

METHOD SELECTION AND ESTIMATING DENSITIES FOR GRASSLAND BUTTERFLIES IN THE  
MIXED-GRASS PRAIRIE

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**Title**

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BUTTERFLIES IN THE MIXED-GRASS PRAIRIE

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**DOCTOR OF PHILOSOPHY**

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## ABSTRACT

Grassland butterflies contribute to the larger group of pollinating insects that are vital for continued plant production and food security in agroecosystems. However, grassland butterflies, along with overall biodiversity, are experiencing widespread declines due to global change factors such as landscape fragmentation, climate change, and agricultural intensification. Research and conservation efforts have therefore increased to mitigate dramatic declines of grassland butterflies. Yet, a large amount of baseline information is unknown for butterflies in the mixed-grass prairie region of the Northern Great Plains, especially for obligate species that wholly depend on grasslands to complete their life cycle. We initiated a study to increase the baseline knowledge and availability of rigorous data to inform conservation by investigating 1) butterfly survey methodology, 2) local and landscape influences on butterfly density, 3) invasive plant species impacts on butterfly communities, and 4) butterfly behavior responses to vegetation. We found that visual encounter surveys (VES) and line-transect distance sampling (LTDS) were complementary methods that should be used in butterfly research to improve the availability of quantifiable data. Even though VES were more efficient at detecting individual butterflies, LTDS provided a rapid way to estimate true density estimates for butterflies. Using LTDS density estimates to model species responses to local and landscape variables, we determined that maintaining large grasslands at the landscape level and promoting heterogeneous plant communities and structure at the local level would help conserve obligate grassland butterflies. One way to promote heterogeneous plant communities is to reduce the cover of invasive plant species. Otherwise, flowering forb and plant species richness decline and butterfly communities shift to fewer obligate butterflies. We can better understand butterfly species' declines by incorporating behavioral surveys at the site level. In doing so, we found that monarch behaviors associated with utilizing a site (e.g., nectaring, ovipositing) did not increase as milkweed (obligate host plant) cover increased because nectar resource availability decline. Overall, maintaining large, heterogeneous grasslands and identifying management strategies like fire and grazing to increase host and nectar resource availability will benefit the largest number of grassland butterflies.

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# **CHAPTER 1. COMPARING TWO STATISTICALLY-BASED BUTTERFLY SURVEY METHODS TO IMPROVE BUTTERFLY CONSERVATION EFFORTS**

## **Abstract**

Declines in pollinators are increasing the need for research that investigates pollinator responses to conservation and management actions. Many methods exist to examine butterfly communities, but methods must produce statistically defensible and rigorous data that can be used for conservation planning and policy. Moreover, researchers are often constrained by study logistical features (i.e., time and money). Choosing methods that maximize data quality while minimizing effort will improve our ability to conduct quality butterfly research. To address this issue, we conducted butterfly surveys with two novel and statistically rigorous methods, visual encounter surveys (VES) and line-transect distance sampling (LTDS), at 31 sites over two years to compare differences in butterfly species richness, detections, detection rates, species accumulation curves, and community composition. Our goal was to guide recommendations for researchers and land managers using methods capable of providing detection functions, important in conservation planning. We found higher species richness and detections with VES compared to LTDS when investigating raw data. Standardizing for time-based effort, VES had 30% higher detection rates than LTDS, however rarefaction curves suggest methods detected new species at similar rates. Butterfly communities were statistically different between methods in multivariate ordinations, but community differences between years was stronger than between methods. When accounting for imperfect detection, true density estimates calculated with LTDS were consistently higher than raw density estimates. We recommend LTDS surveys to produce accurate density estimates for butterfly species, but our data indicates VES methods may be more efficient at detecting butterflies. While VES and LTDS are complementary methods, increasing effort with LTDS may be a viable option if only one method is selected. Regardless of objectives, VES and LTDS should be used in butterfly research because they improve upon previously used methods and incorporate detection functions, allowing them to provide quantifiable data for policy decisions.

## **Introduction**

Grasslands continue to decline and pressures on remaining grasslands continue to increase due to anthropogenic forces (Samson et al. 2004). Consequently, grassland-dependent wildlife are declining,

including most butterfly species (Lalibrete and Ripple 2004, Brennan and Kuvlesky 2005, Hardersen and Corezzola 2014). Conservation plans are vital for protecting populations of butterflies because they are important for pollination (Davis et al. 2008), provide food sources for higher trophic levels (Guppy and Shepard 2001), and act as indicator species for biodiversity and environmental alterations (Blair 1999, Potts et al. 2010, Roy et al. 2015). Climate change and continuing grassland declines have sparked increased interests in rigorous butterfly research (Hellmann et al. 2008, Roth et al. 2014), and several methods are currently used to survey and monitor butterflies (Kral et al. 2018). However, older survey methods commonly only produce population indices, lack statistical rigor (Nowicki et al. 2008), and cannot produce population estimates needed for conservation plans and policies (New et al. 1995, Haddadd et al. 2008, Hamm 2013, Henry et al. 2015). Therefore, more insight into utilizing novel methods for butterfly research (i.e., method strengths and weaknesses) are needed.

Generally, butterfly abundance is estimated with transect-based survey methods. Traditionally, the Pollard walk (PW) method was used to conduct surveys with systematically placed transects to count butterflies in limited, fixed-width transects (Pollard 1977, Pollard and Yates 1993). Because transects are placed in areas with higher probabilities of butterfly detection and rely on raw counts, PW transects are biased, only produce abundance indices, cannot produce accurate population estimates, and fail to account for imperfect detection (Brown and Boyce 1998). For these reasons, most population estimates from PW surveys are inaccurate (Anderson 2001) and can only be used for the same site after many years of surveying, making data difficult to compare across studies (van Swaay 2003). Furthermore, PW surveys are not good at detecting species that occur in low abundances or are not easily observed due to species behavior or vegetation characteristics.

Presence-absence data for species with low abundance or cryptic behavior are commonly determined with area-based search methods. Historically, checklist surveys were used to search an entire area for butterflies, giving preference to areas where butterflies are more likely to be found (Royer et al. 1998). However, checklist surveys are also biased, fail to account for imperfect detection, and can only produce population indices when used for long-term monitoring (Hamm 2013). Despite drawbacks of both PW and checklist surveys, they are still commonly used in butterfly research (Roy and Sparks 2000, Zaman et al. 2015, Kral et al. 2018). However, conservation plans need methods with increased statistical

rigor and detection functions to improve comparability across studies and provide defensible population estimates that can be used in policy formation (Nowicki et al. 2008, Henry and Anderson 2016).

Line-transect distance sampling (LTDS) is an alternative method to PW transects that is more effective at producing true estimates of abundance (Table 1), but it is not yet widely implemented for butterflies (Kral et al. 2018). LTDS has been used to quickly and accurately estimate density and true population abundances for birds (Royle et al. 2004), mammals (Marques and Buckland 2003), and invertebrates (Hamm 2013), while accounting for imperfect detection—an individual is present but not detected (Kellner and Swihart 2014). Observers search along randomly-placed transects and estimate the perpendicular distance of all detected individuals from the transect (Buckland et al. 1993). Distances are used to model detection functions—the probability of detecting an individual from a given distance (Buckland et al. 2001)—and achieve better population estimates by accounting for imperfect detection, as the detection of individuals decreases with increased distance from an observer (Brown and Boyce 1998). Additionally, detection functions can incorporate other site variables that affect detection, such as observer, weather, and plant structure improving abundance estimates (Pocewicz et al. 2009). Moreover, accounting for imperfect detection for all species is important because raw counts and indices generally underestimate true population abundances (Kery and Plattner 2007), and individual species may have differing detection rates (Isaac et al. 2011), especially species that are cryptic or sedentary (Kadlec et al. 2012). Randomly-placed transects and incorporation of detection functions that account for imperfect detection allow LTDS surveys to provide estimates of true abundance, an improvement from traditional PW surveys that are more biased and only provide relative abundances (Brown and Boyce 1998, Haddad et al. 2008, Kral et al. 2018).

Visual encounter surveys (VES) incorporate site variables and multiple site visits in a single season to produce occupancy rates—the probability of a site being occupied by a particular species—for species that are hard to detect (MacKenzie et al. 2005). Unlike checklist surveys that provide presence-absence or relative abundance, VES are a powerful method used to systematically search standardized areas to determine occupancy, along with other data (Table 1; MacKenzie et al. 2006, Kadlec et al. 2012). Although this method was originally developed for herpetofauna (Flint and Harris 2005), benefits associated with VES can easily be applied to grassland butterflies that are hard to detect or occur at low

abundances (Bried and Pellet 2012). Additionally, VES data can be used with detection functions to model occupancy rates (MacKenzie et al. 2002). VES methods improve upon checklist surveys that do not account for imperfect detection, potentially report false absences instead of non-detections, and are unable to estimate probability of occupancy.

Table 1. Response variables, logistical features, and statistical features of commonly used butterfly survey and sampling methods: checklist (CK), Pollard walks (PW), line-transect distance sampling (LTDS), and visual encounter surveys (VES). Table adapted from Kral et al. (2018).

	<b>CK</b>	<b>PW</b>	<b>LTDS</b>	<b>VES</b>
<b>Response Variables</b>				
Occupancy	X	X	X	X
Population indices (relative abundance or density)	X	X	X	X
Presence-absence	X	X	X	X
True population estimates (abundance or density)			X	X
<b>Logistical and Statistical Features</b>				
Detection functions			X	X
Inferential strength		X*	X	X
Low input/effort	X	X	X	X
Randomization	X*	X*	X	X
Replication	X*	X*	X	X
Standardized effort		X*	X	X

\*Depending on how the method is designed and conducted

Increasing the use of rigorous methods that incorporate detection functions create more opportunities for controlled experiments to answer species-specific questions for evidenced-based conservation and planning, while limiting extraneous effort that strain logistical resources (Sutherland et al. 2004, Curtis et al. 2015). Such methods can help improve conservation and preventative planning that can reduce species crisis management when individual butterfly species become too rare to study (New et al. 1995). Therefore, our study objective was to improve our understanding of novel butterfly survey methods by comparing two statistically rigorous surveys, VES and LTDS, which are better suited to produce unbiased, scientifically defensible data for butterfly conservation and policy. By comparing species richness, total detections, detection rates, species accumulation curves, and community composition, we can identify the suitable survey methods for land managers with varying objectives and logistical constraints. These comparisons are focused on methodological features that influence survey efficiency and potential biases, with a smaller emphasis on post-hoc analyses that account for imperfect detection, as methods do this in different ways. By making comparisons in this way, we can elucidate how

methods that produce accurate estimates of density or occupancy can be used separately or complimentary to improve butterfly research and conservation. Furthermore, identifying the benefits of using VES or LTDS methods will help improve standardization in butterfly data collection, an important aspect in local monitoring programs and cross study comparisons (Royer et al. 1998).

## Methods

### Study area

We conducted our research in North Dakota, South Dakota, and Minnesota, USA. Across the region, temperatures vary throughout the year ranging from -17 to 23 °C, averaging 5.5 °C (USDA-NRCS 2015). The warmest temperatures occur in South Dakota in July where the average frost-free period is 136 days; the coldest temperatures occur in central North Dakota in January where the average frost-free period is 122 days. Precipitation falls mostly during the growing season and follows a strong gradient east to west, decreasing from 69 cm to 36 cm. Historically, the plant community in the Dakotas was a gradient of tallgrass prairie in the east to a mixed-grass prairie in the west. The tallgrass prairie was composed mostly of native, warm-season grasses including big bluestem (*Andropogon gerardii* Vitman) and little bluestem (*Schizachyrium scoparium* (Michx.) Nash). Moving west, the mixed-grass prairie was dominated by native, cool-season grasses like green needlegrass (*Nassella viridula* (Trin.) Barkworth) and western wheatgrass (*Pascopyrum smithii* (Rydb.) Á. Löve). Recently, invasive, cool-season grasses, mostly smooth brome (*Bromus inermis* Leyss.) and Kentucky bluegrass (*Poa pratensis* L.), have become dominant in many grasslands, inhibiting native plants (Murphy and Grant 2005). The forb community is similar in both areas, being dominated by asters (*Aster spp.*) and goldenrods (*Solidago spp.*; USDA-NRCS 2015).

We selected sites from U.S. Fish and Wildlife Service (USFWS) refuges throughout the Northern Great Plains. We systematically eliminated sites that did not have enough room to conduct surveys ( $\leq 20$  ha, see survey description below) or were composed entirely of open-water habitats. From the remaining USFWS sites, we randomly selected 20 sites to survey in 2015. After the 2015 field season, we eliminated six sites that lacked butterfly diversity and were highly invaded by exotic plants (>50% canopy cover). Additionally, we expanded our survey efforts to include some privately-owned or non-governmental organization sites in North Dakota, South Dakota, and Minnesota. In 2016, we surveyed 25

sites, and over two years, we surveyed 31 unique sites that ranged in size from 25-350 ha. Regardless of management unit size, we randomly placed two 100 m transects for LTDS and two 200 m × 200 m blocks for VES within each site that were all at least 100 m apart. We adjusted points if necessary (e.g., random point was too close to open water or edge to fit survey area).

### **Butterfly surveys**

We surveyed each site between June and early August, making three site visits per year. Each year, we rotated the time of day and order of surveys to reduce temporal bias. Furthermore, we conducted surveys during optimal field conditions for butterflies: between 0900 and 1800 hours (CDT), when air temperatures were between 21 - 35 °C, winds were under 25 km · hr<sup>-1</sup>, and cloud cover was less than 50% (Royer et al. 1998). For each site, we surveyed two 100 m transects (LTDS method) and two 4 ha plots (VES method). At each study site, surveys were split between two observers, with each observer surveying only one LTDS and one VES. Observers rotated on subsequent visits to reduce observer bias. There were two observers each field season with KK being the primary observer both years.

Observers used LTDS to determine butterfly species richness, total detections, and detection rates. We conducted LTDS surveys by walking 100 m transects marked with a measuring tape at a rate of approximately 10 m · min<sup>-1</sup> (Moranz et al. 2012; Figure 1). Observers recorded all butterfly species detected ahead or to either side of the line, with no constraints on the distance observers could record butterflies. However, special attention was given to detect all individuals on the transect (Buckland et al. 2001). During surveys, observers estimated the perpendicular distance from each individual butterfly to the transect.

Observers used VES to determine butterfly species richness, total detections, and detection rates (Harms et al. 2014; Figure 1). Locating the edges with a GPS unit, observers walked at a rate of approximately 25 m · min<sup>-1</sup> throughout VES plots for 30 min in a zigzag pattern (Hardersen and Corezzola 2014). Our VES blocks were large enough to enclose three 100 m transects with approximately 50 m on each site to create the basis for the zigzag pattern. VES plots are meant to be larger in order to search the site thoroughly and identify as many species as possible. Therefore, observers were not restricted to where they spent their time and could search longer in areas with higher floral resources (Royer et al. 1998). As observers searched, they recorded all butterflies seen within the plot, noting the species and

number. For both LTDS and VES, observers recorded their own data. Additionally, if a butterfly could not be identified, observers suspended the survey, took a picture or attempted to capture the butterfly with a net, and then resumed the survey at the stopped point.

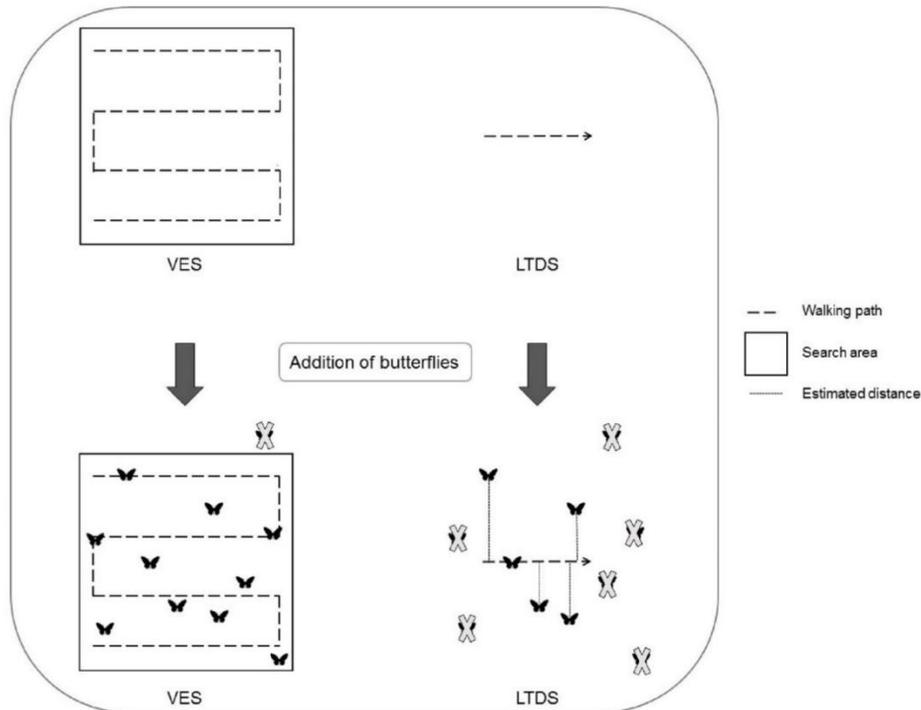


Figure 1. Graphical explanation of visual encounter survey (VES) and line-transect distance sampling (LTDS) methods to visually detect butterflies. Observers walk in a zigzag pattern throughout 4 ha VES plots for 30 min, but they are restricted within the search area. In LTDS plots, observers are only allowed to walk the 100 m transect for approximately 10 min. The area covered in LTDS is variable because detectability differs between species. Using the same butterfly community for each method, observers exclude individuals that fall outside the surveying area (X). Individuals are counted in the VES plot, whereas distances between butterflies and the line are estimated in LTDS.

## Analysis

We first provide a qualitative analysis of our results across all study sites in each year. For each method, we determined the total species richness as the number of species detected across all sites, the total detections across all sites, the detection rate, and the number of unique species detected using one method but not the other (Table 2). To calculate detection rates, we took the total number of detections and divided by the total effort to produce detections per minute.

Next, we used generalized linear mixed-effects modeling (GLMM), rarefaction curves, and multivariate ordination to compare the two methods using count data from each site. GLMM allowed us to

compare single variables (detections per site, detection rates, and species richness per site) between methods. Using the *lme4* package in R, we created GLMMs incorporating site as a random effect to compare single variables between methods at the site level within years ( $n = 20, 25$ ; Kadlec et al. 2012, Bates et al. 2015, R Development Core Team 2015). To investigate species richness further, we used the “specaccum” function to randomly derive sample-based rarefaction curves based on effort, not on the order of species detection, in the *vegan* package for R (Oksanen 2015). Moreover, we used the “specpool” function in the same package with a bootstrap estimator to pool species richness over sites and more accurately estimate species richness (Oksanen 2015).

Finally, we compared the butterfly community composition using multivariate ordination. We first organized our data by totaling counts for every species at each site based on method and year. To standardize the counts by time-based effort, we divided the total counts obtained with the VES method by three to match the effort in the LTDS method. To use the full potential of LTDS data, we also compared the community using species density while accounting for imperfect detection. We calculated true density estimates ( $\text{individuals} \cdot \text{ha}^{-1}$ ) with LTDS data using a combination of key functions and series expansions in Program Distance (Buckland et al. 2001) according to procedures outline by Harms et al. (2014) and Pocewicz et al. (2009). We pooled detections across years and put zeros for species without enough detections to calculate density estimates ( $< 60$ ). We calculated VES density estimates by totaling the number of raw detections for each species and dividing by the total area (4 ha) to give  $\text{individuals} \cdot \text{ha}^{-1}$ . We then used metric multidimensional scaling (MDS) with the “capscale” function and Bray-Curtis distance measures to create community ordination plots in the *vegan* package for R (Kindt and Coe 2005, Oksanen 2015). We used the function “envfit” within the *vegan* package to test how year and method correlated with the butterfly community (Moranz et al. 2012). For each analysis, we set an  $\alpha \leq 0.05$  to indicate statistically significant differences between factors.

## Results

We made  $>7,000$  butterfly detections and observed 37 different butterfly species over two years of data collection (Table 2; Table A1). In 2015, the total species richness across sites and number of unique species were similar between VES and LTDS, but the number of detections and the detection rate were greater with the VES method. The following year, the number of detections and species observed

across all sites were higher for both methods than in 2015, but similar patterns appeared between methods. We still observed a greater number of species, detections, detection rates and unique species in the VES method, with only one unique species detected using LTDS (Table 2).

Table 2. Totals across all sites for butterfly species richness, detections, detection rate, and number of unique species in visual encounter survey (VES) and line-transect distance sampling (LTDS) methods used from North Dakota and South Dakota in 2015-2016. We used the effort (in minutes) for each method in both years with the detections to calculate the detection rate across all sites.

		<b>Totals</b>				
		<b>Species Richness</b>	<b>Detections</b>	<b>Detection Rate (Individuals· min<sup>-1</sup>)</b>	<b>Number of Unique Species</b>	<b>Effort (min)</b>
<b>2015</b>	<b>VES</b>	21	1690	0.47	2	3600
	<b>LTDS</b>	20	320	0.27	1	1200
<b>2016</b>	<b>VES</b>	36	4201	0.93	10	4500
	<b>LTDS</b>	27	1076	0.72	1	1500

The average number of detections per site differed annually ( $z = 5.35$ ,  $df = 89$ ,  $p \leq 0.001$ ), therefore we compared differences between methods within each year. Detections per site (2015:  $z=10.7$ ,  $df = 19$ ,  $p \leq 0.001$ ; 2016:  $z = 1.6$ ,  $df = 24$ ,  $p \leq 0.001$ ) and detection rates (2015:  $z = 8.66$ ,  $df = 19$ ,  $p \leq 0.001$ ; 2016:  $z = 4.49$ ,  $df = 24$ ,  $p = 0.02$ ; Figure 2) were statistically different between methods. The VES method had four times as many detections compared to LTDS surveys despite having only three times more effort (i.e., time spent surveying). Additionally, when controlling for effort, VES had approximately 30% higher detection rates compared to LTDS.

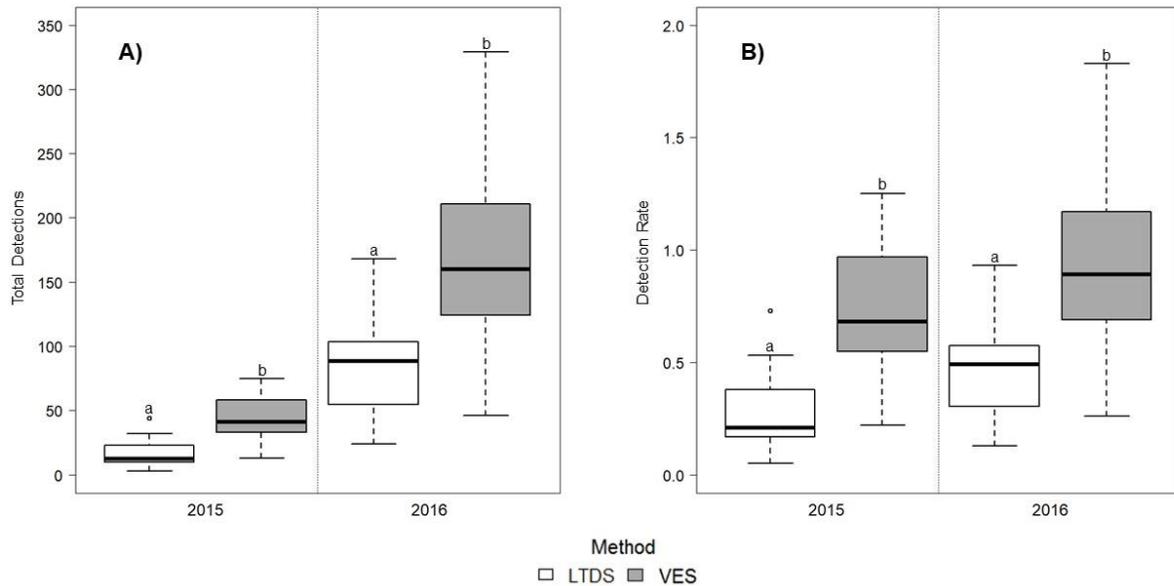


Figure 2. Comparisons between line-transect distance sampling (LTDS) and visual encounter survey (VES) methods for butterfly A) average detections per site and B) average detection rate per site separated by year. Butterfly surveys were conducted across North Dakota and South Dakota in 2015-2016. Differing letters denote statistically significant differences between methods within each year at an  $\alpha \leq 0.05$ . The VES method consistently produced higher numbers in both categories compared to LTDS, even when accounting for effort.

Species richness per site also differed by method annually (2015:  $z = 14.8$ ,  $df = 19$ ,  $p \leq 0.001$ ; 2016:  $z = 9.00$ ,  $df = 24$ ,  $p \leq 0.001$ ; Table A1). However, we allocated three times as much effort in VES surveys (30 min) compared to LTDS surveys (10 min). Rarefaction curves, which account for effort, showed that the average species accumulation for both methods in each year overlapped (Figure 3; Table A1). Overlapping curves indicate that the two methods appear to have accumulated new species at the same rate, at least over the first 1200 (2015) or 1500 (2016) min of effort where we can compare both methods.

