Received: 9 October 2020 | Revised: 26 August 2021 | Accepted: 2 September 2021

DOI: 10.1111/mec.16170

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Repeated sampling of individuals reveals impact of tropical and temperate habitats on microbiota of a migratory bird

Heather R. Skeen[1,2] | Nathan W. Cooper[3,4] | Shannon J. Hackett[2] | John M. Bates[2] | Peter P. Marra[4]

[1]Committee on Evolutionary Biology, University of Chicago, Chicago, Illinois, USA

[2]Negaunee Integrative Research Center, Field Museum of Natural History, Chicago, Illinois, USA

[3]Migratory Bird Center, Smithsonian Conservation Biology Institute, National Zoological Park, Washington, District of Columbia, USA

[4]Department of Biology and McCourt School of Public Policy, Georgetown University, Washington, District of Columbia, USA

Correspondence
Heather R. Skeen, Field Museum Birds, Chicago, Illinois, USA.
Email: skeenhr@gmail.com

Funding information
National Geographic, Grant/Award Number: WW231R-17

Abstract

Migratory animals experiencing substantial change in diet and habitat across the annual cycle may have corresponding shifts in host-associated microbial diversity. Using automated telemetry and radio tags to recapture birds, we examined gut microbiota structure in the same population and often same individual of Kirtland's Warblers (*Setophaga kirtlandii*) initially sampled on their wintering grounds in The Bahamas and subsequently resampled within their breeding territories in Michigan, USA. Initial sampling occurred in March and April and resampling occurred in May, June and early July. The composition of the most abundant phyla and classes of the warblers' microbiota is similar to that of other migratory birds. However, we detected notable variation in abundance and diversity of numerous bacterial taxa, including a decrease in microbial richness and significant differences in microbial communities when comparing the microbiota of birds first captured in The Bahamas to that of birds recaptured in Michigan. This is observed at the individual and population level. Furthermore, we found that 22 bacterial genera exhibit heightened abundance within specific sampling periods and are probably associated with diet and environmental change. Finally, we described a small, species-specific shared microbial profile that spans multiple time periods and environments within the migratory cycle. Our research highlights that the avian gut microbiota is dynamic over time, most significantly impacted by changing environments associated with migration. These results support the need for full annual cycle monitoring of migratory bird microbiota to improve understanding of seasonal host movement ecologies and response to recurrent physiological stressors.

KEYWORDS
annual cycle, Kirtland's Warbler, migration, Nearctic-Neotropical, recapture

1 | INTRODUCTION

A healthy gut microbiome is thought to be both resilient and flexible (Bodawatta et al., 2021; Voolstra & Ziegler, 2020) and may be heavily affected by a variety of extrinsic and intrinsic factors, including host genetics, habitat, and diet (Hird et al., 2015; Rothschild et al., 2018). The composition of a healthy microbiome may change as animals undergo recurrent physiological stressors, such as migration or changing climates across seasons (Carey & Assadi-Porter, 2017; Risely et al., 2018; Sommer et al., 2016). Increased understanding of both resilience and flexibility of gut microbial communities relating to recurrent physiological stressors can further elucidate host adaptation to repetitive stress. Here, we ask what changes and what remains consistent within the gut microbiota of a migratory bird species across multiple time points and locations within the annual cycle.

Species experiencing seasonal variation in habitat, diet, or physiological stressors often exhibit correlated alterations in their microbiota (Drovetski et al., 2019; Maurice et al., 2015; Ren et al.,

2017; Smits et al., 2017; Sommer et al., 2016). Migratory animals may undergo seasonal fluctuations in metabolic needs that, in combination with changing habitats and diets, result in variable microbiota composition across their annual cycles, but the extent to which this occurs remains unclear (Grond et al., 2018; Jenni & Jenni-Eiermann, 1998). Exploring the impact of movement of migratory birds on gut microbial dynamics provides an opportunity to increase understanding of the host-microbe relationships within the context of changing environments and a known, recurrent physiological stress.

Migratory birds are exposed to a variety of habitats and associated novel environmental microbial suites across the annual cycle, which may impact overall gut composition. Recently, several studies have illustrated the effect that environment and movement can have on structuring the host microbiota (Grond, Perreau, et al., 2019; Grond, Santo Domingo, et al., 2019; Hird et al., 2014; Wu et al., 2018). For example, the microbial community differed between the wintering and breeding grounds in migratory geese (Wu et al., 2018), between spring and fall migrants in two passerines (Lewis et al., 2016), and between migratory and nonmigratory barn swallows (*Hirundo rustica*; Turjeman et al., 2020). Additionally, Corl et al. (2020) showed that increased movement, with exposure to more varied environments, results in increased diversity of the gut microbiota in breeding barn owls (*Tyto alba*), and Lewis et al. (2017) found that host microbial communities of birds at a migration stopover site begin to converge with the local environmental microbial suite within 24 h. Contradicting these results, a study of migratory red-necked stints (*Calidris ruficollis*) concluded that only 0.1% of gut microbiota are sourced directly from the environment, and that individual stints in different environments exhibited weak variation in microbial composition (Risely et al., 2017). Further comparison of recent migrants to individuals that had remained on the nonbreeding grounds for a full year identified the bacterial genus *Corynebacterium* as significantly more abundant in migratory individuals than nonmigratory individuals (Risely et al., 2017). Similarly, *Corynebacterium* and the genus *Mycoplasma* were identified as more abundant between a comparison of migratory and nonmigratory sympatrically occurring subspecies of barn swallows (Turjeman et al., 2020).

In addition to the potential impact of novel habitats associated with long-distance movement, physiological adaptations of migratory birds, such as intestinal atrophication, could further affect gut microbiota, possibly by reducing the volume of bacteria that the birds harbor (Grond et al., 2018) or by allowing functionally relevant bacteria to proliferate during active migration (Risely et al., 2018). In addition to environmental factors, host characteristics, such as diet (Song et al., 2020), species (Capunitan et al., 2020), and age (Kreisinger et al., 2017) may play additional roles in structuring the microbiota.

Given the variability of gut microbiota, intrinsic variables such as host genetics, and strong environmental effects, it may be difficult to directly correlate variation in gut microbiota to ongoing biological processes, specific host traits, or environmental factors without temporal sampling across different time points of the annual cycle (Capunitan et al., 2020; Hird et al., 2014; Song et al., 2020). Here, we sampled individuals on their subtropical wintering grounds and recaptured them multiple times on their temperate breeding grounds to better understand local and temporal variability in gut microbiota by reducing sources of variability known to be associated with sampling different individuals and different populations (Baxter et al., 2015; Flores et al., 2014; Hird et al., 2014).

Until now, no migratory songbird has been sampled at multiple time points and locations across their annual cycles. Migratory birds have complicated annual cycles that involve twice-annual movements often spanning thousands of kilometres between stationary breeding and wintering periods. Once captured, researchers typically have no way to relocate or recapture the same individuals outside of the original capture site, especially for species with expansive wintering and breeding ranges and with populations that may number in the millions. This inhibits sampling from the same population, let alone the same individual, at multiple points in the annual cycle. As a result, one must attempt to measure and control for confounding factors, such as between population differences, and account for high interindividual variability (Baxter et al., 2015; Flores et al., 2014; Hird et al., 2014). This inability to study the same individuals across the annual cycle has impeded identification and understanding of variation within birds associated with seasonal movement.

The Kirtland's Warbler (*Setophaga kirtlandii)* provides an unusual opportunity for studying changes across the annual cycle in a migratory species. Their small population size as well as restricted breeding and wintering ranges (Cooper et al., 2019) make it feasible to relocate individuals across seasons (Cooper et al., 2018; Cooper & Marra, 2020). Following substantial population declines, only 167 singing males were recorded in 1974 and again in 1987, based on breeding surveys (Kepler et al., 1996). Through extensive conservation management efforts, the population has increased to approximately 2,300 singing males of which 97% breed across a relatively small area in Michigan's Lower Peninsula. This species winters primarily in the scrub forests of The Bahamas (Cooper, Ewert, et al., 2019), more than 2,000 km south of the breeding grounds. For this study, we radio-tagged individuals on the wintering grounds and then relocated and recaptured the same birds on the breeding grounds in Michigan through the use of automated telemetry towers. We used 16S rRNA next generation sequencing technologies to catalogue the bacterial communities of individuals. Our goals were to: (1) characterize the bacterial diversity of Kirtland's Warblers at three distinct periods of the annual cycle at the population and individual level; (2) evaluate host sex, age, period of annual cycle, and location effect on abundance and diversity of gut microbiota; and (3) determine if a shared bacterial profile for Kirtland's Warblers exists and if so, establish a species-specific pattern.

2 | MATERIALS AND METHODS

2.1 | Initial sample collection in The Bahamas

We captured Kirtland's Warblers on Cat Island, The Bahamas, in March and April of 2017 and 2018 using vocalization playback and mist nets. We classified individuals into two age categories

(SY = second calendar year or ASY = after second year), sexed individuals following Pyle (1997), and attached a USGS metal band and three plastic coloured bands. We then fitted a coded radio-tag (0.35 g, Model = NTQBW-2, Lotek Wireless, Inc.) using a modified leg-loop harness (Rappole & Tipton, 1991). Tags emitted coded pulses at regular intervals (29.3 s), which allowed for individual identification through handheld or automated telemetry receivers (Taylor et al., 2017). After attaching the radio tags, we collected faecal samples by placing birds in a wax paper bag for up to 10 min. We transferred faecal materials from the bag to Whatman FTA Cards (Whatman) using Whatman sterile swabs. Following sample transfer, we stored FTA Cards in airtight containers at room temperature until transportation to and processing within the molecular laboratory. Whatman FTA Cards are stable long term at room temperature, therefore ideal for fieldwork where traditional methods of flash freezing may be unavailable (da Cunha Santos, 2018). The microbial composition recovered from faecal samples stored on FTA cards are comparable to that of flash freezing and storage at −20°C methods (Song et al., 2016).

2.2 | Relocation and recapture in Michigan

We used 11 automated telemetry towers in Michigan erected as part of ongoing Kirtland's Warbler management and research (Cooper et al., 2018; Cooper & Marra, 2020) to detect tagged individuals as they arrived at the majority of breeding sites. Birds arrived between 9 May and 3 June. We downloaded tower data daily and used handheld telemetry to search the few areas not well covered by towers at least every 3 days. We used these data to determine arrival dates in Michigan. Following initial detection, we used handheld telemetry to locate each individual's territory and target them for recapture. Birds were captured an average of 7.7 (SD ± 8.1) days after their first detection in Michigan. We also attempted to recapture individuals towards the end of the breeding season in early July. In May of 2018, we also captured and sampled nontagged Kirtland's Warblers in Michigan to compare microbial variation in individuals known to be from Cat Island with birds that may have wintered on other islands. Regardless of timing, we used identical capture and sampling protocols as those used in The Bahamas (see above).

2.3 | Molecular methods

We isolated DNA from faecal samples stored on Whatman FTA Cards using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen) following the manufacturer's extraction protocol. We included six blank negative controls to account for possible contamination during extraction and polymerase chain reaction (PCR). Following standardized procedures of the Earth Microbiome Project, we used PCR to amplify the V4 region of the 16S microbial small subunit ribosomal RNA (rRNA) gene using the EMP universal primers 515F/806R (Caporaso et al., 2012). We then used the Illumina MiSeq sequencing platform to obtain paired-end 150 base pair reads. We performed DNA extractions at the Field Museum of Natural History. All subsequent molecular work was conducted at the IGM Genomics Centre of the University of California, San Diego.

2.4 | Sequence processing

We processed raw sequence data with the quantitative insights into microbial ecology (QIIME2 version 2019.1) pipeline (Bolyen et al., 2019; Caporaso et al., 2010). In QIIME2, following standard demultiplexing and quality filtering, we generated amplicon sequence variants (ASVs) using the divisive amplicon denoising algorithm (DADA2) (Callahan et al., 2016).

