found giant miscanthus had a biomass yield and subsequent theoretical ethanol yield potential sufficient to be competitive with other biomass crops

CONCLUSIONS

Giant miscanthus dry matter yield ranged from 9.21± 0.346 Mg ha^{-1} yr^{-1} to 22.81 ± 1.023 Mg ha^{-1} yr^{-1} with an average of 16.84 ± 2.526 Mg ha^{-1} yr^{-1} (2010-14) at KBS and giant miscanthus dry matter yield ranged from 9.34 ± 1.322 Mg ha^{-1} yr^{-1} to 15.7 ± 0.898 Mg ha^{-1} yr^{-1} with an average of 12.43 ± 1.487 Mg ha^{-1} yr^{-1} at ARL. The productivity of giant miscanthus was sensitive to water availability, as indicated by the lowest biomass yield in the drought year of 2012 at KBS and Arlington (9.21± 0.346 Mg ha^{-1} and 9.34 ± 1.322 Mg ha^{-1}, respectively) Location had little effect on [Glc] (g $g^{-1}yr^{1}$), [Xyl] (g $g^{-1}yr^{-1}$) and theoretical gravimetric ethanol yield (g $g^{-1}yr^{-1}$) for giant miscanthus biomass. Interestingly, [Glc] (g g^{-1}), [Xyl] (g g^{-1}) and theoretical gravimetric ethanol yield (g g^{-1}) of giant miscanthus biomass produced in the 2012 drought year was higher than non-drought years at the KBS location but not at ARL. Regardless of location, biomass quantity rather than quality played a larger role in theoretical ethanol yield on a land area basis (Mg ha^{-1}). Giant miscanthus had a dry matter yield and subsequent theoretical ethanol yield potential sufficient to be competitive with other biomass crops.

APPENDIX

APPENDIX

Table 4 Herbicide programs of giant miscanthus at KBS and ARL.

Location	Plot	Year	Herbicide(main ingredient)	Herbicide rate	Unit	Note
KBS	Giant Miscanthus	2009	Drive (quinclorac)	0.6	Kg/Ha	post emergence weed
		2010	2,4-D amine	0.9	Kg/Ha	broadleaf weed control
Arlington	Giant Miscanthus	2011	Roundup Power Max	1.7	Kg/Ha	Burndown
			Glyphosate (generic)	3.5	Kg/Ha	Burndown
			2,4-D LV4 Ester	1.1	Kg/Ha	Burndown
		2012	Roundup Power Max	2.9	Kg/Ha	Burndown
			Prowl	1.7	Kg/Ha	Pre-emerge
			2,4-D LV4 Ester	1.1	Kg/Ha	Post-emerge
			Clarity	1.7	Kg/Ha	Post-emerge
		2013	Prowl	0.3	Kg/Ha	Pre-emerge
			Roundup Power Max	1.5	Kg/Ha	Burndown
			2,4-D LV4 Ester	0.8	Kg/Ha	Post-emerge
			FSTransform Plus (adjuvant)	0.8	Kg/Ha	Burndown
		2014	Prowl	2.2	Kg/Ha	Pre-emerge herbicide
			2,4-D LV4 Ester	1.1	Kg/Ha	Pre-emerge herbicide
			Roundup Power Max	2.1	Kg/Ha	Pre-emerge herbicide

Table 5 Fertilizer use and rates of giant miscanthus at KBS and ARL.

Location	Plot	Year	Fertilizer	Fertilizer rate	N rate	P rate	K rate	Unit
KBS	Giant Miscanthus	2009	28% N (28-0-0)	276	77	0	0	Kg/Ha
		2010	28% N (28-0-0)	200	56	0	0	Kg/Ha
		2011	28% N (28-0-0)	200	56	0	0	Kg/Ha
		2012	28% N (28-0-0)	72	20	0	0	Kg/Ha
		2013	28% N (28-0-0)	204	57	0	0	Kg/Ha
		2014	28% N (28-0-0)	204	57	0	0	Kg/Ha
Arlington	Giant Miscanthus	2011	AMS	2	0	0	0	Kg/Ha
			Ammonium Nitrate	165	56	0	0	Kg/Ha
			28% UAN	3	1	0	0	Kg/Ha
		2012	28% UAN	168	57	0	0	Kg/Ha
			Ammonium Nitrate	168	57	0	0	Kg/Ha
		2013	Ammonium Nitrate	168	57	0	0	Kg/Ha

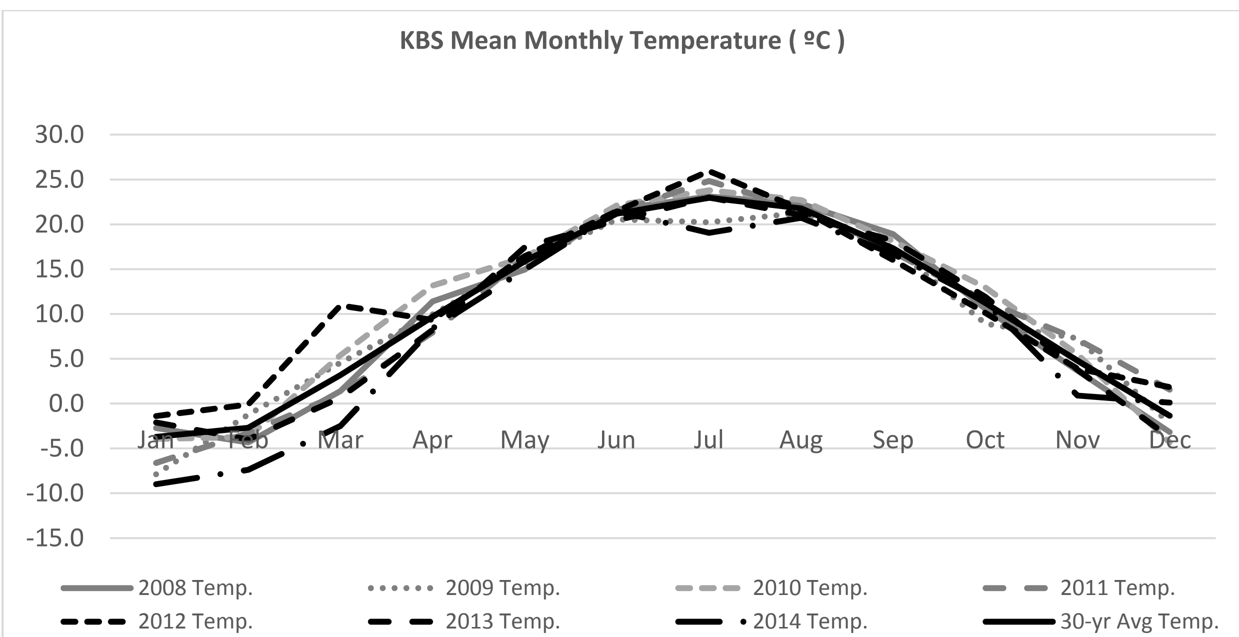

Figure 10 Yearly average air temperature (°C) (2008-14) with 30-years average air temperature (°C) (1985-2014) at KBS.

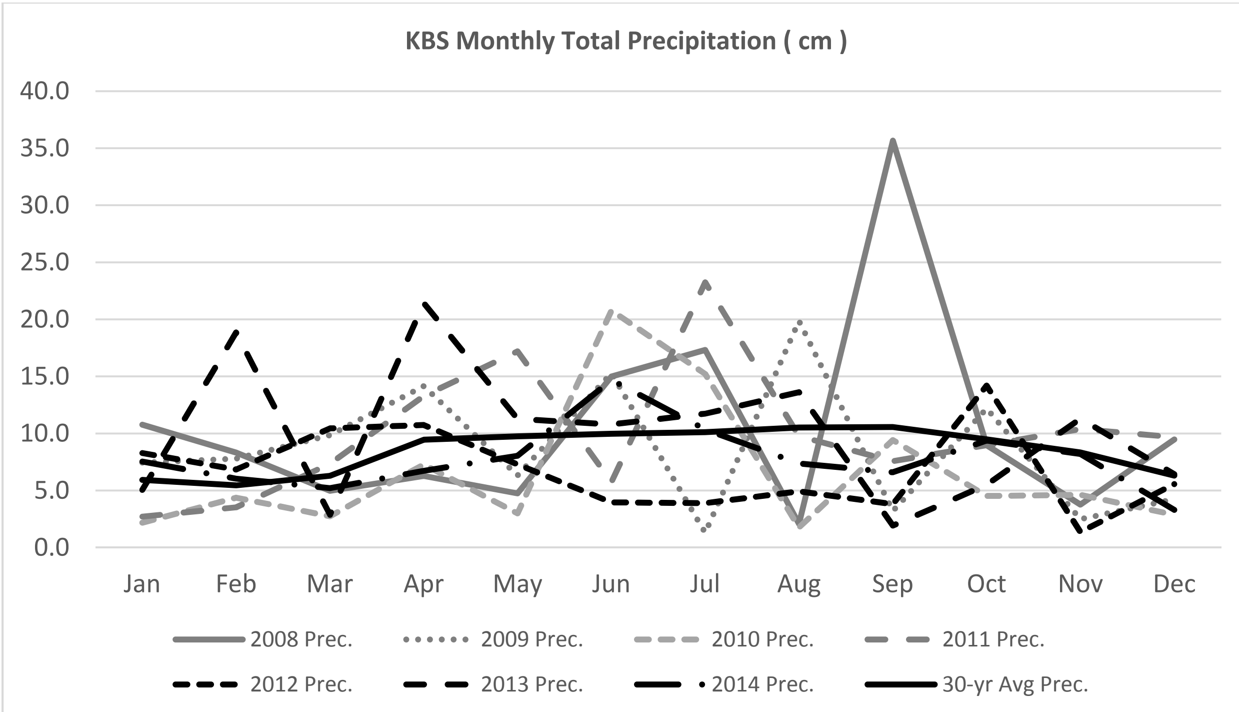

Figure 11 Yearly average precipitation (cm) (2008-14) with 30-years average precipitation (cm) (1985-2014) at KBS.

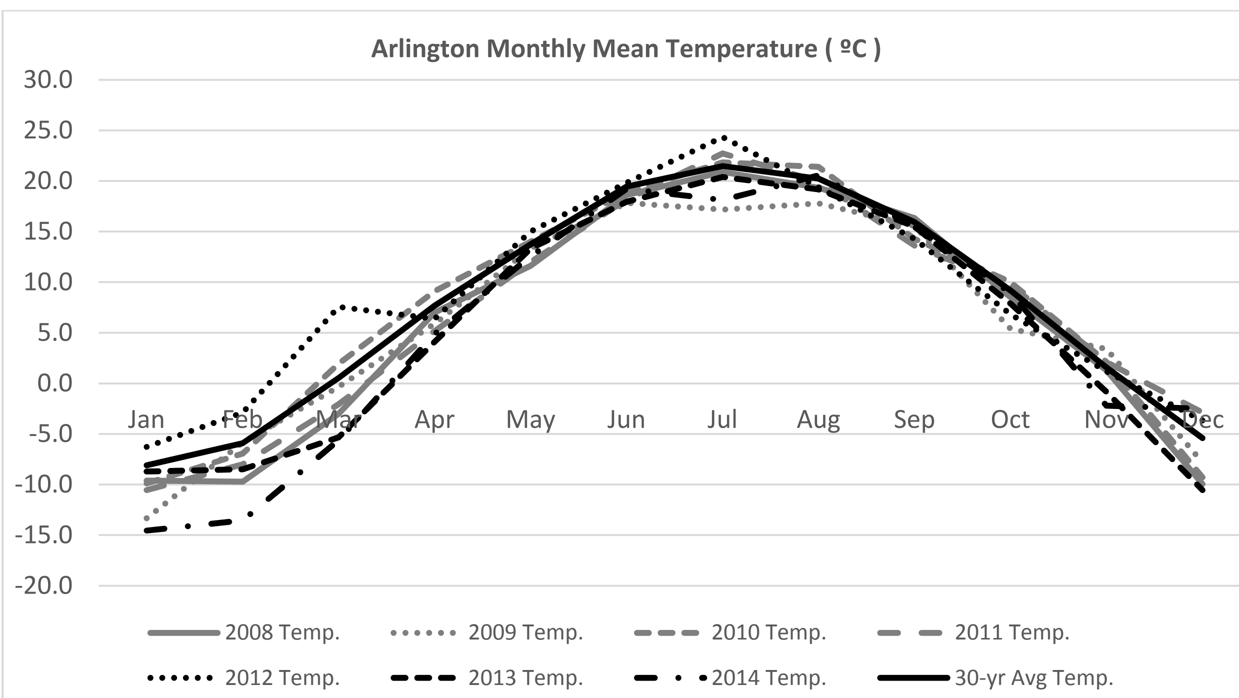

Figure 12 Yearly average air temperature (°C) (2008-14) with 30-years average air temperature (°C) (1985-2014) at ARL.

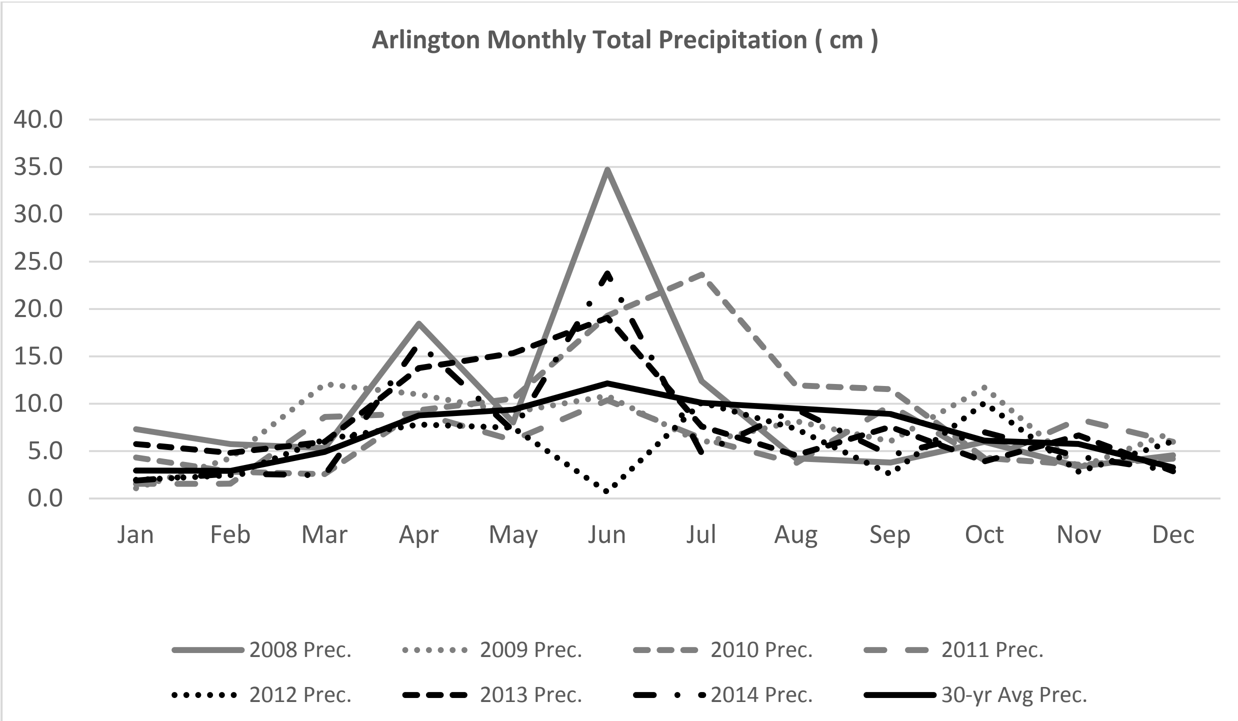

Figure 13 Yearly average precipitation (cm) (2008-14) with 30-years average precipitation (cm) (1985-2014) at ARL.

REFERENCES

REFERENCES

Anderson, Eric. 2010. "Herbicide Phytotoxicity Response and Eradication Studies."

Anderson, Eric, Rebecca Arundale, Matthew Maughan, Adebosola Oladeinde, Andrew Wycislo, Thomas Voigt, Eric Anderson, Rebecca Arundale, and Matthew Maughan. 2011. "Growth and Agronomy of Miscanthus X Giganteus for Biomass Production Growth and Agronomy of Miscanthus X Giganteus for Biomass Production." *Biofuels*, no. 2:1: 71–87.

Brown, R H. 1978. "(1978) Difference in N Use Efficiency in C3 and C4 Plants and Its Implications in Adaptation and Evolution, A" 2 (30).

Carroll, A, and C Somerville. 2009. "Cellulosic Biofuels." *Annu Rev Plant Biol* 60: 165–82. doi:10.1146/annurev.arplant.043008.092125.

Chaves, Manuela M., João P. Maroco, and João S. Pereira. 2003. "Understanding Plant Responses to Drought - From Genes to the Whole Plant." *Functional Plant Biology* 30: 239–64. doi:10.1071/FP02076.

Christian, D. G., A. B. Riche, and N. E. Yates. 2008. "Growth, Yield and Mineral Content of Miscanthus ?? Giganteus Grown as a Biofuel for 14 Successive Harvests." *Industrial Crops and Products* 28 (3): 320–27. doi:10.1016/j.indcrop.2008.02.009.

Clifton-Brown, J C, B Neilson, I Lewandowski, and M B Jones. 2000. "The Modelled Productivity Os Miscanthus." *Industrial Crops and Products* 12: 97–109.

Dondini, Marta, Astley Hastings, Gustavo Saiz, Michael B. Jones, and Pete Smith. 2009. "The Potential of Miscanthus to Sequester Carbon in Soils: Comparing Field Measurements in Carlow, Ireland to Model Predictions." *GCB Bioenergy* 1 (2009): 413–25. doi:10.1111/j.1757-1707.2010.01033.x.

Emerson, Rachel, Amber Hoover, Allison Ray, Jeffrey Lacey, Marnie Cortez, Courtney Payne, Douglas Karlen, et al. 2014. "Drought Effects on Composition and Yield for Corn Stover, Mixed Grasses, and Miscanthus as Bioenergy Feedstocks." *Biofuels* 5 (3): 275–91. doi:10.1080/17597269.2014.913904.

Garlock, Rebecca, Alan Higbee, Scott Bottoms, Quinn Dickinson, Dan Xie, Scott A Smith, Edward Pohlmann, et al. 2016. "Inhibition of Microbial Biofuel Production in Drought Stressed Switchgrass Hydrolysate," 1–15.

Heaton, Emily A. 2008. "Meeting US Biofuel Goals with Less Land : The Potential of Miscanthus," no. 2008: 2000–2014. doi:10.1111/j.1365-2486.2008.01662.x.

Heckathorn, S A, and E H Delucia. 1996. "Retranslocation of Shoot Nitrogen to Rhizomes and

Roots in Prairie Grasses May Limit Loss of N to Grazing and Fire during Drought." *Functional Ecology* 10 (3): 396–400. doi:10.2307/2390289.

Iraki, N M, R a Bressan, P M Hasegawa, and N C Carpita. 1989. "Alteration of the Physical and Chemical Structure of the Primary Cell Wall of Growth-Limited Plant Cells Adapted to Osmotic Stress." *Plant Physiol.* 91: 39–47. doi:10.1104/pp.91.1.39.

Jin, Mingjie, Ming W Lau, Venkatesh Balan, and Bruce E Dale. 2010. "Bioresource Technology Two-Step SSCF to Convert AFEX-Treated Switchgrass to Ethanol Using Commercial Enzymes and Saccharomyces Cerevisiae 424A (LNH-ST)." *Bioresource Technology* 101 (21). Elsevier Ltd: 8171–78. doi:10.1016/j.biortech.2010.06.026.

Keyvan, Shamsi. 2010. "The Effects of Drought Stress on Yield, Relative Water Content, Proline, Soluble Carbohydrates and Chlorophyll of Bread Wheat Cultivars." *Journal of Animal and Plant Sciences* 8 (3): 1051–60.

Larsen, Søren Ugilt, Uffe Jørgensen, Jens Bonderup Kjeldsen, and Poul Erik Lærke. 2014. "Long-Term Miscanthus Yields Influenced by Location, Genotype, Row Distance, Fertilization and Harvest Season." *Bioenergy Research* 7 (2): 620–35. doi:10.1007/s12155-013-9389-1.

Lau, Ming W, and Bruce E Dale. 2009. "Cellulosic Ethanol Production from AFEX-Treated Corn Stover Using Saccharomyces Cerevisiae 424A (LNH-ST)" 106 (5).

Lewandowski, I., J. C. Clifton-Brown, J. M O Scurlock, and W. Huisman. 2000. "Miscanthus: European Experience with a Novel Energy Crop." *Biomass and Bioenergy* 19 (October 2000): 209–27. doi:10.1016/S0961-9534(00)00032-5.

Lewandowski, I., and U. Schmidt. 2006. "Nitrogen, Energy and Land Use Efficiencies of Miscanthus, Reed Canary Grass and Triticale as Determined by the Boundary Line Approach." *Agriculture, Ecosystems and Environment* 112 (4): 335–46. doi:10.1016/j.agee.2005.08.003.

Mostajeran and Rahimi-Eichi. 2009. "Effects of Drought Stress on Growth and Yield of Rice (Oryza Sativa L .) Cultivars and Accumulation of Proline and Soluble Sugars in Sheath and Blades of Their Different Ages Leaves." *Cell* 5 (2): 264–72.

Oa, W, Dafu Wang, Archie R Portis Jr, Stephen P Moose, and Stephen P Long. 2008. "Cool C 4 Photosynthesis : Pyruvate P I Dikinase Expression and Activity Corresponds to the Exceptional Cold Tolerance of Carbon Assimilation in" 148 (September): 557–67. doi:10.1104/pp.108.120709.

Sanford, G. R., Oates, L. G., Jasrotia, P., Thelen, K. D., Robertson, G. P., & Jackson, R. D. (2016). Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA. *Agriculture, Ecosystems and Environment*, *216*, 344–355. http://doi.org/10.1016/j.agee.2015.10.018

Santoro, Nicholas, Shane L Cantu, Carl-erik Tornqvist, Tanya G Falbel, Jenny L Bolivar, Sara E Patterson, Markus Pauly, and Jonathan D Walton. 2010. “A High-Throughput Platform for Screening Milligram Quantities of Plant Biomass for Lignocellulose Digestibility,” 93–102. doi:10.1007/s12155-009-9074-6.

UW Extension. 2005. “Management of Wisconsin Soilsf(Fifth Edition).”

CHAPTER 2

NITROGEN FERTILIZATION RESPONSE IN FIVE BIOENERGY CROP SYSTEMS IN THE NORTH-CENTRAL UNITED STATES

ABSTRACT

The effect of nitrogen fertilization on yield and quality of five perennial bioenergy feedstocks cropping systems: 1) switchgrass (*Panicum virgatum* L.); 2) giant miscanthus (*Miscanthus* × *giganteus* Greef & Deuter ex Hodkinson & Renvoize); 3) a native grass mixture (5 species); 4) an early successional field; and, 5) a restored prairie (18 species); was investigated at two sites in the north-central US region. The study sites were located in Michigan (KBS, 42˚23'47" N, 85˚22'26" W) and Wisconsin (ARL, 43˚17'45" N, 89˚22'48" W). A randomized complete block (RCBD) with 5 replicates and split-plots was used as the experimental design. Nitrogen fertilizer at 0 kg ha^{-1} and 56 kg ha^{-1} was applied to split-plots for each cropping system since 2010. Data were collected for growing seasons from 2010-2014. No dry matter yield response to N fertilization was detected in switchgrass at either location throughout the study. Giant miscanthus exhibited a positive yield response to N fertilization at both KBS and ARL ($P=0.0003$ and $P< 0.0001$). Nitrogen fertilization effect in the polyculture treatments largely depended on which species were dominate in the cropping system with grasses being more responsive than forbs. Giant miscanthus had the highest nitrogen fertilizer use efficiency among five cropping systems evaluated in this study (KBS: 0.03347 Mg kg^{-1} N; ARL: 0.11639 Mg kg^{-1} N). Nitrogen fertilization significantly reduced glucose and xylose levels in biomass from the warm-season grass cropping systems. Total ethanol yield on land area basis (Mg ha^{-1}) was driven more by biomass quantity than quality.

INTRODUCTION

The US Energy Independence and Security Act of 2007 (EISA) mandates the production of 21 billion gallons of biofuel derived from cellulosic feedstock by 2022. Cellulosic biofuels are defined as renewable fuels derived from any cellulose, hemicellulose, or lignin, which originates from renewable biomass, and achieves a 60 percent greenhouse gas emission reduction requirement. Research interests are focused on developing perennial bioenergy cropping systems to provide the bioenergy industry a stable feedstock supply sufficient to meet the EISA mandates. The ultimate goal of renewable energy production is the combination of higher net energy yields and lower GHG emissions.

Soil Carbon Sequestration

Lowering carbon emissions is considered a high priority in most countries around the world today. Agricultural activities account for 6% of the total greenhouse gas emissions in the USA and about 20% globally (Greenhouse Gas Working Group 2010). Many studies reach a consensus that perennial cropping systems have substantially lower greenhouse gas emissions than annual cropping systems (Sainju 2016). One of the ways that perennial cropping systems reduce GHG emissions is by converting atmospheric CO_2 into soil organic carbon (SOC). Perennial cropping systems have dense root systems, which can produce carbon-based exudates. Root exudates play an important role in forming soil aggregates. Aggregates are crucial to the formation of stable, long term SOC. Some researchers have shown that perennial crops facilitate the soil's role as a C sink rather than a C source, especially in the topsoil (Fornara et al. 2016). Whether soil is a carbon sink or source depends on multiple factors, including: land use history, tillage method, fertilization strategies, etc. Among these factors, fertilization strategies seem to attract the most attention from researchers (Kane 2015).

Nitrogen Use Efficiency

Nitrogen fertilization can significantly increase biomass production, which can then lead to carbon sequestration (Jarecki and Lal 2003). However, nitrogen fertilizer production by the Haber-Bosch process is very energy-intensive and relies on fossil fuel such as natural gas. The carbon debt attributable to fertilizer manufacture natural gas use can cancel carbon gains from the increased biomass production if the N fertilization is not managed properly. According to data, agriculture has up to 60% share of global anthropogenic nitrous oxide (N_2O) emissions, which stem from direct emissions from fertilized croplands (IPCC, 2013). Walter et al., (2005) states that higher soil N_2O emissions are largely due to higher N availability in soil associated with higher N fertilizer inputs on annual crops. Compared to annual cropping systems, perennial cropping systems have relatively lower nutrient demands. However, even in perennial crop systems, soil nitrogen reserves may need to be replenished after years of growing in order to maintain a stable yield. Harvest timing for perennial cropping systems is generally after plant senescence, which ensures that most aboveground nutrients are translocated back to the root system. Several researchers have shown that about 30% of plant N can be recycled back to belowground tissue during drought and over 50% without drought (Schwartz & Amasino 2013;Heckathorn & Delucia 1996). A late-fall harvest strategy not only reduces the nitrogen fertilizer requirement needed to replenish soil, but can also mitigate the potential negative environmental impacts (nitrogen leaching and greenhouse gas emissions) excess nitrogen fertilizer brings. One study in central US estimated that low-input cropping systems reduced nitrogen leaching by 15% to 22% and greenhouse gas emissions by 29% to 473% (S. C. Davis et al. 2012).

Perennial Bioenergy Cropping Systems on Marginal Land

Increasing global populations and demand for diets higher in animal protein have increased the need to use productive land for food crop production. The relatively lower nutrient demand by perennial grass cropping systems suggests a potential for their deployment on marginal lands, not suitable for food production.

In addition to providing energy and boosting rural economies, bioenergy cropping systems also provide a variety of ecosystem services such as pest suppression and pollination. Welling et al., (2014) have shown that perennial grasses provide greater ecosystem services compared to annual crop systems. Increased placement of perennial bioenergy crop systems in marginal lands could further enhance these biodiversity functions (Werling et al. 2014; Meehan et al. 2012). Some authors have concluded that perennial crops planted on marginal lands have larger SOC sequestration rates than those planted on fertile land (Division et al. 2000; Lemus & Lal 2005). Current availability of marginal land for growing bioenergy crops is somewhat uncertain since some may currently be used as unimproved grazing land. Nonetheless, Gelfand et al., (2013) estimates that the U.S. Midwest region has the capability of providing 25.3% of the non-grain ethanol mandated by the US Energy Independence and Security Act of 2007, which equates to about 18.6 Gl yr-1.

