

ABSTRACT

KETTENBACH, JESSICA ANN. Floral Resources in Urban Landscapes: Impacts on Pollinator Foraging and Reproduction. (Under the Direction of Dr. Rebecca E. Irwin).

Urbanization is a dominant form of land-use change driving biodiversity loss worldwide. Pollinators are one group of declining ecosystem service providers that deserve particular attention in light of increasing urbanization, especially native bees which benefit humans as dominant pollinators of crops and wildflowers. One mechanism by which urbanization could affect bees is altering floral resource availability either negatively through the conversion of habitat or positively through the planting of domestic gardens, which often contain horticultural plants that may or may not provide sufficient nutrition for the development of bees. The goal of this thesis was to address how urban landscapes affect floral resource availability and bumble bee success, measured as 1) bumble bee catch rates, 2) resource provisioning, and 3) colony productivity. Eusocial, ground nesting bumble bees (*Bombus impatiens*, the common eastern bumble bee) were the focus of this research because these bees are efficient pollinators important for the conservation of plant biodiversity but bumble bees in general may be sensitive to land-use change in urban landscapes. I used paired urban and non-urban study sites in and around Raleigh, NC. In Chapter 1, I explored differences in flower resource availability and community composition, bumble bee catch rates, and bumble bee pollen provisioning in urban and non-urban sites. I analyzed bee pollen load composition because pollen provisions are fed to larvae and used to build the hive structure. Thus, pollen origin may have a large impact on bee reproductive success, if for example, pollen nutrition content varies, or if pollen comes from pesticide-treated plants. Urban sites harbored 2-times higher floral abundance than non-urban sites and differed in community composition. However, despite higher floral resource availability, catch rates of bumble bees did not differ between urban and non-urban sites. Bumble

bees incorporate ornamental species into their diets in both urban and non-urban sites, but pollen ball composition analyses revealed a strong preference for foraging on one naturalized species, *Trifolium repens*. Thus, increased floral resources in urban sites may not directly translate to increased bee abundance or effect foraging choices. In Chapter 2, I related flower community composition and pollen provisioning to colony productivity in urban and non-urban sites, and I used a food-supplementation treatment to test if the degree of food limitation varied with urbanization. I measured colony traits that may correlate with producing reproductive individuals and thus effective population size of bumble bees: colony growth rates, body sizes, worker build up, and the production of sexuals. I found that colonies in non-urban sites grew faster and produced more workers and sexuals than colonies in urban sites, despite higher flower resource availability in urban sites. However, this effect was dependent upon the timing of colony placement into the field. Colonies were not food-limited in either site, but there was an interaction with food supplementation and site type, and food-supplemented colonies in non-urban sites grew the fastest. These results suggest that other factors besides floral resource availability, such as food quality, pathogens, or pesticides, may override food availability in affecting colony success in urban landscapes. Taken together, results from this thesis indicate that urban sites can provide abundant and diverse floral resources but that those resources may not scale to affect bumble colony success. Thus, while urban landscapes may provide forage for native bees, the conservation of native bees in an increasingly urban landscape may depend on access to non-urban habitats.

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Floral Resources in Urban Landscapes: Impacts on Pollinator Foraging and Reproduction

by
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BIOGRAPHY

Jessica Kettenbach was born December 3, 1992, in St. Louis, Missouri. As a child camping trips with her parents, Lawrence and Mary Kettenbach, and her two sisters, Andrea and Amy, got her interested in the natural world. In college, she majored in Biological Sciences at the University of Missouri-Columbia. There, she began studying ecology with Dr. Candace Galen in the Colorado Rocky Mountains. It was this experience that led her to believe in the importance of studying bees in the Anthropocene. In 2015, she was blessed to be accepted to NC State University to continue studying bees with Dr. Rebecca Irwin, and write her Master's thesis on the effect of urbanization on flower and bumble bee communities and bumble bee colony reproduction.

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CHAPTER 1: Increased floral resources do not predict bumble bee catch rates in an urban landscape

ABSTRACT

Urbanization typically results in biodiversity declines, but recently the potential for some urban habitats to hold conservation value has been recognized for certain taxa of concern, including bees. Urban ornamental gardens have the potential to increase bee abundance and species richness, especially under conditions of intermediate disturbance from urbanization. In this study, we evaluated how flowering communities, bumble bee abundances, and bumble bee pollen diets differed in urban and non-urban sites. We also categorized flowering plants to understand functional differences in urban flowering communities and bumble bee pollen foraging. We found that urban and non-urban flowering communities differed in species composition and plant functional categories, with urban sites having more ornamental and native ornamental species flowers and non-urban sites having more native species flowers. While urban sites had 2-times higher flower density, bumble bee catch rates did not increase in urban sites relative to non-urban sites. Across both urban and non-urban habitats, bumble bees showed preference for pollen from the one naturalized plant species, white clover, commonly known as white clover, disproportionately to its relative abundance. Together, the results of this study show that while urbanization can have major impacts on floral communities, these do not necessarily translate to increases in bee abundance or dictate pollen foraging in bumble bees.

INTRODUCTION

Biodiversity is declining worldwide (Barnosky et al., 2011), and the decline of pollinating insects has attracted special attention, as over 87% of flowering species rely on

insects or other animals for pollination (Ollerton et al., 2011). Moreover, pollinating insects are responsible for over 30% of global food production (Klein et al., 2007). One factor that is strongly associated with pollinator declines is habitat loss, fragmentation and degradation, such as intensification of land use for agriculture and urban development (Biesmeijer et al., 2006; Goulson et al., 2005; Potts et al., 2010; Shochat et al., 2006). Rural agricultural areas have decreased in wildlife habitat value over time (Scherr & McNeely, 2008). However, value for conservation of certain types of wildlife is increasingly recognized in urban landscapes (Cane, 2005; Dearborn & Kark, 2010; Gunnarsson & Federsel, 2014; McFrederick & LeBuhn, 2005; Micholap et al., 2017; Sanderson & Huron, 2011), which could be beneficial for increasing habitat connectivity. For pollinators, private gardens are likely the most valuable component of urban conservation (Goddard et al., 2010), because they can harbor high abundances and species richness of flowering plants (Carper et al., 2014; Gunnarsson & Federsel, 2014; Hall et al., 2017; McFrederick & LeBuhn, 2005), especially neighborhoods in higher socio-economic classes (Hope et al., 2003). Native bees may benefit from this “luxury effect” of floral resources in urban habitats (Carper et al., 2014; Goulson et al., 2003; Hope et al., 2003; Matteson et al., 2008; Tonietto et al., 2011) as habitat quality hinges on the availability of nesting and floral resources, and high-value forage increases bumble bee survival and persistence (Carvell et al., 2017).

On the one hand, private and community gardens may increase floral resources in some urban habitats, resulting in increased numbers of foraging bees and thus pollination services. On the other hand, a number of factors may limit their value for urban bee conservation. For example, gardeners may choose to plant non-native ornamentals that have evolved independent of the local pollinator assemblage and have been bred to alter their appearance, a practice that may reduce pollen and nectar nutrients or accessibility (Comba et al., 1999; Corbet et al., 2001).

Bee larval growth has specific nutritive demands (Génissel et al., 2002), and pollen quality and diversity affect bee physiology and immunocompetence (Di Pasquale et al., 2013; Roger et al., 2017). Even within plant genera, there may be large variation in the quality of different ornamental plant varieties as a food source for bees (Garbuzov & Ratnieks, 2014) with certain individual plant species providing higher pollen and/or nectar quantity or quality relative to others (e.g. clover, *Trifolium spp.*, Filipiak et al., 2017). Bees in urban habitats are also exposed to agrochemicals (Botías et al., 2017), and ornamental plants on sale to the public are a significant source of pesticides to pollinators whether growers apply additional chemicals or not (Lentola et al., 2017). Thus, the foraging choices of bees in urban habitats could have a large impact on their health and reproduction.

To understand how the urban landscape affects flower communities and bee populations, we surveyed flower community composition and density, pollen collection by three native bumble bees (*Bombus impatiens*, *B. bimaculatus*, and *B. griseocollis*), and bumble bee abundance estimated as catch rates. *Bombus* spp. are native and highly efficient pollinators (Artz & Nault, 2011; Fisher & Pomeroy, 1989; Goulson & Hughes, 2015; Stubbs & Drummond, 2001; Willmer et al., 1994), have a large impact on plant diversity (Memmott et al., 2004), and may be particularly susceptible to extirpation in urban habitats (Winfrey et al., 2011). Additionally, *Bombus* spp. are generalist foragers and thus should be capable of exploiting the additional resources available in private urban gardens. We predicted that 1) urban gardens would elevate flower abundance and species richness in urban relative to non-urban sites, 2) bees in urban sites would have wider diet breadths and incorporate ornamental species in their diets, and 3) increased floral abundance and diet breadth would lead to increased bumble bee abundance.

Taken together, this work provides insight into how bees exploit urbanized landscapes with regard to foraging preferences.

METHODS

Study system. This study was conducted at sixteen sites in the Raleigh-Durham metropolitan area of North Carolina (RDU) where projected population growth is the second-highest of all cities in the United States (US Census Bureau, 2010), and urban land use is predicted to double or triple by 2060 (Terando et al., 2014). All field work was conducted in the 2016 flowering and flight season. Sites were paired with n=8 urban sites and n=8 non-urban sites (Table S1). The urban sites in this study were defined as single-family residential neighborhoods; the sites contained a range of housing values, styles, and ages, but most were at or above the median housing value for the area (Chatham Co., 2018; Wake Co., 2018). Although we worked in residential neighborhoods, they were classified as urban based on their population density and association within urban metropolitan areas and urban clusters (US Census Bureau, 2010). Non-urban sites included areas such as state parks that were managed for their natural resources. Both site-types contained open and forested areas (mainly pine, hickory, and oak forests). Sites within pairs were the same size and were located <5 km apart to control for landscape-level differences in habitat. The boundaries of sites within pairs were located at least 1 km apart to reduce the probability that bees foraged in both urban and non-urban sites, and site pairs were located at least 5 km apart to reduce the probability that bees could fly among pairs (Darvill et al., 2004; Geib et al., 2015; Knight et al., 2005; Osborne et al., 1999.).

We studied three bumble bee species, *B. impatiens*, *B. bimaculatus*, and *B. griseocollis*. All three species are historical residents of the eastern United States and have medium tongue lengths (Williams et al., 2014). *B. impatiens* and *B. griseocollis* are native in the eastern United

States and found from Maine to Florida, and as far west as Wisconsin, south to Louisiana. *B. bimaculatus* is found from Maine to Florida, and west from southern Missouri to southern Michigan (Williams et al., 2014). *B. impatiens* queens nest underground, while *B. bimaculatus* and *B. griseocollis* nest both underground and on the surface (Williams et al., 2014). *B. impatiens* and *B. bimaculatus* have flight periods from April-October and *B. griseocollis* from May-October (Williams et al., 2014). All three species are considered to have stable populations (IUCN, 2014) and *B. impatiens* may even be expanding its range (Williams et al., 2014).

Flowering community. We monitored the flowering community at each site pair once every other week for 12 weeks from May-August. To estimate flower density at each sampling period, we counted the number of flowers in a 50 x 2 m transect for each microhabitat in the site (garden, lawn, forest, forest edge, roadside, meadow). We used GoogleEarth to define the area of each microhabitat in the sites and extrapolated the microhabitat flower densities from the transects to estimates of microhabitat abundances. To estimate site-wide flower density, we summed all of the microhabitat abundances for that site on a single date and divided by the total site area. To monitor site-wide flowering species richness, we noted any additional flower species that fell outside the transect limits.

We compared site-wide flower density and flower species richness in urban and non-urban sites using log-transformed site-wide flower density on each sampling date by site type (urban vs. non-urban) and sampling date with site pair as a random effect in R version 3.4.1 in R Studio (R Development Core Team 2017) using the ‘lmer’ function from the lme4 package (Bates et al., 2015). All statistical analyses (here and below) were performed in R unless otherwise noted. We used EcoSim Pro to rarefy flower species richness down to the lowest abundance to understand how sampling effort affected flower species richness counts using a

rarefaction randomization algorithm with 1000 iterations (Gotelli & Entsminger, 2000). To visualize how flower community composition differed in the urban and non-urban sites, we used non-metric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity index from the R vegan package (Oksanen et al., 2011).

Additionally, we analyzed the flowering community based on flowering plant category. We used the North Carolina State Cooperative Extension categories to evaluate the proportion of flowering species categories that existed in the sites by assigning each flowering species to one of six categories: native, invasive, naturalized, ornamental, native & ornamental, or undetermined (NC State Cooperative Extension, 2018). We used these categories to understand the functional differences in urban and non-urban flowering communities. We performed MANOVAs in the ‘dplyr’ package in R to evaluate whether the flowering communities were different based on the proportion of the flower density that each flowering plant category comprised, with site type and pair as fixed effects (because random effects are not supported by manova). We then used mixed models with site type as a fixed effect and site pair as a random effect to identify which categories differed based on the proportion of the flowers they represented in the community. We also computed the average rank order for the plant categories by average the ranks for plant categories based on the proportion of the flowering density they comprised.

Bee and pollen collections. Bee collections occurred once every other week at each site pair on the day of flowering measurements. Bees were netted in the air and on flowers and transferred to tubes with mesh tops. When bees were netted on flowers, we noted the species of flower they were collected from. We removed (at the femur) one mesothoracic leg from each worker bee and stored it in 100% EtOH at -20°C for future for genetic analyses (genetic data an

analyses not in this thesis). For bees that were carrying corbicular pollen loads, we removed one pollen ball from each pollen-foraging bumble bee using a dissecting needle and stored it in 0.25 mL of 100% EtOH. All equipment was washed with ethanol between bees to avoid pollen contamination among samples. We netted at each site on each day for approx. 1-4 hrs, and all bee collection estimates were divided by the time netted to control for sampling effort. All recaptured individuals (i.e., bees missing a mesothoracic leg) were released and no pollen or additional legs were removed.

We constructed multiple mixed models to compare differences in bee catch rates (standardized for collecting effort) using the ‘lmer’ function from the lme4 package. The full model included site type (urban vs. non-urban) and sampling week as fixed effects, flower density and richness as covariates, and site pair as a random effect, and all interactions thereof. We used a model selection approach to choose the best model for all bee species combined and for each species separately, based on which model had the lowest AIC value. We separated bee species in the analyses due to phenological differences of the bee species.

Pollen identification and counting. For each corbicular pollen load stored in EtOH, we aliquoted 40 uL of the pollen and EtOH solution and created fixed slides using basic fuchsin dye (Kearns & Inouye, 1993). Pollen ball contents were compared to a pollen library of all flowering plant species found in the sites. All pollen grains on the slides were identified and counted under a compound microscope to the lowest taxonomic level possible (species or genus). Then, the identified pollen was categorized based on the NC State Cooperative Extension categories above.

