ARPHA Preprints

PENSOFT

PREPRINT

Author-formatted, not peer-reviewed document posted on 08/09/2026

DOI: https://doi.org/10.3897/arphapreprints.e214542

Drivers of distance decay of host-associated microbiomes vary by host sociality and microbial association strength

Rebecca Hayes, Brendan J. M. Bohannan, Shalene Jha, Lauren Ponisio

ARPHA Preprints *Author-formatted, not peer-reviewed document posted on 08/09/2026.* DOI: https://doi.org/10.3897/arphapreprints.e214542

Abstract

Spatial patterns in microbial biodiversity can provide valuable insight into the drivers of microbial community variation; however, they are rarely explored in host-associated microbiomes, despite the significance of microbiome variation to host health and fitness. Spatial variation in animal-associated microbiomes can be influenced by several factors, including the strength of the interaction between the host and microbe (microbial association) and the likelihood of social interactions among hosts (host sociality). We investigated the processes that drive host-microbe interaction turnover in wild bees across space and found that they vary with both host sociality and microbial association. Strongly host-associated microbes in solitary bees were geographically structured by opposing processes — interaction rewiring among nearby meadows and turnover in bee species among distant meadows. The net result was that strongly host-associated interactions did not significantly vary with distance in solitary bees. In contrast, for weakly host-associated microbes in solitary bees, interaction turnover was driven solely by turnover in bee species among distant meadows, resulting in a significant relationship between similarity in host-microbe interactions and distance. For strongly host-associated microbes in social bees, networks were geographically structured, exhibiting opposing processes (rewiring among nearby meadows and turnover in both bee and microbial taxa among distant meadows). In contrast, for weakly associated microbes in social bees, networks were geographically structured solely by microbe-driven turnover, which decreased with distance. The net result was that host-microbe interactions did not significantly vary with distance in social bees. Our results demonstrate that both host sociality and host-microbial association strength can influence the drivers of spatial variation in animal-associated microbiomes.

Running head:

DISTANCE DECAY OF STRONG AND WEAK HOST-MICROBE INTERACTIONS

Drivers of distance decay of host-associated microbiomes vary by host sociality and microbial association strength

Rebecca A. Hayes[1*], Brendan J. M. Bohannan[1], Shalene Jha[2,3], Lauren C. Ponisio[1]

1. Institute of Ecology and Evolution
   Department of Biology, University of Oregon
   272 Onyx Bridge
   Eugene, OR USA, 97403

2. Integrative Biology Department
   University of Texas, Austin, Texas, USA

3. Lady Bird Johnson Wildflower Center
   University of Texas, Austin, Texas, USA

⋆ Corresponding author: rhayes7@uoregon.edu

Running head:

DISTANCE DECAY OF STRONG AND WEAK HOST-MICROBE INTERACTIONS

Highlights

- Spatial turnover in bee–microbe interactions depends on host sociality and microbial association strength.
- Similar spatial patterns can emerge from distinct ecological processes underlying host–microbe interaction turnover.
- Interaction rewiring and taxonomic turnover contribute differently to spatial structure across host–microbe networks.
- Host and microbial turnover play contrasting roles in structuring strongly versus weakly associated microbiomes.
- Considering interaction networks can reveal spatial processes that may be obscured when hosts and microbes are studied separately.

Keywords: bee-microbe networks, bee microbiome, distance decay, host-associated microbes, Madrean Sky Islands, microbial biogeography

Introduction

Understanding how and why communities of organisms in different geographic locations are distinct has been a primary objective of ecology since its inception. In addition to stochastic processes, deterministic processes such as dispersal limitation and environmental filtering can drive community composition and spatial turnover, thereby generating biogeographic patterns (Meynard et al. 2013). One of the most fundamental spatial biogeographic patterns is the "distance decay of similarity", which describes the relationship between community compositional turnover and geographic distance (Nekola and White 1999). Communities that are closer together tend to be more similar in composition than those that are farther apart, which has been observed in plants (Nekola and White 1999), animals (Harrison et al. 1992), and free-living microbes (Green et al. 2004), and recent evidence suggests that this pattern may be present in host-associated microbes as well (Suzzi et al. 2023).

However, few recent studies have examined host-associated microbial biogeography, especially in animals (Morelan et al. 2019; Dickey et al. 2021; Neu et al. 2021; Härer and Rennison 2023). Understanding animal-associated microbial biogeography is particularly important, as microbes affect host health, function, population dynamics, and fitness (Engelstädter and Hurst 2009; McFall-Ngai et al. 2013; Parfrey et al. 2018; Rudman et al. 2019). Beyond biogeography, host diet, genotype, and behavioral ecology are well-established drivers of host associated microbiome variation (i.e. Ricigliano et al. 2017; Wu et al. 2021; Wang et al. 2024), however these studies tend to focus on a small geographic scale. The few biogeographic studies of animal-associated microbiomes report variable patterns, attributed to microbial specificity, interaction strength, host identity, and ecology

(Clark et al. 2021). For example, two aspects of host ecology — sociality and degree of host association — have been largely overlooked as drivers of these patterns, despite their potential to explain observed variability. Thus, identifying a host system with variable sociality and strength of host-microbe association is crucial for studying the host ecology's role in microbial biogeography.

Wild bees are ideal for studying host-microbe biogeography because their microbiomes vary in their strength of host-association (Zheng et al. 2018; Voulgari-Kokota et al. 2019), and as host organisms, their communities vary geographically (Orr et al. 2021) and in their social behaviors (social to solitary), influencing how they transmit and acquire microbes. Both social and solitary bees share microbes via flowers but often maintain distinct genus-level microbiomes (McFrederick et al. 2017; Graystock et al. 2017). In social bees, like honey bees (*Apis*) and bumble bees (*Bombus*), the dominant beneficial microbiome members are inherited vertically or acquired through early interactions with nurse bees and later with other colony members (Fig. 1, AI; Kowallik and Mikheyev 2021). These microbes that are consistently found in social hosts can be considered strong associates: they are well-adapted to their host, presumed functionally important and occurring in high abundance, and are primarily transmitted through direct interactions with conspecifics (Fig. 1, AI, thick green arrow; Martinson et al. 2011). Pathogenic bacteria also infect social bees and can be considered strong associates (Cellini et al. 2019; Ramalho et al. 2021; Lang et al. 2022; Mukhtar et al. 2024). This is because pathogenic microbes are often transmitted through adult-adult interactions and are adapted to surviving and replicating in their hosts, much like the dominant gut microbes (Freedman et al. 2024). Thus, their biogeographic patterns are likely similar to those of beneficial strong associates. In both cases, the primary environment of these microbes is within the host, where they reproduce and carry out their presumed functions. However, additional microbes with unique life histories also are detected as members of the bee microbiome.

Beyond these strong host-associates, social bees also acquire additional microbes while foraging on flowers and interacting with the outside environment (Fig. 1, AI, orange arrows). These incidentally-acquired microbes are assumed to be non-host-specific with high inter-individual variation in composition and abundance (Neu et al. 2021; Nelson et al. 2025), with variable functional roles ranging from potentially pathogenic, to neutral, to potentially beneficial (Cariveau et al. 2014; Mockler et al. 2018; Praet et al. 2018; Figueroa et al. 2023). While additional research is required to confirm the role of incidentally-acquired microbes in host function, most studies assume that the impact on host function is less relative to socially transmitted associates. The primary environment of these associates is outside of the host in the surrounding environment: even though they can be detected from sampling the host gut, they are not thought to be reproducing or strongly influencing function. Thus, incidentally-acquired microbes in social hosts can be considered weak associates relative to the strong associates described above. This distinction in primary environment and transmission routes between strong and weak associates could lead to different spatial patterns between the two associate types.

Additionally, bee communities are composed of both social and solitary bee taxa, which have different primary routes of microbial transmission, and so also could vary in their spatial patterns. Despite the fact that solitary bees make up the majority of bee species (Danforth et al. 2019), most of what we know about bee microbes comes from social species (Kwong et al. 2017). Thus, there is an even greater need to explore how microbial host-association type influences biogeographic patterns in solitary bees. Because of their ecological differences, solitary bees could have distinct biogeographical drivers of microbial dissimilarity. Unlike social bees, solitary bees rarely interact with conspecifics until reproduction, long after their microbiome develops (Voulgari-Kokota et al. 2019). This means that they lack the direct, consistent transmission pathways that conserve strong microbial associations in social bees. However, there is growing evidence that solitary bees harbor microbes that may be analogous to the socially-transmitted strong associates (Todorov et al. 2024). Without adult-to-adult transmission routes, solitary hosts' main pathways for strong associate transmission include the inoculation of pollen provisions and nest materials, which transmit microbes to developing larvae, similar to other insects (Fig. 1, AII, green arrows; Gilliam et al. 1984; McFrederick et al. 2014). Pathogens are likely transmitted this way, and similar to social bees, are considered to be strong associates in solitary bees because they are adapted to surviving and replicating in hosts and can likewise be transmitted vertically through pollen provisions or through interacting with shared floral resources (McFrederick and Rehan 2019). Also similar to social bees, many microbes are acquired during foraging and interaction with the environment, and these incidentally acquired microbes make up a larger portion of the solitary bee microbiome compared to social hosts (Fig. 1, AII, orange arrows; Voulgari-Kokota et al. 2019). Because of this, the solitary bee microbiome is expected to be much more spatially variable (reviewed in Voulgari-Kokota et al. 2019). Additionally, the greater phylogenetic diversity of solitary bees compared to social bees likely increases microbial variation, as different genera harbor distinct microbiomes (Kwong and Moran 2016; Graystock et al. 2017; Voulgari-Kokota et al. 2019). Differences in transmission and lifestyle between social and solitary bees may shape biogeography, yet these drivers remain unexplored, despite the existence of many relevant community assembly frameworks.

Leveraging the Island Biogeography framework (MacArthur and Wilson 1967), hosts could be considered microbe "islands", where species presence and abundance reflect dispersal among hosts and environmental filtering within them (Kuris et al. 1980). For strongly host-associated microbes, host-host interactions, whether within a colony or at foraging sites, mediate dispersal among individuals. Thus, physical distance between hosts acts as a dispersal barrier, like an ocean for terrestrial species. Non-random interactions, such as social contact, can result in steep or shallow distance decay depending on the strength of microbial association. For strongly host-associated microbes, across both social and solitary species, sharp distance decay may arise from dispersal requiring host–host contact (Fig. 1, AIII dashed line). In contrast, across both social and solitary species, weakly host-associated microbes that spend multiple generations outside hosts face different and potentially lower dispersal barriers. Soil microbes, for example, are mainly passively dispersed by wind, making distance the primary barrier

(Malard and Pearce 2022). Thus, weakly host-associated microbes that do not need host-host dispersal may display shallow distance decay (Fig. 1, AIV dashed line). The role of environmental filtering in distance decay may also differ between strongly and weakly host-associated microbes. For strongly host-associated microbes, the primary environment is the host. Since hosts are regulated homeostatic "islands", environmental variation within hosts is likely small compared to variation across the external environment, especially within a species. Variation may be greater between host species, but still lower than in the external environment. Thus, environmental selection may be weaker across space, producing shallow distance decay for strong host associates (Fig. 1, AIII dotted line). For weakly host-associated microbes, the primary environment is the external non-host environment, which is more heterogeneous than the host interior. This could produce sharp decay in similarity among weakly host-associated microbes (Fig. 1, AIV dotted line).

