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Social groups constrain the spatiotemporal dynamics of wild sifaka gut microbiomes

Amanda C. Perofsky[1] | Lauren Ancel Meyers[1,2] | Laura A. Abondano[3,4] | Anthony Di Fiore[3,4] | Rebecca J. Lewis[3]

[1]Department of Integrative Biology, The University of Texas at Austin, Austin, Texas, USA

[2]Santa Fe Institute, Santa Fe, New Mexico, USA

[3]Department of Anthropology, The University of Texas at Austin, Austin, Texas, USA

[4]Primate Molecular Ecology and Evolution Laboratory, The University of Texas at Austin, Austin, Texas, USA

Correspondence
Amanda C. Perofsky, Department of Integrative Biology, The University of Texas at Austin, Austin, TX, USA.
Email: amanda.perofsky@nih.gov

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Abstract

Primates acquire gut microbiota from conspecifics through direct social contact and shared environmental exposures. Host behaviour is a prominent force in structuring gut microbial communities, yet the extent to which group or individual-level forces shape the long-term dynamics of gut microbiota is poorly understood. We investigated the effects of three aspects of host sociality (social groupings, dyadic interactions, and individual dispersal between groups) on gut microbiome composition and plasticity in 58 wild Verreaux's sifaka (*Propithecus verreauxi*) from six social groups. Over the course of three dry seasons in a 5-year period, the six social groups maintained distinct gut microbial signatures, with the taxonomic composition of individual communities changing in tandem among coresiding group members. Samples collected from group members during each season were more similar than samples collected from single individuals across different years. In addition, new immigrants and individuals with less stable social ties exhibited elevated rates of microbiome turnover across seasons. Our results suggest that permanent social groupings shape the changing composition of commensal and mutualistic gut microbial communities and thus may be important drivers of health and resilience in wild primate populations.

KEYWORDS
gut microbiome, lemur, longitudinal survey, Madagascar, primate, social network

1 | INTRODUCTION

Mammalian gut microbial communities govern host development, metabolism, immune function, and physiology (Archie & Theis, 2011; Bravo et al., 2011; Chung et al., 2012; Cryan & Dinan, 2012; Ezenwa et al., 2012; Flint & Bayer, 2008; Hooper et al., 2012; Kato et al., 2014; Koch & Schmid-Hempel, 2011; Mackie et al., 1999; McFall-Ngai, 2002; Montiel-Castro et al., 2013; Round & Mazmanian, 2009; Turnbaugh et al., 2007), through interactions that range from commensal and mutualistic to pathogenic. Though a subset of gut microbial lineages are inherited vertically (i.e., mother-to-offspring) (Moeller et al., 2018) and have codiversified with their primate hosts for millions of years (Moeller, Foerster, et al., 2016), primate gut microbiomes are also sensitive to environmental and lifestyle factors, such as dietary change (Gomez et al., 2015; Springer et al., 2017), captivity (Clayton et al., 2016; Delsuc et al., 2014), and habitat degradation (Amato et al., 2013; Barelli et al., 2015). Importantly, social organization and behaviour contribute to microbiome assembly, with mammals and other social animals acquiring a portion of their resident microorganisms

from conspecifics, either through direct physical contact or indirectly through common environmental exposures (Amato et al., 2017; Dill-McFarland et al., 2019; Koch & Schmid-Hempel, 2011; Moeller, Foerster, et al., 2016; Perofsky et al., 2017; Robinson et al., 2019; Song et al., 2013; Tung et al., 2015).

Most diurnal primate species live in relatively stable social groups with one or more individuals of both sexes. Still, considerable variation across group-living primates exists in the structure and demographic composition of those groups, in the frequency of intergroup conflicts, and in the types and patterning of intragroup social interactions. Intrinsic differences in sociality and spatial cohesion across primate taxa are reflected in the degree of gut microbial convergence observed among coresiding group members and between social groups inhabiting the same geographic area (Brito et al., 2019; Degnan et al., 2012; Dill-McFarland et al., 2019; Goodfellow et al., 2019; Lax et al., 2014; Perofsky et al., 2017; Raulo et al., 2018; Tung et al., 2015; Wikberg et al., 2020). Cross-sectional studies indicate that social groups and socioaffiliative interactions facilitate microbial transmission in wild primates (Amato et al., 2017; Moeller, Foerster, et al., 2016; Perofsky et al., 2017; Raulo et al., 2018; Tung et al., 2015; Wikberg et al., 2020), nevertheless knowledge is limited concerning how social structure and individual behaviour affect the longitudinal trajectories of host-associated microbial communities. Given the profound impact of gut microbial communities on host health and ecology, the socially mediated dispersal of mutualistic gut bacteria may confer a selective benefit to group-living and ultimately impact the evolution of animal sociality (Archie & Theis, 2011; Ezenwa et al., 2012; Lombardo, 2008; Montiel-Castro et al., 2013).

In this study, we utilize a rich data set of behavioural, genetic, and demographic data obtained as part of The Sifaka Research Project, a continuous long-term field study of wild lemurs (Verreaux's sifaka, *Propithecus verreauxi*) in western Madagascar, to consider the impact of dynamic contact patterns on the gut microbiome at multiple scales, including between social groups (i.e., at the population-level), among individuals within groups, and within individual hosts. Verreaux's sifaka are folivorous arboreal lemurs that live in small, cohesive, mixed-sex social groups (Leimberger & Lewis, 2017; Richard et al., 1993; Sussman et al., 2012). Sifaka social groups occupy small, highly overlapping home ranges (Lewis, 2005; Richard, 1985) but physical contact between individuals from different groups is infrequent. Sifaka groom with their toothcombs and their tongues rather than digitally, with grooming relationships primarily occurring within social groups (Lewis, 2010; Richard & Nicoll, 1987). Allogrooming may promote the direct oral transfer of gut microbes between individuals (Perofsky et al., 2017; Tung et al., 2015). Sifaka also communicate through scent-marking, in which they rub their throat (males only) or anogenital region (both sexes) on trees (Lewis, 2005, 2006). Males commonly overmark and inspect the scent-marks of both group and extra-group members (Lewis, 2005), thus scent-marking locations may serve as fomites for indirect microbial transmission. While a few past microbiome studies have conducted longitudinal surveys in wild primates (Aivelo et al.,2016; Degnan et al., 2012; Moeller, Foerster, et al., 2016; Raulo et al., 2018; Ren et al.,2015), the present study is unique in its assimilation of fine-grained data on primate community structure, social network relationships, and individual host behaviour and tracking of multiple, coresident animals over several years. Further, because sifaka are behaviourally, ecologically, and evolutionary distinct from anthropoid primates, a common focus of nonhuman primate microbiome studies, our study provides important comparative insights into the roles of biological, social, and ecological forces in shaping the long-term dynamics of primate gut microbiota.

To measure short- and long-term gut microbial plasticity, we assayed sifaka gut microbial communities during three dry seasons over a 5-year period. We collected faecal samples from six social groups on a weekly basis during 1–2 month periods in 2012, 2015, and 2016, with a total of 65 unique individuals (58 of known identity) sampled across the entire study period. We tested two primary hypotheses concerning the sociodemographic determinants of gut microbiome composition: (1) social groups maintain distinct gut microbial signatures over time, despite intergroup dispersal and demographic variability; and (2) the gut microbiota of individual sifaka fluctuate in response to perturbations in their social environment, including intergroup dispersal and variability in social partner choice. The sifaka social groups included in our study occupied overlapping home ranges within a small geographic area (1 km^2) and experienced regular gene flow through inter-group dispersal. Thus, we predicted that social effects are more influential than ecology or genetics in differentiating sifaka gut microbial communities over space and time.

