Evaluating the impacts of local and landscape level site characteristics on bumblebee (*Bombus* spp.) communities along the Flint River riparian zone

By

Alexandrea Morgan Peake

Thesis submitted to the Faculty of the
University of Michigan-Flint
in partial fulfillment of the
requirements for the degree of
Master of Science in Biology

Approved By:

Dr. Rebecca Tonietto
Thesis Adviser
Dept. of Biology

Dr. Heather Dawson
Thesis Adviser
Dept. of Biology

Dr. Lauren Umek
Thesis Adviser
Chicago Park District

University of Michigan-Flint
May 2022

Table of Contents

Table of Figures

Abstract

Flying insect populations, including pollinators such as wild bees, have been declining globally due to anthropogenic changes. Pollinators are important not only for conservation of biodiversity of insects, but also for the conservation of biodiversity of plants. Almost 90% of wild and cultivated plants rely on animal facilitated pollination, including many crops humans rely on for sustenance. Population declines of wild bees has largely been linked to land use changes such as agricultural expansion, but in many cases these insects persist even in intensely industrialized and urban areas. Bees' habitat requirements include suitable nesting habitat, suitable forage availability, and mate availability. In the case of bees in urban areas, drivers of population declines and community structure is unclear. Possible drivers of bee abundance in urban areas include landscape factors such as greenspace, or site characteristics such as floral abundance, floral diversity, quality of floral resources, availability of nesting habitat, soil texture, and soil pollution. Soil texture and soil contaminants could be particularly impactful on ground nesting bees such as species within the genus *Bombus*. Bees in urban areas have oftentimes been studied in parks, natural areas, gardens, and greenroofs but oftentimes bees in urban riparian areas have been overlooked. Riparian areas could be uniquely suited to be important conservation areas for wild bees in urban areas, because they are often left as some of the only intact greenspaces due to difficulty of developing land adjacent to a river.

To study the impacts of landscape factors and site characteristics on *Bombus* communities in a post-industrial urban riparian area, I selected 12 sites within *Bombus* flight range to study where bees were preferentially selecting to be, and what landscape or site characteristics predict this. I captured bees between May and October 2021 and identified all *Bombus* specimens to species. I then used a generalized linear model to find that *Bombus* abundance is predicted by sum of flowering forb cover ($p << 0.05$), total green cover within a 100m radius ($p << 0.05$), and total lawn cover within a 100m radius ($p << 0.05$). For *Bombus* richness I found that only total green cover of the surrounding landscape at 100 m was a significant predictor ($p < 0.05$). For *Bombus* diversity I found that only the Floristic Quality Index was a significant predictor ($p < 0.05$).

To study the impacts of soil texture and soil contamination by heavy metals on *Bombus impatiens*, our most abundantly captured *Bombus* species, I assessed soil texture at each site and heavy metal contamination within soil samples and within individual worker bees and individual worker bee weights. I also assessed heavy metal contamination of *Apis mellifera*, the western honeybee which is not a ground dwelling species, as a negative control as we would not expect it to come into contact with soil contaminants. Soil textures were analyzed using the jar method and ribbon method, and according to both methods each site had a sandy loam soil texture. Because all sites had the same soil texture, this was not used as a predictor in any analysis. Soil heavy metal contamination and individual worker bee heavy metal loads were analyzed using ICP-MS. Heavy metal analyses found for both soils and bees were correlated with Lead (Pb) loads, we performed statistical analyses for lead. Because lead is also a known contaminant in post-industrial cities such as Flint, we expected it to be in the soils. We found lead loads in soil ranging from 13ppm to 76ppm, which is far lower than in many other post-industrial areas. Because lead loads in study sites had a low, limited range the results of this study cannot conclusively say that soil lead contamination is the cause of low worker weights in *Bombus* workers in this case, but it lays out the methods for future studies. Lead in the environment has been shown to have sub-lethal negative effects on *Bombus*, so repetition of this study in more highly polluted areas may show more evidence of this in wild populations than my study.

Chapter 1

Evaluating the impacts of local and landscape level site characteristics on *Bombus* communities in urban riparian areas

Introduction

Pollinators have declined in regions across the globe (Potts et al. 2010). Even in some protected natural areas, flying insect biomass has declined by more than 75% since 1990 (Hallmann et al. 2017). In North America alone, several species of bumblebees have experienced drastic declines (Cameron et al. 2011, Szabo et al. 2012). Bumblebee, (*Bombus spp*.) declines in North America include previously widespread *Bombus* species that are now extirpated in much of their previous ranges (Cameron et al. 2011). For example, *Bombus affinis*, the Rusty Patched Bumblebee used to be abundantly found throughout the midwestern United States, but is now listed as federally endangered and has been extirpated from much of its former range, including the entire state of Michigan (Cameron et al. 2011).

One of the greatest threats to wild pollinators is habitat loss and fragmentation due to human disturbance (Szabo et al. 2012). However, habitat loss is not the only driver of population decline of wild bees (Grixti et al. 2009, Cameron et al. 2011). Dramatic declines in historic ranges of formerly wide spread North American *Bombus* species indicates that there are other drivers of these declines as well, such as greater pathogen prevalence and reduced genetic diversity (Cameron et al. 2011). Such drastic decline of these pollinators is of particular concern as nearly 90% of wild and cultivated plants rely on animal pollination for reproduction globally (Ollerton et al. 2011). These pollination services are vital for conserving biodiversity of wild plants, as well as adding economic value to cultivated crops (Kleijn et al. 2015).

Like all bees, *Bombus* have habitat requirements including suitable forage and nesting habitat within flight range (Harmon-Threatt 2020). Although conservation of existing habitats and habitat restoration can be used to mitigate the impacts of habitat destruction on wild bee communities, habitat requirements of wild bees can oftentimes be met within cities (Hopwood 2008, Tonietto et al. 2011, Hall et al. 2017, Banaszak-Cibicka et al. 2018, Lanner et al. 2019). Even intensely urban areas can support diverse bee communities (Hall et al. 2017). For example, in Chicago, USA Tonietto et al. (2011) found that proportion of non-turfgrass green spaces and plant diversity both supported wild, native bee communities. Lanner (2020) found that communal gardens in Vienna, Austria support wild bee communities, with the main driver being flower frequency within the garden. Banaszak-Cibicka (2018) also found that in Poznań, Poland a large diversified city park was shown to be suitable habitat for wild bees as well. Although parks and green roofs have been studied as pollinator habitats in urban areas (McFrederick and LeBuhn 2006, Tonietto et al. 2011), urban riparian areas have not been adequately studied as sites for pollinator habitats (Golet et al. 2008, Hanula and Horn 2011, Williams 2011, Vossler 2019).

Riparian areas have rarely been studied as candidates for pollinator habitats, and there is currently only one other study on wild bees in urban riparian areas, which was conducted in Argentina (Vossler 2019). Vossler (2019) found that stingless bees foraged from both native and cultivated exotic plants in an urban area with riparian forest in the locality Villa Río Bermejito in the Chaco province of Argentina. Although urban riparian areas have the potential to be valuable wild bee habitats as green spaces in an otherwise urbanized landscape, studying individual site characteristics is necessary to understand how these spaces may provide valuable habitat for wild pollinators and how land management strategies can be utilized to enhance existing riparian areas for wild pollinators. Urban riparian areas are often harder to develop than other spaces, and thus are often left as some of the only unaltered green space in urban areas which makes them great candidates to be pollinator habitat.

Oftentimes remnant habitats or green spaces within urban areas are surrounded by unnatural landscapes such as parking lots or monocultures such as agricultural fields or turfgrass. For animals such as *Bombus* that have a flight range of at least 3 kilometers (Osborne et al. 1999), it is important to consider surrounding landscape factors in addition to study site characteristics. Landscape factors such as total meadow in the surrounding landscape of a study site have been shown to be important factors for *Bombus* richness (Hatfield and LeBuhn 2007). Additionally, in urban areas it has been shown that overall greenspace in the surrounding landscape relates to increased bee abundance and species richness, but only in cases where the greenspace was not dominated by turfgrass monoculture (Tonietto et al. 2011).

In addition to landscape factors, assessment of local floral resource quantity and quality are also important when considering habitat characteristics *Bombus* communities are interacting with. In semi-natural and peri-agricultural landscapes, floral abundance and diversity correlated to an increase of *Bombus* abundance and diversity regardless of habitat type, and *Bombus* community composition was driven by flower abundance (Purvis et al. 2020). Individual workers may select to visit multiple flowering species or only one species of flower often along the same routes (Chittka et al. 1999, Saleh and Chittka 2007). *Bombus* rely on flowers' nectar and pollen for nutrition, and certain species may preferentially visit specific species of flowers (McFrederick and LeBuhn 2006). In an environment with both rewarding and rewardless, co-flowering species, *Bombus* visited both species but rates of switching between flowers was also higher (Katz and Essenberg 2018), and *Bombus* will preferentially visit artificial flowers with higher sucrose concentrations (Cnaani et al. 2006, Katz and Essenberg 2018). Individuals may also travel farther distances for greater nutritional rewards (Heinrich 1976). These findings indicate that *Bombus* have foraging preferences, so floral abundance as well as floral quality may both be important factors for predicting *Bombus* abundance, richness, and diversity. Floral quality, indicated by Coefficients of Conservatism (CC) and Floristic Quality Index (FQI), has been used previously to study relationships between insect and plant communities (Farhat et al. 2014, Peters et al. 2016, Buckles et al. 2019, McNeil et al. 2020). The Floristic Quality Index, calculated and relativized by individual plant CC values, can be used as an indicator of habitat quality, as plants that are more sensitive to disturbance or environmental contaminants, assigned a higher CC score.

To help fill the knowledge gap of how urban riparian areas may or may not be supporting wild *Bombus* communities and which site characteristics may be driving these relationships, I had several research questions each targeting a different landscape or local site characteristic and its relationship to *Bombus* communities. (1) In urban riparian areas, what landscape factors best support *Bombus* communities? Both landscape and local factors have been identified previously as significant predictors of various metrics related to *Bombus* communities (Sivakoff et al. 2018), so I evaluated both landscape (total greenspace within 100m, lawn cover within 100m) and local factors (flowering forb abundance, flowering forb diversity). I hypothesized that total greenspace would be positively correlated to and a significant predictor of *Bombus* abundance, richness, and diversity, while lawn cover will be negatively correlated to and a significant predictor of *Bombus* abundance, richness, and diversity due to typical differences between these categories when looking at availability of nesting sites and floral resources (Tonietto et al. 2011). (2) In urban riparian areas, is floral abundance correlated with *Bombus* abundance, richness, and diversity? I hypothesized that floral abundance would be positively correlated to and a significant predictor of *Bombus* abundance, richness, and diversity (Purvis et al. 2020). (3) In urban riparian areas, does floral quality (quantified by FQI) correlate with *Bombus* abundance, richness, and diversity? I hypothesized that site visits with a higher weighted average FQI would be positively correlated to and be a significant predictor of *Bombus* abundance, richness, and diversity (Farhat et al. 2014).

Methods

Site selection

The Flint River is 142 miles (228.5 km) long with its basin running throughout Oakland, Lapeer, Tuscola, Sanilac, Genesee, Shiawassee, and Saginaw counties in the state of Michigan (Leonardi et al. 2001). Within the city of Flint, located in southeastern MI, the Flint River runs southwest for a stretch of about 14 km, and empties into the Shiawassee River near Saginaw (Leonardi et al. 2001). I selected 12 sites centered around an historic dam downtown, which approximates the halfway point between the stretch of river within the city of Flint. Sites were selected intentionally within potential *Bombus* flight range so I could test where *Bombus* were preferentially selecting to be among the sites, and sites would not act as isolated patches. Sites are each 100m across parallel to the river, and 50m across perpendicular from the river. I selected sites with publicly accessible greenspace within 1.5 km of the next closest site. Distance between sites ranged 37 m - 3.4 km, with a standard deviation of 1.058 km. Each site represents one half hectare of publicly accessible urban greenspace, for which different management regimes include active restoration, turfgrass management, unmanaged forest, fallow meadow, or a combination. Thereof, six sites were within city parks and had a combination of turfgrass management and unmanaged forest or had a combination of turfgrass management and active restoration, or had a combination of turfgrass management, unmanaged forest, and degraded restoration, and six sites on the University of Michigan Flint campus with turfgrass management or a combination of turfgrass management and fallow meadow. I selected sites with varying vegetative structure and management practices so they cumulatively would represent a variety of typical urban riparian areas.

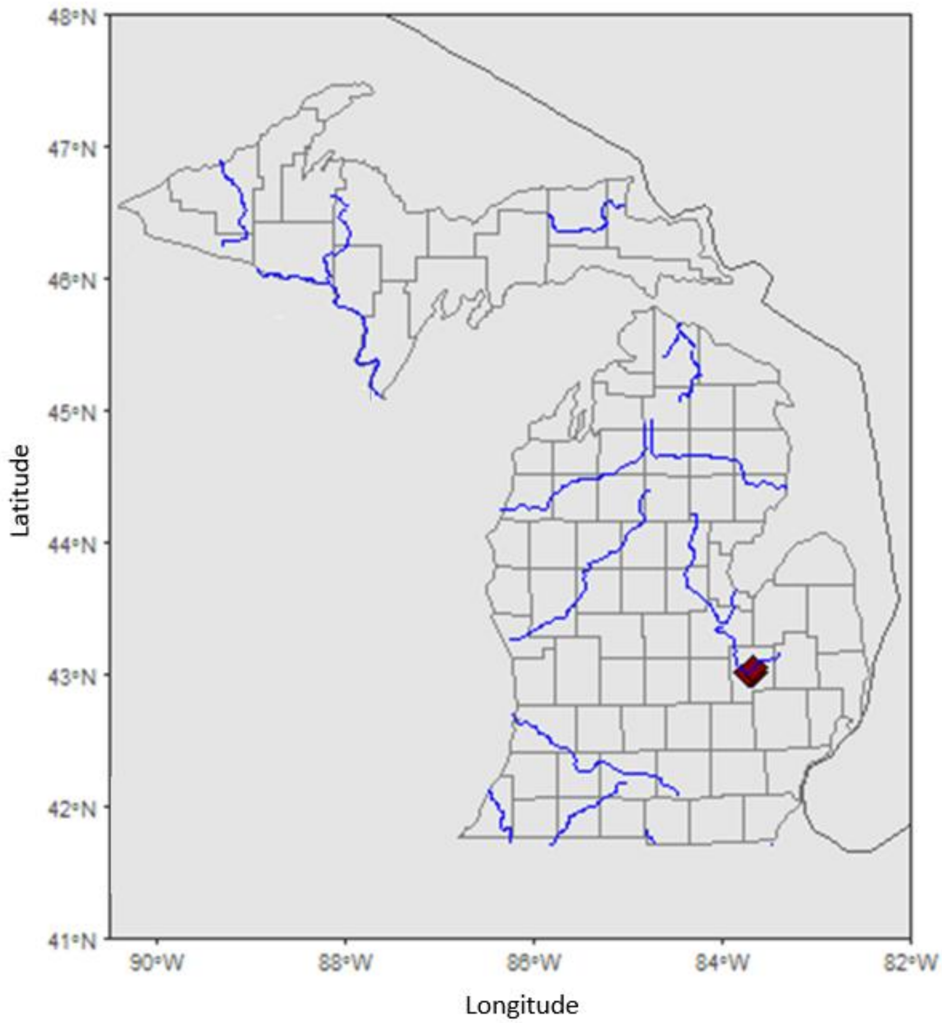

Figure 1: Map of Michigan indicating site locations in Genesee County in relation to the river. Red diamonds in Genesee County indicate study sites used along the Flint River.

