Faecal *n*-alkanes differ significantly between two lemur species reflecting differences in consumed diet

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Abstract

The diet of an animal reflects its species' ecology and local food availability and is often a key metric for monitoring the health and welfare of endangered species. However, determining diets across individuals and through space and time, is an inherent challenge within ecology, being expensive and time-intensive to accomplish with observations. Faeces offer the opportunity for non-invasive sample collection and can provide a snapshot of the ingested diet of the producer. In modern ecology, faecal samples have been a prime target for genetic analyses of diet and the gut microbiome. Here, however, we explore the efficacy of using high molecular weight (HMW) *n*-alkane biomarkers extracted from faeces as dietary tracers. HMW *n*-alkanes are commonly employed in palaeoecological reconstructions. Combining faecal HMW *n*-alkane analyses with gut microbiome composition and detailed foraging data applied to two species of lemur in captivity with access to naturalized forest enclosures, we assess the potential of HMW *n*-alkane profiling in dietary analyses, and therefore modern ecology. Recovered HMW *n*-alkanes are completely dietary in origin and not degraded or influenced by the gut microbiome. Signatures are significantly different between focal lemur species and seasons, reflecting differences in ingested diet. It is possible to infer changes in the relative contributions of major food types, like leaves and fruits, based on the faecal HMW *n*-alkane concentrations. The potential applications for these methods in both captive and wild lemur populations, other primates, and other herbivorous and omnivorous animals are wide and varied. HMW *n*-alkanes are simple to identify and measure; only requiring commonly available and relatively affordable analytical chemistry instruments (e.g. GC-FID). These analyses could open a wide array of modern ecological research possibilities with further research and ground truthing.

Key words

Diet analysis, leaf wax biomarker, *n*-alkane, ring-tailed lemur, Coquerel's sifaka

1. Introduction

The diets of wildlife species reflect their ecological niche and local food availability within a particular habitat (Atsalis, 1999, Curtis, 2003). Thus, dietary analysis has been used extensively to monitor the health and welfare of wildlife species, especially those that are living in threatened habitats (Balko and Underwood, 2005, Beeby and Baden, 2021, Milner et al., 2021). Despite this, the ability to monitor the long-term diets of animals remains inherently challenging. Traditional methods based primarily on behavioural observation are often labour- and time-intensive and therefore expensive to carry out. Such methods also have inherent weaknesses, including observer bias and competence, and the inability to consistently watch the subject animals, especially through the night, in difficult weather conditions and across seasons (Atsalis, 1999). This is made increasingly difficult by unhabituated animals and diverse habitats where plant identification requires extensive background knowledge of the local flora (Holechek et al., 1982).

Studies have shown that analyses of the faecal matter of animals presents a valuable alternative to observational data, with enormous potential in collecting dietary information (Codron et al., 2005b, Kristensen et al., 2011, Murray et al., 2011, Srivathsan et al., 2014, Montanari, 2017, McDermott, 2020). Faeces are a by-product of everyday life and therefore, if collected after voiding, will not impact the daily activities of wildlife and could provide a snapshot of their diet. Unlike behavioural observation, faecal analysis is a non-invasive, potentially inexpensive and less labour-intensive method for dietary analyses. Moreover, it negates the need to rely wholly on labour-intensive and often expensive observational data, which can involve a lot of inaccuracies even with extensive knowledge of the local plant community and well-habituated focal animals which remain within eyesight (Altmann, 1974, Holechek et al., 1982, Nash, 1998, Atsalis, 1999). Therefore, non-invasive faecal sampling could be a particularly valuable asset in research

77 surrounding unhabituated populations, cryptic species and those at risk of extinction (Aylward
78 et al., 2018).
79 To date, faeces have already been shown to have potential in genetic and genomic studies for
80 dietary reconstructions (Bradley et al., 2007, Aylward et al., 2018, Chua et al., 2021, Milner et al.,
81 2021, Rowe et al., 2021), and to determine the gut microbiome, which can also reflect diet (Brice
82 et al., 2019). Yet, techniques established within palaeoclimate, archaeology and agricultural
83 studies, such as lipid biomarkers, remain unexplored within modern ecology. Lipid biomarkers
84 are persistent in the natural environment, produced widely by a range of organisms and are
85 considered recalcitrant. *n*-Alkanes, for example, are a saturated straight-chain hydrocarbon
86 biomarker which display an odd-over-even carbon number preference (Eglinton and Eglinton,
87 2008, Killops and Killops, 2013). High molecular weight (HMW) *n*-alkanes are produced as part
88 of the protective waxy cuticle on the surface of the leaves and fruits of higher plants and are
89 abundant in most biomarker archives (Sachse et al., 2006, Eglinton and Eglinton, 2008). They will
90 therefore be in almost everything a herbivorous animal consumes and are highly unlikely to be
91 altered by the process of digestion given that they are used extensively in palaeoclimate studies
92 to reconstruct climates and vegetation millions of years in the past (Feakins et al., 2005, Sachse
93 et al., 2006, Sachse et al., 2012, Callegaro et al., 2018). They have also been applied in agricultural
94 and archaeological dietary reconstructions (Lichtfouse, 2000, Evershed, 2008, Gill et al., 2009,
95 Vazquez et al., 2021).
96 The lemur (Lemuroidea) superfamily is the most endangered group of mammals in the world
97 (Quéméré et al., 2013, Razafindratsima, 2014, Schwitzer et al., 2014, Schüßler et al., 2018,
98 Estrada et al., 2017), with 94% of species being currently listed as threatened or vulnerable by
99 the IUCN (IUCN, 2024). Lemurs are also the most diverse primate group, consisting of at least
100 100 different species with a diverse array of feeding and life habits (Wright, 2006, Albert-Daviaud
101 et al., 2020). Some species are also routinely maintained in captivity for research and

conservation purposes. Determining the diets of wild lemurs in various habitats has been a longstanding goal of ecologists (Atsalis, 1999, Britt, 2000, Curtis, 2003, Powzyk and Mowry, 2003, Balko and Underwood, 2005, Ratsimbazafy, 2006, Quéméré et al., 2013, Sato et al., 2016, Aylward et al., 2018) with implications for conservation and captive animal welfare, but given the diversity within the group, it is difficult to apply methods like behavioural observation widely. The development of a new tool for monitoring diet could have a lot of potential within conservation monitoring and animal husbandry and could greatly increase our knowledge of this important group of mammals. These techniques need not be restricted to lemurs but could be applied to any animal which reliably consumes plant matter.

Given the widespread use of lipid biomarkers within geology and archaeology, this technique presents great promise within modern ecology. This study will assess the potential of these techniques through a pilot study on captive animals from which we have parallel dietary data from observations and complementary gut microbiome data. This allows for an accurate assessment of these techniques within an ecological context.

Here, we assess the faecal *n*-alkane signatures of two lemur species in captivity: ring-tail lemurs (*Lemur catta*) and Coquerel's sifaka (*Propithecus coquereli*). Ring-tailed lemurs are omnivores (Sauther et al., 1999) and sifakas are frugo-folivores (McGoogan, 2011, Sato et al., 2016), although in Madagascar, both species primarily eat a plant-based diet that varies with season and local availability. The Duke Lemur Center (DLC) in Durham, North Carolina (USA), maintains large populations of both species and allows select groups to gain access to large, multi-acre forested enclosures from spring-fall where they forage *ad libitum* on local and seasonal vegetation in addition to provisioned dietary items (Greene et al., 2022a, Greene et al., 2024). By profiling faecal *n*-alkane and gut microbiome profiles, along with foraging behaviour from lemurs with forest access, we test if faecal *n*-alkanes reflect species and seasonal differences in the lemurs' diets, with no influence from the gut microbiome.

2. Materials and Methods

2.1 Subject Animals

The subject animals included 12 healthy adult captive lemurs representing 2 lemur species; Coquerel's sifakas (*Propithecus coquereli*; 6 individuals in 3 social pairs that variably included their offspring), and ring-tail lemurs (*Lemur catta*; 6 individuals in 3 social pairs that variably included their offspring) housed in Natural Habitat Enclosures (NHEs) at the DLC. All subjects, their social pairs and corresponding NHEs are listed in Table 1. NHEs are large enclosures (0.5 – 6.6 ha) of natural North Carolina Duke Forest (Greene et al., 2022a). At the time of study, lemurs were completely managed in NHEs but had access to indoor housing units or shelters. All individuals were recognisable by unique features; minimally one animal per social pair also bore a radio collar which could be tracked using standard telemetry. Sifakas in NHEs are offered a once-daily provisioned diet of fibrous chow, nuts or beans, orchard vegetables, leafy greens, and local browse; ring-tailed lemurs in NHEs are offered a primate chow daily supplemented with orchard fruits and vegetables twice weekly. Lemurs are free to forage on local vegetation ad libitum (see Supplementary Material for more information). Fresh water is freely available and changed daily.

2.2 Sample Collection

Samples were collected during spring (May – June) and summer (August – September) in 2020 in conjunction with foraging and ranging observations (Greene et al., 2022a, Greene et al., 2024). Each social group was observed for 3 consecutive days per season during all-day follows from dawn to dusk. During focal follows, faeces from focal lemurs were collected within 5 minutes of defecation throughout the day. Samples were scooped from the forest floor into sterile tubes or bags, placed immediately on ice packs, and frozen at -80°C within 2 hours.

Large samples were then aliquoted for *n*-alkane and microbiome profiling. Regarding the former, a portion of each sample was dehydrated to remove the moisture before being stored at -80°C.

A total of 168 faecal samples (92 *L. catta* and 76 *P. coquereli*) were usable for *n*-alkane profiling and were shipped under license to the University of Birmingham, UK on dry ice and stored at -20°C until analysis.

For more details regarding behavioural observation and microbiome profiling see Supplementary Material.

2.3 *n*-Alkane Analysis

All organic sample preparation and analyses were carried out at the University of Birmingham Molecular Climatology (BMC) laboratory using high performance liquid chromatography (HPLC) grade organic solvents.