2 | MATERIALS AND METHODS

2.1 | Study site and study subjects

We collected sifaka behavioural data and faecal samples at Ankoatsifaka Research Station (20°47.69′S, 44°9.88′E) in Kirindy Mitea Biosphere Reserve in western Madagascar (Figure S1; Tables S1 and S2). In this study, we focus on six habituated social groups (I, II, III, IV, V, and VI) inhabiting the Ankoatsifaka trail system during 2011–2016. Since 2007, groups II, III, IV, and V have been the focus of long-term research, whereas genetic and demographic data for all-male bands, solitary individuals, and groups inhabiting the periphery of the study area (I, VI, VII) have been collected opportunistically. Groups I to VI are habituated to the presence of researchers, and most individuals in these groups are marked with nylon collars and tags. Of the 65 individuals sampled in this study, demographic data were available for 58 individuals, social behaviour data were available for 54 individuals in groups II to V, feeding behaviour data were available for 25 individuals in groups II to V, and multilocus genotype data were available for 47 individuals in groups I to VI. We categorized sifaka based on their group affiliation,

age, sex, dispersal status, and group tenure (Table S2), and, for pairwise analyses, scored whether two individuals were close relatives based on multilocus genotype data (see Supporting Information methods). All data collection procedures complied with protocols approved by The University of Texas at Austin Institutional Animal Care and Use Committee (IACUC) and adhered to the legal requirements of Madagascar and the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates (protocol numbers: 05101801, 08110301, AUP-2011-00143, AUP-2014-00361, AUP-2017-00152, AUP-2020-00143). Data were collected and exported under permit approval from Madagascar National Parks (MNP), the Madagascar Ministry of Environment, Forests, and Tourism (MEFT), and the Madagascar Ministry of Higher Education.

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2.2 | Categorization of study subjects

Social group affiliation was determined from direct observation of the study subjects and monthly censuses. For habituated individuals, sex was known from direct observation of external genitalia during annual captures. We assigned individuals to the following age classes: juveniles (1–2 years), subadults (3–4 years), adults (≥5 years); infants (<1 year) were not included in the study. We categorized "residents" as individuals born into the group they were sampled in during a given season or that were present before the start of the long-term study of that group and "immigrants" as individuals that transferred into a group during the long-term study. We used monthly census data to determine the timing of group transfers and the length of group residence ("group tenure") for immigrants. We examined the dispersal histories of "recent immigrants" – individuals that transferred during the year prior to faecal sample collection – and "past immigrants" – long-term but non-natal group members that transferred groups 1–5 years prior to faecal sample collection. "Recent immigrants" included four adult males and one subadult male that dispersed during 2011–2012 and one subadult female that dispersed during 2015–2016. "Past immigrants" included 11 adult males. Methods for sifaka genetic sample collection and genotyping, sifaka behavioural data collection, home range estimation, dietary analysis, and social network analysis are provided in the Supporting Information.

2.3 | Faecal sample collection, processing, and 16S rRNA gene sequencing

We collected faecal samples immediately after defaecation during three dry seasons across 5 years (Table S1). We collected a total of 345 faecal samples from 65 unique individuals, with 161 samples collected from 42 individuals in 2012, 30 samples collected from 30 individuals in 2015, and 154 samples collected from 38 individuals in 2016. Members of groups I to VI were sampled weekly during the 2012 and 2016 collection periods and once during the 2015 collection period. Samples were also collected opportunistically from a solitary individual in 2015 and two all-male bands and group VII in 2016. A total of 31 individual animals were sampled during at least 2 years, and 14 individuals were sampled across 3 years. Faecal samples were collected during individual focal follows ($N = 254$ samples, Figure S2) or during the Sifaka Research Project's annual capture period in 2012 ($N = 43$), 2015 ($N = 30$), or 2016 ($N = 18$) (see Supporting Information for capture methods). We preserved faecal samples in RNAlater (ThermoFisher) and stored them at ambient temperature until their arrival at The University of Texas at Austin, where they were frozen at −80°C until further analysis. We extracted DNA from the majority of 2012 faecal samples ($N = 125$) using a phenol chloroform bead-beating procedure (Goodman et al., 2011) and from the remaining samples ($N = 220$) using the DNeasy Powersoil Kit (Qiagen). For each set of DNA extractions, an empty tube was exposed to laboratory air and processed as a negative control. We amplified the V4 region (254 nt) of the bacterial 16S ribosomal RNA gene with primers 515F and 806R (Caporaso et al., 2012). The resulting amplicons were pooled and paired end (2 × 150) sequenced on the Illumina MiSeq platform at Argonne National Laboratory. Samples extracted with phenol-chloroform bead-beating were sequenced on one MiSeq run, and the remaining samples (all extracted with Powersoil kits) were sequenced on a second run.

We demultiplexed raw Illumina sequence reads with QIIME 1 (Caporaso et al., 2010) and processed demultiplexed reads with DADA2 1.8 in R (Callahan et al., 2016), following the authors' published workflow (https://benjjneb.github.io/dada2/tutorial_1_8.html). DADA2 implements a quality-aware model of Illumina amplicon errors to infer exact biological sequences (i.e., amplicon sequence variants, ASVs). We assigned taxonomic classifications to ASVs based on their best match in the Silva reference database v138 (Glöckner et al., 2017). After taxonomic assignment, we removed 19 putative contaminants (DECONTAM R package, Davis et al., 2018), eukaryotic, chloroplast, and mitochondrial ASVs ($N = 54$), and singletons ($N = 46$). We constructed rarefaction curves of Chao1 species richness and Simpson's diversity with 10 subsamplings at every 1000 reads to verify adequate sequencing depth. We discarded 23 samples with fewer than 3000 sequence reads because rarefaction curves plateaued at this sequencing depth (Figure S3). Lastly, we omitted six samples collected from unmarked individuals in Groups III and VI in 2012. The resultant data set included 315 samples from 58 animals (Table S2) with a total of 3165 unique ASVs ($\bar{x} = 255 \pm 39$ SD per

sample; range: 102–540) and an average sequencing depth of 57,975 reads per sample (21,572 SD; range: 3723–143,656). Across all microbiome samples, 48.2% of phylum resolved ASVs were unclassifiable at the bacteria genus level, consistent with past studies of lemur gut microbiomes (Amato et al., 2018).

2.4 | Gut microbiome diversity and composition

Unless noted otherwise, we conducted microbiome and statistical analyses with the statistical computing software R version 4.0.1 (R Core Team, 2021). In statistical analyses with multiple comparisons, we used the Benjamini–Hochberg procedure to control the false discovery rate (Benjamini & Hochberg, 1995).

2.4.1 | Beta diversity

Prior to estimating pairwise dissimilarities among gut microbial communities, we retained ASVs that appeared more than 30 times in the data set and that were detected in at least two samples, totalling 603 unique sequences. We applied a variance stabilizing transformation (VST), based on a negative binomial mixture model of microbiome count data, to estimate sample-specific normalization factors and rescale ASV counts (*estimateSizeFactors* function with type="poscounts", DESEQ2 package, Love et al., 2014). VST adjusts for unequal library sizes and transforms the ASV count matrix such that variance in an ASV's counts across samples is approximately independent of its mean. VST is preferable to other normalization techniques, such as rarefying or sample proportions, because it retains all available data and adjusts for heteroscedasticity in ASV counts (McMurdie & Holmes, 2014). After normalization, samples contained $\bar{x} = 246 \pm 33$ SD unique ASVs. We quantified variation in microbial community composition by calculating Bray–Curtis dissimilarities and weighted Unifrac distances between samples (PHYLOSEQ package, McMurdie & Holmes, 2013). Principal coordinates analysis (PCoA) was used to visualize ecological distances between microbiome samples from groups I to VI.

2.4.2 | Community state types

We partitioned the taxonomic profiles of 58 sifaka into community state types (CSTs), which are clusters of microbiome samples with similar taxonomic compositions (DiGiulio et al., 2015). We first denoised the Bray–Curtis dissimilarity matrix by extracting the seven most significant eigenvectors obtained from PCoA. We applied partitioning of data around medoids (PAM clustering) with 100 bootstraps and determined the optimal number of clusters via the gap statistic (CLUSTER package, Maechler et al., 2019). PAM clustering of weighted Unifrac distances did not produce distinct CSTs. We used the nonparametric SAMseq method to measure differential abundance of bacterial phyla and genera across Bray–Curtis CSTs (see Supporting Information; Fernandes et al., 2014).

2.4.3 | Random forest classification

We used random forest classifier (RFC) models with 1000 decision trees to predict the year and social group associated with individual microbiome samples. Phylum classified ASVs present in at least 5% of microbiome samples ($N = 405$ of 2732 unique ASVs), and their associated abundances, were used as features in RFC models, with year (2012, 2015, or 2016) or social group (I to VI) as the category for RFC model distinguishability (Supporting Information).