Switchgrasses and miscanthus have been selected as designated bioenergy crops in the USA (Carroll and Somerville 2009). The response of switchgrass and giant miscanthus to N fertilizer application is still unsubstantiated (Biesiada and Koota 2008). The current available studies are not consistent with regard to recommended N rates. Some studies state that there is no significant N effect on yield (Davis et al., 2015; Kering et al., 2013), while others found that N fertilizer significantly increased biomass yield (Stout & Jung 1995; Lemus et al., 2008; Haines et al., 2015)

In order to maximize economic profitability and minimize negative environmental impact, it is imperative to examine the N fertilization effect on potential perennial bioenergy crops. This not only helps to ensure that higher N use efficiency crops are chosen but would also facilitate the adoption of best management practices for N fertilization.

In addition to monocultures of switchgrass and giant miscanthus, an early successional treatment, a native mixed grass stand and a restored prairie were also included as polyculture cropping systems in this study.

Objective of Study

This research was conducted to provide N fertilization response data on perennial bioenergy crops grown in the North-Central US. Specifically, the objectives of the study were to evaluate: 1) the N fertilization effect on the dry matter yield of several bioenergy crop systems including switchgrass, giant miscanthus, a mixed grass stand, an early successional system and a restored prairie in the North-Central US; 2) investigate the effect of N fertilization on glucose and xylose levels of the perennial cropping systems listed above 3) evaluate the effect of N fertilization on the theoretical ethanol yield of the perennial cropping systems listed above; and, 4) determine the N fertilizer use efficiency of the studied crop systems.

MATERIALS AND METHODS

Field locations and experimental design

This study was conducted at two locations: W.K. Kellogg Biological Station in Hickory Corners, Michigan (KBS, 42˚23’47” N, 85˚22’26” W) and the Arlington Agricultural Research Station in Arlington, Wisconsin (ARL, 43˚17’45” N, 89˚22’48” W). The dominant soil series at KBS are the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (Coarse-Loamy, Mixed, Semiactive, Mesic Typic Hapludalfs) series. These soils are well drained with moderate fertility, consisting of mainly alfisols which developed on uplands under forest vegetation (Crum, J.R., 1995). The dominant soil series at ARL is Plano silt loam (Fine-Silty, Mixed, Superactive, Mesic Typic Argiudolls). These soils are well drained with relatively high fertility, consisting of mainly mollisols and developed in loess or other silty material under prairie (UW Extension, 2005).

Field Experiment Design

Experimental fields at both locations were established in 2008. Five perennial cropping systems were planted including: 1) switchgrass, 2) giant miscanthus, 3) a native grass mixture, 4) an early successional field and 5) a restored prairie. In 2010, subplots with or without N fertilizer applications were added to the five perennial cropping systems. Each plot was 27 m wide × 43 m long (0.12 ha) with one 4.5 m wide × 43 m long (0.019 ha) micro plot on both east and west sides of the plot. The previous crop at KBS was alfalfa, while ARL had corn and alfalfa as previous crops.

Establishment and agronomic management

In spring 2008, soil preparation was done at both locations by chisel plow and soil finisher. Miscanthus rhizomes, with one or two active growing points, were hand planted at a depth of

0.1m in late May 2008. The rhizomes were planted on a 0.76m x 0.76m grid spacing. Perennial grass systems including switchgrass, native grasses (five species) and restored prairie (18 species) were planted by a drop spreader (Truax Company, Inc. New Hope, MN) equipped with two culti-pack rollers, in June 2008. The early successional treatment consisted of volunteer plant growth in each season, with no planting activity occurring in this treatment. Agronomic practices used such as fertilizer and herbicide programs, were the best management practices recommended by Michigan State University (MSU) and University of Wisconsin (UW) agronomists and are detailed below. Species planted for native grass cropping systems and restored prairie systems are provided in Table 6 of the appendix.

Weed Control

During the establishment period of switchgrass, giant miscanthus and native grasses, herbicides were applied to avoid weed competition. At KBS, quinclorac at 0.56 kg ha^{-1} was applied as post emergence weed control in mid-May 2009 and 2,4-D amine herbicide for broadleaf weed control at 2.24 kg ha^{-1} was applied in mid-May 2010. At ARL, glyphosate at 1.7 kg ha^{-1} was applied in the switchgrass cropping system in May 2010, and replant giant miscanthus, and native grasses in May 2011. Applications of 2,4-D LV4 ester and quinclorac were applied as pre-emerge herbicide at ARL. Details are listed in Table 7 & 8.

Fertilization and Nutrient Management

No fertilizer was applied the first two years following establishment to reduce potential weed competition. At KBS, N fertilization consisted of 56 kg N ha^{-1} applied in the form of (28-0-0) liquid ammonium urea fertilizer solution (UAN) in early to mid-May and at ARL in the form of liquid ammonium urea fertilizer solution (28-0-0) or granular ammonium nitrate (34-0-0). No P and K fertilizers were applied based on annual fall soil sampling. Table 9 & 10 show details of the nutrient management used for each year.

Harvesting Biomass

The N fertilization and no N fertilization micro-plots were harvested on the same day, within two weeks following the first killing frost of fall (-3.5 ˚C, typically late-October to mid-November). At KBS, a John Deere (John Deere, Moline IL) 7350 self-propelled forage harvester equipped with a John Deere 676 Kemper cutting head was used for biomass harvest. The harvested plant material was tare-weighed in a forage truck to determine harvestable biomass. Cutting height of remaining plant stubble was 15.2 cm in all plots. Grab samples from each plot were placed in paper bags, weighed for wet weight and placed in an air-drying oven at 60 °C until dry, and reweighed to determine moisture content for each plot. A John Deere 7500 self-propelled forage harvester with a 600C series grass header was used for harvest at ARL. The plant material was chopped into a Miller (Art`s Way, Armstrong IA) Pro 8015 dump wagon equipped with load cells to determine harvested biomass weight. Moisture content was obtained by weighing samples and placing in a drying oven at 60 °C until dry. Total dry matter yield was calculated for both locations using the following the equation 1:

Dry Matter Yield (Mg ha^{-1}) = (1- Moisture Content in Percent) × Harvest yield   Equation 1

Theoretical Ethanol Yield Estimate

Fermentable Sugar Determination (Enzymatic Hydrolysis)

After the harvested biomass was dried, about 20-40 mg dry material was ball milled with 7/32 inch (5.56 mm) stainless steel balls (Salem Specialty Ball Co, Canton, CT) until the material became a fine powder (< 1mm). Then, a 1.5 mg subsample of biomass underwent 750 µL 0.25% (wt/vol) NaOH (62.5 mM) pretreatment solution in water bath at 90°C for 3 hours. Where necessary, reactions were neutralized with ~7.5ul 6N Hydrochloric acid. A solution containing 0.5 µL Accellerase 1000 (Genencor, Rochester, NY), 33.3ul 1 M citrate buffer (pH 4.5) plus 10ul 1% w/v sodium azide; 72nL C-Tec2 and 8nL H-tec2 enzymes were added to pretreated subsamples, then incubated for 20h in a rotisserie oven at 50°C. Next, racks were centrifuged and supernatants were transferred to 0.8 mL deep-well plates. Then, enzyme-based assay kits (Megazyme, Ireland) were used to determine glucose (Glc) and pentose (Xyl) content of samples was determined using enzyme-based assay kits. The assay kits for Glucose and Pentose were K-GLUC (Megazyme, Ireland) and K-XYLOSE (Megazyme) respectively(Santoro et al., 2010). Theoretical ethanol yield was calculated based on the empirically derived fermentable Glc and Xyl levels using Equation 2:

$$([Glc] + [Xyl]) * 51.1\% * \text{metabolic yield} = [EtOH]\ (g\ g^{-1}) \qquad \text{Equation 2}$$

Where [Glc] is the glucose concentration of the biomass following pretreatment and enzymatic hydrolysis ($g\ g^{-1}$) and [Xyl] is the xylose concentration of the biomass following pretreatment and enzymatic hydrolysis ($g\ g^{-1}$). The mass conversion of fermentable sugars to ethanol is 51.1%, and metabolic yield equals to the ratio of ethanol to the consumed sugars in the fermentation process divided by 51.1% (Lau & Dale, 2009).

Metabolic yield values for miscanthus (89.7%) were determined using a separate hydrolysis and fermentation (SHF) process and are derived from Jin et al., (2010). Total theoretical ethanol yield ($Mg\ ha^{-1}$) was calculated by multiplying theoretical ethanol yield from equation [2] with its corresponding dry matter yield.

Nitrogen Fertilizer Use Efficiency

Nitrogen Fertilizer Use Efficiency is defined as the ratio of biomass yield gain to nitrogen fertilizer applied. The calculation is shown in equation 3.

$$\text{N fertilizer Use Efficiency} = \frac{Biomass\ Yield\ with\ N\ app - Biomass\ Yield\ without\ N\ app}{N\ rate} \qquad \text{Equation 3}$$

Data analysis

Proc Mixed of SAS 9.4 (SAS 2012, SAS Institute Inc., Cary, NC, USA) was used to evaluate the effect of nitrogen, bioenergy cropping system and location on total biomass yield, biomass quality and theoretical ethanol yield. Different years and locations represent climatic and geological differences. Analysis of variance (ANOVA) was conducted. Year was treated as a random factor and the bioenergy cropping system was the whole plot factor with (+/-) nitrogen as the subplot factor. Block in this study was considered a random effect nested in location and year. Normality of residuals was checked by examining histogram and normal probability plots. Homogeneity of variances was checked by examining a plot of residuals vs. predicted values and side-by-side boxplots. Levene`s test was also used to check homogeneity if necessary. AIC value was the determinant of better model choice. N effects at each year were detected by Fisher`s protected least significant difference (LSD). (α=0.05)

RESULT AND DISCUSSION

Climatology Data Summary

Daily air temperature and precipitation data during the study period (2010-2014) were collected from stations nearest to both field sites. The Arlington University Farm Station and, the Kellogg Biological Station (Gull Lake) were the respective weather stations used for each site. After collecting data, all the data were summarized into monthly mean temperature and precipitation across growth cycle of five perennial cropping systems under this study, then compared with 30-year climatology data. Extreme weather events at both KBS and ARL delayed the establishment of some of the perennial cropping systems. At ARL, giant miscanthus was not established until 2010 due to extreme temperatures over the 2008/2009 winter. Similarly, at KBS, the switchgrass, native grass, and restored prairie systems were spot-reseeded in 2009 following extreme precipitation during the 2008 growing season. A full discussion of weather related establishment details (2008 and 2009) can be found in Sanford et al. (2016). At both locations, monthly average air temperatures did not vary significantly during the growing phase (Table 6 & 7). However, a higher monthly average temperature tendency is noticeable when compared to 30-year average temperatures. July was the hottest month during study years. Generally, KBS monthly average air temperature was higher than Arlington`s during the study period.

It is noteworthy that 2012 was the driest year during the study period and also was drier than the 30-year average at both locations. At KBS, total precipitation during the growing phase in 2010, 2012 and 2014 was 4.8%, 42.7% and 10.9% drier, respectively, than the 30-year average. At Arlington, total precipitation during the growing phase in 2011 and 2012, was 22.9% and 38.8% drier, respectively, than the 30-year average. With the exception of the drought year (2012), June and July tended to be wetter than other months.

Table 6 Monthly mean temperatures (ºC) and precipitation (cm) during the study years compared to the 30-years means (1985-2014) at KBS, MI. The 30-years averages were obtained from NOAA website.

Location	Cropping Phase	Mean Temperature (ºC)						
		Month	2010	2011	2012	2013	2014	30-yrs Avg.
KBS	Growth Phase	Apr	13.2	7.9	9.3	8.2	8.4	9.7
		May	16.3	16.3	16.4	17.4	14.9	15.9
		Jun	22.1	21.4	21.5	20.4	21.4	21.2
		Jul	23.8	24.8	25.9	23.0	19.1	23.0
		Aug	22.7	21.7	21.7	21.1	20.7	21.8
		Sep	18.1	16.9	16.0	18.2	16.7	17.3
	Fall Harvest	Oct	12.9	11.4	10.1	11.9	11.3	11.2
		Nov	5.5	7.2	3.9	3.8	0.9	4.8
	Over-Winter Phase	Dec	-4.3	1.6	1.8	-3.8	0.1	-1.4
		Jan	-3.9	-6.6	-1.4	-2.1	-9.0	-3.7
		Feb	-3.7	-3.3	-0.1	-4.0	-7.4	-2.7
		Mar	5.4	1.1	10.9	0.7	-2.5	3.2

Location	Cropping Phase	Total Precipitation (cm)						
		Month	2010	2011	2012	2013	2014	30-yrs Avg.
KBS	Growth Phase	Apr	7.3	13.3	10.7	21.4	6.7	9.4
		May	3.0	17.2	7.3	11.3	8.1	9.7
		Jun	20.8	5.7	4.0	10.8	14.7	10.0
		Jul	15.2	23.2	3.9	11.7	10.4	10.1
		Aug	1.8	9.8	4.9	13.6	7.4	10.5
		Sep	9.4	7.6	3.8	1.9	6.6	10.6
	Fall Harvest	Oct	4.5	9.0	14.2	5.5	9.4	9.5
		Nov	4.6	10.4	1.4	11.3	8.2	8.3
	Over-Winter Phase	Dec	2.9	9.7	5.6	6.4	3.3	6.3
		Jan	2.2	2.7	8.3	5.1	7.5	5.9
		Feb	4.4	3.5	6.8	18.8	6.0	5.5
		Mar	2.7	7.3	10.4	2.9	5.2	6.3

Table 7 Monthly mean temperatures (ºC) and precipitation (cm) during the study years compared to the 30-years means (1985-2014) at Arlington, WI. The 30-years averages were obtained from NOAA website.

Location	Cropping Phase	Mean Temperature (C)						
		Month	2010	2011	2012	2013	2014	30-yrs Avg.
ARL	Growth Phase	Apr	9.1	5.2	6.4	4.1	4.9	7.7
		May	14.0	12.0	15.0	13.3	12.6	13.7
		Jun	18.9	18.2	19.8	17.9	19.2	19.5
		Jul	21.8	22.7	24.3	20.4	18.1	21.5
		Aug	21.4	19.9	19.4	19.2	20.6	20.2
		Sep	14.3	13.6	14.3	15.5	15.4	15.9
	Fall Harvest	Oct	10.0	9.7	6.8	7.9	8.9	9.2
		Nov	2.1	2.1	1.2	-0.8	-2.2	1.6
	Over-Winter Phase	Dec	-9.3	-2.9	-3.7	-10.6	-2.5	-5.4
		Jan	-9.9	-10.6	-6.3	-8.7	-14.6	-8.1
		Feb	-6.9	-8.0	-2.9	-8.5	-13.6	-5.9
		Mar	1.9	-2.1	7.6	-5.3	-5.6	0.5
Location	**Cropping Phase**	**Total Precipitation (cm)**						
		Month	**2010**	**2011**	**2012**	**2013**	**2014**	**30-yrs Avg.**
ARL	Growth Phase	Apr	9.3	9.0	7.8	13.8	16.4	8.8
		May	10.5	6.1	7.5	15.3	7.1	9.4
		Jun	19.3	10.4	0.7	19.1	23.7	12.1
		Jul	23.6	6.3	10.1	7.6	4.8	10.1
		Aug	11.9	3.7	7.3	4.5	9.4	9.5
		Sep	11.5	9.8	2.6	7.5	4.5	8.9
	Fall Harvest	Oct	4.3	4.0	10.1	3.9	7.0	6.1
		Nov	3.6	8.3	2.8	6.7	4.4	5.7
	Over-Winter Phase	Dec	4.2	6.0	6.0	2.9	2.9	3.3
		Jan	4.3	1.5	2.0	5.7	1.9	2.9
		Feb	2.8	1.5	2.4	4.8	2.6	2.9
		Mar	2.6	8.6	6.2	6.0	2.4	4.9

Table 8 Total growth phase (April - September) precipitation (cm) during study years (2010-14) compared and 30-years average (1985-2014).

	Total Precipitation (cm)					
Location	2010	2011	2012	2013	2014	30-yrs Avg.
KBS	57.5	76.8	34.6	70.8	53.8	60.3
ARL	86.2	45.3	36	67.9	66	58.8

Nitrogen Effect on sugar content and ethanol content

There were few interannual differences of N effect on biomass quality [Glc], [Xyl] and [EtOH] and the differences that were observed did not appear to follow a particular pattern (figure 14). Therefore this study focused on [Glc], [Xyl] and [EtOH] for each cropping system averaged across the studied years.

The interactions between nitrogen fertilization, location and cropping system were not significant on [Glc], [Xyl] and [EtOH] ($P= 0.7516$, $P=0.2313$ and $P=0.6028$, respectively). Strong interactions between cropping system and nitrogen were significant on [Glc], [Xyl] and [EtOH] ($P<0.0001$, $P<0.0001$ and $P<0.0001$, respectively). The significant cropping system × nitrogen fertilization effect on [Glc] was due to the significant negative N responses of native grasses and restored prairie across both locations (-0.01586 g g^{-1}, $P<0.0001$ and -0.01002 g g^{-1}, $P=0.0013$, respectively). The ranking in magnitude of biomass [Glc] reduction in response to N fertilization (descending order) (Table 9) at KBS was 1) native grasses, 2) restored prairie, 3) switchgrass, 4) giant miscanthus, and 5) early successional. ARL had a similar ranking of N responses on [Glc] with the exception that the early successional cropping system up to third in the order ahead of switchgrass and giant miscanthus. The differences of the ranking of N responses on [Glc] was due to higher grass: forb ratio (3.9) of early successional at ARL compared to grass: forb ratio (1.6) at KBS. Song et al., (2011) found that grasses were more responsive to nitrogen fertilizer compared to forbs. Grasses generally have a higher sugar content than forbs (R. J. Garlock et al. 2012). A higher grass: forb ratio in a mixed biomass feedstock can lead to a higher sugar content (Sanford et al. 2017). Structural sugar content can be distinguished by plant species and the maturity of plants.

N fertilization also had a negative effect on biomass [Xyl] in five of six cropping system/location combinations that exhibited a significant N fertilization effect, with the early successional system at ARL being the lone exception. The ranking in magnitude of biomass [Xyl] reduction in response to N fertilization (descending order) at KBS was 1) native grasses, 2) giant miscanthus, 3) switchgrass, 4) early successional and 5) restored prairie. At ARL, the ranking was 1) early successional, 2) switchgrass, 3) giant miscanthus, 4) restored prairie and 5) native grasses. Similar to [Glc], the difference in rank of the early successional system was due to differences in the grass: forb ratio at each respective site. Biomass [EtOH] is dependent upon biomass [Glc] and [Xyl] and as expected, the ranking in magnitude of biomass [EtOH] reduction in response to N fertilization (descending order) followed a similar pattern. At KBS the [EtOH] ranking was 1) native grasses, 2) switchgrass, 3) restored prairie, 4) giant miscanthus and 5) early successional. At ARL, the ranking was 1) early successional, 2) native grasses, 3) restored prairie, 4) switchgrass and 5) giant miscanthus. In figure 16 and 17 of the appendix, the dependency of biomass [EtOH] to [Glc] and [Xyl] is shown using regression. The results show[Glc] having an R^2= 0.9186 and [Xyl] having R^2= 0.8096. Glucose being the dominant monosaccharide in structural plant biomass sugars has also been reported by others (Dien et al. 2006). Negative N responses on [Glc] and [Xyl] cropping system biomass levels can be explained by increasing lignin content attributable to N fertilization (Murozuka et al. 2014). Several other studies also have shown that the lignin content of grasses may be increased by N fertilization (Waramit, Moore, and Heaton 2014; Allison et al. 2012). Dien et al. (2006) stated that glucose content is inversely correlated with lignin content and maturity of plants. Cross-linking between lignin and hemicellulose or pectin reduces the accessibility of enzyme to cell wall constituents which leads to lower [Glc] and [Xyl] from saccharification (Sorek et al. 2014). Another possible reason of negative responses to N fertilization on [Glc] and [Xyl] can be related to an N induced lower leaf to stem ratio. Cruz et al., (2000) found that nitrogen fertilizer reduced leaf to stem ratio of temperate and tropical perennial forage grasses.

However, research on five different energy grass species concluded that nitrogen fertilizer had no effect on cellulose levels and lignin levels of the energy grasses (Adamovics et al. 2016).

Table 9 N responses of averaged glucose content [Glc] (g g-1 yr-1), xylose content [Xyl] (g g-1 yr-1) and ethanol content [EtOH] (g g-1 yr-1) of five cropping systems at KBS and ARL across 2012-2014. N responses are subtraction of averaged [Glc], [Xyl], [EtOH] without N fertilization from the corresponding values with N fertilization. † N effect is significant at α=0.05.

Location	Cropping System	[Glc] ($g\ g^{-1}\ yr^{-1}$)	Pr > F†	[Xyl] ($g\ g^{-1}\ yr^{-1}$)	Pr > F	[EtOH] ($g\ g^{-1}\ yr^{-1}$)	Pr > F
KBS	Switchgrass	-0.00941	0.0322	-0.00787	0.0033	-0.00799	0.0063
	Giant Miscanthus	-0.00427	0.3285	-0.0062	0.02	-0.00484	0.0956
	Native Grasses	-0.02218	<.0001	-0.01078	<.0001	-0.01523	<.0001
	Early Successional	0.00369	0.3975	0.00199	0.4505	0.00263	0.3635
	Restored Prairie	-0.01225	0.0056	-0.00107	0.6862	-0.00615	0.0345
ARL	Switchgrass	-0.0003	0.9451	-0.00654	0.0142	-0.00316	0.2747
	Giant Miscanthus	0.00007	0.9878	-0.00629	0.0182	-0.00288	0.3198
	Native Grasses	-0.00954	0.03	-0.00313	0.2373	-0.00585	0.0442
	Early Successional	0.00595	0.174	0.00823	0.0022	0.00655	0.0245
	Restored Prairie	-0.00779	0.0755	-0.00375	0.1572	-0.00533	0.0664

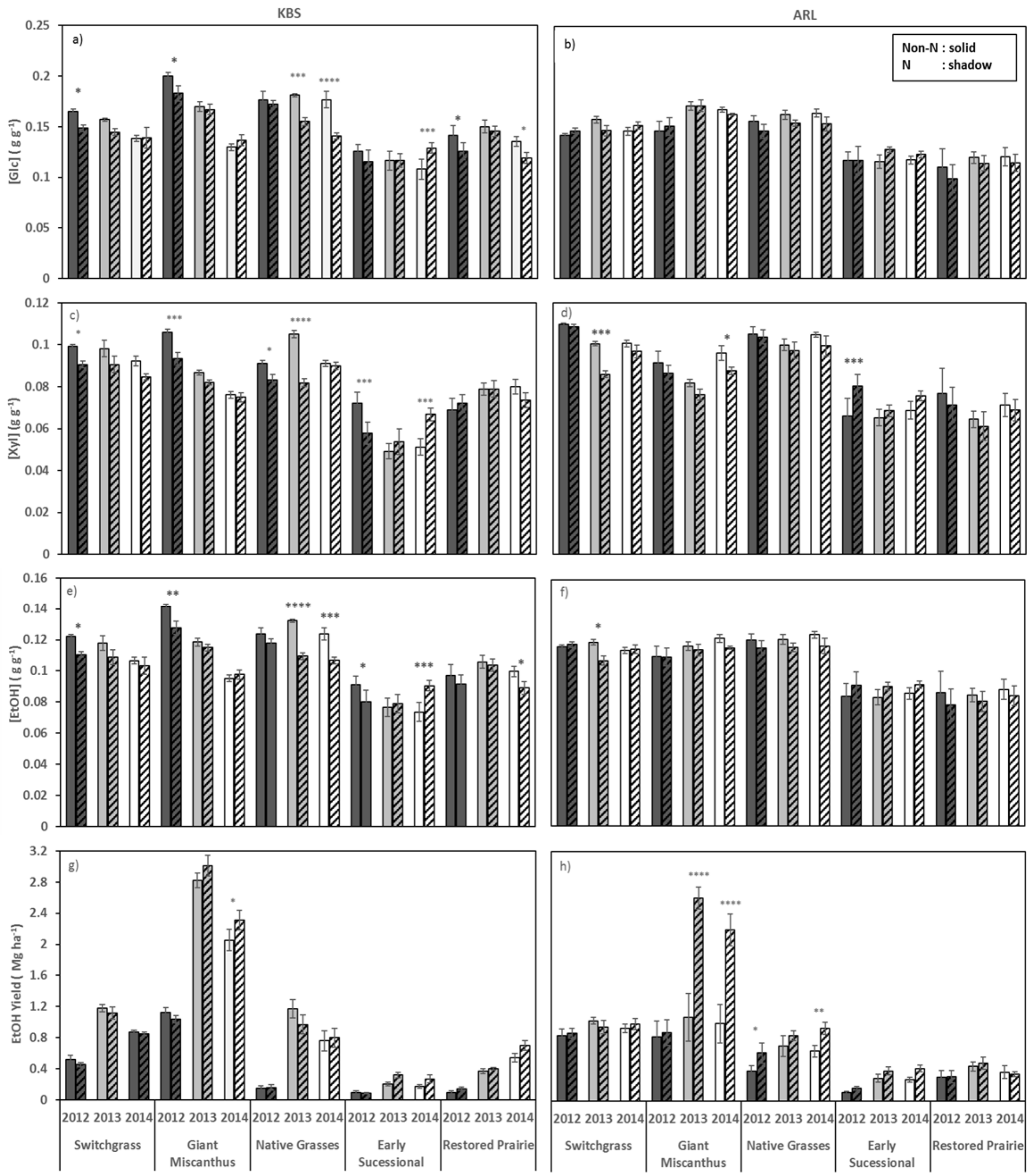

Figure 14 N effects on glucose content [Glc] (g g^{-1}), xylose content [Xyl] (g g^{-1}) and theoretical ethanol concentration [EtOH] (g g^{-1}) and ethanol yield (Mg h^{-1}) during study period (2012-14) at KBS and ARL. Error bars show one standard error from mean. * (α=0.05); ** (α=0.01); *** (α=0.005); **** (α=0.0001).

Nitrogen Effect on Biomass Yield and Ethanol Yield on land areal basis

Some interannual differences of N effect on DM yield (figure 15) were observed in this study. Interestingly, most of the N effect on biomass DM yield appeared in the later years of this study, which was likely caused by nitrogen depletion along years in the plots without nitrogen fertilization. There was only one nitrogen fertilization effect on DM found during the drought year of 2012, which was in native grasses at ARL. Water limitations can restrain N fertilization effects on biomass yield, which suggests that, if forecastable, N fertilizers should not be applied to giant miscanthus under dry growing conditions (Ercoli et al. 1999).

