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Research paper

Balancing biofuel production and biodiversity: Harvesting frequency effects on production and community composition in planted tallgrass prairie

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ABSTRACT

Native perennial grasslands have been proposed as a source of feedstocks for the production of second-generation lignocellulosic biofuels in the Midwestern USA. Although the consequences of some management decisions for biomass production and plant community composition are well understood (e.g. fertilization), less is known about the effects of harvesting frequency. We compared a once- and twice-annual harvesting regime at two restored prairies in southwestern Michigan established with identical seed mixtures as part of a large-scale bioenergy experiment. We determined biomass production and species composition in experimental plots and also measured the availability of light, inorganic nitrogen and soil moisture. The plant communities that established at the two sites differed markedly in composition and there was little evidence of convergence after five years. At the site dominated by warm-season C₄ grasses, single harvests generally produced more biomass than double harvests. By contrast, biomass production was unaffected by harvesting at the more diverse site. Contrary to our prediction that a summer harvest would increase diversity, we found small and subtle effects on plant community composition. This may be due in part to the timing of our harvest treatment. Our results suggest that a single, end-of-season harvest is the best practice for maximizing biomass production in prairies, especially at sites where warm-season grasses dominate. However, at more diverse sites, two harvests can produce the same total biomass and may support other beneficial ecosystem services. This study indicates that in the short term, double harvests are unlikely to affect plant species diversity or community composition in prairie plantings.

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1. Introduction

A number of recent papers have suggested that native perennial grasslands can be used as lignocellulosic feedstocks for the production of biofuel, especially on marginal lands [1–3]. Second-generation lignocellulosic biofuels are made by breaking down

plant fibrous biomass (i.e. leaves, stems, wood) into their component sugars, which are then turned into ethanol, typically by fermentation [4]. Unlike current generations of ethanol derived from corn grain, lignocellulosic biofuels rely on dedicated energy crops, such as native perennial grasses [4,5]. A number of experimental studies have shown that aboveground biomass production in grasslands is related to the relative abundance of highly productive species as well as species richness or diversity [6]. Although productivity of mixed-species perennial grasslands may not outperform the production of managed monocultures of traditional energy crops, they can provide important ecological services such as maintaining pollinator populations or reducing greenhouse gas

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emissions from soils [5,7,8]. Management practices such as fertilization and the frequency or timing of harvesting can affect productivity and community composition of grasslands, and so are likely to affect ecological services dependent on plant biodiversity or composition [9,10]. While the impact of fertilization on biomass production and composition/diversity has been well documented [11–13], relatively few studies have focused on how harvest frequency in grasslands will affect productivity and plant biodiversity in the context of bioenergy production.

Developing management for bioenergy grasslands to assure both consistent high biomass yield and maintenance of the plant community composition necessary for other ecosystem services will be challenging. Fertilization increases productivity, but typically results in declines in species diversity [11,13,14]. The negative effects of fertilization on diversity, however, might be offset by management that increases light availability [9,15,16]. In grasslands, grazing, fire and/or frequent harvests have been shown to prevent the accumulation of plant litter and/or reduce aboveground biomass, which increases light availability and allows certain functional groups (e.g. small-statured forbs) to persist where they might otherwise be excluded [17–19]. Thus, harvesting done early in the growing season could have a positive effect on the abundance of low-statured species, particularly forbs, by reducing the biomass of tall, warm-season, perennial grasses that often dominate grasslands planted for biofuel feedstock [20,21]. However, warm-season grasses may overcompensate for early removal of biomass with increased late-season production [22, 23], but see [24]. If so, then multiple harvests might result in higher total aboveground biomass and dominance by these species rather than an increase in plant diversity or forb abundance. Alternatively, multiple harvests could result in both outcomes: an increase in light allowing the persistence of short-statured species and enhanced regrowth in C_4 grasses due to the reduction of competition with cool-season C_3 grasses.

In Midwestern grasslands grown for lignocellulosic biofuels, the abundance of warm-season C_4 grasses will be a primary determinant of aboveground productivity and the magnitude of the response of productivity to fertilization [25–27]. Understanding the response of C_4 grasses to different harvesting regimes is therefore particularly important for revealing potential tradeoffs between managing for both productivity and species diversity in these systems. Most studies to date have found a single harvest in late fall maximizes the productivity of C_4 grasses relative to harvests at different times of year or higher frequencies [28–31]. The addition of a summer harvest, however, could be attractive to farmers for reasons unrelated to maximizing productivity. For example, in spring and early summer prior to anthesis of the C_4 grasses, prairie biomass is nutritious animal forage [32–34]. Increases in the abundance of subdominant forbs or legumes following summer harvests may also increase the suitability of plant communities for wildlife [35] or enhance pollinators and predatory insects [7].

