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Perennial Bioenergy Crop Yield and Quality Response to Nitrogen Fertilization

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Abstract

At two sites in the North Central USA (Michigan (KBS) and Wisconsin (ARL)), we evaluated the effect of N fertilization on the yield and quality of five perennial bioenergy feedstock cropping systems: (1) switchgrass (*Panicum virgatum* L.), (2) giant miscanthus (*Miscanthus* × *giganteus*), (3) a native grass mixture (5 species), (4) an early successional field (volunteer herbaceous species), and (5) a restored prairie (18 species). In a randomized complete block design with 5 replicates and 2 split plots, N was applied at 0 and 56 kg ha^{-1} to split plots for each cropping system from 2010 to 2016. No yield response to N was detected in switchgrass at either location in any year. Giant miscanthus exhibited a positive yield response to N at both sites (11% at KBS and 83% at ARL). Nitrogen fertilizer addition significantly reduced glucose (KBS 12.9 and 13.8 g kg^{-1} year^{-1}, ARL 11.2 and 9.7 g kg^{-1} year^{-1}) in the native grass mix and restored prairie systems respectively. Nitrogen fertilizer also reduced xylose at KBS in the switchgrasss, native grass mix, and restored prairie (4.9, 7.5, and 5.0 g kg^{-1} year^{-1}). At ARL, N fertilization reduced xylose levels in switchgrass, giant miscanthus, and restored prairie (7.4, 6.8, and 6.2 g kg^{-1} year^{-1}) and increased xylose levels in the early successional system (5.0 g kg^{-1} year^{-1}).

Keywords Biomass yield · Glucose content · Xylose content · Ethanol yield · Perennial grasses

Introduction

Cellulosic biofuels are renewable fuels derived from cellulose, hemicellulose, or lignin from biomass. Research interests are focused on not only developing perennial bioenergy cropping systems to provide the bioenergy industry a stable feedstock supply sufficient to meet EISA mandates but also understanding tradeoffs and synergies in sustainability attributes of alternative bioenergy supply chains [1, 2]. Key sustainability attributes include biomass feedstock quantity and quality for various bioenergy conversion pathways [3], as well as their contributions to ecosystem services such as climate stabilization, water purification, flood mitigation, and biodiversity [4, 5].

Nitrogen fertilization can significantly increase biomass production, which can then lead to carbon sequestration [6]. However, nitrogen fertilizer production, by the Haber-Bosch process, is very energy-intensive and relies on fossil fuel such as natural gas. As a result, the carbon debt accrued in the manufacture of nitrogen fertilizers has the potential to reduce soil carbon gains that may proceed from N fertilization, particularly if fertilizer applications are not managed properly [7]. Agriculture is responsible for up to a 84% of global anthropogenic nitrous oxide (N_2O) emissions, primarily coming from fertilized croplands [8]. Gelfand et al. [9] states that higher soil N_2O emissions are largely associated with annual cropping systems where higher fertilizer rates result in higher soil N availability. While perennial cropping systems tend to have lower nutrient demands than annual crops, bioenergy cropping systems may require supplemental N to maintain stable production over the effective life of the crop.

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Harvest timing for perennial cropping systems is generally after plant senescence, which ensures that most aboveground nutrients are translocated back to the root system and thus conserved for future growth. Several researchers have shown that about 30% of plant N can be recycled back to below ground tissue during drought and over 50% without drought after plant senescence [10, 11]. Jach-Smith and Jackson [12] also noted that N fertilizer applications to switchgrass (*Panicum virgatum* L.) and prairie can diminish soil N conservation ability due to increasing N concentration in biomass rather than yield increase. Excessive N concentration is not a preferable characteristic for biomass feedstock, especially for biomass pretreatment and biofuel production [13, 14]. 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.

Switchgrass and giant miscanthus (*Miscanthus* × *giganteus*) have been identified as important bioenergy crops in the USA [15], with acreage likely to increase for each in coming years. The response of switchgrass and giant miscanthus to N fertilizer application is still unclear however [16], making informed management decisions difficult. Currently, the available studies are inconsistent with regard to recommended N rates. Some studies state that there is no significant N effect on biomass yield of switchgrass and giant miscanthus [17, 18], while others report that N fertilizer significantly increased biomass yields in both switchgrass and giant miscanthus [19–21]. Ruan et al. [7] concluded that an increase in N fertilizer rate from 56 to 196 kg N ha^{-1} would not result in a significant yield increase but would reduce climate benefits in terms of CO_2 emission reductions by half in biomass cropping systems. In order to maximize economic profitability and minimize negative environmental impact, it is imperative to examine the N fertilization effect on both the quantity and the quality of potential perennial bioenergy crops. This not only helps to ensure that high N use efficiency crops are grown for bioenergy but will also facilitate the adoption of best management practices for N fertilization.

A previous study documented the promising perennial cropping system productivity at the same MI and WI sites with biomass yield range from 15.6 to 2.6 Mg ha^{-1} year^{-1} [3] during the establishment stage. This study further exams nitrogen fertilization effects on not only biomass quantity but also quality during the post-establishment, production stage. Here we evaluate N fertilization effects on dry matter yield, glucose and xylose levels, theoretical ethanol yield, and N use efficiency of two monoculture bioenergy cropping systems (switchgrass, giant miscanthus) and three polyculture bioenergy cropping systems (a native grass mixture) (5 species), an early successional field (volunteer herbaceous species), and a restored prairie (18 species) at two upper midwest sites that differ in soil fertility. Glucose and xylose are the primary sugars involved in the biological fermentation conversion of bioenergy feedstocks to biofuel and were chosen as readily obtainable proxies for estimating theoretical ethanol yield.

