The effect of landscape on the distribution and dynamics of pollinators in anthropogenically modified landscapes

By

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ABSTRACT

Insect-mediated pollination supports flowering plants in almost every ecosystem on earth. Consequently, current global declines in pollinators and pollination services have the potential to impact global ecosystem productivity and food security. Most wild plants rely on insect pollinators to produce abundant fruit and seed, and maintain genetic diversity within populations. Declines in insect-mediated pollination services reduce the quantity and quality of seed production. These pollinator declines are associated with causal factors such as parasites, pathogens, and pesticides, but habitat loss is considered to be the primary driver. This global circumstance of pollinator loss has triggered a vibrant investment of communities in pollinator conservation, leading to a call for robust conservation science that investigates pollinator habitat and resource requirements. Research that builds a deeper understanding of the ecological mechanisms that sustain rich pollinator communities and the most functionally important species can tailor restoration practice most effectively to target the limiting factors that shape pollinator diversity in urban and agricultural systems.

My dissertation work investigates the influence of landscape and local site level factors on pollinator communities in urban and agricultural ecosystems. These studies emphasize the influence of temporal variation in nesting and food resources at two scales, by evaluating the impact of neighborhood development on local bee communities in Madison, WI in Chapter 1, and the influence of the temporary resource pulse of cranberry flowers in Central Wisconsin on bumble bee foraging behavior in Chapter 3. I investigate the spatial distribution of floral resource

variables and foraging bumble bees across an urban ecosystem in Chapter 2, and the availability of semi-natural habitat in the landscapes surrounding bee sampling sites at multiple scales in Chapters 1 and also in Chapter 3.

Improving our understanding of the interplay between physical and biological resources, and pollinator population dynamics can help to further the ecological foundation of pollination conservation, in order to maintain biodiversity and bolster food security. Each chapter elucidates ecological mechanisms that drive the spatial and temporal variation in pollinator density. Understanding the relative influence of various landscape factors on ecological processes associated with pollinator habitat use is the most essential goal of this dissertation, and multiple approaches have been applied to that end.

FOUNDATIONS

Most ecosystem functions, like pollination, are derived from interactions between species and the physical environment. Complex ecosystems sustain all extant species, yet the mechanisms contributing to their structure and function are difficult to tease apart in complex, natural systems. In different environmental contexts, the influence of important landscape or site specific factors may vary. The field of landscape ecology has brought ecology together with spatial analysis and scale-explicit study design to ground the conceptual basis of ecological research at the landscape scale. While pattern analysis techniques have stabilized in past decades, research methods to investigate complex inter-specific interactions and ecosystem processes moderated by space and time are still rapidly developing.

Well-designed manipulative experiments are the most effective way to infer causality and understand isolated mechanisms by deductively testing hypotheses which act at particular spatial scales within real-world, social-ecological systems. Manipulative experiments provide the framework that may be used to infer causation by interspersing treatments randomly to replicate experimental units in the study population. Yet, a major disadvantage to this approach is the prior knowledge needed to design the scale of the experiment correctly and to apply the target mechanism of the experiment in isolation. A narrow focus on isolated mechanisms can also distance us from their manifestation in the real world.

Mensurative experiments, heavily utilized in this dissertation, allow us to deductively test hypotheses at specific spatial scales in real-world systems where many ecological mechanisms interact. This type of study relies on the construction and testing of an ad-hoc hypothesis, by

selecting study sites that can be used to contrast the effects of the hypothesized mechanism. This is an important step to understand if a given mechanism applies a strong enough effect to produce a hypothesized, testable pattern, in a given study system. Ecological processes tend to manifest in testable patterns at a finite range of spatial scales (ie. resolution and spatial extent). Therefore, undertaking and communicating the results of landscape ecology studies with explicit definition of spatial and temporal scale is essential. Scale explicitness also helps to ensure results that translate to human land use decisions and ecosystem management.

Understanding the comparative influence of relevant landscape scale and site level factors on ecological processes associated with pollination systems is the most essential goal of this dissertation, and multiple approaches are applied in both urban and agricultural ecosystems.

The quality and amount of habitat in the surrounding landscape are known to influence ecological community composition. However, the sensitivity of species to disturbance varies widely across taxa and based on the intensity of the disturbance. The sensitivity of pollinators to change in land cover caused by urbanization and suburbanization has been investigated in recent studies, but it remains unclear how quickly succession processes may recover lost species richness after a disturbance. The role of surrounding land cover, site level floral resources, and property development history is investigated in **Chapter 1**.

Chapter 2 of this dissertation zooms into the influence of floral resource characteristics on foraging bumble bee presence across commercial, residential, agricultural zones and city parks in Madison, WI. This investigation sheds light on the relative influence of flower cover, flower density, species richness, and prairie and lawn flower species richness as a subset of total

species richness on the number of foraging bumble bees per transect as well as the influence of land use zones, where the baselines for these various factors differ. This study investigates the patchiness of floral resources and foraging bumble bees across the urban landscape, as well as non-stationarity in the influence of floral resource explanatory variables on prediction of foraging bumble bees.

In **Chapter 3**, I assess bumble bee colony density across cranberry marshes nested in high intensity agricultural landscapes, as well as more heterogeneous matrices of forest and semi-natural vegetation, before, during, and after the cranberry bloom. I study how landscape variables and the resource pulse of the cranberry bloom in Central Wisconsin influence the density and representation of bumble bee colonies at marshes. I measured colony representation as a detection rate representing the relative numbers of bumble bees representing colonies on average, in order to illustrate a potential dilution effect caused by the resource pulse, or surrounding land cover. This PhD research utilizes thoughtfully designed mensurative experiments and population genetic analysis to test spatial and temporal ecological hypotheses. The results of these research projects provides pollinator community species richness estimates, bumble bee colony density estimates and landscape effect estimates that may be useful to increase our spatially explicit understanding of insect-derived ecosystem service provisioning.

RESEARCH GOALS

Through this research, I improve our spatially explicit understanding of pollinator movement and resource use, and the influence of landscape structure and change on bee community diversity and bumble bee foraging behavior. I consider the ecological methods and spatial scales through/at which this research has been approached in past studies and implement carefully designed studies that assess pattern at the most mechanistically relevant scales. This work targets processes that occur at local to regional spatial scales, relevant to land use planners and resource managers.

RESEARCH GOAL 1

The first goal is to analyze the influence of surrounding land cover, and land use history on site scale bee species richness (α-diversity) and landscape scale diversity in bee species assemblage (β-diversity) in urban ecosystems. This research will answer the following questions:

1. What are the relative effects of surrounding land cover (i.e. impervious surface, vegetation), and land-use history (i.e. average property development year) on bee community richness.

2. What are the relative effects of surrounding land cover (i.e. impervious surface, vegetation), and land-use history (i.e. average property development year) on landscape level diversity in bee community assemblage.

This research will be submitted to Urban Ecosystems, in 2019 titled, "Urban land cover and property development history influence bee diversity in a rapidly-growing midwestern city"

RESEARCH GOAL 2

The second goal is to investigate the distribution of floral resources across commercial, residential, agricultural zoned land and city parks, and the influence of floral resource variables in prediction of bumble bee foraging. This research will answer the following questions:

1. How are floral resources and foraging bumble bees distributed across the urban landscape?

2. What are the relative effects of land use (i.e. zones) and floral cover, density and flowering plant species richness on bumble bee foraging frequency?

3. Is there non-stationarity in the relationships between floral resource variables and bumble bee density across the urban extent?

This resulting journal article, tentatively titled, "Buzzing around the city: the distribution of floral resources and foraging bumble bees in a city" will be submitted to "Diversity and Distributions" in 2019.

RESEARCH GOAL 3

The third research goal is to investigate the influence of the surrounding landscape and cranberry resource pulse on bumble bee colony density and detection at cranberry marsh sites located in the Central Wisconsin cranberry growing area. This research will answer the following questions:

1. How does surrounding land cover content and configuration influence bumble bee density and colony detection?

2. How does the resource pulse of the cranberry bloom influence bumble bee density and detection?

3. How does surrounding land cover moderate the influence of the resource pulse on bumble bee density and detection?

The forthcoming research article titled, "Woodland and floral richness boost bumble bee density in cranberry resource pulse landscapes" will be published in the journal Landscape Ecology in 2019.

BROADER IMPACTS

This dissertation combines new tools and methodologies to answer pressing questions regarding pollination services in complex, heterogeneous landscapes. Traditional biogeography studies use primarily observation based data, but other types of data can bring different pools of information to pollination research. Landscape genetics is an emerging field that harnesses advancing biological technology to answer landscape ecology questions, through increased power of biological detection at multiple spatial and temporal scales. Similarly, remotely-sensed data provide improved resources to describe underlying physical and biological variability in landscapes. This dissertation combines physical environmental data and species observations or genetic data to investigate pollinator foraging biology and distribution.

CHAPTER 1: BEE ABUNDANCE AND DIVERSITY RESPONSE TO URBANIZATION IN MADISON, WI, USA

INTRODUCTION

Urban ecology is a rapidly developing field, expanding in conjunction with widespread urban development that transforms the Earth's surface, yet urban landscapes are still relatively understudied in comparison to natural and agricultural landscapes. The heterogeneous distributions of semi-natural and built structure that comprise urban landscapes provide habitat resources that sustain urban ecological communities. Increased impervious surface and decreased vegetation often reduce the number of species in urban communities, but supplemental irrigation and cultivated gardens also add biotic diversity and habitat (Rebele 1994). Organisms differ in their sensitivity to urbanization and small, mobile animals like pollinators can thrive despite extremely high rates of habitat disturbance and species turnover, as well as high fine-scale heterogeneity of abiotic and biotic variables (Fetridge et al. 2008; Matteson et al. 2008; Hall et al. 2016; Theodorou et al. 2016). In urban ecosystems, ecological communities are more exposed to habitat fragmentation, habitat loss and loss of interspecific relationships that maintain diversity (Rebele 1994, Rosenzweig 2003). These vulnerabilities carry increased risk for urban communities, for example, urban habitat fragmentation has been associated with the loss of plants and their associated pollinators, especially plants that are reliant on outcrossing by animal pollination (Biesmejer et al. 2006). Urban ecological research that investigates the relationship between ecological community diversity, land cover, and land use history can inform urban

landscape design to increase biodiversity and ecosystem services, in ecotypes that support the majority of the world's human population (Daily et al. 1997).

Native bee populations contribute to the diversity of urban ecosystems by pollinating native plants as well as ornamental and agricultural plants. More than 70% of wild plant populations are pollination limited, meaning that they would produce more fruit and seeds with more pollination (NRC 2007). Diverse and abundant native bee communities improve pollination quantity and quality, and provide pollination services across complementary phenological periods because different bee species are active during different time periods (Hoehn 2008, Garibaldi 2013). As cities grow around the world, urban and suburban agriculture is also on the rise further emphasizing the importance of maintaining urban pollinators to grow food where people live (Hodgson et al. 2011).

Natural and semi-natural habitats tend to support a diverse array of native flowering plants for pollinator food resources, as well as complex bee nesting habitat such as protected cavities, loose soil, and pithy stems (Wojcik et al. 2008). Several studies from around the world observe that habitat simplification in human-dominated ecosystems negatively impacts pollinator populations (Winfree et al. 2011, Gonzales et al. 2013, Martins et al. 2013). Yet, high bee diversity has been noted in cities like New York, NY, USA and Chicago IL, USA (McFrederick and LeBuhn 2006; Matteson et al. 2008; Fetridge et al. 2008). Urban land cover can provide diverse floral resources and phenological consistency, especially when gardens are managed to provide flowers for a longer duration than other surrounding semi-natural ecotypes (Goddard et al 2010, Threlfall et al. 2015). One study observed increased rates of bumble bee colony growth in urban landscapes (Goulson 2002). However, urbanization has also been observed to exclude

cavity-nesting bees, eradicating particular functional guilds of bees associated with this degradation of required habitat (Larsen 2005, Jha and Kremen 2013).

Pollinators' use of diverse types of habitat may account for varying sensitivity of species to particular types of disturbance. Colony-building bees require larger cavities than above-ground nesting solitary bees, that may use embankments, or wood, or pithy stems. Many species of solitary bees dig tunnels into the soil. Man-made structures can in some cases supplement habitat, by providing stone walls, wooden structures, and various other cavities, as well as bare ground and loosened soil, yet these habitats can also be degraded due to soil compaction and habitat simplification due to removal of woody debris and hollow stems. A study of urban areas noted the decline of soil nesting bees compared to above ground cavity nesting bees (Cane et al. 2006). By investigating what aspects of land cover and land use underlie trends in species filtering, we may increase our capacity to restore the resources that are lost along with associated taxa.

This study of bee communities across the cityscape of Madison WI, USA was undertaken to illuminate the influence of land cover and land use history (e.g., property development) on urban bee communities across a rapidly growing mid-sized city. Rather than investigating an urban to rural gradient, we investigate sites randomly distributed throughout the heterogeneous urban extent. Impervious surface frequently excludes suitable nesting habitat (most bees are ground nesting or cavity nesting) as well as foraging resources, however some built environments may provide supplemental habitat as well through cultivated gardens and embedded nesting habitat that includes cavities and non-compacted soil. Impervious surface also limits productive soil that could support floral resources for foraging pollinators, however dense and species rich

gardens may provide attractive food resources for some pollinators. We test three hypotheses: H1) properties developed more recently, in addition to higher surrounding impervious surface, and reduced vegetation cover, decrease the abundance and species richness of bee communities. We specifically expected a stronger influence of the age of property development through the disturbance of soil nesting and above-ground nesting bees that require more local three-dimensional structure above and below ground in spaces more likely to be disturbed than cavity nesting bee habitat (e.g., wood lots or embankments). H2) We expect greater species turnover across sites as a function of distance for smaller bodied bees across the extent of the city, especially for the generally smaller, soil-nesting bees and above ground nesting bees with shorter dispersal distances than cavity nesting bumble bees. Lastly, H3) we expect recent property development will decrease similarity in the diversity in bee community composition in addition to the complex influences of land cover on native bee community assemblage, due to species filtering during community establishment processes. By investigating the influence of land use history as well as land cover on the site scale (α-diversity, H1) and landscape-scale (β-diversity, H2 & H3) diversity of native bee communities, we expect to contribute to the empirical foundations of pollination ecology as it relates to urban ecosystems, restoration, and the spatial and temporal dynamics of bee communities.

METHODS

Study area and sampling design

Madison, Wisconsin is an urban state capital surrounded by agricultural lands in Dane County, one of the fastest growing counties in the Midwest. The primary transition type

occurring in the Madison area for the past century is the conversion of agricultural land to urbanized land around the outer edge of the city (Wegener 2001; Carpenter et al. 2007; Riera et al. 2001). The dominant urban land use is characterized by mixed residential and commercial use area, with small forest patches and city parks distributed throughout the urban area. This 123km^2 central urban zone of Madison includes 45.7km^2 (37.2%) of impervious surface, 29.7km^2 (24.1%) of vegetated space heterogeneously distributed, with the remaining proportion of the landscape covered by Lake Mendota and Lake Monona and other smaller lakes. The city receives semi-frequent rain and severe thunderstorms throughout the summer months, supporting abundant flowering prairie plants where native grasslands have been conserved or restored around the city.

Bee communities were sampled passively in traps across Madison, WI during summer 2013 using a spatially-stratified survey designed to account for changing regional species pools across the urban isthmus. To select sites, a 2.5km^2 grid was overlaid across Madison, WI, then grid cells dominated by lake or rural agricultural-use were excluded, leaving nineteen grid cells dominated by high-density residential and urban land use. Paired sample sites at least 1km apart were selected in each grid cell to contrast different urban terrain characterized by high (>55%) or low (<30%) impervious surface cover within the surrounding 200m. The 38 sample sites sampled during 2013 were selected in a stratified-random manner, then permission from property owners was requested until an appropriate sampling location was identified. Identification of property owners was obtained from the city parcel dataset. Sample sites included primarily residential properties, as well as commercial properties, urban storm water management areas, and city parks. In a second year of urban bee community sampling, ten additional sites were

selected across the urban study extent on City of Madison greenways, and sampled in a similar manner during summer 2016.

Explanatory and response variables

Surrounding land cover

Six-inch resolution digital aerial images were used to classify impervious surface across the urban landscape of Madison, WI. Impervious surface classification included roads, parking lots, house lots, and other permanent built structures. Unsupervised classification was initiated with 30 original classes, which were then clumped into land cover types. This impervious surface layer was added to the City of Madison building footprint and road layers, which helped to recover some impervious surface obscured in aerial imagery by overlying tree canopy to complete a high accuracy digitized layer. Impervious surface was measured as a percentage of the surrounding landscape sector in the 1000m buffers surrounding each study site. Vegetation was estimated using a commonly used Normalized Difference Vegetation Index (NDVI) based on LandSat reflectance in the red and infrared light spectral bands. Average NDVI was calculated within the 1000m buffers surrounding site in Google Earth Engine developer GUI based on a 100% cloud-free LandSat 8 image from June 15th 2016. To investigate hypothesis 3, canopy cover and semi-natural open grassland was digitized within the 1000m buffers surrounding each study site based on 1 m resolution NAIP aerial imagery. Impervious surface, canopy cover, and grassland land cover (%) within the 1000m buffers and 200m buffers surrounding each site were consolidated in a distance matrix for the multiple regression analysis on distance matrices.

