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Land Management And Plant Species Pattern Effects On Grassland Pollinator Services

Leslie Blaine Yellow Hammer

LAND MANAGEMENT AND PLANT SPECIES PATTERN EFFECTS ON
GRASSLAND POLLINATOR SERVICES

by

Leslie Blaine Yellow Hammer

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This thesis, submitted by Leslie B. Yellow Hammer in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

Kathryn Yurkonis

Brett Goodwin

Mia Park

This thesis is being submitted by the appointed advisory committee as having met all of the requirements of the School of Graduate Studies at the University of North Dakota and is hereby approved.

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Leslie B. Yellow Hammer
August 2020

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ABSTRACT

Invertebrate pollinator populations are widely recognized as declining because of anthropogenic activities that include changes in the amount and quality of available habitat, competition with introduced species, increased agricultural chemical use, and climate change. Because habitat and resource availability affect pollinator populations, land management may affect pollinator presence. My first aim was to determine if pollinator communities and habitat characteristics (available bare ground and floral resources) differed between grazed and idle grasslands within the Grand Forks County Grasslands of northeastern North Dakota, USA. Although available bare ground and plant species richness differed between grassland types, floral resources and pollinator communities did not differ between grassland types. My second aim was to determine how the spatial relationships among the plants within a site, in my case for a common prairie forb, affect pollinator visitation and pollinator services. The number of visiting invertebrates increased with plant diversity and visiting invertebrates were more diverse at greater *Ratibida columnifera* densities. Dipterans were by far the most abundant invertebrate visitors and they showed mixed responses to local plant characteristics. This research adds to our understanding of northern grassland pollinator communities and offers insight on how to restore grasslands to maximize pollinator services.

CHAPTER I

BACKGROUND

Pollinator Importance

Approximately 60 to 90% of plant species need animal pollinators to complete sexual reproduction (Klein et al. 2007, Kremen et al. 2007, Ollerton et al. 2011). Such invertebrate pollination services are in high demand to produce crops used for human consumption and for the maintenance of native plant populations (Buchmann et al. 2012, Garibaldi et al. 2013). In the United States there is a clear demand for insect pollinators (Calderone 2012). Common pollinators in agroecosystems, such as those in North Dakota, include species in the insect orders Diptera (flies), Lepidoptera (moths and butterflies), and Hymenoptera (bees, wasps, sawflies, and ants) (Reed 1994, Dickinson & McKone 1992). The most effective pollinators in terms of pollen movement are the bees (Dickinson & McKone 1992, Garibaldi et al. 2013). It is important to note that flies are frequently captured, but their value as pollinators needs to be explored (Kearns et al. 2001). Bees are highly diverse in terms of their life history characteristics and foraging behaviors. Behavior and pollination efficiencies differ among bumblebees, honey bees, and solitary bees (Woodcock et al. 2013). Some studies have found that wild bees drive increases in fruit set over managed honey bees in some agricultural systems (Garibaldi et al. 2013, Mallinger & Gratton 2015).

Threats to Pollinators

However, many insect pollinators, notably bees (Order Hymenoptera, Super family Apoidea, Clade Anthophila), have declined, reducing the effectiveness of their services (Hoehn et al. 2008, Holzschuh et al. 2011, Carvalheiro et al. 2012). While we are still exploring pollinator declines, three main contributors are thought to be the main culprit: habitat loss, degradation, and fragmentation. These affect nesting sites and floral availability (Holzschuh et al. 2016, With and Pavuk 2012, Wratten et al. 2012, Woodcock et al. 2013).

Anthropogenic activities are the main cause of recorded pollinator declines (Potts et al. 2010, Garibaldi et al. 2011, Woodcock et al. 2013). There are many different ways that humans alter landscapes that affect plants and pollinators and some of these changes occur through direct physical modifications of the landscape; others have more indirect effects (Brittain et al. 2009, Schleuning 2009, Bartomeus et al. 2010, Clough et al. 2014). Habitat loss causes wide scale reduction in biodiversity (Chaplin et al. 2000) because habitat conversion (*e.g.*, grassland to row-crop agriculture) reduces the amount of nesting space for pollinators, but it also potentially reduces floral resources on the landscape (Garibaldi et al. 2011). There is a correlation between plant and animal diversity (Murdoch et al. 1972). Habitat loss may cause the remaining habitat to be fragmented, which can further affect pollinators. Pollinators in fragmented sites have been shown to display lower fitness due to decreased food sources, food quality, and nesting sites (Steffan-Dewenter & Tscharntke 2002, Klein et al. 2007). With increased landscape fragmentation, plant communities are subjected to greater edge effects, increased plant

invaders, and overall reduced diversity (Carvalheiro et al. 2010, Potts et al. 2010, With 2012).

Addressing Pollinator Declines

Throughout the world, efforts are underway to bolster native pollinators in grasslands as one strategy to increase pollinator services (Chapin et al. 2000, Albrecht et al. 2007, Scheper et al. 2013, Hardman et al. 2016). Current research implies that bee functional diversity and landscape context can positively affect pollination services (Martins et al. 2015). Pollinator services can be improved in mixed grassland and agricultural landscapes with management to increase floral abundance (Rands & Whitney 2010). Pollinator species richness and abundance often increases when the landscape has more inflorescences and less intensive management (Albrecht et al. 2007, Hardman et al. 2015). In agroecosystems, proximity to natural or semi- natural lands is a predictor of pollinator species richness (Albrecht et al. 2007, Woodcock et al. 2014) and results in greater yields for pollinator dependent crops (Garibaldi et al. 2013).

Historically, fire and grazing were the main large-scale disturbances that affected the composition of grassland landscapes. In current converted landscapes, fire is rarely used and any remaining grasslands are either grazed, hayed, or remain without any annual disturbance (idle). These practices affect floral resources and, likely, the pollinators that occur in these communities. For example, grazed grasslands may have a higher legume presence than idle grasslands because of the need to improve forage quality (Woodcock 2014). In another example of management practices affecting floral resources, Power et al. (2012) found that insect-pollinated forbs, pollinators, and landscape context are linked. Delaney et al. 2015 found that reconstructed and remnant

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prairies were significantly different than old fields, indicating a floral resource difference at site types. Studying how management of natural habitat affects pollinator abundances is key for understanding declines (Power et al. 2012).

Study Aims

At the landscape scale, I aimed to compare floral resources and pollinator communities between grazed and idle grasslands to determine if they differ in how they support pollinator services in a fragmented grassland landscape. Figure 1.1 depicts my understanding of the relationships among environmental factors that affect pollinators. Within a landscape, management can affect habitat quality, which can then affect pollinators. To address management effects, I asked the question how site management affects floral resources, nesting habitat and pollinator communities in northeastern North Dakota. To address this question, I sampled ten grasslands in Grand Forks County, North Dakota, USA that were either grazed (n = 5) or idle (n = 5) in summer 2016 and 2017. Figure 1.2a shows how I established collection points within each site. Figure 1.2b depicts shows how I sampled the vegetation and pollinators at each sample point. I used targeted netting following Xerces Society guidelines (Foltz et al. 2016) to capture pollinators visiting inflorescences within the site. Once collected, I identified the invertebrates to morphospecies and recorded their totals. I used a Principal Component Analysis and a Multivariate Permutation Procedure to determine if the pollinator communities differed between the grazed and idle grasslands. In my analysis, bare ground differed, but invertebrate responses and inflorescence counts were similar between grassland types.

Grassland management can affect pollinators at a landscape scale, but how plants and their associated floral resources are distributed within sites may also affect pollinators and their services (Kadmon & Shmida 1992) on a local scale. Plant communities differ in the amount of species (richness) and communities with higher forb species richness during the season can support more insects in an agricultural landscape (Carvalheiro et al. 2012). When floral resources are low, their local distribution can affect pollinator visitation to individual patches, especially those in transition areas between high quality and low quality patches (With 2002). When we reconstruct grasslands for pollination services, we need to consider how such fine-scale plant pattern affects the pollination services we aim to restore. Figure 1.3 depicts my understanding of the theoretical relationships between pollinator presence and plant association for the Species Pattern and Community Ecology (SPaCE) experiment site based on my review of the literature.

To explore these relationships, my second research question was: Does plant species pattern effect pollinator abundance and services? To address this question, I used the SPaCE experiment plots (see McKenna et al. 2016 for details). The plots are arranged in a randomized complete block design with 5 blocks established at the University of North Dakota's Mekinock Field Station in May 2012. The plots (1 × 1 m) varied in richness (2, 4, 8 species), evenness (0.64, 0.8, and 1), and species pattern (random or aggregated). I focused this analysis on the plant species, *Ratibida columnifera*, to ask how the physical relationships of this forb affected insect visitation and plant seed set. The data for this study was collected in 2014. I calculated eight metrics of plot-scale vegetation growth that could affect pollinator visitation and plant reproductive output

(Figure 1.3). I assessed RC reproductive output by measuring seed biomass from ten randomly selected inflorescences in each plot. During the period of RC peak flowering (July 2014), I sampled invertebrate visitation to each plot with yellow bowl pollinator traps and identified specimens > 3 mm in size to morphospecies. I used multivariate linear models (R v 3.3.2 MuMIn package; v 1.15.6) to determine effects of plant community metrics on invertebrate visitation metrics and RC reproductive output. The number of visiting invertebrates increased with plant diversity and visiting invertebrates were more diverse at greater RC densities. Although plant characteristics affected visitation, invertebrate visitation did not explain seed mass. Seed mass was best explained by the continuity and density of RC patches.

