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Nectar resources affect bird-dispersed microbial metacommunities in suburban and rural gardens

Marion L. Donald[1,2] | **Josie A. Galbraith**[3] | **Daria A. Erastova**[4] | **Anastasija Podolyan**[2] | **Tom E. X. Miller**[5] | **Manpreet K. Dhami**[2]

[1]Department of Biosciences, Rice University, Houston, Texas, USA

[2]Biocontrol & Molecular Ecology, Manaaki Whenua Landcare Research, Lincoln, New Zealand

[3]Department of Natural Sciences, Auckland Museum, Auckland, New Zealand

[4]School of Biological Science, The University of Auckland, Auckland, New Zealand

[5]Program in Ecology and Evolutionary Biology, Department of Biosciences, Rice University, Houston, Texas, USA

Correspondence
Marion L. Donald, Department of Biosciences, Rice University, 6100 Main Street, Houston, Texas 77019, USA.
Email: donaldm@landcareresearch.co.nz

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Abstract

As cities expand, understanding how urbanization affects biodiversity is a key ecological goal. Yet, little is known about how host-associated microbial diversity responds to urbanization. We asked whether communities of microbial (bacterial and fungal) in floral nectar and sugar-water feeders and vectored by nectar-feeding birds—thus forming a metacommunity—differed in composition and diversity between suburban and rural gardens. Compared to rural birds, we found that suburban birds vectored different and more diverse bacterial communities. These differences were not detected in the nectar of common plant species, suggesting that nectar filters microbial taxa and results in metacommunity convergence. However, when considering all the nectar sources present, suburban beta diversity was elevated compared to rural beta diversity due to turnover of bacterial taxa across a plant species and sugar-water feeders. While fungal metacommunity composition and beta diversity in nectar were similar between suburban and rural sites, alpha diversity was elevated in suburban sites, which mirrored the trend of increased fungal alpha diversity on birds. These results emphasize the interdependence of host, vector, and microbial diversity and demonstrate that human decisions can shape nectar microbial diversity in contrasting ways for bacteria and fungi.

INTRODUCTION

Nearly 70% of the world's population is projected to live in urban settings by mid-century (United Nations Department of Economic and Social Affairs Population Division, 2018), and cities are one of a few habitat types that are currently expanding (Angel et al., 2011; Grimm et al., 2008). Understanding how urbanization affects biodiversity is a pressing ecological goal. Despite the general trend of urbanization decreasing biodiversity (Faeth et al., 2011; McKinney, 2008; Seto et al., 2012), some taxonomic groups seem to benefit from it. For instance, urban and suburban areas—particularly gardens—have been documented as hotspots of bird, insect, and plant diversity compared to rural areas (Hope et al., 2003; Kondratyeva et al., 2020; Leveau & Leveau, 2005; Theodorou et al., 2020; Thompson et al., 2003). However, it is not well understood if these diversity trends extend to host-associated microbial communities.

Nectar-feeding birds and insects disperse microorganisms across flowers during visitation and link these individual microbial communities together into a metacommunity (Leibold et al., 2004). Metacommunity diversity arises from the interplay of regional processes, like dispersal, and local drivers, such as habitat type (Leibold et al., 2004; Ricklefs, 1987). Dispersal is essential for microorganisms to reach suitable floral nectar habitats. Thus, these nectar microbial communities may mirror the microbial compositional and diversity patterns carried by the animal dispersal agents. However, when nectar chemistry or other factors that

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may contribute to priority effects are strong (Vannette & Fukami, 2017), local processes should also contribute to shaping metacommunity diversity.

The species sorting concept is commonly invoked to explain metacommunity diversity. It posits that certain habitat types are better suited for specific species (Leibold et al., 2004). Thus, floral nectar may act as a filter, thereby selecting for specific microorganisms and filtering out variation in microbial composition that animal vectors may carry in suburban and rural areas. Indeed, previous studies identified that only subsets of fungal and bacterial taxa vectored by floral visitors established in nectar (Herrera et al., 2010; Vannette & Fukami, 2017). In additional to general filtering by nectar, plant species may select for specific microbial taxa, resulting in host-specific nectar microbial communities (Canto et al., 2017; Fridman et al., 2012; von Arx et al., 2019). Placing this in the urbanized context, which often has increased plant species richness (Tew et al., 2022) and nectar resources of sugar-water bird feeders (which can host also distinct microbial communities from those found in nectar; Lee et al., 2019), we would expect compositional shifts and increased beta diversity (turnover between local communities) in suburban compared to rural metacommunities.

In this study, we addressed the contributions of urbanization and nectar resources in structuring nectar microbial metacommunity composition and diversity. We first characterized the dispersing microbial species pools carried by three species of birds in suburban and rural gardens in Auckland, Aotearoa New Zealand. We then asked (1) how do differences in bird dispersed suburban and rural microbial species pools affect nectar microbial composition and diversity? Specifically, (1a) we first characterized bird dispersed species pools and then asked (1b) if nectar acts as a mirror or a filter? (2) How does increased host richness (i.e. floral species and sugar water feeders) at suburban gardens affect nectar microbial composition and diversity?

EXPERIMENTAL PROCEDURES

Site selection

We initially selected 11 private properties in suburban and rural Auckland, New Zealand (Figure S1). However, while we were able to capture birds at all 11 sites, floral nectar and sugar-water feeders were only available for sampling at five of the eight suburban sites and the three rural sites. We present data from these eight sites (five suburban and three rural) where both bird and nectar samples were collected to link bird-vectored and nectar microbial communities. The suburban and rural classifications were based on the municipal designation by the Auckland District Council. To characterize the land cover within 500 m of each of the sites, we calculated the percentage of each land cover type using 2018 data from the Land Cover Database v.5.0 for Mainland New Zealand (Figure S2). Additional details of the properties and details of this analysis are provided in Appendix S1. In addition to differences in land use, population density within the urban boundary is 1346 people/km^2 compared to 27 people/km^2 in the rural area of the Auckland region (Stats, 2018).

