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Productivity and resistance to weed invasion in four prairie biomass feedstocks with different diversity

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Abstract

High-diversity mixtures of native tallgrass prairie vegetation should be effective biomass feedstocks because of their high productivity and low input requirements. These diverse mixtures should also enhance several of the ecosystem services provided by the traditional monoculture feedstocks used for bioenergy. In this study, we compared biomass production, year-to-year variation in biomass production, and resistance to weed invasion in four prairie biomass feedstocks with different diversity: one species – a switchgrass monoculture; five species – a mix of C_4 grasses; 16 species – a mix of grasses, forbs, and legumes; and 32 species – a mix of grasses, forbs, legumes, and sedges. Each diversity treatment was replicated four times on three soil types for a total of 48 research plots (0.33–0.56 ha each). We measured biomass production by harvesting all plant material to ground level in ten randomly selected quadrats per plot. Weed biomass was measured as a subset of total biomass. We replicated this design over a five-year period (2010–2014). Across soil types, the one-, 16-, and 32-species treatments produced the same amount of biomass, but the one-species treatment produced significantly more biomass than the five-species treatment. The rank order of our four diversity treatments differed between soil types suggesting that soil type influences treatment productivity. Year-to-year variation in biomass production did not differ between diversity treatments. Weed biomass was higher in the one-species treatment than the five-, 16-, and 32-species treatments. The high productivity and low susceptibility to weed invasion of our 16- and 32-species treatments supports the hypothesis that high-diversity prairie mixtures would be effective biomass feedstocks in the Midwestern United States. The influence of soil type on relative feedstock performance suggests that seed mixes used for biomass should be specifically tailored to site characteristics for maximum productivity and stand success.

Keywords: agroenergy, bioenergy, biomass, productivity, switchgrass, tallgrass prairie, weed resistance

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Introduction

Rising global energy use and decreasing fossil fuel reserves have increased the need for renewable sources of energy. Many of the current bioenergy crops (e.g., corn, soybeans, oilseed rape, sugarcane, and willow) require fertilizer and pesticide inputs and compete with food crops for land. These shortcomings have increased interest in alternative bioenergy crops, such as switchgrass (*Panicum virgatum* L.) and *Miscanthus* (*Miscanthus x giganteus*), which are highly productive and can grow on marginal farmland (Lewandowski *et al.*, 2003; Heaton *et al.*, 2004; Khanna *et al.*, 2008). Another viable bioenergy crop, particularly in the Midwestern United

States, is a mixture of native perennial tallgrass prairie vegetation (Hector *et al.*, 1999; Balvanera *et al.*, 2006; Tilman *et al.*, 2006; Cardinale *et al.*, 2007). Experiments focusing on the diversity–productivity relationship suggest that high-diversity prairie mixtures produce more bioenergy than corn on marginal land (Tilman *et al.*, 2006), produce more biomass than perennial monocultures (Tilman *et al.*, 2006; Cardinale *et al.*, 2007; Fornara & Tilman, 2009), and sustain high yields for decades without fertilizer (Glover *et al.*, 2010). While the economic and ecological benefits of high-diversity prairie mixtures for bioenergy seem attractive, more research is needed to determine the feasibility of growing these crops on a production-level scale.

Diversity–productivity experiments suggest that unfertilized high-diversity biomass crops will be more productive than unfertilized low-diversity biomass crops because of greater niche differentiation and/or better facilitation (i.e., the 'complementary effects';

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Loreau & Hector, 2001; Cardinale *et al.*, 2007; but see Hooper *et al.*, 2005 for alternative mechanisms). High-diversity mixtures are more morphologically and phenologically variable than low-diversity mixtures, which should increase total resource acquisition (Wilsey, 2010). For example, high-diversity prairie mixtures have greater variation in root depth and root architecture than low-diversity mixtures, which should increase water and nutrient uptake in these communities (Fornara &Tilman, 2009; Postma & Lynch, 2012). Also, high-diversity mixtures typically have higher functional diversity (i.e., more functional groups: cool-season C_3 grasses, warm-season C_4 grasses, and forbs) than low-diversity mixtures, expanding the time frame in which resources are acquired during the growing season (Diaz & Cabido, 2001; Fargione & Tilman, 2005). One example of enhanced facilitation in high-diversity mixtures is the inclusion of legumes. Legumes form symbiotic associations with nitrogen-fixing rhizobial bacteria. These associations increase nitrogen availability within the community.

High-diversity biomass crops should be more resistant to weed invasion than low-diversity biomass crops because they provide fewer resources for potential invaders (Knops *et al.*, 1999; Levine, 2000; Hooper *et al.*, 2005; Balvanera *et al.*, 2006). For example, Fargione & Tilman (2005) compared five treatments with different diversity and found that the high-diversity mixtures were less susceptible to weed invasion because they captured a greater proportion of available soil nitrates. High-diversity mixtures also tend to have greater absolute cover than low-diversity mixtures, which reduces light availability (Levine, 2000) and helps minimize weed invasion (Davis *et al.*, 2000). Minimizing weed invasion is important for maximizing yield in biomass feedstocks. Although weed invasion increases diversity, the addition of exotic species does not have the same positive influence on productivity as the addition of native species in tallgrass prairie systems (Isbell & Wilsey, 2011). These exotic species may not be adapted to local conditions and occupy space that would otherwise contain prairie species with higher productivity. From a management perspective, the invasion of woody species would be particularly costly if targeted removal is required.

