

Butterfly response to floral resources during early establishment at a heterogeneous prairie biomass production site in Iowa, USA

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Abstract In the Midwestern USA, current biofuel production systems rely on high input monoculture crops that do little to support native biodiversity. The University of Northern Iowa's Tallgrass Prairie Center is investigating the feasibility of cultivating and harvesting diverse mixes of native prairie vegetation for use as a sustainable biofuel in a manner that also conserves biodiversity and protects soil and water resources. In 2009, we established 48 research plots on three soil types at an Iowa site with a uniform history of row crop production. We seeded each plot with one of four treatments of native prairie vegetation: (1) switchgrass monoculture, (2) warm-season grass mix (5 grass species), (3) biomass mix (16 species of grasses, legumes, and forbs), or (4) prairie mix (32 species of grasses, legumes, forbs, and sedges). In 2010, we measured vegetation characteristics and studied butterfly use of the plots to investigate the hypothesis that more diverse plant communities would support a greater abundance and diversity of butterflies. Habitat characteristics varied significantly among the plots by treatment and soil type, and butterflies responded rapidly to variation in floral abundance and richness. Averaged over the entire growing season, butterflies were six times more abundant and twice as species rich in the biomass and prairie mix plots compared to the warm-season grass and switchgrass plots. Our results suggest that implementation of biomass production using diverse mixes of native prairie vegetation on

marginal lands could have positive effects on the maintenance of butterfly populations in agricultural landscapes.

Keywords Bioenergy · Community ecology · Grassland restoration · Lepidoptera · Species richness

Introduction

The destruction, degradation, and fragmentation of grassland habitats has triggered significant butterfly population declines and range contractions throughout Europe (Krauss et al. 2010; Polus et al. 2007; Stefanescu et al. 2010; Thomas et al. 2004) and North America (Forister et al. 2010; Swengel et al. 2010). In the Midwestern USA, the conversion of the native tallgrass prairie ecosystem to row crop agriculture over the past 150 years has been described as one of the most rapid and complete ecological transformations in human history (Samson and Knopf 1994; Mutel 2007; Smith 1998) and has resulted in significant declines in prairie butterfly populations (Swengel et al. 2010). In Iowa, the USA's top producer of corn (*Zea mays*) and soybeans (*Glycine max*), the current agricultural landscape is dominated by row crop monocultures, and more than 99% of the state's tallgrass prairie habitat has been destroyed (Samson and Knopf 1994). In this context, butterfly populations persist in fragmented prairie remnants (Davis et al. 2008; Davis et al. 2007; Vogel et al. 2007), in linear grassland habitats along crop fields, fence rows, roadsides (Ries et al. 2001), and waterways (Reeder et al. 2005; Davros et al. 2006), and in prairie reconstructions (Shepherd and Debinski 2005). These currently available grassland habitats are small, isolated, and generally of lower quality (i.e., less floristically diverse, subject to exotic and woody plant invasions, possessing altered

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disturbance regimes, etc.) compared to intact prairies. Similar habitat conditions for butterflies are likely to persist in the region into the future in the absence of significant changes to current agricultural practices.

One possible development that could trigger a future increase in the amount and quality of grassland habitat in Iowa's landscape would be the large-scale cultivation of native prairie polycultures for use as a lignocellulosic bioenergy feedstock (Fargione et al. 2010; Hill 2009). Biofuel crops, which capture contemporary solar energy during photosynthesis and convert it to harvestable biomass that can be incinerated, converted to liquid fuels, or gasified, are widely viewed as being part of the solution to the global energy challenge (Hill 2009; Koh and Ghazoul 2008). In the Midwestern USA, current biofuel cropping systems produce liquid fuels from food crops grown in monoculture, primarily ethanol from corn but also biodiesel from soybeans (Hill et al. 2006). However, numerous authors have suggested that cultivating low-input high-diversity grassland biomass could have both significant energetic and environmental advantages over corn-based ethanol (Hill 2009; Tilman et al. 2006). From a biodiversity conservation perspective, such cultivation methods would likely provide greatly improved habitat conditions for wildlife (Dale et al. 2010; Fargione et al. 2009; Hill 2009), including butterflies and other beneficial arthropods (Isaacs et al. 2009; Landis and Werling 2010).

While numerous ecological studies have explored relationships between grassland plant and arthropod species richness (Andow 1991; Hawkins and Porter 2003; Knops et al. 1999; Koricheva et al. 2000; Perner et al. 2005; Siemann 1998; Siemann et al. 1998;), few have attempted to do so in the context of modern biofuel production, and those few have compared arthropod abundance and diversity among various candidate biofuel crops (Gardiner et al. 2010; Semere and Slater 2007) without directly manipulating plant diversity or controlling for potentially confounding factors such as site management history or soil type. Likewise, while there is a substantial literature on arthropod community dynamics in response to grassland restoration or abandonment (Stefanescu et al. 2009; Steffan-Dewenter and Tscharrntke 1997; Steffan-Dewenter and Tscharrntke 2001; Summerville et al. 2006), we are aware of no studies that have looked specifically at arthropod response to the establishment of native lignocellulosic biofuel crops in areas previously managed for row crop monocultures.

Here we present the results of a study of butterfly and plant communities during early establishment of a heterogeneous prairie biomass production site in Iowa, USA. In 2009, research plots on a site with a 20 year history of row crop production were seeded with one, five, 16, or 32 species of native prairie vegetation. In 2010, the second growing season after seeding and the first where seeded

native perennials dominated the plant community, we monitored the butterfly and plant communities in the biomass production plots. We chose to study butterflies for several reasons: (1) they are vagile and respond rapidly to environmental change and were thus likely to colonize the site during early establishment, (2) they respond to plant diversity due to their requirements for larval host plants and adult nectar sources, and (3) they serve as indicators of biodiversity and ecosystem health (Ries et al. 2001; Scott 1992; Shepherd and Debinski 2005; Stefanescu et al. 2009). We sought to address the following research questions:

1. How do vegetation structural characteristics and the availability of floral resources vary among four candidate prairie biofuel crops? Are there differences in butterfly abundance, species richness, or community composition among the four treatments?
2. Are there positive relationships between the abundance of floral resources and the abundance of butterflies or between the number of forb species in bloom and the number of butterfly species occurring in biomass production plots?
3. In plots established with identical management practices on different soil types, how does soil influence vegetation characteristics and consequently butterfly abundance, richness, and community composition?

Methods

Study area and design

We conducted our research at a 40 ha site located in the Cedar River Natural Resource Area in southeastern Black Hawk County, Iowa, USA (near 42°23'N and 9°13'W). The site is owned and managed by the Black Hawk County Conservation Board, which leased the land to a local farmer for row crop production from the late-1980s through 2007. In 2008, the lease was transferred to the University of Northern Iowa's Tallgrass Prairie Center to begin a research project investigating the feasibility of cultivating and harvesting native prairie vegetation for use as a biomass energy feedstock.

The site is flat (0–2% slopes) and located entirely within the Cedar River floodplain. It contains six soil types with variable characteristics and suitabilities for row crop production. Only three soil types were included in our research plots: (1) Flagler sandy loam (no flooding, drainage class (DC) = "somewhat excessively drained," corn suitability rating (CSR) = 50), (2) Saude loam (no flooding, DC = "well-drained," CSR = 63), and (3) Spillville/Colland complex (occasional flooding, DC = "somewhat poorly drained," CSR = 60) (Steckley 2006). From this

point forward, we refer to the three soil types listed above as sandy loam, loam, and clay loam, respectively. The statewide average CSR for land planted to corn or soybeans in 2006 was 72 (Secchi et al. 2009), while in Black Hawk County, average CSR for all land is 72 and for cropland is 81 (Hofstrand 2010). Thus, our site consists of non-prime agricultural land that is below average for corn production.

