

Biomass Production a Stronger Driver of Cellulosic Ethanol Yield than Biomass Quality

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ABSTRACT

Many crops have been proposed as feedstocks for the emerging cellulosic ethanol industry, but information is lacking about the relative importance of feedstock production and quality. We compared yield and sugar content for seven bioenergy cropping systems in south-central Wisconsin (ARL) and southwestern Michigan (KBS) during three growing seasons (2012 through 2014). The cropping systems were (i) continuous corn stover (*Zea mays* L.), (ii) switchgrass (*Panicum virgatum* L.), (iii) giant miscanthus (*Miscanthus* × *giganteus* Greef & Deuter ex Hodkinson & Renvoize), (iv) hybrid poplar (*Populus nigra* × *P. maximowiczii* A. Henry 'NM6'), (v) native grass mix, (vi) early successional community, and (vii) restored prairie. A high-throughput pretreatment and fermentation assay showed corn stover with the highest sugar content (213 g glucose kg⁻¹ [Glc] and 115 g xylose kg⁻¹ [Xyl]) followed by the two monoculture perennial grass treatments (154 [Glc] and 88 [Xyl]) and then the herbaceous polycultures (135 [Glc] and 77 [Xyl]). Biomass production and sugar content were combined to calculate ethanol yields. Miscanthus had the highest per hectare ethanol yields (1957 l ha⁻¹ yr⁻¹ ARL, 2485 l ha⁻¹ yr⁻¹ KBS) followed by switchgrass (1091 l ha⁻¹ yr⁻¹ ARL, 1017 l ha⁻¹ yr⁻¹ KBS) and corn stover (1121 l ha⁻¹ yr⁻¹ ARL, 878 l ha⁻¹ yr⁻¹ KBS). Perennial grass cropping systems (i.e., switchgrass and miscanthus) had higher per hectare ethanol yields at both sites relative to diverse systems that included dicots. Despite feedstock differences in fermentable sugars, biomass production was the strongest driver of per hectare ethanol yield.

Core Ideas

- Fermentable sugars were greatest in corn stover > perennial grasses > polycultures.
- Corn stover had the highest ethanol content.
- Miscanthus had the highest ethanol yield potential on a per hectare basis.
- Ethanol yield potential per hectare of switchgrass ≥ corn stover.
- Biomass yield was the strongest driver of per hectare ethanol yield.

CORN GRAIN produced on fertile cropland is used for both human and livestock consumption. However, co-production of distiller's grains notwithstanding, the use of corn grain and fertile cropland to produce fuel remains controversial, driving interest in cellulosic fuels generated from vegetative (non-grain) plant biomass (Biello, 2011; Wetzstein and Wetzstein, 2011; Bonin and Lal, 2012). Bioethanol is important strategically if we are to meet the goals of the U.S. Energy Independence and Security Act of 2007 (Skolrud et al., 2016). While the price of nonrenewable fuel sources are currently low, it is likely that countries will continue to pursue greater energy independence, and reductions in greenhouse gas emissions, by adopting long-term strategies that include cellulosic fuels (Reboredo et al., 2016). Perennial crops in particular, some of which have historically been used for livestock feed, have the potential to promote more sustainable social, environmental, and economic outcomes by reducing dependence on fossil fuels and conserving soils, nutrients, and biodiversity (Gelfand et al., 2013; Landis et al., 2017). Another advantage is these outcomes can be achieved while avoiding both real and perceived competition with food security (Tilman et al., 2006; Dale et al., 2014). Nevertheless, because of its ubiquity on the landscape, agronomic efficiencies, and mature markets (James et al., 2010; Barham et al., 2016), corn stover is currently the main feedstock used for cellulosic ethanol in both the United States and the European Union (Janssen et al., 2013).

The importance of corn stover to the emerging cellulosic ethanol industry makes it an important benchmark crop for comparison against biofuel produced from other candidate biomass crops. However, comparisons are complicated because cropping system inputs (e.g., pesticides and fertilizer) typically are greater for corn than other candidate bioenergy crops, and stover comprises just half of the aboveground corn biomass, whereas all harvestable biomass is collected and used from perennial

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Abbreviations: ANPP, aboveground net primary production; ARL, Arlington Agricultural Research Station; [EtOH], ethanol content; [Glc], glucose content; KBS, Kellogg Biological Station; [Xyl], xylose content.

Published in *Agron. J.* 109:1–12 (2017)

doi:10.2134/agronj2016.08.0454

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METHODS

Study Sites and Experimental Design

bioenergy crops (Owens et al., 2013; Mitchell et al., 2016). Moreover, ethanol yields are likely to differ among cropping systems because both biomass production (Sanford et al., 2016) and sugar content (Garlock et al., 2012; Godin et al., 2013) from harvested crops can vary considerably in time and space.

Crop yields depend on climate, soils, landscape position, and management (Jarchow et al., 2012; Alexopoulou et al., 2015; Sanford et al., 2016). Sanford et al. (2016) showed that over a 6-yr period corn was more productive than a range of perennial crops on highly productive soils in southern Wisconsin when both the grain and stover components were included. However, on less productive soils in southern Michigan, some perennial crops produced similar or greater biomass than corn grain and stover combined. In this experiment, several dedicated perennial crops were capable of producing as much or more biomass than corn stover alone (assuming the corn grain would be directed to non-fuel products), but with fewer costly inputs. Polycultures of perennials that include more than one plant species may improve yield stability (i.e., reduced interannual and spatial variation) via complementarity (Tilman, 1996; Picasso et al., 2011; Stahlheber, K.A., R.D. Jackson, and K.L. Gross. 2017. Plant species diversity and yield stability in experimental fields of perennial bioenergy crops. *Agric. Ecosys. Environ.* [In preparation]) by improving soil health (Robertson et al., 2008; Duran et al., 2016) and increasing pest and pathogen suppression (Werling et al., 2014; Liere et al., 2015; Landis et al., 2017). However, yield stability is likely to come at the expense of attaining the highest possible biomass yields in years with favorable growing conditions (Webster et al., 2010; Duran et al., 2016).

Conversion of biomass to ethanol on a biological platform involves pretreatment to access, separate, and hydrolyze monomeric sugars from biopolymers within biomass (Davison et al., 2013), followed by fermentation of the released sugars to yield ethanol. Similar to biomass production, fermentable sugar content can vary by species, environment, and management practices such as harvest timing (Hedtcke et al., 2014; Garlock et al., 2012; Adler et al., 2009). Numerous pretreatment options and microbial-based fermentation cultures are available, and final ethanol yield for a given species is likely to vary depending on the combination of pretreatment and fermentation options used (Wyman et al., 2013). Moreover, the plant species comprising a particular feedstock can significantly alter pretreatment and conversion efficiencies. For instance, Garlock et al. (2012) found that a feedstock derived from diverse grassland communities yielded more sugars when at least ~60% of the feedstock was grasses; secondary compounds in dicotyledonous species were implicated as inhibitors in this example.