Natural history and study system

Of the nectar-feeding bird species—and therefore nectar microbe dispersal agents—in Auckland, we selected three focal species for this study, which were expected to have sufficient capture rates in the field: tūī (*Prosthemadera novaeseelandiae*), silvereye (*Zosterops lateralis*), and house sparrow (*Passer domesticus*). Of these species, the tūī and silvereye are native to New Zealand, and the house sparrow was introduced in the mid-1800s (Thomson, 2011). Tūī are primarily nectivorous (Bergquist, 1987); silvereyes are primarily insectivorous but also consume large amounts of nectar (Barnett & Briskie, 2007); house sparrows are granivorous, but will opportunistically consume nectar, especially in urbanized areas (Leveau, 2008).

All sites were canvassed for nectar producing plant species that are visited by birds and for sugar-water bird feeders. Two plant species, harakeke—New Zealand flax—(*Phormium tenax*) and bottlebrush (*Callistemon* sp.), were present at both suburban and rural properties. Frangipani (*Hymenosporum flavum*), pōhutukawa (*Metrosideros excelsa*), and sugar-water bird feeders were present only at the suburban gardens. All four plant species are visited by birds and insects. Harakeke and pōhutukawa are native to New Zealand, while frangipani and bottlebrush are introduced species.

Bird and nectar microbial sampling

To characterize the microbial species pools carried by nectar-feeding birds, we sampled the microbial communities at all properties from 9 December 2018 to 8 January 2019. We captured birds using mist nets for 1–3 days at each property (total mist netting effort = 16 days; suburban = 10; rural = 6), depending on capture rates and weather conditions. Prior to banding, birds were allowed to drink from 20% sterile sucrose (w/v) solution to sample the microbial community present on their bills and tongues. In total, we collected samples from 120 birds. Sixty-three were captured at suburban sites (28 silvereyes, 29 sparrows, and 7 tūī), and 54 were captured at rural sites (30 silvereyes, 20 sparrows, and 6 tūī). Additional capture and sampling details are provided in Appendix S1.

To characterize the nectar microbial metacommunity composition and diversity at suburban and rural sites, we collected nectar from plant species and sugar-water feeders present at the eight properties on the same days as the bird sampling. In total, we collected 211 nectar samples from individual flowers. Often only a single tree of each species (bottlebrush, frangipani, Pōhutukawa) was present at the site. We collected a maximum of eight flowers per plant. In the case of harakeke, which grows in a fan shape making individual plants difficult to distinguish, care was taken to select flowers from separate flowering stalks. Sixty-seven nectar samples came from the five suburban sites (44 harakeke, 7 bottlebrush, 8 frangipani, 8 Pōhutukawa) and 144 from the three rural sites (128 harakeke and 16 bottlebrush). When the extracted nectar was less than ca. 50 μl, we washed the nectary with sterile water, which became our sample. We also collected three 1 ml samples from three suburban feeders using a sterile pipette.

DNA extraction, PCR amplification, sequencing, and bioinformatic processing

We characterized microbial communities from bird, nectar, and sugar-water feeder samples with community metabarcoding. Briefly, DNA was extracted, the 16S and ITS1 regions were targeted for bacterial and fungal gene amplification, and sequenced via Illumina MiSeq. Sequence reads were error corrected and assigned to taxonomy via the Ribosomal Database Project classifier (Wang et al., 2007). Additional details are provided in Appendix S1.

STATISCIAL ANALYSES AND VISUALIZATIONS

(1a) Bird-vectored microbial species pools in suburban and rural contexts

To address whether suburban birds host compositionally different, more variable, or richer microbial communities compared to those hosted by rural birds, we characterized the microbial community composition and alpha and beta diversity carried by suburban and rural birds. We calculated Bray–Curtis dissimilarities and visualized them using non-metric multidimensional scaling (NMDS). We selected three dimensions for the visualization of the bacterial communities, as it reduced the stress of the ordination below 0.2, providing a more faithful representation of the relationship across communities. As the combinations of all three axes were qualitatively similar, we just present two axes in our visualization. We used permutational analysis of variance (PERMANOVA) with location set as the blocking effect to determine differences in microbial community composition due to suburban/rural context and bird species. Additionally, we assessed whether samples from the same location clustered on the NMDS (Figure S3).

We characterized microbial alpha diversity using the Chao1 diversity index and beta diversity as the distance of each community within a sample to the group centroid (group = rural or suburban). We tested for differences in alpha and beta diversity between rural and suburban metacommunities using Wilcoxon rank sum tests, as despite transformation the assumptions of nested linear mixed modes were violated. Finally, following a significant effect of urbanization on beta diversity, we used the R-package betapart (Baselga & Orme, 2012) with the Sorensen index to determine the contributions of species turnover and nestedness for significant differences in bacterial beta diversity in the suburban and rural contexts.

(1b) Nectar as a mirror or a filter?

Since plant species can be important in shaping nectar microbial composition and diversity (von Arx et al., 2019), we assessed nectar from plants that were present at both suburban and rural sites. For the two shared plant species, we address the question of whether nectar microbial metacommunities mirror the microbial composition and diversity patterns carried by birds, or if nectar acts as a filter to select for similar composition and diversity across suburban and rural sites. Here, we used the methods described above to visualize the communities and tested for differences in composition, alpha and beta diversities. Details of the statistical tests, models, and R packages used are provided in Appendix S1.