Finally, while past work has often used Bray Curtis dissimilarity to understand compositional differences (Kuris et al. 1980), new metrics such as network decomposition can reveal whether dissimilarity arises from partner turnover or changing interaction patterns across sites, revealing the mechanistic processes guiding composition patterns (Fig. 1B). Traditional microbial dissimilarity indices treat the hosts as static habitats for microbes, while the network decomposition approach treats sites as dynamic "islands" where both hosts and microbes can turnover between islands. This distinction is important because microbes can be transmitted through host-host interactions, which is not accounted for through traditional dissimilarity calculations. Additionally, this technique acknowledges the role of the host as a biotic filter for microbe dispersal, since spatial dissimilarity can arise from shifts in host-microbe interactions between sites with shared species pools separately from the dissimilarity caused by uncommon species pools between sites. Host-microbe interactions are modeled as networks linking host and microbe nodes by microbial presence (Zemenick et al. 2021). Thus, a host-microbe interaction is realized by the detection of a particular microbe within a particular host. Examining interaction turnover provides a mechanistic understanding of how host-microbe communities change through space. Studies have shown that spatial variation in species composition does not always reflect spatial interaction variation (Poisot et al. 2015; Trøjelsgaard et al. 2015). This discrepancy is often driven by "interaction rewiring", which describes the reshuffling of interaction partners among sites with shared species pools (Poisot 2022; Magrach et al. 2023). Interaction rewiring is ubiquitous and is known to influence spatial diversity patterns (Burkle and Irwin 2009; Burkle et al. 2013; CaraDonna et al. 2017). Interaction rewiring increases robustness to disturbances, letting remaining partners switch and maintain function if one mutualist is absent (Vizentin-Bugoni and Maruyama 2023). In the case of bee-microbe interactions, interaction rewiring can translate either to complete switching of partners (i.e. although the microbial associate and host taxon are common to both sites, it associates only with a given host taxon at one of the two sites) or to shifts in the relative dominance structure of microbial associates within host taxa shared between two sites (i.e. although the microbial associate and host taxon are common to both sites, the relative abundance in a given host taxon is different between sites). Interaction rewiring may be particularly important in mediating the spatial

structuring of strongly host-associated microbes, given their essential roles in host function. Conversely, interaction rewiring likely matters less for weakly host-associated microbes, whose presence is more incidental. Interaction rewiring facilitates partner exchange, easing dispersal limitation and enabling microbial association with viable hosts across taxa rather than specific hosts. Although microbial rewiring across life stages and tissues is well-documented (e.g., Parker et al. 2018), the community-wide spatial rewiring of host-microbe interactions in animals remains understudied.

In this study, we examine gut microbiomes of wild bees across two social and four solitary genera to understand how distance decay differs for strongly versus weakly host-associated microbes and how network dissimilarity components contribute to spatial variation. We predict microbial community similarity decays with distance, but that the distance decay of bee-microbe interactions depends on host-association strength (strong vs. weak). Distance decay rates of microbial communities and host-microbe networks may differ between strongly and weakly host-associated microbes, as well as between social and solitary bees, due to ecological differences and the roles of dispersal versus environmental heterogeneity in community assembly. This multi-host analysis examines the drivers of microbial diversity in social and solitary bees across host-association strengths and is one of the first to assess spatial interaction turnover in animal host-microbe networks.

Methods

Site selection and sample collection

In 2018 and 2021, we conducted 10-hour surveys of bee communities in eight high-elevation meadows across seven mountains in the Madrean Sky Islands in Arizona and New Mexico, an ideal system for studying host-associated microbial biogeography (Fig. S5, Table S6, see also Supplementary Methods "Sky Islands history and ecology"). Our focal meadows were at elevations between 2484 and 3178 m above sea level and ranged from 1.16–162.25 ha in area (Table S7). The closest meadows were separated by 1.52 km and the farthest by 553.5 km (Fig. S5, Table S 4). At each meadow, two observers spent 10 hours actively hand-netting bees using sterile collection methods. Bees were stored on dry ice until they were transferred to a $-80°C$ freezer awaiting gut dissection (specific collection methodology described in Supplementary Methods "Host collection").

Defining "strongly" and "weakly" host-associated microbes for social and solitary hosts

Microbial taxa were delineated as Amplicon Sequence Variants (ASVs) of PCR amplified 16S rDNA (see section Bioinformatics and microbe taxonomic assignment below). Because the bee gut microbiome is taxonomically constrained, we classified ASVs as "strongly" or "weakly" host-associated microbes of social and solitary bees using information from previously published literature characterizing the bee microbiome

(Table 1). We defined strongly host-associated microbes as any ASVs matching the families of designated strongly host-associated taxa (strongly host- associated; Table 1). We chose to group these ASVs at the family level to balance the unreliability of calls at lower taxonomic levels while still maintaining a meaningful level of conserved microbial traits relating to their ecological roles. We also chose this family-level grouping because not all of the detected ASVs were resolved fully to species, and because species- and genus-level assignments for these taxa are less reliable than the family level assignments (Johnson et al. 2019). Given that we currently lack information on the microbial traits that relate to microbial host-association strength, these family associations provided a means to group both strongly and weakly host-associated microbes while allowing us to retain more ASVs and avoid over-interpretation of the less-reliable lower taxonomic calls. It is established that different traits are conserved at different microbial taxonomic levels (Martiny et al. 2015), but a complete understanding of how traits map onto taxonomic levels is still unresolved. Although horizontal gene transfer complicates taxonomy-function links, we assume closely related taxa share more traits than distant ones at the family level (Louca et al. 2018; Martiny et al. 2015), a reasonable assumption given what is currently known about the bee microbiome.

Social bee hosts such as honeybees (*Apis* spp.) and bumble bees (*Bombus* spp.) harbor a common set of microbial taxa, consisting of nine groups in *Apis* and seven groups in *Bombus* (social strong associates, Table 1; Meeus et al. 2015; Zheng et al. 2018). We designate these as "strongly host-associated" microbes, since these taxa are usually present in high abundance in healthy social bees throughout space and time and are assumed to be adapted to the host (Meeus et al. 2015; Zheng et al. 2018). These are sometimes referred to as the social "core microbiome" (Meeus et al. 2015), "beneficial"(Todorov et al. 2024), or "obligate symbionts" (Kucuk 2020), but we use "strongly host-associated" to reflect uncertainty in function and occasional presence outside the host (Keller et al. 2021; Tiusanen et al. 2024). While these strong associate groups are commonly found in bee hosts throughout space and time, spatial turnover is still expected. This is because strong associates are primarily restricted to inside the host gut, even though they may be able to survive temporarily outside of the host gut. Host dispersal between meadows in this system is not possible due to the large geographic distance between them, creating the potential for independent diversification via genetic drift, which would result in spatial turnover at the ASV level (Zhang et al. 2024). Thus, strong associates are not considered spatially ubiquitous because they are not distributed uniformly throughout the external environment like many environmental microbes. Pathogens were also included as strongly host-associated because they share transmission routes, are adapted to the host, and likely follow similar biogeographical patterns (pathogenic associates; Table 1). The remaining social bee gut taxa were designated "weakly host-associated", as their primary environment is outside of the host and we lack evidence suggesting host adaptation or functional importance.

The solitary bee microbiome includes several common taxa that differ from those in social bees (Table 1), but are similarly found in high abundance throughout space and time and are assumed to be host-adapted (Voulgari-Kokota et al. 2019), thus we designate these as

microbes "strongly host-associated". We also included pathogenic bacteria known to infect bees in this "strongly host- associated" group (pathogenic associates, Table 1; Cellini et al. 2019; Ramalho et al. 2021; Lang et al. 2022). The remaining solitary bee gut taxa were designated "weakly host-associated", as they lack evidence of host adaptation and conserved routes of transmission between solitary hosts.

We studied six widespread wild bee genera to investigate the spatial patterns of host-associated microbes in both social and solitary hosts. The social host genera included *Bombus* and *Apis* and the solitary hosts included *Andrena*, *Megachile*, *Anthophora*, and *Melissodes* (Table S 5). We grouped individuals at the genus-level because most ecological traits are shared at this taxonomic level ((Roubik 2021)), most bee microbial communities cluster by host genus ((Kwong et al. 2017)), and to improve our replication and characterization for the genus. We focus our analyses on the groups of social and solitary taxa as we have apriori hypotheses about distance decay relationships given past work highlighting distinct microbial spatial patterns in social hosts (Sarkar et al. 2020), and limited host-host interactions in solitary hosts. Further, currently very little is known about the microbiomes of solitary bees, warranting the study of multiple genera, where possible, to characterize this group (Voulgari-Kokota et al. 2019). Thus, species-level differences in behavior and ecology are also important to consider in microbial dynamics ((Goodrich et al. 2016))(for an exploration of these differences in our data, see Table S 8); however, we focus the current study on broader differences between social and solitary taxa and potentially generalizable insight into large-scale microbial spatial dissimilarity patterns

Gut DNA extraction and sequencing

We dissected guts from thawed bees using sterile methods to identify the bee gut microbiome following (Smith et al. 2024). Multiple expert taxonomists identified specimens to the species level (Smith et al. 2021). Gut DNA contents were extracted using Qiagen AllPrep DNA/RNA 96 kits (Qiagen, Valencia, CA), following established methodologies for the use of next-generation sequencing to identify gut microbiomes in bees (Engel et al. 2013; Smith et al. 2024). The $V5 - V6$ region of the 16S rRNA gene was PCR amplified from extracted DNA using the primer pair 799F-mod3 CMGGATTAGATACCCKGG (Hanshew et al. 2013) and 1115R AGGGTTGCGCTCGTTG (Kembel et al. 2014), which optimizes bacterial identification while simultaneously minimizing chloroplast contamination (Hanshew et al. 2013). The amplicons were sequenced using the Illumina MiSeq platform (2x300, V3 reagents) (Illumina, Inc, San Diego, CA) following a dual-index, inline barcoding approach (McFrederick and Rehan 2016). We completed Illumina library preparation in four steps: a round of PCR to produce 16S amplicon, an enzymatic cleaning step, a second PCR round to add Illumina adapters, and finally, a normalization step to standardize between sample wells and plates following (Smith et al. 2024). Our libraries were sequenced using two distinct runs with the MiSeq Reagent Kit v3 with 2 × 300 cycles.

Bioinformatics and microbe taxonomic assignment

After obtaining raw Illumina reads, we processed the paired-end in-line barcodes using QIIME 1 (v2019.1), then demultiplexed and subsequently quality filtered and denoised using QIIME 2 (v2019.1), including DADA2-based chimera removal and error correction, following an established bioinformatic pipeline for assigning taxonomy to bee gut microbes (Bolyen et al. 2019; Smith et al. 2024). Following Smith et al. (2024), we used QIIME1 to process the paired-end in-line barcodes then trimmed the low-quality ends of the reads with QIIME2 and merged data from multiple Illumina runs. We then removed chimeras and reads with more than two expected errors using DADA2 (Callahan et al. 2016) and assigned sequences into amplicon sequence variants (ASVs) by classifying 16S rRNA gene ASVs using the QIIME 2 feature-classifier (Bokulich et al. 2018), trained using the SILVA 138 database (Quast et al. 2012). We clustered at 99 percent sequence similarity to the 799-1115 region of the 16S rRNA gene. We performed several filtering steps to ensure the accuracy of our results, following the sequence cleaning pipeline developed by (Smith et al. 2024). To account for variable sequencing depths, we generated rarefaction curves for each sequencing run and subsampled to 1019 reads (97.70% samples retained) and generated ASV tables for each run. Samples from hosts outside of our focal genera and samples from hosts that were unidentifiable by our taxonomists due to poor condition were excluded from downstream analysis (n=7). Using taxonomic identity, we classified ASVs into "strongly" or "weakly" host-associated categories for both social or solitary hosts.

Constructing bee-microbe networks

We built bee-by-microbe matrices by summing ASV reads per bee to quantify total ASVs per sample and enable read count standardization. We then divided each cell by the row sum to calculate the relative abundance of each ASV per bee, a common method for characterizing bee microbe communities (e.g. (Yun et al. 2018)). We averaged individual relative abundances for each bee species and scaled by their meadow-level abundance, yielding abundance-weighted networks. To quantify interaction dissimilarity between each pair of meadows, we used the betalinkr multi function from the bipartite package (version 2.23) in R (Novotny 2009; Poisot et al. 2012). Because our goal was to decompose network dissimilarity into additive components to compare relative contribution of each component, we used the common denominator partitioning approach. This method uses a common denominator based on all links between network pairs and partitions only the numerator, ensuring additive rewiring and species turnover components (Novotny 2009; Poisot et al. 2012) (Figure 1 B).

Network dissimilarity-distance model specifications

We assumed a Gaussian error distribution for the models. Three MCMC chains were run for 10^5 iterations after an initial burn-in of $5.0x10^4$ iterations using STAN via the R package brms (version 2.23.0) (Bürkner 2017). We used brms's recommended default, uninformative priors: flat over all real numbers for the means of the distributions of the top-level parameters and Student's t-distribution$(3,0,2.5)$ for the variances. Analyses were

conducted in R 4.4.1 (R Core Team 2021). We used standard practices for assessing convergence, including examining the chains, bulk and tail effective sample size, and R-hat values. For the individual components of dissimilarity (rewiring, host-driven turnover, microbe-driven turnover, and complete turnover), we followed the same model specifications outlined in section "Distance decay of bee-microbe interaction network similarity" below, running individual models for each of the components of dissimilarity as the response variable.