2.4.4 | PERMANOVA

We used permutational multivariate analysis of variance (PERMANOVA) to assess variation in gut microbiome beta diversity according to year, social group affiliation, age, sex, and individual identity (Anderson, 2001). We retained samples from individuals in groups I to VI sampled across at least two years and randomly subsampled this reduced data set to retain one sample for each individual in each year. We applied PERMANOVA (1000 permutations) to the Bray–Curtis dissimilarities of 100 subsampled data sets to evaluate the contributions of three sets of predictors to microbial variation (*adonis2* function with marginal effects, VEGAN package, Oksanen et al., 2020). The first model included social group affiliation, year, age, and sex. Because social group affiliation and individual identity are confounded, the second model included individual identity and year, with age and sex removed to avoid model overfitting. The third model included sex, age, and year as predictors. Extraction method had a minor but statistically significant effect on beta diversity among samples collected in 2012 ($R^2 = .04$, $p = .04$, Figure S4), after adjusting for host social group, age, and sex. Thus, we stratified the permutations of multiyear tests by extraction method. For all PERMANOVA tests we report the mean R^2 value and p-value for each variable.

2.5 | Predictors of intergroup microbial clustering

For each study year, we performed Spearman's rank correlations to assess the effects of shared habitat (utilization distribution overlap index, UDOI) and socioaffiliative contact on microbial similarity between social groups. We used Kruskal–Wallis tests and post-hoc Dunn's tests to assess between-group differences in the proportions of plant parts consumed. Methods for home range estimation, social network analysis, and dietary analysis are in the Supporting Information.

2.6 | Comparisons of within- and between-host gut microbial similarity over time

We performed Kruskal–Wallis tests and post-hoc Dunn's tests to assess differences in pairwise microbial dissimilarity among six categories of intra- and interindividual sample comparisons: samples from the same individual collected during the same season, excluding samples

collected fewer than 5 days apart, samples from group coresidents collected during the same season, samples from nongroup members collected during the same season, samples from the same individual collected in different years, samples from group coresidents collected in different years, and samples from nongroup members collected in different years. See Table S3 for the numbers of microbiome samples and pairwise sample comparisons in each category.

2.7 | Predictors of between-individual microbial similarity

We tested the cumulative and annual effects of pairwise grooming, spatial proximity, genetic relatedness, dietary intake, and social group membership on between-host microbial similarity in four social groups (II, III, IV, V) for which extensive demographic, genetic, and behavioural data were available.

2.7.1 | Mantel tests of annual effects

Partial Mantel tests (method = Kendall's tau) were performed to investigate whether social network relationships, while controlling for social group membership, kinship, and shared diet, correlate with pairwise gut microbial similarity in individual study years (Supporting Information).

2.7.2 | Linear regression analysis of cumulative (multiyear) effects

We aggregated genetic, demographic, and behavioural data from the three study years to investigate the combined effects of social relationships, group membership, genetic relatedness, and diet on pairwise gut microbial similarity. Beta generalized linear mixed models (GLMMs) were fit to microbial Bray–Curtis dissimilarities using the GLMMTMB package (Brooks et al., 2017). Models were fit separately to data from sifaka with both social behaviour and genotype data (47 animals, 253 samples) and to data from sifaka with dietary, social behaviour, and genotype data (25 animals, 141 samples). The first data set's full model included covariates for group membership ("same" vs. "different"), genetic relatedness ("related" vs. "unrelated"), grooming network path length, and proximity network path length. The second data set's full model included an additional covariate for dietary distance. We assessed multicollinearity among model covariates using variance inflation factors (VIFs), with VIF >3 indicating collinearity (Zuur et al., 2010). The VIF for proximity exceeded three in the first full model, and VIFs for social group and grooming exceeded seven in both full models. Thus, we tested alternative models that included one of each of the three social network covariates (social group, proximity, and grooming) in combination with genetic relatedness and dietary distance. Models in each set were compared using Akaike information criterion (AIC), with lower values indicating a better fit of the model to the data. In all models, the identities of individual animals in each dyad, year (2012, 2015, or 2016), and DNA extraction method ("Both phenol chloroform", "Both Powersoil", or "Phenol chloroform and Powersoil") were included as random effects.

2.8 | Predictors of intrahost gut microbiome dynamics

2.8.1 | Demographic attributes

We used Beta GLMMs to estimate the effects of demographic predictors on short-term (i.e., within season) and long-term (i.e., between-year) gut microbiome dynamics in individual animals. We used pairwise Bray–Curtis dissimilarities between samples collected from the same individual at different time points to measure temporal stability in individual microbial communities. We excluded individuals with fewer than three microbiome samples and pairwise comparisons for samples collected fewer than 5 days apart, yielding a data set with 252 samples from 43 individuals. Categorical model covariates included the approximate length of time between samples ("within the same season", "one year", "three years", or "four years"), host age ("same age class in both samples", "juvenile to subadult", "subadult to adult," or "juvenile to adult"), host sex ("male" or "female"), and host dispersal status ("recent immigrant in one or both samples" or "resident in both samples"). All VIFs were <2. The identities of individual animals and DNA extraction method were included as random effects. Wilcoxon tests were used to estimate correlations between individual model covariates and intrahost Bray–Curtis dissimilarity.

2.8.2 | Intergroup dispersal

To characterize the timeframe of gut microbial turnover in dispersed individuals, we examined the dispersal histories of "recent immigrants" ($N = 6$) – individuals that transferred groups during the year prior to faecal sample collection – and "past immigrants" ($N = 11$) – non-natal resident group members that immigrated 1–5 years before faecal sample collection and compared the gut microbial communities of both sets of animals to those of long-term group residents. The "recent immigrant" data set included 65 samples among 23 immigrant-resident dyads, and the "past immigrant" data set included 172 samples from 45 immigrant-resident dyads. We tracked microbial exchange between long-term group residents and immigrants by comparing within-season resident-immigrant Bray–Curtis dissimilarity in relation to each immigrant's length of group residence ("group tenure"), with pairwise comparisons limited to microbiome samples collected fewer than 14 days apart. Beta GLMMs were used to measure (1) the relationship between immigrant-resident microbial dissimilarity and immigrant group tenure, and (2) the relationship between male immigrant-resident female microbial dissimilarity and grooming path length or proximity path length between dyads. All models included the identities of individual animals in each dyad and DNA extraction method as random effects.

2.8.3 | Social partner preference

To measure associations between social partner stability and between-year fluctuations in within-host gut microbiota, we focused on long-term residents in social groups for which both microbiome and social behaviour data were available (groups II, III, IV, and V). Methods for social partner stability indices (Kalbitzer et al., 2017; Silk et al., 2012) are in the Supporting Information. This data set included 199 samples from 12 adult and subadult individuals. We used a Beta GLMM to test the effects of proximity-based partner stability index (PSI) and grooming-based PSI on intrahost Bray–Curtis dissimilarity, with the approximate length of time between microbiome samples ("one year", "three years", or "four years") and DNA extraction method included as random effects.

3 | RESULTS

3.1 | Sifaka gut microbiomes exhibit population-level drift through time

Across all study years, Bacteroidetes ($\bar{x}$ = 47.9% reads per sample), Firmicutes ($\bar{x}$ = 28.6%), and Proteobacteria ($\bar{x}$ = 9.2%) were, on average, the most abundant bacterial phyla in sifaka gut microbiomes, followed by Cyanobacteria ($\bar{x}$ = 3.6%), Actinobacteria ($\bar{x}$ = 3.1%), unclassified phyla ($\bar{x}$ = 3.0%), and Verrucomicrobia ($\bar{x}$ = 1.9%) (Figure S5).

The sifaka population experienced substantial, coordinated shifts in gut microbial taxonomic structure over the 5-year study period. In ordination analyses of Bray–Curtis dissimilarities and weighted Unifrac distances (principal coordinates analysis, PCoA), samples clustered according to year, with 2015 microbiomes clustering intermediately between those collected in 2012 and 2016 (Figure 1a, Figure S6A). This pattern was apparent when visualizing the longitudinal trajectories of the six social groups separately (Figure 1b, Figure S6B) and may stem from interannual shifts in food availability (Figures S7 and S8). For example, in 2016, sifaka collectively spent more time foraging on fruit and seeds and less time eating young leaves compared to prior years (Figure S7). Though social groups clustered closely through time and exhibited qualitatively similar trajectories, members of groups IV and V maintained compositionally distinct gut microbiotas from those of sifaka in groups I, II, and III (Bray–Curtis PCoA, Figure 2). The microbiotas of group VI formed a discrete cluster in 2012 but converged in composition with those of groups I, II, and III in 2015 and 2016 (Figure 2, Figure S9). Within social groups, sifaka in groups I, II, III tended to have stable gut microbial communities, while a subset of individuals in groups IV, V, and VI exhibited a high degree of volatility both within and across years (Figure S10). Random forest models trained on phylotype-level information accurately classified samples according to both year and social group (year: kappa = .97, AUC = 0.94; group: kappa = .94, AUC = 0.97).

