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## Density and diversity of bees in the Midwestern agricultural landscape: Influence of surrounding agricultural land use and local plant community characteristics

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DENSITY AND DIVERSITY OF BEES IN THE MIDWESTERN AGRICULTURAL LANDSCAPE:  
INFLUENCE OF SURROUNDING AGRICULTURAL LAND USE  
AND LOCAL PLANT COMMUNITY CHARACTERISTICS

An Abstract of a Thesis  
Submitted  
in Partial Fulfillment  
of the Requirements for the Degree  
Master of Science

Andrew James Ridgway  
University of Northern Iowa  
July 2016

## ABSTRACT

After over a century of large-scale agricultural development in the Midwestern corn-belt of the United States, many wild bee pollinator populations are in rapid decline or extirpated from their historic range. Large-bodied species, such as *Bombus spp.* are especially threatened, but are also valuable as efficient natural pollinators that are capable of effectively pollinating many economically important crops. Bees, therefore, have high conservation value, and pollination research has shifted focus from community inventory to population recovery efforts. These efforts include reducing the effects of habitat fragmentation and destruction using ecological restoration as well as integrating conservation strategies into agricultural land management. This thesis investigates both strategies by analyzing local and landscape-scale vegetation effects on wild bee populations.

At the local scale, I sampled and analyzed bee populations on land planted with perennial tallgrass prairie plants utilized for alternative bioenergy production at the University of Northern Iowa's Cedar River Ecological Research Site. These plantings ranged in diversity from a switchgrass monoculture to a diverse 32-species biofuel feedstock mixture. At the landscape scale, I used existing remote sensing products to examine the effect of surrounding land cover on bee community indices at small organic farming operations throughout Iowa.

I found at the local scale that both bee abundance and diversity increased with plant species richness in biofuel crops, and that temporal stability of floral resources may be a more important factor than sheer abundance of flowering plants. Temporal stability in floral resources refers to the change in abundance of floral food sources for pollinators as well as the degree of overlap in flowering times throughout the growing season. Diverse biofuel feedstocks have a positive effect on the wild bee community and at a site level are capable of supporting a pollinator community similar diversity to small remnant tallgrass prairie communities.

At the landscape scale, bee diversity responded positively to surrounding natural land cover and negatively to agricultural row crops like corn and soy. I was unable, however, to link wild bee abundance to surrounding land cover, perhaps because fine-scale, on-farm factors may have a greater influence on bee abundance, especially for smaller, less mobile species. I conclude that increasing floral abundance and diversity in the landscape is an important step toward recovery of wild bee pollinator communities. It is evident that both degree of isolation from suitable habitat as well as local habitat quality influence pollinator communities of conservation concern.

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This Study by: Andrew James Ridgway

Entitled: Density and Diversity of Bees in the Midwestern Agricultural Landscape:  
Influence of Surrounding Agricultural Land Use and Biofuel Candidate Crops

has been approved as meeting the thesis requirement for the

Degree of Master of Science

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## CHAPTER 1

### LOCAL PLANT COMMUNITIES

#### Introduction

Since 2006, yearly losses of domesticated honey bee (*Apis mellifera*) hives in the United States are over 30% (Watanabe 2013), resulting in subsequent losses in crop yields (Gallai et al. 2008). The causes of this decline are numerous, including parasitism from non-native species (Sammataro et al. 2000), pesticides (Prisco et al. 2013), herbicides (Cameron et al. 2011), climate change (Watanabe 2013), and a reduction in genetic variability (Jaffé et al. 2010). All of these factors contribute to the phenomenon known as Colony Collapse Disorder (CCD), in which an entire honey bee colony suddenly fails (Watanabe 2013). Given these challenges with domesticated bees, it seems prudent to identify an alternative source of pollination services that can be used at both small and large scales.

Wild bees, especially large bumble bees (*Bombus spp.*), are effective pollinators that provide adequate pollination services to pollinator-dependent crops such as watermelon (Winfree et al. 2007). Studies also show that fruit yield increases with the percentage of surrounding natural habitat (Klein et al. 2012), presumably due to wild bee pollination. Unfortunately, the exceptionally efficient bumble bee pollinators are also in drastic decline. Populations of four extensively surveyed *Bombus* species have declined by 96% in recent decades and have been extirpated from a significant portion of their ranges (Cameron et al. 2011). These declines are thought to stem from human-

related habitat destruction, fragmentation, and degradation, which remove floral resources that stable populations require (Roulston and Goodell 2011). Reduction in the availability of floral resources subsequently reduces insect-plant interactions necessary for maintaining high crop productivity levels despite honey bee supplementation (Garibaldi et al. 2013). Unfortunately, except for *Bombus* and *Apis* spp, there is not adequate knowledge of other bee pollinators in the United States to make an assessment of their population trends, although declines are suspected (Cameron et al. 2011) and have been demonstrated in Europe (Biesmeijer et al. 2006).

Recently, demands for increased biofuel feedstock production have led to corn and soybeans being planted on marginal land (Wright and Wimberly 2013), exacerbating the problems associated with habitat fragmentation and degradation. These land use changes reduce habitat availability and quality for a wide range of species, including bees (Kennedy et al. 2013). Clearing trees and brush and tilling fields reduces nesting habitat for all guilds of bee pollinators. Planting large fields of corn not only reduces floral resource availability, but exposes the bees that do manage to persist to sprayed and systemic pesticides (Prisco et al. 2013). In areas where crops do provide floral resources, such as on blueberry farms (Benjamin et al. 2014), these mass flowering crops do not provide the temporal stability required to support many bee pollinator species. Some bees are capable of utilizing only a single flower species by timing their flight period to blooming (Klein et al. 2012) while other, often larger-bodied species require floral food sources throughout the growing season to complete their life cycle

(Hines and Hendrix 2005). An alternative to these practices would be to promote the restoration and management of native vegetation as part of income-producing lands that are managed with ecosystem services in mind (Zilverberg et al. 2014).

Studies at the University of Northern Iowa's Cedar River Ecological Research Site have found that, under certain conditions on marginal land, diverse prairie plantings managed for bioenergy produce just as much biomass as monocultures (Abernathy et al. 2015), in addition to providing habitat for wildlife when managed properly (Myers et al. 2015). The design of this biofuel candidate crop study site allows me the opportunity to examine the effects of plant community and floral resources on the bee community at a crop production scale. We hypothesize that (1) increasing diversity of floral resources will lead to increases in local bee abundance and diversity and (2) bee communities utilizing diverse biofuel crops will more closely resemble those of native prairies than the bee communities using less diverse crops.

If the first hypothesis is supported, we will observe differences in bee community indices such as abundance, species richness, and Shannon-Weiner diversity index not only between treatments, but between plots of the same treatment that vary in their floral resource abundance and diversity. As for the second hypothesis, biofuel candidate crops will be compared to native prairies both in terms of their species richness, but also with the Shannon-Weiner diversity index, which will reveal if the candidate crops are dominated by one or several common species or if the assemblage of species observed is more evenly distributed.

## Methods

### Study Site

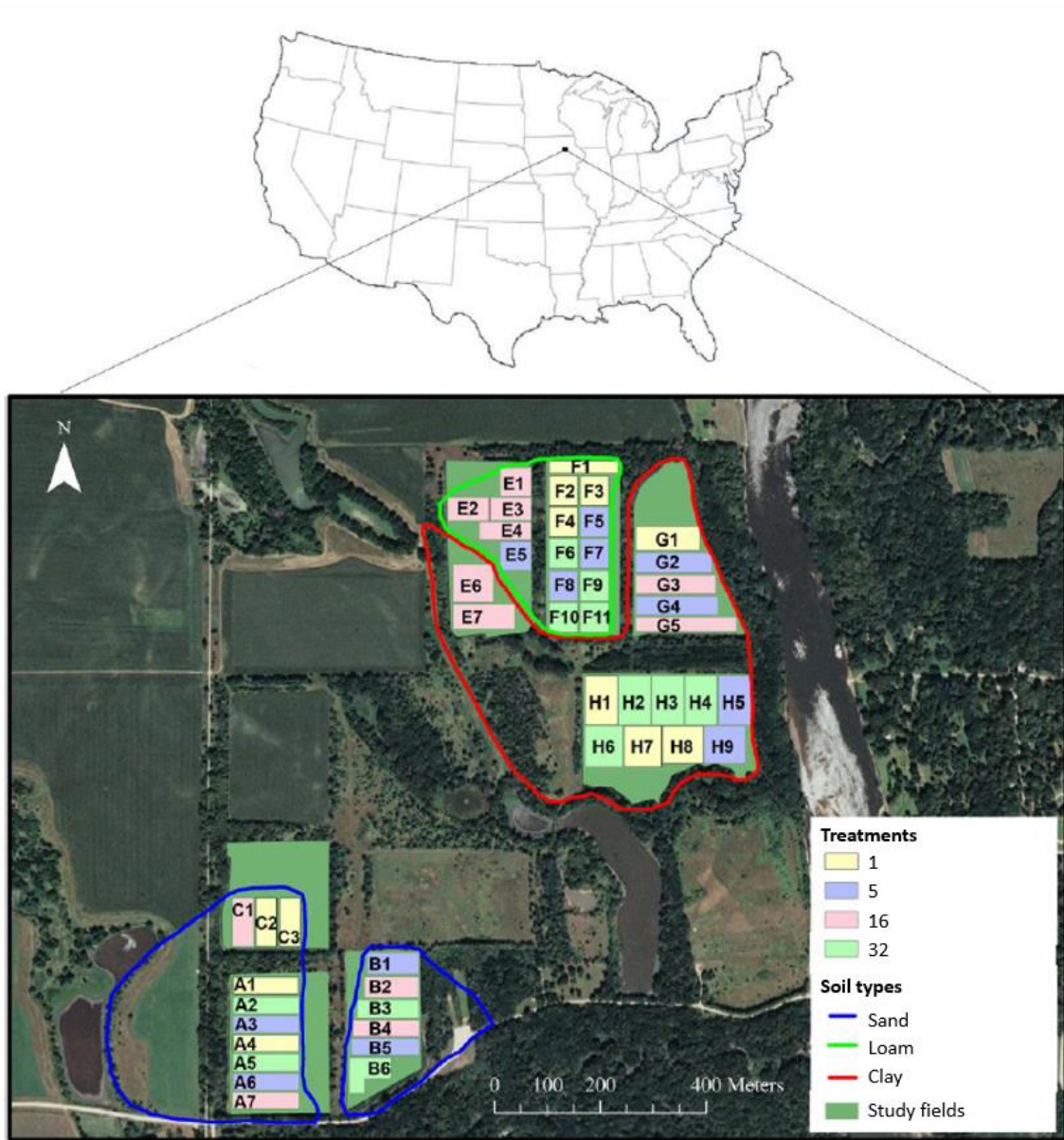
I conducted my research at the Cedar River Ecological Research Site, located in southeastern Black Hawk County, Iowa, USA. This 40 ha area consists of seven agricultural fields that had been farmed with a corn and soybean rotation for over 20 years (Myers et al. 2015). In 2009, the site was restored by seeding 48 different research plots ranging from 0.30-0.56 ha with four mixes of perennial tallgrass prairie plants: 1) Switchgrass (*Panicum virgatum* monoculture), 2) Grasses (five warm-season grasses), 3) Biomass (16 species of prairie grasses and forbs), and 4) Prairie (32 species of prairie grasses and forbs)(Table 1). The plots were arranged so that there are 16 plots (four plots of each treatment) on each of the three soil types represented at the site: Flagler sandy loam (Sand), Waukee loam (Loam), and Spillville-Coland complex (Clay). My research was conducted only on the Flagler sandy loam to minimize variation associated with soil type (Figure 1). This soil has a lower water holding capacity and lower nutrient availability than the other soil types (Myers et al. 2015).