Surrounding land use history

Publicly accessible tax assessment data was obtained from the City of Madison, and property development year was extracted for parcels located within the 1000 m buffer region surrounding each study site. The median property development year (i.e., 'year built') based on the parcels in the 1000m buffer area was used in the analysis to analyze hypothesis 1. For the multiple linear regression analysis on distance matrices test of hypothesis 3, a property development Bray-Curtis distance matrix was constructed based on the mean, median, and more recent development date for parcels extracted for each site within the 200 m and 1000 m buffer.
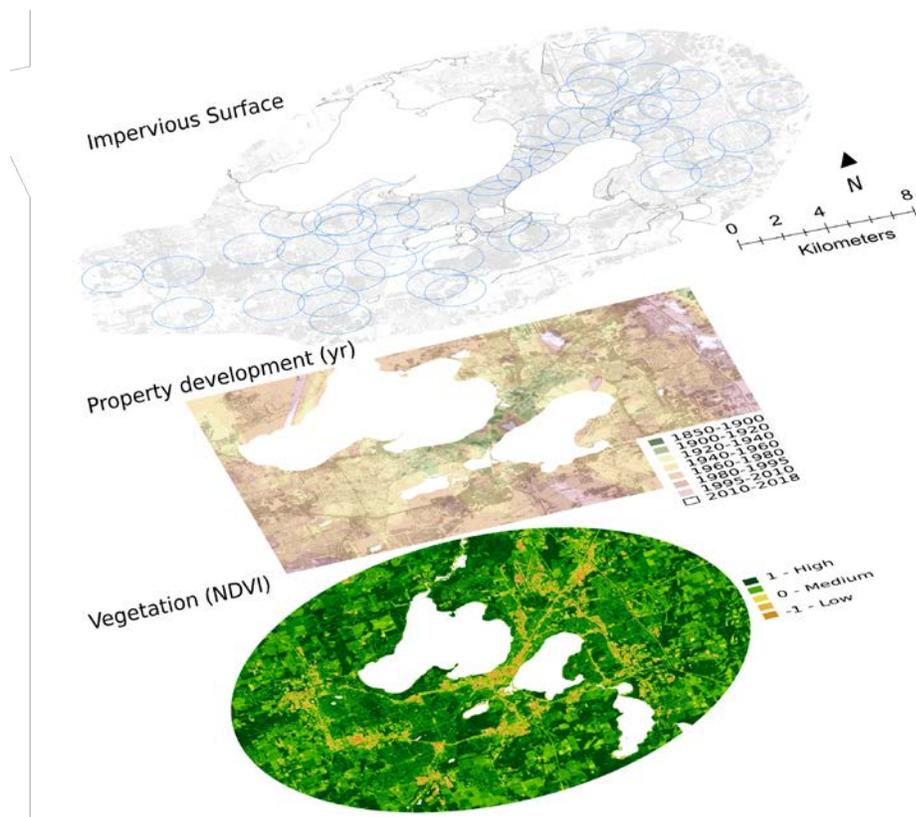
Figure 1. Stacked maps of the land cover and land use history explanatory variables, and 1km buffers surrounding the urban bee community sampling sites. The NDVI map estimates vegetation cover based on 30m resolution LandSat 8 multispectral imagery, the property development layer (krieged surface for visualization) represents 'year built' parcel data from the City of Madison tax assessor database, and the Impervious surface layer represents classified land cover based on 1m resolution NAIP aerial imagery and city spatial data layers.

Bee sampling

Bees were sampled six times during the summer 2013, between early June and late August. Pan traps were distributed approximately every two weeks give or take a few days to optimize clear, sunny weather when bees were foraging. Six bee traps were placed at least 5m apart within a 40m x 40m area, within each site. Two painted dark blue, canary yellow, and white were distributed at each site. Bees were passively trapped in 16oz pan traps filled with soapy water. Traps were suspended either 20cm or approximately 2.5m from the ground to match the height of nearby flowering vegetation.

In 2016, bees were sampled again each month between May and August, using the same painted cups. Traps were distributed for 3 days per sampling event, during which time 10 multi-colored cups were distributed at least 5m apart within a 40m x 40m area, within each site. Bees were identified to species using the discover life online interactive key and the most comprehensive dichotomous key available for *Lasioglossum* (Ascher & Pickering 2013; Gibbs 2011).

Statistical analysis

To test our first hypothesis, regarding the distribution of site-scale (α-diversity) bee abundance and species richness, we used generalized linear regression mixed-models to contrast the effects of the land cover and land use history (i.e. median property development year) explanatory variables on the abundance and richness of the bee community overall, as well as the abundance and richness of bee guilds, using a random effect of sample year in each mixed-effect model. All models for bee abundance were fit by maximum likelihood using a negative binomial distribution using the function 'glmer.nb' in the lme4 R package. This distribution best fit the distribution of bee abundance, compared to gaussian, log-normal, and poisson distributions. Models for overall and soil nesting bee species richness were fit by maximum likelihood for a normal distribution linear regression mixed-model implemented using the 'lmer' function in the lme4 R package. Models for cavity nesting bee species richness and above-ground nesting bee species richness were fit by maximum liklihood, implemented using the penalized quasi-likelihood (PQL) method, for a log-normal distribution. The log-normal mixed models were implemented using the 'glmmPQL.' Top models were selected using AICc model selection implemented using the dredge package in the 'MuMin' R package and multi-model average coefficients based on the 95% confidence set of top models were calculated and reported in the results (Ripley et al. 2018). The Moran's *i* test was used to check for spatial autocorrelation in model fit (i.e., residuals) for each of the final models included in Table 1. Moran's *i* tests were applied using the 'car' R package (Fox et al. 2018).

To investigate the influence of the land cover and land use history on bee community similarity at the landscape scale (β-diversity) in the larger 2013 bee community dataset, we used

multiple regression on distance matrices implemented through the R package 'ecodist' (Legendre and Legendre 1998; Goslee and Urban 2017). This approach allows us to capture the various multifaceted explanatory variables in distance matrices that capture the heterogeneity of land cover and land use history. Compared to other multivariate approaches, this regression framework measures effect size of each explanatory distance matrix to distinguish between competing hypotheses. The land cover distance matrix was calculated based on the proportion of three land cover types, including the impervious surface layer described previously, and two other types: tree canopy, and grassland which were digitized separately within the 200m and 1000m buffers based on 1m resolution NAIP aerial imagery. This analysis uses a non-parametric regression framework to test between competing explanatory variables. The permutation test uses a pseudo-t test to assess significance, rather than using the regression coefficients directly (Goslee and Urban 2017).

RESULTS

Across the season, 1331 bees were surveyed at 38 sites during 2013 and 654 bees were sampled at 10 sites in 2016. In 2013, bees were distributed across families as follows: 31.2% Apidae, 2.5% Andrenidae, 55.1% Halictidae, 7.9% Megachilidae, and 3.2% Collitidae. In 2016, bees were distributed across families: 22.32% Apidae, 9.02% Andrenidae, 44.95% Halictidae, 20.18% Lasioglossum, and 2.6% Megachilidae, 0.09% Colletidae. The 2016 bee sampling provided better coverage of spring mining bees from the Andrenidae family. To test the hypotheses regarding influence of land cover and land use history on nesting habitat guilds of bees, we separated soil-

nesting, cavity-nesting, and above-ground bees, based on available natural history observations. The cavity nesting bees included bumble bees (*Apidae: Bombus*), observed at 17 sites and inclusive of 7 species. Above-ground nesting bees included small carpenter bees (*Ceratina spp.*), yellow faced bees (*Hylaeus spp.*), carder bees, mason bees, and leafcutter bees (*Megachilidae*), and two species of sweat bees observed to nest in decaying wood, *Lasioglossum cressonii* (Mitchell 1960) and *Lasioglossum oblongum* (Sakagami & Michener 1962). Above-ground nesting bees were found at 32 sites and included 22 species. The rest of the bees, including 69 species: long-horned bees (Tribe Eucerini), mining bees (Andrena spp.), green bees, all of the other sweat bees, and others were classified as soil nesting bees, although natural history observation of many species could not be located. Soil nesting bees and bees generally, were found at 36 of the 38 study sites in 2013, and all 10 of the 2016 sites.

The sample sites were surrounded by 6-60% impervious surface, 0-43% natural vegetation, and 0-28% forest in the surrounding 1000m buffer landscape sector. The surrounding 200m landscape sector included 15-90% impervious surface, 0-57% natural vegetation, and 0-60% forest. Average property development year in the surrounding landscape sectors varied between 1920 and 2003.

H1. Does recent property development in addition to more impervious surface and less natural vegetation in the surrounding landscape reduce bee community diversity, especially for cavity nesting bees?

Our generalized linear regression mixed models testing our first hypothesis, that recent property development in addition to increased surrounding impervious surface and decreased natural vegetation reduces the site-scale abundance and of native bees, especially soil and above-ground nesting bees, explained a relatively small amount of variation, yet there were several noteworthy effects (Table 1). The most influential factor in the final models (based on high z-scores) for the full bee community abundance and soil-nesting bee abundance was a positive influence of vegetation. These final models included second explanatory variables of impervious surface, and property development date, respectively. The cavity-nesting bee abundance final model included only a positive influence of impervious surface, and the final above-ground nesting bee abundance model included the negative influence of the median property development year as well as the positive influence of impervious surface.

Table 1. Results of full negative binomial generalized linear mixed models and final AICc-selected negative binomial generalized linear regression models fit by maximum likelihood (Laplace approximation) for bee abundance of a. the full bee community, b. soil-nesting bees, c. cavity-nesting bees, and d. above-ground nesting bees.

a. Bee community: Full model	Coefficient estimates	Standard error	z value	p value	
Random Effect: Sample year	1.22×10^{-13}	3.49×10^{-7}			
Intercept	27.05	7.46	-1.01	0.31	
Impervious surface (%, 1000m)	2.48	0.65	1.60	0.11	
Vegetation (%, 1000m)	2.94	0.74	2.29	0.02	
Median development (yr, 1000m)	0.005	0.004	1.35	0.18	
Bee community: Final model	Coefficients estimates	Standardz value error		p value	Avg Coef (95%)
Intercept	2.53	0.46	5.56	>0.01	-2.31
Impervious surface (%, 1000m)	1.26	0.75	1.69	0.09	1.17
Vegetation (NDVI, 1000m)	1.62	0.66	2.46	0.01	1.46

Median development (yr, 1000m)					0.006

b. Soil-nesting bee community: Full model	Coefficients estimates	Standard error	z value	p value	
Random Effect: Year	0.028	0.17			
Intercept	-10.67	7.60	-1.40	0.16	
Impervious surface (%, 1000m)	0.70	0.70	1.00	0.32	
Vegetation (%, 1000m)	1.75	0.64	2.74	0.01	
Median development (yr, 1000m)	0.0067	0.004	1.72	0.09	

Soil-nesting bee community: Final model	Coefficients estimates	Standard error	z value	p value	Avg Coeff (95%)
Intercept	-15.63	7.30	-2.14	0.03	-11.57
Impervious surface (%, 1000m)					0.91
Natural vegetation (%, 1000m)	1.86	0.63	2.96	0.003	1.96
Median development (yr, 1000m)	0.009	0.004	2.48	0.01	0.01

c. Cavity-nesting bee community: Full model	Coefficients estimates	Standard error	z value	p value	
Random Effect	0.03	0.19			
Intercept	-8.07	7.15	1.88	0.26	
Impervious surface (%, 1000m)	2.85	1.50	1.90	0.05	
Vegetation (%, 1000m)	1.10	1.88	0.59	0.56	
Median development (yr, 1000m)	0.004	0.004	1.14	0.25	

Cavity-nesting bee community: Final model	Coefficients estimates	Standard error	z value	p value	Avg Coeff (95%)
Intercept	0.31	0.55	0.564	0.57	-3.20
Impervious surface (%, 1000m)	2.66	1.45	1.832	0.07	2.85
Natural vegetation (%, 1000m)					1.76
Median development (yr, 1000m)					0.007

d. Above-ground bee community: Full model	Coefficients estimates	Standard error	z value	p value	
Random Effect	0.034	0.19			
Intercept	-8.07	7.15	-1.13	0.26	
Impervious surface (%, 1000m)	2.85	1.50	1.90	0.06	
Vegetation (%, 1000m)	1.10	1.87	0.59	0.56	
Median development (yr, 1000m)	0.004	0.003	1.14	0.25	

Above-ground bee community: Final model	Coefficients estimates	Standard error	z value	p value	Avg Coeff (95%)
Intercept	21.06	12.89	1.63	0.10	10.07
Impervious surface (%, 200m)	1.28	1.33	0.97	0.33	1.18
Vegetation (NDVI, 1000m)					-0.51
Median development (yr, 1000m)	-0.0099	0.0065	-1.51	0.13	-0.0094

The final top models for the species richness of the full bee community, the soil nesting

bee community, both included vegetation as the most prominent positive effect, similar to the

abundance models. While vegetation was the only explanatory variable included in the soil-nesting bee species richness final model, the positive influence of the other two variables (i.e., impervious surface and median development year) were also included in the full bee community final model. No model selection procedure was implemented for the final cavity nesting bee and above ground nesting bee final models, except for elimination of the random effect. The most prominent explanatory variable in the final cavity-nesting bee species richness model was the positive influence of impervious surface, and the negative effect of median property development date was the most prominent variable in the above ground nesting bee model. We used Moran's I test to check for spatial autocorrelation in the model residuals and found no evidence of autocorrelation in model fit for any of the full models or final models.

Table 2. Results of full negative binomial generalized linear mixed models and final AICc-selected negative binomial generalized linear regression models fit by maximum likelihood (Laplace approximation) for bee species richness of a. the full bee community, b. soil-nesting bees, c. cavity-nesting bees, and d. above-ground nesting bees.

a. Bee community: Full model	Coefficient estimates	Error	t value		
Random Effect: Sample year	20.37	4.51			
Intercept	-15.11	97.05	-0.16		
Impervious surface (%, 1000m)	11.05	8.47	1.31		
Vegetation (%, 1000m)	10.20	7.59	1.35		
Median development (yr, 1000m)	0.013	0.049	0.28		
Bee community: Final model F value: 3.036, R^2: 0.18 p = 0.04	Coefficients estimates	Standard error	t value	*p* value	Avg Coef (95%)
Intercept	-153.57	97.67	-1.57	0.12	-94.84
Impervious surface (%, 1000m)	15.04	9.59	1.57	0.12	14.95
Vegetation (NDVI, 1000m)	16.62	8.35	1.99	0.05	16.05
Median development (yr, 1000m)	0.081	0.050	1.61	0.11	0.09
b. Soil-nesting bee community:	Coefficients	Standard	t value		

Full model	estimates	error			
Random Effect: Year	22.76	4.77			
Intercept	183.21	104.64	1.75		
Impervious surface (%, 1000m)	1.87	6.65	0.28		
Vegetation (%, 1000m)	11.36	5.62	2.02		
Median development (yr, 1000m)	-0.088	0.05	-1.65		

Soil-nesting bee community: Final F value: 7.34, R^2: 0.19, p = 0.01	Coefficients estimates	Standard error	t value	p value	Avg Coef (95%)
Intercept	5.33	3.44	1.55	0.13	4.51
Impervious surface (%, 1000m)					6.09
Natural vegetation (%, 1000m)	18.37	6.78	2.71	0.01	18.42

c. Cavity-nesting bee community:	Coefficients estimates	Standard error	df	t value	p value
Random Effect	Int: 0.275	1.12			
Intercept	-4.29	10.10	22	-0.42	0.68
Vegetation (%, 1000m)	1.40	1.71	22	0.82	0.42
Impervious surface (%, 1000m)	2.41	1.22	22	1.98	0.06
Median development (yr, 1000m)	0.0017	0.0051	22	0.34	0.74

Cavity-nesting bee community:	Coefficients estimates	Standard error	t value	p value
Intercept	-20.05	13.52	-1.48	0.15
Impervious surface (%, 1000m)	2.14	1.52	1.41	0.17
Natural vegetation (%, 1000m)	1.70	1.36	1.25	0.22
Median development (yr, 1000m)	0.01	0.0070	1.49	0.15

d. Above-ground bee community:	Coefficients estimates	Standard error	df	t value	p value
Random Effect	1.93×10^{-05}	2.61			
Intercept	10.92	10.06	37	1.09	0.28
Vegetation (%, 1000m)	-0.01	0.90	37	-0.01	0.99
Impervious surface (%, 1000m)	0.58	1.04	37	0.56	0.58
Median development (yr, 1000m)	-0.0051	0.005	37	-0.98	0.33

Above-ground bee community:	Coefficients estimates	Standard error	z value	p value
Intercept	33.44	28.46	1.18	0.25
Impervious surface (%, 1000m)	2.29	3.01	0.76	0.45
Natural vegetation (%, 1000m)	0.62	2.31	0.27	0.79
Median development (yr, 1000m)	-0.02	0.01	-1.09	0.28

H2. Are more geographically distant bee communities more distinct in composition (β-diversity)?

We expect this for the generally smaller, soil-nesting bees in particular.

We tested our second and third hypotheses regarding the distribution of landscape-scale β-diversity of 2013 bee communities using multiple linear regression on distance matrices (Table 2). In our final models fit for the soil-nesting bee community composition and cavity-nesting bee community composition, we observed significant positive effects of geographic distance on community dissimilarity. While this effect was statistically significant in the soil-nesting bee community model, the influence was only weakly significant to explain increased dissimilarity of the cavity-nesting bee community composition. There were no significant effect of geographic distance on bee community composition for the above-ground bees or the full bee community.
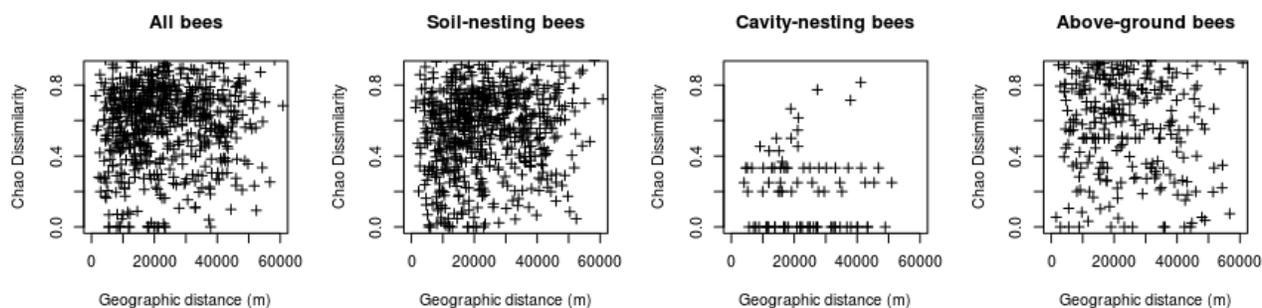

Figure 2. Chao dissimilarity of full bee community and each bee nesting guilds as a function of geographic distance

H3. Land use history (i.e. property development) in addition to surrounding land cover influences the distribution of bee communities at the landscape scale (β-diversity)

While the final models for 2013 bee community composition included significant land cover effects at the 200m landscape scale for the full bee community composition and soil-nesting bee community composition, no significant influence of land cover was observed for the

cavity nesting bee community composition or the above-ground bee community composition. None of the bee community final models included significant effects of property development on community composition.

