Understanding bumble bee community changes and floral use in southern Ontario

Taylor Kerekes

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Abstract

Multiple wild bee species, including bumble bees, are in global decline due to habitat loss, reduced floral resources, and competition with managed honey bees. Monitoring previously surveyed sites is essential to assess ongoing impacts on bumble bee populations and identify trends over time. This research revisited areas in southern Ontario to examine changes in bumble bee abundance, diversity, and floral use (Chapter 1) and explored potential competition for floral resources between bumble bees and honey bees (Chapter 2). Results indicate that while some bumble bee species have increased over the last 50 years, many have declined, leading to an overall loss of diversity. Findings also reveal significant niche overlap between bumble bees and honey bees, suggesting competition for floral resources. This work informs conservation strategies, identifies species requiring focused efforts, and emphasizes the importance of maintaining diverse floral resources to support bumble bee populations.

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Introduction

Pollinators are a vital part of many ecosystems and play an important role by providing the ecosystem service of pollination (Kearns *et al.* 1998). These pollination services are needed for most crops and are crucial in keeping the global food supply stable (Potts *et al.* 2016). Bees have been found to be the most effective group of pollinators and are integral in the pollination of many plant species (Willmer *et al.* 2017). It has been observed that bees are the most common visitors to plant species on many continents, are better at timing their floral visits than other animal pollinators, are more efficient at picking up pollen, and have the greatest single visit pollen disposition (Willmer *et al*. 2017; Phillips *et al.* 2018). Bumble bees (*Bombus*) specifically are excellent pollinators due to their large body size giving them the ability to deposit large amounts of pollen (Nayak *et al*. 2020). However, recent research suggests that bumble bees are in decline globally (Colla and Packer 2008; Jacobson *et al.* 2018; Richardson *et al.* 2019; Janousek *et al*. 2023) due to several threats. These include the loss of habitat including loss of important floral resources (Potts *et al.* 2010) as well as potential competition with managed bees such as the western domesticated honey bee (*Apis mellifera* Linnaeus) (Goulson and Sparrow 2009; MacInnis *et al.* 2023). Understanding the overlap in flower use between managed and wild bees is critical for assessing the risk of pathogen spillover. Having adequate floral resources is especially important for large-bodied generalist bees such as bumble bees due to their need for large amounts of pollen and nectar to obtain proper nutrition (Muller *et al.* 2006; Torné-Noguera *et al.* 2016). Without proper nutrition, bumble bees have been found to have decreased health and reproduction (Thomson 2004; Hudewenz and Klein 2015) and are more susceptible to disease and pathogen infection (Roger *et al.* 2017).

There is evidence that competition for floral resources does occur between bumble and honey bees (Thomson 2004; Goulson and Sparrow 2009; Rogers *et al.* 2013; MacInnis *et al.* 2023). This competition negatively affects bumble bees by reducing pollen and nectar resources, displacing bumble bees off their preferred floral resources, and causing more disease spread between managed and native bees (Elbgami *et al.* 2014; Furst *et al.* 2014; Page and Williams 2023). An emerging threat is pathogen spillover from managed honey bees and commercial bumble bees into wild populations, which can worsen the decline of native bees (Colla *et al*. 2006; Alder *et al.* 2019). Pathogens, such as viruses and parasites, are transmitted at flowers when infected bees leave behind contaminated pollen and nectar, which are then visited by other bees (Alder *et al.* 2019). By further causing nutritional stress on bumble bee colonies, this competition with honey bees may also cause bumble bees to become more susceptible to these diseases and pathogens that are passed to them. More studies are needed to properly assess the impacts of honey bees on bumble bees and other native bees and to determine the ideal floral resources for native bees. It is also important to continue to monitor bumble bee populations and communities' overtime to better understand changing trends in abundance, diversity, and floral use. With this information we can better implement proper conservation practices to prevent further declines.

This thesis addresses these issues by investigating changes in bumble bee abundance, diversity and floral use in southern Ontario over a 50-year period, comparing historical and contemporary datasets. Additionally, it explores the potential impacts of floral resources competition between the western domesticated honey bee (*Apis mellifera*) and native bumble bees, shedding light on how these interactions may contribute to bumble bee population declines.

These findings aim to provide actionable insights for conservation practices that support the recovery and sustainability of bumble bee populations.

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Chapter 1: The changes in bumble bee abundance, diversity, and floral use over time in southern Ontario.

Taylor Kerekes, Sheila R. Colla, Cole Blair, Sarah MacKell, Tiffani Harrison

Author's Contributions:

TK and SRC conceived of the ideas. TK led the development of the field methodology. TK, SM, CB and TH all contributed to the collection of data in the field. TK led data analysis with data cleaning and input from CB. TK led the writing of the manuscript. All authors contributed to editing and gave final approval for inclusion into the dissertation and eventual publication as a peer-reviewed journal article

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Abstract

Bumble bees (*Bombus*) have been documented to be in decline in many temperate regions including Canada. Multiple stressors working in tandem are thought to be contributing to these declines including habitat loss and urbanization, pathogen spillover and disease, climate change, and pesticides. Surveys were done in 2021-2023 in southern Ontario and were compared to historical surveys done in 1971-1973 and 2004-2006. This study works to compare changes over time in the bumble bee community including relative abundance, diversity, and floral use. This study illustrates a persistent absence and decline of some species over the past 50 years along with a continual decline of diversity. Changes in floral use over time can help to explain community variations such as an overall decrease in bumble bee dietary breadth. Although multiple species are experiencing declines, some species are seeing large increases in relative abundance and floral resource use showing a decrease in diversity by having a few species dominating the community. Continued monitoring in southern Ontario is integral to further documenting future bumble bee community changes allowing us to better understand conservation requirements.

Introduction

Globally, the reliance of human diets on crops that depend on pollinators has increased; however wild and managed pollinators have declined in several places (Aizen & Harder 2009; Reilly *et al*. 2020). As well as pollinating crops, bees help maintain biodiversity by contributing to the pollination of wild plants. These plants are important for providing rewards such as pollen and nectar to pollinators (Kearns *et al*. 1998) and providing food and habitat for wildlife from higher trophic levels (Senapathi *et al*. 2015). In urban landscapes, pollinators contribute

significantly by pollinating plants in gardens, parks, and green spaces therefore boosting biodiversity (Lowenstein *et al.* 2015; Hausmann *et al*. 2016; Theodorou *et al.* 2020).

Bumble bees (*Bombus* spp.) specifically, are a well-known and abundant group of pollinators that are important for pollination particularly in northern temperate regions (Goulson 2003), including Canada. Unfortunately, evidence for bumble bee declines has been found in many regions, including in North America (Colla and Packer 2008; Jacobson *et al.* 2018; Richardson *et al.* 2019; Janousek *et al*. 2023). Since biodiversity is known to be a fundamental part of plant-pollinator interactions it is important to maintain diversity in these wild bee communities (Blüthgen & Klein 2011; Winfree et al. 2018; Mathiasson and Rehan 2020). Bumble bees are large and hairy bees, making them efficient pollinators in both natural and agricultural habitats. They can deposit large amounts of pollen and can provide buzz pollination which is the fast contraction of flight muscles that vibrate and dislodge pollen (Nayak *et al.* 2020). They are also important for plant-pollinator networks since they are considered generalist pollinators, and many plants are predominantly pollinated by bumble bees (Goulson *et al.* 2008). Research suggests multiple stressors on bumble bees could be contributing to their declines. These stressors include habitat loss and fragmentation (e.g. increased agriculture land and urbanization), climate change, pesticides, and negative interactions with non-native species, such as competition and the spread of diseases through pathogen spillover (Potts *et al.* 2010; Goulson *et al.* 2015; Herbertsson *et al.* 2016; Soroye *et al.* 2020, Cameron and Sadd 2020).

Although it is thought that bumble bee declines are caused by multiple stressors working in tandem, effort to preserve required habitat is a necessary step to ensure their survival (Goulson et al. 2008). Plant-pollinator interaction networks are impacted by changes in landscapes making them susceptible to anthropogenic changes (Burkle *et al.* 2013; Mathiasson and Rehan 2020). To

provide for themselves and their colonies, it is important that bumble bees have the necessary habitat availability and quality including sufficient pollen and nectar from floral resources, over-wintering areas, and nesting sites. Pollen is essential to bees as it is their main source of protein and lipids (Roulston *et al.* 2000; Vaudo *et al.* 2015) which are important for larval development as well as female reproduction (Genissel *et al.* 2002; Vaudo *et al.* 2020). Nectar provides carbohydrates which are important during overwintering periods and long flight periods such as long foraging trips (Woodard and Jha 2017). The increase in agricultural land and urbanization causes decreases and degradation of bee habitats including floral resources and nesting sites, therefore making them possible threats to bee populations (Potts *et al.* 2010). Conversely some bumble bee species have seen increases in relative abundance (Colla and Packer 2008). Urban landscapes can be a refuge for certain species by providing urban gardens, parks and green spaces compared to rural land which is often monocultures of plants not useful for bees (Hall *et al.* 2016; Baldock et al. 2019; Ayers & Rehan 2021). While some agricultural crops do provide pollen as a source of nutrition for pollinators, these crops often only bloom for short periods of time leading to mass flowering monoculture with few available floral resources beyond this window. This does not provide enough nutrients to satisfy the nutritional needs of pollinators for the entire growing season (Winfree 2010; Spivak *et al.* 2010).

Nutritional stress has been shown to negatively impact bumble bees such as causing lower immunity and therefore increasing susceptibility to disease and parasites as well as leading to slower brood development (Roger *et al.* 2017; Watrous *et al.* 2019). Species with a smaller dietary breadth will see greater losses as they cannot switch to as wide a range of alternative floral resources (Burkle *et al.* 2013; Bartomeus *et al*. 2013). Although bumble bees are generalists, a study by Wood *et al.* (2019) found that declining bumble bee species collected one

third fewer types of pollen than other stable species and that species with broader diets had a buffer against habitat loss due to their ability to use any available floral resources. It has also been noted that habitat degradation could negatively impact larger bodied bees because they require more nutrients therefore need either more floral resources or resources of better quality than do smaller bees (Muller *et al.* 2006). This shows that understanding the floral use and needs of different bumble bee species is integral in conserving diversity.

This study aims to investigate if bumble bee abundance, diversity and floral use in southern Ontario has significantly changed over time. This is done by comparing the current *Bombus* community to historic surveys that were done in the same area in 1971-1973 (MacFarlane 1974) and again in 2004-2006 (Colla and Packer 2008). It is important to sample the same region over long periods of time to properly understand how the bumble bee communities are changing over time. Long-term monitoring data is essential for determining the conservation status of bumble bee species hence it is imperative that historical sites continue to be monitored. Colla and Packer (2008) found that some bumble bee species in this region were declining in relative abundance (*B. affinis* Cresson, *B. pensylvanicus* De Geer. *B. bohemicus* Seidl*, B. fervidus* Fabricius*, B. terricola* Kirby*, B. vagans* Smith*,* and *B. citrinus* Smith) therefore, we continue to monitor this same region to better understand current trends. Since landscape changes such as urbanization and land use modifications can impact bumble bee populations and their habitats (Winfree *et al.* 2009), continued monitoring of previously surveyed areas is crucial for understanding changes in the bumble bee communities. Overall, understanding these trends in abundance and diversity of bumble bees as well as their floral use is essential for future conservation management in human-modified landscapes.

Methods

A comparative study was performed to determine if bumble bee relative abundance, diversity, and floral use has changed over time in southern Ontario, Canada. Historical data that was used as a baseline was taken from Macfarlane (1974) which looked at the ecology of *Bombus* spp. within southern Ontario and Colla and Packer (2008) who surveyed the same sites 30 years later to investigate changes in bumble bee abundance and diversity. This study reassesses select sites from Macfarlane (1974) and Colla and Packer (2008) within the southern region of Guelph and the Town of Belwood as described by MacFarlane (1974). Due to landscape changes and urbanization, one site sampled by MacFarlane (1974) was not resampled by Colla and Packer (2008) because it was no longer accessible and continued to no longer be sampled in the current study.

Bumble bee sampling

To determine bumble bee abundance, diversity, and floral use, four linear transects approximately 1km in length and 3m wide were slowly walked at both the Guelph and Belwood sites. Sites were chosen based on locations surveyed by Colla and Packer (2008), with transects located as close to original survey sites as possible and within boundaries outlined by MacFarlane (1974). Survey frequency differed between time periods with MacFarlane (1974) surveying approximately 3 times per week from mid-April until the first frost in October from 1971-1973, with surveying happening less frequently in 1971. Colla and Packer (2008) surveyed once a month from May to September in 2004-2005 and once a week from April to October in 2006. In this study, to standardize sampling over all three years, both sites were surveyed once a week from April until the first week of October over three years (2021 – 2023). The sites were

surveyed on days between 6ºC and 29ºC and during time periods without precipitation. To reduce the impact of this study on the bumble bee populations, non-lethal sampling was used. While walking the transects, all bumble bees seen within the transect were caught using insect nets, transferred to vials, and placed in coolers to slow bees down for identification to species-level where possible. In the two previous studies, bumble bees that were difficult to identify were brought back to the lab to be identified morphologically. To reduce the impact of our current study, photos were taken for later verification by other experts when necessary. All bees were released at the end of each transect survey to prevent recaptures. If a bee was collected on a flower, that plant was identified to species-level when possible either in the field or photos were taken for later verification using Newcomb's Wildflower Guide (Newcomb 1989).