High-diversity prairie mixtures should also enhance several of the ecosystem services provided by the traditional monoculture feedstocks used for bioenergy. Two concurrent studies at our research site have shown that high-diversity biomass mixtures provide better nesting habitat for birds (Myers *et al.*, 2015) and more resources for butterflies (Myers *et al.*, 2012) than switchgrass monocultures. High-diversity mixtures are also less susceptible to yield loss via specialized pests than

monocultures (Knops *et al.*, 1999). For example, the gall midge pest *Chilophaga virgati* specializes on switchgrass and decreases productivity and fitness in infected monocultures (Boe & Gagne, 2011). Further, high-diversity mixtures should display lower year-to-year variation in any particular ecosystem service than monocultures because they have species with differing levels of stress tolerance (i.e., the insurance effect, Yachi & Loreau, 1999; Hooper *et al.*, 2005). This interspecific variability will ensure a certain level of ecosystem service in extreme climatic years and could help maintain consistent rates of belowground carbon sequestration over the timeframe necessary to mitigate climate change (Hooper *et al.*, 2005).

The potential value of high-diversity prairie mixtures as biomass feedstocks has encouraged some to examine the feasibility of growing these crops on a production-level scale. In particular, three recent studies examined whether Conservation Reserve Program (CRP) lands in Iowa, unfertilized polycultures, and reconstructed prairies might be useful biomass feedstocks. These experiments all supported the potential utility of diverse prairie for bioenergy, finding that CRP land and switchgrass monocultures have similar theoretical ethanol yields (Jungers *et al.*, 2013) and that unfertilized polycultures (31 species, Jarchow & Liebman, 2013) and restored prairies (Zilverberg *et al.*, 2014) are sufficiently productive (9.1 and 7.3 Mg ha^{-1} respectively). However, to the best of our knowledge, no one has compared the productivity and ecosystem services of high-diversity vs. low-diversity prairie mixtures specifically designed for biomass on a production-level scale.

In this study, we compare biomass production, year-to-year variation in biomass production, and resistance to weed invasion in four prairie biomass feedstocks with different diversity (one, five, 16, and 32 species). We predict that the high-diversity treatments (16 and 32 species) will produce more biomass, display lower year-to-year variation in biomass production, and be more resistant to weed invasion than the low-diversity treatments (one and five species).

Materials and methods

Research site

This study was conducted at the Cedar River Ecological Research Site in Blackhawk County, Iowa (42°23N, 92°13W). The 40 ha site is on marginal farmland with a flat slope (0–2%) and a corn suitability rating (CSR) of 50–79 (Natural Resource Conservation Service, 2014). CSR is an index (0–100) that ranks all soils in the state of Iowa based on their potential row crop productivity. There are three soil types at the site: (i) an excessively drained Flagler sandy loam (CSR = 50); (ii) a well-drained

Waukee loam (CSR = 79); and (iii) a somewhat poorly drained Spillville–Coland alluvial complex (CSR = 60; Natural Resource Conservation Service, 2014). The relative amounts of sand, silt, and clay vary between soils: Flagler sandy loam – 73.8% sand, 17.0% silt, and 9.2% clay; Waukee loam – 66.2% sand, 20.9% silt, and 12.8% clay; Spillville–Coland alluvial complex – 42.1% sand, 35.9% silt, and 22.0% clay (Natural Resource Conservation Service, 2014). These soils will henceforth be referred to as the 'sand', 'loam', and 'clay' soils, respectively. The sand soil has the lowest nutrient availability and water holding capacity (Myers *et al.*, 2015; Sherrard *et al.*, 2015). The loam and clay soils have similar nutrient availability but the clay soil has higher water holding capacity (Myers *et al.*, 2015; Sherrard *et al.*, 2015).

In spring 2009, four diversity treatments were seeded at the site: (i) one species – a switchgrass (*Panicum virgatum* L.) monoculture; (ii) five species – a mixture of C_4 grasses; (iii) 16 species – a mixture of C_3 and C_4 grasses, forbs, and legumes; and (iv) 32 species – a mixture of C_3 and C_4 grasses, sedges, forbs, and legumes (see Table S1 for species list). Each diversity treatment contains all species from treatments of lesser diversity plus additional species. Four replicate plots (0.33–0.56 ha each) of each diversity treatment were randomly established on each soil type for a total of 48 research plots (four replicates × four diversity treatments × three soil types; see Sherrard *et al.*, 2015 or Myers *et al.*, 2015 for site map). The size of our plots provides a realistic representation of a production-level biomass crop and should generate reliable estimates of productivity (with minimal edge effects), wildlife use (e.g., Myers *et al.*, 2012, 2015), and susceptibility to weed invasion in the different treatments. To minimize the likelihood of contaminating diversity treatments during establishment, the plots were seeded from least to most diverse using a Truax native seed drill. Prior to seeding, all plots were seeded with Roundup ready soybeans in July 2008 and glyphosate was applied in July/August 2008. Other site management during the study period included: establishment mowing (June 2009) to reduce competition with annual weeds, burning (April 2011), haying (March 2012), and burning (April 2014). A small patch of crown vetch and reed canary grass was treated with glyphosate in 2014 to prevent spread; otherwise, no fertilizers, herbicides, pesticides, weeding, or irrigation have been applied to the treatment plots.