We applied de novo clustering (partitioning around medoids, PAM) to Bray–Curtis dissimilarities to visualize temporal trends in community structure among individual sifaka in six social groups

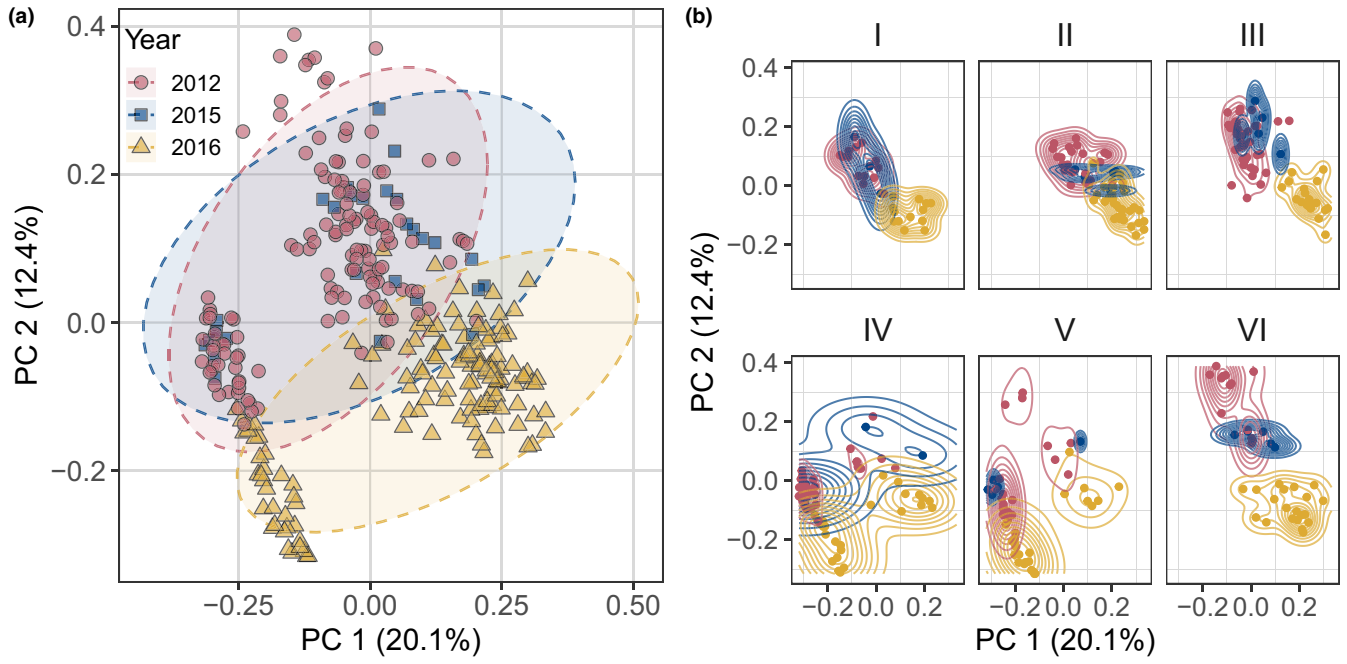

FIGURE 1 Sifaka gut microbiomes exhibit coordinated drift through time. Principal coordinates plots of Bray–Curtis dissimilarities showing ecological distances among 305 microbiome samples collected from 57 sifaka in six social groups (groups I to VI). Point colour and shape indicate study year (red circles: 2012, blue squares: 2015, yellow triangles: 2016). (a) At the population level, samples cluster according to study year, with 2015 samples positioned intermediately between samples collected in 2012 and 2016 (PERMANOVA, R^2 = .06, p = .002). Ellipses represent year-specific 95% confidence intervals assuming a multivariate t-distribution. (b) Within social groups, samples also cluster according to study year. Contours represent year-specific 2D kernel density estimations [Colour figure can be viewed at wileyonlinelibrary.com]

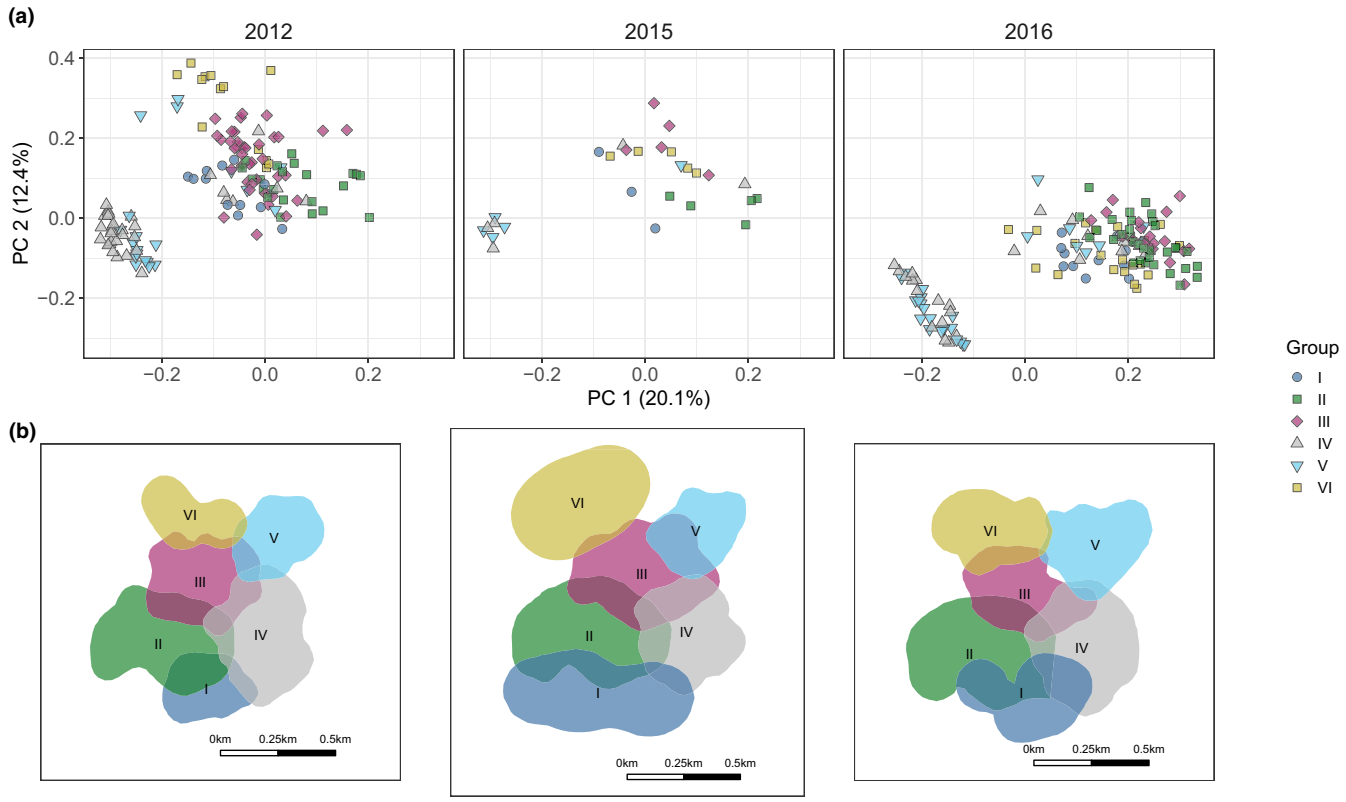

FIGURE 2 Sifaka social groups maintain distinct gut microbiota over time despite overlapping home ranges. (a) Principal coordinate analysis (PCA) of Bray–Curtis dissimilarities showing ecological distances between 305 microbiome samples from 57 Verreaux's sifaka in six social groups (I to VI). Social groups form two to three clusters (PERMANOVA, $R^2 = .24$, $p = .001$). Colour and shape indicate social group affiliation (navy circles, I; green squares, II; purple diamonds, III; grey triangles, IV; cyan inverted triangles, V; yellow squares, VI). (b) Home ranges (95% fixed kernel contours) of six social groups (I to VI) during the year preceding and including each microbiome sampling period. The complete home ranges of group I (navy) and group VI (yellow) could not be estimated because these groups inhabit the periphery of the field site [Colour figure can be viewed at wileyonlinelibrary.com]

(I to VI). Sifaka gut microbiomes aggregated into seven clusters, or community state types (CSTs) (Figure 3, Figure S11) with distinct taxonomic structure and phylotype diversity (Figures S12–S15). Microbiomes assigned to the seventh CST were limited almost exclusively to samples collected from Group VI in 2012. Microbial communities assigned to the seventh CST were enriched with Firmicutes and Actinobacteria and had lower species richness than microbiomes assigned to the other six CSTs (Figure 3, Figures S12–S15). The gut microbial CSTs of individual sifaka fluctuated within each dry season, yet groups IV and V harboured CSTs that were distinct from those of groups I, II, and III, and intraindividual transitions between CSTs tended to temporally coincide among group co-residents (Figure 3). Specifically, group coresidents shared 1–2 CSTs within each season and jointly shifted to a different set of shared CSTs across seasons.