Floral resource assessment

If a bee was caught on a flower and carried pollen within its corbicula, it was assumed that the bee was foraging for pollen on the flower it was observed on, with that flower being identified to species level when possible. Although this may be an inaccurate representation of pollen foraging (bees could be foraging for nectar instead of collecting pollen) this methodology follows MacFarlane (1974) to allow for accurate comparisons. This pollen data were not recorded by Colla and Packer (2008) therefore comparisons were only made between the 1970's and 2020 time periods. Additional floral information was collected at each site in the 2020's. Flowering plants were identified and recorded bi-weekly starting in May, when flowering plants began to bloom, until September. To do this, a 1m x 1m quadrat was randomly tossed once within the 3m transect width every 100m along the 1km. Each open flower within the quadrat was identified to species when possible either in the field or photos were taken. Floral density

was measured by counting the number of open blooming flowers within the quadrat and floral richness was recorded by counting the number of flower species per quadrat. Floral density and richness were then averaged per site per sampling day. Since male bumble bees do not collect pollen, floral use comparisons were done between Macfarlane (1974) and the current data with only queen and worker bumble bee data.

Data analysis

All data analysis was conducted in R (R Core Team, 2022 version 4.1.3). To properly compare the data found in this study to both Macfarlane (1974) and Colla and Packer (2008) all queen data were excluded with only data on male and worker bumble bees being used when looking at relative species abundance and diversity. Because of differing sampling effort each year, relative abundance across all years was determined for each bumble bee species to normalize data and compare between years. This was done by dividing the number of each species found by the total number of bumble bees caught during the sampling period. The relative abundances from 1971-1973 and 2004-2006 were both compared to the relative abundances found in 2021-2023 using two-proportions *z*-tests to determine significant differences. All *z*-tests were conducted by comparing the species proportions. Proportion tests were performed for each species with Chi squared and *p*-values recorded. To get the *z* statistics, the Chi squared values were square rooted (Sokal and Rohlf, 2012). If there was a significant difference found for a species, the direction of that difference (relative abundance increased or decreased) was further investigated using the proportions test in R. A heatmap was created to also show and compare which species were most commonly found within each sampling year.

To investigate species diversity, the Shannon diversity index (*H*) for all three sampling periods was calculated. To examine species evenness, Pielou's evenness index (*J*) was calculated, which measures how evenly individuals are distributed among the species in a dataset, with values closer to 1 indicating greater evenness. Sample size differed across the three sampling periods therefore, rarefaction curves were created to determine if diversity differed among these periods and if the sampling effort could accurately be compared.

Generalized linear models (GLMs) were used to explore whether floral density and richness have an association with bumble bee abundance and diversity within the 2020 sampling period using the Gaussian distribution. To accurately visualize the interaction between bumble bee species and their floral resources, bipartite networks were created for the 1970's and 2020's in R using the 'plotweb' function found in the bipartite package (Memmott 1999; Dormann *et al.* 2009) showing which bee species were found foraging on which floral resources. The function 'networklevel' was used to investigate nestedness and connectance within the network (Dormann *et al.* 2009). Nestedness organises species based on the number of interactions within the community where plants that have fewer pollinator interactions have the most specific interactions and plants with the most interactions have the most general interactions (Almeida-Neto *et al.* 2008). A high degree of nestedness indicates a stable and diverse ecosystem and low nestedness suggests an unstable ecosystem where the loss of key species could lead to the decline of many others. Connectance looks at the proportion of possible interactions between plants and pollinators that are observed in a given ecosystem (Dormann *et al.* 2008; Mathiasson and Rehan 2020). High connectance means that most of the possible interactions are observed, while a low connectance means that only some of the possible interactions are observed.

The function 'specieslevel' from the bipartite package (Dormann *et al.* 2009) was also used to calculate degree, normalized degree, and pollination service index (PSI). The degree gives the number of interactions that one given species has with other species in the network such as the diet breadth of each bee species (the number of floral hosts that bee has visited). The normalized degree takes the degree and normalizes it by accounting for the total number of possible interactions it could have with other species (Bascompte et al. 2003). This is useful because it allows the comparison of different networks regardless of the size or complexity of the networks. The PSI is a measure that assesses the contribution of a pollinator to the plants in the network with a value of 1 showing a species is valuable to the network and a value of 0 showing a noncritical role (Dormann *et al.* 2009).

Results

Bumble bee abundance

In the 2021-2023 sampling period, 2814 individual bees were collected with twelve unique bumble bee species found. In 2004 – 2006 there were 1195 individuals sampled with eleven species found, which was a decrease of one species compared to the 2020's. In the 1971 – 1973 sampling period 3632 individuals were sampled with fourteen unique bumble bee species being found. Due to varying sampling efforts across the different periods, direct comparisons of overall bumble bee abundances are not possible. However, relative abundance within each year can be compared to assess changes across sampling periods. Certain historically abundant species such as *B. vagans*, *B. affinis*, and *B. fervidus* have seen continued declines in their relative abundance from the 1970's to the current sampling period (Figure 1). Conversely, *B. impatiens* Cresson shows a sharp increase in relative abundance over the years, reaching the

highest relative abundance in the 2020's (Figure 1). *Bombus bimaculatus* Cresson also maintains a high relative abundance over time, with it increasing from the 1970's to the early 2000's and decreasing slightly again in the 2020's (Figure 1). There are shifts in community composition from the 1970s to the present sampling period, with most species exhibiting lower relative abundances over time and a few species, such as *B. impatiens*, now dominating the community with significantly higher relative abundances.

When comparing the 1970's sampling period to the early 2000's certain differences are seen as found and documented by Colla and Packer (2008). Firstly, three species are absent from the 2000's sampling period that were previously present including *B. affinis, B. pensylvanicus*, and *B. bohemicus* (Figure 2). There were four species that saw significant decreases in relative abundance including *B. fervidus, B. terricola, B. vagans*, and *B. citrinus* (Figure 2). Significant increases in relative abundances were seen in four species as well, including *B. bimaculatus, B. impatiens, B. rufocinctus* Cresson, and *B. ternarius* Say. When comparing the current surveys with the 2004-2006 data, significant changes continue to be seen. *Bombus affinis, B. pensylvanicus* and *B. bohemicus* continue to be absent from the 2020's sampling period (Figure 2; Table 1). Four species have declined significantly in relative abundance: *B. vagans* and *B. citrinus* who continue to see declines, and *B. bimaculatus* and *B. rufocinctus* who were seen to previously be increasing in relative abundance. Two species*, B. impatiens* and *B. borealis* Kirby, saw a significant increase between these time periods. When looking at the overall trends from the 1970's to the 2020's, we see three species absent *(B. affinis, B. bohemicus, B. pensylvanicus*), four species in decline (*B. fervidus, B. terricola, B. vagans, B. citrinus),* and four species that were increasing in relative abundance (*B. bimaculatus, B. borealis, B. impatiens, B. ternarius)* (Figure 2; Table 1). Although four species are increasing, *B. impatiens* has the highest increase

overall with only 17% of bumble bees found in the 1970's being *B. impatiens* compared to a staggering increase to 62% in the 2020's.

Bumble bee diversity

There were slight differences in species richness between the sampling periods with the 1970's having the highest number of species found (fourteen species), with a reduction in richness in the early 2000's (eleven species), and a slightly higher richness again in the 2020's (twelve species) (Figure 3). We can compare the diversity of each year through rarefaction, showing sufficient sampling effort was done each year (Figure 3). When comparing evenness over years, the 1970's had the highest evenness ($J = 0.82$) meaning it had the most even distribution of species compared to the early 2000's which had a lower evenness ($J = 0.63$). The evenness in the 2020's was the lowest ($J = 0.50$) meaning that species are becoming less evenly distributed over time. To account for both richness and evenness, shannon diversity for all years was found. The 1970's period had the highest diversity (H=2.163), followed by the early 2000's (H=1.504), and the lowest diversity being in the 2020's (H=1.240). Although species richness did not differ much between the different sampling years and increased from the early 2000's to the 2020's (Figure 3), the Shannon diversity index accounts for both species evenness and richness. Since evenness does decline consistently over time periods, this contributes to a lower diversity over time.

Floral assessment

No significant relationship was found between bee abundance and floral density across all sites from 2021-2023 (GLM, $X^2(1) = 30.225$, $t = 0.431$, $p = 0.667$) however floral richness was found to have a significantly positive association with bumble bee abundance (GLM, $X^2(1) = 941.78$, $t = 2.466$, $p = 0.014$) (Figure 4). There was also a significant, positive association between bumble bee diversity and floral richness (GLM, $X^2(2) = 31.223$, $t = 2.660$, $p = 0.009$) and with floral density (GLM, $X^2(2) = 8.867$, $t = 2.336$, $p = 0.021$) (Figure 4).

When looking at the plant-pollinator networks, in the 1970's there were 1458 plant-pollinator interactions which occurred between twelve *Bombus* species and 40 flowering plant species over the three-year study period (Figure 5). Two species*, B. bohemicus* and *B. citrinus*, are absent from the network since no plant interactions were found for these cuckoo bumble bee species. In the current sampling period, there were 2753 plant-pollinator interactions between twelve *Bombus* species and 71 flowering plant species over the three-year study period (Figure 6). Comparing these networks at the community level, the nestedness measure was higher during the 1970's period (20.59) than the current one (12.76) which indicates there was more co-occurrence in the 1970's. Connectance showed a similar trend, being higher in the historical network (0.34) and lower in the current network (0.25). The decline of both of these metrics could be due to an increase in floral diversity seen in the current sampling period, with the same number of bumble bee species visiting a larger diversity of flowers.

Focusing on the bee species interactions in the historical network, only one species (8% of species) interacted with one floral resource, three species (25%) interacted with two to ten floral resources, and eight species (67%) interacted with more than ten floral resources. During the current sampling period one species (8%) interacted with only one floral resource, five species (42%) interacted with two to ten flowers, and six species (50%) interacted with more

than ten floral resources. We can see the number of species interacting with more than ten flowers has declined slightly and more bees are now interacting with two to ten flowers. The breakdown of each species' interactions can be seen in Table 2.

When investigating the individual importance of bee species to the network we looked at diet breadth (degree and normalised degree) and PSIs. The average degree of bee species in the historical network was 13.5 (0.34 normalised degree) and the average in the current network has increased to 16.41 but with a decreased normalized degree of 0.25. The three bee species with the highest diet breadths were the same for both the historical and current networks: *B. impatiens, B. bimaculatus*, and *B vagans.* However, *B. perplexus* Cresson had the same amount of floral resources interactions as *B. vagans* in the 2020's (23) having increased from the 1970's (11). *B. impatiens, B. bimaculatus*, and *B. vagans* also had the highest PSI values for the current sampling period meaning these were key species for this network (Table 2). *Bombus bimaculatus, B. fervidus*, and *B. ternarius* were the species with the highest PSI values in the 1970's indicating that there may be different species that contributed pollination services when comparing networks between time periods (Table 2).

Of the ten bee species found in both networks, five species experienced a decrease in diet breadth when comparing normalised degrees including *B. terricola, B. fervidus, B. griseocollis, B. borealis*, and *B. vagans* (Table 2). Three of these species, *B. terricola, B. fervidus,* and *B. vagans,* have seen significant decreases in relative abundance from the 1970's to the 2020's. For *B. terricola*, three of its top five floral interactions from the 1970s were lost by the 2020s. These include *Medicago sativa*, *Ribes nigrum*, and *Melilotus alba*, while *Echium vulgare* and *Hypericum perforatum* remained in the network. Similarly, *B. fervidus* lost two of its top five interactions from the 1970s: *Symphytum officinale* and *Carduus nutans*, though interactions with

Trifolium pratense, *Echium vulgare*, and *Vicia cracca* persisted. In contrast, *B. vagans* retained most of its top interactions, losing only one: *Hydrophyllum virginianum*. The remaining flowers, *Trifolium pratense*, *Echium vulgare*, *Carduus acanthoides*, and *Symphytum officinale*, were still visited in the 2020s. *Bombus fervidus* was a key species in the 1970's with the second highest PSI value and is no longer key to the 2020's network due to a decrease in PSI therefore indicating it is becoming relatively more specialized in its floral visitation. This PSI decrease could also be caused by a decrease in the abundance of *B. fervidus* meaning they were not observed visiting as many flowers. The PSI values for both *B. terricola* and *B. vagans* have decreased from the historical period to now as well (Table 2). Conversely, five of the ten species have seen increases in diet breadth including *B. perplexus*, *B. rufocinctus, B. bimaculatus, B. impatiens* and *B. ternarius* (Table 2). *Bombus impatiens* shows the largest increase in diet breadth with a previous normalised degree of 0.68 compared to a current of 0.81. Of these five species, three - *B. impatiens*, *B. ternarius* and *B. bimaculatus* are all increasing in relative abundance with *B. perplexus* and *B. rufocinctus* seeing no change in relative abundance since the 1970's.

Floral resource importance was assessed using the degree and the normalized degree, which in this case measures the diversity of visitors to a floral resource. The average degree of floral resources in the historical network was 4.05 (0.34 normalised degree) and the average in the current network was 2.25 (0.25 normalised degree) (Appendix A). During the 1970's the five species with the highest normalized degrees includes *Echium vulgare* (0.92), *Carduus nutans* (0.83)*, Trifolium pratense* (0.83), *Symphytum officinale* (0.67), and *Hypericum perforatum* (0.58). In the 2020's, the five species with the highest normalized degrees includes *Vicia cracca* (0.92), *Cirsium arvense* (0.67), *Lythrum salicaris* (0.58), *Symphytum officinale* (0.58) and

Carduus nutans (0.50). Comparing both networks*, S. officinale* and *C. nutans* are the only floral resource that was key in both networks supporting high numbers of bee diversity. It is important to note that the top five floral resources in both years are all non-native species in North America. Notably, in the 2020's, the genus *Solidago* had a high normalized degree (0.67). These flowerswere identified only to genus, which encompasses many different species including both native and non-native, making it difficult to accurately compare to other flower species within the network.