The DADA2 toolkit statistically infers sample sequences and implements quality control elements including exclusion of singletons, chimera removal, and sequence error elimination.

Using a quality score threshold of 33, we trimmed all sequences outside base pair positions 13 and 145. We based the threshold of quality score on visual assessment of the quality score plots and recommendations in Mohsen et al. (2019). We classified ASV taxonomies using the Silva reference database (Quast et al., 2012, version 132). After classification, we removed all ASVs identified as Archaea, chloroplasts and mitochondria. We aligned all sequences using MAFFT (Katoh & Standley, 2013) and then inferred a phylogeny of all bacterial sequences with FastTree (Price et al., 2010). We identified bacterial contaminants with the R package decontam (Davis et al., 2018). We used six DNA extraction blanks processed in parallel with all other samples as negative controls with the default parameters for frequency-based contaminant determination. Quality control measures resulted in the removal of 10 libraries for poor DNA or PCR yield and 52 contaminant ASVs from the overall data set. Of the 176 total faecal samples, 166 were analysed in the final data set.

2.5 | Normalization of microbial data

Normalization of microbial data accounts for biases introduced by differing library size, technical variability and sampling bias. However, normalization can lead to data loss and may be detrimental to interpretation of results (McMurdie & Holmes, 2014). To ensure that rarefaction does not bias results, we conducted all alpha and beta-diversity analyses on normalized and non-normalized data. For normalization, we rarefied all libraries to 7,000 reads. Results from the normalized data did not qualitatively differ from the non-normalized data. Therefore, we present and discuss the results of rarefied data for diversity analyses. Identification of shared microbes across individuals and sampling periods was conducted on non-normalized data as rarefaction may be ill-suited for detection of ASVs with low abundance in individuals.

2.6 | Statistical analysis

We analysed community alpha diversity using the natural log of observed ASV richness and the Shannon diversity index. For modeling diversity, we used a linear mixed model as implemented in the R package lme4 (Bates et al., 2007) and evaluated the importance of different variables, taking into account the repeated sampling of some birds. We included host age (SY or ASY), sex (male or female), year (2017 or 2018) and sampling period (The Bahamas, first recapture in Michigan, and second recapture in Michigan) as fixed effects and individual host as a random effect. Using lmerTest (Kuznetsova et al., 2015), we generated an ANOVA table from the linear model analysis, and subsequently conducted a posteriori pairwise tests to compare the three sampling periods. Additionally, we conducted a pairwise *t* test to assess differences between tagged and randomly caught birds within the first recapture period of 2018. We tested for the influence of outliers, which appeared to cause a deviation from normality in ASV richness (Shapiro-Wilks test), by repeating the analyses with outliers omitted and obtained very similar results. We also tested for the effect of individual-level random effects with a likelihood ratio test comparing the model with and without individual ID as the random effect term, and we found individuals did not consistently differ from each other. Finally, we used a generalized additive model (GAM) to test the impact of time on alpha diversity of recently arrived birds in Michigan following the end of spring migration with the R package mgcv (Wood, 2012). We used GAMs on Shannon diversity index and the natural log of observed ASV richness of individuals that had been present in Michigan for nine days or less. We determined the day of arrival in Michigan from tower data. We applied the GAM to fit a smoothed curve for days after arrival in Michigan, the predictor variable, to the estimate of variance explained in the alpha diversity metric, the response variable. We determined optimal smoothing parameters for our GAM by examining the minimized generalized cross-validation score of the GAM for all possible smoothing parameters ($k = 3$–10). The GAM estimated a smoothing function of $k = 3$, though all possible k values produced results that are effectively identical. Results presented here, including in Figure 3, are from $k = 3$ for both observed ASV richness and Shannon diversity index.

To examine community differences in the microbiome (beta diversity), we applied permutational multivariate analysis of variance (PERMANOVA) of Bray-Curtis dissimilarity and unweighted UniFrac distances, calculated among individual samples (Anderson, 2017). For variables that showed significant differences in the PERMANOVA analyses, we conducted an a posteriori test to assess differences in dispersion or centroids using PERMDISP. We visualized beta diversity of significant variables using nonmetric multidimensional scaling (nMDS) ordination of the Bray-Curtis measurements. Diversity calculations were implemented using the R packages vegan and phyloseq (McMurdie & Holmes, 2013; Oksanen et al., 2007).

Finally, to ask which taxa differ in abundance across sampling periods, we implemented analysis of composition of microbes with bias correction (ANCOM-BC; Lin & Das Peddada, 2020; Mandal et al., 2015). ANCOM-BC uses the underlying structure of the microbiota data to identify differentially abundant taxa between categories while controlling for false discovery rates. This method applies a library-specific offset term estimated from the observed abundance which is incorporated into a linear regression model, providing the bias correction. We used the R package ANCOMBC to test for differences in abundance of bacterial genera with a significance of $p < .05$ with Bonferroni corrections (Lin & Das Peddada, 2020).

2.7 | Shared microbial profile

We identified a common, species-specific and temporally persistent microbial profile for Kirtland's Warblers following Risely (2020). We defined the species-specific shared microbial profile as ASVs present in over 50% of all individuals in each of the three sampling periods (Astudillo-García et al., 2017; Grond et al., 2017). We studied the shared microbial profile at multiple taxonomic levels using Phylocore (Ren & Wu, 2016). We also identified a temporally persistent microbial profile in birds sampled in triplicate, defined as ASVs found at all three sampling periods within the same bird (Shade & Handelsman, 2012). We calculated the proportion of temporally persistent ASVs to those that are transient and not found at all three sampling periods to identify the average proportion of ASVs that are retained over time.

3 | RESULTS

We collected a total of 166 faecal samples from 116 Kirtland's Warblers at locations throughout Cat Island, The Bahamas ($n = 92$), and Michigan's Lower Peninsula (first recapture $n = 43$, second recapture $n = 18$). Thirty-four birds were sampled twice, once during initial capture in The Bahamas and a second time during first recapture in Michigan. Of those birds, nine individuals were sampled a third time during the second recapture period in Michigan (Table S1). Additionally, 13 nontagged Kirtland's Warblers were sampled in May 2018 in Michigan. Our final data set is composed of 166 sequenced libraries (Table 1) which totaled 5,007,844 reads, with an average 30,168 reads per library (range: 7,022–100,856). We detected 7,426 unique ASVs across all sampled with a mean of 107.3 ± 96.7 (standard deviation [SD]) per library.

3.1 | Bacterial community composition and diversity

Across all samples, bacteria from 37 phyla were detected. Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria composed 91.13% of the total reads; 5.8% of the reads belonged to the 33 remaining phyla and 3.07% of reads did not align to any known bacterial phyla (Figure 1a). *Clostridia* (Phylum Firmicutes), *Gammaproteobacteria* (Phylum Proteobacteria), and *Bacteroidia*

TABLE 1 Individuals sampled per time period, including age (SY, second calendar year; ASY, after second calendar year) and sex (M, male; F, female) breakdown

		Samples	Age			Sex		
Sampling period	Date	Collected	SY	ASY	Unk.	M	F	Unk.
Initial capture (CIB)	29 March–16 April 2017	41	18	22	1	38	3	0
	23 March 23–24 April 2018	51	37	14	0	36	15	0
First recapture (MI1)	20 May–6 June 2017	19	10	9	0	19	0	0
	13 May–26 June 2018	24	18	6	0	23	1	0
Non-tagged birds (MI1)	13–20 May 2018	13	6	6	1	12	0	1
Second recapture (MI2)	2–10 July 2017	8	4	4	0	8	0	0
	1–11 July 2018	10	7	3	0	8	2	0
	Total	166	100	64	2	144	21	1

Note: Numbers reflect libraries included in analyses and do not include those removed for poor sequencing or PCR yield.

(Phylum Bacteroidetes) were the most abundant classes, representing 70.16% of all reads. The mean abundance of most phyla and classes differed between initial sampling in The Bahamas and subsequent samplings in Michigan (Figure 1b, Table S2). The birds shifted from a Firmicutes dominated microbiota in The Bahamas (mean abundance per individual 39.82% [SD, ±13.97%]) and Michigan following arrival (38.12% [SD, ±16.41%]) to Proteobacteria as the most abundant phylum in the second Michigan recapture period (47.07% [SD, ±27.90%]). Bacteroidetes and Actinobacteria were also proportionally more abundant in The Bahamas than in the second Michigan recapture period. Notably, Cyanobacteria represented 1.91% (SD, ±5.93%) of the total microbiota in The Bahamas, but decreased to 0.05% (SD, ±0.23%) by the second recapture period in Michigan.

Alpha diversity was not significantly affected by year, host age or host sex (Table S3). However, the three sampling periods significantly differed (type III ANOVA with Satterthwaite's method; observed richness: $F_{2,116.34} = 14.76$, $p < .0001$; Shannon diversity: $F_{2,126.91} = 29.22$, $p < .0001$). All Bonferroni corrected pairwise comparisons on the fitted values from the linear model were significantly different from each other (observed: Bahamas vs. each recapture period both $p < .0001$, first versus second recapture period, $p = .002$; Shannon diversity: all comparisons: $p < .0001$). Birds in The Bahamas showed higher bacterial diversity compared to either recapture period in Michigan, demonstrated through a comparison of all samples (Figure 2a) as well as with paired sampling of the same individuals (Figure 2b). In the birds sampled in triplicate, alpha diversity varied between first and second Michigan recaptures (Figure 2c). A comparison of tagged and randomly captured birds in the first Michigan sampling period of 2018 revealed no significant differences in alpha diversity (pairwise t test; observed: $p = .13$, Shannon diversity: $p = .22$). Generalized additive models showed that 38.4% of the deviance in observed diversity ($p = .004$) and 10.8% of the deviance in Shannon diversity measures ($p = .354$) could be explained by amount of time spent in Michigan following arrival after spring migration. In the 27 individuals that were found within nine days of arrival to the breeding grounds, we observed a decrease in alpha diversity through the first four to five days followed by an increase in alpha diversity through the ninth day (Figure 3), with each bird represented once in the analysis.

Our results indicate that beta diversity was not significantly affected by age or sex of the birds within the full data set or individual sampling periods (Table 2), with the exception of age in the second Michigan resampling period (unweighted UniFrac: PERMANOVA $p = .0128$, PERMDISP $p = .2213$). Community composition of the microbiota significantly differed by year in the full data set and at each sampling period (Table 2, Figures 4a, S1, S2). Additionally, our PERMANOVA results suggest that microbiota composition differed significantly between sampling periods (Bray-Curtis: $p = .0002$, $R^2 = 0.025$; unweighted UniFrac: $p = .0001$, $R^2 = 0.024$), though the significant unweighted UniFrac result can be explained through variation in spread of the sample composition, rather than with significantly different centroids such as with the Bray-Curtis dissimilarity matrix (PERMDISP; Bray-Curtis $p = 0.7104$, unweighted UniFrac $p = 3.71e\text{-}6$). This indicates that although the abundances of microbiota are significantly different during sampling periods, the taxonomic variation of bacterial lineages present are not. The effect of sampling period on the gut microbiota explained 2.5% and 2.4% of the variation in microbiota composition for Bray-Curtis and unweighted UniFrac respectively. Taken together, all variables tested (sampling period, year, sex, age) explained less than 5% of the total variation in the microbiota (Bray-Curtis: 4.91%, unweighted UniFrac: 4.6%). No consistent changes were observed in the beta diversity of the birds sampled in triplicate (Figure 4b, Figure S3).

