

Evaluating pollinator responses to grassland management techniques

by

David Solomon Stein

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

Program of Study Committee:
Diane M. Debinski, Co-major Professor
John M. Pleasants, Co-major Professor
Richard L. Hellmich
Amy L. Toth

Iowa State University

Ames, Iowa

2017

Copyright © David Solomon Stein, 2017. All rights reserved.

TABLE OF CONTENTS

ABSTRACT	iii
CHAPTER 1: GENERAL INTRODUCTION	1
Thesis Organization.....	4
References	5
CHAPTER 2: ENHANCING NATIVE BUTTERFLY AND FLOWERING PLANT DIVERSITY IN GRASSLANDS VIA COLLABORATIVE ADAPTIVE MANAGEMENT	8
Abstract	8
Introduction	9
Methods	13
Results	16
Discussion	18
Acknowledgments	23
References	24
Figures and Tables	28
CHAPTER 3: EVALUATING NATIVE BEE COMMUNITIES AND BEE HEALTH IN MANAGED GRASSLANDS	37
Abstract	37
Introduction	38
Methods	42
Results	48
Discussion	51
Acknowledgements	57
References	58
Figures and Tables	63
CHAPTER 4: GENERAL DISCUSSION	75
References	79

ABSTRACT

Pollinator species such as butterflies and bees are declining globally primarily due to habitat loss. This is especially concerning within the tallgrass prairie ecoregion of North America, where the land area comprised of native vegetation has declined by an estimated 99%. Thus, tallgrass prairie restoration and management is imperative. The control of invasive plant species such as tall fescue (*Schedonorus phoenix*) is one aspect of grassland management. In our first study we added herbicide and seeding treatments to control tall fescue and enhance plant diversity, and measured how these combined treatments affect the abundance, species richness, and diversity of butterflies and flowering plants. Five sites were each divided into three patches, and each patch was managed using one of three different herbicide and seeding treatments. These treatments included a non-sprayed control patch, a patch sprayed with glyphosate, and a patch sprayed with glyphosate as well as seeded with a mixture of native plants. Floral resource abundance was significantly higher in patches that were sprayed or sprayed and seeded compared to the control patches. In addition, floral species richness and diversity were significantly higher in patches that were sprayed and seeded compared to the control. Butterfly abundance was marginally significantly higher in patches that were sprayed and seeded compared to the control patches. This study indicates that the control of an invasive grass via herbicide treatments and seeding can potentially provide benefits to native pollinator and flowering plant communities. Restoring grasslands with the goal of improving native plant abundance, species richness, and diversity is another aspect of grassland management. In our second study, we test responses of the native bee and flowering plant communities to three grassland management treatments. Twelve sites were selected for bee and floral resource sampling based on their current land management strategy. Four sites were tallgrass prairie

remnants (TGR), four sites were un-grazed restorations (UGR), and four sites were cattle grazed restorations (CGR). Bee community composition was quantified using “bee bowl” and sweep net sampling. Floral resources were measured along transects by counting flowering ramets. Bee health was quantified by measuring relative lipid content in three Halictids, including *Augochlora pura*, *Agapostemon virescens*, and *Halictus ligatus*. Contrary to expectations, bee abundance was significantly higher in UGR sites than TGR sites and floral abundance was significantly higher in CGR sites compared to TGR and UGR sites. There were no differences among treatments in relative lipid content for the three bee species. Within each species, relative lipid content decreased with increased bee mass. Relative lipid content increased over time in *Au. pura* and *H. ligatus* but was primarily due to a decrease in bee mass over time, as was seen for all three species. This study indicates that non-remnant grassland management strategies can positively influence native bee and flowering plant communities, and stresses the inclusion of time and insect mass when evaluating community health.

CHAPTER 1: GENERAL INTRODUCTION

Tallgrass prairie is among the most imperiled ecosystems globally, with decline estimated at 99% of its original total land cover primarily due to industrial agriculture (Samson and Knopf 1994). Habitat loss has not only reduced the land cover of the tallgrass prairie ecosystem, but has also reduced the frequency of the disturbances that they rely on, namely periodic fire and grazing activity (Fuhlendorf and Engle 2004). The lack of these historic disturbance pressures has the potential to further degrade native grasslands into novel grasslands, areas dominated by exotic plant species (Wilsey et al. 2009).

Among the most influential of exotic plant species within the tallgrass prairie ecoregion is tall fescue (*Schedonorus phoenix*), a grass originally introduced to North America as a low maintenance forage plant for cattle (Ball et al. 1991). Tall fescue acts as a monoculture-forming invasive species in grasslands, meaning that it will out-compete and displace native plant species in areas where it establishes. This can affect butterfly communities negatively because it causes a loss of area suitable for oviposition and feeding (Severns and Warren 2008). Through its invasion of large areas, tall fescue reduces litter quality and availability, reducing the spread of prescribed fires (McGranahan et al. 2012), thus reducing the effectiveness of fire as a control for non-native species. The success of tall fescue in grasslands can be attributed to a mutualistic relationship that the plant has with its associated fungal endophyte (*Epichloë coenophiala*), which provides an infected host with drought resistance, and protection from predators (Arachevaleta et al. 1988). This protection from predators can provide challenges to the management of tall fescue. Ingestion of endophyte-infected plants by cattle can lead to fescue toxicosis, a condition which leads to weight loss, decreased milk production, difficulty regulating body temperature, and loss of limbs in cattle (Peters et al. 1992, Paterson et al. 1995) rendering

the management of tall fescue through the second historic disturbance pressure (grazing) ineffective and even potentially dangerous for cattle producers.

In our first study, we examined herbicide application, a third and non-historic disturbance pressure which has been shown to be effective against tall fescue. Herbicide-based control of tall fescue provides benefits for native grass species, while having no effect on larval survival and oviposition preferences of native butterflies (Washburn et al. 1999, Washburn et al. 2000, LaBar 2009, Glaeser and Schultz 2014). However, little is known about how the control of tall fescue through herbicide application affects the surrounding butterfly and flowering plant communities. In this study, we divided five experimental pastures into three equal-sized patches and applied one of three treatments onto each patch. These treatments included spraying an entire patch with glyphosate herbicide and then seeding the patch with a native seed mix (Spray and Seed), spraying an entire patch with glyphosate herbicide without seeding (Spray Only), and a control treatment where no herbicide or seeding occurred in a patch (Control). The goal of this research was to explore how herbicide and seeding treatments used to control an invasive plant species affect both the butterfly and flowering plant communities.

Habitat loss also negatively affects native bee communities, and has been implicated as the driving force for declining bee populations worldwide (Brown and Paxton 2009). The continual conversion of tallgrass prairie into agricultural lands is one piece of this puzzle because land conversion reduces the available habitat for native bees and increases the reliance of native bees on agroecosystems (Kremen et al. 2002). Land conversion provides further pressure toward the restoration of native grasslands within the tallgrass prairie ecoregion. By increasing the abundance, species richness, and diversity of floral resources within restored lands, native bee communities can respond with an increase in abundance, species richness, and diversity (Potts et

al. 2003, Hines and Hendrix 2005, Roulston and Goodell 2011). However, restoring habitat for bees requires more than planting flowering plants. Factors such as consistent seasonal floral levels (Williams et al. 2012), native or exotic status of plants (Morandin and Kremen 2013), and reduced distance between high quality restored lands (Jauker et al. 2009, Kennedy et al. 2013) can all impact native bee community responses to grassland restoration. Additionally, health and nutritional responses of native bees to grassland management treatments have often been overlooked, with the exception of large colonial species (Smith et al. 2016).

In our second study, we evaluated native bee and flowering plant community responses to three types of grassland management treatments. These treatments included 1) tallgrass prairie remnants (TGR), areas of native plant cover that were never converted to agricultural lands, 2) un-grazed restorations (UGR), areas of land that were converted from tallgrass prairie to agriculture, but have since been cleared, re-seeded with native plant species, and managed through prescribed burning, and 3) cattle-grazed restorations (CGR), areas of land that use early intensive grazing from cattle as well as prescribed fire as primary forms of disturbance on the surrounding plant community. We also compared health and nutritional responses of three abundant native sweat bees, *Augochlora pura*, *Agapostemon virescens*, and *Halictus ligatus*, to the same grassland management treatments by comparing lipid content, an indicator of adult bee nutritional status (Smith et al. 2016) of each of the three species.

The research associated with both studies was conducted in the Grand River Grasslands Region of Southern Iowa and Northern Missouri (Figure 1). The Grand River Grasslands is a diverse matrix of agricultural, pastoral, and natural grasslands measuring roughly 28,000 ha in size. This region provides a promising opportunity to explore the responses of pollinator and flowering plant communities to several types of grassland management strategies.

Thesis Organization

This thesis is composed of two chapters, each written with the intention of future publication in scientific journals. Chapter 1 is a general introduction to the research conducted in Chapters 2 and 3. Chapter 2 explores research evaluating the effects of herbicide treatments on butterfly and flowering plant communities in Southern Iowa and Northern Missouri. Chapter 3 explores research evaluating native bee community composition and nutrition in areas managed through different grassland management treatments. Chapter 4 is a general conclusion to the findings of the preceding two chapters and also presents directions for future research.

References

- Arachevaleta, M., C. W. Bacon, C. S. Hoveland, and D. E. Radcliffe. "Effect of the Tall Fescue Endophyte on Plant Response to Environmental Stress." *Agronomy Journal* 81.1 (1989): 83.
- Ball, Don; Lacefield, Garry D.; and Hoveland, Carl S., "The Tall Fescue Endophyte" (1991). Agriculture and Natural Resources Publications. Paper 33.
- Brown, Mark J.f., and Robert J. Paxton. "The Conservation of Bees: A Global Perspective." *Apidologie* 40.3 (2009): 410-16.
- Fuhlendorf, S. D., and D. M. Engle. "Application of the Fire-grazing Interaction to Restore a Shifting Mosaic on Tallgrass Prairie." *Journal of Applied Ecology* 41.4 (2004): 604-14.
- Glaeser, Rachel M., and Cheryl B. Schultz. "Characterizing a Contentious Management Tool: The Effects of a Grass-specific Herbicide on the Silvery Blue Butterfly." *Journal of Insect Conservation* 18.6 (2014): 1047-058.
- Hines, Heather M., and Stephen D. Hendrix. "Bumble Bee (Hymenoptera: Apidae) Diversity and Abundance in Tallgrass Prairie Patches: Effects of Local and Landscape Floral Resources." *Environmental Entomology* 34.6 (2005): 1477-484.
- Jauker, Frank, Tim Diekötter, Franziska Schwarzbach, and Volkmar Wolters. "Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat." *Landscape Ecology* 24.4 (2009): 547-55.
- Kennedy, CM; Lonsdorf, E; Neel, MC; Williams, NM; Ricketts, TH; Winfree, R; et al..(2013). A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology Letters*, 16(5), 584 - 599.
- Kremen, C., N. M. Williams, and R. W. Thorp. "Crop Pollination from Native Bees at Risk from Agricultural Intensification." *Proceedings of the National Academy of Sciences* 99.26 (2002): 16812-6816.
- Labar, Caitlin C., and Cheryl B. Schultz. "Investigating the Role of Herbicides in Controlling Invasive Grasses in Prairie Habitats: Effects on Non-target Butterflies." *Natural Areas Journal* 32.2 (2012): 177-89.
- Mcgranahan, Devan Allen, David M. Engle, Samuel D. Fuhlendorf, James R. Miller, and Diane M. Debinski. "An Invasive Cool-Season Grass Complicates Prescribed Fire Management in a Native Warm-Season Grassland." *Natural Areas Journal* 32.2 (2012): 208-14.

- Morandin, Lora A., and Claire Kremen. "Bee Preference for Native versus Exotic Plants in Restored Agricultural Hedgerows." *Restoration Ecology* 21.1 (2012): 26-32.
- Paterson, J., C. Forcherio, B. Larson, M. Samford, and M. Kerley. "The Effects of Fescue Toxicosis on Beef Cattle Productivity." *Journal of Animal Science* 73.3 (1995): 889.
- Peters, C. W., K. N. Grigsby, C. G. Aldrich, J. A. Paterson, R. J. Lipsey, M. S. Kerley, and G. B. Garner. "Performance, Forage Utilization, and Ergovaline Consumption by Beef Cows Grazing Endophyte Fungus-infected Tall Fescue, Endophyte Fungus-free Tall Fescue, or Orchardgrass Pastures." *Journal of Animal Science* 70.5 (1992): 1550.
- Potts, Simon G., Betsy Vulliamy, Amots Dafni, Gidi Ne'eman, and Pat Willmer. "Linking Bees And Flowers: How Do Floral Communities Structure Pollinator Communities?" *Ecology* 84.10 (2003): 2628-642.
- Roulston, T'ai H., and Karen Goodell. "The Role of Resources and Risks in Regulating Wild Bee Populations." *Annual Review of Entomology* 56.1 (2011): 293-312.
- Severns, P. M., and A. D. Warren. "Selectively Eliminating and Conserving Exotic Plants to save an Endangered Butterfly from Local Extinction." *Animal Conservation* 11.6 (2008): 476-83.
- Smith, Griffin W., Diane M. Debinski, Nicole A. Scavo, Corey J. Lange, John T. Delaney, Raymond A. Moranz, James R. Miller, David M. Engle, and Amy L. Toth. "Bee Abundance and Nutritional Status in Relation to Grassland Management Practices in an Agricultural Landscape." *Environmental Entomology* 45.2 (2016): 338-47.
- Washburn, Brian E. "Improving Northern Bobwhite Habitat by Converting Tall Fescue Fields to Native Warm-Season Grasses." *Wildlife Society Bulletin* (1973-2006) 28.1 (2000): 97-104.
- Washburn, Brian E., Thomas G. Barnes, and Jeffery D. Sole. "No-till Establishment of Native Warm-Season Grasses In Tall Fescue Fields." *Ecological Restoration* 17.3 (1999): 144-49.
- Wilsey, Brian J., Terri B. Teaschner, Pedram P. Daneshgar, Forest I. Isbell, and H. Wayne Polley. "Biodiversity Maintenance Mechanisms Differ between Native and Novel Exotic-dominated Communities." *Ecology Letters* 12.5 (2009): 432-42.
- Williams, Neal M., James Regetz, and Claire Kremen. "Landscape-scale Resources Promote Colony Growth but Not Reproductive Performance of Bumble Bees." *Ecology* 93.5 (2012): 1049-058.

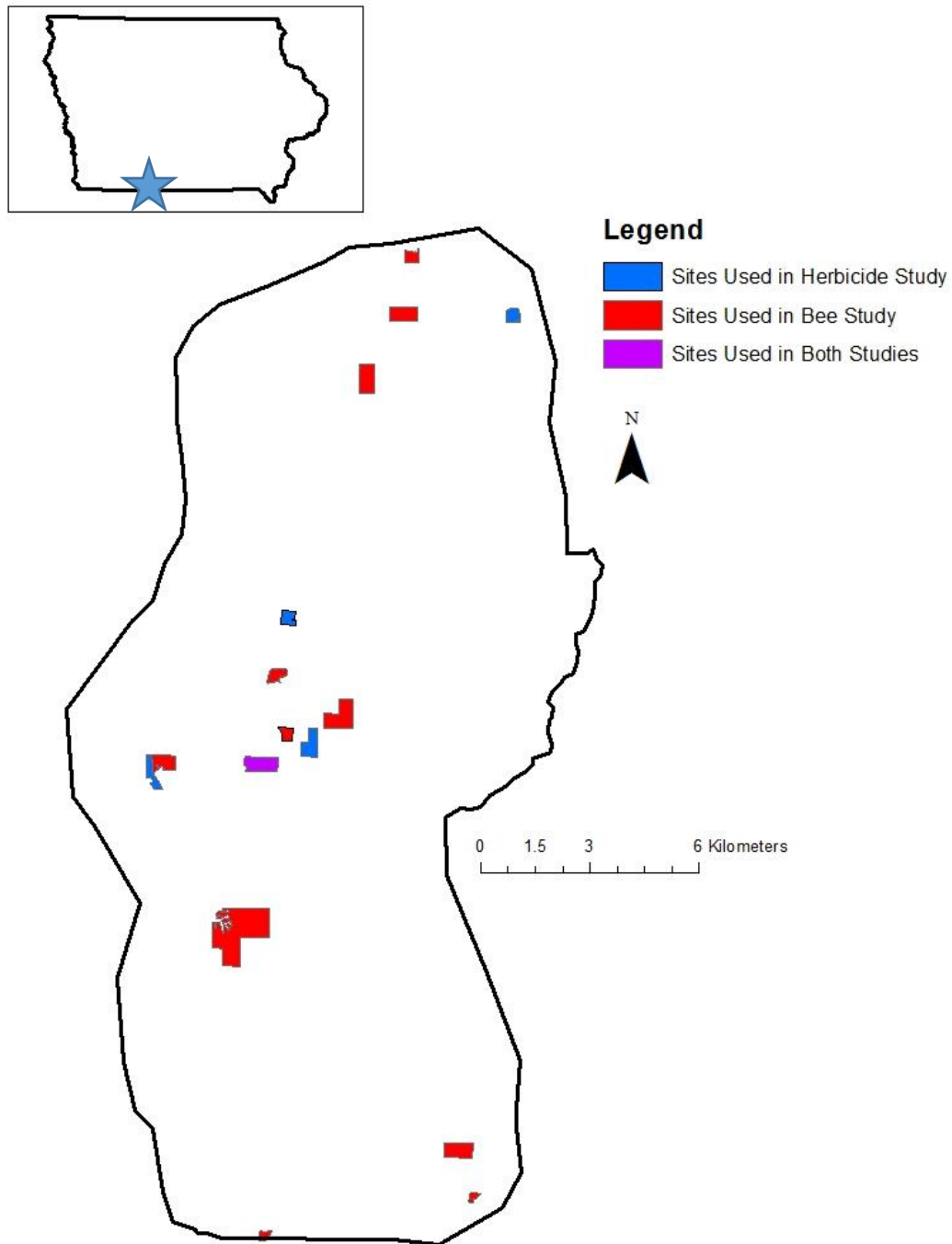


Figure 1: The Grand River Grasslands of Southern Iowa and Northern Missouri with experimental sites used for both the herbicide and bee studies.

CHAPTER 2: ENHANCING NATIVE BUTTERFLY AND FLOWERING PLANT DIVERSITY IN GRASSLANDS VIA COLLABORATIVE ADAPTIVE MANAGEMENT

A paper to be submitted to *Rangeland Ecology and Management*

David S. Stein^{1,2}, Diane M. Debinski^{1,3}, John M. Pleasants¹, James R. Miller⁴, Walter H.
Schacht⁵, Nicholas J. Lyon¹

Abstract

The control and management of invasive plant species poses multiple challenges, including determining how control efforts will affect the larger biotic community. The exotic grass tall fescue (*Schedonorus phoenix*) is a significant threat within the tall grass prairie ecosystem because it competes with forbs that provide the floral resources needed by native pollinators. Fire and grazing are management tools often used to restore native grasslands. Here, in addition to fire and grazing, we have added herbicide and seeding treatments to control tall fescue and enhance plant diversity, and we have measured how these combined treatments affect the abundance, species richness, and diversity of butterflies and flowering plants. We focus here on the herbicide responses because this response occurs more immediately, but we also examine the herbicide plus seeding treatment in the two years post planting. Five sites were each divided into three patches, and each patch was managed using one of three different herbicide and seeding treatments. These treatments included a non-sprayed control patch, a patch sprayed with glyphosate, and a patch sprayed with glyphosate as well as seeded with a mixture of native plants. Floral resource abundance was significantly higher in patches that were

¹ Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa 50011 USA

² Primary researcher and author

³ Corresponding author

⁴ Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61801 USA

⁵ Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68588 USA

sprayed or sprayed and seeded compared to the control. In addition, floral species richness and diversity were significantly higher in patches that were sprayed and seeded compared to the control. Butterfly abundance was marginally significantly higher in patches that were sprayed and seeded compared to the control patches. This study indicates that the control of an invasive grass via herbicide treatments and seeding can potentially provide benefits to native pollinator and flowering plant communities.

Introduction

Tallgrass prairie, like many other grasslands throughout the world, is among the most imperiled ecosystems globally. It has been estimated that total land cover of tallgrass prairie has fallen by 99.9% since the expansion of European settlers over the last two centuries (Samson and Knopf 1994). With this decline, an ecosystem that once covered a third of the North American continent eventually diminished into a patchwork of small remnants that currently dot the landscape. The main contributors to this decline have been the introduction of agricultural practices into the area, and the slow conversion of tallgrass prairie into crop lands and grazing fields for livestock (Swengel 1995).

Agricultural practices have also hindered vital ecosystem services that tallgrass prairies rely on, namely frequent wildfires and grazing by native bison (Fuhlendorf and Engle 2004). Historically, fires provided a necessary disturbance to the tallgrass prairie community by discouraging woody plant invasion and increasing plant community heterogeneity (Copeland et al. 2002). Additionally, grazing by bison provided disturbance to prairies through continual stress by herbivory, leading to a higher diversity of forb species (Joern 2005). Grazing and fire also work in tandem in tallgrass prairie regions. In recently burned sections of grassland the growth of young and highly palatable shoots occurs encourages cattle or bison grazing providing

a period of lower herbivory on older sections with a more established plant community (Fuhlendorf and Engle 2001).

