A MASS FLOWERING CONUNDRUM: DO THE BENEFITS OF CANOLA OUTWEIGHT THE NEGATIVE EFFECTS OF LAND USE CHANGE ON WILD BEES?

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Abstract: In light of the burgeoning human population, the conservation of ecosystems services that sustain agricultural production, such as pollination, is essential. Wild bees are important pollinators of many crops and wild flowering plants. However, agricultural intensification reduces nesting and foraging resources for wild bees and is a major contributor to their decline worldwide. Mass flowering crops may increase foraging resources that support wild bees. In the southern Great Plains canola (*Brassica napus* L.) was introduced in the early 2000s as a rotational crop with winter wheat and greatly increases foraging resources for wild bees in this historically forage-poor region. In turn, wild bees are known to improve canola seed set. The addition of canola may enhance or dilute pollination of wild flowering plants. Despite increased foraging resources from canola, agroecosystems in the southern Great Plains may not provide sufficient nesting habitat for wild bees, potentially outweighing the benefit of increased foraging resources. Our research demonstrated that increased canola cover is associated with increased wild bee abundance and richness across the growing season and wild bee pollination significantly improved canola seed set in this this study system. Three species of wild plants increased in seed set as canola cover increased, suggesting that canola may cause pollination service spillover. We also found that increased anthropogenic land use cover is negatively correlated with wild bee abundance and richness and, further, affected the body size of three wild bee populations. Canola plays an important role in the agroecosystems of the southern Great Plains and may be an important resource to wild bees in this region.

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CHAPTER I

INTRODUCTION AND SYNTHESIS

Introduction

Wild bee status, trends, and threats

Wild bees, those species that are native to a particular region and occur in wild populations, face precipitous declines worldwide (Zattara and Aizen, 2021). Myriad factors contribute to wild bee declines including: climate change (Kelemen and Rehan, 2021; Kerr et al., 2015; Willmer, 2014), pests and pathogens (Bosmans et al., 2018; Colla et al., 2006; Ii and Quandt, 2020; Otterstatter and Thomson, 2008), invasive species (Paini, 2004; Thomson, 2016), land use change and habitat degradation (Kremen et al., 2007), and agrochemical use (Lämsä et al., 2018; Main et al., 2021). Because wild bees provide essential pollination services to wild flowering plants and contribute significant pollination services to entomophilous crops, their conservation is essential to ecosystem functioning and food security (Bailes et al., 2015; Potts et al., 2016; Requier et al., 2019).

Wild bees require nectar and pollen resources from flowering plants, both wild and cultivated, and suitable nesting substrates. Nesting substrates vary by wild bee species, the majority of wild bees nest in exposed soils whereas other species nest in unoccupied cavities or burrows and pithy stems and require additional nesting materials such as mud, resin, leaves, and flower petals (Potts et al., 2005; Torné-Noguera et al., 2014).

Floral resource availability is an important driver of wild bee abundance and diversity. Wild bees require diverse floral morphologies (Fenster et al., 2004). The majority of wild bees have short proboscises and are considered generalist species, visiting open-shaped flowers with short corollas such as those found in the Asteraceae and Brassicaceae families (Inouye, 1980). Other bees, with long proboscises, are considered specialists and can forage on flowers with long or short corollas such as those in the Lamiaceae and Scrophulariaceae families (Inouye, 1980). Diverse floral communities, therefore, may support diverse wild bee communities. Additionally, wild bees require foraging resources throughout their active period which may include only a part of or the entire growing season, depending on the wild bee species. Variability of flowering phenology within the flowering plant community is critical to sustain abundant and diverse wild bee assemblages (Ogilvie and Forrest, 2017). Wild bee abundance is tightly linked to inflorescence density, flower abundance, and nectar and pollen quantity (Hegland and Boeke, 2006; Potts et al., 2003). Robust bee communities require diverse and abundant foraging resources to thrive.

Suitable habitats for wild bees may contract because of climate change (Potts et al., 2010). Climate change may spatially and temporally change resource availability for wild bees (Gordo and Sanz, 2009; Lawson et al., 2018; Rafferty and Ives, 2011). Phenological mismatch, or the decoupling of bee-plant mutualisms because of asynchronous responses to climate change may also threaten both wild bees and wild flowering plant populations (Kudo and Ida, 2013; Petanidou et al., 2014). Warming temperatures were also correlated with decreased bee body size which may further contract wild bee population distribution as body size predicts maximum flight distances (Kelemen and Rehan, 2021). Climate change may have direct effects on wild bees but it may also have indirect effects as it changes resource and nesting habitat availability, agricultural practices, and pest and pathogen distribution (Goulson et al., 2015; Kerr et al., 2015; Owen, 2015; Potts et al., 2010).

Non-native *Apis mellifera* (honey bee) colonies, both managed and feral, increase competition for foraging and nesting resources and increase pest and pathogen spillover intro wild bee populations (Geldmann and González-Varo, 2018; Ii and Quandt, 2020; Manley et al., 2017; Otterstatter and Thomson, 2008; Thomson, 2016). Wild bees, unlike honey bees, exist in small eusocial or semi-social groups or as solitary individuals. Competition with honey bees caused decreased visitation and reproduction in a wild bee species (Hudewenz and Klein, 2015). Pests and pathogens that are spread easily amongst commercially reared honey bees can invade wild bee populations when commercial honey bee hives are used to pollinate various crops nationwide (Colla et al., 2006; Ii and Quandt, 2020).

Land use change and the subsequent habitat fragmentation, degradation, and loss that occurs and is a major contributor to wild bee decline (Goulson et al., 2015). Land use change affects foraging and nesting resources and connectivity and proximity of suitable nesting and foraging habitats for wild bees. One major driver of land use change is agricultural intensification, which is increasing to keep pace with demand for food production for the growing human population (Potts et al., 2016). Agricultural intensification is characterized by homogenization of the landscape as natural and semi-natural areas are converted to crop fields and increased use of agrochemicals. Loss of landscape heterogeneity is linked to decreases in wild bee abundance and diversity (Flores et al., 2018; Jha and Kremen, 2013; Moreira et al., 2015). Habitat fragmentation and increased presence of monocultures require wild bees to forage for longer periods and more frequently, which may be particularly detrimental to female wild bees provisioning nests (Meehan et al., 2011). Simplified landscapes are also linked to decreasing bee body size which affects foraging range and speed, metabolic rate, thermoregulation, survival, fecundity, and pollination efficacy among wild bee species (Elzay and Baum, 2021; Greenleaf et al., 2007; Nagamitsu et al., 2018; Renauld et al., 2016). Agrochemical use has long been linked to lethal and sublethal effects on wild bees including changes and reductions in foraging behaviors and rates, decreased reproduction rates, and increased susceptibility to pathogens (Crall et al., 2018; Doublet et al., 2015; Lämsä et al., 2018; Stanley et al., 2015). Agricultural intensification may contribute significantly to wild bee declines, particularly in regions where agriculture is a major contributor to the regional economy.

Canola in the southern Great Plains

Since the mid-19th century, the southern Great Plains has increasingly been converted to winter wheat fields and rangeland, causing declines in natural and semi-natural habitats. The loss of these habitats, increase in the presence of monocultures of winter wheat, and the use of herbicides in pastures has contributed to declines in wild bee foraging and nesting habitats (Holzschuh et al., 2007). However, in the early 2000s, canola (*Brassica napus*) was introduced as a rotational crop that helps to decrease disease, pest, and weed pressure on winter wheat (Franke et al., 2009). Canola produces nectar and pollen resources that attract wild bees. Via pollination, wild bees enhance canola yield significantly regardless of the presence of commercial honey bee colonies (Zou et al., 2017). In some studies, mass flowering crops (MFC) contributed to increased wild bee abundance and diversity (Hanley et al., 2011; Holzschuh et al., 2013; Rao and Strange, 2012; Rundlöf et al., 2014; Westphal et al., 2003) and increased reproductive rates (Jauker et al., 2012). However, other studies indicate that MFCs may provide resources for wild bees but do not affect wild bee abundance, diversity, or reproduction (Galpern et al., 2017; Kovács-Hostyánszki et al., 2013).

Other effects of mass flowering crops that may impact wild bees include increased agrochemical use to suppress pests and pathogens, changes in the nutrient diversity available to wild bees, and dilution or enhanced pollination services to wild flowering plants nearby. In the southern Great Plains systemic pesticides are used on canola to suppress aphid pests, potentially exposing wild bees that forage on canola fields to harmful neonicotinoids (Franke et al., 2009; Reddy, 2017). Canola provides abundant nectar and pollen but the nutrient diversity available to wild bees foraging on canola is less diverse than were they to forage on diverse wild flowering plants (Carruthers, 2016). Conversely, because canola blooms early in the growing season in the southern Great Plains, the abundant resources it provides before many wild flowering plants are available may be particularly important to wild bees and their reproductive success as many females are provisioning nests early in the growing season (Carruthers, 2016; Jauker et al., 2012; Westphal et al., 2009). Wild bees may preferentially pollinate canola causing a reduction in wild plant pollination and potentially inbreeding depression and population declines among wild flowering plants (Holzschuh et al., 2011). Alternatively, wild bees attracted to canola fields may increase pollination rates of nearby wild flowering plants (Stanley and Stout, 2014).

Results and synthesis

In the following three chapters, I focus on how canola has altered wild bee-plant interactions in the southern Great Plains. In chapter two, I present the effects of land uses, local foraging and nesting resources, and the presence of canola versus wheat on wild bee abundance and generic richness across the growing season. My results suggest that anthropogenic land uses and decreased local foraging resources were correlated with decreased wild bee abundance and generic richness. The presence of canola, however, was correlated with increased bee abundance and generic richness not only during canola bloom but also across the growing season. My results indicate that mass flowering crops, like canola, may play a critical and long-lasting role in supporting wild bees in the southern Great Plains agroecosystem.

In chapter three, I investigate how land use and bee pollination affect canola seed count and total seed weight (g). I found that wild bees contribute to increased canola seed count and total seed weight (g). Land use and wild bee abundance and generic richness interact to affect canola seed set and my results suggest that more research is needed to understand these complex interactions.

In chapter four, I endeavor to understand how canola impacts the pollination of three species of wild flowering plants found in the southern Great Plains. Each of the wild flowering plant species experienced pollen limitation in this agroecosystem. Increased canola cover was correlated with increased seed set in each species of wild flowering plant. My results suggest that canola may foster pollination of wild flowering plants which could have long-lasting effects on wild flowering plant populations in this region.

Conclusions

Agricultural intensification and land use change may contribute to wild bee decline (Potts et al., 2016). However, mass flowering crops may provide critical resources for wild bees in agroecosystems and may even support wild bee-wild flowering plant mutualisms. My results demonstrate that canola may be an important foraging resource for wild bees in the southern Great Plains. In turn, wild bees may improve canola yield. This research provides new avenues in which to continue to investigate the effects of mass flowering crops on wild bees and wild bee-wild flowering plant mutualisms.

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CHAPTER II

CANOLA POSITIVELY INFLUCENCES WILD BEE ABUNDANCE AND RICHNESS

Abstract

Agricultural land use conversion reduces nesting and foraging resources for wild bees and is a major contributor to their decline worldwide. Mass flowering crops (MFCs), however, may increase foraging resources that support wild bees in some agroecosystems. Canola (*Brassica napus* L.) was introduced in the southern Great Plains in the early 2000s as a rotational crop with winter wheat and greatly increases foraging resources for wild bees in this historically forage-poor region. Land use within agroecosystems may influence wild bee abundance and richness by altering nesting and foraging resources available after MFCs senesce. We evaluated how wheat and canola cover, land use, and local forage and nesting resources available alongside fields affected wild bee abundance and generic richness in north-central Oklahoma. We found that canola cover is associated with increased wild bee abundance and generic richness during canola bloom and throughout the growing season. Increased crop cover, developed landscapes, and decreased roadside foraging resources were all negatively correlated with wild bee abundance and generic richness. MFCs may provide critical resources in the early growing season that boost bee abundance and generic richness throughout the active period of wild bees. However, there are complex relationships between land cover and local nesting and foraging resources that affect wild bee abundance and generic richness that warrant more study in this and other agroecosystems.

Introduction

Biodiversity is declining across the globe with species losses documented across taxa (Johnson et al., 2017; Flynn et al., 2009). Land-use change, climate change, habitat fragmentation, modified disturbance regimes, and pollution are some of the causes for these losses (Tittensor et al., 2014). As biodiversity decreases, ecosystems become simplified and ecosystem functioning and ecosystem services are threatened (Duffy, 2003; Loreau et al., 2001; Peterson et al., 1998; Worm et al., 2006).

The loss of species may have particularly dramatic effects on species interactions, such as the flowering plant-pollinator mutualism. Both commercially reared honey bees and wild bees are in decline (Goulson et al., 2015; National Research Council, 2007; Potts et al., 2010). Whereas honey bee declines are well documented, there are fewer studies of declines among the vast majority of wild bee species (but see Biesmeijer et al., 2006; Scheper et al., 2014; Zattara and Aizen, 2021). Wild bees are difficult to monitor because most are solitary and ground nesting or twig nesting species that do not aggregate in large groups. Declines in wild bee populations could have cascading effects throughout ecosystems, particularly on wild flowering plant populations (Potts et al., 2010).

Loss of commercial honey bee colonies and wild bee populations will negatively affect entomophilous cultivated and wild flowering plants. Approximately 87% of all flowering plants require varying degrees of insect pollination (Klein et al., 2007). Almost 75% of the most popular food crops rely on entomophilous pollination services to some extent (IPBES, 2016). Declines in pollination services not only threaten food security but also economic stability among agricultural economies. For example, pesticides decimated wild bee populations in Canada which caused blueberry producers to lose millions of dollars over a decade (Kevan, 1977). Recent estimates of the global monetary value of insect pollination of cultivated plants is between 235 and 577 billion US dollars annually (IPBES, 2016). Declines in honey bees and wild bees have the potential to negatively impact the majority of flowering plants which will decrease food production at a time when human population growth requires increasing crop yields (Tilman et al., 2001)

Pollination services provided by wild bees significantly improve many crop yields (Garibaldi et al., 2013). For example, coffee yields increased with higher diversity of wild bees in Indonesia (Klein et al., 2003). Sour cherry and cucumber yields increased as pollinator diversity increased in Asia (Christmann et al., 2017). Wild bee pollination services have been shown to be adequate for some crops and are required for others that honey bees cannot adequately pollinate (Gardner et al., 2019; Winfree et al., 2008). For example, in a meta-analysis by Garibaldi and colleagues (2013), wild bees significantly increased fruit set in all crop systems but honey bees only increased fruit set in 14% of crop species. Not only do wild bees enhance yield, but they may help decrease economic risk of relying on a single insect pollinator (i.e., honey bees) for pollination services in some crops (Henselek et al., 2016).

Wild bees are the most important pollinators of wild flowering plants. In turn, wild flowering plants provide critical nectar and pollen resources for bees. Wild flowering plants, like bees, are in decline across the globe because of habitat loss, degradation, and fragmentation (Kearns et al., 1998; Potts et al., 2010; Powney et al., 2019). Loss of either plant or bee species will affect the other because of the obligate nature of plant-pollinator mutualisms. More than 60% of all flowering plants already experience pollen limitation (Burd, 1994). Pollen limitation may result in loss of genetic diversity through inbreeding depression (Charlesworth and Charlesworth, 1987). Seeds and fruits from flowering plants are an important food source for birds and small mammals that in turn feed higher trophic levels (Kehoe et al., 2021). A disruption in wild plant-bee mutualisms could potentially have cascading negative effects throughout an ecosystem if it causes seed and fruit set to decrease.

Landscape cover may strongly affect wild bee abundance and diversity by influencing both foraging resources and nesting habitat for wild bees. As agricultural intensification continues to diminish natural and semi-natural landscapes, both nesting and foraging resources may decline (Fahrig et al., 2011; Klein Goldewijk et al., 2017). Flowering plant diversity and abundance are likely important predictors of wild bee communities and may be reduced in agricultural areas (Potts et al., 2003; Roulston and Goodell, 2011). Diverse bee assemblages require diverse floral syndromes as certain bees are adapted to particular floral morphologies. For example, generalist bees often visit open-shaped flowers whereas more specialist species, such as *Bombus* spp., are adapted to forage on flowers with long corollas (Fenster et al., 2004). Additionally, abundance and distribution of floral resources affect wild bee abundance (Hegland and Boeke, 2006; Hines and Hendrix, 2005; Mitchell et al., 2004; Westphal et al., 2003). With the strong link between bees and flowers, flower community characteristics are expected to affect bee abundance and diversity.

Agricultural land uses also reduce nesting habitat for wild bees via increased soil disturbance and compaction from vehicles, decreased areas of natural and semi-natural habitats, and application of agrochemicals (Grundel et al., 2010; Morandin et al., 2007; Potts et al., 2005, 2010). Wild bees require nesting habitat not only for their larvae to develop but also for adults to survive parasites, predators, weather, and other disturbances (Roulston and Goodell, 2011). Different bee species require different nesting substrates including exposed soil, cavities, pithy stems, and unused small mammal burrows, as well as nesting materials such as mud, resin, leaves, and flower petals (Potts et al., 2003). Whereas bumble bees typically nest in previously excavated burrows or cavities, sweat bees nest in loose soil, and leaf-cutter bees lay their eggs in pithy plant stems (Potts et al., 2005; Roulston and Goodell, 2011). In some cases, the availability of desirable nesting habitat can be a stronger predictor of presence and abundance of bees than flower resources (Potts et al., 2005; Steffan-Dewenter and Schiele, 2008). The composition and configuration of the landscape in which bees reside will influence the availability of nesting substrates. Landscape cover may, therefore, directly impact wild bee abundance and diversity through nesting habitat availability.

Agricultural landscapes with more semi-natural areas and even pastures may support greater wild bee diversity. In California, hedgerows between agricultural fields increased wild bee abundance and species richness in adjacent fields, especially among rare, specialist bees (Morandin and Kremen, 2013). Maintaining and restoring hedgerows may promote wild bee communities and their pollination services in nearby fields, increasing fruit and seed set (Blaauw and Isaacs, 2014a). Roadsides with native perennial flowering plants increased wild bee abundance and diversity in Kansas

(Hopwood, 2008). In agricultural landscapes, small patches of forage and nesting resources are important to maintain wild bee communities.

Mass flowering crops (MFCs), such as canola (*Brassica napus*), may add important resources for wild bees in an otherwise forage-poor landscape, particularly in heavily agriculturalized areas (Holzschuh et al., 2013; Westphal et al., 2003). The landscape of the southern Great Plains has been significantly altered over the last century, and the predominant agricultural land uses are pasture and winter wheat (Foley et al., 2005; USDA, 2019). Many producers now rotate winter wheat with canola to reduce pest and disease burdens on winter wheat. Canola production will likely continue as demand for biofuel energy sources and cooking oil tracks with human population growth (Tilman et al., 2001). Canola blooms in the early spring in the southern Great Plains and produces nectar (mean of 2.0µL nectar per flower; Pierre et al., 1999) and pollen resources for pollinators (Bommarco et al., 2012). Seeds of fertilized flowers mature over the early spring and are harvested in June. Although canola is self-compatible and moderately wind-pollinated, insect pollination increases seed weight, oil content, and market value of seeds (Bommarco et al., 2012).