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 soils at KBS are Kalamazoo (fine-loamy, mixed, active, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, active, mesic Typic Hapludalfs) series. These soils are well drained, moderately fertile Alfisols, which developed on uplands under broad leaf forest vegetation [22]. The dominant soil series at ARL is a Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudolls). These soils are well-drained, highly fertile, Mollisols that developed in loess deposits under tallgrass prairie vegetation [3, 23].

Experimental fields at both locations were established in 2008. Five perennial cropping systems were planted including the following: (1) switchgrass, (2) miscanthus, (3) native grasses, (4) an early successional field, and (5) a restored prairie (Table S1). 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) split 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 (see Sanford et al. [24] for further details on the experimental sites).

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 0.1 m in late May 2008. The rhizomes were planted on a 0.76 m × 0.76 m 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. Additional agronomic practices were based on available best management recommendations from Michigan State University (MSU) and University of Wisconsin (UW) agronomists (Sanford et al. [24] for further details). Species planted for native grass cropping systems and restored prairie systems are provided in Table S1 in the supplemental material.

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(Sanford et al. [24] for further details). Details are listed in Tables S2 and S3 in supplemental material.

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 granular ammonium nitrate (34-0-0) or environmentally smart nitrogen (ESN) at the same rate of 56 kg N ha^{-1}. No P and K fertilizers were applied based on annual fall soil test. Table S4 in supplemental material shows details of the nutrient management used for each year.

The N fertilization on main plots (restored prairie without N fertilization on main plots) and no N fertilization split plots (restored prairie with N fertilization on split plots) were harvested on the same day, within 2 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 tractor 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 at harvest for each plot. Before 2014, a John Deere 7500 self-propelled forage harvester with a 600C series grass header was used for harvest at ARL. Since 2014, an AGCO RT120A (AGCO, Beauvais, France) tractor was used for harvest purpose. 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 Eq. 1. Nitrogen Fertilizer Use Efficiency is defined as the ratio of biomass yield gain to applied N fertilizer (Eq. 2).

$$\text{Dry Matter Yield}\left(\text{Mg ha}^{-1}\right) = (1-\text{Moisture Content in Percent}) \times \text{Harvest yield} \tag{1}$$

$$\text{N fertilizer use efficiency} = (\text{Dry matter yield with N}-\text{Dry matter without N}) \div \text{Nitrogen application rate}\left(\text{i.e., 56 kg N ha}^{-1}\right) \tag{2}$$

Theoretical Ethanol Yield Estimates

After the harvested biomass was dried, about 20–40 mg dry material was ball milled with 5.56-mm stainless steel balls (Salem Specialty Ball Co, Canton, CT) until the material became a fine powder (< 1 mm). 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 h. Where necessary, reactions were neutralized with 7.5 μL 6N Hydrochloric acid. A solution containing 0.5 μL Accellerase 1000 (Genencor, Rochester, NY), 33.3 μL 1 M citrate buffer (pH 4.5) plus 10 μL 1% w/v sodium azide, 72 nL C-Tec2, and 8 nL H-tec2 enzymes were added to pretreated subsamples and then incubated for 20 h 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 xylose (Xyl) concentration of samples. The assay kits for glucose and xylose were KGLUC (Megazyme, Ireland) and K-XYLOSE (Megazyme) respectively [25]. Theoretical ethanol yield was calculated based on the empirically derived fermentable Glc and Xyl levels using equation below:

$$([\text{Glc}] + [\text{Xyl}]) \times 0.51 \times \text{metabolic yield} = [\text{EtOH}]\left(\text{g g}^{-1}\right) \tag{3}$$

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 0.51 g g^{-1}, and metabolic yield equals to the ratio of ethanol to the consumed sugars in the fermentation process divided by 51.1% [26]. Metabolic yield values for switchgrass, giant miscanthus, native grasses mix, early successional, and restored prairie (0.897 g g^{-1}) were determined using a separate hydrolysis and fermentation (SHF) process and are derived from Jin et al. [27]. Total theoretical ethanol yield (Mg ha^{-1}) was calculated by multiplying theoretical ethanol yield (g g^{-1}) from equation [3] with its corresponding dry matter yield (Mg ha^{-1}). Theoretical ethanol yields presented here are conservative estimates of ethanol yield and are likely lower than conventional industrial yields.

Data Analysis

Proc Mixed of SAS 9.4 [28] was used to evaluate the effect of nitrogen, bioenergy cropping system, year 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 fixed 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. Akaike Information Criterion (AIC) [29] was the determinant of better model choice. N effects at each combination of year, location, and cropping system were detected by Fisher's protected least significant difference (LSD) by using LSMEANS statement in Proc Glimmix. Pair-wise comparisons were performed on fertilizer use efficiency of total biomass yield, biomass quality, and theoretical ethanol yield. The significance levels of 0.05, 0.01, and 0.001 were reported. R package Hmisc was used to determine significance of correlation coefficients [30].

Results and Discussion

Weather

Daily air temperature and precipitation data during the study period (2010–2016) were collected from stations nearest to both field sites. The Arlington Agricultural Research Station and the Kellogg Biological Station (Gull Lake) were the respective weather stations used for each site. Data were summarized into monthly average air temperature and total precipitation occurring over the growing season and 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 because of extreme cold 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. [24].

