Seasonal shifts in the gut microbiome indicate plastic responses to diet in wild geladas

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ABSTRACT

Animals have evolved numerous strategies to cope with energetic challenges, with dynamic changes to the gut microbiome potentially constituting one such strategy. We tested how proxies of food availability (rainfall) and thermoregulatory stress (temperature) predicted gut microbiome composition of geladas (*Theropithecus geladas*), a grazing, high-altitude primate inhabiting a seasonal environment. The gelada gut microbiome varied across seasons, reflecting more efficient digestion of the primary foods eaten at certain times of year. In rainier periods, the gut was dominated by cellulolytic/fermentative bacteria that specialized in digesting grass, while during dry periods the gut was dominated by bacteria that break down starches found in underground plant parts. Temperature had a smaller, but detectable, effect on the gut microbiome. We found an increase in microbes involved in metabolism and energy production during cold and dry periods, suggesting buffering when thermoregulatory and nutritional stress co-occurred. Our results suggest that the gelada gut microbiome may shift to compensate for host diet and energetic demands.

KEYWORDS: gut microbiome, graminivory, seasonality, thermoregulation, Theropithecus geladas, primates

INTRODUCTION

Obtaining sufficient nutrients is a fundamental challenge for most animals. Yet, the availability and nutritional content of food can vary temporally and spatially in response to changes in climate and geography. Nutritional demands further vary in response to thermoregulatory needs and life history processes, such as growth and reproduction (McNab 2002; Dufour and Sauther 2002). Animals have evolved a variety of behavioral and physiological strategies to cope with these shifting demands, including altered feeding and activity patterns and increased mobilization of stored fat to fuel energetic demands (Doran 1997; Gursky 2000; van Schaik and Brockman 2005; Dias et al. 2011). Recently, the gut microbiome has been proposed as an additional avenue by which animals can cope with changing dietary landscapes and energetic challenges (Candela et al. 2012; David et al. 2014; Amato et al. 2015). The gastrointestinal tract of animals harbors a dense microbial community that helps to break down and ferment plant structural carbohydrates, producing short-chain fatty acids (SCFAs) that can be used as an energy source by hosts (Bäckhed 2011; Flint et al. 2012; White et al. 2014). The absorption of SCFAs in the gut may be particularly important for herbivorous species, such as foregut and hindgut fermenters, which obtain as much as 40-90% of their energy requirements from bacterial degradation of complex plant polysaccharides (Bergman et al. 1965; Udén et al. 1982; Milton and McBee 1983; Popovich et al. 1997). Additionally, variation in gut microbiome composition affects the efficiency of caloric harvest and the metabolic programming of the host (De Filippo et al. 2010; Bäckhed 2011; Krajmalnik-Brown et al. 2012; Tremaroli and Bäckhed 2012; Hanning and Diaz-Sanchez 2015). For instance, in mice (*Mus musculus*) and humans, obese and lean individuals have strikingly different gut microbiota composition, with obese phenotypes being associated with higher energy

extraction from diet and increased lipogenesis (Turnbaugh et al. 2006; Turnbaugh and Gordon 2009; Tseng and Wu 2019).

In wild mammals, the gut microbiome responds rapidly to seasonal and dietary changes (Maurice et al. 2015; Liu et al. 2019; Sun et al. 2016; Amato et al. 2015; Mallott et al. 2018; Ren et al. 2016; Springer et al. 2017), presumably to buffer seasonal energetic challenges (Amato et al. 2015; Sun et al. 2016). For example, a simultaneous increase in bacterial taxa involved in fiber fermentation and in SCFA concentrations during the dry season were suggested to allow Mexican black howler monkeys (*Alouatta pigra*) to maintain energy balance during energetic shortfalls without changes in activity or ranging patterns (Amato et al. 2015). Moreover, gut bacteria increase intestinal absorptive capacity, energy homeostasis, and fat burning during cold periods in mice (Chevalier et al. 2015), and improve digestive efficiency and SCFA production in energetically challenged ruminants living at cold and high-altitude (Zhang et al. 2016; Li et al. 2018). These microbial shifts likely come at some cost. For instance, increases in microbes that improve host metabolism under certain conditions may reduce the abundance of microbes that support host immune function (Amato et al. 2014; Reese and Kearney 2019). However, in seasonal and nutritionally challenging environments, enduring these trade-offs may be necessary for host survival and reproduction.

Geladas (*Theropithecus gelada*) represent an excellent system to investigate the relationship between gut microbiota composition and seasonal variation in host diet and energy needs. Despite being the only graminivorous primate with up to 90% of their diet comprised of grass (Fashing et al. 2014; Jarvey et al. 2018), their gastrointestinal tract appears poorly adapted to this specialization (but see (Wrangham 1980; Venkataraman et al. 2014) for dental, manual, and locomotor adaptations), closely resembling their closest phylogenetic relatives, baboons (*Papio*

93 spp.) – a taxon that is omnivorous (Mau et al. 2011). To compensate, geladas may rely heavily on
94 their gut microbiota to maximize nutrient extraction from grasses, likely through hindgut
95 fermentation (Mau et al. 2011; Trosvik et al. 2018). Moreover, geladas live in a high-altitude,
96 energetically demanding environment that exhibits marked inter- and intra-annual fluctuation in
97 rainfall and temperature (Jarvey et al. 2018; Tinsley Johnson et al. 2018). During rainier months,
98 when grass is abundant, they focus almost exclusively on eating above-ground graminoid leaves
99 and seeds, and during drier months, when grass availability decreases, they shift heavily to
100 underground foods (rhizomes, roots, corms, bulbs) (Hunter 2001; Jarvey et al. 2018). This diet
101 provides distinct challenges. Underground foods are considered a fallback food for geladas since
102 they take additional time and effort to harvest, are harder to process, and are relied upon only when
103 grasses are less abundant (Venkataraman et al. 2014; Jarvey et al. 2018). Despite being considered
104 a fallback food, these underground foods are rich in starches and carbohydrates, suggesting that
105 they contain more nutritional energy than grass (Dominy et al. 2008). This high amount of energy,
106 however, comes at some cost: roots and rhizomes are generally higher in fibers and lignin - and
107 thus harder to digest than grasses. In addition to these nutritional challenges, ambient temperatures
108 frequently drop to near freezing in some months, and the metabolic costs of thermoregulation are
109 known to strongly influence gelada physiology (Beehner and McCann 2008) and the timing of
110 reproduction (Tinsley Johnson et al. 2018; Carrera et al. 2020). Thus, seasonal dietary shifts and
111 temperature variation may entail distinct digestive and thermoregulatory challenges.

112 One previous study on geladas from Guassa, Ethiopia found that gut microbial
113 communities of adult females do indeed shift across seasons (Trosvik et al. 2018), supporting the
114 hypothesis that the gut microbiome may help hosts confront environmental challenges. This study
115 focused on adult females and assessed seasonal variation by separating the samples into two

116 categorical seasons (i.e., rainy, dry). Our study expands on this study by including adult males,
117 incorporating continuous climatic data across several years, and examining proxies of
118 thermoregulatory stress (in addition to diet) as factors that can influence the composition and
119 function of the gelada gut microbiome. Indeed, rainfall and temperature vary independently of
120 each other and represent distinct ecological challenges in gelada ecosystems. Therefore, we were
121 interested in further testing which aspect of gelada ecology more strongly determines seasonal
122 microbiome shifts.

123 We analysed the gut microbiome composition and predicted microbiome function in 758
124 fecal samples across 5 years from 48 adult male and 86 adult female geladas living in the Ethiopian
125 highlands in the Simien Mountains National Park. The Simien Mountains Gelada Research Project
126 (SMGRP) has been collecting detailed climatologic, demographic, and behavioral data from this
127 study population since 2006, allowing us to examine how ecological (rainfall and temperature)
128 and individual (group membership, sex, reproductive status, and age) factors influence gelada gut
129 microbiome composition. We hypothesized that ecological factors would be more strongly
130 associated with variation in the gelada microbiome than individual factors, and that rainfall and
131 temperature would have independent effects. In particular, we expected that rainfall, which is a
132 good proxy for grass availability (Jarvey et al. 2018), would have the strongest effect on the gelada
133 gut microbiome. Specifically, we predicted that the taxonomic changes associated with rainfall
134 would mainly reflect a shift to grass-based versus underground food-based diet, in order to allow
135 individuals to maximize energy extraction from those seasonal foods. We found that the gelada
136 microbiome exhibited drastic shifts related to climatological variables; but individual variables,
137 like age and sex, had minimal effects. Rainfall and temperature exerted independent effects on the
138 microbial composition and predicted function – with rainfall having a stronger effect on the gelada

gut bacteria. High rainfall, which is correlated with grass availability (Jarvey et al. 2018), was associated with more cellulolytic and fibrolytic bacterial taxa, when graminoid leaves were the main food source. Dry periods, which are correlated to underground food consumption (Jarvey et al. 2018), were associated with amylolytic and methanogenic taxa. Cold periods were further characterized by more amylolytic taxa, and hot periods by more methanogenic taxa. In both drier and colder periods, the gut microbiome shifted to predicted functions that suggested increased digestive efficiency, including energy, amino acid and lipid metabolism. Overall, gelada gut microbial composition covaried with diet and temperature in a pattern that suggests plastic but distinct responses to different dietary and metabolic challenges.

RESULTS

The gelada gut microbiome

We identified 3,295 amplicon specific variants (ASVs) in 758 fecal samples (mean±SD=813±243 ASVs per sample, range=92-1730) using deep 16S rRNA gene amplicon sequencing. These 3,295 ASVs came from 16 different phyla, 65 families, and 200 genera (Table S1, Figure 1, Figures S1-S2). Of the 3,295 ASVs, 170 (5%) were present in at least 90% of samples and form what can be considered the "core microbiota" of geladas (Table S2). The four most abundant bacterial phyla were *Firmicutes* (32%), *Kiritimatiellaeota* (formerly called *Verrucomicrobiota subdivision 5;* 26%), *Bacteroidetes* (23%), and *Spirochaetes* (5%) (Table S1, Figure 1A). All microbes assigned to *Kiritimatiellaeota* were part of the *RFP12* family and represent almost one quarter of the gelada gut microbiome (mean 26%, range 0.02%-70%, Figure 1B). Although the metabolic function of *RFP12* remains unknown, those bacteria have been found in high quantities in the gut of some domestic horse (*Equus ferus caballus*) and sheep (*Ovis aries*) populations (Steelman et al. 2012; Costa et al. 2015; Wang et al. 2017) and could have some fermentative function. Other taxa found

at high frequency in the guts of ruminants and herbivorous hindgut fermenters were also prevalent in the gelada gut, including many cellulolytic/fibrolytic (13% *Ruminococcaceae*, 6% *Lachnospiraceae*, 4% *Clostridiales vadinBB60 group*, 1.5% *Fibrobacteraceae*) and fermentative families (*5.3% Rikenellaceae*, 5% *Prevotellaceae*, *4.1% Bacteroidales F082*) (Table S1, Figure 1B and S1). The *Spirochaetes* phylum was mostly composed of *Treponema* (3.5%), a genus involved in lignocellulose degradation (Warnecke et al. 2007).

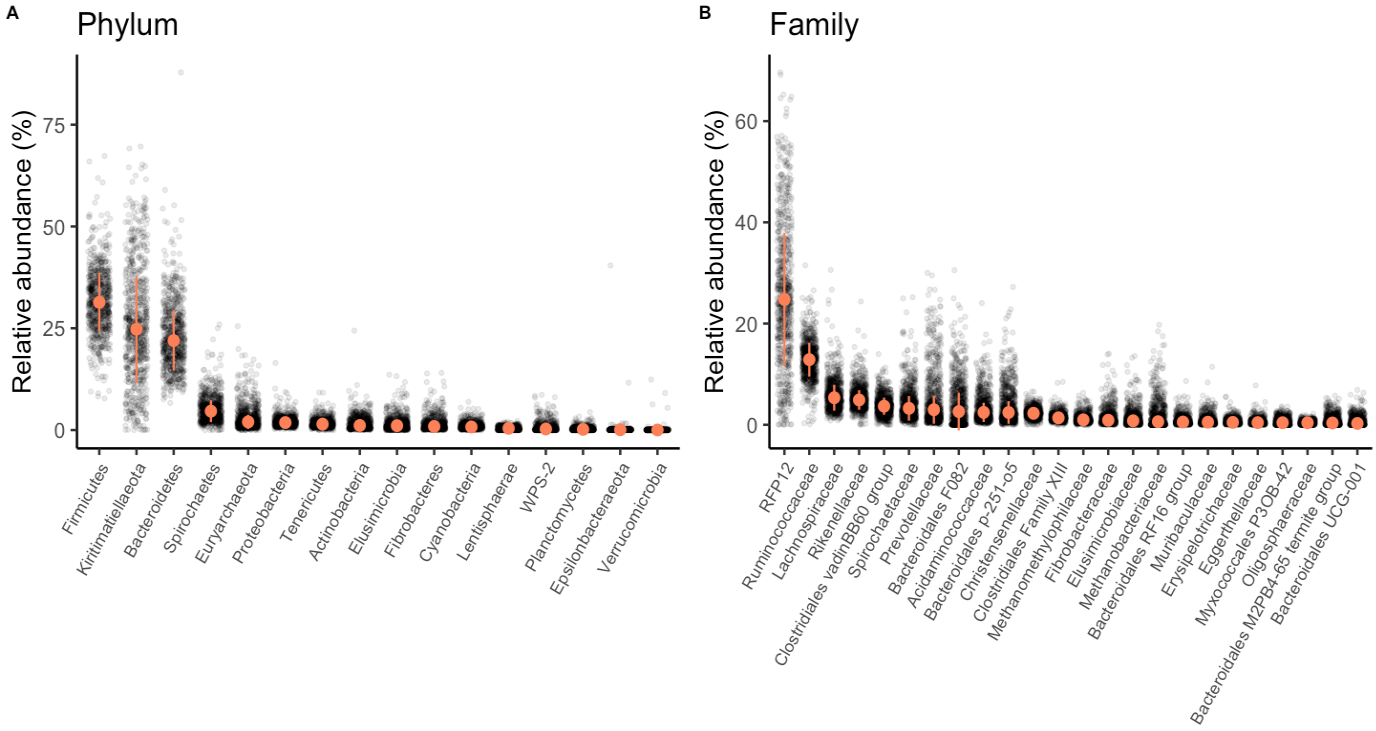

Figure 1. Taxonomic composition of the geladas gut at the phylum and family levels. Relative abundance **(A)** of all bacterial phyla and **(B)** of the 24 most abundant families (whose relative abundance>0.02%) in the gelada feces. The median and median absolute deviation (error limit) are represented in orange.