My work with these two studies aimed to fulfill a gap in knowledge in current literature. I aimed to assess landscape and fine scale factors that affect grassland pollinators in tallgrass prairies. Further studies are needed on multiple levels. To follow up on Chapter 2, I need a better understanding of pollinator roles in grasslands. For example, is there a difference in pollination load between syrphid flies and bees? There is also a need to better understand what influences plant communities at these sites. It is clear there are larger driving forces that overshadow management that affect plant communities that I could not take into account with this study. In Chapter 3, I found that some of the variables I collected did not explain pollinator presence. Future studies are needed to assess effects of the broader landscape on the SPaCE plots. From that, I may be able to determine a better understanding of landscape versus fine-scale effects on pollinator presence. Both studies highlighted the complexity of grassland plant community and pollinator relationships that we aim to reconstruct and manage.
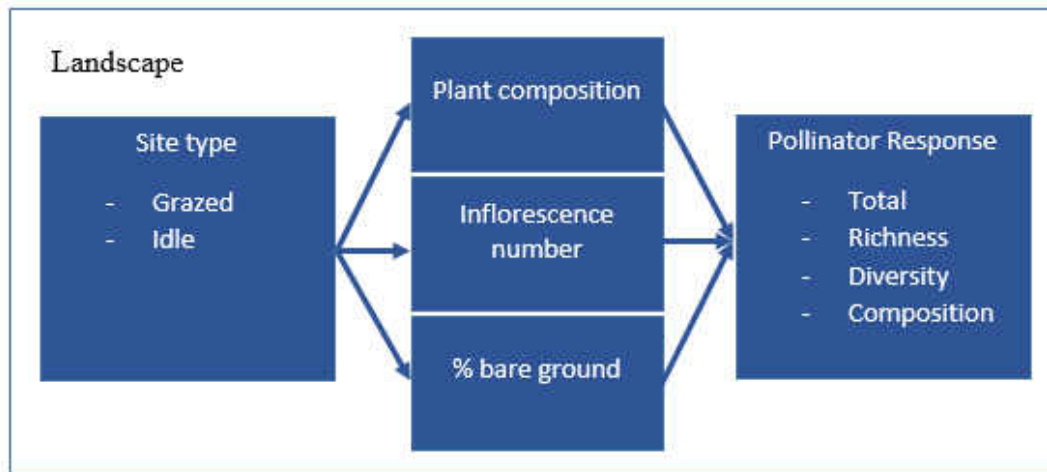
Figure 1.1. A conceptual diagram depicting the effect of site management type (grazed, idle) on vegetative composition, resources (inflorescence number) and nesting habitat (% bare ground) and pollinator response. This assumes that pollinator response does not affect plant composition and inflorescence number. Explanatory variables are plant composition, inflorescence abundance, % bare ground, and pollinator response.
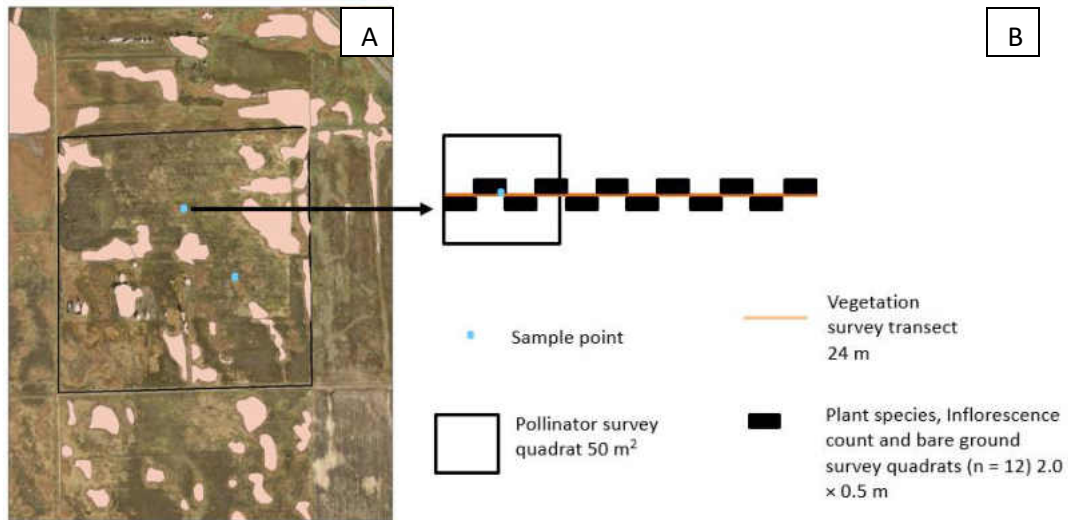
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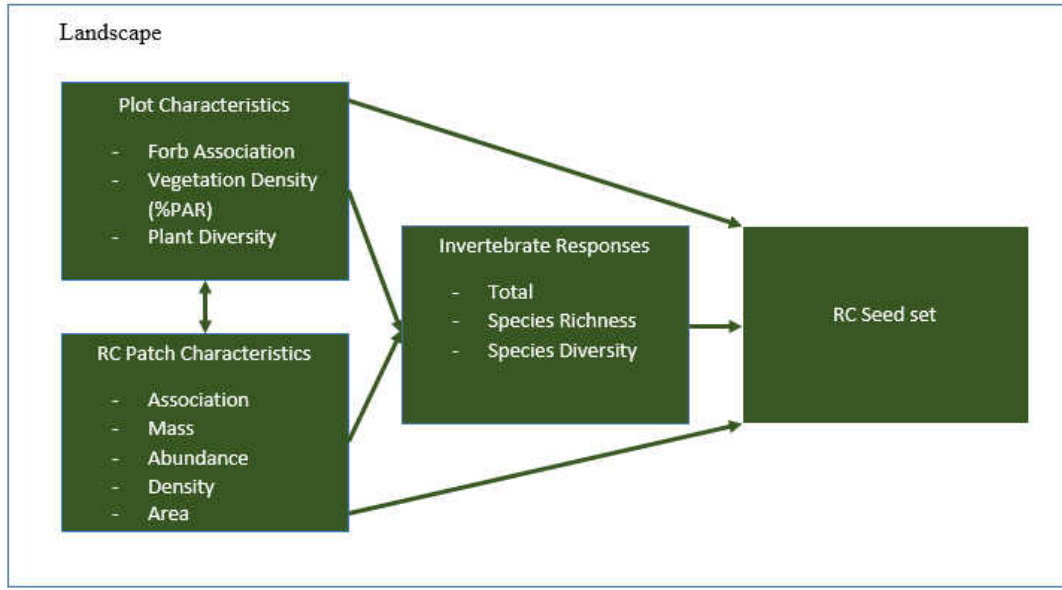
Figure 1.3. A conceptual diagram depicting the effect of local plant community variables (Forb association which is a measurement of floral neighbors, vegetation density which is a measurement of light reaching the soil surface, and plant diversity) and *Ratibida columnifera* (RC) patch characteristics on pollinator abundance and seed set in *Ratibida columnifera* within the SPaCE plots. This theoretical figure represents my understanding of the possible relationships between plant and invertebrate characteristics and how they may affect *Ratibida columnifera* seed set in the SPaCE experimental plots.

CHAPTER II

LANDSCAPE MANAGEMENT EFFECTS ON GRASSLAND POLLINATORS IN
GRAND FORKS COUNTY, ND

Abstract

Invertebrate pollinator populations are widely recognized as declining because of

anthropogenic activities that include changes in the amount and quality of available

habitat, competition with introduced species, increased agricultural chemical use, and

climate change. Because habitat and resource availability affect pollinator populations,

land management may affect pollinator presence. My first aim was to determine if

pollinator communities and habitat characteristics (available bare ground and floral

resources) differed between grazed and idle grasslands within the Grand Forks County

Grasslands of northeastern North Dakota, USA. Although available bare ground and

plant species richness differed between grassland types, floral resources and pollinator

communities did not differ between grassland types. Results suggest that solely

examining management is too simple of an approach when considering pollinator

populations. There is a more complex relationship between sites and the landscape that

need to be considered when investigating pollinator communities.

Introduction

Approximately 60 to 80% of plant species require animal-mediated pollination services

(Klein et al. 2006, Kremen et al. 2007). Many insect pollinators have declined,

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reducing the effectiveness of their services (Hoehn et al. 2008, Potts et al. 2010,

Holzschuh et al. 2011). We do not fully understand all the drivers and their relationships

that may be responsible for this decline (Dixon 2009, Potts et al. 2010). There is a need to

study the drivers of pollinator declines to better quantify and understand their impacts

(Klein et al. 2006, Potts et al. 2010).

A common approach to understanding pollinator declines is to sample habitat and

resource availability (Delaney et al. 2015). Habitat loss, via intensive agriculture, causes

wide scale reduction in biodiversity (Chaplin et al. 2000, Steffan-Dewenter et al. 2005,

Klein et al. 2007) because habitat conversion (*e.g.*, grassland to row-crop agriculture)

reduces the amount of nesting space for pollinators and potentially reduces floral

diversity and resources on the landscape (Klein et al. 2007, Garibaldi et al. 2011, Power

et al. 2012, Otto et al. 2016). Fragmented sites may lead to less fitness in pollinators

because of decreased food sources and nesting sites (Wilcock & Neiland 2002). Because

high plant productivity and diversity increases overall insect diversity (Siemann 1998,

Sutter et al. 2017), increasing nesting sites and floral availability on the landscape may

help bolster native pollinator presence (Klein et al. 2007) in an agricultural landscape.

Insects from many different orders serve as successful pollinators, but most

studies focus on pollinators in the insect orders Hymenoptera, Diptera, Coleoptera, and

Lepidoptera (Buchmann et al. 2012). These orders are commonly recognized to represent

three floral foraging strategies, nectar feeders (Lepidoptera), pollen feeders (Diptera and

Coleoptera), and pollen collectors (Hymenoptera) (Reed 1994). Many studies tend to

focus on bees (Order Hymenoptera, Super family Apoidea, Clade Anthophila) because

they spend a majority of their life collecting pollen and nectar from inflorescences. Bees,

wild and domesticated, are effective pollinators, however, fly (Order Diptera) pollination efficiency remains unknown (Dickinson & McKone 1992). This study aimed to assess inflorescence-visiting invertebrates and it is unclear how they definitively interacted with the plant species they visited.

Current research suggests that bee functional diversity and landscape context affect pollination services (Martins et al. 2015). Studying how habitat management affects pollinator abundances may be one key step in mediating declines (Klein et al. 2007, Potts et al. 2010, Power et al. 2012). Overall ecosystem service improvement in a grassland and agricultural landscape may be achieved by management that increases inflorescence abundance (Rands & Whitney 2010). Traditionally, fire, grazing, and rest (idle) periods dictated grassland community composition. Present-day management rarely includes fire and grazing as a mixed management strategy and, most often, sites remain without any disturbance (idle). These practices affect floral resources and, likely, the pollinators that serve these communities. Grazing management has nuanced effects on pollinator and floral resources. Yoshihara et al. 2008 found that intermediately and overgrazed grasslands had reduced floral diversity. This study aims to assess pollinator and plant communities under grazed and idle management and asked how does site management affect floral resources and pollinator diversity/abundance? I hypothesized that sites with greater floral resources would support more diverse and abundant pollinators.

Methods

Study sites

I sampled pollinator visitation and vegetation in 10 unique sites (Table 2.1, Figure 2.1) in Grand Forks County, ND, USA in summer 2016 and 2017. Sites were under either grazed (n = 5) or idle (n = 5) management. Grazed sites were grazed with cattle for at least two months of the growing season (June to September). Idle sites did not receive any direct defoliating management during the study growing seasons. I sampled each site twice (n = 20) during the peak plant flowering periods (June and July) in each year. In 2016, I first sampled for pollinators June 29th – July 15th. In 2017, plant development was delayed and I first sampled for pollinators July 5th – July 20th, but still during similar bloom conditions as in 2016. Results from the first round of collection in each year are presented here.