First, we used the ‘lmer’ function in the lme4 package in R to test for differences in pollen load composition measured as proportion of pollen grains from each plant category using bumble bee species as a factor and no differences were found among the species ($P > 0.05$). Thus,

all subsequent analyses were performed with pollen balls from all bumble bee species combined. We then employed classical composition analysis (Aebischer et al., 1993) to identify plant categories that bees selected relative to their availability when pollen foraging (e.g., Davis et al., 2012, Jha et al., 2013) using the ‘adehabitatHS’ package (Calenge, 2006). Matrices of floral species availability (the proportion of flowers per plant type per day) were compared to matrices of pollen collection (the proportion of pollen grains per pollen ball). Each individual bee was represented in the matrices so that the proportion of flowers of any given plant category in the site the bee was caught on the day of foraging was compared to the proportion of pollen grains from that bee’s pollen load on that day. Pollen collection was examined by a randomization test (10,000 repetitions) using Wilks’ lambda (λ) to test for preferences for plant categories of all bees, urban-site bees, and non-urban-site bees, respectively. Finally, ranking matrices were created to show the mean difference between the proportion of pollen use and the proportion of flowers to assess the significance of preference for one plant category or species over another (Aebischer et al., 1993; Calenge 2006; Jha et al., 2013). The native ornamentals category was excluded from the non-urban site only analysis because no native ornamentals existed in these sites.

RESULTS

Flowering community. Urban sites had over two times higher floral density compared to non-urban sites ($X^2_1 = 13.0$, $P = 0.005$; Fig. 1a). Moreover, average flower species richness per sampling event was approx. 2.5 times higher in urban compared to non-urban sites ($X^2_1 = 49.99$, $P = 1.5e-12$; Fig. 1b), with urban sites harboring a total of 157 species/morphospecies across all sample dates and non-urban sites harboring 98 species. This difference was statistically significant when the flowering richness data were rarefied, as shown by overlapping 95%

confidence intervals of the rarefied means estimate ($P > 0.05$; Fig. 1D; Fig. 1c). The Shannon's Diversity Index was 3.22 for urban sites and 2.39 for non-urban sites, and the confidence intervals overlapped ($P > 0.05$). The three most common species on a per-flower basis in urban sites were *Lagerstroemia* sp., *Trifolium repens* (hereafter white clover), and *Lantana* sp., making up 34% of the flowering density, while the three most common species in non-urban sites were white clover, *Hypochaeris radicata*, and *Abelia schumannii*, making up 72% of the flowering density.

Both the NMDS and the flowering plant category analysis revealed significant differences in flowering community composition between urban and non-urban sites. First, the NMDS revealed that the flowering communities were different between urban and non-urban sites (Fig. 2), with no overlap in the flowering community space between site-types. The overall stress value of the two-dimensional ordination was 0.25, indicating fair confidence in the ordination. Increasing dimensionality decreased the stress value (e.g., $k = 3$ had stress = 0.129), but the qualitative results were similar. Additionally, the flowering plant-category analysis revealed significant differences in the proportions of the flower density comprised of the different plant categories ($F_{6,62} = 13.0$, $P = 1.87e-09$). Non-urban sites had six-times higher proportions of native flowers ($X^2_1 = 34.26$, $P = 4.82e-09$). Urban sites had two-times higher proportions of native ornamentals ($X^2_1 = 6.12$, $P = 0.013$), and six-times higher proportions of ornamental flowers ($X^2_1 = 40.82$, $P = 1.67e-10$). There was no difference in the proportions of invasive flowers ($X^2_1 = 0.14$, $P = 0.71$), unknown flowers, ($X^2_1 = 2e-4$, $P = 0.99$), or the one naturalized species, *T. repens* ($X^2_1 = 1.71$, $P = 0.19$) in urban and non-urban sites.

Bee and pollen collections. Results suggest that the urban landscape had no effect on bumble bee abundance, estimated as catch rate. Specifically, when we analyzed all bee species

combined, the full model (Table S2a) had the lowest AIC score (Table S2b), but there were no significant variables predicting bumble bee catch rates ($P > 0.05$). When catch rates for three bumble bee species were analyzed separately, the best model for each of them was the one that only included week (Table S2). For *B. impatiens*, week was not a significant predictor of catch rates ($X_1^2 = 0.11$, $P = 0.74$). For *B. bimaculatus* and *B. griseocollis*, sampling week was a significant predictor of catch rates, and catch rates for these species decreased sharply after our second week of sampling ($X_1^2 = 20.57$, $P = 5.75e-06$; $X_1^2 = 17.04$, $P = 3.65e-05$, respectively).

We collected 327 pollen balls with 282 of the pollen balls collected coming from *B. impatiens*, 27 from *B. griseocollis*, and 19 from *B. bimaculatus*. In total, we identified 32 species (or morphospecies) of pollen carried by bees, representing 13.7% of the flowering plant community across all sites. The mean number of pollen grains collected per pollen ball was significantly higher in urban compared to non-urban sites ($X^2_1 = 6.26$, $P = 0.012$), with pollen balls from bees foraging in urban sites having over 200% more pollen grains per ball than from non-urban sites. However, the mean numbers of pollen taxa collected per pollen ball did not differ by site type, with 1.51 pollen taxa collected on average in urban sites and 1.38 in non-urban sites ($X^2_1 = 0.44$, $P = 0.51$).

Pollen in the pollen balls was dominated by the one naturalized species white clover, representing a mean proportion of 0.548 of all pollen grains in urban sites, and 0.748 in non-urban sites. Beyond white clover, we also observed differences in the composition of pollen balls when we classified species by other flowering-plant categories. In particular, bees collected over two-times more pollen from ornamental species in urban compared to non-urban sites (Fig. 4b). Common ornamental species encountered in pollen balls included *Spirea japonica*, *Abelia schumannii*, and *Ligustrum* sp. (Table S3). Common native species encountered in pollen balls

included *Chaemacrista nictans*, *Penstemon laevigatus*, *Oenothera* sp. (Table S3). Surprisingly, the mean proportion of invasive species pollen was 26-times higher in non-urban than urban sites ($X^2_1 = 6.08$, $P = 0.014$; Fig. 4b), with two species in particular, *Lonicera japonica* and *Ampelopsis glandulosa*, making up 99% of the invasive pollen collected.

Bees did not collect pollen into their pollen balls in proportion to what was available. The overall randomization test for plant category preference when urban and non-urban bees were analyzed together was significant ($\lambda = 0.124$, $P = 0.0001$). The ranking in order of preference was naturalized (one species, white clover), ornamental, invasive, unknown, native, and native ornamentals (Table 1a). When we analyzed each site-type separately, the overall randomization test for plant category preference for urban bees was significant ($\lambda = 0.091$, $P = 0.0001$) and for non-urban bees was significant as well ($\lambda = 0.094$, $P = 0.0001$). However, the preference rank orders differed, with urban bees showing the rank order naturalized, ornamental, invasive, native, unknown, and native ornamentals (Table 1b) and non-urban bees showing the rank order naturalized, invasive, ornamental, unknown, native (Table 1c).

DISCUSSION

Urban landscapes can have dramatic effects on species abundances and composition (Somme et al., 2016; Thompson et al., 2003). Prior studies suggest that flowering plant communities can have a major impact on bumble bee abundance in an urban context (Ahrné et al., 2009). However, the results of our study suggest that, despite being generalist foragers, bumble bees may not be responsive ubiquitously to the density and richness of ornamental species in urban habitats. While urban habitats had an average of approx. 2.5 times more flowers per m² than non-urban sites, bumble bee catch rates were similar between urban and non-urban sites. The flowering communities in urban sites were dominated by ornamental species, with

over 90% of flowers site-wide being ornamental. However, it is important to note that even in non-urban sites, there were some ornamental species that we found, either planted or as escaped individuals. On a per flower basis, these ornamentals comprised nearly 25% of the flowering community across all the non-urban sites, underlining the pervasiveness of human influence in this landscape. While the ornamental species were a significant resource for bumble bees in both urban and non-urban sites, bees disproportionately choose to forage on a single floral resource, white clover, suggesting bumble bees prefer this species.

On average, urban sites had greater overall flower density and species richness compared to non-urban sites, and the NMDS showed the flower communities in urban and non-urban sites were different. Urban sites were dominated by ornamental species planted in private gardens and observed flower richness was higher in urban sites. Given that the urban sites that we studied were often at or above the median housing values, our flowering data provide support for the luxury effect on urban floral biodiversity (Hope et al., 2003). Urban sites had six-times higher proportions of ornamental flowers and two-times higher proportions of native ornamental flowers than did non-urban sites, while non-urban sites had six-times higher proportions of native flowers, reflecting that the planting of ornamental flowers by urban homeowners had a large impact on the functional composition of the flowering plant community. Surprisingly, there was no effect of site type on bumble bee catch rates, despite the strong differences in flower density. Some studies show a positive effect of urbanization on bees (Carper et al., 2014) including bumble bees (Carré, 2009), while others show no effect (Baldock et al., 2015). This may reflect that the increased floral resources are alleviating some of the potential negative effects of urbanization, such as impervious surface area (Hülsman et al., 2015), but do not

override these effects. These results provide growing support for studies documenting the effects of urbanization on plant species richness and abundance (Clark et al., 2013; Hope et al. 2003).

Surprisingly, there was no effect of the urban landscape on bumble bee catch rates, despite the strong differences in flower density. Some studies show a positive effect of urbanization on bees (Carper et al. 2014), including bumble bees (Carre 2009), while others show no effect (Baldock et al. 2015). The positive effect of floral resources on bees in some studies may reflect that this increase in floral resources is alleviating potential food limitation (McFrederick & LeBuhn 2006). Bumble bee colonies and productivity of those colonies have been shown to be food limited in some natural systems (Elliot, 2009). However, in cases where increased floral resources are showing no effect, this may indicate that colonies are not food limited (Simao et al., 2018), or that other factors override the benefits of increased food availability in urban landscapes, such as increased heat island effects (Hamblin et al. 2018), increased impervious surface cover (as in Hülsmann et al., 2015), increased exposure to pesticides (Botías et al., 2017; Larson et al., 2013), and changes in the quality of nectar and pollen diets. One challenge with interpreting the catch rate data, as well as data from other studies that simply catch bees or use pan traps to sample bees, is that a demographic response to urbanization cannot be determined. Nest density of social bees determines their effective population size (Darvill et al. 2004) and thus may be a better predictor of inbreeding than catch rates alone (Caballero, 1994; Geib et al. 2015). Additionally, urbanization can limit bumble bee gene flow, at least on a regional scale (Jha et al. 2013), and habitat destruction from urbanization may have triggered bottleneck effects in urban bee populations. Thus, future studies should consider using a population genetics approach to estimate the number of colonies and the effective population size, as well as gene flow and genetic diversity in urban and non-urban sites.

Despite making up only a ~20% of the flowering community, the one naturalized pollen species, *T. repens*, comprised 52% of the pollen diets of urban-site bees and over 90% that of non-urban-site bees. Bees may forage flexibly to select high quality pollen (Reudenauer et al. 2016), and *Trifolium* pollen in particular may be optimally nutritionally balanced for bees. The elemental stoichiometry of the *Trifolium* pollen is ideal with relation to bee development and growth needs (Filipiak et al. 2017) and is higher in amino acid phytosterol (Somme et al., 2015). Thus, foraging on *T. repens* may eliminate the needs for bees to incorporate diverse pollen in their diets in order to obtain adequate nutrition. Another reason for this result may be that bumble bees show high floral constancy, which increases their foraging efficiency. One study of bumble bees in urban habitats in the UK showed that bumble bees selected to forage on plants based on their abundance, which resulted in bumble bees collecting mostly from ornamental plants and not native plants (Hanley et al., 2014). However, pollen collection from *Trifolium* spp. and other members of the Fabaceae over ornamental flowers may be an emerging trend in urban foraging literature, at least for some species of bumble bees in the UK (Somme et al., 2015; Hülsmann et al., 2015; Goulson et al. 2005). Ornamental flowering species in particular can vary greatly in their color and morphology, and thus may be especially difficult for bees to switch between while foraging (Gegear & Lavery 1998, Goulson 2000). Additionally, ornamental varieties may be unattractive to flower-visiting insects (Garbuzov et al., 2014; Garbuzov et al., 2015; Garbuzov et al., 2017.) The results of this study suggest that bees disproportionately select *T. repens* pollen, possibly to efficiently meet their nutritional needs, at least in heterogeneous urban landscapes.

In summary, urban sites had different flowering communities, with much higher flowering densities and species richnesses. Yet, even when urban habitats had more flowers,

bumble bees were not necessarily more abundant. Additionally, despite its relatively low densities, bumble bees showed a strong preference for foraging on one naturalized species, white clover (*T. repens*), over ornamental and other categories of pollen. Although non-native, white clover was clearly an important forage species and one that should likely not be removed from urban landscapes in the creation of lawns and other cover types. Growing native plants for bee forage may be beneficial for bee and plant conservation (Tuell et al. 2008); yet, private gardens continue to use non-native ornamental plants common in popular commercial nurseries, and this study documents that bumble bees do not forage preferentially on these species, although they do incorporate them into their diets. While the increased floral resources in urban sites did not translate to increased bee abundance, it is important to note that urban sites did not have lower bumble bee abundance than non-urban ones. Thus, this study suggests that urban sites may hold conservation value for native bumble bees for forage, but simply continuing to plant more flowers and increase floral density in urban landscapes likely cannot be used as the sole mechanism to encourage bee conservation. Instead, studies should consider how other mechanisms may be interacting with flower density, such as pesticide, heat, and parasite exposure, to affect effective bee population sizes.

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FIGURES

Chapter 1

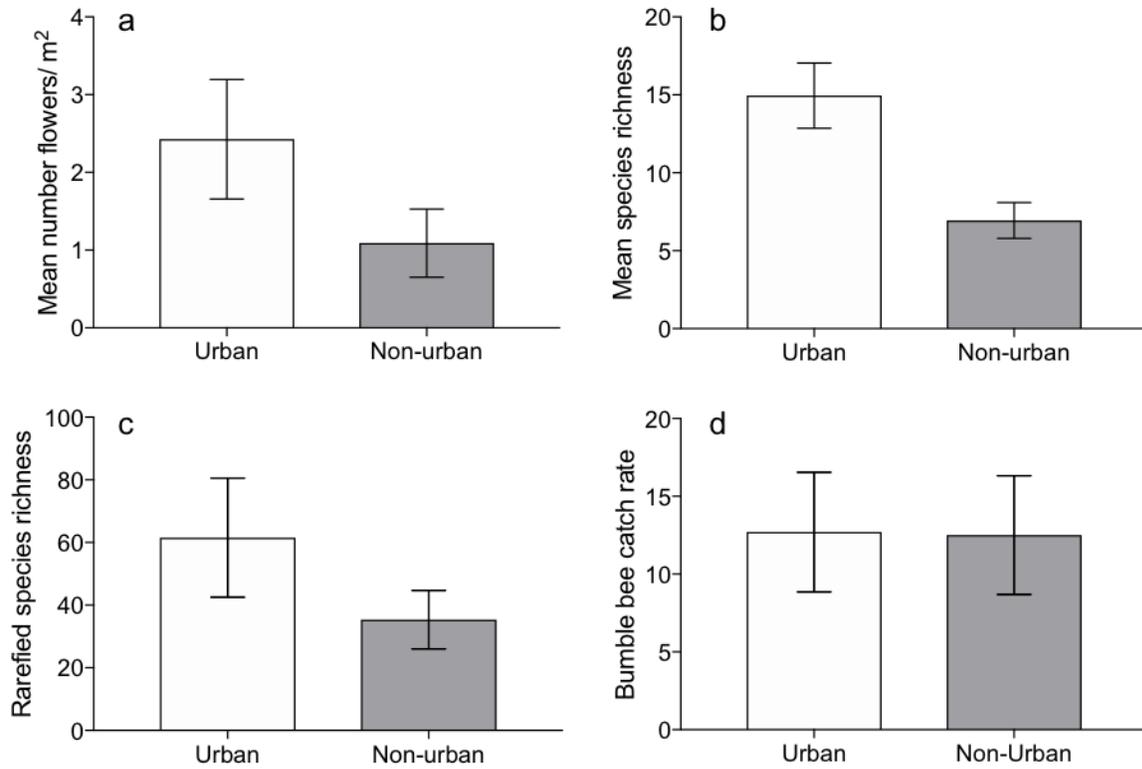


Figure 1. A comparison of urban and non-urban site (a) mean flower density, (b) mean species richness per day, (c) mean rarefied species richness per day, and (d) mean number of bees caught per person/minute per day. In panels a, b, and d, values are means \pm SE. In panel c, values are means \pm CI. Asterisks indicate significant differences between site-types at $P < 0.05$.

