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Environment Shapes the Microbiome of the Blue Orchard Bee, *Osmia lignaria*

RRH: Environmental Drivers of Bee Microbiome

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Abstract
Wild bees encounter environmental microbes while foraging. While environmental context affects bee diversity, little is known about it how affects the wild bee microbiome. We used field surveys in 17 urban gardens to examine whether and how variation in local and landscape habitat features shapes the microbiome of the solitary Blue Orchard Bee, *Osmia lignaria*. We installed *O. lignaria* cocoons at each site, allowed bees to emerge and forage, then collected them. We measured local features of gardens using vegetation transects and landscape features with GIS. We found that in microbiome composition between bee individuals varied by environmental features such as natural habitat, floral resources, and bee species richness. We also found that environmental features were associated with the abundance of bacterial groups important for bee health, such as *Lactobacillus*. Our study highlights complex interactions between environment context, bee species diversity, and the bee-associated microbes.

Keywords Microbiome · Bee-microbe interactions · Horizontal-transmission · Urban gardens

Introduction

An insect hosts a collection of microorganisms, called the microbiome. The microbiome can impact host fitness through impacts to nutrition, growth rate regulation and stress tolerance, and protection against parasites and pathogens [1–5]. While the microbiome is considered important for immunity, it is not known how ecological processes shape and change the microbiome [6]. Insects acquire microbes through vertical transmission, but also through horizontal transmission, from the environment and social interactions [7, 8]. For example, the insect microbiome may be influenced by available diet [8–12] and the specific geographical location where the insect host is found [13–16]. While the impact of habitat context on the insect microbiome has been studied for predatory insects that rely on arthropod prey as food resources [17], systematic studies on the effect of environmental context on the solitary bee microbiome are lacking.

For solitary bees, the ways in which environmental context impacts the microbiome may be especially important because bee decline is attributed, in part, to environmental changes such as loss of floral resources and nesting habitat [18–20]. Multiple qualitative syntheses suggest that environmental changes (such as agricultural intensification and habitat fragmentation) at local and landscape-level scales have population impacts for bees [21–23], likely through changes to floral and nesting resources [24]. The availability of resources may also be important for the microbial associates of bees. For honey bees and bumble bees, a distinctive hindgut microbiome is obtained by direct transmission between members of the same species [25, 26], but for most wild and solitary bees, microbes may also be acquired from the environment. For example, halictid and megachilid bees can acquire *Lactobacillus* bacteria from flowers [27, 28]. Solitary bees may putatively acquire microbes through contact with feces, either from flowers or nesting materials previously visited by other bees, or through direct interactions with other bees while foraging for food and

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nesting materials [29]. Although experiments addressing mechanistic routes of horizontal transmission are few, both bee community composition and resource density at local and landscape scales may be related to the process of microbiome acquisition.

In addition to habitat loss, parasites and pathogens also contribute to bee population declines [18, 30, 31]. But insect-microbe associations can influence the outcome of insect infections by viruses, bacteria, and parasites [32, 33]. For example, the ubiquitous endosymbiont *Wolbachia pipientis* is associated with fitness benefits to *Drosophila melanogaster* flies infected by RNA viruses [34]. For bees, Koch & Schmid-Hempel [35] found that socially transmitted gut microbiota protect bumble bees against a widespread protozoan parasite, *Crithidia bombi*. And experiments in honey bees have found that lactic acid (*Lactobacillus*) bacteria may protect against bacterial infections by *Paenibacillus larvae* and *Melissococcus plutonis* [36, 37]. It is not known if gut microbiota is related to gut parasitism in solitary bees, which unlike social bees, are not associated with a consistent core microbiota [38, 39].

We address the hypothesis that the availability of floral resources, nesting materials, and species composition of the local bee community is related to microbiome variation in solitary bees. Because megachilid bees share bacteria with flowers [28], forage daily for food and nesting materials, and can be artificially incubated to emerge from pupal casings, they can be experimentally manipulated. We used *Osmia lignaria*, a megachilid bee species willing to nest in artificial cavities, as a study organism to test (1) which local and landscape environmental features are associated with the richness and composition of the bee microbiome, (2) which local and landscape environmental features are associated with the abundance of bacterial groups associated with immunity in bees (Gammaproteobacteria, Betaproteobacteria, *Lactobacillus* spp., and *Wolbachia* spp.), and (3) if any bacterial groups are associated with parasite prevalence in our study system.

