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Nest ecology-associated impacts of wastewater on wild bee microbiomes

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ABSTRACT Anthropogenic pollution affects environments differently depending on proximity to the pollution source, exposure route, and species ecology. Thus, understanding an organism's ecological role and exposure route to contaminants is central to assessing pollution impact. Treated municipal wastewater releases contaminants into waterways and alters microbial communities. Plants absorb contaminants and expose animals through foraging and nest-building activities. Nesting ecology differences of ground and wood cavity-nesting bees offer insight into niche-specific susceptibility to pollution. We compared gut microbiomes of directly exposed soil-nesting *Halictus ligatus* and indirectly exposed wood-nesting *Ceratina* spp. upstream and downstream of wastewater. We collected bees, flowers, and soil and analyzed their bacterial microbiomes using 16S rRNA sequencing. The effect from wastewater explained a much larger proportion of the variance in the ground-nesting *H. ligatus* microbiome than wood cavity-nesting *Ceratina* adults, *Ceratina* larvae, and pollen provisions. Conversely, soil and flower microbiomes remained stable, indicating higher stability. These findings suggest that wastewater exposure leads to strong host-associated microbiome disruption, with ground-nesting bees most affected.

IMPORTANCE Because contaminants bind strongly to soil, ground-nesting bees near wastewater are likely most impacted, while wood cavity-nesting bees are likely less impacted since plant uptake of contaminants is species dependent. These results suggest that exposure routes drive contaminant susceptibility, with bee-associated microbes most vulnerable. Because bees are important pollinators and biodiversity contributors, these disruptions point to broader ecological risks in increasingly contaminated landscapes.

KEYWORDS microbiome, pollution, wood cavity-nesting, ground-nesting, effluent, wastewater, wild bees

Urbanization, industrial activity, and agriculture all create pollution and pose a global threat to biodiversity and microbial ecology. Municipal wastewater, a major byproduct of urbanization, exemplifies how human activities introduce contaminants into natural systems. Although treatment plants remove many harmful substances, they often fail to eliminate effluent-associated contaminants, which persist in effluent and accumulate in the environment (1, 2). These effluent contaminants can alter microbial diversity and function in both environmental and host-associated communities, disrupting ecological interactions and potentially compromising plant and animal health (3–6). Furthermore, wastewater effluent creates nutrient-rich environments that are optimal for certain bacteria taxa to exploit and proliferate (7–11) possibly leading to a competitive advantage for other taxa that may serve a role in supporting plant and insect health.

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Lyna Ngor dedicates this work to her father, Daniel Galbraith, whose love and support made this journey possible. As a single mother, Lyna is deeply grateful for the years he spent caring for her son while she pursued her research. This achievement would not have been possible without him.

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Beyond direct contamination of aquatic ecosystems, terrestrial reservoirs such as soil and flowering plants also harbor pollutants along with perturbed microbial communities and mediate exposure of animals to these compounds and their effects. Soil, particularly in urban areas receiving treated effluent, can accumulate contaminants (12) while simultaneously supporting complex microbial networks. Likewise, flowers serve as microbial hotspots, harboring diverse microbial communities that can be shaped by environmental conditions, including pollution (13). Floral-borne microbes form the microbiomes of many native bees (14) and are ecologically important via their effects on floral phenotypes and plant-pollinator interactions (15). However, little is known about how pollution-driven changes in soil and floral microbiota may cascade into higher trophic levels such as the microbial communities of native bees. Understanding how wastewater disrupts microbial communities is, therefore, critical to assessing the broader ecological implications of wastewater pollution.

Bees are ideal models to investigate how environmental pollution affects host microbiomes and how niche-specific susceptibility determines the severity of pollution exposure. Most native bees acquire their gut microbiota from environmental sources rather than through social transmission (16, 17), making them especially vulnerable to pollution-induced shifts in soil and floral microbial communities such as in urban wastewater habitats. Microbiome dysbiosis can impair digestion, immunity, and overall fitness (16), while a stable microbiome has been shown to buffer bees against stressors like metal toxicity (18). Given native bees' diverse nesting ecologies, their exposure to effluent contaminants varies: soil-nesting species, like *Halictus ligatus*, are in direct contact with contaminated soil and floral resources, while cavity-nesting species, like *Ceratina* spp., primarily experience indirect exposure through plant materials. These differences offer a unique opportunity to examine how pollution shapes microbiomes across environments and different host species' ecologies.

In this study, we examine the effects of municipal wastewater pollution on microbial communities in native bees, soil, and flowers from sites up- or down-stream of wastewater treatment plants. By comparing a ground-nesting bee (*H. ligatus*) and a cavity-nesting bee (*Ceratina* spp.) at sites with and without wastewater exposure, we assess how bees' niche-specific susceptibility and exposure routes shape bee-associated microbiota. We also characterize soil and floral microbial communities to understand how environmental reservoirs of microbes and contaminants correlate with bee microbiomes.

MATERIALS AND METHODS

Study system

Halictus ligatus is a soil-nesting bee that excavates nests directly in the ground using its mouthparts (19). Because nesting and brood development occur within soil substrates, *H. ligatus* interacts extensively with soil-associated microbial communities throughout its life cycle. In contrast, *Ceratina* spp. are small carpenter bees that nest within the pith of dead plant stems rather than in soil (20). Like *H. ligatus*, *Ceratina* spp. forage on floral resources and, therefore, encounter microbes associated with pollen and nectar. These differences in nesting environment and foraging context provide an opportunity to examine whether microbial community composition varies in relation to nesting ecology and habitat context.

Experimental design

We sampled 14 riparian sites in California, including 7 downstream sites that receive treated municipal effluent discharge and 7 upstream reference sites located >1 km from effluent outflows to minimize potential overlap in bee foraging ranges (Fig. 1; Table S3). The 1 km buffer was selected based on the nonlinear relationship between bee body size (intertegular span) and foraging distance, which predicts typical foraging distances for *Halictus ligatus* to be well under 1 km and even shorter for *Ceratina* spp., making 1