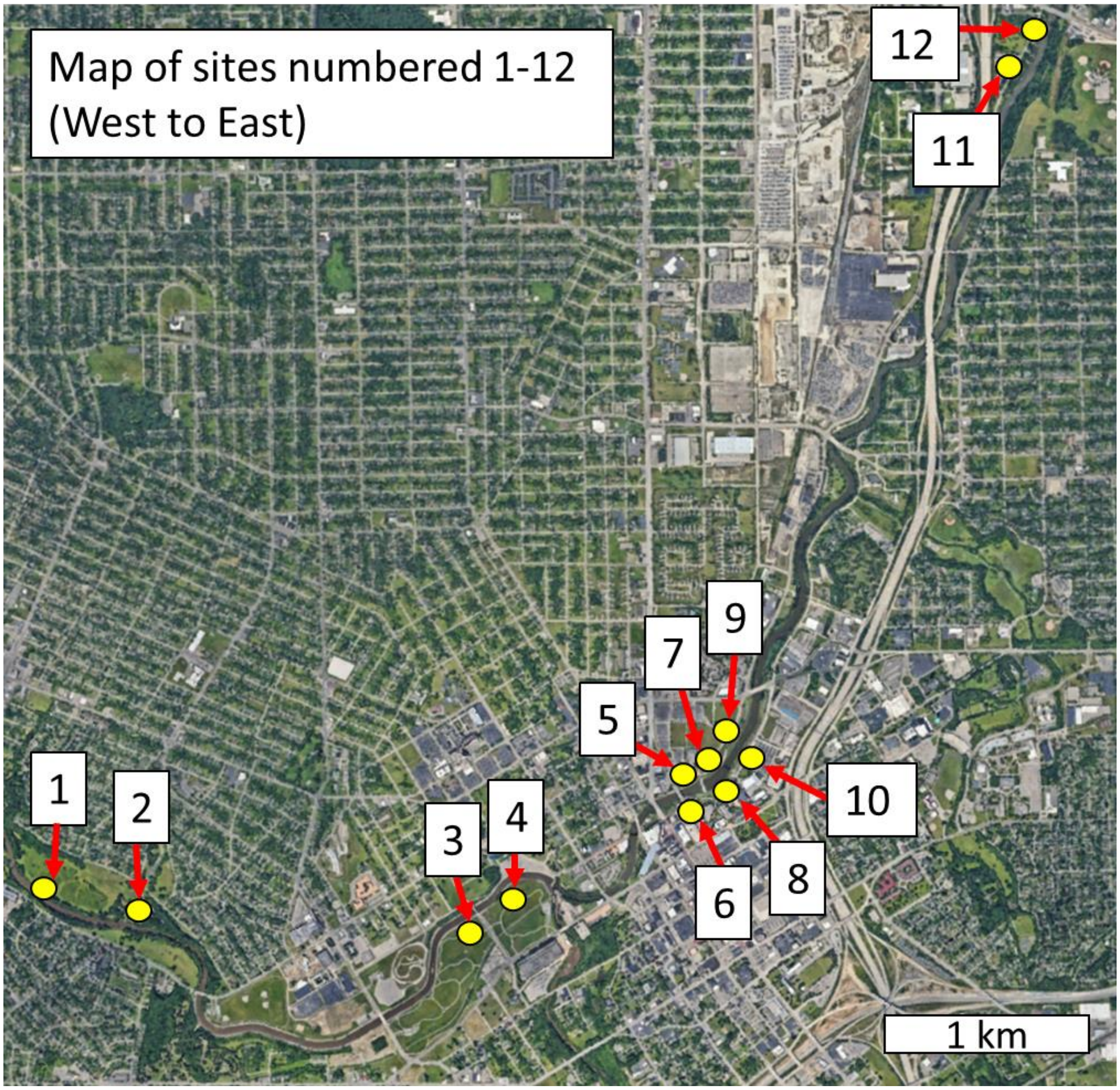

Figure 2: Numbered study sites along the Flint River in Flint, MI.

Landscape analysis

To analyze the landscape within 1000m and 100m radius from the center of each site, I used images from Google Earth, as Google Earth provided images taken during the sampling year (2021). Resolution for these images was 0.15m. I took a screengrab of the landscape and saved it as a Portable Network Graphics (PNG) file. Then, I used Preview software to cut out excess land around the 100m radius circle from the center of the site. I imported this image into the software ImageJ, and used the Color Inspector 3D plugin to select the histogram display mode, select the fewest number of color cells possible, select maximum saturation, and I then manually removed the river and any impervious surface that was being assigned the same color as lawn, and manually separated trees and meadow from the rest of the green space. I then could see cover categories by color, and added percent meadow, percent tree, and percent lawn to get total green space. Because landscape composition does not substantially change throughout one sampling period and due to availability of satellite images, landscape was analyzed only once.

To create an ordination for landscape at each radius I used the data collected using ImageJ software. I created a Non-metric Multidimensional Scaling ordination and tested for vectors using R vegan package version 2.5-7 and MASS. Sites were ordinated according to their landscape composition: tree cover, lawn cover, meadow cover, and total green cover percentages.

Sampling vegetation

I quantified ground cover and the blooming resources available to pollinators at the local scale at each site visit. To do this I ran a 50 meter transect perpendicular to the river, starting at each sampling point at each site. Every 5 meters along the transect I placed a one by one meter quadrat, and visually estimated the ground cover of categories of interest and blooming forbs in that area (Graham et al. 2021). This method totaled in 20 m^2 of ground cover data per half hectare on each sampling date. Categories of interest included: bare soil, flowering forb (total, in addition to the cover of individual blooming species), leaf litter, forbs not presently flowering including leaves, pavement, plants that are not forbs or trees, rock, shrub, trees shorter than chest height, standing dead grass, trash, woody debris, and trees taller than chest height including canopy cover. I identified all presently blooming forbs to species. I visually estimated ground cover according to a modified version of the Braun-Blanquet scale (Wikum and Shanholtzer 1978). Within one 1x1 m quadrat, any cover class of interest with 0% cover was assigned a 0, present but less than 1% cover was assigned a 1, between 1 and 5% cover was assigned a 2, between 5 and 25% was assigned a 3, between 25 and 50% was assigned a 4, between 50 and 75% was assigned a 5, between 75 and 95% was assigned a 6, between 95 and 98% was assigned a 7, and anything over 98% would have been assigned an 8.

The Floristic Quality Index (FQI) was calculated using each plant species Coefficient of Conservatism (CC), which indicates how sensitive the plant is to disturbance and other events or contaminants that result from anthropogenic change (Salter, 2018). I used values provided by University of Michigan's Herbarium, which can be found at https://michiganflora.net/home.aspx. Plants with a higher CC are generally more desirable in remnant and restored sites. Some native plants have a CC of zero and all non-native plants in our study area are not assigned a CC. Non-native plants were not included in FQI and were treated as plants with a CC of zero. I multiplied the CC value of each species by its assigned cover class category for each site visit, and then used these values to calculate a weighted average for each site visit.

Ground cover analysis at the local scale

To create an ordination for ground cover at the local scale I used the ground cover data collected from quadrats on each sampling date. I created a Non-metric Multidimensional Scaling ordination and tested for vectors using R vegan package version 2.5-7 and MASS. Sites were ordinated according to their ground cover composition: tree cover, lawn cover, meadow cover, and bare soil, flowering forb cover, leaf litter, forbs not presently flowering including leaves, pavement, plants that are not forbs or trees, rock, shrub, trees shorter than chest height, standing dead grass, trash, woody debris, and trees taller than chest height including canopy cover.

Sampling Bombus

Between May and October, 2021 I sampled each site for bees using two simultaneous methods. I used two methods, active netting and passive bowl traps, because each sampling method has biases and using multiple methods captures a more cohesive representation of the local bee community (Pei et al. 2021). To do this I walked around each half-hectare, for a half an hour catching any bee I saw within the site. I performed this wandering sample twice on each sampling date, once in the morning and again after noon. Additionally, I walked each 50m transect for 15 minutes per transect, catching any bee I saw within 1m of the transect. Caught bees were be transferred to a jar with soapy water to be killed, and then were later pinned. I only collected bees on days sunny enough to see my shadow when the temperature is above 60 degrees and the wind is below 6 miles per hour, and between the hours of 8am to 5pm (LeBuhn et al. 2003).

Between May and October, on the same days I sampled bees with nets, I set up bowl traps at each sampling point. To do this I placed 10 bowls 5m apart from each other along each transect. Each bowl was one of three colors: blue, yellow, or white, and the color for each spot on each sampling date was haphazardly selected (LeBuhn et al. 2003, Tonietto et al. 2011). In each bowl I poured in soapy water to fill the bowl one centimeter from the bottom. I set up bowls between 8-9 am, and collected them on the same day between 3-4pm. After collection, I strained the content of the bowls into a sieve, then stored the specimens in a whirl pack with site and date information until they were pinned.

Identification

After bees were collected, they were kept in a freezer until they were pinned. I identified specimens within the *Bombus* genus to species using Discover Life keys and comparing to previously identified voucher specimens from a similar region.

Testing for predictors of Bombus abundance, richness, and diversity

I used R and vegan package version 2.5-7 to analyze data. Initially the predictor variables I considered were: floral abundance, floral Shannon diversity, total green cover within 100m radius, total lawn cover within 100m radius, meadow cover within 100m radius, and tree cover within 100m radius. After checking for collinearity using Variance Inflation Factor (VIF) I found a correlation between floral abundance and floral Shannon diversity, and a correlation between total green cover and meadow, and a correlation between total green cover and tree cover. I selected to use floral abundance, total green cover within 100m radius, and lawn cover within 100m radius as the predictor variables for my maximal models. To test for the impacts of flowering forb cover, average forb CC, total green cover within a 100m radius, and total lawn cover within a 100m radius on total bee abundance, *Bombus* abundance, *Bombus* Shannon diversity, and *Bombus* richness. For each response variable, the maximal model included the interactive effects of listed predictors with interactive effects. I used backwards elimination to remove insignificant factors. For all tests except *Bombus* diversity, data were zero-inflated, so I used the Poisson function to account for data being not normally distributed for *Bombus* abundance and richness.

Results

Within a 100m radius of each site, total greenspace as a percent ranged from 31% to 85%, with a median value of 59%. Total greenspace comprised of tree cover, lawn, and fallow meadow cover. Tree cover had a range of 4% to 52%, with a median value of 16%. Lawn cover had a range of 4% to 51%, with a median value of 23%. Meadow cover had a range of 0% to 51%, with a median value of 3%.

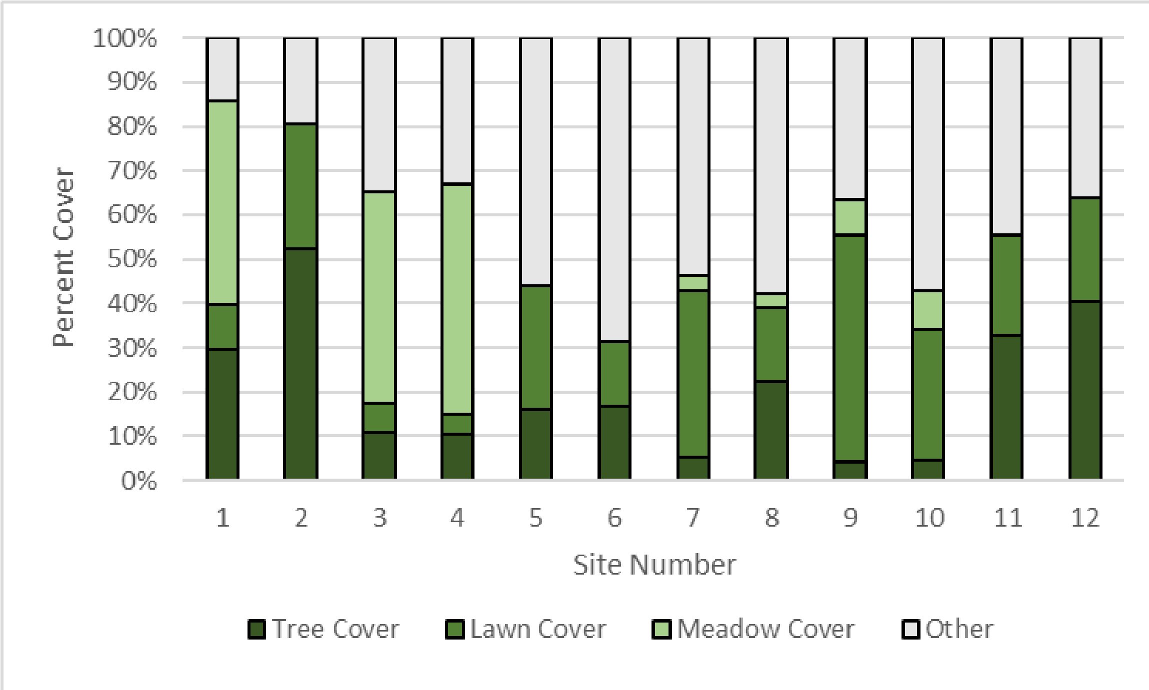

Figure 3: Percent land cover within 100m radius of different cover classes: tree cover, lawn cover, meadow cover, and other. The "other" category includes any cover that is not greenspace, such as the Flint River and impervious surfaces. Sites 1-4 and 11-12 are public parks and sites 5-10 are in part or totally managed by University of Michigan Flint. Data used in this figure was captured and analyzed according to the landscape analysis data described above.

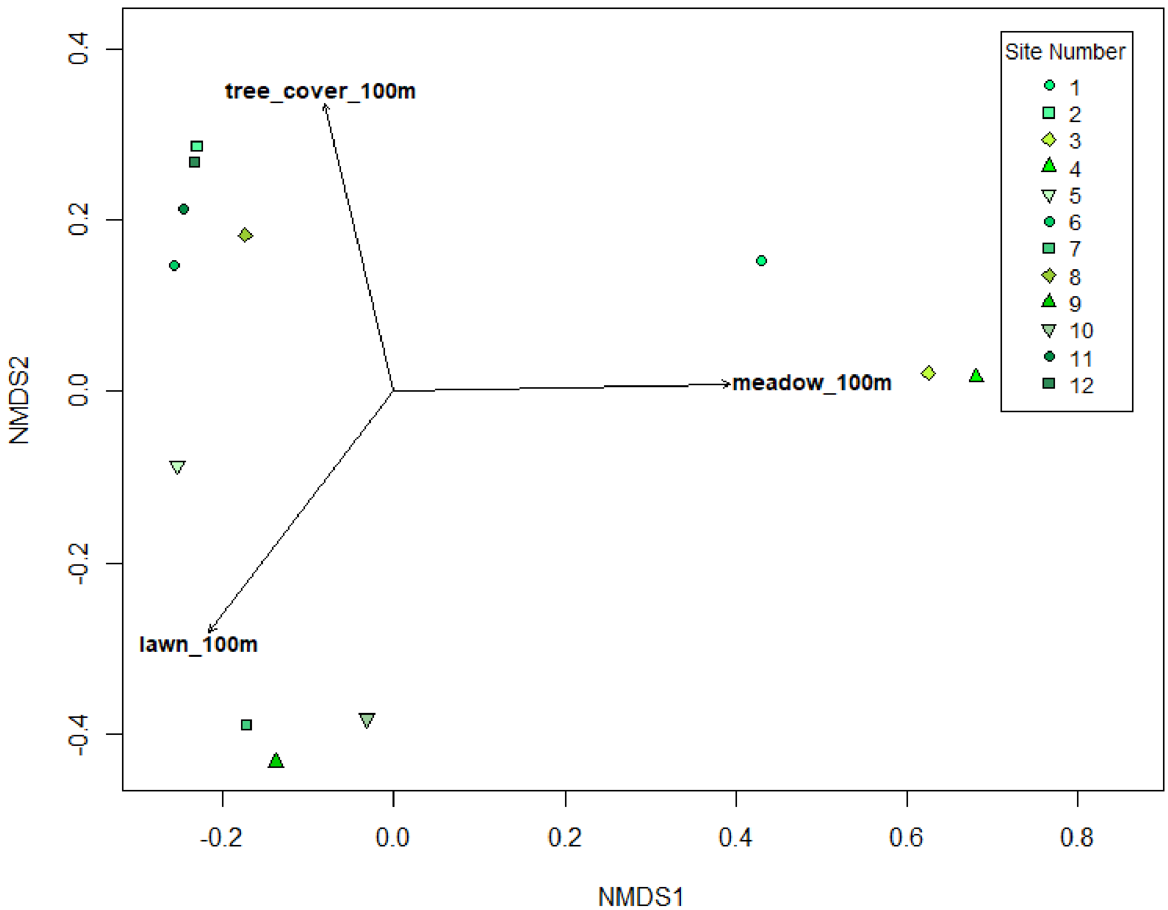

Figure 4: NMDS Ordination with vectors of sites based on land cover within 100m radius. Categories include tree cover, lawn, and meadow. As landscape data did not change throughout my sampling period, each point is representative of the site throughout the sampling period. Data used to create this ordination was captured and analyzed according to the landscape analysis data described above.