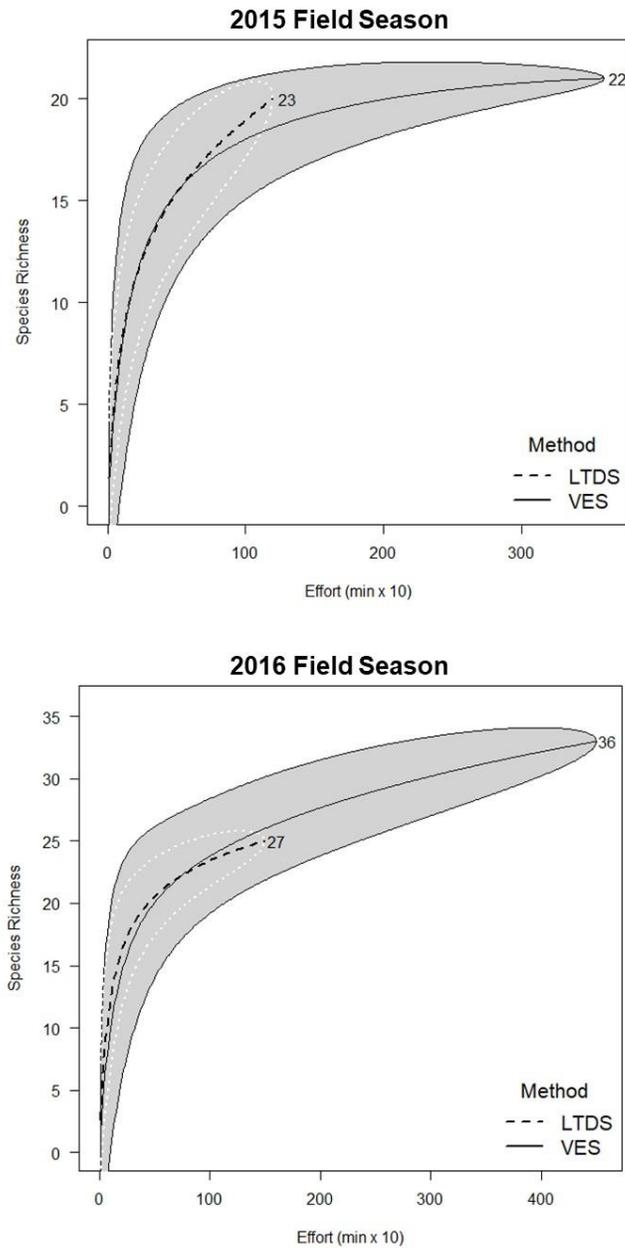


Figure 3. Species accumulation curves for line-transect distance sampling (LTDS) and visual encounter survey (VES) methods used throughout North Dakota and South Dakota in 2015 and 2016. Rarefaction curves are shown for LTDS (dotted lines) and VES (solid lines) with 95% confidence intervals. Additionally, we included the species richness predicted by pooling across survey sites. Generally, the rate of detecting new species did not change between methods, but with more effort allocated to the VES method, more species were found overall.

Ordination results showed a significant difference in the butterfly community between the two methods ( $R^2 = 0.10$ ,  $p < 0.001$ ) and between years ( $R^2 = 0.67$ ,  $p < 0.001$ ) using raw counts (Figure 4).

However, butterfly communities were more similar within the same year than the same methods across

years. No solution could be reached for the ordination using density estimates. However, species density estimates were consistently underestimated with VES (Table 3). To look at the community in a separate way, we also used the number of raw detections from LTDS, VES, along with corrected density estimates to compare the relative community composition (Figure 5). Raw numbers from LTDS and VES produced similar relative community composition. However, the composition of several species (*Speyeria aphorodite*, *Phyciodes tharos*, *Polites mystic*) that made up a small portion of the community using raw numbers made a more substantial contribution with corrected LTDS numbers (Figure 5).

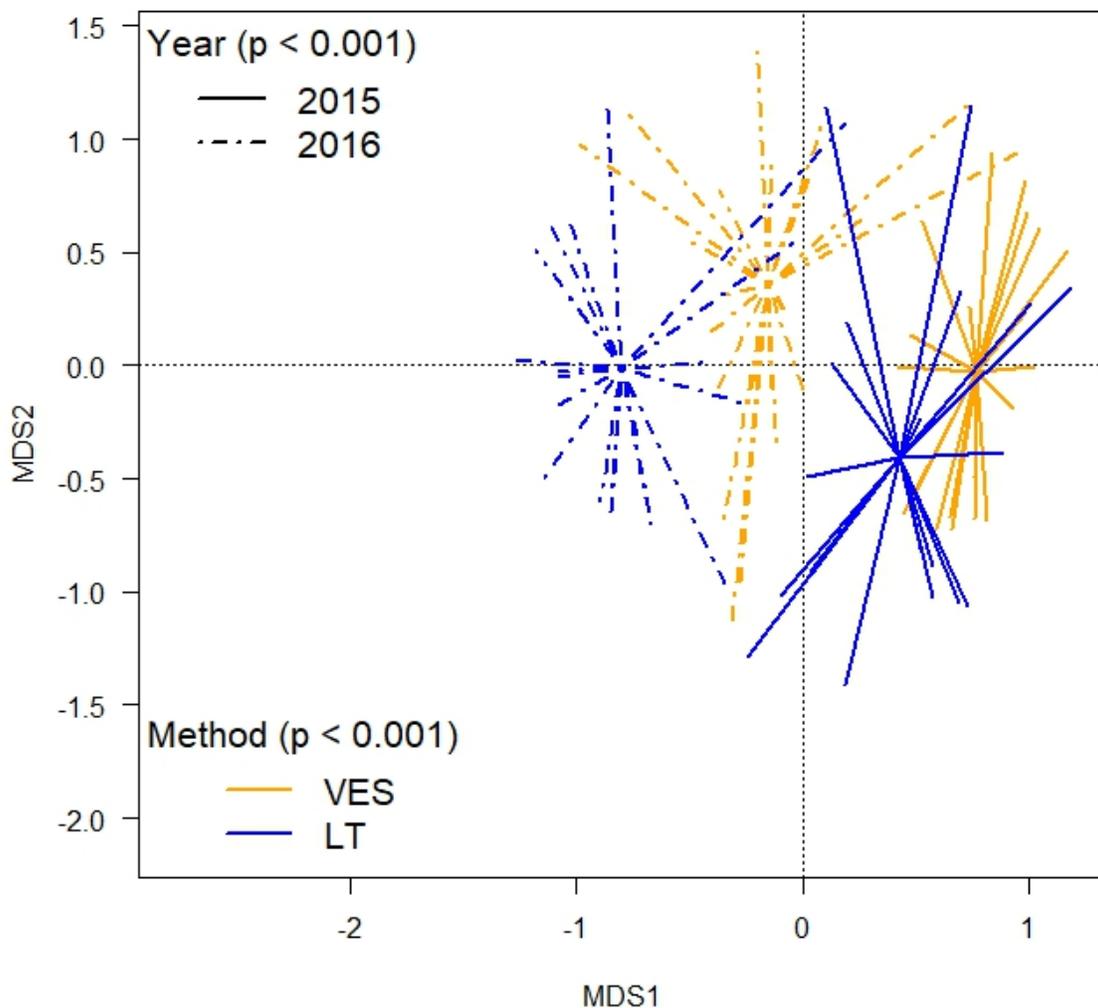


Figure 4. Multidimensional scaling ordination for the butterfly community data collected across North Dakota and South Dakota from 2015-2016 using visual encounter survey (VES) and line-transect distance sampling (LTDS) methods. Site scores are grouped by year (dashed or solid lines) and method (blue or gold lines). The first two axes explained 72% of the variation observed in the data. Year ( $R^2 = 0.67$ ) and method ( $R^2 = 0.10$ ) were both significantly correlated with the ordination, however communities in each year were more closely related to each other than similar methods between years.

Table 3. Density estimates (individuals · ha<sup>-1</sup>) for 12 butterfly species in North Dakota and South Dakota from 2015-2016. Line-transect distance sampling (LTDS) density that accounts for imperfect detection using Program Distance. Visual encounter survey (VES) density was calculated using the raw counts for each species divided by the total area searched (4 ha) to produce individuals · ha<sup>-1</sup>. Densities are compared using the relative percent decrease ( $[(N_{LTDS}-N_{VES})/N_{LTDS}]$ ) from LTDS to VES, as VES consistently underestimated density.

<b>Species</b>	<b>LTDS Density</b>	<b>VES Density</b>	<b>Percent Difference</b>
<i>Boloria bellona</i>	1.25	0.26	79%
<i>Cercyonis pegala</i>	8.41	0.82	90%
<i>Coenonympha tullia</i>	2.36	0.37	84%
<i>Colias eurytheme</i>	6.40	0.63	90%
<i>Colias philodice</i>	4.55	0.62	86%
<i>Danaus plexippus</i>	1.13	0.17	85%
<i>Phyciodes tharos</i>	6.87	0.25	96%
<i>Pieris rapae</i>	2.42	0.36	85%
<i>Plebejus melissa</i>	2.00	0.19	91%
<i>Polites mystic</i>	3.66	0.20	95%
<i>Speyeria aphrodite</i>	5.16	0.24	95%
<i>Speyeria idalia</i>	0.82	0.16	81%
<b>Total</b>	<b>43.78</b>	<b>4.29</b>	<b>90%</b>

### Discussion

Demands for butterfly research are increasing as butterfly populations continue to decline globally (Roth et al. 2014). As these trends persist, research efforts are expanding to try to quantify butterfly populations and to more thoroughly understand their contribution to ecosystem services (Davis et al. 2008, Roy et al. 2015). More frequently, checklist surveys and PW transects are used to collect butterfly data (Pollard and Yates 1993, Droege et al. 1998), but these methods are often biased, fail to account for imperfect detection, and only produce site indices that are inappropriate for creating effective conservation plans (Brown and Boyce 1998, Henry et al. 2015). We compared two novel methods (VES and LTDS) that account for imperfect detection to help guide future research and determine which method matches project objectives and logistics. Moreover, as similar studies have shown, VES had significantly higher detections compared to LTDS surveys (Kadlec et al. 2012). Contrary to expectations, butterfly communities differed between method and year, but annual differences were stronger than method type (Jakubikova and Kadlec 2015), possibly due to annual climatic changes (Oliver et al. 2012). Our original findings were that the VES method produced higher detection rates, even though detection rates were standardized by effort, and the VES and LTDS methods accumulated species at approximately the same rate.

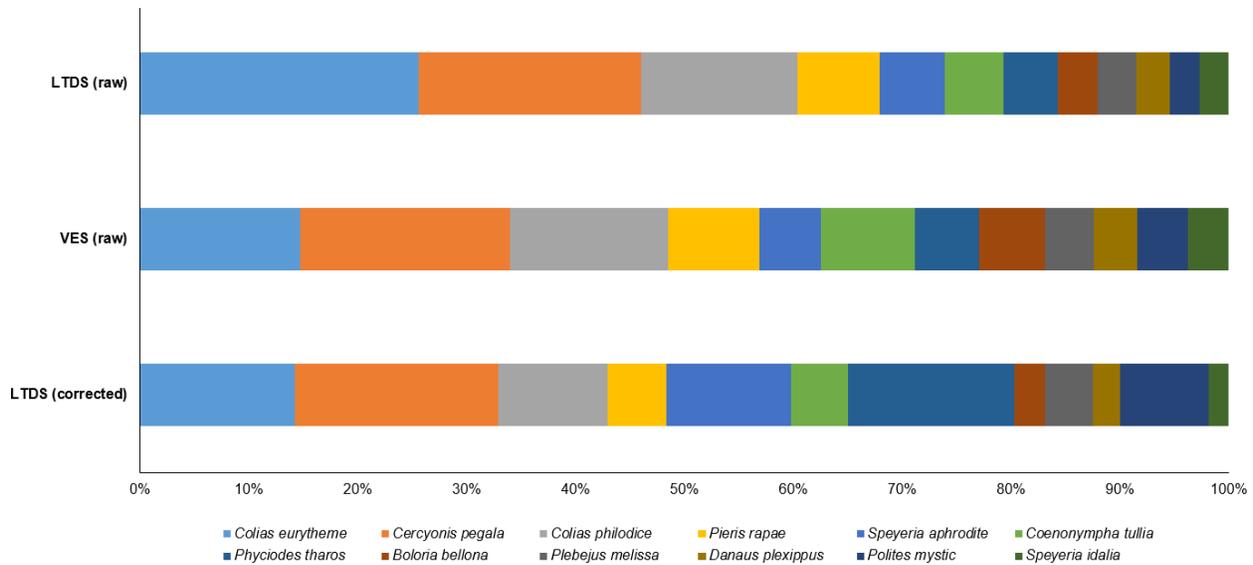


Figure 5. Relative community composition (percent) for 12 butterfly species from surveys conducted across North Dakota and South Dakota in 2015 and 2016. Community composition is presented for line-transect distance sampling (LTDS) or visual encounter survey (VES) methods. Raw detections are used for the VES community, but raw and corrected estimates are both used for LTDS community composition. Species are ranked by their relative abundance from the LTDS raw group. Relative community composition is similar between raw LTDS and VES communities. However, the relative composition of lower abundance species increases with corrected LTDS estimates.

We observed significant differences in the number of detections and species richness between methods, with both response variables being higher in VES compared to LTDS. This was expected, as other studies comparing area searches to line transects reported similar results (Royer et al. 1998, Kadlec et al. 2012). Accounting for effort did not equalize detections between methods, as detection rates were still higher in the VES method. This also occurred in another study where standardizations were based on counts per hour (Royer et al. 1998). In VES, observers can complete more exhaustive searches by allocating more of their time in areas where butterflies are likely to congregate (Kadlec et al. 2012). Since observers are not permitted to wander off the transect in LTDS surveys, they are limited to the area near the transect, which may or may not be suitable for butterflies. The ability to search portions within the standardized area with a higher likelihood of butterflies increases the total number of detections. However, it was the increase in time, not the ability to search in areas butterflies are more likely to congregate, that increased species richness in the VES method. Consequently, species rarefaction curves that accounted for effort were similar between methods.

Butterfly communities varied between method and year. Typically, two different methods will collect similar community composition when used in the same location (Jakubikova and Kadlec 2015), and other studies that compare area searches to line transects detected both similar species and population trends (Kadlec et al. 2012, Roy et al. 2015). Comparing methods that vary more methodologically (e.g., trapping vs. transect counts) are more likely to result in different butterfly communities (Jakubikova and Kadlec 2015), but we did observe changes between methods, most likely due to species abundance changes between methods. Even when accounting for effort, the VES method had more detections of all species and changed the overall composition compared to LTDS. If we were able to compare the entire butterfly community with density estimates from LTDS, this same trend may not have occurred based on the difference between density estimates for each method and the proportional change in species composition. However, annual community differences were still more pronounced compared to method.

Despite our focus on comparing the two methods, one notable result was the variability in butterfly communities between years. Detections were over two times higher in 2016 compared to 2015. Moreover, we detected 15 new species in 2016 that were not detected in 2015. Annual differences could be attributed to changes in observer (Swengel 1990, Royer et al. 1998, Kadlec et al. 2012) or eliminating less diverse sites, but changes are more likely due to yearly fluctuations in climatic variables (Oliver et al. 2012, Roy et al. 2015), especially since one observer was the same both years, a majority of sites remained the same each year, and the relationship between the methods (i.e. community changes and VES producing higher detection rates) was consistent.

LTDS surveys are slowly increasing in popularity because they are used to quantify accurate estimates of density and abundance (Pocewicz et al. 2009, Moranz et al. 2014), which in turn makes it easy to compare across landscapes and projects. Accounting for detected and undetected individuals can greatly impact species estimates and community composition, as we observed with our own data. Transect counts, like LTDS surveys, are also easy to complete and allow observers to cover large areas (Collier et al. 2008), and they are often recommended for long-term monitoring schemes that need quick, reliable data (Royer et al. 1998, Kadlec et al. 2012). Even though LTDS provides butterfly abundances critical for conservation planning, it requires 60 detections per species to calculate accurate abundances

(Buckland et al. 2001). Therefore, studies specifically investigating hard-to-detect and low abundance species should consider using VES to determine occupancy or adding more effort to LTDS.

In many situations, a combination of methods is preferable to collect site lists efficiently and provide accurate population estimates (Royer et al. 1998, Collier et al. 2008, Haddad et al. 2008, Jakubikova and Kadlec 2015). Often, checklist surveys are used once to obtain a list of resident species, with PW methods being used for long-term monitoring (Royer et al. 1998). Likewise, we determined that LTDS surveys should be utilized for quick data collection, conservation plans, and long-term monitoring, as data can be used to determine unbiased estimates of abundance. However, we suggest, if logistics allow, that VES or more LTDS transects should be used on a yearly basis due to annual changes in rare and hard-to-detect species.

We encountered three difficulties when comparing our methods. First, we were unable to compare the entire community using corrected density estimates for both methods. VES density estimates do not account for imperfect detection, therefore they underestimated species density. But, without more species detections, LTDS could only estimate true density estimates for 12 species. Consequently, the disparity between corrected and uncorrected density estimates and number of species used in community analyses prevented unbiased comparisons, and we were only able to compare butterfly surveys based on methodological differences affected species detections and rates rather than how data can be adjusted for imperfect detection and analyzed. Second, it would be interesting to determine how species' detection functions (the probability of detecting an individual from a given distance) differed between methods, but we were unable to compare detection functions because they are calculated in different ways. LTDS detection functions are modeled using counts and distance measurements within various key and series adjustments (Buckland et al. 2001), whereas detection functions for VES are defined and modified in the statistical software (MacKenzie et al. 2002). The third difficulty was the difference in area covered between methods. We chose to standardize methods on time-based effort, but we considered standardizing based on area. However, the area covered by methods varied, especially since LTDS, in practice, covers fluctuating areas due to detectability changes by species (Dennis et al. 2005). Consequently, we found it ineffective to compare methods in this way and chose to standardize by time-based effort.

## Conclusions

Researchers and land managers need efficient methods to survey butterflies to meet research objectives, fit logistical constraints, and provide vital information for conservation efforts, as butterflies are experiencing widespread decline (Hardersen and Corezzola 2014). More importantly, researchers and land managers need methods that incorporate detection functions to produce unbiased, quantifiable data (Nowicki et al. 2008). We compared differences in species richness, detections, and detection rates between VES and LTDS, two methods that incorporate detection functions. We found that VES produced higher detections and detection rates compared to LTDS, possibly because the VES method allows observers to search in areas where butterflies are more likely to be found. Although species richness was higher in VES, rarefaction curves showed that species accumulated similarly between methods, but there were still significant differences in the community between method and year. During the two years of data collection, we found major differences in the number of individuals and species detected due to annual climatic variation. From our results, we found LTDS more appropriate for collecting data to calculate accurate estimates of abundance. Alternatively, VES were more efficient for detecting rare species, producing complete site lists, and calculating occupancy rates. LTDS may be better suited for projects answering broad questions about the butterfly community since they are easier and faster to complete. However, projects with a major focus on rare species should either implement VES or increase LTDS effort. If possible, a combination of methods is helpful for most research projects to improve detection efficiency (VES) and accurate density estimates (LTDS). Then, researchers and land managers can use VES and LTDS methods to collect quantifiable data for rare and abundant species, crucial for improving research and conservation efforts for all butterfly species.

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## CHAPTER 2. MULTI-SCALE CONSIDERATIONS FOR GRASSLAND BUTTERFLY CONSERVATION IN AGROECOSYSTEMS

### Abstract

Global change threatens the persistence of multiple taxonomic groups, including butterflies. While conservation efforts for butterfly populations have consequently increased, they are often hampered by a lack of true density estimates along with a lack of investigation of specific ecological factors that influence density. Our overall objective was to enhance current grassland butterfly conservation efforts by using line-transect distance sampling, a method that accounts for imperfect detection, to calculate true density estimates and model the influence of landscape and local variables on butterfly density in the Northern Great Plains, USA. We calculated true density estimates for five obligate and ten facultative grassland species to produce one of the most extensive datasets for butterfly densities to date. In contrast to most previous research, we found that landscape variables influenced butterfly density more often than local variables. Specifically, the percent cover of perennial grasslands, crop lands, and wetlands appeared in 90% of species models, whereas common local variables— forb richness and invasive plant cover— appeared in 60% of best-ranked models. We expected obligate species' density to decrease as invasive plants increased, but species' responses were mixed based on larval diet, not species habitat associations. Additionally, best-ranked models for *Danaus plexippus* and *Speyeria idalia*, two species of conservation concern with obligate host plant requirements, did not include host plant availability at the local scale. However, landscape variables included in models for these species often determine the presence of local vegetative variables. Our results reiterate the importance of modeling species responses to variables at across multiple scales and the need for conserving large tracts of grasslands. Although our results emphasize the need for conservation at the landscape scale, managing for heterogeneous local scale variables will also help conserve obligate grassland butterflies.

### Introduction

Butterflies provide a variety of ecosystem services including pollination and cultural aesthetics (Davis et al. 2008, Lopez-Hoffman 2010, Cardinale et al. 2012). However, due to their sensitivity to land use alterations and environmental change (Winfree et al. 2011, Roy et al. 2015), butterflies are declining as global change drivers increase (e.g., landscape fragmentation, species habitat simplification, and

climate change; Thomas et al. 2004, Potts et al. 2010). Continued declines in species diversity and abundance can lead to species extinctions (Brook et al. 2008, Urban 2015), with such localized extinctions depending on species' traits that influence specific responses to challenges like land use change (Bartomeus et al. 2018). Therefore, research focused on butterfly conservation has increased to better understand synergistic responses to global change that influence landscape configuration and local resource availability (e.g., Flockhart et al. 2014, Hindle et al. 2015). Still, conservation efforts need to expand and incorporate empirical data and species ecological information necessary for conservation planning to reduce broad- and local-scale declines (Sutherland et al. 2004).

Conservation management plans to protect and bolster butterfly populations are ideally based on scientific data. However, the data used to create butterfly conservation plans is often anecdotal or lacks statistical rigor (Sutherland et al. 2004, Nowicki et al. 2008). Additionally, most research focuses on rare species (e.g., Weibull et al. 2003, Haddad et al. 2008, Longcore et al. 2010), while more abundant or common species are ignored. Common species can also become rare in certain cases (Van Dyck et al. 2009), and without baseline data, land managers and agencies are forced to determine conservation strategies with limited knowledge of those populations (New et al. 1995). Therefore, not only is more butterfly data necessary for all species (Royer et al. 1998) but more rigorously collected and analyzed data is needed to provide accurate estimates that can be incorporated into modeling of resource use and selection (Pocewicz et al. 2009).

Research that increases the amount of data and improves conservation efforts across butterfly species should measure species' responses to ecological variables and provide quantifiable, unbiased information necessary for research-based conservation plans (Sutherland et al. 2004, Kery and Plattner 2007, Kral et al. 2018). Specifically, conservation plans need data that determines species' ecological responses to local and landscape variables (Pocewicz et al. 2009). Butterfly populations are influenced by different variables depending on scale and species (e.g., nectar resources, larval diet, connectivity; Samways 2007). Determining which variables are influential on abundance can inform conservation strategies by identifying advantageous management strategies and specific resources necessary for life stage requirements (Habel et al. 2016). In addition, plans need true density estimates that account for both individuals detected and those not detected but likely present, especially for species of conservation

concern (Isaac et al. 2011). True density estimates provide land managers and policy makers the accurate and defensible population estimates necessary for conservation plans and legislation (New et al. 1995, Pollock et al. 2002, Pellet 2008).

Butterfly density is influenced by both landscape and local variables (Davis et al. 2007), with species' responses often dependent on their life history requirements (e.g., facultative or obligate; Vickery and Herkert 1999). Landscape variables such as land cover are drivers of butterfly density because they regulate species' colonization and extinction (Sweaney et al. 2014, Fourcade et al. 2017, Bartomeus et al. 2018). Common landscape variables that have been shown to influence butterfly density and abundance are road density and crop cover (Davis et al. 2007, Ekroos et al. 2010). However, common local variables shown to influence butterflies include floral abundance, plant community composition, vegetation height, and litter ground cover (Davis et al. 2007, Poyry et al. 2009, Habel et al. 2016). Because local variables often directly measure host and nectar resource availability (Collinge et al. 2003, Wallisdevries et al. 2012, Farhat et al. 2014), they are expected to significantly impact butterfly density more than landscape variables (Mazerolle and Villard 1999, van Halder et al. 2017, but see Sweaney et al. 2014).

Landscape and local variables that impact butterfly density, once identified, can be used to direct management and conservation decisions. Implementing management strategies are often more applicable at the local scale compared to the landscape scale (Cowlshaw et al. 2009). Nonetheless, landscape variables play a critical role in conservation management and can affect or mask local variables and subsequent species' responses (Papankolaou et al. 2017). For example, butterfly density is expected to decrease if local resources are not available at the site level, but species' densities may remain constant if the surrounding landscape provides necessary resources (van Halder et al. 2017). Similar interactions are observed in other obligate grassland species such as Great Prairie-Chickens, where conservation efforts focus on different scales depending on landscape filters and habitat suitability (Hovick et al. 2015). Therefore, modeling species' responses to local and landscape resources at multiple scales is both appropriate and necessary to inform conservation efforts (Pocewicz et al. 2009, Bennett and Gratton 2012).

Conservation efforts for butterflies can be improved through multi-species, multi-scale research that focuses on modeling ecological and management variables of interest (New et al. 1995, Pollock et al.

2002, Pellet 2008, Pocewicz et al. 2009). Our objectives were to 1) quantify true density estimates with line-transect distance sampling (LTDS), and 2) model local and landscape variables that may affect butterfly density in mixed-grass prairies of the Northern Great Plains, USA. Therefore, our results will inform important data gaps in the Northern Great Plains for facultative and obligate grassland butterflies, including species of conservation concern like the monarch (*Danaus plexippus*, L. 1758) and regal fritillary (*Speyeria idalia*, Drury 1773), providing critical data for imperiled pollinators proposed for listing under the Endangered Species Act (USFWS 2017). Our data on both declining and more abundant species will form a crucial foundation for current and future grassland butterfly conservation efforts and policy formation.