It is important to note that species were not randomly selected from a species pool as in many other diversity experiments; rather the species were specifically selected to optimize biomass production at a given level of diversity to make the results more relevant to biofuels production scenarios. Perennial switchgrass monocultures are promoted as an alternative to planting corn for use as a biofuel and are thought to marginally improve habitat value and improve soil stability. The Biomass mix was

designed specifically to grow in tall, dense stands and provide high biomass, but floral resources are available from a limited host of species and may or may not overlap temporally. The Prairie mix more closely resembles what would be used in a diverse prairie reconstruction, with functionally different floral resources as well as flowering times that overlap within the growing season. Of the four tallgrass prairie plant species previously identified as attracting the most diverse and abundant assemblage of pollinators (Harmon-Threat and Hendrix 2015), the Biomass mix contains one of these species (*R. pinnata*), while the Prairie mix contains all four (*A. canescens*, *D. purpurea*, *Z. aurea*) (Table 1). These differences give us the unique opportunity to explore the differences in pollinator assemblages over the course of a growing season between a monoculture in which the only floral resources are weedy invaders, a Biomass mix with boom or bust floral resources, and a Prairie mix designed to attract a diverse bee pollinator community.

I sampled bee diversity and abundance in three of the biofuel treatments, switchgrass, biomass, and prairie, representing 12 of the 16 plots located on the Flagler sandy loam soil. I chose to exclude the five-species grass mix from our experimental design because it has the same floral diversity as the switchgrass monoculture.





**Figure 1:** Map of the Cedar River Ecological Research Site. Each diversity treatment was replicated four times on three soil types (4 vegetation treatments  $\times$  3 soil types  $\times$  4 replicates per soil type = 48 plots total). Each plot is labeled with a unique alphanumeric identifier.

**Table 1:** Species list and seeding rate of each diversity treatment. The number of pure live seeds / m<sup>2</sup> and cost per hectare (USD) for each seed mix are indicated.

Scientific Name	Common Name	FG	1	5	16	32
<i>Panicum virgatum</i>	switchgrass	C <sub>4</sub> grass	561	86	43	32
<i>Andropogon gerardii</i>	big bluestem	C <sub>4</sub> grass		151	151	135
<i>Bouteloua curtipendula</i>	side-oats grama	C <sub>4</sub> grass		86	43	32
<i>Schizachyrium scoparium</i>	little bluestem	C <sub>4</sub> grass		151	151	135
<i>Sorghastrum nutans</i>	indian grass	C <sub>4</sub> grass		86	43	32
<i>Elymus canadensis</i>	canada wildrye	C <sub>3</sub> grass			43	32
<i>Elymus virginicus</i>	virginia wildrye	C <sub>3</sub> grass			43	32
<i>Agropyron smithii</i>	western wheatgrass	C <sub>3</sub> grass			43	32
<i>Sporobolus compositus</i>	tall dropseed	C <sub>4</sub> grass				32
<i>Carex bicknellii</i>	copper shouldered oval sedge	sedge				32
<i>Carex brevior</i>	plains oval sedge	sedge				32
<i>Carex gravida</i>	long-awned bracted sedge	sedge				32
<i>Dalea purpurea</i>	purple prairie clover	legume				16
<i>Desmodium canadense</i>	showy tick-trefoil	legume			38	16
<i>Helianthus grosseserratus</i>	sawtooth sunflower	forb			38	16
<i>Heliopsis helianthoides</i>	oxe-eye sunflower	forb			38	16
<i>Lespedeza capitata</i>	round-headed bush clover	legume			38	16
<i>Oligoneuron rigidum</i>	stiff goldenrod	forb			38	16
<i>Phlox pilosa</i>	prairie phlox	forb				3
<i>Ratibida pinnata</i>	grey headed coneflower	forb			38	16
<i>Astragalus canadensis</i>	milk vetch	legume			38	16
<i>Silphium laciniatum</i>	compass plant	forb			3	3
<i>Amorpha canescens</i>	leadplant	legume				16
<i>Artemisia ludoviciana</i>	prairie sage	forb				16
<i>Baptisia leucantha</i>	white wild indigo	legume				1
<i>Echinacea pallida</i>	pale purple coneflower	forb				16
<i>Eryngium yuccifolium</i>	rattlesnake master	forb				16
<i>Monarda fistulosa</i>	wild bergamot	forb				16
<i>Symphyotrichum laevae</i>	smooth blue aster	forb				16
<i>Symphyotrichum novae angliae</i>	new england aster	forb				16
<i>Tradescantia bracteata</i>	prairie spiderwort	forb				16
<i>Zizia aurea</i>	golden alexander	forb				16
	Pure live seed/m <sup>2</sup>		561	560	829	869
	Total Cost/Hectare		\$158	\$282	\$1,643	\$2,354

### Bee Collection Protocol

I made monthly bee collections from June to August, 2015. Bees were collected from the four replicate plots totaling approximately 2 ha for each treatment. Bees were collected by netting in each of the plots with a sampling intensity equal to one hour/ha in the morning (9am-noon) and one hour/ha in the afternoon (1pm-4pm) for each seed treatment. In order to minimize uneven sampling efforts due to different collectors, each sampling period was broken into 15 minute intervals, which were rotated and sampled by three different collectors. Plots were not explicitly assigned to morning or afternoon; rather the surveys were performed from nearest to farthest plots from the entrance to the area. Since the plots were randomly assigned a seed treatment, the order of surveys was also random. I performed collections between 9:00 AM and 4:00 PM and only in temperatures greater than 15.5°C and with wind speeds less than 32 km/hr (Hendrix *et al.* 2010). During each 15 minute collection period, I haphazardly patrolled the length of the plot and captured all visible bees by net and placed them into ethyl acetate kill jars. Once a bee was captured, I stopped the timer and thus the 15 minutes only accounts for searching time and does not include handling time.

### Floral Surveys

Floral surveys were conducted during each of the surveys months, June, July, and August as part of a complete floral survey of all 48 plots at the Cedar River Ecological Research Site. I quantified floral resources along a permanent 50m transect in each plot. A total of 20 1m<sup>2</sup> quadrats were placed along the transect every two meters,

starting at a random position 1-9 meters from the end point and 1-3 meters right or left of the transect. Within each quadrat, I estimated the number of inflorescences of each flowering species for which there would be a nectar or pollen reward.

#### Bee Processing and Identification

Bees were transferred to the lab and pinned or attached to points. Bees were identified to genus using Michener et al. (1994), Mitchell (1960, 1962), and Arduser (2015). Bees were further identified to species using Mitchell (1960, 1962), Arduser (2015), and with assistance from S.D. Hendrix from The University of Iowa.

#### Statistical Analysis

I used bees collected from all three visits to calculate the community indices (i.e. abundance, species richness, and Shannon-Weiner diversity index) for each experiment plot. I then used one-way ANOVA and Tukey's Honestly Significant Difference (HSD) to compare the difference of these bee indices among the three biofuel treatments (i.e. 1, 16, and 32 species). Similarly, I calculated the flower abundance, species richness, and Shannon-Weiner diversity index for each plot and used one-way ANOVA and Tukey's HSD to evaluate the treatment effect on these flower community indices. Thirdly, I used linear regression to evaluate the correlation between bee and flower community measurements. Data were transformed as appropriate to meet the assumptions of normality and homoscedasticity.

I also compared species richness and diversity values from the biofuel treatments to those found in small hill prairies in Northeast Iowa in 2005 (Hendrix *et al.*

2010). The hill prairies were sampled using the same intensity (1 hour/ha), and therefore the data are comparable to my study. I also used a Monte Carlo simulation of 1000 synthetic “bee communities” (Hendrix *et al.* 2010) from Northwest Iowa prairies to compare with my data. All data were analyzed and figures generated in R version 3.2.3 (R Development Core Team 2016), using the ‘ggplot2’ (Wickham 2009), ‘labdsv’ (Roberts 2016), ‘MASS’ (Venables and Ripley 2002), ‘car’ (Fox and Weisberg 2011), and ‘vegan’ (Oksanen *et al.* 2016) packages.

## Results

In total, we collected 986 bees of 56 species from 19 genera (Table 2). 508 bees were collected during the morning and 478 were from the afternoon collection. The August collection yielded the highest bee abundance (Figure 2) and the July collection yielded the highest number of bee species (Figure 3).

Wild bee abundance was significantly higher in the biomass and prairie treatments than in the switchgrass monoculture, especially early in the season (Figure 2). More bees were collected from the prairie plots than the biomass plots (Table 3), and after accounting for the influence of flower abundance, this difference was significant (Table 4). The Shannon-Weiner diversity index (Figure 5) also showed a similar but not significant positive trend with flower abundance (Figure 4, Table 4). Much of the variation in bee abundance can be explained by flower abundance (Figure 6), although there is still a positive effect the planted diversity treatment on bee abundance despite similar flower abundance (Figure 6, Table 4). Bee abundance in each plot was positively correlated with floral species richness (Table 5), but the effect of floral species richness varied between treatments (Figure 7). Floral species richness had a much larger positive effect on bee abundance in the biomass treatment than either the switchgrass or the prairie treatments (Figure 7).

Wild bee species richness was significantly higher in the biomass and prairie treatments than in the switchgrass monoculture (Table 3, Figure 5). Species richness of bees increased significantly with both flower abundance and species richness within

plots (Table 4, 5). Biomass and prairie plots did not have significantly different species richness in any survey period (Figure 3).

The wild bee species richness values from the biofuel site were comparable to the wild bee species richness values at the small hill prairies (Figure 8). However, Shannon-Weiner diversity values from my site were within the bottom 1<sup>st</sup> percentile of 1000 Monte Carlo simulations drawn from samples of larger prairie remnants. This is due to dominance of several common species at my site that led to a low evenness value (Figure 9). My results show that 41.7% of all wild bee samples were of *Bombus impatiens* and 27.2% were of *Bombus griseocollis*, yielding a low evenness value for the treatments in the Shannon-Weiner calculation.

I was able to identify several flowering species that had particularly strong positive interactions with wild bee abundance. They include *Echinacea pallida* (Figure 10), *Lespedeza capitata* (Figure 11), and *Oligoneuron rigidum* (Figure 12), none of which were identified as bee species by Harmon-Threat and Hendrix (2015). All other flower species either occurred in relatively low abundance or had no significant correlation with bee community indices.

**Table 2:** Total count of each species observed at the Cedar River Ecological Research Site in the summer of 2015 in each candidate crop, as well as the summed count for the whole site.

