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Title: Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA

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Abstract

Biofuels from lignocellulosic feedstocks have the potential to improve a wide range of ecosystem services while simultaneously reducing dependence on fossil fuels. Here, we report on the six-year production potential (above ground net primary production, ANPP), post-frost harvested biomass (yield), and gross harvest efficiency (GHE=yield/ANPP) of seven model bioenergy cropping systems in both southcentral Wisconsin (ARL) and southwest Michigan (KBS). The cropping systems studied were continuous corn (*Zea mays* L.), switchgrass (*Panicum virgatum* L.), giant miscanthus (*Miscanthus × giganteus* Greef & Deuter ex Hodkinson & Renvoize), hybrid poplar (*Populus nigra* × *P. maximowiczii* A. Henry ‘NM6’), a native grass mixture (5 sown species), an early successional community, and a restored prairie (18 sown species). Overall the most productive cropping systems were corn > giant miscanthus > and switchgrass, which were significantly more productive than native grasses ≈ restored prairie ≈ early successional ≈ and hybrid poplar, although some systems (e.g. hybrid poplar) differed significantly by location. Highest total ANPP was observed in giant miscanthus ($35.2 \pm 2.0 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) at KBS during the sixth growing season. Six-year cumulative biomass yield from hybrid poplar at KBS ($55.4 \pm 1.3 \text{ Mg ha}^{-1}$) was high but significantly lower than corn and giant miscanthus (65.5 ± 1.5 , $65.2 \pm 5.5 \text{ Mg ha}^{-1}$, respectively). Hypothesized yield advantages of diversity in perennial cropping systems were not observed during this period. Harvested biomass yields were 60, 56, and 44% of ANPP for corn, perennial grass, and restored prairie, respectively, suggesting that relatively simple changes in agronomic management (e.g. harvest timing and harvest equipment modification) may provide significant gains in bioenergy crop yields. Species composition was an important determinant of GHE in more diverse systems.

Results show that well-established, dedicated bioenergy crops are capable of producing as much biomass as corn stover, but with fewer inputs.

Keywords: corn stover, giant miscanthus, harvest efficiency, hybrid poplar, restored prairie, switchgrass

1. Introduction

Producing biofuels from lignocellulosic feedstocks has the potential to improve social, economic, and environmental goals by increasing energy production and the supply of multiple ecosystem services (Robertson et al., 2008; Meehan et al., 2013) while avoiding the use of food/feed crops such as corn grain. With well-developed harvest, processing, and transportation infrastructure in place, agricultural crop residues such as corn stover and wheat straw are the most common feedstocks currently employed for the production of lignocellulosic ethanol in both the United States and European Union (Janssen et al., 2013). Although abundant (58.3 Tg harvestable US corn stover, Graham et al. 2007), use of annual crop residues may exacerbate the negative environmental externalities of annual grain production (e.g. soil carbon loss, erosion; Blanco-Canqui and Lal, 2007).

As an alternative to annual crops and crop residues, perennial cropping systems have the potential to provide high yields while helping to sequester soil carbon, stabilize climate, and improve water quality (Robertson et al., 2011; Gelfand et al., 2013; Sanford, 2014). Gelfand et al. (2013) for example showed that if fertilized; successional herbaceous vegetation was capable of producing more energy than annual grain crops (65 vs. 41 GJ ethanol energy ha⁻¹ yr⁻¹) with a higher potential to mitigate greenhouse gas emissions (-851 vs. -397 g CO₂eq m⁻² yr⁻²). Moreover, diverse assemblages in perennial cropping systems should promote biodiversity in other trophic levels (Webster et al., 2010; Robertson et al., 2012; Werling et al., 2014) and may

improve long-term yield stability via improved pest suppression and other important ecosystem services (Meehan et al., 2012). Candidate perennial systems include grass monocultures (e.g. switchgrass [*Panicum virgatum* L.] and giant miscanthus [*Miscanthus × giganteus* Greef & Deuter ex Hodkinson & Renvoize]), fast growing woody species (e.g. hybrid poplar [*Populus* spp.] and willow [*Salix* spp.]), and diverse herbaceous assemblages such as those found in restored prairies and successional plant communities (Tilman et al., 2006; Heaton et al., 2008; Vogel et al., 2011; Gelfand et al., 2013).

Switchgrass has received considerable attention in the U.S. as a promising bioenergy crop with yields ranging from of 5 to 8 Mg ha⁻¹ yr⁻¹ for northern-upland ecotypes (Sanderson, 2008; Heaton et al., 2008; Monono et al., 2013) to as high as 21 Mg ha⁻¹ yr⁻¹ reported for northern-lowland ecotypes (Casler et al. 2004). Miscanthus, a promising C₄ grass from Asia, has been grown extensively in the EU and to a lesser extent in the U.S. (Lewandowski et al., 2000; Heaton et al., 2008). Proponents of miscanthus cite its high yield potential (26 to 61 Mg ha⁻¹ yr⁻¹), N fixing capacity, and limited potential to become invasive as key strengths for its use as a biomass crop (Lewandowski et al., 2000; Heaton et al., 2008; Cadoux et al., 2012).

For fast growing woody species such as hybrid poplar and willow, high planting densities and short harvest intervals are often employed to maximize biomass. In a review of short rotation cultural practices for hybrid poplar, Ceulemans and Deraedt (1999) reported planting densities from 15 studies ranging from 1,142 to 111,100 plants ha⁻¹ with a median density of 5,500 plants ha⁻¹. Similarly, harvest intervals from one year to eight years, with a median interval of four years were reported. Poplar yields were higher in coppiced stands than in stands grown from cuttings (20 to 25 Mg ha⁻¹ yr⁻¹).

Native polycultures and successional plant communities may also be viable options for the production of lignocellulosic biofuels. Gelfand et al. (2013) report biomass yields of 3.3 to 5.4 Mg ha yr⁻¹ for unfertilized successional plant communities and 4.8 to 7.9 Mg ha⁻¹ yr⁻¹ for the same system receiving 124 kg N ha⁻¹ yr⁻¹, which exceed yields reported for corn stover in the same region (4.8 Mg ha⁻¹). Jarchow et al. (2012) reported biomass yields from central Iowa of >10 Mg ha⁻¹ for a three species native grass mixture of switchgrass, big bluestem (*Andropogon gerardi* Vitman), and indiangrass (*Sorghastrum nutans* (L.) Nash).