The three-way interaction between nitrogen fertilization, location and cropping system on DM yield was significant, ($P<.0001$) due primarily to different N effects on the DM yield of native grasses at KBS and ARL. At KBS, DM yield of native grasses did not respond to nitrogen fertilization. However, at ARL, DM yield of native grasses with nitrogen fertilization (5.85±0.39 Mg ha^{-1} yr^{-1}) was significantly higher than DM yield of native grasses without nitrogen fertilization (4.38±0.35 Mg ha^{-1} yr^{-1}). Giant miscanthus had significant positive N responses at both KBS and ARL (1.8745 Mg ha^{-1} yr^{-1} and 6.518 Mg ha^{-1} yr^{-1}, respectively), which agrees with Miguez et al. (2008) claiming that miscanthus responded to N fertilization in the post-establishment phase or production phase. The ranking of the magnitude of N responses on DM yield at KBS was 1) giant miscanthus, 2) restored prairie, 3) early successional, 4) native grasses and 5) switchgrass. At ARL, the ranking of N responses on DM was 1) giant miscanthus, 2) native grasses, 3) early successional, 4) restored prairie and 5) switchgrass. Giant miscanthus had the most significant N response on DM yield and switchgrass had the least response among the five cropping systems at both KBS and ARL. Several studies in the US have shown that switchgrass did not respond to N fertilization at rates between 33-224 kg N ha-1 (Mulkey et al., 2006; Keene 2014). Research on switchgrass in southern Iowa has shown that N fertilization improved yields, with the magnitude of the effect declining as N rate increased (Lemus et al. 2008). The lack of a switchgrass yield N response may have been due to sufficient N available to switchgrass by mineralization of soil organic matter in the short-term, coupled with the specie's apparent inherent ability to utilize available soil N. Even though perennial grass systems such as switchgrass can obtain nitrogen through symbiotic relationships with AMF and residual N left by previous crops, outsource nitrogen may still be needed over time to replenish the soil N levels (Van Der Heijden et al. 2006). The duration of this study was sufficient to evaluate N response of the studied crop systems during the establishment and early production phases. However, a longer term evaluation is necessary to determine whether systems may become more responsive to N fertilization over time.

For the polyculture cropping systems, the grass:forb ratio was higher in the restored prairie (3.5:1) than early successional (1.6:1) at KBS (Sanford et al. 2017). Grasses have been found to be more responsive than forbs to N fertilization (Song et al. 2011). The greater responsiveness of grasses to N fertilization may explain the greater N response on DM yield of the restored prairie relative to early successional at KBS. Similarly, the greater N response on DM yield of early successional relative to restored prairie was due to higher grass:forb ratio of early successional (3.9:1) compared to restored prairie at ARL (1.1:1). DM yield across cropping systems tended to increase over time with N fertilization and remain stable or decline over time without N fertilization. Researchers found that N fertilizer additions reduced plant species diversity in grasslands (Borge et al. 2004; Song et al. 2011). Tilman et al.concluded that low input high diversity (LIHD) polycultures had 238% more biomass yield than monocultures, like switchgrass (Tilman, Hill, and Lehman 2006). Therefore, if N fertilization induced reductions in species occur and a polyculture yield advantage as reported by Tilman et al., exists, a long-term N fertilization program could theoretically reduce the productivity of the system. An ideal nitrogen fertilization practice is to synchronize application timing with the need of crop (Dawson, Huggins, and Jones 2008; Kitchen, Goulding, and Shanahan 2008). Identifying optimal application strategies was beyond the scope of this study. Nevertheless, split nitrogen fertilization strategies have been shown to boost biomass yield (Brockman 1971). Overall, nitrogen fertilization effect on biomass yield is a function of multiple factors, like location, precipitation and harvest time. Therefore, N fertilization programs should be tailored to specific regional conditions.

Different nitrogen fertilization effects on EtOH yield on a land area basis of giant miscanthus and native grasses between KBS and ARL led to the significant three-way interaction between nitrogen fertilization, location and cropping system ($P < .0001$). There were no significant N fertilization effects on theoretical ethanol yield between the five cropping systems at KBS. At ARL, EtOH yield of giant miscanthus (1.890±0.217 Mg ha-1 yr-1) and native grasses (0.793±0.063 Mg ha^{-1} yr^{-1}) with N fertilization was significantly higher than without N fertilization (0.960±0.141 Mg ha^{-1} yr^{-1} and 0.576±0.063 Mg ha^{-1} yr^{-1} respectively).

Rankings of N responses on EtOH yield were exactly the same as the ranking of N responses on DM. This is supported by the conclusion of Sanford et al., 2017 that EtOH yield on a land area basis was driven more by feedstock quantity than feedstock quality.

Table 10 N responses of averaged biomass dry matter (DM) yield (Mg ha^{-1} yr^{-1}), ethanol yield ((Mg ha^{-1} yr^{-1}) and nitrogen use efficiency (Mg kg N^{-1}) of five cropping systems at KBS and ARL across 2010-2014. N responses are subtraction of DM, EtOH yield and N use efficiency from corresponding values with N fertilization. † N effect is significant at α=0.05.

Location	Cropping System	DM (Mg ha^{-1} yr^{-1})	Pr > F†	EtOH yield (Mg ha^{-1} yr^{-1})	Pr > F	Mg kg N^{-1}	Pr > F
KBS	Switchgrass	0.01535	0.9759	-0.05029	0.5356	0.00027	0.5356
	Giant Miscanthus	1.8745	0.0003	0.12246	0.1328	0.03347	0.1328
	Native Grasses	0.35689	0.4823	-0.05081	0.5314	0.00637	0.5314
	Early Successional	0.73997	0.1459	0.06752	0.4058	0.01321	0.4058
	Restored Prairie	0.91487	0.0725	0.0796	0.3274	0.01634	0.3274
ARL	Switchgrass	-0.01042	0.9494	0.00357	0.9649	-0.00019	0.9649
	Giant Miscanthus	6.518	<.0001	0.93072	<.0001	0.11639	<0.0001
	Native Grasses	1.4656	0.0042	0.21679	0.0083	0.02617	0.0083
	Early Successional	0.74217	0.1535	0.0963	0.2364	0.01325	0.2364
	Restored Prairie	0.375	0.4445	0.00871	0.9145	0.0067	0.9145

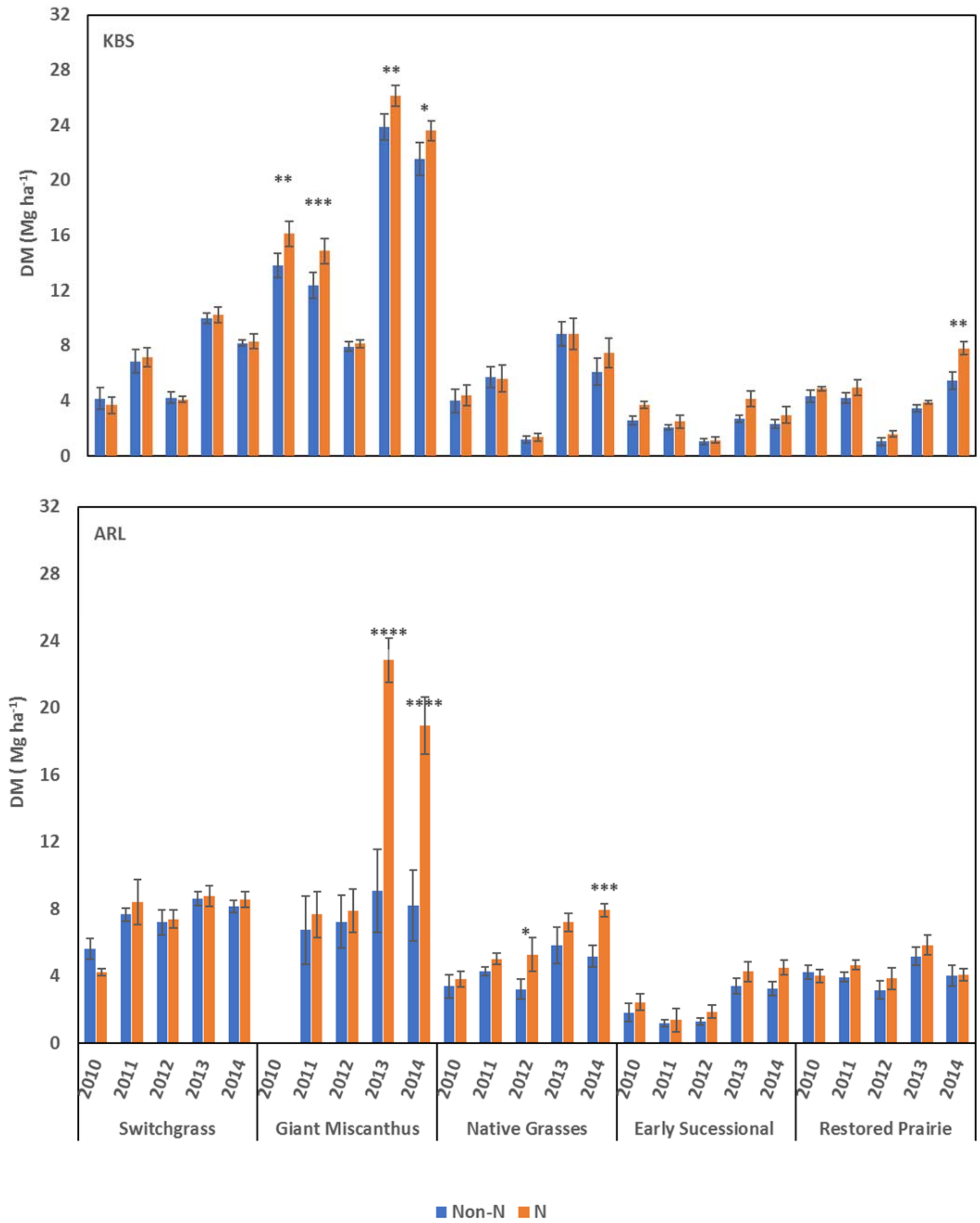

Figure 15 N effects on biomass dry matter yield (Mg ha^{-1}) during study period (2010-14) at KBS and ARL. Error bars show one standard error from the biomass dry matter yield mean. * (α=0.05); ** (α=0.01); *** (α=0.005); **** (α=0.0001).

Nitrogen Fertilizer Use Efficiency

Nitrogen fertilizer use efficiency (Mg kg N^{-1}) mirrored cropping system biomass yield response to N fertilization. Switchgrass had the lowest N fertilizer use efficiency among the five cropping systems at KBS and ARL (0.00027 Mg kg $N^{-1}$and 0.00038 Mg kg N^{-1} respectively). Giant miscanthus was the most efficient in productively utilizing N fertilizer at both locations (0.03347 Mg kg N^{-1} at KBS and 0.11639 Mg kg N^{-1} at ARL). Nitrogen fertilizer use efficiency of giant miscanthus reached 0.35 Mg kg N^{-1} in one reported study (Lewandowski and Schmidt 2006). The literature is unclear regarding N fertilizer use efficiency of switchgrass (Roque Lemus et al. 2008; Ra et al. 2012). Due to a significant difference of N use efficiency in giant miscanthus between KBS and ARL and not for the other cropping systems studied, the interaction between location and cropping system was significant. ($P<.0001$). There was no significant difference in N fertilizer use efficiency between native grasses, early successional and restored prairie at both KBS and ARL. Following giant miscanthus, the second ranked cropping system in N fertilizer use efficiency at KBS was restored prairie followed by early successional system, then native grasses. At ARL, the second ranked system was native grasses followed by early successional system, then restored prairie. Different dominating species led to different rankings of early successional system and restored prairie on N fertilizer use efficiency due to grasses generally having a higher N fertilizer use efficiency than forbs (Xu et al. 2015). The grass:forb ratio of early successional system (1.6) was lower than that of restored prairie (3.5) at KBS, but at ARL the opposite grass:forb ratio was observed with the early successional system (3.9) being higher than the restored prairie (1.1).

CONCLUSION

Nitrogen fertilization increased the productivity of giant miscanthus in several site years but not in polyculture cropping systems (native grasses, early successional and restored prairie) and switchgrass. Dry matter yield of giant miscanthus averaged across 2010-2014 responded positively to N fertilization at both KBS and ARL. Switchgrass, early successional field and restored prairie did not respond to N fertilization when averaged across years. For polycultures cropping systems in this study, only mixed native grasses at ARL had a positive response to N fertilization on averaged biomass yield across 2010-2014. A high grass:forb ratio of restored prairie in 2014 at KBS led to a positive N effect on biomass yield. Nitrogen fertilization significantly reduced [Glc] of native grasses at both KBS and ARL. The [Glc] of switchgrass and restored prairie biomass also responded negatively to N fertilization at KBS. Similarly, the [Xyl] of switchgrass and giant miscanthus biomass responded negatively to N fertilization at both KBS and ARL. The [Xyl] of mixed native grass biomass at KBS responded negatively to N fertilization. The single positive N effect on biomass [Xyl] was found in early successional biomass at ARL, which contributed to the only positive N fertilization effect on [EtOH] also being in early successional biomass at ARL. However, biomass quality in terms of ethanol concentration (g g^{-1}) was more driven by [Glc]. Similar to the results for biomass glucose [Glc]., N fertilization had a negative effect on [EtOH] in the switchgrass, mixed native grasses and restored prairie cropping systems at KBS and mixed native grasses at ARL. N responses on ethanol yield on a land area basis (Mg ha^{-1}) depended more upon biomass quantity than quality. Giant miscanthus was considerably more nitrogen fertilizer use efficient when compared to the other four cropping systems in this study (KBS: 0.03347 Mg kg^{-1} N; ARL: 0.11639 Mg kg^{-1} N). Switchgrass was relatively less nitrogen fertilizer use efficient when compared to the other four cropping systems in this study (KBS: 0.00027 Mg kg^{-1} N; ARL: -0.00019 Mg kg^{-1} N). There were no significant differences in N fertilizer use efficiency between polycultures at both KBS and ARL.

APPENDIX

APPENDIX

Table 11 A detailed species list of five perennial cropping systems under study.

Cropping System	Crop	Planting rate
Switchgrass	Switchgrass (Panicum virgatum L.), "Cave-In-Rock"	7.5 kg ha-1
Giant Miscanthus	Miscanthus x giganteus, "Illinois clone"	17,200 rhizomes ha-1
Native Grasses	Big Bluestem (Andropogon gerardii Vitman)	2.4 kg ha-1
	Canada wild rye (Elymus Canadensis L.)	1.6 kg ha-1
	Indiangrass (Sorghastrum nutans [L.] Nash)	2.4 kg ha-1
	Little Bluestem (Schizachyrium scoparium [Michx.] Nash)	3.2 kg ha-1
	Switchgrass, "Southlow"	1.6 kg ha-1
Early Successional	pre-existing seed bank	n/a
Restored Prairie	Grasses	
	Big Bluestem	1.2 kg ha-1
	Canada Wild Rye	1.2 kg ha-1
	Indiangrass	1.2 kg ha-1
	Junegrass (Koeleria cristata [Ledeb.] Schult.)	0.8 kg ha-1
	Little Bluestem	1.2 kg ha-1
	Switchgrass, "Southlow"	0.8 kg ha-1
	Leguminous forbs	
	Roundhead bushclover (Lespedeza capitata Michx.)	0.4 kg ha-1
	Showy Tick-Trefoil (Desmodium canadense (L.) DC.)	0.4 kg ha-1
	White Wild Indigo (Baptisia leucantha Torr. & Gray)	0.4 kg ha-1
	Non-leguminous forbs	
	Black-eyed Susan (Rudbeckia hirta L.)	0.4 kg ha-1
	Butterfly weed (Asclepias tuberosa L.)	0.4 kg ha-1
	Cup plant (Silphium perfoliatum L.)	0.4 kg ha-1
	Meadow anemone (Aneomone canadensis L.)	0.4 kg ha-1
	New England aster (Symphyotrichum novae-angliae [L.] G.L. Nesom)	0.4 kg ha-1
	Pinnate Prairie coneflower (Ratibida pinnata [Vent.] Barnhart)	0.4 kg ha-1
	Showy goldenrod (Solidago speciosa Nutt.)	0.4 kg ha-1
	Stiff goldenrod (Solidago rigida L.)	0.4 kg ha-1
	Wild bergamot (Monarda fistulosa L.)	0.4 kg ha-1

Table 12 Herbicide use and rate during the period of 2010-2014 at KBS.

Cropping System	Year	Herbicide (main ingredient)	Herbicide rate	Unit	Note
Switchgrass	2009	Drive (quinclorac)	0.6	Kg/Ha	post emergence weed control
	2010	2,4-D amine	2.2	Kg/Ha	broadleaf weed control
Giant miscanthus	2009	Drive (quinclorac)	0.6	Kg/Ha	post emergence weed
	2010	2,4-D amine	0.9	Kg/Ha	broadleaf weed control
Native Prairie	2010	2,4-D amine	0.4	Kg/Ha	broadleaf weed control

Table 13 Herbicide use and rate during the period of 2010-2014 at KBS.

Cropping System	Year	Herbicide (main ingredient)	Herbicide rate	Unit	Note
Switchgrass	2011	Roundup Power Max	1.7	Kg/Ha	post emergence weed control
	2012	Clarity	0.2	Kg/Ha	broadleaf weed control
		2,4-D LV4 Ester	1.2	Kg/Ha	broadleaf weed control
		Quinclorac SPC 75 DF	0.3	Kg/Ha	
	2014	Quinclorac SPC 75 DF	0.6	Kg/Ha	Pre-emerge herbicide spray
		2,4-D LV4 Ester	1.1	Kg/Ha	Pre-emerge herbicide spray
Giant Miscanthus	2011	Roundup Power Max	1.7	Kg/Ha	Burndown
		Glyphosate (generic)	3.5	Kg/Ha	Burndown
		2,4-D LV4 Ester	1.1	Kg/Ha	Burndown
	2012	Roundup Power Max	2.9	Kg/Ha	Burndown
		Prowl	1.7	Kg/Ha	Pre-emerge
		2,4-D LV4 Ester	1.1	Kg/Ha	Post-emerge
		Clarity	1.7	Kg/Ha	
	2013	Prowl	0.3	Kg/Ha	Pre-emerge
		Roundup Power Max	1.5	Kg/Ha	Burndown
		2,4-D LV4 Ester	0.8	Kg/Ha	Post-emerge
		FSTransform Plus (adjuvant)	0.8	Kg/Ha	Burndown
	2014	Prowl	2.2	Kg/Ha	Pre-emerge herbicide
		2,4-D LV4 Ester	1.1	Kg/Ha	Pre-emerge herbicide
		Roundup Power Max	2.1	Kg/Ha	Pre-emerge herbicide
Native Grasses	2011	Roundup Power Max	1.7	Kg/Ha	

Table 14 Fertilizer use and rate during the period of 2010-2014 at KBS.

Cropping System	Year	Fertilizer	Fertilizer rate	N rate	P rate	K rate	Unit
Switchgrass	2010	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2011	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2012	28% N (28-0-0)	72	20	0	0	Kg/Ha
	2013	28% N (28-0-0)	204	57	0	0	Kg/Ha
	2014	28% N (28-0-0)	204	57	0	0	Kg/Ha
Giant Miscanthus	2009	28% N (28-0-0)	276	77	0	0	Kg/Ha
	2010	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2011	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2012	28% N (28-0-0)	72	20	0	0	Kg/Ha
	2013	28% N (28-0-0)	204	57	0	0	Kg/Ha
	2014	28% N (28-0-0)	204	57	0	0	Kg/Ha
Native grasses	2010	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2011	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2012	28% N (28-0-0)	72	20	0	0	Kg/Ha
	2013	28% N (28-0-0)	204	57	0	0	Kg/Ha
	2014	28% N (28-0-0)	204	57	0	0	Kg/Ha
Early Successional	2009	Urea 46%	122	56	0	0	Kg/Ha
	2010	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2011	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2012	28% N (28-0-0)	72	20	0	0	Kg/Ha
	2013	28% N (28-0-0)	204	57	0	0	Kg/Ha
	2014	28% N (28-0-0)	204	57	0	0	Kg/Ha
Restored Prairie	2010	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2011	28% N (28-0-0)	200	56	0	0	Kg/Ha
	2012	28% N (28-0-0)	72	20	0	0	Kg/Ha
	2013	28% N (28-0-0)	204	57	0	0	Kg/Ha
	2014	28% N (28-0-0)	204	57	0	0	Kg/Ha

Table 15 Fertilizer use and rate during the period of 2010-2014 at ARL.

Cropping System	Year	Fertilizer	Fertilizer rate	N rate	P rate	K rate	Unit
Switchgrass	2011	AMS	2	0	0	0	Kg/Ha
	2012	Ammonium Nitrate	165	56	0	0	Kg/Ha
		28% UAN	6	2	0	0	Kg/Ha
		Ammonium Nitrate	168	57	0	0	Kg/Ha
	2013	Ammonium Nitrate	168	57	0	0	Kg/Ha
Giant Miscanthus	2011	AMS	2	0	0	0	Kg/Ha
	2012	Ammonium Nitrate	165	56	0	0	Kg/Ha
		28% UAN	3	1	0	0	Kg/Ha
		28% UAN	168	57	0	0	Kg/Ha
		Ammonium Nitrate	168	57	0	0	Kg/Ha
	2013	Ammonium Nitrate	168	57	0	0	Kg/Ha
Native grasses	2011	AMS	2	0	0	0	Kg/Ha
		Ammonium Nitrate	165	56	0	0	Kg/Ha
	2012	Ammonium Nitrate	168	57	0	0	Kg/Ha
	2013	Ammonium Nitrate	168	57	0	0	Kg/Ha
Early successional	2011	Ammonium Nitrate	165	56	0	0	Kg/Ha
	2012	Ammonium Nitrate	168	57	0	0	Kg/Ha
	2013	Ammonium Nitrate	168	57	0	0	Kg/Ha
Restored Prairie	2011	Ammonium Nitrate	165	56	0	0	Kg/Ha
	2012	Ammonium Nitrate	168	57	0	0	Kg/Ha
	2013	Ammonium Nitrate	168	57	0	0	Kg/Ha

Table 16 Average cropping system biomass yield (Mg ha^{-1} yr^{-1}) within different N fertilization treatments at KBS and ARL from 2010-2014. * Means with a same lower letter in a column within N fertilization practice and location are not significant different. Means with a same upper letter in a row within N fertilization practice and location are not significant different. (α=0.05)

		N		Non		
Location	Cropping System	Mg ha^{-1} yr^{-1}	Group	Mg ha^{-1} yr^{-1}	Group	Pr > F
KBS	Switchgrass	6.70(0.58)	Ab	6.68(0.52)	Ab	0.9759
	Giant Miscanthus	17.77(1.47)	Aa	15.90(1.30)	Ba	0.0003
	Native Grasses	5.52(0.64)	Abc	5.17(0.61)	Abc	0.4823
	Early Successional	2.88(0.28)	Acd	2.14(0.16)	Acd	0.1459
	Restored Prairie	4.61(0.43)	Ad	3.70(0.34)	Ad	0.0725
ARL	Switchgrass	7.43(0.45)	Ab	7.45(0.30)	Aa	0.9494
	Giant Miscanthus	14.33(1.67)	Aa	7.81(0.97)	Ba	<.0001
	Native Grasses	5.85(0.39)	Abc	4.38(0.35)	Bb	0.0042
	Early Successional	3.02(0.33)	Acd	2.18(0.25)	Ab	0.1535
	Restored Prairie	4.49(0.24)	Ad	4.11(0.25)	Ab	0.4445

Table 17 Average cropping system glucose content (g g^{-1} yr^{-1}) within different N fertilization treatments at KBS and ARL from 2012-2014. * Means with a same lower letter in a column within N fertilization practice and location are not significant different. Means with a same upper letter in a row within N fertilization practice and location are not significant different. (α=0.05)

		N		Non		
Location	Cropping System	g g^{-1} yr^{-1}	Group	g g^{-1} yr^{-1}	Group	Pr > F
KBS	Switchgrass	0.144(0.055)	Bb	0.153(0.048)	Ab	0.0322
	Giant Miscanthus	0.162(0.091)	Aa	0.166(0.119)	Aa	0.3285
	Native Grasses	0.156(0.059)	Bab	0.178(0.054)	Aa	<.0001
	Early Successional	0.120(0.070)	Ac	0.117(0.077)	Ac	0.3975
	Restored Prairie	0.130(0.069)	Bc	0.142(0.062)	Ab	0.0056
ARL	Switchgrass	0.148(0.033)	Ab	0.148(0.037)	Ab	0.9451
	Giant Miscanthus	0.162(0.059)	Aa	0.162(0.065)	Aa	0.9878
	Native Grasses	0.151(0.050)	Bab	0.161(0.039)	Aa	0.03
	Early Successional	0.123(0.069)	Ac	0.117(0.055)	Ac	0.174
	Restored Prairie	0.109(0.08)	Ad	0.117(0.100)	Ac	0.0755

Table 18 Average cropping system xylose content (g g^{-1} yr^{-1}) within different N fertilization treatments at KBS and ARL from 2012-2014. * Means with a same lower letter in a column within N fertilization practice and location are not significant different. Means with a same upper letter in a row within N fertilization practice and location are not significant different. (α=0.05)

		N		Non		
Location	Cropping System	g g^{-1} yr^{-1}	Group	g g^{-1} yr^{-1}	Group	Pr > F
KBS	Switchgrass	0.0886(0.0015)	Ba	0.0965(0.0014)	Aa	0.0033
	Giant Miscanthus	0.0834(0.0024)	Ba	0.0896(0.0034)	Aa	0.02
	Native Grasses	0.0850(0.0014)	Ba	0.958(0.0019)	Aa	<.0001
	Early Successional	0.0595(0.0031)	Ac	0.0575(0.0036)	Ac	0.4505
	Restored Prairie	0.0749(0.0022)	Ab	0.0759(0.0026)	Ab	0.6862
ARL	Switchgrass	0.0972(0.0027)	Ba	0.1037(0.0013)	Aa	0.0142
	Giant Miscanthus	0.0835(0.0020)	Bb	0.0898(0.0026)	Ab	0.0182
	Native Grasses	0.1003(0.0023)	Aa	0.1034(0.0016)	Aa	0.2373
	Early Successional	0.0749(0.0024)	Ac	0.0666(0.0032)	Bc	0.0022
	Restored Prairie	0.0671(0.0039)	Ad	0.0708(0.0045)	Ac	0.1572

Table 19 Average cropping system theoretical gravitational ethanol yield (g g^{-1} yr^{-1}) within different N fertilization treatments at KBS and ARL from 2012-2014. * Means with a same lower letter in a column within N fertilization practice and location are not significant different. Means with a same upper letter in a row within N fertilization practice and location are not significant different. (α=0.05)

		N		Non		
Location	Cropping System	g g^{-1} yr^{-1}	Group	g g^{-1} yr^{-1}	Group	Pr > F
KBS	Switchgrass	0.1075(0.0022)	Bab	0.1155(0.0019)	Aab	0.0063
	Giant Miscanthus	0.1135(0.0037)	Aa	0.1183(0.0052)	Aa	0.0956
	Native Grasses	0.1114(0.0018)	Ba	0.1266(0.0021)	Aa	<.0001
	Early Successional	0.0831(0.0034)	Ac	0.0805(0.0038)	Ac	0.3635
	Restored Prairie	0.0947(0.0031)	Bbc	0.101(0.0029)	Ab	0.0345
ARL	Switchgrass	0.1133(0.0018)	Aa	0.1165(0.0011)	Aa	0.2747
	Giant Miscanthus	0.1132(0.0022)	Aa	0.1161(0.0027)	Aa	0.3198
	Native Grasses	0.1161(0.0023)	Ba	0.1219(0.0018)	Aa	0.0442
	Early Successional	0.0912(0.0030)	Ab	0.0846(0.0031)	Bb	0.0245
	Restored Prairie	0.0814(0.0043)	Ab	0.0867(0.0050)	Ab	0.0664

Table 20 Average cropping system theoretical ethanol yield (Mg ha^{-1} yr^{-1}) within different N fertilization treatments at KBS and ARL from 2012-2014. * Means with a same lower letter in a column within N fertilization practice and location are not significant different. Means with a same upper letter in a row within N fertilization practice and location are not significant different. (α=0.05)

		N		Non		
Location	Cropping System	Mg ha^{-1} yr^{-1}	Group	Mg ha^{-1} yr^{-1}	Group	Pr > F
KBS	Switchgrass	0.805(0.077)	Ab	0.855(0.076)	Ab	0.5356
	Giant Miscanthus	2.121(0.226)	Aa	1.998(0.194)	Aa	0.1328
	Native Grasses	0.642(0.108)	Ac	0.693(0.125)	Ab	0.5314
	Early Successional	0.223(0.032)	Ad	0.156(0.017)	Ac	0.4058
	Restored Prairie	0.414(0.063)	Ac	0.334(0.053)	Ac	0.3274
ARL	Switchgrass	0.934(0.041)	Ab	0.931(0.040)	Aa	0.9649
	Giant Miscanthus	1.890(0.217)	Aa	0.960(0.141)	Ba	<.0001
	Native Grasses	0.793(0.062)	Ab	0.576(0.063)	Bb	0.0083
	Early Successional	0.321(0.037)	Ac	0.224(0.029)	Ac	0.2364
	Restored Prairie	0.379(0.040)	Ac	0.370(0.045)	Ac	0.9145

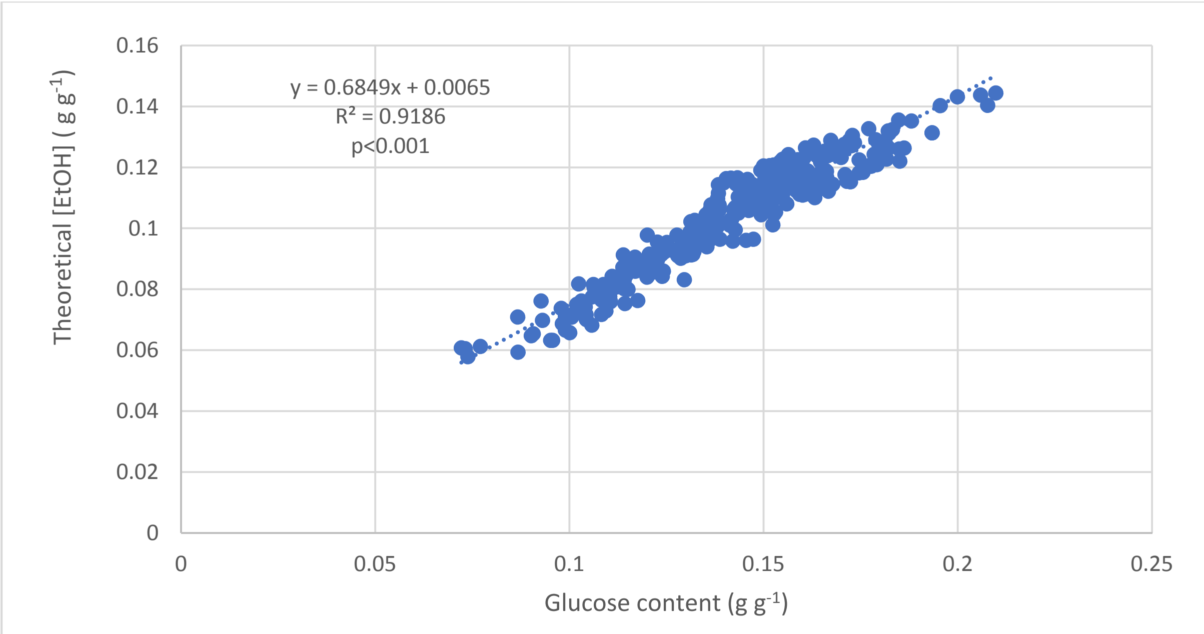

Figure 16 Correlation of theoretical [EtOH] (g g^{-1}) and glucose content (g g^{-1}) for biomass with or without N fertilization at KBS and ARL during the study years from 2012-14 (observation number n=300).