Species	Switchgrass (1)	Biomass (16)	Prairie (32)	Site Total
<i>Agapostemon sericeus</i>	0	0	2	2
<i>Agapostemon splendens</i>	0	0	1	1
<i>Agapostemon virescens</i>	2	0	18	20
<i>Andrena basilicis</i>	0	2	0	2
<i>Andrena dunningi</i>	0	2	0	2
<i>Andrena helianthiformis</i>	0	19	26	45
<i>Andrena herclei</i>	0	1	0	1
<i>Andrena imitatrix</i>	0	1	0	1
<i>Andrena perplexa</i>	0	3	0	3
<i>Andrena sp</i>	0	1	0	1
<i>Augochlora pura</i>	0	1	0	1
<i>Augochlorella aurata</i>	1	1	4	6
<i>Bombus affinis</i>	0	0	1	1
<i>Bombus auricomus</i>	1	10	7	18
<i>Bombus bimaculatus</i>	0	0	5	5
<i>Bombus citrinus</i>	0	0	3	3
<i>Bombus fervidus</i>	1	0	0	1
<i>Bombus griseocollis</i>	24	85	159	268
<i>Bombus impatiens</i>	26	200	185	411
<i>Bombus pennsylvanicus</i>	3	2	6	11
<i>Bombus vagans</i>	0	0	5	5
<i>Certina calcarita</i>	0	1	1	2
<i>Coelioxys rufitarsis</i>	1	3	2	6
<i>Diunomia triangulifera</i>	0	1	0	1
<i>Epioloides pilosula</i>	0	1	0	1
<i>Eucera hamata</i>	0	0	1	1
<i>Halictus confusus</i>	0	0	12	12
<i>Halictus ligatus</i>	6	10	15	31
<i>Halictus parallelus</i>	0	2	2	4
<i>Hylaeus annulatus</i>	1	0	1	2
<i>Hylaeus sp</i>	0	2	0	2
<i>Lasioglossum albipenne</i>	1	0	1	2
<i>Lasioglossum bruneri</i>	1	2	14	17
<i>Lasioglossum coriaseum</i>	0	5	0	5
<i>Lasioglossum cresonii</i>	0	0	1	1

Table Continued



Species	Switchgrass (1)	Biomass (16)	Prairie (32)	Site Total
<i>Lasioglossum mitchelli</i>	0	0	3	3
<i>Lasioglossum pilosum</i>	1	2	6	9
<i>Lasioglossum pruinosum</i>	0	1	2	3
<i>Lasioglossum sagax</i>	0	0	1	1
<i>Lasioglossum</i>				
<i>Sphecodogastra</i>	0	2	0	2
<i>Lasioglossum tegulare</i>	1	0	1	2
<i>Macropis steironematis</i>	1	0	0	1
<i>Megachile brevis</i>	2	2	1	5
<i>Megachile frigida</i>	0	3	3	6
<i>Megachile latimanus</i>	1	11	15	27
<i>Megachile melanophoea</i>	0	0	1	1
<i>Megachile nivalis</i>	0	1	0	1
<i>Megachile petulans</i>	0	3	6	9
<i>Megachile relativa</i>	0	1	0	1
<i>Melissodea apicata</i>	0	1	1	2
<i>Melissodes bidentis</i>	0	2	0	2
<i>Melissodes bimaculata</i>	2	0	6	8
<i>Melissodes comptoides</i>	0	1	1	2
<i>Nomada erigeronis</i>	0	0	3	3
<i>Protandrena bancrofti</i>	0	1	0	1
<i>Pseudopanurgis albitarsis</i>	0	0	2	2
Total Abundance	76	386	524	986
Species Richness	18	35	38	56

**Table 3:** Averaged flower data and summed wild bee data for all three survey session with totals for each treatment type

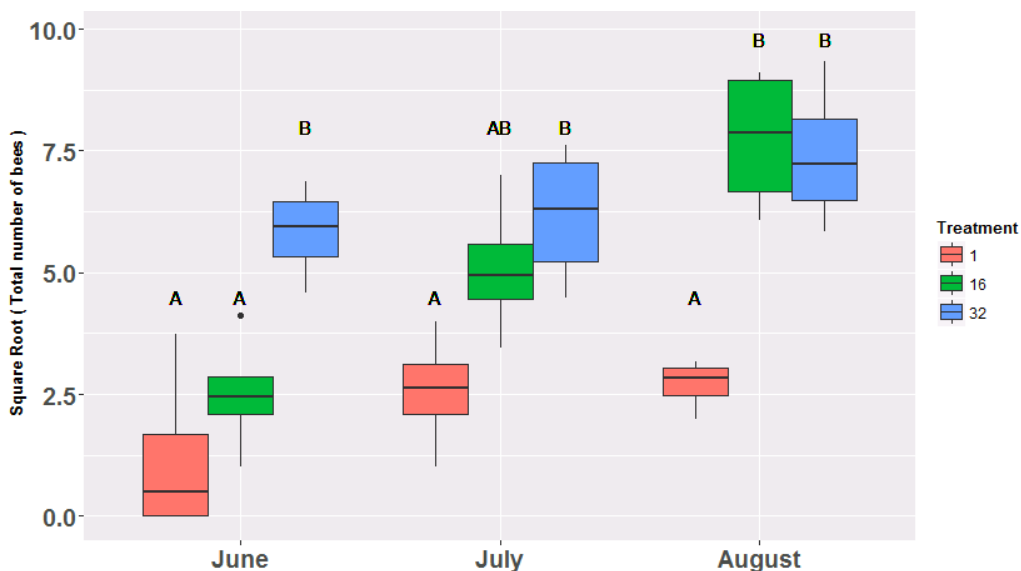
Plot	Treatment	Flower Abundance	Species Richness	Flower Shannon Diversity Index	Bee Abundance	Species Richness	Bee Shannon Diversity Index
A1	Switchgrass	2.333	5	1.292	17	7	1.528
A4	Switchgrass	1.333	3	0.799	29	12	1.960
C2	Switchgrass	0.683	3	0.509	25	5	1.109
C3	Switchgrass	0.333	4	1.211	5	2	0.500
<b>All</b>	<b>Switchgrass</b>	<b>1.171</b>	<b>8</b>	<b>1.307</b>	<b>76</b>	<b>18</b>	<b>1.973</b>
A7	Biomass	3.367	5	1.088	92	20	2.011
B2	Biomass	4.233	7	1.115	106	11	1.327
B4	Biomass	5.550	8	1.296	101	11	1.154
C1	Biomass	1.017	6	1.363	87	19	1.956
<b>All</b>	<b>Biomass</b>	<b>3.542</b>	<b>10</b>	<b>1.503</b>	<b>386</b>	<b>35</b>	<b>1.809</b>
A2	Prairie	2.200	13	1.733	96	20	2.141
A5	Prairie	4.767	15	2.088	142	15	1.762
B3	Prairie	4.783	12	1.730	154	19	1.727
B6	Prairie	2.333	11	1.641	132	23	2.299
<b>All</b>	<b>Prairie</b>	<b>3.521</b>	<b>19</b>	<b>2.362</b>	<b>524</b>	<b>38</b>	<b>2.149</b>

**Table 4:** 2-way ANOVA table showing the effects of flower abundance (per m<sup>2</sup>) and vegetation treatment on wild bee abundance, richness, and Shannon Diversity index (H'). Each data point in the analysis represents 1 of 12 plots in each of three surveys periods, for a total of 36 data points.

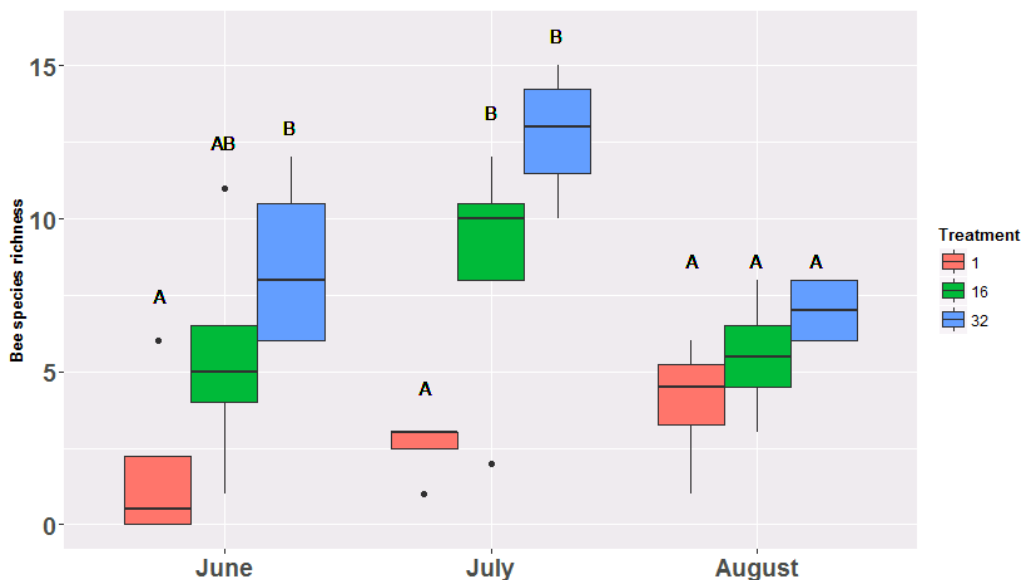
	Bee Abundance			Bee Richness		H'	
	df	F	p	F	p	F	p
<b>Flowers/m<sup>2</sup></b>	1	47.512	<0.0001	12.731	0.0012	1.914	0.177
<b>Treatment</b>	2	11.867	0.0002	9.450	0.0007	3.756	0.0350
<b>Interaction</b>	2	0.324	0.725	0.462	0.634	1.685	0.203
<b>Residuals</b>	30						

**Table 5:** 2-way ANOVA table showing the effects of flower species richness and vegetation treatment on wild bee abundance, species richness, and Shannon Diversity index (H'). Each data point in the analysis represents 1 of 12 plots in each of three surveys periods, for a total of 36 data points.

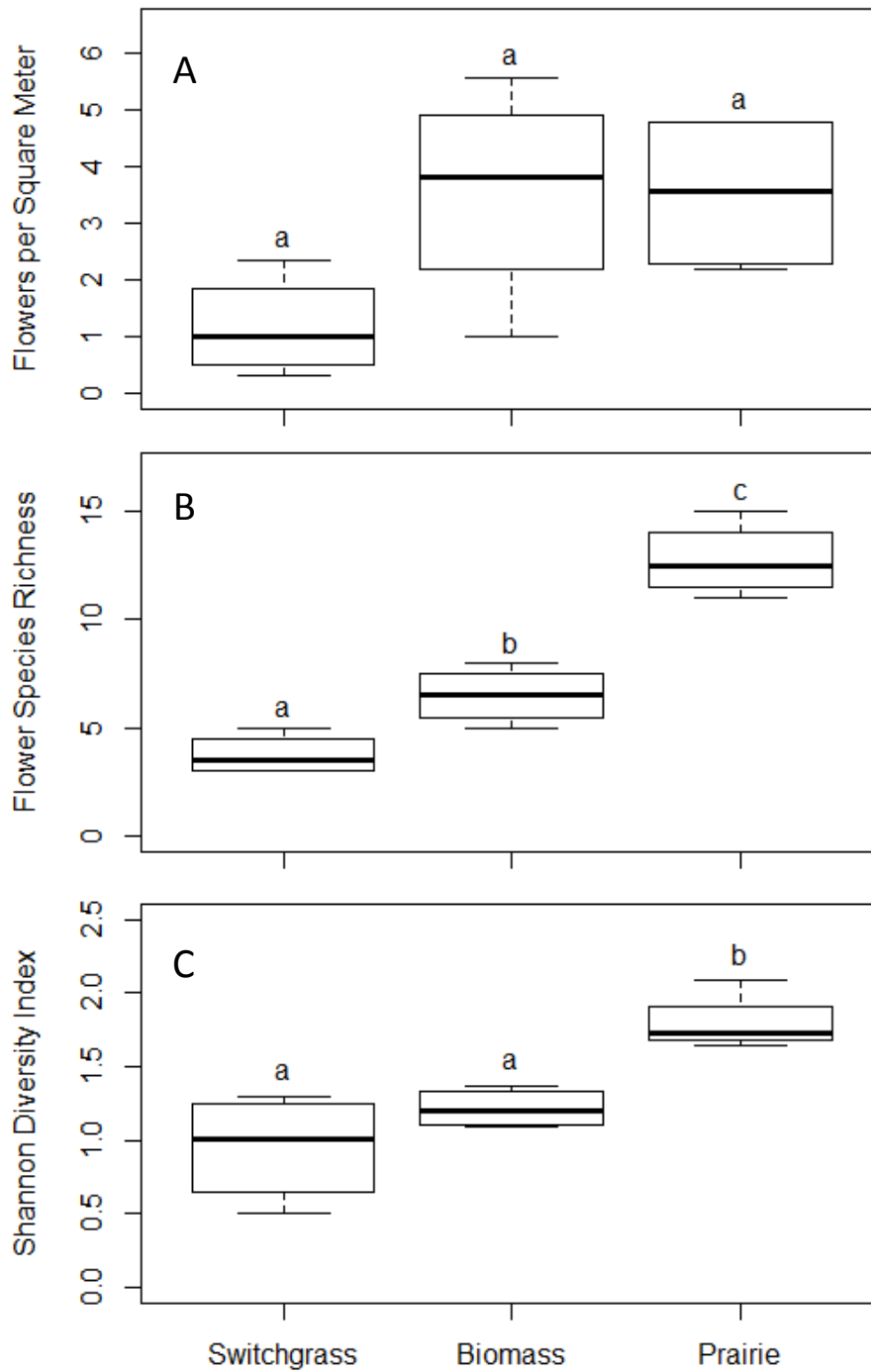
	Bee Abundance			Bee Richness		H'	
	df	F	p	F	P	F	p
<b>Flower Richness</b>	1	49.291	<0.0001	5.562	0.0251	0.189	0.667
<b>Treatment</b>	2	9.258	0.0007	11.797	0.0002	6.932	0.0034
<b>Interaction</b>	2	3.744	0.0353	1.177	0.322	2.290	0.119
<b>Residuals</b>	30						



