



Nitrogen fertilization increases diversity and productivity of prairie communities used for bioenergy

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Abstract

Using prairie biomass as a renewable source of energy may constitute an important opportunity to improve the environmental sustainability of managed land. To date, assessments of the feasibility of using prairies for bioenergy production have focused on marginal areas with low yield potential. Growing prairies on more fertile soil or with moderate levels of fertilization may be an effective means of increasing yields, but increased fertility often reduces plant community diversity. At a fertile site in central Iowa with high production potential, we tested the hypothesis that nitrogen fertilization would increase aboveground biomass production but would decrease diversity of prairies sown and managed for bioenergy production. Over a 3 year period (years 2–4 after seeding), we measured aboveground biomass after plant senescence and species and functional-group diversity in June and August for multispecies mixtures of prairie plants that received no fertilizer or 84 kg N ha⁻¹ year⁻¹. We found that nitrogen fertilization increased aboveground biomass production, but with or without fertilization, the prairies produced a substantial amount of biomass: averaging (\pm SE) 12.2 \pm 1.3 and 9.1 \pm 1.0 Mg ha⁻¹ in fertilized and unfertilized prairies, respectively. Unfertilized prairies had higher species diversity in June, whereas fertilized prairies had higher species diversity in August at the end of the study period. Functional-group diversity was almost always higher in fertilized prairies. Composition of unfertilized prairies was characterized by native C₄ grasses and legumes, whereas fertilized prairies were characterized by native C₃ grasses and forbs. Although most research has found that nitrogen fertilization reduces prairie diversity, our results indicate that early-spring nitrogen fertilization, when used with a postsenescence annual harvest, may increase prairie diversity. Managing prairies for bioenergy production, including the judicious use of fertilization, may be an effective means of increasing the amount of saleable products from managed lands while potentially increasing plant diversity.

Keywords: bioenergy, biofuel, functional-group diversity, grassland, nitrogen fertilization, nonmetric multidimensional scaling, point intercept sampling, prairie, species evenness, species richness

Received 7 April 2012; accepted 3 May 2012

Introduction

The use of perennial plant materials as a renewable source of energy may constitute an important opportunity to improve the environmental sustainability of managed land (Tilman *et al.*, 2009). Currently, the production of energy from agricultural products is primarily in the form of ethanol from corn grain in the United States, which used more than 45% of the domestic corn crop in 2011 (U.S. Department of Agriculture Economic Research Service (USDA ERS), 2012). Concomitantly, using corn grain to produce ethanol has promoted landscape simplification and homogenization through conversion of Conservation Reserve

Program grasslands to annual row crops (Secchi *et al.*, 2009) and has been implicated in increasing environmental damage, such as increased nitrate leaching into water bodies (Donner & Kucharik, 2008) and increased rates of soil erosion (Cox *et al.*, 2011). Collection and conversion of corn stalks and leaves (i.e., stover) and other grain-crop residues could foster the production of lignocellulosic-based biofuels without displacing food and feed crop production (U.S. DOE 2011), but this practice may be undesirable in many situations because of its potential to degrade soil carbon stocks, fertility, and long-term productivity (Linden *et al.*, 2000; Wilhelm *et al.*, 2007; Blanco-Canqui & Lal, 2009; Blanco-Canqui, 2010).

In contrast, perennial prairie vegetation has the potential to be used as a bioenergy feedstock that produces a substantial amount of biomass as well as numerous other ecosystem services. Although creating and managing prairies for bioenergy production are likely to result in prairie systems that differ from

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native or restored prairies in terms of plant community composition, these created prairies would provide numerous ecosystem services including more habitat for wildlife (Fargione *et al.*, 2009) and beneficial insects (Gardiner *et al.*, 2010) and decreased nitrogen, phosphorus, and sediment movement into water bodies (Helmert *et al.*, 2012). Placement of small areas of these prairie systems at strategic locations in landscapes dominated by annual crops may be especially important for maintaining agricultural productivity in the face of emerging soil conservation challenges related to a higher frequency of high intensity precipitation events (National Assessment Synthesis Team, 2001; Angel *et al.*, 2005; Helmert *et al.*, 2012) and from new crop pests, such as the soybean aphid (*Aphis glycines* Matsumura) (Gardiner *et al.*, 2009).

Assessments of the feasibility of using prairie plant biomass as a bioenergy feedstock, such as the U.S. Department of Energy's (U.S. DOE), 2011 "U.S. Billion-Ton Update," have focused on relatively low-yielding unfertilized prairie systems on marginal cropland, including the sand prairies at Cedar Creek Ecosystem Science Reserve (U.S. DOE 2011). Due to the low yields of the prairie systems analyzed, the authors of the "U.S. Billion-Ton Update" suggested that fertilized monocultures of switchgrass (*Panicum virgatum* L.) might be a more suitable bioenergy feedstock due to greater yields in the central Great Plains (U.S. DOE 2011). Using switchgrass as a bioenergy feedstock could provide a variety of ecosystem services not provided by annual row crops, including reduced nutrient pollution and soil erosion (Kort *et al.*, 1998; Parrish & Fike, 2005), but would not provide the increase in native plant and animal diversity that may be desirable for more sustainable bioenergy production (Robertson *et al.*, 2008).