Across the three sampling periods, 22 bacterial genera were identified by ANCOM-BC as differentially abundant, with the majority being significantly more abundant in The Bahamas (Figure 5, Table S4). Ten genera in Phylum Actinobacteria were elevated in The Bahamas with one genus, *Streptomyces*, at higher abundance in the second recapture period in Michigan. Five genera of Phylum Firmictures were significantly more abundant in individuals from The Bahamas. *Bryocella* (Phylum Acidobacteria) was found at higher frequency in the first Michigan sampling period. Phylum Proteobacteria had genera differentially and significantly abundant at all three sampling periods, with *Aureimonas*, *Lysobacter* and an uncultured

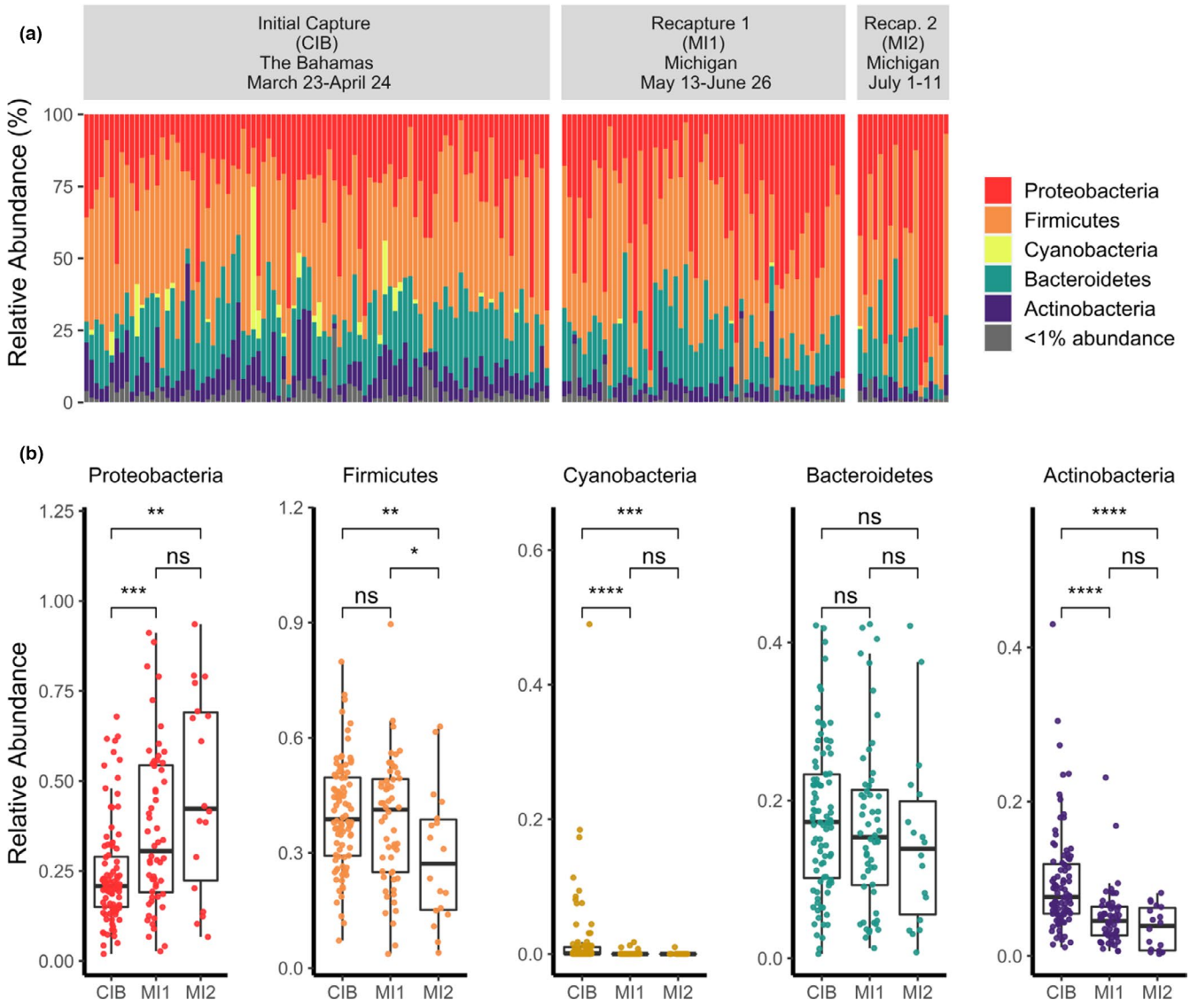

FIGURE 1 Relative abundance of bacterial phyla. (a) Stacked barplots showing the relative abundance of each phylum with each column representing one individual sample, ordered by day of capture and separated by sampling period. Phyla with total abundance less than 1% and unclassified phyla are represented by grey. (b) Relative abundance boxplots of the five most common phyla per individual by sampling period representing the change in relative abundance from Cat Island, The Bahamas (CIB) to the first Michigan recapture period (MI1) and the second Michigan recapture period (MI2). Individual points represent the relative abundance of each phyla per individual per sampling period. Significance levels are pairwise comparisons between sampling periods are shown (ns: $p > .05$; *$p < .05$; **$p < .01$; ***$p < .001$; ****$p < .0001$)

genus of *Beijernickiaceae* at elevated abundances in The Bahamas, *Candidatus* Hamiltonella in the first Michigan sampling period, and *Serratia* at the second Michigan sampling period.

3.2 | Shared microbial profile

We identified 28 ASVs as representing the species-specific shared microbiota of Kirtland's Warblers (Table S5). Two ASVs were from genera *Bifidobacterium* and *Collinsella* of Phylum Actinobacteria. The genus with the most shared ASVs was *Bacteroides* (Phylum Bacteroidetes) with eight. Fourteen ASVs are members of Firmicutes and are from genera *Blautia* (1 ASV), *Eubacterium eligens* (1 ASV), *Eubacterium hallii* (2 ASVs), *Fusicatenibacter* (1 ASV), *Roseburia* (2 ASVs), *Faecalibacterium* (3 ASVs), *Subdoligranulum* (2 ASVs), and two ASVs unclassified at the generic level. Finally, four ASVS from Phylum Proteobacteria are shared with one ASV from each genera *Ralstonia*, *Sutterella*, *Escherichia-Shigella* and *Alkanindiges*. We also identified the temporally persistent ASVs in the birds sampled at all three sampling points. Individuals retained 18–26 ASVs, present at each sampling period, which represented an average of 25.06% (range: 8.58%–50.00%) of ASVs detected per individual per time point.

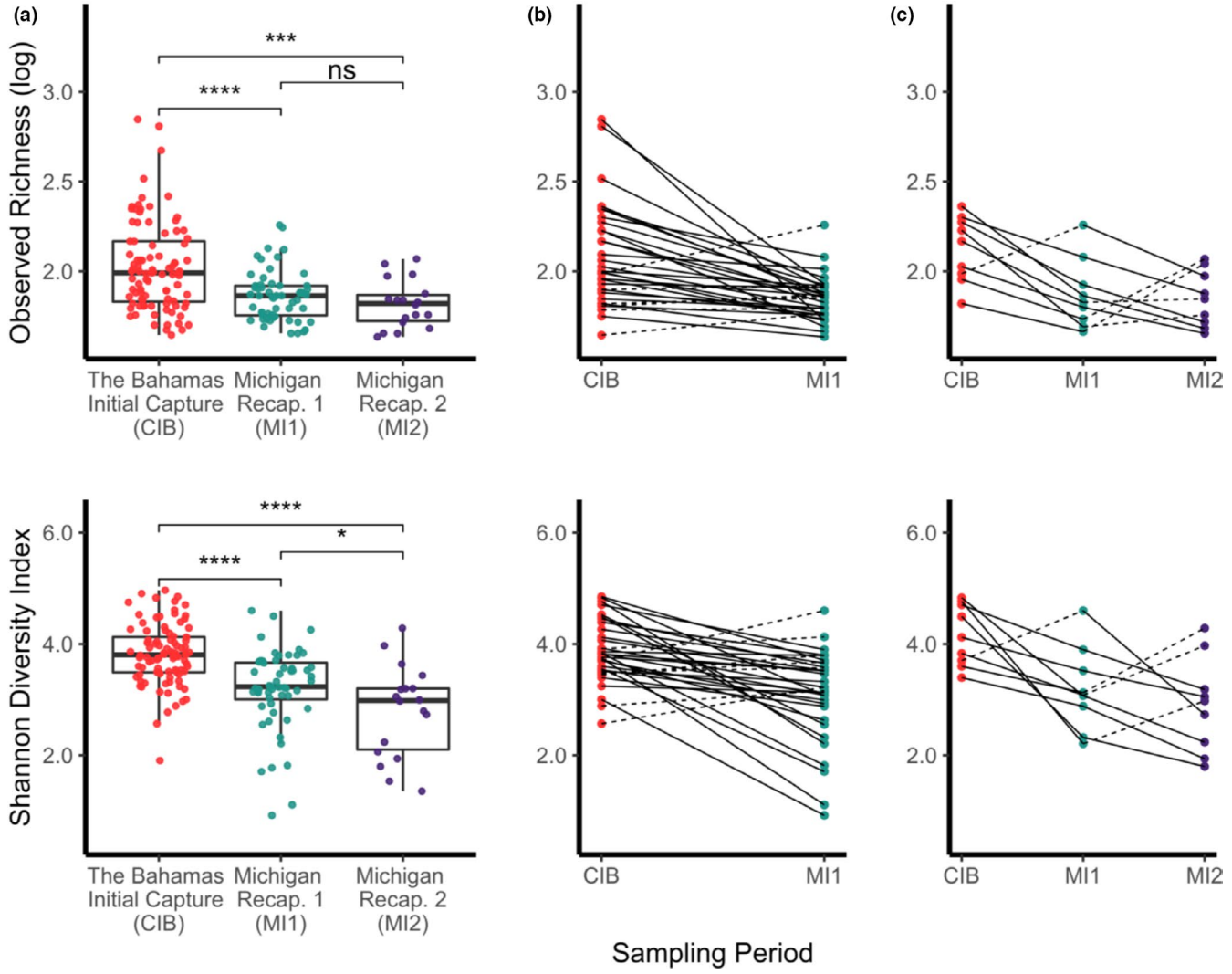

FIGURE 2 Alpha diversity measurements of amplicon sequence variants (ASVs) including observed ASV richness (log transformed, top row) and Shannon diversity index (bottom row) Boxplots of alpha diversity at each sampling period (a). Individual points represent the alpha diversity measure of the individual at that period. Significance levels are pairwise comparisons between sampling periods are shown (ns: $p > .05$; *$p < .05$; **$p < .01$; ***$p < .001$; ****$p < .0001$). Alpha diversity change over time in the individuals sampled two (b) or three times (c). Each line connects the measurements of the same individual between the respective sampling periods. Continuous lines represent a negative change in alpha diversity and dotted lines represent a positive change

4 | DISCUSSION

Significant variation in both the diversity and community composition of Kirtland's Warblers microbiota was observed in individuals and the population as birds migrate from their wintering grounds in The Bahamas to breeding territories in Michigan. Repeated sampling at multiple points across the annual cycle was only possible because we were able to capture, sample, and radio-tag individuals on the wintering grounds and then use automated telemetry to relocate the same individuals thousands of kilometres away on the breeding grounds (Cooper & Marra, 2020). Through the resampling of individuals we removed potential biases associated with sampling multiple populations. Therefore, the effects observed can be attributed to true changes within individuals and our study population. We found that the overall diversity of the microbiota differed significantly between sampling periods and warblers on their wintering grounds had a significantly different and more diverse community of gut microbiota than those on their breeding grounds. We also documented a common, shared microbial profile of Kirtland's Warbler that persisted throughout multiple portions of the annual cycle.