In brief, samples were dehydrated and ground into a fine powder before being extracted by ultrasonicating 3 times with 10 ml dichloromethane:methanol in a 9:1 (v/v) ratio to generate a total lipid extract (TLE). Column chromatography was used to separate the TLE into 4 fractions, of which the first, eluted with *n*-hexane, contained aliphatic hydrocarbons, ie. the *n*-alkane targets of this study.

n-Alkanes were subsequently analysed using an Agilent 7890B GC-FID to determine the concentrations with an external *n*-alkane standard of known concentration (C_{10}-C_{40} even-chained *n*-alkanes, Sigma Aldrich). The front inlet and back detector were held at 330°C throughout. Compressed air was the carrier gas. An Agilent DB1 column (60 m length, 0.25 mm internal diameter, 0.25 μm film thickness) with a 100% dimethylpolysiloxane stationary phase was used. The oven temperature programme held at 70°C for 1 minute, before ramping to 130°C at 30°C/min, then to 320°C at 4°C/min, holding for 10 minutes, for a total runtime of 60.5 minutes. The variability of dominant *n*-alkanes (average chain length, ACL) was calculated using the concentrations of the leaf wax derived odd-chain *n*-alkanes with chain lengths of 21-35 carbon atoms, as these were the most abundant *n*-alkanes in all samples (Equation 1) (Sankelo et al.,

2013). Where X_n represents the carbon number, and C_n represents the abundance of the *n*-alkane for C_{21} – C_{35}.

$$ACL = \frac{\sum X_n C_n}{\sum C_n}$$

[1]

The carbon preference index (CPI) is a numerical representation of the odd-over-even predominance of the *n*-alkanes, in this case the concentration of chain lengths of 25-35 carbon atoms (Equation 2) (Bray and Evans, 1961, Marzi et al., 1993, Killops and Killops, 2013).

$$CPI = \frac{\left[\Sigma C_{odd(C25-33)} + \Sigma C_{odd(C27-33)}\right]}{2\Sigma C_{even(C24-C34)}}$$

[2]

2.4 Statistical Analysis

Data were analysed using R Studio version 4.4.1. The vegan (Oksanen et al., 2025) and ape (Paradis and Schliep, 2019) packages were used to perform Kruskal-Wallis chi-squared tests, principal coordinate analysis (PCoA), on which analysis of variance (ANOVA) tests were carried out. Levels of significance are represented in the text as *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$, unless the result was statistically insignificant ($p > 0.05$).

3. Results

3.1 Faecal *n*-Alkane Signatures

Analysis by GC-FID identified 24 *n*-alkanes from C_{14} to C_{37} within *Lemur catta* and *Propithecus coquereli* faecal samples. The samples displayed an odd-over-even carbon number preference as is typical of plant wax *n*-alkanes (Dove, 1992, Eglinton et al., 1962), with the C_{29} and C_{31} homologues dominating (Figure 1). The number of *n*-alkanes within each sample (*n*-alkane richness) varied only marginally from 22 to 24, and ACL similarly varied only slightly from 29.35 to 28.78. Contrastingly, the CPI varied quite significantly from 3.65 to 53.25, however neither *n*-alkane richness, ACL nor CPI showed a significant difference between lemur, species, or season.

Faecal *n*-alkane concentrations (total ng/g) were significantly different between the two lemur species (p= 0.015*), with *L. catta* concentrations averaging 2.2 times greater than those of *P. coquereli* for the two most abundant homologues (C_{29} and C_{31}). For both species, the total ng/g was significantly different between spring and summer (*L. catta*, p= 0.0089**; *P. coquereli*, p= 0.0038**; Figure 2a). Both dominant *n*-alkane homologues varied significantly in concentration between the individual species (Figure 2b, c; p= 4.48×10^{-9}*** and p= 9.13×10^{-8}***, respectively). The ratio of HMW (C_{27} + C_{29} + C_{31}) to LMW (low molecular weight; C_{15} + C_{17}) *n*-alkanes did not vary significantly between the species (p= 0.12). When regarded individually, neither species displayed a significant difference in the concentration of the C_{29} or C_{31} *n*-alkanes between spring and summer (Figure 2b, c), or the ratio of HMW to LMW *n*-alkanes (*P. coquereli*: p= 0.51, *L. catta*: p= 0.069).

Principal coordinate analysis (PCoA) carried out on a Euclidean matrix of the *n*-alkane concentrations highlighted the species differences in faecal *n*-alkane signatures (Figure 3a), and analysis of variance (ANOVA) indicates that this was significant (p= 0.014*). There was, however, no significant influence of individual lemur (p= 0.087) or season (p= 0.091) on matrix variation. When regarding only *L. catta* (Figure 3b), there was no significant influence of either individual lemur (p= 0.29) or season (p= 0.29) on *n*-alkane variation. Contrastingly, *P. coquereli* (Figure 3c) showed a significant influence of individual lemur (p= 0.0015**), but no influence of season (p= 0.060) on *n*-alkane variation.

Clearly, faecal *n*-alkanes vary significantly by lemur species. There is also a weak influence of both seasonal and individual variation on faecal *n*-alkanes.

3.2 Faecal *n*-Alkanes and Gut Microbiome Composition

Gut microbiome profiles varied significantly between the two lemur species at both the phylum- (p< 2×10^{-16}***) and genus-level (p< 2×10^{-16}***; Figure S4). Alpha diversity indices of gut microbiome composition (taxonomic richness, Shannon entropy, Faith's phylogenetic diversity)

225 reliably demonstrated a strong variation between *Lemur catta* and *Propithecus coquereli*, as well
226 as a strong seasonal trend (Table S3). *n*-Alkane compositions also varied significantly by lemur
227 species (Figure 3). It is therefore essential to determine if the faecal *n*-alkane and gut microbiome
228 datasets covary to assess the influence of the gut microbiome on faecal *n*-alkane signatures.
229 To test for covariation, *n*-alkane indices (n-alkane richness, CPI and ACL) were compared with the
230 gut microbiome alpha diversity indices. The resultant linear relationships are summarised in
231 Table S4. In general, no significant trends were observed between the *n*-alkane indices and gut
232 microbiome alpha diversity, regardless of lemur species or season.
233 Similarly, the influence of the gut microbiome alpha diversity indices on the *n*-alkane PCoA
234 (Figure 3) were also investigated. It was found that neither taxonomic richness nor Shannon
235 entropy had an influence on n-alkane variation (p= 0.31, p= 0.52, respectively), but that Faith's
236 phylogenetic diversity did have an influence on *n*-alkane variation (p= 0.0014**).
237 By the same token, the influence of *n*-alkane richness, CPI and ACL on the phylum- and genus-
238 level gut microbiome PCoA (Figure S4) was investigated and none were found to be significant
239 (Table 2). Additionally, mantel tests comparing both the phylum- and genus-level gut microbiome
240 matrices with the *n*-alkane matrix showed that neither microbiome matrices vary in the same
241 way as the *n*-alkane matrix (r= 0.020, p= 0.29 and r= 0.067, p= 0.06, respectively).
242 These data combined imply that the faecal *n*-alkane signatures and gut microbiome composition
243 vary independently from one another and are not linked.

244 **3.3 Faecal *n*-Alkanes and Foraging Patterns**

245 Both lemur species spent considerable time in their forest enclosures foraging for a frugo-
246 folivorous diet (Figure S1). They had access to a whatever local plant species and plant parts they
247 could find within their forest enclosures, and foraged not only in the tree canopy, but also on the
248 forest floor. A significant difference in foraging patterns (including plant species and plant part)

was found between lemur species ($p < 2x10^{-16}$***), seasons ($p < 2x10^{-16}$***), and individual lemur ($p = 5.11x10^{-12}$***).

Observed foraging patterns vary considerably by lemur species, season and individuals, and therefore it is necessary to consider if these are the driving force behind the observed *n*-alkane variation. In fact, it should be possible to broadly determine if we can track the contribution of different food items in the diet through their faecal *n*-alkane compositions.

The *n*-alkane PCoA (Figure 3) was significantly influenced by the proportion of time spent feeding on leaf matter and the proportion of time spent feeding on fruit matter, both independently (p= 0.0065** and p= 0.0056**, respectively), and when combined (p= 0.00010***). A negative correlation was found between the combined proportion of time spent foraging leaf and fruit matter and PCoA axis 1 (R= -0.25, p= 0.002**). Therefore, there is a general trend of decreasing leaf and fruit foraging reflected in the *n*-alkane PCoA plot along axis 1 (Figure 4).

When *P. coquereli n*-alkane signatures were considered independently, it was found that while fruit foraging had a significant influence on *n*-alkane variation ($p = 1.46x10^{-5}$***), leaf foraging did not (p= 0.807). This influence of fruit foraging is reflected by a positive correlation along axis 1 (R= 0.35, p= 0.0017**). Therefore, *P. coquereli* display a general trend of increasing fruit foraging along axis 1 (Figure 5).

Contrastingly, *L. catta n*-alkane signatures had no influence of either fruit or leaf foraging (p= 0.33 and p= 0.15, respectively). There was, however, a significant influence of dead leaf foraging (p= 0.016*) reflected by a positive correlation along axis 1 (R= 0.29, p= 0.0047**; Figure 6).

Therefore, we can track the proportional contribution of different items to the diet through faecal *n*-alkane compositions.

4. Discussion

4.1 Sources of HMW Faecal *n*-Alkanes

Faecal matter can contain biomarkers from not only the diet, but also the gut microbiome of the source animal (i.e. lemur), and the gastrointestinal tract of the source animal itself (Gill and Bull, 2012). In the case of HMW *n*-alkanes, the main source will be from higher plants (Killops and Killops, 2013). We note there are some records of HMW *n*-alkane production by organisms other than higher plants. Long-chain *n*-alkane lipids exhibiting the same alternating patterns of abundance (as found in plants) have been reported from measurements on the protective coatings of insects (Chikaraishi et al., 2013) and fungal spores (Oró et al., 1966) but the contribution of these sources to our faecal samples is considered negligible compared to the contributions of plants since their biomass is much lower in general and given lemurs in this study were not observed to feed on either of these potential sources (Figure S1). However, we do not know how, or if, microbial degradation from the extensive and well-developed gut microbiota of lemurs will have altered the dietary (plant) *n*-alkanes found in the faecal matter.