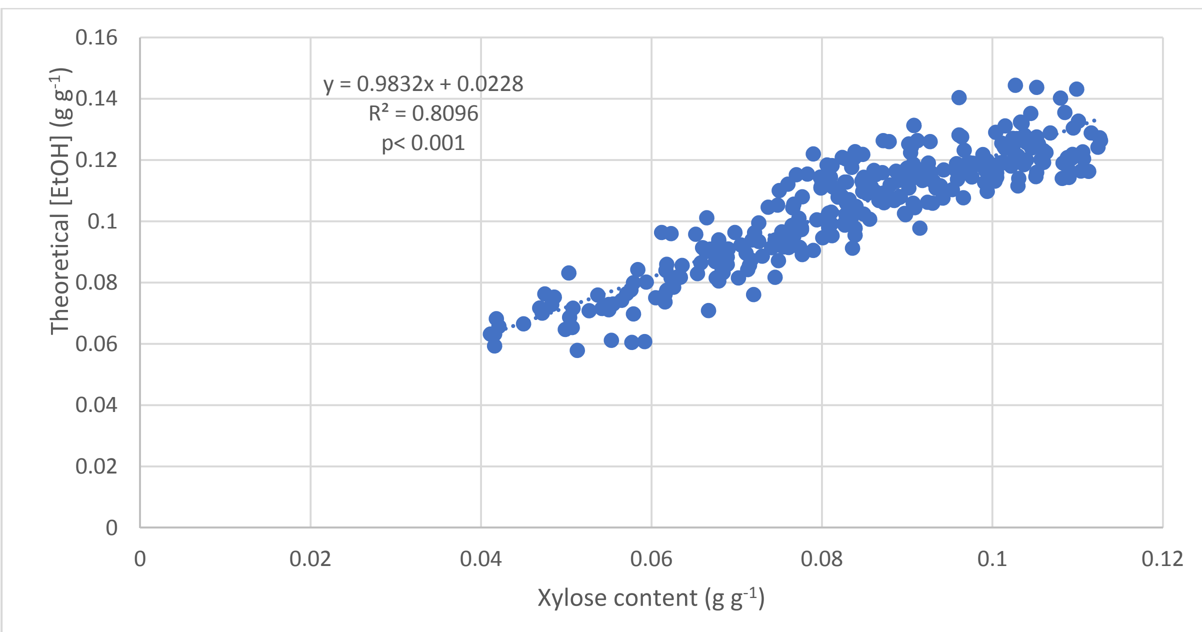

Figure 17 Correlation of theoretical [EtOH] (g g^{-1}) and xylose content (g g^{-1}) for biomass with or without N fertilization at KBS and ARL during the study years from 2012-14 (observation number n=300).

REFERENCES

REFERENCES

Allison, Gordon G., Catherine Morris, Susan J. Lister, Tim Barraclough, Nicola Yates, Ian Shield, and Iain S. Donnison. 2012. "Effect of Nitrogen Fertiliser Application on Cell Wall Composition in Switchgrass and Reed Canary Grass." *Biomass and Bioenergy* 40. Elsevier Ltd: 19–26. doi:10.1016/j.biombioe.2012.01.034.

Biesiada, Anita, and Eugeniusz Koota. 2008. "The Effect of Nitrogen Fertilization on Yield and Quality of Radicchio" 13 (2): 175–80.

Borge, K, D Hultmark, A G Clark, M Rosbash, P T Spellman, P Tzou, G M Rubin, et al. 2004. "Impact of Nitrogen Deposition Grasslands" 303 (March): 1876–80.

Brockman, J S. 1971. "FERTILIZER N AND THE YIELD OF CUT GRASS SWARDS IN Fl," 17–24.

Carroll, A, and C Somerville. 2009. "Cellulosic Biofuels." *Annu Rev Plant Biol* 60: 165–82. doi:10.1146/annurev.arplant.043008.092125.

Cruz, P. & Boval, M., 2000. Effect of Nitrogen on Some Morphogenetic Traits of Effect of Nitrogen on Some Morphogenetic Traits of Temperate. In *Grassland ecophysiology and grazing ecology*. p. 151.

Davis, Morgan P., Mark B. David, Thomas B. Voigt, and Corey A. Mitchell. 2015. "Effect of Nitrogen Addition on Miscanthus ×giganteus Yield, Nitrogen Losses, and Soil Organic Matter across Five Sites." *GCB Bioenergy* 7 (6): 1222–31. doi:10.1111/gcbb.12217.

Davis, Sarah C., William J. Parton, Stephen J. Del Grosso, Cindy Keough, Ernest Marx, Paul R. Adler, and Evan H. Delucia. 2012. "Impact of Second-Generation Biofuel Agriculture on Greenhouse-Gas Emissions in the Corn-Growing Regions of the US." *Frontiers in Ecology and the Environment* 10 (2): 69–74. doi:10.1890/110003.

Dawson, Julie C., David R. Huggins, and Stephen S. Jones. 2008. "Characterizing Nitrogen Use Efficiency in Natural and Agricultural Ecosystems to Improve the Performance of Cereal Crops in Low-Input and Organic Agricultural Systems." *Field Crops Research* 107 (2): 89–101. doi:10.1016/j.fcr.2008.01.001.

Dien, Bruce S., Hans Joachim G Jung, Kenneth P. Vogel, Michael D. Casler, JoAnn F S Lamb, Loren Iten, Robert B. Mitchell, and Gautum Sarath. 2006. "Chemical Composition and Response to Dilute-Acid Pretreatment and Enzymatic Saccharification of Alfalfa, Reed Canarygrass, and Switchgrass." *Biomass and Bioenergy* 30 (10): 880–91. doi:10.1016/j.biombioe.2006.02.004.

Division, Environmental Sciences, Oak Ridge, W M Post, and K C Kwon. 2000. "Soil Carbon

Sequestration and Land-Use Change: Processes and Potential." *Global Change Biology* 6: 317–27.

Fornara, Dario A., Elizabeth-Anne Wasson, Peter Christie, and Catherine J. Watson. 2016. "Long-Term Nutrient Fertilization and the Carbon Balance of Permanent Grassland: Any Evidence for Sustainable Intensification?" *Biogeosciences* 13 (17). Copernicus GmbH: 4975–84. doi:10.5194/bg-13-4975-2016.

Garlock, Rebecca J., Bryan Bals, Poonam Jasrotia, Venkatesh Balan, and Bruce E. Dale. 2012. "Influence of Variable Species Composition on the Saccharification of AFEX??? Pretreated Biomass from Unmanaged Fields in Comparison to Corn Stover." *Biomass and Bioenergy* 37. Elsevier Ltd: 49–59. doi:10.1016/j.biombioe.2011.12.036.

Group, Greenhouse Gas Working. 2010. "Agriculture ' S Role in Greenhouse Gas Emissions & Capture."

Haines, S. A., R. J. Gehl, J. L. Havlin, and T. G. Ranney. 2015. "Nitrogen and Phosphorus Fertilizer Effects on Establishment of Giant Miscanthus." *Bioenergy Research* 8 (1): 17–27. doi:10.1007/s12155-014-9499-4.

Heckathorn, S A, and E H Delucia. 1996. "Retranslocation of Shoot Nitrogen to Rhizomes and Roots in Prairie Grasses May Limit Loss of N to Grazing and Fire during Drought." *Functional Ecology* 10 (3): 396–400. doi:10.2307/2390289.

IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y.

Jarecki, M.K., and R. Lal. 2003. "Crop Management for Soil Carbon Sequestration." *Critical Reviews in Plant Sciences* 22 (6): 471–502. doi:10.1080/713608318.

Jin, Mingjie, Ming W Lau, Venkatesh Balan, and Bruce E Dale. 2010. "Bioresource Technology Two-Step SSCF to Convert AFEX-Treated Switchgrass to Ethanol Using Commercial Enzymes and Saccharomyces Cerevisiae 424A (LNH-ST)." *Bioresource Technology* 101 (21). Elsevier Ltd: 8171–78. doi:10.1016/j.biortech.2010.06.026.

Kane, Daniel. 2015. "Carbon Sequestration Potential on Agricultural Lands: A Review of Current Science and Available Practices."

Keene, Thomas Clarkson, "SWITCHGRASS YIELD AND QUALITY WITH MULTIPLE FERTILIZER APPLICATIONS AND HARVEST DATES" (2014). Theses and Dissertations--Plant and Soil Sciences. Paper 42. http://uknowledge.uky.edu/pss_etds/42

Kering, Maru K., Twain J. Butler, Jon T. Biermacher, Jagadeesh Mosali, and John A. Guretzky. 2013. "Effect of Potassium and Nitrogen Fertilizer on Switchgrass Productivity and Nutrient Removal Rates under Two Harvest Systems on a Low Potassium Soil." *Bioenergy*

Research 6 (1): 329–35. doi:10.1007/s12155-012-9261-8.

Keyvan, Shamsi. 2010. “The Effects of Drought Stress on Yield, Relative Water Content, Proline, Soluble Carbohydrates and Chlorophyll of Bread Wheat Cultivars.” *Journal of Animal and Plant Sciences* 8 (3): 1051–60.

Kitchen, N. R., K. W T Goulding, and J. F. Shanahan. 2008. “Proven Practices and Innovative Technologies for On-Farm Crop Nitrogen Management.” *Nitrogen in the Environment*, 483–517. doi:10.1016/B978-0-12-374347-3.00015-9.

Lemus, R., and R. Lal. 2005. “Bioenergy Crops and Carbon Sequestration.” *Critical Reviews in Plant Sciences* 24 (1): 1–21. doi:10.1080/07352680590910393.

Lemus, Roque, E. Charles Brummer, C. Lee Burras, Kenneth J. Moore, Michael F. Barker, and Neil E. Molstad. 2008. “Effects of Nitrogen Fertilization on Biomass Yield and Quality in Large Fields of Established Switchgrass in Southern Iowa, USA.” *Biomass and Bioenergy* 32 (12): 1187–94. doi:10.1016/j.biombioe.2008.02.016.

Lewandowski, I., and U. Schmidt. 2006. “Nitrogen, Energy and Land Use Efficiencies of Miscanthus, Reed Canary Grass and Triticale as Determined by the Boundary Line Approach.” *Agriculture, Ecosystems and Environment* 112 (4): 335–46. doi:10.1016/j.agee.2005.08.003.

Meehan, Timothy D., Ben P. Werling, Douglas A. Landis, and Claudio Gratton. 2012. “Pest-Suppression Potential of Midwestern Landscapes under Contrasting Bioenergy Scenarios.” *PLoS ONE* 7 (7). doi:10.1371/journal.pone.0041728. doi:10.1016/j.apenergy.2013.06.001.

Murozuka, Emiko, Kristian H. Laursen, Jane Lindedam, Ian F. Shield, Sander Bruun, Jakob Magid, Inge S. Møller, and Jan K. Schjoerring. 2014. “Nitrogen Fertilization Affects Silicon Concentration, Cell Wall Composition and Biofuel Potential of Wheat Straw.” *Biomass and Bioenergy* 64. Elsevier Ltd: 291–98. doi:10.1016/j.biombioe.2014.03.034.doi:10.1104/pp.108.120709.

Ra, Kaien, Fumitaka Shiotsu, Jun Abe, and Shigenori Morita. 2012. “Biomass Yield and Nitrogen Use Efficiency of Cellulosic Energy Crops for Ethanol Production.” *Biomass and Bioenergy* 37. Elsevier Ltd: 330–34. doi:10.1016/j.biombioe.2011.12.047.

Sainju, Upendra M. 2016. “A Global Meta-Analysis on the Impact of Management Practices on Net Global Warming Potential and Greenhouse Gas Intensity from Cropland Soils.” *PLoS ONE* 11 (2): 1–26. doi:10.1371/journal.pone.0148527.

Sanford, G. R., Oates, L. G., Jasrotia, P., Thelen, K. D., Robertson, G. P., & Jackson, R. D. (2016). Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA. *Agriculture, Ecosystems and Environment*, *216*, 344–355. http://doi.org/10.1016/j.agee.2015.10.018

Sanford, Gregg R, Lawrence G Oates, Sarah S Roley, David S Duncan, Randall D Jackson, G Philip Robertson, and Kurt D Thelen. 2017. “Biomass Production a Stronger Driver of Cellulosic Ethanol Yield than Biomass Quality,” no. May: 1–12. doi:10.2134/agronj2016.08.0454.

Santoro, Nicholas, Shane L Cantu, Carl-erik Tornqvist, Tanya G Falbel, Jenny L Bolivar, Sara E Patterson, Markus Pauly, and Jonathan D Walton. 2010. “A High-Throughput Platform for Screening Milligram Quantities of Plant Biomass for Lignocellulose Digestibility,” 93–102. doi:10.1007/s12155-009-9074-6.

Schwartz, C, and R Amasino. 2013. “Nitrogen Recycling and Flowering Time in Perennial Bioenergy Crops.” *Front Plant Sci* 4 (April): 76. doi:10.3389/fpls.2013.00076.

Song, L., X. Bao, X. Liu, Y. Zhang, P. Christie, A. Fangmeier, and F. Zhang. 2011. “Nitrogen Enrichment Enhances the Dominance of Grasses over Forbs in a Temperate Steppe Ecosystem.” *Biogeosciences* 8 (8): 2341–50. doi:10.5194/bg-8-2341-2011.

Sorek, Nadav, Trevor H. Yeats, Heidi Szemenyei, Heather Youngs, and Chris R. Somerville. 2014. “The Implications of Lignocellulosic Biomass Chemical Composition for the Production of Advanced Biofuels.” *BioScience* 64 (3): 192–201. doi:10.1093/biosci/bit037.

Stout, William L., and Gerald a. Jung. 1995. “Biomass and Nitrogen Accumulation in Switchgrass: Effects of Soil and Environment.” *Agronomy Journal* 87: 663. doi:10.2134/agronj1995.00021962008700040010x.

Tilman, D., J. Hill, and C. Lehman. 2006. “Carbon-Negative Biofuels from Low-Input High-Diversity Grassland Biomass.” *Science* 314 (5805): 1598–1600. doi:10.1126/science.1133306.

Van Der Heijden, Marcel G A, Ruth Streitwolf-Engel, Ralph Riedl, Sabine Siegrist, Angelica Neudecker, Kurt Ineichen, Thomas Boller, Andres Wiemken, and Ian R. Sanders. 2006. “The Mycorrhizal Contribution to Plant Productivity, Plant Nutrition and Soil Structure in Experimental Grassland.” *New Phytologist* 172 (4): 739–52. doi:10.1111/j.1469-8137.2006.01862.x.

Waramit, Naroon, Kenneth J. Moore, and Emily Heaton. 2014. “Nitrogen and Harvest Date Affect Developmental Morphology and Biomass Yield of Warm-Season Grasses.” *GCB Bioenergy* 6 (5): 534–43. doi:10.1111/gcbb.12086.

Werling, Ben P, Timothy L Dickson, Rufus Isaacs, Hannah Gaines, Claudio Gratton, Katherine L Gross, Heidi Liere, et al. 2014. “Perennial Grasslands Enhance Biodiversity and Multiple Ecosystem Services in Bioenergy Landscapes.” *Proceedings of the National Academy of Sciences of the United States of America* 111 (4): 1652–57. doi:10.1073/pnas.1309492111.

Xu, Xiaotian, Hongyan Liu, Zhaoliang Song, Wei Wang, Guozheng Hu, and Zhaohuan Qi. 2015.

“Response of Aboveground Biomass and Diversity to Nitrogen Addition along a Degradation Gradient in the Inner Mongolian Steppe, China.” *Scientific Reports* 5 (April). Nature Publishing Group: 10284. doi:10.1038/srep10284.

Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp, doi:10.1017/CBO9781107415324.

CHAPTER 3

ATTRIBUTIONAL LIFE CYCLE ASSESSMENT OF MISCANTHUS AND SWITCHGRASS BIOENERGY CROPPING SYSTEMS IN THE NORTH-CENTRAL US

ABSTRACT

Switchgrass (*Panicum virgatum* L.) and giant miscanthus (*Miscanthus* × *giganteus* Greef & Deuter ex Hodkinson & Renvoize) have been identified as primary dedicated bioenergy crops in the U.S for producing lignocellulosic biofuel feedstock. However, region-specific life cycle analyses for these crops are lacking. We investigated the environmental impacts (global warming potential, acidification potential and eutrophication) of switchgrass and giant miscanthus grown at two sites (Michigan-KBS and Wisconsin-ARL) in the North-Central U.S using a field level life cycle analysis. Switchgrass (-2201.2 kg CO_2-equiv. ha^{-1} and -1675.8 kg CO_2-equiv. ha^{-1} for KBS and ARL, respectively) and giant miscanthus (-5245.9 kg CO_2-equiv. ha^{-1} and -3728.2 kg CO_2-equiv. ha^{-1} for KBS and ARL, respectively) cropping systems at both locations had a net negative global warming potential. The significant displacement of GHG emissions by replacing conventional gasoline with ethanol derived from the harvested biomass was the main driver in net GWP. When omitting gasoline displacement and including only field level agricultural inputs, ΔSOC and soil N_2O emissions, the switchgrass cropping system (-415 kg CO_2-equiv. ha^{-1} yr^{-1}) had a more favorable global warming potential than the miscanthus cropping systems (-306 kg CO_2-equiv. ha^{-1} yr^{-1}) at KBS. Conversely, at ARL, the giant miscanthus cropping systems (97.3 kg CO_2-equiv. ha^{-1} yr^{-1}) had lower global warming potential than the switchgrass cropping (296 kg CO_2-equiv. ha^{-1} yr^{-1}). ΔSOC was the main determinant of global warming potential during the switchgrass and giant miscanthus cultivation phase. Giant miscanthus had a higher acidification potential, eutrophication potential and non-renewable energy depletion than switchgrass. Nitrogen fertilizer manufacture and

production contributed the most significant negative environmental impacts for switchgrass cropping system at both KBS and ARL. For giant miscanthus, diesel emissions associated with harvesting contributed the most significant negative environmental impact at both KBS and ARL due to higher biomass yield of giant miscanthus. Giant miscanthus (KBS: 7.30; ARL: 7.00) had a higher energy return on investment than switchgrass (KBS: 4.27; ARL: 4.80) did at both locations, mainly due to higher biomass yield of giant miscanthus.

INTRODUCTION

Energy security, ecological issues associated with fossil fuel extraction, global warming, and an increasing frequency of extreme weather events, have all contributed to more research efforts being focused on clean energy sources (solar, wind, geothermal, bioenergy). Among those clean energy sources, bioenergy promises a "carbon-neutral" future realized within a relatively short period of time, especially in the transportation fuel sector. First generation biofuels, derived from corn grain or sugar cane, require fertile soil for production and contribute to the food vs. fuel debate (Naik et al. 2010; Thompson, 2012). The global population is expected to reach approximately 9 billion by 2050, and more arable land will be needed for food production to support this large population. This population growth will require that productive arable land use be dedicated to food production rather than fuel (Godfray et al., 2012).

The U.S. renewable fuel standard mandates 16 billion gallons per year of lignocellulosic biofuel by 2022 with the stipulation of at least 60% less greenhouse gas (GHG) emissions compared with a 2005 petroleum fuel baseline (RFS2). Switchgrass (*Panicum virgatum* L.) and giant miscanthus (*Miscanthus* × *giganteus* Greef & Deuter ex Hodkinson & Renvoize) dedicated bioenergy crops fall into the category of lignocellulosic biofuel feedstock. Due to their high yield potential and relatively low fertilizer and pesticide needs during cultivation, they are considered as primary candidates to provide sufficient feedstock to fulfill the federal mandate (McLaughlin, 1992; van der Weijde et al. 2013). Greenhouse gases from agricultural land are mainly nitrous oxide, methane and carbon dioxide. Among them, nitrous oxide (N_2O) is the most potent with a global warming potential 298 times that of CO_2 (IPCC, 2013). If the population continues to grow, agriculture non-CO_2 (CH_4, N_2O) emissions would triple to 15.3Gt CO_2-equiv/yr (Popp, Lotze-Campen, and Bodirsky 2010). From a societal perspective, it is necessary to evaluate the environmental performance of bioenergy cropping systems before deploying them on a large scale. The estimation of GHGs can also lead to the development of better agricultural practices and management strategies in terms of lessening environmental impacts. Life cycle analysis (LCA) has been used as a standard method to estimate environmental impact in the energy sector (Turconi, Boldrin, and Astrup 2013).

Life Cycle Analysis (LCA)

Life Cycle Analysis is a standardized methodology to assess environmental impacts of a product or service guided by ISO 14040(2006) and ISO 14044(2006). This methodology has been used as an important tool for decision making in many industries, such as packaging and manufacturing. It started to gain popularity in the agricultural sector since the Renewable Fuel Standard imposed GHG reductions as a qualification for advanced biofuels(Cherubini and Strømman 2011; Turconi, Boldrin, and Astrup 2013; Borrion, McManus, and Hammond 2012; Guinée et al. 2011).

There are two types of life cycle analysis: attributional LCA and consequential LCA. Attributional LCA focuses only on the flows that cause environmental impacts within a given timeframe. Consequential LCA also accounts for the flows leading to environmental burdens in responses to possible decisions (Ekvall et al. 2016).

Life cycle analysis is a holistic approach that estimates the environmental footprint from every stage of product manufacture or service, beginning from raw material extraction (cradle), manufacturing, distribution, use/reuse, to the end-of-life (grave). According to framework defined by ISO 14040 and ISO 14044 (International Standard Organization, 2006), life cycle analysis consists of four main phases: 1) Goal and scope definition; 2) Life cycle inventory (LCI); 3) Life cycle impact assessment (LCIA); and, 4) Interpretation of results. Life cycle analysis is an iterative process, the result of one phase may affect the prior phase, resulting in revisiting the prior phase. Figure 18, below, shows the concept of four main phases and their connections from ISO 14040.

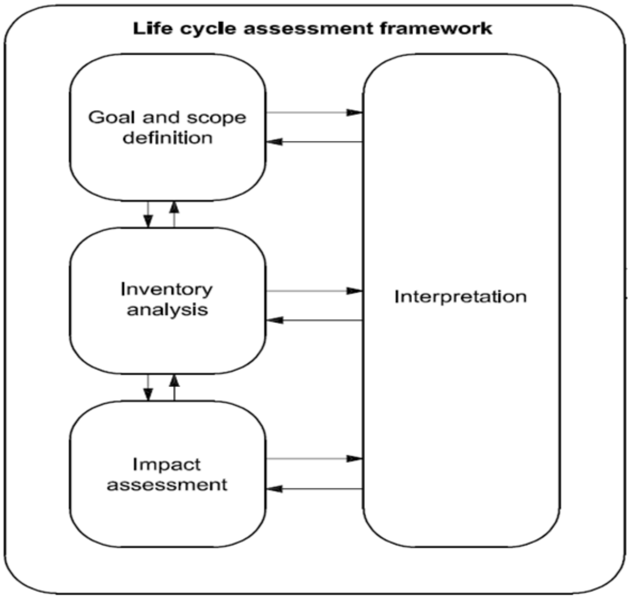

Figure 18 Overview of life cycle analysis phases from ISO 14040

Goal and Scope definition

As the first phase of life cycle analysis, the goal and scope definition phase specifies the goals or aims of the life cycle analysis, which includes explicitly stating the purpose, method, assumptions, intended audience and application of the study. System boundaries and functional units are defined in this phase. The boundaries of the study articulate what should be included and what should be excluded. Temporal and geographical scope are often included for life cycle analysis in the agricultural sector. The functional unit quantifies the service delivered by the product or service, and also establishes the basis from which to collect inputs and outputs. Overall, necessary details and transparency should be given in this phase so as to make sure the study can be validated and useful. The goal and scope phase plays a crucial role in the whole life cycle analysis.

Life Cycle Inventory (LCI)

The second phase of a life cycle analysis is the most labor-intensive phase. Life cycle inventory involves collecting all the inputs and outputs of energy and materials, often referred to as flows, from the defined system in the first goal and scope definition phase. All the data collected must be converted into the previously defined functional unit. The quality of data largely determines the credibility of the study. There are usually two types of data, primary and secondary data.

Life Cycle Impact Assessment (LCIA)

Life cycle impact assessment serves the purpose of linking specific outflows to environmental impacts. Outflows of LCI are summarized by characterization factors and their corresponding environmental impact categories which are expressed in the unit of category indicators. There are several characterization models which connect different impact categories and outflows by category indicators, such as TRACI, and ReCipe. LCIA emphasizes "potential" environmental impacts instead of quantifying impacts precisely.

Interpretation of Results

Based on the goal and scope definition, the results from the life cycle impact assessment are interpreted. Conclusions are drawn by taking a consideration of both results from LCIA and other factors. Recommendations are made for intended audiences. An evaluation includes completeness, and sensitivity and consistency analyses should also be conducted.

