INFLUENCE OF LANDSCAPE COMPOSITION, LANDSCAPE DIVERSITY, AND CONSERVATION MANAGEMENT ON BEE HEALTH VIA A POLLEN NUTRITION MECHANISM

By

Gabriela Marie Quinlan

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ABSTRACT

INFLUENCE OF LANDSCAPE COMPOSITION, LANDSCAPE DIVERSITY, AND CONSERVATION MANAGEMENT ON BEE HEALTH VIA A POLLEN NUTRITION MECHANISM

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Bees are the most important pollinators in agricultural systems, with honey bees (*Apis mellifera* L.) in particular providing the majority of pollination services on commercial farms. However, due to interacting stressors including lack of nutrition and disease, honey bees and other bee species are experiencing elevated loss rates compared to historical records. Access to abundant, high quality, continuous nutrition in the landscape has been suggested as a means of promoting bee health. To test this, I studied honey bee and bumble bee colonies in 12 apiaries that ranged in land cover composition of the surrounding forage landscape. Honey bee colony cluster size and brood area at the end of the summer were most closely related to post-spring pollination colony size and other colony-level variation, whereas bumble bee colony weight, gyne and drone production were related to surrounding land covers. This demonstrates the importance of accounting for potentially confounding honey bee colony variation in landscape-scale studies. To determine if diversity of land covers affected honey bee pollen foraging and colony size, I also measured honey bee colony size and incoming pollen at 12 apiaries located within landscapes of differing land cover diversity, and found that the relationship between land cover diversity, incoming pollen quantity and colony cluster size changed over time. This suggests that land cover diversity alone is insufficient for predicting patterns in honey bee landscape nutrition studies in this region. Conservation Reserve Program (CRP) land may include flowering, herbaceous species in seed mixes, but in states such as Michigan with

abundant forage in unmanaged habitats, it is unclear if CRP investments have unique floral composition, and foraging by honey bees and wild bees. I assessed floral composition and bee visitation on CRP land as compared to analogous unmanaged fields and roadside ditches in 31 triplicate sites. Floral abundance, species richness, native flower abundance, and inflorescence coverage were all higher on CRP land, as were honey bee and wild bee visitation, indicating that herbaceous CRP promotes bee foraging through unique floral composition, namely floral density. By assessing the quantity and quality of incoming pollen at apiaries while concurrently surveying floral communities in nearby grassy-herbaceous forage habitat, I found that crude protein in collected pollen decreased throughout the summer, concurrent with decreasing floral richness and abundance. This suggests pollinator plantings should include protein-rich, late-blooming species in their seed mixes. Because nutrition is closely tied to disease in honey bees, supplementing protein may promote recovery from diseases such as European foulbrood. To compare different approaches to managing this disease, European foulbrood-infected colonies were treated with traditional antibiotics, antibiotics with a soy-based protein supplements, soy-based supplement alone, pollen-based supplement, probiotics, or left untreated. There was no significant difference among non-antibiotic treatments in post-treatment recovery speed or nurse bee physiology, suggesting these supplemental feeding treatments and probiotics provide no treatment benefits for European foulbrood. Based on this research, accounting for colony-level variation is essential in honey bee landscape studies. Adding pollinator conservation habitat with an increased emphasis on late-season, protein-rich pollen species in seed mixes can benefit honey bees and wild bee species. This work provides new insights into the effects of landscapes on honey bee and wild bee foraging, nutrition and health by examining different aspects of these indirect relationships.

Dedicated to the artists who provided the music of my Ph.D.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: THE ROLE OF LANDSCAPES, CONSERVATION HABITAT AND NUTRITION FOR BEE HEALTH

Introduction

Honey bees (*Apis mellifera* L.) are arguably one of the most agriculturally important animals, providing pollination of crops worth billions of dollars annually as well as wax and honey, and supporting beekeepers and growers (Crane 1990; Calderone 2012; US Department of Agriculture National Agricultural Statistics Service 2019a). Despite their agricultural importance and long history of domestication (Ransome 2004), honey bee nutrition is not yet fully understood. Therefore, honey bees are unique from all other managed livestock in that they do not have defined artificial diets and must rely on natural forage (Herbert and Shimanuki 1977; Aupinel et al. 2005). This places honey bees in danger of modern threats such as anthropogenic landscape simplification and intensification (Benton et al. 2003; Newbold et al. 2015; Kovács-Hostyánszki et al. 2017), which can degrade forage landscapes for honey bees, other managed bees and wild bees (Potts et al. 2010; Goulson et al. 2015).

Bee declines and stressors

The number of honey producing colonies in the United States has been declining since the 1950's, according to reports by the National Agricultural Statistics Service (meta-analysis by vanEngelsdorp and Meixner 2010). Surveys by vanEngelsdorp et al. and the Bee Informed Partnership (BIP), likewise show that beekeepers are experiencing unacceptable losses (vanEngelsdorp et al. 2008, 2011; Bruckner et al. 2018). National mean colony overwinter loss in 2017-2018 was 26.4% for commercial beekeepers, according to the BIP survey (Bruckner et al. 2018). In contrast, beekeepers reported acceptable loss rates of 20.6%, a tolerance threshold

which has increased in recent years (Bruckner et al. 2018). Several interacting stressors have been identified as the primary drivers of colony loss, including parasites, pathogens, pesticides, and poor nutrition (vanEngelsdorp and Meixner 2010). Notably, the parasitic mite *Varroa destructor* Anderson & Trueman has led to dramatic losses of honey bee colonies (vanEngelsdorp et al. 2007), necessitating regular treatments to control mite populations (Rosenkranz et al. 2010). Incidence of European foulbrood (EFB), a brood disease, has emerged in recent years in many regions of North America and Europe (Wilkins et al. 2007; Roetschi et al. 2008; McAfee 2018; Dufour et al. 2020) including Michigan (M. Milbrath, unpublished). Beekeepers also report starvation as a leading cause of colony loss (vanEngelsdorp et al. 2008, 2011). While these stressors and loss rates differ by region and across years (vanEngelsdorp and Meixner 2010), in 2017-2018 Michigan had annual total losses of 35.4%, which was below the national average of 47.9% (Bruckner et al. 2018).

Wild pollinators are likely also experiencing declines due to similar stressors to honey bees, including pesticides, pathogens and poor nutrition (Potts et al. 2010; Goulson et al. 2015). While inference on wild bee declines for many species is limited by insufficient longitudinal data, there is now strong evidence for declines and range contractions in many bumble bee species (Koch-Uhuad and Strange 2009; Cameron et al. 2011; Colla et al. 2012; Kerr et al. 2015; Jacobson et al. 2018). However, some bumble bee species, such as *Bombus impatiens* Cresson, are thriving in their native ranges, including Michigan (Colla et al. 2012; Wood et al. 2019). Though research specifically aimed at examining the declines of alternative managed pollinators is limited, there is also evidence that commercially reared conspecific bumble bees have higher pathogen prevalence than associated wild bumble bees (Murray et al. 2013).

Honey bee nutrition

Honey bees obtain all nutrients from flowers: protein, lipids, and micronutrients from pollen and carbohydrates from nectar (Todd and Bretherick 1942; Haydak 1970; Brodschneider and Crailsheim 2010). Pollen feeding promotes hypopharyngeal gland size, the site of brood-food production (Crailsheim and Hrassnigg 1998; DeGrandi-Hoffman et al. 2010).Without pollen, long-term brood production is impossible (Haydak 1935). Pollen nutrition thus ensures normal colony growth and development, making it an important consideration for beekeepers. Much of early honey bee nutrition research focused on the amount of crude protein as a measure of pollen quality (Haydak 1970; Brodschneider and Crailsheim 2010).There is, however, increasing recognition that pollen quality cannot be measured along a single nutrient axis (Di Pasquale et al. 2013; Vaudo et al. 2016). Rather, diet diversity may ensure that honey bees have access to all necessary nutrients, in the appropriate proportions (Haydak 1970). In practice, polyfloral (multiple flower species) pollen diets have been linked to longevity (Schmidt et al. 1987; Di Pasquale et al. 2013, p. 201) and the bees' ability to produce an immune response (Alaux et al. 2010; Di Pasquale et al. 2013) which can moderate the effect of secondary stressors such as pathogens (DeGrandi-Hoffman et al. 2010; Di Pasquale et al. 2013).

Insufficient nutrition is a major stressor to honey bees, as it is directly related to colony growth (Haydak 1935, 1970; Brodschneider and Crailsheim 2010) and individual bee functioning (Schmidt et al. 1987; Brodschneider and Crailsheim 2010; Di Pasquale et al. 2013). Poor nutrition, characterized by limited, monofloral (single species pollen source) or low-protein pollen, also negatively interacts other stressors, including pathogens (DeGrandi-Hoffman et al. 2010; Di Pasquale et al. 2013; Dolezal and Toth 2018), parasites (Huang 2012; Dolezal et al. 2016), and pesticides (Schmehl et al. 2014; Mogren and Lundgren 2016).

Forage landscapes

The best way for honey bees to meet their nutritional needs is through natural (DeGrandi-Hoffman et al. 2016), abundant (Schmidt et al. 1987), polyfloral forage (Alaux et al. 2010; Di Pasquale et al. 2013). While beekeepers may choose to feed protein supplements during certain times of the year, such as spring to promote brood production (Haydak 1970), there is currently no substitute for natural forage (Herbert and Shimanuki 1977; Aupinel et al. 2005). Unfortunately, the availability of natural forage has decreased in recent years (Potts et al. 2010; Goulson et al. 2015; Otto et al. 2016), particularly in key areas of the United States where there are also high needs for pollination (Koh et al. 2016). The last several decades have seen unprecedented rates of anthropogenically-mediated land-use change (Newbold et al. 2015), resulting in lower heterogeneity across multiple scales (Benton et al. 2003; Kovács-Hostyánszki et al. 2017). At the landscape scale, natural forage land covers such as wetlands and grasslands are being are being replaced by crops such as corn and soybean in many regions (Wright and Wimberly 2013; Otto et al. 2016). At the field scale, agricultural intensification results in fewer weeds (Bretagnolle and Gaba 2015), which are an important forage resource in agroecosystems (Requier et al. 2015; Bretagnolle and Gaba 2015).

Economic importance of bees

Concurrent with elevated colony loss rates, there has been an increasing demand for pollination due to increased production of high value, pollination dependent crops (Aizen and Harder 2009). In the United States, honey bee pollination enhances crop production value by an estimated $11.7 billion (Calderone 2012). Most colonies used for crop pollination and honey production are owned by commercial beekeepers (Daberkow et al. 2009), who manage at least

500 colonies each (Bruckner et al. 2018). These beekeepers are often migratory, transporting their colonies around the country to fulfill pollination contracts, produce a honey crop, and/or avoid or recover from stressful conditions (Daberkow et al. 2009). A colony's capacity for pollination depends on its size and health (Delaplane et al. 2000). Thus, the price a beekeeper can fetch for a pollination contract partially depends on colony strength, measured as the number of frames of adult bees (Nasr et al. 1990). For example, almond pollination in California is the largest and highest paying pollination contract in the country, drawing approximately 75% of US colonies annually (US Department of Agriculture National Agricultural Statistics Service 2016a) and paying on average $184 per colony as of 2017 (Goodrich 2018).

Michigan is also an apiculturally important state. It is a leading producer of many pollinator-dependent crops including blueberry, cherry, and apple (Huang and Pett 2010; Bertone 2017). While there is high demand for pollination in these cropping systems, the pollination-dependent crop-producing southwest region of Michigan likely hosts a low abundance of wild bees (Koh et al. 2016). Therefore, there is a heavy reliance on rented honey bee colonies to avoid pollination limitation. In Michigan, honey bees contribute an estimated $1 billion to the fruit and vegetable industry through pollination, annually (Huang and Pett 2010). Often after fulfilling their spring pollination contracts, beekeepers will move colonies into off-farm honey production apiaries in Michigan to produce a honey crop, valued at approximate $9.5 million (US Department of Agriculture National Agricultural Statistics Service 2019a). In Michigan, these feeding apiary locations are typically on private land and selected by the beekeeper based upon property permissions, accessibility, and surrounding forage resources.

Besides honey bees, other bee species such as *B. impatiens* have more recently been developed for their pollination services (Velthuis and Doorn 2004, 2006). Alternative managed

bees may contribute to crop yields by being more efficient pollinators, as seen through *B. impatiens*' ability to buzz-pollinate blueberry flowers (Buchmann 1983; Delaplane et al. 2000; Stubbs and Drummond 2001). They may also have unique functional trait niches, such as *B. impatiens* flying in cooler conditions than honey bees (Tuell and Isaacs 2010). Wild bees also contribute to pollination and have been shown to increase yield independent of the presence of honey bees (Garibaldi et al. 2013). Wild pollinators are more effective than honey bees at pollinating many crops on a per-flower visit (Garibaldi et al. 2013) and are estimated to contribute over $3000 per hectare in crop value through pollination (Kleijn et al. 2015). In some farm situations these different pollinators can be part of an integrated approach, that considers the entire pollination system (Isaacs et al. 2017).

Apiculture in the Midwestern U.S.

The Midwestern US is an area of both apicultural opportunities and challenges. The Northern Great Plains, including Montana, the Dakotas, and Minnesota, is the most important region in the country for honey production, driven primarily by the Dakotas, which consistently produce approximately one-third of the country's honey by volume (US Department of Agriculture National Agricultural Statistics Service 2019a). Due to the Northern Great Plains' abundant natural and crop forage, this region has become a summer feeding ground for approximately 1 million colonies, or about 40% of the country's registered, commercially managed honey bee colonies (Bond et al. 2014; Otto et al. 2016). In these feeding apiaries, colonies produce honey and build up their size for later pollination contracts and queen/package production (Otto et al. 2016). However, forage resources in the Northern Great Plains are being threatened by conversion of beekeeper-identified forage land, including conservation land,

grassland, and wetlands, to corn and soybean fields (Wright and Wimberly 2013; Otto et al. 2016). This threatens the stability of the beekeeping industry nationally and suggests the need for continued investment in forage habitat (Otto et al. 2018).

Similar land conversion is also occurring throughout the corn belt (Wright and Wimberly 2013). Iowa, a famously corn-soy dominated state (US Department of Agriculture National Agricultural Statistics Service 2018) also ranks among the states with the highest colony loss rates in the country (vanEngelsdorp et al. 2011). In Iowa, honey bees kept on intensively cultivated land exhibit poor nutritional physiology, a condition exacerbated by *V. destructor* mites (Dolezal et al. 2016).

The Great Lakes region of the Midwest is agriculturally different from either the Northern Great Plains or the corn belt. Michigan is less dominated by corn-soy rotation agriculture than the rest of the Midwest (US Department of Agriculture National Agricultural Statistics Service 2018), hosting the second highest agricultural diversity behind California (Bertone 2017). Michigan also has one of the smallest average farm sizes of the region at 206 acres, compared to Iowa at 356 acres and North Dakota at 1506 acres (US Department of Agriculture National Agricultural Statistics Service 2019b). Michigan's diverse agriculture and small farm size, interspersed in a matrix of forests, urban, wetlands, and grassy-herbaceous land (US Department of Agriculture National Agricultural Statistics Service 2018), creates a unique landscape for honey bee landscape and nutritional studies.

Bee-supportive land covers

Many different land covers have been shown to benefit honey bees. Landscape-scale studies on the benefit of surrounding land covers to honey bee health often highlight the benefits

of uncultivated or semi-natural, herbaceous land covers such as pasture, grassland, etc. (Naug 2009; Dolezal et al. 2016; Smart et al. 2016b; Alaux et al. 2017). Unlike crop monocultures that only bloom for a short time, seminatural land may provide forage continuity through plant diversity (Carvell et al. 2007). Pollen foragers have been shown to fly greater distances to forage when seminatural land covers are far away (Beekman and Ratnieks 2000) and shorter distances in landscapes when seminatural forage is nearby (Danner et al. 2016). Furthermore, honey bees have been shown to be more concentrated on seminatural habitat in intensive landscapes (Danner et al. 2016). These findings suggest that seminatural, uncultivated land can be a rewarding forage resource for honey bees.

Honey bees have also been shown to benefit from mass flowering crops (Danner et al. 2016; Alaux et al. 2017) and from agriculture generally, mainly due to the availability of weedy species (Requier et al. 2015; Sponsler and Johnson 2015). A study in the Great Lakes region found that the amount of agriculture in the landscape around an apiary contributed to greater food stores (Sponsler and Johnson 2015). The authors proposed that the value of agriculture to honey bees is due to abundant weedy, flowering plants along field margins (Sponsler and Johnson 2015). Another recent study in Michigan found that honey bees forage predominantly on non-native weeds during the summer (Wood et al. 2018). Cultivated land does however present risk of pesticide exposure, even from non-target flowering weeds (Krupke et al. 2012, Mogren and Lundgren 2016). Cultivated land is also often managed as monocultures which only bloom for a short period of time and thus provide dis-continuous forage (Williams et al. 2012; Danner et al. 2016; Dolezal et al. 2019).

Other land covers including urban land, wetlands, and forests have all been identified by various studies as potential forage for honey bees. Honey bees in urban settings collect a

diversity of resources throughout the foraging season (Sponsler et al. 2019). Honey bees have also been observed foraging at higher densities in urban areas, compared to rural areas (Theodorou et al. 2020). However, this is likely due to the higher stocking rates due to urban beekeeping (Theodorou et al. 2020). Indeed, other honey bee landscape studies have identified negative effects of anthropogenic intensification, including urban land (Clermont et al. 2015). Wetlands, which are common in Michigan, host floral species such as purple loosestrife that can provide summer-long forage to honey bees within agroecosystems (Benvenuti et al. 2016). Forests, which have abundant spring-blooming flowers on herbaceous and woody plants, can also support the apicultural industry as colonies are getting started after their overwintering (Hill and Webster 1995).

The range of land covers that have been identified as beneficial to honey bee colonies is likely due to differences in region, timing, landscape structure, land management, and biotic and abiotic conditions across studies. Furthermore, several other interacting factors, from beekeeper management to annual weather (vanEngelsdorp and Meixner 2010) further confound landscape effects. Indeed, beekeeper management has been shown to be more important than landscape features for many colony health outcomes (Sponsler and Johnson 2015). There is therefore a need to understand landscape effects in the context of beekeeper management. Additional regional studies on beneficial land covers for honey bees, which also consider potentially confounding effects, could contribute to the growing body of literature on which land covers and in what contexts those land covers benefit honey bees.

A range of land covers have also been associated with bumble bee foraging. Those identified as beneficial to bumble bees include seminatural land (Rollin et al. 2013; Requier et al. 2019), mass flowering crops (Rollin et al. 2013, p. 201), urban land (Theodorou et al. 2020), and

wetlands (Vickruck et al. 2019). Many of these land covers were likewise identified as beneficial to honey bees, likely because both groups are generalist foragers with broadly similar nutritional requirements (Michener 2007; Vaudo et al. 2015). However, honey bees and bumble bees differ in specific dietary needs, in part due to differences in life histories (Vaudo et al. 2015), with much greater need for nectar foraging by honey bees to support colony overwintering (Seeley and Visscher 1985) and likely a greater need for pollen to support higher brood production (Haydak 1935; Vaudo et al. 2015). Furthermore, recent research shows that bumble bees forage to reach a ratio of protein to lipids (Vaudo et al. 2016), which they have been shown to be capable of optimizing across a range of land covers (Vaudo et al. 2018). Recent research suggests that honey bees may also forage to reach a different protein to lipid ratio (Vaudo et al. 2020). While historic research suggests that honey bees are incapable of distinguishing the protein quantity of pollen (Roulston et al. 2000), recent research shows that honey bees preferentially forage on high protein floral species (Ghosh et al. 2020). It is therefore likely that these two bee groups will respond differently to resources in their foraging landscape. Indeed, in a previous comparative study in France, honey bees and bumble bees foraged differently in landscapes, with honey bees preferring mass-flowering crops and bumble bees showing an intermediate preference between mass-flowering crops and semi-natural habitat, likely due to differences in nectar requirements (Rollin et al. 2013). Studying honey bees and bumble bees, particularly a managed, agriculturally important bumble bee species such as *B. impatiens*, could broaden our understanding of landscape effects on agriculturally important managed, social bees, generally. A study of landscape effects on colonies of both honey bees and *B. impatiens* would also add context to the effect of land covers on honey bees.

Land cover Shannon diversity

Honey bees, as generalist foragers (Winston 1991) and strong dispersers (Beekman and Ratnieks 2000), likely forage in different land covers across landscapes. Wild bees have been shown to adjust foraging throughout the summer, following the bloom phenology of various land covers to achieve resource complementarity at the landscape level (Mandelik et al. 2012). Therefore, it may be useful to characterize forage landscapes using land cover Shannon diversity. The landscape Shannon diversity index treats different land cover types as unique units, so high Shannon diversity landscapes have more, different land covers and/or land covers present at more even proportions (O'Neill et al. 1988).

Land cover diversity may benefit honey bee foraging in many of the same ways in which biodiversity benefits ecosystem functioning, including niche complementarity and sampling effect (Barthlott et al. 2009). Niche complementarity refers to the additional resource space utilized in more diverse systems (Barthlott et al. 2009). Land covers with complementary bloom times fill temporal niche space within the landscape, sustaining bee foraging with continuous resource availability. Sampling effect refers to the increased likelihood of including a high functioning unit in a system with increased diversity (Barthlott et al. 2009). In landscapes, this corresponds with the increased chance of including a highly beneficial land cover as land cover diversity increases. By the same mechanism, a detrimental or low-quality resource could also be more common in more diverse landscapes.

Land cover Shannon diversity has great potential as a landscape metric for honey bee studies, as it characterizes broad scale resource diversity that is likely important to generalist foragers like honey bees. Such a metric could be useful for determining the quality of forage landscapes and for improving recommendations to beekeepers on where to place their apiaries.

Conservation land

Conservation land management is one way of adding forage resources into a landscape to support pollinators (Decourtye et al. 2010), and this has been emphasized recently as one potential solution to the challenges facing pollinators. For example, adding or enhancing pollinator habitat for both honey bees and wild bees is a goal of the Presidential Memorandum on Pollinators (Obama 2014). Honey bees and wild bees both face nutritional stress (Potts et al. 2010), so the addition of pollinator habitat would likely benefit both groups (Evans et al. 2018). The United States has a long history of mixed-use conservation goals (Vincent 2004). Therefore, this practice of using government-funded conservation land to support the beekeeping industry is not unprecedented.

In agroecosystems, historically the largest private-lands conservation program by area and financial investment in the US is the Conservation Reserve Program (CRP). Authorized by the Farm Bill, the US Department of Agriculture (USDA) administers financial support for CRP with oversight through the Farm Service Agency (FSA) and technical support through the Natural Resources Conservation Service (NRCS) (Gray and Teels 2006). The CRP enrollment limit in 2019 was at 24 million acres but is set to increase to 27 million acres by 2023 (Stubbs 2018). The 2018 Farm Bill also authorized $60 billion in spending over 10 years for all federal agriculture conservation support between 2019 and 2028. Of this budget, CRP support was one of the largest line items, with a 10-year budget of $22 billion (Stubbs 2018). CRP has many diverse conservation goals. Originally, it was designed to conserve highly erodible conservation lands (Gray and Teels 2006), which also benefited grassland birds (Johnson 2000). The Conservation Reserve Program has since expanded its goals to target other habitats and wildlife (Gray and Teels 2006). The 2008 Farm Bill made pollinator conservation a primary focus

(Johnson et al. 2008). Pollinator management programs have since focused mainly on wild bees (US Department of Agriculture Farm Service Agency 2008; Vaughan and Skinner 2008). However, many other CRP programs contain herbaceous elements that likely benefit all pollen-eating insects (US Department of Agriculture Farm Service Agency 2015).

Despite extensive financial investment, before 2010 there was very little evidence of the effectiveness of CRP land to pollinator conservation (Winfree 2010; Decourtye et al. 2010). In Europe, the analogous agri-environment schemes (AES) have been questioned for their effectiveness in conserving biodiversity, particularly of rare species (Kleijn et al. 2006, 2011). There is evidence in Europe that AES enhance floral richness and abundance (Knop et al. 2006; Albrecht et al. 2007; Carvell et al. 2007), and that fields managed with an herbaceous element in the seed mix lead to greater abundance and/or richness of bumble bees (Carvell et al. 2004; Pywell et al. 2006; Potts et al. 2009). In the US, the effectiveness of CRP habitat has been most extensively assessed for honey bees in the Northern Great Plains. In these highly intensive landscapes, honey bees benefit from proximity to CRP land (Smart et al. 2016b; Ricigliano et al. 2019), and beekeepers value CRP as forage habitat for colonies (Otto et al. 2016). There is also evidence that wild bees forage on CRP land in this region (Otto et al. 2017).

More research on herbaceous CRP as pollinator forage habitat is still needed. For example, it is necessary to understand how including an herbaceous element in CRP seed mixes, which has been suggested as a means to support pollinators, affect floral community composition throughout the season, which would be important to bee resource continuity. The benefit of CRP to honey bees and wild bees is also yet to be tested in states other than the Dakotas, to my knowledge. The effect of CRP land on pollinator foraging could be affected by differences in landscapes context, and therefore be location-specific. Additionally, the utilization of herbaceous

CRP land by both honey bees and wild bees has not, to my knowledge, been compared to analogous, unmanaged herbaceous habitat. Comparing bee visitation on CRP land to unmanaged habitat would be important for elucidating its relative value as forage habitat. Observing bee interactions with flowers on CRP land and unmanaged land is also important for understanding which elements of floral composition are most important to foragers, to inform future land management decisions.

Pollen from landscape forage resources

The quantity and quality of pollen collected by bees provide a mechanistic link between landscape forage and colony condition. Understanding how pollen quantity and nutritional quality are related to forage in the landscape could inform land management for pollinators and/or identify times that may require supplemental feeding by beekeepers. Previous studies have used colony monitoring tools, including pollen traps and hive scales , to monitor incoming pollen and nectar resources, respectively, in different landscapes (Smart et al. 2017a). These authors identified higher crude protein, pollen species richness, and season-long forage in the more semi-natural uncultivated landscape, compared to more high intensity agriculture. This type of monitoring could be useful in agroecosystems, in which resources can go through dramatic pulses depending upon what is blooming (Williams et al. 2012; Danner et al. 2016; Hemberger and Gratton 2018; Dolezal et al. 2019).

Variation in pollen quality over the season can lead to differences in honey bee health (Di Pasquale et al. 2016). The amount of crude protein, due to its importance in brood rearing, is often considered a primary metric of pollen quality (Schmidt et al. 1987), as is the overall quantity of pollen (Schmidt et al. 1987; Smart et al. 2016a). Pollen richness could also be an

informative measure of pollen quality due to the importance of polyfloral diets to immunocompetence (Alaux et al. 2010, Di Pasquale et al. 2013). Pollen identity can also inform how bees are utilizing landscapes. Previous studies have used pollen metabarcoding to determine which plants bee are foraging on in different landscapes (Smart et al. 2017b; Sponsler et al. 2019) and on which land covers bees are foraging (Smart et al. 2016b). Palynology has also been used to detect temporal shifts in bee diet pollen sources throughout the season (Wood et al. 2018).

Inferring bee land use from identified pollen could be improved through floral surveys, because these can be used to characterize forage landscapes and temporal shifts in forage availability throughout the season (Williams et al. 2012; Smart et al. 2016b). Connecting results from these floral surveys with incoming pollen quantity, quality, and identity could elucidate important forage elements in the landscape and inform areas for improvement and attractive seed mixes for honey bees or other pollinators. No such paired, pollen and floral surveys have been done, to my knowledge, in Michigan. A thorough understanding of how pollen quantity and quality change throughout the season and how they relate to floral availability changes would be valuable to land management and beekeeper management in such an agriculturally and apiculturally important state.

European foulbrood

Nutrition has been shown to interact with disease in honey bees; with insufficient nutrition exacerbating the effects of disease and certain diseases decreasing bees' physiological nutritional condition (Dolezal and Toth 2018). European foulbrood (EFB) is a honey bee brood disease which has been linked to stressful colony conditions, such as insufficient nutrition

(Bailey 1961). European foulbrood is caused by the bacteria *Melissococcus plutonius* (Bailey 1983) and thus has traditionally been treated with antibiotics (Thompson and Brown 2001; Waite et al. 2003b), often prophylactically (Shimanuki et al. 1969; Kochansky 2000). However, as of 2017, the Veterinary Feed Directive began requiring a prescription to obtain antibiotics for all food-producing animals, including honey bees (Food and Drug Administration, Department of Health and Human Services 2015). This has made obtaining antibiotics more difficult for beekeepers. However, concerns with antibiotics include antibiotic resistance, which has developed in American foulbrood, another bacterial honey bee brood disease (Miyagi et al. 2000; Evans 2003) but has not yet developed in European foulbrood (Hornitzky and Smith 1999; Waite et al. 2003a). The inability to harvest honey when treated with antibiotics due to the potential for antibiotic residues in honey (Mutinelli 2003; Bargańska et al. 2011) is also a limitation of antibiotic use. Antibiotics have also been linked to adverse physiological effects in individual honey bees, including gut dysbiosis (Raymann and Moran 2018), for which the consequences are not yet fully understood.

Due to a recognition of the limitations of antibiotics, there is a need for more sustainable treatment alternatives. Alternative methods may include protein supplementation, with or without antibiotics, to address nutrition limitation (Bailey 1961) and negative individual bee physiological effects (Li et al. 2019); probiotics to promote immunity (Evans and Lopez 2004; Daisley et al. 2019); and no-treatment control.

Nurse bees are young adults that tend to the brood and have unique physiology. Nurse bee physiological biomarkers can therefore be informative indicators of colony condition, particularly in complex systems involving nutrition and/or disease (Smart et al. 2016a; Alaux et al. 2018; Ricigliano et al. 2019). Nurse bee condition may inform the mechanism underlying a

treatment's mode of action and indicate future colony condition. Two important organs in nurse bees are the fat body and the hypopharyngeal gland. The fat body is an important energy store as well as the location of vitellogenin production (Kilby 1963; Arrese and Soulages 2010), which plays an important role in aging, caste determination, and immunocompetence (Amdam et al. 2004b). Pollen feeding has been shown to promote the size and/or compounds produced by the fat body (Alaux et al. 2010; Di Pasquale et al. 2013). The hypopharyngeal gland is enlarged in nurse bees, particularly those fed more pollen, and is used for brood food production (Crailsheim and Hrassnigg 1998). Antibiotic use has been shown to decrease nurse bee head weights (Li et al. 2019), a proxy for hypopharyngeal gland size (Crailsheim and Hrassnigg 1998).

European foulbrood has a long history of being a challenge after pollination of Michigan blueberry farms (Wardell 1982). In 2018, more than half of commercial colonies surveyed after blueberry pollination contracts had signs of EFB infection (M. Milbrath, unpublished). Determining the effectiveness of sustainable treatment alternatives for EFB is therefore a priority for Michigan beekeepers. More sustainable treatment alternatives could benefit beekeepers facing EFB globally (Forsgren 2010).

Conclusion

Honey bee colony growth and health are closely tied to the surrounding forage landscape through nutrition at multiple spatial and temporal scales. Managed bumble bees and wild bee communities are likely experiencing similar nutritional stress and may benefit from more rewarding land use (Evans et al. 2018) or may respond differently (Rollin et al. 2013). One of the main threats to the availability of bee forage across the landscape is anthropogenic intensification, which leads to a loss of heterogeneity across scales (Benton et al. 2003; Kovács-

Hostyánszki et al. 2017). Agricultural land conversion decreases broad-scale heterogeneity by reducing the different types of land covers in an area. Agricultural land intensification further decreases heterogeneity at fine scales. The addition of conservation habitat in agroecosystems as a means of pollinator conservation to ameliorate these effects in agroecosystems is supported by theoretical analyses (Benton et al. 2003; Tscharntke et al. 2005) and has growing empirical support (Kleijn et al. 2006; Smart et al. 2016b). In this thesis I explore the connections between land use and bee health through studies to address which land covers and beekeeper management-related metrics best explain variation in honey bee and *B. impatiens* colony outcomes (Chapter 2); how land cover Shannon diversity relates to honey bee colony foraged pollen quantity and colony size (Chapter 3); benefits of CRP land management to bees (Chapter 4); how pollen quantity and quality are related to floral composition on grassy-herbaceous habitats (Chapter 5); and whether alternative treatments for EFB can improve colony recovery from this disease (Chapter 6). Insights from this research will inform land management and colony management in Michigan and add to a broader understanding of how bees respond to landscapes via a nutrition mechanism.

CHAPTER 2: DRIVERS OF HONEY BEE AND BUMBLE BEE COLONY GROWTH IN MICHIGAN

Abstract

Agriculturally important commercially managed pollinators, including honey bees (*Apis mellifera*) and bumble bees (*Bombus impatiens*), rely on natural forage in the surrounding landscape to fulfill their long-term dietary needs. Various land covers have been identified as beneficial to these species, from semi-natural to urban land in different systems, so it is unclear which land covers are important to honey bee and bumble bee colony growth in Michigan. I hypothesized that uncultivated land, including grassy-herbaceous land and wetlands, is beneficial to these bees. To better understand the response of these managed, generalist bees to variation in land use, I measured honey bee colony cluster size and brood area during three years, and bumble bee colony weight at time of reproduction and reproductive outputs in one year at apiaries located across a range of land use in Michigan. I also monitored potentially confounding conditions of honey bee and bumble bee colonies, such as initial colony size. I then used backward step-wise model selection to determine which land covers and management-related conditions best explained the variation in colony outcomes. For honey bees, landscape was not a significant predictor of colony size, whereas colony effects including mid-summer colony size, pests and disease, and year significantly affected brood area in September. Likewise, September colony cluster size was positively correlated with mid-summer cluster size. For bumble bees, colony weight at the time of reproduction was positively correlated with area of staple crops (corn, soy, small grain), wetlands, and urban land. Gyne production was also positively correlated with area of wetlands, whereas drone production was negatively correlated with area of grassy-herbaceous land. My finding that honey bee colony variation is explained by colony

condition more than landscape composition emphasizes the importance of accounting for these potentially confounding effects in honey bee colony field studies. The results for bumble bees further highlight wetlands, and potentially staple crops and urban land as forage resource to conserve in Michigan landscapes to support bees. Overall, these findings add to our understanding of how different landscapes and colony factors affect key social managed pollinators in different ways.

Introduction

The European honey bee, *Apis mellifera* L., is the most economically important pollinator (McGregor 1976; Calderone 2012) across the world, providing pollination of a wide range of food and forage crops. More recently, developments in bee rearing have also made the common eastern bumble bee, *Bombus impatiens* Cresson, commercially available for crop pollination in eastern North America (Velthuis and Doorn 2004, 2006).

Elevated honey bee colony losses over the last several decades are attributed to a number of interacting factors including in-hive factors such as parasites, particularly *Varroa destructor* and disease such as European foulbrood and chalkbrood (vanEngelsdorp et al. 2011; Evans and Schwarz 2011; US Department of Agriculture National Agricultural Statistics Service 2019c). Other, landscape-scale factors such as pesticides (Alburaki et al. 2017), weather (US Department of Agriculture National Agricultural Statistics Service 2019c) and inadequate availability of forage due to habitat loss (Otto et al. 2016) likely exacerbate these effects (Collison et al. 2016; Dolezal and Toth 2018). Landscape-scale studies have identified several different land covers as potentially beneficial to honey bee foraging, health or colony productivity, including urban land (Theodorou et al. 2020), wetlands (Benvenuti et al. 2016), and grassy-herbaceous and semi-

natural land (Dolezal et al. 2016; Smart et al. 2016b). Overall, recent research suggests that many different land covers have the potential to benefit honey bees, but responses may vary geographically, likely due to differences in forage availability and landscape structure. There is therefore a need to conduct honey bee-landscape studies in different geographical regions, to add to the broader understanding of beneficial land covers to honey bees.

Many native species of bumble bee have experienced population declines and range contractions in the United States (Cameron et al. 2011; Colla et al. 2012), potentially attributed to large-scale intensification of land management (Grixti et al. 2009). However, some species, such as *B. impatiens,* seem to be thriving in their native ranges, despite this land-use change (Colla et al. 2012), suggesting that they are able to locate resources in the remaining land-use types. Landscape scale studies on bumble bees have shown positive effects of urban land (Theodorou et al. 2020), wetlands (Vickruck et al. 2019) and semi-natural land (Rollin et al. 2013; Requier et al. 2019) on bumble bee foraging and/or colony performance. Additional studies could clarify how bumble bee colonies respond to these resources under different landscape conditions.

Honey bees and bumble bees, as social, generalist foragers have broadly similar macro- and micro-nutrient dietary requirements (Todd and Bretherick 1942; Michener 2007), with some key areas of divergence. Due to differences in life history, it is unlikely that these two bee guilds would react in the same way to floral resources and forage landscapes (Steffan-Dewenter et al. 2002; Rollin et al. 2013; Vaudo et al. 2015). Indeed, previous studies have found that honey bees and unmanaged bumble bees forage in and respond to the same landscapes in different ways, with unmanaged bumble bees exhibiting foraging behavior intermediate between honey bees and other unmanaged wild bees (Rollin et al. 2013). Therefore, comparing the landscape response of

a locally native, managed species of bumble bee, such as *B. impatiens,* to the response of non-native, managed honey bee*, A. mellifera*, could provide greater insights into how landscapes influence the health of key managed species.

