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Perennial biomass crop establishment, community characteristics, and productivity in the upper US Midwest: Effects of cropping systems seed mixtures and biochar applications

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ABSTRACT

Native perennial plants have potential as bioenergy feedstocks, but their use is currently limited by relatively long establishment times and low biomass yields. Some research suggests that incorporating plant species diversity and applying biochar as a soil amendment might alleviate these limitations by creating a more resilient crop and soil system. The objective of this research was to investigate how 1) seeded plant diversity and 2) biochar soil amendments interact to affect the establishment, yield, and plant species composition of biomass cropping systems during the first four years of growth on productive soils. We measured species emergence, cover, peak and post-frost biomass, and biomass composition for three biomass cropping systems seed mixtures – a switchgrass monoculture, a three-species grass mixture, and a highly diverse mixture of grasses and forbs – either with or without application of a mixed wood gasification biochar (9.3 Mg ha^{-1}). We found that seed mixture had significant effects on nearly every variable measured, with switchgrass monocultures outperforming the two more diverse mixtures by the third year of the experiment (12.0 Mg ha^{-1} in switchgrass, 8.7 Mg ha^{-1} in low diversity plots, and 3.9 Mg ha^{-1} in high diversity plots), despite an initial switchgrass establishment failure. The high diversity plots exhibited poor sown species establishment in the first year due to high weed pressure in a drought year, but continued to improve over time. Biochar application had no consistent effect on plant biomass or community traits, and significantly affected only two community traits, light transmittance and leaf area index. Our results suggest that on productive soils perennial bioenergy productivity may be achieved through selection of one or a few high-yielding grass species, with little or no effect of biochar applications on perennial biomass crop establishment, diversity, or productivity.

1. Introduction

As atmospheric carbon dioxide concentrations increase, there is a growing interest for agricultural practices that mitigate and adapt to global climate change. One such option is to reduce fossil fuel usage through the development of renewable bioenergy from native perennial biomass crops that can be grown over a broad range of conditions. This bioenergy option has several agricultural benefits, including reduced competition with food production and reduced agricultural inputs compared to non-cellulosic crops, as well as improved energy balance and provision of ecosystem services (Lewandowski et al., 2003; Valentine et al., 2012; Werling et al., 2014). Such benefits are particularly important in the Corn Belt of the upper Midwestern US, where conventional agricultural practices in maize (*Zea mays* L.) and soy (*Glycine max* Merr.) cropping systems contribute to widespread soil and water quality degradation (US Environmental Protection Agency, 2015).

Among US native perennials, the C_4 grass switchgrass (*Panicum virgatum* L.) was one of the first species identified by the US Department of Energy as a promising biomass crop, largely due to its broad ecological adaptation and high yield potentials (Parrish and Fike, 2005).While switchgrass and other bioenergy grass monocultures can provide large quantities of biomass and a variety of ecosystem services, incorporating plant diversity into perennial biomass cropping systems

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can further improve ecosystem services such as the provisioning of forage and fuel, wildlife habitat, and ecosystem stability (Tilman et al., 2006; Isbell et al., 2015; Zilverberg et al., 2016). One major challenge to using native perennials is their slow establishment, as it may take up to three years for a stand to fully mature (McLaughlin and Kszos, 2005). During this time, perennial fields may be at increased risk for soil erosion and invasion by undesirable weedy species, both of which can deleteriously affect biomass productivity and other ecosystem services. Increasing seeded plant diversity may help alleviate weed pressure and reduce erosion during the establishment period through rapid ground cover by desirable species (Tilman, 1997; Fargione and Tilman, 2005; Bonin et al., 2014). Others point out that higher plant diversity may reduce ethanol yields (Adler et al., 2009), that plant diversity may decline over time and yields remain unstable (Von Cossel and Lewandowski, 2016), and that ecosystem benefits observed in small-plot diversity experiments may not hold true in large-scale biomass crop plantations (Dickson and Gross, 2015). One potential solution to address these concerns is establishment of a moderately diverse mixture of high-yielding plant species that balances the benefits and challenges of both monocultures and highly diverse plant mixtures (DeHaan et al., 2010; Bonin and Tracy, 2012).

A second option for mitigating greenhouse gases may be biochar production and application to soil (Lehmann, 2007; Laird, 2008). Biochar, a carbon-rich product of biomass pyrolysis, can be used as a soil amendment for multiple purposes such as long-term carbon storage, to improve soil quality, and to increase crop productivity (Ippolito et al., 2012; Laird, 2008). Plant productivity responses to biochar may be affected by a variety of factors, including biochar characteristics, biochar feedstocks, application rate, crop type, and soil properties (Jeffery et al., 2011; Biederman and Harpole, 2013). Much biochar research has focused on traditional annual crops such as maize and wheat (*Triticum aestivum*), and less is known about how biochar amendments may affect perennial biomass cropping systems. Research on the effects of biochar on perennials has often generated equivocal and/or inconsistent results. For example, some experiments report that biochar has positive effects on switchgrass yield (Edmunds, 2012), and that it may improve the height and biomass of big bluestem (*Andropogon gerardii* Vitman), but not the legume sericea lespedeza [*Lespedeza cuneata* (Dum. Cours.) G. Don] (Adams et al., 2013). In contrast, another experiment demonstrated that legume biomass, but not overall community productivity, increased following biochar application in a restored grassland (van de Voorde et al., 2014). Another study suggests that biochar application could increase plant species richness, but in order to observe increases in biomass, the application rate had to be relatively high (56.8 Mg ha^{-1}, Biederman et al., 2017). Meta-analyses suggest that biochar has, on average, a positive effect on cropping system productivity; however, when separated out, studies investigating perennial crops have shown no increase or even a decrease in yield with biochar application (Jeffery et al., 2011; Biederman and Harpole, 2013).

