




Seasonal Effects on the Fecal Microbial Composition of Wild Greater Thick-Tailed Galagos (*Otolemur crassicaudatus*)

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Abstract

Bacterial communities present in the host digestive tract are important for the breakdown and absorption of nutrients required by the host. Changes in diet and the environment are major factors affecting the composition and diversity of the fecal microbiome. In addition to changes in ambient temperature and rainfall, primates living in seasonal temperate environments also need to adapt to seasonal changes in food resource quantity and quality. However, there is a lack of information about the fecal microbiome in African strepsirrhines relative to other primate taxa. We examined the effects of seasonal dietary and environmental changes on fecal microbial alpha diversity and composition in wild greater thick-tailed galagos (*Otolemur crassicaudatus*) at Lajuma Research Centre, South Africa. We collected fecal samples and assessed food availability and weather in summer and winter across 1 year and used 16S rRNA next-generation sequencing to characterise the fecal microbiome of 49 animals. We found significant increases in rainfall, ambient temperature, and food availability in summer compared with winter. However, we found no significant changes in body mass or in the overall diversity of bacterial species present in fecal samples between the two seasons. We found significant decreases in the abundance of certain bacterial families in winter, suggesting a change in diet. Our findings suggest that greater thick-tailed galagos can find food resources to maintain their body mass throughout the year. Our insights into the seasonal fecal microbiome of greater thick-tailed galagos add to the growing knowledge and understanding of fecal microbiomes in primates and how they help primates cope with changes to their environments.

Keywords Bacterial composition · Nonhuman primates · Seasonality · Dietary changes

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Introduction

Primates have adapted to living in a variety of different habitats, such as forests, mountains, and savannahs, and show an array of dietary preferences, such as insectivory, folivory, gummivory, and frugivory (MacKinnon & MacKinnon, 1980). Primates adjust their activity patterns and feeding behaviour in response to changes in their environment (Strier, 2009; Reyna-Hurtado et al., 2018). These developments may include changes to their social structure or habitat use (Chevalier et al., 2015). Changes also may occur in the gut microbiome, which consists of a symbiotic community of microbial organisms residing in the gut of the host individual (Amato et al., 2014). Bacterial communities present in the host digestive tract are linked to changes in the host diet, health, and the extrinsic environment (David et al., 2014; Bjork et al., 2019; Orkin et al., 2019). These communities are important for the breakdown and absorption of nutrients required by the host (Cabana *et al.*, 2018). For example, carbohydrates are fermented and broken down into branched- and short-chain fatty acids (SCFAs; Schepach, 1994; Nicholson et al., 2012), which can be absorbed in the gut and are available as an energy source for the host (Hildebrandt et al., 2009; De Filippo et al., 2010; Wu et al., 2011; Claesson et al., 2012). Carbohydrate fermentation is a critical function of the gut in the catabolism of these important nutrient sources in the metabolic homeostasis of the host (Schepach, 1994; Nicholson et al., 2012; Yuan et al., 2020).

Insects and fruit are common food preferences for primate species as they are high in carbohydrates and nutrients (O'Malley & Power, 2012). Gum exudate is low in nutrients and rare (Irani & Khaled, 2020) but is a common dietary component in many primate species (Caton et al., 2000). Gummivory may be beneficial for an insectivorous primate, providing calcium to complement the phosphorous taken from insects (Génin et al., 2010; Bearder & Martin, 1980). Both gum exudates and insect exoskeletons are comprised of insoluble β -linked complex polysaccharides, which the mammalian gut is incapable of digesting without the assistance of specialized gut microbes and fermentation (O'Malley & Power, 2012). The presence of gut microbes in the gut allows primates to digest and absorb the carbohydrates found in gum and insects exoskeletons (O'Malley & Power, 2012). As a result, fermentation and the digestive abilities of gut microbes are required in the gut for the primate to absorb and use these carbohydrates (Caton et al., 2000; O'Malley & Power, 2012). However, the gut microbiome is sensitive to both the diet of an organism, as well as the prevailing environmental conditions (Xu & Knight, 2015; Kers et al., 2018). Thus, any change in either component will result in a microbiome response to assist an individual in maintaining digestive health (Manor et al., 2020).

Most studies of the influence of seasonal shifts in climate and resource use on the gut microbial composition of primates have been conducted on haplorrhines (Amato et al., 2014; Sun et al., 2016). Very few studies have examined the effect of changing environment on the gut microbiome of African strepsirrhines. Filling this taxonomic gap is important in helping us understand the nutritional and metabolic requirements and physiology of primates.

Two nocturnal strepsirrhine primates are found in South Africa, the southern lesser galago (*G. moholi*) and the thick-tailed greater galago (*Otolemur*

crassicaudatus). While research on *G. moholi* has increased considerably over the past decade (Nowack et al., 2010, 2013; Scheun et al., 2015, 2016), including a study that found significant differences in seasonal bacterial profiles of the fecal microbiome (Long, 2018), very little has been attempted on thick-tailed greater galagos. These galagos inhabit highly seasonal environments in South Africa (Limpopo, Mpumalanga, and KwaZulu-Natal provinces; Hill, 1953), where changes in rainfall and temperature across the four seasons (summer, autumn, winter, spring) result in large changes in resource availability (Hill, 1953). They feed primarily on insects in summer and fruits in winter with gum-feeding occurring throughout the year (Bearder, 1974; Harcourt, 1986; Happold & Happold, 1992). Both insects and fruits provide a range of nutrients, such as phosphorous, sugars, and fats (Scheun et al., 2014; Rothman et al., 2014; O'Malley & Power, 2012). The foregut of thick-tailed greater galagos has evolved to allow fermentation of complex polysaccharides (Cabana et al., 2018). They show morphological and gut adaptations for both accessing and digesting gums and are designated as obligate gum feeders (Smith, 2010; Cabana et al., 2018).

To contribute to our understanding of the effect of seasonality on the gut microbiome in strepsirrhines, we aimed to identify the main microbial taxa found in the fecal microbiome of thick-tailed greater galagos inhabiting an isolated temperate environment in two contrasting seasons. Under the hypothesis that the fecal microbiome is affected by changes in diet, we predict a decrease in microbial taxonomy and relative abundance during winter when a considerable shift in diet occurs in comparison to summer. We also explore potential influences on the fecal microbiome, including seasonal changes in food availability, rainfall, and ambient temperature.