At both locations, monthly average air temperatures did not vary significantly during the growing phase from May to September (Tables S5–S8 in supplemental material). 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 from May to September in 2012, 2013, and 2014 was 48%, 15%, and 13% drier, respectively, than the 30-year average. At Arlington, total precipitation during the growing phase from May to September in 2011, 2012, 2013, 2014, 2015, and 2016 was 46%, 57%, 19%, 25%, 21%, and 2% 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.

Nitrogen Effect on Sugar Content and Estimated Ethanol Content

There were few interannual differences of N effect on biomass quality [Glc], [Xyl], and [EtOH]. However, the differences that were observed did not appear to follow a particular pattern (Fig. 1).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] ($P = 0.8386$, $P = 0.1662$, and $P = 0.6321$, respectively). Strong interactions between cropping system and nitrogen fertilization were significant on [Glc], [Xyl], and [EtOH] ($P = 0.0015$, $P = 0.0038$, and $P = 0.0055$, 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 (− 0.0121 g g^{-1}, $P < 0.0001$ and − 0.0117 g g^{-1}, $P < 0.0001$, respectively).

The ranking in magnitude of biomass [Glc] reduction in response to N fertilization (descending order) (Table 1) at KBS was (1) restored prairie, (2) native grasses mix, (3) switchgrass, (4) early successional, and (5) giant miscanthus. The biomass [Glc] reduction of restored prairie and native grass was significantly different than the biomass [Glc] gain of giant miscanthus at KBS. The ARL site had a similar ranking of N responses on [Glc] with the exception that the native grass mix cropping system moved up to first in the order ahead of restored prairie and switchgrass. At ARL, both giant miscanthus and early successional cropping systems had positive nitrogen response on [Glc]. The negative nitrogen responses on [Glc] of native grasses mix and restored prairie cropping systems were significantly different than the positive nitrogen responses on [Glc] of giant miscanthus and early successional cropping systems at ARL. The responses to N fertilization on [Glc] of early successional cropping system and restored prairie cropping system were both negative, but not significantly different at KBS. At ARL, the positive N fertilization effect on [Glc] in early successional was significantly different than the negative N fertilization effect on [Glc]

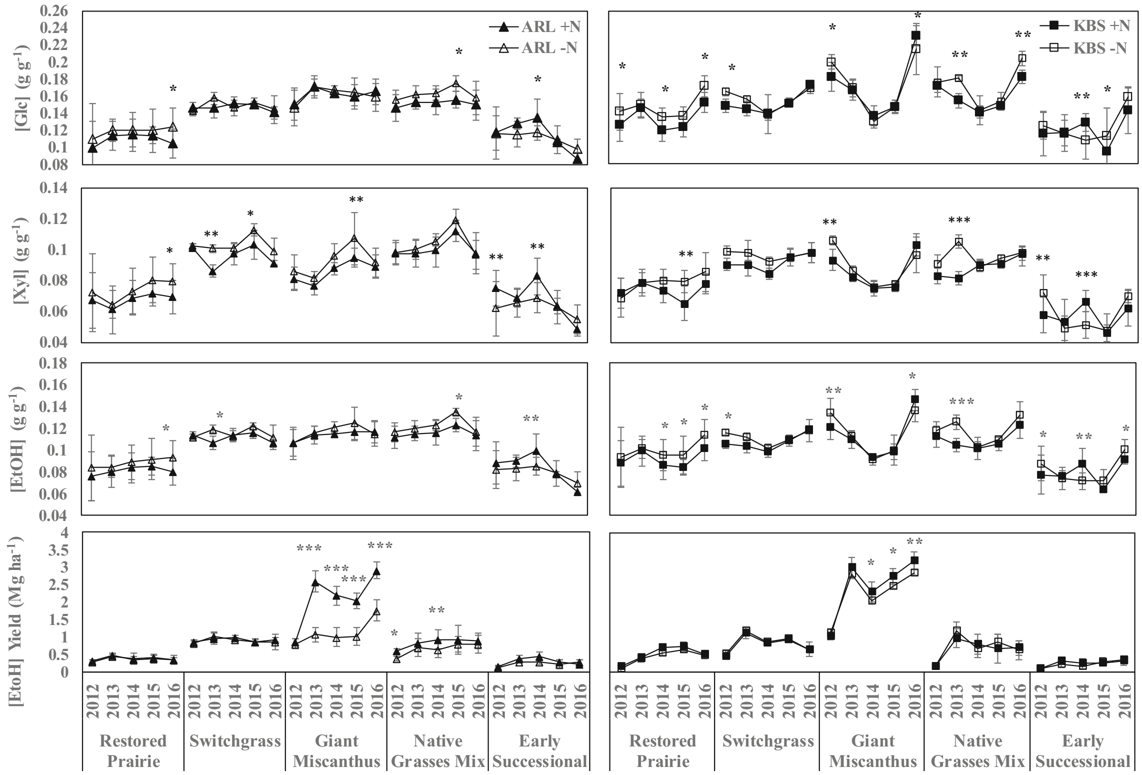

Fig. 1 Yearly glucose content [Glc] (g g^{-1}), xylose content [Xyl] (g g^{-1}), theoretical ethanol content [EtOH] (g g^{-1}), and theoretical ethanol yield on a land area basis (Mg ha^{-1}) of five perennial cropping systems under study with and without nitrogen fertilization at KBS and ARL from 2012 to 2016. *Significant nitrogen fertilization effect within cropping system, year, and location ($P < 0.05$). **Significant nitrogen fertilization effect within cropping system, year, and location ($P < 0.01$). ***Significant nitrogen fertilization effect within cropping system, year, and location ($P < 0.001$)

in restored prairie. This difference was likely due to a higher grass:forb ratio (3.9) of early successional compared to the grass:forb ratio (1.1) of restored prairie at ARL.

