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Linking bacterial diversity to floral identity in the bumble bee pollen basket

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Abstract

Multitrophic interactions are ubiquitous in nature and form the basis of biodiversity. For example, bumble bees visit flowers to collect pollen, on which a variety of bacteria exist. Such bacteria consist of pathogens and mutualists and therefore have consequences for bumble bee colony fitness. However, we still know little about how plant diversity and floral selection by bees translate into the bacterial diversity and composition on the pollen consumed by important pollinators. The aim of this study was to characterize the bacterial and floral alpha and beta diversity from bumble bee corbicula (pollen baskets), identify core communities, and characterize their functional role. We found that bacterial alpha diversity (i.e., the diversity of bacteria determined from the pollen basket of a single bumble bee) was positively correlated with floral pollen alpha diversity (i.e., the diversity of plants from that same pollen basket). Bacterial beta diversity (i.e., bacterial composition) was generally weakly correlated with pollen beta diversity (i.e., floral composition). The abundance of some bacterial genera and pollen families was correlated, specifically *Lactobacillus* and *Acinetobacter* were positively correlated with Asteraceae pollen and negatively correlated with Lamiaceae pollen. The most widespread bacteria (the "core OTU") in bumble bee pollen baskets included both possibly beneficial (*Lactobacillus*) and potentially pathogenic (*Pseudomonas*) taxa, but more core OTU functions were unknown vs. known for bumble bees, illustrating the importance of understanding bee–flower–microbe relationships in natural settings.

KEYWORDS

apidae, asteraceae, bee-flower-microbe interactions, *Bombus*, corbicula, high-throughput DNA sequencing, microbial diversity, plant–pollinator interactions

1 | INTRODUCTION

We are only just beginning to understand the diversity of microbes associated with bee health and reproductive fitness (McFrederick et al., 2017; Steffan et al., 2019). These include viruses, fungi, and bacteria, which compose a wide range of interactions with bees and primarily introduced during foraging and feeding on flowers (Graystock et al., 2017; McArt et al., 2014). Bee–flower–microbe

interactions may have consequences; for example, pathogenic microbes negatively impact bee health (Figueroa et al., 2019; Szabo et al., 2012), or present benefits, such as a healthy gut microbiome that suppresses parasites and other environmental stressors (Klepzig et al., 2009; Mockler et al. 2018). Bees are omnivorous, and incidental consumption of bacteria mixed into pollen and nectar provisions provide essential nutrients for their growth and development (Dharampal et al., 2019; Kwong & Moran, 2016). It is therefore essential to characterize bee–flower–microbe interactions when evaluating which factors influence bee health (Adler et al., 2020; McFrederick et al., 2017).

Microbes (hereafter, this term refers to bacteria only, which are the focus of this study) are ubiquitous in terrestrial environments and interact widely across biotic communities and all trophic levels. Bacteria have many dispersal strategies that depend on other organisms or abiotic forces (e.g., wind). Like many organisms, bees interact with bacteria in a variety of ways, and are host to diverse bacterial assemblages (Engel et al., 2016). Bees interact with bacteria on flowers, and only recently have the bacterial communities they share been characterized (Figueroa et al., 2020; Koch et al., 2013; McArt et al., 2014; McFrederick et al., 2017). This has led to novel questions about how the environment shapes these multitrophic interactions. For example, Donkersley et al. (2018) found that bacterial diversity in bee bread sampled from honey bees (*Apis mellifera*, Family: Apidae) was correlated with land use which varied in floral diversity. In another study, McFrederick and Rehan (2019) used DNA barcoding to identify plant, fungal, and bacterial taxa from pollen provisions of small carpenter bees (*Ceratina*, Family: Apidae) sampled from nests across three climatic regions in Australia. Across the regions, it was found that plant and bacterial alpha and beta diversity co-varied, respectively, illustrating the relationship between pollen and bacterial richness and composition. Plants do host abundant bacterial communities on various structures including flowers (Junker et al., 2011), on pollen (Manirajan et al., 2016) and in nectar (Fridman et al., 2012). The arrival of bacteria and the resultant communities are shaped by complex dispersal, environmental filtering and other community assembly dynamics, and include environmental sources (e.g. the air) (McFrederick et al., 2017), but also insect visitors (Allard et al., 2018; Durrer & Schmid-Hempel, 1994; Frago et al., 2012). In fact, flowers are hotspots for between and among bee species transmission of beneficial and pathogenic bacteria (Graystock et al., 2015).

Bacteria identified from bee provisions are linked to those found within a bee's microbiome (Keller et al., 2020; McFrederick & Rehan, 2016). The health of bees is therefore tied to the availability of such bacteria in their environments (Adler, Barber, et al., 2020). *Lactobacillus*, *Snodgrassella*, and *Gilliamella* are different bacterial genera that have been recorded from flowers (Graystock et al., 2017; McFrederick et al., 2017) and some are core microbiome bacterial symbionts in bumble bees (*Bombus*, Family: Apidae) (Martinson et al., 2011). For example, select *Snodgrassella* and *Gilliamella* species have been shown to assist in reducing pathogenic infections in bumble bees (Graystock et al., 2017; Kwong et al., 2014).

While most research in recent years has focused on the diversity of gut bacteria and their beneficial or pathogenic properties in bees, there has been less focus on the external factors that influence interaction with bacterial communities. Factors such as plant diversity and composition in the landscape will ultimately affect the interactions between bacteria and foraging bees. For example, McFrederick and Rehan (2016) found no direct relationship between flower diversity and gut microbial diversity, but rather that pollen composition has an impact on microbe composition. Specific flower species may harbor more pathogenic taxa (Figueroa et al., 2019). Therefore, the presence of certain flowers may influence a bee's gut microbiome, such that for example, less pathogenic (Adler, Barber, et al., 2020) or more beneficial (Cohen et al., 2020) bacteria are encountered.

Bumble bees are social bees with corbicula (pollen baskets) located on their hind legs. These bees are regarded as important pollinators globally for a wide variety of plants due to their physical characteristics that allow them to easily obtain pollen from different flower types and inhabit a wide variety of habitats (Goulson, 2003). Pollination services are valued across the globe and are important to ecosystem functioning, as well as human health and wellbeing (Vanbergen & Initiative, 2013). Recent data have shown declines in bumble bee species and important factors include habitat loss (such as conversion to agriculture), pathogens and disease (Cameron et al., 2011).

Bumble bees carry gut bacteria that protect against pathogens (Koch & Schmid-Hempel, 2011). Evidence indicates that anti-pathogenic (beneficial) bacteria may reduce microbiome alpha diversity (McFrederick et al., 2014). In honey bees, Graystock et al. (2017) found that the gut and pollen basket varied in bacterial composition. Therefore, analysis of the bacteria associated with the pollen basket will provide a more accurate sense of the direct relationship to the floral environment.

In this study, we characterize the bacterial and plant communities from bumble bee pollen baskets. We specifically tested three hypotheses: first, that the alpha diversity of bacterial communities (i.e., the diversity of bacteria in the pollen basket collected by a bumble bee) is positively correlated with the pollen alpha diversity (i.e., the diversity of plant pollen identified from pollen baskets). Second, the beta diversity of bacteria communities (i.e., bacterial composition) is positively correlated with the pollen beta diversity (i.e., pollen composition). Third, with respect to abundance, the core bacteria (i.e., bacterial genera that occur in over 80% of samples) in pollen baskets co-occur with pollen families.

2 | MATERIALS AND METHODS

2.1 | Field sampling

Bumble bees with visible pollen baskets were collected in peri-urban meadows at the Rouge National Urban Park (RNUP) (43.8190°N,

79.1710°W) in June and September 2017 while conducting wild bee surveys for a multi-year study. RNUP is located at the northeast corner of Toronto, which is Canada's most populous city. While the park contains agricultural and industrial activity, there are many semi-naturalized open meadows where restoration has occurred for over the last 20+ years. The park contains over a quarter of the native flora in the region (Wilson, 2012), including many naturalized and native flowering forbs, grasses, and shrubs, but also significant and spreading invasive species.