Distance decay of microbial similarity

To understand the rates of distance decay for strong and weak associates in social and solitary hosts, we calculated compositional Bray-Curtis dissimilarity for each subset of the 16s ASV table. We determined the correlation between dissimilarity and geographic distance. Samples were combined for analysis across both years because microbial diversity and host diversity did not vary between years (Figures S 6 and S 7). We divided the hosts into social (those in the genus *Apis* and *Bombus*) or solitary (those in the genus *Andrena*, *Anthophora*, *Megachile*, and *Melissodes*). Within each genus, we included only individual species with at least 20 individuals collected in all downstream analyses (Table S 5). We computed an individual Bray-Curtis dissimilarity matrix for the microbe communities in each of the four groups (social strongly host-associated microbes, social weakly host-associated microbes, solitary strongly host-associated microbes, and solitary weakly host-associated microbes), fit exponential distance decay models, and tested for a correlation between dissimilarity and geographic distance for each using Mantel tests with 999 permutations (Oksanen et al. 2025; Baselga et al. 2023).

Distance decay of bee-microbe interaction network similarity

To assess the host's influence on microbial distance decay, we calculated the Bray-Curtis dissimilarity of bee-microbe networks and tested its relationship with geographic distance using Bayesian linear models. This approach quantifies total network dissimilarity between meadows and further partitions it into additive components driving spatial network turnover (Figure 1 B; Novotny 2009; Poisot et al. 2012; Poisot 2022). Because we were interested in whole community turnover (hosts and microbes together) in addition to microbial turnover (microbes alone), we aggregated samples at the species-meadow level rather than the individual level. This allowed both unique and shared species among meadows, which is not possible for individual-level networks because an individual bee can only be in one meadow. The microbial turnover at the individual bee level was captured by the analysis in the previous section ("Distance decay of microbial similarity"), so this analysis incorporates host turnover as a biotic influence on microbial spatial patterns, providing a different perspective. We constructed bee-microbe networks from 16S ASV tables to assess microbial associations at the species-meadow level by averaging individual relative abundances for each bee species and scaling by their meadow-level abundance, yielding abundance-weighted networks (see above section "Constructing bee-microbe networks").

To examine how total network dissimilarity changes with distance, we fit four Bayesian linear models for social and solitary hosts paired with strong or weak associates using the brms package (version 2.23.0) (Bürkner 2017). We modeled total network dissimilarity as a function of geographic distance, with intercepts for meadow identity in each comparison drawn from common distributions (i.e., referred to as random effects in a frequentist framework) to account for the multiple comparisons to each meadow (see above section "Network dissimilarity-distance model specifications" for additional details). If 97% or more of the posterior was above or below zero, we considered this to be strong support for a relationship between the response and explanatory variable under consideration. If 95% or more of the posterior was above or below zero, we considered this to be some support.

Drivers of spatial network dissimilarity

To understand the relative contributions of species turnover versus interaction rewiring to spatial interaction dissimilarity, we employed a network decomposition approach (Fig. 1, BI; Poisot et al. 2012; Poisot 2022). Understanding this distinction is critical because it can help differentiate between spatial patterns due to changing interactions throughout space from patterns due to changing species composition, which provides important ecological context for observed spatial patterns ((Poisot 2022)). In other words, breaking interaction dissimilarity into these two components allows us to understand the extent to which interactions in different meadows vary because some interaction partners are not present at both locations (species turnover component) (Fig. 1, BIII-V), or if the interactions are different because interacting species that are present at both locations have changed their interaction patterns between meadows (interaction rewiring component) (Fig. 1, BII; Trøjelsgaard et al. 2015). The species turnover component can be partitioned further to contextualize which interaction partners drive spatial turnover.

In essence, species turnover between two meadows can occur because 1) bee species are found exclusively in one meadow but not the other (host-driven turnover) (Fig. 1, BIII), 2) microbe ASVs are found only in one meadow but not the other (microbe-driven turnover) (Fig. 1, BIV), or 3) bee-microbe partners occurring in only one of the meadows can be completely missing from the second (complete species turnover) (Fig. 1, BV) (Poisot et al. 2012; Trøjelsgaard et al. 2015). To assess statistical support for the relationship between geographic distance and the bee-microbe network dissimilarity components, we fit individual models with each component of network dissimilarity as a response variable using the brms package (Bürkner 2017) (see above section "Network dissimilarity-distance model specifications" for details).

Results

We successfully sequenced the gut microbial community of 704 individual hosts across our six focal genera (Table S 5). After quality filtering and removal of controls, sequencing depths per sample ranged from 2,342 to 244,862 reads, with a mean of 27,544.7 $\pm$ 27,051.49 reads and a median of 20,495.5 reads. Across all specimens, we recovered 380

unique ASVs. Strongly host-associated microbe families were found in both social and solitary bees, but their presence did vary across genera (Fig. S 4). *Lactobacillaceae* and *Acetobacteriaceae*, strongly host-associate microbes of both social and solitary hosts, were found in multiple samples per genus, across multiple meadows, although there were some differences in which ASVs were present. For example, *Lactobacillus apis*, a strain originally isolated from honey bees, was present in all meadows in *Apis* hosts (Table 3). However for *Megachile* and *Bombus* hosts, it was only found at one and five meadows, respectively. Each host genus had at least one strongly host-associated ASV present in all meadows where it was collected (Table 3). Some *Lactobacillus* ASVs were common across nearly every meadow and all genera, while others were restricted to a few meadows (Fig. S 4). Additionally, geographically widespread clades outside the families defined as strongly host-associated were identified (Fig. S 4A–F, black bars), suggesting additional ASVs may be ubiquitous across bee gut communities and meadows.

Distance decay of microbial community dissimilarity

For both strongly and weakly host-associated microbes of social hosts, our distance decay models supported a shallow, positive relationship between Bray-Curtis dissimilarity and geographic distance (strongly host-associated: mantel $r = 0.1453$, $p = 0.001$; weakly host-associated: mantel $r = 0.1432$, $p = 0.001$) (Fig. 2A), indicating that both types of microbiomes become more dissimilar with increased spatial distance. The strongly and weakly host-associated microbes of solitary hosts similarly showed a shallow, positive distance decay relationship (strongly host-associated: mantel $r = 0.1169$, $p = 0.001$; weakly host-associated: mantel $r = 0.186$, $p = 0.001$) (Fig. 2B). Distance decay model estimates for strongly- and weakly host-associated microbes in social or solitary bees were not significantly different, indicating that host-associated microbes in this system consistently follow a shallow distance decay of similarity relationship, regardless of host sociality or strength of microbial-host association.

Distance decay of bee-microbe network dissimilarity

In contrast, our models investigating network dissimilarity over geographic distance did not support a spatial relationship between total network dissimilarity and distance for the microbe-bee networks of social hosts, for both strongly and weakly host-associated microbes (Fig. 2C, Table 2), indicating that for social hosts, the dissimilarity of both their strongly and weakly host-associated microbes were not spatially structured at the individual host level. For solitary hosts, strongly host-associated microbes also did not show a spatial relationship between network dissimilarity and geographic distance, but we did detect a positive distance-dissimilarity relationship for the weakly host-associated microbes (Fig. 2D, Table 2), indicating that solitary bee interactions with weakly host-associated microbes become more dissimilar with increased distance.

Drivers of social host-microbe network dissimilarity

For social genera, our models found strong support for a geographic structuring of dissimilarity via rewiring for strongly host-associated microbe networks, which decreased with distance, but not for weakly host-associated microbe networks (Fig. 3A, Table 2). Additionally, our models provided strong support for a positive distance-dissimilarity relationship due to complete turnover for social hosts (Fig. 3B, Table 2). We did not find evidence supporting geographic structuring via host-driven turnover or microbe-driven turnover in the social strongly host-associated models (Fig. 3C–D, Table 2). Social weakly host-associated community models revealed geographic structuring exclusively via a negative contribution of microbe-driven turnover (Fig. 3D, Table 2). For these networks, turnover of microbe partners contributed more to dissimilarity at nearby meadows, while turnover of hosts and microbes together accounted for more dissimilarity between distant meadows.

Drivers of solitary host-microbe network dissimilarity

In solitary host-microbe networks, the contribution to dissimilarity by rewiring was negatively related to geographic distance for both strongly host-associated and weakly host-associated microbes (Fig. 4A, Table 2), indicating that partner switching contributed more to dissimilarity at nearby meadows compared to distant meadows for both host-association types. However, the contribution to dissimilarity by complete turnover was geographically-structured for only weakly host-associated microbes, suggesting that the weakly host-associated microbes turnover in tandem with their hosts, however this represents only a small fraction of total dissimilarity (Fig. 4B, Table 2). For both strongly and weakly host-associated microbes of solitary bees, the contribution of host-driven turnover to dissimilarity increased with geographic distance (Fig. 4C, Table 2), indicating that host-driven turnover contributes more to dissimilarity at greater spatial distances in solitary hosts no matter if microbes are strongly or weakly host-associated, implicating host identity as a consistent determinant of spatial dissimilarity for solitary hosts. The dissimilarity due to microbe-driven turnover was not related to geographic distance for either strongly or weakly host-associated microbes in solitary bees (Fig. 4D, Table 2). For the strongly host-associated microbes of solitary bees, the opposing contributions of rewiring and host-driven turnover resulted in the lack of total interaction turnover over distance (Fig. 2D). However, the significant distance-dissimilarity relationship for weakly host-associated microbes of solitary bees was driven by complete turnover (Figs. 2D, 4).

Discussion

We investigated the biogeography of weakly and strongly host-associated microbial communities by examining microbial dissimilarity and network dissimilarity decomposition, offering new insights into the processes underlying distance–decay in community similarity. Microbial communities of social and solitary bees became increasingly dissimilar with distance, consistent with patterns in plants, animals, and free-living microbes (Nekola and

White 1999; Hillebrand 2004; Soininen et al. 2007; Suzzi et al. 2023), though slopes were shallow as in other microbial studies (Meyer et al. 2018). Slopes were similar across host types and association strengths, but decomposition revealed both distinct and shared underlying processes: in social hosts, network dissimilarity was structured by rewiring, complete turnover, and microbe-driven turnover, whereas in solitary hosts it was shaped by rewiring, host-driven turnover, and complete turnover. Thus, similar spatial patterns can emerge from different ecological processes, as shown previously (Brown et al. 2011; Hanson et al. 2012).

Shallow slopes likely reflect both host and methodological factors. Strongly host-associated microbe communities may be stabilized by host-related filtering, consistent with evidence that environmental variables play a smaller role in strongly host-associated microbiomes rather than free-living weakly host-associated microbiomes (Suzzi et al. 2023). Weakly host-associated microbes, being environmentally derived, show broad distributions with limited host dependence. While our study cannot yet distinguish between environmental filtering and dispersal limitation, it provides a framework for future work on host-associated microbial spatial structure.

Spatial structuring of total dissimilarity: divergent turnover mechanisms can negate overall spatial signals

Interaction network dissimilarity revealed more nuanced spatial patterns than composition alone. Distance–decay in interaction dissimilarity was observed only in weakly host-associated microbes for solitary bees, whereas strongly host-associated microbes for solitary bees and both weakly and strongly host-associated microbes for social bees exhibited no overall spatial signal. This suggests that although microbial communities shift across space, many host–microbe associations persist at the host community level, particularly in social bees. Behaviors such as trophallaxis and ecological filters like gut morphology stabilize associations, homogenizing microbiomes within and across generations (Sarkar et al. 2020; Meurville and LeBoeuf 2021; Schmidt and Engel 2021). While total interaction dissimilarity alone quantifies differences, decomposition reveals the ecological mechanisms underlying them, which we found varied systematically with host sociality and microbial lifestyle. Specifically, we found that different turnover components often canceled out, masking spatial signals in total dissimilarity. This means that significant spatial relationships for individual dissimilarity components, when directionally-opposed, can give the appearance of no net interaction turnover throughout space. For example, strongly host- associated microbes of social bees showed decreasing rewiring throughout space but increasing complete turnover with distance. Similarly, strongly host-associated microbes of solitary bees showed decreasing rewiring throughout space but increasing host-driven turnover. In both cases, there was no net interaction dissimilarity over distance, even though we saw spatial relationships for some individual drivers of interaction dissimilarity. While the above opposing dissimilarity components (rewiring versus complete turnover and rewiring versus host-driven turnover) are not necessarily fundamentally opposite ecological processes, this study highlights the

compensatory roles that different dissimilarity components can play in community structuring and their ability to obscure spatial patterns when individual spatial relationships are in opposite directions.

