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Linked networks reveal dual roles of insect dispersal and species sorting for bacterial communities in flowers

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Due to the difficulty of tracking microbial dispersal, it is rarely possible to disentangle the relative importance of dispersal and species sorting for microbial community assembly. Here, we leverage a detailed multilevel network to examine drivers of bacterial community assembly within flowers. We observed flower visitors to 20 focal plant species in a coflowering community in the Sierra Nevada, revealing 289 species of arthropods. We also analyzed bacterial communities on flowers of each species. We found that plant species with similar visitor communities tend to have similar bacterial communities, and visitor identity to be more important than plant relatedness in structuring floral bacterial communities. However, plant species that were hubs of arthropod visitation were not necessarily hubs of floral bacteria, suggesting an important role for species sorting. Across plant species, the composition of flower-visiting Diptera (flies), bees and non-bee Hymenoptera best predicted bacterial species composition on flowers. Taken together, our analyses suggest dispersal is important in determining similarity in microbial communities across plant species, but not as important in determining the overall macrostructure (nestedness, modularity) and microstructure (connectedness based on shared interactors) of the floral bacterial network. A multilevel network approach thus allows us to address features of community assembly that cannot be considered when viewing networks as separate entities.

Keywords: community assembly, dispersal, ecological networks, floral bacteria, flower visitors, metacommunities, multi-level networks, species sorting

Introduction

The strong but variable effects of microbial communities on ecological interactions and host fitness is now well-recognized (Friesen 2013, Sugio et al. 2015). However, it remains difficult to determine the relative influence of factors such as dispersal and species sorting on microbiome assembly, composition and function. This difficulty remains in no small part because it is difficult to track microbial dispersal in natural systems, and as a result, most studies of microbial community assembly use spatial distance as a proxy for dispersal (Venkataraman et al. 2015, Burns et al. 2016), or assume global dispersal. However, it is clear that microbes can be dispersal limited (Peay et al.

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2012). As a result, understanding the relative contribution of microbial dispersal compared to environmental factors has critical applications for understanding the transmission of not only pathogens but beneficial and commensal microbes, as well as the patterns underlying community assembly of complex microbiomes.

One way to assess the relative influences of dispersal and species sorting on community structure is through the use of coupled networks (Fontaine et al. 2011, Pilosof et al. 2017). In this framework, multiple linked networks are sampled and analyzed together. Here, we use diagonally coupled networks (Pilosof et al. 2017) to compare structure between flower visitor networks and floral bacteria networks to examine potential effects of vector dispersal or species sorting on microbial community composition. Comparing network macrostructure (overall structure) and microstructure (within-network structure) between linked networks can indicate processes underlying assembly.

The macrostructure of bipartite ecological networks is the overall structure of the network: based on the documented interactions of two guilds, what shape does the network take? Broadly, the macrostructure of bipartite networks can be nested or modular, which can differentially influence dispersal dynamics (Fig. 1). For example, a floral bacteria network could be modular if microbes are highly specialized and experience strong species sorting, or if their vectors are highly specialized and thereby limit dispersal to few host plant species. In contrast, nested floral bacteria networks may reflect weaker species sorting and dispersal limitation relative to modular networks. Macrostructural properties (e.g. nestedness, modularity) can not only inform the potential for microbial dispersal throughout a network (Tylianakis et al. 2010, Silk et al. 2017), but can be compared across networks to infer the relative importance of dispersal versus species sorting, where significant correlations of network properties between linked flower visitor and floral bacteria networks would suggest the importance of dispersal.

Network microstructural properties can identify host plant species of particular importance for bacterial dispersal dynamics. For example, comparing how plant species are connected based on their shared interactors (i.e. visitors or bacteria) can identify hubs of interaction, which, in human and wildlife systems, have been shown to disproportionately influence disease transmission (Stein 2011, Silk et al. 2017). Hubs can amplify spread by interacting with many other individuals or species (i.e. species with high degree), link otherwise separated modules together (i.e. species with high betweenness centrality), or reduce the average path length connecting two species through shared interactors (i.e. species with high closeness centrality; Silk et al. 2017). If a host plant species is a primary hub of both the flower visitor network and the floral bacteria network (i.e. the networks have