Methods

Characterization of Study Sites

We examined local and landscape characteristics related to floral and nesting resource availability for bees in 17 urban gardens, ranging in size from 444 to 15,525 m^2, each separated by 2 km, across three counties (Monterey, Santa Clara, and Santa Cruz) in the California central coast. In two sampling periods, 1 month apart (early March and early April 2016), we measured local habitat characteristics within a 20 × 20 m plot placed at the center of each garden. We counted and identified all perennial trees and shrubs within the 20 × 20 plot. Then, in each plot we randomly selected four 1 × 1 m plots within which we counted all flowers (from annual crops, weeds, and ornamentals), and assessed percent ground cover of bare soil, grass, herbaceous plants, leaf litter, rocks, and mulch. We also estimated the total garden size. For analysis, values were averaged across the two sampling periods. In all, we measured 10 variables: % rock cover, % mulch cover, % leaf litter, % bare soil, % herbaceous plant cover, species richness of flowers, abundance of flowers, species richness of trees and shrubs, abundance of trees and shrubs, and garden size.

At the landscape scale, we classified the land cover types within a 500 m buffer surrounding each garden with data from the 2011 National Land Cover Database (NLCD, 30 m resolution) [40]. We selected 500 m buffers because while *Osmia lignaria* females have a maximum foraging distance of up to 1200 m [41], they tend to collect more pollen and nectar at flowers near to their nests within 500 m. [42]. We created four land-use categories: (1) natural habitat (composed of deciduous (NLCD number 41), evergreen (42), and mixed forests (43), dwarf scrub (51), shrub/scrub (52), and grassland/herbaceous (71)), (2) open habitat (composed of lawn grass, parks, and golf courses (21)), (3) urban area (composed of low- (22), medium- (23), and high-intensity developed land (24)), and (4) agriculture area (pasture/hay (81) and cultivated crop (82)). Other land cover types that covered < 5% of the total area at each site were not included. We assessed land cover with spatial statistics tools in ArcGIS v. 10.1.

Bee Community Diversity Assessment

We used bee richness data collected for two prior experiments conducted by our research team at these sites [43, 44]. Bee community diversity at each site was measured across six sampling periods between June and September 2013 and between June and September 2015. We used aerial nets for 30 min (not including handling time) each at site and three pan traps for 8 h, and netted and placed traps within the 20 × 20 m vegetation plots. We identified bees using dichotomous keys to genus, and when possible, to species (see Quistberg et al. [43] for details on bee sampling and identification methods). For analysis, values were averaged across sampling periods and then across years for each site. In June 2016, we conducted one visual survey for bees for 30 min at each site to confirm that the relative ranking of species richness and abundance between sites was similar across years.

Bee Installation

Over the course of 3 days in mid-March 2016, we installed *Osmia lignaria* bees at each community garden. We first placed one UV-sterilized binderblock laminate nest (Pollinator Paradise, Parma, ID) at or near the center of each site. Each binderblock was stocked with overwintering cocoons (100 female and 150 males). We applied three sprays of orchard bee attractant on each nest (Crown Bees, Woodinville, WA). Bees were allowed to

emerge and forage for 16 days. We then collected adult female bees. Each bee was placed into a sterile 2-ml vial and immediately stored in dry ice. We also collected a blank, no-template control air sample at each site. We sterilized gloves, forceps, and nets between collecting each bee and between each sampling site with bleach then ethanol. Bees were transported to the lab and into − 80 °C cold storage.

Control Treatment

To confirm that the environment confers unique bacterial communities to foraging bees, we allowed six female bees to emerge from their pupal cocoon casing in a sterile, indoor lab environment in petri dishes. Upon emergence, each female was immediately collected in a sterile 2-ml vial and placed into − 80 °C cold storage. We also removed and collected an additional six females from their pupal casing by cutting individual pupal casings with a blade and removing the female with forceps. We sterilized gloves, blades, and forceps between samples.

Illumina 16s Sequencing

We collected a total of 344 *O. lignaria* (an average of 19 bees per site). We extracted DNA from each sample and 1 control blank per site with the Qiagen "DNeasy Blood and Tissue" extraction kit (Qiagen, Valencia, CA), but with the addition of tissue lysing step using sterile 5-mm stainless steel beads and 0.1-mm glass beads in a Qiagen Tissue Lyser II to ensure extraction of gram positive bacteria [45]. We used whole-insect samples without surface sterilization because previously the bacterial community structure between surface sterilized bees and unsterilized bees has not been significantly different [46]. Library preparation and sequencing (Illumina MiSeq) were performed using previously published protocols [47]. To amplify the 16s rRNA gene, we used the 799F (5′-GAGT TTGATCNTGGCTCAG-3′) and 1115R (5′-GTNTTACNGCGGCKGCTG-3′) primer pair and included negative controls (control blank samples).