4.1 | Community composition

The overarching composition of Kirtland's Warbler microbiota is consistent with that of most wild bird surveys to date, with members of Phyla Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria comprising the majority of all bacteria detected (Dewar et al., 2014; Grond et al., 2018; Lewis et al., 2016). However, the relative abundances of all phyla changed, sometimes dramatically, as the birds

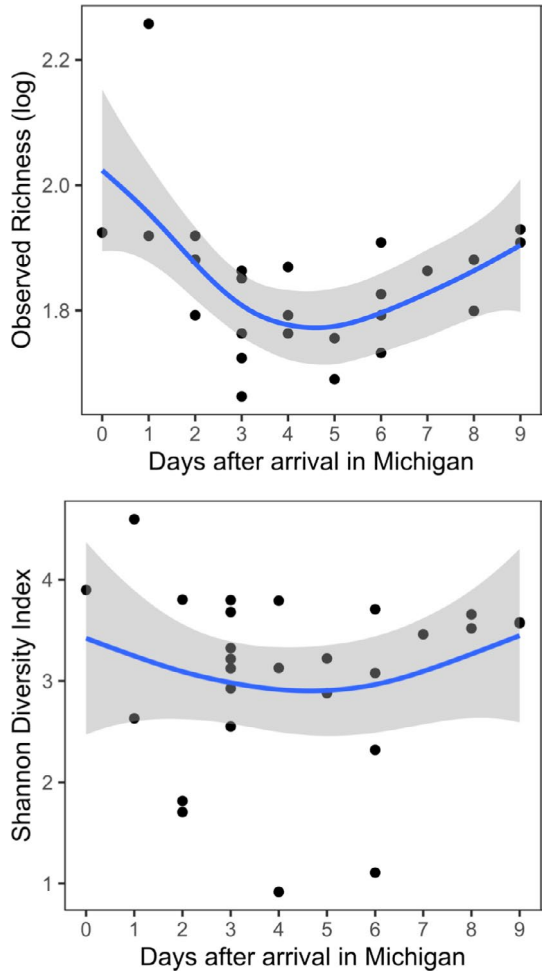

FIGURE 3 Generalized additive model smoothed time series comparing diversity measures (observed richness, top panel; Shannon diversity Index, bottom panel) against day(s) after arriving in Michigan. Each dot represents the diversity measure of an individual bird. The blue line represents the moving average change in diversity over time with the grey area corresponding to a 95% confidence interval

migrated from The Bahamas to Michigan and over time in Michigan. Shifts in major bacterial taxa have been observed previously in migratory birds throughout different points of the annual cycle (Kresinger et al., 2017; Lewis et al., 2016), though this is the first study to resample the same individuals at different points in the annual cycle of a migratory passerine. Population and individual level variation across the annual cycle may reflect difference in presence or abundance of environmental bacteria (Wu et al., 2018) and/or responses to altered diets (Góngora et al., 2021) that in turn favour some bacteria over others or vary with host characteristics and requirements (Kers et al., 2018). Below, we consider plausible examples of each.

4.2 | Environmental effect

The avian gut microbiota frequently reflects the local environment (Hird et al., 2014; Hird et al., 2018; Gillingham et al., 2019; Grond, Perreau, et al., 2019; Grond, Santo Domingo, et al., 2019; Cao et al., 2020, but see Risely et al., 2017), even in cases when migratory birds maintain narrow dietary niches throughout the annual cycle (Wu et al., 2018). We observed evidence of environmental sourcing of microorganisms within the gut microbiota of Kirtland's Warblers. Cyanobacteria, found in marine and brackish waters (Sivonen, 1996), was common in birds in The Bahamas but nearly absent from most individuals in Michigan. Cyanobacteria has previously been found in the gut microbiota of island birds (García-Amado et al., 2018) and is known to be acquired through food (Birrenkott et al., 2004). Kirtland's Warblers probably acquire environmentally derived Cyanobacteria in The Bahamas via food consumption, as most birds were captured within 2 km of the ocean and much of the groundwater on the island is brackish. The most common class of Cyanobacteria detected in Kirtland's Warblers, *Oxyphotobacteria*, is an oxygenic phototroph (Shih et al., 2017). *Oxyphotobacteria* has previously been described in an avian host but is unlikely to provide a host associated function, suggesting the presence of this class is transient and the result of environmental sourcing (Zhu et al., 2020). In addition to *Oxyphotobacteria*, we detected several common environmental, soil-associated bacterial genera, including *Acitomycetospora*, *Aureimonas*, *Solirubrobacter* and *Nocardioides*, as more abundant in birds in The Bahamas (Janssen, 2006; Topp et al., 2000). The presence and abundance of various groups of bacteria associated with The Bahamas when compared to Michigan indicate a strong environmental effect on the gut composition of Kirtland's Warblers.

Variation in microbial community composition of birds is associated with a variety of intrinsic and extrinsic factors, including diet, host genetics, and the environment. Across all samples, we found that sampling period, including movement from The Bahamas to Michigan, accounted for 2.5% (Bray-Curtis) and 2.4% (unweighted UniFrac) of the variation observed. This proportion of dissimilarity between locations is smaller than reported in previous studies (Grond, Perreau, et al., 2019; Grond, Santo Domingo, et al., 2019; Risely et al., 2017) yet is the most significant explanatory factor for observed differences in community composition. Our results contrast with a study which compared the microbiota of co-occurring migratory and resident red-necked stints which identified only slight compositional variation between distinct environments (Risely et al., 2017). This indicates that the response of the avian gut community is not consistently or uniformly impacted by the local environmental suite of microbes.

During migration birds are exposed to varying environments at stopover sites where they could acquire novel microbes (Lewis et al., 2017), possibly resulting in temporarily inflated diversity. However, it is unknown if microbial diversity increases or decreases during active migration or how the microbiota changes following arrival at breeding grounds. Possible adaptations to long distance flight, such as relatively shorter intestinal length and atrophication of intestines during active migration, might result in decreased microbial diversity (Caviedes-Vidal et al., 2007; McWilliams & Karasov, 2005). Using the ability to determine what day individuals arrive in Michigan following migration, we observed variation in microbial diversity over the first nine days following the end of migration, including a slight

TABLE 2 Results of permutational multivariate analysis of variance (PERMANOVA) tests indicating if ASV beta diversity measures are significantly different for the tested variable based on Bray-Curtis dissimilarity and unweighted UniFrac distance metrics

	Bray-Curtis				
	PERMANOVA			PERMDISP	
Variable	Pseudo-*F*	R^2	*Pr*(>*F*)	*f*-value	*p*-value
Sampling period	2.058	0.025	<0.001*	0.343	0.710
Year (full data set)	1.900	0.011	0.002*	1.659	0.200
Year (CIB only)	1.485	0.016	0.019*	0.936	0.336
Year (MI1 only)	2.474	0.044	<0.001*	0.304	0.583
Year (MI2 only)	2.223	0.172	0.003*	0.000	0.984
Sex (full data set)	1.203	0.007	0.137		
Sex (CIB only)	1.035	0.011	0.345		
Sex (MI1 only)	1.169	0.216	0.482		
Sex (MI2 only)	1.425	0.082	0.082		
Age (full data set)	0.929	0.006	0.595		
Age (CIB only)	0.927	0.010	0.681		
Age (MI1 only)	0.926	0.017	0.586		
Age (MI2 only)	1.020	0.060	0.343		
	Unweighted UniFrac				
	PERMANOVA			PERMDISP	
Variable	Pseudo-*F*	R^2	*Pr*(>*F*)	*F*	*p*-value
Sampling period	2.001	0.024	<0.001*	13.514	<.001
Year (full data set)	1.314	0.008	0.121		
Year (CIB only)	2.027	0.022	0.003*	0.180	.673
Year (MI1 only)	1.295	0.025	0.003*	0.7258	.398
Year (MI2 only)	1.541	0.088	0.007*	0.001	.974
Sex (full data set)	1.159	0.007	0.074		
Sex (CIB only)	0.939	0.010	0.737		
Sex (MI1 only)	0.862	0.016	0.695		
Sex (MI2 only)	0.862	0.016	0.695		
Age (full data set)	1.131	0.007	0.099		
Age (CIB only)	0.887	0.010	0.925		
Age (MI1 only)	1.279	0.023	0.013*	1.532	.221
Age (MI2 only)	0.942	0.056	0.615		

Note: Results reported for full data set and within sampling periods for variables year, sex, and age. Asterisks denote statistically significant results of PERMANOVA with Bonferroni correction, $p < .05$. PERMDISP analysis results reported when PERMANOVA results significant. All tests conducted with 999 permutations.

decrease over the first three days before slowly increasing through day nine. During the first few days at their breeding grounds birds may shed transient microbes acquired at stopover sites. This suggests that during spring migration microbial diversity increases due to exposure at stopover sites rather than decreases as an adaptation to long-distance flight. However, sample size per day is small and additional research with larger sample sizes are needed to further assess these results.

Gut microbiota are dynamic, displaying influence of novel microbial pools within 24–48 h of exposure (Capunitan et al., 2020; Grond, Perreau, et al., 2019; Grond, Santo Domingo, et al., 2019; Lewis et al., 2017). Two of our findings further support rapid acclimation to local microbiota. First, we observed no significant variation in the gut microbial diversity in birds sampled during the first recapture period in Michigan when comparing the microbiota of birds known to be from Cat Island and the 12 nontagged birds that may have wintered on other islands. This implies rapid turnover of microbiota sourced from the local Michigan habitat. Second, we observed significant variation in beta diversity between 2017 and 2018 in the full data set, as well as within each sampling period (Table 2,

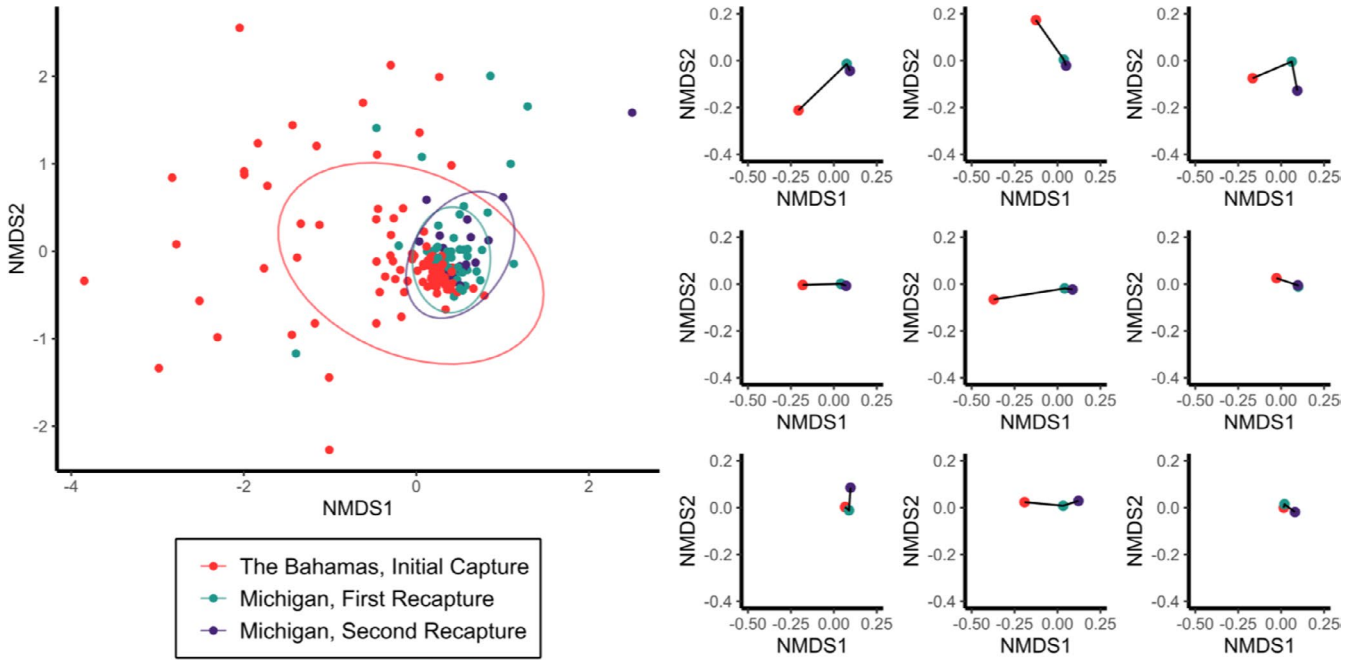

FIGURE 4 (a) Nonmetric multidimensional scaling (nMDS) ordination of Kirtland's Warbler gut microbiome community by sampling period, compared using Bray-Curtis dissimilarity (stress = 0.124). Ellipses show 95% confidence intervals around the centroid of each sampling period. Three outliers were removed from ordination plot for visualization purposes, plot including outliers is shown in Figure S1. (b) Ordination of individual birds sampled in triplicate placed within the nMDS space of all samples, highlighting intraindividual change over time