The objective of this study was to assess both the quantity and quality of available and proposed cellulosic feedstocks for the production of ethanol. To do this we estimated sugar content ([Glc] and [Xyl]) from a digestibility assay of the vegetative biomass from seven potential biomass cropping systems grown for 3 yr at two sites in the north-central United States. We then used these estimates to calculate a theoretical maximum [EtOH] from the sugar content of each feedstock and subsequently scaled [EtOH] using crop production data (early years reported in Sanford et al., 2016) to estimate ethanol yield on an areal basis.

This research was conducted at the U.S. Department of Energy (DOE)-Great Lakes Bioenergy Research Center's (GLBRC) Biofuel Cropping Systems Experiments (BCSE) located at the Arlington Agricultural Research Station in south-central Wisconsin (ARL, 43°17'45" N, 89°22'48" W, 315 m a.s.l.) and the W.K. Kellogg Biological Station in southwestern Michigan (KBS, 42°23'47" N, 85°22'26" W, 288 m a.s.l.). At both sites, cropping systems were established in a randomized complete block design with five blocks in spring 2008. Treatment plots were 27 by 43 m (0.12 ha) with at least 12-m buffers between adjacent plots in any direction. Representing a range of plant diversities (single to >25 species) and chemical inputs (low to high), the cropping systems were: (i) continuous corn, (ii) switchgrass, (iii) miscanthus, (iv) hybrid poplar, (v) native grass mix (five species planted), (vi) early successional community (>25 volunteer species), and (vii) restored prairie (18 species planted; for details see Sanford et al., 2016). We emphasized comparing realistic biomass crop management in an integrated "systems experiment" rather than comparisons of orthogonal components comprising treatments (Drinkwater et al., 2016). At ARL, the field site had been in a dairy forage rotation that included alfalfa (*Medicago sativa* L.), corn, and soybean [*Glycine max* (L.) Merr.] during the previous 8 yr. Prior to establishment in 2008, corn was grown on half of the field (blocks 4 and 5) for 4 yr and alfalfa on the other half (blocks 1, 2, and 3) for 3 yr at ARL. At KBS, the previous crops were alfalfa (blocks 1, 2, 3) and an alfalfa corn rotation (blocks 4 and 5) for the 4 yr prior to establishment.

The dominant soil series at ARL is Plano silt-loam (fine-silty, mixed, superactive, mesic Typic Argiudoll). These soils were formed under tallgrass prairie vegetation in loess deposits over calcareous glacial till and are relatively deep (>1 m), well drained, and have little relief. Thirty-year mean annual temperature and mean annual precipitation at ARL between 1981 and 2010 were 6.8°C and 869 mm, respectively (NWS, 2013). The dominant soil series at KBS is Kalamazoo loam (fine-loamy, mixed, semiactive, mesic Typic Hapludalf). These soils are deep, well drained, and were formed under forest vegetation in loamy outwash overlaying sand and gravel. The mean annual temperature and 30-yr mean annual precipitation at KBS were 9.9°C and 1027 mm, respectively, at the time of the study (MSCO, 2013).

Between 2012 and 2014, growing degree unit (GDU, base 10°C) accumulation (1 April to 31 October) did not vary considerably, with 2012 slightly above and 2013 and 2014 slightly below the 30-yr average at ARL (1563, 1352, and 1341 vs. 1500), and all years slightly below the 30-yr average at KBS (1714, 1613, and 1522 vs. 1743). Yearly and seasonal precipitation varied, particularly in 2012, when drought conditions prevailed and both experimental sites received very low rainfall compared to the 30-yr average. Kellogg Biological Station experienced below-average rainfall throughout the growing season (May through September 2012). In contrast, rainfall at ARL for July approached the 30-yr average while the rest of the growing season was below average. Rainfall at both sites in 2013 and 2014 was very near respective 30-yr averages although the seasonal distribution of precipitation events was quite variable.

Cropping System Establishment and Management

Agronomic decisions about planting densities, hybrid selection, nutrient management, and herbicide application followed local best management practices (see Sanford et al. [2016] for details) as recommended by University of Wisconsin (UW) and Michigan State University (MSU) extension agronomists (Laboski and Peters, 2016; Warncke et al., 2009). Field preparation, consisting of primary (chisel plow) and secondary (soil finisher) tillage, occurred in spring 2008 at both sites. In late June 2008, the perennial grass systems (including switchgrass, native grass mix, and restored prairie) were planted using a drop spreader (Truax Company, Inc., Minneapolis, MN) with two culti-pack rollers. Miscanthus rhizomes with one to two active growing points (industry standard) were hand planted at a depth of 10 cm (76 by 76 cm spacing) in late May 2008. Hybrid poplar cuttings were planted by hand in early May 2008 (1.5 m between plants in-row by 2.4 m between rows). Cuttings averaged 1.3 cm diam. by 25 cm length with a minimum of two active buds and were planted so that no more than 5 cm of the cutting tip was exposed above the soil surface. All planting densities were chosen according to University Extension best management practices with the purpose of maximizing yield at reasonable cost to a producer (Hansen et al., 1993; Renz et al., 2009; Heaton et al., 2011). The early successional treatment, defined as volunteer plant growth each season, required no planting and began with the final tillage pass in spring 2008.

No-till (NT) practices were adopted for each system following initial field preparation in 2008. At both sites, full-season corn hybrids (102–105 d) with advanced traits (e.g., herbicide tolerance and insect resistance) were selected to maximize productivity and remain consistent with local farming practices. Corn was planted annually using a six-row NT corn planter with 76-cm row spacing. Nitrogen application rates for corn were based on the economically optimal yield given the price of N and the value of corn grain (mean return to N) and were adjusted downward if spring soil nitrate tests indicated the presence of residual NO_3^- -N. Nitrogen rate averaged $160 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for both sites over the 3-yr period. Applications of P and K were plot-specific and based on annual fall soil sampling. Specific soil characteristics for each site were presented in Sanford et al. (2016). Because of inconsistent yield response to applied N in perennial grass crops, particularly on fertile soils, an N rate was chosen that would replace the N removed in harvest (Owens et al., 2013; Hong et al., 2014). Nitrogen was applied in the spring at a rate of 56 kg ha^{-1} to the switchgrass, miscanthus, native grass mix, and early successional systems. Hybrid poplar received a single N application in 2010 at a rate of 155 kg N ha^{-1} at KBS and 210 kg N ha^{-1} at ARL. The restored prairie system was not fertilized during the study and no P or K applications were made to the perennial systems.