I randomly selected two sample points (Figure 2.2a) within each site. I used ArcGIS to block out cattail vegetation and tree stands for each site with a 20 m buffer and to generate 2 random points within the site and 20 m buffer. ArcGIS randomly found the point within these buffers and no minimum distance between points was used. I conducted an invertebrate and plant survey for each point (Figure 2.2b). In the event a point needed to be relocated in the field (*e.g.*, inadvertently placed in a wetland with standing water or cattail vegetation), the relocated points were chosen by walking 20 meters from the cattail vegetation or standing water from the original point. Site FA24NE had standing water in both years and the sample points were not positioned in the same location between years.

Invertebrates

I sampled pollinators in a 5 m × 10 m quadrat centered on the site point coordinates (Figure 2.2b) according to Xerces Society for Invertebrate Conservation (Foltz et al. 2016) for a duration of 15 minutes. On clear, sunny days with winds < 8 mph, I used targeted netting to collect invertebrates that were actively visiting flowers during the quadrat sweep. I recorded date, time, and weather conditions (cloud cover, temperature, and wind) at the time of sampling. Collected specimens were pinned and identified to morphospecies. All voucher specimens are deposited in the University of North Dakota invertebrate museum.

Vegetation

At each sample point, I conducted a vegetation survey within the same week of invertebrate collection. I recorded the vegetation composition (species), counted inflorescence stems, and estimated bare ground in twelve- 2 × 0.5 m alternating quadrats along a 24 m transect in the center of the pollinator survey area (Figure 2.2b). From these data, I determined the total number of inflorescences across all survey quadrats, average plant species richness, average % bare ground, and pollinator species richness. Each inflorescence stem was counted individually.

Data analysis

I used mixed model ANOVA (proc mixed, SAS ver. 9.4) with Type (grazed or idle), Year (2016 or 2017), and their interaction as fixed terms and site nested in type as random term to test for land management (type) effects on the number, richness, and diversity of the pollinators collected, inflorescence count (total), plant species richness,

and % bare ground. Total inflorescence count was square root transformed and % bare ground arcsin square root transformed to improve normality.

I conducted a PCA in PC-ORD (Ver. 6.0) on pooled morphospecies counts for each site (counts for both samples were combined) to visualize invertebrate composition in each year. The data matrix was limited to morphospecies present in more than one site and that comprised >1% of the total invertebrates collected in 2016 and >2% of the total invertebrates collected in 2017. I used a MRPP (Multi-Response Permutation Procedure; PC-ORD, Ver. 6.0) on this reduced matrix to test for compositional differences in the invertebrate communities between grazed and idle sites.

<p style="text-align:center">Results</p>

Pollinator responses (count, species richness, and diversity) were similar between grazed and idle grasslands and between years (Table 2.2, Figure 2.3). Not surprisingly, bare ground was higher in grazed sites (Table 2.2, Figure 2.4). Although plant species richness was higher in 2017 than in 2016, plant species richness was higher in grazed sites than in idle sites across both years (Table 2.2, Figure 2.5).

Blooming species during the collection period in 2016 included *Rudbeckia hirta*, *Melilotus officinalis*, *Ratibida columnifera, Cirsium arvense, Rosa arkansana, Asclepias syriaca* (Table 2.3). I collected 757 invertebrates with 350 caught in grazed sites and 407 caught in idle sites. I delineated 93 morphospecies, five of which accounted for 60.4% of the total captures (Table 2.4). I most frequently captured DIPT.007 (*Toxomerus marginatus*) at 37.8% of the total captures. DIPT.040, DIPT.021, and DIPT.102 (Tephritidae) were the next most commonly caught morphospecies at 7.7%, 5.7%, and

5.3% respectively. HYME.058 (*Apis mellifera*) was fifth most commonly caught morphospecies at 4.0%.

Blooming species during the collection period in 2017 included *Rudbeckia hirta*, *Melilotus officinalis*, *Ratibida columnifera, Cirsium arvense, Rosa arkansana, Asclepias syriaca, Symphoricarpos occidentalis, Euphorbia esula* (Table 2.3). I collected 590 invertebrates with 361 captured in grazed sites and 229 captured in idle sites. I delineated 80 morphospecies within this set of specimens. The top 4 morphospecies accounted for 48% of the captures (Table 2.3). DIPT.040 with 24%, DIPT.005 (*Stomoxys calcitrans*) with 6.1%, DIPT.007 (*Toxomerus marginatus*) with 5.9%, and DIPT.102 (Tephritidae) with 5.4% of the captures.

With the 2016 PCA analysis (Table 2.5), the first three axes explained 74% of the variation in the reduced invertebrate matrix (axis 1: Eigenvalue = 4.23, Variance = 35.22, axis 2: Eigenvalue = 2.94, Variance = 24.48, axis 3: Eigenvalue = 1.76, Variance = 14.65). The first axis was most strongly associated with DIPT.102, DIPT.40, and DIPT.053. Compositionally, the invertebrate communities were similar between grazed and idle grasslands in 2016 (MRPP: A = -0.06617, p = 0.81).

The 2017 PCA analysis (Table 2.5) I found the first three axes explain 67% of the variation in the reduced invertebrate matrix (axis 1: Eigenvalue = 3.59, Variance = 29.90, axis 2: Eigenvalue = 2.73, Variance = 22.73, axis 3: Eigenvalue = 1.72, Variance = 14.34). The first axis was most strongly associated with DIPT.040, DIPT.012, and DIPT.005. As with 2016, the invertebrate communities were compositionally similar between grazed and idle grasslands (MRPP: A = 0.02448, p = 0.2400).

Discussion

The diversity and composition of the invertebrate morphospecies community composition was the same between grazed and idle grasslands in 2016 and 2017. Dipterans comprised of the majority of captures for both years, however, it was more apparent in 2017 (Table 2.4). This is not out of the norm for this region (Dickinson and McKone 1992, Reed 1994). However, Reed (1994) found more Apoidea species than any other taxonomic group, whereas I found the most Dipteran morphospecies (51) than Apoidea (12). This discrepancy may be due to seasonal collection differences. Many studies focus on multi-year and complete seasonal collections (June – Sept), however my collections only occurred early in the growing season (June –July).

My results indicate that there is a significant management type effect on bare ground. Solitary bees depend on bare ground for nesting habitat (Klein et al. 2007, Woodcock et al. 2013) which indicates that grazed sites may have more supportive nesting habitat for some solitary bee species than idle sites. More bare ground in grazed management sites is not unique to this study (Elwell et al. 2016). Light intensity grazing was found to have higher species richness and abundance of insect-pollinated species (Yoshihara et al. 2008). This study also found higher plant species richness in grazed sites and in 2017. This echoes findings from Limb et al. (2018) where repeated herbivory in grazed landscapes increased overall plant species richness.

There was a difference in bare ground and plant species richness between management types, but there was no difference in our measured inflorescence resource variable, the count of inflorescence stems. These results indicate that what drives plant diversity and resource availability is more complex than management type. If we aim to

manage sites to support pollinator populations, we need to consider what plant species are present and how they serve pollinators (Nicholls and Altieri 2013, Wood et al. 2015). Simply considering two management types (grazed and idle) does not explain pollinator presence. The site size, quality, and surrounding landscape context are all important pieces of information to consider because the overall landscape context influences invertebrate community structure (Tscharntke et al. 2002)

Site level management type, grazed and idle, did not explain pollinator presence. Diverse types of inflorescences are needed to support diverse invertebrate communities (Dicks et al. 2015), but increasing habitat (floral resources) in an agricultural landscape only serves certain invertebrate species (Wood et al. 2015). Other studies suggest that more intensive management solutions (establishing nesting habitat and planting floral resources) is compensated by the greater pollination efficiency of solitary bees (Woodcock et al. 2013). While these site level studies are insightful, many ignore landscape context. It is important to consider landscape context because the stability of pollination services decreases from natural areas (Garibaldi et al. 2011) which indicate a larger landscape context (Westphal et al. 2003). When we are considering action to address pollinator declines, we need to contemplate landscape sized solutions.

Acknowledgments

Table 2.1. Sample site name, management type, site coordinates, legal description, and soil series. All sites were located within the Grand Forks County Grassland Corridor in Grand Forks County, ND.

Site	Type	Longitude	Latitude	Tract	Soil series
FA12NE	Grazed	-97.2477	47.8301	NE 1/4, 12-150-52	Ojata
FA24NE	Grazed	-97.2423	47.8009	NE 1/4, 24-150-52	Ojata
OA22NE	Grazed	-97.2851	47.8842	NE 1/4, 22-151-52	Ojata-Bearden
OA27W	Grazed	-97.2982	47.8720	W 1/2, 27-151-52	Bearden-Antler
OA36SW	Grazed	-97.2554	47.8410	N1/2 & SW1/4, 36-151-52	Ojata-Bearden
BL18NE	Idle	-97.3496	47.9890	NE 1/4, 18-152-52	Ojata
BL9SE	Idle	-97.3117	47.9966	SE 1/4 9-152-52	Bearden
FE17NW	Idle	-97.2315	48.0759	NW 1/4, 17-153-51	Bearden
LE24SW	Idle	-97.2744	48.1410	SW 1/4 24-154-52	Bearden-Antler
LE31SE	Idle	-97.3671	48.1107	SE 1/4, 31-154-52	Bearden-Antler

Table 2.2. F-values from Mixed model ANOVA of invertebrate (total, species richness, and diversity) and vegetation (% bare ground, total flowers, and plant species richness) response to site management (type) and year.

Source	Num df	df	F	p
Invert Total				
Type	1	8	0.46	0.5151
Year	1	30	0.12	0.7351
Type × Year	1	30	2.77	0.1063
Invert Species Richness				
Type	1	8	1.56	0.2474
Year	1	30	0.04	0.8373
Type × Year	1	30	0.29	0.5939
Invert Simpson's Diversity				
Type	1	8	0.17	0.6894
Year	1	30	0.98	0.3303
Type × Year	1	30	2.35	0.1361
% bare ground				
Type	1	8	20.64	0.0019
Year	1	30	3.22	0.0829
Type × Year	1	30	0.33	0.5684
Total Flowers				
Type	1	8	0.46	0.5171
Year	1	30	0.90	0.3509
Type × Year	1	30	0.05	0.8227
Plant species richness				
Type	1	8	15.64	0.0042
Year	1	30	10.27	0.0032
Type × Year	1	30	0.50	0.4859

Table 2.3. Non-grass and potentially insect pollinated plant species list that were positively identified in 2016 and 2017 and the number of times they were encountered across 22 sample points.