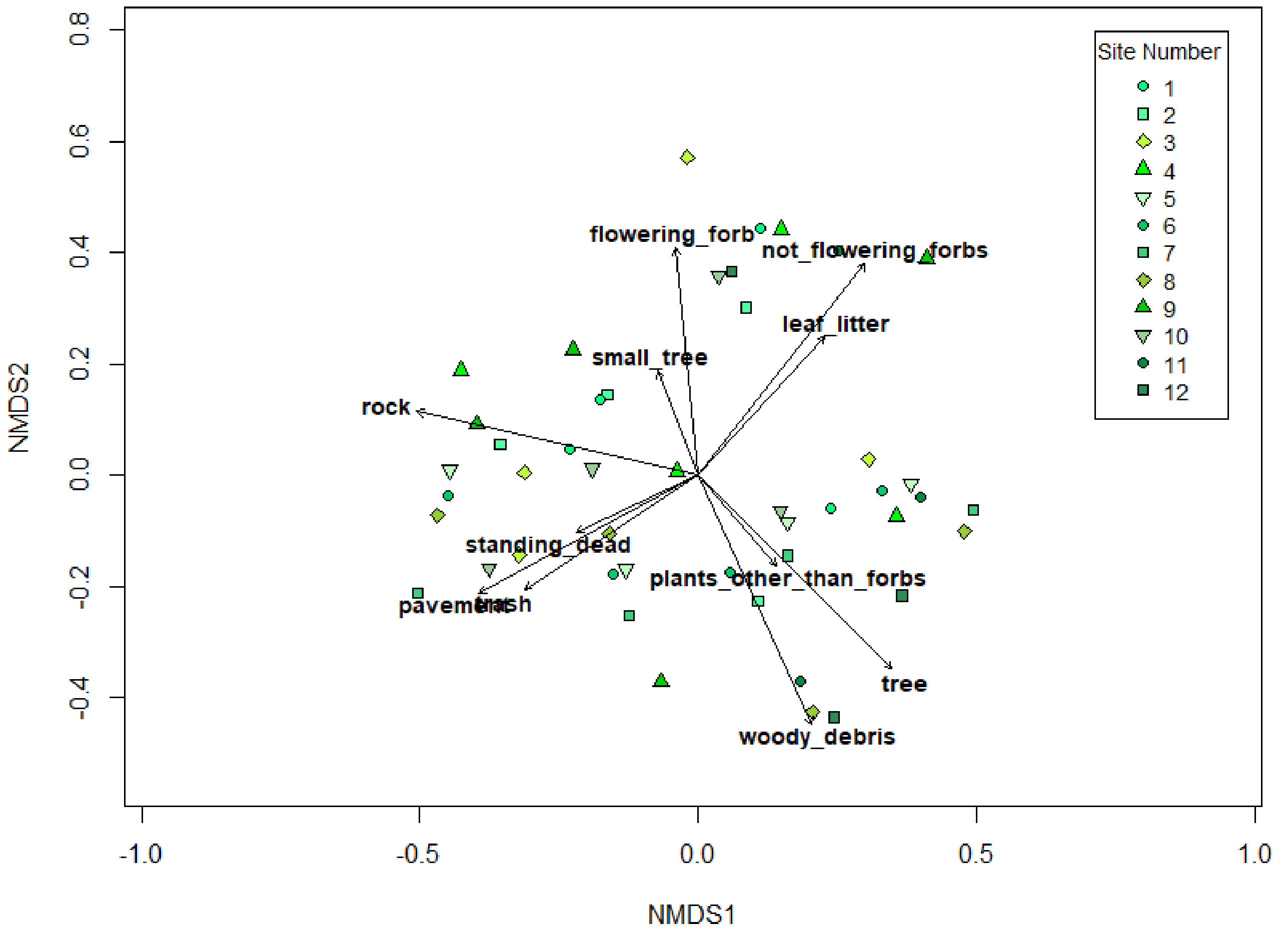

Figure 5: NMDS Ordination with vectors of sites based on land cover within quadrats at each site visit. Each point represents one site visit. Categories include flowering forbs, not presently flowering forbs, leaf litter, small tree, rock, standing dead grass, pavement, trash, plants that are not forbs, tree taller than chest height (including canopy cover) and woody debris.

I assessed floral cover of each site, and identified 96 unique species of flowering forbs. Of these, 26 species were native to southeastern Michigan and had a CC greater than 0. The number of times each species was recorded ranges from 1 time to 153 (*Medicago lupulina*) times, with a median value of 2 times. I used a modified version of the Braun-Blanquet scale to quantify floral abundance and found that sum of floral cover for each individual species throughout the sampling period ranged between 1 and 169 (*Medicago lupulina*), with a median value of 2. Plant species richness at each site ranged from 5 to 33 throughout the season. *Trifolium repens*, or white clover, was found at every site. Forb Shannon diversity was calculated for each individual site visit and ranged from 0 to 2.8 (Site #4, third visit). CC values for forb species found ranged from 0 or unassigned (species not assigned values are not native to Michigan, and were treated as zeros) to 6 (*Myosotis laxa*, which was recorded one time with a cover score of 1 (cover greater > 0 and <1 %)).

I collected a total of 268 *Bombus* specimens throughout the season from netting and bowl traps, and identified them all to species. In order of descending abundance, the species I collected were *Bombus impatiens* (147 individuals), *B. bimaculatus* (65 individuals), *B. fervidus* (30 individuals), *B. griseocollis* (23 individuals), and *B. perplexus* (2 individuals). 253 individuals were collected from active netting (46 from transect method, 207 from wandering method), while 15 were collected from bowl traps.

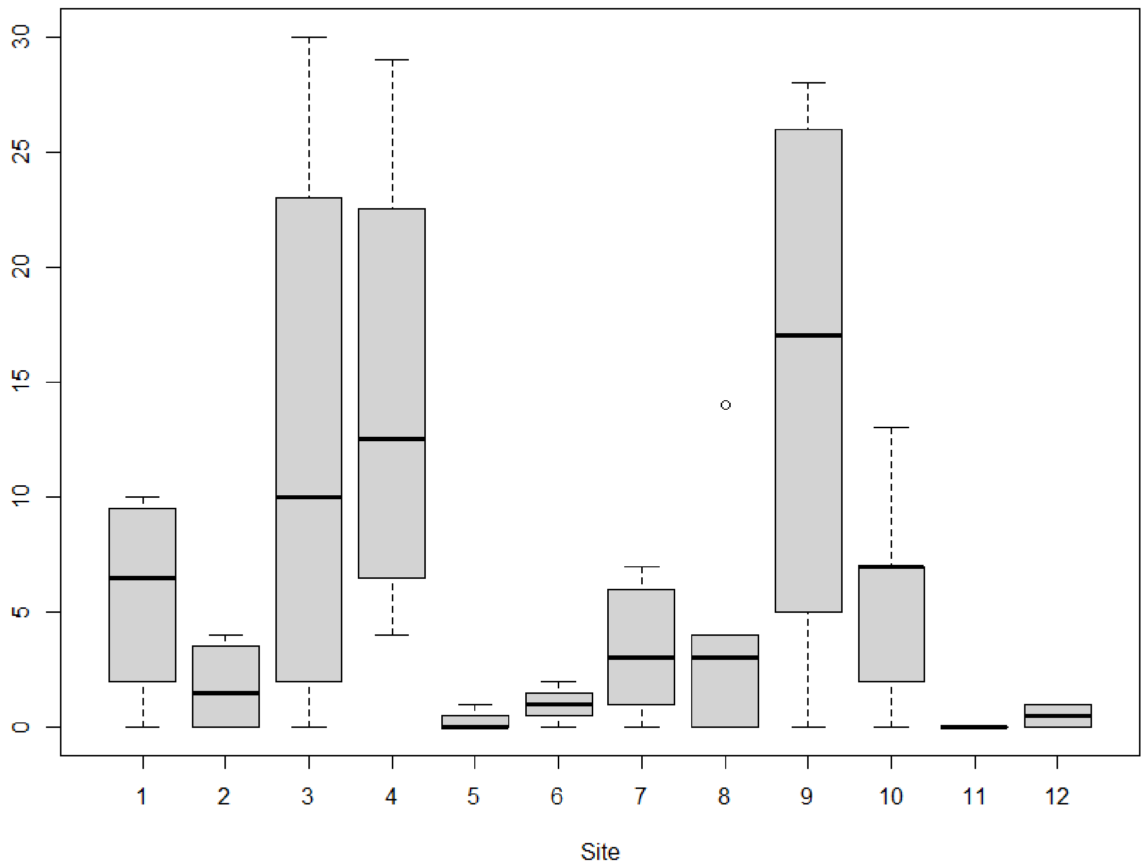

Figure 6: Box and whisker plot of *Bombus* abundance by each of the 12 sites. Bumblebees were collected according to bowl and netting methods described above, between May and October 2021. Note that this figure shows raw abundance and is not relativized by sampling effort.

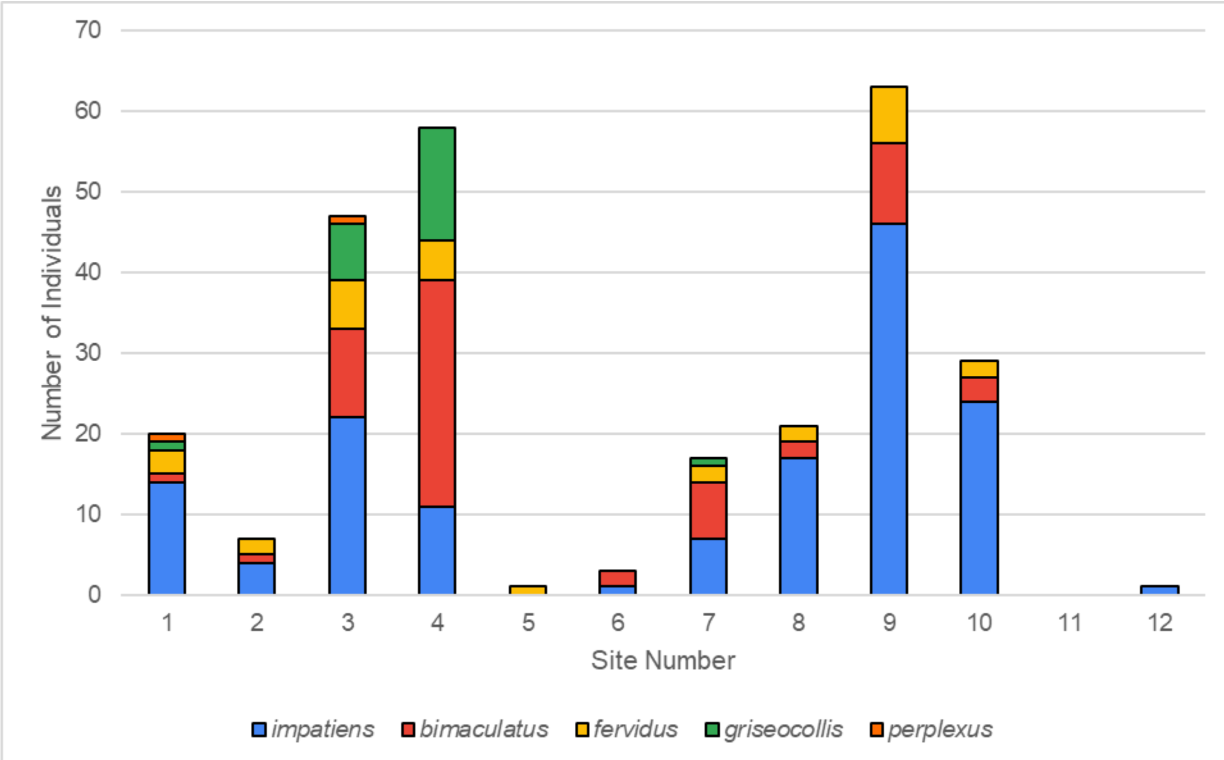

Figure 7: Bar graph of *Bombus* abundance by species at each site. Bumblebees were collected according to bowl and netting methods described above, between May and October 2021. Note that this figure shows raw abundance and is not relativized by sampling effort.

Bombus abundance

To determine significant predictors of *Bombus* abundance, I started with the maximal model and used backwards elimination to find that sum of flowering forb cover (p = 3.11*10^{-12}), total green cover within a 100m radius (p = 6.31*10^{-11}), and total lawn cover within a 100m radius (p = 1.77*10^{-7}) were all significant predictors of *Bombus* abundance with an interactive effect between independent variables.

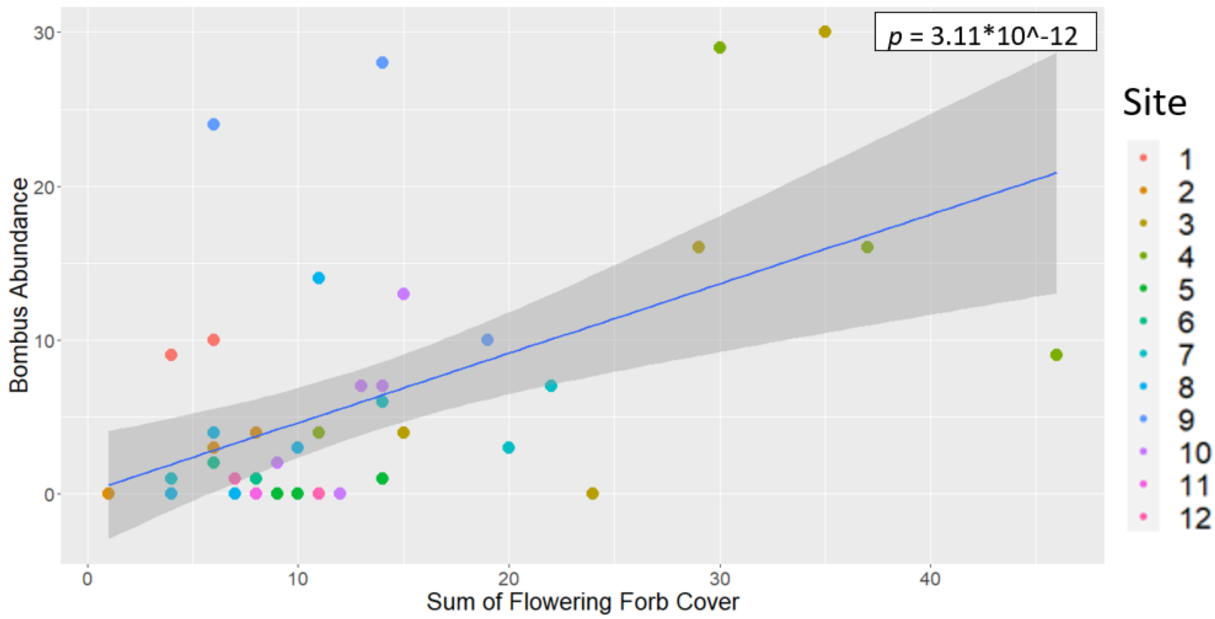

Figure 8: Bombus abundance as predicted by sum of flowering forb cover ($p = 3.11*10^{-12}$). Each point represents one site visit. *Bombus* abundance data was collected according to the netting and bowl trap methods described above. Sum of flowering forb cover was collected using the vegetation sampling methods described above. Correlation was found using a generalized linear model using a Poisson distribution.