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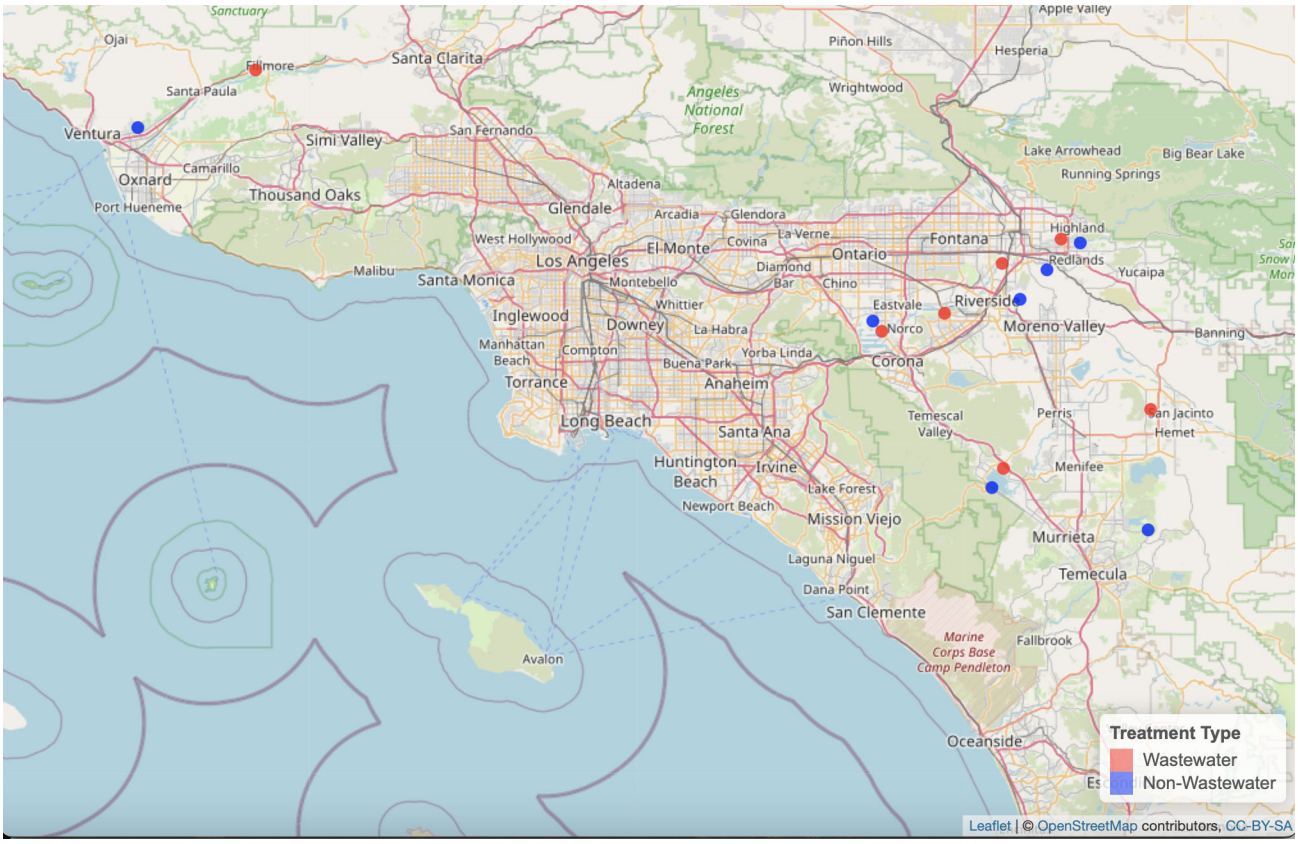


FIG 1 Map of southern California showing 14 riparian sampling sites clustered near Riverside, Ventura, and Redlands. Red circles indicate seven wastewater-associated sites downstream of treated municipal effluent discharges, and blue circles indicate seven non-wastewater reference sites upstream and more than 1 km from effluent outflows.

km a conservative separation between site types (21). At each site, we collected adult *H. ligatus*, adult *Ceratina* spp., *Ceratina* larvae, *Ceratina* pollen provisions, flowers, and surface soil to characterize the microbial communities associated with bee hosts and their immediate environmental contexts. Downstream and upstream sites were sampled during the same sampling season to reduce temporal variation. *H. ligatus* adults were collected while foraging. *Ceratina* nests were collected intact from elderberry stems, and adults, larvae, and pollen provisions were aseptically separated in the laboratory. To represent floral microbial environments, flowers for *H. ligatus* were collected only from plant species on which individuals were directly observed foraging. Flowers for *Ceratina* were collected from the elderberry trees used as nesting substrates, reflecting the nearest and most consistently available floral resource. Surface soil (~3 cm depth) was sampled adjacent to the plants or nesting substrates where bees were collected. At downstream sites, where access permitted, we sampled moist surface soil along visible effluent surface flow paths. We note that chemical contaminant concentrations were not measured, and site type is, therefore, treated as an environmental context variable.

As underground nests are difficult to locate and excavate, we only collected foraging adults of *H. ligatus*. For *Ceratina* spp., we collected the whole nest with adults, larvae, and pollen provisions. Individual *H. ligatus* adults were stored in liquid nitrogen or dry ice during fieldwork and deposited in a −80°C freezer until DNA extraction could be performed. *Ceratina* whole nests (in twigs) were stored in a cool ice box during field work. In the lab, *Ceratina* nests were aseptically dissected. Adults, larvae, and pollen provisions were separated into individual sterile tubes. Overall, we collected 4 types of host samples: *H. ligatus* adults, *Ceratina* spp. adults, *Ceratina* larvae, and *Ceratina* pollen provisions in all of our 14 sites. We analyzed *H. ligatus* and *Ceratina* spp. adult bees,

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Ceratina larvae, and pollen provisions independently. At each site, we collected ~50 mL of flowers on which we found *H. ligatus* foraging. We also collected elderberry tree (*Sambucus mexicana*) flowers from the same trees in which we found *Ceratina* nests. We haphazardly collected surface soil samples at each site into sterile 50 mL Falcon tubes.

Library preparation

We performed surface sterilization of *H. ligatus* adults, *Ceratina* adults, and *Ceratina* larvae by washing with 1% bleach (1 min) followed by DI water twice (2 min). We performed whole gut dissection on *H. ligatus* adults, including the Dufour's gland. Due to their small size, we used the whole abdomen of *Ceratina* adults and intact larvae. To obtain sufficient DNA for extraction, we pooled flower samples by host association and site. We added 10 mL of PBS per 50 mL Falcon tubes. We then agitated the tubes for 15 min using a Corning LSE vortex mixer at medium speed, followed by centrifugation at 18,000 rpm for 10 min to collect bacterial pellets. We used 1,250 mg of soil material for each of the 14 sites. We conducted DNA extraction on adult bee guts and larvae using Qiagen's Blood and Tissue kit, Qiagen Plant Pro kit for *Ceratina* pollen provisions and flower wash, and Qiagen PowerSoil Pro kit for soil samples according to the manufacturer's protocol (Qiagen, Valencia, CA, USA).

For bee guts, we lysed our samples using TissueLyser II Qiagen at 30 Hz for 6 min. Before lysing, we added 20 µL ProK and 180 µL ATL buffer, two 3.2 mm steel beads, and ~100 µL zirconia beads in each sample. After lysing, we incubated our samples for 1 h at 56°C. We added two negative controls that we carried through to all downstream steps. For flowers and soil, we followed the manufacturer's protocol (Qiagen, Valencia, CA). Upon DNA quality verification, we prepared bees and flower wash samples for 16s Illumina barcoded by performing a two-step PCR as previously described (14). In PCR 1, we adapted each sample with its own unique primer sequences for downstream identification using the plant-plastid avoiding 799Fmod3 (CMGGATTAGATACCCKGG) and 1115R (AGGGTTGCGCTCGTTG) primers (see the supplemental methods, Section S1.6, for unique primer sequences). In PCR 2, we completed the Illumina sequencing construct. To ensure an equal molar proportion of each sample input across all samples, we performed PCR normalization using SequelPrep normalization plates (Invitrogen, Carlsbad, CA, USA). Finally, samples were pooled and sent to the University of California, Riverside, Genomic Core for bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) and sequencing services using the NextSeq 2000 P2 600 kit.

For soil samples, we processed extracted DNA for full-length 16S rRNA gene sequencing using the Oxford Nanopore 16S Barcoding kit 24 V14 according to the manufacturer's protocol (Oxford Nanopore, Alameda, CA). As plant-plastid contamination is not problematic for soil samples, which are also extremely diverse and difficult to accurately characterize, the long-read platform allowed us to deeply sequence nearly full-length 16S rRNA gene sequences using 27F and 1492R primers. After the PCR protocol described by the manufacturer, we pooled the amplicons, loaded the MinION flow cell (FLO-MIN114), and sequenced using the MinION Mk1B sequencer. Before starting the sequencing device, we set the minimum qscore to 7 and the base calling model to fast basecalling.