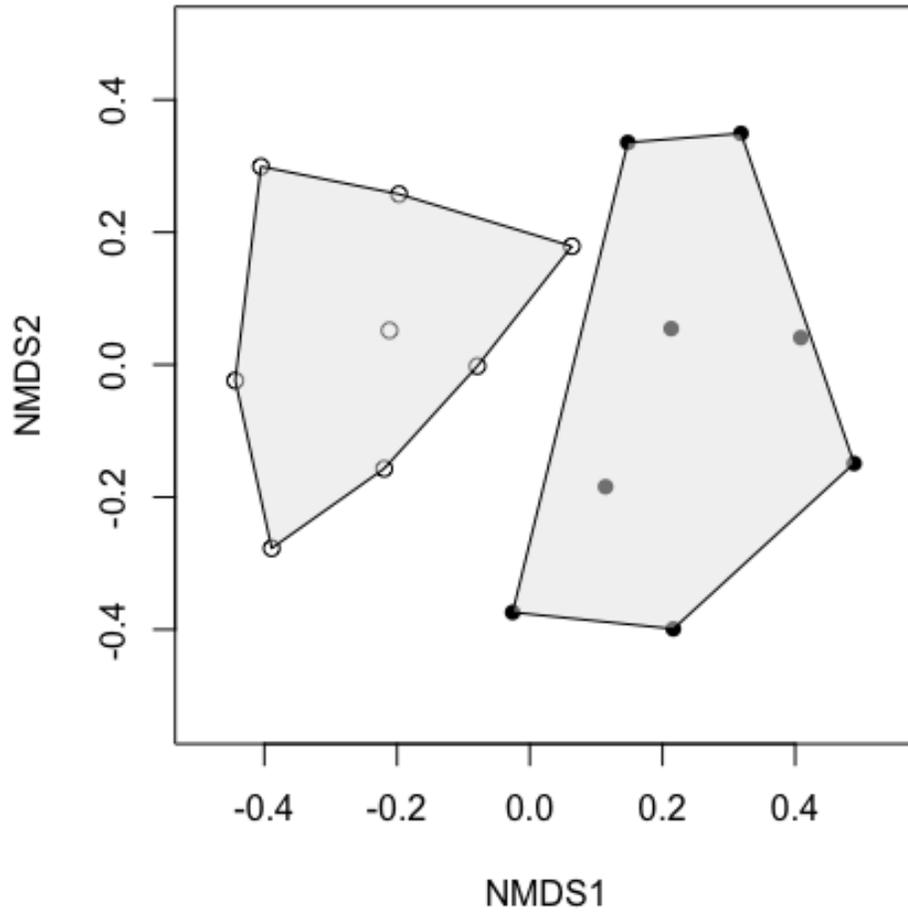


Figure 2. Non-metric multidimensional scaling of urban site (open circles) and non-urban site (closed circles) flowering species communities ($k = 2$).

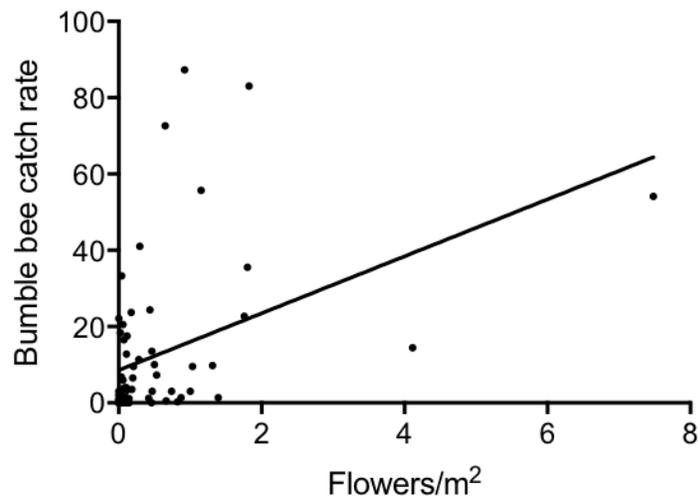


Figure 3. Bumble bee catch rates (bees per person hour) increased significantly with site-wide flower density per day across urban and non-urban sites.

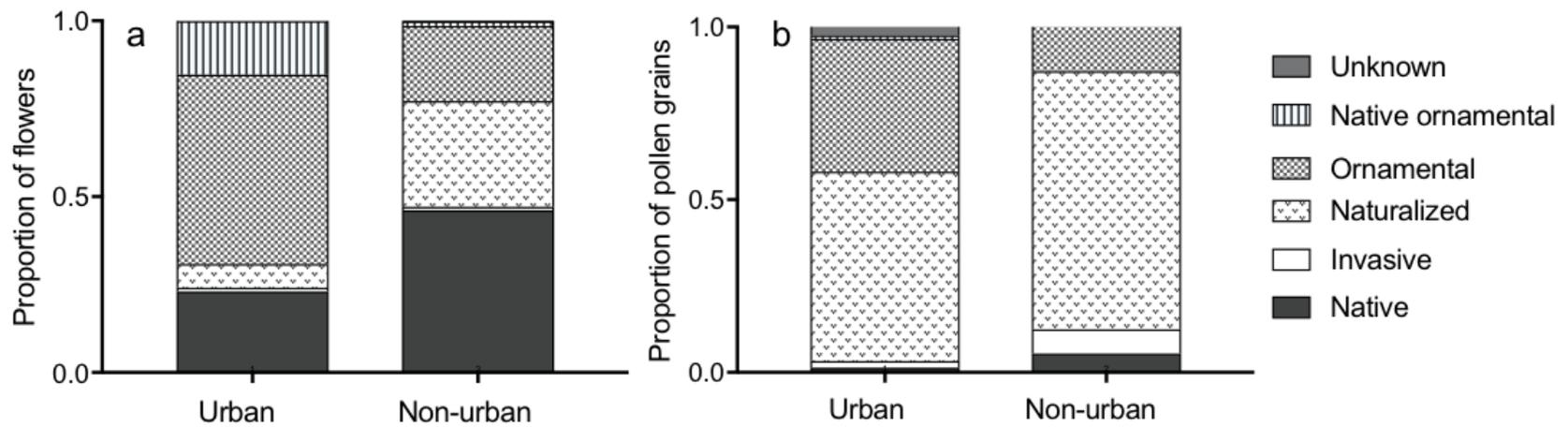


Figure 4. Mean proportions of (a) flowers in the flowering community and (b) pollen grains in pollen balls from each plant category.

TABLES

Table 1. Plant-category ranking matrices where values represent preferences for row plant types over column plant types for pollen preference by plant category for (a) urban and non-urban sites combined, (b) urban sites only, and (c) non-urban sites only. A plus (+) indicates that the plant category in row *i* is used more than the plant category in column *j*, and a negative (-) indicates the plant was used less. Triple signs indicate a significant deviation from random at $P < 0.05$. Usage rank indicates the ranking of plant categories for pollen load composition (by proportion of pollen grains); availability rank indicates the ranking of flower density per plant category, where 1=highest rank.

a	Invasive	Native	Native ornamentals	Naturalized	Ornamental	Unknown	Usage Rank	Availability Rank
Invasive	0	+	+++	-	---	+++	3	5
Native	-	0	+	---	---	-	4	3
Native ornamentals	---	-	0	---	---	-	6	4
Naturalized	+	+++	+++	0	+++	+++	1	1
Ornamental	+++	+++	+++	---	0	+++	2	2
Unknown	---	+	+	---	---	0	5	6

b	Invasive	Native	Native ornamentals	Naturalized	Ornamental	Unknown	Usage Rank	Availability Rank
Invasive	0	---	+++	-	---	+	3	5
Native	+++	0	+	---	---	-	5	3
Native ornamentals	---	-	0	---	---	-	6	4
Naturalized	+	+++	+++	0	+++	+++	1	2
Ornamental	+++	+++	+++	---	0	+++	2	1
Unknown	-	+	+	---	---	0	4	6

c	Invasive	Native	Naturalized	Ornamental	Unknown	Usage Rank	Availability Rank
Invasive	0	+++	-	+++	+++	3	4
Native	---	0	---	---	-	4	2
Naturalized	+	+++	0	+	+++	5	1
Ornamental	---	+++	-	0	+++	1	3
Unknown	---	+	---	---	0	2	5

SUPPLEMENTARY TABLES

Table S1. Site names and locations, with all sites located in Wake County or Chatham County, NC, USA. Type refers to whether it was classified as urban or non-urban. Average property value based on 6 randomly selected properties including single-family homes.

Site name	Type	Pair	North	West	Avg. property value
Lake Crabtree County Park	Wild	CB	35.84328	-78.79473	
Wessex	Urban	CB	35.82807	-78.77986	\$600,881
Jordan Lake State Recreation Area (Crosswinds Campground)	Wild	CW	35.74085	-78.99541	
Heritage Pointe	Urban	CW	35.74405	-78.97262	\$972,853
House Creek Trail	Wild	HCT	35.80745	-78.69238	
Beaver Damn Trail	Urban	HCT	35.80824	-78.67393	\$606,177
Falls Lake State Recreation Area - Holly Point Campground	Wild	HP	36.01132	-78.65356	
Bud Morris Rd.	Urban	HP	36.00637	-78.6184	\$302,057
Lake Benson Park	Wild	LB	35.66981	-78.62599	
The Landings at Lakemoor	Urban	LB	35.67981	-78.64341	\$197,316
William B. Umstead State Park (Reedy Creek Lake)	Wild	RCL	35.83608	-78.74494	
The Lakes at Umstead	Urban	RCL	35.82600	-78.73528	\$1,101,539
Falls Lake State Recreation Area (Rollingview Campground)	Wild	RV	36.01486	-78.72935	
Lakefall Neighborhood	Urban	RV	35.99300	-78.69777	\$354,249
Carl Alwin Schenck Forest	Wild	SF	35.81639	-78.72383	
Stoneridge Neighborhood	Urban	SF	35.82992	-78.71918	\$465,393

Table S2. a) Models testing bee catch rates (bees/person/hour) and b) AIC values for models in a.

a

Model ID	Model
1 (full)	Site type + Flower density + Flower richness + Week + Pair + Site type*Week + Site type*Flower density + Site type*Flower richness + Week*Flower density + Flower density*Flower richness + Week*Flower richness + Site type*Week*Flower density + Site type*Week*Flower richness + Site type*Flower density* Flower richness + Week*Flower density*Flower richness + Site type*Week*Flower density*Flower richness
2	Site type + Flower density + Flower richness + Week + Pair + Site type*Flower density + Site type*Flower richness + Flower density*Flower richness + Site type*Flower density* Flower richness + Site type+ Flower density + Flower richness + Week + Pair + Site type*Week + Site type*Flower richness
3	Site type + Flower density + Flower richness + Week + Pair + Site type*Week + Site type*Flower richness + Week*Flower richness + Site type*Week*Flower richness
4	Site type + Flower density + Flower richness + Week + Pair + Site type*Week + Site type*Flower density + Week*Flower density + Site type*Week*Flower density
5	Site type + Flower density + Week + Pair + Site type*Week + Site type*Flower density + Week*Flower density + Site type*Week*Flower density
6	Site type + Flower richness + Week + Pair + Site type*Week + Site type*Flower richness + Week*Flower richness + Site type*Week*Flower richness
7	Site type + Flower density + Flower richness + Week + Pair
8	Site type * Week + Pair
9	Week + Pair

Table S2 (continued)

b

Model ID	df	All species		<i>Bombus impatiens</i>		<i>Bombus bimaculatus</i>		<i>Bombus griseocollis</i>	
		AIC	Δ AIC	AIC	Δ AIC	AIC	Δ AIC	AIC	Δ AIC
1	1	533.7182	-	148.8698	92.18967	-64.71829	156.95741	-100.5282	164.8383
2	1	548.7636	15.0454	106.9169	50.23677	-141.3348	80.3409	-186.3637	79.0028
3	1	551.1275	17.4093	108.1304	51.45027	-138.0712	83.6045	-170.5552	94.8113
4	1	536.3884	2.6702	94.17163	37.4915	-152.1904	69.4853	-196.5078	68.8587
5	1	555.6622	21.944	83.52419	26.84406	-166.5947	55.081	-212.5124	52.8541
6	1	533.8787	0.1605	101.5815	44.90137	-147.4468	74.2289	-183.3627	82.0038
7	1	552.2018	18.4836	80.14597	23.46584	-187.2537	34.422	-226.6519	38.7146
8	1	551.0771	17.3589	67.66742	10.98729	-204.1314	17.5443	-244.6981	20.6684
9	1	556.5112	22.793	56.68013	-	-221.6757	-	-265.3665	-

Table S3. Species in (a) urban and (b) non-urban sites by plant category. Plants marked with an asterisk * were found in bee pollen loads in that site type, and those with a double symbol ** represented a mean proportion of >0.1 pollen grains in that site type. Mean proportions are given in parentheses for those making up >0.4 of pollen balls in that site type.

a

Plant Type	Family	Species	Found in bee pollen load	Mean prop. in pollen balls	
Invasive	Caprifoliaceae	<i>Lonicera japonica</i>	yes		
	Fabaceae	<i>Albizia julibrissin</i>	yes		
Native	Apiaceae	<i>Daucus carota</i>			
	Apocynaceae	<i>Amsonia</i> sp.			
	Aquifoliaceae	<i>Ilex opaca</i>			
		<i>Ilex</i> sp.			
		Asteraceae	<i>Cirsium</i> sp.		
			<i>Coreopsis</i> sp.		
			<i>Echinacea purpurea</i>		
			<i>Elephantopus</i> sp.		
			<i>Erigeron annuus</i>		
			<i>Erigeron</i> sp.		
			<i>Heracium</i> sp.		
			<i>Hieracium pratense</i>		
			<i>Hypochaeris radicata</i>	yes	
			<i>Krigia</i> sp.		
		<i>Packera</i> sp.			
	<i>Solidago</i> sp.				
	<i>Taraxacum officianale</i>				
	<i>Taraxacum</i> sp.				
	Brassicaceae	<i>Lepidium virginicum</i>			
	Commelinaceae	<i>Commelina communis</i>			
Cornaceae	<i>Cornus</i> sp.				
Ericaceae	<i>Chimaphila maculata</i>				
	<i>Oxydendrum arboreum</i>				
Euphorbiaceae	<i>Euphorbia corollata</i>				
Fabaceae	<i>Chamaecrista nictitans</i>				
	<i>Medicago lupulina</i>				
	<i>Stylosanthes biflora</i>				
	<i>Trifolium pratense</i>				
	<i>Hypericum punctatum</i>				
Hypericaceae					