Published productivity data for mono- and poly-culture perennial bioenergy crops vary considerably (Vogel et al., 2002; Adler et al., 2009; Jarchow et al., 2012). While this stems in part from regional climate and soil differences, variation in production estimates may also reflect the scale, method, and timing of biomass collection. Therefore, to forecast available regional biomass supplies and make informed decisions on the potential tradeoffs between important ecosystem services and biomass production, it is critical to understand the aboveground net primary production (ANPP) potential of candidate bioenergy crops, as well as their realized harvested yields using agricultural equipment likely to be available to producers. Differences between ANPP and yield occur as a result of combined biomass losses through harvest timing (crop senescence and herbivory) and harvest efficiency (cutting height, incomplete collection, transport).

The seven model cropping systems we studied span gradients of perenniality (annual and perennial crops) and diversity (mono- and polycultures). We provide estimates of ANPP, harvestable yield, and gross harvest efficiency (GHE) for a diverse array of cropping systems grown together on agronomically-relevant plots at two locations, one in southcentral Wisconsin and one in southwestern Michigan. While tissue quality and net energy yield are both important

biomass crop response variables, they were beyond the scope of this study. Our objectives were to understand 1) productivity differences among the seven systems, 2) the degree to which ANPP was recovered using standard available harvesting techniques and equipment, 3) whether plant diversity translates to an ANPP advantage in perennial crops, and 4) how long it takes to establish perennial biofuel crops in the North Central U.S.A.

2. Methods

2.1 Study sites and experimental design

This research was conducted at the DOE-Great Lakes Bioenergy Research Center's (GLBRC) Biofuel Cropping Systems Experiments (BCSE) located at the Arlington Agricultural Research Station in southcentral Wisconsin USA (ARL, 43°17'45" N, 89°22'48" W, 315 m a.s.l.) and the W.K. Kellogg Biological Station in southwest Michigan USA (KBS, 42°23'47" N, 85°22'26" W, 288 m a.s.l.). The predominant soil series at ARL is Plano silt-loam (Fine-silty, Mixed, Superactive, Mesic Typic Argiudolls), which are relatively deep (>1 m), well drained soils with little relief that were formed under tallgrass prairie vegetation in loess deposits over calcareous glacial till. Mean annual temperature and mean annual precipitation at ARL between 1981 and 2010 were 6.8 °C, and 869 mm respectively (NWS, 2013). The predominant soil series at KBS is Kalamazoo loam (Fine-Loamy, Mixed, Semiactive, Mesic Typic Hapludalfs). Deep and well-drained, these soils formed under forest in loamy outwash overlaying sand and gravel. The mean annual temperature and mean annual precipitation at KBS between 1981 and 2010 were 9.9 °C and 1027 mm, respectively (MSCO, 2013) (Fig 1 and Table 1).

At both locations, seven candidate bioenergy cropping systems were established in a randomized complete block design with five blocks. The treatment plots are 27 m wide × 43 m long (0.12 ha) with a minimum of 12-m alleyways between adjacent plots in any direction. The

cropping systems were arrayed along gradients of plant diversity (monoculture or polyculture) and chemical inputs (low to high) including: 1) corn (*Zea mays* L.), 2) switchgrass, 3) giant miscanthus, 4) hybrid poplar (*Populus nigra* × *P. maximowiczii* A. Henry ‘NM6’), 5) native grasses, 6) early successional community, and 7) restored prairie (Table 2). Previous crops at ARL consisted of corn (blocks 4 and 5) and alfalfa (*Medicago sativa* L., blocks 1, 2, and 3), while at KBS the previous crop was alfalfa.

2.2 Cropping system establishment and management

Agronomic decisions about planting densities, hybrid selection, nutrient management, and herbicide application followed local best management practices as recommended by University of Wisconsin (UW) and Michigan State University (MSU) extension agronomists. Field preparation began spring 2008 and consisted of primary (chisel plow) and secondary (soil finisher) tillage at both locations. Corn was planted using a six-row NT corn planter with 76-cm row spacing. In late June 2008, the perennial grass systems, including switchgrass, native grasses, and restored prairie were planted using a drop spreader (Truax Company, Inc.) with two culti-pack rollers. Giant miscanthus rhizomes, with one to two active growing points (industry standard), were hand planted at a depth of 10 cm (76 × 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 × 2.4 m between rows). Cuttings averaged 1.3 cm diameter × 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 crop planting densities were chosen according to University Extension best management practice with the purpose of maximizing yield at reasonable cost to a producer. The early successional treatment, which is simply volunteer plant growth each season, began with the

final tillage pass in the spring 2008. No-till practices were adopted for each system following initial field preparation in 2008 (see Table S1 for equipment used in planting operations).

At both locations, full-season corn hybrids with advanced traits (e.g. herbicide and insect resistance) were selected to maximize productivity and remain consistent with local farming practices (Table 2). Nitrogen application rates for corn were based on spring soil tests and averaged $167 \text{ kg N ha}^{-1} \text{ y}^{-1}$ for both locations over the six-year period. Applications of P and K were plot specific and based on annual fall soil sampling (Table 1). Nitrogen was applied in the early summer at a rate of 56 kg ha^{-1} to the early successional treatment beginning in 2009 and to switchgrass, giant miscanthus, and native grasses beginning in 2010, delayed to provide a competitive advantage over weeds during establishment. 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. No P or K applications were made to the perennial systems.

2.3 Estimating aboveground net primary production

Aboveground biomass of corn was measured at crop physiological maturity (signified by a "black layer" located within the base of a kernel). A $1.5 \times 0.65\text{-m}$ ($\sim 1\text{-m}^2$) quadrat was oriented with the long side perpendicular to the row at three pre-determined stations in each plot. All stems within the quadrat were clipped to ground level, cut into manageable pieces, and bagged. Biomass was then placed in an oven at 60°C until dry weight was constant, and ears were shelled to separate seeds from stover. Seed and stover were weighed separately.