## Methods

### Study area and site selection

We collected data primarily in North Dakota with additional sites in South Dakota and Minnesota, USA (Figure 6). Across the region, temperatures vary from -17 to 23 °C, averaging 5.5 °C (USDA-NRCS 2015). The area receives most of its precipitation during the growing season along a strong east to west gradient that averages 69 cm in the east to 36 cm in the west. Historically, the plant community also followed an east-west gradient where tallgrass prairie in the east was composed mostly of native, warm-season grasses, and the western mixed-grass prairie was dominated by native, cool-season grasses (Prosser et al. 2003, USDA-NRCS 2015). Invasive, cool-season grasses such as smooth brome (*Bromus inermis* Leyss.) and Kentucky bluegrass (*Poa pratensis* L.), have invaded grasslands, reducing native plant cover (Murphy and Grant 2005).

We focused site selection on federally managed U.S. Fish and Wildlife Service (USFWS) refuges within our study region that had enough terrestrial area to conduct surveys ( $\geq 20$  ha; Kral et al. *In Review*). From these available sites, we randomly selected 20 sites to survey in 2015, and we expanded our survey efforts to include some privately-owned and non-governmental organization sites in 2016 ( $n = 25$ ) and in 2017 ( $n = 26$ ). We surveyed 35 unique sites in total over three years.

### Butterfly surveys

Within each site, we randomly placed two, 100 m transects. We adjusted points if necessary (e.g., random point was too close to open water or edge to fit survey area). We surveyed each site three

times between June and early August rotating the time of day and order of surveys to reduce temporal biases. We conducted surveys during optimal field conditions for butterflies— between 0900 and 1800 hours (CDT), when air temperatures were between 21 - 35 °C, winds were under 25 km · hr<sup>-1</sup>, and cloud cover was less than 50% to maximize detectability (Royer et al. 1998). Two observers (KCK being the primary observer each year) surveyed two, randomly placed 100 m transects at each site, with each observer surveying one transect. Observers rotated between transects on subsequent visits to reduce observer bias.

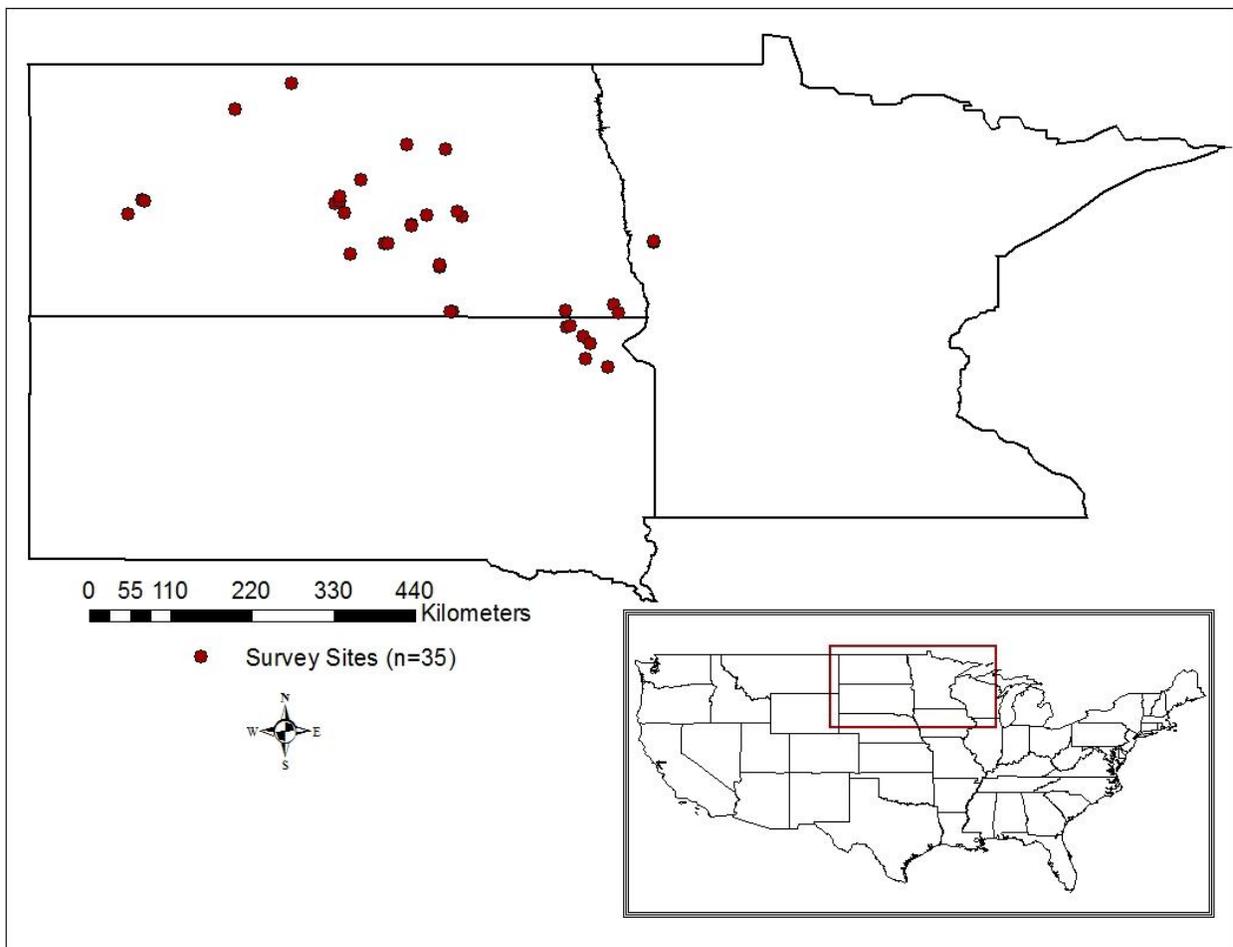


Figure 6. Location of butterfly surveys conducted from 2015-2017 in Minnesota, North Dakota, and South Dakota, USA. In total, we surveyed 35 sites, visiting each site three times in the year(s) it was surveyed.

We conducted butterfly surveys using LTDS (Moranz et al. 2012). Line-transect distance sampling allows us to calculate true density estimates by accounting for individuals observed and those

not observed but likely present by incorporating detection functions (Buckland et al. 2001, Pocewicz et al. 2009). Utilizing detection functions are extremely valuable for butterfly conservation and reduce biases associated with abundance indices (Nowicki et al. 2008), but methods that incorporate detection functions are generally rare in butterfly research (Kral et al. 2018). To meet assumptions of LTDS, observers had to 1) detect all butterflies on the line, 2) detect butterflies at their initial location, and 3) accurately record distances from butterflies to the line (Buckland et al. 2001). Observers were trained and calibrated before each field season on estimating distances. Observers walked the two transects marked with a measuring tape at a slow rate ( $\sim 10 \text{ m} \cdot \text{min}^{-1}$ ) to maximize butterfly detectability. When a butterfly was detected, observers estimated the perpendicular distance from each individual butterfly to the transect. If a butterfly could not be identified, observers marked their stopping point on the transect, took a picture or captured the butterfly with a net, and then resumed the survey at the stopped point.

### **Landscape and local variables**

We created 1,000 and 2,500 m buffers around each survey site (Bergman et al. 2004, Pocewicz et al. 2009) and determined land cover in those buffers using USGS National Land cover datasets in ArcGIS (v. 10.2, ESRI 2013). We used two different sized buffers since butterflies respond to landscape variables at different scales (Krauss et al. 2003). Within each buffer, we quantified the percentage of major cover types including perennial grasslands, wetlands, hay grounds, open water, croplands, and developments (roads, buildings, etc.). We also used the land cover data to calculate landscape development indices (LDI), a measurement of human disturbance, for each site across both buffers to combine land cover and use into one metric (Brown and Vivas 2005). Landscape development indices weigh landscapes with more human development (e.g., croplands, urban areas) higher than landscapes with less development (grasslands, wetlands, open water).

At the local level, we collected site specific plant community data to quantify resource availability for butterflies. We counted the number of flowering ramets in  $2.5 \times 2.5 \text{ m}$  belts along transects during each survey to obtain nectar resource density (Moranz et al. 2012). We also used a Robel pole and  $1 \text{ m}^2$  quadrat frames to measure vegetation height and estimate species canopy cover during peak plant production in late July. Every 20 m along the line transect (6 points), we measured visual obstruction with a Robel pole (Robel et al. 1970) and visually estimated canopy cover to the nearest percent and placed

them in cover class (0-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-95, and 95-100). We also recorded current management strategies as grazed (16 sites), burned and grazed (3 sites), burned and no graze (7 sites), hayed (1 sites), and idled (8 sites) at each site visit and broadly grouped management strategies as disturbance or no disturbance as we did not impose treatments.

### **Density estimates**

We used LTDS data in Program Distance (v. 6.2, Thomas et al. 2010) to model detection functions and calculate true density estimates across all sites (global density) and at the site level (site density) for species detected at least 30 times (Stanbury and Gregory 2009). To increase the number of species with density estimates, we pooled species detections across sites and years (Pocewicz et al. 2009). Additionally, we used Program Distance to quantify effective strip width from our data. Effective strip width (ESW) allows us to determine the approximate area proficiently surveyed for each species, as ESW describes the distance from the transect where the probability of detecting individuals outside that distance is as likely as not detecting individuals inside the distance (Thomas et al. 2002).

Program Distance fits a model detection function to determine the probability of detecting an individual at a certain distance from the transect using the distribution of perpendicular distance estimates from the line transect (Buckland et al. 2001, Powell et al. 2007). Specifically, we used multiple covariate distance sampling (MCDS) to create several models with a combination of half-normal and hazard-rate key functions and cosine and Hermite polynomial series expansions (Thomas et al. 2010, Harms and Dinsmore 2012). Moreover, we incorporated site variables collected prior to each survey such as cloud cover, temperature, and observer to improve density estimates (Pocewicz et al. 2009). We selected models for each species based on Akaike's Information Criterion (AIC) adjusted for small sample sizes ( $AIC_c$ ) and Chi-square ( $\chi^2$ ) goodness-of-fit test scores (Buckland et al. 2001, Burnham and Anderson 2002, Pocewicz et al. 2009). After we selected the best model for each species, we stratified density estimates for each species by site. Additionally, we categorized species based on their dependence on grasslands (facultative or obligate) using descriptions from Glassberg (2001) and Royer (2003). Previously, grassland butterflies have been categorized as either disturbance tolerant or habitat specialists (Reeder et al. 2005, Davis et al. 2007, Smith and Cherry 2014). However, these categories

mask the role disturbance has in maintaining open areas and host plant requirements for grassland specialists (Moranz et al. 2014).

## Analysis

We used generalized linear modeling (GLM) with a Poisson distribution to model the influence of landscape and local variables on butterfly site density using the 'lmer' package in the R statistical environment (Pocewicz et al. 2009, van Dyck et al. 2009, Bates et al. 2015; R Development Core Team 2015). Our *a priori* landscape variables included the cover of perennial grassland, wetland, open water, hay ground, cropland, development, and LDI at the 1,000-m and 2,500-m scale, and our *a priori* vegetation variables included ramet density, visual obstruction, plant species richness, plant species diversity, flowering forb species richness, flowering forb species diversity, percent litter, percent invasive grass species cover (IGC), invasive forb species cover (IFC), host plant cover for *D. plexippus* (milkweeds; *Asclepias* spp.) and fritillary species (violets; *Viola* spp.), and disturbance (Table 4). We tested for correlations among variables and eliminated variables that were highly correlated ( $r \geq 0.60$ ; Marini et al. 2009, Harms et al. 2014). Notably, LDI was negatively correlated with perennial grassland cover and positively correlated with cropland cover, but LDI was not correlated with remaining landscape variables.

We used a hierarchical approach to create butterfly density models, starting at the largest landscape scale and progressively incorporating variables from decreasing scales. We created models by including variables at the 2,500-m landscape scale and compared univariate models to a null model. We joined univariate models to make additive models if  $\Delta AIC_c$  was less than the null and within  $2 \Delta AIC_c$  of the best model (Burnham and Anderson 2002). The best model at the 2,500-m scale became the base model moving forward. We then followed this same procedure of incorporating univariate and additive models at the 1,000-m scale for variables not included and not correlated with variables at the 2,500-m scale. We repeated this process for local variables, comparing null, univariate, and additive models in combination with significant landscape variables from both scales. If univariate models at any scale were less informative than the null model, we did not include those variables in additive models. Final model selections for each species were based on model weights and  $AIC_c$  scores (Burnham and Anderson 2002).

Table 4. Summary statistics and descriptions for landscape and local variables used in density models for butterflies detected in the Northern Great Plains from 2015-2017.

Level	Variable	Mean (SE)	Range	Variable Description
2,500-m landscape scale	NAT	0.63 (0.03)	0.29-0.89	Percent perennial grassland cover in 2,500 m buffers
	WET	0.09 (0.02)	0.00-0.64	Percent wetlands in 2,500 m buffers
	WAT	0.13 (0.01)	0.00-0.40	Percent open water in 2,500 m buffers
	HAY	0.03 (0.01)	0.00-0.09	Percent hay grounds in 2,500 m buffers
	CROP	0.18 (0.03)	0.00-0.58	Percent croplands in 2,500 m buffers
	DEV	0.03 (0.01)	0.00-0.12	Percent developments in 2,500 m buffers
	LDI	1.88 (0.10)	1.03-3.25	Landscape Development Index score based on land use and cover in 2,500 m buffers
1,000-m landscape scale	NAT	0.72 (0.02)	0.37-0.93	Percent perennial grassland cover in 1,000 m buffers
	WET	0.12 (0.03)	0.00-0.85	Percent wetlands in 1,000 m buffers
	WAT	0.12 (0.01)	0.00-0.31	Percent open water in 1,000 m buffers
	HAY	0.02 (0.01)	0.00-0.08	Percent hay grounds in 1,000 m buffers
	CROP	0.11 (0.02)	0.00-0.36	Percent croplands in 1,000 m buffers
	DEV	0.03 (0.01)	0.00-0.11	Percent developments in 1,000 m buffers
	LDI	1.62 (0.07)	1.00-2.50	Landscape Development Index score based on land use and cover in 1,000 m buffers
Local scale	VOR	2.35 (0.13)	1.12-4.08	Average visual obstruction reading to quantity vegetation structure measured at six locations
	RD	165 (14.0)	30.0-419	Average number of flowering forbs in 500 m <sup>2</sup> belt transects
	PR	24.0 (1.10)	9.00-39.0	Average number of individual plant species recorded in 12- 1 m <sup>2</sup> quadrats
	PD	0.81 (0.01)	0.58-0.92	Average plant species diversity measured with Simpson's diversity index in 12- 1 m <sup>2</sup> quadrats
	FR	28.0 (1.31)	13.0-45.0	Average number of individual flowering forb species recorded in 500 m <sup>2</sup> belt transects
	FD	0.77 (0.02)	0.34-0.93	Average flowering forb diversity measured with Simpson's diversity index in 500 m <sup>2</sup> belt transects
	LIT	13.9 (1.22)	1.50-36.8	Average percent litter canopy cover using 12- 1 m <sup>2</sup> quadrats
	PER.NN	42.6 (2.98)	6.97-69.9	Average percent non-native canopy cover using 12- 1 m <sup>2</sup> quadrats
	IGC	39.1 (2.63)	6.81-60.2	Average percent invasive grass canopy cover using 12- 1 m <sup>2</sup> quadrats
	IFC	3.44 (1.16)	0.00-37.1	Average percent invasive forb canopy cover using 12- 1 m <sup>2</sup> quadrats
	ASCSP	0.40 (0.09)	0.00-1.79	Average percent <i>Asclepias</i> spp. canopy cover using 12- 1 m <sup>2</sup> quadrats
	VP	0.31 (0.08)	0.00-1.00	Presence (1) or absence (0) of <i>Viola</i> spp. using 12- 1 m <sup>2</sup> quadrats
DIS	0.43 (0.07)	0.00-1.00	Site managed with disturbance (1) or no disturbance (0)	

## Results

We detected over 2,300 butterflies representing 38 species from 2015-2017 during LTDS surveys. Of the 38 species, 15 met our minimum threshold of 30 detections and were used for density estimates (Table 5). We found the hazard-rate key function with the cosine series expansion to be the best model for 9 of 15 species' density models, while the remaining six used various combinations of hazard-rate and half-normal key functions with the Hermite polynomial series expansion. Global density estimates ranged from 0.29 to 8.70 individuals · ha<sup>-1</sup>, averaging 2.91 individuals · ha<sup>-1</sup> (Figure 7). Effective strip width (ESW) also varied between species, ranging from 1.71 to 11.4 m (Figure 7). Facultative grassland species had an average density of 2.66 individuals · ha<sup>-1</sup> (Figure 7a), whereas obligate grassland species had an average density of 3.40 individuals · ha<sup>-1</sup> (Figure 7b).

Table 5. Scientific and common names for species detected at least 30 times using line-transect distance sampling in the Northern Great Plains from 2015-2017. Habitat associations (obligate or facultative) are included for each species and based on habitat descriptions from Glassberg (2001) and Royer (2003).

<b>Scientific Name</b>	<b>Common Name</b>	<b>Habitat Association</b>	<b>3-Yr Total</b>
<i>Boloria bellona</i> , Fabricius 1775	Meadow fritillary	Obligate	75
<i>Cercyonis pegala</i> , Fabricius 1775	Common wood nymph	Obligate	448
<i>Coenonympha tullia</i> , Muller 1764	Common ringlet	Obligate	112
<i>Colias eurytheme</i> , Boisduval, 1852	Orange sulphur	Facultative	365
<i>Colias philodice</i> , Godart, 1819	Clouded sulphur	Facultative	347
<i>Danaus plexippus</i> , L. 1758	Monarch	Facultative	63
<i>Plebejus melissa</i> , Edwards 1873	Melissa blue	Facultative	113
<i>Phyciodes cocyta</i> , Cramer 1777	Northern crescent	Facultative	38
<i>Phyciodes tharos</i> , Drury 1773	Pearl crescent	Facultative	83
<i>Pieris rapae</i> , L. 1758	Cabbage white	Facultative	132
<i>Polites mystic</i> , Edwards 1863	Long dash	Obligate	63
<i>Speyeria aphrodite</i> , Fabricius 1787	Aphrodite fritillary	Facultative	162
<i>Speyeria cybele</i> , Fabricius 1775	Great spangled fritillary	Facultative	30
<i>Speyeria idalia</i> , Drury 1773	Regal fritillary	Obligate	84
<i>Vanessa cardui</i> , L. 1758	Painted lady	Facultative	134
<b>Total</b>			<b>2,249</b>

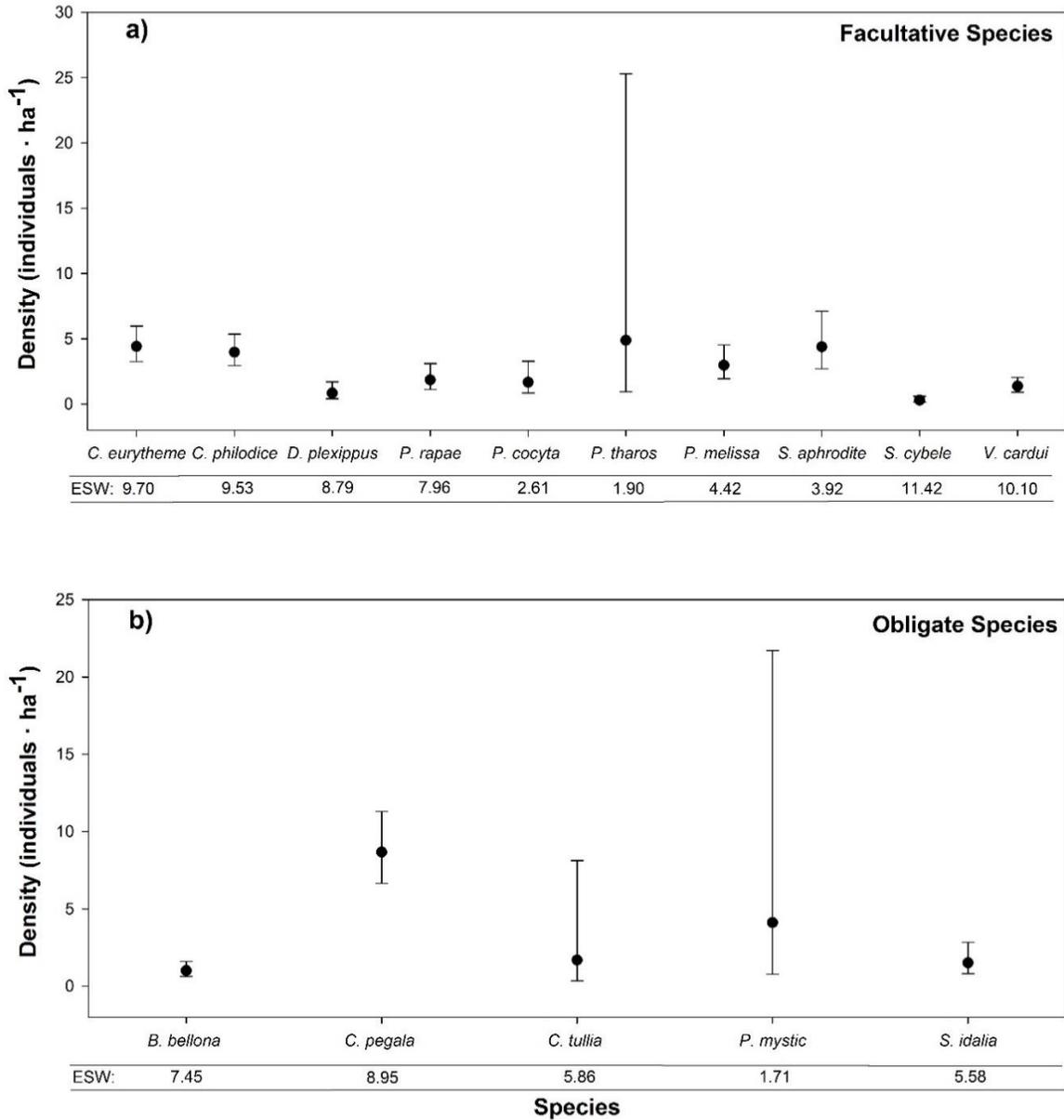


Figure 7. Species density estimates ( $\pm$  95% CI) and effective strip width (ESW) in meters for 15 species in the Northern Great Plains. Estimates were calculated using Program Distance for any species that had over 30 detections from 2015-2017. Species are categorized as Facultative (a) or Obligate (b) grasslands species based on Glassberg (2001).

### Landscape variables

Each species had competitive multivariate models (Table 6; Table A2), and of the 20 variables we measured, 15 were found to be significant for at least one of our focal species. At the landscape level, our best-ranked models for four species included variables exclusively at the 2,500-m scale, and five other species only included variables at the 1,000-m scale (Table 6; Table A2). The remaining six species

included landscape variables from both scales (Table 6; Table A2). LDI, which increases as cropland cover increases and perennial grassland cover decreases, was included in over half of the density models, although the relationship between LDI and species density was mixed for facultative and obligate species (Table 6). As LDI increased, facultative grassland species' densities increased, and obligate species' densities decreased, although *Coenonympha tullia* (obligate species) and *D. plexippus* (facultative species) were exceptions. Wetland cover was also included in over half of our species' models (Table 6). However, conversely to LDI, densities for facultative species decreased as percent wetlands increased, but three of the five obligate species influenced by wetland cover increased as wetland cover increased (Table 6).

### **Local variables**

Important local variables included in our best-ranked models were ramet density, forb and plant species richness, invasive plant species cover, and disturbance (Table 6, Table A2). Ramet density, forb richness, plant richness, and disturbance were included in 10 best-ranked models and positively influenced species density regardless of species' habitat associations (i.e., facultative or obligate). Therefore, density estimates increased with increasing ramet density, forb richness, and plant richness, along with disturbance versus no disturbance (Table 6). Conversely, the influence of invasive plant species (IGC and IFC) on butterfly species were mixed, with no clear patterns among facultative and obligate butterfly density estimates (Table 6). VOR was included in best-ranked models exclusively for two obligate species (i.e., no facultative species were influenced by VOR). However, VOR had mixed impacts on obligate species' density (one positive and one negative), depending on the individual species. Despite predictions, *Boloria bellona* was the only species that included host plant in the best-ranked model. When *Viola* spp. were present, *B. bellona* density increased (Table 6).

Table 6. Results from generalized linear modeling for ten facultative and five obligate grassland butterfly species in the Northern Great Plains from 2015-2017 using statistically significant ( $p \leq 0.05$ ) landscape (2,500-m and 1,000-m scale) and local variables. Species are separated by broad groups of facultative and obligate grassland species. Variables that were influential at the landscape scale include LDI and wetland cover (WET), while influential local variables included forb richness (FR), invasive species cover (IGC, IFC), and disturbance management (DIS).