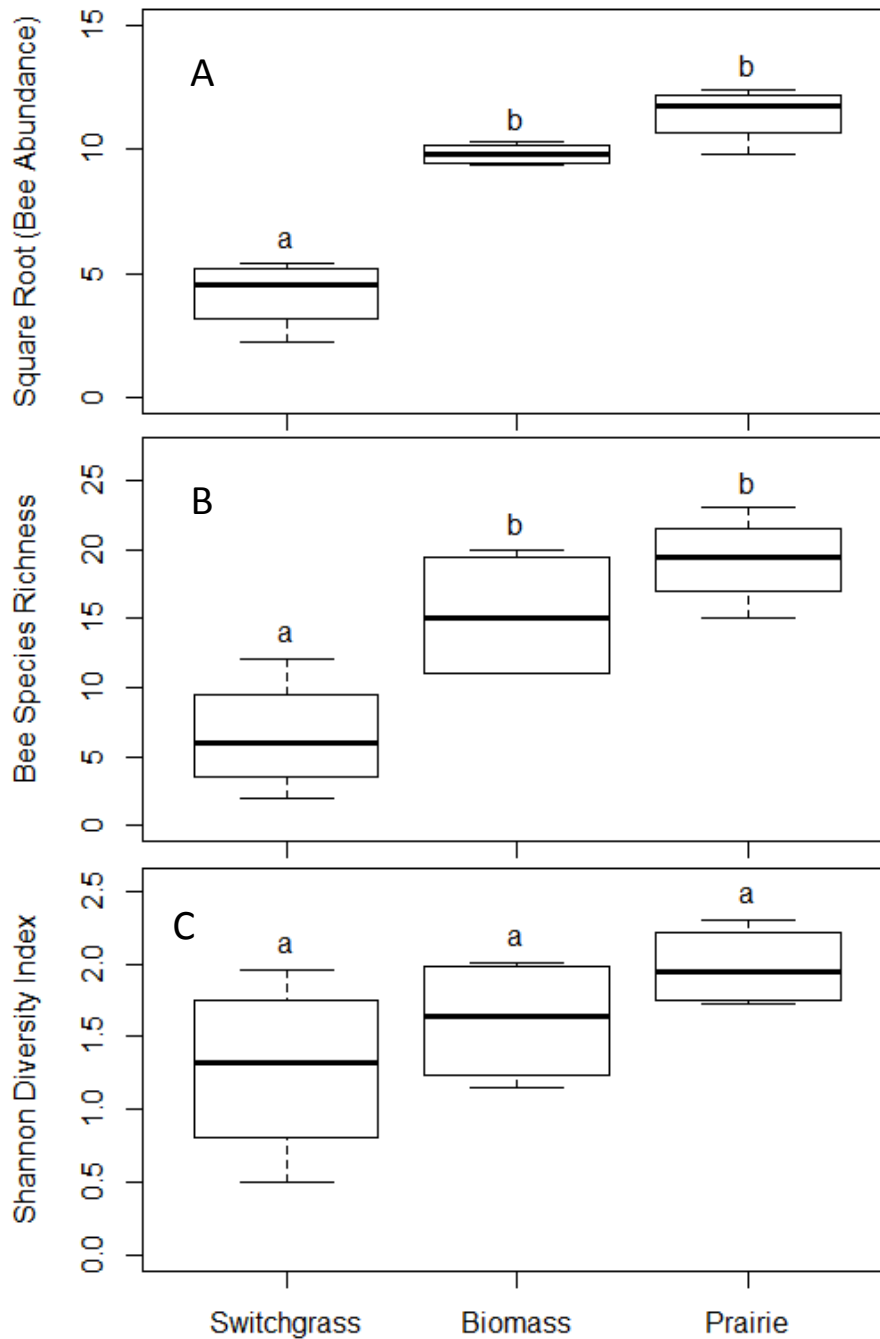
**Figure 2:** Square root transformed wild bee abundance shown chronologically and separated by treatment type. Tukey’s Honest Significant Difference statistics applied to show differences between treatment types in each survey period.



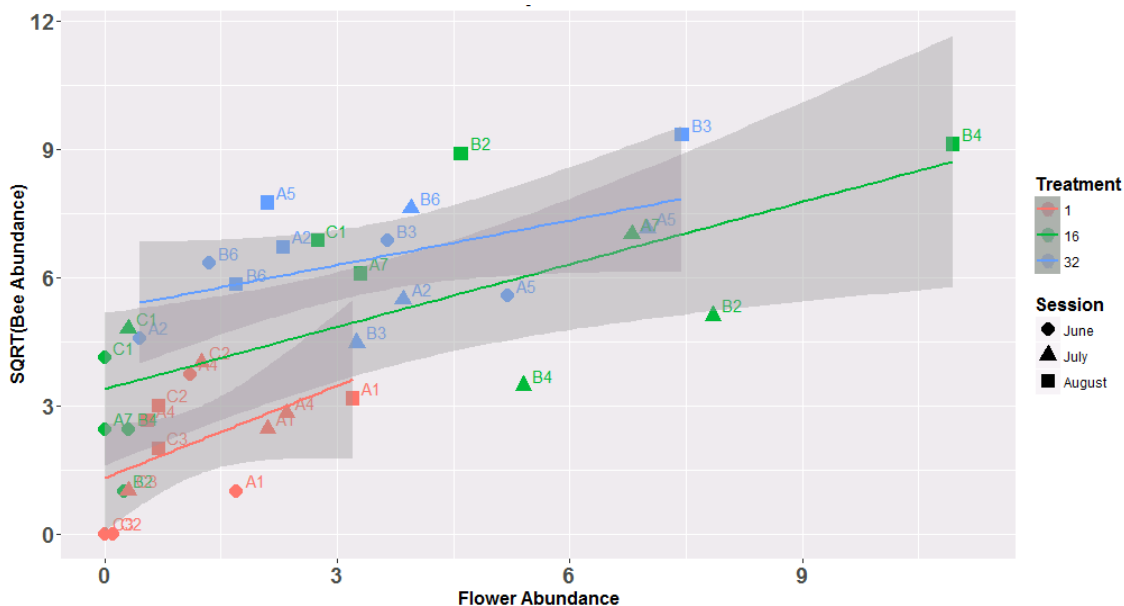
**Figure 3:** Wild bee species richness shown chronologically and separated by treatment type. Tukey’s Honest Significant Difference statistics applied to show differences between treatment types in each survey period.



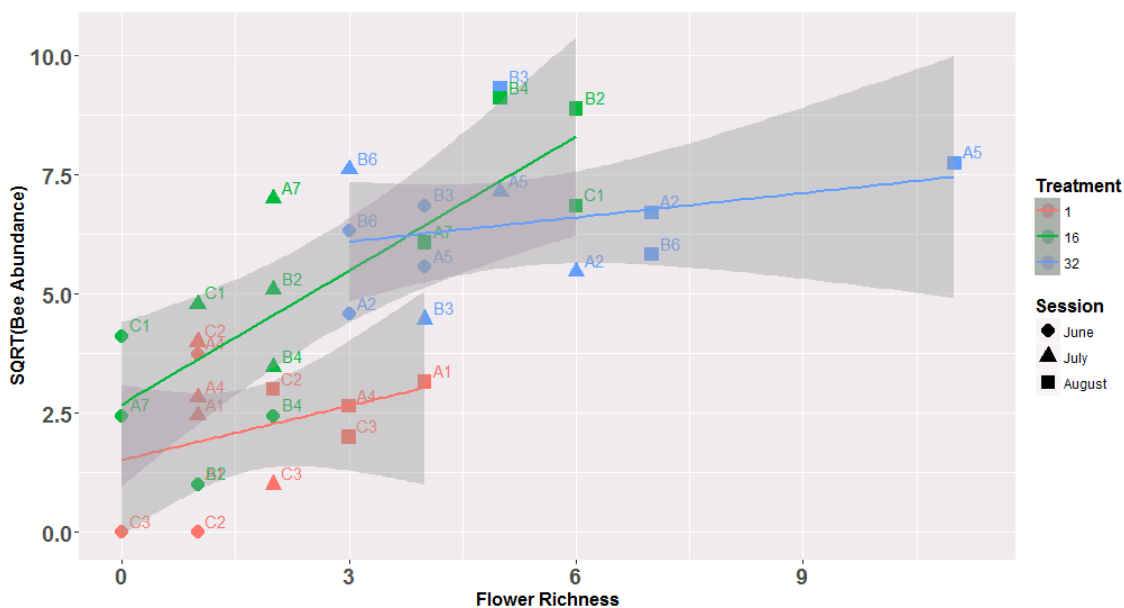
**Figure 4:** Flower abundance (A), richness (B), and Shannon Diversity index (C) for each treatment type. Boxplots represent data aggregated over all three survey periods for all plots in each treatment.



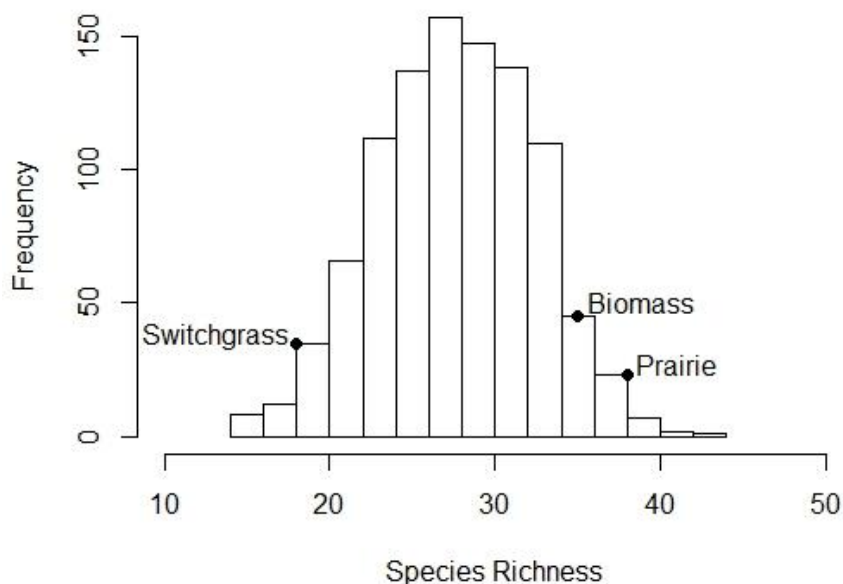
**Figure 5:** Wild bee abundance (A), richness (B), and Shannon Diversity index (C) for each treatment type. Boxplots represent data aggregated over all three survey periods for all plots in each treatment.