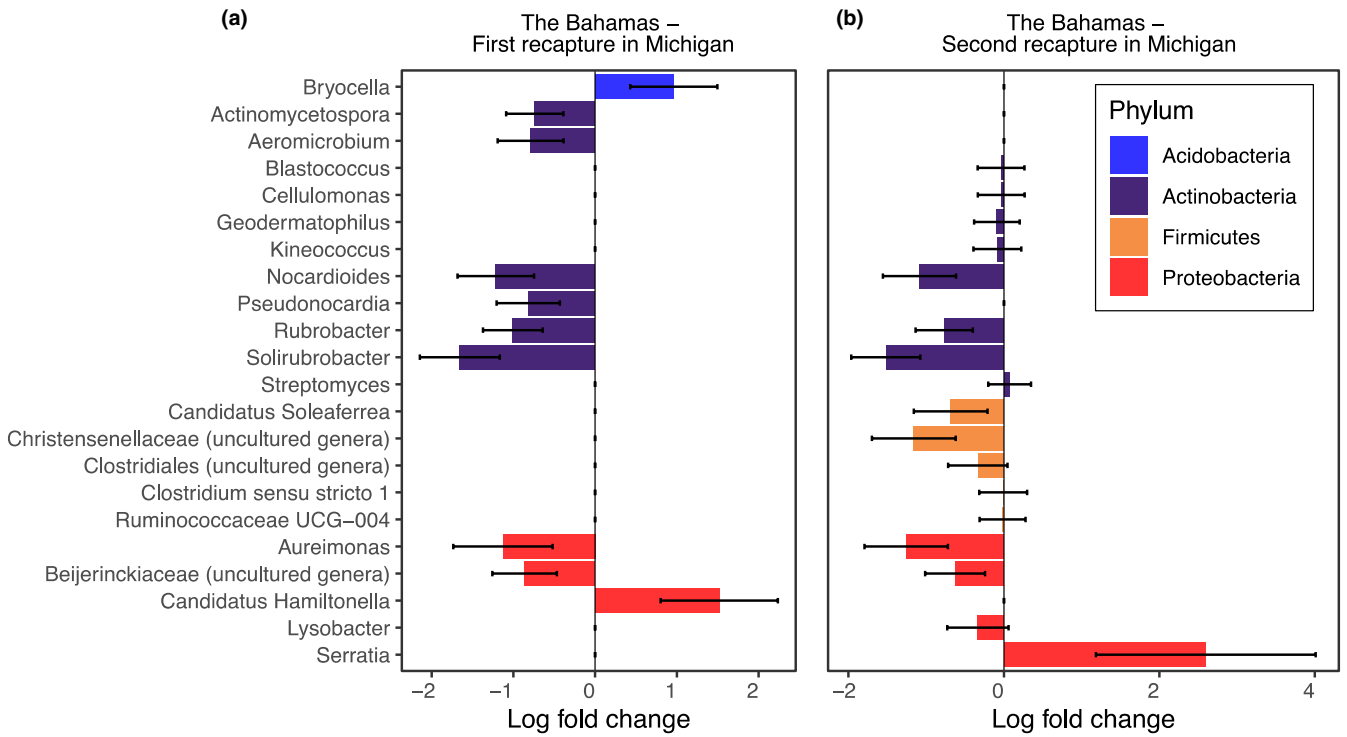

FIGURE 5 Analysis of composition of microbiomes with bias-correction (ANCOM-BC) identified bacterial genera that were differentially abundance at sampling periods. Bars correspond to the effect size (log fold change) of relative abundance of each genera, with negative values associated with an increase in abundance in The Bahamas (both panels) and positive values associated with an increase in abundance in the first recapture period (a) or second recapture period (b) in Michigan. Black bars represent the 95% confidence intervals. Adjusted *p*-values and confidence bounds can be found in Table S5

but for example: PERMANOVA of first Michigan recapture period: Bray Curtis $p < .001$, $R^2 = 0.044$; unweighted UniFrac $p = .003$, $R^2 = 0.025$). Similar results have been observed in greater flamingos (*Phoenicopterus roseus*) during the breeding season, with significant microbial variability within the same site at different years (Gillingham et al., 2019). Environmental microbes often exhibit high turnover over time (Faust et al., 2015). As such, our observations further support significant influence of local environment on the gut microbiota. This highlights the continued need for long term monitoring of microbiota as community-wide differences between years are demonstrable within the same geographic regions.

Though some individuals underwent substantial fluctuations in the gut microbiota structure and diversity over time, the community dissimilarity of the Kirtland's Warblers weakly varied between the first and second recapture periods in Michigan. Large individual fluctuations with community-wide relative stability have been observed in other host species (Hicks et al., 2018; Ren et al., 2016; Risely et al., 2017). These fluctuations observed within a single location may result from changes in host diet, individuals becoming infected with a pathogen or changing physiological demands across seasons.

4.3 | Diet

Dietary shifts throughout the annual cycle often correspond to changes in gut microbiota (Drovetski et al., 2019; Góngora et al., 2021; Ren et al., 2017; Smits et al., 2017). Kirtland's Warblers shift from a fruit-rich diet in The Bahamas to a diet composed primarily of insects in Michigan (Deloria-Sheffield et al., 2001; Wunderle et al., 2010, 2014). Firmicutes and Actinobacteria, which are often associated with frugivorous diets and known to aid in digestion through cellulose and carbohydrate degradation, were more abundant in The Bahamas where the warblers are consuming more fruit (Anand et al., 2012; Segawa et al., 2019). Class *Melainobacteria* (Phylum Cyanobacteria) is prevalent in the guts of herbivorous mammals where it aids in the digestion of plant materials (Di Rienzi et al., 2013). *Melainobacteria* was found in small abundances in some warblers where it may provide a similar role in digesting fruits.

Proteobacteria, often abundant in insectivorous birds and bats (Ben-Yosef et al., 2017; Edenborough et al., 2020) more than doubled in relative abundance from The Bahamas to the second Michigan recapture period. Specific lineages within this phylum support association with an insectivorous diet. Genus *Serratia* was found to be significantly more abundant in the second recapture period in Michigan. *Serratia* are known to produce chitinase which facilitates the degradation of insects' exoskeletons and is found to be abundant in insectivores such as barn swallows (Kreisinger et al., 2017). Similarly, an increased abundance of *Candidatus* Hamiltonella within the first recapture period in Michigan may be a result of insect ingestion. *Hamiltonella* is a symbiotic bacteria of insects, including aphids, which comprise a portion of Kirtland's diet (Deloria-Sheffield et al., 2001; Dykstra et al., 2014). A recent comparison of diet, faecal and intestinal microbiota of bats identified an excess of bacteria associated with food materials in the faecal microbiota compared to the gut microbiota, indicating that fluctuations in the faecal microbiota are not necessarily indicative of compositional changes of colonizing bacteria that are functionally relevant to the host (Ingala et al., 2018). The shift in abundance of Proteobacteria, including genera *Serratia* and *Candidatus* Hamiltonella, is consistent with the insect-rich diet in Michigan, though further examination is needed to identify which bacteria are colonizing the gut in response to a changing diet and which are transient bacteria acquired from food materials.

4.4 | Host

Bacterial taxa presence and abundance may fluctuate in response to host requirements. Phylum Firmicutes has been linked to weight gain, increased nutrient uptake, and metabolic efficiency in birds (Angelakis & Raoult, 2010; Teyssier et al., 2018). Firmicutes, specifically Class *Bacilli* and *Clostridia*, are abundant in migratory birds and may assist with the metabolism of carbohydrates, sugars, and fatty acids, facilitating migration and other energetically demanding activities (Cao et al., 2020; Grond et al., 2017). *Clostridia* and *Bacilli* were the most abundant classes of Firmicutes in Kirtland's Warblers. The abundance of these classes were lower in the second recapture period in Michigan than in the first recapture period or The Bahamas. Initial capture in The Bahamas occurred within the two months prior to the start of spring migration. Around this time birds begin to accumulate fat deposits to sustain them throughout long-distance migration (Fox & Walsh, 2012). At the first recapture in Michigan, individuals are actively seeking and defending breeding territories. Both activities are energetically expensive and associated with increased metabolism, potentially associated with higher abundance of Firmicutes in gut microbiota. It is also possible that the bacteria in early Michigan are residual from The Bahamas and stopover sites (Lewis et al., 2017). Additional sampling is needed to better identify bacterial lineages associated with specific metabolic demands of birds throughout the annual cycle.

Identifying the purpose or response of specific microbes in relation to host behaviours is essential to increasing knowledge of host-microbe interactions. The genera *Corynebacterium* and *Mycoplasma* have been found to be significantly more abundant in both migratory shorebirds and barn swallows when compared to sympatrically occurring, conspecific nonmigratory populations (Risely et al., 2017; Turjeman et al., 2020). Both genera contain pathogenic bacteria that may increase in abundance during the physiological stress of migration. However, Risely et al. (2017) suggested that the abundance of Corynebacterium observed in recent migrants may be due to a possible inflammatory immune response rather than pathogen invasion. In our study, neither *Corynebacterium* nor *Mycoplasma* were significantly associated with any sampling period or host characteristic. These genera were each found in low abundances in less than 50% of birds. Within the recent arrivals to Michigan, we identified two genera of significantly higher abundance – *Bryocella*, an aerobic chemo-organotroph, and *Candidatus* Hamiltonella. Neither genera

are presumed to have an increased abundance due to the physiological stress of migration, rather they are likely to have been acquired through the ingestion of food materials. We found no specific bacterial taxon to be associated with recently migrating individuals that might play a role in or be a response to migration.

Sex specific conditions, such as hormones, behaviours, and reproductive physiology may influence or be influenced by the microbiome (Escallón et al., 2019; Pearce et al., 2017). In the breeding season, close proximity of male and female birds can lead to convergence of microbial composition resulting in reduced variation between males and females (White et al., 2010). We found no significant variation in overall beta diversity between sexes, although female showed slightly higher alpha diversity than males. However, our data set is heavily skewed towards males ($N_{males} = 144$, $N_{females} = 21$) and these results could vary with the addition of more females. In Rufous-collared Sparrows (*Zonotrichia capensis*), cloacal microbiota diversity increased as males transitioned from non-breeding to breeding condition (Escallón et al., 2019), whereas we observed a decrease in diversity in the faecal microbiota of Kirtland's Warblers, which showed a decrease in diversity. These sparrows are nonmigratory and do not experience the same extreme habitat change that the Kirtland's do, which could potentially explain the alpha diversity differences between species.

We generally found no significant compositional differences between age groups in the full data set implying that adult age does not influence the microbiota of these birds. Variation in microbial composition between adults and chicks has been well documented (Grond et al., 2017; Kreisinger et al., 2017; Videvall et al., 2019) but comparisons between age classes of adult wild birds is lacking. However, we did see a difference in beta diversity between SY and ASY in the first recapture period in Michigan. Second year males often do not successfully establish and defend breeding territories against older males which in turn results in these individuals moving at larger spatial scales than territorial adults (Cooper & Marra, 2020). Increased variation is also observed with increased movement in barn owls during the breeding season (Corl et al., 2020). The lack of an established breeding territory and subsequent floating behaviour could result in those individuals being exposed to a different suite of environmental bacteria.