Similar to our findings, Song et al. [31] found that grasses were more responsive to nitrogen fertilizer when compared to forbs. Others have found that structural sugar content differs by plant species and maturity [32] and that grasses generally have a higher sugar contents than forbs [33]. At the same experimental site, Sanford et al. [3] found that a higher grass: forb ratio in mixed biomass feedstocks lead to higher sugar content, despite N application, although they concluded that areal EtOH yields were primarily driven by yield.

N fertilization also had a negative effect on biomass [Xyl] in seven of eight cropping system/location combinations that

Table 1 N responses of averaged glucose content [Glc] (g kg^{-1} year^{-1}), xylose content [Xyl] (g kg^{-1} year^{-1}), ethanol content [EtOH] (g kg^{-1} year^{-1}), dry matter yield [DM] (Mg ha^{-1} year^{-1}) and EtOH yield on land area basis (Mg ha^{-1} year^{-1}) of five cropping systems at KBS and ARL across studied years

	Location	Switchgrass	Giant miscanthus	Native grasses mix	Early successional	Restored prairie
[Glc][a]	KBS	− 5.4abcd[b]	0.7d	*− 12.9a*	− 4.3abcd	*− 13.8a*
	ARL	− 1.4bc	0.2cd	*− 11.2ab*	2.8d	*− 9.7abc*
[Xyl][a]	KBS	*− 4.9ab*	− 2.9ab	*− 7.5a*	− 0.7bc	*− 5.0ab*
	ARL	*− 7.4a*	*− 6.8ab*	− 2.9ab	*5.0c*	*− 6.2ab*
[EtOH][a]	KBS	− 4.7bc	− 1.0cd	*− 13.1a*	− 2.3bcd	*− 8.6ab*
	ARL	− 4.1bc	− 3.0bcd	*− 6.5abc*	3.6d	*− 7.3abc*
DM[a]	KBS	− 0.06a	*1.7b*	0.43ab	0.55ab	1.05ab
	ARL	− 0.06a	*7.5c*	*1.45ab*	0.51ab	0.41ab
EtOH yield[a]	KBS	− 0.04a	*0.2c*	− 0.04a	0.02a	0.07ab
	ARL	0.01a	*1.0d*	*0.18bc*	0.07ab	− 0.01a

Means in italics show N fertilization effect was significant ($P < 0.05$)

[a] N responses are subtraction of averaged [Glc], [Xyl], [EtOH], [DM], and [EtOH] yield without N fertilization from the corresponding values with N fertilization

[b] Means within same measurement followed by a same letter are not significantly different

exhibited a significant N fertilization effect, with the early successional system at ARL being the lone exception that exhibited a significant positive N fertilization effect. The ranking in magnitude of biomass [Xyl] reduction in response to N fertilization (descending order) at KBS was (1) native grasses mix, (2) restored prairie, (3) switchgrass, (4) giant miscanthus, and (5) early successional. At ARL, the ranking was (1) switchgrass, (2) giant miscanthus, (3) restored prairie, (4) native grasses, and (5) early successional. Similar to [Glc], the difference between a positive N fertilization effect in the early successional cropping system and negative N fertilization effect in the restored prairie was due to differences in the grass:forb ratio. Previous studies showed that excess nitrogen had a negative effect on polysaccharide production of plants, which was attributed to source-sink theory [34, 35]. This is in agreement with the negative nitrogen effect on [Glc] and [Xyl] observed in this study.

Biomass theoretical [EtOH] is dependent upon biomass [Glc] and [Xyl] (Eq. 2), 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) restored prairie, (3) switchgrass, (4) early successional, and (5) giant miscanthus. At ARL, the ranking was (1) restored prairie, (2) native grasses, (3) switchgrass, (4) giant miscanthus, and (5) early successional. Similar to the observed cropping system × nitrogen fertilization effect on [Glc], the significant cropping system × nitrogen fertilization effect on [EtOH] was also due to the significant negative N responses of native grasses and restored prairie across both locations (-0.0098 g g^{-1}, $P = 0.0011$and -0.0080 g g^{-1}, $P < 0.0001$, respectively).

In Table 2, the dependency of biomass [EtOH] to [Glc] and [Xyl] is shown using regression. The results show [Glc] has higher correlation coefficient *r* than [Xyl] within five cropping systems, two locations, and two nitrogen treatment. Glucose being the dominant monosaccharide in structural plant biomass sugars has also been reported by others [36]. The negative N effect on [Glc] and [Xyl] levels can be explained by increasing lignin content as a result of N fertilization [37]. Several other studies also have shown that the lignin content of grasses increases with N fertilization [38, 39]. Dien et al. [36] 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 [40]. Another possible reason for the negative response of [Glc] and [Xyl] to N fertilization may be a lower leaf to stem ratio in fertilized plants. Cruz and Boval [41] 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 [42]. It is noteworthy that all significant correlations between DM and [Glc] were positive at ARL and negative at KBS regardless of fertilizer application treatment. This implied that different growing environments can result in a different relationship between biomass dry matter yield and glucose content [3].