Species	2016 (n = 22)	2017 (n = 20)
Taraxacum officinale Weber	14	8
Asclepias syriaca L.	10	6
Cirsium arvense (L.) Scop.	6	11
Cirsium flodmanii (Rydb.) Arthur	5	
Melilotus officinalis (L.) Pall.	5	6
Grindelia squarrosa (Pursh) Dun.	4	9
Euphorbia esula L.	3	3
Helianthus maximiliani Schrad.	2	2
Apocynum cannabinum L.	2	1
Achillea millefolium L.	2	2
Astragalus canadensis L.	1	
Rosa arkansana Porter	1	2
Sonchus arvensis L.	1	5
Symphoricarpos occidentalis Hook.	1	4
Aster simplex Willd.		12
Solidago rigida L.		11
Helianthus rigidus (Cass.)		9
Triglochin palustris L.		8
Salicornia rubra A. Nelson		5
Rudbeckia hirta L.		5
Solidago canadensis L.		4
Ranunculus cymbalaria Pursh		4
Chenopodium album L		3
Medicago lupulina L.		3
Plantago lanceolata L.		3
Rumex maritimus L.		3
Convolvulus arvensis L.		3
Solidago gigantea Ait.		2
Plantago eriopoda Torr.		2
Erigeron strigosus Muhl. ex Willd.		2
Ratibida columnifera (Nutt.) Woot & Standl.		2
Glycyrrhiza lepidota Pursh		2
Ranunculus rhomboideus Goldie		1
Pediomelum argophyllum (Pursh) J. Grimes		1

Table 2.4. The most frequently encountered invertebrate morphospecies (listed by collection code) collected in 2016 and 2017 represented in percentage of total captures and to the finest taxonomic resolution to which they were identified.

Order	Family	Species	Collection Code	2016	2017
Diptera	Muscidae	*Stomoxys calcitrans*	DIPT.005	1.06	6.10
Diptera	Syrphidae	*Toxomerus marginatus*	DIPT.007	37.78	5.93
Diptera	Culicidae	*Culex pipiens*	DIPT.021	5.68	
Diptera	Sarcophagidae		DIPT.022	3.17	
Diptera	Syrphidae	*Helophilus fasciatus*	DIPT.038	1.32	
Diptera			DIPT.040	7.66	24.41
Diptera	Syrphidae	*Parasyphrus* spp.	DIPT.045	1.72	
Diptera	Anthomyiidae	*Leucophora* spp.	DIPT.053	1.45	2.54
Diptera	Tachinidae	*Siphona* spp.	DIPT.058	1.06	
Diptera	Opomyzidae	*Campiglossa* spp.	DIPT.099	3.57	
Diptera	Tephritidae		DIPT.102	5.28	5.42
Diptera	Muscidae		DIPT.MUSCSP3	1.98	
Diptera	Muscidae	*Musca domestica*	DIPT.001		2.37
Diptera	Phoridae		DIPT.012		2.03
Diptera			DIPT.036		2.54
Diptera	Lonchaeidae	*Lonchoptera* spp.	DIPT.072		3.22
Diptera	Dolichopodidae	*Amblypsilopus* spp.	DIPT.075		2.54
Diptera	Tabanidae	*Tabaninae* spp.	DIPT.076		2.03
Diptera	Opomyzidae	*Geomyza* spp.	DIPT.098		3.22
Hymenoptera	Apidae	*Apis mellifera*	HYME.058	3.96	
Hymenoptera	Apidae	*Ceratina* spp.	HYME.059	1.32	

Table 2.5. Principal Component (PC) analysis Eigenvector numbers (species loadings) of

the most frequently encountered morphospecies in 2016 and 2017.