4.5 | Shared microbial profile

Defining the species-specific shared microbial profile is a critical step in understanding the consistent components of often dynamic and complex microbial assemblages but can be hindered by lack of common parameters defining "shared" (Risely, 2020). In this study we define the species-specific shared microbial profile as ASVs found in >50% of all individuals in each of the three sampling periods. These stable components are commonly tied to biological processes within the host and their identification may lead to an increased understanding of host-microorganism interactions and dependencies (Tschöp et al., 2009). Identifying shared microbes can be confounded by environmentally derived, transient bacteria that are common across individuals but play no functional role within the host. By resampling the same individuals and within the same population we establish a shared microbial profile that is persistent across multiple environments and time periods, lessening the probability that transient bacteria are counted as shared. Accordingly, our results are in line with a decreased probability of including location or time period specific bacteria in that none of the bacterial groups we identified as differentially abundant at a specific time period, such as genera *Solirubrobacter* or *Serratia*, overlap with ASVs of the Kirtland's Warbler shared microbial profile.

Identification of microbial taxa that persist with the gut across multiple habitats and time periods will help identify those that may play a role in host biological function. Our analyses identified a group of microbial lineages, including several that probably play a role in digestion and nutrient uptake, as the species-specific shared microbial profile of Kirtland's Warblers. Eight ASVs in genus *Bacteroides* (Phyla Bacteroidetes) were identified as shared across the majority of individuals. *Bacteroides* are common gut microbes in humans that are frequently associated with food material breakdown and production of nutrients and energy (Wexler, 2007). Although common in birds, the exact functions of *Bacteroides* are unknown; however, it is thought they play a similar role in food digestion (Bennett et al., 2013; Grond et al., 2018; Waite & Taylor, 2015). Family Ruminococcaceae (Phyla Firmicutes), contains numerous bacterial species that degrade cellulose (Duncan et al., 2007). Our sampling of Kirtland's Warblers identified seven ASVs from this family that are common throughout the population, including several ASVs from genus *Faecalibacterium*. Similarly, the Greater Sage-Grouse (*Centrocercus urophasianus)* hosts a rich diversity of Ruminococcaceae associated with seasonal variation in foliage consumption (Drovetski et al., 2019). These bacteria may aid in the digestion of the various fruits and berries ingested throughout the year which become a primary diet component on the wintering grounds.

Additionally, through repeated sampling of the same birds at three discrete time periods, we have documented the proportion of ASVs that individuals retain over time. Although several previous studies have described the proportion of core ASVs to total ASVs detected within their study, interpretations may vary depending on the number of birds sampled, laboratory methods and parameters defining shared bacterial taxa, and may therefore not represent the number of core ASVs in each individual (Grond et al., 2017; Lewis et al., 2016). We show that individuals sampled in triplicate retain 18–26 ASVs over time. This represents an average of 25.06% of all lineages detected per individual per sampling point, and we argue it best reflects the proportion of stable, persistent bacteria within an individual. Documenting the species-specific shared microbial profile of Kirtland's Warblers as well as temporally persistent lineages across seasons and changing environments provides model data from which we can begin to understand the extent to which birds depend on their gut microbiota.

5 | CONCLUSION

The ability to study the same individuals and populations throughout the annual cycle greatly enhances our understanding of the consequences of changing environments and seasonal physiological stressors on gut microbiota. We demonstrate that a significant compositional shift occurs in the community structure of gut bacteria as Kirtland's Warblers migrate from The Bahamas to Michigan. Additionally, we describe a species-specific shared microbial profile and the proportion of bacterial lineages retained across three periods of the annual cycle within individuals. Though Kirtland's Warblers were recently removed from the endangered species list after recovering from near extinction, continued management and research is needed for this species to survive (Cooper et al., 2019). In species that have experienced severe population declines, such as Kirtland's Warbler, the subsequent decrease in genomic diversity may leave the species vulnerable to invading pathogens (Radwan et al., 2010). Gut microbiota may be critical in mitigating disease pathogenesis in these species by providing microbially mediated protection against invading pathogens (DeCandia et al., 2020; Ubeda et al., 2017). The symbiotic relationship birds form with their microbiota can confer immunological, developmental and physiological benefits (Grond et al., 2018). Additionally, as anthropogenic influences continue to impact the habitat Kirtland's occupy, the microbiota of the birds may be used as a proxy for individual and population level health (Trevelline et al., 2019. Healthy gut microbiota should be included in the maintenance of threatened and endangered species (Allan et al., 2018; Roth et al., 2019) and this study provides model data as to how species with small population sizes and extreme habitat specialization react to changing environments.

ACKNOWLEDGEMENTS

This work was supported by ARCS Foundation of Illinois, American Ornithological Society, the Women's Board of The Field Museum, James Bond Fund, and National Geographic (no. WW231R-17). USFWS MI Ecological Services Field Office supported tower construction in Michigan. We thank USFWS, USFS, USDA Wildlife Services, and MI DNR for hosting towers and A. Makarewicz and the Davies and Sansote families for providing field housing in Michigan. We also thank Josie, M. Seymour, and F. Wolff for hosting towers and M. Tukeva and the Campaignes for providing field housing on Cat Island. Finally, we thank N. Gottel, C. Moreau, T. Price, M. Ramalho, J. Novembre, B. Trevelline, E. Videvall, and B. Weigel for advice on this study as well as the many field technicians who helped collect samples.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Heather R. Skeen, Nathan W. Cooper, and PPM designed the project with input from Shannon J. Hackett and John M. Bates. Heather R. Skeen and Nathan W. Cooper collected samples and wrote the manuscript. Heather R. Skeen processed samples and analysed data. All authors contributed to manuscript revisions.

DATA AVAILABILITY STATEMENT

The code used in this study is available at https://github.com/skeenhr/KIWA_Microbiome. All sequence data generated for this study are available at the NCBI Sequence Read Archive, accession SRR14778844 and BioProject PRJNA736460. See Table S6 for sample associated metadata.

ORCID

Heather R. Skeen https://orcid.org/0000-0003-3269-031X
Nathan W. Cooper https://orcid.org/0000-0002-4667-1542
John M. Bates https://orcid.org/0000-0002-5809-5941
Peter P. Marra https://orcid.org/0000-0002-0508-7577

REFERENCES

Allan, N., Knotts, T. A., Pesapane, R., Ramsey, J. J., Castle, S., Clifford, D., Foley, J. (2018). Conservation implications of shifting gut microbiomes in captive-reared endangered voles intended for reintroduction into the wild. *Microorganisms*, *6*, 94.–https://doi.org/10.3390/microorganisms6030094

Anand, A., Chattopadhyay, B., & Kandula, S. (2012). Isolation and characterization of cellulose-degrading and xylanolytic bacteria from the short-nosed fruit bat *Cynopterus sphinx*. *Acta Chiropterologica*, *14*, 233–239.

Anderson, M. J. (2017). *Permutational multivariate analysis of variance (PERMANOVA)* (pp. 1–15). Wiley Statsref: Stat. Ref. Online.

Angelakis, E., & Raoult, D. (2010). The increase of *Lactobacillus* species in the gut flora of newborn broiler chicks and ducks is associated with weight gain. *PLoS One*, *5*, e10463.–https://doi.org/10.1371/journal.pone.0010463

Astudillo-García, C., Bell, J. J., Webster, N. S., Glasl, B., Jompa, J., Montoya, J. M., Taylor, M. W. (2017). Evaluating the core microbiota in complex communities: a systematic investigation. *Environmental Microbiology*, *19*, 1450–1462. https://doi.org/10.1111/1462-2920.13647

Bates, D., Sarkar, D., Bates, M. D., & Matrix, L. (2007). *The lme4 package*. R Package Version 2: 74.

Baxter, N. T., Wan, J. J., Schubert, A. M., Jenior, M. L., Myers, P., & Schloss, P. D. (2015). Intra-and interindividual variations mask interspecies variation in the microbiota of sympatric *Peromyscus* populations. *Applied and Environmental Microbiology*, *81*, 396–404. https://doi.org/10.1128/AEM.02303-14

Bennett, D. C., Tun, H. M., Kim, J. E., Leung, F. C., & Cheng, K. M. (2013). Characterization of cecal microbiota of the emu (*Dromaius novaehollandiae*). *Veterinary Microbiology*, *166*, 304–310. https://doi.org/10.1016/j.vetmic.2013.05.018

Ben-Yosef, M., Zaada, D. S., Dudaniec, R. Y., Pasternak, Z., Jurkevitch, E., Smith, R. J., Causton, C. E., Lincango, M. P., Tobe, S. S., Mitchell, J. G., Kleindorfer, S., & Yuval, B. (2017). Host-specific associations affect the microbiome of Philornis downsi, an introduced parasite to the Galápagos Islands. *Molecular Ecology*, *26*, 4644–4656.

Birrenkott, A. H., Wilde, S. B., Hains, J. J., Fischer, J. R., Murphy, T. M., Hope, C. P., Parnell, P. G., & Bowerman, W. W. (2004). Establishing a food-chain link between aquatic plant material and avian vacuolar myelinopathy in mallards (*Anas platyrhynchos*). *Journal of Wildlife Diseases*, *40*, 485–492. https://doi.org/10.7589/0090-3558-40.3.485

Bodawatta, K. H., Freiberga, I., Puzejova, K., Sam, K., Poulsen, M., & Jønsson, K. A. (2021). Flexibility and resilience of great tit (Parus

major) gut microbiomes to changing diets. *Animal Microbiome*, *3*, 1–14.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotech*, *37*, 852–857. https://doi.org/10.1038/s41587-019-0209-9

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581–583. https://doi.org/10.1038/nmeth.3869

Cao, J., Hu, Y., Liu, F., Wang, Y., Bi, Y., Lv, N., Li, J., Zhu, B., & Gao, G. F. (2020). Metagenomic analysis reveals the microbiome and resistome in migratory birds. *Microbiome*, *8*, 1–18. https://doi.org/10.1186/s40168-019-0781-8

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*, 335–336. https://doi.org/10.1038/nmeth.f.303

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, *6*, 1621–1624. https://doi.org/10.1038/ismej.2012.8

Capunitan, D. C., Johnson, O., Terrill, R. S., & Hird, S. M. (2020). Evolutionary signal in the gut microbiomes of 74 bird species from Equatorial Guinea. *Molecular Ecology*, *29*, 829–847. https://doi.org/10.1111/mec.15354

Carey, H. V., & Assadi-Porter, F. M. (2017). The hibernator microbiome: host-bacterial interactions in an extreme nutritional symbiosis. *Annual Review of Nutrition*, *37*, 477–500. https://doi.org/10.1146/annurev-nutr-071816-064740

Caviedes-Vidal, E., McWhorter, T. J., Lavin, S. R., Chediack, J. G., Tracy, C. R., & Karasov, W. H. (2007). The digestive adaptation of flying vertebrates: high intestinal paracellular absorption compensates for smaller guts. *Proceedings of the National Academy of Sciences*, *104*, 19132–19137. https://doi.org/10.1073/pnas.0703159104

Cooper, N. W., Ewert, D. N., Hall, K. R., Rockwell, S. M., Currie, D., Wunderle, J. M. Jr, White, J. D., & Marra, P. P. (2018). Resighting data reveal weak connectivity from wintering to breeding grounds in a range-restricted and endangered long-distance migratory passerine. *Avian Conservation and Ecology*, *13*(1), 1–11. https://doi.org/10.5751/ACE-01159-130109

Cooper, N. W., Ewert, D. N., Wunderle, J. M. Jr, Helmer, E. H., & Marra, P. P. (2019). Revising the wintering distribution and habitat use of the Kirtland's Warbler using playback surveys, citizen scientists, and geolocators. *Endangered Species Research*, *38*, 79–89. https://doi.org/10.3354/esr00937

Cooper, N. W., & Marra, P. P. (2020). Hidden long-distance movements by a migratory bird. *Current Biology*, *30*, 4056–4062.