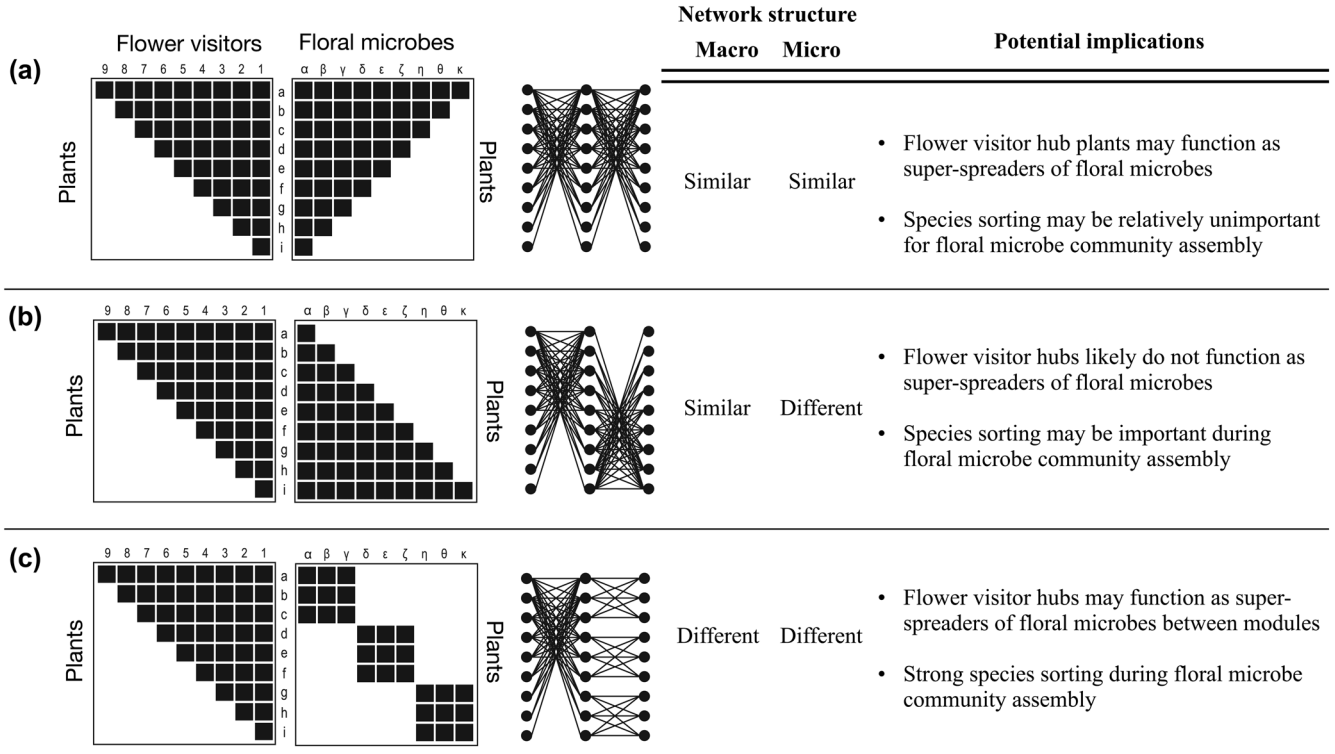

Figure 1. A conceptual framework of a multilevel plant – flower visitor – floral microbe network where for simplicity, each matrix is either perfectly nested or modular. Matrix rows represent plant species (a-i) and columns represent flower visitor (1-9) or floral microbe (α-κ) species. The figure is modified from Fontaine et al. (2011). If floral microbe networks share macrostructure with its associated flower visitor network, they may be linked in such a way that plants occupy similar (a) or dissimilar (b) positions within each network. If floral microbe communities are modular (c), then the relative position of plants in each network would depend on the structure of modules.

similar microstructures), then this host plant species should have a very strong influence on metacommunity structure by facilitating high dispersal rates to other species in the community in an environment that is not very selective, making species sorting weak.

Floral microbial communities offer an ideal system in which to apply a multilevel network approach. Flower visitors strongly influence the composition and abundance of flower microbes (Belisle et al. 2012, Ushio et al. 2015, Morris et al. 2020), although not all flower-inhabiting microbes are dispersed by arthropods. Flower microbial communities change over time (Shade et al. 2013, Aleklett et al. 2014) and influence plant and pollinator health and mediate plant–pollinator interactions (Ngugi and Scherm 2006, Vannette et al. 2013, Junker et al. 2014, McArt et al. 2014, Graystock et al. 2015, Rering et al. 2017). Yet, plant host traits may also exert selection on microbial establishment and growth through multiple mechanisms (Carter and Thornburg 2004, Huang et al. 2012, Block et al. 2019). Although floral microbe community assembly is dependent, in part, on dispersal by flower visitors, the study of floral microbe community assembly has to date been limited to single plant species or coarsely characterized communities of floral visitors, and remains yet to be fully considered in the context of the rich network of plant–flower visitor interactions.

Based on the conceptual framework of multilevel networks proposed by Fontaine et al. (2011), we outline three scenarios in which flower visitor and floral bacteria networks could be linked, and what this structure suggests about dispersal and species sorting. If, like flower visitor networks (Bascompte et al. 2003), floral microbe networks are nested, the networks could be linked in two ways. Plant species may be similarly connected by shared visitors and bacteria, suggesting that dispersal by flower visitors is a stronger force than species sorting in microbial community assembly (Fig. 1a). Alternatively, the networks could be aligned such that plants are not similarly connected by shared visitors and bacteria, which could arise if plant traits filter microbial colonization (species sorting; Fig. 1b). If floral microbe networks are modular, and dispersal by flower visitors is more important than species sorting in floral microbe community assembly, then flower visitor hubs may be important in linking modules in the floral microbe network (Fig. 1c). Since visitors can be important vectors of floral microbes (Belisle et al. 2012, Ushio et al. 2015, Morris et al. 2020), we hypothesized that plant species should be similarly connected by shared visitors and bacteria, and that species with high diversity of visitors should have high diversity of microbes, functioning as hubs of both networks (Fig. 1a). To test this hypothesis, we constructed the first comprehensive snapshot of a host plant–flower visitor–floral microbe network to infer the importance of dispersal and species sorting in this metacommunity. We asked: 1) can variation in bacterial species composition within and across plant species be explained by visitor species composition (after accounting for host plant relatedness)? 2) Do flower visitor and floral bacterial networks have similar macrostructure (i.e. are both similarly nested or modular)? 3) Do flower visitor and floral bacterial networks have similar microstructure (i.e. are visitor hub plants also bacterial hub plants)?

Material and methods

Data collection

Study site

Flower visitor and floral microbe surveys were conducted in a high elevation wet meadow at the University of California's Sagehen Creek Field Station, located within Tahoe National Forest (2400 m, 39°25′11.52″N, 120°18′27.18″W). The meadow is dominated by herbaceous perennial flowering plants and is surrounded by a subalpine old growth pine–fir forest. The focal plant community included the 20 most abundant co-flowering plant species: *Ligusticum grayi* (Apiaceae), *Arnica mollis* (Asteraceae), *Erigeron coulteri* (Asteraceae), *E. glacialis* (Asteraceae), *Senecio triangularis* (Asteraceae), *Lupinus polyphyllus* (Fabaceae), *Trifolium kingii* (Fabaceae), *Lilium parvum* (Liliacae), *Triantha occidentalis* (Liliacae), *Veratrum californicum* (Melanthiaceae), *Platanthera dilatata* (Orchidaceae), *P. sparsiflora* (Orchidaceae), *Castilleja miniata* (Orobanchaceae), *Pedicularis groenlandica* (Orobanchaceae), *Mimulus guttatus* (Phrymaceae), *M. primuloides* (Phrymaceae), *Bistorta bistortoides* (Polygonaceae), *Aconitum columbianum* (Ranunculaceae), *Aquilegia formosa* (Ranunculaceae), and *Drymocallis lactea* (Roseaceae). Flower abundance was quantified by measuring the floral volume of each plant species in the meadow. First, the volume of flowers per stem was measured for 10–30 individuals per species. Then, we counted the number of stems per species within two 0.25-m^2 quadrats placed at 100 random locations within the study site. Finally, we multiplied the average floral volume per stem by the number of stems counted within each quadrat (Supporting information). Floral abundance surveys were done concurrent with visitor and bacterial sampling time windows.