In areas of agricultural development, land conversion and removal of natural ecosystem services leads to the development of novel grasslands, areas with low biodiversity dominated by exotic plant species (Wilsey et al. 2009). On agricultural rangelands, invasive and non-native plant species contribute to a loss of ecosystem services valued at the cost of two-billion dollars annually (DiTomaso 2000). One such exotic plant is the invasive grass tall fescue, *Schedonorus phoenix*. Tall fescue was originally introduced into the tallgrass prairie ecoregion as a low maintenance forage species for cattle, and now covers over 35 million acres of the Midwestern and Southern United States (Ball et al. 1991). Although this grass was established as an important staple for cattle production, over time the negative attributes of tall fescue proved to be counterproductive, both agriculturally and ecologically. Tall fescue acts as a host to a fungal endophyte (*Epichloë coenophiala*) as part of a mutually beneficial symbiotic relationship providing the plant with drought resistance, protection from predators, and larger growth (Arachevaleta et al. 1988). However, when endophyte-infected tall fescue is ingested by cattle, a condition known as fescue toxicosis can occur (Peters et al. 1992). Beef cattle with fescue toxicosis can display multiple symptoms including lower milk production, decreased weight, difficulties regulating body temperature, and loss of extremities by way of gangrenous sores (Paterson et al. 1995, Ball et al. 1991). Ecologically, tall fescue can have negative impacts on the surrounding tallgrass prairie regions where it establishes. Tall fescue that has been infected with its associated endophyte has been shown to decrease the abundance and diversity of the surrounding arthropod community (Rudgers and Clay 2008). Areas of grassland dominated by

fescue have also been shown to negatively affect communities of butterfly pollinators by reducing the abundance of host plants for oviposition (Severns and Warren 2008).

In the Grand River Grasslands region of Southern Iowa and Northern Missouri, tall fescue is a significant ecological threat to the remnants of high quality prairie and an economic threat to the local cattle industry, the dominant agricultural activity in the area. Previous attempts at controlling tall fescue within the Grand River Grasslands region have primarily focused on the combined use of prescribed burning and cattle grazing. This system, known as patch-burn-grazing divides a pasture into several patches and invasive species are potentially controlled through the use of yearly patch burnings, followed by grazing by local cattle (Vermeire et al. 2004). By only burning one patch per year, this system is designed to have cattle graze primarily on new shoot growth (Allred et al. 2011). However, tall fescue can alter fire regimes in areas that it has invaded by reducing litter accumulation, thus lowering the overall spread of prescribed burns (McGranahan et al. 2012). For this reason, we decided to test a collaborative adaptive management strategy to control tall fescue, a strategy implemented through multiple institutions and landowners in response to previous management findings. (Olsson et al. 2004, Armitage 2005). Our goal was to strategically apply herbicide and seeding treatments to subsections of pastures to reduce the fescue cover and augment the warm season grass and forb cover. Application of herbicides such as glyphosate has been shown to lead to a significant increase of native warm season grasses in areas formerly dominated by tall fescue (Washburn et al. 1999, Washburn et al. 2000). Wildlife and pollinator communities also reap the benefits of herbicide use. Areas treated with herbicide to control for tall fescue provide higher quality feeding and nesting habitat for northern bobwhite quails as compared to prescribed burns, disking, and other invasive plant control techniques (Madison et al. 2001). Furthermore,

applications of herbicides in prairie regions show little to no effect on larval survival and oviposition preference in native butterflies (LaBar 2009, Glaeser and Schultz 2014).

In this study, we compare butterfly and flowering plant community responses to three treatments used for the control of invasive tall fescue within pastures located in the Grand River Grasslands region of Southern Iowa and Northern Missouri. Each pasture was subdivided into three patches and these patches were adaptively managed to apply treatments to maximize the effects of treatments to improve the native plant community diversity and cover. Treatments included general application of glyphosate in a section of an experimental pasture (spray only), application of glyphosate in a section of an experimental pasture followed by a seed mix application (spray and seed), and no treatment (control). Because community responses to herbicide application for both native butterflies and flowering plants have not been thoroughly studied, a major goal of this experiment was to establish a dataset of prairie butterflies and flowering plants, collected over multiple years post-treatment. We focus our analysis on community data two years post-treatment testing the following hypotheses:

- i. As a result of the decreased competition resulting from decreased land cover of invasive tall fescue, total flowering plant abundance, species richness, and diversity will be greater in patches that were treated with herbicide as compared to the un-treated control patches.
- ii. With an increase in flowering plant abundance, total butterfly abundance, species richness, and diversity will be greater in patches treated with herbicide as compared to the un-treated control patches.

- iii. Over time, the spray and seed patches will become even more diverse in plant community than the spray only patches, resulting in higher total butterfly abundance, species richness, and diversity.

Methods

Study Sites

Five pastures within the Grand River Grasslands region of Southern Iowa and Northern Missouri were selected for this study (Table 1.) Pastures were selected based on a high relative level of invasion by tall fescue as compared to other experimental pastures in the region. Three pastures, Gilleland, Lee Trail Road, and Pyland West, were previously used as a part of a multi-year patch-burn-graze experiment (Moranz et al. 2012, Delaney et al. 2016), with burning on a three year cycle. The additional two pastures, Besh and Richardson 2, were previously used for harvesting hay. Like the grazed pastures, these two pastures are managed by burning on a three year cycle and haying annually. Each pasture was divided into three equally sized sub-units called patches, ranging in size from 13 to 28 acres, and a different treatment was applied to each patch. The patch designated as the control treatment was selected based on having the highest cover of native grasses and forbs and lowest cover of tall fescue. The “spray only” treatment was applied to the patch with intermediate cover of native grasses, forbs and tall fescue. The “spray and seed” treatment was applied to the patch with the lowest cover of native grasses and forbs and the highest cover of tall fescue. All five experimental pastures were treated with glyphosate in their appropriate patches during the fall of 2014. Our goal in applying the herbicide in the fall was to reduce the tall fescue cover while minimizing negative effects on native vegetation. During fall, tall fescue is still green, whereas native vegetation has senesced

and is therefore less likely to take up the herbicide into living tissues. The spray and seed patch was planted with a native seed mix following glyphosate application.

Butterfly Sampling

Butterflies were sampled in 2015 and 2016 during a 2.5 month sampling season lasting from early June to mid-August. In order to account for the two butterfly emergence periods, which last from June-July and July-August (Schlicht 2007, Moranz et al. 2012), each of the five experimental pastures were sampled for butterflies twice during the field season using a transect method modified from Thomas (1983). Using this method, two permanent 100 meter long transects were established in each individual patch throughout the five experimental pastures, providing six transects per pasture. Sampling occurred through the use of a modified Pollard walk method (Pollard 1977). Observers would travel from one end of the transect to the other over a ten minute period while simultaneously identifying and recording each butterfly that they came across within a 5x5 meter field of vision directly in front of the observer. Butterflies that could not be identified were captured using a net, and identified in the lab. Butterfly sampling events were conducted between 0930-1830 hours Central Standard Time when temperatures were between 21 and 35 degrees Celsius, wind speed was below 16 kilometers per hour, and total cloud cover was below 80 percent (Moranz et al. 2012, Delaney 2014).

Floral Resource Sampling

Floral resources were sampled in 2015 and 2016 during the same 2.5 month sampling season as the butterflies, lasting from early June to mid-August; each site was sampled for floral resources twice during the field season. Floral resources were sampled along the same permanent 100 meter transects that were used for butterfly sampling. Sampling of floral resources occurred through the use of a modified Pollard walk (Pollard 1977). Each transect was

sub-divided into five 20 meter long sections, with flowering plants surveyed in five 1x20 meter quadrats that were located to one side of the transect. Observers traveled along each section of the transect while identifying plant species and counting each ramet, or flowering stem that was located within the sampling quadrat (Moranz et al. 2012, Delaney et al. 2015). Plants that could not be identified in the field were collected and later identified in the lab. Floral resource sampling occurred at the same time as butterfly sampling, so sampling events were conducted between 0930-01830 hours when temperatures were between 21 and 35 degrees Celsius, wind speed was below 16 kilometers per hour, and total cloud cover was below 80 percent (Moranz et al. 2012, Delaney 2014).

Statistical Analysis

The goal of statistical analysis was to determine the response of butterfly and flowering plant abundance, species richness, and diversity to herbicide and seeding treatments two years post treatment. Because adult butterfly lifespans are generally 2-3 weeks (Schlicht 2007), we assumed that butterflies surveyed on separate survey dates were in fact different individuals. Thus, to calculate butterfly abundances, the total number of individuals observed on each patch was summed across the sampling season, to create a total butterfly count for each of the patch treatments. Richness was defined as the total number of species observed on each patch across the entire sampling season. We used the Shannon diversity index as the measurement of butterfly diversity and this was calculated using butterfly abundances for the entire sampling season.

Flowering plant abundance was calculated similarly to butterfly abundance. The total number of flowering stems observed in each section of each transect was summed across the season. Species richness was defined as the total number of species observed within a patch

across the entire sampling season. Shannon diversity was used as the measure of flowering plant diversity within a patch.

We used the R software package (R 3.3.1) to construct linear mixed effects models (LMER) with site as a random variable that tested for differences in butterfly and flowering plant abundance, species richness, and diversity among patches by treatment.

Results

Butterfly Community

In 2015, the first year post-herbicide treatment, a total of 807 butterflies belonging to 24 species were observed across all five experimental pastures. In 2016, two years post-herbicide treatment, a total of 930 individual butterflies belonging to 31 species were counted across all five pastures. Between the first and second year of post-herbicide butterfly sampling, we observed a 15.2% increase in total butterfly counts and a 29.2% increase in butterfly species counts. Two Iowa Species of Greatest Conservation Need were observed in 2015 (Iowa Department of Natural Resources), including the Monarch (*Danaus plexippus*) and the Regal Fritillary (*Speyeria idalia*), accounting for 30 individuals and 3.7% of total butterflies sampled. Four Iowa Species of Greatest Conservation need were found in 2016, including the Gorgone Checkerspot (*Chlosyne gorgone*), Monarch (*Danaus plexippus*), Regal Fritillary (*Speyeria idalia*), and Wild Indigo Duskywing (*Erynnis baptisiae*), accounting for 10 individuals and 1.1% of total butterflies sampled.

In 2016, two years after treatments, butterfly abundance was marginally significantly higher in the spray and seed patches as compared to the control ($t=2.20$, $df=8$, $p=0.06$) (Figure 1). However, no significant differences were found in species richness between the sprayed patches and the control patches ($F=1.92$, $df=2,8$, $p=0.21$). There was a significant difference in

Shannon diversity of butterflies, with the spray only treatment being significantly lower than the control ($t=-2.40$, $df=8$, $p=0.04$) (Figure 1).

Flowering Plant Community

In 2015, the first year following treatment of the experimental pastures, a total of 22,106 individual flowering ramets belonging to 42 species were observed across all experimental pastures. This included 18,972 individuals of 17 species classified as non-native or introduced plant species amounting to 85.8% of total flowering ramets counted. Native plants accounted for 3134 individual flowering ramets belonging to 25 species, and 14.2% of total flowering ramets surveyed. In 2016, the second year sampled following the herbicide treatment of the experimental pastures, a total of 71,922 flowering ramets belonging to 47 species were observed. This included 54,103 individuals belonging to 20 species classified as non-native or introduced plant species, comprising 75.2% of total flowering ramets surveyed. Native plants accounted for 17,822 individual flowering ramets belonging to 27 species, and 24.8% of total flowering ramets surveyed. Native flowering plants comprised 16.1% of total flowering plants in control patches, 23.6% of all flowering plants in spray only patches, and 29.1% of flowering plants in spray and seed patches. Across all pastures, total abundance of flowering ramets increased by 224.5% and the number of flowering plant species increased by 1.1% between 2015 and 2016. Between 2015 and 2016, flowering ramet abundance increased by 72.7% in the control patches, 282.8% in the spray only patches, and 330.7% in the spray and seed patches.

In 2016, two years post-treatment, floral abundance was significantly higher in the spray only treatment compared to the control ($t=2.82$, $df=4.82$, $p=0.04$) (Figure 2). Floral abundance was also significantly higher in the spray and seed treatment compared to both the control and the spray only treatments ($t=5.46$, $df=4.82$, $p<0.01$). Species richness in the spray and seed

patches was also significantly higher compared to the control ($t=3.77$, $df=8$, $p=0.01$) (Figure 2). Similarly, Shannon diversity in the spray and seed patches was significantly higher compared to the control patches ($t=2.52$, $df=8$, $p=0.036$) (Figure 2). Non-native floral abundance was significantly higher in spray only patches ($t=2.38$, $df=8.12$, $p=0.04$) and spray and seed patches ($t=4.27$, $df=8.12$, $p<0.01$) as compared to the control (Figure 3). Non-native floral species richness was significantly higher in spray and seed patches ($t=2.29$, $df=12$, $p=0.04$) as compared to the control (Figure 3). Native floral abundance was significantly higher in the spray only patches ($t=4.52$, $df=8.33$, $p<0.01$) as compared to the control. Additionally, native floral abundance was significantly higher in the spray and seed patches as compared to the spray only patches ($t=2.47$, $df=7.92$, $p=0.04$) (Figure 4). Native floral species richness was significantly higher in the spray and seed patches as compared to the control ($t=3.56$, $df=8$, $p=0.01$) (Figure 4). Native floral species richness was also significantly higher in the spray and seed patches as compared to the spray only patches ($t=2.52$, $df=8$, $p=0.04$) (Figure 4).

Discussion

Butterfly Community Trends

The results of this study indicate that the use of collaborative adaptive management to control tall fescue can have a measurable effect on the plant and pollinator communities within two years post-treatment. We originally hypothesized that the removal of tall fescue through the use of herbicide treatments would allow for an increase in flowering plants, thus leading to new nectar and oviposition opportunities for native butterflies to utilize, leading to greater overall butterfly abundance. Butterfly communities reacted positively to the use of herbicide as an exotic plant species controlling agent. Two years after herbicide treatments, total butterfly abundance was marginally significantly higher the spray and seed patches as compared to the

control. The increase in abundance in the spray and seed patches is being primarily driven by habitat generalist butterfly species. Although little research has been conducted on herbicide-pollinator interactions in a tall grass prairie ecosystem, these results seem to fall in line with similar studies conducted in other areas of North America. One such study involved the removal of the invasive shrub Chinese privet from riparian forests. Controlling Chinese privet with both mechanical removal and herbicide treatments resulted in increased butterfly abundance and diversity (Hanula and Horn 2011). Additionally, a similar study conducted in the sagebrush steppe ecoregion used fire and herbicide to control cheatgrass and juniper encroachment and led to similar positive trends for the native butterfly community (McIver and Macke 2014).

A departure from previous studies however came from the butterfly species richness and diversity findings. Species richness showed no significant differences between any of the treatments. Additionally, species richness appeared to be non-significantly lower in spray only patches, while the control and spray and seed patches were relatively comparable in the number of species found. Butterfly diversity showed a similar trend, with spray only patches being significantly less diverse than control patches. Patches that were sprayed and seeded showed an intermediate level of diversity between the two. Thus, our predictions for increased species richness and diversity of butterflies were not supported. One reason for this could be the timeframe in which this study took place. Previous studies have indicated that large shifts in prairie plant community composition can occur over a three year cycle (Camill et al. 2004). However, it is likely that the two year time frame for this study was not enough for new host and nectar plants to establish well enough in order to attract more butterfly species in herbicide treated areas.

Species of greatest conservation need, such as the Regal Fritillary (*Speyeria idalia*) also have the potential to benefit from herbicide application. The foraging behavior of regal fritillaries has been shown to be influenced by areas of prairie that have recently been burned. Regal fritillaries will often seek out recently burned areas in order to take advantage of new nectar resources afforded there (Moranz et al. 2014). In areas that have been invaded with tall fescue, effects of fire could be diminished (Brooks et al. 2004). Models show that fire behavior is reduced in magnitude in areas that are dominated by tall fescue. This reduction is driven by low levels of available litter needed for fuel and high levels of moisture (McGranahan 2013). By reducing tall fescue cover through the application of herbicide, fire could potentially spread more easily throughout a treated patch, providing a key benefit for a species of greatest conservation need.

Flowering Plant Community Trends

Two years following application, the flowering plant community showed a positive response to the application of herbicide and seeding throughout the experimental pastures. We originally hypothesized that treating patches with herbicide would lead to higher levels of flowering plant abundance, species richness, and diversity. Total abundance of flowering plants was significantly higher in areas that were sprayed with herbicide as compared to the control. Additionally spray and seed patches had significantly higher flowering plant abundances as compared to both the spray only and control patches. Flowering plant species richness was significantly higher in spray and seed patches as compared to the control, and the spray only patches had an intermediate level of species richness. Flowering plant diversity followed a similar trend. Spray and seed patches were significantly more diverse than the control patches, whereas the spray only patches had an intermediate level of diversity. Our results for the

flowering plant community indicate that our initial hypotheses were correct. The increase in flowering plant abundance, species richness, and diversity is most likely a result of herbicide application intended to reduce the level of tall fescue in the experimental pastures. Preliminary results show that total percent cover of tall fescue has declined in the spray and spray and seed patches (Lyon 2017). Lack of tall fescue within the experimental pastures may have led to a decreased level of competition and less plant cover, allowing for the establishment of additional flowering plant species. The addition of native seeds in the spray and seed patches likely led to further plant establishment, leading to an increased level of abundance, species richness, and diversity of flowering plants.

Although our treatments were originally implemented in order to reduce the total amount of an invasive plant species cover within the experimental pastures, herbicide treatments may have also benefitted non-native flowering plant species. Total abundance and species richness of the non-native flowering plant community showed a similar trend to the total flowering plant community. Total abundance of non-native flowering plants was significantly higher in patches that were sprayed only and sprayed and seeded as compared to the control patches. Non-native flowering plant species richness was also significantly higher in patches that were sprayed and seeded as compared to the control patches, with the spray only patches having an intermediate level of species richness. However, native flowering plants also appeared to have benefitted from the herbicide treatments, with flowering ramet abundance and species richness being significantly higher in the spray and seed patches. This shows that the herbicide treatments are benefitting both native and non-native flowering plants due to reduced competition from tall fescue.

Implications and Future Research

The findings of this research indicate that the application of collaborative adaptive management may have the potential to alter the composition of plant and butterfly communities. Although the herbicide application was used primarily to control and manage invasive tall fescue, over two years it changed both the pollinator and plant communities. Butterfly communities showed a positive response, with the total abundance of individuals highest in spray and seed patches. The flowering plant community showed an even stronger response. Both total abundance and species richness were higher in patches that were sprayed with herbicide and seeded as compared to untreated control patches. Although further monitoring is needed, this study supports the use of fall herbicide application as a legitimate management strategy in controlling tall fescue in Midwestern tall grass prairie restoration and management.

One direction for future studies that we would recommend would be to incorporate responses of other pollinators, such as bees. This would allow the comparison of bee and butterfly community responses to determine whether these treatments represent a positive improvement for the pollinator community as a whole, or whether different pollinator communities are reacting differently. Additionally, long-term monitoring of such experiments is essential. As discussed earlier, the community composition of tallgrass prairie can change considerably after three or more years (Cavill et al. 2004, Delaney et al. 2016). Thus, both the flowering plant and butterfly communities can continue to shift in experimental pastures over time. Continued monitoring of invasive species such as tall fescue will also be crucial to determine whether these plant cover changes can be sustained. As demonstrated earlier, flowering non-native plant species benefitted from the initial herbicide treatment, leading to

higher abundances and species richness. As these non-native forb species continue to establish, additional herbicide or burning treatments may be needed for their control.

Acknowledgments

This research was supported by Iowa Agricultural Experiment Station project IOW05392, and through funding provided by the Iowa State Wildlife Competitive Grants Program in Cooperation with the U.S. Fish and Wildlife Service (State Wildlife Grant SWG-C #14CRDWBKReed-0011). Thank you to Logan Crees, Ray Moranz, Emma Stivers, Nick Lyon, Josh Rusk, Scott Nelson, and Jaime Coon for their assistance during field seasons. A final thank you to the Iowa Department of Natural Resources for partnering in application of the management to these sites and allowing us access to the study sites.

References

- Allred, Brady W., Samuel D. Fuhlendorf, David M. Engle, and R. Dwayne Elmore. "Ungulate Preference for Burned Patches Reveals Strength of Fire-grazing Interaction." *Ecology and Evolution* 1.2 (2011): 132-44.
- Armitage, Derek. "Adaptive Capacity and Community-Based Natural Resource Management." *Environmental Management* 35.6 (2005): 703-15.
- Arachevaleta, M., C. W. Bacon, C. S. Hoveland, and D. E. Radcliffe. "Effect of the Tall Fescue Endophyte on Plant Response to Environmental Stress." *Agronomy Journal* 81.1 (1989): 83.
- Ball, Don; Lacefield, Garry D.; and Hoveland, Carl S., "The Tall Fescue Endophyte" (1991). Agriculture and Natural Resources Publications. Paper 33.
- Bray, Tanya. "Nectar-Seeking Visits by Butterflies in a Tallgrass Prairie Remnant in Eastern Nebraska." *Transactions of the Nebraska Academy of Sciences* 21 (1994): 63-72.
- Brooks, Matthew L., Carla M. D'antonio, David M. Richardson, James B. Grace, Jon E. Keeley, Joseph M. Ditomaso, Richard J. Hobbs, Mike Pellant, and David Pyke. "Effects of Invasive Alien Plants on Fire Regimes." *BioScience* 54.7 (2004): 677.
- Camill, Philip, Mark J. Mckone, Sean T. Sturges, William J. Severud, Erin Ellis, Jacob Limmer, Christopher B. Martin, Ryan T. Navratil, Amy J. Purdie, Brody S. Sandel, Shano Talukder, and Andrew Trout. "Community- And Ecosystem-Level Changes in a Species-Rich Tallgrass Prairie Restoration." *Ecological Applications* 14.6 (2004): 1680-694.
- Copeland, Tanya E., William Sluis, and Henry F. Howe. "Fire Season and Dominance in an Illinois Tallgrass Prairie Restoration." *Restoration Ecology* 10.2 (2002): 315-23.
- Davis, Jessica D., Stephen D. Hendrix, Diane M. Debinski, and Chiara J. Hemsley. "Butterfly, Bee and Forb Community Composition and Cross-taxon Incongruence in Tallgrass Prairie Fragments." *Journal of Insect Conservation* 12.1 (2007): 69-79.
- Ditomaso, Joseph M. "Invasive Weeds in Rangelands: Species, Impacts, and Management." *Weed Science* 48.2 (2000): 255-65.
- Delaney, J.T., "Utilizing novel grasslands for the conservation and restoration of butterflies and other pollinators in agricultural ecosystems" (2014). Graduate Theses and Dissertations. Paper 14097.
- Delaney, J. T., K. J. Jokela, and D. M. Debinski. "Seasonal Succession of Pollinator Floral Resources in Four Types of Grasslands." *Ecosphere* 6.11 (2015).