Parasite Detection

We screened all *O. lignaria* bees with genus-specific primers for the presence of the neogregarine protozoan *Apicystis* spp., the trypanosomatid protozoean *Crithidia* spp., and the fungal *Aspergillus* spp., a group that includes both common non-pathogenic environmental fungi and strains that manifest as Stonebrood disease in some bee species. We used parasite specific primers and conditions for genus-level identification (Table S1). Products were run alongside a standard ladder on a 1% agarose gel stained with GelRed to confirm amplicon size. Each assay included a negative and positive control.

Data Analysis

We used QIIME 2 (2019.10) to demultiplex and filter 16s sequence libraries [48]. First, we visualized and trimmed the low-quality ends of the reads with QIIME2, then used DADA2 [49] to remove chimeras, remove reads with more than two expected errors, and assign sequences into amplicon sequence variants (ASVs). We used the qiime2 feature-classifier [50] and trained to the 799–1115 region of the 16S gene with the SILVA database [51]. We filtered out features from the resulting ASV table that corresponded to contaminants as identified in our blanks (such as *Propionibacterium*) [52] or were present at only one read (singletons). After filtering, we retained 340 samples and observed an average of 25,064 sequence counts (± 1051.63 SE) across all samples. To account for variable sequencing depth, we subsampled to 1746 reads per sample using alpha rarefaction curves (Fig. S1). This allowed us to retain most samples (14 bee specimens excluded) and capture the majority of sequence diversity found in our samples. After subsampling, we retained 326 samples. We used the MAFFT aligner [53] and FastTree v2.1.3 in QIIME 2 to generate a phylogenetic tree of our sequences [54]. We used the resulting tree and ASV tables to tabulate weighted UniFrac distance matrices (phylogenetic distances, weighted by abundance) [55] for beta diversity comparisons (Fig. 1).

We used QIIME 2 and the R statistical environment [56] to conduct and visualize analyses on alpha and beta diversity. To avoid co-linearity between independent variables, we performed a variable selection process. We divided variables into four groups (floral resources, nesting resources, landscape composition, and bee community diversity) and ran Pearson's correlations within groups to identify correlated ($P < 0.05$) variables within groups. We selected variables that were correlated with the largest number of other variables in that group for subsequent analysis and that were previously found to be ecologically meaningful in describing pollinator diversity in the same field system [43]. We selected two variables reflective of floral resources at a site (abundance of annual flowers and the abundance of perennial trees and shrubs), one variable describing nesting materials (% bare soil, as soil is used by *Osmia lignaria* construct nests), one variable describing the landscape cover (% natural cover within 500 m), and one variable describing the bee community (bee richness). The natural log transformed variables that did not meet conditions of normality. To test for multicollinearity, we calculated a variance inflation factor (VIF) using the car package [57] and found each predictor had a VIF score below 2.

To compare the microbiome communities of bees allowed to forage and bees from our control treatment, we used QIIME2 to first test for homogeneity of dispersion using "permdisp" and then performed a pairwise permutation-based, nonparametric test (PERMANOVA, permutations = 999) [58]

