UNIVERSITY BUSINESS ACADEMY IN NOVI SAD
FACULTY OF ECONOMICS AND ENGINEERING MANAGEMENT
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CONTEMPORARY APPROACHES IN ENHANCING HONEY BEE (*Apis mellifera*) NATURAL HABITATS

DOCTORAL DISSERTATION

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	This area represents a model landscape for other agricultural suitable parts and can be used to understand how bees respond to agricultural intensification and may provide valuable insights into the future of pollinator health. For this doctoral thesis, we examined the responses of both wild and managed bees to row-crop agriculture, by investigating population, colony, and individual metrics of health both longitudinally over time and spatially, across landscapes with different extents of agricultural industrialization. In addition, we explored two ways in which landscape diversity may help to mitigate bee health declines in monoculture crop landscapes: diversified fruit and vegetable farming, and perennial habitat. Overall, we found that landscape diversity, not honey bee presence, positively influence the wild bee community. In contrast, managed honey bees had a positive response to row-crop agriculture with higher populations and colony health in landscapes with more production of corn and soybean; however, these colonies ultimately declined in the late season, i.e., post-crop senescence. Diversified farming through fruit and vegetable production resulted in small increases in abundance and richness of a subset of the wild bee community during parts of the season. Honey bee colonies and individual bees were healthier on fruit and vegetable farms compared to monocrop soybeans; however, honey bees still declined in the late season. Native perennial habitat was able to mitigate late season honey bee declines and may be a promising habitat type able to support both wild and managed bees in heavily cultivated row-crop agricultural systems. These studies underline the importance of landscape and farm diversity in supporting the health of honey bee (*Apis mellifera*).
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ABSTRACT

Honey bee populations are declining across Europe with populations particularly at risk in the west where vast areas of land have been converted into extensive row-crop agricultural systems, resulting in homogenous landscapes with reduced forage availability. These declines are problematic as bees are an essential part of maintaining natural ecosystems, and honey bees contribute to the pollination of over a hundred crops. Vojvodina has been identified as a critical area for pollinator conservation and is an ideal location to study agriculture-related bee declines. This area represents a model landscape for other agricultural suitable parts and can be used to understand how bees respond to agricultural intensification and may provide valuable insights into the future of pollinator health. For this doctoral thesis, we examined the responses of both wild and managed bees to row-crop agriculture, by investigating population, colony, and individual metrics of health both longitudinally over time and spatially, across landscapes with different extents of agricultural industrialization. In addition, we explored two ways in which landscape diversity may help to mitigate bee health declines in monoculture crop landscapes: diversified fruit and vegetable farming, and perennial habitat. Overall, we found that landscape diversity, not honey bee presence, positively influence the wild bee community. In contrast, managed honey bees had a positive response to row-crop agriculture with higher populations and colony health in landscapes with more production of corn and soybean; however, these colonies ultimately declined in the late season, i.e., post-crop senescence. Diversified farming through fruit and vegetable production resulted in small increases in abundance and richness of a subset of the wild bee community during parts of the season. Honey bee colonies and individual bees were healthier on fruit and vegetable farms compared to monocrop soybeans; however, honey bees still declined in the late season. Native perennial habitat was able to mitigate late season honey bee declines and may be a promising habitat type able to support both wild and managed bees in heavily cultivated row-crop agricultural systems. These studies underline the importance of landscape and farm diversity in supporting the health of honey bee (*Apis mellifera*).

1. INTRODUCTION

Human caused adaptations of natural ecosystems have led to global impacts on biodiversity, including insects, with one of the most extreme causes being the conversion of natural landscapes into row-crop agricultural systems (Halfawi et al., 2022). Some of the most notable declines in insect biodiversity have been observed with bees. In the Europe, declines of bees are well documented, with the lowest abundance of bees observed in regions which are committed to extensive agricultural production of large monoculture commodity row-crops such as corn and soybeans (Puvača et al., 2021).

In addition to worldwide reductions in bee populations, the global stock of managed honey bees is growing slower than the demand for agricultural production (Lika et al., 2021; Taric et al., 2019; Zakour & Bienefeld, 2014). In the Europe, beekeepers are experiencing high annual losses of managed honey bee colonies, with losses regularly exceeding the acceptable rate. In the past decade the beekeepers frequently lose as many as 60% or more of their annual stock of honey bees, a level that is considered four times higher than what is considered sustainable for beekeeping and substantially greater than colony losses historically reported for this region (Kahono et al., 2018; Kovačić et al., 2020; Neov et al., 2019, 2021; Page & Fondrk, 1995; Papa et al., 2022).

Declines in wild and managed bees are alarming as they provide an essential ecosystem service through pollination; the services provided support 35% of the global food supply (Puvača, 2018). In addition to honey bees, wild bees can be efficient pollinators of crops, contributing to 20% of crop pollination requirements. Despite their contributions, the demand for agricultural production outweighs the supply (i.e., abundance) of bees (Al Naggar et al., 2018; Frunze et al., 2021; Halfawi et al., 2022).

Multiple interacting factors drive wild and managed bee declines, including pesticides, disease, and reduced nutritional resources, all of which stress bees in complex and poorly understood ways (Goulson et al., 2015). In extensive agricultural systems where resource and habitat abundance and diversity are limited, effects of these stressors are likely exacerbated (O'Neal et al., 2018). Simplified, large-scale agricultural systems have been suggested to create one of the most inhospitable conditions for both wild and managed bees. The invention of herbicide tolerant crops as well as the widespread use of insecticides such as neonicotinoids has resulted in vast areas of land committed to crop production with very little presence of weeds or pests (McNeil et al., 2020). These types of systems have been popularized as "green deserts" or "agricultural deserts" for bees. Although stressors are not independent in their effects on bees, it has been argued that the main driver of decline in bee populations in agricultural systems is caused by a reduction in resource availability because of habitat loss and landscape conversion (Becher et al., 2013; Engel et al., 2016; Puvača, 2018).

In addition, wild bees in agricultural landscapes already under stress from resource limitation may be further impacted by the presence of managed honey bee colonies, which are often integrated for pollination services (Chakrabarti et al., 2015). Although honey bees are essential for crop pollination, in the Europe they are a non-native pollinator that can result in negative impacts on wild bee communities through competition for floral resources or transmission of disease (Corbet et al., 1991; Halfawi et al., 2022). Despite evidence for some negative effects of honey bees on wild bees, the severity of their impact is not well-understood, and has been variable across studies, particularly in agricultural landscapes. There is still a critical knowledge gap concerning how the presence of managed bees affects wild bee communities, specifically the interaction of honey bee presence within agricultural landscapes on wild bee abundance, richness and diversity (Mallinger et al., 2017). Furthermore, throughout an entire growing season, it is not clear how large scale agricultural systems effect honey bee health. Longitudinal studies are important because floral resources are highly seasonally variable, and many crops are in bloom for only a small fraction of the growing season (Park et al., 2015). For efforts to improve the health of wild and managed bees in agricultural landscapes to be effective (Spivak et al., 2011), it is vital to fully understand to what degree extensive agricultural land conversion impacts bees, as well as the potential interaction between honey bees and wild bees in these settings (Patel et al., 2021).

The region of Vojvodina is an ideal location to study agriculture-related bee declines, as it has been identified a critical area for pollinator conservation (Prodanović et al., 2019). The region of Vojodina is over 70% committed to farm operations with 59% in production of annual crops (predominately corn and soybean), creating a unique opportunity to study bee declines in the context of large scale farming (Vapa-Tankosić et al., 2020). With demands for agricultural production expected to increase, many regions worldwide may undergo extensive conversion of the landscape, similar to what has already occurred in Vojvodina. Vojvodina thus represents a futuristic or model landscape for other parts of the world (Prodanović et al., 2019; Vapa-Tankosić et al., 2020). Results from these studies in Vojvodina can be used to understand how bees respond to extensive agricultural land conversion and may provide valuable insights into the future of pollinator health. The goal of this dissertation is to assess to what degree agricultural development and landscape diversity affect bee communities and honey bee health, as well as assess the how the presence of honey bees may affect wild bees in this type of agroecosystem (Nicholls & Altieri, 2013).

2. REVIEW OF LITERATURE

2.1. The honey bee (*Apis mellifera*)

The honey bee, *Apis mellifera*, belongs to the insect order Hymenoptera, which features over 100,000 species of sawflies, wasps, ants and bees (Seeley & Morse, 1976). Most insects within the order Hymenoptera exhibit haplodiploid sex determination (males from unfertilized haploid eggs and females from fertilized diploid eggs) which is thought to be a basis for the evolution and maintenance of eusociality (Beekman & Ratnieks, 2000). Hymenoptera diverged from Diptera and Lepidoptera over 300 million years ago to form an ancient lineage of bees that evolved in tropical Eurasia and migrated north and west, reaching Europe at the end of the Pleistocene, 10,000 years ago (Yu et al., 1984).

The honey bee genus (*Apis L.*) is the most well recognised of all insects due to the component species services to agriculture, pollination and mankind (Page & Peng, 2001). This genus includes the giant honey bees (*Apis dorsata* and *Apis laboriosa*), the dwarf honey bees (*Apis florae* and *Apis andreniformis*), the eastern hive bees, (*Apis cerana*, *Apis nigrocinca*, *Apis koschevnikovi*, *Apis nuluensis*) and the western hive bees *Apis mellifera*, for which there are over 24 different breeds (Halfawi et al., 2022).

Apis mellifera can be grouped into four bio geographical branches: African (A), Oriental (O), Northern Mediterranean (C) and West European (M). European honeybees (M-lineage) are thought to have survived the last glacial period in two refugia, one on the Iberian peninsula and one on the Balkan peninsula (C-lineage) (Garnery et al., 1992). After the glacial retraction 10,000 years ago the honeybees re-colonized Europe with the M-lineage occupying north and west Europe and the C-lineage occupying central Europe. Geographical barriers such as the Alps maintained the differentiation of subspecies (Garnery et al., 1992).

Only *A. mellifera* is found in the UK (Hawkins & Martin, 2021), and there is evidence that the subspecies *A. m. mellifera* travelled into Britain across the European land bridge well before 8500BP (Thompson, 2010). In fact, it has been shown that the honeybee's range was closely linked with hazel and lime distribution (Ruttner, 1988). In 6500BP oak and hazel forests extended as far north as Skye in the west and Buchan in the east so as environmental conditions eased honeybees could have travelled with the advancing tree lines. Wild honeybees could have reached Britain from remnant populations in France within 1100 years, if they were to swarm once every second year and travel a conservative 1.5 km to their new colony site (Jules & Shahani, 2003).

2.2. Bee keeping

Honey storing insects are all social and living in colonies, most of which are bees but wasps and ants also have this ability. The evolution of honey bees led to two very advanced cavity nesting species who's nest would contain numerous parallel combs: *Apis cerana* and *Apis mellifera* (Yang et al., 2010). By forming clusters within the cavity these two species developed the ability to survive cold winters and therefore extended their distribution. *Apis mellifera* has been and is the most important species to man (Haldane & Spurway, 1954). Indeed, this specie is both productive and amenable to management. It is often called the European honey bee or the western honey bee even though it is not native to Europe (Kotthoff et al., 2013).

A honey bee colony represents tens of thousands of individuals divided into three main categories (Schmickl & Crailsheim, 2004):

The queen: she is the central element of the colony by ensuring its survival. Through pheromone secretion she regulates the colony's activities and ensures the cohesion of the worker bees. But mostly she is the only one capable of laying eggs providing future worker bees that will forage food for the colony among many other tasks. Shortly after hatching, the young queen leaves the colony for her mating flight. She returns to the hive mated and begins to lay eggs (1500 – 3000 eggs a day) (Bloch et al., 1996).

The drones: They hatch mainly over spring and their main known tasks consist in mating a queen during her mating flight. The mating process is lethal to the drones (Page et al., 1995).

The worker bees: They represent the bulk of the colony, around 30 000 in a healthy hive. They ensure the survival of the colony by many aspects: the maintenance of the hive (cleaning the bottom board, the empty cells, etc.), breeding the larvae, building the combs, protecting the hive, foraging food. The task they are given is function of their age and the colony's needs (Kimura et al., 2011).

The Dadant beehive is the model used by a large majority of beekeepers in Europe (Figure 1). It is divided into two main parts: the brood box in which the queen lays the eggs, constituting the brood and the honey super in which the queen cannot go because of a bee excluder (a grid with holes of a precise diameter letting the worker bees through only). The queens' access is reduced to the brood box and therefore workers use the honey super to store the collected nectar. It is this box that the beekeeper will harvest (Wakjira et al., 2021).

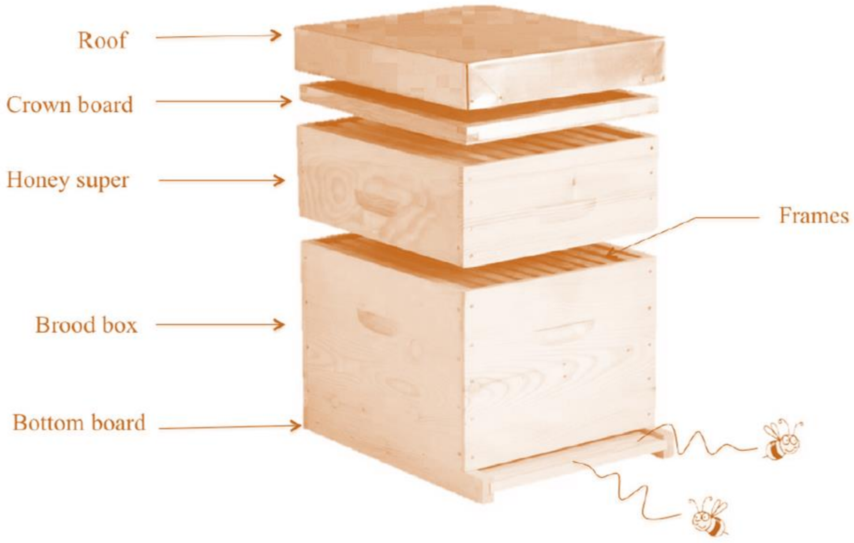

Figure 1. Structure of a Dadant hive.

The colony is segmented into three parts (Horng, 2011):

The adult population: Mainly found in the brood box it can also spread to the honey super when it is populous. The foraging bees come and go throughout the day, it mostly depends of the climate (temperature, precipitation, wind), the environment (resource availability) and the colony's needs (Meikle et al., 2019).

The brood: It represents the reproductive investment of the colony, it is composed of all the future colony population: eggs, larvae, and pupae in capped brood. In the hive the brood nest is found in the middle on the central frames of the brood box. This organisation allows the brood to stay in an environment with its optimal temperature (34-35°) and hygrometry (50-60%). The development of a worker bee lasts approximately 21 days (Allen, 1958).

The honey reserves: Composed of the nectar and pollen foraged by the worker bees. Nectar foragers returning to the hive pass their loads to younger bees through trophallaxis. It is then deposited in the combs where it will be processed by other bees into honey (Requier et al., 2015).

Returning pollen foragers store their loads in empty cells close to the area of the nest (Figure 2).

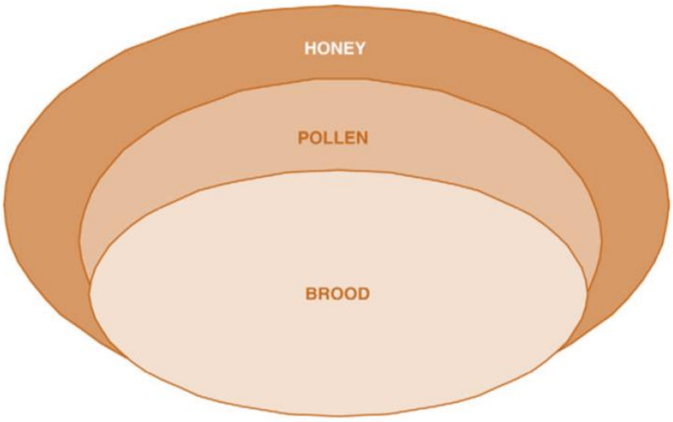

Figure 2. A diagram of a comb drawn from near the centre of a honeybee nest

Several naturally occurring events take place during a colony's' life (Dreller & Tarpy, 2000):

The queens' death: It can occur accidently or naturally. The colony is considered as orphan. If there are queen cells or a young queen (that hasn't been mated yet) the hive is said to be in a "requeening" process. On the contrary if there is no queen to be the worker bees will start laying eggs, giving birth to drones only. The hive is considered as a "drone colony" and will collapse (Gilley, 2001).

Swarming: It occurs mainly in spring but also throughout summer. Healthy and populous colonies may choose to swarm: they will set up queen cells and the previous queen will leave the hive with many worker bees in order to settle somewhere else. Many factors can provoke swarming: the environment, anthropogenic disturbances and some species are genetically susceptible to swarming (tropical bees) (Charlwood & Jones, 1980).

Starvation: When the environmental resources are scarce or when the climate does not allow worker bees to forage, the colonies development is directly affected. Starvations may have carry-over effects on the dynamic of the colonies for the rest of the season (Hunt et al., 2004).

Disease: Many diseases and parasites infections can weaken a colony by attacking the brood or the adults. Among the most common disease are the European & American foulbroods (*Melissococcus plutonius*, *Paenibacillus larvae*). It is known as a bacterial brood disease lethal to the colonies if no treatment is carried out. Another parasite destroying the brood is the wax moth that settles in combs, slowly developing into a plague that will force the colony to leave its hive (Halfawi et al., 2022; Martin, 2001; Puvača et al., 2021). Regarding the adult population, the most devastating parasite is the famous Varroa destructor. It is an external parasite that attacks both adult and pupae. It is native to Asia where it's natural host, the Asian honeybee (*Apis cerana*) lives. The mite rarely negatively affects *Apis cerana* since it has developed some natural defences against it. Varroa's host shift to *Apis mellifera* resulted in a devastating decrease of *Apis mellifera* colonies that did not have the natural defences to fight Varroa destructor (Martin, 2001).

Predation: Honey bees are attractive prey for many predators, birds, spiders, insects, but the current focus has been given to the Asian wasp (Rondeau et al., 2018). This imported predator, *Vespa velutina*, was first seen in France and in Europe in 2005. It is a well-known honey bee predator, against which *Apis mellifera*, unlike *Apis cerana*, has not been trained to fight. *Vespa velutina* feeds on honey bees, mostly forager bees, coming back to the hive with pollen and nectar. It beheads it's pray, removes its wings and legs and brings the thorax back to its colony (Rondeau et al., 2018).

Honey bee forage both pollen and nectar to meet their food requirements. Nectar or honeydew represents their natural source of carbohydrates which allows them to meet their energetic expenses (Lika et al., 2021; Puvača, 2018). Foragers collect nectar from the flowers, transport it to the hive and store it into sealed cells as honey. During the returning flight the transformation process of nectar into honey starts (Puvača, 2018).

On the other hand, pollen is the only natural protein and lipid source for honey bees. It is consumed both by adults and larvae and is often consumed shortly after being brought back to the hive. Honey bees mix regurgitated nectar with pollen and store it in small quantities the mixture is called beebread (Halfawi et al., 2022). The weight of pollen in the amount of honey reserves of a bee colony is minor. Regardless of its weight pollen plays a key role in the accumulation of honey reserves. The pollen intake will influence the brood size and in fine the number of bee workers. Added to this indirect effect pollen influences positively bee health and is therefore crucial for the colony resilience to diseases (Frias et al., 2016).

The impact of pollination service on agricultural production is widely acknowledged. Pollination consists in pollen transfer from the anther to the stigma of a same or different flower (Frias et al., 2016). This is the first step in the fertilisation process. Among various dissemination agents different animals can contribute to this step among which the invertebrates and more specifically insects (Becher et al., 2013; Corbet et al., 1991; Horng, 2011; Kimura et al., 2011; Puvača, 2018).

Honey bees are considered as the main insect pollinator in agricultural landscapes (Quigley et al., 2019). This is due to the high number of individuals within one nest. As mentioned earlier in this report, in Europe 84%, meaning 150 grown crops, directly depend on insect pollination (Iwasaki & Hogendoorn, 2021). At the international scale, 70% of the crops grown for human consumption, corresponding to 87 of the 124 crops grown directly for human consumption rely on animal pollination to produce and/or increase its production. The level of crop dependency to insect pollination varies from a crop to another (Eeraerts et al., 2019; Holzschuh et al., 2008; Iwasaki & Hogendoorn, 2021).

Losing all pollinators would have sizeable effects on international food security (Tscharntke et al., 2012), leading to the average reduction of 8% of the agricultural production (van der Sluijs & Vaage, 2016). However this scenario should be considered with care since a major part of the calories used in human consumption come from crops that are not dependent on pollination such as wheat, rice and corn (Richards, 2001).

2.3. Composition of landscape

Honey bees forage pollen and nectar on specific plants: melliferous plants. A melliferous plant produces substances that can be collected by insects and turned into honey (Eeraerts et al., 2019; Frias et al., 2016; Gilley, 2001; Halfawi et al., 2022; Iwasaki & Hogendoorn, 2021; Lika et al., 2021; Martin, 2001; Prodanović et al., 2019; Puvača, 2018; Quigley et al., 2019; Rondeau et al., 2018). Many plants are melliferous however not all produce both nectar and pollen that can be harvested by honey bees, for instance rapeseed and sunflower produce both nectar and pollen. In the landscape melliferous plants can be grown as well as wild (Kotthoff et al., 2013).

In order to ensure its survival, reproduction and development honey bee colonies require a large diversity of melliferous plants (Gratzer et al., 2021). In the current agricultural context the landscape is almost entirely composed of agricultural land thus the largest food supply for honey bees comes from field crops, vegetable growing and grasslands. Melliferous field crops are mainly: oilseed crops such as Rapeseed (*Brassica napus* L.) and Sunflower (*Helianthus annuus* L.), protein crops such as faba beans (*Vicia faba* L.) and others such as buckwheat (*Fagopyrum esculentum* M.). Field crops are commonly grown for their grain on vast areas of land with minimum labour (Gratzer et al., 2021). Their blooming period occurs massively on a very short period of time. These crops are very attractive for beekeepers because of their high melliferous potential, however the intensive use of crop protection products endangers honey bees. Many vegetable plants such as pumpkins, carots, onions and many others, are melliferous despite their scarce blooming. Grasslands for animal consumption usually host several melliferous plants such as alfalfa (*Medicago sativa* L.) and white (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) (Masierowska et al., 2018).