2016 Species	PC1	PC2	PC3	2017 Species	PC1	PC2	PC3
DIPT.007	0.286	-0.342	-0.242	DIPT.076	0.094	-0.558	-0.051
DIPT.102	-0.422	-0.129	-0.303	DIPT.007	-0.420	-0.699	-0.243
DIPT.099	-0.096	0.128	-0.591	DIPT.102	-0.780	0.254	0.051
DIPT.045	0.247	-0.411	-0.220	DIPT.001	-0.259	-0.648	-0.401
HYME.058	-0.363	-0.348	0.144	DIPT.040	-0.957	0.181	0.032
HYME.059	-0.286	-0.367	0.295	DIPT.005	-0.752	-0.337	-0.347
DIPT.021	0.262	-0.272	-0.405	DIPT.036	0.246	0.045	0.616
DIPT.040	-0.409	-0.266	0.034	DIPT.053	0.196	0.738	-0.490
DIPT.MUSC3	0.240	-0.389	0.142	DIPT.098	-0.088	-0.061	0.103
DIPT.022	0.106	-0.313	0.129	DIPT.072	-0.158	0.807	-0.519
DIPT.005	-0.030	0.170	-0.019	DIPT.075	-0.523	0.177	0.663
DIPT.053	-0.389	-0.052	-0.378	DIPT.012	-0.917	0.252	0.189
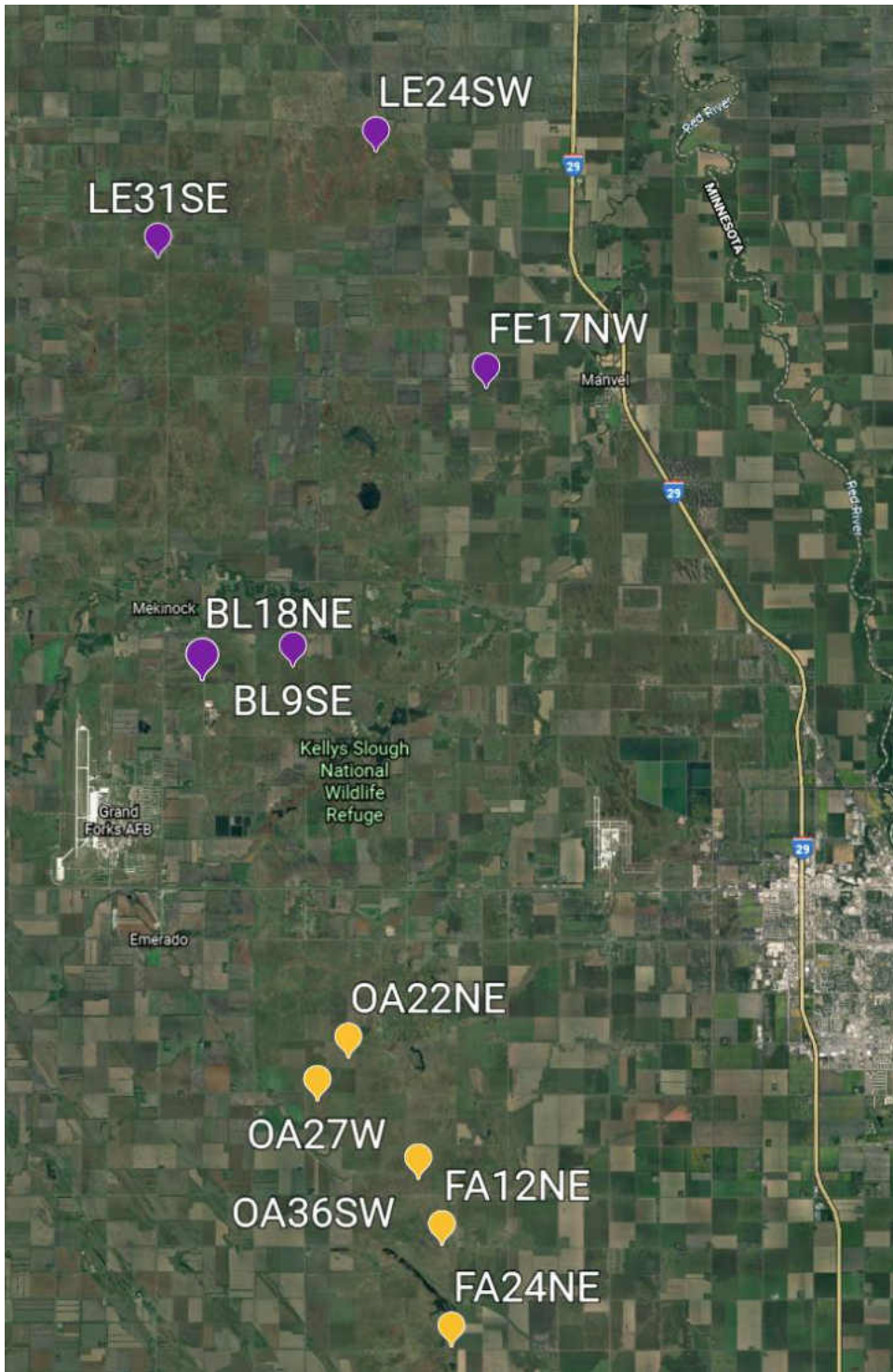
Figure 2.1. A map of the sample points (n = 10). The purple points were idle and the yellow points were grazed. The samples points were located in Eastern Grand Forks County, west of Grand Forks, ND and east of the Air Force Base near Emerado, ND.
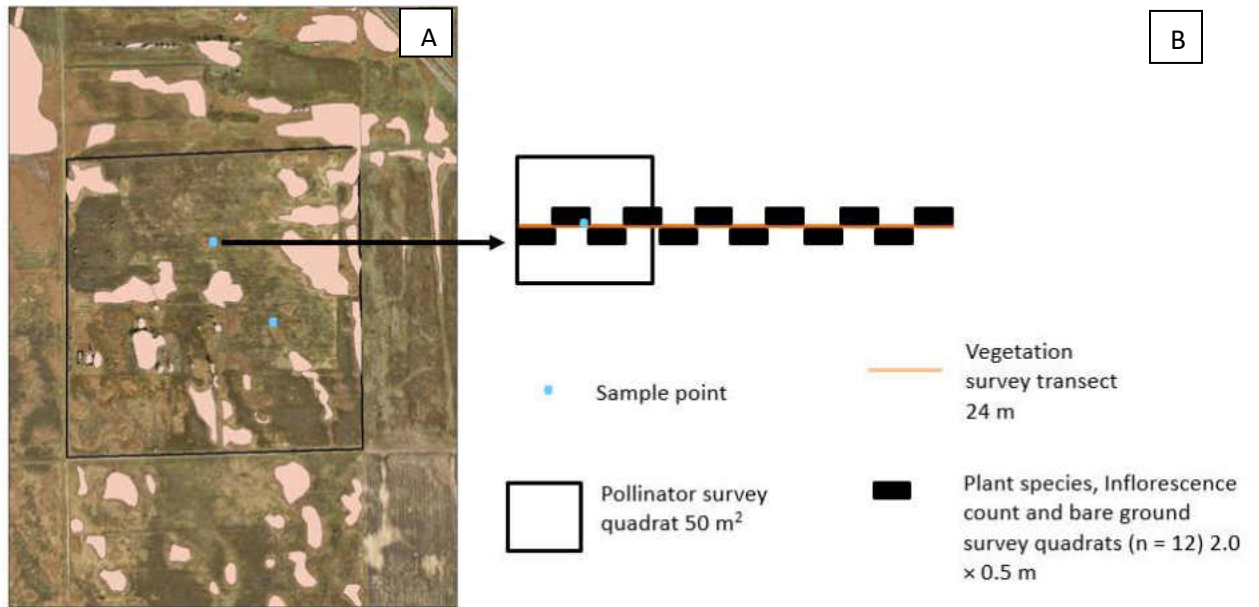
Figure 2.2. Site sample point layout. A) Representative image of a two sample points (blue) randomly placed within upland, non-wetland (wetlands = pink shading) habitat within the site. B) A 5m × 10 m (50 m^2) quadrat was used to conduct a pollinator survey. The plant community was surveyed using 12 - 2 × 0.5 m plots (filled black rectangles) distributed along a 24 m transect (orange line) in the center of the pollinator survey polygon (black square).
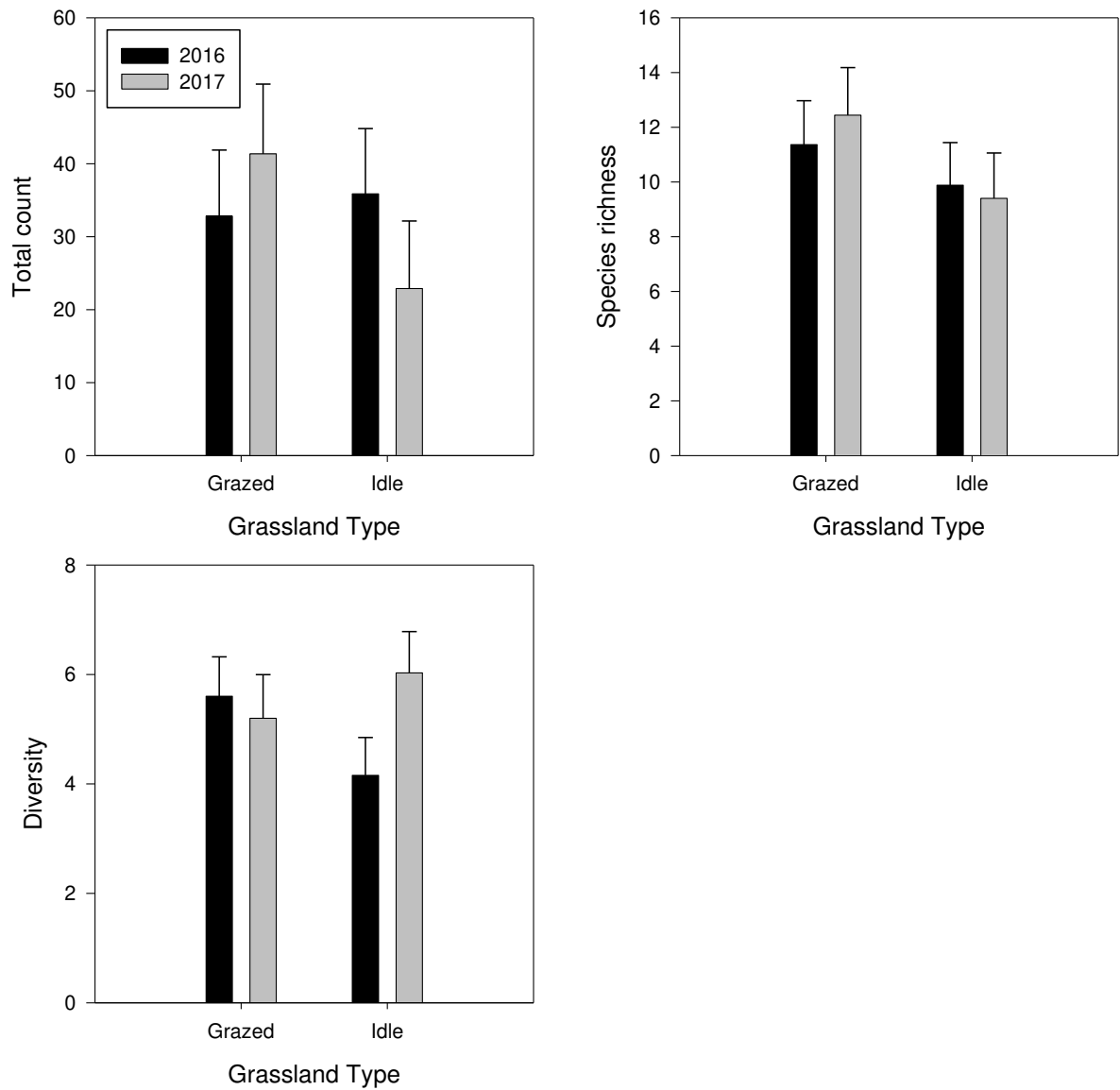
Figure 2.3. Mean (+SE) invertebrate count, species richness and diversity response to grassland type (grazed or idle) for each year 2016 (black) and 2017 (grey).
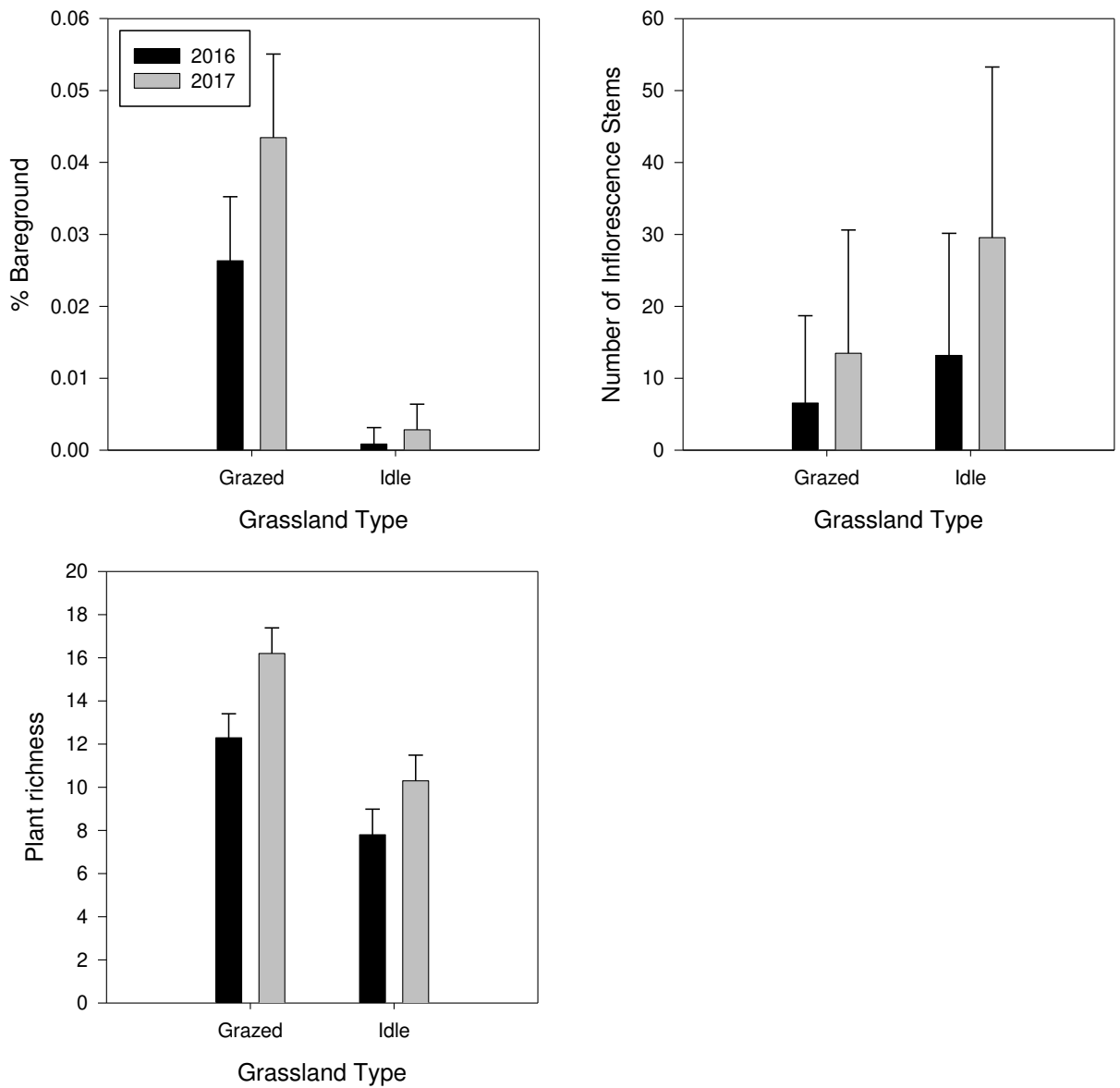
Figure 2.4. Mean (+SE) % bare ground, plant species richness, and number of inflorescence stems response to grassland type for each year 2016 (black) and 2017 (grey).
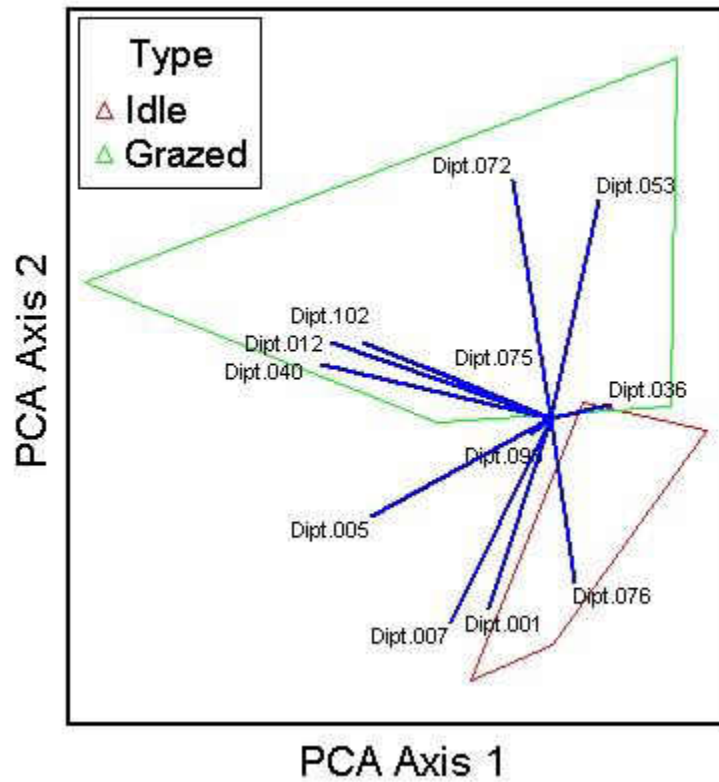
Figure 2.5. Visualization of the Principal Component Analyses ordination on the 2016 invertebrate morphospecies community composition matrix. Morphospecies composition was similar between idle (red polygon) and grazed (green polygon) grasslands (MRPP: A = -0.06617, p = 0.81).
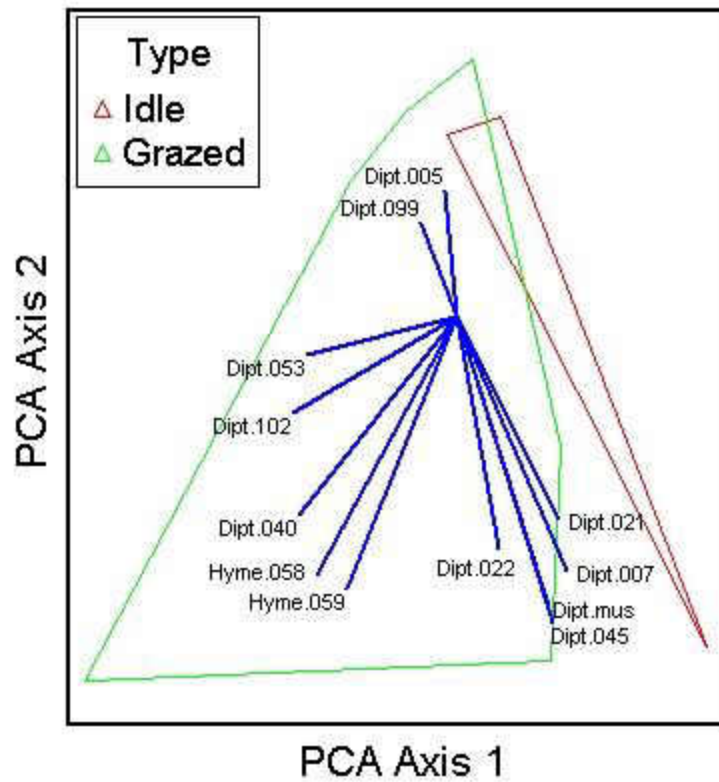
Figure 2.6. Visualization of the Principal Component Analyses ordination on the 2017 invertebrate morphospecies community composition matrix. Morphospecies composition was similar between idle (red polygon) and grazed (green polygon) grasslands (MRPP: A = 0.02448, p = 0.2400).