Methods

Study Site

Lajuma Research Centre (23°02'16.2"S, 29°26'35.4"E) is situated in the Soutpansberg Mountains in the Limpopo Province in the North-Eastern region of South Africa. It has a highly seasonal, temperate environment, comprised of montane forests and savannah woodland habitats (Van Wyk & Smith, 2001; Von Maltitz et al., 2003). Summer is from October to March, and winter is from late April to September. Temperatures fluctuate from 0 °C to 22 °C in the dry, winter months when overnight frost is common in more open areas and 12 °C to 36 °C in the wet, summer months (Mostert, 2010; Durowoju et al., 2019). While rainfall generally peaks in January and February, almost no rainfall is seen in June and July (median = 0 insects per capture). The vegetation grows from October to April, whereas the less favourable season for vegetation growth is from May to September. Five of the six species of primates found in South Africa live sympatrically at this location while several natural predators exist for galagos (Cuozzo et al., 2021; Allan et al., 2022).

Animal Captures and Faecal Sampling

We trapped thick-tailed greater galagos in a 3-km² area at Lajuma along transects from a northwest to southeast direction in June 2017, December 2017/January 2018, March 2018, and May/June 2018. We aimed to collect samples from at least 20 adults (10 males and 10 females; Table A1) in each trapping period. Each night, we focused on one area of the trapping range of approximately 50–100 m². In each sampling area, we set between four and nine traps, depending on the density of galagos and the proximity of the traps to one another. A licenced veterinarian was present during all animal capture and sampling events. We set Havahart® traps (Woodstream Corporation Inc., Lancaster, PA) baited with small amounts of peanut butter and banana before sunset (between 17h00 and 18h30, depending on the season) and checked them at sunrise (between 04h30 and 06h00, depending on the season). To digest gum, galagos use gut fermentation, a process that requires prolonged gut retention times (Caton et al., 2000). With a gut retention time of >10 hours (Long et al., 2021) and their preference for being most active late at night, it is unlikely that the samples we collected from trapped individuals changed due to the ingestion of the bait that we set out. We cleaned empty traps with a solution of 99% rubbing alcohol and water and closed them until the next trapping session. If an animal was present in the trap, we used a capture bag to collect it, identified it by using a microchip transponder reader (ID100 Trojan, EURO I.D., Weilerswist, Germany), then placed it into a pet travel box and translocated it to the field laboratory. We collected fecal samples for seed availability assessment from the trap once the animal was safely placed in the travel box.

Once at the field lab, we anesthetized the animal with an intramuscular injection of zolazepam and tiletamine (Zoletil®, Virbac, South Africa). We maintained the level of anesthesia with inhaled isoflurane in oxygen, administered intermittently via a face mask. Once anesthetized, we weighed each animal using a table-top scale. If we did not detect a microchip, we inserted a transponder microchip subcutaneously, and recorded the transponder identity number as the animal's ID. The vet checked reproductive status for females (pregnant, lactating, not pregnant). We recorded male reproductive status as either mating (June to August) or not mating (summer months) depending on the time of year. We inserted a FLOQ® fecal swab (FLOQSwabs®, Copan Group, Brescia, Italy) into the rectum to retrieve a sufficient volume of fecal matter to discolour the swab. Once removed, we cut off the head of the swab and placed it immediately into a 2-ml sterile tube, then stored it immediately in a -4 °C freezer. Once transported back to the University of Pretoria, approximately one week after the start of sampling, we stored the samples in a -80 °C freezer until DNA isolation.

We released captured animals where we caught them, on the same day we removed from the traps, as soon as they were completely conscious, and the veterinarian was satisfied with their health condition.

Seasonal Food Availability

We collected insect, gum, and seed samples to reflect the annual food availability for galagos. We collected insect and gum data from the environment and ascertained seed data from ingested fruit. We collected insects from September 2017 to June

2018. We set up two light traps at the field site before sunset approximately 15-m apart on consecutive days before and after trapping sessions in areas frequented by galagos. We placed traps before sunset. We set one light trap on the ground to estimate the abundance of ground dwelling insects, and the second 2 m above the ground to assess the abundance of flying insects. We set each light above a collecting chamber with a hole in the lid preventing most insects from escaping from the trap. We checked traps every morning at sunrise and sprayed all trapped insects with 70% ethanol, counted, and, where possible, identified them. We stored all specimens in 90% ethanol until we analysed food availability. For the analysis, we took the cumulative value of insects from both light traps.

We collected gum exudate samples monthly from 50 trees (10 trees per sampling section). We selected trees after we identified trees frequented by galagos or with gum exudates (personal observations by J.B. Millette and C. Long). We scraped gum exudates off the tree bark using a multitool and placed the sample in a sample bag. We stored these samples in a -20°C freezer until the food availability analysis.

We collected all fecal matter excreted by trapped individuals from July 2017 to June 2018 (winter: $N = 12$, summer: $N = 25$, winter 2: $N = 32$). We used a subset of each fecal sample to determine the presence and number of seeds. We dried the feces and extracted seeds by using tweezers. We took each seed to represent one fruit except for multiple *Ficus* seeds, which we identified as one fruit per fecal sample (Long et al., 2021). We counted the seeds retrieved and identified them to genus if possible.

Seasonal Weather Differences

We captured rainfall and ambient temperature data by using the on-site weather station (Davis Instruments sensor suite linked via wireless to a Davis Pro 2™ console, Measurement and Console Systems CC, Cape Town, South Africa), where conditions were analogous to those experienced by the sampled individuals. The Ndlovu Node of the Northeastern Mountain Observatories project of the South African Environmental Observation Network (SAEON) made these data available. We used data collected every hour to calculate the mean (\pm standard deviation; SD) daily ambient temperature ($^{\circ}\text{C}$) and daily cumulative rainfall (mm) total from June 2017 to August 2017 (winter 1), November 2017 to February 2018 (summer), and May to July 2018 (winter 2).