The species composition of each diversity treatment was selected based on its potential utility as a biomass feedstock. Switchgrass was chosen as the monoculture because it has been recommended as a bioenergy crop by the U.S. Department of Energy (McLaughlin *et al.*, 1999). We used source identified class yellow label seed for the switchgrass monoculture to ensure that the genotype of all seeds originated from remnant prairies in Iowa. The 'yellow tag' designation indicates that the Iowa Crop Improvement Association has verified the seed source in accordance with standards set by the Association of Official Seed Certifying Agencies (AOSCA). In pilot research, we found that switchgrass plots grown from Iowa 'yellow tag' seed produced more biomass than plots grown from cultivar seed (D. Smith, pers. obs.). The five C_4 grass treatment was selected because all five species are highly productive in tallgrass prairies. We used Iowa 'yellow tag' seed for the five species in this treatment as well. The 16-species treatment was

chosen based on nine *a priori* criteria: (i) a statewide distribution; (ii) high aboveground biomass production; (iii) availability of Iowa 'yellow tag' seed; (iv) ease of establishment from seed; (v) ability to maintain standing vegetation through winter; (vi) ability to grow in a variety of soil moisture conditions; (vii) variable phenologies and life histories – species that produce biomass at different times; (viii) long life span; and (ix) ability to coexist with other species. Many of the species in the 32-species treatment were selected based on the above criteria; however, some were selected because they are commonly seeded species in native tallgrass prairie restorations. The seeding rate of the one- and five-species treatments was 561 pure live seeds m^{-2} (Table S1), which was based on recommendations for establishing switchgrass as a bioenergy crop (Natural Resource Conservation Service, 2009). The 16- and 32-species treatments contained the same number of graminoid seeds as the one- and five-species treatments plus seeds of other functional groups for a total of 829 and 869 pure live seeds m^{-2}, respectively. These seeding rates are consistent with recommendations for prairie restorations in Iowa. Because our diversity treatments are perennial, they do not need to be reseeded after establishment.

Climate data

During our five-year study (2010–2014), the average growing season (April–October) temperature for the region was 16.9 °C and the average growing season precipitation was 698 mm (data collected from nearest weather station: Waterloo Airport, 15.5 km, Fig. S1). The site experienced a drought in 2012 (growing season precipitation = 443.2 mm). The clay and loam soil experienced severe flooding in spring 2013 (clay: submerged for ~two weeks, max height = 1.8 m; loam: submerged for two days, max height = 50 cm) and spring 2014 (clay: submerged for one week, max height = 1.3 m; loam: submerged for two days, max height = 30 cm). The sand soil did not experience flooding during the study.

Experimental design

To compare biomass production between treatment combinations, we harvested biomass in each year of the study (2010–2014) between August 25 and September 27 (dates within this range differ between years based on the timing of plant senescence). This is the timing of maximum yield in switchgrass biomass crops (Heaton *et al.*, 2004). In 2010–2012, ten 0.1-m^2 quadrats were randomly selected in each plot and all standing biomass was cut to ground level. The duff layer (senesced vegetation from the previous year) was omitted from harvest. In 2013 and 2014, we increased the quadrat size to 0.3 m^2 to obtain more plant tissue. After harvest, the biomass was divided into functional groups: C_4 grasses, C_3 graminoids, forbs, legumes, and weeds dried to a constant mass (min. 65 °C for 72 h) and weighed. Harvested biomass was used to estimate plot-level productivity in Mg ha^{-1}. We used the portion of weeds from the harvested biomass to estimate % weed biomass in each plot. Any species that was not included in the

original seed mix of that plot was classified as a weed. Consequently, a weed could either be a species from another diversity treatment ('treatment' weeds) or a species that was not seeded at the site ('nontreatment' weeds). We acknowledge that our low-diversity treatments have a higher probability of containing 'treatment' weeds than our high-diversity treatments with this approach. For example, the 32-species treatment, by definition, can not contain any 'treatment' weeds. To account for this bias, we performed an additional statistical analysis that compared % weed biomass between treatments using 'nontreatment' weeds only. 'Nontreatment' weed biomass was estimated from the basal area coverage of each weed group in 2014 (see below).

To examine changes in species composition over the five-year study, basal area coverage of every species was measured each year in July. Two 10 m transects were established in random positions in each plot (one transect oriented North–South, one transect oriented East–West). A 0.1-m^2 quadrat (20 cm × 50 cm) was placed at one meter intervals along each transect and basal area coverage of each seeded species was estimated one inch above the ground by comparing the total area of live material to 0.006 cm^2 standardized squares. From 2010 to 2013, the presence of weeds was noted during this analysis but not quantified. We modified this design in 2014 and quantified the basal area coverage of every weed species to characterize the relative % of 'treatment' vs. 'nontreatment' weeds. The % of bare ground was measured in 2012–2014 during this sampling period to assess vulnerability to weed invasion. Ground covered in plant litter was not classified as bare ground. % bare ground was higher in 2014 because of the spring burn that year.

Statistical analysis

Aboveground biomass, % weed biomass, and % bare ground were analyzed with repeated measures ANOVAs with diversity treatment and soil type as fixed factors and year as the repeated measure. Aboveground biomass met the assumption of normality, but % weed biomass and % bare ground were log (1+x)- and square-root–transformed, respectively, to meet this assumption. All three measures violated the homogeneity of variance assumption. Aboveground biomass and weed biomass were corrected using the Greenhouse–Geisser epsilon ($\varepsilon = 0.630$ and 0.708 respectively). % bare ground data was corrected using the Huynh Feldt correction because the Greenhouse–Geisser correction was too conservative for these data ($\varepsilon > 0.75$; Girden, 1992). All post hoc analyses were performed according to Loftus & Masson (1994) using confidence intervals calculated according to Hollands & Jarmasz (2010).

To correct for bias associated with differences in the amount of 'treatment' weeds between diversity treatments, we compared the % of 'nontreatment' weeds between treatment combinations using a 2-way ANOVA with diversity treatment and soil type as fixed factors. This analysis was performed on 2014 data only as this was the only year in which the basal area coverage of 'treatment' vs. 'nontreatment' weeds was quantified.

To examine year-to-year variation in biomass production, we calculated coefficients of variation for each treatment combina-

tion and compared these coefficients using ANOVA with diversity treatment as a fixed factor and soil type as a random factor.