4.1.1 Gut Microbiome: Direct and Indirect Effects

To account for the lack of bodily enzymes to digest complex dietary fibre, mammals have adapted diverse gut microbiomes to enable efficient breakdown and digestion (Gill and Bull, 2012). In turn, this diverse community of bacteria within the host animal has the potential to degrade molecules in food matter, even those such as *n*-alkanes which are generally considered indigestible and inert, potentially resulting in a reduced carbon preference index due to preferential degradation of odd-chain lengths (Thomas et al., 2021). In addition, there is strong evidence that the gut microbiome composition is heavily influenced by digested diet (Fogel, 2015, David et al., 2014, Greene et al., 2018, McKenney et al., 2018, Greene et al., 2020, Bornbusch et al., 2022). We might expect to see this reflected in the faecal *n*-alkane signatures, even though the link between the two is indirect. The potential of faecal *n*-alkanes for dietary analysis will be significantly reduced, or completely negated, if we cannot rule out or account for

297 the possibility of direct microbial degradation of the target molecules, or indirect covariation in
298 the datasets.
299 In general, analyses showed that there was a distinct lack of correlation between the gut
300 microbiome and *n*-alkane datasets, both in terms of comparisons between bacterial alpha
301 diversity indices and indices of *n*-alkane variation (Table S4), and in terms of the gut microbiome
302 and *n*-alkane matrices. In fact, mantel tests showed a strong lack of similarity between the *n*-
303 alkane signatures and phylum- and genus-level variation (p= 0.29 and p= 0.06, respectively).
304 Therefore, the faecal *n*-alkane signatures and gut microbiome compositions vary independently
305 from one another, and any microbial degradation of the *n*-alkanes occurring during digestion is
306 negligible.
307 By the same token, given that little similarity was found between gut microbiome composition
308 and faecal *n*-alkane signatures, we can conclude that the dietary influence on the gut
309 microbiome composition is not indirectly reflected in the faecal *n*-alkane composition. The most
310 likely reason for this is that these changes are not instantaneous. Rather, changes in the gut
311 microbiome reflecting dietary changes occur on a more seasonal or annual level (David et al.,
312 2014, Bornbusch et al., 2022, Greene et al., 2022b). However, some studies suggest that in some
313 lemur species, minor changes can be reflected in a change in gut microbiome composition within
314 days (Greene et al., 2018). It is much more realistic to expect that the gut microbiome will not
315 reflect one-off consumption of novel dietary items, but rather broad-scale seasonal changes in
316 diet like the proportion of leaf and fruit matter.
317 In summary, the faecal *n*-alkanes and the gut microbiome composition do not co-vary. We
318 observe negligible-to-no interference of the gut microbiota on the *n*-alkanes during digestion,
319 and no indirect covariation resulting from changes in the diet altering the gut microbiome.
320 Therefore, we can infer that changes in faecal *n*-alkane signatures will inform on changes in the
321 dietary inputs, rather than gut microbiome contributions to their signatures.

4.1.2 Diet

Given that it has been shown that the gut microbiome is neither directly nor indirectly influencing the faecal *n*-alkane signatures observed here, the faecal *n*-alkane signatures must represent plant matter in the ingested diet. Faecal *n*-alkane signatures display typical plant wax characteristics such as an odd-over-even carbon number preference and a predominance of the C_{29} and C_{31} homologues (Figure 1) (Eglinton and Eglinton, 2008, Bush and McInerney, 2013). Additionally, we observe a high carbon preference index and distinct similarities between the *n*-alkane and foraging datasets.

Therefore, there is no indication that these signatures did not originate from higher plants and are highly likely to provide clear inferences as to their dietary origin.

4.2 Faecal *n*-Alkane Signatures: Implications for Dietary Analysis

Simpler dietary systems, such as those of livestock, or animals in areas with little plant diversity have been profiled with relative ease from faecal *n*-alkane compositions and microscopic analysis of faeces, each combined with analysis of plant food samples for comparison (Storr, 1960, Dove and Mayes, 1991, Dove, 1992, Ferreira et al., 2005, Jin et al., 2006, Hueblin et al., 2016, Sawyer, 2020, Andriarimalala et al., 2021). The dietary diversity of lemurs, even in captivity with access to forest enclosures, however, is much more varied and diverse. This study observed a foraged diet of up to 42 different plant species, and 32 different provisioned items (Tables S1, S2). In wild lemurs, these numbers will likely be even higher given the diversity of Madagascar's flora (Antonelli et al., 2022). Figure 7 shows a compilation of *n*-alkane profiles from a range of different temperate plant types, along with the faecal *n*-alkane signatures of the lemurs in this study, and samples of the two types of 'chow' they are provisioned (Table S2). While the profiles of each plant type are uniquely different, in a mixture within faecal matter, combined with the similarly profiled chows, it is unlikely that any inferences as to plant species or type would be possible. It has also been documented that *n*-alkane profiles vary significantly through the growth and

expansion of young leaves, but remain relatively stable once leaves have matured (Piasentier et al., 2000, Bush and McInerney, 2013). There is great variation in consumption of young and mature leaves within this study and therefore we posit that identification of plant types and plant parts would not be possible.

While identification of individual plant species and plant parts may not be discernible from faecal *n*-alkane profiles, principal coordinate analysis of faecal *n*-alkane variation showed that, regardless of lemur species, we can infer changes in the combined proportion of leaves and fruits to 99.9% confidence (p= 0.00010***; Figure 4). Firstly, could this be an important method for monitoring captive diets of lemurs (or other herbivorous/omnivorous primates), especially in naturally forested enclosures such as those at the Duke Lemur Center. Secondly, in wild populations, these analyses could help to provide key information regarding the species' intrinsic ecology and feeding niches.

4.2.1 Applications within Captivity

These techniques have been clearly shown to have potential for dietary analysis and discrimination in a captive setting. Within captivity there are many potential applications for these techniques, which could help to monitor and enrich the lives of captive animals and increase their overall wellbeing. Moreover, captive lemurs have the potential to provide insights into wild lemur behaviours which cannot be directly observed. Therefore, developing methods such as this to help provide these insights could be integral to our long-term understanding of this group, and other animals this technique could be applied to.

In terms of animal husbandry and care, these techniques have the potential to enable enrichment and development of the captive diet to increase animal health and wellbeing. Many facilities that house captive animals are moving towards more naturalised housing conditions that provide greater space and foraging opportunities (Greene et al., 2022a) and improve animal welfare. Faecal dietary monitoring can augment animal observation to more comprehensively

determine what vegetation animals are choosing to supplement their provisioned diets through foraging activities. This could be particularly insightful for managed populations of nocturnal species kept on a normal light cycle. As a result, there could be potential to incorporate a greater proportion of particular foodstuffs into their provisions that they might be missing in order to better emulate a wild environment.

These techniques also have the potential to inform on the behaviours of wild populations. For example, how foraging patterns change and evolve with regard to external factors. This technique could give the freedom and opportunity to track how foraging patterns change on a seasonal to annual basis, in response to environmental and weather conditions, with group size, or home range area. Ultimately, knowledge of these responses in a captive setting will provide a solid basis for hypothesising how wild populations will react in similar circumstances and inform new research questions for wild studies.

4.2.2 Applications to the Wild

This is a pilot study testing the potential of these methods within modern ecology for dietary analysis using captive animals with detailed foraging and ranging data to inform our results. With further development and testing, there is great potential for this method to be applied to wild populations in conjunction with other monitoring methods. It is, however, important to note that local calibrations would be imperative to gaining useful and applicable information. Firstly, captive lemurs will never be fully representative of their wild counterparts. It is difficult to completely mimic the wild environment and provide accurate dietary provisions. Additionally, plant species vary in *n*-alkane compositions between temperate and tropical or sub-tropical environments (Bush and McInerney, 2013), and Madagascar is highly diverse in plant flora also (Antonelli et al., 2022). Therefore, for this work to be directly applicable to Malagasy forests and wild lemurs, *in-situ* ground truthing would be required.

Once ground truthed, this technique could help us to greatly develop our understanding of a species' feeding ecology, especially in the case of elusive, nocturnal or newly discovered species. Additionally, it could be possible to shed light on niche partitioning of sympatric species within a habitat and between seasons, helping to understand how they coexist and share or compete for resources. This could also determine if there are any co-dependent/co-evolutionary relationships otherwise unknown.

In eventuality, the use of faecal *n*-alkane analysis, in combination with a range of environmental parameters and organism-specific data, could provide clarity on the influence of anthropogenic climate change and habitat degradation on the diets and health of lemurs. This will rely, not only on food availability, but their intrinsic ecology and how that interplays with local environmental conditions. The response of a species to environmental and food stress will differ based on their intrinsic ecology. Some species may change their feeding patterns to reflect differing food availability within their environment. A switch to a more folivorous diet is often noted in some frugivorous lemur species outside of Madagascar's fruiting season, given the unreliability of tree fruiting patterns and lack of sufficient material to survive on (Donati et al., 2017, Crowley and Godfrey, 2019). For other species, a reliance on so-called 'fallback foods' has been observed, involving turning to foods otherwise considered low quality in the face of food stress (LaFleur and Gould, 2009, Naughton-Treves et al., 1998). Potentially, an increase in reliance on these behaviours could be indicated by these analyses.

5. Conclusions

Clearly, faecal *n*-alkanes differ significantly between the two lemur species in this study. These *n*-alkane signatures experience little-to-no degradation by the gut microbiome, and no indirect influence from changes in diet altering the gut microbiome composition, leading us to the conclusion that the faecal *n*-alkanes recovered are wholly dietary in origin.

Identification of individual plant species and plant parts is not possible given the diversity of plant matter consumed making *n*-alkane profiles indistinguishable from one another. However, it is possible to infer changes in the proportion of leaves and fruit consumed. This could have numerous applications within both captive and wild lemur populations.

The use of these *n*-alkane data in combination with *n*-alkane compound-specific $\delta^{13}C$ values could further the conclusions possible from these analyses. Commonly used to reconstruct changes in vegetation regimes in the past (Hughen et al., 2004, Feakins et al., 2005, Krull et al., 2006, Kristensen et al., 2011), lemur faecal *n*-alkane $\delta^{13}C$ values could reflect changes in the relative contributions of C_3 and C_4 plant material in the diet. Given that C_3 and C_4 plants utilise different photosynthetic pathways resulting in characteristic $\delta^{13}C$ values, these studies have been conducted before (Jones et al., 1979, Codron et al., 2005a, 2005b, Wittmer et al., 2010, Montanari, 2017, de Lira et al., 2021) and therefore have great potential here.

Additionally, given the potential of faecal *n*-alkanes shown here, there is no reason that this technique should be limited to lemurs, or even primates. Provided thorough ground truthing pilot studies are carried out, and the focal species is herbivorous (or an omnivore which reliably consumed a large quantity of plant matter), this technique could be applied across the animal kingdom. Insights into the feeding ecology and niches of many organisms, and the functioning of many habitats could be achieved.

Finally, there is also no need to limit the scope of target biomarkers to just *n*-alkanes. Other plant waxes, such as *n*-fatty acids, could have the potential to provide increasing insights into diets. Furthermore, *n*-fatty acids are not only produced by higher plants, but also bacteria, and therefore these compounds could inform on the interplay between ingested diet, gut microbiome composition and intrinsic ecology. Other, wholly bacterial-origin biomarkers, such as 3-hydroxy fatty acids (Wang et al., 2016, 2021) could enrich the biomarker dataset and provide greater clarity on the origin of observed patterns.

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Data Availability

All data is archived at https://doi.org/10.25500/edata.bham.00001324.

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691

692 **Tables**

693 Table 1: Subject animals included in this study, including social pair and Natural Habitat Enclosure

694 at the DLC.