DNA Isolation and Next-Generation Sequencing

We extracted the total fecal bacterial DNA from 49 individuals (males: $N = 28$; females: $N = 21$) using the DNeasy® PowerSoil® Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. We assessed the quality of DNA using a QuBit 2.0 Fluorometer (Invitrogen, Carlsbad, CA). Inqaba Biotec Industries (Pty) Ltd (Muckleneuk, Pretoria) conducted PCR amplification, library preparation, next-generation sequencing, and data process and analysis. They created bacterial gene libraries by using the PacBio Full-Length 16S Amplification, SMRTbell® Library Preparation and Sequencing protocol. Briefly, the workflow implements two PCR rounds, the first with universal primer-tailed 16S primers (27F; 1492R) and the

second with PacBio Barcoded Universal Primers. They performed the first round of amplification with an initial denaturation at 95 °C for 30 s, 23 cycles of denaturing (95 °C for 30 s), annealing (55 °C for 30 s), and elongation (72 °C for 60 s). At each PCR step they generated amplicons using a KAPA HiFi PCR kit (Kapa Biosystems, Wilmington, MA) and quantified them on a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA). The second round of amplification used the same thermocycler instructions. They prepared libraries by using SMRTbell Template Prep kit (Pacific Biosciences, Menlo Park, CA) and purified them using AMPure® PB beads (Pacific Biosciences).

We sequenced the prepared libraries on the PacBio Sequel system (PacBio, <http://www.pacbio.com>). We processed the raw subreads through the SMRTlink (v7) Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40). The minimum sequence read length was set at >150 bp. We identified and removed the chimeric sequences and, finally, generated FASTQ files through vsearch (<https://github.com/torognes/vsearch>). We identified taxa using QIIME2 (Bolyen et al., 2019). We assigned the taxonomy of each gene sequence by using the Silva SSU NR database (v132.1; Quast et al., 2013), picking operational taxonomic units (OTUs) using a 97% similarity cutoff. We normalized the data by reducing the sequence reads to 10,000 per sample.

Statistical Analyses

We performed statistical analyses in R (R Core Team, 2020). We set significance at $p < 0.05$, except where we corrected p -values for multiple testing by using the Bonferroni correction. We report means plus SD for parametric data and medians plus interquartile ranges for nonparametric data.

Seasonal Analysis

We split the data for food availability into the three sampling seasons: June 2017 to August 2017 (winter 1); November 2017 to February 2018 (summer); and May to July 2018 (winter 2). We assessed seasonal differences in insect count, gum mass, and seed count data using Kruskal-Wallis tests with a chi-square distribution as the data were not normally distributed. We used Dunn's *post-hoc* tests to test for differences between seasons. For insects, we analysed the cumulative number of insects from both light traps per month. For gum mass, we analysed the sum of the gum mass from all trees per month. We also tested for seasonal differences (between winter 1, summer, and winter 2) in body mass for both sexes by using Kruskal-Wallis tests with chi-squared distribution.

If the Kruskal-Wallis analysis found seasonal differences, we used post-hoc Dunn's tests to test for differences between the three seasons. Before analysis, we used the Levene's test to determine that variances were significantly different for both rainfall and ambient temperature. If seasonal differences were found after the Kruskal-Wallis analysis, we performed post hoc Dunn's tests to determine which seasons experienced the highest ambient temperature and rainfall.

We compared relative abundances of the phyla present across the three seasons (winter 1, summer, winter 2) using a Kruskal-Wallis test and post hoc Dunn's tests. We assessed the seasonal presence of the most predominant microbial families by performing Kruskal-Wallis tests with post hoc Dunn's tests. We created Venn diagrams by using Venny 2.1 (Olivieros, 2015) to illustrate the unique and shared bacterial families between seasons. As there was little variation between the bacterial families present between winter 1 and winter 2, we combined the data. We used rarefaction curves to evaluate the depth of sequencing (Fig. A1). We used Analysis of Similarities (ANOSIM) to compare gut microbial abundance (the total number of bacteria present) between seasons using the *anosim* function in the "vegan" package. We tested the effects of sex and seasonal variation (winter 1, summer, winter 2) on the microbial composition using a permutational multivariate analysis of variance (PERMANOVA) using the *adonis2* function of the "vegan" package. We set the number of permutations at 9999. We used a Mann-Whitney *U* test to test for seasonal differences in bacterial diversity (the number of bacterial taxa present). We used the Kruskal-Wallis test to assess differences in the relative abundance (log₁₀ transformed) of genera between winter 1, summer, and winter 2 and Dunn's test to identify which seasons significantly differed.

We used linear mixed models (LMMs) to identify factors contributing to gut microbial composition. The fixed parameters were daily insect count, gum volume, seed count, rainfall, ambient temperature, sex, and reproductive status. We set Animal ID as a random variable to control for nonindependence of samples from the same animal. We ran the linear model by using the *lmer* function in the "lme4" package (Bates, 2010). Before modelling, we normalized the dataset by using the *preProcess* function. After assessing the variance inflation factor (VIF > 3; Zuur & Ieno, 2016), we removed ambient temperature as a fixed variable owing to collinearity. Finally, we investigated Spearman's correlations between the most abundant phyla and specific environmental factors to assess their effects on the fecal microbial community composition within galagos.

Ethical Note

We received ethical clearance to conduct the study from the Research and Ethical Sciences Committee at the South African National Biodiversity Institute's (SANBI) National Zoological Gardens (NZG; Project 18/26), the Animal Ethics Committee of the University of Pretoria (Project V037-17), and Animal Protocol Approval 181 Assurance D16-00388 from the IACUC office of the University of Colorado, which reviewed our research protocols. The authors declare there is no conflict of interest.

Data Availability Statement Data can be viewed or uploaded from the National Centre of Biotechnology Information <https://ncbi.nlm.nih.gov> by using the reference SUB13752167.

Results

Seasonal Food Availability

We captured 2414 insects from June 2017 to June 2018. The number of insects captured varied significantly with season (Kruskal-Wallis: $\chi^2 = 23.12$, degree of freedom [df] = 2, $p < 0.001$). Insects were significantly more abundant during summer (102 capture events) than winter 1 (56 capture events) and winter 2 (48 capture events) with the highest number of insects found in December 2017 and the lowest in June 2018 (Fig. 1a). Winter 2 also had significantly higher abundance of insects than winter 1 (Fig. 1a).

We found significant variation in gum availability between seasons ($\chi^2 = 14.69$, df = 2, $p < 0.0001$) with the highest gum mass in summer ($n = 69$, median = 0.5 ml, interquartile range = 0.4–0.7 ml) and the lowest in winter 1 ($n = 48$; median = 0.2 ml, interquartile range = 0.1–0.6 ml; Fig. 1b). Gum volume in summer was significantly higher than winter 2. We also found significantly lower gum volumes in winter 1 compared with winter 2 (Fig. 1b).