Nitrogen Effect on Biomass and Ethanol Yield

Some interannual differences of N effect on DM yield (Fig. 2) 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 split plots without nitrogen fertilization. There was no significant nitrogen fertilization effect on DM found during the drought year of 2012 at any combination of cropping system and location. 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 [43]. The three-way interaction between nitrogen fertilization, location, and cropping system on DM yield was significant ($P < 0.0001$) 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 (6.38 ± 1.65 Mg ha^{-1} year^{-1}) was significantly higher than DM yield of native grasses without nitrogen fertilization (4.93 ± 1.34 Mg ha^{-1} year^{-1}, $P = 0.0086$). Giant miscanthus had significant positive N responses at both KBS and ARL (1.72 Mg ha^{-1} year^{-1} and 7.50 Mg ha^{-1} year^{-1}, respectively), which agrees with Miguez et al. [44] claiming that giant miscanthus responded to N fertilization in the post-establishment phase or production phase. Based on field visual observations, the N fertilized giant miscanthus stands looked in better health than the non-fertilized ones, which increased survival during winter. The ranking of the magnitude of N responses (descending order) 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 (descending order) 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 USA have shown that switchgrass did not respond to N fertilization at rates between 33 and 224 kg N ha^{-1} [45, 46]. Research on switchgrass in southern Iowa has shown that N fertilization improved yields, with the magnitude of the effect declining as N rate increased [47]. 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

Table 2 Correlation coefficient between ethanol content [EtOH] and glucose content [Glc], ethanol content [EtOH] and xylose content [Xyl], biomass dry matter (DM) and glucose content [Glc], biomass dry matter (DM) and xylose content [Xyl], ethanol yield on a land areal basis (EtOH yield) and biomass dry matter (DM) and xylose content (Xyl), and ethanol yield on a land areal basis (EtOH yield) and ethanol content (EtOH)

		[EtOH]-[Glc]	[EtOH]-[Xyl]	DM-[Glc]	DM-[Xyl]	EtOH yield-DM	EtOH yield-[EtOH]
ARL							
Switchgrass	+N	*0.85*	*0.85*	0.23	− 0.24	*0.92*	0.37
	−N	*0.92*	*0.78*	0.23	0.02	*0.95*	*0.47*
Giant miscanthus	+N	*0.91*	*0.74*	*0.49*	0.14	*0.98*	*0.56*
	−N	*0.91*	*0.87*	0.21	0.13	*0.98*	0.35
Native grasses mix	+N	*0.93*[a]	*0.89*	0.18	− 0.01	*0.94*	*0.43*
	−N	*0.95*	*0.91*	0.03	− 0.03	*0.97*	0.24
Early successional	+N	*0.98*	*0.95*	0.01	− 0.13	*0.9*	0.39
	−N	*0.98*	*0.96*	− 0.25	− 0.11	*0.93*	0.14
Restored prairie	+N	*0.98*	*0.94*	*0.44*	0.15	*0.9*	*0.7*
	−N	*0.98*	*0.96*	*0.4*	0.24	*0.87*	*0.74*
KBS							
Switchgrass	+N	*0.97*	*0.83*	*− 0.5*	− 0.25	*0.97*	− 0.23
	−N	*0.96*	*0.74*	*− 0.45*	− 0.13	*0.98*	*− 0.22*
Giant miscanthus	+N	*0.99*	*0.92*	− 0.28	− 0.5	*0.5*	*0.54*
	−N	*0.99*	*0.93*	*− 0.49*	*− 0.75*	*0.85*	− 0.05
Native grasses mix	+N	*0.95*	*0.63*	*− 0.48*	0.11	*0.99*	− 0.22
	−N	*0.97*	*0.68*	− 0.22	0.35	*0.97*	0.11
Early successional	+N	*0.98*	*0.92*	− 0.18	− 0.36	*0.89*	0.19
	−N	*0.97*	*0.87*	0.2	− 0.22	*0.91*	*0.46*
Restored prairie	+N	*0.98*	*0.91*	− 0.32	− 0.3	*0.97*	− 0.1
	−N	*0.96*	*0.87*	− 0.2	0.19	*0.98*	0.13

[a] Correlation coefficients in italics are significant correlated ($P < 0.05$)

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 [48]. Lee et al. [49] noted that the variability of switchgrass and giant miscanthus yield responses to N fertilizer were higher between sites than within sites, mainly due to different initial soil N content. With moderate fertility soil, it may take years to show significant positive yield response to N fertilizer. 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 [3]. Grasses have been found to be more responsive than forbs to N fertilization [31]. The greater