Flower visitor communities

During a ten-day window in July 2015, each of the 20 focal plant species was observed for all flower-visiting arthropods (regardless of their size or perceived pollination efficiency) during fourteen 30-min time periods (7 h of observation per species, 140 total observation hours). Observations for plant species were randomly assigned to time of day (08:00–17:00 h) and to one of five observers. On observation days, each species was observed once in the morning and once in the afternoon. The first ten minutes of each observation window focused on the observation and collection of micro-flower visitors using short focal length binoculars as a visual aid. The final twenty minutes were used to collect any flower visitors that could be seen with the unaided eye. Each flower-visiting arthropod observed during these time windows was collected and identified to the lowest taxonomic level possible by the authors and taxonomic experts (Acknowledgements).

Floral microbial communities

At two time-points during the flower visitor observations, 15 flowers of each plant species were collected for floral microbe community assessment (30 flowers per species for a total of 600 flowers). Flowers of each species were chosen haphazardly such that they were representative of the whole study meadow and life stages of the flower. Flowers were placed in sterile plastic bags and frozen at −20°C then −80°C until processing.

Floral microbial DNA was isolated by placing five flowers per species into phosphate buffer saline (PBS), sonicating for 10 min, vortexing briefly, and then collecting resulting microbe + PBS solution by filtering through autoclaved cheesecloth (Shade et al. 2013). The solution was centrifuged at 3000 rpm for 10 min at 20°C, and the resulting pellet was used for extraction. This procedure should sample both flower surface microbes and nectar-inhabiting microbes (for species that produce nectar). Floral microbial DNA was extracted from each sample using the Qiagen DNeasy 96 Blood and Tissue Kit following the manufacturer protocols. Extracted DNA was sent to the Microbiome Resource Center (Halifax, Nova Scotia, Canada) for amplicon library preparation and MiSeq Illumina sequencing using ITS2 for fungi and 16S V4–V5 (515F and 926R; GTGYCAGCMGCCGCGGTAA and CCGYCAATTYMTTTRAGTTT) for bacteria (Parada et al. 2016, Walters et al. 2016). Amplification of chloroplast DNA in 16S reactions was reduced with pPNA PCR blockers (GGCTCAACCCTGGACAG; PNA Bio, Newbury Park, CA).

Sequence data were processed using default parameters of the dada2 ver. 1.16 (Callahan et al. 2016). Briefly, sequences were filtered and truncated at 260 and 220 bp for forward and reverse reads, respectively. Sequences were error-corrected and merged, then chimeras were removed using consensus method. Taxonomy was assigned using 'assignTaxonomy' in the Silva v138 training set (Quast et al. 2012) using default parameters. ASV and taxonomy tables were assembled and analyzed in R (<www.r-project.org>) using the phyloseq package (McMurdie and Holmes 2013).

Few reads from the ITS primers were recovered, perhaps due to uneven or low fungal abundance, so only 16S amplicon data were utilized for analyses. From these amplicons, Cyanobacteria and chloroplast ASVs were removed. Following removal of chloroplast DNA, no sequences were recovered from DNA extraction controls. Samples yielding no sequences were not interpreted to be sterile, but instead to have DNA concentrations that are below the detection threshold, given the sequence protocols utilized in the study. In total, 6999 bacterial ASVs were detected. To remove bias of uneven sequence depth across samples, all samples were rarified to an even depth of 500 reads to retain as many samples as possible while reducing ASV loss. We based this decision on sampling curves, which indicate that 500 reads captures saturating ASV Shannon diversity and much of the ASV richness of samples (Supporting information). After rarefaction, 4060 OTUs were represented in the dataset.

Data analysis

Variation in bacterial communities within and across co-flowering plant species

To assess whether plant species hosted different bacterial communities, we compared multivariate community dispersion and species composition of floral bacteria across samples using the functions 'betadispr' and 'adonis' based on Bray–Curtis distances (based on the rarified read abundance data), using the R package vegan (Oksanen et al. 2017). Random forest, an estimator method that fits decision tree classifiers on subsets of the data, was used to assess the contribution of bacterial ASVs in distinguishing the bacterial species composition among different plant species and implemented using 'randomForest' (Liaw and Wiener 2002). To examine if the relative abundance of known floral bacteria varied across sampled plant species, we compared the average abundance of reads from three bacterial families (Bacillaceae, Enterobacteriaceae, Moraxellaceae) that have been previously isolated from nectar across plant species (Alvarez-Perez et al 2012).

To examine if flower visitor and microbial communities were correlated at the plant species level, Jaccard and Bray–Curtis dissimilarity matrices were constructed using the 'vegdist' function based on presence–absence and rarified read abundance or arthropod abundance data, respectively (Oksanen et al. 2017). A Mantel test was used to examine the correlations between matrices. To evaluate whether particular groups of flower visitors were more strongly associated with variation in floral bacterial community patterns, the analysis was repeated using dissimilarity matrices based on different subsets of the flower visitor community: bees (39 species), non-bee Hymenoptera (120 species), Diptera (95 species), and Coleoptera (35 species). See the Supporting information for a breakdown of the visitor community across plant species.

Since a correlation between visitor and bacterial communities could be due to bacteria and visitors tracking similar plant traits, we constructed a plant phylogenetic distance matrix to serve as a proxy for similarity in functional plant traits (Supporting information). Relatedness of the focal plant species was determined by reconstructing a phylogeny with the Interactive Tree of Life (Letunic and Bork 2007), which was converted to a phylogenetic distance matrix using the package ape (Paradis et al. 2004).

Flower visitor and floral bacterial network architecture

Network macrostructure

To compare the macrostructural properties of the flower visitor and floral bacteria networks, we assessed the degree to which each network was nested and modular. Nested networks have a core of generalist species that interact, and highly specialized species tend to interact with subsets of the generalist core (Bascompte et al. 2003). Network nestedness was estimated using the 'nested' function in bipartite (Dormann et al. 2008, 2009) with methods 'NODF2,' which considers only the presence of interactions, and 'weighted NODF,' which also accounts for interaction frequencies.