- Delaney, John T., Raymond A. Moranz, Diane M. Debinski, David M. Engle, and James R. Miller. "Exotic-Dominated Grasslands Show Signs of Recovery with Cattle Grazing and Fire." *Plos One* 11.11 (2016).
- Fuhlendorf, Samuel D., and David M. Engle. "Restoring Heterogeneity on Rangelands: Ecosystem Management Based on Evolutionary Grazing Patterns." *BioScience* 51.8 (2001): 625.
- Fuhlendorf, S. D., and D. M. Engle. "Application of the Fire-grazing Interaction to Restore a Shifting Mosaic on Tallgrass Prairie." *Journal of Applied Ecology* 41.4 (2004): 604-14.
- Glaeser, Rachel M., and Cheryl B. Schultz. "Characterizing a Contentious Management Tool: The Effects of a Grass-specific Herbicide on the Silvery Blue Butterfly." *Journal of Insect Conservation* 18.6 (2014): 1047-058.
- Hanula, James L., and Scott Horn. "Removing an Exotic Shrub from Riparian Forests Increases Butterfly Abundance and Diversity." *Forest Ecology and Management* 262.4 (2011): 674-80.
- "Iowa Wildlife Action Plan." Iowa Department of Natural Resources. (2015).
- Joern, Anthony. "Disturbance By Fire Frequency And Bison Grazing Modulate Grasshopper Assemblages In Tallgrass Prairie." *Ecology* 86.4 (2005): 861-73.
- Labar, Caitlin C., and Cheryl B. Schultz. "Investigating the Role of Herbicides in Controlling Invasive Grasses in Prairie Habitats: Effects on Non-target Butterflies." *Natural Areas Journal* 32.2 (2012): 177-89.
- Lyon, Nicholas J. "Mean Fescue Percent Cover." Unpublished Data (2017).
- Madison, L. Andrew. "Effectiveness of Fire, Disking, and Herbicide to Renovate Tall Fescue Fields to Northern Bobwhite Habitat." *Wildlife Society Bulletin* (1973-2006) 29.2 (2001): 706-12.
- Mcgranahan, Devan Allen, David M. Engle, Samuel D. Fuhlendorf, James R. Miller, and Diane M. Debinski. "An Invasive Cool-Season Grass Complicates Prescribed Fire Management in a Native Warm-Season Grassland." *Natural Areas Journal* 32.2 (2012): 208-14.
- Mcgranahan, Devan A., David M. Engle, James R. Miller, and Diane M. Debinski. "An Invasive Grass Increases Live Fuel Proportion and Reduces Fire Spread in a Simulated Grassland." *Ecosystems* 16.1 (2013): 158-69.
- McIver, James, and Euell Macke. "Short-Term Butterfly Response to Sagebrush Steppe Restoration Treatments." *Rangeland Ecology & Management* 67.5 (2014): 539-52.

- Moranz, R. A. 2010. "The effects of ecological management on tallgrass prairie butterflies and their nectar sources." Dissertation. Oklahoma State University, Stillwater, Oklahoma.
- Moranz, Raymond A., Diane M. Debinski, Devan A. Mcgranahan, David M. Engle, and James R. Miller. "Untangling the Effects of Fire, Grazing, and Land-use Legacies on Grassland Butterfly Communities." *Biodiversity and Conservation* 21.11 (2012): 2719-746.
- Olsson, Per, Carl Folke, and Fikret Berkes. "Adaptive Co-management for Building Resilience in Social-Ecological Systems." *Environmental Management* 34.1 (2004): 75-90.
- Paterson, J., C. Forcherio, B. Larson, M. Samford, and M. Kerley. "The Effects of Fescue Toxicosis on Beef Cattle Productivity." *Journal of Animal Science* 73.3 (1995): 889.
- Peters, C. W., K. N. Grigsby, C. G. Aldrich, J. A. Paterson, R. J. Lipsey, M. S. Kerley, and G. B. Garner. "Performance, Forage Utilization, and Ergovaline Consumption by Beef Cows Grazing Endophyte Fungus-infected Tall Fescue, Endophyte Fungus-free Tall Fescue, or Orchardgrass Pastures." *Journal of Animal Science* 70.5 (1992): 1550.
- Pollard, E. "A Method for Assessing Changes in the Abundance of Butterflies." *Biological Conservation* 12.2 (1977): 115-34.
- Rudgers, Jennifer A., and Keith Clay. "An Invasive Plant-fungal Mutualism Reduces Arthropod Diversity." *Ecology Letters* 11.8 (2008): 831-40.
- Samson, Fred, and Fritz Knopf. "Prairie Conservation in North America." *BioScience* 44.6 (1994): 418-21.
- Schlicht, Dennis W., John C. Downey, and Jeffrey C. Nekola. *The Butterflies of Iowa*. Iowa City: U of Iowa, 2007.
- Severns, P. M., and A. D. Warren. "Selectively Eliminating and Conserving Exotic Plants to save an Endangered Butterfly from Local Extinction." *Animal Conservation* 11.6 (2008): 476-83.
- Swengel, Ann B. "Effects of Fire and Hay Management on Abundance of Prairie Butterflies." *Biological Conservation* 76.1 (1996): 73-85.
- Thomas, J.A. "A Quick Method for Estimating Butterfly Numbers during Surveys." *Biological Conservation* 27.3 (1983): 195-211.
- Vermeire, Lance, Robert Mitchell, Samuel Fuhlendorf, and Robert Gillan. "Patch Burning Effects on Grazing Distribution." *Journal of Range Management* 57.3 (2011): 248-52.
- Washburn, Brian E. "Improving Northern Bobwhite Habitat by Converting Tall Fescue Fields to Native Warm-Season Grasses." *Wildlife Society Bulletin* (1973-2006) 28.1 (2000): 97-104.

Washburn, Brian E., Thomas G. Barnes, and Jeffery D. Sole. "No-till Establishment of Native Warm-Season Grasses In Tall Fescue Fields." *Ecological Restoration* 17.3 (1999): 144-49.

Wilsey, Brian J., Terri B. Teaschner, Pedram P. Daneshgar, Forest I. Isbell, and H. Wayne Polley. "Biodiversity Maintenance Mechanisms Differ between Native and Novel Exotic-dominated Communities." *Ecology Letters* 12.5 (2009): 432-42.

Figures and Tables

Table 1: Experimental pastures used in the collaborative adaptive management experiment.

Site	Site Code	Area (Acres)	2016 Stocking Rate (AUM)	Harvest Method
Besh	BSH	40	N/A	Hayed once in August 2014
Gilleland	GIL	77	0.97	Early intensive stocking of cattle from April through July
Lee Trail	LTR	84	1.33	Early intensive stocking of cattle from May through July
Pyland West	PYW	44	0.73	Continuous Stocking of Cattle from April through September
Richardson 2	RCH2	35	N/A	Hayed once in August 2014

Site code indicates a shorthand name of an experimental pasture used in data analysis. Harvest method refers to the way that vegetation is annually removed from the experimental pasture. Stocking rate is measured in AUM, animal unit months.

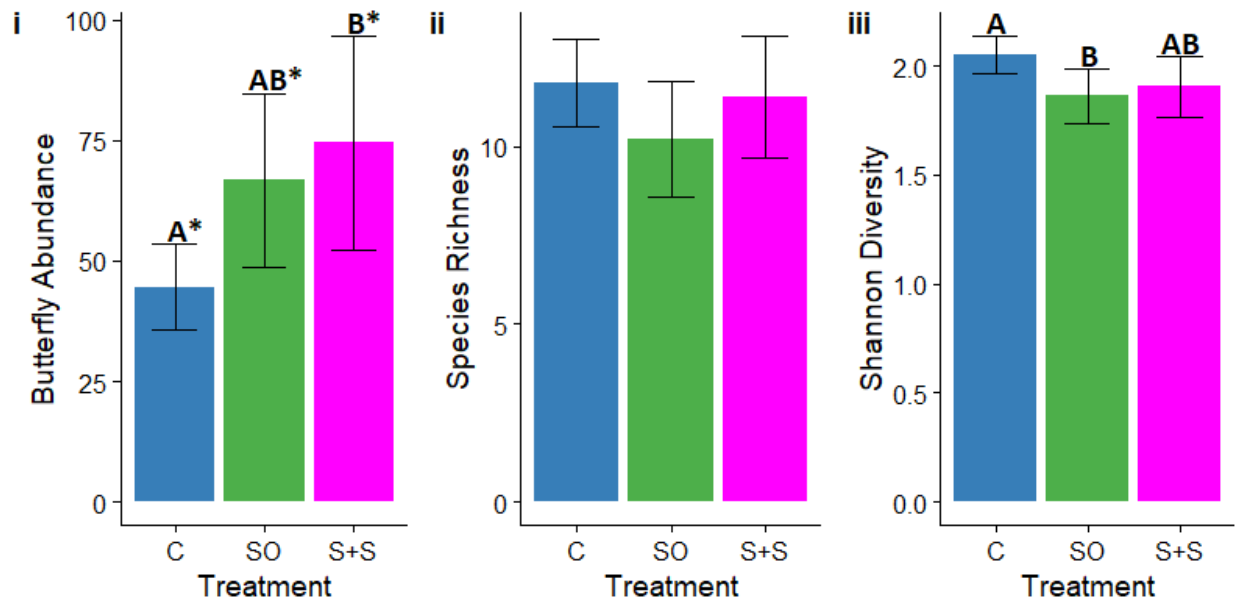


Figure 1: Measures of i) butterfly abundance, ii) species richness, and iii) diversity among three different treatments. C indicates control patches, SO indicates spray only patches that were treated with herbicide, and S+S indicates spray and seed patches that were treated with herbicide and seeded with a native seed mix. Letters indicate statistically significant differences at the $P < 0.05$ level. Letters with asterisks (*) indicate statistically significant differences at the $0.05 < P < 0.1$ level. Bars indicate standard error.

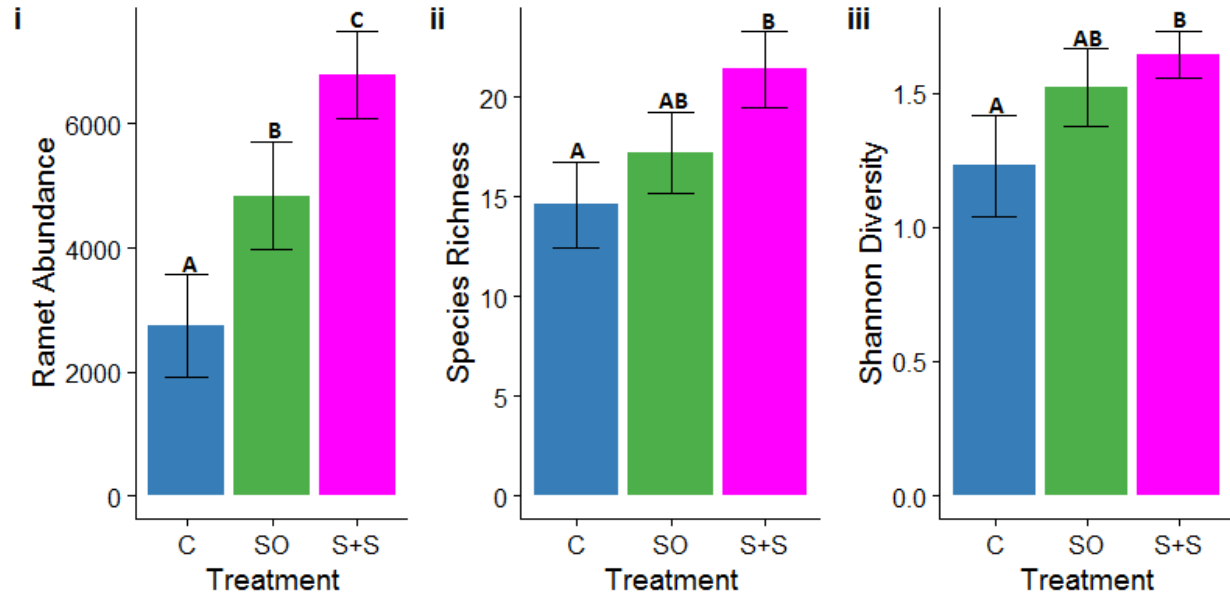


Figure 2: Measures of average i) abundance of flowering ramets ii) flowering plant species richness, and iii) flowering plant diversity among three different treatments. C indicates control patches, SO indicates spray only patches that were treated with herbicide, and S+S indicates spray and seed patches that were treated with herbicide and seeded with a native seed mix. Different letters indicate statistically significant differences at the $P < 0.05$ level. Bars indicate standard error.

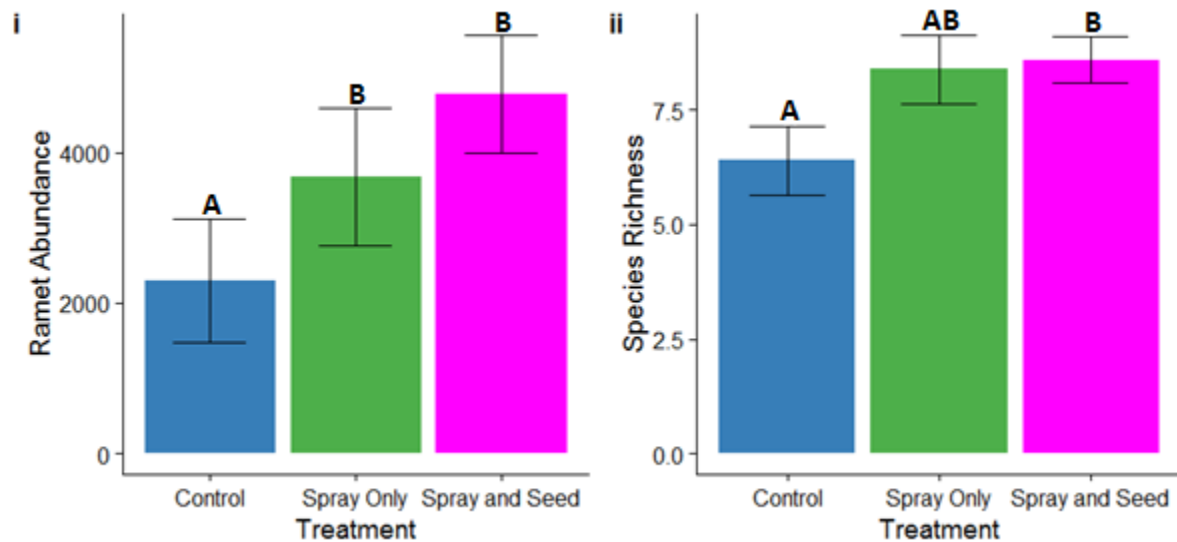


Figure 3: Differences in i) non-native flowering ramet abundance and ii) non-native flowering plant species richness between three different treatments. Control indicates patches where no treatment was applied, Spray only indicates patches that were treated with herbicide, and Spray and Seed indicates patches that were treated with herbicide and seeded with a native seed mix. Different letters indicate statistically significant differences at the $P < 0.05$ level. Bars indicate standard error.

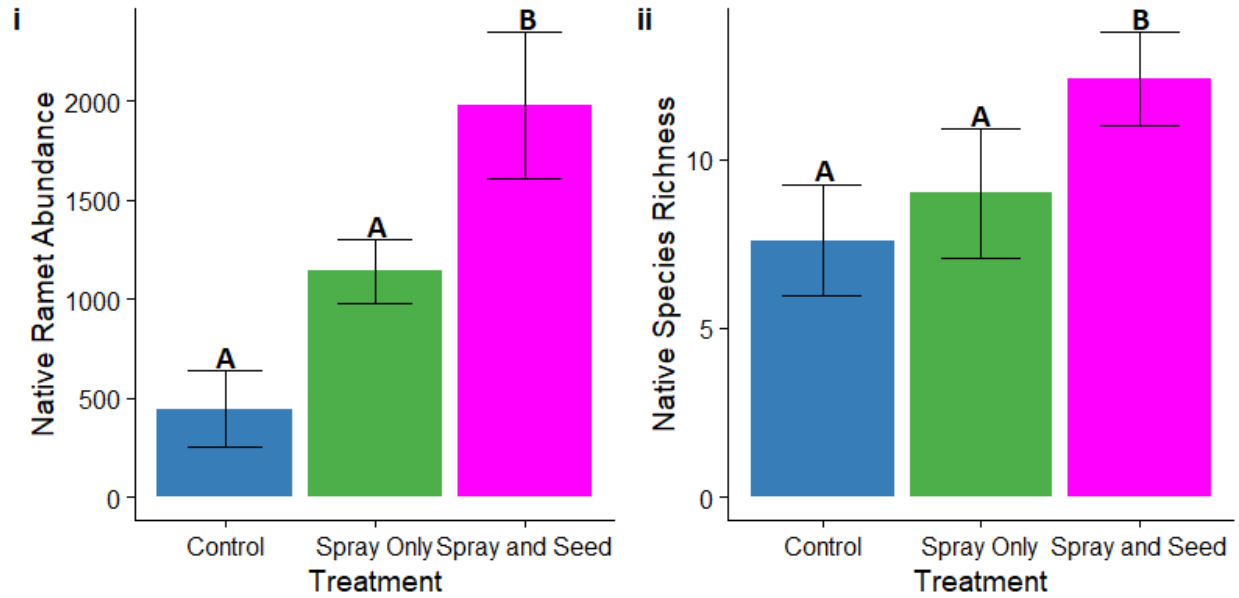
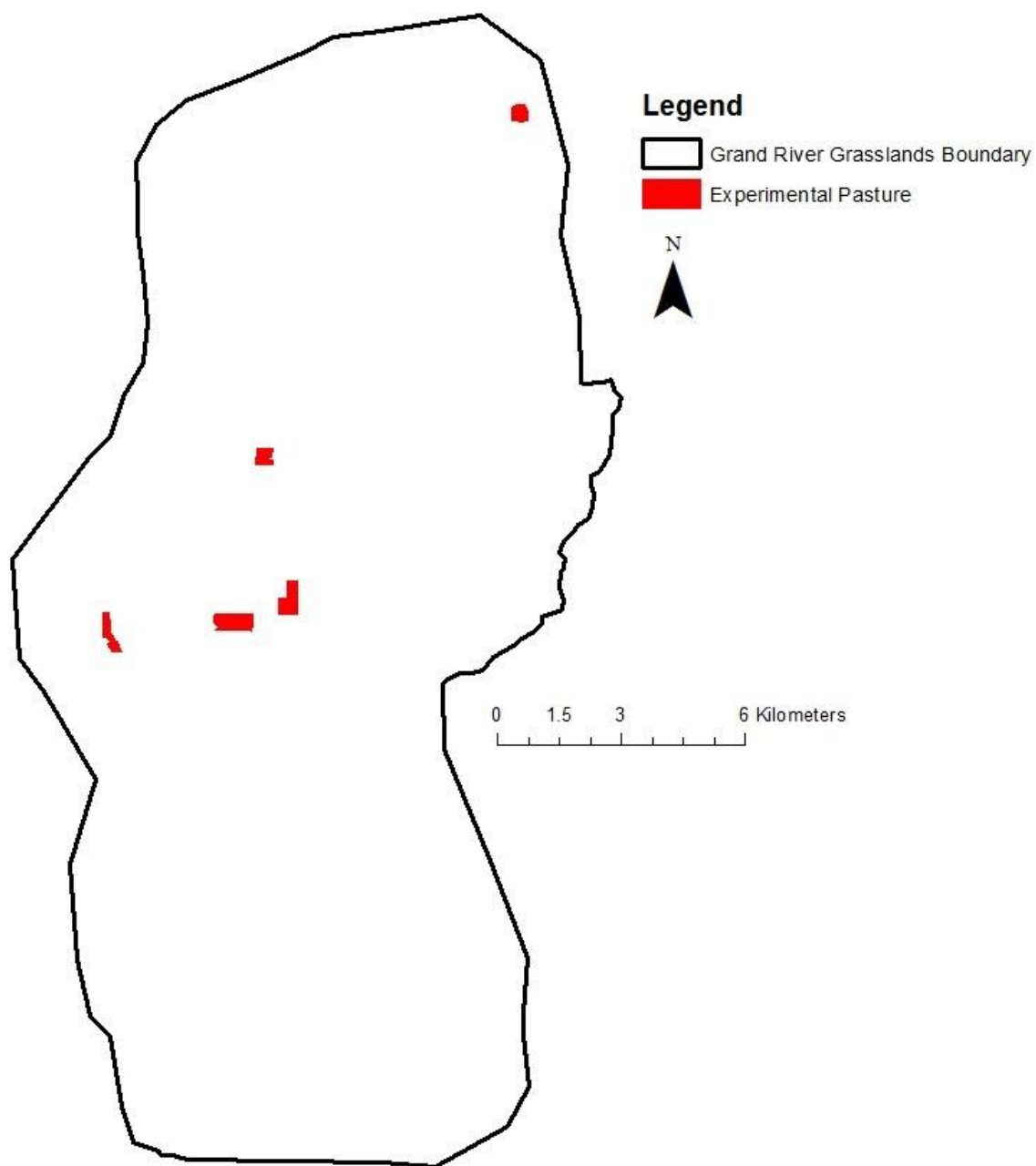
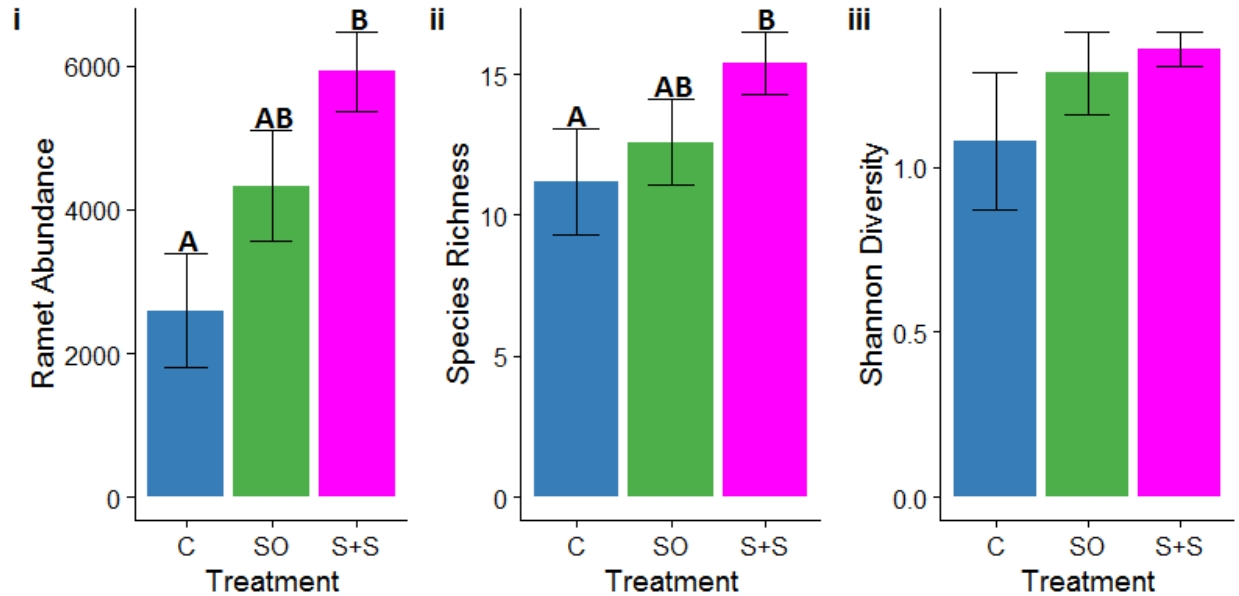


