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# Effect of urbanization and parasitism on the gut microbiota of Darwin's finch nestlings

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## Abstract

Host-associated microbiota can be affected by factors related to environmental change, such as urbanization and invasive species. For example, urban areas often affect food availability for animals, which can change their gut microbiota. Invasive parasites can also influence microbiota through competition or indirectly through a change in the host immune response. These interacting factors can have complex effects on host fitness, but few studies have disentangled the relationship between urbanization and parasitism on an organism's gut microbiota. To address this gap in knowledge, we investigated the effects of urbanization and parasitism by the invasive avian vampire fly (*Philornis downsi*) on the gut microbiota of nestling small ground finches (*Geospiza fuliginosa*) on San Cristóbal Island, Galápagos. We conducted a factorial study in which we experimentally manipulated parasite presence in an urban and nonurban area. Faeces were then collected from nestlings to characterize the gut microbiota (i.e. bacterial diversity and community composition). Although we did not find an interactive effect of urbanization and parasitism on the microbiota, we did find main effects of each variable. We found that urban nestlings had lower bacterial diversity and different relative abundances of taxa compared to nonurban nestlings, which could be mediated by introduction of the microbiota of the food items or changes in host physiology. Additionally, parasitized nestlings had lower bacterial richness than nonparasitized nestlings, which could be mediated by a change in the immune system. Overall, this study advances our understanding of the complex effects of anthropogenic stressors on the gut microbiota of birds.

**KEYWORDS**
gut microbiome, host parasite interactions, invasive species, parasitism, species interactions, urban ecology

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## 1 | INTRODUCTION

The gut microbiota is a community of microorganisms inhabiting an organism's digestive tract that can affect host physiology and health (Gomaa, 2020; Grond et al., 2018). Specific gut bacterial taxa, such as *Ruminococcus*, can aid in host digestion by breaking down indigestible food compounds, such as cellulose, and increase digestive efficiency in humans (Arumugam et al., 2011; Thursby & Juge, 2017). Furthermore, variation in diet, such as a plant-based diet versus a meat-based diet, can shape host gut microbiota as it shifts in response to nutrient availability (Gomaa, 2020; Zhu et al., 2015). Additionally, the host's immune system can interact with the gut microbiota, specifically by shaping its community composition (Hooper et al., 2012). For example, Kato et al. (2014) found that knockout mice who are deficient in B-cell production have lower gut bacterial diversity than non-knockout mice. The gut microbiota can also initiate the production of immune cells to help maintain or develop an effective immune response (Zhang et al., 2017). Studying the dynamic relationships between physiological processes and gut microbiota will be continually important, especially in the light of increasing anthropogenic change, which can directly and indirectly affect these factors.

Parasites and pathogens, which also affect host health, can directly and indirectly affect microbial communities in the gut (Stensvold & Van Der Giezen, 2018). Within the gut, parasites can directly affect the microbiota by competing with or consuming commensal bacteria (Abt & Pamer, 2014). Parasites might also indirectly affect the gut microbiota via the host's immune system. For example, gut parasites (e.g. *Heligmosomoides polygyrus*) can activate a non-specific mucosal immune response (e.g. T and Th2 cells), which can select for or against bacterial taxa (Rausch et al., 2018). However, parasites located outside the gut might also elicit a systemic immune response that could affect the bacterial community in the gut. Fish with ectoparasitic fluke (*Dactylogyrus lamellatus*) infections have lower gut bacterial diversity than uninfected fish, which was likely mediated by elevated expression of immune genes related to the IgM antibody, Toll-like receptor 3, and major histocompatibility factor II responses (Wang et al., 2023). Knutie (2020) found that parasitic nest fly abundance is negatively correlated with gut bacterial diversity and positively correlated with IgY antibody levels, in eastern bluebirds (*Sialia sialis*). Overall, most of these nonmodel organism studies are correlational, and thus, the causal effects of parasitism on gut microbiota are not well-understood.

Factors related to urbanization, such as changes in food availability, can also influence the gut microbiota of wild hosts (Berlow et al., 2021; Phillips et al., 2018; Teyssier et al., 2020). Studies have found that urban hosts have a larger diet breadth, which can result in greater diversity of the gut bacterial community (Gadau et al., 2019; Littleford-Colquhoun et al., 2017). Nonurban water dragons (*Intellagama lesueurii*) feed primarily on invertebrates but urban water dragons feed on invertebrates and plant material, which results in higher gut bacterial diversity. These effects are either because plants introduce additional bacterial taxa or a larger diet breadth selects for different bacteria to aid in digestion. If the latter is supported, a more diverse gut microbial community could help hosts be more equipped to deal with environmental change (Littleford-Colquhoun et al., 2017). In contrast, the gut of house sparrows (*Passer domesticus*) consuming a nonurban diet, which is rich in protein, is correlated with higher bacterial diversity than urban sparrows whose diet is poor in protein (Teyssier et al., 2020). While increased gut bacterial diversity is generally assumed to be beneficial to hosts, in some cases, such as in organisms with highly specialized diets, lower bacterial diversity can be associated with better health outcomes (Shade et al., 2017). Urban coyotes consume carbohydrate-rich anthropogenic food items and have higher bacterial diversity than their rural counterparts but are in poorer body condition and have increased prevalence of the parasite *Echinococcus multilocularis* (Sugden et al., 2020). Ultimately, these effects of urbanization and other stressors related to human activity (e.g. invasive parasites) are complex and could result in positive or negative implications for host health.