We used nonmetric multidimensional scaling (NMDS) to examine changes in species composition in the five-, 16-, and 32-species treatments on each soil type over the five-year study. We used the Manhattan dissimilarity index after comparing it to other dissimilarity indices with the rank index function in R. A 2-dimensional solution was used after comparing stress and goodness of fit. Permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was used to test for significant differences between diversity treatments, years, and soil types.

All statistics were performed using the 'VEGAN' package (v. 2.0-10; Oksanen et al., 2013), the 'EZ' package (v.4.2-2; Lawrence, 2013), or the 'NLME' package (v. 3.1-117; Pinheiro et al., 2014) of R (v. 3.1.1; R Core Team, 2014).

Results

Biomass production

Aboveground biomass production differed between diversity treatments, soil types, and years (Figs 1 and S2, Table 1). On average, more biomass was produced in the one-species treatment (8.24 Mg ha^{-1} yr^{-1}) than the five-species treatment (7.17 Mg ha^{-1} yr^{-1}, Fig. 1). The 16- and 32-species treatments produced 8.03 Mg ha^{-1} yr^{-1} and 7.91 Mg ha^{-1} yr^{-1}, respectively, which did not differ significantly from the other two diversity
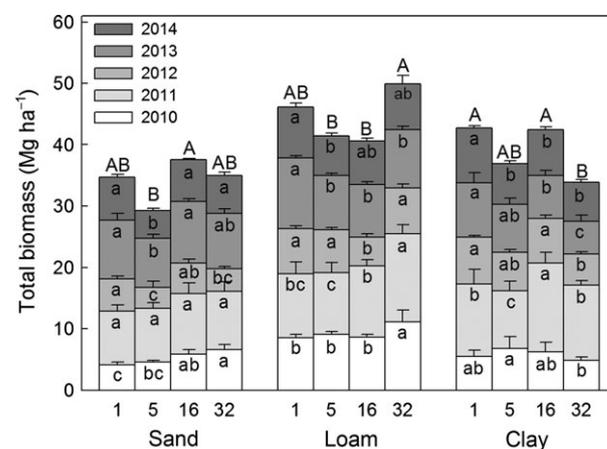
Fig. 1 Cumulative biomass production of each soil type × diversity treatment combination during the five-year study (2010–2014). The bars in each stack represent mean annual biomass production (+ 1SE). *Post hoc* analyses compare biomass production between diversity treatments within a soil type. Capital letters indicate significant differences in cumulative biomass production between diversity treatments over the five-year study. Lower case letters indicate significant differences in biomass production between diversity treatments in a given year.

Table 1 Repeated-measures ANOVA comparing aboveground biomass, % weed biomass, and % bare ground between treatment combinations. 'Plot' represents variation between factors (diversity treatment and soil type) and 'Within' represents variation within factors across the repeated measure (year)

	Biomass			% weed biomass†			% bare ground‡		
	df	MS	F	df	MS	F	df	MS	F
Plot									
Diversity treatment (T)	3	12.86	2.88*	3	0.0052	10.68***	3	15.97	12.13***
Soil type (S)	2	86.44	12.41***	2	0.0026	5.34**	2	0.11	0.09
T × S	6	13.60	3.05*	6	0.0001	0.28	6	2.38	1.81
Residuals	36	4.45		36	0.0005		36	1.32	
Within									
Year (Y)	4	196.20	52.24***	4	0.0028	9.22***	2	496.80	366.60***
T × Y	12	8.89	2.37***	12	0.0001	3.32***	6	7.80	5.77***
S × Y	8	21.37	5.69***	8	0.0003	1.13	4	4.40	3.24*
T × S × Y	24	2.36	0.63	24	0.0005	1.67*	12	0.40	0.327
Residuals	144	3.76		144	0.0003		72	1.40	

Reported values are: degrees of freedom (df), mean squares (MS), and *F*-statistics (*F*).
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
†Data log(1+x)-transformed.
‡Data square-root-transformed.

treatments. More biomass was produced on the loam soil (8.90 Mg ha^{-1} yr^{-1}) than on the sand soil (6.82 Mg ha^{-1} yr^{-1}, Fig. 1). Biomass production on the clay soil (7.79 Mg ha^{-1} yr^{-1}) did not differ significantly from the other two soil types. More biomass was produced in 2011 than in 2010, 2012, and 2014 (Figs 1 and S2). Biomass production in 2013 did not differ significantly from any other year.

The rank order of the four diversity treatments differed between soil types (treatment × soil term Table 1). On the sand soil, the 16-species treatment produced more biomass than the five-species treatment but not more than the one- or 32-species treatments (Fig. 1). On the loam soil, the 32-species treatment produced more biomass than the five- and 16-species treatments but not more than the one-species treatment (Fig. 1). On the clay soil, the one- and 16-species treatments produced more biomass than the 32-species treatment but not more than the five-species treatment (Fig. 1).

The diversity treatment that produced the most biomass varied between years (treatment × year term Table 1). In 2011, the 16- and 32-species treatments produced more biomass than the one- and five-species treatments (Fig. S2). In 2013, the one-species treatment produced more biomass than the five- and 32-species treatments but not more than the 16-species treatment. In 2014, the one-species treatment produced more biomass than the five-species treatment, but not more than the 16-and 32-species treatments. In 2010 and 2012, all diversity treatments produced the same amount of biomass.

Year-to-year variation in biomass production differed between soil types (*F* = 7.007; *P* < 0.05). The coefficient of variation for biomass production across years was 0.292 on the loam soil, 0.403 on the clay soil, and 0.381 on the sand soil. Year-to-year variation in biomass production did not differ between diversity treatments (*F* = 1.609; *P* = 0.284); however, there was a nonsignificant trend suggesting that variability increased with diversity. Specifically, the coefficient of variation for each diversity treatment was as follows: one-species: 0.332, five-species: 0.333, 16-species: 0.373, 32-species: 0.398.