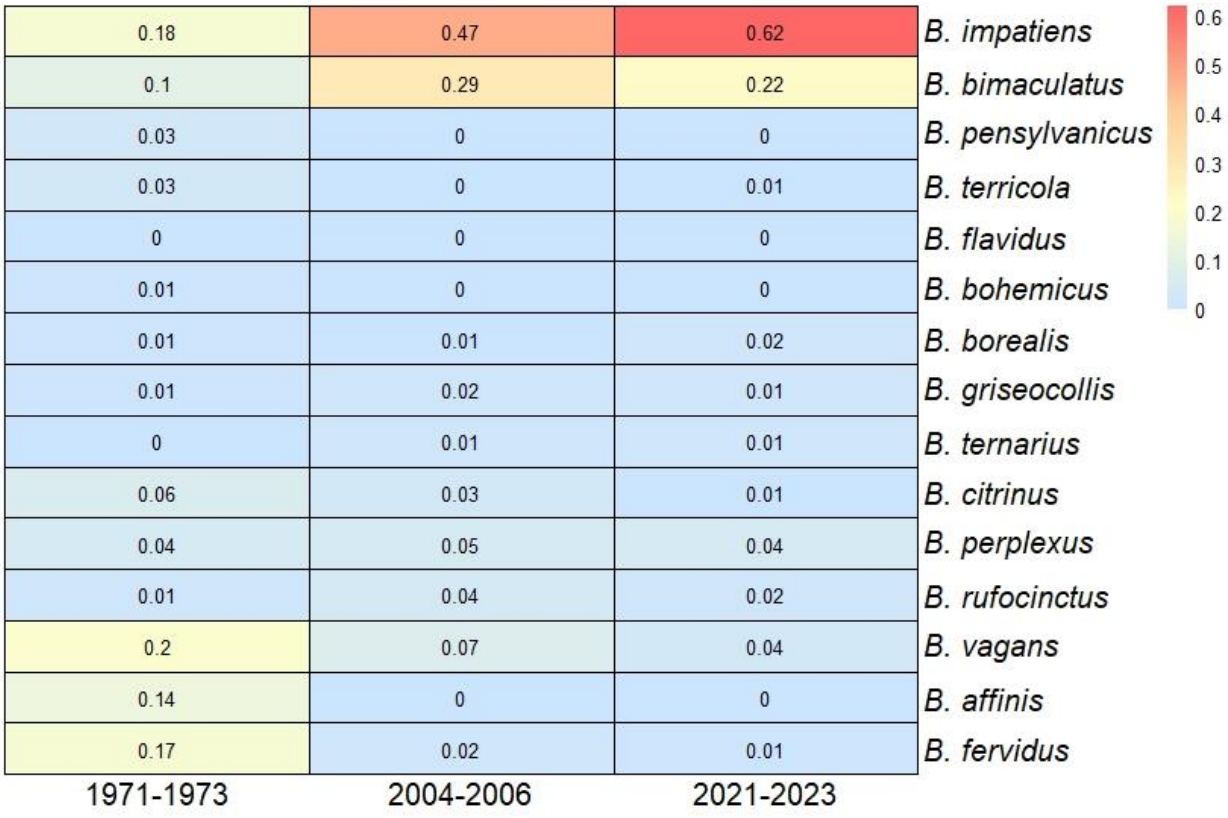

Figure 1: Heatmap showing the relative abundance of *Bombus* species across the three study periods: 1971-1973, 2004-2006, and 2021-2023. Relative species abundance ranges from 0 to 0.6 as indicated by the color legend on the right. Warmer colors (yellow to red) represent higher relative abundance, while cooler colors (blue) represent lower abundance. Species are grouped based on similarity in relative abundance trends across time periods.

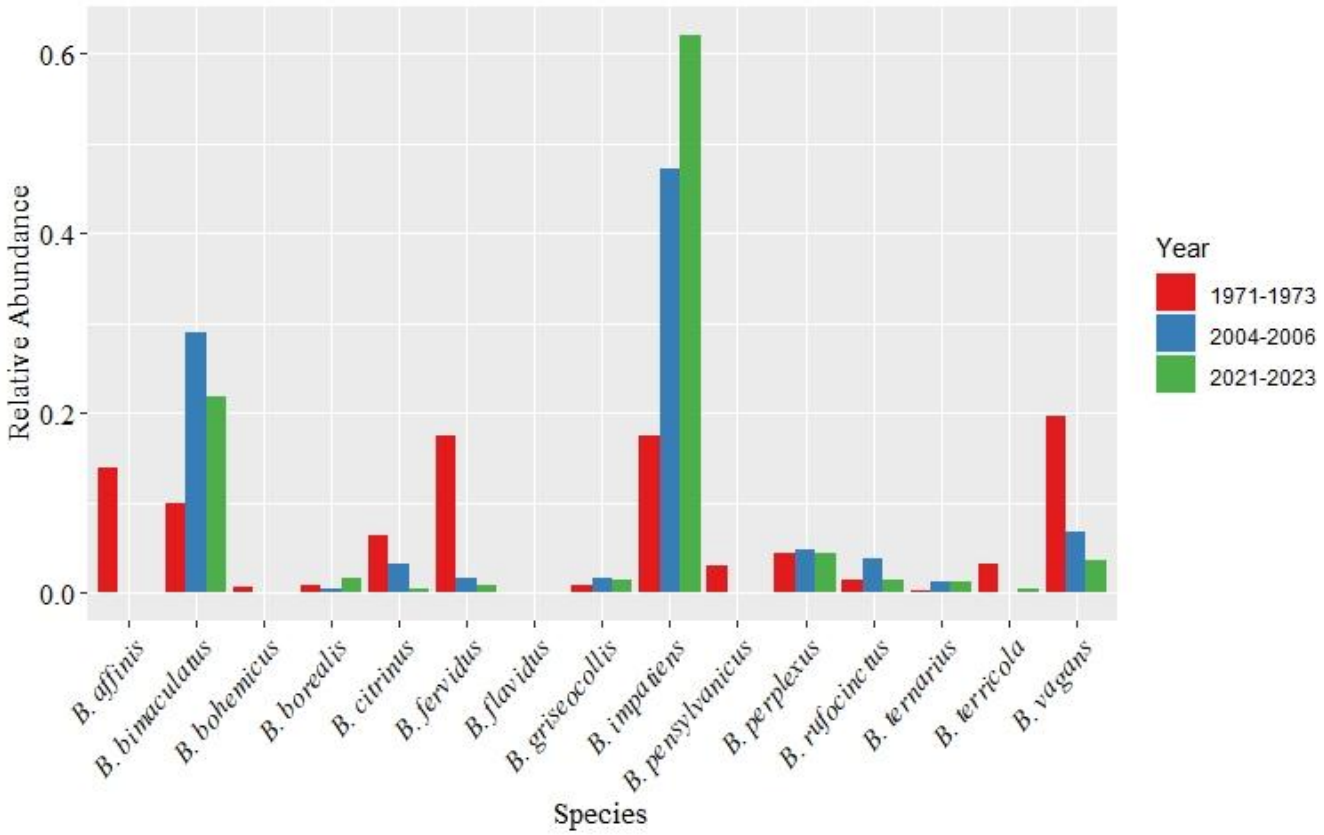

Figure 2: Comparing the relative abundances of each *Bombus* species found at all sites from 1971-1973 (red, n=3632), 2004-2006 (blue, n=1195), and 2021-2023 (green, n=2814).

Table 1: *p*-values for the relative abundance comparisons of each *Bombus* species comparing both historical time periods to 2021-2023. *p*-values less than 0.05 are considered significant.

	1971 – 1973		**2004 – 2006**	
Species	***p*-value**	**Direction of change**	***p*-value**	**Direction of change**
B. affinis	<0.001*	Decrease	N/A	No change
B. pensylvanicus	<0.001*	Decrease	N/A	No change
B. bohemicus	<0.001*	Decrease	N/A	No change
B. terricola	<0.001*	Decrease	0.058	No change
B. fervidus	<0.001*	Decrease	0.074	No change
B. vagans	<0.001*	Decrease	<0.001*	Decrease
B. citrinus	<0.001*	Decrease	<0.001*	Decrease
B. flavidus	0.898	No change	1.00	No change
B. perplexus	0.992	No change	0.709	No change
B. rufocinctus	0.673	No change	<0.001*	Decrease
B. griseocollis	0.144	No change	0.457	No change
B. borealis	0.002*	Increase	0.004*	Increase
B. bimaculatus	<0.001*	Increase	<0.001*	Decrease
B. impatiens	<0.001*	Increase	<0.001*	Increase
B. ternarius	<0.001*	Increase	1.00	No change

*shows significance ($p < 0.05$)

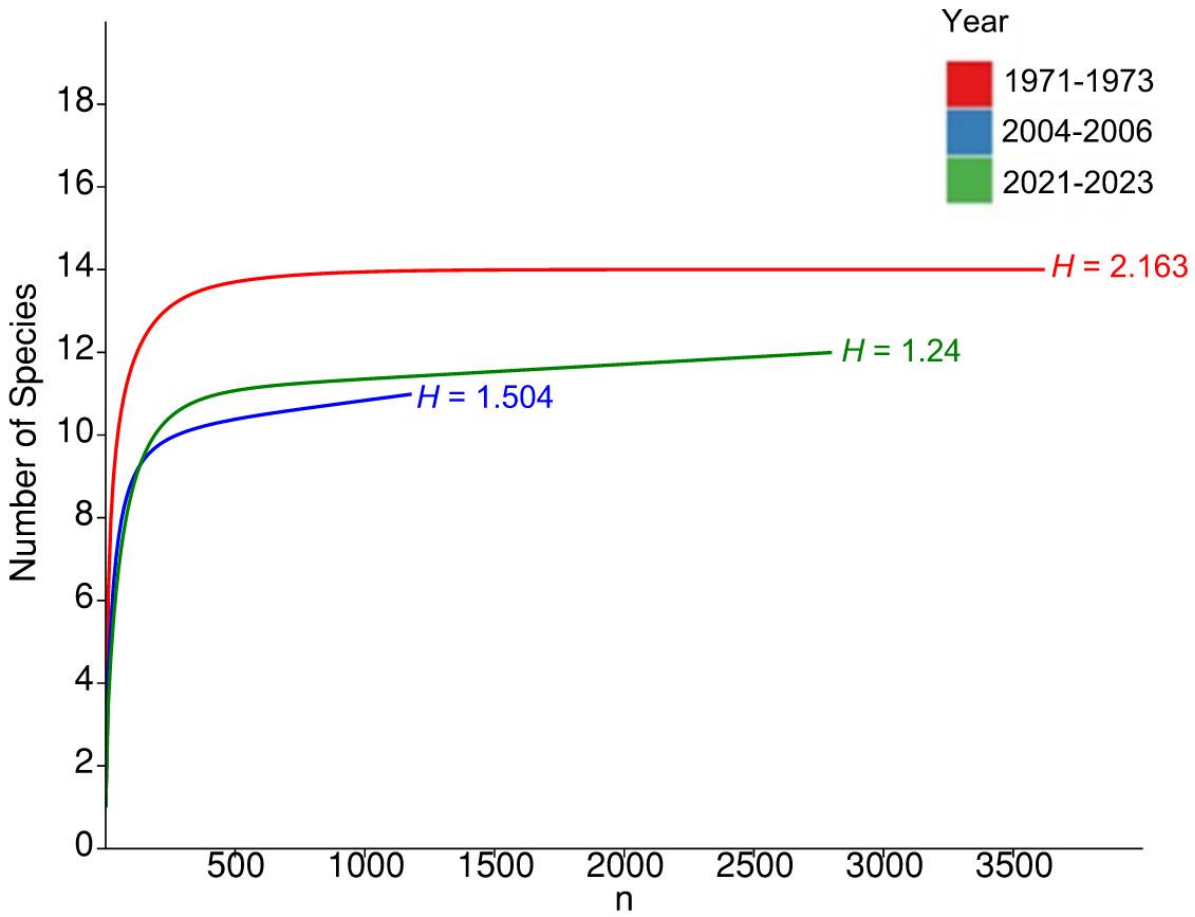

Figure 3: Individual rarefaction curves showing the number of individuals sampled (n) compared to the number of species found in 1971-1973 (red), 2004-2006 (blue), and 2021-2023 (green) with Shannon diversity indices for each year indicated.