We established 48 research plots ranging in size from 0.30 to 0.56 ha in seven existing row crop fields

(3.7–6.1 ha). Each plot was seeded with one of four treatments of native vegetation: (1) switchgrass monoculture, (2) warm-season grass mix (five grass species), (3) biomass mix (16 species of grasses, legumes, and forbs), or (4) prairie mix (32 species of grasses, legumes, forbs, and sedges) (Table 1). Four plots of each treatment were established on each of the three soil types (4 seed mix treatments × 3 soil types × 4 replicates of each seed mix treatment per soil type = 48 plots total).

Table 1 Species composition, seeding rates, and native species establishment in four experimental prairie biomass production treatments in Black Hawk County, Iowa, USA

Scientific name	Functional group	Switchgrass		Warm-season grass mix		Biomass mix		Prairie mix	
		SR ^a	% est ^b	SR	% est	SR	% est	SR	% est
<i>Panicum virgatum</i>	C4 grass	561	97.5	86	40.4	43	12.5	32	27.1
<i>Andropogon gerardii</i>	C4 grass	0	0	151	55.8	151	42.9	135	43.7
<i>Bouteloua curtipendula</i>	C4 grass	0	0	86	61.3	43	50.0	32	39.2
<i>Schizachyrium scoparium</i>	C4 grass	0	0	151	55.4	151	34.2	135	38.7
<i>Sorghastrum nutans</i>	C4 grass	0	0	86	33.7	43	10.4	32	30.0
<i>Agropyron smithii</i>	C3 grass	0	0	0	0	43	0	32	0
<i>Astragalus canadensis</i>	Legume	0	0	0	0	38	3.3	16	2.9
<i>Desmodium canadense</i>	Legume	0	0	0	0	38	0	16	7.5
<i>Elymus canadensis</i>	C3 grass	0	0	0	2.1	43	29.2	32	37.5
<i>Elymus virginicus</i>	C3 grass	0	0.8	0	7.1	43	21.7	32	29.2
<i>Helianthus grosseserratus</i>	Forb	0	0.8	0	0.8	38	5.8	16	5.0
<i>Heliopsis helianthoides</i>	Forb	0	2.5	0	2.9	38	70.8	16	49.6
<i>Lespedeza capitata</i>	Legume	0	0	0	0	38	2.9	16	10.0
<i>Oligoneuron rigidum</i>	Forb	0	0	0	0.4	38	6.7	16	10.4
<i>Ratibida pinnata</i>	Forb	0	1.7	0	0.8	38	39.2	16	26.3
<i>Silphium laciniatum</i>	Forb	0	0	0	0	3	7.1	3	7.1
<i>Amorpha canescens</i>	Legume	0	0	0	0	0	0	16	2.9
<i>Artemisia ludoviciana</i>	Forb	0	0	0	0	0	0	16	2.9
<i>Baptisia leucantha</i>	Legume	0	0	0	0	0	0	16	0.4
<i>Carex bicknellii</i>	Sedge	0	0	0	0.4	0	0.4	32	2.9
<i>Carex brevior</i>	Sedge	0	0	0	0	0	0	32	6.7
<i>Carex gravida</i>	Sedge	0	0	0	0	0	0	32	1.3
<i>Dalea purpurea</i>	Legume	0	0	0	0	0	0	16	7.5
<i>Echinacea pallida</i>	Forb	0	0	0	0	0	2.5	16	21.2
<i>Eryngium yuccifolium</i>	Forb	0	0	0	0	0	0	16	2.5
<i>Monarda fistulosa</i>	Forb	0	0	0	0	0	0.4	16	9.2
<i>Phlox pilosa</i>	Forb	0	0	0	0	0	0	3	1.7
<i>Sporobolus compositus</i>	C4 grass	0	0	0	0	0	0	32	10.0
<i>Symphyotrichum laevis</i>	Forb	0	0	0	0.4	0	0	16	12.5
<i>Symphyotrichum novae-angliae</i>	Forb	0	0	0	0	0	0	16	4.2
<i>Tradescantia bracteata</i>	Forb	0	0	0	0	0	2.1	16	0.8
<i>Zizia aurea</i>	Forb	0	0	0	2.1	0	4.2	16	27.9

^a SR = rate (seeds per m²) at which plots were seeded with each species

^b % est = mean percentage of 0.1 m² quadrats containing seedlings of each species in June 2010

Vegetation establishment and management

To ensure a uniform management history, all fields to be seeded as part of the research project were managed for soybean production in 2008. Soybeans were harvested in the fall, after which the fields were treated with a broad-leaf herbicide to control weeds. In May 2009, plots were seeded sequentially from least to most diverse to avoid seed contamination using a no-till grass drill. Plots were mowed at a height of 10 cm in July 2009 to reduce competition with annual weeds (Williams et al. 2007). No fertilizer, herbicides, pesticides, or irrigation were applied to the plots at any time after seeding, nor were the plots burned prior to the 2010 growing season.

To assess vegetation establishment, we sampled plant community composition in all plots from 11 to 14 June 2010. We randomly located two 10 m transects in each plot and identified all native plants and weeds within ten 0.1 m² quadrats placed at 1 m intervals along each transect.

Vegetation characteristics and floral resource surveys

From 19 May to 2 June 2010, we sampled vegetation composition and structure in the research plots. We established one 50 m transect in each plot and measured vegetation characteristics in 15 1 m² quadrats placed at 3 m intervals along each transect. Within each 1 m² quadrat, we measured litter depth (mm) at each corner of the sampling frame and recorded heights (cm) of the tallest living and dead grass and forb. We measured vegetation height-density (cm) by recording visual obstruction readings on a 1.2 m Robel pole placed in the center of the quadrat (Robel et al. 1970). One reading was taken in each of the four cardinal directions from a height of 1 m at a distance of 4 m from the pole. Within each 1 m² quadrat, ground cover and canopy coverage of plant functional groups were measured in two 0.1 m² quadrats (15 × 2 = 30 per plot) placed in the outside corners of the 1 m² frame. Percent bare ground and litter and canopy coverage of standing dead vegetation and live grasses and forbs were estimated using Daubenmire cover classes (Daubenmire 1959).

We also sampled floral resources in each plot during five survey periods from June through September 2010. Using the same 50 m transects described above, we randomly selected a starting point 0–9 m from the transect endpoint, then randomly located 20 1 m² quadrats 1, 2, or 3 m to the right or left of the transect centerline at 2 m intervals along the length of the transect. We recorded the number of forb species in bloom, number of flowering ramets (individual stems emerging from the ground), and number of inflorescences of each species in each quadrat. We sampled

each plot once during each of the five survey periods. To avoid temporal bias, within each survey period we randomly selected (without replacement) one plot of each treatment in each of two soil types per survey day. Thus, all 48 plots were surveyed over 6 days (eight plots per day) over the duration of each survey period.

Butterfly surveys

We conducted visual surveys of butterflies along the same 50 m transects used in the floral resource surveys. Surveyors walking at a pace of 10 m per minute (5 min survey time per plot) recorded all butterflies observed within a 3 m window around the observer. Butterflies were identified on the wing when possible. Otherwise, they were captured with a net and identified in hand or sacrificed and transported to the lab in a glassine envelope for subsequent identification. Timers were stopped to handle butterflies and record data. We categorized the behavior of each butterfly encountered as either: (1) searching (flying erratically over the plot), (2) feeding on a nectar source, (3) resting (resting on vegetative parts of a plant or flushed from vegetation), or (4) mating or courting. For those butterflies observed feeding, we also recorded the plant species being fed upon. Surveys were conducted between 1,000 and 1,900 h on warm (20–36°C), at least partly sunny days (<75% cloud cover) with winds <16 km/h. Each plot was surveyed ten times (twice during each of the five survey periods) between June and September 2010. Within each survey period, we randomly selected 16 plots per survey day, so it took 6 days to sample all 48 research plots twice during each survey period.