Species	Individual	Sex	Social Pair	Enclosure (ha)	Number of samples
Propithecus coquereli	Beatrice	Female	Elliot	NHE6	13
	Elliot	Male	Beatrice		8
	Gertrude	Female	Remus	NHE5	15
	Remus	Male	Gertrude		18
	Gisela	Female	Rupert	NHE2	12
	Rupert	Male	Gisela		9
				Total	76
Lemur catta	Alena	Female	Stewart	NHE6	11
	Stewart	Male	Alena		16
	Brigitta	Female	Nikos	NHE5	25
	Nikos	Male	Brigitta		16
	Sophia	Female	Randy	NHE2	12
	Randy	Male	Sophia		12
				Total	92
				TOTAL	168

695

696 Table 2: The significance of gut microbiome alpha diversity indices between lemur species and

697 season. Significant ($p < 0.05$) and **Not Significant** ($p > 0.05$) relationships are highlighted.

Alpha diversity index	Variable	*p*-value
Taxonomic richness	Lemur species	4.76×10^{-7}***
	Season	4.50×10^{-5}***
Shannon entropy	Lemur species	**0.17**
	Season	7.53×10^{-5}***
Faith's phylogenetic diversity	Lemur species	$<2.2 \times 10^{-16}$***
	Season	0.00047***

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Figures

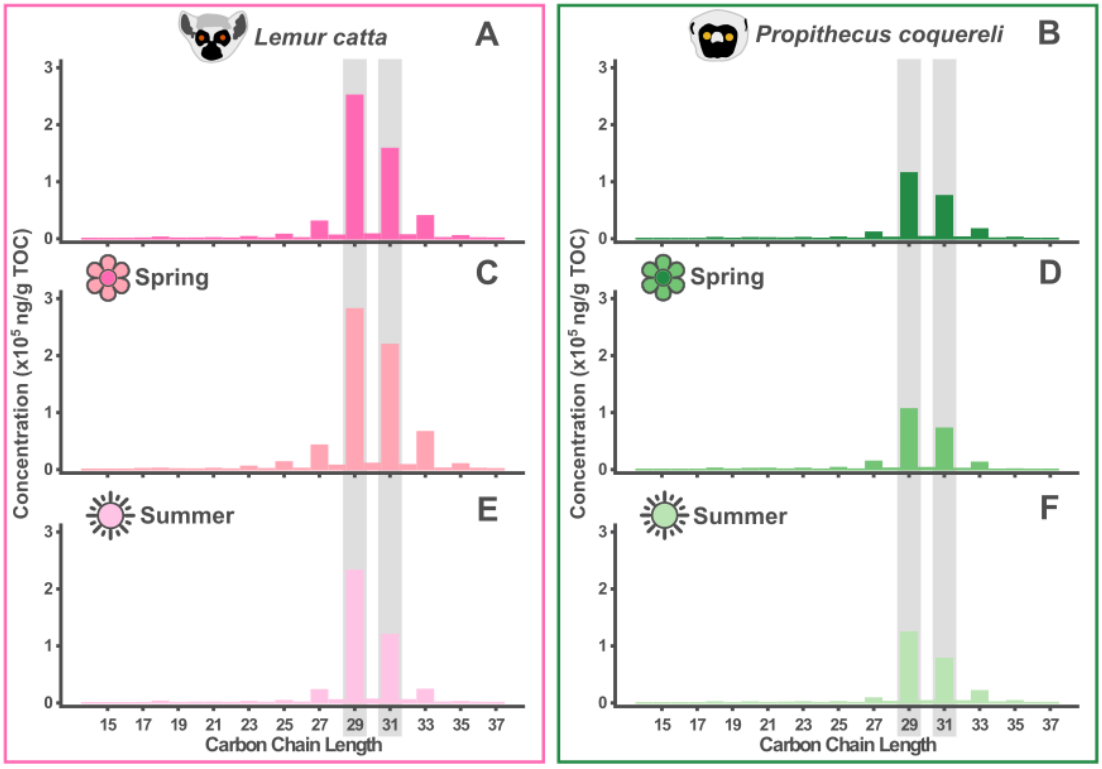
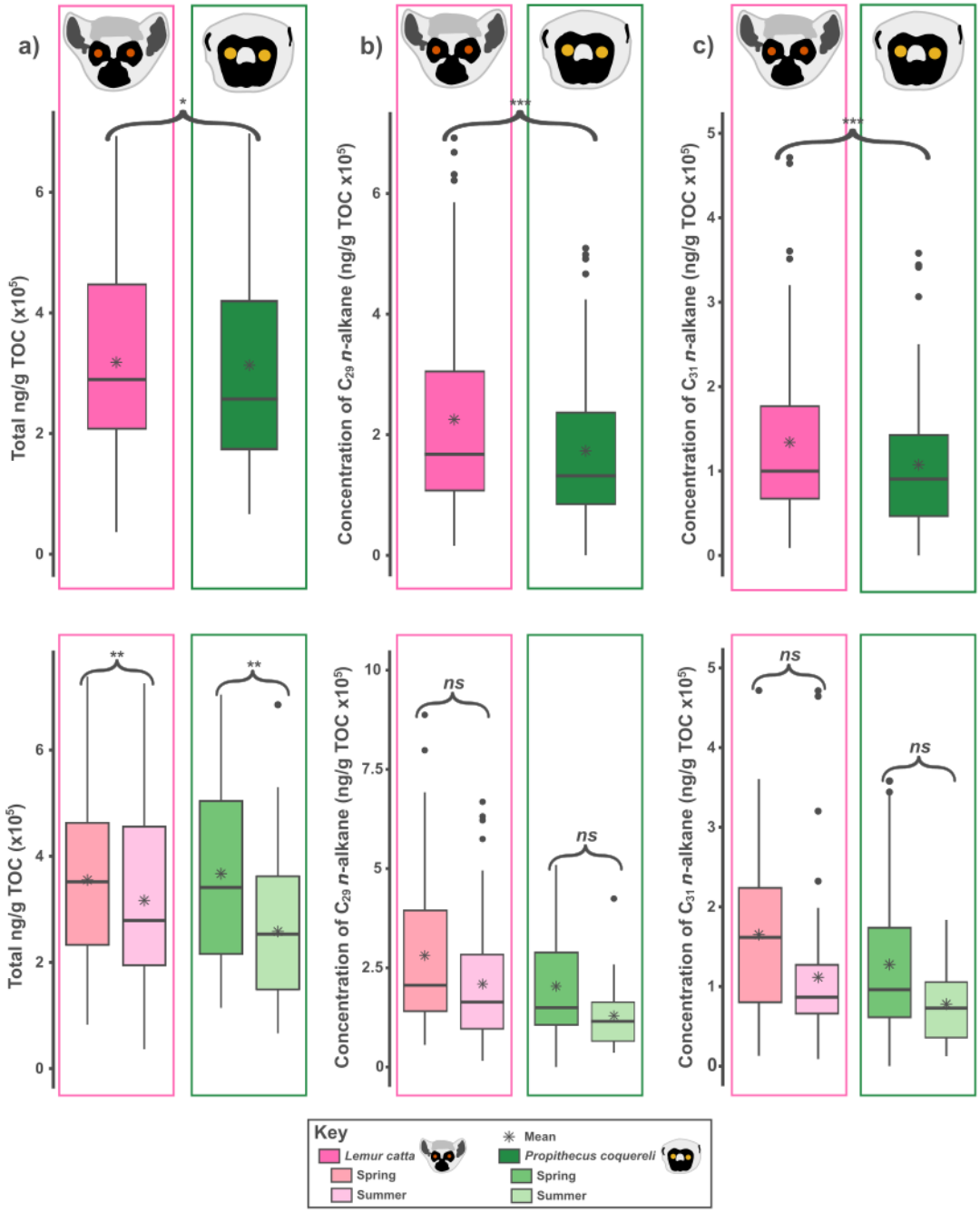


Figure 1: The concentration in ng/g TOC of *n*-alkanes extracted from 92 *Lemur catta* and 76 *Propithecus coquereli* faecal samples. Panels A, B) give the average concentration of all faecal samples; panels C, D) give the average for measurements on faecal samples collected during the Spring (May-June); panels E, F) give the average for measurements on faecal samples collected during the summer (August-September).



706

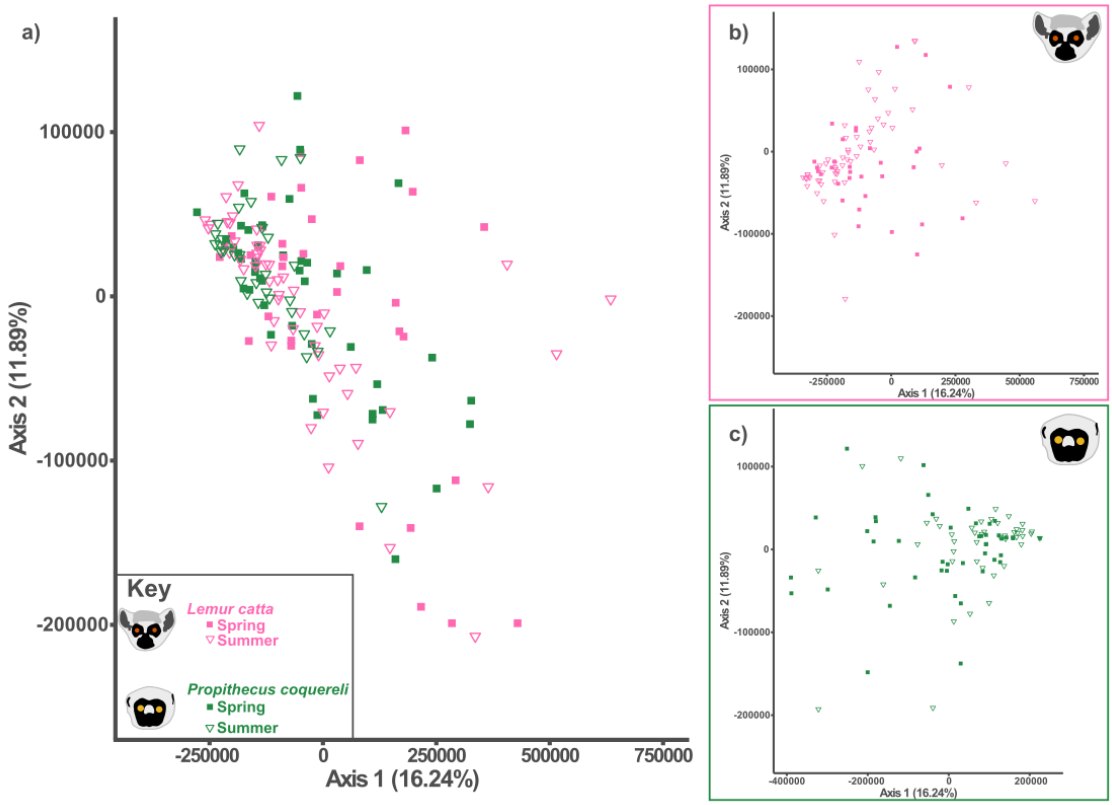
707 **Figure 2**: Variation in *n*-alkane concentrations by species and season. a) the total ng/g TOC of *n*-