(2) Does nectar resource richness affect nectar microbial composition and diversity?

To determine whether nectar resource richness affects nectar microbial composition and diversity, we included all nectar samples (from plant species and sugar water feeders) to ask whether composition responded to nectar resource identity across rural and suburban sites. The higher richness of plant species in our urban compared to rural sites is biologically relevant and this trend has also been documented in the northern hemisphere (Hope et al., 2003; Tew et al., 2022). We also used pairwise PERMANOVA to identify significant differences in microbial composition across host plant species and sugar water feeders. This analysis relied on the R-package pairwiseAdonis (Martinez Arbizu, 2020). We also assessed whether these microbial samples clustered by site (Figure S4). We calculated the Chao1 alpha diversity index for each host (plant species and sugar-water feeder) within the rural and suburban contexts and the distance to centroid for each of the hosts within each context. We then tested for differences in alpha and beta diversity of microbial communities across hosts in suburban and rural sites using Kruskal–Wallis and Dunn tests with a Holm correction for repeated sampling. Finally, following a significant effect of urbanization on beta diversity, we used the R-package betapart with the Sorensen index to determine the contributions of species turnover and nestedness across the suburban and rural contexts.

Microbial compositional differences across birds, plants, and sugar-water feeders

To identify differences in composition across host species and sugar-water feeders, we characterized the taxonomic composition within microbial communities carried by the three bird species, four host plant species, and sugar-water feeders by pooling all samples by host identity. We converted the sequence counts to relative abundance within phyloseq (McMurdie & Holmes, 2013) and characterized genera that comprised less than 10% of the total relative abundance across all host types to be low abundance for this visualization.

RESULTS

(1a) Suburban avian-vectored bacterial species pools are compositionally distinct and have higher turnover compared to rural bacterial species pools; suburban fungal species pools are richer but similar in composition across contexts

Compared to rural bird species, suburban bird species hosted different bacterial but not fungal community compositions (Figure 1A bacterial: suburban/rural: $F = 7.179$, $R^2 = 0.067$, $p = 0.019$; bird species $F = 1.595$, $R^2 = 0.029$, $p = 0.015$; suburban/rural × - bird species: $F = 1.342$, $R^2 = 0.025$, $p = 0.13$; B fungal: urban $F = 1.996$, $R^2 = 0.028$, $p = 0.17$; bird species: $F = 1.289$, $R^2 = 0.037$, $p = 0.066$; urban × bird species: $F = 1.443$, $R^2 = 0.041$, $p = 0.50$). Alpha and beta diversity of bacterial and fungal communities differed in their responses to suburban and rural bird hosts. Suburban bacterial communities hosted by birds had higher beta diversity across the communities compared to those hosted by rural birds (Figure 2A, $\chi^2 = 7.78$, df $= 1$, $p = 0.005$). This difference in beta diversity was primarily driven by bacterial species turning over, rather than the rural community being nested within the suburban community (Figure S5A, mean ± sd dissimilarity: 85.9% ± 12%;