In the early successional and restored prairie polycultures, species composition was determined at peak standing crop (mid-August). The long side of a 2.0 by 0.5-m quadrat was placed in an East–West direction at a distance of 2.1 m from three pre-determined sampling stations in each plot. Each year quadrat placement relative to the sampling station changed (e.g., Northeast, Northwest, Southeast, Southwest) so that biomass was not harvested in the same location twice within four consecutive years. All plant biomass rooted in the quadrat was identified, clipped to ground level and bagged for dry matter (DM) determination.

Harvesting Biomass

Grain and biomass harvests were performed using commercial-grade agricultural equipment. At both sites, ~40% of total corn stover was collected shortly after grain harvest using a flail-chopper/forage-wagon combination, leaving ~10 cm of residual stubble height. Samples were collected from each plot for moisture content determination by oven-drying at 60°C until weight was stable, and subtracting the dry weight from the initial weight. All yields were corrected to 100% DM. Harvest of switchgrass, miscanthus, native grass mix, early successional, and restored prairie occurred within 2 wk following the first killing frost of autumn (-3.5°C , typically after mid-October) using the most appropriate equipment available at each study site. At ARL, biomass was cut and windrowed, then chopped with a self-propelled forage harvester into a dump wagon equipped with load cells. Biomass at KBS was cut directly using a self-propelled forage harvester. The biomass was chopped into a forage truck equipped with load cells or weighed using the local grain-truck scale. Cutting height at both sites left 15 cm of residual stubble and harvest efficiency averaged 60% for switchgrass and native grass mix, 50% for miscanthus and early successional, and 40% for restored prairie (Sanford et al., 2016). All yields were corrected to 100% DM as described above.

Hybrid poplar biomass was harvested after its sixth growing season in early December 2013 at ARL and mid-January 2014 at KBS. At ARL, hybrid poplar trees were cut by hand ~10 cm above the soil surface and then all biomass was chipped into a truck. At KBS, trees were harvested using a hydraulic cutting shear ~2 cm above the soil surface with biomass chipped into a truck. At both sites, biomass was weighed field moist using truck scales and yields were corrected to a DM basis as described in the previous paragraph.

Sugar Content Assays

Glucose content and [Xyl] of biomass was determined using a high-throughput analytical platform designed to screen plants for desirable agronomic traits (Santoro et al., 2010). Biomass grinding and weighing were performed by a custom-designed robot (Labman Automation Ltd., Middlesbrough, UK). Samples of dried plant material (20–40 mg) were loaded manually into 2-mL screw-cap microtubes (Sarstedt, Nümbrecht, Germany) along with three 5.56-mm stainless steel balls (Salem Specialty Ball Co, Canton, CT). The tubes were placed into racks and positioned in the robot where pulverization was accomplished by ball milling. The length of the milling time was adjusted sufficient to reduce the sample to a fine powder. A 1.5-mg biomass subsample was transferred to a barcoded 1.4-mL polypropylene microtube sealed with a thermoplastic elastomer cap (Micronic brand, Aston, PA) and 750 μL of pretreatment solution (NaOH [62.5 mM]) was pipetted into each tube and then placed into a water bath at 90°C for 3 h. Where necessary, reactions were neutralized with ~7.5 μL of 6 M HCl. Next, 50 μL of 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 all tubes. Enzymatic hydrolysis was done in a final volume of 0.8 mL using an enzyme concentration of $50 \text{ g protein kg}^{-1}$ glucose. Tubes were placed in racks and incubated for 20 h in a rotisserie oven at 50°C . Racks were centrifuged and supernatants

were transferred to 0.8 mL deep-well plates. The [Glc] and [Xyl] of samples were determined using enzyme-based assay kits (Megazyme, Ireland). The [Glc] was assayed with the glucose oxidase/peroxidase (GOPOD) method (K-GLUC, Megazyme, Ireland) using 4 μL of the supernatant of the digestion reaction mixture and 64 μL of the GOPOD assay reagent. The [Xyl] was assayed enzymatically (K-XYLOSE, Megazyme) using 8 μL of sample and 62 μL K-XYLOSE of assay reagent (for details see Santoro et al., 2010).

Ethanol Content and Yield Calculations

Ethanol content [EtOH] was calculated based on biomass [Glc] and [Xyl] as:

$$[\text{EtOH}] = ([\text{Glc}] + [\text{Xyl}]) \times 0.51 \times \text{metabolic yield}$$

Per Lau and Dale (2009), a theoretical maximum conversion factor 0.51 g EtOH g^{-1} sugar was used for the mass conversion of sugars to ethanol content. Hence, metabolic yield was the ratio of ethanol to the consumed sugars in the fermentation process divided by 0.51 g g^{-1} (Lau and Dale, 2009). Metabolic yield values were determined using a separate hydrolysis and fermentation (SHF) process and were derived from Jin et al. (2010) for switchgrass (0.897 g g^{-1}) and Jin et al. (2012) for corn stover (0.931 g g^{-1}). Metabolic yield for other perennial biomass feedstocks was assumed to be the same as switchgrass (0.897 g g^{-1}). Ethanol yields on an areal basis were calculated by combining site-specific biomass crop production estimates with site and crop [EtOH] and converting to volume based on an EtOH density of 0.7893 g cc^{-1} .

Data Analyses

Linear mixed effect models (PROC GLIMMIX, SAS v9.3, SAS, 2012) were used to compare [Glc], [Xyl], [EtOH], DM production, and EtOH yields by cropping system within site across years and cropping systems within years and sites. Year, and blocks nested within site were treated as random effects. The residual option in the random effects statement (random _residual_, PROC GLIMMIX, SAS v9.3) was used to account for temporal autocorrelation between quality components, and biomass and ethanol yields within a given field plot. The unstructured covariance matrix (type = un) was selected based on goodness of fit (BIC), allowance for variance inequality, and relevance to the experimental data for all but per hectare ethanol yields where a first order autoregressive covariance structure was used [type = ar(1)]. Fisher's protected LSD was used for least squares means separation when fixed effects were significant ($P \leq 0.05$) in a given analysis.

All systems except hybrid poplar were harvested annually and results are presented on a yearly basis. The hybrid poplar system average was calculated as the 6-yr average annual growth spanning from 2008 (date of planting) to the single winter harvest of 2013.

Scatterplots with least square fits for each cropping system with sites and years were examined to explore relationships among response variables.

Coefficients of variation (CVs) were calculated within sites and cropping systems and across years. Significance of differences between CVs were evaluated using the asymptotic test

of Feltz and Miller (1996), as implemented in the R package cvequality (v 0.1.1, Marwick and Krishnamoorthy 2016, <http://cran.r-project.org/package=cvequality>). Significance of correlation coefficients was determined using the rcorr() function in the R package Hmisc (v 4.0-2, Harrell and Dupont (2017), <http://cran.r-project.org/package=Hmisc>).