Dietary changes

To examine how seasonal variation in rainfall and temperature was associated with changes in the gelada gut microbiome, we used measures of true climatic conditions, including monthly

178 cumulative rainfall (an appropriate proxy of grass availability in the Simiens: (Jarvey et al. 2018))
179 and average monthly minimum temperature (a proxy of thermoregulatory constraint: (Beehner and
180 McCann 2008; Tinsley Johnson et al. 2018)). At the level of within-sample community diversity
181 ("alpha diversity"), we found that cumulative rainfall was positively associated with Shannon
182 evenness (Table 1, Figure 2A,C) but had no effect on bacterial richness or Faith's phylogenetic
183 diversity (Table S3, Figure S3). Thus, rainfall was associated with the relative abundance of ASVs
184 within a sample but not the absolute number of ASVs or their phylogenetic diversity.
185
186 **Table 1.** Determinants of alpha diversity, as measured by the Shannon index.

Fixed factor	Estimate	SE	95% confidence interval	LRT	P-value
Sex (male)	-0.12	0.04	[-0.20 ; -0.05]	9.27	**0.002**
Age	0.01	0.02	[-0.02 ; 0.04]	0.49	0.484
Cumulative rainfall	0.05	0.02	[0.02 ; 0.08]	12.13	**<0.001**
Min temperature	0.00	0.01	[-0.03 ; 0.03]	0.04	0.848
Sequencing depth	0.07	0.01	[0.04 ; 0.10]	24.24	**<0.001**

187 Parameters and tests are based on linear mixed models of 758 samples and 131 individuals, controlling for individual identity and
188 unit membership. Factors with p-values less than 0.05 are highlighted in bold.

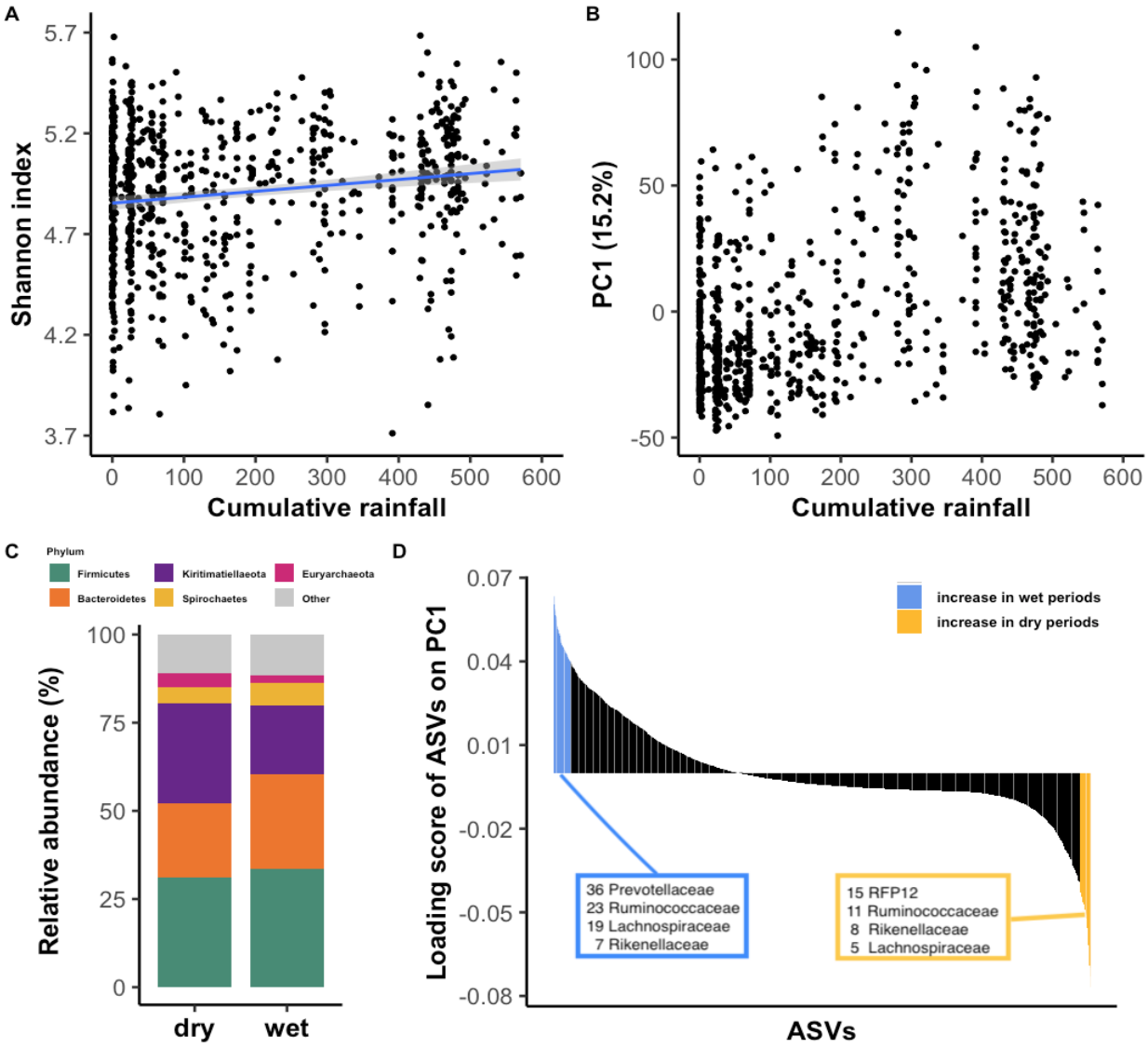

Figure 2. Rainfall structures the gelada gut microbiome. (A) Partial residual plot of Shannon alpha diversity index according to cumulative rainfall (in mm). Black dots represent the partial residuals from the LMM (i.e. showing the association between cumulative rainfall and alpha diversity, while controlling for all other predictors). The blue line and confidence intervals come from a linear regression (for representation only). Seven outlier samples (with a particularly low Shannon index) were omitted for clarity of representation. **(B)** Visualization of between-sample dissimilarity (based on Aitchison distance) on the first principal component (PC1) according to cumulative rainfall. **(C)** Compositional barplot of the five most abundant phyla in the dry (<100mm of rain in the past month, N=362) and wet (>200mm of rain in the past month, N=282) samples (cumulative rainfall was converted to a categorical variable for representation purposes). **(D)** Loading scores of each amplicon sequence variant (ASV) on the first principal component. ASVs with a loading score >0.4 (characteristics of the wet season) and <-0.4 (characteristic of the dry season) are colored.

Cumulative rainfall significantly explained 3.3% of the overall compositional dissimilarity - or beta diversity - between samples (as measured by Aitchison distance) (Table 2), which was nonetheless less than that explained by two demographic variables, individual identity and unit (social group) membership (20% and 6%, respectively; Table 2). The first principal component of beta diversity, which explained 15% of variation, was strongly associated with rainfall ($r=0.43$, $t=12.93$, $df=756$, $p<0.001$, Figure 2B). The ASVs that loaded positively on PC1 (i.e. correlated with higher rainfall, Figure 2D) were primarily from the families *Prevotellaceae*, *Ruminococcaceae*, and *Lachnospiraceae* (Table S4 and S5). By contrast, the ASVs that loaded negatively on PC1 (i.e. more abundant in low rainfall, Figure 2D) belonged to the family *RFP12* and a different subset of *Ruminococcaceae* that were not abundant during the wet season (Table S4 and S5).

Table 2. Results of PERMANOVA testing for the predictors that significantly structure the gut microbiome of geladas, using 10,000 permutations and the Aitchison dissimilarity distance between samples. The R-squared values indicate the amount of between-sample variation explained by each variable.

Factor	R2 (%)	P-value
Individual[1]	20.25	**<0.001**
Sequencing depth[2]	3.77	**<0.001**
Unit[2]	5.84	**<0.001**
Cumulative rainfall[2]	3.30	**<0.001**
Min temperature[2]	0.33	**<0.001**
Sex[2]	0.23	**0.012**
Age[2]	0.19	0.045

[1] We first fit a model with individual identity as the only predictor in a PERMANOVA to estimate the sole effect of individual identity at explaining the overall gut composition of samples.

[2] We then fit a second PERMANOVA model where all other predictors were fit, stratifying on individual identity to control for pseudoreplication of samples from the same individual.

Cumulative rainfall predicted the relative abundance of gut microbes at all taxonomic levels and was significantly associated with the relative abundance of 63% of bacterial families tested (59-81% of taxa at other taxonomic levels, Figure 3, $p_{BH}<0.05$). Thus, across most taxa, there was a clear contrast in the relative abundance of gut bacteria between the wet and dry periods (Table S6, Figure 4). In wetter periods, there was an increase in several important fermentative families from the *Bacteroides* order (including *Prevotellaceae* and *Bacteroidaceae),* as well as in several cellulolytic/fibrolytic taxa (*Lachnospiraceae, Fibrobacteraceae, Spirochaetaceae* and several genera from the *Ruminococcaceae*; Figure 4 and 5A), suggesting improved digestive efficiency of plant cell wall polysaccharides at a time when the gelada diet consists mainly of grasses. In particular, nine *Prevotella* genera as well as the *Bacteroides* genus were higher during wetter periods than drier periods (Table S6, Figure 4B). There was also an increase in several proficient cellulolytic genera (e.g. *Senegalimassilia*, *Butyrivibrio*, *Saccharofermentans*, *Cellulosilyticum*, *Marvinbryantia*) (Table S6, Figure 4B). By contrast, the dry season was characterized by an increase in amylolytic genera (*Succinivibrio*; *Streptococcus* and *Pirellulaceae p-1088-a5 gut group*), in several efficient sugar-fermenting families (*Victivallales vadinBE97*, *Christensenellaceae*), and in the methane-producer *Methanobrevibacter*, a genus known to increase the rate of fermentation and digestive efficiency (Table S6, and Figures 4 and 5B). Consistent with our beta diversity analyses, we also found an increase in the relative abundance of the *RFP12* family during the dry season (Table S6, Figure 5B).

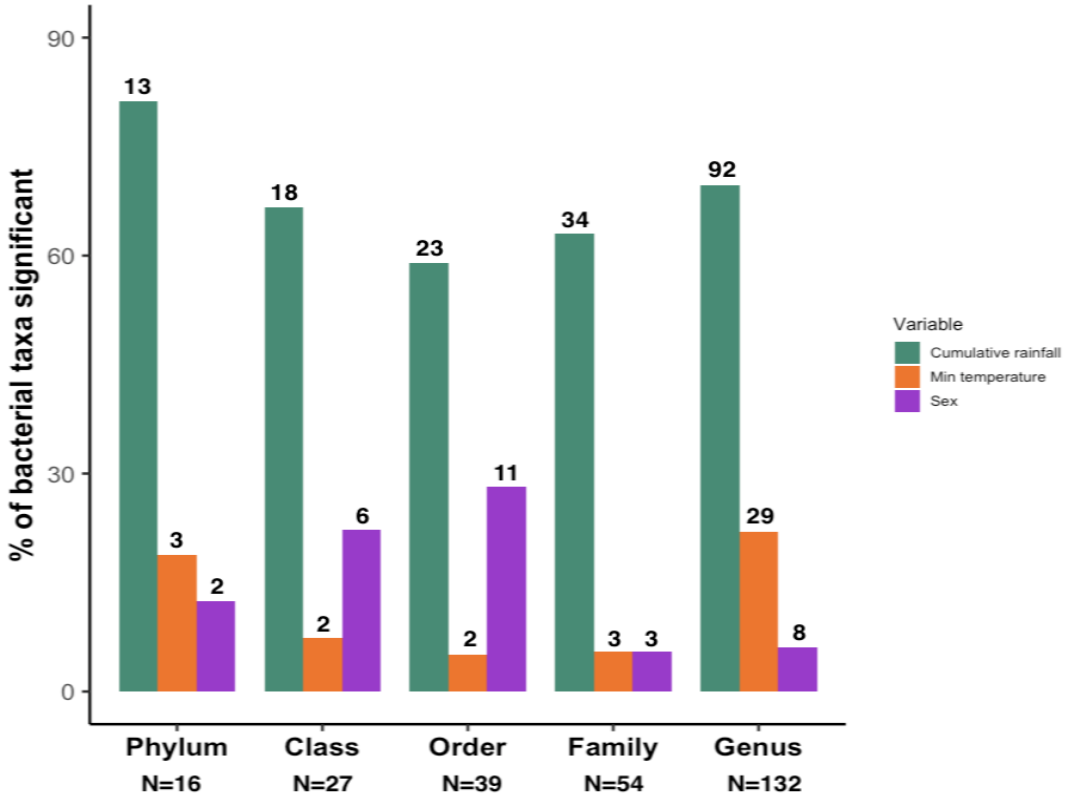

Figure 3. Rainfall exerts the strongest effect on bacterial relative abundance. Percent of taxa that are significantly associated (p_{BH}<0.05) with rainfall (purple bars), temperature (orange bars), or sex (green bars), across five taxonomic levels. For a given bacterial taxa, the significance of each predictor was assessed using a negative binomial GLMM fitted on the count of this taxa per sample (controlling for sequencing depth as an offset factor, and including individual and unit membership as random effects). Only taxa with $p_{BH} < 0.05$ were considered significant. The numbers above the bars depict the number of taxa significantly differentially abundant, while the numbers below indicate the total taxa measured per level. Age was not significantly associated with relative abundance of any taxa at any level.

