



Parasitism of urban bumble bees influenced by pollinator taxonomic richness, local garden management, and surrounding impervious cover

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Abstract

As urban areas continue to expand globally, animal biodiversity is likely to experience altered habitat conditions, resource levels, and pathogen dynamics, with critical implications for insect pollinators. Specifically, local and regional land management may impact pollinator infectious disease prevalence, and this may be particularly relevant in urban garden systems where local food production depends on crop pollination. Further, because multi-host parasites can be spread across insect pollinator communities with varying impacts on host species, changes in pollinator community composition can lead to ‘amplification’ or ‘dilution’ effects, whereby increases in pollinator diversity could lead to higher or lower rates of parasitism, respectively. In this study, we investigate how urban garden management and regional landscape composition structure disease dynamics for a critical native pollinator, the bumble bee *Bombus vosnesenskii*. Parasite prevalence in our gardens was low in *Nosema ceranae* (1.06%) and *Nosema bombi* (1.06%), and *Crithidia expoekii* (0.5%), while we observed higher prevalence of *Crithidia bombi* (18%) and *Apicystis bombi* (6.4%). We found that gardens with higher pollinator taxonomic richness had significantly lower prevalence of *Apicystis bombi* in *B. vosnesenskii* hosts, providing evidence for the dilution effect. We also found that the prevalence of the parasite *Crithidia bombi* was significantly higher in gardens with higher mulch use and in gardens surrounded by greater proportions of impervious urban cover. Overall, we document a wide range of stressors facing urban bees, and show that parasitism is mediated by local ground management, regional land use, and pollinator community composition.

Keywords *Bombus vosnesenskii* · Urban gardens · Disease ecology · Landscape epidemiology · Dilution effect · Land use

Introduction

Infectious disease transmission is a major driver of animal population dynamics and can even lead to local extinction events (Thomas et al. 2004), especially in the context of global change (Altizer et al. 2013). Global change processes, such as human-mediated land use change, can impact disease transmission by altering a number of local and landscape factors, such as host population and community dynamics (Johnson et al. 2013) and resource distributions across the landscape, both of which can influence pathogen transmission (Graystock et al. 2020). This is because concentrating individuals in limited natural habitat can promote the spread of parasites through close contact, and may add to other health challenges experienced by wildlife in human-altered environments (Murray et al. 2019). Further, habitat loss can result in changes to animal community composition, which

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can alter levels of susceptibility and patterns of transmission in the host community (Johnson et al. 2013). For example, under the dilution effect, it is hypothesized that sites with greater host diversity promote frequent transmission into low suitability hosts, thereby decreasing the pathogen load observed in target species (Keesing et al. 2006). Alternatively, higher host diversity could effectively increase the overall density of susceptible hosts, and potentially lead to higher rates of parasitism in the target species through the ‘amplification’ effect (Keesing et al. 2006). Thus, landscapes that experience rapid habitat alteration, and resulting shifts in pollinator diversity (e.g., Cusser et al. 2018) may be especially likely to exhibit variation in host community composition with potential impacts on disease dynamics.

Urban landscapes in particular create a potent combination of biodiversity loss and resource limitation, which can alter and potentially exacerbate disease transmission (Murray et al. 2019). While urban areas often include parks, green spaces, and community gardens, which provide food and nesting resources for many animals (Baldock et al. 2019), animal community composition can be highly heterogeneous across these same spaces, with community members varying in their suitability for multi-host parasites (Johnson et al. 2013). The expansion and intensification of human development can also impede the movement of animals across the landscape, altering their ability to locate necessary resources, leading to physiological stress at the individual and population level (Harrison and Winfree 2015), and potentially resulting in increases in zoonotic disease prevalence (Jones et al. 2008). These disease dynamics may also be impacted by animal foraging patterns, where urbanization can aggregate individuals in limited patches of suitable habitat (Matteson and Langellotto 2009), while landscape-level reductions in floral resources can draw mobile foragers from greater distances to visit resource-rich patches (Pope and Jha 2018), potentially altering patterns of disease spread. Given high resource heterogeneity and resulting shifts in animal community composition, it is critical to examine how urban land use influences the disease dynamics of key animal ecosystem service providers, such as pollinators.

Urban landscapes can have varying effects on animal pollinators depending on habitat management and spatial context (reviewed in Wenzel et al. 2020). Within urban areas, parks and other green spaces with greater semi-natural cover can serve as critical refugia for pollinators (Baldock et al. 2015; Hall et al. 2017). Overall, landscape-scale factors, like the presence of nearby buildings (Matteson and Langellotto 2010), proportion of urban habitat cover (Plascencia and Philpott 2017), or interactions between local (e.g., urban garden) and regional natural habitat cover (ex. Ballare et al. 2019) have been shown to influence bee richness and community composition. Local vegetation management is also critical, and past studies have found that urban bee

taxonomic richness responds negatively to canopy cover, but positively to floral resource area (Matteson and Langellotto 2010), floral abundance (Plascencia and Philpott 2017), and floral richness (Lowenstein et al. 2015). Bee taxonomic richness also responds positively to the availability of wild or unmanaged areas within gardens, likely due to increased nest site availability (Matteson and Langellotto 2010; Threlfall 2015). Previous work at our study gardens has found that both cavity-nesting and ground-nesting bees respond positively to the presence of bare ground (Quistberg et al. 2016), which indicates that bare ground has both direct (ground-nesting sites) and indirect impacts on bee communities, which could explain why bare ground is often found to predict bee richness in urban areas (Ballare et al. 2019).