Cooper, N. W., Rushing, C. S., & Marra, P. P. (2019). Reducing the conservation reliance of the endangered Kirtland's warbler through adaptive management. *The Journal of Wildlife Management*, *83*, 1297–1305. https://doi.org/10.1002/jwmg.21714

Corl, A., Charter, M., Rozman, G., Toledo, S., Turjeman, S., Kamath, P. L., Getz, W. M., Nathan, R., & Bowie, R. C. K. (2020). Movement ecology and sex are linked to barn owl microbial community composition. *Molecular Ecology*, *29*, 1358–1371.

da Cunha Santos, G. (2018). FTA cards for preservation of nucleic acids for molecular assays: a review on the use of cytologic/tissue samples. *Archives of Pathology & Laboratory Medicine*, *142*, 308–312. https://doi.org/10.5858/arpa.2017-0303-RA

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*, 226. https://doi.org/10.1186/s40168-018-0605-2

DeCandia, A. L., Brenner, L. J., King, J. L., & vonHoldt, B. M. (2020). Ear mite infection is associated with altered microbial communities in genetically depauperate Santa Catalina Island foxes (*Urocyon littoralis catalinae*). *Molecular Ecology*, *29*, 1463–1475.

Deloria-Sheffield, C. M., Millenbah, K. F., Bocetti, C. I., Sykes, P. W. Jr, & Kepler, C. B. (2001). Kirtland's Warbler diet as determined through fecal analysis. *The Wilson Journal of Ornithology*, *113*, 384–387.

Dewar, M. L., Arnould, J. P., Krause, L., Dann, P., & Smith, S. C. (2014). Interspecific variations in the faecal microbiota of *Procellariiform* seabirds. *FEMS Microbiology Ecology*, *89*, 47–55.

Di Rienzi, S. C., Sharon, I., Wrighton, K. C., Koren, O., Hug, L. A., Thomas, B. C., Goodrich, J. K., Bell, J. T., Spector, T. D., Banfield, J. F., & Ley, R. E. (2013). The human gut and groundwater harbor non-photosynthetic bacteria belonging to a new candidate phylum sibling to Cyanobacteria. *Elife*, *2*, e01102.

Drovetski, S. V., O'Mahoney, M. J., Matterson, K. O., Schmidt, B. K., & Graves, G. R. (2019). Distinct microbiotas of anatomical gut regions display idiosyncratic seasonal variation in an avian folivore. *Animal Microbiome*, *1*, 1–11. https://doi.org/10.1186/s42523-019-0002-6

Duncan, S. H., Louis, P., & Flint, H. J. (2007). Cultivable bacterial diversity from the human colon. *Letters in Applied Microbiology*, *44*, 343–350. https://doi.org/10.1111/j.1472-765X.2007.02129.x

Dykstra, H. R., Weldon, S. R., Martinez, A. J., White, J. A., Hopper, K. R., Heimpel, G. E., Asplen, M. K., & Oliver, K. M. (2014). Factors limiting the spread of the protective symbiont Hamiltonella defensa in Aphis craccivora aphids. *Applied and Environmental Microbiology*, *80*, 5818–5827. https://doi.org/10.1128/AEM.01775-14

Edenborough, K. M., Mu, A., Mühldorfer, K., Lechner, J., Lander, A., Bokelmann, M., Couacy-Hymann, E., Radonic, A., & Kurth, A. (2020). Microbiomes in the insectivorous bat species *Mops condylurus* rapidly converge in captivity. *PLoS One*, *15*, e0223629. https://doi.org/10.1371/journal.pone.0223629

Escallón, C., Belden, L. K., & Moore, I. T. (2019). The cloacal microbiome changes with the breeding season in a wild bird. *Integrative Organismal Biology*, *1*, 581–617. https://doi.org/10.1093/iob/oby009

Faust, K., Lahti, L., Gonze, D., De Vos, W. M., & Raes, J. (2015). Metagenomics meets time series analysis: unraveling microbial community dynamics. *Current Opinion in Microbiology*, *25*, 56–66. https://doi.org/10.1016/j.mib.2015.04.004

Flores, G. E., Caporaso, J. G., Henley, J. B., Rideout, J. R., Domogala, D., Chase, J., Leff, J. W., Vázquez-Baeza, Y., Gonzalez, A., Knight, R., Dunn, R. R., & Fierer, N. (2014). Temporal variability is a personalized feature of the human microbiome. *Genome Biology*, *15*, 531. https://doi.org/10.1186/s13059-014-0531-y

Fox, A. D., & Walsh, A. (2012). Warming winter effects, fat store accumulation and timing of spring departure of Greenland White-fronted Geese *Anser albifrons flavirostris* from their winter quarters. *Hydrobiologia*, *697*, 95–102. https://doi.org/10.1007/s10750-012-1173-2

García-Amado, M. A., Shin, H., Sanz, V., Lentino, M., Martínez, L. M., Contreras, M., Michelangeli, F., & Domínguez-Bello, M. G. (2018). Comparison of gizzard and intestinal microbiota of wild neotropical birds. *PLoS One*, *13*, e0194857. https://doi.org/10.1371/journal.pone.0194857

Gillingham, M. A. F., Béchet, A., Cézilly, F., Wilhelm, K., Rendón-Martos, M., Borghesi, F., Nissardi, S., Baccetti, N., Azafzaf, H., Menke, S., Kayser, Y., & Sommer, S. (2019). Offspring microbiomes differ across breeding sites in a panmictic species. *Frontiers in Microbiology*, *10*, 35. https://doi.org/10.3389/fmicb.2019.00035

Góngora, E., Elliott, K. H., & Whyte, L. (2021). Gut microbiome is affected by inter-sexual and inter-seasonal variation in diet for thick-billed murres (*Uria lomvia*). *Scientific Reports*, *11*, 1–12.

Grond, K., Lanctot, R. B., Jumpponen, A., & Sandercock, B. K. (2017). Recruitment and establishment of the gut microbiome in arctic shorebirds. *FEMS Microbiology Ecology*, *93*, fix142. https://doi.org/10.1093/femsec/fix142

Grond, K., Perreau, J. M., Loo, W. T., Spring, A. J., Cavanaugh, C. M., & Hird, S. M. (2019). Longitudinal microbiome profiling reveals impermanence of probiotic bacteria in domestic pigeons. *PLoS One*, *14*, e0217804.

Grond, K., Sandercock, B. K., Jumpponen, A., & Zeglin, L. H. (2018). The avian gut microbiota: community, physiology and function in wild birds. *Journal of Avian Biology*, *49*, e01788. https://doi.org/10.1111/jav.01788

Grond, K., Santo Domingo, J. W., Lanctot, R. B., Jumpponen, A., Bentzen, R. L., Boldenow, M. L., Brown, S. C., Casler, B., Cunningham, J. A., Doll, A. C., Freeman, S., Hill, B. L., Kendall, S. J., Kwon, E., Liebezeit, J. R., Pirie-Dominix, L., Rausch, J., & Sandercock, B. K. (2019). Composition and drivers of gut microbial communities in Arctic-breeding shorebirds. *Frontiers in Microbiology*, *10*, 2258. https://doi.org/10.3389/fmicb.2019.02258

Hicks, A. L., Lee, K. J., Couto-Rodriguez, M., Patel, J., Sinha, R., Guo, C., Olson, S. H., Seimon, A., Seimon, T. A., Ondzie, A. U., Karesh, W. B., Reed, P., Cameron, K. N., Lipkin, W. I., & Williams, B. L. (2018). Gut microbiomes of wild great apes fluctuate seasonally in response to diet. *Nature Communications*, *9*, 1–18. https://doi.org/10.1038/s41467-018-04204-w

Hird, S. M., Carstens, B. C., Cardiff, S. W., Dittmann, D. L., & Brumfield, R. T. (2014). Sampling locality is more detectable than taxonomy or ecology in the gut microbiota of the brood-parasitic Brown-headed Cowbird (*Molothrus ater*). *PeerJ*, *2*, e321.

Hird, S. M., Ganz, H., Eisen, J. A., & Boyce, W. M. (2018). The cloacal microbiome of five wild duck species varies by species and influenza a virus infection status. *mSphere*, *3*, 1–15. https://doi.org/10.1128/mSphere.00382-18

Hird, S. M., Sánchez, C., Carstens, B. C., & Brumfield, R. T. (2015). Comparative gut microbiota of 59 neotropical bird species. *Frontiers in Microbiology*, *6*, 1403. https://doi.org/10.3389/fmicb.2015.01403

Ingala, M. R., Simmons, N. B., Wultsch, C., Krampis, K., Speer, K. A., & Perkins, S. L. (2018). Comparing microbiome sampling methods in a wild mammal: fecal and intestinal samples record different signals of host ecology, evolution. *Frontiers in Microbiology*, *9*, 803. https://doi.org/10.3389/fmicb.2018.00803

Janssen, P. H. (2006). Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Applied and Environmental Microbiology*, *72*, 1719–1728. https://doi.org/10.1128/AEM.72.3.1719-1728.2006

Jenni, L., & Jenni-Eiermann, S. (1998). Fuel supply and metabolic constraints in migrating birds. *Journal of Avian Biology*, *29*(4), 521–528. https://doi.org/10.2307/3677171

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780. https://doi.org/10.1093/molbev/mst010

Kepler, C. B., Irvine, G. W., DeCapita, M. E., & Weinrich, J. (1996). The conservation management of Kirtland's Warbler *Dendroica kirtlandii*. *Bird Conservation International*, *6*, 11–22.

Kers, J. G., Velkers, F. C., Fischer, E. A., Hermes, G. D., Stegeman, J. A., & Smidt, H. (2018). Host and environmental factors affecting the intestinal microbiota in chickens. *Frontiers in Microbiology*, *9*, 235. https://doi.org/10.3389/fmicb.2018.00235

Kreisinger, J., Kropáčková, L., Petrželková, A., Adamkova, M., Tomášek, O., Martin, J. F., Michálková, R., & Albrecht, T. (2017). Temporal stability and the effect of transgenerational transfer on fecal microbiota structure in a long distance migratory bird. *Frontiers in Microbiology*, *8*, 50. https://doi.org/10.3389/fmicb.2017.00050

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2015). *Package "lmerTest": R package (Version, 2.0)*.

Lewis, W. B., Moore, F. R., & Wang, S. (2016). Characterization of the gut microbiota of migratory passerines during stopover along the northern coast of the Gulf of Mexico. *Journal of Avian Biology*, *47*, 659–668. https://doi.org/10.1111/jav.00954

Lewis, W. B., Moore, F. R., & Wang, S. (2017). Changes in gut microbiota of migratory passerines during stopover after crossing an ecological barrier. *The Auk*, *134*, 137–145. https://doi.org/10.1642/AUK-16-120.1

Lin, H., & Das Peddada, S. (2020). Analysis of compositions of microbiomes with bias correction. *Nature Communications*, *11*, 3514. https://doi.org/10.1038/s41467-020-17041-7

Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R., & Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial Ecology in Health & Disease*, *26*, 27663. https://doi.org/10.3402/mehd.v26.27663

Maurice, C. F., Knowles, S. C., Ladau, J., Pollard, K. S., Fenton, A., Pedersen, A. B., Turnbaugh, P. J. (2015). Marked seasonal variation in the wild mouse gut microbiota. *The ISME Journal*, *9*, 2423–2434. https://doi.org/10.1038/ismej.2015.53

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*, e61217. https://doi.org/10.1371/journal.pone.0061217

McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Computational Biology*, *10*, e1003531. https://doi.org/10.1371/journal.pcbi.1003531

McWilliams, S. R., & Karasov, W. H. (2005). *Birds of two worlds: the ecology and evolution of migration* (pp. 67–78). Smithsonian Institution Press.