Table S3 (continued)

	Iridaceae	<i>Sisyrinchium angustifolium</i>	
	Lamiaceae	<i>Scutellaria</i> sp.	
	Malvaceae	<i>Hibiscus</i> sp.	
	Mazaceae	<i>Mazus japonicus</i>	
		<i>Mazus</i> sp.	
	Melastomaceae	<i>Rhexia mariana</i>	
	Oleaceae	<i>Jasmine</i> sp.	
	Oxalidaceae	<i>Oxalis stricta</i>	
		<i>Oxalis violacea</i>	
	Paeoniaceae	<i>Paeonia</i> sp.	
	Phytolaccaceae	<i>Phytolacca americana</i>	
	Plantaginaceae	<i>Penstemon laevigatus</i>	yes
	Ranunculaceae	<i>Aquilegia canadensis</i>	
	Rosaceae	<i>Potentilla canadensis</i>	
		<i>Potentilla</i> sp.	
		<i>Prunus</i> sp.	
	Rubiaceae	<i>Diodia virginiana</i>	
		<i>Houstonia caerulea</i>	
		<i>Houstonia purpurea</i>	
		<i>Mitchella repens</i>	
	Scrophulariaceae	<i>Mimulus alatus</i>	
		<i>Nuttallathus texanus</i>	
	Solanaceae	<i>Solanum carolinense</i>	yes
	Violaceae	<i>Viola palmata</i>	
		<i>Viola</i> sp.	
		Fabaceae	
Native ornamentals		<i>Vinca</i> sp.	
	Apocynaceae		
	Asteraceae	<i>Rudbeckia hirta</i>	
		<i>Rudbeckia</i> sp.	
	Campanulaceae	<i>Lobelia</i> sp.	yes
	Crassulaceae	<i>Sedum</i> sp.	
	Ericaceae	<i>Rhododendron</i> sp.	
		<i>Vaccinium</i> sp.	yes
	Lamiaceae	<i>Salvia</i> sp.	
	Magnoliaceae	<i>Magnolia grandiflora</i>	yes
	Passifloraceae	<i>Passiflora</i> sp.	yes
	Plumbaginaceae	<i>Plumbago auriculata</i>	

Table S3 (continued)

Naturalized Ornamental	Polemoniaceae	<i>Phlox carolina</i> <i>Phlox nivalis</i> <i>Phlox</i> sp.		
	Rosaceae	<i>Prunus serotina</i> <i>Rosa</i> sp.	yes	
	Solanaceae	<i>Ruellia caroliniensis</i>		
	Fabaceae	<i>Trifolium repens</i>	yes	0.55
	Adoxaceae	<i>Viburnum plicatum</i>		
	Amaranthaceae	<i>Celosia</i> sp.		
	Amaryllidaceae	<i>Narcissus</i> sp.		
	Araceae	<i>Zantedeschia rehmannii</i>		
	Asparagaceae	<i>Hosta</i> sp. <i>Liriope muscari</i> <i>Yucca</i> sp.		
	Asphodelaceae	<i>Hemerocallis</i> sp.	yes	
	Asteraceae	<i>Centaurea</i> sp. <i>Dahlia</i> sp. <i>Leucanthamum</i> sp. <i>Stokesia laevis</i> <i>Tagetes</i> sp. <i>Unknown</i> <i>Zinnia</i> sp.	yes	
	Balsaminaceae	<i>Impatiens</i> sp.		
	Begoniaceae	<i>Begonia</i> sp.		
	Boraginaceae	<i>Lithodora diffusa</i> <i>Lithodora</i> sp.		
	Cannaceae	<i>Canna</i> sp.		
	Caprifoliaceae	<i>Abelia schumannii</i> <i>Kolkwitzia amabilis</i> <i>Scabiosa columbaria</i>	yes yes	0.044
	Caryophyllaceae	<i>Dianthus</i> sp. <i>Tradescantia pallida</i>		
	Geraniaceae	<i>Pelargonium</i> sp.		
	Hamamelidaceae	<i>Loropetalum</i> sp.		
	Hydrangeaceae	<i>Duetzia</i> sp. <i>Hydrangea</i> sp.		
	Iridaceae	<i>Iris germanica</i> <i>Iris pseudacorus</i> <i>Iris</i> sp.		

Table S3 (continued)

Lamiaceae	<i>Salvia nemorosa</i>	yes	
	<i>Salvia splendens</i>	yes	
	<i>Stachys byzantina</i>	yes	
Liliaceae	<i>Tulipa</i> sp.		
Lythraceae	<i>Lagerstroemia</i> sp.		
Oleaceae	<i>Ligustrum</i> sp.	yes	0.042
	<i>Syringa</i> sp.		
Onagraceae	<i>Oenothera speciosa</i>	yes	
Orchidaceae	<i>Unknown</i>		
Paeoniaceae	<i>Paeonia</i> sp.		
Plantaginaceae	<i>Veronica persica</i>		
Rosaceae	<i>Photinia</i> sp.		
	<i>Spiraea japonica</i>	yes	0.17
Rubiaceae	<i>Gardenia jasminoides</i>		
	<i>Gardenia</i> sp.	yes	
Sapindaceae	<i>Acer japonicum</i>		
	<i>Acer palmatum</i>		
Scrophulariaceae	<i>Buddleja</i> sp.		
Solanaceae	<i>Calibrachoa</i> sp.		
	<i>Petunia</i> sp.		
Verbenaceae	<i>Lantana</i> sp.		
	<i>Verbena</i> sp.		
Violoideae	<i>Viola</i> sp.		
-	UK 9 8.10.2016		
	UK 1 8.26.2016		
	UK 11 4.22.16		
	UK 13 4.22.16		
	UK 13 5.12.16		
	UK 17 5.26.16		
	UK 2 5.12.16		
	UK 26		
	UK 29 4.15.16		
	UK 29 6.7.16		
	UK 37 5.19.2016		
	UK 38 5.19.2016		
	UK 4 5.3.2016		
	UK 5 4.22.16		
	UK 5 6.16.16		
	UK 6 5.17.16		

Table S3 (continued)

UK 7 4.22.16
UK 7 5.27.2016
UK 7 6.16.16
UK 8 5.26.16
UK 8 6.20.2016
UK 8 8.10.2016
UK 8 9.9.2016
UK 9 5.26.16
UK 9 9.9.2016
UK MJ 8.19.2016

Table S3 (continued)

b

Plant Type	Family	Species	Found in bee pollen load	Mean prop. in pollen balls
Invasive	Fabaceae	<i>Albizia julibrissin</i>	yes	
		<i>Lespedeza sp.</i>		
	Caprifoliaceae	<i>Lonicera japonica</i>	yes	
	Vitaceae	<i>Ampelopsis glandulosa</i>	yes	0.07
Native	Apiaceae	<i>Daucus carota</i>		
	Araliaceae	<i>Aralia sp.</i>		
	Asteraceae	<i>Achillea sp.</i>		
		<i>Cirsium vulgare</i>		
		<i>Elephantopus carolinianus</i>		
		<i>Erigeron annuus</i>		
		<i>Erigeron sp.</i>		
		<i>Eupatorium perfoliatum</i>		
		<i>Helenium amarum</i>		
		<i>Hypochaeris radicata</i>	yes	
		<i>Krigia montana</i>		
		<i>Krigia sp.</i>		
		<i>Pyrrhopappus carolinianus</i>		
		<i>Solidago sp.</i>		
		<i>Taraxacum officianale</i>		
		<i>Taraxacum sp.</i>		
	<i>Vernonia noveboracensis</i>			
	Bignoniaceae	<i>Campsis radicans</i>		
	Brassicaceae	<i>Lepidium virginicum</i>		
	Colchinaceae	<i>Uvalaria perfoliata</i>		
	Commelinaceae	<i>Commelina communis</i>		
Ericaceae	<i>Vaccinium arboreum</i>			
	<i>Vaccinium sp.</i>	yes		
Fabaceae	<i>Centrosema virginianum</i>			
	<i>Chamaecrista nictitans</i>	yes		
	<i>Medicago lupulina</i>			
	<i>Trifolium campestre</i>			
	<i>Trifolium pratense</i>			
	<i>Triodanis perfoliata</i>	yes		

Table S3 (continued)

	<i>Vicia angustifolia</i>	
Gentianaceae	<i>Sabatia angularis</i>	
Geraniaceae	<i>Geranium carolinum</i>	yes
	<i>Hypericum</i>	
Hypericaceae	<i>punctatum</i>	
	<i>Sisyrinchium</i>	
Iridaceae	<i>angustifolium</i>	
	<i>Lamium</i>	
Lamiaceae	<i>amplexicaule</i>	
	<i>Salvia</i> sp.	yes
	<i>Scutellaria</i>	
	<i>integrifolia</i>	
Mazaceae	<i>Mazus japonica</i>	
	<i>Mazus</i> sp.	
	<i>Amianthium</i>	
Melanthiaceae	<i>muscitoxicum</i>	
Melastomataceae	<i>Rhexia</i> sp.	
Onagraceae	<i>Oenothera biennis</i>	yes
	<i>Oenothera</i> sp.	yes
Oxalidaceae	<i>Oxalis stricta</i>	
	<i>Oxalis violacea</i>	
	<i>Phytolacca</i>	
Phytolaccaceae	<i>americana</i>	
	<i>Penstemon</i>	
Plantaginaceae	<i>laevigatus</i>	yes
Ranunculaceae	<i>Ranunculus</i> sp.	
	<i>Thalictrum</i>	
	<i>thalictroides</i>	
Rosaceae	<i>Duchesnea indica</i>	
	<i>Potentilla canadensis</i>	
	<i>Potentilla fruticosa</i>	
Rubiaceae	<i>Diodia virginiana</i>	
	<i>Gallium</i> sp.	
	<i>Houstonia caerulea</i>	
	<i>Houstonia pusilla</i>	
	<i>Houstonia</i> sp.	
	<i>Nuttallanthus</i>	
Scrophulariaceae	<i>texanus</i>	
Solanaceae	<i>Solanum carolinense</i>	yes
Violaceae	<i>Viola bicolor</i>	
	<i>Viola palmatta</i>	
Asteraceae		yes

Table S3 (continued)

		Fabaceae		
Naturalized	Fabaceae	<i>Trifolium repens</i>	yes	0.75
Ornamental	Adoxaceae	<i>Viburnum</i> sp.		
	Asparagaceae	<i>Hosta</i> sp.		
		<i>Liriope muscari</i>		
	Caprifoliaceae	<i>Abelia schumannii</i>	yes	0.1
	Hydrangeaceae	<i>Duetzia</i> sp.		
	Verbenaceae	<i>Verbena</i> sp.		
Unknown		UK 1 6.15.16		
		UK 1 8.11.2016		
		UK 2 7.5.2016		
		UK 2 8.26.2016		
		UK 25 4.26.16		
		UK 3 8.11.2016		
		UK 4 5.2.2016		

CHAPTER 2: Elevated floral resources do not translate to bumble bee colony productivity in urban habitats

ABSTRACT

Urbanization has many effects on ecosystems, including changing primary productivity and flower availability, which can have cascading effects on consumers. For bees, urban gardens can provide abundance and diverse floral resources, which can increase the abundance and diversity of foraging bees. While most studies use catch rates to estimate population size, for social bees, effective population size is better predicted by the success of individual colonies in producing sexual individuals. In this study, we used *Bombus impatiens* colonies to test the effects of elevated flowering resources in urban relative to non-urban sites and food supplementation on correlates of bumble bee colony fitness, including colony weight gain, worker build up, worker body mass, and the production of sexual individuals (males and new queens). We found that urban sites had six-times higher floral resource density and compositional differences in the floral assemblages. Bees actively collected pollen in both urban and non-urban sites, but their pollen preferences differed. In urban sites, bees primarily foraged on ornamental plant category while in non-urban sites bees foraged on one naturalized species, *Trifolium repens*, and these foraging patterns were not solely based on floral availability. We found that despite exploiting the increase in resource availability in urban sites, colonies fared better in non-urban sites based on weight change rate, and only non-urban colonies benefited from food supplementation. These results suggest that other factors, including nutritional quality, pathogens, pesticides, or heat-island effects, may override food availability in predicting bumble bee colony success in urban habitats.

INTRODUCTION

Variation in resource availability can be an important factor affecting the population dynamics of organisms (Mittelbach et al., 2001). On the one hand, increased resource availability can propagate up food webs to affect the productivity, reproduction, and population dynamics of higher trophic levels in both terrestrial (Anderies et al., 2007) and aquatic ecosystems (Weber & Brown, 2013). Field experiments exploiting natural variation in resource availability as well as those using direct food supplementation have shown that various demographic parameters can be limited by resources in animals (Adler, 1998; Goldstein et al., 2016; Puida & Paglia, 2015), birds (Anderies et al., 2007), fish (Anderson, 1988; Jones, 1986), and insects (Silva et al., 2011; Pinheiro et al., 2002; Rohlf & Hoffmeister, 2004). On the other hand, a variety of ecological factors can interact with or override any benefits of increased resource availability for populations, including abiotic factors, variation in the quality of the food resource base and the timing in which it is provided, and top-down predation or parasitism (Anderies et al., 2007; Fortini et al., 2010; Nicolle et al., 2011; Rohlf & Hoffmeister, 2004). Thus, experiments are critical in determining the importance of food resource availability on the productivity and reproduction of populations, especially those of conservation concern.

Resource availability on the landscape can be strongly affected by global environmental change. There are compelling examples of how climate change, species invasions, and land-use change have affected primary productivity and plant species composition (Alstad et al., 2016; Christian, 2001; Hall et al., 2003; Levine, 2000; Panetta et al., 2018; Root et al., 2003; Shochat et al., 2006). For example, land-use change associated with the conversion of non-urban habitats into urban ones can be associated with reduced plant species richness, increased density of remaining species, especially those of horticultural value, and increased abundance of species

that exploit the urban environment, and competitive exclusion of native species (Shochat et al., 2006). However, the level of urban development can also have a strong effect on floral resources (Matteson & Langelotto, 2012). For example, in areas of low-level urban development, remnant forest fragments can provide food, shelter and substrate resources to a wide variety of animals (Brown & Freitas 2002), and private gardens can harbor high abundances and species richness of flowering plants (Carper et al., 2014; Gunnarsson & Federsel, 2014; Hall et al. 2017; Hope et al. 2003; McFrederick & LeBuhn, 2005). What remains unresolved in many of these instances is how these changes in plant and floral resources, both positive and negative, may be affecting higher trophic levels. Here we used natural variation in floral resource availability in urban and non-urban habitats combined with a food supplementation experiment to assess their effects on the growth and performance of native bumble bees.