Both metrics range from 0 to 100, where 100 indicates a perfectly nested network. Modular networks are more partitioned and are composed of species groups that interact more strongly with each other than with other species in the community (Olesen et al. 2007, Poisot 2013). Modularity was assessed using the 'compart' and 'computeModules' functions in bipartite. The first counts the number of distinct groups of plants and visitors or bacteria that interact only with each other, and not any other members in the community ('compartments'). The second modularity metric ('Modularity Q') identifies modules in which 'within-module interactions are more prevalent than between-module interactions,' but without requiring that modules are discrete compartments (Dormann and Strauss 2014). Modularity Q ranges from 0 (less modular) to 1 (more modular).

Because network size affects measures of nestedness and modularity (Nielsen and Bascompte 2007, Fründ et al. 2016), and because there were many more bacterial ASVs than species of flower visitors (4060 versus 289), the floral bacteria network was rarefied by sampling randomly without replacement for 289 bacterial OTUs. The 95% confidence intervals of the rarefied bacterial network (based on 1000 rarefied networks) were used to determine whether metrics calculated for the floral bacteria network differed significantly from the flower visitor network. We also report metrics quantified from the un-rarefied bacterial network for comparison.

Network microstructure

Next, we compared the position of plant species in the flower visitor and floral bacteria networks. For each plant species, we compared the proximity to the nested core in each network using the 'nestedrank' index of the 'specieslevel' function in bipartite. We then assessed whether plant species that are hubs of the flower visitor network are also hubs of the floral bacteria network by comparing two metrics: 1) degree (richness of interaction partners) and 2) weighted closeness centrality (a measure of how closely linked a species is to all other species in the network; Silk et al. 2017) for plants in terms of their interactions with flower visitors and associations with floral bacteria. Closeness centrality was used instead of betweenness centrality due to the low levels of modularity in each network. Proximity to nested core, degree, and weighted closeness centrality were computed in bipartite with the 'specieslevel' function and indices 'degree', and 'weighted closeness.' Linear models were used to assess whether indices were significantly correlated across network types.

All analyses were performed in R ver. 3.3.3 and 3.6.3 (<www.r-project.org>).

Results

We observed 289 species of arthropods visiting the 20 focal plant species. Interactions between arthropods and plants comprised 695 unique links and 4364 total interactions. Accumulation curves reveal that we observed many, but not all, members of the flower visitor community and interactions between plants and flower visitors (Supporting information). The rarefied bacterial network included 4060 ASVs of bacteria isolated from the 20 plant species. There were 7558 unique links between bacteria and plants and 55 440 total interactions. See the Supporting information for degree distributions.

Variation in bacterial communities within and across co-flowering plant species

Plant species differed in floral bacteria species composition (Fig. 2; Supporting information; perMANOVA $R^2 = 0.29$, $p = 0.001$) but did not significantly differ in dispersion of bacterial species composition ($F_{19} = 1.3$, $p = 0.194$). Bacterial taxa that best distinguished plant species included representatives from the classes Alphaproteobacteria, and Gammaproteobacteria (Supporting information).

At the plant species level, Mantel tests revealed significant correlations between flower visitor dissimilarity and floral bacterial dissimilarity (Fig. 3a; $R^2 = 0.36$, $p = 0.003$). Patterns were consistent and qualitatively similar when either Jaccard or Bray–Curtis dissimilarities were used (Table 1); results based on Jaccard distances are presented below. There was a significant correlation between flower visitor dissimilarity and floral bacterial dissimilarity for each subgroup of flower visitors considered separately, but correlation strength varied by group (Fig. 3b–e). The strongest correlation was between flies and floral bacteria ($R^2 = 0.26$, $p = 0.023$), followed by bees ($R^2 = 0.22$, $p = 0.011$), then non-bee Hymenoptera ($R^2 = 0.19$, $p = 0.036$). There was no significant correlation between beetles and floral bacteria ($R^2 = 0.14$, $p = 0.090$).

Plant phylogenetic distance was not correlated with bacterial community dissimilarity ($R^2 = -0.01$, $p = 0.513$).

Flower visitor and floral bacterial network architecture

Network macrostructure

The plant–floral bacteria network was significantly more nested than the plant–flower visitor network (Fig. 4a–b). Both networks were composed of only one compartment, and did not have significantly different levels of Modularity Q (Fig. 4c–d). Metrics based on the rarefied plant–floral bacteria matrix did not differ qualitatively from metrics based on the un-rarefied matrix (caption of Fig. 4).

Network microstructure

Plant species comprising the nested core of the flower visitor network were not necessarily in the nested core of the floral bacteria network (Fig. 5a–b); there was no significant correlation between plant nested rank in both networks (Fig. 5c; $R^2 = -0.02$, $p = 0.45$). Similarly, plants that were hubs of the flower visitor network were not necessarily hubs of the floral bacteria network. There was no significant relationship between plant degree (number of interaction partners) in the visitor and bacterial webs (Fig. 5d; $R^2 = -0.05$, $p = 0.952$). There was a positive correlation between the closeness

