Social relationships: key to gut microbiome composition in wild redfronted lemurs?

Dissertation

For the award of the degree

"Doctor rerum naturalium" (Dr.rer.nat.)

Division of Mathematics and Natural Sciences

at the Georg-August-University Göttingen

in the doctoral program Behavior and Cognition

at the Georg-August University School of Sciences (GAUSS)

Submitted by

Sonia Tatiana Murillo Corrales

From San José, Costa Rica

Göttingen, 2021

Thesis committee:

Dr. Claudia Fichtel, Behavioral Ecology & Sociobiology Unit, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

Prof. Dr. Rolf Daniel, Department of Genomics and Applied Microbiology, Institute of Microbiology and Genetics, Georg-August Universität, Göttingen.

PD Dr. Oliver Schülke, Department of Behavioral Ecology, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, Georg-August Universität, Göttingen.

Members of the examination board:

Reviewer: **Dr. Claudia Fichtel,** Behavioral Ecology & Sociobiology Unit, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

Reviewer: **Prof. Dr. Rolf Daniel,** Department of Genomics and Applied Microbiology, Institute of Microbiology and Genetics, Georg-August Universität, Göttingen.

Members of the examination board:

PD Dr. Oliver Schülke, Department of Behavioral Ecology, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, Georg-August Universität, Göttingen.

PD Dr. Christian Roos Primate Genetics Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

Dr. Verena Behringer Endocrinology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

Prof. Dr. Stefanie Pöggeler, Department of Genetics of Eukaryotic Microorganisms, Institute of Microbiology and Genetics, Georg-August Universität, Göttingen.

Date of oral examination: December 17th, 2021.

Table of contents

1 General introduction

1.1 Gut microbiome and the gastrointestinal tract

The bodies of animals are colonized inside and outside by trillions of microbial cells and viruses, and are collectively known as the microbiome or microbiota (Whitman, Coleman and Wiebe, 1998; Clemente *et al.*, 2012; McKenney, Koelle, *et al.*, 2018). The microbiome colonizes all body sites in connection with the external environment such as, skin, eyes, vagina, respiratory tract, and the gastrointestinal tract (Clemente *et al.*, 2012; Huttenhower *et al.*, 2012; Janiak *et al.*, 2021). More recently, body sites previously thought to be sterile have associated microbiome as well, like the bladder and the placenta (Aagaard *et al.*, 2014; Olaniyi *et al.*, 2020; Perez-Carrasco *et al.*, 2021). Depending on the body site, the microorganisms may include bacteria, archaea, viruses, fungi, helminths, and protozoa (Caporaso *et al.*, 2011; Lukeš *et al.*, 2015; Laforest-Lapointe and Arrieta, 2018; McKenney, Koelle, *et al.*, 2018).

The gut microbiome are the prokaryotic and eukaryotic communities inhabiting the gastrointestinal tract of an animal (Clemente *et al.*, 2012). The composition and diversity of the endosymbiotic community differs depending on the site of the gastrointestinal tract due to differences in physicochemical and nutritional conditions (Gu *et al.*, 2013; Yasuda *et al.*, 2015; Pereira and Berry, 2017). The small or proximal intestine is characterized by low levels of easily degradable nutrients, low pH and higher levels of oxygen. It is mostly colonized by facultative anaerobes (He *et al.*, 1999; Zoetendal *et al.*, 2012). Conversely, the large or distal intestine has lower oxygen levels, higher pH and less bile salts, promoting organisms that are strict anaerobes but contributing to higher cell density and diversity compared to the proximal intestine (Gu *et al.*, 2013; Pereira and Berry, 2017). The intestinal mucosa and the lumen differ as well (Figure 2) (Yasuda *et al.*, 2015). The secretion of mucus by the epithelial tissue produces the intestinal mucosa, thereby providing particular nutrients for bacteria and allowing the generation of low diversity biofilms (Eckburg *et al.*, 2005; De Weirdt and Van De Wiele, 2015). Phages can also be found in the intestinal mucosa (Barr *et al.*, 2013; Muniesa and Jofre, 2014). Invaginations of the epithelium produce the crypts which have a different partial oxygen pressure and higher concentrations of host

glycans, like mucins (Pédron *et al.*, 2012; Lee *et al.*, 2013). The lumen, delimited by the mucus layer, has a greater bacterial diversity than the crypts, and the other members of the microbiome such as protozoa, helminths, fungi, viruses, phages and archaea are localized here (Drudy *et al.*, 2004; Lukeš *et al.*, 2015; Nash *et al.*, 2017; Nkamga, Henrissat and Drancourt, 2017; Shkoporov *et al.*, 2019). Investigating the microbiome from fecal samples reflects the microbiome of the mucosa and lumen from the colon (Zoetendal *et al.*, 2012; Gu *et al.*, 2013; Yasuda *et al.*, 2015).

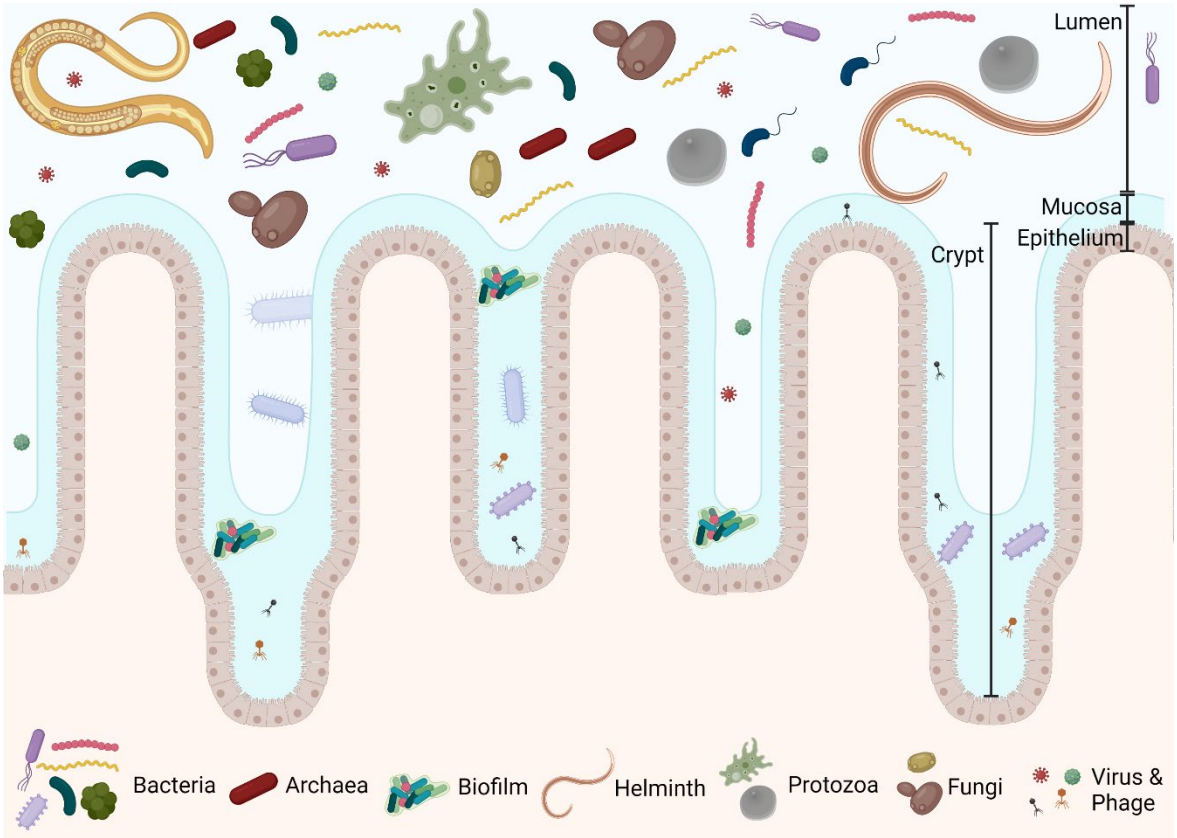


Figure 1. Structure of the colon and the inhabiting microbiome. Created with BioRender.com.

Complex interactions occur in the gut microbiome between the microorganisms composing the community, with the host and with microorganisms outside the host. This results in highly dynamic systems and variation between individuals and within-individuals over time (Parfrey, Walters and Knight, 2011; Miller, Svanbäck and Bohannan, 2018; Björk *et al.*, 2019). Furthermore, the gut microbiome is highly host-specific, for example it varies between species, and even between individuals of the same species living in difference geographical regions (Ley *et al.*, 2008; Yatsunenko *et al.*, 2012; Amato *et al.*, 2013, 2019). The factors shaping these microbial

communities are not fully understood, thus this research avenue needs to be further followed (Clemente *et al.*, 2012; Falony *et al.*, 2016; Miller, Svanbäck and Bohannan, 2018).

1.2 Importance of the gut microbiome

The effect of the gut microbiome on the health of the host is so relevant that the gut microbiome has been called the "forgotten organ" (O'Hara and Shanahan, 2006; Clemente *et al.*, 2012). The term dysbiosis meaning an imbalance, disturbance or dysfunction of the gut microbiota is used to try to understand the relationship between changes in gut microbiome composition and/or functionality and disease. However, defining what is a "healthy" or "normal" gut microbiome is challenging because of the inherent dynamics of the community (Hooks and O'Malley, 2017; Wei *et al.*, 2021). Most of the understanding of the importance of the gut microbiome and its impact on the health of the host comes from data collected in experimental studies in laboratory animals or human research (Stothart, Palme and Newman, 2019). Thus, this section summarizes this research to build-up the relevance of investigating the gut microbiome.

1.2.1 Resistance to infection

The gut microbiome protects against infections by promoting the healthy development of the intestinal epithelium and the gut-associated lymphoid tissues, and by providing a barrier to infection (Round and Mazmanian, 2009; McKenney, Koelle, *et al.*, 2018). For instance, mice without gut microbiome (germ-free) have structural deficiencies of the intestinal epithelium affecting cell turnover and its immunological functions, such as production of cytokines and expression of the major histocompatibility complex (Abrams, Bauer and Sprinz, 1963). Furthermore, germ-free mice have reduced levels of secretory immunoglobulin A (sIgA), defective gut-associated lymphoid tissues, and mesenteric lymph nodes leading to a higher susceptibility to infection by bacterial, viral and parasitic pathogens (Falk *et al.*, 1998; Bouskra *et al.*, 2008; Round and Mazmanian, 2009; Clemente *et al.*, 2012). Additionally, the gut bacterial communities provide a barrier to infection or "colonization resistance" by competing for resources, binding sites, producing antibiotics, immune activation and increasing tightening of the junctions between epithelial cells (Maier and Hentges, 1972; Zachar and Savage, 1979; Bansal *et al.*, 2010; Estrela, Whiteley and Brown, 2015). For instance, it was

shown in rodents that protists can protect against bacterial infection through immune activation (Chudnovskiy *et al.*, 2016).

1.2.2 Programming of the immune response

The gut microbiome is essential for the early development of the innate and adaptive immune responses for distinguishing between self- and non-self-molecules determining proper immune response activation (Chu and Mazmanian, 2013; Arrieta *et al.*, 2014). The innate immune response can identify microorganisms through microbe-associated molecular patterns (MAMPs) using Toll-like receptors (TLRs) and elicit inflammatory responses to prevent infections (Chu and Mazmanian, 2013). The MAMPs of the gut microbiome train TLRs to discern between commensal and pathogenic microorganisms, thus promoting tolerance to commensal microorganisms and avoiding inflammatory responses (O'Hara and Shanahan, 2006; Round and Mazmanian, 2009). Similarly, the commensal microbiota trains the T cell populations from the adaptive immune response to discriminate between self- and non-self-molecules in order to be capable to determine when to elicit an immune response (Lee and Mazmanian, 2010; Lathrop *et al.*, 2011; van Tilburg Bernardes *et al.*, 2020). The relevance of these interactions between the gut microbiome and the immune system are further sustained by inflammatory bowel diseases in humans (Clemente *et al.*, 2012; Arrieta *et al.*, 2014). For instance, conditions like Crohn's disease and ulcerative colitis have been linked to dysregulation of the immune response acting on particular members of the gut microbiome and shifts in the microbial communities (Round and Mazmanian, 2009; Morgan *et al.*, 2012; Gevers *et al.*, 2014; Hoarau *et al.*, 2016; Sokol *et al.*, 2017; van Tilburg Bernardes *et al.*, 2020). Moreover, early exposure of the immune system to certain members of the gut microbiome or metabolites produced by the gut microbiome could play a role in the prevention of allergic diseases (Arrieta *et al.*, 2014; Fujimura *et al.*, 2016). This is emphasized when comparing industrialized and non-industrialized countries, where higher sanitation standards, antibiotic and antiparasitic treatments were shown to affect the gut microbiome and associate with a higher prevalence of asthma and allergies (Ege *et al.*, 2011; Graham-Rowe, 2011; Fujimura *et al.*, 2016). The hygiene hypothesis states that the lack of exposure to helminths and protozoa impacts the proper development of the immune response, which results in increasing the incidence of immunological disorders in industrialized countries (Parfrey, Walters and Knight, 2011; Chabé, Lokmer and Ségurel, 2017;

Laforest-Lapointe and Arrieta, 2018). Additionally, germ-free mice showed an increase of T cell populations associated with allergies (Smith and Garrett, 2011). Furthermore, some autoimmune disorders have been linked to interactions between the immune response and the gut microbiome, such as diabetes type 1, multiple sclerosis, and rheumatoid arthritis (Lee and Mazmanian, 2010; Clemente *et al.*, 2012).

1.2.3 Nutrition and metabolism

The gut microbiome is essential for the digestion of diet, energy harvest and the production of essential metabolites. These processes are taking place through trophic interactions (Bäckhed *et al.*, 2005; Gill *et al.*, 2006; A. J. Johnson *et al.*, 2019). Enzymes for the digestion of complex polysaccharides, such as fiber and resistant starch, are absent in an animal's body but are provided by the members of the gut microbiome (McKenney, Koelle, *et al.*, 2018). Bacterial enzymes hydrolyze polysaccharides, oligosaccharides, and disaccharides to their constituent sugars, which are then fermented for energy uptake by microorganisms. The short-chain fatty acids (SCFAs) propionate, acetate, and butyrate, which are products of these fermentations, are absorbed by the host to produce energy and contribute to a healthy function of the intestinal epithelium (Topping and Clifton, 2001; Gill *et al.*, 2006; Qin *et al.*, 2010). Archaea use the H_2 from fermentations for methanogenesis and remove the end product, which affects the efficiency of polysaccharide digestion (Stams, 1994; Rychlik and May, 2000; Gill *et al.*, 2006). Additionally, the gut microbiome produces essential vitamins for the host, such as A, C, K and the B-vitamins (Qin *et al.*, 2010; McKenney, Koelle, *et al.*, 2018). Moreover, bacteria degrade dietary and host-derived amino acids, and urea into ammonia which is used by the bacteria and the host for protein synthesis (McKenney, Koelle, *et al.*, 2018; McKenney, O'Connell, *et al.*, 2018). Finally, the gut microbiome plays a major role in processing xenobiotics, like diet-derived bioactive compounds protecting the host. In addition, therapeutic drugs can also be processed, which might affect the effects of pharmaceuticals (Gill *et al.*, 2006; Spanogiannopoulos *et al.*, 2016). Less is known about protists and helminths, but flagellates can help digest cellulose in termites and ciliates are essential for digestion in the rumen (Parfrey, Walters and Knight, 2011). Moreover, anaerobic gut fungi from the phylum *Neocallimastigomycota*, are important symbionts from the gut of ruminants and other herbivores for the degradation of plant fibers to obtain nutrients from diet (Gruninger *et al.*, 2014; Wang *et al.*, 2019). Further indications of the impact of the gut

microbiome on metabolism are the changes observed when sub-therapeutic doses of antibiotics are given as early life promoters in animal husbandry to increase adiposity (Cho *et al.*, 2012; Economou and Gousia, 2015). Conversely, microbiome immaturity has been linked to undernutrition in children and a new area of research investigates how to treat acute malnutrition with microbiota-directed complementary food (Blanton *et al.*, 2016; Gehrig *et al.*, 2019).