Habitat overlap and intergroup encounters may facilitate microbial exchange between social groups. For example, in 2012, samples from Group V cluster with those from both Groups IV and VI, which occupy home ranges adjacent to group V. However, we did not find strong statistical support for either mechanism (Figure S16). To assess the contribution of diet to observed patterns of between-group microbial similarity, we profiled the feeding behaviour of 25 individuals in groups II, III, IV, and V during the 6 months prior to and including each period of faecal collection. Though we found some statistically significant between-group differences in each year (Figure S7), patterns in dietary intake did not align with the consistent microbial clustering of groups IV and V from groups I, II, and III.

3.2 | Social groups constrain the longitudinal dynamics of sifaka gut microbiomes

Across all samples, social group membership explained 23.5% of observed variation in gut microbial composition (PERMANOVA, Bray–Curtis metric, $p = .001$), while study year, host sex, and host age collectively explained 11.3% of variation (year: $R^2 = .06$, $p = .002$; sex: $R^2 = .03$, $p = .01$; age: $R^2 = .02$, $p = .3$). In a separate model that partitioned variance according to host identity and year, longitudinally sampled sifaka exhibited substantial inter-individual differences, with individual identity and year explaining 52.8% and 5.2% of microbial variation, respectively (identity, $p = .001$; year, $p = .002$). When we replaced individual identity with age and sex, neither of these variables contributed significantly to microbiome beta diversity (age: $R^2 = .04$, $p = .21$; sex: $R^2 = .02$, $p = .1$). Thus,

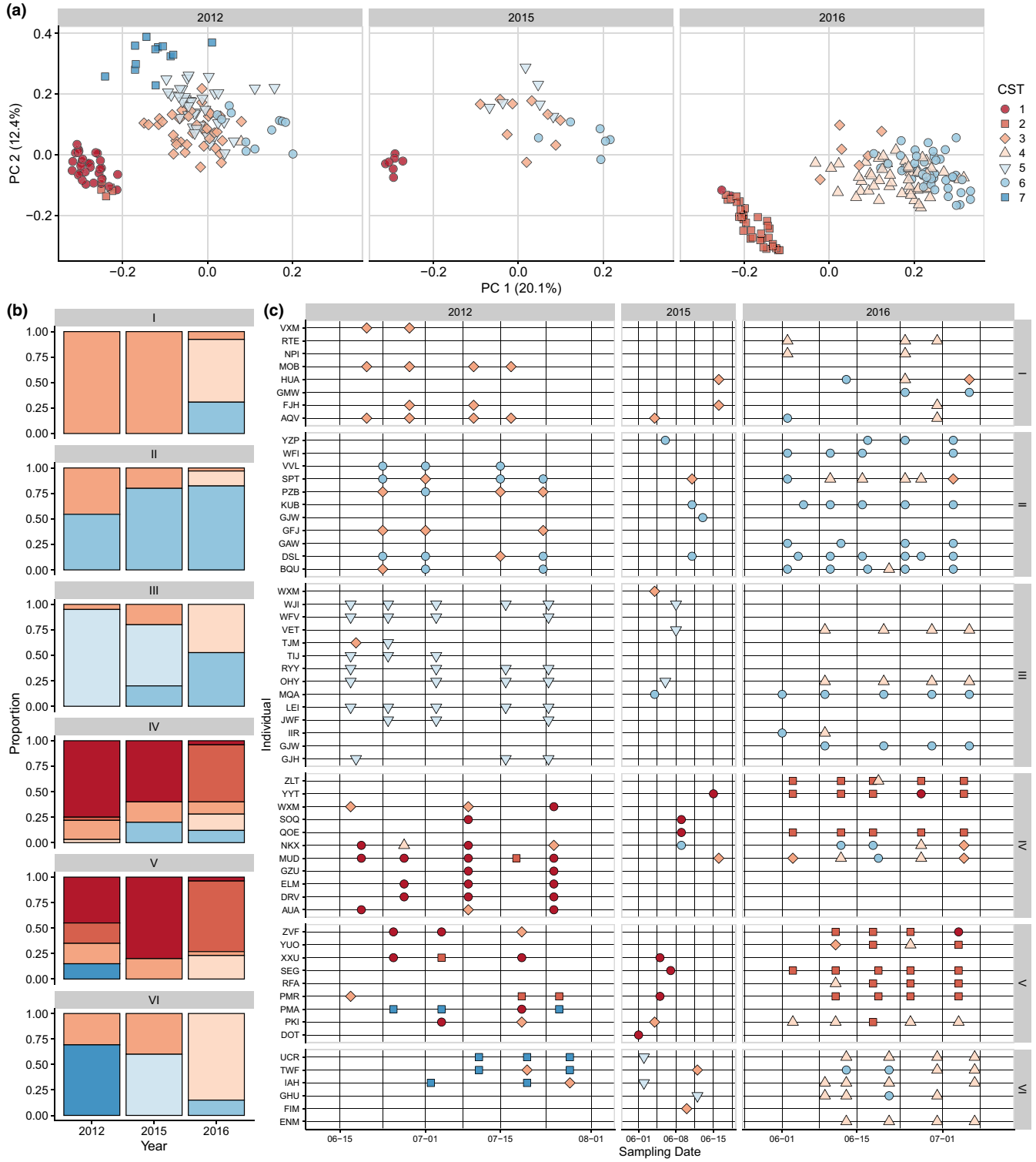

FIGURE 3 Temporal dynamics of sifaka gut microbial communities. (a) Principal coordinate analysis (PCA) of Bray–Curtis dissimilarities showing ecological distances between 305 microbiome samples from 57 sifaka in six social groups (I to VI). Sample colour and shape indicate community state type (CST) assignment. (b) Bar plots show the proportion of microbiome samples classified into each of seven CSTs detected. Samples are facetted by social group affiliation (groups I to VI) and aggregated by study year (2012, 2015, and 2016). (c) Gut microbial CST time course for 57 individual sifaka in groups I to VI during sample collection periods in 2012, 2015, and 2016 [Colour figure can be viewed at wileyonlinelibrary.com]

individual-level microbial signatures do not appear to stem from physiological differences between sexes or age groups.

To decouple the confounding effects of individual identity and social group membership on gut microbial dynamics, we compared microbial dissimilarities between samples collected from the same individual animal, from group coresidents, and from nongroup members, during the same dry season and across study years. In support of group-level effects, pairs of microbiome samples from group coresidents collected