Chapter III

EFFECTS OF FINE-SCALE PLANT COMMUNITY STRUCTURE ON POLLINATOR
SERVICES PROVIDED TO *RATIBIDA COLUMNIFERA*

Abstract

Aims We aim to reconstruct grasslands for pollination services, but we know relatively

little about how fine-scale plant pattern affects pollinator visitation and plant fitness. I

asked whether the spatial relationship of *Ratibida columnifera* (hereafter RC) in

reconstructed grassland communities affected pollinator visitation and whether pollinator

visitation and plant species pattern affected RC seed production. I test the hypothesis that

increasing RC spatial association would increase invertebrate visitation and plant

reproductive output.

Methods I assessed invertebrate visitation (morphospecies count, richness, and diversity)

to experimental plots (1×1 m; n = 34) containing RC within the Species Pattern and

Community Ecology experiment in Grand Forks County, North Dakota, USA via bowl

traps during peak flowering. I quantified RC distribution, abundance, mass, and seed set.

Important Findings The number of visiting invertebrates increased with plant diversity

and visiting invertebrates were more diverse at greater RC densities. Dipterans were by

far the most abundant invertebrate visitors and they showed mixed responses to plant

characteristics. While a Dolichopodidae (DIPT.105), *Toxomerus marginatus* (DIPT.007),

and an *Agapostemon* spp. (HYME.065) were affected by RC characteristics, DIPT.036,

unidentified dipteran, was most affected by overall plot characteristics. Although plant

characteristics affected visitation, invertebrate visitation did not explain seed mass. Seed mass was best explained by the continuity and density of RC patches. Results suggest that fine scale plant association affects seed set and potentially plant recruitment in reconstructed grasslands. In effort to attract pollinators for services, managers need to be attentive to the role that plant competition plays in determining seed set, which may be stronger than any role of the pollinators themselves.

Introduction

It is well established that pollinators are declining worldwide (Cane and Tepedino 2001, Goulson et al. 2008, Cameron et al. 2011, Koh et al. 2016) and that substantial gaps exist between habitat availability and pollinator abundance through North American grasslands (Koh et al. 2016). Grassland reconstruction efforts aim to return perennial plant species to heavily disturbed sites and, by turn, restore ecosystem services to these areas. One key question is how such efforts serve grassland pollinators (Dixon 2009). A multitude of studies have considered what steps can be taken to improve pollinator visitation to reconstructed grasslands (Woodcock et al. 2014). These include augmenting sites with specific species (Woodcock et al. 2014, Harmon-Threatt and Hendrix 2015, Otto et al. 2017) and managing sites with a mixture of grazing, mowing, and fire (Harmon-Threatt and Chin 2016, Tonietto and Larkin 2018, Wojcik et al. 2018, Buckles and Harmon-Threatt 2019). One aspect of this process that has received relatively little attention is to what extent the spatial arrangement of plant species in a community affects pollinator visitation and subsequent plant seed set (Goulson 2000, Charpentier 2001).

Grassland pollinators include bumblebees (*Bombus* spp.), domesticated honey bees (Apidae), small wild bees (Megachillidae, Halictidae, Andrenidae, Colletidae,

Melittidae, and Stenotritidiae), countless dipterans (71 known anthophilous families), some lepidopterans and coleopterans (Forup and Memmott 2005, Otto et al. 2016). Surprisingly, little difference between pollinator communities visiting reconstructed and remnant grasslands in England (Forup and Memmott 2005, Forup et al. 2008) and along roadsides in Iowa (Hopwood 2008), but this may result from intensity of surrounding land use which is known to regulate composition in heavily disturbed landscapes (Clough et al. 2014).

Within restored sites, pollinator visitation is affected by the presence, density, and composition of floral resources. Generally, pollinators use visual and floral cues when selecting forage sites, and communities with higher forb species richness support more insects (Carvalheiro et al. 2012). But the pollinator visitation response is more nuanced than this, affected by the local density and composition of available floral resources that pollinators encounter during foraging bouts (Ohashi and Thomson 2009). Different pollinator species forage on different plants as well (Pearce et al. 2012). In a categorical analysis of plant population and patch effects on floral visitation across 10 European plant species, the area and density of floral patches affected flower visitation and seed set for plants in small populations (Dauber et al. 2010). Bumblebees (*Bombus* spp.) foraging in *Brassica napus* (Canola) spent more time in larger patches (on the order of meters) than smaller patches (Cresswell and Osborne 2004). Presumably, individual flowers are less likely to be revisited in patches with more floral resources (higher floral density) and, therefore, are more likely to be visited because they maximize the foraging effort (Ohashi and Yahara 2002). These effects of plant community composition and structure are likely pollinator specific. In a study of visitation to 1.5 × 1.5 m plots established in a Norwegian

grassland, beetle, bumblebee, and muscoid fly visitations were positively affected by plot-scale inflorescence density, while syrphid fly visitations were more strongly positively affected by plant richness (Hegland and Boeke 2006). There is also evidence to support the hypothesis that plants compete for floral visitors with individuals of same and other species. Within the Norwegian grassland study, floral visitation was affected by conspecific and heterospecific floral density. Visitations to bumblebee pollinated species were more strongly affected by heterospecific floral density, whereas a predominately fly visited plant species was positively affected by conspecific floral density (Hegland et al. 2009). This pollinator specific attraction to different aspects of plant community composition (density, composition) was additionally supported in Lazaro and Totland (2010).

The challenge for managers is how to assemble grasslands to ensure that pollinators optimize plant reproductive output. At the landscape scale, pollinator visitation and resulting seedset in *Salvia* was affected by the number of linear landscape elements (hedgerows) that connected sites (Cranmer et al. 2012). Within sites, plant reproductive output was maximized as pollinator visits increased with patch area and density for a pool of Norwegian plant species (Dauber et al. 2010). But even within the studies that assess plant patch characteristics, few assess the effects of plant structure on pollinator visitation and plant reproductive output at the scales over which plants compete and allocate their resources.

To move forward with our understanding of the interaction between restoration activities and pollinator networks, managers need to connect pollinator and seed set studies with fine-scale plant community studies. Plant seed set is directed by competitive

interactions that affect plant resource allocation as well as the plant-pollinator interactions that affect pollinator visitation and successful pollination. Ohashi and Thomson (2009) surmised that the spatial configuration of plants can affect the foraging behavior efficiency where plants distributed in more "loose" patches requires a greater foraging investment and potentially less efficient pollination than those in more "compact" patches of the same larger scale plant density. Either through direct and differential plant conspecific and heterospecific interactions for pollinator effects (Hegland et al. 2009). Changes in inter and intraspecific relationships among plant species in a community can also affect seed set. At fine-scales (0.25 m^2), altering plant species pattern and increasing patch connectivity reduces plant productivity (biomass) and potentially seed set (McKenna and Yurkonis 2016, Seahra et al. 2016). When considering restoration activities for pollinators, there is a balance between effects of pollinators and vegetation characters on plant fitness and managers need to understand their relative effects when aiming to improve grasslands for pollinator services.

With this study, I aimed to test the effect of plant species pattern on pollinator visitation and plant reproductive output. My objective was to determine whether the spatial association (spatial pattern) of *Ratibida columnifera* (RC) affects pollinator visitation and reproductive output (seed set). I hypothesized that with increased *Ratibida columnifera* association, there will be a corresponding increase in pollinator visitors and reproductive output. *Ratibida columnifera* is a mid-season flowering forb commonly found throughout the Great Plains of North America. A study of the closely related species *Ratibida pinnata* suggests that species in this genus are pollinated by generalists and specialists (Dickinson and McKone 1992). Pollination occurs primarily in the

morning and *Ratibida pinnata* is known to be visited a number of native bees (8) and flies (Dickinson and McKone 1992, Tooker et al. 2006). In particular, the Syrphid fly, *Toxomerus marginatus*, the most common Syrphid in Minnesota, is a notably frequent visitor to *Ratibida pinnata* (Dickinson and McKone 1992, Tooker et al. 2006) and *Ratibida pinnata* is a known host for Tachinidae flies *Gymnoclytia occidua* (Tooker et al. 2006). I hypothesized that I would find a similar range of invertebrate visitors to *Ratibida columnifera*.

Methods

Study site

I sampled invertebrate visitation and *Ratibida columnifera* (RC) seed set in the Species Pattern and Community Ecology (SPaCE) experiment. The SPaCE experiment is a randomized complete block (n = 5) reconstructed grassland experiment that contains plots (1 × 1 m) that varied in initial plant species richness (2 to 8 species) and evenness (0.64 to 1.0) established at the University of North Dakota's Mekinock Field Station (Lat 47.9620, Long -97.4517) in May 2012. Plots were planted with greenhouse grown transplants distributed into a pre-determined 8 × 8 array of individuals (see McKenna and Yurkonis 2016 for further details). For the purposes of this study, I assessed visitations to weeded plots that contained RC in mixture with other species (n = 34; number of planted RC individuals ranged from 8 to 56 in mixtures).