In this paper we take advantage of a unique experiment begun by the Great Lakes Bioenergy Research Center (GLBRC) to compare performance of alternative bioenergy crops in large field trials. We used fields planted with a native prairie seed mixture at two sites in southwest Michigan using methods and agronomic practices likely to be implemented by commercial growers of biofuel feedstocks. The two sites had different land-use history and soil fertility, allowing us to test if these attributes affect responses to harvest. In this study, we address the following questions: (1) How does the frequency of harvesting affect total productivity and community composition at these sites? And (2) What is the relationship between species composition and response to harvesting? The answer to the latter question can only be inferred as we have not

manipulated species composition within the context of our experimental treatments, and with only two sites we have no replication of the site differences. Insights from this comparison, however, will be of use to guide prediction and modeling efforts if restored prairie communities are to be considered as a source for lignocellulosic biofuel production.

2. Materials and methods

2.1. Study sites

We conducted our study at two sites in southwestern Michigan – Lux Arbor (42°28'23" N, 85°26'50" W) and Marshall Farm (42°26'37" N, 85°18'34" W) – that were used in the establishment of biofuel cropping systems by GLBRC. Though managed in the same way for this experiment, the two sites differed in soil type, total soil resource pools and prior agricultural management (Table 1). In 2009, both sites were prepared for conversion to prairie with the application of a non-selective foliar herbicide and one year of no-till soybean cultivation (see [online supplementary material](#) for detailed agronomic information). The same mix of prairie species (Table 2) was sown into both sites in June 2010 using a no-till seed drill. The seed mix was selected to provide a balance between biomass production of high-yielding grass species, diversity of important tallgrass prairie functional groups and affordability for seeding large sites. Neither site was fertilized after planting. Annual mechanical harvest for biomass began in the fall of 2011, after the plants had time to establish.

Our study was conducted from 2012 to 2014. Growing season precipitation differed among the three years; in 2012 it was well below average, particularly from May–July, whereas 2013 and 2014 had more typical rainfall (Fig. S1). Drought conditions in 2012 ranged from abnormally dry to severe drought according to the National Drought Mitigation Center (<http://droughtmonitor.unl.edu/>). In the following years, by contrast, no drought conditions occurred during the growing season. We consider in our interpretation of the harvesting effects on production the influence of large inter-annual differences in precipitation.

2.2. Multiple harvest experimental design

To determine the effects of multiple harvests on biomass production and species composition, in 2012 we established a harvest frequency experiment at both sites. Six replicate blocks were placed at least 25 m apart in each site. Each block had two 5 × 5 m treatment plots, one of which was harvested twice each growing season (July & late September – double harvest treatment) and the other was harvested once (late September – single harvest treatment). These harvesting times were chosen to coincide with when farmers and ranchers in the North Central US might cut warm-season grasses for forage (summer, when grasses are in the early boot stage) and when harvests for biofuel feedstocks occur (fall) [29,32,34,36]. Our summer harvest falls within the range of times typical for haying pastures containing mixed warm-season grasses [37–40]. The fall experimental harvests took place one to two weeks before the mechanical harvest of the entire field. In each plot, a 0.5 × 2 m area in the plot center was harvested by hand, leaving stubble approximately 10 cm tall to mimic the biomass removal of mechanical harvests. The harvested biomass was sorted to species in the field, oven-dried and weighed. The remaining area of the treatment plot was cut to 10 cm height with a weed-whacker and raked to remove the biomass, also mimicking the effects of mechanical harvest.

The experimental harvests were done annually from 2012 to 2014; however, the location of the treatment blocks within the field

Table 1
Site characteristics of the two restored prairies used for the experimental harvesting study. Harvested yield is based on the whole-field mechanical harvest in 2014 (see Appendix S1). Total soil C and N were taken in 2009 prior to the start of the experiment; pH is from 2013. All other values were determined from the single harvest treatment in 2014. Where reported, values are means \pm 1 SEM (N = 6). See Table 3, as well as Tables S2 and S4 in online supporting materials for additional years and treatments.