Michigan agroecosystems are highly heterogeneous. One might therefore expect different responses from colonies to land covers compared to recent studies conducted in much larger scale farms that are more common in the Dakotas, Minnesota, Iowa, etc. (Dolezal et al. 2016; Smart et al. 2016b; Otto et al. 2018; Vickruck et al. 2019). Due to the context-specific nature of various land covers and the impact of land management across landscapes, assessing the effect of various land covers and management-related factors on honey bee and bumble bee colonies in the more diverse landscapes of Michigan can add to a broader understanding of bees' responses to land covers.

To determine land cover effects on honey bees and bumble bees in Michigan, in this study, I aim to: 1) Determine which land covers and colony condition factors best explain variation in honey bee colony cluster size and brood area, and 2) Determine which land covers and management-related factors best explain variation in bumble bee colony weight at the time of reproduction, gyne production, and drone production. I expect that colony development of honey bees and bumble bees will be positively associated with uncultivated land covers, including grassy herbaceous fields and wetlands. My findings could inform land management practices for supporting pollinators in heterogeneous agroecosystems and support beekeeper decision-making by helping to identify beneficial land covers for locating apiaries and/or detrimental areas which might require additional management such as supplemental feeding.

Methods

Sites

In the summers of 2015-2017 I assessed the performance of managed honey bee and bumble bee colonies at several locations across Michigan. In 2015, I assessed 4 apiaries (sites A, C, F, and G), in 2016 I assessed 10 apiaries (sites A-J), and in 2017 I assessed 12 apiaries (sites A-L) (Figure 2.1). Apiary locations were selected from my collaborating beekeepers' existing apiary locations to be spatially independent within a 3.2 km (2 mile) radius (Walther-Hellwig and Frankl 2000; Smart et al. 2016b) and to capture various Michigan land covers, at different proportions within the buffer radii of the apiaries. Land covers that were quantified within these areas were wetlands, grassy-herbaceous fields (hay, wildflower, switchgrass, fallow cropland, shrubland, grassland, and pasture), staple crops (soybean, corn, and small grains), non-staple crops (e.g., vegetables, tree fruits, and vineyards), urban, forests, and NA (water or no data) (Figure 2.2). The land cover composition of these study sites were largely representative of the surrounding land use, though the study sights represented a higher proportion of forest, and lower proportion of urban land compared to the region, and did not capture as much variance in grassy-herbaceous fields or urban land compared to the region (Table B1). I used the 30 m^2 resolution Cropland Data Layer (US Department of Agriculture National Agricultural Statistics Service 2016b) in R Studio version 3.6.3 (R Core Team 2020), using the raster (Hijmans et al. 2020) and rgeos (Bivand et al. 2019) packages to categorize and calculate the area of these land covers within the 3.2 km radius.

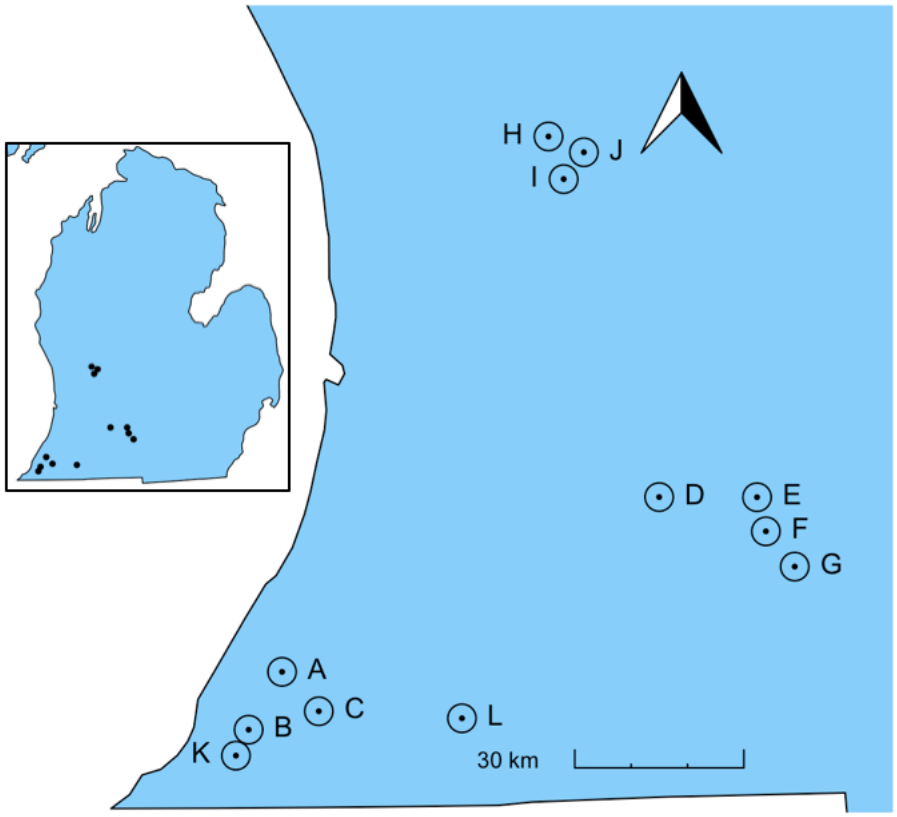

Figure 2.1. Labeled apiary site locations in Michigan, with locations indicated by black points, surrounded by the 3.2 km buffer distance over which the area of land covers was calculated.

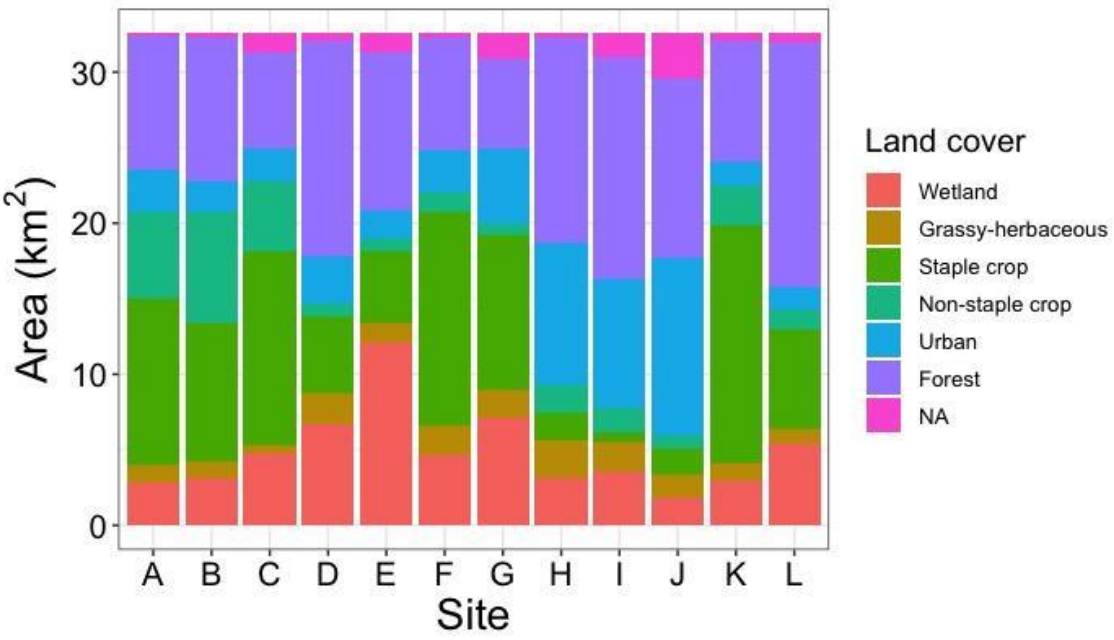

Figure 2.2. Area (km^2) of seven simplified land covers within 3.2 km of each of the 12 apiaries sampled in Michigan. Land cover categories are wetlands, grassy-herbaceous fields (hay, wildflower, switchgrass, fallow cropland, shrubland, grassland, and pasture), staple crops (soybean, corn, and small grains), non-staple crops (e.g., vegetables, tree fruits, and vineyards), urban, forests, and NA (water or no data).

Honey bee colonies

In 2015-2017 I assessed a total of 312 commercially managed migratory honey bee colonies for their growth (cluster size and brood area), queen status, *Varroa destructor* infestation, and signs of brood disease. I examined 12 colonies per apiary: 4 apiaries in 2015 (48 colonies), 10 apiaries in 2016 (120 colonies), and 12 apiaries in 2017 (144 colonies). In July of each year at the start of their time in the apiaries, I assessed colonies for cluster size (Nasr et al. 1990) during the day, *V. destructor* infestation (Macedo et al. 2002), and visual signs of brood disease. In September I assessed colony survival, queen status, cluster size, area of capped brood (Delaplane et al. 2013), *V. destructor* infestation, and visual signs of brood disease. Colonies were being managed for honey production, and thus were kept in double-deep brood boxes, with

medium supers added/ removed for honey. In some of the colonies, cluster size may have been limited by the hive equipment provided by my collaborating beekeeper, however no correction factor was applied as all colonies were similarly managed and therefore subject to similar constraints. Furthermore, it is unclear how different colonies would have responded if given more room.

Bumble bee colonies

In 2017, three commercial research bumble bee colonies of *B. impatiens* (Koppert Biological Systems Inc., Howell, MI) were placed in each of the 12 apiary locations (36 colonies). Research colonies had one queen and approximately 50 workers and included a queen excluder to prevent gynes from exiting the colony when they were produced later in the season. Colonies were blocked by initial weight before being put in the field. The colonies were removed from the field after reproductive males were observed exiting any colony (mid-August, approximately 6.5 weeks post-placement). Colonies were stored at -20°C until processing. For processing, colonies were weighed and the number of gynes (adults and pupae) and drones (adults and pupae) were recorded (Heinrich 2004).

Statistical analysis

All analyses were completed in R Studio version 3.6.3 (R Core Team 2020). To determine differences in honey bee colonies among years and sites, colonies were treated as the unit of replication and correlated variance among colonies within site, year or site within year was accounted for through the use of random effects in the models used for analysis. Differences between years in colony July and September cluster size, *V. destructor* infestation, and

September capped brood area were determined using analysis of variance (ANOVA) on general linear mixed effects models (GLMM) using the lme4 package (Bates et al. 2015). Year was included as a three-level predictor variable as I anticipated no continuous temporal trends, site was included as a random intercept (12 levels) to account for location-specific variability, and the interaction between site and year was used as a random intercept (26 levels), to account for within-year apiary-level variability. To determine between-site differences in honey bee colonies, each response variable was regressed in GLMMs with site as the fixed effect, year as a fixed effect, and the interaction between site and year as a random intercept. Year was treated as a fixed effect to account for among year variability rather than a random effect because there were only three levels, and because new colonies were enrolled each year. To determine between-year and between-site differences in July and September honey bee visible brood disease, the same model structure was used in generalized linear mixed effects models (Bates et al. 2015) with a binomial distribution. To separate the effect of site or year from the inherent design variance, the full model was compared to a null model using ANOVA. For between-year differences, the null model only included the random effects (site and the interaction of year and site), and for the between-site differences the null model included the random intercepts and year as a fixed effect. Post-hoc Tukey's multiple comparisons between sites and years were determined using the multcomp package (Hothorn et al. 2020).

To determine which land covers and which colony variables had a significant effect on September honey bee colony size and capped brood area, I conducted backward step-wise model selection, using the lmerTest package (Kuznetsova et al. 2019). All honey bee colony data and land cover data were scaled and centered prior to model selection using the scale function (R Core Team 2020) to assist with model convergence and variable comparison. Honey bee

colonies which did not survive or in which I observed a queen event (queen loss or replacement) in September were excluded from model selection (51 colonies). The global models for honey bee September cluster size and capped brood area were additive GLMMs with July cluster size, July and September *V. destructor* counts, July and September presence/absence of brood disease, the six, non-NA land covers (wetlands, grassy-herbaceous fields, staple crops, non-staple crops, urban, and forests), and year as fixed effects to account for between year variation. Random effects for both models were a random intercept of site and a random intercept of the interaction between year and site. To treat site as the unit of replication by accounting for inherent design variance among colonies, random effects and year were force-retained in the final selected model. The variance inflation factor (VIF) was tested for selected fixed effects using the usdm package (Naimi et al. 2014). A VIF score of < 4.0 for each was used to indicate multicollinearity (Hair et al. 2010). Quality of the selected model was assessed by the coefficient of determination using the r2glmm package for partial R^2 values (Jaeger 2017) and the MuMIn package for conditional R^2 values of the entire model (Bartoń 2019). Selected models were compared to the global model using ANOVA and by calculating the correlation coefficient (Bartoń 2019) and Akaike information criterion (AIC) score (R Core Team 2020) of each model.

I also assessed between-site differences in bumble bee August colony weight, number of gynes, and number of drones using ANOVA, with colony as my replicate and site as the fixed effect. Bumble bee colonies were only studied in 2017, in 12 sites so no year or site within year variation needed to be accounted for using random effects structure. No bumble bee data were excluded from analysis due to confounding effects. I then conducted model selection on scaled and centered data for bumble bee August colony weight, number of gynes, and number of drones. Each of the three bumble bee colony outcomes (colony weight, gyne production, and

drone production) were regressed in a global model with initial colony weight and the six non-NA land covers as fixed effects. To treat site as the unit of replication, a random intercept of site was also in these bumble bee global models and force-retained in the variable reduction process, to account for inherent design variance. Assessment of the selected bumble bee models was the same as for the honey bee models, described above.

Results

Honey bee colonies

In July, colony cluster size was 15.04 ± 6.07 frames of bees. July cluster size was larger in 2016 than 2015 by about 8 frames of bees and larger than 2017 by about 5 frames of bees ($F_{2, 23.36}$=15.24, p<0.01). There were no site differences in July cluster size ($F_{11,11.54}$=1.69, p=0.19). Honey bee September cluster size was on average 14.89 ± 4.32 frames of bees. September cluster size was larger in 2016 ($F_{2,22.99}$=7.33, p<0.01) by about three frames of bees. There were no differences in September cluster size between sites ($F_{11,11.56}$=0.47, p=0.89). Honey bee September capped brood area was on average 2.46 ± 1.05 full deep frames of capped brood. 2015 had the lowest September capped brood area at 1.29 ± 0.73 frames, followed by 2017 at 2.40 ± 0.84 frames, and 2016 at 3.00 ± 1.00 frames ($F_{2, 23.46}$=24.05, p<0.01). There were no differences in September capped brood area between sites ($F_{11,11.86}$=0.96, p=0.52).

Average *Varroa destructor* July infestation was 0.20% ± 0.50% with a maximum infestation level of 5%. The July infestation did not differ between years ($F_{2,21.82}$=1.14, p=0.34) or sites ($F_{11,10.52}$=0.89, p=0.58). Mean September *V. destructor* infestation was 2.96% ± 4.28% with a maximum infestation level of 29.33%. 2016 had greater September *V. destructor*

infestation, 4.27% higher than 2015 and 2.96% higher than 2017 ($F_{2,22.94}$=7.51, p<0.01). There were no site differences in September *V. destructor* infestation ($F_{11,11.21}$=1.30, p=0.34).

There were 59 cases of visibly identified brood disease observed in July and 58 observed in September across all years. There were no year differences in likelihood of a colony exhibiting July brood disease ($X^2_{2,5}$=2.89, p=0.24), but there were differences among sites ($X^2_{11,16}$=34.00, p<0.01). Site D exhibited more July brood disease than sites A, B, C, H, I, or J, and site F had greater July brood disease expression than site C. September brood disease was greater in 2016 than 2017 ($X^2_{2,5}$=7.51, p=0.02), but there were no site differences ($X^2_{11,16}$=0.00, p=1.00).

The best selected model for final, September cluster size only included initial, July colony size. Year was dropped during parameter elimination, but I chose to include it in the final model to account for heterogeneity inherent to the experimental design. September cluster size was positively correlated (β=0.24) with July cluster size (Figure 2.3) and had a weak correlation (R^2=0.05). The selected model (AIC=648.46, R^2_c=0.33) was qualitatively better than the global model (AIC=682.06, R^2_c=0.36); the selected model was more parsimonious than the global model while fitting the September cluster size data equally well ($X^2_{10,17}$=8.23, p=0.61). For September capped brood area, July cluster size, July *V. destructor* infestation, September brood disease, and year were all included in the selected model. September capped brood area was weakly positively correlated with July cluster size (β=0.20, R^2=0.04) and weakly negatively correlated with July *V. destructor* infestation (β=-0.17, R^2=0.04) and presence of September brood disease (β=-0.37, R^2=0.03) (Figure 2.4). The VIF of July cluster size and *V. destructor* infestation were both 1.02. The selected model (AIC=630.10, R^2_c=0.39) was more parsimonious than the global model (AIC=653.44, R^2_c=0.42) and both fit the September capped brood area data equally well ($X^2_{8,17}$=11.56, p=0.17).

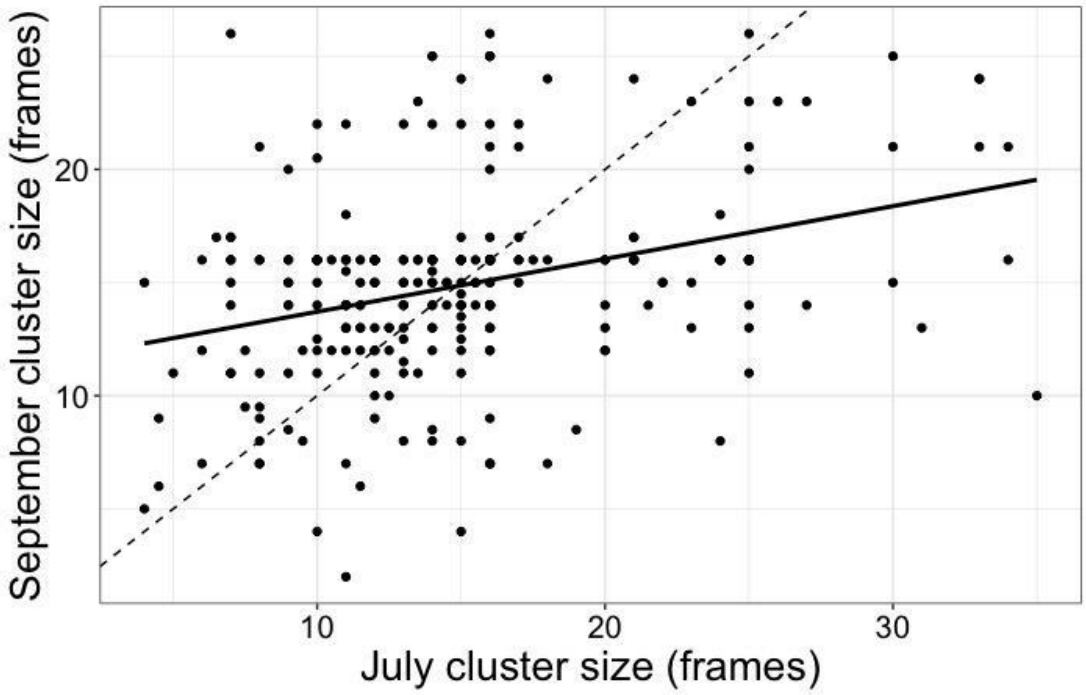

Figure 2.3. Correlation between honey bee colony September cluster size and July cluster size in frames of adult bees, which was selected as the best model for September cluster size (AIC=648.46, R^2_c=0.33). The solid line indicates the line of best fit, while the dashed, 1:1 line represents no change in colony size from July to September.

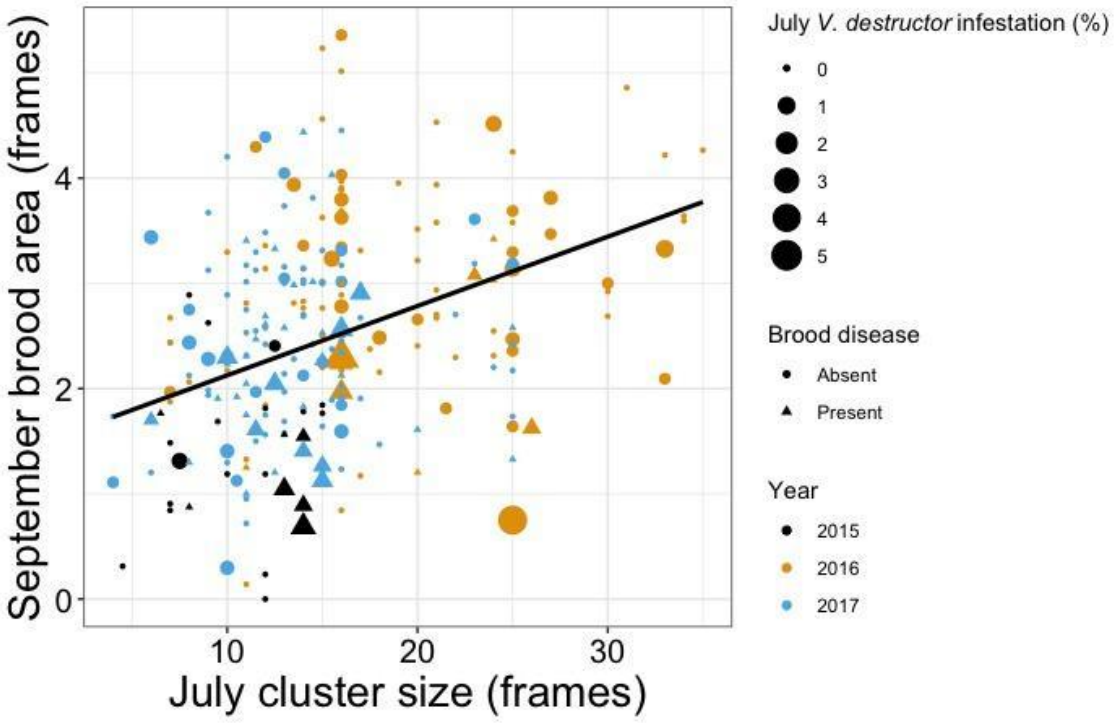

Figure 2.4. Correlation between July cluster size in frames of adult honey bees and September brood area in frames of brood, illustrated with line of best fit (AIC=630.10, R^2_c=0.39). The July *Varroa destructor* infestation (%) is indicated by point size, brood disease presence by a triangle and absence by a circle, and year is indicated by color. These factors were selected in the best model for honey bee September brood area.

Bumble bee colonies

Initial colony weight in July was 689.67 ± 41.39 g, which did not differ by site ($F_{11,35}$=1.80, p=0.11), by design. By time of reproduction in August, bumble bee colonies were 736.63 ± 103.75 g. These weights still did not differ between sites ($F_{11,35}$=1.07, p=0.42). The average number of gynes produced by bumble bee colonies was 20.31 ± 25.72, while on average 112.81 ± 77.23 drones were produced per colony. Neither gyne production ($F_{11,35}$=1.02, p=0.46) nor drone production ($F_{11,35}$=0.99, p=0.48) differed by site.

The best selected model for bumble bee colony weight at time of reproduction included the area of non-staple crops (β=0.62, R^2=0.19), wetlands (β=0.51, R^2=0.13), and urban land

(β=0.52, R^2=0.14). These land covers were each moderately, positively correlated with bumble bee colony weight and each had approximately the same magnitude of effect on colony weight (Figure 2.5). The VIF was approximately 2 for area of staple crops, wetlands, and urban land. The selected model was superior (AIC=111.28, R^2_c=0.20) to the global model (AIC=117.96, R^2_c=0.22) in parsimony, and both these models fit the colony weight data equally well ($X^2_{4,10}$=1.92, p=0.75).

Gyne production (log scale) was best modeled by the area of wetlands (β=0.32, R^2=0.12), with a positive correlation (Figure 2.6). The selected model (AIC=102.34, R^2_c=0.12) was more parsimonious than the global model (AIC=115.70, R^2_c=0.22), and both fit the gyne production data equally well ($X^2_{6,10}$=2.57, p=0.86). However, when the site with the greatest amount of wetland was excluded from analysis, a random-effects-only model was selected as the best model for gyne production. Drone production was best modeled by the area of grassy-herbaceous land. Drone production was negatively correlated with the area of grassy-herbaceous land (β=-0.41, R^2=0.16) (Figure 2.7). The selected model (AIC=106.41, R^2_c=0.16) was better than the global model (AIC=118.36, R^2_c=0.21), as it was more parsimonious while both fit the drone production data equally well ($X^2_{6,10}$=3.64, p=0.73).

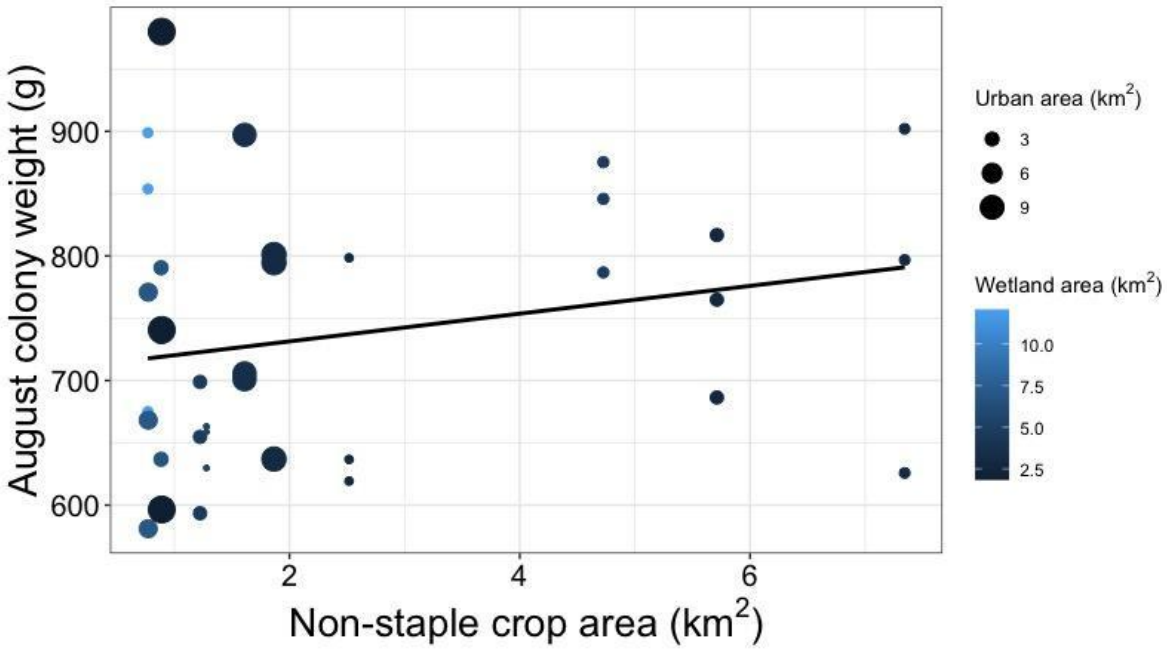

Figure 2.5. Correlation between area of non-staple crops (km^2) and bumble bee colony August (time of reproduction) weight (g), illustrated with line of best fit. Area of urban land (km^2) is indicated by point size, and area of wetlands (km^2) is indicated by point color. These factors were selected in the best model for bumble bee colony weight at time of reproduction (AIC=111.28, R^2_c=0.20).

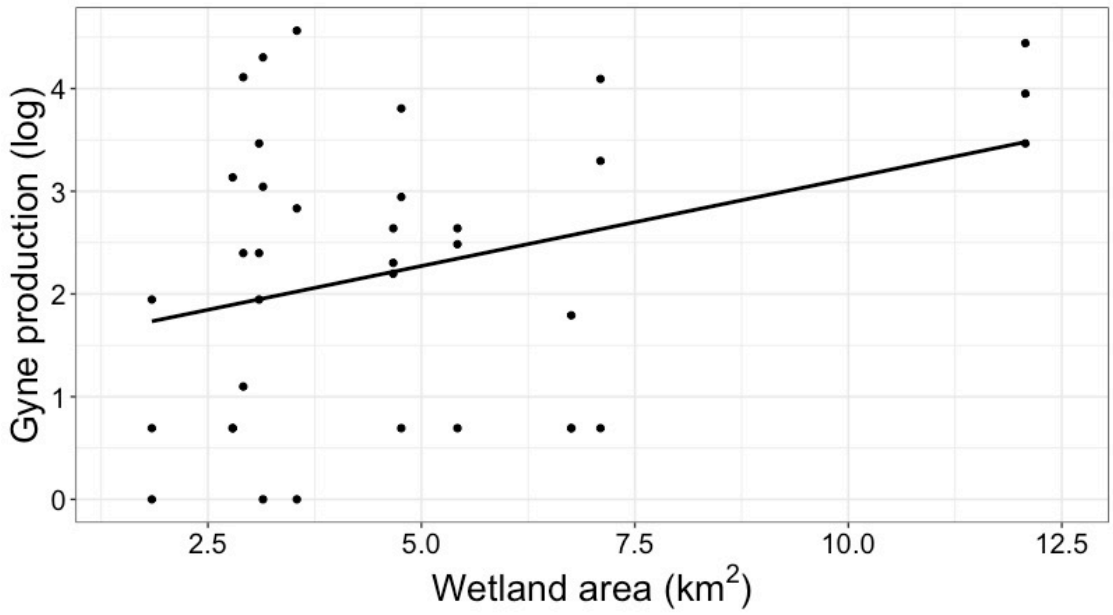

Figure 2.6. Correlation between area of wetlands (km^2) and bumble bee colony gyne production illustrated with line of best fit (AIC=102.34, R^2_c=0.12).

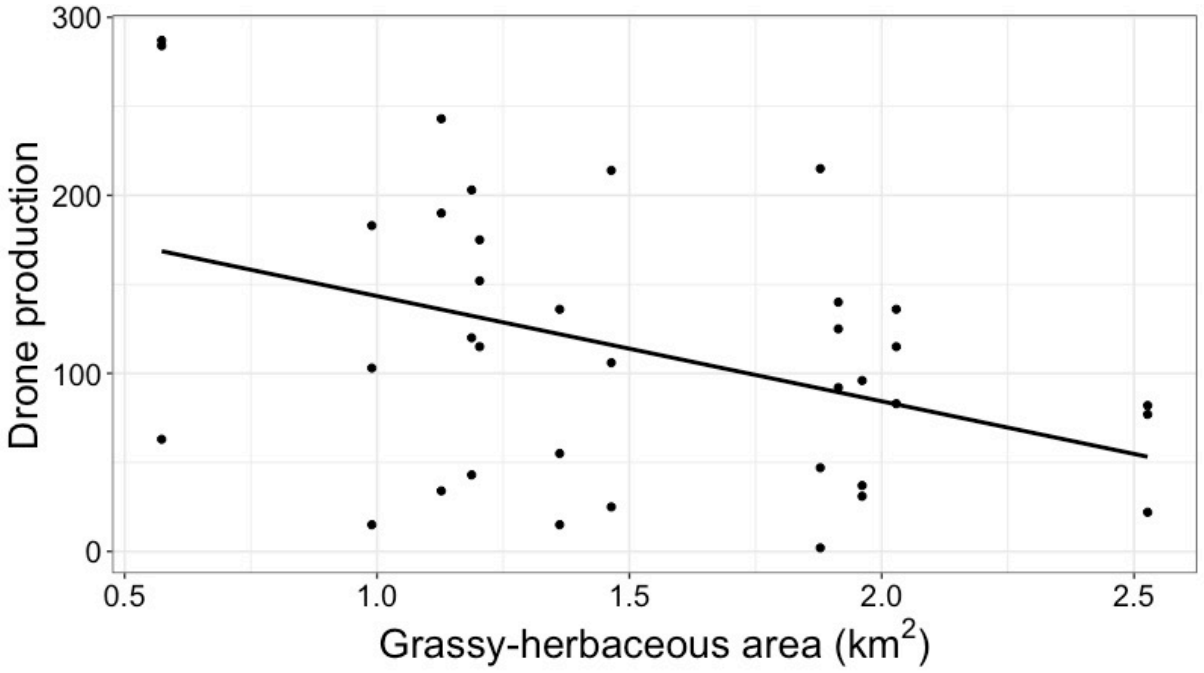

Figure 2.7. Correlation between area of grassy-herbaceous fields (km^2) and bumble bee colony drone production, illustrated with the line of best fit (AIC=106.41, R^2_c=0.16).

Discussion

Honey bee colonies

September colony cluster size and capped brood area were more closely associated with colony-related factors than land covers. There were also no between-site differences in September colony cluster size or capped brood area, despite apiaries being surrounded by different land use. This finding contradicts recent landscape scale studies in other systems, that found a significant effect of various land covers on honey bee colony outcomes including colony survival and bee physiological condition (Clermont et al. 2015; Dolezal et al. 2016; Smart et al. 2016b). This disparity could be due to different outcome metrics, more standardized colony conditions, or a region-specific greater effect of forage landscape. My finding highlights the importance of accounting for colony-level factors when investigating landscape level effects on honey bee colonies. The finding that in-hive colony conditions, rather than landscapes, were

important for driving honey bee colony size aligns with primary concerns expressed by beekeepers (vanEngelsdorp et al. 2011; US Department of Agriculture National Agricultural Statistics Service 2016a). In a 2009-2010 survey, commercial beekeepers ranked issues related to queens and mites first and second, respectively, while starvation was ranked fifth (vanEngelsdorp et al. 2011) among causes of colony mortality. Likewise, the US Department of Agriculture colony survey includes forage and starvation in a category, "other," along with weather, queen failure, and hive damage, to which Michigan beekeepers only attributed 1.8% of colonies affected by said stressor in the summer of 2015 (US Department of Agriculture National Agricultural Statistics Service 2016a).

The initial July colony cluster size accounted for more of the variability in September colony cluster size than any of the land cover variables. While this indicates that colonies which start larger stay larger, colonies generally dwindled over the summer and the correlation was weak. Summer honey production apiaries for honey bees in Michigan are meant to provide oases of recovery, productivity, and growth after colonies have provided crop pollination (Bond et al. 2014). It is therefore concerning that colonies were smaller after being in feeding apiaries for three months. The regression line between July cluster size and September cluster size intersects the 1:1 line (no change) at 15 frames of bees (Figure 2.3). My collaborating beekeeper typically kept colonies in 10-frame, double-deep brood boxes (containing~15 frames) and often removed honey supers in August, which may have limited the growth of colonies which started larger in July. This truncation of colony size may have been one of the reasons I was unable to observe an effect of landscape on the honey bee colonies. It is also possible that I am observing the greatest colony cluster size that can be expected of colonies in this region, given available forage. This is supported by another honey bee landscape study in Ohio, a similar study system, which matched

the range of colony cluster sizes I observed (Sponsler and Johnson 2015). Given a greater range of landscape, which were not available and thus not represented in this study, it is possible I could have observed a more pronounced effect. A third possibility is that these colonies may not be exhibiting a response to land covers if in Michigan the differences in forage quality among these land covers is indistinguishable to honey bees. It is possible that many of the land covers in Michigan host abundant volunteer flowering plant species that honey bees forage upon (Requier et al. 2015) (Chapter 5). This highlights the need for field-scale floral surveys to characterize the forage quality range of herbaceous land covers (Chapter 4). Finally, it is also possible that I had insufficient power to detect an effect of landscape, which could be resolved with the addition of more sites.

I also found that September colony capped brood was better accounted for by colony factors, including July size, *V. destructor* infestation, and September brood disease, as well as between-year differences, than any of the land covers. As with September colony size, September colony capped brood area was also positively correlated with July colony size. Large colonies may be able to collect more forage to supporting greater brood production (Brodschneider and Crailsheim 2010). Late-summer capped brood area has been linked to overwintering survival as well as the colony's capacity to service almond pollination contracts (Smart et al. 2016a), which at an average of $184 per colony as of 2017, is a high paying pollination contract (Goodrich 2018). Therefore, capped brood is an important metric, but was not related to any land covers in my study. July *V. destructor* infestation and presence of brood disease in September were both negatively correlated with September cluster size. Observing a negative effect of July *V. destructor* infestation, even though infestation was at most 5%, reinforces the need for diligent *V. destructor* control and supports the most recent treatment

threshold recommendation of 3% (Honey Bee Health Coalition 2018). Likewise, beekeepers should monitor brood and consider management interventions to mitigate late-summer brood disease (Chapter 6).