Most of the seeds originated from fig (*Ficus* sp) and jojoba (*Simmondsia chinensis*) fruits. We found no significant variability between seasons ($\chi^2 = 1.43$, df = 2, $p = 0.489$); however, winter 2 had the highest count of seeds present in the feces (Fig. 1c).

There was no significant difference in body mass between seasons for both males (Kruskal-Wallis: $\chi^2 = 4.34$, df = 2, $p = 0.11$) and females ($\chi^2 = 0.31$, df = 2, $p = 0.86$; Table I).

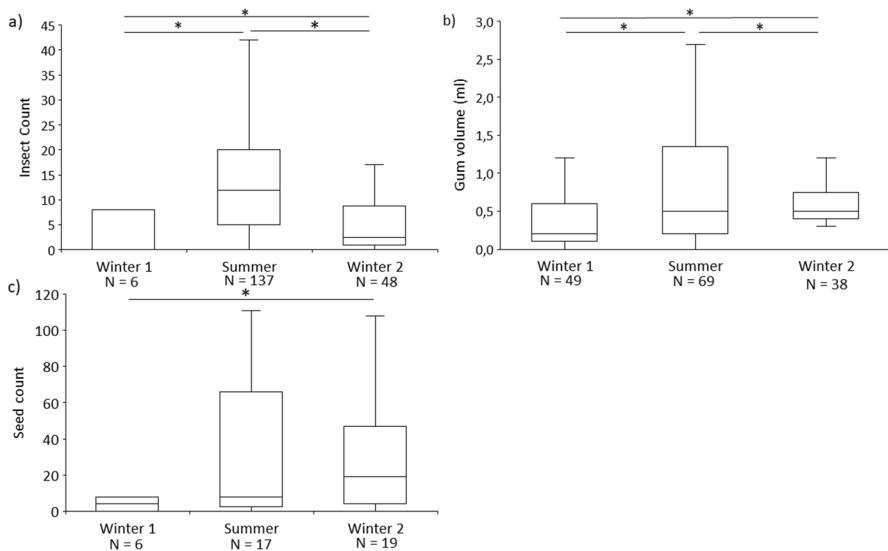


Fig. 1 Seasonal differences in **a**) insect count, **b**) gum volume, and **c**) seed count in winter 1 (June – August 2017), summer (November 2017 – February 2018), and winter 2 (May 2018 – July 2018) at Lajuma Research Centre in South Africa. Asterisks show significant differences between seasons ($p < 0.05$ based on Kruskal-Wallis analyses followed by post hoc tests). Boxplots show the median and interquartile range, whiskers show the standard deviation, and points show the outliers.

Table 1 Body mass of male and female galagos (*Otolemur crassicaudatus*) across seasons at Lajuma Research Centre in South Africa. Numbers in brackets indicate sample size

Sex	Mean body mass ± SD (g)		
	Winter 1 (June to August 2017)	Summer (November 2017 to February 2018)	Winter 2 (May to July 2018)
Male	1029.8 ± 327 (19)	1131.1 ± 165.9 (15)	1298.5 ± 109.7 (7)
Female	922.8 ± 177.7 (12)	907.9 ± 211.6 (15)	919.3 ± 219.3 (13)

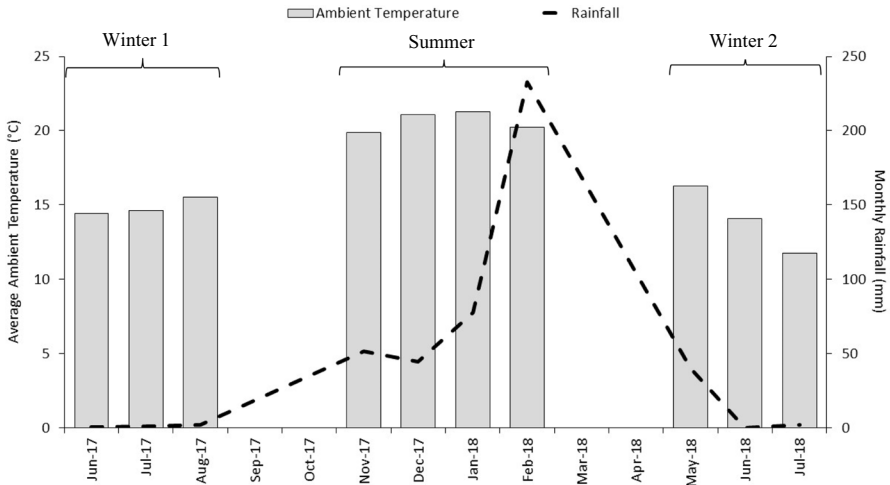


Fig. 2 Weather patterns from June 2017 to July 2018 at Lajuma Research Centre in South Africa. The line indicates the cumulative monthly rainfall (mm). Data for months September and October 2017 and March and April 2018 have been omitted, because they were not used in the analyses.

Seasonal Weather Analysis

Rainfall was significantly higher in the summer season ($\chi^2 = 7.03$, $df = 2$, $p = 0.029$; post hoc Tukey HSD: $p = 0.044$), with peak levels observed in February 2018 (mean: 8.2 mm per day; Fig. 2) compared with winter 2, which received <1 mm of precipitation per day. Summer was not significantly higher than winter 1 (mean: 1.2 mm per day; Tukey HSD: $p = 0.281$). Ambient temperatures were significantly higher in summer (range = 9.9–35.6 °C; mean = 20.58 ± 4.59 °C; $\chi^2 = 24.72$, $df = 2$, $p < 0.001$) than in winter 1 (range = 4.4–29.6 °C; mean = 14.85 ± 0.59 °C; Tukey HSD: $p < 0.001$) and winter 2 (Tukey HSD: $p < 0.001$; Fig. 2).

Diversity of Bacterial Taxa

We sequenced 75 fecal samples. After excluding samples with low read numbers, we used 49 samples in analyses, from 15 adult males and 16 adult females (mean: females = 1.38 ± 0.62 ; males = 1.40 ± 0.83 samples from each individual). In total,

we identified 17,269 sequences, ranging from 100–376 bp, and 295 unique OTUs with a 97% sequence identity threshold. We detected 12 phyla and 53 families in the fecal samples. We identified all taxa to at least family level, 136 taxa to genus level, and 72 to species level. Neither season (PERMANOVA: $F = 0.984$, $R^2 = 0.021$, $p = 0.444$) nor sex ($F = 0.593$, $R^2 = 0.013$, $p = 0.695$) had a significant effect on the diversity of the bacterial populations in the galago feces.