As generalist foragers, bumble bees are prominent and important pollinators of both wildflowers and crop plants worldwide (Cameron et al., 2011; Kremen et al., 2002; Memmott et al., 2004). There are mounting concerns about the declines of many bumble bee species (Cameron et al., 2011; Kearns et al., 1998; Ploquin et al., 2013), and variation in habitat loss and floral food resources have been implicated in those declines (Biesmeijer et al., 2006; Bommarco et al., 2012; Grixti et al. 2009). While private and community gardens may increase flower availability and diversity, these floral resources may not be a panacea for urban bumble bee conservation. For example, bee larval growth has specific nutritive demands (Genissel et al., 2002), and pollen quality and diversity affect bee physiology and immunocompetence (Di Pasquale et al., 2013; Roger et al., 2017). However, gardeners may choose to plant non-native ornamentals that have evolved independent of the local pollinator assemblage and have been bred to alter their appearance, a practice that may reduce pollen and nectar nutrients or

accessibility (Comba et al., 1999; Corbet et al., 2001). Even within genera, there may be large variation in the quality of different ornamental plant varieties as a food source for bees (Garbuzov & Ratnieks, 2014). Bees in urban habitats are also exposed to agrochemicals (Botias et al., 2017), and ornamental plants on sale to the public are a significant source of pesticides to pollinators whether growers apply additional chemicals or not (Lentola et al., 2017). Thus, the management practices of urban gardeners, and even the foraging choices of bees in urban habitats could have a large impact on bee reproductive success.

To understand how urbanization affects flower communities and bee reproduction, we monitored flower density and species richness and performed a field experiment testing colony growth rates of the common eastern bumble bee, *Bombus impatiens*. Within both urban and non-urban sites, we included colonies that we did and did not experimentally supplement with nectar and pollen to directly test for food resource limitation as a function of habitat type. *Bombus* spp. have a large impact on plant diversity (Memmott et al., 2004) and may be particularly susceptible to extirpation in urban habitats (Winfrey et al., 2011). However, *Bombus* spp. are typically generalist foragers and thus should be capable of exploiting floral resources available in urban landscapes. Specifically, we addressed the following questions: (1) How do urban habitats vary in floral density and community composition? (2) How does the urban landscape affect the amount and composition of pollen collected by foraging bees? And (3) to what degree does urbanization affect bee colony growth rates and the magnitude of food limitation?

METHODS

STUDY SYSTEM

This study was conducted at twelve sites in the Raleigh-Durham metropolitan area (RDU hereafter) of North Carolina, USA where projected population growth is the second-highest of all

cities in the United States (US Census Bureau, 2010), and urban land use is predicted to double or triple by 2060 (Terando et al., 2014). All field work was conducted in the 2017 flowering and flight season. Sites were paired with $n = 6$ urban sites and $n = 6$ non-urban sites (Table S1). The urban sites in this study were defined as single-family residential neighborhoods; the sites contained a range of housing values, styles, and ages, but most were at or above the median housing value for the area (Chatham Co., 2018; Wake Co., 2018). Although we worked in residential neighborhoods, they were classified as urban based on their population density and association within urban metropolitan areas and urban clusters (US Census Bureau, 2010). Non-urban sites were city, state and county parks that were managed for their natural resources, which were mainly pine, hickory, and oak forests. Sites within pairs were the same size and were located at least 1 km but less than 5 km apart to control for landscape-level differences in habitat, while reducing the probability that bees foraged in both urban and non-urban sites. Site pairs were located at least 5 km apart to reduce the probability that bees could fly among pairs (Darvill et al., 2004; Geib et al., 2015; Knight et al., 2005; Osborne et al., 1999).

Bumble bees are eusocial insects that live in colonies with a single queen. Queens are produced near the end of the flowering season, mate, and overwinter before nesting in the spring. The queen rears a first brood of workers who mature and help rear subsequent workers, males, and new queens. The size of the colony, including the number of workers, eggs, larvae, nesting materials and food provisions, while not reproductive success per se, is a precursor to reproductive success – producing new queens and males (Muller & Schmid-Hempel, 1992). *B. impatiens* is native in the eastern United States and found from Maine to Florida, and as far west as Wisconsin, south to Louisiana (Williams et al., 2014). *B. impatiens* queens nest underground (Williams et al., 2014) and have flight periods from April-October. It is important to note that *B.*

impatiens is considered to have stable populations (IUCN, 2014), and may even be expanding its range (Williams et al., 2014). However, how the urban landscape affects *B. impatiens* reproduction is relatively unknown and was investigated here. Most studies use bee catch rates as a proxy for bee abundance and population dynamics; yet, for social insects such as bumble bees, the number of colonies that reproduce is a better estimate of effective population size (Caballero, 1994; Geib et al., 2015). Thus, this study will provide insight into how urban landscapes affect colony level reproduction in social bees.

FIELD METHODS

Flowering community. We monitored the flowering community at each site pair once per week from May-July. To estimate flower density at each sampling period, we counted the number of flowers in a 50 x 2 m transect for each microhabitat in the site (garden, lawn, forest, forest edge, roadside, meadow). We used GoogleEarth to define the area of each microhabitat in the sites and extrapolated the microhabitat flower densities from the transects to estimates of microhabitat abundances. To get estimates of site-wide flower density, we added all of the microhabitat abundances for that site on a single date and divided by the total site area. To monitor site-wide flowering species richness, we noted any additional flower species that fell outside the transect limits.

Colony growth and food limitation. We used commercial colonies of *B. impatiens* (BioBest Canada, Leamington, Canada) to test for differences in colony growth in urban vs. non-urban sites and as a function of food (pollen and nectar) supplementation. We used colonies with one queen and an average of 15 workers (range of 6-27 workers). We removed three workers from each colony and screened them for the parasite *Crithidia bombi*, a widespread trypanosome intestinal parasite of bumble bees (Shykoff & Schmid-Hempel, 1991a), using methods in

Richardson et al. (2015). Only uninfected colonies were used in this study to prevent spread of the parasite to resident bee populations. In each of the 12 sites, we placed three randomly selected colonies, and one of the colonies in each site was randomly assigned to the food supplementation treatment for the entirety of its life cycle to test for food limitation on colony growth in urban and non-urban sites. For all colonies, we recorded the initial number of workers and the initial colony weight before transporting the colonies to their respective field sites. Colonies were placed into the field on two dates: May 2, 2017 (Group A: 24 colonies in three site pairs) and May 24, 2017 (Group B: 24 colonies in the remaining 3 site pairs; Table S1).

In the field, colonies were placed in forested areas atop small, individual elevated platforms constructed from plasterboard and PVC pipes affixed to the ground using rebar. Colony boxes were secured to the platform using bungee cords. Each colony was protected from overheating by a corrugated plastic tent, and Tanglefoot © was used on the platform legs to prevent ant infestation. Within sites, colonies were placed approx. 10 m apart, and all colony entry points faced southeast. Prior to placing colonies in the field, all colonies were fed 400 mL of 30% sucrose solution and 5 g of pollen dough (fresh pollen collected by honeybees in Raleigh, NC mixed with 30% sucrose solution) for one week. For colonies not in the food supplementation treatment, the sucrose solution was removed after 3 days to prevent shock-related colony death and no additional sugar solution or pollen were provided. For colonies in the food supplementation treatment, they were given 400 mL of 30% sucrose solution and 5 g of pollen dough once per week. Any sugar solution or pollen that was not used the prior week was removed and refreshed weekly.

Once per week under red light after sunset, we photographed and weighed each colony to estimate colony growth. In addition, once per week on warm, clear days and during peak worker

bee foraging hours (10 am-2 pm), we monitored the activity at each colony for one hour, recording the number of workers that left the colony, the number of workers that returned to the colony, and whether returning workers carried corbicular pollen loads or not. After the one-hour period, we closed the colony doors and captured returning workers to collect one corbicular pollen load from each of 3-5 workers. For each corbicular pollen load, we weighed it, suspended it in 0.5 mL EtOH solution, and aliquoted 20 μ L of the pollen and EtOH solution to create fixed slides using basic fuschin dye (Kearns & Inouye, 1993). All pollen grains were compared to a pollen library of all flowering plant species found in the sites, identified and counted under a compound microscope to the lowest taxonomic level possible (species or genus).

Colonies were left in the field until we observed drones emerging or the production of queen pupal cells (approx. 3-6 weeks after placing colonies in the field), to avoid releasing reproductives from commercially reared colonies. Upon removing colonies from the field, they were placed in a -30°C freezer until processing. We weighed all colonies in the lab for a final colony weight, and we counted the number of workers, males, queens, larvae, and eggs. For each colony, we randomly selected up to 20 workers, removed the right forewing and measured radial cell length as an estimate of bee size (Harder 1982), and we are in the process of screening these workers for *Nosema* spp., external mites, tracheal mites, and Canopid fly larvae (Malfi & Raulston, 2014). We also counted and weighed (en masse) any other arthropods found inside the colony boxes and identified them to order or family.

STATISTICAL ANALYSES

Because bee colonies were placed in the field on two separate dates, resulting in Group A and Group B sites (Table S1), we analyzed data from the two groups separately for all analyses unless otherwise noted because of differences in the phenological progression of flowering in the

sites and colony formation. All statistical analyses were performed in R version 3.4.1 (R Development Core Team 2017) unless otherwise noted.

Flowering community. We compared site-wide flower density and flower species richness in urban and non-urban sites using the log-transformed value of site-wide flower density on each sampling date by site type (urban vs. non-urban) and sampling date with site and site pair as random effects using the lmer function from the lme4 package (Bates et al., 2015). We used EcoSim Pro to rarefy flower species richness down to the lowest common abundance and used a rarefaction randomization algorithm with 1000 iterations to understand how sampling effort affected flower species richness counts and to estimate flower diversity using Shannon's Diversity Index (Gotelli & Entsminger, 2000). To visualize how flower species community composition differed in the urban and non-urban sites, we used non-metric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity index from the R vegan package (Oksanen et al., 2011). The NMDS combined data across both Group A and B sites to insure enough site-level data for the analysis.

Additionally, we analyzed the flowering community based on flowering plant category. We used the North Carolina State Cooperative Extension categories to evaluate the proportion of flowering species types that existed in the sites by assigning each flowering species to one of six categories: native, invasive, naturalized, ornamental, native & ornamental, or undetermined (NC State Cooperative Extension 2017). We used these categories to understand the functional differences in urban and non-urban flowering communities and bumble bee foraging. We performed MANOVAs using the manova function in the 'dplyr' package in R to evaluate whether the flowering communities were different based on the proportion of the flower density that each flowering plant category comprised, with site type and pair as fixed effects, (because

random effects are not supported by manova). We then used mixed models to identify which categories were significantly different from each other using site type as a fixed effect and site pair as a random effect. We also computed the average rank order for the plant categories by site type using the proportion of the total flower density at that site on that date that each category comprised, and then averaging the ranks for plant categories for urban sites and non-urban sites.

Colony foraging and pollen ball composition. We analyzed whether bees returning to the colony box were carrying pollen balls or not (binary) using a mixed model with site type, group and food supplementation treatment as factors and site pair and colony as random factors. We calculated the rate of pollen collection as the total number of pollen-carrying bees returning to colony during the monitoring period times the average weight of the pollen balls collected for that monitoring period, times two (to account for both pollen balls each pollen-collecting bee carried), divided by the total number of minutes in the monitoring period. We used mixed models to compare the rates of pollen collection in urban vs. non-urban sites with sampling date and average flower density as covariates, food supplementation treatment as a fixed effect nested within site type, and site pair and colony as random effects using the `lmer` function from the ‘lme4’ package in R.

The identified pollen in pollen balls was categorized based on the NC State Cooperative Extension categories. We used the ‘adehabitatHS’ package (Calenge, 2006) to perform classical composition analysis (as in Aebischer et al., 1993) to identify which plant categories bees selected when pollen foraging by comparing what each bee selected to what was available in the site when pollen foraging (e.g., Davis et al., 2012.; Jha et al., 2013). The analysis compared matrices of floral species availability (the proportion of flowers per plant category per day) to matrices of pollen collection (the proportion of pollen grains per pollen ball for the categories).

Each individual bee was represented in the matrices so that the proportion of flowers of any given plant category in the site the bee was caught on the day of foraging was compared to the proportion of pollen grains from that bee's pollen load on that day. Pollen collection was examined by a randomization test (10,000 repetitions) using Wilks' lambda (λ) to test for preferences for plant category of all bees, urban-site bees, and non-urban-site bees, respectively. We then created ranking matrices to show the mean difference between the proportion of pollen use and the proportion of flowers to assess the significance of preference for one plant category over another (Aebischer et al., 1993; Calenge, 2006; Jha et al., 2013).

Colony growth and food limitation. To understand how the urban landscape affected colony productivity, we calculated the rate of colony weight change as final weight minus the weight of other arthropods in the colony minus the initial weight of the colony divided by the total number of days in the field. We used mixed models using the lmer function in the lme4 package with site type (urban vs. non-urban) as a factor, average site-wide flower density for each site (flowers/m²) as a covariate, site pair as a random effect, and food supplementation as a factor nested within site type to understand effects on the rate of colony weight change rate, average worker body mass (estimated using radial cell length), worker build up (final number of workers minus initial number of workers divided by days in the field). Sexual productivity (the number of males produced plus 3.9 times the number of queens produced to account for the cost ratios of queens to males (Bourke, 1997) was analyzed with a mixed model with site type (urban vs. non-urban) as a factor, average site-wide flower density for each site (flowers/m²), worker build up rate, and colony weight change rate as covariates, site pair as a random effect, and food supplementation as a factor nested within site type . Sexual productivity was only analyzed for

Group A colonies because Group B colonies generally performed poorly (see Results) and many did not produce sexuals even upon decline of the flowering season.

Colony arthropod associates. We calculated the biomass accumulation rate of arthropod associates by using the total weight of arthropod associates present when the colony was removed from the field divided by the number of days the colony was in the field. We used a mixed model to understand how arthropod biomass accumulation related to the weight change rates of the colonies (excluding arthropod biomass) using site type and food supplementation nested within site type as fixed effects and site pair as a random effect.

RESULTS

Flowering community. We found significant differences in some aspects of the flowering community between urban and non-urban sites, but the significance of those effects varied based on when colonies were placed into the field and thus when we started sampling flowers. For the sites in which colonies were placed into the field earlier (Group A), flower density was nearly six-times higher in urban compared to non-urban sites ($X^2_1 = 4.19$, $P = 0.041$; Fig. 1A). However, no difference in flower density was observed in Group B sites between urban and non-urban sites ($X^2_1 = 2.15$, $P = 0.14$; Fig. 1B). In a similar vein, mean flower species richness per day was 2.4-times higher in urban compared to non-urban sites for Group A ($X^2_1 = 4.62$, $P = 0.03$; Fig. 1C), representing 16.25 more species, on average, in urban sites. However, when we examined rarefied species richness, we found no difference between urban and non-urban sites for Group A as shown by overlapping confidence 95% intervals of rarefied means (2-9 for non-urban sites, 2-4 for urban sites; $P > 0.05$; Fig. 1D), suggesting that the higher species richness observed in urban sites was driven by sampling (i.e., increased flower density in urban sites). For Group B

sites, there were no differences in species richness (Fig. 1E) or rarefied species richness between urban and non-urban sites (95% CI for rarefied means 1-6 for urban sites, 2-9 for non-urban sites; $P > 0.05$; Fig. 1F). The NMDS showed little overlap in flowering communities of urban and non-urban sites (Fig. 2; Table S2). The overall stress value of the two-dimensional ordination was 0.189 indicating fair confidence in the model. Increasing dimensionality decreased the stress value (e.g., $k = 3$ had stress = 0.052), but the qualitative results were similar.