Given the complex effects of human activity on hosts, few studies have examined the influence of multiple synergistic anthropogenic factors, such as urbanization and invasive parasitism, on host gut microbiota. The Galápagos Islands of Ecuador provide an ideal study system to investigate these complex effects. Since 1979, the number of residents and tourists has increased, leading to changes in the island's natural habitat. These changes include the introduction of non-native species, including parasites (Kerr et al., 2004; Wikelski et al., 2004). More specifically, the avian vampire fly (*Philornis downsi*; hereon, vampire fly) was introduced to the Galápagos in the past several decades and is found on nearly all islands, including human-inhabited islands such as San Cristóbal. Adult flies are nonparasitic but lay their eggs in birds' nests where the hematophagous larvae feed on nestling hosts and brooding mothers (Fessl et al., 2001, 2006; Fessl & Tebbich, 2002). Several studies have found that the vampire fly can have detrimental effects on the survival of nestling Darwin's finches (Fessl et al., 2010; Kleindorfer et al., 2021; Kleindorfer & Dudaniec, 2016; Knutie et al., 2016; Koop et al., 2011, 2013; McNew & Clayton, 2018; O'Connor et al., 2010). However, a recent study found that urban finches on San Cristóbal Island are less affected by and more resistant to the vampire fly than nonurban finches (Knutie et al., 2023), who suffer up to 100% mortality due to the fly (Koop et al., 2013; O'Connor et al., 2014). For nonurban finches, the vampire fly does not affect their gut microbiota (Addesso et al., 2020; Knutie, 2018; Knutie et al., 2019). However, because urban finches are more resistant to the fly and have fewer parasites (Knutie et al., 2023), and immunological resistance can interact with gut microbiota, parasitism may cause a greater change on the microbiota of urban finches compared with nonurban finches. To date, no studies have causally explored whether parasitism and urbanization interact to affect the gut microbiota of hosts.

The goal of this study was to quantify the effects of avian vampire flies and urbanization on the gut microbiota of nestlings of the small ground finch (*Geospiza fulignosa*) in 2018 and 2019. Specifically, we experimentally manipulated parasite abundance in

urban and nonurban finch nests and then characterized the gut microbiota (i.e. alpha and beta diversity, community composition, and relative abundance of taxa). Because diet can influence the gut microbiota (Davidson et al., 2020), and urban finches have a more diverse diet than nonurban finches (De León et al., 2019), we hypothesize that urban nestlings will have a different gut microbiota than nonurban nestlings (Loo, Dudaniec, et al., 2019; Loo, García-Loor, et al., 2019). Because immunological resistance can be linked to the gut microbiota (Hooper et al., 2012), we predict parasitism will affect and ultimately change the gut microbiota of urban nestlings. We also predict that parasitism will not affect the gut microbiota of nonurban nestlings, since previous research has found there is no effect of parasitism on nonurban birds. Further investigation will allow for a more thorough understanding of environmental change on the gut microbiota of small ground finch nestlings.

## 2 | METHODS

### 2.1 | Study system

We conducted the study between February–May 2018 and 2019 (during the breeding season) in the arid lowlands of San Cristóbal (557 $km^2$) in the Galápagos Islands. We used the capital city of Puerto Baquerizo Moreno (0°54′09″ S, 89°36′33″ W) as our urban area (hereon, urban area), which is the only large town on San Cristóbal and the second largest city in the Galápagos archipelago with a human population of 7199 (INEC, 2016). The urban area primarily consists of impermeable concrete or stone surfaces and human-built structures in altered landscapes. The urban study area measured 0.79 $km^2$ (~1.2 × 0.62 $km^2$) and included tourist and residential zones. We used Jardín de Opuntias (0°56′18″ S, 89°32′54″ W) as our nonurban area (hereon, nonurban area), which is a Galápagos National Park site located 4.5 km southeast of the urban area. This site consisted of vegetated natural habitats with no unnatural impermeable surfaces present. Our nonurban study area measured 0.21 $km^2$ and covered 1.4 km of the main trail and 0.15 km to each side.

Small ground finches are abundant at both field sites (Harvey et al., 2021). Small ground finches build domed-shaped nests in native and non-native trees and shrubs as well as human-built structures, depending on the location. On San Cristóbal Island, they lay 1–4 eggs per clutch (mean = 2.84 eggs) and have an average of 2.83 nestlings per brood across urban and nonurban areas. However, in some years, nests in urban areas contained more eggs than nests in nonurban areas, yielding higher nestling survival (Harvey et al., 2021).