At phylum level, the fecal microbiome of the galago population was dominated by Actinobacteria (65%), Bacteroidetes (15%), Firmicutes (14%), Proteobacteria (3%), with unclassified (>1%; Fig. A2). We detected several other phyla, including Cyanobacteria, Fusobacteria, Planctomycetes, and Tenericutes, which represented less than 1% of the total taxa present. We detected ten phyla in samples from winter 1 and winter 2 and eight in samples from summer (Fig. 3).

The relative abundance of Actinobacteria significantly differed between all seasons (winter 1 = 6%, summer = 69%, winter 2 = 82%; Kruskal-Wallis: $\chi^2 = 47.08$, $df = 2$, $p < 0.001$). Winter 2 had a significantly higher relative abundance of Actinobacteria than summer and winter 1 (post hoc Dunn's test: $p < 0.001$) and made up 82% of the total bacterial population. Summer had a significantly higher relative abundance of Actinobacteria than winter 1 (Dunn's test: $p < 0.001$). An inverse relationship was present between Bacteroidetes and Firmicutes; with Bacteroidetes increasing in summer (winter 1 = 4%, summer = 19%, winter 2 = 7%) and Firmicutes relative abundance highest in winter 1 (winter 1 = 44%, summer = 5%; winter 2 = 4%); however, season did not have a significant impact on the abundance of Bacteroidetes ($\chi^2 = 1.90$, $df = 2$, $p = 0.387$). Firmicutes showed significant differences between all seasons ($\chi^2 = 52.96$, $df = 2$, $p < 0.001$; Fig. 3).

The most prominent families identified were Bifidobacteriaceae (62%), Prevotellaceae (13%), Clostridiaceae (6%), and Micrococcaceae (3%; Fig. A3). Overall,

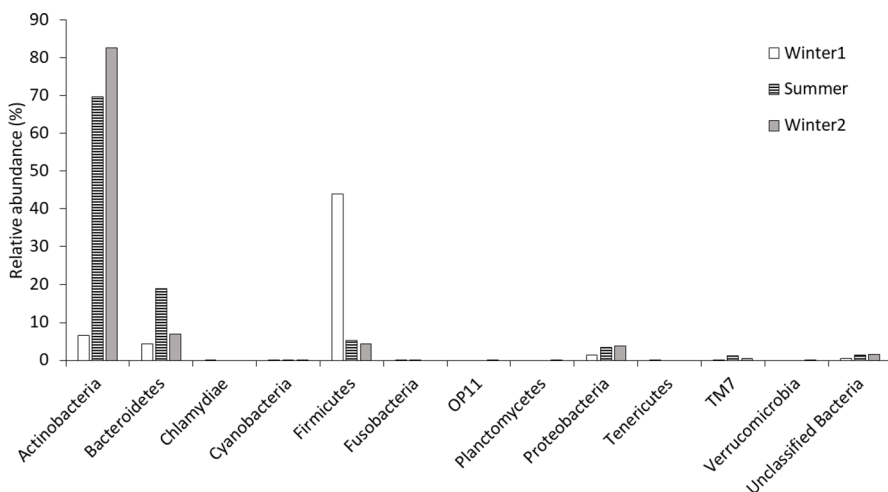


Fig. 3 Relative abundance of phyla in the feces of galagos (*O. crassicaudatus*) in summer ($n = 25$) and winter ($n = 24$) collected at the Lajuma Research Centre in South Africa. Data are mean \pm standard deviation.

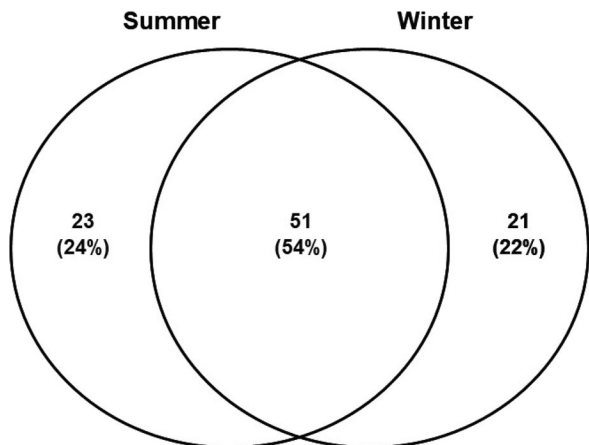
galagos share 51 bacterial families between seasons. Bifidobacteriaceae was the most dominant family for each individual throughout each sampling period (Fig. A4). In summer, we observed 23 families, including Neisseriaceae, Gemellaceae, and Rhodospirillaceae; in winter, we observed 21 families, including Peptostreptococcaceae, Turicibacteraceae, and Lactobacillaceae (Fig. 4; Table A1).

The most abundant genera comprised >85% of the total. *Bifidobacterium* (55%), *Prevotella* (14%), *Clostridium* (6%) were the most dominant genera detected with lesser contributions from *Staphylococcus*, *Arthrobacter*, *Gardnerella*, and unclassified genera of Proteobacteria, Micrococcaceae, and Bacteria (Fig. 5). *Bifidobacterium* bacteria increased significantly during summer (Kruskal-Wallis: *Bifidobacterium*: $\chi^2 = 23.66$, $df = 2$; $p < 0.001$) compared with both winter 1 (Dunn's test: $Z = 4.79$; $p < 0.001$) and winter 2 ($Z = 0.78$; $p < 0.001$). *Prevotella* also significantly increased in summer ($\chi^2 = 22.02$, $df = 2$; $p < 0.001$) compared with the relative abundance in winter 1 ($Z = 4.70$; $p < 0.001$) and winter 2 ($Z = 1.78$; $p < 0.001$), while unclassified Micrococcaceae decreased in summer ($t = 2.81$, $df = 2$; $p = 0.011$). *Clostridium* bacteria were only present in the winter 2 samples, whereas *Gardnerella*, unclassified *Micrococcaceae*, and *Staphylococcus* were absent from the winter 2 samples altogether.

In winter, the most prominent species found in females were *Bifidobacterium* sp. (50%), *Clostridium perfringens* (10%), and *Prevotella* unclassified (3%). In the males, the most prominent species were *Bifidobacterium* sp. (19%), *Clostridium perfringens* (17%), and *P. ruminicola* (5%). In summer, the most abundant species in both sexes were *Bifidobacterium* sp., *Prevotella* sp., and *P. ruminicola*.