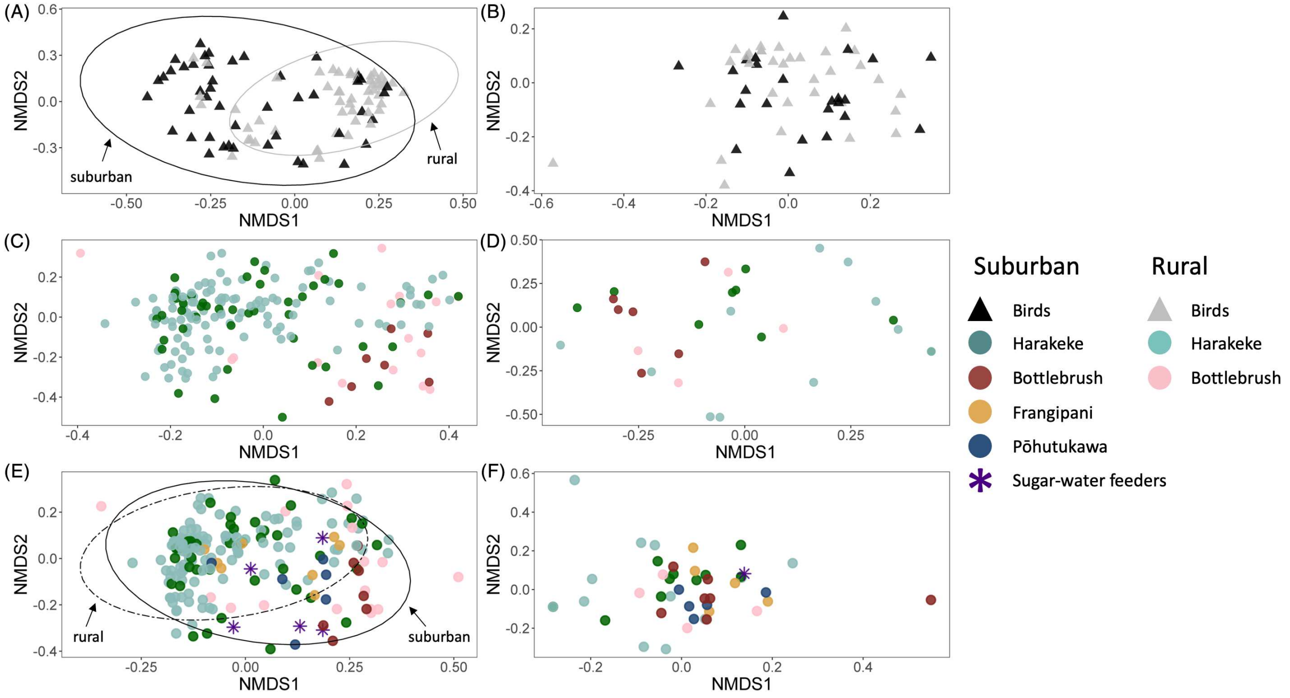

FIGURE 1 NMDS ordination bacterial (A, C, E) and fungal (B, D, F) microbial communities. The first row depicts bird-vectored microbial species pools in suburban (black) and rural (grey) sites. The second row shows the microbial communities from nectar types that are shared across suburban and rural contexts (harakeke and bottlebrush). And the third row shows microbial communities from all the nectar sources (suburban samples are shown in dark colours and rural samples are in light colours). The ellipses represent 95% confidence intervals when the groupings are statistically significant ($p < 0.05$).

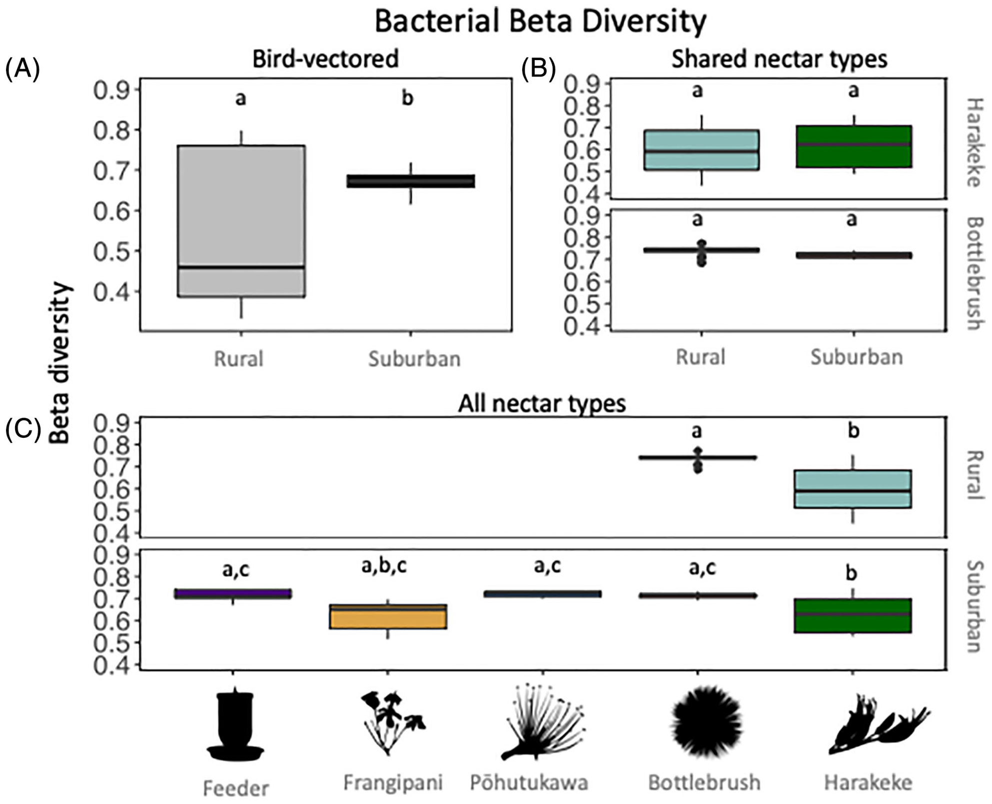

FIGURE 2 Bacterial beta diversities across bird, shared nectar, and all nectar samples (A–C). Letters denote significance of $p < 0.05$

turnover: 76.4% ± 18%; nestedness: 9.5% ± 10.9%). Bacterial richness (alpha diversity) was similar between the suburban and rural bird-associated microbial communities (Figure S6A, $p = 0.66$). Fungal communities hosted by suburban birds had higher richness compared to communities hosted by rural birds (Figure 3A, $p = 0.0087$). Fungal beta diversity was similar across communities hosted by suburban and rural birds (Figure S7A, $\chi^2 = 0.79$, df $= 1$, $p = 0.37$).

(1b) Nectar can act as a filter and a mirror

Despite clear differences in the composition and diversity of the bacterial communities carried by suburban and rural birds, bacterial communities of harakeke and bottlebrush nectar, which were common to both garden types, were compositionally similar across suburban and rural sites (Figure 1B bacterial: urban $F = 1.610$, $R^2 = 0.0085$, $p = 0.056$). Yet, the bacterial communities clustered by plant species (Figure 1B host species: $F = 8.785$, $R^2 = 0.046$, $p = 0.001$), which was irrespective of whether the plant host species were in suburban or rural gardens (suburban/rural × host species: $F = 1.168$, $R^2 = 0.006$, $p = 0.23$). Fungal communities showed no response to host plant species, suburban/rural context, or their interaction (Figure 1D, fungal: suburban/rural: $F = 2.022$, $R^2 = 0.062$, $p = 0.22$; host species: $F = 1.934$, $R^2 = 0.059$, $p = 0.067$; suburban/rural × host species: $F = 0.758$, $R^2 = 0.0232$, $p = 0.76$). Additionally, comparing bacterial richness and beta diversity between rural and suburban gardens, we did not detect any strong differences in bottlebrush or harakeke nectars (Figure S6B, $p > 0.05$). Finally, we find 62% of bacterial taxa carried by the three species of birds are shared with bottlebrush and harakeke nectars.