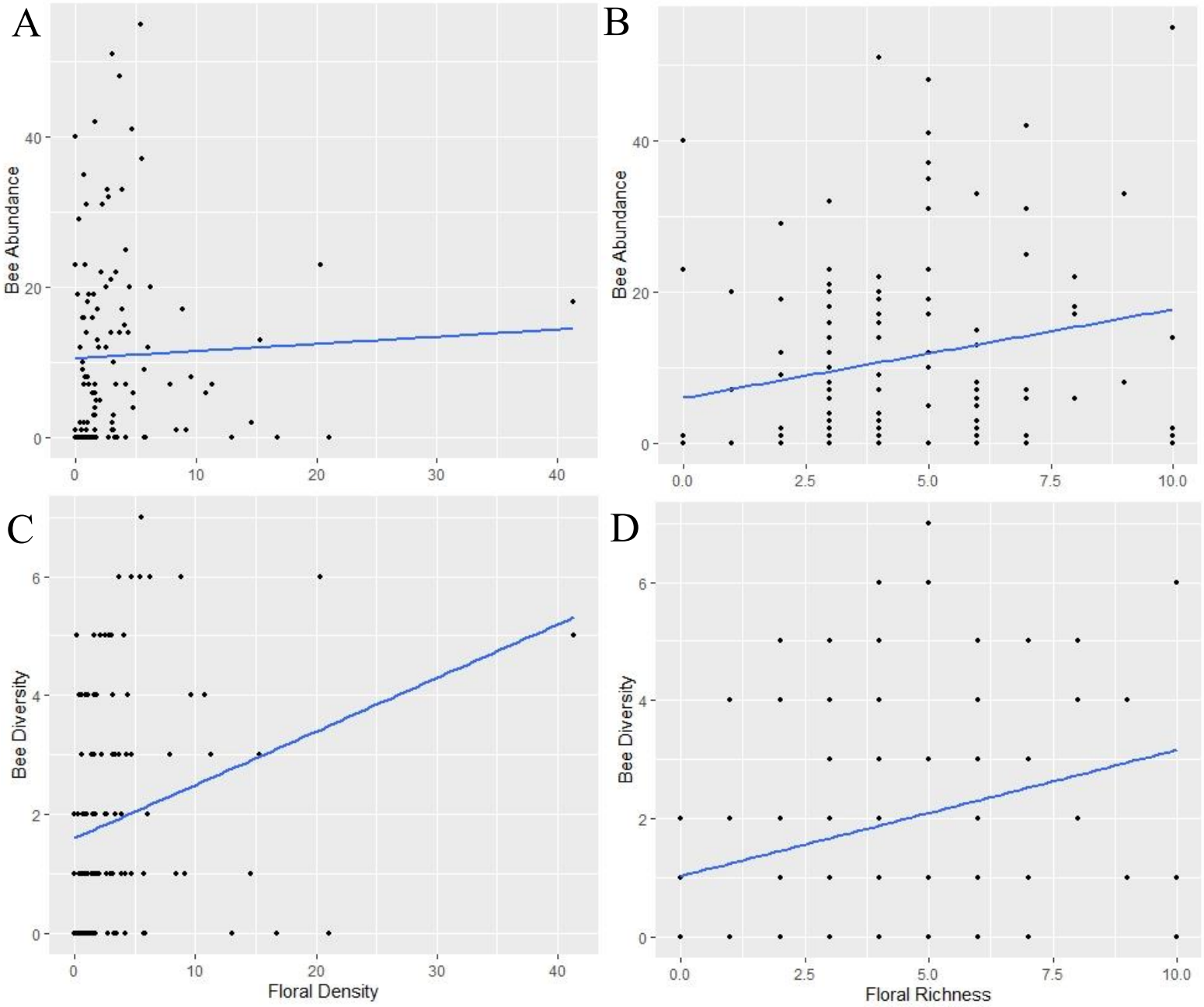

Figure 4: Plots of the relationships between floral richness and density and bumble bee abundance and diversity from all sites in 2021 - 2023, showing**:A:** No significant association between bee abundance and floral density (GLM, $X^2(1) = 30.225$, $t = 0.431$, $p = 0.667$) **B**: Bee abundance was positively associated with floral richness (GLM, $X^2(1) = 941.78$, $t = 2.466$, $p = 0.014$). **C**: Bee diversity was positively associated with floral density (GLM, $X^2(2) = 8.867$, $t = 2.336$, $p = 0.021$). **D:** Bee diversity was positively associated with floral richness (GLM, $X^2(2) = 31.223$, $t = 2.660$, $p = 0.009$).

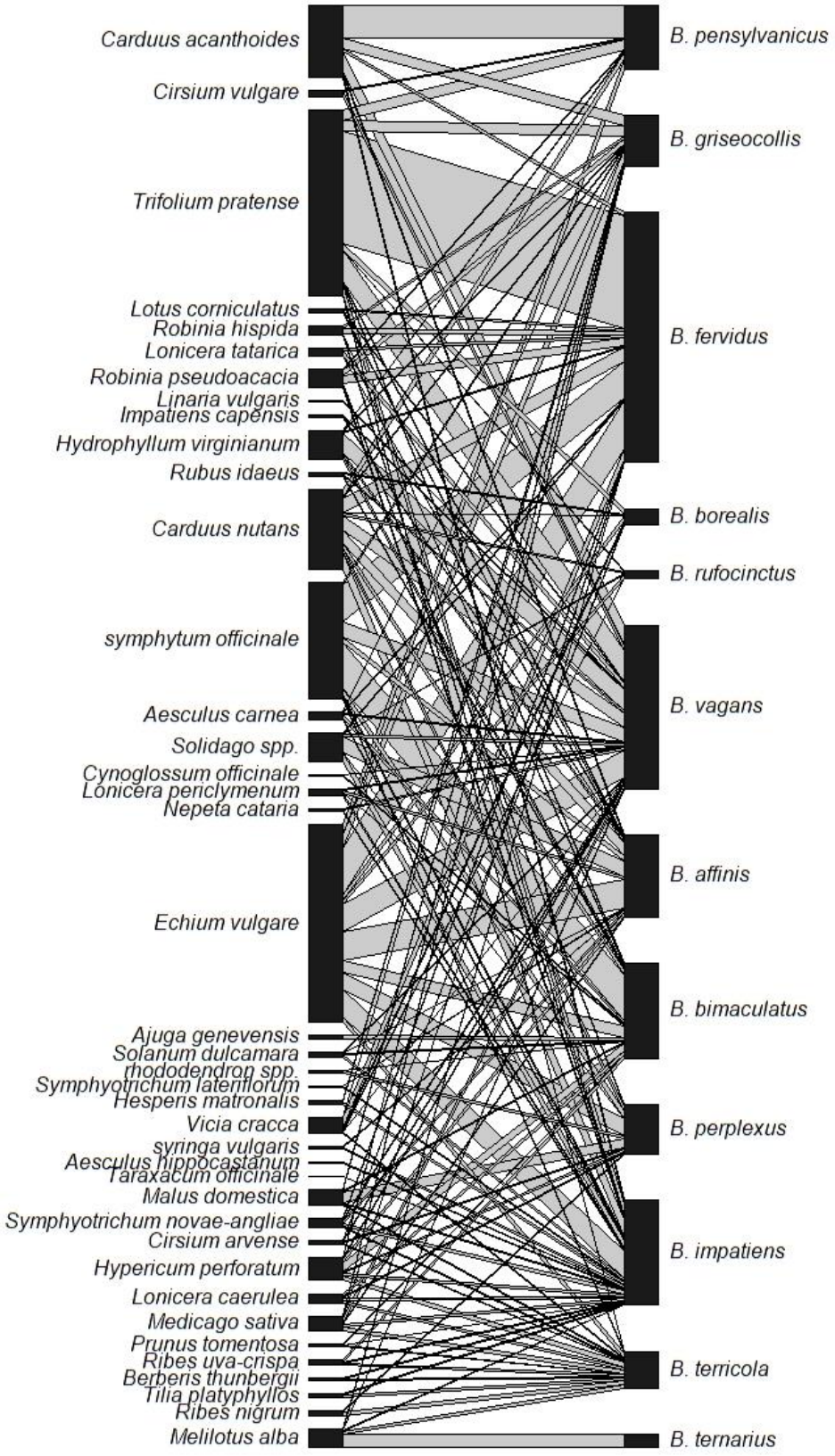

Figure 5: *Bombus* floral interaction network from MacFarlane (1974) data illustrating all *Bombus* and floral resource interactions from queens and workers. Bees and flowers are arranged based on their similarity in interactions patterns. Thicker lines represent more frequent interactions between a given bee and flower species.

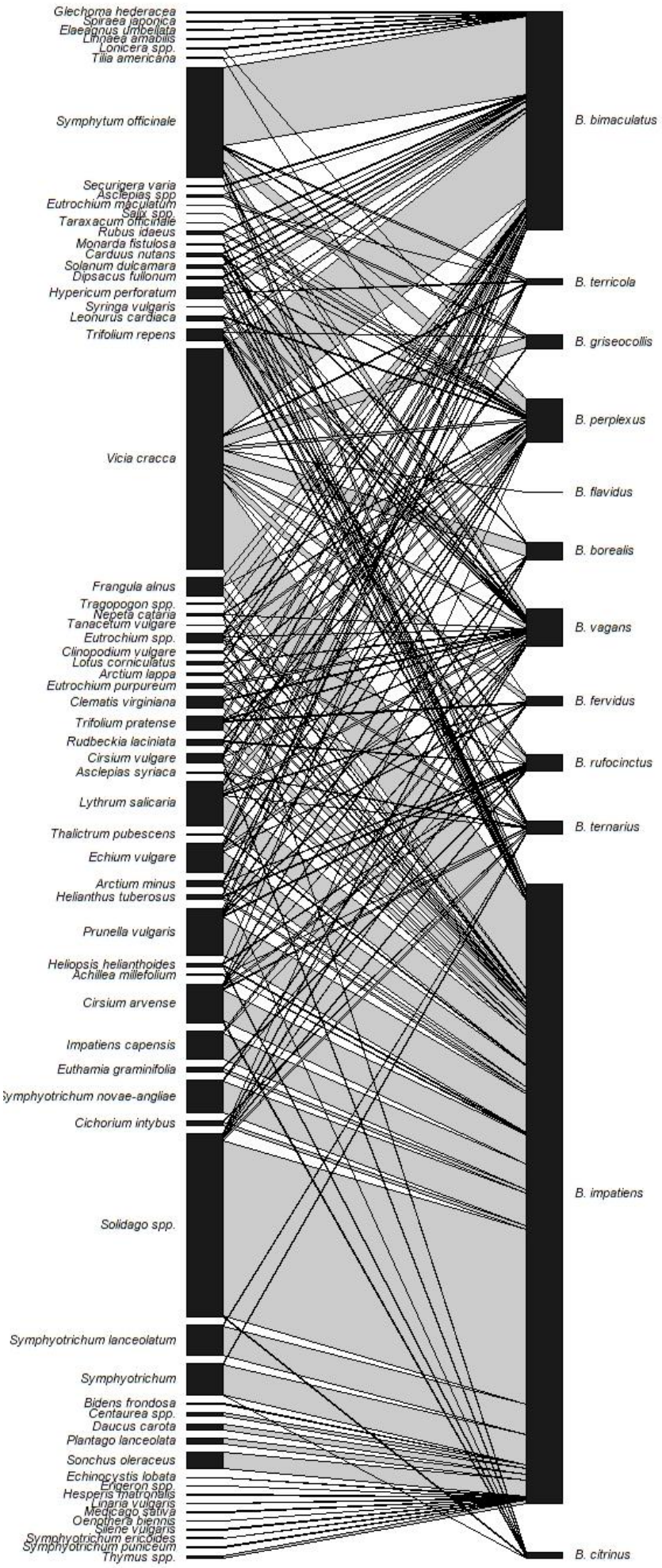

Figure 6: *Bombus* floral interaction network from 2021-2023 illustrating all *Bombus* and floral resource interactions from queens and workers. Bees and flowers are arranged based on their similarity in interactions patterns. Thicker lines represent more frequent interactions between a given bee and flower species.

Table 2: The degree, normalized degree, and pollination service index (PSI) for each *Bombus* species in the historical time period (1971-1973) and the current survey period (2021-2023).

	Degree		**Normalized degree**		**PSI**	
Species	**Historical**	**Current**	**Historical**	**Current**	**Historical**	**Current**
B. affinis	17	N/A	0.425	N/A	0.224	N/A
B. pensylvanicus	7	N/A	0.175	N/A	0.273	N/A
B. terricola	16	10	0.400	0.149	0.289	0.091
B. flavidus	N/A	1	N/A	0.015	N/A	0.002
B. citrinus	N/A	7	N/A	0.104	N/A	0.085
B. fervidus	16	8	0.400	0.119	0.439	0.029
B. vagans	24	23	0.600	0.343	0.299	0.109
B. perplexus	11	23	0.275	0.343	0.243	0.199
B. borealis	6	7	0.150	0.104	0.074	0.059
B. rufocinctus	5	11	0.125	0.164	0.021	0.079
B. griseocollis	14	6	0.350	0.089	0.113	0.132
B. bimaculatus	18	33	0.450	0.493	0.336	0.499
B. impatiens	27	54	0.675	0.806	0.223	0.782
B. ternarius	1	14	0.025	0.209	0.714	0.104

Discussion

There have been major differences in bumble bee community composition over the sampling periods with declines in bumble bee relative abundances for multiple species and an overall loss of evenness and diversity over time. There were also select species that have experienced significant increases in relative abundance over time, contributing to the decline in species evenness. These findings align with what has been seen in studies that have explored bumble bee declines in other areas of North America (Grixti *et al.* 2009; Cameron *et al.* 2011; Richardson *et al.* 2019; Jacobson *et al.* 2018). Although there are species that are increasing, many bumble bee species are becoming less common leading to the loss of diversity that continues to be seen. In the 2020 sampling period, one more species was found compared to Colla and Packer (2008) however; the relative abundance of many of the species is decreasing. The higher diversity found in the early 2000's was due to bumble bee abundances being more evenly spread amongst different species, with current data showing *B. impatiens* to be the most commonly occurring species and the majority of other species found being much less common.

The significant increase in the relative abundance of *B. impatiens* in the current sampling period, coupled with the decline of several other bumble bee species, is an indication of biotic homogenization. This process, characterized by the dominance of a few widespread species and the concurrent loss of species richness and evenness (McKinney and Lockwood 1999; Olden *et al.* 2004). Homogenized ecosystems are less capable of recovering from phenomena such as environmental changes (Olden *et al*. 2004) therefore, environmental stressors such as climate change or habitat loss could cause bumble bee communities to become less resilient. As such, maintaining a diverse community is crucial for both bumble bee conservation and for the overall health of ecosystems that rely on these pollinators (Katumo *et al.* 2022).