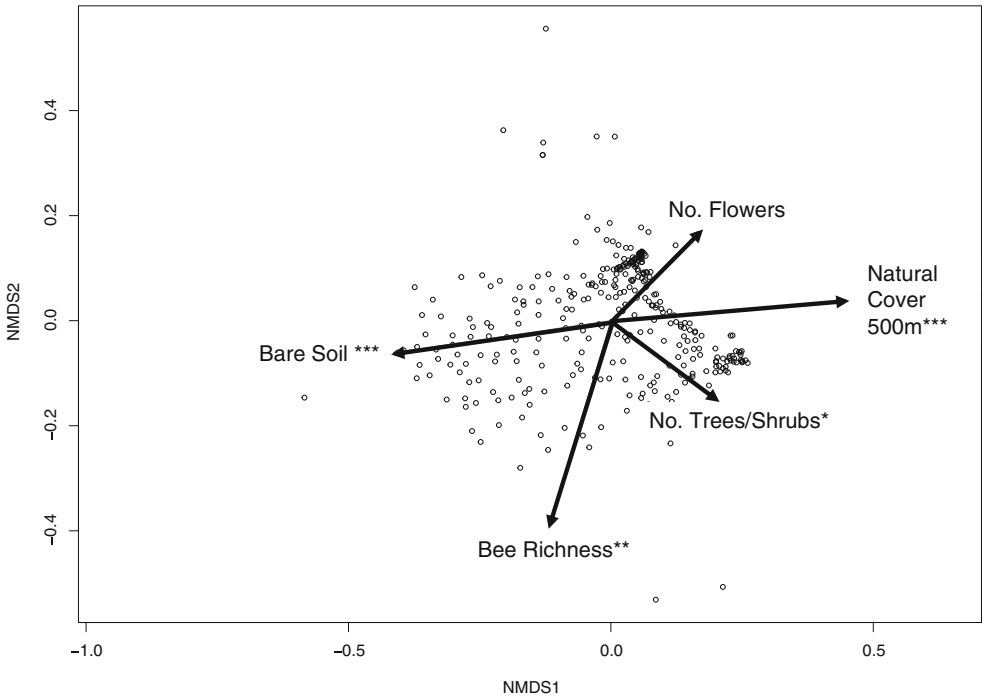

Fig. 1 NMDS plot of Weighted Unifrac abundances. Each sample represents the microbial community of bees from experimental, foraging treatments in urban gardens. Each vector reflects the variables correlated with differences between microbial communities. Significance values were determined by ADONIS function. (Signif. codes: "***" 0.001, "**" 0.01, "*" 0.05, " " 1)

on the weighted Unifrac distance matrices (which accounts for phylogenetic relatedness between ASVs). We visualized differences by plotting 95% confidence ellipses around each centroid on nonmetric multidimensional scaling (NDMS) plots using "ordiellipse" function in vegan package [59] and ggplot2 [60]. To analyze how floral abundance, tree and shrub abundance, natural cover within 500 m, bare soil, and bee richness are related to the variance between microbiome communities of experimental foraging bees at different sites, we first performed non-metric multidimensional scaling weighted by abundance using metaMDS in the ecodist package in R [61]. We partitioned the weighted Unifrace distance matrices between sources of vegetative variation with the vegan package by applying "adonis" on the dissimilarity distance matrix and used "envfit" to fit floral abundance, tree and shrub abundance, natural cover, bare soil, and bee richness onto the ordination. We then compared the dissimilarity between vegetation communities and the bee microbiome communities at each site using a Mantel test, with 999 permutations. We first calculated dissimilarity between vegetation communities at each site using the Bray-Curtis method with the "vegdist" function in the vegan package. The vegetation community matrix consisted of the following variables: % natural cover (500 m), abundance of trees and shrubs, bare soil groundcover (%), and flower abundance. We then calculated the dissimilarity between microbiome samples with the Bray-Curtis method. We obtained a Mantel statistic describing the correlation between matrices based on the Pearson method and plotted the correlogram with the mgram function in ecodist.

To analyze how site factors are associated with microbe diversity and parasite prevalence, for each bee we calculated relative ASV abundance for bacterial sequences found in the following groups: class *Gammaproteobacteria*, order *Betaproteobacteriales,* genus *Lactobacillus*, and genus *Wolbachia*. We selected these groups because, putatively, they are associated with parasitism outcomes [35–37]. We used linear models with the lm function in R to examine relationships between bacterial groups and the site variables (floral abundance, tree/shrub abundance, % natural cover within 500 m, % bare soil, and bee richness). We tested combinations of these variables using the glmulti package [62], with bacterial abundance of Gammaproteobacteria, *Lactobacillus*, and *Wolbachia* as our response variables. For models where the AICc for top models was within 2 points of the next best model, we ran model averages with the MuMIn package [63]. When the best models shared the same significant predictors as model averages, and we reported output from best models.

We indicated parasitism for each bee specimen with binary (0/1) prevalence data for each parasite. To examine the relationship between the prevalence of each parasite and the (scaled) abundance of each bacterial group, we used a generalized linear mixed effect model with a random effect of site and a binomial distribution using the glmer function in R. We then used the q2-composition plugin in QIIME 2 to perform an analysis of composition of microbiomes (ANCOM) [64] to test for which bacterial taxa differ between parasitized and non-parasitized bees. We ran three tests, for which taxa that differ between groups with/without *Apicystis*, with/without *Crithidia*, and with/without *Aspergillus*.