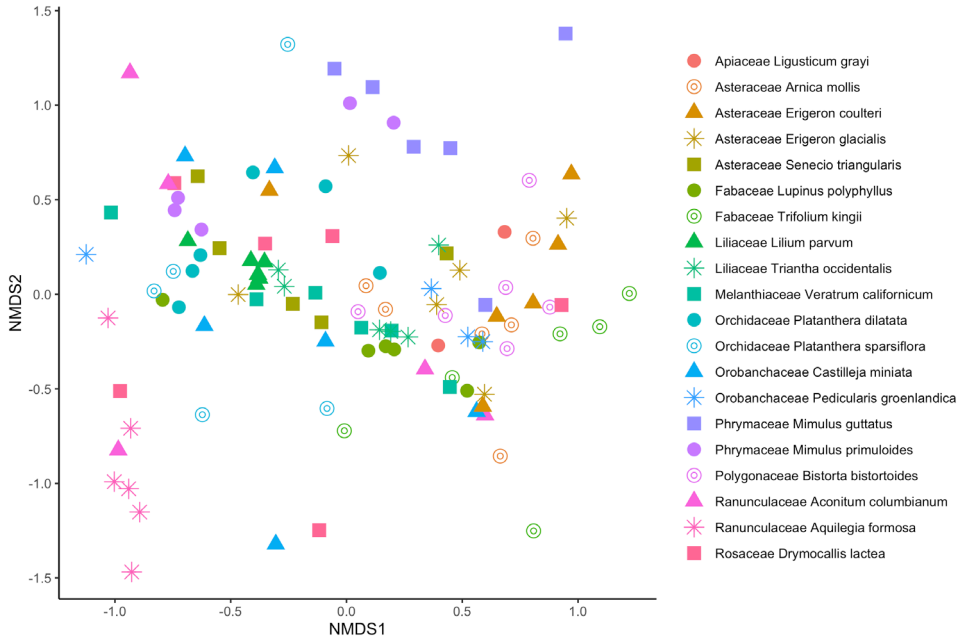

Figure 2. Nonmetric multidimensional scaling plot of floral bacterial communities, with NMDS based on Bray–Curtis dissimilarities, with three dimensions and stress = 0.18. Points with unique combination of color and shape correspond to different plant species.

centrality of plant species in the flower visitor network and floral bacteria network, but this was only marginally significant (Fig. 5e; $R^2 = 0.12$, $p = 0.074$).

Discussion

We found that plant species with similar visitor communities tend to have similar bacterial communities, suggesting that visitor identity may be important in structuring floral bacterial communities. While both the plant–bacteria and plant–visitor networks were significantly nested, plants that were hubs of visitors were not necessarily hubs of floral bacteria, suggesting an important role for species sorting (most similar to scenario depicted in Fig. 1b). Taken together, the results suggest that dispersal by flower visitors is important in determining similarity in microbial communities across plant species, but not as important in determining plant–floral bacteria associations (the structure of the floral bacteria network).

Using only analyses of community dissimilarity, a typical approach in microbial community analysis, we would have concluded that visitation is the major driver of bacterial species composition in floral hosts. However, analyzing these same data using the network approach offered the additional insight that processes other than insect-mediated dispersal are clearly important in structuring floral bacterial communities. For example, *Ligusticum grayi*, an umbel, was the plant

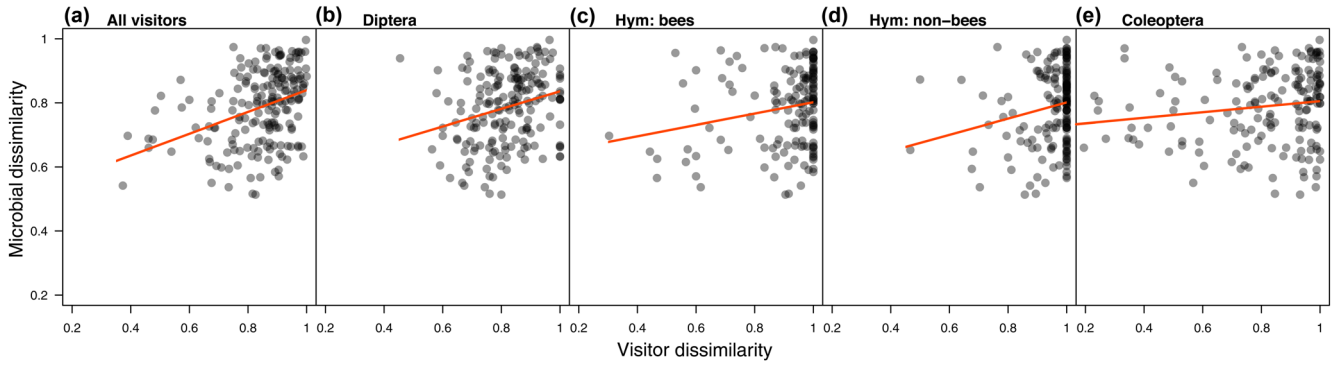

Figure 3. Across plant species, there was a significant correlation between flower visitor and floral bacteria communities for (a) all visitors, (b) Diptera (fly) visitors, (c) Hymenoptera (bee) visitors, (d) non-bee Hymenoptera and (e) Coleoptera (beetle) visitors. Points represent dissimilarity values between plant species based on either dissimilarity in microbial community composition and floral visitor community. The line in (a) represents the full visitor community while the lines in (b–e) represent that these are only portions of the full visitor community.

Table 1. Results of Mantel tests for correlations between floral bacterial communities and plant phylogeny and/or flower visitor communities based on dissimilarity matrices using Jaccard or Bray–Curtis distances. Values are R^2 values and significance levels (* for $p \leq 0.05$, ** for $p \leq 0.01$). All correlations were positive.

Mantel test	Jaccard	Bray–Curtis
Plant phylogeny	0.27	0.26
Whole visitor community	0.35**	0.36**
Bee community	0.22*	0.21*
Non-bee Hymenoptera community	0.19*	0.20*
Fly community	0.26*	0.26*
Beetle community	0.14	0.16

species with the greatest degree and closeness centrality in the floral visitor network (Fig. 5), likely due to its accessible morphology. Yet, *L. grayi* was characterized by a low degree and great distance from the nested core in plant–bacterial networks. In contrast, plant species in the Asteraceae (e.g. *Erigeron* spp.) were close to the nested core and showed high closeness centrality in plant–microbe network, but no consistent patterns in the flower visitor network. Taken together, these patterns suggest that despite a likely role for visitor communities in microbial dispersal, distinct processes influence how plant species are connected by shared visitors versus shared bacteria. Instead of simply reflecting insect-mediated dispersal, the evidence presented here supports the hypothesis that bacterial community assembly is also driven by species sorting processes that may include plant volatile composition (Burdon et al. 2018), floral microenvironment, UV exposure, nectar chemistry, competitive interactions, and historical contingency (Chappell and Fukami 2018). For example, bacteria in the Moraxellaceae, which includes the dominant nectar bacterium *Acinetobacter*, were found in many plant species (Supporting information), particularly in plants that present copious nectar (e.g. *Castilleja miniata* and *Aquilegia formosa*). This pattern may reflect species sorting at flowers or dispersal by nectar foragers. More broadly, we suggest that the linked network approach can offer significant insight into factors that structure microbial communities across host plants that differ both in frequency of visitation and species input, and environmental characteristics compared to the analyses typically applied to microbial community data. Specifically, the use of community similarity or clustering-based algorithms may overlook biologically meaningful features of complex multivariate data, particularly when multilevel network information is available.