No prior publications have investigated interactions between biochar amendments and perennial biomass cropping systems seeded diversity, presenting a gap in knowledge on how these two management choices may impact the viability of bioenergy cropping systems. Here we ask whether 1) biochar soil amendments will influence native perennial crop establishment and plant growth, 2) if planting more diverse perennial cropping systems (switchgrass monoculture vs. low-diversity mixture vs. high-diversity mixture) will result in greater aboveground productivity, and finally, 3) if biochar applications will act synergistically with seeded species diversity to further boost productivity.

2. Materials and methods

2.1. Study site and experimental design

The experiment was established at Armstrong Memorial Research and Demonstration Farm, located near Lewis, IA (41.311250, −95.179493) on land that had previously been planted in a maize-soy rotation. Experimental plots ranged from flat to slightly sloping, and were comprised primarily of loess-derived silt loams and silty clay loams. The soil types within the experiment included Marshall (Fine-silty, mixed, superactive, mesic Typic Hapludolls), Exira (Fine-silty, mixed, superactive, mesic Typic Hapludolls), Clarinda (Fine, smectitic, mesic Vertic Argiaquolls), and Ackmore-Colo-Judson complex (complex of Fine-silty, mixed, superactive, mesic Cumulic Endoaquolls, Cumulic Hapludolls, and Mollic Fluvaquents) (Soil Survey Staff, 2014). These soils contained predominantly silt, as well as 4–160 g kg^{-1} clay and 40–390 g kg^{-1} sand; other soil properties are described in Table 1.

Table 1
Average physical and chemical properties of the soils prior to biochar application at Armstrong Farm in IA, USA.

Properties	0–15 cm	15–30 cm	0–30 cm
Bulk density (g cm^{-3})	1.22	1.21	1.21
pH	6.43	6.05	6.24
Electrical conductivity(μS cm^{-1})	128.45	85.07	106.76
N (%)	0.20	0.12	0.16
C (%)	1.95	1.32	1.63
C:N	9.88	11.11	10.50

A split-plot design was used with the whole plots (0.330 ha in size) receiving seed mixture treatments and the subplots (0.165 ha) receiving biochar treatments. The whole-plot cropping system treatments were: (1) a switchgrass monoculture (SG) established under a maize companion crop (following Hintz et al., 1998), (2) a low-diversity (LD) polyculture of high-yielding native grass species, and (3) a high-diversity (HD) polyculture of prairie grasses and forbs. These three cropping systems were designed to be managed differently based on sown species diversity, similar to Tilman et al. (2006); in this case, the two lower diversity treatments received higher fertilizer inputs than the high diversity treatment. There were four replications of each cropping system. The split-plot treatment, biochar, was randomly applied to one half of each whole plot in late October of 2011 at a rate of 9.3 Mg ha^{-1} (dry weight equivalent) and immediately incorporated to a depth of 15 cm by chisel plow tillage followed by disking. The biochar was generated from the gasification of mixed wood (primarily *Quercus, Ulmus* and *Carya* spp. woodchips with particle sizes 0.1–2000 mm) at ~600 °C (ICM, Inc., Colwich, KS, USA). Detailed information concerning this biochar, including chemical properties, can be found in Fidel et al., 2017a; and Fidel et al., 2017b. Previous chemical and thermogravimetric analyses showed that the biochar was alkaline (pH 8.8), and contained 29% ash, 16% volatile matter, and 55% fixed carbon, 63% total C, 2.7% total H, 0.6% total N, 0.06% total P and 0.86% total K on a dry weight basis (Fidel et al., 2017b).

The switchgrass cultivar used for the monoculture was ‘Liberty,’ a recently released biomass-type cultivar adapted to the US Midwest and Great Plains that was developed at the USDA-ARS in Lincoln, Nebraska (Vogel et al., 2014). The LD mixture was a 45:45:10 mixture of big bluestem (‘Bonanza’ and ‘Goldmine’), indiangrass (*Sorghastrum nutans* L., ‘Scout’ and ‘Warrior’), and sideoats grama (*Bouteloua curtipendula* (Michx.) Torr. ‘Butte’), respectively. The HD mixture was comprised of 44 species of native perennial grasses, sedges, forbs, and legumes that would be typically seeded in a prairie restoration (See SI Table). Seeds for the LD and HD mixtures were obtained from Diversity Farms (Dedham, IA, USA).