Year was also a significant predictor of September capped brood area. This finding indicates that temporal dynamics can be more variable than spatial dynamics, highlighting the necessity to conduct this type of study over multiple seasons. Year differences could be reflective of differences in weather (Beyer et al. 2018), pollen and nectar availability, or effects that occurred during spring pollination such as pesticide exposure. In the Midwest, capped brood has been positively correlated with the amount of available forage (Smart et al. 2016a). However, the range of uncultivated forage found by Smart et al. in the Dakotas, analogous to grassy-herbaceous land cover in this study, exceeded the greatest amount of grassy-herbaceous land here by 1.5-12.8-fold. Given the foraging needs and patterns of honey bees, colonies in the current study might have shown more significant capped brood response to land covers if they had access to greater uncultivated forage (Smart et al. 2016a) or other large areas with abundant floral resources (Rollin et al. 2013). However, such land covers are not available in Michigan landscapes and thus not represented in my study. Another study in Ohio, a state with more similar landscape composition to Michigan, found no significant predictors of capped brood area (Sponsler and Johnson 2015). This supports the explanation I suggested for the lack of variation in colony cluster size, that the range of landscapes in the Great Lakes states provide insufficient opportunity to see variation in capped brood production.

The inference that can be drawn from these data are limited by the experimental design limitations. I chose to work with a commercial colonies to reflect realistic conditions that commercial honey bee colonies may experience, and to only work with a single collaborating

beekeeper to limit variation in management and colony history. However, this greatly limits the inference that should be drawn from these data. This study is inherently confounded by the management and colony history and these data only represents a short time period in the life cycle of these colonies. Furthermore, site locations, and thus the range of land covers were limited by apiary location permissions. Therefore, these results should be interpreted with caution as they may not be representative of honey bee colonies in Michigan as a whole.

Bumble bee colonies

Unlike honey bee colonies, *B. impatiens* colony weight, gyne production, and drone production were each correlated with land cover variables. I found that bumble bee colony weight at the time of reproduction was positively correlated with the area of non-staple crops, wetland, and urban land. Many species of bumble bees have been experiencing range constrictions and population declines over the past decade (Grixti et al. 2009; Cameron et al. 2011), linked to anthropogenic land use change, including urbanization and agricultural intensification. However, *B. impatiens* is still very successful across its range in the eastern US and Canada (Colla et al. 2012; Wood et al. 2019). My findings that *B. impatiens* benefits from urban and non-staple crop lands contributes to the growing body of work that shows that certain species of bumble bees benefit from land covers associated with anthropogenic land use change (Persson et al. 2015; Theodorou et al. 2020). Resource pulses provided by non-staple crops can offer important forage to bumble bee colonies (Hemberger and Gratton 2018), and urban green spaces may likewise provide bumble bee habitat (Ahrné et al. 2009). Wetlands host a number of bee-supportive plants (Oeetel 1980) and have been identified as important forage habitat for bumble bees in Midwestern agroecosystems (Vickruck et al. 2019). It is possible that wetlands

provide excellent forage to bumble bees in ways that were not available to honey bees in Michigan. For example, wetlands host a number of flowering species more beneficial to bumble bees than honey bees such as *Liatris spicata, Lobelia siphilitica, Mimulus ringens,* and *Tradescantia ohiensis* (Clark 2012), none of which are extremely common in Michigan. An assessment of bumble bee and/or honey bee-collected pollen could elucidate if either bee species is foraging extensively on wetland floral species (Chapter 5).

My data also showed that bumble bee colony gyne production was positively correlated with the area of wetlands (Figure 2.6). However, the correlation between wetlands and number of gynes produced heavily depended upon a single site that was surrounded by about 12 km^2 of wetlands. When that site was removed from analysis, there was no correlation between gynes and any land cover. A broader range of surrounding area of wetlands should be investigated to determine the benefit of wetlands to bees. Generally, access to seminatural land has been shown to enhance bumble bee colony reproductive ability by providing pollen and nectar forage (Requier et al. 2019). My observation that wetlands are important for colony weight as well as colony reproductive capacity suggest that Michigan wetlands provide temporally continuous forage resources, which is essential to producing reproductive individuals (Williams et al. 2012; Rundlöf et al. 2014). Future studies could investigate the effect of wetlands further with floral assessments.

It is interesting that wetlands were positively related to colony weight and gyne production but not related to drone production. This could represent a trade-off in reproductive investment by the colony (Duchateau et al. 2004) or could be a consequence of floral resource temporal availability (Williams et al. 2012). Grassy-herbaceous land also only represented at most 7.8% of the total land area surrounding my study sites. While this was representative of

grassy-herbaceous land distribution in Michigan, it is unclear if the negative relationship observed between colony drone production and grassy-herbaceous land area would be similar had I included sites where grassy-herbaceous land was the dominant land cover type. Exploring bumble bee colony response across a larger distribution of grassy-herbaceous land would help elucidate this relationship.

Conclusions

While *A. mellifera* and *B. impatiens* are both social, generalist bees (Winston 1991; Williams et al. 2014) and are very common in Michigan (Wood et al. 2019; US Department of Agriculture National Agricultural Statistics Service 2019c), each responded to land covers differently. *Bombus impatiens* colony weight and reproductive output were associated with area of various land covers, while *A. mellifera* cluster size and capped brood area were more closely associated with colony-level factors. My findings suggest that it is essential to account for colony variation in honey bee landscape studies. Future studies should investigate whether abundant, weedy flowering plants are providing sufficient forage to honey bees in Michigan across land cover types by monitoring field-level floral availability and resource foraging (Chapter 5). Such a finding would indicate the need to preserve habitat heterogeneity within land covers (Kovács-Hostyánszki et al. 2017). My findings show that the native bumble bee, *B. impatiens,* is sensitive to land cover composition, and that bee-supportive land covers such as wetlands should continue to be investigated for their potential to provide forage for managed and non-managed pollinators.

CHAPTER 3: THE RELATIONSHIPS BETWEEN LANDSCAPE DIVERSITY, HONEY BEE FORAGED POLLEN, AND COLONY SIZE VARY OVER TIME

Abstract

Abundant pollen forage can support honey bee colony growth, and different land covers, such as cropland, wetlands and forests, each probably host unique floral communities with different bloom phenology. Land cover diversity may therefore provide honey bees with complementary forage resources. Land cover diversity may also increase the likelihood of access to beneficial land covers through sampling effect. However, to my knowledge, land cover diversity has not yet been examined for its effects on honey bee foraging or colony growth. Therefore, to determine the relationship between land cover diversity, pollen resources, and colony size (both adult bees and brood), I assessed the amount of incoming pollen and colony size throughout the summer in apiaries placed along a gradient of land cover diversity. Using general linear mixed models and path analysis, I determined that land cover Shannon diversity had a variable, and marginally significant relationship with the amount of incoming pollen and colony growth. There was some indication that colonies surrounded by landscapes of intermediate Shannon diversity had larger cluster sizes, and was associated with greater quantities of foraged pollen in late August. Brood area was also positively correlated with the amount of incoming pollen. Land cover identity, underlying change in Shannon diversity could explain this variable relationship. The marginal significance of land cover Shannon diversity to honey bee health outcomes suggests the need for further investigation into the utility of this metric for honey bee landscape studies.

Introduction

Commercial honey bee colonies are often rented for pollination services on pollinator dependent crops (Southwick and Southwick 1992; Calderone 2012), and the rental price of a colony for pollination is largely dependent upon its size (Goodrich 2018). Crop pollination is often stressful to honey bee colonies, in part due to limited access to adequate nutrition in monocultures (Girard et al. 2012). It is therefore important for colonies, between pollination contracts, to have access to forage landscapes with abundant, consistent flower resources so they can grow and produce honey. Colony growth, measured by the adult bee population (cluster size) as well as the amount of immature larvae (area of brood) is closely related to the amount of incoming forage, particularly pollen, (Brodschneider and Crailsheim 2010) provided by the surrounding forage landscape.

Land cover diversity at the broad landscape scale could be useful for determining forage landscape quality (Benton et al. 2003; Kovács-Hostyánszki et al. 2017). This is particularly relevant in agroecosystems, in which resources may go through drastic temporal pulses of providing resources for honey bees, based upon bloom phenology of various land covers (Danner et al. 2016; Dolezal et al. 2019). Honey bees, as generalist foraging insects (Winston 1991) and strong dispersers (Beekman and Ratnieks 2000) are capable of taking advantage of a wide range of resources that occur across landscapes. Furthermore, honey bees can socially recruit nest-mates to quality forage resources, making them highly capable of finding and taking advantage of rewarding resources in the forage landscape (von Frisch 1967). Land cover diversity may therefore provide a bees-eye-view of the overall suitability of landscapes.

Shannon diversity, an evenness-corrected measure of richness (Shannon 1948), is often used to quantify species diversity. It has been used in bee studies as an index of floral diversity at

the field scale (Carson et al. 2016). Shannon diversity has also been identified as a useful metric for characterizing landscape structure in broader spatial scale studies (O'Neill et al. 1988), where higher land cover Shannon diversity indicates more land cover types at more even proportions. To my knowledge, using land cover Shannon diversity is a novel technique for assessing forage landscape quality for positioning apiaries. Land cover Shannon diversity has previously been used to assess landscape effects on generalist insects (Jonsen and Fahrig 1997). Jonsen and Fahrig found that land cover Shannon diversity was correlated with greater richness and abundance of generalist insects, and I expect landscapes with higher Shannon diversity will similarly benefit honey bee colonies.

Land cover Shannon diversity may benefit honey bee foraging and thus colony size through a number of mechanisms. Additional land covers and land cover evenness could increase forage availability by providing complementary blooming resources that increase the temporal stability of pollen availability. At higher Shannon diversity it also becomes increasingly likely, through sampling effect, that beneficial land covers are included (Barthlott et al. 2009). An increase in forage land covers, associated with higher land cover Shannon diversity, would also result in increased connectivity, which could facilitate foraging (Taylor et al. 1993; Jonsen and Fahrig 1997). Indeed, pollen foragers have been shown to forage shorter distances in more complex landscapes (Steffan-Dewenter and Kuhn 2003).

Determining indirect relationships between external factors such as landscape metrics and colony outcomes is inherently challenging with traditional regression models. One form of multiple regression that can elucidate indirect relationships is path analysis (Wright 1918, 1921). This approach allows for an *a priori* set of hypothesized correlations among variables to be explored by partitioning variance from the data into the specified relationships (Scheiner 2001).

Such an analysis approach is common in ecological field studies (Scheiner 2001), and was suitable for exploring causal relationships between land cover Shannon diversity, foraged pollen quantity, and colony cluster size and brood area.

Michigan is a very agriculturally and apiculturally important state in the Great Lakes region of the United States. It hosts the second highest diversity of agricultural crops in the United States behind California (Bertone 2017), and honey bee pollination is valued at $1 billion annually (Huang and Pett 2010). Many commercial beekeepers come to Michigan in spring for pollination contracts, then their colonies remain in feeding yards through the summer to produce honey and build up colony size for other pollination contracts throughout the year (US Department of Agriculture National Agricultural Statistics Service 2019a). These feeding yards are important for recovering through the summer from stressful pollination contracts and to prepare for almond pollination the following spring, when beekeepers earned on average $184 per strong colony in 2017 (Goodrich 2018). Michigan is also unique from the rest of the region in its small average farm size (US Department of Agriculture National Agricultural Statistics Service 2019b). This results in agricultural fields being interspersed in a matrix of urban land, wetlands, grassy-herbaceous fields, and forests (US Department of Agriculture National Agricultural Statistics Service 2018). Throughout the state, the composition and diversity of these land covers vary, making Michigan an ideal place to examine how land cover diversity affects honey bee foraging and subsequent colony size.

I aimed to determine if land cover Shannon diversity is related to honey bee pollen forage and colony size. The objectives of this study were to: 1) Determine the relationship between land cover Shannon diversity and the amount of foraged pollen from July through September, 2) Determine how colony size (cluster size and brood area) varied with land cover Shannon

diversity throughout the summer, and 3) Determine the indirect relationships between land cover Shannon diversity, amount of foraged pollen and colony size. I hypothesize that increased Shannon diversity will have a positive effect on colony size by providing increased pollen abundance.

Methods

In 2018, I assessed 16 commercial honey bee colonies each of 12 apiaries (192 colonies) in southwest Michigan at three time points throughout the summer; July, August, and September. Two of the 16 colonies per apiary were outfitted with pollen traps (Superior Pollen Traps Mann Lake, Hackensack, MN) (24 colonies) that were set to collect pollen every two weeks between the July and September colony inspections for a total of four collections: early August, late August, early September, and late September. Apiary locations were chosen to range in Shannon diversity of surrounding land cover and were spatially independent at a 4 km foraging range, overlapping minimally in one case (Figure 3.1). Land cover Shannon diversity was calculated in R version 3.6.3 (R Core Team 2020) using the vegan package (Legendre et al. 2018) based on six land covers: urban, wetland, staple crop (corn, soy, small grains), other crop (tree fruit, vineyards, vegetables, etc.), grassy-herbaceous (pasture, grassland, fallowed cropland, etc.), and forest. These broad land use categories were collated from the 2018 Cropland Data Layer (US Department of Agriculture National Agricultural Statistics Service 2018) and had their areas calculated using the raster (Hijmans et al. 2020) and rgeos packages (Bivand et al. 2019) (Table 3.1).

Shannon diversity ranged from 1.14 to 1.66 across the sites, with an average diversity of 1.42 and variance of 0.04 (Figure 3.1). This represented the range of land cover Shannon

diversity in this region, which ranged from 0.30 to 1.70, with an average diversity of 1.37 and variance of 0.07, based upon a random sample of 1000, 4 km radii, generated using the sp package (Pebesma et al. 2020) (Figure C1). The mean diversity of my sites was similar to the regional mean ($F_{1,887}$=0.31, p=0.58). Both were likewise similar in variance ($F_{11,875}$=0.55, p=0.27). Land cover Shannon diversity was positively correlated with the area of non-staple cropland (ρ=0.66, t_{10}=2.81, p=0.02) and negatively correlated with the area of forests based on Pearson's product-moment correlation (ρ=-0.87, t_{10}=-5.65, p<0.01).

Table 3.1. Landscape composition of each of the 12 study sites, with land cover Shannon diversity, calculated from the area of six land covers in km^2: urban, wetland, staple crop (corn, soy, small grains), other crop (tree fruit, vineyards, vegetables, etc.), grassy-herbaceous (pasture, grassland, fallowed cropland, etc.), and forest.

Shannon diversity	Urban	Wetland	Staple crop	Other crop	Grassy-herbaceous	Forest
1.14	2.27	2.97	1.92	6.25	3.13	33.31
1.14	2.11	9.96	1.14	0.62	5.67	30.65
1.15	1.60	2.60	3.38	3.93	5.08	33.46
1.35	2.45	3.67	25.25	3.54	1.91	12.76
1.35	13.69	4.67	1.03	1.63	4.47	22.78
1.37	15.52	3.36	2.50	1.09	3.89	19.92
1.42	14.57	5.23	2.22	1.32	4.79	21.83
1.50	3.02	12.19	2.56	5.86	4.30	21.44
1.60	3.95	6.88	18.90	8.18	2.32	8.43
1.66	3.23	7.54	11.94	12.04	2.68	11.74
1.66	4.44	3.02	15.14	8.98	6.16	12.29
1.66	3.96	4.39	12.15	6.81	6.49	16.14

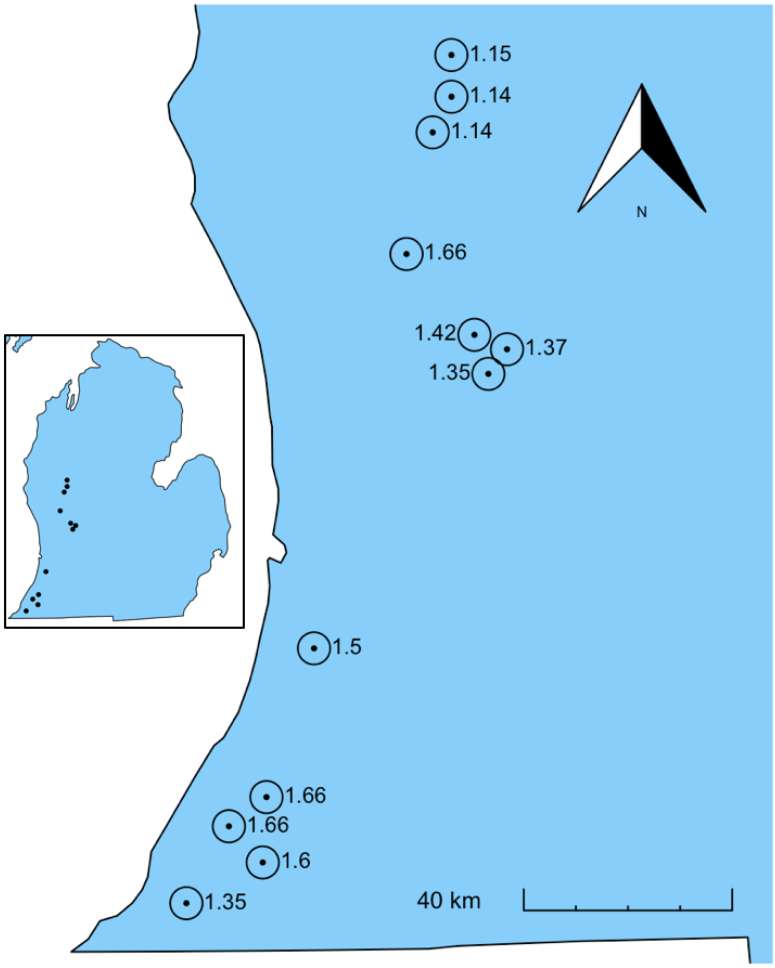

Figure 3.1. Map of research apiaries in Michigan with points representing apiary locations and circles representing the 4 km range, over which land cover Shannon diversity was calculated. Land cover Shannon diversity is shown for each site.

All colonies were managed by a single collaborating commercial beekeeper, to keep the colony management practices consistent. Enrollment criteria specified that colonies must meet the following characteristics: be larger than 5 frames of adult bees, alive, queen-right, have below 3% *Varroa destructor* infestation (Macedo et al. 2002), and show no visual signs of brood disease. During each of the three monthly assessments, I recorded cluster size of each colony in frames of bees (Nasr et al. 1990) during the day. I also monitored for potentially confounding health variables and only included colonies if they met the aforementioned criteria. In

September, the 24 colonies with pollen traps were further assessed for area of capped brood using the method outlined in Delaplane et al. (2013). Pollen was collected for 72 hours every two weeks between July and September. Pollen was transported at room temperature from the field, stored at -20°C and weighed. Samples from colonies that died or where foragers found alternative entrances into the colony were excluded from analysis.

To summarize temporal and spatial differences in pollen weights and colony cluster size I used repeated measures analysis of variance (ANOVA) on general linear mixed effects models (GLMM). Temporal differences in pollen weight between the four biweekly sampling rounds were calculated by treating sampling round as a fixed effect and colony nested within apiary as a random intercept, with a compound symmetry error distribution (Bates et al. 2015). Multiple comparisons between apiaries were then calculated using Tukey's method in the multcomp package (Hothorn et al. 2020). In the same way, between-apiary differences in pollen weight within each biweekly sampling round were calculated using repeated measures ANOVA on GLMMs with round as a fixed effect to account for design variation because it only had four levels and colony as the random effect. Tukey's multiple comparison was then used as a post-hoc test. Site differences within round were likewise assessed on the stratified data. Differences in colony cluster size between sampling month, apiary and apiary within month were likewise calculated using repeated measures ANOVA on GLMMs, but using a first order autoregressive error structure in the package nlme (Pinheiro et al. 2020).

To determine the relationship between amount of surrounding land cover Shannon diversity and amount of incoming pollen, the weight of incoming pollen for each colony at each collection was regressed with the interaction of land cover Shannon diversity as a second order polynomial, and sampling round in a repeated measures GLMM with colony nested within

apiary as the random effect and a compound symmetry error structure (Kuznetsova et al. 2019). A polynomial of land cover Shannon diversity was used because data visualization suggested a higher order relationship. The second order polynomial GLMM was tested against the first-order model using Akaike information criterion, corrected for small sample size (AIC_c) model selection (Burnham and Anderson 2007). A cut-off value of $\Delta AIC_c \geq 2.0$ was used to distinguish differentiable models (Burnham and Anderson 1998, 2007). Deciding between a compound symmetry and first order autoregressive error structure was also determined using AIC_c model selection. Trends in the relationship between pollen weight and land cover Shannon diversity were further assessed by stratifying the data by sampling period and treating apiary as a random intercept. Coefficient of determination values (R^2_c) for these models were calculated using the MuMIn package (Bartoń 2019), and partial R^2 using the Nakagawa and Schielzeth approach were calculated using the r2glmm package (Jaeger 2017).

In the same way, to determine the relationship between cluster size and surrounding land cover Shannon diversity, I conducted a repeated measures GLMM with a compound symmetry error structure. Colony cluster size was regressed with the interaction of land cover Shannon diversity, as a second order relationship, and sampling month (3 levels) as fixed effects and colony nested within apiary as the random effects. The same was done within sampling month, with apiary as the random effect. Likewise, to determine the relationship between area of September capped brood and land cover Shannon diversity, September area of capped brood was also regressed in GLMM with land cover Shannon diversity and apiary as the random effect.

To determine correlations between the amount of foraged pollen and colony cluster size and brood area, August and September colony cluster size and September brood area were each

regressed with each of the preceding bi-weekly pollen weights, collected by that colony, in a GLMM with apiary as the random effect.

These individual regressions were combined to determine indirect relationships among land cover Shannon diversity, incoming pollen weight, and colony cluster size and brood area (Table 3.2) based on *a priori* expectations using path analysis in the lavaan package (Rosseel 2012). Incoming pollen for each biweekly sample round (< 2 colonies/ apiary), the September brood area data (< 2 colonies/ apiary, same colonies as pollen colonies), and the colony cluster size for each month (< 16 colonies/ apiary, including the pollen-trapped colonies) were all used for path analysis. To account for repeated measures, apiary was specified as the clustering identity in the design specification through the survey package (Lumley 2020), and incorporated into the path analysis using the lavaan.survey package (Oberski 2016). Modifications were made to the path analysis based upon F-tests to analyze model fit using the lavaan.survey package, and modification indices suggested by the lavaan package.

Table 3.2. Path analysis model relating September colony cluster size and brood area to land cover Shannon diversity (abbreviated as SD) and pollen weights. Asterisks indicate variables added to the original *a priori* model based upon modification indices assessment.

September colony cluster size	
Regression responses	Regression predictors
Late August pollen weight	SD
Early September pollen weight	SD
September cluster size	SD + Late Aug. pollen + Early Sept. pollen
Residual correlation responses	Residual correlation predictors
Early August pollen weight	Late Aug. pollen
Late August pollen weight	Early Sept. pollen
Early August pollen weight	Early Sept. pollen
September cluster size	Early Sept. pollen*
September colony brood area	
Regression responses	Regression predictors
Early August pollen weight	SD*
Late August pollen weight	SD
Early September pollen weight	SD
September cluster size	SD + Early Aug. pollen* + Late Aug. pollen + Early Sept. pollen
Residual correlation responses	Residual correlation predictors
Early August pollen weight	Late Aug. pollen
Late August pollen weight	Early Sept. pollen
Early August pollen weight	Early Sept. pollen

Results

Pollen weight

Pollen weight collected per colony through the four sampling periods was highly variable, ranging from 3.45 to 272.08 g, with an average of 81.48 ± 61.98 g. The overall amount of incoming pollen decreased over time between August and September, with colonies collecting less pollen in late September than late August by 72 g and early September by 82 g ($F_{3,55.06}$=9.82, p<0.01) (Figure 3.2). Overall, pollen weights did not vary significantly by apiary ($F_{11,11.64}$=0.74, p=0.69), nor did they vary by apiary within each biweekly sampling round (early August: $F_{10,19}$=0.45, p=0.89, late August: $F_{11,23}$=1.31, p=0.32, early September: $F_{10,19}$=0.45, p=0.89, late September: $F_{7,14}$=3.12, p=0.08).

The repeated measures ANOVA of pollen weights with land cover Shannon diversity, was optimized at a land cover Shannon diversity of 1.38, but was not significant ($F_{2,25.07}$=2.13, p=0.14). Bi-weekly sampling round was also non-significant ($F_{1,49.78}$=1.74, p=0.17). However the interaction of land cover Shannon diversity and sampling round was significant ($F_{2,50.41}$=2.68, p=0.02), suggesting that the relationship between pollen weight and land cover Shannon diversity changes with time. The overall model was a good fit for the data (R^2_c=0.56) but the partial R^2 values were low, with the two strongest relationships, the interaction between pollen collected in late August and the polynomial elements of land cover Shannon diversity, both having a coefficient of determination below 0.05. Within each biweekly sampling round, Shannon diversity of forage landscapes was only significantly correlated with the weight of honey bee collected pollen in late August. Early August pollen weight was not correlated with land cover Shannon diversity ($F_{1,18}$=1,68, R^2_c=0.08, p=0.21) (Figure 3.2A). Late August pollen weight was negatively correlated with Shannon diversity, though marginally ($F_{1,22}$=4.45,

R^2_c=0.16, p=0.05); for each 0.1 unit increase in Shannon diversity, pollen weight decreased by 12.08 g (Figure 3.2B). Incoming pollen weight was not correlated with Shannon diversity in early September ($F_{1,10.17}$=0.19, R^2_c=0.33, p=0.67) (Figure 3.2C), or in late September, though the non-significance was marginal ($F_{1,13}$=4.46, R^2_c=0.24, p=0.05) (Figure 3.2D). For each 0.1 unit increase in land cover Shannon diversity, the weight of pollen collected in late September increased by 8.55 g.

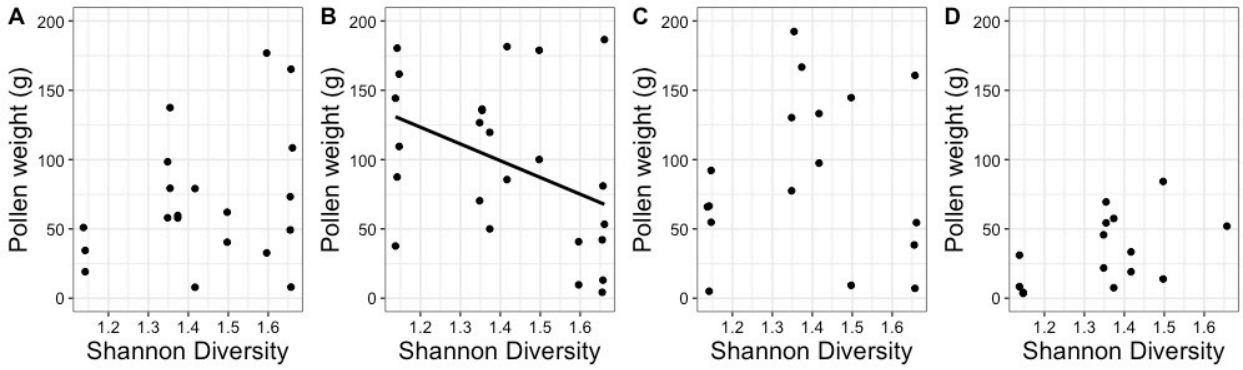

Figure 3.2. Pollen weight collected by honey bees in relation to land cover Shannon diversity of the surrounding landscape at each biweekly sampling round: early August (A), late August (B), early September (C), and late September (D). Best fit lines are shown when the correlation is significant.

Cluster size

Based on the colony inclusion criteria I used, by the September inspection, only 101 of the 192 colonies were included for analysis. Within this cohort of colonies, colony sizes ranged from 5.69 to 31.75 deep frames of adult bees (based upon a correction factor of 0.68 for medium frames), with average cluster sizes of 18.16 ± 5.56, 16.11 ± 4.30, 13.51 ± 3.29 frames for July, August, and September, respectively. Colony size decreased throughout the summer ($F_{2,240}$=86.30, p<0.01), with colonies being on about 2.3 frames smaller each month (Figure 3.3).

While apiaries varied in cluster size overall ($F_{11,157}$=2.67, p<0.01), this variation was not related to cluster size, as the time series model relating colony cluster size and the polynomial of land cover Shannon diversity showed no significant relationship with land cover Shannon diversity ($F_{2,9.00}$=0.19, p=0.83). Sampling month was also non-significant in this model ($F_{2,244.10}$=1.11, p=0.33), but the interaction of land cover Shannon diversity and month was marginally non-significant ($F_{4,245.31}$=2.17, p=0.07). While this overall time series model had a strong coefficient of determination (R^2_c=0.52), none of the partial R^2 values were over 0.01.

When examining colony cluster size within sampling month, apiaries were significantly different in the initial colony size in July ($F_{11,168}$=3.56, p<0.01), and these differences were not correlated with land cover Shannon diversity ($F_{1,10.04}$=0.34, R^2_c=0.17, p=0.57) (Figure 3.3A). There were also between-apiary differences in colony size in August ($F_{11,128}$=2.62, p<0.01), and colony size in August was also not correlated with land cover Shannon diversity ($F_{1,5.54}$=0.83, R^2_c=0.15, p=0.40) (Figure 3.3B). In September, colony cluster size varied by apiary ($F_{11,100}$=2.15, p=0.02), showing a curvilinear relationship with Shannon diversity ($F_{1,6.86}$=6.61, R^2_c=0.13, p=0.03) that was optimized at a Shannon diversity of 1.33 (Figure 3.3C).

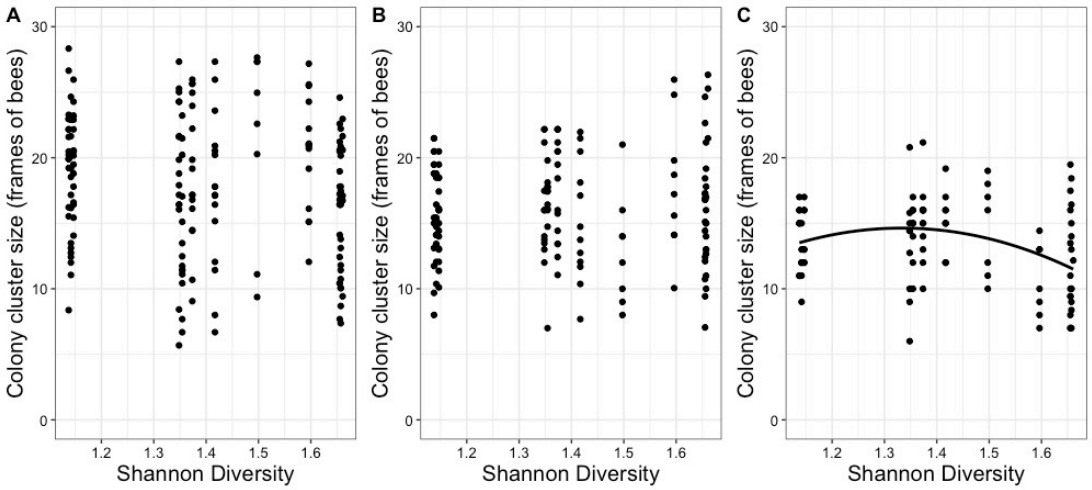

Figure 3.3. Colony cluster size in relation to land cover Shannon diversity of the surrounding landscape at each monthly assessment: July (A), August (B), and September (C). Best fit lines are shown when the correlation is significant.

Capped brood

Of the 24 colonies with pollen traps, 15 met the inclusion criteria and were assessed for area of capped brood in September. Capped brood area ranged from 0.66 to 6.69 full, deep frames, with a mean of 3.16 ± 1.68 frames. There was no difference in the area of capped brood between apiaries ($F_{10,14}$=4.04, p=0.10) and no correlation between land cover Shannon diversity and area of capped brood ($F_{1,9.09}$=2.00, p=0.19).

Effect of pollen weight on colony cluster size and capped brood

Area of brood was correlated with the weight of incoming pollen at two time points throughout the study in early August and early September (Table 3.3). For every additional 10 g of incoming pollen in early August, September capped brood area increased by about 13% of a frame, and the correlation was strong ($F_{1,3.02}$=11.22, R^2_c=0.90, p=0.04). The effect size of early September pollen on September brood was much smaller, requiring 500 additional grams of

pollen to see a similar effect as early August pollen on September brood area, but the correlation was still strong ($F_{1,3.35}$=47.94, R^2_c=0.98, p<0.01).

Table 3.3. Correlation between incoming pollen weight at each prior biweekly sampling round and honey bee colony August and September cluster size and colony September brood area. For each model, the effect size (β), which is the change in frames of bees or brood for each additional gram of pollen, degrees of freedom (df), the F-value and p-value are provided. P-values with an asterisk indicate significance at $\propto$<0.05.

Colony health	Pollen weight	β	df	F-value	p-value
August cluster size	Early August pollen weight	0.01	1, 17	0.08	0.78
	Late August pollen weight	0.01	1, 20	0.72	0.41
September cluster size	Early August pollen weight	-0.01	1, 8.09	0.08	0.79
	Late August pollen weight	0.03	1, 13	4.53	0.05
	Early September pollen weight	0.01	1, 11.27	2.17	0.17
	Late September pollen weight	-0.04	1, 9	1.49	0.25
September brood area	Early August pollen weight	0.44	1, 3.02	11.22	0.04 *
	Late August pollen weight	0.04	1, 8.47	0.03	0.86
	Early September pollen weight	0.01	1, 3.35	47.94	<0.01 *
	Late September pollen weight	0.37	1, 3.13	1.03	0.38

Path analysis

Path analysis of step-wise correlations among the interacting components revealed a network of links between September colony cluster size (df=11, p=0.36) and brood area (df=11, p=0.60) with land cover Shannon diversity and foraged pollen weight. Of the multiple comparisons for September cluster size, only late August pollen weight was significantly predicted by land cover Shannon diversity (β=-0.48, z=-2.35, R^2=0.22, p=0.02). While not significant, September cluster size (R^2=0.07) showed a negatively relationship with land cover Shannon diversity (β=-0.43, z=-0.93, p=0.35), a positive relationship with late August pollen weight (β=0.28, z=1.02, p=0.31), and a negatively relationship with early September pollen weight (β=-0.22, z=0.44, p=0.66). I also found that early September pollen weight had a non-significant negative relationship with Shannon diversity (β=-0.46, z=-1.48, R^2=0.15, p=0.14).

In the path analysis model for September brood area, September brood area was significantly, positively correlated with early August pollen weight (β=0.44, z=2.54, p=0.01) and non-significant related to Shannon diversity (β=0.21, z=0.76, p=0.45), late August pollen weight (β=-0.30, z=-0.83, p=0.41) and early September pollen weight (β=0.23, z=0.82, p=0.41) (R^2=0.39). Early August pollen weight was non-significantly positively related with Shannon diversity (β=0.22, z=1.26, R^2=0.04, p=0.21). Late August pollen weight was non-significantly negatively related to land cover Shannon diversity (β=-0.40, z=-1.79, R^2=0.16, p=0.07), as was early September pollen weight (β=-0.31, z=-0.80, R^2=0.08, p=0.42).

Discussion

The relationship between land cover Shannon diversity and incoming pollen and colony size of honey bees was highly variable. Time series analyses on the effects of land cover

Shannon diversity on pollen weight and cluster size indicated that the relationships changed over time. Previous studies in agroecosystems have likewise found that the effect of landscape metrics on bees can change over time during a season (Hemberger and Gratton 2018; Dolezal et al. 2019). This highlights the advantages of monitoring of colonies throughout the season, to detect changes over time. Colony monitoring tools, such as pollen traps can be useful implements for such data collection (Smart et al. 2017a).

Within each sampling period, when there were relationships between resource collection and landscape diversity, the direction and magnitude of the effects varied. For example, pollen weight was negatively correlated with land cover Shannon diversity in late August, but this relationship was positive in late September. However, the significance of both these relationships was marginal, with weak correlation. This finding contradicted my expectations that land cover Shannon diversity would be positively correlated with incoming pollen weight throughout the summer. It is possible that this reflects a confounding effect of the identity of land covers that make up the Shannon diversity index. Because different land covers can provide pulses of resources at a landscape level based upon their bloom phenology (Danner et al. 2016; Dolezal et al. 2019), one may expect to see this type of variation in incoming pollen, associated with an important pollen forage land cover. In this study, land cover Shannon diversity was positively correlated with the area of non-staple cropland and negatively correlated with the area of forests. The natural history of these land covers does not suggest they are driving the patterns in this study. Forests have abundant spring blooming flowers, but closed canopies later in the summer and fall, making them unlikely forage resources for honey bees (Taki et al. 2013; McCabe et al. 2019). Non-staple crops in this region are also comprised primarily of spring blooming fruit and vegetable crops (US Department of Agriculture National Agricultural Statistics Service 2018). It

is possible that weeds are growing in the margins of the non-staple cropland (Requier et al. 2015; Sponsler and Johnson 2015), however this would not explain the variable nature of the observed relationship. Additionally, neither non-staple cropland or forests are shown as significant land cover predictors of colony health in this state (Chapter 2). It is also possible that these land covers interact with land cover Shannon diversity to create these variable relationships, as landscape effects can interact to affect pollinators across spatial scales (Kennedy et al. 2013; Moreira et al. 2015). While this study was designed to determine whether land cover Shannon diversity can predict suitability for on honey bees, it does not seem to be an effective landscape metric in this region. Future studies integrating land cover Shannon diversity with individual land cover forage quality may improve prediction of landscape effects on honey bee colonies.