Table 2. Results of multiple regression on distance matrices (MRDM) for a. the full bee community, b. soil-nesting bees, c. cavity-nesting bees, and d. Vegetation/wood-nesting bees.

a. Bee community dissimilarity: full model F value: 2.78, R^2: 0.02, p value: 0.08	Regression coefficients	p value
Intercept	5.55×10^{-1}	0.81
Geographic distance (m)	1.03×10^{-6}	0.28
Property development (yr)	8.61×10^{-4}	0.18
Land cover (200m, %)	-1.83×10^{-4}	0.04 *
Land cover (1000m, %)	1.53×10^{-4}	0.43
Bee community: final model F value: 9.69, R^2: 0.02, p value: 0.09	Regression coefficients	p value
Intercept	0.57	0.03 *
Land cover (200m, %)	-1.86×10^{-4}	0.03 *
b. Soil nesting bee community: full model F value: 4.16, R^2: 0.03, p value: 0.02	Regression coefficients	p value
Intercept	4.92×10^{-1}	1
Geographic distance (m)	2.16×10^{-6}	0.04 *
Property development (yr)	7.51×10^{-4}	0.26
Land cover (200m, %)	-1.70×10^{-4}	0.04 *
Land cover (1000m, %)	-1.26×10^{-4}	0.50
Soil nesting bee community: final model F value: 7.39, R^2: 0.02, p value: 0.01	Regression coefficients	p value
Intercept	4.91×10^{-1}	0.97
Land cover (200m, %)	-1.70×10^{-4}	0.04 *
Geographic distance (m)	2.18×10^{-6}	0.04 *
c. Cavity nesting bee community: full model F value: 5.81, R^2: 0.07, p value: 0.04	Regression coefficients	p value
Intercept	3.76×10^{-1}	0.95
Geographic distance (m)	5.07	0.08 .
Property development (yr)	-2.55	0.63
Land cover (200m, %)	-4.30×10^{-4}	0.92
Land cover (1000m, %)	-3.60×10^{-3}	0.60
Cavity nesting bee community: final model F value: 3.81, R^2: 0.03, p value: 0.07	Regression coefficients	p value

Intercept	3.72×10^{-1}	0.96
Geographic distance (m)	5.23×10^{-6}	0.07 .
d. Above-ground bee community: full model F value: 1.59, R^2: 0.01, p value: 0.36	Regression coefficients	p value
Intercept	8.13×10^{-1}	0.11
Geographic distance (m)	-1.84×10^{-6}	0.21
Property development (yr)	-1.65×10^{-4}	0.48
Land cover (200m, %)	3.33×10^{-4}	0.68
Land cover (1000m, %)	-3.71×10^{-4}	0.17
Above-ground bee community: final model F value: 2.13, R^2: 0.00, p value: 0.14	Regression coefficients	p value
Intercept	7.69×10^{-1}	0.27
Land cover (1000m, %)	-4.00×10^{-4}	0.14

DISCUSSION

Site-scale bee community richness and the urban landscape

Bees from each of the three nesting-guilds were observed throughout the City of Madison, yet the cavity-nesting bee abundance and richness models primarily included the positive influence of impervious surface within the surrounding 1000m landscape sector. The positive influence of impervious surface in the cavity bee models as well as other models, may result from positive sampling bias, since there is less semi-natural land cover to compete with the passive traps. The prominence of the positive influence of vegetation productivity (i.e., NDVI) in the overall and soil nesting bee models suggests a bottom up limitation of floral resources on overall bee community abundance and richness. While differences were observed between the bee habitat guild models, these results suggests that in general, each nesting guild of bees is able to utilize habitat within the most urbanized landscapes of the city consistent with findings of

other studies (Theodorou 2016; Hall et al. 2016). The positive influence of surrounding vegetation on the full bee community and soil-nesting bees may support the explanation that this habitat type provides both uncompacted soil and an increase of flowering forbs that bees use as a food resource.

A negative influence of recent property development was observed in the full models for the soil-nesting bees, and cavity-nesting bees, yet only found in the final models of the later above-ground nesting bee abundance and species richness models. The negative influence of median property development date on above-ground nesting bees, but not in any of the other final bee community models, evidences differential impacts of high urbanization. In older neighborhoods, increased prevalence of complex structure in residential yards (e.g., stone walls, garden boundaries, decaying wood) may improve habitat for above ground nesting bees specifically. This result aligns with our expectation that above-ground nesting bees would be sensitive to the loss of complex three-dimensional nesting habitat as well. While this influence could be caused by the influence of disturbance to the nesting habitat used by this bee guild, we should additionally consider other modifications to the landscape structure in more recently developed urban areas compared to more established neighborhoods, such as abundance of floral resources in established gardens.

Geographic distribution of bee communities

While geographic distance did not explain the dissimilarity of the full bee community, it contributed a statistically significant explanation of dissimilarity in soil-nesting bee community

composition and a weakly significant influence on cavity-nesting bee community composition. This result confirmed our hypothesis that generally smaller, soil-nesting bee communities would vary more across the extent of the city. Past studies have confirmed that bee foraging distances are generally correlated with body size, contributing to the patchy distribution of smaller bee species (Steffan-Dewenter et al. 2002; McKinney 2008). A recent study of pollinator community composition around cotton farms in Texas found no geographic pattern of isolation by distance in Hymenopteran communities, while they observed these effects for beetles and other more movement limited insect taxa, however this study was also conducted across a larger spatial extent and in an agricultural setting rather than an urban landscape (Cusser et al. 2018).

Influence of land cover and property development on urban bee community composition

In models for the community similarity of the full bee community as well as each nesting habitat guild, property development was not a significant factor. Surrounding land cover significantly influenced the full bee community and soil-nesting bee community dissimilarity at the 200m scale, while it was included in the final model for above-ground bee community dissimilarity at the 1000m scale. While the influence of land cover significantly influenced the dissimilarity of species assemblages, these factors did not explain much of the variation overall. High species richness of bees was observed across the city, as well as patchy distributions of rare species.

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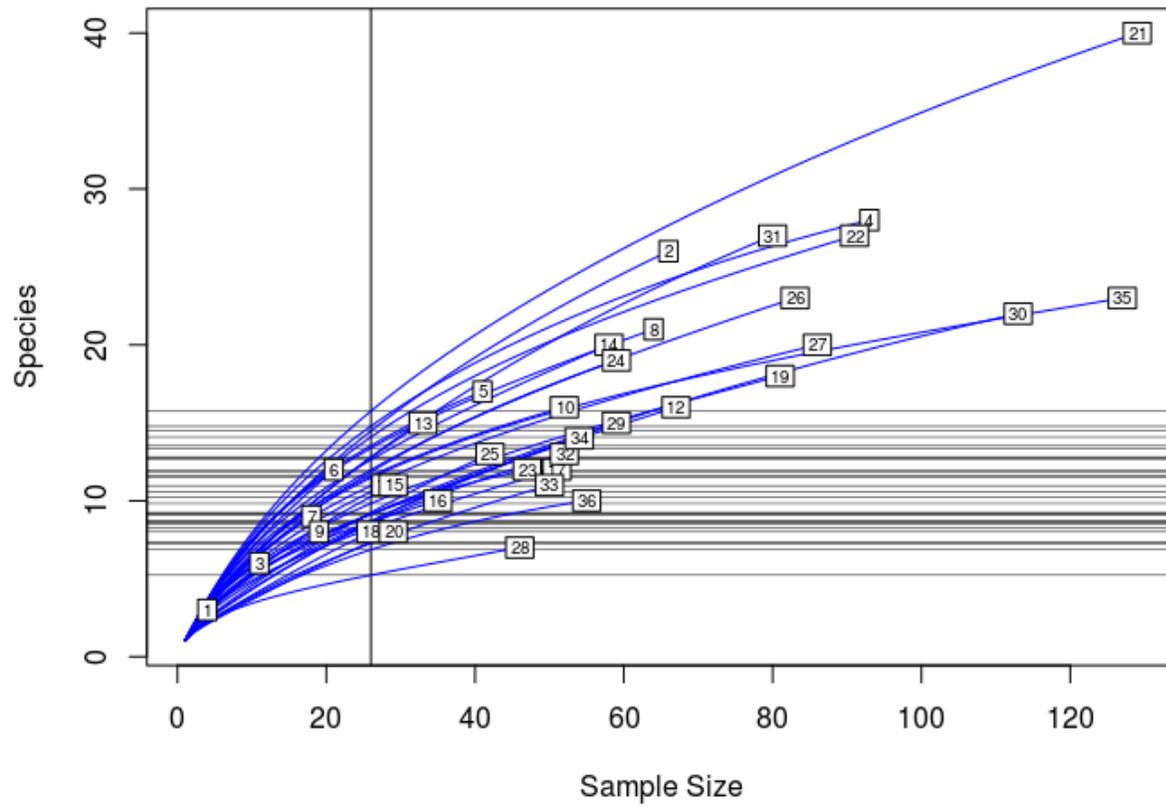
Supplemental Figure 1. Individual-based rarefaction curves and n=26 cutoff for site species richness.

Supplemental Table 1

<u>Bee species</u>
Agapostemon sericeus
Agapostemon virescens
Andrena bisalicis
Andrena crataegi
Andrena erythronii
Andrena milwaukensis
Andrena nasonii
Andrena nigrae
Andrena nuda
Andrena phaceliae
Andrena wellesleyana
Anthidium maculifrons
Anthidium manicatum
Anthidium oblongatum
Apis mellifera
Augochlora pura
Augochlorella aurata
Augochlorella persimilis
Augochlorella sp
Augochloropsis fulgada
Bombus affinis
Bombus bimaculatus
Bombus fervidus
Bombus griseocollis
Bombus impatience
Bombus rufosphinctus
Bombus sandersoni
Ceratina calcarata
Ceratina dupla
Ceratina mikmaqi
Ceratina strenua
Coelioxys spp
Colletes spp
Eucera atriventris
Florilegus condiginus
Halictus confusus
Halictus ligatus
Halictus parallelus
Halictus rubicundus
Hylaeus affinus

Hylaeus annulatus
Hylaeus floridanus
Hylaeus mesillae
Hylaeus rudbeckiae
Lasioglossum albepenne
Lasioglossum anomalum
Lasioglossum atwoodi
Lasioglossum atwoodii
Lasioglossum bruneri
Lasioglossum cattelae
Lasioglossum coreopsis
Lasioglossum corerulevum
Lasioglossum coriaceum
Lasioglossum cressoni
Lasioglossum divergens
Lasioglossum egregium
Lasioglossum hartii
Lasioglossum illinosese
Lasioglossum imatatum
Lasioglossum leucozonium
Lasioglossum lineatulum
Lasioglossum michiganense
Lasioglossum nigrovirde
Lasioglossum nymphacorum
Lasioglossum obscurum
Lasioglossum paraforbesii
Lasioglossum pilosum
Lasioglossum pruinosum
Lasioglossum spp
Lasioglossum tegulare
Lasioglossum timothyi
Lasioglossum versans
Lasioglossum weems
Lasioglossum weemsi
Lasioglossum zephyrum
Lasioglossum zonulum
Megachile inimica
Megachile latimanus
Megachile melanophoea
Megachile mendica
Megachile relativa
Melissodes agilis
Melissodes bimaculatus

Melissodes boltonae
Melissodes comunis
Melissodes dentiventris
Melissodes druriellus
Melissodes rustica
Melissodes tinctus
Melissodes trinodis
Nomada affabilis
Nomada articulata
Nomada cressoni
Nomada illinoensis pygmaea
Osmia sp.
Sphecodes sp.
Stelis louisae
Stelis nitida

CHAPTER 2: BUZZING AROUND THE CITY: THE EFFECT OF LOCAL AND LANDSCAPE FACTORS ON SOCIAL BEE OCCURRENCE IN MADISON, WI

ABSTRACT

Urbanization diminishes the extent of uncompacted, productive soil and the abundance of native plant species, yet urban landscapes still support diverse pollinator communities because their habitat is maintained within the urban environment. Floral cover, flower species richness, and native plant richness are all factors known to influence pollinator foraging behavior and health, and these factors vary across landscapes, including heterogeneous urban extents. This study assesses how floral resources and bumble bees are distributed across the city of Madison, WI and how floral resource explanatory variables predict the distribution of foraging bumble bees. We conducted a systematic transect survey of bumble bees across Madison, WI. The resulting data associated with ~5,500 non-lethally surveyed bumble bees were analyzed with regard to floral resource variables as well as underlying land use zoning on more than 700 transects. We used variograms and correlograms to investigate spatial structure in floral resource variables and bumble bee counts, then we used a generalized linear model predicting transect bee counts based on floral cover, density, species richness and lawn and prairie species richness on the distribution of foraging bees, and found significant positive influence of flower cover, species richness, and weakly significant positive influence of prairie and lawn species richness on foraging bee counts within the model as well as the land use zoning categorical variable. We employed a geographically-weighted regression model to explore non-stationarity in effects of

floral resource explanatory variables across the study extent, to suggest more context specific reasoning for the distribution of foraging bumble bees across the city.

INTRODUCTION

Pollination is an essential ecosystem service that enables the production of fruits, nuts, and seeds, and maintains the diversity of most plant populations in almost all global ecosystems. However, global declines of many pollinators have triggered international interest in conservation science to address risk factors and isolate the most influential variables that promote bee community resilience. Floral abundance and species richness are known factors that influence bumble bee colony growth and foraging distance (Jha and Kremen 2013, Crone et al. 2016). These resources are generally assumed to decrease in urban ecosystems compared to forb-rich semi-natural land cover (e.g. prairie), yet urban ecosystems likely provide intermediate amounts of floral resources that surpass other types of natural land cover (e.g. arid ecotypes or forb-poor grassland) (Goddard et al 2010, Threlfall et al. 2015). Comprehensive investigation of the distribution of floral resources and foraging bumble bees across an urban extent may illuminate which aspects of floral resource distribution shape bumble bee foraging patterns most across an urban ecosystem.

Foraging for food is an energy intensive process that must be continuously accomplished to sustain bumble bee colonies through the growing season. Bumble bees do not store much surplus food within the colonies, so constant, efficient foraging is a necessity. Bumble bees are central place foragers, known to fly long distances to visit resource rich floral assemblages. Landscape scale floral resources influence bumble bee foraging distances in studies based in

multiple landscapes (Jha and Kremen 2013, Pope et al. 2018). However, bumble bee activities and resource use are difficult to assess at broad landscape scales and are known to differ depending on the composition of the landscape. Spiesman et al. (2016) found no influence of landscape scale floral resources on bumble bee colony growth, when local resource-rich floral dominance was high.

Landscape-scale studies have been conducted to confirm the importance of floral cover and native plant species richness to sustain bee communities, further consideration of the distribution of these factors may help to illuminate the degree of their influence in heterogeneous urban ecosystems. Many studies focus on components of urban landscapes in isolation, such as city parks or residential gardens, rather than the comprehensive urban landscape. Developing a thorough understanding of the distribution of important foraging resources and their use by foraging bees in different ecosystems is important to improve the capacity of landscapes to sustain robust pollinator populations. Comparing the influence of various influential factors on the distribution of bumble bee foragers can help to tune conservation guidelines to the context of a particular ecotype.

This study investigates the spatial distribution of floral resources and foraging bumble bees around the city of Madison, and tests the predictive capacity of four aspects of floral resource distribution to estimate the distribution of foraging bumble bees. We include interactions between a land use categorical variable and the floral resource explanatory variables to test if the effect of floral resource descriptors on bumble bee foragers differs based on land use context. We then explore patterns of non-stationarity in the effect size of floral resource explanatory variables across the study extent. The results of this study identify what aspects of

floral resource distribution confer the strongest influence on observed frequency of bumble bee foragers, informing conservation practitioners based on local context.

METHODS

Study area

We conducted a systematic transect survey across a 125km^2 extent (including lakes, which were not surveyed) covering the city of Madison, WI, USA to map the distribution of floral resources and foraging bumble bees in July and August of 2013 and 2014. A XXkm2 square on each side of the city was surveyed between July 1st and August 31st in 2013, and the intervening area across the downtown urban extent was surveyed between July 1st and August 31st of 2014. The full study extent included the city of Madison, located on an isthmus bounded by a northern and southern lake, as well as the exurban agricultural land immediately adjacent to the eastern and western edges of the city. Several high density urbanized patches of mixed commercial/residential zones were located within both eastern and western residential zones, as well as the center of the grid (downtown Madison). Agricultural land was primarily located on the eastern and western edges of the city, across an approximately 6km x 3km of surveyed terrain on each edge. The majority of the surveyed terrain was comprised by residential land use, characterized by a moderate range of impervious surface (~20-30% based on 1 m resolution classification), and grass lawn, with scattered patches of woodland and semi-natural grassland or restored prairie scattered throughout the extent.