Switchgrass seeds were no-till drilled into cultivated soil in May 2012 using a Great Plains Drill 1006 N T drill (Great Plains Manufacturing, Salina, KS, USA) in 19-cm width rows at a rate of ~323 pure live seed (PLS) m^{-2}, which equated to 6.7 kg ha^{-1}, between rows of a maize companion crop. The same seeding rate was used for the LD and HD cropping systems and equated to 15.1 kg ha^{-1}. These mixes were broadcast and then cultipacked using a Vicon seeder (Kverneland Group, Klepp Stasjon, Norway) and a Brillion cultipacker (Landoll

Corp., Marysville, KS, USA). The maize companion crop was seeded into SG plots on the same day at a planting density of 64,000 seeds ha^{-1} with 76-cm width rows. Due to poor stand establishment in 2012, SG plots were directly reseeded into the original SG plots with a no-till drill on 1 May 2013 at a seeding rate of 8.6 kg ha^{-1}, this time without a maize companion crop. Reseeded SG plots were prepared with quinclorac (Paramount®, BASF, Research Triangle Park, NC, USA) applied at a rate of 0.6 l ha^{-1} and glyphosate (Roundup®, Monsanto, St. Louis, MO, USA) applied at a rate of 2.3 l ha^{-1}.

Nitrogen in the form of urea was surface applied at a rate of 56 kg N ha^{-1} to all perennial plots in 2014, and to only SG and LD plots in 2015. Thus, the HD plots received less overall N, with the goal of managing these plots as low input systems in a manner similar to that suggested by Tilman et al. (2006). Weed control was accomplished by mowing vegetation to a height of 20 cm 2–3 times over the summers of 2012 and 2013. The HD plots were also mowed once in August 2014 to reduce continued heavy weed pressure. In addition to plot-wide weed control, large thistle patches, composed primarily of Canada thistle (*Cirsium arvense* (L.) Scop.), within a few plots were controlled by spot mowing or hand-spraying aminopyralid (Milestone®, Dow AgroSciences, Indianapolis, IN, USA). In 2015, 2,4-D (2,4-dichlorophenoxyacetic acid) was applied to the grass-only treatment plots (SG and LD plots), while the HD plots were mowed once in early summer.

2.2. Plant measurements

Plant frequency of occurrence was measured using the frequency grid method (Vogel and Masters, 2001). The presence of any vegetation was noted within each 15-cm x 15-cm cell of a 25-cell grid. Grids were flipped four times to generate a count of 100 cells, and repeated twice in each subplot. Plant establishment was measured using the grid count in October 2012, but no attempt was made to identify plant species. In 2013, 2014, and 2015, frequency grids were used to estimate plant emergence during May, and the numbers of cells containing grasses and forbs were recorded separately.

Plant species composition and relative cover were visually estimated in June from 2013 to 2015, and in October in 2013 (as a proxy for end-of-season biomass). Relative cover data were collected using two sets of four randomly placed 0.25-m^2 quadrats within each subplot for all June sampling dates. Data within each set of four subsamples were compiled, resulting in two subsamples of relative cover for each subplot. The one exception was in October 2013, when only one set of four quadrats was collected, resulting in a single sample per subplot.

Stand heights were measured on 18 June 2013, 15 July 2014, and 13 July 2015 by measuring the canopy height at eight random locations within each subplot. Photosynthetically active light transmittance (i.e., the percentage that reaches the soil surface) and leaf area index (LAI) were estimated on 2 July 2013, 15 July 2014 and 13 July 2015 with an AccuPAR LP-80 Ceptometer (Decagon Devices, Pullman, WA, USA) by placing the sensor on the ground parallel with the soil and averaging five measurements across a 1-m space concurrently with a sensor located above the canopy. Four sets of measurements were taken within each subplot.

In 2014 and 2015, peak biomass was measured in August and post-frost biomass was collected in November and December. No post-frost biomass was collected in 2014 from the HD plots, due to a late August mowing for weed control. In each subplot, all biomass within two 0.25-m^2 quadrats was hand-harvested at a cutting height of 10 cm and sorted to sown grasses, sown forbs (HD plots only), and unsown weedy plants. Biomass was dried at 60 °C for a minimum of 48 h and weighed. After the 2013 through 2015 seasons, plots were mowed in late fall or early spring, prior to plant regrowth.

The 2015 post-frost sown species biomass was ground to 1-mm particle size and analyzed using near-infrared reflectance spectroscopy (NIRS) to assess composition (neutral detergent fiber [NDF], acid detergent fiber [ADF], acid detergent lignin [ADL], hemicellulose, cellulose, nitrogen, and ash) and estimate potential ethanol yield using a Model 6500 near-infrared spectrometer (FOSS North America, Eden Prairie, MN, USA). The NIRS calibrations (average $r^2 = 0.8$) and ethanol calculations were developed by the ARS laboratory in Lincoln Nebraska for warm season grasses using procedures similar to those described by Vogel et al. (2011).