1.2.4 Gut-brain axis

The gut-brain axis is the bidirectional communication between the gastrointestinal tract and the brain and is involved in the etiology of several psychiatric disorders (Cryan *et al.*, 2019). The brain communicates with the gut through neural signals with the vagal and spinal nerve pathways, neurotransmitters, and endocrinologically through the hypothalamic-pituitary-adrenal (HPA) axis. In turn, the gut communicates with the brain through SCFAs, gut peptides, immunomodulatory signals, cytokines, the HPA axis and neuromodulatory metabolites (Figure 2) (Rogers *et al.*, 2016; Cryan *et al.*, 2019). It has been shown that the gut microbiome plays a key role in the communication with the brain. For instance, studies in germ-free mice showed that colonization by the gut microbiome is essential for early life neurodevelopment because these mice have an exaggerated stress response to mild stressors, increased motor activity, reduced anxiety, abnormal social behaviors, issues with non-spatial memory, and affected pain signaling (Sudo *et al.*, 2004; Amaral *et al.*, 2008; Diaz Heijtz *et al.*, 2011; Gareau *et al.*, 2011; Desbonnet *et al.*, 2014). SCFAs, metabolites produced by bacteria, have a myriad of effects on the brain (Cryan *et al.*, 2019). They induce the innate immune system by altering the levels of cytokines which can affect the brain (Macfarlane and Macfarlane, 2003). Their immunomodulatory properties are so relevant that germ-free mice have altered immune responses in the central nervous system (Erny *et al.*, 2015). Furthermore, SCFAs can interact with nerve cells, or cross the blood brain barrier and access the brain, modulating behavior and brain development (MacFabe *et al.*, 2007; Kimura *et al.*, 2011; MacFabe, 2012). Moreover, SCFAs regulate the production of gut peptides by the enteroendocrine cells, thus altering the gut-brain hormonal communication (Wren and Bloom, 2007; Schéle *et al.*, 2013). The gut microbiome can also produce neuromodulatory metabolites like histamine, acetylcholine, catecholamines, and GABA (γ-aminobutyric acid) (Stephenson and Rowatt, 1947; Barrett *et al.*, 2012; Thomas *et al.*, 2012; Schretter *et al.*, 2018; Sudo, 2019). It plays a

major role in the regulation of the tryptophan metabolism, a diet-derived amino acid essential for the synthesis of serotonin (5-HT) (Rogers *et al.*, 2016). The gut microbiome utilizes tryptophan for growth, thereby affecting its availability, but also alters host enzymes involved in its degradation (Milligan *et al.*, 1978; Clarke *et al.*, 2013). Serotonin has vast effects in the body, and variations in its levels have been implicated in psychiatric disorders and depressive symptoms (Yano *et al.*, 2015; Rogers *et al.*, 2016; Cryan *et al.*, 2019).

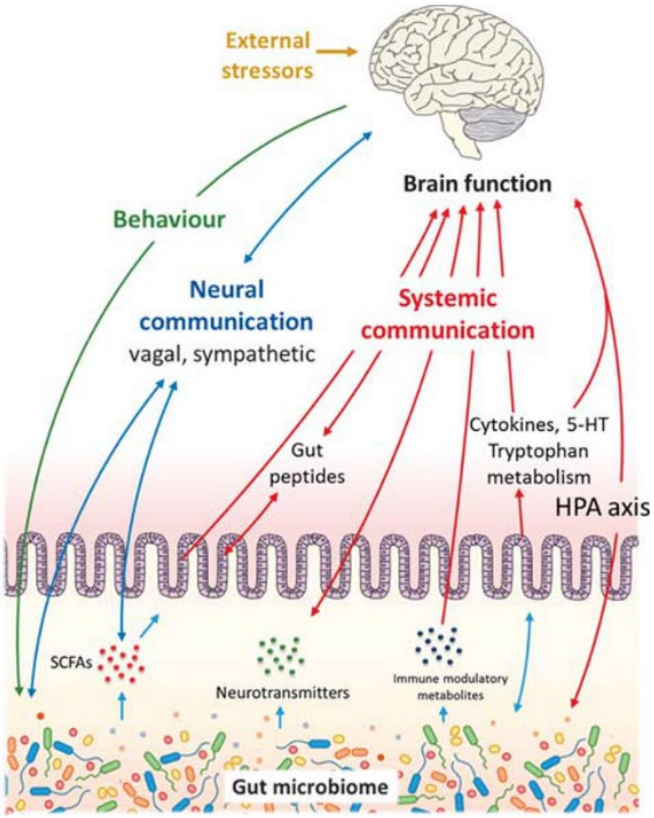


Figure 2. Interacting pathways between the gut microbiome and the brain conforming the gut-brain axis. Taken from (Rogers *et al.*, 2016)

The hypothalamic-pituitary-adrenal (HPA) axis is the non-neuronal major player of the microbiota-gut-brain axis and coordinates the response to stress (Cryan *et al.*, 2019). Stress is any acute threat to the homeostasis of an organism, these threats may be physical or psychological, and arise from inside or outside the body. Thus, the HPA axis aims to defend the stability or homeostasis of the environment (Mayer, 2000). Homeostatic changes induce the release of corticotrophin releasing factor (CRF) from the hypothalamus, which in turn activates the anterior pituitary gland to secrete adrenocorticotrophic hormone (ACTH), then ACTH promotes the production of

glucocorticoids by the adrenal glands (Figure 3). Glucocorticoids suppress the immune response, increase blood sugar, and enhanced fat and protein metabolism to prepare the body to the “fight or flight” response (Mayer, 2000; Coutinho and Chapman, 2011; Cryan *et al.*, 2019; Frankiensztajn, Elliott and Koren, 2020). The effects of the gut microbiome on the HPA axis are due to the induction of inflammation or as side effects of the regulation of serotine metabolism, which also regulates the HPA axis (Cryan *et al.*, 2019; Frankiensztajn, Elliott and Koren, 2020). For example, the increase of gut permeability due to the action of glucocorticoids, allows the translocation of gut bacteria beyond the intestinal lumen, paradoxically inducing pro-inflammatory cytokines and activating the HPA axis (Demaude *et al.*, 2006; Frankiensztajn, Elliott and Koren, 2020). Mice exposed to immobilization stress presented changes in gut microbiota composition with higher abundances of *Proteobacteriota* and gut inflammation (Jang *et al.*, 2018). Furthermore, mice submitted to maternal separation to induce stress exhibit enhance HPA axis activity, but after treatment with probiotics glucocorticoid induced changes were prevented (Fukui *et al.*, 2018). However, the direct effects of the gut microbiome on HPA axis need to be further investigated, although the close relationship between the brain, the immune response and the HPA axis makes them difficult to disentangle.

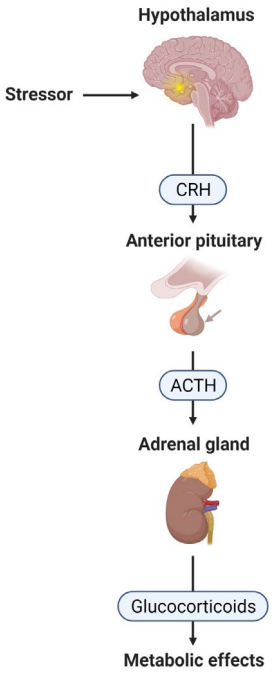


Figure 3. Scheme of the hypothalamic-pituitary-adrenal (HPA) axis. Created with BioRender.com.

1.3 Identifying the members of the gut microbiome

Many of the microorganisms that are forming the gut microbiome are difficult to cultivate but advent of high-throughput sequencing technologies made it possible to investigate them (Clemente *et al.*, 2012; Knight *et al.*, 2018). Depending on the aim of the study, different sequencing techniques can be applied which in turn provide diverse types of information regarding the community and its members (Knight *et al.*, 2018). Marker gene analysis, the method used for this study, provides a broad overview of the microbiome. In this approach, primers are designed to target a domain conserved gene, which has a region of high variability that allows phylogenetic identification (Knight, 2016; Knight *et al.*, 2018). The gold standard for investigating gut bacterial and archaeal communities is the amplification of the 16S rRNA gene encoding for the 30S ribosomal subunit, which is unique to prokaryotes, has several hypervariable regions providing specificity for species resolution and is relatively short (Arrieta *et al.*, 2014; Fukuda *et al.*, 2016). It is recommended to use separate set of primers for analyzing bacteria and archaea to increase detection capacity and resolution (Bahram *et al.*, 2019). In turn, marker gene analysis of the gut protozoa and helminths is performed by amplifying the 18S rRNA gene from the small ribosomal subunit, which have several alternating hypervariable (V1 – V9) and conserved regions (Stoeck *et al.*, 2010; Bradley, Ian M; Pinto, Ameet; Guest, 2016; Gogarten *et al.*, 2020). The V4 and the V9 regions are the most frequently used ones (Stoeck *et al.*, 2010; Choi and Park, 2020). The internal transcribed spacer (ITS) region of the nuclear ribosomal RNA is used for the study of the gut fungal communities or the gut mycobiome (Toju *et al.*, 2012; Nilsson *et al.*, 2019). The ITS region is relatively long, thus two subregions can be amplified ITS1 and ITS2. The ITS2 is preferred because it has more universal primer sites and lower length variation (Tedersoo *et al.*, 2015). One common bias for all marker genes is that the selected gene region for the amplification and the primers used can predispose the detection of certain taxa, thus primer choice should be made cautiously, and data interpretations should consider this inherit bias of the method (Klindworth *et al.*, 2013; Arrieta *et al.*, 2014; Tedersoo *et al.*, 2015; J. S. Johnson *et al.*, 2019).

The entire community is investigated by amplifying the marker gene directly from extracted DNA, thus named DNA-based marker gene analysis (Arrieta *et al.*, 2014; Knight, 2016). In RNA-based marker gene analyses the potential active community is

investigated by performing RNA extraction from the sample, reverse transcribing the RNA to cDNA, and amplifying the marker gene from the generated cDNA (Figure 4). This approach studies the community which is actively replicating thus avoiding biases from the amplification of dormant or dead cells and reducing the effect of differences in the numbers of 16S rRNA operon and copy variants (Větrovský and Baldrian, 2013; Berkelmann *et al.*, 2018; De Vrieze *et al.*, 2018; J. S. Johnson *et al.*, 2019).

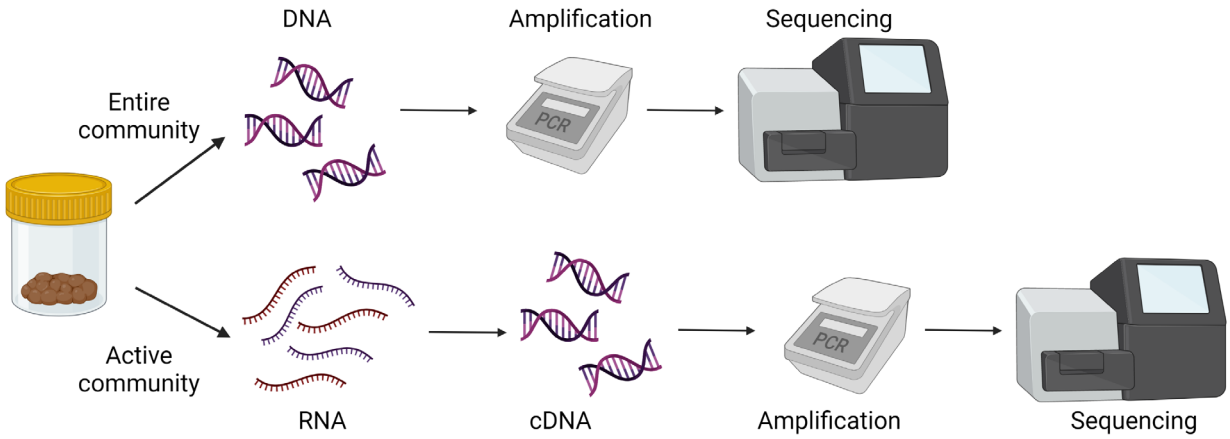


Figure 4. Schematic overview of the differences in the sample processing for DNA-based marker gene (entire community) and RNA-based marker gene (active community) analysis. Created with BioRender.com.

The microbiome can be investigated through determining its variation with alpha and beta diversity measures and/or studying the differentially abundant taxa (Knight *et al.*, 2018). Alpha diversity quantifies taxa diversity within a sample, whereas beta diversity evaluates differences between samples (Xia, Sun and Chen, 2018). Differences in taxon abundances and composition can be investigated at any taxonomical level, from phylum to species, even to the level of amplicon sequence variants (ASVs) (Knight *et al.*, 2018). An amplicon sequence variant is the exact nucleotide sequence amplified and sequenced during marker gene analysis (Callahan, McMurdie and Holmes, 2017; Knight *et al.*, 2018; Nearing *et al.*, 2018). ASVs enables the detection of single nucleotide differences in the sequence allowing to determine strain-level differences in a species (Callahan, McMurdie and Holmes, 2017). Other omic-methods investigating nucleotide sequences are shotgun metagenomics and metatranscriptomics. In metagenomics, all microbial genomes in a sample are sequenced thus providing information regarding gene content and allowing the construction of metagenome assembled genomes (Quince *et al.*, 2017; Pasolli *et al.*, 2019). Metatranscriptomics is

used to assess the functional community by sequencing the RNA transcripts present in the sample (Heintz-Buschart and Wilmes, 2018).

1.4 Factors shaping the gut microbiome

The complex interplay between the gut microbiome and the host also means that host intrinsic and extrinsic factors shape the diversity and composition of the microbial community. This section summarizes the literature regarding the drivers of the gut microbiome.