However, consistent with mirroring the increased alpha diversity carried by birds, suburban harakeke nectar hosted higher fungal richness (Figure 3B, $p = 0.009$) and fungal richness in bottlebrush nectar samples was also consistently high, although not statistically significant (Figure 2F, $\chi^2 = 1.39$, df $= 1$, $p = 0.28$), possibly due to the small sample size. Fungal beta diversity was not significantly different across suburban and rural environments for harakeke nor bottlebrush nectar samples (Figure S7B, $p > 0.05$).

(2) Suburban gardens have different compositions and higher turnover of bacterial taxa due to species sorting across the nectar resources

Considering all nectar and sugar-water feeder samples, we found suburban and rural contexts and nectar

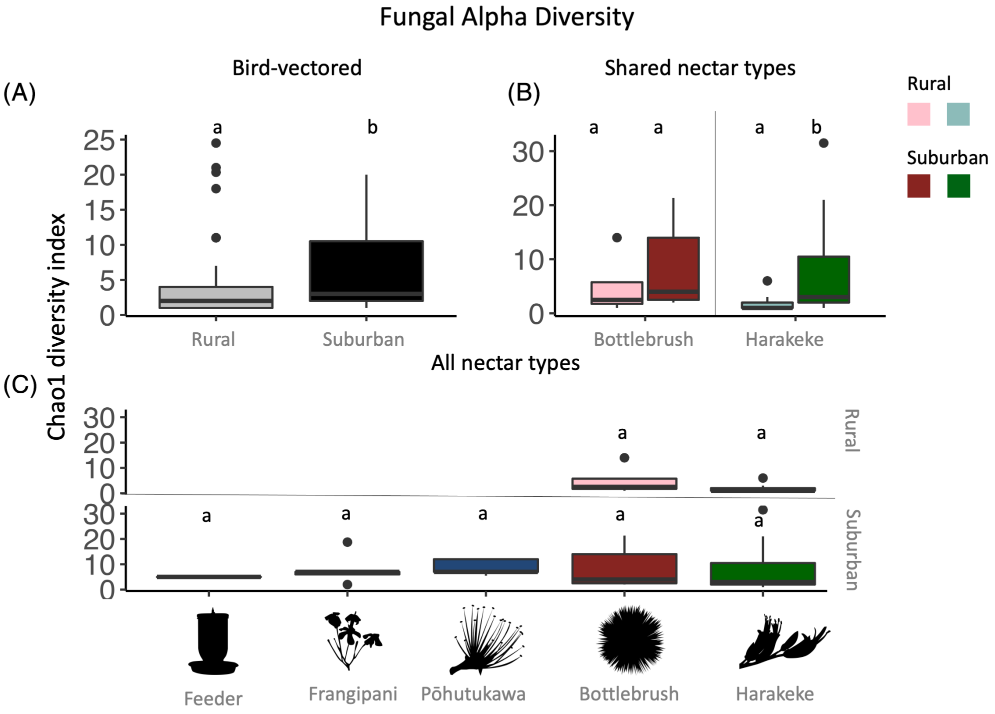

FIGURE 3 Fungal alpha diversities across bird, shared nectar, and all nectar samples (A–C). Letters denote significance of $p < 0.05$.

resource type to be important drivers of bacterial community composition (Figure 1C, suburban/rural: $F = 2.560$, $R^2 = 0.012$, $p = 0.003$; nectar resource: $F = 3.906$, $R^2 = 0.0732$, $p = 0.001$), but their interaction was not a significant driver ($F = 1.1667$, $R^2 = 0.0055$, $p = 0.23$). Fungal community composition was significantly affected by nectar resource (Figure 1C, PERMANOVA: $F = 1.35$, $R^2 = 0.11$, $p = 0.02$), but not by urbanization context ($F = 1.47$, $R^2 = 0.03$, $p = 0.08$) nor the interaction between urbanization context and nectar resource ($F = 1.30$, $R^2 = 0.03$, $p = 1$).

These compositional drivers were further confirmed with significant differences detected between bacterial community compositions for harakeke—pōhutukawa, harakeke—bottlebrush, and bottlebrush—frangipani comparisons (Table S1); however, fungal communities were not significantly different across nectar resources (Table S2, Figure S5C). Bacterial beta diversities were different across several of the nectar resources. Specifically, suburban sugar-water feeder, pōhutukawa and bottlebrush samples had higher beta diversities than suburban harakeke samples did (Figure 2C, Dunn test $p < 0.05$). Rural bottlebrush samples also had higher beta diversity when compared to rural harakeke samples (Figure 2C, Dunn test $p < 0.05$). These differences in beta diversity (mean ± sd: 81.9% ± 19.3%) were primarily due to bacterial taxonomic turnover (67.85% ± 24.59%), rather than to nestedness (14.06% ± 13.5%) (Figure S5B).

Alpha diversities of fungal and bacterial communities were similar across sugar-water feeders and plant species (fungal: Figure 3C; bacterial: Figure S6C, Dunn test $p > 0.05$), with one exception. Rural harakeke nectar samples hosted higher bacterial richness than did rural bottlebrush nectar samples (Figure S6C, Dunn test $p < 0.05$).