Rewiring, host-driven turnover, and complete turnover influence spatial structuring of solitary associate networks

In solitary strongly host-associated networks, rewiring declined while host-driven turnover increased with distance, canceling each other and producing no overall signal. This could be explained by the fact that strongly host-associated microbes may switch among hosts locally, but such rewiring may become rarer at greater distances, potentially due to environmental differences, dispersal limits, or phylogenetic divergence among distant hosts (Handy et al. 2023; Borah and Beckman 2025; Fuzessy and Pizo 2025). Strongly host-associated microbes of solitary hosts may also be less specialized than those of social hosts, relying more on opportunistic transmission (Voulgari-Kokota et al. 2019). However, the ecological meaning assigned to rewiring comes with some uncertainty, as apparent shifts in interaction structure could potentially result from stochastic variation in relative abundance data rather than true reshuffling of host-microbe interactions. Still, because we detected a significant decline in the dissimilarity due to rewiring along our spatial gradient in all but the weak associates of social bees, it does appear that there is spatial structuring attributed to the rewiring component. Whether this is due to spatially-structured shifts in relative abundance within a host, or due to true reorganization of interactions between sites, our results indicate that changes in interactions among shared host-microbe partners between sites contributes to spatial dissimilarity.

For solitary weakly host-associated microbes, the contribution of rewiring to spatial dissimilarity was shallower compared to the solitary strongly host-associated microbes, consistent with their environmental acquisition and transient nature. By contrast, host-driven turnover increased with distance for both strongly and weakly host-associated microbes, reflecting host community turnover and echoing the “hosts as islands” concept. Solitary hosts differ more strongly among sites than social hosts, likely due to the lack of social transmission, making host identity a primary filter of microbial composition (Voulgari-Kokota et al. 2019). Although host turnover was higher in solitary genera compared to social bees (Fig. S 9), it remains unclear whether host-driven turnover is genuinely stronger in solitary systems or undetectable in social hosts here. Overall, decomposing dissimilarity shows that while compositional turnover was similar across social and solitary hosts and strongly and weakly-associated microbes, the underlying mechanisms differ.

Rewiring, complete turnover, and microbe-driven turnover influence spatial structuring of social associate networks

Social strongly host-associated networks exhibited spatial structure from rewiring and complete turnover. Rewiring was also a negative driver of spatial dissimilarity for the

strongly and weakly host-associated microbes of solitary hosts, reflecting the importance of host switching as a determinant of microbial dissimilarity at smaller spatial scales. Complete turnover increased with distance, suggesting host filtering shapes social strongly host-associated microbe biogeography (Mallott and Amato 2021). This may be due to the fact that despite limited overall host turnover, particular host species differed in traits and behaviors that influenced which strains of strong associates were able to persist, producing paired host–microbe turnover. Such filtering is consistent with central-place foraging and spatial variation in floral or nesting resources (Ogilvie and Forrest 2017). Microbe-driven turnover structured weakly host-associated microbes of social bees, with higher turnover at shorter rather than longer distances. When hosts were similar across nearby meadows, dissimilarity arose primarily from local microbial differences, suggesting that environmental heterogeneity drives this pattern. Localized gut microbial communities are documented in other studies (Couch and Epps 2022), underscoring the role of fine-scale environmental variation in shaping larger spatial patterns. Alternatively, among similar hosts, dispersal limitation is expected to be reduced for all gut microbes, so particular microbial identity may play an outsize role in assembly at the local scale. Given the scope of this study, we do not disentangle the relative roles of dispersal limitation and environmental heterogeneity, but suggest this investigation for future work.

Establishing the "environment" and a framework for host–microbe biogeography

A key challenge in applying ecological assembly theory to host-associated systems is defining what constitutes the "environment." For host-associated microbes, the environment spans two nested scales: the external (e.g. landscape, floral resources, soil microbes) and the internal host environment (e.g. gut physiology, immune function). These scales interact — for example, external environmental conditions influence host diets, which in turn shape gut microbiota (Youngblut et al. 2019). Our findings suggest both scales are important: spatial changes in host composition and host filtering both shape microbial presence and interactions. These dynamics highlight the host's role not only as a passive substrate but also as an active ecological filter that shapes microbial associations. Additionally, host-associated microbial distance decay patterns could potentially be obscured by undersampling, similar to free-living microbes and macroorganisms, but we do not explicitly explore the role of undersampling in this study (Meyer et al. 2018; Cam et al. 2002; Clark et al. 2021). These outstanding challenges highlight the necessity for further work to develop maximally informative methods and perspectives to study host-associated microbial biogeography.

Conclusion

This study underscores the importance of distinguishing among different types of host-microbe relationships in understanding microbial biogeography. Although strongly and weakly host-associated taxa exhibited similar spatial turnover rates at the compositional

level, the processes driving these patterns were markedly different. Likewise, the drivers of spatial dissimilarity differed between hosts with varying socialities. Without accounting for variation in host dependence among microbes and ecological differences in host interactions, we risk masking critical ecological mechanisms behind community structure. By jointly analyzing compositional and interaction dissimilarity and decomposing the latter into meaningful ecological components, we reveal how different processes operate across a spectrum of microbial lifestyles and host social systems. In doing so, we take an important step toward a more mechanistic and predictive understanding of host-associated microbial biogeography.

Acknowledgements

We thank Martin Hauser, Robbin Thorp, Terry Griswold, John Ascher, Kristal Watrous, Jason Gibbs, Joel Gardner, and Lincoln Best for bee specimen identification, and Hamutahl Cohen and Jocelyn Zorn for specimen and 16S library preparation. Field collections were made possible by Hanna Scardina, Julie Fowler, Kaysee Arrowsmith, Marilia Gaiarsa, Magda Argueta Guzman, Jess Mullin, Paul Markley, Dayna de la Cruz, Stefania Steele, Corey Andrikopoulos, Claire Healey, Hamutahl Cohen, Alejandro Santillana-Fernandez, and Melory Brandao.

Author Contributions

Rebecca A. Hayes: Conceptualization, Data curation, Investigation, Methodology, Formal analysis, Validation, Visualization, Writing - original draft, Writing - review and editing Brendan J. M. Bohannan: Conceptualization, Methodology, Writing - review and editing Shalene Jha: Conceptualization, Methodology, Project administration, Funding acquisition, Writing - review and editing Lauren C. Ponisio: Conceptualization, Data curation, Methodology, Project administration, Supervision, Funding acquisition, Writing - review and editing

Data Accessibility

Raw data, code, and data products for reproducing all analyses are on Github (https://github.com/hayesrebecca/microbeBiogeography), enabling full replication of figures and statistics. Microbial sequence data are available from NCBI SRA BioProject PRJNA1269117.

Artificial Intelligence (AI) Use Statement

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.

The authors used ChatGPT 5.5 for grammar and style suggestions to improve readability of original manuscript text.

Conflict of Interest Statement

The authors have declared that no competing interests exist.

Funding Statement

This work was funded by NSF PCE Grant 2009075 to SJ and LCP. RAH was supported by NSF GRFP 2137533. BJMB thanks the Gordon and Betty Moore Foundation (https://doi.org/10.37807/GBMF10001).

Bibliography

Adler, Lynn S, Rebecca E Irwin, Scott H McArt, and Rachel L Vannette. 2021. "Floral Traits Affecting the Transmission of Beneficial and Pathogenic Pollinator-Associated Microbes." *Current Opinion in Insect Science* 44: 1–7.

Baker, Allan J. 2008. "Islands in the Sky: The Impact of Pleistocene Climate Cycles on Biodiversity." *Journal of Biology* 7: 32.

Baselga, Andres, David Orme, Sebastien Villeger, et al. 2023. *Betapart: Partitioning Beta Diversity into Turnover and Nestedness Components*. https://CRAN.R-project.org/package=betapart.

Bokulich, Nicholas A, Benjamin D Kaehler, Jai Ram Rideout, et al. 2018. "Optimizing Taxonomic Classification of Marker-Gene Amplicon Sequences with QIIME 2's Q2-Feature-Classifier Plugin." *Microbiome* 6 (1): 1–17.

Bolyen, Evan, Jai Ram Rideout, Matthew R Dillon, et al. 2019. "Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2." *Nature Biotechnology* 37 (8): 852–57.

Borah, Binod, and Noelle G Beckman. 2025. "Interaction Rewiring Can Buffer the Reduction of Long-Distance Dispersal of Seeds When Large Birds Go Extinct: An Allometric Perspective." *Oikos* 2025 (3): e10946.

Brown, Calum, Richard Law, Janine B Illian, and David FRP Burslem. 2011. "Linking Ecological Processes with Spatial and Non-Spatial Patterns in Plant Communities." *Journal of Ecology* 99 (6): 1402–14.

Burkle, Laura A, John C Marlin, and Tiffany M Knight. 2013. "Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function." *Science* 339 (6127): 1611–15.

Burkle, Laura, and Rebecca Irwin. 2009. "The Importance of Interannual Variation and Bottom–up Nitrogen Enrichment for Plant–Pollinator Networks." *Oikos* 118 (12): 1816–29.

Bürkner, Paul-Christian. 2017. “brms: An R Package for Bayesian Multilevel Models Using Stan.” *Journal of Statistical Software* 80 (1): 1–28. https://doi.org/10.18637/jss.v080.i01.

Callahan, Benjamin J, Paul J McMurdie, Michael J Rosen, Andrew W Han, Amy Jo A Johnson, and Susan P Holmes. 2016. “DADA2: High-Resolution Sample Inference from Illumina Amplicon Data.” *Nature Methods* 13 (7): 581–83.

Cam, Emmanuelle, James D Nichols, James E Hines, John R Sauer, Russell Alpizar-Jara, and Curtis H Flather. 2002. “Disentangling Sampling and Ecological Explanations Underlying Species–Area Relationships.” *Ecology* 83 (4): 1118–30.

Cameron, Sydney A, JB Whitfield, CL Hulslander, WA Cresko, SB Isenberg, and RW King. 1996. “Nesting Biology and Foraging Patterns of the Solitary Bee Melissodes Rustica (Hymenoptera: Apidae) in Northwest Arkansas.” *Journal of the Kansas Entomological Society*, 260–73.

CaraDonna, Paul J, William K Petry, Ross M Brennan, et al. 2017. “Interaction Rewiring and the Rapid Turnover of Plant–Pollinator Networks.” *Ecology Letters* 20 (3): 385–94.

Cariveau, Daniel P, J Elijah Powell, Hauke Koch, Rachael Winfree, and Nancy A Moran. 2014. “Variation in Gut Microbial Communities and Its Association with Pathogen Infection in Wild Bumble Bees (Bombus).” *The ISME Journal* 8 (12): 2369–79.

Cellini, Antonio, Valentino Giacomuzzi, Irene Donati, et al. 2019. “Pathogen-Induced Changes in Floral Scent May Increase Honeybee-Mediated Dispersal of Erwinia Amylovora.” *The ISME Journal* 13 (4): 847–59.

Clark, Dave R, Graham JC Underwood, Terry J McGenity, and Alex J Dumbrell. 2021. “What Drives Study-Dependent Differences in Distance–Decay Relationships of Microbial Communities?” *Global Ecology and Biogeography* 30 (4): 811–25.

Clement, Stephen L. 1973. “The Nesting Biology of Melissodes (Eumelissodes) Rustica (Say), with a Description of the Larva (Hymenoptera: Anthophoridae).” *Journal of the Kansas Entomological Society*, 516–25.

Couch, Claire E, and Clinton W Epps. 2022. “Host, Microbiome, and Complex Space: Applying Population and Landscape Genetic Approaches to Gut Microbiome Research in Wild Populations.” *Journal of Heredity* 113 (3): 221–34.

Danforth, Bryan N., Robert L. Minckley, and John L. Neff. 2019. *The Solitary Bees: Biology, Evolution, Conservation*. Princeton University Press. http://www.jstor.org/stable/j.ctvd1c929.