Explanatory variables

An ~400m transect was surveyed within each accessible 400m x 400m cell of a 125km^2 grid. Within each grid cell, an observer walked a 400m long and 10m wide transect, at which time the observer recorded a list of flowering plants in anthesis, and mapped point occurrences of bumble bees. Tracks were mapped for spatial analysis conducted following the transect survey. When bumble bees were observed within 5m of the observer, they were mapped and their species was recorded along with the plant morphospecies they were visiting in most cases. Most native plants were identified to species, however many ornamental plants and rare plants were identified by morphospecies alone.

The floral resource explanatory variables collected included percent cover, density, flowering plant species richness, and prairie and lawn species richness. During the transect walk, the observer estimated the total percentage area of the transect that supported flowering vegetation, as well as the average density of flowers within that area. After completion of the data collection, all flower morphospecies were classified as lawn, prairie, or garden species based on the ecotype where they were most often observed. The lawn species included morphospecies that commonly persisted in areas with mowed lawn, including white clover, red clover, birds-foot trefoil, crown vetch, dandelion, heal-all (*Prunella vulgaris*), sweet clover, plantains (*Plantago sp.*), and chickweed (*Cerastium sp.*). Prairie species included species commonly found in restored prairie ecotypes (as well as gardens), but require complete or reduced mowing to produce flowers such as Joe-pye weed, Boneset, Cone flower, milkweed, rosinweed, cup plant, vervain, Queen Anne's lace and others. Most other species were classified as garden species found primarily in managed gardens.

Transects were mapped on city parcel spatial data layers, and each transect was assigned a categorical land use zoning type, loosely based on city zoning surrounding the parcels where the transect was surveyed. Commercial use was prioritized, so that mixed residential and commercial zones were coded as commercial. Residential zones were the most common land use category. Transects that passed through city parks were coded as city parks. Agricultural zoning qualifications in the city parcel data were much less accurately linked to observed land use, so agricultural land use zoning was determined based on transect survey observation.

Statistical analysis

We investigated the scale of spatial autocorrelation in bee count data as well as the floral resource explanatory variables using variograms and correlograms. Variograms and correlograms estimate the variance and Moran's i correlation metrics between classes of points separated by increasing spatial lags to illustrate the scales at which spatial autocorrelation is observed and where it dissipates. Signals at the most distant lags should be ignored because they are based on very few comparisons.

The number of bumble bees foraging on each transect was modeled using a generalized linear regression model to estimate the effect of floral resource explanatory variables and land use zoning on bumble bee frequency to test hypotheses 1. The variance in count data was greater than the mean, indicating a pattern of over-dispersion that breaks the assumptions associated with the Poisson distribution. As there was no reason to assume the error structure associated with the negative binomial distribution, the robust quasi-poisson fitting was used for the Poisson model to

accommodate overdispersion commonly observed in count datasets. This fitting adjusts the standard error with an appropriate scalar, although the coefficient estimates remain similar to the standard Poisson model. Interactions between land use type and each floral resource variable were included to test hypothesis 2.

Robust standard error was calculated separately for each coefficient estimate in the glm model using the "sandwich" package in R (Zeileis et al. 2018). The robust standard error for heteroskedasticity was used (ie. vcovHC) with White's estimator for large sample sizes (ie. "HC0"). This method is tailored to take into account leverage points.

Before fitting the model, the variance inflation factor of the explanatory variables was calculated to ensure that multicollinearity would not overestimate the variance explained by the model. All VIF scores fell below 2, well below commonly used cut-off values of 5 and 10.

The Moran's i test of residuals based on spatially weighted 10 nearest neighbor points was used to check for spatial structure in the residuals of the model, and the alternative hypothesis that existing spatial structure in the dataset was not captured in the model. However, the test statistic ranging between -1 and 1, was calculated to be 0.062 (p < 0.001), very close to zero indicating only a very small proportion of the variance in bee frequency was explained by spatial structure with a ten nearest point (k = 10) neighborhood covering an approximately 1.5 km radius neighborhood. Similarly small spatial structure was observed using a 25 or 100 point neighborhood, with moran's I test statistics estimated as 0.016 p = 0.037 or 0.013 p = 0.001.

In the final component of the study, we employed a local Poisson model fitted by the geographically-weighted quasi-Poisson regression method to investigate non-stationarity in floral resource coefficient estimates across the study extent. This exploratory statistical method is a

type of local regression whereby generalized linear regression models are fit across the study extent based on a moving window, and regression coefficients are calculated for each data point. This analysis was accomplished using the 'lctools' R package. Local regression coefficients were calculated based on an adaptive geographic window including the 50 closest points and each included data point is weighted by geographic distance.

RESULTS

Overall, 784 transect were surveyed, including 408 residential transects, 222 commercial transects, 77 agricultural transects, and 77 transects through city parks. Across the transects 5574 foraging bumble bees were documented. The mean and median transect lengths was 389m and 409m, respectively, while the minimum and maximum transects were 210m and 664m. Floral cover, density, species richness, as well as the number of prairie and lawn species were documented, and while the range of each of these variables was similar across each of the land use zoning types, the distribution of values for several of these explanatory variables differed between land cover zoning categories (Figure 1). Transects in agricultural zoned areas and city parks were characterized by a more uniform distribution of high and low flower cover compared to commercial and residential transects which were more frequently characterized by low flower cover. Commercial and residential transects included more low and high values of species richness, while agricultural and city park transects included more high values of lawn and prairie species compared to commercial and residential transects.
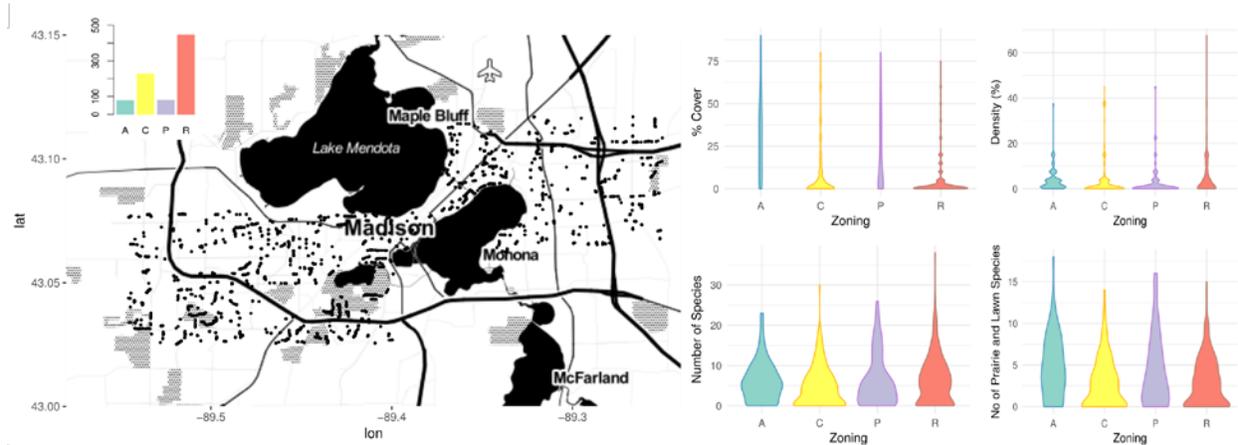
Figure 1. Map of the study extent and point locations of bumble bees observed along the transects. The upper left bar chart shows the number of transects sampled of each land use category, while the fiddle plots on the right show the distribution of flower resource explanatory variables by land use categorical variable zoning levels.

Floral resources were more spatially autocorrelated than bees

Positive spatial autocorrelation was observed in bee count data up to about 5 km, and in floral resource variables up to about 2 or 3 km. Maximum Moran's i estimates at very short distances reached 0.1, while floral resource variables reached a higher maximum of just over 0.15, which indicate that only a small proportion of the variation could be attributed to local spatial autocorrelation.
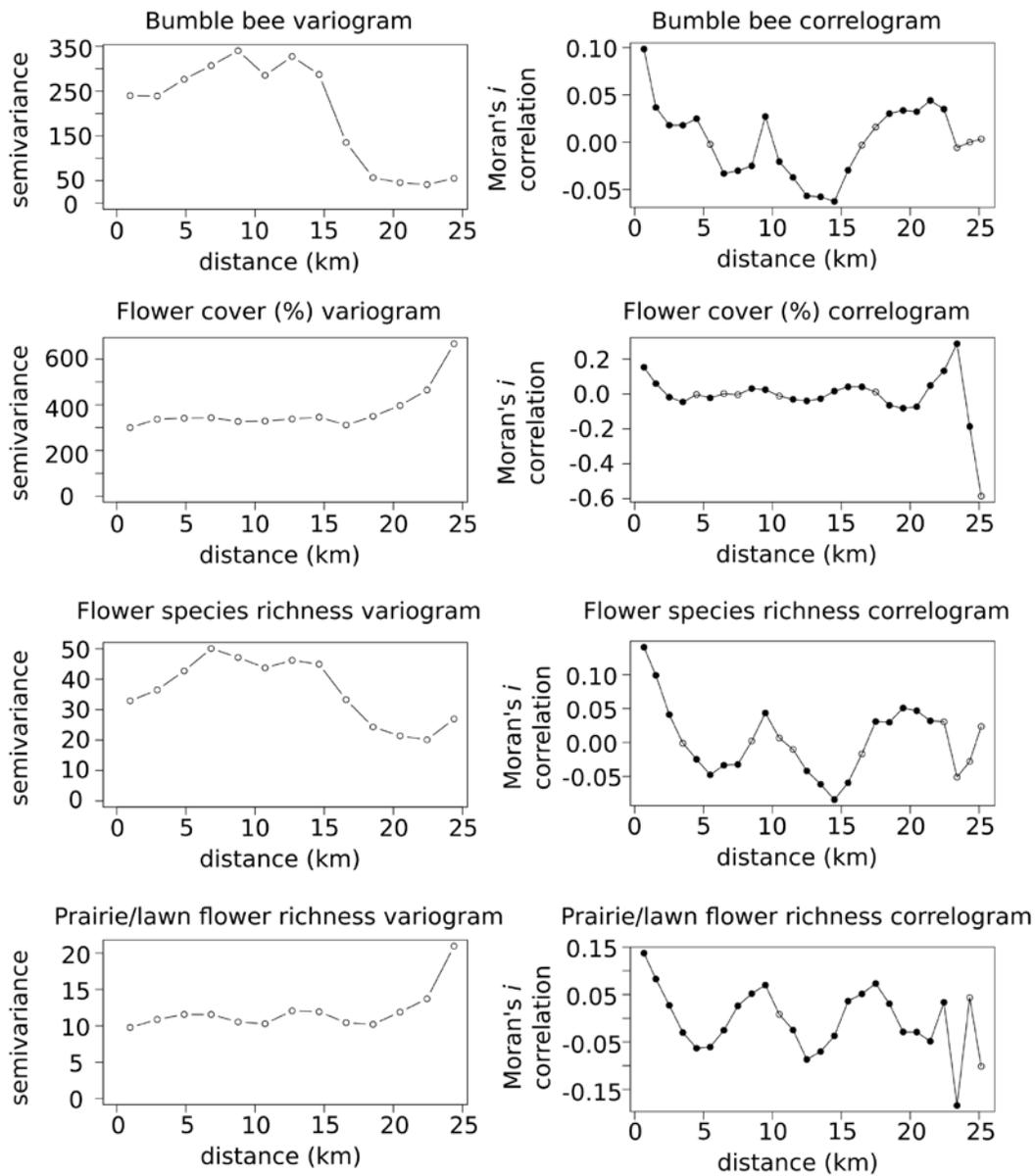
Figure 2. Variograms and correlograms depict the spatial structure of variance across scales within the study extent. Variograms illustrate the semi-variance between transects separated by increasing spatial lags within the dataset, and correlograms plot the Moran's i correlation statistic

The most bee-attractive flower species were prairie and lawn species

The most 14 most-visited flower species achieving over 1% of bee visits accounted for ~70% of all bumble bee foraging documented in our survey. Top visited species included, goldenrod (*Solidago spp.*) (12.4%), white clover (*Trifolium repens*) (12.2%), thistle (*Cirsium spp.*) (11.2%), bee balm (*Monarda spp.*) (10.4%), garden mints *Mentha spp.* (5.6%), spirea (*Spirea spp.*) (5.5%), purple coneflower (*Echinacea purpurea*) (4.0%), and silphiums (Silphium spp.), Russian sage (*Perovskia atriplicifolia*), Birds-foot trefoil *(Lotus corniculatus*), knapweed (*Centaurea spp.*), crown vetch (*Secuigera varia*), joe pye weed (*Eutrochium purpureum*), red clover (*Trifolium pretense*), each supported between 1-3% of the bee visits. Most of these species were classified as either lawn or prairie species rather than garden species. While these species were commonly distributed across the city, there were other common species that were rarely visited, including hosta (Hosta spp.), bell flower (Campanula latifolia), and lillies (Lilium spp.). The most visited flowers classified as garden types included, mint, russian sage, and spirea.

Table 1. Most common species of each flower ecotype group

Lawn	Prairie	Garden
Trifolium repens	Daucus carota	Hosta spp.
Lotus corniculatus	Cirsium spp.	Lilium spp.
Taraxacum officinale	Rudbeckia hirta	Lilium lancifolium
Melilotus officeinalis	Cichorium intybus	Campanula latifolia
Phlox spp.	Echinacea purpurea	Calendula officinalis
Oxalis stricta	Monarda fistulosa	Leucanthemum vulgare
Securigera varia	Liatris pycnostachya	Perovskia atriplicifolia
Cerastium spp.	Solidago canidensis	Spirea spp.
Trifolium pretense	Erigeron spp.	Rosa spp.
Plantago lanceolate	Achillea millefolium	Impatiens spp.

Floral cover and diversity positively influenced the number of foraging bumble bees

The quasi-Poisson generalized linear regression model fit for the transect count data evidenced significant influence of flower cover as well as species richness, and a weakly significant influence of prairie and lawn flower species richness on the bee count data across the transects (Table 2). Additionally, the categorical factor of land use zoning, showed significant differences in the number of foraging bees where city parks supported 4 times more bumble bee foragers than agricultural transects on average, and residential and commercial transects supported about twice as many bees as agricultural transects. The effect size of floral resource explanatory variables are contrasted in terms of their unit increase influence on bee count in Figure 2.

Interactions between land use type and each floral resource variable were tested, yet interactions were only weakly significant in the full model, and when non-significant resource variable and zone class interaction subsets were eliminated, the weak significance dissipated, and more variance was explained by the primary floral resource factors.

Table 2. Global model summary information for the quasi-Poisson fit regression model used to test the influence of floral resource explanatory variables and land use zoning on foraging bumble bee transect count data.

Model summary

Factors	Estimate	Robust error	p-value
Intercept	0.330	0.225	0.194
Cover	0.016	0.003	0.000 ***
Species richness	0.042	0.011	0.000 ***
Wild species richness	0.037	0.023	0.073 .
Commercial	0.755	0.222	0.004 **
Park	1.463	0.251	0.000 ***

Residential	0.628	0.223	0.015 *

Null deviance: 12821.6 on 783 degrees of freedom
Residual deviance: 9085.1 on 777 degrees of freedom

Figure 3. Transect polygon layer symbolized by (a) land use zoning type (b) log base 10 of the number of foraging bees counted on each transect (2 = 100 bees) and (c) the fitted bee count estimates based on the final glm model presented in Table 1.
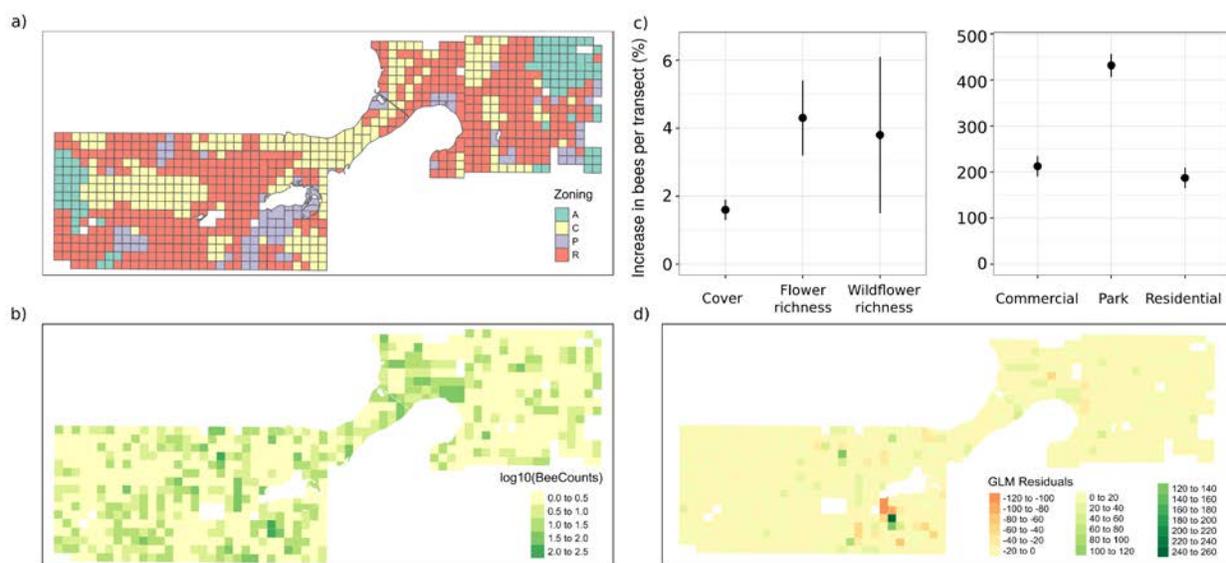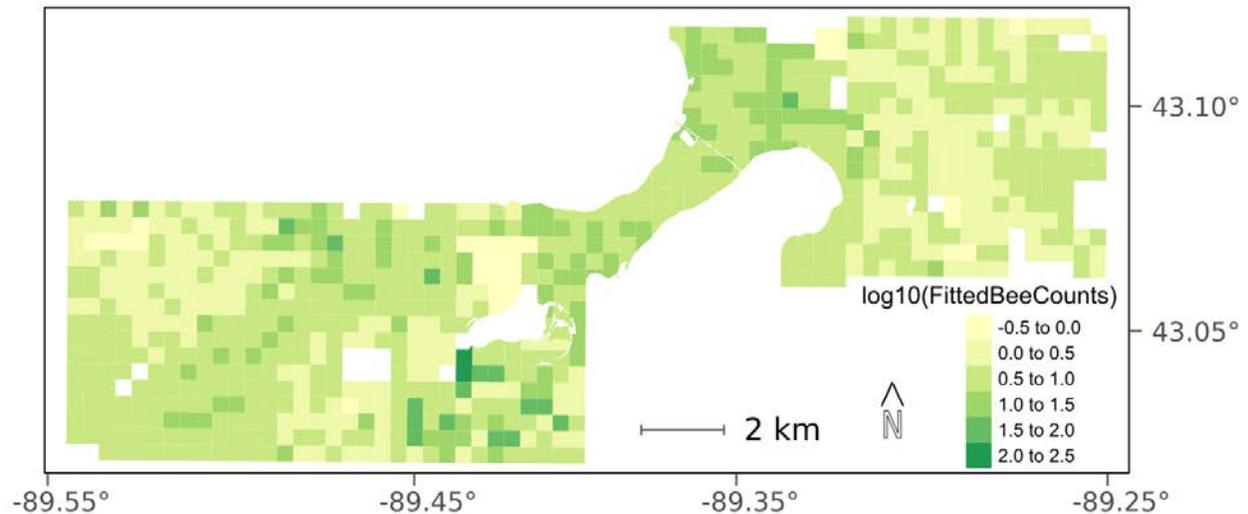

Figure 4. Generalized linear regression model residuals across the study extent, depicting departure between the raw bee count data and bee count results predicted by the glm model.