H. ligatus adults

To determine if differences in microbiome structure were related to total bacterial load, we used qPCR to compare 16S rRNA gene copy number (absolute abundance) between wastewater and non-wastewater samples in the soil-dwelling *H. ligatus* adults. We performed qPCRs in triplicate using the same primers that we used for library preparation: 799Fmod3 and 1115R. Cycle times were converted to copy numbers using Gblock sequences (see the supplemental methods, Section S1, and Fig. S7) from 16S rRNA gene region for the bee-associated *Apilactobacillus* (22) as standard curves. A triplicate dilution series was included in the standard curve for all reactions. Amplification efficiency was between 90% and 110% in all of our reactions.

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Bioinformatics Qiime2 for bees and flowers

We processed the 16S rRNA gene amplicons using the Qiime2 pipeline (23). We excluded any reads with a quality score below 35, and we binned exact amplicon sequence variants (ASVs) using DADA2 (24). We used the SILVIA 138 database (25) and QIIME2's scikit-learn (26) to assign taxonomy to the ASVs. We removed reads that were assigned to chloroplasts and mitochondria from our data set. We also eliminated any ASV that showed up only once (singletons). To avoid any potential reagent contaminant, we used the decontam package in Qiime2 (23) to filter out any reads that showed up in our blank controls with a cutoff threshold of 0.5, which was 534 reads (0.001%) of the total reads (51,522,200 reads). In total, we retained 51,521,666 reads after quality controls for our downstream analysis (see the supplemental methods, Section S1, and Fig. S1).

Nanopore 16S amplicons using EPI2ME and Docker

We processed the 16S rRNA gene amplicon sequencing data using the epi2me-labs/wf-16s pipeline (v1.4.0) via Nextflow (v23.04.2) with Docker. Nanopore FASTQ files were quality filtered at a Q score threshold of 7, followed by reference database preparation using default SILVA and NCBI taxonomy databases. Taxonomic classification was performed with Minimap2, and microbial abundance tables and summary reports were generated. Negative controls yielded fewer than 10 reads and were excluded from downstream analysis.

Alpha refraction, alpha and beta diversity, and ALDEx2 taxonomic differential abundance

In the bee-associated samples, we separated our data into four groups by tissue source: adult *H. ligatus*, adult *Ceratina* spp., *Ceratina* larvae, and *Ceratina* pollen provisions. Using QIIME2, we rarefied each of these data sets and chose a sampling depth at a point in which the observed features in the rarefaction graph leveled off. Sampling depth was set for the following: *H. ligatus* = 9,000 reads, *Ceratina* adult = 9,000 reads, *Ceratina* larvae = 20,000 reads, and *Ceratina* pollen provision = 50,000 reads (Table S1). We set the sampling depth for our flower and soil samples at 5,000 and 200,000 reads, respectively.

We assessed alpha diversity (observed ASVs, Pielou's evenness) using Kruskal-Wallis tests. For beta diversity, we computed Bray-Curtis dissimilarities from rarefied ASV tables and visualized patterns with NMDS using metaMDS() in the vegan R package (27). We used PERMANOVA via adonis (vegan) to test for significant differences in community composition between wastewater and non-wastewater sites for each sample type. To assess within-group variability, we applied beta dispersion tests using betadisper() and permutest() (vegan), which evaluate heterogeneity in Bray-Curtis distances within site types. We used Moran's I to test spatial autocorrelation in Shannon diversity. Differential abundance was assessed using ALDEx2 (QIIME 2 plugin) with FDR < 0.1 (28, 29) which applies compositional modeling and Monte Carlo sampling to detect ASVs enriched by treatment. As a complementary exploratory analysis, we also calculated genus-level baseline prevalence in non-wastewater samples as a proxy for taxon commonness or "core-ness" and compared it with wastewater-associated log2 fold change in mean relative abundance within each sample group (see Fig. S10 and S11).

RESULTS

Quality control

A total of 77 out of 584 samples (13.18%) were excluded due to low sequencing depth based on alpha rarefaction thresholds. Across all filtering steps, we removed 5.99×10^7 reads, the vast majority of which were lost during rarefaction subsampling. An additional 1.58×10^5 reads were removed due to contamination or classification as non-target sequences (e.g., blanks, chloroplasts, mitochondria). After filtering, we retained 1.27×10^7 high-quality reads for analysis. ASV reads were separated by host types such as *H. ligatus*, *Ceratina* adults, larvae, and pollen provision (Table S1).

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qPCR for *H. ligatus*

To assess whether wastewater exposure altered absolute bacterial load in *H. ligatus* adults, we quantified 16S rRNA gene copy number via qPCR (n = 118). Across field sites, total bacterial load in *H. ligatus* showed substantial variability within and among sites but did not differ significantly between wastewater and reference conditions. The log-normal mixed model indicated overlapping 95% confidence intervals for wastewater and non-wastewater EMMs, and the Type (site-condition) fixed effect was not significant (see Fig. S8). Observed copy numbers spanned more than four orders of magnitude across sites; however, this variation was driven primarily by site-level differences captured by the random intercept rather than by wastewater exposure. These results suggest that while wastewater strongly altered microbial community composition, it did not produce a detectable shift in total bacterial estimated community size in adult *H. ligatus* guts.

NMDS ordination analysis and beta diversity analyses

Our beta diversity analyses revealed that the abdominal microbial communities of *H. ligatus* adults exhibited the strongest response associated with proximity to wastewater, with PERMANOVA explaining 24.74% of the variation in Bray-Curtis dissimilarities between site types (R^2 = 0.2474, P < 0.001; Fig. 2A; Table S2). *Ceratina* spp. adults also showed significant but smaller treatment differences (R^2 = 0.0135; P < 0.05; Fig. 2A, Table S2). Similarly, *Ceratina* larvae and pollen provision microbiomes differed by treatment, with R^2 values of 0.0415 and 0.0403, respectively (Fig. 2B and C, Table S2). In contrast, no significant treatment differences were detected in flower and soil microbiomes (Table S2, Fig. S8 and S9), P > 0.1, suggesting higher environmental stability.

To complement these findings, we examined within-group variability using the betadisper() and permutest() functions in the vegan R package. Wastewater exposure significantly increased beta dispersion in *H. ligatus* adults ($F_{1,84}$ = 34.85, P = 0.001; Fig. S4.1) and Ceratina pollen provisions ($F_{1,107}$ = 10.09, P = 0.003; Fig. S4.4), indicating greater microbial heterogeneity under contamination. Ceratina larvae showed a marginal increase in dispersion ($F_{1,147}$ = 3.99, P = 0.055; Fig. S4.3), while Ceratina adults ($F_{1,116}$ = 2.82, P = 0.109; Fig. S4.2), floral washes (P = 0.91; Fig. S4.5), and soil samples ($F_{1,10}$ = 2.11, P = 0.159; Fig. S4.6) did not differ significantly.

Together, these complementary analyses demonstrate that wastewater exposure not only shifts the average microbial composition (PERMANOVA) in sensitive hosts like *H. ligatus* and *Ceratina* brood stages but also increases variability in microbial structure (beta dispersion), particularly in *H. ligatus* that are in close contact with contaminated soils. These findings emphasize both the directional and stochastic impacts of contamination on host-associated microbial communities. Importantly, PERMANOVA results were not solely attributable to dispersion differences, as shown in Table S2 and Fig. S4.