1.4.1 Phylogeny

Phylogeny studies the evolutionary history of species (Choudhuri, 2014). Host phylogeny is one of the major drivers of gut microbiota composition and diversity, where conspecifics hosts have more similar microbiomes than those from different host species while hosts from the same taxonomic order have more similar gut microbiome (Ley *et al.*, 2008; Nishida and Ochman, 2018; Amato *et al.*, 2019; Youngblut *et al.*, 2019; Gogarten *et al.*, 2021). Even in conspecific hosts living separately, such as humans from different geographical regions or animals either captive or in the wild, the gut microbiome is more similar between conspecifics (Ley *et al.*, 2008). At high taxonomic levels (family or genus) very few bacteria are shared between non-conspecifics, thus suggesting they are constrained to specific host clades (Groussin *et al.*, 2017; Youngblut *et al.*, 2019). This phylogenetic signal between gut microbiome and host phylogeny is stronger in mammals than in non-mammals, particularly for the order *Artiodactyla*, possibly due to the evolution of complex forestomachs (Nishida and Ochman, 2018; Youngblut *et al.*, 2019). In vertebrates, host phylogeny only predicts composition but not alpha and beta diversity therefore its proposed that host phylogeny has a greatest impact on microbial taxa prevalence. But in mammalians clades, variations in microbial diversity and composition according to host phylogeny were detected (Youngblut *et al.*, 2019). Furthermore, it has been determined that in mammals the influence of phylogenetic signal is greater than the one from diet as more closely related species possess more similar gut communities despite having different diets (Nishida and Ochman, 2018). These concordances between gut microbiome similarity and phylogenetic relatedness has been termed phylosymbiosis, and has been detected in many hosts (Brooks *et al.*, 2017; Kartzinel *et al.*, 2019; Rojas *et al.*, 2021). An exception are bats and flying birds where only weak correlations between

host phylogeny and gut microbiome were detected, also in birds, bacterial taxa are broadly shared between hosts. It was proposed that this apparent loss of host gut microbiome specificity compared to other vertebrates is due to physiological changes associated with flying (Song *et al.*, 2020).

1.4.2 Diet

So far, diet is the second most important predictor of gut microbiota composition and diversity (Ley *et al.*, 2008; Nishida and Ochman, 2018; Zmora, Suez and Elinav, 2018; Kartzinel *et al.*, 2019; Youngblut *et al.*, 2019). Dietary changes have driven the evolution of new vertebrate species by producing physiological adaptations for energy harvesting from diet. For instance, the change to a plant-based diet meant the enlarging of the foregut or the hindgut to lengthen gut retention times allowing bacteria to ferment complex plant polysaccharides (Ley *et al.*, 2008; Clauss, Hume and Hummel, 2010; Rojas *et al.*, 2021). These physiological and dietary changes caused adaptive challenges for the gut microbial communities as well. The gut microbiome interacts directly with dietary nutrients that might promote or inhibit their growth, and the capacity to use a specific nutrient as energy source provides competitive advantages in the community (Zmora, Suez and Elinav, 2018). For instance, microbial taxa able to hydrolyze complex polysaccharides harbor the required functions to colonize the gut of herbivores (Youngblut *et al.*, 2019). Carnivores, omnivores and herbivores have different gut microbial community composition and diversity, and herbivores have the highest genus level richness of the three (Ley *et al.*, 2008; Nishida and Ochman, 2018). It has been detected that diet is a stronger predictor of alpha and beta diversity than composition. Thus, diet affects microbial diversity by determining the functional guilds required for digestion of specific food types (Youngblut *et al.*, 2019). Although, the gut microbiome of animals can converge according to diet category, this only happens at lower taxonomic levels (phylum) (Groussin *et al.*, 2017; Nishida and Ochman, 2018). An exception is the gut microbiome of flying birds and bats, which shows little or no correlation to diet, and it is suggested that this is due to physiological changes associated to flying (Song *et al.*, 2020). Notable differences are also detected between individuals and within individuals of the same species following dietary changes. For example, in humans differences have been detected between individuals eating plant-based or animal-based diets (David *et al.*, 2014). In addition, humans from rural settings consuming more fiber-rich diets have higher alpha diversity

and different microbial taxa compared to individuals from industrialized countries with a fat-rich diet (De Filippo *et al.*, 2010; Yatsunenko *et al.*, 2012; Schnorr *et al.*, 2014; Clemente *et al.*, 2015; Martínez *et al.*, 2015). In non-human primates seasonal fluctuations affecting food availability and feeding behaviors associate to changes in gut microbiome diversity and composition (Amato *et al.*, 2014; Ren *et al.*, 2016; Jagsi *et al.*, 2017; Hicks *et al.*, 2018). Finally, lower alpha diversity and differences in microbial taxa have been detected in captive animals compared to their wild counterparts, which is partly explained by the access to different diets (Uenishi *et al.*, 2007; Clayton *et al.*, 2016).

1.4.3 Immune response

The immune response maintains the homeostasis and mutualistic relationship between the host and the gut microbiome by balancing the tolerance to the microorganisms and preventing their overexploitation of resources and translocation from the intestinal lumen to tissues (Chu and Mazmanian, 2013; Zheng, Liwinski and Elinav, 2020). In healthy individuals, the immune response to the gut microbiome is localized at the mucosal surface (Figure 1). The mucus layer separates the microorganisms from the intestinal epithelium and induces tolerance of immune cells towards commensals (Belkaid and Naik, 2013; Shan *et al.*, 2013). Additionally, intestinal and pancreatic secretory cells secrete antimicrobial peptides that also mobilize immune cells, all contributing to the control of the microbial populations (Biragyn *et al.*, 2002; Ahuja *et al.*, 2017; Ehmann *et al.*, 2019). Recognition of microbial signals through pattern recognition receptors by the innate immune response, such as TLRs, shape the gut microbiome, prevent inflammation by regulating microbial abundances or may induce anti-inflammatory mechanisms for tolerance (Fulde *et al.*, 2018; Erturk-Hasdemir *et al.*, 2019; Zheng, Liwinski and Elinav, 2020). Production of IgA by B cells during the adaptive immune response maintains a diverse and balance microbiota (Suzuki *et al.*, 2004). For instance, IgA preferentially coats bacteria which can induce colitis (Palm *et al.*, 2014). These are just a few examples of the mechanisms how the immune response maintains homeostasis with the gut microbiome and many more remain to be investigated.

1.4.4 Heritability

The study of heritability of the gut microbiome aims to determine the impact of the host's genetic variation on the microbial community composition and diversity attempting to link changes in microbial taxa to the host health and fitness (Grieneisen *et al.*, 2021; Kurilshikov *et al.*, 2021). In humans, very few heritable microbial taxa have been detected having low and varying estimates of heritability, and no heritability of alpha diversity has been identified (Goodrich *et al.*, 2014; Turpin *et al.*, 2016; Kurilshikov *et al.*, 2021). Genes that were shown to be linked to microbial heritability, were associated to the host's nutritional preferences as well as metabolic, immunological, and psychiatric traits (Blekhman *et al.*, 2015; Kurilshikov *et al.*, 2021). Conversely, in yellow baboons (*Papio cynocephalus*) 97% of single-taxon, alpha and beta diversity phenotypes are heritable with low and varying heritability estimates (Grieneisen *et al.*, 2021). The discrepancies found between hosts arise from different study designs, since the research performed in baboons was longitudinal, which made it feasible to identify a masking of the effect by age, season, and diet (Grieneisen *et al.*, 2021).

1.4.5 Social relationships

Dispersal processes happen in the gut microbiome connecting the gut communities from other different hosts directly or indirectly through social relationships (Miller, Svanbäck and Bohannan, 2018; Sarkar *et al.*, 2020). Group living facilitates social transmission of microorganisms through interactions such as grooming, kissing, mating, hugging, sleeping together, cohabitation, and food sharing (Sarkar *et al.*, 2020). For example, humans sharing or having shared a household have more similar gut microbiomes than related individuals who never cohabited (Rothschild *et al.*, 2018). Moreover, it was determined that the gut microbiome of twins becomes more dissimilar when they live apart (Xie *et al.*, 2016). Co-housing of laboratory mice makes their gut microbiome composition and diversity more similar, whereas mice from the same strain but located in different cages have more different gut microbiomes (Rogers *et al.*, 2014; Hoy *et al.*, 2015; Caruso *et al.*, 2019). In wild primates, group membership is a predictor of gut microbiome similarity in yellow baboons, Verreaux's sifakas (*Propithecus verreauxi*), ring-tailed lemurs (*Lemur catta*), black howler monkeys (*Alouatta pigra*), white-faced capuchins (*Cebus capucinus*), mangabeys (*Cercocebus atys atys*), chimpanzees (*Pan troglodytes schweinfurthii*) and redbellied lemurs (*Eulemur*

rubriventer) (Degnan *et al.*, 2012; Bennett *et al.*, 2016; Amato *et al.*, 2017; Grieneisen *et al.*, 2017; Raulo *et al.*, 2017; Springer *et al.*, 2017; Gogarten *et al.*, 2018; Orkin, Webb and Melin, 2019). Also, in black-and-white colobus monkeys (*Colobus vellerosus*) the gut microbiome of individuals in different groups became more dissimilar after a group fission event (Goodfellow *et al.*, 2019). Social networks or duration in body contact shaped gut microbiome similarity in yellow baboons, chimpanzees, Verreaux's sifakas, Welsh mountain ponies (*Equus ferus caballus*) and wild mice (*Apodemus sylvaticus*) (Tung *et al.*, 2015; Moeller *et al.*, 2016; Amato *et al.*, 2017; Perofsky *et al.*, 2017; Antwis *et al.*, 2018; Raulo *et al.*, 2021). Furthermore, female to male gut microbiome transmission and increase similarity was detected in marmosets (*Callithrix jacchus*) after pairing (Zhu *et al.*, 2020). Effects have also been identified in non-mammals, for instance changes in alpha diversity of the cloacal microbiota from rufous-collared sparrows (*Zonotrichia capensis*) and barn swallows (*Hirundo rustica*) have been detected during the breeding season and with increased social contacts, respectively (Levin *et al.*, 2016; Escallón, Belden and Moore, 2019). Moreover, social bees and bumblebees possess different bacterial taxa than solitary bees, and social transmission of commensal bacteria can protect or reduced their susceptibility to pathogens (Koch and Schmid-Hempel, 2011; Martinson *et al.*, 2011; Schwarz, Moran and Evans, 2016). Social relationships may also impact gut microbiome indirectly through their positive and negative influence on the host's physiological and immune response (Kappeler, Cremer and Nunn, 2015). For instance, social support can help buffer the impact of stressful events whereas social isolation and social status are stress loads for an individual (Cohen *et al.*, 1992; Ostner, Kappeler and Heistermann, 2008; Young *et al.*, 2014; Snyder-Mackler *et al.*, 2016; Wittig *et al.*, 2016a). In contrast, group living increases the risk of infection by parasites and individuals with more social contacts have a higher infection risk (MacIntosh *et al.*, 2012; Kappeler, Cremer and Nunn, 2015; Rimbach *et al.*, 2015; Springer *et al.*, 2016). The impact of social behaviors on host's health and parasite transmission was further investigated in this thesis (chapter 4).

1.4.6 Age

Host age differences in gut microbiome composition and diversity have been reported at early and late stages of life. Research performed in humans determined colonization by bacteria start *in utero* and that, the meconium, an infant's first fecal sample, already

has a low bacterial diversity (Breitbart *et al.*, 2008; Koenig *et al.*, 2011). After delivery, infants are colonized by the vaginal microbiota and this event has an impact on the development of the immune response and the intestinal epithelium (Bouskra *et al.*, 2008; Arrieta *et al.*, 2014). Due to the exposure to breast milk, solid food, contact with the mother's skin, contact with other individuals, and the environment, the gut microbiome further changes (Arrieta *et al.*, 2014; Wampach *et al.*, 2017; Guevarra *et al.*, 2019; Petrullo *et al.*, 2019). In humans, the bacterial community of children under 3 years of age is highly fluctuating, has a lower alpha diversity compared to adults, and varies widely between individuals (De Filippo *et al.*, 2010; Koenig *et al.*, 2011; Yatsunenko *et al.*, 2012). Furthermore, the microeukaryotic community presents more intra- and inter-variability than the prokaryotic counterpart (Wampach *et al.*, 2017; Ward *et al.*, 2018). In animals, it remains to be determined at which age the gut microbiome of an infant stabilizes, although it is known from piglets (*Sus domesticus*) and calves (*Bos taurus*) that weaning has major effects on the future development of the gut microbiome (Malmuthuge and Guan, 2017; Guevarra *et al.*, 2019). In Rhesus macaques (*Macaca mulatta*) the gut microbiome of infants <1 year of age is significantly different from the one of adults suggesting a faster maturation of the gut microbiome in non-human primates (Rhoades *et al.*, 2019; Janiak *et al.*, 2021). In humans, it has been detected that the gut microbiome of individuals becomes more unique with age, and uniqueness associated to better health outcomes (Wilmanski *et al.*, 2021). Conversely, other studies identified lower alpha diversity and a higher variability in composition possibly due to an increase in pathobionts (Claesson *et al.*, 2011; Jackson *et al.*, 2016). The effect of host's age has been difficult to determine in wild animals. Differences in composition and diversity according to age categories were reported in rufous mouse lemurs (*Microcebus rufus*), ring-tailed lemurs, Rhesus macaques, African buffalos (*Syncerus caffer*), chinstrap penguins (*Pygoscelis antarctica*) and spotted hyenas (*Crocuta crocuta*) (Aivelo, Laakkonen and Jernvall, 2016; Barbosa *et al.*, 2016; Bennett *et al.*, 2016; Heitlinger *et al.*, 2017; Janiak *et al.*, 2021). In western lowland gorillas, differences between age classes were detected only during the dry season (Pafčo *et al.*, 2019). Conversely, in chimpanzees and redbellied lemurs no effect of age class was detected (Degnan *et al.*, 2012; Raulo *et al.*, 2017).

1.4.7 The HPA axis

The hypothalamic-pituitary-adrenal axis produces glucocorticoids as a physiological response to overcome stressors (Heistermann, Palme and Ganswindt, 2006; Tetel *et al.*, 2018; Lu *et al.*, 2019). This response can alter the gut barrier, motility and suppress immune activation consequently affecting the gut microbiome (Heistermann, Palme and Ganswindt, 2006; Bailey *et al.*, 2011; Tetel *et al.*, 2018; Lu *et al.*, 2019). For example, this exposure has been associated to a decrease in bacterial richness and diversity and to changes in the abundances of certain bacterial taxa in mice (Bailey *et al.*, 2011). Also, increase intestinal permeability allows the translocation of bacteria from the lumen to other tissues activating inflammatory responses and increasing susceptibility to infections by pathogens (Bailey *et al.*, 2010, 2011; Vlčková *et al.*, 2018).