While a number of studies have evaluated drivers of urban pollinator communities (Ballare et al. 2019; Lowenstein et al. 2015; Matteson and Langellotto 2010; Threlfall 2015; Youngsteadt et al. 2015), far less is known about pathogen dynamics in these communities. It is clear that urban areas can provide suitable habitat for bees (e.g., Baldock et al. 2019); however, some of the factors previously found to promote pollinator diversity and abundance (e.g., floral abundance, Plascencia and Philpott 2017), may have secondary impacts on pollinator pathogen status. Cohen et al. (2022) found that parasite richness in *Bombus vosnesenskii* was lower in sites with high flowering density and high bee richness. Other studies have shown that bumble bees experience higher rates of *C. bombi* and *Nosema bombi* infection in urban compared to natural environments (Goulson et al. 2012; Theodorou et al. 2016). Though higher host population density has been proposed as a mechanism for increased parasitism in urban bees (Goulson et al. 2012; Theodorou et al. 2016), semi-field experiments have shown contrasting results, where virus prevalence positively correlated with host density at field-realistic levels, but protozoan prevalence only correlated with host density at very low densities unlikely to be found naturally in the field (Bailes et al. 2020). This indicates that a broader perspective is needed to understand the relative role of host community composition and fine scale habitat management on pathogen prevalence in heterogeneous landscapes. Given increasing levels of habitat and biodiversity alteration in urban areas, it is essential to investigate the role of both host composition and habitat management on pathogen dynamics within urban bee communities (Penczykowski et al. 2016; Grass et al. 2018).

In this study, we quantify the impacts of local and landscape habitat management on pathogen prevalence in bumble bees across a gradient of urban garden landscapes. Specifically, we leverage a model urban garden study system spanning more than 95 km across the rapidly urbanizing California central coast (Egerer et al. 2018; Philpott and Bichier 2017). Understanding pollinator disease dynamics is particularly urgent in urban gardens given the importance

of native pollinators to garden crop production and local food security (Cohen et al. 2017). We focus our pathogen investigation on a key crop pollinator within the region (Kremen et al. 2002) and a common member of the urban garden pollinator community, the yellow-faced bumble bee, *Bombus vosnesenskii* (Quistberg et al. 2016; Plascencia and Philpott 2017; Cohen et al. 2017). We targeted three taxa of generalist protozoan gut parasites, which each have broad host ranges among bees (Graystock et al. 2020), and have detrimental fitness effects on their hosts. Specifically, we screened for neogregarines (*A. bombi*), trypanosomatids (*C. bombi* and *C. expoekii*), and microsporidians (formerly *Nosema spp.* recently reclassified as *Vairimorpha*, Tokarev et al. 2020). All three taxa have strong evidence for deposition and dispersal on flowers in experimental (Graystock et al. 2015; Figueroa et al. 2019) and natural settings (Graystock et al. 2020). We predict that local management intensity will mediate parasitism, where gardens with low intensity ground cover and greater floral resources will support lower bumble bee pathogen prevalence. We also predict that community-level pollinator taxonomic richness will negatively correlate with bumble bee pathogen prevalence, indicative a ‘dilution’ effect where richness decreases primary host parasite prevalence.

Materials and methods

Study region and garden metrics

We conducted our study in June and early July of 2016 in 20 urban gardens across three counties in the California central coast (Monterey, Santa Clara, and Santa Cruz). Each site consisted of an urban community garden (0.10 to 3.84 acres) separated from other gardens by > 2 km. All gardens are organically managed and have produced food for between 2–50 yrs. The study region hosts ~1 million people and is heterogeneous in its urban cover as well as in the management of its urban garden landscapes. Thus the study gardens vary in local vegetation management, local ground and canopy cover, and landscape composition, among other characteristics (Egerer et al. 2019).

At the local scale, we measured vegetation and ground cover management within a 20 × 20 m plot placed at the center of each garden (as per Cohen et al. 2021). Specifically, we measured canopy cover with a convex spherical densiometer at the center of the plot, and 10 m to the North, South, East, and West. We counted and identified all trees and shrubs in the plot and noted the number of individuals in flower. In each plot, we randomly selected eight 1 × 1 m quadrats within which we identified all herbaceous plants (except grasses) to morphospecies, measured the height of the tallest non-woody vegetation, noted which species were

in flower and counted their flowers, and assessed percent ground cover of bare soil, grass, herbaceous plants, leaf litter, rocks, mulch, and straw. These measures were averaged across the 8 quadrats at each site.