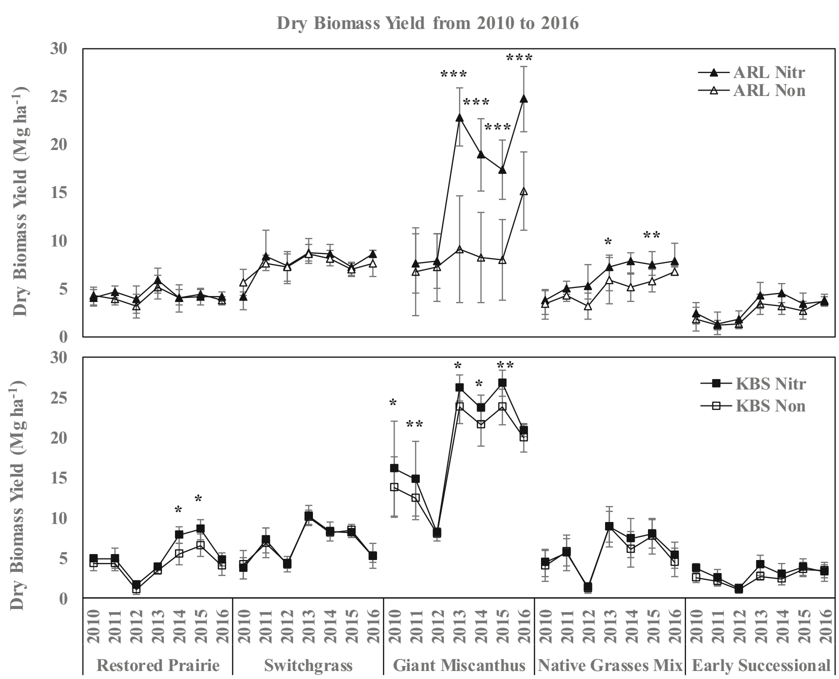

Fig. 2 Yearly dry biomass yield (Mg ha^{-1}) of five perennial cropping systems under study with and without nitrogen fertilization at KBS and ARL from 2010 to 2016. *Significant nitrogen fertilization effect within cropping system, year, and location ($P < 0.05$). **Significant nitrogen fertilization effect within cropping system, year, and location ($P < 0.01$). ***Significant nitrogen fertilization effect within cropping system, year, and location ($P < 0.001$)

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 the early successional system relative to restored prairie was due to higher grass:forb ratio of the early successional system(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.

Others have found that N fertilizer additions reduced plant species diversity in grasslands [50]. Tilman et al. [51] concluded that low input high diversity (LIHD) polycultures had 238% more biomass yield than monocultures, like switchgrass. Therefore, if N fertilization induced reductions in species, it would reduce the polyculture yield advantage reported by Tilman et al. [51], and consequently, a long-term N fertilization program could reduce productivity of the system. An ideal nitrogen fertilization practice is to synchronize application timing with the need of crop [52, 53]. Identifying optimal application strategies were beyond the scope of this study. Nevertheless, split nitrogen fertilization strategies have been shown to boost biomass yield [54]. Overall, the nitrogen fertilization effect on biomass yield is a function of multiple factors, including soil type, precipitation, and harvest time. Therefore, N fertilization programs should be tailored to specific regional conditions.

A statistically significant three-way interaction between nitrogen fertilization, location, and cropping system ($P <$ 0.0001) was found on EtOH yield on a land area basis. This three-way interaction was caused by different nitrogen response of EtOH yield on a land area basis of native grasses mix between KBS (P = 0.9409) and ARL (P < 0.0001). Significant fertilization effects on theoretical ethanol yield on a land area basis were found at both KBS and ARL. At KBS, EtOH yield of giant miscanthus (2.45 ± 0.86 Mg ha^{-1} year^{-1}) with N fertilization was significantly higher than without N fertilization (2.25 ± 0.71 Mg ha^{-1} year^{-1}). At ARL, EtOH yield of giant miscanthus (2.11 ± 0.78 Mg ha^{-1} year^{-1}) and native grasses (0.83 ± 0.14 Mg ha^{-1} year^{-1}) with N fertilization was significantly higher than without N fertilization (1.12 ± 0.37 Mg ha^{-1} year^{-1} and 0.66 ± 0.17 Mg ha^{-1} year^{-1} respectively). Rankings of N responses on EtOH yield were exactly the same as the ranking of N responses on DM with a lone exception that switchgrass had higher EtOH yield response than restored prairie at ARL. This supports the conclusion of Sanford et al. [3] that EtOH yield on a land area basis was driven more by feedstock quantity than feedstock quality. Overall, giant miscanthus had the highest N fertilization effect on EtOH yield among the five cropping systems at both sites.

Nitrogen Fertilizer Use Efficiency

Nitrogen fertilizer use efficiency (Mg kg N^{-1}) mirrored cropping system biomass yield response to N fertilization. Switchgrass had the zero N fertilizer use efficiency among the five cropping systems at KBS and ARL. Giant miscanthus was the most efficient in productively using N fertilizer at both locations (0.03 Mg kg N^{-1} at KBS and 0.11 Mg kg N^{-1} at ARL). Nitrogen fertilizer use efficiency of giant miscanthus reached 0.35 Mg kg N^{-1} in one reported study [55]. The literature is unclear regarding N fertilizer use efficiency of switchgrass [47, 56]. 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 ($P <$ 0.0001). There was no significant difference in N fertilizer use efficiency between native grasses, early successional and restored prairie at either KBS or 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 mix followed by early successional system and 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 [57]. 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).

Conclusions

Nitrogen fertilization increased or prevented a reduction in 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 2010–2016 responded positively to N fertilization at both sites. 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 2010–2016. 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 sites. The [Glc] of restored prairie biomass also responded negatively to N fertilization at KBS. Similarly, the [Xyl] of switchgrass and restored prairie biomass responded negatively to N fertilization at both sites. The [Xyl] of mixed native grass biomass at KBS and giant miscanthus biomass at ARL 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 kg^{-1}) was more driven by [Glc]. Similar to the results for biomass glucose [Glc], N fertilization had a negative effect on theoretical [EtOH] in the native grass mix and restored prairie cropping systems at KBS and restored prairie 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 0.03 Mg kg^{-1} N; ARL 0.11 Mg kg^{-1} N). The results indicate switchgrass as an optimal bioenergy feedstock crop for low input marginal land systems.