Site limitations, due to the landscape composition of southwest Michigan and beekeeper property permissions, introduced the potentially confounding effect of land cover Shannon diversity being correlated with forests and non-staple crops. Likewise, most of the high Shannon diversity sites were in the south. While this spatial autocorrelation could have also confounded incoming pollen weight, through differences in weather or other abiotic effects, including latitude as a predictor in exploratory data analysis did not improve any of the models. Still, the inferences that can be drawn from this study are limited by constraints of site limitations as well as limitations associated with working with a single collaborating beekeeper. I chose to work with a single collaborating beekeeper to introduce less variation in management and colony history. However, this decision also limited site accessibility and limits broader inference into how land cover Shannon diversity affects honey bee colonies, generally. Future studies on land cover Shannon diversity in different regions, with various different beekeepers could broaden the scope of inference.

Late September pollen weight was positively correlated with land cover Shannon diversity in accordance with my expectations, though the effect was marginally non-significant. Late September was also the sample period with the least incoming pollen. It is therefore possible that land cover Shannon diversity reflects resource heterogeneity at the landscape scale (Kovács-Hostyánszki et al. 2017) and has a more pronounced effect when pollen forage is limited. Michigan experiences seasonal depletion in floral abundance and richness in seminatural fields throughout the summer (Chapter 5), and the marginal significance of these results suggests the need for sampling apiaries with a wider range in land cover Shannon diversity. Further adaptation of this metric in honey bee landscape nutrition studies could further clarify the relationship and elucidate the utility of this metric.

September colony cluster size, which determines capacity to fulfill future pollination contracts (Nasr et al. 1990; Goodrich 2018), was largest in apiaries surrounded by intermediate levels of land cover Shannon diversity, however once again the significance was marginal and the correlation was weak, particularly given the higher order polynomial model. September colony cluster size and late August pollen weight were positively correlated, though marginally non-significant, suggesting that greater pollen weight collected in late August promotes larger colonies by the end of the foraging season for honey bees in this region. This is biologically supported, as the time between late August pollen sampling and September colony assessments (about one month) was about the time it takes for a generation of bees to develop (Winston 1991). The curvilinear relationship between land cover Shannon diversity and September cluster size is not a clear indirect effect of pollen quantity, as I hypothesized. It was predicted that intermediate land cover Shannon diversity maximizes September cluster size by moderating the negative correlation between late August incoming pollen weight and land cover Shannon

diversity. It was also expected that there would be a positive correlation between late September incoming pollen weight and land cover Shannon diversity, but this was not supported by either the direct correlations or the path analysis. Alternatively, intermediate land covers may provide colonies with forage of higher quality, not seen by assessing pollen quantity alone (Chapter 5). In this study, the sites with intermediate land cover Shannon diversity also had high proportions of urban land and staple crops. These land covers likely host abundant weedy species in marginal habitat, along roadsides and fields, that could be providing high quality forage resources (Requier et al. 2015; Sponsler and Johnson 2015) (Chapter 5). Colony cluster size also to decreased throughout the summer. This could have been an artifact of my collaborating beekeeper removing honey supers in August, which might have limited the space available to the colonies and prevented detection of more pronounced effect of colony growth. Future studies should provide colonies with space *ad libitum* as well as analyze colonies in the spring, when honey bees have increased populations growth rates, to improve the opportunity to observe a more pronounced effect of land cover Shannon diversity.

The area of capped brood was likewise not related to land cover Shannon diversity, either through a direct correlation or path analysis. Area of capped brood was, however, strongly correlated with incoming pollen weight. The amount of brood can be thought of as stored protein (Haydak 1935) and an intermediate step in producing adult bees. The more direct mechanistic relationship between pollen and capped brood than pollen and adult bees may explain the stronger correlation and significance of these relationships. The area of capped brood at the end of the season is also an important measure for the future health of a honey bee colony after the summer at an apiary site (Smart et al. 2016a). Area of capped brood may therefore be a viable

measure of colony response to the landscape that is less affected by confounding factors than colony cluster size.

Overall, these results suggest that land cover Shannon diversity is not an effective metric for characterizing landscapes for honey bee nutrition studies. The relationship between land cover Shannon diversity and foraged pollen was highly variable as was the relationship with September colony cluster size. These marginally significant results suggest further investigation into suitable landscape metrics, to elucidate how and when land cover composition affects honey bee colony growth. Such insights could improve landscape classification schemes for honey bee nutrition studies and recommendations to beekeepers for apiary locations.

CHAPTER 4: CONSERVATION RESERVE PROGRAM LAND HAS UNIQUE FLORAL COMPOSITION THAT PROMOTES HONEY BEE AND WILD BEE FORAGING

Abstract

Bee conservation has become a topic of global concern, particularly in agroecosystems where pollination is highly valued. Over a decade ago, bees and other pollinators were made a priority of the Conservation Reserve Program (CRP), a federal conservation program for private agricultural lands. Despite large financial investment, few studies have measured the benefit of CRP to bees. To determine if CRP land provides distinct floral resources and has more foraging bees than unmanaged habitats, I compared CRP fields to nearby paired unmanaged fields and roadside ditches in Michigan. CRP fields had higher floral abundance, species richness, and more native floral species compared to unmanaged fields or ditches. CRP fields also had a greater density of honey bees and wild bees than either unmanaged fields or roadside ditches, associated with greater inflorescence coverage. *Monarda fistulosa* was the most visited flower species on CRP fields by both honey bees and wild bees. In addition, honey bees were most concentrated on melliferous genera such as *Solidago, Asclepias,* and *Melilotus*, while wild bees were most concentrated on *Trifolium, Arctium,* and *Helianthus*. These findings demonstrate the benefit of managing herbaceous CRP land for pollinators, to provide resources to both wild bees and honey bees. Insights from this study could be used to enhance the composition of future conservation program investments to benefit pollinators in the Great Lakes region of the United States.

Introduction

Land sparing is a long-standing conservation solution to promoting ecosystem services (Phalan et al. 2011; Kremen and Merenlender 2018) and such set-aside programs can help provide habitat for taxa of concern while also supporting ecosystem services (Tscharntke et al. 2012; Grass et al. 2019). In the United States the federal government provides funding for various agricultural conservation programs on private land through the Farm Bill. Historically, the largest of these programs by both area and funding investment was the Conservation Reserve Program (CRP). This was established started in 1985 to address soil conservation and water quality concerns and consequently much of the habitat funded by this program was planted with native grasses that also benefitted grassland birds. Since its inception, CRP has spawned several offshoot programs with diverse conservation objectives (Gray and Teels 2006).

In 2008, pollinator conservation became an explicit objective of CRP (Johnson et al. 2008). Adding or enhancing pollinator habitat is also a goal of the national pollinator plan (Obama 2014). Habitats in agroecosystems are often of low quality for bees and other pollinators due the intensive land use for food and fiber production (Kremen et al. 2002; Goulson et al. 2015; Kovács-Hostyánszki et al. 2017). Limitation of floral resources is addressed in the CRP by adding herbaceous flowering plants to the seed mixes (Vaughan and Skinner 2008). CRP programs without an herbaceous element, such as grassy soil conservation programs, are not likely to benefit pollinators due to a lack of forage. Before 2010, there had been little evidence that pollinator-focused CRP programs were having their intended effect (Winfree 2010). Since then, a few studies have shown that wild bees forage upon CRP land (Otto et al. 2017) and honey bee colonies benefit from proximity to CRP land (Smart et al. 2016b; Ricigliano et al. 2019). Analogous agricultural pollinator conservation programs have been more extensively studied in

Europe and the UK through agri-environment schemes (AES). These have shown benefits to pollinator richness and abundance, likely driven by enhanced floral richness and abundance (Knop et al. 2006; Albrecht et al. 2007; Carvell et al. 2007). Many UK studies have likewise shown greater abundance and/or richness of bumble bees when fields are managed with flowering herbaceous plants in the seed mix (Carvell et al. 2004; Pywell et al. 2006; Potts et al. 2009). However, the benefit of conservation land to pollinators is not seen in all contexts and the magnitude of effect varies based on species, country, and conservation method (Kleijn et al. 2006). Therefore, additional studies are needed in areas where the benefits of CRP have not been explored.

Many reviews have highlighted the need for research to clarify the impact of conservation practices on pollinators (Kleijn et al. 2006, 2011; Winfree 2010). To date, there have been limited assessments of the effectiveness of pollinator-focused CRP habitat to either wild bees or honey bees in Michigan (Blaauw and Isaacs 2014), despite their importance to the state's economy (Huang and Pett 2010; Calderone 2012; Garibaldi et al. 2013). Conservation schemes can have large regional differences in effectiveness (Kleijn et al. 2006), making region-specific assessments necessary. Indeed, Michigan agroecosystems are uniquely diverse (Bertone 2017), with the second smallest average farm sizes of the Midwestern US states (NASS 2019a) and the lowest proportion of cropped agricultural land across the Midwest region (Callahan 2012). This makes it distinct from many of the corn-soy dominant landscapes found throughout the rest of the region where previous studies on conservation land and pollinators have been conducted (Smart et al. 2016b; Otto et al. 2017; Ricigliano et al. 2019). The agricultural regions of Michigan also have many floristically rich unmanaged areas that do not receive federal compensation but may provide valuable pollinator forage. For example, fallowed crop fields,

pastureland, and hay fields may all have abundant volunteer flowering species (Requier et al. 2015; Sponsler and Johnson 2015; Bretagnolle and Gaba 2015) similar to that of CRP land. Edge habitats such as the extensive network of roadside ditches could also support pollinator forage (Hopwood 2008; Smart et al. 2016b). Early successional volunteer flower species tend to be promoted in these ephemeral habitats through the mowing regimes (Hopwood 2008). Compared to CRP fields and analogous unmanaged fields, roadsides have a higher proportion of edge habitat, which can have unique effects on plant communities (Angold 1997; Otto et al. 2014) and may also be beneficial for connecting habitat so pollinators can exploit larger nectar and pollen-rich areas (Spellerberg 1998; Ries et al. 2001).

CRP represents a huge financial investment for the United States. The most recent Farm Bill authorized a 10-year budget of $22 billion for CRP (Stubbs 2018). The 2019 annual rental value of CRP enrollment in Michigan was over $15 million (US Department of Agriculture Farm Service Agency 2019). It is therefore important for the continuation of this program to determine whether pollinator habitat is having the desired effect. Michigan is a critical area for pollinator conservation, with recent evidence of declines in bumble bee species (Wood et al. 2019). Southwest Michigan in particular has been identified as an area with high pollination demand from spring and summer-blooming crops but with low wild pollinator habitat suitability (Koh et al. 2016). Likewise, Michigan agriculture relies on the beekeeping industry (Bianco et al. 2014) and is one of the most important states for honey bees, ranking fourth in the Midwest in honey production (NASS 2019b) and summer colony numbers (NASS 2019c). Even though CRP pollinator habitat is aimed at benefiting both wild bees and honey bees (US Department of Agriculture Farm Service Agency 2008, 2015; Vaughan and Skinner 2008), many studies comparing conservation land to unmanaged land have only focused on the impact on either wild

bees or honey bees (Pywell et al. 2006; Carvell et al. 2007; Smart et al. 2016b; Ricigliano et al. 2019). Honey bees and wild bees are known to respond to land use and floral composition in different ways (Steffan-Dewenter et al. 2002; Tuell et al. 2008; Rollin et al. 2013; Otto et al. 2017). Therefore, if the goal of CRP is to benefit both groups, it will be important to study their foraging behavior on CRP land simultaneously.

It is not only important to understand the flowering plants and pollinators that are on CRP land, but also the interactions between plants and pollinators. One approach is through bipartite networks (Memmott 1999) that offer a way to display and analyze these interactions. Previous studies have shown that habitat restoration can affect the structure of these networks, making them more similar to old natural sites (Forup and Memmott 2005; Forup et al. 2007). This approach can be used to determine whether CRP management alters honey bee and wild bee plant interactions, as compared to unmanaged fields by increasing the diversity of interactions. Studying plant-pollinator interactions on these different field types can also identify floristic characteristics important to foraging by either bee group, to inform future conservation land management.

This study aims to determine if floral communities are unique on CRP as compared to analogous unmanaged habitats and if bees forage more on CRP as a result. To my knowledge, this is the first comparative study to assess the floral plant communities, pollinator visitors and plant-pollinator interactions on CRP habitat as compared to unmanaged fields. The objectives of this study were to: 1) Compare floral composition in different habitats (CRP land, unmanaged fields, and ditches) throughout the summer, 2) Determine honey bee and wild bee plant selection in different habitats throughout the summer, and 3) Compare the network of bee-plant interactions in these different habitats. I expected CRP to have unique, consistent floral

composition, as compared to unmanaged fields and to have higher honey bee and wild bee visitation, with more sharing of resources between honey bees and wild bees.

Methods

This study was carried out during the summer of 2018 at 31 sites in Michigan (Figure 4.1). Candidate CRP fields were identified using USDA-Farm Service Agency land use records (Inter-agency Agreement 16IAMRECRPHBTA1). CRP fields were chosen that fit the following three criteria: they were at least 8 km apart to ensure independent bee communities (Greenleaf et al. 2007), they was enrolled in a CRP programs with an herbaceous element (CP-1, CP-2, CP4D, CP-10, CP-25, CP-38E, or CP-42) (US Department of Agriculture Farm Service Agency 2008, p. 42, 2015), and landowner permission was obtained. These focal CRP fields were then paired with a similarly sized unmanaged field and a roadside ditch within a 1 km buffer. Unmanaged fields were identified as pasture, barren, fallowed cropland, non-alfalfa hay, grass, switchgrass, or clover/wildflower using the 2016 Cropland Data Layer (US Department of Agriculture National Agricultural Statistics Service 2016b) and were not under a CRP management program of any kind.

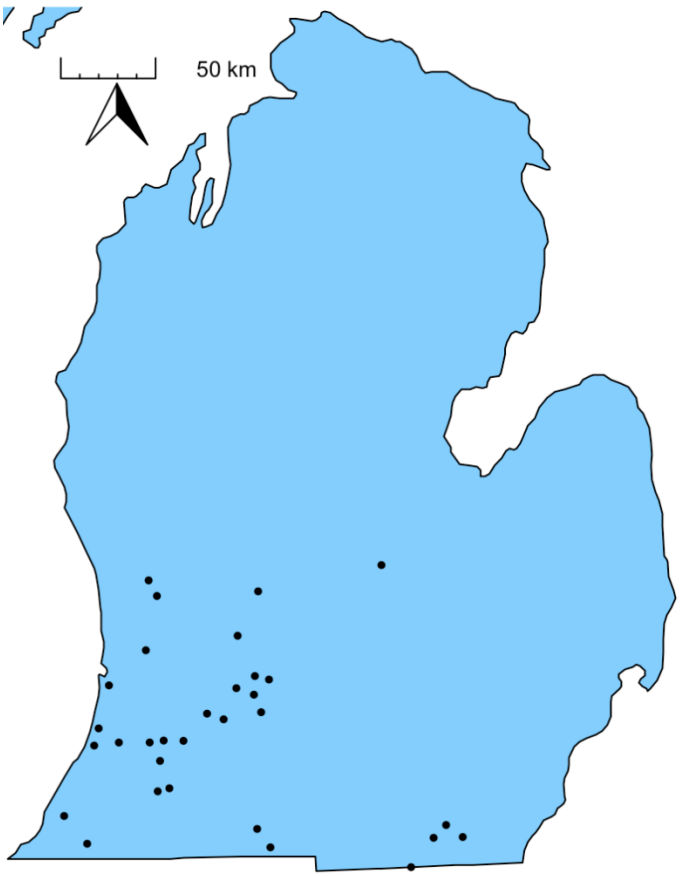

Figure 4.1. Locations of 31 sites across Michigan where CRP land, ditches, and grassy fields were sampled for plant communities and bees foraging.

In each of the three habitats, two, 20 m x 2 m transects were sampled once per month in July, August, and September to quantify flowering plants and bees at each site. The start location of each transect within the CRP and unmanaged fields was selected using a random number generator to select the direction and number of paces into the field. From this start location, transects ran north. Roadside transects started from the CRP-property owner's mailbox and extended north or east along both sides of the road.

Within each transect, I recorded inflorescence coverage, floral identity, and abundance. Inflorescence coverage was visually estimated based on the percent of the transect area covered by inflorescences. Floral abundance was measured as the number of stems with flowering heads.

The floral abundance of native plants was measured as the number of native species' stems with flowering heads per transect, with their native status based on the USDA plants database for Michigan (US Department of Agriculture Natural Resources Conservation Service 2019b). Floral richness was calculated as the number of unique species per transect.

I noted the presence of honey bees and net-collected wild bees visiting flowers for five minutes along each transect, noting the flower species with which the bee was interacting. Honey bees were identified on the wing while all other bees were net-collected, pinned, and retained in the Isaacs lab collection of the AJ Cook MSU Entomology Arthropod Research Collection. Wild bees that were missed during a net-collection attempt were noted and counted as a wild bee visit.

All data analyses were completed in R studio version 3.6.3 (R Core Team 2020). Floral and bee data for the two transect locations within each habitat were averaged at each site and log transformed after adding 1 to correct for heavy skew. To determine whether the habitats differed in inflorescence coverage, floral abundance, native floral abundance, and floral richness I used analysis of variance (ANOVA) (R Core Team 2020) with general mixed effects models (GLMM), using the nlme package (Pinheiro et al. 2020). These models were fit with a first order autoregressive correlation structure, with habitat and sampling month, as a numeric variable to account for temporal variation, as fixed effects and treatment nested within site as random intercepts. Means separation was conducted using Tukey's HSD (R Core Team 2020). To compare floral composition between habitats within months, data were by stratified and habitat was treated as a fixed effect and site as a random intercept. To determine independence of floral communities between the three habitat types, permutational multivariate analysis of variance (PERMANOVA) was completed using the adonis function (Legendre et al. 2018), with site and habitat as the independent variables. Dispersion for each habitat was then calculated using the

vegan package (Legendre et al. 2018). This was done on the overall, summer-long data, as well as by month. Floral species communities for the three habitats were visually compared using the vegan package to calculate a dissimilarity matrix (Legendre et al. 2018) and the stats package to do classical non-metric multidimensional scaling (CMDS), to reduce data to two dimensions (R Core Team 2020). To compare summer-long floral communities using CMDS, plant species counts were summed for the pair of transects within each site and habitat, across the three sampling months. To compare floral communities within month, species counts were summed within each site-month transect pair. Convex hulls were then used to visualize differences among the three habitat types in floral communities.

Honey bee and wild bee foraging was compared among habitats using ANOVA (R Core Team 2020) on GLMMs using the nlme package (Pinheiro et al. 2020). Differences in bee abundance between habitats were identified using a first order autoregressive errors with repeated measures design, by treating habitat and sampling month, a numeric time variable, as fixed effects and treatment nested within site as a random intercept. Means separation was assessed using Tukey's HSD from the stats package (R Core Team 2020). This was also done within each sampling month. Likewise, to determine differences among sampling month in bee abundance within CRP land, round was set as a fixed effect and site was set as a random intercept. The mean numbers of honey bees and wild bees per averaged transect pair were each regressed with transect variables in seven candidate models and Akaike's Information Criterion with a correction for small sample size (AICc) model selection from the bbmle package (Bolker and R Development Core Team 2017) was used to select most parsimonious model. For both honey bees and wild bees, the seven models included a null intercept model, habitat type, inflorescence coverage, floral richness, floral abundance, native flowering plant abundance, and

number bees of the opposite group (e.g., wild bees for the honey bee model), as a fixed effect for each model, site as a second fixed effect, and sampling month as random intercepts. Habitat was not included as a fixed effect in all candidate models, as in the aforementioned models to account for inherent habitat differences, because the goal of this analysis was to compare the effect of habitat, among floral characteristics as a factor for driving bee visitation. The random effect of site by habitat was also excluded, as it explained no additional variance. Model separation was considered sufficient at a ΔAICc value of 2.0 or greater (Burnham and Anderson 1998, 2007). The same model selection approach was done for each bee group within each habitat type.

To interpret honey bee and wild bee interactions with flowering species within each habitat, I summarized visitation data using bipartite networks with the bipartite package (R Core Team 2020). Within these networks, the number of honey bees, wild bees, and plant counts for each plant species were summed across sites and rounds within habitat type. Number of shared partners (shared floral species by honey bees and wild bees) was calculated as a network analysis metric using the bipartite package (R Core Team 2020), though these networks were primarily used to visualize the data, and no statistical comparisons were made between the three network metrics. Honey bee and wild bee visitation were correlated using a general linear model (R Core Team 2020). Preference was determined as the abundance of bees, corrected for floral species abundance (Alldredge and Ratti 1992), by dividing the number of bees visiting a flower species by the overall abundance of that flower species (Morandin and Kremen 2013). This was done for honey bees and wild bees separately, overall and within each habitat type.

Results

Floral composition

Transects were highly variable in floral composition. The mean transect inflorescence coverage was 9.4 ± 15.3% with a minimum of 0% and a maximum coverage of 90%. Floral abundance on each transect ranged from 0 to 2428 flowering stems with a mean of 102.9 ± 190.1 flowering stems. Native floral abundance ranged from 0 to 715 flowering stems, with a mean of 42.5 ± 95.7 flowering stems. Species richness ranged from 0 to 16 species with a mean of 4.4 ± 3.2 species. Separating this dataset by habitat type, I found that CRP fields had greater inflorescence coverage ($F_{2, 60}$=32.98, p<0.01), floral abundance ($F_{2, 60}$=21.83, p<0.01), native floral abundance ($F_{2, 60}$=80.85, p<0.01), and floral richness ($F_{2, 60}$=23.30, p<0.01) than the other two habitats across the entire season, as well as within each month (Figure 4.2). The unmanaged fields were similar to ditches in inflorescence coverage (95% CI=-0.07-0.70, p=0.13), floral abundance (95% CI=-0.09-0.84, p=0.14), and floral richness (95% CI=-0.13-0.21, p=0.84), but unmanaged fields had on average 99.7% more native flowering plants than ditches across the season (95% CI=0.21-1.17, p<0.01), seemingly driven by greater native floral abundance on unmanaged fields in September (z=3.29, p<0.01).

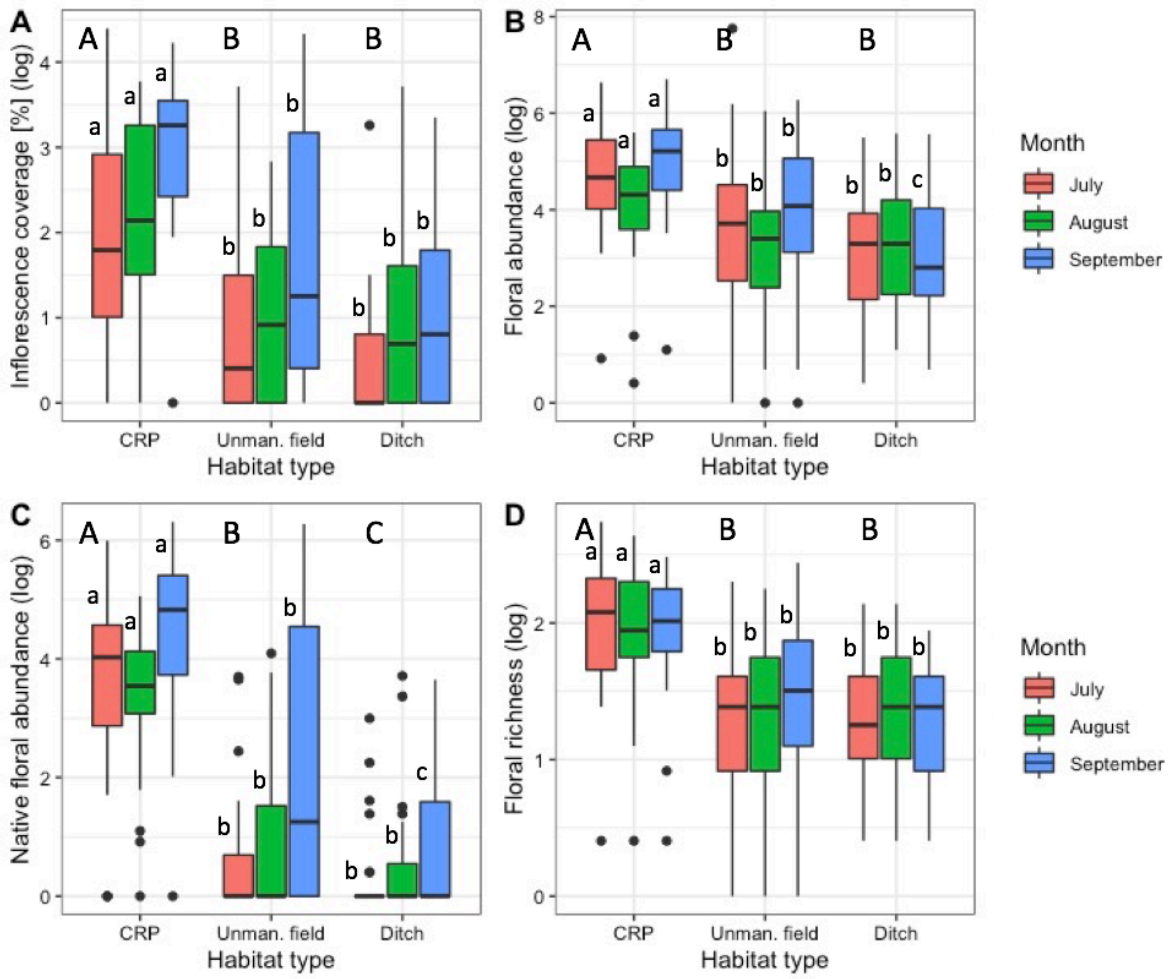

Figure 4.2. Percent inflorescence coverage (A), floral abundance (B), native floral abundance (C), floral richness (D), per averaged transect pair, each on the log scale compared among three habitat types. Data were collected from Conservation Reserve Program, unmanaged habitat, or ditches in Michigan during 2018. Significant differences between habitats overall at $\propto<0.05$ are indicated with different capital letters, and significant differences between habitats within each month at $\propto<0.05$ are indicated with different lowercase letters.

Using PERMANOVA, I found that floral communities were distinct between habitats ($F_{2, 91}=7.40$, $R^2=0.14$, $p=0.01$). Floral species community on CRP properties had distinct centroids from that of roadside ditches ($F=12.26$, $R^2=0.17$, $p<0.01$), as well as unmanaged fields ($F=2.85$, $R^2=0.05$, $p<0.01$), even though the floral communities in the CRP land shared multidimensional space with unmanaged fields in the CMDS (Figure 4.3A). Additionally, the dispersion was

similar for CRP and unmanaged fields (95% CI=-269.02-302.67, p=0.99), but ditch habitat had tighter clusters than both CRP (95% CI=-568.49- -1.50, p=0.05) and unmanaged fields (95% CI=15.97-587.66, p=0.04), suggesting lower variance in the floral community composition on CRP fields. Within each month, the CRP floral community centroids were distinct from both unmanaged fields (July= $F=1.84$, $R^2=0.03$, $p<0.01$, August= $F=2.60$, $R^2=0.04$, $p<0.01$, September= $F=4.70$, $R^2=0.07$, $p<0.01$) and ditches (July= $F=5.88$, $R^2=0.09$, $p<0.01$, August= $F=5.82$, $R^2=0.09$, $p<0.01$, September= $F=16.16$, $R^2=0.21$, $p<0.01$). Dispersion among the three habitats was not significantly different in July ($F_{2, 90}=1.70$, $p=0.19$) or August ($F_{2, 91}=0.86$, $p=0.43$). However, in September the plant communities in ditches had less dispersion than either CRP land or unmanaged land ($F_{2, 91}=9.75$, $p<0.01$), with CRP land and unmanaged land having similar dispersion (95% CI=-223.97-26.68, p=0.15) (Figure 4.3D).

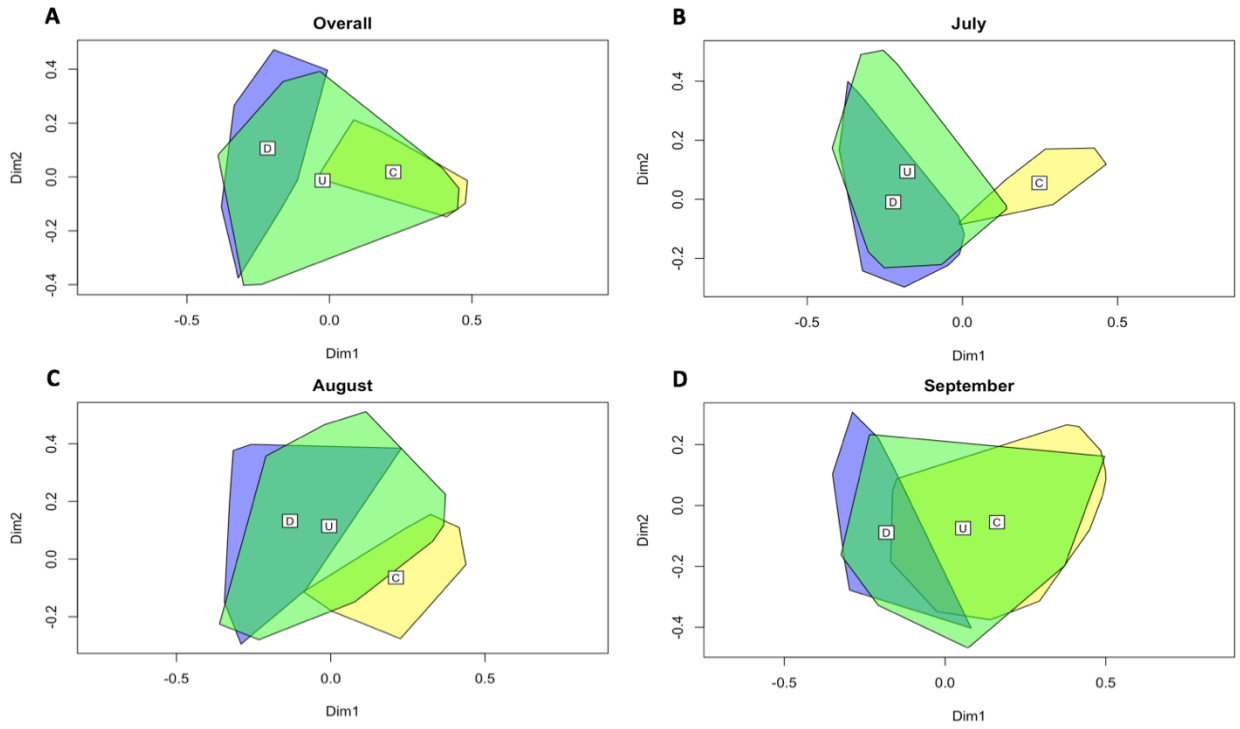

Figure 4.3. Floral species communities of three habitats in Michigan (CRP = C/yellow, Unmanaged field = U/green, and ditches = D/blue) across the whole summer (A) and by month (July=B, August=C, September=D). Floral species abundance was used to orient the unique site-habitat communities using Principal Coordinates Analysis. For the overall community plot (A), species counts were summed across two transects per site-habitat and across the three sampling months. For the monthly plots (B-D) species counts were summed across the two transects within each site-habitat.

Bee foraging

Within each transect, bee abundance ranged from 0 to 25 honey bees, with an average of 0.7 ± 2.6 bees. Wild bees ranged from 0 to 22 bees per transect with an average of 0.6 ± 1.9 per transect. Honey bees ($F_{2, 60}$=9.81, p<0.01) and wild bees ($F_{2, 60}$=5.31, p=0.01) were both more abundant in CRP habitats than either unmanaged fields or ditches (Figure 4.4). CRP land had 20.2% more honey bees (95% CI=-0.37-0.00, p=0.06) than unmanaged fields and 39.9% more honey bees (95% CI=-0.52- -0.15, p<0.01) than ditches. There were 22.1% more wild bees (95% CI=-0.36- -0.04, p=0.01) on CRP land than unmanaged fields and 18.5% more wild bees on CRP

land than ditches (95% CI=-0.33- -0.01, p=0.04). There were no significant differences observed between unmanaged fields and ditches for honey bees (95% CI=-0.04-0.34, p=0.14) or wild bees (95% CI=-0.19-0.13, p=0.90). Within sampling months, honey bees were observed more often on CRP land than other habitats in August ($F_{2,60}$=3.37, p=0.04) and September ($F_{2,60}$=7.90, p<0.01), and wild bees were observed more on CRP land than other habitats in July ($F_{2,60}$=10.08, p<0.01) (Figure 4.4). On CRP land, honey bee abundance was higher in September than July ($F_{2,60}$=3.86, p=0.03) while wild bee abundance was higher in July than September ($F_{2,60}$=7.59, p<0.01).

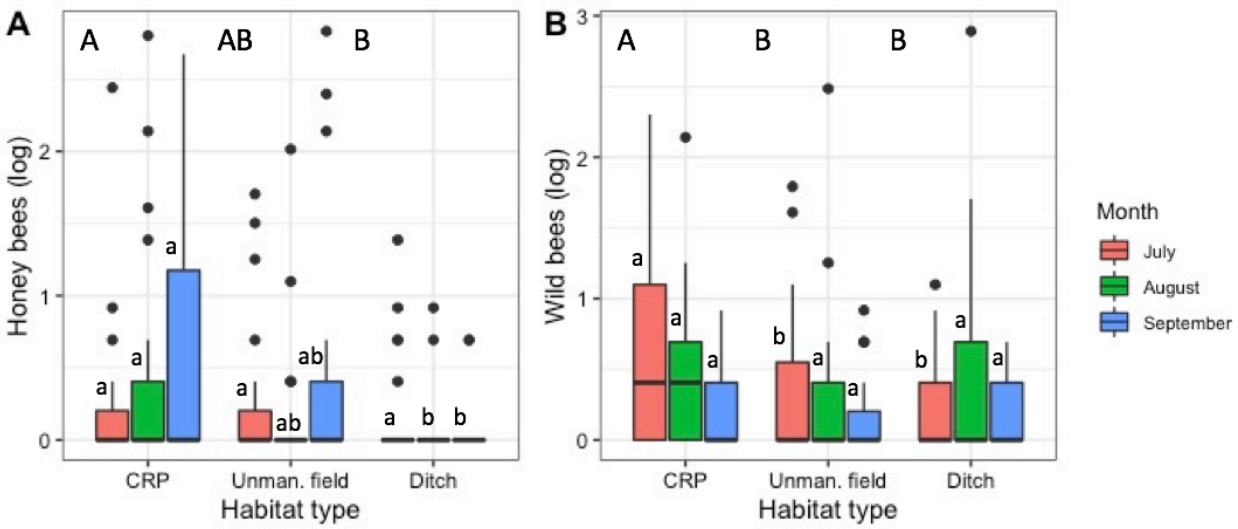

Figure 4.4. Honey bee (A) and wild bee (B) forager abundance per averaged transect pair, each on the log scale, compared among three habitat types in Michigan during 2018: Conservation Reserve Program, unmanaged habitat, and ditches. Significant differences between habitats overall at $\propto$<0.05 are indicated with different capital letters, and significant differences between habitats within each month at $\propto$<0.05 are indicated with different lowercase letters.

Inflorescence coverage was the best predictor of visitation by both honey bees and wild bees. For each percent increase in inflorescence coverage, honey bee visitation increased by 0.20% ($F_{1, 269.53}$=70.43, R^2_c=0.28). For wild bees, a percent increase in inflorescence coverage

increased their abundance by 0.13% ($F_{1, 272.02}$=41.01, R^2_c=0.19). (Figure 4.5). For honey bees, the model of percent inflorescence was differentiable from all six other proposed models and accounted for nearly all the likelihood weight (w=1, df=6, ΔAICc=17.3). For wild bees, percent inflorescence coverage also accounted for the majority (96%) of the likelihood weights (w=0.96, df=6, ΔAICc=6.6).

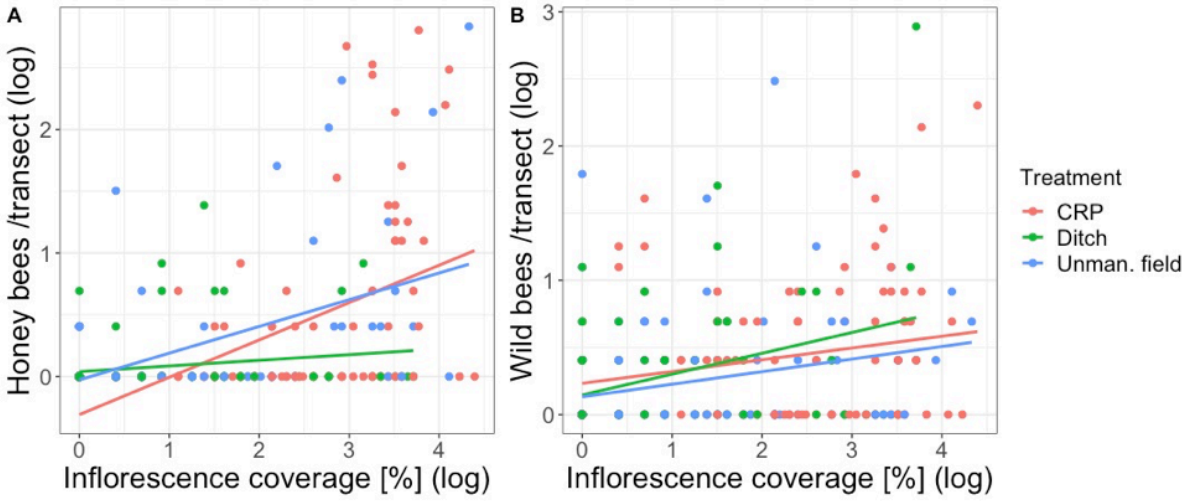

Figure 4.5. Relationship between percent inflorescence coverage and the number of honey bees and wild bees per averaged transect pair, in three habitats in Michigan: Conservation Reserve Program habitat, unmanaged habitat, and ditches both on log scales. The lines depict the best fit lines for the three habitat types.