RESULTS

While some interannual differences were observed in our response variables (Fig. 1), variability was relatively low from year to year, especially in sugar content responses, so we focused our interpretations at the cropping systems level across years. A significant site \times cropping system interaction was observed for all response variables, so results were analyzed and shown separately for each site.

Sugar and Ethanol Content

The corn stover system, the only annual crop in the experiment, exhibited the highest [Glc] and [Xyl] across the 3 yr of the experiment (Fig. 1A–1D). The perennial grass systems (i.e., switchgrass, native grass mix, and miscanthus) were the next highest yielding groups for [Glc] and [Xyl] and hybrid poplar was the lowest. The 3-yr average cropping system ranking for [Glc] was relatively consistent across sites (Fig. 1A and 1B) with corn stover having the highest concentrations ($^3203 \text{ g kg}^{-1}$) and hybrid poplar the lowest (87 g kg^{-1}). The main [Glc] differences between ARL and KBS were related to the magnitude of [Glc] in the corn stover (ARL = 223 and KBS = 203 g kg^{-1} , $P < 0.0001$) and restored prairie systems (ARL = 117 and KBS = 142 g kg^{-1} , $P < 0.0001$).

The system average relative ranking of [Xyl] differed slightly by site (Fig. 1C and 1D). Over the 3-yr period, average [Xyl] at ARL ranged from a low of 49 g kg^{-1} for hybrid poplar to a high of 120 g kg^{-1} for corn stover. At KBS average [Xyl] for the 3-yr period ranged from a low of 45 g kg^{-1} for hybrid poplar to a high of 110 g kg^{-1} for corn stover. The main [Xyl] variability between sites was the difference in the native grass mix (ARL = 100 and KBS = 85 g kg^{-1} , $P = 0.0038$) and early successional (ARL = 75 and KBS = 59 g kg^{-1} , $P = 0.0061$) systems.

The feedstock produced from the restored prairie and early successional systems contained several broadleaf plant species in addition to grasses (Table 1) and subsequently had lower levels of [Glc] and [Xyl] than the grass-only feedstocks. At ARL, grasses contributed 3.9 times more biomass to early successional productivity than did forbs, as compared to KBS where the grass/forb ratio (1:6) was less pronounced (Table 1).

A significant site \times cropping system interaction was observed for [EtOH]. At ARL, 3-yr average [EtOH] ranged from a low of 62 g kg^{-1} for hybrid poplar to a high of 162 g kg^{-1} for corn stover (Fig. 1E). At KBS, 3-yr average yields ranged from a low of 61 g kg^{-1} for hybrid poplar to a high of 149 g kg^{-1} for corn stover (Fig. 1F). The main difference between sites was the magnitude of [EtOH] for corn stover (ARL = 162, and KBS = 149 g kg^{-1} , $P < 0.0001$) and restored prairie (ARL = 86, and KBS = 100 g kg^{-1} , $P < 0.0001$). The only woody perennial system in our experiment, hybrid poplar, had very consistent [EtOH] across both sites (Fig. 1E and 1F).

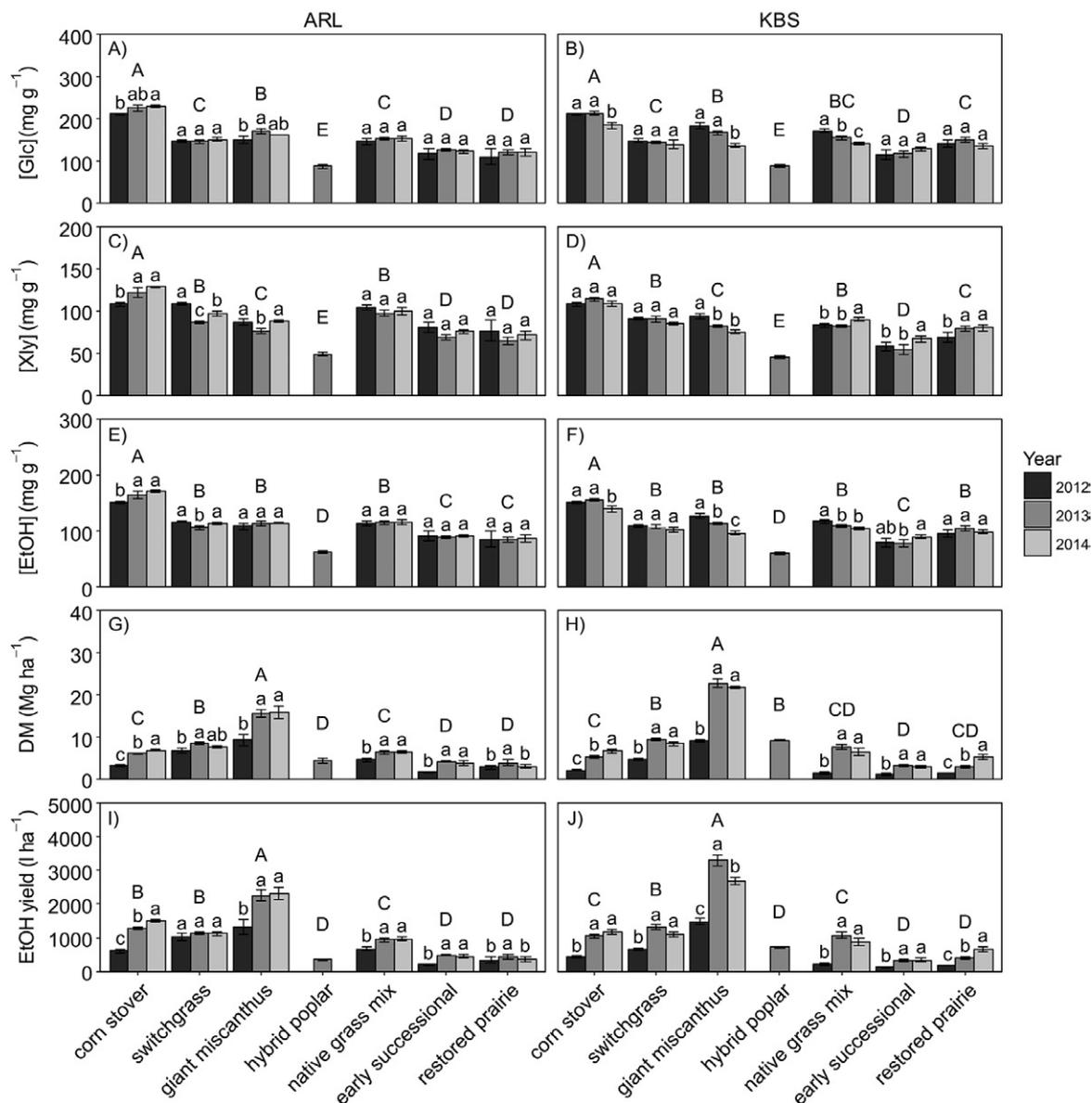


Fig. 1. (A, B) Annual [Glc], (C, D) [Xyl], (E, F) [EtOH], (G,H) biomass production, and (I,J) ethanol yields at ARL and KBS, respectively. Lowercase letters indicate significant differences between years within system and site ($p < 0.05$), while uppercase letters indicate cropping system differences within a site across years ($p < 0.05$).