Data analysis

To describe vegetation structural characteristics within each plot, we used the data from the 15 quadrats to calculate plot means for the following variables: forb and grass percent cover, height of tallest living forb and grass, litter and bare ground percent cover, and Robel pole visual obstruction readings. We tested for variation in vegetation structural characteristics by treatment and soil type using analysis of variance (ANOVA) or non-parametric Kruskal–Wallis tests as appropriate.

To assess floral resources, we tallied the total number of forb species observed in bloom in each plot over the entire growing season and compared treatment species richness, non-treatment species richness, and total forb species richness by treatment using ANOVA or Kruskal–Wallis tests. We also summed the total flower abundance within each plot over the five survey periods and compared total abundance of treatment, non-treatment, and

total flowers using Kruskal–Wallis tests. Finally, we assessed variation in flower abundance over the course of the growing season within each treatment using non-parametric Quade tests with the five survey periods as a grouping factor and plot as a blocking factor.

We calculated average butterfly abundance (mean of two surveys) and total species richness (total number of species observed in two surveys) for each plot within each of the five survey periods. We used the criteria presented in Vogel et al. (2010) to classify species into grassland habitat specialist, grassland habitat generalist, or woodland habitat guilds. We used repeated measures multivariate ANOVA to compare butterfly abundance and richness by treatment and soil type over the five survey periods. Where MANOVA indicated significant main effects, we performed a posteriori pair-wise multiple comparisons.

We tested for differences in butterfly community composition by treatment and soil type and in floral assemblages within each treatment by soil type using permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001). For these analyses, we pooled butterfly or floral abundances within each plot over the entire field season and applied a square-root transformation to reduce the influence of dominant species (Legendre and Gallagher 2001). We generated a distance matrix using the Bray-Curtis dissimilarity measure, performed 9,999 permutations, and applied pair-wise a posteriori tests of significant main effects and/or interaction terms. In the butterfly community analysis, we used PERMDISP (Anderson 2006) to test for homogeneity of multivariate dispersions by treatment. We used similarity percentages analysis (SIMPER) to identify species most responsible for dissimilarity in community composition among groups and non-metric multidimensional scaling to visualize patterns of variation in butterfly community composition among the treatment \times soil groups (Clarke and Warwick 2001).

We assessed butterfly response to floral resources by examining the relationships between (1) average butterfly abundance and total flower abundance and (2) total butterfly species richness and total number of forb species in bloom within each of the five survey periods using least-squares linear regression. Butterfly and flower abundance values were $\log(x + 1)$ -transformed prior to analysis. Finally, we tested for variation in butterfly behavior by treatment using one-way ANOVA; behavioral data were arcsine-root transformed prior to analysis. Analyses were performed using SYSTAT 12.0 (SYSTAT Software Inc., Chicago, Illinois, USA), PAST (Hammer et al. 2001), and PRIMER 6 (version 6.1.13) with PERMANOVA + (version 1.0.3) (PRIMER-E Ltd., Plymouth PL1 3DH, UK) software.

Results

Comparison of vegetation characteristics and floral resources by treatment and soil type

We found significant variation in vegetation structure, composition, and the abundance of floral resources among the four prairie biomass production treatments. Percent forb cover (ANOVA, $F_{3,36} = 9.30$, $P < 0.001$) and height of the tallest living forbs (ANOVA, $F_{3,36} = 13.92$, $P < 0.001$) were significantly greater in the biomass and prairie mix treatments than in the switchgrass and warm-season grass plots, whereas percent grass cover was greater in the warm-season grass plots than in any of the other treatments (ANOVA, $F_{3,36} = 4.78$, $P = 0.007$).

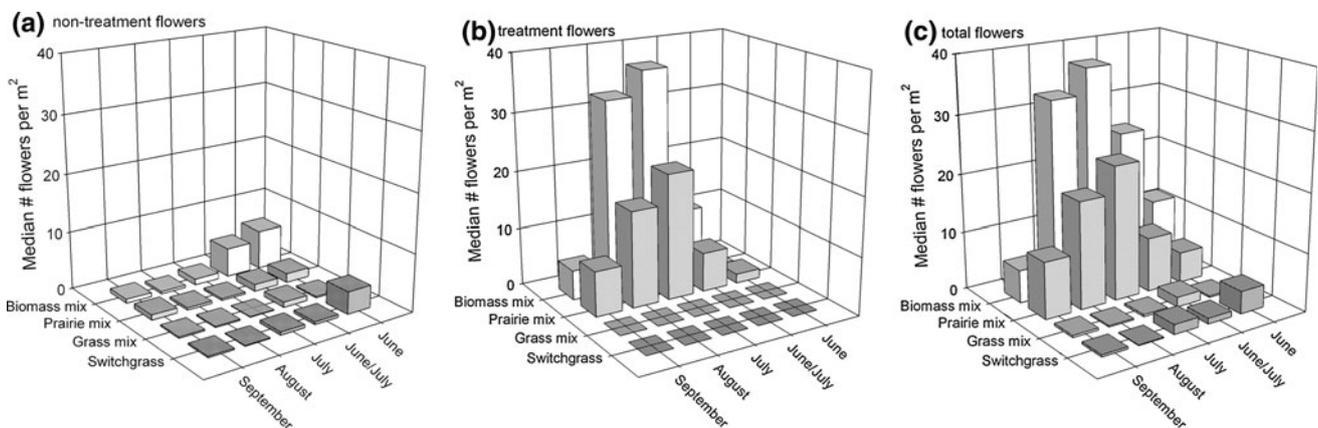
Many structural characteristics of the vegetation varied significantly among soil types. In general, vegetation on the excessively drained sandy loam was sparser with less litter accumulation and shorter vegetation heights compared to vegetation on the loam or clay loam soils. For example, the ratio of litter coverage to bare ground (ANOVA, $F_{2,36} = 7.85$, $P = 0.001$) and Robel visual obstruction readings (Kruskal–Wallis, $df = 2$, $H = 25.75$, $P < 0.001$) were significantly lower on the sandy loam than on the clay loam, and the height of living grasses (ANOVA, $F_{2,36} = 14.46$, $P < 0.001$) and forbs (ANOVA, $F_{2,36} = 24.82$, $P < 0.001$) were significantly shorter on the sandy loam than on the other soil types.

We recorded 53 forb species (16 treatment species, 37 non-treatment species) in bloom during the 2010 growing season (Table 2). Pooled over the entire growing season, the mean total number of species observed in bloom was significantly higher in the biomass and prairie mix plots compared to the switchgrass and warm-season grass plots (Kruskal–Wallis, $df = 3$, $H = 36.94$, $P < 0.001$). As anticipated treatment species were more species rich in the prairie mix plots than the biomass plots, which were richer than the warm-season grass or switchgrass plots (Kruskal–Wallis, $df = 3$, $H = 40.31$, $P < 0.001$). However, non-treatment species richness was significantly greater in the biomass mix than prairie mix, which was richer than switchgrass but not warm-season grass mix (ANOVA, $F_{3,36} = 15.35$, $P < 0.001$).