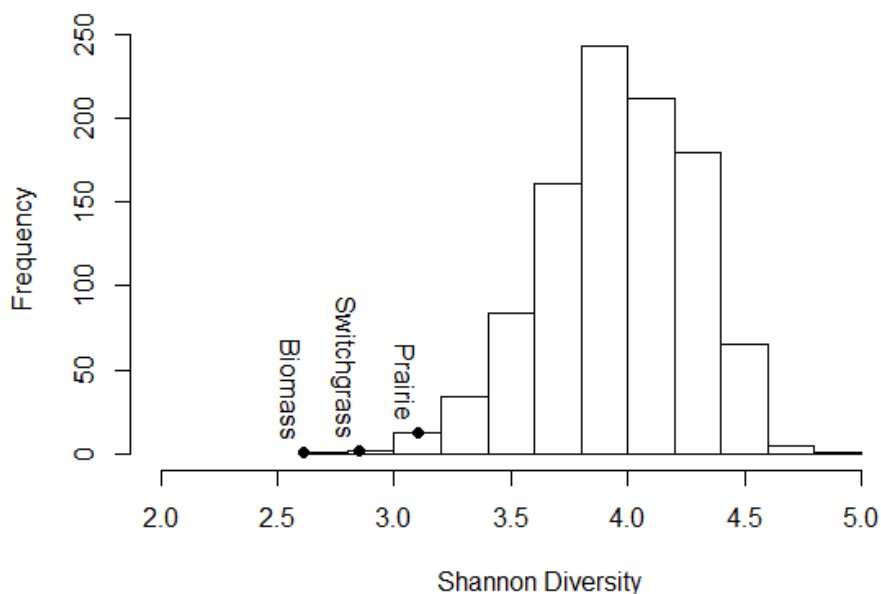
**Figure 6:** Square root transformed wild bee abundance from each plot in each survey session as a function of flowers/m<sup>2</sup> within each plot during the same survey session. Trend lines represent the linear model fit to each treatment type.



**Figure 7:** Square root transformed wild bee abundance from each plot in each survey session as a function of observed flower species richness within each plot during the same survey session. Trend lines represent the linear model fit to each treatment type, and gray shading depicts the 95% confidence interval for the line.

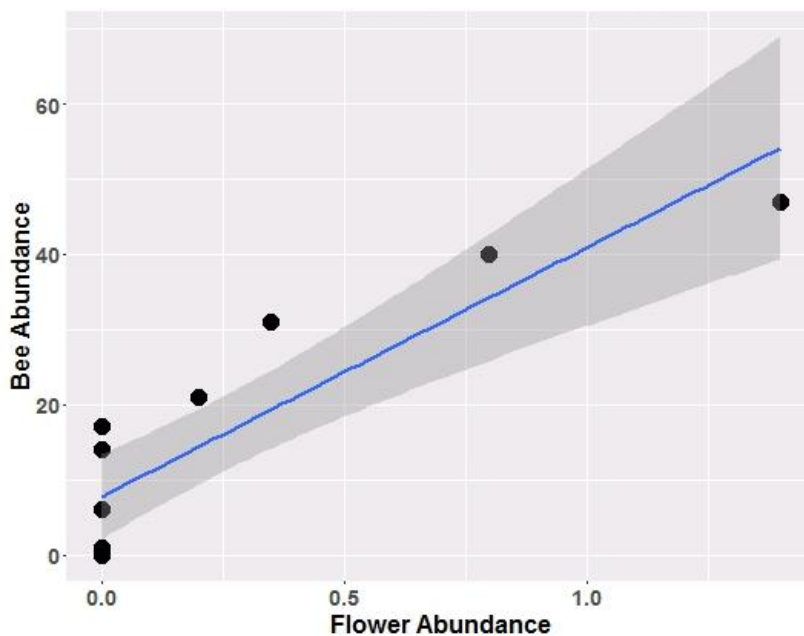


**Figure 8:** Distribution of 1,000 species richness measurements of the wild bee community at large remnant prairie reserves generated by Monte Carlo simulation with the location of the species richness for three biofuel treatment types indicated.

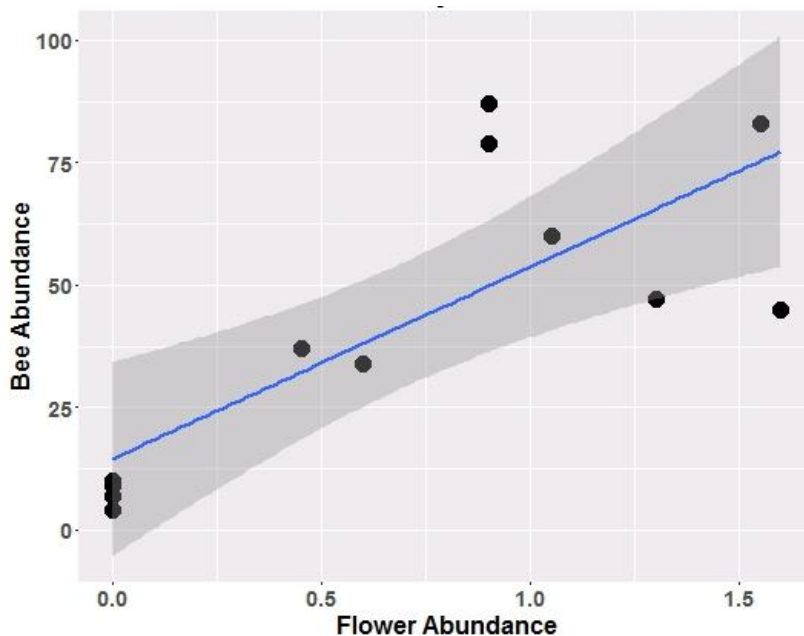


**Figure 9:** Distribution of 1,000 Log<sub>2</sub> Shannon Weiner diversity indices of the wild bee community at large remnant prairie reserves generated by Monte Carlo simulation with the location of the diversity indices for three biofuel treatment types indicated. All biofuel treatment types are located within the 1<sup>st</sup> percentile

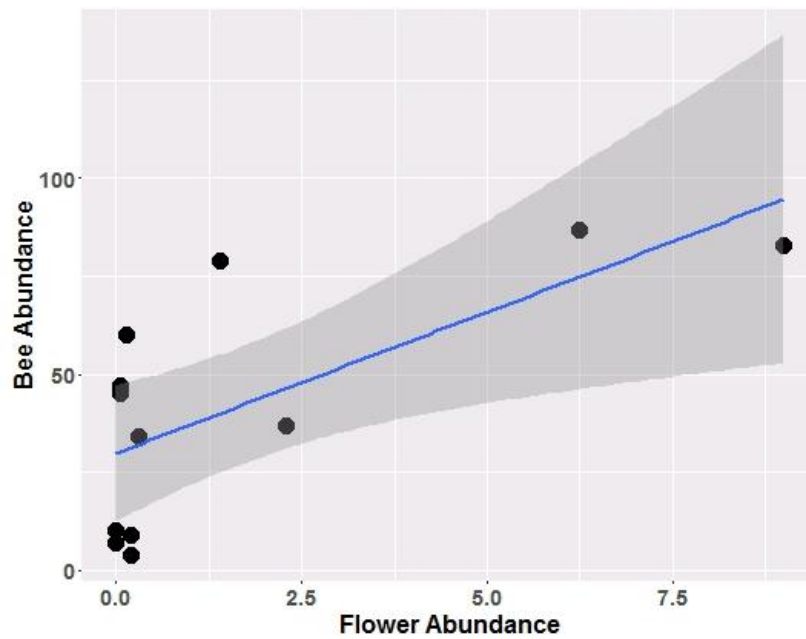




**Figure 10:** Bee abundance in each plot as a function of *Echinacea pallida* abundance. Floral abundance is expressed as inflorescences/m<sup>2</sup> observed during a survey and bee abundance is the total number of bees collected during that same month's survey period.



**Figure 11:** Bee abundance in each plot as a function of *Lespedeza capitata* abundance. Floral abundance is expressed as inflorescences/m<sup>2</sup> observed during a survey and bee abundance is the total number of bees collected during that same month's survey period.



**Figure 12:** Bee abundance in each plot as a function of *Oligoneuron rigidum* abundance. Floral abundance is expressed as inflorescences/m<sup>2</sup> observed during a survey and bee abundance is the total number of bees collected during that same month's survey period.

## Discussion

The wild bee communities utilizing biofuel candidate crops at the Cedar River Ecological Research Site vary significantly at a plot scale in response to floral resources available in each plot. Despite only marginally significant variations in flower abundance between treatments, bee abundance was higher in the biomass and prairie treatments than the switchgrass (Table 3). Furthermore, the difference in wild bee abundance between biomass and prairie plots may be biologically significant; the biomass and prairie treatments show similar abundance in all months except June, when the prairie treatment had 463% higher abundance compared to the biomass treatment (Figure 2). Thus, the prairie treatment shows a stable wild bee population throughout the growing season while the biomass treatment starts off with abundance values nearly equivalent to the switchgrass treatment in June but eventually increase to high relative abundance in August. These differences may indicate that bee abundance and, in turn, stable pollinator presence may be driven by floral resource abundance (Table 4) as well as flower species richness (Table 5). The prairie treatment plots may also provide refuge for pollinators during times of scarce resources, especially early in the growing season, allowing persistence of pollinators to support the population boom in July and August when the biomass treatments reach their peak flower abundance (Figure 2).

Species richness of wild bees may be influenced by total site flower species richness as well as other habitat factors that were not controlled for in this study design. The treatment of the site, partly as a prairie restoration and partly as a biofuel

feedstock, may contribute to the lack of difference in bee species richness between treatment types. As a result of the low-input approach to site management, absence of effort to maintain the switchgrass treatment as a pure monoculture allows invasion of plant species from nearby diverse mixes as well as weedy invasion from other sources, which accounts for 100% of flower abundance and species richness within switchgrass plots. In addition, many species collected at the site have estimates flight ranges greater than the size of individual plots, and several greater than the entire site (Benjamin et al. 2014). Bee populations persisting at the site because of floral resource abundance and temporal stability in the diverse mixes may occasionally utilize plant species invading switchgrass plots and obscure the differences in bee species richness I expected to observe within treatments with changes in flower abundance and richness (Table 4, Table 5).

Due to the randomized treatment distribution of these plots I may be able to observe the effects of fragmentation on the bee community and the possible additive effects that high-diversity plantings can have on cheaper, lower diversity areas. Biomass and prairie treatments show statistically similar flower abundance (Figure 2A) and subsequent bee abundance (Figure 3A). In plot C1 (Figure 1), which is adjacent to only switchgrass fields (C2, C3, A1) I observed lower flower abundance and wild bee abundance than all other biomass plots, although it did have similar diversity measures (Table 3). Similarly, in plot A2, which is surrounded by switchgrass (A1, A4) and a warm-season grass mixture (A3) I observed the lowest flower abundance and wild bee

abundance of all prairie plots. Conversely, prairie lot with the greatest floral and bee abundance (B3) is flanked by the two biomass plots (B2, B3) with the greatest floral and bee abundance (Table 3), eluding to the possible additive effects of higher diversity seed treatments on ones of lower diversity. These observations may be important for future management strategies. The biomass mix is designed to provide high yield as a biomass feedstock and can be made more valuable as pollinator habitat by its proximity to a higher diversity area. Pockets of a field or field margins consisting of a more expensive (Table 1) high-diversity seed mix could increase the ecosystem services of areas that are seeded with a cheaper mix designed to provide greater yield.