I calculated eight metrics of plot-scale vegetation growth that could affect pollinator visitation and plant reproductive output. In May 2014, I mapped the vegetation within each plot by recording the dominant (≥ 50% cover) species within each cell of a 64 – 12.5 × 12.5 cm cell grid established over each plot. I used these maps to quantify RC

basal area (m^2) and the proportion of all possible neighborships that occurred among RC

individuals (RC association) and among all planted forbs (Forb association) for each plot

with the program QRULE (Gardner and Urban 2007). In June 2014, I sampled

Photosynthetically Active Radiation (PAR) in each plot (AccuPAR LP-80 Ceptometer,

Meter Group, Inc., Pullman, WA, USA) and calculated the % PAR reaching the soil

surface as a measure of growing season vegetation density. In September 2014, I

harvested, sorted to species, dried, and weighed all plant biomass (described in McKenna

and Yurkonis 2016) in order to calculate plot-scale Simpson's Diversity (plant diversity).

RC mass (g) data was used to calculate the proportion of RC biomass produced within

each plot (RC abundance), an area adjusted RC density measurement (RC density = RC

mass / RC basal area).

I assessed RC reproductive output by measuring seed biomass from ten randomly

selected inflorescences in each plot. In plots with less than ten inflorescences, I collected

no more than 50% of inflorescences present (4 of 34 plots). In July 2014, I covered

selected inflorescences with a fine mesh after flowering and for the seed maturation

period to ensure that seeds did not scatter until processing. Focal inflorescences were

harvested in September 2014 and dried for 48 hours at 60˚ C. Seeds were removed from

any residual chaff, dried further for 2 hours at 60˚ C, and weighed. I quantified RC

reproductive output as the average seed mass per inflorescence head within each plot.

Pollinator sampling

During the period of RC peak flowering (July 2014), I sampled invertebrate

visitation to each plot with yellow (9 cm base × 18.2 cm diameter × 4.5 cm depth bowls)

pollinator traps. Each pollinator trap contained a saturated salt solution and was

positioned at inflorescence level on the edge nearest the established RC within each plot.

I activated the traps on clear, sunny days. After 24 hours, collected specimens were

rinsed and stored in 70% ethanol. I identified specimens > 3 mm in size to morphospecies

within each invertebrate order and presumed non-pollinating morphospecies (six total;

three Hymenoptera, one Hemiptera, one Opiliones, and one Orthoptera) were excluded

from further analyses. Nine of the retained morphospecies were confirmed as potential

pollinators through additional sweep net sampling of RC floral visitors in summer 2015. I

calculated the number of collected potential pollinators, morphospecies richness, and

morphospecies Simpson's diversity ($1/D$) for each plot with the reduced morphospecies

dataset. I identified the most frequent morphospecies to the finest taxonomic resolution

possible (genus or species). I captured over 80 individuals of each of four morphospecies,

and, together, they comprised 63% of total captures. These included one Syrphid fly

(DIPT.007, Syrphidae: *Toxomerus marginatus*), a Phorid fly (DIPT.036; Phoridae), a

Dolichopodid fly (DIPT.105; Dolichopodidae), and one Hymenopteran (HYME.065;

Halictidae: *Lasioglossum* spp.).

Data analysis

Multivariate linear models (R v 3.3.2 MuMIn package; v 1.15.6) were used to

determine effects of plant community structure metrics (RC association, RC area (m^2),

RC mass (g), RC abundance, RC density (g/m^2), Forb association, PAR, Plot diversity)

on invertebrate visitation metrics (number, richness, and diversity of visitors). I

additionally used plant and invertebrate metrics to determine effects of plant community

and invertebrate metrics on plant reproductive output (ave. seed mass/head). I used AIC_c

model averaging and best subsets to identify best models and predictors (R ver. 3.3.2

MuMIn package; ver. 1.15.6). RC association, forb association, and RC abundance values were aricsin squareroot transformed prior to analysis. If the top model was not separated from other competing models by >2 AIC_c units, the model with the lowest number of covariates and lowest AIC_c was selected. Invertebrate diversity and seed mass were modeled based on a Gaussian distribution. I assessed model fit using alternative distributions for count based responses. The linear model was based on a negative binomial distribution for total invertebrate count and DIPT.105 and a Poisson distribution for invertebrate richness, DIPT.007, HYME.065, and DIPT.036.

<div align="center">Results</div>

The reduced dataset contained 49 morphospecies (n = 996) representing five insect orders. I most frequently captured Dipterans (19 morphospecies, 65% of captures). However, I captured the greatest number of Hymenopteran morphospecies (20 morphospecies, 27% of captures). Less frequently captured was the order Coleoptera (6 morphospecies, 6%), Lepidoptera (3 morphospecies, 0.3%), and Hemiptera (2 morphospecies, 0.4%) individuals.

The number of insects captured was positively affected by plot scale plant diversity (Tables 3.1, 3.2; Figure 3.1). The number of morphospecies (invertebrate richness) arriving to the bowls was not affected by any of the measured plot characteristics. However, the diversity of invertebrates arriving to the bowls was affected by RC density (Table 3.1, Figure 3.1).

Visitation by the most frequently encountered morphospecies was a species-specific response (Table 3.1). Two of the four most prevalent species were affected by RC. Morphospecies DIPT.105 was best explained by RC abundance (Table 3.2).

Morphospecies DIPT.036 was less explained by RC, but best explained best by the plot characteristics %PAR, plant diversity, and forb association. DIPT.036 had a strong positive relationship with plant diversity and %PAR (Table 3.2, Figure 3.3).

Although plot characteristics affected visitation, the best seed set model did not include any invertebrate metrics. In this case, seed set was most strongly determined by the density and spatial relationships of the RC plants. RC reproductive output (seed mass) was explained with three models with ΔAIC_c less than 2, all of which included RC adjusted density and RC association. The top model included both adjusted RC density and RC association, the remaining two models included a measure of invert diversity (Table 3.2), and the latter two models were 1.53 ΔAIC_c apart, indicating that the first model described the data best. In all cases the adjusted RC density had the strongest effect, which was positive (Tables 1, 2). The more continuous patterned plots resulted in reduced seed mass (Figure 3.2).

Discussion

I tested for effects plot scale plant characteristics and plant species (RC) patch characteristics on pollinator visitation and plant reproductive output. My objective was to determine whether the spatial association of RC affected pollinator visitation and reproductive output. With pollinator visitation, the absolute number of invertebrates captured was positively affected by plot scale plant diversity. Invertebrate richness was not affected by any of the measured plot characteristics. RC density positively affected invertebrate diversity. I found a species-specific response with four frequently encountered morphospecies, with two (DIPT.105, HYME.065) of those affected by RC characteristics. DIPT.036 had a strong positive relationship with plant diversity and

%PAR. Plant reproductive output was most strongly determined by the density and spatial relationship of RC plants.

Grassland pollinators cover a wide range of orders. In this study, I collected a majority of Dipterans (65%). Dickinson and McKone 1992 and Reed 1994 found that the majority of invertebrates visiting their grassland plots were also flies, specifically syrphid and bombyliidae flies. Whereas I found DIPT.105 (258, Dolichopodidae), HYME.065 (*Agapostemon*), DIPT.007 (103, *Toxomerus marginatus*), but arguably the majority of captures were flies. These differences may be due to scale, where I sampled 1×1 m plots and the other studies sampled a larger area (Pearce et al. 2012). Syrphid flies were common from end of June to end of September (Reed 1994). However, the temporal range of this study only analyzed one day in July 2014. Reed 1994 found that with *Ratibida pinnata*, the ratio of percent insect species to the percent of total collections was 2.24, one of the lowest ratios in the study. This suggests that RC may not be a preferred forage inflorescence for some pollinators (Pearce et al. 2012), but it certainly is an important forage plant. However, in a study of a suite of helanthiae plants, the closely related *Ratibida pinnata* was most visited by the most number of native bees (8) and flies (Dickinson and McKone 1992, Tooker et al. 2006). *Toxomerus marginatus*, the most common Syrphid in Minnesota, was a notably frequent visitor, but syrphids could not be determined to be efficient pollinators (Dickinson and McKone 1992, Tooker et al. 2006) which may explain why plant competition drove reproductive output.

Some, but not all, plot characteristics determined visits. Plant diversity had a positive effect on overall invertebrate count which is supported in many other studies (Panzer & Schwartz 1998, Potts et al. 2010). Florally diverse natural and semi-natural

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areas in an agricultural setting stabilize pollination services (Garibaldi et al. 2011). RC

mass, density, and abundance did have an effect on visitors. The density and mass of RC

increased over all invertebrate diversity. This follows similar findings (Seimann 1998)

where greater plant productivity increases arthropod species richness. However,

visitations to a closely related species *Ratibida pinnata* were visited by generalists and

specialists. Limitations on seed set due to insufficient pollinators is unlikely for *Ratibida*

pinnata (Dickinson and McKone 1992).

Results suggest that fine scale plant association affects pollinator services and

resulting plant recruitment in reconstructed grasslands. Average seed mass increased

when *Ratibida columnifera* (RC) was more isolated from intraspecific neighbors.

Increased plant competition decreases resources available for seed production in larger

RC patches. Intraspecific competition in RC was predicted best by models that

incorporated water resource usage (Vargas-Mendoza and Fowler 1998). Pollinators

foraging in contiguous patches for RC may decrease time spent at each flower, resulting

in less efficient pollination, however, in small foraging patch size it is easier to find

unvisited inflorescence (Goulson 2000). RC may not be a preferred forage plant. Pearce

et al. (2012) found that *A. mellifera* does not visit RC as much as it does other

wildflowers.

These results also suggest that factors that affect pollinator richness on the

landscape needs further exploration at larger scales (Power et al. 2012). Further

investigation on forage selection for each forb species may provide additional insights

into the observed patterns. Our research site is situated in an agricultural landscape and is

surrounded by active farm fields. Many other projects found small natural habitat

increases pollinator presence and increased agricultural plant reproductive output

(Garbaldi et al. 2013, Dicks et al. 2015). Further study is needed to see if the

phenomenon is replicated in this landscape, too.

Acknowledgements

Table 3.1. Top explanatory models for invertebrate and seed set response variables. The

list of models for each response was truncated at < 2.00 ΔAIC_c for brevity.

Model	df	AICc	ΔAIC_c	Weight
Invertebrate count				
Plant diversity	3	236.21	-	0.71
Plant diversity + PAR	4	237.98	1.77	0.29
Invertebrate diversity				
RC density	3	125.13	-	0.62
RC density + Plot diversity	4	126.10	0.97	0.38
DIPT.105 abundance				
RC abundance	3	198.10	-	0.13
DIPT.007 abundance				
RC density	2	152.22	-	0.30
RC density + RC mass	3	152.35	0.13	0.29
RC density + RC area	3	153.44	1.22	0.17
RC area	2	153.91	1.69	0.13
RC abundance + RC density	3	154.20	1.98	0.11
DIPT.036 abundance				
Plant diversity + PAR	3	135.41	-	0.43
Plant diversity + PAR + Forb association	4	136.82	1.41	0.21
Plant diversity + PAR + RC mass	4	137.22	1.81	0.18
Plant diversity + PAR + RC abundance	3	137.24	1.83	0.17
HYME.065 abundance				
RC mass + RC abundance	3	167.04	-	0.12
Seed mass				
RC density + RC association	4	-136.25	-	0.52
RC density + RC association + Invert count	5	-134.93	1.32	0.27
RC density + RC association + Invert diversity	5	-134.37	1.88	0.20

Table 3.2. Model averaged coefficients ± SE (conditional average) across the top models for invertebrate count, diversity, and seed mass.