Davis Jr, Lloyd R, and Wallace E LaBerge. 1975. “The Nest Biology of the Bee Andrena (Ptilandrena) Erigeniae Robertson (Hymenoptera: Andrenidae).” *Biological Notes* 95.

735 Dickey, Jonathan R, Rachel A Swenie, Sophia C Turner, et al. 2021. “The Utility of
736 Macroecological Rules for Microbial Biogeography.” *Frontiers in Ecology and Evolution* 9:
737 633155.

738 Doeke, Mehmet Ali, Maryann Frazier, and Christina M Grozinger. 2015. “Overwintering
739 Honey Bees: Biology and Management.” *Current Opinion in Insect Science* 10: 185–93.

740 Engel, Philipp, Rosalind R James, Ryuichi Koga, Waldan K Kwong, Quinn S McFrederick,
741 and Nancy A Moran. 2013. “Standard Methods for Research on *Apis Mellifera* Gut
742 Symbionts.” *Journal of Apicultural Research* 52 (4): 1–24.

743 Engelstädter, Jan, and Gregory DD Hurst. 2009. “The Ecology and Evolution of Microbes
744 That Manipulate Host Reproduction.” *Annual Review of Ecology, Evolution, and*
745 *Systematics* 40: 127–49.

746 Figueroa, Laura L, Ben M Sadd, Amber D Tripodi, et al. 2023. “Endosymbionts That
747 Threaten Commercially Raised and Wild Bumble Bees (Bombus Spp.).” *Journal of*
748 *Pollination Ecology* 33: 14.

749 Freedman, Elisha H, Oliva K Davies, Mark I Stevens, and James B Dorey. 2024.
750 “Widespread Wolbachia Infection Is Correlated with Increased mtDNA Diversity in Native
751 Bees Across the Fijian Archipelago.” *Symbiosis* 94 (1): 19–29.

752 Fuzessy, Lisieux Franco, and Marco Aurélio Pizo. 2025. “Navigating a Changing World: On
753 the Significance of Rewiring for Mutualistic Interactions, Caveats and Future Directions.”
754 *Oikos*, e11230.

755 Gilliam, Martha, Stephen L Buchmann, and Brenda J Lorenz. 1984. “Microbial Flora of the
756 Larval Provisions of the Solitary Bees, Centris Pallida and Anthophora Sp.” *Apidologie* 15
757 (1): 1–10.

758 Goodrich, Julia K, Emily R Davenport, Jillian L Waters, Andrew G Clark, and Ruth E Ley.
759 2016. “Cross-Species Comparisons of Host Genetic Associations with the Microbiome.”
760 *Science* 352 (6285): 532–35.

761 Graystock, Peter, Sandra M Rehan, and Quinn S McFrederick. 2017. “Hunting for Healthy
762 Microbiomes: Determining the Core Microbiomes of Ceratina, Megalopta, and Apis Bees
763 and How They Associate with Microbes in Bee Collected Pollen.” *Conservation Genetics*
764 18: 701–11.

765 Green, Jessica L, Andrew J Holmes, Mark Westoby, et al. 2004. “Spatial Scaling of
766 Microbial Eukaryote Diversity.” *Nature* 432 (7018): 747–50.

767 Griswold, Terry, James Herndon, and Victor Gonzalez. 2015. “First Record of the Orchid
768 Bee Genus Eufriesea Cockerell (Hymenoptera: Apidae: Euglossini) in the United States.”
769 *Zootaxa* 3957 (August): 342–46. https://doi.org/10.11646/zootaxa.3957.3.7.

770 Handy, Madeline Y, Dino L Sbardellati, Michael Yu, Nicholas W Saleh, Madeleine M
771 Ostwald, and Rachel L Vannette. 2023. “Incipiently Social Carpenter Bees (Xylocopa) Host
772 Distinctive Gut Bacterial Communities and Display Geographical Structure as Revealed by
773 Full-Length PacBio 16S rRNA Sequencing.” *Molecular Ecology* 32 (6): 1530–43.

774 Hanshew, Alissa S, Charles J Mason, Kenneth F Raffa, and Cameron R Currie. 2013.
775 “Minimization of Chloroplast Contamination in 16S rRNA Gene Pyrosequencing of Insect
776 Herbivore Bacterial Communities.” *Journal of Microbiological Methods* 95 (2): 149–55.

777 Hanson, China A, Jed A Fuhrman, M Claire Horner-Devine, and Jennifer BH Martiny. 2012.
778 “Beyond Biogeographic Patterns: Processes Shaping the Microbial Landscape.” *Nature*
779 *Reviews Microbiology* 10 (7): 497–506.

780 Härer, Andreas, and Diana J Rennison. 2023. “The Biogeography of Host-Associated
781 Bacterial Microbiomes: Revisiting Classic Biodiversity Patterns.” *Global Ecology and*
782 *Biogeography* 32 (6): 931–44.

783 Harrison, Susan, Sally J Ross, and John H Lawton. 1992. “Beta Diversity on Geographic
784 Gradients in Britain.” *Journal of Animal Ecology*, 151–58.

785 Hillebrand, Helmut. 2004. “On the Generality of the Latitudinal Diversity Gradient.” *The*
786 *American Naturalist* 163 (2): 192–211.

787 Johnson, Jethro S, Daniel J Spakowicz, Bo-Young Hong, et al. 2019. “Evaluation of 16S
788 rRNA Gene Sequencing for Species and Strain-Level Microbiome Analysis.” *Nature*
789 *Communications* 10 (1): 5029.

790 Katoh, Kazutaka, and Daron M Standley. 2013. “MAFFT Multiple Sequence Alignment
791 Software Version 7: Improvements in Performance and Usability.” *Molecular Biology and*
792 *Evolution* 30 (4): 772–80.

793 Keller, Alexander, Quinn S McFrederick, Prarthana Dharampal, Shawn Steffan, Bryan N
794 Danforth, and Sara D Leonhardt. 2021. “(More Than) Hitchhikers Through the Network: The
795 Shared Microbiome of Bees and Flowers.” *Current Opinion in Insect Science* 44: 8–15.

796 Kembel, Steven W, Timothy K O’Connor, Holly K Arnold, Stephen P Hubbell, S Joseph
797 Wright, and Jessica L Green. 2014. “Relationships Between Phyllosphere Bacterial
798 Communities and Plant Functional Traits in a Neotropical Forest.” *Proceedings of the*
799 *Natural Academy of Sciences U.S.A* 111 (38): 13715–20.

800 Kowallik, Vienna, and Alexander S Mikheyev. 2021. “Honey Bee Larval and Adult
801 Microbiome Life Stages Are Effectively Decoupled with Vertical Transmission Overcoming
802 Early Life Perturbations.” *MBio* 12 (6): e02966–21.

803 Kucuk, RA. 2020. “Gut Bacteria in the Holometabola: A Review of Obligate and Facultative
804 Symbionts.” *Journal of Insect Science* 20 (4): 22.

Kuris, Armand M, Andrew R Blaustein, and Jose Javier Alio. 1980. “Hosts as Islands.” *The American Naturalist* 116 (4): 570–86.

Kwong, Waldan K, Luis A Medina, Hauke Koch, et al. 2017. “Dynamic Microbiome Evolution in Social Bees.” *Science Advances* 3 (3): e1600513.

Kwong, Waldan K, and Nancy A Moran. 2016. “Gut Microbial Communities of Social Bees.” *Nature Reviews Microbiology* 14 (6): 374–84.

Lang, Haoyu, Huijuan Duan, Jieni Wang, et al. 2022. “Specific Strains of Honeybee Gut Lactobacillus Stimulate Host Immune System to Protect Against Pathogenic Hafnia Alvei.” *Microbiology Spectrum* 10 (1): e01896–21.

Louca, Stilianos, Martin F Polz, Florent Mazel, et al. 2018. “Function and Functional Redundancy in Microbial Systems.” *Nature Ecology and Evolution* 2 (6): 936–43.

MacArthur, Robert H, and Edward O Wilson. 1967. *The Theory of Island Biogeography*. Vol. 1. Princeton university press.

Magrach, Ainhoa, Maddi Artamendi, Paula Dominguez Lapido, Clara Parejo, and Encarnacion Rubio. 2023. “Indirect Interactions Between Pollinators Drive Interaction Rewiring Through Space.” *Ecosphere* 14 (6): e4521.

Malard, Lucie A, and David A Pearce. 2022. “Bacterial Colonisation: From Airborne Dispersal to Integration Within the Soil Community.” *Frontiers in Microbiology* 13: 782789.

Mallott, Elizabeth K, and Katherine R Amato. 2021. “Host Specificity of the Gut Microbiome.” *Nature Reviews Microbiology* 19 (10): 639–53.

Martinson, Vincent G, Bryan N Danforth, Robert L Minckley, Olav Rueppell, Salim Tingek, and Nancy A Moran. 2011. “A Simple and Distinctive Microbiota Associated with Honey Bees and Bumble Bees.” *Molecular Ecology* 20 (3): 619–28.

Martiny, Jennifer BH, Stuart E Jones, Jay T Lennon, and Adam C Martiny. 2015. “Microbiomes in Light of Traits: A Phylogenetic Perspective.” *Science* 350 (6261): aac9323.

McCormack, John E, Huateng Huang, and L Lacey Knowles. 2009. “Sky Islands.” *Encyclopedia of Islands* 4: 841–43.

McFall-Ngai, Margaret, Michael G Hadfield, Thomas CG Bosch, et al. 2013. “Animals in a Bacterial World, a New Imperative for the Life Sciences.” *Proceedings of the National Academy of Sciences* 110 (9): 3229–36.

McFrederick, Quinn S, and Sandra M Rehan. 2016. “Characterization of Pollen and Bacterial Community Composition in Brood Provisions of a Small Carpenter Bee.” *Molecular Ecology* 25 (10): 2302–11.

McFrederick, Quinn S, and Sandra M Rehan. 2019. “Wild Bee Pollen Usage and Microbial Communities Co-Vary Across Landscapes.” *Microbial Ecology* 77 (2): 513–22.

McFrederick, Quinn S, Jason M Thomas, John L Neff, et al. 2017. “Flowers and Wild Megachilid Bees Share Microbes.” *Microbial Ecology* 73: 188–200.

McFrederick, Quinn S, William T Wcislo, Michael C Hout, and Ulrich G Mueller. 2014. “Host Species and Developmental Stage, but Not Host Social Structure, Affects Bacterial Community Structure in Socially Polymorphic Bees.” *FEMS Microbiology Ecology* 88 (2): 398–406.

McLaughlin, Steven P. 1994. “An Overview of the Flora of the Sky Islands, Southeastern Arizona: Diversity, Affinities, and Insularity.” *United States Department of Agriculture Forest Service General Technical Report RM*, 60–70.

Mee, Lauren, and Seth M Barribeau. 2023. “Influence of Social Lifestyles on Host–Microbe Symbioses in the Bees.” *Ecology and Evolution* 13 (11): e10679.

Meeus, Ivan, Laurian Parmentier, Annelies Billiet, et al. 2015. “16S rRNA Amplicon Sequencing Demonstrates That Indoor-Reared Bumblebees (Bombus Terrestris) Harbor a Core Subset of Bacteria Normally Associated with the Wild Host.” *PLOS One* 10 (4): e0125152.

Meurville, Marie-Pierre, and Adria C LeBoeuf. 2021. “Trophallaxis: The Functions and Evolution of Social Fluid Exchange in Ant Colonies (Hymenoptera: Formicidae).” *Myrmecological News* 31.

Meyer, Kyle M, Hervé Memiaghe, Lisa Korte, David Kenfack, Alfonso Alonso, and Brendan JM Bohannan. 2018. “Why Do Microbes Exhibit Weak Biogeographic Patterns?” *The ISME Journal* 12 (6): 1404–13.

Meynard, Christine N, Sébastien Lavergne, Isabelle Boulangeat, et al. 2013. “Disentangling the Drivers of Metacommunity Structure Across Spatial Scales.” *Journal of Biogeography* 40 (8): 1560–71.

Michener, Charles D. 1953. “The Biology of a Leafcutter Bee (Megachile Brevis) and Its Associates.” *University of Kansas Science Bulletin* 35 (3): 1659.

Mockler, Blair K, Waldan K Kwong, Nancy A Moran, and Hauke Koch. 2018. “Microbiome Structure Influences Infection by the Parasite Crithidia Bombi in Bumble Bees.” *Applied and Environmental Microbiology* 84 (7): e02335–17.