Alpha-diversity: richness and evenness

We assessed microbial alpha diversity using both Pielou's evenness and species richness (observed ASVs) across adult *H. ligatus*, adult *Ceratina* spp., *Ceratina* larvae, pollen provisions, floral washes, and soil samples. All diversity metrics were calculated from rarefied ASV tables using QIIME 2 and analyzed with Wilcoxon rank-sum tests in R using the vegan, ggplot2, and ggpubr packages. Evenness differed significantly only in *H. ligatus* adults, with wastewater samples showing reduced community evenness (W = 1,330, P < 0.001; Cliff's delta = −0.45), suggesting dominance by a few taxa in wastewater treatment (Fig. S5). No significant differences in evenness were detected in *Ceratina* adults (W = 1,309, P = 0.2254), larvae (W = 2,403, P = 0.1899), pollen provisions (W = 1,572, P = 0.5958), flower washes (P = 0.79), or soil (W = 25, P = 0.7789) (Fig. S5). These results highlight a host-specific response in microbial balance under wastewater exposure, with *H. ligatus* being the most sensitive. Species richness remained stable across all sample types. No significant differences in observed ASVs were found between wastewater and non-wastewater sites for any host or environmental matrix (all P > 0.05;

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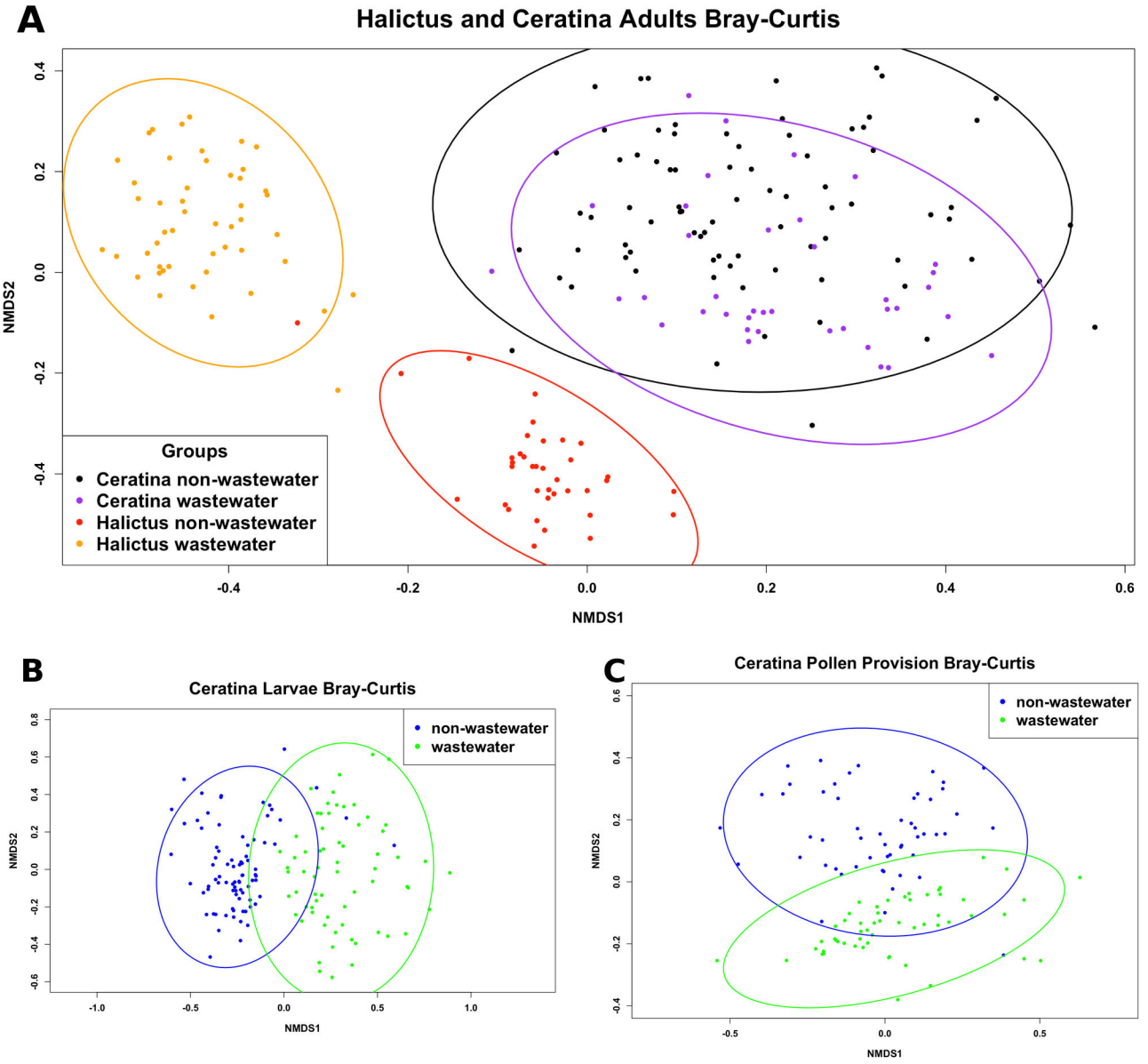


FIG 2 NMDS ordination of Bray-Curtis dissimilarities across wastewater and non-wastewater sites for microbial communities from bee, larvae, and pollen provisions showed distinct clusters, while soils and flowers showed no distinction between site types. Each point represents an individual sample; ellipses indicate 95% confidence intervals for group centroids. (A) Gut microbiomes of adult *Halictus ligatus* and *Ceratina* spp. show strong separation by treatment in *H. ligatus* (PERMANOVA R^2 = 0.2474, P < 0.001), and weaker but significant separation in *Ceratina* adults (R^2 = 0.0135, P < 0.05). (B) *Ceratina* larval gut communities differ significantly between site types (R^2 = 0.0415, P < 0.001), with moderate centroid separation. (C) Pollen provisions show modest but significant compositional differences (R^2 = 0.0403, P < 0.001), suggesting environmental input from contaminated floral sources.

Fig. S6). This indicates that while the relative abundance distribution may shift in *H. ligatus*, the overall number of microbial taxa remains unchanged.

Aldex2 differential abundance

Our differential abundance analysis showed that *Enterococcus* was the most differentially abundant microbe in *H. ligatus* adults from wastewater environments, while *Wolbachia, Lactobacillus,* and *Gilliamella* dominated in non-wastewater environments (Fig. 3A). In contrast, *Ceratina* adults showed *Wolbachia, Lactobacillus,* and *Gilliamella* were more abundant in wastewater environments (Fig. 3B). *Ceratina* larvae, *Lactobacillus, Gilliamella,*