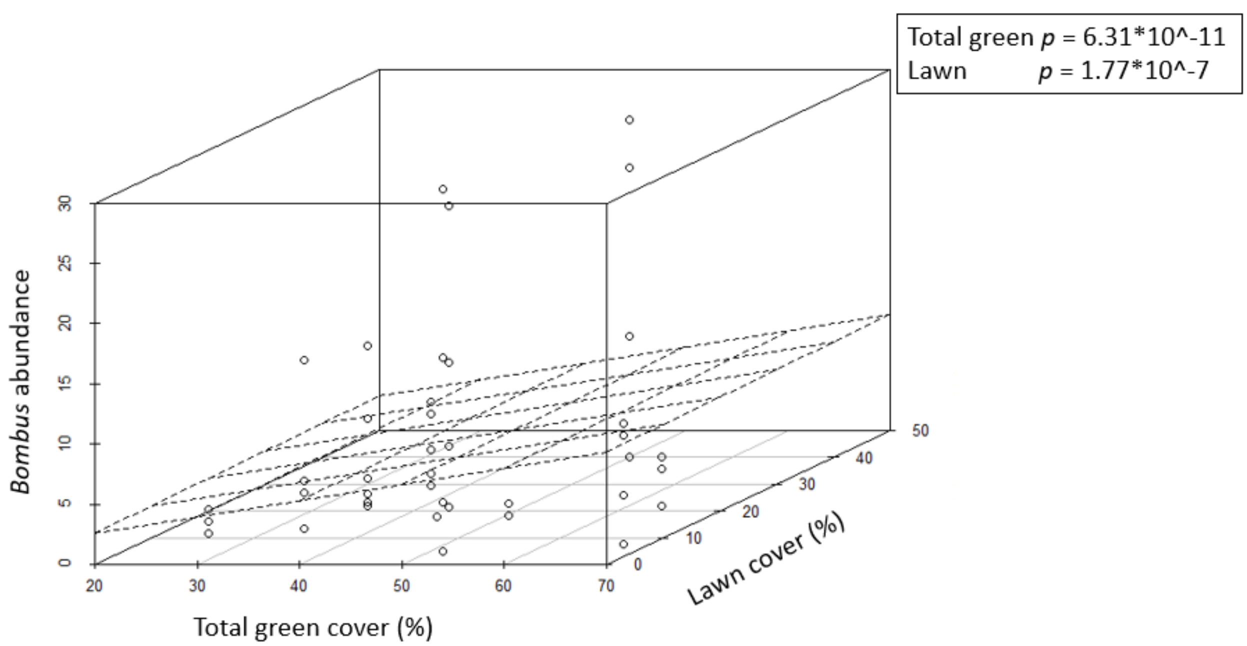

Figure 9: Bombus abundance as predicted by total green cover within 100m radius and lawn cover within 100m radius ($p = 6.31*10^{-11}$; $p = 1.77*10^{-7}$). Each point represents one site visit. *Bombus* abundance data was collected according to the netting and bowl trap methods described above. Total green cover and lawn cover were found using the landscape analysis methods described above. Correlation was found using a generalized linear model using a Poisson distribution.

Bombus richness

To determine significant predictors of *Bombus* richness, I started with the maximal model and used backwards elimination to find that of all tested predictors, only total green cover proportion of the surrounding landscape at 100 m was a significant predictor of *Bombus* richness (p = 0.0214).

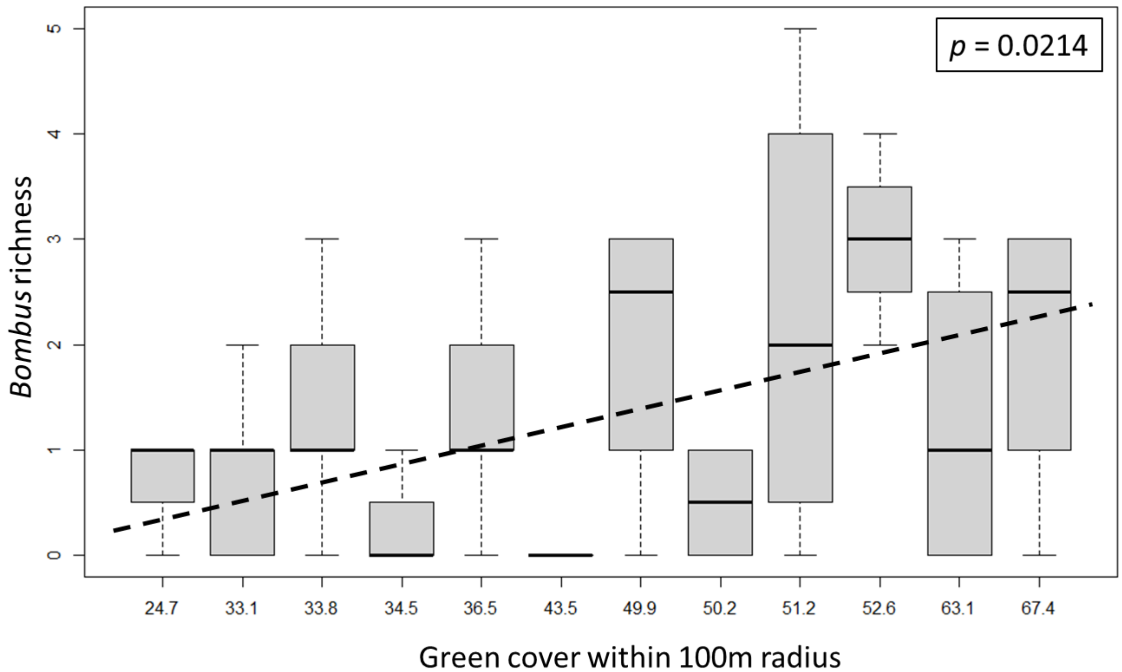

Figure 10: Bombus richness as predicted by total green cover within 100m radius (p = 0.0214). *Bombus* richness data was collected according to the netting and bowl trap methods described above. Specimens were identified according to Discover Life keys. Green cover data was found according to the landscape analysis methods described above. Correlation was found using a generalized linear model using a Poisson distribution.

Bombus diversity

To determine significant predictors of *Bombus* diversity, I started with the maximal model and used backwards elimination to find that weighted average of FQI was a significant predictor of *Bombus* diversity (p = 0.0158).

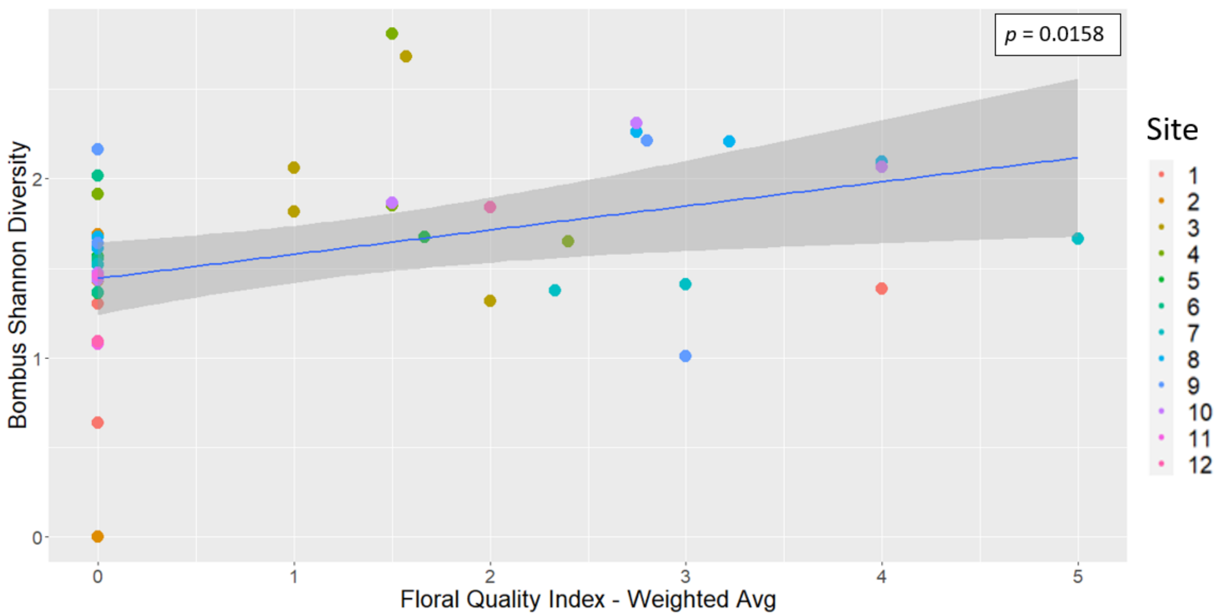

Figure 11: Bombus diversity predicted by a weighted average of Floral Quality Index (FQI) (p = 0.0158). *Bombus* specimens were collected according to the netting and bowl trap methods described above. Specimens were identified according to Discover Life keys. *Bombus* Shannon diversity was calculated using R vegan package version 2.5-7. Floral quality index was calculated using a weighted average of blooming flower CC and blooming flower quantity described in the vegetation analysis above.

Discussion

Many studies on bee conservation efforts target landscapes already intensely altered by humans, and we know that these altered landscapes can support diverse bee communities (Gathmann et al. , Albrecht et al. 2010, Batáry et al. 2010, Haaland et al. 2011, Williams et al. 2015). Restoration efforts have the potential to prevent further loss of insect pollinators, but urban greenspaces including roofs, grasslands, and even vacant lots have all also been shown to be important for urban pollinator communities' support and conservation (Tonietto et al. 2011, Fiedler et al. 2012, Hall et al. 2017, Sivakoff et al. 2018, Buchholz et al. 2020, Turo et al. 2021). Natural areas support higher species richness of arthropod taxa when compared to intensively managed areas (Albrecht et al. 2010), but even ornamental and crop plant communities have the potential to support bee abundance and species richness throughout the season whereas natural landscapes oftentimes have a scarcity of flowers in dry summer months (Frankie et al. 2009, Leong et al. 2016). I expected to find less than 10 species of *Bombus* at these study sites, and I found five. All five species are generalists, which was not surprising considering the quality of habitats at these sites and available forage.

Although *Bombus* have a flight range of up to several kilometers (Osborne et al. 1999), landscapes can be studied at the local scale using a radius as small as 100m (Sivakoff et al. 2018). Through this study, I found that *Bombus* abundance was predicted by flowering forb cover, total green cover within a 100m radius, and lawn cover within a 100m radius, but not by weighted FQI. This indicates that forb abundance as well as nearby greenspace are both important for *Bombus* abundance. These results were somewhat different than what I hypothesized—I initially hypothesized that total lawn within a 100m radius would be negatively correlated to *Bombus* abundance, but that was not the case here. I found that lawn within a 100m radius was positively correlated with *Bombus* abundance, and I think these results may differ from previous findings (Tonietto et al. 2011) because anecdotally, the lawn at these study sites was not as intensively managed as many other traditional lawns, like those used by Tonietto in 2011. Less intensive management, particularly by not using herbicides and by mowing infrequently allows weedy forbs to bloom, and thus provide forage for *Bombus*.

I found that *Bombus* richness was predicted by total green cover within 100m radius, but not by forb cover, weighted FQI, or lawn within 100m radius. I hypothesize that green cover may be the only significant predictor because it may reflect the availability of nesting habitat. Larger greenspaces within intensely urbanized areas may provide a larger number of suitable nesting habitats (Banaszak-Cibicka et al. 2016). One limitation of this study is that I did not identify or quantify suitable nesting habitats. Moving forward, similar studies should aim to identify and quantify suitable nesting sites in the area.

I found that *Bombus* Shannon diversity was predicted by the weighted average of FQI at each site visit, and not by sum of flowering forb cover, total green cover within 100m radius, or lawn cover within 100m radius. Because diversity is more reflective of community structure when compared to richness, I hypothesize that diversity and richness had different predictors because while more available nesting habitat (possibly reflected by total green cover) may support a broader range of species, a higher average of higher quality floral resources (reflected by weighted FQI) may support *Bombus* species that may be more selective when foraging. Additionally, plants with higher FQI reflect higher quality habitats, thus further supporting more sensitive *Bombus* species relative to more common *Bombus* species. FQI scores are related to a plant species' sensitivity to disturbance, with higher scores indicating higher sensitivity to disturbance. FQI scores from the plant community reflect conservation value of a given habitat in regards to its plant community (Bowers and Boutin 2008).

Although total green cover is important and we should strive to maintain or expand the persisting green space in urban areas, it is not the only important factor when considering how to support wild bees in urban areas, and particularly in urban riparian areas. Habitat connectivity and local floral resource abundance support bee community functional diversity and species traits (Buchholz et al. 2020). At this scale with sites mostly within *Bombus* flight range of each other, these habitats are fairly well connected in regards to *Bombus.* Generally, restoration has positive effects on bee communities (Harmon-Threatt and Chin 2016, Tonietto and Larkin 2018), but simple land management strategies such as not using herbicide, or mowing less frequently could also provide the floral resources necessary to support abundant *Bombus* communities, regardless of whether the upcoming lawn weeds are native or non-native (Turo et al. 2021). Analysis of the ground cover at the local scale in my study shows relationships between trees and woody debris, and pavement and trash. These results highlight implications of ground cover and how management strategies could improve habitats for native bees. For example, raking woody debris could provide more bare ground for possible nesting habitat or targeting trash cleanup could be particularly effective in areas with high proportions of pavement. These characteristics also varied throughout the season, so management strategies should be thoughtfully planned and executed for what is best appropriate at that time of the season. Including high-quality flowers in gardens or small patches of managed land could also help further support diverse *Bombus* communities, even when high-quality flowers are rare in the landscape when compared to lower-quality flowers. Within urban areas, particularly shrinking cities where vacant lots are prominent in the landscape, management could benefit wild bee and wasp communities (Sivakoff et al. 2018, Turo and Gardiner 2021).

Supplemental Information

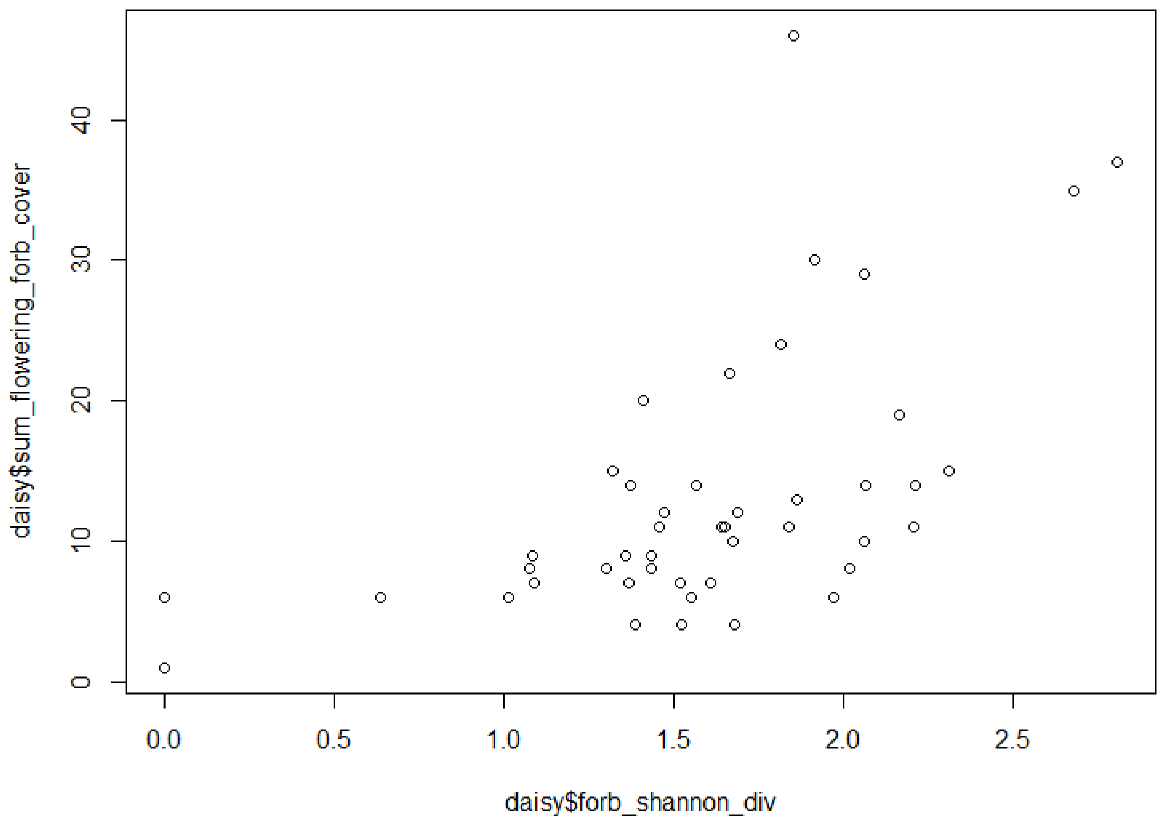

Figure 12: Sum of flowering forb cover is significantly correlated to forb Shannon diversity (p = 4.843*10^{-5}).