Morelan, Ian A, Christopher A Gaulke, Thomas J Sharpton, Rebecca Vega Thurber, and Dee R Denver. 2019. “Microbiome Variation in an Intertidal Sea Anemone Across Latitudes and Symbiotic States.” *Frontiers in Marine Science* 6: 7.

Mukhtar, Salma, M Amine Hassani, Tracy Zarrillo, Zhouqi Cui, George W Sundin, and Quan Zeng. 2024. “The Role of Foraging Pollinators in Assembling the Flower Microbiota and

874 Transmitting the Fire Blight Pathogen Erwinia Amylovora." *Environmental Microbiology* 26
875 (10): e16702.

876 Nekola, Jeffrey C, and Peter S White. 1999. "The Distance Decay of Similarity in
877 Biogeography and Ecology." *Journal of Biogeography* 26 (4): 867–78.

878 Nelson, Annika S, McKenna J Larson, and Tobin J Hammer. 2025. "Core Symbionts, Age at
879 Inoculation and Diet Affect Colonization of the Bumblebee Gut by a Common Bacterial
880 Pathogen." *Journal of Animal Ecology* 94 (5): 985–98.

881 Neu, Alexander T, Eric E Allen, and Kaustuv Roy. 2021. "Do Host-Associated Microbes
882 Show a Contrarian Latitudinal Diversity Gradient? Insights from Mytilus Californianus, an
883 Intertidal Foundation Host." *Journal of Biogeography* 48 (11): 2839–52.

884 Norden, Beth B. 1984. "Nesting Biology of Anthophora Abrupta (Hymenoptera:
885 Anthophoridae)." *Journal of the Kansas Entomological Society*, 243–62.

886 Novotny, Vojtech. 2009. "Beta Diversity of Plant–Insect Food Webs in Tropical Forests: A
887 Conceptual Framework." *Insect Conservation and Diversity* 2 (1): 5–9.

888 Ogilvie, Jane E, and Jessica RK Forrest. 2017. "Interactions Between Bee Foraging and
889 Floral Resource Phenology Shape Bee Populations and Communities." *Current Opinion in*
890 *Insect Science* 21: 75–82.

891 Oksanen, Jari, Gavin L. Simpson, F. Guillaume Blanchet, et al. 2025. *Vegan: Community*
892 *Ecology Package*. https://vegandevs.github.io/vegan/.

893 Orr, Michael C, Alice C Hughes, Douglas Chesters, John Pickering, Chao-Dong Zhu, and
894 John S Ascher. 2021. "Global Patterns and Drivers of Bee Distribution." *Current Biology* 31
895 (3): 451–58.

896 Parfrey, Laura Wegener, Corrie S Moreau, and Jacob A Russell. 2018. "Introduction: The
897 Host-Associated Microbiome: Pattern, Process and Function." *Molecular Ecology* 27 (8).

898 Parker, Aimée, Melissa AE Lawson, Laura Vaux, and Carmen Pin. 2018. "Host-Microbe
899 Interaction in the Gastrointestinal Tract." *Environmental Microbiology* 20 (7): 2337–53.

900 Plowright, RC, and TM Laverty. 1984. "The Ecology and Sociobiology of Bumble Bees."
901 *Annual Review of Entomology* 29 (1): 175–99.

902 Poisot, Timothée. 2022. "Dissimilarity of Species Interaction Networks: Quantifying the
903 Effect of Turnover and Rewiring." *Peer Community Journal* 2.

904 Poisot, Timothée, Elsa Canard, David Mouillot, Nicolas Mouquet, and Dominique Gravel.
905 2012. "The Dissimilarity of Species Interaction Networks." *Ecology Letters* 15 (12): 1353–61.

906 Poisot, Timothée, Daniel B Stouffer, and Dominique Gravel. 2015. "Beyond Species: Why
907 Ecological Interaction Networks Vary Through Space and Time." *Oikos* 124 (3): 243–51.

Praet, Jessy, Anneleen Parmentier, Regula Schmid-Hempel, Ivan Meeus, Guy Smagghe, and Peter Vandamme. 2018. “Large-Scale Cultivation of the Bumblebee Gut Microbiota Reveals an Underestimated Bacterial Species Diversity Capable of Pathogen Inhibition.” *Environmental Microbiology* 20 (1): 214–27.

Price, Morgan N, Paramvir S Dehal, and Adam P Arkin. 2010. “FastTree 2–Approximately Maximum-Likelihood Trees for Large Alignments.” *PLOS One* 5 (3): e9490.

Quast, Christian, Elmar Pruesse, Pelin Yilmaz, et al. 2012. “The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools.” *Nucleic Acids Research* 41 (D1): D590–96.

R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/.

Ramalho, Manuela de O, Zoe Kim, Steven Wang, and Corrie S Moreau. 2021. “Wolbachia Across Social Insects: Patterns and Implications.” *Annals of the Entomological Society of America* 114 (2): 206–18.

Ricigliano, Vincent A, William Fitz, Duan C Copeland, et al. 2017. “The Impact of Pollen Consumption on Honey Bee (Apis Mellifera) Digestive Physiology and Carbohydrate Metabolism.” *Archives of Insect Biochemistry and Physiology* 96 (2): e21406.

Roubik, David W. 2021. “Bees: Ecological Roles.” In *Encyclopedia of Social Insects*. Springer.

Rudman, Seth M, Sharon Greenblum, Rachel C Hughes, et al. 2019. “Microbiome Composition Shapes Rapid Genomic Adaptation of Drosophila Melanogaster.” *Proceedings of the National Academy of Sciences* 116 (40): 20025–32.

Sarkar, Amar, Siobhán Harty, Katerina V-A Johnson, et al. 2020. “Microbial Transmission in Animal Social Networks and the Social Microbiome.” *Nature Ecology and Evolution* 4 (8): 1020–35.

Schmidt, Konstantin, and Philipp Engel. 2021. “Mechanisms Underlying Gut Microbiota–Host Interactions in Insects.” *Journal of Experimental Biology* 224 (2): jeb207696.

Smith, Eric A, Hoang Q Vuong, Delaney L Miller, Audrey J Parish, Quinn S McFrederick, and Irene LG Newton. 2020. “Draft Genome Sequences of Four Saccharibacter Sp. Strains Isolated from Native Bees.” *Microbiology Resource Announcements* 9 (10): 10–1128.

Smith, Gordon P, Hamutahl Cohen, Jocelyn F Zorn, Quinn S McFrederick, and Lauren C Ponisio. 2024. “Plant–Pollinator Network Architecture Does Not Impact Intraspecific Microbiome Variability.” *Molecular Ecology* 33 (7): e17306.

Smith, Gordon P, Joel Gardner, Jason Gibbs, et al. 2021. “Sex-Associated Differences in the Network Roles of Pollinators.” *Ecosphere* 12 (12): e03863.

943 Soininen, Janne, Robert McDonald, and Helmut Hillebrand. 2007. “The Distance Decay of
944 Similarity in Ecological Communities.” *Ecography* 30 (1): 3–12.

945 Spector, Sacha. 2002. “Biogeographic Crossroads as Priority Areas for Biodiversity
946 Conservation.” *Conservation Biology* 16 (6): 1480–87.
947 https://doi.org/https://doi.org/10.1046/j.1523-1739.2002.00573.x.

948 Su, Qinzhi, Qinglin Wang, Xiaohuan Mu, et al. 2021. “Strain-Level Analysis Reveals the
949 Vertical Microbial Transmission During the Life Cycle of Bumblebee.” *Microbiome* 9: 1–14.

950 Suzzi, Alessandra L, Michael Stat, Troy F Gaston, and Megan J Huggett. 2023. “Spatial
951 Patterns in Host-Associated and Free-Living Bacterial Communities Across Six Temperate
952 Estuaries.” *FEMS Microbiology Ecology* 99 (7): fiad061.

953 Tiusanen, Mikko, Antoine Becker-Scarpitta, and Helena Wirta. 2024. “Distinct
954 Communities and Differing Dispersal Routes in Bacteria and Fungi of Honey Bees, Honey,
955 and Flowers.” *Microbial Ecology* 87 (1): 100.

956 Todorov, Svetoslav Dimitrov, Marcos Vinício Alves, Gisana Cristina Alves Bueno, Virgínia
957 Farias Alves, and Iskra Vitanova Ivanova. 2024. “Bee-Associated Beneficial Microbes—
958 Importance for Bees and for Humans.” *Insects* 15 (6): 430.

959 Trøjelsgaard, Kristian, Pedro Jordano, Daniel W Carstensen, and Jens M Olesen. 2015.
960 “Geographical Variation in Mutualistic Networks: Similarity, Turnover and Partner Fidelity.”
961 *Proceedings of the Royal Society B* 282 (1802): 20142925.

962 Vizentin-Bugoni, Jeferson, and Pietro Kiyoshi Maruyama. 2023. “To Rewire or Not to Rewire:
963 To What Extent Rewiring to Surviving Partners Can Avoid Extinction?” *Journal of Animal*
964 *Ecology* 92 (9): 1676–79.

965 Voulgari-Kokota, Anna, Quinn S McFrederick, Ingolf Steffan-Dewenter, and Alexander
966 Keller. 2019. “Drivers, Diversity, and Functions of the Solitary-Bee Microbiota.” *Trends in*
967 *Microbiology* 27 (12): 1034–44.

968 Wang, Kang, Ming Zheng, Minqi Cai, et al. 2024. “Possible Interactions Between Gut
969 Microbiome and Division of Labor in Honey Bees.” *Ecology and Evolution* 14 (8): e11707.

970 Williamson, Elisabeth, Kelly Hill, Katja Hogendoorn, and Raphael Eisenhofer. 2025. “The
971 Bacterial Community Associated with the Solitary Resin Bee Megachile Tosticauda
972 Throughout Its Life Cycle.” *FEMS Microbiology Ecology* 101 (4): fiaf023.

973 Winfree, Rachael, Neal M Williams, Jonathan Dushoff, and Claire Kremen. 2014. “Species
974 Abundance, Not Diet Breadth, Drives the Persistence of the Most Linked Pollinators as
975 Plant-Pollinator Networks Disassemble.” *The American Naturalist* 183 (5): 600–611.

976 Wright, Karen W, Kelly B Miller, and Hojun Song. 2020. “A Molecular Phylogeny of the Long-
977 Horned Bees in the Genus Melissodes Latreille (Hymenoptera: Apidae: Eucerinae).” *Insect*
978 *Systematics and Evolution* 52 (4): 428–43.

Wu, Jiaqiang, Haoyu Lang, Xiaohuan Mu, et al. 2021. “Honey Bee Genetics Shape the Strain-Level Structure of Gut Microbiota in Social Transmission.” *Microbiome* 9 (1): 225.

Youngblut, Nicholas D, Georg H Reischer, William Walters, et al. 2019. “Host Diet and Evolutionary History Explain Different Aspects of Gut Microbiome Diversity Among Vertebrate Clades.” *Nature Communications* 10 (1): 1–15.

Yun, Ji-Hyun, Mi-Ja Jung, Pil Soo Kim, and Jin-Woo Bae. 2018. “Social Status Shapes the Bacterial and Fungal Gut Communities of the Honey Bee.” *Scientific Reports* 8 (1): 2019.

Zemenick, Ash T, Rachel L Vannette, and Jay A Rosenheim. 2021. “Linked Networks Reveal Dual Roles of Insect Dispersal and Species Sorting for Bacterial Communities in Flowers.” *Oikos* 130 (5): 697–707.

Zhang, Zhengyi, Yulong Guo, Mingsheng Zhuang, et al. 2024. “Gut Microbiome Diversity and Biogeography for Chinese Bumblebee Bombus Pyrosoma.” *Msystems* 9 (7): e00459–24.

Zheng, Hao, Margaret I Steele, Sean P Leonard, Erick VS Motta, and Nancy A Moran. 2018. “Honey Bees as Models for Gut Microbiota Research.” *Lab Animal* 47 (11): 317–25.