In the MANOVA testing for significant differences in the flowering community based on the proportion of flowers in the flowering community in each flowering plant category showed a significant difference between urban and non-urban sites ($F_{6,51} = 5.84$, $P = 0.00011$). Native species comprised higher proportions of the flowering density in non-urban sites than in urban sites ($X^2_1 = 14.47$, $P = 0.00014$). Native ornamental plants made up a larger proportion of flowers in urban sites ($X^2_1 = 5.56$, $P = 0.018$). There was no difference in the proportion of the community made up by the one naturalized species, *T. repens* ($X^2_1 = 0.17$, $P = 0.68$). Urban sites had 4x higher proportions of ornamental flowers than did non-urban sites ($X^2_1 = 19.73$, $P = 8.9e-06$). Surprisingly, invasive plants comprised higher proportions of the flowering density in non-urban sites than in urban sites ($X^2_1 = 3.9$, $P = 0.05$). There was no difference in the proportion of flowers from unknown plants in urban vs. non-urban sites ($X^2_1 = 2e-04$, $P = 0.99$). For urban sites the average rank of plant categories by their flower density (from greatest to least proportion of the community) was ornamental, naturalized, native, native ornamental, unknown, and invasive (Table 1). For non-urban sites, the average rank of plant categories by flower density was invasive, native ornamental, ornamental, unknown, native, and naturalized.

Colony foraging and pollen ball composition. Food supplementation treatment ($X^2_1 = 5.31$, $P = 0.021$) but not site type ($X^2_1 = 1.93$, $P = 0.16$) or group ($X^2_1 = 1.17$, $P = 0.28$) predicted whether a bee carried pollen or not. Surprisingly, only 35% of unfed bees returning to the colony carried pollen balls while 45% of food-supplemented bees carried pollen balls. Foraging rates were 1.6-times higher for Group A colonies compared to Group B colonies ($X^2_1 = 18.13$, $P < 0.0001$), and average foraging rate was positively correlated with rate of weight change ($X^2_1 = 11.09$, $P = 0.0009$). For Group A colonies, pollen collection rates were 1.3-times higher in non-urban compared to urban sites ($F_{1,30} = 6.20$, $P = 0.018$; Fig. 3A). Moreover, the covariate flower density was significantly associated with pollen foraging rate ($F_{1,30} = 19.34$, $P = 0.0001$), with increasing flower density associated with increased foraging rate. For Group B sites, there were no significant differences in pollen collection rates between urban and non-urban sites ($F_{1,50} = 0.05$, $P = 0.83$; Fig. 3B). Flower density was positively associated with pollen collection rates ($F_{1,45} = 11.39$, $P = 0.002$), and there was no significant interaction between site-type and flower density ($F_{1,44} = 0.30$, $P = 0.58$).

In total, we collected 266 pollen balls and identified 28 species (or morphospecies) of pollen in them. Overall, *T. repens* was the most-collected pollen species and represented an average of 41% of pollen grains across all habitats, with 45% in urban and 38% in non-urban habitats. Common ornamental species collected included *Spirea japonica*, *Abelia schumannii*, and *Ligustrum* sp. Common native species collected included *Campsis radicans*, *Amsonia* spp., and *Passiflora incarnata*. Surprisingly, collection of invasive species pollen was two orders of

magnitude greater in non-urban sites compared to urban sites, with pollen collected from *Lonicera japonica*, *Pueraria montana*, and *Albizia julibrissin*, and 28% of pollen collected in non-urban sites came from ornamental species.

Bees did not collect pollen in proportion to what was available (Fig. 3C-D). The overall randomization test including both groups (A and B) and both site types (urban and non-urban) was significant ($\lambda = 0.17$, $P = 0.011$). The overall ranking of pollen categories, from most to least preferred, was native ornamentals, naturalized (one species, *Trifolium repens*), ornamental, invasive, native, and unknown (Table 1). There was not sufficient data to further analyze groups and site types separately using all pollen categories so we chose the top 3 pollen categories from the overall analyses (native ornamental, naturalized, and ornamental) and compared preferences of urban and non-urban site bees respective to these three plant categories. For the non-urban bees, the preference ranking was native ornamental, naturalized, then ornamental ($\lambda = 0.51$, $P = 0.00001$). For urban bees, the ranking was native ornamental, ornamental, then naturalized, but the randomization test was non-significant ($\lambda = 0.96$, $P = 0.3$).

Colony growth and food limitation. Rate of colony weight change was 14-times higher in Group A compared to Group B colonies ($X^2_1 = 11.82$, $P = 0.0006$). For group A, site type was a significant predictor of weight change rate ($X^2_1 = 9.89$, $P = 0.0017$). Surprisingly, we found that non-urban colonies gained weight 5-times faster than urban colonies (Fig. 4A). Site type was not a significant predictor of worker build up rates ($X^2_1 = 1.22$, $P = 0.27$). Average flower density was a significant covariate and was positively correlated with the weight change rate ($X^2_1 = 7.33$, $P = 0.0068$; Fig. 4A) but not worker build up rates ($X^2_1 = 0.81$, $P = 0.37$) in group A. Group A colonies were not food-limited for worker build up rate ($X^2_1 = 0.11$, $P = 0.95$). Additionally for

Group A, there was an interaction between food supplementation treatment (fed or unfed) and site type, such that fed colonies in urban sites did not change weight significantly faster or slower, but colonies in non-urban sites changed weight significantly faster if they received food supplementation ($X^2_1 = 6.65$, $P = 0.036$; Fig. 4a,b). This interaction did not exist with respect to worker build up rates ($X^2_1 = 1.01$, $P = 0.60$). Finally for group A colonies, average body mass was not food-limited by site type ($X^2_1 = 1.78$, $P = 0.41$), but urban worker bees were bigger, on average, by approx. 10 mg ($X^2_1 = 5.24$, $P = 0.022$), and average site flower density was positively correlated with larger worker body size ($X^2_1 = 3.99$, $P = 0.046$).

For Group B colonies, site type was not a significant predictor of weight change rate ($X^2_1 = 0.14$, $P = 0.71$) but was a significant predictor of worker build up ($X^2_1 = 3.97$, $P = 0.046$). Worker build up rates were higher in urban sites ($X^2_1 = 3.97$, $P = 0.046$). Average site flower density was a significant positive covariate of worker build up ($X^2_1 = 5.09$, $P = 0.024$). Group B colonies were not food-limited for weight change rate by site type ($X^2_2 = 4.5$, $P = 0.11$), and average flower density was not a significant covariate ($X^2_1 = 0.64$, $P = 0.42$). For group B colonies, worker bees were not food-limited for body size ($X^2_1 = 0.42$, $P = 0.81$), and neither site type ($X^2_1 = 0.90$, $P = 0.34$) or average site flower density ($X^2_1 = 1.05$, $P = 0.31$) were significant predictor of worker bee body size.

When we examined the production of sexuals (males and queens), we found that in Group A, five colonies produced males only, two colonies produced queens only, and one colony

produced both queens and males. Colonies producing sexuals made between 1-186 males and 1-9 queens. Of the colonies in Group A that produced at least some sexuals, two were fed, non-urban colonies; three were unfed, non-urban colonies; two were unfed urban colonies, and two were fed urban colonies. In Group B, three colonies produced drones, but none produced queens. One was a non-urban, fed colony, one was a non-urban unfed colony, and the third was a fed, urban site colony. Neither site type ($X^2_1 = 0.42$, $P = 0.52$), average flower density ($X^2_1 = 0.002$, $P = 0.99$), nor food supplementation ($X^2_1 = 3.96$, $P = 0.14$) had any effect on the sexual productivity rate of Group A colonies. There was also no correlation between the weight change rate of the colony ($X^2_1 = 0.31$, $P = 0.55$) or worker build up rates ($X^2_1 = 0.0$, $P = 0.99$) and the sexual productivity score.

Colony arthropod associates. When we examined other arthropod associates dwelling within the field colonies, we found 2 members of the Araneae (adults), 18 Blattodea (adults), 521 Diptera (Stratiomyidae, larvae), 1 Hymenoptera, and 362 Lepidoptera (larvae and adults). The rate at which arthropod biomass accumulated in the colonies (weight of arthropod associates divided by days in the field) correlated positively with the weight gain rate of the colony (i.e., larger colonies also had larger biomass of arthropod associates ($X^2_1 = 7.02$, $P = 0.0081$)), but there was no effect of site type ($X^2_1 = 0.97$, $P = 0.33$) or food supplementation ($X^2_1 = 1.63$, $P = 0.20$).

DISCUSSION

Food limitation can have severe consequences on productivity and reproduction at higher trophic levels (Mittelbach et al., 2001), and global environmental change, including urbanization of the landscape, can strongly affect food availability, which could have cascading effects on

consumers. We found that despite having access to higher floral density in urban sites, Group A colonies on average grew faster in non-urban sites and were not food-limited for rate of weight change, suggesting that factors apart from flower density alone are impeding colony growth in urban sites. On the other hand, rate of weight change in Group B colonies was not affected by either flower density or site type, and in general, Group B colonies exhibited lower foraging and growth rates than Group A colonies. Moreover, colonies were not food-limited in either site type for Group B colonies. Combined, these data suggest that the urban landscape can negatively affect the productivity of bumble bee colonies in early founding colonies, but not through changes in food limitation. The mechanisms by which the urban landscape may be affecting the colonies are unknown but are discussed below and will be explored in future research.

On average, flower density and flower species richness were higher in urban sites compared to non-urban sites, suggesting that human alteration of the landscape, particularly planting ornamental gardens, is a main driver of flower communities in this area. Even the non-urban sites had some plantings of ornamental or native ornamental plant species, which at times drove the flower density and species richness of these sites as well. These results parallel results of our previous study of these sites (Chapter 1), as well as those of Carper et al. (2014). The results are also similar to other studies of urban flower communities, which find that planting gardens can have a major impact on local flower richness and abundance (Somme et al., 2016; Thompson et al., 2003) with subsequent increase of biodiversity (Gaston et al. 2005), and more abundant and species-rich bumble bee communities (Micholap et al., 2017; Plascencia & Philpott, 2017; Wojcik et al., 2008). The higher flower density and species richness observed in Group A compared to Group B sites may have been driven by phenology and the later start to sampling flowers in Group B sites. In particular, the delayed sampling meant we missed the

commonly planted, early-blooming shrubs that contribute substantially to floral density, (e.g. *Rhododendron* sp., *Rosa* sp.).

Flower density, not site type, was most important in predicting pollen provisioning rates, although site-type was also significant in the analysis, suggesting that there are aspects of the urban landscape that encouraged pollen provisioning rates independent of flower density. These results are similar to those of Kaluza et al. (2016), wherein bee foraging was highest in suburban gardens compared to forests and plantations. A higher percentage of food-supplemented bees carried pollen, which may indicate that the nectar resources provided were sufficient while the pollen resources provided were not., or that larger colonies had more workers available for food provisioning. Differences in spatial distribution of flowers in urban compared to non-urban sites may also be important in determining foraging and pollen provisioning rates as well. For example, when resources are patchier, bees may spend longer flying to find patches of flowers (Westphal et al., 2006). While quantification of spatial spread of resources was beyond the scope of this study, flower distributions in urban sites tended to be patchier as flowers were grouped in gardens, while flowers in non-urban sites were more evenly distributed across the landscape, and thus it is possible bees spent more time traveling to and/or locating flowers there. Yet, while group B colonies were not exposed to more flowers in urban sites, bee foraging rates were not different in urban vs. non-urban sites, suggesting the bees may compensate for differences in resource spread if it is an important factor.

In contrast to a study of the bee assemblage of northern California (Frankie et al., 2005), and another in southern New Jersey and central California (Williams et al., 2011) the bumble bees in this study preferentially selected ornamental species over native or native ornamental species, at least when pollen foraging. Wild bees in our previous study (Chapter 1) preferred

foraging on *Trifolium repens*, and other studies also indicate a preference for *Trifolium* spp. or other members of the Fabaceae (Hülsman et al., 2015; Goulson et al. 2005), which may provide higher-quality resources (Filipiak et al., 2017; Somme et al., 2015). This variation may reflect the generalist nature of bumble bees, or year-to-year variation in floral resources or bee preferences. Thus, this study provides evidence that bumble bees can act as urban exploiters with respect to resource provisioning.

For Group A, non-urban colonies grew much faster than urban-site colonies with respect to colony weight, despite their being much higher flower resources in urban sites. Worker build up, however, was not affected by site type, which likely reflects that bees store nectar and pollen and create their hive using pollen as well. Thus, while bees can continue accumulating mass of pollen, nectar, and larvae in the colony, worker build up is limited by the development of the larvae. Worker build up rate was the only predictor of sexual productivity, which may reflect that workers produced males, and/or that the costly production of queens only initiated after a sufficient number of workers were present to care for the larvae. In contrast to our results, similar studies using experimental colonies find that colony growth is positively correlated with floral resource diversity and abundance (e.g., Crone & Williams, 2016; Lanterman & Gooddell, 2018; Williams et al., 2012,). Thus, our results that increased resources in urban sites do not necessarily translate to bee productivity, suggest other factors, perhaps the types of flowers available in these sites, competition from other flower-visiting insects, abiotic factors, pesticides (e.g. Whitehorn et al. 2012, Larson et al., 2013) or pathogens may be limiting bumble bee colony productivity and potentially population dynamics. For example, urban sites are hotter than non-urban sites. Bumble bees in particular have a low thermal tolerance compared to other native bees and appear particularly intolerant of warming (Hamblin et al. 2017; Kerr et al. 2015). In

the field, this translated to lower abundances of bees in general, species community composition change, and a temperature-only model that predicted some bumble bees have lower abundances in urban sites than natural sites in Raleigh, NC (Hamblin et al. 2018). Changes in temperature change the behavior of the bees as well. When temperatures are too high, bumble bees have to spend time wing fanning (Vogt, 1986) which takes away from time spent doing more productive activities. Some studies have found temperature to decrease foraging activity and colony traffic of bumble bee hives, resulting in reduced colony development (Kwon & Saeed, 2003).

Additionally, insecticides used on common foraging habitat, such as *T. repens*, negatively influences bumble bee colony growth (Larson et al. 2013), and insecticide usage has been negatively related to bumble bee abundance, especially in urban gardens in France (Muratet & Fontaine, 2014). Additionally, the exposure of bees to pesticides may be elevated in urban habitats and (Botías et al., 2017; Lentola et al., 2017) and may increase their susceptibility to parasites and disease (Goulson et al. 2015). Future directions of this work include identification and quantification of pesticides in nest material as well as infection from *Nosema* spp., a microsporidian parasite that may decrease colony activity and queen oocytes (Shykoff & Schmid-Hempel, 1991b).