The LMM we fitted to explore which predictors influenced bacterial composition had moderate variation (marginal $R^2 = 0.26$). It showed that bacterial composition was most influenced by insects ($\beta = 2.62$, standard error (SE) = 6.40, $t = 0.41$), gum ($\beta = -3.33$, SE = 10.23, $t = -0.33$), rainfall ($\beta = -21.56$, SE = 42.56, $t = -0.51$), and seeds ($\beta = 0.68$, SE = 0.82, $t = 0.82$). We found significant effects of rainfall, insects, gum, and seeds (Table II). Reproductive status showed no significant effect ($\chi^2 = 1.12$, $df = 4$, $p = 0.89$; Table II). Ambient temperature, gum mass, and insect

Fig. 4 Venn diagram demonstrating shared and unique bacterial families found in galagos (*O. crassicaudatus*) during summer and winter in the Lajuma Research Centre in South Africa. The top number is the number of unique families. The number in brackets is the percentage of the total families unique to the season.



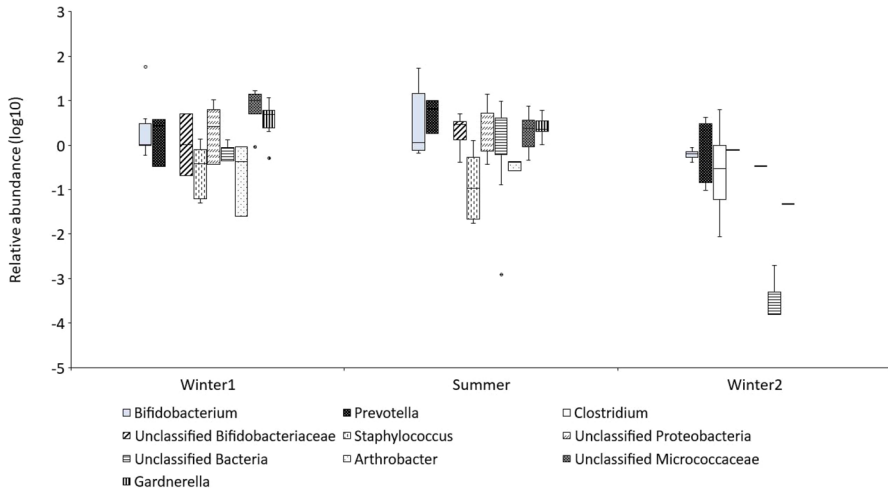


Fig. 5 Seasonal differences in the relative abundance (log₁₀ transformed) of the ten most abundant genera in the fecal microbiome of galagos (*O. crassicaudatus*) at Lajuma Research Centre, South Africa, between June 2017 and July 2018. Lines show the median, boxes the interquartile range, whiskers the standard deviation, and points outliers.

availability were positively correlated with Proteobacteria (Fig. 6). Rainfall and seed count were negatively correlated with Bacteroidetes (Fig. 6b, d).

Discussion

We found that the fecal microbial abundance of galagos is dominated by a small selection of bacterial genera; however, we can see the effects of seasonal and dietary changes as several taxa are able to thrive in a certain season while not in another. We found seasonal changes in ambient temperature and rainfall, which matched our findings that insects and gum volumes were more abundant in summer but less so in winter. Our findings are not fully consistent with the

Table II Results of a linear mixed model of the predictors driving variation in fecal bacterial composition of galagos (*Otolemur crassicaudatus*) at Lajuma Research Centre, South Africa, from May 2017 to July 2018

Predictors	β	SE	χ^2	p	CI (2.5%; 97.5%)
Intercept	421.14	251.91			-135.01; 977.33
Rainfall	-21.56	42.56	14.46	0.02	-115.53; 72.40
Insects	2.62	6.40	14.55	0.02	-11.52; 16.76
Gum	-3.33	10.23	14.61	0.02	-25.91; 19.25
Seeds	0.68	0.82	14.05	0.03	-1.14; 2.49
Reproduction-			1.12	0.89	
Mating	-146.58	244.64			-686.68; 393.53
None	-72.53	191.80			-495.99; 350.93
Lactating	270.22	215.52			-205.61; 746.04

hypothesis that changes in season (dietary and environmental) influence changes in the galago fecal microbiome. However, our results might indicate the plasticity of the microbiota in galagos to cope with seasonal changes. First, we found significant effects of rainfall, insects, and gum on the bacterial composition. Interestingly, we found no significant seasonal differences in body mass. Second, we did not find significant differences in bacterial abundance and diversity between seasons. *Bifidobacterium* was the most dominant genus throughout the study period. Nevertheless, we did identify several unique bacterial taxa present in each season, such as *Clostridium*. We found an inverse relationship between the population of Firmicutes and Bacteroidetes in seasons, whereby there was a lower abundance of Firmicutes in summer while Bacteroidetes thrived as a phylum.

The lack of significant variation in body mass between seasons may be linked to the consistent food reserves of gum and fruit during winter to sustain the galago population. The galago population also may reduce their activity in winter to preserve energy levels. Testing this hypothesis will require data on their activity budgets across seasons.

Rainfall was an important driver of the galago microbiome composition. This pattern is similar to that in geladas (*Theropithecus gelada*; Baniel et al., 2021), where increased rainfall had a positive effect on the presence of cellulolytic degraders necessary to breakdown cell walls and fermenters (such as *Prevotellaceae* and *Bacteroidales*) required for the breakdown of polysaccharides into simple sugars (Flint et al., 2008). We observed a significant increase in *Prevotella* during summer. *Prevotella*, which is important in carbohydrate metabolism, is associated with a carbohydrate and plant-based diet (Wu et al., 2011; Amato et al., 2014; Kovatcheva-Datchary et al., 2015; Gorvitovskaia et al., 2016). High densities of *Prevotella* are found in primate species, which have a largely plant-based diet, such as the common marmoset (*Callithrix jacchus*; Sheh et al., 2012) and golden snub-nosed monkey (*Rhinopithecus roxellanae*; Zeng et al., 2022). It is possible that the increase in *Prevotella* was a result of a shift to a high-fat and -sugar diet of insect and gum. As the presence of *Prevotella* is linked to carbohydrate consumption and glucose metabolism, the high numbers of this genus in the galago population are possibly linked to the nondigestible food matter that can be broken down by *Prevotella* to allow for galagos to extract nutrients.