Field sampling was conducted in eight meadow sites, each of which were approximately 50 × 50 m and were located between 155 to 4680 m apart (mean 2510 m ± 1460 m *SD*). In July and August, bees were netted by two surveyors every 7–10 days at each site for 30 min, until 100 bumble bees having large pollen baskets were caught. Each sampling event occurred on clear, non-windy days between 9:00 to 18:00 EDT. Collected bees were identified to five species (*B. impatiens*, *B. bimaculatus*, *B. griseocollis*, *B. rufocinctus*, *B. borealis*) and curated in the MacIvor lab collections at the University of Toronto Scarborough.

2.2 | Bacterial DNA extraction, amplification and sequencing

For each bumble bee sampled, the pollen basket from one hindleg was removed using sterilized forceps. DNA from the pollen samples was extracted following the QIAGEN DNeasy PowerSoil Kit protocol (Qiagen). Following extractions, samples were tested for DNA yield using a NanoDrop 1000 Spectrophotometer. Six samples that were below the minimum DNA yield were omitted. We did not include positive (e.g., mock bacterial communities) or negative (e.g., microbiota of fresh bumble bees having not yet visited a flower) controls. We did not know which bacterial species would be present in the pollen baskets, and we did not have access to any colonies or newly emerged bumblebees to obtain samples of their microbiota.

For each pollen basket DNA extract, the V4 region of the 16S rRNA gene was amplified using the 515F (5′-GTGCCAGC MGCCGCGGTAA-3′) and the 806R (5′-GGACTACHVGGGTWTC TAAT-3′) primers. Sequencing of amplicons was performed using the Illumina HiSeq 2500 platform (Illumina). Sequence reads were quality trimmed using Mothur v1.35.1 (http://mothur.org) (Corby-Harris et al., 2014) and then merged using FLASH v1.2.11 (https://ccb.jhu.edu/software/FLASH) (Magoč & Salzberg, 2011). The UCHIME algorithm (https://drive5.com/usearch/manual/uchime_algo.html) (Edgar et al., 2011) implemented in USEARCH was used to identify and remove chimeras. Following this, USEARCH was used to cluster quality reads at 97% with UPARSE (http://www.drive5.com/usearch/manual/uparseotu_algo.html) into operational taxonomic units (OTUs). Singleton OTU's were removed. 16S Greengenes sequences (http://greengenes.secondgenome.com) were referenced to assign OTUs to a genus. This was performed using the RDP Classifier algorithm (Wang et al., 2007) implemented in the QIIME package (http://qiime.org).

2.3 | Phylogenetic reconstruction

Multiple sequence alignment of OTU representative sequences was conducted using PyNAST (Caporaso et al., 2010). Following this, phylogenetic reconstruction was performed using maximum likelihood implemented in FastTree 2 (Price et al., 2010).

2.4 | Flower identification from pollen

A sample was taken from each pollen basket sample (described above) and mixed with fuchsin gel (Kearns & Inouye, 1993) on a microscope slide to identify plants (to family). From each sample, 100 pollen grains were counted and identified from a single view plane under a light microscope at 100× magnification and repeated three times for a total of 300 pollen grains per pollen sample (MacIvor et al., 2014). Pollen families were determined using a pollen synoptic collection curated in the MacIvor lab of over 200 plant species found in the region.

2.5 | Pollen and bacterial alpha and beta diversity

All calculations of community composition and diversity were performed using R v3.6.2 (R Core Team, 2019). Plant and bacterial alpha diversity were measured using Hill numbers (${}^{q}_{D}$), and Hill numbers adapted to measure phylogenetic diversity (${}^{q}D(T)$) (Chao et al., 2010). Taxonomic richness ($S = {}^{0}D$), Gini-Simpson index ($GS = {}^{2}D$), Faith's phylogenetic diversity ($PD = {}^{0}D(T)$), and Rao's Q ($Q = {}^{2}D(T)$) were used. Taxonomic indices were computed using the *vegan* package (Oksanen et al., 2018) and phylogenetic indices were computed using the *iNextPD* package (Hsieh et al., 2016). We wanted to limit conservatism in the multiple comparison test, and so we chose ${}^{2}D$ instead of ${}^{1}D$. ${}^{1}D$ does not put weight on rare nor abundant species, whereas ${}^{2}D$ puts more weight on relative abundances and therefore would provide better inference when paired with ${}^{0}D$ than ${}^{1}D$. Abundance data were rarified and interpolated or extrapolated to 90% sampling coverage to account for bias in alpha diversity estimates due to unequal sequencing reads among samples. We calculated 90% sampling coverage point estimates for taxonomic alpha diversity using the *iNEXT* package (Hsieh & Chao, 2017), and the *iNextPD* package for phylogenetic alpha diversity. One specimen was removed from analysis because computed bacterial alpha diversity values were an outlier, and therefore 92 *Bombus* individuals remained for further processing (*B. impatiens* = 73, *B. bimaculatus* = 9, *B. griseocollis* = 7, *B. rufocinctus* = 2, *B. borealis* = 1). Rarefaction/extrapolation curves for bacterial taxonomic alpha diversity and phylogenetic alpha diversity are presented in Figures S1 and S2, respectively.

Plant and bacterial taxonomic beta diversity were measured as Sørensen dissimilarity (D_S) and Bray–Curtis dissimilarity (D_B) using the *vegan* package. Bacterial phylogenetic beta diversity was measured as *UniFrac* and *weighted-UniFrac* using the *phyloseq* package (McMurdie & Holmes, 2013). Community data matrices were

Hellinger transformed prior to calculation of distance matrices. All bacterial diversity calculations were also completed using a community data matrix consisting of the core OTU (e.g., occurred in at least 80% of samples).

All bumble bee, pollen, and bacterial OTU data used in our analyses are available on FigShare (Sookhan et al., 2020, https://doi.org/10.6084/m9.figshare.13208234).

2.6 | Statistical analysis

All statistical analyses were performed using R (R Core Team, 2019). Linear mixed models (LMMs) were constructed to test the magnitude and significance of the effect of pollen and bacterial alpha diversity. LMMs were implemented using the *lme4* package (Bates et al., 2015). Random intercepts included the month of sampling, site identity, and bee species identity; the latter fit as a random intercept instead of a fixed effect because of unequal numbers of individuals between species. Response and independent variables were scaled to have a mean of zero and a standard deviation of one so that model estimates represented standardized regression coefficients. Additionally, marginal and conditional R^2 (R^2_m and R^2_c) developed by Nakagawa and Schielzeth (2013) were used to assess the proportion of variance explained by pollen alpha diversity (R^2_m), and pollen alpha diversity and the random terms (R^2_c). Eight additional bee specimens were removed for alpha diversity analyses. For these specimens, 90% sampling coverage point estimation was biased due to extrapolation surpassing double the number of sequence reads (Hsieh & Chao, 2017). Therefore, 85 bumble bee individuals remained (*B. impatiens* = 69, *B. bimaculatus* = 9, *B. griseocollis* = 4, *B. rufocinctus* = 2, *B. borealis* = 1).

Partial distance-based redundancy analysis (partial db-RDA) was used to assess the significance of the correlation between pollen and bacterial beta diversity. Partial db-RDAs were constructed using the *vegan* package. Ordinations from pollen beta diversity metrics were calculated using principal coordinates analysis (PCoA). Pollen PCoA axes were used to constrain ordinations on bacterial distance matrices while accounting for the effect of bee species identity, month of sampling and site identity. Backwards stepwise elimination was used to optimize constrained ordinations using the *vegan* package, followed by variance partitioning on the optimized model. This was done to calculate the amount of variance in bacterial beta diversity explained solely by pollen beta diversity using the marginal correlation between pollen and bacterial beta diversity (R^2_{adj}). The overall significance of the optimized constrained ordination was determined using a permutation test (999 permutations) developed by Legendre et al. (2011).

Kendall rank correlations were used to assess the magnitude and significance of co-occurrence between pollen and core bacterial OTUs. Alpha diversity, beta diversity, and co-occurrence analyses were completed with pollen resolved to family. Bacteria were resolved to OTU (total or core OTU) for alpha and beta diversity analysis, and to the genus level for co-occurrence analysis. Core OTUs that were not resolved to the genus level were removed for co-occurrence analysis. To correct for multiple comparisons, FDR adjusted *p*-values were calculated (Benjamini & Hochberg, 1995) and a false discovery rate of 5% was used as a threshold. Multiple comparison correction was calculated using the *qvalue* package (Stoney et al., 2019).