When comparing my site to remnant prairies (Hendrix *et al.* 2010) I find that the wild bee species richness of our site is comparable to those found at both large and small prairies (Figure 7). This suggests that my site contains the necessary diversity of resources (flora, nesting, etc.) to support diverse wild bee communities that are capable of providing pollination services to the surrounding landscape. The biomass and prairie treatments contribute to this diversity, but the switchgrass treatment does not seem to have the potential to foster a native bee community. In agreement with my second hypothesis, the high diversity treatments do resemble native prairies in terms of their wild bee species richness.

The decidedly different Shannon Diversity values of my study site compared to large and small remnants is not a result of a lack of species, but rather an issue of disproportionate abundances (Figure 8). My species composition was dominated by

two species of *Bombus* (*B. impatiens* and *B. griseocollis*) which accounted for 68.9% of the total bees collected. As a result, evenness (as part of the Shannon Diversity Index) values are extremely low for many of my experimental plots and the treatments do not compare well to surveys of native prairies. *B. impatiens* and *B. griseocollis* may represent species that are capable of quickly colonizing a site and building up substantial populations. Other species that are not capable of flying as far or reproducing as quickly may lag behind in their colonization and population growth relative to these two *Bombus* species. I predict that over time, with appropriate management, species evenness will increase as floral resources stabilize, more species become established, population sizes grow, and niches are filled in community. Long-term studies are needed to observe if this prediction will hold true, however.

Further research is needed to discover temporal patterns in wild bee abundance. While this study estimates intra-seasonal variability due to different flowering patterns, it was conducted during a single field season and does not track inter-annual variability. Of particular interest is whether the populations of *Bombus spp.* continuously cause low evenness values or if the 2015 field season was an anomaly. It would be beneficial to track the changes in abundance, richness, and diversity indices as a restoration and potential biofuel crop ages.

In conclusion, I have determined that high diversity biofuel crops are capable of supporting abundant and diverse pollinator populations, relative to lower diversity alternatives. As pressure to produce biofuels leads to land conversion, we are placed at

a crossroads where we have the opportunity to invest in cropping systems that provide wildlife habitat as well as ecosystem services (Zilverberg et al. 2014). Once industries are developed to gather, distribute, and process diverse grassland plant communities in a biofuels production context, we may see positive effects on wild bee abundance, diversity, and their subsequent ecological services they provide. Incorporating ecological principles into the management of working land may help to recover or at least stall the loss of diversity in areas of high agricultural land conversion such as the Midwestern United States.

## CHAPTER 2

### LANDSCAPE BEE COMMUNITY ANALYSIS

#### Introduction

Recent studies have found that for farms that rely upon pollinators, surrounding landscape may directly influence the bee communities at the farm (Kennedy et al. 2013). Agricultural intensification in the surrounding landscape can decrease bee abundance and diversity by destroying nesting and floral food resources as well as impacting them negatively in other ways, such as with exposure to pesticides like neonicotinoids (Prisco et al. 2013). Organic farming techniques that avoid pesticide use and provide small habitat oases are not sufficient to provide a refuge for pollinators in a landscape dominated by conventional agriculture (Brittain et al. 2010).

Not all types of wild bees respond in the same way to agricultural intensification. Large pollinators, such as *Bombus spp.* tend to be more efficient pollinators (Winfree et al. 2007), but require large areas with abundant floral resources to support their large colonies (Roulston and Goodell 2011). Thus, removal of large tracts of habitat for agricultural use, which would either reduce the quantity or temporal stability of floral resources, has been shown to negatively impact these large species (Cameron et al. 2011). Smaller species such as *Lasioglossum spp.* have relatively limited flight ranges and may actually respond positively to surrounding agricultural land cover on a small scale. For example, on blueberry farms, small species are capable of timing their flight



period to coincide with the short time that the plants are in bloom (Benjamin et al. 2014).

As a result of recent honey bee declines (Watanabe 2013), there is an increased value placed on the pollination services provided by wild bees (Gallai et al. 2008). Understanding the effect of landscape scale habitat modification within an agricultural context is an important step in planning for the utilization of wild bees as crop pollinators. In light of recent increases in row-crop agriculture (Wright and Wimberly 2013), I aim to identify the differences in bee communities at habitat oases with various degrees of isolation from natural habitat resulting from variation in surrounding row crop agriculture.

I analyzed the pollinator communities on small vegetable farms to determine if the surrounding landscape influences the wild bee communities that these farms rely upon for pollination services. I hypothesize that surrounding conventional agricultural land cover is negatively correlated with bee community indices such as abundance, species richness, and Shannon diversity index, while natural features that provide floral resources and nesting habitat (e.g. grassland, forest) are positively correlated with these community indices. I also predict that abundance of larger species will show greater correlation with these ratios than smaller species that may rely on specific on-farm factors and less on the surrounding landscape.

## Methods

### Study Sites

I conducted my research at nine small vegetable farms across the state of Iowa (Figure 1). Two of the farms are located in northeastern Iowa, four are located in south-central Iowa (near Des Moines), and three are located in east-central Iowa (near Iowa City). All of the farms are certified organic or adhere to organic practices, so wild bee populations on the farms were not influenced by on-farm pesticide use, though pesticide use on adjacent farms could have influenced these sites.

### Bee Collection Protocol

Farms were sampled once a month from May to August, 2015. The schedule was subject to change based on weather, as I only collected when the forecast predicted temperatures greater than 15.5°C and wind speeds less than 32 km/hr. Bees were collected using a combination of pan trapping and sweep netting techniques. I established a grid in a central location on each farm that encompassed a total area of 750 m<sup>2</sup> in which we evenly distributed 18 pan trap poles. During each collection period, a white, blue, or yellow fluorescent cup was randomly placed on each pan trap pole and filled approximately half full with soapy water, left for 24 hours, and collected the next day at approximately the same time. Bees were sweep netted for 20 minutes within 5m of the pan trapping area on one of the two days we visited the farm. Once a bee was captured we stopped the timer and thus the 20 minutes only accounts for searching time and does not include handling time.

### Bee Processing and Identification

Bee samples were transferred back to the lab, cleaned with 70% ethanol and pinned or attached to points. Bees were identified to genus using Michener et al. (1994), Mitchell (1960, 1962), and Arduser (2015).

### Landscape Analysis

I analyzed land cover within 1km of our 750m<sup>2</sup> pan trapping grid using ArcGIS. The land-cover data came from the Natural Resources Geographic Information System (NRGIS) maintained by the Iowa Department of Natural Resources (DNR). The High Resolution Land-Cover map was produced using aerial images collected in 2007, 2009, and 2010. It has a spatial resolution of one meter. Land was classified into 15 cover types based on these aerial images using remote sensing techniques. For the purposes of my analysis, I grouped these 15 land-cover types into the following categories: 1) All of the forest categories were grouped as "Forest" (Coniferous Forest, Deciduous Short, Deciduous Medium, and Deciduous Tall); 2) Grass 1 and Grass 2 were grouped together as "Grassland"; 3) Corn and Soybeans were grouped together as "Row-Crop". These groupings represent the three most abundant cover types and also three very different habitat types for bees, in terms of both floral resource availability and nesting substrate (Lentini et al. 2012). 4) Additionally, I also summed the Forest and Grassland areas together as the "Natural Habitat" land-cover to look at their combined effect on wild bee communities. Other cover class such as wetland, water, barren/fallow, and structures were not included in the analysis, as none exceeded 0.6% of total area

around farms. Roads/impervious were also not included in the analysis, although the coverage averaged 3.3% of total area, including one instance of 11.0%

### Statistical Analysis

Survey periods in which not all farms were sampled were excluded from the data for analysis to maintain a constant sampling effort among farms. I calculated the community indices (i.e. abundance, species richness, and Shannon-Weiner diversity index) for each sampling event and farm. I then used one-way repeated measures multiple regression ANOVA to compare the difference of these bee indices among landscape scale factors within 1km of the farm. I also combined the data from all four months to calculate the overall community indices for each farm over the entire sampling season. I used linear regression to examine the correlation between the bee community indices and landscape factors. To determine if landscape influenced community composition, I used a PERMANOVA to examine the correlation between land-cover type and bee community composition, pooled across all four months. I also used the pooled four month data to calculate NMDS scores using a Manhattan distance metric, and used one-way ANOVA to determine if surrounding agricultural land-cover influenced NMDS scores. I used a Manhattan distance metric to calculate NMDS scores, which quantifies abundance of each species as well as includes data from species that are absent in one sample and present in another. Using this method, samples that both lack the same species may be considered more similar and may appear gravitate closer together in multidimensional space. I chose to use the Manhattan model, which

includes joint absences, because it is capable of providing greater resolution in situations where many species are rare or narrowly distributed. In addition, sparse samples tend to inflate measures which exclude joint absences (Anderson et al. 2011).

Since body size and flight ability may change the influence of different cover types (Benjamin et al. 2014), I separated the wild bees into two groups for a secondary analysis using the size class criteria from Benjamin et al. (2014). Large bees comprised 205 of the total individuals and were from the genera *Andrena*, *Anthophora*, *Bombus*, *Eucera*, *Megachile*, *Melissodes*, *Nomada*, *Stelis*, and *Tetraloniella*. Small bees comprised 895 of the total individuals and were from the genera *Agapostemon*, *Augochlora*, *Augochlorella*, *Halictus*, *Hylaeus*, and *Lasioglossum*. *Lasioglossum* spp. represented 623 of the total bees collected. I used the same repeated measures one-way ANOVA, NMDS, and PERMANOVA tests to compare abundance of these groups of bees to landscape scale factors within 1km of the farm.

## Results

In total, I collected 1100 wild bees from 15 different genera from the pan traps and 81 wild bees from 16 different genera from sweep netting. The total number of wild bees trapped at each farm over the whole season ranged from 28 to 240, and number of wild bee genera ranged from 5 to 11. Percentage of surrounding traditional corn and soybean agriculture within 1km ranged from 14.5% to 77.4%. Grassland ranged from 7.1% to 58.9% and forest ranged from 1.9% to 22.4%.

Wild bee genera richness and Shannon diversity index varied significantly with surrounding cover types (Table 6). Both indices increased with surrounding grassland (Figure 13) and forest (Figure 14) land cover and decreased with increasing corn/soy (Figure 15) agricultural land cover. Wild bee abundance was not significantly influenced by any of the surrounding landscape factors (Table 6). When I analyzed data subsets separately for large and small wild bee categories, neither group showed results that were qualitatively different from the trends observed in the whole data set. There was a slight but non-significant difference in the response of large and small bees to agricultural land cover. Small bees tended to be more negatively by increasing agricultural dominance than large bees were (Figure 16), but this difference was not significant.

NMDS analysis revealed no significant shift in community composition as a result of changes in surrounding land cover, shown by an absence of distinctly clustered

groups in figure 17. PERMANOVA results (Table 7) confirmed no significant shift in the community composition as a result of changing land cover in the surrounding landscape.