Local models illustrated non-stationarity

The geographically weighted generalized linear regression analysis used to explore heteroskedasticity, showed some fluctuation of floral resource explanatory variable effect size across the study extent. Floral cover was the most consistently strong positive influence on bumble bee foraging counts across the study extent, consistent with the global quasipoisson generalized linear model. Density was the only non-significant floral resource explanatory variable. The slight positive influence of density was also widespread and highest in the agricultural and commercial areas.

While overall floral morphospecies richness and the richness of prairie and lawn flower morphospecies was positively correlated, this overall and non-managed subset of flower richness was distributed differently across land use zones. Commercial and residential areas sustained higher floral richness than parks and agricultural areas, however, parks and agricultural transects sustained rather higher richness of the lawn and prairie species subset. Across the study extent,

the positive influence of overall species richness was more widespread and disappeared only in places where the positive influence of prairie and lawn species richness was particularly strong. These areas where prairie and lawn species richness stood out as the stronger effect in the glm regression model included areas with large, species-rich restored prairies (West – UW Madison Arboretum; East – Heritage Prairie, Elvehjem Park) surrounded by residential areas.
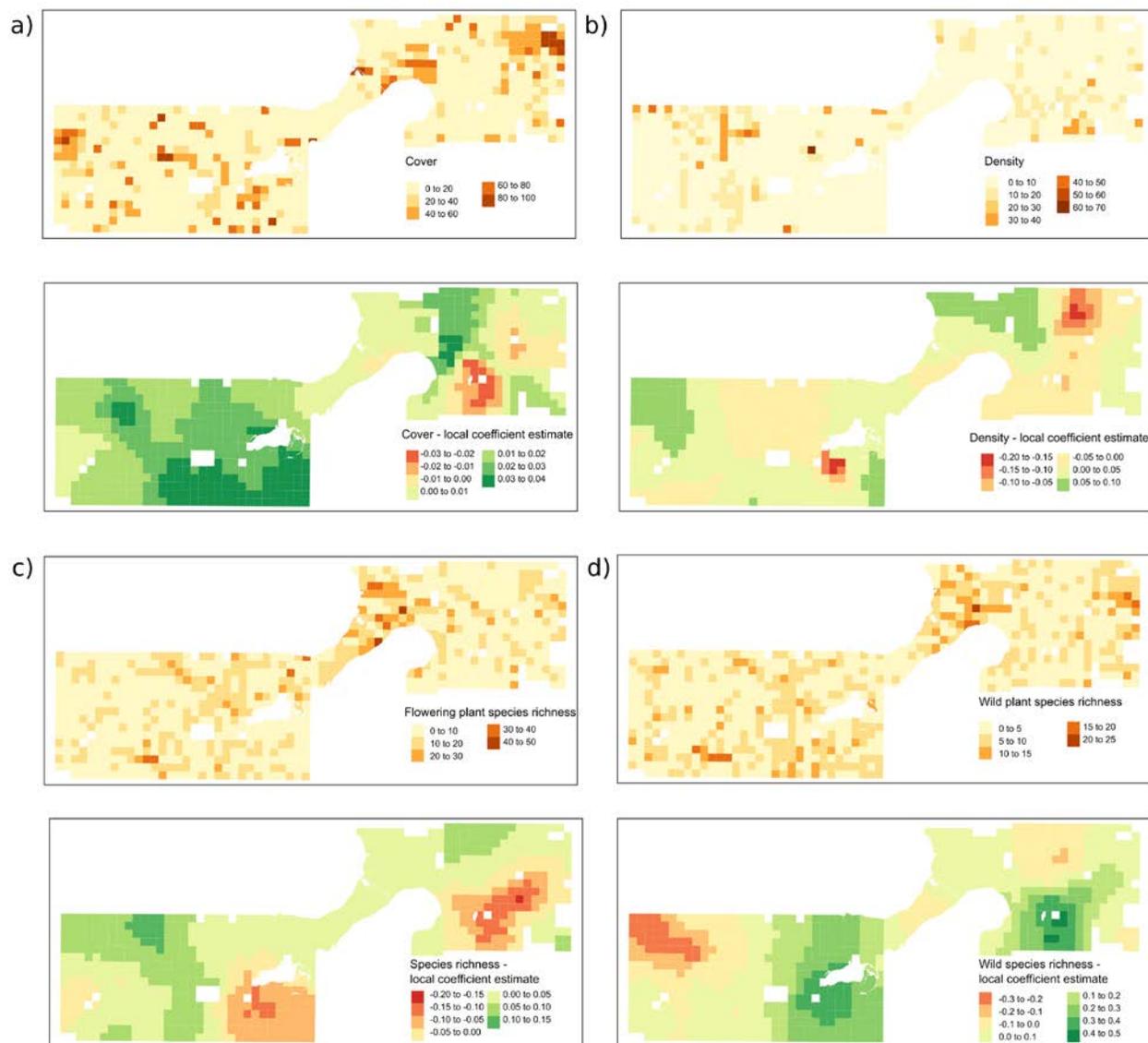
Figure 5. Raw explanatory variables and coefficient estimate results of geographically weighted regression mapped across study extent for a) % flower cover b) % flower density c) flower species richness d) wildflower species richness. Coefficient estimates can be interpreted as, -0.3 = 26%, -0.2 = 18%, -0.1=10% less bees, 0.1 =11%, 0.2 = 22%, 0.3 = 35%, 0.4 = 49 % more bees.

DISCUSSION

The results of the study illustrate the distribution of foraging bumble bees with regard to the most favored floral species, scales of spatial autocorrelation present in the bee and floral resource variables, and also provide evidence to support the positive influence floral cover and species richness explanatory variables across the study extent, as well as some heteroscedasticity in the influence of these floral resource variables.

Flower species and foraging bumble bees

Many past studies have recorded particular preference for high resource flower species by foraging bees. Gardeners and pollinator enthusiasts have identified bee-attractive flowers and supported the use of native plants in pollinator gardens for many years (Tuell et al. 2008, Williams et al. 2015). However, the benefit of lists has also been questioned, as many diverse flower species and are likely useful to bees and other factors like consistent availability of resources through the season might be more important factors to consider (Garbuzov and Ratnieks 2014). The dramatic differences in visitation rates between flower species underscored the variation in capacity of flower species to provision foraging bumble bees. While many concerns should be considered to optimize floral species assemblage for pollinator habitat, selection of bee-attractive species is an important consideration.

Global models and heteroskedasticity in the influence of floral resources
on the number of foraging bumble bees

In the global glm regression model, we estimated average effect sizes of floral cover, density, overall floral richness, and prairie and lawn floral richness for prediction of foraging

bumble bee counts. This approach is useful to test our hypotheses that our explanatory variables provided useful predictive capacity across our study extent, and consistent with other studies, we observed that these explanatory floral variables help predict bee distribution. However, we also expected that the influence of particular variables might differ based on local context. For instance, where floral resources are abundant and specious, the highest quality floral resource patches likely attract the most bees. Investigating the residuals of the model could help to identify locations that surpass our expectations of foraging quality, and locations which fail to meet our predictions. In our map of global model residuals we can see some high and low predictions of bumble bee foraging, especially several very attractive transects at the UW Madison Arboretum. We can hypothesize about what might cause this additional variation in bumble bee foraging, and set up new studies to test these hypotheses, but we also can explore potential heteroscedasticity of our predictor variables using geographically-weighted regression. This comprehensive investigation of fluctuation in the effect sizes of floral resource explanatory variables across the study extent contextualizes some differing results observed in studies that focus only on a particularly ecotype within cities or exurban ecotypes.

In the global model, we tested for evidence of interactions between land use zoning class and floral resource variables, but finally none were statistically significant ($\alpha=0.05$). Yet, in the full model and through the model selection process, several interactions were weakly significant, including, the interaction between city parks and native flower species richness, boosting the number of foraging bumble bees when they occurred together

Floral cover and density

Loss of habitat including nesting and foraging resources are likely the most important factors contributing to loss of pollinators around the world (Goulson et al. 2008, Winfree et al. 2009, Potts et al. 2010, Williams and Osborne 2009). Yet, it is unclear in what contexts various aspects of floral abundance and distribution may be most critical for local bees (Crone and Williams 2016, Spiesman et al. 2016). The geographically weighted regression results in this study the pervasive positive influence of flower cover, and the slight positive influence of flower density – which is most apparent in agricultural zones and commercial zones. Floral resources are a primary bottom-up limiting factor for pollinator populations, but local circumstances of distribution and phenology should be considered in depth. Studies located in resource pulse landscapes have observed beneficial results of late blooming mass-flowering crops, but not early blooming mass-flowering crops, suggesting increased floral resources as colonies are reaching maximum size has a much different than a bump in floral food resources while the colony is still growing. Bumble bees store very little extra food resources in the nest, so continuous access to floral resources during the season is crucial. Recent studies of foraging distance have observed variation in foraging distance in response to floral cover in the surrounding landscape and that documented flexibility is likely essential for bumble bees to respond to heterogeneity in the spatial and temporal distribution of resources (Jha and Kremen 2013; Vasseur et al., 2013).

Several recent studies have isolated the efficiency of foragers in resource collection as the primary driver of colony growth (Spiesman et al. 2016). One study found no influence of landscape scale floral resources when local resources were high, and in this case found high flower species dominance as the primary driver of colony growth (Spiesman et al. 2016). The association between transect scale floral cover and foraging bumble bees seems quite clear, and

persistently positive across the study extent. It's possible that the spatial scale at which density was estimated, and the heterogeneity within a transect, obscured the usefulness of this metric.

Floral species richness, floral type, and foraging bumble bees

Floral richness positively influenced the count of foraging bumble bees more than the richness of prairie and lawn species alone. While many common lawn and prairie species were highly attractive to foraging bumble bees and supported the majority of foraging visits, there were highly bee-attractive garden plants as well, including mints, Russian sage, and spirea. As micronutrients may vary greatly between plant species, the need for diverse floral resources may vary based on the nutritional content of the available assemblage.

In two parts of the city, the influence of native plant species diversity overcame the influence of overall species richness in the geographically weighted regression results. This phenomena appeared to result in compensatory negative coefficients in the overall floral species richness variable, where the prairie and lawn species subset surpassed it as a strong influential explanatory variable. These areas represented localities where species rich prairies were nested in residential communities. While it seems that, generally floral richness is an important predictor, of improved foraging resource quality, in some circumstances prairie and lawn species richness provides more explanatory capacity and better encapsulates the relative quality of floral resources. This result underscores the value of pollinator resources that in relatively large extents of restored prairie inside the urban matrix.

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Chapter 3: Woodland and floral richness boost bumble bee density in cranberry resource pulse landscapes

Abstract

Native pollinators provide an important ecosystem service for many pollination-dependent fruit crops, but require nesting and foraging resources in proximity to target crop plants. Additionally, pollination services provided by wild bees may fluctuate temporally due to foraging preferences that shift based on surrounding habitat and floral resources throughout the season. This study investigates how landscape context influences bumble bee density and fluctuation around and during the resource pulse created by the target crop bloom. We sampled bumble bees at fourteen cranberry marshes before, during, and after the cranberry bloom in central Wisconsin. We quantified floral richness and surrounding land cover and assessed their effects on individual and colony density and detection using linear regression. Forest in the surrounding area best predicted increased individual and colony density during all temporal extents. The interspersion of meadow through the surrounding landscape and the total bog edge were also useful to explain positive covariation in top regression models for bumble bee colony density, while floral richness on site was a useful second factor for explanation of increased forager density. Overall, colony density and detection did not change between the pre- and post-bloom period and resource pulse, controlling for important landscape scale effects. Yet, in the multiple variate regression models for relative change in colony density, increased meadow interspersion and decreased open-shrub bog explained some increase in colony density. While sites with high meadow interspersion in the surrounding landscape had lower average detection probabilities, suggesting some dilution

effect, landscapes with more surrounding meadow and less clumpy forest experienced increased colony detection probability during the crop bloom. We suggest maintaining forest in the surrounding landscape to promote local bumble bee colony density and increasing floral richness on site to attract foraging bees. While colonies may be somewhat diluted by surrounding interspersed meadow, marshes located in landscapes with more surrounding meadow experienced a bigger increase in detection probabilities of colonies during the resource pulse, corroborating our observation that cranberry floral resources are attractive to bumble bees.

INTRODUCTION

As increasing crop yields have plateaued in past years, the idea of ecological intensification has emerged aiming to reduce pollution and energy input, while bolstering yield and biodiversity through optimized restoration (Bommarco et al. 2013; Garnett et al. 2013; Grab et al. 2017). Studies show that native bee visitation already provides measurable pollination services to many important agricultural crops in addition to pollination services supplied by managed pollinators, such as the European honey bee (Hoehn et al. 2008; Garibaldi et al. 2011). A meta-analysis of agricultural systems around the world showed that in many cases, pollination supplementation by honeybees could not replace the supplemental benefits of native bee pollination regardless of supply (Garibaldi et al. 2014). However, adjusting the management of agroecosystems through supplementation of floral resources or natural habitat has been shown to increase wild pollinator richness and abundance and improve agricultural yield in several studies (Pywell et al. 2015; Blaauw and Isaacs 2014; Iverson et al. 2014). Cranberry growers rely on pollination from managed bees as well as wild bees to transfer pollen between plants to increase

berry production. Since US cranberry production is valued at over $250 million, there is clear motivation to bolster yields including by enhancement of native pollinator habitat. Cranberry (*Vaccinium macrocarpon*, Ericaceae), is a crop native to North America and indigenous wild populations are abundant throughout Wisconsin bog ecosystems. Industrial production is concentrated in the central part of Wisconsin, the top cranberry producing US state, accounting for more than 57% of US production (USDA 2010; Kashian and Peterson 2013).

Bumble bees are important contributors to high-quality pollination services for cranberry. They are a common polylectic (i.e. generalist) pollinator, and the only social bees native to North America. Bumble bees are especially successful pollinators of cranberry because they buzz pollinate, increasing pollen removal from the poricidal anthers (Cane and Schiffhauer 2003; King and Buchmann 2003). Cranberries require pollination to develop, and while abiotic factors such as wind and agitation provide some base level of pollination services, bees pollinate the flowers much more effectively, leading to a substantial increase in yield (Gaines-Day and Gratton 2015). While many types of bees visit cranberry flowers (Gaines-Day and Gratton 2016), interspecific comparisons identified bumblebees as the most effective pollinators from a mix of solitary and social bees (Cane and Schiffhauer 2003). Bumble bees carried more than three times as much pollen and honey bees were more often observed collecting nectar alone (Broussard 2011). Additionally, bumble bees provide the advantage of foraging in slightly more adverse weather conditions, with lower sensitivity to temperature and light conditions. This is an especially advantageous characteristic as most cranberry production occurs in cool, north temperate climates.

The simplifying influence of agricultural intensification on the structure of rural landscapes is associated with declines in biodiversity around the world. However, agricultural crops that are cultivated at high density and bloom *en masse*, simultaneously create a large resource pulse, that can boost the populations of species who may benefit from the supplement of food or habitat resources (Williams et al. 2012; Diekotter et al. 2013; Riedinger et al. 2014; Rundlöf et al. 2014). In central Wisconsin, the resource pulse of the cranberry bloom significantly impacts the floral resources available to bees. Mass-flowering landscapes, in which a cultivated crop blooms at once over a limited time frame, are known to influence pollinator density, including the density of bumble bees, creating both positive spillover effects (Hanley et al. 2011; Kovacs-Hostyanszki et al. 2013) as well as competition between co-blooming plants (Holzschuh et al. 2016; Diekotter et al 2010; Stanley and Stout 2014). Although the timing of the resource pulse (Williams et al. 2012; Riedinger et al. 2014; Rundlöf et al. 2014; Kallioniemi et al. 2017) and adequacy of mass-blooming plants as food resources for pollinators must be considered. Oilseed rape, an early blooming mass-flowering crop has been observed to attract nesting queen bumble bees, then fail to adequately provision worker bees reducing colony survival (Galpern et al. 2017). Additionally, food resources may not necessarily bolster local populations if there is not enough nesting habitat available (Williams et al. 2012).