Crop Production

We observed a highly significant site \times cropping system effect ($P < 0.0001$) in the analysis of biomass yield. Over the 3-yr period, average biomass yields at ARL ranged from a low of 3.3 Mg ha^{-1} for the restored prairie system to a high of 13.7 Mg ha^{-1} for miscanthus (Fig. 1G). At KBS, average 3-yr yields ranged from a low of 2.5 Mg ha^{-1} for the early successional system to a high of 18.0 Mg ha^{-1} for miscanthus (Fig. 1H). At both sites, switchgrass yields were just above 7.5 Mg ha^{-1} and corn stover yields averaged close to 5.0 Mg ha^{-1} .

Results reported in Sanford et al. (2016) for the 2012 and 2013 growing seasons showed that harvest efficiencies (HE = yield/aboveground net primary production [ANPP]) for all of the cropping systems, with the exception of hybrid poplar, were comparable and ranged from 0.4 for corn stover and prairie to 0.6 for switchgrass and native grass mix. Corn stover ANPP was 10.2 Mg ha^{-1} , with 4.1 Mg ha^{-1} recovered at harvest. For switchgrass 7.0 Mg ha^{-1} was recovered of 11.3 Mg ha^{-1} of

ANPP and for miscanthus 12.3 Mg ha^{-1} of the 23 Mg ha^{-1} of ANPP was recovered. With an average N rate in corn of $160 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, and stover accounting for approximately 30% of N removed by the crop (i.e., $48 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; Bundy, 1998), both harvest efficiencies and N application rates were comparable between corn and the herbaceous perennial cropping systems.

The hybrid poplar system, unlike the other cropping systems, was harvested once during the duration of the study. Six-year annualized hybrid poplar average biomass yields were significantly different between sites (ARL = 4.4 and KBS = $9.2 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, $P = 0.0004$). The ARL hybrid poplar yields were equivalent to those of the restored prairie and early successional systems despite substantial hybrid poplar damage caused by *Marssonina* spp. leaf spot fungus (Sanford et al., 2016). At KBS, the hybrid poplar performed markedly better, with yields second only to the miscanthus cropping system (Fig. 1G and 1H).

Table 1. Grass (G) and forb (F) biomass in early successional and restored prairie systems as a percentage of total plot biomass. Dominant grass and forb species are presented in Table 1 of Sanford et al. (2016).

Site†	System	2012			2013			2014			System average		
		Grass	Forbs	G/F	Grass	Forbs	G/F	Grass	Forbs	G/F	Grass	Forbs	G/F
g DM m ⁻²													
ARL	Early successional	135.2a	67.3b	2.0	332.6a	63.2b	5.3	667.1a	175.8b	3.8	390.5a	100.9b	3.9
	Restored prairie	64.5a	74.1a	0.9	108.1a	112.6a	1.0	143.2a	122.9a	1.2	107.5a	99.5a	1.1
KBS	Early successional	19.4a	24.2a	0.8	60.8a	59.4a	1.0	73.9a	22.8b	3.2	54.1a	34.5b	1.6
	Restored prairie	35.5a	15.8b	2.2	94.5a	36.6b	2.6	97.6a	15.6b	6.3	75.8a	21.7b	3.5

† ARL = Arlington, WI; KBS = Kellogg Biological Station, MI. Means within system and year followed by a common letter are not significantly different $P \leq (0.05)$.

Ethanol Yield

Differences in ethanol yield per hectare depended on site. At ARL, average yields over 3 yr ranged from a low of 339 L ha⁻¹ for hybrid poplar to a high of 1957 L ha⁻¹ for miscanthus (Fig. 1I). At KBS, average per hectare ethanol yields ranged from a low of 257 L ha⁻¹ for the early successional system to a high of 2485 L ha⁻¹ for miscanthus (Fig. 1J). System rankings were similar across study sites with the exception of (i) differences between ARL and KBS in the ranking of the hybrid poplar system and (ii) the magnitude of ethanol yield in the miscanthus system (hence the significant site × system effect).

Variability of Ethanol Yield Components

We estimated ethanol yield per hectare as a multiplicative relationship between biomass yields and [EtOH]. The CVs for these components reflect their relative impact on overall ethanol yields. In nearly all cases, variability was higher for biomass yields than for [EtOH] (Table 2). The sole exceptions were the restored prairie at ARL, which had the most variable [EtOH] of any system, and the hybrid poplar at KBS, which had the least variable

biomass of any system. Consequently, variance in ethanol yield matched variance in biomass production to a greater extent than [EtOH] (Fig. 2).

At ARL, biomass yield and [Glc] were positively correlated in all systems except for the hybrid poplar, although not all correlations were statistically significant (Table 2, Fig. 3A). Conversely, at KBS biomass yield and [Glc] were negatively correlated, although again not always with statistical significance (Table 2, Fig. 3B). In contrast, relationships between biomass yield and [Xyl] were less consistent and less frequently significant (Table 2, Fig. 3C and 3D).

DISCUSSION

Ethanol Yields Driven by Biomass Production

Biomass supply is the main bottleneck in the siting of cellulosic biorefineries (Carolan et al., 2007; Eranki and Dale, 2011) and ultimately, development of the nascent bioeconomy (Richard, 2010). The large amount of capital needed to plan and build a biorefinery requires a known and dedicated supply of feedstock, with known characteristics, be committed to

Table 2. Coefficients of variation (CV) for ethanol content and biomass yield, and correlations between biomass and sugar content for seven bioenergy cropping systems grown at two sites.

Site†	System	Biomass	[EtOH]‡	Biomass-[Glc]	Biomass-[Xyl]
			CV	correlation coefficient	
ARL	Continuous corn	32.1%	7.1%§	0.55*	0.64*
	Switchgrass	13.9%	6.2%**	0.19	-0.48
	Miscanthus	29.6%	7.7%§	0.56*	-0.06
	Native grass mix	20.6%	7.6%***	0.26	-0.38
	Hybrid poplar	28.2%	9.8%§	-0.95*	-0.93*
	Early successional	42.0%	12.6%***	0.20	-0.44
	Restored Prairie	37.6%	22.2%	0.59*	0.37
KBS	Continuous corn	43.2%	6.6%§	-0.53*	0.09
	Switchgrass	29.9%	7.7%§	-0.16	-0.02
	Miscanthus	36.5%	12.7%***	-0.58*	-0.73**
	Native grass mix	58.8%	6.2%§	-0.69**	0.05
	Hybrid poplar	5.2%	8.6%	-0.76	-0.31
	Early successional	46.9%	16.1%***	0.02	-0.09
	Restored Prairie	56.0%	11.0%‡	-0.16	0.37

* Significance of difference between biomass and ethanol coefficients of variation (Feltz and Miller, 1996): $P \leq (0.05)$.