	Lux Arbor	Marshall farm
Soil Type	Fine-loamy, mixed, mesic Typic Hapludalfs	Coarse-loamy, mixed, mesic Typic Hapludalfs
Area of Planted Prairie (ha)	13	11
Land use 1987–2008	row crops	CRP grassland
Harvested Yield (Mg/ha)	5.00	3.95
Total Soil C, 0–10 cm (g kg^{-1})	16.38 \pm 0.84	26.05 \pm 2.39
Total Soil N, 0–10 cm (g kg^{-1})	1.62 \pm 0.07	2.24 \pm 0.21
Soil pH	6.19 \pm 0.05	6.44 \pm 0.13
Soil Moisture, 0–12 cm (%)	30.9 \pm 0.9	14.4 \pm 2.3
Ammonium, 0–10 cm (g kg^{-1})	2.56 \pm 0.26	6.03 \pm 0.88
Nitrate, 0–10 cm (g kg^{-1})	0.16 \pm 0.08	0.33 \pm 0.11
Species Richness	12.3 \pm 1.0	13.8 \pm 0.8
Species Evenness (Simpson's $E_{1/D}$)	0.15 \pm 0.02	0.19 \pm 0.02
Proportion Planted Species by 2014	0.99 \pm 0.003	0.85 \pm 0.05
Proportion C ₄ Grasses	0.89 \pm 0.10	0.69 \pm 0.09

Table 2
Species added as seed to the prairie plantings at LAR and MF. The overall seeding rate was approximately 7.85 kg/ha; seeding rates for each species are presented below. Seventy-one percent of the mixture was made up of grasses with the remainder legumes and forbs. Nomenclature follows: Voss, E. G. & A. A. Reznicek (2012) Field Manual of Michigan Flora. University of Michigan Press, Ann Arbor, MI. Abbreviations were used in displaying ordination results (Online Supplementary Material).

Species	Abbrev.	Common name	Functional group	Seeding rate (kg ha^{-1})
<i>Andropogon gerardii</i>	ANOGE	Big Bluestem	C ₄ grass	0.56
<i>Elymus canadensis</i>	ELYCA	Canada Wild Rye	C ₃ grass	2.24
<i>Panicum virgatum</i>	PANVI	Switchgrass	C ₄ grass	0.56
<i>Schizachyrium scoparium</i>	SCHSC	Little Bluestem	C ₄ grass	1.12
<i>Sorghastrum nutans</i>	SOSNU	Indian Grass	C ₄ grass	1.12
<i>Asclepias syriaca</i>	ASCSY	Common Milkweed	forb	0.07
<i>Asclepias tuberosa</i>	ASCTU	Butterfly Milkweed	forb	0.04
<i>Symphyotrichum oolentangiense</i>	ASTAZ	Sky Blue Aster	forb	0.07
<i>Chamaecrista fasciculata</i>	CASFA	Partridge Pea	legume	0.28
<i>Coreopsis lanceolata</i>	CRLLA	Lance-leaf Coreopsis	forb	0.14
<i>Echinacea purpurea</i>	RUDPU	Purple Coneflower	forb	0.28
<i>Eryngium yuccifolium</i>	ERYYU	Rattlesnake Master	forb	0.21
<i>Heliopsis helianthoides</i>	HEFHE	False Sunflower	forb	0.28
<i>Monarda fistulosa</i>	MOAFI	Wild Bergamot	forb	0.07
<i>Penstemon digitalis</i>	PEEDI	Foxglove Beardtongue	forb	0.07
<i>Ratibida pinnata</i>	RATPI	Yellow Coneflower	forb	0.21
<i>Rudbeckia hirta</i>	RUDHU	Black-eyed Susan	forb	0.21
<i>Rudbeckia triloba</i>	RUDTR	Brown-eyed Susan	forb	0.17
<i>Verbena stricta</i>	VEBST	Hoary Vervain	forb	0.14

were moved each year to avoid repeated harvests. Replicate blocks were located in the same general location each year to maintain a similar distribution of blocks across the site. Relocation of the plots, rather than sampling the same area repeatedly, prevents us from making conclusions about the long-term effects of multiple vs. single harvest, but allowed us to evaluate the effects of harvesting treatment in three very different years. We are also able to judge the effects of different plant species' responses to disturbance or their interactions on the response of productivity to multiple harvests, rather than long-term effects that might build up over three years of two harvests (e.g. changes in soil resource availability). Thus, our double harvest treatment represents three independent replicates (years) of one multiple harvest rather than the cumulative response over three years.

To determine how a mid-summer harvest impacted short-term resource availability, we measured light, soil moisture and soil inorganic nitrogen in both treatments. Photosynthetically active radiation (PAR) was measured above and below the vegetation canopy of each treatment plot before and after harvests at midday on cloudless days with an AccuPAR LP-80 light wand (Decagon Devices; Pullman, WA, USA). We measured soil moisture in the field periodically with Hydrosense II probes (Campbell Scientific; Logan, UT, USA) inserted 12 cm deep. Soil samples of the upper 10 cm were

also taken to measure inorganic nitrogen. We performed KCl extractions and measured ammonium and nitrate ion concentrations via colorimetric continuous flow analysis with an ALPKEM Model 3550 Analytical Flow Solution IV analyzer (OI Analytical; College Station, TX, USA) [41].