Bumble bees (Hymenoptera: Apidae: *Bombus*) are central-place foragers and are known to be relatively vagile dispersers, resilient to habitat fragmentation and capable of maintaining pollination services in complex and resource-limited landscapes (Heard et al. 2007). However, bumble bees require cavities to protect their colonies and prefer to forage on diverse floral assemblages including native plants, thus they can be inadvertently extirpated from simplified

landscapes lacking these habitat requirements (Williams et al. 2012; Westphal et al. 2006). An increase in forested land within the surrounding landscape is one of the most common factors associated with increased colony density in some examined landscapes, yet forested landscapes with limited floral resources have exhibited the reverse association in other investigated study extents (Dramstad 1996; Jha and Kremen 2013; but see Kallioniemi et al. 2017). The positive association between forested land cover and bumble bee colony density is attributed to the assumed increase in abandoned rodent burrows and other cavity spaces that provide nesting habitat (O'Connor et al. 2012). Studies that assess the relative importance of various components of landscape composition and landscape configuration related to nesting and floral resources for bumble bee colony density in various regional contexts provide important information to inform ecological intensification efforts to improve crop yields.

In recent years, pollination ecologists have become increasingly interested in how habitat loss and disruption of habitat configuration independently impact pollinator populations. Processes including habitat fragmentation or concentration of land cover types associated with simplification of landscapes may cause additional disruption of pollinator activity. Attention to each distinct component of landscape structure may improve the capacity of landscape managers to specifically address limitations associated with particular aspects of land use change related to either landscape content or configuration (Fahrig 2003; Hadley and Betts 2012; Nicolson and Wright 2017). Yet a recent major meta-analysis has discovered that habitat loss effects are far more often observed than effects of land cover configuration effects on species declines (Fahrig 2017). In some cases configuration metrics may effectively summarize relatively subtle types of habitat that covary with the shape of more pronounced land cover types (e.g. riparian edge).

Several pollination studies suggest that improving aspects of landscape composition that contribute to bee habitat could be an effective way to promote indigenous pollination services on site in an agricultural context (Brosi et al. 2008; Carvell et al. 2011; Blaauw and Isaacs 2014). Isolating and testing the independent contributions of landscape content and configuration variables is useful to distinguish which mechanisms act as limiting factors in a particular landscape context.

Recent studies have also emphasized the importance of landscape level floral resources and native plant richness in attracting foraging bumble bees (Jha and Kremen 2013; Kallioniemi et al. 2017). Sites with increased floral richness were observed to attract bumble bees into longer foraging trips in one study (Jha and Kremen 2013), and in others a higher density of foraging bumble bees was measured in association with habitats providing increased diverse floral resources (Carvell et al. 2011; Kallioniemi et al. 2017). Kallioniemi et al. (2017) observed positive effects of late blooming mass-flowering crops on bumble bee populations and negative effects of early blooming mass-flowering crops, as well as a positive association with linear elements associated with increased semi-natural habitat and floral resources.

Despite the known benefits of forested land and flowering plant assemblages on bumble bee colony density and abundance, few studies simultaneously investigate the influence of these factors on bumble bee foragers over the course of a mass-bloom in a pulse resource landscape (Riedinger et al. 2014; Rundlöf et al. 2014; Kallioniemi et al. 2017). This study aimed to assess the following hypotheses (see Predictions and Rationale in Figure 1):

H1) The number of bumble bee colonies foraging at cranberry marshes is higher at

locations with a lower proportion of agriculture in the surrounding landscape and more

woodland and other semi-natural habitat associated with floral resources, during both the pre- and post-bloom periods and the floral resource pulse;

H2) Colony detection is higher at marshes located in forested landscapes compared to agricultural landscapes because more colonies are located in closer proximity to the forested landscape marshes. In other words, we expect to find more bees per colony at forested landscape sites compared to sites located in areas of intensive agricultural cultivation;

H3) colony density (A1) increases or (A2) decreases during the resource pulse due to increased attraction to the concentrated resource or dilution across landscapes supplied with abundant cranberry pollen and nectar resources or displacement by honeybees;

H4) colony detection (A1) increases or (A2) decreases during the resource pulse due to a higher dependence of colonies on cranberry resources or conversely due to dilution or displacement by honeybees

H5) landscape and local site factors moderate the change in density of bumble bee individuals and colonies foraging at cranberry marshes between the pre- and post-bloom periods and floral resource pulse reducing the influence of the resource pulse on density and detection in landscapes with more forest and semi-natural habitat

This will inform us if, and where bumble bee foraging patterns shift in relation to the pollination of a native monoculture crop. We use microsatellite DNA to assign colonies to non-lethally sampled bumble bees to focus our study on the effective population size and derive important additional information about foraging distribution based colony detection rates.
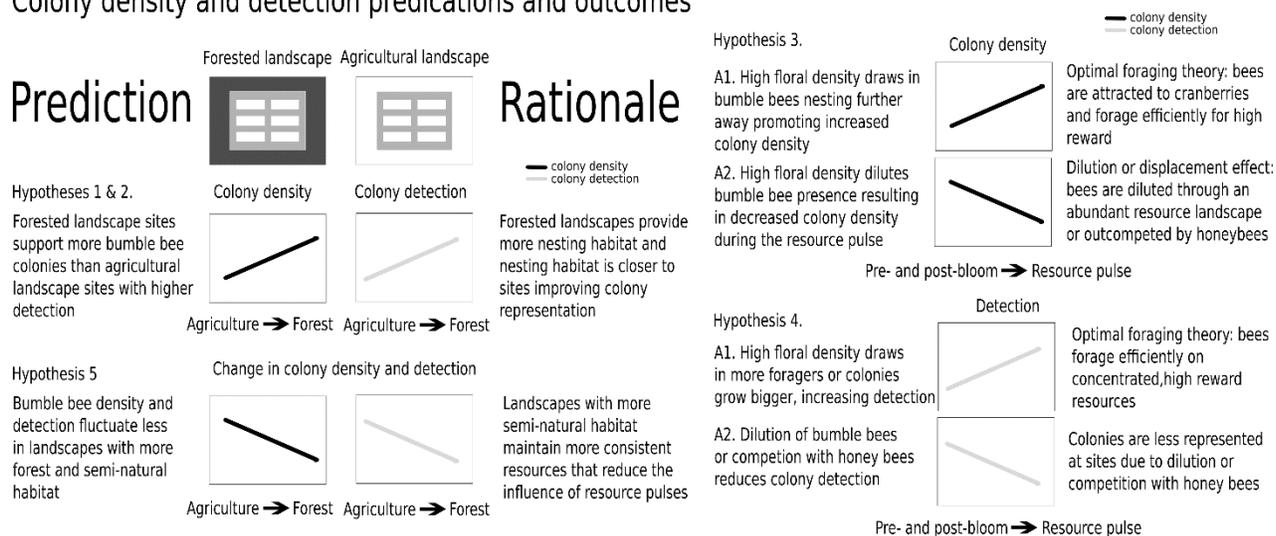
Fig1. Study expectations of higher colony density (black) and detection (grey) in cranberry landscapes across a habitat gradient, as well as alternative hypotheses regarding changes in colony density and detection observed during the pulse in resources. Detection is the proportion of estimated total colonies observed relative to the total estimate, e.g. low detection occurs with increased proportion of single colony observation events.

METHODS

Study area

The study included fourteen cranberry marsh sites in Central Wisconsin, between Tomah, Wisconsin, USA (44.0°N, -90.5°W) and Stevens Point, Wisconsin, USA (44.5°N,-89.5°W). This study extent is located within the Wisconsin Department of Natural Resources Central Sand Plains ecological landscape. Deep sand deposits formed the well-drained, permeable soil, frequently characterized by low nutrient and low available water capacity. The water table is very close to the surface, and low lying terrain is frequently poorly drained with shallow surface to 15

foot deep peat deposits (Wisconsin Department of Natural Resources 2017). The landscape surrounding the marshes is largely comprised of a mosaic of cropland, pine, oak, and aspen woodlots, wooded swamps, peat bogs, patchy residential properties and managed grasslands. Many of the historical wetlands were drained for agriculture. North of the study extent, the proportion of swamps and bogs in the landscape increases compared to this central sand plain ecoregion. Cranberry marshes in this landscape were constructed as rows of rectangular, excavated beds surrounded by mowed dikes and irrigation canals connected to natural and man-made reservoirs with varying amounts of riparian and prairie habitat. Rich prairie floral assemblages are sometimes cultivated on site by cranberry growers.

Site selection and landscape characterization

Cranberry marshes within the study extent were identified and overlaid on a classified land cover raster to select fourteen marsh sites in a stratified-random design. Seven marsh sites were selected from high intensity agricultural landscapes and seven sites from more heterogeneous landscapes with more woodland and other semi-natural land cover types. We produced the site-selection land cover raster using the unsupervised classification of 1m resolution aerial photos into 30 classes, followed by reclassification of similar classes to best identify cultivated land, forested land, and other land cover types (USDA 2013). Landowners or marsh managers were contacted to request permission to sample bumble bees during the summer. The average nearest neighbor distance between sites was 4.3km and the average distance between sites was 37km. All sites were at least 2.5km apart, and the seven agricultural landscape marshes were well interspersed with the seven forested landscape marshes. A full map is not

included to protect grower anonymity, but a partial map is shown to illustrate the interspersion of agricultural and forested landscape marsh sites (Figure 2).

Cranberry marsh sites
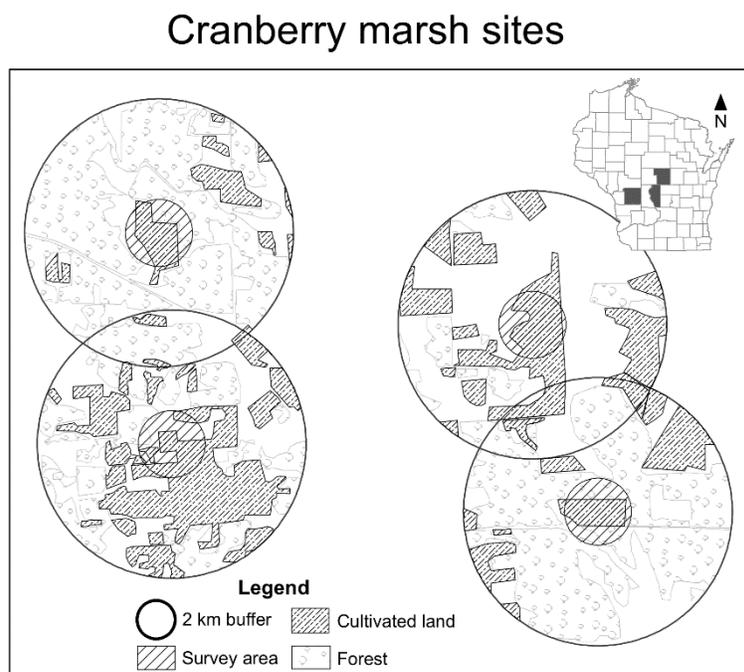
Fig2. Map of several cranberry marsh study sites nested in forested and intensive agricultural landscapes located in central Wisconsin.

Following site selection, lowland forest, open meadow-perennials, open-shrub bog, and cultivated land were digitized manually based on 1m resolution aerial photos in the 2km landscape sectors surrounding the study marsh sites to measure the land cover content and configuration as an explanatory variable. This landscape sector area aims to cover at least twice the average foraging distances of bumble bees based on the assessment of previous studies (Darvill et al. 2004; Carvell et al. 2012; Redhead et al. 2016). Cultivated land was identified based on the relatively homogeneous texture and geometrical shapes, and open meadow-

perennial land cover was distinguished by the highly heterogeneous texture compared to mowed areas, and the lighter, yellowish shade compared to bogs or swampy areas. The land cover of much of the surrounding landscape was ground truthed during the sampling period to improve accuracy. This digitized vector layer was used to quantify the landscape content in terms of the proportion of each land cover class within the surrounding 2km landscape sector. The 2km landscape sectors surrounding the agricultural landscape marshes were comprised of 28-75% cultivated land, 3-33% forest, 2-21% meadow, and 0-10% bog, compared to the marshes nested in lowland forest landscapes that were surrounded by 5-25% cultivated land, 38-61% lowland forest, 2-27% meadow, and 0-15% bog. The proportion of agricultural land and forest in the surrounding landscape were negatively correlated with a Pearson correlation coefficient of 0.63, while the amount of meadow and bog varied more within the groups rather than between them (Online resource 1). Landscapes with lower proportions of cultivated land selected from high intensity agricultural landscapes were generally near large reservoirs of water or lakes rather than woodland and other semi-natural terrestrial land cover types.

Several aspects of landscape configuration also contribute to the composition of the landscape and distribution of resources available to pollinators. We calculated these metrics using Fragstats 3.0 (McGarigal et al. 2012), after converting the vector layers for lowland forest, open meadow-perennials, open-shrub bog, and cultivated land into 20m x 20m resolution raster layers and combining them in ESRI ArcGIS 10.0 (ESRI 2011). The first configuration metric related to the distribution of cultivated land measures field size as Mean Patch Size (MPS). Many marshes consistently expand as established farm businesses grow and develop, yet larger field sizes result in a reduction of semi-natural field borders, and create a larger interior:edge

ratio. The Mean Patch Size (MPS) metric allows us to test if the reduction of semi-natural verges and increase of interior:edge ratio resulting from larger size farms predict changes in bumble bee colony density better than the proportion of cultivated land area alone.

The other four landscape configuration metrics were land cover class aggregation metrics relating to the distribution of lowland forest, open meadow-perennial, and open-shrub bog across the landscape, as well as the overall heterogeneity. The second metric selected related to the distribution of wooded land was the Clumpiness Index for wooded land, as forested habitat is often positively associated with nesting habitat and negatively associated with foraging habitat due to displacement of flowering forbs. This metric was selected to test if the spatial distribution of woodland had a significant explanatory capacity related to the bolstering of bumble bee density across the landscape. The Clumpiness Index (CLUMPY), concerns the frequency with which the adjacent cells are of similar or different class types. This index is written to isolate the configuration component from the area, to provide an index of fragmentation (McGarigal et al. 2012). Third, we selected interspersion of open meadow - perennial land cover, measured using the Interspersion and Juxtaposition Index (IJI). This metric was selected to consider the hypothesis that interspersion of meadow would distribute floral resources more widely across the landscape, decreasing the search time between foraging patches. The Interspersion and Juxtaposition Index (IJI) relates to the likelihood of cells of a certain class to be located in the same patch and uses the size distribution of patches as well as the number of patches to deliver a metric of fragmentation. Wetland edge can support increased floral resources due to dependable soil moisture so this measure of Total Edge length (TE) was included for the open-shrub bog land cover class to test if this landscape feature contributed a measurable influence on bumble

bee density and fluctuation. Finally, we summarized the overall heterogeneity of the landscape using the landscape level contagion metric (Li and Reynolds 1993), a widely used metric which subsumes both dispersion and interspersion, based on the probability of finding adjacent dissimilar cells (McGarigal 2012).

Bumble bee sample collection

The bee and flower sampling effort was standardized across the marshes. We surveyed a roughly 500m radius sector of the landscape, dominated by cultivated cranberry marsh at each site that included at least two marsh edges. This sampling area encompassed the whole marsh for the smaller marshes, while it constrained the surveyed area for the larger marshes, which sometimes covered remarkably extensive areas. Bee sampling was conducted by walking around the beds, dikes, or semi-natural marsh edges with blooming flowers, and sampling any foraging bumblebees using a hand net, both on the cranberry beds as well as on surrounding edge vegetation. Bumble bees were identified to species as often as possible in the field (Williams et al. 2014). The sampling occurred during three two-week periods which occurred before the cranberry bloom between May 18th and June 6th, during the cranberry bloom between June 18th and July 4th, and after the cranberry bloom between July 18th and July 31st, 2015. Bees were sampled for 12 person hours spread over two or three visits, on sunny days between 9am-5pm. During the post-bloom period, the sampling time was decreased to three person hours per marsh spread over two days, as bumble bees reached peak abundance and were constrained to smaller foraging areas after the cranberry bloom finished. More bumble bees were collected in the post-

bloom period than the other periods. Sampling effort (i.e. time) spent in each marsh was kept equal so that the number of sampled bees would be proportional to bumble bee density. As bumble bee frequency can be highly variable, sampling effort was maximized, and constrained to sunny, non-windy conditions distributed over two or three periods during each survey so that these variables would not impact forager detection.

Two of the marshes purchased commercial bumble bee colonies housed in protected cardboard boxes (Koppert, Howell, MI, USA) to supplement pollination during the season. Individuals from these nests were sampled directly as reference sisters in order to identify which bees in the study came from the commercial colonies rather than wild bumble bee nests surrounding the sites.

Floral richness and evenness sampling

A single site floral richness survey was conducted between the bloom and post-bloom sampling periods for the marsh study area. The same sampling area was used at each marsh for the bee survey and the floral richness survey, constrained to a 250m radius sector of the landscape to standardize the area surveyed across the marshes. This full site survey targeted all of the flowering plants in anthesis in mid-July (see Online resource 2 for common species). All blooming species were sampled and identified to species or morpho-species.

Molecular methods

Sampled bumble bees were anesthetized using CO_2 and one middle leg was clipped to provide a non-lethal DNA sample. Upon collection, bees were identified to species and the

flower on which they foraged was recorded. The bee tissue sample was frozen in 95% (vol/vol) ethanol upon return to the laboratory at the University of Wisconsin, Madison, WI, USA.

A 5% chelex solution was used to extract bee DNA, from a small piece of the sampled middle leg, then 13 highly polymorphic microsatellite loci were amplified to cluster individuals into colonies based on high probability of full sisterhood (Walsh et al. 1991). Amplifications were conducted using 10µL PCRs containing 60ng of template DNA, 0.125-0.5µM of each primer, 0.5 units of *Taq* DNA polymerase (Fisher Scientific), 1X reaction buffer (included in the Fisher Scientific *Taq* batch), 250µM of each dNTP, and 2.25-3.75mM of $MgCl_2$. Thermocycling conditions consisted of an initial denaturation stage at 94°C for 2 min, followed by 30 cycles at 94°C for 45s, 55-63°C for 30s, and 72°C for 30s, and a final extension at 72 °C for 10min. One primer from each primer pair was labelled with FAM, HEX, NED, or PET dye to allow multiplex PCR reactions. Primers used included B125, B124, B116, BL15, BT30, B119, B126, B72, B59, B63, BT28, B62, B52 (Estoup et al. 1995; Funk 2006; Stolle et al. 2009).