Diversity can foster increases in the productivity of prairie communities. In a meta-analysis examining diversity-productivity relationships, Cardinale *et al.* (2007) found that in 79% of the studies the most diverse communities produced more biomass than the average of the component monocultures. There was no relationship between community diversity and productivity in 21% of the studies, but the most diverse communities never had lower productivity than the average of the component monocultures (Cardinale *et al.*, 2007). However, in managed systems, including those used for bioenergy feedstock production, it is desirable for diversity to increase productivity above the yield of the most productive species (i.e., transgressive overyielding), which occurred in 12% of the cases examined by Cardinale *et al.* (2007). In the remaining studies reviewed by Cardinale *et al.* (2007), yield of the most diverse systems did not differ from the most productive monoculture in 63% of

cases, and more diverse systems produced less biomass than the most productive monoculture in 25% of cases.

Fertilization, particularly nitrogen fertilization, is generally effective at increasing aboveground biomass production in most ecosystems, including prairies (Reich *et al.*, 2003; Suding *et al.*, 2005; LeBauer & Treseder, 2008), but often reduces diversity (Suding *et al.*, 2005; Michalet *et al.*, 2006) and shifts species compositions to those species that are better competitors for light rather than soil resources (Tilman, 1988). When grown in monocultures in nutrient-poor soil, relative growth rates of nonleguminous prairie seedlings were stimulated by nitrogen fertilization, with forbs having the strongest response followed by C₃ grasses then C₄ grasses, and legumes not consistently stimulated by nitrogen fertilization (Reich *et al.*, 2003). It may be expected, therefore, that forbs and C₃ grasses would become more abundant in prairies with nitrogen fertilization. C₃ grasses, particularly exotic C₃ grasses, often become dominant in fertilized prairies and grasslands (Wedin & Tilman, 1996; Suding *et al.*, 2005; Foster *et al.*, 2009; Pan *et al.*, 2010). Forbs are often a more minor component of reconstructed prairies whether fertilized or not (Baer *et al.*, 2002; Polley *et al.*, 2005; Foster *et al.*, 2009; Pan *et al.*, 2010), and legumes become less abundant with nitrogen fertilization (Jarchow & Liebman, 2012).

Shifts in the species composition of prairie communities can affect both bioenergy feedstock suitability and ecosystem services that are provided. The stiff and nutrient-poor stems of C₄ grasses have been found to be effective at reducing sediment, nitrogen, and phosphorus losses through surface runoff when incorporated into riparian buffers (Lee *et al.*, 1999) and have high theoretical maximum ethanol yields (Jarchow *et al.*, in press). C₃ grasses begin growth early in the spring (Jarchow & Liebman, 2012) when the potential for nitrate leaching is highest (Heggenstaller *et al.*, 2009), but have relatively low higher heating values and high ash concentrations (Jarchow *et al.*, in press), making them less desirable as bioenergy feedstocks. Forbs, including legumes, are important sources of food for wildlife and beneficial insects (Fiedler *et al.*, 2008; Fargione *et al.*, 2009).

In this study, we tested the hypothesis that nitrogen fertilization would increase the aboveground productivity of prairie systems managed for bioenergy production but would decrease diversity. We tested our hypothesis by measuring aboveground biomass production and characterizing plant community composition, including species and functional-group diversity, in fertilized and unfertilized prairie systems that were harvested annually late in the growing season, a harvest regime that has been recommended for herbaceous

bioenergy feedstock production (Adler *et al.*, 2006, U.S. DOE 2011).

Materials and methods

Study site and experimental design

We conducted the study in Boone County, IA, on the Iowa State University Agronomy and Agricultural Engineering Research Farm (41°55'N, 93°45'W). Soils at the site were primarily Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls). The 60 year mean growing season (April through November) precipitation near the site was 72 cm. Growing season precipitation in 2008, 2009, 2010, and 2011 was 115 cm, 75 cm, 116 cm, and 61 cm, respectively. Late-spring to early-summer flooding occurred in 2008 and summer flooding occurred in 2010.

Prior to initiation of the field experiment in 2008, the site was used for corn and soybean production and was planted with soybean in 2007. Soil sampling to 15 cm in November 2007 indicated mean soil pH was 6.7, mean organic matter concentration (via dry combustion analysis with a conversion factor of 1.724 from total carbon to organic matter [Schumacher, 2002]) was 51 g kg⁻¹, mean extractable phosphorus concentration (via Bray-1 procedure) was 11 mg kg⁻¹, and mean extractable potassium (via Mehlich-3 procedure) was 141 mg kg⁻¹. Because the prairie treatments discussed here were components of a larger bioenergy cropping-systems experiment, phosphorus and potassium were added in May 2008 to all treatments including the prairie treatments to ensure that sufficient phosphorus and potassium were available for annual-crop growth. Phosphorus was added at a rate of 78 kg P₂O₅ ha⁻¹ (34 kg P ha⁻¹). Potassium was added at a rate of 146 kg K₂O ha⁻¹ (122 kg K ha⁻¹).