Taking a closer look at declines, *B. affinis, B. pensylvanicus* and *B. bohemicus* have not been found at the sampling sites in the last 50 years. *Bombus fervidus, B. terricola, B. vagans,* and *B. citrinus* were present in the 2020's but have seen significant declines since the 1970's. Two of the declining species, *B. bohemicus* and *B. citrinus*, are parasitic cuckoo bumble bees known for invading other species nests for reproduction (Koch *et al.* 2015) with the former preferring *B. affinis* and *B. terricola* and the latter preferring *B. impatiens, B. bimaculatus*, and *B vagans* as hosts (Williams *et al.* 2014). The most likely threat to these species is the loss of their preferred host species which does apply to *B. bohemicus* and can explain its absence from the current sampling periods. *B. citrinus* however, has multiple host preferences that have not seen declines meaning that its decline is most likely attributed to something other than host availability. Something of note is that cuckoo bumble bees do not produce workers of their own therefore making it less likely to observe these species compared to other bumble bee species in general.

There are many other possible causes for declines such as pathogen spillover from managed bees causing higher pathogen loads in bumble bees. Research on pathogens has shown that bumble bee species with declining populations have had a higher prevalence of pathogen loads including *B. affinis, B. pensylvanicus* and *B. terricola* (Cameron *et al.* 2016). This could help explain why species like *B. pensylvanicus* and *B, terricola* continue to decline in southern Ontario, while *B. affinis* remains absent. which could help to explain why these species continue to see declines in southern Ontario. An additional explanation for decline could be climate change. The climate in North America has warmed substantially since 1974 and the decline of bumble bee species could be related to shrinking range limits (Kerr *et al.* 2015). Both the study done in the early 2000's and the current one took place after this significant warming, and this

context may help explain changes in the relative species abundances observed across the sampling periods. Due to the warmer climate, there has been evidence for latitudinal and elevation shifts for multiple species such as *B. impatiens, B. vagans, B. ternarius* and *B. terricola* (Kerr *et al*. 2015; Nooten and Rehan 2020). Pesticides are also a well-known problem when it comes to bee health, especially neonicotinoids. In bumble bees, there have been observations of exposure to neonicotinoids which have caused a reduction in learning, foraging ability and homing ability (Goulson *et al.* 2015; Stanley *et al.* 2016). These would be of particular concern for bumble bees that prefer agricultural areas such as *B. pensylvanicus* and *B. fervidus* (Richardson *et al.* 2019) and may explain why they have seen decreases since the sampling done in the 1970's, mainly *B. pensylvanicus* which has been absent from sampling in the last 50 years. These findings are consistent with those reported by Burkle et al. (2013) who found population declines in *B. pensylvanicus* causing it to have a very reduced role in the plant-pollinator network within their study area.

This study demonstrates lower nestedness and connectance in the current floral network, likely due to the increased floral diversity observed. In the 1970s, many bumble bee species visited a wide range of available flowers, resulting in a more interconnected network. In contrast, the current period shows a greater variety of flower species, with each bumble bee species interacting with a narrower selection from among them, leading to a more diffuse network structure. We know that a lower nestedness can mean that the ecosystem is becoming less stable and is more prone to the loss of species (Duan *et al.* 2023). The lower connectance could also indicate that bumble bee species are missing therefore some interactions are missing from the network. This is consistent with the absence of *B. affinis, B. pensylvanicus* and *B. bohemicus* whose interactions were not present in the current sampling periods network. As we lose more

species and bumble bee diversity becomes lower, the more likely we are to continue to see individual species loss. We also see an overall decrease in dietary breadth in the 2020's based on the normalized degree values found. Three of these species are also seen to be in decline including *B. fervidus, B. vagans*, and *B. terricola*. *Bombus terricola* had lost three out of its top five floral interactions from the 1970's by the 2020's, *B. fervidus* lost two, and *B. vagans* lost only one. This suggests that *B. terricola* and *B. fervidus* could be experiencing decreased dietary breadth due to the loss of preferred foraging resources. In contrast, *B. vagans* retained most of its key floral interactions, therefore it's also possible that the current available floral resources are not abundant enough to sustain its colonies. Since nutritional stress can have many negative impacts on bumble bees (Burkle *et al.* 2013; Bartomeus *et al.* 2013; Wood *et al*. 2019), the decrease in dietary breadth of these species could be a large factor contributing to their declines and make them less resilient to other potential stressors.

On top of a decrease in dietary breadth, the diversity of bumble bees visiting each flower is declining and the most preferred flowers have changed over time. This shift is thought to be driven by changes in availability and abundance of their preferred plants. Due to climate change and habitat loss, the floral resources which are available during the flight periods of bumble bee species may have changed over time. Because of this change, bumble bees will need to align their foraging activity with the flowering periods of alternate plant species (Goulson *et al.* 2015). Although it is often thought that native plants are the best option for our native bees, the bumble bees in this study were more likely to be found on non-native species (Gibson *et al*. 2019). This could be because of the abundance of non-native species in the area causing the bumble bees to turn to these resources in the absence of native plants. It is important to not rule out non-native plants in our landscapes if they are providing the needed sustenance to our native species when

other resources are limited (Harmon-Threatt and Kremen 2015), however it has been found that certain at-risk species including *B. terricola* and *B. pensylvanicus* are correlated with areas that provide more native floral resources in the spring (Liczner and Colla 2020). Because at-risk species may be more reliant on our native plant species than are those with more stable populations, it is important to continue to promote the restoration and planting of these resources.

Declines are often the focus for many studies however, it is important to also address increases in abundance and investigate what is allowing certain species to thrive. *Bombus impatiens* saw a significant increase since the early 2000's and makes up the largest proportion of bees caught in the current sampling period. This is consistent with recent findings (Colla *et al.* 2012; Jacobson *et al.* 2018) and could be attributed to its early emergence and prolonged flight period compared to other species. Having a very long season of activity, emerging early, and persisting later into the fall than other species (Williams *et al.* 2014), could help in its maintenance of a larger population. It has had a large increase in dietary breadth over time which could play a major role in its capacity to use floral resources, adapt to urbanization, and shift to the most available floral resources (Jacobson *et al.* 2018). Another explanation for these drastic increases could be based around body size. Research has found that *B. vagans* and *B. terricola* were declining in New Hampshire and workers were 19% larger than two increasing species, *B. impatiens* and *B. ternarius* (Nooten and Rehan 2020). Similar trends were seen in the current study for these four species therefore, declines could be correlated with a smaller body size which may be contributing to these species' ability to better adapt to land use changes. *Bombus impatiens* is also used as a managed pollinator commercially with populations being established outside of the native ranges potentially due to escapes from greenhouses (Looney *et al*. 2019).

This could then also mean individuals escaping from greenhouses could be increasing wild populations.

Although no significant overall association was found between bumble bee abundance and floral density, this variable had a significant positive relationship with floral richness. Additionally, bumble bee diversity showed positive relationships with both flower richness and density. This suggests that having a variety of flower species present within bumble bee habitat will have a positive impact on bumble bee abundance. It also shows that a higher abundance and variety of flowers could contribute to higher bumble bee diversity. This aligns with research that has found in agricultural landscapes, regardless of agricultural cover, floral richness can increase wild bee diversity (Lane *et al.* 2020). The same findings can be seen within urbanized environments with local plant gardens and communities being important in providing floral resources and that floral richness was a top predictor of wild bee abundance in these landscapes (Gerner and Sargent 2022). Evidence of floral partitioning among more common bumble bee species (Ye et al. 2024) suggests that floral richness may impact bee diversity and abundance by providing a wider variety of floral resources, allowing more bumble bee species to coexist and fulfill their foraging needs. This evidence supports the importance of not only planting more floral resources to conserve bumble bees, but also a variety of floral species as well.

One major limitation of this study, along with the two historical studies, is that there were a limited number of sites sampled within southern Ontario. This means that our data is representative of a relatively small region in the area. Additional floral sampling along the transect and sampling a larger area could also be favourable, with quadrats being done every 50m instead of every 100m. The use of relative abundance, though widely used, can cause biases in cologicla studies. There may be bias toward species detection that is not related to the actual

abundance and size of the population (Richardson *et al.* 2019). For example, differences in phenology among bumble bee species – such as variations in colony emergence and duration – can influence the likelihood of detecting certain species. This means that species that emerge later in the season may be underrepresented in the data. Lastly, while this study includes data on both nectar and pollen foragers, it does not account for whether the flowers in the dataset provide the adequate amount of pollen and nectar and necessary nutrients for bumble bee survival and reproduction. This lack of specificity means we may not fully capture the importance of certain flowers to the bumble bees nutritional needs.

Overall, we have seen the decline of bumble bee abundance for certain species and diversity in southern Ontario as well as many changes in floral use such as declines of dietary breadth and floral visitation diversity over a 50-year period. Based on these results we may attribute bumble bee declines at least partially to the loss of and availability of their floral resources and further research is needed to understand how the various causes of decline may be working in tandem on different species. Plant-pollinator networks not only rely on the floral resources to be stable, but also the pollinators to maintain a functioning ecosystem. Possible ways to mitigate negative effects to wild bees have been proposed such as planting flowering plants, including trees and shrubs, near agricultural fields and crops which has been shown to increase the abundance and diversity of wild bees in these areas (Nicholls and Altieri 2013). To maintain diverse and abundant bumble bee communities, it is important to restore flowering plants within bee flight ranges in fragmented or resource-poor landscapes since these plants are imperative when it comes to bee survival. This will offer the nutrition needed for bees to provide for their offspring (Blaauw and Isaacs 2014). Studies such as this one allow us to better understand the trends in bumble bee communities as well as their floral interactions making them

fundamental for the conservation and management of these species. It is recommended that this study be repeated in 10-20 years to continue to assess the bumble bee community in this region. Bumble bees continue to see overall declines in southern Ontario, indicating that they continue to need conservation efforts more than ever to bring back diverse and healthy populations.

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Chapter 2: Floral use competition between honey bees and bumble bees within an urban community

Taylor Kerekes, Sarah MacKell, Hadil Elsayed, Sheila R. Colla

Author's Contributions:

All authors contributed to conceiving the ideas. SM and HE led the development and collection of the data. TK analyzed all samples (i.e. pollen identification) and data. TK led data analysis and the writing of the manuscript. All authors contributed to editing and gave final approval for inclusion into the dissertation and eventual publication to a peer-reviewed journal article.

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Abstract

The global reliance on managed honey bees (*Apis mellifera*) for crop pollination has raised concerns about their impact on native pollinator species, including bumble bees (*Bombus* spp.). This study examines the extent of floral resource overlap between honey bees and bumble bees in urban areas of the Greater Toronto Area (GTA), Ontario, Canada, with a focus on understanding how competition for floral resources may influence bumble bee foraging behavior and niche partitioning. Pollen samples were collected from 541 bees, representing seven *Bombus* species, across ten urban sites with varying honey bee abundances. We analyzed floral visitation patterns and compared bumble bee floral use at sites with high and low honey bee abundance to assess the potential impact of competition. Our results show significant overlap in the floral resources used by honey bees and bumble bees, with bumble bees interacting with a slightly broader range of floral genera. Despite this high overlap, no significant difference was found in the overall floral genera visitation of bumble bees between sites with different honey bee abundances. However, some floral genera were more frequently visited by bumble bees at sites with lower honey bee abundance, indicating possible niche partitioning in response to competition. The findings highlight the potential for competition between honey bees and bumble bees to influence floral resource use, with implications for bumble bee health and conservation. The study underscores the need for conservation strategies that promote native floral diversity and manage honey bee placement to minimize negative impacts on wild bee populations. By providing insights into the dynamics of pollinator interactions, this research contributes to our understanding of the ecological balance between managed and wild pollinators in urban environments.

Introduction

The western domesticated honey bee (*Apis mellifera* Linnaeus) is the most common managed pollinator worldwide (Potts *et al.* 2010) and is often highlighted as a main conservation focus (Colla and MacIvor 2017). Despite concerns over Colony Collapse Disorder (CCD) which has caused significant regional declines, particularly in the United States (vanEngelsdorp *et al.* 2009; Steinhauer *et al.* 2018), the global population of honey bees has been increasing in recent decades (Aizen and Harder 2009; Zattara and Aizen 2021). In addition to the rise in pollinator-dependent crops observed from 1961 to 2009, recent years have continued this trend, with global agriculture becoming ever more reliant on these crops (Aizen and Harder 2009; Aizen *et al.* 2019). Despite this growing dependency, honey bee colonies alone may not be able to meet the increasing demand for pollination services, as studies have shown that the expansion of honey bee populations has not kept pace with the escalating agricultural needs (Aizen and Harder 2009; Garibaldi *et al.* 2013). Due to the increasing crop demand for insect pollinators, it is important to turn our attention to wild bees, such as bumble bees (*Bombus* Latreille), which have been shown to contribute more to pollination than honey bees for many crops (Wahengbam *et al.* 2019**;** Reilly *et al.* 2020). Unlike honey bees, there is evidence of global declines in many bumble bee species (Colla and Packer 2008; Jacobson *et al.* 2018; Richardson *et al*. 2019). Research suggests that managed honey bees may be contributing to these declines in bumble bees in two main ways: floral use competition and pathogen spillover (Goulson and Sparrow 2009; Fürst *et al.* 2014; MacInnis *et al.* 2023).