Unlike other systems where bacterial dispersal is difficult or near impossible to track, our flower visitor–plant–floral

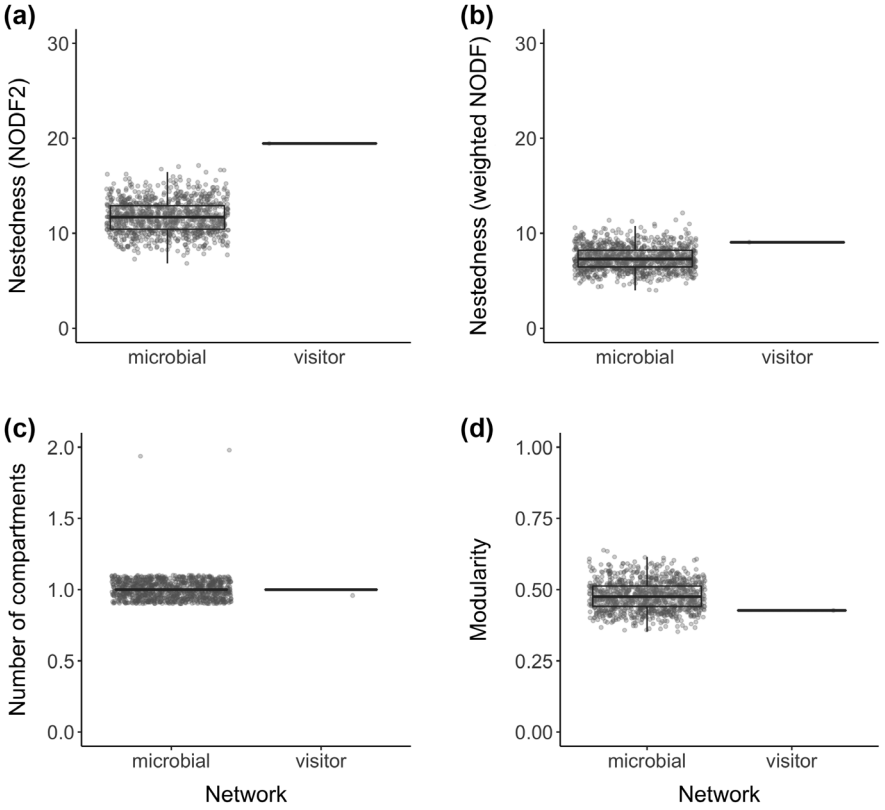

Figure 4. The floral bacteria network was significantly more nested than the flower visitor network considering un-weighted nestedness (a) and weighted nestedness (b). The flower visitor and floral bacteria web had the same number of compartments (c) and average modularity score (d). For the bacterial network, metrics calculated with entire network were similar to the rarefied network: nestedness = 11.67, weighted nestedness = 7.39, number of compartments = 1, and modularity Q = 0.43. Microbial networks were resampled 1000 times and all iterations are shown.