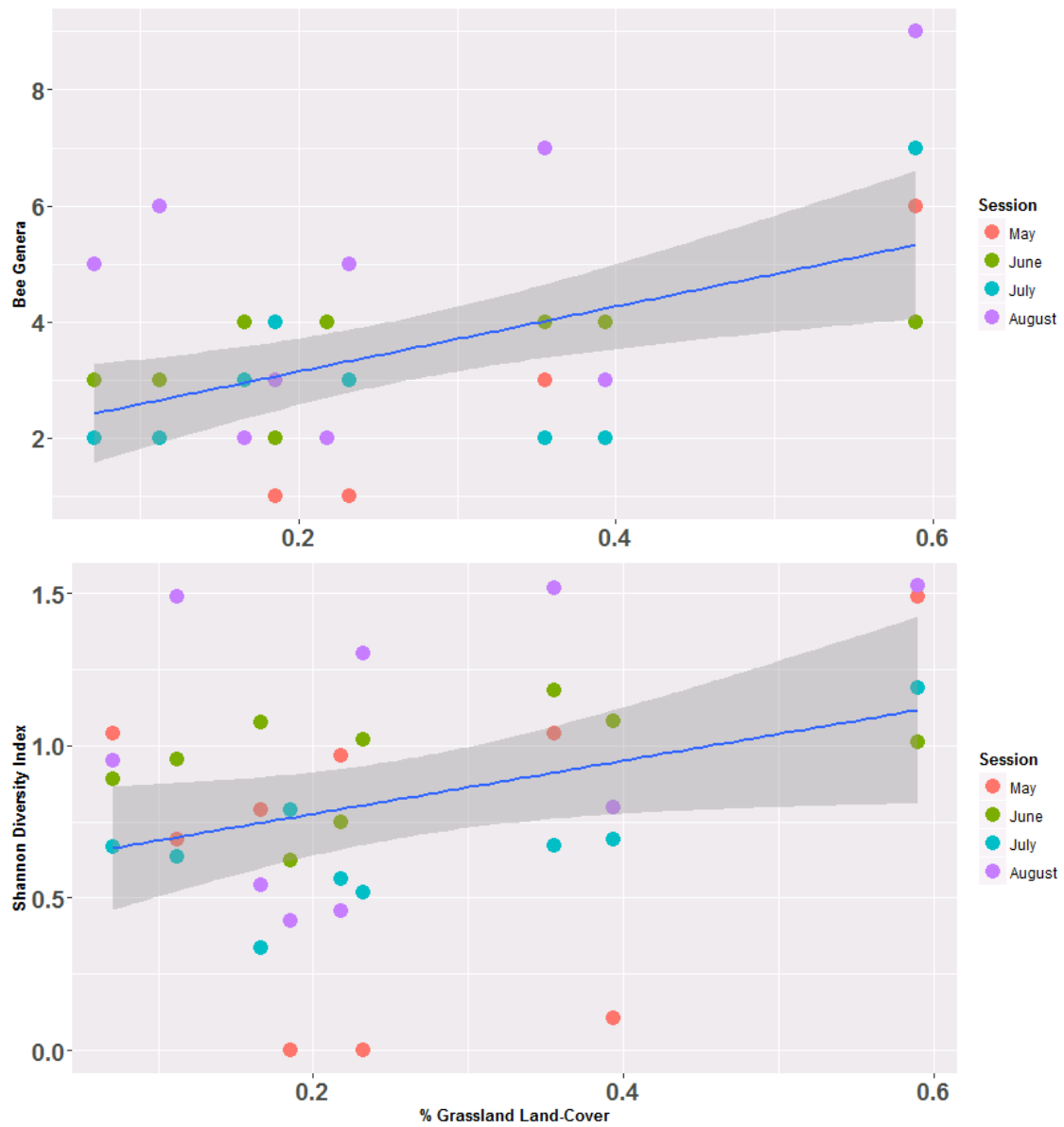
**Table 6:** Repeated measures ANOVA table showing the response of wild bee abundance, richness, and Shannon Diversity ( $H'$ ) as they relate to surrounding land use within 1 km of the pan trapping grid. Grassland represents summed Grass 1 and Grass 2. Forest is comprised of all deciduous and coniferous forest. Agriculture represents all land that was conventionally farmed as corn or soybeans.

Source	df	Abundance		Richness		$H'$	
		F	p	F	p	F	p
<b>Grassland</b>	1	0.099	0.755	15.92	0.00041	5.816	0.0224
<b>Forest</b>	1	2.973	0.095	4.762	0.03904	5.353	0.0280
<b>Agriculture</b>	1	0.689	0.413	5.860	0.02198	2.411	0.1314
<b>Residuals</b>	29						

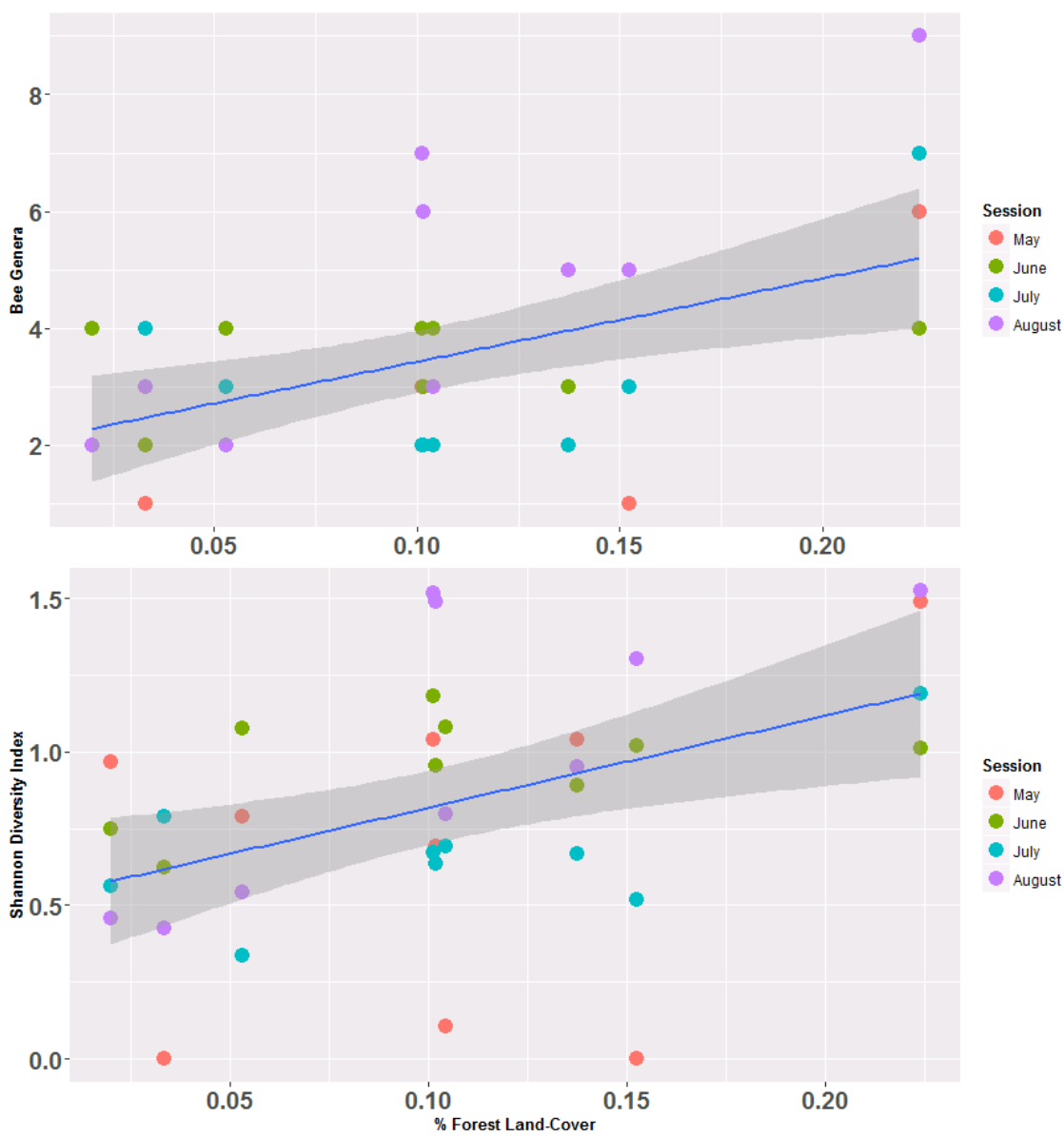
**Table 7:** PERMANOVA analysis output showing interactions between changes in wild bee community composition as they relate to surrounding land use within 1 km of pan trapping grid. Grassland represents summed Grass 1 and Grass 2. Forest is comprised of all deciduous and coniferous forest. Natural is the sum of grassland and forest. Agriculture represents all land that was conventionally farmed as corn or soybeans.

Source	df	F	p
<b>Agriculture</b>	1	0.49585	0.7642
<b>Forest</b>	1	0.74827	0.5563
<b>Grassland</b>	1	0.29406	0.8835
<b>Residuals</b>	5		