Honey bees can cause pollen and nectar depletion in local communities (Cane and Tepedino 2017), thereby forcing wild bees to spend more time foraging over greater distances (Thomson 2004) and displacing them from their preferred pollen hosts (Roubik and Villanueva-Gutierrez 2009). Honey bees are not only competing for resources but also changing the

composition of habitats by pollinating non-native plants which can outcompete native plants (Barthell *et al.* 2001; Mallinger *et al.* 2017). This can have further negative effects on native bees who rely on native plants for optimal nutrition and cause a decrease in wild bee species richness (MacKell *et al*. 2023). Because bumble bees and honey bees are both generalists, they have a high chance of foraging on similar floral species causing competition for these resources (Goulson and Sparrow 2009). Generalist species require multiple diet choices to obtain proper nutrition which has been found to highly influence bumble bee health, body size, and reproduction (Thomson 2004; Goulson and Sparrow 2009; Elbgami *et al.* 2014; Page and Williams 2023). Niche partitioning, the process of competing species being driven to different patterns of resource use (Casanelles-Abella *et al.* 2023), has been seen as a response from wild bees which have high niche overlap and competition with honey bees (Valido *et al.* 2019). This could drive wild bees to becoming more specialized in the resources used and/or switching to alternate resources if they are available (Inouye 1978**;** Walther-Hellwig *et al*. 2006).

There is also growing concern that managed honey bees transfer parasites and pathogens to native bee populations (Fürst *et al.* 2014). Research has shown that the spread of diseases such as Deformed Wing Virus (DWV) and *Nosema ceranae* Fries et al. (1996), which are prevalent in managed honey bee colonies, can infect bumble bees leading to population declines and weakened immunity in these wild pollinators (Fürst *et al.* 2014; Graystock *et al.* 2013). Pathogens can be spread between honey bees and bumble bees when bees from different species interact at the same floral resources where pathogens can be transmitted through contaminated pollen and nectar (Singh *et al.* 2010; Graystock et al. 2013). They can also be spread when pathogens are deposited on the flowers by infected honey bees and subsequently picked up by visiting bumble bees (McMahon *et al.* 2015; Alger *et al.* 2019). The competitive displacement of

bumble bees by the more aggressive foraging behaviour of honey bees further increases the risk of pathogen transmission, as stressed bumble bees may have compromised immune systems, making them more susceptible to infections (Goulson *et al.* 2015).

Here we investigate the floral use of both bumble bees and honey bees which may be competing for floral resources within the Greater Toronto Area (GTA), Ontario, Canada. Given the large presence of managed honey bees in urban areas (Matteson *et al.* 2008) and the resulting possible floral competition, it is essential to assess how this may be impacting our native bees (MacInnis *et al.* 2023). We assessed niche overlap between bumble bees and honey bees and compared bumble bee floral use at sites with a high and low relative abundance of honey bees present. Comparing bumble bee floral use at sites with varying relative abundances of honey bees provides insights into how competition influences resource partitioning and habitat use. This research is aimed at contributing to a deeper understanding of pollinator dynamics and highlight the importance of creating pollinator-friendly environments that support diverse bee communities.

Methods

Pollen identification

In total, 541 pollen samples were collected from May to August in 2019 within ten different urban sites around the Greater Toronto Area including two on university campuses, one in a botanical garden, and seven in urban parks. Sites were sampled every 7-10 days with bees caught using 30 pan traps (put out for 8 hours) and sweep netting (30 minutes 3x per day). Refer to MacKell *et al.* (2023) for full site location information and bee sampling methodology.

Samples include pollen collected by bumble bees (277 pollen samples) and honey bees (264 pollen samples) caught at each site. Sites were categorized based on relative honey bee abundance, which was determined by calculating the proportion of honey bees to the total number of bees observed at each site. The average relative honey bee abundance was calculated across all sites. Sites with relative abundance below this average were classified as having low honey bee abundance, while those above the average were classified as having high honey bee abundance. As a result, five sites were identified as having high relative honey bee abundance, and the remaining five were classified as having low relative abundance.

Pollen samples were collected by swiping each bee specimen (as described in MacKell *et al.* 2023) with a piece of fuchsin jelly to collect any pollen on the corbicula and body, which was then stored in a centrifuge tube. During analysis, each piece of fuchsin jelly was picked up using a new tooth pick to ensure no cross contamination between samples. Pollen samples were then heated to allow grains to expand, thoroughly mixed with the fuchsin jelly and mounted on slides. Pollen was identified morphologically to genus using McAndrews *et al.* (1973) and Crompton and Wajtas (1993) as well as using a reference collection from the MacIvor Lab (University of Toronto Scarborough). Online resources including the pollen grain identification guide on Discover life (Delaney n.d), the Global Pollen Project (Martin and Harvey 2017) the Cornell Pollen Grains Reference Library (Cornell University 2014), and Quebec's *Ouvrage de référence photographique de grains de pollen non acétolysés* (Girard 2014) were also used, to compare pollen grains to more recent records of plants in North America. Pollen grains were identified to the genus level rather than species due to the high morphological similarity among species, which makes reliable species-level identification challenging using light microscopy (Mander and Punyasena 2014; Keller *et al.* 2015).

To properly identify pollen load grains methods similar to those used by Russo and Danforth (2017) were followed. Each slide was scanned under a microscope at 400x, side to side, with a minimum of 3 randomly selected lines/transects being observed across each slide to account for non-random clumping. A minimum of 300 pollen grains were identified per slide when possible. When less than 300 pollen grains were present on a slide, all pollen grains were identified. Pollen that amounted to 3% or less of the total amount of pollen grains identified per slide were classified as contaminants (Russo and Danforth, 2017).

Data analysis

All statistics were conducted in R (R Core Team, 2022, 4.1.3). To visually compare floral genera overlap between *Bombus* and *A. mellifera*, a bipartite network was created using the 'plotweb' function found in the bipartite package (Memmott 1999; Dormann *et al.* 2009). The function 'networklevel' was used to investigate connectance and niche overlap within the network. Connectance looks at the proportion of possible interactions within the network with high connectance showing the most possible interactions observed and low showing only a portion of the possible interactions observed (Dormann *et al*. 2008**;** Mathiasson and Rehan 2020).

The function 'specieslevel' from the bipartite package was used to calculate degree, normalized degree, and proportional generality. The degree illustrates the number of interactions that the bees have with the floral genera, the normalized degree takes this degree and accounts for the total number of possible interactions it could have with other species. This allows the comparison of different values regardless of the size and complexity of each dataset (Bascompte

et al. 2003). The proportional generality is a quantitative measure of the normalized degree, looking at the number of potential floral interactions between bees and floral genera.

A heatmap was created using the pheatmap package to show the relationship between *A. mellifera* and various *Bombus* species and their association with different flower genera. To create an accurate heatmap that accounts for the varying number of observations per bee species, all bee-floral visitation data was normalized by dividing floral interactions by the total interactions for each species. To further explore the floral genera overlap between *Bombus* and *A. mellifera*, the Morisita-Horn index (MH) was used. This index compares the similarities between two samples, in this instance the floral resources used by *Bombus* and *A. mellifera*, while taking abundances into account. This index was also used to compare *A. mellifera* floral use to each individual *Bombus* species as well. For the purposes of this paper, we consider an MH value over 0.8 high overlap, MH between 0.3-0.8 indicate moderate overlap, and an MH of less than 0.3 inidcates low overlap.

A boxplot was created to compare the proportion of *Bombus* floral visitations at sites with both low and high *A. mellifera* abundance to examine if bumble bees were foraging on more or less flower genera at each. Proportions were calculated at each site by dividing the number of *Bombus* visits to each flower genus to the total number of visits at each site. A Mann-Whitney U test was used to test if there was a significant difference found in these floral visitations between high and low *A. mellifera* abundance sites. A bar graph was created to show the proportion of visits from *Bombus* on each flower genera at high and low *A. mellifera* abundance sites. Multiple *z*-tests were performed to investigate if there were significant differences in how often *Bombus* were found visiting each flower genera at high and low *A. mellifera* abundance sites. All *z*-tests were done by comparing proportions of visitations for each flower genera at each site.

Results

Floral Overlap

In total, 821 floral interactions were identified, 434 interactions with *Bombus* and 387 interactions with *A. mellifera*. The data specifically focuses on seven *Bombus* species: *Bombus bimaculatus* Cresson, *Bombus citrinus* Smith, *Bombus griseocollis* De Geer, *Bombus impatiens* Cresson, *Bombus pensylvanicus* De Geer, *Bombus rufocinctus* Cresson, and *Bombus vagans* Smith. The 821 plant-pollinator interactions occurred between any *Bombus* species or *A. mellifera* with 37 flowering plant genera (Figure 1). When comparing *Bombus* species floral resource use to that of *A. mellifera*, a large amount of overlap was found when looking at the floral genera that were visited by each group. Investigating these interactions at a community level, connectance was 0.797 showing that a high proportion of the possible interactions between bees and flowers are occurring, indicating a well-connected and densely interacting community. Niche overlap was 0.826 showing high overlap in resources between *A. mellifera* and *Bombus*.

Looking closer at specific floral interactions, *Apis mellifera* had a degree value of 25 (0.676 normalized degree) compared to *Bombus* that had a degree value of 34 (0.919 normalized degree). This shows that *Bombus* interacted with more flower genera than *A. mellifera*, with very few flower genera used exclusively by *A. mellifera*. Looking at the proportional generality, *A. mellifera* had a lower generality (0.819) than *Bombus* (0.959) indicating that *Bombus* is more generalized in its interactions within the network. Something important to keep in mind is that this network contains seven different *Bombus* species compared to only one *Apis* species, *A. mellifera*. This could help to explain why *Bombus* is more diverse and generalized in its

interactions in these results. It is then important to also investigate relationships between each *Bombus* species compared to *A. mellifera*.

To examine floral visitation patterns, a heatmap was created to compare the visitation frequencies of each Bombus species with those of A. mellifera (Figure 2). Plant genera that had high visitation from both *A. mellifera* and at least one *Bombus* species were *Cichorium*, *Salix*, *Taraxicum*, *Trifolium* and *Vicia*. *Apis mellifera* had overlap in floral genera visitation with *B. citrinus* and *B. impatiens* on *Cichorium*, *B. rufocinctus* on *Trifolium*, and *B. griseocollis* when foraging on *Salix*, *Taraxicum,* and *Vicia* (Figure 2). From this we can see that based on the plant genera *A. mellifera* favoured the most, multiple *Bombus* species could also often be found foraging on those same genera.

To further investigate the similarity in diet between *Bombus* and *A. mellifera* the Morisita-Horn (MH) index was used. When comparing all *Bombus* species to *A. mellifera* MH = 0.826, indicating the use of similar resources and high overlap when looking broadly at the floral interactions. To better understand the individual *Bombus* species interactions with *A. mellifera*, MH index was calculated for each bumble bee species (Table 1). Two species showed high overlap over (MH > 0.8) including *B. impatiens* and *B. rufocinctus*. Four species had moderate overlap (MH 0.3-0.8): *B. griseocollis*, *B. bimaculatus*, *B. citrinus*, and *B. vagans*. One species, *B. pensylvanicus*, had low overlap (MH < 0.3).

Bombus floral use at high vs low relative honey bee abundance sites

There was no significant difference found when comparing bumble bee relative floral visitation rates at sites with high relative honey bee abundance compared to sites with low

abundance (Mann-Whitney U test: $p = 0.169$) (Figure 3). This shows that bumble bees are visiting a similar number of flower genera at both high and low honey bee abundance sites. Proportions were used for the number of floral genera visited by bumble bees per site to account for varying numbers of observations at each site. Taking a closer look at each individual floral genus that bumble bees foraged from at high and low honey bee abundance sites (Figure 4), 22 flower genera were found to have significantly different floral visitation from bumble bees at high vs low honey bee sites (Table 2). Conversely, 11 genera did not have significantly different floral visitation from bumble bees at high vs low honey bee sites (Table 2). It is important to note that there are many flower genera that were only visited by bumble bees at either high or low honey bee sites and not both. This could indicate that when honey bees are present in higher abundance, bumble bees lean away from certain flower genera. This could include *Crataegus*, *Vicia*, *Cichorium*, *Prunella*, *Pastinaca*, *Potentilla*, *Rudbeckia*, *Salvia*, and *Tanacetum* which were all only visited by bumble bees at low honey bee sites (Figure 4). This could also indicate that these floral genera were not present at all sites including high honey bee sites. Conversely, *Lotus*, *Ranunculus*, and *Salix* were only visited by bumble bees at high honey bee abundance sites which could show that bumble bees turn to these resources when more honey bees are present.