Wild floral resources are all the resources that are not cropped by humans: weeds, hedges, woods, grass strips, etc. Starting from the end of the 2nd world war, European and National agricultural landscape have been strongly modified in order to meet the growing food requirements. The regrouping of agricultural land led to farm expansion and a progressive decrease of semi natural habitats, hedges and grasslands that would only take up land needed for growing food. Land use intensification led to a shift in the spatial organisation of the landscape with obvious effects on agrobiodiversity. The fragmentation of the semi natural habitats, appropriate for nesting, feeding, mating, etc., causes the loss, in quality and quantity, of favourable habitats for biodiversity. All the processes combined: fragmentation, homogenisation, decrease of semi natural habitats, intensification progressively lead to the erosion of the agrobiodiversity.

A strong diversity of wild floral resources can be encountered in the grass strips along the roads or the fields. However, their intensive mowing progressively reduces their occurrence and limits their attractiveness for pollinators.

Together with the landscape changes, agricultural practices became more intensive with an increase in pesticide use depriving pollinators from vital floral resources. For instance, cereal fields are not very attractive for honeybees (Decourtye et al., 2010), however the weeds they host have widely been recognised as extremely interesting for the pollen supply of honey bee colonies (Odoux et al., 2012). The intensive weeding and in particular the use of pesticide or the thorough cleaning of the seeds is leading to their decline, excluding them from the core of the field and reducing their growth to the field margins (González-Varo & Vilà, 2017).

2.4. Weakening of honey bees

Recent public and scientific interest for honey bees occurred when the sharp disappearance of worker bees from a colony was described as colony collapse disorder (Vercelli et al., 2021). From there on, research efforts have focused on improving colony health and management techniques and identifying possible causes of colony collapse disorder (Paris et al., 2018). The population of honey bees are decreasing worldwide, this phenomena has been detected in Europe, many parts of the USA and in Asia (Adjlane et al., 2016).

In Europe the number of colonies decreased from 21 million in 1970 to 15.5 million in 2007. Between 1985 and 2005, for 18 European countries the mean rate of colony losses reached 16%. Considering the extent of this decline it was defined as: Colony Collapse Disorder (CCD) (J. D. Ellis et al., 2010; Watson & Stallins, 2016; G. R. Williams et al., 2010).

Since 1975, the number of publications related to honey bee colony losses has increased exponentially. To explain honey bee decline many factors have been proposed, they can be grouped into three broad categories of causes: Parasites and Pathogens, Genetic diversity and vitality and Environmental stress (Watanabe, 2008).

This third group accounts for about 31.3% of the publications on honey bee colony losses, it is composed of three different subgroups: Pesticides, flower availability and habitat loss (Noël et al., 2020).

The pesticide subgroup shows over 56% of the literature occurrence frequency, since honey bees extensively forage on flower-blooming crops such as rapeseed, maize (*Zea mays* L.) and sunflower, they are exposed to a high number of pesticides. The increase in pesticide uses has largely been blamed for honeybee colonies losses due to their lethal composition (Odemer et al., 2018). A recent law was voted prohibiting the use of neonicotinoids insecticides by 2018. Neonicotinoids are systemic insecticides, the three most virulent molecules being: Imidacloprid, thiametoxam and clothianidin. These insecticides in a sub lethal concentration will alter the behaviour of bees and thus reduce the survival of entire colonies. Moreover, honey bees cannot taste neonicotinoids and therefore are not repelled by them. Exposing social bees to these insecticides presents a sizeable hazard (Odemer et al., 2018).

Habitat loss is sometimes referred as a cause of honeybee colony losses. Habitat loss acts negatively on biodiversity through a decrease of nesting and foraging sites (Siede et al., 2018).

Though floral resources without doubt have an impact on the honey bee colony survival which is totally dependent on the honey reserves stored, there is no demonstrated evidence of a direct link between floral resources decrease and honey bee colony losses (Halfawi et al., 2022; Lika et al., 2021; Puvača, 2018; Puvača et al., 2021).

2.5. Feed sources for honey bee colonies

In an intensive cereal farming system, the reserve accumulation of honey bee colonies follows a seasonal pattern connected to the blooming period of the main mass flowering crops being rapeseed and sunflower. Honey bees forage on a wide diversity of flowers, however when the mass flowering crops are available they focus their foraging effort using them. Unfortunately, these mass flowering crops are highly seasonal and result in the occurrence of a ‘dearth period’, with a severe decrease in honey reserves, between the two peak flowering period of respectively rapeseed and sunflower (Lika et al., 2021; Sperandio et al., 2019).

The severe food depletion during May and June compels honey bees to forage on wild floral resources (Al-Ghamdi et al., 2021).

Several landscape elements have been found to contribute favourably to the reserve of the colony such as the woody elements and the weeds in a landscape. The woody elements and the weeds represent a major part of the pollen intake, more than 60% of the average pollen mass brought back to the hive (Abou-Shaara, 2017).

Few studies have focused only on the dearth period, though some elements have been pointed out, such as the possible positive contribution of flax (*Linum usitatissimum*) during this food shortage (Hoover et al., 2022). And on the other hand the negative effect of sunflower, blooming only later, taking up agricultural land without providing resources. However later in the season, during its blooming period, sunflower represents a major resource for pollinators, accountable for the main honey harvest for beekeepers (Breed et al., 2012).

Weeds constitute the bulk of the honeybee pollen diet during the dearth period (Breed et al., 2012; Burden et al., 2019). Arable weed species such as red poppy (*Papaver rhoeas*) act as an important food resource for biodiversity protection, in particular birds and insects. However, this central food resource is difficult to preserve considering that its optimal habitat is in crop fields. The occurrence of arable weeds has been declining as well as the species richness in which they occur. They are now disappearing from the core of the fields progressively confined to the field margins that act as refugee for weeds that can no longer survive in core fields. Thus, edges and woody habitats are considered as crucial landscape elements when focusing on biodiversity and honey bee survival (Thom et al., 2016).

Regarding some important features of the landscape, no clear consensus has been reached concerning its effect on the amount of reserve (Taha et al., 2021). Urban areas were proved to have a positive effect, whereas others highlighted its negative correlation to the number of resources in the hive. Some authors focused on the amount of food produced around an apiary to determine what crops would provide most resources for honeybee. They showed that arable land is the poorest regarding the amount and diversity of nectar (Thom et al., 2018). On the other hand, calcareous grassland, and neutral grassland are the habitats that produce the most nectar (Bromenshenk et al., 2015). Though the number of available resources around the apiary could not yet be correlated to the number of reserves in the hives. We suspect a carry-over effect of the dearth period on the colony dynamics: the food shortage (May and June) would impact the colony later in the season. During the dearth period it has been showed that the woody elements act as a buffer for the population decrease, decrease which commonly occurs between the two mass flowering crops. Thus, could be suspected that there would be more foraging bees and thus more food brought back to the hive when woody elements and weeds are abundant (Johansen, 1977; Simon-Delso et al., 2014).

2.6. Honey bee activity density in agricultural fields

The use of colored pan traps is a cost-effective, simple, and efficient technique to passively quantify insect communities, including bees. When used for studying pollinators, pan traps (or 'bee-bowls') can be a useful tool for monitoring bee communities, but honey bees may be underrepresented, possibly due to a bias toward smaller bee species (Sabbahi et al., 2005). Honey bee presence in pan traps may be lower compared to net samples and direct observations; however, the presence of smaller bees which are susceptible to pan traps (*Lasioglossum* spp.) has also been observed to be lower compared to other sampling methods (St. Clair et al., 2020). Thus, the potential for using pan traps in assessing honey bee activity may be unnecessarily underutilized (Gaines-Day & Gratton, 2016).

Despite a potential bias, honey bees have appeared in colored pan traps, although in low numbers, even in crops that do not require animal-mediated pollination. Studies conducted in regions where honey bees are native or feral colonies are abundant have found pan traps effective at capturing honey bees (Kasina et al., 2009). However, in regions where honey bees are not native or feral colonies absent, very small numbers of honey bees are captured, likely due to an absence of a stable honey bee population or ineffective usage of traps to capture honey bees (e.g., traps placed on the ground rather than elevated). In general, pan traps estimate activity-density, that is the movement of an insect through a landscape coupled with its population density (Rucker et al., 2012). Assuming honey bees are present, factors that affect the activity of a honey bee within a sampled area would affect the abundance of honey bees captured in a pan trap, therefore, the number of honey bees captured per trap is an indicator of activity-density (Banaszak, 1992). The usefulness of pan trapping as an accurate method of estimating honey bee activity-density is not well understood (Palmer-Jones et al., 1962).

Pan trapping has been identified as a method which captures the greatest activity-density of a pollinator community in agricultural fields compared to sampling methods used by applied entomologists to study insect pests of crops (e.g., yellow sticky traps and non-target sweep netting). Although these studies confirmed the presence of honey bees in crop fields using pan traps, they revealed a low level of honey bee activity-density, with honey bee foragers contributing a small percentage (0.005%) of the entire bee community (Hall & Reboud, 2019). However, these studies were conducted in areas in which it was not known whether honey bee colonies were present. Additionally, these studies sampled for a limited time period, potentially missing changes in seasonal activity-density of honey bees in relation to available flowering resources within or around the crop field (Hall & Reboud, 2019).

Declines in wild bee biodiversity are documented worldwide. These have been attributed to multifactorial stressors including environmental toxins, pathogens, reduced forage availability, and climate change (Gill & O'Neal, 2015). Highly developed agricultural systems result in reduced landscape diversity, which may reduce diversity and abundance of wild bee communities. In the U.S., wild bee populations are particularly at risk in regions of the Upper Midwest where vast areas of the landscape have been converted for the annual production of row crop agriculture (primarily corn and soybeans). In addition to reduced wild bee populations, honey bee (*Apis mellifera* L.) colony losses have mounted in this region, with beekeepers in the Midwest frequently losing 60% or more of their colonies annually (McCravy, 2018).

These declines are particularly problematic as wild bees are an essential part of maintaining natural ecosystems and honey bees contribute to pollination of over 150 crops. Increased demand in food supply has resulted in greater dependence on honey bee pollination services (McCravy, 2018). With unsustainably high colony losses, honey bees are unable to meet crop production needs. As a result, there is an increasing reliance on wild bees, in addition to managed bees, for pollination services, which are more efficient in some cropping systems (Yi et al., 2012).

Crop management plans that integrate wild and managed bees can reduce pollination costs for farmers and ensure long term stability of pollination services (Isaacs et al., 2017). However, in systems where wild bee communities and honey bees overlap there is potential for competition of floral resources and transmission of disease. Many studies have investigated the effects of landscape intensification on wild bee communities, and the impacts honey bees can have on wild bees. However, a critical knowledge gap about how landscape and honey bee presence interact to impact wild bee communities exists (Isaacs et al., 2017). Many species of wild bees already under stress from agricultural industrialization could be negatively impacted by additional competition with managed honey bees, further exacerbating population declines (Halfawi et al., 2022).

Corn and soybean do not require insect pollination studies have revealed that corn and soybean fields can house over 50 species of pollinators, including honey bees. How this community responds to variation within the surrounding landscape is not clear (Otto et al., 2016). In soybeans, surrounding landscape has been shown to influence pest and beneficial insects. In other agricultural systems, surrounding landscape influences pollinators. Higher plant diversity in a surrounding landscape can increase both pollinator and natural enemy abundance and richness. If extensive farming is associated with reductions in resource diversity and/or abundance, then it would be expected, landscapes committed to high proportions of annual production of corn and soybean would pose the highest risk of conflict between wild and managed bees compared to resource-rich areas (Otto et al., 2016). To advance efforts of conserving bee biodiversity and maintaining a sustainable future food supply it is vital to understand the impacts of surrounding land use on wild bees, particularly in areas where the landscape is dominated by agriculture (Schulte et al., 2017).

Additionally, because of our reliance on honey bees for agricultural pollination, it is a necessity to understand how vulnerable wild bee populations are to impacts from honey bees in agricultural landscapes (Main et al., 2020).

2.7. Honey bee habitat conditions in agricultural landscapes

As human population grows, habitat loss from anthropogenic landscape changes threatens the health and existence of many species (Dolezal, St. Clair, et al., 2019). An ever-increasing demand for food and biofuels following human population expansion requires more land be dedicated to agricultural production (Brosi et al., 2008). Global land use has shifted to meet this demand, with natural areas and smaller scale agricultural enterprises transformed into high-yielding monocultures, but with some cost (Buchori et al., 2019). Monocultures can have substantial negative environmental effects on soil, water, and air quality, and when coupled with the removal of native, non-crop habitat, this form of agriculture is associated with declines in pollinator populations. This conversion is provoking concerns for reduced pollination of crops and wild plants that could lead to reductions in agricultural production and ecosystem service delivery (Buchori et al., 2019).

Worldwide, honey bees (*Apis mellifera*) are the most economically important pollinator of crops, with honey bee colonies in the United States alone responsible for over €15 billion per year (Hung et al., 2018). Like other bee species, honey bees are challenged by environmental stresses that reduce colony survival, with state-wide losses as high 60% depending upon their location (Hung et al., 2018). This rate is higher than beekeepers consider sustainable, resulting in increased costs for contracted pollination services. These losses are associated with multiple, potentially interacting, stressors, including pest/pathogen pressure, pesticide exposure, and nutritional shortages, all associated with anthropogenic influence (Paudel et al., 2015; Puvača, 2018).

How do honey bees respond to landscapes that become increasingly dominated by extensive agriculture, particularly of crops considered to have limited nutritional benefit? Nationwide surveys have shown some of the worst colony losses occur in the Midwestern United States, a region of major agricultural production (Aizen & Harder, 2009). Further, agricultural land use has been associated with lower amounts of protein in stored pollen, lower honey production, and decreased physiological health of honey bees. Conversion of non-cropped land to crops has been linked to a decline in suitability for productive apiaries and several key metrics of honey bee health and productivity where agricultural intensification has recently increased (Abbasi et al., 2021; Aizen & Harder, 2009).

While the popular press has evocatively described regions that are agriculturally productive but devoid of biodiversity as “green deserts”, corn and soybean fields can host dozens of pollinator species (Abbasi et al., 2021). Further, increases in cropland can correlate with improvements in key honey bee growth metrics like food accumulation, as mass flowering crops or non-crop plants growing in field edges can provide forage for honey bees and wild bees (Duan et al., 2008). Thus, it remains unclear whether intensely farmed landscapes are overall net-positive or net-negative for managed pollinators such as honey bees. Studies of honey bees’ responses to crop production that do not explore seasonal exposure to landscape features may miss changes in phenology that can be significant for colony and individual honey bee health. Determining the net effects of agriculture upon honey bee survival requires multi-season, longitudinal studies of replicated, researcher-controlled colonies embedded in multiple agroecosystems (Duan et al., 2008).

A longitudinal study of colony growth and bee nutrition in one of the most extensively farmed areas of the world in USA, a perennial leader in the production of corn and soybean, with 92.6% of the state dedicated to agriculture and 72.9% planted with annual crops (Brown & Paxton, 2009). Despite this general lack of landscape diversity, variation in land use within the state can explain the abundance and diversity of key members of the insect community found within soybean fields. By placing bee colonies next to soybean fields and comprehensively studying their response to variation in land use surrounding these fields, we can understand how honey bees respond to a highly intensified agricultural landscape and begin to forecast the future of honey bee health in other regions undergoing similar agricultural intensification (Havard et al., 2019). Analogous longitudinal approaches can be used to assess intensification in other cropping systems (Kumar et al., 2018).

2.8. Honey bee health and diseases

Bees are an essential component of ecosystems providing a pivotal service through the pollination of a wide variety of plants, including economically important crops. However, wild bee populations have declined at local and regional scales, and managed honey bees are also facing high colony losses (Dolezal & Toth, 2018).

Wild and managed bees are affected by interacting environmental stressors, such as diseases, inadequate nutrition, and exposure to pesticides as a result of agricultural intensification (Lee et al., 2015). Worldwide, habitat conversion due to transformation of landscapes into row-crop agricultural systems is cited as a primary driver of wild and managed bee declines (Raymann & Moran, 2018).

Land used for agriculture can reduce natural and semi-natural habitat creating a scarcity in floral diversity and abundance that affects pollinator abundance and health (Mayack et al., 2022). Although mass-flowering monocultures may provide transient forage for some bee species, the simplified landscape and post-crop bloom results in a paucity of floral abundance. Such loss of resource diversity can lead to sub-optimal bee nutrition resulting in a compromised bee immune system and poor overall health (Wilkins et al., 2007).

Honeybees in the EU have a range of diseases and parasites, some of which are novel like *Varroa*, and some of which act in combination with novel parasites to reduce colony health (Mayack et al., 2022).

2.8.1. *Varroa*

The Varroa mite (*Varroa destructor*) arrived in the Europe in 1992 and is an ectoparasite which if left unchecked leads to colony death. The Varroa mite causes direct negative effects by damaging developing honey bee larvae and pupae by sucking their hemolymph and reducing their hatching weight (Gregorc & Sampson, 2019). Bees parasitized in this way usually begin foraging earlier and have a significantly reduced life span which may be due to decreased learning abilities, impaired navigation ability and consequently a lower probability of returning to the colony (DeGrandi-Hoffman & Curry, 2004).

Indirect effects of the Varroa mite are called Varroosis. This occurs when the varroa mite acts as a vector for viruses, most notably Kashmir bee virus (KBV), Slow paralysis virus (SPV), Acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV), and Deformed wing virus (DWV) (Rinderer et al., 2001). Indeed, honey bees can have multiple infections simultaneously, although it is not known what effect this has on the honey bees' physiology (Gregorc & Sampson, 2019).

The arrival of Varroa mites is the 'biggest catastrophe to befall apiculture'. Indeed, Varroosis is now considered to be the most destructive disease of honey bees worldwide and the major cause of winter colony loss (Arechavaleta-Velasco & Guzmn-Novoa, 2001; DeGrandi-Hoffman & Curry, 2004; Drijfhout et al., 2005; Locke et al., 2012; F. Rinkevich et al., 2017; F. D. Rinkevich, 2020).

2.8.2. *Nosema* spp.

After Varroosis, Nosemosis is one of the most prevalent adult honey bees diseases. The microsporidian *Nosema apis* is correlated with reduced lifespan of individual bees, reduced performance of colonies, and increased winter mortality (Pettis et al., 2012). In extreme cases it can even cause the death of colonies (Giersch et al., 2009). In 2004 another Nosema species, Nosema ceranae, was found in the honey bee (Y. Chen et al., 2008; Y. Zhang et al., 2021). *N. ceranae* doesn't exhibit the classic symptoms of *N. apis* such as crawling bees or dysentery but early research has found it to be more pathogenic (Botías et al., 2012; Bravo et al., 2017; Y. P. Chen & Huang, 2010; Forsgren & Fries, 2010; Li et al., 2017; Paxton, 2010).

2.8.3. Foulbrood in bees

There are two types of Foul Brood; American (AFB) and European (EFB) (Bailey, 1959). Both foul broods are a serious problem for beekeepers and are a notifiable disease, meaning beekeepers must report the infection to the local inspectorate at the National Bee Unit and treatment must be sought (Forsgren, 2010).

AFB always leads to the destruction of the colony. Where European Foul brood (EFB) is detected, three potential avenues can be explored: 1) Treatment of the colony with oxytetracycline, 2) A shook swarm method or 3) Destruction of the colony (de Graaf et al., 2006; Hansen & Brødsgaard, 1999).

EFB is caused by the bacterium *Melissococcus plutonius*. EFB affects mainly unsealed brood, killing larvae when they are 4-5 days old, leaving a decomposing larva twisted around the wall of the cell (Forsgren, 2010). If the comb is sealed affected larvae can be identified by sunken cell cappings. Where a high proportion of cells are affected, the brood pattern appears patchy and gives off a foul odour giving the disease its name. It is thought that outbreak of the disease may be due to colony stress (Forsgren, 2010).

American foulbrood is caused by the spore forming bacterium *Paenibacillus larvae* (Ratnieks, 1992). AFB affects larvae in the early stages (between 12 and 36 hours after hatching) and bacteria colonise the midgut resulting in the breakdown of the larva into a 'brownish, semi-fluid, glue-like' state. It is most easily identified by sunken cappings and a 'ropey mass' inside the cell. Spores are then distributed into the colony and are swallowed by the next host. The spores are incredibly infectious and hardy, being able to retain infectiousness for up to 35 years (Y. Chen et al., 2008; Poppinga et al., 2012). It is for this reason that control demands the destruction of the colony (Albo et al., 2003, 2003; Belloy et al., 2007; de Graaf et al., 2006; Fries et al., 2006; Fries & Raina, 2003; Spivak & Reuter, 2001).

2.8.4. Tracheal mites

Acarine is caused by the tracheal mite *Acarapis woodi* which infests the tracheal of adult honeybees, where it feeds on hemolymph and can act as a vector of viruses (Otterstatter & Whidden, 2004). It is thought to have been the cause of 'Isle of Wight disease' and the widespread colony losses in the early 1920s. Significant infestation by tracheal mites can lead to high levels of bee mortality, poor overwinter survival and individual bees may show symptoms of disorientation, dysentery and an inability to fly (Delaplane, 1992; Gary & Page, 1987). In the EU, only low levels of acarine are seen, which may be due to the widespread use of miticides to control *Varroa* which also controls *Acarapsis woodi* (Danka & Villa, 2003, 2005; M. D. Ellis & Baxendale, 1997; Harrison et al., 2001; Page & Gary, 1990).

2.8.5. Pesticides

There are a large number of pesticides in use in the environment and they have a countless of effects on honey bees, both direct and indirect, depending on dose, and state of contact (Goulson, 2013). Standard LD50 tests to assess safety of these products do not currently take into account potential sub-lethal effects (Pettis et al., 2013).

In honey bees pesticide application has been shown to; impair navigation, foraging and communication of the position of food resources within the hive, cause lack of co-ordination, bees to become preoccupied with self-cleaning, trembling and abdomen cleaning and foragers to fail to return to the colony (Mesnage & Antoniou, 2018). Also, it has been noted reduced egg laying, early supersedure, increased queen cell rejection and reduced ovarian weight in queen bees (Poquet et al., 2016). Decreased levels of house cleaning is also noted in honey bees and is of particular concern due to the high level of disease blighting some colonies (Nicolopoulou-Stamati et al., 2016).

2.8.6. Herbicides

Herbicides, bactericides and fungicides have all been found in honey and pollen. Little recent literature exists on the effect of these compounds on honey bee health, but in feeding trial certain herbicides were shown to vary widely in their toxicity and seriously reduce or eliminate brood production (Morton et al., 1972). Individual studies of fungicides showed they had little effect on honey bees, however when the combined effect of an azole fungicide and the insecticide deltamethrin was examined a significant effect on honey bee thermoregulation was found (Jumarie et al., 2017). A major concern is how best to test the interplay between all the chemicals the honey bees are exposed to (Cullen et al., 2019). In a study of pollen samples, 45 pesticides including toxic metabolites belonging to seven chemical classes of insecticides were found together with fungicides and herbicides. In some cases, fungicides have been shown to increase the already high toxicity of certain insecticides (Motta et al., 2020; Vázquez et al., 2020).