Species	Landscape Variables										Local Variables								
	L DI	2,500-m scale				1,000-m scale					VOR	RD	FR	PR	LIT	VP	IGC	IFC	DIS
<b>Facultative</b>																			
<i>Colias eurytheme</i>																			
<i>Colias philodice</i>																			
<i>Danaus plexippus</i>	-																		
<i>Pieris rapae</i>	+																		
<i>Phyciodes cocyta</i>																			
<i>Phyciodes tharos</i>	+																		
<i>Plebejus melissa</i>																			
<i>Speyeria aphrodite</i>																			
<i>Speyeria cybele</i>																			
<i>Vanessa cardui</i>	+																		
<b>Obligate</b>																			
<i>Boloria bellona</i>																			
<i>Cercyonis pegala</i>	-																		
<i>Coenonympha tullia</i>																			
<i>Polites mystic</i>																			
<i>Speyeria idalia</i>																			

## Density models for species of conservation concern

The final best-ranked density models for species of conservation concern did not share any landscape or local variables. *Danaus plexippus* density was negatively influenced by LDI (2,500-m scale), percent hay (1,000-m scale), and percent water (1,000-m scale), but no local features improved our models (Figure 8; Table A2). At the largest scale (2,500-m), *D. plexippus* density decreased with increasing LDI (Figure 8). Similarly, as hay ground and open water cover increased in the landscape, *D. plexippus* density decreased (Figure 8). Unlike *D. plexippus*, *S. idalia* density was influenced by both landscape and local variables (Figure 8; Table A2). *Speyeria idalia* density increased as the cover of wetland (1,000-m scale) increased and as VOR decreased at the local scale (Figure 8).

## Discussion

Effective conservation planning and management of butterfly populations relies on rigorous, quantitative data and informed modeling (Sutherland et al. 2004, Haddad et al. 2008). We quantified density estimates for 15 grassland butterflies in the Northern Great Plains, USA, using LTDS and identified local and landscape variables affecting these populations. Our results indicate that landscape variables were more informative in determining butterfly densities than local variables, which contrasts many previous findings (Mazerolle and Villard 1999, Pocewicz et al. 2009). Overall, our most informative landscape variables included LDI and wetland cover. Four facultative and two obligate species responded negatively while three obligate species responded positively to increasing wetland cover. For LDI, six facultative and one obligate responded positively and one facultative and two obligate species responded negatively to increasing LDI. At finer, local scales, forb richness and ramet density positively influenced butterfly density. Specifically, densities for *D. plexippus* increased as LDI and the cover of hay ground and water decreased. Additionally, densities for *S. idalia* increased as the cover of wetlands at the landscape level increased and vegetation height at the local scale decreased. Neither of these species of conservation concern were influenced by their obligate host plants, although landscape-level variables could affect local host plant availability (e.g., Flockhart et al. 2014). The variability in species responses stresses the importance of managing at the local scale for variability and heterogeneity while reducing fragmentation at the landscape scale (Robinson et al. 2014, Hovick et al. 2015).

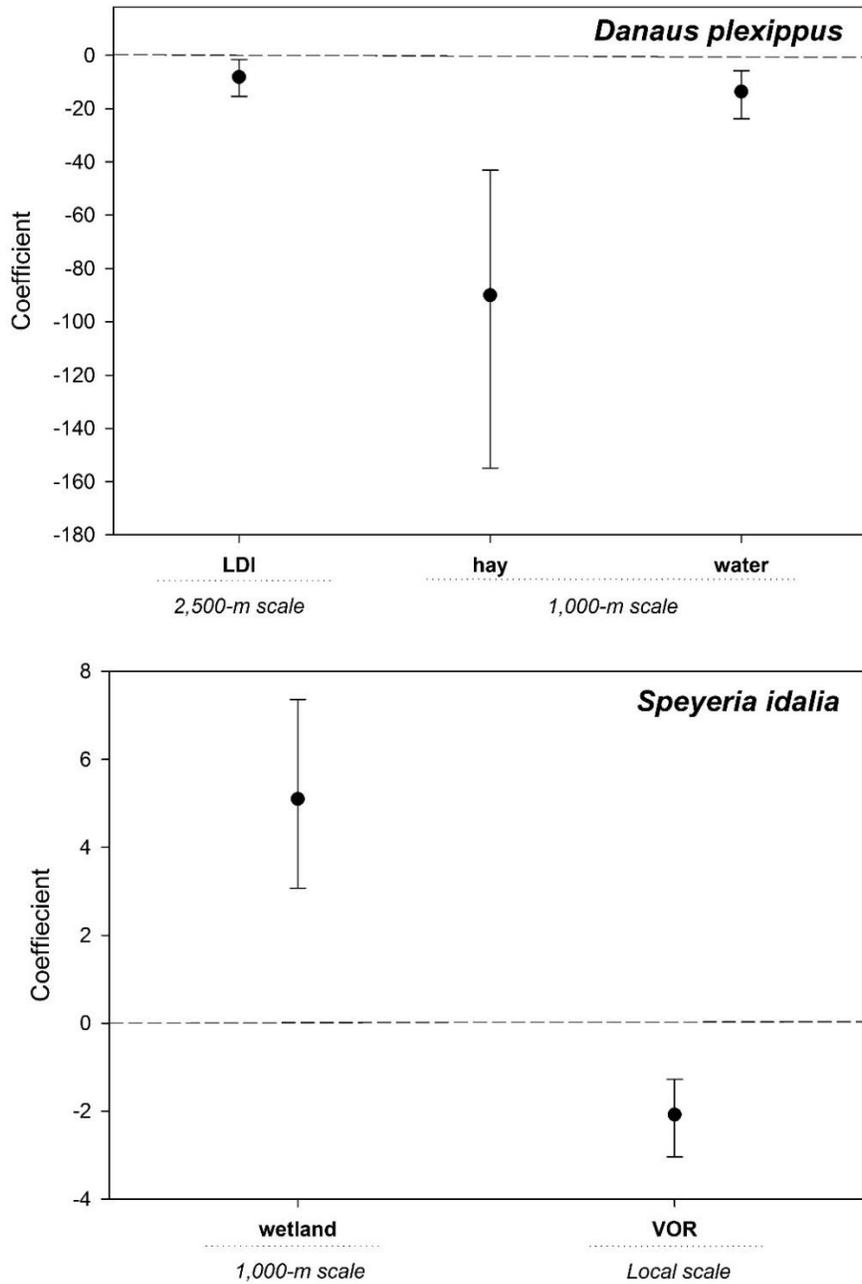


Figure 8. Coefficients ( $\pm$  95% CI) from the best-ranked species density models based on  $AIC_c$  scores and model weight for *Danaus plexippus* and *Speyeria idalia*, two species of conservation concern, using data collected in the Northern Great Plains from 2015-2017. Variables are designated as either landscape (2,500-m or 1,000-m scales) or local scales.

We provide true density estimates for 10 facultative and 5 obligate grassland butterflies in the Northern Great Plains. True density estimates are rare for butterflies (Kral et al. 2018) but allow researchers and land managers to incorporate detection functions which improves our ability to monitor populations, compare across studies, and provide accurate numbers for conservation planning and policy

(Brown and Boyce 1998, Pellet 2008). Our density estimates for several species were much lower than true density estimates from another Northern Great Plains study which found densities for species included in our study to be over 100 individuals  $\cdot$  ha<sup>-1</sup> (Bendel et al. 2018). However, estimates for species of conservation concern (*D. plexippus* and *S. idalia*) were much more similar. All of our density estimates were under 10 individuals  $\cdot$  ha<sup>-1</sup>, similar for overlapping species in Idaho and Iowa that also used distance sampling (Pocewicz et al. 2009, Moranz et al. 2012).

Landscape variables were more common in our models than local variables. LDI was included in models for two-thirds of our species. As LDI increased, seven species' densities increased (six facultative and one obligate), and three species' densities decreased (one facultative and two obligate). Since species were mostly facultative grassland butterflies, they are expected to respond well to disturbance and may use crops for nectar and larval resources (Bartomeus et al. 2018). Wetlands were included in nine best-ranked models for species density. Obligate species that are expected to utilize moist prairie to find host plants responded positively to wetlands (Glassberg 2001, Cozzi et al. 2008), but most facultative species had lower densities as wetland cover increased. Facultative butterflies may avoid wetlands in the region due to increases in predatory odonates (Batzer and Wissinger 1996) or microclimate changes between wetlands and uplands (Turlure et al. 2010). But without necessary species' information and microclimate data, we are unable to better inform our models. Another likely explanation for reduced densities with increasing wetlands depends on diet. Several facultative species utilize alfalfa (*Medicago sativa* L.) for host and nectar resources (Royer 2003), but alfalfa is unavailable in wetlands due to moisture levels (USDA-NRCS 2017). Landscape variables are commonly expected to be a secondary influence on butterfly density compared to local variables (Mazerolle and Villard 1999, van Halder et al. 2017), but our results showed how landscape variables influence local vegetation variables related to species' habitat quality (van Halder et al. 2017).

Local variables were not consistently included in species' best-rank models. Host plant was only included for one obligate species, and ramet density was included for three facultative species, although we expected increasing these variables would increase many species' densities (Curtis et al. 2015, Habel et al. 2016). Additionally, vegetation height, measured with VOR, influenced two obligate species, one positively and one negatively. Structure is a significant variable for obligate grassland species across

multiple taxa, with varying structure supporting diverse communities (Davis 2004), and butterflies were no exception. We expected invasive plants to negatively influence obligate grassland butterflies because invasive plants reduce native plant species used for host and nectar resources (Wolf et al. 2003, Moranz et al. 2012). Yet, species responses to invasive grasses (IGC) and invasive forbs (IFC) could not be explained by habitat associations. Invasive plants had mixed effects on facultative and obligate species, but we could discern patterns when grouped by larval diet. Larvae that consume Poaceae positively responded to IGC, whereas larvae that consume Asteraceae positively responded to IFC. At low to medium levels of invasion, invasive plants can provide larval and adult resources, but problems are likely to occur as invasion levels increase (Kalarus and Nowicki 2015). Because they relate to species' habitat quality, local variables are expected to influence butterfly density (Collinge et al. 2003, Poyry et al. 2009, Wallisdevries et al. 2012, Farhat et al. 2014), but the realized relationship changes between species and specific traits.

Density models for *D. plexippus* and *S. idalia* did not include their host plants—milkweed and violets, respectively—contrary to expectations (Flockhart et al. 2014, Caven et al. 2017). However, we only measured host plant availability at the local scale, and landscape variables included for both species can relate to host plant availability. For example, *D. plexippus* was the only facultative species negatively influenced by LDI. Increasing cropland cover often coincides with increased herbicide use, which decreases milkweed availability in these landscapes (Flockhart et al. 2014). Similarly, *S. idalia* was positively influenced by wetlands at the landscape scale and decreasing vegetation height (VOR) at the local scale. Wetlands are often associated with violets (Glassberg 2001), and lower VOR is better for ovipositing females (Habel et al. 2016). Although *D. plexippus* and *S. idalia* did not respond to their host plants at the local level directly, they are considered stronger dispersers (Sekar 2011) and may be responding to resource availability at broader scales, demonstrating the importance of modeling at multiple scales.

Our goal for modeling grassland butterfly density estimates was to find important connections and generate future hypothesis testing. We do not mean for our models to be predictive because we did not validate our models (Roberts et al. 2017), but this does not discount their ability to aid butterfly species' conservation. Concerns often exist that models may not always directly align with resource availability.

For instance, fewer bees are found in bee bowls placed in flower-dense areas because bees can use adjacent flowers and are less attracted to traps (Baum and Wallen 2011). We could be observing similar relationships with some of our density models. *Polites mystic* are associated with wet areas (Glassberg 2001), but we found they had a negative relationship with the cover of wetlands. Since transects did not go through wetlands, we could be observing a negative region where *P. mystic* were not near line transects because they were in wetlands. However, remaining variables in the *P. mystic* model biologically made sense for the species. Future modeling efforts may benefit from mapping local resource patches to increase the ability of models to include even more scales (i.e., local plant, patch resource, landscape cover) and discern how smaller-scale resource patch heterogeneity influence butterflies, as cover at the landscape scale may be measured too coarsely (Mac Nally et al. 2003, Bartomeus et al. 2018).

### **Conclusions**

Research to improve conservation efforts for butterflies is increasing, but accurate density estimates are still mostly unavailable for butterflies in the USA (Kral et al. 2018, but see Henry et al. 2015, Bendel et al. 2018). We calculated true density estimates for 15 grassland butterfly species and modeled their densities using landscape and local variables to improve conservation strategies and generate future research questions. We found landscape variables were more influential than local variables, unlike previous research (van Halder et al. 2017). Overall, densities for species of conservation concern (*D. plexippus* and *S. idalia*) and obligate grassland butterflies decreased in areas with more croplands. Local scale management is still vital to maintain ramet density and generate structural diversity to improve habitat quality for obligate species such as *S. idalia*. However, even if local host and nectar resources are available, they may not sustain butterflies if the surrounding landscape adversely impacts a species (Krauss et al. 2003, Robinson et al. 2014). Our results reiterate the importance of maintaining local resources for butterflies but highlight the significance and necessity of conserving large, intact landscapes with minimal crop cover to positively influence obligate grassland butterfly density.

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## CHAPTER 3. BUTTERFLY COMMUNITY RESPONSES TO KENTUCKY BLUEGRASS INVASIONS

### Abstract

Rangeland management strategies impact biodiversity, the quality and quantity of ecosystem services, and overall rangeland resiliency. Previous management strategies, coupled with climate change, have led to exotic plant species invasions by Kentucky bluegrass (*Poa pratensis*; bluegrass) in the Northern Great Plains, USA. Bluegrass invasions are expected to have detrimental impacts on ecosystem services provided by rangelands. Yet, few have investigated how bluegrass invasions influence pollinators, who provide arguably one of the most important ecosystem services. We measured the impact of bluegrass invasions on the grassland butterfly community, an indicator of biodiversity and provider of pollination services. We found that bluegrass had an overall negative effect on butterfly communities. Specifically, the butterfly community shifted to more facultative grassland species and fewer obligate grassland species as bluegrass cover increased. However, bluegrass did not significantly impact total butterfly abundance. Additionally, plant species diversity and flowering forb species richness also decreased as bluegrass cover increased. Overall, bluegrass invasion led to butterfly and plant community simplification, signaling a loss of biodiversity and potentially ecosystem services. Although bluegrass invasions were predicted to reduce plant species diversity and impact pollinators, our research is the first to quantify how grassland butterflies are negatively impacted by bluegrass. Resource managers should adopt management strategies that restore ecological processes like fire and grazing to reduce bluegrass cover, improve nectar and host resources for obligate grassland butterflies, and increase butterfly species richness. In doing so, the ability of rangelands to support vital ecosystem services provided by butterflies will improve.

### Introduction

Rangelands provide a wide array of ecosystem services including food and fiber, pollination, nutrient and water cycling, and recreation (Havstad et al. 2007). As a result, land managers utilize strategies that allow them to capitalize on ecosystem services of interest. However, the way in which individuals manage rangelands in the United States has changed dramatically, even within the last century (Grant et al. 2009, Fuhlendorf et al. 2012). Prior to the Dust Bowl in the early 1930s, rangelands were commonly overgrazed to maximize livestock production (Hurt 1985). After the Dust Bowl,

overgrazing was discouraged to reduce rangeland degradation and prioritize increased rangeland sustainability (Holechek et al. 2004). This management shift was successful at reducing soil erosion and promoting sustainable livestock production with moderate, uniform grazing. However, uniform disturbance reduces heterogeneity and biodiversity (Hovick et al. 2015). Currently, resource managers are adopting management strategies to conserve patterns and processes of rangelands by restoring fire and grazing disturbances to increase heterogeneity and improve rangeland biodiversity (Fuhlendorf et al. 2012, Bowman et al. 2016). Each broad management change has had consequences for plant communities, grassland-dependent wildlife, and the provisioning of ecosystem services.

Resource managers in the Northern Great Plains removed livestock grazing and promoted rest as a management strategy starting in the mid-1930s to improve grassland bird resources (Grant et al. 2009). This management strategy was widely implemented on public lands to increase dense nesting cover for waterfowl and upland game bird production because livestock production was viewed at odds with wildlife conservation (Naugle et al. 2001, Murphy and Grant 2005, Bahm et al. 2011, Bullock et al. 2016). Although no longer a broadly applied policy after the 1990s (Grant et al. 2009), years of rest (idle management) created significant changes in these rangelands, including increased invasive plant species (Toledo et al. 2014, Limb et al. 2018). Plant community composition on US Fish and Wildlife Service (USFWS) properties are dominated by several invasive species, including Kentucky bluegrass (*Poa pratensis* L.) which composes approximately 30% of the plant community on USFWS properties (Kobiela et al. 2017).

Kentucky bluegrass (hereafter bluegrass) is an exotic, cool-season grass that has invaded and spread across rangelands in the Northern Great Plains over the last several decades (DeKeyser et al. 2015). Bluegrass cover has increased both in rangelands with a history of idle management commonly found on public lands (Bahm et al. 2011) and on private lands that have been continuously grazed (Murphy and Grant 2005, Toledo et al. 2014). Bluegrass invades rangelands with both management strategies because it grows early in the spring before native cool-season grasses, the dominant vegetation in the region (Prosser et al. 2003), and spreads rapidly mainly via vegetative reproduction (Cully et al. 2003). Quickly spreading rhizomes create a thick thatch layer that helps bluegrass outcompete native plant species for water, nutrients, and light (Pierson et al. 2002, DeKeyser et al. 2013).

Consequently, bluegrass forms monocultures that can have major consequences on biodiversity (Cully et al. 2003, DeKeyser et al. 2015).

Conservation efforts focused on reducing biodiversity declines and improving the delivery of ecosystem services frequently emphasize negative impacts of invasive species (Cardinale et al. 2012, Bezemer et al. 2013). Invasive plant species homogenize plant communities, displace native wildlife (Washburn et al. 2000, Gerber et al. 2008), and reduce the delivery of ecosystem services (Clavel et al. 2011, Hanley et al. 2015). Bluegrass invasions are no different and have been found to reduce plant diversity (DeKeyser et al. 2013) and disrupt water and nutrient cycling (Pierson et al. 2002). Even though bluegrass can seasonally provide valuable forage for livestock (Schlaepfer et al. 2011), bluegrass invasions are expected to have severe consequences for wildlife habitat availability (Toledo et al. 2014). However, few studies have directly assessed the impacts of bluegrass on higher trophic levels such as butterflies.

Butterflies, along with other pollinators, are a major conservation concern (Lebuhn et al. 2013). Pollinator responses to invasive species are not well understood (Pejchar and Mooney 2009), but invasive species are expected to negatively impact butterflies and may even act in synergy with other drivers of declines such as fragmentation (Keeler et al. 2006). Moreover, the impacts of invasive species on regulating services like pollination—valued at over \$215 billion globally (Vanbergen et al. 2013)—may be more detrimental than invasive species impacts on provisioning services (Pejchar and Mooney 2009). Consequently, researchers, resource managers, and policy makers need to understand how butterflies respond to invasive plant species to improve conservation efforts and more thoroughly understand the wider impacts of invasive species (Bezemer et al. 2013). Additionally, butterflies can be used to identify ecological consequences of bluegrass invasion and determining how bluegrass impacts overall biodiversity. Butterflies are regularly used as bio-indicators (Thomas 2005, Potts et al. 2010, Roy et al. 2015, Habel et al. 2016) because they are sensitive to environmental factors and respond to vegetation shifts before other organisms (Mortimer et al. 1998, Borschig et al. 2013).

The goal of our research was to quantify whether bluegrass invasions influence butterflies in rangelands of the Northern Great Plains. Invasive plant species are expected to influence butterflies (Bezemer et al. 2013, Hanley et al. 2015), but not all species will respond the same to invasive plant

species and plant community homogenization (Filz et al. 2013, Habel et al. 2016, Kral et al. *In Review*). Therefore, our objective was to evaluate the entire butterfly community to determine how species or groups of species respond to varying levels of bluegrass invasion. Our results can help determine how bluegrass has impacted biodiversity in rangelands. Moreover, our results can help identify specific butterfly species that may be useful as indicators of biodiversity or bluegrass invasion, directing management strategies and conservation planning.

## Methods

### Study area

We collected butterfly and vegetation data in North Dakota, South Dakota, and Minnesota (Figure 9). In the region, temperatures average 5.5 °C, varying throughout the year from -17 to 23 °C. The majority of precipitation falls during the growing season and follows a strong gradient east to west, decreasing from 69 cm to 36 cm (USDA-NRCS 2015). The plant community was historically tallgrass prairie in the east and mixed-grass prairie in the west. The tallgrass prairie was largely composed of native, warm-season grasses (*Andropogon gerardii* Vitman, *Schizachyrium scoparium* (Michx.) Nash), and the mixed-grass prairie was largely composed of native, cool-season grasses (*Nassella viridula* (Trin.) Barkworth, *Pascopyrum smithii* (Rydb.) Á. Löve). The forb community is similar across prairie types, being dominated by *Aster spp.* and *Solidago spp.* (USDA-NRCS 2015). However, invasive, cool-season grasses such as *Bromus inermis* Leyss. and bluegrass have become dominant in many grasslands throughout the region (Murphy and Grant 2005).

### Survey sites

We surveyed 20 sites in 2015, 25 sites in 2016, and 26 sites in 2017 for a total of 35 unique sites over three years of data collection. We selected a majority of our survey sites from U.S. Fish and Wildlife Service (USFWS) refuge lands, but we also surveyed some privately-owned or non-governmental organization areas in our study region (for full details see Kral et al. *In Review*). Most sites were managed with different combinations of cattle grazing and prescribed fire, however several sites had idle management. Because of inherent differences in site history and management, bluegrass cover ranged from 3-36% canopy cover (Table A3).

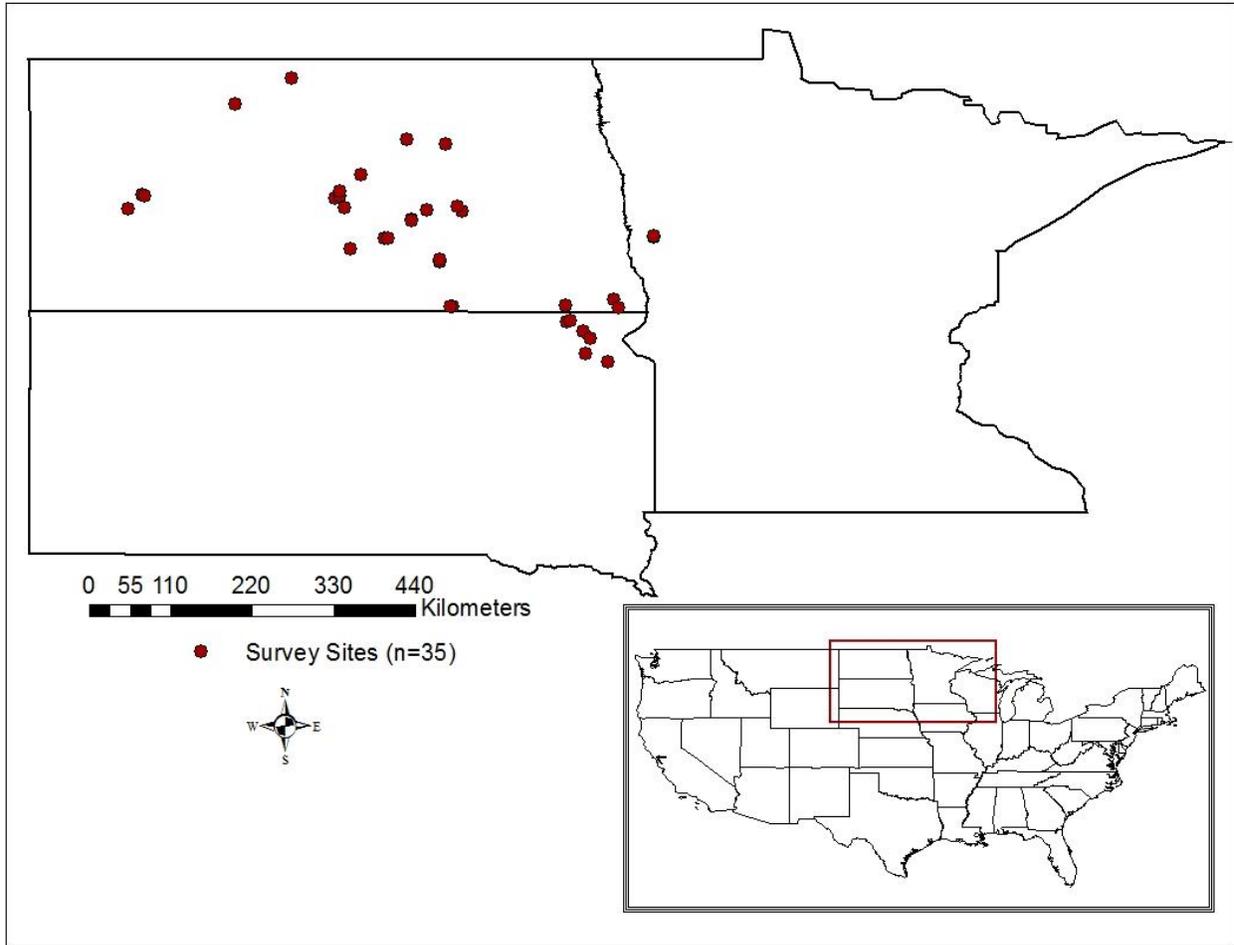


Figure 9. Location of butterfly surveys conducted from 2015-2017 in the Northern Great Plains, USA. In total, we surveyed 35 sites, visiting each site three times in the year(s) it was surveyed.

### Butterfly surveys

We collected butterfly community data including species composition, richness, diversity, and abundance using two different methods at each site. We randomly placed two, 100-m line transects (Moranz et al. 2012) and two, 4-ha visual encounter survey blocks (Hardersen and Corezzola 2014, Harms et al. 2014) at each site. Each year, we surveyed sites three times between June and early August. We rotated the time of day and order of surveys to reduce temporal bias. Furthermore, we conducted surveys between 0900 and 1800 hours (CDT) during optimal field conditions for butterflies: air temperatures between 21 - 35 °C, winds under 25 km · hr<sup>-1</sup>, and cloud cover less than 50% (Royer et al. 1998). If a butterfly could not be identified, observers suspended their survey, and took a picture or attempted to capture the butterfly with a net, and then resumed the survey at the stopped point. During

each site visit, two observers (KCK being the primary observer each year) surveyed one line transect and one survey block each. On subsequent visits, observers rotated to reduce observer bias.