Across most habitats, the inflorescence coverage was the best model for predicting both honey bee and wild bee visitation (Table 4.1). However, for honey bees on ditches, the best model was floral abundance (w=0.84, df=6, ΔAICc=4.3). Additionally, for wild bees on CRP land the inflorescence model was not differentiable from native plant abundance (w=0.47, df=6, ΔAICc=0.5). Inflorescence coverage was selected as the best model for honey bee visitation in CRP land (w=0.89, df=6, ΔAICc=5.2) and unmanaged fields (w=0.99, df=6, ΔAICc=11.1) and for wild bee visitation on CRP land (w=0.37, df=6, ΔAICc=2.8), unmanaged fields (w=0.65, df=6, ΔAICc=2.3), and ditches (w=0.66, df=6, ΔAICc=2.2). For each percent increase in

inflorescence coverage, honey bee visitation increased by 0.30% on CRP land, 0.22% on unmanaged fields, but only 0.06% on ditches (Figure 4.5A), and wild bee visitation increased by 0.17% on CRP land, 0.12% on unmanaged fields, and 0.14% on ditches (Figure 4.5B).

Table 4.1. Akaike information criterion model selection results for determining honey bee and wild bee visitation drivers on the overall data, and data stratified by habitat type. The varying fixed effect for each model, ΔAICc values, degrees of freedom (df), and model weights are provided for each candidate model.

Habitat	Model	ΔAICc	df	weight
Overall	Honey bees			
	Inflorescence coverage	0.0	6	1
	Floral abundance	17.3	6	<0.01
	Native floral abundance	24.5	6	<0.01
	Floral richness	45.0	6	<0.01
	Habitat type	46.2	7	<0.01
	Wild bee abundance	51.0	6	<0.01
	Null	60.1	5	<0.01
	Wild bees			
	Inflorescence coverage	0.0	6	0.96
	Floral abundance	6.6	6	0.04
	Native floral abundance	9.6	6	0.01
	Floral richness	16.6	6	<0.01
	Honey bee abundance	27.0	6	<0.01
	Habitat type	29.5	7	<0.01
	Null	35.8	5	<0.01
Conservation Reserve Program	Honey bees			
	Inflorescence coverage	0.0	6	0.89
	Floral abundance	5.2	6	0.07
	Native floral abundance	6.9	6	0.03
	Wild bee abundance	7.5	6	0.02
	Null	14.6	5	<0.01
	Floral richness	16.8	6	<0.01
	Wild bees			
	Native floral abundance	0.0	6	0.47
	Inflorescence coverage	0.5	6	0.37
	Honey bee abundance	2.8	6	0.12
	Floral abundance	4.6	6	0.05
	Floral richness	10.1	6	<0.01
	Null	10.1	5	<0.01

Table 4.1 (cont'd)

Habitat	Model	ΔAICc	df	weight
Unmanaged fields	Honey bees			
	Inflorescence coverage	0.0	6	0.99
	Native floral abundance	11.2	6	<0.01
	Floral abundance	11.3	6	<0.01
	Floral richness	17.2	6	<0.01
	Wild bee abundance	17.9	6	<0.01
	Null	20.5	5	<0.01
	Wild bees			
	Inflorescence coverage	0.0	6	0.65
	Floral abundance	2.3	6	0.21
	Honey bee abundance	4.8	6	0.06
	Native floral abundance	5.7	6	0.04
	Floral richness	6.8	6	0.02
	Null	7.0	5	0.02
Ditches	Honey bees			
	Floral abundance	0.0	6	0.84
	Floral richness	4.3	6	0.10
	Inflorescence coverage	5.9	6	0.04
	Null	9.3	5	0.01
	Native floral abundance	11.3	6	<0.01
	Wild bee abundance	11.3	6	<0.01
	Wild bees			
	Inflorescence coverage	0.0	6	0.66
	Floral abundance	2.2	6	0.22
	Native floral abundance	5.2	6	0.05
	Floral richness	5.2	6	0.05
	Null	8.0	5	0.01
	Honey bee abundance	10.0	6	<0.01

Honey bee and wild bee visitation to plots were positively correlated ($F_{1, 274.77}$=11.58, R^2_c=0.13, p<0.01), as were honey bee and wild bee abundance by plant species ($F_{1, 55}$=10.81, adj-R^2=0.15, p<0.01). Honey bee and wild bee interactions with flowering plants across the three habitats (Figure 4.6) revealed similarities and differences in foraging behavior between the two bee groups. The most commonly visited flower species was *Solidago altissima,* which was visited primarily by honey bees in September and was found most commonly on CRP land and in unmanaged fields. This was followed by *Monarda fistulosa,* which was visited approximately equally by both bee groups and found almost entirely on CRP land. These two dominant host plants were followed by *Euthamia graminifolia*, which was mainly visited by honey bees and only observed on CRP land and unmanaged fields, and *Cichorium intybus,* which was primarily visited by wild bees and mostly observed in ditches. Ditches contained the highest proportion of flowering plants that were not observed being visited by any bee (66.7%), followed by CRP land (57.9%), and then unmanaged fields (54.5%).

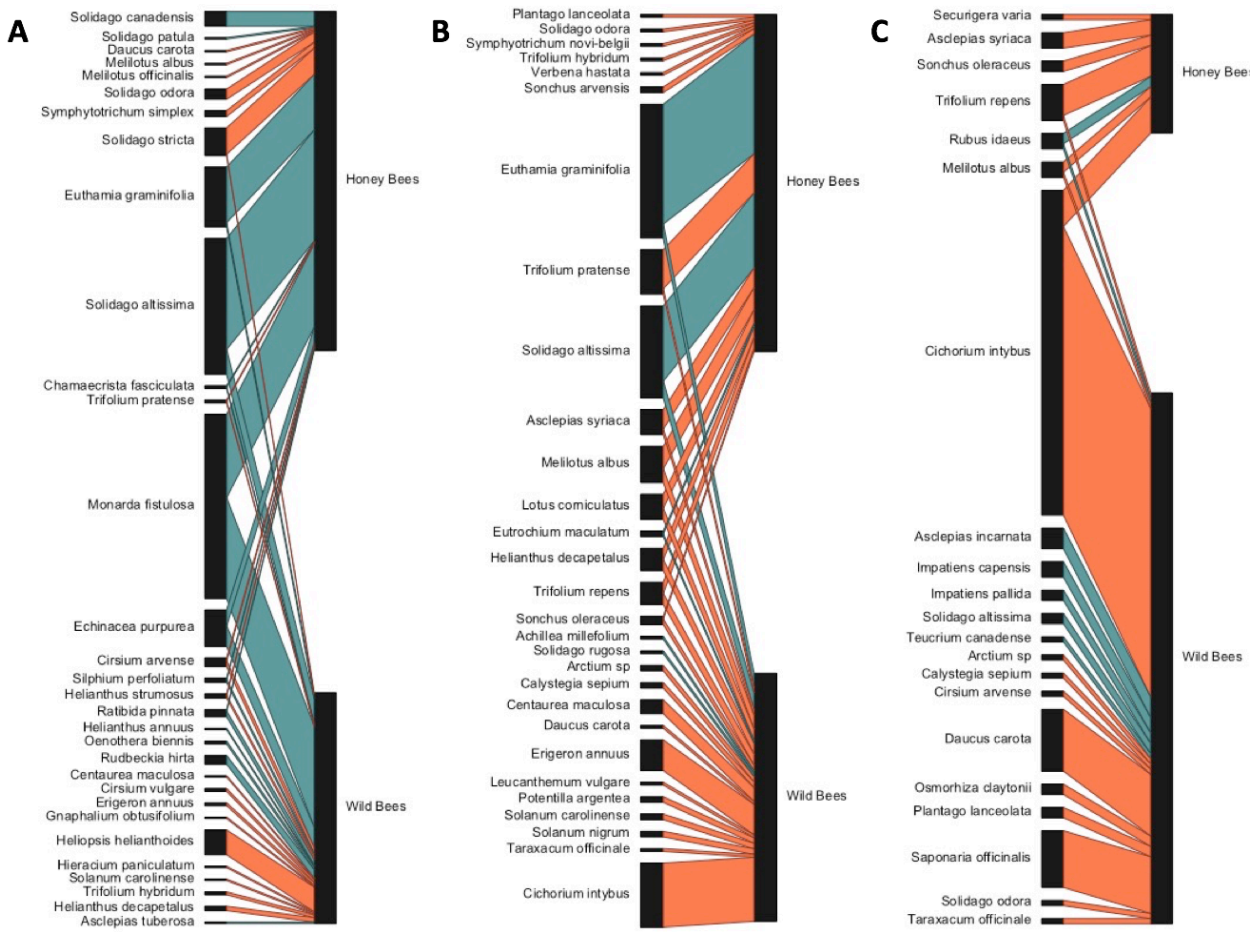

Figure 4.6. Interactions between flowering plants and two types of bees across three habitats sampled in Michigan: Conservation Reserve Program herbaceous habitat (A), unmanaged fields (B), and ditches (C). The width of the line connecting the bees with flowers indicates the number of collections of each type of bee on each floral species. Blue lines indicate an interaction with a native flowering species and orange lines indicate an interaction with a non-native species.

Honey bees had a slightly greater proportion of interactions with native flowering plants (71.5%) than wild bees (42%). Most bee interactions with native plants occurred on CRP land (316 interactions), which was much greater than unmanaged fields (85 interactions) or ditches (15 interactions). There were approximately equal numbers of interactions with native plants in July and August with 100 and 109 interactions, respectively, and 207 interactions with native plants in September. Honey bees and wild bees shared the most flower species on CRP land (11

species), followed by unmanaged fields (10 species), and the lowest number of shared flower species on ditches (4 species).

Honey bee and wild bee floral preferences were not correlated, overall ($F_{1, 55}$=0.44, adj-R^2=-0.01, p=0.51). I found that honey bees preferred flowering species that were observed at greater abundance than those preferred by wild bees (Table 4.2). The most visited flowering species by both species were not also the most preferred flowering species (Table 4.2). There were multiple non-native species that dominated floral preferences of both bee groups overall and in each habitat (Table 4.2). Most flowering plant species preferred by wild bees in ditches and by honey bees on CRP were native.

Table 4.2. Flower species preference of honey bees and wild bees across three habitats sampled in Michigan: Conservation Reserve Program herbaceous habitat, unmanaged fields, and ditches, with abundance of the bee visitor, total abundance of the flower species, and preference, calculated as bee abundance divided by flower abundance. Flower species native to Michigan are indicated with an asterisk, and flower species which were also identified as having the top five most bee visitors within the habitat/bee group are in bold.

Habitat	Bee type	Flower species	Bee visitors	Flower count	Preference
Overall	Honey bee	*Rubus idaeus**	2	10	0.200
		Asclepias syriaca	10	85	0.118
		Euthamia graminifolia*	81	954	0.085
		*Solidago patula**	1	12	0.083
		Melilotus albus	11	141	0.078
	Wild bee	*Solanum nigrum*	2	2	1.000
		*Asclepias incarnata**	4	6	0.667
		Arctium sp.	3	5	0.600
		Potentilla argentea	2	11	0.182
		Gnaphalium obtusifolium	1	6	0.167

Table 4.2 (cont'd)

Habitat	Bee type	Flower species	Bee visitors	Flower count	Preference
CRP land	Honey bee	*Solidago patula**	1	7	0.143
		Solidago stricta	18	231	0.078
		*Solidago canadensis**	10	144	0.069
		Euthamia graminifolia*	38	569	0.067
		Melilotus albus	1	28	0.036
	Wild bee	*Trifolium hybridum*	2	11	0.182
		Gnaphalium obtusifolium	1	6	0.167
		Helianthus decapetalus	3	21	0.143
		Cirsium vulgare	2	19	0.105
		*Helianthus annuus**	1	24	0.042
Unmanaged field	Honey bee	***Asclepias syriaca***	7	38	0.184
		Melilotus albus	8	71	0.113
		Euthamia graminifolia*	43	385	0.112
		Verbena hastata	1	12	0.083
		Helianthus decapetalus	4	104	0.038
	Wild bee	*Solanum nigrum*	2	1	2.000
		Arctium sp.	2	3	0.667
		Potentilla argentea	2	6	0.333
		Cichorium intybus	23	192	0.120
		Calystegia sepium	2	24	0.083
Ditch	Honey bee	***Asclepias syriaca***	3	15	0.200
		Rubus idaeus*	2	10	0.200
		Melilotus albus	2	42	0.048
		Sonchus oleraceus	2	65	0.031
		Trifolium repens	6	536	0.011
	Wild bee	***Asclepias incarnata****	4	6	0.667
		Arctium sp.	1	2	0.500
		*Impatiens pallida**	2	13	0.154
		*Teucrium canadense**	1	8	0.125
		*Rubus idaeus**	1	10	0.100

Discussion

My results provide strong evidence for the benefits of CRP land management for supporting bees through their unique floral composition, particularly greater inflorescence coverage. Conservation management of farmland has previously been shown to enhance plant and pollinator biodiversity (Kleijn et al. 2006), and various Farm Bill programs have been shown to be successful in providing benefits to a range of wildlife through habitat enhancements (Haufler et al. 2005). However, to my knowledge this is the first comparative assessment to identify multiple floral characteristics including inflorescence coverage, floral abundance, native floral abundance, floral richness, and floral community composition that are unique to herbaceous CRP land and show evidence of CRP fields as attractive forage for both honey bees and wild bees.

In addition to having greater floral abundance and richness, CRP fields were unique from the other two habitat types in their plant community composition. This is an important finding as it demonstrates that management on herbaceous CRP has a measurable impact on the composition of floral communities. The two field habitat types, CRP and unmanaged fields had similar and wider spread in multidimensional space than ditches, overall and in September. This contradicts my expectation that CRP land would have lower species composition variation due to prescribed conservation practice implementation and management including a recommended seed mix. These two field habitats comprised several different and potentially highly variable land covers (e.g. pasture, fallow cropland) and conservation programs, though each had an herbaceous element. This could explain some of the variation in fields. Differences between fields and ditches may also be due to the high proportion of edge habitat in ditches. Edge effects are known to cause unique habitat conditions that can influence their suitability for bees (Angold

1997; Otto et al. 2014). The overall differences in dispersion between habitat types seems to be primarily driven by ditches having a narrower spread than either CRP land or unmanaged fields in September. This may be because early fall species, such as *Solidago* spp. were more likely to grow in fields, potentially introducing additional floral community variation between sites.

Both honey bees and wild bees were observed foraging more on CRP fields than the other unmanaged habitats, overall. This suggests that the financial investment of the CRP program is having the intended benefit to create rewarding habitat for these pollinators. Other studies have demonstrated the benefit of herbaceous agricultural enhancements for bees (Carvell et al. 2004; Pywell et al. 2006; Potts et al. 2009), and within these habitats the floral abundance (Albrecht et al. 2007; Hopwood 2008), inflorescence coverage (Delmas et al. 2014), and floral richness (Knop et al. 2006; Albrecht et al. 2007) have each been shown to support bees. In this study, the attractiveness of CRP land can be attributed to its greater inflorescence coverage, which was the strongest predictor of honey bee and wild bee abundance. That the two bee groups were positively affected by the same floral metric and were positively correlated with one another in plot and floral species visitation, suggests that similar management strategies would benefit them both. Similar findings have been reported in other parts of the Midwest (Evans et al. 2018). This provides additional support for adding floral resources to the environment to support managed and wild bees, alike. This study was not designed to assess the effects of competition between honey bees and wild bees. However, due to concern surrounding this subject (Steffan-Dewenter and Tscharntke 2000; Goulson and Sparrow 2009; Mallinger et al. 2017) future studies should assess competition on conservation land as compared to unmanaged land, if the objective of the conservation land is to protect native wildlife.

Both of the most commonly foraged upon species, *S. altissima* and *M. fistulosa*, were found in greatest abundance on CRP land. *Monarda fistulosa* was foraged upon similarly by wild bees and honey bees. Other recent research has highlighted the attractiveness of *M. fistulosa* to foraging bees (Rowe et al. 2018), recommending its inclusion in seed mixes for pollinator habitat in the Great Lakes region. There were more floral species visited by both honey bees and wild bees on CRP land and grassy-herbaceous fields. Greater sharing of resources in CRP land and unmanaged fields may be due to their similar floral community composition, which includes species attractive to both species. Due to concerns about pathogen transmission on shared flowers between honey bees and wild bees (Graystock et al. 2015), more flower sharing may be detrimental. Much work is still needed to determine the potential risk of honey bee- wild bee interactions on pollinator plantings and how to mitigate those risks. There was no correlation in floral preference between bee groups, however. This suggests that plantings could be designed to align with the floral preferences of either honey bees or wild bees and to target conservation for a specific group of insects.

Honey bees were observed foraging most often on *Solidago* species and were shown to prefer these plants. These are late season, mass blooming flowers and their dominance during late summer likely accounts for the significantly higher floral abundance and inflorescence coverage. Honey bees often recruit nest mates to high quality forage sources (von Frisch 1967; Beekman and Lew 2008; Couvillon et al. 2014). In particular, mass-blooming flowers have been shown to be attractive to honey bee foragers (Beekman and Ratnieks 2000; Danner et al. 2016). The much lower abundance of *Solidago* and the smaller areas of ditches may explain the low observations of honey bees. In Michigan, *Solidago* provides an important nectar resource late in the season while forager populations are high (Pellett 1920; Oeetel 1980). In addition to

Solidago, honey bees showed preference for a number of melliferous plants including *Asclepias, Melilotus,* and *Rubus* that have been listed previously as good nectar sources (Pellett 1920).

The most commonly foraged upon plants by wild bees, *M. fistulosa* and *C. intybus,* were also common in this study and grew in high abundance aggregations. *Cichorium intybus* is a good source of pollen and nectar (Oeetel 1980), but unlike the other commonly foraged upon species, it was primarily found in ditches. *Cichorium intybus* bloomed throughout the summer, making it not only an attractive plant but also a reliable resource for wild bees. This supports previous assertions that weeds can be valuable forage resources for bees (Requier et al. 2015; Bretagnolle and Gaba 2015). Wild bees showed a preference for a range of flowering plants such as *Trifolium, Arctium,* and *Helianthus,* which showed no clear patterns in life history, likely due to the broad life histories of wild bees as a group. Based on the abundance of floral resources throughout the season, it is likely that the lower wild bee abundance in September was due to the bees' life cycles (Linsley 1958; Williams et al. 2014), rather than the lack available forage.

Honey bees interacted with native plants at slightly greater rates than wild bees. This finding is not unprecedented; honey bees can show preference for native species in certain contexts (Morandin and Kremen 2013), and wild species have been shown to utilize non-native species (Tepedino et al. 2008; Harmon-Threatt and Kremen 2015). However, honey bees, as an introduced species are often associated with non-native plant species (Hanley and Goulson 2003; Requier et al. 2015; Bretagnolle and Gaba 2015). It should be noted that while native and nonnative flowering species seem equally attractive, especially to honey bees, native plants provide additional ecosystem functions that may support the long-term persistence of a pollinator planting, in addition to other ecosystem services such as biological pest control and pollination of crop plants (Charles and Dukes 2007; Isaacs et al. 2009).

Foraging by these pollinators was positively correlated with inflorescence coverage, highlighting the importance of developing conservation plantings with high forb seed density and effective establishment methods. Mass blooming flower species such as *Solidago* and *M. fistulosa* supported a large number of bees of both types in these CRP plantings, so I recommend these species be included in seed mixes. While I did not include the effect of surrounding landscape matrix in this study, it is likely that landscape context is plays an important role in the bees' local populations and their utilization of these different habitats (Tscharntke et al. 2005). Future research should assess bee foraging on CRP land across a land use gradient, to determine how local site quality and the larger landscape quality interact. In this study, I found compelling evidence that both honey bees and wild bees utilize CRP-funded plantings across Michigan, driven by floral enhancements that can be linked to the specific management decisions of the landowners. These findings provide insight into effective management for pollinators within farm landscapes and establish a foundation for future research on CRP's impact on pollinator health and pollination services.

CHAPTER 5: SEASONAL PROTEIN DEPLETION IN HONEY BEE COLLECTED POLLEN IS CONCURRENT WITH DECLINING FORAGE QUALITY IN GRASSY-HERBACEOUS HABITATS

Abstract

Nutrition limitation driven by low quality forage habitat is often cited as a major stressor for honey bees (*Apis mellifera* L.). To determine how the composition of non-cropped habitat in landscapes surrounding honey bee colonies affects the nutritional value of bee-collected pollen, I collected pollen from colonies in Michigan every two weeks over the summer, while concurrently assessing forage in the surrounding grassy-herbaceous habitat. Collected pollen was analyzed for crude protein content, and pollen sources were identified using DNA metabarcoding. There was a gradual decrease in crude protein of collected pollen over the summer, concurrent with decreasing floral richness, abundance, and abundance of the most dominant floral species in grassy-herbaceous habitats within the surrounding landscape. Bloom phenology of common, high protein flowering plant species in early summer such as *Sinapis alba* and *Plantago* sp., followed by abundant, low protein flowering plant species in late summer such as *Solidago* sp. and *Ambrosia* sp. on grassy-herbaceous habitat is likely underlying the decrease in protein of foraged pollen in this region. These results show that non-cropped land supports honey bee pollen foraging and highlights the importance of further investments in forage plantings, particularly of late season, high protein forage to support honey bees and the beekeeping industry.

Introduction

Global pollinator declines pose a risk to food security and human nutrition (van der Sluijs and Vaage 2016; Food and Agriculture Organization 2019; Aizen et al. 2019). Honey bees are the most economically important managed pollinator (Klein et al. 2007; Calderone 2012), and the only major livestock animal that cannot be entirely sustained by commercial feed, therefore requiring access to forage for a complete diet. Collecting sufficient nutrients from the surrounding forage landscape is key to producing healthy, robust colonies (Haydak 1970; Brodschneider and Crailsheim 2010; DeGrandi-Hoffman et al. 2016). Colony condition, in turn, determines honey production (Harbo 1986) and pollination service capacity (Nasr et al. 1990; Goodrich 2018). Recent decades have seen greater demand for honey bee pollination globally (Aizen and Harder 2009), while crop acreage has increased in key apicultural regions, to the detriment of potential bee forage (Otto et al. 2016). Honey bee feeding yards with abundant, nutritious natural forage promote colony health and growth (Haydak 1970; Brodschneider and Crailsheim 2010; DeGrandi-Hoffman et al. 2016). However, there is a lack of agreement on which types of land uses provide this forage. Some studies have demonstrated a positive correlation between proportion of semi-natural uncultivated forage land and the summer-long performance of colonies (Naug 2009; Smart et al. 2016b). However, in other studies uncultivated land is not found to benefit honey bees (Sponsler and Johnson 2015) (Chapter 2).

One explanation for this inconsistency is the effect of uncultivated land on colony health may be too indirect to detect, and these effects may be further confounded by other factors such as beekeeper management (Chapter 2). Therefore, directly assessing how uncultivated land affects pollen nutrition, the presumed intermediate step to colony growth, is important for understanding what resources these habitats provide for bees. Previous studies have used pollen

traps for colony monitoring and detected differences in forage composition and/or nutritional quality over time (Sponsler et al. 2019) and in different landscapes (Smart et al. 2017a). Honey bee pollen nutrition is important for immune function, demographic structure, and colony size (DeGrandi-Hoffman et al. 2008; Alaux et al. 2010; Khoury et al. 2013; Di Pasquale et al. 2013). In particular, pollen protein helps determine colony size by contributing to larval growth and development (Haydak 1935; Brodschneider and Crailsheim 2010; Khoury et al. 2013). Floral richness can also be a measure of sufficient nutrition (Di Pasquale et al. 2013); polyfloral forage (forage from multiple different flowering species) supports not only high protein diets but also promotes the health and survival of individual bees (Alaux et al. 2010; Di Pasquale et al. 2013). Pollen quantity is also important for colony health because pollen consumption has been correlated with survival, brood production, and individual bee physiological condition (Schmidt et al. 1987; Crailsheim and Hrassnigg 1998; DeGrandi-Hoffman et al. 2008).

Another explanation for inconsistency among published studies in the effect of uncultivated land on honey bee colony health is the heterogeneity in forage quality on uncultivated land, both among fields and over time. Therefore, conducting floral surveys to understand the variability and composition of floral resources within uncultivated land could also inform the underlying mechanism of their value to bees (Williams et al. 2012; Smart et al. 2016b). Floral composition likely varies based on landscape context, land-use history, management history, and biotic and abiotic factors (Perring et al. 2016; Zirbel et al. 2017, 2019). Additionally, in agroecosystems, forage resource quality and quantity may go through drastic pulses based on bloom phenology (Williams et al. 2012; Danner et al. 2016; Hemberger and Gratton 2018; Dolezal et al. 2019). Understanding the changes in field-scale habitat quality over time through floral composition surveys, coupled with assessments of pollen protein, taxonomic

richness, and quantity could elucidate specific areas and time periods of pollen nutritional shortcomings within the region. Such insights could direct management decisions to address these concerns. Furthermore, insufficient nutrition is also one of the primary stressors impacting unmanaged bees (Potts et al. 2010; Goulson et al. 2015), so methods for determining fine scale variation in landscape level nutrition would likely also benefit wild bees.

In this study, I examined the quantity and quality of honey bee-collected pollen across apiary sites in Michigan through the summer. Michigan is an agriculturally- and apiculturally-important state (US Department of Agriculture National Agricultural Statistics Service 2019a, c) that differs from other states in the Midwest where similar studies have been conducted due to its highly diverse agriculture (Bertone 2017), small farm size (US Department of Agriculture National Agricultural Statistics Service 2019b), and unique biotic and abiotic conditions. I tested whether floral composition in grassy-herbaceous habitat within the foraging range of the apiaries was related to the quality and quantity of collected pollen. Specifically, I investigated the following objectives: 1) Determine temporal variation in incoming pollen quantity and quality (pollen crude protein content, pollen taxonomic richness, and pollen weight), 2) Determine whether pollen quantity and quality are correlated with surrounding landscape floral composition (richness, total floral abundance, abundance of the most dominant species), and 3) Compare the most common floral taxa collected by honey bees to the most common flowering species growing in grassy-herbaceous habitat within the surrounding forage area, to determine if honey bee foraging is correlated with floral availability in grassy-herbaceous habitat. I predict that pollen quantity and quality would vary over the summer, related to the floral characteristics of grassy-herbaceous forage habitats surrounding apiaries. I also anticipated that this would be

supported by a positive correlation between the relative rank of pollen taxa and the taxa blooming in grassy herbaceous habitats.

Methods

Pollen collection

In the summers of 2015-2017 I trapped incoming pollen from commercial honey bee colonies in multiple locations throughout Michigan (Figure 5.1). In 2015, pollen was collected from two colonies in each of four apiaries (8 colonies; sites A, C, F, and G), in 2016 from ten apiaries (20 colonies; sites A-J), and in 2017 from 12 apiaries (24 colonies; sites A-L). Apiary locations were consistent in amount of grassy-herbaceous habitat (ex. fallowed fields, pastureland, wildflower, conservation land, roadside ditches) (2.42 km^2 - 7.20 km^2) within a 4-km radius around each apiary (Table D1). Research apiaries were as spatially separated, with 4-km buffers overlapping minimally in two cases: between apiaries J and I, and B and K (Figure 5.1).

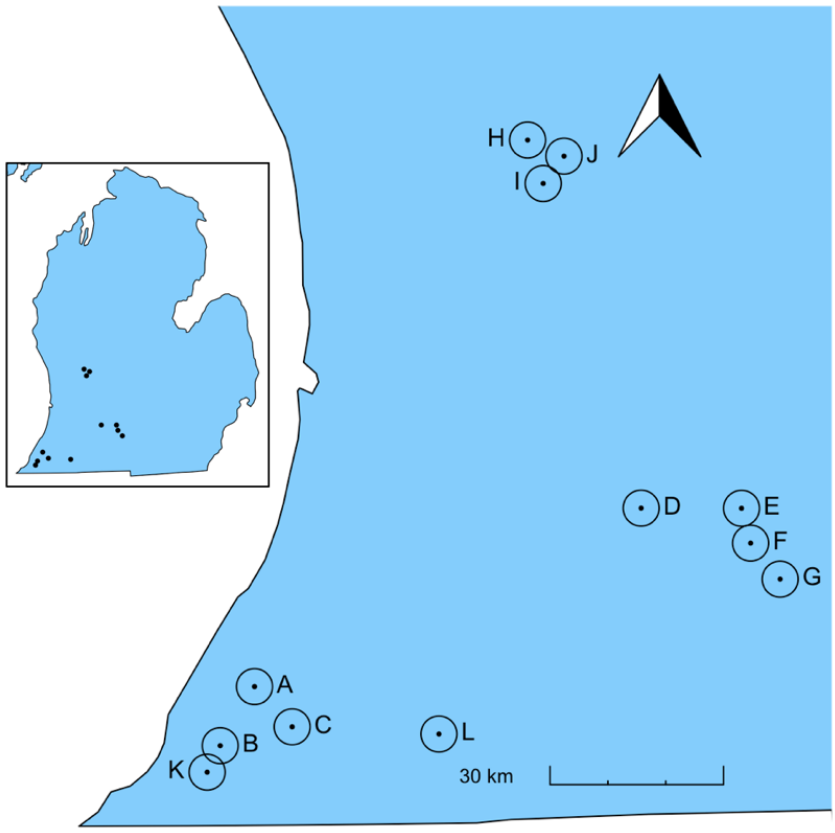

Figure 5.1. Map of the 12 study sites in Michigan, with the dots indicating the locations of the apiaries and the surrounding circle representing the 4-kilometer buffer around each apiary. In 2015, sites A, C, F, and G were studied, in 2016 sites A-J were studied, and in 2017 sites A-L were studied.

Every 2 weeks, from early July to early September, pollen traps were activated (Superior Pollen Traps, Mann Lake, Hackensack, MN) on the colonies for a 72-hour period. In 2015, pollen was collected five times, in 2016 pollen was collected four times (no early July collection), and in 2017 pollen was collected four times (no late August collection). Thirty-six samples were not obtained due to insufficient quantity of collected pollen due to colony death or foragers bypassing the traps (180 collected samples total). In 2015, 29 pollen samples were assessed, in 2017, 65 samples were assessed, and in 2017, 86 samples were assessed. The fresh weight of each pollen sample was determined, after which pollen was stored at -20°C for later sample preparation. Pollen from each colony by sample date was analyzed independently using

the following process. A 15 g subsample of the homogenized pollen was dried at 60ºC for 60 hours. After drying, the pollen was ground with a mortar and pestle and a 1 g subsample of the prepared pollen was sent to the USGS National Fish Health Research Laboratory in Kearneysville, WV for DNA sequencing, and the remaining prepared pollen was sent to Midwest Labs in Omaha, NE for percent crude protein content analysis (AOAC 990.03).

Pollen DNA sequencing

Pollen composition was estimated by paired-end sequencing of the internal transcribed spacer (ITS) region of the nuclear ribosomal locus, as described by Cornman et al. (2015). Reads were trimmed of adapters and low-quality bases with the bbduk package (Joint Genome Institute 2019), specifying a minimum kmer size of 10 to detect adapter matches and a requiring a minimum Phred-scaled quality score of 10. Reads less than 150 nt after trimming were discarded, and only intact read pairs were retained. Forward reads were clustered at 97% identity using vsearch (Rognes et al. 2016). Cluster representatives were then scaffolded with their reverse reads using an arbitrary gap size of 25 N's. Scaffolds were then reclustered at 97% to account for variation contributed by the reverse read, producing an initial set of operational taxonomic units (OTUs). Each read of these OTUs s was aligned to the NCBI nucleotide database (download date 3/19/2018) with the Basic Local Alignment Search Tool (BLAST+ v. 2.3.0).

Taxonomic assignment of OTUs was based on the concordance of two methods: the lowest common ancestor (LCA) method (Huson et al. 2007), which is based on the distribution of global alignment scores for each OTU and the RDP Classifier program (Wang et al. 2007), which identifies kmer frequencies that differentiate taxa from a training set. For LCA, OTUs

were assigned to the lowest taxonomic entity encompassing all species scoring within 3% of the highest bit score for that OTU (summed across the reads of each pair). Species level assignments were demoted to genus if the average percent identity of matches was less than 95%; genus level assignments were demoted to family if matches were less than 90% on average.

For this study, OTUs were retained for analysis if they were assigned to the same genus by both methods and had a minimum of 10 reads mapped at a stringency of 97%, with no more than five skipped bases at read ends and fewer than five indel positions within the alignment (Table D2). OTU read abundance was not drawn from the vsearch cluster size but instead estimated by mapping reads to OTUs with bowtie2 (Langmead and Salzberg 2012). Reads were mapped using the "local" mapping mode, which is more permissive to mismatches at read edges while still imposing a minimum alignment score. The "score-min" parameter for local mapping was set to "G,80,8" (see Langmead and Salzberg (2012) for details of the alignment method).

Flower transects

To determine the available floral resources for honey bees in the landscape, I conducted floral surveys within the 4-km buffer surrounding each apiary from July to September each year (2015-2017). I counted and identified all actively flowering, herbaceous stalks at a total of 731 transect locations from 2015-2017 (average of 28.11 ± 0.80 transect locations/apiary/year), with different transect locations chosen each year. Transects were chosen in floristically-rich grassy-herbaceous habitats. Each transect was sampled once per month (July, August, September) for a total of three visits per year.

The start location of each transect was randomized using a random number generator to select the entering direction and number of paces into the field, and the transect ran north where

possible. In 2015 and half of 2016, transects were 25 m by 2 m, while in half of 2016 and all of 2017, transects were conducted over a 20 m by 2 m area, a 22% difference in total area. The two different 2016 lengths were equally interspersed temporally and among fields.

Statistical Analysis

All statistical analyses were completed in R-studio version 3.6.3 (R Core Team 2020). The measured characteristics of sampled pollen included protein, taxonomic richness, and weight. Pollen protein is the percent crude protein in the pollen sample. Pollen taxonomic richness was calculated as the sum of total unique taxa identified per sample. Pollen weight was the fresh weight of the entire pollen sample in grams and was log transformed to reduce strong skew. To determine seasonal differences in pollen protein, taxonomic richness, and weight, these variables were assessed using repeated measures analysis of variance (ANOVA) on separate general linear mixed effects models (GLMM) with compound symmetry using the lme4 package (Bates et al. 2015). Bi-weekly sample period, as a factor, and year were treated as fixed effects. Year was included as a fixed effect to account for design variance and only had three levels so it could not be included as a random effect. Apiary by year (26 levels= 4+10+12 apiaries) and unique colony identity (54 levels= 26 apiaries x 2 pollen traps/ apiary + 2 changed colonies) were treated as random intercepts to account for the repeated measures design. A compound symmetry error structure was chosen due to the low variance explained by unique colony identity in all of the models. Apiary was excluded as a random effect, because it caused convergence issues and singular model fit when included in addition to the random intercept of apiary by year. To determine seasonal differences within each year, bi-weekly sample period was set as a fixed effect, while apiary and unique colony identity were random effects. Likewise, yearly differences

were calculated using a compound symmetry error structure and setting year as a fixed effect, while sample period (5 levels), apiary by year (26 levels), and unique colony identity (54 levels) were random intercepts to account for repeated measures. Differences in incoming pollen among apiaries were calculated using compound symmetry variance structure with apiary (12 levels) and year (3 levels) set as a fixed effect and colony identity (54 levels) and bi-weekly sampling period (5 levels) as random intercepts to account for repeated measures. All of these analyses were also completed with data stratified by year, apiary, and/or sampling period. Post-hoc pairwise Tukey comparisons were then conducted to determine how pollen protein, taxonomic richness, and weight varied between sampling periods, years, or apiary (Hothorn et al. 2008). I also tested for correlations between pollen weight, protein, and taxonomic richness using Pearson's product-moment correlation (R Core Team 2020).