2.8.7. Contaminations in apiculture

Varroacides are an unsurprising source of contamination within honey bee hives as they are used long-term for Varroa control (Traynor et al., 2021). Water soluble varroacides such as formic acid, oxalic acid and cymiazole can be found dissolved in honey but are not soluble in beeswax so do not accumulate (Mullin et al., 2010). Lipid soluble varroacides, however, such as bromopropylate, coumaphos and fluvalinate are stable and accumulate in colonies over time (Jiménez et al., 2005). Investigations have showed that bees from contaminated hives contain varroacides in the fat tissue of their bodies (Simone-Finstrom et al., 2017). The effect of these residues on honey bee health is not known. Nowadays is advocated the use of natural acaricides such as thymol and organic acids such as oxalic and formic acid which do not leave significant residues if used properly (Murcia Morales et al., 2020).

2.8.8. Intensification of agriculture

Agricultural intensification is most detrimental to solitary bee species as they rely on native vegetation for nesting habitat and local flower plants. Honey bees are only effected by landscape context at a larger scale, as they are housed in hives and can forage at up to 10km away (Tscharntke et al., 2005). The most important parameters for honey bees are insecticide use and agricultural intensification through monoculture, improved grassland, regular mowing and cutting and practices that result in fewer flowers (Le Féon et al., 2010).

Honey bee health was studying the in Latin America. It has been suggested that a major cause of the apparent health of honey bees in Latin America is the low income agriculture that is practiced there (Le Féon et al., 2013). It is characterised by a small heterogeneous field system on small farms in fragmented landscapes with low nitrogen and pesticide application. One major factor is that honey bees found in Latin America are Africanized honey bees and have a naturally higher level of hygienic behaviour (Klaus et al., 2021). Consequentially they have lower levels of *Varroa* and they never surpass the critical level (N. M. Williams & Kremen, 2007).

However, in Brazil where Africanized bees are also found, there has been expansion of crops for agrofuels and increased use of pesticides, and here large-scale losses are becoming increasing common (Kline & Joshi, 2020). No colony collapse disorder style losses have been reported yet, but beekeepers do report an increase in the severity of *Nosema* and *Varroa* (Emmerson et al., 2016; Freitas et al., 2009; N. M. Williams et al., 2010).

2.8.9. Honey bee future

It appears that in modern beekeeping it is increasingly necessary to manage honey bees as farm animals rather than as a semi-domesticated species; i.e. treating them for pests and diseases, selectively breeding and importing specimens and enabling an increasing intensification of methods (Andrews, 2019). However the honey bee has never really been considered wholly domesticated. This is due to its multiple mating breeding strategy at remote drone congregations, which is difficult to control and adapt. Artificial insemination and queen rearing are still not widespread in the smaller scale bee keeping (Carroll & Kinsella, 2013). Indeed, there is an increasing gulf between the more intensive methods of some honey bee farmers and the hobbyists, who instead of treading the path to total domesticity would prefer to see a return to 'old fashioned' beekeeping. This movement has been dubbed 'natural beekeeping' and has attracted a lot of support from new beekeepers prompted to take up the hobby as a result of media reports of the decline of the honey bee and a future pollinator crisis (Tlak Gajger et al., 2021).

3. AIM OF THE RESEARCH

3.1. Objectives of the research

- To establish whether honey bee activity and density in agricultural fields can be accurately estimated with trapping methods
- To determine effect of soybean fields on the colony growth, productivity, and nutritional health of bees
- To investigate whether different vegetable farms have influence on the bee community and overall health and productivity of honey bee colonies.

4. MATERIALS AND METHODS

4.1. Selection of the experimental sites

During the summers of 2019 and 2020, we identified 18 and 20 soybean fields in Vojvodina region. All soybean fields were planted with pesticidal seed treatments; as well as with a fungicidal seed treatment, while some of the fields were planted with an insecticide and fungicide. No insecticides were applied to soybean foliage or in fields directly surrounding soybeans and weeds were managed with glyphosate. Both years we surveyed soybean developmental growth stage to evaluate at what time points flowers were present within the fields. Growth stages in which flowers were present spanned the R1 (at least one open flower at any node on the main stem) to R4 (pods 2 cm at four uppermost nodes, flowers still present on main stem) stages.

4.2. Honey bee apiary placement

To determine if the activity-density of honey bees in fields varies with the presence of a nearby apiary, four colonies were placed 3 m from a field edge of a subset of soybean fields. Of our 18 fields in 2019, we randomly selected 10 soybean fields to receive an apiary Hive (+). We compared honey bee activity-density in fields with apiaries to those without an apiary Hive (-). To confirm that our Hive (-) sites did not have any other managed apiaries present within 1.6 km of the field. In addition to the registry, we also scouted all fields directly neighbouring our experimental field to validate that no honey bee apiaries were present. These efforts resulted in eight Hive (-) locations. For the Hive (+) sites, apiaries were transported to soybean fields after 90% of the corn and soybean had been planted. Apiaries remained at soybean field edges throughout the season until. Of the 20 fields in 2020, we repeated the design used in 2019, selecting 10 soybean fields to receive an apiary Hive (+) and 10 fields which were Hive (-).

4.3. Sampling honey bee activity-density

To estimate honey bee activity-density, we used colored pan. We were interested in which of the common trap colors were more attractive to honey bees, so each post contained traps painted either fluorescent yellow, fluorescent blue, or white. Each field had three posts with three traps of each color (nine traps total) placed 10 m apart and 10 m into a soybean field in a row that ran parallel to the field edge and was adjacent to the honey bee colonies when present. Traps were deployed for 24 hour, every other week, on days with low cloud cover, no precipitation, and low to no wind (<10 mph). During each collection, the pan traps were adjusted on the post so that their height was level with the soybean plant canopy. Each trap was filled with a soap-water solution consisting of 3% and 97% water. We sampled bees for 13 weeks. In 2020, we repeated the 2019 sampling design and added an additional three posts into the grassy perimeter of the field to test whether placement of the trap affected honey bee activity-density. We sampled bees for 13 weeks. All estimates of honey bee activity-density were calculated as honey bees per trap.

4.4. Sampling the bee community

To quantify wild bee abundance, richness, and diversity within soybean fields we used pan traps. Each field had 3 posts with 3 bowls (9 bowls total) placed 10 m apart and 10 m into the soybean field. At Hive (+) sites, bee-bowls were placed on the same field edge where honey bee colonies were present.

During each year, we sampled bees every other week for 13 weeks. Collections were made on days with low cloud cover, no precipitation, and low to no wind (<10 mph). During each collection, the bee-bowls were adjusted on the post so that their height was level with the soybean plant canopy. Bee-bowls were deployed for 24 hour, with each bowl filled with a 3% aqueous soap-water solution. Each field was considered the experimental unit, with bee-bowls as sub-samples, therefore all collections within a date were combined and all measures of abundance and richness are represented at the field level.

Individuals were identified to genus using the dichotomous key and to species using the online dichotomous key "Discover Life". All bees were identified to species, with the exception of the genus *Lasioglossum* which were identified to subgenus.

4.5. Apiary inspection regime

At each site, apiaries were inspected on a biweekly basis. During each inspection, each colony within an apiary was weighed and additional hive boxes were added when those present reached approximately 75% capacity. The mass of these additional hive boxes was weighed before inspection, allowing the calculation of weight added by bee-forage only. Immature bee population was estimated by capped pupae area (cm^2) in each colony via photography. In 2020, adult bee populations were estimated based on fractional estimates of sides of a frame covered in bees (i.e., 'frame sides'. At each inspection, queen presence was determined by observation of the queen or eggs in a colony; if the queen was determined to be absent, a new queen from the same source was provided within 1 week. Monthly quantification of Varroa desctructor mites was performed via alcohol wash. At the beginning of all experiments, mite load (mites per 300 bees) for every colony was zero. Mite levels remained below this threshold throughout the season, but thymol was applied beginning in the last week of August to prevent mite infestation from confounding the effects of our experimental. During each inspection, a 15 mL tube was filled with worker bees collected from frames of exposed larvae (i.e., putative nurse bees), placed on ice and transported back to the laboratory and frozen at -80° C until further processing. In addition to assessing each colony at an apiary, the adjacent soybean field was assessed for its growth and development using methods developed by, to determine when and to what extent the crop was blooming.

4.6. Concentrations of lipids

To measure colony lipid levels of nurse bees, sampled bees from each date were processed. Approximately 50 nurse bees, by mass, were homogenized in liquid nitrogen, and approximately 0.25 g of homogenate was subsampled and weighed. Lipid content was quantified via phosphor-vanilin spectrophotometric assay and lipid calculated as mg lipid/mg bee mass.

4.7. Collection of pollen

To quantify pollen collected by honey bees in each cultivation category, a colony was randomly chosen within each apiary to receive a pollen trap. This trap was attached to the front of the hive and requires foraging bees to pass through a plastic plate which releases pollen from the bees and is collected in a pan. Although pollen collection may vary by colony, pollen traps were only added to one colony per apiary to reduce overall stress to colony growth at an apiary. Each trap was open for 24 hour each week.

A sub-sample of 2 g was extracted from each pollen sample collected on each day and sorted by pellet color. The sorted pellets were weighed, dissolved in

Caberla's solution with fuschin dye and mounted onto glass slides. Pollen was identified to the lowest possible taxonomic unit or morphospecies using light microscopy to observe morphological features. To validate pollen identification, pollen was also collected from all flowering plants found near each site during collection days and compared to mounted specimens.

In 2019, we found clover pollen to be the most abundant pollen collected by honey bees (60.4%) in the pollen traps. To assess when clover was blooming, we created two 10 m^2 plots around a patch of white clover (*Trifolium repens*) at the Bee and Wasp Research Apiary. We sampled blooms per m^2 once per week starting 12 July, when clover blooms were at maximum abundance, and continued through September.

4.8. Grassland access rescue experiment

To evaluate if the decline in honey bee health metrics could be prevented or reversed, we kept a separate set of colonies (n=10) at an independent agricultural site in 2020 monitored changes in weight beginning July. The colony represented the experimental unit, with the treatment being the availability of late-summer forage. Colonies were sourced and maintained as described with the exception that inspections occurred weekly and did not include brood or bee assessments. A sample of putative nurse bees was collected biweekly to assess individual lipid content. Three weeks after colonies reached their peak weight, half (n=5) were randomly selected and moved to new location. This location was not insulated from crop production, as 36% of the land within 1.6 km from the colonies was comprised of corn and soybean. Colonies were inspected weekly when all were moved back to the research apiary in preparation for overwintering. Though it is not quantifiably comparable to pollen trap data from the other experiments, we qualitatively assessed the presence and blooming status of flowering forbs present along a 60 m linear transect at this site on a weekly basis. A blooming forb was considered any plant with at least one stem in anthesis within 10 m on either side of the transect.

4.4. Statistical analysis

Data obtained will be subjected to two-way analysis of variance (ANOVA) techniques. The data were analyzed by using STATISTICA 13 statistical software. To investigate whether honey bee activity-density varied with the presence of honey bee colonies, we performed a t-test with pooled variance comparing Hive (+) sites to Hive (-) sites. The sampling days were combined such that the analysis is compares honey bees per trap per site. To explore whether activity-density of honey bees differed between pan traps 10 m inside the soybean field compared to traps placed 10 m in the exterior grassy perimeter, we performed a t-test with pooled variance. For this test, we used only data from the Hive (+) sites pooled across the season, such that the analysis compared honey bees per trap per site. To explore which pan trap color was most attractive to honey bees we performed a mixed model analysis of variance with trap color as the main effect and site-year as a random effect. Data consisted of only trap collections from the interior of the soybean field at Hive (+) sites. Honey bee activity-density was pooled across the season, such that the analysis compared honey bees per trap color per site. We used least squared comparison of means with Tukey adjustment to evaluate post-hoc comparisons of trap colors.

To examine how honey bee activity-density in soybean fields varied across the season and with soybean phenology, we performed a mixed model analysis of variance with date as a main effect and site-year as a random variable. For this analysis, we analyzed honey bees per trap per site for Hive (+) sites only. We used least squared comparison of means with Tukey adjustment to evaluate post-hoc comparisons of sampling dates.

5. RESULTS AND DISCUSSION

Activity-density of honey bees was observed to be significantly higher ($p < 0.05$) at Hive (+) soybean fields compared to Hive (-) fields (Figure 3). At locations where apiaries were placed, there was no observed difference in activity-density between traps placed 10 m inside the soybean field compared to those placed 10 m inside the grassy perimeter of the field (Figure 4). The amount of honey bees in the pan traps varied significantly by color ($p < 0.05$) with blue traps capturing significantly more honey bees than both yellow and white traps (Figure 5). Activity-density varied by date ($p < 0.05$), with significantly more bees captured in traps (after soybeans ceased blooming) compared to all other dates including before and during soybean bloom (Figure 6 and Table 1).

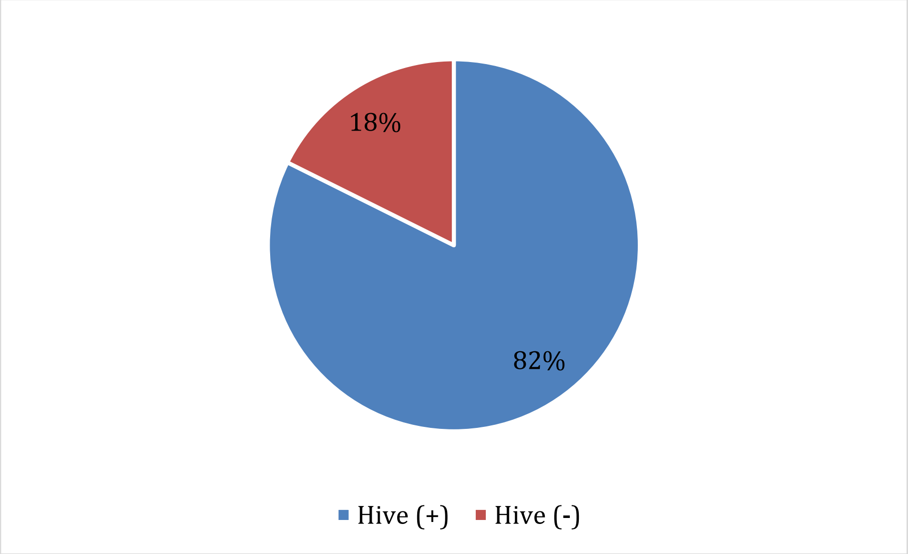

Figure 3. Mean honey bee activity-density in pan traps inside soybean fields, %.

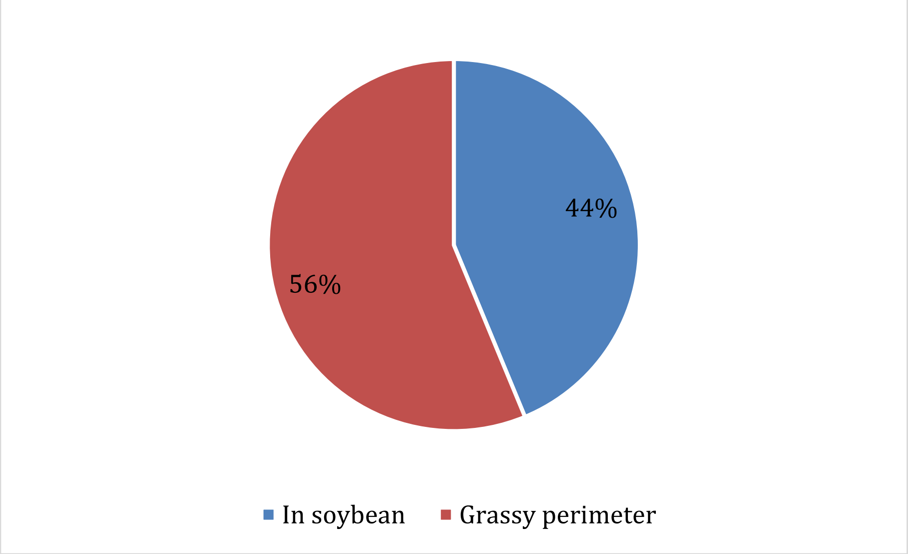

Figure 4. Mean honey bee activity-density at Hive (+) soybean fields with pan traps, %.

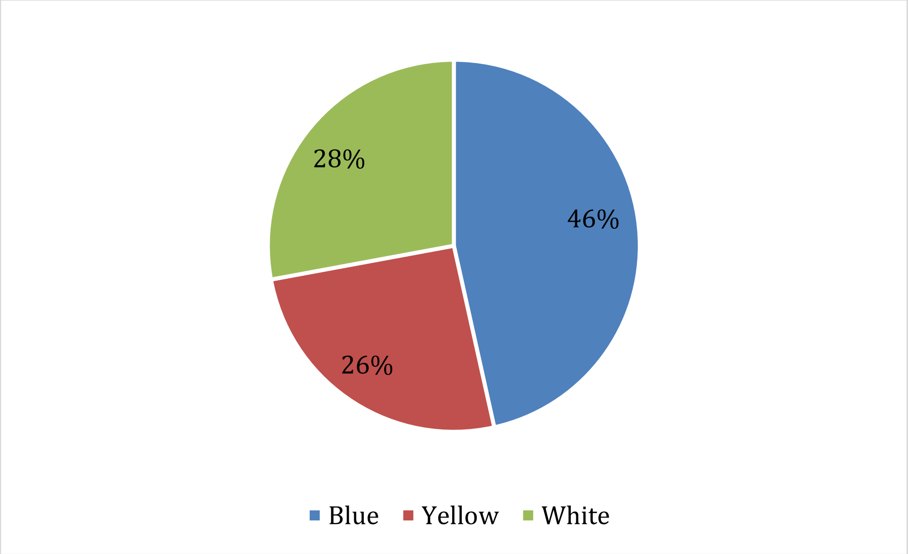

Figure 5. Mean honey bee activity-density (bees per trap color per site) in soybean fields, %.

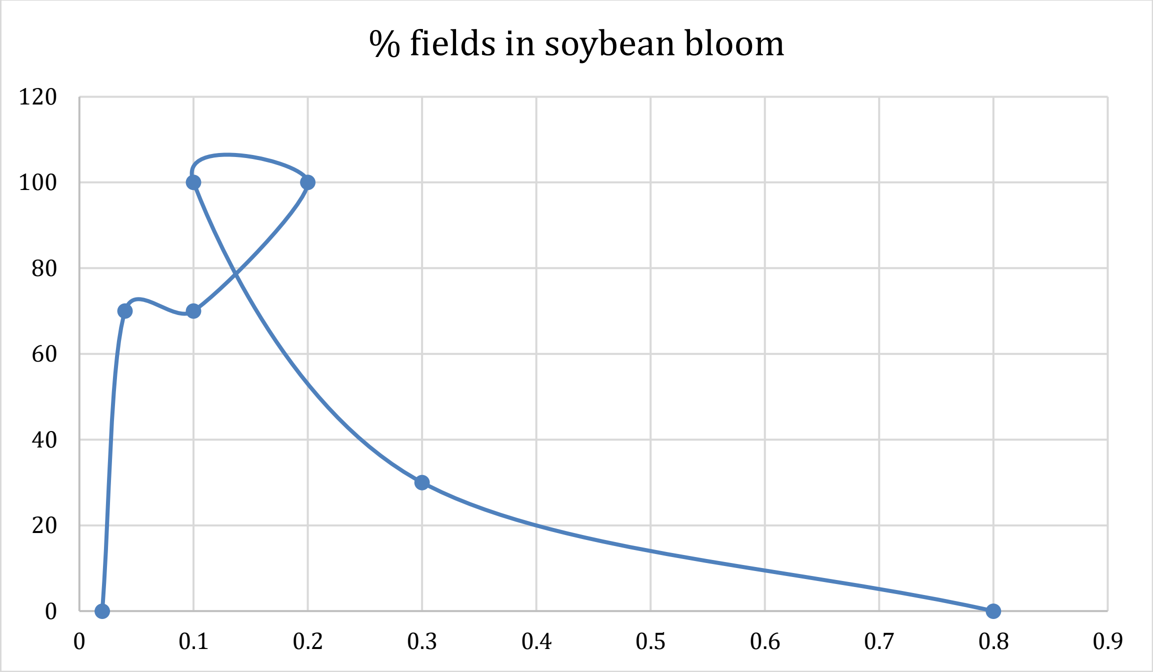

Figure 6. Seasonal honey bee activity-density inside Hive (+) soybean fields.

There were no observable differences in honey bee activity-density based on field type (soybean vs prairie) ($p > 0.05$), distance from the apiary ($p > 0.05$), or date ($p > 0.05$) (Figure 7). There were no interactions between field type and trap distance ($p > 0.05$), field type and date ($p >0.05$), and date and trap distance ($p > 0.05$). Likewise, there was no observable interaction of field type by trap distance by date ($p >0.05$).

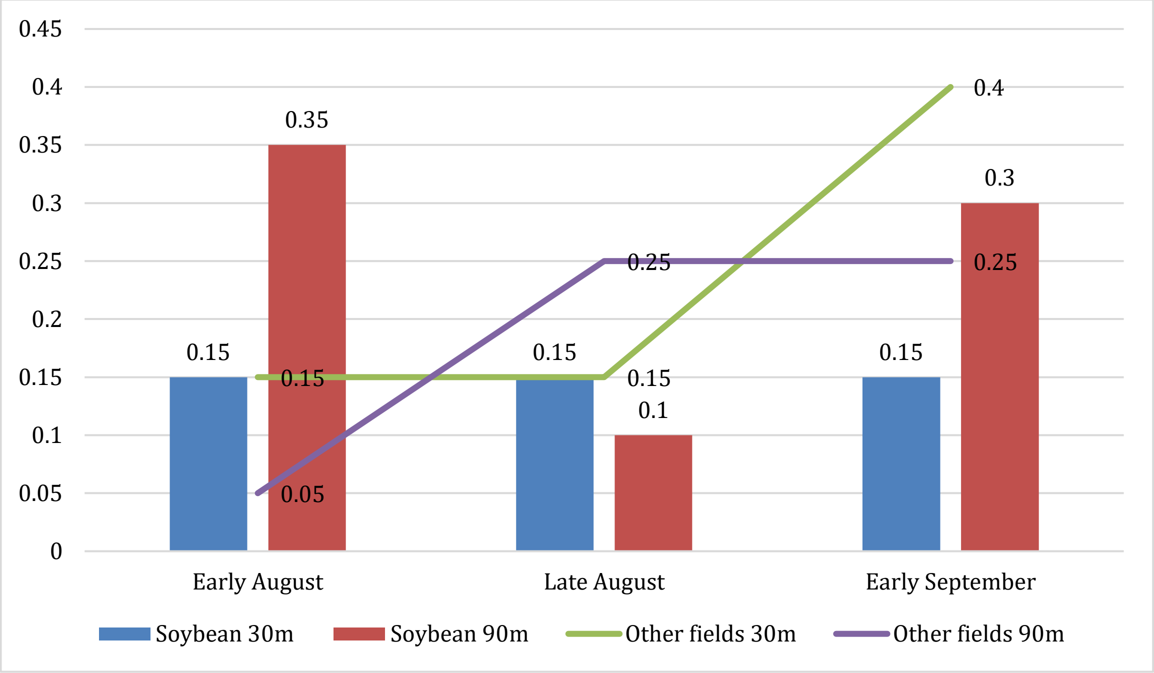

Figure 7. Mean seasonal honey bee activity-density in pan traps in soybean and other fields.