Results

Microbiome Composition between Control and Foraging Groups

For the microbiomes of bees allowed to forage and bees reared or dissected in sterile environments, dispersion was significantly heterogenous between control and treatment groups ($n = 332$, $p < 0.001$, $F = 15.720$, $p = 999$). There was also significant difference between the composition of microbiomes in bees from each treatment group. (Figure S2, $n = 332$, $p < 0.001$, $q < 0.0001$, pseudo-$F = 10.460$, $p = 999$).

We found 979 distinct ASVs across all foraging and control bees from all sites ($n = 326$), with variation in relative abundance across samples (Fig. 2). The mean ASV richness across specimens was 55.38 ± 2.423 (SE). The bacterium present in the greatest number of forager samples and present in the highest relative abundance across all samples were unknown strains in the *Acinetobacter* group, which are associated with soil and water (Fig. 2). Acinetobacter were 2.6-fold more abundant than the second most abundant bacterium, *Erwinia*, a genus in the Enterobacteriaceae that is associated with plants. *Wolbachia* was the 25th most abundant bacteria in foraging bees, present in 18 foraging bee specimens (out of 344 bees), and not present in control bees. The bacteria present in the greatest number of control samples and present in highest relative abundance across all samples were unknown strains in the Enterobacteriaceae group. Other dominant bacteria in the control samples included Corynebacterium, which were found in both foraging and control bees, and those in the Roseiflexaceae and Chitinophagaceae groups, environmental bacteria commonly found in soil, water, plants, and nature but interestingly only found in our control samples. We found *Bacillus* in high abundance in both our forager and control samples; these bacteria are resistant to disinfectants and possibly present in the dishes in which the bees were emerged.

Beta Diversity and Environmental Context

Environmental variables related to perennial floral resources, landscape context, and bee diversity correlated with differences in microbiome composition of foraging bees. Variance in the dissimilarity matrix were significantly explained by percent natural cover within 500 m ($R^2 = 0.021$, $p < 0.001$), the number of trees and shrubs in the garden ($R^2 = 0.007$, $p < 0.05$), and bee species richness ($R^2 = 0.011$, $p < 0.01$) and bare soil ($R^2 = 0.022$, $p < 0.001$) (Fig. 1). We found a significant relationship between the environmental dissimilarity matrix