### **Vegetation surveys**

We collected plant community data to quantify cool-season grass invasion and resource availability for butterflies. We recorded species and counted the number of flowering ramets along eight, 100 m transects in 5-m belts during each site visit to obtain nectar resource density (Moranz et al. 2012). We established belts around 100-m line transect and three more belts in each 4-ha survey block. During peak plant production in late July, we estimated average plant species canopy cover at each site using 30, 1 m<sup>2</sup> quadrat frames. We placed six evenly distributed quadrat frames along each line transect and nine evenly distributed quadrat frames within survey blocks. We then visually estimated absolute canopy cover to the nearest percent and placed them in cover classes (0-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-95, and 95-100). Although we were largely interested in the cover of invasive cool-season grasses, we collected composition and canopy cover for all plant species.

### **Landscape cover**

We collected landscape cover data around each site to quantify the influence on the butterfly community. We created 1,000 m buffers around each survey site (Pocewicz et al. 2009, Perovic et al. 2015) and determined land cover using USGS National Land cover datasets in ArcGIS v. 10.2 (ESRI 2013). Within each buffer, we calculated the percentage of major cover types including perennial grassland, wetland, hay ground, open water, cropland, and development (roads, buildings, etc.).

### **Analysis**

We conducted all our statistical analyses in the R (v. 3.1.3) statistical environment for Windows (R Development Core Team 2015). We determined butterfly community composition at each site by identifying all species detected by either method within each year. Additionally, we categorized species based on their dependence on grasslands (facultative or obligate) using descriptions from Glassberg (2001) and Royer (2003). To be included in our community analysis, a species had to be detected at least twice, since we were interested in rarer species but wanted to remove species with limited detections (Blanchet et al. 2014). Then we summed abundance data for each species over site visits in each year (Davis et al. 2008) and averaged species relative site abundance over the three years of data collection

for our multivariate analysis. We used non-metric multidimensional scaling (NMDS) with Bray-Curtis distance measures to create community ordination plots in the *vegan* package (Kindt and Coe 2005, Oksanen 2015). In NMDS, site scores are plotted as points, and the distances between sites allow us to determine the similarity or dissimilarity of the butterfly community (Moranz et al. 2012; Oksanen 2015). Sites that are closer together in ordination space are more similar. Moreover, species are also plotted as points next to sites where they are relatively more abundant. We considered three-dimensional ordinations with stress values under 0.20 to be acceptable (Clark 1993).

We used the function “envfit” within the *vegan* package to test how the ordinated butterfly community was influenced by vegetation variables (ramet density, bluegrass canopy cover, smooth brome (*Bromus inermis*) canopy cover, flowering forb species richness, flowering forb species diversity, plant species richness, and plant species diversity) and landscape variables (perennial grassland, wetland, hay ground, open water, cropland, and development cover; Table A3). Before variables were used in our community analysis, we tested for correlations and eliminated correlated variables ( $r^2 \geq 0.60$ ; Marini et al. 2009, Harms et al. 2014). Flowering forb species richness and diversity were highly correlated ( $r^2 = 0.60$ ), along with plant species richness and diversity ( $r^2 = 0.72$ ). However, flowering forb species richness and diversity were not correlated with plant species richness and diversity ( $r^2 = 0.31$  and  $0.27$ , respectively). We chose to include forb species richness and plant species richness in our analysis, eliminating forb and plant diversity, based on previous research (see Moranz et al. 2012). At the landscape scale, perennial grassland and cropland cover were negatively correlated ( $r^2 = -0.78$ ), so we only included perennial grassland cover. If vegetation or landscape variables were significantly related with the butterfly community ( $\alpha \leq 0.05$ ), we included them as vectors in our ordination plots (Moranz et al. 2012).

Next, we used generalized linear mixed-effect modeling (GLMM) to quantify the influence of bluegrass (fixed effect) on single variables. Whereas other studies have used univariate analyses to inform individual species responses or treatment effects (e.g., Moranz et al. 2012, Hovick et al. 2015), we used GLMM to determine relationships with variables of interest (i.e., plant and butterfly community metrics and bluegrass cover). GLMM allowed us to determine how single vegetation variables used in the community ordination and butterfly variables including butterfly species richness, butterfly species

diversity, total butterfly abundance, obligate grassland butterfly richness, obligate grassland butterfly abundance, and facultative grassland butterfly abundance responded to bluegrass cover (Table A3). Using the *lmer* package, we created GLMMs with Gaussian and Poisson distributions, depending on variable distribution, to compare univariate variables to bluegrass cover at the site level (Bates et al. 2015). We used log-transformations as necessary to normalize variables in models with Gaussian distributions (Crawley 2013). Similar to the multivariate analysis, we summed abundance data for each species over site visits in each year to get relative site abundance (Davis et al. 2008), but instead of averaging at the site level, we incorporated year and site as random factors in GLMMs (Price et al. 2005). For each analysis, we set an  $\alpha \leq 0.05$  to indicate statistically significant differences between factors.

## Results

We detected over 11,000 butterflies representing 45 species from 2015-2017. Of these 45 species, we utilized 39 species that met our minimum threshold of being detected at least twice (Table 7). Overall, we detected 12 obligate grassland butterflies (4,173 detections) and 27 facultative grassland butterflies (6,917 detections; Table 7).

### Butterfly community responses to bluegrass

The butterfly community was influenced by three vegetation variables including bluegrass cover ( $R^2 = 0.23$ ,  $p = 0.02$ ), flowering forb species richness ( $R^2 = 0.52$ ,  $p = 0.001$ ), and plant species richness ( $R^2 = 0.28$ ,  $p = 0.004$ ; Figure 10). This means that the butterfly community composition changed as these variables increased or decreased. Sites with relatively greater abundance of obligate grassland butterflies were associated with greater flowering forb species richness and less bluegrass cover, including the threatened *Hesperia dacotae* (HESDAK; Figure 10). Similarly, *Speyeria idalia* (SPEIDA), a species of conservation concern, was associated with greater plant species richness and less bluegrass cover.

Sites with a larger proportion of facultative butterfly species such as *Colias philodice* (COLPHI) and *Pieris rapae* (PIERAP) were associated with decreasing flowering forb and plant species richness and increasing bluegrass cover (Figure 10). One obligate grassland species, *Cercyonis pegala* (CERPEG), was associated with less flowering forb species richness compared to other obligate species which were correlated with greater flowering forb richness (Figure 10). However, *C. pegala* was the most

abundant obligate grassland species (Table 7) and may respond differently to plant community dynamics compared to other obligate species that occur at lower abundances.

Table 7. Species list, six-letter codes, classification (obligate or facultative), and total detections for each year, and cumulative three-year total for all butterflies detected at least twice in butterfly surveys conducted in the Northern Great Plains, USA, from 2015-2017. Classifications are based on species' dependence on grasslands using descriptions from Glassberg (2001) and Royer (2003).

<b>Species</b>	<b>Six-letter code</b>	<b>Classification</b>	<b>2015</b>	<b>2016</b>	<b>2017</b>	<b>3-Yr Total</b>
<i>Boloria bellona</i>	BOLBEL	Obligat	31	215	104	350
<i>Boloria selene</i>	BOLSEL	Obligat	1	1	4	6
<i>Celastrina neglecta</i>	CELNEG	Facultative	0	2	1	3
<i>Cercyonis pegala</i>	CERPEG	Obligat	477	1104	809	2390
<i>Charidryas gorgone</i>	CHAGOR	Obligat	0	19	13	32
<i>Coenonympha tullia</i>	COETUL	Obligat	167	275	201	643
<i>Colias eurytheme</i>	COLEUR	Facultative	60	1437	204	1701
<i>Colias philodice</i>	COLPHI	Facultative	429	568	538	1535
<i>Danaus plexippus</i>	DANPLE	Facultative	124	104	109	337
<i>Epargyreus clarus</i>	EPACLA	Facultative	0	1	2	3
<i>Euptoieta claudia</i>	EUPCLA	Obligat	0	45	48	93
<i>Glaucopsyche lygdamus</i>	GLALYG	Facultative	31	57	23	111
<i>Hesperia dacotae</i>	HESDAK	Obligat	0	1	1	2
<i>Junoia coenia</i>	JUNCOE	Facultative	0	2	0	2
<i>Limenitis archippus</i>	LIMARC	Facultative	0	0	25	25
<i>Limenitis arthemis</i>	LIMART	Facultative	0	1	2	3
<i>Lycaena hyllus</i>	LYCHYL	Facultative	0	6	9	15
<i>Oeneis uhleri</i>	OENUHL	Obligat	0	2	14	16
<i>Papilio glaucus</i>	PAPGLA	Facultative	0	2	5	7
<i>Papilio polyxenes</i>	PAPPOL	Facultative	0	22	37	59
<i>Phyciodes batesii</i>	PHYBAT	Facultative	0	13	9	22
<i>Phyciodes cocyta</i>	PHYCOC	Facultative	13	76	55	144
<i>Phyciodes tharos</i>	PHYTHA	Facultative	186	208	69	463
<i>Pieris rapae</i>	PIERAP	Facultative	190	224	127	541
<i>Plebejus melissa</i>	LYCMEL	Facultative	83	136	251	470
<i>Polites mystic</i>	POLMYS	Obligat	71	120	70	261
<i>Polites peckius</i>	POLPEC	Obligat	6	2	8	16
<i>Polites themmistocles</i>	POLTHE	Obligat	10	22	18	50
<i>Pyrgus communis</i>	PYRCOM	Facultative	0	1	13	14
<i>Pontia protodice</i>	PONPRO	Facultative	0	1	2	3
<i>Satyroides eurydice</i>	SATEUR	Facultative	10	15	3	28
<i>Satyrrium titus</i>	SATTIT	Facultative	4	27	6	37
<i>Speyeria aphrodite</i>	SPEAPH	Facultative	44	308	298	650
<i>Speyeria atlantis</i>	SPEATL	Facultative	0	0	5	5
<i>Speyeria cybele</i>	SPECYB	Facultative	15	51	33	99
<i>Speyeria idalia</i>	SPEIDA	Obligat	10	151	153	314
<i>Vanessa atalanta</i>	VANATA	Facultative	22	54	31	107
<i>Vanessa cardui</i>	VANCAR	Facultative	1	2	528	531
<i>Vanessa virginiensis</i>	VANVIR	Facultative	0	2	0	2

The butterfly community was also influenced by wetland cover at the landscape level ( $R^2 = 0.30$ ,  $p = 0.005$ ; Figure 10). Notably, sites with a larger relative abundance of *Satyrrium titus* (SATTIT) and

*Limenitis arthemis* (LIMARC), two facultative species, were associated with greater wetland cover (Figure 10). Overall, as bluegrass cover increased both the plant community and the butterfly community became relatively more homogenized.

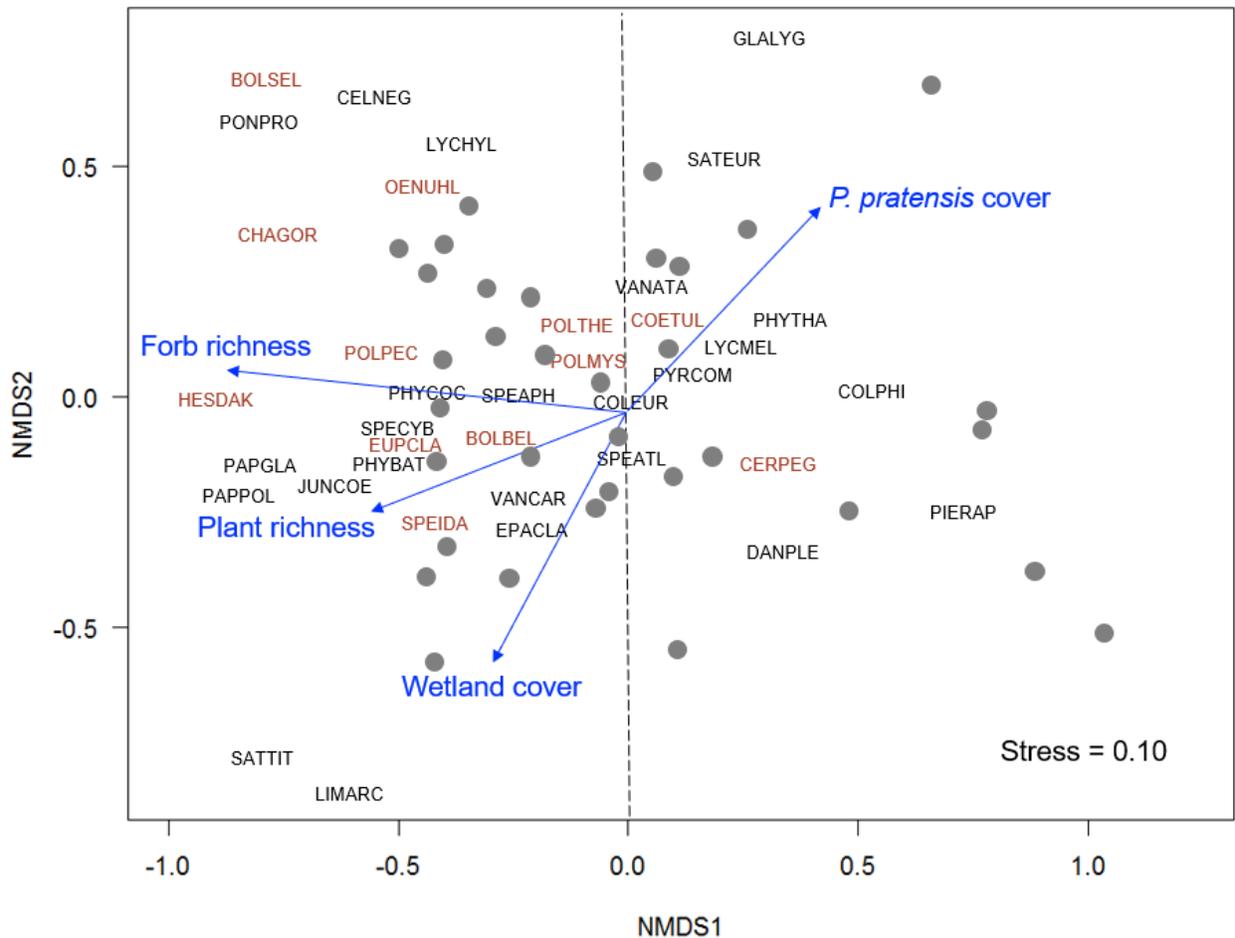


Figure 10. Non-metric multidimensional scaling ordination for the butterfly community detected from 2015-2017 in the Northern Great Plains, USA. Six-letter codes for obligate grassland species (red) and facultative grassland species (black) are shown (see Table 1 for full scientific names) along with site scores (gray circles). Species nearest to sites represent which species were more prominent at those sites. We then used envifit to determine if vegetation vectors were correlated site butterfly communities. Flowering forb richness (Forb richness), plant species diversity (Plant diversity), and Kentucky bluegrass canopy cover (*P. pratensis* cover) were statistically significant ( $p \leq 0.05$ ) vegetation vectors for the community. Additionally, the percent cover of wetlands (Wetland cover) was also a statistically significant landscape vector ( $p \leq 0.05$ ). The plant community and nectar resources became more homogenized as Kentucky bluegrass cover increased. The butterfly community correspondingly went from more obligate grassland species to more facultative grassland species as bluegrass cover increased.

### Univariate responses to bluegrass

Bluegrass canopy cover was significantly correlated with univariate plant and butterfly community metrics (Figure 11). Plant species diversity ( $t = -3.16$ ,  $df = 66$ ,  $p = 0.002$ ; Figure 11A) and flowering forb

species richness ( $t = -1.97$ ,  $df = 66$ ,  $p = 0.05$ ; Figure 11B) decreased as bluegrass cover increased. Additionally, butterfly species richness ( $t = -2.27$ ,  $df = 66$ ,  $p = 0.03$ ; Figure 11C) and obligate butterfly species abundance ( $z = -8.65$ ,  $df = 66$ ,  $p \leq 0.001$ ; Figure 11D) decreased as bluegrass cover increased. We did not find a statistically significant relationship between bluegrass cover and the other plant and butterfly variables including total butterfly abundance and facultative species abundance (Figure A1).

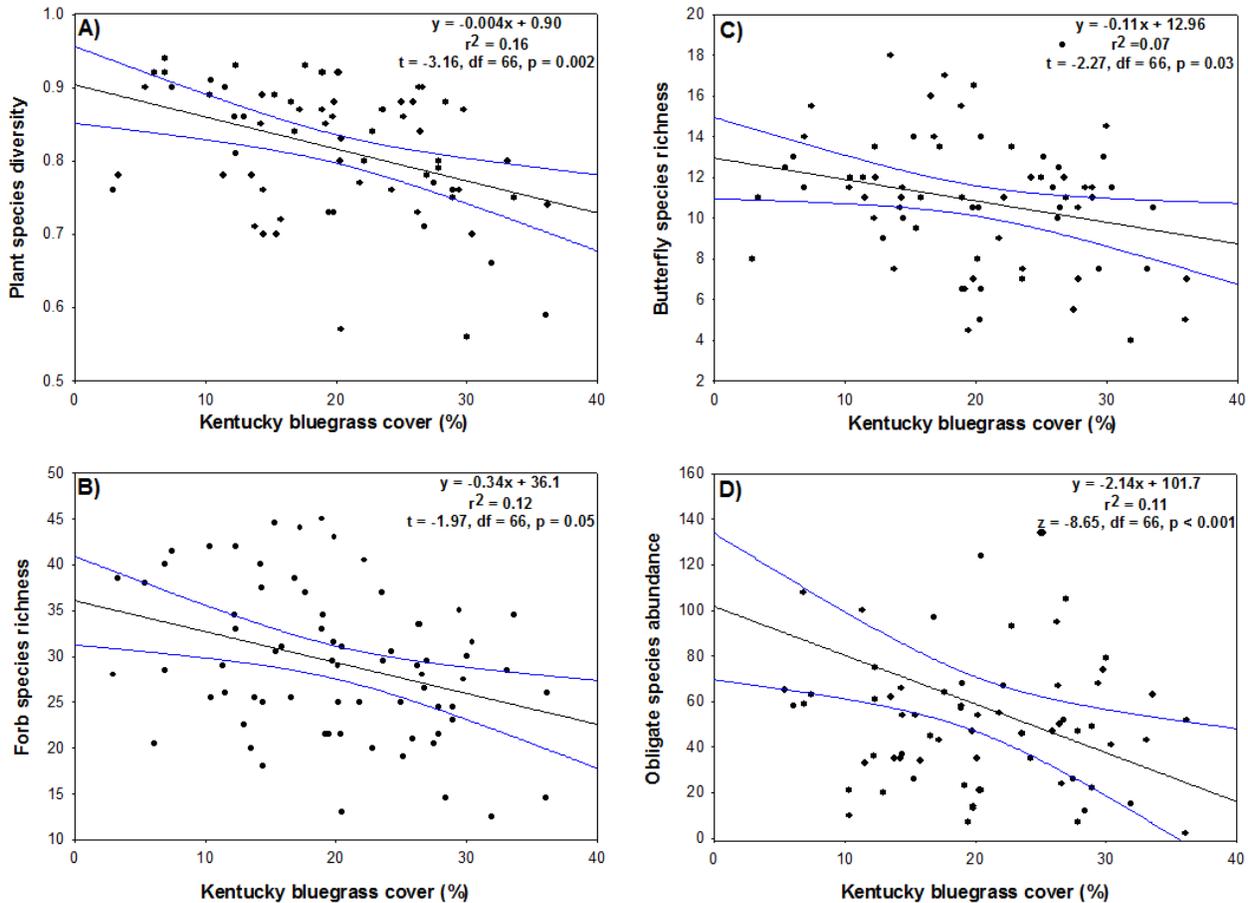


Figure 11. Plant and butterfly community responses to Kentucky bluegrass (*Poa pratensis*) canopy cover from butterfly and vegetation surveys completed in the Northern Great Plains, USA, from 2015-2017. Kentucky bluegrass cover had a negative relationship with plant species Simpson's diversity index (A), flowering forb species richness (B), butterfly species richness (C), and obligate species abundance (D). Generalized linear model results are included for each variable, along with 95% confidence bands (blue lines). Models for plant species diversity, flowering forb species richness, and butterfly species richness were created using the Gaussian distribution, but the obligate species abundance model utilized the Poisson distribution. Note differing scales of each dependent variable. As Kentucky bluegrass cover increased the plant and butterfly communities became more homogenized and the abundance of obligate grassland butterfly species declined.

## Discussion

Idle management strategies previously used in the Northern Great Plains have had considerable ramifications for the plant community, biodiversity, and ecosystem processes (Limb et al. 2018). Bluegrass invasions are a prominent example as upwards of 30% canopy cover are now common on public lands (Kobiela et al. 2017). However, little is known about how plant invasions impact pollinators (Pejchar and Mooney 2009). We found that increasing bluegrass cover had negative impacts on obligate grassland butterflies including *H. dacotae* and *S. idalia*, species of conservation concern. As bluegrass cover increased, facultative grassland butterflies became dominant and obligate grassland butterflies decreased. However, declines in the number of obligate species did not equate to reductions in total butterfly abundance. Total butterfly abundance, along with facultative species abundance, was not related to bluegrass cover. Negative responses to bluegrass in the butterfly community were mirrored in the plant community. Sites with higher bluegrass cover had lower plant species diversity and flowering forb richness. Collectively, more bluegrass translated to reduced host and nectar resource availability and homogenized butterfly communities. Similar results have been reported for native plants and arthropods in Europe (Gerber et al. 2008). Simplified butterfly communities that shift from obligate to facultative communities likely provide less pollination services and could jeopardize food security (Pejchar and Mooney 2009, Borschig et al. 2013). Consequently, management strategies should focus on reducing invasive grass cover and improving biodiversity.

Butterfly communities in our ordination were driven by bluegrass canopy cover. Overall, the butterfly community transitioned to more facultative species and fewer obligate species as bluegrass cover increased. The environmental vectors showed how increasing bluegrass was negatively correlated with plant species richness, creating a bottom-up effect on the butterfly community (Keeler et al. 2006, Vila et al. 2016). Obligate species declined as bluegrass cover increased and were replaced by generalist facultative species that can tolerate plant community changes (Moranz et al. 2012, Farhat et al. 2014). Conversely, increased plant and forb richness provided host and nectar resources for species with stricter resource requirements (Ekroos et al. 2010). This could explain why more butterfly species, including facultative species, were found as these environmental vectors increased.

Obligate grassland species and overall species richness increased as wetland cover at the landscape level increased. Wetland cover offers another form of perennial cover and increases compositional heterogeneity at the landscape scale (Fahrig et al. 2011), much like plant diversity and flowering forb richness increase heterogeneity at the local scale. Higher compositional heterogeneity supports communities with taxonomic diversity because more niches exist for species (Perovic et al. 2015). In landscapes with increasing wetland cover, obligate grassland species that require mesic prairie such as *S. idalia* increased (Glassberg 2001, Royer 2003), but butterfly species such as *S. titus* and *L. arthemis* that require willows (*Salix* spp.), commonly found in wetlands in the Northern Great Plains (Royer 2003, van der Kamp and Hayashi 2009), also increased. Unexpectedly, crop cover did not influence the butterfly community. We expected increasing crop cover would shift the community from obligate to facultative species as observed in other agroecosystems (e.g., Ekroos et al. 2010, Borschig et al. 2013), but crop cover may be too low or irregular to detect in our study region. In our study, crop cover averaged 11% (Table A3), whereas studies where crop cover significantly impacted the butterfly community averaged 51% (see Ekroos et al. 2010).

Our regression analysis allowed us to examine univariate responses to bluegrass invasion that may be helpful to understanding community responses. Butterfly species richness declined as bluegrass cover increased, but total butterfly abundance was not significantly correlated with bluegrass cover. Invasive species are expected to negatively impact butterfly abundance (Kalarus and Nowicki 2015), so we separated butterfly abundance between obligate and facultative species. This allowed us to determine that only obligate butterfly species abundance decreased as bluegrass cover increased. Therefore, although butterfly species richness declined, we did not detect a significant decrease in overall abundance due to either increased abundance of facultative species or because obligate species represent a smaller proportion of the community ( $\bar{x} = 36\%$ ; Table A3). Similar patterns of reduced richness, declines in specialist species, and stable or fluctuating overall abundance have been observed for butterfly communities responding to human disturbance gradients in Japan (Kitahara and Fujii 1994) and butterflies across the UK (Brereton et al. 2010).

When invasive plants reduce butterfly species richness, especially obligate species, functional homogenization diminishes the ability of the butterfly community to provide ecosystem services (Clavel et

al. 2011, Borschig et al. 2013). For example, invasive woody plants reduce pollination services in forested systems (Pejchar and Mooney 2009, Florens et al. 2010). We did not measure ecosystem services like pollination directly, but we can infer from our results potential declines in ecosystem services provided by butterflies. With increased bluegrass cover, fewer butterfly species are observed, and non-market cultural services are reduced (Hanley et al. 2015). Additionally, we would also expect the grassland butterfly communities' contribution to pollination services to decline because species that can withstand bluegrass invasion (facultative, more generalists species) are expected to be less efficient pollinators (Madjidian et al. 2008). Although bees contribute more to pollination (Rader et al. 2016), some grassland forbs are exclusively pollinated by butterflies (Bloch et al. 2006). Consequently, obligate grassland butterfly declines and shifts in the community threatened ecosystem services provided by rangelands.