For each transect I calculated flowering plant species richness, total abundance, and most dominant species abundance. These measures were not corrected based on transect length because no comparisons are made between individual transects. Plant species richness was calculated as the sum total of all unique species per transect. Total floral abundance was the count of all flowering stalks within the transect. Dominant species abundance was the count of the most abundant flowering herbaceous plant species at each transect and is henceforth referred to as floral dominance. Floral richness, abundance, and dominance were all log-transformed to reduce strong skew. To investigate any inter-apiary differences in pollen protein, taxonomic richness and weight within year associated with the respective difference in apiary, I assessed inter-site differences in floral richness, abundance, and dominance, within the year of interest, using separate ANOVAs on GLMMs with a first order autoregressive structure (Pinheiro et al. 2020). Floral richness, abundance, or dominance were each the response variables, site and

numeric sampling month were the fixed effects and unique transect identity (726 levels= 731 transects - 5 with no flowers) was the random effect to account for repeated sampling. Tukey's multiple comparisons contrasts were calculated using the multcomp package (Hothorn et al. 2020). I also used GLMMs to determine if pollen percent protein, richness, or weight were directly correlated with floral richness, abundance, or dominance in grassy-herbaceous habitat within the 4-km radius of each apiary, within the same month and year. Data from both pollen and floral datasets were averaged by common site, month, and year. Data were averaged to common month because transect observations only occurred once per month, while pollen collections occurred more than once per month in July and August. Then, each of the three pollen characteristics was regressed in a separate GLMM with each of the three floral transect characteristics, year (3 levels) and sampling month (3 levels) as fixed effects and site (12 levels) as a random effect, with a compound symmetry variance structure. An autoregressive structure was also tested but was determined to be inferior to the compound symmetry model based on AICc scores. Coefficient of determination values (R^2_c) were calculated using the MuMIn package (Bartoń 2019). The same analysis was also done, without sampling month as a fixed effect as well as stratified by month, to determine the effect of month on the correlation between grassy-herbaceous forage and incoming pollen characteristics. Difference in transect floral richness, abundance, and dominance from July to September were calculated using GLMMs with a first order autoregressive error structure using the nlme package (Pinheiro et al. 2020) with sampling month as a numeric fixed effect to specify the time series, year (3 levels) as fixed effect to account for among year variance and site (12 levels), site by year (26 levels), and unique transect identity (726 levels) as random intercepts.

Taxonomic rank concordance

To determine if pollen collection by colonies was proportional to the most abundant blooming flowers in the landscape, I conducted correlation analysis between the commonness (rank) of pollen taxa identified with DNA metabarcoding and the commonness (rank) of taxa identified in the transects (Smart et al. 2017b). For taxa identified by DNA metabarcoding, ranking was based on the number of OTU reads, multiplied by total pollen sample weight, to reflect the abundance of each taxa. For transect flower observations, ranking was based on the observed number of plants of each species. Pollen collections and transects which shared a common site, sampling month, and year were compared. These paired ranks were regressed with one another in a compound symmetry error structure GLMM with year (3 levels) and sampling month (3 levels) as fixed effects and site (12 levels) as a random intercept (Bates et al. 2015). The same was also done within each month, with site and year as random intercepts.

Results

Pollen crude protein

Across all pollen samples, percent crude protein ranged from 11.7% to 31.3%. Overall, mean protein decreased from July to September ($F_{4, 124.30}$=78.61, p<0.01), and this trend was consistent for each year (Figure 5.2A). Protein also decreased each year of the study ($F_{2, 20.40}$=22.97, p<0.01) but not significantly between 2015-2016 (Figure 5.2A). Protein was higher in 2016 than 2017 for colonies in apiaries A ($F_{2,3.39}$=21.34, p=0.01), G ($F_{1,10.21}$=7.21, p=0.02), and J ($F_{2,20.64}$=20.64, p<0.01), when apiaries were analyzed independently. Overall, apiaries did not differ in protein ($F_{11, 30.86}$=1.03, p=0.44), though in 2016 apiary A was 4.11% higher in

protein than apiary I ($z=-3.44$, $p=0.01$). There was also no difference between apiaries in late August and early September, when protein was lowest ($F_{11, 28.52}=1.84$, $p=0.09$).

These pairwise apiary and year differences in the stratified pollen data were not also seen in any of the corresponding transect floral data. In 2016, floral abundance ($F_{9,242}=1.44$, $p=0.17$) and dominance ($F_{9,242}=1.33$, $p=0.22$) were not significantly different among sites. In 2016, floral richness, while different among the sites ($F_{9,242}=2.34$, $p=0.02$), was higher in site C than sites A ($t=3.99$, $p<0.01$), F ($t=3.60$, $p=0.01$), G ($t=4.22$, $p<0.01$), H ($t=3.47$, $p<0.01$), I ($t=3.71$, $p<0.01$) and J ($t=3.83$, $p<0.01$), rather than sites A, which had higher crude protein. Pollen protein was weakly negatively correlated with pollen taxonomic richness ($\rho=-0.22$, $t_{172}=-2.93$, $p<0.01$) and log-transformed pollen sample weight ($\rho=-0.17$, $t_{171}=-2.29$, $p=0.02$).

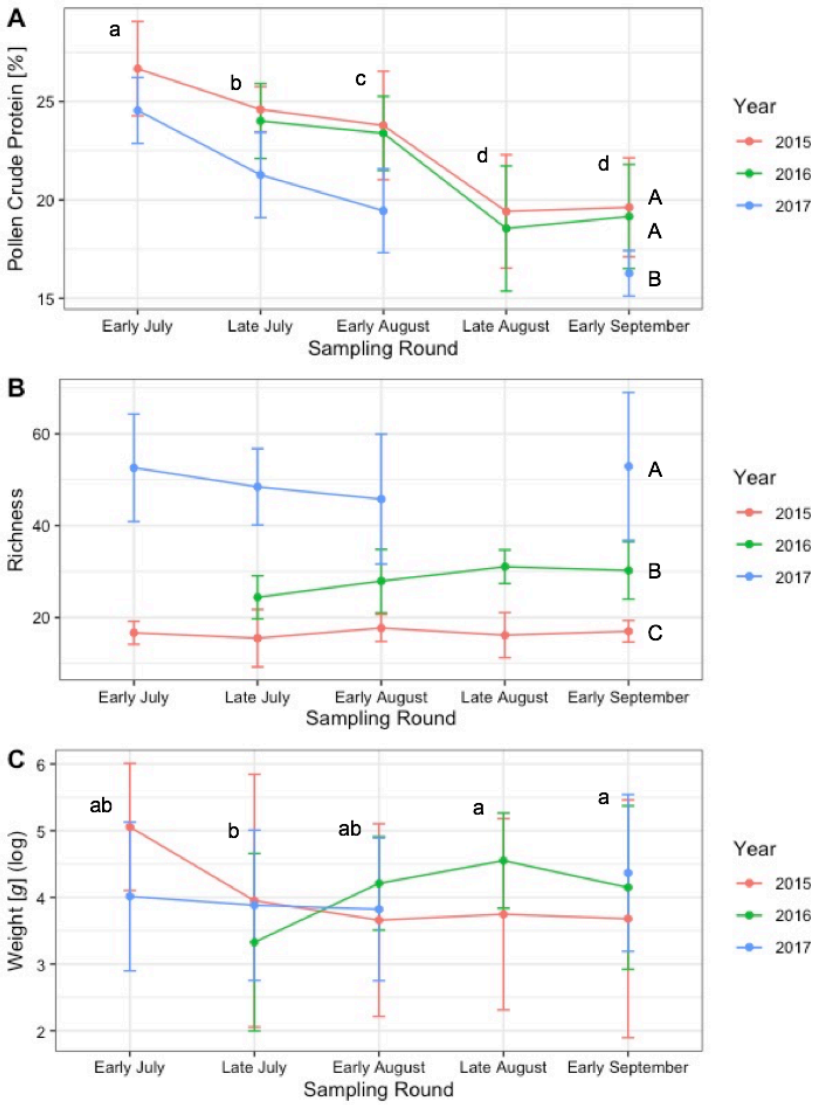

Figure 5.2. Variability in composition and weight of pollen collected by honey bees in Michigan: pollen percent crude protein (A), pollen taxonomic richness (B), and pollen weight in grams (log transformed) (C), measured every two weeks from early July to early September. Different lowercase letters indicate statistically significant differences in pairwise analysis of variance comparisons between biweekly sampling rounds, with data pooled across years. Different uppercase letters indicate statistically significant differences in pairwise analysis of variance comparisons of pollen metric between years, with data pooled across biweekly sampling rounds.

Percent protein was not correlated with transect floral richness ($F_{1, 51.15}$=0.32, p=0.57).

However, when sampling month was excluded from the model, pollen percent protein was

positively correlated with transect floral richness ($F_{1, 65}$=12.51, R^2_c=0.24, p<0.01). In the pooled data, percent protein increased by 0.07% for each 1% increase in transect species richness, due to transect richness being log-transformed (Figure 5.3). For example, the model predicts that when species richness increases from 5 to 10 species, percent protein increased by 5.02, while increasing from 15 to 20 species resulted in only a 2.09 increase in percent protein. The positive correlation between protein and floral richness was found in 2016 ($F_{1, 19.88}$=4.63, p=0.04) and 2017 ($F_{1, 34}$=11.41, p<0.01), however there was no trend in 2015 ($F_{1, 7}$=0.20, p=0.67) when I had fewer sites to sample. Transect floral richness ranged from 0 to 22 species, with an average of 5.12 ± 3.02 species per transect. Transect floral richness decreased stepwise throughout the summer ($F_{1, 1413}$ =64.85, p<0.01) (Figure 5.3).

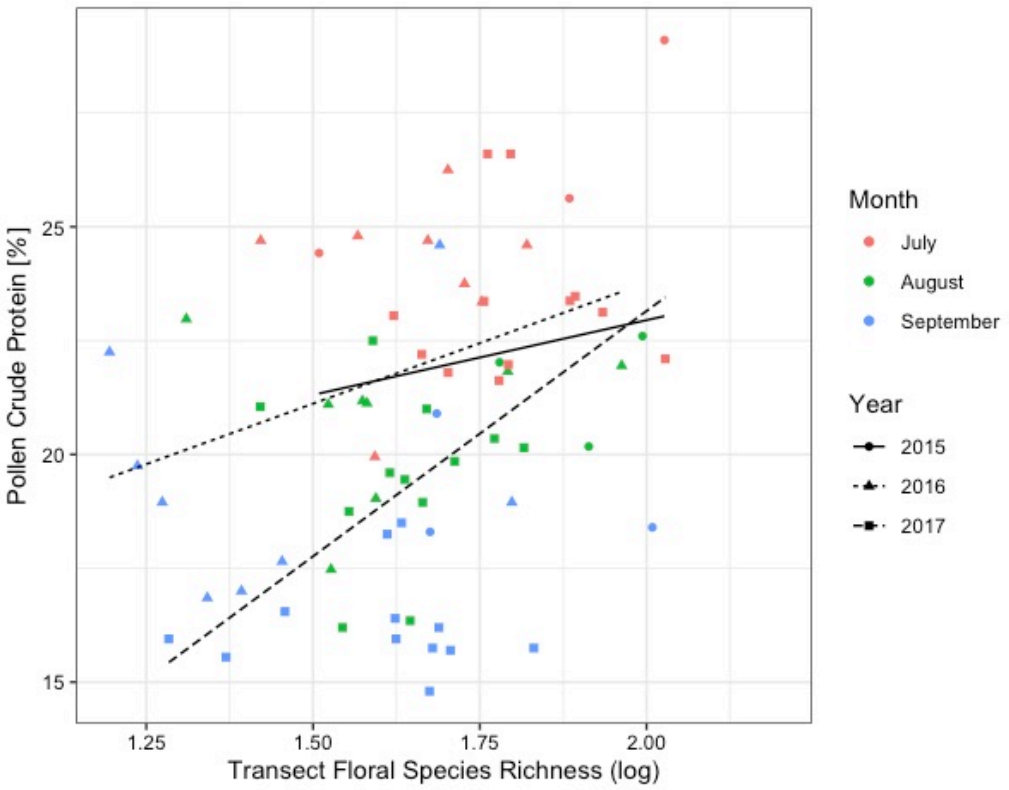

Figure 5.3. Crude protein levels of pollen collected by honey bees in apiaries distributed across Michigan regressed against transect floral species richness (log transformed) in grassy-herbaceous habitat surrounding the apiaries. Different colors show different sampling months and different shapes indicate different years, with lines showing the best fit model for each year.

Percent protein was also not correlated with transect floral abundance ($F_{1, 62.68}$=0.00, p=1.00), unless sampling month was excluded from the model. When this was done, percent protein was positively correlated with transect floral abundance ($F_{1, 65}$=7.44, R^2_c=0.19, p<0.01). When data were pooled across sampling months, for each 1% increase in transect flowering plant abundance, percent crude protein increased by 0.02. So, adding 50 plants at 100 plants per transect would result in an 0.70 increase in percent protein but only a 0.20 increase in percent protein when increasing from 400 to 450 plants. This positive trend was observed in 2016 ($F_{1,17.91}$=5.13, p=0.04) and 2017 ($F_{1, 34}$=4.28, p=0.05), but not 2015 when fewer sites were sampled ($F_{1,7}$=0.16, p=0.70). The abundance of flowering plants in the transects ranged from 0 to

4301 plants, with an average of 147.6 ± 231.81 flowering plants per transect. Floral abundance was highest in July and decreased throughout the summer ($F_{1, 1413}$=50.42, p<0.01).

Protein was likewise not correlated with transect floral dominance($F_{1, 62.13}$=0.02, p=0.90), except when sampling months were pooled ($F_{1, 65}$=4.29, R^2_c=0.15, p=0.04). Protein increased by 0.01% for each 1% increase in transect flowering plant abundance. There was no correlation between protein and floral dominance within any year (2015: $F_{1,7}$=0.02, p=0.89; 2016: $F_{1,18.55}$=3.80, p=0.07; 2017: $F_{1, 34}$=1.98, p=0.17). Floral dominance ranged from 0 to 4300 plants of the most dominant species, with an average of 97.35 ± 189.87 plants of these species in each transect. Transect floral dominance also decreased throughout the summer ($F_{1, 1413}$=30.63, p<0.01).

The overall correlations between protein composition in pollen and transect floral richness, abundance, and dominance seem to be driven by the concurrent decrease in floral richness, abundance, and dominance throughout the summer. This was seen in the lack of correlation between protein and transect floral richness, abundance, and dominance when accounting for sampling month in the model, compared to the significant correlation between pollen protein and floral richness, abundance and dominance seen when pooling data throughout the summer.

Pollen taxonomic richness

Taxonomic richness in pollen ranged from 9 to 100 taxa identified per sample. Overall, mean pollen taxonomic richness was not different among the sampling rounds ($F_{4, 154.69}$=2.38, p=0.05) (Figure 5.2B). This trend held for 2015 and 2017, but in 2016 there were differences in taxonomic richness between rounds ($F_{3, 55.14}$=6.01, p<0.01) with late July having approximately 6.6 fewer species than late August (z=3.86, p<0.01) and 5.8 fewer species than early September

(z=3.38, p<0.01). Pollen taxonomic richness increased across the three years of the study ($F_{2, 24.06}$=95.58, p<0.01) (Figure 5.2B). From 2015 to 2016, an average of 11.6 additional species were detected per sample (z=3.92, p<0.01) and from 2016 to 2017 an average of 21.5 additional species were detected per sample (z=10.58, p<0.01). This longitudinal increase in taxonomic richness was also reflected in every apiary, except E ($F_{1,2.21}$=2.67, p=0.23) and H ($F_{1,1.92}$=15.19, p=0.06). There were no between-apiary differences in taxonomic richness overall ($F_{11, 37.98}$=1.05, p=0.43), though in 2016, apiary A was higher in pollen taxonomic richness than apiary D by 8.6 taxa (z=-3.52, p=0.01) and F by 8 taxa (z=-3.27, p=0.03); apiary E was also higher in pollen taxonomic richness than apiary D by 8.6 taxa (z=3.52, p=0.01) and apiary F by 8 taxa (z=-3.27, p=0.03). As noted above, floral abundance, richness, and dominance around site A and E in 2016 were not higher than other sites. Pollen richness was not correlated with log-transformed pollen weight (ρ=0.05, t_{176}=0.68, p=0.50).

Pollen taxonomic richness was also not correlated with floral richness, abundance, or dominance overall, nor within any year. When the pollen and transect data were stratified by month, there was also no correlation between pollen richness and transect floral richness, abundance, or dominance in any month highlighting the independence of pollen composition from these metrics.

Pollen weight

Pollen samples collected per colony ranged from 2.17 g to 725.92 g per 72-hour collection event. Mean pollen weight increased slightly in late season, overall ($F_{4, 121.91}$=3.74, p<0.01), with late August and early September pollen samples weighing about 0.6 g more on average per 72-hour sampling event than late July (Figure 5.2C). This end-of-summer increase in

weight seems primarily driven by the 2016 pollen samples ($F_{3,44.07}$=5.59, p<0.01), which had less incoming pollen in late July than early August through early September; there were no sampling round differences in either the 2015 ($F_{4, 12.31}$=1.46, p=0.27) or 2017 samples ($F_{3,59.22}$=1.92, p=0.14). Pollen weight was not different between years ($F_{2, 16.76}$=0.03, p=0.97). No differences between years were observed when data were stratified by apiary either, except for apiary A ($F_{2,19.27}$=3.74, p=0.04), which had about 2.7 g more pollen collected in 2017 than 2015 (z=-2.24, p=0.06) or 2016 (z=-2.48, p=0.03). Pollen weight was not different between apiaries overall ($F_{11, 35.77}$=1.67, p=0.12), though in 2015 ($F_{3,6.52}$=8.80, p=0.01), apiary G was lower in incoming pollen weight than apiary A (z=3.23, p<0.01), C (z=5.00, p<0.01), and F (z=3.12, p<0.01). Corresponding differences between sites in floral richness ($F_{3,116}$=4.63, p<0.01), abundance ($F_{3,116}$=5.04, p<0.01), and dominance ($F_{3,116}$=5.16, p<0.01) included G having higher richness (t=2.67, p=0.04), abundance (t=2.83, p=0.03) and dominance (t=2.64, p=0.04) than sites A and higher dominance than site C (t=2.71, p=0.04) in 2015.

Pollen weight was not correlated with floral richness, abundance, or dominance overall, nor within any year. When data were stratified by month, pollen weight was not correlated with transect floral richness, abundance, or dominance in any of the months.

Taxonomic rank concordance

Molecular analysis identified various native and non-native plant species as being dominant in the pollen collected by honey bees at the sampled apiaries. I identified *Lythrum* as a dominantly collected pollen genus across the entire season, being second most common in July, first in August, and fifth in September (Figure 5.4). *Trifolium* was also found to be an important pollen source throughout the summer. *Sinapis alba* and *Plantago* sp. were identified as important

early season pollen sources, whereas more *Solidago* and *Ambrosia* were collected later in the summer (Figure 5.4).

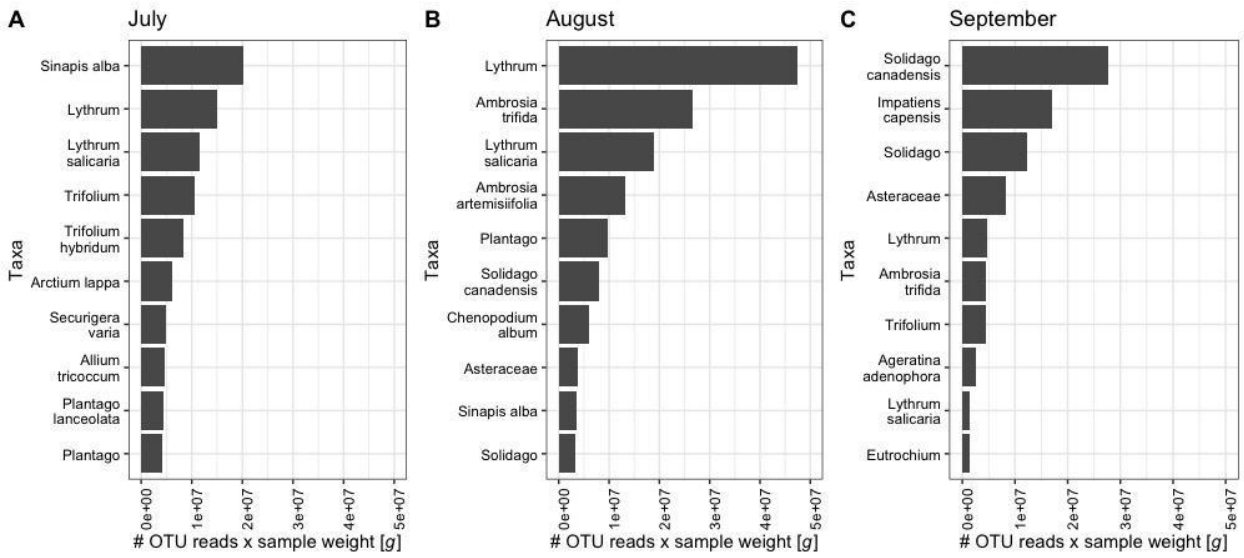

Figure 5.4. Top ten most common taxa collected by honey bee colonies in Michigan during each month of the summer, as identified based on molecular Operational Taxonomic Unit (OTU) reads, summed over sampling rounds, years, and apiaries and multiplied by the weight of the pollen from which the sample was taken.

In grassy-herbaceous habitat surrounding my study apiaries, *Daucus carota* and *Trifolium pratense* were consistently identified as a dominantly blooming species. In July and August, *Plantago lanceolate, Centaurea maculosa, Erigeron annuus, Lotus corniculatus,* and *Monarda fistulosa* were all among the top 10 most abundantly blooming species. In September, *Solidago* species became more dominant in the landscape and *Cichorium intybus* was also a commonly blooming species (Figure 5.5).

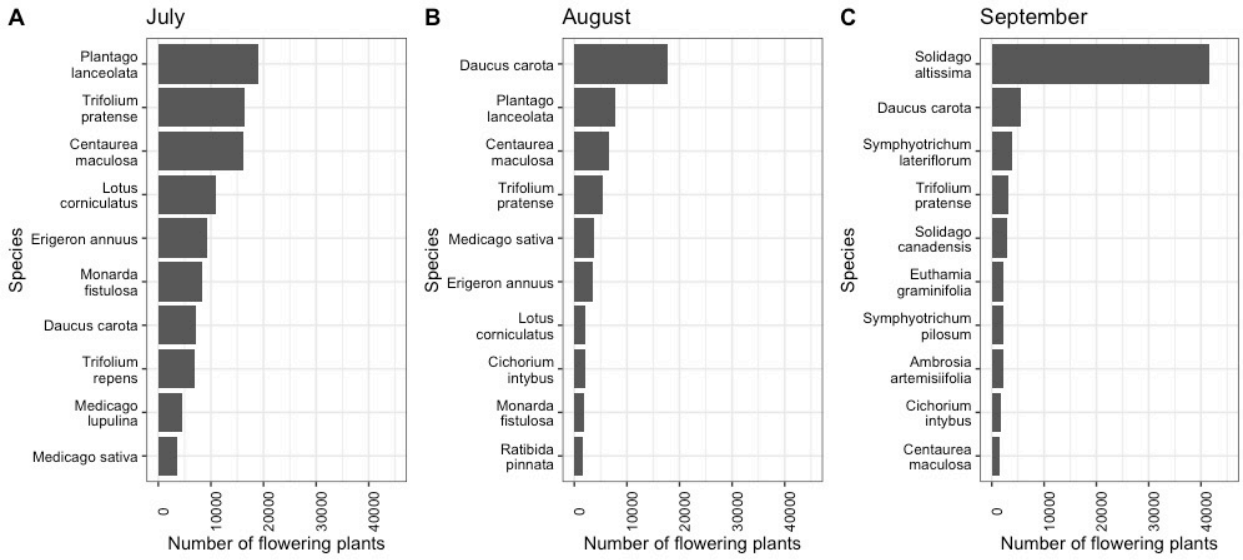

Figure 5.5. Abundance of the top ten most common flowering species observed within transect samples taken in grassy-herbaceous habitats in Michigan during each month of the summer, summed over years and sites.

In spite of *Trifolium, Plantago,* and *Solidago* appearing as common genera in both incoming pollen and field transects, there was very low taxonomic rank concordance between pollen OTUs and floral abundance in grassy-herbaceous habitat in the surrounding forage area ($F_{1,261.8}$=0.10, p=0.75). There was also no significant correlation in rank of species when data were stratified by month in July ($F_{1, 95.53}$=0.33, p=0.57), August ($F_{1, 78}$=2.02, p=0.16) or September ($F_{1,82.00}$=2.16, p=0.15).

Discussion

Analysis of honey bee-collected pollen from apiaries across Michigan revealed temporal variation in percent protein, taxonomic richness, and weight. Notably, pollen protein, which plays a critical role in colony brood rearing, colony population stability, and growth (Haydak 1935; Schmickl and Crailsheim 2001; Brodschneider and Crailsheim 2010) decreased

consistently from July to September each year. Protein levels between 20-25% are sufficient to support honey bee adult survival and brood rearing (Schmidt et al. 1987). Mean percent protein was within this range from early July through early August, but dropped below this level in most apiaries from late August through early September. This late season drop in protein may affect colony health, particularly because this is when winter bees are being raised. Winter bees longevity, and thus colony overwintering success, strongly depends upon protein status (Amdam and Omholt 2002; Amdam et al. 2004a). It is possible that this shift is due to honey bees changing their foraging behavior to preferentially collect less protein later in the season, to optimize a their protein to lipid ratios (Vaudo et al. 2020), or foraging for other nutrients (Bonoan et al. 2017, 2018), based upon seasonal colony needs and shifting bee physiology. However, this seems unlikely as honey bees ability to detect, and preferentially forage for protein in pollen is not currently well documented (Roulston et al. 2000; Ghosh et al. 2020). Additionally, a previous study in Arizona found that honey bee collected pollen protein and lipid levels did not vary between spring and fall (DeGrandi-Hoffman et al. 2018), suggesting this could be a regional phenomenon driven by floral composition. Further research is needed on honey bee dietary needs, seasonal shifts in dietary needs and how these affect foraging behavior.

My finding that pollen protein levels are positively correlated with the richness, abundance, and dominance of floral resources in grassy-herbaceous habitats in the landscapes around apiaries suggests that investment in management of these resources could have a direct benefit for honey bee nutrition. Indeed, a recent study underscores the importance of conservation forage plantings to improve colony health (Ricigliano et al. 2019). Based upon the positive relationship between biodiversity and ecosystem functioning, high floral richness theoretically provides foragers increased access to highly nutritious options. It could also

improve season-long floral availability and complementarity in nutrients (Barthlott et al. 2009). While I found higher floral richness was associated with improved pollen resource collection between months, a positive correlation between floral composition and pollen composition was not seen within months. This suggests that bloom phenology of early summer, high protein plants is driving the overall pattern. Studies in other agroecosystems have reported seasonal changes in incoming pollen weight and protein associated with inconsistency in forage availability due to crop bloom phenology (Dimou and Thrasyvoulou 2009; Decourtye et al. 2010; Odoux et al. 2012; Requier et al. 2015; Di Pasquale et al. 2016).

The approach of combining incoming pollen analysis with field floral observations throughout the summer provides some insight into how floral composition on non-cropped fields, which is inherently more variable than cropland, affects honey bee pollen quantity and quality. For example, it is likely that these high protein, early season plants are also common, volunteer species. If rare floral species, specific to grassy-herbaceous habitat were underlying the phenological patterns, one might expect to see an effect of floral diversity on pollen protein within month due to sampling effect increasing the likelihood of including such a rare, high functioning species (Barthlott et al. 2009). These high protein species likely also bloom across land covers and there may be other early summer high protein species in other land covers. This highlights the need to not only consider floral richness and abundance, but floral resource quality in pollinator habitat management. It also suggests that Michigan beekeepers should consider management interventions such as supplementing protein in late-summer, as all apiaries in this study experienced similar late-summer protein depletion and even sites surrounded by grassy-herbaceous habitat with greater floral richness, abundance, and/or dominance did not collect greater pollen protein.

The importance of common, early summer, high protein species in grassy-herbaceous habitat for honey bee foraging is further supported by the identity of taxa observed in the pollen and transects. *Sinapis alba* and *Plantago* sp., which were both important early-season pollen species, have pollen crude protein of 24.5% (Szczêsna 2006) and 23.9% (*P. lanceolata*) (Sharma et al. 1993), respectively, which is within the biologically important crude protein range of 20%-25% (Schmidt et al. 1987). *Plantago* was commonly observed within grassy-herbaceous habitat early in the summer but decreased in abundance throughout the summer, a trend that was also seen in the honey bee collected pollen. *Lythrum* and *Trifolium* were important pollen sources throughout the summer, but both were less abundant in pollen in September than July and August, and *Trifolium* decreased in abundance in grassy-herbaceous habitat throughout the summer. Species of *Trifolium* range in crude protein, but many common species are above 30% (Roulston et al. 2000). *Solidago* and *Ambrosia*, which were collected and observed in grassy-herbaceous habitats later in the summer, were of lower pollen protein quality. *Solidago* crude protein in current climactic conditions is typically below 13% (Ziska et al. 2016). *Ambrosia* crude protein is likewise low, at 13.4% and has been associated with reduced longevity in honey bees (Schmidt et al. 1987). Honey bee foragers do not seem to collect pollen on the basis of protein content (Roulston et al. 2000). Foragers in my study are thus likely foraging on whatever is blooming abundantly at the time, leading to the decrease in late-summer pollen protein. These observations align with previous studies which found common, dense-blooming volunteer flowering plants seem to play an important role in honey bee foraging (Requier et al. 2015; Bretagnolle and Gaba 2015). There was not strong concordance between taxa observed in in grassy-herbaceous habitat and in honey bee foraged pollen. This supports my earlier assertion that the lack of correlation between pollen protein and floral richness, abundance, or dominance

within month is in part due to honey bees also foraging in other land covers. Notably, *Lythrum salicaria,* an invasive species (Midwest Invasive Species Network 2019), was commonly detected via pollen reads but was not often observed in grassy-herbaceous habitat. *Lythrum salicaria* could have been very abundant in wetlands (Benvenuti et al. 2016), where I did not sample. Part of the lack of concordance could also have been due to misidentification of transect taxa. For example, *S. altissima* and *S. canadensis*, which are notoriously difficult to distinguish without genetic testing (Semple et al. 2015), could have affected the correlation with bee-collected pollen identification. However, I chose to not change the field data to avoid arbitrary reassignment of identifications.

The only temporal differences in pollen taxonomic richness were between sampling rounds in 2016 and between years. The lack of overall seasonal pattern in taxonomic richness may be because honey bees exhibit forage constancy (Donaldson-Matasci and Dornhaus 2014), typically aggregating on whichever dominant species is blooming within the foraging landscape (Gilpin et al. 2019). This could keep incoming pollen richness relatively constant. Additionally, no correlations were found between incoming pollen richness and any of the grassy-herbaceous floral richness, abundance, or dominance metrics, which may be due to a sufficient abundance and richness of flowers in the landscape, particularly for a generalist species such as honey bees (Vaudo et al. 2015). The yearly increase in taxonomic richness is most likely due to differences in weather affecting plant species blooming, rather than the addition of forage, because it was seen across sites. This demonstrates the necessity to conduct these types of studies over multiple seasons.

Less pollen was brought back in late July than late August or early September, with this result being driven by the 2016 pollen samples. The amount of incoming pollen being relatively

consistent across time and space suggests that colonies are stimulated to collect pollen in response to the colony's needs, as driven by brood pheromone signals (Pankiw et al. 1998). Confounding factors, such as colony size (Khoury et al. 2013) could thus explain the lack of correlation between pollen weight and floral richness or abundance in grassy-herbaceous habitat. Indeed, the only difference between apiaries in quantity of foraged pollen was site G bringing in less pollen than all other apiaries in 2015. However, site G was also lower in floral abundance and dominance than other sites in 2015, suggesting unique confounding effects at that site. Pollen weight staying relatively constant while pollen protein decreased throughout the season supports my suggestion that foragers are indiscriminately collecting to reach quantity targets from floral resources of decreasing protein quality in the landscape. This indiscriminate foraging behavior could also explain the unexpected negative correlation between pollen protein and pollen weight/richness.

In conclusion, I found that grassy-herbaceous habitat in Michigan supports a richness of dense-blooming floral species, which are likely to be visited by foraging honey bees and contribute to colony nutrition. In this region, decreased floral richness and abundance in these habitats in late summer is associated with decreased pollen protein. Therefore, habitat enhancements should focus on seeding or planting high protein August and September-blooming species. There are few late summer, high protein, native plants that honey bees were found to forage upon and which could be incorporated into pollinator habitat plantings. These include *Allium* sp. and Polygonaceae (Liolios et al. 2015; US Department of Agriculture Natural Resources Conservation Service 2019b). This result suggests the need for forage pasture plantings of clover or buckwheat, to provide honey bees with late-season pollen forage in this area (Roulston et al. 2000; Carreck and Williams 2002). A recent study in Iowa also observed

less honey bee collected pollen in September and advised implementing fall forage (Zhang et al. 2020). It is likely that other parts of the Midwest are likewise experiencing similar temporal dynamics in floral nutrition for honey bees, due to similarly intensive agroecosystems, and could benefit from honey bee habitat enhancements in the late summer and fall.

CHAPTER 6: NEITHER PROTEIN SUPPLEMENTS NOR PROBIOTICS SPEED UP RECOVERY OF HONEY BEE COLONIES FROM EUROPEAN FOULBROOD IN THE FIELD

Abstract

European foulbrood (EFB) is a disease of honey bees (*Apis mellifera*, L.) that in the United States has traditionally been treated with antibiotics. Due to challenges obtaining antibiotics, the inability to harvest honey for a period after their use, negative physiological effects on bees, and the potential for the development of antibiotic resistance, more sustainable in-hive treatments for EFB are needed. To test this, I assessed colonies naturally infected with EFB in the field that were either left untreated, treated with traditional antibiotics, antibiotics with a soy-based protein supplement, soy-based supplement alone, pollen-based supplement, or probiotics. I compared EFB infection severity among treatments every two weeks to track recovery speed. Colonies treated with antibiotics were almost fully recovered within two weeks, at the end of the treatment course. Two weeks later, all groups showed similar recovery. There were also no significant differences among treatments in the colony size six weeks after treatment. Furthermore, nurse bee physiology, measured by head and fat body weights, were not different among treatments, suggesting no physiological effects of any of the treatments. Based on these findings, different forms of protein supplements and probiotics were not as effective as antibiotics in reducing EFB. Furthermore, none of the alternative treatments enhanced recovery speed or colony size compared to untreated colonies. These results support the expectation that EFB symptoms can be rapidly reduced through antibiotic treatment, but nutritional supplements do not affect the time-course of symptoms of this disease.

Introduction

Growers of pollinator dependent crops often supplement pollination services through the addition of managed honey bees rented from beekeepers (Aizen and Harder 2009). Pollination contracts can, however, be stressful on honey bee colonies, as those kept on farms may experience higher pesticide exposure and lower nutritional diversity making them more susceptible to various pathogens (Collison et al. 2016; Dolezal and Toth 2018). For example, there has long been evidence of brood disease, European foulbrood (EFB), associated with honey bee pollination of blueberry (Wardell 1982), and more cases of EFB have been reported globally over the past few decades (Wilkins et al. 2007; Roetschi et al. 2008). Beekeepers whose colonies pollinate blueberry crops across North America have begun to experience higher levels of brood disease in recent years (McAfee 2018; Dufour et al. 2020). EFB has recently been observed at high rates after Michigan blueberry pollination contracts, with 23% of colonies sampled in 2018 exhibiting signs of EFB and 56% in 2019 (M. Milbrath, unpublished).

EFB is a bacterial disease caused by *Melissococcus plutonius* (Bailey 1983). This pathogen is found even in healthy colonies (Forsgren et al. 2005; Budge et al. 2010), but it is not well understood under what conditions EFB becomes expressed. Some early research suggested expression could be stress-related, such as having insufficient nutrition (Bailey 1961). Third to fifth instar *A. mellifera* larvae show visible signs of infection which include corkscrewing, yellowing, melting, and exposed trachea, typically followed by larval death seen as sunken/irregular holes in the cappings or rubbery scaling (Bailey 1961; Forsgren 2010). Larval death results in a disruption of the brood cycle, with downstream implications for colony demography and cluster size (Russell et al. 2013).

Antibiotics are typically used to treat EFB (Thompson and Brown 2001; Waite et al. 2003b). Some commercial beekeepers have also historically used antibiotics prophylactically before blueberry pollination contracts to prevent EFB (Shimanuki et al. 1969; Kochansky 2000). However, in 2015 the US Food and Drug Administration (FDA) released a Veterinary Feed Directive that required a prescription to obtain antibiotics for all food animals, including honey bees starting in 2017 (Food and Drug Administration, Department of Health and Human Services 2015). This mandate has made obtaining antibiotics more difficult for beekeepers. There are also other limitations of antibiotics. For example, antibiotic resistance has developed in other honey bee bacterial diseases (Miyagi et al. 2000; Evans 2003), although resistance has not yet been reported in *M. plutonius* (Hornitzky and Smith 1999; Waite et al. 2003a). Other concerns include antibiotic residues in honey, which would make the honey unmarketable for human consumption (Mutinelli 2003; Bargańska et al. 2011). Antibiotics can also have negative physiological effects on adult honey bees through gut dysbiosis (Raymann and Moran 2018) leading to decreased metabolism (Zheng et al. 2017) and decreased immunity (Kwong et al. 2017; Raymann et al. 2017). Consequently, there is interest in alternative approaches to managing EFB that would avoid these potential issues.