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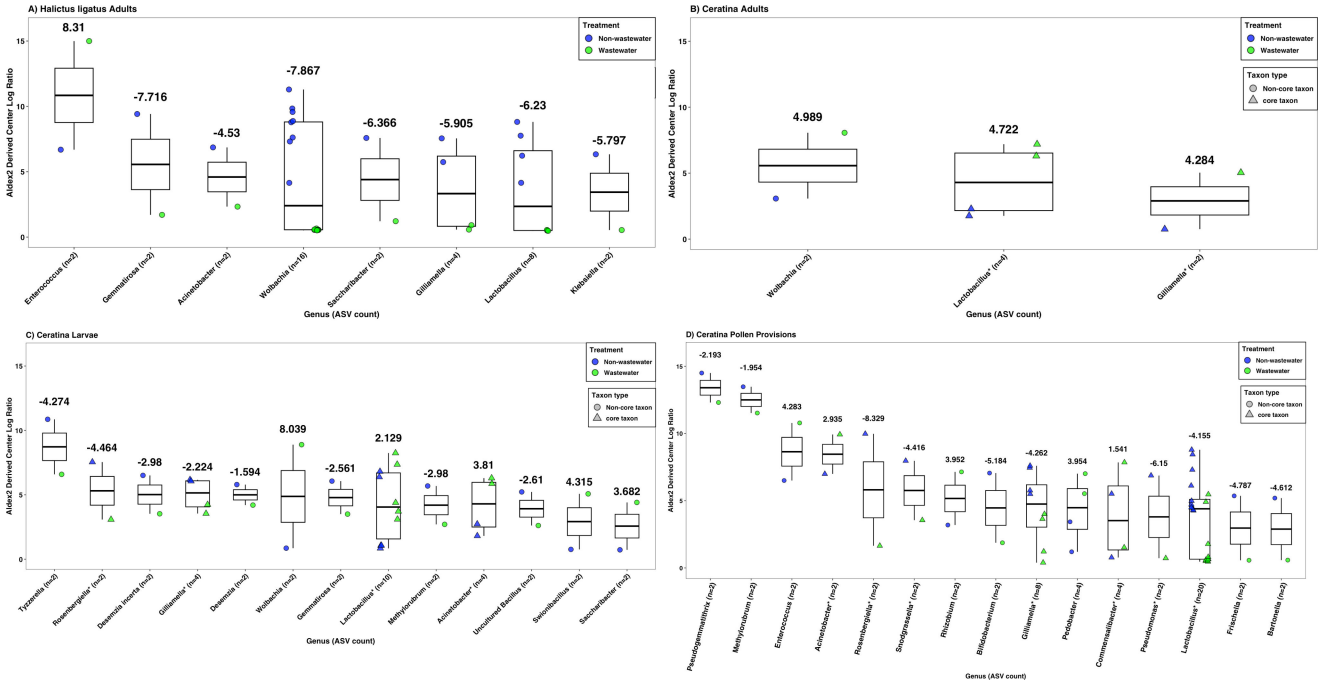


FIG 3 Boxplots show ALDEx2-derived rab.win values for differentially abundant ASVs grouped by genus in (A) *Halictus ligatus* adults, (B) *Ceratina* adults, (C) *Ceratina* larvae, and (D) *Ceratina* pollen provisions. Relative abundance refers to the proportion of sequencing reads assigned to each ASV within a sample before ALDEx2 compositional transformation. The plotted rab.win values are ALDEx2-estimated within-group relative abundance values generated from its centered log-ratio-based workflow, not raw read proportions. Points (n = x) represent individual ASVs assigned to each genus. Numeric labels on top of each boxplot data point indicate the mean difference in rab.win values for each genus, calculated as wastewater minus non-wastewater. Positive values indicate higher abundance in wastewater-associated samples, whereas negative values indicate higher abundance in non-wastewater samples. Asterisks denote previously identified *Ceratina* core taxa.

and *Tyzzerella* were more abundant in non-wastewater environments (Fig. 3C). In *Ceratina* pollen provisions, samples had higher differential abundance of *Lactobacillus* and *Gilliamella* in non-wastewater habitats (Fig. 3D).

Spatial autocorrelation Moran I using beta diversity ordinations

We only conducted spatial autocorrelation for sample types that showed significant *P*-values < 0.05 from our PERMANOVA result. Our analysis of spatial autocorrelation was assessed using Moran's I for sample types with significant PERMANOVA results ($P < 0.05$; Table S4). *Ceratina* larvae exhibited strong positive spatial autocorrelation ($I = 0.4523$, $P < 0.001$). In contrast, *Halictus ligatus* adults ($I = -0.0613$, $P = 0.001$) and *Ceratina* pollen provisions ($I = -0.0339$, $P = 0.001$) showed significant negative spatial autocorrelation. *Ceratina* adults did not display significant spatial structure ($I = -0.0114$, $P = 0.175$; Fig. S3).

DISCUSSION

Our comparison of bee-associated microbes between site types, with and without exposure to wastewater effluent, indicates that microbes of ground-nesting bees (*Halictus ligatus*) showed the strongest association with wastewater exposure more than wood-cavity-nesting bees (*Ceratina* spp.). This result was driven by a major reduction in wild bee-associated microbes such as *Wolbachia, Lactobacillus,* and *Gilliamella* in *H. ligatus*, as well as in *Ceratina* larvae and pollen provisions exposed to wastewater effluent, while these same microbial taxa were elevated in wastewater-associated *Ceratina* adults. Notably, we do not know whether *H. ligatus* has a well-defined core microbiome. If *H. ligatus* lack a core microbiome, this may increase its susceptibility to colonization by environmentally derived and opportunistic taxa.

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We found that *Enterococcus* was highly abundant in wastewater-associated *H. ligatus* adults. This pattern likely reflects the ability of *Enterococcus* to thrive in the nutrient-rich environment associated with wastewater discharge (7–11, 29). Although we did not sample wastewater effluent directly, *Enterococcus* was among the five most abundant taxa detected on floral surfaces in wastewater sites (Fig. S2.5), suggesting that flowers may represent one plausible environmental route of transmission. *Enterococcus* was also detected in soil but was not dominant and was not significantly differentially abundant in our soil differential abundance analysis. Thus, while soil cannot be excluded as a potential route of transmission for bee-colonizing microbes, our data provide stronger support for floral exposure as a plausible route in this system.

However, shared floral exposure alone is unlikely to explain the strong enrichment of *Enterococcus* in *H. ligatus* because *Ceratina* adults from the same sites did not show a similar increase. Instead, the increase in *Enterococcus* together with declines in *Lactobacillus* and *Gilliamella* in wastewater-associated *H. ligatus* is more consistent with host-specific microbiome restructuring under wastewater exposure. In contrast, *Ceratina* hosts maintained core symbionts, including *Lactobacillus* and *Gilliamella*, which were elevated in wastewater-associated adults. This difference may reflect reduced exposure to contaminated soil due to their wood cavity-nesting life history, as well as the persistence of established *Ceratina*-associated core taxa under wastewater-associated conditions.

Furthermore, other *Ceratina* core bacteria such as *Acinetobacter* exhibit broad ecological tolerance, occurring in both floral environments and wastewater systems (30–33), which may facilitate their persistence in wastewater-associated *Ceratina*. Consistent with this broad ecological distribution (30–33), we qualitatively observed greater floral resource availability at wastewater-associated sites, which could provide additional opportunities for the persistence of these taxa; however, floral availability was not rigorously quantified. Although wild bees' core gut microbiome functional studies remained sparse, in managed bees, reductions in core gut-associated taxa are often linked to impaired digestive or immune-associated functions (16, 18) but comparable functional evidence remains limited for solitary wild bees. As we did not characterize wastewater chemistry or effluent microbiomes at our sites, any mechanistic interpretation of wastewater-associated taxa remains provisional and inferred from other systems.

Although *Ceratina* adults, larvae, and pollen provision microbial communities differed significantly across site types (Fig. 2; Fig. S2.2 to S2.4), key microbial taxa showed stage-specific responses to wastewater exposure. In *Ceratina* adults, core taxa including *Lactobacillus* and *Gilliamella* (34) were more abundant under wastewater exposure, reinforcing the persistence of core taxa despite environmental disturbance. However, this increase likely reflects more than stability alone. One possibility is compensatory proliferation, in which tolerant core symbionts expand after disturbance and help maintain microbiome stability. To further evaluate this pattern, we used baseline prevalence in non-wastewater samples as a simple measure of taxon commonness or "core-ness" and compared it with wastewater-associated abundance change. This analysis (Fig. S10 and S11) did not support a broad increase of highly prevalent genera across hosts and life stages in *Ceratina*; rather, highly prevalent genera tended to decline in wastewater-associated *H. ligatus* adults, while the focal core-associated genera *Lactobacillus* and *Gilliamella* increased in wastewater-associated *Ceratina* adults. Thus, we interpret the *Ceratina* adult pattern as possible maintenance or enrichment of specific core taxa, rather than direct evidence of generalized compensatory proliferation. In contrast, the decline of these same taxa in *H. ligatus* may indicate niche release, where reduction of bee-associated microbes creates ecological space for *Enterococcus* to increase.