708 alkanes extracted by species and season, b) the concentration of the C_{29} *n*-alkane by species and

709 season, and c) the concentration of the C_{31} *n*-alkane by species and season. *** denotes

710 significance at $p < 0.001$, ** denotes significance at $p < 0.01$, * denotes significance at $p < 0.05$ and

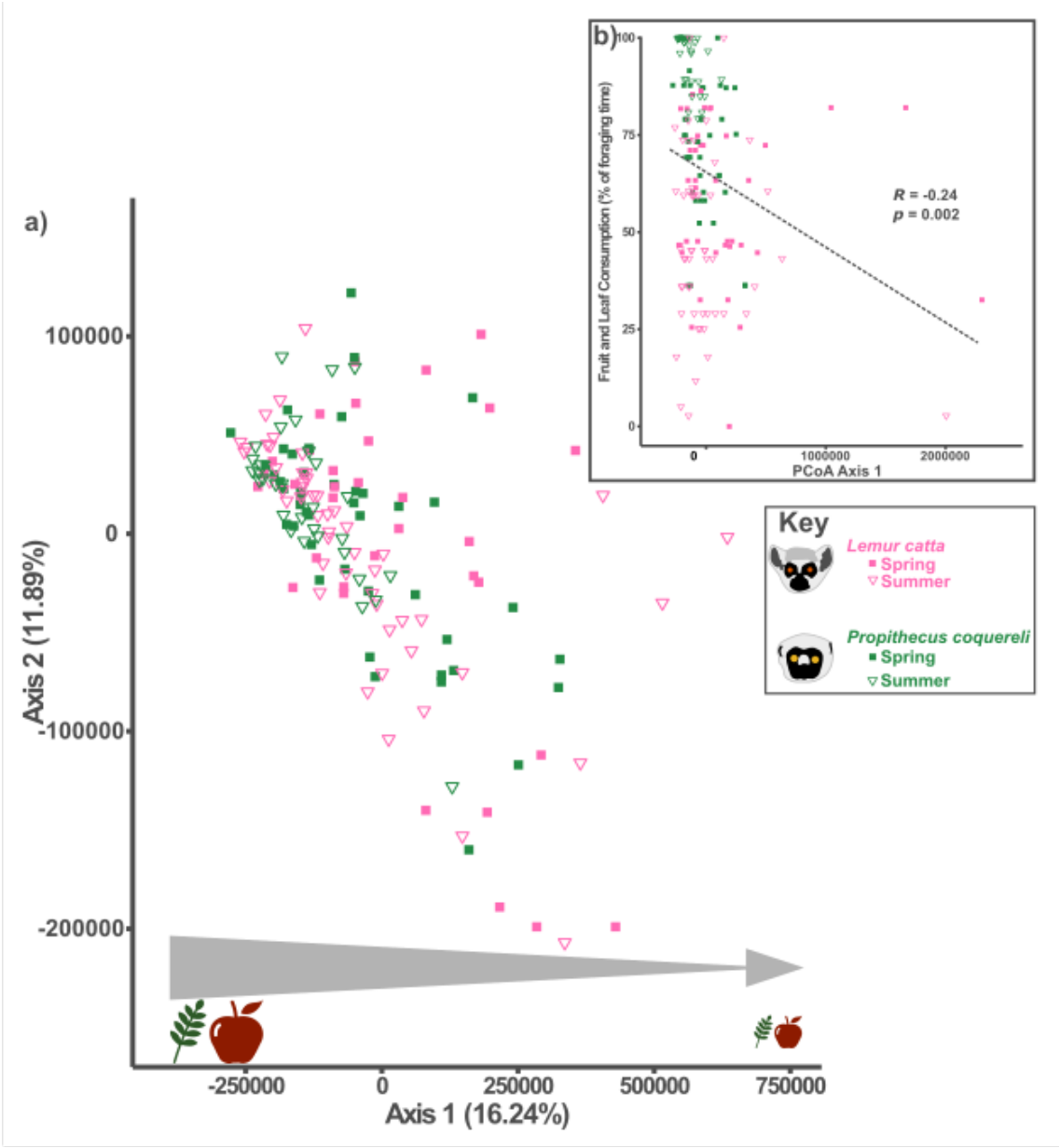
711 *ns* denotes no significance.



712

713 **Figure 3**: Principle Coordinate Analysis (PCoA) plots of faecal *n*-alkanes from a) all samples

714 analysed, b) all *Lemur catta* samples, and c) all *Propithecus coquereli* samples.



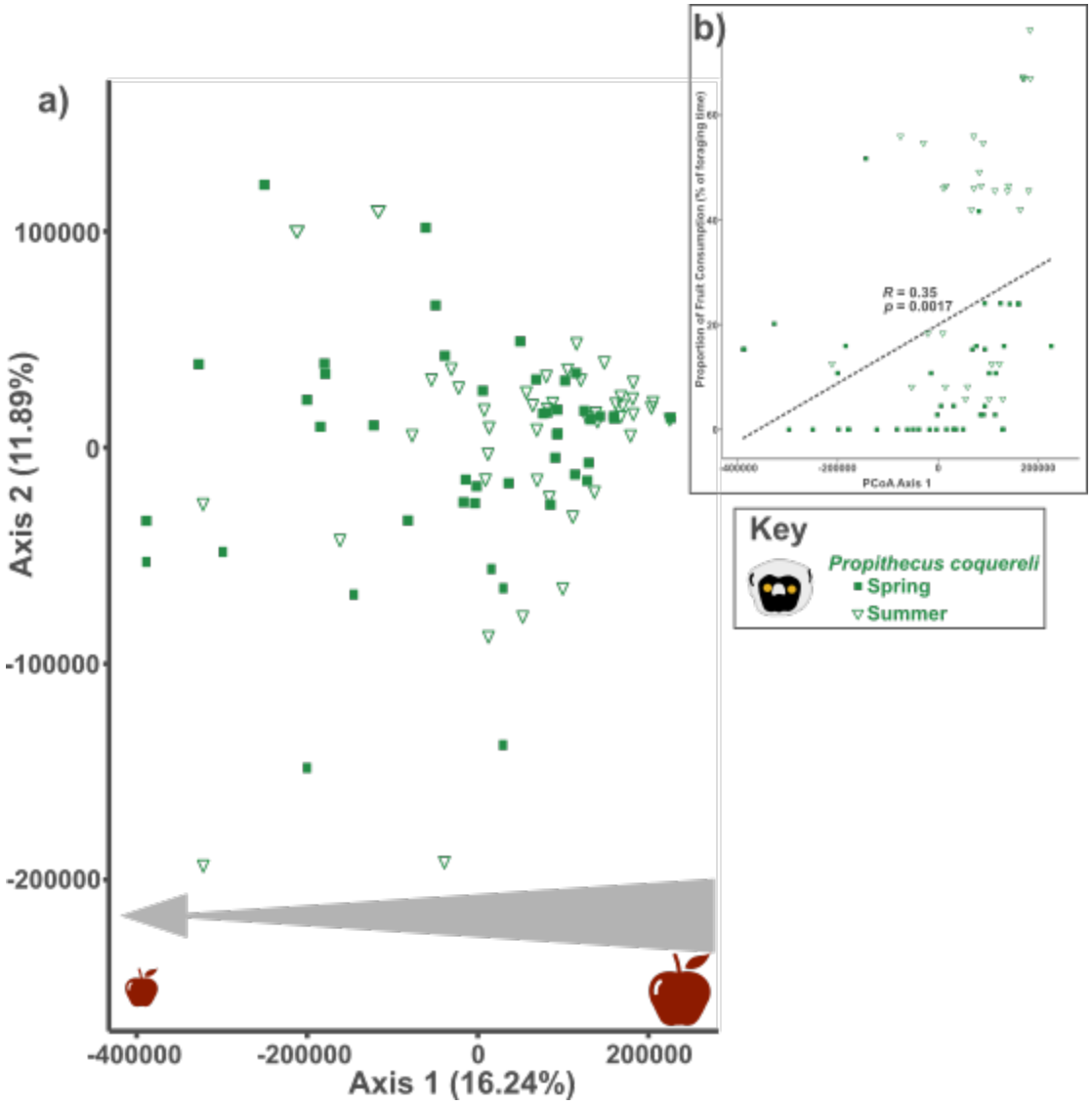
715

716 **Figure 4**: The effect of fruit and leaf consumption on faecal *n*-alkane variation in the study lemurs

717 displayed on a) a Principle Coordinate Analysis (PCoA) plot of the *n*-alkane signatures; b) (inset)

718 the relationship between PCoA Axis 1 and the proportion of fruit and leaf consumption (*p*=

719 0.002**).



720

721 **Figure 5**: The effect of fruit consumption on the faecal *n*-alkane variation in *Propithecus coquereli*

722 displayed on a) a Principle Coordinate Analysis plot of the *n*-alkane signatures; b) (inset) the

723 relationship between PCoA Axis 1 and the proportion of fruit consumption (*p*= 0.0017**).