** Significance of difference between biomass and ethanol coefficients of variation (Feltz and Miller, 1996): $P \leq (0.01)$.

*** Significance of difference between biomass and ethanol coefficients of variation (Feltz and Miller, 1996): $P \leq (0.001)$.

† ARL = Arlington, WI; KBS = Kellogg Biological Station, MI.

‡ [EtOH], ethanol content; [Glc], glucose content; [Xyl], xylose content.

§ Significance of difference between biomass and ethanol coefficients of variation (Feltz and Miller, 1996): $P \leq (0.0001)$.

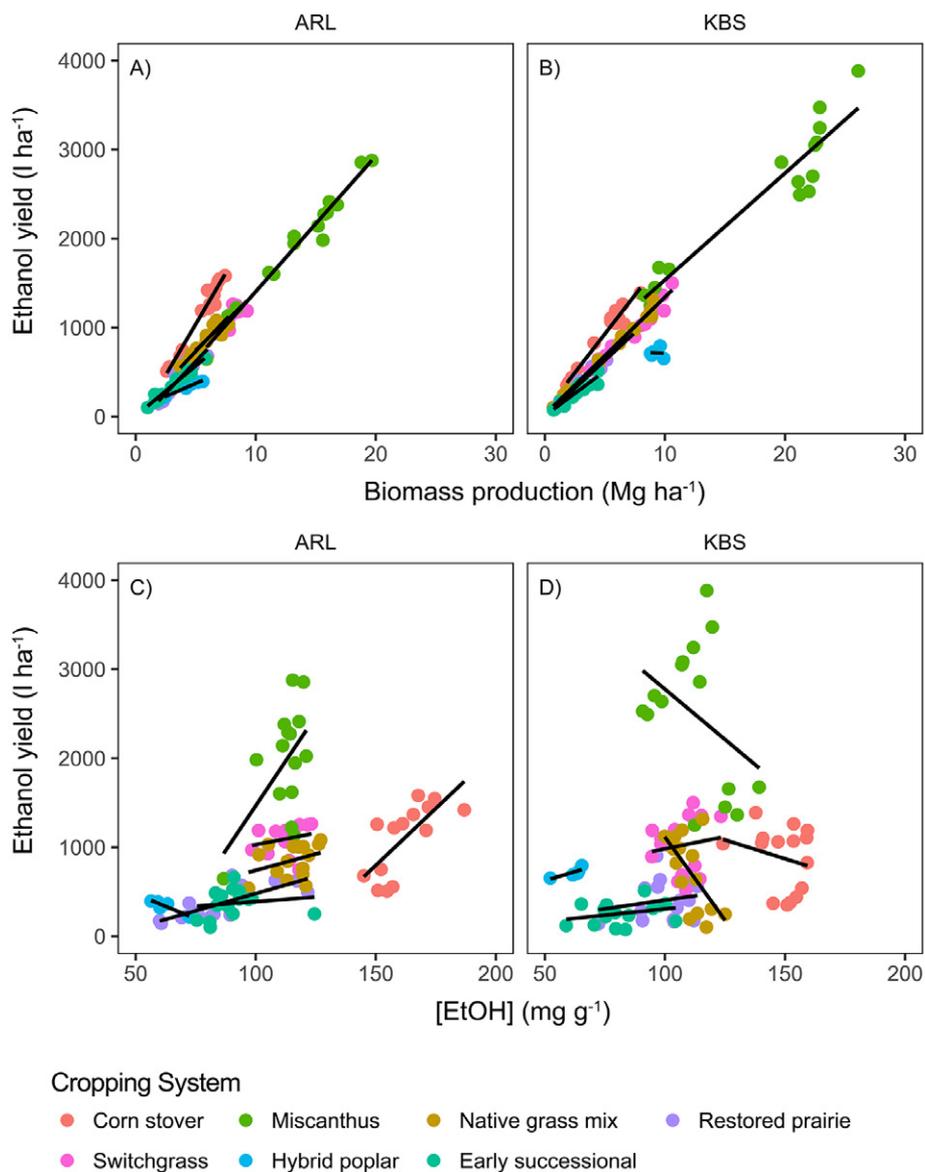


Fig. 2. Scatterplots with least squares fits for ethanol yield vs. biomass production at (A) Arlington Agricultural Research Station (ARL), and (B) Kellogg Biological Station (KBS), as well as ethanol yield vs. [EtOH] at (C) ARL, and (D) KBS.

the refinery (Sendich and Dale, 2009). However, transportation costs of low-density herbaceous crops can be prohibitive, so some have pointed to a particular radius around a proposed biorefinery—so-called “fuelsheds”—as the key hectares for biomass supply (Kim and Dale, 2015). Gelfand et al. (2013) assumed an average biomass supply of 8 Mg ha⁻¹ could be harvested from fertilized herbaceous species growing on presently fallow lands in the north-central region of the United States and found that at these biomass production levels, 35 biorefinery possibilities with fuelshed radii of 80 km existed in this 10-state area. Biomass production estimates for the lowest yielding cropping systems in our experiment (fertilized early successional fields and unfertilized restored prairie ~3 Mg ha⁻¹) indicates low potential for biorefinery siting based on these systems alone. However, it should be possible to ensure a consistent supply of biomass by developing a diversity of feedstock sources including switchgrass monocultures and corn stover (~6 Mg ha⁻¹), mixed grass stands (~6 Mg ha⁻¹), and miscanthus (~16 Mg ha⁻¹) into a landscape mosaic surrounding a biorefinery. In addition, the resulting land

use and land cover mosaic would likely provide significantly higher levels of other ecosystem services such as higher crop yields in pollinator-dependent crops (Liere et al., 2015), better wildlife habitat (Robertson et al., 2011), and climate stabilization (Oates et al., 2016) among others (Landis et al., 2017; Ventura et al., 2012). Moreover, where the feasibility and availability of feedstock supply align, these fuelsheds could be expanded with a distributed network of preprocessing and aggregation depots where pretreatment and densification occur (Eranki et al., 2011). The construction and operation of these depots would have the additional benefit of providing significant economic stimulus to rural economies and alternative markets for agricultural residues (Egbenewe-Mondzozo et al., 2013).