### 2.2 | Experimental manipulation of parasites

We searched field sites daily for evidence of nest building by small ground finches. Once eggs were laid, we checked nests every other day until the nestlings hatched. Prior to egg hatching, we experimentally manipulated fly abundance via adding approximately 10 mL of 1% permethrin solution (Permacap; hereon, fumigated) or water (hereon, sham-fumigated) to the nest. Within 2 days of nestling hatching, we treated nests again to ensure the removal of all parasites. Briefly, we removed the contents of the nests (including the nestlings, unhatched eggs and the nest liner), and treated them with either a permethrin solution or water by spraying approximately 5 mL into the nest where the larvae live. After treatment, we returned the dry nest liner, and placed nestlings back into the nest. We used permethrin because it is highly effective at removing vampire flies from the nests (Kleindorfer & Dudaniec, 2016). However, studies have found that permethrin can have sublethal effects on nestling birds (Bulgarella et al., 2020); therefore, we ensured that nestlings did not come into contact with the insecticide. Additionally, adults returned to their nests with no cases of abandonment from the treatment (Knutie et al., 2016; Koop et al., 2013).

### 2.3 | Sample collection

We banded nestlings at 6–8 days old with a unique colour band combination and a numbered metal band (National Band and Tag). We collected faecal samples from nestlings opportunistically at this time. To collect faecal samples, we removed nestlings from the nest and held them over a sterile weigh boat until they defecated. We then moved the faecal sample from the tray to a sterile tube, placed it on ice in the field for up to 6 h and then stored it in a −20°C freezer until we extracted the bacterial DNA. We transported the samples to the University of Connecticut and stored them in a −80°C freezer for downstream 16S rRNA gene sequencing. Although studies show that the bacterial community in avian faeces does not always represent the entire digesta of the host (e.g. in the cecum; Wilkinson et al., 2017), faecal samples are generally representative of the bacterial community in the large intestines (Videvall et al., 2018; Wilkinson et al., 2017) and are used when hosts cannot be euthanized. We collected and examined nests for *P. downsi* when they were empty; the full data set was reported in Knutie et al. (2023).

### 2.4 | Bacterial DNA extraction and sequencing

We extracted total DNA from faeces using a ZymoBIONICS DNA kit and sent DNA extractions to the University of Connecticut Microbial Analysis, Resources and Services for sequencing with an Illumina MiSeq platform and v2 2 × 250 base pair kit (Illumina, Inc.). We also sequenced a laboratory extraction blank to control for kit contamination. We conducted bacterial inventories via amplification of the V4 region of the 16S rRNA gene using primers 515F and 806R and with Illumina adapters and dual indices (Kozich et al., 2013). We used the DADA2 (version 1.22.0) pipeline (Callahan et al., 2016) in R (version 4.2.0) to process sequence data. After quality assessment, we trimmed sequences to

remove low-quality read areas and chimeric reads. We classified amplicon sequence variant (ASV) taxonomies using RDP's Naive Bayesian Classifier (Wang et al., 2007) with the Silva reference database (version 138.1; Quast et al., 2012). After classification, we removed sequences identified as chloroplast and mitochondria from the data set. We identified and removed likely bacterial contaminants with the package *decontam* (Davis et al., 2018) in R using the kit extraction blank that was processed in parallel with the other samples as a control. Sequences were aligned using the *DECIPHER* package (version 2.22.0) in R (Wright, 2015), and a generalized time-reversible maximum likelihood tree of the remaining ASVs was constructed with the *phangorn* package version 2.9.0 (Schliep, 2011). The ASV table, taxonomic information, phylogeny and sample metadata were joined for bacterial community analyses using the package *phyloseq* (McMurdie & Holmes, 2013). We filtered the feature table to retain samples with at least 1500 total reads, which reduced the data set to 58 samples containing 2526 unique ASVs. The resulting data set had an average of 51,056 ± 6178 reads per sample (min: 1732; max: 294,720). For alpha and beta diversity analyses, we rarefied samples to the sample with the lowest read count (1732). The filtered data set contained 1448 ASVs after random subsampling.

## 2.5 | Statistical analyses

### 2.5.1 | Alpha diversity

We used the R package *vegan* (Oksanen et al., 2022) to compute alpha diversity (observed ASV richness, Shannon diversity index) on the filtered data set. Observed richness describes the number of observed species, and the Shannon diversity index is an estimator of species richness and species evenness, which describes the distribution of abundance across the species. We ran generalized linear mixed effects models with a negative binomial error structure for observed ASV richness and a Gaussian distribution for Shannon diversity using the glmmTMB package (Brooks et al., 2017). Location (urban, nonurban), parasite treatment (fumigated, sham-fumigated) and the interaction between location and parasite treatment were considered in all models. Because the microbiota of finches can vary across years (Michel et al., 2018), we also included year (2018 and 2019) as a covariate in all models. Nest identification was included as a random effect in all models. We used the Anova function in the *car* package (Fox & Weisberg, 2018) to determine significance.

### 2.5.2 | Beta diversity

To deal with unequal sequence coverage, we used cumulative-sum scaling normalization on the data set using the R package *metagenomeseq* (Paulson et al., 2013) following the methods in Maraci et al. (2021). We then log ($x+0.0001$) transformed the data and later corrected the transformed values by subtracting the log of the pseudo count (Thorsen et al., 2016). Dissimilarity matrices were computed based on Bray–Curtis (Bray & Curtis, 1957), unweighted UniFrac (Lozupone & Knight, 2005), and weighted UniFrac (Lozupone et al., 2007). To visualize the dissimilarities between nestlings based on parasite treatment and urbanization, we used a principal coordinate analysis (PCoA) using the ordinate function in the *phyloseq* package (McMurdie & Holmes, 2013). To determine the effect of parasitism and urbanization on beta diversity metrics, we used PERMANOVAs with parasite treatment, location, year (2018, 2019), and the interaction between parasite treatment and location as fixed effects and beta diversity metrics as response variables. We included nest identification as a random effect in all models. For these analyses, we used the adonis2 function with the *vegan* package (Oksanen et al., 2022).