At the landscape scale, we calculated the proportion cover of four main land use categories within 2 km buffers surrounding each garden with data from the 2011 National Land Cover Database (NLCD, 30 m resolution) (Homer et al. 2015). Specifically, we selected 2 km buffer zones as this is the largest landscape scale that has been shown to be predictive of resource usage for bumble bees (Steffan-Dewenter et al. 2002), which are among the larger bees found in our study system; this buffer size is also used in many other urban bee studies (Ballare et al. 2019; Egerer et al. 2017; Quistberg et al. 2016). We categorized land use into four categories (as per past studies in this urban garden system, e.g. Philpott and Bichier 2017) and calculated the proportion of area represented for each category in the 2 km buffer: semi-natural habitat (deciduous, evergreen and mixed forests, dwarf scrub, shrub/scrub, and grassland/herbaceous), urban habitat (low, medium, and high intensity developed land), agricultural habitat (including pasture/hay and crop), and open developed habitat (including lawn grass, park, and golf courses). Other land cover types covered < 5% of the total area and were not included. Additionally, we estimated total garden size by ground-truthing GPS points around the garden and running spatial statistics tools in ArcGIS v.10.1 (ESRI, Redlands, CA).

Pollinator survey

We focused our study on one of the most common bumble bees in our study system, *B. vosnesenskii*, which is an annual, primitively eusocial, central-place forager native to the Pacific Coast of the United States (Thorp et al. 1983) and is also a critical crop and greenhouse pollinator for the region (Strange 2015). The species is often found in urban greenspaces, where it displays relatively generalist foraging behaviors (McFredrick and LeBuhn 2006). In this study, we collected *B. vosnesenskii* individuals between the 27th of June and 11th of July 2016 (as described in O’Connell et al. 2020). We captured 10 *B. vosnesenskii* individuals in each garden with a net and then transferred them to a jar with ethyl acetate. Individuals were then placed into 5 mL test tubes where they were submerged in a 95% ethanol solution. We rinsed the forceps with 95% ethanol between the processing of each individual to avoid contamination. A few of the samples experienced ethanol leakage and degradation, leaving a total of 189 bees for subsequent analysis (mean = 9.45, SE = 0.08, per garden).

To characterize the broader pollinator community, visual surveys were conducted in July 2016 within a 20 × 20 m plot at each of the gardens. Within this plot, four 4 × 20 m transects

spaced 5 m apart and running E-W in each plot were walked at a pace of 1 transect per 3.5 min (as described in Cohen et al. 2021). Surveys were conducted with a one hour total observation time between 0:900 and 16:00 on sunny days with less than 50% cloud cover. We recorded all pollinating insects visiting flowers within the plot, including bees, butterflies, wasps, and flies, and identified each individual to the finest taxonomic resolution possible (Table S4). Each of the observers were trained using a combination of field guides, online resources, and physical examples of specimens collected at these sites in previous years (Cohen et al. 2021). Pollinating insects were identified to morpho-group and morpho-species categories, which is common in visual surveys of pollinators in taxonomically diverse localities (Prendergast and Ollerton 2021; Theodorou et al. 2020; O'Connor et al. 2019), and consistent with past work in this garden system (Cohen et al. 2021; O'Connell et al. 2020). The morpho-groups and morpho-species (Theodorou et al. 2020) categories are listed in Supplemental Table 4, along with an example reference for previously reported infections of each parasite. To ensure the estimate of taxonomic richness was as conservative as possible, and given the coarse nature of the visual survey, we used raw bee taxonomic richness and pollinator taxonomic richness in the statistical tests opposed to Chao1 or ACE richness estimates.

Parasite detection

Prior to *B. vosnesenskii* dissection, the lab workspace was sterilized using a 10% bleach solution, dissection tools were heat sterilized at 300 °C for > 30 s, and each bee was placed on a micro-foam platform covered in a fresh sheet of parafilm. Bees were pinned to the dissection platform with a single pin through the ventral mesosoma, legs were pinned away from the dissection site to further prevent contamination from the outside of the body. An incision was made along the left side of the body and across the first sternite using iris scissors before pinning the ventral portion and exposing the body cavity. The ethanol-saturated gut tissue was removed from the body cavity and dried at room temperature for one hour prior to storage at -20 °C. Gut tissue was homogenized in 100 uL DEPC treated water using a motorized pellet pestle; 20uL of the homogenate was retained as a voucher and stored at -80 °C and remaining 80 uL sample was used for a DNA extraction following a standard salting out method (Mullins et al. 2020), and was further purified through isopropyl precipitation.