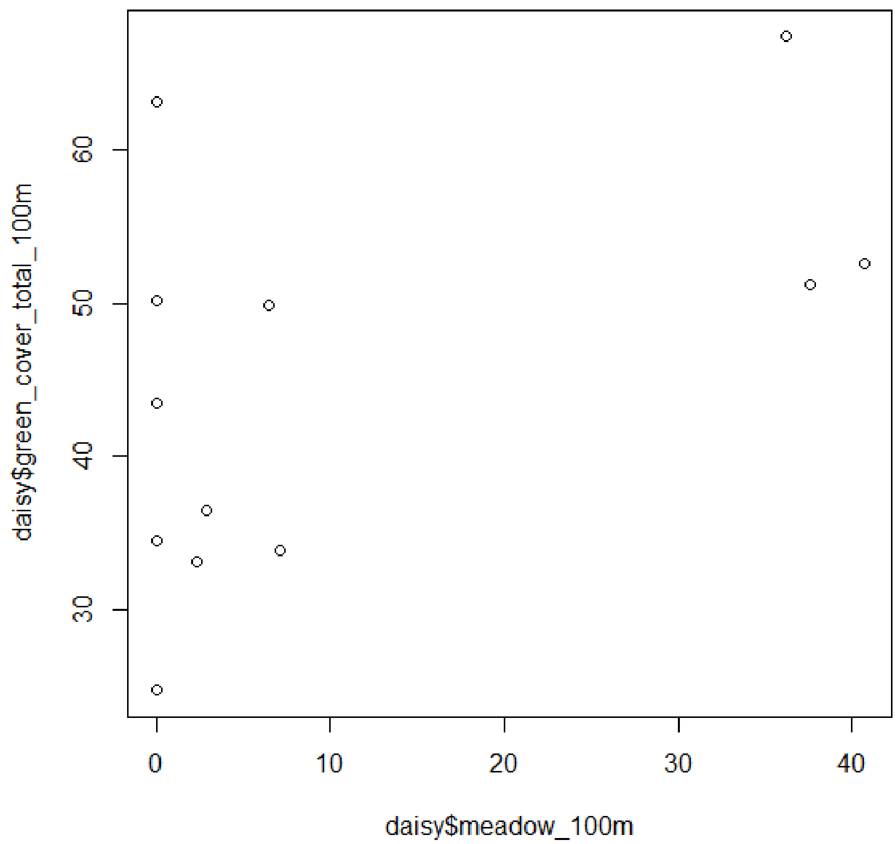

Figure 13: Total green cover within 100m radius is significantly correlated to meadow cover within 100m radius (p = $8.973*10^{-5}$).

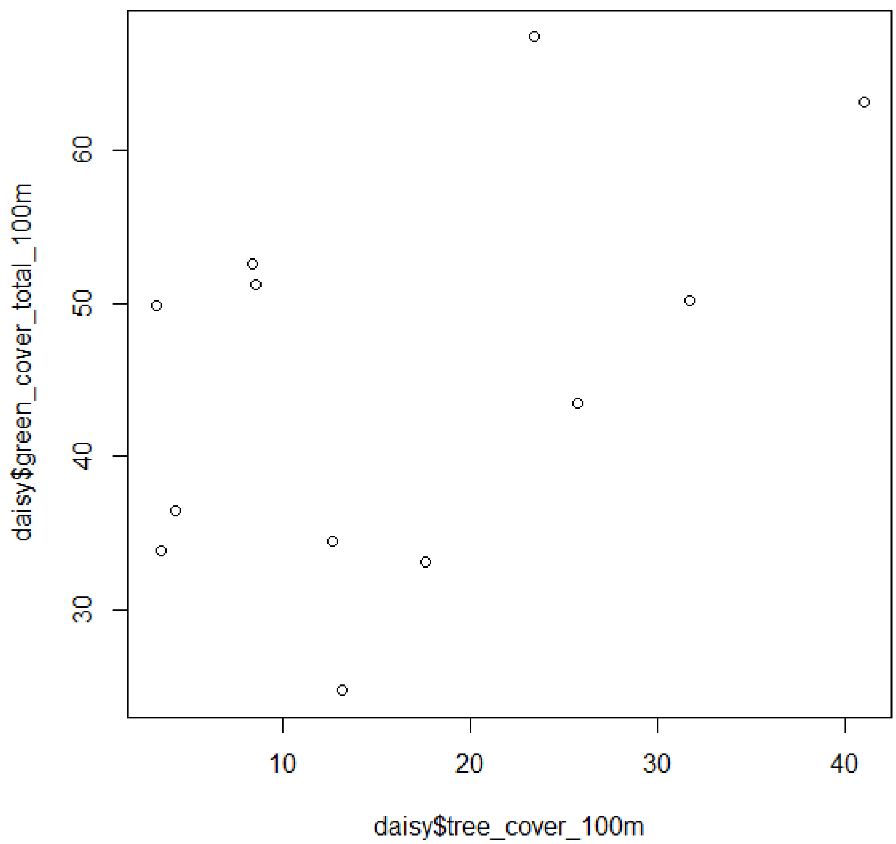

Figure 14: Total green cover within 100m radius is significantly correlated to tree cover within 100m radius (*p* = 0.0003612).

Chapter 2

Methods for assessing relationships between soil heavy metal concentrations and bee heavy metal concentrations and bee worker weight

Introduction

Heavy metal contaminants pose risk for animal health (Wang et al. 2018). Heavy metals such as Lead and Mercury may enter ecosystems through air pollution, water pollution (Evers et al. 2005) or through the food chain (Cristol et al. 2008). Heavy metals are prevalent contaminants in aquatic systems, and through food webs can transfer to terrestrial systems (Cristol et al. 2008). Once present, primary producers, such as aquatic or semi-aquatic plant species may uptake heavy metals, but the ability to uptake them varies between each species, and if the plants are consumed by an animal higher on a higher trophic level then contaminants may bioaccumulate within higher-level organisms (Evers et al. 2005, Bonanno et al. 2017). For example, heavy metals have been shown to bioaccumulate in long-lived animals and may negatively impact reproductive success in birds, mustelids, and humans (Grove and Henny 2008, Jackson et al. 2011, Henriques et al. 2019).

Both managed and wild pollinators have experienced drastic declines from their historic population sized and ranges (Grixti et al. 2009, Cameron et al. 2011, Szabo et al. 2012). Habitat loss and fragmentation certainly contribute to these declines, but do not fully explain these patterns (Potts et al. 2010, Szabo et al. 2012). Pesticide use and pathogens are likely other contributing factors, but heavy metals such as Lead have also been shown to have sub-lethal negative effects as well (Cameron et al. 2011, Sivakoff et al. 2020). Heavy metals such as Cadmium, Lead, and Zinc have also been linked to low wild bee species abundance and diversity in polluted environments (Moron et al. 2012). Pollution of heavy metals play a role in pollinator decline, but we need to further study bee communities in polluted areas to better understand the impacts of heavy metal contamination on local populations (Moron et al. 2012). The presence of heavy metals such as lead or mercury can enter the environment through industrial pollution, and it has already been shown that environmental contamination of lead negatively correlates to larval abundance in bumblebee colonies (Sivakoff et al. 2020). Many species of bee nest in the ground including many *Bombus* species, although bee nesting information is generally understudied (Harmon-Threatt 2020). Bee species nesting in the ground may be particularly impacted by these contaminants if they are present in the soil.

Pesticides including insecticides are commonly used in agricultural fields, and their use has oftentimes been indicated as a cause of both managed and wild bee decline (Arena and Sgolastra 2014, Fairbrother et al. 2014). Insecticides often persist in the environment years after their initial application which can continuously expose insect communities to the pesticide and pollute waterways (van der Sluijs et al. 2013). Similarly, heavy metals do not biodegrade and continue to persist in the environment (Mohammed et al. 2011). Lead in the environment negatively correlates to number of *Bombus impatiens* workers and larva within manufactured hives (Sivakoff et al. 2020).

Heavy metals persist in the environment and do not degrade, and are often a result of legacy effects of industrialization even decades after factories shut down. Heavy metals often persist in the soil, as factories often emitted heavy metals via air pollution. Although heavy metals persist in the environment, dams can act as contaminant sinks within rivers for both pesticides and heavy metals (Conrad et al. 2021). In polluted environments, soil contaminants are found in higher concentrations in dam sediments than in terrestrial soils (Conrad et al. 2021). Persisting contaminants may move through the environment via water runoff. These findings indicate that dams may act as contaminant sinks within rivers for persisting contaminants, thus preventing contamination from moving farther downstream (Conrad et al. 2021).

Flying insects including pollinators have been declining in regions all across the world (Potts et al. 2010, Hallmann et al. 2017). In addition to the issues facing non-native, managed honey bees in North America, native bees including several species of bumblebees have been observed to have declining populations as well due to habitat loss and other various reasons (Winfree et al. 2009, Szabo et al. 2012, Fairbrother et al. 2014, Havard et al. 2019). Although habitat loss and fragmentation are large contributors to declining populations of native bees, there are other factors contributing to these declines as well (Szabo et al. 2012). Other drivers may include pesticide use, pathogen spillover from commercial bumblebee colonies, or other still unidentified causes (Potts et al. 2010, Szabo et al. 2012, Kopit and Pitts-Singer 2018). One study found that in the *Bombus* subgenus Latreille (*Bombus affinis, Bombus terricola, Bombus pensylvanicus*) herbicide density, insecticide density, and change in human population density (correlated to vegetable greenhouse density which was used as a proxy for commercial bumblebee density) did not explain the decline of *Bombus affinis*, the first species of bumblebee to be declared as federally endangered in the continental United States (Szabo et al. 2012). Another possible environmental contaminant impacting *Bombus* populations could be heavy metals (Sivakoff et al. 2020). Post-industrial shrinking cities often have swaths of land no longer being used for industry, but often have persisting contaminants in the environment such as Lead (Pb).

Presence of heavy metals and other contaminants also impact invertebrates, such as benthic communities (Winner et al. 1980). Some aquatic invertebrates cannot survive in polluted streams, while others continue to persist (Winner et al. 1980, Rosenberg et al. 1986). Aquatic insect communities indicate the severity of pollution in streams, and can be indicators of stream health (Rosenberg et al. 1986). Heavy metals may also be present in and bioaccumulate in predatory terrestrial invertebrates such as spiders, although in some instances it is unclear if this is due to consumption of emergent aquatic insects or deposition in the floodplain (Cristol et al. 2008). Heavy metal pollution also negatively impacts terrestrial invertebrates, such as solitary wild bee abundance and diversity (Moron et al. 2012).

Most species of wild bees nest in the ground (Harmon-Threatt 2020), which may be another point of exposure to contamination for these species. Because pesticides and heavy metals are known to persist particularly in soil, nesting in the soil may provide an additional avenue of contaminant exposure for ground nesting bees when compared to managed colonies or species of bee that do not nest in the ground. The presence of heavy metals in the environment negatively correlates to larval abundance in manufactured bumblebee colonies, however, this study used commercially available bumblebee colonies that were nesting in manufactured hives, not wild bees nesting in the ground (Sivakoff et al. 2020). There are currently no studies on the impacts of soil Lead concentrations and wild ground-nesting bees such as *Bombus impatiens.* I hypothesize there may be similar or larger consequences for wild bumble bees nesting in the ground.

Ground-nesting species such as *Bombus impatiens*, the Common Eastern Bumble Bee, rely on their habitats for suitable ground nesting sites. Queens select a suitable cavity in the ground such as cavities dug by mice, and lay their eggs to start their colony. However, managed honey bees such as *Apis mellifera* live in manufactured, often wooden, hives above ground. Because they do not have the additional avenue of exposure of coming into contact with the soil, I hypothesize that soil Lead concentration would have no correlation to *Apis mellifera* worker weight or Lead concentration. However, because *Bombus impatiens* would be regularly coming into contact with soil contamination, I hypothesize that *Bombus* worker weight would be negatively correlated to soil Lead concentration because we know that Lead in the environment has negative, sub-lethal effects on *Bombus* (Sivakoff et al. 2020). Additionally, I hypothesize that *Bombus impatiens* Lead concentration would be positively correlated to soil Lead concentration.

Although we know that many species of wild bees nest in the ground including species in the *Bombus* genus (Harmon-Threatt 2020), little is known about how heavy metals such as Lead may be coming into contact with individuals and affecting them and how soil texture may impact suitability of potential nesting habitats. To study the relationships between soil heavy metal contamination and bees, I asked the following questions. (1) Is soil Lead concentration a predictor of *Bombus impatiens* worker weight? I hypothesize that there will be a significant negative correlation between Lead concentration and *Bombus impatiens* worker weight. *Bombus impatiens* often nest in cavities in the ground, and if there is soil Lead contamination then individuals may be exposed in their nest. In commercial colonies (nesting in boxes, rather than wild colonies nesting in the ground), Lead in the environment was negatively correlated with the number of workers and larva in the hive, so we know that there are sub-lethal negative affects of Lead in the environment on *Bombus impatiens* (Sivakoff et al. 2020). (2) Is soil Lead concentration a predictor of *Apis mellifera* worker weight? I hypothesize that there will be no relation—*Apis mellifera* do not nest in the ground, so that would not be a contact point of exposure to Lead for these individuals. (3) Does soil Lead concentration predict *Bombus impatiens* worker Lead concentration? I hypothesize that there will be a significant positive correlation, because *Bombus impatiens* oftentimes nest in the ground. (4) Does soil Lead concentration predict *Apis mellifera* worker Lead concentration? I hypothesize there will be no correlation because *Apis mellifera* do not nest in the ground.

Methods

Site selection

The Flint River in Michigan, USA, is 142 miles (228.5 km) long and its basin runs throughout Oakland, Lapeer, Tuscola, Sanilac, Genesee, Shiawassee, and Saginaw counties (Leonardi et al. 2001). Within the city of Flint, in southeastern MI, the Flint River runs for a stretch of about 14 km. Historically, Flint was home to several industrial factories that utilized Lead and other contaminants in its processing (Rosner 2016). These factories were often right on the waterfront, and their operations lead to pollution of the river, soil, and air (Rosner 2016). In 2014 the City of Flint switched its drinking water source to the Flint River, and due to lack of implementing corrosion control the water corroded historic lead pipes, leading to the Flint water crisis that is still ongoing (Pieper et al. 2017). Although the Flint River itself was not the source of lead causing the Flint water crisis, due to the legacies of industrialization along the river it is known to contain contaminants such as Lead (Leonardi et al. 2001, Pieper et al. 2017).