These opposing patterns also suggest that differences in host physiology, diet, and nesting-related exposure shape how wastewater-associated disturbance is expressed across hosts. Although both bee taxa forage on flowers, *Ceratina* adults may be buffered by wood cavity-nesting, whereas ground-nesting *H. ligatus* experiences greater

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contact with contaminated substrates. Notably, opposing trends across life stages, where *Lactobacillus* remains elevated but *Gilliamella* decreases in larvae, and both taxa decrease in pollen provisions while increasing in adults under wastewater exposure, further highlight the importance of exposure pathways, as immature stages and pollen provisions remain directly associated with environmentally derived substrates.

Native bees acquire their gut microbiomes from the environment (17), including flowers, soil, and nest materials, which differ substantially in chemical composition, pH, and temperature (35, 36), creating heterogeneous microbial habitats. Spatial autocorrelation analyses revealed significant negative autocorrelation in *H. ligatus* adults and *Ceratina* pollen provisions, indicating that nearby samples were more compositionally dissimilar than expected by chance. This pattern is consistent with our differential abundance results, in which these same sample types showed the strongest wastewater-associated shifts in dominant taxa. In *H. ligatus*, wastewater exposure was associated with enrichment of *Enterococcus* and reduction of common bee-associated taxa, whereas *Ceratina* pollen provisions showed declines in key core taxa under wastewater exposure. Together, these results suggest that wastewater-associated filtering may act heterogeneously within-sites, producing patchy microbial restructuring rather than a uniform site-level response. This interpretation may also be influenced by our sampling design, as inclusion of multiple individuals from various nests or sites could amplify local contrasts among nearby samples. As a result, site-level effects should be interpreted as broad environmental patterns that contain substantial within-site heterogeneity, rather than as a simple spatial gradient. In contrast, the positive autocorrelation observed in *Ceratina* larvae likely reflects host relatedness coupling with shared nest environments, where related individuals develop within a more uniform microbial context.

The contrasting association between wastewater exposure and *Wolbachia* abundance in *H. ligatus* vs *Ceratina* exemplifies the potential influence of nest ecology with *H. ligatus* nesting in the ground encountering potential contaminated soil directly, while *Ceratina* nesting in wood stems may avoid interacting with potential contaminated soil. Furthermore, *Wolbachia*, an endosymbiotic bacterium, can hijack host physiological function and drive its own proliferation by manipulating sex ratios to favor infected females (37). While various strains and abundances of *Wolbachia* were found in many solitary bee species (32, 35, 36), they were not part of the *Ceratina* core gut microbiome. Notably, *Wolbachia* remained stable in *Ceratina* across wastewater vs non-wastewater sites but were highly responsive in *H. ligatus*, which is supported by a major difference in *H. ligatus* $R^2 = 0.25$ vs *Ceratina* $R^2 = 0.0135$ even though both were statistically significant $P < 0.05$.

Given the known sensitivity of *Wolbachia* to antibiotics (38), its decline in *H. ligatus*, a ground-nesting bee, may be associated with wastewater-related contaminants in soil. However, with only 16S data, we cannot assess Wolbachia strain-specific differences between *H. ligatus* and *Ceratina*, and host-specific factors such as nesting ecology and exposure pathways likely also contribute to this pattern. These findings highlight divergent microbial responses based on host nesting ecology, where the extent of exposure to contaminated materials appears to be the key risk factor: *Ceratina* nests in wood cavities, while *H. ligatus* directly contacts contaminated soil.

The movement of effluent-derived contaminants through the ecosystem results in a concentration gradient between waterways and other discharge sites, surrounding soils and groundwater, and plant materials. Even though antibiotics and effluent contaminants can translocate into plant roots, stems, and leaves (34, 39, 40), their accumulation in floral tissues is minimal or undetectable (41). This limited floral contamination likely reduces effluent contaminant exposure in *Ceratina*, whose nesting and foraging behaviors minimize direct contact with contaminated substrates. In contrast, *H. ligatus* nests in sandy-clay mixes, firm silt, and hardened clay (19) that are rich in Al^{3+}, Ca^{2+}, and Fe^{3+}. These soils have high cation exchange capacity, allowing strong binding of antibiotics (42). Additionally, in wastewater-impacted habitats, continuous input of effluent contaminants further enriches soils with substances that have strong antibiotic

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binding capacity, leading to chronic exposure for ground-nesting bees. This chemical exposure may favor antibiotic-resistant taxa like *Enterococcus*, which was dominant in *H. ligatus* guts but rare in soil, further supporting a chemically driven selection process.

In *Ceratina* adults, the wastewater-associated difference was statistically significant but explained only a small proportion of community variation (R^2 = 0.0135), suggesting a modest compositional shift rather than strong evidence of major microbiome disruption. Several of the taxa that changed significantly in association with wastewater exposure have previously been identified as members of the core *Ceratina* microbiome (32). *Ceratina* larvae and pollen provisions also showed statistically significant differences in microbial community composition between wastewater and non-wastewater sites. *Ceratina* larvae core gut microbiome showed mostly similar patterns as in *Ceratina* adults affected by wastewater with a significant increase in abundance of *Lactobacillus* and *Acinetobacter*. On the contrary, *Gilliamella* was significantly reduced in wastewater-exposed *Ceratina* larvae (Fig. 3C) while significantly increased in wastewater-exposed *Ceratina* adults. In comparison, *Ceratina* pollen provisions exposed to wastewater showed a significant reduction in 8 taxa of its 13 core microbial taxa including *Lactobacillus, Gilliamella*, *Rosenbergiella*, *Pseudomonas*, *Snodgrassella*, *Commensalibacter*, *Acinetobacter*, *Mythylobacterium* (32) (Fig. 3D), suggesting host physiology during larval development may modulate microbial colonization (22). These results suggest that while *Ceratina* adults may avoid direct environmental impact, the microbial communities associated with larvae and pollen provisions are more susceptible to wastewater exposure, with some core taxa demonstrating stability while others show significant decline. Notably, while wastewater exposure was significantly associated with microbial shifts ($P < 0.05$), site-level differences accounted for a relatively minor portion of the total community variance ($R^2 \approx 0.01$), leaving the broader ecological and functional implications of these modest compositional changes yet to be determined.

The differences in *Ceratina* adults, larvae, and pollen provision R^2 values (Table S2) from our PERMANOVA showed different susceptibilities across life stages of *Ceratina* bees. *Ceratina* pollen provisions and larvae were more affected by wastewater pollution than adults. This heightened vulnerability could be due to larvae's direct exposure to contaminated pollen, which serves as their primary food source during development as shown by the declining abundance of one of its core taxa *Rosenbergiella*. *Ceratina* adults, on the other hand, demonstrated greater stability, possibly due to more developed detoxification systems, a broader foraging range, and/or the stability of key microbial taxa such as *Lactobacillus* across wastewater and non-wastewater habitats (Fig. 3). These differences emphasize the need to consider the entire life cycle when evaluating the ecological risks posed by contaminants, as microbes can affect development in some (43), but not all, bees (44).