We screened for parasites using a multi-plex PCR which detects DNA from three genera of microparasites known to infect bumble bees: neogregarines (*Apicystis* spp.), trypanosomatids (*Crithidia* spp.), and microsporidians (*Vairimorpha* spp.) (Mullins et al. 2020; Tripodi et al. 2018). The PCR reaction mix consisted of 0.8 µM of each parasite

detection primer, 0.4 µM of a bee positive control primer set, 1.3× buffer, 2.0 mM MgCl₂, 0.8 µM total dNTPs, 1 unit *Taq* (Genesee Scientific, Apex polymerase), 1.2 µL DNA sample, with DEPC treated water to adjust the volume to a total of 25 µL (Mullins et al. 2020). Each reaction batch included at least one positive control sample for each of the parasite groups in the screen as well as a negative control using water in place of DNA. The PCR reaction followed an initial denaturation step of 94 °C for 2 min, followed by 10 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 45 s then 30 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 45 s and finally an extension at 72 °C for 5 min (Mullins et al. 2020). PCR products were visualized through gel electrophoresis using 2–2.5% agarose in TAE buffer, stained with SybrSafe (Life Technologies Corporation, Carlsbad, CA). Fragment lengths were estimated with a 100 bp DNA ladder (GeneRuler ThermoScientific, Waltham, MA). Gel electrophoresis was run for 30 min at 100 V to allow separation of bands, with each sample possibly containing bands for neogregarines (357 bp), trypanosomatids (584 bp), microsporidians (270–316 bp), as well as the expected bee-positive control (233 bp) (Mullins et al. 2020). Because positive controls occasionally failed to amplify one or more parasite taxa, each reaction with a failed positive control was repeated, and all results were verified through a minimum of two reactions.

For species-level parasite identification, in samples that tested positive for either trypanosomes or microsporidians, we used a second multi-plex PCR to distinguish between the two common trypanosomatids using species-specific cytochrome B primers (for *C. bombi* and *C. expoeikii*), and between the two common microsporidians using species-specific primers for the RNA polymerase subunit (RPBS) (for *N. ceranae* and *N. bombi*) (Graystock et al. 2020; Schmid-Hempel and Tognazzo 2010; Tripodi et al. 2018). All samples that tested positive for Neogregarines were Sanger sequenced using ITS primers, followed by identification through NCBI BLAST, and alignment to verified sequences for *A. bombi* (Accession #FN546182.1) and the recently described *Apicystis cryptica* (Accession #MF998086.1) (Schoonvaere et al. 2020).

Statistical Analysis

To identify relationships between bee pathogen prevalence and the local, landscape, and community characteristics of hosts and food resources, we used generalized linear mixed effect models (*lme4* package in R) (Bates 2010) with site as a random effect, and a binomial distribution given the binary response format of parasite prevalence (presence/absence) (Zuur et al. 2009). Given the extremely low prevalence of *N. bombi*, *N. ceranae*, and *C. expoeikii* (1.06%, 1.06%, & 0.05% of all bee individuals tested, respectively), statistical

analysis and model selection was not possible for these species; instead, we focused our modeling on the prevalence of *C. bombi* and *A. bombi* as response variables. All explanatory variables were organized into four categories: landscape composition, floral resources, nesting resources, and pollinator community composition. Landscape composition variables were strongly correlated (Supplemental Table 1) as expected from a single raster, and thus only urban land cover was included (Pearson correlation with natural cover = -0.801 , $P = 0.00000137$) as urban cover is frequently used in urban pollinator studies (Plascencia and Philpott 2017). We prioritized retaining floral and nesting resource variables that had been found to be important predictors of the pollinator community in past studies, including number of flowers as a measure of food resource availability (Cohen et al. 2021) and proportion mulch cover as an indicator of ground-nesting access and management intensity (Egerer et al. 2017; Splawski et al. 2014). Because pollinator community composition variables were also correlated (Supplemental Table 1), we prioritized retaining the most inclusive pollinator community variable, pollinator taxonomic richness, which included wasps, butterflies, flies, and native and managed bees (as in Cohen et al. 2021) as bee-associated pathogens have recently been found in hoverflies (Bailes et al. 2018) and syrphid flies (Anderson 2019) and thus it is likely that non-bee pollinators play a role in transmission and dispersal of bee-associated pathogens (Bailes et al. 2018). We evaluated correlations across variable categories and failed to find strong collinearity, for example, pollinator taxonomic richness was not related to local floral richness ($R = -0.0242$), floral abundance ($R = 0.4136$), or mulch cover ($R = -0.3806$). Further, we ran Pearson's correlations within each of the categories to ensure that the selected the variable correlated ($P < 0.05$) with a large number of other variables in each category (Supplemental Table 1). The *car* package (Fox and Weisberg 2011) was then used to ensure Variance Inflation Factor (VIF) scores were maintained below a conservative cut-off of 2 for the four remaining predictor variables.

Thus, our global prevalence models for *A. bombi* and *C. bombi* included the four predictor variables: proportion of urban land-cover within 2 km, number of flowers, proportion of mulch cover, and pollinator richness. As an additional measure, we also ran this model with bee taxonomic richness, bee abundance, and pollinator abundance, instead of pollinator taxonomic richness, and found similar results (Supplemental Table 2); we primarily focus on pollinator taxonomic richness as this variable has been a significant predictor of ecosystem function in this system previously (Cohen et al. 2021) and most directly connects to our assumptions about the mode of transmission for the parasites. This global model then underwent a model selection process with the MuMIn package (Barton 2012). MuMIn

generates new models with every combination of explanatory variable included in the initial global model. Each model output is then ranked using Akaike Information Criterion (AIC), which estimates the relative quality of the model based on the goodness of fit to the data, while penalizing models for potential over-parameterization (Barton 2012). In cases where multiple MuMIn generated multiple models that were within 2 AICc points, each composite model was included in a weighted model average (Table 2, Table 3).