TABLE 1 Relative occupancy and abundance of pollen families from bumble bee pollen baskets used in analysis. Relative abundances are pooled across samples or averaged across samples for each pollen family

Family	Occupancy	Abundance (pool)	Abundance (mean)
Fabaceae	82.609	58.793	58.721
Asteraceae	50.000	18.641	18.659
Oxalidaceae	65.217	11.338	11.171
Ranunculaceae	9.783	2.969	2.924
Balsaminaceae	20.652	2.737	2.716
Lamiaceae	33.696	2.141	2.116
Apiaceae	14.130	1.968	2.006
Brassicaceae	8.696	1.048	1.329
Geraniaceae	2.174	0.342	0.337
Caprifoliaceae	2.174	0.018	0.018
Malvaceae	1.087	0.004	0.004

3 | RESULTS

3.1 | Plant pollen

A total of 11 plant families (see Table 1) were identified from the pollen baskets of the bumble bees. Dominant families include Fabaceae (common examples in RNUP include vetch and clover), Asteraceae (coneflowers, goldenrod, aster), and Oxalidaceae (wood sorrel) which were widespread across specimens and locally abundant. Balsaminaceae (jewelweed), Lamiaceae (mints, bee balm), and Apiaceae (goutweed, wild carrot and parsnip) were common across samples but locally sparse. The remaining five families were rare across bee individuals and locally sparse (Table 1).

3.2 | Bacteria

Across the 92 samples considered in the analysis, there were a total of 795,367 sequence reads amounting to 3,992 OTUs; of these, a total of 17 core OTU were identified. This accounted for 569,278 reads which was 71.574% of all sequence reads. 10 of the 17 core OTU were assigned to a genus which resulted in the identification of nine core bacterial genera (see Table 2). *Lactobacillus* was the dominant genus, followed by *Kingella*, *Pantoea*, *Acinetobacter*, and then *Pseudomonas*. The four remaining genera accounted for <2% of core OTU sequence reads.

TABLE 2 Bacterial core OTUs identified to genus. Relative abundances are pooled across samples or averaged across samples for each bacterial genus

Genus	Possible role in bumble bees	Occupancy	Abundance (pooled)	Abundance (mean)
Lactobacillus	Fermentation in bee gut, lactic acid production (McFrederick et al., 2017)	100.000	71.167	31.056
Kingella	Antagonistic toward plant pathogenic fungi (Berg & Hallmann, 2006)	97.826	10.838	27.867
Pantoea	Ubiquitous (Walterson & Stavrinides, 2015); blight-inhibitor on flowers and ferments lactose in bee gut (Loncaric et al., 2009)	98.913	7.301	12.889
Acinetobacter	Ubiquitous; inhibits plant pathogens (Liu et al., 2007), symbiont in bee gut inhibits American foulbrood (Evans & Armstrong, 2006)	89.130	6.643	9.043
Pseudomonas	Plant pathogen (Pattemore et al., 2014) and negative effect on bees (Meikle et al., 2012)	89.130	2.431	6.589
Candidatus Phlomobacter	Malformation of fruits (Tanaka et al., 2006); transmitted by insects (Danet et al., 2003)	80.435	0.639	4.676
Sphingomonas	Found in bee gut (Donkersley et al., 2018), but unknown function (Ma et al., 2019)	97.826	0.467	3.826
Agrobacterium	Ubiquitous; No information	81.522	0.266	2.570
Halomonas	Found in bee gut, but unknown function (Raymann et al., 2017)	80.435	0.250	1.484

3.3 | Alpha and beta diversity

Only 1 of 16 correlation tests for alpha diversity was significant which was that pollen family taxonomic richness had a positive effect on total bacterial OTU Gini-Simpson diversity (See Table 3 for a tabular summary). For this correlation, R^2_m was 0.089, and R^2_c was 0.151, and therefore, pollen alpha diversity was important as it accounted for more than half of explained variation. Further, the variance of site identity was 0.229 standard deviations, species identity was 0.111 standard deviations, and the variance of the month factor was 0. Thus, among the random terms, it was found that only the location of sampling and bee species identity were important. Pollen family alpha diversity did not have a significant effect on core bacterial OTU alpha diversity (Table 3).

Across all samples, there were marginal correlations between pollen family and bacterial OTU beta diversity, with 6 of 8 correlation tests determined to be marginally significant for total OTUs (see Table 4 for a tabular summary). At most, pollen family beta diversity explained 1.4% of the variation observed in total bacterial OTU beta diversity. Compared to pollen family beta diversity marginal correlations, species identity and month of sampling were weaker, and site identity was stronger. This trend was also observed for marginal correlations with bacterial core OTU beta diversity. We found 4 of 8 correlation tests were marginal significant between pollen family and bacterial core OTU beta diversity (Table 4). Pollen family Sørensen diversity had a significant marginal correlation with bacterial core OTU Sørensen, Bray–Curtis and Weighted-UniFrac distance. The marginal correlation with total bacterial OTU Bray–Curtis and bacterial core OTU weighted-UniFrac was moderately strong, explaining 10.8% and 15.7% of the variation, respectively. In addition, pollen family Bray–Curtis distance had a significant marginal correlation with bacterial core OTU Sørensen distance.

TABLE 3 Effect of pollen family alpha diversity on bacterial OTU alpha diversity

Bacteria	Pollen	Estimate	Df	*t* value	*p* value	*q* value	Species	Month	Site	R^2_m	R^2_c
a) All OTU											
^0D	^0D	0.233	79.851	2.174	.033	0.106	0.000	0.000	0.083	0.053	0.060
^0D	^2D	0.200	83.000	1.862	.066	0.106	0.000	0.000	0.000	0.040	0.040
^2D	**^0D**	**0.304**	**81.980**	**2.897**	**.005**	**0.039***	**0.111**	**0.000**	**0.229**	**0.089**	**0.151**
^2D	^2D	0.111	82.757	1.020	.310	0.362	0.000	0.000	0.252	0.012	0.073
$^0D(T)$	^0D	0.217	81.659	2.011	.048	0.106	0.000	0.000	0.137	0.046	0.064
$^0D(T)$	^2D	0.204	82.970	1.899	.061	0.106	0.000	0.000	0.105	0.041	0.052
$^2D(T)$	^0D	0.096	77.706	0.856	.395	0.395	0.156	0.222	0.325	0.008	0.170
$^2D(T)$	^2D	0.109	81.670	1.008	.317	0.362	0.000	0.188	0.333	0.011	0.146
b) Core OTU only											
^0D	^0D	0.154	90.000	1.483	.142	0.485	0.000	0.000	0.000	0.024	0.024
^0D	^2D	0.090	90.000	0.856	.394	0.631	0.000	0.000	0.000	0.008	0.008
^2D	^0D	0.192	83.865	1.858	.067	0.485	0.489	0.253	0.000	0.030	0.278
^2D	^2D	0.042	87.667	0.408	.685	0.782	0.491	0.208	0.000	0.001	0.237
$^0D(T)$	^0D	0.139	90.000	1.333	.186	0.485	0.000	0.000	0.000	0.019	0.019
$^0D(T)$	^2D	0.068	90.000	0.647	.519	0.692	0.000	0.000	0.000	0.005	0.005
$^2D(T)$	^0D	0.128	87.409	1.176	.243	0.485	0.351	0.381	0.360	0.013	0.320
$^2D(T)$	^2D	0.015	85.983	0.147	.883	0.883	0.284	0.256	0.000	0.000	0.133

Note: Alpha diversity was measured using the first and third taxonomic and phylogenetic Hill numbers. "Estimate" is the standardized regression coefficients estimated from linear mixed models. Significant effects are bolded and denoted with an asterisk ($q < 0.05$). "Species," "Month," and "Site" are random terms estimated in standard deviations. "R^2_m" and "R^2_c" are the marginal and conditional R^2 from the linear mixed models.

3.4 | Co-occurrence

Across the correlations between pollen families and bacterial genera, 6.1% were significant (see Table 5). *Acinetobacter and Lactobacillus* were positively correlated with Asteraceae, and negatively correlated with Lamiaceae (Figure 1; significant Kendall correlations given in Table 5). In addition, *Sphingomonas* was positively correlated with Asteraceae and Balsaminaceae.