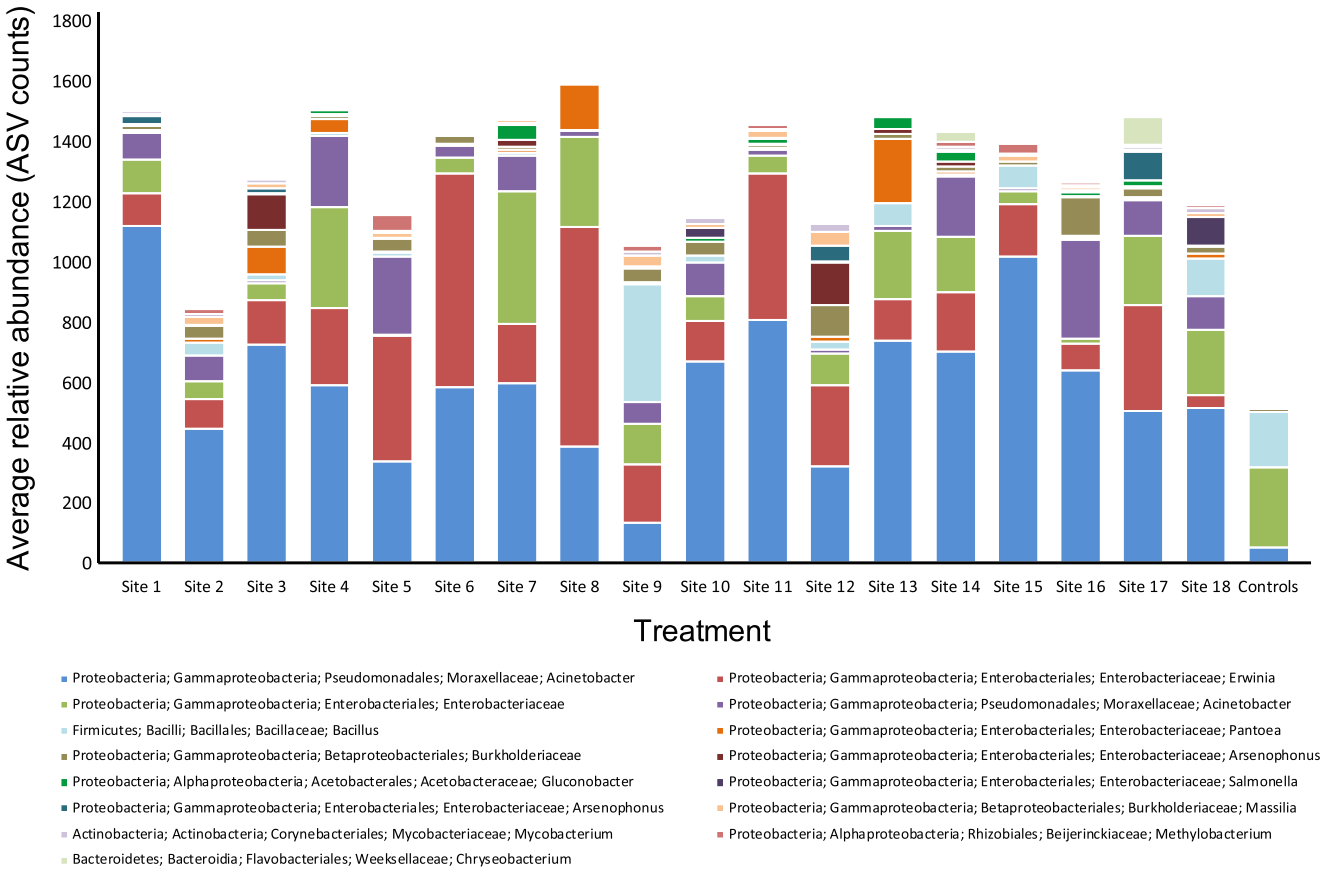

Fig. 2 The relative abundance of the 15 most dominant gut microbiome bacteria, averaged across bee specimens at a site. Each site represents a garden site, whereas control bees are those who emerged in sterile lab conditions. Taxonomic classification was performed based on SILVA

and the bee microbiome dissimilarity matrix ($r = 0.065$, $p < 0.01$): the positive but small coefficient r indicates a weak correlation between distance matrices, suggesting that microbial communities from bees from similar environments are more similar to one another than those in bees from dissimilar vegetation groups (Fig. S3).

Alpha Diversity and Environmental Context

Environmental variables were significantly associated with rarefied ASV abundance (Table 1). *Wolbachia* abundance was higher in sites with higher bee richness ($t = 2.627$, $p < 0.05$, Fig. 3a) and a higher percentage of natural cover in the landscape (500 m) ($t = 2.321$, $p < 0.05$, Fig. 3b). Gammaproteobacteria abundance was not significantly predicted by any environmental variable. *Lactobacillus* abundance was higher in sites with higher floral abundance ($t = 2.405$, $p < 0.05$) (Fig. 3c). Betaproteobacteria abundance was higher in sites with higher bee richness ($t = 2.634$, $p < 0.05$) (Fig. 3d).

Parasite Prevalence

Crithidia, *Apicystis*, and *Aspergillus* were present at varying rates in bees across the sites (Table 2), however, parasitism was not related to the abundance of Betaproteobacteria, Gammaproteobacteria, *Lactobacillus*, or *Wolbachia*. ANCOM analysis found no taxa with significant differential abundance across parasitized and non-parasitized bees.

Discussion

The composition of the bee microbiome varied by environmental variables related to resource availability, landscape context, and bee diversity. While environmental features such as diet and geography are more influential than host genetics for microbiome variation in humans, mammals, and flies [65], our study is the first to confirm that environmental variation plays an important role in shaping the microbiome and the relative abundance of particular bacterial groups in bees. For example, *Acinetobacter* bacteria associated with soil and water were a dominant bacterium the greatest number of forager samples. These bacteria (speculatively) may be common in *Osmia* bees because they use mud to construct their nests. This is in contrast to previous study by Cariveau et al., which found that agricultural land-use has little to no impact on the microbial communities in social bumble bees [66]. Our study design differs because we examined the impact of fine-scale, local variables, such as floral abundance and groundcover characteristics, whereas Cariveau et al. [66] addressed the impact of categorical habitat types on microbiome composition. In addition, it may be easier to detect and compare the impact of environmental variables on the microbiome of this species of megachilid bees because they lack the core bacteria commonly found in social bees such as honey bees and bumble bees. Furthermore, megachilid bees have shorter foraging ranges than social bees and they can be manipulated to emerge and forage within the same time frame across multiple sites.