Consistent with previous findings, we found relatively small variation in the sugar content and thus [EtOH] of our candidate feedstocks relative to variation in crop production. Jungers et al. (2013), for example, working in conservation grasslands in Minnesota, found that variation in ethanol yield per hectare was almost exclusively the result of biomass yield variability rather

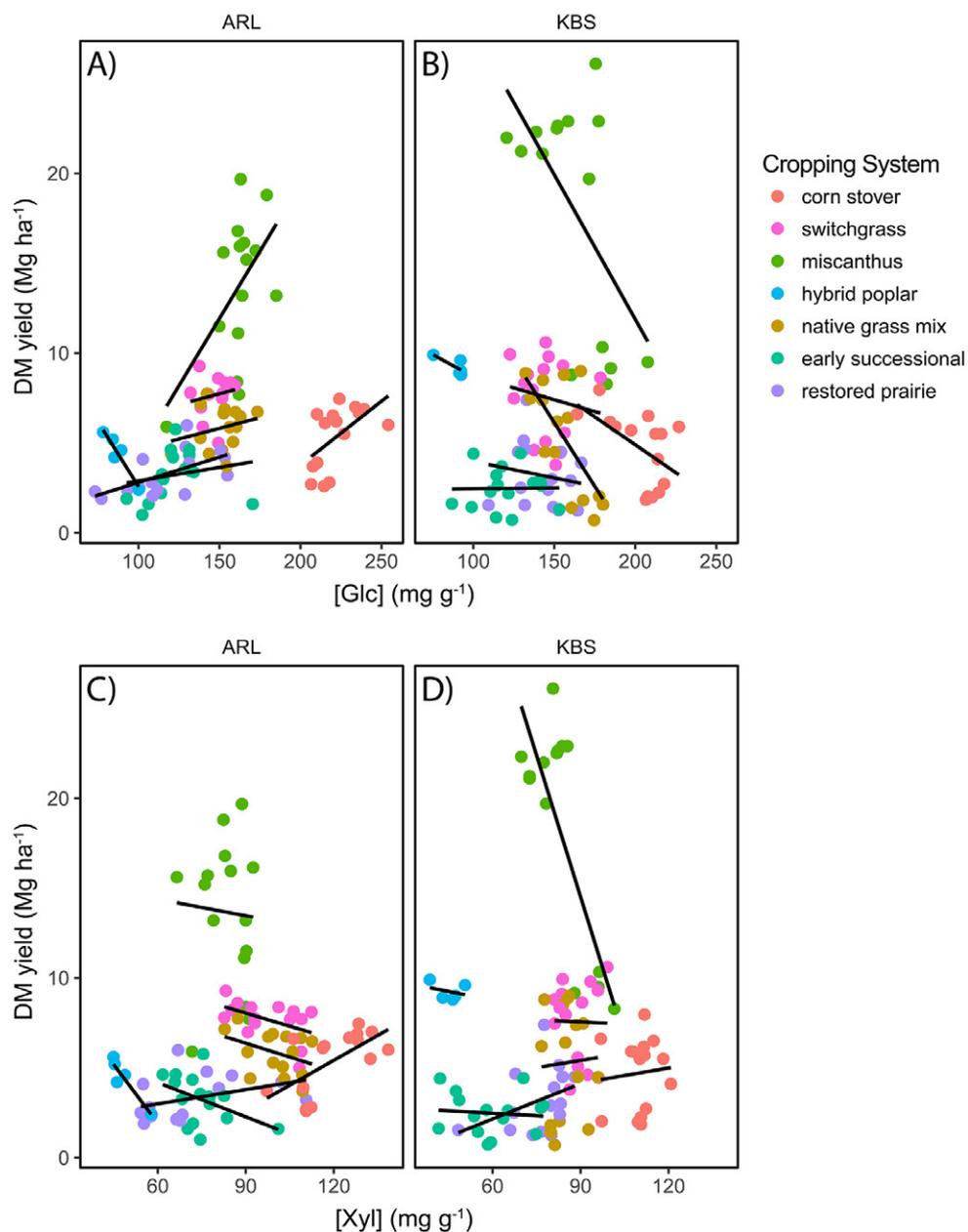


Fig. 3. Scatterplots with least squares fits for biomass production vs. [Glc] at (A) Arlington Agricultural Research Station (ARL), and (B) Kellogg Biological Station (KBS), as well as biomass production vs. [Xyl] at (C) ARL, and (D) KBS.

than biomass quality. Tumbalam et al. (2016) also concluded that corn stover yields were more important than corn stover quality in determining areal ethanol yields. In an extension of these studies, we found this relationship largely held both within and among a variety of feedstock production systems. The relative insensitivity of ethanol yields to biomass quality is critical to the scenario described above, where multiple cropping systems are feeding into a biorefinery, because it allows the pretreatment and conversion processes to be more or less agnostic with respect to what type of biomass is delivered to the depot for pretreatment and densification.

Biomass yields reported here and previously (Sanford et al., 2016) are consistent with the range of yields reported across the U.S. Midwest and elsewhere for similar bioenergy cropping systems (James et al., 2010; Propheter and Staggenborg, 2010; Jarchow et al., 2012; Johnson et al., 2013). Godin et al. (2013)

for example, showed that miscanthus was as productive as corn silage in trials in Europe, and James et al. (2010) reported miscanthus yields of $22 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ in the north-central United States. The relatively low biomass yields we report from the polyculture systems are consistent with Griffith et al. (2011) who reported a 0.6 to $1.4 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ yield decline in polycultures relative to monocultures across two locations in Oklahoma.

We observed contrasting relationships between biomass yields and sugar concentrations at our two sites. At the more productive ARL site, yields and sugar content were positively correlated, which bodes well for efforts to improve ethanol production in comparable environments. Yield and sugar content were frequently negatively correlated at the less productive KBS site, as well as in the highly stressed hybrid poplar system at ARL (Sanford et al., 2016). It is likely that bioenergy cropping systems will be grown on marginal land, and thus in frequently stressed

environments (Tilman et al., 2009). If the relationship between yield and sugar content holds across less productive environments, management for bioenergy production may need to consider sugar content and availability in addition to biomass yields.