Results

Garden management, pollinator community & parasite prevalence

The urban gardens included in this study varied considerably in landscape composition. Within 2 km of the gardens, the proportion urban cover ranged from 7.77–97.3% (mean 61.43%, \pm SE 6.98); within the gardens, flower abundance ranged from 3.25–273.75 (mean 64.19, \pm SE 18.43), the proportion of mulch cover ranged from 0–58.5% (mean 23.14%, \pm SE 4.09), and pollinator taxonomic richness ranged from 4–13 species (mean 9.64%, \pm SE 0.65). Moderate rates of parasitism were seen for the neogregarine *A. bombi* ($N = 12/188$; 6.4%). Sequences of samples that tested positive for Neogregarines were ~99% identical to the reference sequence FN546182.1 and are thus identified as *A. bombi* (Schoonvaere et al. 2020). The microparasite with the greatest prevalence was the trypanosome *C. bombi* ($N = 35/188$; 18.6%). The closely related *C. expoekii* was only detected in only one individual. Two microsporidian species were also detected, with low rates of parasitism: *N. bombi* ($N = 2/188$; 1.06%), and *N. ceranae* ($N = 2/188$; 1.06%).

Model selection

Prevalence of *A. bombi* was significantly and negatively predicted by pollinator taxonomic richness ($z = -3.408$, $P = 0.000654$), which was the single variable present in the top model (Table 1; Fig. 1C; Supplemental Table 3). The average of the best models (within 2 AICc points) showed the same patterns, with a significant negative effect of pollinator taxonomic richness ($z = -2.32$; $P = 0.0204$) and non-significant effects of floral abundance, proportion mulch cover, and proportion urban cover (Table 2). The global model also reflected the same results (Supplemental Table 5). Because pollinator taxonomic richness was a significant predictor, we also substituted this term with bee taxonomic richness ($z = -3.204$; $P = 0.00136$), bee abundance ($z = -3.104$; $P = 0.00191$), or pollinator abundance ($z = -2.695$; $P = 0.00704$), we find the same significant

Table 1 Results of *Apicystis bombi* Generalized Linear Modeling model selection. ‘Top Model’ is the model with the overall lowest overall AIC score. ‘Averaged Best Model’ is the full average of allcomposite models within $\Delta 2$ AIC from MuMIN model selection. Significance codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Adjusted R^2 available for component models of Averaged Best Model in Table S3

Model	Variable	Estimate	Standard Error	Z-Value	Pr(> z)	Adjusted R^2	AIC
Top Model	Pollinator Taxonomic Richness	-0.9649	0.2831	-3.408	0.000654***	0.2205	73.5
Averaged Best Model	Pollinator Taxonomic Richness	-0.9313	0.2937	3.147	0.00165**		78
	Floral Abundance	0.2419	0.2721	0.882	0.37775		
	Proportion Mulch Cover	0.2548	0.4043	0.625	0.53187		
	Proportion Urban Cover	0.1381	0.3916	0.350	0.72637		

negative effect on *A. bombi* prevalence as these pollinator terms are collinear (Supplemental Table 1).

Parasite prevalence for *C. bombi* was significantly positively predicted by both the proportion of mulch cover within the gardens ($z = 2.993$; $P = 0.0027$) and the proportion of urban cover within 2 km ($z = 2.242$; $P = 0.02494$) and these were the only two variables in the top model (Table 2; Fig. 1A, B). The average of the best models (within 2 AICc points) also indicated the same significant positive effect of mulch cover ($z = 2.709$; $P = 0.00675$) and urban cover ($z = 2.111$; $P = 0.03475$) and also included the non-significant predictors, pollinator taxonomic richness and floral abundance (Table 2). The global model also reflected the same results (Supplemental Table 5).

Discussion

In this study, we show that the pollinator community, local garden management, and landscape composition each play a role in structuring pathogen prevalence for an essential native pollinator, the bumble bee *B. vosnesenskii*. We show that the prevalence of the neogregarine *A. bombi* was correlated with the local pollinator community, where gardens with higher pollinator taxonomic richness experienced lower parasite prevalence, suggesting a disease dilution effect (Ostfeld and Keesing 2000). In contrast, the prevalence of the parasitic trypanosome *C. bombi* in *B. vosnesenskii* increased with greater

mulch cover, an indicator of agricultural intensity (Kratschmer et al. 2019) within the garden, and greater urbanization at the 2 km scale, an indicator of landscape-level urban development. Both of these predictors capture a strong element of habitat modification, and reveal a negative impact of both local and landscape management on pollinator disease prevalence. The microsporidians *N. bombi* and *N. ceranae* were rare in this study, but similar to prevalence seen in *B. vosnesenskii* in other portions of California (*N. bombi* 1.2% prevalence; Cordes et al. 2012).