Figures and Tables

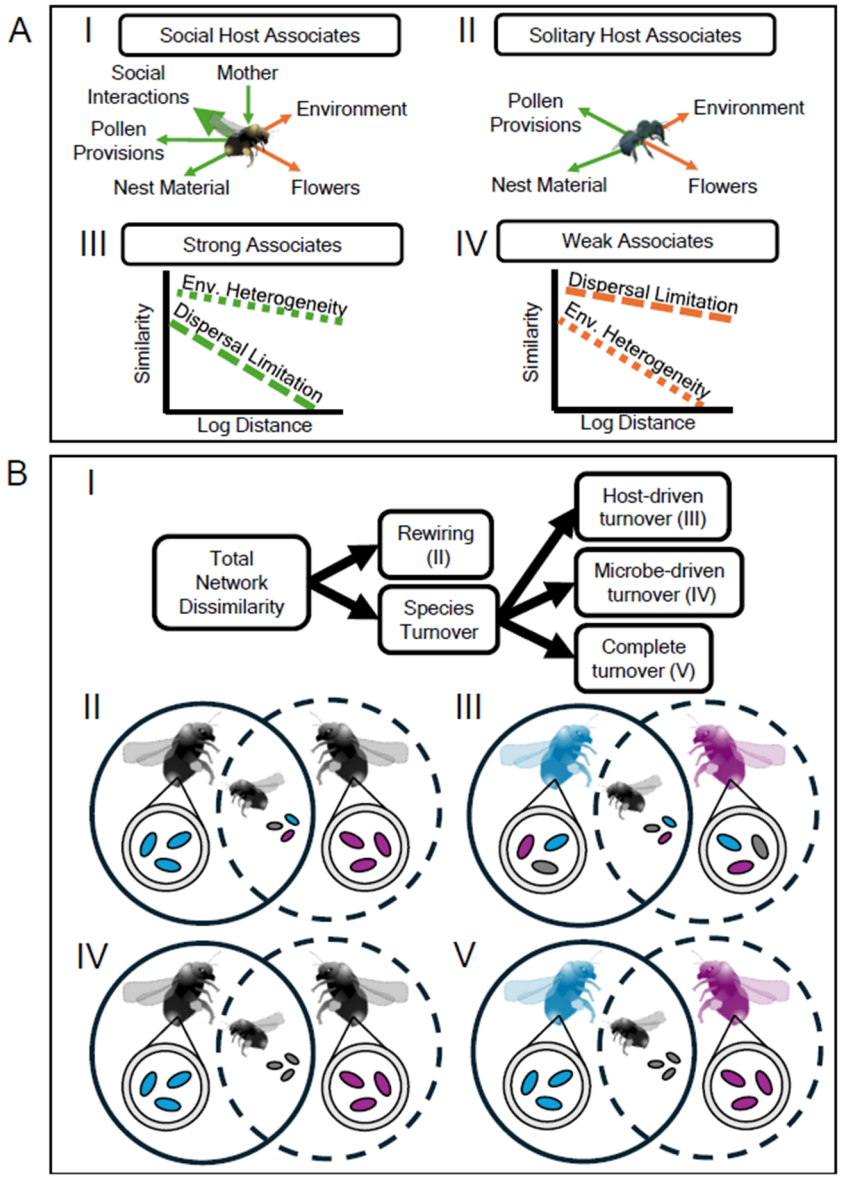

Figure 1: Conceptual framework for spatial variation in host-microbe interactions. ***A*** *Hypothesized drivers of the microbial distance-dissimilarity relationship vary by host sociality and strength of interaction.* ***AI*** *Social hosts transmit strong associates (green arrows) maternally, through host interactions, or via pollen provisions and nesting material. Social interactions are represented by thick arrows to highlight their increased importance relative to other routes.* ***AII*** *Solitary hosts transmit strong associates (green arrows) through pollen provisions and nesting material. Microbes with weak host relationships (orange arrows) are transmitted through shared floral resources or environmental interactions.* ***AIII–IV*** *Microbial distance decay of similarity is influenced by dispersal limitation (dashed lines) and environmental heterogeneity (dotted lines).* ***AIII*** *Strong associates may show steep distance decay due to host-limited dispersal or shallow decay due to a stable gut environment throughout space.* ***AIV*** *Weak associates may exhibit steep decay due to variable external environments or shallow decay if dispersal is not host-dependent.* ***B***

Host-microbe network dissimilarity can be decomposed into four components. ***BI*** *Total dissimilarity includes rewiring and species turnover, further divided into host-driven, microbe-driven, and complete turnover.* ***BII–V*** *Simplified examples illustrate:* ***BII*** *rewiring — same species present but altered interactions,* ***BIII*** *host-driven turnover — microbes remain, but host species differ,* ***BIV*** *microbe-driven turnover — hosts remain, but microbes differ, and* ***BV*** *complete turnover — no shared hosts or microbes.*

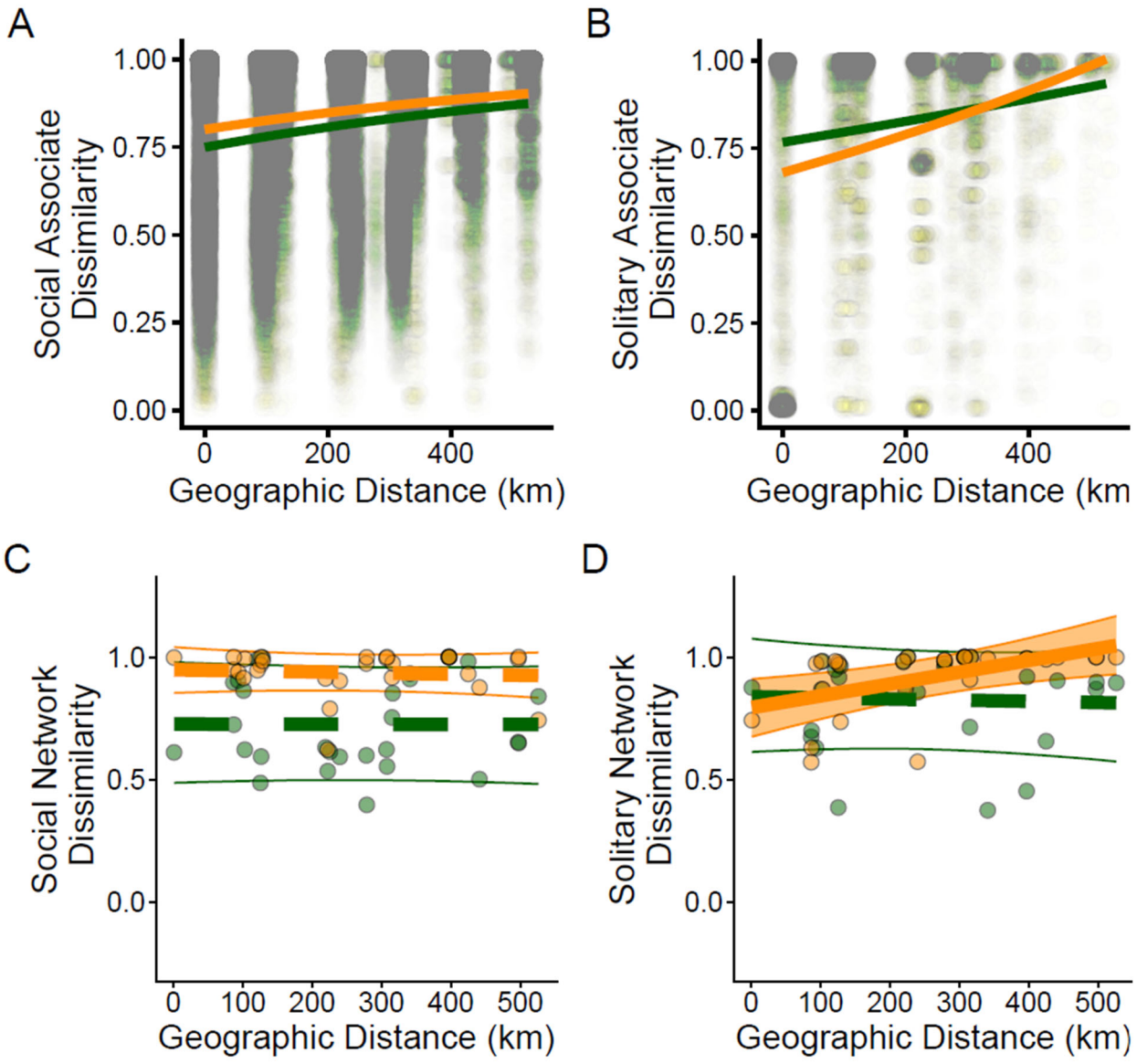

Figure 2: Distance decay of host-associated microbial communities and bee–microbe networks.
Distance decay relationships for strong (green) and weak (orange) host-associated microbe communities and bee-microbe networks in ***A, C*** *social hosts and solitary hosts* ***B,***

__D__. Pairwise microbial Bray-Curtis dissimilarity over distance for __A__ social and __B__ solitary hosts. Total network dissimilarity between meadows for __C__ social and __D__ solitary hosts. Model slope estimates are shown as solid lines when 97–100% of the posterior density was above or below zero and as dashed lines otherwise. Credible interval bands are 95% intervals. Credible interval ribbons are shaded to indicate 97–100% of the posterior density was greater or less than zero.

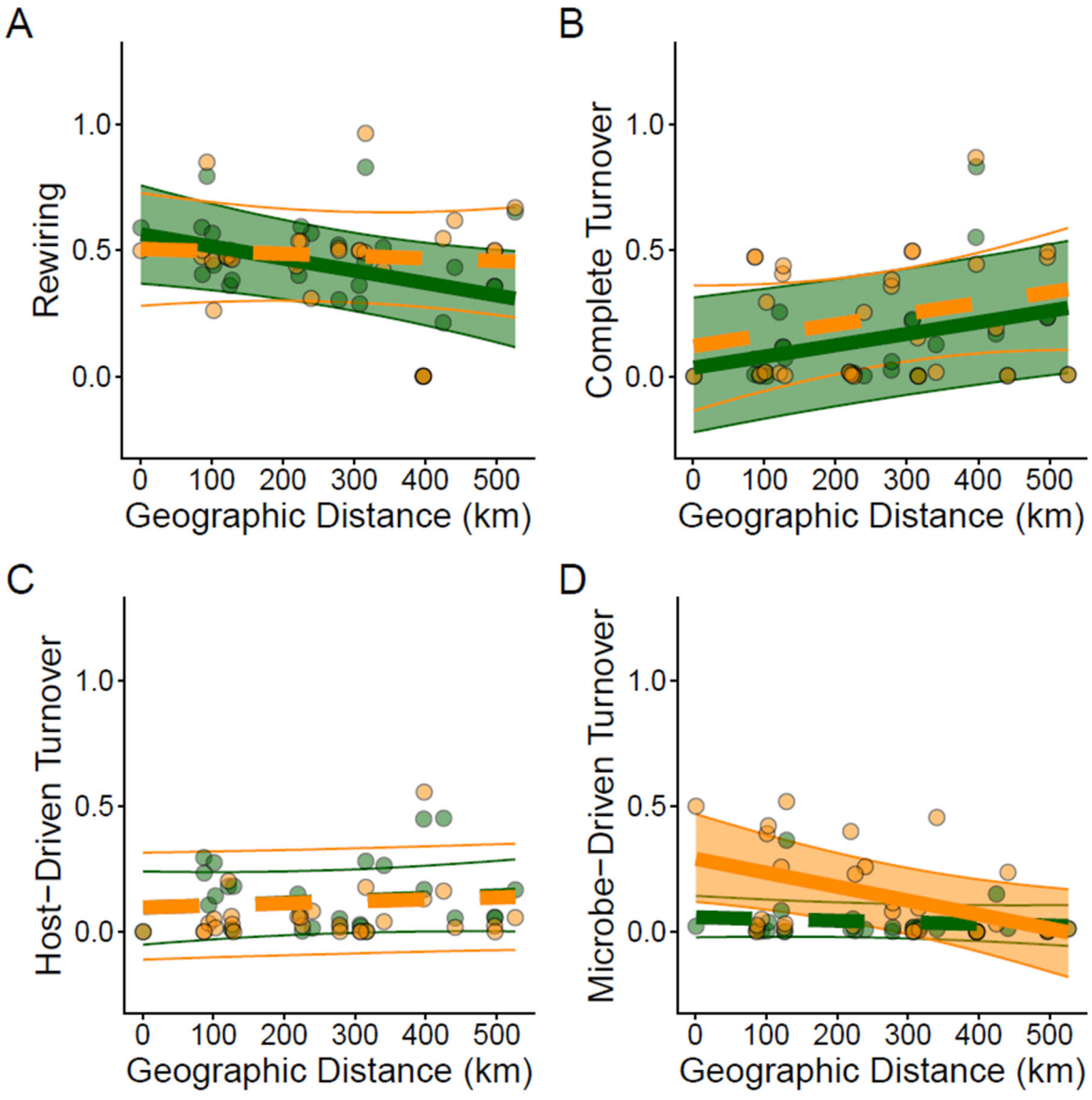

Figure 3: Distance decay of network dissimilarity components in social bee-microbe networks.
__A__ Network dissimilarity due to rewiring. __B__ Network dissimilarity due to complete species turnover. __C__ Network dissimilarity due to host-driven species turnover. __D__ Network dissimilarity due to microbe-driven species turnover. Strong associate microbe networks are represented in green, while weak associate microbe networks are represented in

orange. Model slope estimates are shown as solid lines when 97–100% of the posterior density was above or below zero and as dashed lines otherwise. Credible interval bands are 95% intervals. Credible interval ribbons are shaded to indicate 97–100% of the posterior density was greater or less than zero.