Like other MFCs, canola provides significant resources to support pollinator communities (Hanley et al., 2011). In Oklahoma, canola blooms before many native wildflowers and may help boost resources available to early emerging pollinator populations (Stanley and Stout, 2013; Westphal et al., 2003). As canola senesces, however, wild flowering plants are likely less abundant than canola and may not be adequate to support wild bee populations. Galpern and colleagues (2017) observed that although canola sites in Canada had higher populations of *Bombus* spp. queens while canola was in bloom, after senescence, *Bombus* spp. worker populations were significantly lower in canola sites than sites without canola (Galpern et al., 2017).

Wild bees play an important role in the pollination of wild flowering plants and many cultivated crops, particularly MFCs. Understanding how agricultural landscapes with MFCs affect wild bee abundance and richness is critical to sustain wild bee communities, particularly as the demand for agricultural production of MFCs increases with the growing human population. Here, we evaluate the changes in wild bee abundance and generic richness as a function of landscape cover at varying spatial scales, local foraging and nesting resource availability, time across the growing season, and presence of winter wheat or canola. To understand how landscape cover structured wild bee communities, we employed ordination.

Methods

Site locations

A total of 46 sites, 23 adjacent to canola fields and a corresponding 23 adjacent to winter wheat fields were selected across north-central Oklahoma in 2017, 2018, and 2019. Fourteen sites were selected in 2017 and sixteen sites were selected in both 2018 and 2019. In a given year, selected sites were separated by at least 20km to ensure samples collected at each site represented independent wild bee populations. Sites differed each year due to crop rotation between canola and winter wheat, as determined by producers. Each site measured approximately 800m x 800m.

Bee sampling and spatial scales

To test the effects of landscape composition on wild bee abundance, generic richness and evenness, we deployed pan trap arrays for a period of seven days, once per month between May and October during 2017, 2018, and 2019. Two pan trap arrays were placed adjacent to each canola and winter wheat site, approximately 200m from the field corner.

Each pan trap array contained three pan traps (500 mL plastic beakers), with one painted each of the three different colors: fluorescent yellow, fluorescent blue, and white; this combination of colors has been shown to be effective for capturing bee diversity (Droege et al., 2010; Toler et al., 2005; Westphal et al., 2008). The pan traps were filled with 100% propylene glycol. For every liter of propylene glycol, several ml of Dawn® Original Blue dish washing detergent (Proctor & Gamble, Cincinnati, Ohio) were added to reduce the surface tension of the propylene glycol so that bees that land on the liquid are more likely to be captured and less likely to be able to fly off of pan traps. To prevent the loss of samples during periods of rain, four small holes were made in the upper 2cm of the pan traps to allow excess liquid to leak out. The pan trap array was secured to a 1.2m rebar stake with a wood platform for holding the pan traps. The pan trap platform was adjustable and was moved to the height of nearby canola plants. Samples were collected from pan trap arrays weekly and placed in individual 70ml scintillation vials filled with 70% ethanol. Samples were removed from ethanol, rehydrated using warm water, washed in soapy water, rinsed and dried using forced air. Once dried, bee samples were pinned or pointed and keyed to genus or species, when possible (Michener, 2000; Michener et al., 1994).

We selected five nested spatial scales that corresponded to typical flight distances of varying groups of bees including, but not limited to: small solitary bees (*e.g. Lasioglossum* spp.), medium sized solitary bees (*e.g. Osmia* spp.), large solitary bees (*e.g. Andrena* spp.), and very large solitary and social bees (*e.g. Anthophora* spp. and *Bombus* spp.; Zurbuchen et al., 2010b). Nested spatial scales consisted of circles with radii of 500m, 1000m, 1500m, 2000m, and 3000m that were centered on each site.

Land uses across the study area were downloaded from CropScape (USDA, 2019). We downloaded Crop Data Layers (CDLs), a raster layer that gives annual crop and land use specific land cover data with 30m pixel resolution. We then uploaded all necessary rasters to ArcGIS. Using ArcGIS, we created unique pixel counts of all crop and land use cover at the five spatial scales. Pixel counts were converted to total area in hectares. All land uses were then categorized into resources that are relevant to wild bee foraging and nesting. The land use categories were bee forage crops (i.e., crops that bees are known to visit for either pollen, nectar, or both; canola, sorghum, millet, corn, cotton, herbs, peas, soybeans, dry beans, sunflowers, and alfalfa), non-bee forage crops (winter wheat, rye, barley, triticale, sod grass, non-alfalfa hay, and oats), forests, development (roads, homes, oil and natural gas developments, etc.), pasture, and fallow (fallow, idle, and barren fields).

We measured local foraging and nesting resources using ten 1m x 1m Daubenmire quadrats along one 100m transect along the edge of two sides of each site once per month from May through October each year (Daubenmire, 1959). Using six cover ranges 0-5%, >5-25%, >25-50%, >50-75%, >75-95%, and >95-100%, cover of the following types was recorded: flowering forbs, non-flowering forbs, flowering shrubs, non-flowering shrubs, grass, bare ground, detritus, and rock. We estimated local forage resource availability outside of crop fields using flowering forbs and non-flowering forbs and potential nesting habitat using bare ground cover.

Statistical analysis

One-way ANOVAs were used to test the difference in wild bee abundance and generic richness as a function of year, month, and crop type (canola or wheat). We analyzed bee community structure and the effects of local foraging and nesting resources and land use cover on bee abundance and generic richness using non-metric multidimensional scaling (NMDS) using the “vegan” package in the R software program (Oksanen et al., 2015; R Core Team, 2020). We used the results of the NMDS model to inform the variables included in the mixed effect models. We used generalized mixed-effects models to test the effects of land cover and local foraging and nesting resources on wild bee abundance and generic richness using Poisson distribution. Because land cover types were highly correlated, each model only included one land cover type. For this reason, interactions between land cover types were not included in the models. Site, month, and year were included as random effects in each model. We used forward selection to test models and AIC values to select the best fit model for bee abundance and generic richness. We confirmed that the models fit assumptions by testing for linearity, homogeneity of variance, and the normality of residuals. We performed simple linear regression between each variable selected from the results of the NMDS against bee abundance and generic richness. We applied the Bonferroni correction ($\alpha = 0.005$) to the simple regressions to control for false positives that may occur (Bonferroni, 1936).

Generalized and linear mixed-effects models were performed using the *lme4* package (Bates et al., 2015). All analyses were performed in the R software program.

Results

We collected a total of 11,849 individual wild bees, with more bees collected in 2017 (5,842) and 2018 (5,184) than in 2019 (823). The majority fell into the following genera: *Lasioglossum* (51%), *Agapostemon* (31%), *Augochlora* (3%), and *Melissodes* (3%). We collected several singleton individuals in the following genera: *Hesperapis, Hoplitis, Osmia,* and *Habropoda.* A total of 35 genera were collected across all years. Thirty genera were collected in 2017 and 26 were collected in each 2018 and 2019. Bee abundance and generic richness were positively correlated (Figure 1; $F_{1,274} = 266.52$, $p < 0.0001$). Bee abundance was significantly greater in 2017 and 2018 compared with 2019 (Figure 2A; $F_{2,274} = 13.34$, $p < 0.0001$). Generic richness was also significantly greater in 2017 and 2018 compared to 2019 (Figure 2B; $F_{2,274} = 14.26$, $p < 0.0001$). Based on the genera accumulation curve, we sampled a sufficient number of sites to capture bee generic richness in this study system (Figure 3).

Bee abundance and generic richness differed significantly between the months of May through October (Figure 4). Among canola sites, bee abundance decreased significantly from May through October (Figure 4A; $F_{1, 132} = 18.90$; $p < 0.0001$). Among wheat sites, bee abundance significantly decreased from May through October (Figure 4B; $F_{1, 132} = 5.32$; $p = 0.0002$). Bee generic richness decreased significantly from May through October in both canola and wheat sites (Figures 4C and 4D; $F_{1, 132} = 18.86$; $p < 0.0001$, $F_{1, 132} = 6.20$; $p < 0.0001$, respectively).

We compared bee abundance and generic richness between canola and wheat sites. Bee abundance was significantly greater at canola sites compared to wheat sites (Figure 5A; $F_{1,274} = 9.15$, $p = 0.0027$). Generic richness was significantly greater around canola fields compared with wheat fields (Figure 5B; $F_{1,274} = 16.90$, $p < 0.0001$). We rarefied the number of genera by total individuals collected at each site and found that sites adjacent to canola fields were more diverse compared to sites adjacent to wheat fields (Figure 6). Using ordination, we found that wild bee communities adjacent to canola fields were also more similar to each other compared to wild bee communities adjacent to wheat fields (Figure 7).

The NMDS model defined the local foraging and nesting resources and land use variables that structured the wild bee communities at each site (Figure 8; Table 1). Simple linear regressions were also used to determine the direction of the correlation between bee abundance, generic richness, and all foraging and nesting resources and land use variables (Table 1). Area of bee-forage crops at 500m and area of development at 3000m were significantly correlated with decreasing bee abundance. The following variables: Bare ground cover, area of development at 3000m, and area of fallow cropland at 3000m were significantly correlated with bee generic richness. Both bare ground cover and area of development at 3000m were negatively correlated with bee generic richness but the area of fallow cropland at 3000m was positively correlated with bee generic richness. Once Bonferroni corrections were applied, the area of development at 3000m was the only significant variable to impact bee abundance and the area of fallow cropland at 3000m was the only significant variable to affect bee generic richness. We found that the model that included generic richness, area of bee-forage crops at the 500m spatial scale, and area of bare ground as fixed effects and site and year as random effects was most predictive of bee abundance (AIC = 6226; Table 2). The most predictive model of bee generic richness included bee abundance, area of developed land at the 500m spatial scale, and the interaction between abundance and area of developed land as fixed effects and site and year as random effects (AIC = 1204; Table 3). We also found that the smallest spatial scale, 500m, was the most predictive of bee abundance and generic richness in the mixed effects models relative to all other spatial scales.

Discussion

Canola impacts wild bee communities in this study system. Bee communities adjacent to canola fields were both more diverse and abundant than those adjacent to wheat fields. Canola not only attracted more genera but also similar genera at each canola site relative to wheat sites, suggesting that canola may drive, in part, wild bee communities in this agroecosystem. In this system, canola is an important foraging resource for wild bees. Among canola sites we sampled, both bee abundance and generic richness decreased significantly from May to June, coinciding with canola senescence. Relative to wheat sites, which also had a steep decline in bee abundance and generic richness from May to June, canola sites maintained greater bee abundance and generic richness throughout the growing season. This suggests that wild bees may be attracted to canola during bloom and remain even after canola senescence. The presence of canola influences wild bee communities throughout the growing season in this agroecosystem. These results support findings from other studies of the effects of MFCs on wild bee populations (Hanley et al., 2011; Holzschuh et al., 2013; Jauker et al., 2012; Stanley and Stout, 2014; Westphal et al., 2003, 2009). Canola provides abundant foraging resources at a time when many wild flowering plants may not be available and, therefore, may boost resources to wild bees at a critical time during early emergence. Presumably, the effects of canola may be detectable throughout the growing season as canola increases resources available to female bees that are provisioning brood cells, potentially improving reproductive success causing subsequent increases in wild bee populations. Resource density and proximity to nesting sites is known to significantly impact brood cell count among wild bee species (Jauker et al., 2012; Klein et al., 2004; Zurbuchen et al., 2010).

Despite the benefit of canola as a foraging resource, agricultural land cover reduces nesting resources available to wild bees (Cusser et al., 2016; Le Féon et al., 2010; Saturni et al., 2016; Wratten et al., 2012). In our study system, very little natural and semi-natural habitat remain. Large monocultures of largely non-flowering crops, such as winter wheat, have decreased the diversity of land cover across the landscape. A less diverse landscape can contribute to decreased bee abundance and diversity (Blaauw and Isaacs, 2014b; Flores et al., 2018; Grab et al., 2019; Martins et al., 2015). In this system increased bare ground cover, bee-forage and non-bee-forage crop area, and developed land area were correlated with decreased wild bee abundance and generic richness. Several factors contribute to these findings. Increased agricultural land area is correlated with increased disturbance, agrochemical use, and habitat fragmentation, all of which negatively impact wild bees. In a meta-analysis of the effects of disturbance on wild bees, agricultural land conversion, tilling, and pesticide use all reduced wild bee abundance and richness (Winfree et al., 2009). The strongest negative effects were found in landscapes where very little natural and semi-natural habitat remained (Winfree et al., 2009). With little semi-natural habitat in the southern Great Plains, any disturbance may have a strong negative effect on wild bees.

Agrochemicals are known to negatively affect wild bee health and behavior and, therefore, may impact bee abundance and richness (Kovács-Hostyánszki et al., 2017; Rundlöf et al., 2015). In the southern Great Plains, the addition of canola in the early 2000s coincided with the increased use of broad-spectrum insecticides because of novel aphid pests that attack canola during canola bloom (Franke et al., 2009; Reddy, 2017). Because many producers apply insecticides at the beginning of canola bloom, wild bees may experience lethal and sublethal effects of insecticides when foraging on canola (Peterson et al., 2021; Rundlöf et al., 2015; Shires et al., 1984). Even if broad-spectrum insecticides do not negatively impact wild bee health, they are known to cause changes in foraging behavior among wild bees (Crall et al., 2018; Lämsä et al., 2018; Stanley et al., 2015). Additionally, the application of agrochemicals by heavy equipment may disturb roadside foraging and nesting habitat, making it undesirable for wild bees. The synergistic effects of agrochemical use in this agroecosystem may contribute to the pattern of decreased bee abundance and generic richness in areas with increasing cover of crops where agrochemicals may be used.

Many of the individual bees we collected were small-bodied with relatively short maximum flight distances and connectivity of habitat patches may be particularly important for these bees. More than 50% of the bees we collected belong to the genus *Lasioglossum* with documented maximum flight distances of less than 500m (Westrich, 2006). Sites with increased crop cover may not support wild bee populations with short flight distances. Increased crop cover is correlated with decreased nesting resources and fewer natural and semi-natural areas and bees with short flight distances may avoid these landscapes (Tepedino et al., 1984). Therefore, increased crop cover, even if it consists of bee-forage crops, was correlated with decreased wild bee abundance and generic richness.

Bee generic richness was positively correlated with fallow cropland. Fallow cropland may be an important nesting habitat for wild bees in this study system. The majority of bee genera we collected were ground-nesting genera and may utilize fallow cropland for nesting habitat and foraging habitat throughout the growing season. However, fallow cropland may be converted to bee-forage or non-bee-forage cropland in subsequent years, reducing the availability of nesting and foraging resources for wild bees. In a study of varying agricultural landscape composition, Holzschuh and colleagues (2009) found that fallow strips did not enhance wild bee abundance and richness when only cereal crops surrounded the fallow strips. They hypothesized higher quality nesting resources (such as those found in fallow strips) may not benefit wild bees if the surrounding landscape does not also provide foraging resources. Our results are compelling because they suggest that canola may provide important foraging resources and fallow cropland may provide important nesting and foraging habitat throughout the growing season.

Local foraging and nesting resources did not have a strong impact on wild bee abundance and generic richness in this system, with the exception of bare ground cover. Whereas we hypothesized increased bare ground cover provide nesting resources for wild bees and therefore increase wild bee abundance and richness, we found that there was a strong negative correlation between bare ground cover and bee abundance and generic richness. These results may be explained by the quality of bare ground rather than the quantity. For example, high quality bare ground where bees are more likely to nest is not densely packed or routinely disturbed. Bare ground around canola and wheat fields in the southern Great Plains is highly disturbed and compacted by repeated vehicle use, agrochemical application, grading, and tilling. All of these mechanisms of disturbance reduce nesting habitat for ground nesting wild bees (Winfree et al., 2009). More than 70% of wild bee species are ground nesters and the majority of individuals we collected belonged to ground nesting genera. Highly disturbed bare ground may discourage nesting and result in the decreased bee abundance and diversity we found (Michener et al., 1958; Potts and Willmer, 1997). Additionally, this pattern may be due to the facts that higher percentages of bare ground cover represent a loss of floral resources. Sites with high percentages of bare ground cover had consistently low forb cover. The available bare ground in this study system may be of low quality and associated with poor floral resource availability, contributing to declines in both bee abundance and generic richness.

The negative impacts of agricultural land use conversion on wild bees are manifold (Kennedy et al., 2013; Potts et al., 2010) however, in this system, canola may provide critical foraging resources for wild bees. Indeed, canola sites sustained greater bee abundance and generic richness throughout the growing season compared to wheat sites, indicating that the pulse of floral resources provided by canola positively influences bee communities beyond the period of canola bloom. Undoubtably, there are more variables that we did not test that also likely affect wild bee communities within agroecosystems in the southern Great Plains, such as agrochemical application, connectivity of foraging and nesting habitat patches, and frequency and intensity of disturbances. The diverse nesting and foraging requirements of different wild bee species suggest that further studies would benefit from considering individual species or guilds of bees. Agricultural land use will continue to affect wild bee communities and understanding the specific mechanisms through which agricultural landscapes drive wild bee abundance and richness is important to sustain wild bee communities within agroecosystems.

Acknowledgements

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Tables and Figures

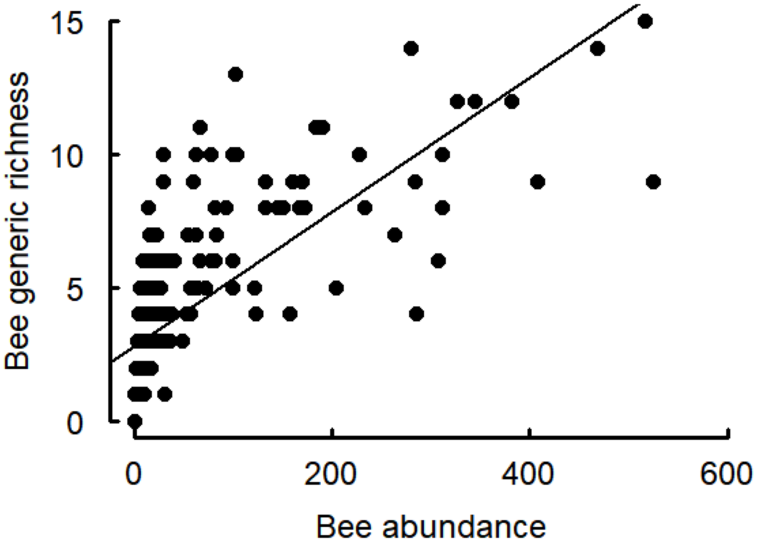

Figure 1. Bee abundance and generic richness at the site level were tightly correlated. Generic richness increased significantly as bee abundance increased ($F_{1,274} = 266.52$, $p < 0.0001$). Black line represents the linear regression model.