Firmicutes, which are associated with producing high-energy SCFAs (Schleifer, 2009), increase in relative abundance when individuals increase their dietary dependence on fruits. In contrast, Bacteroidetes abundance increases when fiber consumption increases and the consumption of high-glycemic index sugars decreases (De Filippo et al., 2010; Ley, 2010; Wu et al., 2011; Nagpal et al., 2018). The increase in Firmicutes densities in this study occurred during the cold, winter months when fruit consumption increased. This increase may lead to a rise in SCFA production (Houtman et al., 2022; Kapoor et al., 2023). An increase in Bacteroidetes in summer may occur in response to an increase in insect consumption and the need to digest hard exoskeletons (Wu et al., 2011; Rothman et al., 2014). In winter, we also saw an increase in the phylum Firmicutes. The ratio of Firmicutes to Bacteroidetes we observed may lead to increased fermentation functionality. Such functionality, which would improve energy

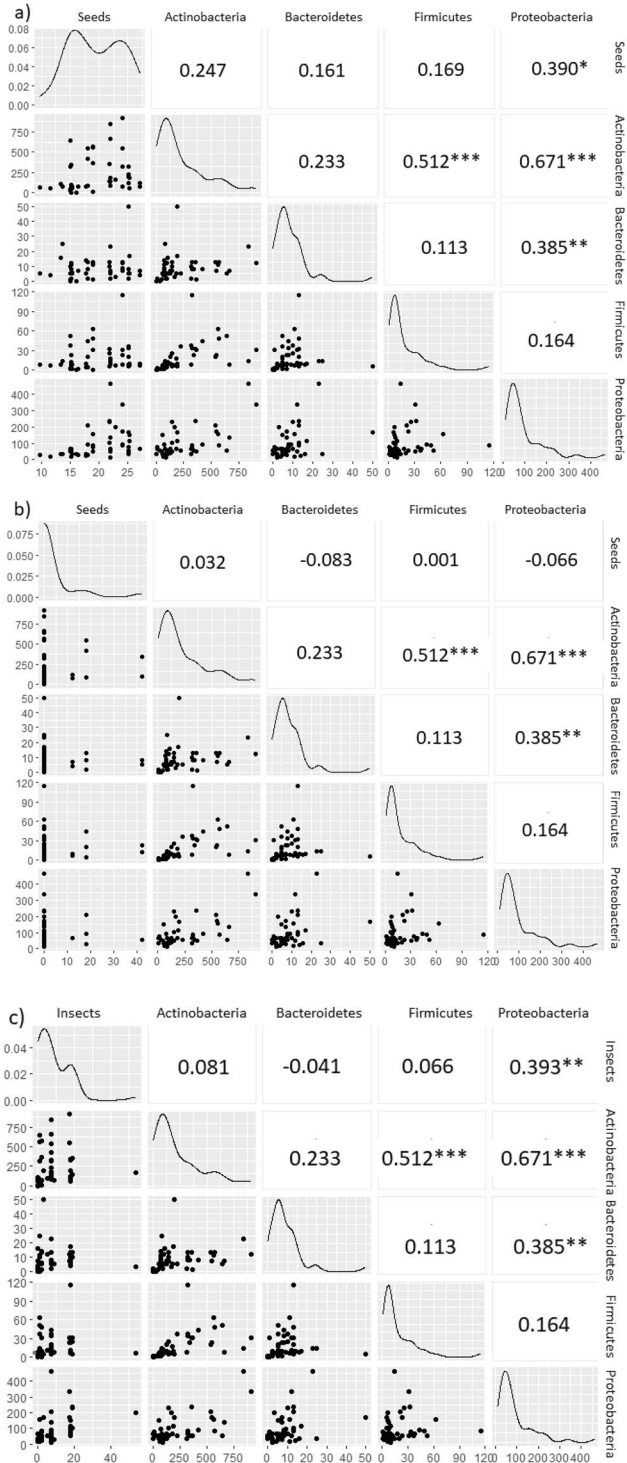
Fig. 6 Correlations between the most abundant phyla (Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria) in the fecal microbial community and environmental factors. **a)** ambient temperature, **b)** rainfall, **c)** insect availability, **d)** seed count, **e)** gum volume, in galagos (*Otolemur crassicaudatus*) sampled at Lajuma Research Centre in South Africa between June 2017 and July 2018. Each element of the matrix shows the correlation coefficient (the number situated in the blank box), a scatter plot and line graph of plotted variables. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

production and has been noted in wild, black howler monkeys (*Alouatta pigra*; Amato et al., 2014) and Javan lorises (*Nycticebus javanicus*; Cabana et al., 2018).

A major role of the anatomical and physiological aspects of the galago's gut and the major taxa characterized in the fecal microbiome is to ferment food matter (Nash, 1986; Langer & Clauss, 2018). Gut fermentation is a critical mechanism in several galago species that allows for the breakdown and absorption of nutrients from low-quality and difficult-to-digest foods that are necessary for survival in the less ideal conditions of winter (Nash, 1986). Due to the high-energy demands placed on galagos in winter, when temperature and resource quality are low and mating activity occurs, it is important that gut microbiome health is optimized to ensure nutrient and energy absorption. Fermentation spaces are present in the foregut of *G. moholi* and galagos to elongate the gut passage time and increase the volume of food matter broken down (Caton et al., 2000). The phylum Firmicutes, the family Bifidobacteriaceae, and the genus *Clostridium* are primary fermenters and may contribute to the fermentation abilities in the gut of galagos. Interestingly, *Clostridium* is only present in winter. *Clostridium* can cause harm when an individual's health is compromised (McClane et al., 2012). *Clostridium perfringens*, the most common species found in the fecal gut microbiota of galagos in this study, is an opportunistic pathogen that can form part of the mucosa lining in the gastrointestinal tract (Pluvinage et al., 2019). During the study, we found two animals with dental pathologies in winter, which may be associated with the presence of *Clostridium* during this season.

The presence of the genus *Clostridium* in winter, while absent in summer, could suggest a role in fermentation and the breakdown of cellulose and dietary fiber, as in Tibetan macaques (*Macaca thibetana*; Sun et al., 2016). *Clostridium* is commonly found in decaying vegetation, soil, and plays a role in the fermentation of polysaccharides. Similarly, an increase in *Clostridiales* and a decrease in *Bacteroides* was determined in rats (*Rattus norvegicus*) fed fruit pectin (Licht et al., 2010). We found a slight increase in unclassified Proteobacteria genera in winter. As Proteobacteria plays a role in energy acquisition, this increase may be a coping mechanism during cold weather when energy loss is inevitable (Koren et al., 2012; Amato et al., 2014).