2.3. Statistical analysis

Statistical analyses were performed using the MIXED procedure in SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA), with the significance set at $P = 0.05$. All biomass, relative cover, canopy height, and transmittance values were square root-transformed to meet normality and equal variance assumptions. All data were analyzed using a repeated measures split-plot analysis of variance, shown in Eq. (1):

$$Y_{ijkl} = \mu + M_i + Bj + MB_{ij} + T_k + TM_{ik} + TB_{jk} + \delta_{il} + \alpha_{ijl} + e_{ijkl} \tag{1}$$

where Y_{ijkl} is the dependent variable (yield or another plot attribute measured), M_i is whole- plot main effect (cropping system seed mix diversity), Bj is the split-plot effect (biochar application), MB_{ij} is their interaction effect, T_k is the effect of time (years 2013–2015), TM_{ik} is the interaction of time and seed mixture, TB_{jk} is the interaction of time and biochar application, δ_{il} is the whole-plot error term α_{ijl} is the split-plot error term, and e_{ijkl} is the random error ascribed to each observation. All effects were treated as fixed, except for error terms. Significance was set at $P = 0.05$, and pairwise comparisons were examined using t-tests of the differences of least square means.

3. Results

3.1. Weather

The growing season weather in 2012, which was the establishment year, was characterized by severe drought and heat stress (Table 2). The growing season precipitation was approximately 60% of historic average amounts, while the average growing season temperature was 3.3 °C (~20%) warmer than the 30-year average. The growing season weather patterns in 2013 and 2015 were near average, while 2014 was 45% wetter than average. However, the winter of 2013–2014 was exceptionally cold, with mean air monthly temperatures from November through February 11–29% lower than historic average temperatures for these months. The late harvest in 2015 was also due to a late first freeze date (20 Nov 2015, about one month later than average).

3.2. Plant establishment - frequency of occurrence

No biomass was harvested in 2012, the establishment year; instead, frequency grids were used to assess plant growth. No attempt to quantify sown species and unsown ("weedy") species was made during these measurements. However, the SG plots were observed to have very few plants besides the maize companion crop, with switchgrass seedlings present in less than 1% of grid cells. Hence reseeding switchgrass in 2013 was necessary. The LD and HD plots had higher plant establishment ($P < 0.001$), with approximately 85% of grid cells containing at least one sown plant.

There were interactions between year and cropping systems for both grasses ($P = 0.0148$) and forbs ($P < 0.0001$), driven by changes in SG plots compared to LD and HD plots. There were no impacts from biochar application. The 2013 switchgrass replanting (1 May 2013) was done without a maize companion crop, and was more successful than the initial planting, with grass seedlings present in 25% of SG grid cells four weeks after replanting. However, this count was lower than grass frequency counts from the LD and HD plots (56%). The SG plots also

Table 2
Monthly average temperatures and total precipitation throughout the growing season during the experiment and the 30-year historic values at Armstrong Farm in Iowa.

	2012		2013		2014		2015		30-year Average	
Month	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)	Temp. (°C)	Precip. (mm)
Mar.	13.33	55.6	2.06	36.8	0.39	20.1	4.71	12.7	2.61	55.1
Apr.	13.67	25.9	8.67	128.5	9.17	111.3	10.83	143.5	9.44	86.1
May	20.28	61.2	16.78	180.1	16.72	88.4	15.29	111.3	16.06	115.6
June	24.06	113.8	22.39	152.7	21.39	252.2	21.87	135.1	21.39	115.8
July	27.94	0.0	24.00	11.4	21.00	57.9	22.77	108.2	23.50	115.8
Aug.	23.44	65.0	23.39	32.5	22.44	281.2	20.86	145.8	22.11	98.6
Sept.	18.28	41.4	20.67	111.3	17.39	158.8	20.50	121.2	19.33	92.5
Oct.	10.94	87.1	10.50	126.2	11.61	114.8	12.48	33.3	10.89	61.5
Average (°C)	19.00	–	16.06	–	15.00	–	16.16	–	15.67	–
Total (cm)	–	450.1	–	779.5	–	1077.7	–	811.0	–	740.9

contained fewer forbs than LD or HD plots in 2013.

In May 2014, SG plots had the highest grass count (62% of grid cells contained grass), with LD plots in the middle (50% of grid cells), and HD plots with the lowest (28% of grid cells). By May 2015, all three cropping systems had similar grass frequencies (an average of 91% of grid cells contained grasses) and forb frequencies (52%).

3.3. Species composition – relative cover

As plots established over the three years observed (stand years 2–4), sown species cover increased ($P < 0.0001$; Figs. 1 and 2; Table 3a). Biochar application had no effect on the relative cover of sown grasses, forbs, or unsown weedy species in early summer. In contrast, cropping system seed diversity impacted both sown grass and weedy species cover ($P < 0.0001$ for both, Table 3a & b). Sown forb cover in the HD plots also increased from 2013 to 2015 (not applicable to SG or LD, Table 3c). Sown grass cover in SG plots, which initially was low (< 5% in 2013), increased to > 30% by 2015. Grass cover in LD and HD plots also increased, but the increases were slower than in SG plots. There was an interaction between cropping system and year ($P < 0.0001$) for grass cover, which was due the rapid increase in cover in the SG plots. The LD plots initially had significantly higher grass cover than the SG and HD plots, but grass cover in SG plots increased to equal that found in LD plots by 2014 and 2015. Weed species cover also showed an interaction between cropping system and year ($P < 0.0001$), as weed cover within the SG plots was low early (likely due to management practices for maize) but increased by 2015 to equal that in LD and HD plots.