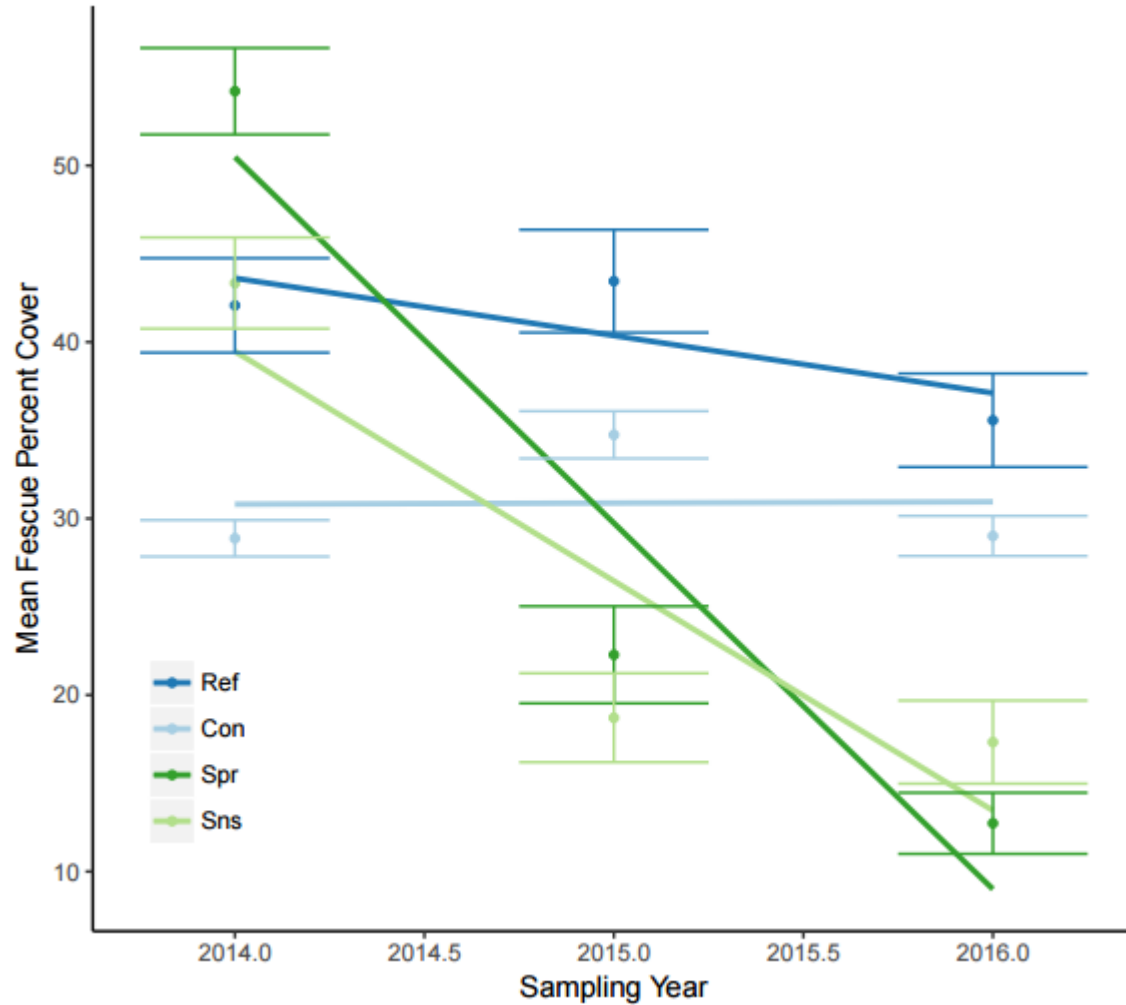
Figure 4: Differences in i) native flowering ramet abundance and ii) native flowering plant species richness between three different treatments. Control indicates patches where no treatment was applied, Spray only indicates patches that were treated with herbicide, and Spray and Seed indicates patches that were treated with herbicide and seeded with a native seed mix. Different letters indicate statistically significant differences at the $P < 0.05$ level. Bars indicate standard error.



Supplemental Figure 1: The Grand River Grasslands region of Southern Iowa and Northern Missouri. Pastures used in the herbicide experiment are shown in red.



Supplemental Figure 2: Measures of average i) abundance of flowering ramets belonging to butterfly-visited flowering plant species ii) butterfly-visited flowering plant species richness, and iii) butterfly-visited flowering plant diversity among three different treatments. C indicates control patches, SO indicates spray only patches that were treated with herbicide, and S+S indicates spray and seed patches that were treated with herbicide and seeded with a native seed mix. Different letters indicate statistically significant differences at the $P < 0.05$ level. Bars indicate standard error.



Supplemental Table 3: Measures of average percent cover by tall fescue in four grassland management treatments. Ref refers to reference sites not used in the herbicide experiment. Con refers to control patches, Spr refers to spray only patches, and Sns refers to spray and seed patches. Bars indicate standard error. Adapted from unpublished data (Lyon 2017).

Supplemental Table 1: Total species counts of butterflies sampled among 3 herbicide treatments summed across the 2016 field season.

	Control	Spray Only	Spray and Seed
<i>Anatrytone logan</i>	0	0	1
<i>Ancyloxypha numitor</i>	0	0	1
<i>Boloria bellona</i>	18	17	20
<i>Celastrina neglecta</i>	1	0	0
<i>Cercyonis pegala</i>	9	3	5
<i>Chlosyne gorgone*</i>	0	1	0
<i>Chlosyne nycteis</i>	0	0	3
<i>Colias eurytheme</i>	47	105	112
<i>Colias philodice</i>	38	43	45
<i>Cupido comyntas</i>	22	60	57
<i>Danaus plexippus*</i>	2	1	0
<i>Epargyreus clarus</i>	5	0	3
<i>Erynnis baptisiae*</i>	1	0	1
<i>Euphyes vestris</i>	2	0	0
<i>Junonia coenia</i>	3	12	12
<i>Limenitis archippus</i>	1	1	0
<i>Limenitis arthemis</i>	1	0	0
<i>Lycaena hyllus</i>	1	2	2
<i>Megisto cymela</i>	1	0	0
<i>Papilio cresphontes</i>	0	3	3
<i>Papilio glaucus</i>	0	0	1
<i>Papilio polyxenes</i>	3	7	12
<i>Phyciodes tharos</i>	10	18	24
<i>Pieris rapae</i>	48	49	57
<i>Polites peckius</i>	2	1	2
<i>Polites themistocles</i>	1	6	0
<i>Polygonia interrogationis</i>	1	0	0
<i>Speyeria idalia*</i>	1	1	2
<i>Speyeria cybele</i>	4	3	5
<i>Vanessa atalanta</i>	1	1	4
<i>Vanessa virginiensis</i>	0	0	1

Asterisks (*) indicate Iowa species of greatest conservation need.

Supplemental Table 2: Total counts of flowering ramets summed by species among 3 herbicide treatments sampled during the 2016 field season.

	Status	Butterfly Use	Control	Spray Only	Spray and Seed
<i>Achillea millefolium</i>	N	*	9	22	42
<i>Apocynum cannabinum</i>	N	*	0	0	10
<i>Asclepias hirtella</i>	N		0	3	0
<i>Asclepias tuberosa</i>	N	*	0	5	1
<i>Baptisia alba</i>	N		0	6	2
<i>Brassica napus</i>	E		0	1	0
<i>Carduus nutans</i>	E	*	0	0	1
<i>Chamaecrista fasciculata</i>	N	*	0	81	134
<i>Cichorium intybus</i>	E		0	1	110
<i>Convolvulus arvensis</i>	E	*	3	0	0
<i>Daucus carota</i>	E	*	1034	1631	2459
<i>Desmodium canadense</i>	N	*	0	0	5
<i>Dianthus armeria</i>	E		12	748	1018
<i>Erigeron annuus</i>	N	*	174	3912	5420
<i>Helianthus grosseserratus</i>	N	*	3	0	1
<i>Hypericum punctatum</i>	N		0	18	3
<i>Leucanthemum vulgare</i>	E	*	14	663	1429
<i>Linum sulcatum</i>	N		0	0	10
<i>Lobelia spicata</i>	N	*	43	20	36
<i>Lotus corniculatus</i>	E	*	6916	8267	6803
<i>Medicago lupulina</i>	E	*	57	110	198
<i>Medicago sativa</i>	E	*	1	1	18
<i>Melilotus albus</i>	E	*	0	6	27
<i>Melilotus officinalis</i>	E	*	23	33	50
<i>Monarda fistulosa</i>	N	*	575	7	45
<i>Oxalis stricta</i>	N	*	0	10	10
<i>Pastinaca sativa</i>	E	*	7	20	27
<i>Plantago lanceolata</i>	E		65	735	1105
<i>Potentilla arguta</i>	N		2	22	10
<i>Potentilla simplex</i>	N	*	0	10	18
<i>Prunella vulgaris</i>	N	*	31	83	40
<i>Pycnanthemum tenuifolium</i>	N		550	699	1936
<i>Ratibida pinnata</i>	N	*	16	0	80
<i>Rosa multiflora</i>	E		1	0	0
<i>Rudbeckia hirta</i>	N	*	25	185	380
<i>Ruellia humilis</i>	N		68	12	26

Supplemental Table 2 Continued.

	Status	Butterfly Use	Control	Spray Only	Spray and Seed
<i>Silene latifolia</i>	E		0	0	7
<i>Solanum carolinense</i>	N		51	10	27
<i>Teucrium canadense</i>	N	*	104	28	8
<i>Trifolium hybridum</i>	E		3	289	51
<i>Trifolium pratense</i>	E	*	1191	1935	4999
<i>Trifolium repens</i>	E	*	2192	4042	5799
<i>Verbascum blattaria</i>	E		0	0	1
<i>Verbena hastata</i>	N	*	187	467	487
<i>Verbena stricta</i>	N	*	126	101	872
<i>Verbena urticifolia</i>	N		0	0	89
<i>Vernonia fasciculata</i>	N	*	245	8	209

Status indicates whether a species is exotic (E) or native (N). Asterisks (*) under butterfly use indicate whether butterfly species use this plant species as a nectar source (Bray 1994, Davis et al. 2007, Moranz et al. 2010).

CHAPTER 3: EVALUATING NATIVE BEE COMMUNITIES AND BEE HEALTH IN MANAGED GRASSLANDS

A paper to be submitted to *Environmental Entomology*

David S. Stein^{1,2}, Diane M. Debinski¹, John M. Pleasants¹, Amy L. Toth¹

Abstract

Native pollinators are incredibly important for providing vital services in agroecosystems; however, their numbers are declining globally. Bees are the most efficient and diverse members of the pollinator community and therefore are key elements of native pollinator communities. It is imperative that management strategies be implemented that positively affect native bee community composition and health. Here we test responses of the native bee and flowering plant communities to land management treatments in the context of grasslands in the upper Midwestern US, a critical area with respect to bee declines. Twelve grassland sites were selected to examine floral resources and wild bee communities based on differences in current land management strategy. Four sites were designated as tallgrass prairie remnants (TGR), 4 sites were designated as un-grazed restorations (UGR), and 4 sites were designated as cattle grazed restorations (CGR). Bee community composition was quantified using “bee bowl” and sweep net sampling. Floral resources were measured along nectar transects. Total bee abundance was significantly higher in UGR sites than TGR sites. Floral abundance was significantly higher in CGR sites compared to TGR and UGR sites. We also examined relative lipid content as a bee health indicator in three sweat bees (Halictidae), *Augochlora pura*, *Agapostemon virescens*, and *Halictus ligatus*. There were no differences among sites in relative

¹ Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011 USA

² Primary researcher and author

lipid content for the three bee species. We also explored relationships between bee nutritional factors, pollinator mass, and time. Within each species, relative lipid content decreased with increased bee mass. Relative lipid content increased over time in *H. ligatus*, but was primarily due to a decrease in bee mass over time. This study indicates that non-remnant grassland management strategies can positively influence native bee and flowering plant communities, and stresses the inclusion of time and pollinator mass when evaluating community health.

Introduction

Pollinators are unparalleled in importance with respect to the ecosystem services that they provide, and our understanding of their contributions both ecologically and economically has been expanding over the past several decades. Among the biotic pollinators, the most efficient by far are bees (Batra 1995). Given their importance, it is concerning that native and domesticated bee populations are declining globally (Potts et al. 2010). Many factors have contributed to this unfortunate trend within native bee communities. The effects of disease have recently been quantified as drivers in native pollinator community declines (Vanbergen et al. 2013). *Nosema bombi* in particular, is a pathogenic fungus introduced to North America from Europe that infects native *Bombus* species, leading to reduced fitness and declining populations (Otti and Schmid-Hempel 2007, Cameron et al. 2011). *Bombus* species are under additional pathogenic stress from increased contact with domesticated *Apis mellifera* populations, potentially giving rise to exotic diseases such as deformed wing virus and *Nosema ceranae* (Furst et al. 2014). Insecticides such as neonicotinoids can have potentially detrimental effects on native bee species (Goulson 2013). Sub-lethal doses of neonicotinoid insecticides can reduce cognitive functions and feeding ability in *Bombus*, and slow growth in colonies (Whitehorn et al. 2012, Goulson 2013).

Although disease and insecticides have the potential to reduce populations of native bees, the driving force of worldwide decline is habitat loss (Brown and Paxton 2009). The continual conversion of natural lands into agricultural systems can have wide-ranging negative impacts on native bee and pollinator communities as well as bee-dependent crop systems (Kremen et al. 2002). Thus, continued dependence of agricultural pollination on native bees makes the restoration of natural habitats imperative. This is especially true within the tallgrass prairie ecoregion of North America. Since the introduction of industrial agriculture, total land cover of native grassland in this region of the continent has declined by an estimated 99% (Samson and Knopf 1994) and recent increases in agricultural activity have only exacerbated this trend (Wright and Wimberly 2013). Restoring native prairie plant communities can help to reverse this trend. By increasing the abundance, species richness, and diversity of flowering plants on a landscape, it is generally thought that the native bee community will respond with its own increase in abundance, species richness, and diversity (Potts et al. 2003, Hines and Hendrix 2005, Roulston and Goodell 2011). However, simply planting flower plant species is not enough; floral resources must be available throughout the season (Williams and Kremen 2012, Persson and Smith 2013). Seasonal floral resource shortages have the potential to lead to nutritional stress in pollinators (Delaney et al. 2015). Thus, it is important to examine pollinator responses throughout the season. The native or exotic status of flowering plants is also important to native bees. Although bees will visit exotic flowering plants, preference is shown toward native plants (Morandin and Kremen 2013). Location of restorations can also have an effect on native bee populations. Low quality landscapes in a close vicinity to high quality tallgrass prairie restorations or remnants can limit the dispersal and foraging capacities of native bees (Jauker et al. 2009, Kennedy et al. 2013).

While community composition has been assessed in many studies, fewer studies have attempted to also look into health indicators, such as nutritional state, as an aspect of native bee conservation. The Grand River Grasslands Region of Southern Iowa and Northern Missouri presents a promising opportunity for monitoring native bee community composition and health under different grassland management conditions. Previous grassland management studies related to health in the Grand River Grasslands have been conducted on large colonial species such as *Apis mellifera* and *Bombus* species (Smith et al. 2016) but data on smaller, socially variable species are lacking. This region is home to a diverse matrix of agricultural, pastoral, and natural grasslands. Previous research in this region has identified three unique grassland management treatments that compose a significant portion of this landscape matrix (Delaney et al. 2015). These include tallgrass prairie remnants (TGR,) which are defined as areas of native tallgrass prairie cover which were never plowed and converted into agricultural lands during the settlement of Europeans in the area and which are currently managed through regular prescribed burnings,. Un-grazed restorations (UGR) are areas of land that were once plowed in order to use for agricultural production, but have since been cleared, re-seeded with native tallgrass prairie plants, and managed through prescribed burns to restore the original plant community. Cattle-grazed restorations (CGR) are areas of land that use both grazing by cattle and fire as methods of disturbance for managing the native plant community.

In this study, we compare bee and flowering plant community responses to the aforementioned three grassland management treatments found within the Grand River Grasslands Region of Southern Iowa and Northern Missouri. Health of native bees found in each grassland management treatment was also examined. Lipid content and change in lipid content over the growing season have been used as indicators of adult bee nutritional health (Smith et al.

2016). Here, we compare nutritional indicators (lipid mass and relative lipid mass) of three common native bee species in the family Halictidae (sweat bees): *Augochlora pura*, *Agapostemon virescens*, and *Halictus ligatus*. Although phylogenetically closely related, these species differ significantly in terms of sociality. The level of sociality has the potential to affect bee responses to environmental stress, with the general prediction that social species are more “buffered” from environmental change (Wang et al. 2016). Further, due to changes in plant phenology and floral availability over the sampling season, it is worth considering nutritional changes over time in native bee species. *Augochlora pura* is a purely solitary bee, although food and salt availability can lead to very loose aggregations of individuals (Barrows, 1974). This species creates nests in rotting wood, and consists of two to three generations over its flight season, the last of which overwinters in a state of ovarian diapause (Stockhammer 1966, Michener 1974). *Agapostemon virescens* is a ground-nesting bee, and has a mixed or communal social strategy, meaning that numerous females can share a common nest, but there is no division of labor as seen in colonial eusocial species (Roberts 1973, Eickwort 1981). Despite a lack of clear division of labor, individuals have been observed taking on roles guarding a nest while other nest-mates foraged (Abrams and Eickwort 1981). Although literature on *Ag. virescens* is scarce, other members of the genus *Agapostemon* have shown to produce two generations per year with mated females overwintering as adults (Holm, 2017). *Halictus ligatus* is a ground-nesting, primitively eusocial bee, meaning this species will form small colonies with a social and reproductive hierarchy based on aggressive behavior (Richards and Packer 1994). Overwintered females of *H. ligatus* will emerge in early summer and establish a nest, giving rise to a new brood of daughters. This species will generally produce three broods in one year, with the last

brood overwintering as adults (Litte 1977, Holm 2017). Following two years of data collection, we test the following hypotheses:

- i. Tallgrass prairie remnants will support a higher abundance as well as higher species richness and diversity of bees compared to both un-grazed and cattle-grazed restorations.
- ii. Tallgrass prairie remnants will have a higher abundance of floral resources available for the native bee community, as well as higher species richness and diversity compared to both un-grazed and cattle-grazed restorations.
- iii. Nutritional indicators (relative lipid content) will be higher in bees collected from tallgrass prairie remnants compared to those collected from both un-grazed and cattle-grazed restorations.

Methods

Study Sites

A total of 12 field sites were selected within the Grand River Grasslands of Southern Iowa and Northern Missouri (Table 1). Each site was designated as one of three treatments based on their historic land use. These treatments included the following: Tallgrass prairie remnants (TGR) including Ringgold North, Ringgold Southeast, Luisi, and Parsons; Un-grazed restorations (UGR) including Kellerton House, Kellerton Tauke, TNC (The Nature Conservancy) Forb and TNC Cemetery; and Cattle-grazed restorations (CGR) including Kellerton North, Sterner, Lee Trail, and Pyland West.