I identified ten sites, each a half-hectare of publicly accessible urban riparian greenspace along the Flint River, all with public access and varying management styles and land use histories. I selected sites with a variety of vegetative structure and management practices to get a diverse sample of urban riparian areas, to capture the variety across the river. Two sites were never developed and are partially remnant riparian forest, two sites used to be fully developed as an industrial site, but post-factory demolishment has been restored. The two restored sites have a cement cap over where the factory used to be, covered with 2-10 feet of replacement soil, then were seeded with native plants and are now mostly managed like a meadow. The remaining six sites are a mixture of turfgrass and impervious surfaces, and no longer have remnant forest. All sites have some degree of lawn management such as mowing. Management strategies across sites include active restoration, turfgrass management, unmanaged forest, fallow meadow, or a combination of these strategies. Each site is a half-hectare in area, and the dimensions of the sites are 100m parallel to the river, and 50m perpendicular to the river. I intentionally selected sites within *Bombus* and *Apis* flight range so that sites would not act as isolated patches (Eckert 1933, Osborne et al. 1999).

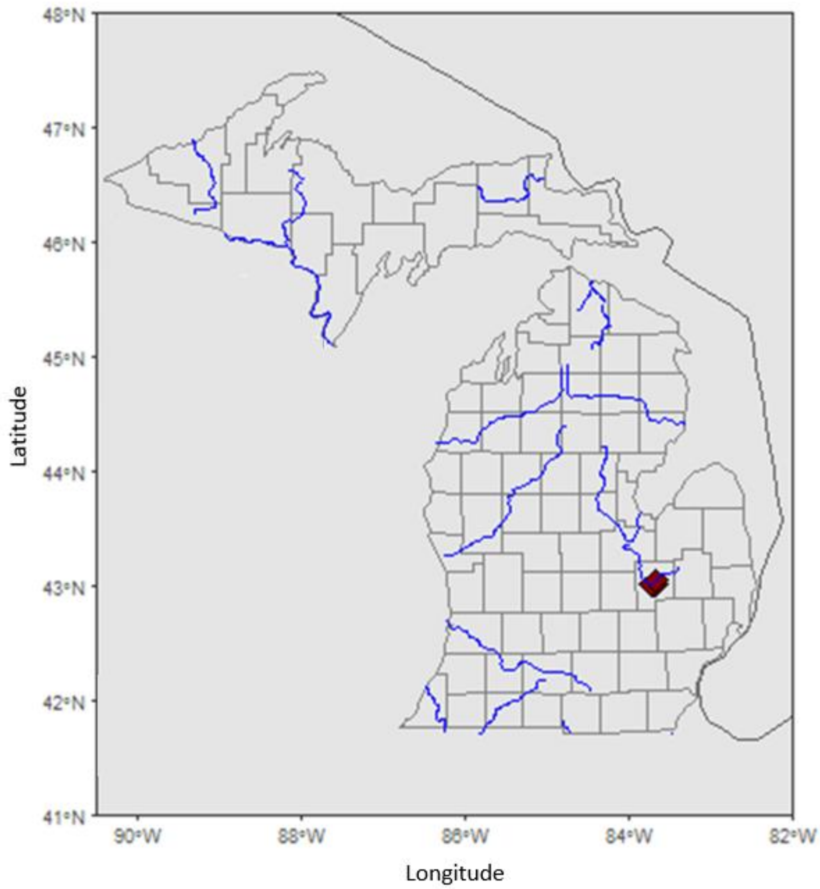

Figure 15: Map of Michigan, USA indicating sites within Genesee County along the Flint River.

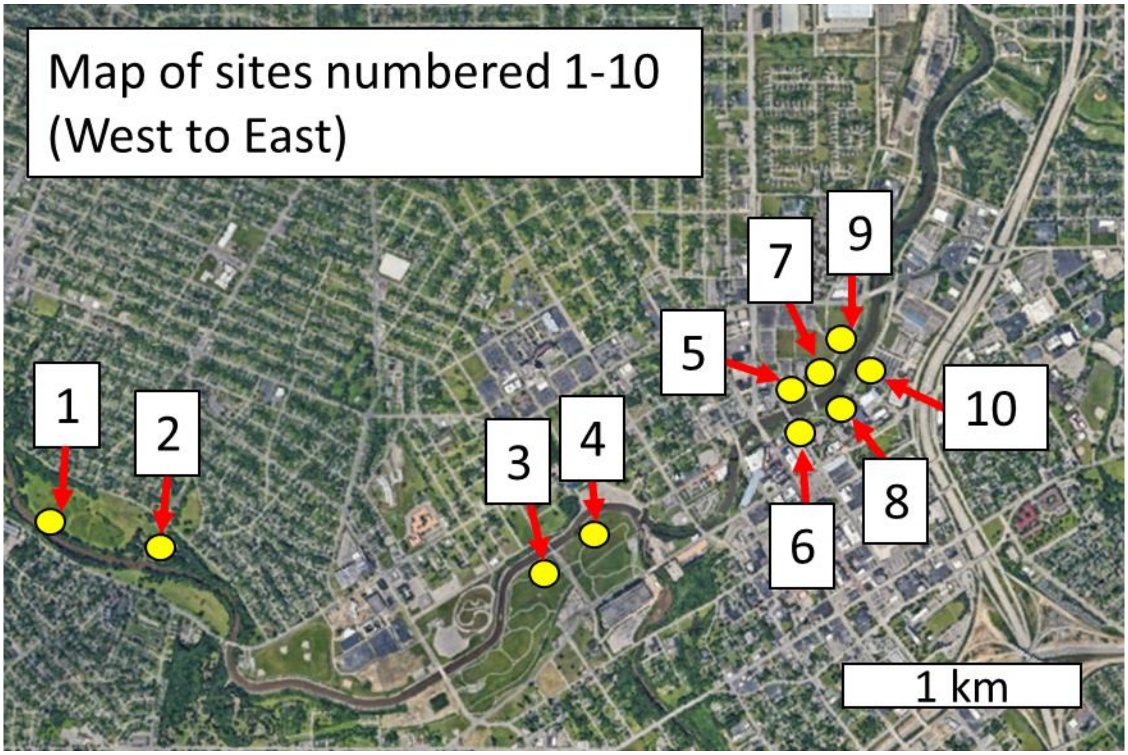

Figure 16: Map of study sites along the Flint River within Genesee County.

Sampling Bombus and Apis

Between spring and fall (May through October) 2021 I sampled each site for bees using two simultaneous methods. Although I wanted to focus on *Bombus* for this study, I still used two simultaneous to capture a cohesive representation of the local bee community (Pei et al. 2021). I used both active netting, as well as passive bowl traps. To perform active netting of bees, I walked around each half-hectare, for a half an hour catching any bee I saw within the site. I performed this wandering sample twice on each sampling date, once in the morning and again after noon. I also walked each 50m transect for 15 minutes per transect, catching any bee I saw within 1m of the transect. Caught bees were be transferred to a jar with soapy water to be killed, and then were later pinned. I only collected bees on days sunny enough to see my shadow when the temperature is above 60 degrees and the wind is below 6 miles per hour, and between the hours of 8am to 5pm.

Between May and October, on the same days I sampled bees with nets, I set up bowl traps at each sampling point. To do this I placed 10 bowls 5m apart from each other along each transect. Each bowl was one of three colors: blue, yellow, or white, and the color for each spot on each sampling date was haphazardly selected. In each bowl I poured in soapy water to fill the bowl one centimeter from the bottom (Tonietto et al. 2011). I set up bowls between 8-9 am, and collected them on the same day between 3-4pm. After collection, I strained the content of the bowls into a sieve, then stored the specimens in a whirl pack with site and date information until they were pinned.

Identification

After bees were collected, they were kept in a freezer until they were pinned. Bees were shaken in a jar of soapy water, and were then rinsed in clean running water. Then bees were dried and pinned. I identified *Apis mellifera* specimens and specimens in the *Bombus* genus to species using Discover Life keys.

Apis mellifera and *Bombus impatiens* workers were analyzed for heavy metal content.

Inductively coupled plasma–mass spectrometry (ICP-MS) was performed at the Michigan State University Veterinary Diagnostic Laboratory (Michigan, USA) (Buchweitz, personal communication, 2022). I used approximately 10x the dry tissue mass of nitric acid and a 95 °C oven to digest whole bees. I then diluted the digested samples with water to 100x the dried tissue mass.

Elemental analyses were conducted using an Aligent 7900 ICP-MS (Agilent, Santa Clara, CA) following the methods of Wahlen et al. (2005). An aliquot of each diluted tissue digest and calibration standard was diluted 20-fold with a solution containing 0.5 % EDTA and Triton X-100, 1 % ammonium hydroxide, 2 % butanol, with 5 ppb of scandium and 7.5 ppb germanium, rhodium, indium, and bismuth as internal standards (Buchweitz, personal communication, 2022). The ICP-MS was tuned to yield a minimum of 7,500 cps sensitivity for 1 ppb yttrium (mass 89), less than 1.0 % oxide level as determined by the 156/140 mass ratio and less than 2.0 % double charged ions as determined by the 70/140 mass ratio (Buchweitz, personal communication, 2022). Elemental concentrations were calibrated using a 6-point linear curve of the analyte-internal standard response ratio (Buchweitz, personal communication, 2022). Standards were obtained from Inorganic Ventures (Christianburg, VA) and standard reference materials (SRM) included National Institute of Standards and Technology Bovine Liver (SRM 1577c) and Mussel (SRM 2976) were used as controls (Buchweitz, personal communication, 2022).

Heavy metal contamination in soils

I collected two cups (480 mL) of soil from each site. To do this I used a soil corer to collect one cup of soil from two haphazardly selected points within the site, totaling at least 2 cups of soil for each site. Soil collection points were selected haphazardly, but from points that seemed representative of the site—not on the edge of the site, or next to walkways. I mixed the two cups of soil in a bag and labelled it with site information, repeating this process for each site. In the field, I analyzed soil texture be feel according to the USDA (USDA-NRCS 1999). Once soil was brought back to the lab, I also analyzed soil texture according to the jar method.

Soil samples were analyzed for heavy metal content at the Kansas State University Soil Testing Laboratory. Soil samples were ground using a Dynacrush grinder with flailers and a 2mm sieve. DTPA extraction for Fe, Zn, Cu, and Mn uses the method described on pp. 41-42 (Dahnke 1975) in "Recommended Chemical Soil Test Procedures for the North Central Region". Analysis was performed using an Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer, manufactured by Varian Austrailia Pty Ltd, Mulgrave, Vic Australia or a Model 3110 Flame Atomic Absorption (AA) Spectrometer from Perkin Elmer Corp., Norwalk, CT (Hargrave, personal communication, 2022). Nitric Digest for Heavy Metals (Fe, Zn, Cu, Mn, Cd, Cr, Ni, Pb). Adapted from Sposito, et.al., 1982. Soil Sci. Soc. Am. J. 46, 260-264. ". Analysis is done by an Inductively Coupled Plasma (ICP) Spectrometer, Model 720-ES ICP Optical Emission Spectrometer, manufactured by Varian Austrailia Pty Ltd, Mulgrave, Vic Australia or a Model 3110 Flame Atomic Absorption (AA) Spectrometer from Perkin Elmer Corp., Norwalk, CT (Hargrave, personal communication, 2022).

Results

All sites had a sandy loam soil texture according to both soil texture methods used. Soil Lead concentration ranged between 13.5 – 76.5 ppm, with a median value of 24.2 ppm. We tested other heavy metals, but Lead was correlated with every heavy metal that was present in detectable levels except Iron and Copper.

I collected 97 *Bombus impatiens* workers and 230 *Apis mellifera* workers. The range of number of *Bombus impatiens* workers caught from each site throughout the season is 0 – 23 individuals. The range of number of *Apis mellifera* workers caught from each site throughout the season is 1 – 62. 80 *Bombus impatiens* workers were caught while performing wandering active netting, 13 were caught while performing transect active netting, and 5 were captured using passive bowl trapping. 176 *Apis mellifera* workers were caught while performing wandering active netting, 50 were caught while performing transect active netting, and 4 were caught from passive bowl traps.

Lead concentrations in *Bombus impatiens* workers was found at a range between 0.01 – 9.3 ppm, with a median value of 0.3 ppm. Lead concentrations in *Apis mellifera* workers was found at a range between 0.03 – 11.9 ppm, with a median value of 0.4 ppm. Iron and copper were not related to *Bombus* worker weight, *Bombus* Iron or Copper levels, *Apis* worker weight, or *Apis* Iron or Copper levels, respectively.

Soil Lead concentration and Bombus heavy metal concentration

I found no correlation between soil Lead concentration and *Bombus impatiens* worker heavy metal concentration (p = 0.7981), however, the individual worker with the highest Lead concentration was collected from the site with the highest Lead concentration. This opposes my initial hypothesis that soil Lead concentration would be a predictor of and positively correlated with *Bombus impatiens* worker Lead concentration because they often nest in the ground.

Soil Lead concentration and Bombus worker weight

I found that soil Lead concentration was a significant predictor of *Bombus impatiens* worker weight, and soil Lead concentration was negatively correlated with *Bombus impatiens* worker weight (p = 0.008165). This confirms my initial hypothesis that soil Lead concentration would be a predictor of and be negatively correlated with *Bombus impatiens* worker weight.

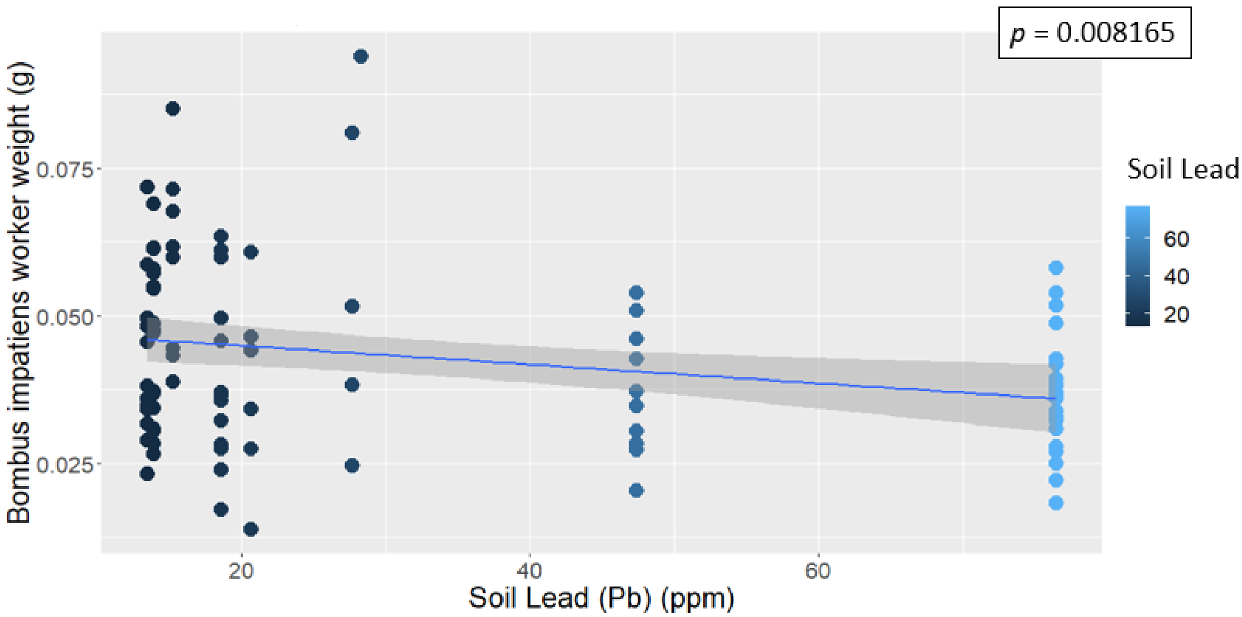

Figure 17: Bombus impatiens worker weight is predicted by soil Lead concentration. There is a negative linear relationship between *Bombus impatiens* worker weight and soil Lead concentration (p = 0.008165). *Bombus* specimens were collected according to netting and bowl trap methods described above between May and October 2021. Soil samples were collected according to the soil sampling methods described above. Soil Lead concentration was found according to the methods described above at Kansas State University's soil testing laboratory.