These effects have been mostly studied in humans and laboratory animals, but few studies exist for wild animals. The measurement of glucocorticoid metabolites in feces (fGCM) is an approach to determine the effects of HPA axis activation due to stressors on the gut microbiome of wild populations (Heistermann, Palme and Ganswindt, 2006; Stothart *et al.*, 2016; Vlčková *et al.*, 2018). Until now, no covariation of fGCM levels with alpha or beta diversity has been detected in eastern grey squirrels or western lowland gorillas (Vlčková *et al.*, 2018; Stothart, Palme and Newman, 2019). However, positive correlations were observed with specific taxa in gorillas (Vlčková *et al.*, 2018). Furthermore, long-term effects of stressors were detected from hair cortisol measurements in squirrels and yellow-legged gull chicks (*Larus michahellis*) implanted with corticosterone who had a lower alpha diversity and different predominant bacterial taxa compared to controls (Stothart *et al.*, 2016; Noguera *et al.*, 2018).

1.4.8 Sex

Differences in gut microbiome composition and diversity associated to sex may arise from the effect of gonadal hormones and sexual dimorphic immunity (Elderman, de Vos and Faas, 2018; Tetel *et al.*, 2018). For example, castration of mice decreased sex differences in gut microbiome, administration of testosterone propionate to newborn female rats decreased alpha diversity and ovariectomy of adult female rats shifted the *Firmicutes*-to-*Bacteroidetes* ratio (Yurkovetskiy *et al.*, 2013; Moreno-Indias *et al.*, 2016). Moreover, in several animal species, the innate and adaptive immune

response is lower in males than in females. This sexual dimorphic immunity is an effect of the X chromosome having many genes regulating the immune response and the mediation of the immune function by androgens, oestradiol and progesterone (Klein and Flanagan, 2016). Clear distinctions in microbiome composition and diversity have been found in laboratory animals, although these signatures vary between genotype and diet (Bolnick *et al.*, 2014; Xiao *et al.*, 2015; Org *et al.*, 2016; Sheng *et al.*, 2017). Differing results have been found in humans. Some studies reported a considerable effect of sex, while others detected none or a modest effect (Dominianni *et al.*, 2015; Falony *et al.*, 2016; Haro *et al.*, 2016). Similar differences have been reported in wild animals. In western lowland gorillas sex differences were only detected for immature individuals during the wet season, and those detected in chimpanzees could also be attributed to dietary changes (Degnan *et al.*, 2012; Pafčo *et al.*, 2019). However, no effect of sex was detected in redbellied lemurs, Rhesus macaques and eastern grey squirrels (*Sciurus carolinensis*) (Stothart *et al.*, 2016; Raulo *et al.*, 2017; Janiak *et al.*, 2021). Only in rufous mouse lemurs host sex was an important variable although they discussed that variations in home range size or social contacts between sexes could have impacted the gut microbiome (Aivelo and Norberg, 2017). The close interplay between age, diet, body mass index, genotype and reproductive condition increase the difficulty to detect sexual dimorphism in the gut microbiome (Elderman, de Vos and Faas, 2018).

1.4.9 Environment and seasonality

Dispersal processes between the environment and the host's gut microbiome are also taking place, although they have been difficult to estimate thus far (Miller, Svanbäck and Bohannan, 2018). The impact of the environment on the gut microbiome can either be measured by detecting transmission of microorganisms from the environment to the host or by environmental changes of the host habitat, e.g., seasonality. For instance, for wild animals, seasonal shifts impact available food and water sources, thus suggesting seasonality as a predictor for differences in gut microbiome composition and diversity as observed before in wild mice, Tibetan macaques (*Macaca thibetana*) and pandas (*Ailuropoda melanoleuca*) (Maurice *et al.*, 2015; Sun *et al.*, 2016; Wu *et al.*, 2017). However, in other cases in which correlations with dietary shifts were detected, an impact of seasonality was also distinguished i.e. in black howler monkeys, white faced capuchins, and Verreaux's sifakas (Amato *et al.*, 2014; Springer *et al.*,

2017; Orkin *et al.*, 2019). Similarly, longitudinal studies in great apes (*Gorilla gorilla gorilla*, *Gorilla beringei beringei* and *Pan troglodytes troglodytes*), geladas (*Theropithecus geladas*) and yellow baboons detected an effect of environmental factors such as rainfall, and temperature (Ren *et al.*, 2016; Hicks *et al.*, 2018; Baniel *et al.*, 2021). Other research does signal a direct uptake of microorganisms from the environment. In laboratory animals, most of the variance of the gut microbiome is explained by the location in different cages, despite having the same conditions, materials and diet (Hufeldt *et al.*, 2010; Hoy *et al.*, 2015; van Tilburg Bernardes *et al.*, 2020). Furthermore, an influence of habitat type on gut microbiome has been reported for black howler monkeys, ring-tailed lemurs and *Eulemur spp*. (Amato *et al.*, 2013; Bennett *et al.*, 2016; Umanets *et al.*, 2018). In yellow and anubis baboons (*Papio anubis*) inhabiting a hybrid zone in Kenya, host environments explained most of the variation, and microbial population differences were predicted by soil chemical properties and site geology (Grieneisen *et al.*, 2019). Furthermore, in the gut microbiome of Weddell's saddleback tamarins (*Leontopithecus weddelli*) soil dwelling bacteria was detected according to functional predictions (Garber *et al.*, 2019). Finally in humans, genetically unrelated individuals sharing a household have significant microbiota similarity (Rothschild *et al.*, 2018). Nevertheless, it is complicated to disentangle the effect of microbes present in the environment compared to shared diets or lifestyles between study subjects which might be driving these differences. These relationships can only be studied in higher detail when investigating the microbial communities of the host's environment.

1.4.10 Interactions between members of the gut microbiota

Interactions such as competition for resources, trophic chains, predation, and mutualism are happening between the prokaryotes (bacteria and archaea), microeukaryotes (fungi and protozoa), macroeukaryotes (helminths), and viruses comprising the gut microbiome (Parfrey, Walters and Knight, 2011; Laforest-Lapointe and Arrieta, 2018; McKenney, Koelle, *et al.*, 2018; Cortés *et al.*, 2019). For the aim of this study, only literature regarding interactions between prokaryotes and eukaryotes was reviewed. In mice, gut fungi can benefit from the presence of certain bacteria whereas they can also antagonize the growth of other bacterial taxa, and in human infants, inverse correlations between fungal and bacterial alpha diversity were detected (Fujimura *et al.*, 2016; van Tilburg Bernardes *et al.*, 2020). SCFAs produced by gut

bacteria can inhibit the growth of the yeast, *Candida albicans* (Noverr and Huffnagle, 2004). Furthermore, through mechanisms yet undescribed, co-colonization with fungi and bacteria of germ-free mice associate to a higher colitis severity (van Tilburg Bernardes *et al.*, 2020). Investigations in humans and laboratory animals detected associations between helminthic and protist parasites and changes in diversity, composition and bacterial taxa in the gut (Glendinning *et al.*, 2014; Cortés *et al.*, 2019; Berry *et al.*, 2020). *Blasytocystis* sp. is a highly prevalent protozoa in industrialized (0.5-30%) and non-industrialized (30-100%) countries, which has been associated to higher bacterial alpha diversity and changes in bacterial taxa composition (Audebert *et al.*, 2016; Beghini *et al.*, 2017; Laforest-Lapointe and Arrieta, 2018). It is proposed that *Blasytocystis* sp. may alter the bacterial communities by predation of bacteria (Laforest-Lapointe and Arrieta, 2018). Furthermore, dogs infected with *Giardia* had significant shifts in bacteria composition and taxa abundances (Berry *et al.*, 2020). In wild animals, these associations have also been reported in yellow-necked mice (*Apodemus flavicolis*), western chimpanzees, western lowland gorillas, rufous mouse lemurs, and *Eulemur spp.* with varying results regarding the correlations with alpha diversity, beta diversity and bacterial taxa (Kreisinger *et al.*, 2015; Aivelo and Norberg, 2017; Vlčková *et al.*, 2018; Renelies-Hamilton *et al.*, 2019; de Winter *et al.*, 2020). Moreover, comparative research of the gut microbiome from nonhuman primates detected positive correlations between bacterial and eukaryotic diversity (Mann *et al.*, 2020).

1.5 Redfronted lemurs (*Eulemur rufifrons*)

Redfronted lemurs (*Eulemur rufifrons*), also known as redfronted brown lemurs or Bennet's brown lemur, are cathemeral and arboreal primates found in western and eastern Madagascar (Pereira *et al.*, 1990; Donati *et al.*, 2001; Johnson *et al.*, 2020). They live in small multimale-multifemale groups that are socially tolerant but with one dominant male, who monopolizes social interactions with females, and several subordinate males (Pereira *et al.*, 1990; Ostner and Kappeler, 1999; Fichtel, Schnoell and Kappeler, 2017). They experience periods of social instability with immigration, emigration, and eviction of group members. For instance, females can be aggressively evicted during the mating (May - June) and/or birth (September - October) seasons (Ostner, Kappeler and Heistermann, 2008; Kappeler and Fichtel, 2012b). Additionally,

males can perform group takeovers, and the dominant male in the group sires most of the infants (Kappeler and Port, 2008; Port, Clough and Kappeler, 2009). These periods of instability are social stressors activating the HPA axis and possibly impacting the gut microbiome (Ostner and Kappeler, 1999; Ostner, Kappeler and Heistermann, 2002, 2008; Kappeler and Fichtel, 2012b). They perform auto- and allogrooming, including their anogenital regions, with a buccal structure named the toothcomb (Barton, 1987). This mechanism can promote the uptake of microorganisms from the fur of other individuals and thus contribute to the dispersal of gut microbial communities between individuals (Clough, 2010; Perofsky *et al.*, 2017). Their guts are inhabited by a diverse eukaryotic community including helminths and protozoa prevalent over the entire year, thus complex interactions between prokaryotes and eukaryotes are happening in their guts (Clough, 2010; Peckre *et al.*, 2018; Gogarten *et al.*, 2020). Redfronted lemurs are mainly frugivorous, they may also feed on flowers, leaves, fungi, and/or insects according to the seasonal changes in food availability (Ostner, Kappeler and Heistermann, 2008; Schnoell and Fichtel, 2013). Furthermore, these periods of lower food availability pose adaptative challenges activating the physiological stress response that may impact the gut microbiome (Ostner, Kappeler and Heistermann, 2008; Koch *et al.*, 2017). Their more common predators are raptors, the fossa (*Cryptoprocta ferox*), and dogs, however they also suffer from poaching events by humans (Fichtel and Kappeler, 2002). Altogether, these lemurs provide a unique opportunity to study the impact of social relationships on the gut microbiome while studying the influence of interactions with the host, the relationships between gut members and interaction with ecological determinants.

1.6 Aim of the study

Social individuals live in groups with conspecifics in spatial proximity interacting through social behaviors such as, grooming, mating, breeding, competition, and aggression. Group-living provides benefits and disadvantages to an individual, which can impact their health (Kappeler, Cremer and Nunn, 2015). Reduced predation risk, access to resources, social support, social learning, and contact immunity through exposure to low doses of pathogens, are some of the assets from group-living (Ugelvig and Cremer, 2007; Ezenwa *et al.*, 2016; Wittig *et al.*, 2016b; Peckre *et al.*, 2018). Social bonds, regular positive interactions, and associations between parties, may help individuals to deal with stressors and reduce HPA axis activation (Young *et al.*, 2014).

Conversely, individuals living in groups may have to compete for food, mates, and social status (Kappeler, Cremer and Nunn, 2015). These factors impact caloric intake and may produce chronic activation of the HPA axis decreasing the immune function (Ceacero *et al.*, 2012; Cavigelli and Caruso, 2015). Moreover, group-living increases the exposure to transmissible diseases and the susceptibility to infection when members are genetically related (Ezenwa *et al.*, 2016). However, members of the gut microbiome may also be transmitted through social relationships, thus making them essential for the individual's development of the gut microbial community (Tung *et al.*, 2015; Sarkar *et al.*, 2020).

The main aim of this study was to determine the drivers of gut microbiome composition and diversity in wild redfronted lemurs focusing on the impact of social relationships. Their influence was investigated directly through determining the possibility of transmission of the gut microbiome, and indirectly by addressing the influence of HPA axis activation due to social stressors. These was achieved through three studies.

1) Dietary shifts and social interactions drive temporal fluctuations of the gut microbiome from wild redfronted lemurs (Chapter 2).

In this study, first the members of the gut microbiome of redfronted lemurs including the bacteria, archaea, fungi, protists, and helminths present and their temporal fluctuations over one year were analyzed. Additionally, the potential active bacterial community was investigated and was compared to the entire bacterial community. Moreover, the seasonal associated factors such as diet, affiliative interactions, and precipitation, which could correlate to the shifts in the bacterial entire and active community, were determined

2) Multiscale study of temporal drivers of gut microbiome composition from wild redfronted lemurs (Chapter 3).

In this project the drivers of gut microbiome were studied following concepts from metacommunity theory Thus, interactions between the host and the microbiota, among members of the microbiota, and between microorganisms from other conspecifics through social interactions were explored.

3) Parasites in a social world – Lessons from primates (Chapter 4).

Most of the knowledge about the impact of social relationships on the transmission of microorganisms derives from investigating the transfer of pathogens or parasites through social interactions. Therefore, this study is a literature review regarding the social transmission of parasites and the impact of social behaviors on non-human primates, to further understand the link between social behaviors and their influence on the gut microbiome and the host.

1.7 Experimental design

The four groups, A (n= 5 - 8), B (n= 5 - 10), F (n= 6 - 7) and J (n= 11), of redfronted lemurs investigated live in Kirindy Forest, Western Madagascar (44° 39′ E, 20° 03′ S). Here, the German Primate Center operates a research field station since 1993 within a forestry concession managed by the Centre National de Formation, d'Etudes et de Recherche en Environnement et Foresterie (CNFEREF) from Madagascar. The redfronted lemurs' groups have been investigated since 1996 through population censuses and daily behavioral observations, additionally individuals are captured regularly to mark them with identifying collars and one female from each group is marked with a radio collar for localization. This long-term data collection allows the knowledge of group members age (Kappeler and Fichtel, 2012a).

Behavioral and fecal sample collection was performed for one year, from May 2018 until April 2019 to follow the temporal fluctuations of the gut microbiome. This time encompasses the dry season from May 2018 until October 2018, followed by the rainy season from November 2018 until March 2019, and April 2019 which is the transition and beginning of the next dry season (Kappeler and Fichtel, 2012a). Also, it considered the mating season from May until June and the birth season from September until October (Ostner and Heistermann, 2003; Ostner, Kappeler and Heistermann, 2008; Kappeler and Fichtel, 2012b). Only two births happened during the study period, one individual in group A and another in group B, the latter disappeared in a lapse of two months. To determine social and feeding behaviors continuous focal observations for 30 minutes were collected in the morning from 7:30 – 11: 00 and in the afternoon from 14:00 – 17:00. Furthermore, daily collection of precipitation data was performed for assessing environmental changes and available water sources. Fecal samples were collected only during the mornings from 7:30 until 11:00 for studying the members of the gut microbiome and measuring levels of fecal glucocorticoids from the same sample. Marker gene analysis was used to identify the bacteria (16S rRNA), archaea

(16S rRNA), fungi (ITS2), protists (18S rRNA), and helminths (18S rRNA) present using MiSeq Ilumina sequencing and an in-house developed amplicon pipeline (Berkelmann *et al.*, 2020). Levels of fecal glucocorticoid metabolites (fGCM) were measured using a standardized enzyme immunoassay (Heistermann, Palme and Ganswindt, 2006; Ostner, Kappeler and Heistermann, 2008). All data and statistical analyses were performed in R.