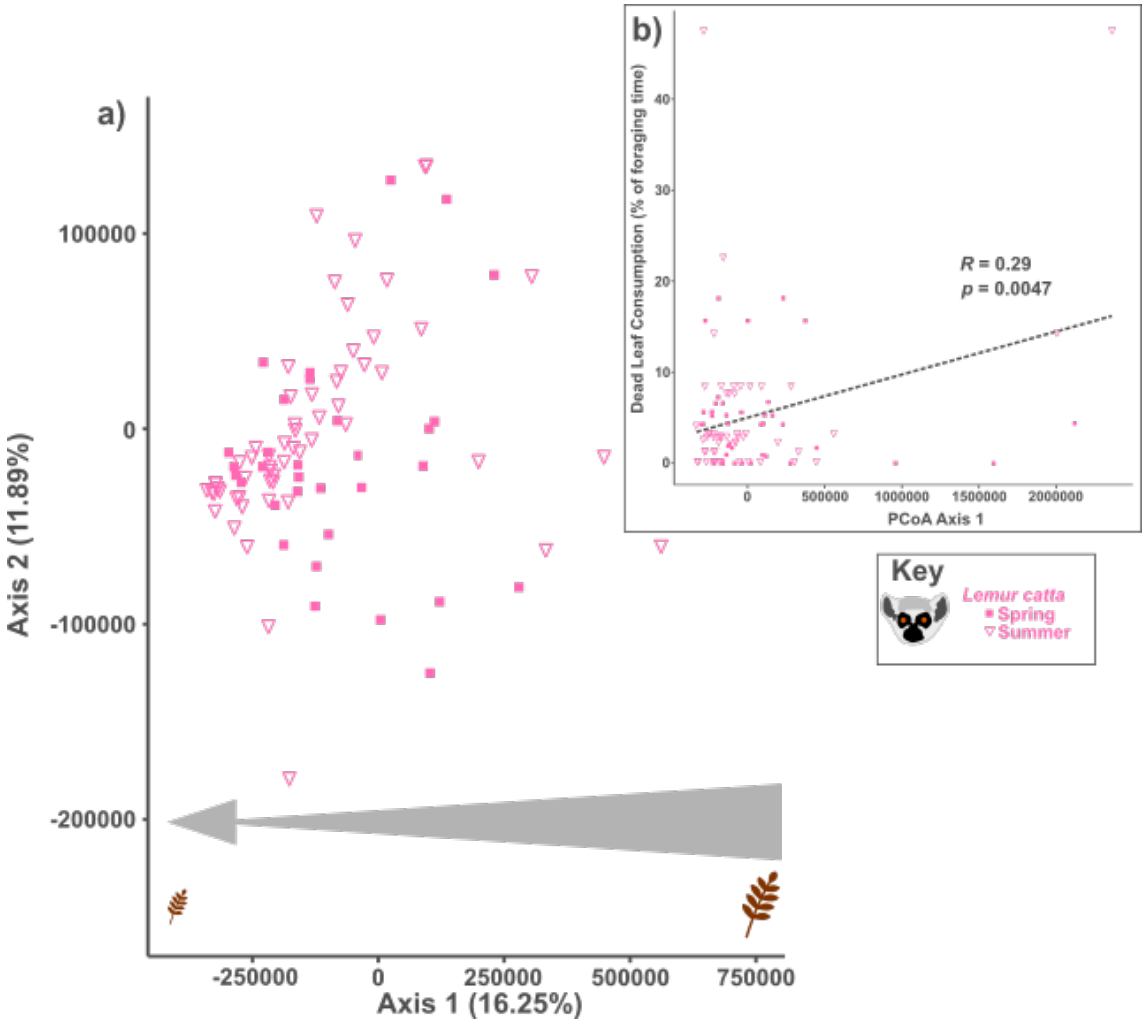
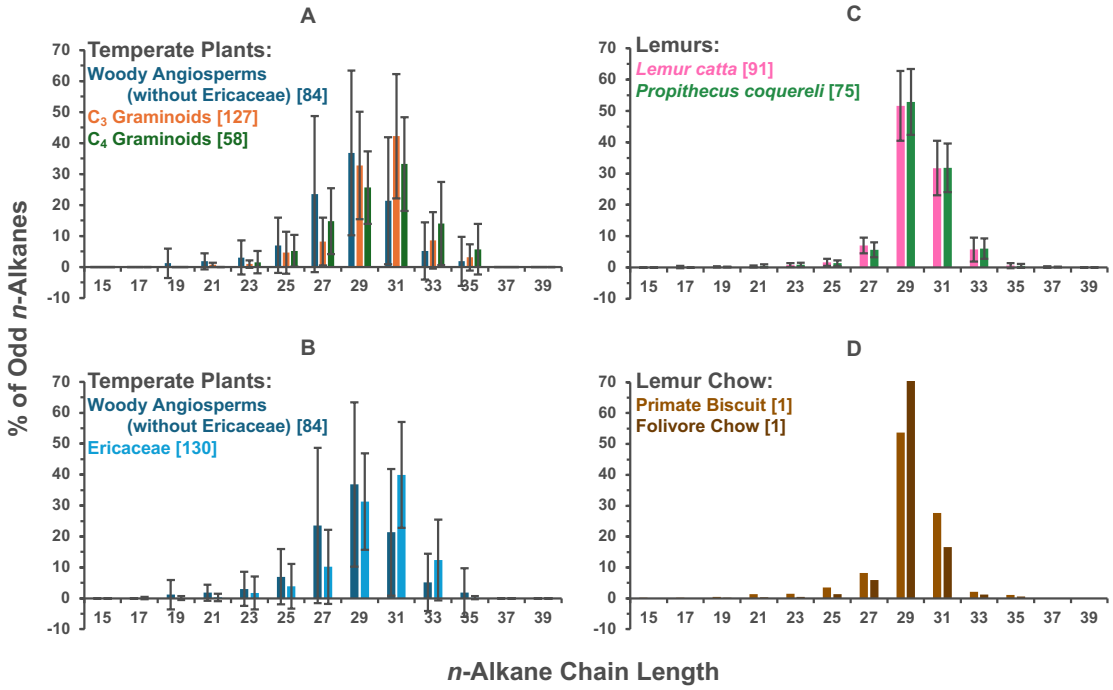


Figure 6: The effect of dead leaf consumption on faecal *n*-alkane variation in *Lemur catta* displayed on a) a Principle Coordinate Analysis (PCoA) plot of the *n*-alkane signatures, with b) (inset) the relationship between PCoA Axis 1 and the proportion of dead leaf consumption (*p*= 0.0047**).



729

730 **Figure 7**: Average *n*-alkane distributions. (A) Temperate woody angiosperms (without Ericaceae),

731 temperate C_3 graminoids, and temperate C_4 graminoids. (B) Temperate woody angiosperms

732 (without Ericaceae), and Ericaceae. (C) *Lemur catta* and *Propithecus coquereli* faeces. (D) Primate

733 biscuit (Mazuri® Leaf-Eater Primate Diet, Biscuit 5M02) and folivore chow (Lab Diet® Monkey

734 Diet 5038). Numbers in square brackets are sample sizes. Error bars are ±1 standard deviation.

735 Data presented in A-B are from Bush and McInerney (2013).

736

Supplementary Materials

Methods: Collection of Behavioural Data

To enable accurate interpretations of faecal *n*-alkane compositions, complementary foraging data were collected, which will allow for direct comparisons between the observed signatures and dietary intake. Foraging data were collected in conjunction with previous projects (Greene et al., 2022, Greene et al., 2024). Social pairs of lemurs were observed for three consecutive days during all-day follows from dawn to dusk each in spring (April-May) and summer (August-September) in 2020. At sunrise, the social pair was tracked to their sleeping site. Observations began once the group was located, were paused midday, resumed in the early afternoon, and continued until it was too dark to record foraging data or until the lemurs had stopped foraging for > 20 minutes. Observations were paused for heavy storms. Most observational data were collected between 6:30-11:30 and from 14:00-19:00, seasonally dependent.

Because the members of the social pairs foraged in close proximity, the behaviour of the focal female and male were concurrently recorded. The start and stop time of each foraging bout, plant part (e.g., young leaves, fruits, flowers) and plant identification, if possible, were all recorded. Plant identities were assigned based on visual assessments of leaf, flower, fruit, and bark appearance. Identifications were checked against an in-house database and against Google Photos to compare photographs to online databases.

Foraging bouts ended after 10 seconds of no foraging activity. Individuals were recorded as 'out-of-view' when they could not be seen, or behaviour could not be assessed. On rare occasions, it was challenging to record behaviour from both individuals concurrently and females were followed while males were placed on 'out-of-view'. Occasionally, lemurs high in the canopy were not visible, but foraging behaviour was recorded due to the consistent dropping of items to the forest floor, including leaves, flower petals, nut casings, and fruit skins. Using this regimen, 505.1

hours of animal observation was collected on 36 days, split evenly between the spring and summer.

From raw data, the percentage of time lemurs spent foraging on non-provisioned items was determined. For each lemur on each observation day, the total time spent foraging on individual plant parts was divided by the total foraging time.

Methods: DNA Extraction and Analysis

To evaluate and assess the potential impact of the gut microbiome on the results in this study, gut microbiome data were also generated for each sample analysed for *n*-alkanes. This will allow for direct comparisons between the two datasets, enabling us to rule out or account for the influence of bacteria on the *n*-alkane dataset, and therefore the interpretations gleaned.

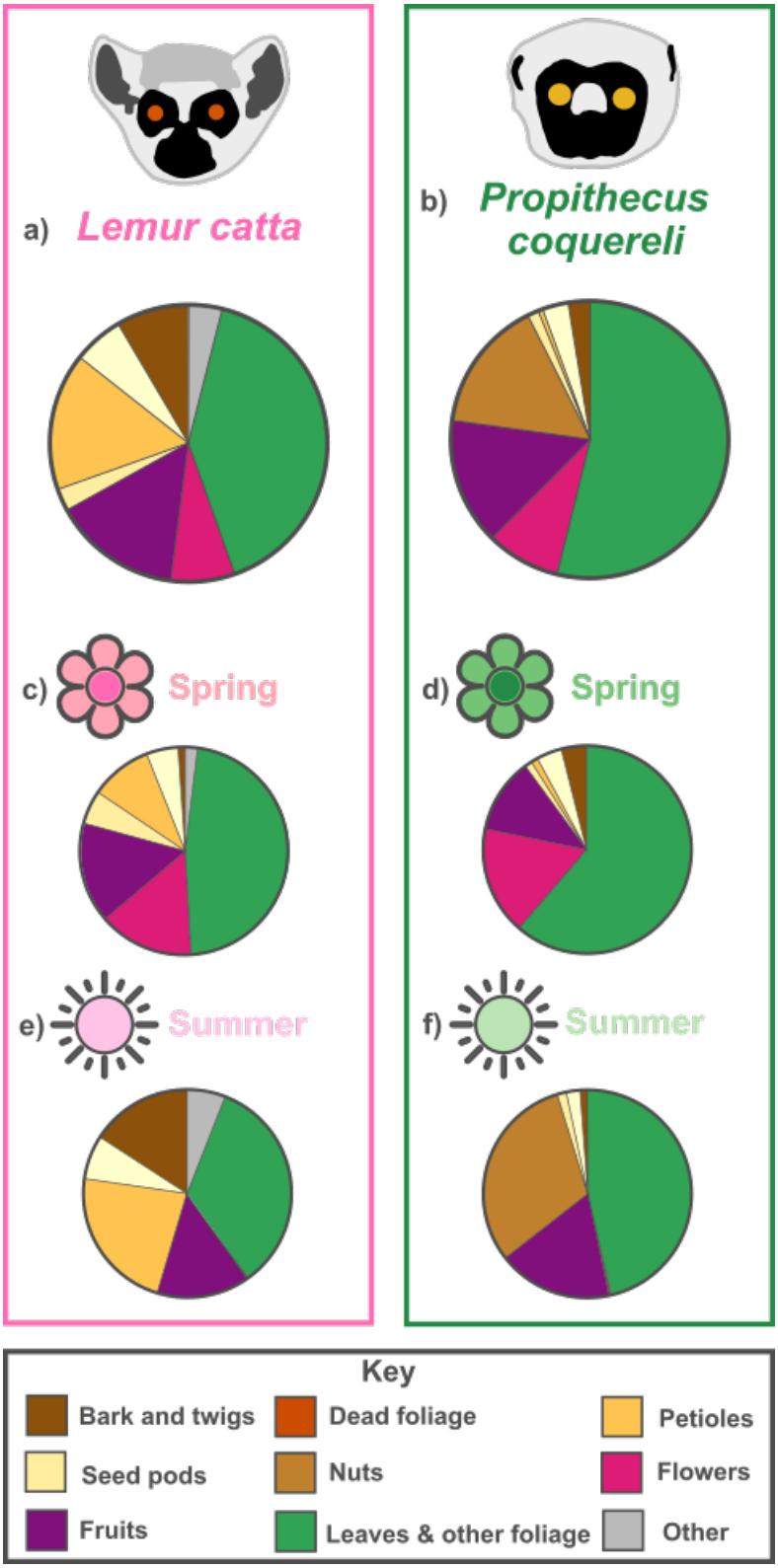
We determined gut microbiome structure following established protocols (Greene et al., 2020) #. In brief, gDNA was extracted from aliquots of frozen faecal samples using commercial kits (DNeasy PowerSoil or PowerSoil Pro kits by Qiagen, Hilden Germany), adding a heat-blocking step (at 50-60°C for 10 minutes) prior to bead-beating. Aliquots of gDNA were shipped, on dry ice, to the Argonne National Lab (Lemont, Illinois) for preparation and sequencing of the 16S rRNA gene using the 515F/806R primers, 150x150 base pair, paired-end reads, and Illumina's MiSeq platform. Raw sequences were analyzed using QIIME 2 (Bolyen et al., 2019), where they were demultiplexed, filtered for quality, and assigned to amplicon sequence variants (ASVs) at 100% sequence fidelity. ASVs were identified by comparison to the SILVA 138 database (Quast et al., 2013) . We determined the relative abundance of taxa at the phylum and genus level for statistical analyses. Alpha diversity metrics, including Observed Features (richness), Shannon entropy (evenness), and Faith's Phylogenetic Diversity, were also computed, with rarefication to 12,000 reads per sample at the time of metric computation.