Grass-based Systems had Higher Sugar Content than Grass–Forb Mixtures

Our results and those of others indicate that the majority components of feedstocks within localized fuelsheds should be grasses rather than forbs to maintain relatively high pretreatment and conversion efficiency (Garlock et al., 2012). Among the non-woody systems in our study, structural sugar content ([Glc] and [Xyl]) decreased as (i) species diversity increased, and (ii) the grass-forb ratio of the feedstock decreased. Others have also reported species differences in feedstock [Glc] and [Xyl], often noting similar trends related to diversity and plant functional group. For example, Kumar et al. (2012) found significant differences in [Glc] and [Xyl] among planted and volunteer species in conservation buffers in the Pacific Northwest. Jabbour et al. (2014), reported lower [Glc] and conversion efficiencies in four common forb species compared to corn stover, and Adler et al. (2009) reported that biofuel yield per unit land area decreased by 77% as plant species richness increased from 3 to 12.8 species per square meter, corroborating our findings of lower [EtOH] for the polyculture treatments in general. This compounded result stemmed from the combination of greater biomass yield and greater [EtOH] as the ratio of grass relative to broadleaf species increased. Similarly, Garlock et al. (2012) found that mixed-species feedstocks with high grass composition tended to have higher structural sugar contents and were more digestible than forb-dominated feedstocks resulting in higher saccharification yields.

These findings align with the results from the mixed grass–forb systems in our study. Differences in [EtOH] between the early successional and restored prairie systems at ARL and KBS in large part arose from the grass–forb ratio in each system and the effect of species composition on sugar concentrations. At ARL, for example, where [EtOH] was higher in the early successional system, the grass–forb ratio was 3.9 compared to just 1.1 in the restored prairie. However, [EtOH] was higher at KBS in the restored prairie system where the grass–forb ratio was 3.5 compared to 1.6 in the early successional system. In addition to favorable sugar profiles (Garlock et al., 2012) and high yield potential (Jarchow et al., 2012; James et al., 2010; Sanford et al., 2016), many bioenergy grasses produce considerable belowground biomass, which has been linked to soil C stabilization (Liebig et al., 2008; Sanford, 2014). Efforts to design polyculture bioenergy systems should therefore consider the ecosystem services that a target species provides (e.g., climate mitigation, crop pollination, and flood mitigation) in addition to its yield potential and ethanol conversion efficiency.

The [EtOH] of poplar was significantly lower than our herbaceous crops, which may be related to the particular NaOH pretreatment used in our analytical protocol. Fortunately, alternative pretreatment processes to the alkaline conditions we used are available and known to perform better with woody species (da Costa Sousa et al., 2009; Wyman et al., 2013). Moreover, in some regions, hybrid poplar offers advantages relative to herbaceous feedstocks including drought tolerance and duration of feedstock storage (Sannigrahi et al., 2010).

Edaphic and Growing Season Conditions Influence Ethanol Yields

In addition to feedstock diversity and species composition, edaphic conditions may affect [Glc] and [Xyl]. For example, Wyman et al (2013) reported significant differences in [Glc] and [Xyl] in the same poplar variety grown at different sites. Tumbalam et al. (2016) and Gao et al. (2011) both reported significant site effects on corn stover [Glc] and attributed these effects to differences in growing conditions. We found very little difference in [Glc] and [Xyl] related to site, although significant differences in EtOH yields occurred in response to the effect edaphic conditions had on biomass production. For example, 3-yr average EtOH yields for corn stover were higher at ARL than KBS by 243 L ha⁻¹ although switchgrass EtOH yields were quite comparable. The coarse-textured Alfisols at KBS, which are subject to occasional periods of water stress, coupled with a slightly milder climate, provided a production advantage for the perennial switchgrass cropping system over corn that was not observed on the more productive and drought-tolerant Mollisols at ARL. In addition to a slight difference in cropping system ranking, there were also differences in the magnitude of ethanol yields within cropping systems between the two sites. The ethanol yield disparity for corn stover arose from the higher corn grain yield at ARL. Despite the high per hectare ethanol yields in miscanthus, the KBS advantage was related to the fact that the ARL miscanthus stand was two growing seasons younger than the KBS stand as a result of overwintering stand loss in 2008 at ARL (Sanford et al., 2016).

Biomass Crop Production is Key, but Other Considerations are Important

At both sites, miscanthus was the highest yielding system in terms of both biomass and ethanol yield per hectare, reinforcing its appeal as a candidate bioenergy feedstock (Johnson et al., 2013; Heaton et al., 2004). However, the resilience of miscanthus with regard to insect, disease, and cold in North America remains an open question. Evaluating North American miscanthus, Bradshaw et al. (2010) reported infestations of yellow sugarcane aphid (*Sipha flava*) and corn leaf aphid (*Rhopalosiphum maidis*), both of which are known vectors of potyviruses. Similarly, Falter and Voigt (2014) screened 13 fungal species on detached miscanthus leaves and identified four that were infectious. In addition to potential insect and disease issues, cold sensitivity in miscanthus is frequently reported, with up to 50% mortality documented when soil temperatures at the depth of planting reach -3.5°C (Clifton-Brown and Lewandowski, 2000; Lewandowski et al., 2000; Zub and Brancourt-Hulmel, 2010). In a modeling study, Kucharik et al. (2013) showed that soils in the north-central region had temperatures below -3.5 and -6.0°C in greater than 75 and 50% of years, respectively, between 1978 and 2007. Similar to our experience, Johnson et al. (2013) reported cold-related establishment difficulties with miscanthus in Minnesota and suggested that these issues may limit the use of the crop in colder climates. Also concerning is the potential for miscanthus to become invasive (Raghu et al., 2006). Given the limited genetic base and cold hardiness in commercially available miscanthus rootstock, widespread adoption of giant miscanthus carries significant risks. These risks as well as similar consideration with any dedicated bioenergy feedstock must be factored into any deployment decision.

CONCLUSIONS

While there is significant effort allocated toward improving biomass quality to improve the feasibility of biological conversion to ethanol, it was clear from our analysis that crop production was the main driver of ethanol yields. Our results also indicated that less productive sites might result in anti-quality responses of many dedicated biomass crops, particularly in stressful growing conditions. Unfortunately, growing conditions are predicted to be more stressful in the future. Emphasis on growing dedicated bioenergy crops on so-called “marginal lands” is likely to exacerbate this situation. Cropping systems dominated by grasses had higher sugar content, and therefore higher ethanol content, so species composition is an important secondary consideration when planting dedicated biomass plots. Finally, while biomass yields clearly drove ethanol yields on a per hectare basis, it is important to consider how the choice of cropping system is likely to influence ecosystem services other than profitability, especially if yield differences between competing feedstocks are not high.

ACKNOWLEDGMENTS

Thanks to S.K. Hamilton, N. Santoro, A.P. Butz, C.C. McMinn, C. Menick, J.T. Simmons, S.J. Sippel, J.O. Sustachek, J.R. Tesmer, S.L. VanderWulp, and numerous other field and lab personnel who made this study possible. Funding was provided by the DOE-Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494) and the DOE OBP Office of Energy Efficiency and Renewable Energy (DE-AC05-76RL01830), and at KBS the NSF Long-term Ecological Research Program and Michigan State University AgBioResearch.

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