Mohsen, A., Park, J., Chen, Y. A., Kawashima, H., & Mizuguchi, K. (2019). Impact of quality trimming on the efficiency of reads joining and diversity analysis of Illumina paired-end reads in the context of QIIME1 and QIIME2 microbiome analysis frameworks. *BMC Bioinformatics*, *20*, 1–10. https://doi.org/10.1186/s12859-019-3187-5

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., Suggests, M. A. (2007). The vegan package. *Community Ecology Package*, *10*, 719.

Pearce, D. S., Hoover, B. A., Jennings, S., Nevitt, G. A., & Docherty, K. M. (2017). Morphological and genetic factors shape the microbiome of a seabird species (*Oceanodroma leucorhoa*) more than environmental and social factors. *Microbiome*, *5*, 1–16. https://doi.org/10.1186/s40168-017-0365-4

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS One*, *5*, e9490. https://doi.org/10.1371/journal.pone.0009490

Pyle, P. (1997). *Identification guide to North American birds: a compendium of information on identifying, ageing, and sexing" near-passerines" and passerines in the hand*. Slate Creek Press.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*, D590–D596. https://doi.org/10.1093/nar/gks1219

Radwan, J., Biedrzycka, A., & Babik, W. (2010). Does reduced MHC diversity decrease viability of vertebrate populations? *Biological Conservation*, *143*, 537–544.

Rappole, J. H., & Tipton, A. R. (1991). New harness design for attachment of radio transmitters to small passerines (Nuevo Diseño de Arnés para Atar Transmisores a Passeriformes Pequeños). *Journal of Field Ornithology*, *62*(3), 335–337.

Ren, T., Boutin, S., Humphries, M. M., Dantzer, B., Gorrell, J. C., Coltman, D. W., McAdam, A. G., & Wu, M. (2017). Seasonal, spatial,

and maternal effects on gut microbiome in wild red squirrels. *Microbiome*, *5*, 163. https://doi.org/10.1186/s40168-017-0382-3

Ren, T., Grieneisen, L. E., Alberts, S. C., Archie, E. A., & Wu, M. (2016). Development, diet and dynamism: longitudinal and cross-sectional predictors of gut microbial communities in wild baboons. *Environmental Microbiology*, *18*, 1312–1325. https://doi.org/10.1111/1462-2920.12852

Ren, T., & Wu, M. (2016). PhyloCore: A phylogenetic approach to identifying core taxa in microbial communities. *Gene*, *593*, 330–333. https://doi.org/10.1016/j.gene.2016.08.032

Risely, A. (2020). Applying the core microbiome to understand host–microbe systems. *Journal of Animal Ecology*, *89*(7), 1549–1558. https://doi.org/10.1111/1365-2656.13229

Risely, A., Waite, D. W., Ujvari, B., Hoye, B. J., & Klaassen, M. (2018). Active migration is associated with specific and consistent changes to gut microbiota in Calidris shorebirds. *Journal of Animal Ecology*, *87*, 428–437.

Risely, A., Waite, D., Ujvari, B., Klaassen, M., & Hoye, B. (2017). Gut microbiota of a long-distance migrant demonstrates resistance against environmental microbe incursions. *Molecular Ecology*, *26*, 5842–5854. https://doi.org/10.1111/mec.14326

Roth, T. L., Switzer, A., Watanabe-Chailland, M., Bik, E. M., Relman, D. A., Romick-Rosendale, L. E., Ollberding, N. J. (2019). Reduced gut microbiome diversity and metabolome differences in rhinoceros species at risk for iron overload disorder. *Frontiers in Microbiology*, *10*, 2291. https://doi.org/10.3389/fmicb.2019.02291

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Costea, P. I., Godneva, A., Kalka, I. N., Bar, N., Shilo, S., Lador, D., Vila, A. V., Zmora, N., Pevsner-Fischer, M., Israeli, D., Kosower, N., Malka, G., Wolf, B. C., … Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature*, *555*, 210–215. https://doi.org/10.1038/nature25973

Segawa, T., Fukuchi, S., Bodington, D., Tsuchida, S., Mbehang Nguema, P. P., Mori, H., Ushida, K. (2019). Genomic analyses of *bifidobacterium moukalabense* reveal adaptations to frugivore/folivore feeding behavior. *Microorganisms*, *7*, 99. https://doi.org/10.3390/microorganisms7040099

Shade, A., & Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology*, *14*, 4–12. https://doi.org/10.1111/j.1462-2920.2011.02585.x

Shih, P. M., Hemp, J., Ward, L. M., Matzke, N. J., & Fischer, W. W. (2017). Crown group Oxyphotobacteria postdate the rise of oxygen. *Geobiology*, *15*, 19–29. https://doi.org/10.1111/gbi.12200

Sivonen, K. (1996). Cyanobacterial toxins and toxin production. *Phycologia*, *35*, 12–24. https://doi.org/10.2216/i0031-8884-35-6S-12.1

Smits, S. A., Leach, J., Sonnenburg, E. D., Gonzalez, C. G., Lichtman, J. S., Reid, G., Knight, R., Manjurano, A., Changalucha, J., Elias, J. E., Dominguez-Bello, M. G., & Sonnenburg, J. L. (2017). Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science*, *357*, 802–806. https://doi.org/10.1126/science.aan4834

Sommer, F., Ståhlman, M., Ilkayeva, O., Arnemo, J. M., Kindberg, J., Josefsson, J., Newgard, C. B., Fröbert, O., & Bäckhed, F. (2016). The gut microbiota modulates energy metabolism in the hibernating brown bear *Ursus arctos*. *Cell Reports*, *14*, 1655–1661. https://doi.org/10.1016/j.celrep.2016.01.026

Song, S. J., Amir, A., Metcalf, J. L., Amato, K. R., Xu, Z. Z., Humphrey, G., Knight, R. (2016). Preservation methods differ in fecal microbiome stability, affecting suitability for field studies, *Msystems*, *1*(3), 1–12. https://doi.org/10.1128/mSystems.00021-16

Song, S. J., Sanders, J. G., Delsuc, F., Metcalf, J., Amato, K., Taylor, M. W., Mazel, F., Lutz, H. L., Winker, K., Graves, G. R., Humphrey, G., Gilbert, J. A., Hackett, S. J., White, K. P., Skeen, H. R., Kurtis, S. M., Withrow, J., Braile, T., Miller, M., … Knight, R. (2020). Comparative analyses of vertebrate gut microbiomes reveal convergence between birds and bats, *Mbio*, *11*(1), 1–14. https://doi.org/10.1128/mBio.02901-19

Taylor, P., Crewe, T., Mackenzie, S., Lepage, D., Aubry, Y., Crysler, Z., Finney, G., Francis, C. M., Guglielmo, C. G., Hamilton, D. J., Holberton, R. L., Loring, P. H., Mitchell, G. W., Norris, D. R., Paquet, J., Ronconi, R. A., Smetzer, J. R., Smith, P. A., Welch, L. J., & Woodworth, B. K. (2017). The Motus Wildlife Tracking System: a collaborative research network to enhance the understanding of wildlife movement. *Avian Conservation and Ecology*, *12*(1), 1–11. https://doi.org/10.5751/ACE-00953-120108

Teyssier, A., Lens, L., Matthysen, E., & White, J. (2018). Dynamics of gut microbiota diversity during the early development of an avian host: Evidence from a cross-foster experiment. *Frontiers in Microbiolog*, *9*, 1524. https://doi.org/10.3389/fmicb.2018.01524

Topp, E., Mulbry, W. M., Zhu, H., Nour, S. M., & Cuppels, D. (2000). Characterization of s-triazine herbicide metabolism by a *Nocardioides sp*. isolated from agricultural soils. *Applied and Environmental Microbiology*, *66*, 3134–3141.

Trevelline, B. K., Fontaine, S. S., Hartup, B. K., & Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B: Biological Sciences*, *286*, 20182448. https://doi.org/10.1098/rspb.2018.2448

Tschöp, M. H., Hugenholtz, P., & Karp, C. L. (2009). Getting to the core of the gut microbiome. *Nature Biotechnology*, *27*, 344–346. https://doi.org/10.1038/nbt0409-344

Turjeman, S., Corl, A., Wolfenden, A., Tsalyuk, M., Lublin, A., Choi, O., Kamath, P. L., Getz, W. M., Bowie, R. C. K., & Nathan, R. (2020). Migration, pathogens and the avian microbiome: A comparative study in sympatric migrants and residents. *Molecular Ecology*, *29*, 4706–4720. https://doi.org/10.1111/mec.15660

Ubeda, C., Djukovic, A., & Isaac, S. (2017). Roles of the intestinal microbiota in pathogen protection. *Clinical & Translational Immunology*, *6*, e128. https://doi.org/10.1038/cti.2017.2

Videvall, E., Song, S. J., Bensch, H. M., Strandh, M., Engelbrecht, A., Serfontein, N., Hellgren, O., Olivier, A., Cloete, S., Knight, R., & Cornwallis, C. K. (2019). Major shifts in gut microbiota during development and its relationship to growth in ostriches. *Molecular Ecology*, *28*, 2653–2667.

Voolstra, C. R., & Ziegler, M. (2020). Adapting with microbial help: microbiome flexibility facilitates rapid responses to environmental change. *BioEssays*, *42*, 2000004. https://doi.org/10.1002/bies.202000004

Waite, D. W., & Taylor, M. (2015). Exploring the avian gut microbiota: current trends and future directions. *Frontiers in Microbiology*, *6*, 673. https://doi.org/10.3389/fmicb.2015.00673

Wexler, H. M. (2007). Bacteroides: the good, the bad, and the nitty-gritty. *Clinical Microbiology Reviews*, *20*, 593–621.

White, J., Mirleau, P., Danchin, E., Mulard, H., Hatch, S. A., Heeb, P., Wagner, R. H. (2010). Sexually transmitted bacteria affect female cloacal assemblages in a wild bird. *Ecology Letters*, *13*, 1515–1524. https://doi.org/10.1111/j.1461-0248.2010.01542.x

Wood, S. (2012). *mgcv: Mixed GAM Computation Vehicle with GCV/AIC/REML smoothness estimation*.

Wu, Y., Yang, Y., Cao, L., Yin, H., Xu, M., Wang, Z., Liu, Y., Wang, X., & Deng, Y. E. (2018). Habitat environments impacted the gut microbiome of long-distance migratory swan geese but central species conserved. *Scientific Reports*, *8*, 1–11. https://doi.org/10.1038/s41598-018-31731-9

Wunderle, J. M. Jr, Currie, D., Helmer, E. H., Ewert, D. N., White, J. D., Ruzycki, T. S., Parresol, B., & Kwit, C. (2010). Kirtland's Warblers in anthropogenically disturbed early-successional habitats on Eleuthera, The Bahamas. *The Condor*, *112*, 123–137.

Wunderle, J. M. Jr, Lebow, P. K., White, J. D., Currie, D., & Ewert, D. N. (2014). Sex and age differences in site fidelity, food resource

tracking, and body condition of wintering Kirtland's Warblers (*Setophaga kirtlandii*) in The Bahamas. *Ornithological Monographs*, *2014*(80), 1–62.

Zhu, C., Xu, W., Tao, Z., Song, W., Liu, H., Zhang, S., & Li, H. (2020). Effects of Rearing Conditions and Sex on Cecal Microbiota in Ducks. *Frontiers in Microbiology*, *11*, 1–11. https://doi.org/10.3389/fmicb.2020.565367

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Skeen, H. R., Cooper, N. W., Hackett, S. J., Bates, J. M., & Marra, P. P. (2021). Repeated sampling of individuals reveals impact of tropical and temperate habitats on microbiota of a migratory bird. *Molecular Ecology*, 00, 1–17. https://doi.org/10.1111/mec.16170