Model term	Invertebrate count	Invertebrate diversity	Seed mass
Intercept	3.138 ± 0.133***	4.781 ± 0.548***	8.875e-02 ± 2.209e-02***
Plant diversity	0.083 ± 0.036*	0.244 ± 0.199	
PAR	0.451 ± 0.497		
RC density		0.005 ± 0.001**	7.343e-05 ± 3.387e-05*
RC association			-6.098e-02 ± 1.870e-02**
Invertebrate total			-7.734e-04 ± 6.775e-04
Invertebrate diversity			3.314e-03 ± 3.724e-03

$p < 0.05 =$ *, <0.01**, <0.001***

Table 3.3. Model averaged coefficients ± SE (conditional average) across the top models

for the most frequent species.

Model term	DIPT.105	DIPT.007	DIPT.036	HYME.065
Intercept	2.492 ± 0.187***	1.088 ± 0.244***	-0.250 ± 0.539	1.735 ± 0.181***
Plant diversity			0.265 ± 0.108*	
PAR			3.184 ± 1.450*	
RC mass		-0.144 ± 0.092	-0.090 ± 0.102	-0.218 ± 0.094*
RC density		0.00130 ± 0.000608*		
RC area		-0.707 ± 0.556		
RC abundance	-2.920 ± 0.981**	-0.645 ± 0.989	-1.104 ± 1.294	3.036 ± 0.969**
Forb association			-0.489 ± 0.454	

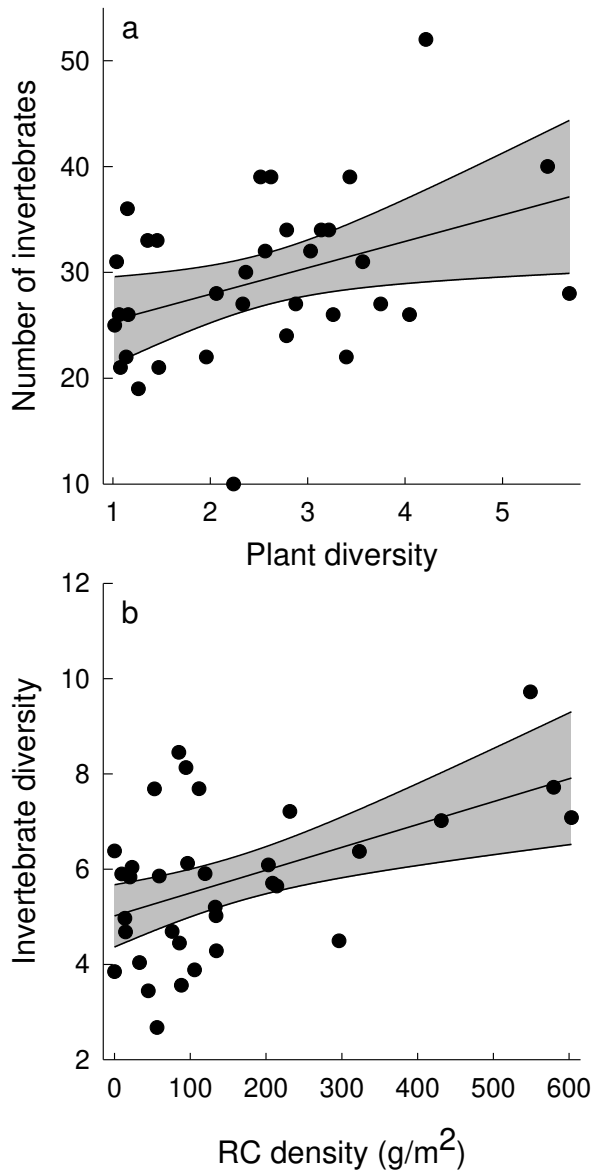$p < 0.05 = $ *, <0.01**, <0.001***

Figure 3.1. Predicted effect of (a) plot-scale plant diversity on the number of invertebrates captured and (b) RC plant density (g/m^2) on captured invertebrate diversity with their 95% confidence intervals (shaded band in each panel). I captured invertebrates with yellow bowl traps placed adjacent to 1×1 m mixtures of tallgrass prairie plant species in during peak flowering in July 2014. Observed invertebrate responses are plotted (symbols) for reference.
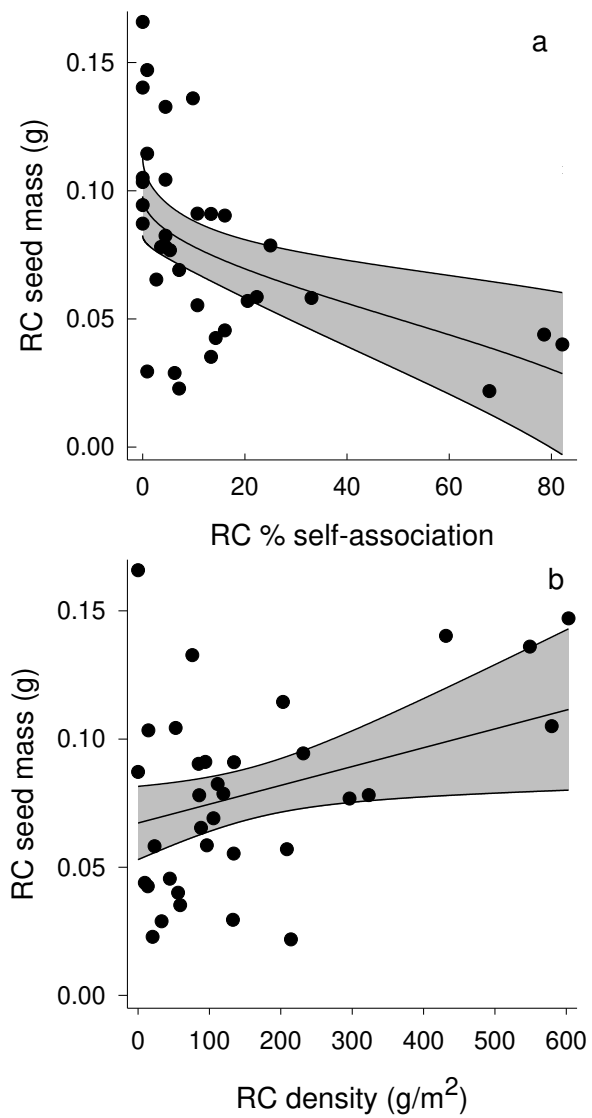
Figure 3.2. Predicted effect of (a) RC aggregation (% self-association) and (b) RC density (g/m^2) on the average RC seed mass (g) per inflorescence with their 95% confidence intervals (shaded band in each panel). Observed seed mass values are plotted (symbols) for reference.
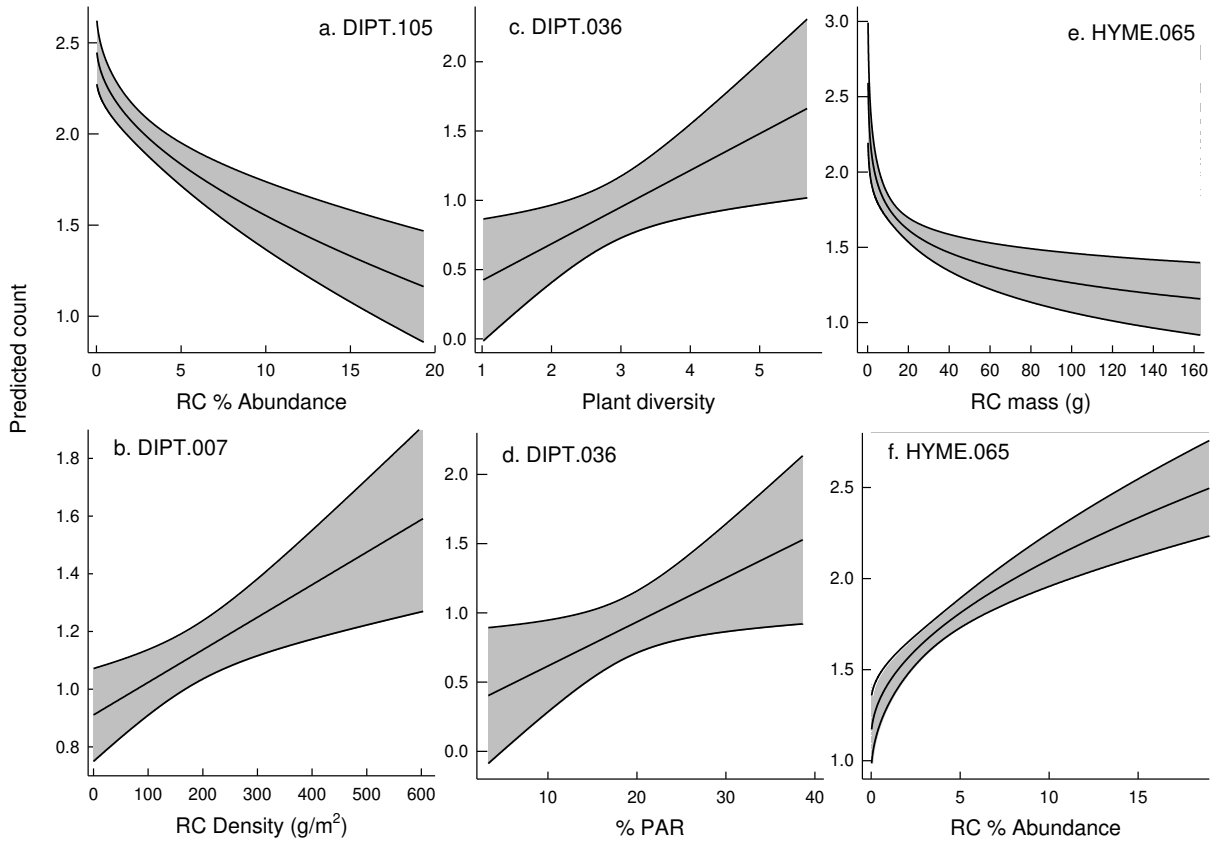
Figure 3.3. Predicted effect of (a) plant diversity and (b) percentage soil surface
photosynthetic active radiation (PAR) on the number of Dipt.036 individuals with their
95% confidence intervals (shaded band in each panel).

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