2.3. Data analysis

Because community composition differed between the two sites and among years, we characterized variation in plant community composition using several different approaches. First, we examined patterns using non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarities in community composition with the *metaMDS* function of the 'vegan' R package [42]. Next, we tested the importance of site and year using permutational analysis of variance (PERMANOVA) models of the Bray-Curtis dissimilarities [43]. Lastly, we performed indicator species analysis with the *signassoc* function of the 'indicspecies' R package [44]. Interactions between site and year were highly significant, and plant communities at the two sites were very different. Therefore, we conducted the above ordination and PERMANOVA analyses within each site individually, as well as for each year within site, to explore the effects of a summer harvest on plant communities in the fall. For each site-

level NMDS analysis, we determined which species were responsible for the separation of plots in the ordination space by using the *envfit* function to evaluate linear relationships between individual species abundances and NMDS scores.

We analyzed differences in total productivity, productivity of specific life form groups (cool-season C₃ grass, warm-season C₄ grass, non-leguminous forb, legume), Simpson's evenness ($E_{1/D}$) [45], species richness and environmental measurements using ordinary standard least squares models with site, year, harvest frequency and their interactions as factors. Linear mixed-effects models (using packages 'lme4' and 'lmerTest') including a random term for block within year indicated that a significant amount of variance was never explained by the random factor, so standard linear models were used without a term for block. We checked for normality of residuals using Shapiro-Wilk tests and normal Q-Q plots. For the analyses of biomass within life-form groups, transformations (*n*-root) were applied in order to meet assumptions of linear models. All values presented in this manuscript have been back-transformed to the units of measurement for ease of interpretation. All analyses were performed in R 3.1.2.

3. Results

3.1. Differences in plant community trajectories by site

Although initiated from the same seed mixture and in the same year, prairies at Lux Arbor (LAR) and Marshall Farm (MF) differed dramatically in species richness, Simpson's evenness and plant community composition (Table 1, Fig 1). In the first year of the experiment (2012), species included in the prairie seed mixture ('planted species') generally made up 50–65% of the harvested biomass at both sites, although the sites differed in the abundance of planted species in subsequent years. At LAR the proportion of

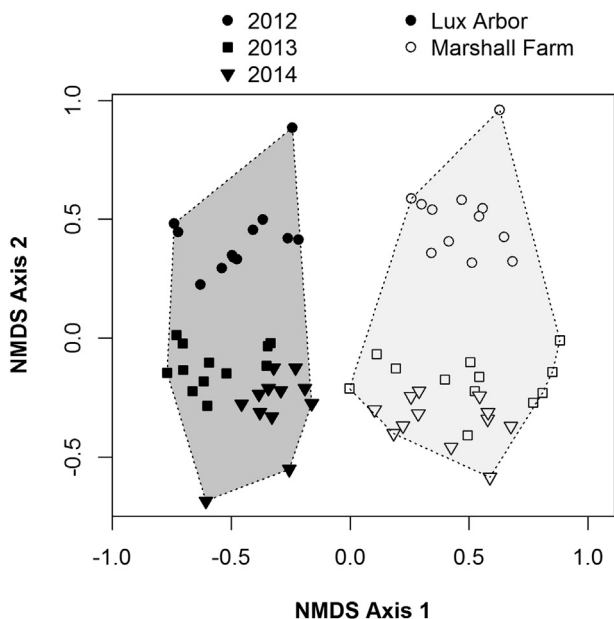


Fig. 1. Non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarity matrix of species composition and abundance illustrating differences in the prairie communities at LAR and MF sites and changes over time. Data from the summer and fall biomass data are combined for the plots harvested twice. Closed and open symbols differentiate the two sites, and symbol shapes represent years. The interaction between site and year was highly significant in a PERMANOVA ($F_{2,66} = 5.18$, $p < 0.001$). Final stress of the five-dimensional solution = 0.10 after 20 iterations. See Fig. S2 in online Supporting Information for a corresponding plot of species scores.

sown species increased to >90% by 2014, mostly due to the increasing abundance of warm-season C₄ grasses (Table 1). By contrast, the relative abundance of planted species increased more slowly at MF (Table S2).