Individual bees were categorized by species based on catch identification and confirmed with the non-metric multidimensional scaling (NMDS) multivariate statistical technique. Bees were genotyped and separated by species and only individuals with more than eight genotypes were used in subsequent analyses. Alleles were scored manually using GENEMARKER (Softgenetics). Full sisters were removed, and genotypes of each species were tested to ensure necessary assumptions were met. Microsatellite loci showed no evidence of null alleles. Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) assumptions were met for all loci in each common species using a Bonferroni-Holm correction for multiple comparisons applied to a significance level of $p<0.05$.

Colony assignment and genetic mark recapture

Full sisters from each site were assigned unique colonies identification using the maximum likelihood program, COLONY 2.0 (Wang 2004; Jones and Wang 2010). The colony assignment algorithm was utilized for haploid-diploid species, allele frequencies were updated every 1000 iterations, and a 1% genotyping error rate was used for all loci. The total number of colonies at each site was estimated using the genetic mark recapture R package, capwire. This package uses the recapture rate of individuals (or colony mates in this case) to estimate population size. This genetic mark recapture method treats the resampling of sisters as repeat observations of an individual colony, through either the equal capture model or the two innate rates model. In this study, similar to past studies (see Goulson et al. 2010), the estimations produced by both models were highly correlated. A maximum likelihood bootstrapping method included in the package was used to compare the fit of the equal capture model (ECM) and two-innate rates model (TIRM). However, the two innate rates model was preferred based on the maximum likelihood tool, across 1000 bootstrap runs. The two innate rate model represents the hypothesis that closer colonies will be resampled at higher rates than more distant colonies (see Goulson 2010; Jha and Kremen 2012).

Commercial bumble bees

Two marshes included in the study purchased supplemental bumble bee colonies as well as honey bees during the season. We sampled workers from the supplemental hives, to allow the identification and removal of field sampled bees from our study that originated from these supplemental colonies. Individual worker bees were sampled from 118 supplemental bumble bee nests that showed signs of activity at one forest landscape marsh and 31 active nests at one

agricultural landscape marsh. When colonies were assigned, based on the COLONY full sister clustering, some of the sister bees representing different supplemental nests were identified as full or half-sisters. The supplemental bees were removed from all study analyses, since the main focus of the investigation targeted wild bumble bee colonies. The supplemental bees made up a larger proportion of the sampled bees at the marsh in the intensive agricultural landscape (41%) compared to the high woodland landscape (14%), yet the supplemental bees comprised a minority of the sample in both cases. The detection probability of the supplemental colonies at the intensive agricultural marsh in the sampled bees was also much higher.

Statistical analysis

The detection probabilities of bumble bee colonies were calculated per site and sampling period as the proportion of colonies observed relative to the total estimated number of nests using the TIRM genetic-mark recapture method. The change in colony density was calculated as the ratio of the number of colonies estimated during the resource pulse relative to the number of colonies estimated from the pre- and post-bloom surveys, when the pulse colony estimate is divided by the change in detection rate (See Table 1).

Linear regression analysis was first used to evaluate how landscape composition, configuration and floral richness explained individual and colony density for the full season, pre- and post-bloom periods, and during the resource pulse using R statistical software (R core team 2013). Subsequently, the same method was utilized to evaluate how these factors influenced colony detection, the change in individual and colony density between the pre- and post-bloom

periods and the resource pulse, and the change in colony detection. Standardized linear regression coefficients or betas and their 95% confidence windows were calculated based on the univariate linear regression models.

To address hypotheses 1, 2, and 5, the best multiple variate linear regression models were identified based on all landscape composition and configuration variables as well as site scale floral richness using AICc model selection and multi-model averaging (Symonds and Moussalli 2011; Case 2015). After the top model was discovered using AICc model selection and tested for multicollinearity, a more stringent selection process was conducted to reduce multicollinearity amongst the suite of potential model factors. Explanatory variables with a Variance Inflation Factor (VIF) over 2.5 were eliminated, corresponding to a correlation of R^2=0.6 between variables. Standardized beta coefficients from all top models within a subset within Δ4 AICc were averaged across models in which they appeared as well as the whole subset (Table 2). Model selection and multi-model averaging processes were conducted using the MultMod R package (Barton 2017). We checked for spatial autocorrelation in model fit using Moran's I correlogram statistics conducted using the R package to test for the presence of a spatial pattern in model residuals.

To address hypotheses 3 and 4, we tested for a change in colony density and detection between the pre- and post- bloom period and the resource pulse period using multiple variate linear regression models to control for important landscape factors that contributed to top models for colony density (i.e. proportion of forest, proportion of meadow, meadow interspersion, bog edge, and floral richness) and detection (i.e. interspersion of meadow and floral richness) while testing for a categorical effect of the change in phenological period.

RESULTS

We sampled over 1500 bumble bees at 14 cranberry marshes. In mid-summer, during the floral richness survey, we recorded 16 to 50 flowering plant species in anthesis at each marsh. The bumble bees sampled represented six common species, *B. impatiens*, *B. bimaculatus*, *B. vagans*, *B. ternarius*, *B. griseocollis*, *B. rufosphinctus*, as well as three occassional species *B. perplexus*, *B. terricola*, *B. auricomus.*

Colony density and detection across a landscape gradient

On average, we sampled 112 bees per site across the full season (Table 1). The full season colony estimates ranged between 92 and 533 colonies, with an average and standard deviation of 165 ± 75 colonies estimated at the intensive agricultural sites and 343 ± 130 colonies estimated at the lowland forest sites. We sampled more individual bumble bees from sites nested in landscapes with more lowland forest (156 ± 60) compared to the intensive agricultural landscape sites (62 ± 31) surrounded by more cultivated land, however, there was not a difference in the colony detection probabilities measured at the study sites between the agricultural landscape (0.30 ± 0.08) and forest landscape sites (0.28 ± 0.12). The proportion of cultivated land and the proportion of forest in the surrounding landscape covaried inversely, and forest explained the most total variance in both individual and colony density during the pre- and post-bloom extent ($R^2 = 0.58$, $R^2 = 0.51$, respectively) as well as the full season ($R^2 = 0.56$, $R^2 = 0.68$), and resource pulse ($R^2 = 0.54$, $R^2 = 0.78$) compared to any other single factor. As

expected, the negative influence of cultivated land area also explained a large proportion of the variation in both individual and colony density during each period (Figure 3).

Bumble bee colony density and detection

Total colony density estimates
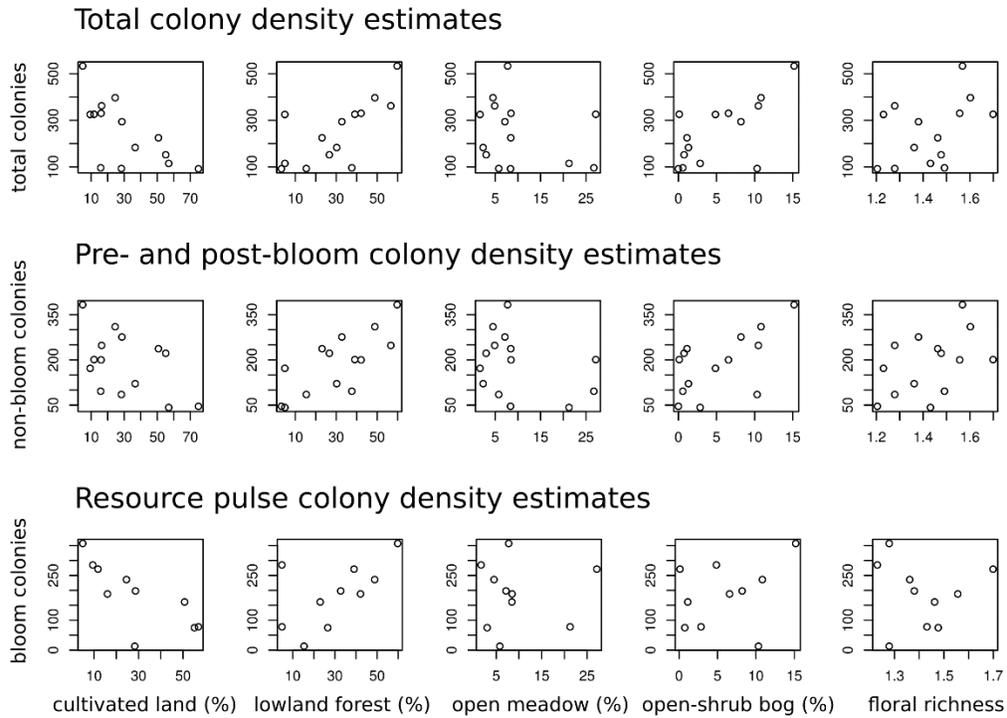
Fig3. Colony density estimates during the full season, pre- and post-bloom periods, and during the floral resource pulse displayed relevant to landscape content and study site floral richness.

A. Landscape composition and bumble bee density
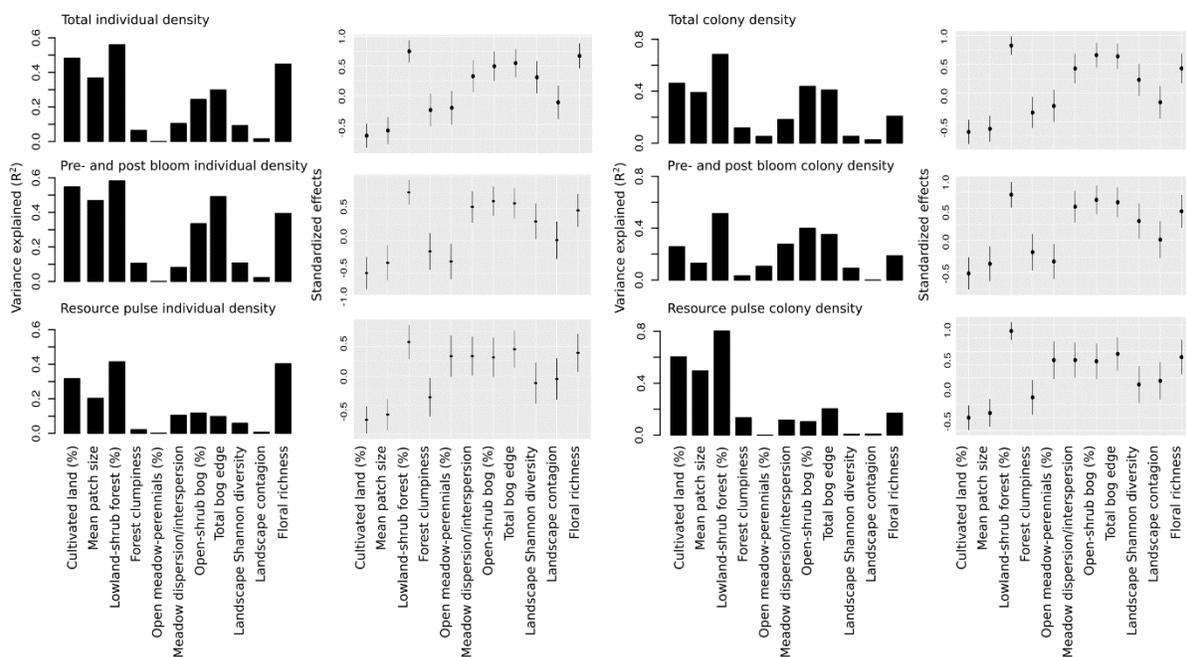
B. Resource pulse shifts in bumble bee density and detection probability
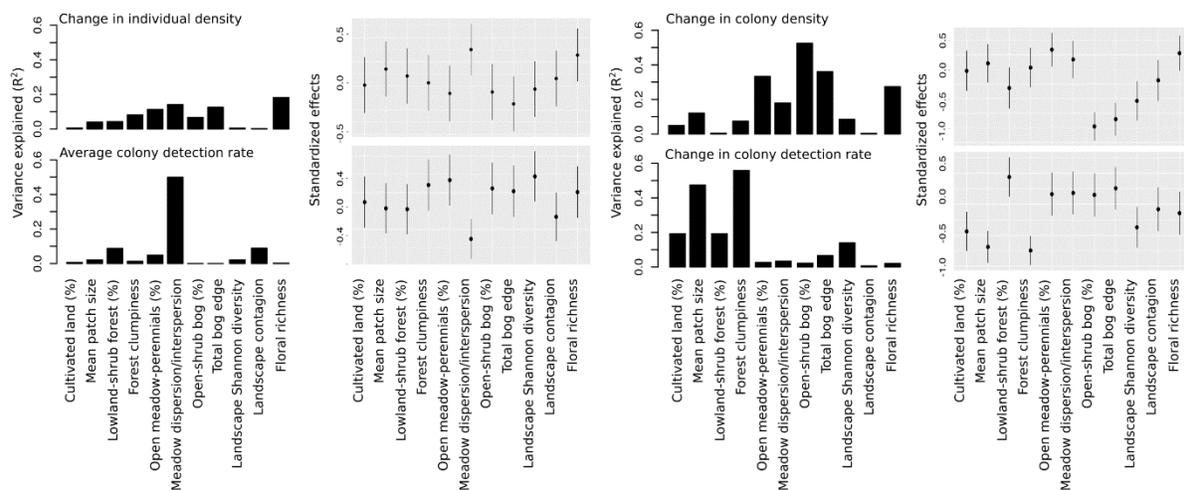
Fig4. Landscape content and configuration variables explain variation in the full season, pre- and post-bloom periods, and resource pulse densities of individual bees and estimated bumble bee colonies. The upper panel illustrates the variance in individual foraging bee density and colony density in terms of R^2, as well as the standardized beta coefficients and their standard error from

univariate linear regressions. The lower panel shows the influence of landscape on average detection as well as change in individual colony density and colony detection.

To investigate the evidence for hypotheses 1 and 2, we examined the influence of all aspects of landscape composition and configuration on bumble bee density and detection alongside each other through independent univariate linear regressions (Figure 4) as well as multiple variate regression models (Table 2). Standardized linear regression coefficients or betas and their 95% confidence windows were calculated based on the univariate linear regression models to provide a direct comparison of the variable effect sizes in terms of the standard deviation of the explanatory variable, meaning a change of one standard deviation of the explanatory variable will result in a change of the response variable equal to the effect size times the standard deviation of the response variable. In addition to the influence of forest land cover on colony density, the proportion of open-shrub bog in the surrounding landscape stood out as a positive predictor of bumble bee individual and colony density during the full season and pre- and post-bloom periods, but not during the resource pulse (Figure 3). Across the full season and during the pre- and post-bloom periods, the proportion of forest, total edge length of bog, and the interspersion of meadow contributed to the top multiple variate linear regression models which explained 85% of the variance in full season colony density and 76% of the variance in pre- and post-bloom colony density (Table 2). In addition to the proportion of forest and bog in the surrounding landscape, floral richness was an important predictor of individual bumble bee abundance throughout the season (Figure 4). The negative influence of interspersion of meadow was the most important predictor of average colony detection probabilities, inversely influencing

the ratio of observed colonies to estimated colonies, meaning colonies were more often represented by single individuals at sites surrounded by more interspersed meadow (Table 1).

Colony density and detection during the resource pulse

On average, 35 ± 17 individual bees were sampled per agricultural landscape marsh during the pre- and post-bloom periods, resulting in 30 ± 15 colonies identified and 147 ± 97 estimated, on average with standard deviations. We captured 89 ± 35 individual bees during the pre- and post-bloom period surveys at lowland forest marshes, resulting in 71 ± 27 colonies identified and 230 ± 94 estimated, on average. The detection probabilities per site, calculated as the proportion of nests observed relative to the total estimated number of nests using the TIRM genetic-mark recapture method, ranged between 0.12 to 0.41 in the pre- and post-bloom periods, with an average detection probability of 0.24 ± 0.10 for intensive agricultural sites and 0.32 ± 0.08 for high woodland sites during the pre- and post-bloom period.

During bloom, 26 ± 17 individual bees were sampled at intensive agricultural marshes on average, resulting in 22 ± 14 observed colonies, 105 ± 74 colonies estimated, and a detection probability of 0.32 ± 0.14, on average. We sampled 71 ± 37 individuals from forest landscape marshes during the bloom, resulting in 57 ± 28 observed colonies, 267 ± 63 estimated colonies, and a detection probability of 0.28 ± 0.09. Two intensive agricultural and two lowland forested sites were dropped in statistical analyses for this period, due to sampling ratios insufficient to estimate a total number of colonies, resulting in a sample size of n=10 for the bloom colony

density analyses and the change in detection and density analyses, and detection probabilities ranged between 0.18 and 0.53 during the resource pulse.

To evaluate hypotheses 3 and 4, we used multiple variate linear regression models for colony density and detection to control for important quantitative landscape factors while testing for a significant influence of phenological period represented by at categorical variable. The other explanatory variables considered in the models included the proportion of surrounding forest, proportion of surrounding meadow, meadow interspersion, bog edge, and floral richness for the colony density analysis, and interspersion of meadow and floral richness for the colony detection model. There was no evidence of any perceivable change in colony density or detection between the pre- and post-bloom period and the resource pulse period.

Landscapes and fluctuation in bumble bee foraging dynamics

Landscape variables did not explain the change in individual bumble bee density well in univariate linear regressions, yet the combined negative influence of the proportion of open shrub bog and positive influence of high floral richness explained 76% of the variation in relative change in bumble bee forager density between the two periods (Table 2). The best multivariate model for the change in bumble bee colony density between the pre- and post-bloom periods and the resource pulse included the positive effect of floral richness, as well as a negative contribution of the proportion of open-shrub bog (Table 2).