4 | DISCUSSION

Flower-associated microbial communities are shaped by dynamic and complex environmental factors that include bee pollinator-mediated dispersal (Durrer & Schmid-Hempel, 1994; Keller et al., 2020). As technologies and tools to sequence the DNA of these microscopic communities expand, its utility among pollination ecologists to ask fundamental questions about bee–flower–microbe relationships is growing. In this study, we tested three hypotheses on the relationship between flower-associated microbial communities represented in the pollen baskets of bumble bees. First, pollen alpha diversity was correlated with a single measure of total bacterial OTU alpha diversity and was not correlated with widespread bacterial OTU ("core OTU") alpha diversity. Therefore, weak support was found for the first hypothesis, but not when core OTUs were considered. Second, pollen beta diversity was weakly correlated with total and core OTU beta diversity; and so, moderate support was found for the second hypothesis. These findings provide evidence that the flowering plant families visited impact the taxonomic and phylogenetic composition of core bacterial OTU communities. Third, the abundance of some core bacterial genera and pollen families was correlated. Therefore, support was found for the final hypothesis that multiple core bacterial genera were positively correlated with Asteraceae and negatively correlated with Lamiaceae. This evidence suggests that flowering plant families vary in the extent to which they act as reservoirs of core bacterial genera that are transferred to bumble bees.

Our results show that bumble bees visiting more different flowers in a single foraging trip lead to the acquisition of more diverse bacterial communities. For social bumble bee workers whose primary role is to provide food for the colony, there may be a benefit to visiting multiple flower types in a single foraging trip from the perspective of diversifying the bacteria in pollen baskets brought back to the colony. Predominantly visiting one flower type might lead to not only a higher probability of nutritional deficiency or pollen toxicity, but also pathogens or missing key beneficial bacteria, since there is evidence certain taxa are associated with certain floral traits (Adler et al., 2020) and species (Figueroa et al., 2019). Although the bee–bacteria mutualisms present within a bees' gut microbiota will predominantly arise from workers exchanging microbes by handling nest provisions and other within-colony interactions via vertical transmission, more work is needed to resolve the relative contribution of environmentally sourced bacteria on the health of individual bumble bees in a colony, as well as solitary wild bees (Voulgari-Kokota et al., 2019).

TABLE 4 Correlation between a) bacterial OTU beta diversity and b) core bacterial OTU beta diversity with pollen family

Bacteria	Pollen	Estimate	*df*	F stat	*p* value	*q* value	Species	Month	Site
a) All OTU									
Sorensen	**Sorensen**	**0.008**	**1, 76**	**1.718**	**.004**	**0.008***	**0.000**	**0.004**	**0.082**
	Bray Curtis	**0.009**	**1, 76**	**1.774**	**.003**	**0.008***	**0.000**	**0.010**	**0.084**
Bray Curtis	**Sorensen**	**0.010**	**1, 76**	**1.848**	**.002**	**0.008***	**0.006**	**0.004**	**0.047**
	Bray Curtis	**0.010**	**1, 76**	**1.831**	**.006**	**0.010***	**0.006**	**0.013**	**0.049**
UniFrac	**Sorensen**	**0.005**	**1, 76**	**1.448**	**.023**	**0.031***	**−0.001**	**0.007**	**0.090**
	Bray Curtis	**0.014**	**2, 75**	**1.629**	**.001**	**0.008***	**−0.001**	**0.012**	**0.096**
W UniFrac	Sorensen	0.009	1, 76	1.725	.068	0.078	0.006	0.007	0.082
	Bray Curtis	0.007	1, 76	1.568	.107	0.107	0.005	0.026	0.085
b) Core OTU only									
Sorensen	Sorensen	0.007	1, 76	1.364	.192	0.192	−0.022	−0.006	0.089
	Bray Curtis	**0.108**	**3, 74**	**2.743**	**.001**	**0.004***	**−0.011**	**0.009**	**0.074**
Bray Curtis	**Sorensen**	**0.024**	**1, 76**	**2.549**	**.017**	**0.034***	**0.014**	**−0.009**	**0.026**
	Bray Curtis	**0.034**	**2, 75**	**2.117**	**.008**	**0.021***	**0.008**	**0.009**	**0.029**
UniFrac	Sorensen	0.011	1, 76	1.575	.157	0.179	−0.016	0.001	0.031
	Bray Curtis	**0.157**	**4, 73**	**3.210**	**.001**	**0.004***	**0.005**	**0.007**	**0.030**
W UniFrac	Sorensen	0.019	1, 76	2.283	.057	0.091	0.011	−0.009	0.027
	Bray Curtis	0.009	1, 76	1.610	.133	0.178	0.009	0.019	0.033

Note: Beta diversity was measured using the Sorensen, Bray Curtis, UniFrac and Weighted-UniFrac indices. "Estimate" is the marginal correlations from partial RDA. Significant effects are bolded and denoted with an asterisk ($q < 0.05$). "Species," "Month," and "Site" are the marginal correlations of terms accounted for before estimating the correlation between pollen and bacterial OTU beta diversity.

TABLE 5 Kendall correlation between pollen families and bacterial genera. Only significant effects are displayed ($p < .05$)

	Bacteria								
	ACI	AGR	CAP	HAL	KIN	LAC	PAN	PSE	SPH
Pollen									
Apiaceae									
Asteraceae	0.267					0.266			0.261
Balsaminaceae									0.252
Brassicaceae									
Caprifoliaceae									
Fabaceae									
Geraniaceae									
Lamiaceae	−0.291					−0.345			
Malvaceae									
Oxalidaceae									
Ranunculaceae									

Abbreviations: ACI, *Acinetobacter*; AGR, *Agrobacterium*; CAP, *Candidatus Phlomobacter*; HAL, *Halomonas*; KIN, *Kingella*; LAC, *Lactobacillus*; PAN, *Pantoea*; PSE, *Pseudomonas*; SPH, *Sphingomonas*.

4.1 | Transmission and functional role of core bacteria

Contrary to our expectations, well-known taxa from studies of bumble bee gut microbiota were not included in the core bacteria communities, such as *Snodgrassella* and *Gilliamella*. The primary reservoirs for both these bacteria types are through social activity within the colony and transmission among nest mates; however, Koch et al. (2013) posited that *Gilliamella* could be transferred horizontally on flowers. We did not find any evidence of this pathway, despite finding other very common gut microbiota (e.g., *Lactobacillus*) in the pollen baskets that have had pathways (via flowers) confirmed (McFrederick et al., 2012). Other bacteria genera that were identified in our study

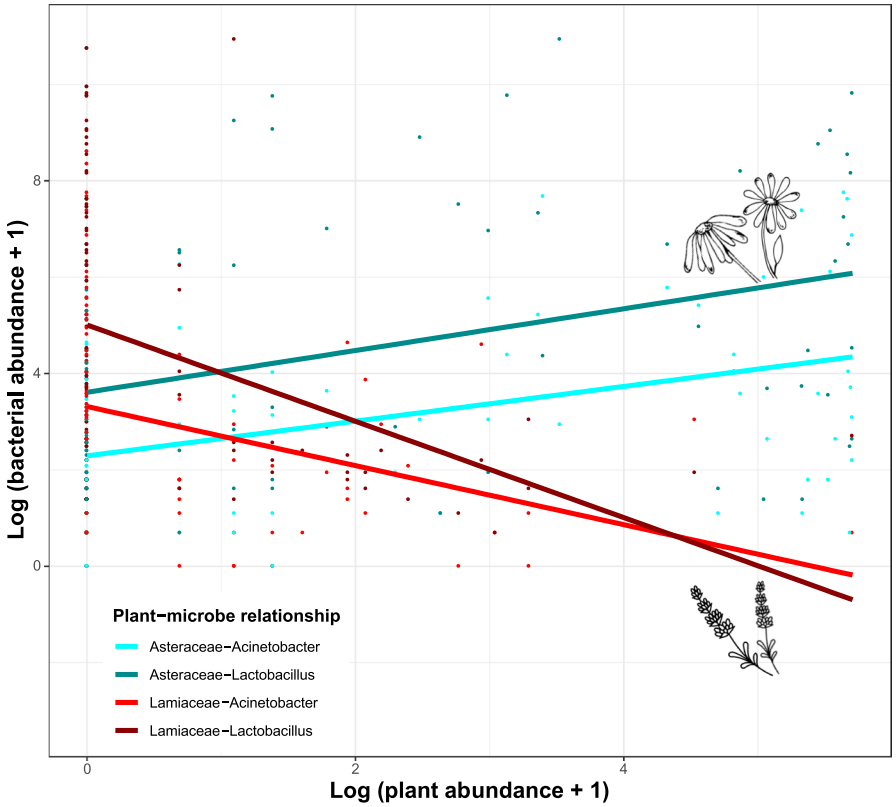

FIGURE 1 The correlation between presumed beneficial bacteria (*Lactobacillus* and *Acinetobacter*) and flowering plant types (Asteraceae and Lamiaceae) commonly found in bumble bee pollen baskets

are highly speciose and ubiquitous in the environment having host associations across plants, insects, and even humans (e.g., *Pantoea*; Walterson & Stavrinides, 2015).