Our results show the value of reducing bluegrass cover to improve butterfly species richness and rangeland biodiversity. Invasive species are difficult to remove once they have been introduced, but reducing invasive species cover in a tropical forest and prairie fen wetland was successful at increasing butterfly species richness and diversity (Florens et al. 2010, Fiedler et al. 2012). Nevertheless, selecting appropriate management strategies to reduce invasive species and promote the butterfly community can be difficult. Resource managers regularly use herbicides to reduce bluegrass cover (Bahm et al. 2011), but herbicides also reduce the cover of desirable plants and butterfly survival and fitness (Russell and Schultz 2010, Wagner et al. 2017). Alternatively, restoring historic disturbance regimes (fire and grazing) is one way to reduce bluegrass cover in rangelands with a history of rest (Figure A2; Bahm et al. 2011, Kral et al. *In Review*) and increase vegetation and structural heterogeneity (Diacon-Bolli et al. 2012), while maintaining livestock production (Fuhlendorf et al. 2012). Historic disturbance regimes increase native plant diversity, forb richness, and benefit butterfly diversity when applied at the correct spatial-temporal scale (Kral et al. 2017). Nonetheless, management changes may not produce immediate results in heavily-invaded rangelands without additional efforts (Fiedler et al. 2012) because land-use legacies have lasting impacts on plant communities (Moranz et al. 2012).

We can utilize our results to suggest butterfly communities that can be used as indicators of functioning rangelands with increased biodiversity. For instance, communities composed of more obligate species (e.g., *S. idalia*) and fewer facultative, habitat generalist (e.g., *C. philodice*, *P. tharos*) would be

expected to have increased biodiversity, and likely less bluegrass cover. However, problems do exist using butterflies as indicators for specific taxa (Brereton et al. 2011). For example, butterfly diversity was negatively correlated with bee diversity in Iowa (Davis et al. 2008). Nonetheless, developing trends and potential insights for how the butterfly community relates to biodiversity in rangelands and bluegrass cover is imperative for identifying consequences of invasion, encouraging proactive management, and restoring ecological processes to increase the delivery of ecosystem services.

### **Conclusions**

Rangelands provide a variety of ecosystem services (Havstad et al. 2007), but management strategies to simultaneously benefit multiple ecosystem services are often viewed as contradictory (e.g., wildlife habitat or livestock production; Bullock et al. 2016). Management decisions on public lands in the Northern Great Plains assisted widespread invasion of exotic grasses like bluegrass (Grant et al. 2009), but no research is available on how bluegrass impacts pollinators, an important group for providing ecosystem services and food security. We surveyed the butterfly community along a gradient of bluegrass invasion (3-36% canopy cover) to determine how bluegrass invasions have impacted imperiled pollinators like butterflies and overall biodiversity. Bluegrass cover negatively impacted the butterfly community, and the butterfly community transitioned from more obligate and facultative grassland species to being dominated by several facultative species as bluegrass cover increased. Additionally, the availability of host and nectar resources for butterflies decreased as bluegrass cover increased. Even though bluegrass provides some benefits to rangelands (e.g., early spring forage), it negatively impacts the butterfly community and plant species diversity. We can infer from our results that supporting and cultural ecosystem services provided by butterflies are reduced when butterfly communities are homogenized. Therefore, management strategies that promote decreasing bluegrass cover while increasing plant species diversity and flowering forb richness are necessary for conservation of obligate grassland butterflies such as *S. idalia* and *H. dacotae* and continued ecosystem services in rangelands.

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## CHAPTER 4. IMPROVING MONARCH CONSERVATION EFFORTS BY INCORPORATING FIELD BEHAVIORS

### Abstract

Monarchs (*Danaus plexippus*) are one of the most recognized butterflies, and population declines have made them a species of major conservation concern. Yet, behavioral field observations are rare. Incorporating behaviors into monarch monitoring protocols can help connect landscape and behavioral ecology to ultimately benefit species conservation. Our objectives were to quantify monarch activity budgets, evaluate how behaviors differ across sites, and determine how monarchs transition between behaviors in the Northern Great Plains, USA. We opportunistically made 15-minute field behavioral observation surveys of wild monarchs and recorded behaviors including basking, flying, mating, nectaring, ovipositing, and resting. We also collected plant community data including flowering ramet density and plant species composition and canopy cover. Over two years, we observed 15 male and 35 female monarch butterflies. During observation surveys, the amount of time spent in each behavior was different, with monarchs spending most of their time flying and nectaring. Although we expected more ovipositing and nectaring at sites with higher milkweed (*Asclepias* spp.) cover, we observed more ovipositing on sites with a moderate cover of milkweed and more nectaring as milkweed cover decreased. As milkweed cover increased, invasive grasses also increased and reduced plant species diversity and available nectar resources important for adult survival. Planting more milkweed is a viable conservation option in agricultural areas without milkweed, but conservation practices in more natural areas may be better suited to identify management strategies like fire and grazing to increase the availability of milkweed for larvae and non-milkweed nectar resources for adults.

### Introduction

Eastern North American monarchs (*Danaus plexippus*) are one of the most easily recognized butterflies in the United States (Gustafsson et al. 2015). They have gained notoriety due to their distinct dependence on milkweed (*Asclepias* spp.), their exclusive larval host plant (Pleasants and Oberhauser 2013), and for their long, annual migrations which extend from central Mexico to the Canadian border (Swengel 1995, Brower 1996, Prysby and Oberhauser 2004). Their impressive migration has made them culturally important (Lopez-Hoffman et al. 2010), but interests in monarchs has exponentially grown as

population numbers have drastically decreased over the last two decades (Badgett and Davis 2015). The total area occupied by overwintering monarchs, a measurement of the population, has decreased from around 9 ha in 1999 to just over 1 ha in 2014 (Pleasants et al. 2017). Precipitous declines have garnered substantial support from the public to increase the amount of research and applied conservation efforts for monarchs (Diffendorfer et al. 2014, Davis and Dyer 2015). Although research has increased to better understand monarchs and utilize public support, many questions remain on how to best address declines and apply effective management strategies for this valued species (Davis and Dyer 2015).

Monarch declines are likely due to a combination of factors including climate change, habitat loss and fragmentation, disease, and loss of milkweed host plants due to agricultural intensification (Fischer et al. 2015, Flockhart et al. 2015, Harvell et al. 2015). Monarch conservation efforts are focused on addressing a suite of threats, but identifying which threats have the greatest impact on monarchs can be helpful to decrease declines more effectively. Recently, controversy has arisen over which threat is responsible for monarch declines with two leading hypotheses: the milkweed limitation hypothesis and fall migration hypothesis (see Ries et al. 2015, Inamine et al. 2016, Pleasants 2017, Pleasants et al. 2017). The milkweed limitation hypothesis posits that agricultural intensification and the use of herbicide-resistant crops has diminished the amount of milkweed on the landscape and planting more milkweed will be a viable way to address declines (Pleasants et al. 2017). The fall migration hypothesis posits that monarchs do well during the breeding season but experience increased mortality during migrations back to overwintering grounds, and conservation strategies should focus on improving resources for migrating monarchs (Badgett and Davis 2015, Inamine et al. 2016).

Generally, butterflies are surveyed as a part of monitoring efforts and to quantify responses to ecological changes and management (Parmesan et al. 1999, Powell et al. 2007, Moranz et al. 2012, Roy et al. 2015). The type and amount of available data in the US for monarchs is unique in that thousands of volunteers collect egg, larvae, and adult data each year through various citizen science programs (Ries and Oberhauser 2015). This wealth of knowledge has allowed researchers to monitor population trends across their entire range (e.g., Howard and Davis 2015, Pleasants et al. 2017), and has been extremely valuable for conservation. Most monitoring and survey efforts produce abundance estimates, but

researchers and citizen science programs can enhance the inferential power of their surveys by including behavioral aspects in monitoring protocols (Pickens and Root 2009, Knowlton and Graham 2010).

Researchers can use behaviors, similar to using abundance estimates, to determine how butterflies are influenced by local vegetation variables (e.g., Pickens and Root 2009). However, behaviors provide an important detail unavailable with abundance data: how do butterflies use or behave at different sites? When behaviors are not quantified, some uncertainty exists if butterflies are benefiting from being at a particular site (e.g., reproducing, nectaring) or merely unable to leave due to mobility or connectivity issues (Pickens and Root 2009). For species with high dispersal abilities like monarchs (Sekar 2011), connectivity may not be as much of an issue, but integrating behaviors improves our capacity to distinguish between fly-over sites (i.e., where monarchs are observed but do not stay) and utilized sites (i.e., where monarchs are observed and complete part of their life history). For example, butterflies are more likely to be observed nectaring and ovipositing when host plant density and availability increases (Schultz et al. 2012). At larger scales, incorporating behaviors into landscape-level monitoring protocols connects landscape and behavioral ecology to determine how individuals choose resources or move through landscapes (Lima and Zollner 1996, Knowlton and Graham 2010), all of which can guide conservation and management decisions (Pickens and Root 2009).

Most monarch behavioral research has focused on navigation for migration (e.g., Brower 1996, Reppert et al. 2010), oviposition (e.g., Tschenn et al. 2001, Casagrande and Dacey 2007), larval survival (e.g., Nail et al. 2015), and overwintering (e.g., Howard et al. 2010, Brower et al. 2011). Additionally, long-term field behavioral data for adult monarchs has been collected at a migration stop-over site in South Carolina since 1996 (McCord and Davis 2010, McCord and Davis 2012). However, less is known about how summer breeding adults interact with vegetation variables. A study in Washington State was one of the first to observe how field behaviors of adult monarchs during the summer breeding season responded to characteristics at a single site, with the overall goal of better informing conservation efforts (James 2016).

Collecting behavior data in conjunction with abundance data is one way to increase our basic monarch knowledge, understand how management decisions impact monarch site utilization, and improve ecosystem-based management decisions for conservation (Pickens and Root 2009). Our

objectives were to initiate a behavioral study on breeding monarch across a range of sites in the Northern Great Plains to 1) quantify activity budgets from ethograms and determine how monarchs allocate their time 2) evaluate how local and landscape features affect monarch activity budgets across multiple sites, and 3) calculate transitional matrices to identify behavioral flow patterns—the likelihood that a butterfly transitions from one behavior to another. Understanding monarch behaviors and predicting responses to local and landscape variables will be invaluable as monarch conservation efforts continue to increase (Badgett and Davis 2015).

## Methods

### Behavioral field observation surveys

We opportunistically made behavioral observation surveys on wild monarchs encountered during other field surveys (hereafter butterfly surveys) for grassland butterfly species (see Kral et al. *In Review* for survey details). We had 29 potential survey sites located in North Dakota, South Dakota, and Minnesota, USA. A majority of sites were managed by the US Fish and Wildlife Service, but we also conducted surveys on lands privately owned. Most sites were managed with different combinations of cattle grazing and prescribed fire, however several sites had idle management (Table A4). We conducted scheduled butterfly surveys for approximately two hours on three separate occasions each year during optimal conditions for butterflies (see Royer et al. 1998, Kral et al. *In Review*).

When a monarch was detected, we suspended our butterfly surveys to collect behavioral field observations and created ethograms—a list of pre-determined behaviors—for individual monarchs (McCord and Davis 2010). We conducted 15-minute behavioral observation surveys (Pickens and Root 2009), categorizing behaviors into 20-second intervals using standard *a priori* behaviors identified in other monarch surveys (Pliske 1975, McCord and Davis 2010), including basking, courtship, flying, mating, nectaring, ovipositing, and resting (Table 8). If an individual transitioned to another behavior within a 20-second timeframe, we recorded the dominant behavior. To avoid interfering with butterflies, we remained at least 20 m away from individuals during observation surveys and used binoculars to observe monarchs that were not easily visible. Additionally, we recorded the time of day, weather conditions, and sex of the monarch during each observation. If applicable, we also noted the plant species used for basking, nectaring, ovipositing, or resting.

Table 8. Description for each *a priori* behavior used for wild monarch field observations from 2016-2017 in North Dakota, South Dakota, and Minnesota, USA. Behaviors and descriptions modified from Pliske (1975) and McCord and Davis (2010).

<b>Behavior</b>	<b>Description</b>
Basking	Adult is slowly moving wings back-and-forth while resting on a substrate
Courtship	More than one individual displaying courtship behaviors
Flying	Individual moving across landscape
Mating	Male and female joined at the abdomen
Nectaring	Adult on flowering forb with proboscis extended
Ovipositing	Female resting on vegetation with abdomen flexed laying eggs
Resting	Stationary adult neither nectaring nor basking

### **Vegetation and landscape variables**

We collected local vegetation variables while conducting butterfly surveys to test whether the plant community influenced monarch behavior. We counted the number of flowering ramets along 8- 100 m transects in 5-m belts established for our butterfly surveys to obtain average nectar resource density at each site (Moranz et al. 2012). We also estimated average plant species canopy cover during peak plant production in late July using 30- 1 m<sup>2</sup> quadrat frames. We visually estimated canopy cover to the nearest percent and placed them in cover classes (0-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-95, and 95-100). We collected canopy cover for all plant species, but we were particularly interested in milkweed cover, as it is the sole host plants for monarchs and expected to increase monarch activity (Pleasants et al. 2017). Although common to collect milkweed stem density (Pleasants and Oberhauser 2013), our surveys were not exclusive to monarchs, and we therefore collected more generalized plant data. In addition to vegetation variables, we also created 1,000 m and 2,500 m buffers around each survey site (Bergman et al. 2004, Pocewicz et al. 2009), and determined land cover based on USGS National Land cover datasets in ArcGIS (v. 10.2, ESRI 2013). We quantified the percentage of major cover types including perennial grassland, wetland, hay ground, open water, cropland, and development (roads, buildings, etc.) within each buffer.

### **Analysis**

We first used individual ethograms to quantify activity budgets for all individuals where behavioral observation surveys were recorded for at least 10 minutes (Richer et al. 1997, Peixoto and Bensen 2009). Then we calculated the mean duration, frequency, and proportion of time monarchs spent in each

behavior (Wang and Messing 2003). We arcsine-transformed our data and tested whether the proportion of time spent in each behavior differed over all monarchs using an analysis of variance (ANOVA; Crawley 2013, Vankosky and VanLaerhoven 2015), with a post-hoc Tukey test to distinguish statistically significant differences between behaviors in the R statistical environment (R Development Core Team 2015). We also tested for differences in activity budgets between males and females, month of observation (June, July, or August), and time of observation (morning, mid-day, afternoon) using multiple analysis of variance (MANOVA) in R (R Development Core Team 2015, Vankosky and VanLaerhoven 2015). We pooled observation times by morning (9:00-11:59), mid-day (12:00-14:59), and afternoon (15:00-17:00) in order to increase the number of samples in each group, compared to separating observations by each hour of the day.

Next, we compared the average proportion of time monarchs from the same site spent in each behavior in relation to local vegetation and landscape variables using multivariate ordination. This allowed us to evaluate how site variables influence monarch behavior at the site level, not the individual level. All multivariate ordination procedures were conducted with the *vegan* package in R (Oksanen 2009). We used metric multidimensional scaling (MDS) with the “capscale” function and Bray-Curtis distance measures to create ordination bi-plots for average site activity budgets (Stafford et al. 2012). We removed mating from potential behaviors because it was rare and only occurred at two sites. Using the function “envfit”, we tested how monarch activity budgets at the site level related to vegetation variables that included ramet density, forb species richness and diversity, plant species richness and diversity, Kentucky bluegrass (*Poa pratensis*) canopy cover, smooth brome (*Bromus inermis*) canopy cover, invasive forb canopy cover, and milkweed canopy cover, along with landscape variables that included percent cover of perennial grassland, crop land, hay ground, open water, and wetland (Table A4). We tested for correlations among variables to eliminate any redundancies in our analysis ( $r \geq 0.60$ ). For all analyses, we set an  $\alpha < 0.05$  to indicate statistically significant differences.

Finally, we used individual ethograms to quantify how field behaviors were organized using first-order Markovian transition matrices (Goodman 1968, Wang and Messing 2003). Transitional matrices allow us to count the number and type of transitions between behaviors (behavioral flow) and depict typical behavioral observation surveys by calculating the probability of different behaviors. We

constructed matrices with rows and columns representing preceding and following behaviors, respectively. In each cell, we entered the total number of times a transition between behaviors was observed. A butterfly could not transition to the same behavior (i.e., flying to flying). Additionally, if a behavior lasted for the entire observation (e.g., mating), no transitions were counted. We created one matrix using ethograms for all monarchs, but we also constructed matrices using either male or female ethograms to evaluate the potential impact of sex on behavioral transitions. Then we used maximum likelihood estimates to calculate expected values for the matrix using the *markovchain* package in R (R Development Core Team 2015, Spedicato et al. 2015). We used a likelihood ratio test (G-Test) using the *DescTools* package in R to assess the significance of each matrix (Wang and Keller 2002, Signorell et al. 2017).

## Results

We recorded behaviors for 51 wild monarchs over two summers for a total of 13 hours of observation. Of the 51 individuals, we observed 35 females, 15 males, and 1 undetermined, which was included in our overall totals but excluded from any analyses differentiating by sex. We made monarch behavioral observation surveys at 16 of the 29 sites we visited, with over 50% of our observation surveys occurring at five sites in northeast South Dakota (Figure 12). We observed our first monarchs on June 19 and June 5 in 2016 and 2017, respectively. Since we collected monarch behavior data opportunistically, we did not continue monarch behavioral observation surveys into the fall, as our other butterfly surveys were completed by mid-August each year. Consequently, our last monarch observation surveys occurred on August 5, 2016 and August 8, 2017. We completed more observation surveys in August (24 of 51 surveys) than any other month, even though we only collected data for approximately one week in August. Additionally, we completed more observation surveys in the morning (27 of 51 surveys) compared to the mid-day or afternoon (Figure A3).

### Activity budgets

We quantified activity budgets and found a significant difference in the percentage of time allocated for each behavior when pooling across all monarchs ( $F_{5, 300} = 11.8, p < 0.001$ ). Monarchs spent the most time flying, with individuals spending an average 36% of behavioral observation surveys flying (Figure 13). However, monarchs also spent significantly more time nectaring and resting compared to

basking, mating, and ovipositing, the latter three behaviors only comprising 20% of surveys (Figure 13). We did not observe any courtship behaviors.

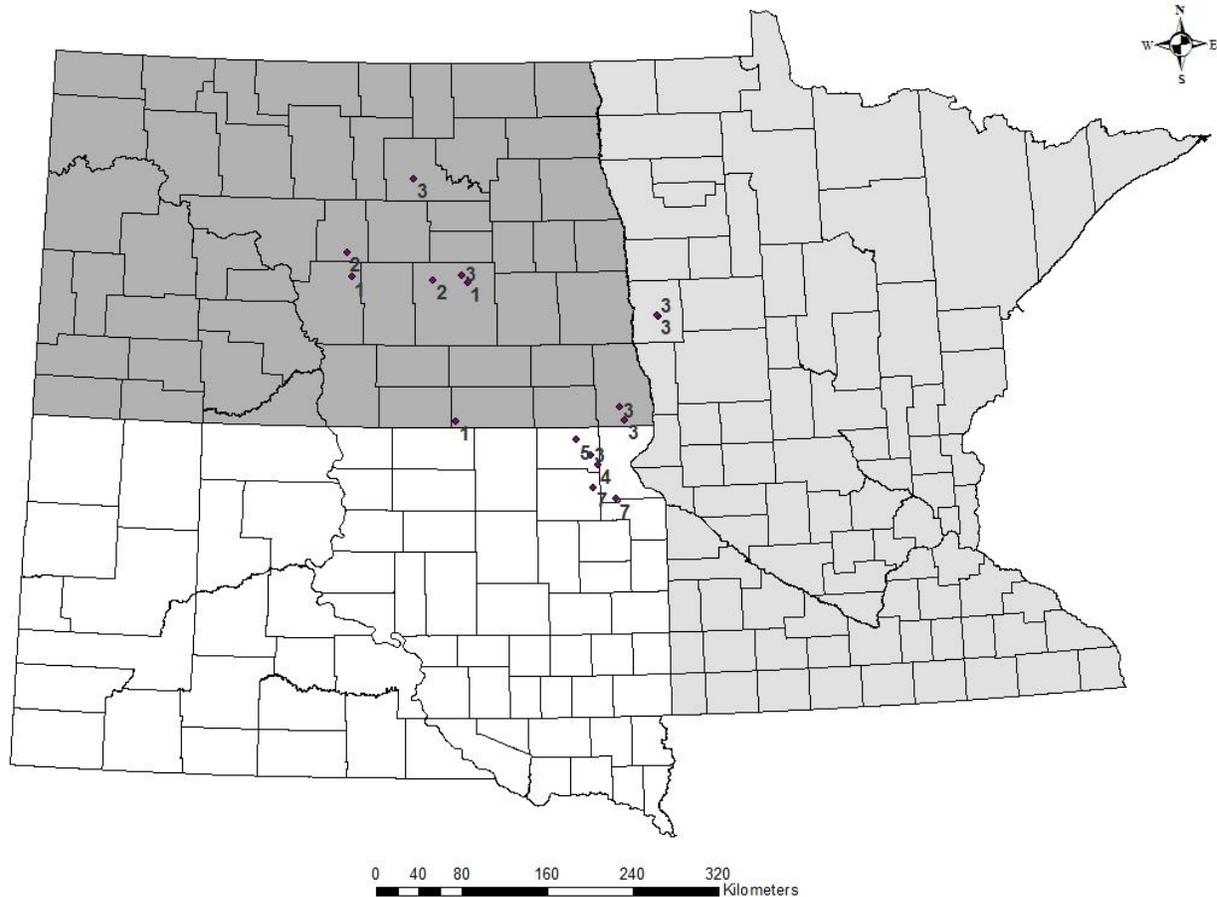


Figure 12. Survey locations and number of monarch behavioral observation surveys from 2016-2017 in North Dakota, South Dakota, and Minnesota, USA. We observed 51 monarchs in total, with most observation surveys occurring in northeast South Dakota.

We then delved into behaviors further by examining how activity budgets differed based on sex, observation month, and observation time. Unlike behaviors across all monarchs, we did not find significant differences in activity budgets between sexes ( $F_{48,2} = 0.79$ ,  $p > 0.05$ ), observation months ( $F_{48,2} = 1.37$ ,  $p > 0.05$ ), or observation times ( $F_{48,2} = 1.73$ ,  $p > 0.05$ ). Although not statistically significant, we did not observe any monarchs basking or mating in June, altering the activity budget compared to July and August. All other behaviors, besides courtship, were observed in July and August at relatively similar proportions quantified for all monarchs (i.e., most time was spent flying, nectaring, and resting with a

smaller proportion spent basking, mating, and ovipositing; Figure A3). Interestingly, we only observed mating during the afternoon. Mating accounting for 33% of afternoon activity budgets, even though we only observed two separate mating events (four individuals total). Each mating event lasted for the entirety of the 15-minute observation survey. Consequently, averaged over the 12 monarchs observed in the afternoon, mating became a substantial proportion of activity budgets. We did not have sufficient statistical evidences that males and females behaved differently or that behaviors changed depending on the month or time of day. However, these outcomes were likely a result of our limited sample size (Figure A3).

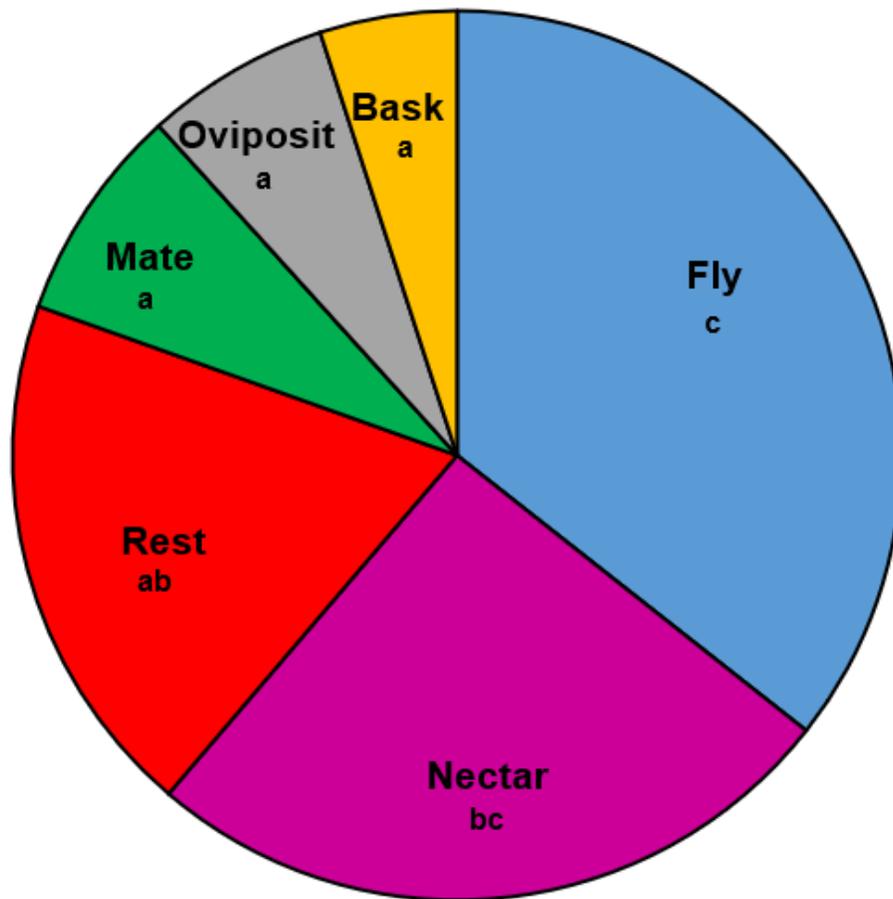


Figure 13. The average percent of time spent in each behavior during monarch behavioral observation surveys from 2016-2017 in the Northern Great Plains, USA. Statistically significant differences occurred between the amount of time spent in each behavior ( $F_{5, 300} = 11.8, p \leq 0.001$ ). During observation surveys, most monarchs were either flying, nectaring, or resting, with a smaller portion of time spent basking, mating, and ovipositing. Different letters correspond to a difference at the  $p \leq 0.05$  level calculated from the post-hoc Tukey test.