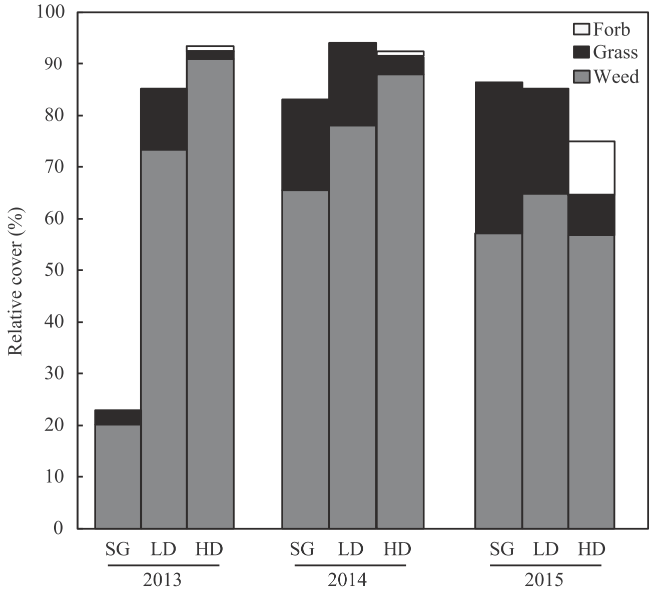

Fig. 1. Relative cover in whole plots (n = 4) in late May-early June, divided into grasses, sown forbs (high diversity plots only), and weeds, from 2013 to 2015. Notes: Low switchgrass emergence and establishment in 2012 prompted reseeding in 2013. Relative cover may not sum to 100%, indicating bare ground.

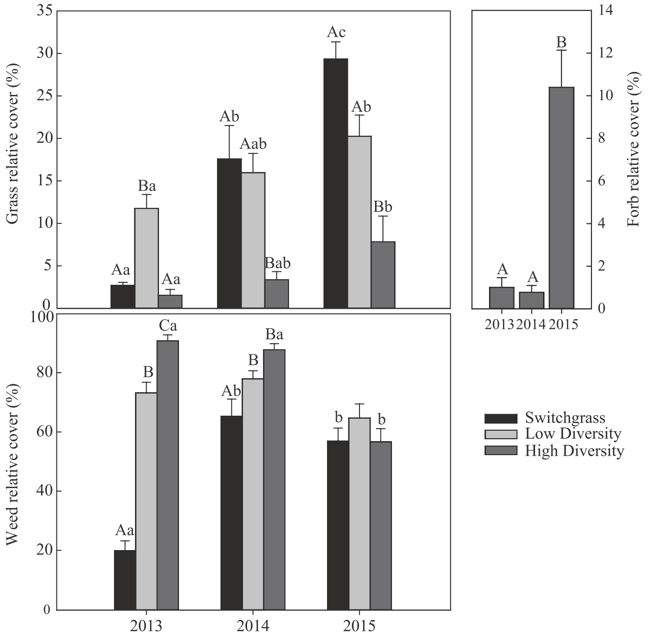

Fig. 2. Total plant relative cover in whole plots (n = 4) in late May-early June, partitioned into grasses, sown forbs (high diversity plots only), and weeds, from 2013 to 2015. Notes: Low switchgrass emergence and establishment in 2012 prompted reseeding in 2013. Total relative cover may not sum to 100%, indicating bare ground. Pair letters represent significant differences of least squared means ($P < 0.05$) within a year across the three cropping systems, and lowercase letters represent significant differences within a cropping system across the three years.

Relative cover was also calculated in October 2013 as an alternative to post-frost plant biomass. The SG and LD plots had similar cover under sown grasses (24% of ground cover), while grass cover in HD plots was lower (3.5%, $P = 0.0002$). By contrast, HD plots had the most weedy species cover ($P = 0.0092$), while SG and LD plots had equivalent weedy species cover.

3.4. Stand characteristics – mid-season height and light interception

Stand characteristic measurements are presented in Table 4. Plant canopy height increased for all three cropping systems as the stands

Table 3
Summary of ANOVA results of year, seed mixture, biochar application, and their interactions on the relative cover of a) sown grasses, b) unsown weedy species, and c) forbs (high diversity plots only).

a) Grass	Num DF	Den DF	F Value	Pr > F
Year	2	36	55.35	< 0.0001
Seed Mix	2	18	21.99	< 0.0001
Year*Seed Mix	4	36	9.65	< 0.0001
Biochar	1	36	0.75	0.6312
Year*Biochar	2	36	2.14	0.1340
Seed Mix*Biochar	2	36	0.2	0.6312
b) Unsown	Num DF	Den DF	F Value	Pr > F
Year	2	36	20.96	< 0.0001
Seed Mix	2	18	46.62	< 0.0001
Year*Seed Mix	4	36	30.32	< 0.0001
Biochar	1	36	3.2	0.0898
Year*Biochar	2	36	0.22	0.7955
Seed Mix*Biochar	2	36	0.82	0.4296
c) Forbs (HD only)	Num DF	Den DF	F Value	Pr > F
Year	2	12	30.52	< 0.0001
Biochar	1	12	0.14	0.7358
Year*Biochar	2	12	1.25	0.3220

Table 4
Mid-season (July) canopy height, light transmission (T), and leaf area index (LAI) for each cropping system and year. There was no effect of biochar application on canopy height. Capital letters represent significant differences within a year across the three cropping systems, and lowercase letters represent significant differences within a cropping system across the three years. Values are the sample mean ± one standard error; n = 4. SG: switchgrass monocultures; LD: low diversity grass mixture; HD: high diversity grass and forb mixture.