Honey bee activity-density was significantly positively associated with the density of colonies nearby or within soybean fields ($p < 0.05$); however, only 10.8% of the variation in activity-density was explained by the number of colonies present (Figure 8).

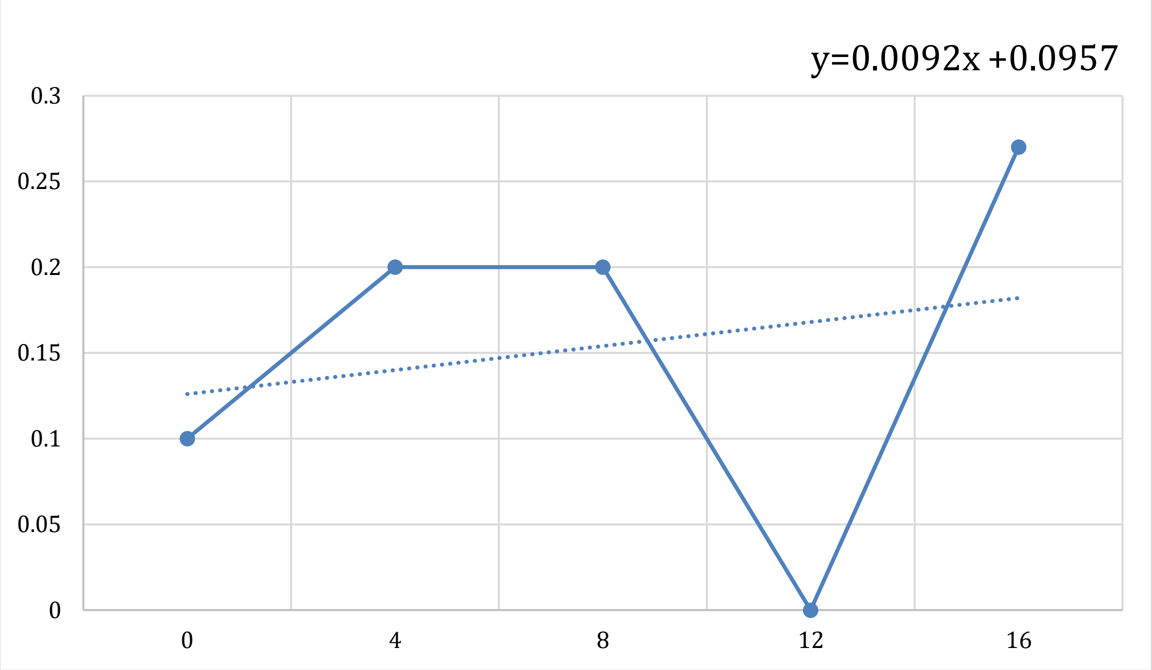

Figure 8. Correlation of colony number with the observed honey bee activity-density per trap per soybean site per day.

Contrary to other studies suggesting honey bees are not captured in pan traps, we found that these traps can be an effective method to measure honey bee activity-density in agricultural fields. These results suggest that, in regions such as Vojvodina, pan traps can be used to measure activity-density of honey bee colonies placed within a focal crop field. Although we observed a positive association of honey bee activity-density with the number of colonies present within 90 m of pan traps, the relationship was not particularly strong, perhaps due to low replication. Nonetheless, our results suggest there may be some usefulness in using pan traps to predict the density of colonies nearby and this relationship deserves further investigation. The use of multiple colors (blue, yellow, and white) is usually recommended to capture a diverse community of bee species. We found blue traps captured the most honey bees. For studies specifically targeting honey bee activity-density, blue traps may be the most effective.

The placement of a pan trap can affect how many pollinators are captured. For honey bees in soybeans, the placement of pan traps within the field or within the grassy perimeter adjacent to the field did not affect the estimate of activity-density when colonies were present at field edges. Furthermore, when colonies were embedded within the field, trap distance from the colonies did not affect the estimate of activity-density. These observations suggest that when honey bee colonies are present near or within a large crop field (>20 ha), the activity of foragers is spread evenly throughout the field. However, in our study, because we sampled only 30 and 90 m from the apiary, we cannot infer anything about the activity-density of honey bees in the entire field. Reduced activity-density may occur at greater distances than 90 m from an apiary. Alternatively, it is possible that a difference did occur, and the pan traps just failed to capture those differences. An additional caveat of our study is the reduced replication of soybean sites in experiment two, which may have failed to capture enough variation in trap distance to see significant effects. Further studies should aim to tease apart how honey bee activity-density estimates vary with trap distance from an apiary by examining multiple distances with increased site replication alternative floral attractants. In late September, we observed honey bee activity-density in soybeans nearly triple. This is not likely due to an increase in the population of bees within the colony as colonies in this region are typically declining in mass, developing bee, and adult bee populations after the beginning of August. Instead, it is probable that the increased bee captures in late September were a result of decreased flowering resource availability in soybean fields. In late September, soybeans have typically senesced to the point of no longer having leaves. The high density of honey bees observed in a soybean field at that time could be due to the pan traps being perceived as the only source of flowers and as a highly attractive beacon to the bees at a time when no other floral attractants were present. This explanation supports the idea that pan traps are biased towards collecting more bees when floral diversity and abundance is low. In this study, we were unable to keep pan traps in soybean fields into late September because our traps interfered with the crop harvest. To better understand the relationship between activity-density estimates from pan traps and the resource availability in soybeans (St. Clair et al., 2020), future studies should focus on assessing activity into the late season. Although pan traps may overestimate the activity-density of honey bees when floral resources decline, these data still serve as a valuable representation of honey bee foraging behaviour (Mendoza-García et al., 2018). Peaks in honey bee abundance in pan traps may provide useful information about when honey bees face forage limitations in crops and other field types, but additional studies are necessary to parse out the differences in activity due to forage limitation compared to other possible reasons (Halfawi et al., 2022).

We further investigated if variation in foraging resources affects activity-density by comparing honey bee activity-density inside soybean fields (where plant diversity is limited to the crop) to other fields (where plant diversity varied between 10-12 species across sampling dates). We did not observe a difference in activity-density of honey bees in pan traps in other fields compared to soybeans, suggesting floral diversity did not drive honey bee activity. At the time points we compared activity-density of honey bees in soybean to other fields (i.e., August and early September), soybean plants still had flowering resources available within the fields, meaning floral abundance may have been equivalent. Due to the generalist nature of honey bee foraging and honey bee preference for legumes, it may be that honey bees were able to readily utilize the abundant resources available from monocultures of soybean. It is possible that in the Vojvodina cropping system, abundance rather than diversity of floral resources is a more important driver of honey bee activity-density. This justification would explain why pan traps only over estimated activity after crops ceased blooming, a time when both floral abundance and diversity were low in soybean. Further studies are necessary to better understand the interaction between resource availability and honey bee activity-density. Thus, it is important to provide a word of caution about using pan-traps under conditions of extremely low resource availability, as our data from soybeans in late September suggest inflated activity-density estimates under such extreme conditions.

Results from these experiments provide value on the usefulness of pan traps as a method of quantifying honey bee activity-density in extensive agricultural landscapes. With increases in crop production and the demand for honey bee pollination services occurring concurrent to widespread declines in managed honey bee colonies, there is a need for improved methods to gain insight into the effects agricultural systems have on honey bees. In our study, pan traps gave estimates of activity-density, but they were not necessarily synonymous with foraging activity. While some studies have used pan-trap collected bees to assess foraging resources, for honey bees, we found this unlikely to be effective, because honey bee collected pollen was usually washed off in the traps, and the bees often regurgitated the contents of their honey stomachs when they drowned in the traps. However, we were able to demonstrate that pan traps can be used as a tool to gain insights into honey bee activity-density within crop fields within 90 m of an apiary. Pan traps may be most useful in estimating local colony presence; however, when colonies are present, activity-density estimated by pan traps does not strongly correlate with number of colonies present. Our data also suggest restrictions associated with pan traps; at times when floral abundance and diversity are low, pan-traps can lead to inflated estimations of activity-density. Thus, we suggest that pan-trapping can have applications in easily and quantitatively estimating presence-absence of honey bee colonies as well as identifying times of extreme resource limitation. Such applications could be useful in identifying the presence of nearby honey bee colonies in studies estimating wild bee activity in the landscape, choosing landscape conservation enhancements that target critical resource gaps for bees, and identifying when honey bee colonies may need to be moved to landscapes with more resources or provided supplemental feed by beekeepers (Abasi & Daneshyar, 2020; Beura et al., 2014; Kahono et al., 2018).

Regarding apiaries, they were heavier in landscapes with high cultivation than low cultivation (Mullin et al., 2010). In both years, apiaries kept adjacent to soybean fields in high cultivation landscapes were heavier $p < 0.05$ (Figure 9), with marginally higher immature bee populations $p > 0.05$ (Figure 10), and higher adult bee populations $p < 0.05$ (Figure 11) than those in low cultivation landscapes. All metrics of colony growth varied significantly within a year. We also detected interactions between cultivation category and sampling week for apiary weight ($p < 0.05$) and adult bee populations ($p < 0.05$), discussed below. However, weight ($p >0.05$) and immature population ($p > 0.05$) did not vary by year. We did not observe a significant difference in nurse bee nutritional state, as estimated by lipid content, between cultivation categories $p > 0.05$ (Figure 12) or sampling years ($p > 0.05$), and there was no interaction between landscape categories and sampling week ($p > 0.05$).

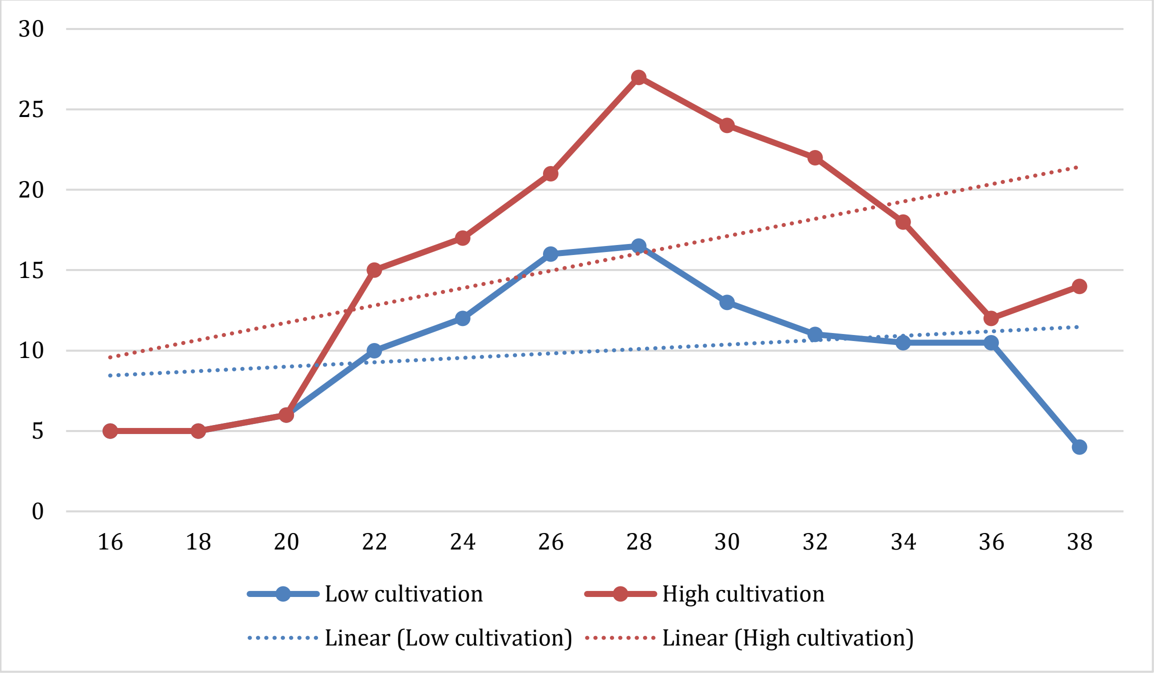

Figure 9. Apiary averaged hive weight, kg.

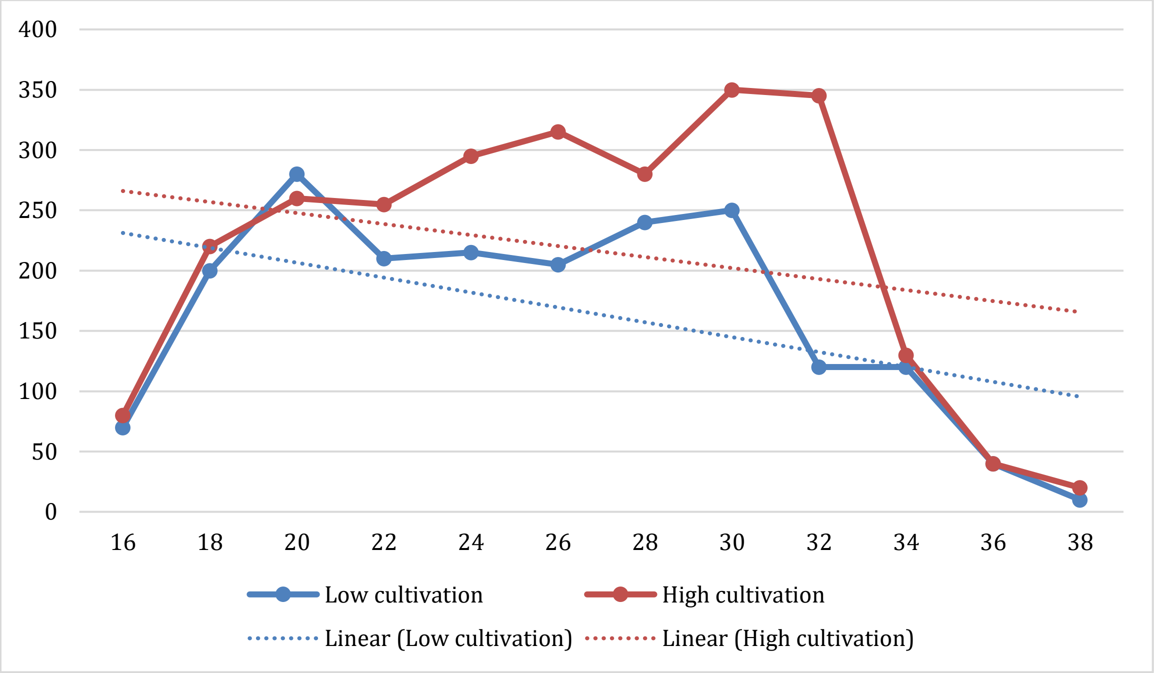

Figure 10. Mean immature bee population, cm^2.

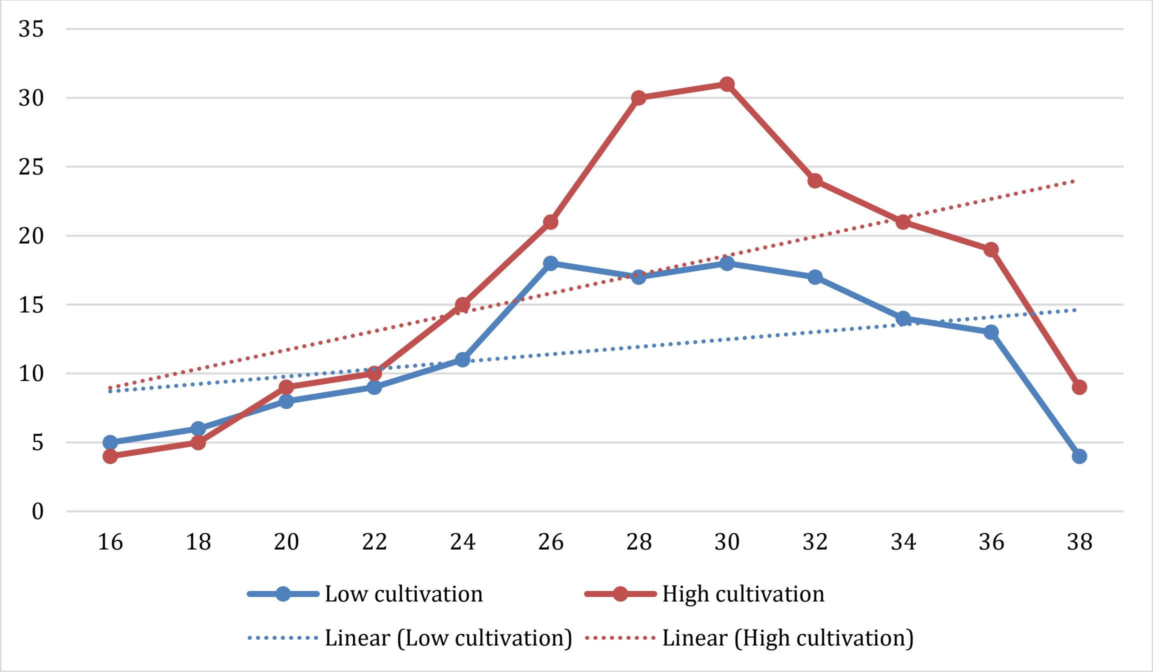

Figure 11. Mean adult bee population in frame sides.

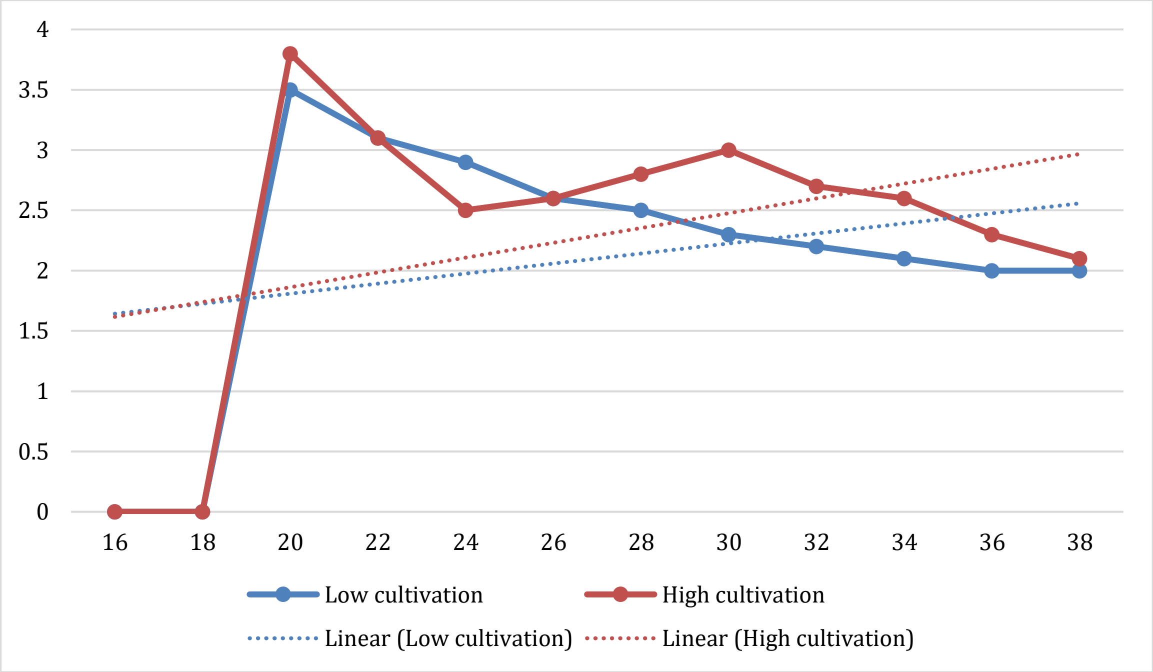

Figure 12. Mean nurse bee lipid content, %.

Apiaries and individual bee health declined drastically in late summer. To further understand the temporal dynamics of colony growth and decline in light of the interaction between cultivation category and weeks when weight was estimated, we calculated rates at which apiaries gained weight (from initial weight to the seasonal maximum), and lost weight (seasonal maximum to the end of our observations) (Figure 13). Apiaries surrounded by high cultivation gained and lost weight at greater rates than those in low cultivation landscapes (Figure 13). The rates of gain and loss were nearly identical within a cultivation category (Figure 13). Apiaries in both cultivation categories began to lose weight after 10 weeks at rates that were similar to the rates at which they gained weight, such that by mid-October all apiaries returned to their initial weight. Similar patterns of gains and declines were observed in immature and adult bee populations (Belloy et al., 2007). These declines began nearly two months before sub-freezing temperatures that terminate all flowering resources; therefore, the significantly faster rate at which colonies lost weight in high cultivation landscapes may put them at an increased risk for nutritional deficit and overwinter starvation.

However, despite the differences in weight decline, lipid concentration of worker bees did not differ by cultivation category, but only by date ($p < 0.05$). Regardless of where apiaries were located, lipid content of nurse bees was highest at the initiation of the experiment and declined throughout the weeks of our monitoring. This is noteworthy as the final sampling period occurred in mid-October, when honey bee colonies in temperate regions enter a pre-overwintering stage commonly associated with increased lipid stores.

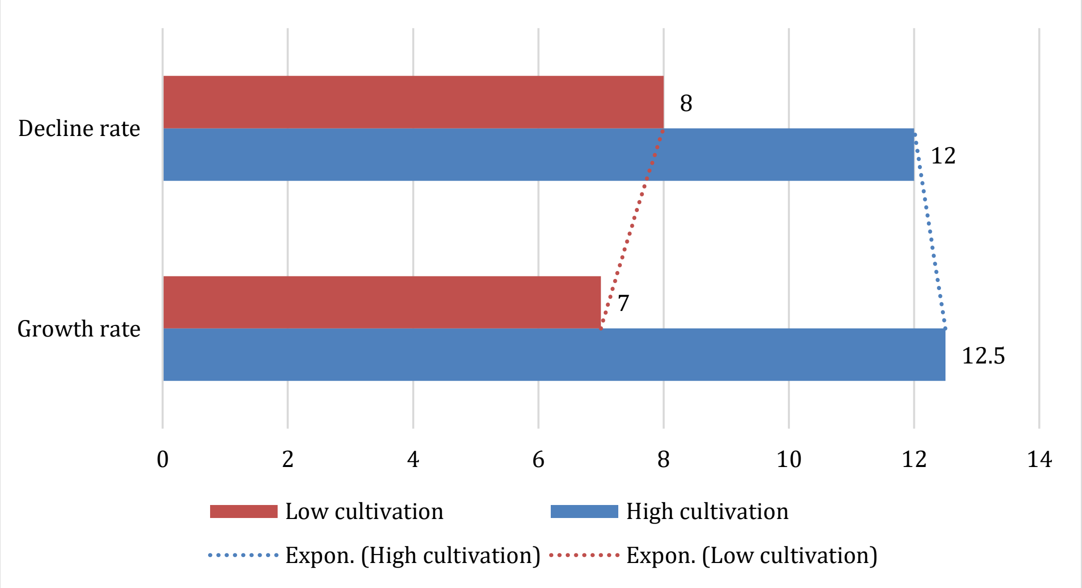

Figure 13. Rate of weight change, kg/month.