For perennial crops, aboveground biomass was estimated at peak standing crop, usually mid-August. The long side of a $2.0 \times 0.5\text{-m}$ quadrat was placed in an east-west direction at 3 pre-determined stations in each plot in switchgrass, native grasses, early successional, and restored

prairie, and a 1.5×0.65 -m quadrat was oriented in the same direction at the 3 stations for giant miscanthus. All plant biomass rooted in the quadrat was clipped to ground level and bagged. Biomass was placed in an oven at 60 °C until dry weight was constant. The dry weight was determined and recorded.

Each December, 2008 through 2012, two trees were harvested from each hybrid poplar plot. Variation in early stand growth can be substantial (Nelson et al., 2012). To account for growth variation, trees were stratified into two diameter classes and one tree from each size class was randomly chosen to be harvested for the given year. Since the form of stem taper will change over time, three pre-labeled trees at each of three stations were also measured for basal diameter, diameter at 15-cm height, and primary stem height, but were not harvested. To predict biomass for the plot, an allometric equation was developed based on the relationship between harvested biomass and the best fit measured metric (Zhang et al., 2015; Arevalo et al., 2011; Fang et al., 2007). At KBS, the best predictive model across years was the relationship between harvested biomass and diameter at 15-cm (mean $r^2 = 0.93$), while at ARL it was basal diameter in 2008 and 2009 (mean $r^2 = 0.89$), diameter at 15-cm in 2010 ($r^2 = 0.93$), and primary stem height for 2011 and 2012 (mean $r^2 = 0.89$). In 2013, trees were harvested and biomass calculated by weight. Yearly woody growth increment was calculated as the mean biomass for the given year minus biomass from the previous year.

2.4 Harvesting biomass

Grain and biomass harvests were carried out using production-level agricultural equipment to estimate yield. At both locations, corn grain was harvested from each plot using a six-row grain combine and weighed using a grain wagon with load cells. Grain moisture was determined using an electronic grain moisture meter. Corn stover was collected shortly after

grain harvest using a flail-chopper/forage-wagon combination (ARL: 2008 to 2013 and KBS: 2011 to 2013) or standard round-baler (KBS: 2008 to 2010) leaving ~10 cm of residual stubble height. Grab samples were collected from each plot for moisture content determination and all yields were corrected to 100% dry matter (DM).

In an effort to maximize biomass production while minimizing nutrient removal, harvest of switchgrass, giant miscanthus, native grasses, early successional, and restored prairie occurred within two weeks following the first killing frost of fall (-3.5°C , typically mid-October) using the most appropriate equipment available at each study location. Moisture at harvest timing was not considered a critical factor in this study because chopped/densified material has been proposed as a promising avenue for handling lignocellulosic biomass post-harvest. 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 and weighed using the local grain-truck scale. Cutting height at both locations left 15 cm of residual stubble. Samples of biomass were collected from each plot for moisture determination and all yields were corrected to 100% DM.

Hybrid poplar biomass was harvested 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 with biomass chipped into a truck. At both locations, biomass was weighed field moist using truck scales, sub-samples were collected and oven dried to determine moisture, and yields were corrected to 100% DM (see Table S1 for harvest equipment).

2.5 Calculating gross harvest efficiency

Gross harvest efficiency (GHE) was calculated by dividing harvested yield by ANPP to give the fraction of peak standing biomass that was mechanically collected. This metric estimates the sum of biomass losses to crop senescence (leaf drop), herbivory (leaf loss), cutting height (residue left in the field), and mechanical inefficiencies (yield loss during the harvest process).

2.6 Data analysis

Linear mixed effects models (Proc Mixed, SAS v9.3) were used to evaluate ANPP as a function of location (ARL and KBS) and bioenergy system (1 to 7) over the 2008 to 2013 growing seasons. Comparisons between locations and bioenergy systems were made both including and excluding grain in the estimate of biomass produced by the corn system. Location and system, as well as their interaction terms, were treated as fixed effects. Year and block both nested within location as well as the interaction term of system by block nested within location were treated as random effects. To further account for and constrain variability related to differential establishment success between 2008 and 2010 a parameter called *period* was created. Period was a two-level factor: level 1 corresponded to the seasons before and including the first year in which system establishment was successful (no further planting required) and level 2 corresponded to all subsequent seasons. The resulting linear mixed effects model was:

$$Z_{iklm} = \mu + S_i + P_j + SP_{ij} + L_k + SL_{ik} + B(L)_{kl} + SB(L)_{ikl} + Y(L)_{km} + \epsilon_{iklm}$$

where μ = overall mean, S_i = the fixed effect of cropping system i ($i = 1, \dots, 7$), P_j = the fixed effect of period j (j = establishing, established), SP_{ij} = the fixed effect of cropping system i in period j , L_k = the fixed effect of location k (k = ARL, KBS), SL_{ik} = the fixed effect of cropping system i at location k , $B(L)_{kl}$ = the random error attributable to block l ($l = 1, \dots, 5$) at location k , $SB(L)_{ikl}$ = the random error attributable to cropping system i in block l at location k , $Y(L)_{km}$ = the random error attributed to year m ($m = 2008, \dots, 2013$) at location k . Note that all replicates of cropping system i at location k would have been in the same phase during year m .

The “repeated” statement (Proc Mixed, SAS v9.3) was used to account for temporal autocorrelation between yields (subject = SB(L)_{ikl}). The unstructured covariance matrix (type = un) was selected based on goodness of fit (BIC), allowance for variance inequality, and relevance to the experimental data. The inclusion of the repeated statement resulted in significant model improvement ($P < 0.0001$, log likelihood ratio test).