Specimen Collection: Bee Bowls

Native bee sampling took place over two years, 2015-2016, with each sampling period lasting from late May until early August. Bees were sampled every two weeks within each field

season. Bee specimens were captured using colored pan traps, or “bee bowls.” A 100-meter North-to-South transect which served as the bee-bowl sampling area was established in a location not impeded by fences or topographic barriers within each TGR and UGR site. In CGR sites, this transect was constructed along the outside of the pasture fence accessible by foot for 100 meters, in order to limit negative interactions from cattle. Six 1-meter tall bamboo poles were placed evenly along each 100-meter sampling transect and 3.25 fluid ounce plastic cups, or bee bowls, were attached to the side of each of the bamboo poles using a fastening mechanism. Each bee bowl was colored blue, white, or yellow, colors that fall within the bees’ range of vision. Each transect had two replicates of each bee bowl color. Bee bowls were then filled to the top with a soap and water solution in order to break surface tension and prevent bees from flying out of the traps (Droege et al. 2010, Grundel et al. 2011). Bee bowls were left in the field for a sampling period of 24 hours, after which they were collected, capped, and brought into the lab for specimen extraction. The sampling of native bees only occurred when temperatures were greater than 18 degrees Celsius, and there was no precipitation.

After collection, the contents of each bee bowl were filtered, and excess soap and water solution was discarded. Captured arthropod specimens were examined, and non-bee individuals were removed. Bees were then sterilized using distilled water and a 75% ethanol solution, and air-dried. Each bee was then grouped by their sampling site and capture date, and assigned an identification number for future analysis. Bees were identified to species level, with the exception of individuals belonging to genus *Lasioglossum*, which were identified to sub-genus. Following identification, specimens were frozen until lipid analysis or pinning.

Specimen Collection: Sweep Netting

Sweep net sampling was used to sample bees that generally do not get captured through bee bowl sampling. These include large bees, most commonly belonging in genera *Bombus*, and *Xylocopa* (Roulston et al. 2007). Sweep net sampling events occurred once during the 2015 field season in late July, and twice during the 2016 field season in late June and late July. Sweep net sampling events were not conducted during the first half of June, in order to avoid the capture of emerging *Bombus* queens, and disrupting new colony formation. Sweep net sampling events occurred over a standardized 20-minute sampling period, where an observer would walk throughout a pasture, in a non-specific pattern, seeking out large bees. Bees were captured using a sweep net, and then transferred into site-labelled “kill jars” where specimens were euthanized using diluted ethyl acetate. After capture, bees were immediately brought back to the lab and frozen until identification could occur.

Floral Resource Sampling

Floral resources were sampled in order to provide information about food availability in bee sampling areas. Floral resources were sampled in 2015 and 2016, with sampling events taking place at the same time bee bowl sampling occurred. Floral resource sampling was conducted using a modified Pollard walk technique (Pollard 1977). A 1x100 meter quadrat was constructed on one side of the permanently-established bee-sampling transect. Observers travelled from one end of the transect to the other, counting every flowering ramet, or stem that was located within the sampling quadrat, and identifying them to species level (Moranz et al. 2012, Delaney et al. 2015). Plants that could not be identified in the field were collected, and identified in the lab at a later date.

Lipid Assessment

Bees were selected for lipid assessment based on species availability within each treatment, meaning that a species needed to be abundant in each grassland management treatment in order to be analyzed. Three highly abundant species were selected from among the specimens captured through bee bowl sampling in the 2015 field season, *Augochlora pura*, *Agapostemon virescens*, and *Halictus ligatus*. No species were selected from the 2016 field season due to low numbers of captured specimens. We chose 36 individuals from each species, with each treatment having 12 individuals. Bees were randomly selected from the pool of sampled specimens for lipid analysis. Before lipid extraction, whole bees were weighed using a microbalance in order to provide total mass to calculate relative lipid content following quantification. Lipids were extracted using methods adapted from those described in Toth and Robinson, 2005. Individual bees were submerged in liquid nitrogen, and ground into a coarse powder. Each ground bee was then transferred into a specimen-specific vial, where 5 ml of a 2:1 chloroform: methanol solution was added to each vial to induce lipid extraction. Lipids were extracted at room temperature for a period of 18 hours. Contents of each vial were filtered through glass wool and transferred into a separate sterile specimen-specific vial. Lipid extract solutions were increased to a standardized volume of 6 ml by adding additional 2:1 chloroform: methanol solution. Lipid solution (30 μ l) was transferred to a specimen-specific test tube, where all liquid was evaporated through the use of compressed air flow. Sulfuric acid (200 μ l) was then added to each test tube, and immediately submerged in boiling water for 10-minutes. Following boiling, 2 ml of a vanillin solution was added into each test tube, vortexed and placed away from light for 15 minutes. Each test tube was vortexed once more, and 200 μ l of the liquid contents of each tube were added twice to species-specific 96-well spectrophotometry plates.

Total lipid content was then quantified with a spectrophotometer using a colorimetric assay at 525 nm, where absorbance readings collected from each bee sample were fitted along a standard curve of pure cholesterol (Smith et al. 2016).

Statistical Analysis: Community Data

The goal of statistical analysis was to compare the total abundance, species richness, and diversity of native bees and flowering plants on each of the grassland management treatments over two-years. To calculate abundance, bee specimens collected through bee bowl and sweep net sampling were summed across experimental site and grassland management treatment within both 2015 and 2016. Species richness was defined as the total number of species or members of *Lasioglossum* sub-genera, collected in each site and grassland management treatment within each sampling year. We used the Shannon diversity index to calculate native bee diversity in each year (Smith et al. 2016). Flowering plant abundance was calculated similarly to native bee abundance. The total number of flowering stems counted were summed across experimental site and treatment for both field seasons. Species richness was defined as the total number of flowering plant species identified in each experimental site and each management treatment. The Shannon diversity index was used in order to calculate flowering plant diversity (Smith et al. 2016).

We used the R software package (R 3.3.1) to construct generalized linear mixed effects models (GLMER) assuming a Poisson distribution, using site and year as random effects to test for significant differences in native bee and flowering plant abundances and species richness among TGR, UGR, and CGR treated grasslands. Shannon diversity for each grassland management treatment for native bees and flowering plants was compared using linear mixed effects models (LMER) using site and year as random effects. To compare changes in bee and

floral resource abundance over time, two linear mixed effects models (LMER) were constructed using abundance and sampling day as explanatory variables, and year and site as random effects. All results from GLMER and LMER tests were graphed using the R-package “ggplot2” for visual interpretation. Ordination of community composition of bees and butterflies was conducted through the PC-ORD software package (PC-ORD 6.08) to visualize community relationships in two-dimensional space.

Statistical Analysis: Lipid Data

The goal of statistical analysis of lipid data was to compare relative lipid content for *Augochlora pura*, *Agapostemon virescens*, and *Halictus ligatus* among grassland management treatments. Concentration of lipids within extract solutions was estimated by fitting absorbance readings along a standard curve for pure cholesterol. Estimated concentrations of total lipids within the extract solutions allowed for total lipid mass to be calculated for each bee specimen. Relative lipid content was calculated by dividing lipid mass, by the total mass of each specimen collected prior to lipid extraction.

We used the R software package (R 3.3.1) to construct linear mixed effects models (LMER) using site as a random effect, for each individual species to test for significant differences in relative lipid content among each grassland management treatment. In order to examine the effects of phenology on the nutrition of native bees, linear mixed effects models with site as a random effect were constructed for each individual species to test for significant differences in bee mass, lipid mass, and relative lipid content over all sampling dates. Body size was also examined in order to provide insight as to whether total mass influenced bee lipid content. Another linear mixed effects model was constructed with site as a random effect to test for significant differences in lipid mass relative to total mass of bee specimens.

Visually, the exploratory models appeared as follows:

1. $X \sim \text{Specimen Collection Date} + (1|\text{Experimental Site})$

2. $Y \sim \text{Total Bee Mass} + (1|\text{Experimental Site})$

Within these models, X represents bee mass, total lipid mass or relative lipid content, and Y represents total lipid mass. All results from the constructed linear mixed effects models were graphed using the R-package “ggplot2” for visual interpretation.

Results

Community Data

Over two sampling seasons, a total of 1363 individual bee specimens belonging to 49 species and 3 *Lasioglossum* sub-genera were collected (Supplemental Table 1). Over the two sampling periods, 292 specimens belonging to 26 species were collected from TGR sites, the most common of which was *Augochlora pura*, totaling 28.0% of bees collected. A total of 625 specimens were collected from UGR sites belonging to 41 species, the most common of which was the *Lasioglossum* sub-genus, *Lasioglossum (Dialictus)*, totaling 29.4% of bees collected. A total of 446 specimens belonging to 37 species were collected from CGR sites, the most common of which was again the *Lasioglossum* sub-genus *Lasioglossum (Dialictus)*, totaling 34.8% of bees collected. Bee bowl sampling over two sampling seasons accounted for 1290 collected individuals or 94.6% of total specimens. Sweep net sampling over two sampling seasons accounted for 73 collected individuals or 5.4% of total specimens.

Over two sampling seasons, a total of 45,038 individual flowering ramets belonging to 68 species were counted and identified (Supplemental Table 2). Native plant species consisted of 16,951 flowering ramets belonging to 50 species, comprising 37.6% of the total flowering ramets counted. Exotic plant species consisted of 28,087 flowering ramets belonging to 18 species,

comprising 62.4% of total flowering ramets counted. TGR sites over two years had a total of 9,180 flowering ramets, with native species comprising 79.2% of total floral abundance. UGR sites over two years had a total of 8,278 flowering ramets, with native species comprising 87.3% of total floral abundance. CGR sites over two years had a total of 27,580 flowering ramets, with native species comprising 8.9% of total floral abundance.

Over the two field seasons of 2015 and 2016, the total abundance of bees collected was significantly higher in UGR sites as compared to TGR sites ($z=1.98$, residual $df=19$, $p=0.05$) (Figure 2). However, no significant differences were found in CGR sites compared to TGR sites ($z=1.53$, residual $df=19$, $p=0.13$) or CGR sites compared to UGR sites ($z=-0.46$, residual $df=19$, $p=0.65$). Bee abundance decreased significantly over sampling date ($F=13.21$, $df=1$, 84.3 , $p<0.01$). No significant differences were found in species richness for UGR sites ($z=1.59$, residual $df=19$, $p=0.11$) or CGR sites ($z=1.53$, residual $df=19$, $p=0.13$) compared to TGR sites, or for UGR and CGR sites compared to each other ($z=-0.06$, residual $df=19$, $p=0.96$). Similarly, no significant differences were found in Shannon diversity for UGR sites ($t=1.17$, $df=21$, $p=0.25$) or CGR sites ($t=1.19$, $df=21$, $p=0.25$) compared to TGR sites, or for UGR and CGR sites compared to each other ($t=0.10$, $df=21$, $p=0.99$). Differences in the native bee community composition did not clearly separate out in ordination space by treatment type (Figure 5), but we did find that TGR sites exhibited the highest differentiation, or level of variability in the bee community, among sites.

Over the two field seasons of 2015 and 2016, the total abundance of flowering ramets was significantly higher in CGR sites compared to TGR sites ($z=2.72$, residual $df=19$, $p<0.01$), and UGR sites ($z=2.50$, residual $df=19$, $p=0.01$) (Figure 3). Flowering ramet abundance increased significantly over sampling date ($F=14.77$, $df=1$, 101.48 , $p<0.01$). Species richness

was marginally significantly higher in CGR sites as compared to TGR sites ($z=1.68$, residual $df=19$, $p=0.09$), but not compared to UGR sites ($z=0.29$, residual $df=19$, $p=0.78$) (Figure 3). No significant differences were found in Shannon diversity for UGR sites ($t=1.82$, $df=9$, $p=0.10$) or CGR sites ($t=0.08$, $df=9$, $p=0.94$) compared to TGR sites, or for UGR and CGR sites compared to each other ($t=1.75$, $df=9$, $p=0.12$). Differences in plant community composition, however, can be seen in ordination space between un-grazed and cattle grazed sites (Figure 6). Over two years TGR and UGR sites have formed an overlapping cluster, while CGR sites remained distinct, indicating a unique community composition of floral resources.

When floral abundance was restricted to native flowering plant species commonly used by bees as a source of nectar over the two field seasons of 2015 and 2016, flowering ramet abundance was significantly lower in CGR sites as compared to TGR sites ($z=-2.04$, residual $df=19$, $p=0.04$) and UGR sites ($z=-3.52$, residual $df=19$, $p<0.01$) (Figure 4). Species richness of flowering plants was significantly higher in UGR sites compared to TGR sites ($z=2.02$, residual $df=19$, $p=0.04$) and CGR sites ($z=-3.54$, residual $df=19$, $p<0.01$) (Figure 4). Shannon diversity of flowering plants was marginally significantly lower in CGR sites compared to UGR sites ($t=-1.93$, $df=9$, $p=0.08$) (Figure 4).

Nutritional Indicators

A total of 108 bees were used for the lipid extraction process, with each species having 36 individuals consisting of 12 individuals from each treatment. We focused on analyzing lipid content relative to body mass. No significant differences were found when comparing relative lipid content among the three grassland management for any of the tested species of bee (Figure 7). Additionally, no significant differences were found when comparing total lipid mass and total bee mass among grassland management treatment for any of the tested species of bee.

Although not significant, *Augochlora pura* and *Agapostemon virescens* found in un-grazed restorations had the highest average levels of relative lipid content. Individuals of *H. ligatus* collected from TGR sites had generally higher relative lipid content compared to those collected from the other two treatments (Figure 7).

Sampling date was used to explore changes in bee mass, lipid mass, and relative lipid content over time. A significant negative correlation between bee mass and sampling date was shown for *Au. pura* ($F=5.57$, $df=1$, 32.72 , $p=0.02$), and *H. ligatus* ($F=7.02$, $df=1$, 34 , $p=0.01$) (Figure 8). *Halictus ligatus* showed a significant positive correlation between lipid mass and sampling date ($F=4.98$, $df=1$, 34 , $p=0.03$) (Figure 9). A significant positive correlation was shown between relative lipid content and sampling date for *H. ligatus* ($F=9.40$, $df=1$, 34 , $p<0.01$) (Figure 10). No significant correlation was shown between relative lipid content and sampling date for *Au. pura* ($F=2.53$, $df=1$, 32.56 , $p=0.12$) or *Ag. virescens* ($F=1.02$, $df=1$, 34 , $p=0.16$).

Bee mass was used to examine changes in lipid mass over varying body sizes, a result of larval nutrition. *Agapostemon virescens* showed a significant positive correlation between lipid mass and bee mass ($F=7.44$, $df=1$, 34 , $p=0.01$) (Figure 11). No significant correlation between lipid mass and bee mass was shown for *Au. pura* ($F<0.01$, $df=1$, 34 , $p=0.97$) or *H. ligatus* ($F=2.15$, $df=1$, 34 , $p=0.15$). When the correlation between relative lipid content and bee mass was plotted, negative trends were seen in *Au. pura* and *H. ligatus*, while *Ag. virescens* showed a positive trend (Figure 12).

Discussion

Bee Community Trends

The results of this study indicate that the application of different grassland management strategies can have an effect on the surrounding native plant and bee community. Despite our

original hypothesis, native bees were more abundant in un-grazed restorations than tallgrass prairie remnants, and although not significant, cattle-grazed restorations also had a higher average number of bees collected than the remnants. However, these findings are similar to community data of other insects, namely beetles, having greater abundances in restorations rather than remnant prairies (Larsen and Work 2003). Ultimately, it is unknown what is causing lower bee abundance in TGR sites, further analysis is needed to identify potential site-related variables that could be driving this trend. We also predicted that bee species richness and Shannon diversity would be higher in tallgrass prairie remnants compared to un-grazed and cattle-grazed restorations, however this was not the case, as no significant differences were found among any of the grassland management treatments. These findings are a departure from previous insect community studies within tallgrass prairies, where tallgrass prairie remnants generally contained the highest number of species and the greatest level of diversity (Bomar 2001, Nemec and Bragg 2008). Interestingly, studies of butterflies, another pollinator, also show greater numbers of species and diversity in tallgrass prairie remnants rather than restorations (Shepherd and Debinski 2005). However, community trends of butterflies are not always good predictors of native bee community responses (Davis et al. 2007).

Flowering Plant Community Trends

Results of the flowering plant community analysis followed a different pattern than the bee community. In contrast to our original hypothesis, cattle-grazed restorations had significantly higher abundances of flowering ramets compared to both tallgrass prairie remnants as well as un-grazed restorations. The abundance of non-native species has the tendency to make CGR sites “novel grasslands” (Wilsey et al. 2011). Within novel grasslands, aboveground biomass is primarily composed of exotic species (Wilsey et al. 2009), potentially giving rise to

an increased total abundance of flowering ramets. Again, contrary to our predictions, TGR sites had the lowest number of flowering species counted whereas CGR sites had the highest. An aspect of cattle-grazed restorations within the Grand River Grasslands which sets them apart from both tallgrass prairie remnants and un-grazed restorations is the addition of exotic floral resources in combination with existing areas of native flowering plant species, potentially explaining the high level of species richness. We hypothesized that Shannon diversity would be higher in tallgrass prairie remnants compared to un-grazed and cattle-grazed restorations, but our results do not support this trend. UGR sites, although not significantly, had higher levels of diversity compared to TGR and CGR sites, departing from previous literature (Polley et al.). A reason for this increased diversity could be related to the re-seeding techniques involved in the restoration of UGR sites which are not present in TGR or CGR sites.

We compared community data from species of flowering plant that are commonly used by bees as a source of nectar (Holm 2017), illustrating the relationships between the native plant and bee communities. Native flowering plant abundance was significantly lower in CGR sites as compared to TGR and UGR sites. However, TGR sites, while non-significant do have lower average levels of bee-visited flowering plant abundance. This follows a similar trend to the bee abundance results, showing that high nectar plant abundance can result in high bee abundance. Species richness of bee-visited flowering plants showed similar results. UGR sites had the highest number of bee-visiting flowering plants, being significantly higher than both TGR and CGR sites. This illustrates that areas high in the number of flowering plant species used by bees, can be a predictor for high levels of bee abundance. This illustrates why CGR sites, with higher total floral resources, had lower amounts of bees compared to UGR sites.

Bee Nutritional Indicators

Despite our original hypothesis, no significant differences were found in nutritional indicators for any of the tested species, indicating that grassland management treatment does not have a large effect on one aspect of the nutritional health of the native bee community. However, *H. ligatus* showed non-significantly higher relative lipid content in TGR sites. Potential reasons for this trend could include both food availability and climate. *Halictus ligatus* has a shifting level of eusociality based on environmental conditions which could lead to changing resource partitioning strategies within individual colonies (Richards and Packer 1996) possibly giving rise to individuals with higher lipid content, potentially more resistant to environmental stress. Floral abundance and species richness is significantly lower in TGR sites compared to CGR sites, and average diversity is lower compared to UGR sites, which could provide enough stress to colonies located in TGR sites to shift resource partitioning priorities. However, it is important to note that we did not examine other aspects of nutritional health such as protein content or micronutrients, which could differ based on floral resource differences between treatments.

Although no significant differences were found in total nutritional indicators across treatments, an exploratory study comparing each grassland management treatment across sampling date and bee mass did reveal some interesting relationships. A significant positive relationship was observed when comparing lipid mass to bee mass in *Ag. virescens*, meaning that as the average mass of captured *Ag. virescens* increased, so did the average lipid mass. This correlation could be driven by the life history of this species. Since bee body mass is determined by larval nutrition (Smith et al. 2016), individuals that were well nourished as larvae are more likely to have higher levels of lipids as adults, possibly due to a competitive edge resulting from a large body size. A negative correlation between relative lipid content and bee mass is shown

by *Au. pura* and *H. ligatus*. This correlation is consistent with trends identified in previous studies describing relative lipid content over variations in mass in *Bombus impatiens* (Couvillon et al. 2011).

Examining trends in bee mass, lipid mass, and relative lipid content over time also provided insight into the chronology of bee nutrition. We examined bee mass throughout the sampling period to determine changes in body size over time, and found that *Au. pura* and *H. ligatus* showed significant negative correlations between mass and time, meaning the average size of bees is generally decreasing over the sampling season. This is most likely due to a decrease in large, overwintered individuals that were active primarily in the early part of the sampling season, along with an increase of smaller and younger bees (Stockhammer 1966, Litte 1977). We found significantly positive-rates of change in relative lipid content over time in *H. ligatus*, a departure from previous studies that indicated both decreases and stagnations in relative lipid content over time in bees (O’Niell et al. 2015). The change in relative lipid content over time can potentially be explained through decreasing body mass and increasing lipid mass in *H. ligatus*, due to overwintering preparation.

Implications and Future Directions for Research

This study provided a solid foundation for describing the community composition and health of native bees within a grassland matrix in the tallgrass prairie ecoregion. Overall, native bees responded to different grassland management practices in different ways, with significantly higher numbers being found in un-grazed restorations compared to tallgrass prairie remnants. The flowering plant resources available to pollinators are driven by large numbers of exotic flowering ramets, leading the novel cattle-grazed pastures to have significantly higher levels of floral abundance and species richness. However, when examining only plants used by native

bees, we see that un-grazed restorations have the highest level of available resources, in terms of total flowering ramets and total flowering plant species and un-grazed restorations also have the highest abundance of native bees. Pollinator health and nutrition cannot be viewed through a one size fits all lens. Nutritional indicators such as lipid content in bees need to be evaluated over different scales. Using total bee mass as one such scale stressed a positive correlation between total bee mass and lipid content for *Agapostemon virescens*. Time also provides an important dimension for assessing the nutritional status of bees. Our results show that for some species, bee mass, lipid mass, and relative lipid content increased over time within the summer season. Thus, without providing temporal or body size context, nutritional analysis can be inconclusive.