### 2.5.3 | Relative abundance of bacterial taxa

We calculated the relative abundances of phyla and genera from the unrarefied data set. For analyses, we lumped phyla and genera with mean abundances <1% into the 'Other' category. These data stringency limited analyses to the top three most abundant phyla (Proteobacteria, Firmicutes, Actinobacteriota) and the top 12 most abundant genera (Table 1). We ran nonparametric Kruskal–Wallis tests using the kruskal.test function in R to compare abundances of bacterial taxa between locations (urban, nonurban), parasite treatments (sham-fumigated, fumigated), and locations within the parasitized (sham-fumigated) treatment group. We adjusted *p*-values for false discovery rate with a Benjamini–Hochberg correction where significance was determined as $p_{adj} < .05$.

# 3 | RESULTS

## 3.1 | Bacterial diversity

Nestlings from fumigated nests had higher bacterial richness than nestlings from sham-fumigated nests (Figure 1a, $\chi^2 = 4.04$, $p = .04$). Shannon diversity index was not significantly affected by parasite treatment (Figure 1b, $\chi^2 = 1.10$, $p = .29$). Urbanization did not significantly affect observed richness (Figure 1c, $\chi^2 = 2.30$, $p = .13$), but did influence the Shannon index (Figure 1d, $\chi^2 = 7.50$, $p = .006$). Nonurban nestlings had higher Shannon diversity index values than urban nestlings. Observed richness and Shannon index were not affected by the interaction between parasite treatment and urbanization (Figure S1, Observed richness: $\chi^2 = 0.28$, $p = .60$, Shannon Index: $\chi^2 = 0.21$, $p = .65$). The year that samples were collected (2018, 2019) did not significantly affect the observed richness (Table S1, $\chi^2 = 2.55$, $p = .11$) or Shannon index ($\chi^2 = 0.38$, $p = .54$).

**TABLE 1** Kruskal–Wallis chi-squared ($\chi^2$) test statistics and *p*-values for comparisons of bacterial taxa across location (urban, nonurban), parasite treatment (sham-fumigated, fumigated), and location (urban, nonurban) across parasitized (sham-fumigated only) nests. *p*-values were adjusted for false discovery rate with a Benjamini–Hochberg correction where significance was determined as $p_{adj} < .05$. Significant differences between groups are represented in bold.

| | Location | | Parasite treatment | | Location (sham-fumigated only) | |
|---|---|---|---|---|---|---|
| **Taxonomic group** | $\chi^2$ | $p_{adj}$ | $\chi^2$ | $p_{adj}$ | $\chi^2$ | $p_{adj}$ |
| Phylum | | | | | | |
| Actinobacteriota | 4.14 | .06 | 4.03 | .18 | 0.68 | .55 |
| Firmicutes | **13.74** | **.0008** | 0.65 | .42 | **9.63** | **.004** |
| Proteobacteria | **8.35** | **.01** | 0.93 | .42 | **9.63** | **.004** |
| Other (<1%) | 1.31 | .25 | 1.35 | .42 | 0.00 | .48 |
| Genus | | | | | | |
| *Campylobacter* | 0.65 | .55 | 2.18 | .56 | 0.69 | .53 |
| *Candidatus* Arthromitus | **10.94** | **.01** | 1.94 | .56 | 5.71 | .11 |
| *Clostridium* sensu stricto *1* | 0.46 | .59 | 0.36 | .71 | 2.62 | .28 |
| *Corynebacterium* | 1.17 | .44 | 1.07 | .56 | 0.04 | .85 |
| *Cronobacter* | 0.14 | .71 | 4.59 | .42 | 1.49 | .36 |
| *Enterococcus* | 0.27 | .66 | 1.11 | .56 | 0.21 | .76 |
| *Erysipelatoclostridium* | **6.62** | **.03** | 0.98 | .56 | 3.32 | .22 |
| *Escherichia-Shigella* | 1.06 | .44 | 0.90 | .56 | 0.09 | .82 |
| *Klebsiella* | **7.13** | **.03** | 0.74 | .56 | 6.76 | .11 |
| *Kocuria* | 1.57 | .44 | 0.05 | .82 | 3.30 | .22 |
| *Ligilactobacillus* | 1.30 | .44 | 0.07 | .82 | 0.72 | .53 |
| *Rothia* | **7.29** | **.03** | 0.17 | .80 | 2.10 | .32 |
| Other (<1%) | 4.07 | .11 | 1.79 | .56 | 1.83 | .33 |