During metamorphosis, bees undergo gut reorganization in which the larval gut is shed [67]. However, we found that bees reared in sterile environments are not "blank slates," but are host to a microbiome that is significantly different in composition to the microbiome of foraging bees, but with an important caveat: the results from our statistical analyses indicate that differences in microbiome composition between treatment and control groups may be an artifact of heterogenous dispersions between groups. In particular, permutational multivariate analysis of variance (PERMANOVA) is not robust to unbalanced designs [68], and indeed our sampling sizes varied greatly between treatment and control groups. Therefore, we suggest more research on how bacteria are acquired after bee emergence from overwintering states to determine how the adult bee microbiome is shaped by interactions with the environment.

We expected to find that Betaproteobacteria, Gammaproteobacteria, *Wolbachia*, and *Lactobacillus* would be associated with lower prevalence of parasites because of previous research on these associations [34, 35]. While our study does not confirm the role of the microbiome in defense against parasites, it is possible that horizontal transmission of some bacterial symbioses may still be important for bee health, but research that explores the mechanistic underpinnings of this relationship is needed. Our study does highlight

Table 1 Results of GLM model selection for bacterial groups

Dependent variable	Predictor(s)	AIC	R^2	df
Gammaproteobacteria	None	–	–	–
Betaproteobacteria	Bee richness *	208.728	.3316	15
Lactobacillus	Floral abundance*	134.023	0.278	15
*Wolbachi*a	Natural cover 500 m (%)*, bee richness *	132.123	0.451	15

[§] All relationships featured in the table are positive, there were no negative predictors. (Signif. code: "*" 0.05)
Table shows significant variables from the best model selected[§]

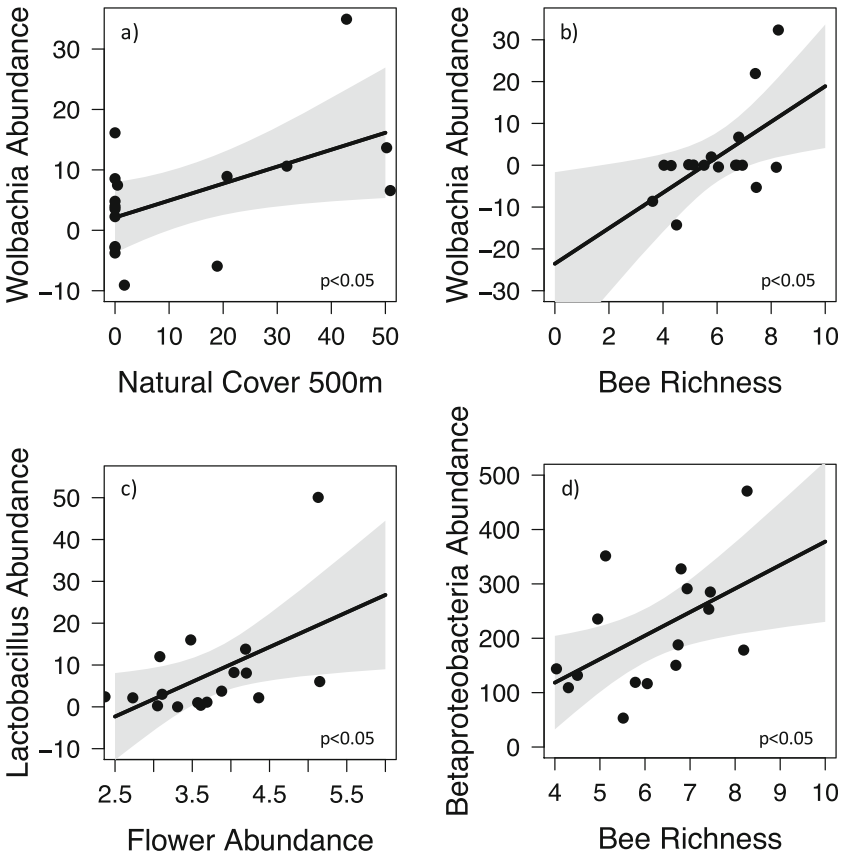

Fig. 3 Results of GLM model selection for bacterial groups. Graphs show significant variables from the best model predicting the average abundance of *Wolbachia* spp. (a, b), *Lactobacillus* spp., (c), and Betaproteobacteria (d)

that bee community richness influences the relative abundance of certain bacterial groups (*Wolbachia*, Betaproteobacteria). While some bacteria are known to be transmitted horizontally [28, 69], it is unclear how bee species richness facilitates the association between bacteria and the bees. An increase in bee species may increase the likelihood of within and between species interactions during foraging trips for food and nesting materials, facilitating bee-bee transmission of bacteria. It may also increase the likelihood that a foraging bee is exposed to materials previously touched by other bees or exposed to bee feces left in the environment. Other environmental features may influence both bee richness and bee-microbe interactions but were not included in this study, such as variation in climate, which likely impacts physiological interactions.