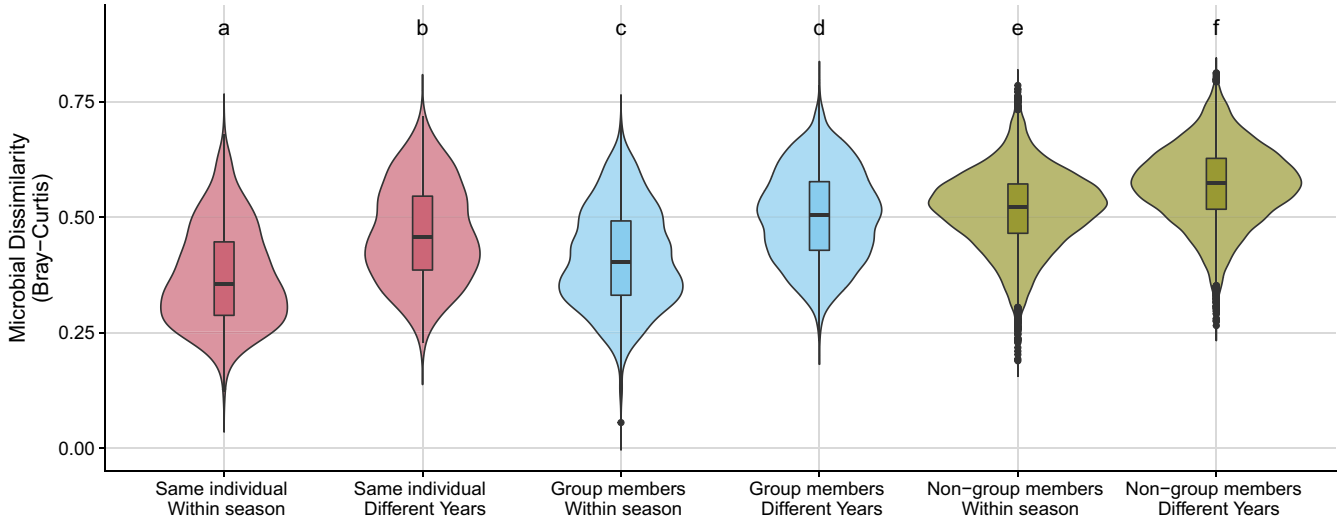

FIGURE 4 Sifaka gut microbiome dynamics vary according to individual identity, social group membership, and the length of time between samples. Bray–Curtis dissimilarities between gut microbiomes sampled within and between sample collection periods in 2012, 2015, and 2016. Different letters indicate significant differences between categories (Kruskal–Wallis and post-hoc Dunn's tests, adjusted $p < .05$). Whiskers inside each violin extend to points within 1.5 IQRs of the lower and upper quartile for the distribution, and the centre bar represents the median [Colour figure can be viewed at wileyonlinelibrary.com]

during the same season were more similar in composition than pairs of samples collected from the same individual in different years (Kruskal–Wallis and post-hoc tests, $p < .05$; Figure 4). When we limited pairwise analyses to samples collected only one year apart, distances between microbiome samples collected from the same individual in different years were not significantly different to those between samples collected from group coresidents during the same dry season ($p > .05$).

Social, dietary, and genetic divisions between social groups may jointly contribute to persistent group-level microbiome signatures (Figure 2; Figures S7, S8, S17, and 18). In individual study years, close social partners harboured more similar gut microbiota, after controlling for group membership, genetic relatedness, and diet (partial Mantel tests, $p < .05$; Table S4). In the aggregated three-year data set, group membership was the strongest predictor of shared microbiome composition (Beta GLMMs, Tables S5 and S6). When we replaced group membership with grooming path length or proximity path length, individuals with stronger social relationships (i.e., shorter distances in the grooming or proximity social networks) had more similar gut microbiota (Figure S19), with grooming producing a better model fit than proximity (Table S5). In the subset of individuals for which genetic, dietary, and social behaviour data were available, the best fit model included group membership and dietary distance (Table S6).

3.3 | Age, sex, and social environment impact individual gut microbiome stability

3.3.1 | Demographic predictors of within-host gut microbiome dynamics

We used microbial dissimilarities between samples collected from the same animal at different time points to quantify the within-season and between-year dynamics of intrahost gut microbial communities. Among 43 longitudinally sampled individuals, intrahost Bray–Curtis dissimilarity predictably increased with the length of time between samples, with microbiome samples collected during the same dry season exhibiting greater compositional similarity than samples collected in different years (Beta GLMM, Table 1, Figure S20). Individual animals that transitioned from being "juveniles" to being "adults" during the study exhibited greater compositional turnover than individuals that remained in the same age class, the gut microbiotas of long-term group residents were more stable than those of recent immigrants, and male sifaka harboured more volatile microbiomes than female sifaka (Beta GLMM, Table 1, Figure S20).

3.3.2 | Immigrants acquire gut bacteria from the residents of their new social groups

Within social groups, the gut microbiotas of recent immigrants – individuals that transferred groups during the year prior to faecal sample collection – were distinguishable from those of long-term coresidents (Bray–Curtis, Wilcoxon test, $p < .0001$; Figure 5 and Figure S21). Recent immigrants appeared to continuously acquire gut bacteria from long-term group residents during their first year of group residence, with the degree of microbial similarity between recent immigrants and residents increasing with immigrants' length of group tenure within the first year after dispersal (Bray Curtis, Beta GLMM, $z = -2.87$, $p = .004$; Figure 5 and Figure S21). In contrast, past immigrants – dispersed males that had resided in groups for longer than one year – maintained a consistent level of microbial similarity to other coresidents over time (Figure S22). Resident female sifaka sometimes encourage the entrance of subordinate males into their groups via grooming (Lewis, 2008). However, microbial dissimilarity between 15 pairs of female residents and male immigrants

TABLE 1 Determinants of intrahost gut microbiome stability. Results from Beta GLMM with Bray–Curtis dissimilarity as the response variable

Explanatory variable	Coefficient estimate	95% CI	Effect size	*p*-value
Time between samples				
Same season	–	–		
One year	0.31	0.20, 0.41	5.64	**<.001**
Three years	0.14	0.00, 0.29	1.95	**.05**
Four years	0.33	0.22, 0.43	6.05	**<.001**
Age				
No change	–	–		
Juvenile to subadult	0.20	−0.06, 0.46	1.50	.13
Subadult to adult	−0.09	−0.28, 0.10	−0.90	.4
Juvenile to adult	0.22	0.05, 0.40	2.5	**.01**
Sex				
Female	–	–		
Male	0.18	0.01, 0.34	2.14	**.03**
Dispersal status				
Resident	–	–		
Recent immigrant	0.19	0.03, 0.35	2.33	**.02**

Note: Mean coefficient estimates, 95% confidence intervals, *z*-values, and *p*-values are shown for fixed-effect parameters. Individual identity and DNA extraction method were included as random effects. Bolded relationships are significant at $p < .05$.

$\sigma = 26.1$; Residual df = 697.

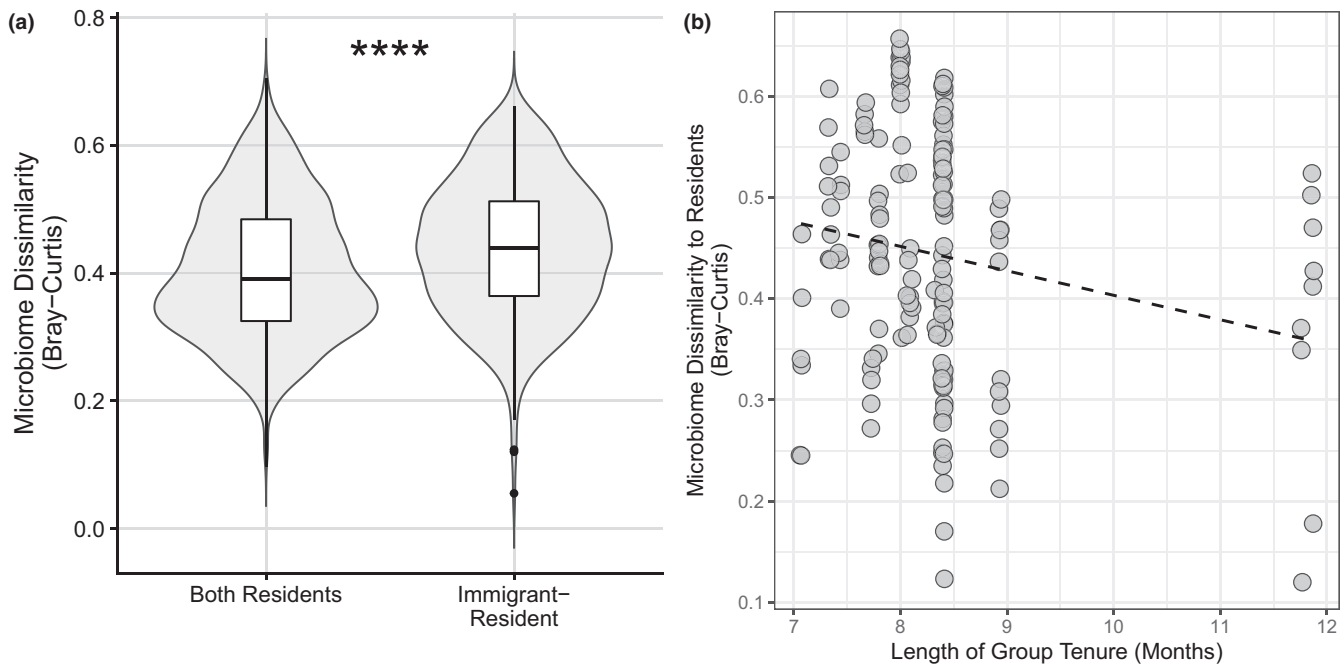

FIGURE 5 Immigrants exchange gut bacteria with their new social groups. (a) Within social groups, pairs of long-term coresidents share more bacterial phylotypes than pairs of recent immigrants and residents (Bray–Curtis, Wilcoxon test, $p < .0001$). Whiskers inside each violin extend to points within 1.5 IQRs of the lower and upper quartile for the distribution, and the centre bar represents the median. (b) Immigrant-resident Bray–Curtis microbial dissimilarity decreases with the length of time an immigrant has resided in the group (Beta GLMM, $z = -2.87$, $p = .004$). Points represent Bray–Curtis dissimilarities between 23 recent immigrant-resident pairs residing in the same social group during the same dry season. The dashed line shows the linear trend of immigrant-resident microbial dissimilarity as a function of length of immigrant group tenure. Points were jittered for clarity