Objective of this study

Input data of most currently available agricultural LCA studies are generated from simulation models (secondary data), such as EPIC and GREET. Life cycle analysis studies based on empirical field data (primary data) are limited. The objective of this study was to conduct an attributional LCA with empirical field measured data (primary data) to investigate the environmental impacts of switchgrass and miscanthus dedicated perennial cropping systems for bioenergy feedstock production, in the Northcentral United States. Since the primary advantage associated with the adoption of biofuel is substantial GHGs reduction, the study mainly focuses on the environmental impact category of global warming potential. Additionally, we assessed eutrophication potential and acidification potential to broaden the scope of environmental impact assessment beyond a single response variable (Hennig and Gawor 2012). The 1997 Intergovernmental Panel on Climate Change report estimated that about 30% of applied N fertilizer may runoff from the agriculture system. Biofuel production can potentially be unfavorable in terms of eutrophication and acidification potential, and therefore it is highly necessary to include these two impact categories (Blottnitz & Curran 2007). Contribution analysis was conducted to identify "hotspots" during the biomass feedstock production phase. Sensitivity analysis was also conducted to see which parameter was more powerful in changing the total global warming potential. The potential uses of this study include but are not limited to public policy making support and in providing further research direction in academia.

MATERIALS AND METHODS

Field locations and experimental design

This study was conducted at two locations: W.K. Kellogg Biological Station in Hickory Corners, Michigan (KBS, 42˚23’47” N, 85˚22’26” W) and the Arlington Agricultural Research Station in Arlington, Wisconsin (ARL, 43˚17’45” N, 89˚22’48” W). The dominant soil series at KBS are the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (Coarse-Loamy, Mixed, Semiactive, Mesic Typic Hapludalfs) series. These soils are well drained with moderate fertility, consisting of mainly alfisols which developed on uplands under forest vegetation (Crum, J.R., 1995). The dominant soil series at ARL is Plano silt loam (Fine-Silty, Mixed, Superactive, Mesic Typic Argiudolls). These soils are well drained with relatively high fertility, consisting of mainly mollisols and developed in loess or other silty material under prairie (UW Extension, 2005).

Field Experiment Design

Experimental fields at both locations were established in 2008. Perennial grass (C_4) cropping systems were planted including switchgrass and giant miscanthus. The experimental design for this study was a randomized complete block design with 5 blocks at both locations. Each plot was 27 m wide × 43 m long (0.12 ha). The previous crop at KBS was alfalfa, while ARL had corn and alfalfa as the previous crops.

Goal and Scope definition

Goals

The goal of this attributional life cycle analysis was to identify which perennial cropping system, switchgrass or miscanthus, performs better in terms of three different environmental impact categories: 1) global warming potential (GWP); 2) eutrophication potential (EP) and 3) acidification potential (AP) in the Northcentral U.S. Region. The results will assist growers and policy makers in making informed decisions as to which cropping system is more suitable in the region. Moreover, this study attempts to find “risk-hotspots” in the cropping systems to identify specific practice improvements. It intends to facilitate informed decision making by growers in choosing appropriate perennial cropping systems and improving management of the cropping systems in terms of environmental performance. The functional unit of this LCA study is one hectare of arable land per year. Cherubini et al. (2011) suggests that unit of arable land should be considered first as functional unit when biomass come from dedicated energy crops. This study has been carried out in conformance with the instructions in ISO 14040 (2006) and ISO 14044 (International Standard Organization, 2006).

Temporal and Geographic Scope

The time-frame of this study is from the spring of 2008, when the cropping system experiments were established, through the harvest of 2015 and assuming keeping the same agronomic practice during established phase till 2023. Geographically, the study covers both locations, KBS and ARL, to represent a broad spectrum of soil and weather variability in the Northcentral US region.

System Boundary

There are different pathways of converting bioenergy feedstock into biofuels and each feedstock has its optimum pathway due to different chemical and physical characteristics (Dale & Ong 2012). With the focus on agricultural aspects, this study only accounts for the agronomic production phase (cradle to farm gate) instead of cradle-to-grave. The cradle for this study includes the extraction and production of all material and energy inputs. Biomass harvest is the defined end point of this study, with a gasoline offset credit calculated on empirical biomass yield and fermentable sugar content of the harvested biomass. After harvesting, transportation to storage facilities on the farm and pretreatment facilities, pretreatment process and bio-refinery phase are excluded from this study because they will vary significantly between growers and therefore may confound comparative results between systems. Capitol input, such as labor use and agricultural buildings are commonly disregarded since they can be used for a relatively long period of time and also serve other duties, and therefore contribute insignificant environmental impacts (Muench et al. 2013; Bessou et al. 2013).

Co-products and allocation

In addition to the main service generated by the system (biofuels), co-products which offer other services beyond the system also commonly exist. The method used to allocate input costs between products and co-products can play a crucial role in accurately estimating environmental impacts of the product and service under study. The main co-product of the cropping systems under this study is the residual plant material remaining after harvesting. Residuals can be burned for heat-energy, used as feedstock for other products, or are can be cycled back into soil as a nutrient or carbon source. All residuals were assumedly returned back to the soil, so residual allocation procedures were not necessary for this study, which is consistent with other studies on giant miscanthus (Brandão, Milà i Canals, and Clift 2011). The allocation method used in many published LCA studies on perennial cropping systems is based on a physical basis and some LCA’s don`t clearly indicate the allocation method used (Bessou et al. 2013). In order to be consistent, this study didn`t allocate impacts to coproducts and focused solely on the biofuel produced from harvested giant miscanthus and switchgrass biomass.

Data quality

All data used in this report was evaluated using the pedigree matrix from Weidema (1998). The scores each data source received are given next to the values provided the inventories Table 25 to Table 36 in the appendix.

Life Cycle Inventory (LCI)

As many field measured data as possible were used in the LCI phase. A summarized LCI table can be found in Table 41 of the appendix. Carefully tracked and recorded data of material inputs, agronomic activities and biomass mechanical yield are located in the online dataset (Link: data.sustainability.glbrc.org).

Establishment and agronomic management

Soil preparation

Generally, soil preparation was less intense at KBS than ARL. In spring 2008, soil preparation was done for both crops by a soil finisher followed by a cultimulcher at KBS. John Deere (John Deere, Moline IL) 7520 tractor with JD 726 15'9" soil finisher was adopted for soil finishing for both crops at KBS. JD 6420 Tractor with JD 970 12 ft cultimulcher was used for packing soil after planting giant miscanthus and before seeding switchgrass at KBS. At ARL, the primary tillage was chisel plowing, but the secondary tillage was done by using a disk, followed by a cultivator and culti-packer.

Seeding

Switchgrass

There are many commercial varieties of switchgrass available to growers. Cave-in-rock was the one used in this study. This variety is suitable for Michigan and Wisconsin growing conditions. Switchgrass was solid seeded in 2008. Due to heavy rain and seed washing off plots later in the spring of 2008, switchgrass was replanted in 19.05 cm rows at KBS in 2009. Switchgrass was also replanted at ARL in bare areas based on poor (<40%) crop frequency ratings in 2012. Switchgrass seed production data were obtained from the Gabi (Thinkstep, Leinfelden-Echterdingen, Germany) database.

Giant Miscanthus

Miscanthus rhizomes, with one or two active growing points, were hand planted at a depth of 0.1m in a 0.76 x 0.76m grid pattern in late-May 2008 at both locations. Due to serious winter kill, Miscanthus was replanted at ARL in 2010. A culti-packer was pulled over the plot to provide sufficient rhizome/soil contact after planting. Since the Gabi database does not have miscanthus rhizome production data, rhizome production data were obtained from a study on miscanthus propagation (Shemfe et al. 2016). Shemfe et al., (2016) estimated 100 kg ammonium nitrate ha^{-1}, 40 kg K_2O ha^{-1} and 575 l diesel ha^{-1} were used for miscanthus rhizome multiplication with a multiplication ratio of 1:14.

Seeding rates are located in Table 25 & 26 and planting machinery use can be found in Table 33, 34,37 and 38 in the appendix.

Weed Control

During the establishment period of switchgrass and miscanthus, herbicides usually have to be applied to minimize weed competition. Weed control strategies were generally similar between the two crops at both locations. After planting, switchgrass was flail mowed, using the JD 7420a tractor and JD 115 flail mower twice, in August and September at KBS. Miscanthus was cultivated with a Case IH (Case IH, Goodfield IL) 183 S-tine 6 row cultivator at KBS. Quinclorac at 0.56 kg ha^{-1} was applied post emergence for both crops in May 2009 at KBS. Then, there were 2 passes of mowing conducted in July 2009 with JD 6420 tractor and JD 115 15' flail mower at KBS. After establishment, 2,4-D amine herbicide was applied at 2.24 kg ha^{-1} for broadleaf weed control at KBS. At ARL, glyphosate at 1.7 kg ha^{-1} was applied pre-green up in the switchgrass cropping system in May 2010, and replant giant miscanthus in May 2011. Applications of 2, 4-D LV4 ester and quinclorac were applied as pre-emerge herbicide at ARL. Herbicide products and rates are located in Table 37 & 38 in the appendix.

Fertilization and Nutrient Management

No fertilizer was applied during the stand establishment period to minimize weed competition. Since 2010, 56 kg N ha^{-1} was applied in the form of (28-0-0) liquid ammonium urea fertilizer solution or granular urea was applied early to mid-May for both crops at KBS. At ARL, 56 kg N ha^{-1} was applied in the form of (28-0-0) liquid ammonium urea fertilizer solution or granular ammonium nitrate (34-0-0). No P and K were applied as recommended based on annual fall soil sampling. Table 29 and 30 show details of annual fertilization and nutrient management.

Harvesting

Switchgrass and miscanthus harvest used the same equipment, which for this study involved a field chop method. The field chopper saves machine field trips compared to a mow, rake, and bale method, which in turn reduces the system energy requirement. In addition, it offers a bulk material to the biorefinery plant that eliminates bale string removal and minimizes subsequent feedstock grinding. However, a potentially lower biomass density may increase transportation costs. Bulk consolidation could be a way to increase biomass density and reduce transportation cost. (Sustainable Bioenergy Production,7.6)

At KBS, a JD 7350 self-propelled forage harvester equipped with a JD 676 Kemper cutting head was adopted for miscanthus and switchgrass biomass harvest. The harvested material was chopped into a tare-weighed truck which was reweighed to determine harvestable biomass yield.

Cutting height of remaining plant stubble was 15.2 cm in all plots. Grab samples from each plot were placed in paper bags and weighed to determine wet weight. The sample bags were then placed in an air-dry oven at 60 °C until dry and reweighed to determine moisture content for each plot. A JD 7500 self-propelled forage harvester with a 600C series grass header was used at ARL. Plant material was chopped into a Miller (Art`s Way, Armstrong IA) Pro 8015 dump wagon equipped with load cells to determine harvested biomass weight. Moisture content was determined as described above. For purpose of the LCA, all the harvested biomass material was assumed to be dumped in a bunker silo near the field. Since 2010, after 2 years' establishment, switchgrass was harvested during fall at both locations. Biomass harvest happened within two weeks after a killing frost (-3.5 ˚C, typically late-October to mid-November) to optimize nutrient translocation back to roots.

The chop and ensile harvesting strategy had the diesel use rate at 5.44 l Mg^{-1} (Kumar et al. 2007; Sokhansanj et al. 2006). See appendix Table 40, for annual yield results of the switchgrass and giant miscanthus cropping systems.

Climate Data

Daily air temperature and precipitation data during the study period (2010-2015) were collected from the Arlington University Farm Station and, the Kellogg Gull Lake Biological Station. After collecting data, all the data were summarized into monthly mean temperature and precipitation levels and compared with 30-year climatology data.

Biogenic CO2

The CO2 generated from biomass during the biorefinery phase and biofuel combustion phase are considered autotrophic. So, the uptake of CO2 through photosynthesis and locked in the harvested biomass was not taken into account.

Net Soil Organic Carbon Change (SOC)

Soil organic carbon change (ΔSOC) due to direct land use change (dLUC) from the previous corn or soybean and alfalfa rotation to switchgrass and miscanthus is the major determinant of carbon balance for bioenergy feedstock production. It usually takes a long-term duration for SOC to reach a new equilibrium once the dLUC occurs. Since this 7-year study does not have enough time to detect any meaningful ΔSOC, data from an adjacent long-term research site were used instead because they have a similar field history. Gelfand et al. concluded that net soil organic carbon change for 20-years alfalfa cultivation is -1220±920 kg CO2-equiv/hectare (Gelfand et al. 2013). Values from the Gelfand study were used to reflect ΔSOC in this study.

Estimating N_2O emissions

The N_2O emission estimation protocol used in this study has been described by Oates et al. (Oates et al. 2016). In sum, while soil temperatures were consistently > 0 °C, nitrous oxide (N_2O) fluxes were measured twice monthly, as well as immediately following precipitation events (Robertson et al., 2000) using vented static chambers (Livingston & Hutchinson, 1995). The chambers had a 28.45 cm diameter and 18 cm headspace height and were inserted 5 cm below the soil surface. Chamber lids were modified with a septum for gas extraction and a vent to allow for chamber pressure equilibration. Tubing was attached to the vent prior to capping and hung inside the chamber to reduce possible crosswind induced loss of gas from the chamber vent. Headspace gas from within the chambers was extracted immediately following lid placement with a 30-mL nylon syringe and a 23-gauge needle. Three subsequent extractions were made at 20-min intervals over a 60-min period. Glass 5.9 mL Exetainer vials (Labco Limited, Buckinghamshire, UK) were flushed with 20 mL of extracted sample and then overcharged with 10 mL of sample to facilitate sample extraction for analysis (Parkin &

Venterea, 2010). Field standards (1 ppm N_2O) and ambient air were also loaded into vials at this time to assess ambient GHG concentrations and potential storage-vial degradation in the period between sampling and analysis. Samples were analyzed by gas chromatography using an electron capture detector (micro-ECD, Agilent 7890A GC System, Santa Clara, CA, USA). "Carbon dioxide (CO2) accumulation was used to evacuate compromised vial through a visual inspection process which could result in deletion of a single observation in a series, or the removal of the entire series. Samples passing visual inspection were analyzed with the HMR package (v0.3.1, Pedersen 2012) in the R statistical environment ("warm puppy" version, R Core Team, 2013). Briefly, the method fits trace gas concentration time series with either a nonlinear model (Hutchinson and Mosier 1981) or linear regression, or identifies the series as a null flux. When the 95% confidence interval of a nonlinear flux estimate did not include the corresponding linear flux estimate, the nonlinear estimate was used for that series; in all other cases, linear flux estimates were used. Daily fluxes were aggregated to an annual scale by linearly interpolating between consecutive sampling dates and integrating (Smith & Dobbie, 2001)."

Details of Yearly GHGs can be found at Table 39.

Field Machinery Use

Diesel consumption of machinery for agronomic activities was taken into account. Flows of diesel production and combustion for field machinery were obtained from the Gabi software database (PE international). Diesel consumption was calculated for each type of field machine used according to 2015 MSU Machine and work Rate Estimates (Stein 2015) and University of Minnesota Machine cost estimates (Lazarus 2015). During a perennial crop establishment year, agronomic activities are more frequent than the following years. Therefore, diesel consumption during this period was amortized into a typical stand life-15 years. Details of diesel consumption for each farming practice are present in Table 31, 32, 35 and 36.

Fossil fuel displacement

This study assumed that ethanol produced by the harvested biomass was used to replace gasoline. The calculation of gasoline displacement was based on the energy ratio of one volumetric unit of ethanol and one volumetric unit of gasoline, which was 0.7:1(U.S Deparment of Energy 2014). GHG emissions of gasoline was estimated at 93.1 g CO_2-equiv./MJ gasoline by US. EPA (EPA, 2010).

Gabi Software Models

Gabi 6.0 was used to build the LCA models (Figure 19-22) in this study with as much primary data as possible. Flows like production and combustion of diesel, production of fertilizer and pesticide are based on Gabi Professional+Extension 2012 Database.

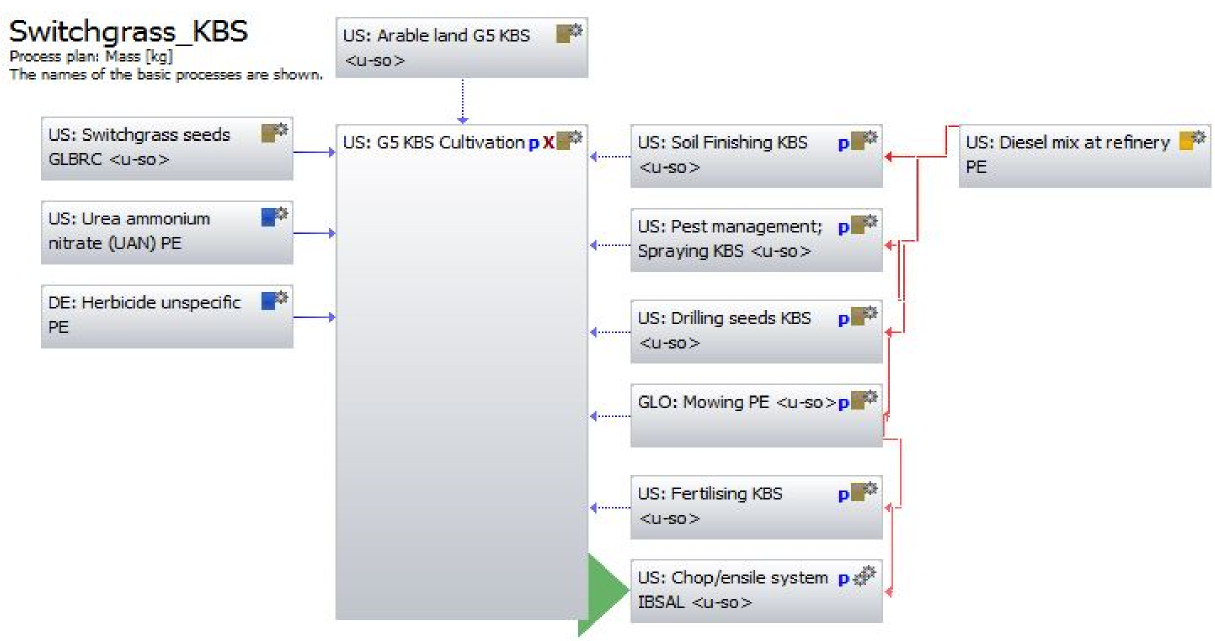

Figure 19 Cultivation phase model in Gabi 6.0 for switchgrass at KBS from year 2008 to 2022. Boxes represent individual unit processes. Arrow width is based on mass (kg) value of flows. Details of model inputs are in appendix.

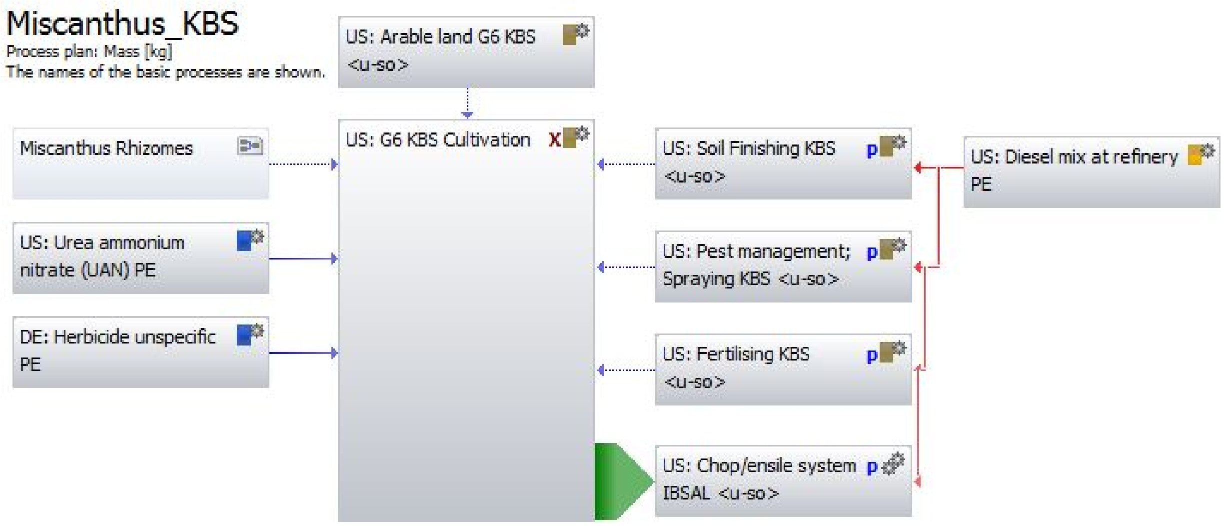

Figure 20 Cultivation phase model in Gabi 6.0 for giant miscanthus at KBS from year 2008 to 2022. Boxes represent individual unit processes. Arrow width is based on mass (kg) value of flows. Details of model inputs are in appendix.

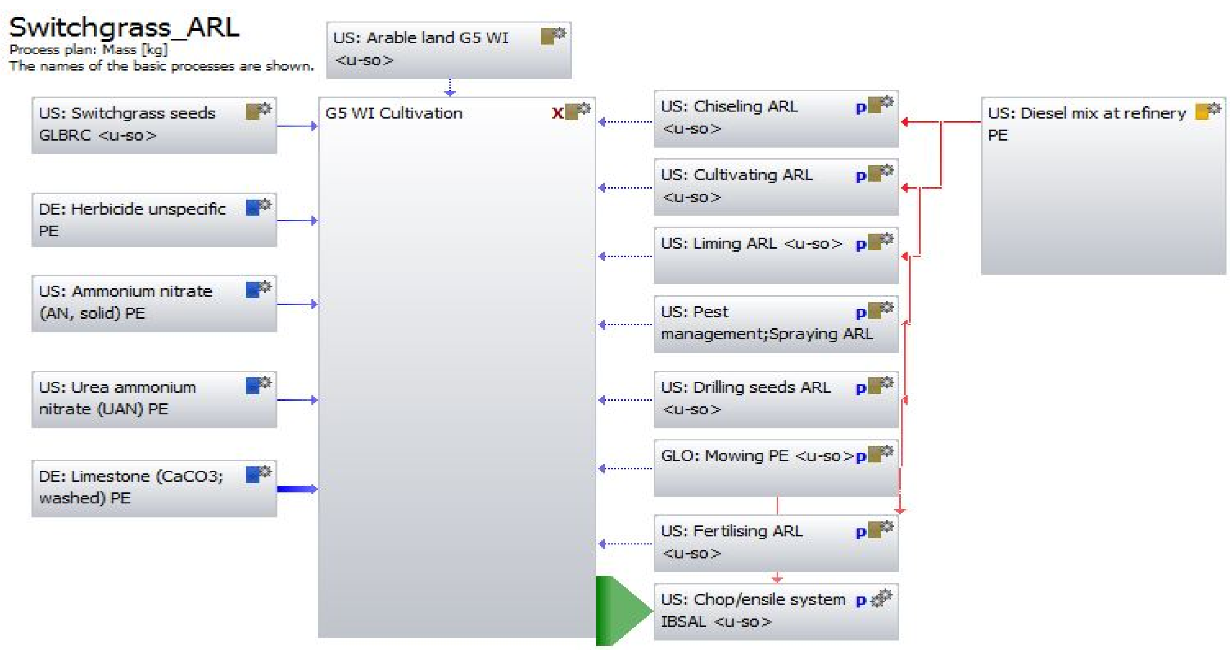

Figure 21 Cultivation phase model in Gabi 6.0 for switchgrass at ARL from year 2008 to 2022. Boxes represent individual unit processes. Arrow width is based on mass (kg) value of flows. Details of model inputs are in appendix.

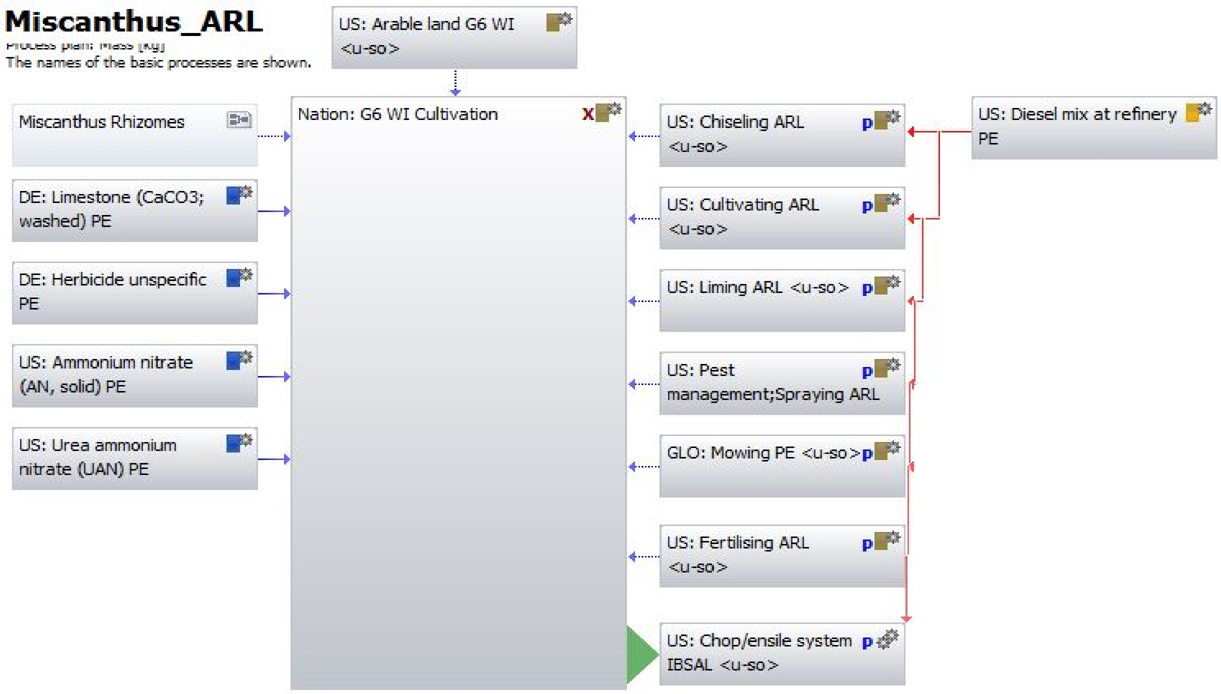

Figure 22 Cultivation phase model in Gabi 6.0 for giant miscanthus at KBS from year 2008 to 2022. Boxes represent individual unit processes. Arrow width is based on mass (kg) value of flows. Details of model inputs are in appendix.

Life cycle impact assessment

Midpoints impact categories vs. Endpoints impact categories

Along the cause-effect chain, there are two links or levels: midpoint and endpoints. Midpoints, prior to endpoints, reflect the potential problem areas in the system. Midpoint characterization indicators include global warming potential, eutrophication potential, acidification potential (air or aqueous), etc. Endpoints, which focus even further downstream, look at potential damage that could be caused by the midpoints. Endpoint characterization indicators include human health, climate change, etc. Both midpoints methodology and endpoint methodology have pros and cons. Midpoint methodology brings less uncertainty and is easier to quantify than endpoints methodology (Bare, 2002). For purposes of this study midpoint methodology is emphasized. Figure 23 shows the midpoints and endpoints categories and their relationships.