The type of forage used by apiaries did not vary by location but varied during the season. Apiaries at every location began our experiment with the same average weight but reached different seasonal maximums, suggesting that variation in land use between the cultivation categories contributed to available forage (Belloy et al., 2007). Honey stores are the greatest contributor to hive weight, derived from foragers focused on collecting nectar over other material (e.g., pollen, water, propolis). The design of this experiment does not allow us to determine how much a specific plant contributed to honey production, but there is indirect evidence suggesting several plants were nectar sources when colonies were gaining weight. Colonies in high cultivation landscapes were surrounded by significantly more soybean (and thus field edges) than those in low cultivation landscapes $p < 0.05$ (Figure 14).

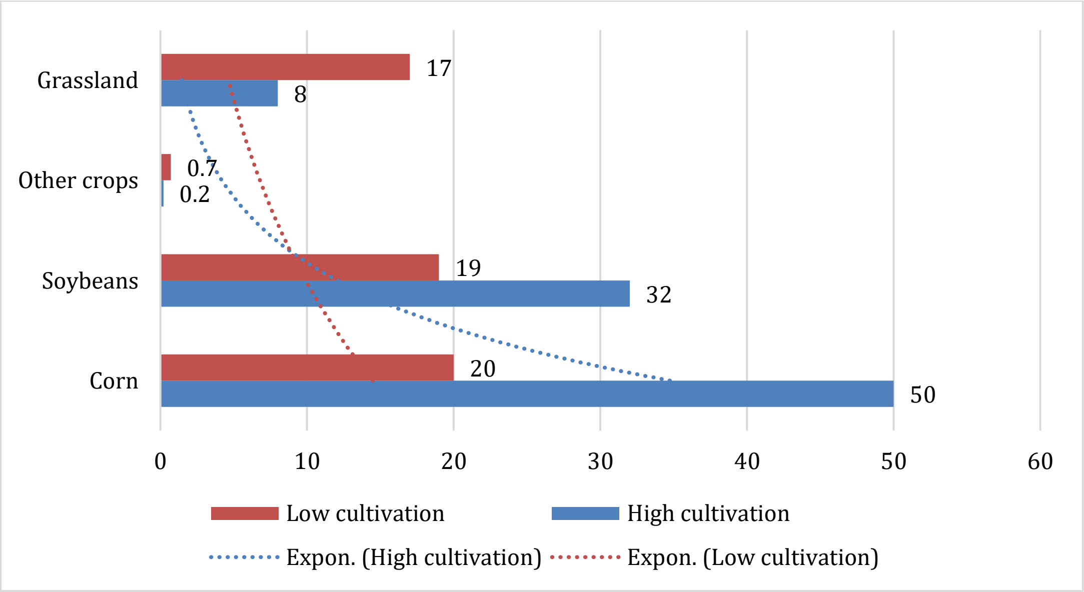

Figure 14. Landscape features of experimental site, %.

Field edges are likely to contain a higher abundance of clover, a resource which has previously been identified as a significant source of nectar for honey production. During our experiment, the period of greatest colony weight gain occurred when clover was in bloom (Figure 15).

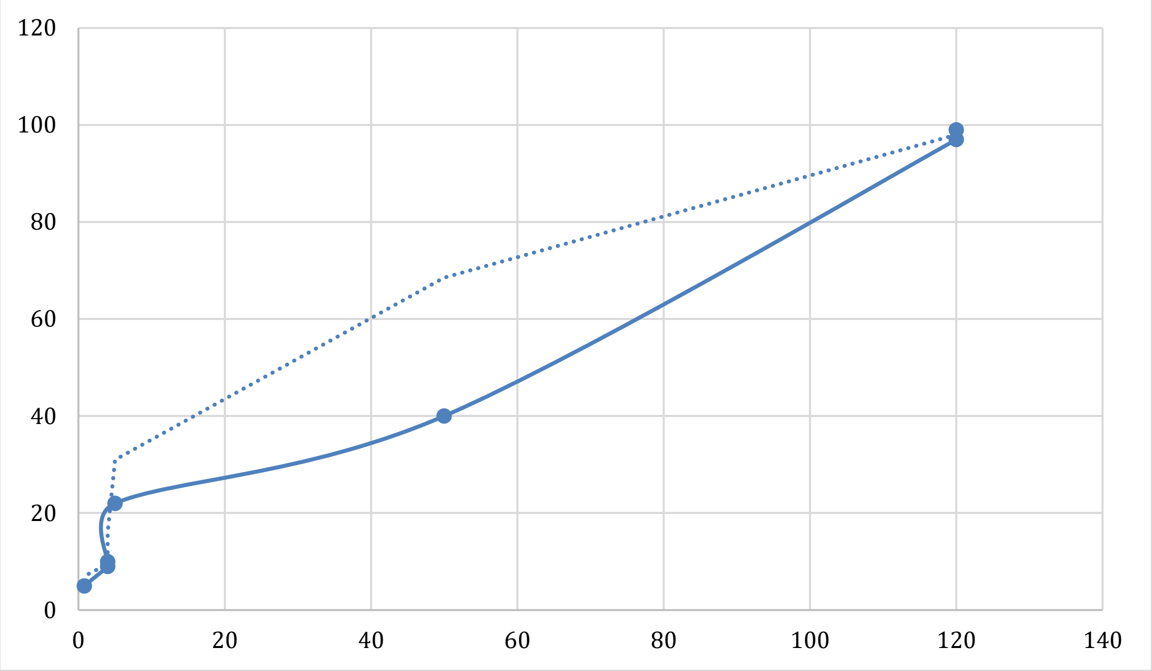

Figure 15. Fields in soybean bloom, %.

However, this period also occurred when most soybean fields adjacent to our apiaries were blooming. Although soybeans have been bred for self-pollination, the flowers sometimes produce nectar used by honey bees for honey production, though nectar production varies by cultivar and growing conditions. Nectar foragers incidentally encounter pollen during foraging, and observations from stored honey within our colonies revealed traces of both soybean and clover pollen. Although traces of both plants' pollens were present in honey, these observations do not allow us to determine when and to what degree a single plant contributed to overall honey production. Overall, these observations suggest that colonies in the high cultivation landscapes may have grown heavier and at a faster rate because more nectar forage was available.

Conversely, apiaries in the low cultivation landscapes may have had more alternative sources of forage available later in the season such that their weight loss occurred at a slower rate than those in the high cultivation landscapes (Zhang et al., 2020). We tracked the collection of pollen by colonies in these apiaries to determine if this type of forage provided insight into whether flowering resources varied by cultivation category. We did not observe differences in the amount of pollen collected between the landscape categories $p > 0.05$ (Figure 16), nor the pollen types collected

($p > 0.05$). No soybean pollen was detected in pollen traps at any apiary. Pollen was collected primarily from clover (*Trifolium* spp.) and secondarily from partridge pea (*Chamaecrista fasciculata*), with the remaining 17.2% comprised of 25 species.

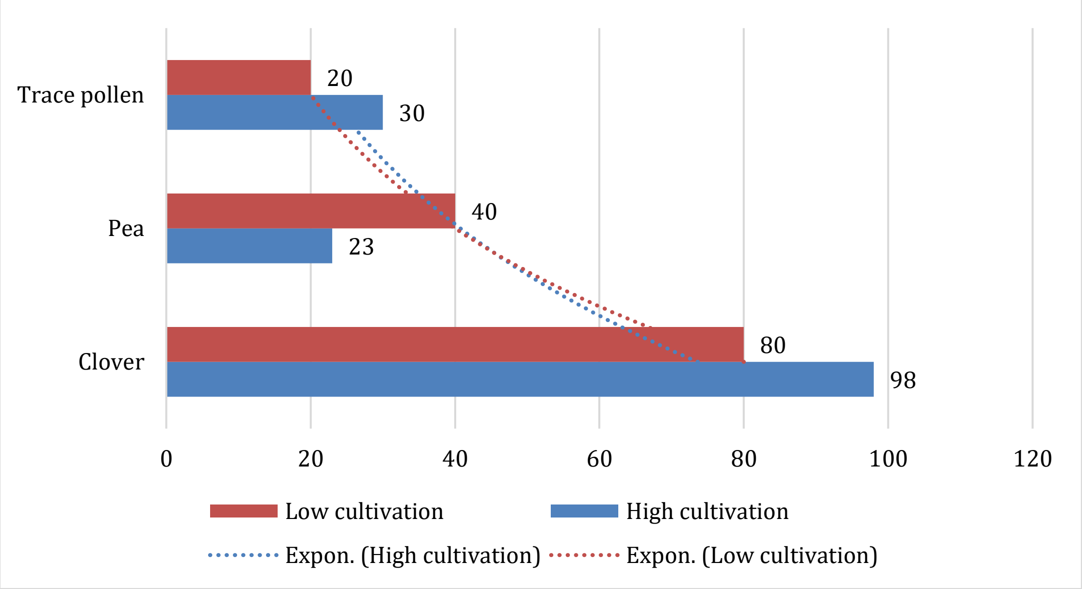

Figure 16. Average mass of collected pollen, g.

Although our analysis of pollen collected by honey bees did not help explain potential differences in forage availability between the two landscape categories, they provided insight into why apiaries in both categories lost weight at the same time. Clover (*Trifolium* spp.) was the most common pollen source for our apiaries and is also a common nectar source for honey bees and is likely to have contributed substantially to differences in colony weight (Sponsler et al., 2017). Flower production of both clover and soybean declined dramatically (Figure 15). Without a substantial source of flowering resources during late August and September, honey bees would be left with only their stored honey and pollen as a food source. The larger colonies in the highly cultivated landscapes may have lost weight at a faster rate than those in the lower cultivated landscapes simply because their greater populations consumed their honey stores at a faster rate than smaller colonies (Pearson & Braiden, 1990; Zhang et al., 2022).

Providing colonies access to prairie reverses late summer declines in weight and lipids. We conducted a separate experiment to determine if declines in honey bee weight and health could be prevented by providing access to prairie habitat. We selected prairie because it is comprised of flowering plants that bloom during the late summer to early fall and are not commonly found in purely agricultural landscapes. Many prairie plant species are attractive to pollinators, and a subset bloom when we observed colony decline in our first experiment.

For this experiment, we focused on honey bee colonies as the experimental unit, and used ten colonies of similar population and weight, established at an agricultural location (Zhang et al., 2021). After three consecutive weeks of weight loss after the mid-summer mass peak, a random selection of five colonies were moved to a reconstructed tallgrass prairie, with the remaining colonies kept at the agricultural site. After the relocation, these colonies not only ceased losing weight (Figure 17) but became heavier than those remaining at the agricultural site ($p < 0.05$). Colonies remaining at the agricultural site continued to decrease in weight and ended the season significantly lower than their summer maxima $p < 0.05$ (Figure 17).

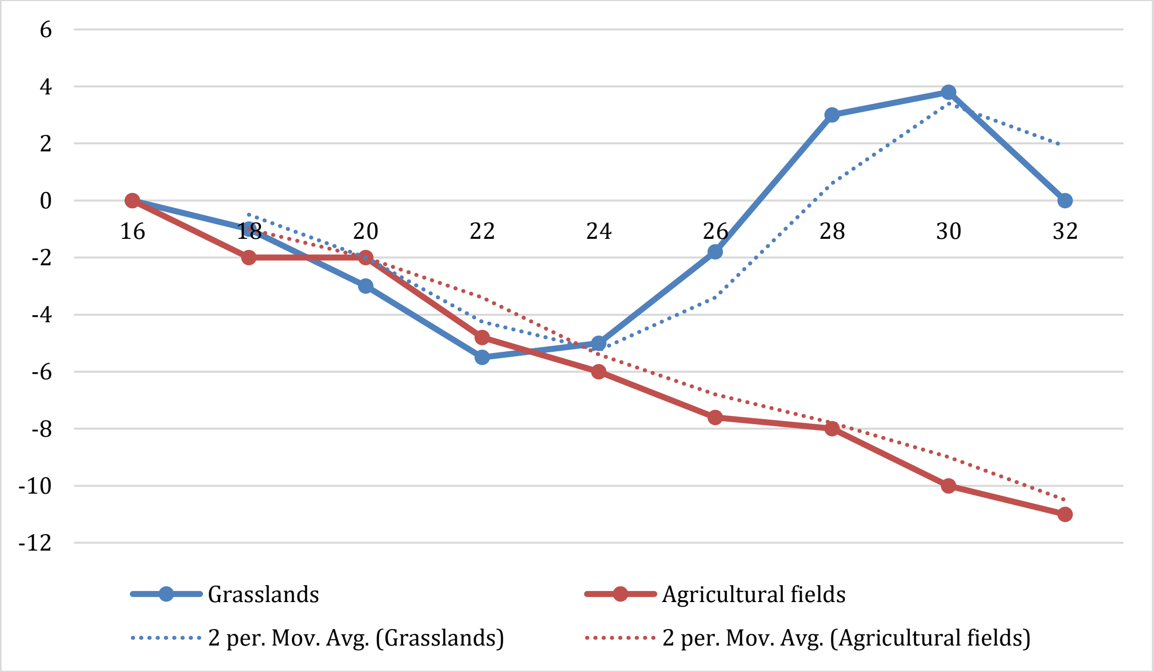

Figure 17. Colony weight, kg.

In contrast, colonies with access to prairie ended the season with a weight that reached their summer maxima (Figure 17). In addition, colonies placed in prairie contained nurse bees with significantly higher lipid content at week 40 than those that remained at the agricultural site $p < 0.05$ (Figure 18).

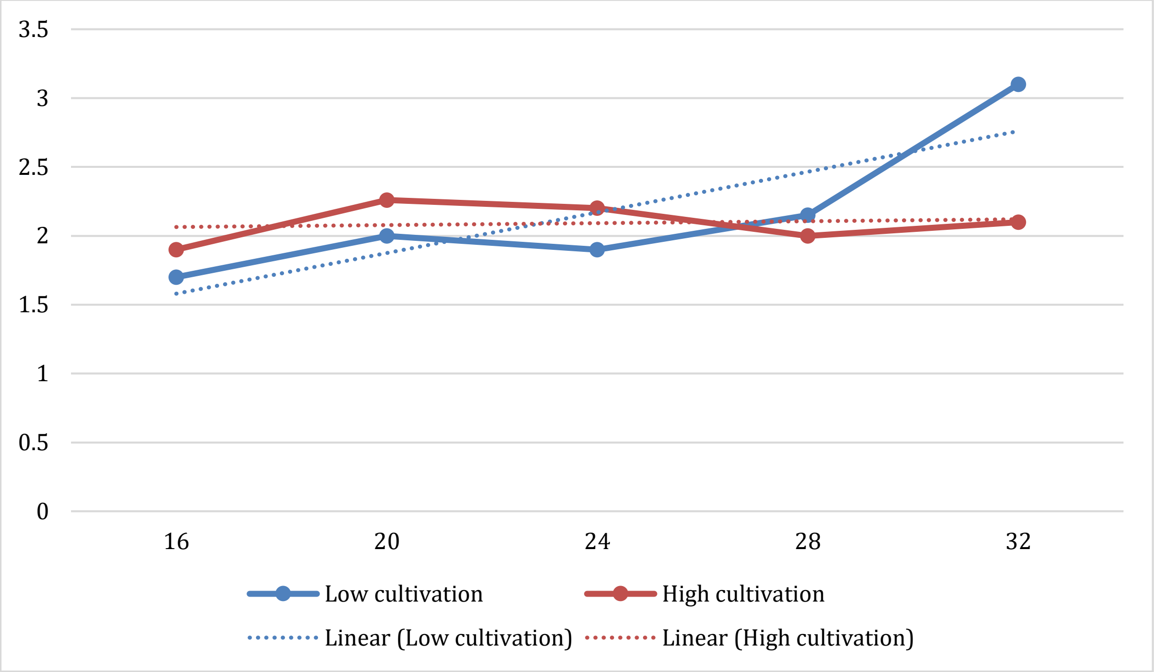

Figure 18. Average percentage of lipid content in nurse bee, %.

Overall, our results demonstrate that some highly cultivated landscapes can provide short-term gains in colony growth but can also fail to support colony health across the entire growing season, especially in the critical pre-overwintering period. This longitudinal perspective on honey bee health helps to clarify the dynamics of honey bee responses to landscape and forage availability, especially given previous, sometimes conflicting reports suggesting both positive and negative impacts of extensive farming on honey bee health. In both high and low cultivation landscapes, colonies relied upon a startlingly limited number of plants, primarily clover, for pollen, suggesting agricultural landscapes as a whole do not provide a diverse pollen resource for bees. Bees use pollen as their primary source of proteins, lipids, and micronutrients. Further, honey bees are generalist pollinators and prefer mixed-pollen diets. Polyfloral pollen diets are associated with longer honey bee lifespan, increased resilience against pathogens, and can interact with their response to pesticide exposure (Di Pasquale et al., 2013). Colony reliance on a limited pollen diet may contribute to honey bees stress in agroecosystems; first, access to pollen only occurs for part of the season; second, even when pollen is most abundant, the lack of diversity may produce colonies that are less tolerant of other stressors (Dolezal, Carrillo-Tripp, et al., 2019). While we report evidence consistent with studies that reveal a positive response between annual crop production and colony health, the uniform decline late in the growing season supports the findings of other studies suggesting that agricultural lands are detrimental to bee health. While honey bees can survive long periods of forage dearth, like winters in temperate climates, the responses we observed are not consistent with healthy colonies. Before the overwintering period has begun, colonies had lost on average 53% of their total maximum weight, bringing their food stores to a dangerously low level unlikely to allow survival during the winter in a temperate climate, let alone produce a harvestable honey crop. Further, the lipid content of nurse bees at the end of the season was reduced, suggesting individual bees were not transitioning to a physiological state for successful overwintering. By the end of the growing season, adult bees in an overwintering state should have high fat stores; for example, experimentally-stimulated winter bees exhibit 43-59% higher lipid stores than summer controls. In contrast, the lipid concentration for bees kept in both of our cultivation categories changed in similar magnitude, but in the opposite direction, declining by 49%. Even if colonies were able to reduce populations to a level that could survive on the existing stored resources, or if supplemental food source were added, the remaining bees may not be physiologically capable of surviving. To what extent the colonies we tracked in these experiments capture the physiological state of commercially-managed honey bees is not clear, as we did not provide a supplemental food source, a common practice for managed colonies experiencing a lack of forage.

Pesticide exposure is a significant stressor experienced by bees in agricultural landscapes, and since 2000, insecticide use on soybean has increased, due in part to the invasive soybean aphid. Although we did not control for insecticide use within our experiments, we did not observe evidence of direct, lethal exposure to insecticides in any of our colonies. On the contrary, colonies performed better in areas of higher cultivation, particularly during a period when insecticide use to prevent aphid outbreaks is recommended. Furthermore, no foliar insecticides were applied to any of the adjacent soybean fields, though applications could have occurred in the surrounding landscape, possibly leading to sub-lethal exposure. Thus, we cannot rule out a possible interaction between sub-lethal exposures to insecticides and forage availability contributing to the nutritional deficiencies in nurse bees. Future experimental work is needed to better understand the interaction between nutritional stress and sub-lethal pesticide exposure in a field setting.

Based on species accumulation curves generated from our data, the sampling efforts accounted for 77.5% of the potential species that could be found in soybean and 83.3% of the potential species in vegetable fields (Figure 19). The nonmetric multidimensional scaling plots produced polygons connecting the perimeter distributions of sites in the nonmetric multidimensional scaling plots constructed from the pollinators collected inside vegetable and soybean fields (Figure 20). The pMANOVA indicated no significant difference in bee communities between the farm types ($p > 0.05$), although several species were unique to the different farm types. There was a significant difference in the bee communities across years ($p < 0.05$). There was no interaction between farm type and year ($p > 0.05$). Species that were collected in only one farm type were often rare species. We observed a total of 42 taxa in soybean and 36 taxa in vegetable fields.

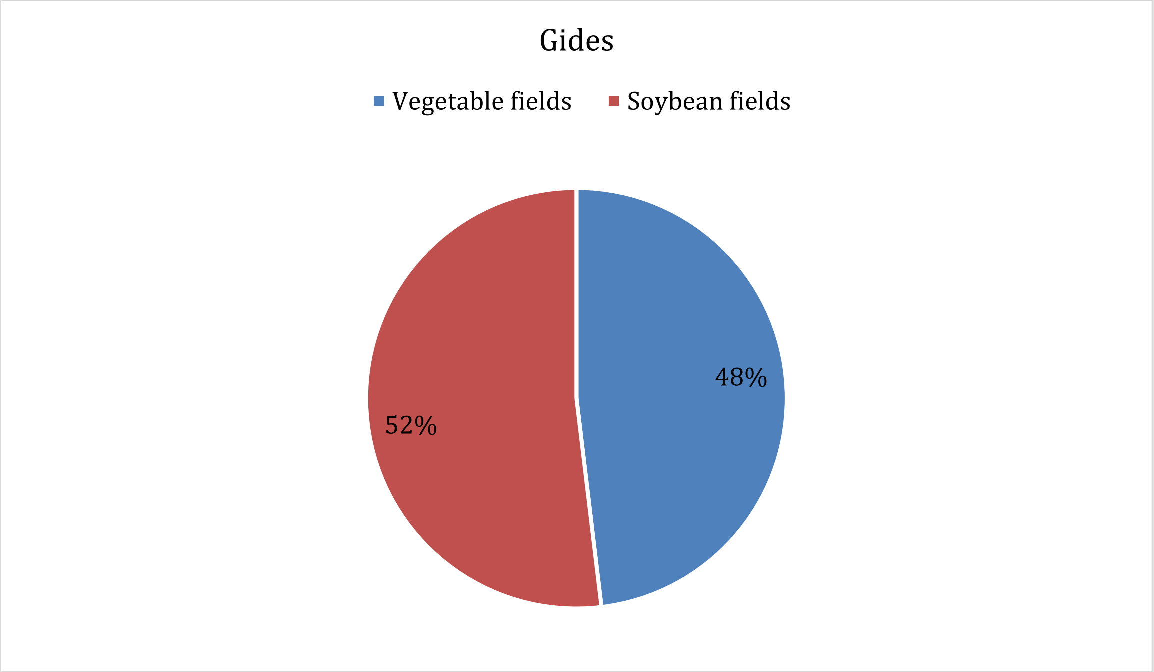

Figure 19. Sample size based rarefaction.