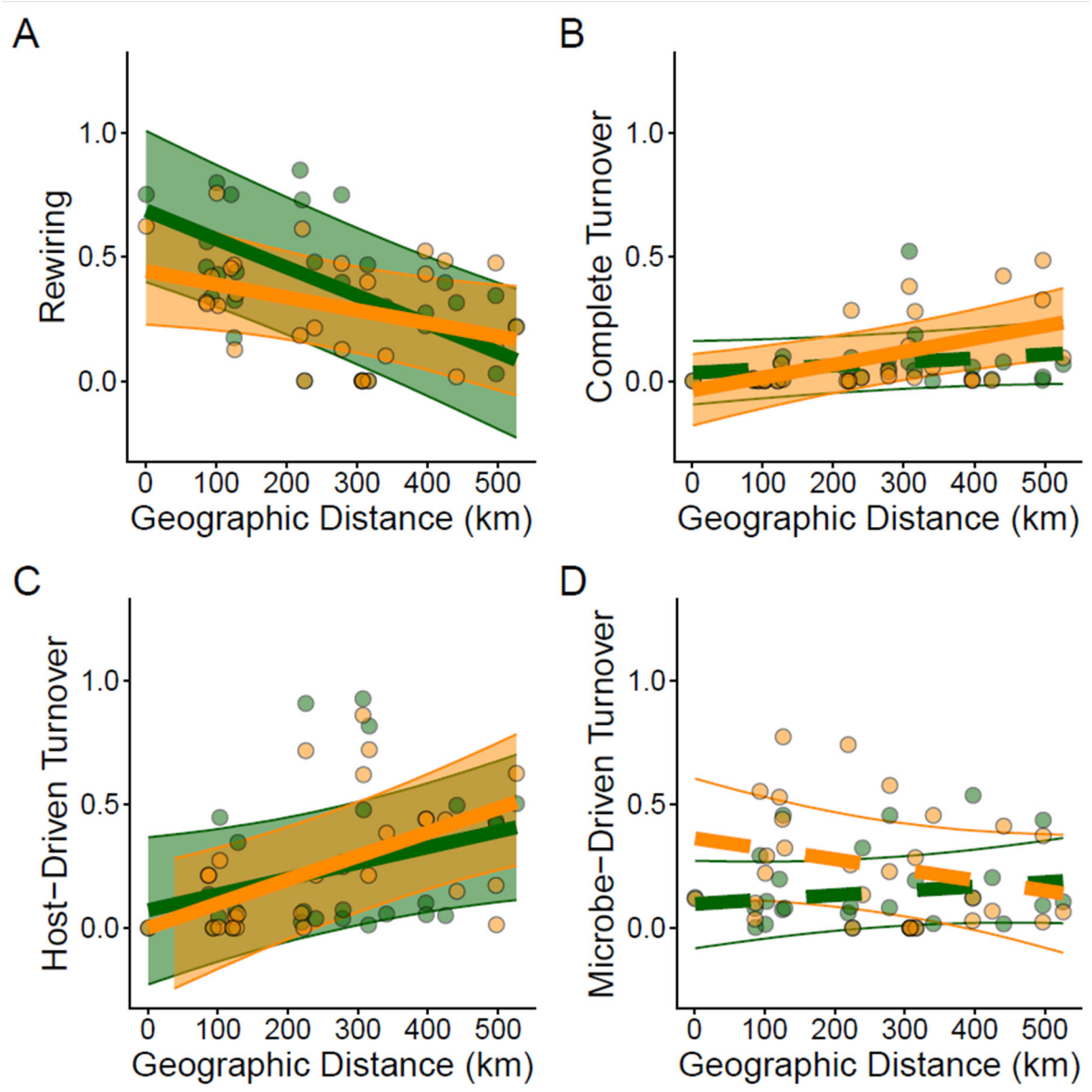

Figure 4: Distance decay of network dissimilarity components in solitary bee–microbe networks.
A *Network dissimilarity due to rewiring.* ***B*** *Network dissimilarity due to complete species turnover.* ***C*** *Network dissimilarity due to host-driven species turnover.* ***D*** *Network dissimilarity due to microbe-driven species turnover. Strong associate microbe networks are represented in green, while weak associate microbe networks are represented in orange. Model slope estimate lines are solid if [97–100%) posterior density was greater or*

less than zero, and otherwise dashed. Credible interval bands are 95% intervals. Credible interval ribbons are shaded to indicate [97–100%) posterior density was greater or less than zero.

Table 1: Strong microbial associates and potential pathogens of social and solitary bees.

Taxa are reported at the finest taxonomic level described in the cited literature. Potentially pathogenic taxa are considered common to both social and solitary hosts.

Family	Genus	Species	Citation
Strong Social			
Lactobacillaceae	Lactobacillus Firm-4		(Zheng et al. 2018; Kwong et al. 2017)
Lactobacillaceae	Lactobacillus Firm-5		
Bifidobacteriaceae	Bifidobacterium		
Neisseriaceae	Snodgrassella	S. alvi	
Neisseriaceae	Gilliamella	G. apicola	
Orbaceae	Frischella	F. perrara	(Zheng et al. 2018)
Bartonellaceae	Bartonella	B. apis	
Acetobacteraceae	Commensalibacter	C. 'Alpha 2-1'	
Bifidobacteriaceae	Bombiscardovia		(Kwong et al. 2017)
Orbaceae	Schmidhempelia		
Strong Solitary			
Acetobacteraceae			(Voulgari-Kokota et al. 2019)
Bacillaceae			
Burkholderiaceae			
Clostridiaceae			
Comamonadaceae			
Enterobacteriaceae			
Lachnospiraceae			
Lactobacillaceae			
Methylobacteriaceae			
Moraxellaceae			
Sphingomonadaceae			
Oxalobacteraceae			

Family	Genus	Species	Citation
Potential Pathogen			
Anaplasmataceae	Wolbachia		(Ramalho et al. 2021)
Erwiniaceae	Erwinia		(Cellini et al. 2019)
Hafniaceae	Hafnia	H. alvei	(Zheng et al. 2018; Lang et al. 2022)

Table 2: Geographic distance effects on bee–microbe network turnover.
Geographic distance model parameter estimates are shown for each component of network dissimilarity, including lower (LB) and upper (UB) 95% credible intervals, $\hat{R}$, bulk and tail effective sample sizes (ESS), and the proportions of posterior samples greater than and less than zero. Bolded response variables indicate strong model support. All $\hat{R}$ values were within the acceptable range for chain convergence.

Host association	Response variable	Estimate	SE	LB-95%	UB-95%	$\hat{R}$	Bulk ESS	Tail ESS	$p > 0$	$p < 0$
Social Hosts										
Strong Associates	Total Network Turnover	0	2.00E-04	-3.00E-04	3.00E-04	1	88280.6	75738.7	0.46	0.54
	Rewiring	-5.00E-04	2.00E-04	-9.00E-04	0	1	207345.7	109992.7	0.02	0.98
	Host-driven Turnover	1.00E-04	1.00E-04	-2.00E-04	4.00E-04	1	164506.4	110541.6	0.76	0.24
	Microbe-driven Turnover	-1.00E-04	1.00E-04	-2.00E-04	1.00E-04	1	150822.7	100101.8	0.22	0.78
	Complete Turnover	4.00E-04	2.00E-04	1.00E-04	8.00E-04	1	152214.6	97478.9	0.99	0.01
Weak Associates	Total Network Turnover	0	1.00E-04	-3.00E-04	2.00E-04	1	145518.9	105517.1	0.35	0.65
	Rewiring	-1.00E-04	3.00E-04	-6.00E-04	4.00E-04	1	223021.4	113174.3	0.35	0.65
	Host-driven Turnover	1.00E-04	1.00E-04	-1.00E-04	2.00E-04	1	156909.5	112588.9	0.84	0.16

Host association	Response variable	Estimate	SE	LB-95%	UB-95%	$\hat{R}$	Bulk ESS	Tail ESS	$p > 0$	$p < 0$
	Microbe-driven Turnover	-6.00E-04	2.00E-04	-0.0001	-1.00E-04	1	182809.7	110478.3	0.01	0.99
	Complete Turnover	4.00E-04	3.00E-04	-2.00E-04	0.0011	1	187332.3	110814.8	0.92	0.08
Solitary Hosts										
Strong Associates	Total Network Dissimilarity	-1.00E-04	2.00E-04	-5.00E-04	4.00E-04	1	159513.6	110058.3	0.39	0.61
	Rewiring	-0.0012	3.00E-04	-0.0016	-6.00E-04	1.0001	72288.8	70321.2	0	1
	Host-driven Turnover	6.00E-04	3.00E-04	0	0.0013	1	186727.6	116689.6	0.98	0.02
	Microbe-driven Turnover	2.00E-04	2.00E-04	-2.00E-04	6.00E-04	1	180904.1	108549.1	0.82	0.18
	Complete Turnover	1.00E-04	1.00E-04	-1.00E-04	4.00E-04	1	146742.5	97605.2	0.9	0.1
Weak Associates	**Total Network Dissimilarity**	5.00E-04	2.00E-04	1.00E-04	8.00E-04	1	194631.5	105139.1	1	0
	Rewiring	-5.00E-04	3.00E-04	-0.0011	0	1	158700.0	105942.1	0.03	0.97
	Host-driven Turnover	0.001	3.00E-04	4.00E-04	0.0016	1.0001	108656.2	99766.3	1	0
	Microbe-driven Turnover	-4.00E-04	3.00E-04	-0.001	2.00E-04	1	232265.0	113815.0	0.07	0.93
	Complete	5.00E-	2.00	2.00	9.00	1	16051	10742	1	0

Host association	Response variable	Estimate	SE	LB-95%	UB-95%	$\hat{R}$	Bulk ESS	Tail ESS	$p > 0$	$p < 0$
	Turnover	04	E-04	E-04	E-04		8.8	1.0		

1064

1065 ***Table 3: Ubiquitous host-associated ASVs across bee genera and sampling meadows.***
1066 *ASVs shown were detected in at least one individual at every meadow where the*
1067 *corresponding host genus was collected. Bolded ASVs were not included among the*
1068 *putative strongly host-associated microbial families of either social or solitary bees defined*
1069 *in Table 1.*

Host Genus	ASV Order	ASV Family	ASV Genus	ASV Species
Apis	Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium*	
	Lactobacillales	Lactobacillaceae	*Lactobacillus*	
	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus apis*
	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus kullabergensis*
	Acetobacterales	Acetobacteraceae	*Commensalibacter*	
	Burkholderiales	Neisseriaceae	*Snodgrassella*	
	Orbales	Orbaceae		
	Orbales	Orbaceae	*Frischella*	*Frischella perrara*
	Orbales	Orbaceae	*Gilliamella*	
Bombus	Lactobacillales	Lactobacillaceae	*Lactobacillus*	
	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus kosoi*
Melissodes	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus kosoi*
	Enterobacterales			
Anthophora	Acetobacterales	Acetobacteraceae		
	Enterobacterales			
Andrena	Rickettsiales	Anaplasmataceae	*Wolbachia*	

Host Genus	ASV Order	ASV Family	ASV Genus	ASV Species
	Enterobacterales			
Megachile	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*Lactobacillus kosoi*
	Enterobacterales			

1070

1071

1072