Pooled over the entire growing season, the total number of flowers in bloom was greater in the biomass mix than the prairie mix, and in the prairie mix than either the switchgrass or warm-season grass mix, which did not differ from one another (Kruskal–Wallis, $df = 3$, $H = 33.98$, $P < 0.001$). The abundance of treatment flowers followed the same pattern (Kruskal–Wallis, $df = 3$, $H = 37.04$, $P < 0.001$); however, there was no significant difference in the abundance of non-treatment flowers (Kruskal–Wallis, $df = 3$, $H = 7.45$, $P = 0.059$) among the four treatments.

Table 2 Species richness of forbs in bloom and percentage of total flowers represented by seeded native species in four experimental prairie biomass production treatments across five survey periods at a site in Black Hawk County, Iowa, USA

	June	June/July	July	August	September	Growing season
Number of species in bloom (treatment/non-treatment/total)						
Switchgrass	1/10/11	2/6/8	2/6/8	3/3/6	2/3/5	5/17/22
Warm-season grass mix	1/12/13	1/12/13	2/6/8	2/7/9	3/3/6	4/20/24
Biomass mix	4/14/18	4/14/18	5/9/14	6/7/13	5/4/9	8/24/32
Prairie mix	6/9/15	8/9/17	8/8/16	7/10/17	9/7/16	15/25/40
All plots	8/21/29	9/23/32	10/14/24	10/14/24	10/10/20	16/37/53
Percent of total inflorescences from seeded treatment species						
Switchgrass	0.3	9.7	23.4	20.5	10.2	5.9
Warm-season grass mix	0.1	3.6	19.5	29.8	15.5	5.4
Biomass mix	7.8	65.6	93.4	98.0	91.0	72.0
Prairie mix	25.9	61.7	96.3	95.7	90.1	78.2
All plots	10.0	53.4	89.8	95.6	85.2	65.9

**Fig. 1** Median number of **a** non-treatment species flowers, **b** treatment species flowers, and **c** total flowers in four experimental prairie biomass production treatments over the 2010 growing season at a site in Black Hawk County, Iowa, USA

Forb species richness and floral abundance and composition varied over the course of the growing season within the different treatments. Generally, non-treatment species richness and flower abundance were greater in the June and June/July survey periods and declined over the course of the growing season (Table 2; Fig. 1a). This pattern was statistically significant in the switchgrass (Quade test, $F_{4,44} = 3.90$, $P = 0.009$) and biomass mix (Quade test, $F_{4,44} = 12.12$, $P < 0.001$) but not in the warm-season grass or prairie mix ($P > 0.05$) and was driven primarily by the abundance of *Melilotus officinalis* and *Melilotus alba* during these periods (Table 3). In contrast, treatment species flowers were more abundant in July and August than during the June, June/July, or September survey periods (Table 2; Fig. 1b) in all treatments (switchgrass, Quade test, $F_{4,44} = 3.00$, $P = 0.028$; biomass, Quade test, $F_{4,44} = 11.53$, $P < 0.001$; prairie, Quade test, $F_{4,44} = 9.60$, $P < 0.001$) except the warm-season grass mix ($P = 0.068$).

By July, > 90% of all flowers in biomass and prairie mix plots consisted of seeded native species (Table 2). The offset seasonal peaks in non-treatment and treatment species flower abundance reduced the variation in total flower abundance over the course of the growing season (Fig. 1c). Total flower abundance varied seasonally only in the switchgrass (Quade test, $F_{4,44} = 3.00$, $P = 0.018$) and biomass mix (Quade test, $F_{4,44} = 2.80$, $P = 0.037$), with total flower abundance (primarily *M. officinalis* and *Erigeron strigosus*) in switchgrass greater in June than any other month and total flower abundance (primarily *Heliopsis helianthoides*, *Desmodium canadense*, and *Ratibidia pinnata*) in biomass mix greater in July and August than September (Table 3). Total flower abundance did not vary significantly over the growing season in the warm-season grass ($P = 0.45$) or prairie mix ($P = 0.24$) treatments.

Within treatments, the species composition of forbs in bloom varied significantly among soil types. In switchgrass

Table 3 Most abundant forbs in bloom in four experimental prairie biomass production treatments at a site in Black Hawk County, Iowa, USA

Species	Switchgrass	Warm-season grass mix	Biomass mix	Prairie mix	Peak abundance
<i>Astragalus canadensis</i>			0.17	0.09	2
<i>Chamaechrista fasciculata</i> ^a			0.08	0.14	4
<i>Desmodium canadense</i>			6.84	4.97	4
<i>Echinacea pallida</i>				0.16	1
<i>Erigeron strigosus</i> ^a	0.58	0.44	0.53	0.13	1,2,3
<i>Eryngium yuccifolium</i>				0.07	3
<i>Helianthus grosseserratus</i>			0.19	0.07	5
<i>Heliopsis helianthoides</i>	0.15	0.13	9.96	5.65	3
<i>Medicago lupulina</i> ^a			0.06		1
<i>Medicago sativa</i> ^a			0.05		1
<i>Melilotus alba</i> ^a		0.10	1.58	0.19	1,2
<i>Melilotus officinalis</i> ^a	2.16	1.87	4.16	2.71	1
<i>Monarda fistulosa</i>				0.25	3
<i>Oligoneuron rigidum</i>			0.36	0.34	5
<i>Phlox pilosa</i>				0.60	1
<i>Potentilla recta</i> ^a			0.07		1
<i>Ratibida pinnata</i>			0.94	0.66	3
<i>Solidago canadensis</i> ^a	0.11	0.09	0.09	0.07	4,5
<i>Symphytotrichum laevis</i>				0.80	5
<i>Symphytotrichum novae-angliae</i>				0.06	5
<i>Symphytotrichum pilosum</i> ^a	0.14	0.09	0.11	0.21	5
<i>Trifolium hybridum</i> ^a			0.07	0.05	1,2
<i>Trifolium pratense</i> ^a			0.26		2

Values indicate the mean number of inflorescences per m² for each species indicated. Means were calculated using the plot means for each of the 12 plots of each treatment over each of the five survey periods (total N = 60 for each treatment). All species with mean abundance greater than 0.05 inflorescences per m² are listed. “Peak abundance” indicates the time of year during which flowers of the listed species were most abundant at the site: 1 = June, 2 = late June/early July, 3 = July, 4 = August, 5 = September. If a particular species peaked in abundance at different times in different treatments, both survey periods are reported

^a Non-treatment species were not included in the seed mix when the site was established in 2009

(PERMANOVA, $F_{2,36} = 2.83$, $P = 0.006$) and warm-season grass plots (PERMANOVA, $F_{2,36} = 2.03$, $P = 0.012$), floral assemblages were significantly different only between sandy loam and loam plots. *Melilotus officinalis* was the dominant species on loam plots but was completely absent from sandy loam plots, whereas *E. strigosus* was the dominant species on sandy loam but was found in low abundance on loam plots. Soil effects on flower species composition were even more pronounced in the biomass (PERMANOVA, $F_{2,36} = 5.55$, $P < 0.001$) and prairie mix (PERMANOVA, $F_{2,36} = 3.25$, $P = 0.004$). In the biomass treatment, floral assemblages were significantly different from one another on all soil types, while in the prairie mix floral assemblages were similar on the loam and clay loam soils, both of which differed from the floral assemblage on sandy loam. In both treatments, *D. canadense*, *Chamaecrista fasciculata*, *Phlox pilosa*, and *E. strigosus* were among the most abundant species on sandy loam, whereas

H. helianthoides and *R. pinnata* were dominant on the clay loam. The abundance of all of these species was intermediate on the loamy soil, whose floral assemblage was also characterized by the highest abundance of the non-treatment *M. officinalis* and *M. alba*.