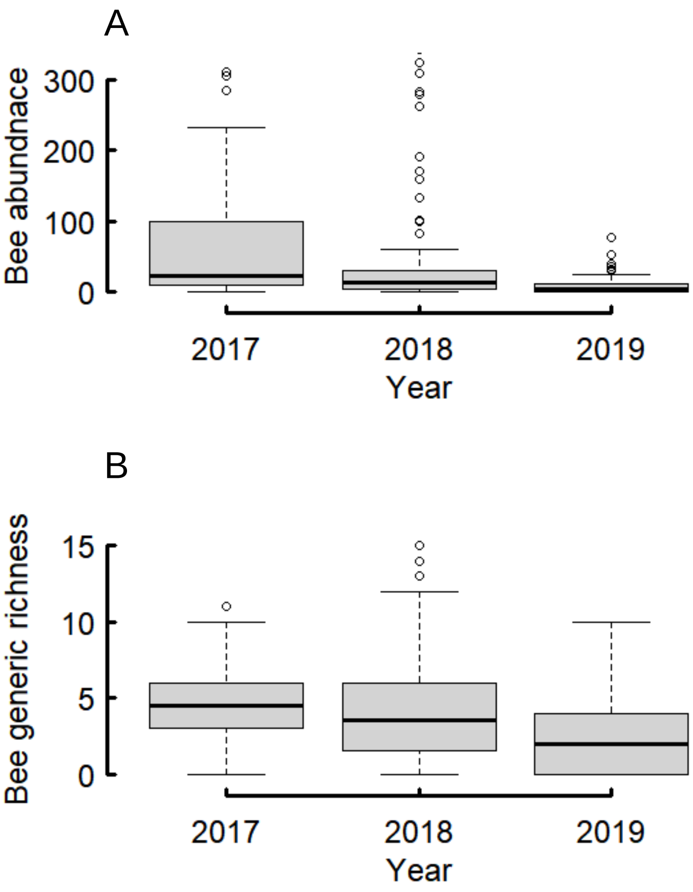

Figure 2. Bee abundance was significantly greater in 2017 and 2018 compared with 2019 (A; $F_{2,274} = 13.34$, $p < 0.0001$). Generic richness was also significantly greater in 2017 and 2018 compared to 2019 (B; $F_{2,274} = 14.26$, $p < 0.0001$). Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers.

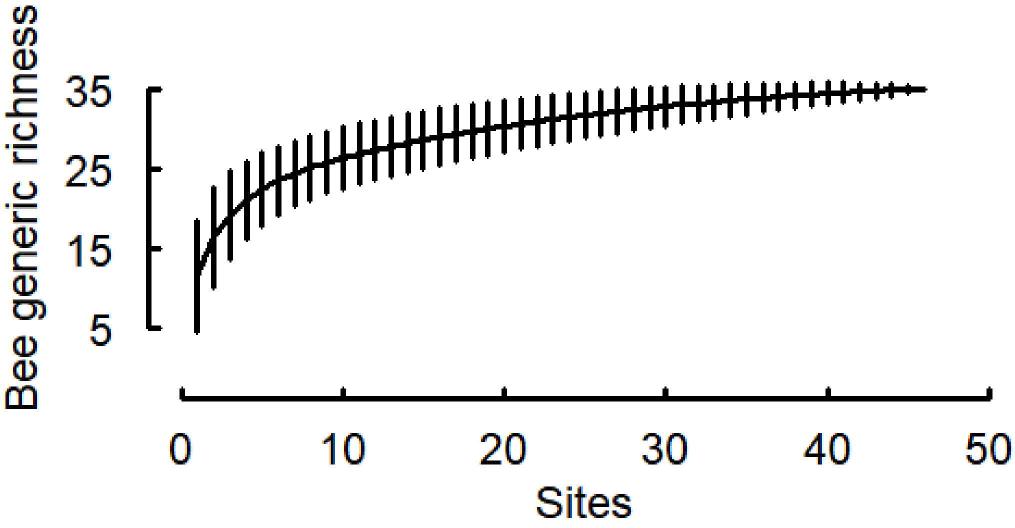

Figure 3. Bee generic richness accumulation curve of all bee genera collected across all 46 sites. The curve indicates that we sampled this study system sufficiently. Black bars represent confidence intervals at each site.

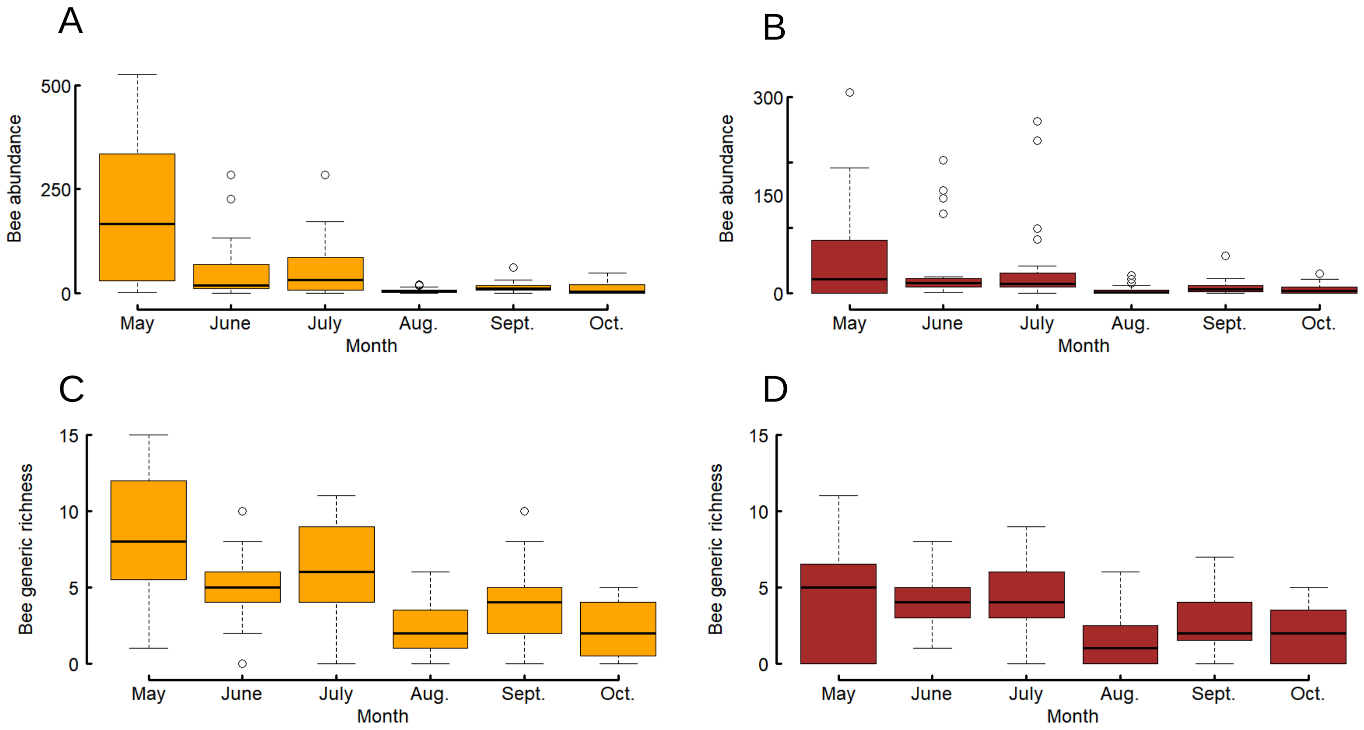

Figure 4. Bee abundance at canola sites differed significantly between the months of May through October (A; $F_{1,132} = 18.90$, $p < 0.0001$). Bee abundance at wheat sites differed significantly between the months of May through October (B; $F_{1,132} = 5.32$, $p = 0.0002$). Generic richness at canola sites differed significantly between the months of May through October (C; $F_{1,132} = 18.86$, $p < 0.0001$). Generic richness at wheat sites differed significantly between the months of May through October (D; $F_{1,132} = 6.20$, $p < 0.0001$). Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers.

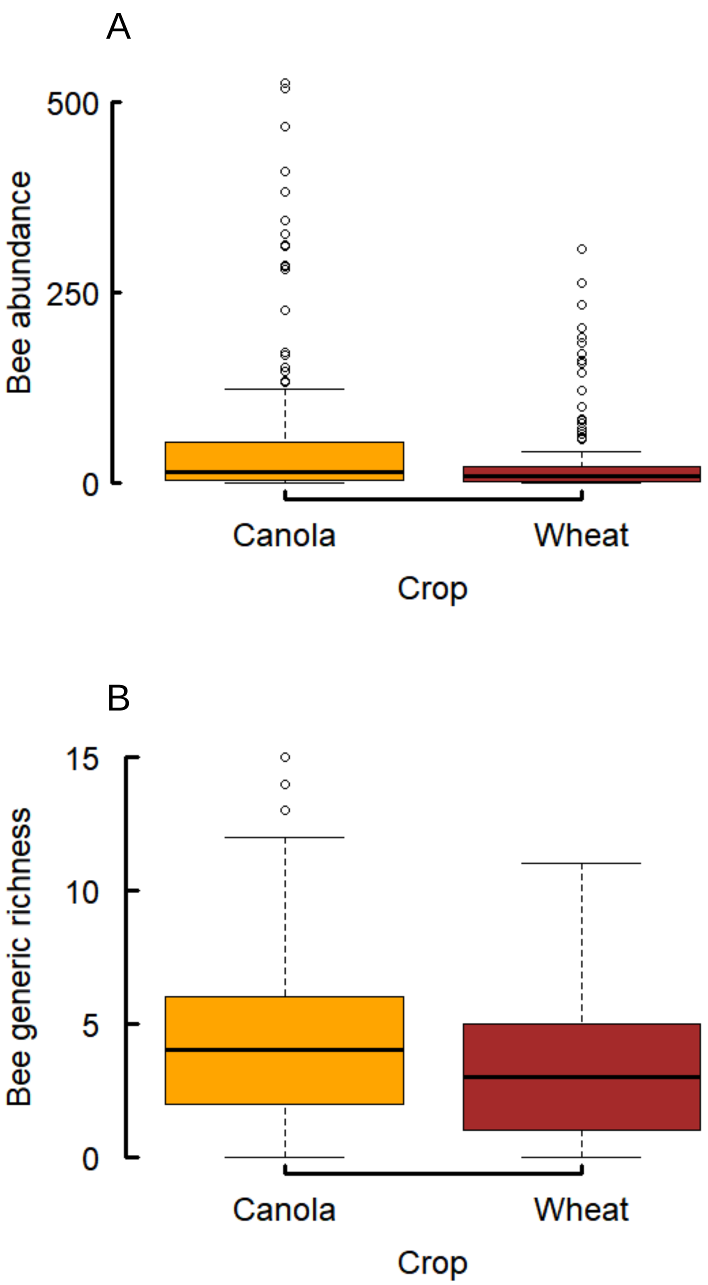

Figure 5. Bee abundance was significantly greater around canola fields compared with wheat fields (A; $F_{1,274} = 9.15$, $p = 0.0027$). Generic richness was significantly greater around canola fields compared with wheat fields (B; $F_{1,274} = 16.90$, $p < 0.0001$). Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers.

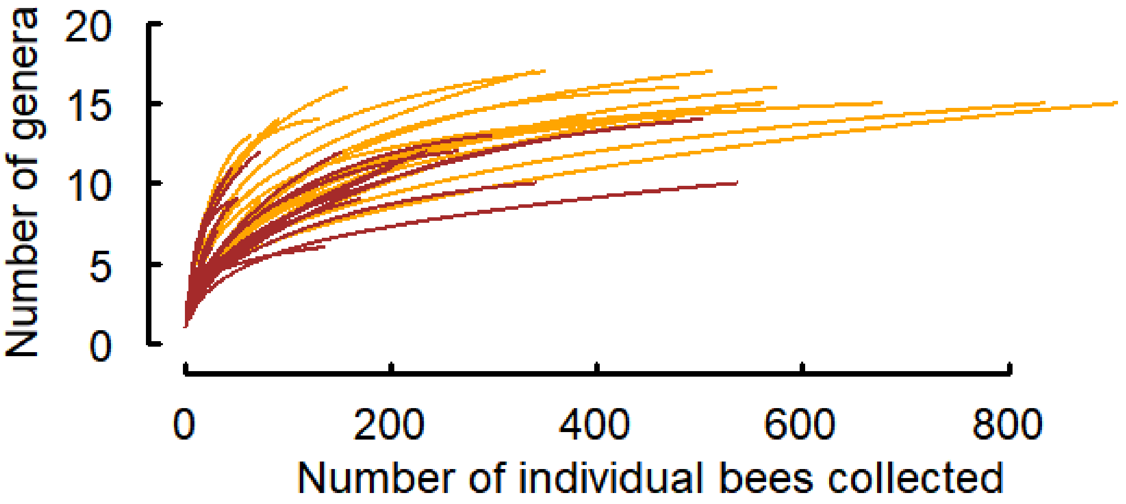

Figure 6. Bee generic rarefaction curves as a function of number of individual bees collected. Yellow curves represent canola sites and red curves represent wheat sites.

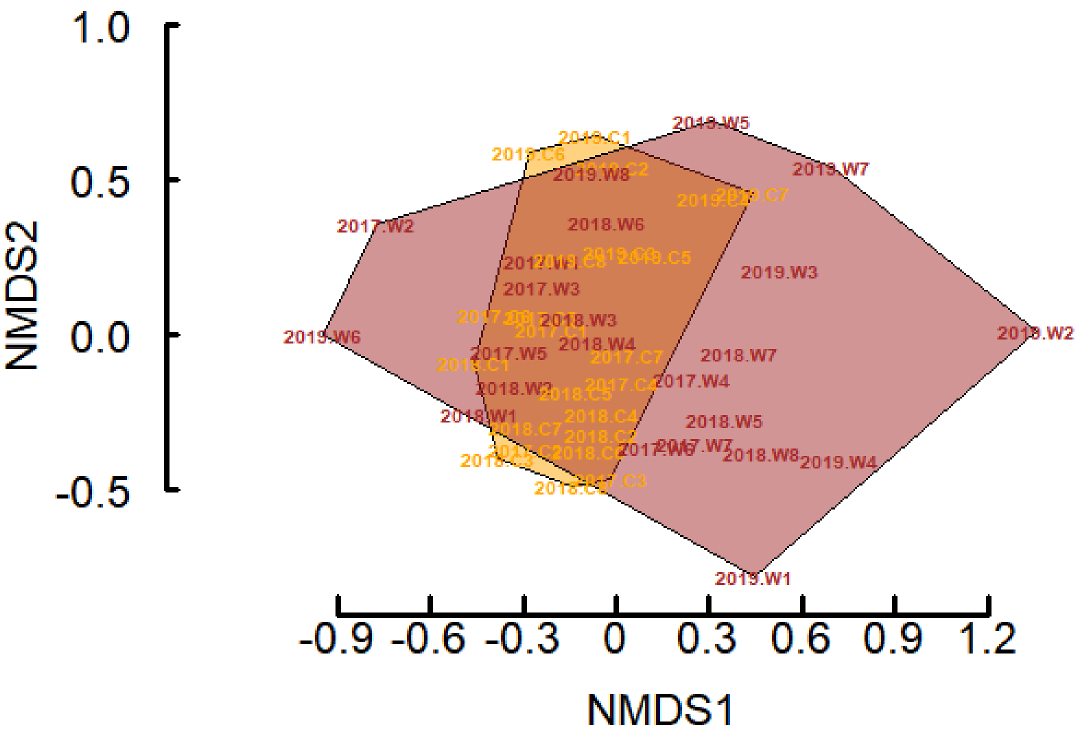

Figure 7. NMDS model representing sites relative to one another. Canola sites are labeled by year and site number in yellow and wheat sites are labeled by year and site number in red. The yellow polygon includes all canola sites and the red polygon includes all wheat sites.

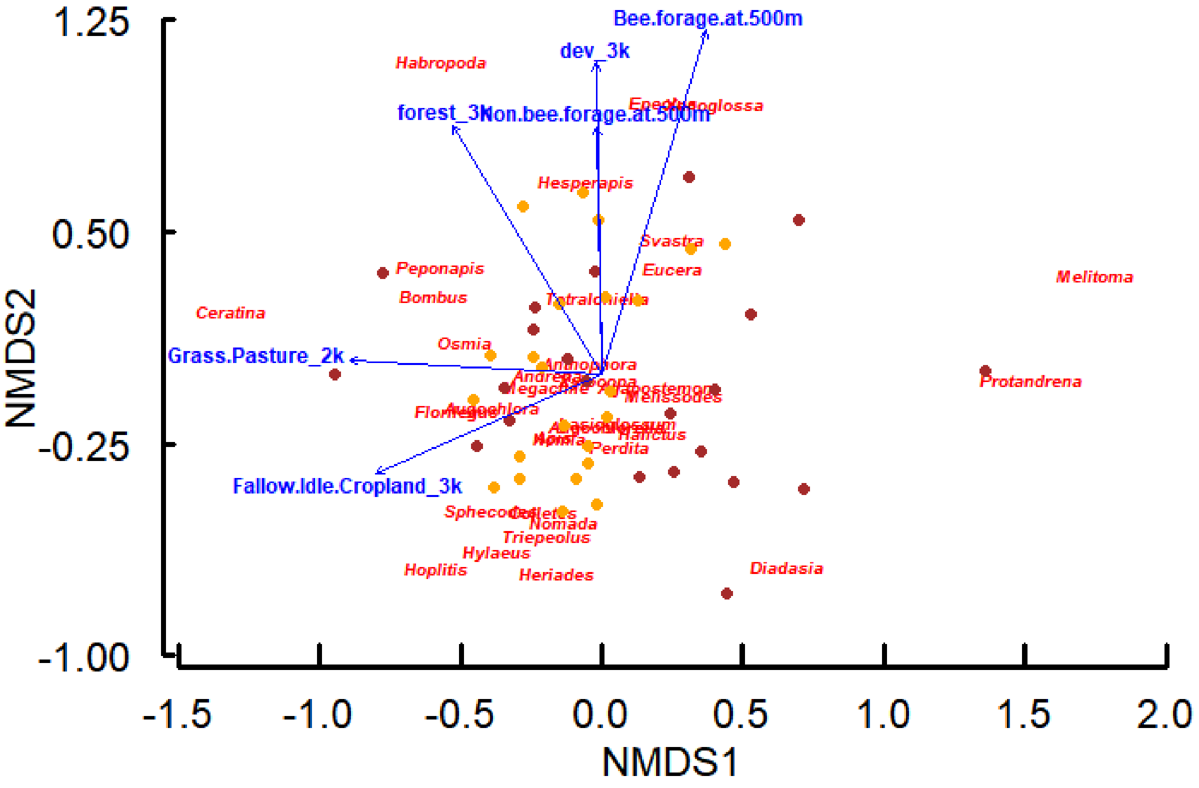

Figure 8. NMDS model representing foraging and nesting resources and land use variables as they affect the structure of the bee community. Significant variables are indicated by blue arrows and identified in blue. Increased arrow length represents increased strength of the effect of each variable on bee generic richness. Canola sites are indicated by yellow dots and wheat sites are indicated with red dots. Bee genera are in red.

Table 1. Local foraging and nesting resources and land use variables and the measured contribution to the NMDS model and linear regression models between each variable and bee abundance and generic richness. Significant variables are indicated with asterisks: * : *p* < , 0.05; ** : *p* < , 0.01; ***: *p* < , 0.001. Bold terms indicate linear regression models were still significant after applying the Bonferroni correction (*p* <0.005).