Although much of the work detailing beneficial interactions with bacteria in bumble bees results from study of *Snodgrassella* and *Gilliamella*, core bacteria having potentially beneficial functions for bumble bees were well represented in our sample, including *Acinetobacter* and *Lactobacillus*. These genera include species that are known symbionts of the bee gut microbiome that are obtained during foraging and feeding (McFrederick et al., 2012, 2017). *Acinetobacter* is particularly well-known from honey bee larvae in which it inhibits the growth of *Paenibacillus larvae*, the cause of American foulbrood in honey bees (Evans & Armstrong, 2006) and associated with brood mortality in the red mason bee, *Osmia bicornis* (Voulgari-Kokota et al., 2020). *Lactobacillus* are ubiquitous members of the bee gut microbiome (Praet et al., 2018) having a functional role in fermentation and the production of lactic acid (McFrederick et al., 2018), which provides additional protection against the *Paenibacillus larvae* (Forsgren et al., 2010). *Lactobacillus* also produces hydrogen peroxide, which is known to inhibit fungal growth and pathogens (Arredondo et al., 2018).

Bees acquire pathogenic bacteria during floral visits and feeding (Adler et al., 2018; Koch et al., 2017; McArt et al., 2014). Pathogens can also be deposited onto flowers (Figueroa et al., 2019; Pattemore et al., 2014), and transferred within and between bee species (Graystock et al., 2015; Huang et al., 1986). One core bacteria genus recorded in our study was *Pseudomonas*, within which some species are pathogens of bees and others of plants, and that are transmitted between foraging bees and flowers (Meikle et al., 2012). For example, *Pseudomonas apiseptica* picked up from flowers is known to cause septicemia-related death in bumble bees (Cankaya & Kaftanoglu, 2006) and honey bees (Bailey, 1965). As well, Donati et al. (2018) demonstrated experimentally that bumble bees transferred the plant pathogen *Pseudomonas syrinage* from flowers that were inoculated to healthy flowers. Parmentier et al. (2018) found that *Pseudomonas* did not occur in the guts of bumble bee larvae and was rare in workers, speculating that the presence in workers is related to foraging. Ultimately, we cannot confirm whether *Pseudomonas* in our study include pathogenic species, as many common *Pseudomonas* species are non-pathogenic and exist within floral systems (e.g., in nectar: Álvarez-Pérez et al., 2012; Fridman et al., 2012).

Other core bacteria identified in our study appear to be neutral or have unknown functions in relation to bees. Graystock et al., (2017) identified *Sphingomonas* as core bacteria in the pollen provisions of small carpenter bees (*Ceratina*), and Anjum et al. (2018) found that it was part of the core gut microbiome of honey bees. Ma et al. (2019) found *Sphingomonas* abundance was low in honey bee pupae. But, when pupae were parasitized by *Tropilaelaps mercedesae* mites (Family: Laelapidae), relative abundance of *Sphingomonas* increased which was correlated with a decrease in relative abundance of non-core bacteria. Ma et al. (2019) did not test if this association was causal, or if the decrease in abundance of non-core bacteria negatively affected the health of pupae. Therefore, they refrained from assigning a functional role to *Sphingomonas*. In our study, we found *Sphingomonas* was positively correlated with Balsaminaceae

and Asteraceae in bumble bee pollen baskets, the latter relationship also recorded for goldenrod (Solidago: Asteraceae) from nest provisions of megachilid bees by Voulgari-Kokota et al. (2019). Further, Kim et al. (1998) found *Sphingomonas* in the seeds, leaves, and flowers of 11 plant families, and therefore, it may be common in the direct pollination environment. More research is needed to determine its functional role in bee–flower–microbe interactions and potential negative impacts on bee health.

Another core bacteria genus identified whose functional role in bees is not clear are *Kingella*. This genus includes species (e.g., *Kingella kingae*) that are known from endophytic isolates from roots of plants and have antagonistic properties toward plant pathogenic fungi (Berg & Hallmann, 2006). This species might serve as one example of microbial groups that inevitably serve bees as a source of nutrition and are consumed by larva feeding on pollen and nectar provisions. Indeed, it has been suggested that much of the pollen microbial community are simply fed to developing bees, inevitably digested and representing an important component of the bumble bee diet. This "microbivory" in bees has been demonstrated across six families (Steffan et al., 2019). A more developed understanding of the diversity of bacteria interacting with bees, and their ecological and behaviour transmission pathways will improve knowledge of their functional contribution to bee health and targeted conservation tactics (e.g., where and at what point to intervene in management of pathogenic bacteria).

4.2 | Floral and bacterial resources: A potential trade-off in foraging?

A positive correlation between *Lactobacillus* and *Acinetobacter* (and *Sphingomonas*) with Asteraceae illustrates an interesting bee–flower–microbe interaction (bumble bee—*Lactobacillus/Acinetobacter*—Asteraceae) deserving of more research attention and indicative of an emergent and understudied driver of well-known bee–flower mutualisms. Despite some Asteraceae (e.g., dandelion; *Taraxacum*) being toxic to bumble bees when the sole source of food (Vanderplanck et al., 2020), many Asteraceae provide ample pollen and nectar that are nutritious and attractive to bumble bees (Hicks et al., 2016) and Asteraceae were present in 50% of all pollen samples in our study (Table 1). LoCascio et al. (2019) showed bumble bees fed Asteraceae pollen from different genera (sunflower; *Helianthus*, or goldenrod; *Solidago*) had reduced levels of the gut pathogen, *Crithidia bombi*, that were 20–40 times less than controls. In a follow-up study from the same research group, chemical mechanisms by which Asteraceae pollen suppressed *C. bombi* were evaluated and none were found to be significant (Adler, Fowler, et al., 2020). In yet another study, Mockler et al. (2018) showed that bumble bees having higher levels of *Lactobacillus* in their gut microbiome led to reduced infection rates of *C. bombi*. With our identification of a positive correlation between *Lactobacillus* and Asteraceae, we offer a link between these research studies that suggest bumble bee floral preference could be partly determined by pursuit of individual and colony-level microbiome inoculation. To investigate trade-offs in this bee–flower–microbe interaction, we recommend further research to determine: (a) whether a causal link can be established between Asteraceae taxa and presumably beneficial bacteria (*Lactobacillus/Acinetobacter*); (b) if the presence/abundance of these key bacteria in pollen baskets leads to the presence/abundance of the same bacteria in the bee microbiome; (c) whether there is generality in this bumble bee—*Lactobacillus/Acinetobacter*—Asteraceae relationship (i.e., are there differences at the species level?); and (d) to what extent vertically transmitted and environmentally sourced bacteria provide beneficial functions in the bumble bee microbiome.