### **Local and landscape influences on activity budgets**

We used multivariate ordination to determine how activity budgets—the percentage of time spent in each behavior—at the site level responded to local and landscape variables. Sites are plotted as points, and the nearest behavior label represents which behavior made up the largest proportion of time at each site (Figure 14). If sites are located between two behaviors, both behaviors contribute to activity budgets. Monarch activity budgets were significantly ( $p \leq 0.05$ ) influenced by three local variables, plotted as environmental vectors, but landscape variables were not included in our final ordination ( $p > 0.05$ ). The environmental vectors show the strength and direction of each variable. Monarch activity budgets with a higher percentage of resting and basking were correlated with increasing milkweed and smooth brome cover (Figure 14). Sites where monarchs spent more time nectaring were correlated with high plant species diversity. However, monarchs that spent a majority of their time ovipositing and flying were not highly correlated with any one individual vector. Individuals that spent more time ovipositing and flying were located in the middle of the ordination plot where sites had a moderate cover of both milkweed and smooth brome (Figure 14; Figure A4).

### **Behavioral transitional matrices**

Behavioral transitional matrices allowed us to visualize the average behavioral observation survey. We found that monarchs transitioned from one behavior to at least one other behavior during observation surveys, except for the four individuals mating (see above). Consequently, mating was not included in transitional matrices (Figure 15). Behavioral flow patterns constructed from transitional matrices were non-random (i.e., monarchs transitioned to certain behaviors or combination of behaviors consistently over observation surveys), and flow patterns were significant for all monarchs combined ( $G = 329$ ,  $df = 16$ ,  $p < 0.001$ ), females ( $G = 204$ ,  $df = 16$ ,  $p < 0.001$ , Figure 15A) and males ( $G = 83.2$ ,  $df = 9$ ,  $p < 0.001$ , Figure 15B). Female behavioral flow patterns were more complex than male behavioral flow patterns because females had an additional behavior with ovipositing (Figure 15A). Females did not transition from basking to nectaring (Figure 15A), and males did not transition between basking and nectaring in either direction (Figure 15B). Collectively, monarchs initially observed in any behavior had the highest probability of transitioning to flying (0.68) or nectaring (0.50) during observation surveys. Conversely, monarchs had a much lower probability of transitioning to basking or ovipositing (Figure 15).

For example, if we detected a male monarch nectaring, we would most likely observe a transition to flying. From there, the monarch would likely transition back to nectaring. A smaller probability exists that a male monarch initially detected nectaring would transition to resting (Figure 15B).

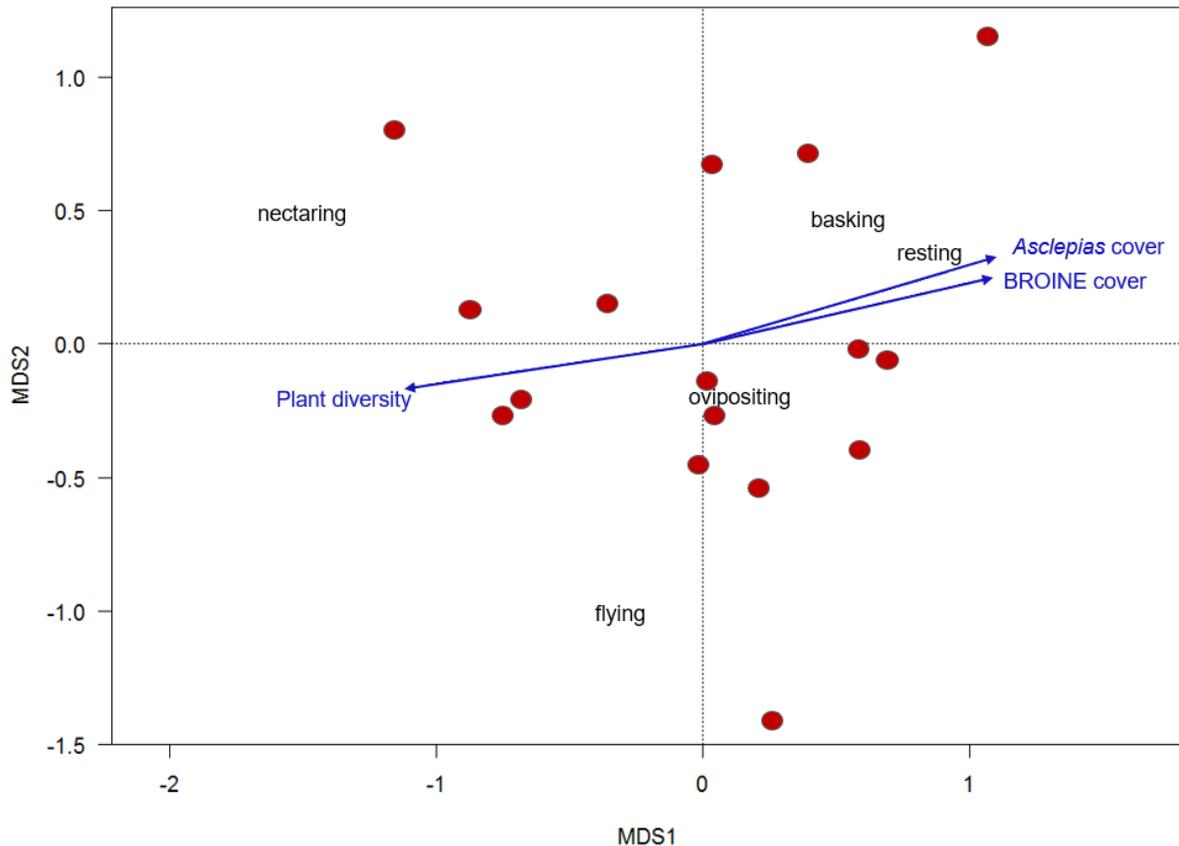


Figure 14. Multidimensional scaling ordination using Bray-Curtis distance for monarch behaviors observed during the breeding season from 2016-2017 in the Northern Great Plains, USA. The first two axes explained 78% of the variation. Site scores are represented with red circles. We used envifit to determine that sites with higher milkweed (*Asclepias* cover) and smooth brome (BROINE cover) cover were associated with monarchs that spent more time basking and resting. As site plant diversity increased, individuals spent more of their time nectaring.

We can increase our ability to visualize behavioral observation surveys by including the average duration (seconds) and average frequency of behaviors per observation survey (Figure 15). On average, monarchs spent 134 seconds in each behavior before transitioning, and resting had the longest average duration at 215 seconds. Therefore, even though monarchs were less likely to transition to resting, if they were observed in this behavior, they remained resting longer compared to the other behaviors.

Additionally, most behaviors occurred at least two separate times during observation surveys (Figure 15).

Flying and nectaring had the highest average frequency per observation with over three occurrences, while resting had the lowest frequency at just under 2 (Figure 15). This means that monarchs, besides individuals mating, transitioned to the same behavior multiple times during surveys (i.e., individuals did not restrict themselves to one behavior and transitioned back-and-forth between behaviors during 15-minute observations).

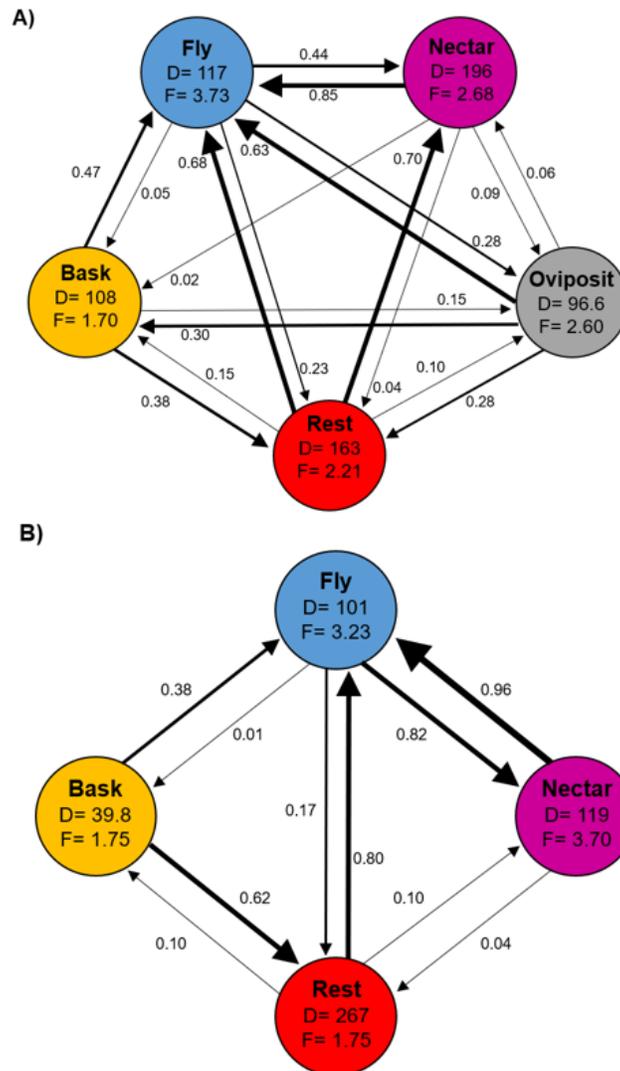


Figure 15. Behavior transitions for females (A, n= 33) and male (B, n= 13) monarchs from surveys conducted in 2016-2017 in the Northern Great Plains, USA. If monarchs transitioned to another behavior during observation surveys, we included an arrow going from the first to second behavior and the probability of that transition occurring using our data to calculate maximum likelihood estimates. Transitions with a higher probability of occurring have thicker arrows. For each behavior, we included the average duration in seconds (D) and average frequency per behavioral observation survey (F) for each behavior.

## Discussion

Behavioral studies for wild butterflies are infrequently conducted (Peixoto and Benson 2009), even though behavioral data at the site level can improve conservation efforts beyond what is provided by abundance estimates (Knowlton and Graham 2010, James 2016). By connecting behaviors to local and landscape variables, we can determine if and how butterflies utilize sites (e.g., nectaring, reproduction, fly-over). We conducted behavioral field observations for summer breeding monarchs in the Northern Great Plains, USA, an area located on the northwestern edge of the main breeding population of eastern North American monarchs (Flockhart et al. 2013). Monarchs spent most of their time flying, nectaring, or resting compared to basking, ovipositing, and mating. Although we may associate flying with unsuitable sites (i.e., flying to leave areas without resources), we found that flying often occurred before and after other behaviors necessary for monarch success (e.g., nectaring, ovipositing). At the site level, we expected oviposition would increase as milkweed cover increased, but the proportion of time spent ovipositing was highest at moderate amounts of milkweed cover. Additionally, nectaring individuals were associated with increasing plant species diversity and lower milkweed cover. Sites with high milkweed cover also had more invasive grass cover, which reduces nectar resources. Consequently, a trade-off appears to exist between milkweed cover and nectar resource availability on sites we surveyed. Although some conservation efforts are directed towards increasing the number of milkweed stems in agricultural areas (Pleasants et al. 2017), efforts should also consider disturbance practices (fire and grazing) in natural and non-agricultural landscapes to improve availability of nectar and oviposition resources at the same site.

Flying made up the highest percentage of monarch activity budgets. We observed flying more often than other behaviors because it covers different types of flight behaviors such as patrolling and oviposition site searching. Patrolling was the most common behavior observed in behavioral field observations of wild monarchs at one site in Washington State (James 2016). However, we did not include it in our *a priori* behaviors because we were unsure how often we would observe this behavior and if we would be able to distinguish this behavior from other types of flight. Whereas our *a priori* behaviors have distinct differences (Table 8), discerning between different flight behaviors requires more experience and may not be easily adaptable for novice observers. After flying, nectaring made up the

largest proportion of activity budgets. Individuals flying are expected to nectar frequently to meet energy requirements (Hirota and Obara 2000), so we would expect these two behaviors to dominate activity budgets, especially over behaviors that may not occur every day like ovipositing and mating.

Activity budgets at the site level had mixed responses to milkweed cover. Monarchs that spent more time ovipositing were unexpectedly associated with moderate milkweed cover, not sites with the highest amount of milkweed. However, females often lay eggs in low density milkweed patches because milkweed quality is improved, they can avoid natural enemies (Zalucki and Lammers 2010, Pitman et al. 2017), and enough milkweed is available to ensure larval survival (Nail et al. 2015). Additionally, monarchs spent more time nectaring as plant diversity increased and milkweed cover decreased. Sites with more milkweed also had increased coverage of smooth brome, an invasive grass that reduces plant species diversity including nectar resources (Hendrickson and Lund 2010). Monarch activity is expected to increase as milkweed increases (Pleasants et al. 2017) but not if nectar resources are unavailable (James 2016). Consequently, monarchs may be optimizing the cover of milkweed with available nectar resources when ovipositing (Figure A4), not just selecting sites with the highest amount of milkweed.

We created transitional matrices and behavioral flow patterns to understand how monarchs typically behave during observational surveys. We expected monarchs would not transition as often and remain longer in behaviors like ovipositing or nectaring compared to flying. Some of these assumptions were supported, as nectaring had the second longest average duration. However, behavioral flow patterns showed the importance of flying. Collectively, monarchs had the highest probability of transitioning to flying from any other behavior. Thus, even though flying may be perceived as a behavior indicating a site is unsuitable (i.e., fly-over site), behavioral flow patterns demonstrate how flying is interconnected with these behaviors (Hirota and Obara 2000). Therefore, if behaviors are utilized in monarch counts, with behavior at the time of detection being noted instead of longer observational surveys (e.g., McCord and Davis 2010), our results indicate that flying should not prematurely categorize a site as lacking necessary resources.

Interestingly, the sex ratio of individuals from behavioral observation surveys was female biased 70:30. Typically, sex ratios are male biased, with males making up nearly 60% of the population (McCord and Davis 2010, Davis and Rendon-Salinas 2010, James 2016). Recently, male biased sex ratios have

created concerns that female monarchs may face more declines compared to male monarchs (McCord and Davis 2012), especially since females are more negatively impacted by an obligate protozoan parasite *Ophryocystis elektroscirrha* (OE; Davis and Rendon-Salinas 2010). Monarchs in northern latitudes are less infected with OE, so we would not expect to detect the same impacts on sex ratios (Altizer et al. 2015, Flockhart et al. 2018). Nonetheless, our observed sex ratio may be the result of our ability to maintain visuals of males versus females since we did not record the sex of failed observation surveys.

Monarch behavior at the site level changed in response to measured vegetation variables. Therefore, disturbance management (fire, grazing, haying) has the ability to alter monarch behaviors and optimize host and nectar resource availability. Our results cannot verify either the fall migration hypothesis or milkweed limitation hypothesis (see Pleasants et al. 2017), but they do add another aspect to consider in monarch conservation management. Natural and non-agricultural areas are important for monarch conservation (Nail et al. 2015, Pitman et al. 2017), but planting more milkweed in these areas may not improve monarch numbers since the focus of milkweed loss is in agricultural areas (Pleasants et al. 2017). Furthermore, more milkweed in these natural and non-agricultural areas could increase OE prevalence and increase predation (Zalucki and Lammers 2010, Altizer et al. 2015), without increasing the amount of ovipositing and nectar resources. Fire and haying disturbance have already been used to increase host plant availability for monarchs (Baum and Sharber 2012, Alcock et al. 2016). Consequently, we argue that disturbance management could also be used, with the correct temporal and spatial scale, to increase nectar resources (Towne et al. 2005), increase native plant diversity (Hendrickson and Lund 2010), and improve milkweed availability to provide ovipositing and nectar resources for adults and enough milkweed resources to sustain larvae all at the same site (Figure 16).

Future surveys on breeding monarchs should incorporate several changes to better inform conservation efforts. Flying should be separated into multiple categories that may allow us to detect behavioral differences between sexes including male patrolling and female oviposition site searching. Additionally, flying could also be separated into distinct behaviors we noted in the field such as intra- and inter-species fleeing and chasing. Since our behavioral observation surveys were opportunistically collected during other butterfly surveys, we did not include several factors that may help discern

behavioral differences such as testing individuals for OE and noting wing condition (Hiorta and Obara 2000, Goehring and Oberhauser 2002, Bradley and Altizer 2005). Additionally, future research should connect activity budgets with fitness (e.g., fecundity, larval survival). We determined how site variables influence activity budgets, but we do not know how activity budgets affect overall success. For example, how do differences in overall activity budgets (more nectaring vs. more resting) relate to adult fitness and subsequent larval success? Observing monarchs repeatedly throughout their life at the same site and assessing monarch survival at each life stage could elucidate the connection between behaviors, success, and ecological variables (Sih et al. 2004, Lind and Cresswell 2005, Berger et al. 2012).

### **Conclusions**

Monarch butterflies are proposed for listing under the Endangered Species Act (USFWS 2017), yet behavioral studies for summer-breeding monarchs that can connect behaviors to landscape level management practices are rare (Knowlton and Graham 2010, James 2016). We completed behavioral field observation surveys to determine how monarch activity budgets responded to local and landscape variables and gained a better understanding of monarch site utilization necessary for conservation efforts (Pickens and Root 2009). We observed 51 monarchs over two years and found that individuals spent most of their time flying, nectaring, and resting. Contrary to expectations, we did not observe more monarchs mating, nectaring, and ovipositing as milkweed cover increased. However, activity budgets did change from nectaring to resting as milkweed cover increased. Increases in milkweed cover corresponded with increases in smooth brome cover, an invasive grass that reduces plant species diversity. Our results suggest monarchs are adjusting activity budgets based on the availability of adult nectar resources and larval host plants. However, more research is necessary to discern how activity budgets relate to lifetime fitness. Instead of planting more milkweed in natural areas, conservation efforts should focus on utilizing disturbance to increase availability of both milkweed plants and nectar resources within the same site. Because larval survival increases in natural areas (Nail et al. 2015), improving resource availability has the potential to reduce monarch declines in these areas.



Figure 16. Removing fire and grazing increases the cover of milkweed (*Asclepias* spp.) plants in rangelands. However, invasive species like smooth brome (*Bromus inermis*) also increase, decreasing plant species diversity and producing large, indistinct stands of milkweed and smooth brome (A; photo by KCK), which can make it difficult to concurrently find oviposition sites and nectar resources. Restoring ecological processes like fire can reduce invasive plant species and increase both host and nectar resource availability (B; photo by TJH).

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## APPENDIX

Table A1. Averaged counts ( $\pm$ SE) for each butterfly species detected in North Dakota and South Dakota. Columns give a per site average for species surveyed using either line-transect distance sampling (LTDS) or visual encounter surveys (VES) completed in either 2015 and 2016. In 2015 20 sites were sampled using both methods, and in 2016 there were 25 sites. In both years each site was surveyed using VES for three times the effort that it was surveyed using LTDS.

Species	2015				2016			
	LTDS		VES		LTDS		VES	
	mean	SE	mean	SE	mean	SE	mean	SE
<i>Boloria bellona</i>	0.3	0.04	1.25	0.16	1.64	0.13	6.96	0.48
<i>Boloria selene</i>	-	-	0.05	0.01	-	-	0.04	0.01
<i>Celastrina neglecta</i>	-	-	-	-	0.04	0.01	0.04	0.01
<i>Cercyonis pegala</i>	3.6	0.16	20.25	0.61	7.56	0.42	36.60	2.25
<i>Chlosyne gorgone</i>	-	-	-	-	0.20	0.03	0.56	0.06
<i>Coenonympha tullia</i>	0.8	0.04	7.55	0.56	2.08	0.11	8.92	0.41
<i>Colias eurytheme</i>	0.3	0.03	2.70	0.21	12.80	0.39	44.68	1.27
<i>Colias philodice</i>	3.45	0.17	18.00	0.58	4.56	0.14	18.16	0.42
<i>Danaus plexippus</i>	1.1	0.10	5.10	0.29	0.68	0.05	3.48	0.18
<i>Epaygyreus clarus</i>	-	-	-	-	-	-	0.04	0.01
<i>Euptoieta claudia</i>	-	-	-	-	0.32	0.04	1.48	0.15
<i>Glaucopsyche lygdamus</i>	0.05	0.01	1.50	0.17	0.52	0.05	1.76	0.14
<i>Hesperia dacotae</i>	-	-	-	-	-	-	0.04	0.01
<i>Junonia coenia</i>	-	-	-	-	-	-	0.08	0.01
<i>Limenitis arthemis</i>	-	-	-	-	-	-	0.04	0.01
<i>Lycaena hyllus</i>	-	-	-	-	0.16	0.01	0.08	0.01
<i>Oeneis uhleri</i>	-	-	-	-	-	-	0.08	0.02
<i>Papilio glaucus</i>	-	-	-	-	-	-	0.08	0.02
<i>Papilio polyxenes</i>	-	-	-	-	0.16	0.02	0.72	0.04
<i>Phyciodes batesii</i>	-	-	-	-	0.12	0.02	0.40	0.03
<i>Phyciodes coccyta</i>	0.05	0.01	0.60	0.11	1.00	0.09	2.04	0.17
<i>Phyciodes tharos</i>	1.85	0.12	7.45	0.47	1.08	0.07	7.24	0.72
<i>Pieris rapae</i>	1.85	0.12	7.65	0.59	2.36	0.18	6.60	0.48
<i>Plebejus melissa</i>	0.65	0.07	3.50	0.38	1.28	0.11	4.16	0.23
<i>Polites mystic</i>	0.5	0.05	3.05	0.20	1.00	0.07	3.80	0.23
<i>Polites peckius</i>	-	-	0.30	0.05	-	-	0.08	0.01
<i>Polites themistocles</i>	0.2	0.04	0.30	0.07	0.20	0.04	0.68	0.07
<i>Pontia protodice</i>	-	-	-	-	-	-	0.04	0.01
<i>Pyrgus communis</i>	-	-	-	-	0.04	0.01	-	-
<i>Sayrodes eurydice</i>	0.1	0.02	0.40	0.05	0.04	0.01	0.56	0.08
<i>Satyrium titus</i>	0.05	0.01	0.15	0.02	0.16	0.02	0.92	0.10
<i>Speyeria aphrodite</i>	0.45	0.06	1.75	0.15	2.68	0.18	9.64	0.65
<i>Speyeria cybele</i>	0.05	0.01	0.70	0.07	0.64	0.05	1.40	0.08
<i>Speyeria idalia</i>	0.05	0.01	0.45	0.05	1.32	0.12	4.72	0.32
<i>Vanessa atalanta</i>	0.15	0.02	0.95	0.05	0.36	0.04	1.80	0.09
<i>Vanessa cardui</i>	0.05	0.01	-	-	0.04	0.01	0.04	0.01
<i>Vanessa virginiensis</i>	-	-	-	-	-	-	0.08	0.01

Table A2. Model outputs for 15 butterfly species found in the Northern Great Plains between 2015-2017 that met minimum detection thresholds for density estimation. AIC<sub>c</sub> scores, ΔAIC<sub>c</sub>, model weight, and the number of parameters (k) are included for the top model and the null model at each level. Best-ranked models and competitive model (≤2 ΔAIC<sub>c</sub>) are included for each level. If additional variables at each level did not improve AIC<sub>c</sub> scores, they were not included in the table, and only the null model appears.

Species		AIC <sub>c</sub>	ΔAIC <sub>c</sub>	weight	k
<b>Facultative species</b>					
<i>Colias eurytheme</i>					
2,500 Landscape					
	null	290	39	0	1
1,000 Landscape					
	WET	251	0	0.64	2
	WET+HAY	252	1	0.36	3
	null	290	39	0	1
Local					
	RD+FR	186	0	1	3
	null	290	104	0	1
Final model: WET+RD+FR					
<i>Colias philodice</i>					
2,500 Landscape					
	DEV	296	0	0.99	2
	null	305	9	0.01	1
1,000 Landscape					
	LDI+WET+WATER	220	0	1	4
	null	305	85	0	1
Local					
	FORBRICH+DIS	222	0	1	4
	null	305	83	0	1
Final model: DEV+LDI+WET+WAT+FR+DIS					
<i>Danaus plexippus</i>					
2,500 Landscape					
	LDI	140	0	0.89	2
	null	145	5	0.13	1
1,000 Landscape					
	HAY+WAT	111	0	0.98	3
	null	145	34	0	1
Local					
	null	145	0	1	1
Final model: LDI+HAY+WAT					
<i>Pieris rapae</i>					
2,500 Landscape					
	LDI+HAY	254	0	1	3
	null	311	57	0	1
1,000 Landscape					
	null	311	0	0	1
Local					
	PR	233	0	0.95	2

Table A2. Model outputs for 15 butterfly species found in the Northern Great Plains between 2015-2017 that met minimum detection thresholds for density estimation (continued). AIC<sub>c</sub> scores, ΔAIC<sub>c</sub>, model weight, and the number of parameters (k) are included for the top model and the null model at each level. Best-ranked models and competitive model (≤2 ΔAIC<sub>c</sub>) are included for each level. If additional variables at each level did not improve AIC<sub>c</sub> scores, they were not included in the table, and only the null model appears.