2 Dietary shifts and social interactions drive temporal fluctuations of the gut microbiome from wild redfronted lemurs

Tatiana Murillo[1,2], Dominik Schneider[2], Claudia Fichtel[1*] and Rolf Daniel[2*]

[1]Behavioral Ecology and Sociobiology Unit, German Primate Center, Göttingen, Germany

[2]Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, Georg-August-University of Göttingen, Göttingen, Germany

*These authors contributed equally.

Author contributions:

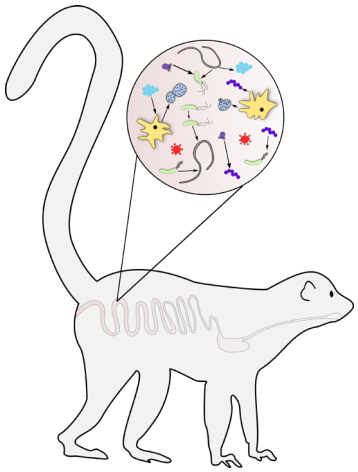
RD and CF designed and conceived the study.

TM collected data and samples.

TM and DS prepared and analyzed the data.

All authors revised and approved the manuscript.

Citation: Murillo, T., Schneider, D., Fichtel, C. & Daniel, R. Dietary shifts and social interactions drive temporal fluctuations of the gut microbiome from wild redfronted lemurs. *ISME COMMUN.* **2,** 3 (2022). https://doi.org/10.1038/s43705-021-00086-0



ISME

www.nature.com/ismecomms

ARTICLE OPEN

Dietary shifts and social interactions drive temporal fluctuations of the gut microbiome from wild redfronted lemurs

Tatiana Murillo [1,2], Dominik Schneider [2], Claudia Fichtel [1,3] and Rolf Daniel [2,3]✉

Animals living in highly seasonal environments adapt their diets accordingly to changes in food availability. The gut microbiome as an active participant in the metabolization of the host's diet should adapt and change with temporal diet fluctuations, but dietary shifts can be short-term and, hence, difficult to detect in cross-sectional studies. Therefore, we performed a longitudinal study combining repeated sampling of fecal samples with observations of feeding behavior in wild redfronted lemurs. We amplified taxonomical marker genes for assessing the bacteria, archaea, protozoa, helminths, and fungi, as well as the active bacterial community inhabiting their gut. We found that the most abundant protozoans were *Trichostomatia* and *Trichomonadida*, and the most abundant helminths were *Chromadorea*. We detected known members of the gut mycobiome from humans but in low abundances. The archaeal community is composed only of members of *Methanomethylophilaceae*. The predominant phyla in the entire bacterial community were *Bacteroidota* and *Firmicutes* while the most abundant genera harbor so far unknown bacteria. Temporal fluctuations at the entire community level were driven by consumption of fruits and flowers, and affiliative interactions. Changes in alpha diversity correlated only with the consumption of flowers and leaves. The composition of the entire and active bacterial community was not significantly different, but the most abundant taxa differed. Our study revealed that monthly changes in the bacterial community composition were linked to fruit and flower consumption and affiliative interactions. Thus, portraying the importance of longitudinal studies for understanding the adaptations and alterations of the gut microbiome to temporal fluctuations.

ISME Communications; https://doi.org/10.1038/s43705-021-00086-0

INTRODUCTION

The gut microbiome is a complex fluctuating microbial ecosystem comprising prokaryotic and eukaryotic microorganisms playing a pivotal role in immunity, physiology, metabolism, and susceptibility to disease of the host [1, 2]. Investigations of factors driving these fluctuations help to understand how this ecosystem adapts to the changing conditions, and the potential effects these variations have on the health and fitness of their hosts [2–4].

Essential nutrient cycling processes of the gut ecosystem occur between the host diet, the microorganisms, and the host itself [1, 2, 4]. Bacteria catalyze the fermentation of dietary fiber and starch into short-chain fatty acids and monosaccharides taken up by the host and other microorganisms [1, 2]. They also provide ammonia for protein synthesis by metabolizing essential and non-essential amino acids [2]. The host diet shapes the microbial gut communities and the presence of certain microorganisms is crucial for proper degradation and uptake of nutrients from diet and the resilience of the gut ecosystem [1, 2, 4]. Therefore, the gut microbial ecosystem of wild animals living in highly seasonal environments should be capable of adapting to dietary changes following fluctuations in food availability and seasonality [3–5]. Research in wild mice (*Apodemus sylvaticus*), Tibetan macaques (*Macaca thibetana*) and pandas (*Ailuropoda melanoleuca*) found marked seasonal variations in the gut microbiome composition and diversity associated with environmental fluctuations affecting food availability [5–7]. Furthermore, cross-sectional studies in black howler monkeys (*Alouatta pigra*), white faced capuchins (*Cebus capucinus*), and Verreaux's sifakas (*Propithecus verreauxi*) determined these fluctuations correlate with changes in foraging and feeding behaviors [8–10]. Nonetheless, by sampling only representative months of each season, short-term dietary and gut microbiome shifts might be undetected [4]. In the Hadza hunter-gatherers, a seasonal cycling of the gut microbiome following seasonal changes in their diets between fruit foraging and hunting was detected [11]. Longitudinal studies in great apes (*Gorilla gorilla gorilla*, *Gorilla beringei beringei* and *Pan troglodytes troglodytes*) and geladas (*Theropithecus geladas*) determined seasonal fluctuations of the gut microbiome correlate with rainfall, temperature, and food availability [12, 13]. While a time series study in baboons (*Papio cynocephalus)* detected a highly dynamic gut microbiome varying according to the group's diet, rainfall, and the quality of the water sources [14]. Thus, enhancing the importance of time series analysis in wild animals to determine how the gut microbial communities adapt to seasonal changes [4, 8, 12, 14].

[1]Behavioral Ecology and Sociobiology Unit, German Primate Center, Göttingen, Germany. [2]Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, Georg-August-University of Göttingen, Göttingen, Germany. [3]These authors contributed equally: Claudia Fichtel, Rolf Daniel.
✉email: rdaniel@gwdg.de

Received: 23 June 2021 Revised: 12 December 2021 Accepted: 21 December 2021
Published online: 19 January 2022

To our knowledge, all taxonomic profiling studies in wild animals focus on the amplification of 16 S rRNA gene from DNA, hence studying the entire community. This approach can be biased by the number of 16 S rRNA operons and the presence of dormant or dead cells in the sample [15]. Conversely, when amplifying the 16 S rRNA transcripts, only the bacterial community that is actively replicating is investigated, providing insights into the potentially active community [16]. This approach can provide better proxies into the functional metabolic changes that the gut microbiome undergoes as a response to seasonality [2, 4].

We performed a longitudinal analysis of the entire and active gut bacterial community in a wild primate, the redfronted lemur (*Eulemur rufifrons*). Their habitat, Kirindy Forest in Madagascar, is highly seasonal having a cold dry season from April to October and a warm rainy season from November to March [17, 18]. These environmental conditions cause changes in the availability of food and water sources, posing adaptive challenges for these animals [19–21]. Redfronted lemurs are mainly frugivorous but consume leaves, and flowers following seasonal fluctuations, and adjust their drinking behavior according to the available water sources [20, 21]. Hence, these redfronted lemurs are a suitable study system to characterize temporal fluctuations in the gut microbiome composition. Moreover, they possess a high eukaryotic parasite richness with variations in their monthly prevalence as detected from morphological studies, suggesting complex prokaryotic and eukaryotic interactions occur in their guts [18, 22, 23]. However, their gut mycobiome is still unexplored despite its potential metabolic importance [24, 25].

For 1 year, we collected up to three fecal samples per month for each individual and conducted regular animal focal observations to determine their dietary composition and affiliative interactions. Since previous research suggested that social group and home range can also impact the gut microbiome [10, 26], we studied only one group consisting of five individuals to control for these potential confounding factors. To characterize the microbiome composition, we assessed the entire and active bacterial community as well as other inhabitants of the gut, including *Protozoa*, helminths, *Fungi*, and *Archaea*. We hypothesize that by using a longitudinal approach, we [1] determine temporal fluctuations in composition and diversity of the bacterial entire and active community correlate to monthly changes in diet and affiliative interactions, [2] find no significant differences between the entire and the active bacterial communities, and [3] detect temporal changes in the abundances of the eukaryotic community.

METHODS

Sample, behavioral, and environmental data collection

This study was conducted at the research station of the German Primate Center in Kirindy Forest, Western Madagascar (44°39′E, 20°03′S) from May 2018 to April 2019 [17]. Samples and data were collected over 1 year from five redfronted lemurs belonging to the same group; three adult females (FLucF, FTorF, and FMayF), one juvenile female (FBonF) and one adult male (FCaiM) (Supplementary Table S1). Fecal samples were collected in RNAlater (Thermofisher Scientific, Massachusetts, USA) from the forest floor immediately after defecation between 7:30 and 11:00, stored at −20 °C in the field station and later at −80 °C in Germany. A total of 142 samples were collected, with an average of two samples per individual per month (Supplementary Table S1). Behavioral data was collected by continuous focal observations for 30 min in the morning (7:30–11:00) and afternoon (14:00–17:00). Feeding behaviors were recorded by protocolling their duration and the ingested food item (leaves, flowers, or fruits). For affiliative interactions, we protocolled the duration of grooming and body contact behavior. Environmental data (daily temperature and precipitation) were collected at the field station with a Tropos data logger (Lambrecht meteo, Göttingen, Germany).

DNA extraction and amplification of taxonomic marker genes

DNA extractions were performed with the PowerSoil DNA isolation kit (Qiagen, Hilden, Germany) using 150 mg fecal sample following the manufacturer´s instructions but including a bead beating step of 6.5 m/s and 24 × 2 for 20 s using FastPrep-24^{TM}5G (MP Biomedicals, California, USA). PCR reactions for all taxonomical marker genes were performed in triplicates with the primers and thermocycling protocols listed in the Supplementary Table S2 and included a negative control without DNA template and a positive control [27–32]. Triplicates per sample were pooled equimolar, purified, and sequenced as in [33].

RNA extraction and cDNA synthesis

RNA was extracted from 250 mg fecal sample using the RNeasy Power Microbiome kit (Qiagen) following the manufacturer´s instructions, and according to the protocol from [33].

Bioinformatic processing of amplicon data

Paired-end reads were quality-filtered with Fastp0.20.0 [34] using default settings with the addition of an increased per base phred score of 20, base pair corrections by overlap (-c), as well as 5′- and 3′-end read-trimming with a sliding window of 4, a mean quality of 20 and minimum sequence length of 50 bp. Quality-controlled reads were merged with PEARv0.9.11 [35] and primer-clipping was performed with cutadapt2.5 [36] with default settings. VSEARCH2.14.1 [37] was used for size-sorting, size-filtering (16 S rRNA ≥ 300 bp; 18 S rRNA ≥ 250 bp; ITS2 ≥ 140 bp) and dereplication. The sequences were denoised with UNOISE3 [38] using default settings and chimeras were removed with UCHIME3 (*de novo* followed by reference-based) [39] leading to the final set of amplicon sequence variants (ASVs). Then all reads were mapped against the ASVs and taxonomy was assigned with a minimum identity of 90% using BLAST2.9.0 + [40] against different databases according to the taxonomical marker gene. The databases were SILVA SSU 138 NR [41] for 16 S rRNA, PR^2 SSU rRNA [42] for 18 S rRNA and UNITE 8.2 [43] for ITS2. Best hits were only accepted if $\left(\frac{\%identity+\%coverage}{2}\right) \geq 93$ following the recommendation of SILVA database [41]. Best blastn hit identity for bacterial species <98.7% or genus <94.5% were corrected to unclassified [44]. Functional predictions were performed using Faprotax1.2.3 [45] for the bacterial 16 S rRNA gene data after beforementioned filters were applied. All sequencing statistics are presented in Supplementary Table S3.

Data visualization and statistical analysis

Data visualization and statistical analysis were performed using Rv3.6.2 [46] and RStudiov1.20.5033 [47] by using the packages ampvis2 [48], ape [49], stringr [50], reshape2 [51], viridis, data.table [52], tidyverse [53], and ggplot2 [54]. Datasets for barcharts, heatmaps, and linecharts were normalized using GMPR [55], whereas data was rarefied for diversity and multivariate analysis (Supplementary Table S3). A phylogenetic tree was generated by aligning all sequences with MAFFTv7.407-1 [56] at 100 iterations, calculated using FastTreeMPv2.1.7 [57] and midpoint-rooted using FigTree v1.4.4 [58] for estimating Faith´s phylogenetic diversity (PD) with the package picante [59].

For the 18 S rRNA gene amplicon analysis of eukaryotic parasites and symbionts, samples with <9000 reads were excluded leaving 115 samples. ASVs from the kingdoms previously reported as inhabitants of the gastrointestinal tract of animals: *Cercozoa*, *Ciliophora*, *Metazoa*, *Apicomplexa*, *Lobosa*, *Conosa,* and *Metamonada* were analyzed [23, 60]. For the ITS2 dataset samples with <7000 reads after quality-filtering samples were removed leaving 125 samples for analysis.

ANCOM analysis to estimate differential taxa between seasons. To determine bacterial genera with significant different relative abundances between seasons, we used ANCOM 2.1 [61] and the packages exactRankTests [62], nlme [63], compositions [64], and readr [65] by using the repeated measures model with season as main variable and individual as random effect, and 0.7 as threshold of the *W* statistic.

Multivariate analysis to study temporal changes in β-diversity. Principal coordinate analyses (PCoA) using weighted UniFrac distances (WUnifrac) [66, 67] were calculated in ampvis2 [48]. To test for correlations of the behavioral and environmental variables an environmental fit with 999 permutations was calculated and corrected for repeated sampling by using strata as individual with vegan [66]. A PERMANOVA test was calculated with the adonis function from the vegan package to test for significant differences between individual β-diversity calculated as WUnifrac. Mantel tests using Spearman correlations were calculated with the vegan package to estimate correlations between β-diversity from WUniFrac distances and time between sample collection.