We also found a negative correlation for *Lactobacillus* and *Acinetobacter* with Lamiaceae (mints). Mint oils are well-known to exhibit anti-bacterial properties (Hammer et al., 1999). Park et al. (2019) investigated the anti-bacterial properties of *Agastache rugosa* ("Korean mint") and found flower extracts exhibited greater anti-bacterial properties than other parts of the plant. Hammer et al. (1999) reported that oils extracted from mints inhibited *Acinetobacter baumanii*, a human pathogen. Whether, this inhibitory ability extents broadly to *Acinetobacter* or to *Lactobacillus* is unknown. Despite the negative association with these bacteria, Lamiaceae was still well represented in pollen baskets presumably because it is highly attractive to bees due to its nectar rich flowers (Garbuzov & Ratnieks, 2014). The attractivity of flowers to bees as determined by morphology, chemistry, and other plant attributes has been well studied and remain the focus of significant and important research. However, the accumulation of bacterial communities on flowers may represent an underlying mechanism in floral preference, visitation rates and timing by bees, and ultimately trade-offs in foraging activity driven by bacteria, pollen and nectar rewards (Figure 1). Demonstrating the importance of diverse foraging opportunities for bee health and reproductive fitness in decision-making will vastly improve by filling the gaps in our understanding of bee–flower–microbe interactions. These approaches answer the call for ecologists to better characterize the multitrophic nature of the complex interactions and systems we study (Seibold et al., 2018).

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AUTHOR CONTRIBUTIONS

JSM, MY conceived the idea of the study. MY and AL collected the data. ST, AL, and MY completed the molecular work. MY, AL, and

JSM completed the flower and bee identifications. NS and ST led the statistical analysis. NS and JSM led the writing of the manuscript and the revision with all other authors contributing. All authors have read and commented on the final version of the revised manuscript.

DATA AVAILABILITY STATEMENT

All bee species, plant family, and bacterial OTU data used in our analysis is available on the FigShare data repository platform (Sookhan et al., 2020, https://doi.org/10.6084/m9.figshare.13208234).

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REFERENCES

Adler, L. S., Barber, N. A., Biller, O. M., & Irwin, R. E. (2020). Flowering plant composition shapes pathogen infection intensity and reproduction in bumble bee colonies. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(21), 11559–11565. https://doi.org/10.1073/pnas.2000074117

Adler, L. S., Fowler, A. E., Malfi, R. L., Anderson, P. R., Coppinger, L. M., Deneen, P. M., Lopez, S., Irwin, R. E., Farrell, I. W., & Stevenson, P. C. (2020). Assessing chemical mechanisms underlying the effects of sunflower pollen on a gut pathogen in bumble bees. *Journal of Chemical Ecology*, 1–10. https://doi.org/10.1007/s10886-020-01168-4

Adler, L. S., Irwin, R. E., McArt, S. H., & Vannette, R. L. (2020). Floral traits affecting the transmission of beneficial and pathogenic pollinator-associated microbes. *Current Opinion in Insect Science*, *44*, 1–7.

Adler, L. S., Michaud, K. M., Ellner, S. P., McArt, S. H., Stevenson, P. C., & Irwin, R. E. (2018). Disease where you dine: Plant species and floral traits associated with pathogen transmission in bumble bees. *Ecology*, *99*(11), 2535–2545. https://doi.org/10.1002/ecy.2503

Allard, S. M., Ottesen, A. R., Brown, E. W., & Micallef, S. A. (2018). Insect exclusion limits variation in bacterial microbiomes of tomato flowers and fruit. *Journal of Applied Microbiology*, *125*(6), 1749–1760. https://doi.org/10.1111/jam.14087

Álvarez-Pérez, S., Herrera, C. M., & de Vega, C. (2012). Zooming-in on floral nectar: A first exploration of nectar-associated bacteria in wild plant communities. *FEMS Microbiology Ecology*, *80*(3), 591–602. https://doi.org/10.1111/j.1574-6941.2012.01329.x

Anjum, S. I., Shah, A. H., Aurongzeb, M., Kori, J., Azim, M. K., Ansari, M. J., & Bin, L. (2018). Characterization of gut bacterial flora of *Apis mellifera* from north-west Pakistan. *Saudi Journal of Biological Sciences*, *25*(2), 388–392. https://doi.org/10.1016/j.sjbs.2017.05.008

Arredondo, D., Castelli, L., Porrini, M. P., Garrido, P. M., Eguaras, M. J., Zunino, P., & Antúnez, K. (2018). Establishment of characteristic gut bacteria during development of the honeybee worker. *Applied Environmental Microbiology*, *78*(8), 2830–2840.

Bailey, L. (1965). Susceptibility of the honey bee, *Apis mellifera* Linnaeus, infested with *Acarapis woodi* (Rennie) to infection by airborne pathogens. *Journal of Invertebrate Pathology*, *7*(2), 141–143. https://doi.org/10.1016/0022-2011(65)90025-X

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B*, *57*(1), 289–300.

Berg, G., & Hallmann, J. (2006). Control of plant pathogenic fungi with bacterial endophytes. In B. J. E. Schulz C. J. C Boyle & T. N. Sieber (Eds.), *Microbial root endophytes* (pp. 53–69). Springer.

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., & Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(2), 662–667. https://doi.org/10.1073/pnas.1014743108

Cankaya, N. E., & Kaftanoglu, O. (2006). An investigation on some diseases and parasites of bumblebee queens (*Bombus terrestris* L.) in Turkey. *Pakistan Journal of Biological Sciences*, *9*(7), 1282–1286. https://doi.org/10.3923/pjbs.2006.1282.1286

Caporaso, J. G., Bittinger, K., Bushman, F. D., DeSantis, T. Z., Andersen, G. L., & Knight, R. (2010). PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics*, *26*(2), 266–267. https://doi.org/10.1093/bioinformatics/btp636

Chao, A., Chiu, C.-H., & Jost, L. (2010). Phylogenetic diversity measures based on Hill numbers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1558), 3599–3609.

Cohen, H., McFrederick, Q. S., & Philpott, S. M. (2020). Environment shapes the microbiome of the blue orchard bee, Osmia lignaria. *Microbial Ecology*, *80*(4), 1–11.

Corby-Harris, V., Maes, P., & Anderson, K. E. (2014). The bacterial communities associated with honey bee (*Apis mellifera*) foragers. *PLoS One*, *9*(4), e95056. https://doi.org/10.1371/journal.pone.0095056

Danet, J. L., Foissac, X., Zreik, L., Salar, P., Verdin, E., Nourrisseau, J. G., & Garnier, M. (2003). "*Candidatus Phlomobacter fragariae*" is the prevalent agent of marginal chlorosis of strawberry in French production fields and is transmitted by the planthopper *Cixius wagneri* (China). *Phytopathology*, *93*(6), 644–649.

Dharampal, P. S., Carlson, C., Currie, C. R., & Steffan, S. A. (2019). Pollen-borne microbes shape bee fitness. *Proceedings of the Royal Society B*, *286*(1904), 20182894. https://doi.org/10.1098/rspb.2018.2894

Donati, I., Cellini, A., Buriani, G., Mauri, S., Kay, C., Tacconi, G., & Spinelli, F. (2018). Pathways of flower infection and pollen-mediated dispersion of *Pseudomonas syringae* pv. Actinidiae, the causal agent of kiwifruit bacterial canker. *Horticulture Research*, *5*(1), 1–13. https://doi.org/10.1038/s41438-018-0058-6

Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., & Wilson, K. (2018). Bacterial communities associated with honeybee food stores are correlated with land use. *Ecology & Evolution*, *8*(10), 4743–4756. https://doi.org/10.1002/ece3.3999

Durrer, S., & Schmid-Hempel, P. (1994). Shared use of flowers leads to horizontal pathogen transmission. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *258*(1353), 299–302.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, *27*(16), 2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Engel, P., Kwong, W. K., McFrederick, Q., Anderson, K. E., Barribeau, S. M., Chandler, J. A., Cornman, R. S., Dainat, J., de Miranda, J. R., Doublet, V., Emery, O., Evans, J. D., Farinelli, L., Flenniken, M. L., Granberg, F., Grasis, J. A., Gauthier, L., Hayer, J., Koch, H., … Dainat, B. (2016). The bee microbiome: Impact on bee health and model for evolution and ecology of host-microbe interactions. *American Society for Microbiology*, *7*(2), e02164–e2215. https://doi.org/10.1128/mBio.02164-15

Evans, J. D., & Armstrong, T. N. (2006). Antagonistic interactions between honey bee bacterial symbionts and implications for disease. *BMC Ecology*, *6*(1), 1–9. https://doi.org/10.1186/1472-6785-6-4

Figueroa, L. L., Blinder, M., Grincavitch, C., Jelinek, A., Mann, E. K., Merva, L. A., Metz, L. E., Zhao, A. Y., Irwin, R. E., McArt, S. H., & Adler, L. S. (2019). Bee pathogen transmission dynamics: Deposition, persistence and acquisition on flowers. *Proceedings of the Royal Society B*, *286*(1903), 20190603. https://doi.org/10.1098/rspb.2019.0603

Figueroa, L. L., Grab, H., Ng, W. H., Myers, C. R., Graystock, P., McFrederick, Q. S., & McArt, S. H. (2020). Landscape simplification

shapes pathogen prevalence in plant-pollinator networks. *Ecology Letters*, *23*(8), 1212–1222. https://doi.org/10.1111/ele.13521

Forsgren, E., Olofsson, T. C., Váasquez, A., & Fries, I. (2010). Novel lactic acid bacteria inhibiting *Paenibacillus* larvae in honey bee larvae. *Apidologie*, *41*(1), 99–108.