Plots were 27 × 61 m and were arranged as a randomized complete block design with four replicates. Prairie treatments comprised a multispecies mixture that received no fertilizer or the same mixture fertilized with 84 kg N ha⁻¹ year⁻¹. Both prairie treatments were seeded with the same custom seed mix obtained from Prairie Moon Nursery (Table 1) (Winona, MN, USA), which contained 31 species, including C₃ and C₄ grasses and leguminous and nonleguminous forbs (hereafter legumes and forbs, respectively). All species were perennial and were sourced from within 240 km of Boone County, IA. The composition of the seed mix by weight was 12% C₃ grasses, 56% C₄ grasses, 8% legumes, and 24% forbs. The prairies were seeded on 19 May 2008 with a Truax[®] drill (FLEXII Series Grass Drill; Truax Company, Inc., New Hope, MN, USA). The fertilized prairie treatment received no fertilizer in the first year because we expected weeds to respond more strongly than prairie plants to fertilization during the establishment phase. Both prairie treatments were mowed on 14 July and 7 August 2008 for weed control and were not harvested for biomass in 2008. Nitrogen fertilizer was applied to the fertilized prairie treatment as ammonium nitrate (34% N) on 17 April 2009 and 29 March 2010, and as urea-ammonium nitrate (32% N) on 11 April 2011.

Data collection and analyses

Aboveground biomass was harvested after plant senescence in mid-October in 2009–2011. Biomass was first harvested with a self-propelled forage harvester (John Deere model 5830; John Deere Co., Moline, IL, USA) to a cut height of between 8 and 20 cm. Fresh weights of the biomass removed from the plots were determined in the field. A subsample of biomass from each plot was then taken for moisture determination, dried at 60 °C for at least 2 days, and weighed. The remaining above-ground biomass was determined by harvesting four 0.28 m² samples to ground height, drying the samples at 60 °C for at least 4 days, and weighing the samples. Biomass values for each experimental unit were calculated on the basis of summing machine- and hand-harvested samples.

To determine the composition of the fertilized and unfertilized prairies, the point intercept method (Jonasson, 1988) was used in mid-June and mid-August of 2009, 2010, and 2011. Eight 1 m² areas were sampled per plot at each sampling period. Two samples, with randomly determined locations that excluded the exterior 2 m of the plot, were taken in each of the four quadrants of the plot. At each sampling location, a pin was dropped 12 times for a total of 96 pin drops per plot. With each pin drop, we recorded the species identity and number of hits of all living plant tissue. Senesced plant tissue was not recorded. Plant cover was determined by dividing the number of hits by the total number of pin drops.

We used Simpson's diversity index, Simpson's evenness index, and species number per plot to characterize prairie species diversity. We also used Simpson's diversity index to characterize prairie functional-group diversity, which we divided into C₃ grasses, C₄ grasses, legumes, and forbs. We used Simpson's diversity index because it is less sensitive to rare species than other indices such as Shannon's diversity index. Of the 58 species found in the plots, only 15 species had percent covers greater than 1% at any of the sampling periods (Table S1), indicating that the remaining 48 species had relatively small effects on the functioning of the prairie treatments. Trees were excluded from our functional-group analysis, even though we found cottonwood (*Populus deltoides* Bartram ex Marsh.) seedlings in both the unfertilized and fertilized prairie plots, because they represented less than 0.1% plant cover on all sampling dates. Simpson's diversity (1/*D*) was calculated as the inverse of $D = \sum p_i^2$ where p_i is the proportional abundance of the *i*th species or functional group. Simpson's evenness was calculated by dividing Simpson's species diversity index by the species number.

We analyzed the aboveground biomass using repeated-measures analysis of variance that assessed differences among years with a compound symmetry covariance model (SAS release 9.2; SAS Institute, Cary, NC, USA). Species and functional-group diversity, species evenness, and species number were analyzed with repeated-measures ANOVA using an unstructured covariance model that accounted for the two levels of repeated measures (i.e., sampling twice per year over 3 years). Pairwise comparisons between unfertilized and fertilized prairie treatments were performed using contrasts. All values were considered significant at $P \leq 0.05$. Change in species diversity, evenness, and richness within the unfertilized

Table 1 Species list, functional-group identity, and seeding rates of prairie seeding mix. The same mix was used for the unfertilized and fertilized prairies