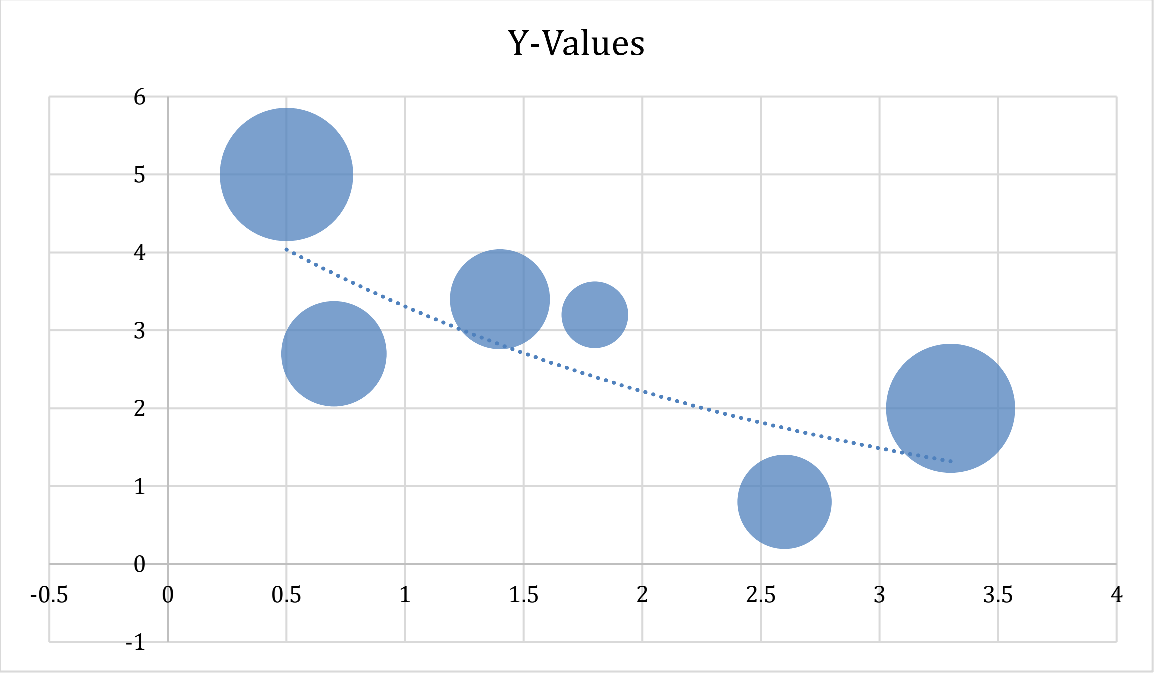

Figure 20. Scaling plot of the pollinator community.

There were no observable differences in the total abundance ($p > 0.05$) or total richness ($p > 0.05$) in the overall community of bees collected between vegetable and soybean fields. Across the entire season an average of 117 individuals for an average of 13 species per farm were collected in soybean field. Within vegetable field, an average of 120 individuals for an average of 14 species were collected per field. Bee abundance and richness did not vary by year, however both varied significantly by month ($p < 0.05$). There were no significant interactions of farm type and month with bee abundance ($p > 0.05$); however, there were significant interactions with bee richness ($p < 0.05$). We observed greater total richness in vegetable fields than soybean fields ($p < 0.05$).

We further investigated whether groups of bees differed in their responses to farm type by subdividing the bee community into common, uncommon, and rare taxa. We classified nine taxa as common, nineteen taxa as uncommon, and twenty one taxa as rare. Collectively, common taxa comprised 92.8% of the entire wild bee community, with uncommon and rare taxa comprising 6.1% and 1.1% respectively. We did not observe a difference in abundance or richness of common taxa between field types ($p > 0.05$). A higher richness of common bee taxa was observed in soybean field compared to vegetable field in the month of August ($p < 0.05$). Richness, but not abundance, of common taxa varied significantly across years $p < 0.05$ (Figure 21).

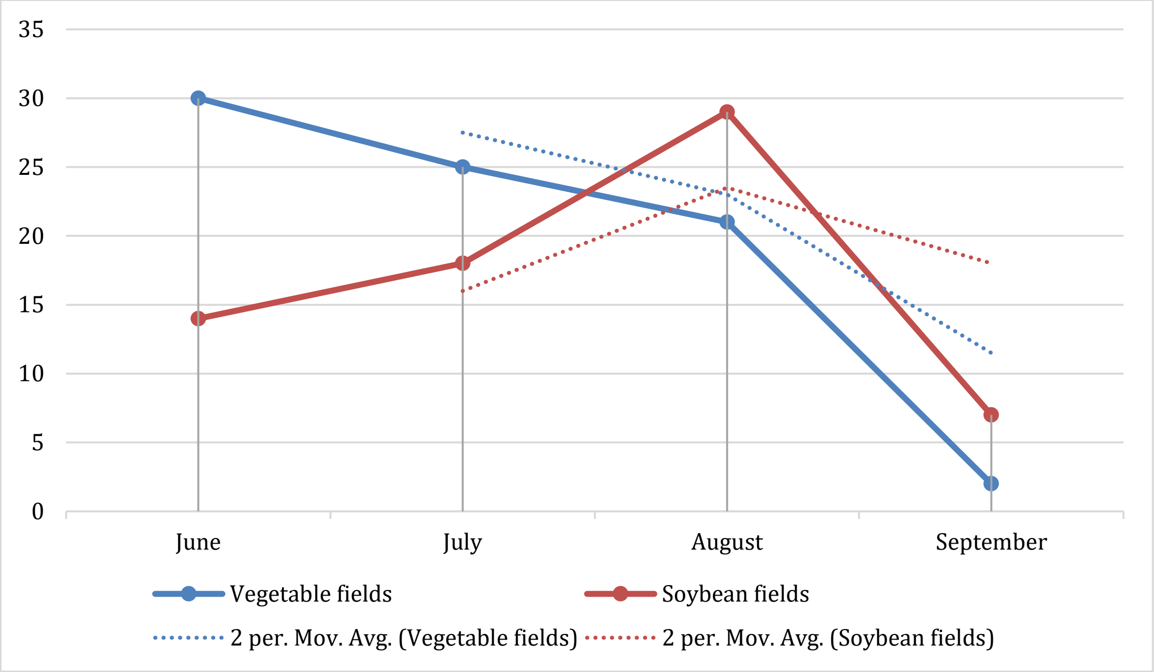

Figure 21. Mean common bee abundance of common taxa.

Abundance and richness of common taxa varied across sampling months $p > 0.05$ (Figure 22).

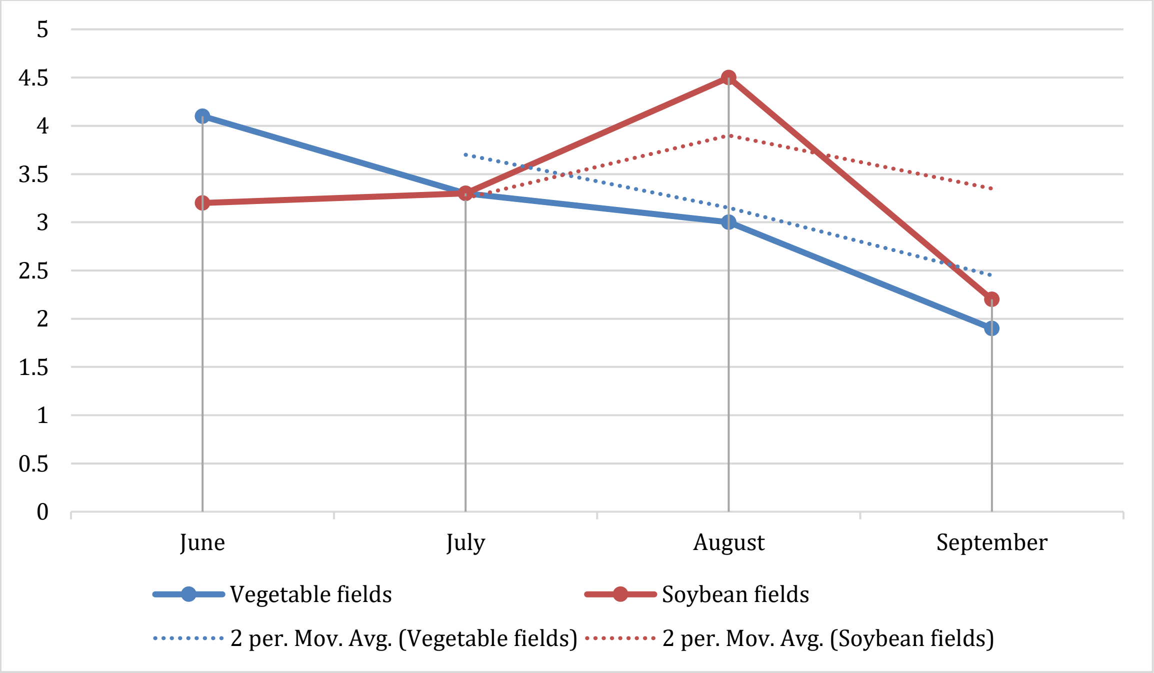

Figure 22. Mean common bee richness.

There were no observable differences in the abundance of uncommon taxa (Figure 23); however, richness of uncommon taxa was significantly higher in vegetable fields compared to soybean fields (Figure 24).

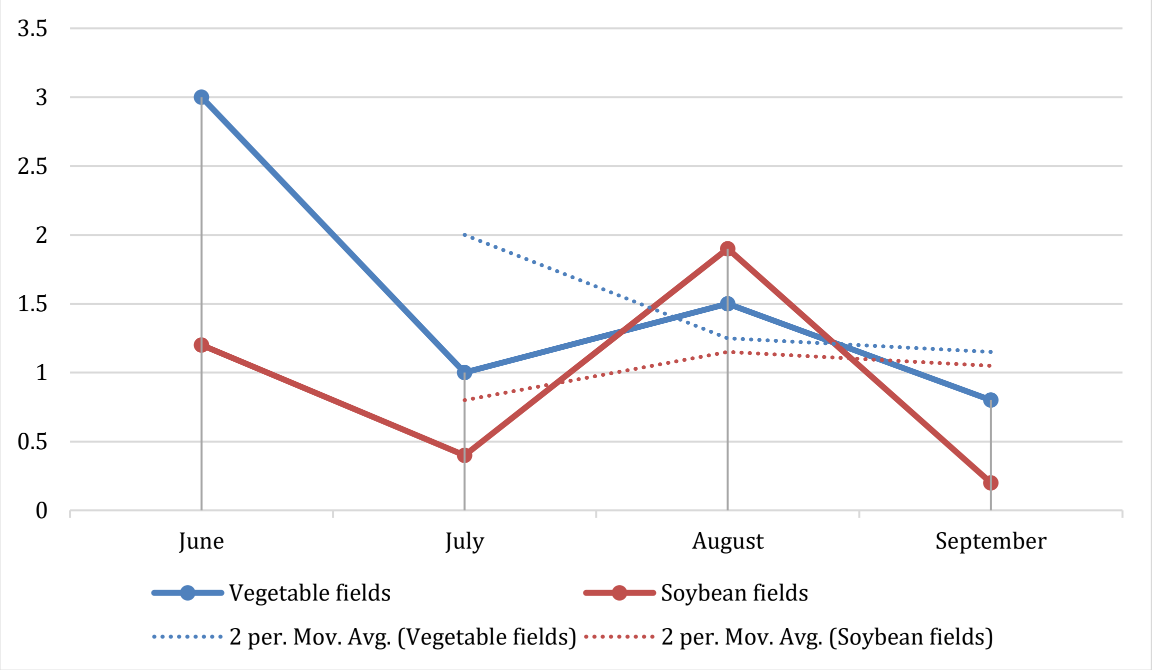

Figure 23. Mean uncommon bee abundance.

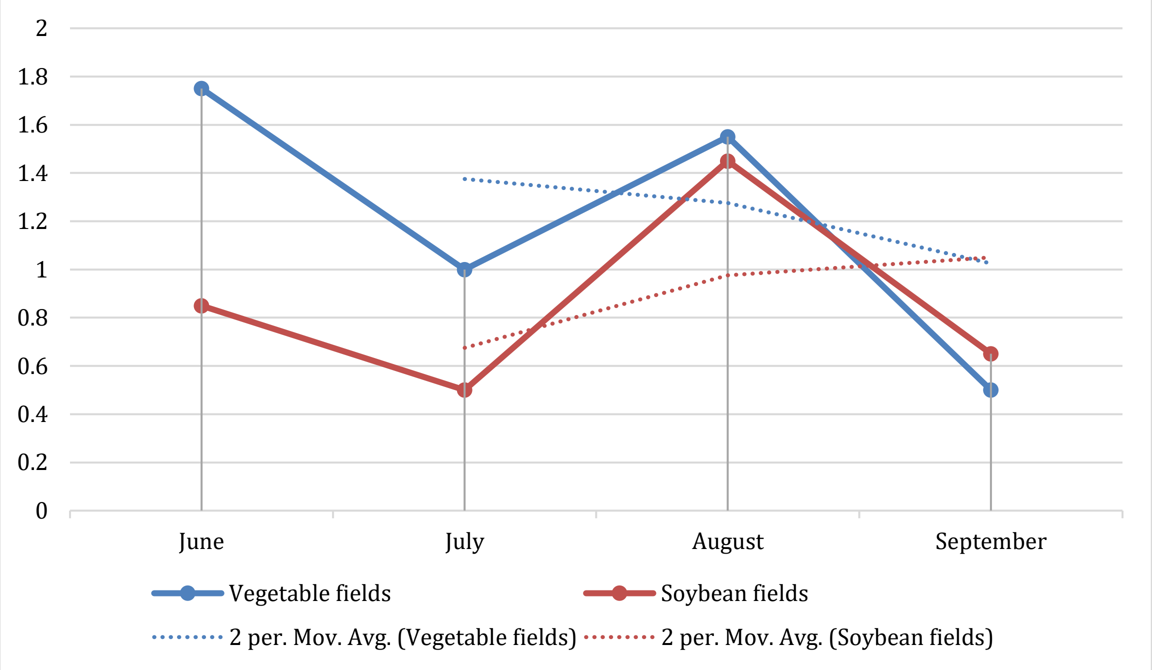

Figure 24. Mean uncommon bee richness.

Uncommon bee taxa abundance and richness were significantly greater in vegetable fields compared to soybean fields during the month of June. Uncommon bee taxa abundance and richness did not vary by year; however, abundance and richness did vary across months. There were no observable interactions of farm type and month on uncommon bee abundance or richness.

Rare bee taxa abundance and richness did not differ between farm types p >0.05 (Figure 25 and Figure 26).

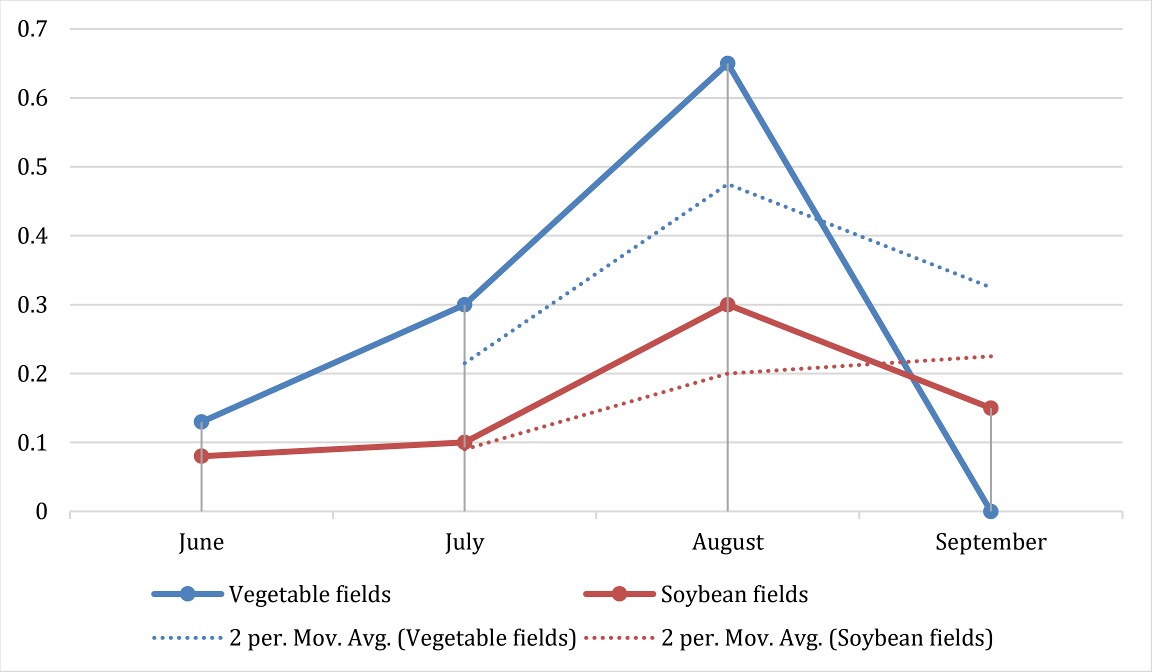

Figure 25. Mean rare bee abundance.

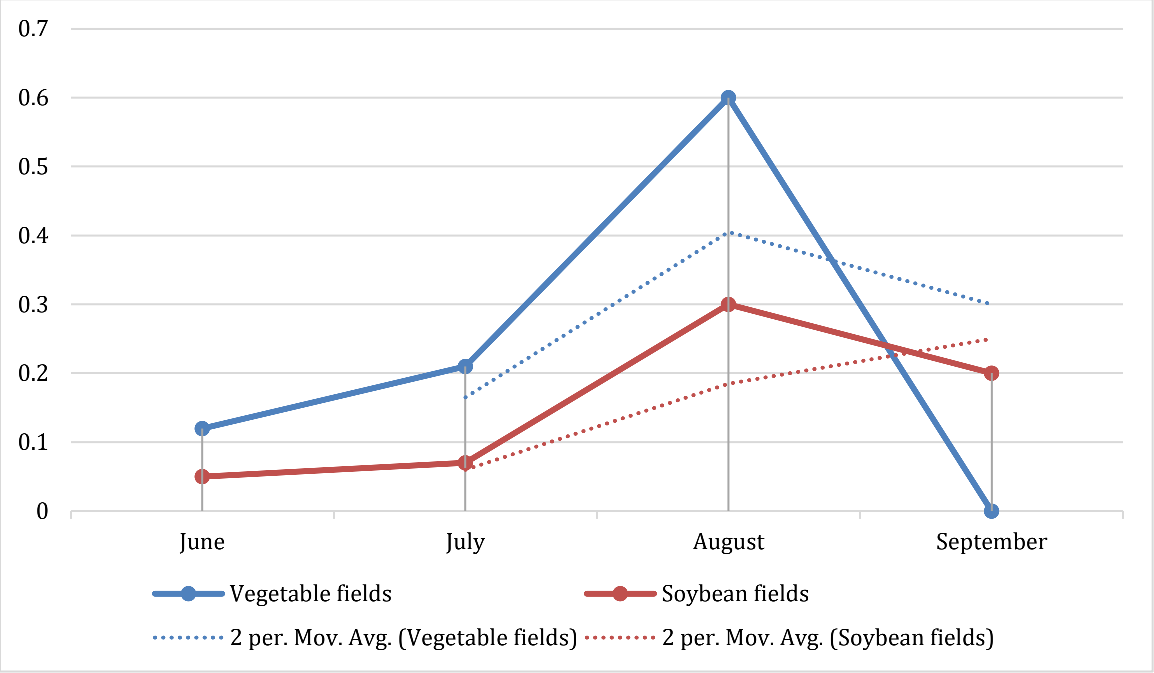

Figure 26. Mean rare bee richness.

However, abundance and richness of rare bees was significantly greater in vegetable fields compared to soybean fields during August. Abundance and richness of rare bee taxa varied across sampling months but did not vary by year. There were no interactions of farm type and month on the abundance or richness of rare wild bees.

Colony weight did not vary significantly by field type ($p > 0.05$). Colony weight varied significantly by date, year, and all interactions of date, year, and farm type for data combined from both years, apart from a farm type by year interaction. The field type by date interaction was observed on 8-July when colonies were significantly heavier in vegetable fields (Figure 27) and at the end of the season on 18-October ($p < 0.05$).

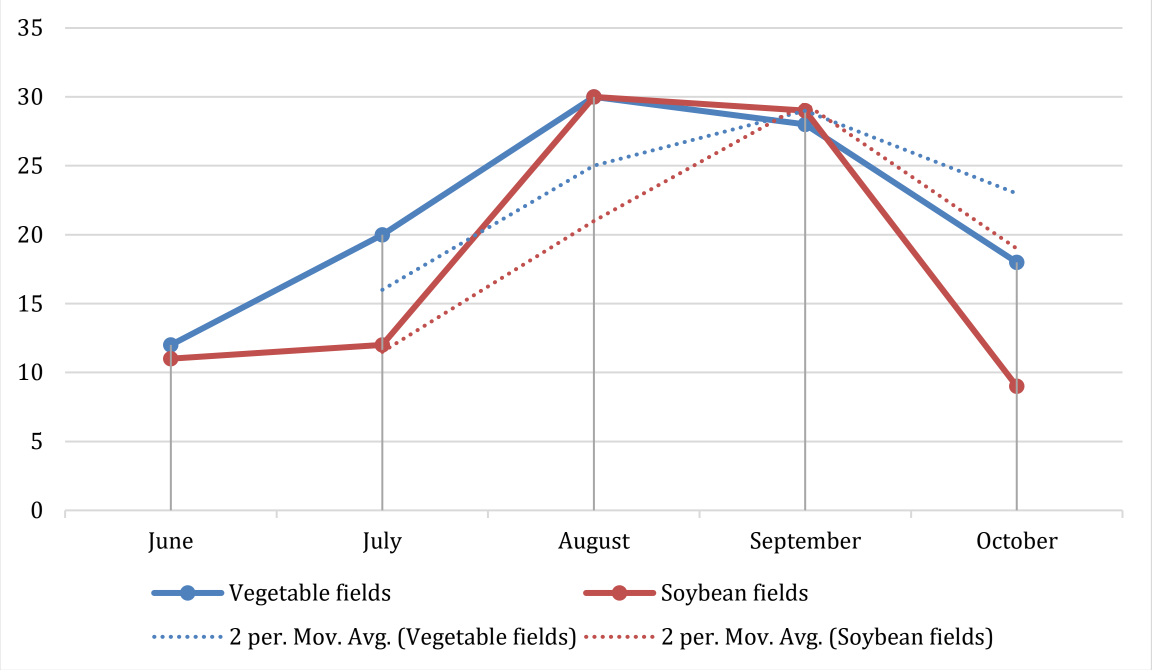

Figure 27. Mean colony weight, kg.