Environmental variable	Contribution to NMDS model	Simple regression (*p* value)	
		Bee abundance	Bee generic richness
Flowering forbs	0.469	0.390	0.819
Grass	0.540	0.348	0.347
Bare ground	0.115	0.234	0.023*
Area of bee-forage crops at 500m	0.001***	0.035*	0.156
Area of non-bee-forage crops at 500m	0.035*	0.189	0.476
Area of development at 3000m	0.006**	**0.003****	0.018*
Area of fallow at 3000m	0.030*	0.203	**0.005****
Area of forest at 3000m	0.009**	0.126	0.665
Area of pasture at 2000m	0.024*	0.457	0.199

Table 2. Results for generalized linear mixed-effects models of bee abundance for the best fit model. Fixed effects variables included bee generic richness, bare ground cover, and area of bee-forage crops at the 500m scale. Variables that are significant are in bold. All variables were significant.

Response variable	Fixed effects	Estimate	SE	*z* value	*p* value
Bee abundance	**Intercept**	**1.199**	**0.421**	**2.844**	**0.0044**
	Bee generic richness	**0.323**	**3.233 e^{-3}**	**100.017**	**<0.0001**
	Bare ground cover	**9.812 e^{-3}**	**8.632 e^{-4}**	**11.368**	**<0.0001**
	Area of bee-forage crops at 500m scale	**6.327 e^{-4}**	**6.336 e^{-5}**	**9.985**	**<0.0001**

Table 3. Results for generalized linear mixed-effects models of bee generic richness for the best fit model. Fixed effects variables included bee abundance, area of developed land at the 500m scale, and the interaction between abundance and developed land area. Variables that are significant are in bold.

Response variable	Fixed effects	Estimate	SE	*z* value	*p* value
Bee generic richness	**Intercept**	**0.876**	**0.224**	**3.913**	**<0.0001**
	Bee abundance	**3.107 e^{-3}**	**5.559 e^{-4}**	**5.589**	**<0.0001**
	Developed land at 500m scale	**0.039**	**0.011**	**3.567**	**0.0004**
	Bee abundance * Developed land at 500m scale	-1.032 e^{-4}	8.304 e^{-5}	-1.243	0.214

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CHAPTER III

THE IMPACTS OF LAND USE AND WILD BEE ABUNDANCE AND RICHNESS ON CANOLA PRODUCTIVITY

Abstract

Canola (*Brassica napus* L.) is a rotational crop planted with winter wheat in the southern Great Plains. Canola is an important biofuels crop and although self-compatible, increases seed set with bee pollination. Canola significantly increases the foraging resources for wild bees, particularly early in the growing season and compared to winter wheat. The addition of abundant pollen and nectar may benefit wild bee populations which, in turn, may also improve canola seed set by providing pollination services. Wild bees have been documented to improve canola seed set in other agroecosystems even when managed honey bees are present. To test the effects of wild bees on canola in this study system, we performed a pollen limitation study on canola grown in north-central Oklahoma. We modeled canola seed count and total seed weight as a function of land use cover and wild bee abundance and generic richness at five nested spatial scales. Insect pollination significantly improved both seed count and total seed weight in this system. The best fit seed count model included wild bee abundance, generic richness, and the area of bee forage crop cover at the largest spatial scale. Two models best fit total seed weight. The first included wild bee abundance, generic richness, and area of forest cover at the smallest spatial scale and the second included wild bee abundance, generic richness, and the area of canola cover at the largest spatial scale. Wild bee abundance and generic richness combined with land use affects canola productivity in this agroecosystem. Additional research is needed to understand the effects of landscape characteristics on wild bee communities and how these effects influence mass flowering crop productivity in other agroecosystems.

Introduction

Pollinators, particularly bees, provide essential pollination services to approximately 87% of wild flowering plants and 35% of cultivated crops (Klein et al., 2007; Potts et al., 2016). Ongoing and unsustainable annual losses of honey bee colonies threaten agricultural production worldwide. Indeed, managed honey bee colony losses in the United States between April 2018 and April 2019 exceeded 40% (Bruckner et al., 2019). Recent estimates of the worldwide monetary value of bee pollination of cultivated plants are between 235 and 577 billion US dollars annually (IPBES, 2016). As the human population continues to grow, increasing demands on agricultural production and declines of managed honey bees and their pollination services may negatively impact food production, leading to food scarcity (Bryan et al., 2018; Tilman et al., 2001).

Wild bee species are experiencing precipitous declines worldwide further disrupting pollination services across agricultural and natural ecosystems (Goulson et al., 2015). Declines among wild bumble bees (*Bombus* spp.) are well documented across Europe and the United States (Biesmeijer et al., 2006; Cameron et al., 2011; Grixti et al., 2009; Jacobson et al., 2018). Solitary wild bees are more difficult to monitor and, with little historical data to compare to current populations, the extent of declines among solitary wild bee species is poorly understood. Wild bees not only supplement pollination of cultivated crops that are pollinated by managed honey bees but may also enhance crop productivity in more cultivated crop species than honey bees (Garibaldi et al., 2013). Wild bees improve production in some crops because honey bees are ineffective or unable to pollinate certain flower morphologies (Christmann et al., 2017; Greenleaf and Kremen, 2006; Holzschuh et al., 2012; Klein et al., 2003). By diversifying the profile of pollinators providing pollination services to crops, wild bee species also reduce economic risk due to the ongoing annual losses of honey bees colonies (Henselek et al., 2016).

Declines in managed honey bees and wild bees are caused by myriad synergistic factors including foraging and nesting habitat loss, fragmentation, and degradation, agrochemical exposure, parasites and pathogens and climate change (Goulson et al., 2015). Factors may interact with one another to increase negative impacts on bees. For example, several parasites and pathogens including *Nosema ceranae* (microsporidian fungus)*, Varroa destructor* (ectoparasitic mite)*, Acarapis woodi* (tracheal mite), and *Aethina tumida* (small hive beetle) infect managed honey bee colonies and can spillover into nearby wild bee populations (Colla et al., 2006; Otterstatter and Thomson, 2008; Tehel et al., 2016). As habitat loss continues in agricultural landscapes, fewer patches of floral resources are available for managed honey bees and wild bees, increasing the likelihood of interactions and the rate of pathogen and parasite exposure to wild bee populations (Otterstatter and Thomson, 2008).

More than 40% of the Earth’s surface has been converted for agricultural uses (Fahrig et al., 2011; Klein Goldewijk et al., 2017). The demand for crops that benefit from bee pollination (*e.g.,* oils and fruits) has far outpaced the demand for wind-pollinated crops (*e.g.,* cereals) and overall crop diversity within agricultural landscapes is declining (Aizen et al., 2019). Agricultural intensification, often characterized by expanding monocultures, coincides with a loss of pollinator nesting and foraging habitat across large areas (Hendrickx et al., 2007). Even in agricultural landscapes where crops that benefit from bee pollination or mass flowering crop (MFC) production are incorporated, intensively managed large monocultures rely on the use of harmful agrochemicals, only temporarily provide foraging resources, and limit the availability of nesting habitat for wild pollinators. However, agricultural landscapes with greater crop diversity or more natural and semi-natural area may contain more foraging and nesting resources which, in turn, may support greater wild bee richness and abundance (reviewed by Kovács-Hostyánszki et al., 2017). For example, hedgerows between agricultural fields in California increased native bee abundance and species richness in adjacent fields, especially among rare, specialist bees (Guzman et al., 2019; Morandin and Kremen, 2013).

Agricultural yields have been shown to improve if the surrounding landscape supports greater abundance and richness of wild bees (Blaauw and Isaacs, 2014). Plant and field level productivity in several crops including apples (Blitzer et al., 2016), canola (Woodcock et al., 2019), coffee (Klein et al., 2003), and watermelon (Kremen et al., 2002) increase as diversity within wild bee communities increases. However, other studies indicate that this pattern does not persist across all crop varieties and ecosystems (Quinn et al., 2017; Sardiñas and Kremen, 2015; Steffan-Dewenter and Leschke, 2003). Therefore, understanding the effects of landscape composition on wild bee communities and the effects of wild bee communities on plant level productivity among specific agricultural crops is critical to gain a comprehensive understanding of the role of wild bees as pollinators of agricultural crops in the context of ongoing wild bee declines.

Diverse and abundant foraging resources are critical to support diverse wild bee communities (Hegland and Boeke, 2006; Hines and Hendrix, 2005; Mitchell et al., 2004; Potts et al., 2003; Roulston and Goodell, 2011; Westphal et al., 2008). Foraging resource availability from the previous year may be an important predictor of reproductive success from the previous year and, therefore, an indicator of bee abundance in the current year (Potts et al., 2003). Wild bee nesting habitats are important not only for larval development but also for adults to survive parasites, predators, adverse weather conditions, and other disturbances (Roulston and Goodell, 2011). Different bee species require different nesting substrates including exposed soil, cavities excavated in wood, pithy plant stems, and unused small mammal burrows and other types of existing cavities, as well as nesting materials such as mud, resin, leaves, and flower petals (Potts et al., 2005, 2005; Roulston and Goodell, 2011). In some cases, the availability of suitable nesting habitat can be a stronger predictor of abundance and richness of bees than flower resources (Potts et al., 2005; Steffan-Dewenter and Schiele, 2008). It is unclear the degree to which landscape composition and configuration influence wild bee communities but these factors undoubtably affect nesting resource availability which is expected to be an important predictor of wild bee abundance and diversity.

Distances between wild bee nesting and foraging habitats may influence wild bee communities. Common wild solitary bee species have relatively short flight distances of only a couple hundred meters (Gathmann and Tscharntke, 2002; Zurbuchen et al., 2010a). Estimates of flight distances based on body length and experimental measurements suggest that many small and medium sized solitary bees (*e.g.,* *Lasioglossum, Andrena,* and *Colletes* spp.) fly between 100 and 1,000m whereas larger and social bees (*e.g. Bombus* spp.) may fly between 200 and 2,800m (Beil et al., 2008; reviewed by Zurbuchen et al., 2010a). Large monocultures may increase distances between nesting habitat and foraging patches, which may negatively affect reproductive success by increasing the number of trips required to provision brood cells, limiting the number of brood cells created, and increasing exposure to pesticides (Garibaldi et al., 2011; Ricketts et al., 2008; Zurbuchen et al., 2010b). Even in monocultures of mass flowering crops, the nectar and pollen of a single crop species may not fulfill the nutritional needs of wild bees and may only serve to further increase the distance needed to travel to acquire nutrients from diverse nectar and pollen sources (Woodard and Jha, 2017).

In historically agricultural landscapes like those in the southern Great Plains, landscape characteristics may have a stronger effect on plant level production than in more semi-natural and natural landscapes. Winter wheat and managed rangelands are the dominant land use in this study system. Canola (*Brassica napus*) was recently introduced as rotational crop that helps reduce disease, pest, and weed pressure on wheat (Franke et al., 2009). The addition of a MFC to a wheat and grassland dominated system may drastically increase the floral resources available to wild bees. Canola blooms in the early spring, before most wild flowering plants, producing abundant nectar and pollen resources (Bommarco et al., 2012; Pierre et al., 1999). Although canola is self-compatible and moderately wind-pollinated, bee pollination increases seed weight, oil content, and market value of seeds (Bommarco et al., 2012). The yield of canola increases by more than 10% with the use of honey bee colonies for pollination services

(Lindström et al., 2016; Manning and Wallis, 2005; Rosa et al., 2011; Shakeel and Inayatullah, 2013). In other agroecosystems, wild bee pollination increased canola seed weight regardless of the presence of commercial honey bees (Zou et al., 2017). There is not a sufficient understanding of how much wild bee pollination improves canola yield in other agroecosystems such as the southern Great Plains but, in the context of ongoing honey bee colony losses, wild bees may play a critical, synergistic role in canola production.

To test the importance of pollinators to canola production, we compared seed set (seed count and total seed weight) between racemes with and without bee pollination. We employed generalized linear and mixed effect models to evaluate the effects of bee abundance and generic richness and landscape composition on canola productivity (canola seed count and total seed weight). The results are discussed in the context of the contribution of wild bees to agricultural production.

Methods

Site selection

A total of 23 unique canola fields (hereafter, sites) were selected across north-central Oklahoma in 2017, 2018, and 2019. Seven sites were selected in 2017 and eight sites were selected in both 2018 and 2019. Sites differed each year due to crop rotation between canola and winter wheat, as determined by producers. Each site measured approximately 800m x 800m and was located at least 3000m from other sites. The separation distance of 3000m prevented overlap among sites in terms of access by individual bees (Zurbuchen et al., 2010a). Most producers utilized commercial honey bee colonies for pollination services during peak canola bloom (mid-April – mid-May).

Canola seed set and production

Plant level production was measured at each of the 23 sites along two 300m transects separated by 50m. The transects were at least 200m from field edges and extended towards the center of the field. On each transect at 75m, 150m, and 300m, six individual racemes on six separate canola plants (one raceme per plant) were selected to test plant level production via seed count and seed weight. At each distance, three racemes were bagged using fine mesh produce bags and marked with flagging tape at the base of the raceme. The fine mesh material allowed airflow, gas exchange, and sunlight to pass through but prevented the pollinators from reaching the reproductive organs of the flowers (Delaplane et al., 2013). The three remaining racemes were marked with flagging tape but left exposed to ambient pollination as a control. After inflorescences senesced on each bagged raceme, bags were removed to allow seed pods to develop. Upon seed pod maturation, the racemes were collected. Seeds were separated from pods, dried (Yamato Gravity Convection Oven DVS 600 ®) for 48 hours at 60 °C and weighed. Seed set was measured by counting the total number of seeds produced on each raceme (Elmor C1 Seed Counter ®) and the weight of all seeds per raceme to the nearest 0.001g was recorded.

Bee abundance and generic richness

Native bee communities were passively sampled with pan traps at each site for seven-day intervals throughout four weeks of peak canola bloom (mid-April – mid-May). Pan traps are commonly used for sampling bee communities (Roulston et al., 2007;

Saunders and Luck, 2013) but may be biased towards collecting smaller species, such as halictids (Toler, 2005). There is some evidence of an inverse relationship between pan trap capture rates and floral resource availability (Baum and Wallen, 2011; Roulston et al., 2007; Toler, 2005), but pan traps are still considered an effective and efficient method for sampling the bee community. Each pan trap array contained three pan traps (500 mL plastic beakers), with one painted each of three different colors: fluorescent yellow, fluorescent blue, and white; this combination of colors has been shown to be effective for capturing bee richness (Toler et al., 2005; Westphal et al., 2008; Droege et al., 2010). The pan traps were filled with 100% propylene glycol. For every liter of propylene glycol, several ml of Dawn® Original Blue dish washing detergent (Proctor & Gamble, Cincinnati, Ohio) was added to reduce the surface tension of the propylene glycol so that bees that landed on the liquid were more likely to be captured and less likely to be able to fly off of pan traps. To prevent the loss of samples during periods of rain, four small holes were made in the upper 2cm of the pan traps to allow excess liquid to leak out. The pan trap array was secured to a 1.2m rebar stake with a wood platform for holding the pan traps (Figure 1). The pan trap platform was adjustable and was moved to the approximate height of nearby canola plants. Samples were collected from pan trap arrays weekly and placed in individual 70ml scintillation vials filled with 70% ethanol.

In the laboratory, samples were removed from the 70% ethanol, rehydrated using warm water, washed in soapy water for five minutes, rinsed with warm water, and dried using forced air. Once dried, samples were keyed to family and representative individuals of each unique bee specimen were pinned or pointed and identified to genus and species, whenever possible.

Foraging and nesting resources across spatial scales

Five spatial scales were selected for the categorization of land uses within the study area. Nested spatial scales were selected to correspond to typical flight distances of varying groups of bees including, but not limited to: small solitary bees (*e.g.*, *Lasioglossum* spp.), medium sized solitary bees (*e.g., Osmia* spp.), large solitary bees (*e.g.*, *Andrena* spp.), very large solitary bees (e.g. *Anthophora* spp.), and large social bees (*e.g., Bombus* spp.; Zurbuchen et al., 2010b). The nested spatial scales measured 500m, 1000m, 1500m, 2000m, and 3000m radii and were centered on each site (Figure 2).

Land uses across the study area were downloaded from CropScape (USDA, 2019) at each nested spatial scale at each site for the year of and the year prior to when canola seed set (both seed count and total seed weight) was estimated to evaluate the effects of both current and previous year resources on native bees and plant level production. We downloaded Crop Data Layers (CDLs) as a raster layer that gives annual crop and land use specific land cover data with 30m pixel resolution. Using ArcGIS, we created unique pixel counts of all crop and land use cover at the seven spatial scales. Pixel counts were converted to total area in hectares. All land uses were then categorized into resources that are relevant to wild bee foraging and nesting. The land use categories were bee forage crops (i.e. crops that bees are known to visit for either pollen and nectar or both including canola, sorghum, millet, corn, cotton, herbs, peas, soybeans, dry beans, sunflowers, and alfalfa (USDA, 2017), canola (separated because it was the most common bee forage crop), non-bee forage crops (winter wheat, rye, barley, triticale, sod grass, non-alfalfa hay, and oats), forests, wetlands, development (roads, homes, oil and natural gas developments, etc.), pasture, and fallow (fallow and barren).

Statistical analyses

To evaluate if canola plant level productivity was influenced by wild bee abundance and richness, we used t-tests. Generalized mixed-effects models were used to test the effects of bee abundance and generic richness and land use on canola seed count using Poisson distribution. Linear mixed-effects models were used to test the effects of bee abundance and generic richness and land use on canola seed weight (g). Because land use metrics were highly correlated with one another at all spatial scales, mixed-effects models were constructed with single land use categories at a single scale as the fixed effect and site and year as random effects. All models used were random slope models to account for variations between sites and years and to reduce type I error (Schielzeth and Forstmeier, 2009). Because of the strong effect of bee abundance and generic richness on both seed count and total seed weight, both bee community metrics and the interaction between them were included in all models. This also prevented an unnecessarily high number of models. Appendix A includes all tested models. All analyses were performed in the R software program (R Development Core Team, 2005). Generalized and linear mixed-effects models were performed using the *lme4* package (Bates et al., 2015).

Results

Seed set as a function of pollination treatment, distance, and year

Across all years and sites, pollinator exclusion (bagged treatment) had a significantly negative effect on seed count (Figure 3A; $F_{1,\,566} = 49.18$; $p < 0.0001$) and total seed weight (g; Figure 3B; $F_{1,\,566} = 45.53$; $p < 0.0001$).

Among canola racemes in the open treatment, both seed count and total seed weight were significantly higher at greater distances into canola fields (g; Figure 4A & 4B; $F_{2,\,285} = 5.60$; $p < 0.004$; $F_{2,\,285} = 7.26$; $p = 0.0008$, respectively). Post-hoc analysis revealed that both seed count and total seed weight (g) were significantly greater at the 150m and 300m distances relative to the 75m distance ($p < 0.0511$).

Among canola racemes in the open treatment, seed count did not vary significantly by year (Figure 5A; $F_{2,\,285} = 1.85$; $p = 0.159$). Total seed weight (g) also did not vary significantly between years (Figure 5B; $F_{2,\,285} = 1.21$; $p = 0.3010$)

Bee community

Bee abundance varied significantly between years with 117 individuals collected in 2017, 757 collected in 2018, and 113 collected in 2019 (Figure 6A; $F_{2,\,20} = 3.963$; $p = 0.0355$). We collected 21 unique genera during the sampling periods between 2017 and 2019. Generic richness varied significantly between years with seven genera collected in 2017, 18 in 2018, and 11 in 2019 (Figure 6B; $F_{2,\,20} = 9.428$; $p = 0.0013$). The most common genus during each year was *Lasioglossum* followed by *Andrena* and *Agapostemon.* We caught several uncommon genera including: *Osmia, Xenoglossa, Hyleus,* and *Heriades.* Post hoc analysis revealed that bee abundance and generic richness were significantly higher in 2018 compared to both 2017 and 2019.