Annual yields from 2010 to 2013 were analyzed with a simplified version of the ANPP model that did not include the period term. By 2010 the majority of cropping systems at the two locations were beyond the point of needing additional planting or startup maintenance. Hybrid poplar was not included in this analysis as it was harvested just once at the end of the six-year period. Cumulative biomass yields were calculated between 2008 and 2013 and hybrid poplar yields were compared to those of the other bioenergy systems using this metric. Additional yield comparisons during the 2008 and 2009 growing seasons were made with descriptive statistics.

3. Results

3.1 Bioenergy system productivity

Annual net primary production was significantly different among systems over the six-year study for both total (including grain) and vegetative biomass ($P < 0.0001$, Fig. 2). While there was no significant location effect, there was a highly significant location by system interaction for total biomass ($P < 0.0001$) indicating that systems performed differently between locations when corn grain was factored into biomass calculations.

At both locations, total corn biomass (grain + stover) and giant miscanthus were the most productive systems. At ARL, corn was significantly more productive than giant miscanthus while at KBS there was no difference between the two. Average ANPP values for corn and giant miscanthus were 23.3 and 16.2 Mg ha⁻¹ yr⁻¹ at ARL, and 18.9 and 19.5 Mg ha⁻¹ yr⁻¹ at KBS,

respectively. The greatest total ANPP of 35.2 Mg ha⁻¹ was observed in giant miscanthus at KBS in 2013. Giant miscanthus ANPP at ARL during the same season was similar at 33.2 Mg ha⁻¹. The ordering of the remaining systems differed somewhat between the two locations. At ARL, switchgrass ANPP was significantly lower than giant miscanthus (10.8 Mg ha⁻¹ yr⁻¹), but similar to native grasses at 8.5 Mg ha⁻¹ yr⁻¹ (Fig. 2).

At KBS, there were two main groupings of bioenergy systems based on ANPP. The first and most productive included giant miscanthus and corn (grain + stover) while the second included switchgrass, restored prairie, native grasses, and early successional. Annual net primary production in these four systems was not significantly different. Switchgrass ANPP was 7.5 Mg ha⁻¹ yr⁻¹, followed by restored prairie (7.0 Mg ha⁻¹ yr⁻¹), and native grasses and early successional, both of which averaged 6.7 Mg ha⁻¹ yr⁻¹.

Interannual variability in ANPP between 2008 and 2013 was quite high at both locations with a general trend of increasing biomass over time in the perennial systems (particularly the monocultures) punctuated by a large decline in production during the drought-stricken 2012 growing season, most evident at KBS (Fig. 2).

3.2 Harvested yields

Though much lower, harvested yields closely followed the trends observed for ANPP, including those related to interannual variability (Fig. 3). The 2008 and 2009 growing seasons were omitted from the statistical analysis of yield because corn and early succession were the only two treatments consistently harvestable during these first two growing seasons (Fig. 3). Similar to the analysis of ANPP, there was no significant location effect, but there was a highly significant system effect for total (including grain) and vegetative biomass from 2010 to 2013 ($P < 0.0001$). The interaction of location with system was also significant, but only for total

biomass yield ($P=0.0058$). The main differences between ARL and KBS were related to the order and magnitude of biomass yield in the corn and giant miscanthus systems.

At ARL, the highest yielding system was total corn biomass at $16.6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, which was significantly higher than giant miscanthus ($11.9 \text{ Mg ha}^{-1} \text{ yr}^{-1}$). Switchgrass was the next most productive system at $6.9 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ followed by native grasses ($5.2 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) and restored prairie ($3.7 \text{ Mg ha}^{-1} \text{ yr}^{-1}$). The early successional system was significantly less productive than switchgrass and native grasses and similar to the restored prairie at $2.8 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ between 2010 and 2013.

At KBS, the highest yielding system was giant miscanthus at $15.6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$. This was significantly higher than both total corn biomass ($12.3 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) and switchgrass ($6.0 \text{ Mg ha}^{-1} \text{ yr}^{-1}$), which were in turn higher than native grasses ($4.4 \text{ Mg ha}^{-1} \text{ yr}^{-1}$), restored prairie ($2.8 \text{ Mg ha}^{-1} \text{ yr}^{-1}$), and early successional ($2.6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$).

Biomass moisture content for each location, system, and year is presented in Table S2.

3.3 Hybrid poplar ANPP

Annual assessment of ANPP in the hybrid poplar system showed rapid biomass accumulation between 2008 and 2010 (Fig. 4). This growth was similar between the two locations, though slightly higher at ARL with 0.4 , 7.8 and $13.4 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ produced in 2008, 2009, and 2010, respectively compared to 0.3 , 4.9 , and $11.8 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ at KBS for the same seasons. Biomass production at the two locations diverged beginning in 2011. At KBS, continued strong growth resulted in ANPP rates of 10.2 , 16.6 , and $11.6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ for 2011, 2012, and 2013, respectively while growth rates at ARL were just 2.7 and $5.3 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ for the 2011 and 2012 seasons. Annual net primary production estimates for the 2013 season at ARL suggested an overall loss of standing biomass of -3.1 Mg ha^{-1} from the previous season. These

divergent growth patterns resulted in average ANPP rates that were more than twice as high at KBS for the six-year period: 9.2 Mg ha⁻¹ yr⁻¹ for KBS and 4.4 Mg ha⁻¹ yr⁻¹ for ARL.

3.4 Cumulative bioenergy system productivity and yield

Bioenergy system ANPP and yields were summed over the six growing seasons to provide a comparison with hybrid poplar, as well as to integrate production potential from system establishment through to persistence phases. Location was not significant in this analysis although both system and its interaction term with location were significant ($P < 0.0001$).

At ARL, total corn biomass was the most productive system over the six growing seasons, with cumulative ANPP values of 140.0 Mg ha⁻¹ and cumulative yields close to 99.3 Mg ha⁻¹. In terms of ANPP, giant miscanthus was the second most productive system at ARL (81.0 Mg ha⁻¹) followed by switchgrass and native grasses at 64.5 and 50.9 Mg ha⁻¹. The least productive system at ARL between 2008 and 2013 was hybrid poplar at 26.5 Mg ha⁻¹. Bioenergy system rankings based on harvested yield were different from ANPP and are shown in Figure 5.