In the future, additional continuous environmental and climatic variables could be incorporated into the analysis of both community and nutritional data. Average levels of floral abundance, species richness, and diversity during sampling events could provide further insight into the shifting levels of relative lipid content in captured specimens. Similarly, temporal analysis could also be applied to assess the seasonal succession of bee species over sampling periods. Finally, within the Grand River Grasslands, butterfly community composition is well documented (Debinski et al. 2011, Moranz et al. 2012) however butterfly nutrition has yet to be studied. Conducting lipid analyses on butterflies would allow a comparison of responses between different pollinator groups. Grassland management treatments could also be examined to determine even finer dimensions of the effects of management on vegetation structure and composition and the effects of vegetation structure and composition on pollinator communities. Factors such as the age of grassland restoration (Tonietto et al. 2016) and grazing intensity (Kruess and Tscharnke 2002) have been explored, and could be expanded upon using similar models to those described in this study. Additional bee data could be collected and functional

groups related to nesting, feeding, and social strategy could be analyzed in place of species for both community composition and lipid analysis. Native bees are a very diverse group of organisms, and thus the research opportunities involving this group of insects are just as varied.

Acknowledgements

This research was supported by Iowa Agricultural Experiment Station project IOW05392 and through funding provided by the Iowa State Wildlife Competitive Grants Program in Cooperation with the U.S. Fish and Wildlife Service (State Wildlife Grant SWG-C #14CRDWBKReed-0011). Thank you to Logan Crees, Ray Moranz, Emma Stivers, Nick Lyon, Josh Rusk, Scott Nelson, Jaime Coon, Hilary Haley, and Randy Arndt for their assistance during field seasons. Additional thanks to Miranda Salsbery, Anna Broderick, Ashley St. Clair, Ed Hsieh, and Katie Rey for their assistance in lab and statistical analysis. A final thank you to the Iowa Department of Natural Resources and The Nature Conservancy for collaborating on the management of these study sites.

References

- Abrams, Judith, and George C. Eickwort. "Nest Switching and Guarding by the Communal Sweat Bee *Agapostemon Virescens* (Hymenoptera, Halictidae)." *Insectes Sociaux* 28.2 (1981): 105-16.
- Barrows, Edward M. "Aggregation Behavior and Response to Sodium Chloride in Females of a Solitary Bee, *Augochlora Pura* (Hymenoptera: Halictidae)." *The Florida Entomologist* 57.2 (1974): 189.
- Batra, S. W.t. "Bees and Pollination in Our Changing Environment." *Apidologie* 26.5 (1995): 361-70.
- Bomar, Charles R. "Comparison of Grasshopper (Orthoptera: Acrididae) Communities on Remnant and Reconstructed Prairies in Western Wisconsin." *Journal of Orthoptera Research* 10.1 (2001): 105-12.
- Brown, Mark J.f., and Robert J. Paxton. "The Conservation of Bees: A Global Perspective." *Apidologie* 40.3 (2009): 410-16.
- Cameron, S. A., J. D. Lozier, J. P. Strange, J.B. Koch, N. Cordes, L. F. Solter, and T. L. Griswold. "Patterns of Widespread Decline in North American Bumble Bees." *Proceedings of the National Academy of Sciences* 108.2 (2011): 662-67.
- Couvillon, Margaret J., Jennifer M. Jandt, Jennifer Bonds, Bryan R. Helm, and Anna Dornhaus. "Percent Lipid Is Associated with Body Size but Not Task in the Bumble Bee *Bombus Impatiens*." *Journal of Comparative Physiology A* 197.11 (2011): 1097-104.
- Davis, Jessica D., Stephen D. Hendrix, Diane M. Debinski, and Chiara J. Hemsley. "Butterfly, Bee and Forb Community Composition and Cross-taxon Incongruence in Tallgrass Prairie Fragments." *Journal of Insect Conservation* 12.1 (2007): 69-79.
- Debinski, Diane M., Raymond A. Moranz, John T. Delaney, James R. Miller, David M. Engle, Laura B. Winkler, Devan A. Mcgranahan, Robert J. Barney, James C. Trager, Andrew L. Stephenson, and Molly K. Gillespie. "A Cross-taxonomic Comparison of Insect Responses to Grassland Management and Land-use Legacies." *Ecosphere* 2.12 (2011).
- Delaney, J.T., "Utilizing novel grasslands for the conservation and restoration of butterflies and other pollinators in agricultural ecosystems" (2014). Graduate Theses and Dissertations. Paper 14097.
- Delaney, J. T., K. J. Jokela, and D. M. Debinski. "Seasonal Succession of Pollinator Floral Resources in Four Types of Grasslands." *Ecosphere* 6.11 (2015).

- Droege, Sam, Vincent J. Tepedino, Gretchen Lebuhn, William Link, Robert L. Minckley, Qian Chen, and Casey Conrad. "Spatial Patterns of Bee Captures in North American Bowl Trapping Surveys." *Insect Conservation and Diversity* 3.1 (2010): 15-23.
- Eickwort, George C. "Aspects of the Nesting Biology of Five Nearctic Species of *Agapostemon* (Hymenoptera: Halictidae)." *Journal of the Kansas Entomological Society*, vol. 54, no. 2, 1981, pp. 337–351.
- Fürst, M. A., D. P. McMahon, J. L. Osborne, R. J. Paxton, and M. J. F. Brown. "Disease Associations between Honeybees and Bumblebees as a Threat to Wild Pollinators." *Nature* 506.7488 (2014): 364-66.
- Goulson, Dave. "An Overview of the Environmental Risks Posed by Neonicotinoid Insecticides." *Journal of Applied Ecology* 50.4 (2013): 977-87.
- Grundel, Ralph, Krystalynn J. Frohnapple, Robert P. Jean, and Noel B. Pavlovic. "Effectiveness of Bowl Trapping and Netting for Inventory of a Bee Community." *Environmental Entomology* 40.2 (2011): 374-80.
- Hines, Heather M., and Stephen D. Hendrix. "Bumble Bee (Hymenoptera: Apidae) Diversity and Abundance in Tallgrass Prairie Patches: Effects of Local and Landscape Floral Resources." *Environmental Entomology* 34.6 (2005): 1477-484.
- Holm, Heather. *Bees: An Identification and Native Plant Forage Guide*. Minnetonka, MN: Pollination, 2017.
- Jauker, Frank, Tim Diekötter, Franziska Schwarzbach, and Volkmar Wolters. "Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat." *Landscape Ecology* 24.4 (2009): 547-55.
- Kennedy, CM; Lonsdorf, E; Neel, MC; Williams, NM; Ricketts, TH; Winfree, R; et al..(2013). A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology Letters*, 16(5), 584-599.
- Kremen, C., N. M. Williams, and R. W. Thorp. "Crop Pollination from Native Bees at Risk from Agricultural Intensification." *Proceedings of the National Academy of Sciences* 99.26 (2002): 16812-6816.
- Kruess, Andreas, and Teja Tscharntke. "Grazing Intensity and the Diversity of Grasshoppers, Butterflies, and Trap-Nesting Bees and Wasps." *Conservation Biology* 16.6 (2002): 1570-580.
- Larsen, Kirk J., and Timothy W. Work. "Differences in Ground Beetles (Coleoptera: Carabidae) of Original and Reconstructed Tallgrass Prairies in Northeastern Iowa, USA, and Impact of 3-year Spring Burn Cycles." *Journal of Insect Conservation* 7.3 (2003): 153-66.

- Litte, Marcia. "Aspects of the Social Biology of the Bee *Halictus Ligatus* in New York State (Hymenoptera, Halictidae)." *Insectes Sociaux* 24.1 (1977): 9-36.
- Morandin, Lora A., and Claire Kremen. "Bee Preference for Native versus Exotic Plants in Restored Agricultural Hedgerows." *Restoration Ecology* 21.1 (2012): 26-32.
- Michener, Charles D. *The Social Behavior of the Bees: A Comparative Study*. Cambridge, MA: Belknap of Harvard UP, 1974.
- Moranz, Raymond A., Diane M. Debinski, Devan A. Mcgranahan, David M. Engle, and James R. Miller. "Untangling the Effects of Fire, Grazing, and Land-use Legacies on Grassland Butterfly Communities." *Biodiversity and Conservation* 21.11 (2012): 2719-746.
- Nemec, Kristine T., and Thomas B. Bragg. "Plant-Feeding Hemiptera and Orthoptera Communities in Native and Restored Mesic Tallgrass Prairies." *Restoration Ecology* 16.2 (2008): 324-35.
- O'Neill, Kevin M., Casey M. Delphia, and Theresa L. Pitts-Singer. "Seasonal Trends in the Condition of Nesting Females of a Solitary Bee: Wing Wear, Lipid Content, and Oocyte Size." *PeerJ* 3 (2015).
- Otti, Oliver, and Paul Schmid-Hempel. "Nosema Bombi: A Pollinator Parasite with Detrimental Fitness Effects." *Journal of Invertebrate Pathology* 96.2 (2007): 118-24.
- Potts, Simon G., Betsy Vulliamy, Amots Dafni, Gidi Ne'eman, and Pat Willmer. "Linking Bees And Flowers: How Do Floral Communities Structure Pollinator Communities?" *Ecology* 84.10 (2003): 2628-642.
- Potts, S., Biesmeijer, J., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W. 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*. 25:345–353.
- Pollard, E. "A Method for Assessing Changes in the Abundance of Butterflies." *Biological Conservation* 12.2 (1977): 115-34.
- Richards, Miriam H., and Laurence Packer. "Trophic Aspects of Caste Determination in *Halictus Ligatus*, a Primitively Eusocial Sweat Bee." *Behavioral Ecology and Sociobiology* 34.6 (1994): 385-91.
- Richards, Miriam H., and Laurence Packer. "The Socioecology of Body Size Variation in the Primitively Eusocial Sweat Bee, *Halictus Ligatus* (Hymenoptera: Halictidae)." *Oikos* 77.1 (1996): 68.
- Roberts, R. B. *Bees of Northwestern America: Agapostemon* (Hymenoptera: Halictidae). 1973.

- Roulston, T'ai H., Stephen A. Smith, and Amanda L. Brewster. "A Comparison of Pan Trap and Intensive Net Sampling Techniques for Documenting a Bee (Hymenoptera: Apiformes) Fauna." *Journal of the Kansas Entomological Society* 80.2 (2007): 179-81.
- Roulston, T'ai H., and Karen Goodell. "The Role of Resources and Risks in Regulating Wild Bee Populations." *Annual Review of Entomology* 56.1 (2011): 293-312.
- Russo, Laura, Nelson Debarros, Suann Yang, Katriona Shea, and David Mortensen. "Supporting Crop Pollinators with Floral Resources: Network-based Phenological Matching." *Ecology and Evolution* 3.9 (2013): 3125-140.
- Samson, Fred, and Fritz Knopf. "Prairie Conservation in North America." *BioScience* 44.6 (1994): 418-21.
- Shepherd, Stephanie, and Diane M. Debinski. "Evaluation of Isolated and Integrated Prairie Reconstructions as Habitat for Prairie Butterflies." *Biological Conservation* 126.1 (2005): 51-61.
- Smith, Griffin W., Diane M. Debinski, Nicole A. Scavo, Corey J. Lange, John T. Delaney, Raymond A. Moranz, James R. Miller, David M. Engle, and Amy L. Toth. "Bee Abundance and Nutritional Status in Relation to Grassland Management Practices in an Agricultural Landscape." *Environmental Entomology* 45.2 (2016): 338-47.
- Stockhammer, Karl A. "Nesting Habits and Life Cycle of a Sweat Bee, *Augochlora Pura* (Hymenoptera: Halictidae)." *Journal of the Kansas Entomological Society* 39.2 (1966): 157-92.
- Tonietto, Rebecca K., John S. Ascher, and Daniel J. Larkin. "Bee Communities along a Prairie Restoration Chronosequence: Similar Abundance and Diversity, Distinct Composition." *Ecological Applications* (2016): 705-17.
- Toth, A. L. "Nutritional status influences socially regulated foraging ontogeny in honey bees." *Journal of Experimental Biology* 208.24 (2005): 4641-649.
- Vanbergen, Adam J., The Insect Pollinators Initiative. "Threats to an Ecosystem Service: Pressures on Pollinators." *Frontiers in Ecology and the Environment* 11.5 (2013): 251-59.
- Wang Y, Kaftanoglu O, Brent CS, Page RE, Amdam GV. Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (*Apis mellifera* L.). *Journal of Experimental Biology*. 2016 Apr 1; 219(7): 949-59.
- Whitehorn, P. R., S. O'connor, F. L. Wackers, and D. Goulson. "Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production." *Science* 336.6079 (2012): 351-52.

- Winfree, R. 2010. The Conservation and Restoration of Wild Bees. *Annals of NY Academy of Sciences*. 1195:169-197.
- Winfree, Rachael, Neal M. Williams, Jonathan Dushoff, and Claire Kremen. "Native Bees Provide Insurance against Ongoing Honey Bee Losses." *Ecology Letters* 10.11 (2007): 1105-1113.
- Wright, C. K., and M. C. Wimberly. "Recent Land Use Change in the Western Corn Belt Threatens Grasslands and Wetlands." *Proceedings of the National Academy of Sciences* 110.10 (2013): 4134-139.
- Wilsey, Brian J., Terri B. Teaschner, Pedram P. Daneshgar, Forest I. Isbell, and H. Wayne Polley. "Biodiversity Maintenance Mechanisms Differ between Native and Novel Exotic-dominated Communities." *Ecology Letters* 12.5 (2009): 432-42.
- Wilsey, Brian J., Pedram P. Daneshgar, and H. Wayne Polley. "Biodiversity, Phenology and Temporal Niche Differences between Native- and Novel Exotic-dominated Grasslands." *Perspectives in Plant Ecology, Evolution and Systematics* 13.4 (2011): 265-76.

Figures and Tables

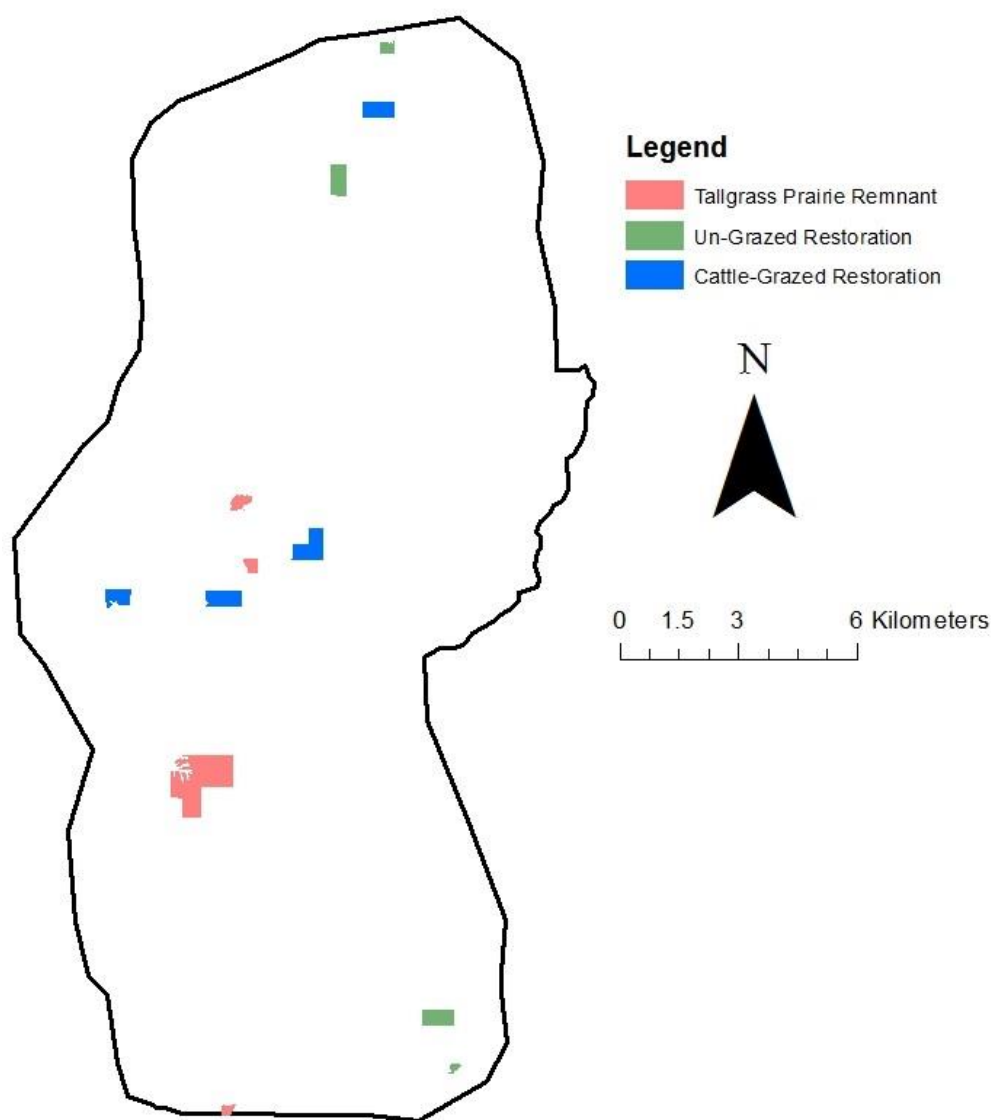


Figure 1: Experimental sites used for floral resource sampling and bee specimen collection. Black boundary denotes the area of the Grand River Grasslands Region of Southern Iowa and Northern Missouri.

Table 1: Experimental sites used for bee specimen collection and floral resource sampling.

Experimental Site	Treatment	Treatment Code	Location	Area (ha)
Luisi	Tallgrass Prairie Remnant	TGR	Harrison Co. MO	156
Parsons	Tallgrass Prairie Remnant	TGR	Harrison Co. MO	6.80
Ringgold North	Tallgrass Prairie Remnant	TGR	Ringgold Co. IA	17.8
Ringgold Southeast	Tallgrass Prairie Remnant	TGR	Ringgold Co. IA	12.0
Kellerton House	Un-Grazed Restoration	UGR	Ringgold Co. IA	11.8
Kellerton Tauke	Un-Grazed Restoration	UGR	Ringgold Co. IA	31.6
TNC Cemetery	Un-Grazed Restoration	UGR	Harrison Co. MO	3.60
TNC Forb	Un-Grazed Restoration	UGR	Harrison Co. MO	32.7
Kellerton North	Cattle-Grazed Restoration	CGR	Ringgold Co. IA	32.0
Lee Trail Road	Cattle-Grazed Restoration	CGR	Ringgold Co. IA	31.0
Pyland North	Cattle-Grazed Restoration	CGR	Ringgold Co. IA	32.0
Sterner	Cattle-Grazed Restoration	CGR	Ringgold Co. IA	46.9

Treatment code indicates the type of grassland management treatment implemented on each site. TGR indicates tallgrass prairie remnants, UGR indicates un-grazed restorations and CGR indicates cattle-grazed restorations.

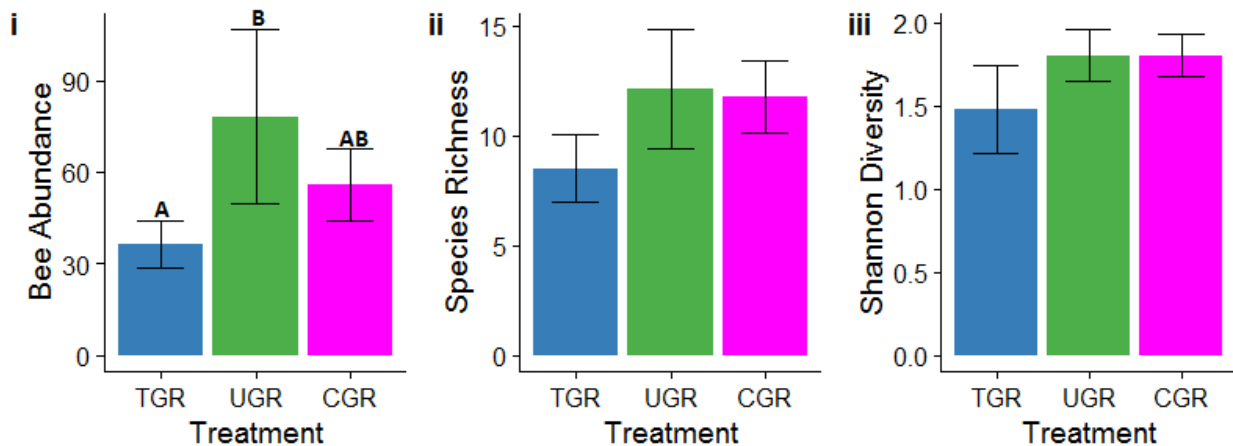


Figure 2: Measures of i) average bee abundance, ii) average bee species richness, and iii) bee shannon diversity among three grassland management treatments. TGR indicates tallgrass prairie remnants, UGR indicates un-grazed restorations, and CGR indicates cattle-grazed restorations. Different letters indicate statistically significant differences at the $P < 0.05$ level. Bars indicate standard error.