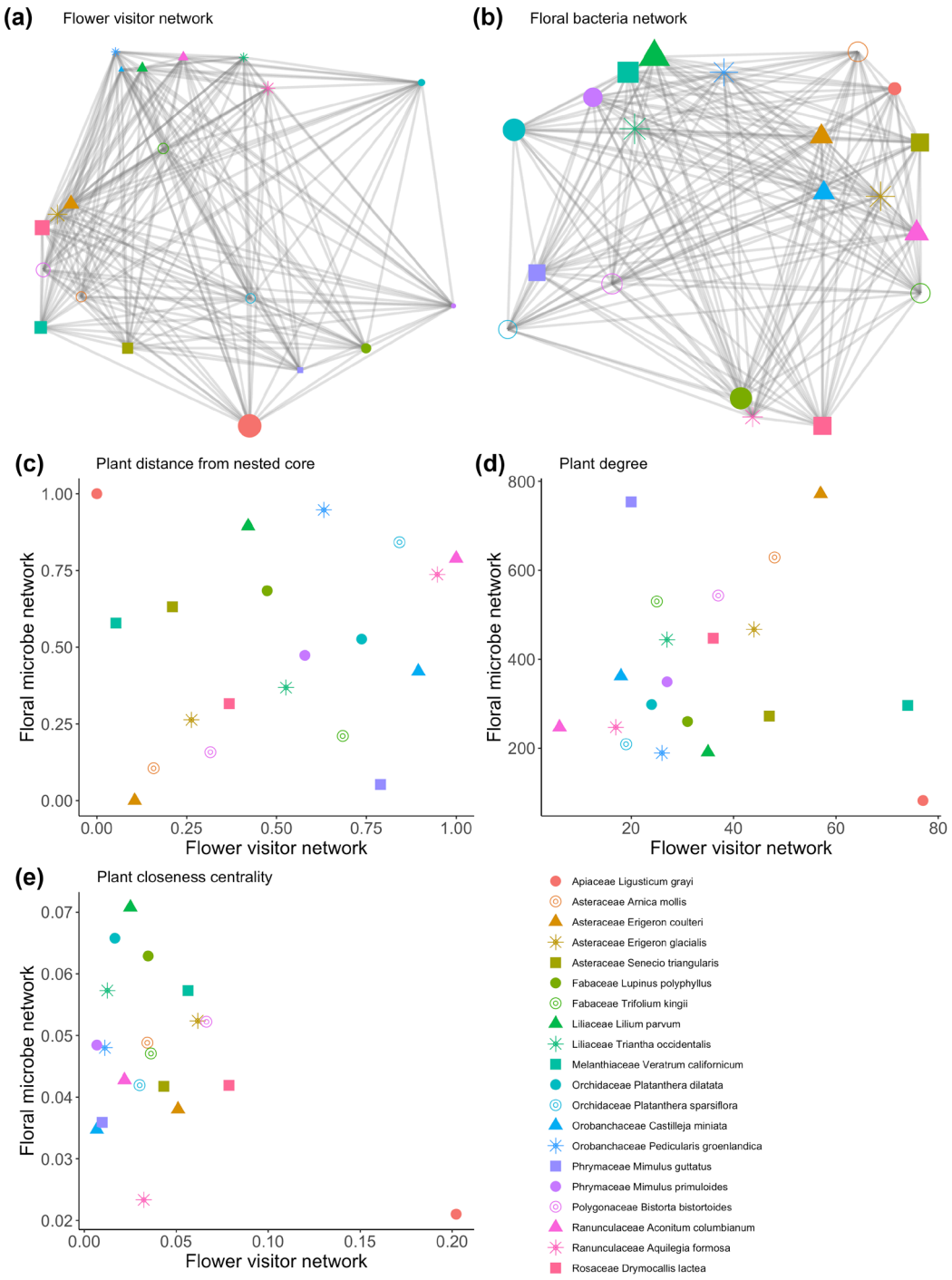

Figure 5. Comparison of plant species network position (microstructure) in flower visitor and floral bacteria networks. The flower visitor network (a) had a different microstructure than the floral bacterial network (b). The size of the symbol representing plant species in (a) and (b) are scaled by weighted closeness centrality measures, where larger symbols represent a higher weighted closeness centrality. (c) Plants in closer proximity to the nested core of the flower visitor network (closer to 0) were not necessarily close to the nested core of the floral bacteria network. (d) Plant species with a high number of flower visitor species (degree) were not necessarily associated with a high number of bacterial ASVs. (e) The closeness centrality of plant species was not related in flower visitor and floral bacteria web. Points represent a single value for each plant species.

bacteria system allowed us to construct a detailed network of potential microbial vectors to plant hosts. Previous studies have used visitor guilds or observed single plant species to link visitor composition to variation in microbial community structure (de Vega et al. 2009, Herrera et al. 2009, Samuni-Blank et al. 2014), but this study offers detailed species-level identification of floral visitors including predators and parasitoids across a relatively diverse coflowering plant community. This rich dataset allowed us to examine the relative contribution of each taxonomic guild of floral visitors (Fig. 3b–e, Table 1). Although past studies of floral microbe communities have tended to focus on only the primary pollinators as microbe vectors (McArt et al. 2014), our study suggests that flies (Diptera), and to a lesser degree, non-bee Hymenoptera are also important vectors of microbes (Fig 3b, d). These results suggest that insects that are poor vectors of pollen could be just as effective in vectoring microbes as pollinators, as suggested previously (Samuni-Blank et al. 2014, Zemenick et al. 2018, Morris et al. 2020). Flies are known to harbor diverse and heterogeneous microbial communities (Park et al. 2019). In the current study, main fly visitor families were diverse and varied among plant species, including known flower visiting families such as Scathophagidae, Muscidae, Syrphidae and Anthomyiidae (Pont 1993, Tooker et al. 2006, Šifner 2008). Although the current approach can identify key taxonomic groups that effectively link all flower types through microbial dispersal, interactions between visitor and floral morphology that make insect species inconsistent vectors of bacteria (e.g. flies contact the stigma in some plant species, but not all) may not be detectable. Therefore, future work studying the dispersal or community assembly of floral microbes should not be limited to visitors presumed to be the most efficient pollinators. Flower visitors that are not pollinator mutualists may still have ecologically important interactions with flowers by vectoring diverse microbes. More generally, this result emphasizes that vector identity is important, but often not considered in metacommunity studies.

The multilevel network framework can be a useful tool to understand the ecology and assembly of linked communities. By building a multilevel plant–flower visitor–floral microbe network as a model system, we show that plant species with similar visitor communities tend to have similar bacterial communities, and that the identity of visitors (disperser) is important in structuring floral bacterial communities. Further, we show that plants were differently connected by shared visitors versus shared bacteria in the flower visitor and floral bacteria networks, suggesting an important role for habitat filtering. Ultimately, dissecting the relative importance of environmental filtering and dispersal to microbial assembly in flowers will require experimental validation. However, our results suggest that dispersal is important in determining similarity in microbial communities across plant species, but not as important in determining structural features of plant–floral microbe network. Using a uniquely tractable system to estimate dispersal (via frequency of visitation), we demonstrate that a multilevel network approach is therefore useful in estimating the relative importance of dispersal and other factors of assembly (such as habitat filtering or historical contingency) that cannot be addressed considering networks as separate entities.

Data accessibility statement

Data are available from the NCBI under BioProject PRNJA699375 and code are available from the Dryad Digital Repository <https://doi.org/10.25338/B8P35> (Zemenick et al. 2021).

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References

Aleklett, K. et al. 2014. The microbial ecology of flowers: an emerging frontier in phyllosphere research. – Botany 92: 253–266.

Alvarez-Perez, S. et al. 2012. Zooming-in on floral nectar: a first exploration of nectar-associated bacteria in wild plant communities. – FEMS Microbiol. Ecol. 80: 591–602.

Bascompte, J. et al. 2003. The nested assembly of plant–animal mutualistic networks. – Proc. Natl Acad. Sci. USA 100: 9383–9387.

Belisle, M. et al. 2012. Flowers as islands: spatial distribution of nectar-inhabiting microfungi among plants of *Mimulus*