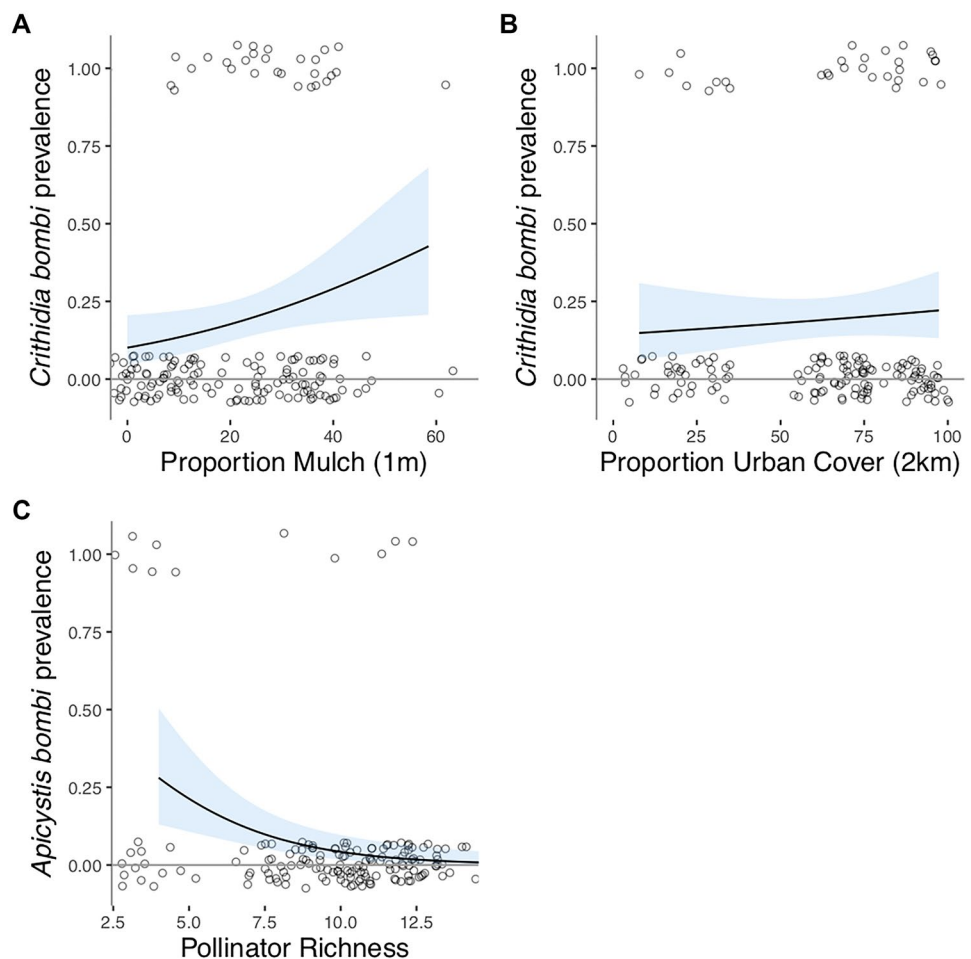
Apicystis bombi prevalence is indicative of a dilution effect in urban gardens

We found that gardens with greater pollinator taxonomic richness experienced lower *A. bombi* prevalence. This result is consistent with the classic ‘dilution effect’ in disease ecology, where increases in the broader host taxonomic richness act to reduce the overall parasite burden on target hosts, in this case *B. vosnesenskii* (Keesing et al. 2006). While there is broad evidence that the dilution effect plays a role in many host–pathogen systems (Civitello et al. 2015), this is one of the first reports of a dilution effect in the multi-host parasites of pollinators (Cohen et al. 2017). The dilution effect assumes that species vary in their reservoir competence (the ability to support and transmit parasites), that low-competence hosts reduce parasite burden on the target species, and that populations of the target species are generally stable and resilient

Table 2 Results of *Crithidia bombi* Generalized Linear Modeling model selection. ‘Top Model’ is the model with the overall lowest overall AIC score. ‘Averaged Best Model’ is the full average of allcomposite models within $\Delta 2$ AIC from MuMIN model selection. Signif. codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Model	Variable	Estimate	Standard Error	Z-Value	Pr(> z)	Adjusted R^2	AIC
Top Model	Proportion Mulch Cover	0.7957	0.2659	2.993	0.00277**	0.1249	150.5
	Proportion Urban Cover	0.5687	0.2536	2.242	0.02494*		
Averaged Best Model	Proportion Mulch Cover	0.4902	0.27662	2.709	0.00675**		151.42
	Proportion Urban Cover	0.54902	0.258	2.111	0.03475*		
	Floral Abundance	0.05658	0.13565	0.415	0.678		
	Pollinator Taxonomic Richness	-0.02656	0.10897	0.242	0.80859		