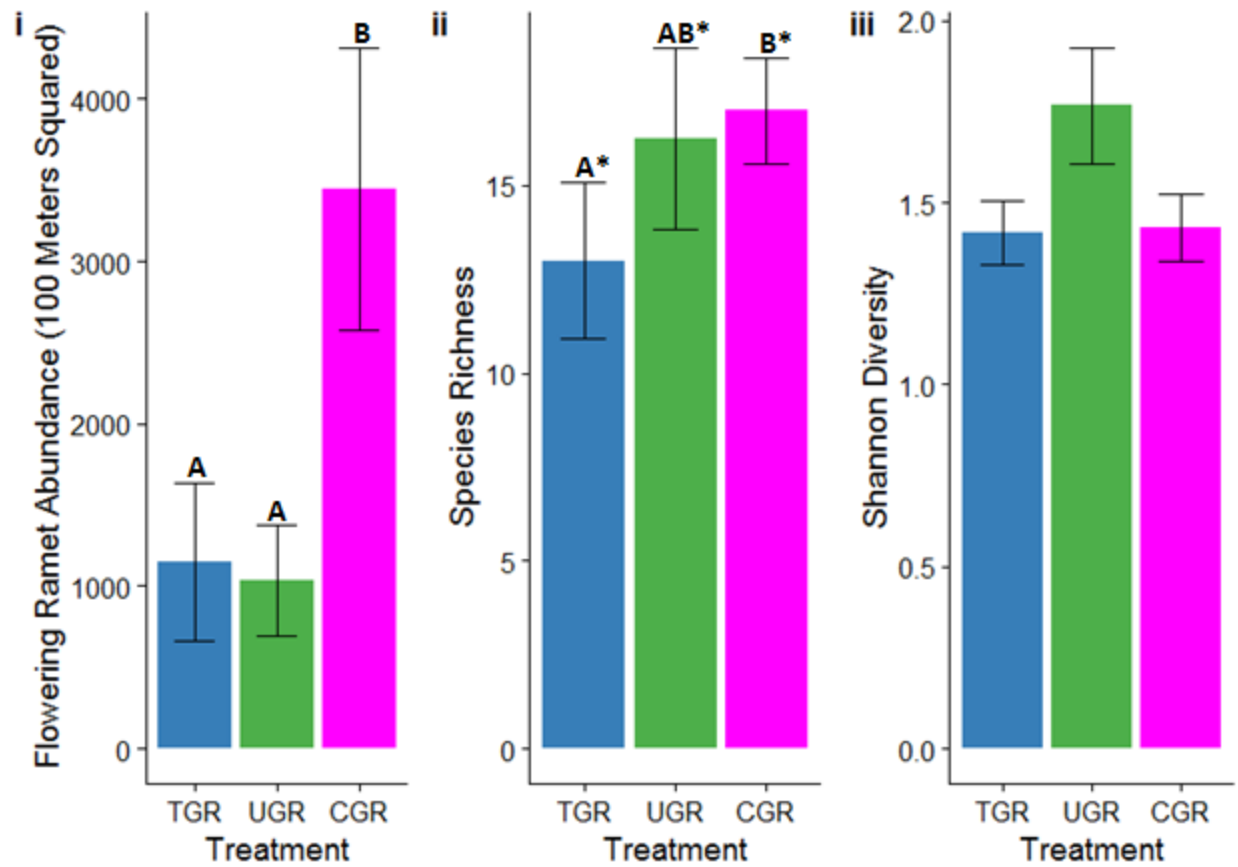


Figure 3: Measures of i) average flowering ramet abundance, ii) average flowering plant species richness, and iii) flowering plant shannon diversity among three grassland management treatments. TGR indicates tallgrass prairie remnants, UGR indicates un-grazed restorations, and CGR indicates cattle-grazed restorations. Different letters indicate statistically significant differences at the $P < 0.05$ level, letters with asterisks indicate marginal significant differences at $0.05 < P < 0.1$ level. Bars indicate standard error.

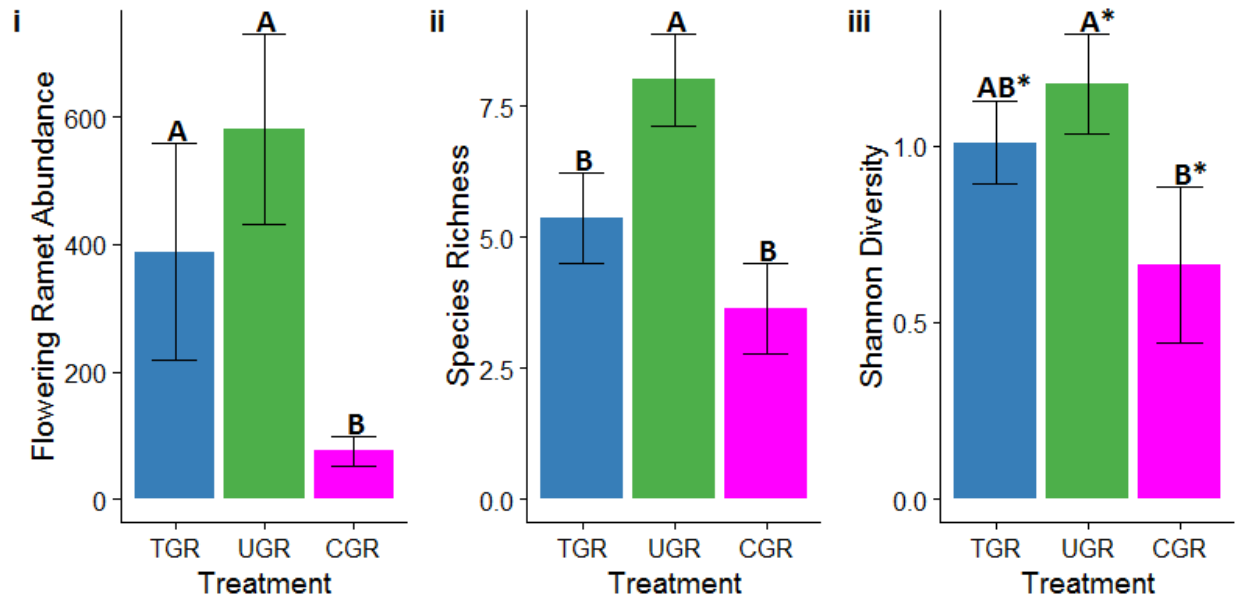


Figure 4: Measures of i) average bee-visited flowering ramet abundance, ii) average bee-visited flowering plant species richness, and iii) bee-visited flowering plant shannon diversity among three grassland management treatments. TGR indicates tallgrass prairie remnants, UGR indicates un-grazed restorations, and CGR indicates cattle-grazed restorations. Different letters indicate statistically significant differences at the $P<0.05$ level, letters with asterisks indicate marginal significant differences at $0.05<P<0.1$ level. Bars indicate standard error.

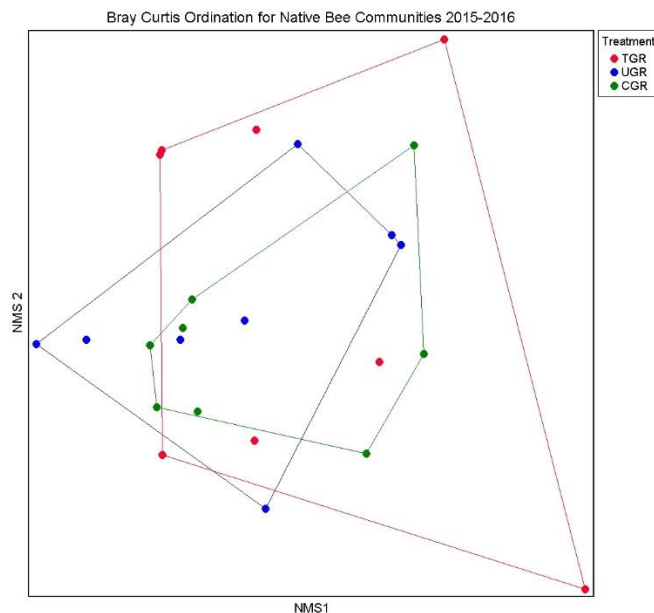


Figure 5: Bray-Curtis ordination of floral resources among three grassland management treatments. TGR indicates tallgrass prairie remnants, UGR indicates un-grazed restorations, and CGR indicates cattle-grazed restorations.

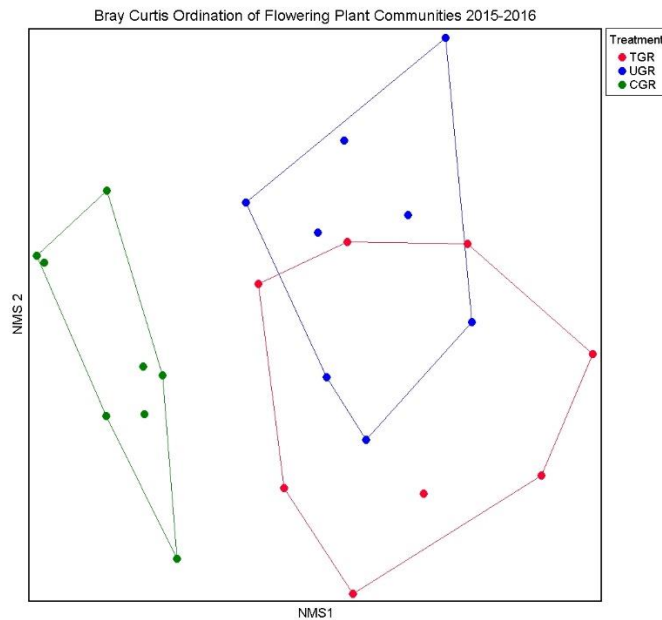


Figure 6: Bray-Curtis ordination of floral resources among three grassland management treatments. TGR indicates tallgrass prairie remnants, UGR indicates un-grazed restorations, and CGR indicates cattle-grazed restorations.

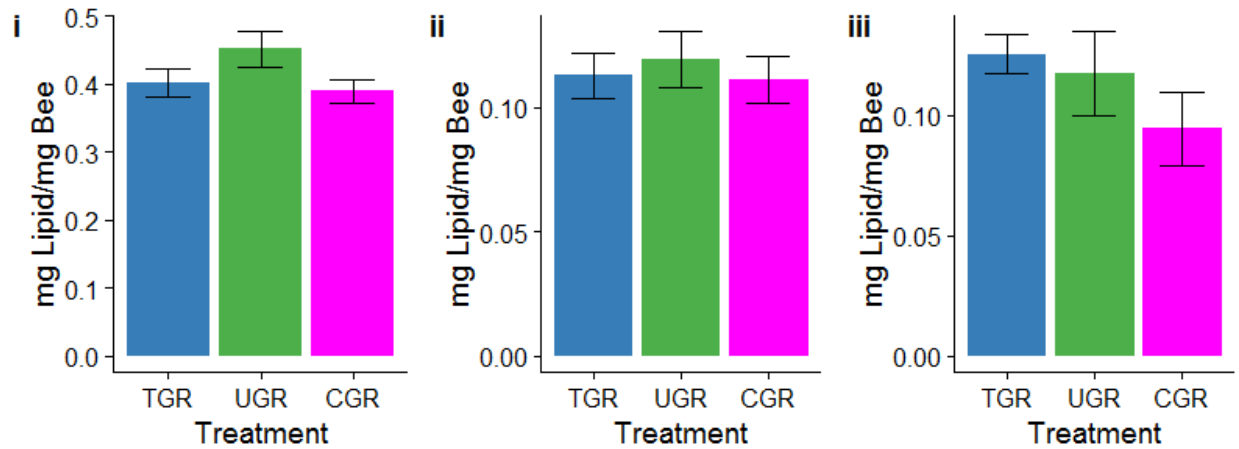


Figure 7: Relative lipid content in i) *Augochlora pura*, ii) *Agapostemon virescens*, and iii) *Halictus ligatus* among three grassland management treatments. TGR indicates tallgrass prairie remnants, UGR indicates un-grazed restorations, and CGR indicates cattle-grazed restorations. No significant differences were found among the treatments. Bars indicate standard error.

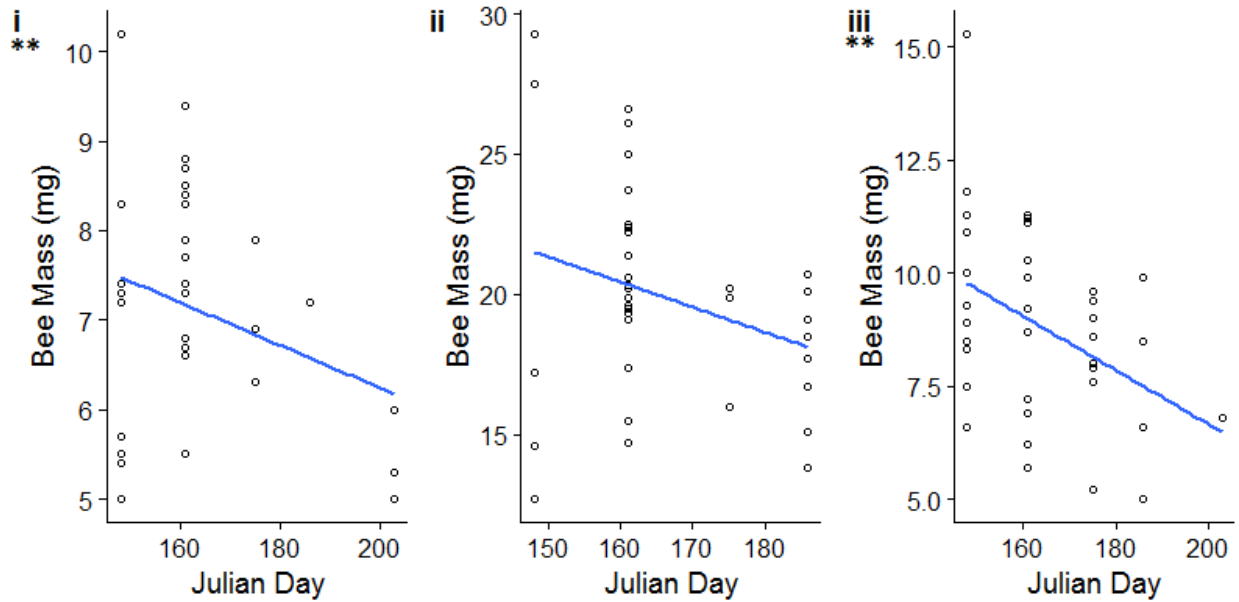


Figure 8: Correlations between bee mass and Julian Day in i) *Augochlora pura*, ii) *Agapostemon virescens*, and iii) *Halictus ligatus*. Asterisks indicate significance where (**) indicates significance at the $P < 0.05$ level.

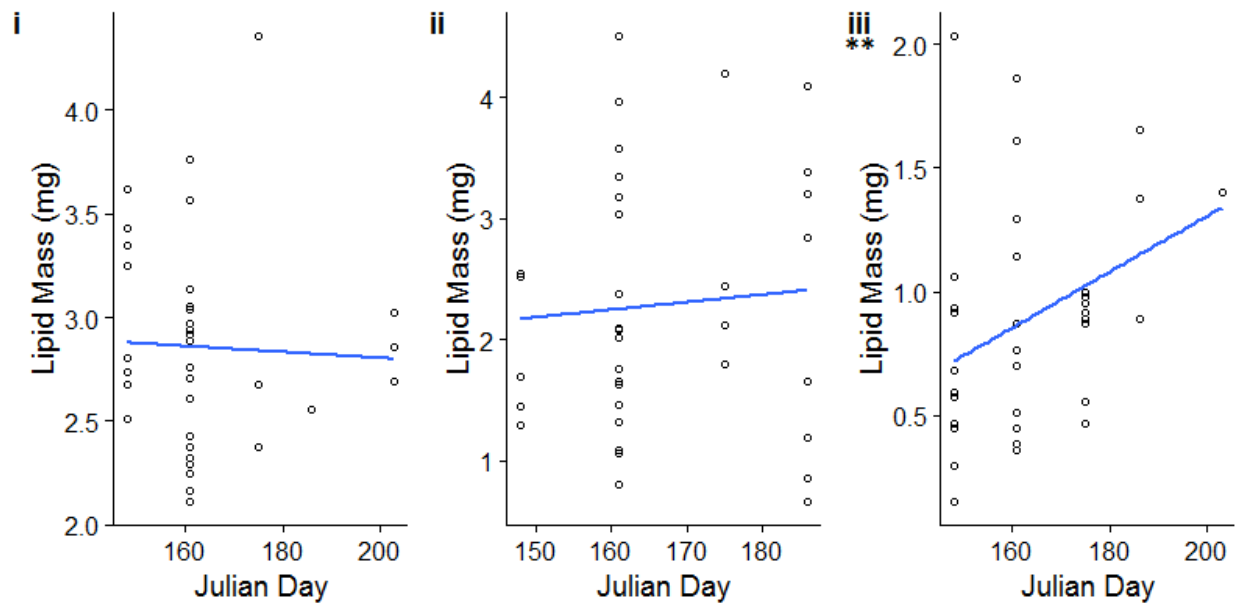


Figure 9: Correlations between lipid mass and Julian Day in i) *Augochlora pura*, ii) *Agapostemon virescens*, and iii) *Halictus ligatus*. Asterisks indicate significance where (**) indicates significance at the $P < 0.05$ level.

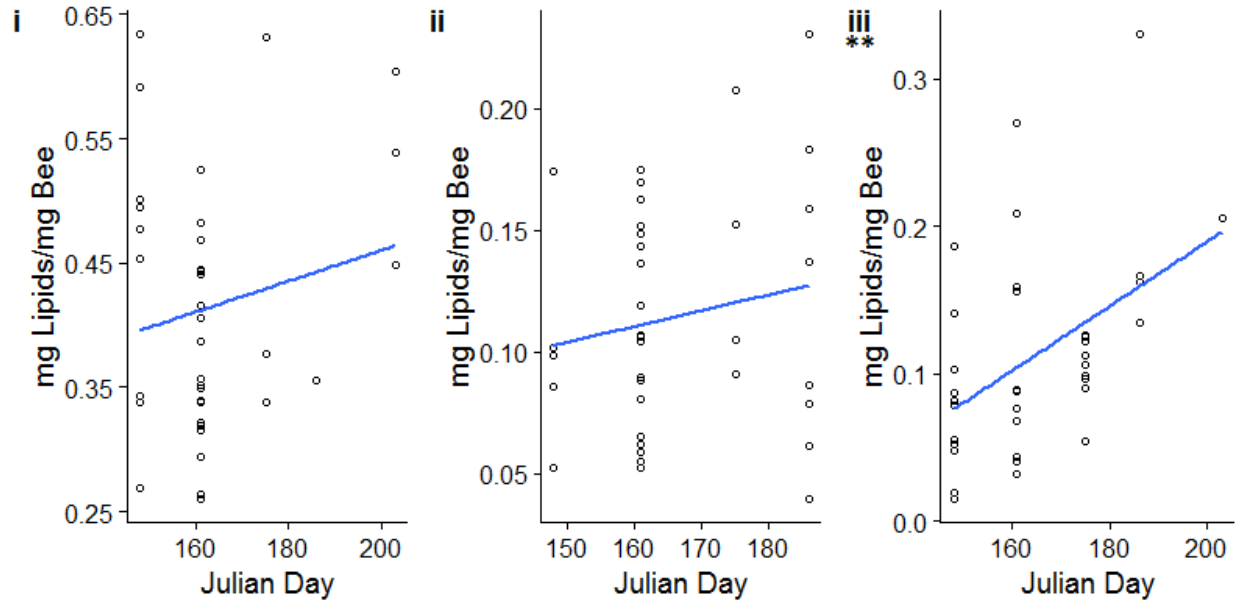


Figure 10: Correlations between relative lipid content and Julian Day in i) *Augochlora pura*, ii) *Agapostemon virescens*, and iii) *Halictus ligatus*. Asterisks indicate significance where (**) indicates significance at the $P < 0.05$ level.

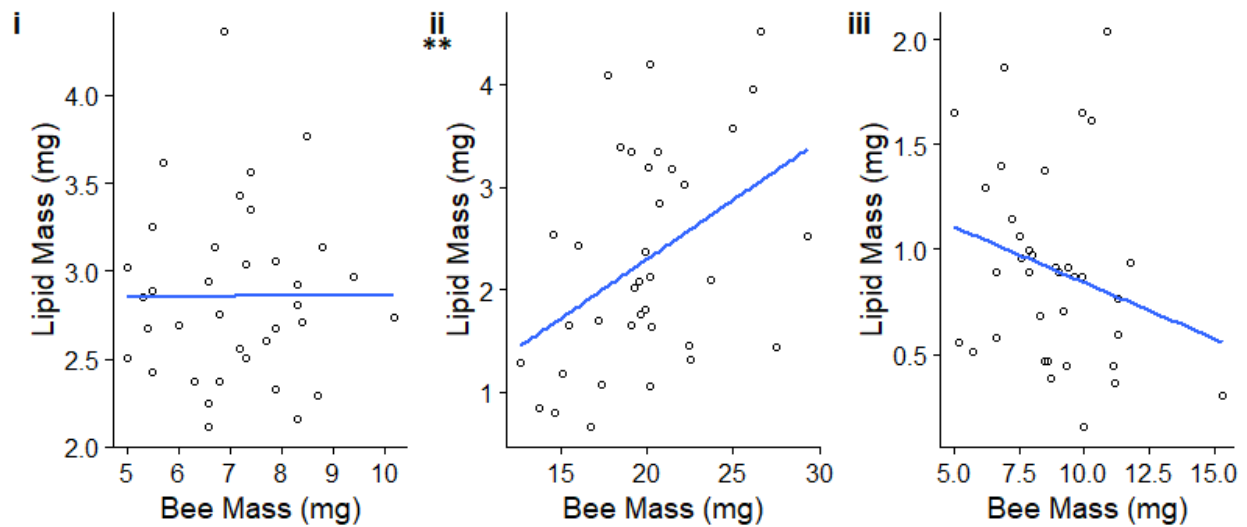


Figure 11: Correlations between lipid mass and total bee mass in i) *Augochlora pura*, ii) *Agapostemon virescens*, and iii) *Halictus ligatus*. Asterisks indicate significance where (**) indicates significance at the $P < 0.05$ level.