Species Latin name	Common name	Functional group	Seeding rate ¹ Seeds m ⁻²	% by weight
<i>Amorpha canescens</i>	Lead plant	Legume	8	1.0
<i>Andropogon gerardii</i>	Big bluestem	C ₄ grass	99	20.0
<i>Asclepias tuberosa</i>	Butterfly milkweed	Forb	5	2.5
<i>Aster novae-angliae</i>	New England aster	Forb	16	0.5
<i>Astragalus canadensis</i>	Canadian milk vetch	Legume	4	0.5
<i>Baptisia leucantha</i>	White wild indigo	Legume	2	2.0
<i>Dalea purpurea</i>	Purple prairie clover	Legume	19	2.5
<i>Desmanthus illinoensis</i>	Illinois bundleflower	Legume	3	1.5
<i>Desmodium canadense</i>	Showy ticktrefoil	Legume	4	1.5
<i>Echinacea pallida</i>	Purple prairie coneflower	Forb	8	3.0
<i>Elymus canadensis</i>	Canada wildrye	C ₃ grass	31	12.0
<i>Eryngium yuccifolium</i>	Rattlesnake master	Forb	6	1.5
<i>Helianthus laetiflorus</i>	Showy sunflower	Forb	5	0.7
<i>Helianthus maximiliani</i>	Maximilian's sunflower	Forb	5	0.7
<i>Heliopsis helianthoides</i>	Early sunflower	Forb	6	2.0
<i>Lespedeza capitata</i>	Round-head lespedeza	Legume	8	2.0
<i>Liatris pycnostachya</i>	Prairie blazing star	Forb	8	1.5
<i>Monarda fistulosa</i>	Wild bergamot	Forb	26	0.8
<i>Panicum virgatum</i>	Switchgrass	C ₄ grass	55	8.0
<i>Pycnanthemum virginianum</i>	Common mountain mint	Forb	33	0.3
<i>Ratibida pinnata</i>	Yellow coneflower	Forb	22	1.5
<i>Rudbeckia hirta</i>	Black-eyed Susan	Forb	46	1.0
<i>Schizachyrium scoparium</i>	Little bluestem	C ₄ grass	59	8.0
<i>Silphium integrifolium</i>	Rosin weed	Forb	0.3	0.5
<i>Silphium laciniatum</i>	Compass plant	Forb	0.5	1.5
<i>Solidago rigida</i>	Stiff goldenrod	Forb	20	1.0
<i>Sorghastrum nutans</i>	Indiangrass	C ₄ grass	119	20.0
<i>Sporobolus heterolepis</i>	Prairie dropseed	C ₄ grass	4	0.5
<i>Tradescantia ohimensis</i>	Ohio spiderwort	Forb	6	1.5
<i>Vernonia fasciculata</i>	Common ironweed	Forb	9	0.8
<i>Veronicastrum virginicum</i>	Culver's root	Forb	8	0.2

¹Estimated seeding rate. Grass seed weights are presented as pure live seed (PLS) amounts.

and fertilized prairies from June 2009 to August 2011 were assessed using regression analyses that included a first-order autoregressive [AR(1)] covariance model (SAS release 9.2; SAS Institute). We used nonmetric multidimensional scaling (NMS) to visualize the species compositions with Bray–Curtis distance measures in PC-ORD (McCune & Mefford, 2011). Data were square root transformed before NMS analysis to reduce the effects of the dominant species, which can have disproportionate effects without transformation (Faith *et al.*, 1987).

Results

The unfertilized prairie treatment produced 10.5, 9.6, and 7.2 Mg ha⁻¹ of aboveground biomass in 2009, 2010, and 2011, respectively, whereas the fertilized prairie treatment produced 13.0, 13.9, and 9.7 Mg ha⁻¹ of aboveground biomass in those years (Figure 1). Thus,

the fertilized prairie produced 24%, 44%, and 34% more aboveground biomass than the unfertilized prairie in 2009, 2010, and 2011, respectively ($P_{\text{treatment}} < 0.0001$, $P_{\text{year}} < 0.0001$, $P_{\text{treatment} \times \text{year}} = 0.008$).

Nitrogen fertilization had more complex effects on prairie diversity than on aboveground biomass production. Species richness was generally higher in the unfertilized prairie than the fertilized prairie ($P_{\text{treatment}} = 0.0005$), but the differences between treatments varied over the sampling period ($P_{\text{date}} = 0.061$, $P_{\text{year}} = 0.02$, $P_{\text{treatment} \times \text{date}} = 0.0009$, $P_{\text{treatment} \times \text{year}} = 0.18$, $P_{\text{treatment} \times \text{date} \times \text{year}} = 0.36$) (Figure 2a). Species richness was always higher in the unfertilized prairie than fertilized prairie in August of all years and was also higher in June 2011. Regressions of species richness from June 2009 to August 2011 indicated that there was no increase in richness for

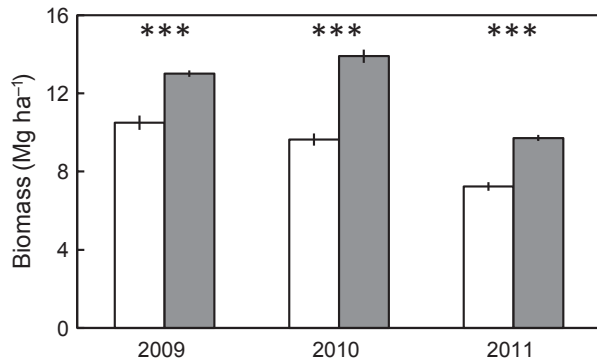


Fig. 1 Total aboveground biomass (\pm SE) of unfertilized (white) and fertilized (gray) prairies in 2009, 2010, and 2011 ($n = 4$). Asterisks above treatments indicate differences between treatments within years ($*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$).

either the unfertilized ($P = 0.14$) or fertilized ($P = 0.36$) prairies over the course of the study. Simpson's evenness differed between treatments ($P_{\text{treatment}} = 0.03$), between the June and August sampling dates ($P_{\text{date}} = 0.001$, $P_{\text{treatment} \times \text{date}} < 0.0001$), and among the 3 years of the study ($P_{\text{year}} = 0.03$, $P_{\text{treatment} \times \text{year}} = 0.14$) (Figure 2b). There was no difference in species evenness between treatments in June ($P = 0.20$). In August, however, species evenness was higher in the fertilized prairie ($P = 0.03$). Species evenness decreased from June 2009 to August 2011 in the unfertilized prairie ($P < 0.0001$), but showed no trend in the fertilized prairies ($P = 0.96$).