Comparison of butterfly abundance and species richness by treatment over time

We recorded 2 110 butterfly observations representing 31 species (Table 4) from June through September 2010. The average number of butterflies observed per survey ($F_{3,36} = 105.5$, $P < 0.001$; Table 5) and the total number of butterfly species observed per survey period ($F_{3,36} = 79.5$, $P < 0.001$; Table 5) were significantly greater in the biomass and prairie mix plots compared to the switchgrass and warm-season grass plots (Hotelling’s T-square pair-wise comparisons, all $P < 0.001$; Fig. 2).

Table 4 Butterfly species and number of individuals observed during transect surveys of prairie biomass production plots in Black Hawk County, Iowa, USA

Scientific name	Habitat ^a	Switchgrass	Warm-season grass mix	Biomass mix	Prairie mix	Total
Family Papilionidae						
<i>Papilio polyxenes</i>	hg	1	0	4	7	12
<i>Papilio glaucus</i>	w	1	0	0	1	2
Family Pieridae						
<i>Colias eurytheme/philodice</i> ^b	hg	107	119	332	365	923
<i>Pieris rapae</i>	hg	3	9	8	5	25
<i>Eurema lisa</i>	hg	0	6	7	4	17
<i>Pontia protodice</i>	hg	0	0	1	0	1
<i>Nathalis iole</i>	hg	3	0	0	0	3
Family Nymphalidae						
<i>Vanessa atalanta</i>	hg	20	11	229	183	443
<i>Danaus plexippus</i>	hg	12	18	71	75	176
<i>Phyciodes tharos</i>	hg	19	25	38	31	113
<i>Junonia coenia</i>	hg	10	6	23	28	67
<i>Vanessa cardui</i>	hg	2	1	6	10	19
<i>Limenitis archippus</i>	hs	0	1	1	6	8
<i>Polygonia comma</i>	w	1	2	4	1	8
<i>Speyeria cybele</i>	hs	1	0	6	1	8
<i>Asterocampa celtis</i>	w	1	1	2	3	7
<i>Euptoieta claudia</i>	hg	0	1	0	2	3
<i>Vanessa virginiensis</i>	hg	0	0	2	1	3
<i>Polygonia interrogationis</i>	w	1	0	1	0	2
<i>Speyeria idalia</i>	hs	1	0	0	1	2
<i>Asterocampa clyton</i>	w	0	0	0	1	1
<i>Nymphalis antiopa</i>	w	0	1	0	0	1
Family Lycaenidae						
<i>Everes comyntas</i>	hg	11	24	96	75	206
<i>Celastrina neglecta</i>	hg	3	1	7	4	15
<i>Strymon melinus</i>	hg	0	1	9	2	12
<i>Lycaena hyllus</i>	hs	1	0	0	0	1
Family Hesperidae						
<i>Hylephila phyleus</i>	hg	2	4	9	5	20
<i>Pyrgus communis</i>	hg	0	1	1	3	5
<i>Atalopedes campestris</i>	hg	0	0	0	3	3
<i>Ancyloxypha numitor</i>	hg	0	0	1	1	2
<i>Erynnis funeralis</i>	hg	0	0	2	0	2
Individuals observed		200	232	860	818	2110
Species observed		19	18	23	25	31

Each of the 12 plots within each treatment was sampled 10 times during five survey periods between June and September 2010. Abundance values are the summed totals within each treatment across the five survey periods

^a Butterflies were classified as habitat-specialist (hs), habitat-generalist (hg), or woodland (w) species using the criteria applied by Vogel et al. 2010

^b Observations of *Colias eurytheme* and *Colias philodice* were pooled and considered one species in analyses

However, butterfly abundance and species richness did not vary significantly between the biomass and prairie mix or between the switchgrass and warm-season grass treatments (Hotelling's T-square, all $P > 0.05$; Fig. 2). Soil type did not influence butterfly abundance or species richness as a main effect (Table 5).

Butterfly abundance (Fig. 2a) and species richness (Fig. 2b) varied over the course of the 2010 growing season; however, the pattern of change was complex with

significant treatment and soil interactions (Table 5). Butterfly abundance increased significantly over the course of the growing season in the biomass and prairie mix plots, peaking in either July or August depending on soil type. In contrast, there was little variation in butterfly abundance in the switchgrass and warm-season grass plots on two soil types, with only a slight increase on the clay loam soil in August. Species richness peaked in the biomass and prairie mix plots in September, while

Table 5 Repeated measures analysis of variance comparing average butterfly abundance and total species richness by vegetation treatment and soil type over five survey periods during the 2010 growing season

	df	Average butterfly abundance			Total butterfly species richness		
		MS	F-ratio	P	MS	F-ratio	P
<i>Between subjects</i>							
Treatment	3	542.64	105.49	<0.001	73.87	79.50	<0.001
Soil	2	6.35	1.23	0.303	2.26	2.44	0.102
Treatment × soil	6	6.44	1.25	0.304	1.17	1.27	0.296
Error	36	5.14			0.93		
<i>Within subjects</i>							
Time	4	39.39	12.79	<0.001	13.86	12.61	<0.001
Time × treatment	12	11.41	3.70	<0.001	3.29	3.00	0.001
Time × soil	8	14.44	4.69	<0.001	1.91	1.74	0.095
Time × treatment × soil	24	10.40	3.38	<0.001	2.23	2.03	0.006
Error	144	3.08			1.10		

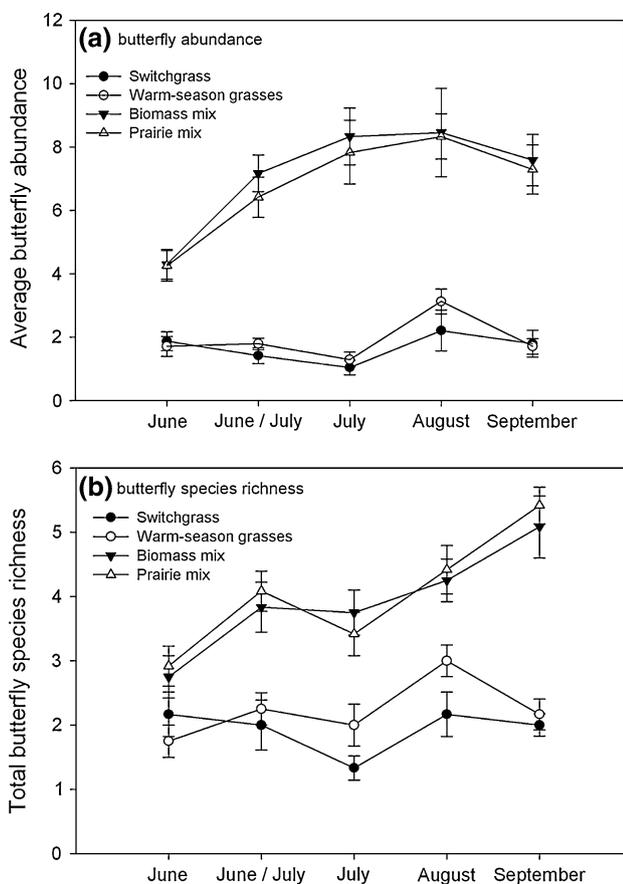


Fig. 2 Butterfly **a** average abundance and **b** total species richness (mean ± SE) in in four experimental prairie biomass production treatments during the 2010 growing season. *N* = 12 for each vegetation treatment