Microbial compositional differences across birds, plants, and sugar-water feeders

Floral nectar, sugar-water feeders, and birds all hosted *Sphingomonas* and *Raoutella* in relatively high abundances. Sugar-water feeders, frangipani, and pōhutukawa (the host types only present at suburban gardens) hosted higher abundances of specific bacterial taxa (*Pantoea*, *Mesoaciditoga*, *Spongiimonas*, *Caloribacterium*, *Neokomagatea*, and *Asaia*) than did bottlebrush or harakeke nectar samples (Figure 4). These taxonomic differences within communities of the additional suburban nectar resources likely underly the observed increase in bacterial beta diversity in suburban compared to rural gardens. Notably, the fungal taxa *Kabatiella* dominated the sugar-water feeder sample and was also found across nectar and bird samples in both suburban and rural environments. Suburban harakeke and bird samples hosted *Candida*, *Rhodoporidium*, *Hannaella*, *Exophiala*, and *Pseudozyma*, all

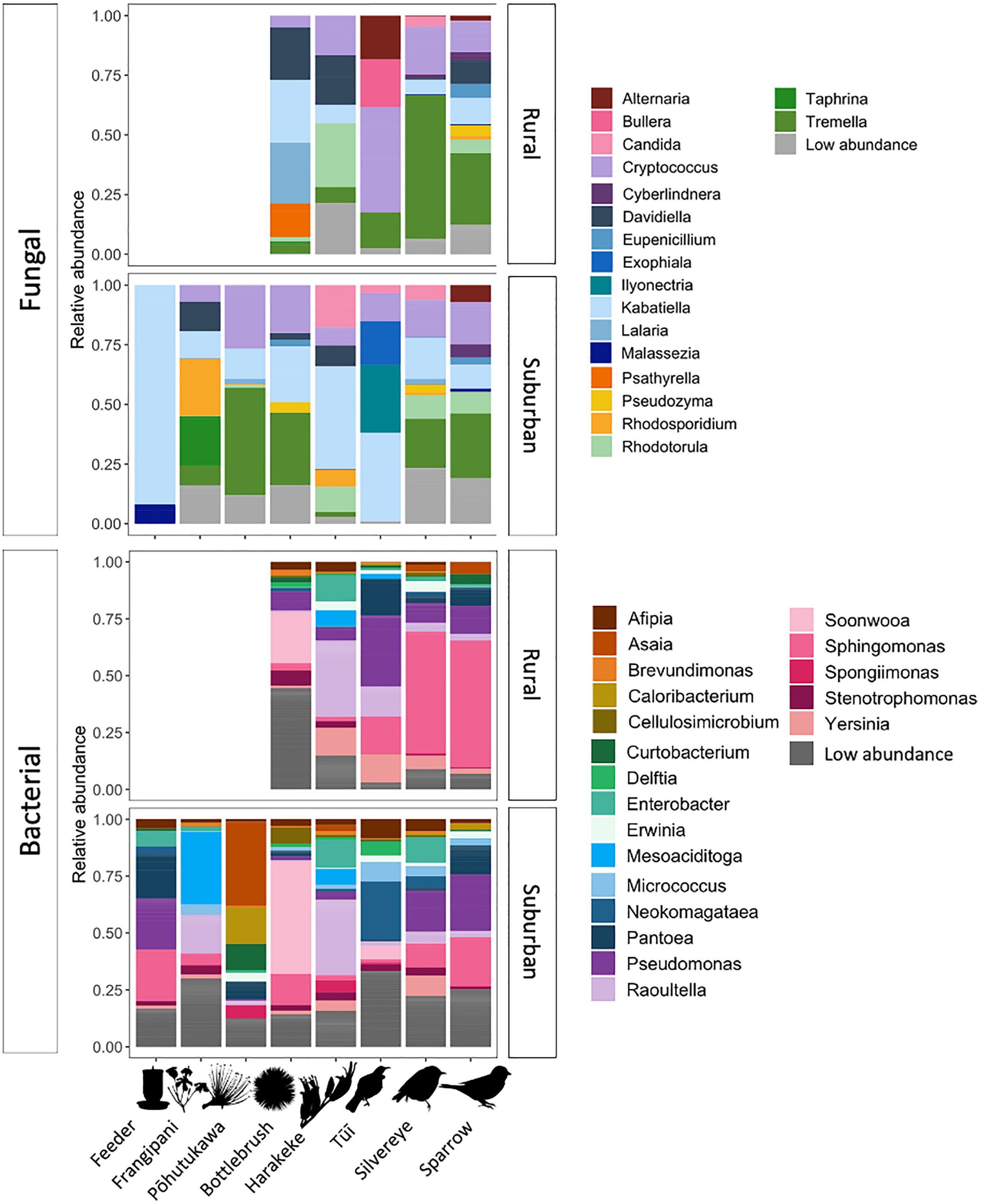

FIGURE 4 Relative abundance of fungal and bacterial genera that were greater than 10% of the total abundance across feeders, frangipani, pōhutukawa, bottlebrush, harakeke, tūī, silvereye, and sparrow. Genera that were <10% of the total relative abundance are grouped as low abundance. Genera are shown in different colours, note that colours repeat across fungal and bacterial groupings.

of which likely account for the increase in fungal richness in these suburban samples. Low-abundance bacterial and fungal taxa were common across samples from all hosts. Consistent with earlier findings that bird species is an important factor for bacterial composition, we also find that the proportion of shared bacterial taxa with nectarvaries across the three bird species. Specifically, tūī shared 84.2% of taxa with the nectar resources, while silvereyes and sparrows shared 74.1% and 69.5%, respectively. Birds also hosted fungal and bacterial taxa that were not commonly seen in nectar samples.