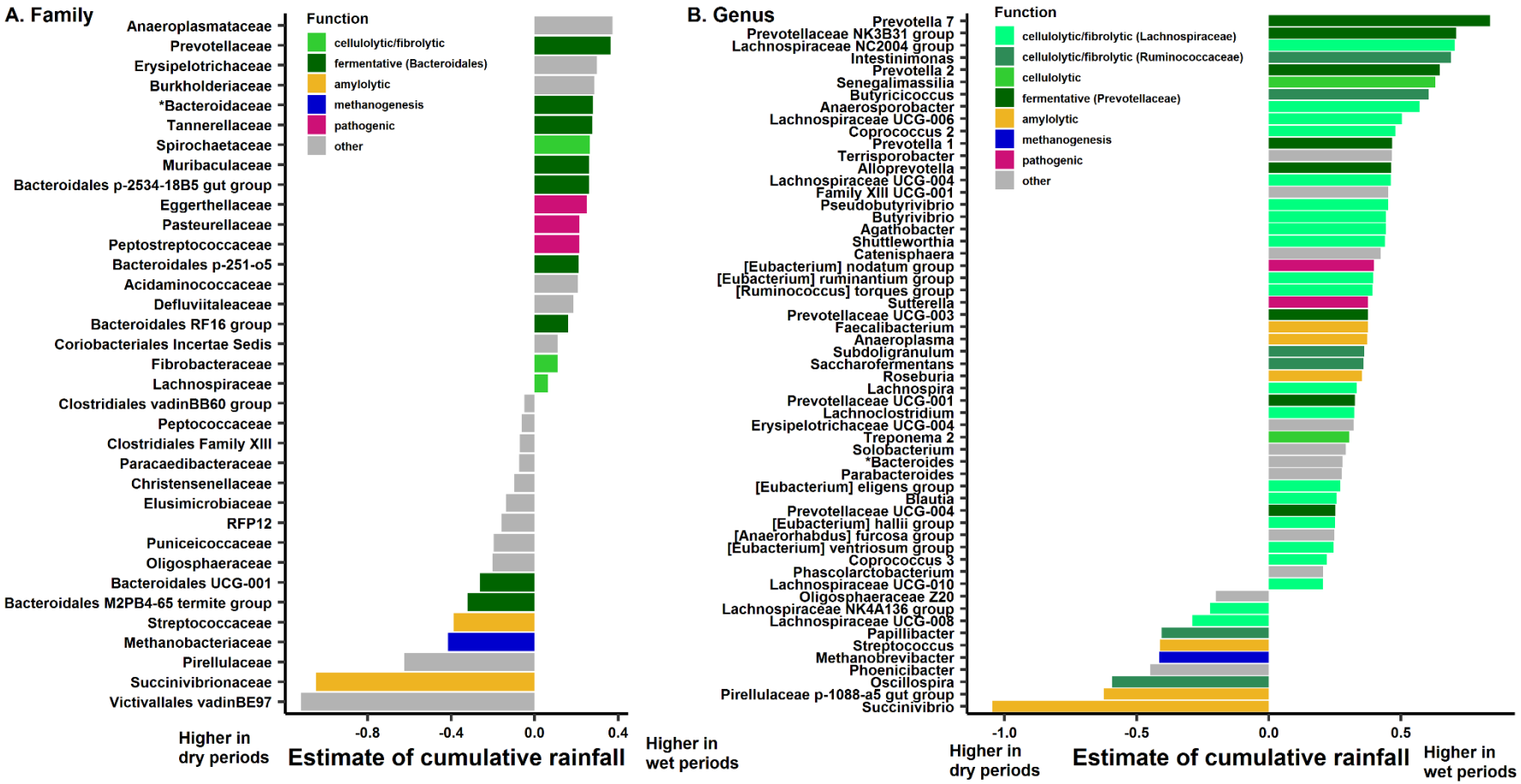

Figure 4. Rainfall predicts the relative abundance of many bacterial taxa. (A) Families and **(B)** Genera that are found differentially abundant according to cumulative rainfall. The estimate for each taxa of the cumulative rainfall effect comes from a negative binomial GLMM fitted on the count of this taxa per sample (controlling for sequencing depth as an offset factor, and including individual and unit membership as random effects). Taxa starting with "*" were fit with a binomial model instead. Only taxa with $p_{BH} < 0.05$ were considered significant. For ease of representation on panel B, only genera with effect sizes > |0.2| are represented. The full list of differentially abundant genera can be found in Table S8. Assignment of the "broad function" of a family or genus is for representation only, and is a simplification of the various functions subsumed within each taxonomic group.

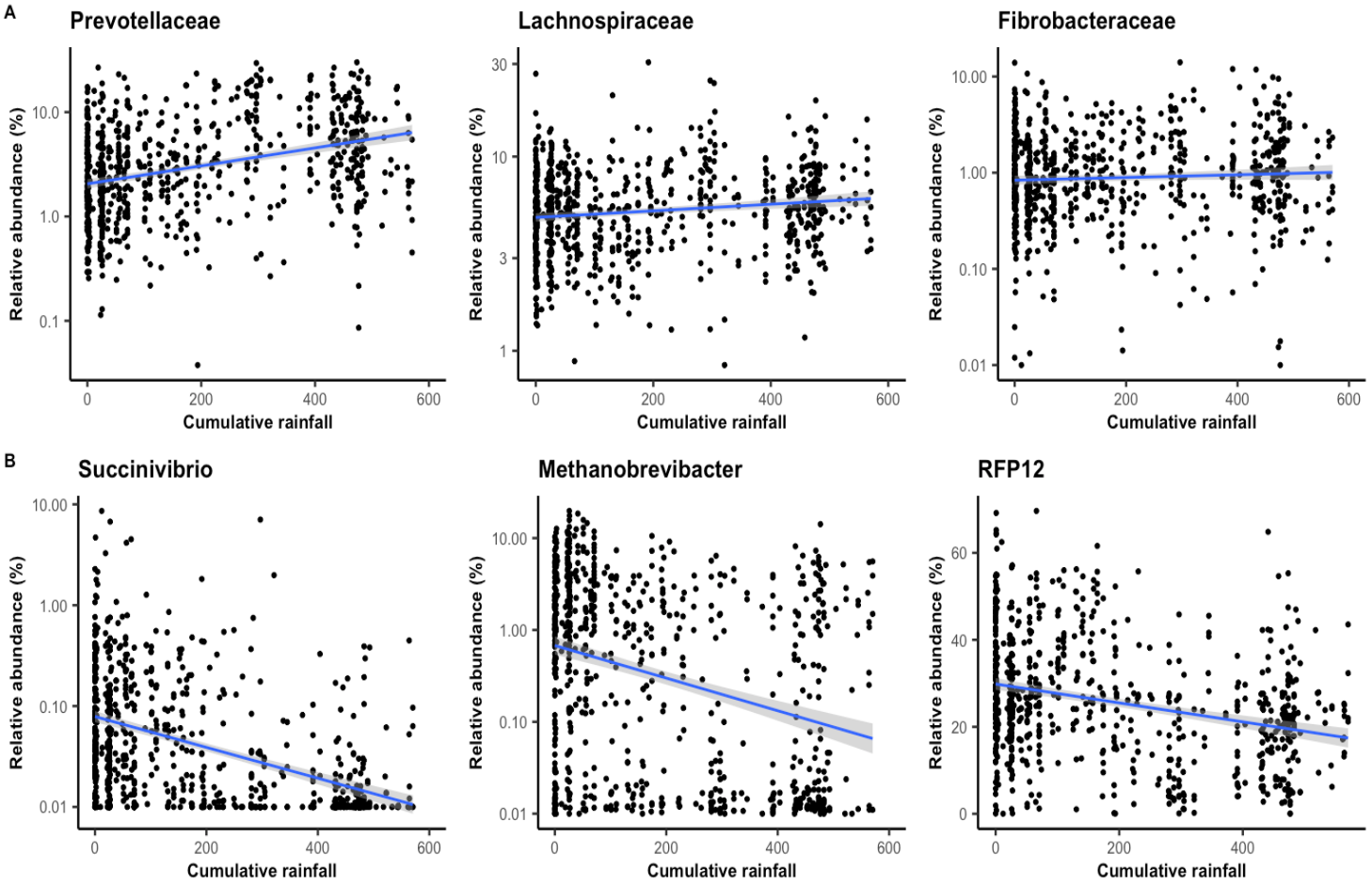

Figure 5. Relative abundance in six bacterial taxa (family or genus) that are significantly associated with rainfall. (A) more abundant during the wet season and **(B)** more abundant during the dry season. Note that the tick marks on the y-axis are spaced on a log10 scale (except for RFP12 which is plotted on a raw scale because of its high abundance). The blue line and confidence intervals come from a linear regression (for representation only). The significance of those effects have been estimated using negative binomial GLMMs including individual and unit membership as random effects.

The taxonomic changes associated with rainfall also corresponded to changes in the predicted function of the gelada gut microbiome (as assessed by PICRUSt2: (Douglas et al. 2019), NSTI mean±SD=0.60±0.13). During the wetter periods, functional changes tended to reflect the activity of the cellulolytic and fermentative bacterial taxa. Microbial pathways involved in the transport of molecules through bacterial membranes (e.g. ions, sugars, lipids, peptides), DNA replication and repair, and cell motility (Tables S7-S8, Figure 6, S4 and S5A) increased. We further found an increase in the metabolism of sugars (e.g. starch and sucrose metabolism, fructose, mannose, and galactose) (Figure S4 and S5A). Such activity probably reflects the exportation of sugar-cleaving enzymes and cellulosome complex across the outer membrane(Biddle et al. 2013; White et al. 2014) of fibrolytic bacteria (complex polysaccharides are too big to penetrate directly inside bacteria and have to be cleaved first) and the absorption of the soluble oligosaccharides back across the bacterial membrane (Biddle et al. 2013; White et al. 2014).

During drier periods, the gelada gut harbored a greater abundance of bacterial genes involved in energy, amino acid, and lipid metabolism (Tables S7-S8 and Figure 6A,C). In particular, cellular energy production and cellular activity were enhanced during this period, as evidenced by increases in pathways involved in the citric acid cycle, oxidative phosphorylation, and fatty acid synthesis and metabolism (Figure S4 and S5B). Other energy metabolism pathways also increased during drier periods, including the methane pathway and the carbon fixation pathways, which are important for generating energy in anaerobic bacteria (Figure S4 and S5B). Finally, drier periods were associated with an increase in functions related to the synthesis of proteinogenic amino acids (e.g. tryptophan), the translation and synthesis of proteins (Figure S4), and the synthesis of lipopolysaccharide.