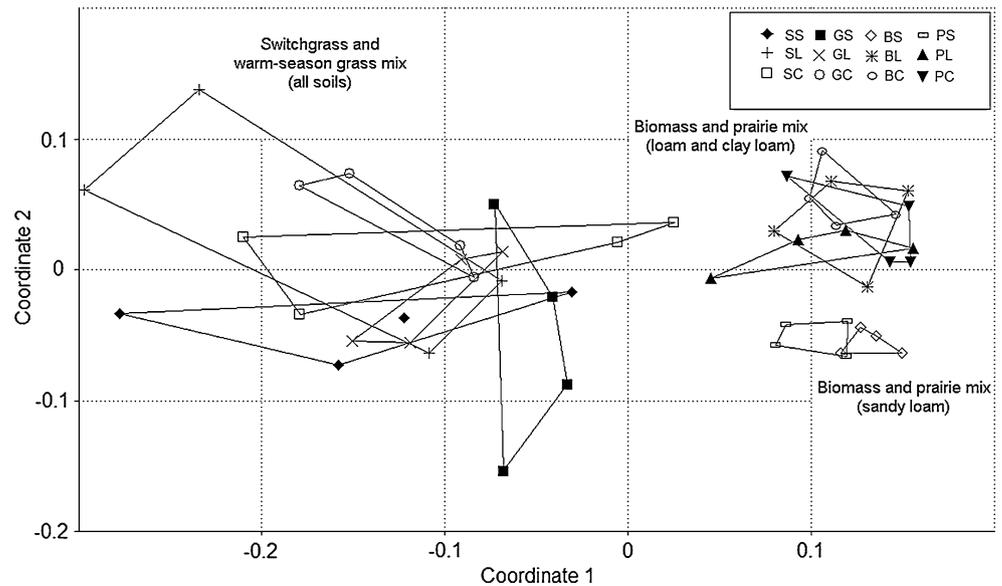
there was little variation in species richness over the growing season in the switchgrass and warm-season grass plots (Fig. 2b).

Comparison of butterfly community composition by treatment and soil type

Butterfly community composition varied significantly by treatment (PERMANOVA, $F_{3,36} = 8.98$, $P < 0.001$) and soil type ($F_{2,36} = 2.65$, $P = 0.006$), and there was a significant treatment × soil type interaction ($F_{6,36} = 1.68$, $P = 0.017$). The effect of treatment showed a consistent pattern across all soil types. Butterfly community composition in the biomass and prairie mix was significantly different from the communities found in the switchgrass and warm-season grass plots (pair-wise comparisons, all $P < 0.001$; Fig. 3); however, there were no differences in community composition between the biomass and prairie mix nor between the switchgrass and warm-season grass mix ($P > 0.05$). Most common species were several times more abundant and many rarely encountered species were more frequently recorded in the biomass and prairie mixes compared to the grass treatments (Table 4). Within treatments, butterfly assemblages were more variable among the switchgrass and warm-season grass plots than among the biomass and prairie mix plots (PERMDISP, $F_{3,44} = 9.17$, $P < 0.001$; Fig. 3).

The significant treatment × soil type interaction was revealed by comparing butterfly community composition within each treatment across the three soil types. There was no significant variation in butterfly community composition in switchgrass or warm-season grass plots ($P > 0.05$; Fig. 3) across soil types; however, community composition in the biomass and prairie mix varied significantly between the excessively drained sandy loam and the well-drained loam or poorly drained clay loam soils. In the biomass mix, species assemblages on loam and clay loam plots did not differ from one another ($P = 0.083$) but were significantly different from those found on sandy loam ($P = 0.029$,

Fig. 3 Non-metric multidimensional scaling of butterfly community composition among the twelve treatment \times soil type groups. The first letter of the two-letter group codes refers to treatment: S = switchgrass, G = warm-season grass mix, B = biomass mix, and P = prairie mix; the second refers to soil type: S = sandy loam, L = loam, and C = clay loam



$P = 0.027$, respectively). In the prairie mix, community composition on the loam and clay loam plots was similar ($P = 0.554$); however, community composition was significantly different between the clay loam and sandy loam plots ($P = 0.028$) and nearly so between the loam and sandy loam plots ($P = 0.061$).

Similarity percentages analysis of the difference in community composition between biomass and prairie mix plots on sandy loam versus those on loam or clay loam revealed that about 50% of the dissimilarity among groups was accounted for by variation in the abundance of the six most common species (Table 6). *Vanessa atalanta*, *Danaus plexippus*, and *Junonia coenia* were more abundant in plots on loam and clay loam, whereas *Colias eurythemelphidice*, *Everes comyntas*, *Phyciodes tharos* were proportionally more abundant on sandy loam plots. Differences in the presence/absence or relative abundance of many less common or rarely encountered species accounted for the other 50% of dissimilarity among groups (Table 6).

Butterfly response to floral resources

There was a highly significant positive linear relationship between butterfly abundance and flower abundance during all five survey periods. The relationship was weakest early in the growing season (June: $y = 0.20x + 0.41$, $r^2 = 0.29$, $P < 0.001$; June/July: $y = 0.33x + 0.38$, $r^2 = 0.41$, $P < 0.001$), strongest in July (Fig. 4; $y = 0.53x + 0.17$, $r^2 = 0.82$, $P < 0.001$) when flower abundance peaked, and then weakened slightly in August ($y = 0.35x + 0.45$, $r^2 = 0.62$, $P < 0.001$) and September ($y = 0.49x + 0.36$, $r^2 = 0.67$, $P < 0.001$) as flower abundance declined. There was also a highly significant positive linear relationship between butterfly species richness and the number of forbs

in bloom in all survey periods except June ($r^2 = 0.025$, $P = 0.281$). The strength of the relationship increased over the course of the growing season (June/July: $y = 0.28x + 1.96$, $r^2 = 0.31$, $P < 0.001$; July: $y = 0.31x + 1.62$, $r^2 = 0.27$, $P < 0.001$; August: $y = 0.33x + 2.17$, $r^2 = 0.46$, $P < 0.001$) and was strongest in September (Fig. 5; $y = 0.49x + 1.78$, $r^2 = 0.53$, $P < 0.001$) when butterfly species richness peaked.

Butterfly behavior also varied significantly among treatments. More butterflies observed in biomass and prairie mix were actively feeding compared to the switchgrass and warm-season grass mix (ANOVA, $F_{3,36} = 13.50$, $P < 0.001$). Conversely, the proportions of butterflies observed searching (ANOVA, $F_{3,36} = 5.60$, $P = 0.002$) or resting on or being flushed from vegetation (ANOVA, $F_{3,36} = 5.53$, $P = 0.003$) were significantly less in the biomass mix than in switchgrass or the warm-season grass mix.

Discussion

We studied butterfly use of four candidate lignocellulosic prairie biofuel crops and found significant differences in butterfly abundance, species richness, and community composition among treatments with different levels of plant diversity and floral resources. Throughout the growing season, biomass and prairie mix plots had more species of forbs in bloom and more abundant floral resources for butterflies compared to the warm-season grass mix and switchgrass plots. Contrary to our expectations, in all survey periods except September, flowers were more abundant in the biomass mix than the prairie mix. This observation was driven primarily by the abundance of *H. helianthoides*, which was seeded at twice the rate in the biomass mix

Table 6 Similarity percentages (SIMPER) analysis of species contributing to dissimilarity in butterfly community composition in the biomass and prairie mix plots on sandy loam versus loam and clay loam soils