Differences in plant community composition between the two sites persisted from 2012 to 2014, regardless of harvest treatment and despite successional change in species composition within each site (Fig 1). At both LAR and MF, composition in the drought year (2012) was distinct from composition in years with higher rainfall (2013, 2014). Removing the drought year (2012) from the PERMANOVA, however, still resulted in a model with a significant effect of year, and a site by year interaction ($p < 0.05$). Indicator species analysis revealed key species that distinguished the sites and exemplified the inter-annual changes (Table S3). Distinctive species at LAR in 2012 were mostly weedy grasses (*Lolium* and *Digitaria* spp.) whereas MF had higher frequencies of the planted perennial forb *Coreopsis lanceolata* and a few unplanted, weedy forbs and C₃ grasses. In later years, LAR was characterized by planted C₄ prairie grasses and *Chamaecrista fasciculata* (a planted, annual legume). A mixture of unplanted, non-native legumes and C₃ grasses distinguished MF in the later years (Table S3).

3.2. Harvesting effects on resources and productivity

As expected, harvesting significantly increased light availability, but this effect declined in the months following harvest (Table 3). There were no effects of harvesting on soil moisture or extractable ammonium concentrations, although there was a short-term increase in extractable nitrate following June harvests (Table S4). In both years when soil nitrate levels were measured, summer-harvested plots overall had nearly 50% higher nitrate levels compared to plots that were not harvested, although this difference was primarily driven by the strong response at MF (Table S4).

Despite similar changes in resource availability, the magnitude and direction of the harvesting effect on biomass production varied across sites and years (site * harvest freq * year: $F_{2,60} = 7.41$, $p = 0.001$). Consistent with drought conditions in 2012, biomass production in 2012 was significantly lower than in 2013 and 2014 (year: $F_{2,60} = 36.39$, $p < 0.001$). This depression in production due to drought was more evident at MF; LAR 2014 was not significantly more productive than 2012 (site * year: $F_{2,60} = 4.99$, $p = 0.009$). Despite these differences among years, harvest frequency did not affect biomass yield at MF. At LAR, plots that were harvested once (fall only) had higher total yield than those that were harvested twice in 2013 and 2014, but not 2012 (Fig 2). Dividing plants according to life form revealed differing biomass production responses to harvesting among C₃ grasses, C₄ grasses, forbs and

Table 3

Light availability, measured as the proportion of ambient PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$) at the soil surface, in plots assigned to the two harvest treatments over time. Multiple measures were taken in each plot; values are replicate means \pm SEM (N = 6). June light measurements were taken following the summer harvest (21–28 June 2013). September light measurements were taken prior to the fall harvests (18–25 Sept 2013 and 18 to 22 Sept 2014).

	Lux Arbor reserve		Marshall farm	
	Double harvest	Single harvest	Double harvest	Single harvest
2013				
June	0.22 \pm 0.03	0.26 \pm 0.01	0.24 \pm 0.04	0.28 \pm 0.03
July	0.69 \pm 0.03	0.17 \pm 0.02	0.82 \pm 0.02	0.11 \pm 0.02
August	0.30 \pm 0.02	0.09 \pm 0.01	0.35 \pm 0.03	0.16 \pm 0.03
September	0.22 \pm 0.06	0.10 \pm 0.02	0.14 \pm 0.03	0.12 \pm 0.03
2014				
July	0.63 \pm 0.02	0.21 \pm 0.02	0.68 \pm 0.02	0.08 \pm 0.02
August	0.35 \pm 0.14	0.19 \pm 0.08	0.63 \pm 0.26	0.15 \pm 0.06

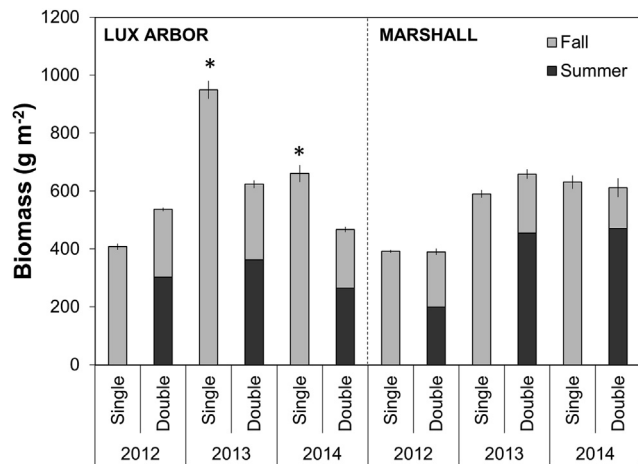


Fig. 2. Biomass production from the prairie communities at Lux Arbor and Marshall Farm by year and harvest frequency. In the double harvest treatment, the bar is divided to indicate biomass harvested in the summer (Late June to early July) and in the fall (September). A three-way interaction between site, year, harvest treatment was significant in an ANOVA ($F_{2,60} = 7.41$, $p = 0.001$). Asterisks above bars designate significant ($p < 0.05$) pairwise contrasts between the single and double harvest treatment within each site and year (p -values adjusted for a family of 6 tests with Dunn-Sidak's correction).

legumes (Fig. S3). C₃ grasses had higher biomass in plots harvested twice, and the opposite was true for C₄ grasses in the two typical rainfall years but not 2012 (Table S5). The biomass production of non-leguminous forbs was unaffected by harvest treatment, and legumes were highest overall in the plots harvested twice (Table S5).