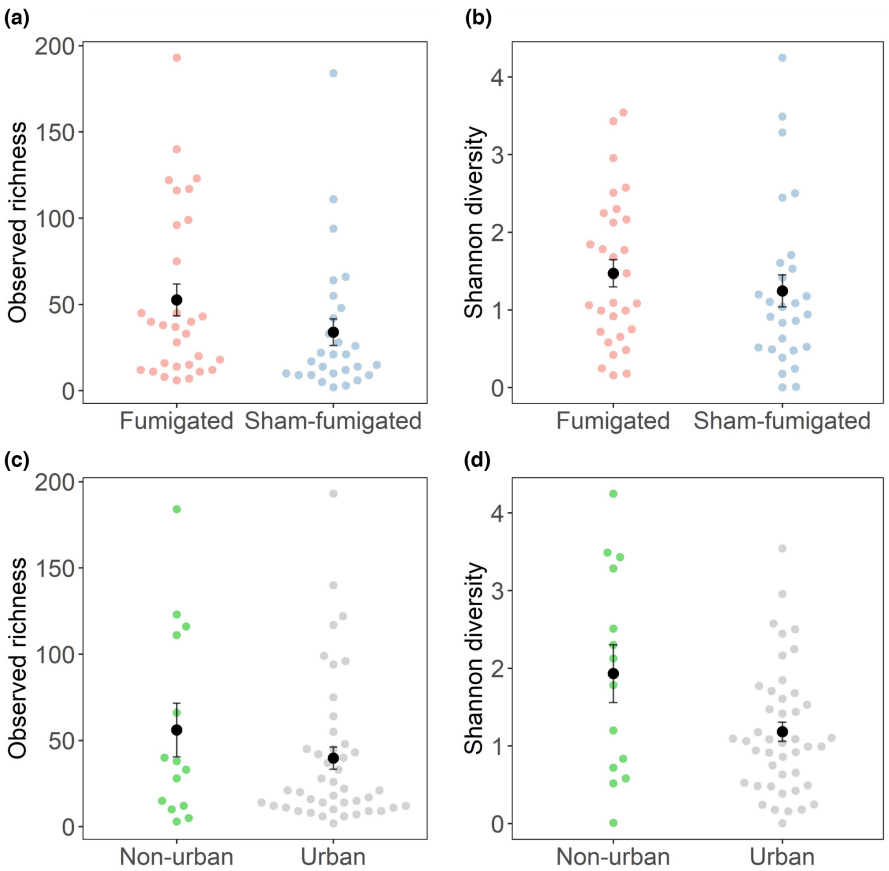


**FIGURE 1** Effect of parasite treatment (sham-fumigated, fumigated) on (a) observed ASV richness and (b) Shannon diversity of the microbiota in nestling small ground finches. Individuals from fumigated nests are represented by red circles and individuals from sham-fumigated nests are represented by blue circles. Effect of location (urban, nonurban) on (c) the observed ASV richness and (d) Shannon diversity of the microbiota in nestling small ground finches. Individuals from nonurban nests are represented by green circles and individuals from urban nests are represented by grey circles. Black circles denote the mean values (±SE) of birds from each treatment.

### 3.2 | Bacterial community structure and membership

Parasite treatment, urbanization, and the interaction between the two variables did not significantly affect Bray–Curtis dissimilarity, unweighted UniFrac, or weighted UniFrac distances (Figure S2a, Table S2). There was also no effect of year (2018, 2019) on Bray–Curtis dissimilarity, weighted UniFrac, and unweighted UniFrac distances (Table S2).

### 3.3 | Microbiota taxonomic composition

There were 2526 unique ASVs present in the samples. Twenty-four phyla were identified, and samples were dominated by Firmicutes (52.6%), Proteobactiera (35.1%) and Actinobacteriota (8.5%; Figure 2). All other phyla had mean relative abundances of <1%. Across the 58 samples, there were 551 genera identified and 12 genera had relative abundances >1%. The most commonly observed genera were *Candidatus* Arthromitus (22.3%), *Escherichia-Shigella* (13.8%), *Enterococcus* (13.5%), *Ligilactobacillus* (9.1%), *Klebsiella* (7.8%), *Cronobacter* (4.8%), *Erysipelatoclostridium* (2.3%), *Rothia* (2.2%), *Clostridium* sensu stricto *1* (1.9%), *Campylobacter* (1.6%), *Corynebacterium* (1.4%) and *Kocuria* (1.1%).

At the phylum level, location significantly affected the relative abundance of Firmicutes and Proteobacteria (Table 1). Specifically, nestlings from urban sites had higher relative abundances of Firmicutes ($\chi^2 = 13.74$, $p_{adj} = .008$) and lower abundances of Proteobacteria ($\chi^2 = 8.35$, $p_{adj} = .01$) than nestlings from nonurban field sites (Figure 2). At the genus level, location significantly affected the abundance of *Candidatus* Arthromitus, *Klebsiella*, *Erysipelatoclostridium*, and *Rothia* (Table 1, Figure S3). *Candidatus* Arthromitus was highly abundant in urban nestlings, but rarely observed in faecal samples collected from nonurban nestlings (Figure 3, $\chi^2 = 10.94$, $p_{adj} = .01$). Conversely, *Klebsiella* ($\chi^2 = 7.13$, $p_{adj} = 0.03$) and *Erysipelatoclostridium* ($\chi^2 = 6.62$, $p_{adj} = .03$) were observed at higher abundances in faecal samples from nonurban nestlings (Figure 3). The genus *Rothia* was more common in nestlings from urban nests and was rarely observed in nonurban nestlings ($\chi^2 = 7.29$, $p_{adj} = .03$). This trend was difficult to visualize due to two individuals that had very high relative abundance values (Figure S4: one nonurban nestling: 41.89% *Rothia*, and one urban nestling: 63.43% *Rothia*). Additional outliers were detected and removed using the boxplot.stats function in R (nonurban:

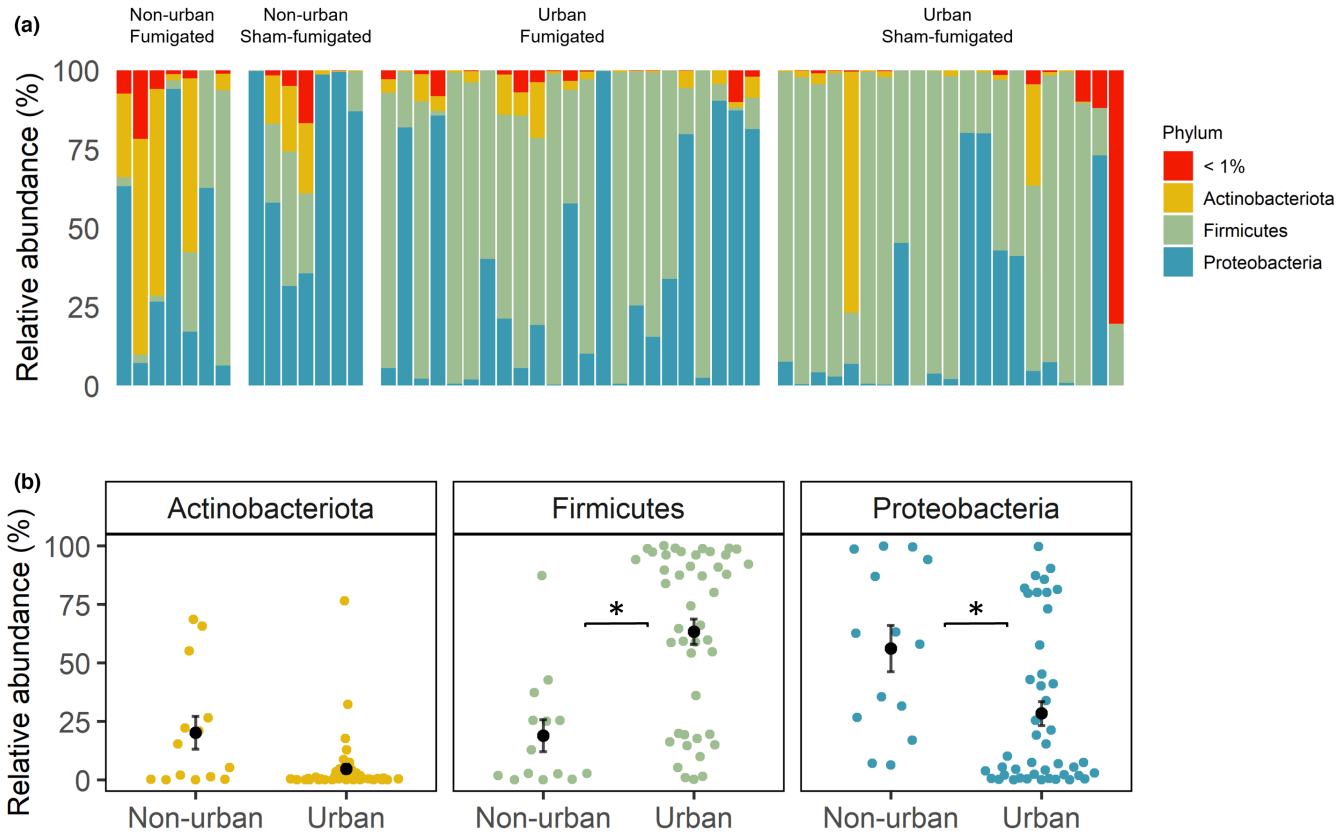


FIGURE 2 (a) Proportional abundance of bacterial phyla across location (urban, nonurban) and parasite treatment (fumigated, sham-fumigated). Each bar represents a sample from an individual bird. Samples are divided into treatment groups as follows: nonurban, fumigated: $n = 7$ nestlings; nonurban, sham-fumigated: $n = 7$; urban, fumigated: $n = 23$; urban, sham-fumigated: $n = 21$. Phyla with <1% relative abundance are collapsed into the category '<1% abundance'. (b) Relative abundance of the three most common phyla separated by location (urban, nonurban). Individual points represent the relative abundance of each phyla from an individual nestling. Black circles denote the mean (±SE) relative abundances across treatments.