Seed set as a function of bee community and landscape characteristics

At the largest spatial scale (3000m radius), the model including area of bee forage crops, bee abundance, bee generic richness, and the interactions between each factor as fixed effects was the best predictor of canola seed count (Table 1; AIC =18832; ΔAIC = 0.0; w = 0.999). Total seed weight at the largest spatial scale was best predicted with a model including the area of canola crop with bee abundance, bee generic richness, and the interactions between each factor (Table 2; AIC = 449.3, ΔAIC = 0.0; w = 0.772).

At the 2000m scale, the model including area of bee forage crops, bee abundance, bee generic richness, and the interactions was the best predictor of seed count (AIC = 18837, ΔAIC = 0.0; w = 0.999). However, total seed weight was more difficult to predict using mixed effect models. The model that fit the data best included the area of fallow and idle cropland from the previous year and bee abundance and bee generic richness (AIC = 452.8, ΔAIC = 0.0; w = 0.362). Models including the area of non-bee forage crops from the previous year and the area of canola were both within 2.0 ΔAIC of the first model.

At the 1500m scale, the model including the area of canola from the previous year along with bee abundance, bee generic richness and the interaction between the factors as fixed effects was the best predictor of canola seed count (AIC = 18976; ΔAIC = 0; w = 0.463). At the 1500m scale, total seed weight was best predicted in a model including the area of grass and pastureland, bee abundance, bee generic richness, and their interactions (AIC = 453.8; ΔAIC = 0; w = 0.409). Two additional models fell within

2.0 ΔAIC of the best fit model for total seed weight. One model included the area of fallow and idle cropland and the other included the area of developed land.

At the 1000m scale, seed count was best predicted using a model that included the area of non-bee forage crops, bee abundance, bee generic richness, and their interactions (AIC = 18914; ΔAIC = 0; w = 0.999). Total seed weight was best predicted using a model that included the area of fallow and idle cropland, bee abundance, bee generic richness, and their interactions (AIC = 453.5; ΔAIC = 0; w = 0.470).

At the smallest scale, 500m radius, seed count was best predicted by a model including the area of canola from the previous year, bee abundance, bee generic richness, and their interactions (AIC = 18902; ΔAIC = 0; w = 0.501). A second model was equally predictive of seed count and it included the area of bee-forage crops from the previous year, bee abundance, bee generic richness, and their interactions (AIC = 18902; ΔAIC = 0; w = 0.471). A model including the area of forest, bee abundance, bee generic richness, and their interactions was most predictive of total seed weight at the 500m scale (Table 2; AIC = 447; ΔAIC = 0; w = 0.826).

Across all spatial scales, the model including the area of bee forage crops at the 3000m scale, bee abundance, bee generic richness, and their interactions was the best predictor of seed count (Table 1). Across all spatial scales, two models were within two ΔAIC of one another in predicting total seed weight. A model including the area of forest at the 500m scale, bee abundance, bee generic richness, and their interactions was the best predictor of total seed weight (Table 2). A model including the total area of canola crop at the 3000m scale with bee abundance, bee generic richness, and their interactions predicted total seed weight within two ΔAIC (Table 2).

Discussion

Pollination services provided by bees positively affected canola seed set in this study system despite canola cultivars being self-compatible (Bommarco et al., 2012). These results are consistent with previous studies that found that commercial honey bees and wild bees increase canola production (Lindström et al., 2016; Zou et al., 2017). Indeed, increased seed weight likely improves oil content and, therefore, yield for producers (Ricketts et al., 2008). Thus, canola productivity will benefit from a greater abundance of bees. Previous research has found that wild bees may improve pollen deposition and seed set in canola regardless of the presence of honey bees (Garibaldi et al., 2013). Although wild bees and honey bees populations are declining, wild bees may provide synergistic pollination services with honey bees that could offer insurance for canola production (Henselek et al., 2016). In the context of increasing demand for crops that benefit from bee pollination, understanding the effects of wild bee communities on crop production, independent of honey bees, is important to develop approaches that support production.

Contrary to our predictions, monocultures of canola did not correlate with greater abundance or generic richness of wild bees, and, in fact, resulted in significantly reduced wild bee abundance and generic richness. Although not significant, both seed count and total seed weight increased as bee abundance and generic richness increased. Our results add to existing literature that suggests landscape composition affects MFC productivity indirectly via its direct effects on wild bee abundance and generic richness (Bommarco et al., 2010; Garibaldi et al., 2013; Potts et al., 2010; Winfree et al., 2009).

This study system is unique because it has historically been composed of wheat and managed rangeland and the recent addition of canola as a rotating crop with wheat significantly increased floral resources. Wheat-canola rotation may influence the wild bee community and the pollination services they provide to canola and wild flowering plants (Epplin and Peeper, 2011). Wild bee populations in the southern Great Plains may have been constrained by the homogenous wheat-rangeland agricultural matrix with few patches of floral resources available (Holzschuh et al., 2007). The addition of canola to this agroecosystem added a significant yet short-lived pulse of floral resources that may benefit wild bee populations (Grass et al., 2016; Holzschuh et al., 2007). Although canola provides novel floral resources, canola rotation does not affect the already limited area of semi-natural and natural habitat where wild bees most likely nest and forage throughout the growing season. The abundance of forage resources offered by canola may temporarily boost wild bee populations as they are attracted to canola fields. However, wild bee populations then likely experience a steep decline in floral resource availability after canola senesces.

Canola was introduced into the study system in the early 2000s to help decrease the disease, pest, and weed pressure on continuous wheat fields (Bushong et al., 2012; Lofton et al., 2010) and has led to an overall increase in pesticide use in this system as canola attracts many species of aphid pests (Franke et al., 2009; Reddy, 2017). The majority of wheat-canola producers use pyrethroid pesticides to suppress aphid outbreaks in canola, often sprayed heavily around the time of flowering when aphid outbreaks are common (Reddy, 2017). This poses a significant risk to wild bees as the lethal and non-lethal effects of pyrethroids on wild bees are well documented across agroecosystems (Baron et al., 2014; Desneux et al., 2007; Dietzsch et al., 2019; Oliver et al., 2015; Peterson et al., 2021; Thatheyus and Selvam, 2013). It is unknown if the benefits of the floral resource pulse provided by canola to wild bees outweigh the negative effects of pyrethroids used during peak canola bloom (Reddy, 2017).

Floral communities are strong drivers of wild bee communities (Potts et al., 2003; Roulston and Goodell, 2011). Nectar quality and diversity, floral diversity, and ratio of pollen to nectar energy are important predictors of bee community structure and bee diversity (Potts et al., 2003). Wild flowering plants are frequently eliminated from wheat-rangeland agroecosystems to improve forage for cattle, reduce pests, and reduce competition with crops, often drastically reducing forage resources for wild bees (Black et al., 2011; Fuhlendorf et al., 2009). Our results suggest that even landscapes with MFCs may not provide the necessary nectar and pollen diversity to support wild bee communities. In this study, canola made up the greatest proportion of bee forage crops. Despite canola providing abundant nectar and pollen, the nutrition profile of canola nectar and pollen, in particular, may not provide wild bees with the nutrients required for nest provisioning (Carruthers, 2016). Even if canola provides important forage resources for wild bees, it only blooms between mid-April and mid-May. The brief surge of floral resources in this study system provided by canola may initially offer abundant forage for wild bees but the limited wild flowering plant resources available later in the growing season after canola senescence may not sustain the wild bees that visited canola fields.

Agrochemical use negatively impacts wild bees, and herbicides are increasingly used on genetically modified crop varietals, including canola, that are resistant to herbicides and are associated with decreased pollinator densities (Bohnenblust et al., 2016; Shaw et al., 2011). Broad-spectrum insecticides, such as pyrethroids, are used to suppress pest outbreaks and attack the nervous system of insects, including wild bees (Desneux et al., 2007). In studies of canola treated with pyrethroid insecticides, wild bee reproductive rates and survival declined (Rundlöf et al., 2015), foraging rates decreased (Dietzsch et al., 2019), and larval development decreased (Klaus et al., 2021). The timing of application of pyrethroids in this system, which peaks during canola bloom, likely negatively impacts wild bees and potentially pollination services to canola and wild flowering plants (Franke et al., 2009; Reddy, 2017).

Spatial scales affected which model best predicted seed count and total seed weight. Total seed weight models varied more at different spatial scales than count models. We included several spatial scales to account for varying maximum flight distances of wild bees that occur in this study region. Seed count was consistently predicted by models that included area of bee forage crops or area of canola across spatial scales, suggesting that bee forage crop area may have similar effects on wild bee species of different body sizes that vary in maximum flight distances. Total seed weight, however, was predicted by different land use categories at nearly every spatial scale. These results suggest that other factors not included in these models may be important in predicting seed weight, such as fertilizer use, cultivar, or pollinator visitation metrics (visitation rate, pollen deposition, etc.; Ashman et al., 2004).

In conclusion, landscape composition and wild bee abundance and generic richness have strong yet complex effects on canola seed count and total seed weight. The southern Great Plains represents a unique study system in which canola provides abundant floral resources for wild bees in a predominantly wheat-rangeland agroecosystem. The long history of agricultural land use in the southern Great Plains has likely limited nesting habitat and floral resources after canola senescence for wild bees. Additionally, canola rotation has increased the use of broad-spectrum insecticides in this study system which are known to negatively impact wild bees. Wild bees remain important contributors to canola production and conserving wild bee populations by increasing nesting habitat and season-long floral resources within this system may benefit wild bees and indirectly improve canola productivity. Future research should consider additional landscape characteristics such as patch size and connectivity and if these characteristics support wild bee populations and improve canola productivity. Additional research could evaluate which specific genera of wild bees visit canola and what landscape characteristics are associated with the presence and absence of these genera. Finally, it may particularly beneficial to understand if complementary MFC plantings improve floral resource availability to wild bees within this system.

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Tables and Figures

Figure 1. Pan trap array consisted of three pan traps (500 mL plastic beakers). Pan traps were painted one of the three colors: fluorescent yellow, fluorescent blue, and white. Pan traps were secured to a wooden platform and attached to 1.2m rebar stake. Pan trap platform was adjustable and moved to the approximate height as nearby canola plants throughout sampling period.

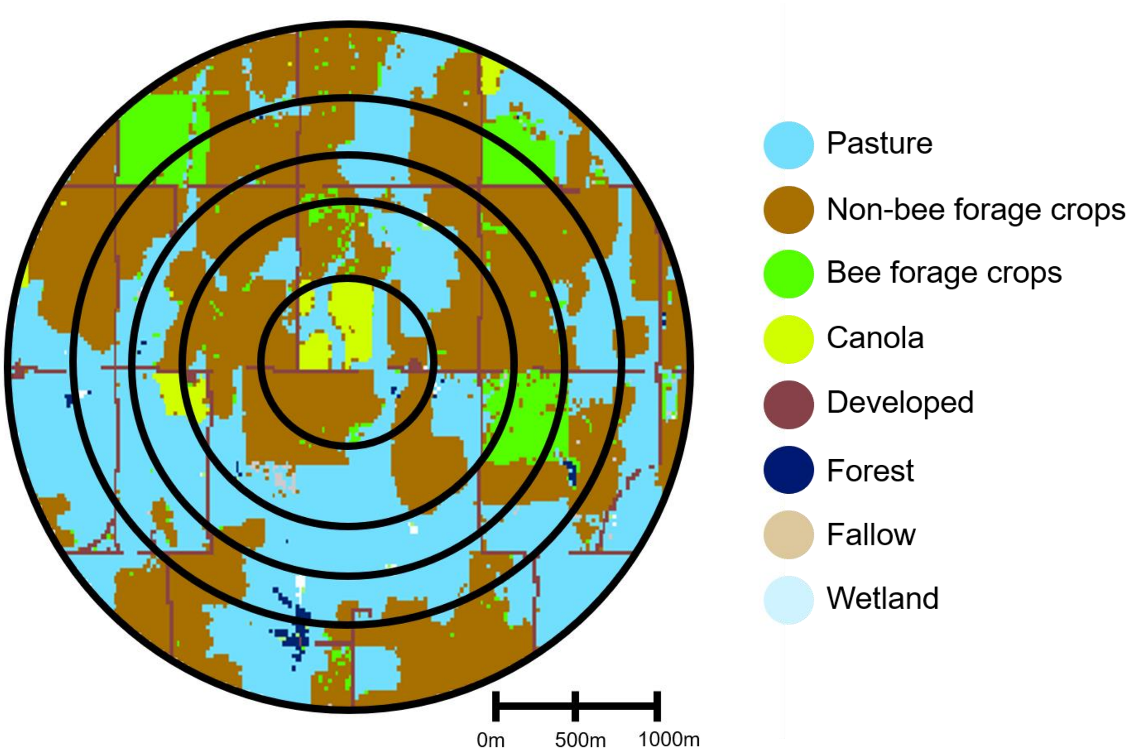

Figure 2. Example of spatial scales and land use categories taken from CropScape raster layer. Canola field is located at the center of the figure in yellow surrounded by the 500m, 1000m, 1500m, 2000m, and 3000m radii scales indicated in black.

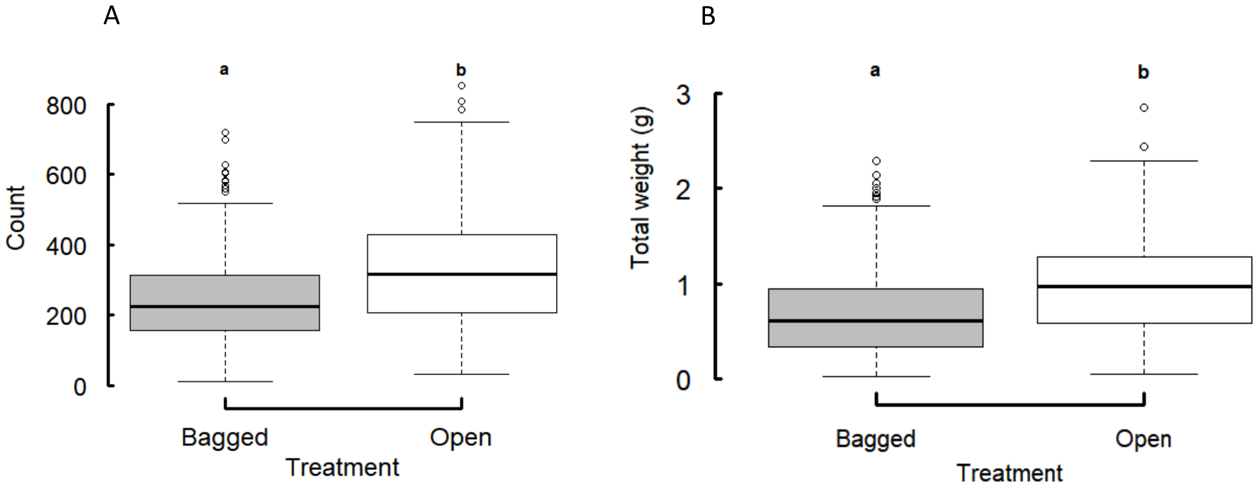

Figure 3. Seed count in open-pollinated canola racemes was significantly greater than in bagged canola racemes (A) Total seed weight (g) in open-pollinated racemes was significantly greater than in bagged plants (B). Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers. Significance is indicated by lowercase letters.

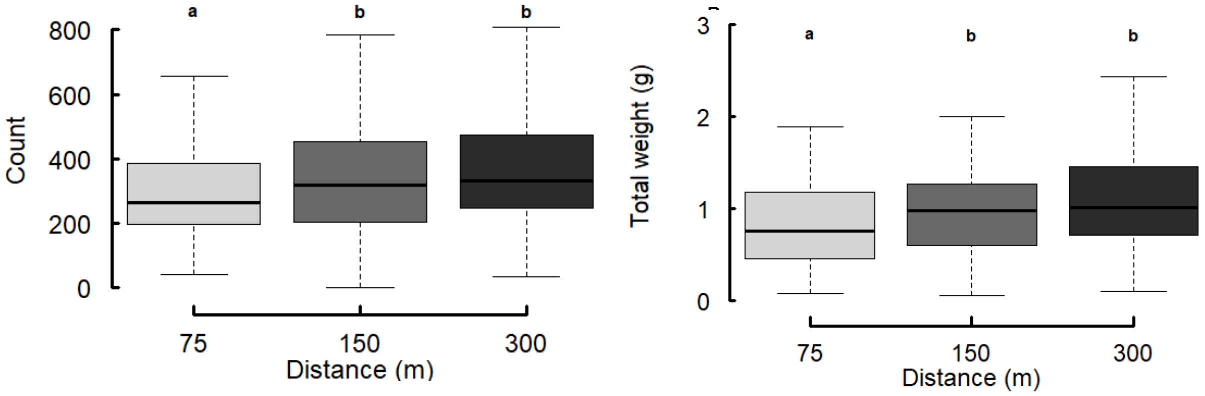

Figure 4. Seed count within the open treatment group was significantly greater at 150m and 300m relative to 75m distance into canola fields (A).Total seed weight (g) within the open treatment group was significantly greater at 150m and 300m relative to 75m into canola fields (B). Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers. Significance is indicated by lowercase letters.

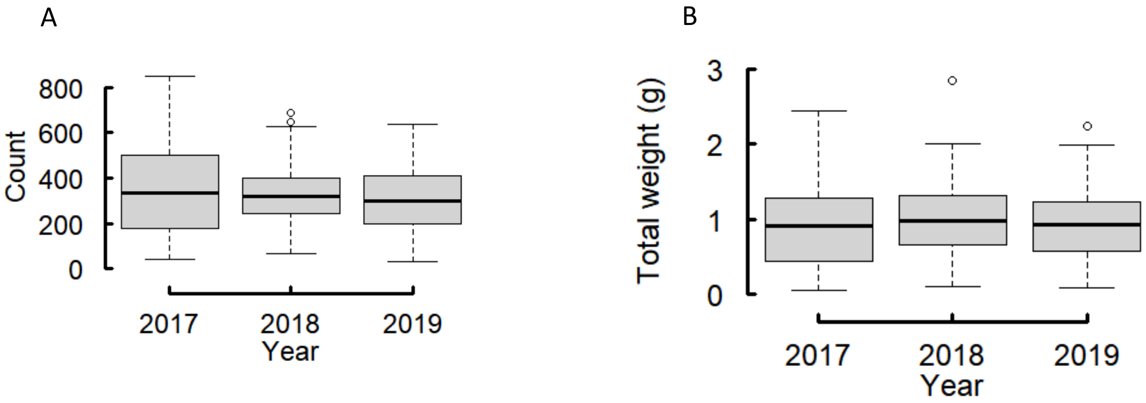

Figure 5. Seed count and total seed weight (g) did not vary significantly by year (). Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers.