Year	Cropping System	Height (cm)	T (%)	LAI
2013	SG	16.61 ± 0.95 Aa	48.25 ± 0.20 Bb	1.68 ± 0.04 Aa
	LD	39.29 ± 2.88 Ba	24.13 ± 0.28 Ac	3.48 ± 0.03 Ba
	HD	32.35 ± 2.52 ABa	21.38 ± 0.44 Ab	3.73 ± 0.04 Ba
2014	SG	135.53 ± 4.49 Aab	8.59 ± 0.41 Aa	5.19 ± 0.02 Ac
	LD	122.03 ± 4.85 Ac	6.84 ± 0.38 Aa	5.79 ± 0.01 Ab
	HD	112.63 ± 9.02 Ac	6.61 ± 0.39 Aa	5.49 ± 0.01 Ab
2015	SG	146.77 ± 2.26 Cb	10.21 ± 0.27 Aa	4.29 ± 0.02 Bb
	LD	91.77 ± 2.49 Bb	13.88 ± 0.30 ABb	3.72 ± 0.01 ABa
	HD	74.16 ± 5.34 Ab	18.18 ± 0.25 Bb	2.94 ± 0.02 Aa

became older ($P < 0.0001$), but leveled out by the second year. This is reflected in an interaction between cropping system and year ($P < 0.0001$), driven by a slight decrease in canopy heights of LD and HD plots from 2014 to 2015, while the SG canopy height remained approximately the same during these two years. Similar to grass relative cover, SG canopy height was the shortest of the three cropping systems in 2013 (16.6 cm), but was the tallest in 2015 (147 cm). There was no effect of biochar application on canopy height.

Light transmittance through the canopy decreased as stands aged and canopies closed ($P < 0.0001$). While all treatments had less light penetrating the canopy in mature than juvenile stands, there was again an interaction between cropping system and year, with juvenile SG plots having the highest transmittance (48%) in 2013, but the lowest transmittance by the time they matured in 2015. Biochar application also decreased transmittance by approximately 9% ($P = 0.0397$).

Similar to light transmittance, LAI was also affected by year ($P < 0.0001$) and the cropping system by year interaction ($P = < 0.0001$). The SG plot LAI was lower than LD and HD plot LAI in 2013, while all three cropping systems had similar LAI in 2014. In 2015, LAI was the largest in the SG plots and smallest in the HD plots. LAI was also affected by biochar application, and biochar subplots had a larger LAI (4.8 vs. 3.7; $P = 0.0162$).

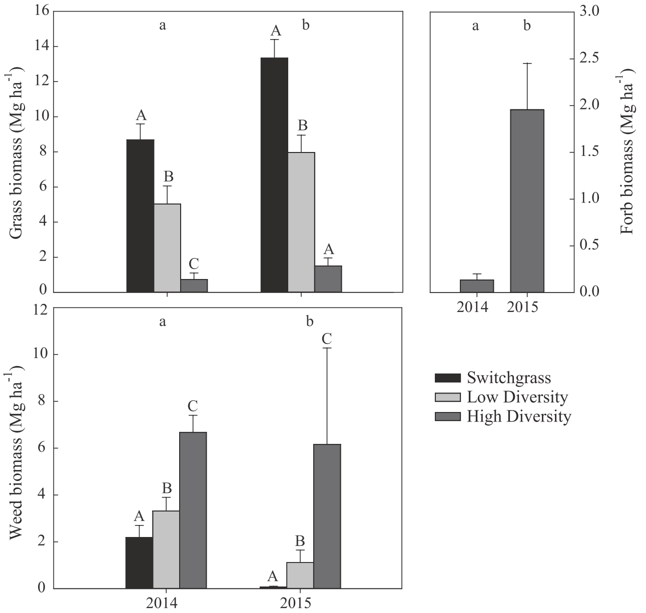

Fig. 3. Peak biomass (dry weight) for each cropping system in whole plots (n = 4) in mid-August, divided into grasses, forbs (high diversity plots only), and weeds, for 2014 and 2015. Capital letters represent significant differences of least squared means ($P \leq 0.05$) within a year across the three cropping systems, and lowercase letters represent significant differences between 2014 and 2015.