Although we observed no significant differences in microbial communities in flower surface washes and soil from wastewater vs non-wastewater sites, it is important to note that similar taxonomic profiles do not necessarily imply functional redundancy. Even though soil samples were rarefied at a higher sequencing depth than bee and flower samples, the soil alpha rarefaction curves did not plateau (Fig. S1.6), suggesting that high soil microbial diversity and/or platform-specific sensitivity may have limited our ability to detect wastewater-associated taxonomic shifts. Importantly, we did not make direct statistical comparisons between soil microbiomes and bee or flower microbiomes; wastewater effects were evaluated within each sample type, with soil comparisons made only between wastewater and non-wastewater soils generated using the same Nanopore workflow, and bee and flower comparisons made within the Illumina data set. Soil and flowers from wastewater and non-wastewater environments may host microbial communities with overlapping taxonomic identities yet diverge in their gene expression (45). Thus, functional shifts may still occur in the absence of clear taxonomic differentiation. Also, our surface layer soil sampling effort may have limited our ability to assess effluent contaminants' impact on soil microbes. Future studies incorporating soil from greater depth and metatranscriptomic sequencing may provide higher taxonomic

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resolution and, more importantly, uncover functional shifts. This approach could offer better insight into how wastewater influences not only microbial community composition but also potential functional consequences for native bees and surrounding ecosystems.

While the field of ecotoxicology has consistently demonstrated that wastewater effluent is impacting the microbial communities of nearby fauna, findings are often focused on organisms that lack distinctive niche-specific susceptibility between their own species (6, 46). For instance, aquatic-associated insects like mosquitoes are an insightful system in assessing wastewater effluent impact (3) with implication in disease vectors, but their ecological niche which determines their exposure to effluent contamination is similar between species. In contrast, our research leverages the distinct nesting habitats (soil-nesting vs wood cavity-nesting) of two related bee taxa, to associate the intensity of exposure to wastewater-contaminated substrates with the extent of wastewater-associated disturbance to the host microbiome. This pioneers the exploration of ecological niche-specific vulnerability to wastewater effluent exposure and its implications for native bees.

A key limitation of this study is that wastewater exposure was inferred from site position relative to effluent discharge rather than measured chemically or microbiologically. Future work integrating effluent sequencing and contaminant profiling will be critical for establishing mechanistic links between wastewater-associated sites and bee-associated microbiomes. Another limitation is that we examined only one ground-nesting species (*H. ligatus*) and one cavity-nesting species (*Ceratina* spp.), so species identity and nesting ecology cannot be fully separated. Direct functional tests will be needed to determine whether the compositional shifts observed here translate into altered immunity, nutrition, or fitness.

Conclusion

Research in municipal wastewater effluent has primarily focused on the effects of effluent contaminants on specific study systems including soil, water, plants, and aquatic-associated animals ranging from mosquitoes to fishes. Here, we offer a new perspective. By leveraging niche-specific susceptibility in native bees, we ask how municipal wastewater effluent impacts the environment based on animal ecological niche. We present the first investigation into how wastewater effluent affects microbial communities differently in related host species but with distinct exposure to wastewater effluent due to their nesting strategies. Our results show that ground-nesting bees, particularly *Halictus ligatus*, are severely impacted, while cavity-nesting *Ceratina* exhibit significant effects in larvae and pollen provisions with a significant decline of one of its core taxa as well as variable responses across developmental stages. The bacterial taxa driving these changes differ between habitats, with wastewater-exposed environments dominated by wastewater-associated bacteria, and non-wastewater sites characterized by bee- and flower-associated bacteria. These findings highlight the potential for species-specific host ecology to moderate exposure to and microbial effects of a ubiquitous anthropogenic disturbance. Consideration of these differences could help prioritize at-risk taxa in ecological risk assessments and conservation efforts.

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DATA AVAILABILITY

The 16S rRNA gene amplicon sequencing data and associated metadata generated in this study are available through the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1543218.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental figures (AEM01381-26-s0001.docx). Fig. S1 to S11.
Supplemental material (AEM01381-26-s0002.docx). Supplemental methods and Tables S1 to S4.

REFERENCES

1. Zhang T, Li B. 2011. Occurrence, transformation, and fate of antibiotics in municipal wastewater treatment plants. Crit Rev Environ Sci Technol 41:951–998. https://doi.org/10.1080/10643380903392692
2. Hedgespeth ML, Sapozhnikova Y, Pennington P, Clum A, Fairey A, Wirth E. 2012. Pharmaceuticals and personal care products (PPCPs) in treated wastewater discharges into Charleston Harbor, South Carolina. Sci Total Environ 437:1–9. https://doi.org/10.1016/j.scitotenv.2012.07.076