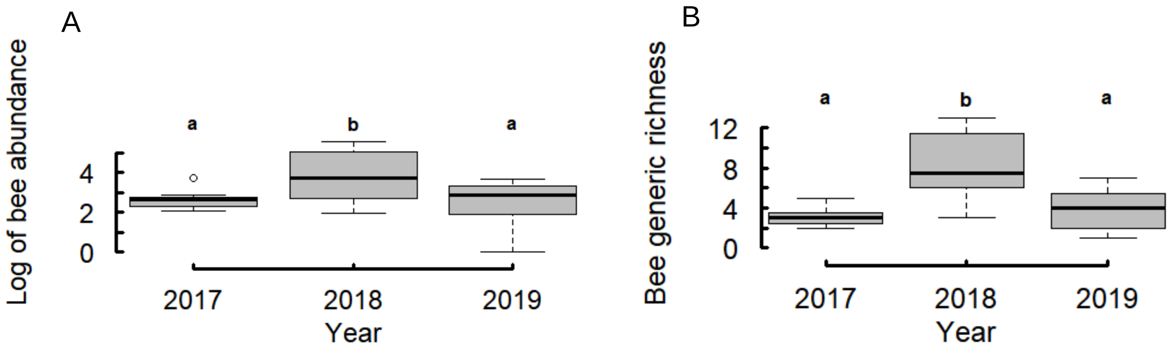

Figure 6. Log of bee abundance varied significantly by year (A). Bee generic richness also varied significantly by year (B). Both bee abundance and generic richness were significantly higher in 2018 relative to 2017 and 2019. Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers. Significance is indicated by lowercase letters.

Table 1. Results for generalized linear mixed-effects models of canola seed count for the best fit model. Fixed effects variables included land use characteristics, bee abundance, bee generic richness, and the interactions between those factors.

Response variable	**Fixed effect variable**	**Estimate**	**SE**	***z* – value**	***p – value***
Seed count (3000m)	Intercept	6.159	0.318	19.382	<2e-16
	Area of bee forage crops	-7.308e-4	3.949e-4	-1.850	0.064
	Bee abundance	-0.088	0.056	-1.560	0.119
	Bee generic richness	0.051	0.010	5.203	<2e-16
	Area of bee forage crops * bee abundance	1.087e-4	6.978e-5	1.557	0.119
	Area of bee forage crops * bee generic richness	1.412e-4	1.095e-5	12.893	<2e-16
	Bee abundance * bee generic richness	-1.193e-3	2.308e-4	-5.170	<2e-16
	Area of bee forage crops * bee abundance * bee generic richness	-1.378e-5	1.309e-6	-10.525	0.001

Table 2. Results for the top two linear mixed-effects models of total canola seed weight (g). Fixed effects variables include area of canola at the 3000m scale and area of forest at the 500m scale, bee abundance, bee generic richness, and the interactions between those factors.

Response variable	**Fixed effect variable**	**Estimate**	**SE**	***t* – value**
Total seed weight (g; 3000m)	Intercept	0.721	0.127	5.664
	Area of canola	2.967e-3	1.210e-3	2.451
	Bee abundance	0.034	0.012	2.834
	Bee generic richness	0.051	0.010	5.203
	Area of canola * bee abundance	-7.376e-4	2.594e-4	-2.843
	Area of canola * bee generic richness	-2.738e-4	4.460e-4	-0.614
	Bee abundance * bee generic richness	-3.585e-3	1.251e-3	-2.865
	Area of canola* bee abundance * bee generic richness	8.939e-5	3.944e-5	2.267
Response variable	**Fixed effect variable**	**Estimate**	**SE**	***t* – value**
Total seed weight (500m)	Intercept	0.988	0.082	12.047
	Area of forest	-0.016	0.037	-0.428
	Bee abundance	0.008	0.007	1.037
	Bee generic richness	-0.002	0.027	-0.063
	Area of forest * bee abundance	-0.019	0.007	-2.945
	Area of forest * bee generic richness	0.009	0.010	0.893
	Bee abundance * bee generic richness	-0.0006	0.0008	-0.747
	Area of forest * bee abundance * bee generic richness	0.003	0.0010	2.935

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CHAPTER IV

CANOLA SUPPORTS WILD BEE POLLINATION OF WILD PLANTS ACROSS SPATIAL SCALES

Abstract

Agricultural land use affects wild bee-plant mutualisms, increasing the incidence of pollen limitation among wild flowering plants. Over time, pollen limitation may have a negative impact on wild flowering plant populations. Mass flowering crops (MFCs) increase foraging resource availability to wild bees in historically forage-poor agroecosystems. Increased foraging resources by way of MFCs, may enhance or dilute pollination of wild flowering plants growing nearby. Canola (*Brassica napus* L.) produces abundant nectar and pollen and was recently introduced as a rotational crop with wheat in the southern Great Plains. We tested the effects of novel MFCs on wild bee-plant mutualisms by evaluating seed set of three wild flowering plants, G*aillardia pulchella* (Foug.; Indian blanket)*, Verbesina encelioides* (Cav.; cowpen daisy), and *Salvia azurea* (Michx ex Lam.; azure blue sage), across a gradient of MFC cover. We also tested pollen limitation of each wild flowering plants found in this system. Each species experienced increased seed set with increased canola cover, suggesting that canola may enhance pollination services to wild flowering plants. However, each species also demonstrated pollen limitation. MFCs may play an important role in supporting wild bees and wild flowering plants in some agroecosystems by providing foraging resources for bees and by enhancing pollination services to wild flowering plants. However, other studies have found that MFCs caused dilution of pollination services to wild flowering plants. Our results show that MFC support wild bee-plant mutualisms, particularly in historically forage-poor agroecosystems. In the context of ongoing wild bee declines, understanding of the effects of MFCs on wild bee-plant mutualisms is important in the

conservation of wild bees and wild flowering plants. By supporting both wild bees and wild flowering plants in agroecosystems, MFC yields may also increase.

Introduction

Flowering plants, both cultivated and wild, experience pollen limitation. Pollen limitation is the lack of or insufficient deposit of conspecific pollen reducing seed count, weight, or quality in flowering plants (Ashman et al., 2004; Burd, 1994). Many empirical studies show that pollen supplementation improves seed set and seed quality (Ashman et al., 2004; Burd, 1994; Larson and Barrett, 2000), indicating the pressures of pollen limitation on flowering plants. Pollen limitation may occur if plants are self-incompatible (Lloyd and Schoen, 1992). Without the ability to self-fertilize, self-incompatible plants rely solely on the deposition of conspecific pollen. Flowering plants that are capable of self-fertilization may not be as pollen limited as those that are self-incompatible. Many annuals are self-compatible and will self-fertilize in the absence of sufficient out-crossed pollen or lack of pollinators (Knight et al., 2005; Porcher and Lande, 2005). Autogamy may, therefore, insulate self-compatible plants from pollen limitation. However, self-compatibility may increase inbreeding depression within a population, reducing genetic diversity over time (Charlesworth and Charlesworth, 1987; Schemske and Lande, 1985). Outcrossing enables flowering plant populations to avoid inbreeding depression and may allow populations to more rapidly adapt to environmental changes (Charlesworth and Charlesworth, 1987; Crow, 1992; Lande and Schemske, 1985). Pollinators are an important source of outcrossed pollen for self-compatible plants.

Specialist flowering plant species are likely more pollen limited than generalist species. Tightly coevolved plant-pollinator mutualisms are obligate and require their specific partner(s) for effective seed set (Vázquez and Aizen, 2004). For example, long-tongued pollinators (*e.g.,* hawkmoths, bumble bees, and hummingbirds) are the only pollinators equipped to reach the nectaries of long-spurred flowers (*e.g., Angraecum sesquipedale* and the genus *Aquilegia;* Darwin, 1888; Whittall and Hodges, 2007). The majority of pollinators are short-tongued and are not effective pollinators of flowers with long-corollas. Should few long-tongued pollinators occur within a pollinator community, specialist long-corolla flowers relying on those pollinators may be more pollen limited compared to open, short-corolla flowers, such as Asteraceae. Short-lived species are expected to be less pollen limited than long-lived flowering plants (Knight et al., 2005). One reason for this is that the majority of autogamous plant species are short-lived annuals or monocarps (Aarssen, 2000). Another reason may be that, among annuals, we expect any pollen limitation to significantly impact fitness and therefore, selection for traits that reduce pollen limitation will be stronger in monocarpic and annual species than in polycarpic and perennial species that are more resilient to variations in pollen deposition and pollinator populations from year to year (Calvo and Horvitz, 1990; Primack and Hall, 1990; Vamosi and Otto, 2002). Monocarpic and annual populations may be more threatened than polycarpic and perennial populations by decreases in pollinator populations.

Pollen limitation among wild flowering plants is affected by landscape composition (Ferreira et al., 2013). In agroecosystems, increased natural area can lead to increased pollinator abundance and richness, which indirectly reduces pollen limitation among both cultivated and wild flowering plants (Cusser et al., 2016). Highly modified agricultural landscapes, particularly those with large monocultures, reduce connectivity between optimal foraging and nesting habitats for pollinators and may isolate populations or impede pollinators’ ability to move between patches and, over time, cause extirpations within these systems (Garibaldi et al., 2011; Ricketts et al., 2008; Zurbuchen et al., 2010). Several studies have documented a relationship between landscape composition and pollen limitation of cultivated plants, although the relationship may be weak (Cusser et al., 2016; Klein et al., 2003). The effects of landscape composition on pollen limitation of wild flowering plants growing in margins of agroecosystems is not well-understood. Because wild flowering plants may be more pollen limited than cultivated plants, which are frequently bred to be, at least in part, autogamous, the effects of landscape composition and pollen limitation may be more pronounced.

Wild flowering plants may experience changes to pollen limitation in the presence of mass flowering crops (MFCs) such as canola (*L.; Brassica napus*). Some MFCs cause pollination services to be diluted if pollinators forage on crops and not on wild flowering plants (Holzschuh et al., 2013). Dilution of pollination services to wild flowering plants may cause decreased seed set and inbreeding depression as populations decline and potentially rely more on self-fertilization. *Primula veris* (L.; cowslip) seed set decreased as the percentage of canola increased across the landscape, suggesting a dilution of bee pollination services (Holzschuh et al., 2011). In the southern Great Plains, canola blooms early in the growing season and thus pollination dilution may occur more frequently in early blooming wild flowering plants. Late blooming plants may experience pollination spillover services once canola has senesced and bees must rely on wild flowering plants for forage (Woodcock et al., 2016). Herbertsson and colleagues (2017) found fruit set in *Fragaria vesca* (L.; woodland strawberry), which blooms after canola, was highest in areas with low abundance of wild flowering plants and high abundance of canola, suggesting that canola promotes spillover pollination services to other wild flowering plants, especially when the landscape has limited heterogeneity. However, they did not observe any differences in pollination of *Trifolium pratense* (L.; red clover), which also flowers after canola (Herbertsson et al., 2017). There is increasing evidence that MFCs may alter wild flowering plant-pollinator mutualisms potentially changing populations of both mutualist partners. Understanding pollination service spillover or dilution to wild flowering plants across areas with different amounts of canola on the landscape is critical to predict these population level effects on wild flowering plants, and begin to understand the implications for the pollinators that rely on them.

In the southern Great Plains, canola was introduced in the early 2000s as a rotational crop with winter wheat to reduce disease, pest, and weed burdens (Bushong et al., 2012; Lofton et al., 2010). Canola, with its abundant nectar and pollen resources, greatly increased forage availability to pollinators, particularly in the early growing season (mid-April to mid-May) when many wild flowering plants may not be in bloom. Pollinators, therefore, may be attracted to agroecosystems with increased canola cover (Grass et al., 2016; Holzschuh et al., 2007). However, the brief surge of foraging resources provided by canola may not sustain pollinator populations as canola flowers senesce by mid-May. Pesticide use in the southern Great Plains has increased with the addition of canola because of several species of aphids. Outbreaks of aphid pests on canola coincides with canola flowering and many producers use pyrethroid pesticides to combat the outbreaks (Franke et al., 2009; Reddy, 2017). The lethal and sublethal effects of pyrethroids on pollinators are well documented (Dietzsch et al., 2019;

Peterson et al., 2021; Thatheyus and Selvam, 2013). Whether the benefit of increased forage resources exceeds that of the detriment of increased insecticide use in this study system is unknown. More research is necessary to understand the myriad impacts canola may have on pollinator populations in this study system.

Among pollinators, managed and wild bees are the dominant pollinator to the majority of entomophilous cultivated crops and many wild flowering plants (Klein et al., 2007; Ollerton, 2017; Potts et al., 2016). Declines of managed honey bees and wild bee populations are well documented across the world (Biesmeijer et al., 2006; IPBES, 2016; Potts et al., 2010; Zattara and Aizen, 2021). Bee declines may exacerbate pollen limitation that may already occur within both entomophilous crops and wild flowering plants. Although many crops are bred to be autogamous, crop yields increase when out-crossing occurs via bee pollination including: canola (Araneda Durán et al., 2011; Halinski et al., 2018; Perrot et al., 2018; Shakeel and Inayatullah, 2013), cotton (Stein et al., 2017), almonds (Sáez et al., 2020; in a study focused on a self-compatible variety), strawberries (MacInnis and Forrest, 2019) and many more. Bee pollination increases seed set among wild flowering plants, as well (*e.g.,* Albrecht et al., 2012; Klein et al., 2007; Schmucki and de Blois, 2009). Should bees continue to decline, the disruption of bee-plant mutualisms may cause seed set to decrease among wild flowering plants and entomophilous crops. This disruption may cause plants to rely on autogamy more frequently which may ultimately lead to reduced genetic diversity and possible declines among plant populations. In the context of land use change and expansion of MFCs, it is important to understand these changes on wild flowering plant reproductive success within agroecosystems. We compared seed set between open-pollinated and hand-pollinated treatments within three common wild flowering plant species, G*aillardia pulchella* (Foug.; Indian blanket)*, Verbesina encelioides* (Cav.; cowpen daisy), and *Salvia azurea* (Michx ex Lam.; azure blue sage) to test if each species experiences pollen limitation in this study system. To evaluate if timing of bloom determines pollen limitation, we included one early blooming species, *G. pulchella,* and two late blooming species, *V. encelioides* and *S. azurea.* To evaluate if floral morphology determines pollen limitation, we included one long-corolla species, *S. azurea,* and two short-corolla species, *G. pulchella* and *V. encelioides.* To test the effects of a MFC on seed set of each study species, we modeled the effects of canola cover on seed count and seed weight of each study species at three increasing spatial scales.

Methods

Site selection

A total of nine unique sites were selected, four in 2018 and five in 2019. Sites were selected such that each site varied in the area of canola crop cover within three nested spatial scale radii: 500m, 1000m, and 2000m to test how canola area affected pollination services to the three study species (Table 1). Sites differed between years due to crop rotation between canola and winter wheat, which is determined by producers. Each site was separated by a distance of at least 3000m to limit overlap between sites and access by individual bees (Zurbuchen et al., 2010).

Land use at each site was downloaded from Cropscape (USDA, 2019) at each nested spatial scale for the year in which the site was used. We downloaded Crop Data Layers (CDLs) as raster layers. CDLs give annual crop and land use cover data with a

30m pixel resolution. Using ArcGIS, we created unique pixel counts of canola cover data at each of the three nested spatial scales. Pixel counts were then converted to total area in hectares. We calculated percent cover of canola within each nested spatial scale.

Study species

We selected three common wild flowering plant species for this study. We selected one species that blooms early in the growing season, *G. pulchella*, and two that bloom late in the growing season, *V. encelioides* and *S. azurea.* We originally planned to use *Monarda citriodora* (Cerv. Ex Lag; lemon beebalm), but many of them failed in the greenhouse during the spring of 2018 so we used *S. azurea* in 2019. *Gaillardia pulchella* and *V. encelioides* have an open floral morphology with short corollas and a wide variety of bees that may pollinate them whereas *S. azurea* has a tubular floral morphology with a longer corolla and a smaller guild of bees that are capable of pollinating it.

Verbesina encelioides is a native annual Asteraceae found primarily in the south-central United States, blooming late in the growing season, from July to September. It reaches a height of 30-90 cm and is ramose with a varying number of 3-5 cm diameter yellow flower heads (Correll and Johnston, 1979). *Verbesina encelioides* is self-compatible but may also be cross-pollinated by various generalist pollinators (Feenestra and Clements, 2008).

Gaillardia pulchella is a native annual Asteraceae found throughout the south-central United States and blooms early in the growing season, from May to June (Hammond et al., 2007). *Gaillardia pulchella* reaches a height of approximately 30-60 cm with a solitary red and yellow flower head that is typically 3.8-7.6 cm in diameter (Hammond et al., 2007). *Gaillardia pulchella* is self-incompatible and is typically pollinated by a wide variety of short-tongued bees, flies, and soldier beetles (Cantharidae; Heywood, 1986, Stoutamire, 1977).

Salvia azurea is a perennial that occurs across the Midwest and eastern United States. *Salvia azurea* reaches approximately 1-1.5 meters in height with whorled, blue inflorescences that bloom late in the growing season, between August and October. It has a deep corolla and is primarily pollinated by specialist, long-tongued bees such as bumble bees (Claßen-Bockhoff et al., 2004; Grant and Grant, 1964). Carpenter bees (*Xylocopa virginiaca*) are also documented as pollinators of *Salvia* spp. (Celep et al., 2014; Claßen-Bockhoff et al., 2004; Grant and Grant, 1964; Ott et al., 2016) but are also well documented nectar robbers so their contribution to pollination of *S. azurea* is not well understood (Adler and Irwin, 2005; Irwin et al., 2010; Varma et al., 2020). Although self-compatible, outcrossing is correlated with increased seed set among *Salvia* spp. (Ott et al., 2016).

We sowed at least 200 seeds of each species in vermiculite in late winter and transplanted 108 individual seedlings to 15.24 cm diameter pots containing a potting mix (Sta-Green Potting Mix plus Fertilizer® 10% N, 8%P, 8%K). Plants matured in a greenhouse until flower buds emerged on plants and nearly opened. We determined both *V. encelioides* and *G. pulchella* were mature and ready for the pollination experiment once each plant produced one or more inflorescences. *Salvia azurea* produced many flowers per plant arranged in whorls along a stalk. Once the bottom most whorl of inflorescences bloomed and the upper whorl buds appeared, plants were deemed mature enough for the pollen limitation experiment. Individual inflorescences were selected on each plant immediately upon opening. Each experimental plant was labeled at the site of the experimental inflorescence with waterproof paper labels identifying plant number, treatment group, site location, and date. The labels were attached to the stem of the plant with paper clips (Figure 1).

To test pollen limitation, we used three replicate inflorescences on three separate plants at each site in each of the three treatments: hand-pollinated, open-pollinated, and bagged. Because *S. azurea* only produces a maximum of four seeds per inflorescence (Haque and Ghoshal, 1981), we used three inflorescences on each experimental plant for each pollination treatment. To prevent pollination in the bagged treatment group, we used bridal veil cloth (1 mm^2 openings) to prevent pollinators accessing inflorescences. We created bridal veil cloth pouches that were zip-tied onto the stem below the experimental inflorescence. Bridal veil cloth prevented ambient pollination by insects but allowed sunlight and gas exchange (Delaplane et al., 2013). The hand-pollinated treated inflorescences were hand-pollinated using donor pollen from additional greenhouse-grown plants that were not used as part of the experimental treatments. Pollen from donor plant anthers was collected with a cotton swab by brushing the cotton swab across the anthers approximately 10 times. Donor pollen on the cotton swab was then brushed across the experimental plant’s stigma approximately 10 times (Wesselingh, 2007). We hand-pollinated experimental inflorescences in the greenhouse immediately before placing plants at the field sites. Finally, the open-pollinated treated inflorescences were left exposed to insect pollinators. For each study species, three plants per treatment were placed adjacent to sites at 10m intervals. To evaluate the effects of canola cover on seed set of each experimental species, we used only the open-pollinated treatment plants.