Non-urban colonies were food-limited only for weight change rate, and food-supplemented colonies in non-urban sites grew significantly faster than both fed and unfed urban colonies. These results suggest, again, that some other factor besides food limitation affects colonies in urban sites, given that fed urban colonies did not attain the weight of fed non-urban colonies. Bumble bees may often be food-limited in non-urban sites for some fitness correlates. For example, in a study of *B. appositus* colonies in subalpine meadows, nectar supplementation increased production of new queens but not of workers and males (Elliott, 2009). What is

interesting, however, is that certain characteristics of urban landscapes may diminish the effects of increased resource availability. For example, in birds, food supplementation by bird feeders in urban habitats has mostly positive effects but may not override the increased disease transmission caused by increased traffic on forage (Robb et al. 2008). Our results support that other factors besides food availability are limiting urban colony growth in urban habitats and parallels the results and problems with food supplementation of birds in urban areas.

Timing of initial colony placement had a major impact on colony growth and productivity. For Group B, it was clear that most colonies were in decline by the time they were removed from the field, with 9 of the 12 unfed colonies in Group B actually losing weight during the time they were in the field. In a study of food deprivation in *B. terrestris*, early food availability was crucial to long-term colony development (Rotheray et al. 2017). One explanation for the lack of flower density effects on colony growth in Group B is that the bees may have had access to flowering resources outside the site or permission boundaries, or that some flowers counted are not visited by bumble bees. Additionally, even if group B colonies had had access to the same *number* of flowers as group A, it was later in the season, and thus open flowers may have had depleted pollen resources. Our results suggest further support that early nest establishment is crucial to the development and productivity of bumble bee colonies.

In this study, native ornamentals were the most-preferred pollen forage for bumble bees, which was counter to results in prior research (Chapter 1). We suspect this difference may have been driven by the fact that we previously only caught bees foraging where we had access, whereas the bee colonies may have foraged in places where we did not have access. Yet, these results support similar studies that report bees use garden plants to supplement their diets (e.g. Harmon-Threatt & Kremen 2015). Additionally, the results of this and our previous study in

these sites (Chapter 1) indicate that bees also preferentially forage on white clover, *T. repens*. *T. repens* is a common forb in much of the United States, including the southeast, growing mostly in lawns and along roadsides, and is typically considered to be a weed. However, *T. repens* may be optimally nutritionally balanced for bees based on elemental composition (Filipiak et al., 2017), indicating that bees forage for quality of resources, as other studies have found (Somme et al., 2015; Vaudo et al., 2017). Taken together, these results suggest that bumble bees can thrive independent of community composition of flowers by regulating their foraging choices.

A number of caveats are important to the interpretation of these results, especially with respect to the use of commercial as opposed to local *B. impatiens*. First, it is important to note that the commercial nest boxes may have affected survival and invasion by arthropods. The nest boxes may be more susceptible to invasion, or at least invasion by a different suite of species, than the typical bumble bee nest, especially in urban habitats (Goulson et al., 2002). Second, the plastic commercial nest boxes may have increased temperature or humidity inside the colony, with potential reproduction or fitness costs, especially for Group B colonies, which were in the field during warmer weeks (data not shown). Third, the commercially-reared bees used in this study tended to collect pollen somewhat differently from wild bumble bees in our previous research from these sites (Chapter 1). Specifically, the wild bumble bee assemblage collected most of their pollen provisions from the one naturalized species, *T. repens*, in both urban and non-urban sites, whereas for the commercial bees, native ornamentals were preferred to *T. repens*. However, the commercial bumble bees still collected *T. repens* pollen in higher proportions to its availability in the sites, and thus, the difference may also represent year-to-year variation in pollen foraging rather than a difference in pollen preference.

In summary, even when the urban habitats in our study contained more flowers, bees inhabiting them did not necessarily fair better than bees in relatively more natural areas. On the flip side, bee colonies in urban habitats did survive to produce workers and some sexuals. Thus, the urban habitat can hold conservation value for native bees, but our data suggest that planting flowers alone should not be the sole management strategy. A number of factors may affect the relationship between resource availability and colony growth, including pesticide use, pathogen infestation rates, urban heat island effects, and the spatial arrangement or quality of floral resources. Mechanistic exploration of these factors may provide insight that will guide urban planning that supports native bee conservation.

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FIGURES

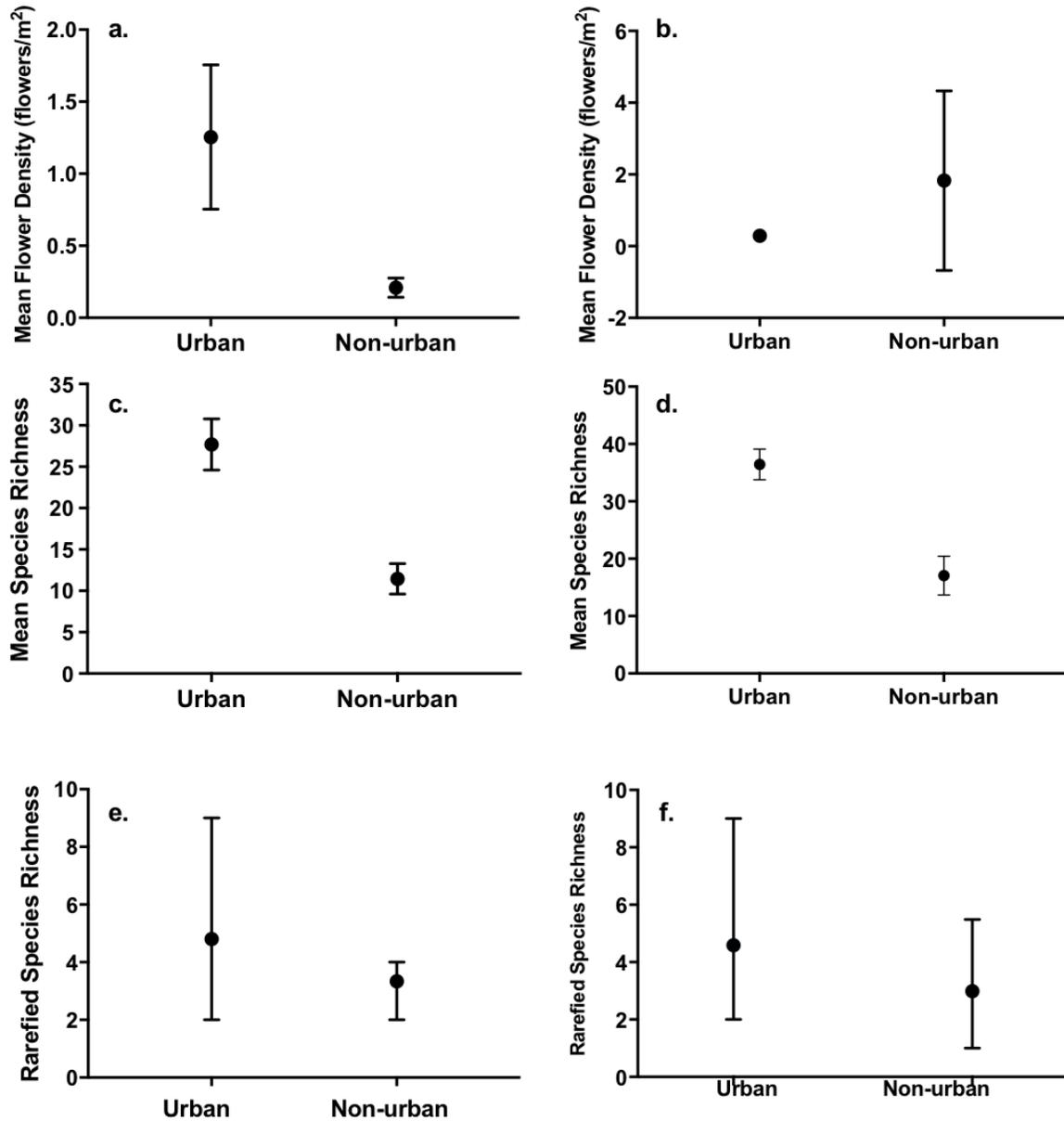


Figure 1. Flowering communities in urban and non-urban sites in Groups A (left) and B (right): (a and b) site-wide flower density (flowers/m²), (c and d) site-wide flower species richness, and (e and f) rarefied flower species richness. All points and bars for panels a-d are means \pm SE and for e-f are 95% CI.

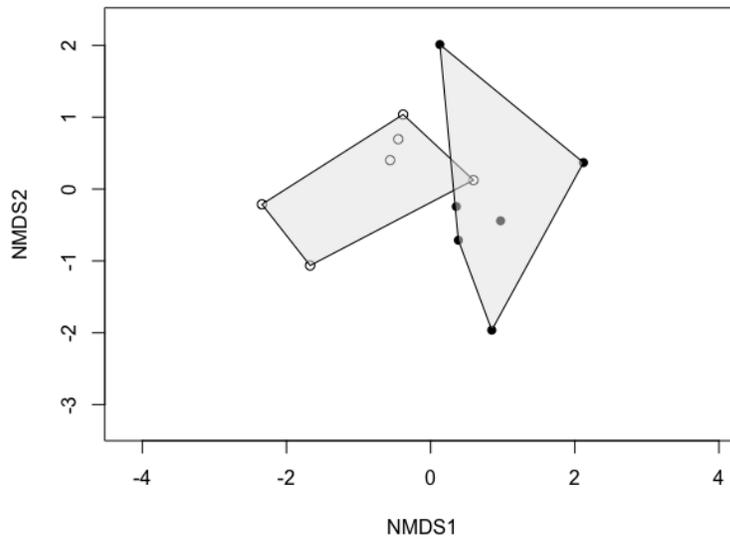


Figure 2. NMDS of the flowering community for non-urban (open circle) and urban sites (closed circle). There was little overlap in the flowering community composition between the two site-types ($k=2$).

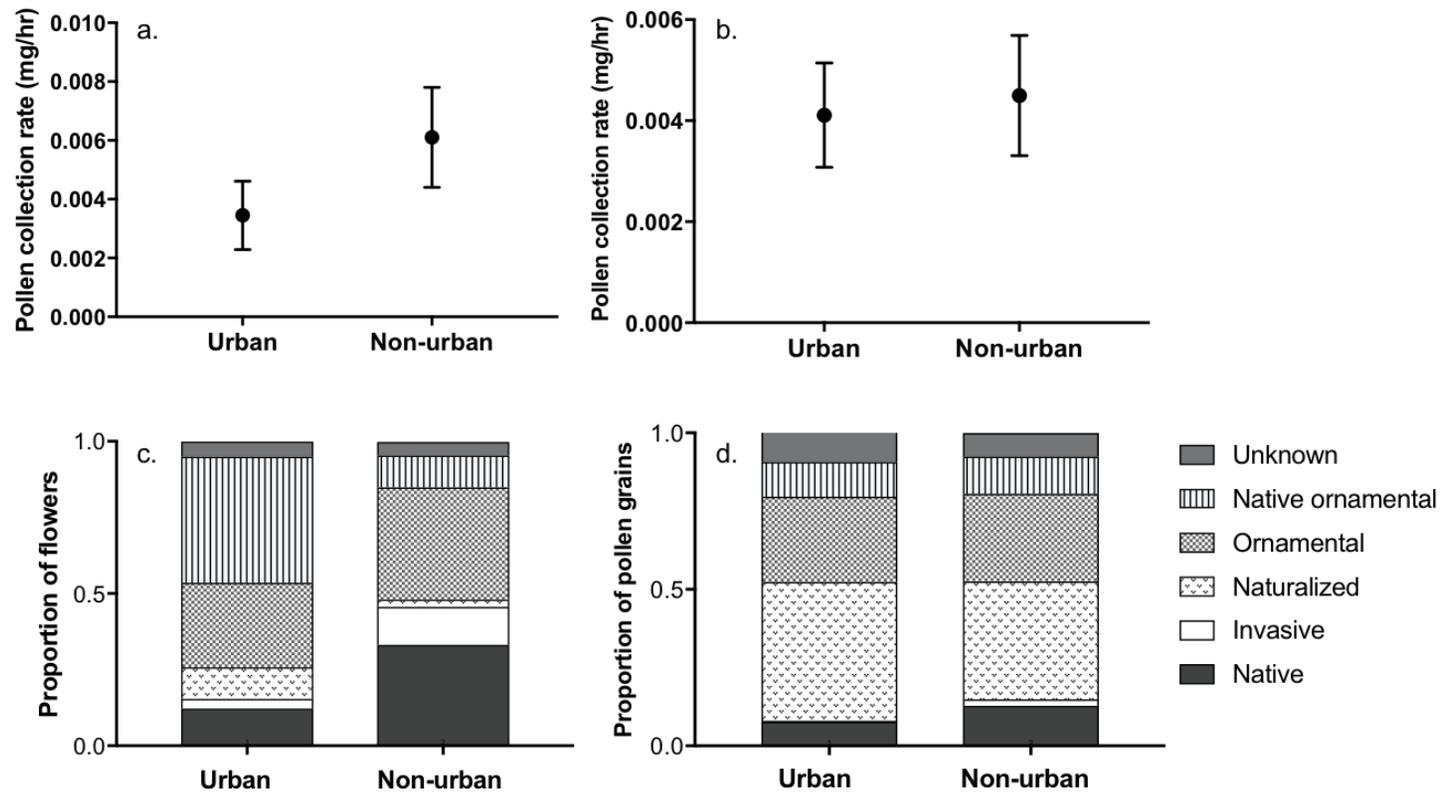


Figure 3. Foraging rates (mg pollen/hour) for (a) Group A colonies and (b) Group B colonies, and plant category proportions for (c) flowers in the community and (d) proportion of pollen grains in pollen balls.

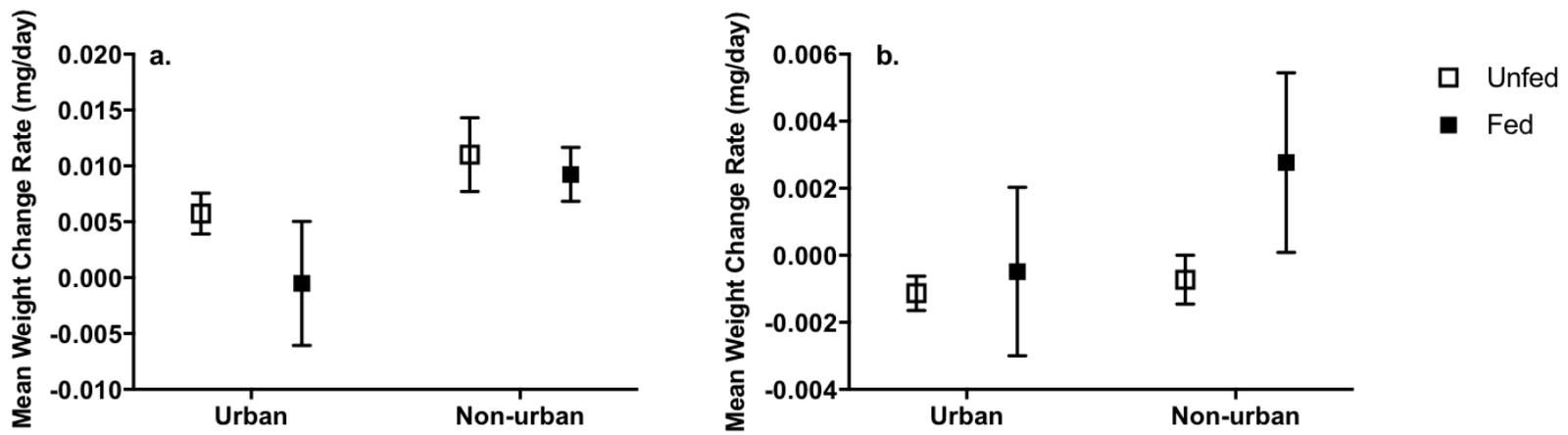


Figure 4. Mean rate of weight change (mg/day) \pm SE in urban and non-urban sites for colonies in (a) Group A and (b) Group B.