Weed biomass

In the basal area coverage survey conducted at the end of the five-year study (2014), most weeds were 'non-treatment' weeds (species that were not seeded in any treatment at the site). Nontreatment weeds represented 82.8% (one-species), 74.2% (five-species), 83.3% (16-species), and 100% (32-species) of total weed coverage.

Percent weed biomass ('treatment' + 'nontreatment' weeds) differed significantly between diversity treatments, soil types, and years (Fig. 2, Table 1). Weed biomass was higher in the one-species treatment than in the five-, 16-, and 32-species treatments (7.33%, 3.10%, 2.46%, and 2.53% respectively, Table 1). 'Nontreatment' weed biomass was also higher in the one-species treatment than in the five-, 16-, and 32-species treatments (*F* = 8.611, *P* < 0.001, only 2014 data analyzed). Weed biomass was higher on the clay soil (5.47%) than on the sand soil (2.84%, Fig. 2, Table 1). Weed biomass was
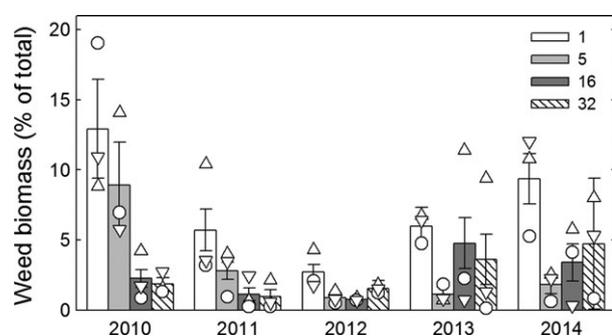
Fig. 2 Percent weed biomass in each diversity treatment. Bars represent mean % weed biomass ($\pm$ 1SE) of each diversity treatment pooled across soil types and symbols represent mean % weed biomass within a soil type (circle = sand; down triangle = loam; up triangle = clay). Standard error bars omitted from within soil type means for clarity.

Table 2 Three factor nonparametric PERMANOVA reporting differences in species composition between treatments, soil types, and years in the 5-, 16-, and 32-species treatments

	df	MS	F
Year (Y)	4	0.843	9.45*
Soil type (S)	2	0.846	9.48*
Diversity treatment (T)	2	3.412	38.25*
Y × S	8	0.322	3.61*
Y × T	8	0.267	2.99*
S × T	4	0.232	2.60*
Y × S × T	16	0.102	1.14
Residuals	135	0.089	

*$P < 0.001$.
Reported values are as follows: degrees of freedom (df), mean squares (MS), and F-statistics (F).

3.25% on the loam soil, which did not differ significantly from either other soil type. Weed biomass was higher in 2010 (during the early establishment of the site) than in 2011 and 2012. Weed biomass increased in 2013 and 2014 after flooding on the loam and clay soils (Fig. 2).

The significant treatment × soil type × year term for weed biomass (Table 1) was likely driven by severe flooding on the clay soil in 2013. On the clay soil in 2013, weed biomass was highest in the 16- and 32-species treatments (Fig. 2). In contrast, weed biomass was highest in the one-species treatment on the sand and loam soils in most years.

Bare ground

Percent bare ground differed between diversity treatments and years (Table 1). There was less bare ground in the 32-species treatment than in the one-, five-, and 16-species treatments (Fig. S3). There was significantly more bare ground in 2014 (85.8%) than in 2013 (18.7%) and significantly more bare ground in 2013 than in 2012 (13.1%). Percent bare ground was higher in 2014 because of the spring burn.

Differences in % bare ground between diversity treatments varied across years (treatment × year term, Table 1). In 2012 and 2014, % bare ground was lowest in the 16- and 32-species treatments but in 2013, % bare ground was lowest in the five-species treatment (Fig. S3). Percent bare ground was highest in the one-species treatment every year.

Species composition

The species composition of the five-, 16-, and 32-species treatments changed over the five-year study (Table 2, Fig. 3). The species composition of the 16- and 32-spe-
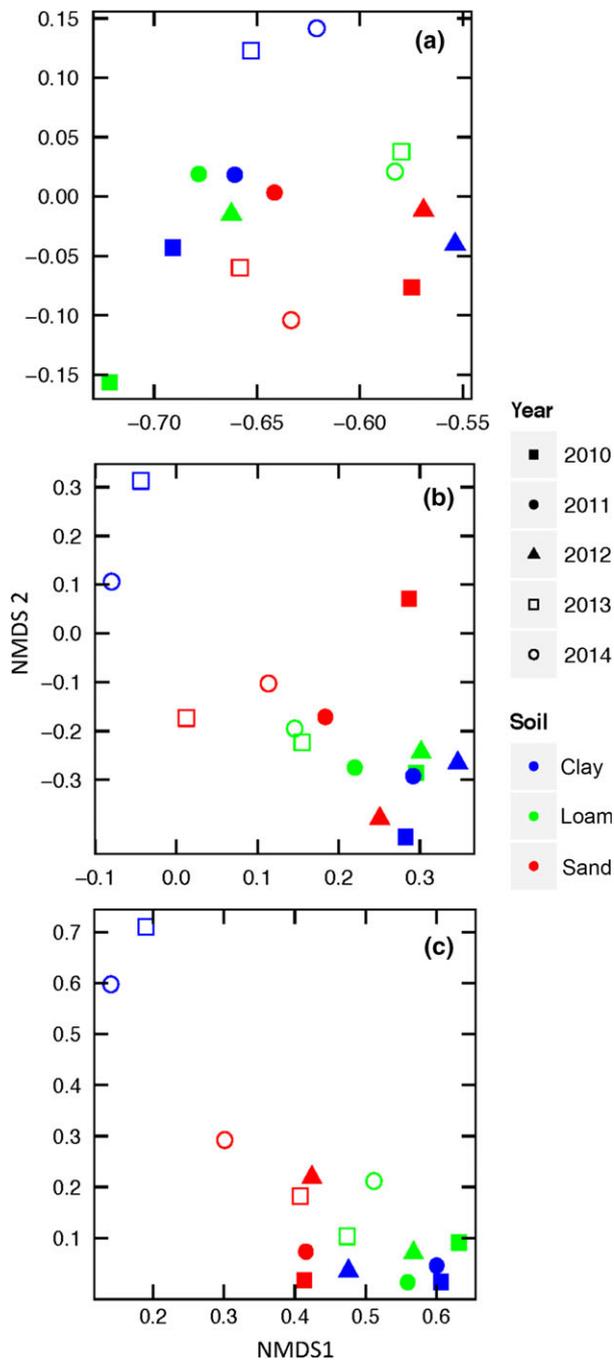
cies treatments also differed between soil types (Fig. 3). The most dramatic change in species composition occurred in the 16- and 32-species treatments on the clay soil after the flooding in 2013 (Fig. 3).