DISCUSSION

As nectar microorganisms can play key roles in mediating plant–pollinator interactions (Herrera et al., 2013; Schaeffer & Irwin, 2014; Vannette et al., 2013), understanding how urbanization affects nectar microbial metacommunity diversity is important. We asked if nectar microbial composition and diversity mirrors the patterns found on avian dispersers and if additional floral taxa and sugar-water feeders in suburban gardens affect microbial metacommunity composition and diversity. Our results showed that while suburban birds hosted different and more diverse bacterial communities than rural birds did, nectar bacterial communities did not mirror these microbial compositional and diversity patterns carried by dispersal agents. This suggests that nectar can act as a strong filter, selecting for compositionally similar communities across suburban and rural gardens. Additional plant hosts and sugar-water feeders at suburban gardens contributed to differences in bacterial community composition and increased suburban bacterial beta diversity. However, rural bottlebrush nectar samples had higher bacterial beta diversity than di rural harakeke nectar samples, indicating that host types, rather than urbanization alone, drives the increase in beta diversity. Placing these results in the broader context of ongoing urbanization, it is likely that increased nectar-producing plant diversity along with the addition of sugar-water feeders may coincide with shifts in microbial community composition and increased nectar microbial beta diversity due to microbial species sorting across plant species and sugar-water feeders.

Urbanization can have varied impacts on plant, animal and microbial diversity, and our findings of differing responses of bird- and nectar-associated microbial diversity emphasize this and identify underlying drivers. Similar to a previous study that identified increased species taxonomic turnover of avian gut microbiomes in urbanized compared to rural areas (Phillips et al., 2018), we found bacterial communities carried by suburban birds were more variable than those carried by rural birds. However, the nectar microbial communities across suburban and rural gardens generally did not mirror the diversity of these bird associated communities, suggesting that nectar can act as a strong filter. This result supports previous findings of nectar as a filter, selecting for specific fungal (Herrera et al., 2010) and bacterial (Vannette & Fukami, 2017) taxa from the larger microbial species pool carried by floral visitors, such as birds and bees. This filtering may be due to high osmotic pressure, low nitrogen (Letten et al., 2018), and various antimicrobial compounds in nectar (Roy et al., 2017), all of which may select for a specific subset of fungal and bacterial taxa able to physiologically tolerate and grow under these conditions. Additionally, as bottlebrush and harakeke nectars in suburban and rural gardens hosted similar communities, our findings suggest that nectar inhabiting microorganisms represent a consistent subset of microbial taxa across both suburban and rural environments.

A notable exception to the general similarities in diversity between shared plant taxa in suburban and rural gardens was the elevated fungal richness in suburban gardens. This was significantly increased for harakeke and results trended in the same direction for the limited number of bottlebrush nectar samples. These differences appear to be driven by the presence of *Candida* and *Rhodoporidium* taxa in the case of suburban harakeke and *Pseudozyma* and low abundance taxa for bottlebrush samples. While certain *Candida* yeast species can be human pathogens, other species are readily isolated from floral nectar (Belisle et al., 2012).

Microbial species sorting across different host types has been identified across a wide range of host species (Cleary et al., 2019; Engel et al., 2018) and can be an important driver of beta diversity within metacommunities (Leibold et al., 2004). Our findings that bacterial communities associate with specific plant hosts and sugar-water feeders and increased nectar bacterial metacommunity beta diversity in suburban compared to rural gardens align with the predictions of the species sorting paradigm. Further, we found that differences in beta diversity are due to microbial species sorting across host types rather than to nestedness of the microbial communities (where the rural metacommunity is a strict subset of the suburban one). Our findings of species sorting across host taxa agree with previous studies on nectar microbial communities associated with neotropical (Canto et al., 2017), temperate (Fridman et al., 2012), and desert plant species (von Arx et al., 2019), as well as on sugar-water bird feeders (Lee et al., 2019) that have also identified host-specific microbial communities. Microbial species sorting may be driven by disperser identity (de Vega et al., 2021) and nectar characteristics of different plant species, which likely vary in their sugar and nitrogen concentrations and secondary metabolites, which may function as antimicrobial compounds (Roy et al., 2017). Quantification of dispersal rate by different species of nectar-feeding birds and experimental studies are necessary to determine the roles these different bird species play and which aspects of nectar act as agents of selection on the microbial communities (Álvarez-Pérez et al., 2021).

We detected varied relative abundances of specific bacterial genera across plant species and sugar-water feeders. These differences likely underlie the observed microbial species turnover. Specifically, we identified higher relative abundance of the bacterial genera *Pantoea*, *Mesoaciditoga*, *Spongiimonas*, *Curtobacterium*, *Neokomagatea*, and *Asaia* in the pōhutukawa, frangipani, and sugar-water feeder samples from suburban gardens compared to nectar samples from rural gardens. *Pantoea* was common in our sugar-water feeder samples, and species in this genus have been identified previously from floral nectar samples (Álvarez-Pérez et al., 2012; Samuni-Blank et al., 2014). *Asaia* was found in higher relative abundance in pōhutukawa nectar samples compared to samples from the other hosts, and *Neokomagatea* was found in relatively high abundance in sugar-water feeders, tūī, and silvereye samples in suburban compared to samples from rural gardens. These two genera are in the acetic acid family and have been associated with reduced sugar concentration and pH in nectar (Vannette et al., 2013) and sugar-water (Lee et al., 2019; Tucker & Fukami, 2014). Hummingbirds have been shown to consume less nectar when the pH is low with negative consequences for plant seed set (Vannette et al., 2013), and these findings may extend to this plant–pollinator system.