Alternatives to traditional antibiotic use have been explored previously (Waite et al. 2003b; Thompson et al. 2006; Roetschi et al. 2008), the most common of which are the shook swarm method and in severe cases colony destruction to prevent future outbreaks. The shook swarm method, which involves transferring bees on to new equipment, with or without subsequent antibiotic treatment has been found to be effective at preventing recurrence of EFB in the UK (Waite et al. 2003b; Thompson et al. 2006; Wilkins et al. 2007). However, in Switzerland, where the use of antibiotics is restricted and the shook swarm method and colony

destruction are utilized exclusively, cases of EFB have increased precipitously in recent decades (Roetschi et al. 2008). Other treatments, not yet tested for effectiveness against EFB, could be effective based on their use in different disease systems. The combination of antibiotics with a soy-based supplement may be more effective than antibiotics alone, by counteracting the negative physiological effects of antibiotics on adult bees (Li et al. 2019). Generally, access to sufficient nutrition, particularly protein, can enhance the immune response and disease resistance in *A. mellifera* (Alaux et al. 2010; DeGrandi-Hoffman and Chen 2015; Dolezal and Toth 2018), for example, this has been demonstrated in deformed wing virus (DeGrandi-Hoffman et al. 2010) and *Nosema ceranae* (Di Pasquale et al. 2013). Furthermore, because EFB may a nutrition stress-induced disease, addressing this stressor may lead to faster recovery (Bailey 1961; Bailey and Ball 1991). Natural pollen has been linked with lower pathogen loads, including deformed wing virus, black queen cell virus, and *Nosema* (DeGrandi-Hoffman et al. 2010, 2016), so feeding pollen-based supplements to colonies has the potential to reduce EFB infections. Probiotics have also been proposed in different systems to address immune concerns in bees (Wu et al. 2013b). There is some evidence that nonpathogenic bacteria can cause larvae to mount an immune response (Evans and Lopez 2004), and probiotics have been shown to reduce other bacterial brood pathogen loads (Daisley et al. 2019). Japanese honey bee gut bacteria isolates have also been shown to control *M. plutonius in vitro* (Wu et al. 2013a), but to my knowledge, probiotics have not been field tested for their potential to reduce EFB. Finally, it has been suggested that EFB is a self-limiting disease, capable of clearing spontaneously as infected larvae die and are ejected under natural brood-rearing cycles (Bailey 1960). In human medicine, watchful waiting is suggested as a treatment approach for certain diseases, such as pediatric ear infections which are likewise capable of clearing on their own; antibiotics are only prescribed in certain cases

and/or after a certain interval of time (Lieberthal et al. 2013). Watchful waiting may likewise be advisable in certain cases for EFB.

While the effects of treatments on recovery from EFB are expected to be seen at the colony level, honey bee colonies as super organisms can buffer against stressors (Straub et al. 2015) making effects only apparent only at the individual bee level. Nurse bee biomarkers may help to elucidate the mechanism underlying colony response to stressors, and they may provide early detection of stress in such a complex system. Nurse bee biomarkers of colony stress have been used in various studies including nutrition and disease (Di Pasquale et al. 2013) and nutrition and antibiotic use (Li et al. 2019). Two nurse bee organs important for immunity and brood health are the hypopharyngeal gland and the fat body. Nurse bee hypopharyngeal glands are the site of brood food production. The size of the hypopharyngeal gland is related to nutritional condition and the amount of brood (Crailsheim and Hrassnigg 1998). Hypopharyngeal gland size could therefore be informative for detecting treatment effects on EFB, if EFB is in fact a nutritionally-stressed disease. Previous studies have found that nurse bees fed antibiotics develop lighter heads, a proxy for hypopharyngeal gland size (Crailsheim and Hrassnigg 1998), but protein feeding along with antibiotics can reverse those effects (Li et al. 2019). Protein feeding, for example with soy- or pollen-based supplements, can increase hypopharyngeal gland development (Maurizio and Hodges 1950; Standifer 1967). The nurse bee fat body is an important organ, associated with aging and acting as the site of immune protein synthesis and energy for brood food production (Amdam and Omholt 2002). Supplemental protein feeding has been shown to increase the size of the fat body (Maurizio and Hodges 1950), as have polyfloral pollen (pollen from multiple different flower species) diets (Kazimierczak-Baryczko and Szymaś 2006).

Due to the concerns about long-term use of antibiotics, more sustainable approaches to managing this disease are needed. This study was aimed at assessing the effectiveness of various sustainable in-hive treatments to EFB, as compared to traditional antibiotics and an untreated control. To do so, every two weeks I assessed infection severity of EFB-infected colonies treated with traditional antibiotics, antibiotics with a soy-based protein supplements, soy-based supplement alone, pollen-based supplement, probiotics, or colonies left untreated. I also wanted to determine secondary treatment effects on the colony. Therefore, I measured colony cluster size before and six weeks after treatments were implemented. I also measured nurse bee fat body size and head weights to determine whether the treatments affected these biomarkers of colony health.

Methods

Treatments and experimental design

I tested for differences in severity of EFB infection among honey bee colonies at multiple apiaries that were randomly assigned to six different treatments. These colonies were assessed every two weeks after the start of treatment. The six different in-hive treatments were: (1) antibiotic, (2) antibiotics and a soy-based supplement, (3) a soy-based supplement alone, (4) a 15% pollen-based supplement, (5) probiotics, and (6) a no-treatment control in which the colony was opened but nothing was added (Table 6.1). Each treatment was replicated in 10 colonies in a blocked design across four different apiaries, managed by two different beekeepers (Figure 6.1). One beekeeper managed two apiaries, with four replicates of each treatment in each apiary, and the other beekeeper managed two apiaries with a single replicate of each treatment in each apiary. Both collaborating commercial beekeepers rent colonies to growers for highbush

blueberry (*Vaccinium corymbosum* L.) pollination. The colonies were moved to the blueberry fields in mid-May 2019 and then moved into my research apiaries after bloom in late June, where they remained through early August, during which time I completed this study.

Table 6.1. Product descriptions used in the six, in-hive treatments, with trade name, active ingredient and concentration, supplier and location, and application method.

Product	Treatment	Trade name	Active ingredient	Supplier	Application
Antibiotic	1 & 2	Tetra Bee Mix	Oxytetracycline hydrochloride	Dadant & Sons Hamilton, IL	Per label, 200 mg every 4-5 days for a total of 3 treatments
Soy Supplement	2 & 3	Bee-Pro+		Mann Lake Hakensack, MN	Maintained at 0.9 kg/colony
Pollen Supplement	4	Global Patties 15%		Global Patties Butte, MT	Maintained at 0.9 kg/colony
Probiotic	5	SuperDFM		Strong Microbials Milwaukee, WI	Northern apiaries: per label, 1, 15 mL application Southern apiaries: 2, 15 mL applications, 5 days apart

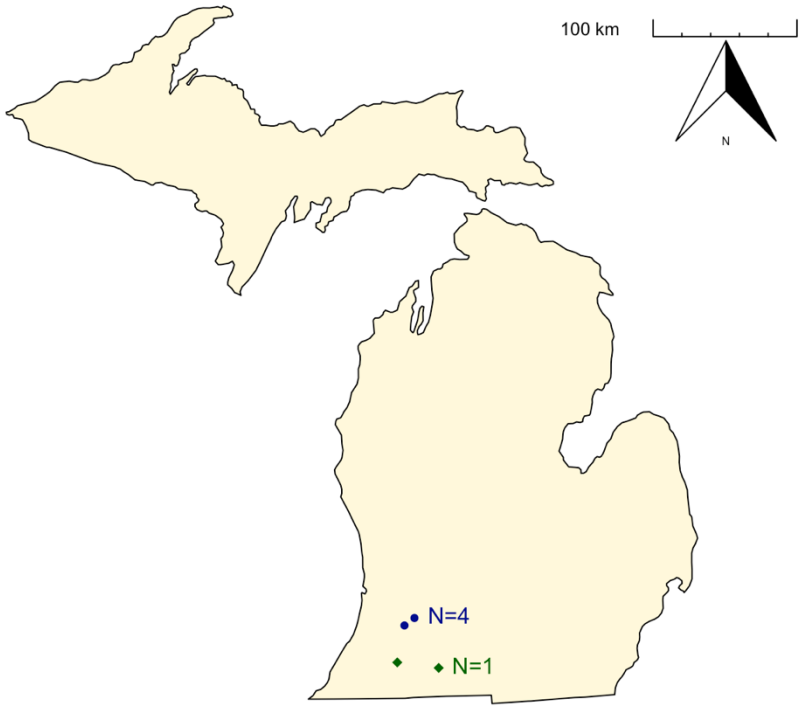

Figure 6.1. Apiary site locations for comparing treatments against European foulbrood of honey bees. The two northern most sites, indicated with blue circles each had four replicates of each treatment, and the two southern most sites, indicated with green diamonds each had one replicate of each treatment. The northern and southern sites were each managed by a different beekeeper.

Upon colonies arriving in the apiaries after blueberry pollination, each was inspected for visually detectable signs of EFB. Signs of EFB included third to fifth instar larvae which showed yellowing, corkscrewing, melting, exposed trachea, sunken/irregular holes in the cappings, or rubbery scaling (Figure 6.2). Colonies were rated on an ordinal scale based upon number of cells exhibiting signs of disease. Colonies with no diseased cells received a 0 score, 1-10 diseased cells received a 1, 11-100 diseased cells received a 2, and greater than 100 diseased cells received a 3. This ranking system was developed by North American researchers during a working group meeting on ongoing European foulbrood research (University of British Columbia, 2018, unpublished). Diagnosis of infected colonies was confirmed by sending sampled larvae to the USDA-ARS Beltsville Laboratory, where the presence of *M. plutonius* was

confirmed by light microscopy (Hornitzky and Wilson 1989). Queen-right colonies, with *V. destructor* infestation below 2%, based on an alcohol wash (Fries et al. 1991), and an EFB severity rating of at least 2 were enrolled in the study. Within each apiary, colonies were either randomized (for the single replicate apiaries) or randomized once blocked by severity level (for the four replicate apiaries) into each of the six treatments. Treatments were applied per the label instructions (Table 6.1).

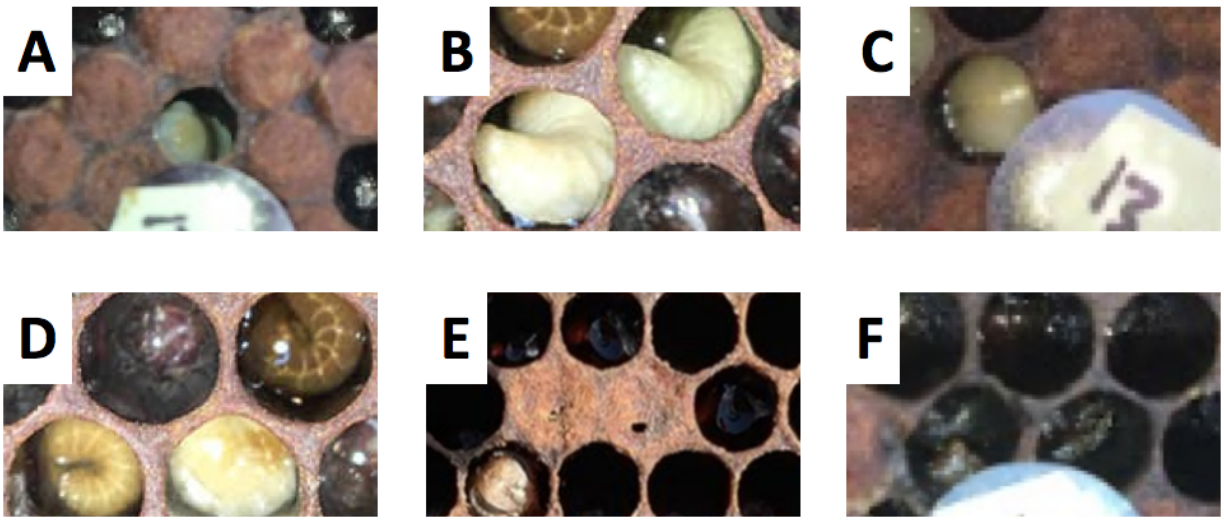

Figure 6.2. Visual signs of European foulbrood, from left to right: yellowing (A), corkscrewing (B), melting (C), exposed trachea (D), sunken/irregular holes in the cappings (E) and rubbery scaling (F). Photographs by G. Haynes, montage by G. Quinlan.

Colony inspections occurred every two weeks from enrollment to six weeks post-enrollment. At each post-enrollment inspection, EFB infection severity was recorded for the entire colony, as well as colony survival and queen status, as potentially confounding effects. At the six-week follow up, *V. destructor* infestation levels were also assessed.

Treatment effects on colony cluster size

At enrollment and at the six-week follow up the colony cluster size, in number of frames of adult bees per colony, was also recorded (Nasr et al. 1990) during the day.

Nurse bee physiology

At enrollment before treatment and at the first post-treatment inspection (four weeks post enrollment), a sample of 18 nurse bees per colony was obtained by shaking a frame of brood vigorously and scooping bees which remained on the frame. Nurse bees were immediately put on dry ice, then transferred to -80°C until processing. Nurse bee heads were weighed, and fat body size was quantified using the ether wash method, as described in Wilson-Rich et al. (2008) (David et al. 1975; Doums et al. 2002).

Statistical Analysis

All statistical analyses were completed in R-studio version 3.6.3 (R Core Team 2020). To determine differences in infection severity between treatments, analysis of variance (ANOVA) using general cumulative link models (CLM) was done using the ordinal package (Christensen 2019). Within each round, treatments were compared for EFB status with apiary, nested within beekeeper, as independent variables. Post-hoc Tukey's pair-wise comparisons between treatments were then done using the emmeans package (Lenth et al. 2020). Colony size change from enrollment to six weeks post-enrollment was calculated for each colony, and these data were analyzed using ANOVA, with treatment and apiary nested within beekeeper as independent variables (R Core Team 2020). To determine whether incidence of chalkbrood or small hive beetle differed among treatments, I preformed Chi-square tests within each two-week sampling

round (R Core Team 2020). Differences among treatments and sampling rounds in colony nurse bee fat body and head weights were analyzed with colony as the replicate, by averaging measures of nurse bees taken at the same time, from the same colony. Due to time constraints, of the 10 colonies per treatment and 18 nurse bees collected from each colony, a subset of 4-10 colonies per treatment and 1-18 nurse bees per colony were assessed and averaged for nurse bee fat body and head weight (Table 6.2). Pre- versus post-treatment change in these colony averages of nurse bee fat body and head weights were then calculated for each colony. Change in fat body and head weights were calculated to account for potential inter-colony variation in these organs associated with genetic differences, as fat body size has been shown to vary between colonies with different levels of intra-colony genetic diversity (Wilson-Rich et al. 2012). Differences between rounds for nurse bee fat body and head weights were calculated using ANOVA, with treatment and colony nested within apiary, nested within beekeeper as independent variables (R Core Team 2020). Differences among treatments within sampling round and in change in nurse bee fat body and head weights were calculated using ANOVA, with apiary nested within beekeeper as independent variables. To determine if colony nurse bee fat body or head weight change was significantly different from zero in each treatment, I conducted separate one-sample *t*-tests using the weight change data.

Table 6.2. Number of colonies and number of nurse bee samples per colony (mean and standard deviation) that were assessed and averaged for nurse bee fat body and head weights. Sample numbers are given for each treatment and for each sampling period (pre-treatment and post-treatment).

Metric	Treatment	Pre-treatment		Post-treatment	
		Colonies	Nurse bees	Colonies	Nurse bees
Fat body weight	Antibiotic	8	7.13 ± 3.56	8	4.50 ± 2.78
	Antibiotic & soy	7	4.71 ± 2.93	6	5.00 ± 3.10
	Soy supplement	6	8.00 ± 4.10	8	5.25 ± 3.11
	Pollen supplement	7	6.43 ± 3.21	10	6.00 ± 3.16
	Probiotic	9	7.89 ± 3.86	9	4.89 ± 2.85
	Control	7	6.57 ± 3.05	9	5.00 ± 3.00
Head weight	Antibiotic	7	8.43 ± 7.14	6	2.33 ± 0.82
	Antibiotic & soy	7	4.00 ± 3.51	4	3.00 ± 1.41
	Soy supplement	4	7.50 ± 7.33	5	2.80 ± 0.45
	Pollen supplement	7	6.86 ± 5.73	5	2.20 ± 0.84
	Probiotic	8	7.88 ± 7.02	6	2.17 ± 0.75
	Control	6	6.83 ± 5.42	6	2.83 ± 0.41

Results

Effect of treatments on European foulbrood infection

At enrollment, 29 colonies had an EFB severity rating of 2, and 31 colonies had a severity rating of 3. Severity was not significantly different among treatments upon enrollment (X^2_5=4.22, p=0.52) (Figure 6.3). Colonies which were observed experiencing a queen event or which did not survive during the trial were excluded from the analysis (11 colonies). *Varroa destructor* was sufficiently controlled in the research colonies, with infestation rates below 2% for the entire experiment. Two-weeks post enrollment, after the conclusion of the treatment courses, there was a significant difference among treatments in the levels of EFB (X^2_5=25.38, p<0.01). Colonies which were treated with antibiotics, including both the antibiotic only treatment and the antibiotic with soy-based supplement treatment, had greater recovery than those which were not treated with antibiotics (Figure 6.3). Among the non-antibiotic treated

colonies, 5% had completely recovered, 26% had a severity rating of 1, 46% had a severity rating of 2 and 23% had a severity rating of 3. In contrast, 75% of all the antibiotic treated colonies had completely recovered and contained no signs of affected brood, 13% (2 colonies) had a severity rating of 1 and 13% had a severity rating of 2. By four weeks post-enrollment, which was two-weeks after treatment had concluded, 64% of all colonies showed no signs of disease. At this assessment, all antibiotic-only treated colonies fully recovered, resulting in a numerically singular Hessian matrix, meaning the effect of this treatment could not be estimated due to lack of variation. When the antibiotic-only treatment was excluded, there was no significant difference among any of the other treatments (X^2_4=6.49, p=0.17). Of the non-recovered colonies, 22% had a severity rating of 1, 8% had a severity rating of 2 and 5% had a severity rating of 3. Six weeks post-enrollment, four weeks after the conclusion of treatment, there was significant differences among treatments (X^2_5=14.22, p=0.01); the pollen-based supplement had a resurgence of the EFB signs. While all other treatment groups had recovered to an average severity rating between 0 and 1, 60% of the pollen-based supplement colonies had a severity rating of 2. This made the pollen-based supplement group higher in EFB severity than the control (β=3.57, z=2.98, p=0.03) or antibiotic-only (β=3.46, z=3.00, p=0.03) treatments.

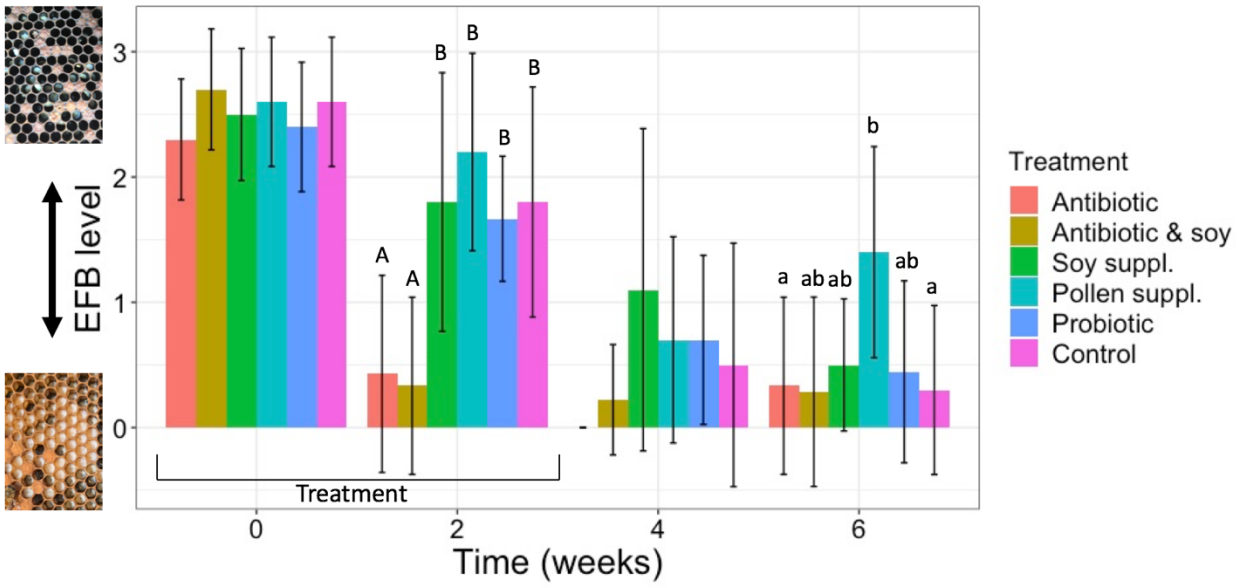

Figure 6.3. Mean severity of European foulbrood (EFB) in *Apis mellifera* colonies, before and after different treatments. Treatments were applied between week 0 and week 2 sampling rounds, indicated by "Treatment" below the bars for those weeks. EFB severity is rated on an ordinal scale: level 0: 0 diseased cells, level 1: 1-10 diseased cells, level 2: 11-100 diseased cells, and level 3: >100 diseased cells. Bars represent the standard deviation around the mean. Differences in severity ratings between treatments, within each sampling round are indicated by different letters. The effect of the antibiotic only treatment in week 4 could not be estimated due to full recovery in all colonies and lack of variation. Photo credit: Grace Haynes.

Treatment effects on colony cluster size

Upon enrollment, colony cluster size was on average 14.17 ± 3.31 frames of adult bees, and it increased slightly to 15.65 ± 6.80 frames after six weeks. There was no difference in colony growth between the different treatments ($F_{5, 54}$=1.84, p=0.12).

Nurse bee physiology

At the start of the experiment, nurse bee fat body weights ranged from 1.57 mg to 25.48 mg, with an average of 12.60 ± 5.94 mg. At the first post-treatment inspection, four weeks post enrollment, the fat body weights ranged from 5.19 mg to 33.71 mg and averaged 18.33 ± 6.63

mg. Nurse bee fat bodies were significantly heavier after the treatment than before treatment ($F_{1,93}$=20.40, p<0.01) (Figure 6.4). There was no significant difference among treatments pre-treatment ($F_{5,43}$=0.75, p=0.59), post-treatment ($F_{5,49}$=0.99, p=0.43), or in the change in fat body size pre- versus post-treatment ($F_{5,37}$=0.42, p=0.83). The only change in fat body size that was significantly different from zero was in the probiotics treatment (t_7=3.11, p=0.02), with an average weight increase of 7.94 ± 7.23 mg. Fat body weights increased the least in colonies treated with pollen-based supplement, with an average increase of only 2.29 ± 8.28 mg. This was even less than the control treatment which had an average increase of 4.15 ± 5.98 mg. The average increases in the other treatments were 5.89 ± 13.90 mg for the soy-based supplement treatment, the antibiotic treatment at 6.43 ± 6.92 mg, then the antibiotic with soy-based supplement treatment at 7.23 ± 9.79 mg.

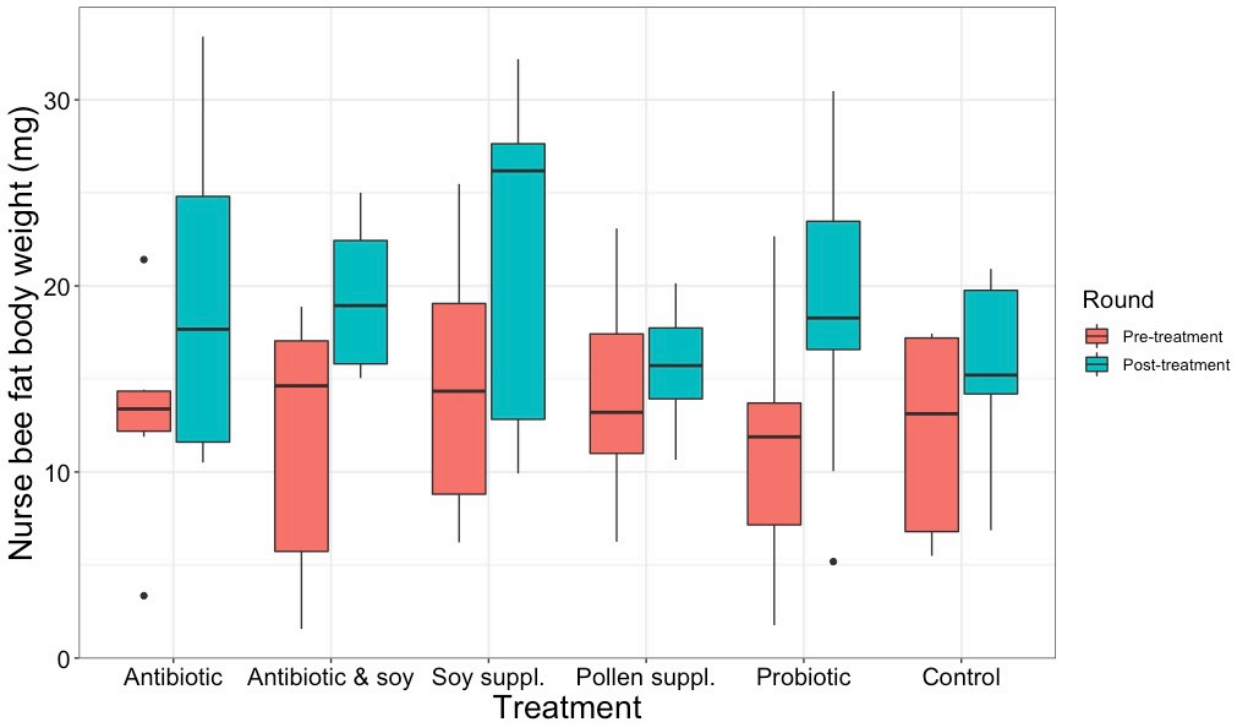

Figure 6.4. Weight (mg) of fat bodies in nurse bees sampled pre- and post-treatment with various antibiotic and nutritional colony treatments to manage European foulbrood. Boxplots depict the data distribution, with the center line indicating the median, and box representing the quartile ranges.

Nurse bee head weights ranged from 9.88 mg to 13.94 mg and averaged 12.34 ± 0.82 mg pre-treatment and from 10.43 mg to 14.29 mg, averaging 12.45 ± 0.92 mg at the first post-treatment inspection, four weeks post enrollment. The head weights did not change significantly during the experiment ($F_{1,70}$=0.69, p=0.42). There were also no differences among treatments pre-treatment ($F_{5,38}$=0.62, p=0.68), post-treatment ($F_{5,31}$=0.77, p=0.58), or in nurse bee head weight change pre- versus post-treatment ($F_{5,20}$=0.91, p=0.50). None of the changes in nurse bee head during the experiment were significantly different from zero for any treatment (Figure 6.5). However, on average the control colonies (-0.19 ± 0.15), soy-based supplement (-0.42 ± 0.64 mg), and pollen-based supplement colonies (-0.34 ± 0.88 mg) had lower nurse bee head weights post-treatment, while the antibiotic (0.61 ± 1.03), antibiotics with soy-based supplement (0.17 ±

0.58 mg), and probiotic colonies (0.42 ± 0.95 mg) each had heavier nurse bee heads post-treatment.

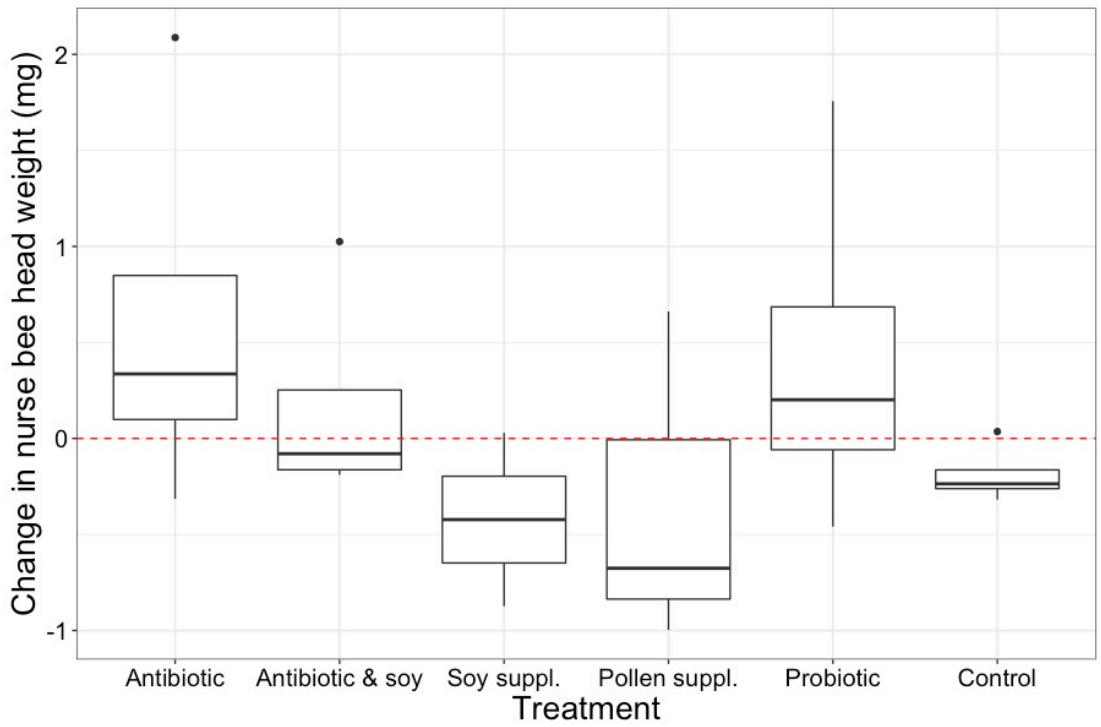

Figure 6.5. Change in the weights (mg) of honey bee nurse bee heads pre- versus post-treatment with various antibiotic and nutritional treatments. No change (y=0) is indicated with the dashed, horizontal line.

Discussion

In this study, I found initial benefits of antibiotics, where the levels of EFB expression in honey bees were reduced within two weeks, immediately after the treatment course. Within four weeks (two weeks post treatment course), however, there were no differences among treatments, and recovery maintenance was the same for antibiotic-treated and untreated colonies. I also found no significant difference among treatments in colony growth, indicating that the treatments provided no benefit or harm to colony size in EFB-infected colonies. None of these treatment

alternatives were as effective as antibiotics at clearing signs of EFB, nor were they any better than the untreated control. Therefore, these various supplemental feeding methods and the use of this probiotic do not seem effective as EFB treatment options in the field. General conclusions on the effectiveness of protein supplements and probiotics should not be drawn from this study. I chose to assess commercially available products to reflect what is available to beekeepers, rather than manipulating treatments along a single axis. It is unclear how the composition of the products in this study affected their ability to treat EFB. Further assessment of other alternative, sustainable treatment methods for EFB are thus still necessary and could benefit from well-defined and manipulated active ingredients/ substance.

In this study, the addition of in-hive treatment alternatives were costly interventions that provide little benefit or even negative effects. Such negative effects were seen in the pollen-based supplement colonies, which recovered at the same speed as the control colonies but later increased in disease severity four weeks post treatment. The colonies fed pollen-based supplement also had the smallest gain in nurse bee fat body weight and the greatest decrease in head weights of any of the treatments, though neither was significant. Lighter fat bodies can be an indicator of immune stress (Wilson-Rich et al. 2008; Alaux et al. 2010), which might be expected in diseased colonies. Lighter heads are correlated with smaller hypopharyngeal glands (Crailsheim and Hrassnigg 1998), which are important for brood food production. Brood food production may be particularly important in combating a brood disease such as EFB, particularly if EFB expression is related to nutritional stress (Bailey 1961).

The lack of difference in colony cluster size between treatments six weeks post-enrollment shows that any difference in recovery speed between treatments did not have a significant short-term effect on colony size, though it is unclear from this study what the long-

term effects of slower recovery might be in non-antibiotic treated colonies. Colony size is an important outcome for commercial beekeepers whose income from pollination contracts depends on colony size (Goodrich 2018). This finding also demonstrates that none of these treatments incur any additional benefits to colony growth, despite not having an effect on EFB recovery speed. It is important to note that during this time of year in Michigan, honey bee colonies have access to abundant, natural forage within their flight range (Chapter 5). Future studies could assess the impact of these different in-hive treatments on EFB recovery speed and colony in systems where forage is more limited.

The nurse bee physiological metrics that I measured were not affected by any of the antibiotic or nutritional treatments tested in this study. The lack of effect on nurse bee fat body or head weight further supports the conclusion that the tested alternative treatments did not affect colonies, as compared to untreated colonies. Furthermore, the lack of a treatment response suggests no downstream implications on colony health from any of the treatments. Nurse bee fat bodies increased in size during the study, and although seasonal increase in fat body size is expected in preparation for overwintering, such a notable increase so early in the summer is unexpected (Shehata et al. 1981). Only in the probiotic treatment did I find that nurse bees had fat body weight change that was significantly different from zero. Probiotics may increase weight gain by enhancing metabolism, which would allow for greater access to nutrients (Zheng et al. 2017). However, this finding should be interpreted cautiously as there were no treatment differences post-treatment or pre-treatment. Therefore, change may be an artifact of slightly lower pre-treatment weights. In application, it is unclear how artificially-induced larger fat bodies would affect colony functioning. For example, larger fat bodies in nurse bees could inhibit aging and the eventual transition to foraging (Alaux et al. 2018), which would be

detrimental to colony nutrition. I did not observe either lighter fat bodies or head weights in the antibiotic treated colonies, as found by (Li et al. 2019). It is possible that the effect of antibiotics on nurse bees is not very long lasting, as Li et al. (2019) collected nurse bees 7 days, rather than 14 days post-treatment. There was also large variation in my physiology data, suggesting a need for a larger sample size and/or marking of newly eclosed nurse bees to ensure consistency in the age of bees collected.

My finding that colonies receiving alternative treatments were no better than the untreated colonies at clearing EFB, along with the lack of treatment effect on colony cluster size and the absence of significant effects on nurse bee physiology, suggests that these forms of supplemental feeding or probiotics should not be used to address EFB incidence. This also suggests that the expense of using them is not warranted for affecting colony recovery from EFB. Antibiotics are still the most effective form of EFB treatment, clearing two weeks faster than any of the other tested treatments. Further studies should be conducted to determine viable and cost-effective treatment alternatives to antibiotics for treating EFB symptoms.

APPENDICES

APPENDIX A: Record of deposition of voucher specimens

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _2020-04_____

Author and Title of thesis:
Gabriela Marie Quinlan: Influence of landscape composition, landscape diversity, and conservation management on bee health via a pollen nutrition mechanism.

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-species	Life stage	Quantity	Preservation
Apidae	*Apis mellifera*	adult	1	pinned
Apidae	*Bombus impatiens*	adult	1	pinned

APPENDIX B: Supplementary material for chapter 2

Table B1. Land cover composition of wetlands, grassy-herbaceous fields (e.g. hay, wildflower, and pasture), staple crops (soybean, corn, and small grains), non-staple crops (e.g., vegetables, tree fruits, and vineyards), urban, and forests within 3.2 km of the 12 research apiaries (sites) as compared to the surrounding region, based upon 500 randomly generated centroid locations and 2 km buffers generated using the sp package in R (Pebesma et al. 2020). Ranges, means and variances are given in km^2. Analysis of variance (ANOVA) or Kruskal-Wallis rank sum test (for comparisons with unequal variance) was used to compare means of the study sites and the region (F-statistic or X^2 value, degrees of freedom and p-values provided). Two-sided F-tests were used to compare variances between study sites and the region (F-statistic, degrees of freedom and p-values provided). Asterisks indicate p-values significant at an $\alpha<0.05$.