Fig. 1 Significant drivers of *Crithidia bombi* (A, B) and *Apicystis bombi* (C) prevalence in *B. vosnesenskii* at urban gardens in the California central coast. In all plots, the parasite detection status for each bee is plotted (1 = presence, 0 = absence) and slightly jittered to reduce data overlap and improve visualization. The regression lines show the predicted parasite prevalence following a binomially distributed generalized linear model with shading indicating the 95% confidence interval. *Crithidia bombi* prevalence was higher in gardens with more intensive mulch use (A), and in gardens surrounded by higher urban cover (B). Panel C shows an inverse relationship between pollinator taxonomic richness and *Apicystis bombi* prevalence, indicative of a disease dilution effect



to local extirpation (Huang et al. 2015). There is still considerable contention in the literature concerning the generality of the dilution effect and it is clear that the magnitude and direction of the effect are context-dependent (Rohr et al. 2020). For example, the ability for a host species to impact disease dynamics in the system is dependent on their relative abundance as well as their competence as hosts (Rohr et al. 2020). Similarly, parasite transmission is dependent on interactions among host species, in our system likely through shared flower visitation (Graystock et al. 2015).

Specifically, while the transmission mode for *A. bombi* has not been experimentally determined, there is sufficient evidence that *A. bombi* is a multi-host parasite and that fecal–oral transmission is viable. Ingestion of *A. bombi* spores leads to true infection (Graystock et al. 2015), and oocysts are found in the gut, in fecal samples, and on flowers (Graystock et al. 2015). Although *A. bombi* primarily infects bumble bees (Lipa and Triggiani 1996; Graystock et al. 2014) it has been reported in commercial honeybees (Lipa and Triggiani 1996; Ravoet et al. 2014; Schulz et al. 2019), as well as several solitary bee species, including *Osmia*, *Andrena*, and *Xylocopa* species (Graystock et al. 2020) (Supplemental Table 4). Further, our best model

incorporated all insect pollinators, not simply bees. While our results and those of related studies are suggestive of non-target transmission effects, further investigation of non-collinear pollinator community metrics are required to characterize the role of each taxon in the field. A finer taxonomic resolution would also improve our understanding of the role of community assembly in parasite dynamics, but it is clear that even at a coarse taxonomic scale, our estimates of pollinator taxonomic richness correlate with healthier bumble bee populations.

Interestingly, in addition to having a large number of potential hosts, it is possible that *A. bombi* host species show variation in their host competency, and thus prevalence may be driven by the abundance of select competent hosts (Figuroa et al. 2019; Graystock et al. 2020). In other words, there is potential for ‘identity effects’ (Rohr et al. 2020), where certain hosts are extremely competent, and drive the dynamics of infection for the community. Figuroa et al. (2019) found that the diet breadth of *B. impatiens*, the dominant bumble bee species in the eastern US, moderated infection prevalence across the bee community; generalist foraging in one species led to lower infection prevalence overall. Our study species, *B. vosnesenskii*, plays a similar

dominant and generalist role in the west coast of the US (McFredrick and LeBuhn 2006), suggesting a potential role for ‘identity effects’ in field-level pathogen dynamics. The model selection process revealed that bee abundance and pollinator abundance were each significant predictors of *A. bombi* prevalence, and richness and abundance terms were strongly correlated (*Pollinator Richness: Pollinator Abundance*; Pearson’s correlation coefficient = 0.822). It is possible that sites with higher pollinator abundance also included taxa with lower host competence, which could be another potential mechanism for the dilution effect in this system. To develop greater insight into the mechanisms underlying dilution effects in pollinator communities, next steps could include lab and field-level quantification of variation in host competency and transmission dynamics within and among species in the community (e.g., Manley et al. 2015).

Crithidia bombi infection is influenced by anthropogenic habitat modification at multiple spatial scales

We found that the prevalence of the trypanosome *C. bombi* was driven by two common features of human habitat modification, local ground cover and landscape-level urban development. Specifically, we found that the proportion of mulch cover within gardens (which significantly negatively correlated with bare ground, Pearson = -0.853), and the proportion of urban habitat cover within 2-km of the gardens (which significantly negatively correlated with natural habitat cover, Pearson = -0.801) were both positively related to *C. bombi* prevalence. With respect to landscape habitat cover in the study region, past survey-based bumble bee work has found that areas with less natural cover are often nest site limited for bumble bees (McFredrick and LeBuhn 2006). Molecular studies have also shown that *B. vosnesenskii* nesting densities are lower in landscapes with high levels of impervious cover and higher in landscapes with high levels of natural woodland cover (Jha and Kremen 2013). Additionally, because bumble bees are central-place foragers, local nest-site limitation directly impacts foraging by requiring longer flight distances (Harrison and Winfree 2015), and thus potentially introduces additional nutritional and metabolic stress. *B. vosnesenskii* in particular are known to travel greater distances while foraging in response to seasonal food scarcity (Pope and Jha 2018), and in response to preferred foraging habitat (Jha and Kremen 2013). Increased metabolic stress from foraging can lead to decreased immunocompetence (Konig and Schmid-Hempel 1995; Doums and Schmid-Hempel 2000), and can lead to higher susceptibility to *C. bombi* (Deshwal and Mallon 2014). Overall, these results suggest that nest site limitation and possible resulting stress could increase pathogen susceptibility in urban landscapes.