Colony detection was on average lower at sites with increased interspersion of meadow in the surrounding landscape. Forest clumpiness explained a decrease in colony detection during

the resource pulse and proportion of meadow explained an increase in colony detection rates between the non-bloom and bloom periods, meaning colony detection or the proportion of observed colonies relative to the estimated total number of colonies increased during the mass-bloom at sites surrounded by less expansive extents of forest and/or more surrounding meadow.

DISCUSSION

The results of this study confirm the first main study hypothesis that the proportion of forested land in the surrounding landscape is associated with increased bumble bee colony density (hypothesis 1), yet surrounding forest cover was not associated with increased colony detection (hypothesis 2). Instead, the interspersion of meadow in the surrounding landscape was associated with an inverse influence on colony detection. There was no evidence for either alternative tested for increased or decreased colony density or detection between the pre- and post-bloom period and the resource pulse (hypotheses 3 and 4). But, increased floral richness on site was the most useful factor explaining an increased number of bumble bee foragers and colonies during the resource pulse, alongside the negative influence of surrounding open-shrub bog in the case of change in colony density. Investigation of landscape scale patterns of covariance between surrounding land cover and change in colony density and detection confirmed hypothesis 5. Colony detection decreased more at marshes with more clumpy forest and larger average agricultural patch sizes during the resource pulse, suggesting that colonies located in more homogeneous landscapes perhaps stayed closer to their nest sites, or that more

distant colonies were perhaps less likely to visit these sites. Study results corroborate several recent foraging studies that indicate an influence of landscape scale distribution of floral resources on bumble bee foraging behavior (Jha and Kremen 2013; Redhead et al. 2016; Hemberger and Gratton 2018).

Influence of landscape on colony density

A high proportion of lowland forest or a low proportion of cultivated land in the surrounding landscape explained the most variation in bumble bee colony density at the cranberry marshes during each part of the season. This result provides further evidence that simplification of agricultural landscapes heavily modified for human use can threaten the resources available to support native pollinator habitat (Carvell et al. 2012; Jha and Kremen 2013; Redhead et al. 2016). While foraging resources impact colony growth and foraging range, it is reasonable to expect that land cover associated with nesting habitat would be the primary driver of colony density. The association between forest and wild pollination services is also supported by a previous study across the same ecoregion that provided evidence that honey bee hive abundance is correlated with cranberry yield in the low woodland cranberry landscapes of Central Wisconsin, but not in high woodland landscapes (Gains-Day and Gratton 2016). Wisconsin landscapes generally are characterized by a relatively high proportion of woodland and high floral richness especially in mid-summer through the early fall season due to summer rains and flower-rich wetland and prairie plant communities. This northern region of the USA is also characterized by a cooler climate, associated with high bumble bee species richness

(Williams et al. 2012; Lozier et al. 2011). These factors likely contribute to a relatively high regional population of bumble bees, and in combination with the pulse of cranberry floral resource availability during the early summer, likely promote the importance of nesting habitat as the major limiting factor for colony density. This study uncovered seemingly comparable total colony estimates compared to a similar study in the United Kingdom of two common bumble bee species with similar detection rates. Goulson et al. (2010) sampled *B. lapidarius* and *B. pascuorum,* observing 30 and 36 colonies on average for each species across the sites, and estimating the presence of 114 and 88 total colonies per site, on average, for these two common species across the sites.

Total edge length of open-shrub bog and meadow interspersion were useful contributing factors to the top models of colony density during both the full season as well as the pre- and post-bloom period models. While wetland edge differs from riparian forest, the increased soil moisture in both of these habitats likely increases the chances of late season floral resources. Riparian forest in addition to oak woodland stood out as a useful explanatory factors of colony density in a recent California study (Jha and Kremen 2013). Since highly vagile bumble bees can fly long distances to forage, they are not considered to be an "area sensitive" species, meaning that while some species are very sensitive to the core area of patches, they can use the resources of habitat patches regardless of patch size. It is unclear if isolation between foraging patches may influence bumble bee foraging behavior, however foraging plasticity and preference for floral richness over floral cover in another recent study (Jha and Kremen 2013) seems to suggest this may not be the reason for the positive influence of meadow interspersion rather than the proportion of meadow in the surrounding landscape. We suspect that lower flower abundance

and richness in some of the larger expanses of open meadow - perennial patches is the most likely explanation. Some of the landscapes included in our study with higher meadow contagion were observed to be dominated by grasses with less flowering forbs.

Meadow interspersion simultaneously explained decreased colony detection rates, meaning more colonies were represented by single individuals. We expect that high quality floral resource patches distributed heterogeneously across meadow areas may draw foraging bumble bees away from our sites, which could explain the decreased detection, and dilution of foraging bumble bees in landscapes with increased floral resources distributed in semi-natural meadow and grassland.

Influence of landscape on foraging fluctuation

Several alternative impacts of mass-flowering crops have been observed on bumble bee growth and population density such as boosted colony growth or dilution of native pollinators (Riedinger et al. 2014; Rundlöf et al. 2014; Kallioniemi et al. 2017; conversely; Holzschuh et al. 2016; Galpern et al. 2017), underscoring the fact that the influences of surrounding landscape composition and resources likely vary based on the regional context, phenology, and biotic community (Sardinas and Kremen 2015). Despite the import of honey bee colonies to enhance cranberry pollination during the mass-bloom, there was no evidence of a statistically significant drop in detection during the resource pulse period, controlling for the effects of landscape factors. Colony density also did not change significantly between the non-bloom and mass-bloom periods. As bumble bee colonies do not relocate, an increase or decrease in colony density would

imply the arrival of more distant colonies at the marshes or dilution or displacement of local colonies.

While the proportion of lowland forest in the surrounding landscape best explained individual and colony density of bumble bees during all temporal extents, the influence of the surrounding landscape was more closely matched by the positive influence of floral richness on site for individual bumble bee density compared to colony density, especially during the resource pulse period, even though the bumble bees were mostly sampled on flowering cranberry during this period. Floral richness has been shown to provide important nutrients for developing bee larvae (Genissel et al. 2002; Tasei and Aupinel 2008), potentially explaining the importance of floral richness in predicting foraging bee abundance, especially during the period with the most abundant floral resource availability overall. While high floral richness has been observed to incentivize longer foraging trips (Jha and Kremen 2013) and correspond with increased colony growth (Goulson et al. 2002), the increased floral abundance of mass-flowering crops have also been observed to explain increased colony growth (Williams et al. 2012; Riedinger et al. 2014; Rundlöf et al. 2014; Kallioniemi et al. 2017), at least for late-flowering crops. High floral dominance has additionally been observed as the best predictor of colony growth and reproduction in another recent study (Spiesman et al. 2017).

In our study, the proportion of open-shrub bog in the surrounding landscape was the primary factor negatively influencing the change in bumble bee colony density during the resource pulse. This may be because soil saturation on one hand prevents the use of underground cavities for nesting habitat, yet simultaneously wetland edge provides a potentially more consistent source of floral resources throughout the season to provision local colonies.

Additionally, the change in colony density was positively influenced by meadow interspersion in the surrounding landscape as well as floral richness. While meadow interspersion may also provide increased floral richness, it is also associated with lower colony detection, potentially contributing greater uncertainty in its effect.

Colony detection probability is influenced by colony size, survival, and distance from site, but also, foraging behavior and the dependence of colonies on floral resources at the site. In this study, change in detection, calculated between the non-bloom and bloom temporal extents should be most strongly influenced by the change in the dependence of colonies foraging at the site, as the colonies do not change nest location during the season and both periods include sampling of the late season colonies near peak abundance. The change in colony detection rates did not differ much based on the proportion of forest or cultivated land or floral richness, but colony detection rates decreased more during the mass-bloom at marshes surrounded by larger expanses of forest and larger sized farms. This may suggest that foraging workers from more distant colonies located around extensive forest patches or agricultural areas continued to make long foraging trips throughout their range, yet the colony dependence and the majority of colony individuals tended to shift toward other resources, potentially closer to nest sites. In an experimental project located across the same study extent, bumble bee foraging trip duration was observed to decrease by 22% during the resource pulse (Hemberger and Gratton 2018). Colonies located in these landscapes may have been foraging at longer distances than colonies located in more heterogeneous landscapes, and the resource pulse may provide more convenient resources.

CONCLUSIONS

This study provides further evidence that the landscape context dramatically influences the density of bumble bee colonies as well as fluctuation in foraging patterns throughout the pre- and post-bloom periods. Developing the contextual understanding of bumble bee foraging behavior depending on landscape content and configuration is important to predict and enhance the resources available to wild populations and even to improve the benefits derived from their ecosystem services. As wild pollinators are known to contribute uniquely and persistently to fruit yield, harnessing the mechanisms that promote these gains can improve the efficiency and production of farms. Native bee contribution is estimated to influence the yield of most fruit and nut crops that account for 35% of food production around the world, and demand for these crops is on the rise (Klein et al. 2007; Aizen and Harder 2009). We conclude that ecological intensification through conservation and restoration of interspersed forest and floral resources could promote native bumble bee populations and relieve dependence on managed honey bees to some degree.

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Table 1. Bumble bee colony estimates.

Site	pre- and post-bloom				resource pulse				change in detection (δd)	change in density	Full season			
	N_{Ind}	N_{Col}	N_{Tot}	d_1	N_{Ind}	N_{Col}	N_{Tot}	d_2			N_{both}	N_{Ind}	N_{Col}	N_{Tot}
A1	10	9	46	0.20	4	4	-	-	-	-	0	14	13	92
A2	29	27	207	0.12	41	30	75	0.4	0.30	1.11	3	70	54	152
A3	42	35	121	0.29	16	16	-	-	-	-	2	58	49	183
A4	23	17	42	0.40	24	19	78	0.24	1.66	1.12	0	47	36	115
A5	33	26	85	0.31	11	7	13	0.53	0.57	0.27	0	44	33	93
A6	50	45	276	0.16	40	36	198	0.18	0.90	0.8	6	90	75	294
A7	60	52	237	0.22	48	41	161	0.25	0.86	0.79	11	108	82	225
F1	104	86	310	0.28	87	71	236	0.30	0.92	0.83	15	191	142	397
F2	74	64	172	0.37	66	52	285	0.18	2.04	0.81	6	140	109	325
F3	85	64	192	0.32	104	83	266	0.31	1.03	1.31	13	189	134	330
F4	54	39	96	0.41	0	0	-	-	-	-	0	54	39	96
F5	50	45	248	0.18	53	51	-	-	-	-	8	103	88	362
F6	148	118	383	0.31	78	69	357	0.19	1.59	0.58	10	226	176	533
F7	98	74	194	0.39	92	72	237	0.40	0.98	0.96	12	190	135	357

Notes: The number of sampled workers during the temporal extent (N_{Ind}), the number of colonies observed within the specified sampling extent identified by Colony full sibship reconstruction (N_{Co}), the total number of colonies observed within each temporal extent estimated using the Capwire TIRM model estimation (N_{Tot}). Detection probabilities (d) are the proportion of colonies observed relative to the estimated total number of colonies (N_{Col}/N_{Tot}) as calculated as in (Goulson et al. 2010). The change in detectability (δd) is calculated as ($d1/d2$). The change in colony density during the resource pulse considers detectability, and is calculated as change in colony density = ($N_{Tot}2/\delta d$)/$N_{Tot}1$. N_{both} is the number of colonies observed during both the pre- and/or post-bloom sampling extent and the bloom sampling.

Table 2. Top and average multiple variate linear regression model betas (standardized linear coefficients), standard error, and model summary for individual and colony density estimates, detection, and change in individual and colony density and detection during the resource pulse.

Model	Landscape content factors					Landscape configuration factors					Site factor	Model summary
	%Ag	%F	%M	%B	SD	Ag,MPS	F,C	M,IJI	B,TE	L,H	Flw R	R^2
Full season colony estimates												
Top model		0.61±0.12						0.28±0.13	0.34±0.14			0.85
Avg (ΔAIC<4)		0.71					-0.17	0.28	0.33		0.22	
Pre- and post-bloom colony estimates												
Top model		0.64±0.18						0.40±0.18				0.67
Avg (ΔAIC<4)		0.56	-0.55	0.19			0.32	0.42	0.41		0.51	
Resource pulse colony estimates												
Top model		1.01±0.12	0.38±0.12									0.91
Avg (ΔAIC<4)		0.98	0.37									
Change in colony density												
Top Model		-0.70±0.18						0.48±0.18				0.76
Avg (ΔAIC<4)		-0.77				0.49		-0.54	0.48			
Full season individual estimates												
Top Model		0.54±0.13					-0.27±0.13				0.59±0.13	0.86
Avg (ΔAIC<4)		0.57	-0.20				-0.26		0.22		0.55	
Pre- and post-bloom individual estimates												
Top Model		0.46±0.11						0.42±0.10			0.44±0.10	0.91
Avg (ΔAIC<4)		0.44				-0.18	-0.18	0.39			0.45	
Resource pulse individual estimates												
Top Model		0.51±0.18									0.50±0.18	0.65
Avg (ΔAIC<4)		0.52	-0.32								0.57	
Change in individual density												
Top Model											0.41±0.26	0.18
Avg (ΔAIC<4)		0.34	0.34	0.07				0.38	-0.43	0.05	0.46	
Average detection												
Top Model								-0.75±0.30			0.28±0.30	0.47
Avg (ΔAIC<4)								-0.64				
Change in detection												
Top Model		0.44±0.21					-0.88±0.20					0.73
Avg (ΔAIC<4)		0.44			-0.52	-0.67	-0.75					

Notes: These top models were selected using AICc selection, based on the following terms: the proportion of cultivated land, the proportion of lowland forest, proportion of open meadows-perennials, and proportion of open-shrub bog in the surrounding 2km landscape sector, the Shannon diversity of different land cover

types (L_SD), and also the landscape configuration descriptors: mean patch size of agricultural lands, forest clumpiness index, meadow interspersion/dispersion, and total edge of open-shrub bog measured in the surrounding 2km landscape sector, and $\log_{10}$ corrected floral richness

Online resource 1.
Cranberry marsh landscape composition and site variables.

Site	Landscape content factors					Landscape configuration factors				Local site factor	
	%Ag	%F	%M	%B	SD	Ag, MPS	F, C	M, IJI	B, TE	L, H	Flw R
A1	74.93	3.16	8.38	0	0.8	55.39	0.93	56	0	61.65	16
A2	55.12	26.61	3.0	0.75	1.1	57.73	0.94	77.77	0.02	56.86	30
A3	36.64	30.21	2.35	1.32	1.24	17.71	0.92	61.42	0.08	50.22	23
A4	56.94	4.89	21.27	2.86	1.17	35.78	0.91	86.36	0.25	54.86	27
A5	28.30	15.35	5.78	10.33	1.41	25.41	0.94	0.86	2.71	45.91	19
A6	28.62	32.74	7.14	8.21	1.46	29.98	0.95	79.63	1.07	44.88	24
A7	50.71	23.04	8.44	1.13	1.24	33.54	0.92	83.66	0.04	96.74	29
F1	24.55	48.86	4.51	10.84	1.32	23.74	0.93	91.44	1.74	49.23	40
F2	9.42	60.57	1.66	4.86	1.08	8.45	0.89	53.82	1.04	56.34	17
F3	11.76	39.07	27.15	0.12	1.31	24.63	0.96	45.60	0.00	52.56	50
F4	15.76	37.61	26.73	0.58	1.36	24.76	0.96	49.49	0.02	50.65	31
F5	16.34	56.66	4.89	10.49	1.25	20.54	0.94	70.53	1.31	52.74	19
F6	4.91	59.74	7.74	15.18	1.2	10.29	0.89	84.02	7.47	51.07	37
F7	16.03	42.13	8.49	6.57	1.4	13.43	0.93	68.08	1.52	44.13	36

Surrounding landscape context descriptors, including proportion of cultivated land (%Ag), forest (%F), meadow (%M), and bog (%B), and the Shannon Diversity Index of land cover types (SD), as well as land cover configuration descriptors, including mean patch size of agricultural parcels (A, MPS), forest clumpiness index (F, C), meadow interspersion/dispersion index (M, IJI), riparian edge (B, TE), and landscape homogeneity (L, H) were calculated based on digitized aerial photographs. Floral richness (Flw R) was recorded during a mid-summer survey on site, and plant samples were brought back to UW-Madison campus for identification.

Online resource 2.
Common flowering plants. This table presents the flowering plants species that occurred at more than 40% of the cranberry marsh sites during the flowering plant richness survey that was conducted immediately following the floral resource pulse bumble bee sampling period

Most common flower species blooming at the cranberry marshes
Achillea millefolium
Asclepias syriaca
Berteroa incana
Centaurea stoebe
Cerastium nutans
Cirsium arvense
Cirsium muticum
Crepis tectorum
Erigeron strigosus
Euphorbia corollata
Hypericom perforatum
Leucanthemum vulgare
Lotus corniculatus
Lysimachia lanceolata
Melilotus officinalis
Mellilotus albus
Oenothera biennis
Persicaria maculosa
Plantago rugelii
Potentilla argentea
Prunella vulgaris
Rudbeckia hirta
Rumex acetosella
Silene latifolia
Solidago canidensis
Trifolium pratense
Trifolium repens
Vaccinium macrocarpon
Verbascum thapsus
Vicia americana

CONCLUSIONS

The results of these studies, consistent with leading pollinator conservation science emphasize the importance of floral resources and nesting habitat to promote bee populations and pollination ecosystem services in urban and rural ecosystems. Conservation efforts should focus on maintaining complex structural habitat in woodlands as well as established residential neighborhoods in urban ecosystems. Floral cover and presence of resource rich species are commonly shown to regulate pollinator diversity and abundance, and these factors are shown to influence bee foraging behavior.