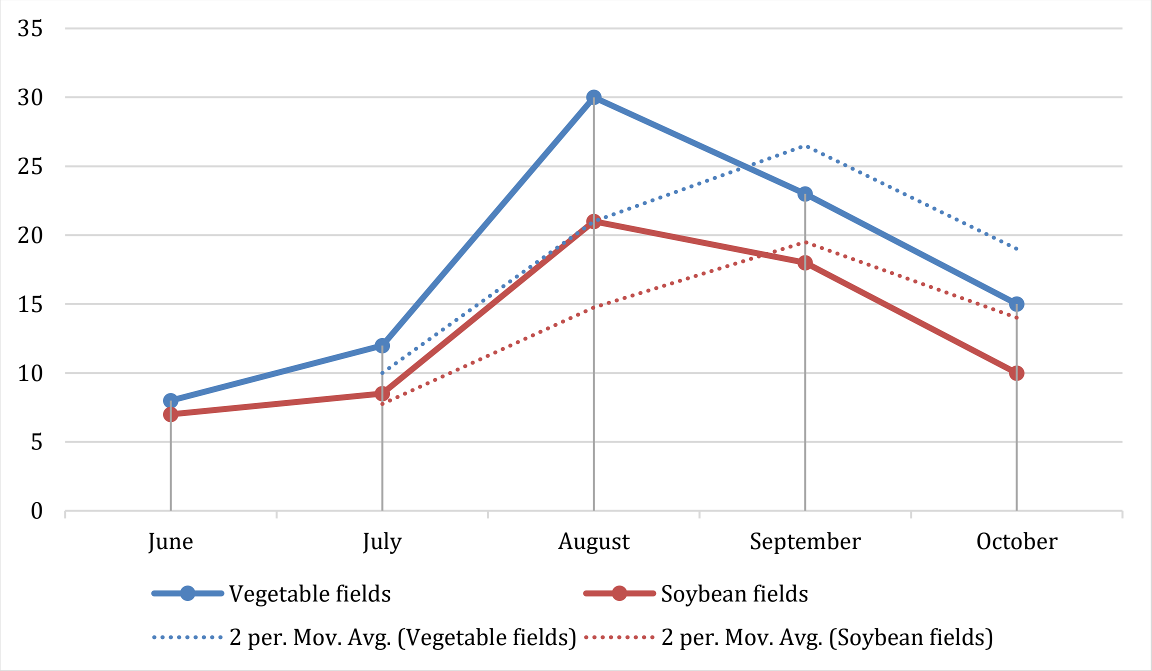

Figure 28. Mean colony weight, kg.

The heavier weight in vegetable prior to overwintering sparked an interest in investigating the late season changes in colonies more closely, as we may have overlooked subtle changes by only using a subset of the colony data. Therefore, we analyzed the earlier data separately and observed significant differences in several honey bee colony metrics between the two field types. Colonies in vegetable fields had higher weight ($p < 0.05$), with weight varying significantly by date (Figure 28). Colonies were significantly heavier in vegetable fields compared to soybean fields starting in August and remained heavier than soybean fields through October. No overall difference in brood production between the farm types was observed; however, brood production varied by date (Figure 29).

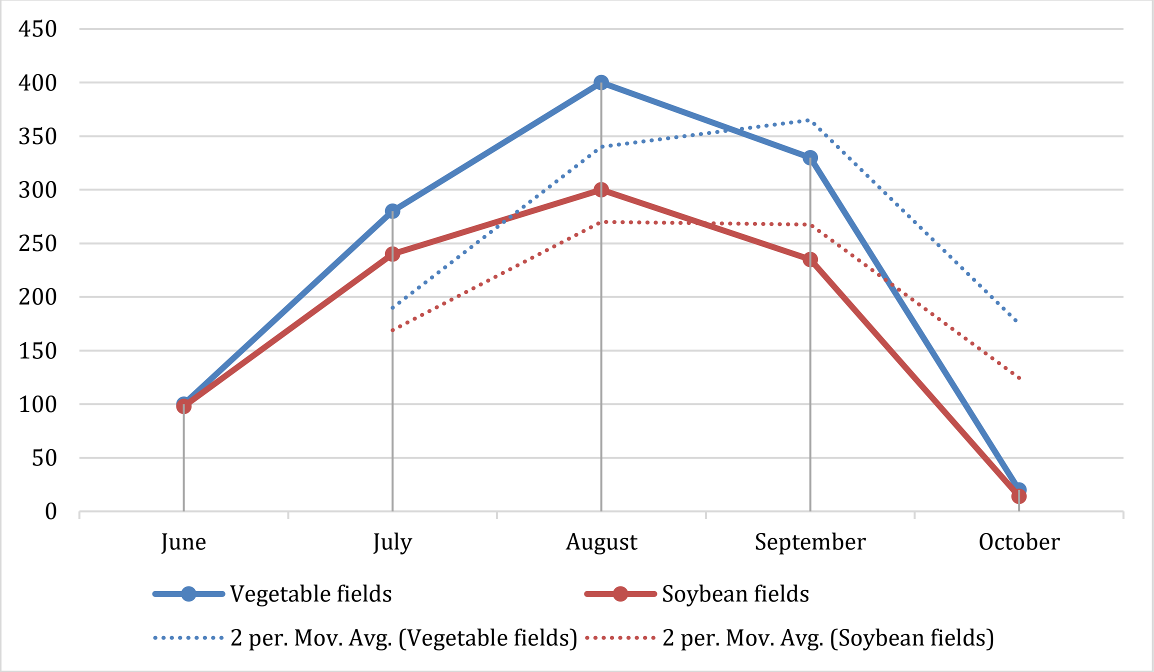

Figure 29. Mean colony capped brood area, cm^2.

Brood production was significantly higher in July and August. There were no interactions of date and field type with brood production. More frame sides of bees were produced in colonies in vegetable fields and varied by date (Figure 30), with no interactions of date and field type. There were significantly more frame sides of bees in colonies in vegetable fields in July, and August ($p < 0.05$).

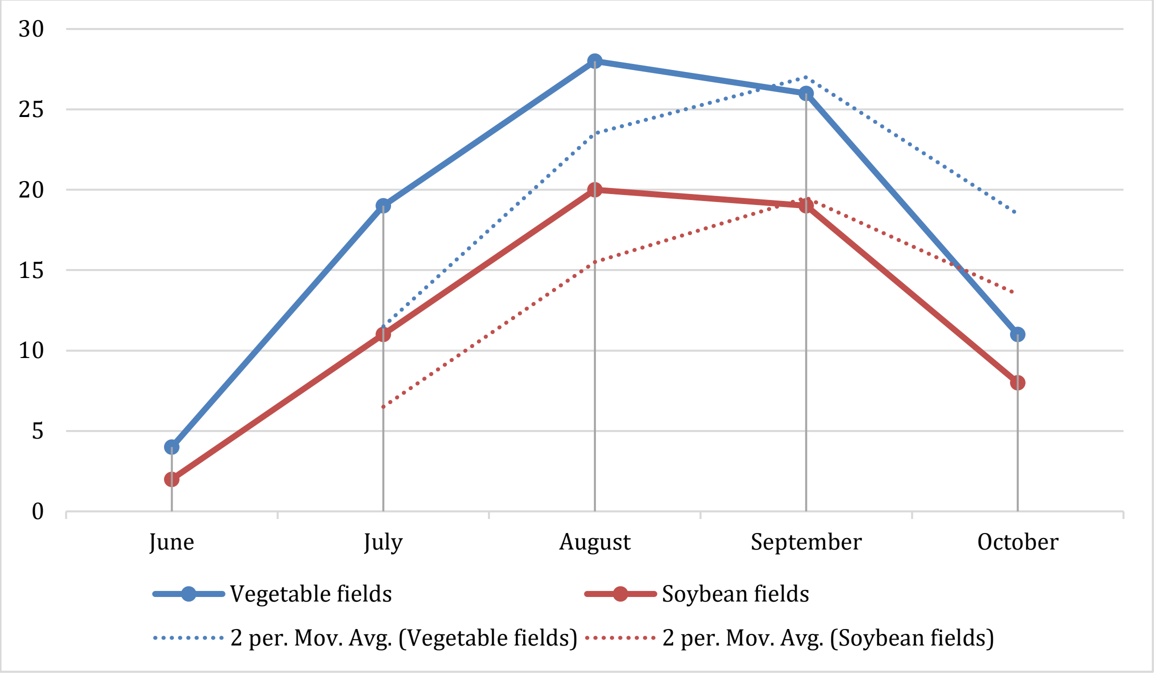

Figure 30. Mean colony frame sides of bees.

There were no overall differences in total lipid content of honey bees between colonies in soybean and vegetable fields ($p > 0.05$); however, prior to overwintering lipid content was significantly higher in honey bees in colonies in vegetable fields (Figure 31). Honey bee lipid content varied by date ($p < 0.05$) with lipids highest at the start of the season in June and then decreasing in July with no change throughout the remainder of the season regardless of field type. There were no interactions of treatment and date ($p > 0.05$).

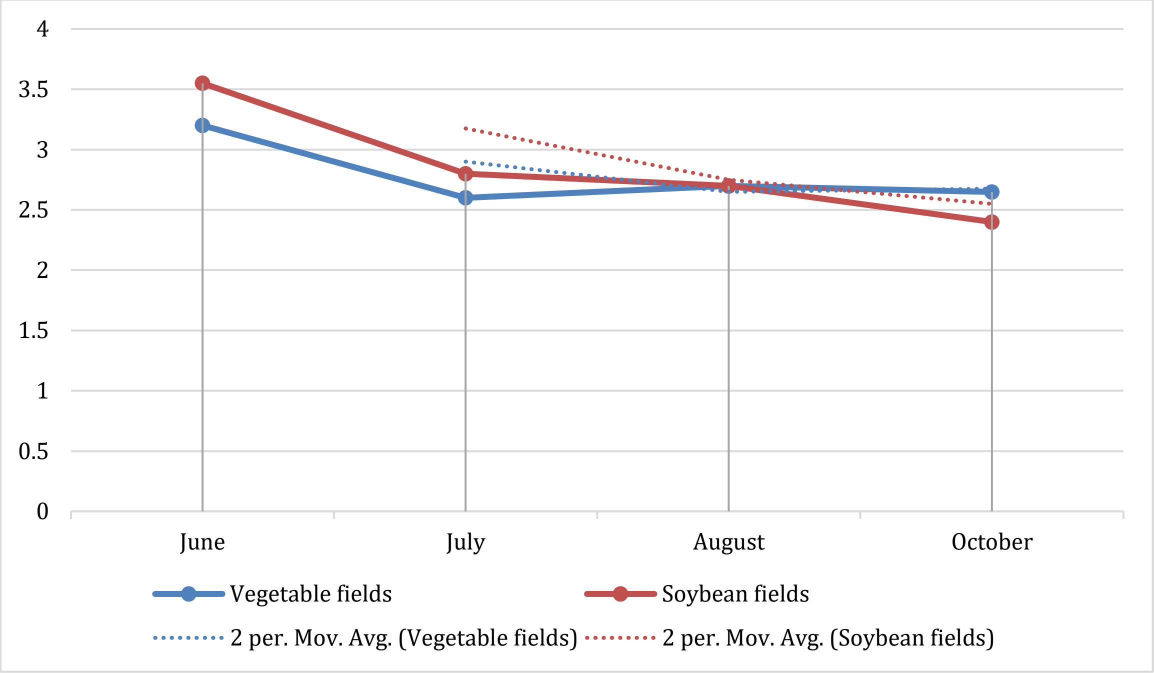

Figure 31. Mean honey bee lipid content, %.

This study presents novel insights into whether there is potential for bee conservation through diverse farming in a monoculture landscape. Our data support the hypothesis that diversified fruit and vegetable farms, even when found in a landscape that consists of extensive monoculture crops, can benefit honey bee health. Specifically, our results show vegetable fields supported increased colony growth and individual nutritional state from honey bees collected from within a managed colony. Because the fruit and vegetable farms we studied are characterized by increased plant diversity and abundance throughout the season, our data suggest that diversified farming may benefit honey bees through increased forage availability.

In addition to a nutritional enhancement of honey bees in fruit and vegetable farms over soybean monocultures, we observed differences in the abundance and diversity of some wild bees between the two farm types. Increased plant diversity through cropping of exotic fruit and vegetables hosted a more species rich community of uncommon bees. The most pronounced increases in uncommon bee biodiversity in vegetable fields were seen during month of June, where abundance and richness were greater. It may be that the plants present on vegetable fields during the early season provided sufficient nesting and forage resources for more specialized or sensitive wild bee species at a time when corn and soybean fields were not yet planted or in an early growth stage. However, not all bees responded the same to diversified farming. Although there were no main effects of diversified farming on rare bees, abundance and richness of rare bees was greater in vegetable fields during the month of August. It may be that during late season forage limitations, vegetable fields provide a necessary requirement to support species. Common bees were near ubiquitous in our study and are regularly found in the Europe, often associated with landscapes impacted by human disturbance. These common bees were found in high abundance in both farm types throughout the season. Surprisingly, there was a higher richness of common bee species found within soybean fields compared to vegetable fields during the month of August. This is a time when soybeans are flowering and potentially provide a forage resource for bees, suggesting a subset of bees may have become habitat and dietary generalists that may be well adapted to living in highly disturbed landscapes and thrive on resources available in agricultural systems. If declines in bee populations continue as they have in recent years the future of crop pollination success will likely depend upon incorporating both wild and managed bees into pollination management plans. These results suggest cropping diversity through fruit and vegetable farming can increase bee biodiversity beyond common agricultural species, although the effects are not overwhelming. In heavily cultivated systems addition of landscape diversity through small scale cropping diversity has the potential to support increased biodiversity of less common but not truly rare species. We suggest that diversified farming, in addition to an increase in more native, perennial habitat may be required in both farm types to support rare ecotone species which require resources from non-crop habitat at some point in their development.

With respect to managed honey bees, our data suggest on-farm diversity can have subtle, but significant impact on the health and fitness of honey bees. Across both years, honey bee colonies in vegetable fields had higher colony weight at the end of the season prior to overwintering. Colonies in vegetable fields had higher colony weight, bee populations throughout much of the season, and produced more brood at individual dates throughout the season. Although the surrounding agricultural landscape can provide abundant resources, these data suggest honey bees benefit from being housed in the vegetable fields. The morphology and behaviour of honey bees allows them to utilize many plant species more readily as forage, making them a 'supergeneralist' compared to other bee species. Unlike many wild bee species which only forage roughly 500 meters from their nesting site, honey bees can forage long distances and utilizing both the resources directly available from the diversity of vegetable fields and in the soybean surrounding those farms. Taken together with the varied results from wild bees with respect to common, uncommon, and rare bee taxa, this study suggests that farm practices that benefit honey bees are not necessarily a good indicator of how wild bee communities in general will respond. As other studies have previously suggested, honey bees cannot be indiscriminately used as an "indicator species" to extrapolate wild bee response to anthropogenic landscape change, especially in areas such as the Europe where honey bees are exotic to the landscape and likely utilizing different foraging resources compared to many wild bees.

Although honey bees gained some measurable benefits from being in vegetable fields compared to soybean fields, there was nonetheless a precipitous decline in weight of colonies in the late summer regardless of farm type. This resulted in colonies from both farm types entering the winter with honey stores below what is considered adequate to sustain them. An additional challenge for honey bees kept at either farm type is indicated by our lipid analysis. Honey bees store fat in the form of vitellogenin in preparation for overwintering, therefore, lipid stores of bees in the colony are an indicator of colony overwintering potential. Although honey bee total lipid content was higher in vegetable fields than soybean fields, by October, even the highest lipid levels observed were below what would be considered adequate for successful overwintering indicating that neither fields type is ideal for long term success of honey bee colonies.

In our study sites, all fields were surrounded by a matrix of extensive monoculture. Fruit and vegetable farms can have a measurable, though modest impact on a few key health indicators for honey bees and support elevated richness of uncommon species of wild bees through additional resources. Our results suggest there is potential for positive effects of increased forage and habitat through the floral resources found in diversified farms, but for greater benefits to be realized, the land area in diversified farming and type of resources provided may need to be more extensive and pollinator targeted. Integration of native, perennial habitat is a promising possibility for enhancing forage in an agricultural landscape.

5.1. Honey bee in agricultural service

The honey bee, *Apis mellifera*, belongs to the insect order Hymenoptera, which features over 100,000 species of sawflies, wasps, ants and bees. Most insects within the order Hymenoptera exhibit haplodiploid sex determination (males from unfertilized haploid eggs and females from fertilized diploid eggs) which is thought to be a basis for the evolution and maintenance of eusociality. Hymenoptera diverged from Diptera and Lepidoptera over 300 million years ago to form an ancient lineage of bees that evolved in tropical Eurasia and migrated north and west, reaching Europe at the end of the Pleistocene, 10,000 years ago (Halfawi et al., 2022; Puvača et al., 2021).

The honey bee genus (*Apis L.*) is the most well recognised of all insects due to the component species services to agriculture, pollination and mankind. This genus includes the giant honey bees (*Apis dorsata* and *Apis laboriosa*), the dwarf honey bees (*Apis florae* and *Apis andreniformis*), the eastern hive bees, (*Apis cerana*, *Apis nigrocinca*, *Apis koschevnikovi*, *Apis nuluensis*) and the western hive bees *Apis mellifera*, for which there are over 24 different breeds (Dolezal, Carrillo-Tripp, et al., 2019; Sponsler et al., 2017; G. Zhang et al., 2022).

Apis mellifera can be grouped into four bio geographical branches: African (A), Oriental (O), Northern Mediterranean (C) and West European (M). European honeybees (M-lineage) are thought to have survived the last glacial period in two refugia, one on the Iberian Peninsula and one on the Balkan peninsula (C-lineage). After the glacial retraction 10,000 years ago the honeybees re-colonized Europe with the M-lineage occupying north and west Europe and the C-lineage occupying central Europe. Geographical barriers such as the Alps maintained the differentiation of subspecies (Y. P. Chen & Huang, 2010; Forsgren & Fries, 2010; Spivak & Reuter, 2001).

Only *A. mellifera* is found in the UK, and there is evidence that the subspecies *A. m. mellifera* travelled into Britain across the European land bridge well before

8500BP. In fact, it has been shown that the honeybee's range was closely linked with hazel and lime distribution. In 6500BP oak and hazel forests extended as far north as Skye in the west and Buchan in the east so as environmental conditions eased honeybees could have travelled with the advancing tree lines. Wild honeybees could have reached Britain from remnant populations in France within 1100 years, if they were to swarm once every second year and travel a conservative 1.5 km to their new colony site (Hawkins & Martin, 2021; Noël et al., 2020; Thompson, 2010).

5.2. Apicultural and honey bee roles in sustainable agriculture

Honey storing insects are all social and living in colonies, most of which are bees but wasps and ants also have this ability. The evolution of honey bees led to two very advanced cavity nesting species who's nest would contain numerous parallel combs: *Apis cerana* and *Apis mellifera*. By forming clusters within the cavity these two species developed the ability to survive cold winters and therefore extended their distribution. *Apis mellifera* has been and is the most important species to man. Indeed, this specie is both productive and amenable to management. It is often called the European honey bee or the western honey bee even though it is not native to Europe (Fries et al., 2006; Page & Gary, 1990; Paxton, 2010; Spivak & Reuter, 2001; G. Zhang et al., 2020, 2021).

A honey bee colony represents tens of thousands of individuals divided into three main categories:

The queen: she is the central element of the colony by ensuring its survival. Through pheromone secretion she regulates the colony's activities and ensures the cohesion of the worker bees. But mostly she is the only one capable of laying eggs providing future worker bees that will forage food for the colony among many other tasks. Shortly after hatching, the young queen leaves the colony for her mating flight. She returns to the hive mated and begins to lay eggs (1500 – 3000 eggs a day).

The drones: They hatch mainly over spring and their main known tasks consist in mating a queen during her mating flight. The mating process is lethal to the drones.

The worker bees: They represent the bulk of the colony, around 30 000 in a healthy hive. They ensure the survival of the colony by many aspects: the maintenance of the hive (cleaning the bottom board, the empty cells, etc.), breeding the larvae, building the combs, protecting the hive, foraging food. The task they are given is function of their age and the colony's needs.

The Dadant beehive is the model used by a large majority of beekeepers in Europe. It is divided into two main parts: the brood box in which the queen lays the eggs, constituting the brood and the honey super in which the queen cannot go because of a bee excluder (a grid with holes of a precise diameter letting the worker bees through only). The queens' access is reduced to the brood box and therefore workers use the honey super to store the collected nectar. It is this box that the beekeeper will harvest.

The colony is segmented into three parts:

The adult population: Mainly found in the brood box it can also spread to the honey super when it is populous. The foraging bees come and go throughout the day, it mostly depends on the climate (temperature, precipitation, wind), the environment (resource availability) and the colony's needs.

The brood: It represents the reproductive investment of the colony, it is composed of all the future colony population: eggs, larvae, and pupae in capped brood. In the hive the brood nest is found in the middle on the central frames of the brood box. This organisation allows the brood to stay in an environment with its optimal temperature (34-35°) and hygrometry (50-60%). The development of a worker bee lasts approximately 21 days.

The honey reserves: Composed of the nectar and pollen foraged by the worker bees. Nectar foragers returning to the hive pass their loads to younger bees through trophallaxis. It is then deposited in the combs where it will be processed by other bees into honey.

Returning pollen foragers store their loads in empty cells close to the area of the nest.

Several naturally occurring events take place during a colony's' life:

The queens' death: It can occur accidently or naturally. The colony is considered as orphan. If there are queen cells or a young queen (that hasn't been mated yet) the hive is said to be in a “requeening” process. On the contrary if there is no queen to be the worker bees will start laying eggs, giving birth to drones only. The hive is considered as a “drone colony” and will collapse.

Swarming: It occurs mainly in spring but also throughout summer. Healthy and populous colonies may choose to swarm: they will set up queen cells and the previous queen will leave the hive with many worker bees in order to settle somewhere else. Many factors can provoke swarming: the environment, anthropogenic disturbances and some species are genetically susceptible to swarming (tropical bees).

Starvation: When the environmental resources are scarce or when the climate does not allow worker bees to forage, the colonies development is directly affected. Starvations may have carry-over effects on the dynamic of the colonies for the rest of the season.