Because species diversity is a combination of species richness and evenness, diversity differed among the treatments ($P = 0.007$), but there were also strong interaction effects over the course of the study ($P_{\text{date}} = 0.01$, $P_{\text{year}} = 0.006$, $P_{\text{treatment} \times \text{date}} = 0.0006$, $P_{\text{treatment} \times \text{year}} = 0.002$, $P_{\text{treatment} \times \text{date} \times \text{year}} = 0.006$). Species diversity was always higher in the unfertilized prairie than the fertilized prairie in June (Figure 2c). In August, however, species diversity was higher in the unfertilized prairie in 2009 but became higher in the fertilized prairie by 2011 (Figure 2c). Species diversity in the unfertilized prairies decreased over the study period ($P < 0.0001$), whereas species diversity did not exhibit consistent trend in the fertilized prairies ($P = 0.58$). Only 15 species had percent cover values greater than 1% at any point during the study period and therefore were most important in affecting prairie composition (Table S1).

Prairie community composition was strongly affected by nitrogen fertilization (Figure 3). The three axes of the NMS ordination, which had a stress value of 6.3, separated out important changes in the prairie communities due to the effects of nitrogen fertilization, sampling date, and year. Axis 1 separated the data primarily

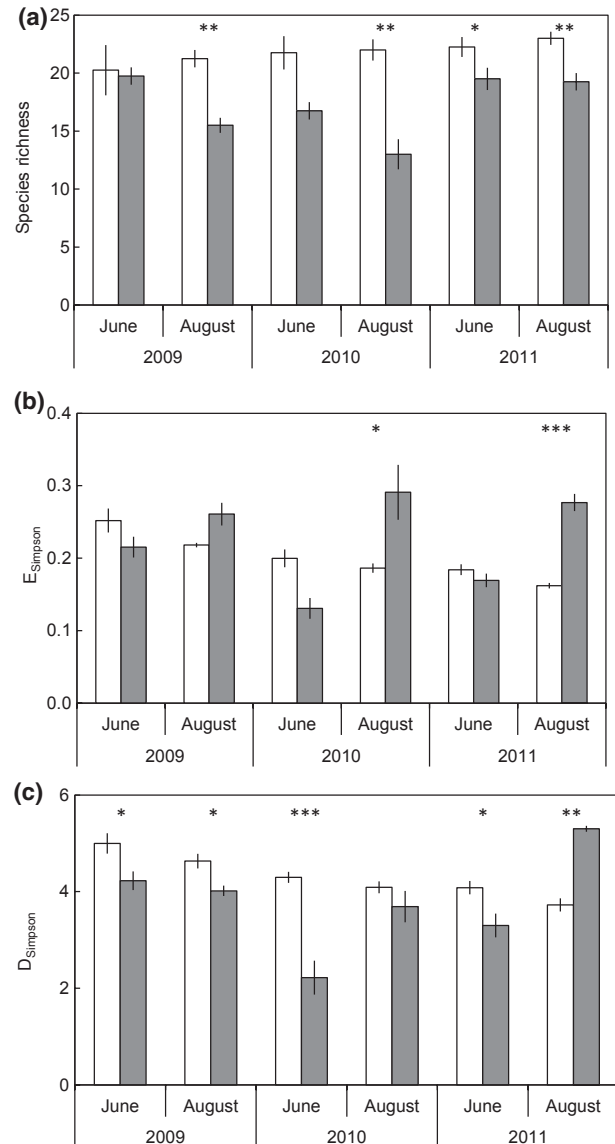
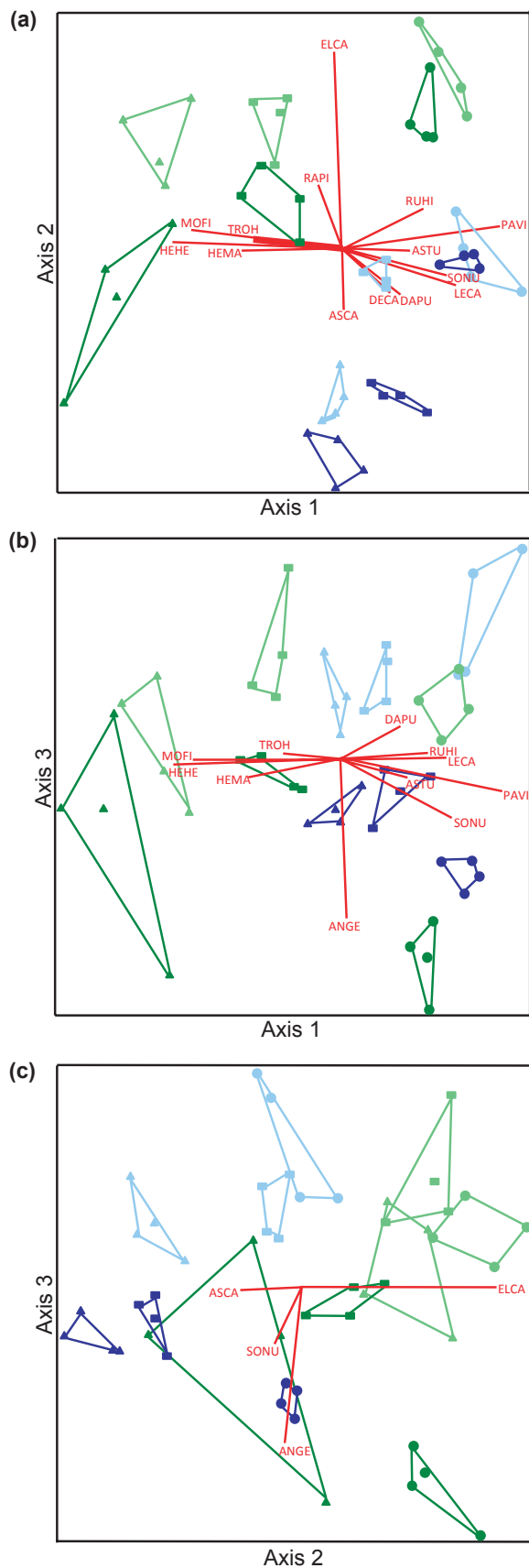