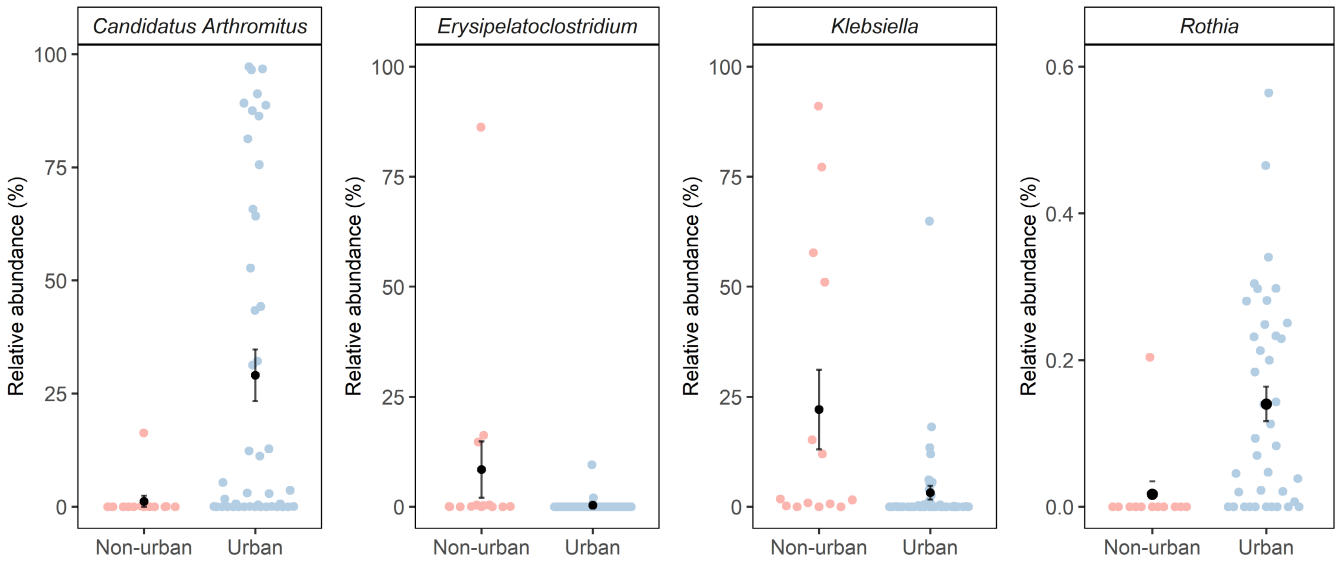


**FIGURE 3** Relative abundance of the four genera (*Candidatus* Arthromitus, *Klebsiella*, *Erysipelatoclostridium*, and *Rothia*) that significantly varied by location (urban, nonurban). Individual points represent the relative abundance of each genera from an individual nestling. Black circles denote the mean (±SE) across treatments.

1.25%, urban nestlings: 8.36%, 2.77%, 2.15%, 0.73%). Location (urban, nonurban) still significantly affected the relative abundance of *Rothia* with the two greatest outliers removed (Figure S4: $\chi^2 = 10.11$, $p_{adj} = .01$) and with the five additional outliers identified by the boxplot.stats function removed (shown in Figure 3, $\chi^2 = 12.57$, $p_{adj} = .005$).

Parasite treatment did not significantly influence the relative abundance of the three most abundant phyla or the abundance of any of the 12 most abundant genera (Table 1). When analysing only nestling samples from sham-fumigated nests, there was still a significant effect of location on the abundance of Firmicutes and Proteobacteria (Figure S5). However, there was no effect of urbanization on the abundance of the top 12 genera within the sham-fumigated nests (Table 1).

## 4 | DISCUSSION

Our study used a factorial experiment to determine the main and interactive effects of urbanization and parasitism on the gut microbiota of nestling small ground finches across two years. Although we did not find an interactive effect of urbanization and parasitism on the microbiota, we did find main effects of each variable. Contrary to our prediction, urban nestlings had lower bacterial diversity (Shannon index) than nonurban nestlings. Parasitized (sham-fumigated) nestlings had lower bacterial richness than nonparasitized (fumigated) nestlings. However, parasitism did not affect the Shannon index and urbanization did not affect observed richness, which suggests that parasitism affects the number of bacterial taxa but not evenness of those taxa, whereas urbanization influences gut microbiota evenness. This explanation is supported by our finding that urbanization, but not parasite treatment, affects the relative abundance of several bacterial phyla and genera. We hypothesized that urban nestlings would have higher bacterial diversity because of their wide breadth of food items, including human-processed food, compared with a primarily insect-rich diet in nonurban nestlings. This hypothesis was based on several studies that found an increase in gut bacterial diversity in response to urbanization (Berlow et al., 2021; Knutie et al., 2019; Littleford-Colquhoun et al., 2019; Phillips et al., 2018) and because diet can influence the gut microbiota (Bodawatta et al., 2022). However, we found that urban nestlings had lower bacterial diversity than nonurban nestlings, as found in Teyssier et al. (2018). One possible explanation is that the human-related food items select for particular bacterial taxa that dominate the microbiota, leading to fewer taxa in the gut. Teyssier et al. (2020) found that adult house sparrows (*P. domesticus*) experimentally fed an urban diet had lower gut bacterial diversity. Knutie (2020) found that food supplementation with yellow mealworm beetle (*Tenebrio molitor*) larvae increased the gut bacterial diversity of eastern bluebirds. Thus, an insect-rich diet that is high in protein in nonurban nestlings might maintain high bacterial diversity compared to diets of human-based foods in urban nestlings. Another possible explanation is that the food itself is introducing bacteria into the gut of the nestlings (Grond et al., 2018; Videvall et al., 2019) because human food items have different microbiota (Jarvis et al., 2018). To test these hypotheses, future studies may consider sequencing the microbiota of specific diet items and comparing these results with the gut microbiota of finches.