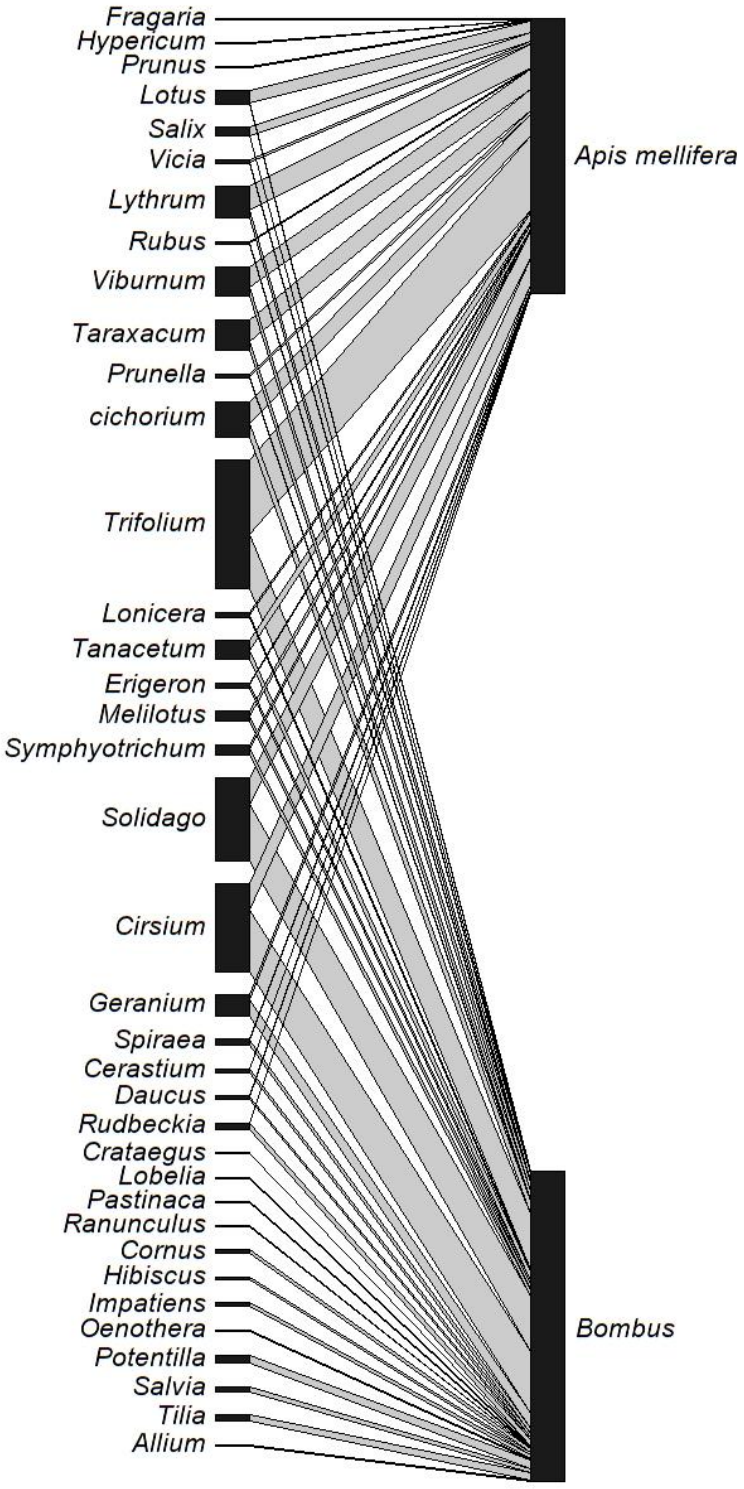

Figure 1: Floral networking comparing flower genera visitation of *Bombus* spp. and *A. mellifera*. Larger lines indicate a higher interaction between the bee and flower.

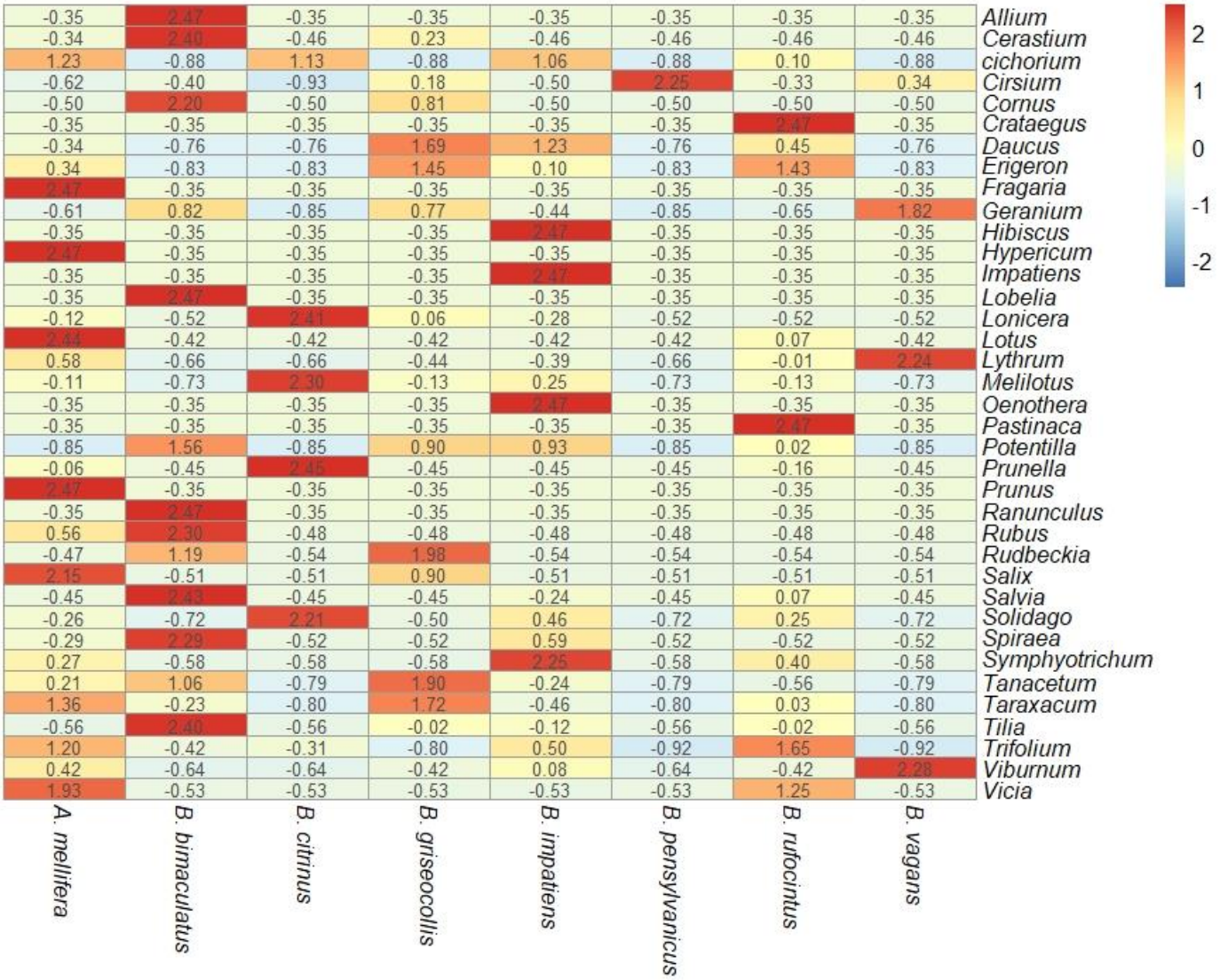

Figure 2: Heatmap showing the flower genera visited by each *Bombus* species and *A. mellifera*. The colour gradient indicates the relative frequency of bee visits to each flower genus, with red indicating higher visitation rates and blue representing lower visitation rates. Each cell contains normalized counts of individuals bees observed visiting a specific flower genus, allowing for comparison across different species and flower types.

Table 1: Horn's Index results looking at floral niche overlap of each *Bombus* spp. compared to *A. mellifera*.

***Bombus* Species**	**Horn's Index**
B. bimaculatus	0.383
B. citrinus	0.336
B. griseocollis	0.403
B. impatiens	0.8
B. pensylvancius	0.172
B. rufocinctus	0.883
B. vagans	0.368

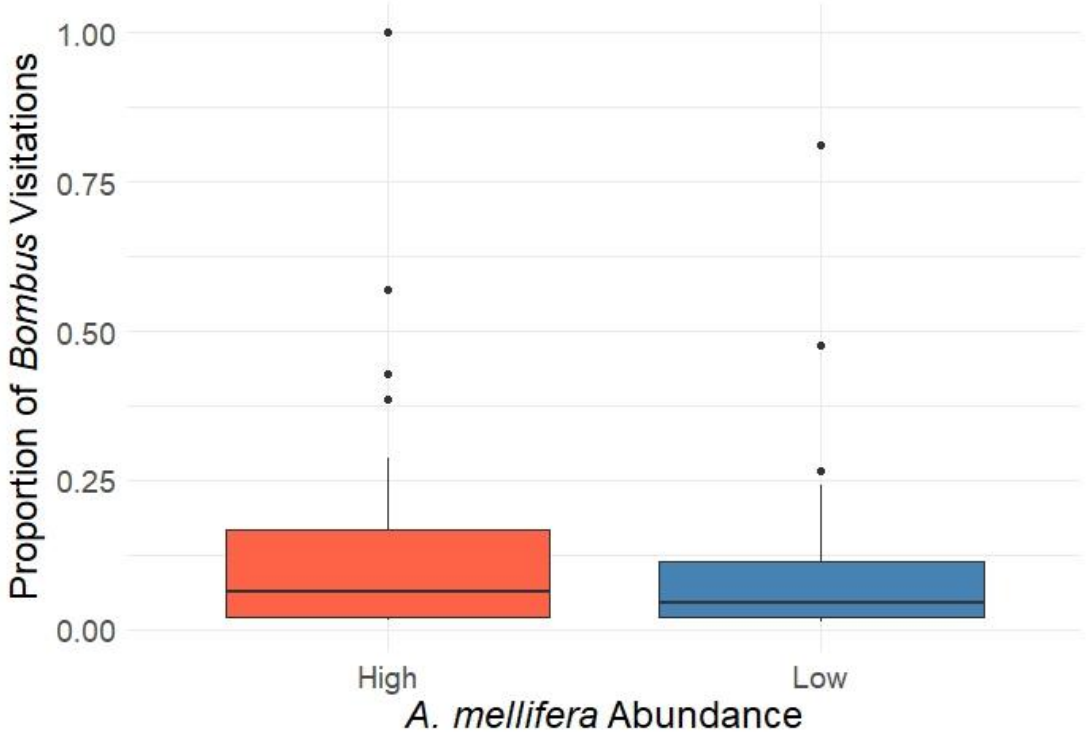

Figure 3: Comparing the proportion of *Bombus* floral genera visitations recorded at sites with both high (red) and low (blue) relative *A. mellifera* abundance.

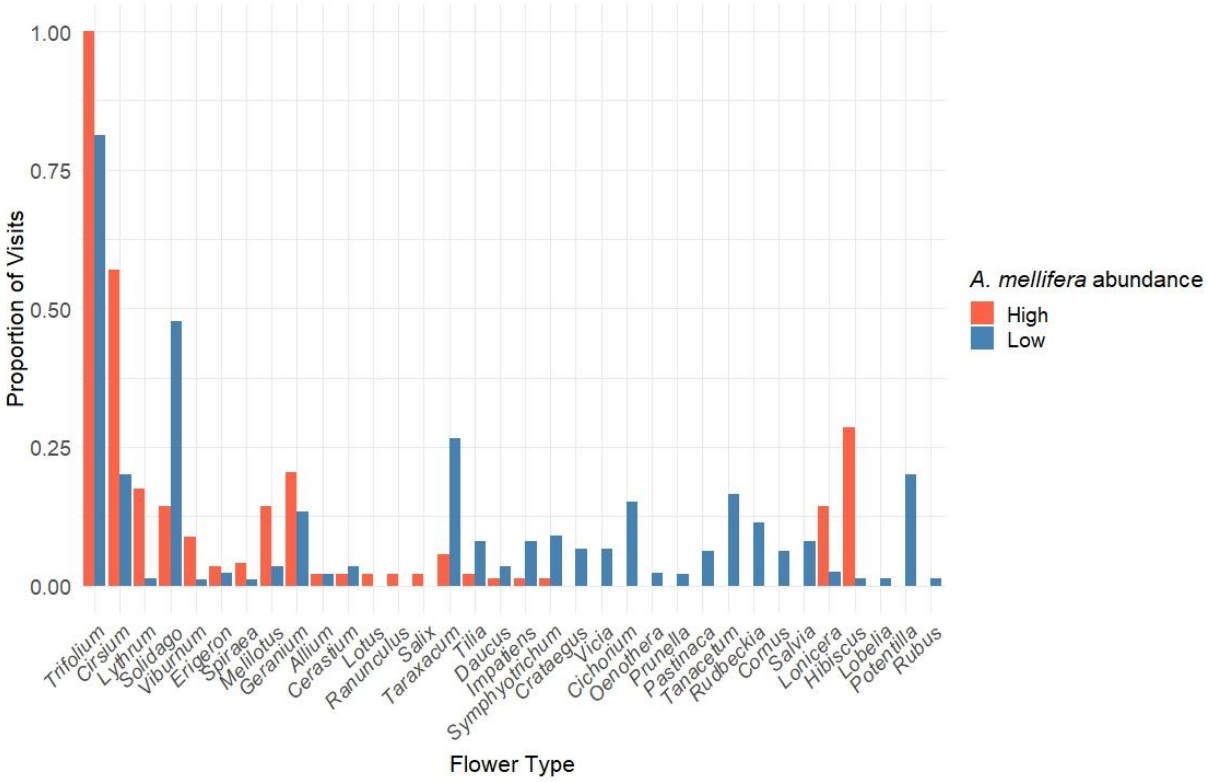

Figure 4: Comparing the proportion of *Bombus* visits to different flower genera at high (red) and low (blue) relative *A. mellifera* abundance sites.

Table 2: Results of proportion *z*-tests looking at the difference in *Bombus* visitation rates to each flower genera at high and low relative *A. mellifera* abundance sites.

Floral Genera	***p*-value**
Cichorium	<0.001*
Cirsium	<0.001*
Cornus	<0.001*
Crateaegus	<0.001*
Hibiscus	<0.001*
Impatiens	<0.001*
Lonicera	<0.001*
Lythrum	<0.001*
Melilotus	<0.001*
Pastinaca	<0.001*
Potentilla	<0.001*
Prunella	0.002*
Rudbeckia	<0.001*
Salvia	<0.001*
Solidago	<0.001*
Spiraea	<0.001*
Symphyotrichum	<0.001*
Tanacetum	<0.001*
Taraxacum	<0.001*
Tilia	<0.001*
Viburnum	<0.001*
Vicia	<0.001*
Allium	1
Cerastium	0.3
Daucus	0.077
Erigeron	0.417
Geranium	0.163
Lobelia	0.074
Lotus	0.243
Oenothera	0.139
Ranunculus	0.243
Rubus	0.073
Salix	0.243

*Shows significance ($p < 0.05$)

Discussion

When comparing the floral resource use of bumble bees and honey bees within the Greater Toronto Area, high overlap is found with bumble bees, as a group, visiting slightly more flower genera than honey bees. Since there is much variation in visitation patterns among bumble bee species, it is important to also look at each individual bumble bee species in comparison to honey bees as well. It was found that multiple bumble bee species had high flower genera overlap with honey bees, including *B. impatiens*, *B. rufocinctus*, *B. griseocollis* and *B. citrinus*. Many other studies have also suggested that floral overlap between honey bees and bumble bees could be contributing to bumble bee declines (Thomson 2006; Elbgami *et al.* 2014; Thomson 2016; Wojcik *et al*. 2018). Both honey bees and bumble bees are generalist foragers which have the potential to compete for floral resources (Thomson 2004; Goulson and Sparrow 2009; Rogers *et al.* 2013; MacInnis *et al.* 2023), this can explain the high niche overlap found in this study.

