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 (Figuerola 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 (Figuerola 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 microbiota diversity, but rather that pollen composition has an impact on microbiota composition. Specific flower species may harbor more pathogenic taxa (Figuerola 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 microbiota 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, 80.3786°W).
97.1710°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 x 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 observers 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′-GTGCCAGCMGCCGCGGTAA-3′) and the 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers. Sequencing of amplicons was performed with 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) (Magoc & 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 reference 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 100x 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 (Hill), and Hill numbers adapted to measure phylogenetic diversity (PD) (Chao et al., 2010). Taxonomic richness (S) = 2D, Gini-Simpson index (GS = 2D), Faith’s phylogenetic diversity (PD = 2D(T)), and Rao’s Q (Q = 2D(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 2D instead of 1D. 2D does not put weight on rare nor abundant species, whereas 1D puts more weight on relative abundances and therefore would provide better inference when paired with 2D. Abundance data were rarefied 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 (D1) and Bray–Curtis dissimilarity (D2) 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
Hellingter 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 R² (R²m and R²c) developed by Nakagawa and Schielzeth (2013) were used to assess the proportion of variance explained by pollen alpha diversity (R²m), and pollen alpha diversity and the random terms (R²c). 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 (R²oββ). 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).

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, Pantoaea, Acinetobacter, and then Pseudomonas. The four remaining genera accounted for <2% of core OTU sequence reads.
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 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           |
| Pantoae     | 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            |
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 | R²m | R²c |
|----------|--------|----------|----|---------|---------|---------|---------|-------|------|------|------|
| a) All OTU | | | | | | | | | | | |
| 0      | 0 | 0.233 | 79.851 | 2.174  | .033    | 0.106   | 0.000   | 0.000 | 0.083 | 0.053 | 0.060 |
| 0      | 2 | 0.200 | 83.000 | 1.862  | .066    | 0.106   | 0.000   | 0.000 | 0.040 | 0.120 | 0.073 |
| 2      | 0 | 0.304 | 81.980 | 2.897  | .005    | 0.039*  | 0.111   | 0.000 | 0.229 | 0.089 | 0.151 |
| 2      | 2 | 0.111 | 82.757 | 1.020  | .310    | 0.362   | 0.000   | 0.000 | 0.252 | 0.012 | 0.073 |
| 0D(T)  | 0 | 0.217 | 81.659 | 2.011  | .048    | 0.106   | 0.000   | 0.000 | 0.137 | 0.046 | 0.064 |
| 0D(T)  | 2 | 0.204 | 82.970 | 1.899  | .061    | 0.106   | 0.000   | 0.000 | 0.105 | 0.041 | 0.052 |
| 2D(T)  | 0 | 0.096 | 77.706 | 0.856  | .395    | 0.395   | 0.156   | 0.222 | 0.325 | 0.008 | 0.170 |
| 2D(T)  | 2 | 0.109 | 81.670 | 1.008  | .317    | 0.362   | 0.000   | 0.000 | 0.188 | 0.333 | 0.146 |
| b) Core OTU only | | | | | | | | | | | |
| 0      | 0 | 0.154 | 90.000 | 1.483  | .142    | 0.485   | 0.000   | 0.000 | 0.024 | 0.024 | 0.024 |
| 0      | 2 | 0.090 | 90.000 | 0.856  | .394    | 0.631   | 0.000   | 0.000 | 0.000 | 0.008 | 0.158 |
| 2      | 0 | 0.192 | 83.865 | 1.858  | .067    | 0.485   | 0.489   | 0.253 | 0.000 | 0.000 | 0.000 |
| 2      | 2 | 0.042 | 87.667 | 0.408  | .685    | 0.782   | 0.491   | 0.208 | 0.000 | 0.000 | 0.000 |
| 0D(T)  | 0 | 0.139 | 90.000 | 1.333  | .186    | 0.485   | 0.000   | 0.000 | 0.000 | 0.191 | 0.273 |
| 0D(T)  | 2 | 0.068 | 90.000 | 0.647  | .519    | 0.692   | 0.000   | 0.000 | 0.000 | 0.000 | 0.000 |
| 2D(T)  | 0 | 0.128 | 87.409 | 1.176  | .243    | 0.485   | 0.351   | 0.381 | 0.360 | 0.013 | 0.320 |
| 2D(T)  | 2 | 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. "R²m" and "R²c" are the marginal and conditional R² 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 microbial communities expand, 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).
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

### 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 | 0.004   | 0.008*  | 0.000   | 0.004 | 0.082 |
|          | Bray Curtis | 0.009    | 1.76 | 1.774 | 0.003   | 0.008*  | 0.000   | 0.010 | 0.084 |
|          | Bray Curtis | 0.010    | 1.76 | 1.848 | 0.002   | 0.008*  | 0.006   | 0.004 | 0.047 |
|          | UniFrac |          |    |        |         |         |         |       |      |
|          | Sorensen | 0.005    | 1.76 | 1.448 | 0.023   | 0.031*  | −0.001  | 0.007 | 0.090 |
|          | Bray Curtis | 0.014    | 2.75 | 1.629 | 0.001   | 0.008*  | −0.001  | 0.012 | 0.096 |
|          | Bray Curtis | 0.009    | 1.76 | 1.725 | 0.068   | 0.078   | 0.006   | 0.007 | 0.082 |
|          | Bray Curtis | 0.007    | 1.76 | 1.568 | 0.107   | 0.107   | 0.005   | 0.026 | 0.085 |
| b) Core OTU only |        |          |    |        |         |         |         |       |      |
| Sorensen | Sorensen | 0.007    | 1.76 | 1.364 | 0.192   | 0.192   | −0.022  | −0.006 | 0.089 |
|          | Bray Curtis | 0.108    | 3.74 | 2.743 | 0.001   | 0.004*  | −0.011  | 0.009 | 0.074 |
|          | Bray Curtis | 0.024    | 1.76 | 2.549 | 0.017   | 0.034*  | 0.014   | −0.009 | 0.026 |
|          | UniFrac |          |    |        |         |         |         |       |      |
|          | Sorensen | 0.011    | 1.76 | 1.575 | 0.157   | 0.179   | −0.016  | 0.001 | 0.031 |
|          | Bray Curtis | 0.157    | 4.73 | 3.210 | 0.001   | 0.004*  | 0.005   | 0.007 | 0.030 |
|          | Bray Curtis | 0.019    | 1.76 | 2.283 | 0.057   | 0.091   | 0.011   | −0.009 | 0.027 |
|          | Bray Curtis | 0.009    | 1.76 | 1.610 | 0.133   | 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 |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Apiaceae | 0.267 |     |     |     |     |     |     |     |     |
| Asteraceae |     | 0.266 |     |     |     |     |     |     |     |
| Balsaminaceae |     |     | 0.261 |     |     |     |     |     |     |
| Brassicaceae |     |     |     | 0.252 |     |     |     |     |     |
| Caprifoliaceae |     |     |     |     | 0.291 |     |     |     |     |
| Fabaceae |     |     |     |     |     | 0.345 |     |     |     |
| Geraniaceae |     |     |     |     |     |     | 0.195 |     |     |
| Lamiaceae |     |     |     |     |     |     |     | 0.345 |     |
| Malvaceae |     |     |     |     |     |     |     |     | 0.291 |
| 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
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 other 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 syringae 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

**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
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 understood 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 (Vanderplank 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, Clinkhilda 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 Astastache 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 attractiveness 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 multirrophic 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
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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SUPPORTING INFORMATION

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

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