3.3. Harvesting effects on diversity and community composition

To measure the effects of a summer harvest on plant community composition, we focused on the abundances of species and life-form groups in the fall. Because total biomass differed between treatments due to the removal of approximately half the plant material in June (Fig 2), we expressed abundance as the proportion of harvested biomass. Consistent with patterns in total biomass discussed previously, harvesting twice increased the proportional abundance of legumes in fall at both LAR and MF (Fig 3). In contrast, single harvests favored the relative abundance of C₄ grasses (Fig 3).

Species richness in the fall harvests was lower in the double harvest plots in 2013 and 2014, (freq * year: $F_{2,60} = 6.41$, $p = 0.003$), despite higher overall species richness (harvest freq: $F_{1,60} = 35.23$, $p < 0.001$; Table 4). Overall species evenness was reduced in the double harvest plots in 2012 at Lux Arbor, but unaffected in other years and at MF (site * year * freq: $F_{2,60} = 3.45$, $p = 0.038$; Table 4). In the fall harvests, species evenness also varied among sites, years and harvest treatments (site * year * freq: $F_{2,60} = 4.82$, $p = 0.011$; Table 4). Similar to the overall pattern, in the fall species evenness was lower in the double harvest plots in 2012 at LAR, but it was higher in 2013. At MF and LAR in 2014 species evenness was unaffected by harvesting.

There were subtle, yet significant effects of double harvests on community composition in the fall of 2012 at LAR, but not the two later years, (PERMANOVA freq. * year: $F_{2,35} = 7.09$, $p < 0.001$). In 2012 the plots that had been harvested in the summer had proportionally more *Sorghastrum nutans* in September, and those that had not been harvested had abundant *Lolium perenne* (Fig. S4). In later years, there was very little difference between the fall species composition of the single and double harvest treatments (Fig. S4). Unlike at LAR, there were no significant effects of a summer harvest

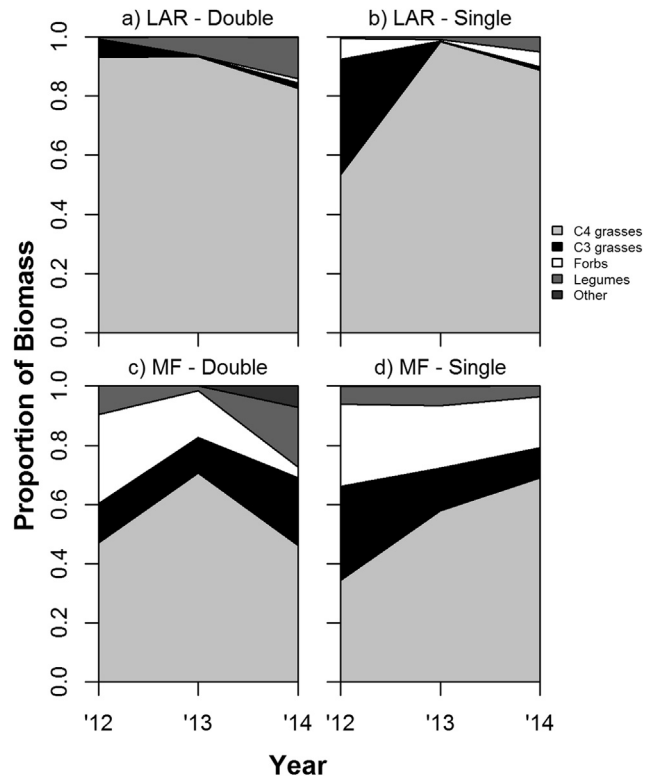


Fig. 3. Proportional abundance of different plant life-form groups in the September harvest, illustrating differences in community composition following a summer harvest (double harvest treatment) compared to plots that were not harvested in summer (single harvest treatment). LAR = Lux Arbor, MF = Marshall Farm.

on fall composition at MF. Despite this, there were some indicator species that were distinctive in the double harvest treatment at each site, even in years where there was no overall difference in composition (Table S6). *Chamaecrista fasciculata*, a native annual legume, was a significant indicator species in the double harvests at LAR in 2013. *Schizachyrium scoparium* distinguished double harvest treatments at both sites in 2014, and the presence of *Lolium perenne* in the fall was indicative of the single harvest treatment in two of the three years (Table S6).