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3. Pennington MJ, Prager SM, Walton WE, Trumble JT. 2016. Culex quinquefasciatus larval microbiomes vary with instar and exposure to common wastewater contaminants. Sci Rep 6:21969. https://doi.org/10.1038/srep21969
4. Millar EN, Surette MG, Kidd KA. 2022. Altered microbiomes of aquatic macroinvertebrates and riparian spiders downstream of municipal wastewater effluents. Sci Total Environ 809:151156. https://doi.org/10.1016/j.scitotenv.2021.151156
5. Carles L, Wullschleger S, Joss A, Eggen RIL, Schirmer K, Schuwirth N, Stamm C, Tlili A. 2022. Wastewater microorganisms impact microbial diversity and important ecological functions of stream periphyton. Water Res 225:119119. https://doi.org/10.1016/j.watres.2022.119119
6. Mills M, Mollenkopf D, Wittum T, Sullivan MP, Lee J. 2024. One health threat of treated wastewater discharge in urban Ohio rivers: implications for surface water and fish gut microbiome and resistome. Environ Sci Technol 58:13402–13414. https://doi.org/10.1021/acs.est.3c09070
7. Da Silva MF, Tiago I, VerÃssimo A, Boaventura RAR, Nunes OC, Manaia CM. 2006. Antibiotic resistance of enterococci and related bacteria in an urban wastewater treatment plant. FEMS Microbiol Ecol 55:322–329. https://doi.org/10.1111/j.1574-6941.2005.00032.x
8. Chhimwal M, Srivastava RK, Mudgal A, Khan S. 2023. Metagenomic decoding and oxidation pond-based treatment of domestic wastewater using 16 s V3-V4 rRNA sequencing tool. Int J Environ Sci Technol 20:12695–12706. https://doi.org/10.1007/s13762-022-04647-1
9. Jing K, Li Y, Li J, Jiang C, Li Y, Yao C. 2024. Antibiotic biotransformation potential of biofilms in streams receiving treated wastewater effluent: biodegradation mechanism and bacterial community structure. Chemosphere 367:143636. https://doi.org/10.1016/j.chemosphere.2024.143636
10. Takebe F, Hirota K, Nodasaka Y, Yumoto I. 2012. *Brevibacillus nitrificans* sp. nov., a nitrifying bacterium isolated from a microbiological agent for enhancing microbial digestion in sewage treatment tanks. Int J Syst Evol Microbiol 62:2121–2126. https://doi.org/10.1099/ijs.0.032342-0
11. Rebah FB, Tyagi RD, Prévost D, Surampalli RY. 2002. Wastewater sludge as a new medium for rhizobial growth. Water Qual Res J 37:353–370. https://doi.org/10.2166/wqrj.2002.022
12. Richter E, Hecht F, Schnellbacher N, Ternes TA, Wick A, Wode F, Coors A. 2015. Assessing the ecological long-term impact of wastewater irrigation on soil and water based on bioassays and chemical analyses. Water Res 84:33–42. https://doi.org/10.1016/j.watres.2015.07.013
13. Wiśniewska K, Przemieniecki SW, Krawczyk K, Hoffmann A, Piwowarczyk R. 2025. Impact of pollution on microbiological dynamics in the pistil stigmas of *Orobanche lutea* flowers (*Orobanchaceae*). Sci Rep 15:3382. https://doi.org/10.1038/s41598-024-84717-1
14. McFrederick QS, Rehan SM. 2016. Characterization of pollen and bacterial community composition in brood provisions of a small carpenter bee. Mol Ecol 25:2302–2311. https://doi.org/10.1111/mec.13608
15. Vannette RL. 2020. The floral microbiome: plant, pollinator, and microbial perspectives. Annu Rev Ecol Evol Syst 51:363–386. https://doi.org/10.1146/annurev-ecolsys-011720-013401
16. Koch H, Schmid-Hempel P. 2011. Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. Proc Natl Acad Sci USA 108:19288–19292. https://doi.org/10.1073/pnas.1110474108
17. McFREDERICK QS, Wcislo WT, Taylor DR, Ishak HD, Dowd SE, Mueller UG. 2012. Environment or kin: whence do bees obtain acidophilic bacteria? Mol Ecol 21:1754–1768. https://doi.org/10.1111/j.1365-294X.2012.05496.x
18. Rothman JA, Leger L, Kirkwood JS, McFrederick QS. 2019. Cadmium and selenate exposure affects the honey bee microbiome and metabolome, and bee-associated bacteria show potential for bioaccumulation. Appl Environ Microbiol 85:e01411–19. https://doi.org/10.1128/AEM.01411-19
19. Michener CD, Bennett FD. 1977. Geographical variation in nesting biology and social organization of Halictus ligatus. Univ Kans Sci Bull 51:233–260.
20. McIntosh M. 1996. Nest-substrate preferences of the twig-nesters *Ceratina acantha, Ceratina nanula* (Apidae) and *Pemphredon lethifer* (Sphecidae). J Kans Entomol Soc 69:216–231.
21. Greenleaf SS, Williams NM, Winfree R, Kremen C. 2007. Bee foraging ranges and their relationship to body size. Oecologia 153:589–596. https://doi.org/10.1007/s00442-007-0752-9
22. Vuong HQ, McFrederick QS. 2019. Comparative genomics of wild bee and flower isolated lactobacillus reveals potential adaptation to the bee host. Genome Biol Evol 11:2151–2161. https://doi.org/10.1093/gbe/evz136
23. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9
24. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869
25. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219
26. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas J, Passos A, Cournapeau D. 2011. Scikit-learn: machine learning in Python. J Mach Learn Res 12:2825–2830. https://doi.org/10.5555/1953048.2078195
27. Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E. 2025. Vegan: community ecology package (2.7-1)
28. Jiang P, Green SJ, Chlipala GE, Turek FW, Vitaterna MH. 2019. Reproducible changes in the gut microbiome suggest a shift in microbial and host metabolism during spaceflight. Microbiome 7:113. https://doi.org/10.1186/s40168-019-0724-4
29. Fernandes AD, Macklaim JM, Linn TG, Reid G, Gloor GB. 2013. ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. PLoS One 8:e67019. https://doi.org/10.1371/journal.pone.0067019
30. Gaca AO, Lemos JA. 2019. Adaptation to adversity: the intermingling of stress tolerance and pathogenesis in Enterococci. Microbiol Mol Biol Rev 83:e00008-19. https://doi.org/10.1128/MMBR.00008-19
31. Vaughan M, Hoffman Black S. 2007. Enhancing nest sites for native bee crop pollinators. Agroforestry note 34. Xerces society for invertebrate conservation, 4828 SE Hawthorne Boulevard, Portland, OR 97215
32. Graystock P, Rehan SM, McFrederick QS. 2017. Hunting for healthy microbiomes: determining the core microbiomes of Ceratina, Megalopta, and Apis bees and how they associate with microbes in bee collected pollen. Conserv Genet 18:701–711. https://doi.org/10.1007/s10592-017-0937-7
33. Aleklett K, Hart M, Shade A. 2014. The microbial ecology of flowers: an emerging frontier in phyllosphere research. Botany 92:253–266. https://doi.org/10.1139/cjb-2013-0166
34. Pennington MJ, Rothman JA, Dudley SL, Jones MB, McFrederick QS, Gan J, Trumble JT. 2017. Contaminants of emerging concern affect *Trichoplusia ni* growth and development on artificial diets and a key host plant. Proc Natl Acad Sci USA 114:E9923–E9931. https://doi.org/10.1073/pnas.1713385114
35. Martinson VG, Danforth BN, Minckley RL, Rueppell O, Tingek S, Moran NA. 2011. A simple and distinctive microbiota associated with honey bees and bumble bees. Mol Ecol 20:619–628. https://doi.org/10.1111/j.1365-294X.2010.04959.x
36. McFrederick QS, Wcislo WT, Hout MC, Mueller UG. 2014. Host species and developmental stage, but not host social structure, affects bacterial community structure in socially polymorphic bees. FEMS Microbiol Ecol 88:398–406. https://doi.org/10.1111/1574-6941.12302
37. Werren JH, Baldo L, Clark ME. 2008. Wolbachia: master manipulators of invertebrate biology. Nat Rev Microbiol 6:741–751. https://doi.org/10.1038/nrmicro1969
38. Endersby-Harshman NM, Axford JK, Hoffmann AA. 2019. Environmental concentrations of antibiotics may diminish *Wolbachia* infections in *Aedes aegypti* (Diptera: Culicidae). J Med Entomol 56:1078–1086. https://doi.org/10.1093/jme/tjz023
39. Stockwell VO, Duffy B. 2012. Use of antibiotics in plant agriculture. Rev Sci Tech OIE 31:199–210. https://doi.org/10.20506/rst.31.1.2104
40. Zhang C, Xue J, Cheng D, Feng Y, Liu Y, Aly HM, Li Z. 2019. Uptake, translocation and distribution of three veterinary antibiotics in Zea mays L. Environmental Pollution 250:47–57. https://doi.org/10.1016/j.envpol.2019.03.110
41. Pérez DJ, Doucette WJ, Moore MT. 2022. Contaminants of emerging concern (CECs) in Zea mays: uptake, translocation and distribution tissue patterns over the time and its relation with physicochemical properties and plant transpiration rate. Chemosphere 288:132480. https://doi.org/10.1016/j.chemosphere.2021.132480

42. MacKay AA, Canterbury B. 2005. Oxytetracycline sorption to organic matter by metal-bridging. J Environ Qual 34:1964–1971. https://doi.org/10.2134/jeq2005.0014
43. Dharampal PS, Carlson C, Currie CR, Steffan SA. 2019. Pollen-borne microbes shape bee fitness. Proc R Soc B 286:20182894. https://doi.org/10.1098/rspb.2018.2894
44. Brar G, Ngor L, McFrederick QS, Torson AS, Rajamohan A, Rinehart J, Singh P, Bowsher JH. 2025. High abundance of lactobacilli in the gut microbiome of honey bees during winter. Sci Rep 15:7409. https://doi.org/10.1038/s41598-025-90763-0
45. Liu Z, Klümper U, Liu Y, Yang Y, Wei Q, Lin J-G, Gu J-D, Li M. 2019. Metagenomic and metatranscriptomic analyses reveal activity and hosts of antibiotic resistance genes in activated sludge. Environ Int 129:208–220. https://doi.org/10.1016/j.envint.2019.05.036
46. Boukerb AM, Noël C, Quenot E, Cadiou B, Chevé J, Quintric L, Cormier A, Dantan L, Gourmelon M. 2021. Comparative analysis of fecal microbiomes from wild waterbirds to poultry, cattle, pigs, and wastewater treatment plants for a microbial source tracking approach. Front Microbiol 12:697553. https://doi.org/10.3389/fmicb.2021.697553

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