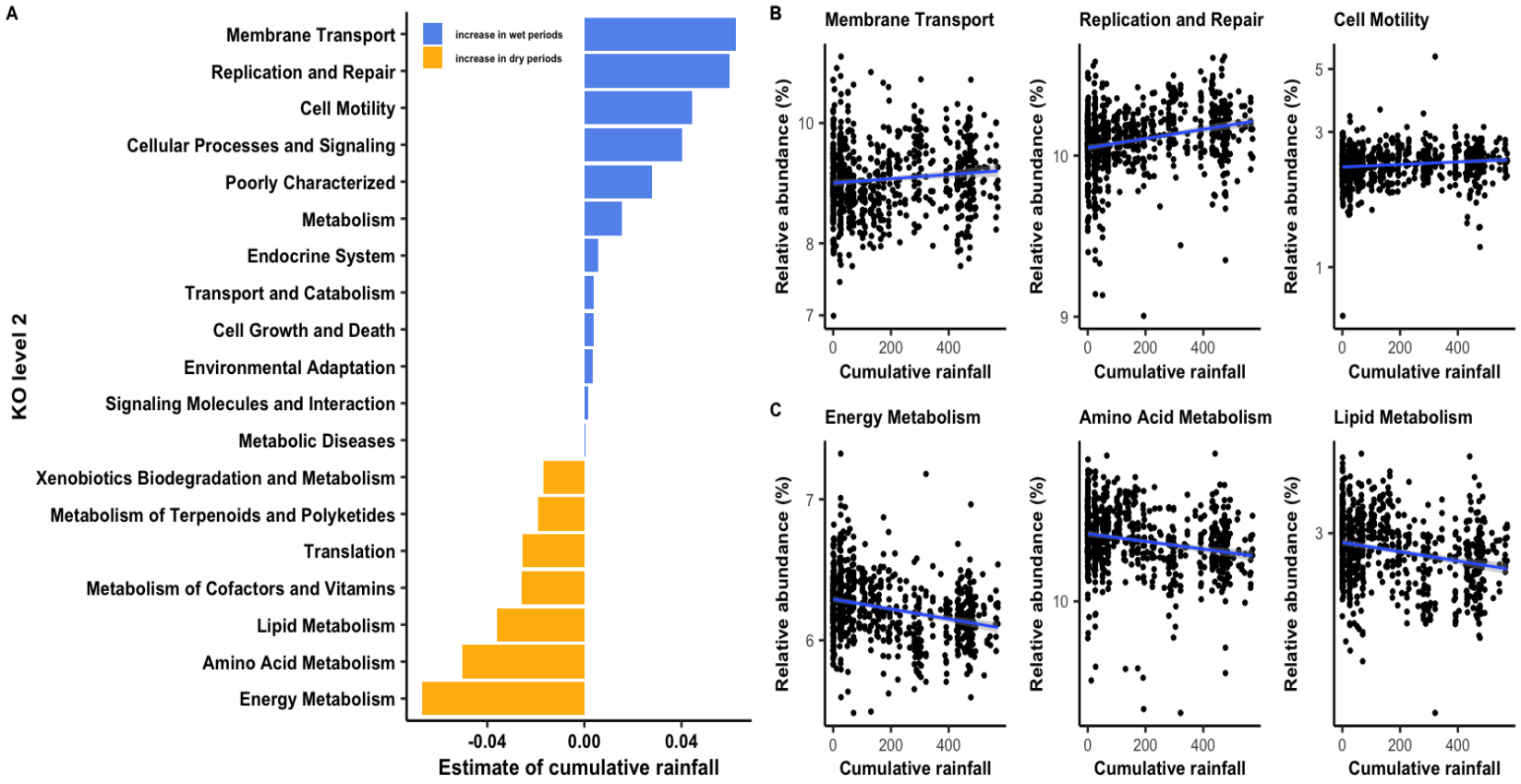

Figure 6. Rainfall predicts the functional profile of the gut microbiome. (A) Bacterial pathways at level 2 of KEGG Orthology (KO) that are differentially abundant according to cumulative rainfall (in mm). The estimate of the "rainfall" effect for each pathway comes from a LMM fitted on the relative abundance of each pathway per sample. Only pathways with $p_{BH}<0.05$ are reported. Relative abundance of the three most enhanced functional pathways during **(B)** the wet season and **(C)** the dry season according to monthly cumulative rainfall. Note that the tick marks on the y-axis are spaced on a log10 scale. The blue line and confidence intervals come from a linear regression (for representation only). The significance of the rainfall effect effects per pathway have been estimated using LMMs including individual and unit membership as random effects.

Temperature

Compared to rainfall, minimum temperature had a much smaller impact on the gut microbiome. Average minimum temperature did not influence any metric of alpha diversity (Table 1 and S3, Figure S6A), and explained only 0.33% of the variation in beta diversity (Table 2, Figure S6B). Changes in temperature were significantly associated with the relative abundance of 5% of the families (5-22% at other taxonomic levels; Figure 3; $p_{BH}<0.05$). More specifically, colder temperatures were characterized by a greater abundance of two amylolytic genera (*Lactobacillus* and *Streptococcus*); in several sugar-fermenting (*Hydrogenoanaerobacterium*, *Clostridium sensu stricto 1, Coprococcus 1*) and cellulose-degrading bacteria (*Marvinbryantia* and two genera from the *Ruminococcaceae* family) (Table S6, Figure S7). By contrast, hotter temperatures were associated with an increase in *Verrucomicrobia*, in the methane-producer *Methanobrevibacter*, and in several cellulolytic/fibrolytic genera from the *Ruminococcaceae and Lachnospiraceae* families (Table S6, Figure S7).

Similar to our taxonomic analysis, we found that temperature had a much smaller effect of the predicted function of the gelada gut microbiome (Tables S7-S8, Figure S8). During colder periods, we found a predicted increase in bacterial pathways involved in lipid metabolism and energy production (notably in oxidative phosphorylation pathway; Figure S8). Other pathways that increased during colder periods involved DNA repair and recombination and the bacterial secretion system. During hotter weather, pathways were more-poorly characterized and less specific, with predicted increases in methane metabolism and ABC transport (a membrane transporter).

Sex, reproductive state, and age

The gut microbiome of females exhibited higher alpha diversity compared to males, regardless of the metric (richness, evenness, and Shannon index) (Table 1 and S3, Figure S9A). Across samples however, sex explained little between-sample variation (i.e. <1%) (Table 2, Figure S9B). We detected a handful of bacterial taxa that were differentially abundant according to sex (Table S6, Figure 3). At the phylum level, females harboured more *Verrucomicrobia* and *Proteobacteria* (particularly from class *Gammaproteobacteria*, *Deltaproteobacteria* and *Alphaproteobacteria*). At the family and genus levels, females had more taxa involved in lactic acid metabolism (*Lactobacillaceae*, *Anaerovibrio)*, cellulolysis (*Saccharofermentans*) and regulation of glucose and fat transport (*Erysipelatoclostridium*). Males, on the other hand, only harboured more *Pirellulales*. No predicted metabolic pathway was found to be differentially abundant with sex (Tables S7-S8).

Female reproductive state did not influence any alpha diversity metric (Table S9, Figure S10A) and was not a significant factor influencing beta diversity between samples (Table S10, Figure S10B). Very few taxa were differentially abundant according to female reproductive state (Table S11 and S12). Pregnant females harboured more *Verrucomicrobiota* (class *Verrucomicrobiae*) and *Epsilonbacteraeota* than cycling and lactating females (Table S12). In particular, the genus *Helicobacter* (within the family *Epsiolonbacteroaeto*) was highly prevalent in pregnant females (Table S12), is a presumed pathogen. No predicted metabolic pathways were found to differ based on reproductive state (Table S13-S14). Age did not influence any metric of alpha diversity (Table 1 and S3, Figure S11A) or beta diversity (Table 2, Figure S11B), and no bacterial taxa (Table S6) or predicted metabolic pathways (Table S7-S8) were differentially abundant between young and old adults.

DISCUSSION

Our findings are consistent with the hypothesis that changes in the gelada gut microbiome may help animals cope with the altered food availability and increased thermoregulatory demands associated with seasonality. First, the gelada gut microbiome was highly plastic and responded rapidly to seasonal fluctuations in climate – particularly rainfall (a proxy for available foods). Second, an increase in predicted bacterial functions involved in energy, amino acid, and lipid metabolism during both drier and colder periods suggested increased production of SCFAs, and more efficient digestion in energetically and thermoregulatory challenging periods. We further found that individual identity and social group explained nearly a third of the variation of the gelada microbiome, while other individual traits such as sex, reproductive state, and age had little effect on gut microbiome composition and function.

Rainfall was the strongest ecological factor influencing changes in the gelada gut microbiome, explaining ~3.3% of overall microbiome composition. In particular, cellulolytic/fibrolytic and fermentative bacterial taxa increased during wetter periods when grass, which is mostly composed of cellulose, was the primary food source, while amylolytic and methanogenic bacterial taxa increased during drier periods, when geladas incorporated more starch (i.e. amylose) and lignified food into their diet. This effect of rainfall on the gut microbiome was strong, despite the fact that geladas exhibit only moderate dietary changes (i.e. from only grass to less grass and more underground organs - but from the same plant species) compared to other mammals living in more seasonal environments, e.g. that switch from ripe fruits to more folivorous diets (black howler monkeys: Amato et al. 2015; gorillas, *Gorilla gorilla gorilla* and *G. beringei beringei*: Gomez et al. 2016; Hicks et al. 2018). This pattern highlights the importance of the gut microbiome for geladas in processing their unique diet across seasons.

The efficiency of grass digestion in wet periods seems to rely on a syntropy between the first cellulolytic degraders (*Ruminococcaceae*, *Lachnospiraceae*, *Fibrobacteraceae*, *Spirochaetes)* and a high diversity of secondary fermenters (*Prevotellaceae* and *Bacteroidales)*, which all increase in abundance during the wet season. The first degraders attach first to the plant cell walls and hydrolyse cellulose, hemicellulose, and xylan into smaller polysaccharides and oligosaccharides (Biddle et al. 2013; White et al. 2014), while secondary fermenters ferment those soluble polysaccharides into more simple sugars (Flint et al. 2008, 2012). *Ruminococcaceae* and *Lachnospiraceae* are the two main cellulolytic taxa in mammalian gut and are commonly increasing in prevalence when animals eat more leaves and plants (Amato et al. 2015; Springer et al. 2017). In terms of secondary fermenters, *Prevotella* are widely known for their role in breaking down non-cellulosic polysaccharides and pectin (Flint et al. 2012; White et al. 2014). They are the major constituent (~70%) of rumen bacteria (van Gylswyk 1990), and commonly increase in high fiber or fruit diets (Rampelli et al. 2015; Kovatcheva-Datchary et al. 2015; Gomez et al. 2016; Springer et al. 2017). Members of *Bacteroidales* - and particularly from the *Bacteroides* genus - have some of the largest repertoires of carbohydrate degrading activities and are able to ferment a broad range of plant polysaccharides (Salyers et al. 1977; Comstock and Coyne 2003; Flint et al. 2012; El Kaoutari et al. 2013). The increase in these cellulolytic/fibrolytic taxa and the high versatility of the secondary fermenters likely allow geladas to optimally extract nutrients from grasses eaten during wet periods.

In contrast, during drier periods, when geladas relied more on underground storage organs, we found a corresponding increase in microbial families involved in amylolytic and saccharolytic activities (*Succinivibrionaceae*, *Streptococcaceae*, *Christensenellaceae*). Interestingly, *Succinivibrionaceae* also increased during periods of energetic stress in Tibetan macaques

(*Macaca thibetana*) (Sun et al. 2016) and during the dry season in the Hazda hunter gatherers of Tanzania (Smits et al. 2017), suggesting that it might help hosts cope with diet-related energy shortfalls. The gelada microbiome during the dry season was also characterized by an increase in *Methanobrevibacter*, a genus containing hydrogenotrophic archaea that converts hydrogen and formate into methane (Miller et al. 1982). The simultaneous enrichment of efficient hydrogen-producers (e.g. *Christensenellaceae (Morotomi et al. 2012)*, *Hydrogenoanaerobacterium*: *(Song and Dong 2009)*) and formate-producers (*Succinivibrionaceae*: *(O'Herrin and Kenealy 1993)*), combined with methanogens during the dry season suggest that these taxa work together in syntropy to improve the efficiency of polysaccharide fermentation from starch in the gut in dry periods (Samuel and Gordon 2006; Basseri et al. 2010). In mice and humans, a higher abundance of methanogenic archaea was found to increase calorie harvest from diet, facilitate SCFA production by other fermentative bacteria, and stimulate lipogenesis (Samuel and Gordon 2006; Zhang et al. 2009; Basseri et al. 2010; Mathur et al. 2013).

Finally, drier periods were also characterized by a large increase in the *RFP12* family (i.e. ~30% versus ~18% in wetter periods) from the *Kiritimatiellaeota* phylum. The *RFP12* family remains poorly characterized but is increasingly recognized as being a keystone bacterial group in the hindgut of horses (Steelman et al. 2012; Costa et al. 2015; Edwards et al. 2020), and a common inhabitant of the rumen of sheep or cattle (Wang et al. 2017; De Mulder et al. 2017; Ribeiro et al. 2017). This suggests that it might be a keystone bacterial group for the digestion of some underground food components commonly eaten by the geladas during dry periods.

At the functional level, bacterial genes involved in energy, amino acid, and lipid metabolism increased in prevalence during the dry season. In particular, metabolic pathways linked to cellular respiration, methanogenesis, and carbon fixation pathways of prokaryotes

403 became more common, strongly suggesting that both bacterial energy production and cellular
404 activity were stimulated during this time. One interpretation of this data is that the increase in
405 cellular activity simply reflects a dietary switch to starch, which is easier to hydrolyse than
406 cellulose, and thus might more readily provoke a stimulation of bacterial activity and carbohydrate
407 fermentation. Alternatively, the stimulation of bacterial energy metabolism and cellular activity
408 could reflect a higher production of SCFAs by gut bacteria, supplying the host with additional
409 energy in periods of nutrient restriction (when relying on fallback foods) (Russell and Rychlik
410 2001; Zhang et al. 2016). Similar increases in predicted bacterial energy metabolism have been
411 found in energetically challenging environments (e.g. high altitude) in several other mammalian
412 species and were correlated with higher SCFA production (Zhang et al. 2016; Li et al. 2018).
413 Analysis of fecal SCFA profiles in geladas would help to identify if this is also the case in this
414 high-altitude species.