4. Discussion

Although planted with the same seed mixture in the same year and managed using the same agronomic methods, the prairie communities that developed at these two sites were significantly different in species composition and diversity. Species composition and abundance changed over time in both sites, yet there was no evidence that communities were converging five years post-planting. These site differences may be due to persistent effects of past land use and/or site conditions (see Table 1) prior to the restoration plantings. Several studies have shown that past land use has strong and long-lasting effects on restoration outcomes, even relative to the effect of abiotic site conditions [46,47]. With only two sites, we cannot identify the exact mechanisms causing the observed differences in species composition and productivity at these two sites. Based on other studies and the observed higher proportion of unplanted species in one of our sites; however, it appears that prior land use will likely be an important determinant of both the overall productivity and diversity of sites planted to prairie for bioenergy and how these respond to harvesting. This highlights the challenges of making strong inferences about the

Table 4

Summary of plant community diversity metrics for each year and site (mean \pm SEM). Values for the double harvest treatment are presented with the summer and fall communities separate (i.e. biomass harvested in the summer vs. the fall) and for both harvests combined.

Site	Year	Harvest	Species evenness (Simpson's $E_{1/D}$)	Species richness
LUX	2012	Double - Combined	0.20 \pm 0.03	12.83 \pm 1.11
		Double – Summer	0.30 \pm 0.04	8.33 \pm 0.56
		Double – Fall	0.17 \pm 0.03	9.33 \pm 0.80
		Single - Fall	0.35 \pm 0.03	7.83 \pm 0.95
LUX	2013	Double - Combined	0.17 \pm 0.03	11.67 \pm 1.09
		Double – Summer	0.21 \pm 0.03	10.67 \pm 1.28
		Double – Fall	0.26 \pm 0.04	6.00 \pm 0.26
		Single - Fall	0.13 \pm 0.01	9.50 \pm 0.43
LUX	2014	Double - Combined	0.14 \pm 0.2	18.17 \pm 1.01
		Double – Summer	0.17 \pm 0.02	15.50 \pm 1.23
		Double – Fall	0.24 \pm 0.03	8.67 \pm 0.56
		Single - Fall	0.15 \pm 0.02	12.33 \pm 0.95
MF	2012	Double - Combined	0.39 \pm 0.07	18.67 \pm 1.48
		Double – Summer	0.45 \pm 0.08	12.17 \pm 0.79
		Double – Fall	0.32 \pm 0.04	15.33 \pm 1.41
		Single - Fall	0.33 \pm 0.04	16.50 \pm 0.85
MF	2013	Double - Combined	0.29 \pm 0.04	17.50 \pm 1.84
		Double – Summer	0.32 \pm 0.04	15.00 \pm 1.34
		Double – Fall	0.24 \pm 0.03	11.67 \pm 1.65
		Single - Fall	0.24 \pm 0.03	14.17 \pm 0.54
MF	2014	Double - Combined	0.19 \pm 0.02	17.50 \pm 1.84
		Double – Summer	0.20 \pm 0.02	15.50 \pm 0.99
		Double – Fall	0.26 \pm 0.04	9.67 \pm 0.92
		Single - Fall	0.19 \pm 0.02	13.83 \pm 0.79

impacts of different management on the production of lignocellulosic bioenergy crops [3,5,48]. Our results show that even sites in close geographic proximity with similar management are likely to develop distinct plant communities.

This variation in species composition between the sites and having repeated this experiment for three years does allow us to examine how/if different life-form groups respond to harvesting and provide insights into how species composition may impact the response to different harvesting regimes. For example, at LAR, where highly productive warm-season (C_4) grasses dominated, harvesting twice negatively affected total annual biomass production. However, at the more diverse MF site with higher forb and legume abundance, total biomass yields were similar from single (fall) and double (summer + fall) harvests.

4.1. Responses of C_4 versus C_3 grasses

We expected that C_4 grasses might respond positively to a summer harvest because of the increase in resources made available (light and soil nitrate in our study) by removing competition with cool-season (C_3) grasses and forbs just prior to the peak of C_4 grass growth. Previous studies have found that early spring fires encourage growth of C_4 relative to C_3 grasses, due to reduced light competition and nutrient conditions that are more favorable for C_4 species [49–51]. In contrast to our predictions, the total biomass of C_4 grasses in plots harvested twice did not exceed that produced in plots harvested once, and in fact was lower in all years but the first (2012). This is despite the increase in resources following harvest. Likewise, except for the first year, C_4 grasses made up an equivalent or lower percentage of the biomass compared to C_3 grasses in the fall harvest in both double and single harvest plots.