In the 16- and 32-species treatments, years in which *Andropogon gerardii* (big bluestem) and *Sorghastrum nutans* (Indian grass) had high basal area coverages were years with high productivity and years in which *Schizachyrium scoparium* (little bluestem) had high basal area coverage were years with low productivity (Fig. 4). The basal area coverages of *Desmodium canadense* (showy tick-trefoil) and *Heliopsis helianthoides* (oxeye sunflower) decreased after 2011. The basal area coverage of *Panicum virgatum* (switchgrass) increased after the flooding in 2013 (Fig. 4).
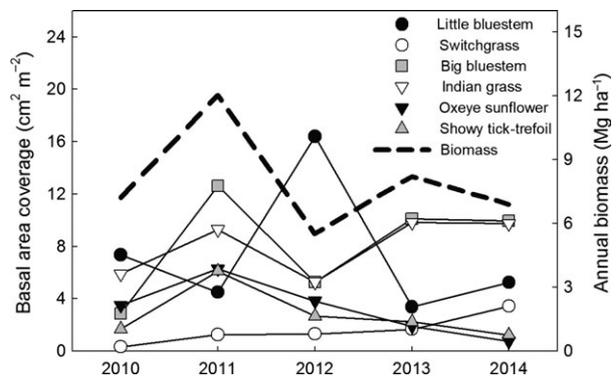
Discussion

Diversity–productivity experiments have helped foster the hypothesis that high-diversity prairie mixtures would be effective bioenergy crops (e.g., Tilman *et al.*, 2006). To test this hypothesis, we compared biomass production, year-to-year variation in biomass production, and resistance to weed invasion in four treatments of tallgrass prairie vegetation with different diversity. Our results indicate that high-diversity prairie mixtures produce the same amount of biomass as a switchgrass monoculture and are more resistant to weed invasion on a range of soil types. Collectively, these results support the conclusion that high-diversity prairie mixtures would be effective biomass feedstocks in the Midwestern United States. In contrast with the insurance effect (Yachi & Loreau, 1999; Hooper *et al.*, 2005), we found that year-to-year variation in biomass production was equal in all diversity treatments.

In contrast to other diversity–productivity experiments, biomass production did not increase with species

Figure 3 (a), (b), (c)

Fig. 4 Basal area of all species with coverages >5 cm^2 m^{-2}. Values represent the mean basal area coverage of species in the 16- and 32-species treatment plots on all three soil types. Mean annual biomass production of all treatment combinations is provided for reference.

Fig. 3 Nonmetric multidimensional scaling (NMDS) plot depicting changes in community composition in the five-, 16-, and 32-species treatments during the five-year study. The NMDS plot was separated by treatment for clarity: 5-species (a), 16-species (b), and 32-species (c) treatment. Soil type is represented with different colors (blue = clay; green = loam; red = sand), and year is represented by symbol. 2D-stress: 0.16, linear R^2: 0.981, nonmetric R^2: 0.926.

diversity in our study. The most likely reason for this distinction was that our seed mixes were specifically designed for their potential value as biomass feedstocks

whereas most diversity–productivity studies are based on random species assemblages (e.g., Hector *et al.*, 1999; Tilman *et al.*, 2006; Cardinale *et al.*, 2007). A synthesis of diversity–productivity experiments found that high-diversity mixtures often produce more biomass than monocultures on average (i.e., over-yielding is common), but rarely produce more biomass than the most productive monoculture (i.e., transgressive over-yielding is rare; Cardinale *et al.*, 2007). Because switchgrass is a highly productive monoculture (McLaughlin *et al.*, 1999), our experimental design was perhaps more consistent with a test of transgressive over-yielding. Based on this comparison, the equal productivities of the 16- and 32-species treatments and the switchgrass monoculture actually supports the value of these high-diversity mixtures for bioenergy (Sanderson *et al.*, 2004). The estimated yields of our high-diversity treatments (average = 7.8 Mg ha^{-1} yr^{-1}) were twice those reported for low input high-diversity prairies in the US Billion Ton Update (3.9 Mg ha^{-1} yr^{-1}) and are consistent with reported yields for unfertilized diverse prairies in Iowa (9.1 Mg ha^{-1} yr^{-1}; Jarchow & Liebman, 2013). The estimated yield of our switchgrass monocultures (8.24 Mg ha^{-1} yr^{-1}) was higher than those reported for unfertilized fields of the 'Cave in Rock' cultivar in southern Iowa (3.9 Mg ha^{-1}; Lemus *et al.*, 2008) and comparable to the average productivity of 20 fertilized switchgrass cultivars on fertile (CSR = 75) soils in southern Iowa (Lemus *et al.*, 2002).