Fig. 2 Species diversity parameters (\pm SE) including species richness (a), Simpson's species evenness (b), and Simpson's species diversity ($1/D$) (c) of unfertilized (white) and fertilized (gray) prairies in June and August of 2009, 2010, and 2011 ($n = 4$). Asterisks above treatments indicate differences between treatments within years and sampling date ($*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$).

based on the abundance of forbs (to the left) and some C_4 grasses and legumes (to the right) (Figure 3a). The unfertilized prairie samples were clustered in the center and right on Axis 1 due to the greater abundance of C_4 grasses and legumes. The fertilized prairie samples spanned Axis 1, and moved from right to left from 2009 to 2011 as the prairie became more dominated by forbs such as oxeye sunflower (*Heliopsis helianthoides* (L.) Sweet) and bergamot (*Monarda fistulosa* L.).



The abundance of Canada wildrye (*Elymus canadensis* L.), a C_3 grass, was the vector most important for separating samples on Axis 2, and it was negatively associated with Canadian milkvetch (*Astragalus canadensis* L.), a legume. The unfertilized prairie generally had lower abundances of Canada wildrye than the fertilized prairie and higher abundances of Canadian milkvetch. The abundance of Canada wildrye was generally higher in June than August and generally decreased from 2009 to 2011 for both the unfertilized and fertilized prairies (Figure 3a). The presence of legumes, including round-head lespedeza (*Lespedeza capitata* Michx.), purple prairie clover (*Dalea purpurea* Vent.), and showy ticktrefoil (*Desmodium canadense* (L.) DC.), had a strong effect between Axes 1 and 2 (to the lower left), and the unfertilized prairie samples were clustered in this region (Figure 3a).

Axis 3 separated the June from the August samples based on plants that were most abundant early (to the top) or late (to the bottom) in the growing season (Figure 3a,b). The presence of big bluestem (*Andropogon gerardii* Vitman), a C_4 grass, was most strongly associated with the samples in August.

As demonstrated by the NMS ordination, the functional-group composition of the unfertilized and fertilized prairies differed greatly ($P_{\text{treatment}} = 0.0002$), and there were strong interactive effects over the course of the study ($P_{\text{date}} = 0.07$, $P_{\text{year}} = 0.01$, $P_{\text{treatment} \times \text{date}} = 0.003$, $P_{\text{treatment} \times \text{year}} < 0.0001$, $P_{\text{treatment} \times \text{date} \times \text{year}} = 0.001$) (Figure 4). Functional-group diversity, in contrast to species diversity, was higher in the fertilized prairie than unfertilized prairie for all dates except June 2010 (Figure 5). Functional-group evenness was lower in the unfertilized prairie because they consistently were dominated by C_4 grasses (Figure 4). In the unfertilized prairie, C_4 grasses comprised 62%, 57%, and 69% of the vegetation cover in June 2009, 2010, and 2011, respectively, and 70%, 77%, and 77% of the cover in August of those years. The fertilized prairie had more equal

Fig. 3 Nonmetric multidimensional scaling (NMS) ordination of unfertilized (blue hues) and fertilized (green hues) prairie communities in June (less saturated colors) and August (more saturated colors) in 2009 (circles), 2010 (squares), and 2011 (triangles). The ordination is three dimensional and is presented as Axes 1 and 2 (a), Axes 1 and 3 (b), and Axes 2 and 3 (c). Biplot vectors shown have R^2 values of at least 0.25. Species codes used are ANGE *Andropogon gerardii*, ASCA *Astragalus canadensis*, ASTU *Asclepias tuberosa*, DAPU *Dalea purpurea*, DECA *Desmodium canadense*, ELCA *Elymus canadensis*, HEMA *Helianthus maximiliani*, HEHE *Heliopsis helianthoides*, LECA *Lespedeza capitata*, MOFI *Monarda fistulosa*, PAVI *Panicum virgatum*, RAPI *Ratibida pinnata*, RUHI *Rudbeckia hirta*, SONU *Sorghastrum nutans*, and TROH *Tradescantia ohiensis*.