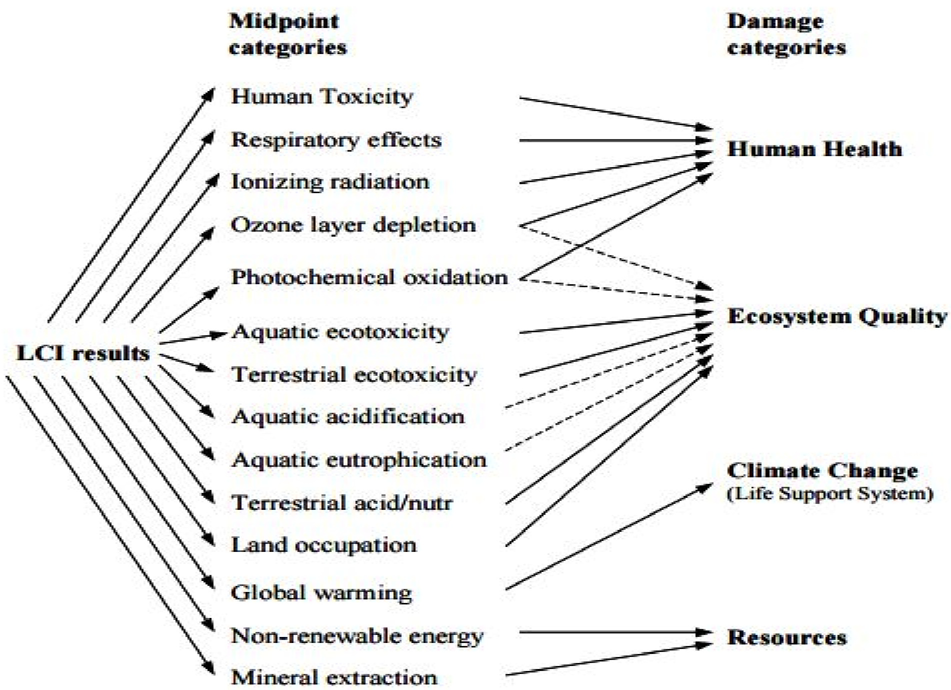

Figure 23 Impact categories and pathways adapted from the IMPACT 2002+ methodology (European Commission - Joint Research Centre - Institute for Environment and Sustainability 2010) .

Characterization model

The adopted model for LCIA in this study is TRACI 2.1: the tool for the reduction and assessment of chemical and other environmental impacts, which was developed by the U.S. Environmental Protection Agency and is most suitable for studies conducted in United States. Global warming potential, eutrophication potential and acidification potential (air) were of interest in this study. Midpoint impact indicators are calculated by multiplying the mount of stressor emission and its potency and then summing to a total. Equation 3, is adapted from TRACI 2.1 and depicts the calculation of a midpoints impact indicator for a specific impact category (Bare, 2012).

$$\mathrm{Pi} = \sum_i Mi \times \mathrm{CFi} \qquad \text{Equation 3}$$

where Pi=the potential impact of all stressors(i) for the impact category of concern, CFi=Characterization factor of stressor(i) for the impact category of concern, Mi= the mass of stressor(i).

Energy return on investment

Equation 4 was used to calculate energy return on invest (EROI) to evaluate the efficiency of non-renewable energy production from switchgrass and giant miscanthus during the production phase. Ethanol yield potentials for switchgrass and giant miscanthus are published in Sanford et al. 2017. Switchgrass ethanol yield was 134.21 l Mg^{-1} at KBS and 142.2 l ha^{-1} at ARL. Ethanol yield from giant miscanthus was 138.44 l Mg^{-1} at KBS and 143.21 l Mg^{-1} at ARL. The published lower heating value of ethanol (21.2 MJ l^{-1}) was used this study (Schmer et al. 2008) to make energy balance comparisons between cropping systems. Renewable energy acquired was calculated by multiplying the lower heating value of ethanol by the ethanol yield potentials from switchgrass and giant miscanthus. Average biomass yield of switchgrass and giant miscanthus are presented in Table 40 of the appendix.

$$\text{EROI} = \frac{Renewable\ Energy\ Acquired}{Non{-}renewable\ energy\ required} \qquad \text{Equation 4.}$$

RESULTS AND DISCUSSION

Climatology Data Summary

Extreme weather events at both KBS and ARL delayed the establishment of some cropping systems. At ARL, giant miscanthus was not successfully established until 2010 due to extreme temperatures over the 2008/2009 winter. Similarly, at KBS, the switchgrass was spot-reseeded in 2009 following extreme precipitation during the 2008 growing season. A full discussion of weather related establishment complication (2008 and 2009) can be found in Sanford et al. (2016).

At both locations, monthly average air temperatures did not differ significantly during the growing phase (Table 21 & 22). However, a higher monthly average temperature tendency was noticeable when compared to 30-year average temperatures. July was the hottest month during study years. Generally, KBS monthly average air temperature was slightly higher than Arlington`s during the study period.

It is noteworthy that 2012 was the driest year during the study period and was also drier than the 30-year average at both locations. At KBS, total precipitation during the growing season in 2010, 2012, 2014 and 2015 was 7.7%, 44.5%, 13.6% and 16.7% respectively drier than the 30-year average. At Arlington, total precipitation during growing phase in 2011 and 2012 was 24.2% and 39.8% respectively drier than the 30-year average. With the exception of the 2012 drought year, June and July tended to have more precipitation than the other months.

Table 21 Monthly mean temperatures (ºC) and precipitation (cm) during the study years (2010-15) compared to the 30-years means (1986-2015) at KBS, MI. The 30-years averages were obtained from NOAA website.

Location	Cropping Phase	Mean Temperature (ºC)							
		Month	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	30-yrs Avg.
KBS	Growth Phase	Apr	13.2	7.9	9.3	8.2	8.4	9.0	9.8
		May	16.3	16.3	16.4	17.4	14.9	16.3	15.9
		Jun	22.1	21.4	21.5	20.4	21.4	19.2	21.1
		Jul	23.8	24.8	25.9	23	19.1	20.7	23.1
		Aug	22.7	21.7	21.7	21.1	20.7	20.3	21.8
		Sep	18.1	16.9	16	18.2	16.7	19.1	17.8
	Fall Harvest	Oct	12.9	11.4	10.1	11.9	11.3	11.1	11.3
		Nov	5.5	7.2	3.9	3.8	0.9	7.2	5.0
	Over-Winter Phase	Dec	-4.3	1.6	1.8	-3.8	0.1	4.2	-1.1
		Jan	-3.9	-6.6	-1.4	-2.1	-9	-3.2	-3.1
		Feb	-3.7	-3.3	-0.1	-4	-7.4	-2.0	-2.5
		Mar	5.4	1.1	10.9	0.7	-2.5	5.1	3.3

Location	Cropping Phase	Total Precipitation (cm)							
		Month	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	30-yrs Avg.
KBS	Growth Phase	Apr	7.3	13.3	10.7	21.4	6.7	3.6	9.5
		May	3	17.2	7.3	11.3	8.1	0.0	9.9
		Jun	20.8	5.7	4	10.8	14.7	20.7	9.5
		Jul	15.2	23.2	3.9	11.7	10.4	11.3	10.0
		Aug	1.8	9.8	4.9	13.6	7.4	11.9	11.5
		Sep	9.4	7.6	3.8	1.9	6.6	4.4	11.9
	Fall Harvest	Oct	4.5	9	14.2	5.5	9.4	3.8	9.1
		Nov	4.6	10.4	1.4	11.3	8.2	5.7	8.4
	Over-Winter Phase	Dec	2.9	9.7	5.6	6.4	3.3	5.7	6.6
		Jan	2.2	2.7	8.3	5.1	7.5	2.5	6.5
		Feb	4.4	3.5	6.8	18.8	6	1.9	4.4
		Mar	2.7	7.3	10.4	2.9	5.2	5.1	6.2

Table 22 Monthly mean temperatures (ºC) and precipitation (cm) during the study years (2010-15) compared to the 30-years means (1986-2015) at Arlington, WI. The 30-years averages were obtained from NOAA website.

Location	Cropping Phase	Month	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	30-yrs Avg.
			Mean Temperature (ºC)						
ARL	Growth Phase	Apr	9.1	5.2	6.4	4.1	4.9	8.3	8.3
		May	14	12	15	13.3	12.6	15.6	14.5
		Jun	18.9	18.2	19.8	17.9	19.2	20.4	20.0
		Jul	21.8	22.7	24.3	20.4	18.1	21.0	22.2
		Aug	21.4	19.9	19.4	19.2	20.6	21.3	20.9
		Sep	14.3	13.6	14.3	15.5	15.4	17.7	16.5
	Fall Harvest	Oct	10	9.7	6.8	7.9	8.9	10.4	9.7
		Nov	2.1	2.1	1.2	-0.8	-2.2	2.3	2.7
	Over-Winter Phase	Dec	-9.3	-2.9	-3.7	-10.6	-2.5	-0.2	-4.0
		Jan	-9.9	-10.6	-6.3	-8.7	-14.6	-6.7	-6.8
		Feb	-6.9	-8	-2.9	-8.5	-13.6	-2.8	-5.1
		Mar	1.9	-2.1	7.6	-5.3	-5.6	4.4	1.4

Location	Cropping Phase	Month	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	30-yrs Avg.
			Total Precipitation (cm)						
ARL	Growth Phase	Apr	9.3	9	7.8	13.8	16.4	11.1	9.3
		May	10.5	6.1	7.5	15.3	7.1	10.6	9.3
		Jun	19.3	10.4	0.7	19.1	23.7	8.0	12.1
		Jul	23.6	6.3	10.1	7.6	4.8	12.8	10.6
		Aug	11.9	3.7	7.3	4.5	9.4	10.4	10.5
		Sep	11.5	9.8	2.6	7.5	4.5	15.2	8.0
	Fall Harvest	Oct	4.3	4	10.1	3.9	7	6.9	5.8
		Nov	3.6	8.3	2.8	6.7	4.4	12.1	5.8
	Over-Winter Phase	Dec	4.2	6	6	2.9	2.9	8.5	4.4
		Jan	4.3	1.5	2	5.7	1.9	2.5	3.4
		Feb	2.8	1.5	2.4	4.8	2.6	1.4	3.4
		Mar	2.6	8.6	6.2	6	2.4	5.4	5.8

Table 23 Total growth phase (April - September) precipitation (cm) during study years (2010-15) compared and 30-years average (1986-2015).

Location	2010	2011	2012	2013	2014	2015	30-yrs Avg.
	Total Precipitation (cm)						
KBS	57.5	76.8	34.6	70.8	53.8	51.9	62.3
ARL	86.2	45.3	36	67.9	66	68.1	59.8

Life Cycle Impact Assessment and Interpretation

One of the objectives of this LCA study is to identify the potential risk hotspots of switchgrass and giant miscanthus during the biomass production phase. Contribution analyses were conducted for this purpose at both study locations.

Global Warming Potential

Figure 24, reveals that switchgrass and giant miscanthus cropping systems at both locations had a net negative global warming potential (-5245.9 kg CO_2-equiv. ha^{-1} and -3728.2 kg CO_2-equiv. ha^{-1} for KBS and ARL, respectively), which indicates net carbon removal from the atmosphere. Consistent with Schmer et al., (2008), the main driver for the net negative global warming potential for both systems was the significant displacement of GHG emissions by replacing conventional gasoline with ethanol derived from the harvested biomass. When focusing only on field level agricultural inputs, ΔSOC and soil N_2O emissions, both switchgrass and miscanthus cropping systems at KBS still had negative global warming potentials (-415 kg CO_2-equiv. ha^{-1} yr^{-1} and -306 kg CO_2-equiv. ha^{-1} yr^{-1}, respectively). In contrary, when omitting gasoline displacement, switchgrass and giant miscanthus had a positive global warming potential at ARL (296 kg CO_2-equiv. ha^{-1} $yr^{-}$and 97.3 kg CO_2-equiv. ha^{-1} yr^{-1}). Sinistore et al. (2015) reported in their research that global warming potential during switchgrass biomass production ranged from -22.2 kg CO_2-equiv. ha^{-1} yr^{-1} to -6.84 kg CO_2-equiv. ha^{-1} yr^{-1} when converted to a land area basis. The differences between this study and the research conducted by Sinistore et al. (2015) is attributable to different ΔSOC and N_2O emission values. Gelfand et al. (2013) concluded a similar global warming potential (-520 ± 920 kg CO_2-equiv. ha^{-1} yr^{-1}) from alfalfa production. Figure 35 shows that dLUC drove the switchgrass cropping system and miscanthus cropping system to be carbon sinks at KBS. At Arlington, even though switchgrass and giant miscanthus cropping system`s carbon emissions are positive, dLUC sequestered a considerable amount of carbon. Fertilizer manufacture/production, (no matter which product) and N_2O emission were the two biggest contributors to global warming potential for both studied cropping systems at both locations. During nitrogen fertilizer manufacture, carbon dioxide emission from energy combustion (mainly natural gas) and nitrous oxide emission from nitric acid production are two major GHGs emissions (Wood and Cowie 2004). In this study, emission factors of UAN for KBS and ammonium nitrate (UA) for ARL are 1.85 kg CO_2-equiv. kg UAN^{-1} and 3.11 kg CO_2-equiv. kg UA^{-1}, respectively. Wood et al. (2004) summarized that AN and UAN have emission factors of 2.56 kg CO_2-equiv. kg UA^{-1} and 1.84 kg CO_2-equiv. kg UAN^{-1}, which are similar to this study. N_2O emissions were closely related to fertilizer use (Oates et al. 2016). The switchgrass and giant miscanthus cropping systems had higher global warming potential at ARL than at KBS due to higher N_2O emissions at ARL. Diesel consumption of harvesting equipment was calculated on a biomass yield basis in this study. Table 40 shows giant miscanthus had yielded nearly three times more than switchgrass at KBS, which lead to nearly three times the diesel combustion emissions for harvesting of giant miscanthus relative to switchgrass. Because there was not a large difference of N_2O emissions between switchgrass and giant miscanthus cropping systems at KBS, harvest equipment diesel emissions of giant miscanthus harvest resulted in a greater giant miscanthus global warming potential than switchgrass. At ARL, with a lower relative yield of giant miscanthus than switchgrass, N_2O emissions from the switchgrass cropping system were higher enough to result in the switchgrass cropping system having the higher agricultural input and soil factors global warming potential than giant miscanthus.

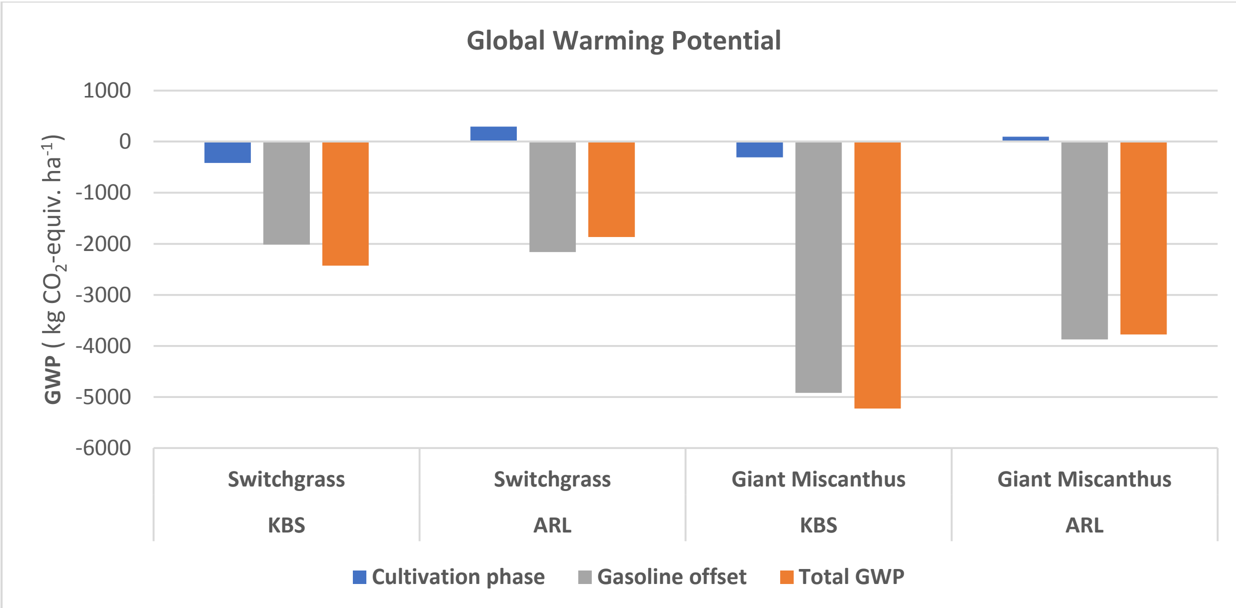

Figure 24 Global warming potential on 100 years horizon for switchgrass and giant miscanthus cultivation phase amortized to 15-years production with gasoline offsets at both KBS and ARL. Results are from TRACI 2.1 (U.S. EPA) calculations.

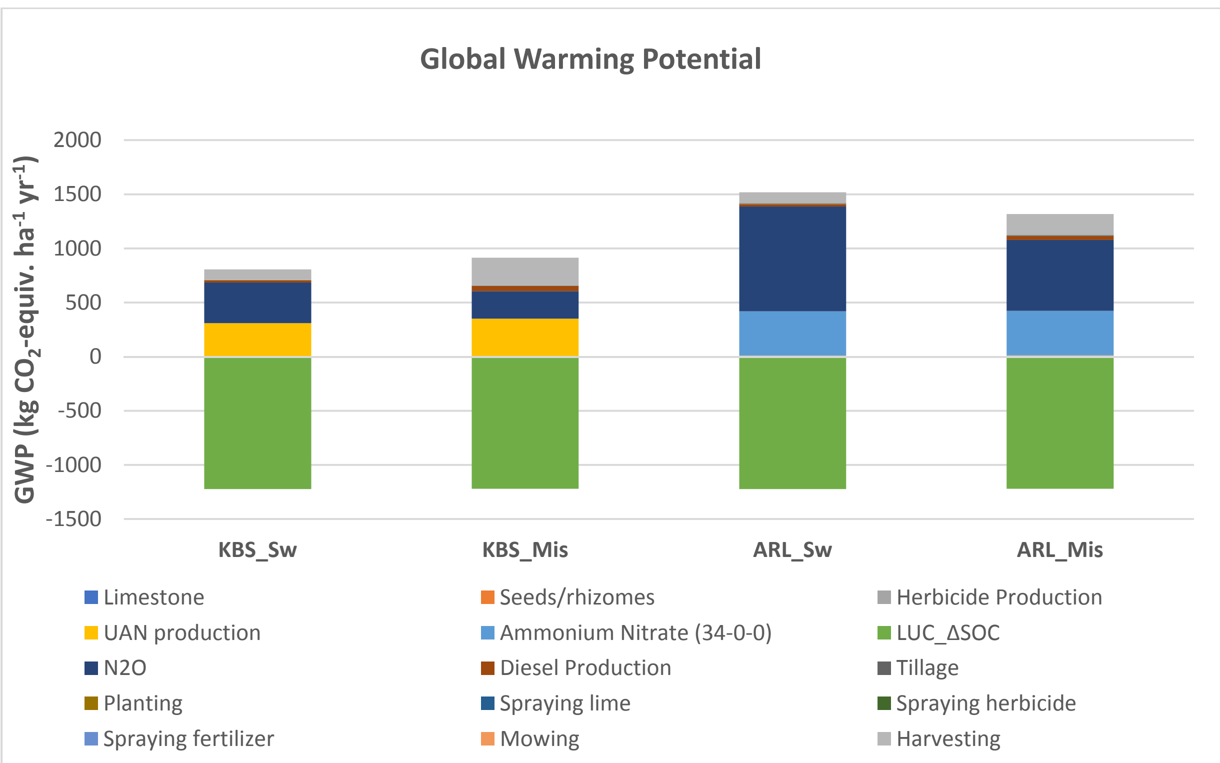

Figure 25 Distribution analysis on global warming potential on 100 years horizon for switchgrass and giant miscanthus cultivation phase amortized to 15-years production (year 2008-2022) at both KBS and ARL. Results are from TRACI 2.1 (U.S. EPA) calculations. KBS_Sw= switchgrass at KBS; KBS_Mis= giant miscanthus at KBS; ARL_Sw= switchgrass at ARL; ARL_Mis= giant miscanthus at ARL.

Acidification Potential

Acidification is caused by accumulating acidifying substances, like ammonia (NH_3), sulfur dioxide (SO_2) and nitrogen oxide (NOx) in the lower atmosphere leading to “acid rain”. The acidifying substances are attributable to fertilizer use and combustion of diesel during farming activities (Brentrup et al. 2004). Figure 26 shows that the switchgrass cropping system (77.1 H^+ moles-equiv. ha^{-1} yr^{-1}) had a low acidification potential relative to giant miscanthus (170 H^+ moles-equiv. ha^{-1} yr^{-1}) at KBS. Similarly, at ARL, the switchgrass cropping system (82.7 H^+ moles-equiv. ha^{-1} yr^{-1}) also had a lower acidification potential than giant miscanthus (134 H^+ moles-equiv. ha^{-1} yr^{-1}). Sinistore et al. (2015) reported that switchgrass production using 60 kg N ha-1 yr^{-1} application rate had a similar acidification potential to what we found in this study. Similarly, Brandao et al., (2011) reported giant miscanthus feedstock production having a similar AP as this study. Fertilizer use in biomass production has been identified as the main cause of acidification and eutrophication, especially nitrogen fertilizer (Cherubini et al. 2010). However, fertilizer use, whether liquid UAN or granular ammonium nitrate (34-0-0)) contributed the second most acidification potential in the two cropping systems of our study. Due to diesel emissions, harvesting practices were the biggest AP contributors at both KBS and ARL. Higher biomass yield of giant miscanthus lead to higher diesel consumption for harvesting of giant miscanthus than switchgrass, which in turn resulted in a higher AP for giant miscanthus.

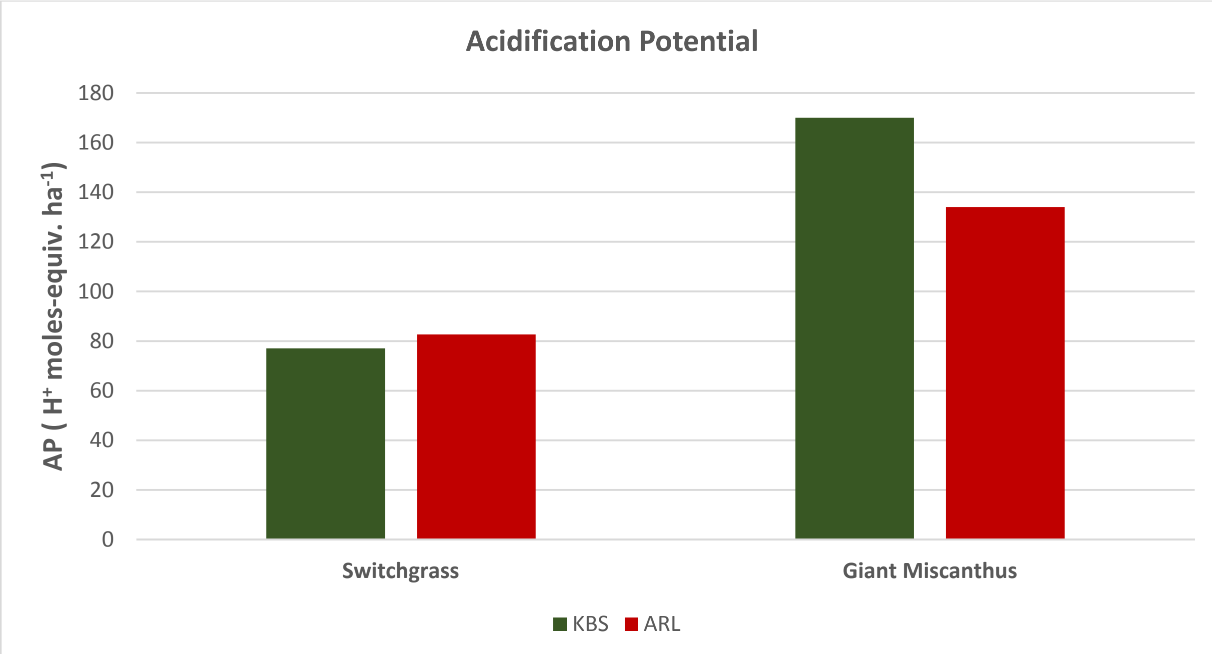

Figure 26 Acidification potential for switchgrass and giant miscanthus cultivation phase amortized to 15-years production at both KBS and ARL. Results are from TRACI 2.1 (U.S. EPA) calculations.

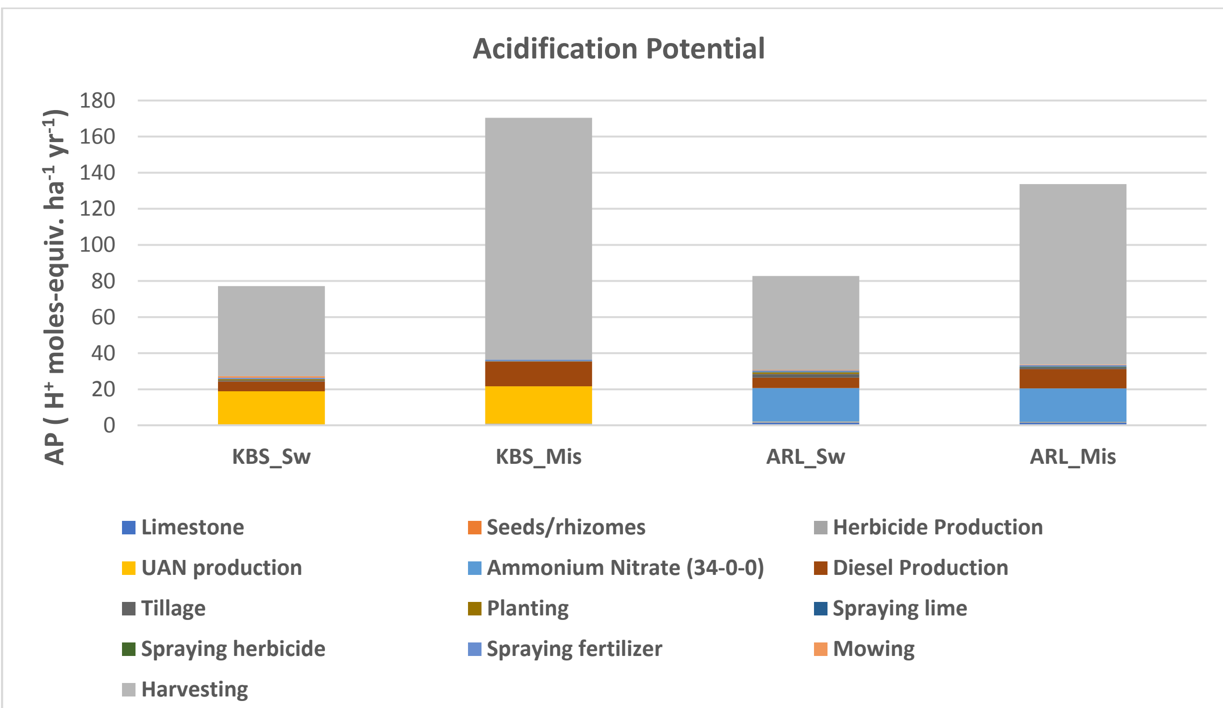

Figure 27 Distribution analysis on acidification potential for switchgrass and giant miscanthus cultivation phase amortized to 15-years production (year 2008- 2022) at both KBS and ARL. Results are from TRACI 2.0 (U.S. EPA) calculations. KBS_Sw= switchgrass at KBS; KBS_Mis= giant miscanthus at KBS; ARL_Sw= switchgrass at ARL; ARL_Mis= giant miscanthus at ARL.

Eutrophication Potential

Eutrophication is induced by excess nutrient depositions, such as NH_3, NO_3-N and NOx, which eventually leads to anoxia (Brentrup et al. 2004). The main factor causing eutrophication has been determined to be fertilization during biomass feedstock production (Hasler et al. 2015). In figure 28, the switchgrass cropping system (0.18 kg N-equiv. ha^{-1} yr^{-1}) had lower eutrophication potential than the giant miscanthus cropping system (0.186 and 0.12 kg N-equiv. ha^{-1} yr^{-1}) respectively at KBS and ARL. Eutrophication potential in other studies on switchgrass and giant miscanthus production ranged from negative -8.81 kg N-equiv. ha^{-1} yr^{-1} to positive 2.35 kg N-equiv. ha^{-1} yr^{-1} (Brandão, Milà i Canals, and Clift 2011; Sinistore et al. 2015). The eutrophication potential from this study falls into the range of that published for other studies (Brentrup et al. 2004; Sinistore et al. 2015). Fertilizer use was the main contributor to eutrophication potential for the switchgrass cropping system at both locations. Eighty eight percent of switchgrass' eutrophication potential was found to be attributable to N fertilization (Cherubini et al. 2010). Harvesting practice also contributed considerable eutrophication potential for switchgrass at both locations. For giant miscanthus, harvesting practice was the biggest eutrophication potential contributor at both locations due to higher biomass yield. Diesel production, mowing, and limestone production also contributed significantly to the eutrophication potential for both the switchgrass and miscanthus cropping systems.