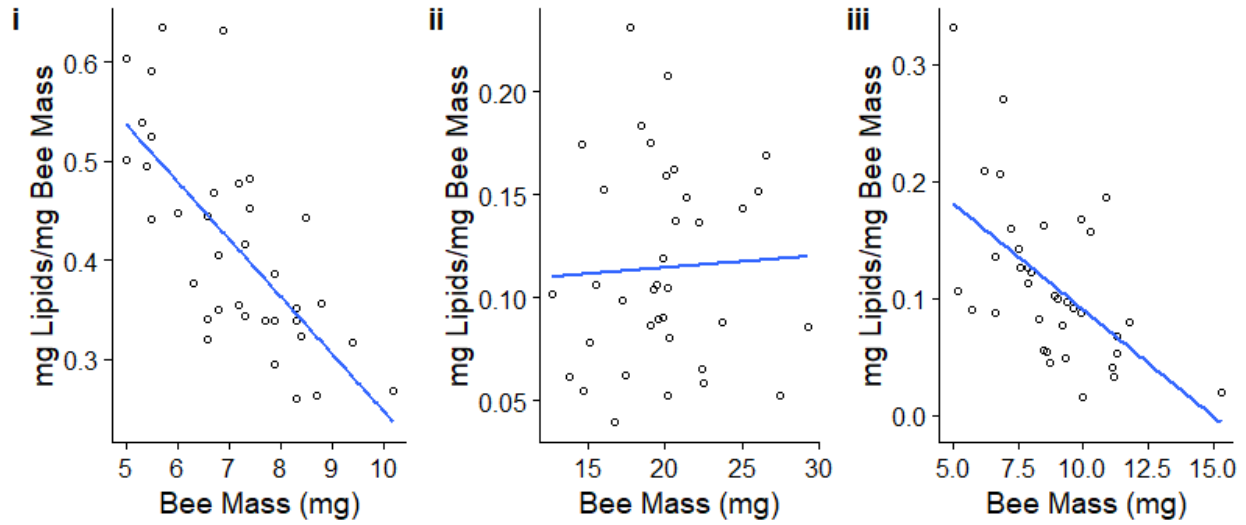
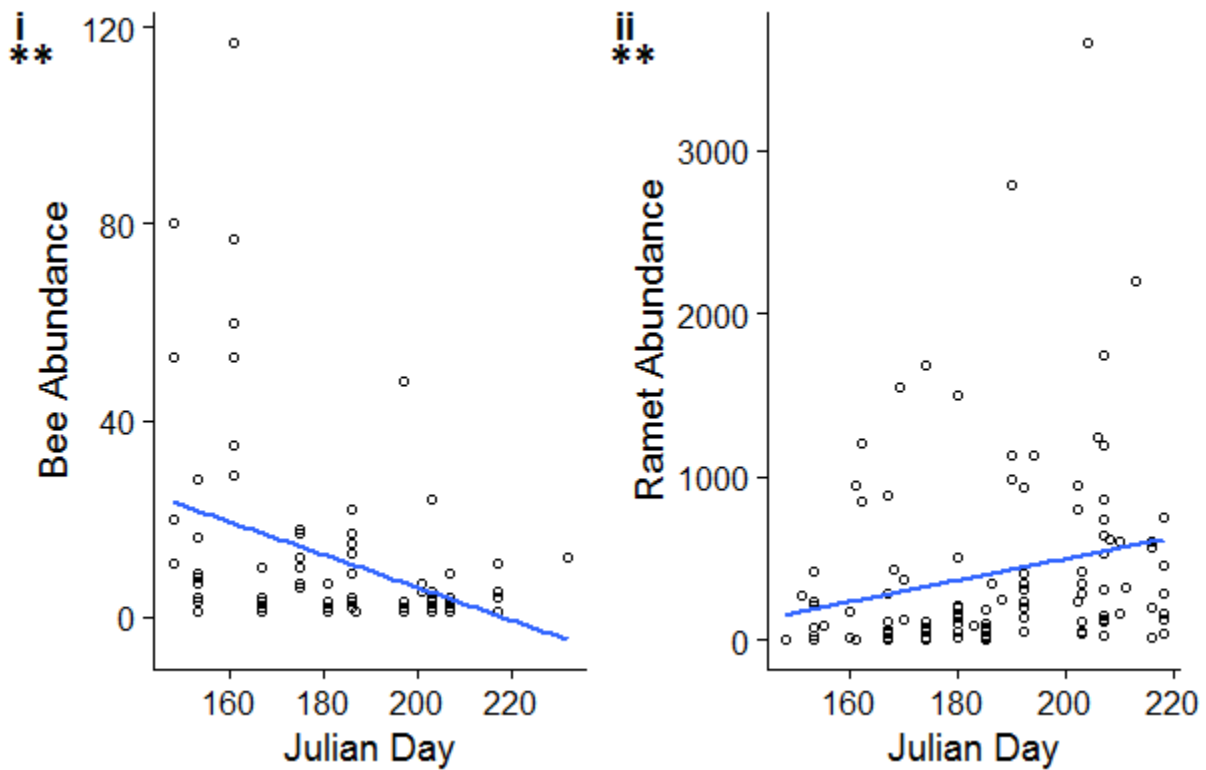


Figure 12: Correlations between relative lipid content and total bee mass in i) *Augochlora pura*, ii) *Agapostemon virescens*, and iii) *Halictus ligatus*



Supplemental Figure 1: Correlations between i) bee abundance and ii) flowering ramet abundance across Julian Day. Asterisks indicate significance where (**) indicates significance at the $P < 0.05$ level.

Supplemental Table 1: Total counts of bees collected from bee bowl transects and sweep net sampling among 3 grassland management treatments collected in 2015 and 2016.

	TGR		UGR		CGR	
	2015	2016	2015	2016	2015	2016
<i>Agapostemon sericeus</i>	0	0	0	0	1	0
<i>Agapostemon texanus</i>	0	1	0	0	5	2
<i>Agapostemon virescens</i>	26	4	111	18	84	5
<i>Andrena arabis</i>	0	0	0	1	0	0
<i>Andrena milwaukeeensis</i>	0	0	0	1	0	0
<i>Andrena rudbeckiae</i>	0	0	1	0	0	0
<i>Andrena sp.</i>	1	0	0	0	0	0
<i>Apis mellifera</i>	0	0	0	0	0	1
<i>Augochlora pura</i>	26	56	26	27	27	19
<i>Augochlorella aurata</i>	5	0	1	1	5	2
<i>Augochlorella persimilis</i>	25	5	5	1	16	2
<i>Augochloropsis metallica</i>	1	0	0	0	3	0
<i>Bombus auricomus</i>	0	0	0	0	0	1
<i>Bombus bimaculatus</i>	0	1	14	5	8	0
<i>Bombus griseocollis</i>	12	2	0	9	1	6
<i>Bombus impatiens</i>	0	0	2	0	1	0
<i>Bombus pennsylvanicus</i>	1	0	1	1	2	0
<i>Bombus perplexus</i>	0	1	0	1	2	0
<i>Ceratina calcarata</i>	2	3	2	0	7	0
<i>Ceratina dupla</i>	4	6	3	0	3	3
<i>Ceratina strenua</i>	7	1	3	2	1	0
<i>Coelioxys sayi</i>	0	0	0	0	1	0
<i>Dieunomia heteropoda</i>	0	0	0	0	1	0
<i>Dieunomia triangulifera</i>	1	0	0	1	0	0
<i>Dufourea marginata</i>	0	0	0	1	0	0
<i>Eucera hamata</i>	7	0	45	11	5	1
<i>Halictus confusus</i>	0	0	4	1	3	0
<i>Halictus ligatus</i>	38	3	37	28	38	7
<i>Halictus parallelus</i>	0	1	6	1	2	1
<i>Halictus rubicundus</i>	0	0	2	0	4	0
<i>Heriades carinata</i>	0	0	1	0	0	0
<i>Heriades variolosa</i>	0	0	1	0	2	0
<i>Hoplitis pilosifrons</i>	0	1	2	0	2	0
<i>Hoplitis producta</i>	0	0	7	0	0	0
<i>Hoplitis spoliata</i>	0	0	2	0	0	0
<i>Hoplitis truncata</i>	0	0	7	0	2	0
<i>Hylaeus affinis</i>	2	0	5	0	2	0
<i>Hylaeus annulatus</i>	1	0	1	0	0	0

Supplemental Table 1 Continued.

	TGR		UGR		CGR	
	2015	2016	2015	2016	2015	2016
<i>Hylaeus fedorica</i>	0	0	1	0	0	0
<i>Hylaeus messilae</i>	0	0	1	0	1	0
<i>Hylaeus modestus</i>	0	1	3	0	1	0
<i>Hylaeus schwarzii</i>	1	0	0	0	0	0
<i>Lasioglossum (Dialictus)</i>	5	36	151	33	105	50
<i>Lasioglossum</i> <i>(Hemihalictus)</i>	2	1	3	17	0	4
<i>Lasioglossum</i> <i>(Lasioglossum)</i>	0	1	2	4	1	1
<i>Melissodes agilis</i>	0	0	1	4	0	2
<i>Melissodes bimaculata</i>	0	0	0	1	0	1
<i>Melissodes trinodis</i>	0	0	0	3	0	0
<i>Nomia universitatis</i>	0	0	1	0	0	0
<i>Osmia distincta</i>	0	0	0	0	1	0
<i>Xylocopa virginica</i>	0	0	1	0	0	1

Supplementary Table 2: Abundances of flowering ramets found in 3 grassland management treatments collected in 2015 and 2016.

	Status	TGR		UGR		CGR	
		2015	2016	2015	2016	2015	2016
<i>Achillea millefolium</i> *	N	32	12	14	24	27	21
<i>Agrimonia gryposepala</i>	N	1	26	0	0	0	0
<i>Asclepias purpurascens</i>	N	2	0	0	0	0	0
<i>Asclepias incarnata</i> *	N	0	0	0	2	0	0
<i>Asclepias verticillata</i>	N	0	0	0	0	3	0
<i>Asclepias hirtella</i>	N	0	0	0	0	1	0
<i>Asclepias syriaca</i> *	N	0	295	0	0	0	0
<i>Asclepias tuberosa</i> *	N	35	0	8	2	0	0
<i>Baptisia alba</i> *	N	0	1	0	0	3	6
<i>Brassica napus</i>	E	0	0	0	0	0	1
<i>Chamaecrista fasciculata</i> *	N	269	28	615	62	5	25
<i>Cichorium intybus</i>	E	0	0	2	0	0	6
<i>Cirsium discolor</i>	N	0	0	1	0	0	0
<i>Coreopsis palmata</i>	N	0	0	42	1	0	0
<i>Coreopsis tripteris</i>	N	0	0	0	1044	0	0
<i>Dalea purpurea</i> *	N	0	0	17	94	0	0
<i>Daucus carota</i>	E	62	1458	244	275	457	620
<i>Desmodium canadense</i> *	N	14	0	20	87	0	0
<i>Dianthus armeria</i>	E	0	6	22	0	9	72
<i>Drymocallis arguta</i>	N	0	5	10	3	0	23
<i>Echinacea pallida</i> *	N	0	0	8	0	0	0
<i>Erigeron strigosus</i>	N	6	270	125	478	513	668
<i>Eryngium yuccifolium</i> *	N	0	0	5	38	0	0
<i>Euphorbia corollata</i>	N	49	0	0	0	0	0
<i>Helianthus grosseserratus</i>	N	1	3	5	227	0	0
<i>Heliopsis helianthoides</i> *	N	0	0	0	3	0	0
<i>Hypericum punctatum</i>	N	12	57	7	0	0	0
<i>Leucanthemum vulgare</i>	E	0	0	0	64	1	6
<i>Liatris spicata</i> *	N	0	0	97	105	0	0
<i>Lobelia spicata</i>	N	1	83	0	12	1	20
<i>Lotus corniculatus</i>	E	0	0	13	29	4872	6915
<i>Medicago lupulina</i>	E	0	20	0	28	0	630
<i>Melilotus albus</i>	E	11	131	1	3	0	105
<i>Melilotus officinalis</i>	E	9	0	6	91	44	38
<i>Monarda fistulosa</i> *	N	129	127	267	801	0	28
<i>Oxalis stricta</i>	N	0	1	0	0	82	3
<i>Parthenium integrifolium</i> *	N	0	0	0	3	0	0
<i>Pastinaca sativa</i>	E	3	46	0	2	0	0

Supplementary Table 2 Continued.

	Status	TGR		UGR		CGR	
		2015	2016	2015	2016	2015	2016
<i>Penstemon digitalis</i> *	N	0	0	0	27	0	0
<i>Persicaria maculosa</i>	E	0	0	0	0	0	6
<i>Plantago lanceolata</i>	E	0	0	0	0	0	474
<i>Potentilla recta</i>	E	0	0	0	0	1	0
<i>Prunella vulgaris</i>	N	0	19	0	0	62	22
<i>Pycnanthemum pilosum</i>	N	296	38	31	4	0	0
<i>Pycnanthemum tenuifolium</i>	N	0	3189	0	177	85	54
<i>Ratibida pinnata</i> *	N	428	1181	241	1537	3	1
<i>Rosa arkansana</i> *	N	0	26	0	0	0	0
<i>Rubus sp.</i>	N	0	14	0	0	0	0
<i>Rudbeckia hirta</i> *	N	304	200	261	227	154	56
<i>Ruellia humilis</i>	N	0	5	0	0	24	2
<i>Silphium integrifolium</i>	N	52	0	21	238	0	0
<i>Silphium laciniatum</i>	N	0	2	0	0	0	0
<i>Silphium perfoliatum</i>	N	0	0	28	19	0	0
<i>Sisyrinchium angustifolium</i>	N	0	4	0	0	0	2
<i>Solanum carolinense</i>	N	0	17	0	16	11	235
<i>Solidago canadensis</i>	N	0	0	13	75	0	0
<i>Stellaria graminea</i>	E	0	0	0	0	0	63
<i>Taraxacum officinale</i>	N	0	0	0	0	0	4
<i>Teucrium canadense</i>	N	0	4	0	0	9	8
<i>Tradescantia bracteata</i> *	N	8	16	39	43	0	0
<i>Trifolium hybridum</i>	E	0	0	0	29	1	12
<i>Trifolium pratense</i>	E	33	45	90	138	1479	1167
<i>Trifolium repens</i>	E	0	89	10	2	3948	4138
<i>Verbena hastata</i> *	N	0	0	0	0	0	27
<i>Verbena stricta</i> *	N	0	1	0	0	118	101
<i>Verbena urticifolia</i>	N	0	0	0	0	1	20
<i>Vernonia fasciculata</i> *	N	0	4	2	3	14	13
<i>Veronica arvensis</i>	E	0	0	0	0	60	0

Status indicated whether a plant species is native or exotic. N indicates a native flowering plant species. E indicates an exotic flowering plant species. Asterisks (*) indicate plant species commonly visited by bees.

CHAPTER 4: GENERAL DISCUSSION

The experiments described in this thesis provide results that describe how invertebrate communities respond to multiple herbicide management techniques. In Chapter 2, we focused mainly on how butterfly and flowering plant communities responded to grassland management through herbicide treatments. We applied three herbicide treatments at a patch level, a spray and seed treatment with glyphosate and a native seed mix, a spray only with application of only glyphosate, and an untreated control. We found that the abundance of butterflies reacted positively to the herbicide treatments, being marginally significantly higher in the spray and seed patches as compared to the control. The responses of butterfly communities to herbicide management that we observed followed trends similar to other studies of butterflies and invasive plant species in North America (Hanula and Horn 2011, McIver and Macke 2014). The flowering plant community's response to herbicide was much more pronounced than the butterfly community, with abundance, species richness, and diversity all showing significant positive responses to herbicide treatment, primarily in the spray and seed treated patches. This was most likely attributed to the lack of competition from reduced tall fescue cover. However, reducing tall fescue cover benefitted exotic flowering plant species as well, leading to significantly higher levels of floral abundance and species richness in spray and seed patches.

Chapter 2 also provides reasoning for further study and analysis of community responses to herbicide treatments. The results in this chapter were derived from the butterfly and flowering plant community two years post-herbicide treatment. Additional monitoring is crucial due to the potential for large turnover in prairie plant community composition over a three year cycle (Camill et al. 2004). Other pollinators could also be analyzed in future studies, namely native and managed bees. Non-native flowering plants could also be examined in further studies

because they responded favorably to herbicide treatments of tall fescue. Additional herbicide or burning treatments may need to be implemented to control these invasive flowering plants, furthering the need for monitoring.

In Chapter 3, we explored the native bee and flowering plant community responses to different grassland management treatments, namely tallgrass prairie remnants, un-grazed restorations, and cattle-grazed restorations. The native bee community reacted favorably to un-grazed restorations, with bee abundances showing significantly higher values than in tallgrass prairie remnants. These results are similar to abundance data of non-bee insects (Larsen and Work 2003). Bee species richness and diversity were also highest in un-grazed restorations, departing from previous findings on insect communities (Bomar 2001, Nemec and Bragg 2008). Flowering plants responded quite differently, with cattle-grazed restorations having significantly higher flowering ramet abundance than tallgrass prairie remnants and un-grazed restorations, driven mainly by high numbers of exotic flowering plant species. Similarly, cattle-grazed restorations also had the highest species richness, again attributed to high numbers of exotic flowering plant species on top of existing areas of native flowering plants. From this study, we observed that bee community did not follow similar trends to the overall flowering plant community responses to grassland management treatment.

In Chapter 3, we also examined health differences in native bees found in each of the grassland management treatments by comparing lipid content in *Augochlora pura*, *Agapostemon virescens*, and *Halictus ligatus*. We found no significant differences in relative lipid content among the grassland management treatments for any of the bee species. This indicates that grassland management treatment is not a contributing factor in determining the nutritional status of native bees. We also explored how nutritional status of three species of native bees changed

with body size and time within the field season. Lipid mass in *Ag. virescens* increased with increasing body mass. *Augochlora pura* and *H. ligatus* showed a significant decrease in mass over the sampling period. Lipid mass increased significantly in only *H. ligatus* over the sampling period, while showing no significant trends in the other two species. Only *Halictus ligatus* had relative lipid content that increased significantly over the sampling period, primarily due to decreasing body mass and increasing lipid mass. This study illustrates that bee nutritional responses of native bees are not determined by grassland management treatment, but are rather influenced by a complex combination of life history and chronological variables

The results of Chapter 3 suggest many opportunities for further research. Additional environmental, community, and time-based variables could be added to community data to explore how both the native bee and flowering plant communities respond over time within each grassland management treatment. Further lipid assessment could be applied not only to additional bee species, but to other pollinators such as butterflies. Grassland management treatments could also be examined in more detail. Previous studies examining grassland restoration age and grazing intensity have been explored on pollinator communities (Kruess and Tscharntke 2002, Tonietto et al. 2016) and could be expanded upon within the context of the Grand River Grasslands. Further analysis of bee species data related to social, feeding, and nesting strategies could also be applied.

Grassland management is multi-faceted and site-specific responses can be variable. Each management treatment can be designed to accomplish specific restoration goals, but evaluating the interactions among these responses is critical. The results from this thesis show that community responses from pollinators and flowering plants were variable, but that herbicide and seeding have the potential to restore grassland plant and insect communities to a more native

state. However, when designing future grassland management projects, it is vital to consider these multifaceted community responses.

References

- Bomar, Charles R. "Comparison of Grasshopper (Orthoptera: Acrididae) Communities on Remnant and Reconstructed Prairies in Western Wisconsin." *Journal of Orthoptera Research* 10.1 (2001): 105-12.
- Camill, Philip, Mark J. Mckone, Sean T. Sturges, William J. Severud, Erin Ellis, Jacob Limmer, Christopher B. Martin, Ryan T. Navratil, Amy J. Purdie, Brody S. Sandel, Shano Talukder, and Andrew Trout. "Community- And Ecosystem-Level Changes in a Species-Rich Tallgrass Prairie Restoration." *Ecological Applications* 14.6 (2004): 1680-694.
- Hanula, James L., and Scott Horn. "Removing an Exotic Shrub from Riparian Forests Increases Butterfly Abundance and Diversity." *Forest Ecology and Management* 262.4 (2011): 674-80.
- Kruess, Andreas, and Teja Tscharntke. "Grazing Intensity and the Diversity of Grasshoppers, Butterflies, and Trap-Nesting Bees and Wasps." *Conservation Biology* 16.6 (2002): 1570-580.
- Larsen, Kirk J., and Timothy W. Work. "Differences in Ground Beetles (Coleoptera: Carabidae) of Original and Reconstructed Tallgrass Prairies in Northeastern Iowa, USA, and Impact of 3-year Spring Burn Cycles." *Journal of Insect Conservation* 7.3 (2003): 153-66.
- McIver, James, and Euell Macke. "Short-Term Butterfly Response to Sagebrush Steppe Restoration Treatments." *Rangeland Ecology & Management* 67.5 (2014): 539-52. Web.
- Nemec, Kristine T., and Thomas B. Bragg. "Plant-Feeding Hemiptera and Orthoptera Communities in Native and Restored Mesic Tallgrass Prairies." *Restoration Ecology* 16.2 (2008): 324-35.
- Tonietto, Rebecca K., John S. Ascher, and Daniel J. Larkin. "Bee Communities along a Prairie Restoration Chronosequence: Similar Abundance and Diversity, Distinct Composition." - *Ecological Applications* (2016): 705-17.