Urban living also affected the relative abundance of bacterial genera and phyla in nestlings. For example, urban nestlings had higher abundances of the phylum Firmicutes and the genus *Candidatus* Arthromitus. Higher levels of Firmicutes in urban nestlings could be driven by the high-fat content of anthropogenic food sources available to finches in urban environments. For example, high-fat diets are associated with increased Firmicutes abundance in rodents and humans (Murphy et al., 2015; Turnbaugh

et al., 2008). These specific taxonomic changes in the gut microbiota can also facilitate functional changes in host physiology. Specifically, bacterial species from the phylum Firmicutes can aid in nutrient uptake and metabolism in chickens (Li et al., 2016; Zheng et al., 2016), which might be required for human-processed food. *Candidatus* Arthromitus is a well-studied, segmented filamentous bacterium that is nonpathogenic and attaches to the intestinal wall (Snel et al., 1995). Across host taxa, *Candidatus* Arthromitus influences the innate and adaptive immune responses in the gut (Macpherson & McCoy, 2015; Suzuki et al., 2004). Specifically, Liu et al. (2023) found that the relative abundance of *Candidatus* Arthromitus is positively correlated with the innate immune response (e.g. T-lymphocytes) during avian development. In our system, urban finch nestlings upregulate the expression of genes related to the T-lymphocyte production (Knutie et al., 2023), which might explain why we observed higher relative abundance of *Candidatus* Arthromitus in urban nestlings.

Given that past studies have not found an effect of parasitism on gut microbiota of nonurban finches (Addesso et al., 2020; Knutie, 2018), we did not expect to find different results in our nonurban nestlings. However, we found an overall effect of parasitism on the gut microbiota across both locations, with parasitized nestlings having lower bacterial diversity (via observed richness) than nonparasitized nestlings. One possible explanation for the contradictory results is that other species of Darwin's finches (e.g. medium ground finch [*Geospiza fortis*] and common cactus finch [*Geospiza scandens*]), host different gut microbial communities due to their different diets (e.g. cactus flower pollen, different seed types; De León et al., 2014). The interactions between the immune system and gut microbiota can be determined by which microbes are recognized by immune molecules. Thus, a change in the gut microbiota in small ground finches, but not medium ground finches or common cactus finches, in response to parasitism could be because their specific members of the microbiota are recognized and removed by the immune system, a process known as host filtering (Mallott & Amato, 2021). We also hypothesized that only urban nestlings would be affected by parasitism since they are more resistant to parasites compared to nonurban nestlings (Knutie et al., 2023). This resistance is potentially related to expression of type 1 interferon (IFN) genes, which can activate natural killer cells and macrophages that can destroy bacteria (Perry et al., 2005). Since the bacterial diversity metrics of both urban and nonurban nestlings were affected by parasitism, this suggests that both populations are having a general response to the parasite that interacts with the gut microbes. To establish a causal relationship, further investigation is required to understand the interaction between gut microbiota and immune responses to parasitism.

Overall, our study suggests that both parasitism and urbanization affect the gut microbiota of small ground finches. Since these anthropogenic factors also affect the health of finches in the Galápagos Islands (Harvey et al., 2021; Knutie et al., 2023), the next question is whether the microbiota are mediating these effects or influencing other traits, such as the immune system, in developing finches. To causally test these interactions, an experimental manipulation of the gut microbiota is necessary, either with the introduction of relevant bacterial taxa or a disruption of the gut microbiota with antibiotics. Although birds of the Galápagos Islands have been directly affected by human presence, such as the introduction of parasites and changes in diet (De León et al., 2019; Wikelski et al., 2004), many indirect effects that are more difficult to study, such as those on the gut microbiota, could have important implications for the fitness of many endemic birds.

## AUTHOR CONTRIBUTIONS

Sarah A. Knutie was involved in conceptualization, supervision and funding acquisition; Sarah A. Knutie and Grace J. Vaziri were involved in experimental methodology; Ashley C. Love was involved in analyses; Gabrielle Solomon, Ashley C. Love, Grace J. Vaziri, Johanna Harvey, Taylor Verrett, Kiley Chernicky, Shelby Simons, Lauren Albert and Sarah A. Knutie were involved in investigation; Ashley C. Love was involved in visualization; Jaime A. Chaves and Sarah A. Knutie were involved in project administration; Gabrielle Solomon, Ashley C. Love and Sarah A. Knutie were involved in Writing—original draft; All authors were involved in writing—review & editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

## OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at [http://www.doi.org/10.6084/m9.figshare.24151974].

## DATA AVAILABILITY STATEMENT

Supporting information has been made available online. Data are available at FigShare (doi: 10.6084/m9.figshare.24151974) and sequences have been uploaded to GenBank (BioProject accession number: PRJNA1018585).

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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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