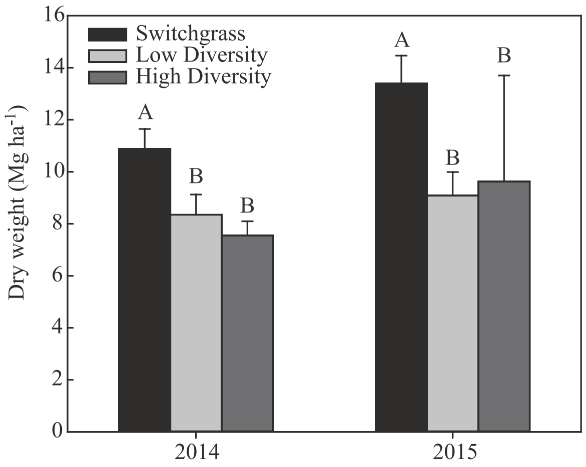

Fig. 4. Peak biomass total yields (dry weight) for 2014 and 2015 for each cropping system in whole plots (n = 4).

3.5. Biomass

3.5.1. Peak biomass

Sown species peak biomass increased for all three cropping systems between 2014 and 2015 (Figs. 3 and 4; $P < 0.0001$). Estimated amounts were lowest in HD plots and highest in SG plots ($P < 0.0001$), with no effects due to biochar application. In the HD plots, the majority of the sown species biomass gain was due to an increase in sown forb biomass, which went from 0.13 Mg ha^{-1} to 1.95 Mg ha^{-1}. Weedy species biomass decreased during the same period ($P < 0.0001$), particularly within SG and LD plots, which received a 2,4-D application in the spring of 2015 ($P = 0.0004$). Total biomass (sown plus weedy species) was only affected by cropping system, with SG plots containing 40% more total biomass than HD and LD plots (Fig. 4, $P = 0.0133$).

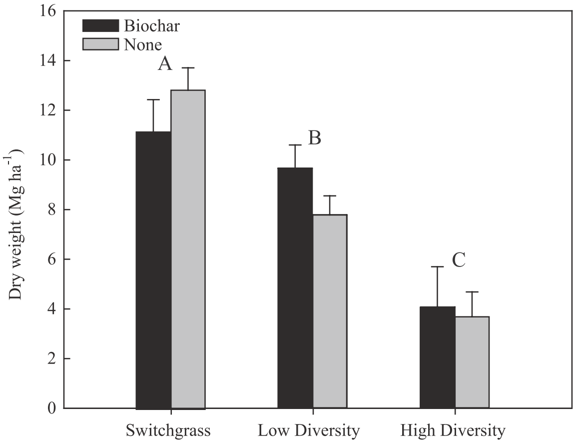

Fig. 5. Post-frost yields (dry weight) for 2015 for each cropping system and by biochar treatment.

3.5.2. *Post-frost biomass*

The post-frost biomass estimates followed similar trends to those for peak biomass. Between 2014 and 2015, sown plant yields increased by 36% ($P = 0.0084$), weedy species biomass decreased by 85% ($P < 0.0001$), and SG plots yielded 38% more sown biomass ($P = 0.0157$) and 14% less weedy species biomass ($P = 0.0007$) than LD plots. There was also an interaction between cropping system and biochar for total biomass ($P = 0.0492$), where the biochar application increased total biomass within LD plots but not in SG plots.

In 2015 (Fig. 5), HD plots had the lowest estimated total biomass (3.9 Mg ha^{-1}, $P = 0.0004$) and sown biomass ($P < 0.0001$), while SG plots had three times more total biomass (12.0 Mg ha^{-1}) and six times more sown biomass. Yields from LD plots were intermediate, with a total biomass of 8.7 Mg ha^{-1}. SG plots also had the least amount of weedy species biomass ($P < 0.0001$).

Analysis of post-frost sown species biomass composition in 2015 revealed that biochar application had no effect on any components measured, while cropping system had significant impacts on three variables (Table 5). When compared to biomass from SG and LD plots, the HD plots had lower neutral detergent fiber and hemicellulose contents, but higher ash content ($P < 0.05$ for all three variables). Additionally, compared with SG, plants in the LD plots had significantly less ash. While there were some differences in cell wall component proportions among the three cropping systems, the potential ethanol production for all three was not significantly different and averaged 47.6 ml of ethanol kg^{-1} of dry biomass (Table 5).

4. Discussion

Cropping system had a consistent effect on nearly every variable measured. In contrast to some plant research (Hector et al., 1999; Tilman et al., 2001), the HD cropping system plots in the current experiment did not outperform the less diverse cropping systems (SG and LD). As discussed by Dickson and Gross (2015) and Von Cossel and Lewandowski (2016), realistic seeding rates and challenges in weed control can obscure the purported benefits of diverse plantings, especially during the establishment period. In our experiment, while the switchgrass (SG) monocultures experienced a poor initial establishment year, resown plots exceeded sown biomass production in LD and HD plots within three seasons of the initial planting date. While the percent ground cover was higher in HD plots than SG plots, most of this increase was due to weedy species, demonstrating the difficulties in maintaining large-scale diverse plantings at low weed levels. In our study, broadleaf weeds were abundant in all plots but were easily controlled via herbicides in grass-only cropping systems (SG and LD). Furthermore, our results also demonstrate higher overall quality of biomass (lower ash and higher hemicellulose and neutral detergent fiber contents) harvested from the SG and LD plots relative to biomass harvested from the HD plots, traits which are desirable for bioenergy production. Our research suggests that biofuel agroecosystems may be best established by planting just a few high-performing species, rather than a diverse mixture of native prairie species.