Species	Contribution ^a	Cumulative % ^b	Abund ^c	Abund ^d _{l, c}
<i>Vanessa atalanta</i>	5.95	17.66	2.20	4.73
<i>Everes comyntas</i>	3.36	27.62	3.44	2.04
<i>Colias eurytheme/philodice</i>	2.46	34.93	5.97	4.98
<i>Phyciodes tharos</i>	1.92	40.62	2.04	1.35
<i>Danaus plexippus</i>	1.74	45.78	2.31	2.40
<i>Junonia coenia</i>	1.68	50.77	1.03	1.41
<i>Hylephila phyleus</i>	1.46	55.12	0.25	0.67
<i>Vanessa cardui</i>	1.44	59.39	0.38	0.67
<i>Pieris rapae</i>	1.44	63.65	0.18	0.57
<i>Strymon melinus</i>	1.42	67.87	0.59	0.15
<i>Eurema lisa</i>	1.41	72.04	0.63	0.36
<i>Papilio polyxenes</i>	1.24	75.73	0.18	0.49
<i>Celastrina neglecta</i>	1.21	79.32	0.25	0.53
<i>Limenitis archippus</i>	1.12	82.64	0.43	0.19
<i>Asterocampa celtis</i>	0.80	85.03	0.25	0.19
<i>Pyrgus communis</i>	0.72	87.18	0.25	0.13
<i>Speyeria cybele</i>	0.72	89.32	0.00	0.32
<i>Polygonia comma</i>	0.69	91.36	0.13	0.21
<i>Ancyloxypha numitor</i>	0.58	93.08	0.25	0.00
<i>Vanessa virginiensis</i>	0.43	94.36	0.00	0.19
<i>Erynnis funeralis</i>	0.39	95.51	0.13	0.06
<i>Atalopedes campestris</i>	0.34	96.53	0.00	0.15
<i>Asterocampa clyton</i>	0.29	97.39	0.13	0.00
<i>Euptoieta claudia</i>	0.28	98.22	0.00	0.13
<i>Papilio glaucus</i>	0.16	98.71	0.00	0.06
<i>Pontia protodice</i>	0.15	99.16	0.00	0.06
<i>Polygonia interrogationis</i>	0.14	99.58	0.00	0.06
<i>Speyeria idalia</i>	0.14	100.00	0.00	0.06
Total dissimilarity	33.71			

^a Contribution of species to index of dissimilarity among groups

^b Cumulative % dissimilarity accounted by species at each row position

^c Mean abundance in biomass and prairie mix in plots on sandy loam

^d Mean abundance in biomass and prairie mix in plots on loam and clay loam

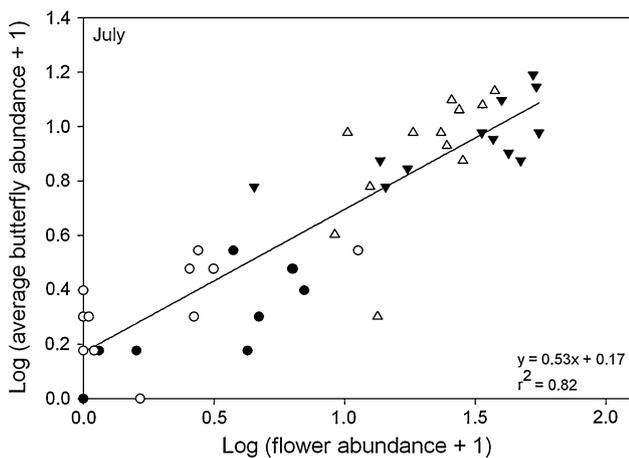


Fig. 4 Relationship between flower abundance and average butterfly abundance in prairie biomass production plots during July 2010

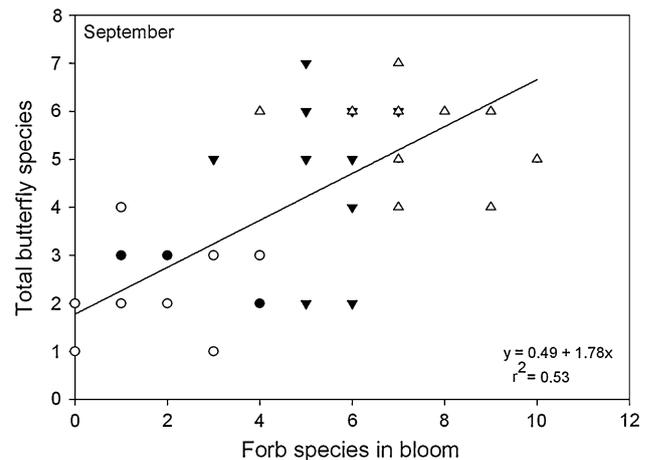


Fig. 5 Relationship between number of forb species in bloom and butterfly species richness in prairie biomass production plots during September 2010

compared to the prairie mix, during the June/July, July, and August survey periods. Averaged over the entire growing season, butterflies were approximately six times more abundant and twice as species rich in the biomass and prairie mix plots compared to the warm-season grass mix and switchgrass plots. However, at this stage of establishment, there were no significant differences in butterfly abundance, species richness, or community composition between the biomass and prairie mix treatments.

We found that floral abundance was a strong predictor of butterfly abundance, consistent with previous studies which have reported similar relationships between butterfly abundance and percent forb cover (Vogel et al. 2007; Reeder et al. 2005) or number of ramets in bloom (Reeder et al. 2005; Shepherd and Debinski 2005). While some authors have reported positive associations between butterfly and plant species richness (Ries et al. 2001; Steffan-Dewenter and Tscharntke 1997), others have found no significant relationship between these variables (Hawkins and Porter 2003; Shepherd and Debinski 2005). At the small spatial extent of our study, we found strong positive relationships which strengthened as the growing season progressed between the number of forb species in bloom and butterfly species richness.

Our results suggest that choice of biofuel feedstocks will have profound effects on butterfly communities and on the maintenance of biodiversity in agricultural landscapes in general and support the conceptual model of habitat value presented by Fargione et al. (2009). Further, we expect that if biomass production using the treatments we studied were implemented at a larger a field scale, differences in butterfly communities between them would be even more pronounced. In our study, single fields contained multiple plots each of which was randomly assigned a treatment, creating a heterogeneous mosaic of patches with different vegetation characteristics within each field. Based on the differences in butterfly behavior we observed among the various treatments, we suspect that larger switchgrass or warm-season grass plots would be used less frequently by fewer species if they were not in close proximity to areas with diverse nectar sources as found in the biomass or prairie mix plots in our study.

In all treatments, butterfly assemblages were dominated by habitat generalists. Habitat generalists accounted for 98.1% of all butterflies observed, and six dominant species (*C. eurythemelphilodice*, *V. atalanta*, *E. comyntas*, *D. plexippus*, and *P. tharos*) accounted for 88.2% of all observations. To date, most documented prairie butterfly declines in the Midwestern USA involve site-restricted, habitat specialists (Swengel et al. 2010; Schlicht and Orwig 1998); however, recent studies in Europe indicate that many formerly common, widespread, generalist butterflies (Thomas et al. 2004; Van Dyck et al. 2009) and moths

(Conrad et al. 2006) have declined significantly in recent decades across landscapes characterized by long-term, intense human activity.

While widespread habitat generalists have historically received little attention from conservationists (Gaston and Fuller 2007), we believe there is value in providing improved habitat conditions for common species in Iowa's intensively farmed landscape. For example, *D. plexippus*, a widespread migratory species with specialized host plant (*Asclepias* sp.) requirements but generalist feeding habits, was frequently detected at our site and is common throughout Iowa; however, there is growing concern about the species' status due to its complex, migratory life cycle and the intense habitat pressures at both its breeding sites in the USA and its overwintering sites in Mexico (Brower et al. 2006; Davis and Rendon-Salinas 2009). In the Midwestern USA, the extent and quality of *D. plexippus* breeding habitat has been greatly reduced by agricultural intensification, especially the conversion of native prairie to row crop monocultures and the frequent application of chemical herbicides which kill larval host plants and adult nectar sources (Brower et al. 2006).