Tables

Table 1. Plant-category ranking matrices where values represent pollen foraging preferences for row plant categories over column plant categories for a) all sites, b) non-urban sites, and c) urban sites. A plus (+) indicates that the plant category in row *i* is used more than the plant category in column *j*, and a negative (-) indicates the plant was used less. Triple signs indicate a significant deviation from random at $P < 0.05$. Usage rank indicates the ranking of plant categories for pollen load composition (by proportion of pollen grains); availability rank indicates ranks of flower density per plant category, where 1=highest rank.

a	Invasive	Native	Native ornamentals	Naturalized	Ornamental	Unknown	Usage Rank	Availability Rank
Invasive	0	+	-	---	-	-	4	1
Native	-	0	---	---	---	+	5	6
Native ornamentals	+	+++	0	+++	+++	+++	1	3
Naturalized	+++	+++	+++	0	+	+++	2	5
Ornamental	+	+++	+++	-	0	+++	3	2
Unknown	+	-	+	---	---	0	6	4

b	Native ornamentals	Naturalized	Ornamental	Usage Rank	Availability Rank
Native ornamentals	0	+	+++	1	3
Naturalized	-	0	+	2	2
Ornamental	---	-	0	3	1

c	Native ornamentals	Naturalized	Ornamental	Usage Rank	Availability Rank
Native ornamentals	0	+	+	1	3
Naturalized	-	0	-	2	1
Ornamental	-	+	0	3	2

SUPPLEMENTARY TABLES

Table S1. Site names and locations, with all sites located in Wake County or Chatham County, NC, USA. Type refers to whether it was classified as urban or non-urban. Group refers to when colonies were placed in the field (and thus when flower sampling started). Group A colonies were placed in the field on May 2, 2017 and Group B colonies on May 23, 2017.

Site name	Type	Group	Pair	North	West	Avg. property value
Lake Crabtree County Park	Non-urban	B	CB	35.84328	-78.79473	
Wessex	Urban	B	CB	35.82807	-78.77986	\$600,881
Jordan Lake State Recreation Area (Crosswinds Campground)	Non-urban	B	CW	35.74085	-78.99541	
Heritage Pointe	Urban	B	CW	35.74405	-78.97262	\$972,853
House Creek Trail	Non-urban	A	HCT	35.80745	-78.69238	
Beaver Damn Trail	Urban	A	HCT	35.80824	-78.67393	\$606,177
Falls Lake State Recreation Area - Holly Point Campground	Non-urban	A	HP	36.01132	-78.65356	
Bud Morris Rd.	Urban	A	HP	36.00637	-78.6184	\$302,057
Lake Benson Park	Non-urban	B	LB	35.66981	-78.62599	
The Landings at Lakemoor	Urban	B	LB	35.67981	-78.64341	\$197,316
Falls Lake State Recreation Area (Rollingview Campground)	Non-urban	A	RV	36.01486	-78.72935	
Lakefall Neighborhood	Urban	A	RV	35.99300	-78.69777	\$354,249

Table S2. Species in (a) urban and (b) non-urban sites by plant category. Plants marked with an asterisk * were found in bee pollen loads in that site type, and those with a double symbol ** represented a mean proportion of >0.1 pollen grains in that site type. Mean proportions are given in parentheses for those making up >0.3 of pollen balls in that site type.

a

Plant Type	Family	Species	Found in bee pollen load	Mean prop. in pollen balls	
Invasive	Caprifoliaceae	<i>Lonicera japonica</i> *	yes		
	Fabaceae	<i>Albizia julibrissin</i> *	yes		
Native		<i>Pueraria montana</i>			
	Plantaginaceae	<i>Plantago lanceolata</i>			
	Apiaceae	<i>Daucus carota</i>			
	Asteraceae	<i>Cirsium vulgare</i>			
		<i>Coreopsis</i> sp.			
		<i>Echinacea purpurea</i>			
		<i>Erigeron annuus</i>			
		<i>Erigeron</i> sp.			
		<i>Hypochaeris radicata</i>			
		<i>Krigia</i> sp.			
		<i>Packera</i> sp.			
		<i>Solidago</i> sp.			
		<i>Taraxacum</i> sp.			
		Brassicaceae	<i>Lepidium virginicum</i>		
			<i>Commelina</i>		
		Commelinaceae	<i>communis</i>		
	Euphorbiaceae	<i>Euphorbia corollata</i>			
	Fabaceae	<i>Medicago lupulina</i>			
		<i>Stylosanthes biflora</i>			
		<i>Trifolium pratense</i>			
Hypericaceae	<i>Hypericum</i>				
	<i>punctatum</i>				
	<i>Sisyrinchium</i>				
Iridaceae	<i>angustifolium</i>				
Lamiaceae	<i>Scutellaria</i> sp.				
Malvaceae	<i>Hibiscus</i> sp.				
Mazaceae	<i>Mazus japonicus</i>				
	<i>Mazus</i> sp.				
Oxalidaceae	<i>Oxalis stricta</i>				
	<i>Oxalis violacea</i>				
Phytolaccaceae	<i>Phytolacca</i>				
	<i>americana</i>				

Table S2 (continued)

	Plantaginaceae	<i>Penstemon laevigatus</i>		
	Rosaceae	<i>Potentilla canadensis</i>		
		<i>Potentilla</i> sp.		
	Rubiaceae	<i>Diodia virginiana</i>		
		<i>Houstonia caerulea</i>		
	Solanaceae	<i>Solanum carolinense</i>		
	Violaceae	<i>Viola palmata</i>		
		<i>Viola</i> sp.		
Native ornamental	Asteraceae	<i>Echinacea purpurea</i>		
		<i>Eutrochium fistulosum</i>		
		<i>Helianthus</i> sp.		
		<i>Rudbeckia hirta</i>		
		<i>Rudbeckia</i> sp.		
	Cactaceae	<i>Opuntia</i> sp.		
	Campanulaceae	<i>Lobelia</i> sp.		
		<i>Lonicera sempervirens</i>		
	Crassulaceae	<i>Sedum</i> sp.		
	Ericaceae	<i>Rhododendron</i> sp.		
		<i>Vaccinium</i> sp.*	yes	
	Fabaceae	<i>Baptisia australis</i>		
	Lamiaceae	<i>Salvia</i> sp.*	yes	
	Magnoliaceae	<i>Magnolia grandiflora</i>		
	Passifloraceae	<i>Passiflora incarnate</i> *	yes	
	Plumbaginaceae	<i>Plumbago auriculata</i>		
	Polemoniaceae	<i>Phlox carolina</i>		
		<i>Phlox</i> sp.		
	Rosaceae	<i>Rosa</i> sp.*	yes	
Solanaceae	<i>Ruellia caroliniensis</i>			
Naturalized Ornamental	Fabaceae	<i>Trifolium repens</i> **	yes	0.45
	Amaranthaceae	<i>Celosia</i> sp.		
	Araceae	<i>Zantedeschia</i> sp.		
	Asparagaceae	<i>Hosta</i> sp.*	yes	
		<i>Liriope muscari</i>		
		<i>Yucca</i> sp.		
	Asphodelaceae	<i>Hemerocallis</i> sp.		
	Asteraceae	<i>Leucanthamum</i> sp.		
		<i>Stokesia laevis</i>		

Table S2 (continued)

	<i>Tagetes</i> sp.	
	<i>Unknown</i>	
	<i>Zinnia</i> sp.	
Balsaminaceae	<i>Impatiens</i> sp.	
Begoniaceae	<i>Begonia</i> sp.	
Berberidaceae	<i>Nandina domestica</i>	
Boraginaceae	<i>Lithodora diffusa</i>	
	<i>Lithodora</i> sp.	
Cannaceae	<i>Canna</i> sp.	
Caprifoliaceae	<i>Abelia schumannii</i> *	yes
Caryophyllaceae	<i>Dianthus</i> sp.	
	<i>Tradescantia pallida</i>	
Hamamelidaceae	<i>Loropetalum</i> sp.	
Hydrangeaceae	<i>Hydrangea</i> sp.	
Iridaceae	<i>Gladiolus</i> sp.	
	<i>Iris</i> sp.*	yes
Lamiaceae	<i>Lavendula</i> sp.	
	<i>Nepeta</i> sp.	
	<i>Salvia splendens</i>	
	<i>Stachys byzantina</i>	
	<i>Lagerstroemia</i>	
Lythraceae	<i>indica</i> *	
Oleaceae	<i>Ligustrum</i> sp.	yes
Onagraceae	<i>Guara</i> sp.	
	<i>Oenothera speciosa</i>	
Rosaceae	<i>Spiraea japonica</i> **	yes
	<i>Gardenia</i>	
Rubiaceae	<i>jasminoides</i>	
Scrophulariaceae	<i>Buddleja</i> sp.*	yes
Solanaceae	<i>Calibrachoa</i> sp.	
	<i>Petunia</i> sp.	
Verbenaceae	<i>Lantana</i> sp.	
	<i>Verbena</i> sp.	
Violoideae	<i>Viola</i> sp.	
Unknown	<i>UQ1CBUJUNE15</i>	
	<i>UQ1CBUJune6</i>	
	<i>UQ1CWUJUNE27</i>	
	<i>UQ1HCTJUNE12</i>	
	<i>UQ1HCTUJULY14</i>	
	<i>UQ1HCTUJUNE21</i>	

Table S2 (continued)

UQ1LBU7June
UQ1LBUJUNE19
UQ1LBUJune29
UQ1RVJune9
UQ1RVU20JUNE
UQ1RVUJULY14
UQ1RVUJuly3
UQ2CBUJune6
UQ2CWUJUNE27
UQ2HCTUJULY14
UQ2LBU7June
UQ2LBUJUNE19
UQ2RVU20JUNE
UQ2RVUJULY14
UQ3CBUJune6
UQ3CWUJUNE27
UQ3LBU7June
UQ3RVU20JUNE
UQ3RVUJULY14
UQ4LBU7June
UQ5LBU7June
UQ6LBU7June

Table S2 (continued)

b

Plant Type	Family	Species	Found in bee pollen load	Mean prop. in pollen balls
Invasive	Fabaceae	<i>Lespedeza sp.</i>		
		<i>Pueraria montana*</i>	yes	
	Caprifoliaceae	<i>Lonicera japonica*</i>	yes	
Native	Vitaceae	<i>Ampelopsis glandulosa</i>		
	Apiaceae	<i>Cicuta maculata</i>		
	Asteraceae	<i>Daucus carota</i>		
		<i>Achillea millefolium</i>		
		<i>Chrysogonum virginianum*</i>	yes	
		<i>Cirsium vulgare</i>		
		<i>Erigeron annuus</i>		
		<i>Erigeron sp.</i>		
		<i>Hypochaeris radicata*</i>	yes	
		<i>Krigia sp.</i>		
		<i>Pseudognaphalium obtusifolium</i>		
		<i>Pyrrhopappus carolinianus</i>		
		<i>Solidago sp.</i>		
		<i>Taraxacum officianale</i>		
		<i>Taraxacum sp.</i>		
	Uknown			
	Balsaminaceae	<i>Impatiens capensis</i>		
	Brassicaceae	<i>Lepidium virginicum</i>		
		<i>Commelina communis</i>		
	Commelinaceae	<i>Oxydendrum arboreum</i>		
Ericaceae	<i>Vaccinium arboreum</i>			
	<i>Vaccinium sp.**</i>	yes		
	<i>Euphorbia corrollata</i>			
Euphorbiaceae	<i>Medicago lupulina</i>			
Fabaceae	<i>Trifolium campestre</i>			
	<i>Trifolium pratense</i>			
	<i>Triodanis perfoliata</i>			
	<i>Wisteria frutescens</i>			

Table S2 (continued)

		Unknown		
	Gentianaceae	<i>Sabatia angularis</i>		
	Geraniaceae	<i>Geranium carolinum</i>		
		<i>Hypericum punctatum</i>		
	Hypericaceae	<i>Sisyrinchium angustifolium</i>		
	Iridaceae	<i>Lamium amplexicaule</i>		
	Lamiaceae	<i>Salvia</i> sp.		
		Unknown		
		<i>Scutellaria integrifolia</i>		
	Mazaceae	<i>Mazus japonica</i>		
		<i>Mazus</i> sp.		
	Melastomataceae	<i>Rhexia</i> sp.		
	Onagraceae	<i>Guara</i> sp.		
		<i>Oenothera biennis</i>		
		<i>Oenothera</i> sp.		
	Oxalidaceae	<i>Oxalis stricta</i>		
		<i>Phytolacca americana</i>		
	Phytolaccaceae	<i>Penstemon laevigatus</i>		
	Plantaginaceae	<i>Ranunculus</i> sp.		
	Ranunculaceae	<i>Potentilla canadensis</i>		
	Rosaceae	<i>Diodia virginiana</i>		
	Rubiaceae	<i>Houstonia caerulea</i>		
		<i>Houstonia</i> sp.		
	Solanaceae	<i>Solanum carolinense</i>		
Native ornamental	Bignoniaceae	<i>Baptisia australis</i>		
	Apocynaceae	<i>Asclepias tuberosa</i>		
		<i>Asclepias variegata</i>		
		<i>Bignonia capreolata</i>		
Naturalized Ornamental	Fabaceae	<i>Trifolium repens</i> **	yes	0.38
	Asparagaceae	<i>Hosta</i> sp. *	yes	
		<i>Liriope muscari</i>		
	Asteraceae	<i>Kalimeris pinnatifida</i>		
		<i>Liatris</i> sp.		
	Berberidaceae	<i>Nandina domestica</i>		
	Caprifoliaceae	<i>Abelia schumannii</i> *	yes	

Table S2 (continued)

	Lamiaceae	<i>Monarda didyma</i>
	Lythraceae	<i>Lagerstroemia indica</i>
	Xanthorrhoeaceae	<i>Kniphofia</i> sp.
	Verbenaceae	<i>Verbena</i> sp.
Unknown		UK1CBWJUNE15
		UK1CBWJUNE6
		UK1CWWJULY11
		UK1CWWJUNE2
		UK1HCTWMAY30
		UK1HPWJULY3
		UK2CBWJUNE6
		UK2CWWJUNE2
		UK2CWWJUNE27
		UK2HCTWJUNE12
		UK3CBWMAY25
		UK3HCTWMAY30
		UK3HPWMay11
		UK4CBWJUNE6
		UK4HCTWJULY5
		UK4HPWMay11
		UK5CBWJUNE6
		UK9CBWJUNE6
		UKLBWJULY13
		UKYELLOWTREE