At KBS, cumulative ANPP was highest in giant miscanthus (116.8 Mg ha⁻¹) followed by total corn biomass (106.1 Mg ha⁻¹) (Fig. 5). Hybrid poplar was significantly more productive at KBS than at ARL with ANPP values at KBS equivalent to those for switchgrass (55.4 Mg ha⁻¹ for hybrid poplar and 45.1 Mg ha⁻¹ for switchgrass). The productivity of hybrid poplar at KBS was even more apparent in terms of yield, with significantly greater harvested biomass than switchgrass, native grasses, early successional, and prairie (Fig. 5). Giant miscanthus and total corn yields were roughly 10 Mg ha⁻¹ greater than hybrid poplar at KBS between 2008 and 2013.

3.5 Gross harvest efficiency

Of the seven bioenergy cropping systems evaluated, corn grain had the highest overall GHE (0.85 to 0.90 for KBS and ARL, respectively) followed by total corn biomass (0.72 and

0.56 for ARL and KBS, respectively) (Table 3). GHE for the remaining systems were consistent, with higher values for grass-based systems (0.54 for switchgrass, giant miscanthus, and native grasses) compared to mixed grass and forb systems (0.43 for early successional and restored prairie)(Table 3).

3.6 Cropping system establishment complications

Perennial biomass crop establishment was complicated by weather events during the 2008 and 2009 growing seasons at both ARL and KBS. At ARL, in spite of vigorous first year growth (2008), giant miscanthus suffered greater than 95% mortality over winter. Giant miscanthus re-planting occurred in May 2010, delaying the first harvest at ARL until the fall of 2011. At KBS, above average rainfall shortly after seeding (June/July 2008, Fig. 1), caused significant flooding and stand loss in the switchgrass, native grass mix, and native prairie. Re-seeding was required in 2009, delaying first harvest of these systems at KBS until 2010.

As a result of these establishment difficulties, corn and the early successional treatment were the only two systems to consistently generate harvested biomass at both locations during the first two growing seasons (2008 and 2009). Early successional ANPP and yields were highest in the first year but declined by the second year at both locations (Figs. 2 and 3). Cumulative yields during the main establishment window (2008 and 2009) of the two experiments was 34.9 (13.4), 3.3, 4.4, 6.7, and 5.9 Mg ha⁻¹ for total corn biomass (stover), switchgrass, native grasses, early successional, and restored prairie, respectively at ARL and 16.2 (2.7), 2.9, and 3.8 Mg ha⁻¹ for total corn biomass (stover), giant miscanthus, and early successional, respectively at KBS.

4. Discussion

4.1 Dedicated bioenergy crops were highly productive

Once well established, dedicated bioenergy crops were capable of producing as much biomass as corn, with significantly fewer inputs. Differences between high-yielding perennial systems (e.g. giant miscanthus, hybrid poplar, switchgrass) and corn were less pronounced on our less productive site, highlighting the importance of climate and edaphic conditions in regional considerations of bioenergy crop deployment. While Alfisols in southwestern Michigan were adequate for annual crop growth, occasional periods of water stress coupled with a slightly milder climate provided a production advantage for perennial cropping systems over corn that was not observed on our more productive south central Wisconsin Mollisols.

Although corn yields were generally (but not always) lower at KBS than ARL, biomass yields were similar between the two locations for many of the perennial systems and consistent with the findings of others. Prophet and Staggenborg (2010), for example, reported no yield differences among corn stover, big bluestem, or switchgrass on Mollisols in northeastern Kansas. Furthermore, Godin et al. (2013) showed that giant miscanthus was as productive as corn silage in trials in Europe, and James et al. (2010) report values from the North Central U.S. of 11.2 and 22.4 Mg ha⁻¹ yr⁻¹ for hybrid poplar and giant miscanthus, respectively, compared to 11.7 Mg ha⁻¹ yr⁻¹ for total corn biomass. These findings are consistent with our observations of high biomass production in giant miscanthus (ARL and KBS) and hybrid poplar (KBS), which were often equal to or greater than that of total corn biomass.

Divergent hybrid poplar production between our sites was mainly the result of high fungal disease pressure in Wisconsin and limited resistance in the hybrid poplar clone NM6. While early biomass production (2008 to 2010) was similar between the two locations, warm temperatures and above average rainfall in 2010 in Wisconsin provided favorable conditions for *Marssonina* spp. leaf spot fungus (hereafter marssonina). Marssonina is common in hybrid

poplar, but severe outbreaks such as occurred at ARL in 2010 can cause early leaf drop and tree dieback. Furthermore, with repeated infections the trees become susceptible to other diseases, insects, and winter injury (Ostry, 1985; Erickson et al., 2004). In 2010 *Marssonina* infestation peaked in mid-August at ARL, with complete defoliation by 15 September. The disease was then present at some level in each subsequent growing season. The most affected plots began to lose trees to canker (various species) and winterkill and at the time of harvest as little as 22% of the initial stand was alive (ARL: blocks 5). Ostry (1985) reported that before 1978/1980, *Marssonina brunea* was of only minor importance in northern Wisconsin and Minnesota, but after 1980 they noted marssonina leaf spot reached epidemic proportions each subsequent year, with six-year survival rates ranging from 8 to 83% depending on the clone evaluated. Commercially available hybrid poplar clones are limited to a handful of standards that differ in their growth and disease resistance profiles. Continued development of hybrid poplar germplasm is needed to provide disease resistance to common pathogens while maintaining or improving biomass yield potential.