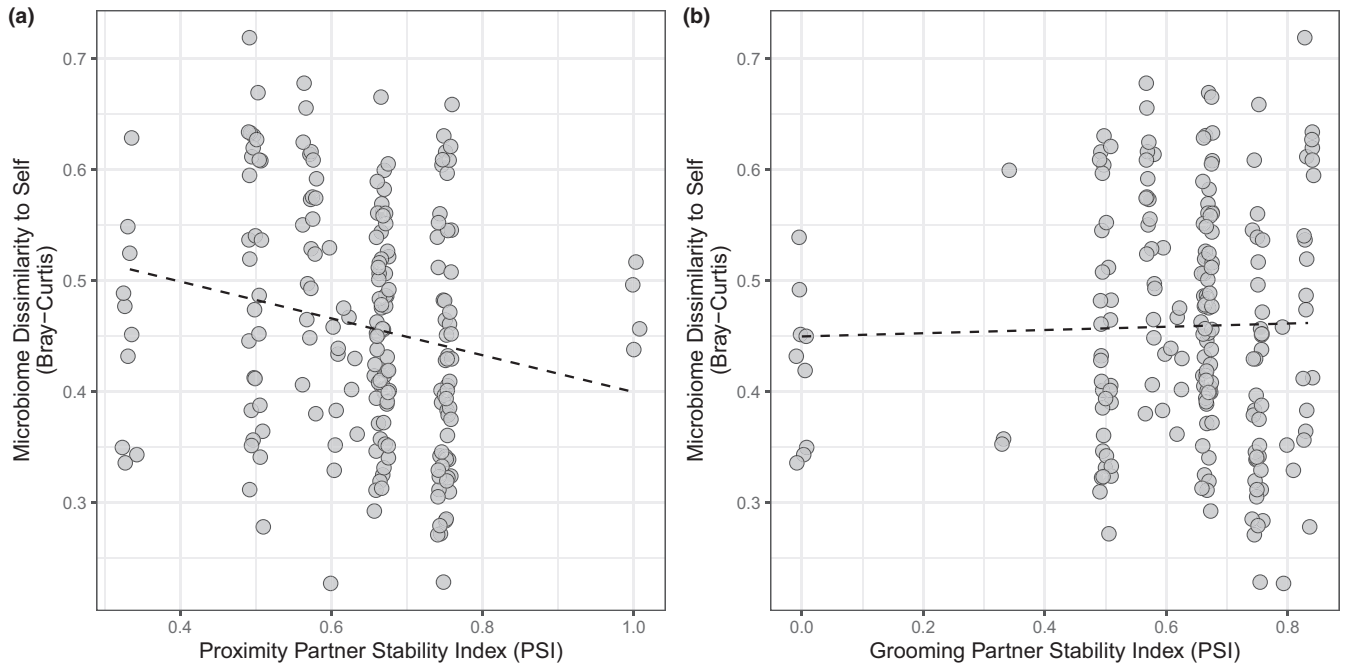

FIGURE 6 Sifaka with persistent social ties exhibit greater gut microbiome stability. Partner stability indices (PSI) were computed for 12 individual animals based on (a) proximity and (b) grooming. For proximity interactions, individuals with less frequent switching of preferred social partners (high PSI) exhibited greater within-host gut microbial stability (lower Bray–Curtis dissimilarity between microbiome samples) than individuals that frequently switched their top partners across years (Beta GLMM, $z = -3.19$, $p = .001$). Grooming PSI was not significantly associated with intrahost gut microbiome dynamics ($z = 0.14$, $p = .89$). Dashed lines show the linear trend of within-host microbial dissimilarity as a function of social partner stability (PSI). Points were jittered for clarity

did not correlate with social network distance based on close spatial proximity or grooming (Beta GLMMs, both behaviours, $p > .1$).

3.4 | Consistency in close social partners promotes intrahost gut microbiome stability

We assessed the impact of social partner preference, measured via partner stability indices (PSI; Figure S23) on gut microbiome dynamics for 12 adults and subadults that were long-term residents of groups II, III, IV, and V. When considering social partner stability based on close proximity, individuals with lower PSI values (i.e., those that more frequently switched preferred social partners across years) experienced greater volatility in gut microbiome composition than individuals whose close social partners were consistent over time, after controlling for the length of time between samples (Beta GLMM, $z = -3.19$, $p = .001$, Table S7, Figure 6). Switching of close grooming partners was not associated with intrahost gut microbial plasticity ($z = 0.14$, $p = .89$, Table S7, Figure 6).

4 | DISCUSSION

Many diurnal primates live in social groups with individuals typically interacting frequently and intimately within their own group and rarely with individuals outside of their group. Social groups and socioaffiliative interactions promote gut microbial convergence between individuals (Moeller, Foerster, et al., 2016; Perofsky et al., 2017; Tung et al., 2015), yet we lack a clear understanding of how dynamic social networks impact longitudinal changes in gut microbiome composition, from the population to the individual host level. Over the course of three dry seasons in a five-year period, the six Verreaux's sifaka groups in our study maintained distinct gut microbial signatures, with the taxonomic changes of individual communities changing in tandem among coresiding group members. Demographic attributes, including age and sex, as well as perturbations in one's social environment, such as switching close social partners and dispersing between groups, were also associated with compositional shifts in intrahost gut microbiota.

We detected a strong temporal trend in gut microbiome composition, with samples from 2015 clustering intermediately between those collected 2012 and 2016. Consistent with past longitudinal microbiome surveys in Hadza hunter-gatherers (Smits & Leach, 2017), chimpanzees (Degnan et al., 2012; Moeller, Foerster, et al., 2016), baboons (Grieneisen et al., 2017), and mouse lemurs (Aivelo et al., 2016), sifaka microbiome samples collected during the same dry season were more similar in composition than those collected in different years. This pattern was evident at the population (between-group) level, within social groups, and in individual animals, signifying the retention and management of core resident bacterial taxa and the gradual turnover of less prevalent transient taxa (Degnan et al., 2012), due to recent environmental or social exposures (Grieneisen et al., 2017). The sifaka population's coordinated drift in gut microbial composition, even with infrequent physical

contact between social groups, suggests that local environmental conditions are a principal force in shaping wild primate gut microbiomes (Grieneisen et al., 2019). The field site encompasses a 1 km^2 area; thus, we speculate that sifaka in different social groups share a common "microenvironment" and are subject to similar interannual changes in food availability.

Microbial communities from the six social groups aggregated into two to three clusters, with Groups IV and V clustering separately from groups I, II, and III in each year. Given the small geographic area inhabited by the sifaka study population and the infrequency of direct socioaffiliative contact between groups, we predicted that the consistent microbial clustering of particular groups stems from shared environment or similar dietary intake (Degnan et al., 2012; Yatsunenko et al., 2012). Sifaka social groups, on average, consumed different proportions of food items, and groups with greater habitat overlap harboured more similar gut microbiota in some years of our study, but the statistical support for these mechanisms was weak and did not explain the consistent clustering of groups IV and V over time. We did not find a statistically significant association between group-level microbial clustering and social network distance, as has been observed in primate species with more frequent intergroup contacts, such as colobus monkeys (Wikberg et al., 2020). Though shared space use and intergroup microbial clustering were not strongly correlated, scent marking in areas of home range overlap could provide a conduit for horizontal transmission between groups if the length of time between scent mark deposition and subsequent investigation is short enough so that gut bacteria are still viable on the substrate after deposition. If this horizontal transmission between groups occurs, gut microbiome variance within groups and convergence between groups could be at least partially affected by the extent of interaction with other groups, including scent mark investigation and overmarking during intergroup encounters.