The biomass and prairie mix plots in our study provided *D. plexippus* with a variety of abundant nectar sources over the entire growing season. We observed *D. plexippus* feeding on *Echinacea pallida* in June; *E. pallida*, *H. helianthoides*, and *Monarda fistulosa* in July; *Cirsium altissimum* and *Solidago canadensis* in August; and *Helianthus grosseserratus*, *Oligoneuron rigidum*, *S. canadensis*, *Symphotrichum laevis*, and *Symphotrichum novae-angliae* in September. The availability of abundant nectar sources throughout the growing season in our biomass production plots sharply contrasts the habitat conditions found in corn and soy fields, and the consistent seasonal availability of high quality nectar sources may support *D. plexippus* at critical times during its complex migratory cycle. For example, abundant nectar sources in June and early July support breeding individuals whose offspring later colonize the eastern USA (Miller et al. 2011), and nectar sources in August and September allow migrating individuals to accumulate sufficient lipid stores to survive the overwintering period in Mexico (Brower et al. 2006). While the degree to which agricultural intensification and the consequent reduction in forbs is currently affecting *D. plexippus* populations is unclear (Brower et al. 2006), our observations suggest that implementation of biomass production systems using diverse mixes of native species on marginal lands could provide abundant nectar sources and improved habitat conditions for *D. plexippus* and other generalist nectar-feeding butterflies. Adoption of lignocellulosic biomass production using native prairie species at a larger scale in the Midwestern USA could diversify the agricultural landscape and help to relieve extinction debt

(Krauss et al. 2010) or prevent future declines of common species (Gaston and Fuller 2007).

Habitat specialists accounted for <1% of all butterflies observed, though we did observe a few less common specialist species (*Limenitis archippus*, *Lycaena hyllus*, *Speyeria cybele*, and *Speyeria idalia*) in the biomass production plots. Habitat specialists were encountered more frequently in the biomass and prairie mix plots than the switchgrass and warm-season grass plots, and they were observed with increasing frequency as the growing season progressed. Community assembly models predict that a particular species from a regional species pool will become part of a local community if it is first able to disperse to the site and then encounters suitable environmental conditions to meet its resource requirements (Zobel 1997; Leibold et al. 2004; Summerville et al. 2006). Our results indicate that a number of habitat specialist butterflies successfully arrived at the site in just the second year after seeding and the first growing season dominated by native perennials, but continued monitoring is required to determine if these species will successfully meet their resource requirements to be able to colonize the site and increase in abundance over time (Leibold et al. 2004). For example, we observed *S. idalia*, a prairie-specialist and species of special concern in Iowa, in two formal surveys and on several opportunistic occasions at our site. The rapid arrival of *S. idalia* suggests that the site could potentially provide suitable habitat for a declining prairie-specialist, particularly if targeted efforts were undertaken to increase the abundance of the species' larval host plants (*Viola petatifida*) (Debinski and Kelly 1998). Generally, the degree to which biomass production sites using native vegetation will be colonized by habitat-specialist butterflies will depend a number of factors including (1) species life history characteristics and dispersal ability (Summerville et al. 2006), (2) landscape context and isolation from existing remnant populations (Shepherd and Debinski 2005; WallisDeVries and Ens 2008), and (3) and site characteristics (e.g., plant community composition, vegetation structural characteristics, host plant and nectar availability) as they are affected by management practices (e.g., initial seed mix, fire management, harvesting timing and methods, etc.) over time.

We believe that establishment and management of native prairie vegetation for biomass production over relatively large areas that involves rotational haying and harvest over multiple years may be a beneficial form of management for many butterflies, potentially including habitat specialist species. Currently, prescribed fire is the dominant form of habitat management for Iowa grasslands, yet the abundance and richness of butterflies, including many specialist species, appears to be positively associated with time since burning (Swengel 1996; Vogel et al. 2010). In comparisons of burned versus hayed prairies, most

metrics of total and specialist butterfly abundance and richness favor cutting (Swengel 1996; Swengel and Swengel 2001). Lands managed for prairie biomass production would likely experience reduced burn frequency, with periodic harvests replacing fire as the dominant management action.

Management for prairie biomass generally involves harvest in late-fall after the flight period of most adult butterflies is complete, so mortality of adult butterflies during harvest (Dover et al. 2010) are likely to be minimal. However, fall biomass harvests may potentially affect butterflies directly by causing mortality of immobile, immature life stages or indirectly by causing changes in vegetation structure, composition, and flowering phenology in subsequent growing seasons (Humbert et al. 2009; Johst et al. 2006; Pywell et al. 2011). Future research is needed to identify species most susceptible to harvest, to develop harvest methods that minimize impacts on immature life stages (Humbert et al. 2010), and to characterize vegetation and butterfly response to cutting in subsequent years. Long-term studies are also needed to investigate the effects of harvest frequency on plant and butterfly communities and to develop rotational harvest regimes that leave unburned, unharvested refugia (Humbert et al. 2010; Swengel and Swengel 2007) for overwintering species on the landscape each year while maximizing biomass production. Such long-term studies are critical to determine whether areas managed for biomass production act as sources or sinks for particular butterfly species.

Finally, we observed significant effects of soil type on butterfly community composition (but not abundance or species richness) mediated by soil-induced changes in vegetation characteristics. In the biomass and prairie mixes, butterfly community composition varied significantly between the very dry sandy loam (characterized by less productive, shorter, more sparse vegetation) and more moist loam and clay loam (characterized by more productive, taller, more dense vegetation), with *C. eurythemel philodice*, *E. comyntas*, and *P. tharos* proportionally more abundant on the sandy loam and *V. atalanta*, *D. plexippus*, and *J. coenia* more abundant on the loam and clay loam. Within our site, butterfly species responded to variation in vegetation composition among soil types even though the plots had identical seed mixes and management histories. For example, *H. helianthoides*, an important nectar source for many adult butterflies, was the dominant treatment species in the loam and clay loam plots but was much less abundant on the sandy loam. As a result, *V. atalanta*, which was feeding on *H. helianthoides* during over 70% of observations of this species during our study, was among the most dominant species in the loam and clay loam plots but was underrepresented in the sandy loam plots. Conversely, both *E. comyntas* and one of its larval host plants

(*D. canadense*) were several times more abundant on the sandy loam than the loam or clay loam plots.

If biomass production using native species were implemented at a large scale, the relatively large size of potential biomass production plots relative to the existing small, highly fragmented, often linear grassland habitat patches that characterize Iowa's current landscape means that they would be more likely to encompass multiple soil types or other gradients of environmental variation which promote habitat heterogeneity. Large, heterogeneous sites supporting diverse plant communities that produce abundant nectar sources over the entire growing season should offer improved habitat conditions better able to support diverse butterfly communities compared to current agricultural practices (Benton et al. 2003; Fargione et al. 2009; Schlicht and Orwig 1990).

In conclusion, our research demonstrates that habitat characteristics varied widely among four candidate lignocellulosic prairie biofuel crops during early establishment, and that floral abundance and richness were strong predictors of butterfly abundance and richness, respectively. We found significant variation in butterfly abundance, richness, and community composition among treatments in just the second growing season after seeding, and we predict that these differences will become more pronounced over time. Thus, we plan to continue to monitor our research site over time in order to: (1) document successional changes in plant and butterfly communities, (2) determine if proportions of habitat generalist and habitat specialist butterflies change over time, (3) determine whether vegetation characteristics and butterfly communities in the biomass and prairie mix plots diverge over time, and (4) assess the effects of future biomass harvests on plant and butterfly communities. While our findings are limited to butterflies during the establishment phase of a prairie biofuel project, they provide preliminary evidence supporting the idea that biofuel production systems based on diverse mixtures of native prairie species can contribute to the maintenance of biodiversity in agricultural landscapes.

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