We did not detect common bird pathogens, such as *Salmonella*, *Chlamydophila*, or *Aspergillus* in sugar-water feeders, which suggests that sugar-water may not be a suitable habitat. Indeed, similar to floral nectar, sugar-water may constitute a harsh environment for non-specialized microorganisms. However, *Pseudomonas* was detected in moderate abundance in the sugar-water feeder samples, as well as on all three bird species and in pōhutukawa, bottlebrush, and harakeke nectar samples. This bacterial genus is cosmopolitan, and previous work has isolated it from nectar samples (Álvarez-Pérez et al., 2012) and identified some species that can cause disease in birds (Walker et al., 2002) and plants (Serizawa et al., 1989). Additionally, the fungal plant pathogen *Kabatiella* dominated the sugar-water feeder. The presence of putative plant pathogens in feeders may result in increased dispersal opportunity, and thus disease spread. Yet, we add a note of caution to this interpretation as following post-processing for fungal taxa only a single sugar-water feeder sample remained. To our knowledge, our study is the first to characterize microbial communities across floral nectar, sugar-water feeders, and birds in the southern hemisphere. Interestingly, we did detect a higher proportion of shared bacterial taxa between nectar resources and tūī, a nectar-specialist bird species, compared to the proportion hosted by silvereyes and sparrows. The influence of pollinator behaviour and ecology on nectar microbial communities remains understudied and our results suggest that this is an important question to be addressed in future research. Notably, while present in our data, we did not detect a high relative abundance of the bacterial genus *Acinetobacter* nor of the fungal genus *Metschnikowia*, which are both common in floral nectar samples in the northern hemisphere (Álvarez-Pérez et al., 2012; Álvarez-Pérez & Herrera, 2013; Belisle et al., 2012; Fridman et al., 2012; Jacquemyn et al., 2013; Vannette & Fukami, 2017), suggesting there may be biogeographical patterns of nectar microbial diversity.

As with any observational study, we interpret our results with respect to several caveats and limitations. First, we focused on nectar-feeding birds, as they are an important guild of pollinators in New Zealand (Anderson, 2003). However, insects are also key pollinators and visit all the flowering plants in this study. Recent work has linked nectar microbial communities with pollinator guilds (de Vega et al., 2021; Morris et al., 2020), and these microbes constitute part of the larger dispersing species pool that was not considered within this study. Including insect-vectored microbes could increase overall taxonomic richness but would likely not change our findings, as any insect-vectored microbes are currently included within samples from floral nectar and sugar-water feeders. Second, our sampling of the nectar microbial communities was limited to gardens in suburban and rural Auckland, with limited sampling of sugar-water feeders. Additionally, our analyses include five suburban and three rural gardens and plant species and sugar-water feeders that were present only at the suburban gardens. An unbalanced study design, often a feature of ecological surveys, could affect diversity estimates, we address this concern by comparing harakeke and bottlebrush nectar samples (which were collected from both suburban and rural gardens) to test for differences that arise from suburban and rural gardens, while controlling for plant identity. However, in order to be biologically realistic, we then include the nectar microbial communities from the plant species and sugar-water feeders present only at the suburban gardens to understand how increased host richness affects nectar microbial metacommunities. Future research should expand data collection to include multiple cities and their adjoining suburban and rural areas to determine how generally these findings apply. Care should also be taken when selecting these sites as environmental factors may have nonlinear effects. It would also be particularly useful to expand data collection on nectar microbial communities in the southern hemisphere to understand whether the biogeographical patterns of potentially restricted ranges observed here hold.

CONCLUSIONS

In summary, we show that plant species and sugar-water feeders harbour specific consortia of bacteria, indicative of filtering from the larger species pools vectored by birds, and that additional plant species and sugar-water feeders drive the increased bacterial taxonomic turnover across the nectar bacterial metacommunity in suburban compared to rural environments. In contrast, fungal communities in suburban nectar samples showed increased fungal richness, yet similar beta diversity across the communities. While we detected microorganisms that can influence plant attractiveness, the consequences of this increased bacterial beta diversity and fungal richness in suburban areas on plant–pollinator interactions remain unknown. In light of the recent finding that pollinator-dependent plants in urbanized areas experience the greatest pollen limitation globally (Bennett et al., 2020), future work on the impacts of nectar microorganisms on pollination in urbanized areas is an important line of inquiry. Effects of urbanization on biodiversity can vary based on the taxonomic groups considered and human decisions. Selecting diverse plants for urban gardens and provisioning birds with sugar-water feeders shape the host plant and bird vector species in differing ways, leading to contrasting consequences on nectar microbial diversity.

AUTHOR CONTRIBUTIONS

Marion L. Donald, Manpreet K. Dhami, and Josie A. Galbraith designed the study, Marion L. Donald, Manpreet K. Dhami, Josie A. Galbraith, and Daria A. Erastova performed the field study, Anastasija Podolyan developed and optimized duplexed amplicon PCR, Marion L. Donald processed the samples, analysed the data with input from Manpreet K. Dhami and Tom E. X. Miller and wrote the manuscript with input from all co-authors. All authors gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Land use classification data from the Land Cover Database v.5.0 for Mainland New Zealand (DOI: 10.26060/W5B4-WK93). Datasets are available in the FigShare Repository at https://figshare.com/projects/Urbanization_increases_microbial_diversity_in_a_nectar_microbial_metacommunity/100367. Sequence data are available at NCBI SRA under BioProject no. PRJNA839845.

ETHICS STATEMENT

All animal procedures related to this study were approved by the University of Auckland Animal Ethics Committee (Ref. 002100) and the Department of Conservation wildlife permit (69319-FAU).

ORCID

Marion L. Donald https://orcid.org/0000-0001-9077-8411

Josie A. Galbraith https://orcid.org/0000-0003-3869-7977

Daria A. Erastova https://orcid.org/0000-0003-1947-2123

Anastasija Podolyan https://orcid.org/0000-0002-8332-2683

Tom E. X. Miller https://orcid.org/0000-0003-3208-6067

Manpreet K. Dhami https://orcid.org/0000-0002-8956-0674

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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