It is important to investigate how this overlap may negatively impact bumble bees and understand how it could be contributing to declines. One study found that a higher abundance of honey bees caused a decrease in nectar availability at sites where resources were already low (Page and Williams 2023). This means that if resources are already scarce at a site, competition with honey bees can make it increasingly difficult for other native bees such as bumble bees to gain the nutrition needed. Bumble bees require a large amount of pollen and nectar to meet their nutritional needs therefore constraints to their nutrition can be more detrimental to them than it would be to smaller bodied bees (Goulson *et al.* 2002; Torné-Noguera *et al.* 2016). It has been found that when bumble bees are in competition with honey bees, workers have smaller body sizes which could indicate reduced pollen consumption therefore causing growth limitations

within the colony (Goulson and Sparrow 2009). A study by Thomson (2004) found that bumble bees had reduced foraging rates near honey bee hives and had lower larval and gyne production when in greater competition with honey bees. This further suggests that high niche overlap can have negative impacts on bumble bee growth and reproduction.

Overall, there was no difference found between bumble bee floral visitation rates (considered collectively across all *Bombus* species) at sites with high honey bee abundance compared to sites with low honey bee abundance. This may suggest that although there is high overlap in floral resource use with honey bees, bumble bees do not seem to be foraging less when honey bee abundance is high. If bumble bees do continue to forage on floral resources that may be depleted by honey bee visitation (Page and Williams 2023), the impacts of nutritional stress on bumble bee colonies could worsen. The passage of pathogens and parasites from honey bees could also increase if they have high overlap in their floral resources (Furst *et al.* 2014). It is known that undergoing nutritional stress can make bumble bees more susceptible to diseases, therefore floral competition and disease spread from honey bees could work in tandem to contribute to bumble bee declines (Furst *et al*. 2014; Roger *et al.* 2017).

Although niche overlap between bumble bees and honey bees was high, some species such as *B. pensylvanicus* had low overlap. *Bombus pensylvanicus* is a species that is in decline and it is listed as Vulnerable on the IUCN Red List of Threatened Species (Hatfield *et al.* 2015). Due to the rarity of this species at the study sites (MacKell *et al*. 2023), it should be emphasized that there were few pollen samples collected. This small dataset could mean we may not be getting an accurate picture of the floral use of this species, therefore giving the impression of low niche overlap with honey bees. Something else to consider is that *B. pensylvanicus* might be displaced from its preferred floral resources by honey bees. Research has found that bumble bee species

could be narrowing their diet breadth to avoid competition with honey bees (Walther-Hellwig *et al.* 2006; Magrach *et al.* 2017). Although some studies state that bumble bees can become more generalized when in competition for resources (Fontaine *et al.* 2008), there has been evidence that bumble bees can adapt to high honey bee abundance by becoming more specialized in their floral resource use (Page and Williams 2023).

When looking closer at the specific flower genera visited, there are many genera that had varying visitation from bumble bees at sites with high and low honey bee abundance. This could indicate niche partitioning which in this case could look like bumble bees shifting their diet at sites with higher honey bee abundance due to exclusion from certain flowers (Thomson 2021). Since interspecific disease transmission can happen when visiting the same flowers, niche partitioning by bumble bees could help to reduce this transmission by foraging on different resources (Adler *et al.* 2021). By shifting to floral resources not as commonly used by honey bees, bumble bees may be able to minimize competition (Valdovinos *et al*. 2013). However, if adequate floral resources are not available to accommodate this shift, bees could end up collecting fewer resources therefore seeing increasing effects of competition (Rogers *et al.* 2013; Page and Williams 2023).

There are certain limitations within this study that are important to keep in mind. This data only takes floral genera into account, therefore different patterns could be seen if flowers were able to be identified to species. By looking closer at flower species visitation, a better insight into overlap and potential niche partitioning when competition is found could be investigated. Certain data presented was pooled to compare multiple bumble bee species to honey bees. Although other studies draw conclusions from similar comparisons (Thomson 2006; Elbgami *et al.* 2015) it is important to consider that different bumble bee species can vary in their

response to stressors (Cameron and Sadd 2020). For results in this study that do investigate comparisons at the bumble bee species level, there are fewer observations for each bumble bee species compared to honey bees meaning results should be interpreted with caution.

Overall, we can see here that there may be a high overlap between bumble bees and honey bees for floral resources. Because of the negative effects this competition can have on wild bees, including bumble bees, it is important to look at ways to mitigate negative impacts. One potential approach is planting native flowering plants within urban communities and urban green spaces, including trees and shrubs (Baldock *et al.* 2019). Community gardens have been shown to increase the abundance and diversity of wild bees, as these environments provide critical foraging and nesting habitats that are often lacking in urbanized areas (Hall *et al.* 2017; Baldock *et al.* 2019). To maintain diverse and abundant wild bee communities, it is important to restore native flowering plants within bee flight ranges in fragmented or resource-poor landscapes since these plants are imperative when it comes to wild bee survival. This will offer the nutrition needed for bees to provide for their offspring (Blaauw and Isaacs 2014) and allow more flexibility to bees if they are forced to turn to floral resources not used by honey bees. In addition to restoring native flora, rewilding urban spaces can involve reducing lawn areas, increasing the diversity of plant species, and creating pollinator corridors that connect isolated green spaces. These actions not only benefit wild bees but also enhance overall urban biodiversity and resilience (Aronson *et al.* 2017). Regarding honey bee competition, keeping honey bees out of urban areas where they could cause changes to native floral resources and relying more on native bees as managed pollinators could lessen the competition that wild bees are facing when located in habitats with colonies of managed honey bees.

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Conclusion

The objective of this MSc research was to continue to monitor changes in bumble bee abundance, diversity and floral use in historically surveyed areas (Chapter 1) and to investigate floral resources use competition between bumble bees and honey bees (Chapter 2). This research was done to better understand bumble bee floral use in southern Ontario and to better understand its potential impacts on declines. Chapter 1 highlights the continued decline in bumble bee relative abundance and diversity at sites in southern Ontario that were previously sampled. Significant decreases in relative abundance are seen in multiple species which also aligns with other studies investigating these trends (Grixti *et al.* 2009; Cameron *et al.* 2011; Richardson *et al.* 2019; Jacobson *et al.* 2018). Alternately, some bumble bee species such as *B. impatiens* have seen significant increases in relative abundance. Despite this increase in some species, many bumble bees are facing nutritional stress and competition for floral resources, exacerbated by factors such as habitat loss and pathogen spillover (Potts *et al.* 2010; Goulson *et al.* 2015; Herbertsson *et al.* 2016; Cameron and Sadd 2020). This study also highlights the change in floral resources use, showing the potential for a decrease in dietary breadth for some declining species. Since adequate floral resources are needed within flight ranges to support their nutritional needs, planting and restoring these resources could help to mitigate some declines. Future research should focus on expanding the sampling area and assessing the combined effects of various stressors on bumble bee populations. This research provides valuable insights into long-term trends in bumble bee diversity and relative abundance, highlighting the importance of ongoing monitoring and adaptive conservation strategies to preserve vital pollinators.

Chapter 2 suggested significant floral resource overlap between bumble bees and honey bees in the Greater Toronto Area, with bumble bees visiting slightly more flower genera. High

overlap was observed among several bumble bee species, including *B. impatiens*, *B. rufocinctus*, *B. griseocollis*, and *B. citrinus*, which aligns with previous research indicating floral competition as a contributing factor to bumble bee declines (Thomson 2006; Elbgami *et al*. 2014; Thomson 2016; Wojcik *et al.* 2018). Although bumble bee floral visitation rates were similar between sites with high and low honey bee abundance, the competition for resources still poses risks. The potential for nutritional stress due to depleted floral resources, along with the increased risk of pathogen transmission, highlights the harmful effects of honey bee presence on bumble bees (Page and Williams 2023; Fürst *et al*. 2014). The study also suggests that while some bumble bee species like *B. pensylvanicus* might appear to have low overlap, this could be due to small sample sizes or displacement by honey bees, indicating the need for further investigation into species-specific responses (Walther-Hellwig et al. 2006; Magrach et al. 2017). To mitigate negative impacts, enhancing urban and agricultural landscapes with diverse native flowering plants can support wild bee populations by providing essential nutritional resources and reducing reliance on managed honey bees (Nicholls and Altieri 2013; Blaauw and Isaacs 2014). Future research should take a closer look not only at floral species visited by both bumble bees and honey bees and gather more information on individual bumble bee species to better compare each to honey bee floral use.

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Appendices

Appendix A: Floral network plant species comparison results

Table 1: Showing the degree and normalized degree for each plant species in the historical survey period (1971-1973) and current survey period (2021-2023).

	Degree		Normalized degree	
Species	**Historical**	**Current**	**Historical**	**Current**
Achillea millefolium	--	2	--	0.167
Arctium lappa	--	2	--	0.167
Arctium minus	--	5	--	0.417
Aesculus carnea	5	--	0.417	--
Aesculus hippocastanum	1	--	0.083	--
Ajuga genevensis	1	--	0.083	--
Asclepias spp	--	2	--	0.167
Asclepias syriaca	--	3	--	0.250
Berberis thunbergii	2	--	0.167	--
Bidens frondosa	--	1	--	0.083
Carduus acanthoides	6	--	0.500	--
Carduus nutans	10	6	0.833	0.500
Centaurea spp.	--	1	--	0.083
Cichorium intybus	--	2	--	0.167
Cirsium arvense	4	8	0.333	0.667
Cirsium vulgare	2	5	0.167	0.417
Clematis virginiana	--	3	--	0.250
Clinopodium vulgare	--	3	--	0.250
Cynoglossum officinale	2	--	0.167	--
Daucus carota	--	1	--	0.083
Dipsacus fullonum	--	3	--	0.250
Echinocystis lobata	--	1	--	0.083
Echium vulgare	11	6	0.917	0.500
Elaeagnus umbellata	--	1	--	0.083
Erigeron spp.	--	1	--	0.083
Euthamia graminifolia	--	2	--	0.167
Eutrochium spp.	--	6	--	0.500
Eutrochium maculatum	--	1	--	0.083
Eutrochium purpureum	--	4	--	0.333
Frangula alnus	--	3	--	0.250

Glechoma hederacea	--	1	--	0.083
Helianthus tuberosus	--	3	--	0.250
Heliopsis helianthoides	--	4	--	0.333
Hesperis matronalis	2	1	0.167	0.083
Hydrophyllum virginianum	6	--	0.500	--
Hypericum perforatum	7	5	0.583	0.417
Impatiens capensis	2	3	0.167	0.250
Leonurus cardiaca	--	4	--	0.333
Linaria vulgaris	1	1	0.083	0.083
Linnaea amabilis	--	1	--	0.083
Lonicera caerulea	4	--	0.333	--
Lonicera periclymenum	4	--	0.333	--
Lonicera tatarica	3	--	0.250	--
Lonicera spp.	--	2	--	0.167
Lotus corniculatus	3	3	0.250	0.250
Lythrum salicaria	--	7	--	0.583
Malus domestica	5	--	0.417	--
Medicago sativa	6	1	0.500	0.083
Melilotus alba	5	--	0.417	--
Monarda fistulosa	--	3	--	0.250
Nepeta cataria	2	5	0.167	0.417
Oenothera biennis	--	1	--	0.083
Plantago lanceolata	--	1	--	0.083
Prunella vulgaris	--	6	--	0.500
Prunus tomentosa	3	--	0.250	--
rhododendron spp.	2	--	0.167	--
Ribes nigrum	2	--	0.167	--
Ribes uva-crispa	3	--	0.250	--
Robinia hispida	4	--	0.333	--
Robinia pseudoacacia	5	--	0.417	--
Rubus idaeus	3	3	0.250	0.250
Rudbeckia laciniata	--	4	--	0.333
Salix spp.	--	1	--	0.083
Securigera varia	--	2	--	0.167
Silene vulgaris	--	1	--	0.083
Solanum dulcamara	4	3	0.333	0.250
Solidago spp.	6	8	0.500	0.667
Sonchus oleraceus	--	1	--	0.083

Spiraea japonica	--	1	--	0.083
Symphyotrichum	--	3	--	0.250
Symphyotrichum ericoides	--	1	--	0.083
Symphyotrichum lanceolatum	--	2	--	0.167
Symphyotrichum lateriflorum	2	--	0.167	--
Symphyotrichum novae-angliae	5	3	0.417	0.250
Symphyotrichum puniceum	--	1	--	0.083
Symphytum officinale	8	7	0.667	0.583
Syringa vulgaris	2	1	0.167	0.083
Tanacetum vulgare	--	1	--	0.083
Taraxacum officinale	1	1	0.083	0.083
Thalictrum pubescens	--	2	--	0.167
Thymus spp.	--	1	--	0.083
Tilia americana	--	2	--	0.167
Tilia platyphyllos	2	--	0.167	--
Tragopogon spp.	--	2	--	0.167
Trifolium pratense	10	5	0.833	0.417
Trifolium repens	--	6	--	0.500
Vicia cracca	6	11	0.500	0.917