Experimental plants of *G. pulchella* and *V. encelioides* were placed at sites for a total of 48 hours during the summer of 2018 and 2019. Experimental plants of *S. azurea* were placed at sites for a total of 48 hours during the summer of 2019 only. *Gaillardia pulchella* experimental plants were placed at sites on the 25th of June in 2018 and the 26th of June in 2019. *Verbesina encelioides* experimental plants were placed at sites on the 14th of July in 2018 and the 9th of August 2019. *Salvia azurea* experimental plants were placed at sites on the 7th of August in 2019. After the 48 hours, experimental plants were collected and returned to the greenhouse so that seeds could mature. Seeds were collected from plants by October of 2018 and 2019, respectively. Seeds were then dried (Yamato Gravity Convection Oven DVS 600 ®) at 60°C for 48 hours and immediately weighed and counted using a seed counter adjusted to each seed size (Elmor C1 Seed Counter ®).

Statistical analyses

To evaluate if treatments affected seed count and seed weight, we performed one-way ANOVAs. Generalized mixed-effects models were used to test the effects of canola area on seed count using Poisson distribution. Linear mixed-effects models were used to test the effects of canola area on seed weight (mg). All analyses were performed in the R software program (R Development Core Team, 2005). Generalized and linear mixed-effects models were performed using the *lme4* package (Bates et al., 2015).

Results

Seed count of *V. encelioides* varied significantly between pollination treatments ($F_{2,78}$ = 17.95, $p < 0.0001$; Figure 2A). Post-hoc analyses revealed hand-pollinated *V. encelioides* set significantly more seeds than either the open-pollinated or the bagged plants ($p = 0.0026$, $p < 0.0001$; respectively). Open-pollinated plants produced significantly more seeds than the bagged treatment plants ($p = 0.0357$).

Seed weight of *V. encelioides* varied significantly between pollination treatments ($F_{2,78}$ = 11.36, $p < 0.0001$; Figure 2B). Post-hoc analysis revealed that hand-pollinated *V. encelioides* set significantly heavier seeds than both open-pollinated and bagged treatment plants ($p = 0.0213$, $p < 0.0001$; respectively). There was no difference in seed weight between open-pollinated and bagged treated *V. encelioides* plants.

Seed count of *G. pulchella* varied significantly by pollination treatments ($F_{2,78}$ = 12.28, $p < 0.0001$; Figure 3A). Post-hoc analyses revealed that hand-pollinated *G. pulchella* set significantly more seeds than the open-pollinated or bagged treatments ($p = 0.0416$, $p < 0.0001$; respectively). Open-pollinated *G. pulchella* plants produced significantly more seeds than bagged plants ($p < 0.0001$).

Seed weight of *G. pulchella* plants varied significantly between pollination treatments ($F_{2,78}$ = 197.3, $p < 0.0001$; Figure 3B). Post-hoc analysis revealed that hand-pollinated *G. pulchella* set significantly heavier seeds than the bagged treatment plants ($p < 0.0001$). The open-pollinated *G. pulchella* plants set significantly heavier seeds than the bagged treatment plants ($p < 0.0001$). There was no difference in seed weight between the open-pollinated and hand-pollinated treated *G. pulchella* plants.

Seed count of *S. azurea* varied significantly by pollination treatment ($F_{2,42}$ = 20.96, $p < 0.0001$; Figure 4A). Post-hoc analyses revealed that bagged, open-pollinated, and hand-pollinated treatments each varied significantly, with seed count increasing for each treatment group ($p = 0.0189$, $p < 0.0001$, $p < 0.0001$; respectively).

Seed weight of *S. azurea* varied significantly by treatment ($F_{2,42} = 34.93$, $p < 0.0001$; Figure 4B). Post-hoc analysis revealed that hand-pollinated *S. azurea* were significantly heavier than the open-pollinated and bagged treatment plants ($p = 0.0015$, $p < 0.0001$; respectively). There was no difference in seed weight between bagged and open-pollinated treated *S. azurea* plants.

Seed count and seed weight of *V. encelioides* increased as percent cover of canola increased, particularly at the smallest spatial scale (500m radius); however, the trend was not significant ($F_{1,25} = 3.008$, $p = 0.0952$; $F_{1,25} = 1.624$, $p = 0.2143$; Figure 5A and 5B). When we considered only the 2018 sample year, both seed count and seed weight of *V. encelioides* increased significantly as percent cover of canola increased at the 500m scale ($F_{1,10} = 10.90$, $p = 0.0080$; $F_{1,10} = 17.02$, $p = 0.0020$). Seed count and seed weight of *G. pulchella* increased as percent cover of canola increased at the 500m radius scale; however, the trend was not significant ($F_{1,25} = 1.612$, $p =0.2158$; $F_{1,25} = 1.48$, $p = 0.2351$; Figure 5C and 5D). When we considered only the 2018 sample year, seed count increased significantly as percent cover of canola increased at the 500m scale ($F_{1,10} = 4.96$, $p = 0.0501$). In 2018 seed weight of *G. pulchella* increased significantly as percent cover of canola increased at the 500m scale ($F_{1,10} = 31.22$, $p = 0.0002$). Finally, seed weight of *S. azurea* increased significantly as percent cover of canola increased at all spatial scales, including the 500m scale ($F_{1,13} = 31.34$, $p = 0.0001$; Figure 6).

Discussion

Pollen limitation among wild flowering plants is common, however, chronic pollen limitation can lead to population declines via inbreeding depression as plants rely heavily on autogamy (Ashman et al., 2004; Uyenoyama and Waller, 1991). All three study species exhibited pollen limitation as seed count was significantly greater in the hand-pollinated treatment compared to the open treatment. Both *V. encelioides* and *S. azurea* demonstrated pollen limitation based on seed weight as seed weight was significantly greater in the hand-pollinated treatment compared to the open-pollinated treatment. Autogamy allows for plant reproduction when pollinators are limited or absent. However, reproductive failure can be common when plants self-pollinate, such as for *Clarkia xantiana* ssp. *Xantiana* (Moeller et al., 2011). Self-pollination may also limit genetic variation which may reduce resilience to pollen limitation within wild flowering plant populations (Charlesworth and Charlesworth, 1987; Schemske and Lande, 1985). Inbreeding depression within flowering plant populations can result in increased frequency of deleterious alleles (Husband and Schemske, 1996), reduced seed count and weight (Cardoso, 2004), and reduced germination success and survivorship (Rodger and Johnson, 2013). Indeed, *G. pulchella*, which is self-incompatible, may be at increased risk of population declines relative to the other study species because it experiences pollen limitation in this system and cannot rely on autogamy. *Gaillardia pulchella, V. encelioides,* and *S. azurea* may experience inbreeding depression and its negative effects should out-crossing decrease due to a lack of pollinators in this study system.

Neither bloom time nor morphological specialization predicted pollen limitation in this study. *Gaillardia pulchella* blooms from May to June and was placed in the field in June, closely following canola bloom whereas *V. encelioides* blooms from July to September and was placed in the field in July of 2018 and August of 2019. Both species are in the Asteraceae family and have morphologically open-shaped inflorescences with short corollas which attract a wide range of bees. As canola also has morphologically open-shaped inflorescences, the generalist bees visiting canola may more readily pollinate wild flowering plants with similarly open-shaped inflorescences (Ashman et al., 2004). *Salvia azurea*, which blooms from August to October and was placed in the field in August, has a more specialized inflorescence morphology, with a longer corolla better suited for long-tongued bees (Ashman et al., 2004). The only early blooming species we studied, *G. pulchella,* did not exhibit pollen limitation via seed weight measurements, although it did via the seed count measurements. This may suggest that wild flowering plants that bloom immediately following canola could experience increased pollination rates in this system. Increased seed weight is correlated with improved germination and recruitment among many flowering plants (Leishman et al., 1995). Even if seed count indicated pollen limitation, seed weight and the resulting germination and recruitment that it may yield may be sufficient to sustain plant populations and diminish the possibility of inbreeding depression.

Land use change is linked to greater rates of pollen limitation, particularly among plants that rely on specialist pollinators (Bennett et al., 2020). In highly modified landscapes such as this study system, where wild bee abundance and richness may be reduced, pollen limitation likely occurs among many wild flowering plant species

(Bennett et al., 2020; Garibaldi et al., 2011). Wild flowering plants may be restricted to small patches along roadsides and in few semi-natural areas in agroecosystems. These populations may be more susceptible to reduced outcrossing and decreased drift between populations and, therefore, increased reliance on self-pollination (reviewed by Leimu et al., 2010). This may lead to inbreeding depression, potentially leading to population declines. Out-crossing may also be reduced if small, fragmented patches are not as attractive to pollinators (Sih and Baltus, 1987).. Ongoing pollen limitation may directly affect wild flowering plant populations and indirectly affect pollinator communities that forage on and pollinate those plants.

Our results suggest that canola may cause pollination service spillover to some wild flowering plant species in this study system. As the area of canola increased, seed weight increased for each study species, significantly so for both *V. encelioides* and *G. pulchella* in 2018 and for *S. azurea* in 2019. *Verbesina encelioides* and *G. pulchella* seed count increased as area of canola increased in 2018. Canola may attract pollinators to a particular area (Bennett et al., 2020; Blitzer et al., 2012), and once canola senesced, wild bees as central place foragers may remain in the area and provide pollination services to nearby wild flowering plants (Bronstein, 1995; Westrich, 1996). Spillover of pollination services have been documented across agricultural and natural systems (Bänsch et al., 2021; Blitzer et al., 2012; Hegland, 2014). Such spillover effects have also been documented for predator-prey interactions (reviewed by Rand et al., 2006). Beneficial parasitoids spilled over into agricultural orchards that were surrounded by a greater proportion of semi-natural landscape (Inclan et al., 2015). Changes in seed set between years may be indicative of stochastic fluctuations within bee populations year to year. More research is needed to evaluate which factors contribute to variable responses between years.

Canola may play a valuable role in sustaining wild bee populations in otherwise resource-limited agroecosystems or, alternatively, may attract wild bees to locations that cannot support wild bees after canola senesces and thus act as wild bee population sinks (Westphal et al., 2009). Agricultural intensification has led to severely reduced nesting and foraging resources for wild bees (Potts et al., 2010); however, MFCs such as canola may replenish foraging resources for wild bees (Holzschuh et al., 2013). Canola only adds foraging resources for a brief period of time early in the growing season in our study system. Interestingly, seed weight of *G. pulchella* did not indicate pollen limitation. As the area of canola increased, seed set of *G. pulchella* also increased significantly. These results together may suggest that canola attracts bees that then spillover and pollinate *G. pulchella* plants nearby. Our results suggest a complex relationship between pollination services to wild flowering plant populations and the surrounding landscape.

Pollinator spillover resulting from canola may be critical in an agroecosystem where wild flowering plant species are already pollen limited. In this study system, each study species was pollen limited and increased in seed count or weight as canola area increased. Continuous pollen limitation and resulting population declines may be more likely in agroecosystems without MFCs such as canola (Stanley and Stout, 2014). Additionally, canola is rotated with winter wheat in this study system to reduce weed, disease, and pest burdens that occur when wheat is grown continuously. The presence of canola may, therefore, reduce the need for herbicides that are used with continuous wheat that are harmful to wild flowering plants. This may be particularly beneficial to wild flowering plant populations surrounding canola fields as fewer herbicides will be used which may indirectly benefit wild bee populations. However, the advent of canola as a rotational crop in the southern Great Plains increased the use of broad-spectrum insecticides that are used by 90% of producers at the time of canola bloom (Reddy, 2017). Any benefit from the reduction of herbicides to wild flowering plants may be negated if bee abundance and richness decrease due to increased insecticide use. More studies are needed to understand how canola and changing agrochemical use may impact wild flowering plants and wild bees in the southern Great Plains.

As agricultural intensification increases to match the burgeoning human population, MFCs may change how wild bees forage in agroecosystems. MFCs may attract wild bees and even sustain their populations in otherwise forage-limited areas. Wild bees may provide critical pollination services to fragmented wild flowering plant populations, insulating the plant populations from population declines caused by sustained pollen limitation. However, more research is required to understand how MFCs affect plant-bee relationships across this and other study systems. As agricultural intensification continues and MFCs are incorporated into some agroecosystems, understanding the complex relationship between landscape characteristics, wild flowering plant populations and wild bee populations is critical to conserve wild flowering plants and wild bees.

Acknowledgements

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Tables and Figures

Figure 1. Experimental *G. pulchella* plant with tag indicating experimental inflorescence, treatment, date, and study site.

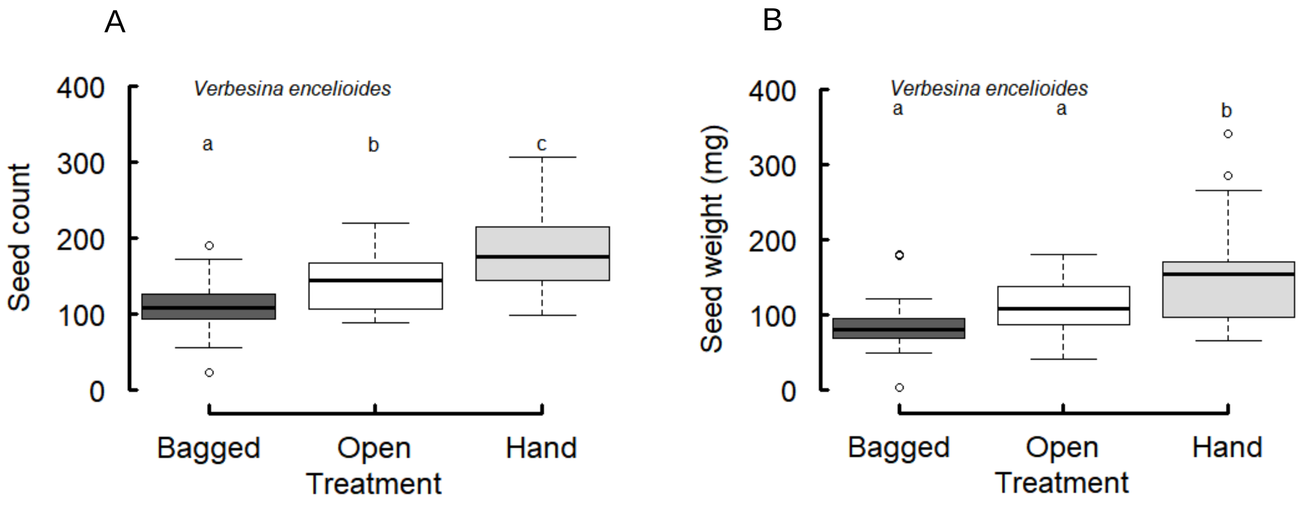

Figure 2. Pollination treatments significantly affected seed count (A) and seed weight (mg; B) of *V. encelioides.* Seed count was significantly greater in each successive treatment: bagged, open-pollinated and hand-pollinated treatments. Seed weight was significantly greater in the hand-pollinated treatment relative to the bagged and open-pollinated treatments. Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers.

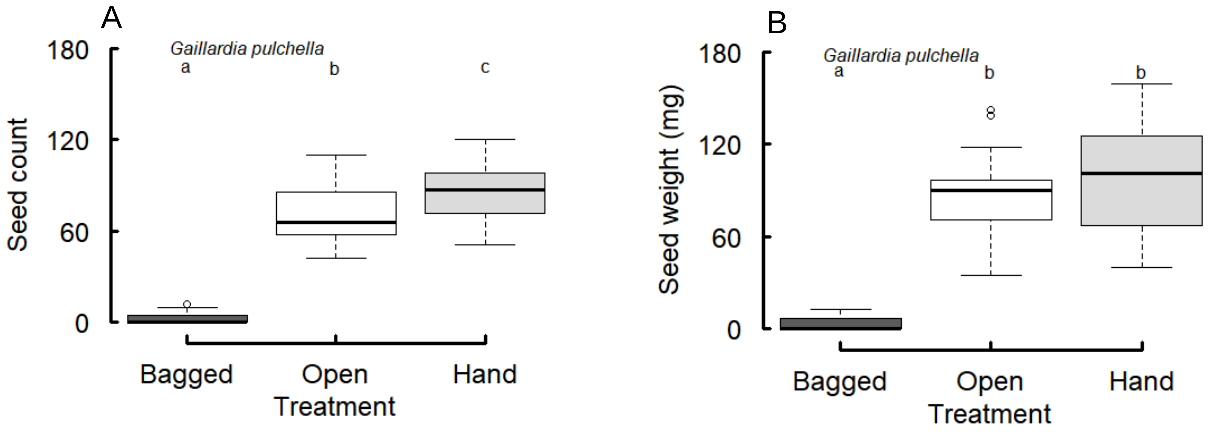

Figure 3. Pollination treatments significantly affected seed count (A) and seed weight (mg; B) of *G. pulchella.* Seed count was significantly greater in each successive treatment: bagged, open-pollinated and hand-pollinated treatments. Seed weight was significantly greater in the hand-pollinated and open-pollinated treatments relative to the bagged treatments. Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers.

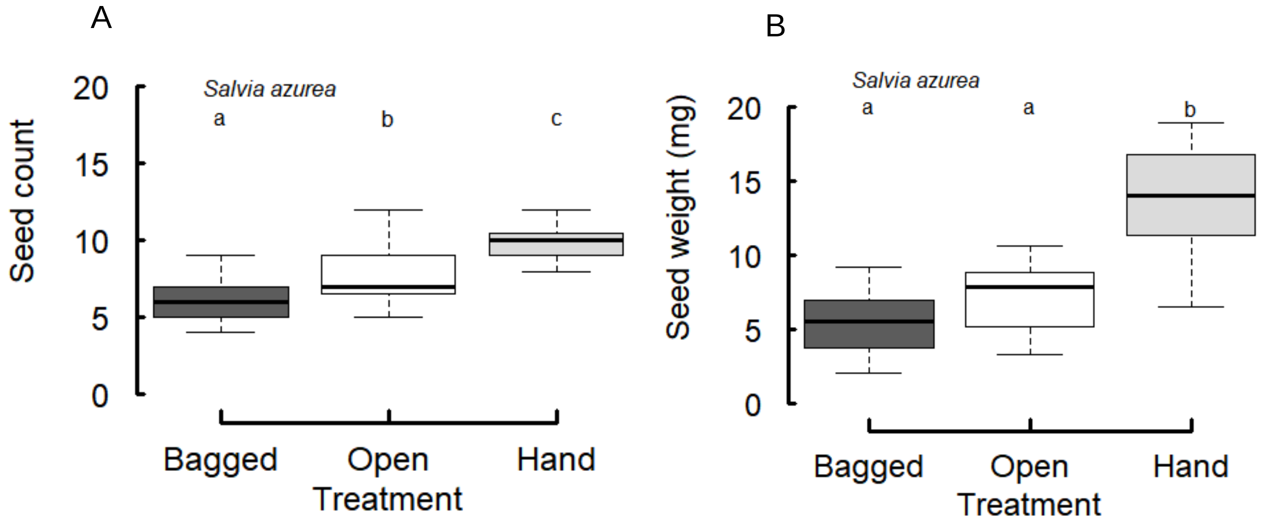

Figure 4. Pollination treatments significantly affected seed set (A) and seed weight (mg; B) of *S. azurea.* Seed count was significantly greater in each successive treatment: bagged, open-pollinated and hand-pollinated treatments. Seed weight was significantly greater in the hand-pollinated treatment compared to the bagged and open-pollinated treatments. Boxes represent interquartile range, bold black lines represent medians, whiskers represent minimums and maximums, and empty circles represent outliers.