Linear mixed model for estimating the effects on bacterial composition. The effects of feeding behaviors and affiliative interactions on the bacterial composition of the entire bacterial community were tested by fitting a Linear Mixed Model (LMM) with lme4 [68]. The model included monthly feeding rates (min/h) on fruits, leaves, or flowers, and affiliative interactions (min/h) per individual as test predictors, and mean monthly precipitation (mm) as control predictor. Taxa with abundances <0.5% in a sample were removed to account for index hopping during sequencing [69]. To deal with data compositionality, the microbial proportions of each sample were centered log-ratio transformed [70]. The random intercepts effects of individual, taxon, sample, and taxon nested within individual (taxon-individual) were included, the latter to account for individual specific microbial compositions. Random slopes for all predictors in taxon, individual, and taxon-individual were included, excluding flower feeding rates for taxon-individual [71]. Parameters for the correlations between random intercepts and slopes within taxon and taxon-individual were included [71] but not within individual because they were unidentifiable [72]. Assumptions of normally distributed and homogeneous residuals were checked visually with QQ-plots of residuals and residuals plotted against fitted values which revealed no obvious deviations. No issues of collinearity were detected by calculating Variance Inflation Factors using car [73] on a model lacking the random effects (maximum: 1.203). The crucial terms in this model were the random slopes within taxon representing the taxon-specific effects of the test predictors and were tested with a permutation test by shuffling the labels of taxa within sample [74, 75]. As a test statistic, we used the difference between the log likelihoods of the full model and simpler models. One of the simpler models lacked all random slopes within the sample except that of precipitation allowing a full-null model comparison by testing the combined effects of all test predictors. The others lacked the individual random slopes (except precipitation) within taxon allowing to test their individual contribution. A total of 1000 permutations including the original data as one permutation were conducted, and *p*-values were calculated as the proportion of permutations that revealed a test statistic at least as large as that of the original data. If an individual random slope effect was significant, then the effect of the respective predictor differs between taxa. The 20 taxa differing most from the average effect across all taxa, meaning they had the largest absolute values of the respective Best Linear Unbiased Predictors (BLUPs), were inspected [76]. Model stability was assessed by dropping individuals one at time, fitting the full model to each of the subsets, and then comparing the estimates derived with those obtained for the full model revealing it was acceptable. Residuals for each combination of taxon and predictor were plotted verifying the presence of linear trends.

Linear mixed models for estimating effects on alpha diversity. The effects of feeding behaviors and affiliative interactions on alpha diversity for the entire and active bacterial community were estimated by fitting a LMM using lme4 [68], MuMIn [77], and visualized with sjPlot [78]. The response variable was PD, which was log-transformed for the model of the active community. Affiliative interactions were log-transformed to achieve a more symmetrical distribution and avoid influential cases, and all predictors were z-transformed to facilitate model convergence. We included individual identity as a random intercept effect and the random slopes of all fixed effects into individual identity to keep the type I error at the nominal level of 5% [71]. For estimating the significance of the test predictors, a null model excluding the test predictors was calculated and then compared to the full model using a likelihood ratio test. We determined the effect of single fixed effects using likelihood ratio tests comparing the full model with reduced models removing one fixed effect at a time [71]. Model assumptions and collinearity (DNA: 1.203; RNA: 1.205) were checked as in the LMM for bacterial composition with no obvious deviations from these assumptions. Model stability was assessed as described above.

Procrustes analysis. Procrustes analysis and significance testing with protest were performed using vegan [66] to test for correlations between the plant material detected from the 18 S rRNA gene amplicons and the entire bacterial community from calculated PCoAs of Bray Curtis dissimilarity matrices in ampvis2 [48]. Only those samples with >1000 reads for *Archaeplastida* were analyzed, leaving 97 samples after rarefaction. The same test was used to determine significant differences between the composition of the entire and active bacterial community from the PCoAs from WUnifrac distances. A summary of all statistical results is depicted in Supplementary Table S4.

Gene alignments and phylogenetic tree from eukaryotic data

Sequence alignments were done with MUSCLE [79] with UPGMA and default settings. Phylogenetic trees were calculated with the Maximum Likelihood method, Tamura-Nei model, and 1000 bootstrap in MEGA X [80]. The 18S rRNA gene and ITS2 sequences from representative nematodes and *Fungi* were retrieved from GenBank database [81].

Data deposition

The 16 S rRNA gene and transcripts, 18 S rRNA gene, and ITS2, paired-end raw reads were deposited in the National Center for Biotechnology Information Sequence Read Archive (SRA) under the Bioproject PRJNA694983. SRA numbers are in Supplementary Table S1.

RESULTS

Composition of the redfronted lemur gut microbiome

The most abundant bacterial phyla in the five redfronted lemurs were constant throughout the sampling period with varying relative abundances; these were *Bacteroidota* (30.6% ± 7.6), *Firmicutes* (30.0% ± 8.2), *Proteobacteria* (12.3% ± 6.5), *Spirochaetota* (8.7% ± 2.5) and *Verrucomicrobiota* (6.3% ± 2.2) (Fig. 1A and Supplementary Table S5). These were consistent for all individuals exempting an increase of *Firmicutes* (55.7%) and *Proteobacteria* in February for FBonF, and an increase of *Fusobacteriota* (9.9%) in January for FLucF.

The taxa detected from the amplification of the 18 S rRNA gene were *Metazoa* (56.9% ± 22.7), *Streptophyta* (21.3% ± 13.2), *Fungi* (6.6% ± 6.1), *Ciliophora* (9.6% ± 7.2), and *Metamonada* (1.9% ± 1.5) with a total of 3.1% ± 2.9 unclassified reads (Fig. 1B and Supplementary Table S6). The most abundant orders previously reported as eukaryotic parasites detected were *Chromadorea*, *Trichostomatia,* and *Trichomonadida* (Fig. 1C). *Chromadorea* highest abundances were in October (94.4%) and lowest in February (40.0%). While *Trichostomatia* increased in February (53.6%), and *Trichomonadida* in May (7.3%). Overall, ASVs classified as *Chromadorea* showed high diversity, indicating a diverse nematode community (Supplementary Fig. S1).

To study fungal gut communities, we analyzed the ITS2 region. A total of 71% ± 16.8 of sequences were unclassified to Kingdom, thus demonstrating a lack of information from Malagasy fungal organisms in databases (Fig. 1D and Supplementary Table S7). When studying the gut mycobiome the separation between symbionts and environmental fungi using metagenomic approaches is challenging [24, 82]. Especially in redfronted lemurs, who feed on *Fungi* and plants, which potentially harbor fungal pathogens. Thus, we extracted only those fungal genera described before as gut symbionts [24]. We detected these genera in relative abundances <1%: *Cryptococcus*, *Agaricus*, *Candida*, *Saccharomyces*, *Malassezia,* and *Clavispora* whereas other genera like *Cladosporium*, *Aspergillus*, *Fusarium,* and *Penicillium* were present in relative abundances >1%. In a phylogenetic analysis calculated from the 20 most abundant unclassified ASVs against ITS2 sequences from some of the fungal genera described as inhabitants of the gut mycobiome only one ASV was phylogenetically related to *Cladosporium* (Fig. 1E). Also, no similar sequences were detected in the NCBI database.

The archaeal community was assessed with 16 S rRNA gene analysis in a smaller set of samples using two different sets of primers aiming to recover sequences of different lineages. In both cases, only *Methanomethylophilaceae* was identified (Supplementary Tables 8 and 9). Thus, the archaeal community has a low diversity and comprises members also known from the gut of great apes and humans [83, 84].

Temporal variations of the entire gut bacterial community composition

The five most abundant genera comprise mostly novel organisms for which only classification at the family level was possible

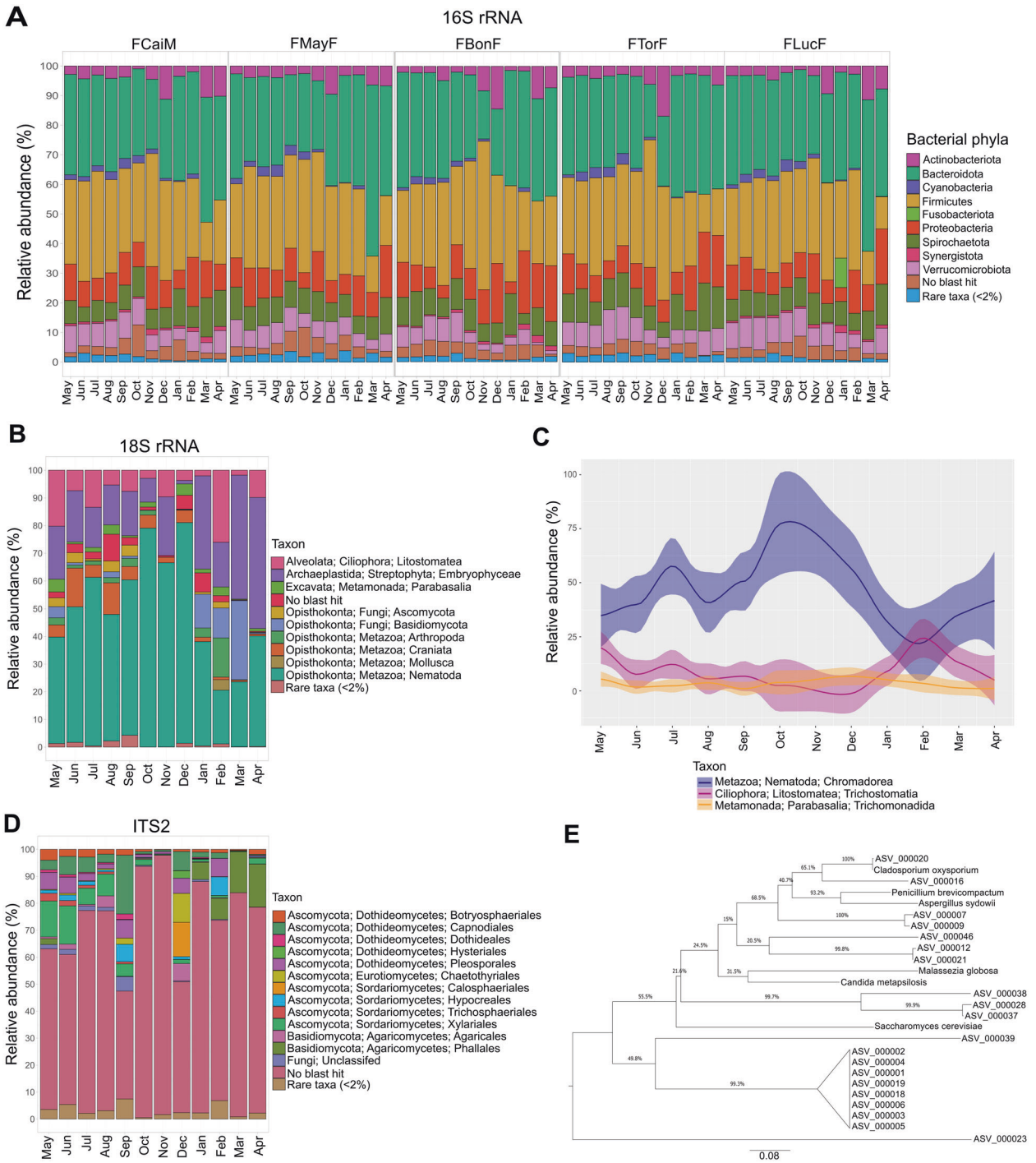


Fig. 1 Prokaryotic and eukaryotic communities from the gut of redfronted lemurs during the study period from May 2018 until April 2019. A Monthly relative abundances of bacterial phyla for the five studied individuals as determined from 16 S rRNA gene sequencing. Bar charts depict relative abundances of bacterial phyla from normalized counts for each individual per month. All phyla with abundances <2% were grouped as rare taxa. **B** Eukaryotic organisms detected in the fecal samples through 18 S rRNA gene sequencing. Bar charts show monthly relative abundances of eukaryotic classes from normalized counts. All phyla with abundances <2% were grouped as rare taxa. **C** Monthly fluctuations in the relative abundances of *Chromadorea*, *Trichostomatia*, and *Trichomonadida*. Linecharts depict relative abundances of normalized counts of the detected eukaryotic parasites or endosymbionts in the fecal samples. **D** Fungal organisms detected in the fecal samples through ITS2 sequencing. Barcharts display monthly relative abundances of fungal orders from normalized counts. All taxa with abundances <2% were unified as rare taxa. **E** Maximum likelihood phylogenetic tree of the unclassified ITS2 ASVs against representative *Fungi*.

(Fig. 2A). These genera belong to the four families of *Prevotellaceae* (14.6% ± 7.4), *Spirochaetaceae* (8.9% ± 3.1), *Rikenellaceae* (5.7% ± 4.1) and *Kiritimatiellae* (5.1% ± 2.4). The fifth most abundant genus was *Sutterella* (3.9% ± 2.3). All showed monthly fluctuations in their abundances, which were not always consistent among individuals (Supplementary Fig. S2). The top 20 most abundant genera also presented monthly and individual differences in their relative abundances (Supplementary Fig. S3). A PERMANOVA test confirmed the β-dissimilarities were significantly different between individuals ($p < 0.002$).