Notably, sifaka social groups maintained distinct gut microbial signatures over the 5-year study period, despite changes in group membership and size and the addition, loss, and replacement of group members' constituent microbial taxa. Social partners act as bacterial reservoirs in sifaka and other primates (Brito et al., 2019; Lombardo, 2008; Moeller, Caro-Quintero, et al., 2016; Perofsky et al., 2017; Tung et al., 2015), thus we hypothesized that within-group social cohesion drives the emergence of distinct, stable microbial signatures across sifaka social groups. The microbial community state types (CSTs) of individual animals fluctuated within each dry season, yet sifaka residing in the same social group maintained similar taxonomic profiles and experienced compositional shifts in tandem. An animal's social group affiliation could be accurately predicted based on gut microbiome composition alone, even with microbiomes sampled up to five years apart. As observed in the fur microbiomes of Egyptian fruit bats (Kolodny et al., 2019), gut microbiomes from different sifaka in the same social group sampled closely in time were more compositionally similar than those from the same individual animal sampled across different years, indicating that social groups may be more influential than individual-level processes in shaping gut microbial communities.

Extending prior cross-sectional studies in primates, our longitudinal analysis also demonstrates that social networks and social relationships shape sifaka gut microbial communities through time, even when accounting for confounding factors, such as kinship, shared diet, and habitat overlap. However, the relative importance of direct physical contact, such as grooming, and indirect fomite transmission in socially mediated microbial dispersal remains unclear. In the multiyear analysis of factors contributing to between-individual microbiome composition, grooming relationships were more predictive of pairwise microbial similarity than the duration of close proximity between individuals. When estimating the potential impact of social ties on within-host gut microbiome dynamics, sifaka with consistent social relationships over time had more stable gut microbiota, though this association was evident only when focusing on relationships based on close proximity. Allogrooming and mutual grooming occur less frequently than other dyadic behaviours, which may explain the lack of a statistical association between grooming partner stability and intrahost gut microbial turnover in Verreaux's sifaka. Due to the small size and cohesiveness of sifaka social groups, spatial proximity and grooming are strongly interrelated, and both behaviours probably contribute to sustained microbial transfer between group coresidents.

Our results additionally demonstrate that individual-level characteristics, such as physiological development, sex, and dispersal behaviour are associated with the degree of volatility in intrahost gut microbiota. As primates physically mature and form new social connections, their gut microbiome assembly undergoes an ecological succession-like process (Koenig et al., 2011; Palmer et al., 2007; Yatsunenko et al., 2012). In humans, chimpanzees, and sifaka, adults harbour significantly greater microbial richness than younger individuals (Degnan et al., 2012; Perofsky et al., 2017; Yatsunenko et al., 2012), and, in this study, sifaka that remained in the same age class across study years – juvenile, subadult, or adult – experienced a lower degree of gut microbial turnover than individuals that matured to adulthood. The gut microbiotas of male sifaka were more temporally variable than those of female sifaka, which could be attributed to behavioural or physiological differences (Grieneisen et al., 2020) or the confounding of sex with dispersal status. Male sifaka scent mark and inspect the scent marks of other sifaka more frequently than females (Lewis, 2005), which may expose them to a greater diversity of microbial phylotypes (Perofsky et al., 2017), and an experimental study of female-male marmoset pairs found that females transmit more microbes to their partners than males do, potentially due to sex biases in anogenital investigation and grooming (Zhu et al., 2020). In sifaka, subadult males and clean-chested adult males (staining of the sternal scent gland is an indicator of male dominance) (Lewis & van Schaik, 2007) explore scent marks from nongroup members during intergroup encounters (Lewis, 2005) and might be expected to have greater microbial turnover than other group members that inspect scent marks less frequently.

The gut microbiomes of recently dispersed sifaka were more volatile than those of long-term group residents. Dispersal occurs in the majority of group-living mammals (Greenwood, 1980) and comprises both the permanent departure of prereproductive individuals away from their birth group (natal dispersal) and the subsequent transfer between groups throughout an individual's lifetime (secondary or breeding dispersal) (Leimberger & Lewis, 2017). In Verreaux's sifaka, females sometimes disperse but tend to be philopatric (Lawler et al., 2003), whereas males generally disperse from their natal group between 3 and 6 years of age (Kubzdela, 1997). Secondary transfer between groups occurs frequently (Leimberger & Lewis, 2017; Richard et al., 1993), with male sifaka sometimes immigrating into groups as pairs (Leimberger & Lewis, 2017; Richard et al., 1993) and transferring males rarely joining groups more than two home ranges away from their previous group (Richard et al., 1993). Because sifaka live in such small social groups, immigration, emigration, and group fission events have the potential to not only profoundly impact group size and composition (Lewis, 2008) but to also perturb the gut microbiotas of long-term residents and dispersing individuals.

Sifaka that dispersed to new social groups may acquire gut bacteria from long-term group residents (Degnan et al., 2012; Grieneisen et al., 2017; Perofsky et al., 2017) through direct or indirect social contacts (e.g., grooming, sequential scent-marking) or shared environmental exposures. Our findings suggest that sustained microbial transfer occurs between recent immigrants and resident group members within the first year after dispersal, with an immigrant's length of group tenure predicting its degree of gut microbial similarity to coresident group members. Male immigrants with group tenures longer than 1 year maintained a more consistent level of microbial convergence with other coresidents, contrasting with a study in baboons (Grieneisen et al., 2017), which found that the microbiotas of immigrant males continue to assimilate to those of other group members over the course of several years. Resident female sifaka are known to facilitate the entrance of subordinate males via grooming to promote group stability and reduce infanticide (Lewis, 2008), but we did not find an association between pairwise microbial similarity and the strength of grooming relationships between recent male immigrants and resident females.

5 | CONCLUSIONS

In summary, we found that the spatiotemporal dynamics of wild sifaka gut microbiomes are scale-dependent (Miller et al., 2018; Robinson et al., 2019), with coordinated shifts through time exhibited at the population and social group level (Kolodny et al., 2019). The population-wide temporal trend may stem from shared environmental conditions among groups residing in close proximity, while persistent social group signatures are consistent with the high degree of social cohesion within sifaka social groups and the infrequency of direct physical contact between groups. Dispersal between groups and social partner stability are also associated with turnover in intrahost gut microbial communities, underscoring that individual behaviour, in addition to higher-level social structure, modifies exposure and susceptibility to commensal bacteria over time. Our findings contribute further evidence that permanent social groups and host social interactions facilitate the development and perpetuation of diverse commensal and mutualistic microbial communities across generations (Moeller, Foerster, et al., 2016). Though the fitness impacts of the "social microbiome" (the collective microbial metacommunity of an animal social group) (Sarkar et al., 2020) are just beginning to be explored, the social transmission of gut microbiota could promote host health by facilitating the acquisition of beneficial bacteria (Lombardo, 2008; Tung et al., 2015) and the maintenance and replenishment of microbiome diversity (Browne et al., 2017; Burns et al., 2017). Strain tracking, through high resolution shotgun metagenomic sequencing, could establish a causal link between primate social networks and gut microbiome transmission (Brito et al., 2019; Sarkar et al., 2020) and clarify whether microbes that are socially transmitted more frequently or effectively provide important services to their hosts or are detrimental to host health (Moeller et al., 2018; Tung et al., 2015).

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AUTHOR CONTRIBUTIONS

Amanda C. Perofsky, Rebecca J. Lewis and Lauren Ancel Meyers designed the research. Amanda C. Perofsky collected faecal samples, conducted faecal DNA extractions, analysed microbial and behavioural data, and wrote the manuscript. Rebecca J. Lewis collected ear tissue samples and behavioural and demographic data. Laura A. Abondano and Anthony Di Fiore conducted ear tissue DNA

extractions and genotyping analyses. All authors edited drafts of the manuscript and gave their final approval for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

16S rRNA sequence data have been deposited in the NCBI Sequence Read Archive (BioProject PRJNA756780). Custom R scripts and data inputs (e.g., R phyloseq objects, sifaka metadata) to reproduce the main results and figures can be accessed in GitHub (https://github.com/aperofsky/sifaka-temporal-microbiome) and the Dryad Digital Repository (https://doi.org/10.5061/dryad.v6wwpzgws).

ORCID

Amanda C. Perofsky https://orcid.org/0000-0001-7341-9193

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