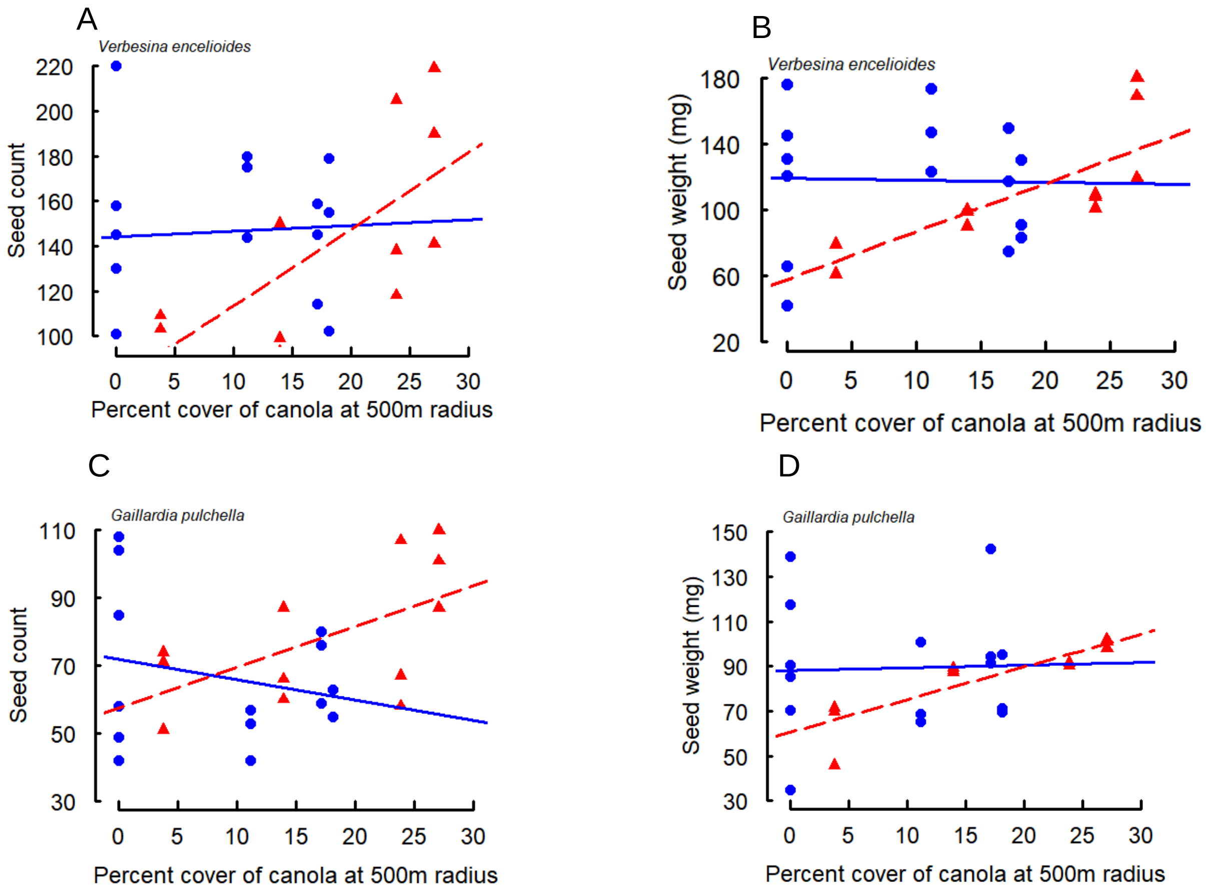

Figure 5. Seed count (A) and seed weight (mg; B) of *V. encelioides* and seed count (C) and seed weight (mg; D) of *G. pulchella* as percent cover of canola at 500m radius spatial scale. Red triangles represent seeds collected in 2018 and blue circles represent seeds collected in 2019. Red dashed lines represent linear model regression lines for seeds collected in 2018. Blue lines represent linear model regression lines for seeds collected in 2019.

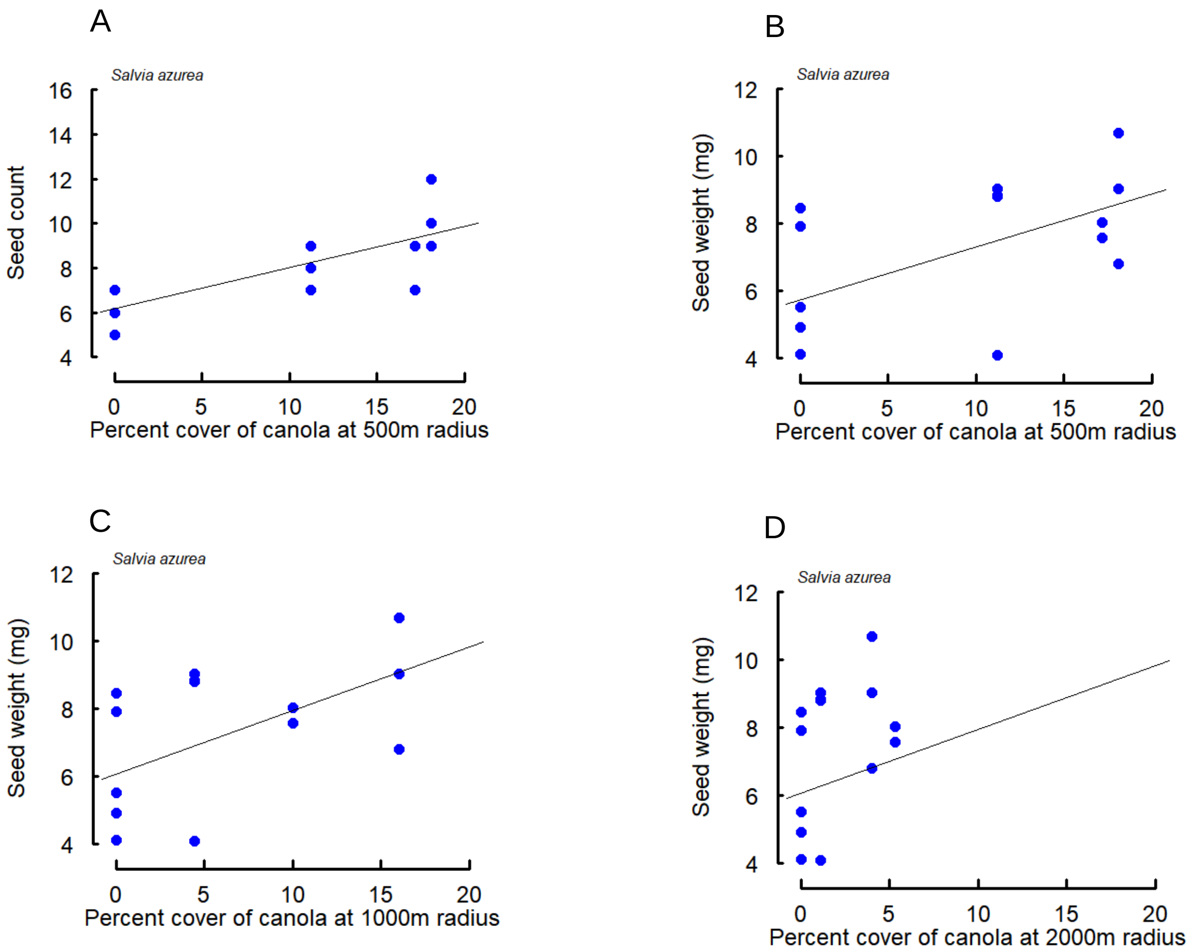

Figure 6. Seed count (A) and seed weight (mg; B) of *S. azurea* as percent cover of canola increased at 500m radius spatial scale during 2019. Seed weight of *S. azurea* as percent cover of canola increased at the 1000m (C) radius spatial scale and the 2000m radius spatial scale (D). The black lines represent linear model regression lines.

Table 1. Canola cover at 500m, 1000m, and 2000m radii spatial scales at each study site during each year of the study. Sites are ordered from greatest to least canola cover at the 2000m radius spatial scale within each year.

		Percent canola area at varying spatial scales		
Site	Year	500m	1000m	2000m
C2	2018	27.11	18.48	4.93
C1	2018	23.88	10.11	2.77
C5	2018	13.96	7.50	2.30
C3	2018	3.80	2.20	0.78
C5	2019	17.19	10.03	5.29
C7	2019	18.11	16.05	4.01
C8	2019	11.19	4.44	1.11
W7	2019	0	0	0.01
W8	2019	0	0	0

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APPENDICES

Appendix A. Comparison of AIC values from all models used to examine canola seed count and total seed weight (g) at each spatial scale tested. Fixed effect variables were selected after data exploration. Models were selected using ΔAIC values. Models with ΔAIC of less than two are in bold within each spatial scale. Weights of each model are also included.

<table>
<tr><td>Seed count at 3 KM</td><td>Model</td><td>AIC</td><td>ΔAIC</td><td>weights</td></tr>
<tr><td>Null</td><td>count ~ Fallow.Idle.Cropland_3k + (1 + babund | site) + (1 + babund | year)</td><td>19291</td><td>457.8</td><td><0.001</td></tr>
<tr><td>C1</td><td>count ~ Fallow.Idle.Cropland_3k * babund + (1 + babund | site) + (1 + babund | year)</td><td>19293</td><td>460.5</td><td><0.001</td></tr>
<tr><td>c2</td><td>count ~ Fallow.Idle.Cropland_3k * babund * brich + (1 + babund |site) + (1 + babund | year)</td><td>18973</td><td>140.9</td><td><0.001</td></tr>
<tr><td>c3</td><td>count ~ P_bee_3k * babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18951</td><td>119.2</td><td><0.001</td></tr>
<tr><td>c4</td><td>count ~ bee_3k * babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18832</td><td>0.0</td><td>0.999</td></tr>
<tr><td>c5</td><td>count ~ Canola_3k * babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18932</td><td>100.6</td><td><0.001</td></tr>
<tr><td>c6</td><td>count ~ P_Canola_3k * babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18948</td><td>116.3</td><td><0.001</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="5">Count at 2K</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Cnull</td><td>count ~ Fallow.Idle.Cropland_2k * babund + (1 + babund | site) + (1 + babund | year)</td><td>19292</td><td>453.6</td><td><0.001</td></tr>
<tr><td>C2</td><td>count ~ P_bee_2k + babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18978</td><td>140.2</td><td><0.001</td></tr>
<tr><td>C3</td><td>count ~ bee_2k * babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18837</td><td>0.0</td><td>0.999</td></tr>
<tr><td>C4</td><td>count ~ Canola_2k * babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18957</td><td>119.3</td><td><0.001</td></tr>
<tr><td>C5</td><td>count ~ P_Canola_2k * babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18962</td><td>136.7</td><td><0.001</td></tr>
<tr><td>C6</td><td>count ~ Fallow.Idle.Cropland_2k * babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18974</td><td>136.7</td><td><0.001</td></tr>
<tr><td colspan="5"></td></tr>
<tr><td colspan="5">Seed count 1500m</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Null</td><td>count ~ bee_1500 + babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18979</td><td>3</td><td>0.118</td></tr>
<tr><td>C1</td><td>count ~ P_bee_1500 + babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18979</td><td>3</td><td>0.090</td></tr>
<tr><td>C2</td><td>count ~ Fallow.Idle.Cropland_1500 + babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18979</td><td>3</td><td>0.091</td></tr>
<tr><td>C3</td><td>count ~ Canola_1500 + babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18979</td><td>3</td><td>0.098</td></tr>
<tr><td>C4</td><td>count ~ P_Canola_1500 + babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18976</td><td>0.0</td><td>0.463</td></tr>
<tr><td>C5</td><td>count ~ dev_1500 + babund * brich + (1 + babund | site) + (1 + babund | year)</td><td>18979</td><td>3</td><td>0.140</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="5">Seed count 1K</td></tr>
</table>

C1	count ~ P_wind_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	18972	58.0	<0.001
C2	count ~ Fallow.Idle.Cropland_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	18971	56.2	<0.001
C3	count ~ P_Fallow.Idle.Cropland_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	18966	51.8	<0.001
C4	count ~ Canola_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	18955	40.7	<0.001
C5	count ~ P_Canola_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	18929	14.5	<0.001
C6	count ~ Grass.Pasture_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	18965	50.9	<0.001
C7	count ~ P_Grass.Pasture_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	18977	62.7	<0.001
C8	**count ~ wind_1k * babund * brich + +(1 + babund | site) + (1 + c8: babund | year)**	**18914**	**0.0**	**0.999**
Seed count 500m				
Null	count ~ P_Fallow.Idle.Cropland_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	18955	53.5	<0.001
C1	count ~ Fallow.Idle.Cropland_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	18980	78.7	<0.001
C2	count ~ Canola_500 + babund * brich * (1 + babund | site) + (1 + babund | year)	18931	29.7	<0.001
C3	**count ~ P_Canola_500 + babund * brich + (1 + babund | site) + (1 + babund | year)**	**18902**	**0.0**	**0.501**
C4	count ~ wind_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	18924	22.5	<0.001
C5	count ~ P_wind_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	18936	33.8	<0.001
C6	count ~ bee_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	18908	5.8	0.028
C7	**count ~ P_bee_500 * babund * brich + (1 + babund | site) + (1 + babund | year)**	**18902**	**0.1**	**0.471**
Total seed weight at 3 KM				
C1	totmass ~ P_Canola_3k * babund * brich + (1 + babund | site) + (1 + babund | year)	458.3	9.0	0.008
c2	**totmass ~ Canola_3k * babund * brich + (1 + babund | site) + (1 + babund | year)**	**449.3**	**0.0**	**0.772**
c3	totmass ~ bee_3k * babund * brich + (1 + brich | site) + (1 + brich | year)	458.4	9.1	0.008
c4	totmass ~ P_bee_3k * babund * brich + (1 + brich | site) + (1 + brich | year)	454.9	5.7	0.045
c5	totmass ~ Fallow.Idle.Cropland_3k * babund * brich + (1 + brich | site) + (1 + brich | year)	453.1	3.9	0.112
C6	totmass ~ P_Fallow.Idle.Cropland_3k * babund * brich + (1 + brich | site) + (1 + brich | year)	454.5	5.3	0.055
Total seed weight at 2km				
Cnull	**totmass ~ Canola_2k * babund * brich + (1 + babund | site) + (1 + babund | year)**	**453.9**	**1.7**	**0.153**

C1	totmass ~ P_Canola_2k * babund * brich + (1 + babund | site) + (1 + babund | year)	459.0	6.8	0.012
C2	totmass ~ Fallow.Idle.Cropland_2k * babund * brich + (1 + babund | site) + (1 + babund | year)	454.3	2.1	0.127
C3	totmass ~ dev_2k * babund * brich + (1 + brich | site) + (1 + brich | year)	457.8	5.6	0.022
C4	**totmass ~ P_Fallow.Idle.Cropland_2k + babund * brich + (1 + brich | site) + (1 + brich | year)**	**452.8**	**0.0**	**0.362**
C5	totmass ~ wind_2k * babund * brich + (1 + brich | site) + (1 + brich | year)	455.0	2.2	0.122
C6	**totmass ~ P_wind_2k * babund * brich + (1 + brich | site) + (1 + brich | year)**	**454.0**	**1.2**	**0.202**
Seed weight at 1500m				
Null	totmass ~ Canola_1500 * babund * brich + (1 + babund | site) + (1 + babund | year)	456.9	3.1	0.085
C1	totmass ~ P_Canola_1500 * babund * brich + (1 + babund | site) + (1 + babund | year)	458.8	5.0	0.033
C2	**totmass ~ dev_1500 * babund * brich + (1 + brich | site) + (1 + brich | year)**	**455.4**	**1.6**	**0.180**
C3	totmass ~ wind_1500 * babund * brich + (1 + brich | site) + (1 + brich | year)	461.8	8.0	0.007
C4	**totmass ~ Fallow.Idle.Cropland_1500 * babund * brich + (1 + babund | site) + (1 + babund | year)**	**455.0**	**1.2**	**0.224**
C5	totmass ~ P_Fallow.Idle.Cropland_1500 * babund * brich + (1 + babund | site) + (1 + babund | year)	460.9	7.1	0.012
C6	**totmass ~ Grass.Pasture_1500 * babund * brich + (1 + babund | site) + (1 + babund | year)**	**453.8**	**0.0**	**0.409**
C7	totmass ~ P_Grass.Pasture_1500 * babund * brich + (1 + babund | site) + (1 + babund | year)	458.0	4.2	0.050
Seed weight 1K				
Null	totmass ~ Canola_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	459.3	5.8	0.025
C1	totmass ~ P_Canola_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	456.5	3.0	0.150
C2	totmass ~ dev_1k * babund * brich + (1 + brich | site) + (1 + brich | year)	458.2	4.7	0.044
C3	totmass ~ wind_1k * babund * brich + (1 + brich | site) + (1 + brich | year)	460.4	7.0	0.014
C4	**totmass ~ Fallow.Idle.Cropland_1k * babund * brich + (1 + babund | site) + (1 + babund | year)**	**453.5**	**0.0**	**0.470**
C5	totmass ~ P_Fallow.Idle.Cropland_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	460.5	7.0	0.014
C6	totmass ~ Grass.Pasture_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	455.7	2.3	0.152
C7	totmass ~ P_Grass.Pasture_1k * babund * brich + (1 + babund | site) + (1 + babund | year)	455.4	2.0	0.176
Seed weight 500m				
Null	totmass ~ Canola_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	461.5	14.5	<0.001
C1	totmass ~ P_Canola_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	455.2	8.2	0.014

C2	**totmass ~ forest_500 * babund * brich + (1 + brich | site) + (1 + brich | year)**	**447.0**	**0.0**	**0.826**
C3	totmass ~ wind_500 * babund * brich + (1 + brich | site) + (1 + brich | year)	456.9	9.9	0.005
C4	totmass ~ Fallow.Idle.Cropland_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	451.3	4.3	0.098
C5	totmass ~ P_Fallow.Idle.Cropland_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	460.6	13.7	<0.001
C6	totmass ~ Grass.Pasture_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	454.3	7.3	0.021
C7	totmass ~ P_Grass.Pasture_500 * babund * brich + (1 + babund | site) + (1 + babund | year)	453.4	6.4	0.034

VITA

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Dissertation: A MASS FLOWERING CONUNDRUM: DO THE BENEFITS OF CANOLA OUTWEIGHT THE NEGATIVE EFFECTS OF LAND USE CHANGE ON WILD BEES?

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Biographical:

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