Soil Lead concentration and Apis worker concentration

I found that soil Lead concentration was a significant predictor of *Apis* worker Lead concentration, and there was a positive correlation (p = 0.00259). This opposes my initial hypothesis that there would be no relationship between soil Lead concentration and *Apis mellifera* worker weight.

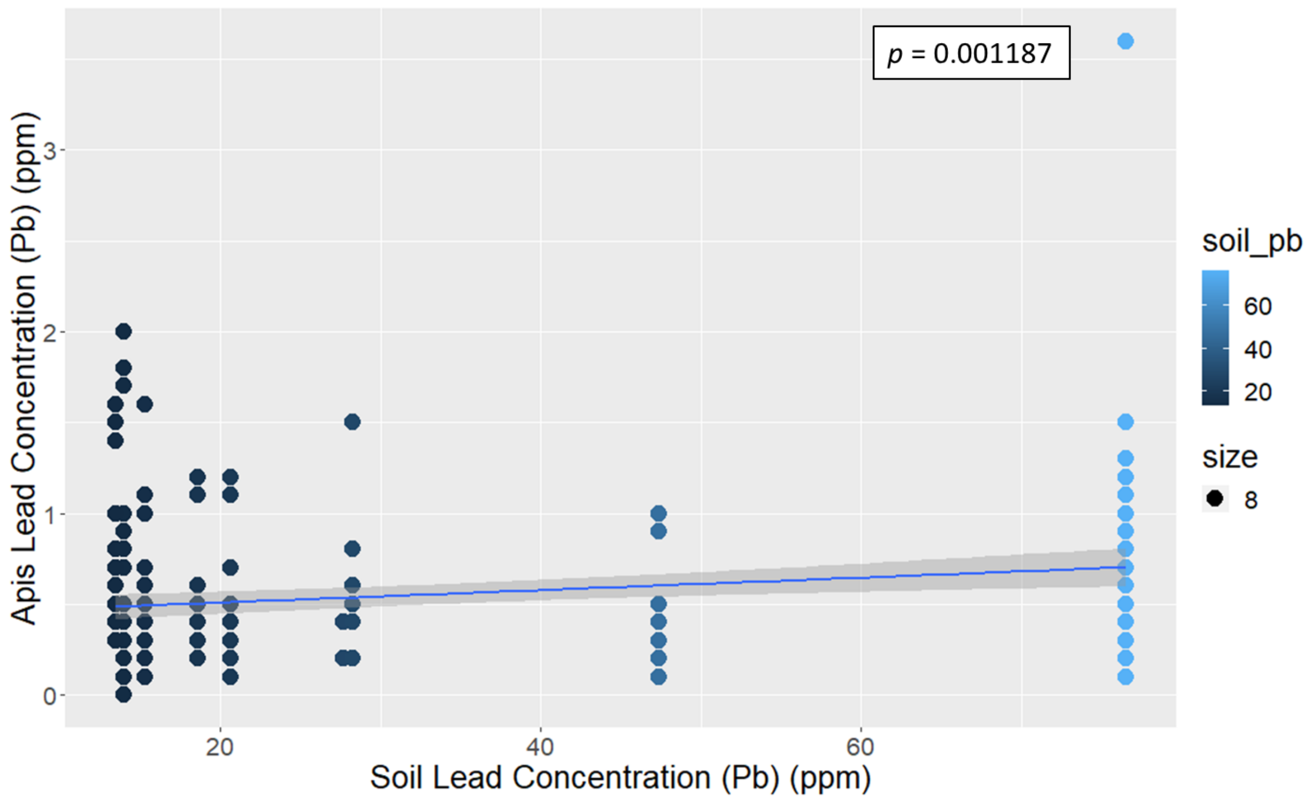

Figure 18: Apis mellifera Lead concentration is predicted by soil Lead concentration. There is a positive linear relationship between *Apis mellifera* Lead concentration and soil Lead concentration (p = 0.00259). *Apis* specimens were collected using the netting and bowl trapping methods described. *Apis* Lead concentration and soil Lead concentration were found using the heavy metal analyses described above. Soil samples were collected according to the methods described above.

Soil Lead concentration and Apis worker weight

I found that there was no correlation between soil Lead concentration and *Apis mellifera* worker weight (p = 0.496). This supports my hypothesis that there would be no relation between soil Lead concentration and *Apis mellifera* worker weight.

Discussion

I found that all the sites used in this study had a sandy loam soil texture, which is suitable for many species of ground nesting wild bees (Harmon-Threatt 2020). Soil texture is important for ground nesting bees because species that dig their own nests may not be able to in compacted or slag soils, and other ground dwelling species nest in cavities created by rodents (Harmon-Threatt 2020). Sandy loam is suitable for digging by bees and rodents.

Because most plants do not uptake heavy metals in any meaningful amount, and especially would not move heavy metals within the plant to any pollen, heavy metals of plants were not considered. Bees would not be coming into contact with heavy metals via flowers or pollen. Soil contaminants in study sites were also at suitable levels for bees nesting in the soil. The Lead values found in the soil at the study sites are all low and within EPA designated safe levels for direct contact in non-residential areas, especially for being in post-industrial locations (Sharma et al. 2014). Specifically, the Direct Contact

Criteria (DCC) for non-residential area soil Lead values is 330 ppm (https://www.michigan.gov/documents/deq/deq-rrd-chem-CleanupCriteriaTSD_527410_7.pdf). Because our soil Lead values are incredibly lower than the DCC value of 330 ppm, this indicates that the soils found in these riparian areas in Flint are safe for non-residential purposes such as recreational activities. However, all soil Lead concentrations were higher than the average soil Lead concentration for Genesee Co., MI as reported by the USGS (https://mrdata.usgs.gov/geochem/county.php?place=f26049&el=Pb&rf=upper-midwestern), thus highlighting a need for more accurate and updated soil contaminant information.

I hypothesized that soil Lead concentration would be a predictor of and positively correlated to *Bombus* Lead concentration, but I found no correlation. From a human health perspective, the biggest takeaway from this is to wash your hands after coming into contact with heavy metal contamination. Bees were not intaking contaminants from soil, and after being washed there were no correlations between *Bombus* heavy metal contamination and soil contamination.

I also hypothesized that soil Lead concentration would be a predictor of and negatively correlated to *Bombus* worker weight. I hypothesized this because Lead in the environment is known to have sub-lethal effects on *Bombus impatiens* (Sivakoff et al. 2020), and I found that soil Lead concentration was a predictor of *Bombus* worker weight, and there was a negative correlation between soil Lead and worker weight. These findings support my hypothesis, however, because there was no correlation between soil Lead and *Bombus* Lead concentrations, this may be an artifact of something else, possibly another heavy metal not tested for or another type of contaminant. Because lead concentration in bees was found as a concentration (ppm), we know that it is relativized by weight so it would not be a result of differences in weight. We know lead in the environment has sub-lethal negative effects on *Bombus* abundance and richness at the site level (Moron et al. 2012), and heavy metals in the environment negatively correlates to the number of workers and larva in commercial *Bombus impatiens* colonies in another shrinking city. However, individual *Bombus* worker weight had not previously been explicitly studied in regards to lead exposure or concentration. Perhaps if study sites would have had a broader range of Lead contamination there would have been more profound effects of higher lead concentrations.

I hypothesized that there would be no relationship between soil Lead concentration and *Apis* Lead concentration, as *Apis mellifera* do not nest in the ground. Contrary to my hypothesis, I found that there was a significant positive correlation between soil Lead and *Apis* Lead concentration. Because *Apis mellifera* do not nest in the ground, there must be another point of contact for Lead contamination in *Apis mellifera*. Moving forward I would advise the analysis of other possible sources of contamination: perhaps Lead is still a contaminant in the air, and dust is on flower heads preferentially foraged on by *Apis*? If this is the case, the numerous hairs on *Bombus* could possibly be shielding their body from Lead dust particles. I hypothesized there would be no correlation between soil Lead concentration and *Apis mellifera* worker weight, which was supported. However, this was surprising that there was no correlation while soil Lead concentration was a predictor of *Apis* Lead concentration. These animals may be coming into contact with lead from somewhere else. For example, Cristol et al, 2008 found that terrestrial spiders had higher concentration of mercury contamination compared to fish. However, they concluded that more research was needed to identify if the exposure is from consumption of emergent aquatic insects or from mercury deposition in the floodplain (Cristol et al. 2008).

Despite pollution and alterations to the environment by humans, cities still support rich and diverse wild bee communities (Hall et al. 2017, Sivakoff et al. 2018, Turo et al. 2021). Management practices and site characteristics of urban greenspaces can be utilized to further provide support for native bee communities (Turo et al. 2021). For example, in Chicago, USA an increase in greenspace was found to support wild bee abundance and richness when greenspace was not dominated by turfgrass (Tonietto et al. 2011). Recreational parks have also been identified as important for wild bee communities in intensely urbanized cities (Banaszak-Cibicka et al. 2018). Even vacant lots have been identified as greenspaces capable of supporting wild bee communities in urban areas (Turo et al. 2021).

Cities can provide suitable habitat for wild bees (Hall et al. 2017). Riparian areas in particular tend to be hard to develop areas, and oftentimes host the remaining remnant habitat in urban areas. Although these riparian areas could be important for bee conservation in cities, their characteristics have been rarely studied in regards to wild bee support (Vossler 2019). Wild bees are still found in post-industrial cities, but legacy effects of industrial pollution could negatively impact wild bee communities (Moron et al. 2012, Sivakoff et al. 2020). It's important to understand the legacy effects of human land-use change to know how to best mitigate these impacts to enhance wild pollinator support.

There are several limitations to this study. Although I tested soil Lead concentrations, I did not test flowerheads or any other possible points of exposure. Additionally, all the soil Lead concentrations were low compared to what we expected, considering Flint's history of industrial pollution. Low soil Lead concentrations are great in regards to human health implications and ecosystem health, but for the purposes of this study the limited soil Lead range was not particularly helpful when discerning impacts on bees. The contrasting results between *Bombus* and *Apis* ultimately infer that either exposure to Lead from the soil is not main point of contact between Lead and these bees, or there are artifacts from another contaminant impacting *Bombus* weight.

Moving forward, I would advise individuals to either consider a broader swath of heavy metals in their analyses, consider other contaminants such as pesticides in their analyses, or consider alternative points of exposure. Additionally, I would encourage lab experiments on the impacts of Lead exposure to bees at different life stages. Because *Bombus* nest in the ground, there could be potential for more impactful exposure to Lead at the egg and larval stages. Based on differences by taxa, I would advocate for testing bees with different natural histories, for example soil nesters and cavity nesters or carpenter bees or generalist and specialist foragers.

Supplemental Information

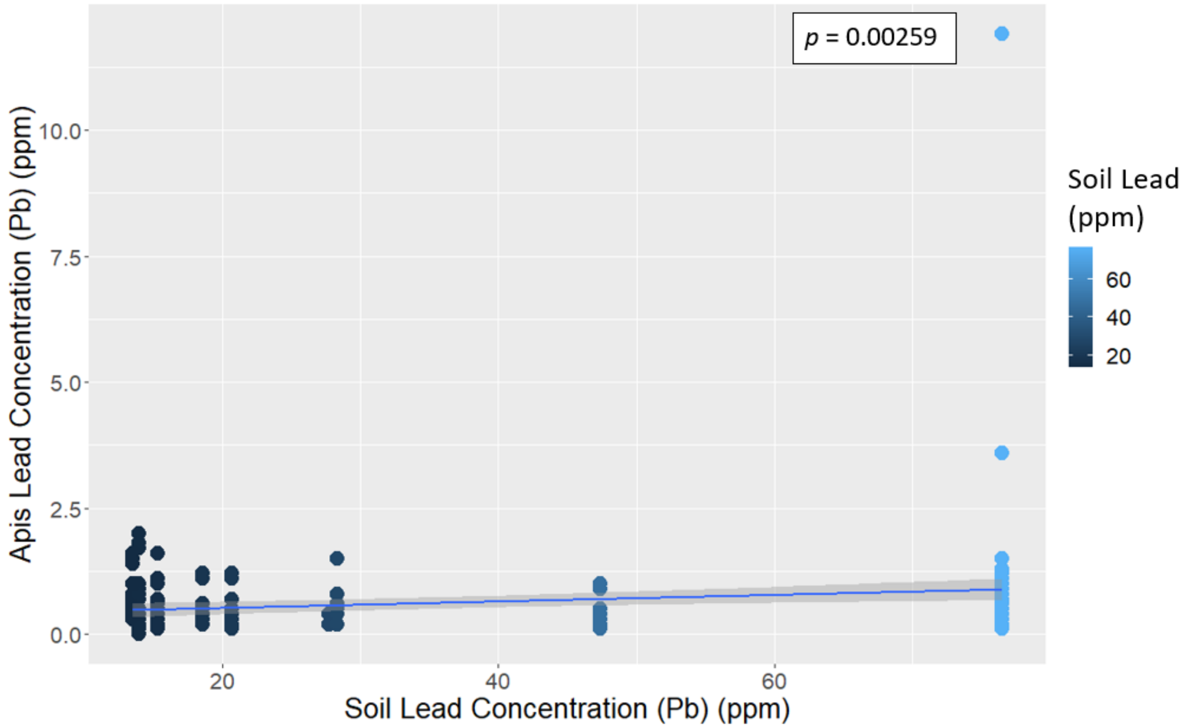

Figure 19: Apis lead concentration is correlated to soil lead concentration. This data is shown without the outlier removed (p = 0.00259).

Sources

Albrecht, M., B. Schmid, M. K. Obrist, B. Schüpbach, D. Kleijn, and P. Duelli. 2010. Effects of ecological compensation meadows on arthropod diversity in adjacent intensively managed grassland. Biological conservation **143**:642-649.

Arena, M., and F. Sgolastra. 2014. A meta-analysis comparing the sensitivity of bees to pesticides. Ecotoxicology (London) **23**:324-334.

Banaszak-Cibicka, W., H. Ratyńska, and Ł. Dylewski. 2016. Features of urban green space favourable for large and diverse bee populations (Hymenoptera: Apoidea: Apiformes). Urban forestry & urban greening **20**:448-452.

Banaszak-Cibicka, W., L. Twerd, M. Fliszkiewicz, K. Giejdasz, and A. Langowska. 2018. City parks vs. natural areas - is it possible to preserve a natural level of bee richness and abundance in a city park? Urban ecosystems **21**:599-613.

Batáry, P., A. Báldi, M. Sárospataki, F. Kohler, J. Verhulst, E. Knop, F. Herzog, and D. Kleijn. 2010. Effect of conservation management on bees and insect-pollinated grassland plant communities in three European countries. Agriculture, ecosystems & environment **136**:35-39.

Bonanno, G., J. A. Borg, and V. Di Martino. 2017. Levels of heavy metals in wetland and marine vascular plants and their biomonitoring potential: A comparative assessment. Science of the Total Environment **576**:796-806.

Bowers, K., and C. Boutin. 2008. Evaluating the relationship between floristic quality and measures of plant biodiversity along stream bank habitats. Ecological indicators **8**:466-475.

Buchholz, S., A. K. Gathof, A. J. Grossmann, I. Kowarik, and L. K. Fischer. 2020. Wild bees in urban grasslands: Urbanisation, functional diversity and species traits. Landscape and urban planning **196**:103731.

Buckles, B. J., A. N. Harmon-Threatt, and G. Peralta. 2019. Bee diversity in tallgrass prairies affected by management and its effects on above- and below-ground resources. The Journal of applied ecology **56**:2443-2453.