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Table S1 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}

Table S2 Herbicide use and rate during the period of 2010-2016 at KBS

Cropping System	Year	Herbicide (main ingredient)	Herbicide rate	Unit	Note
Switchgrass	2009	Drive (quinclorac)	0.6	kg ha^{-1}	post emergence weed control
	2010	2,4-D amine	2.2	kg ha^{-1}	broadleaf weed control
Giant miscanthus	2009	Drive (quinclorac)	0.6	kg ha^{-1}	post emergence weed
	2010	2,4-D amine	0.9	kg ha^{-1}	broadleaf weed control
Restored Prairie	2010	2,4-D amine	0.4	kg ha^{-1}	broadleaf weed control

Table S3 Herbicide use and rate during the period of 2010-2016 at ARL

Cropping System	Year	Herbicide (main ingredient)	Herbicide rate (kg ha^{-1})	Note
Switchgrass	2011	Roundup Power Max	1.7	post emergence weed control
	2012	Clarity	0.2	broadleaf weed control
		2,4-D LV4 Ester	1.2	broadleaf weed control
		Quinclorac SPC 75 DF	0.3	Pre-emerge herbicide spray
	2014	Quinclorac SPC 75 DF	0.6	Pre-emerge herbicide spray
		2,4-D LV4 Ester	1.1	Pre-emerge herbicide spray
Giant Miscanthus	2011	Roundup Power Max	1.7	Burndown
		Glyphosate (generic)	3.5	Burndown
		2,4-D LV4 Ester	1.1	Burndown
	2012	Roundup Power Max	2.9	Burndown
		Prowl	1.7	Pre-emerge
		2,4-D LV4 Ester	1.1	Post-emerge
		Clarity	1.7	broadleaf weed control
	2011	Roundup Power Max	1.7	Burndown
	2013	Prowl	0.3	Pre-emerge
		Roundup Power Max	1.5	Burndown
		2,4-D LV4 Ester	0.8	Post-emerge
		FSTransform Plus (adjuvant)	0.8	Burndown
	2014	Prowl	2.2	Pre-emerge herbicide
		2,4-D LV4 Ester	1.1	Pre-emerge herbicide
		Roundup Power Max	2.1	Pre-emerge herbicide
Native Grasses	2011	Roundup Power Max	1.7	Burndown

Table S4 Nitrogen fertilizer use and rate during the period of 2010-2016 at KBS and ARL

Cropping System	Year	KBS			ARL		
		Fertilizer	Fertilizer (kg ha^{-1})	N rate (kg ha^{-1})	Fertilizer	Fertilizer (kg ha^{-1})	N rate (kg ha^{-1})
Switchgrass	2010	28% N (28-0-0)	200	56			
	2011	28% N (28-0-0)	200	56	NH_4NO_3	165	56
	2012	28% N (28-0-0)	72	20	NH_4NO_3	168	57
	2013	28% N (28-0-0)	204	57	NH_4NO_3	168	57
	2014	28% N (28-0-0)	204	57			
	2015	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56
	2016	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56
Giant Miscanthus	2009	28% N (28-0-0)	276	77			
	2010	28% N (28-0-0)	200	56			
	2011	28% N (28-0-0)	200	56	NH_4NO_3	165	56
	2012	28% N (28-0-0)	72	20	NH_4NO_3	168	57
	2013	28% N (28-0-0)	204	57	NH_4NO_3	168	57
	2014	28% N (28-0-0)	204	57	NH_4NO_3	168	57
	2015	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56
	2016	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56
Native Grasses Mix	2010	28% N (28-0-0)	200	56			
	2011	28% N (28-0-0)	200	56	NH_4NO_3	165	56
	2012	28% N (28-0-0)	72	20	NH_4NO_3	168	57
	2013	28% N (28-0-0)	204	57	NH_4NO_3	168	57
	2014	28% N (28-0-0)	204	57	NH_4NO_3	168	57
	2015	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56
	2016	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56
Early Successional	2009	Urea 46%	122	56			
	2010	28% N (28-0-0)	200	56			
	2011	28% N (28-0-0)	200	56	NH_4NO_3	165	56
	2012	28% N (28-0-0)	72	20	NH_4NO_3	168	57
	2013	28% N (28-0-0)	204	57	NH_4NO_3	168	57
	2014	28% N (28-0-0)	204	57	NH_4NO_3	168	57
	2015	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56
	2016	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56
Restored Prairie	2010	28% N (28-0-0)	200	56			
	2011	28% N (28-0-0)	200	56	NH_4NO_3	165	56
	2012	28% N (28-0-0)	72	20	NH_4NO_3	168	57
	2013	28% N (28-0-0)	204	57	NH_4NO_3	168	57
	2014	28% N (28-0-0)	204	57	NH_4NO_3	168	57
	2015	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56
	2016	28% N (28-0-0)	200	56	ESN (44-0-0)	127	56

Table S5 Monthly total precipitation (mm) during the study years compared to the 30-years averages (1981-2010) at KBS, MI. The 30-years averages were obtained from