Past studies have also found that higher mulch use locally can negatively impact the abundance of ground-nesting bee species in urban systems (Ballare et al. 2019; Plascencia and Philpott 2017). Our focal species, *B. vosnesenskii*, typically nests in pre-existing cavities including rodent burrows and abandoned bumble bee nests (Thorpe et al. 1983), often residing below ground for insulation. Mulch is thought to create a physical barrier which may deter new burrows from being excavated (Julier and Roulston 2009; Splawski et al. 2014), and may obscure or destroy existing cavities and thus prevent bumble bees from nesting locally. Both of these forms of habitat modification may reduce ground nest site availability over time (McFredrick and LeBuhn 2006; Harrison and Winfree 2015). In addition, mulching causes soil disturbance (Julier and Roulston 2009), and gardeners using large amounts of mulch may also visit the gardens more frequently and cause additional disturbance through more intensive weed management (Egerer et al. 2018). Mulching also leads to changes in the arthropod community at multiple trophic levels (Bruggisser et al. 2010), and this work has revealed that ground cover is an important predictor of parasitism for both managed honeybees and native bumble bees. However, it is also possible that mulch use is simply a proxy for a more direct factor influencing parasitism. Within this study system, Egerer et al. (2018) demonstrated that mulch cover was predictive of a variety of soil properties, including soil organic matter, water holding capacity, and soil carbon. These factors can improve soil moisture availability (Egerer et al. 2018) and could subsequently influence floral signals and cues for pollinators, floral resource quality metrics such as nectar concentration (ie. Descamps et al. 2021), and could indirectly impact bee foraging patterns and parasite transmission. Overall, our study and others indicate that ground cover management plays an important, if indirect, role in mediating pathogen prevalence in urban bee communities.

At the landscape scale, our finding of greater *C. bombi* prevalence in more urbanized landscapes resonates with a number of past studies which have also found higher rates of *C. bombi* infection in urban areas (Goulson et al. 2012; Theodorou et al. 2016). Goulson et al. (2012) found that urbanization led to higher rates of *C. bombi*, *N. bombi*, and *A. bombi* infection in the host *Bombus terrestris*. However, *C. bombi* infection actually decreased in more urban habitats for the host *Bombus pascuorum*, indicating that the impacts of urbanization are context-dependent and that infection rates likely involve multiple interacting drivers. Past studies have suggested that bee density promotes higher rates of parasitism in urban areas (Theodorou et al. 2016; Goulson et al. 2012), which could lead to higher rates of intercolonial and interspecific transmission (Theodorou et al. 2016). Our models do not support a host density relationship for *C. bombi* infection, as all pollinator community variables including bee abundance were non-significant and absent

from the top models. However, there is evidence from our system that foraging effort of our *B. vosnesenskii* individuals is concentrated within gardens relative to outside of the gardens (O'Connell et al. 2020). Specifically, O'Connell et al. (2020) analyzed pollen loads from the same bee samples used in the current study, and found that foraging patterns differed across the gradient of urban cover at our sites. Bees foraging at gardens with higher urban cover were less likely to forage for floral resources outside of the garden, indicating that foraging effort is concentrated more locally within more urbanized landscapes (O'Connell et al. 2020). Thus we suggest that urban garden habitat patches may act to concentrate bumble bee foraging effort (as in Matteson and Langelotto 2009), and this could lead to higher disease contact risk for the resident bumble bee population.

Conclusions

Our results show that taxonomically diverse pollinator communities experience lower rates of parasitism, and that practices which promote diversity, such as providing nesting habitat for bees by leaving bare ground and planting flowers for forage could go a long way in supporting the health of the pollinator community. Additionally, our findings support growing evidence that human disturbance within urban habitats is related to higher parasite prevalence in wildlife populations. This is especially concerning for native pollinators given that they provide essential pollination services, not only to rural agricultural systems, but also for urban gardens (Cohen et al. 2021), which are critical for addressing food security within cities and underserved communities (Lin and Egerer 2020). Further, it has become clear that species-rich urban green-spaces like urban gardens provide essential foraging and nesting resources to pollinators inhabiting such highly modified landscapes (O'Connell et al. 2020; Lowenstein et al. 2015). In order to best preserve the mutually beneficial relationship between urban gardens and pollinators, our results indicate that management of these green spaces should consider the reduction of excess mulch, as we found mulch to have unintended negative consequences for pollinator health. We also urge urban developers, landscapers, and gardeners to consider preservation of natural habitat within urban areas, as these efforts to improve habitat for diverse pollinator communities will likely also benefit pollinator health, with implications for longer-term population persistence and ecosystem service provision.

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Availability of data and material Will be made available on DRYAD repository upon publication.

Code availability Will be made available on GitHub upon publication.

Declarations

Conflicts of interest/Competing interests We have no conflicts of interest to report.

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