4.2 Improving gross harvest efficiency should increase perennial biomass yields

Gross harvest efficiencies suggest improved perennial crop yields are possible by optimizing crop management practices, harvest strategies, and mechanical efficiencies. Bioenergy harvest strategies vary between multiple cut and single cut systems, with the latter usually executed after a killing frost to maximize crop nutrient resorption (Beale and Long, 1997; Vogel et al., 2002; Heaton et al., 2009). However, many perennial bioenergy crops lose significant dry matter to senescence and herbivory between peak standing biomass and a post-frost harvest, greatly reducing the total biomass and energy yield (Vogel et al., 2002; Lewandowski and Heinz, 2003). For example, Lewandowski and Heinz (2003) working in

southern Germany, reported bioenergy yield losses of ~15% when *Miscanthus* harvest was delayed from December to February and an additional loss of 13% between February and March. The practice of delaying harvest until early spring is primarily touted for the economic advantages realized with lower drying costs because late fall weather can significantly interfere with drying and baling biomass in temperate regions. In spite of this advantage, Lewandowski and Heinz (2003) found that harvesting earlier (December) resulted in greater CO₂ equivalent savings despite the high cost of drying.

While maximum resorption of aboveground nutrients may not occur until late fall (Beale and Long, 1997; Heaton et al., 2009), some evidence suggests the process is non-linear, with the majority of nutrients absorbed well before a killing frost (Wilson et al., 2013), especially when little to no N is applied (Jach-Smith and Jackson, 2015). Immediate biomass gains might therefore be realized by strategically timing biomass harvest between peak standing crop and a killing frost. An intermediate timing would also provide added flexibility to bale or green-chop biomass depending on available equipment and local demand. In spite of slightly different harvesting techniques at ARL and KBS, only minor differences were observed between GHE of perennial cropping systems. It is likely that combining the cutting and collection of biomass at harvest into a single pass would further provide a means to increase yields by reducing mechanical loss of biomass. While different timing and/or harvest practices were not evaluated in this study, the implication of increasing GHE is important for improving perennial cropping system competitiveness with annual row crops and potentially increasing the willingness of growers to establish such systems. For example, increasing GHE to a conservative value of 0.5 as a result of optimized harvest timing and mechanical efficiency would have added roughly 1.8 Mg ha⁻¹ to 2013 switchgrass yields at ARL and 1.7 Mg ha⁻¹ to 2013 prairie yields at KBS.

4.3 Benefits of diverse plant assemblages not realized during first six growing seasons

The inverse relationship we observed between plant diversity and ANPP suggests that the decision to establish a polyculture should be made based on objectives other than biomass production such as carbon sequestration, habitat conservation, biodiversity, or other ecosystems services. Perennial polycultures can improve soil quality and sequester C (Lemus and Lal, 2005), have lower greenhouse gas emissions (Oates et al., 2015), support greater numbers of predatory arthropods, pollinators, bird species (Werling et al., 2014), increase water holding capacity through root carbon inputs and improved soil structure (Sanford, 2014), reduce nitrate leaching (Robertson et al., 2013), and provide a buffer against overland soil erosion (Helmets et al., 2012). If biomass production is a primary objective, then systems such as early successional vegetation, which require minimal inputs, should be considered as a low risk option that can produce more biomass than perennial monocultures during early establishment.

Results reported by others on polyculture productivity have been mixed. Some have observed lower productivity among more diverse systems similar to our results (Johnson et al., 2010, Griffith et al., 2011) while others have found that polyculture performance can equal or exceed that of monocultures (Tilman et al., 2006; Jarchow et al., 2012; Gelfand et al., 2013). Gelfand et al. (2013), for example, reported high biomass yields of 7.9 and 5.4 Mg ha yr⁻¹, with and without fertilizer respectively. Establishment time was a major factor influencing the consistency of their findings: in their 23-year study, biomass production from the early successional community did not consistently exceed 5 Mg ha⁻¹ yr⁻¹ until the 8th field season due to the delayed presence of high productivity species. It is possible that, because of the relatively short duration of our study, we have yet to observe the long term yield potential of the native grasses, early successional, and restored prairie systems. Biomass production notwithstanding,

the substantial ecosystem services realized in polyculture systems can be compelling (Meehan et al., 2012; Gelfand et al., 2013; Werling et al., 2014) and require consideration in land management decisions.

4.4 Perennial bioenergy crop establishment successes and setbacks

Setbacks during the first few years of this study highlight the potential risks and challenges associated with establishing perennial bioenergy crops. Because many of these crops are slow to establish, an accurate stand assessment (*sensu* Vogel and Masters, 2001) is often delayed until fall of the first year or later. This was the case at KBS where the full impact of episodic 2008 precipitation was not apparent until stand frequency counts were made in the spring of 2009. Stand loss at KBS was caused by excessive runoff that washed seed from planted fields. While the brillion-type planter used in this study was very effective, and is widely used for native grass and prairie seeding throughout the region, the prerequisite tillage and shallow seeding depth provided little physical protection to the seed. In retrospect, no-till planting may have prevented some of the stand loss at KBS by reducing overall soil disturbance (no pre-plant tillage) and ensuring sufficient planting depth for seed protection.

Another challenge of establishment was the loss of giant miscanthus during the first winter at ARL. Overwintering losses during establishment have been reported by others (Clifton-Brown and Lewandowski, 2000; Lewandowski et al., 2000; Zub and Brancourt-Hulmel, 2010). Clifton-Brown and Lewandowski (2000) reported that the high risk of winter kill was the main obstacle for production of giant miscanthus in northern Europe. Zub and Brancourt-Hulmel (2010) and Clifton-Brown et al. (2001) reported 50% rhizome mortality for *M. giganteus* and *M. sinensis* at -3.5 and -6.0 °C, respectively. During the first winter at ARL, for 22 days (12 February to 1 April) soil temperatures (0 to 5 cm) were lower than -3.5 °C for more than 5 h. On

17 of those days, soil temperature dropped below -6°C with a low of -13.6°C recorded on 12 March 2009. In addition, there was a brief period between 28 February and 3 March when soil temperature below -3.5°C extended to more than 10 cm deep.

Kucharik et al. (2013) modeled wintertime soil temperatures in the North Central U.S. (including North and South Dakota, Minnesota, and Wisconsin) to show that regional soils experienced 10 cm soil temperatures below -3.5 and -6.0°C in greater than 75% and 50% of years, respectively, between 1978 and 2007. Soil temperatures encountered during the first winter of this study are thus common in the North Central U.S. and may limit giant miscanthus establishment in this region until winter hardy germplasm is available. Kucharik et al. (2013) also reported that giant miscanthus winter survival would increase as the thickness of residual biomass remaining in the field increased. While lack of winter cover may have contributed to the stand loss at ARL, nearby plots planted with the same rhizomes exhibited the same mortality rates, despite winter cover.