Species		AIC <sub>c</sub>	ΔAIC <sub>c</sub>	weight	k
	null	311	78	0	1
	Final model: LDI+HAY+PR				
<hr/>					
<i>Phyciodes cocyta</i>					
	2,500 Landscape				
	HAY	182	0	0.97	2
	null	189	7	0.03	1
	1,000 Landscape				
	LDI	165	0	0.99	2
	null	189	24	0	1
	Local				
	IGC	97	0	1	2
	null	189	92	0	1
	Final model: HAY+LDI+IGC				
<hr/>					
<i>Phyciodes tharos</i>					
	2,500 Landscape				
	LDI	377	0	0.90	2
	null	477	100	0	1
	1,000 Landscape				
	null	477	0	1	1
	Local				
	FB+IGC+IFC	211	0	1	4
	null	477	266	0	1
	Final model: LDI+FR+IGC+IFC				
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<i>Plebejus melissa</i>					
	2,500 Landscape				
	DEV+HAY	306	0	1	3
	null	343	37	0	1
	1,000 Landscape				
	LDI+WET	182	0	1	3
	null	343	153	0	1
	Local				
	DIS+LIT	145	0	0.92	4
	null	343	198	0	1
	Final model: DEV+HAY+LDI+WET+DIS+LIT				
<hr/>					
<i>Speyeria aphrodite</i>					
	2,500 Landscape				
	WAT+WET	735	0	1	3
	null	856	121	0	1
	1,000 Landscape				
	null	854	0	1	1

Table A2. Model outputs for 15 butterfly species found in the Northern Great Plains between 2015-2017 that met minimum detection thresholds for density estimation (continued). AIC<sub>c</sub> scores, ΔAIC<sub>c</sub>, model weight, and the number of parameters (k) are included for the top model and the null model at each level. Best-ranked models and competitive model (≤2 ΔAIC<sub>c</sub>) are included for each level. If additional variables at each level did not improve AIC<sub>c</sub> scores, they were not included in the table, and only the null model appears.

Species		AIC <sub>c</sub>	ΔAIC <sub>c</sub>	weight	k
	Local				
	RD+PR+ LIT+IFC+DIS	201	0	1	7
	null	856	656	0	1
	Final model: WAT+WET+RD+PR+LIT+IFC+DIS				
<hr/>					
<i>Speyeria cybele</i>					
	2,500 Landscape				
	null	38	0	1	1
	1,000 Landscape				
	WAT	30	0	0.79	2
	LDI+WAT+DEV	32	2	0.21	4
	null	38	8	0	1
	Local				
	FR	29	0	0.75	2
	FR+PR	31	2	0.25	3
	null	38	9	0	1
	Final model: WAT+FR				
<hr/>					
<i>Vanessa cardui</i>					
	2,500 Landscape				
	LDI+HAY	148	0	1	3
	null	191	43	0	1
	1,000 Landscape				
	null	191	0	1	1
	Local				
	IGC	109	0	1	3
	null	191	82	0	1
	Final model: LDI+HAY+IGC				
<hr/>					
<b>Obligate Species</b>					
<hr/>					
<i>Boloria bellona</i>					
	2,500 Landscape				
	WAT	158	2	0.43	2
	null	156	0	0.57	1
	1,000 Landscape				
	LDI+WET	121	0	0.81	3
	null	156	35	0	1
	Local				
	RD+ VP	84.5	0	1	4
	null	156	71.5	0	1
	Final model: LDI+WET+RD+VP				
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<i>Cercyonis pegala</i>					
	2,500 Landscape				
	LDI+HAY+WAT+DEV	848	0	1	5

Table A2. Model outputs for 15 butterfly species found in the Northern Great Plains between 2015-2017 that met minimum detection thresholds for density estimation (continued). AIC<sub>c</sub> scores, ΔAIC<sub>c</sub>, model weight, and the number of parameters (k) are included for the top model and the null model at each level. Best-ranked models and competitive model (≤2 ΔAIC<sub>c</sub>) are included for each level. If additional variables at each level did not improve AIC<sub>c</sub> scores, they were not included in the table, and only the null model appears.

Species		AIC <sub>c</sub>	ΔAIC <sub>c</sub>	weight	k	
<i>Coenonympha tullia</i>	2,500 Landscape	null	999	151	0	1
	1,000 Landscape	WET	599	0	1	2
		null	999	400	0	1
	Local	FR+IGC	148	0	1	3
		null	999	851	0	1
	Final model: LDI+HAY+WAT+DEV+WET+FR+IGC					
<hr/>						
<i>Coenonympha tullia</i>	2,500 Landscape	null	289	0	1	1
	1,000 Landscape	LDI+WET+DEV	173	0	1	4
		null	289	116	0	1
	Local	DIS+IFC	150	0	0.94	4
		null	289	139	0	1
	Final model: LDI+WET+DEV+DIS+IFC					
<hr/>						
<i>Polites mystic</i>	2,500 Landscape	null	256	13	0.01	1
	1,000 Landscape	WET	222	0	1	2
		null	256	34	0	1
	Local	VOR+LIT+IGC	148	0	1	4
		null	256	108	0	1
	Final model: WET+VOR+LIT+IGC					
<hr/>						
<i>Speyeria idalia</i>	2,500 Landscape	null	148	0	0.75	1
	1,000 Landscape	WET	125	0	0.72	2
		WET+LDI	127	2	0.26	3
		null	148	23	0	1
	Local	VOR	90	0	1	2
		null	148	58	0	1
Final model: WET+VOR						

Table A3. Summary statistics and descriptions for butterfly and vegetation variables utilized in multivariate and generalized linear modeling analyses for surveys conducted in the Northern Great Plains, USA, from 2015-2017.

<b>Variable</b>	<b>Mean (SE)</b>	<b>Range</b>	<b>Description</b>
<b>Vegetation variables</b>			
Kentucky bluegrass cover	20.0 (0.96)	2.92-36.2	Average percent Kentucky bluegrass ( <i>Poa pratensis</i> ) canopy cover using 30- 1 m <sup>2</sup> quadrats at each site
Smooth brome cover	18.7 (1.29)	0.34-43.1	Average percent smooth brome ( <i>Bromus inermis</i> ) canopy cover using 30- 1 m <sup>2</sup> quadrats at each site
Forb richness	29.0 (0.95)	13.0-45.0	Average number of individual flowering forb species recorded in 500 m <sup>2</sup> belt transects. Richness was a simple count of the number of species.
Forb diversity	0.78 (0.01)	0.35-0.94	Average forb diversity measured with Simpson's diversity index ( $D = 1 - \sum_i^s p_i^2$ ) in 500 m <sup>2</sup> belt transects
Plant richness	22.5 (0.97)	6.00-39.0	Average number of individual plant species recorded in 30- 1 m <sup>2</sup> quadrats at each site. Richness was a simple count of the number of species.
Plant diversity	0.82 (0.01)	0.56-0.94	Average plant diversity measured with Simpson's diversity index ( $D = 1 - \sum_i^s p_i^2$ ) in 30- 1 m <sup>2</sup> quadrats
Ramet density	171.9 (15)	18.4-563	Average number of flowering ramets in 8- 500 m <sup>2</sup> belt transects at each site
<b>Landscape variables</b>			
Perennial grassland cover	0.72 (0.02)	0.37-0.93	Percent perennial grassland cover in 1,000 m buffers
Wetland cover	0.12 (0.03)	0.00-0.85	Percent wetland cover in 1,000 m buffers
Water cover	0.12 (0.01)	0.00-0.31	Percent open water cover in 1,000 m buffers
Hay ground cover	0.02 (0.01)	0.00-0.08	Percent hay ground cover in 1,000 m buffers
Crop land cover	0.11 (0.02)	0.00-0.36	Percent cropland cover in 1,000 m buffers
Development cover	0.03 (0.01)	0.00-0.11	Percent development cover in 1,000 m buffers
<b>Butterfly variables</b>			
Butterfly richness	11.0 (0.38)	4.00-19.0	Mean number of individual butterfly species detected per site. Richness was a simple count of the number of species.
Butterfly diversity	0.78 (0.01)	0.39-0.92	Mean butterfly diversity measured with Simpson's diversity index ( $D = 1 - \sum_i^s p_i^2$ ) at each site
Total abundance	156 (9.11)	28.0-386	Cumulative number of individual butterflies detected at each site
Obligate abundance	58.0 (6.00)	2.00-355	Cumulative number of obligate grassland butterflies detected at each site
Facultative abundance	97.0 (6.02)	10.0-236	Cumulative number of facultative grassland butterflies detected at each site
Obligate proportion	0.36 (0.06)	0.06-0.91	Proportion of obligate butterfly abundance over the total abundance

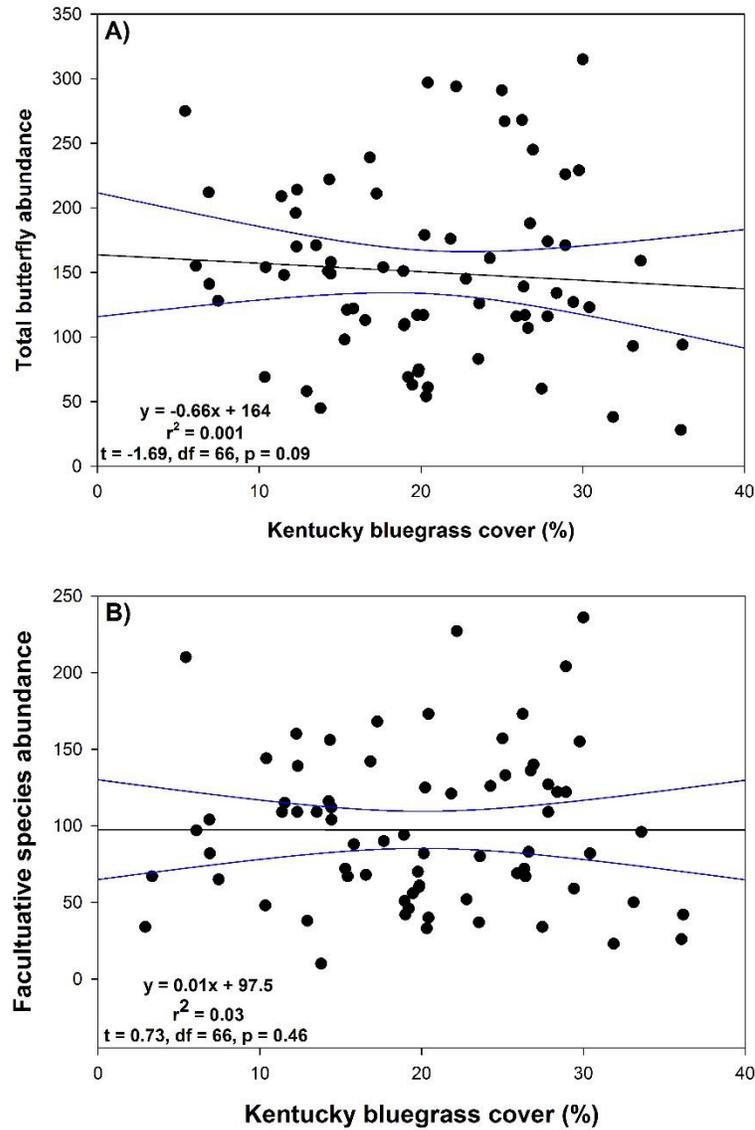


Figure A1. Butterfly abundance responses to Kentucky bluegrass (*Poa pratensis*) cover from butterfly surveys completed in the Northern Great Plains, USA, from 2015-2017. Kentucky bluegrass cover did not significantly reduced total butterfly abundance (A) or facultative species abundance (B). Generalized linear model results are included, along with 95% confidence bands (blue lines). Kentucky bluegrass cover was not correlated with total butterfly abundance or facultative grassland species abundance.

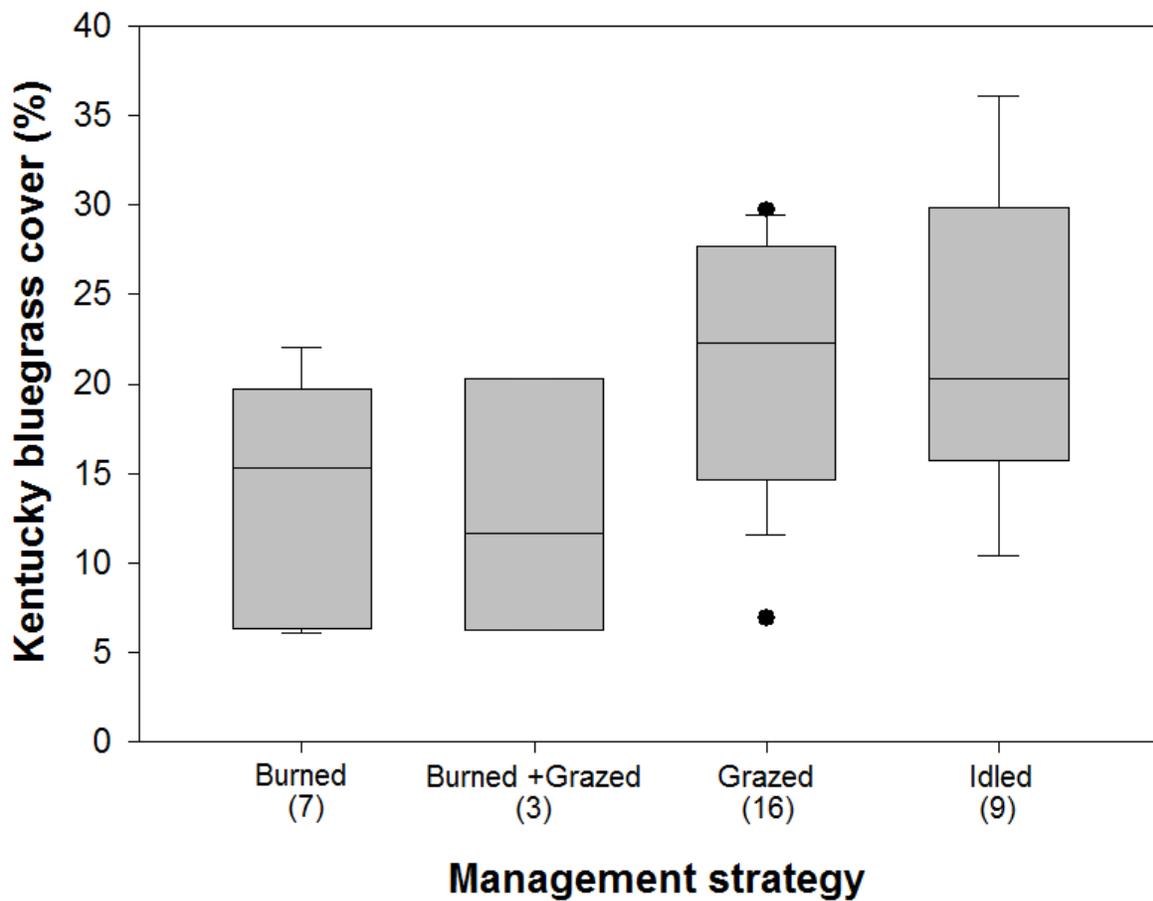


Figure A2. Boxplot and results from an analysis of variance to determine how management strategy impacts the canopy cover of Kentucky bluegrass (*Poa pratensis*) from vegetation surveys completed in the Northern Great Plains, USA, from 2015-2017. No significant differences ( $F_{4, 30} = 2.08, p = 0.11$ ) were detected, but sample sizes for some of the management strategies are very small, influencing our results. The total number of sites with each strategy are listed in parentheses under each management types. We did not impose management strategies on the 35 sites where we collected data. On average, sites that were burned or burned and grazed had lower Kentucky bluegrass cover.

Table A4. Site values for mean flowering milkweed (*Asclepias* spp.) density (per 500 m<sup>2</sup>), mean milkweed canopy cover (%), mean smooth brome (*Bromus inermis*) canopy cover (%), plant species diversity using Simpson's diversity index ( $D = 1 - \sum_i^s p_i^2$ ), number of completed behavioral observation surveys, and management strategy broadly categorized as burned, burned+grazed, idled, and grazed.

Site	Milkweed density	Milkweed cover	Smooth brome cover	Plant diversity	Completed observations	Management
Arrowwood 14	1.20	0.32	8.55	0.88	3	Grazed
Arrowwood 28	0.09	0.08	25.9	0.79	1	Burned
Berwald	6.63	1.09	22.0	0.87	7	Grazed
Bien	0.13	0.00	25.9	0.84	5	Grazed
Biggs	0.36	0.17	16.0	0.91	3	Grazed
Blue	0.54	0.42	1.18	0.84	3	Burned
Buffalo	0.10	0.72	8.13	0.85	4	Burned+Grazed
Chase	0.13	0.00	17.5	0.74	0	Grazed
CL	0.29	0.14	18.6	0.85	0	Grazed
Davis 1	1.63	0.37	2.33	0.89	0	Burned+Grazed
Davis 8	0.33	0.00	2.85	0.86	2	Burned+Grazed
GLT	1.82	0.08	6.32	0.73	0	Grazed
Hartleben	0.97	0.25	12.2	0.88	3	Idled
HB	0.08	0.14	1.67	0.89	0	Burned
Ilo 1	0.00	0.00	15.2	0.63	0	Idled
Ilo 2	0.00	0.00	11.5	0.83	0	Grazed
Larson 12	8.16	0.70	26.4	0.72	0	Grazed
Larson 4	0.44	0.00	37.7	0.70	0	Idled
Lazy m	0.08	0.14	32.5	0.77	1	Grazed
Lazy m2	0.00	0.00	34.4	0.77	0	Grazed
Manning	0.00	0.00	0.33	0.91	0	Grazed
Melass	1.88	0.02	16.7	0.85	3	Idled
Rath	15.57	1.77	43.3	0.75	1	Idled
Red iron	3.60	0.91	11.9	0.89	3	Idled
Stem	0.42	0.13	1.04	0.90	3	Burned
Sully	5.19	0.44	0.72	0.92	0	Burned
Waubay	0.42	1.18	28.3	0.86	7	Idled
Weber	11.75	1.79	17.9	0.79	0	Grazed
Winberg	7.04	0.74	24.1	0.84	2	Burned

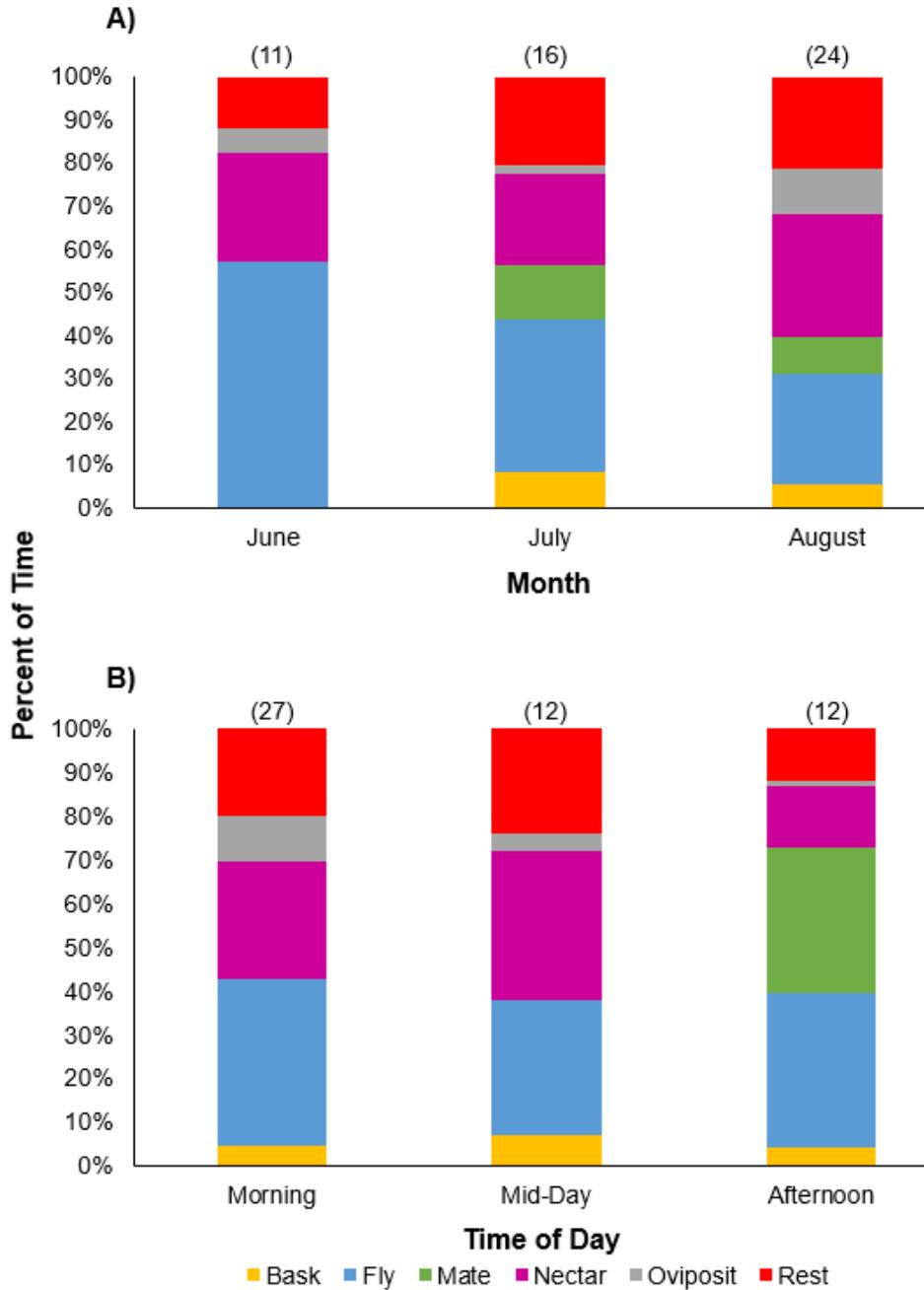


Figure A3. The average percent of time in each month (A) and time of day (B) for behaviors observed during monarch behavioral observation surveys from 2016-2017 in Northern Great Plains, USA. The number of observation surveys are listed in parentheses above each category. No significant differences were observed based on the month or time of day. However, more monarchs were observed during August in the morning, and mating was only observed during the afternoon, providing guidance for future surveys.

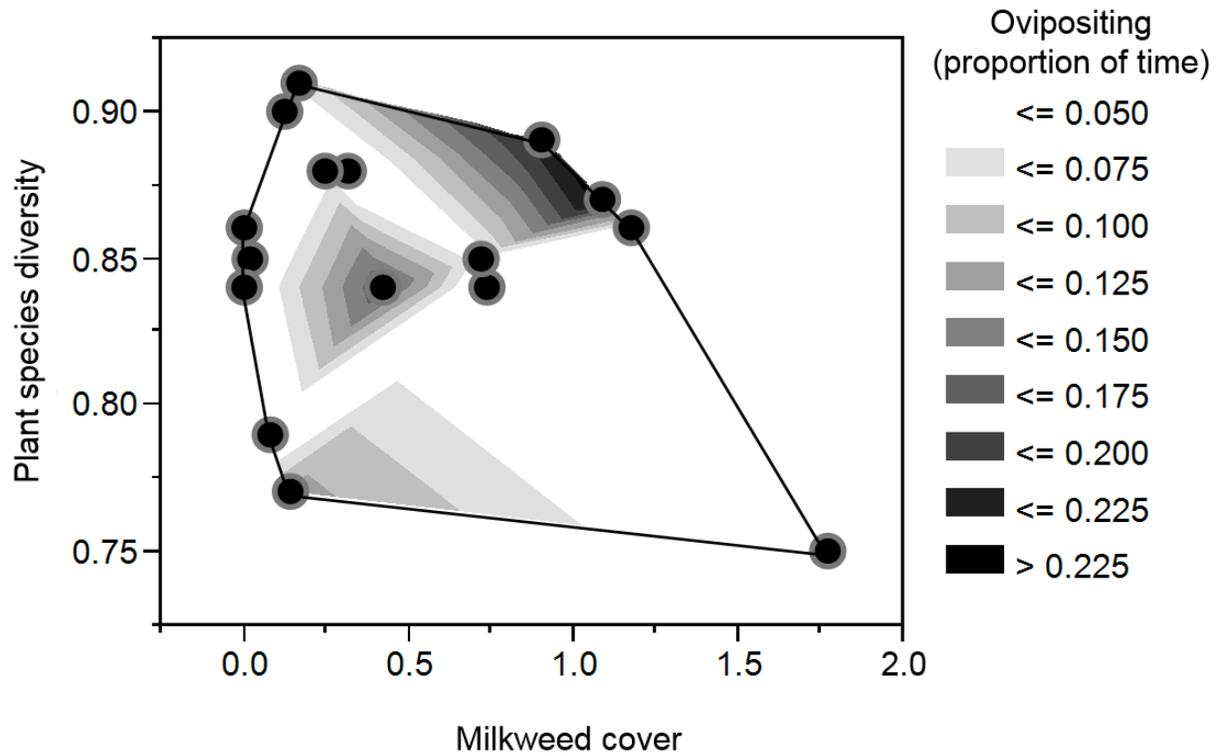


Figure A4. Proportion of time spent ovipositing for monarchs observed in the Northern Great Plains, USA, from 2016-2017. Black circles represent sites. Activity budgets included more ovipositing at moderate amounts of milkweed and higher plant species diversity. Adults need nectar resources, but areas with a higher cover of milkweed, which may be more desirable for ovipositing, do not have available nectar resources. Monarchs appear to be optimizing available oviposition sites and available nectar resources.