Unlike in most other primate host species, such as black howler monkeys (Amato et al., 2014) and ring-tailed lemurs (*Lemur catta*; Bennett et al., 2016), *Bifidobacterium* was the most abundant genus in galagos over the entire sampling period. *Bifidobacterium* is a commensal genus that plays a key role in carbohydrate metabolism and also is beneficial to the host health by protecting the gut mucosal lining from harmful bacteria (Pinzone et al., 2012; Ghouri et al., 2014; Turrone et al., 2014). *Bifidobacterium* also has been associated with the degradation of arabinogalactan (Crociani



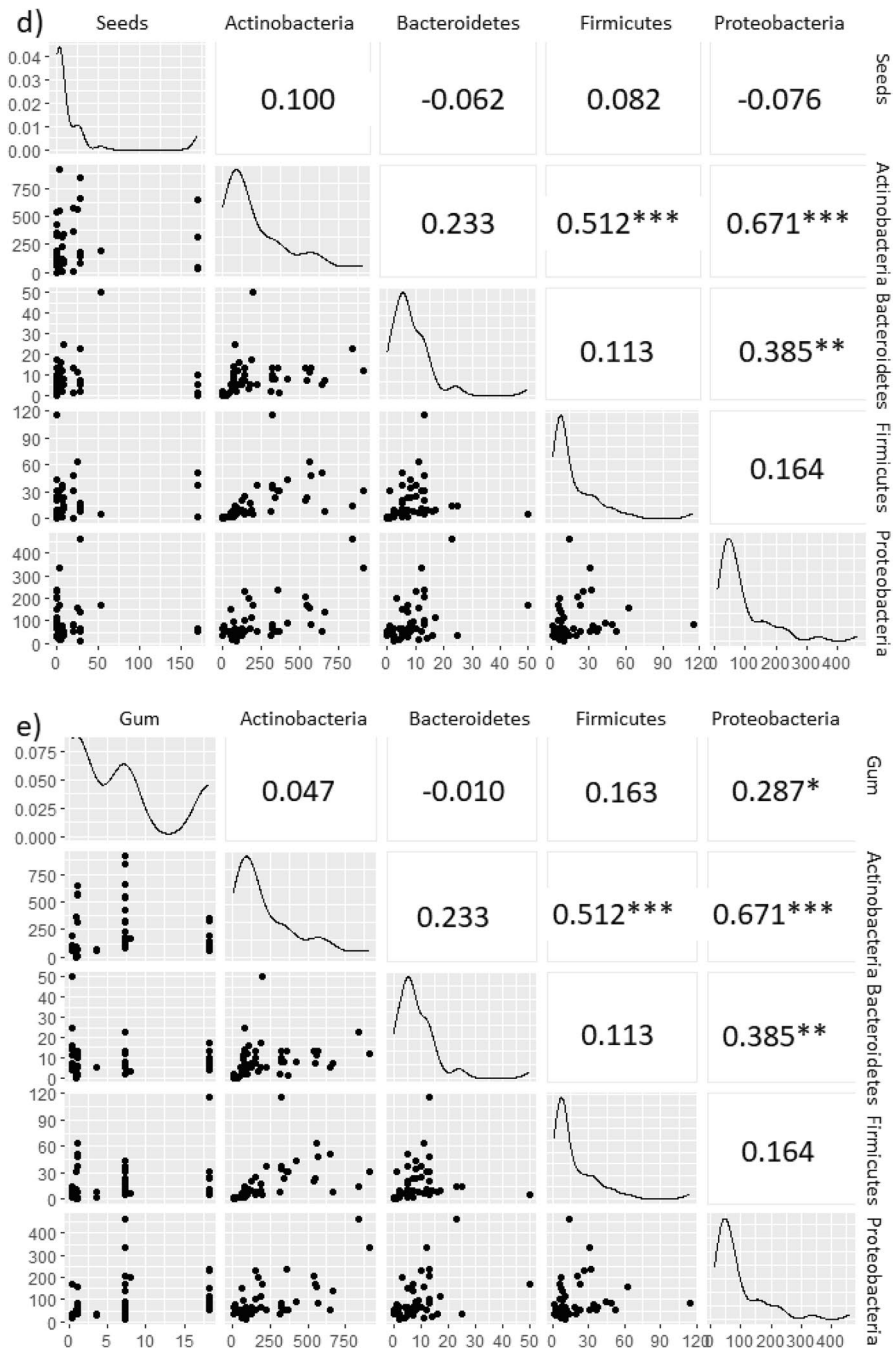


Fig. 6 (continued)

et al., 1994; Grieshop et al., 2002; Bornbusch et al., 2019; Long, 2018) and was abundant in the fecal microbiome in the lesser galago (*G. moholi*; Long, 2018) during the winter when gum exudates were the main food source. Certain genes in the genome of *B. aesculapii* are necessary for the breakdown of β -arabinans in gum exudates in *Acacia enegal* trees that common marmosets (*Callithrix jacchus*) eat (Zhu et al., 2021). *Bifidobacterium* also was prevalent in the fecal microbiome of exudativorous emperor tamarins (*Sanguinus imperator*; Duranti et al., 2017). A deeper assessment of the function of the fecal microbiome may provide more insight about whether *Bifidobacterium* also plays a functional role in the degradation of gum in galagos.

Conclusions

We assessed the gut microbial community in thick-tailed greater galagos, nocturnal primates living in a temperate environment and depending on food resources generally found in low quantities. Our results show the variation and versatility in the microbial community in the gut of galagos and their symbiotic relationship with their host. The microbiota appears to be able to shift with the changes in diet to allow the galagos to gain the nutrients they need from their food resources. Food availability in the study site appears to be influenced by rainfall. With climate change progressing, this may lead to decreases in rainfall, which may weaken the food resources currently available to galagos. Approaches, such as shotgun metagenomics coupled with more refined characterization of bacterial diversity and metabolic function, would further enhance our understanding of the relationship between diet and microbial communities (Troci et al., 2022). Overall, by identifying the gut microbial profile for galagos, this study provides insight into the ecology and the relationship between host and microbiome and demonstrates the importance of this relationship during changes in the environment.

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