Another factor that might have impacted our ability to detect a positive effect of diversity on productivity was the high nutrient content of our soils. Many diversity–productivity experiments are conducted on low nutrient soil (Lambers *et al.*, 2004; Tilman *et al.*, 2006, 2012; Fornara & Tilman, 2009; Isbell *et al.*, 2011; Jungers *et al.*, 2013), which increases the likelihood of detecting the benefits of niche differentiation and facilitation for

biomass production in high-diversity mixtures (e.g., Dybzinski et al., 2008). For example, reported values of initial soil nitrogen (N) at Cedar Creek Ecosystem Science Reserve, home of the Biodiversity II experiment, range from 0.09 to 1.1 g kg^{-1} (Wedin & Tilman, 1993) and 0.378 – 0.701 g kg^{-1} (Tilman, 1987). At the beginning of our study, total N in the surface soil (0–15 cm) was 2.13 g kg^{-1}, 2.00 g kg^{-1}, and 1.28 g kg^{-1} in the clay, loam, and sand soils, respectively (Sherrard et al., 2015). This indicates that our lowest N soils had ~16% higher N than the highest N soils at Cedar Creek Ecosystem Science Reserve. Because of our higher initial soil N content, it may take longer than five years for nutrient depletion to begin limiting productivity in the low-diversity treatments. Supporting this interpretation, long-term diversity–productivity studies have shown that the superior yields of high-diversity vs. low-diversity mixtures often become more pronounced with time (Cardinale et al., 2007; Fornara & Tilman, 2009).

Our results indicate that soil type influences the relative productivity of our biomass feedstocks, as the rank order of the four diversity treatments differed between soil types (treatment × soil type term, Table 1). Other diversity–productivity studies have noted that soil fertility can influence the relationship between species richness and productivity (Hooper et al., 2005; Balvanera et al., 2006; Ma et al., 2010), which might explain some of the variation observed in our study. In natural systems, low phosphorous/high potassium soils, such as our loam soil (Myers et al., 2015; Sherrard et al., 2015), tend to support communities of greater species richness (Janssens et al., 1998). This could explain the strong performance of our 32-species treatment on the loam soil (Fig. 1). From a management perspective, the contrasting performance of our four diversity treatments on different soil types suggests that seed mixes designed for bioenergy must be specifically tailored to the soil characteristics of a site for maximum productivity and stand success.

The five-species treatment performed poorly on all three soil types suggesting that a C$_4$ grass mixture is not an ideal biomass feedstock on marginal farmland in the Midwestern United States. Our results are consistent with Wilsey (2010), who used the same five-species mixture and found that it produced less biomass than switchgrass and big bluestem monocultures (nonsignificant trend). The low productivity of the five-species treatment in our study may have been caused by higher rates of N depletion in this treatment. In a concurrent study examining plant tissue N content, switchgrass plants in the five-species treatment had lower leaf N, lower photosynthesis, lower chlorophyll content, and lower capacity for light capture (FvFm) than switchgrass plants in other diversity treatments (Sherrard

et al., unpub ms). Also supporting this interpretation, the poor performance of the five-species treatment was most evident on the sand soil (Fig. 1), which had the lowest initial N content and the highest probability of ultimately becoming N deficient. The 16- and 32-species treatments contain legumes, which have likely slowed the rate of N depletion in these treatments. The switchgrass monoculture does not contain legumes, but big bluestem, Indian grass, and little bluestem all have faster rates of N uptake than switchgrass (Fargione & Tilman, 2006), which might account for a slower rate of N depletion in the monoculture. The low productivity of the five C$_4$ grass mixture is disappointing because Conservation Reserve Program (CRP) land in Iowa often has a similar species composition and has the potential to be a large existing source of biomass for bioenergy (Adler et al., 2009; Jungers et al., 2013).

Although we had two floods (2013 and 2014) and a drought year (2012) during the study period, our results did not support the hypothesis that high-diversity mixtures have more consistent annual yields than low-diversity mixtures (i.e., the insurance effect, Yachi & Loreau, 1999; Hooper et al., 2005). Instead, we detected a nonsignificant trend of higher year-to-year variation in biomass production with increasing diversity. Pfisterer & Schmid (2002) suggest that species-poor systems can be more resistant to disturbance than species-rich systems because they are statistically less likely to contain a species that will be greatly affected by disturbance and because the positive effects of niche differentiation may be minimized in disturbance years. Switchgrass is drought and flood tolerant, which could be why this treatment maintained the most consistent year-to-year biomass production in our study. Conversely, the 16- and 32-species treatments contained species that were less resistant to disturbance. The species composition of these treatments changed rapidly after the drought and floods at our site (Fig. 3), which likely influenced the productivity of these treatments. In terms of ecosystem services, our results suggest that high diversity does not necessarily ensure more consistent year-to-year production in biomass feedstocks. This is particularly true for feedstocks grown on marginal farmland in a floodplain.

Establishment time, annual precipitation, and changes in species composition may have contributed to year-to-year variation in biomass production during the five-year study. 2011 was the year in which biomass production was highest (Fig. S2) because there was high rainfall (Knapp & Smith, 2001), no flooding, and it was not during the early establishment of the site. Other years were less productive because they were either early in site establishment (2010), a drought year (2012), or a flood year (2013 and 2014). Changes in basal area

coverage of big bluestem and Indian grass may have influenced aboveground biomass production in the 16- and 32-species treatments (Fig. 4). Flooding on the clay soil in 2013 and 2014 reduced the abundance of these two highly productive species and likely reduced biomass production in these years. Oxeye sunflower and showy tick-trefoil are both early establishment species (Camill et al., 2004) and their decreasing abundance over the course of the study may be part of the reason that biomass production was higher in 2011 than in 2012–2014.