Table 2 Prevalence rates for each parasite. Standard error is calculated as the sample standard deviation divided by the square root of the sample size

(Binomial variances)

	No. detected	n	Percent (± SE) infected
Crithidia spp.	98	344	45.01 ± 1.33
Apicystis spp.	155	344	28.49 ± 0.87
Aspergillus spp.	272	344	79.07 ± 0.94

A weakness of this study is that bee species richness was not measured when *Osmia lignaria* trap nests were set out, so we exercise caution with interpretation of this data, although our visual pollinator survey confirmed that the relative ranking of species richness between sites was similar across years. Our findings are further complicated because we examined relative abundance, not absolute abundance of bacteria in the microbiome. Bacterial richness estimates based on rarefaction should be taken with a grain of salt, as rarefaction may result in false positives [70]. Furthermore, we found that neither bee richness nor any environmental features predict the abundance of the socially transmitted Gammaproteobacteria, a large class of bacteria. More work is needed to determine the importance of horizontal transmission for the microbiome of the bee.

We contribute to the growing body of literature highlighting the dual role of flowers in mediating bee-microbe interactions. On one hand, flower diversity can benefit bees by providing food and promoting immune responses [71, 72]. But flowers can also host parasites and viruses, which can be florally transmitted within and between bee species [73, 74]. Finally, flowers act as bacterial transmission hubs [27, 28]. It has even been proposed that *Wolbachia* endosymbionts may be horizontally transmitted between bees at flower sites [75], as *Wolbachia* has been isolated from pollen samples [27]. We found that increased floral abundance in our field sites was associated with greater counts of *Lactobacillus* in *Osmia*

lignaria, suggesting that the environment plays a role in shaping *Lactobacillus*-bee interactions. One question remaining is how lactic acid bacteria influence bee health and fitness. Because some *Lactobacillus* species protect bees against fungal parasites in laboratory settings [36, 37], we were surprised to find no association between lactic acid bacteria and the fungus *Aspergillus*. We suggest more research on the possible protective benefits of the *Lactobacillus*, as it has been proposed as medicinal probiotic for bees [76]. Finally, while bee-friendly initiatives recommend growing flowers for bees, we must first address the fitness tradeoffs between immunity benefits, microbial associations, and exposure to flower-associated parasites and pathogens for bee health. Future experiments examining which flower species and which flower traits are important for parasite, pathogen, and microbial associations may reveal some of these tradeoffs and inform decisions around which flowers to plant for bees.

While other studies have confirmed the presence of *Crithidia* and *Aspergillus* (Stonebrood) in megachilid bees, this is the first report of the neogregarine *Apicystis* in a species from the genus *Osmia*. The impacts of these three parasites for megachilids are largely unknown, and our findings only confirm that they are present. Because *Osmia lignaria* is increasingly adopted for commercial pollination, it is important to know if these parasites actively replicate, infect, and harm megachilid bees. These parasites were not found in control bees dissected from their cocoons. Suggesting they may have acquired them from the environment. Finally, although we examined if microbial composition is associated with reduced parasite prevalence, we did not assess how bee fitness is directly impacted by environmental context. For example, local and landscape features in an agricultural site can impact bee health directly through variability in food quality and quantity, or indirectly through impacts to bee physiology. Although local and landscape features in agricultural landscapes are associated with fitness-related measures such as bee size, bee fat content, and nesting density [77], the role of the microbiome remains elusive. We suggest more research on how the microbiome impacts the nutritional state of bees [7, 78]. This work may reveal how changes in the microbiome associated habitat changes could impact bee health.

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Author Contributions HC acquired funding for the research, led study design, fieldwork, lab work, and coordinated manuscript writing and publication. QSM contributed to field research design, provided physical and financial access to laboratory equipment, supervised laboratory methods, and contributed to the manuscript. SMP contributed to field research design, fieldwork logistics, data analysis, and manuscript writing.

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Data Accessibility We have uploaded our code, data, and metadata to a dryad repository at https://doi.org/10.6086/D1H094. We added sequencing data to SRA, accession #PRJNA613243.

Compliance with Ethical Standards

Competing Interest The authors declare that they have no competing interests.

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