The response of plant communities to disturbances often depends upon the timing and magnitude of these events and how this interacts with resource supply and demand. The dates of our summer harvest (late June/early July) may have been late enough in the growing season that there was no advantage for C_4 species. Studies examining the impact of fire on grassland communities have found that the functional group responses depend on when a

site is burned. For example, prescribed burning that has been shown to increase C_4 grasses abundance typically occurs earlier in the spring, prior to the onset of C_4 growth [41,49,52–54]. Even though harvesting is not an exact replacement for fire [[55–57], but see [58]], timing can have large effects on the community and life-form group response [55,59]. In grasslands grown for bioenergy, a summer harvest (whether for forage or biofuel) is unlikely to have the same benefits for C_4 growth as an early spring burn, due to these differences in timing relative to plant growth.

4.2. Species diversity and community composition

The effect of summer harvest on species diversity and composition in these two sites were subtle, as might be expected in response to a single summer harvest event. Although overall species composition differed between the plots harvested once and twice, much of this was due to detecting a distinct plant community in the summer harvest, rather than a response of the remaining community to the increase in available light. This difference may be a result of small-statured and early-season species being detected in the summer harvest, and 'absent' from the fall harvest because they had senesced by September when all of the plots were harvested.

However, it seems that legumes did benefit from the removal of biomass in the summer, particularly *Chamaecrista fasciculata*, a native annual. In 2014, the smaller-statured C_4 grass *Schizachyrium scoparium* (little bluestem) was also more abundant at both sites in the fall following a summer harvest. Thus, individual species appeared to benefit from the removal of competition by tall dominant grasses, but without dramatic changes in subsequent richness or diversity. Other studies have found that legumes respond positively to increased cutting frequency and/or grazing [60–63], yet this response is likely dependent on regional context as well as disturbance intensity [10]. Additionally, a single summer harvest may not be enough to see effects on diversity or composition that have been observed elsewhere [55,64–66]. Jungers et al. [67], however, found no impact of a repeated annual harvest on plant species richness or diversity in conservation grasslands across

Minnesota, suggesting that some communities may be resistant to change.

4.3. Significance for restoration and management

The observed differences in species composition between our two study sites, despite their being established with the same methods, provides insights into how prior land use and site conditions can affect the trajectory of restoration. Variation in the outcome of grassland restoration is correlated with many different factors, from site history, to environmental attributes and the broader landscape context [46,47,68,69]. Working in a similar regional setting across southwestern Michigan, Grman, et al. [46,70] found in a comparison of 27 + restored tallgrass prairies that history (particularly site age) was often more important than local site conditions in determining current species composition and restoration outcomes. Although in our study we cannot separate the effects of landscape context, history and fertility on the restoration outcome/community composition, the differences in management prior to restoration (row-crop agriculture at LAR and conservation grassland at MF) in the decades preceding our study likely played a major role. Agricultural practices such as tillage, chemical use and the presence or absence of cover crops can have long-lived effects on the seedbank [71,72], and the recruitment of these non-sown species can contribute heavily to site-by-site differences in the outcome of sowing grassland plants as they did in our study [73]. A single year of no-till soybean cultivation was unlikely to remove the effects from decades of different management on the seedbank.

Given that the relative proportion of C₄ grass biomass largely predicts ethanol yield from diverse grasslands [27], our results suggest a single, late-season harvest will be best practice if maximizing biomass production is the primary objective, especially at sites where warm-season grasses are highly dominant (>90%). However, at more diverse sites, multiple harvests may result in similar total biomass production and biofuel yield, while providing other beneficial ecosystem services such as pollination or soil nitrogen retention (by increasing legumes). Farmers and land managers may choose to harvest bioenergy feedstocks twice for many reasons. Spring or summer harvests are useful livestock forage due to their higher nutrient content, and could allow farmers to maintain diverse land uses [32–34]. Refiners may also incentivize earlier cuttings to ensure a more continuous supply of feedstocks throughout the year, especially if storage options are limited [74,75].

5. Conclusions

The objectives of this study were to determine how the frequency of harvesting affects total productivity and community composition in native grasslands planted for biofuel and evaluate if differences in plant community composition could affect the responses of a site to harvesting. Overall we found no positive effects of combining a summer and fall harvest on total biomass production, although we only observed reduced production at a site with a high dominance of C₄ grasses (>90%). This negative effect on productivity was much less noticeable in a year where there was a significant regional drought, highlighting the importance of replicating our experiment in years with different conditions during the growing season. Our results also suggest that performing two annual harvests is unlikely to have major negative impacts on plant species diversity or community composition, at least in the short-term. The different responses to harvest we observed in this experiment suggests that sites planted with prairie species and managed for bioenergy production may offer farmers flexibility for use of their crop and adaptability to changing environments and markets.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.biombioe.2016.06.012>.

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