Our results suggest that weed biomass was influenced by variation in % bare ground, and to lesser extent, variation in soil N between treatment combinations. The one-species treatment had the highest % bare ground (Fig. S3), which likely contributed to higher weed biomass in this treatment (Fig. 2; Levine, 2000). In 2014, the five-species treatment had the same % bare ground as the one-species treatment but fewer weeds suggesting that bare ground was not the only factor influencing weed biomass at our site. Weed biomass may have been lower in the five-species treatment because there is less soil N to facilitate weed invasion in this treatment. This interpretation is consistent with our previous conclusion that efficient N uptake by other species in this diversity treatment (Fargione & Tilman, 2006) has accelerated the rate of N depletion. The presence of nitrogen-fixing legumes should make the 16- and 32-species treatments more vulnerable to weed invasion, but higher plant coverage in these treatments (Fig. S3) offsets this vulnerability. Weed invasion can reduce yield in bioenergy crops (Palmer & van der Maarel, 1995) because an increase in exotic species diversity does not have the same positive influence on productivity as an increase in native species diversity in tallgrass prairie systems (Isbell & Wilsey, 2011).

Management implications

For landowners interested solely in biomass production, our results suggest that a switchgrass monoculture is the best choice for a biomass feedstock. It has the lowest seed cost (one-species: \$158 ha^{-1}; five-species: \$282 ha^{-1}; 16-species: \$1643 ha^{-1}; 32-species: \$2354 ha^{-1}), it is productive on a variety of soils (Fig. 1), and it maintains consistent annual yields because of high resistance to disturbance. Two weaknesses of a switchgrass monoculture for bioenergy are that it is more susceptible to weed invasion (Fig. 2) and that it will likely require more fertilizer than high-diversity prairie bioenergy crops to maintain our reported yields. This study was conducted on relatively high N soil and not of sufficient length to showcase N depletion

in the one-species treatment but such an effect would likely occur with annual fall harvests.

For landowners interested in additional ecosystems services, the 16-species treatment would be the best choice. This mixture is highly productive and should maintain high yields with minimal fertilizer because of enhanced niche differentiation and facilitation (Loreau & Hector, 2001; Cardinale et al., 2007). This mixture provides better habitat for birds and pollinator resources for butterflies than a switchgrass monoculture (Myers et al., 2012, 2015) and annual post frost harvests should not affect the species and functional group composition (Jungers et al., 2013). For landowners that are particularly interested in ecosystem services, perhaps at the expense of some productivity, the 32-species treatment would be the best choice. This treatment would be a good candidate for multifunctional on farm use (e.g., the STRIPS program in Iowa - which integrates prairie strips with row crops in watersheds to reduce nutrient runoff and erosion, or, the Buffer Initiative in Minnesota). The additional diversity of this treatment should increase nutrient retention and provide even better habitat for wildlife (Myers et al., 2012, 2015). However, this mixture should not be planted at sites that flood frequently. Flooding alters the species composition of this treatment, which will reduce the diversity-based environmental benefits of the costly seed mix. For example, white wild indigo was the only legume that survived the 2013 and 2014 floods on the clay soil.

In our study, we used a site management strategy that maximized stand establishment and habitat value for wildlife. Establishment mowing and burning helps control weed abundance and fosters productivity in prairie restorations (Smith et al., 2010). Harvesting biomass in spring maintains fall and winter habitat for birds (Fargione et al., 2009) but reduces biomass yield relative to fall harvest. State, federal, and private landowners seeking to balance the provisioning of ecosystem services (e.g., wildlife habitat, soil and water conservation, and recreation) with economic returns would likely use a comparable management model. Consequently, our results might apply best to county-owned recreational land or CRP land (Adler et al., 2009).

Landowners that prioritize biomass production would likely use a different management strategy (e.g., no burning/complete, annual fall harvests immediately after stand establishment) resulting in different productivity, weed resistance, and wildlife benefit values than those reported in our study and in Myers et al. (2012, 2015). Sites that are not burned early in establishment would have more weed biomass than our research plots, but the differences between diversity treatments reported in our study (Fig. 2) would likely still persist because

switchgrass monocultures naturally provide more light to invading weeds than high-diversity prairie mixtures (Fig. S3). Although we hayed our site in spring, we estimated productivity from quadrats harvested in fall, and therefore, our data should provide a realistic estimate of fall biomass production values. However, ground-level hand clipping can overestimate harvestable biomass with field-scale baling, which leaves ~12 cm stubble (Zilverberg *et al.*, 2014). Future research at the site will include baling to examine the % reduction in biomass production across treatments. Fall harvests also remove more tissue N than spring harvests (Dohleman *et al.*, 2012), which would accelerate the rate of soil N depletion (particularly in biomass feedstocks that lack legumes) and ultimately reduce yield.

In conclusion, our results suggest that high-diversity mixtures of native prairie vegetation would be effective biomass feedstocks in the Midwestern United States. In comparison to one of the leading bioenergy crops in the United States (a switchgrass monoculture), these mixtures produce the same amount of aboveground biomass, display similar year-to-year consistency in their biomass production values, and are more resistant to weed invasion. Companion studies at our site suggest that high-diversity mixtures also provide better habitat and resources for wildlife (Myers *et al.*, 2012, 2015). Future research at the site will examine rates of belowground carbon sequestration, which could represent another significant advantage of high-diversity vs. low-diversity biomass feedstocks.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Rainfall patterns during the five-year study.

Figure S2. Average biomass production in each diversity treatment across soil types.

Figure S3. The percentage of bare ground in each diversity treatment.

Table S1. Species list and seeding rates of the four diversity treatments