415 While it is clear that diet shifts during drier periods, it remains unknown if (and to what
416 extent) geladas are nutritionally or energetically constrained during this time. Grass availability
417 declines and geladas spend more time foraging and digging for underground plant parts during the
418 dry season (Hunter 2001; Jarvey et al. 2018). Such underground foods are usually considered
419 fallback foods because individuals rely on them only when grass is less available and because they
420 require long processing times (Venkataraman et al. 2014; Jarvey et al. 2018). However, one study
421 (Hunter 2001) found that geladas obtain just as much, or even more, calories from underground
422 storage organs as they do from grass. Whether this increased caloric intake is offset by increased
423 foraging costs is currently unknown. However, even if increased foraging costs were
424 demonstrated, our data suggests that the gut microbiota may increase digestive efficiency from

425 starchy food and thereby help geladas maintain or improve energetic status during the dry season.
426 Future studies on seasonal changes in energy balance will help resolve this issue.

427 In contrast to the effect of rainfall, we found mixed evidence for the effect of temperature
428 on the gut microbiome. Temperature only explained ~0.33% of variation in the gelada gut
429 microbiome composition. Furthermore, few taxa shifted in abundance between the coldest and
430 hottest months, and most taxa affected by temperature were also affected by rainfall (although the
431 reverse was not true). This might be explained by the fact that rainfall (and thus diet) still covary
432 with temperature to some extent (Pearson's correlation coefficient = 0.20): geladas rely the most
433 on underground foods in the hot-dry season (Feb to May) and the most on grass on the cold-wet
434 season (Jun to Sep) (Jarvey et al. 2018). The cold-dry season (Oct to Jan), however, displays a
435 mixed pattern of diet and temperature: grass availability is still high in Oct-Nov (following the
436 rainy season) but decreases markedly in Dec-Jan (Jarvey et al. 2018; Tinsley Johnson et al. 2018).
437 These two months are thus characterized by the introduction of underground foods in the diet and
438 are also incidentally the coldest months of the year, making them likely the most challenging times
439 for geladas (compounding nutritional and thermoregulatory challenges). Accordingly, cold periods
440 were characterized by an increase in two amylolytic and lactate-producing taxa (*Streptococcus*,
441 *Lactobacillus*), presumably to more efficiently extract starch from the underground foods. At the
442 functional level, the energy and lipid metabolism of bacteria were also stimulated in the cold
443 months, further suggesting some role of gut bacteria in stimulating host digestive efficiency and
444 energy metabolism during thermoregulatory-demanding times.

445 These seasonal changes that increase energy production during colder periods may come
446 at some cost. Such trade-offs have been proposed where shifts that benefit one aspect of host
447 physiology consequently lead to a decrease in other microbes that may also be necessary for the

host. For example, microbes that promote host digestive efficiency and energy metabolism may also promote inflammation or even suppress immune function (Vijendravarma et al. 2015; Reese and Kearney 2019). We did not detect any obvious evidence of these tradeoffs in geladas, but future work that incorporates detailed host immunological and functional microbial data is needed to help determine if such trade-offs exist.

Finally, the present study found that the gelada gut microbiome was largely explained by individual identity (20%), a pattern consistent with data from a range of vertebrates (Bik et al. 2016; Antwis et al. 2018; Trosvik et al. 2018; Kolodny et al. 2019), including humans (Costello et al. 2009; Human Microbiome Project Consortium 2012). However, the effect of social group was lower in geladas than reported for other social mammals (geladas: 6.0% vs. e.g. yellow baboon, *Papio cynocephalus*: 18.6% of variation explained (Tung et al. 2015), black howler monkey: 14% (Amato et al. 2017), ring-tailed lemurs, *Lemur catta*: 21 % (Bennett et al. 2016), Welsh Mountain ponies: 14%: (Antwis et al. 2018)). The combination of large individual effects with weak unit effects closely resembles data reported for the Guassa gelada population (Trosvik et al. 2018), suggesting a general, but consistent gelada pattern. The weak unit-level effects may result from the unique social system of geladas: because social units often aggregate into large bands whose composition change regularly, geladas may be characterized by a higher rate of inter-unit microbial transmission compared with other primates. Future studies should explore in more detail the intra-individual fluctuation in gut microbiome composition, and whether group differences in ranging patterns may explain these differences.

Other individual predictors, namely age, sex, and female reproductive state, had a very limited effect on the gut microbiome, mirroring results in other mammals (yellow baboons: (Tung et al. 2015; Ren et al. 2016), ring-tailed lemurs: (Bennett et al. 2016), Verreaux's sifakas,

Propithecus verreauxi: (Springer et al. 2017), chimpanzees, *Pan troglodytes schweinfurthii*: (Degnan et al. 2012), rhesus monkeys, *Macaca mulatta*: (Adriansjach et al. 2020), Welsh Mountain ponies (Antwis et al. 2018), domestic dog, *Canis lupus familiaris*: (Mizukami et al. 2019), but see black howler monkeys: (Amato et al. 2014) or Egyptian fruit bats, *Rousettus aegyptiacus*: (Kolodny et al. 2019)). Although female geladas harbored higher microbial richness than males, this resulted in minimal differences in gut microbial composition and predicted function. Compared to males, females had higher abundance of *Proteobacteria* and *Lactobacillus*. These two bacterial taxa that were previously reported to increase during pregnancy and lactation in humans and primates (Koren et al. 2012; Mallott and Amato 2018), and that act as early colonizers of the infant gut (Matsumiya et al. 2002; Martín et al. 2007; Shin et al. 2015). Additionally, pregnant female geladas harbored more *Helicobacter*, a potentially pathogenic genus (Chichlowski et al. 2008; Gao et al. 2018). An increase in potentially pathogenic microbes in pregnant females was also observed in black howler monkeys (Amato et al. 2014) and was hypothesized to be the consequence of a trade-off between reproduction and immunity. These dynamics warrant further investigation.

Overall, the gut microbiome of geladas seems to be highly plastic and can respond rapidly to changes in host diet and thermoregulatory demands. Stimulation of bacteria cellular activity could allow geladas to maintain adequate or even improved energetic balance during dry and cold periods. Our study adds to an increasing body of literature suggesting that the gut microbiota is an important system providing dietary and metabolic flexibility for the host and might be a key factor influencing the acclimatization to changing environments (Candela et al. 2012; Alberdi et al. 2016; Macke et al. 2017). In addition to fostering phenotypic plasticity, the gut microbiome is increasingly hypothesized to contribute to host evolution and speciation (Amato 2016; Alberdi et

al. 2016; Macke et al. 2017) given the strong host phylogenetic signal in mammalian microbiome composition and function (Groussin et al. 2017; Amato et al. 2019) and evidence of microbiome heritability (Goodrich et al. 2014; Blekhman et al. 2015; Waters and Ley 2019). To the extent that microbiomes affect host phenotypes under selection, they will also affect host evolutionary trajectories. In the case of geladas, a shift in gut microbiome composition was probably an important adaptive mechanism that allowed members of the *Theropithecus* genus to adopt a specialized dietary niche and diversify rapidly from *Papio* ~5 million years ago (Jablonski 2005). Contrary to host adaptive genetic mutations, which occur over the course of many generations, the gut microbiota can shift in response to changes in host diet in a matter of days (David et al. 2014). Given that the common ancestor of *Theropithecus* and *Papio* was omnivorous (Jolly 1970; Dunbar 1976), dietary flexibility provided by the gut microbiome may have been an important first step allowing members of *Theropithecus* to exploit new grassland habitats in East Africa, leading to the evolution of a specialized diet and, ultimately, further genetic and phenotypic adaptation. Future research in geladas and other mammals with peculiar dietary adaptations will further uncover how the gut microbiota influences host ecology, fitness, and the evolution of wild animal populations, and determine how an adaptable and heritable microbial community might have played a key role in supporting expansion into new habitats.

MATERIAL & METHODS

Study population and fecal sample collection

We collected fecal samples from a wild population of geladas living in the Simien Mountains National Park, in northern Ethiopia (13º15′N, 38º00’E). Samples were collected over a four-year period between Jan 2015 and Feb 2019. Geladas live in multi-level societies, where reproductive units (comprising a leader male, several adult females, their offspring and occasionally 1–2

follower males) and bachelor groups (comprising between 1-10 young adult males) form the smallest levels of the society, that forage and sleep together in a "band" sharing the same homerange (Snyder-Mackler et al. 2012). Since Jan 2006, the Simien Mountains Gelada Research Project (SMGRP) has collected demographic and behavioral data on over 200 individuals from two bands. All individuals are habituated to human observers on foot and are individually recognizable. Dates of birth of individuals were established using a combination of known (N=42) and estimated (N=89) birth dates. Estimated birth dates were calculated by using the mean individual age at major life-history milestones in our population (e.g. sexual maturation or first birth for females and canine eruption for males) (Beehner et al. 2009; Roberts et al. 2017). Birth dates of unknown immigrant males were estimated using an established protocol based on body size and other age-related morphological characteristics (Beehner et al. 2009). Here, we focused only on samples from adult males and females. Adult males were included when they reached 7 years of age. At this age, males have reached adult body size in stature but not in weight (Beehner et al. 2009; Lu et al. 2016), and most males have dispersed into a non-natal group (i.e. 96% of our male samples, males could thus be leaders, followers, bachelors or natals). Adult females were included after they had experienced their first sex skin swelling, a marker of reproductive maturation (which is around 4.65 years old in our population (Roberts et al. 2017)).

Fecal samples of known adult and subadult male and female subjects were collected regularly and opportunistically during the study period. Immediately upon defecation, approximately 1.5 g of feces was collected in 3 ml of RNA later (Vlčková et al. 2012; Blekhman et al. 2016), stored at room temperature for up to two months, and subsequently shipped to the University of Washington (UW). At UW, samples were stored at -80°C until the sequencing libraries were prepared. A total of 758 samples (620 female samples, 138 male samples) were

collected from 131 individuals (83 females, 48 males) (mean±SD=5.79±6.14 samples per individual, range=1-21) from 28 reproductive units and 4 bachelors groups (mean±SD= 4.69±2.97 number of individuals sampled per unit, range=1-11).

The reproductive state of females at the date of sample collection was assigned based on daily monitoring of individuals for the status of sex skin swellings and the birth of infants. We assigned the three reproductive states as follows: (1) Cycling began at the first sign of postpartum sex skin swelling and ended when a female conceived - with conception defined as 183 days (mean gestation length) before the birth of a subsequent infant (Roberts et al. 2017). (2) Pregnancy started on the date of conception and ended the day before parturition. (3) Finally, lactation started on the day of parturition and ended the day before the female's first postpartum swelling. Lactating females were further categorized as being in early lactation (infant <1 year old) or late lactation (infant >1 year old). When testing the effect of reproductive state, late lactating females were removed from the lactating category to include only females that were still nursing at the time of sample collection (females resume cycling when infants are ~1.5 year old in our population, which is presumably accompanied by infant weaning around the same time (Roberts et al. 2017)). Furthermore, because pregnant females can abort their fetus during male takeover of their reproductive unit (Roberts et al. 2012), some pregnancies might have been misidentified as cycling based on our method of back-calculating from the date of birth. We therefore removed cycling females that experienced a takeover in the previous 6 months before the date of sample collection (N=55 samples) to avoid any misclassification of reproductive state in our analyses.

Study site and climatic data

563 The study area is located at 3200m above sea level and is characterized as an Afroalpine grassland
564 ecosystem, consisting of grassland plateaus, scrublands, and Ericaceous forests (Puff and
565 Nemomissa 2005). Fecal samples were collected across the year, with roughly equal coverage
566 across seasons (244 in cold-dry, 298 in cold-wet and 216 in hot-dry season as defined above). As
567 part of the long-term monitoring of the SMGRP, daily cumulative rainfall and minimum and
568 maximum temperature are recorded on a near-daily basis. We used the total cumulative rainfall
569 over the 30 days prior to the date of fecal sample collection as a proxy for grass availability at the
570 time of sample collection (Jarvey et al. 2018). In addition, we used the average minimum daily
571 temperatures in the 30 days preceding the date of sample collection as a proxy of thermoregulatory
572 constraints. The average minimum temperature is less correlated with cumulative monthly rainfall
573 than the average maximum temperature in the previous 30 days (correlation coefficient: 0.25
574 versus -0.56) and, more importantly, is more likely to reflect the physiological effect of
575 thermoregulation on the body (Beehner and McCann 2008; Tinsley Johnson et al. 2018).
576
577 ***DNA extraction, sequencing, and data processing***
578 We prepared 16S sequencing libraries using the protocols developed and optimized by the Earth
579 Microbiome Project and the University of Minnesota Genomics Core (UMGC; (Gohl et al. 2016)).
580 We extracted microbial DNA from the fecal samples using Qiagen's PowerLyzer PowerSoil DNA
581 Isolation kit (Qiagen #12855) following the standard protocol. We amplified the hypervariable V4
582 region of the 16S rRNA gene using PCR primer set 515F
583 (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA) and
584 806R
585 (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACNVGGGTWTCTAA

T) from The Human Microbiome Project and a dual-indexing approach (Gohl et al. 2016). Details of the amplification protocol can be accessed at https://smack-lab.com/protocols/. The first PCR round aimed at amplifying the V4 region. Each 25 µl PCR reaction well consisted of 12.5 µl of Nebnext Ultra II Q5 mastermix, 1.0 µl of each primer, and 25 ng of total DNA in 10.5 µl of nuclease-free water. PCR was performed in an Eppendorf thermocycler with a 100°C heated lid using the following cycling steps: an initial denaturing for 5 min at 95°C; followed by 15 cycles of 20 s at 98°C, 15 s at 62°C, 60 s at 72°C; and a final hold at 4°C. We cleaned up the PCR reaction with a 2:1 ratio of SPRI beads to PCR amplified DNA. The second PCR round aimed at adding a unique index primer combination to molecularly barcode each sample. We took 4 µl of product from the first PCR and added 6 µl of Nebnext Ultra II Q5 mastermix and 1 µl of of n5 and n7 indexing primers, with each sample being assigned a unique n5/n7 index primer combination. This 12 µl reaction was placed in an Eppendorf thermocycler with a 100°C heated lid, denatured for 5 min at 95°C, and amplified with 10 cycles of 20 s at 98°C, 15 s at 55°C, and 60 s at 72°C with a final hold at 4°C. After a 2:1 SPRI bead clean-up, amplification of the V4 region was confirmed in a few samples using an AATI fragment analyzer, and all libraries were quantified using a qubit fluorometer. The libraries were then pooled in roughly equimolar amounts (each with their own unique indexing primer combination), spiked with 10% PhiX to increase library complexity, and sequenced together on a single Illlumina NovaSeq 6000 SP 250 bp paired-end sequence flowcell.