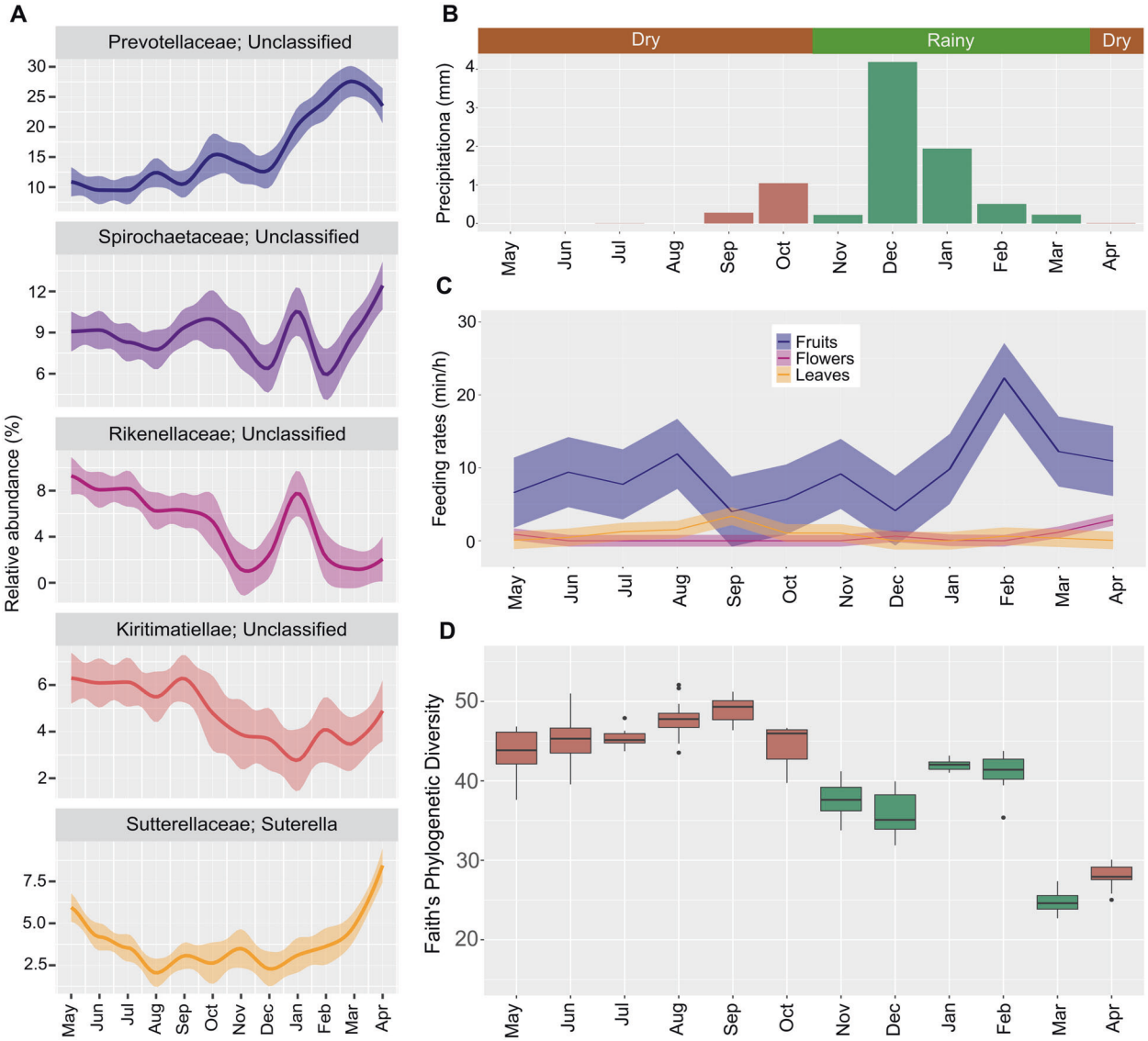


Fig. 2 Monthly fluctuations in most abundant bacterial genera and alpha diversity detected in fecal samples from redfronted lemurs from May 2018 to April 2019. A Top 5 most abundant bacterial genera and their monthly changes for all studied individuals. Line charts display relative abundances from normalized counts. **B** Mean monthly precipitation calculated from records of daily precipitation and seasons from the study period. **C** Monthly feeding rates on fruits, leaves, and flowers determined through behavioral focal observations. **D** Monthly variations in alpha diversity measured by Faith's Phylogenetic Diversity Index of all studied individuals.

Seasons were defined following previous publications [17], however, during our study rainfall increased at the end of the dry season (Fig. 2B), and feeding behaviors varied across months (Fig. 2C). Alpha diversity increased during the dry season with a maximum between August and October (Fig. 2D). The PD value fluctuated during the whole rainy season and was lower compared to the dry season. Monthly alpha diversity changes followed the same pattern in all individuals (Supplementary Fig. S4).

ANCOM analysis revealed that 75 genera showed significant differential abundance between dry and rainy seasons (Supplementary Fig. S5). We focused on taxa classified with relative abundances ≥1% (Fig. 3A).

Mean monthly precipitation, consumption of fruits, leaves, and/or flowers and the rate of affiliative interactions correlated to the temporal variations in β-diversity. Samples from the dry season clustered together unlike the samples from the rainy season (Fig. 3B), and season ($p = 0.001$), precipitation ($p = 0.001$), feeding on fruits ($p = 0.003$), leaves ($p = 0.044$), flowers ($p = 0.001$), and affiliative interactions ($p = 0.006$).

The LMM detected taxon-specific effects (full-null model comparison; permutation test: $p = 0.001$) of flower ($p = 0.001$) and fruit feeding ($p = 0.001$), and affiliative interactions ($p = 0.043$) on the overall bacterial community composition (Supplementary Table S10) the following: exhibited significant correlations. We thus inspected the 20 taxa for which the taxon-specific effect deviated most from the average effect across all taxa for each significant predictor (Fig. 3C and Supplementary Fig. S6–9).

A time series analysis of WUnifrac distances against time between sample collection confirmed temporal variations on individual level (Supplementary Fig. S10 and Supplementary Table S11). Thus, the longer the timespan between the samples, the more dissimilar were the gut bacterial communities.

The LMM for the alpha diversity (full-null model comparison: $p = 0.003$) detected an effect of feeding on leaves ($p = 0.055$, Fig. 4B) which correlated with an increase in PD, while the rates of flower

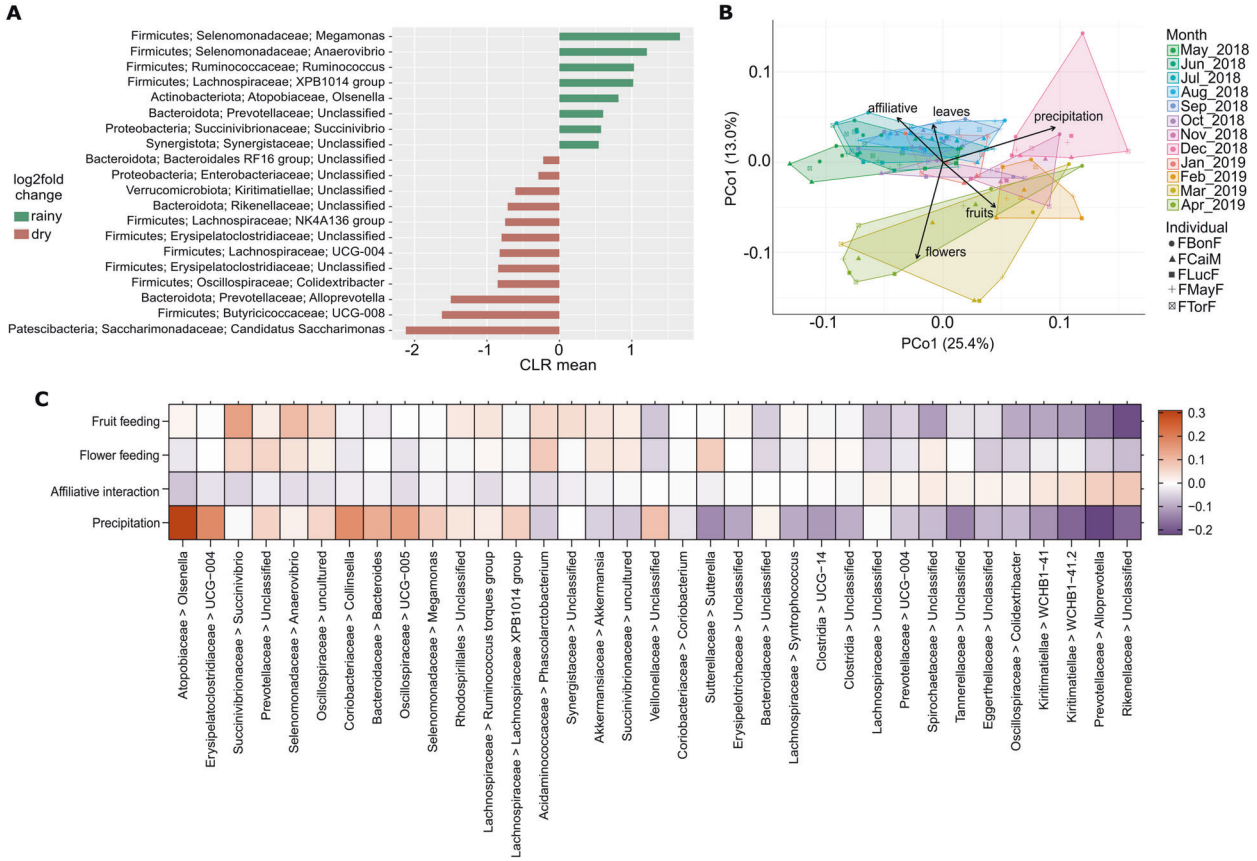


Fig. 3 Seasonal variations of bacterial genera, beta diversity, and composition of the entire gut bacterial community of redfronted lemurs from May 2018 until April 2019. A Log2f fold changes in the mean abundances of bacterial genera between dry and rainy season as detected with ANCOM 2.1. **B** PCoA based on weighted Unifrac of the bacterial community and environmental fit analysis depicting significant correlations between temporal fluctuations in beta diversity and the environmental, diet and social factors investigated. **C** Heatmap showing the 20 bacterial genera for which taxon-specific effects differed most from the average across all taxa as detected in a LMM estimating the effects of diet and affiliative interactions on community composition. The image displays the test predictors for which an effect was detected, feeding on flowers and fruits, and affiliation rates. Precipitation was included as the control predictor. Positive effects are depicted with orange, whereas negative effects are colored in purple.

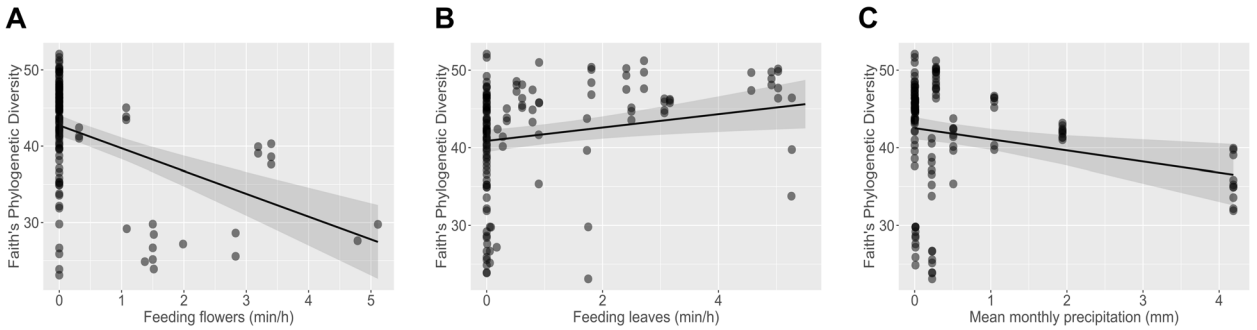


Fig. 4 Environmental and dietary factors driving the monthly fluctuations in alpha diversity of the entire bacterial community measured with the Faith's Phylogenetic Diversity index. A Monthly rates of flower consumption (min/h) correlate negatively with alpha diversity. **B** Monthly rates of leaves feeding (min/h) correlate positively with a higher alpha diversity. **C** Mean monthly precipitation correlates negatively with alpha diversity. The effects of diet, affiliation rates and precipitation were determined with a LMM.

consumption and mean monthly precipitation correlated negatively with PD (flowers: $p = 0.002$; Fig. 4A; monthly rainfall: $p = 0.039$, Fig. 4C, Supplementary Table S12). An effect of dietary changes on bacterial community composition was further confirmed by significant correlations from the plant diet deduced from the 18 S rRNA gene analysis (Supplementary Fig. S11A) to the fluctuations of the bacterial community (Supplementary Fig. S11B) ($p = 0.001$; Supplementary Fig. S11C).

Potential active bacterial community in the redfronted lemur gut

The potentially active bacterial communities were analyzed from one sample per individual per month. The five most abundant phyla detected in the active community were *Firmicutes* (56.1% ± 13.1), *Bacteroidota* (16.5% ± 6.1), *Actinobacteriota* (9.9% ± 4.4), *Proteobacteria* (5.2% ± 2.2), and *Spirochaetota* (4.9% ± 2.2) (Supplementary Fig. S12A). The five most abundant genera were *Colidextribacter*

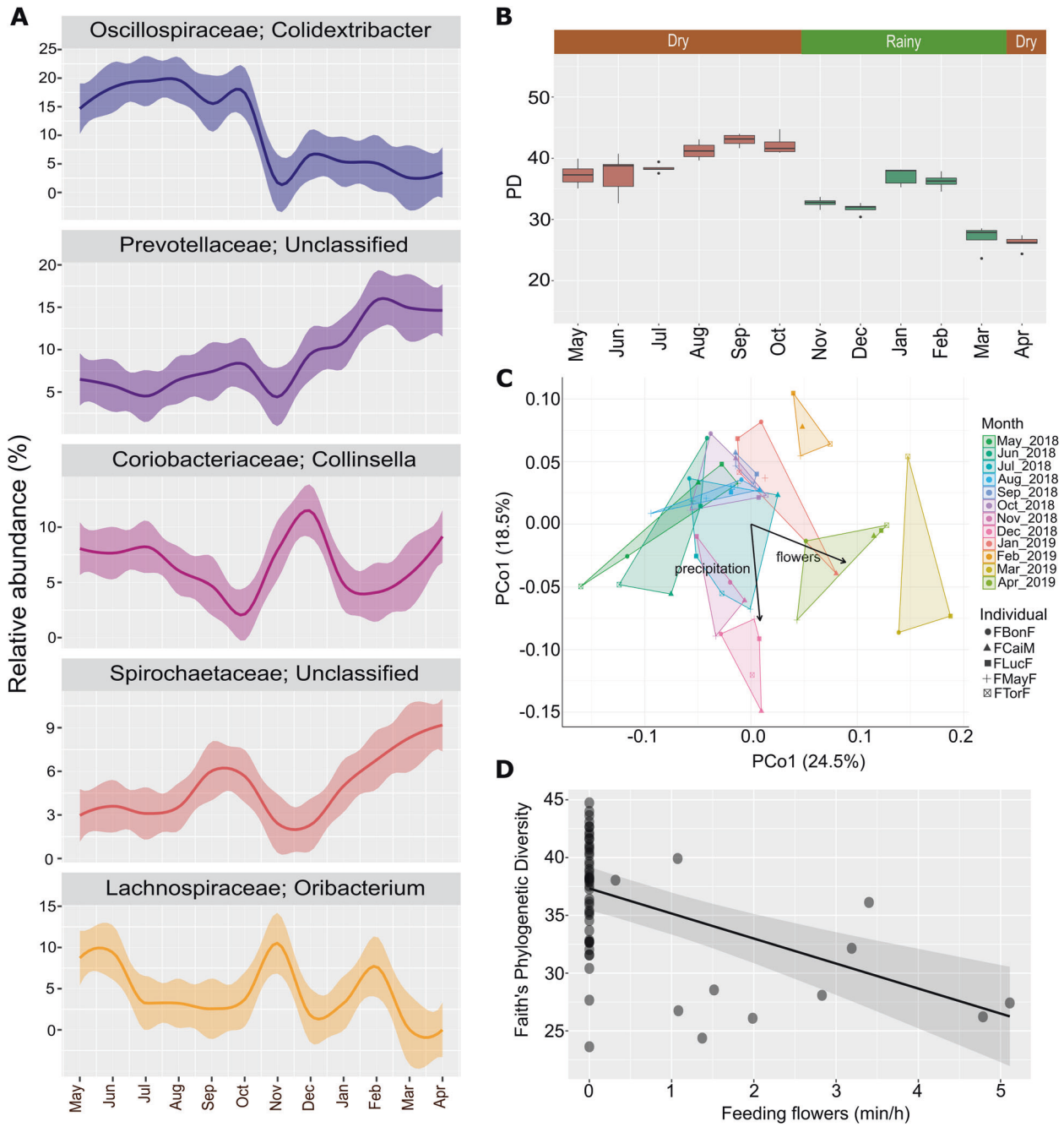


Fig. 5 Monthly fluctuations in the bacterial composition and alpha diversity of the active bacterial community in fecal samples from redfronted lemurs from May 2018 to April 2019. A Top five most abundant bacterial genera and their monthly changes for all studied individuals. Line charts display relative abundances from normalized counts. **B** Monthly variations in alpha diversity measured by Faith's Phylogenetic Diversity Index in all studied individuals. **C** PCoA from WUnifrac of the bacterial community and environmental fit analysis depicting significant correlations between temporal fluctuations in beta diversity and the environmental, diet and social factors investigated. **D** Monthly rates of flower consumption (min/h) correlate negatively with alpha diversity. The effects of diet, affiliation rates and precipitation on alpha diversity were determined with an LMM.