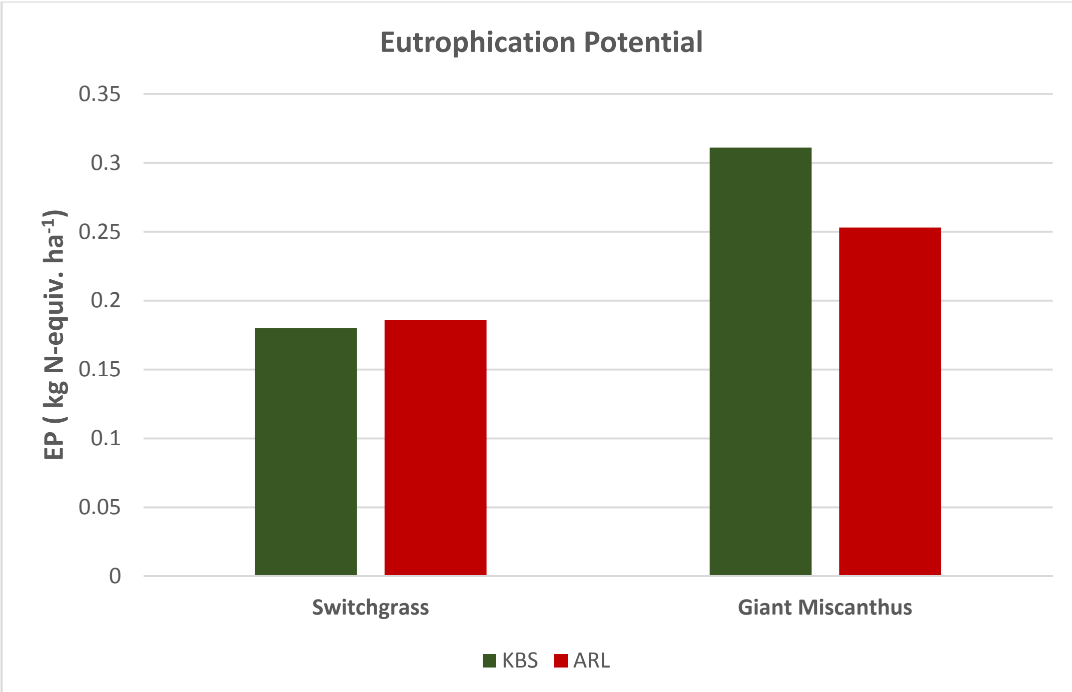

Figure 28 Acidification potential for switchgrass and giant miscanthus cultivation phase amortized to 15-years production at both KBS and ARL. Results are from TRACI 2.1 (U.S. EPA) calculations.

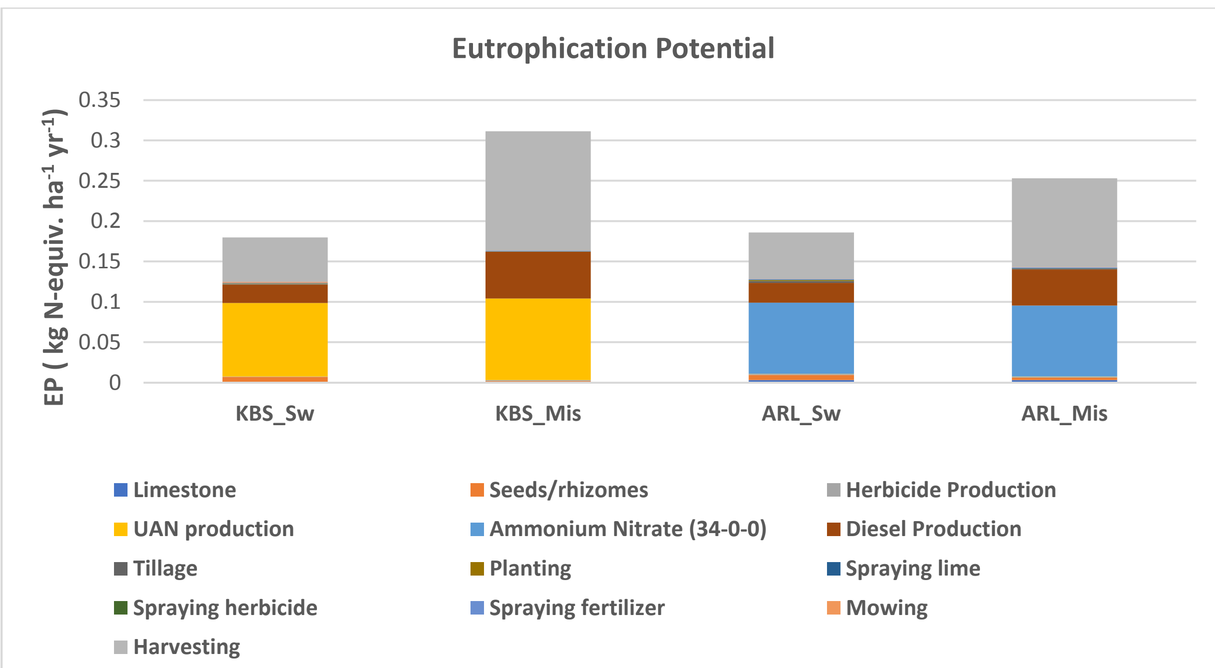

Figure 29 Distribution analysis on eutrophication potential for switchgrass and giant miscanthus cultivation phase amortized to 15-years production (year 2008-2022) at both KBS and ARL. Results are from TRACI 2.1 (U.S. EPA) calculations. KBS_Sw= switchgrass at KBS; KBS_Mis= giant miscanthus at KBS; ARL_Sw= switchgrass at ARL; ARL_Mis= giant miscanthus at ARL.

Non-renewable energy depletion

This LCA study involves two potential biofuel feedstock production systems, and as such, non-renewable energy depletion is a relevant factor to evaluate and EROI. Cropping systems at KBS (Switchgrass: 4490.09 MJ ha^{-1} yr^{-1}; giant miscanthus: 7265.71 MJ ha^{-1} yr^{-1}) required more non-renewable energy than ARL (Switchgrass: 4412.53 MJ ha^{-1} yr^{-1}; giant miscanthus: 5859.66 MJ ha^{-1} yr^{-1}). Energy inputs used in this study are consistent with that reported for other studies, which ranged from 2340 MJ ha^{-1} yr^{-1} to 6451 MJ ha^{-1} yr^{-1} for switchgrass (Schmer et al. 2008) and averaged 3860 MJ ha^{-1} yr^{-1} for miscanthus (Ercoli et al. 1999). The giant miscanthus cropping system used more energy than switchgrass at both KBS and ARL mainly due to higher diesel consumption for harvesting of giant miscanthus. Nitrogen fertilizer manufacture/production was the largest contributor of non-renewable energy for the switchgrass cropping system at both locations, regardless of which fertilizer source was used. Schmer et al. (2008) estimated that N fertilizer consumes 67% of agricultural energy input during switchgrass cultivation. Harvesting was the second greatest contributor to non-renewable energy depletion for the switchgrass cropping system at both locations. Farming practices, like planting, tillage, spraying herbicides and lime application generally only happen during the first 1-2 years of a perennial crop stand, which results in less energy use relative to N fertilization and harvesting activities which occur annually. Since diesel consumption for harvesting biomass was primarily affected by biomass yield, harvesting practice consumed most non-renewable energy for giant miscanthus at both locations due to higher giant miscanthus biomass yield. More energy inputs required for giant miscanthus rhizome production relative to switchgrass seed production also contributed significantly. It is noteworthy that KBS had a higher energy requirement than ARL did. In summary, the main nitrogen fertilizer source at KBS was UAN and at ARL, ammonium nitrate was used. The manufacturing processes of these two different nitrogen fertilizer products have different non-renewable energy consumption values. Giant miscanthus had higher energy returned on energy invested during cultivation phase at both locations mainly due to its higher biomass yield.

Nitrogen fertilizer manufacture/production is very energy-intensive process, which in turn increases the potential for causing negative environmental impacts, like global warming potential, acidification potential, and eutrophication potential (Meeusen and Weidema 2000). Nitrogen fertilizer use strategies influence how much N will be absorbed by crops or lost in forms of greenhouse gases (N_2O) or leaching (NO_3^-). The ideal situation is the nitrogen application timing synchronizing with crop needs and avoiding applying nitrogen before heavy rainfall events.

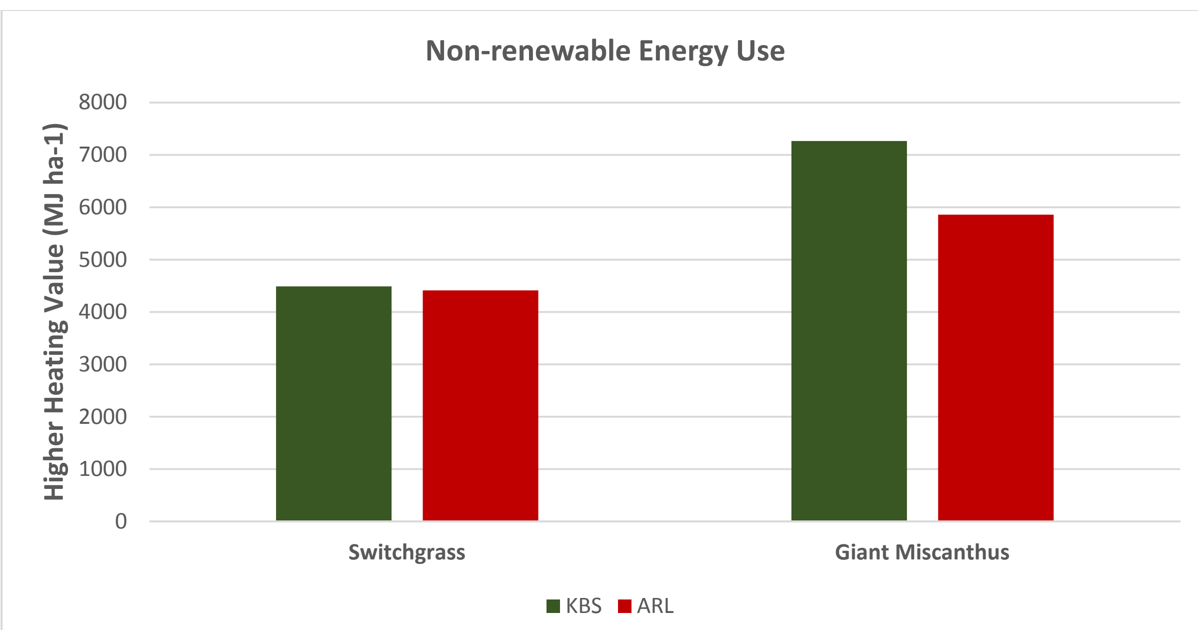

Figure 30 Non-renewable energy use for switchgrass and giant miscanthus cultivation phase amortized to 15-years production at both KBS and ARL.

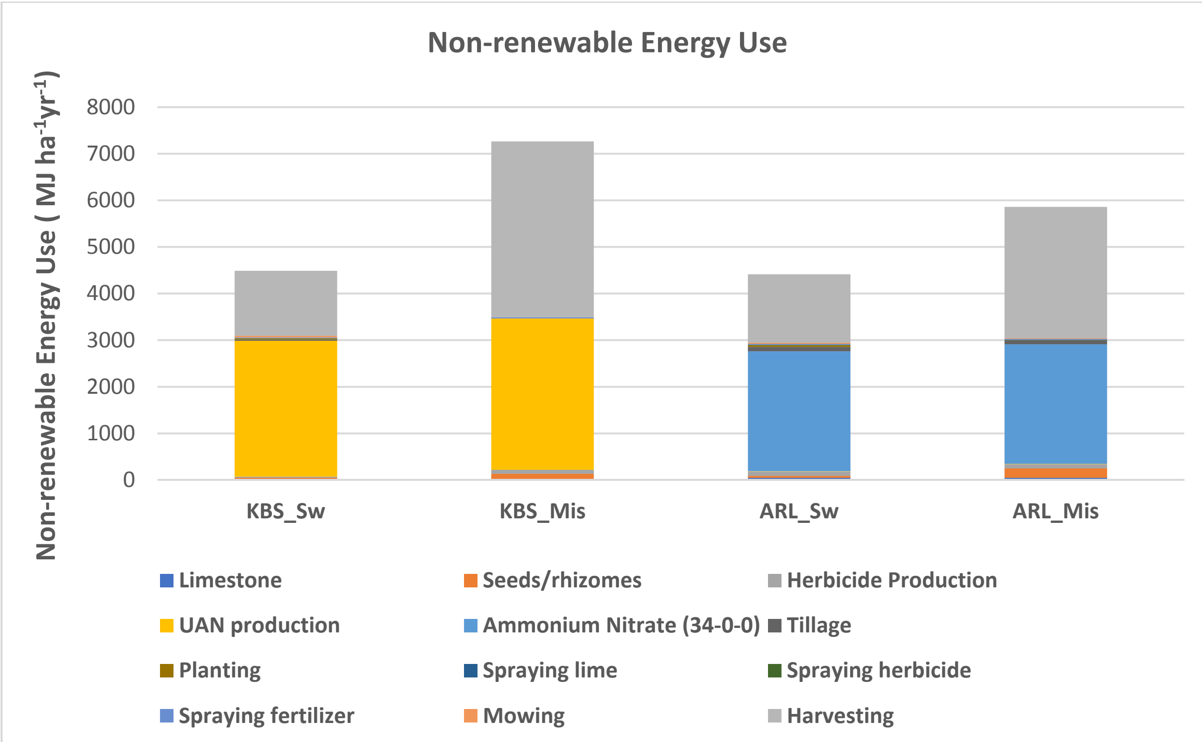

Figure 31 Distribution analysis on eutrophication potential for switchgrass and giant miscanthus cultivation phase amortized to 15-years production (year 2008-2022) at both KBS and ARL. Results are from Gabi 6.0 calculation. KBS_Sw= switchgrass at KBS; KBS_Mis= giant miscanthus at KBS; ARL_Sw= switchgrass at ARL; ARL_Mis= giant miscanthus at ARL.

Table 24 Energy return on investment (EROI) for switchgrass and giant miscanthus cultivation phase amortized to 15-years production.

	EROI	
Location	Switchgrass	Miscanthus
KBS	4.27	7.30
ARL	4.80	7.00

Sensitivity Analysis for GWP

Sensitivity analysis is an important tool to determine which parameters have more power to change the overall result. Sensitivity analysis was conducted for GWP to examine its sensitivity to different variables including: ΔSOC; N_2O emission; and, fertilizer application rate. Cherubini et al. (2010) and Bessou et al. (2013) found that SOC, N_2O and fertilizer are important GWP contributors in the agricultural sector. We evaluated a range of variable changes between +30% to -30%. Sensitivity was determined by the slopes in figure 32.

Global warming potential was most sensitive to ΔSOC for both switchgrass and giant miscanthus cropping systems at both locations. Soil organic carbon change plays a critical role in total carbon balance during bioenergy feedstock production (Shemfe et al. 2016; Brandão, Milà i Canals, and Clift 2011). Management practices, such as no-tillage and winter cover crops in annual crop systems, can help preserve soil organic carbon (West et al. 2002; Lal 2003). Global warming potential was less sensitive to N fertilizer rate than N_2O emissions for the studied cropping systems with exception being the giant miscanthus cropping system at KBS. N_2O emissions are generally associated with nitrogen fertilizer application rates (Hoben et al. 2011). Applying a nitrification inhibitor can be a way to reduce N_2O emissions (Dobbie and Smith 2003).

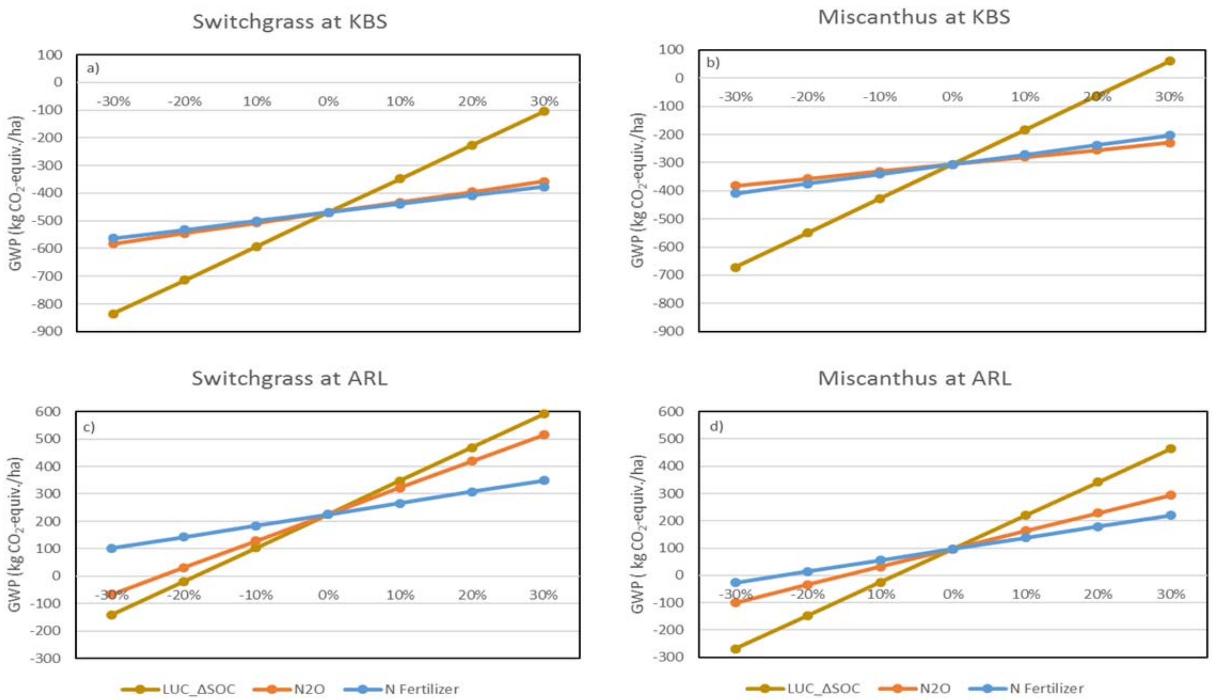

Figure 32 Sensitivity analysis on global warming potential (GWP) on 100 years horizon responses to LUC_SOC, N_20 emission and N fertilizer use for switchgrass and giant miscanthus cultivation phase amortized to 15-years production (year 2008-2022) at KBS and ARL. Results are from TRACI 2.1 (U.S. EPA) calculations. Variables change from +30% to -30%.

Completeness Checks

Completeness checks serve the purpose of filling data gaps. A rigorous check makes it easier to track data necessary to fully meet the goal and scope of the study. Table 44 shows that all data are complete.

Consistency Checks

All assumptions being made in this study are consistent with normal agricultural practices, such as harvest/storage method. A full list of assumptions for this study is given in the appendix. Majority of data are field data from GLBRC database, other are from Gabi database and literature and specific for U.S. Age of the data is the most recent data. Overall, the consistency of data is adequate for this LCA study.

CONCLUSION

Switchgrass (-2201.2 kg CO_2-equiv. ha^{-1} and -1675.8 kg CO_2-equiv. ha^{-1} for KBS and ARL, respectively) and giant miscanthus (-5245.9 kg CO_2-equiv. ha^{-1} and -3728.2 kg CO_2-equiv. ha^{-1} for KBS and ARL, respectively) cropping systems at both locations had a net negative global warming potential. Gasoline displacement from bioethanol production and ΔSOC associated with land use change to a perennial system were the greatest factors improving net GWP. When omitting gasoline displacement and including only field level agricultural inputs, ΔSOC and soil N_2O emissions, the switchgrass cropping system (-415 kg CO_2-equiv. ha^{-1} yr^{-1}) had a lower global warming potential than the giant miscanthus cropping system (-306 kg CO_2-equiv. ha^{-1} yr^{-1}) at KBS and, conversely, the giant miscanthus cropping system (97.3kg CO_2-equiv. ha^{-1} yr^{-1}) had lower global warming potential than the switchgrass cropping system (296 kg CO_2-equiv. ha^{-1} yr^{-1}) at ARL. Nitrous oxide soil emissions and nitrogen fertilizer manufacture were the biggest global warming potential contributors in each system. Giant miscanthus cropping systems had a higher acidification potential, eutrophication potential and non-renewable energy use than switchgrass cropping systems at both KBS and ARL. However, giant miscanthus was favorable in energy return over invest (EROI) during the production phase due to higher biomass yield. For the switchgrass cropping system, fertilizer manufacture/production was the biggest contributor of acidification potential, eutrophication potential and non-renewable energy use at both KBS and ARL. For the giant miscanthus cropping system, harvesting activities contributed the most acidification potential, eutrophication potential and non-renewable energy use at both KBS and ARL due to higher biomass yield of giant miscanthus.

Because the quality of life cycle analysis is heavily reliant on data quality and assumptions are inevitably made, evaluations of additional switchgrass and miscanthus production systems in other environments would be helpful in evaluating and comparing these important bioenergy cropping systems across a range of regional growing conditions.

APPENDIX

APPENDIX

Table 25 Switchgrass variety and seeding rates at KBS and ARL for life cycle inventory.

Location	Crop	Variety	Year	Seeding Rate	Unit	Data Quality
KBS	Switchgrass	Cave in Rock	2008	7.5	kg ha^{-1}	1,1,1,1,1
			2009	7.5	kg ha^{-1}	1,1,1,1,1
Arlington			2008	7.5	kg ha^{-1}	1,1,1,1,1
			2009	6.0	kg ha^{-1}	1,1,1,1,1

Table 26 Switchgrass variety and seeding rates at KBS and ARL for life cycle inventory.

Location	Crop	Variety	Year	Seeding Rate	Unit	Data Quality
KBS	Giant Miscanthus	M. giganteous	2008	17215.9	rhizome ha^{-1}	1,1,1,1,1
ARL			2008	17215.9	rhizome ha^{-1}	1,1,1,1,1
			2010	3942.6	rhizome ha^{-1}	1,1,1,1,1
			2012	4304.0	rhizome ha^{-1}	1,1,1,1,1
			2013	4304.0	rhizome ha^{-1}	1,1,1,1,1

Table 27 Switchgrass herbicide use and rates at KBS and ARL for life cycle inventory.

Location	Crop	Year	Herbicide (main ingredient)	Herbicide rate	Unit	Note	Data Quality
KBS	Switch grass	2009	Drive (quinclorac)	0.6	kg ha^{-1}	Post-emerge	1,1,1,1,1
		2010	2,4-D amine	2.2	kg ha^{-1}	Broadleaf-post	1,1,1,1,1
Arlington		2008	Honcho plus(glyphosate)+AMS	0.8	kg ha^{-1}	Burdown	1,1,1,1,1
			Honcho plus(glyphosate)+AMS	0.8	kg ha^{-1}	Pre-emerge	1,1,1,1,1
		2009	2,4-D ester	2.2	kg ha^{-1}	Post-emerge	1,1,1,1,1
			Mirage Plus(glyphosate)+AMS	1	kg ha^{-1}	Pre-emerge	1,1,1,1,1
		2010	2,4-D ester	0.7	kg ha^{-1}	Post-emerge	1,1,1,1,1

Table 27 (cont`d)

Arlington	Switchgrass	2011	Roundup Power Max	1.7	kg ha^{-1}	Post-emerge	1,1,1,1,1
		2012	Clarity (3,6-dichloro-o-anisic acid)	0.2	kg ha^{-1}	Broadleaf-post	1,1,1,1,1
			2,4-D LV4 Ester	1.2	kg ha^{-1}	Broadleaf-post	1,1,1,1,1
			Quinclorac SPC 75 DF	0.3	kg ha^{-1}	Broadleaf-post	1,1,1,1,1
			Quinclorac SPC 75 DF	0.6	kg ha^{-1}	Pre-emerge	1,1,1,1,1
		2014	2,4-D LV4 Ester	1.1	kg ha^{-1}	Pre-emerge	1,1,1,1,1

Table 28 Giant miscanthus herbicide use and rates at KBS and ARL for life cycle inventory.

Location	Crop	Year	Herbicide (main ingredient)	Herbicide rate	Unit	Note	Data Quality
KBS	Giant miscanthus	2009	Drive (quinclorac)	0.6	kg ha^{-1}	Post-emerge	1,1,1,1,1
		2010	2,4-D amine	0.9	kg ha^{-1}	Broadleaf-post	1,1,1,1,1
Arlington		2008	Dual II Magnum(S-metolachlor)	3.2	kg ha^{-1}	Pre-emerge	1,1,1,1,1
			2,4-D Ester 8 oz./ac	0.4	kg ha^{-1}	Broadleaf-post	1,1,1,1,1
		2009	Honcho plus(glyphosate)+AMS	0.8	kg ha^{-1}	Pre-emerge	1,1,1,1,1
			2,4-D Ester	0.7	kg ha^{-1}	Pre-emerge	1,1,1,1,1
			Glyphosate	1.7	kg ha^{-1}	Pre-emerge	1,1,1,1,1
		2010	Dual II Magnum+AMS	1.6	kg ha^{-1}	Pre-emerge	1,1,1,1,1
			2,4-D Ester	1.5	kg ha^{-1}	Post-emerge	1,1,1,1,1
			Roundup Power Max	1.7	kg ha^{-1}	Burndown	1,1,1,1,1
			Glyphosate (generic)	3.5	kg ha^{-1}	Burndown	1,1,1,1,1
		2011	2,4-D LV4 Ester	1.1	kg ha^{-1}	Burndown	1,1,1,1,1

Table 28 (cont`d)

			Roundup Power Max	2.9	kg ha^{-1}	Pre-emerge	1,1,1,1,1
			Prowl(pendimethalin)	1.7	kg ha^{-1}	Pre-emerge	1,1,1,1,1
			2,4-D LV4 Ester	0.7	kg ha^{-1}	Pre-emerge	1,1,1,1,1
			2,4-D LV4 Ester	0.7	kg ha^{-1}	Post-emerge	1,1,1,1,1
		2012	Clarity	1.7	kg ha^{-1}	Rescue App	1,1,1,1,1
Arlington	Giant miscanthus		Prowl(pendimethalin)	0.3	kg ha^{-1}	Pre-emerge	1,1,1,1,1
			Roundup Power Max	1.5	kg ha^{-1}	Burndown	1,1,1,1,1
			2,4-D LV4 Ester	0.8	kg ha^{-1}	Post-emerge	1,1,1,1,1
		2013	FS Transform Plus (adjuvant)	0.8	kg ha^{-1}	Burndown	1,1,1,1,1
			Prowl(pendimethalin)	2.2	kg ha^{-1}	Pre-emerge	1,1,1,1,1
			2,4-D LV4 Ester	1.1	kg ha^{-1}	Pre-emerge	1,1,1,1,1
		2014	Roundup Power Max	2.1	kg ha^{-1}	Pre-emerge	1,1,1,1,1

Table 29 Switchgrass fertilizer use and rates at KBS and ARL for life cycle inventory.