We analyzed the resulting data using the Quantitative Insights Into Microbial Ecology 2 (QIIME2) platform (Caporaso et al. 2010; Hall and Beiko 2018). After trimming low quality bases from the de-multiplexed reads, we merged overlapping paired-end reads, and denoised the sequencing data by filtering and correcting Illumina amplicon sequencing errors using the Divisive Amplicon Denoising Algorithm 2 (DADA2: (Callahan et al. 2016)) plugin incorporated in

609 QIIME2. DADA2 infers sequences exactly resulting in amplicon sequence variants (ASVs).
610 Forward and reverse reads were trimmed to 220 and 180 bases, respectively, to remove the low-
611 quality portion of the sequences. The forward and reverse reads were then merged together and
612 chimeric sequences were removed. Only samples with more than 20,000 reads were retained for
613 analysis (following observation of rarefaction curves, Figure S12). After filtering, trimming,
614 merging, and chimera removal, we retained a total of 348,390,395 reads across the 758 fecal
615 samples (459,618±815,020 reads per sample, range=20,109-10,735,588). ASVs were
616 taxonomically assigned using the q2-feature classifier in QIIME2 against version 132 of the
617 SILVA database (updated December 2017) (Quast et al. 2013) based on 100% similarity.
618 Uninformative taxonomic assignments of ASVs found in SILVA (e.g. "wallaby metagenome",
619 "unassigned bacteria", etc.) were converted to "NA" to simplify analysis at higher taxonomic
620 levels. All ASVs belonging to the order *WCHB1-41* (phylum *Kiritimatiellaeota*) were not assigned
621 at the family level in the SILVA classification. However, in the Greengene classification (version
622 13_8) all ASVs from this order in the gelada gut were assigned to the *RFP12* family. Thus, we
623 attribute the family *RFP12* to all ASVs from the order *WCHB1-41* in SILVA classification.
624
625 ***Statistical analyses***
626 The count and taxonomy files generated by QIIME2 were imported into R version 3.5.2 (Team
627 and Others 2013) using the qiime2R package (Bisanz 2008) and analyzed using the phyloseq
628 package (McMurdie and Holmes 2013). The majority of the 19,606 ASVs in our dataset were
629 found at very low frequency or only in one sample (71% of ASVs were found in only one sample
630 and 6.2% of ASVs were not assigned at the phylum level). Thus, we further filtered the count table
631 to retain only ASVs that had at least 500 reads in total in the dataset (i.e. 0.00014% relative

abundance) to eliminate potentially artifactual sequences. With this filtering criteria, only 3,295 ASVs remained, with all of them assigned at the phylum level and most (97%) observed in at least two samples (Figure S13). Most ASVs could be taxonomically assigned to the class and order levels (~99%), with assignments decreasing at the family (85%) and genus (61%) levels.

The use of rarefaction (i.e. subsampling of the read count in each sample to a common sequencing depth) has been discouraged due to the loss of information and precision (McMurdie and Holmes 2014), as well as the use of count normalization methods from the RNA-seq field (e.g. DESeq2 or edgeR). However, microbiome datasets are more sparse (zero-inflated) and more asymmetrical than genetic expression datasets (Gloor et al. 2017; Weiss et al. 2017). Thus, we used a compositional approach when possible (e.g. centered-log-ratio normalization of the counts and Aitchison distance for beta diversity analysis) (Gloor et al. 2016, 2017), controlling for sample sequencing depth in multivariate analyses to account for repeated samples from the same individual.

We replicated alpha- and beta-diversity analyses using traditional rarefaction methods to facilitate comparisons with other studies. To generate the rarefied dataset, we randomly sampled 20,000 reads from the raw fastq files of each sample and processed this new rarefied dataset into the DADA2 pipeline. This dataset was further filtered to remove the low frequency ASVs (i.e. ASVs not included in the pool of 3,295 ASVs retained in the full dataset). This resulted in a dataset containing the same 758 samples, with 2853 ASVs and with relatively homogenous sequencing depth (18205±1415 reads per sample, range=7460-19444).

All mixed models described below were run using either the lmer (for linear mixed models, LMMs) or glmer (for binomial and negative binomial generalized linear mixed models, GLMMs) functions of the lme4 package (Bates et al., 2014). All quantitative variables (i.e. cumulative

rainfall, averaged temperature, and age) were z-transformed to have a mean of zero and a standard deviation of one to facilitate model convergence. The significance of the fixed factors was tested using a likelihood ratio test, LRT (assuming an asymptotic chi-square distribution of the test statistic) via the drop1 function. To test for significant pairwise differences between levels of multilevel categorical variables (i.e. reproductive state), *post hoc* Tukey's Honest Significant Difference tests were carried out using the multcomp package in R (Hothorn et al. 2008).

Alpha-diversity analyses

We calculated three measures of alpha diversity: observed richness (the total number of different ASVs in a sample), Shannon diversity index (accounts for both richness and evenness of ASVs in a sample), and Faith's phylogenetic diversity (accounts for phylogenetic distance between bacterial species, using the picante package (Kembel et al. 2010)). We modeled each alpha diversity metric using linear mixed models: (i) as a function of age, sex, cumulative monthly rainfall, average monthly minimum temperature, and sequencing depth of the sample (N=758 samples), and (ii) as a function of reproductive state (cycling, early lactating and pregnant), age, cumulative monthly rainfall, and average monthly minimum temperature in samples collected from females (N=439). Individual identity and unit membership were included as random effects to control for individual and unit repetition across samples. We also ran the same models on the rarefied dataset (Table S15).

Beta-diversity analyses

We then assessed how the same predictors were associated with between-sample community dissimilarity. To account for differences in sequencing depth between samples, the counts were

normalized using the centered-log-ratio (CLR) method (and using a pseudocount of 0.65 for zero counts) from the "compositions" package (van den Boogaart and Tolosana-Delgado 2008). We then calculated the Aitchison distance between samples (i.e. simply the Euclidean distance between samples after clr transformation of the counts) (Aitchison et al. 2000) and conducted a Principal Component Analysis (PCA) (function "prcomp") to visually represent between-samples dissimilarity according to the predictors. This approach has been recommended for microbiome datasets (Gloor et al. 2017), and allows for the projection of each sample onto individual principal components (PCS) and the variable loadings of ASVs onto each PC. While the first axis of variation correlated mostly with rainfall (Figure 1C), the second PCA axis was correlated with sequencing depth, and explained 11% of the variation (Figure S14). We used Permutational Multivariate Analysis of Variance (PERMANOVA) tests to assess the effect of the predictors on the Aitchison distance between samples (using using 10,000 permutations and the "adonis2" function from the "vegan" package (Oksanen et al. 2010)). We ran three different models: (1) including all samples where we tested only the effect of individual identity and sequencing depth, (2) including all samples where we tested the effect of unit, age, sex, cumulative monthly rainfall, average monthly minimum temperature, and sequencing depth of the sample and (3) including only female samples where we tested the effect of unit, reproductive state, age, cumulative monthly rainfall and average monthly minimum temperature. In models 2 and 3, individual identity was included as a blocking factor ("strata") to control for repeated sampling. We also replicated beta diversity analysis on the rarefied dataset. We ran PERMANOVA tests using three complementary pairwise dissimilarity metrics (Bray-Curtis distance, unweighted and weighted UniFrac distances) to assess between-sample variation according to the same predictors (the same three models). Beta diversity results remained qualitatively similar (Table S16).

701

702 Differential abundance testing

703 We examined how our predictors were associated with differential abundance of bacteria (at the
704 phylum, class, order, family and genus levels) using negative binomial GLMMs. Compared to
705 LMMs, negative binomial mixed models are better equipped to handle over-dispersed and zero-
706 inflated distributions that often characterize microbiome datasets (Zhang et al. 2018). They also
707 facilitate tests of several independent predictors while taking into account longitudinal designs
708 including random effects. We first aggregated the counts (i.e. the number of reads per taxa and per
709 sample) at the taxonomic level of interest. Only taxa that had an average relative abundance across
710 samples ≥0.01% were tested. Then, for a given taxa, the count per sample was modeled as a
711 function of: (1) age, sex, cumulative monthly rainfall and averaged monthly minimum temperature
712 (all samples), or (2) female reproductive state, age, cumulative monthly rainfall and averaged
713 monthly minimum temperature (female samples only). The log-transformed number of reads per
714 sample was included as an offset term to control for variation in sequencing depth across samples.
715 Individual identity and unit membership were included as random effects in all models. When
716 negative binomial models failed to converge in some taxa, we converted the counts in
717 presence/absence and modeled them with binomial GLMMs. Benjamini-Hochenberg corrected p-
718 values < 0.05 were considered statistically significant.

719

720 Functional profiling of microbiota

721 We estimated the bacterial and archaeal genes present in the metagenomes of each sample using
722 Phylogenetic Investigation of Communities by Reconstruction of Unobserved States version 2
723 (PICRUSt2) (Douglas et al. 2019). In brief, ASVs were aligned to reference sequences using

724 HMMER (Finn et al. 2011) and placed into a reference tree using EPA-NG (Barbera et al. 2019)
725 and gappa (Czech 2019). PICRUSt2 normalizes for multiple 16S gene copies in bacteria using
726 castor, a hidden state prediction tool (Louca and Doebeli 2018). The normalized data were used to
727 predict gene family profiles, and mapped onto gene pathways using MinPath (Ye and Doak 2009).
728 We followed the default protocols outlined on the PICRUSt2 GitHub page
729 (https://github.com/picrust/picrust2/wiki). We investigated the predicted gene families using the
730 Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology (KO) database. The accuracy of
731 the PICRUSt2 predictions for each sample were assessed by calculating the weighted Nearest
732 Sequence Taxon Index (NSTI) score, a measure of how similar the bacteria from the sample are
733 to reference genome sequences. The average NSTI value across all samples was high in geladas
734 (mean±SD=0.60±0.13) compared to other mammals (Douglas et al. 2019), so the results of this
735 analysis should be interpreted with caution. Five ASVs (out of 3295) had a NSTI score>2 and were
736 removed from our final predictions. The association between the relative abundance of functional
737 categories as estimated by PICRUSt2 and the predictors (on all samples or female samples only)
738 were examined using LMMs. Only functional pathways that had $\geq 0.1\%$ relative abundance across
739 samples were tested. Individual identity and unit membership were included as random effects in
740 all models.
741
742 **DATA AVAILABILITY**
743 All 16S sequence data used in this study are available at the NCBI Sequence Read Archive
744 (https://www.ncbi.nlm.nih.gov/) under BioProject ID PRJNA639843. Data and code (including
745 how to run the QIIME2 pipeline on our data) are available at:
746 https://doi.org/10.5281/zenodo.3932310.

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AUTHOR CONTRIBUTIONS

Conceptualization, Data curation, Formal analysis, Investigation, Visualization: A.B, A.L., N.S.M; Methodology: A.B., S.S., A.M., R.P.; Writing – Original Draft: A.B, A.L., N.S.M; Writing – Review & Editing: all authors; Funding Acquisition: A.L.,N.S.M.,J.C.B., T.J.B.,L.R.; Supervision: A.L. and N.S.M.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Adriansjach J, Baum ST, Lefkowitz EJ, van Der Pol WJ, Buford TW, Colman RJ. 2020. Age-related differences in the gut microbiome of Rhesus macaques. *J Gerontol A* **75**:1293-1298

Aitchison J, Barceló-Vidal C, Martín-Fernández JA, Pawlowsky-Glahn V. 2000. Logratio analysis and compositional distance. *Math Geol* **32**:271–275.

Alberdi A, Aizpurua O, Bohmann K, Zepeda-Mendoza ML, Gilbert MTP. 2016. Do vertebrate gut metagenomes confer rapid ecological adaptation? *Trends Ecol Evol* **31**:689–699.

Amato KR. 2016. Incorporating the gut microbiota into models of human and non-human primate ecology and evolution. *AJPA* **159**:196-215.

Amato KR, G Sanders J, Song SJ, Nute M, Metcalf JL, Thompson LR, Morton JT, Amir A, J McKenzie V, Humphrey G, Gogul G, Gaffney J, L Baden A, A O Britton G, P Cuozzo F, Di Fiore A, J Dominy N, L Goldberg T, Gomez A, Kowalewski MM, J Lewis R, Link A, L Sauther M, Tecot S, A White B, E Nelson K, M Stumpf R, Knight R, R Leigh S. 2019. Evolutionary trends in host physiology outweigh dietary niche in structuring primate gut microbiomes. *ISME J* **13**:576–587.