	Monthly Total Precipitation (mm)							
Month	2010	2011	2012	2013	2014	2015	2016	30 years Average
Jan	21.9	23.7	82.9	82.4	63.5	13.3	19.9	56.2
Feb	43.8	35.2	68.3	188.5	69.1	9.7	16.7	45.4
Mar	27.5	73.4	104.3	17.1	47	30.5	92.7	63.5
Apr	73	132.8	107.5	195.9	68.2	30.5	90.8	87.1
May	133.5	171.9	33.53	62	104.3	147.3	110.6	98.4
Jun	205.5	57.5	39.7	102.1	155	40.6	33.4	88.6
Jul	141.9	232.4	38.9	98.6	64.2	124.2	142.5	92.2
Aug	17.5	97.8	77.4	131.6	41.5	147.4	192.3	100.7
Sep	94.3	75.9	64.3	20.9	60.1	50.8	35.7	106.1
Oct	45.2	89.9	41.6	65.2	102.7	37.7	85.7	81.6
Nov	46.1	103.9	3.4	92.4	81.5	50.6	48.1	78.3
Dec	28.8	97	109	61.1	21	54.1	24.4	65.4

Table S6 Monthly mean temperatures (ºC) during the study years compared to the 30-years averages (1981-2010) at KBS, MI. The 30-years averages were obtained from NOAA website

	Monthly Mean Air Temperature (ºC)							
Month	2010	2011	2012	2013	2014	2015	2016	30 years-Average
Jan	-3.94	-5.75	-1.40	-2.11	-7.05	-5.44	-2.41	-3.84
Feb	-3.70	-3.25	-0.10	-4.00	-7.42	-6.25	-2.04	-2.45
Mar	5.56	1.10	10.93	0.27	-2.49	3.55	6.57	2.75
Apr	13.16	7.68	9.35	8.13	9.01	8.96	9.35	9.63
May	16.64	16.28	17.05	17.50	14.61	18.28	15.46	15.59
Jun	22.09	21.40	21.50	20.42	21.47	20.22	21.40	20.80
Jul	24.10	24.82	25.93	23.01	19.94	20.79	23.57	22.88
Aug	22.71	21.69	21.64	21.07	21.74	21.08	23.39	21.96
Sep	18.13	16.87	17.35	18.05	16.68	19.73	19.87	17.79
Oct	12.92	11.42	11.00	11.90	11.46	13.41	14.14	11.18
Nov	5.52	7.19	4.27	3.79	3.33	9.56	9.34	5.08
Dec	-4.34	1.56	1.83	-3.04	2.40	5.55	-3.31	-1.47

Table S7 Monthly total precipitation (mm) during the study years compared to the 30-years averages (1981-2010) at ARL, WI. The 30-years averages were obtained from NOAA website

	Monthly Total Precipitation (mm)							
Month	2010	2011	2012	2013	2014	2015	2016	30 years-Average
Jan	43.2	15.2	19.6	57.3	18.8	9.3	20	35.4
Feb	28.1	25.4	24.4	48	26.3	26	9.8	37.5
Mar	25.8	85.9	62.3	59.7	24.5	9.8	108.8	62.5
Apr	92.9	89.8	77.9	137.5	163.5	162.4	37.4	114.1
May	105.5	55.1	74.7	153.5	71.4	111.9	87.6	120.8
Jun	192.8	103.6	6.6	190.9	237.7	79.7	104.2	155.1
Jul	236.2	63.2	108.3	75.9	47.9	80.3	164.9	131.3
Aug	119.4	37.1	73.5	45.4	94.4	110	138.8	134.5
Sep	115.4	98	25.6	75.6	45.4	144.8	156.6	122.2
Oct	42.7	40.1	100.8	39.2	70	49.8	85.7	82.1
Nov	35.6	83.5	28.2	66.6	44.2	123.2	41.3	74.1
Dec	41.8	59.7	60.2	28.6	29.2	86.3	32.9	50.3

Table S8 Monthly mean temperatures (ºC) during the study years compared to the 30-years averages (1981-2010) at ARL, WI. The 30-years averages were obtained from NOAA website

	Monthly Mean Air Temperature (ºC)							
Month	2010	2011	2012	2013	2014	2015	2016	30 years-Average
Jan	-9.90	-10.56	-6.28	-8.72	-14.54	-8.03	-7.96	-8.34
Feb	-6.97	-8.02	-2.91	-8.49	-13.56	-12.61	-3.81	-6.09
Mar	1.92	-2.04	7.54	-5.34	-5.63	0.46	3.53	0.43
Apr	9.10	5.18	6.39	4.10	4.88	8.31	7.08	7.62
May	14.01	11.99	14.99	13.36	12.55	14.86	14.31	14.14
Jun	18.91	18.17	19.82	17.93	19.21	18.60	20.32	19.36
Jul	21.85	22.72	24.32	20.37	18.10	20.31	21.85	21.52
Aug	21.37	19.93	19.41	19.18	20.54	19.81	21.39	20.43
Sep	14.28	13.63	14.25	15.59	15.44	18.97	17.73	16.19
Oct	10.00	9.70	6.91	7.91	8.92	10.46	11.33	9.32
Nov	2.10	2.08	1.16	-0.83	-2.23	4.90	6.27	1.87
Dec	-9.26	-2.90	-3.65	-10.54	-2.52	1.24	-6.09	-5.23