Land cover	Range		Mean		Variance		ANOVA or K-W	F-test
	Sites	Region	Sites	Region	Sites	Region		
Wetland	1.84-12.07	0.10-12.62	4.84	3.99	7.81	5.48	$F_{1,511}=1.56$, p=0.21	$F_{11,499}=1.43$, p=0.31
Grassy-herb.	0.57-2.53	0.05-6.56	1.52	1.67	0.30	1.29	$X^2_1=0.02$, p=0.88	$F_{11,499}=0.23$, p=0.01*
Staple crop	0.68-15.82	0.00-25.64	7.82	10.29	26.27	43.50	$F_{1,511}=1.65$, p=0.20	$F_{11,499}=0.60$, p=0.34
Other crop	0.77-7.34	0.01-11.79	2.47	2.29	4.93	4.22	$F_{1,511}=0.08$, p=0.77	$F_{11,499}=1.17$, p=0.62
Urban	1.48-11.73	0.85-31.32	4.37	4.98	12.35	39.34	$X^2_1=5.14$, p=0.02*	$F_{11,499}=0.20$, p<0.01*
Forest	5.94-16.19	0.21-25.92	10.61	7.88	12.00	19.46	$F_{1,511}=4.52$, p=0.03*	$F_{11,499}=0.62$, p=0.37

APPENDIX C: Supplementary material for chapter 3

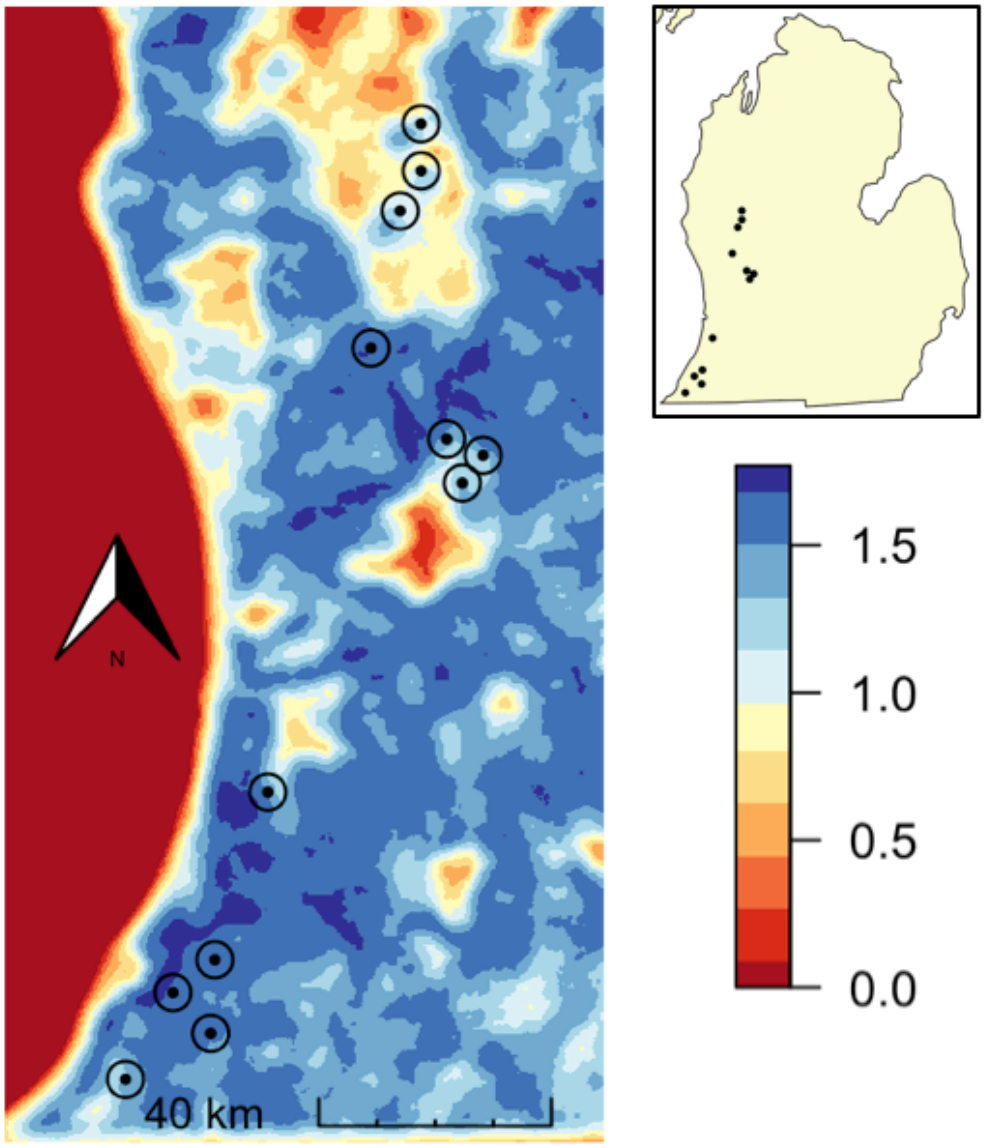

Figure C1. Land cover Shannon diversity heat map of southwest Michigan, based on seven simplified land covers from the 2018 Cropland Data Layer: urban, wetland, staple crop (corn, soy, small grains), other crop (tree fruit, vineyards, vegetables, etc.), grassy-herbaceous (pasture, grassland, fallowed cropland, etc.), forest, and NA (water or no data) and calculated over 4 km. Study apiaries locations are shown as points, surrounded by a 4 km radius. Study apiary locations surrounded by low land cover Shannon diversity (dark red) were limited by yard accessibility eg. Grand Rapids, MI in the center and Huron-Manistee National Forest to the north. Map code modified from whuber 2015.

APPENDIX D: Supplementary material for chapter 5

Table D1. Land use by area in square kilometers, within 4 km of each apiary including grassy-herbaceous habitat (hay, wildflower, switchgrass, fallow cropland, shrubland, grassland, and pasture, Conservation Reserve Program land, and roadside ditches), agriculture, forest, urban, and wetlands. Data layers were collated and had their area calculated in R (R Core Team 2020) using the raster (Hijmans et al. 2020) and rgeos (Bivand et al. 2019) packages, from the 2016 Croplands Data Layer (US Department of Agriculture National Agricultural Statistics Service 2016b), Conservation Reserve Program data, provided by the USDA Farm Service Agency through Inter-agency Agreement 16IAMRECRPHBTA1, and the All Roads data layer through Michigan Open Data (Michigan GIS Open Data 2019). Due to overlapping data layers from different sources and the exclusion of non-applicable land area (water and no data) some sites' land use does not sum to the area of a 4-km radius circle.

Site	Grassy-herbaceous	Agriculture	Forest	Urban	Wetland
A	4.29	25.89	13.17	4.52	4.29
B	3.41	27.37	12.89	3.03	4.62
C	2.42	27.27	9.22	4.04	7.22
D	4.97	8.24	21.46	6.42	10.05
E	3.96	8.88	16.19	3.00	17.76
F	6.25	21.87	12.13	4.98	7.42
G	5.32	17.09	9.57	8.53	10.30
H	7.20	4.48	22.48	14.60	4.88
I	6.18	3.24	22.82	13.90	4.98
J	5.30	4.73	20.11	15.52	3.40
K	3.54	27.87	12.81	2.53	4.57
L	3.00	16.28	20.52	2.34	8.08

Table D2. Pollen species identified with metabarcoding organized by year, in alphabetical order. Each species has an indication if the species was excluded and the reason for exclusion, either the species showed up in 10 or fewer reads per sample, or the species was a non-flowering species. Non-flowering species do produce spores that were likely picked up while the bees were collecting water. 67 angiosperm genera identified, of which 63 are present in Michigan according to the USDA plants database. Two others, Hydrangea and Capsicum, are very common garden plants. Heliotropium is in adjacent states and seems likely to be present in southern Michigan. The last genus is Kraschennikovia, which occurs in the Dakotas and could be a contaminant or erroneous assignment. This genus was censored anyways for having less than 10 counts. Thus, despite using assignment methods that were not constrained to the Michigan flora, the final assignments were in fact entirely consistent with that flora.

Year	Species	Excluded	Reason
2015	*Ageratina adenophora*	no	
2015	*Alopecurus aequalis*	yes	Under 10 reads
2015	*Amaranthus tuberculatus*	yes	Under 10 reads
2015	*Ambrosia artemisiifolia*	no	
2015	*Ambrosia trifida*	no	
2015	*Amorpha apiculata*	yes	Under 10 reads
2015	*Anemone canadensis*	no	
2015	*Arctium lappa*	no	
2015	*Artemisia absinthium*	no	
2015	*Bassia scoparia*	yes	Under 10 reads
2015	*Bolboschoenus caldwellii*	yes	Under 10 reads
2015	*Brassica napus*	no	
2015	*Brassica nigra*	yes	Under 10 reads
2015	*Brassica oleracea*	yes	Under 10 reads
2015	*Carduus acanthoides*	yes	Under 10 reads
2015	*Chamaecrista nictitans*	no	
2015	*Cirsium arvense*	no	
2015	*Dalea purpurea*	yes	Under 10 reads
2015	*Dupontia fisheri*	yes	Under 10 reads
2015	*Glycyrrhiza lepidota*	yes	Under 10 reads
2015	*Helianthus annuus*	no	
2015	*Hesperis sibirica*	no	
2015	*Hydrangea paniculata*	no	
2015	*Hydrophyllum tenuipes*	no	
2015	*Impatiens capensis*	no	
2015	*Phalaris arundinacea*	yes	Under 10 reads
2015	*Pisum sativum*	no	

Table D2 (cont'd)

Year	Species	Excluded	Reason
2015	*Ranunculus septentrionalis*	yes	Under 10 reads
2015	*Raphanus sativus*	no	
2015	*Rhamnus davurica*	yes	Under 10 reads
2015	*Rudbeckia hirta*	yes	Under 10 reads
2015	*Sagittaria montevidensis*	yes	Under 10 reads
2015	*Securigera varia*	no	
2015	*Sinapis alba*	no	
2015	*Sium suave*	yes	Under 10 reads
2015	*Sonchus arvensis*	no	
2015	*Sonchus megalocarpus*	yes	Under 10 reads
2015	*Sparganium eurycarpum*	no	
2015	*Symphyotrichum cordifolium*	no	
2015	*Trifolium hybridum*	no	
2015	*Zea mays*	no	
2015	*Zizia aurea*	yes	Under 10 reads
2016	*Achillea alpina*	yes	Under 10 reads
2016	*Ageratina adenophora*	no	
2016	*Agrimonia*	no	
2016	*Amaranthus*	no	
2016	*Ambrosia*	no	
2016	*Ambrosia artemisiifolia*	no	
2016	*Ambrosia trifida*	no	
2016	*Anemone*	yes	Under 10 reads
2016	*Anemone hupehensis*	no	
2016	*Arctium*	no	
2016	*Artemisia absinthium*	no	
2016	*Asparagus*	no	
2016	*Asparagus oligoclonos*	no	
2016	*Asteraceae*	no	
2016	*Atriplex*	yes	Under 10 reads
2016	*Bassia scoparia*	no	
2016	*Berteroa*	no	
2016	*Berteroa incana*	no	
2016	*Bidens*	no	
2016	*Capsicum*	no	
2016	*Centaurea*	yes	Under 10 reads
2016	*Centaurea stoebe*	no	
2016	*Cephalanthus*	yes	Under 10 reads

Table D2 (cont'd)

Year	Species	Excluded	Reason
2016	*Chenopodium*	no	
2016	*Chenopodium album*	no	
2016	*Cichorium*	yes	Under 10 reads
2016	*Cichorium intybus*	no	
2016	*Cirsium*	no	
2016	*Clematis*	no	
2016	*Clematis ligusticifolia*	no	
2016	*Clematis terniflora*	no	
2016	*Cucumis sativus*	no	
2016	*Cuscuta*	yes	Under 10 reads
2016	*Erigeron canadensis*	yes	Under 10 reads
2016	*Eupatorium*	no	
2016	*Eupatorium perfoliatum*	yes	Under 10 reads
2016	*Euthamia*	yes	Under 10 reads
2016	*Euthamia graminifolia*	no	
2016	*Eutrochium*	no	
2016	*Eutrochium dubium*	no	
2016	*Fabaceae*	no	
2016	*Fagopyrum esculentum*	no	
2016	*Grindelia*	yes	Under 10 reads
2016	*Helenium autumnale*	no	
2016	*Helianthus*	no	
2016	*Helianthus annuus*	no	
2016	*Heliotropium curassavicum*	yes	Under 10 reads
2016	*Hydrangea*	no	
2016	*Hydrangea paniculata*	yes	Under 10 reads
2016	*Hylotelephium*	no	
2016	*Impatiens*	no	
2016	*Impatiens capensis*	no	
2016	*Krascheninnikovia*	yes	Under 10 reads
2016	*Laportea*	no	
2016	*Lathyrus*	no	
2016	*Lotus*	no	
2016	*Lotus corniculatus*	no	
2016	*Lythrum*	no	
2016	*Lythrum salicaria*	no	
2016	*Medicago sativa*	no	
2016	*Melilotus*	no	

Table D2 (cont'd)

Year	Species	Excluded	Reason
2016	*Nepeta cataria*	yes	Under 10 reads
2016	*Oenothera*	yes	Under 10 reads
2016	*Oxalis stricta*	yes	Under 10 reads
2016	*Persicaria*	no	
2016	*Persicaria pensylvanica*	no	
2016	*Phytolacca*	no	
2016	*Phytolacca americana*	no	
2016	*Plantago*	no	
2016	*Plantago lanceolata*	no	
2016	*Ranunculaceae*	no	
2016	*Raphanus sativus*	no	
2016	*Rhaponticum*	no	
2016	*Rhaponticum uniflorum*	yes	Under 10 reads
2016	*Rosa*	yes	Under 10 reads
2016	*Rubus*	yes	Under 10 reads
2016	*Senecio*	yes	Under 10 reads
2016	*Silphium perfoliatum*	no	
2016	*Solanum*	yes	Under 10 reads
2016	*Solanum dulcamara*	yes	Under 10 reads
2016	*Solidago*	no	
2016	*Solidago canadensis*	no	
2016	*Sonchus*	no	
2016	*Sonchus arvensis*	yes	Under 10 reads
2016	*Sparganium*	yes	Under 10 reads
2016	*Sparganium eurycarpum*	yes	Under 10 reads
2016	*Spiraea*	no	
2016	*Symphyotrichum*	no	
2016	*Symphyotrichum cordifolium*	no	
2016	*Symphyotrichum novae-angliae*	yes	Under 10 reads
2016	*Tanacetum*	no	
2016	*Trifolium*	no	
2016	*Trifolium pratense*	no	
2016	*Trifolium repens*	no	
2016	*Triticum*	yes	Under 10 reads
2016	*Urtica*	yes	Under 10 reads
2016	*Verbascum*	no	
2016	*Vernonia*	no	
2016	*Zea mays*	no	

Table D2 (cont'd)

Year	Species	Excluded	Reason
2017	*Acer*	yes	Under 10 reads
2017	*Acer tataricum*	yes	Under 10 reads
2017	*Achillea*	yes	Under 10 reads
2017	*Adoxaceae*	no	
2017	*Agastache*	yes	Under 10 reads
2017	*Ageratina*	no	
2017	*Ageratina adenophora*	no	
2017	*Agrimonia*	no	
2017	*Alismataceae*	no	
2017	*Allium*	no	
2017	*Allium tricoccum*	no	
2017	*Alopecurus*	yes	Under 10 reads
2017	*Alternaria*	yes	Under 10 reads
2017	*Amaranthaceae*	yes	Under 10 reads
2017	*Amaranthus*	no	
2017	*Amaranthus tuberculatus*	no	
2017	*Amaryllidaceae*	no	
2017	*Ambrosia*	no	
2017	*Ambrosia artemisiifolia*	no	
2017	*Ambrosia trifida*	no	
2017	*Anacardiaceae*	no	
2017	*Andropogon*	no	
2017	*Anemone*	no	
2017	*Anemone canadensis*	yes	Under 10 reads
2017	*Anethum*	no	
2017	*Apiaceae*	no	
2017	*Arctium*	no	
2017	*Arctium lappa*	no	
2017	*Artemisia*	yes	Under 10 reads
2017	*Artemisia absinthium*	no	
2017	*Asparagus oligoclonos*	no	
2017	*Asteraceae*	no	
2017	*Astragalus*	yes	Under 10 reads
2017	*Balsaminaceae*	no	
2017	*Begoniaceae*	yes	Under 10 reads
2017	*Berteroa*	no	
2017	*Berteroa incana*	no	
2017	*Bidens*	no	

Table D2 (cont'd)

Year	Species	Excluded	Reason
2017	*Bidens frondosa*	no	
2017	*Brassica*	no	
2017	*Brassicaceae*	no	
2017	*Bryaceae*	yes	Non-flower species
2017	*Buddleja officinalis*	no	
2017	*Calystegia*	yes	Under 10 reads
2017	*Caprifoliaceae*	yes	Under 10 reads
2017	*Capsella*	yes	Under 10 reads
2017	*Capsicum*	yes	Under 10 reads
2017	*Capsicum annuum*	no	
2017	*Carduus*	no	
2017	*Carduus acanthoides*	no	
2017	*Carduus crispus*	yes	Under 10 reads
2017	*Carex*	yes	Under 10 reads
2017	*Centaurea*	no	
2017	*Centaurea stoebe*	no	
2017	*Cephalanthus*	no	
2017	*Cerastium*	yes	Under 10 reads
2017	*Chelidonium*	no	
2017	*Chenopodiaceae*	yes	Under 10 reads
2017	*Chenopodium*	no	
2017	*Chenopodium album*	no	
2017	*Chilodonellidae*	yes	Under 10 reads
2017	*Cichorium*	yes	Under 10 reads
2017	*Cichorium intybus*	no	
2017	*Cicuta*	no	
2017	*Cirsium*	no	
2017	*Cirsium arvense*	no	
2017	*Cirsium vulgare*	no	
2017	*Clematis*	no	
2017	*Clematis virginiana*	no	
2017	*Convolvulus arvensis*	yes	Under 10 reads
2017	*Coreopsis*	yes	Under 10 reads
2017	*Cornaceae*	yes	Under 10 reads
2017	*Crepis*	yes	Under 10 reads
2017	*Cucumis*	yes	Under 10 reads
2017	*Cucumis sativus*	yes	Under 10 reads
2017	*Cucurbita pepo*	yes	Under 10 reads

Table D2 (cont'd)

Year	Species	Excluded	Reason
2017	*Cyperaceae*	yes	Under 10 reads
2017	*Dactylis glomerata*	yes	Under 10 reads
2017	*Dalea purpurea*	no	
2017	*Dasiphora*	yes	Under 10 reads
2017	*Dasiphora fruticosa*	yes	Under 10 reads
2017	*Datura stramonium*	no	
2017	*Daucus*	no	
2017	*Daucus carota*	no	
2017	*Decodon*	yes	Under 10 reads
2017	*Decodon verticillatus*	no	
2017	*Dianthus*	yes	Under 10 reads
2017	*Diplotaxis*	no	
2017	*Dipsacus*	no	
2017	*Dipsacus fullonum*	yes	Under 10 reads
2017	*Doellingeria umbellata*	no	
2017	*Dulichium*	no	
2017	*Echinacea*	yes	Under 10 reads
2017	*Echinacea angustifolia*	no	
2017	*Erigeron annuus*	no	
2017	*Erucastrum gallicum*	yes	Under 10 reads
2017	*Erysimum*	yes	Under 10 reads
2017	*Eupatorium*	no	
2017	*Eupatorium perfoliatum*	no	
2017	*Eustoma exaltatum*	yes	Under 10 reads
2017	*Euthamia*	no	
2017	*Euthamia graminifolia*	no	
2017	*Eutrochium*	no	
2017	*Eutrochium dubium*	yes	Under 10 reads
2017	*Fabaceae*	no	
2017	*Fagaceae*	no	
2017	*Fagopyrum*	no	
2017	*Fagopyrum esculentum*	no	
2017	*Fallopia convolvulus*	yes	Under 10 reads
2017	*Gastrostyla steinii*	yes	Non-flower species
2017	*Glycine*	no	
2017	*Glycine max*	no	
2017	*Grindelia*	yes	Under 10 reads
2017	*Grindelia nana*	yes	Under 10 reads

Table D2 (cont'd)

Year	Species	Excluded	Reason
2017	*Hartmannellidae*	yes	Non-flower species
2017	*Helianthus*	no	
2017	*Helianthus annuus*	no	
2017	*Heliopsis helianthoides*	yes	Under 10 reads
2017	*Heliotropiaceae*	yes	Under 10 reads
2017	*Hesperis*	no	
2017	*Hydrangea*	no	
2017	*Hydrangea paniculata*	no	
2017	*Hydrangeaceae*	no	
2017	*Hypericaceae*	yes	Under 10 reads
2017	*Hypericum prolificum*	no	
2017	*Hypochaeris*	yes	Under 10 reads
2017	*Hypochaeris radicata*	no	
2017	*Imbribryum*	yes	Under 10 reads
2017	*Imbribryum blandum*	yes	Non-flower species
2017	*Impatiens*	yes	Under 10 reads
2017	*Impatiens capensis*	no	
2017	*Iva*	no	
2017	*Iva xanthiifolia*	yes	Under 10 reads
2017	*Juglans*	yes	Under 10 reads
2017	*Lactuca*	yes	Under 10 reads
2017	*Lamiaceae*	no	
2017	*Laportea canadensis*	yes	Under 10 reads
2017	*Leonurus*	yes	Under 10 reads
2017	*Lespedeza*	yes	Under 10 reads
2017	*Liatris*	yes	Under 10 reads
2017	*Lonicera*	yes	Under 10 reads
2017	*Lotus*	no	
2017	*Lotus corniculatus*	no	
2017	*Lotus japonicus*	yes	Under 10 reads
2017	*Lythraceae*	no	
2017	*Lythrum*	no	
2017	*Lythrum salicaria*	no	
2017	*Malvaceae*	yes	Under 10 reads
2017	*Medicago*	yes	Under 10 reads
2017	*Medicago lupulina*	yes	Under 10 reads
2017	*Medicago sativa*	no	
2017	*Melilotus*	no	

Table D2 (cont'd)

Year	Species	Excluded	Reason
2017	*Melilotus albus*	yes	Under 10 reads
2017	*Melilotus officinalis*	no	
2017	*Mentha*	yes	Under 10 reads
2017	*Molluginaceae*	yes	Under 10 reads
2017	*Monarda*	no	
2017	*Monarda fistulosa*	no	
2017	*Muridae*	yes	Under 10 reads
2017	*Nepeta cataria*	yes	Under 10 reads
2017	*Nuphar*	no	
2017	*Nuphar variegata*	no	
2017	*Nymphaea*	no	
2017	*Nymphaea odorata*	no	
2017	*Nymphaeaceae*	no	
2017	*Oenothera*	yes	Under 10 reads
2017	*Oxalis*	yes	Under 10 reads
2017	*Parthenocissus quinquefolia*	no	
2017	*Penthorum*	no	
2017	*Persicaria*	no	
2017	*Persicaria amphibia*	yes	Under 10 reads
2017	*Persicaria sagittata*	yes	Under 10 reads
2017	*Persicaria viscosa*	no	
2017	*Phacelia tanacetifolia*	no	
2017	*Phalaris arundinacea*	yes	Under 10 reads
2017	*Phleum pratense*	no	
2017	*Phryma*	yes	Under 10 reads
2017	*Physalis*	no	
2017	*Phytolacca*	no	
2017	*Phytolaccaceae*	yes	Under 10 reads
2017	*Plantaginaceae*	no	
2017	*Plantago*	no	
2017	*Plantago lanceolata*	no	
2017	*Poaceae*	no	
2017	*Polygonaceae*	no	
2017	*Populus deltoides*	yes	Under 10 reads
2017	*Potamogeton amplifolius*	no	
2017	*Potamogetonaceae*	yes	Under 10 reads
2017	*Quercus*	no	
2017	*Ranunculaceae*	no	

Table D2 (cont'd)

Year	Species	Excluded	Reason
2017	*Raphanus*	yes	Under 10 reads
2017	*Raphanus sativus*	yes	Under 10 reads
2017	*Ratibida*	no	
2017	*Rhamnaceae*	yes	Under 10 reads
2017	*Rhamnus*	no	
2017	*Rhaponticum*	yes	Under 10 reads
2017	*Rhaponticum uniflorum*	no	
2017	*Rhus*	no	
2017	*Rhus copallinum*	no	
2017	*Rosa*	no	
2017	*Rosa acicularis*	no	
2017	*Rosa carolina*	no	
2017	*Rosaceae*	no	
2017	*Rubiaceae*	no	
2017	*Rubus*	no	
2017	*Rudbeckia*	no	
2017	*Rudbeckia hirta*	yes	Under 10 reads
2017	*Rudbeckia laciniata*	no	
2017	*Rumex*	yes	Under 10 reads
2017	*Sagittaria*	no	
2017	*Salix*	yes	Under 10 reads
2017	*Sambucus*	no	
2017	*Sambucus canadensis*	no	
2017	*Sapindaceae*	yes	Under 10 reads
2017	*Saururaceae*	no	
2017	*Schoenoplectus*	yes	Under 10 reads
2017	*Schoenoplectus tabernaemontani*	no	
2017	*Scrophulariaceae*	no	
2017	*Securigera*	no	
2017	*Securigera varia*	no	
2017	*Senecio*	yes	Under 10 reads
2017	*Silene*	no	
2017	*Silphium*	no	
2017	*Silphium perfoliatum*	no	
2017	*Sium*	yes	Under 10 reads
2017	*Sium suave*	no	
2017	*Smallanthus*	yes	Under 10 reads

Table D2 (cont'd)

Year	Species	Excluded	Reason
2017	*Smallanthus maculatus*	no	
2017	*Solanaceae*	no	
2017	*Solanum*	yes	Under 10 reads
2017	*Solidago*	no	
2017	*Solidago canadensis*	no	
2017	*Solidago rugosa*	yes	Under 10 reads
2017	*Sonchus*	no	
2017	*Sonchus arvensis*	no	
2017	*Sorghum*	yes	Under 10 reads
2017	*Sparganium eurycarpum*	yes	Under 10 reads
2017	*Spathidiidae*	yes	Non-flower species
2017	*Sphagnum*	yes	Under 10 reads
2017	*Spiraea*	yes	Under 10 reads
2017	*Staphyleaceae*	yes	Under 10 reads
2017	*Symphyotrichum*	no	
2017	*Symphyotrichum cordifolium*	no	
2017	*Symphyotrichum novae-angliae*	no	
2017	*Symphyotrichum subulatum*	no	
2017	*Syringa*	no	
2017	*Syringa josikaea*	yes	Under 10 reads
2017	*Tanacetum vulgare*	no	
2017	*Taraxacum*	no	
2017	*Tephroseris*	yes	Under 10 reads
2017	*Tephroseris integrifolia*	yes	Under 10 reads
2017	*Tetradium daniellii*	no	
2017	*Thalictrum*	yes	Under 10 reads
2017	*Tilia*	yes	Under 10 reads
2017	*Trifolium*	no	
2017	*Trifolium nigrescens*	no	
2017	*Trifolium occidentale*	yes	Under 10 reads
2017	*Trifolium pallescens*	yes	Under 10 reads
2017	*Trifolium pratense*	yes	Under 10 reads
2017	*Trifolium repens*	no	
2017	*Urtica*	yes	Under 10 reads
2017	*Verbascum*	no	
2017	*Verbascum macrocarpum*	yes	Under 10 reads
2017	*Verbena hastata*	yes	Under 10 reads
2017	*Verbesina*	no	

Table D2 (cont'd)

Year	Species	Excluded	Reason
2017	*Vernonia*	no	
2017	*Vicia*	yes	Under 10 reads
2017	*Vitaceae*	no	
2017	*Xanthium*	no	
2017	*Zea*	no	
2017	*Zea mays*	no	
2017	*Zinnia violacea*	no	

Table D3. Mean and standard deviation (SD) of OTU sample counts by year, site, and sampling round.

Year	Site	Round	Mean	SD
2015	A	early July	709.24	4513.39
2015	A	early July	167.19	872.66
2015	A	late July	55.10	237.15
2015	A	late July	874.55	4513.78
2015	A	early August	440.83	1967.65
2015	A	early August	261.29	1332.60
2015	A	late August	162.14	1024.24
2015	A	late August	1433.88	7656.18
2015	A	early September	2065.40	10612.37
2015	A	early September	1200.07	6063.18
2015	C	early July	1125.88	5409.12
2015	C	early July	66.29	423.42
2015	C	late July	718.12	3990.84
2015	C	late July	151.57	896.71
2015	C	early August	366.86	1403.61
2015	C	early August	505.88	3270.74
2015	C	late August	291.50	1254.46
2015	C	late August	463.14	2396.92
2015	C	early September	1405.31	7390.00
2015	C	early September	77.21	489.67
2015	F	early July	454.81	2065.24
2015	F	early July	54.07	221.29
2015	F	early August	87.45	545.79
2015	F	early August	120.86	465.28
2015	F	late August	58.81	358.30
2015	F	late August	1875.00	7350.79
2015	F	early September	600.60	3622.47
2015	G	early August	1504.07	8819.45
2016	A	late July	21.29	124.98
2016	A	late July	144.41	946.82
2016	A	early August	42.03	223.74
2016	A	early August	91.95	597.38
2016	A	late August	181.21	1434.67
2016	A	late August	65.94	340.06
2016	A	early September	217.73	1295.82
2016	A	early September	309.04	2516.03

Table D3 (cont'd)

Year	Site	Round	Mean	SD
2016	B	late July	180.82	1529.26
2016	C	early August	276.80	1882.54
2016	D	late July	156.87	1018.89
2016	D	late July	479.46	3061.09
2016	D	early August	575.13	4135.78
2016	D	early August	275.04	2126.44
2016	D	late August	560.60	3245.28
2016	D	late August	619.31	4186.61
2016	D	early September	370.47	2254.60
2016	D	early September	193.64	1095.89
2016	E	late July	324.34	2126.89
2016	E	late July	40.81	184.32
2016	E	early August	543.60	3950.58
2016	E	early August	134.78	1019.74
2016	E	late August	122.69	637.72
2016	E	late August	371.29	1947.14
2016	E	early September	88.27	603.23
2016	E	early September	349.95	2482.46
2016	F	late July	27.14	106.23
2016	F	late July	30.38	124.99
2016	F	early August	564.68	4504.79
2016	F	early August	120.06	939.07
2016	F	late August	398.10	2071.83
2016	F	late August	309.68	1534.80
2016	F	early September	158.91	1215.67
2016	F	early September	103.83	772.28
2016	G	late July	126.60	825.49
2016	G	late July	398.35	3063.11
2016	G	early August	301.05	2181.81
2016	G	early August	141.16	1129.16
2016	G	late August	192.71	878.95
2016	G	late August	312.98	2201.20
2016	G	early September	118.57	635.77
2016	G	early September	327.61	1830.35
2016	H	late July	137.10	813.93
2016	H	late July	309.82	2336.18
2016	H	early August	921.25	5985.52
2016	H	early August	208.95	1400.69

Table D3 (cont'd)

Year	Site	Round	Mean	SD
2016	H	late August	324.33	1410.09
2016	H	late August	182.73	900.20
2016	H	early September	60.82	463.23
2016	H	early September	194.79	1312.51
2016	I	late July	449.11	3027.63
2016	I	late July	189.93	1359.61
2016	I	early August	467.96	3075.86
2016	I	early August	534.67	3294.05
2016	I	late August	153.72	1108.92
2016	I	late August	367.89	1903.95
2016	I	early September	155.87	848.13
2016	I	early September	104.88	576.76
2016	J	late July	1.47	4.62
2016	J	late July	540.47	3786.75
2016	J	early August	380.83	2418.36
2016	J	early August	509.71	3669.61
2016	J	late August	109.07	567.44
2016	J	late August	235.58	1180.66
2016	J	early September	150.40	1083.06
2016	J	early September	32.51	231.69
2017	A	early July	79.69	755.05
2017	A	early July	84.46	640.44
2017	A	late July	27.71	208.65
2017	A	late July	139.29	1127.73
2017	A	early August	21.40	182.84
2017	A	early August	63.26	679.38
2017	A	early September	187.49	1376.48
2017	A	early September	102.02	540.70
2017	B	early July	73.11	585.22
2017	B	early July	60.28	352.00
2017	B	late July	37.07	271.46
2017	B	early August	96.25	755.84
2017	B	early September	85.12	684.87
2017	B	early September	62.18	417.60
2017	C	early July	71.26	474.04
2017	C	early July	109.88	976.62
2017	C	late July	91.05	994.00
2017	C	late July	40.03	286.07

Table D3 (cont'd)

Year	Site	Round	Mean	SD
2017	C	early August	82.81	557.37
2017	C	early August	95.56	1058.62
2017	C	early September	90.57	601.92
2017	C	early September	71.78	403.25
2017	D	early July	121.41	985.88
2017	D	late July	89.43	1179.25
2017	D	late July	52.10	409.29
2017	D	early August	84.60	605.27
2017	D	early August	70.82	624.98
2017	D	early September	125.58	827.22
2017	D	early September	94.88	619.66
2017	E	early July	97.74	771.76
2017	E	early July	65.78	378.17
2017	E	late July	23.57	123.90
2017	E	late July	68.94	561.15
2017	E	early August	0.18	0.91
2017	E	early August	92.15	680.67
2017	E	early September	45.28	290.42
2017	E	early September	90.33	689.34
2017	F	early July	18.50	138.87
2017	F	early July	129.38	1330.76
2017	F	late July	86.79	501.43
2017	F	late July	65.79	486.97
2017	F	early August	35.68	192.69
2017	F	early August	126.14	826.09
2017	F	early September	70.36	355.35
2017	F	early September	35.27	204.42
2017	G	early July	162.51	1915.64
2017	G	early July	86.09	895.20
2017	G	late July	136.90	1850.56
2017	G	late July	45.06	526.80
2017	G	early August	36.28	353.63
2017	G	early August	178.01	1898.26
2017	G	early September	51.84	374.73
2017	G	early September	88.81	560.49
2017	H	early July	84.04	735.72
2017	H	early July	69.94	776.30
2017	H	late July	39.40	258.52

Table D3 (cont'd)

Year	Site	Round	Mean	SD
2017	H	early August	73.45	473.10
2017	H	early August	92.81	737.07
2017	H	early September	113.61	796.80
2017	H	early September	109.25	830.40
2017	I	early July	91.07	877.55
2017	I	early July	99.33	953.96
2017	I	early August	107.75	843.14
2017	I	early August	158.12	1157.25
2017	I	early September	28.22	184.62
2017	I	early September	61.40	484.18
2017	J	early July	96.65	945.61
2017	J	early July	102.15	1003.06
2017	J	late July	67.18	422.39
2017	J	late July	54.92	304.31
2017	J	early August	68.24	404.50
2017	J	early August	56.93	362.03
2017	J	early September	70.61	513.76
2017	J	early September	0.42	2.83
2017	K	early July	35.77	234.44
2017	K	early July	71.85	510.01
2017	K	late July	83.51	618.69
2017	K	late July	113.22	819.07
2017	K	early August	84.20	632.38
2017	K	early August	57.93	305.68
2017	K	early September	84.00	512.94
2017	K	early September	111.11	1011.99
2017	L	early July	39.24	284.91
2017	L	early July	19.74	145.80
2017	L	late July	82.75	594.24
2017	L	late July	66.08	659.27
2017	L	early August	69.87	513.68
2017	L	early August	101.37	1127.26
2017	L	early September	113.64	838.85

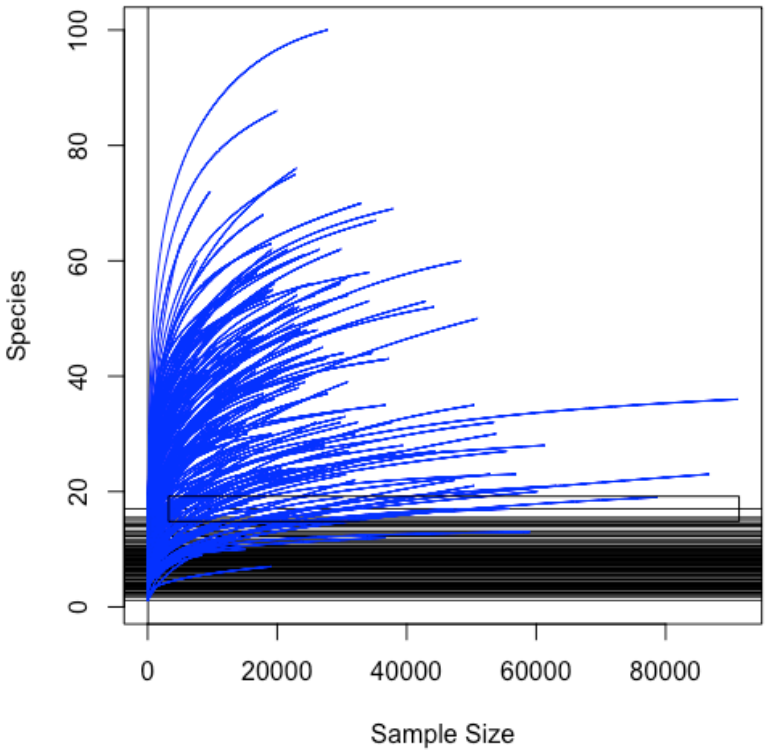

Figure D1. Rarefaction curves for taxa identified by DNA metabarcoding, indicating the species accumulation by sample size.

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