Amato KR, Leigh SR, Kent A, Mackie RI, Yeoman CJ, Stumpf RM, Wilson BA, Nelson KE, White BA, Garber PA. 2015. The gut microbiota appears to compensate for seasonal diet bariation in the wild black howler monkey (*Alouatta pigra*). *Microbial Ecology*. **69**:434-443.

Amato KR, Leigh SR, Kent A, Mackie RI, Yeoman CJ, Stumpf RM, Wilson BA, Nelson KE, White BA, Garber PA. 2014. The role of gut microbes in satisfying the nutritional demands of adult and juvenile wild, black howler monkeys (*Alouatta pigra*). *Am J Phys Anthropol* **155**:652–664.

Amato KR, van Belle S, Di Fiore A, Estrada A, Stumpf R, White B, Nelson KE, Knight R, Leigh SR. 2017. Patterns in gut microbiota similarity associated with degree of sociality among sex classes of a neotropical primate. *Microb Ecol* **74**:250–258.

Antwis RE, Lea JMD, Unwin B, Shultz S. 2018. Gut microbiome composition is associated with spatial structuring and social interactions in semi-feral Welsh Mountain ponies. *Microbiome*. **6**:207.

Bäckhed F. 2011. Programming of host metabolism by the gut microbiota. *Annals of Nutrition and Metabolism*. **2**:44-52.

Barbera P, Kozlov AM, Czech L, Morel B, Darriba D, Flouri T, Stamatakis A. 2019. EPA-ng: Massively parallel evolutionaryplacement of genetic sequences. *Systematic Biology*. **68**:365-369.

Basseri RJ, Basseri B, Chong K, Youdim A, Low K, Hwang LJ, Mathur R, Khalili T, Soffer EE, Chang C, Pimentel M. 2010. W1367 Intestinal methane production in obese humans is associated with higher body mass index. Gastroenterology. **138**:S-708

Bates D, Maechler M, Bolker B, Walker S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7.

Beehner JC, Gesquiere L, Seyfarth RM, Cheney DL, Alberts SC, Altmann J. 2009. Testosterone related to age and life-history stages in male baboons and geladas. *Horm Behav* **56**:472–480.

Beehner JC, McCann C. 2008. Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (*Theropithecus gelada*). *Physiol Behav* **95**:508–514.

Bennett G, Malone M, Sauther ML, Cuozzo FP, White B, Nelson KE, Stumpf RM, Knight R, Leigh SR, Amato KR. 2016. Host age, social group, and habitat type influence the gut microbiota of wild ring-tailed lemurs (*Lemur catta*). *American Journal of Primatology*. **78**: 883-892.

Bergman EN, Reid RS, Murray MG, Brockway JM, Whitelaw FG. 1965. Interconversions and production of volatile fatty acids in the sheep rumen. *Biochem J* **97**:53–58.

Biddle A, Stewart L, Blanchard J, Leschine S. 2013. Untangling the genetic basis of fibrolytic specialization by *Lachnospiraceae* and *Ruminococcaceae* in diverse gut communities. *Diversity* **5**:627–640.

Bik EM, Costello EK, Switzer AD, Callahan BJ, Holmes SP, Wells RS, Carlin KP, Jensen ED, Venn-Watson S, Relman DA. 2016. Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. *Nat Commun* **7**:10516.

Bisanz JE. 2018. qiime2R: Importing QIIME2 artifacts and associated data into R sessions. Version 0 99 13.

Blekhman R, Goodrich JK, Huang K, Sun Q, Bukowski R, Bell JT, Spector TD, Keinan A, Ley RE, Gevers D, Clark AG. 2015. Host genetic variation impacts microbiome composition across human body sites. *Genome Biol* **16**:191.

Blekhman R, Tang K, Archie EA, Barreiro LB, Johnson ZP, Wilson ME, Kohn J, Yuan ML, Gesquiere L, Grieneisen LE, Tung J. 2016. Common methods for fecal sample storage in field

studies yield consistent signatures of individual identity in microbiome sequencing data. *Scientific Reports*. **6**:31519.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**:581–583.

Candela M, Biagi E, Maccaferri S, Turroni S, Brigidi P. 2012. Intestinal microbiota is a plastic factor responding to environmental changes. *Trends Microbiol* **20**:385–391.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**:335–336.

Carrera SC, Sen S, Heistermann M, Lu A, Beehner JC. 2020. Low rank and primiparity increase fecal glucocorticoid metabolites across gestation in wild geladas. *Gen Comp Endocrinol* **293**:113494.

Chevalier C, Stojanović O, Colin DJ, Suarez-Zamorano N, Tarallo V, Veyrat-Durebex C, Rigo D, Fabbiano S, Stevanović A, Hagemann S, Montet X, Seimbille Y, Zamboni N, Hapfelmeier S, Trajkovski M. 2015. Gut microbiota orchestrates energy homeostasis during cold. *Cell*. **136**:1360-1374

Chichlowski M, Sharp JM, Vanderford DA, Myles MH, Hale LP. 2008. *Helicobacter typhlonius* and *Helicobacter rodentium* differentially affect the severity of colon inflammation and inflammation-associated neoplasia in IL10-deficient mice. *Comp Med* **58**:534–541.

Comstock LE, Coyne MJ. 2003. *Bacteroides thetaiotaomicron*: a dynamic, niche-adapted human symbiont. *BioEssays*. **25**:926-929

Costa MC, Silva G, Ramos RV, Staempfli HR, Arroyo LG, Kim P, Weese JS. 2015. Characterization and comparison of the bacterial microbiota in different gastrointestinal tract compartments in horses. *Vet J* **205**:74–80.

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. 2009. Bacterial community variation in human body habitats across space and time. *Science* **326**:1694–1697.

David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**:559–563.

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* **107**:14691–14696.

Degnan PH, Pusey AE, Lonsdorf EV, Goodall J, Wroblewski EE, Wilson ML, Rudicell RS, Hahn BH, Ochman H. 2012. Factors associated with the diversification of the gut microbial communities within chimpanzees from Gombe National Park. *Proc Natl Acad Sci U S A* **109**:13034–13039.

De Mulder T, Goossens K, Peiren N, Vandaele L, Haegeman A, De Tender C, Ruttink T, van de Wiele T, De Campeneere S. 2017. Exploring the methanogen and bacterial communities of rumen environments: solid adherent, fluid and epimural. *FEMS Microbiol Ecol* **93**:fiw251.

Dias PAD, Rangel-Negrín A, Canales-Espinosa D. 2011. Effects of lactation on the time-budgets and foraging patterns of female black howlers (*Alouatta pigra*). *Am J Phys Anthropol* **145**:137–146.

Dominy NJ, Vogel ER, Yeakel JD, Constantino P, Lucas PW. 2008. Mechanical properties of plant underground storage organs and implications for dietary models of early hominins. *Evol Biol* **35**:159–175.

Doran D. 1997. Influence of seasonality on activity patterns, feeding behavior, ranging, and grouping patterns in Tai chimpanzees. *Int J Primatol* **18**:183–206.

Douglas GM, Maffei VJ, Zaneveld J, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI. 2019. PICRUSt2: An improved and extensible approach for metagenome inference. *bioRxiv*. 672295

Dufour DL, Sauther ML. 2002. Comparative and evolutionary dimensions of the energetics of human pregnancy and lactation. *Am J Hum Biol* **14**:584–602.

Dunbar RIM. 1976. Australopithecine diet based on a baboon analogy. *J Hum Evol* **5**:161–167.

Edwards JE, Shetty SA, van den Berg P, Burden F, van Doorn DA, Pellikaan WF, Dijkstra J, Smidt H. 2020. Multi-kingdom characterization of the core equine fecal microbiota based on multiple equine (sub)species. *Animal Microbiome* **2**:1-16.

El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B. 2013. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev Microbiol* **11**:497–504.

Fashing PJ, Nguyen N, Venkataraman VV, Kerby JT. 2014. Gelada feeding ecology in an intact ecosystem at Guassa, Ethiopia: Variability over time and implications for theropith and hominin dietary evolution. *Am J Phys Anthropol* **155**:1–16.

Finn RD, Clements J, Eddy SR. 2011. HMMER web server: interactive sequence similarity searching. *Nucleic Acids Research*. **39**:W29-37

Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA. 2008. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nature Reviews Microbiology*.

Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. 2012. Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* **3**:289–306.

Gao J-J, Zhang Y, Gerhard M, Mejias-Luque R, Zhang L, Vieth M, Ma J-L, Bajbouj M, Suchanek S, Liu W-D, Ulm K, Quante M, Li Z-X, Zhou T, Schmid R, Classen M, Li W-Q, You W-C, Pan K-F. 2018. Association between gut microbiota and *Helicobacter pylori*-related gastric lesions in a high-risk population of gastric cancer. *Front Cell Infect Microbiol* **8**:202.

Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. 2017. Microbiome datasets are compositional: and this is not optional. *Frontiers in Microbiology*. **8**:2224

Gloor GB, Macklaim JM, Vu M, Fernandes AD. 2016. Compositional uncertainty should not be ignored in high-throughput sequencing data analysis. *Austrian Journal of Statistics*. **45**:73-87

Gohl DM, Vangay P, Garbe J, MacLean A, Hauge A, Becker A, Gould TJ, Clayton JB, Johnson TJ, Hunter R, Knights D, Beckman KB. 2016. Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. *Nat Biotechnol* **34**:942–949.

Gomez A, Rothman JM, Petrzelkova K, Yeoman CJ, Vlckova K, Umaña JD, Carr M, Modry D, Todd A, Torralba M, Nelson KE, Stumpf RM, Wilson BA, Blekhman R, White BA, Leigh SR. 2016. Temporal variation selects for diet–microbe co-metabolic traits in the gut of *Gorilla* spp. *ISME J* **10**:514–526.

Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, van Treuren W, Knight R, Bell JT, Spector TD, Clark AG, Ley RE. 2014. Human genetics shape the gut microbiome. *Cell*. **159**:789-799

Groussin M, Mazel F, Sanders JG, Smillie CS, Lavergne S, Thuiller W, Alm EJ. 2017. Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. *Nat Commun* **8**:14319.

Gursky S. 2000. Effect of seasonality on the behavior of an insectivorous primate, *Tarsius spectrum*. *Int J Primatol* **21**:477–495.

Hall M, Beiko RG. 2018. 16S rRNA Gene Analysis with QIIME2. *Methods Mol Biol* **1849**:113–129.

Hanning I, Diaz-Sanchez S. 2015. The functionality of the gastrointestinal microbiome in non-human animals. *Microbiome*. **3**:51

Hicks AL, Lee KJ, Couto-Rodriguez M, Patel J, Sinha R, Guo C, Olson SH, Seimon A, Seimon TA, Ondzie AU, Karesh WB, Reed P, Cameron KN, Lipkin WI, Williams BL. 2018. Gut microbiomes of wild great apes fluctuate seasonally in response to diet. *Nat Commun* **9**:1786.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biom J* **50**:346–363.

Human Microbiome Project Consortium. 2012. Structure, function and diversity of the healthy human microbiome. *Nature* **486**:207–214.

Hunter CP. 2001. Ecological determinants of gelada ranging patterns (*Theropithecus gelada*). University of Liverpool.

Jablonski NG. 2005. *Theropithecus*: The rise and fall of a primate genus. Cambridge University Press.

Jarvey JC, Low BS, Pappano DJ, Bergman TJ, Beehner JC. 2018. Graminivory and fallback foods: annual diet profile of geladas (*Theropithecus gelada*) living in the Simien Mountains National Park, Ethiopia. *Int J Primatol* **39**:105–126.

Jolly CJ. 1970. The Seed-Eaters: a new model of hominid differentiation based on a baboon analogy. *Man* **5**:5–26.

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**:1463–1464.

Kolodny O, Weinberg M, Reshef L, Harten L, Hefetz A, Gophna U, Feldman MW, Yovel Y. 2019. Coordinated change at the colony level in fruit bat fur microbiomes through time. *Nat Ecol Evol* **3**:116–124.

Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, Gonzalez A, Werner JJ, Angenent LT, Knight R, Bäckhed F, Isolauri E, Salminen S, Ley RE. 2012. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* **150**:470–480.