Location	Plot	Year	Fertilizer	Fertilizer rate	N rate	P rate	K rate	Unit	Data Quality
		2010	28% N (28-0-0)	200	56	0	0	kg ha^{-1}	1,1,1,1,1
		2011	28% N (28-0-0)	200	56	0	0	kg ha^{-1}	1,1,1,1,1
		2012	28% N (28-0-0)	72	20	0	0	kg ha^{-1}	1,1,1,1,1
KBS	Switchgrass	2013	28% N (28-0-0)	204	57	0	0	kg ha^{-1}	1,1,1,1,1
		2014	28% N (28-0-0)	204	57	0	0	kg ha^{-1}	1,1,1,1,1
		2015	28% N (28-0-0)	204	57	0	0	kg ha^{-1}	1,1,1,1,1
		2016	28% N (28-0-0)	204	57	0	0	kg ha^{-1}	1,1,1,1,1

Table 29 (cont`d)

Arlington	Switch grass		AMS	2	0	0	0	kg ha^{-1}	1,1,1,1,1
		2011	Ammonium Nitrate (34-0-0)	165	56	0	0	kg ha^{-1}	1,1,1,1,1
			28% UAN	6	2	0	0	kg ha^{-1}	1,1,1,1,1
		2012	Ammonium Nitrate (34-0-0)	168	57	0	0	kg ha^{-1}	1,1,1,1,1
		2013	Ammonium Nitrate (34-0-0)	168	57	0	0	kg ha^{-1}	1,1,1,1,1
		2014	Ammonium Nitrate (34-0-0)	168	57	0	0	kg ha^{-1}	1,1,1,1,1
		2015	Polymer Coated Urea (44-0-0)	128	56	0	0	kg ha^{-1}	1,1,1,1,1
		2016	Polymer Coated Urea (44-0-0)	128	56	0	0	kg ha^{-1}	1,1,1,1,1

Table 30 Giant miscanthus fertilizer use and rates at KBS and ARL for life cycle inventory.

Location	Plot	Year	Fertilizer	Fertilizer rate	N rate	P rate	K rate	Unit	Data Quality
KBS	Giant Miscanthus	2009	28% N (28-0-0)	276	77	0	0	kg ha^{-1}	1,1,1,1,1
		2010	28% N (28-0-0)	200	56	0	0	kg ha^{-1}	1,1,1,1,1
		2011	28% N (28-0-0)	200	56	0	0	kg ha^{-1}	1,1,1,1,1
		2012	28% N (28-0-0)	72	20	0	0	kg ha^{-1}	1,1,1,1,1
		2013	28% N (28-0-0)	204	57	0	0	kg ha^{-1}	1,1,1,1,1
		2014	28% N (28-0-0)	204	57	0	0	kg ha^{-1}	1,1,1,1,1
		2015	28% N (28-0-0)	204	57	0	0	kg ha^{-1}	1,1,1,1,1
		2016	28% N (28-0-0)	204	57	0	0	kg ha^{-1}	1,1,1,1,1

Table 30 (cont`d)

Arlington	Giant Miscanthus		AMS	2	0	0	0	kg ha^{-1}	1,1,1,1,1
			Ammonium Nitrate (34-0-0)	165	56	0	0	kg ha^{-1}	1,1,1,1,1
		2011	28% UAN	3	1	0	0	kg ha^{-1}	1,1,1,1,1
			28% UAN	168	57	0	0	kg ha^{-1}	1,1,1,1,1
		2012	Ammonium Nitrate (34-0-0)	168	57	0	0	kg ha^{-1}	1,1,1,1,1
		2013	Ammonium Nitrate (34-0-0)	168	57	0	0	kg ha^{-1}	1,1,1,1,1
		2014	Ammonium Nitrate (34-0-0)	168	57	0	0	kg ha^{-1}	1,1,1,1,1
		2015	Polymer Coated Urea (44-0-0)	128	56	0	0	kg ha^{-1}	1,1,1,1,1
		2016	Polymer Coated Urea (44-0-0)	128	56	0	0	kg ha^{-1}	1,1,1,1,1

Table 31 Farming practice activities and diesel consumption for switchgrass at KBS for life cycle inventory (Stein 2015)

Location	Crop	Year	Field activity	Field Pass No.	Diesel Consumption	Unit	Data Quality
KBS	Switch grass	2008	Soil finishing	2	7.18	kg ha^{-1}	2,1,1,1,2
			Cultimulcher	1	3.58	kg ha^{-1}	2,1,1,1,2
			Planting	1	6.33	kg ha^{-1}	2,1,1,1,2
			Mowing	2	1.42	kg ha^{-1}	2,1,1,1,2
		2009	Spraying	1	1.09	kg ha^{-1}	2,1,1,1,2
			Mowing	1	1.42	kg ha^{-1}	2,1,1,1,2
		2010	Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
			Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Spraying	1	1.09	kg ha^{-1}	2,1,1,1,2
		2011	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2012	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2013	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2014	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2015	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2016	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		Gabi input data	Soil finishing	0.13	0.48	kg ha^{-1}	2,1,1,1,2
			Cultimulcher	0.07	0.24	kg ha^{-1}	2,1,1,1,2
			Planting	0.07	0.42	kg ha^{-1}	2,1,1,1,2
			Mowing	0.20	0.28	kg ha^{-1}	2,1,1,1,2
			Spraying	0.13	0.15	kg ha^{-1}	2,1,1,1,2
			Fertilising	0.87	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	6.68 Mg	30.4	kg ha^{-1}	2,1,1,1,2

Table 32 Farming practice activities and diesel consumption for giant miscanthus at KBS for life cycle inventory (Stein 2015).

Location	Crop	Year	Field activity	Field Pass No.	Diesel Consumption	Unit	Data Quality
KBS	Giant Miscanthus	2008	Planting	1	0	kg ha^{-1}	2,1,1,1,2
			Cultivating	1	3.58	kg ha^{-1}	2,1,1,1,2
		2009	Spraying	1	1.09	kg ha^{-1}	2,1,1,1,2
			Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
		2010	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Spraying	1	1.09	kg ha^{-1}	2,1,1,1,2
		2011	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2012	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2013	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2014	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2015	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2016	Fertilising	1	1.09	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		Gabi input data	Planting	0.07	0	kg ha^{-1}	2,1,1,1,2
			Cultivating	0.07	0.24	kg ha^{-1}	2,1,1,1,2
			Spraying	0.13	0.15	kg ha^{-1}	2,1,1,1,2
			fertilising	0.87	1.02	kg ha^{-1}	2,1,1,1,2
			harvesting	17..91 Mg	81.6	kg ha^{-1}	2,1,1,1,2

Table 33 Farming machinery use for switchgrass at KBS for life cycle inventory.

Location	Crop	Field activity	Machine
KBS	Switchgrass	Soil finishing	JD 7520 tractor and JD 726 soil finisher for weed control
		Cultimulcher	JD 6420 tractor and JD 970 cultimulcher (12 ft)
		Planting	hired Three Rivers, MI, www.nativeconnection.net
		Mowing	JD 7420a tractor and JD 115 15' flail mowe
		Spraying	JD 5220 tractor+top air sprayer 30ft boom and TurboTeeJet 11003 Flat Fan Nozzle
		Fertilising	JD 5220 tractor+top air sprayer 30ft boom and TurboTeeJet 11003 Flat Fan Nozzle
		Harvesting	JD 7350 self-propelled forage harvester equipped with a JD676 forage head

Table 34 Farming machinery use for giant miscanthus at KBS for life cycle inventory.

Location	Crop	Field Activity	Machine
KBS	Giant Miscanthus	Cultivating	John Deere 726 15'9" soil finisher
		Spraying	JD 5220 tractor+top air sprayer 30ft boom
		Planting	Hand planting 36 rows 30in space rootstock 32
		Fertilising	JD 5220 tractor+top air sprayer 30ft boom and TurboTeeJet 11003 Flat Fan Nozzle
		Harvesting	JD 7350 self-propelled forage harvester equipped with a JD676 forage head

Table 35 Farming practice activities and diesel consumption for switchgrass at ARL for life cycle inventory (Lazarus 2015).

Location	Crop	Year	Field activity	Field Pass No.	Diesel Consumption	Unit	Data Quality
ARL	Switchgrass	2008	Chiseling	1	5	kg ha^{-1}	2,1,1,1,2
			Mowing	1	1.42	kg ha^{-1}	2,1,1,1,2
			Liming	1	0.94	kg ha^{-1}	2,1,1,1,2
			Disking	2	11.56	kg ha^{-1}	2,1,1,1,2
			Field digging	1	8.59	kg ha^{-1}	2,1,1,1,2
			culti-packing	1	2.66	kg ha^{-1}	2,1,1,1,2
			planting	1	3.67	kg ha^{-1}	2,1,1,1,2
			Spraying	1	0.94	kg ha^{-1}	2,1,1,1,2
		2009	spraying	2	0.94	kg ha^{-1}	2,1,1,1,2
			replanting	1/3	1.16	kg ha^{-1}	2,1,1,1,2
		2010	spraying	2	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2011	Fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Spraying	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2012	Fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2013	Fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2014	Fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2015	Fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2

Table 35 (cont`d)

		2016	Fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
		2016	Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
ARL	Switchgrass	Gabi input data	Chiseling	0.07	0.33	kg ha^{-1}	2,1,1,1,2
			Soil-finishing	0.4	1.52	kg ha^{-1}	2,1,1,1,2
			Planting	0.09	0.31	kg ha^{-1}	2,1,1,1,2
			Mowing	0.07	0.1	kg ha^{-1}	2,1,1,1,2
			Spraying	0.27	0.38	kg ha^{-1}	2,1,1,1,2
			Liming	0.07	0.06	kg ha^{-1}	2,1,1,1,2
			Fertilising	0.8	0.75	kg ha^{-1}	2,1,1,1,2
			Harvesting	6.96 Mg	31.7	kg ha^{-1}	2,1,1,1,2

Table 36 Farming practice activities and diesel consumption for giant miscanthus at ARL for life cycle inventory (Lazarus 2015).

Location	Crop	Year	Field activity	Field Pass No.	Diesel Consumption	Unit	Data Quality
ARL	Giant Miscanthus	2008	Liming	1	0.94	kg ha^{-1}	2,1,1,1,2
			Chiseling	1	5	kg ha^{-1}	2,1,1,1,2
			Disking	2	10.92	kg ha^{-1}	2,1,1,1,2
			culti-packed	1	2.51	kg ha^{-1}	2,1,1,1,2
			Hand-planting	1	0	kg ha^{-1}	2,1,1,1,2
			spraying	1	0.94	kg ha^{-1}	2,1,1,1,2
		2009	Spraying	1	0.94	kg ha^{-1}	2,1,1,1,2
			Mowing	1	1.34	kg ha^{-1}	2,1,1,1,2
		2010	Hand-planting	0.23	0	kg ha^{-1}	2,1,1,1,2
			Spraying	2	0.94	kg ha^{-1}	2,1,1,1,2
		2011	fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Spraying	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2012	fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Spraying (1/4)	1	0.94	kg ha^{-1}	2,1,1,1,2
			Planting (1/4)	1	0	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2013	fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2014	fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2

Table 36 (cont`d)

ARL	Giant Miscanthus	2015	fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		2016	fertilising	1	0.94	kg ha^{-1}	2,1,1,1,2
			Harvesting	1	4.55	kg Mg^{-1}	2,1,1,1,2
		Gabi input data	Hand-Planting	0.08	0	kg ha^{-1}	2,1,1,1,2
			Chiseling	0.07	0.31	kg ha^{-1}	2,1,1,1,2
			Soil-finishing	0.2	0.9	kg ha^{-1}	2,1,1,1,2
			liming	0.07	0.06	kg ha^{-1}	2,1,1,1,2
			Mowing	0.07	0.09	kg ha^{-1}	2,1,1,1,2
			Spraying	0.33	0.3	kg ha^{-1}	2,1,1,1,2
			fertilising	0.87	0.71	kg ha^{-1}	2,1,1,1,2
			harvesting	13.39 Mg	61	kg ha^{-1}	2,1,1,1,2

Table 37 Farming machinery use for switchgrass at ARL for life cycle inventory. N/A: not available.

Location	Crop	Field Activitiy	Machine
ARL	Switchgrass	Disking	N/A
		Soil-digging	N/A
		culti-packing	N/A
		Planting	JD 6420 with JD 780 NT Drill
		Mowing	N/A
		Spraying	Massey Ferguson - 6490+Miller Pro 1000 - 45' pull behind sprayer
		Fertilising	Case IH - MX 150 +Valmar 5500 - 40' vacuum spreader
		Harvesting	JD 7500 - Chopper+Miller Pro 8015 Dump Wagon

Table 38 Farming machinery use for giant miscanthus at ARL for life cycle inventory. N/A: not available.

Location	Crop	Field Activitiy	Machine
ARL	Giant Miscanthus	Chiseling	N/A
		Planting	N/A
		Mowing	N/A
		Spraying	Bobcat Sprayer
		Cultivating	N/A
		Fertilising	Case IH 684 tractor - MX 150 +Valmar 5500 - 40' vacuum spreader
		Harvesting	JD 7500 - Chopper+Miller Pro 8015 Dump Wagon

Table 39 N_2O emission for switchgrass and giant miscanthus cultivation phase at KBS and ARL from year 2011-2014.

N_20 g/ha/yr		2011	2012	2013	2014	Average	Std dev
G5	KBS	2441.5	728.6	1089.1	772.9	1258.0	805.2
	ARL	2914.6	3873.9	3556.0	2654.0	3249.6	562.9
G6	KBS	2090.6	336.4	502.2	479.9	852.3	828.8
	ARL	1171.0	1418.5	3304.8	2909.7	2201.0	1063.6

Table 40 Dry biomass yield for switchgrass and giant miscanthus at KBS and ARL amortized to 15-years production from year 2008-2022.

Year	Switchgrass (Mg ha^{-1})		Giant miscanthus (Mg ha^{-1})	
	KBS	ARL	KBS	ARL
2010	3.51	5.35	13.95	N/A
2011	6.42	7.10	16.37	10.56
2012	4.70	6.88	9.21	9.34
2013	9.53	8.46	22.81	15.70
2014	8.46	7.75	21.86	14.10
2015	7.46	6.25	23.28	17.26
15-years Average	6.68	6.96	17.91	13.39

Table 41 Inputs for switchgrass and giant miscanthus amortized to 15-years production (year 2008-2022) at both KBS and ARL. KBS_Sw= switchgrass at KBS; KBS_Mis= giant miscanthus at KBS; ARL Sw= switchgrass at ARL; ARL_Mis= giant miscanthus at ARL.

	KBS_Sw	**KBS_Mis**	**ARL_Sw**	**ARL_Mis**
Material inputs (kg ha^{-1} yr^{-1})				
Limestone			719	719
Seeds (kg) /rhizomes (*pieces*)	1	1150	0.9	1760
Herbicide Production	0.19	0.66	0.71	0.66
UAN production	167	186	0.4	0.4
Ammonium Nitrate (34-0-0)			132	132
LUC_ΔSOC	1220	1220	1220	1220
N2O	1.26	0.852	3.25	2.2
Diesel consumption (l ha^{-1} yr^{-1})				
Tillage	0.60	0.21	2.16	1.95
Planting	0.53		0.75	
Spraying lime			0.04	0.05
Spraying herbicide	0.06	0.06	0.18	0.22
Spraying fertilizer	0.43	0.43	0.52	0.52
Mowing	0.71		0.25	0.25
Harvesting	36.30	97.40	37.90	72.80
Total Diesel consumption (l ha^{-1} yr^{-1})	38.62	98.10	41.80	75.78

Table 42 Pedigree matrix used to assess the quality of data sources, modified from Weidema (1998).

Indicator score	**1**	**2**	**3**	**4**	**5(default)**
Reliability	Verified date based on measurements	Verified data partly based on assumptions or non-verified data based on measurements	Non-verified data partly based on qualified estimates	Qualified estimate (e.g. by industrial expert)	Non-qualified estimates
Completeness	Representative data from all sites relevant for the market considered, over an adequate period to even out normal fluctuations	Representative data from > 50% of the sites relevant for the market considered, over an adequate period to even out normal fluctuations	Representative data from only some sites (<< 50%) relevant for the market considered or > 50% of sites but from shorter periods	Representative data from data only one site relevant for the market considered or some sites but from shorter periods	Representativeness unknown or data from a small number of sites and from shorter periods
Temporal correlation	Less than 3 years of difference to the time period of the dataset	Less than 6 years of difference to the time period of the dataset	Less than 10 years of difference to the time period of the dataset	Less than 15 years of difference to the time period of the dataset	Age of data unknown or more than 15 years of difference to the time period of the dataset
Geographical correlation	Data from area under study	Average data from larger area in which the area under study is included	Data from area with similar production conditions	Data from area with slightly similar production conditions	Data from unknown or distinctly different area (North America instead of Middle East, OECD-Europe instead of Russia)

Table 42 (cont`d)

Further technological correlation	Data from enterprises,processes and materials under study	Data from processes and materials under study (i.e. identical technology) but from different enterprises	Data from processes and materials under study but from different technology	Data on related processes or materials	Data on related processes on laboratory scale or from different technology

Table 43 Major Assumptions for this LCA study.

Assumptions
No allocation method was applied for this LCA study.
Herbicide unspecific was used as herbicide production process unit for the cropping systems from Gabi database.
Fertilizer use rate was consistent for the whole 15-years life span of the crops at both locations since crops established.
ΔSOC by direct land use were the same for switchgrass and giant miscanthus at both locations.
Average yields from 2010-2015 represented 15-years average
Diesel consumption of chop and ensile harvesting system were the same for switchgrass and giant miscanthus, which is 5.44 l Mg^{-1} biomass.
Diesel consumption of cut and bale harvesting system were the same for switchgrass and giant miscanthus, which is 6.09 l Mg^{-1} biomass

Table 44 Input date completeness check list for Gabi 6.0 model.

Cropping system	Life Cycle Stage	Data availability	Complete	Required Action
Switchgrass	Cultivation	Yes	Yes	No
	Harvest/storage	Yes	Yes	No
Miscanthus	Cultivation	Yes	Yes	No
	Harvest/storage	Yes	Yes	No

REFERENCES

REFERENCES

Bessou, Cecile, Claudine Basset-Mens, Thierry Tran, and Anthony Benoist. 2013. "LCA Applied to Perennial Cropping Systems: A Review Focused on the Farm Stage." *International Journal of Life Cycle Assessment* 18 (2): 340–61. doi:10.1007/s11367-012-0502-z.

Borrion, Aiduan Li, Marcelle C. McManus, and Geoffrey P. Hammond. 2012. "Environmental Life Cycle Assessment of Lignocellulosic Conversion to Ethanol: A Review." *Renewable and Sustainable Energy Reviews* 16 (7). Elsevier: 4638–50. doi:10.1016/j.rser.2012.04.016.

Brandão, Miguel, Llorenç Milà i Canals, and Roland Clift. 2011. "Soil Organic Carbon Changes in the Cultivation of Energy Crops: Implications for GHG Balances and Soil Quality for Use in LCA." *Biomass and Bioenergy* 35 (6): 2323–36. doi:10.1016/j.biombioe.2009.10.019.

Brentrup, F., J. Küsters, H. Kuhlmann, and J. Lammel. 2004. "Environmental Impact Assessment of Agricultural Production Systems Using the Life Cycle Assessment Methodology." *European Journal of Agronomy* 20 (3): 247–64. doi:10.1016/S1161-0301(03)00024-8.

Cherubini, Francesco, and Gerfried Jungmeier. 2010. "LCA of a Biorefinery Concept Producing Bioethanol, Bioenergy, and Chemicals from Switchgrass." *International Journal of Life Cycle Assessment* 15 (1): 53–66. doi:10.1007/s11367-009-0124-2.

Cherubini, Francesco, and Anders Hammer Strømman. 2011. "Life Cycle Assessment of Bioenergy Systems: State of the Art and Future Challenges." *Bioresource Technology* 102 (2). Elsevier Ltd: 437–51. doi:10.1016/j.biortech.2010.08.010.

Dale, Bruce E., and Rebecca G. Ong. 2012. "Energy, Wealth, and Human Development: Why and How Biomass Pretreatment Research Must Improve." *Biotechnology Progress* 28 (4): 893–98. doi:10.1002/btpr.1575.

Dobbie, Ke, and Ka Smith. 2003. "Impact of Different Forms of N Fertilizer on N 2 O Emissions from Intensive Grassland." *Nutrient Cycling in Agroecosystems* 67 (1): 37–46. doi:10.1023/A:1025119512447.

Ekvall, Tomas, Adisa Azapagic, Göran Finnveden, Tomas Rydberg, Bo P. Weidema, and Alessandra Zamagni. 2016. "Attributional and Consequential LCA in the ILCD Handbook." *International Journal of Life Cycle Assessment* 21 (3): 293–96. doi:10.1007/s11367-015-1026-0.

Ercoli, L., M. Mariotti, A. Masoni, and E. Bonari. 1999. "Effect of Irrigation and Nitrogen Fertilization on Biomass Yield and Efficiency of Energy Use in Crop Production of Miscanthus." *Field Crops Research* 63 (1): 3–11. doi:10.1016/S0378-4290(99)00022-2.

European Commission - Joint Research Centre - Institute for Environment and Sustainability. 2010. “International Reference Life Cycle Data System (ILCD) Handbook : Analysing of Existing Environmental Impact Assessment Methodologies for Use in Life Cycle Assessment.” *European Commission*, 115. doi:10.2788/38479.

Godfray, H Charles J, John R Beddington, Ian R Crute, Lawrence Haddad, David Lawrence, James F Muir, Jules Pretty, Sherman Robinson, Sandy M Thomas, and Camilla Toulmin. 2012. “The Challenge of Food Security.” *Science* 327 (February): 812. doi:10.4337/9780857939388.

Guinée, Jeroen B, Reinout Heijungs, Gjalt Huppes, Alessandra Zamagni, Paolo Masoni, Roberto Buonamici, Tomas Ekvall, and Tomas Rydberg. 2011. “Life Cycle Assessment: Past, Present, and Future.” *Environmental Science & Technology* 45 (1): 90–96. doi:10.1021/es101316v.

Hasler, K., S. Bröring, S. W F Omta, and H. W. Olfs. 2015. “Life Cycle Assessment (LCA) of Different Fertilizer Product Types.” *European Journal of Agronomy* 69. Elsevier B.V.: 41–51. doi:10.1016/j.eja.2015.06.001.

Hennig, Christiane, and Marek Gawor. 2012. “Bioenergy Production and Use: Comparative Analysis of the Economic and Environmental Effects.” *Energy Conversion and Management* 63. Elsevier Ltd: 130–37. doi:10.1016/j.enconman.2012.03.031.

Hoben, J. P., R. J. Gehl, N. Millar, P. R. Grace, and G. P. Robertson. 2011. “Nonlinear Nitrous Oxide (N2O) Response to Nitrogen Fertilizer in on-Farm Corn Crops of the US Midwest.” *Global Change Biology* 17 (2): 1140–52. doi:10.1111/j.1365-2486.2010.02349.x.

Kumar, Amit, and Shahab Sokhansanj. 2007. “Switchgrass (Panicum Vigratum, L.) Delivery to a Biorefinery Using Integrated Biomass Supply Analysis and Logistics (IBSAL) Model.” *Bioresource Technology* 98 (5): 1033–44. doi:10.1016/j.biortech.2006.04.027.

Lal, R. 2003. “Global Potential of Soil Carbon Sequestration to Mitigate the Greenhouse Effect.” *Critical Reviews in Plant Sciences* 22 (February 2015): 151–84. doi:10.1080/713610854.

Lazarus, William F. 2015. “Machinery Cost Estimates.” *University of Minnesota Extension*, 1–8. http://www3.extension.umn.edu/sites/default/files/download/Machinery Cost Estimates June 2015.pdf.

Meeusen, M.J.G, and B.P. Weidema. 2000. *Agricultural Data for Life Cycle Assessments. European Invitational Expert Seminar on Life Cycle Assessment of Food Products*. Vol. 2. doi:10.2788/49570.

Muench, Stefan, and Edeltraud Guenther. 2013. “A Systematic Review of Bioenergy Life Cycle Assessments.” *Applied Energy* 112. Elsevier Ltd: 257–73. doi:10.1016/j.apenergy.2013.06.001.

Naik, S. N., Vaibhav V. Goud, Prasant K. Rout, and Ajay K. Dalai. 2010. "Production of First and Second Generation Biofuels: A Comprehensive Review." *Renewable and Sustainable Energy Reviews* 14 (2): 578–97. doi:10.1016/j.rser.2009.10.003.

Oates, Lawrence G., David S. Duncan, Ilya Gelfand, Neville Millar, G. Philip Robertson, and Randall D. Jackson. 2016. "Nitrous Oxide Emissions during Establishment of Eight Alternative Cellulosic Bioenergy Cropping Systems in the North Central United States." *GCB Bioenergy* 8 (3): 539–49. doi:10.1111/gcbb.12268.

Robertson, G P. 2000. "Greenhouse Gases in Intensive Agriculture: Contributions of Individual Gases to the Radiative Forcing of the Atmosphere." *Science* 289 (5486): 1922–25. doi:10.1126/science.289.5486.1922.

Sanford, G. R., Oates, L. G., Jasrotia, P., Thelen, K. D., Robertson, G. P., & Jackson, R. D. (2016). Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA. *Agriculture, Ecosystems and Environment*, *216*, 344–355. http://doi.org/10.1016/j.agee.2015.10.018

Sanford, Gregg R, Lawrence G Oates, Sarah S Roley, David S Duncan, Randall D Jackson, G Philip Robertson, and Kurt D Thelen. 2017. "Biomass Production a Stronger Driver of Cellulosic Ethanol Yield than Biomass Quality," no. May: 1–12. doi:10.2134/agronj2016.08.0454.

Schmer, M R, K P Vogel, R B Mitchell, and R K Perrin. 2008. "Net Energy of Cellulosic Ethanol from Switchgrass." *Proceedings of the National Academy of Sciences* 105 (2): 464–69. doi:10.1073/pnas.0704767105.

Shemfe, Mobolaji B., Carly Whittaker, Sai Gu, and Beatriz Fidalgo. 2016. "Comparative Evaluation of GHG Emissions from the Use of Miscanthus for Bio-Hydrocarbon Production via Fast Pyrolysis and Bio-Oil Upgrading." *Applied Energy* 176. Elsevier Ltd: 22–33. doi:10.1016/j.apenergy.2016.04.113.

Sinistore, Julie C., Douglas J. Reinemann, R. César Izaurralde, Keith R. Cronin, Paul J. Meier, Troy M. Runge, and Xuesong Zhang. 2015. "Life Cycle Assessment of Switchgrass Cellulosic Ethanol Production in the Wisconsin and Michigan Agricultural Contexts." *Bioenergy Research* 8 (3): 897–909. doi:10.1007/s12155-015-9611-4.

Sokhansanj, Shahab, Amit Kumar, and Anthony F. Turhollow. 2006. *Development and Implementation of Integrated Biomass Supply Analysis and Logistsics Model (IBSAL). Biomass and Bioenergy*. Vol. 30. doi:10.1016/j.biombioe.2006.04.004.

Stein, Dennis. 2014. "2014 Custom Machine and Work Rate Estimates." *MSU*, no. 13: 1–4.

Thompson, Paul. 2012. "The Agricultural Ethics of Biofuels: The Food vs. Fuel Debate." *Agriculture* 2 (4): 339–58. doi:10.3390/agriculture2040339.

Turconi, Roberto, Alessio Boldrin, and Thomas Astrup. 2013. "Life Cycle Assessment (LCA) of Electricity Generation Technologies: Overview, Comparability and Limitations." *Renewable and Sustainable Energy Reviews* 28. Elsevier: 555–65. doi:10.1016/j.rser.2013.08.013.

U.S Deparment of Energy. 2014. "Alternative Fuels Data Center – Fuel Properties Comparison." *Energy Efficiency & Renewable Energy*, 1–4. http://www.afdc.energy.gov/publications/search/keyword/?q=alternative fuel price report.

UW Extension. 2005. "Management of Wisconsin Soilsf(Fifth Edition)."

Van der Weijde, T, C L Alvim Kamei, A F Torres, W Vermerris, O Dolstra, R G Visser, and L M Trindade. 2013. "The Potential of C4 Grasses for Cellulosic Biofuel Production." *Front Plant Sci* 4 (May): 107. doi:10.3389/fpls.2013.00107.

West, Tristram O, and Gregg Marland. 2002. "A Synthesis of Carbon Sequestration, Carbon Emissions, and Net Carbon Flux in Agriculture: Comparing Tillage Practices in the United States." *Agriculture, Ecosystems & Environment* 91 (1–3): 217–32. doi:10.1016/S0167-8809(01)00233-X.

Wood, Sam, and Annette Cowie. 2004. "For Fertiliser Production .," no. June.