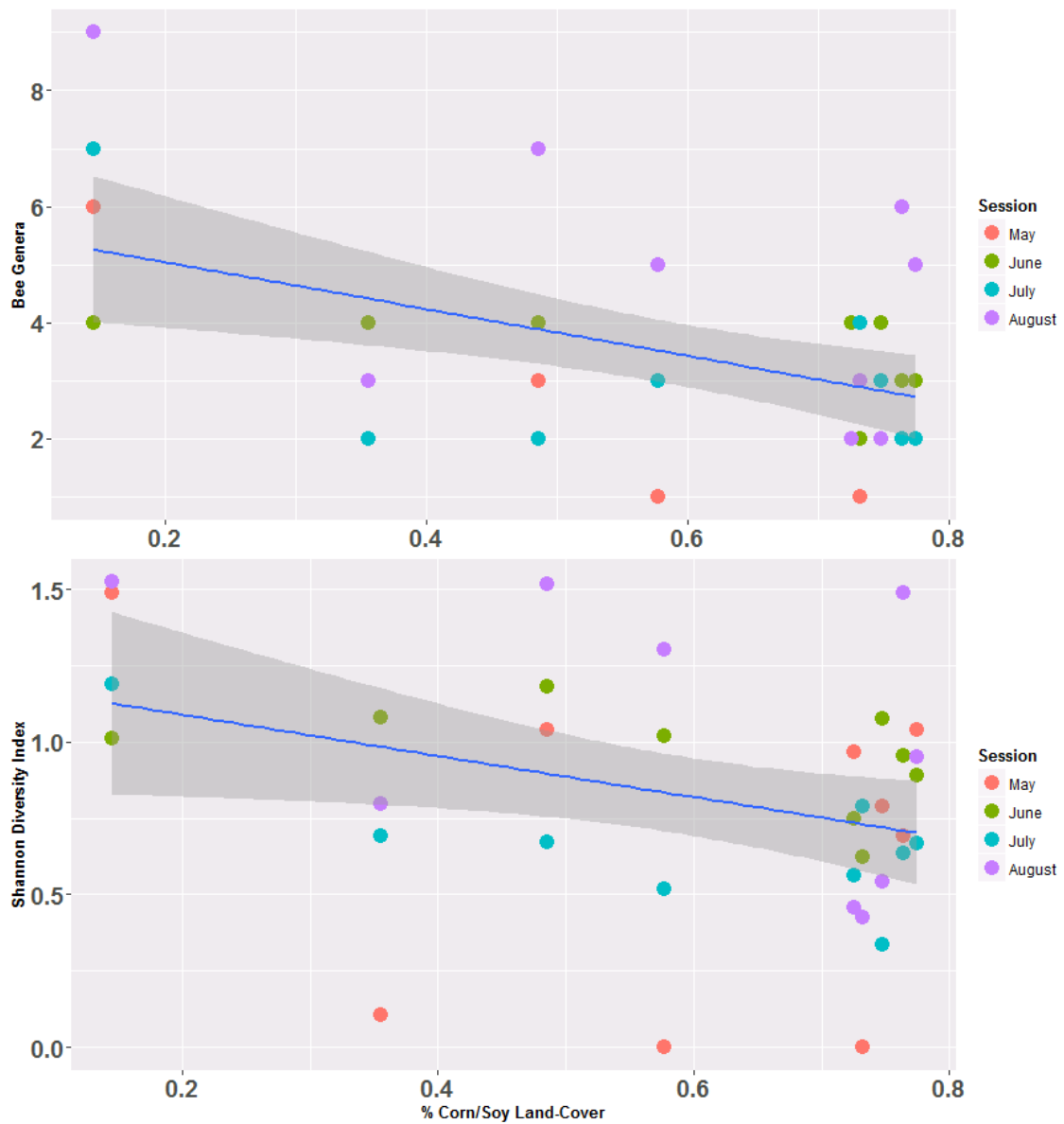




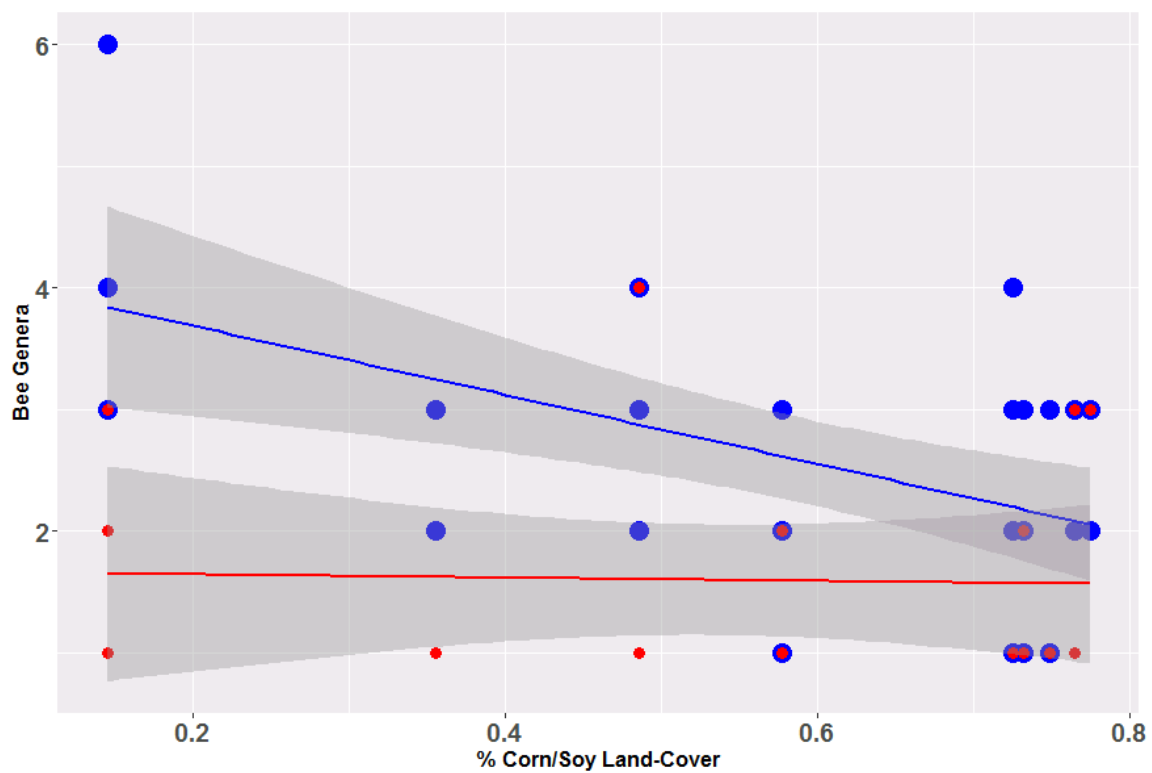
**Figure 13:** Wild bee genera richness and Shannon Diversity index from each farm in each survey session as a function of percentage grassland cover within 1km<sup>2</sup>. Trend lines represent the linear model fit with a 95% confidence interval.



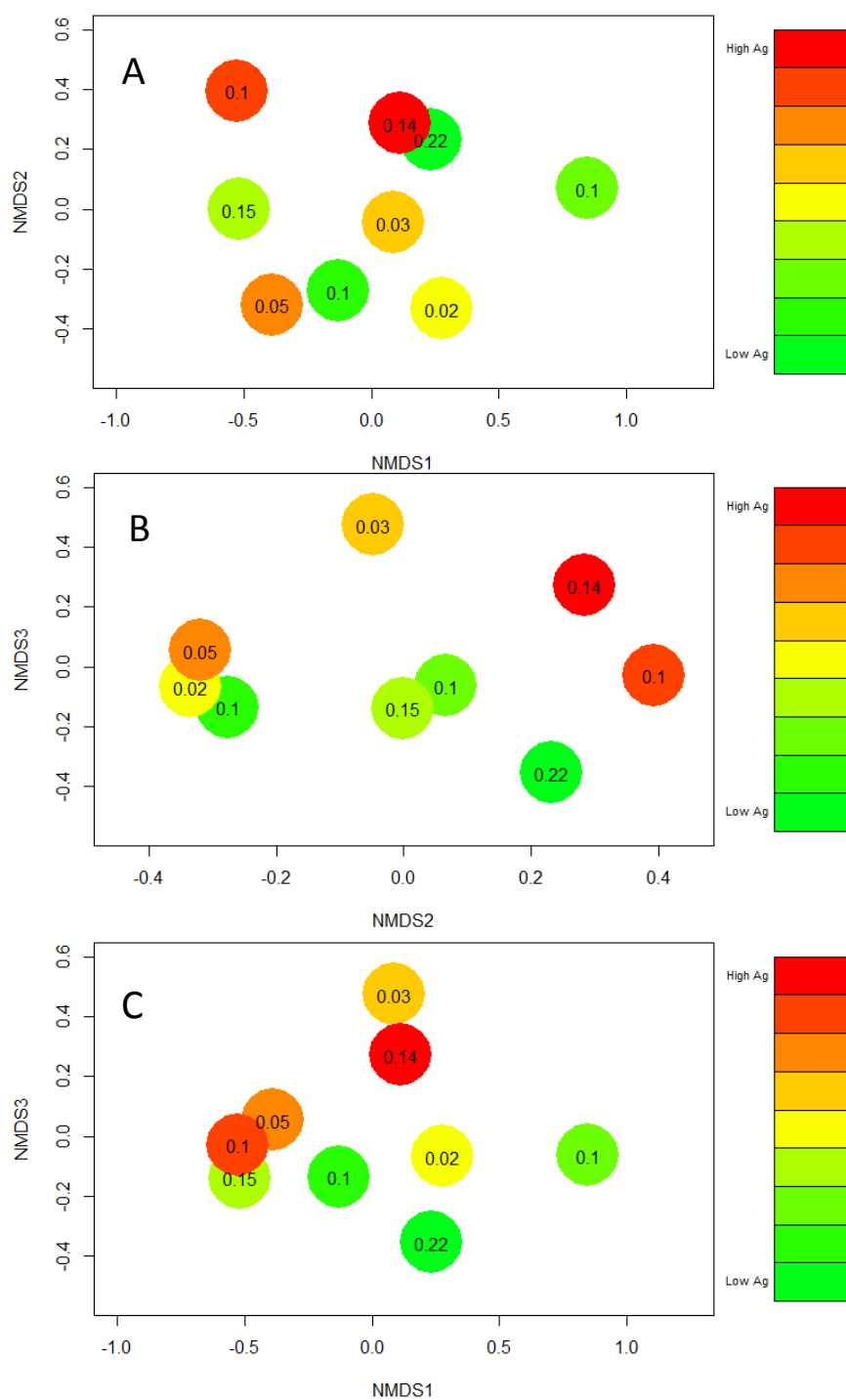
**Figure 14:** Wild bee genera richness and Shannon Diversity index from each farm in each survey session as a function of percentage forest cover within 1km<sup>2</sup>. Trend lines represent the linear model fit with a 95% confidence interval.



**Figure 15:** Wild bee genera richness and Shannon Diversity index from each farm in each survey session as a function of percentage corn/soy cover within 1km<sup>2</sup>. Trend lines represent the linear model fit with a 95% confidence interval.



**Figure 16:** Genus richness for small (blue) and large (red) bees from each farm in each survey session as a function of percentage corn/soy cover within 1km<sup>2</sup>. Trend lines represent the 95% confidence interval of a non-standard model fit for each group.



**Figure 17:** Axes 1 and 2 (A), 2 and 3 (B) and 1 and 3 (C) of a 3-dimensional non-metric multidimensional scaling analysis with overlain species vectors and ranked relative corn/soy land-cover within 1km<sup>2</sup> represented by color. Values located within each wild bee community represent proportion of forest land-cover.

## Discussion

As I hypothesized, the number of genera increased with grassland and forest cover. This could be the result of a combination of increased nesting substrate and floral resources near those farms. Undisturbed fallow fields, prairies, and wetlands provide an important refuge for ground or grass-dwelling species while forest areas provide a different suite habitat such as rotten wood, cavities, beetle holes, and bare ground (Arduser 2015). *Augochlora pura*, for example, is a species which nests only in rotten wood (Arduser 2015) and was only found at the location that contained the highest percentage of forest cover within 1km.

Contrary to my hypothesis, abundance values for wild bee communities showed no correlation with landscape factors within 1km (Table 6). I predicted that larger species may show some correlation while smaller species may not, and I was unable to show evidence of such a correlation. This may be due to the high relative abundance of small bees collected during the surveys. Of the 1100 wild bees, 623 were of the genus *Lasioglossum* and 201 were of the genus *Agapostemon*. The lack of large species could be a result of the homogenous landscape characteristic of Iowa which has driven the declines of many of our large-bodied species, particularly *Bombus spp.* (Cameron et al. 2011). To determine the effect of landscape on the abundance of bees, I believe that increasing sampling effort and/or changes in techniques to monitor exclusively large bees may be necessary to observe these differences.

No significant interactions were revealed through the NMDS analysis, although the proximity of certain communities may give insight into the effects of habitat heterogeneity on wild bee community composition (Figure 17). Axis 3 tends to group wild bee communities with low percentage of surrounding agriculture closer together. Axis 2 separates bee communities with higher percentage of forest from those with lower percentage forest. Due to the abundance of common species, rare species may have been underrepresented in the community samples. Different techniques or increased sampling effort may be necessary to observe differences in community composition using NMDS analysis.

In conclusion, land use practices in the surrounding landscape do affect the number of species in a farm, but may not strongly affect the number of individual bees or the community composition. The notable absence of large species, such as bumble bees, may have obscured the effect of landscape characteristics on total abundance, however. Small bee species, which comprised 81% of our total bees, are likely impacted by on-farm habitat characteristics more than landscape-scale factors. Our inability to detect differences in bee abundance in the large bee community could be a result of small sample size or, as other research suggests (see Cameron et al. 2011, Potts et al. 2010), a vestige of impoverished bee pollinator communities caused by over a century of agricultural intensification. Current bee communities, composed of small species, presumably persist in conjunction with spatially and temporally limited floral resources in a fragmented landscape (Kwaiser and Hendrix 2008). In order to benefit pollinators,

future land management strategies will need to consider habitat heterogeneity and connectivity in order to improve metrics of wild bee communities (M'Gonigle et al. 2015).



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