Our study differs markedly from others reporting giant miscanthus productivity during establishment in that rhizomes were direct-planted rather than grown in the greenhouse and transplanted into the field. This method was chosen to emulate how farmers might establish large acreages of giant miscanthus given constraints on labor, facilities, and transplanting equipment. Although giant miscanthus establishment was successful at KBS, second-year yields (2009) were relatively low (2.9 Mg ha^{-1}). Prophet and Staggenborg (2010), working in northeast Kansas, reported first year yields (year of planting) of 3 to 4 Mg ha^{-1} and second year yields of 12 to 14 Mg ha^{-1} using transplants. Similarly, Maughan et al. (2011) reported average yields of 16.5 Mg ha^{-1} in the second year of their trial using transplants. The use of greenhouse grown transplants helps explain the discrepancy between our findings (low establishment yields and stand loss) and

those of others. Lewandowski et al. (2000) corroborate this conclusion by indicating that mechanical propagation may result in variable degrees of emergence (~70%). In some instances however, even establishment with transplants can yield variable results. Maughan et al. (2011) reported first winter survival rates of 25% for a giant miscanthus stand started from transplants in central Illinois, while Boersma and Heaton (2014) found no difference in survival or yield of rhizome vs. plug transplants at three research farms in Iowa. When 70% or more of the plants establish successfully in the first growing season, the issue of variable emergence may be of little concern as tillers will fill in stand gaps and plant populations will rebound with time (Lewandowski et al. 2000).

Three growing seasons passed before harvest was possible for all seven bioenergy systems at both of our locations. In the best case scenario, harvestable biomass would be available by the second growing season for warm season grass crops (Vogel et al. 2002). While this was the case for the native systems at ARL, and giant miscanthus at KBS, native system harvest was delayed until the third year at KBS, and the fourth year for giant miscanthus harvest at ARL; in both cases to accommodate re-planting. These delays may negatively affect a producer's willingness to grow cellulosic bioenergy crops if sufficient safeguards and/or subsidies are not available to them.

5. Conclusions

We found that 1) perennial bioenergy crops were highly productive relative to intensively managed corn, 2) harvested yields lagged ANPP by as much as 60% with the implication that reducing this discrepancy may be an immediate option to increase bioenergy system yields, 3) production benefits associated with high diversity polycultures were not realized in the six-year period of this study, and 4) while bioenergy crop establishment should be possible within two to

three years, physiological constraints and seasonal complications may significantly increase the length of the establishment window.

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Table 1. Baseline soil physical and chemical characteristics at Arlington Agricultural Research Station (ARL) and Kellogg Biological Station (KBS) including soil organic carbon (SOC) determined via dry combustion, available phosphorus (P) and potassium (K), and exchangeable calcium (Ca) and magnesium (Mg). Numbers represent the mean and standard error (in parentheses) for each parameter.

	Horizon	Bulk			Sand	Silt	Clay	SOC	P [†]	K [‡]	Ca [§]	Mg [§]
		pH	Density (Mg m ⁻³)	Texture								
	(cm)				g kg ⁻¹				mg kg ⁻¹			
ARL	0-10	6.6	1.3	silt loam	91 (5)	661 (5)	248 (3)	22.4 (0.3)	151 (11)	189 (10)	1578 (27)	458 (5)
	10-25	6.4	1.4	silt loam	81 (4)	654 (4)	265 (3)	18.3 (0.3)	100 (9)	110 (6)	1594 (32)	466 (7)
	25-50	6.3	1.4	silty clay loam	73 (6)	626 (6)	301 (4)	9.1 (0.3)	35 (3)	88 (4)	1497 (34)	555 (14)
	50-100	6.1	1.4	silty clay loam	101 (8)	592 (7)	308 (3)	4.8 (0.3)	46 (1)	103 (5)	1531 (34)	575 (8)
KBS	0-10	6.1	1.6	sandy loam	626 (18)	311 (14)	64 (6)	14.3 (0.3)	35 (2)	90 (5)	885 (40)	118 (6)
	10-25	6.0	1.7	sandy loam	602 (16)	320 (14)	79 (5)	8.4 (0.3)	31 (2)	91 (5)	823 (38)	125 (6)
	25-50	6.1	1.8	sandy loam	704 (20)	184 (17)	112 (8)	3.4 (0.2)	37 (2)	89 (4)	910 (37)	122 (6)
	50-100	6.1	1.5	sand	880 (11)	63 (9)	58 (5)	1.4 (0.1)	35 (2)	86 (5)	823 (37)	122 (6)

[†] Bray P1 extract, (0.3N NH₄F in 0.025N HCl)

[‡] ARL: Bray P1 extract, (0.3N NH₄F in 0.025N HCl), KBS: (1N NH₄OAc at pH 7.0)

[§]Exchangeable Ca and Mg (1N NH₄OAc, pH 7.0)

Table 2. Agronomic details for bioenergy systems at ARL and KBS. Five year average planting date, seeding rate, and N application rate are included for continuous corn.