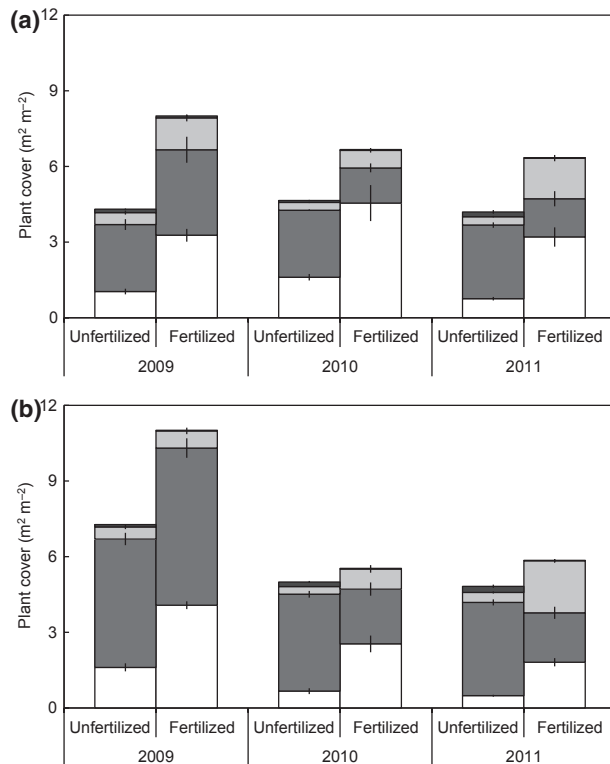


Fig. 4 Plant cover (\pm SE) of C_3 grasses (white), C_4 grasses (dark gray), forbs (light gray), and legumes (black) in the unfertilized and fertilized prairies in June (a) and August (b) of 2009, 2010, and 2011 ($n = 4$).

abundances among C_3 grasses, C_4 grasses, and forbs, although legumes comprised less than 1% of the fertilized prairie throughout the study period (Figure 4). By 2011, the composition of the fertilized prairie treatment was 51% C_3 grasses, 24% C_4 grasses, and 25% forbs in June, and 31% C_3 grasses, 33% C_4 grasses, and 35% forbs in August.

Discussion

As we expected, aboveground biomass production was higher in fertilized prairies. Nitrogen fertilization increased aboveground biomass production by 24%–44%, which is within the range of stimulation due to fertilization found in other studies. Turner *et al.* (1997) reported that in native tallgrass prairie in eastern Kansas, fertilization at a rate of $100 \text{ kg N ha}^{-1} \text{ year}^{-1}$ increased aboveground biomass 57% in annually burned sites, which were more nitrogen limited, and 15% in unburned sites, which were less nitrogen limited. Nitrogen fertilization at $100 \text{ kg N ha}^{-1} \text{ year}^{-1}$ increased aboveground biomass production by >50% in 1–3 year-old reconstructed prairies in southern Minnesota, and by ~40% in prairies older than 3 years (Camill *et al.*, 2004).

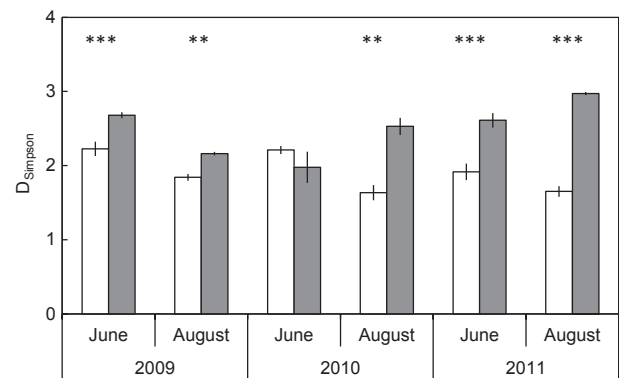


Fig. 5 Simpson's functional-group diversity ($1/D$) of unfertilized (white) and fertilized (gray) prairies in June and August of 2009, 2010, and 2011 ($n = 4$). Asterisks above treatments indicate differences between treatments within years and sampling date (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

With or without nitrogen fertilization, the prairies grown in the present study produced a substantial amount of aboveground biomass, with a mean of 12.2 Mg ha^{-1} for the fertilized treatment and a mean of 9.1 Mg ha^{-1} for the unfertilized treatment. Therefore, the fertilized prairie observed in the present study produced substantially more aboveground biomass than the perennial systems referred to in the "U.S. Billion-Ton Update": more than three times that of sand prairies (3.9 Mg ha^{-1}), more than double that of 'managed native prairie' (5.6 Mg ha^{-1}), and 9% more than the upland switchgrass forage variety 'Shawnee' (11.2 Mg ha^{-1}) (U.S. DOE 2011). The unfertilized prairie observed in our study, while producing less aboveground biomass than the fertilized prairie, still produced considerably more than the estimates of prairie biomass used in the "U.S. Billion-Ton Update". Our results suggest that prairies have the potential to be a more productive biofuel feedstock than previously stated.