aurantiacus, a hummingbird–pollinated shrub. – Microb. Ecol. 63: 711–718.
Block, A. K. et al. 2019. Specialized naphthoquinones present in *Impatiens glandulifera* nectaries inhibit the growth of fungal nectar microbes. – Plant Direct 3: e00132.
Burdon, R. C. et al. 2018. Bacteria colonising *Penstemon digitalis* show volatile and tissue-specific responses to a natural concentration range of the floral volatile linalool. – Chemoecology 28: 11–19.
Burns, A. R. et al. 2016. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. – ISME J. 10: 655–664.
Callahan, B. J. et al. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. – Nat. Methods 13: 581–583.
Carter, C. and Thornburg, R. W. 2004. Is the nectar redox cycle a floral defense against microbial attack? – Trends Plant Sci. 9: 5–9.
Chappell, C. R. and Fukami, T. 2018. Nectar yeasts: a natural microcosm for ecology. – Yeast 35: 417–423
de Vega, C. et al. 2009. Yeasts in floral nectar of some South African plants: quantification and associations with pollinator type and sugar concentration. – S. Afric. J. Bot. 75: 798–806.
Dormann, C. F. et al. 2008. Introducing the bipartite package: analysing ecological networks. – Interaction 1(0.2413793).
Dormann, C. F. et al. 2009. Indices, graphs and null models: analyzing bipartite ecological networks. – Open Ecol. J. 2(1).
Dormann, C. F. and Strauss, R. 2014. A method for detecting modules in quantitative bipartite networks. – Methods Ecol. Evol. 5: 90–98.
Fontaine, C. et al. 2011. The ecological and evolutionary implications of merging different types of networks. – Ecol. Lett. 14: 1170–1181.
Friesen, M. L. 2013. Microbially mediated plant functional traits. – Mol. Microb. Ecol. Rhizosphere 1: 87–102.
Fründ, J. et al. 2016. Sampling bias is a challenge for quantifying specialization and network structure: lessons from a quantitative niche model. – Oikos 125: 502–513.
Graystock, P. et al. 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. – Proc. R. Soc. B 282: 20151371.
Herrera, C. M. et al. 2009. Yeasts in floral nectar: a quantitative survey. – Ann. Bot. 103: 1415–1423.
Huang, M. et al. 2012. The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (E)-b-caryophyllene, is a defense against a bacterial pathogen. – New Phytol. 193: 997–1008.
Junker, R. R. et al. 2014. Density-dependent negative responses by bumblebees to bacteria isolated from flowers. – Apidologie 45: 467–477.
Letunic, I. and Bork, P. 2007. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. – Bioinformatics 23: 127–128.
Liaw, A. and Wiener, M. 2002. Classification and regression by randomForest. – R News 2: 18–22.
McArt, S. H. et al. 2014. Arranging the bouquet of disease: floral traits and the transmission of plant and animal pathogens. – Ecol. Lett. 17: 624–636.
McMurdie, P. J. and Holmes, S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. – PLoS One 8: e61217.
Morris, M. M. et al. 2020. Microbial abundance, composition and function in nectar are shaped by flower visitor identity. – FEMS Microbiol. Ecol. 96: fiaa003.
Ngugi, H. K. and Scherm, H. 2006. Biology of flower-infecting fungi. – Annu. Rev. Phytopathol. 44: 261–82.
Nielsen, A. and Bascompte, J. 2007. Ecological networks, nestedness and sampling effort. – J. Ecol. 95: 1134–1141.
Oksanen, J. et al. 2017. vegan: community ecology package. – R package ver. 2.4-3. <https://CRAN.Rproject.org/package=vegan>.
Olesen, J. M. et al. 2007. The modularity of pollination networks. – Proc. Natl Acad. Sci. USA 104: 19891–19896.
Parada, A. et al 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. – Environ. Microbiol. 18: 1403–1414.
Paradis, E. et al. 2004. APE: analyses of phylogenetics and evolution in R language. – Bioinformatics 20: 289–290.
Park, R. et al. 2019. Microbial communities of the house fly *Musca domestica* vary with geographical location and habitat. – Microbiome 7: 147.
Peay, K. G. et al. 2012. Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. – Mol. Ecol. 21: 4122–4136.
Pilosof, S. et al. 2017. The multilayer nature of ecological networks. – Nat. Ecol. Evol. 1: 1–9.
Poisot, T. 2013. An a posteriori measure of network modularity. – F1000Research 2: 130.
Pont, A. C. 1993. Observations on anthophilous Muscidae and other Diptera (Insecta) in Abisko National Park, Sweden. – J. Nat. Hist. 27: 631–643.
Quast, C. et al. 2012. The SILVA ribosomal RNA gene database project: improved data processing and we-based tools. – Nucleic Acids Res. 4: 590–596.
Rering, C. C. et al. 2017. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. – New Phytol. 220: 750–759
Samuni-Blank, M. et al. 2014. The role of abiotic environmental conditions and herbivory in shaping bacterial community composition in floral nectar. – PLoS One 9: e99107.
Shade, A. et al. 2013. Unexpected diversity during community succession in the apple flower microbiome. – mBio 4: 1–12.
Šifner, F. 2008. A catalogue of the Scathophagidae (Diptera) of the Palaearctic region, with notes on their taxonomy and faunistics. – Acta Entomol. Mus. Nationalis Pragae 48(1): 111–196.
Silk, M. J. et al. 2017. Using social network measures in wildlife disease ecology, epidemiology, and management. – BioScience 67: 245–257.
Stein, R. A. 2011. Super-spreaders in infectious diseases. – Int. J. Infect. Dis. 15: e510–e513.
Sugio, A. et al. 2015. Plant–insect interactions under bacterial influence: ecological implications and underlying mechanisms. – J. Exp. Bot. 66: 467–478.
Tooker, J. F. et al. 2006. Floral host plants of Syrphidae and Tachinidae (Diptera) of central Illinois. – Ann. Entomol. Soc. Am. 99: 96–112.
Tylianakis, J. M. et al. 2010. Conservation of species interaction networks. – Biol. Conserv. 143: 2270–2279.
Ushio, M. et al. 2015. Microbial communities on flower surfaces act as signatures of pollinator. – Sci. Rep. 5: 1–7.
Vannette, R. L. et al. 2013. Nectar bacteria, but not yeast, weaken a plant–pollinator mutualism. – Proc. R. Soc. B 280: 1–7.

Venkataraman, A. et al. 2015. Application of a neutral community model to assess structuring of the human lung microbiome. – mBio 6: e02284-14.

Walters, W. et al. 2016. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. – Msystems 1(1): e00009–15.

Zemenick, A. T. et al. 2018. Legitimate visitors and nectar robbers of *Aquilegia formosa* have different effects on nectar bacterial communities. – Ecosphere 9: e02459.

Zemenick, A. T. et al. 2021. Data from: Linked networks reveal dual roles of insect dispersal and species sorting for bacterial communities in flowers. – Dryad Digital Repository, <https://doi.org/10.25338/B8P35M>.