4.1. *Monocultures vs. polycultures*

A well-managed monoculture cropping system of a productive biomass variety such as ‘Liberty’ switchgrass may ultimately yield more biomass once stands are mature, but monocultures are also at increased risk for stand failure, as occurred initially in this experiment, and generally provide fewer ecosystem services (Hector and Bagchi, 2007; Gamfeldt et al., 2008). Perennial grass crops typically require several years to reach full biomass production and typically do not produce a harvestable crop during the establishment year. One method to reduce the economic risk of growing switchgrass is to plant maize as a companion crop during the establishment year (Hintz et al., 1998; Anderson et al., 2016). However, under the severe drought conditions that prevailed in 2012, the maize companion crop apparently outcompeted the emerging switchgrass for moisture, causing substantial seedling mortality. Our results demonstrate that planting a cash crop companion during the switchgrass establishment year may provide additional income but also brings additional risk in dry years.

Diverse cropping systems carry their own benefits and risks. Cropping systems with higher species diversity may be able to withstand extreme weather events (e.g., drought) due to a “portfolio effect,” where the poor performance of some species is balanced by the improved performance of others (Lehman and Tilman, 2000; Isbell et al., 2015). Thus, incorporating some diversity in bioenergy cropping systems may provide early stand benefits such as more rapid ground cover and reductions in weedy species abundances. However, increasing plant diversity also can create challenges in weed control; for example we found high weed pressure in the most diverse mixture (HD) may have reduced sown species abundances and biomass. In addition, weed control in the HD plots could only be accomplished through repeated mowing, which may alter community composition and stand

Table 5
Post-frost biomass composition of sown species collected from the three cropping systems in 2015. Capital letters represent a significant difference across the three cropping systems for a given variable; no letter indicates no difference. SG: switchgrass monocultures; LD: low diversity grass mixture; HD: high diversity grass and forb mixture.

Cropping System	NDF[a] g kg^{-1}	ADF[b]	ADL[c]	Hemicellulose	Cellulose	Nitrogen	Ash	Ethanol mL kg^{-1}
SG	872.5 A	531.8	76.6	340.7 B	455.3 B	2.5	30.6 B	43.8
LD	845.9 A	521.1	64.3	324.8 B	456.8 B	2.0	13.4 A	50.8
HD	764.3 B	515.2	86.4	249.1 A	428.8 A	3.6	43.1 C	48.2

[a] Neutral detergent fiber.
[b] Acid detergent fiber.
[c] Acid detergent lignin.

characteristics such as height, light transmittance, and LAI, in a manner that is distinct from weed control through broadleaf herbicide application.

4.2. Limited effects of biochar

Biochar had no consistent effects on plant community composition, height, or biomass yields, generally agreeing with a meta-analysis that examines biochar effects on perennial species (Biederman and Harpole, 2013). Although plant community composition was not measured during the establishment year in this study, biochar may have had an impact on plant emergence or other characteristics in the establishment year. Allaire et al. (2015) found that switchgrass yields increased by up to 10% with biochar application during establishment in a dry summer, hypothesizing that biochar increased soil moisture levels. If the plant communities were challenged by drought after the establishment year, it is conceivable that biochar might affect community composition and/or biomass production. Thus, it is possible that in our study, no consistent biochar effect was observed from 2013 to 2015 because good soils with adequate fertilizer and rainfall meant that neither fertility nor water limited plant growth, creating relatively low-stress growth conditions upon which biochar could not significantly improve.

While generating only minimal aboveground changes, biochar application may still have positive belowground influences on characteristics such as soil organic carbon, soil nitrogen, and soil moisture retention, even in fertile Midwestern US soils (Laird et al., 2010) similar to those present in the current experiment. Indeed, biochar increased soil C in all cropping systems, and tended to increase soil moisture both during in 2014 and in parallel laboratory incubations using soils collected from the same location as the current study (Fidel, 2015). Furthermore, biochar significantly reduced N_2O emissions from continuous corn cropping systems and also slightly reduced N_2O emitted from SG plots located at the same field site studied here, implying potential reductions in system N losses (Fidel, 2015). Thus, while the biochar impacts on bioenergy cropping system performance indicators examined within this paper were non-significant, biochar application may have additional benefits worth consideration.

4.3. Implications for native bioenergy cropping systems

Our results suggest that careful selection of highly productive seed mixtures for biomass cropping system is critical for the establishment of plant communities and optimization of biomass production during the first four years of stand establishment. Results specifically highlighted a trade-off that managers should consider when choosing cropping system seed mixes: switchgrass monocultures can provide higher biomass yields under ideal conditions ≥4 years after establishment, whereas native grass polycultures are likely to be more resilient to stressors. In addition, establishing native perennial cropping systems can increase overall landscape diversity and provide more ecosystem services compared to a landscape dominated by maize (Landis et al., 2008; Werling et al., 2014). Based on this experiment, one to three high-performing grass species may balance establishment success, yields, biomass composition, and ease of management for plant communities managed as biomass crops. More research is needed to assess the efficacy of other polycultures and to determine how cropping system diversity and biochar application can be optimized for specific climates and soils.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eja.2018.08.009.

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