Nitrogen fertilization, in contrast to our hypothesis and the findings of other researchers, did not consistently reduce prairie diversity. The unfertilized prairie had higher species diversity than the fertilized prairie in June due to small increases in both species richness and evenness. Species diversity was higher in the fertilized prairie in August 2011, however, due to greater evenness. Functional-group diversity was also almost always higher in the fertilized prairie than the unfertilized prairie in both June and August due to increased functional-group evenness even though the legumes never comprised more than 1% of the plant cover in the fertilized prairie.

A decline in species diversity with nitrogen fertilization is often associated with increased dominance by C_3

grass species, often exotics, under a variety of management regimes, including annual burning, annual mowing, and no aboveground biomass removal (Wedin & Tilman, 1996; Suding *et al.*, 2005; Pan *et al.*, 2010). We observed very low (< 0.1%) abundance of exotic C₃ grasses, and although native C₃ grasses were more dominant in the fertilized than unfertilized prairie in our study, C₃ grass abundance did not increase and generally decreased as the study progressed. Forbs, such as oxeye sunflower and bergamot, became more abundant in the fertilized prairie, which is also in contrast to other studies, which have found forb abundance to either remain constant or decrease with nitrogen fertilization (Patrick *et al.*, 2008; Pan *et al.*, 2010).

The timing of the application of nitrogen fertilizer is likely to influence its effects on species and functional-group diversity. In managing our prairies for bioenergy production, we applied nitrogen in a single dose in early spring. The timing of nitrogen fertilization varied among other studies, including multiple applications during the growing season (Wedin & Tilman, 1996) and application as a slow-release fertilizer (Patrick *et al.*, 2008; Pan *et al.*, 2010), but consistently resulted in decreased diversity.

The marked increase in forb abundance with nitrogen fertilization in prairies managed with an annual, postsenescence harvest is a unique aspect of our results relative to other studies. We hypothesize that the combination of management strategies used here resulted in increased forb abundance. Spring nitrogen fertilization stimulated C₃ grass growth, which accelerated C₃ grass phenology and dominance, thereby reducing the growth of C₄ grasses (Jarchow & Liebman, 2012). Because the prairies were not harvested within the growing season, the fertilized C₃ grasses flowered and began senescence earlier in the growing season than unfertilized C₃ grasses, as evidenced by their decreased abundance in August compared with June (Figure 4). The forbs may have had sufficient light resources later in the growing season to become better established in the fertilized prairie and therefore become more abundant over the course of the study, which has been found to be an important factor in enhancing forb abundance in other prairies (Williams *et al.*, 2007). Alternatively, the native C₃ grasses used in this study may be less aggressive than the exotic C₃ grasses that become dominant in other prairies. Additional research examining the probable interactive effects of nitrogen fertilization and the timing of biomass harvest on prairie composition is necessary.

Prairie community composition is not static. Compositional changes have been found to be greatest in the first year of prairie establishment, however, and become

much smaller after 3 years (Camill *et al.*, 2004). We have presented data from years 2 to 4 after prairie establishment. We recognize that long-term monitoring of the effects of nitrogen fertilization and annual harvest on the prairie systems described here is necessary to more completely assess community dynamics. Furthermore, additional research is needed to determine if similar results are found for prairies that are seeded into sites, such as old fields, that have greater perennial weed seed and bud banks; for prairies that are seeded with a different seed mix; and for established prairies that are subsequently fertilized.

Nonetheless, most research has found that nitrogen fertilization reduces prairie diversity, but we observed that early-spring nitrogen fertilization, when used in combination with a postsenescence annual harvest, may increase prairie diversity by increasing species and functional-group evenness. Furthermore, we have found that fertilized prairie can produce a substantial amount of aboveground biomass, similar to the productivity of monocultures of fertilized switchgrass. Consequently, we believe that managing prairies for bioenergy production, including the judicious use of nitrogen fertilization, may be an effective means of increasing the amount of saleable biomass from managed lands while also increasing plant diversity.

Acknowledgements

Funding for this research was provided by the ConocoPhillips Company, Iowa State University's College of Agriculture and Life Sciences, a research training fellowship from Iowa State University's Department of Agronomy, a graduate fellowship from Iowa State University's Plant Sciences Institute, and a grant from the U.S. EPA's National Center for Environmental Research (NCER) STAR Program. We thank David Sundberg, Shane Bugeja, Madeline Tomka, Brent Beelner, and Céline Guignard for technical assistance in the field and Brian Wilsey and Jon Hobbs for assistance with data analyses and revisions of the manuscript.

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Supporting Information

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

Table S1. Mean percent cover (SE) of all species with percent covers $\geq 1\%$ on any sampling date from June 2009 to August 2011 for the unfertilized and fertilized prairies.

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