Cameron, S. A., J. D. Lozier, J. P. Strange, J. B. Koch, N. Cordes, L. F. Solter, and T. L. Griswold. 2011. Patterns of widespread decline in North American bumble bees. Proceedings of the National Academy of Sciences - PNAS **108**:662-667.

Chittka, L., J. D. Thomson, and N. M. Waser. 1999. Flower Constancy, Insect Psychology, and Plant Evolution. Die Naturwissenschaften **86**:361-377.

Cnaani, J., J. D. Thomson, and D. R. Papaj. 2006. Flower Choice and Learning in Foraging Bumblebees: Effects of Variation in Nectar Volume and Concentration. Ethology **112**:278-285.

Conrad, S. R., S. A. White, I. R. Santos, and C. J. Sanders. 2021. Assessing pesticide, trace metal, and arsenic contamination in soils and dam sediments in a rapidly expanding horticultural area in Australia. Environmental geochemistry and health **43**:3189-3211.

Cristol, D. A., R. L. Brasso, A. M. Condon, R. E. Fovargue, S. L. Friedman, K. K. Hallinger, A. P. Monroe, and A. E. White. 2008. The movement of aquatic Mercury through terrestrial food webs. Science **320**:335-335.

Dahnke, W. C. 1975. Recommended chemical soil test procedures for the North Central Region.

Eckert, J. E. 1933. The Flight Range of the Honeybee. Illus. Journal of agricultural research (Washington, D.C.) **47**:257.

Evers, D. C., N. M. Burgess, L. Champoux, B. Hoskins, A. Major, W. M. Goodale, R. J. Taylor, R. Poppenga, and T. Daigle. 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. Ecotoxicology **14**:193-221.

Fairbrother, A., J. Purdy, T. Anderson, and R. Fell. 2014. Risks of neonicotinoid insecticides to honeybees. Environmental toxicology and chemistry **33**:719-731.

Farhat, Y. A., W. M. Janousek, J. P. McCarty, N. Rider, and L. L. Wolfenbarger. 2014. Comparison of butterfly communities and abundances between marginal grasslands and conservation lands in the eastern Great Plains. Journal of insect conservation **18**:245-256.

Fiedler, A. K., D. A. Landis, and M. Arduser. 2012. Rapid Shift in Pollinator Communities Following Invasive Species Removal. Restoration ecology **20**:593-602.

Frankie, G. W., R. W. Thorp, J. Hernandez, M. Rizzardi, B. Ertter, J. C. Pawelek, S. L. Witt, M. Schindler, R. Coville, and V. A. Wojcik. 2009. Native bees are a rich natural resource in urban California gardens. California agriculture (Berkeley, Calif.) **63**:113-120.

Gathmann, A., H. J. Greiler, and T. Tscharntke. Trap-nesting bees and wasps colonizing set-aside fields: succession and body size, management by cutting and sowing. Oecologia **98**.

Golet, G. H., T. Gardali, C. A. Howell, J. Hunt, R. A. Luster, W. Rainey, M. D. Roberts, J. Silveira, H. Swagerty, and N. Williams. 2008. Wildlife Response to Riparian Restoration on the Sacramento River. California Bay-Delta Authority Science Program and the John Muir Institute of tthe Environment, San Fransisco Estuary & Waatershed.

Graham, K. K., J. A. Perkins, A. Peake, M. Killewald, J. Zavalnitskaya, J. K. Wilson, and R. Isaacs. 2021. Wildflower plantings on fruit farms provide pollen resources and increase nesting by stem nesting bees. Agricultural and forest entomology **23**:222-231.

Grixti, J. C., L. T. Wong, S. A. Cameron, and C. Favret. 2009. Decline of bumble bees (Bombus) in the North American Midwest. Biological conservation **142**:75-84.

Grove, R. A., and C. J. Henny. 2008. Environmental contaminants in male river otters from Oregon and Washington, USA, 1994-1999. Environmental Monitoring and Assessment **145**:49-73.

Haaland, C., R. E. Naisbit, and L.-F. Bersier. 2011. Sown wildflower strips for insect conservation: a review. Insect conservation and diversity **4**:60-80.

Hall, D. M., G. R. Camilo, R. K. Tonietto, J. Ollerton, K. Ahrné, M. Arduser, J. S. Ascher, K. C. R. Baldock, R. Fowler, G. Frankie, D. Goulson, B. Gunnarsson, M. E. Hanley, J. I. Jackson, G. Langellotto, D. Lowenstein, E. S. Minor, S. M. Philpott, S. G. Potts, M. H. Sirohi, E. M. Spevak, G. N. Stone, and C. G. Threlfall. 2017. The city as a refuge for insect pollinators. Conservation biology **31**:24-29.

Hallmann, C. A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Muller, H. Sumser, T. Horren, D. Goulson, and H. de Kroon. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. Plos One **12**.

Hanula, J. L., and S. Horn. 2011. Removing an invasive shrub (Chinese privet) increases native bee diversity and abundance in riparian forests of the southeastern United States. Insect Conservation and Diversity **4**:275-283.

Harmon-Threatt, A. 2020. Influence of Nesting Characteristics on Health of Wild Bee Communities. Annual Review of Entomology, Vol 65 **65**:39-+.

Harmon-Threatt, A., and K. Chin. 2016. Common Methods for Tallgrass Prairie Restoration and Their Potential Effects on Bee Diversity. Natural areas journal **36**:400-411.

Hatfield, R. G., and G. LeBuhn. 2007. Patch and landscape factors shape community assemblage of bumble bees, Bombus spp. (Hymenoptera: Apidae), in montane meadows. Biological conservation **139**:150-158.

Havard, T., M. Laurent, and M.-P. Chauzat. 2019. Impact of Stressors on Honey Bees (Apis mellifera; Hymenoptera: Apidae): Some Guidance for Research Emerge from a Meta-Analysis. Diversity (Basel) **12**:7.

Heinrich, B. 1976. The Foraging Specializations of Individual Bumblebees. Ecological monographs **46**:105-128.

Henriques, M. C., S. Loureiro, M. Fardilha, and M. T. Herdeiro. 2019. Exposure to mercury and human reproductive health: A systematic review. Reproductive Toxicology **85**:93-103.

Hopwood, J. L. 2008. The contribution of roadside grassland restorations to native bee conservation. Biological conservation **141**:2632-2640.

Jackson, A. K., D. C. Evers, M. A. Etterson, A. M. Condon, S. B. Folsom, J. Detweiler, J. Schmerfeld, and D. A. Cristol. 2011. MERCURY EXPOSURE AFFECTS THE REPRODUCTIVE SUCCESS OF A FREE-LIVING TERRESTRIAL SONGBIRD, THE CAROLINA WREN (THRYOTHORUS LUDOVICIANUS). Auk **128**:759-769.

Katz, E. J., and C. J. Essenberg. 2018. The effect of the dispersion of rewarding and rewardless flowers on visitation and constancy by bumblebees (Bombus impatiens). Journal of pollination ecology **23**:119-126.

Kleijn, D., R. Winfree, I. Bartomeus, L. G. Carvalheiro, M. Henry, R. Isaacs, A. M. Klein, C. Kremen, L. K. M'Gonigle, R. Rader, T. H. Ricketts, N. M. Williams, N. L. Adamson, J. S. Ascher, A. Baldi, P. Batary, F. Benjamin, J. C. Biesmeijer, E. J. Blitzer, R. Bommarco, M. R. Brand, V. Bretagnolle, L. Button, D. P. Cariveau, R. Chifflet, J. F. Colville, B. N. Danforth, E. Elle, M. P. D. Garratt, F. Herzog, A. Holzschuh, B. G. Howlett, F. Jauker, S. Jha, E. Knop, K. M. Krewenka, V. Le Feon, Y. Mandelik, E. A. May, M. G. Park, G. Pisanty, M. Reemer, V. Riedinger, O. Rollin, M. Rundlof, H. S. Sardinas, J. Scheper, A. R. Sciligo, H. G. Smith, I. Steffan-Dewenter, R. Thorp, T. Tscharntke, J. Verhulst, B. F. Viana, B. E. Vaissiere, R. Veldtman, C. Westphal, and S. G. Potts. 2015. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nature Communications **6**.

Kopit, A. M., and T. L. Pitts-Singer. 2018. Routes of Pesticide Exposure in Solitary, Cavity-Nesting Bees. Environmental Entomology **47**:499-510.

Lanner, J., S. Kratschmer, B. Petrović, F. Gaulhofer, H. Meimberg, and B. Pachinger. 2019. City dwelling wild bees: how communal gardens promote species richness. Urban ecosystems **23**:271-288.

LeBuhn, G., T. Griswold, R. Minckley, S. Droege, T. a. Roulston, J. Cane, F. Parker, S. Buchmann, V. Tepedino, N. Williams, C. Kremen, and O. Messenger. 2003. A standardized method for monitoring Bee Populations – The Bee

Inventory (BI) Plot.

Leonardi, J. M., W. J. Gruhn, and D. Michigan. Fisheries. 2001. Flint River assessment: Joseph M. Leonardi and William J. Gruhn. Ann Arbor :: Michigan Dept. of Natural Resources, Fisheries Division.

Leong, M., L. C. Ponisio, C. Kremen, R. W. Thorp, and G. K. Roderick. 2016. Temporal dynamics influenced by global change: bee community phenology in urban, agricultural, and natural landscapes. Global change biology **22**:1046-1053.

McFrederick, Q. S., and G. LeBuhn. 2006. Are urban parks refuges for bumble bees Bombus spp. (Hymenoptera : Apidae)? Biological Conservation **129**:372-382.

McNeil, D. J., E. McCormick, A. C. Heimann, M. Kammerer, M. R. Douglas, S. C. Goslee, C. M. Grozinger, and H. M. Hines. 2020. Bumble bees in landscapes with abundant floral resources have lower pathogen loads. Scientific reports **10**:22306-22312.

Mohammed, A. S., A. Kapri, and R. Goel. 2011. Heavy Metal Pollution: Source, Impact, and Remedies. Pages 1-28. Springer Netherlands, Dordrecht.

Moron, D., I. M. Grzes, P. Skorka, H. Szentgyorgyi, R. Laskowski, S. G. Potts, and M. Woyciechowski. 2012. Abundance and diversity of wild bees along gradients of heavy metal pollution. Journal of Applied Ecology **49**:118-125.

Ollerton, J., R. Winfree, and S. Tarrant. 2011. How many flowering plants are pollinated by animals? Oikos **120**:321-326.

Osborne, J. L., S. J. Clark, R. J. Morris, I. H. Williams, J. R. Riley, A. D. Smith, D. R. Reynolds, and A. S. Edwards. 1999. A Landscape-Scale Study of Bumble Bee Foraging Range and Constancy, Using Harmonic Radar. The Journal of applied ecology **36**:519-533.

Pei, C. K., T. J. Hovick, C. A. Duquette, R. F. Limb, J. P. Harmon, and B. A. Geaumont. 2021. Two common bee-sampling methods reflect different assemblages of the bee (Hymenoptera: Apoidea) community in mixed-grass prairie systems and are dependent on surrounding floral resource availability. Journal of insect conservation **26**:69-83.

Peters, V. E., K. U. Campbell, G. Dienno, M. García, E. Leak, C. Loyke, M. Ogle, B. Steinly, and T. O. Crist. 2016. Ants and plants as indicators of biodiversity, ecosystem services, and conservation value in constructed grasslands. Biodiversity and conservation **25**:1481-1501.

Pieper, K. J., M. Tang, and M. A. Edwards. 2017. Flint Water Crisis Caused By Interrupted Corrosion Control: Investigating "Ground Zero" Home. Environmental science & technology **51**:2007-2014.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution **25**:345-353.

Purvis, E. E. N., M. L. Meehan, and Z. Lindo. 2020. Agricultural field margins provide food and nesting resources to bumble bees (Bombus spp., Hymenoptera: Apidae) in Southwestern Ontario, Canada. Insect conservation and diversity **13**:219-228.

Rosenberg, D. M., H. V. Danks, and D. M. Lehmkuhl. 1986. IMPORTANCE OF INSECTS IN ENVIRONMENTAL-IMPACT ASSESSMENT. Environmental Management **10**:773-783.

Rosner, D. 2016. Flint, Michigan: A Century of Environmental Injustice. American journal of public health (1971) **106**:200-201.

Saleh, N., and L. Chittka. 2007. Traplining in Bumblebees (Bombus impatiens): A Foraging Strategy's Ontogeny and the Importance of Spatial Reference Memory in Short-Range Foraging. Oecologia **151**:719-730.

Sharma, K., N. T. Basta, and P. S. Grewal. 2014. Soil heavy metal contamination in residential neighborhoods in post-industrial cities and its potential human exposure risk. Urban ecosystems **18**:115-132.

Sivakoff, F. S., S. P. Prajzner, and M. M. Gardiner. 2018. Unique bee communities within vacant lots and urban farms result from variation in surrounding urbanization intensity. Sustainability (Basel, Switzerland) **10**:1926.

Sivakoff, F. S., S. P. Prajzner, and M. M. Gardiner. 2020. Urban heavy metal contamination limits bumblebee colony growth. Journal of Applied Ecology **57**:1561-1569.

Szabo, N. D., S. R. Colla, D. L. Wagner, L. F. Gall, and J. T. Kerr. 2012. Do pathogen spillover, pesticide use, or habitat loss explain recent North American bumblebee declines? Conservation Letters **5**:232-239.

Tonietto, R., J. Fant, J. Ascher, K. Ellis, and D. Larkin. 2011. A comparison of bee communities of Chicago green roofs, parks and prairies. Landscape and Urban Planning **103**:102-108.

Tonietto, R. K., and D. J. Larkin. 2018. Habitat restoration benefits wild bees: A meta-analysis. Journal of Applied Ecology **55**:582-590.

Turo, K. J., and M. M. Gardiner. 2021. Effects of urban greenspace configuration and native vegetation on bee and wasp reproduction. Conservation biology **35**:1755-1765.

Turo, K. J., M. R. Spring, F. S. Sivakoff, Y. A. Delgado de la flor, M. M. Gardiner, and L. Garibaldi. 2021. Conservation in post-industrial cities: How does vacant land management and landscape configuration influence urban bees? The Journal of applied ecology **58**:58-69.

USDA-NRCS. 1999. Identify Soil Texture by Feel.

van der Sluijs, J. P., N. Simon-Delso, D. Goulson, L. Maxim, J.-M. Bonmatin, and L. P. Belzunces. 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. Current opinion in environmental sustainability **5**:293-305.

Vossler, F. G. 2019. Native and ornamental exotic resources in pollen loads and garbage pellets of four stingless bees (Apidae, Meliponini) in an urban environment with riparian native forest. Anais da Academia Brasileira de Ciências **91**:e20190360-e20190360.

Wikum, D. A., and G. F. Shanholtzer. 1978. Application of the Braun-Blanquet cover-abundance scale for vegetation analysis in land development studies. Environmental management (New York) **2**:323-329.

Williams, N. M. 2011. Restoration of Nontarget Species: Bee Communities and Pollination Function in Riparian Forests. Restoration Ecology **19**:450-459.

Williams, N. M., K. L. Ward, N. Pope, R. Isaacs, J. Wilson, E. A. May, J. Ellis, J. Daniels, A. Pence, K. Ullmann, and J. Peters. 2015. Native wildflower plantings support wild bee abundance and diversity in agricultural landscapes across the United States. Ecological applications **25**:2119-2131.

Winfree, R., R. Aguilar, D. P. Vazquez, G. Lebuhn, and M. A. Aizen. 2009. A Meta-Analysis of Bees' Responses to Anthropogenic Disturbance. Ecology (Durham) **90**:2068-2076.

Winner, R. W., M. W. Boesel, and M. P. Farrell. 1980. INSECT COMMUNITY STRUCTURE AS AN INDEX OF HEAVY-METAL POLLUTION IN LOTIC ECOSYSTEMS. Canadian Journal of Fisheries and Aquatic Sciences **37**:647-655.