System	crop	Loc	Planting date	Crop and variety [†]	seed/plant rate [‡]	N rate (kg ha ⁻¹)	First N application	Weed control	Average harvest date
1	corn	ARL	5 May	DK5259, P35F40, DK5259, DK5259, P35F40, FS53TV4	84,000 sds ha ⁻¹	150	2008	herbicides	Oct. 22 - grain
									Oct. 23 - stover
		KBS	6 May	DK5259, DK5259, DK5259, DK5259, DK5259, DK5259	70,900 sds ha ⁻¹	168	2008		Nov. 4 - grain
									Nov. 17 - stover
2	switchgrass	ARL	24 June 2008	Switchgrass (<i>Panicum virgatum</i> L.), “Cave-In-Rock”	7.5 kg ha ⁻¹	56	2010	herbicides	Oct. 27
		KBS	19 June 2008 [§]						Nov. 10
3	giant miscanthus	ARL	May 13, 2008 [#]	<i>Miscanthus x giganteus</i> , “Illinois clone”	17,200 rhizomes ha ⁻¹	56	2011	herbicides	Oct. 24
		KBS	May 21, 2008				2010		Nov. 22
4	hybrid poplar	ARL	May 9, 2008	<i>Populus nigra</i> × <i>P. maximowiczii</i> , NM6	2,778 cuttings ha ⁻¹	210	2010	herbicides/	Dec. 2013
		KBS	May 1, 2008			155		oat cover	Jan. 2014
5	native grasses	ARL	June 24, 2008	Big bluestem (<i>Andropogon gerardii</i> Vitman)	2.4 kg ha ⁻¹	56	2010	herbicides	Oct. 27
				Canada wild rye (<i>Elymus Canadensis</i> L.)	1.6 kg ha ⁻¹				
				Indiangrass (<i>Sorghastrum nutans</i> [L.] Nash)	2.4 kg ha ⁻¹				
		KBS	June 19, 2008	Little bluestem (<i>Schizachyrium scoparium</i> [Michx.] Nash)	3.2 kg ha ⁻¹				Nov. 12
				Switchgrass, “Southlow”	1.6 kg ha ⁻¹				
6	early successional	ARL	n/a	Plant community defined by pre-existing seed bank and novel recruitment	n/a	56	2009	None	Oct. 27
		KBS							Nov. 11

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[†] Hybrid specifics are listed in order of season, 2008 to 2013.

Table 3. Gross harvest efficiency (YLD:ANPP) of bioenergy systems at ARL and KBS. Numbers represent the mean and standard error (in parentheses) for each system. The samples size for system averages vary by crop, year, and location as a result of establishment lag.

			Year						
Location	System	Crop	2008	2009	2010	2011	2012	2013	System average
ARL	1	corn (whole)	0.71	0.74	0.82	0.76	0.70	0.60	0.72(0.03)
		grain			0.90	0.94	0.89	0.87	0.90(0.01)
		stover			0.70	0.57	0.44	0.37	0.52(0.07)
	2	switchgrass		0.41	0.60	0.64	0.64	0.41	0.54(0.05)
	3	giant miscanthus				0.52	0.42	0.48	0.47(0.03)
	4	hybrid poplar						0.90	
	5	native grasses		0.45	0.53	0.55	0.87	0.49	0.58(0.08)
	6	early successional	0.45	0.35	0.51	0.38	0.57	0.47	0.46(0.03)
	7	restored prairie		0.53	0.54	0.42	0.76	0.40	0.53(0.06)
	Season Average		0.58(0.13)	0.50(0.07)	0.66(0.06)	0.60(0.06)	0.66(0.06)	0.51(0.06)	
Location Average								0.59(0.03)	

KBS	1	corn (whole)	0.39	0.49	0.60	0.64	0.60	0.63	0.56(0.04)
		grain	0.82	0.75	0.93	0.86	0.89	0.84	0.85(0.03)
		stover	0.02	0.25	0.26	0.32	0.36	0.41	0.27(0.06)
	2	switchgrass			0.46	0.58	0.61	0.80	0.61(0.07)
	3	giant miscanthus		0.27	0.54	0.59	0.59	0.66	0.53(0.07)
	4	hybrid poplar						0.89	
	5	native grasses			0.49	0.46	0.29	0.64	0.47(0.07)
	6	early successional	0.33	0.19	0.41	0.35	0.38	0.43	0.35(0.04)
	7	restored prairie			0.37	0.44	0.30	0.32	0.35(0.03)
	Season Average		0.39(0.16)	0.39(0.10)	0.51(0.07)	0.53(0.06)	0.50(0.07)	0.59(0.07)	
Location Average								0.50(0.03)	

Figure 1. Monthly accumulated growing degree days (GDD) at A) ARL and B) KBS and precipitation (precip) at C) ARL and D) KBS between 2008 and 2013 (1 April to 31 October), as well as 30-year climate normals (1981-2010) for each location. Growing degree units calculated between 10 (base) and 30 (max) °C. Normals are from the Wisconsin and Michigan state climatology offices respectively. Growing season weather data logged daily at both ARL and KBS.

Figure 2. Aboveground net primary production (ANPP) at A) ARL and B) KBS between 2008 and 2013 (hybrid poplar not shown). Giant miscanthus was lost at ARL during the 2008/2009 winter and replanted in 2010. Corn grain and stover were not separated during the first two seasons at ARL. Error bars show $\pm$ standard error (SE). Letters indicated differences in average system ANPP within a location ($\alpha=0.1$).

Figure 3. Harvested biomass yields at A) ARL and B) KBS between 2008 and 2013 (hybrid poplar not shown). KBS corn stover harvest delayed in 2008 and 2009 until the following spring due to early fall snow. Giant miscanthus was lost at ARL during the 2008/2009 winter and replanted in 2010. At KBS, 2009 was a replanting year for switchgrass, native grasses, and restored prairie so no yields were collected. Error bars show $\pm$ standard error (SE). Letters indicated differences in average system yield within a location between 2010 and 2013 ($\alpha=0.1$).

Figure 4. Mean above ground net primary production (ANPP) growth curves for hybrid poplar at ARL (●) and KBS (◆) between 2008 and 2013. Error bars show $\pm$ standard error (SE), n=5.

Figure 5. Six year cumulative above ground net primary production (ANPP) at A) ARL and B) KBS and harvested biomass yields at C) ARL and D) KBS. Error bars show $\pm$ standard error (SE). Letters indicated differences in cumulative system yield within a location ($\alpha=0.1$).

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Figure 5. Six-year cumulative above ground net primary production (ANPP) at A) ARL and B) KBS and harvested biomass yields at C) ARL and D) KBS. Error bars show $\pm$ standard error

(SE). Letters indicated differences in cumulative system yield within a location ($\alpha=0.1$).