Results: Foraging Patterns

Both species foraged for diverse plant items (Figure S1). They had access to whatever native plant species and plant parts they could find within their forest enclosures, and foraged not only in the tree canopy, but also on the forest floor. Foraging was in addition to their provisioned diets. A total of 42 different plant species were foraged, 30 by *P. coquereli*, and 34 by *L. catta*, of which 21 were common in both lemur species' diet (Table S1). A list of consumed items provisioned by caretakers is provided in Table S2.

Principle coordinate analysis of the foraging patterns (Figure S2) confirmed a strong influence of species ($p < 2x10^{-16}$***) and season ($p < 2x10^{-16}$***), along with a strong influence of individual lemur ($p = 5.11x10^{-12}$***). This analysis showed that, while *L. catta* foraged more plant species than *P. coquereli*, the individuals within the species were less variable and more homogeneous than those of *P. coquereli*, given the spread of the species clusters observed within the PCoA plot (Figure S2).

Foraging patterns vary considerably by species, season and individuals. This is potentially the driving force of patterns observed within the faecal *n*-alkane signatures.



802

803 **Figure S1** Mean dietary intake as a proportion of time spent foraging for different food items. a)
804 all Lemur catta focal observations, b) all Propithecus coquereli focal observations, c,d) Spring
805 (May-June) focal observations of Lemur catta and Propithecus coquereli, respectively, e,f)
806 Summer (August-September) focal observations of Lemur catta and Propithecus coquereli,
807 respectively.

808

Table S1 The plants and plant parts foraged by the subject species in this study. YL = young leaves, ML = mature leaves, G/W = weeds and grasses, DL = dead leaves, UF = unripe fruit, SRF = semi-ripe fruit, RF = ripe fruit, FL = flowers, DF = dead flowers, PT = petioles, SP = seed pods, DSP = dead seed pods, NT = nuts, BK = bark, TWIG = twigs, CONE = pine cones, SOIL = soil. * indicates were identification was tentative. ** indicates provisioned browse.

Common name	Latin name	Plant part foraged		
		Propithecus coquereli		*Lemur catta*
American Beech	*Fagus grandifolia*			PT
Aspen*	*Populus tremuloides* and *grandidentata*			YL
Bamboo grass				G, SP
Black Locust	*Robinia pseudoacacia*			YL, SP, DSP
Blackberry	*Rubus occidentalis*	YL		YL, ML, RF
Blackgum	*Nyssa sylvatica*	YL, UF, RF		YL, ML, PT
Boneset	*Eupatorium perfoliatum*	BUDS		
Box Elder	*Acer negundo*			SRF
Bush Clover	*Lespedeza cuneata*	YL, ML, FL, PT		YL, ML
Chamber Bitter	*Phyllanthus urinaria*	YL		
Common Greenbrier	*Smilax rotundifolia*	SP		YL
Common Vetch	*Vicia sativa*	SP		
Dogfennel	*Eupatorium capillifolium*	YL		
Dogwood	*Cornus florida*			YL, UF, PT, FL
Eastern Red Cedar	*Juniperus virginiana*	ML, BK		
Eastern Redbud	*Cercis canadensis*	YL, ML, DSP		YL, SP
Grapevine	*Vitis rotundifolia*	YL, ML, RF		YL, ML, DL, TWIG, UF, RF
Hackberry*	*Celtis occidentalus*			RF, DSP
Hickory	*Carya glabra* and *ovata*	YL		YL, PT
Hillside Blueberry	*Vaccinum pallidum*	ML		YL, ML, PT
Honeysuckle	*Lonicera Japonica*	YL		RF, DRF, FL
Hornbeam	*Carpinus caroliniana*	ML		YL, ML, DL, TWIG

Japanese silverberry/Autumn Olive	*Elaeagnus umbellate*	ML		ML, UF
Loblolly Pine	*Pinus taeda*	YL, DL, BK		YL, ML, DL, BK, TWIG, CONE SHARDS
Magnolia	*Magnolia* spp.			PT
Mimosa**	*Acacia dealbata*	YL, ML, stems, SP, TWIG, BK		YL
Morning Glory	*Ipomoea* spp.			YL, FL
Partridge Pea	*Chamaecrista fasciculata*	SP		
Poison Ivy	*Toxicodendron radicans*	YL, ML		
Privet	*Ligustrum sinense*			UF
Red Maple	*Acer rubrum*	YL, ML, PT, BK, TWIG		YL, ML, DL, PT, BK, TWIG
Rhomboid Mercury	*Acalypha rhomboidei*	YL		
Selfheal	*Prunella vulgaris*			FL
Sourwood	*Oxydendrum arboreum*			YL, ML, FL
Sweetgum	*Liquidambar styraciflua*	YL, ML, DL, UF, PT, BARK		YL, ML, DL, PT
Tulip Poplar	*Liriodendron tulipifera*	YL, ML, UF, FL, BARK		YL, ML, FL, TWIG
Viburnum	*Viburnum* spp.	ML, UF		ML, UF, RF, PT
Virginia Creeper*	*Parthenocissus quinquefolia*	YL		
White Clover	*Trifolium repens*	ML or FL?		YL?
White Oak	*Quercus alba*	YL, ML, DL, PT, BK, NT		YL, DL, PT, TWIG, NT
Winged Sumac**	*Rhus copallinum*	ML, YL, stems, BUDS, BK		
Yellow Wood Sorrel	*Oxalis stricta*			YL
Total species	**42**	**29**		**32**

Table S2 The food items provisioned by the Duke Lemur Center to the subject species, and those of which they were documented to consume during the course of observations indicated yes (Y) or no (N).

819

Common name	Documented consumption (Y/N)	
	Propithecus coquereli	*Lemur catta*
Apple	Y	Y
Banana	N	Y
Black bean	Y	Y
Broccoli	Y	N
Cantaloupe	N	Y
Carrot	Y	Y
Cashew	Y	N
Cauliflower	Y	Y
Celery	Y	Y
Chickpea	Y	N
Collards	Y	Y
Corn	Y	Y
Craisin	Y	Y
Cucumber	Y	N
Folivore chow (Lab Diet® Monkey Diet 5038)	Y	Y
Green onion	Y	Y
Jam	Y	N
Kale	Y	Y
Kidney bean	Y	Y
Kiwi	N	Y
Leek	Y	N
Primate biscuit (Mazuri® Leaf-Eater Primate Diet, Biscuit 5M02)	Y	Y
Papaya	N	Y
Peanut	Y	Y
Pineapple	Y	Y
Purple cabbage	Y	Y
Lettuce	Y	Y
Sweet potato	Y	N