Frago, E., Dicke, M., & Godfray, H. C. J. (2012). Insect symbionts as hidden players in insect-plant interactions. *Trends in Ecology and Evolution*, *27*(12), 705–711. https://doi.org/10.1016/j.tree.2012.08.013

Fridman, S., Izhaki, I., Gerchman, Y., & Halpern, M. (2012). Bacterial communities in floral nectar. *Environmental Microbiology Reports*, *4*, 97–104. https://doi.org/10.1111/j.1758-2229.2011.00309.x

Garbuzov, M., & Ratnieks, F. L. (2014). Quantifying variation among garden plants in attractiveness to bees and other flower-visiting insects. *Functional Ecology*, *28*(2), 364–374. https://doi.org/10.1111/1365-2435.12178

Goulson, D. (2003). *Bumblebees: their behaviour and ecology*. Oxford University Press.

Graystock, P., Goulson, D., & Hughes, W. O. H. (2015). Parasites in bloom: Flowers aid dispersal and transmission of pollinator parasites within and between bee species. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1813), 20151371.

Graystock, P., Rehan, S. M., & McFrederick, Q. S. (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. *Conservation Genetics*, *18*(3), 701–711. https://doi.org/10.1007/s10592-017-0937-7

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*, *86*(6), 985–990. https://doi.org/10.1046/j.1365-2672.1999.00780.x

Hicks, D. M., Ouvrard, P., Baldock, K. C. R., Baude, M., Goddard, M. A., Kunin, W. E., Mitschunas, N., Memmott, J., Morse, H., Nikolitsi, M., Osgathorpe, L. M., Potts, S. G., Robertson, K. M., Scott, A. V., Sinclair, F., Westbury, D. B., & Stone, G. N. (2016). Food for pollinators: Quantifying the nectar and pollen resources of urban flower meadows. *PLoS One*, *11*(6), e0158117. https://doi.org/10.1371/journal.pone.0158117

Hsieh, T. C., & Chao, A. (2017). Rarefaction and extrapolation: Making fair comparison of abundance-sensitive phylogenetic diversity among multiple assemblages. *Systematic Biology*, *66*(1), 100–111.

Hsieh, T. C., Ma, K. H., & Chao, A. (2016). iNEXT: An R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, *7*(12), 1451–1456.

Huang, H. C., Richards, K. W., & Kokko, E. G. (1986). Role of the leafcutter bee in dissemination of *Verticillum alboatrum* in alfalfa. *Phytopathology*, *76*(1), 75–79.

Junker, R. R., Loewel, C., Gross, R., Dötterl, S., Keller, A., & Blüthgen, N. (2011). Composition of epiphytic bacterial communities differs on petals and leaves. *Plant Biology*, *13*, 918–924. https://doi.org/10.1111/j.1438-8677.2011.00454.x

Kearns, C. A., & Inouye, D. W. (1993). *Techniques for pollination biologists*. University Press of Colorado.

Keller, A., McFrederick, Q. S., Dharampal, P., Steffan, S., Danforth, B. N., & Leonhardt, S. D. (2020). (More than) Hitchhikers through the network: The shared microbiome of bees and flowers. *Current Opinion in Insect Science*, *44*, 8–15.

Kim, H., Nishiyama, M., Kunito, T., Senoo, K., Kawahara, K., Murakami, K., & Oyaizu, H. (1998). High population of *Sphingomonas* species on plant surface. *Journal of Applied Microbiology*, *85*(4), 731–736. https://doi.org/10.1111/j.1365-2672.1998.00586.x

Klepzig, K. D., Adams, A. S., Handelsman, J., & Raffa, K. F. (2009). Symbioses: A key driver of insect physiological processes, ecological interactions, evolutionary diversification, and impacts on humans. *Environmental Entomology*, *38*(1), 67–77. https://doi.org/10.1603/022.038.0109

Koch, H., Abrol, D. P., Li, J., & Schmid-Hempel, P. (2013). Diversity and evolutionary patterns of bacterial gut associates of corbiculate bees. *Molecular Ecology*, *22*(7), 2028–2044. https://doi.org/10.1111/mec.12209

Koch, H., Brown, M. J., & Stevenson, P. C. (2017). The role of disease in bee foraging ecology. *Current Opinion in Insect Science*, *21*, 60–67. https://doi.org/10.1016/j.cois.2017.05.008

Koch, H., & Schmid-Hempel, P. (2011). Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(48), 19288–19292. https://doi.org/10.1073/pnas.1110474108

Kwong, W. K., Engel, P., Koch, H., & Moran, N. A. (2014). Genomics and host specialization of honey bee and bumble bee gut symbionts. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(31), 11509–11514. https://doi.org/10.1073/pnas.1405838111

Kwong, W. K., & Moran, N. A. (2016). Gut microbial communities of social bees. *Nature Reviews Microbiology*, *14*, 374–384. https://doi.org/10.1038/nrmicro.2016.43

Legendre, P., Oksanen, J., & ter Braak, C. J. F. (2011). Testing the significance of canonical axes in redundancy analysis. *Methods in Ecology and Evolution*, *2*(3), 269–277. https://doi.org/10.1111/j.2041-210X.2010.00078.x

Liu, C. H., Chen, X., Liu, T. T., Lian, B., Gu, Y., Caer, V., Xue, Y. R., & Wang, B. T. (2007). Study of the antifungal activity of *Acinetobacter baumannii* LCH001 in vitro and identification of its antifungal components. *Applied Microbiology & Biotechnology*, *76*(2), 459–466. https://doi.org/10.1007/s00253-007-1010-0

LoCascio, G. M., Aguirre, L., Irwin, R. E., & Adler, L. S. (2019). Pollen from multiple sunflower cultivars and species reduces a common bumblebee gut pathogen. *Royal Society open science*, *6*(4), 190279.

Loncaric, I., Heigl, H., Licek, E., Moosbeckhofer, R., Busse, H. J., & Rosengarten, R. (2009). Typing of *Pantoea agglomerans* isolated from colonies of honey bees (*Apis mellifera*) and culturability of selected strains from honey. *Apidologie*, *40*(1), 40–54.

Ma, S., Yang, Y., Jack, C. J., Diao, Q., Fu, Z., & Dai, P. (2019). Effects of *Tropilaelaps mercedesae* on midgut bacterial diversity of *Apis mellifera*. *Experimental and Applied Acarology*, *79*(2), 169–186. https://doi.org/10.1007/s10493-019-00424-x

MacIvor, J. S., Cabral, J. M., & Packer, L. (2014). Pollen specialization by solitary bees in an urban landscape. *Urban Ecosystems*, *17*(1), 139–147. https://doi.org/10.1007/s11252-013-0321-4

Magoč, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, *27*(21), 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Manirajan, B. A., Ratering, S., Rusch, V., Schwiertz, A., Geissier-Plaum, R., Cardinale, M., & Schnell, S. (2016). Bacterial microbiota associated with flower pollen is influenced by pollination type, and shows a high degree of diversity and species-specificity. *Environmental Microbiology*, *18*(12), 5161–5174. https://doi.org/10.1111/1462-2920.13524

Martinson, V. G., Danforth, B. N., Minckley, R. L., Rueppell, O., Tingek, S., & Moran, N. A. (2011). A simple and distinctive microbiota associated with honey bees and bumble bees. *Molecular Ecology*, *20*(3), 619–628.