Disease: Many diseases and parasites infections can weaken a colony by attacking the brood or the adults. Among the most common disease are the European & American foulbroods (*Melissococcus plutonius*, *Paenibacillus larvae*). It is known as a bacterial brood disease lethal to the colonies if no treatment is carried out. Another parasite destroying the brood is the wax moth that settles in combs, slowly developing into a plague that will force the colony to leave its hive. Regarding the adult population, the most devastating parasite is the famous Varroa destructor. It is an external parasite that attacks both adult and pupae. It is native to Asia where it’s natural host, the Asian honeybee (*Apis cerana*) lives. The mite rarely negatively affects *Apis cerana* since it has developed some natural defences against it. Varroa’s host shift to *Apis mellifera* resulted in a devastating decrease of *Apis mellifera* colonies that did not have the natural defences to fight Varroa destructor.

Predation: Honey bees are attractive prey for many predators, birds, spiders, insects, but the current focus has been given to the Asian wasp. This imported predator, *Vespa velutina*, was first seen in France and in Europe in 2005. It is a well-known honey bee predator, against which *Apis mellifera*, unlike *Apis cerana*, has not been trained to fight. *Vespa velutina* feeds on honey bees, mostly forager bees, coming back to the hive with pollen and nectar. It beheads it’s pray, removes its wings and legs and brings the thorax back to its colony.

Honey bee forage both pollen and nectar to meet their food requirements. Nectar or honeydew represents their natural source of carbohydrates which allows them to meet their energetic expenses. Foragers collect nectar from the flowers, transport it to the hive and store it into sealed cells as honey. During the returning flight the transformation process of nectar into honey starts.

On the other hand, pollen is the only natural protein and lipid source for honey bees. It is consumed both by adults and larvae and is often consumed shortly after being brought back to the hive. Honey bees mix regurgitated nectar with pollen and store it in small quantities the mixture is called beebread. The weight of pollen in the amount of honey reserves of a bee colony is minor. Regardless of its weight pollen plays a key role in the accumulation of honey reserves. The pollen intake will influence the brood size and in fine the number of bee workers. Added to this indirect effect pollen influences positively bee health and is therefore crucial for the colony resilience to diseases.

The impact of pollination service on agricultural production is widely acknowledged. Pollination consists in pollen transfer from the anther to the stigma of a same or different flower. This is the first step in the fertilisation process. Among various dissemination agents different animals can contribute to this step among which the invertebrates and more specifically insects.

Honey bees are considered as the main insect pollinator in agricultural landscapes. This is due to the high number of individuals within one nest. As mentioned earlier in this report, in Europe 84%, meaning 150 grown crops, directly depend on insect pollination. At the international scale, 70% of the crops grown for human consumption, corresponding to 87 of the 124 crops grown directly for human consumption rely on animal pollination to produce and/or increase its production. The level of crop dependency to insect pollination varies from a crop to another.

Losing all pollinators would have sizeable effects on international food security, leading to the average reduction of 8% of the agricultural production. However this scenario should be considered with care since a major part of the calories used in human consumption come from crops that are not dependent on pollination such as wheat, rice and corn.

5.3. Landscape diversity

Honey bees forage pollen and nectar on specific plants: melliferous plants. A melliferous plant produces substances that can be collected by insects and turned into honey. Many plants are melliferous however not all produce both nectar and pollen that can be harvested by honey bees, for instance rapeseed and sunflower produce both nectar and pollen. In the landscape melliferous plants can be grown as well as wild.

In order to ensure its survival, reproduction and development honey bee colonies require a large diversity of melliferous plants. In the current agricultural context the landscape is almost entirely composed of agricultural land thus the largest food supply for honey bees comes from field crops, vegetable growing and grasslands. Melliferous field crops are mainly: oilseed crops such as Rapeseed (*Brassica napus* L.) and Sunflower (*Helianthus annuus* L.), protein crops such as faba beans (*Vicia faba* L.) and others such as buckwheat (*Fagopyrum esculentum* M.). Field crops are commonly grown for their grain on vast areas of land with minimum labour. Their blooming period occurs massively on a very short period of time. These crops are very attractive for beekeepers because of their high melliferous potential, however the intensive use of crop protection products endangers honey bees. Many vegetable plants such as pumpkins, carots, onions and many others, are melliferous despite their scarce blooming. Grasslands for animal consumption usually host several melliferous plants such as alfalfa (*Medicago sativa* L.) and white (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.).

Wild floral resources are all the resources that are not cropped by humans: weeds, hedges, woods, grass strips, etc. Starting from the end of the 2nd world war, European and National agricultural landscape have been strongly modified in order to meet the growing food requirements. The regrouping of agricultural land led to farm expansion and a progressive decrease of semi natural habitats, hedges and grasslands that would only take up land needed for growing food. Land use intensification led to a shift in the spatial organisation of the landscape with obvious effects on agrobiodiversity. The fragmentation of the semi natural habitats, appropriate for nesting, feeding, mating, etc., causes the loss, in quality and quantity, of favourable habitats for biodiversity. All the processes combined: fragmentation, homogenisation, decrease of semi natural habitats, intensification progressively lead to the erosion of the agrobiodiversity.

A strong diversity of wild floral resources can be encountered in the grass strips along the roads or the fields. However, their intensive mowing progressively reduces their occurrence and limits their attractiveness for pollinators.

Together with the landscape changes, agricultural practices became more intensive with an increase in pesticide use depriving pollinators from vital floral resources. For instance, cereal fields are not very attractive for honeybees, however the weeds they host have widely been recognised as extremely interesting for the pollen supply of honey bee colonies. The intensive weeding and in particular the use of pesticide or the thorough cleaning of the seeds is leading to their decline, excluding them from the core of the field and reducing their growth to the field margins.

5.4. Deterioration of bees population

Recent public and scientific interest for honey bees occurred when the sharp disappearance of worker bees from a colony was described as colony collapse disorder. From there on, research efforts have focused on improving colony health and management techniques and identifying possible causes of colony collapse disorder. The population of honey bees are decreasing worldwide, this phenomena has been detected in Europe, many parts of the USA and in Asia.

In Europe the number of colonies decreased from 21 million in 1970 to 15.5 million in 2007. Between 1985 and 2005, for 18 European countries the mean rate of colony losses reached 16%. Considering the extent of this decline it was defined as: Colony Collapse Disorder (CCD).

Since 1975, the number of publications related to honey bee colony losses has increased exponentially. To explain honey bee decline many factors have been proposed, they can be grouped into three broad categories of causes: Parasites and Pathogens, Genetic diversity and vitality and Environmental stress. This third group accounts for about 31.3% of the publications on honey bee colony losses, it is composed of three different subgroups: Pesticides, flower availability and habitat loss.

The pesticide subgroup shows over 56% of the literature occurrence frequency, since honey bees extensively forage on flower-blooming crops such as rapeseed, maize (*Zea mays* L.) and sunflower, they are exposed to a high number of pesticides. The increase in pesticide uses has largely been blamed for honeybee colonies losses due to their lethal composition. A recent law was voted prohibiting the use of neonicotinoids insecticides by 2018. Neonicotinoids are systemic insecticides, the three most virulent molecules being: Imidacloprid, thiametoxam and clothianidin. These insecticides in a sub lethal concentration will alter the behaviour of bees and thus reduce the survival of entire colonies. Moreover, honey bees cannot taste neonicotinoids and therefore are not repelled by them. Exposing social bees to these insecticides presents a sizeable hazard.

Habitat loss is sometimes referred as a cause of honeybee colony losses. Habitat loss acts negatively on biodiversity through a decrease of nesting and foraging sites.

Though floral resources without doubt have an impact on the honey bee colony survival which is totally dependent on the honey reserves stored, there is no demonstrated evidence of a direct link between floral resources decrease and honey bee colony losses.

5.5. Honey bee nutrition

In an intensive cereal farming system, the reserve accumulation of honey bee colonies follows a seasonal pattern connected to the blooming period of the main mass flowering crops being rapeseed and sunflower. Honey bees forage on a wide diversity of flowers, however when the mass flowering crops are available they focus their foraging effort using them. Unfortunately, these mass flowering crops are highly seasonal and result in the occurrence of a 'dearth period', with a severe decrease in honey reserves, between the two peak flowering period of respectively rapeseed and sunflower.

The severe food depletion during May and June compels honey bees to forage on wild floral resources.

Several landscape elements have been found to contribute favourably to the reserve of the colony such as the woody elements and the weeds in a landscape. The woody elements and the weeds represent the major part of the pollen intake, more than 60% of the average pollen mass brought back to the hive.

Few studies have focused only on the dearth period, though some elements have been pointed out, such as the possible positive contribution of flax (*Linum usitatissimum*) during this food shortage. And on the other hand the negative effect of sunflower, blooming only later, taking up agricultural land without providing resources. However later in the season, during its blooming period, sunflower represents a major resource for pollinators, accountable for the main honey harvest for beekeepers.

Weeds constitute the bulk of the honeybee pollen diet during the dearth period. Arable weed species such as red poppy (*Papaver rhoeas*) act as an important food resource for biodiversity protection, in particular birds and insects. However, this central food resource is difficult to preserve considering that its optimal habitat is in crop fields. The occurrence of arable weeds has been declining as well as the species richness in which they occur. They are now disappearing from the core of the fields progressively confined to the field margins that act as refugee for weeds that can no longer survive in core fields. Thus, edges and woody habitats are considered as crucial landscape elements when focusing on biodiversity and honey bee survival.

Regarding some important features of the landscape, no clear consensus has been reached concerning its effect on the amount of reserve. Urban areas were proved to have a positive effect, whereas others highlighted its negative correlation to the number of resources in the hive. Some authors focused on the amount of food produced around an apiary in order to determine what crops would provide most resources for honeybee. They showed that arable land is the poorest regarding the amount and diversity of nectar. On the other hand, calcareous grassland, broadleaved woodland and neutral grassland are the habitats that produce the most nectar. Though the number of available resources around the apiary could not yet be correlated to the number of reserves in the hives. We suspect a carry-over effect of the dearth period on the colony dynamics: the food shortage (May and June) would impact the colony later in the season. During the dearth period it has been showed that the woody elements act as a buffer for the population decrease, decrease which commonly occurs between the two mass flowering crops. Thus, could be suspected that there would be more foraging bees and thus more food brought back to the hive when woody elements and weeds are abundant.

5.6. Concentration of honey bees in agricultural fields

The use of colored pan traps is a cost-effective, simple, and efficient technique to passively quantify insect communities, including bees. When used for studying pollinators, pan traps (or ‘bee-bowls’) can be a useful tool for monitoring bee communities, but honey bees may be underrepresented, possibly due to a bias toward smaller bee species. Honey bee presence in pan traps may be lower compared to net samples and direct observations; however, the presence of smaller bees which are considered to be susceptible to pan traps (*Lasioglossum* spp.) has also been observed to be lower compared to other sampling methods. Thus, the potential for using pan traps in assessing honey bee activity may be unnecessarily underutilized.

Despite a potential bias, honey bees have appeared in colored pan traps, although in low numbers, even in crops that do not require animal-mediated pollination. Studies conducted in regions where honey bees are native or feral colonies are abundant have found pan traps effective at capturing honey bees. However, in regions where honey bees are not native or feral colonies absent, very small numbers of honey bees are captured, likely due to an absence of a stable honey bee population or ineffective usage of traps to capture honey bees (e.g., traps placed on the ground rather than elevated). In general, pan traps estimate activity-density, that is the movement of an insect through a landscape coupled with its population density. Assuming honey bees are present, factors that affect the activity of a honey bee within a sampled area would affect the abundance of honey bees captured in a pan trap, therefore, the number of honey bees captured per trap is an indicator of activity-density. The usefulness of pan trapping as an accurate method of estimating honey bee activity-density is not well understood.

Pan trapping has been identified as a method which captures the greatest activity-density of a pollinator community in agricultural fields compared to sampling methods used by applied entomologists to study insect pests of crops (e.g., yellow sticky traps and non-target sweep netting). Although these studies confirmed the presence of honey bees in crop fields using pan traps, they revealed a low level of honey bee activity-density, with honey bee foragers contributing a small percentage (0.005%) of the entire bee community. However, these studies were conducted in areas in which it was not known whether honey bee colonies were present. Additionally, these studies sampled for a limited time period, potentially missing changes in seasonal activity-density of honey bees in relation to available flowering resources within or around the crop field.

Declines in wild bee biodiversity are documented worldwide. These have been attributed to multifactorial stressors including environmental toxins, pathogens, reduced forage availability, and climate change. Highly developed agricultural systems result in reduced landscape diversity, which may reduce diversity and abundance of wild bee communities. In the U.S., wild bee populations are particularly at risk in regions of the Upper Midwest where vast areas of the landscape have been converted for the annual production of row crop agriculture (primarily corn and soybeans). In addition to reduced wild bee populations, honey bee (*Apis mellifera* L.) colony losses have mounted in this region, with beekeepers in the Midwest frequently losing 60% or more of their colonies annually.

These declines are particularly problematic as wild bees are an essential part of maintaining natural ecosystems and honey bees contribute to pollination of over 150 crops. Increased demand in food supply has resulted in greater dependence on honey bee pollination services. With unsustainably high colony losses, honey bees are unable to meet crop production needs. As a result, there is an increasing reliance on wild bees, in addition to managed bees, for pollination services, which are more efficient in some cropping systems.

Crop management plans that integrate wild and managed bees can reduce pollination costs for farmers and ensure long term stability of pollination services. However, in systems where wild bee communities and honey bees overlap there is potential for competition of floral resources and transmission of disease. Many studies have investigated the effects of landscape intensification on wild bee communities, and the impacts honey bees can have on wild bees. However, a critical knowledge gap about how landscape and honey bee presence interact to impact wild bee communities exists. Many species of wild bees already under stress from agricultural industrialization could be negatively impacted by additional competition with managed honey bees, further exacerbating population declines.

Corn and soybean do not require insect pollination studies have revealed that corn and soybean fields can house over 50 species of pollinators, including honey bees. How this community responds to variation within the surrounding landscape is not clear. In soybeans, surrounding landscape has been shown to influence pest and beneficial insects. In other agricultural systems, surrounding landscape influences pollinators. Higher plant diversity in a surrounding landscape can increase both pollinator and natural enemy abundance and richness. If extensive farming is associated with reductions in resource diversity and/or abundance, then it would be expected, landscapes committed to high proportions of annual production of corn and soybean would pose the highest risk of conflict between wild and managed bees compared to resource-rich areas. To advance efforts of conserving bee biodiversity and maintaining a sustainable future food supply it is vital to understand the impacts of surrounding land use on wild bees, particularly in areas where the landscape is dominated by agriculture. Additionally, because of our reliance on honey bees for agricultural pollination, it is a necessity to understand how vulnerable wild bee populations are to impacts from honey bees in agricultural landscapes.

5.7. Territory restrictions for honey bees in agricultural terrains

As human population grows, habitat loss from anthropogenic landscape changes threatens the health and existence of many species. An ever-increasing demand for food and biofuels following human population expansion requires more land be dedicated to agricultural production. Global land use has shifted to meet this demand, with natural areas and smaller scale agricultural enterprises transformed into high-yielding monocultures, but with some cost. Monocultures can have substantial negative environmental effects on soil, water, and air quality, and when coupled with the removal of native, non-crop habitat, this form of agriculture is associated with declines in pollinator populations. This conversion is provoking concerns for reduced pollination of crops and wild plants that could lead to reductions in agricultural production and ecosystem service delivery.

Worldwide, honey bees (*Apis mellifera*) are the most economically important pollinator of crops, with honey bee colonies in the United States alone responsible for over €15 billion per year. Like other bee species, honey bees are challenged by environmental stresses that reduce colony survival, with state-wide losses as high 60% depending upon their location. This rate is higher than beekeepers consider sustainable, resulting in increased costs for contracted pollination services. These losses are associated with multiple, potentially interacting, stressors, including pest/pathogen pressure, pesticide exposure, and nutritional shortages, all associated with anthropogenic influence.

How do honey bees respond to landscapes that become increasingly dominated by extensive agriculture, particularly of crops considered to have limited nutritional benefit? Nationwide surveys have shown some of the worst colony losses occur in the Midwestern United States, a region of major agricultural production. Further, agricultural land use has been associated with lower amounts of protein in stored pollen, lower honey production, and decreased physiological health of honey bees. Conversion of non-cropped land to crops has been linked to a decline in suitability for productive apiaries and several key metrics of honey bee health and productivity where agricultural intensification has recently increased.

While the popular press has evocatively described regions that are agriculturally productive but devoid of biodiversity as “green deserts”, corn and soybean fields can host dozens of pollinator species. Further, increases in cropland can correlate with improvements in key honey bee growth metrics like food accumulation, as mass flowering crops or non-crop plants growing in field edges can provide forage for honey bees and wild bees. Thus, it remains unclear whether intensely farmed landscapes are overall net-positive or net-negative for managed pollinators such as honey bees. Studies of honey bees’ responses to crop production that do not explore seasonal exposure to landscape features may miss changes in phenology that can be significant for colony and individual honey bee health. Determining the net effects of agriculture upon honey bee survival requires multi-season, longitudinal studies of replicated, researcher-controlled colonies embedded in multiple agroecosystems.

A longitudinal study of colony growth and bee nutrition in one of the most extensively farmed areas of the world in USA, a perennial leader in the production of corn and soybean, with 92.6% of the state dedicated to agriculture and 72.9% planted with annual crops. Despite this general lack of landscape diversity, variation in land use within the state can explain the abundance and diversity of key members of the insect community found within soybean fields. By placing bee colonies next to soybean fields and comprehensively studying their response to variation in land use surrounding these fields, we can understand how honey bees respond to a highly intensified agricultural landscape and begin to forecast the future of honey bee health in other regions undergoing similar agricultural intensification. Analogous longitudinal approaches can be used to assess intensification in other cropping systems.

5.8. Overview of honey bees diseases occurrence

Bees are an essential component of ecosystems providing a pivotal service through the pollination of a wide variety of plants, including economically important crops. However, wild bee populations have declined at local and regional scales, and managed honey bees are also facing high colony losses.

Wild and managed bees are affected by interacting environmental stressors, such as diseases, inadequate nutrition, and exposure to pesticides as a result of agricultural intensification. Worldwide, habitat conversion due to transformation of landscapes into row-crop agricultural systems is cited as a primary driver of wild and managed bee declines.

Land used for agriculture can reduce natural and semi-natural habitat creating a scarcity in floral diversity and abundance that affects pollinator abundance and health. Although mass-flowering monocultures may provide transient forage for some bee species, the simplified landscape and post-crop bloom results in a paucity of floral abundance. Such loss of resource diversity can lead to sub-optimal bee nutrition resulting in a compromised bee immune system and poor overall health.

Honeybees in the EU have a range of diseases and parasites, some of which are novel like *Varroa*, and some of which act in combination with novel parasites to reduce colony health.

6. CONCLUSIONS

The research presented in this thesis investigates bee responses to land cover diversity within a landscape dominated by row-crop agriculture by assessing honey bees. The results provide clarity on several previously unanswered questions in this active area of research.

Usefulness of pan traps as an accurate method of assessing honey bee activity-density in an agricultural landscape was investigated. Despite previous research suggesting pan traps are not effective at capturing honey bees, we have found evidence that pan traps can be used to assess honey bee activity-density and these estimates provide a representation of the presence of honey bee colonies with a potential to estimate number of colonies nearby. We also demonstrate limitations to the sensitivity of this method; we did not detect differences in activity-density in field types, in different locations within a field, nor based on distance from the colony. It is possible that activity-density of honey bees across these contexts truly did not differ. Further studies are necessary to tease apart whether the lack of difference is attributable to a lack of trap effectiveness vs a true lack of difference in honey bee activity level in the landscape.

We investigated whether proportion of corn and soybean production in the surrounding landscape of a soybean field affected the community of bees present within the field. Additionally, because there has been growing concern about the impact of managed honey bees on wild bee populations, we explored how the presence of honey bee colonies in fields surrounded by high and low proportions of corn and soybean affected wild bee communities. We found that richness and diversity of the overall bee community were greater in fields surrounded by landscapes with lower proportions of corn and soybean. However, not all bee taxa responded in the same way. Common bee species had no response to proportion crop production in the surrounding landscape. Bees classified as uncommon and rare were more abundant, species rich, and diverse in soybean surrounded by a low proportion crop production. Specifically, uncommon bees were positively associated with increasing amounts of woodland in the surrounding landscape, whereas rare bees were positively associated with proportion grassland in the surrounding landscape. Overall, there was no observable effect of honey bee presence on the wild bee community. These results are important because they may help to inform conservation management decisions, suggesting that interactions with honey bees are less important than landscape composition in shaping the wild bee community in extensive agroecosystems.

Further health and productivity of honey bee colonies in soybean fields surrounded by high and low proportions of corn and soybean production was investigated. Honey bee colonies had more adult bees, immature bees, and heavier weight in fields surrounded by higher production of corn and soybean. Despite honey bee colonies being more productive when surrounded by row-crop, colony population, size, and nutritional state declined precipitously in the late season, a time corresponding to the senescence of soybean and clover blooms. This suggests that while areas of high corn and soybean production may support bursts of colony growth during certain feed periods of the season, colony health cannot be maintained throughout the season, with colonies ending in a “famine” state that indicated they were too weak to survive. In a follow-up study, we showed these declines are not inevitable in an agricultural landscape when bee colonies are given access to grass fields. Providing honey bee colonies access to native perennial habitat during this critical time reversed late season decline of honey bee colonies and individual bee nutritional state. These results are significant because they provide clarity on previously conflicting reports about both positive and negative responses of honey bees to extensive agricultural production. This study reveals the subtle and complex feast/famine dynamics of honey bee colonies experiencing seasonal forage fluctuations in highly cultivated landscapes. These results also suggest landscape enhancements with native habitat can provide benefits to non-native honey bees, which is an important finding because it opens the door to a way forward that can address both conservation and agricultural priorities.

Also, we have examined whether local farm diversity through the production of vegetables can also serve as a viable option for boosting bee community and honey bee colony health. We focused on vegetable farms that grew a diverse mixture of crops, some of which bloomed in the late season and could potentially provide valuable resources to wild and managed bees. For bee community, the largest responses observed were with uncommon bee species, which were more species rich in vegetable fields, especially in June. Honey bee colonies in vegetable fields had higher adult and immature bee populations and were heavier compared to colonies in soybean fields. Although colonies were more productive in diverse vegetable fields, colonies still declined precipitously in population, size, and nutritional health during the late season critical period, suggesting both farm types do not offer necessary late season resources for honey bees. Overall, these results suggest diversified farming can offer some modest benefits to bees.

Further research should focus on understanding to what degree the incorporation of native lasting habitat within extensive agricultural systems can support both wild and managed bees.

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