Tomato	Y		Y
White beans	Y		N
Total: 32	**24**		**21**

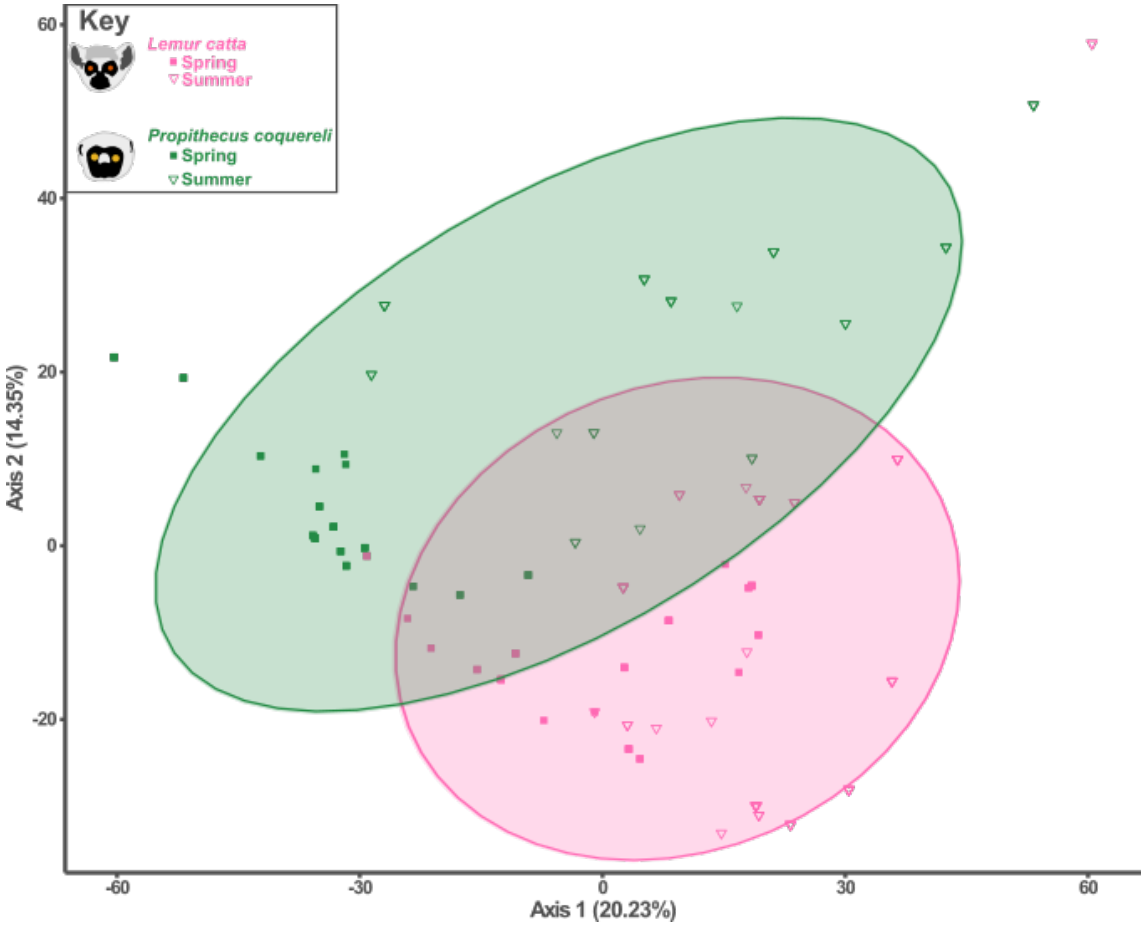


Figure S2 Principle Coordinate Analysis (PCoA) plot of the foraging behaviour of the lemurs in this study with ellipses drawn at the 95% confidence level for multivariate *t*-distribution.

Results: Gut Microbiome Composition

Both species displayed different gut microbiome profiles at both the phyla- and genus-level (Figure S3). When alpha diversity indices were calculated, it was shown that, in general, they vary significantly by species and season (Table S3). Additionally, principle coordinate analysis (PCoA) demonstrated a very strong influence of lemur species ($p < 2x10^{-16}$***) on both the phyla- and genus-level diversity (Figure S4). At the phyla-level there was no influence of individual

lemur (p= 0.404), but a very significant influence at the genus-level (p= 4.52x10^{-7}***). Season had no influence on either phyla- or genus-level variation (p= 0.217, p= 0.717, respectively). Lemur species was the primary driver of gut microbiota variation. This is potentially the driving force of observed faecal *n*-alkane patterns.

Table S3 The significance of gut microbiome alpha diversity indices between lemur species and season. Significant (p< 0.05) and Not Significant (p> 0.05) relationships are highlighted.

Alpha diversity index	Variable	*p*-value
Taxa richness	Lemur species	4.76x10^{-7}***
	Season	4.50x10^{-5}***
Shannon entropy	Lemur species	0.17
	Season	7.53x10^{-5}***
Faith's phylogenetic diversity	Lemur species	<2.2x10^{-16}***
	Season	0.00047***

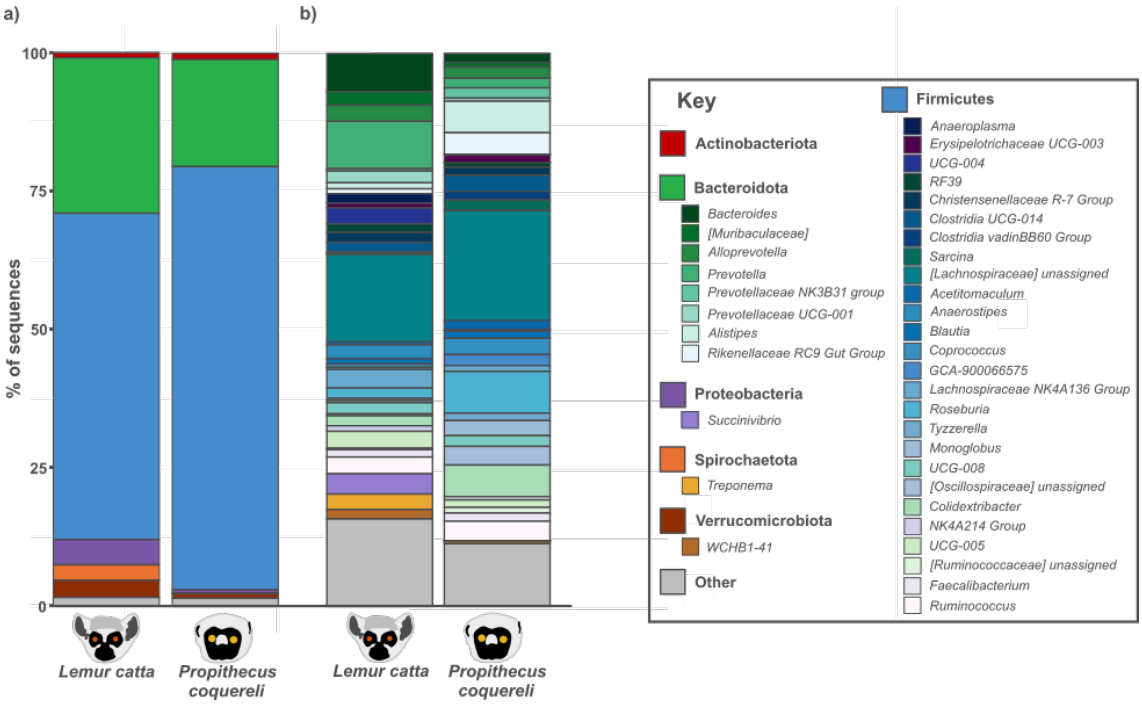
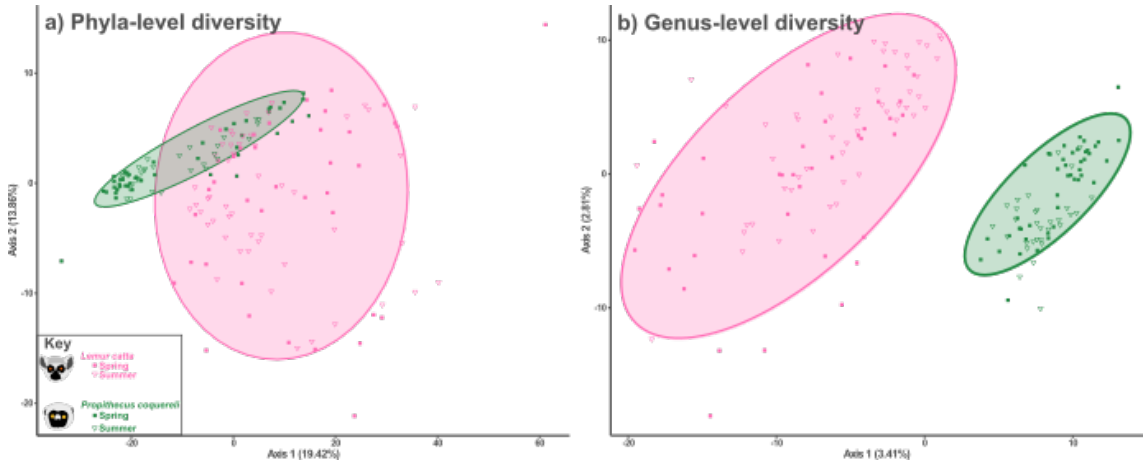


Figure S3 Average gut microbiome compositions of Lemur catta and Propithecus coquereli at a) the phyla level, and b) the genus level. Only those phyla representing >1% of sequences are presented, those <1% of sequences are grouped into 'Other'.



840 **Figure S4** Principle Coordinate Analysis (PCoA) plots of the gut microbiome diversity at a) phyla

841 level, and b) genus level. Ellipses are drawn at the 95% confidence level for multivariable t-

842 distribution.

843

844 **Results: Faecal n-Alkanes and Gut Microbiome Composition**

845 **Table S4** The relationship between n-alkane indices and gut microbiome alpha diversity indices.

846 Significant ($p < 0.05$) and Not Significant ($p > 0.05$) relationships are highlighted.

				***n*-Alkane Indices**		
				***n*-Alkane Richness**	**Carbon Preference Index (CPI)**	**Average Chain Length (ACL)**
Gut Microbiome Alpha Diversity Indices	**Taxa Richness**	*Lemur catta*	All	p= 0.4 R= 0.089	p= 0.28 R= -0.11	p= 0.0036 R= 0.3
			Spring	p= 0.026 R= 0.36	p= 0.15 R= -0.24	p= 0.003 R= 0.46
			Summer	p= 0.56 R= -0.083	p= 0.3 R= 0.15	p= 0.048 R= 0.73
		Propithecus coquereli	All	p= 0.37 R= -0.1	p= 0.27 R= -0.13	p= 0.37 R= 0.1
			Spring	p= 0.99 R= -0.0016	p= 0.44 R= -0.12	p= 0.24 R= 0.18
			Summer	p= 0.17 R= -0.25	p= 0.54 R= -0.11	p= 0.71 R= -0.07
	Shannon Entropy	*Lemur catta*	All	p= 0.15 R= 0.15	p= 0.43 R= -0.084	p= 0.00029 R= 0.37
			Spring	p= 0.02 R= 0.37	p= 0.3 R= -0.17	p= 0.001 R= 0.51
			Summer	p= 0.93 R= 0.013	p= 0.43 R= 0.11	p= 0.25 R= 0.16
			All	p= 0.19	p= 0.43	p= 0.32

		Propithecus coquereli		*R*= -0.15	*R*= -0.093	*R*= 0.12
			Spring	*p*= 0.83 *R*= -0.033	*p*= 0.85 *R*= 0.03	*p*= 0.21 *R*= 0.19
			Summer	*p*= 0.058 *R*= -0.34	*p*= 0.059 *R*= -0.34	*p*= 0.42 *R*= -0.15
	Faith's Phylogenetic Diversity	*Lemur catta*	All	*p*= 0.7 *R*= 0.041	*p*= 0.2 *R*= -0.14	*p*= 0.034 *R*= 0.22
			Spring	*p*= 0.11 *R*= 0.26	*p*= 0.16 *R*= -0.23	*p*= 0.1 *R*= 0.27
			Summer	*p*= 0.21 *R*= -0.18	*p*= 0.8 *R*= 0.036	*p*= 0.43 *R*= 0.11
		Propithecus coquereli	All	*p*= 0.28 *R*= -0.13	*p*= 0.43 *R*= -0.092	*p*= 0.13 *R*= 0.18
			Spring	*p*= 0.81 *R*= -0.037	*p*= 0.85 *R*= -0.029	*p*= 0.052 *R*= 0.3
			Summer	*p*= 0.15 *R*= -0.26	*p*= 0.28 *R*= -0.2	*p*= 0.59 *R*= -0.1

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