McArt, S. H., Koch, H., Irwin, R. E., & Adler, L. S. (2014). Arranging the bouquet of disease: Floral traits and the transmission of plant and animal pathogens. *Ecology Letters*, *17*(5), 624–636. https://doi.org/10.1111/ele.12257

McFrederick, Q. S., Mueller, U. G., & James, R. R. (2014). Interactions between fungi and bacteria influence microbial community structure in the *Megachile rotundata* larval gut. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1779), 20132653.

McFrederick, Q., & Rehan, S. M. (2016). Characterization of pollen and bacterial community composition in brood provisions of a small carpenter bee. *Molecular Ecology*, *25*, 2302–2311. https://doi.org/10.1111/mec.13608

McFrederick, Q. S., & Rehan, S. M. (2019). Wild bee pollen usage and microbial communities co-vary across landscapes. *Microbial Ecology*, *77*(2), 513–522. https://doi.org/10.1007/s00248-018-1232-y

McFrederick, Q. S., Thomas, J. M., Neff, J. L., Vuong, H. Q., Russell, K. A., Hale, A. R., & Mueller, U. G. (2017). Flowers and wild megachilid bees share microbes. *Invertebrate Microbiology*, *73*, 188–200. https://doi.org/10.1007/s00248-016-0838-1

McFrederick, Q. S., Vuong, H. Q., & Rothman, J. A. (2018). *Lactobacillus micheneri* sp. nov., *Lactobacillus timberlakei* sp. nov. and *Lactobacillus quenuiae* sp. nov., lactic acid bacteria isolated from wild bees and flowers. *International Journal of Systematic and Evolutionary Microbiology*, *68*(6), 1879–1884.

McFrederick, Q. S., Wcislo, W. T., Taylor, D. R., Ishak, H. D., Dowd, S. E., & Mueller, U. G. (2012). Environment or kin: Whence do bees obtain acidophilic bacteria? *Molecular Ecology*, *21*(7), 1754–1768. https://doi.org/10.1111/j.1365-294X.2012.05496.x

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Meikle, W. G., Mercadier, G., Guermache, F., & Bon, M. (2012). *Pseudomonas* contamination of a fungus-based biopesticide: Implications for honey bee (Hymenoptera: Apidae) health and Varroa mite (Acari: Varroidae) control. *Biological Control*, *60*(3), 312–320. https://doi.org/10.1016/j.biocontrol.2011.12.004

Mockler, B. K., Kwong, W. K., Moran, N. A., & Koch, H. (2018). Microbiome structure influences infection by the parasite Crithidia bombi in bumble bees. *Applied and environmental microbiology*, *84*(7). https://doi.org/10.1128/AEM.02335-17

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*(2), 133–142.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. M., Szoecs, E., & Wagner, H. (2018). *vegan: Community ecology package (version 2.5-3) [Computer software]*.

Park, C. H., Yeo, H. J., Baskar, T. B., Park, Y. E., Park, J. S., Lee, S. Y., & Park, S. U. (2019). In vitro antioxidant and antimicrobial properties of flower, leaf, and stem extracts of Korean mint. *Antioxidants*, *8*(3), 75. https://doi.org/10.3390/antiox8030075

Parmentier, A., Meeus, I., Nieuwerburgh, F. V., Deforce, D., Vandamme, P., & Smagghe, G. (2018). A different gut microbial community between larvae and adults of a wild bumblebee nest (*Bombus pascuorum*). *Insect Science*, *25*(1), 66–74. https://doi.org/10.1111/1744-7917.12381

Pattemore, D. E., Goodwin, R. M., McBrydie, H. M., Hoyte, S. M., & Vanneste, J. L. (2014). Evidence of the role of honey bees (*Apis mellifera*) as vectors of the bacterial plant pathogen *Pseudomonas syringae*. *Australasian Plant Pathology*, *43*(5), 571–575. https://doi.org/10.1007/s13313-014-0306-7

Praet, J., Parmentier, A., Schmid-Hempel, R., Meeus, I., Smagghe, G., & Vandamme, P. (2018). Large-scale cultivation of the bumblebee gut microbiota reveals an underestimated bacterial species diversity capable of pathogen inhibition. *Environmental Microbiology*, *20*(1), 214–227. https://doi.org/10.1111/1462-2920.13973

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 – Approximately Mmaximum-Likelihood trees for large lignments. *PLoS One*, *5*(3), e9490.

R Core Team (2019). *R: A language and environment for statistical computing (Version 3.6.2) [Computer software]*. R Core Team.

Raymann, K., Shaffer, Z., & Moran, N. A. (2017). Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. *PLoS Biology*, *15*(3), e2001861. https://doi.org/10.1371/journal.pbio.2001861

Seibold, S., Cadotte, M. W., MacIvor, J. S., Thorn, S., & Müller, J. (2018). The necessity of multitrophic approaches in community ecology. *Trends in Ecology & Evolution*, *33*(10), 754–764. https://doi.org/10.1016/j.tree.2018.07.001

Sookhan, N., MacIvor, J. S., Lorenzo, A., Tatsumi, S., & Yuen, M. (2020). Data used in: Linking bacterial diversity to floral identity in the bumble bee pollen basket. FigShare Data Repository. https://doi.org/10.6084/m9.figshare.13208234

Steffan, S. A., Dharampal, P. S., Danforth, B. N., Gaines-Day, H. R., Takizawa, Y., & Chikaraishi, Y. (2019). Omnivory in bees: Elevated trophic positions among all major bee families. *The American Naturalist*, *194*(3), 414–421. https://doi.org/10.1086/704281

Stoney, J. D., Bass, A. J., Dabney, A., & Robinson, D. (2019). *qvalue: Q-value estimation for false discovery rate control (Version 2.18.0) [Computer software]*.

Szabo, N. D., Colla, S. R., Wagner, D. L., Gall, L. F., & Kerr, J. T. (2012). Do pathogen spillover, pesticide use, or habitat loss explain recent North American bumblebee declines? *Conservation Letters*, *5*(3), 232–239. https://doi.org/10.1111/j.1755-263X.2012.00234.x

Tanaka, M., Nao, M., & Usugi, T. (2006). Occurrence of strawberry marginal chlorosis caused by "*Candidatus Phlomobacter fragariae*" in Japan. *Journal of General Plant Pathology*, *72*(6), 374–377. https://doi.org/10.1007/s10327-006-0308-6

Vanbergen, A. J., & Initiative, T. I. P. (2013). Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment*, *11*(5), 251–259.

Vanderplanck, M., Gilles, H., Nonclercq, D., Duez, P., & Gerbaux, P. (2020). Asteraceae paradox: Chemical and mechanical protection of *Taraxacum* pollen. *Insects*, *11*(5), 304. https://doi.org/10.3390/insects11050304

Voulgari-Kokota, A., Ankenbrand, M. J., Grimmer, G., Steffan-Dewenter, I., & Keller, A. (2019). Linking pollen foraging of megachilid bees to their nest bacterial microbiota. *Ecology and Evolution*, *9*(18), 10788–10800. https://doi.org/10.1002/ece3.5599

Voulgari-Kokota, A., McFrederick, Q. S., Steffan-Dewenter, I., & Keller, A. (2019). Drivers, diversity, and functions of the solitary-bee microbiota. *Trends in Microbiology*, *27*(12), 1034–1044. https://doi.org/10.1016/j.tim.2019.07.011

Voulgari-Kokota, A., Steffan-Dewenter, I., & Keller, A. (2020). Susceptibility of red mason bee larvae to bacterial threats due to microbiome exchange with imported pollen provisions. *Insects*, *11*(6), 373. https://doi.org/10.3390/insects11060373

Walterson, A. M., & Stavrinides, J. (2015). *Pantoea*: Insights into a highly versatile and diverse genus within the Enterobacteriaceae. *FEMS Microbiology Reviews*, *39*(6), 968–984. https://doi.org/10.1093/femsre/fuv027

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267.

Wilson, S. (2012). *Canada's wealth of natural capital: Rouge National Park*. David Suzuki Foundation. Retrieved from https://davidsuzuki.org/wp-content/uploads/2012/09/rouge-national-park-canada-wealth-natural-capital.pdf

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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