952 Kovatcheva-Datchary P, Nilsson A, Akrami R, Lee YS, De Vadder F, Arora T, Hallen A, Martens
953 E, Björck I, Bäckhed F. 2015. Dietary fiber-induced improvement in glucose metabolism is
954 associated with increased abundance of *Prevotella*. *Cell Metab* **22**:971–982.
955 Krajmalnik-Brown R, Ilhan Z-E, Kang D-W, DiBaise JK. 2012. Effects of gut microbes on nutrient
956 absorption and energy regulation. *Nutr Clin Pract* **27**:201–214.
957 Li H, Qu J, Li T, Wirth S, Zhang Y, Zhao X, Li X. 2018. Diet simplification selects for high gut
958 microbial diversity and strong fermenting ability in high-altitude pikas. *Appl Microbiol*
959 *Biotechnol* **102**:6739–6751.
960 Louca S, Doebeli M. 2018. Efficient comparative phylogenetics on large trees. *Bioinformatics*.
961 Lu A, Bergman TJ, McCann C, Stinespring-Harris A, Beehner JC. 2016. Growth trajectories in
962 wild geladas (*Theropithecus gelada*). *Am J Primatol* **78**:707–719.
963 Macke E, Tasiemski A, Massol F, Callens M, Decaestecker E. 2017. Life history and eco-
964 evolutionary dynamics in light of the gut microbiota. *Oikos* **126**:508–531.
965 Mallott EK, Amato KR. 2018. The microbial reproductive ecology of white-faced capuchins
966 (*Cebus capucinus*). *Am J Primatol* **80**:e22896.
967 Martín R, Heilig GHJ, Zoetendal EG, Smidt H, Rodríguez JM. 2007. Diversity of the *Lactobacillus*
968 group in breast milk and vagina of healthy women and potential role in the colonization of the
969 infant gut. *J Appl Microbiol* **103**:2638–2644.
970 Mathur R, Amichai M, Chua KS, Mirocha J, Barlow GM, Pimentel M. 2013. Methane and
971 hydrogen positivity on breath test is associated with greater body mass index and body fat. *The*
972 *Journal of Clinical Endocrinology & Metabolism*. **98**:E698-E702
973 Matsumiya Y, Kato N, Watanabe K, Kato H. 2002. Molecular epidemiological study of vertical
974 transmission of vaginal *Lactobacillus* species from mothers to newborn infants in Japanese, by
975 arbitrarily primed polymerase chain reaction. *Journal of Infection and Chemotherapy*. **8**:43-49
976 Mau M, Johann A, Sliwa A, Hummel J, Südekum K-H. 2011. Morphological and physiological
977 aspects of digestive processes in the graminivorous primate *Theropithecus gelada*—a
978 preliminary study. *Am J Primatol* **73**:449–457.
979 McMurdie PJ, Holmes S. 2014. Waste not, want not: why rarefying microbiome data is
980 inadmissible. *PLoS Comput Biol* **10**:e1003531.
981 McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis and
982 graphics of microbiome census data. *PLoS One* **8**:e61217.

McNab BK. 2002. The physiological ecology of vertebrates: a view from energetics. Cornell University Press.

Miller TL, Wolin MJ, Conway de Macario E, Macario AJ. 1982. Isolation of *Methanobrevibacter smithii* from human feces. *Appl Environ Microbiol* **43**:227–232.

Milton K, McBee RH. 1983. Rates of fermentative digestion in the howler monkey, *Alouatta palliata* (primates: ceboidea). *Comp Biochem Physiol A Comp Physiol* **74**:29–31.

Mizukami K, Uchiyama J, Igarashi H, Murakami H, Osumi T, Shima A, Ishiahra G, Nasukawa T, Une Y, Sakaguchi M. 2019. Age-related analysis of the gut microbiome in a purebred dog colony. *FEMS Microbiol Lett* **366**.

Morotomi M, Nagai F, Watanabe Y. 2012. Description of *Christensenella minuta* gen. nov., sp. nov., isolated from human faeces, which forms a distinct branch in the order *Clostridiales*, and proposal of *Christensenellaceae* fam. nov. *International journal of systematic and evolutionary microbiology* **62**: 144-149.

O'Herrin SM, Kenealy WR. 1993. Glucose and carbon dioxide metabolism by *Succinivibrio dextrinosolvens*. *Appl Environ Microbiol* **59**:748–755.

Oksanen J, Blanchet FG, Kindt R, Legendre P, O'hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2010. Vegan: community ecology package. R package version 1.17-4.

Popovich DG, Jenkins DJ, Kendall CW, Dierenfeld ES, Carroll RW, Tariq N, Vidgen E. 1997. The western lowland gorilla diet has implications for the health of humans and other hominoids. *J Nutr* **127**:2000–2005.

Puff C, Nemomissa S. 2005. Plants of the Simen: a flora of the Simen Mountains and surroundings, northern Ethiopia. National Botanic Garden (Belgium).

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**:D590–6.

Rampelli S, Schnorr SL, Consolandi C, Turroni S, Severgnini M, Peano C, Brigidi P, Crittenden AN, Henry AG, Candela M. 2015. Metagenome sequencing of the Hadza hunter-gatherer gut microbiota. *Curr Biol* **25**:1682–1693.

Reese AT, Kearney SM. 2019. Incorporating functional trade-offs into studies of the gut microbiota. *Curr Opin Microbiol* **50**:20–27.

1013 Ren T, Grieneisen LE, Alberts SC, Archie EA, Wu M. 2016. Development, diet and dynamism:
1014 longitudinal and cross-sectional predictors of gut microbial communities in wild baboons.
1015 *Environmental Microbiology*. **18**:1312-1325
1016 Ribeiro GO, Oss DB, He Z, Gruninger RJ, Elekwachi C, Forster RJ, Yang W, Beauchemin KA,
1017 McAllister TA. 2017. Repeated inoculation of cattle rumen with bison rumen contents alters
1018 the rumen microbiome and improves nitrogen digestibility in cattle. *Sci Rep* **7**:1276.
1019 Roberts EK, Lu A, Bergman TJ, Beehner JC. 2017. Female reproductive parameters in wild
1020 geladas (*Theropithecus gelada*). *Int J Primatol* **38**:1–20.
1021 Roberts EK, Lu A, Bergman TJ, Beehner JC. 2012. A Bruce effect in wild geladas. *Science*
1022 **335**:1222–1225.
1023 Russell JB, Rychlik JL. 2001. Factors that alter rumen microbial ecology. *Science* **292**:1119–1122.
1024 Salyers AA, Vercellotti JR, West SE, Wilkins TD. 1977. Fermentation of mucin and plant
1025 polysaccharides by strains of *Bacteroides* from the human colon. *Applied and Environmental*
1026 *Microbiology*. **33**:319-322
1027 Samuel BS, Gordon JI. 2006. A humanized gnotobiotic mouse model of host-archaeal-bacterial
1028 mutualism. *Proceedings of the National Academy of Sciences*. **103**:10011-10016
1029 Shin N-R, Whon TW, Bae J-W. 2015. *Proteobacteria*: microbial signature of dysbiosis in gut
1030 microbiota. *Trends Biotechnol* **33**:496–503.
1031 Smits SA, Leach J, Sonnenburg ED, Gonzalez CG, Lichtman JS, Reid G, Knight R, Manjurano A,
1032 Changalucha J, Elias JE, Dominguez-Bello MG, Sonnenburg JL. 2017. Seasonal cycling in the
1033 gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science* **357**:802–806.
1034 Snyder-Mackler N, Beehner JC, Bergman TJ. 2012. Defining higher levels in the multilevel
1035 societies of geladas (*Theropithecus gelada*). *Int J Primatol* **33**:1054–1068.
1036 Song L, Dong X. 2009. *Hydrogenoanaerobacterium saccharovorans* gen. nov., sp. nov., isolated
1037 from H2-producing UASB granules. *International journal of systematic and evolutionary*
1038 *microbiology* **59**: 295-299.
1039 Springer A, Fichtel C, Al-Ghalith GA, Koch F, Amato KR, Clayton JB, Knights D, Kappeler PM.
1040 2017. Patterns of seasonality and group membership characterize the gut microbiota in a
1041 longitudinal study of wild Verreaux's sifakas (*Propithecus verreauxi*). *Ecology and Evolution*.
1042 **7**:5732-5745.

1043 Steelman SM, Chowdhary BP, Dowd S, Suchodolski J, Janečka JE. 2012. Pyrosequencing of 16S
1044 rRNA genes in fecal samples reveals high diversity of hindgut microflora in horses and
1045 potential links to chronic laminitis. *BMC Vet Res* **8**:231.
1046 Sun B, Wang X, Bernstein S, Huffman MA, Xia D-P, Gu Z, Chen R, Sheeran LK, Wagner RS, Li
1047 J. 2016. Marked variation between winter and spring gut microbiota in free-ranging Tibetan
1048 Macaques (*Macaca thibetana*). *Sci Rep* **6**:26035.
1049 Team RC, Others. 2013. R: A language and environment for statistical computing.
1050 Tinsley Johnson E, Snyder-Mackler N, Lu A, Bergman TJ, Beehner JC. 2018. Social and
1051 ecological drivers of reproductive seasonality in geladas. *Behav Ecol* **29**:574–588.
1052 Tremaroli V, Bäckhed F. 2012. Functional interactions between the gut microbiota and host
1053 metabolism. *Nature* **489**:242–249.
1054 Trosvik P, de Muinck EJ, Rueness EK, Fashing PJ, Beierschmitt EC, Callingham KR, Kraus JB,
1055 Trew TH, Moges A, Mekonnen A, Venkataraman VV, Nguyen N. 2018. Multilevel social
1056 structure and diet shape the gut microbiota of the gelada monkey, the only grazing primate.
1057 *Microbiome* **6**:84.
1058 Tseng C-H, Wu C-Y. 2019. The gut microbiome in obesity. *J Formos Med Assoc* **118:**S3–S9.
1059 Tung J, Barreiro LB, Burns MB, Grenier J-C, Lynch J, Grieneisen LE, Altmann J, Alberts SC,
1060 Blekhman R, Archie EA. 2015. Social networks predict gut microbiome composition in wild
1061 baboons. *Elife* **4:** e05224
1062 Turnbaugh PJ, Gordon JI. 2009. The core gut microbiome, energy balance and obesity. *J Physiol*
1063 **587**:4153–4158.
1064 Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. 2006. An obesity-
1065 associated gut microbiome with increased capacity for energy harvest. *Nature* **444**:1027–1031.
1066 Udén P, Rounsaville TR, Wiggans GR, van Soest PJ. 1982. The measurement of liquid and solid
1067 digesta retention in ruminants, equines and rabbits given timothy (*Phleum pratense*) hay. *Br J*
1068 *Nutr* **48**:329–339.
1069 van den Boogaart KG, Tolosana-Delgado R. 2008. "compositions": a unified R package to analyze
1070 compositional data. *Computers & Geosciences*. **34**:320-338
1071 van Gylswyk NO. 1990. Enumeration and presumptive identification of some functional groups of
1072 bacteria in the rumen of dairy cows fed grass silage-based diets. *FEMS Microbiol Ecol* **6**:243–
1073 261.

van Schaik CP, Brockman DK. 2005. Seasonality in primate ecology, reproduction, and life history. *Seasonality in primates: studies of living and extinct human and non-human primates* 3–20.

Venkataraman VV, Glowacka H, Fritz J, Clauss M, Seyoum C, Nguyen N, Fashing PJ. 2014. Effects of dietary fracture toughness and dental wear on chewing efficiency in geladas (*Theropithecus gelada*). *Am J Phys Anthropol* **155**:17–32.

Vijendravarma RK, Narasimha S, Chakrabarti S, Babin A, Kolly S, Lemaitre B, Kawecki TJ. 2015. Gut physiology mediates a trade-off between adaptation to malnutrition and susceptibility to food-borne pathogens. *Ecology Letters*. **18**:1078-1086.

Vlčková K, Mrázek J, Kopečný J, Petrželková KJ. 2012. Evaluation of different storage methods to characterize the fecal bacterial communities of captive western lowland gorillas (*Gorilla gorilla gorilla*). *J Microbiol Methods* **91**:45–51.

Wang J, Fan H, Han Y, Zhao J, Zhou Z. 2017. Characterization of the microbial communities along the gastrointestinal tract of sheep by 454 pyrosequencing analysis. *Asian-australas J Anim Sci* **30**:100–110.

Warnecke F, Luginbühl P, Ivanova N, Ghassemian M, Richardson TH, Stege JT, Cayouette M, McHardy AC, Djordjevic G, Aboushadi N, Sorek R, Tringe SG, Podar M, Martin HG, Kunin V, Dalevi D, Madejska J, Kirton E, Platt D, Szeto E, Salamov A, Barry K, Mikhailova N, Kyrpides NC, Matson EG, Ottesen EA, Zhang X, Hernández M, Murillo C, Acosta LG, Rigoutsos I, Tamayo G, Green BD, Chang C, Rubin EM, Mathur EJ, Robertson DE, Hugenholtz P, Leadbetter JR. 2007. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* **450**:560–565.

Waters JL, Ley RE. 2019. The human gut bacteria *Christensenellaceae* are widespread, heritable, and associated with health. *BMC Biol* **17**:83.

Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, Lozupone C, Zaneveld JR, Vázquez-Baeza Y, Birmingham A, Hyde ER, Knight R. 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*. **5**:27.

White BA, Lamed R, Bayer EA, Flint HJ. 2014. Biomass utilization by gut microbiomes. *Annu Rev Microbiol* **68**:279–296.

Wrangham RW. 1980. Bipedal locomotion as a feeding adaptation in gelada baboons, and its implications for hominid evolution. *J Hum Evol* **9**:329–331.

1105 Ye Y, Doak TG. 2009. A parsimony approach to biological pathway reconstruction/inference for
1106 genomes and metagenomes. *PLoS Computational Biology*. **5**:e1000465.
1107 Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, Parameswaran P, Crowell MD,
1108 Wing R, Rittmann BE, Krajmalnik-Brown R. 2009. Human gut microbiota in obesity and after
1109 gastric bypass. *Proceedings of the National Academy of Sciences*. **106**:2365-2370
1110 Zhang X, Pei Y-F, Zhang L, Guo B, Pendegraft AH, Zhuang W, Yi N. 2018. Negative binomial
1111 mixed models for analyzing longitudinal microbiome data. *Frontiers in Microbiology*. **9**:1683.
1112 Zhang Z, Xu D, Wang L, Hao J, Wang J, Zhou X, Wang W, Qiu Q, Huang X, Zhou J, Long R,
1113 Zhao F, Shi P. 2016. Convergent evolution of rumen microbiomes in high-altitude mammals.
1114 *Curr Biol* **26**:1873–1879.