(11.5% ± 8.1), *Prevotellaceae*—Unclassified (8.8% ± 4.8), *Collinsella* (6.7% ± 3.4), *Spirochaetaceae*—Unclassified (4.8% ± 2.8), and *Oribacterium* (4.5% ± 4.6) (Fig. 5A and Supplementary Fig. S12A). The top 20 most abundant genera were also investigated, which presented monthly and individual differences in their relative abundances (Supplementary Fig. S13).

The ANCOM analysis revealed that 40 genera exhibited significantly different relative abundances between seasons. Most exhibited abundances <1% or were only classifiable to order level (Supplementary Fig. S14) leaving only *Bacteroidales* group RF16 with lower abundances in the rainy season, whereas *Lachnospiraceae* group XPB1014 and *Fusobacterium* had higher abundances in the rainy season. PD was higher during the dry season and more variable during the months of the rainy season, like at the entire community level (Fig. 5B). The PCoA did not show seasonal clustering (Fig. 5C). However, the environmental fit analysis detected correlations of season ($p = 0.007$), feeding on flowers ($p = 0.003$) and precipitation ($p = 0.013$) with the monthly alterations of the bacterial community (Fig. 5C). Mantel correlation tests of the β-dissimilarities and the timespan between sample

collection for each individual were significant (Supplementary Table 13). Only feeding on flowers ($p = 0.002$) was associated with an effect in alpha diversity correlating with a decrease (Fig. 5D, Supplementary Table S14).

For comparison of entire with active communities a PCoA from WUnifrac with the reduced sample size was calculated also at entire community level. Precipitation ($p = 0.003$) and feeding on fruits ($p = 0.056$) and flowers ($p = 0.002$) were significantly correlated (Supplementary Fig. S15A). The comparison between the PCoAs from the entire and active community with the protest test from the Procrustes analysis detected significant correlations ($p = 0.001$). Thus, they were not significantly different (Supplementary Fig. S15B).

Functional predictions performed for the active community assigned 51.7% of the ASVs to an entry of the Faprotax database. Chemoheterotrophy (21.6% ± 3.6) and fermentation (21.3 ± 3.7) were the most abundant metabolisms, with a peak during the rainy season from October to January coinciding with an increase in fruit feeding (Fig. 2C and Supplementary Fig. S16).

DISCUSSION

Our longitudinal approach coupled with a dense sampling regime and behavioral data allowed us to detect in detail the temporal fluctuations of the gut microbial communities from redfronted lemurs. We determined the entire bacterial community changed accordingly to a higher consumption of fruits and flowers, and variations in affiliative interactions. Hence, the bacterial community quickly adapted to monthly changes in the diet but also to the host social behavior. Moreover, we characterized the potentially active bacterial community, which also underwent temporal fluctuations that correlated but only to flower consumption. The overall composition of the entire and the active bacterial communities were not significantly different, but the most abundant genera differed. The eukaryotic communities also presented temporal fluctuations and includes undescribed organisms.

Unknown genera inhabit the gut microbiome of redfronted lemurs

The most abundant bacterial phyla identified were *Bacteroidota* and *Firmicutes* similarly to other primates and humans [12, 85, 86]. *Spirochaetota* was also detected in high abundances, coinciding with previous reports from other primates and a cross-sectional study from the same species [86, 87]. A previous study in the same lemur species detected only low abundances of treponemes but higher abundances of *Cyanobacteria* and *Firmicutes* [87]. However, in this study, samples were not placed in preservation solution for a time span of 12 h, which might have altered the bacterial community [87].

The impossibility to classify the most abundant bacteria to taxonomic resolutions below family level highlights the presence of yet unclassified microorganisms in the gut of redfronted lemurs, as described in other non-human primates [86]. While the classifiable taxa are reported inhabitants of the gut from humans and other non-human primates [8, 11, 13, 86, 88]. Genera from *Prevotellaceae* and *Spirochaetaceae*, have been associated to plant-based diets providing pathways for their metabolization [11, 85, 86]. *Rikenellaceae* ferments carbohydrates and proteins [89]. Taxa from *Verrucomicrobiota* have been reported as mucin-utilizers [11]. Less is known about the metabolic role of *Sutterella*, a common inhabitant of the human gut [90].

The potential active bacterial community has a lower alpha diversity and differs in the most abundant taxa

The potential active community had higher relative abundances of *Firmicutes* and a lower alpha diversity compared to the entire community. There are several possible explanations for the lower alpha diversity detected. First, redundancy of metabolisms in the bacterial community due to a pool of phylogenetically different community members capable to degrade the same substrates, which are not all active at the same time [2, 16, 91]. Second, community resilience, with other members in dormant stages that allow further functional adaptations when the environmental conditions change [16, 91]. Third, differences in the copy numbers of 16 S rRNA genes between taxa, inflating the abundance of a taxa but not portraying the actual functional scenario [15, 16, 91]. However, the entire and active community are not significantly different and follow similar temporal fluctuations. Therefore, studying only the entire community provides insights into the temporal fluctuations of the gut microbiome, but studying the active community indicated functionally important active taxa can go unnoticed because of their lower abundances at entire community level. Regarding the most abundant genera detected differing from the entire community, there is no information about the metabolism of *Colidextribacter*, while *Collinsella* and *Oribacterium* are polysaccharide degraders coinciding with the lemurs' diet [92–94].

The functional predictions from the active community indicated an increase in fermentation and chemoheterotrophy during the rainy season possibly associated to the higher consumption of fruits and flowers [8, 9, 12, 14]. However, we did not detect an augmentation of cellulolytic metabolism correlating with leaf consumption during the dry season. Since we performed metabolic predictions from taxonomy, we consider this is caused by the limited and biased metabolic information for certain taxa.

Dietary changes have an effect in the temporal fluctuations of the gut microbiome

The collection of behavioral data and the dietary assessment performed with the 18 S rRNA gene data allowed us to confirm temporal fluctuations of the gut microbiome correlate to dietary changes. We detected differentially abundant taxa for the rainy season, correlations of flower and fruit consumption to the temporal variations in β-diversity, and taxon-specific effects of flower and fruit consumption in bacterial composition. Flowers and fruits are high in non-structural polysaccharides like mono- and disaccharides, but flowers contain more protein whereas fruits have a higher lipid content [95–97]. The positively affected taxa by the consumption of these plant parts coincide with these observations, since they are reported fermenters of mono- and disaccharides, like *Succinivibrio, Oscillospiraceae,* and *Prevotellaceae*, while *Anaerovibrio*, metabolizes glycolipids [85, 98–100]. Furthermore, *Succinivibrio* and *Anaerovibrio* produce succinate from their fermentations which in turn is the energy source of *Phascolarctobacterium*, another positively affected taxon [98, 99, 101]. The correlation of flower consumption with a lower alpha diversity suggests that a diverse gut microbial community is not needed for the digestion of flowers, coinciding with their less complex biochemical composition [96, 97].

Against our expectations, consumption of leaves only correlated to β-diversity changes from the dry season but did not influence the overall bacterial composition. However, leaf consumption correlated to higher alpha diversities, also reported in the Hadza community and baboons [11, 14]. As leaves have complex structural polysaccharides like hemicellulose, cellulose, and lignin, this indicates that a more diverse bacterial community is needed for the processing of the structural polysaccharides from a leaf diet [97].

Social interactions have an effect in the temporal fluctuations of the gut microbiome

Affiliative interactions correlated to the changes in β-diversity and influenced the overall gut microbiome composition. Lemurs use their toothcomb to groom themselves and others, by doing so, they can uptake microorganisms present on their furs and anogenital regions [102]. *Rikenellaceae, Alloprevotella, Kiritimatiellae*—WCHB1–41, and

Spirochaetaceae were positively affected by affiliative interactions, indicating that they are transmitted via affiliative interactions. Social interactions correlated with β-diversity fluctuations in the dry season as well. During this period, social behaviors like mating, birth, and social thermoregulation to cope with the low temperatures occur, increasing microbe transmission [19, 103]. Nonetheless, there were no births during our study period suggesting social thermoregulation played a more important role [19].

Correlations between precipitation and temporal fluctuations of the gut microbiome

Precipitation correlated with the fluctuations in β-diversity and a lower alpha diversity during the rainy season. Redfronted lemurs drink from waterholes and temporary ponds during the rainy season, whereas in the dry season, they drink from partially dry water holes in the river having higher microbial loads [20]. Higher precipitation resulting in water sources with lower microbial loads decreased alpha diversity and correlated to changes in β-diversity. Thus, taxa with higher abundances in the dry season could be ingested from drinking at the river waterholes like *Kiritimatiellae*—WCHB1–41, which was impacted negatively by higher precipitation and has been previously isolated from environmental water suggesting transmission from water sources [104].

Gut of redfronted lemurs is inhabited by a great diversity of molecularly uncharacterized helminths and protozoa

The gut of all individuals harbored helminth and protozoan organisms over the entire year. These were classified only at the order level because they had high identity but low coverage to parasites of humans or livestock at higher taxonomical resolution. We detected a high prevalence of the *Chromadorea* and suspect most are from *Lemuricola vauceli* or *Callistoura* of *Oxyuridae*; however, genetical information from the V4 region of these organisms is absent in databases [18, 22]. This high prevalence has been previously detected morphologically but not in other metabarcoding studies [18, 22, 23]. Furthermore, our phylogenetic analysis detected other families like *Trichuridae* and *Strongyloididae* confirming a great diversity of nematodes inhabiting these lemurs [22].

The sequences detected from *Trichostomatia* possibly belong to *Balantidium*, following previous microscopical reports of this lemur species [22, 23]. Moreover, the identified *Trichomonadida*, possibly a novel organism, was not detected before in microscopical studies, only in amplicon-based reports [22, 23]. The differences in the taxa detected between this study and a previous metabarcoding report might be due to the amplification of different regions of the 18 S rRNA gene, we used the V4 while in other studies the V3-V4 and V3-V5 were investigated [23, 31].

The gut mycobiome of redfronted lemurs is comprised by novel fungi

We detected in low relative abundances fungal genera described as human gut symbionts, suggesting the gut mycobiome of redfronted lemurs has low abundances and diversity, as reported in humans [24]. The majority of the ASVs were unclassifiable, even after performing a phylogenetic analysis of the most abundant sequences against representative fungi, confirming the deficiency in genomic information from fungal organisms found in Madagascar and the gut of wild-living animals [24, 82]. The observed variation of the unclassified taxa between months could portray changes in the gut mycobiome. Nonetheless, it should be considered that some of the detected taxa might derive from diet, as redfronted lemurs fed on fungi or fungal plant pathogens [25, 82].

CONCLUSION

Fruit and flower consumption, affiliative interactions and water sources are important drivers of the temporal fluctuations of the gut bacterial communities from redfronted lemurs. Thus, displaying how this bacterial community adapts to the host diet and behavior following temporal changes. Eukaryotic gut communities also fluctuate monthly and are very diverse. Our results affirm intricate host-microbiome interactions in the gut of redfronted lemurs are affected by the host diet, precipitation, and social behavior. To our knowledge, this is the first 1-year study combining thorough sampling and individual behavioral data collection allowing the detection of direct links between temporal fluctuations of bacterial taxa and consumption of specific food items and social behavior. Longitudinal studies as the one performed here capture better the effects of seasonality on the fluctuations of the gut microbiome, diet, and social behaviors than cross-sectional approaches.

REFERENCES

1. McKenney EA, Koelle K, Dunn RR, Yoder AD. The ecosystem services of animal microbiomes. Mol Ecol. 2018;27:2164–72.
2. Miller ET, Svanbäck R, Bohannan BJM. Microbiomes as metacommunities: understanding host-associated microbes through metacommunity ecology. Trends Ecol Evol. 2018;33:926–35.
3. Johnson AJ, Vangay P, Al-Ghalith GA, Hillmann BM, Ward TL, Shields-Cutler RR, et al. Daily sampling reveals personalized diet-microbiome associations in humans. Cell Host Microbe. 2019;25:789–802.
4. Björk JR, Dasari M, Grieneisen L, Archie EA. Primate microbiomes over time: Longitudinal answers to standing questions in microbiome research. Am J Primatol. 2019;81:1–23.
5. Sun B, Wang X, Bernstein S, Huffman MA, Xia DP, Gu Z, et al. Marked variation between winter and spring gut microbiota in free-ranging Tibetan Macaques (Macaca thibetana). Sci Rep. 2016;6:1–8.
6. Wu Q, Wang X, Ding Y, Hu Y, Nie Y, Wei W, et al. Seasonal variation in nutrient utilization shapes gut microbiome structure and function in wild giant pandas. Proc R Soc B Biol Sci. 2017;284:20170955.
7. Maurice CF, Knowles SCL, Ladau J, Pollard KS, Fenton A, Pedersen AB, et al. Marked seasonal variation in the wild mouse gut microbiota. ISME J. 2015;9:2423–34.
8. Amato KR, Leigh SR, Kent A, Mackie RI, Yeoman CJ, Stumpf RM, et al. The gut microbiota appears to compensate for seasonal diet variation in the wild black howler monkey (Alouatta pigra). Microb Ecol. 2014;69:434–43.
9. Orkin JD, Campos FA, Myers MS, Cheves Hernandez SE, Guadamuz A, Melin AD. Seasonality of the gut microbiota of free-ranging white-faced capuchins in a tropical dry forest. ISME J. 2019;13:183–96. 22.
10. Springer A, Fichtel C, Al-Ghalith GA, Koch F, Amato KR, Clayton JB, et al. Patterns of seasonality and group membership characterize the gut microbiota in a longitudinal study of wild Verreaux's sifakas (Propithecus verreauxi). Ecol Evol. 2017;7:5732–45.
11. Jagsi R, Jiang J, Momoh AO, Alderman A, Giordano SH, Buchholz TA, et al. Seasonal cycling in the gut microbiome of the Hadza Hunter-Gatherers of Tanzania. Science. 2017;357:802–6.
12. Hicks AL, Lee KJ, Couto-Rodriguez M, Patel J, Sinha R, Guo C, et al. Gut microbiomes of wild great apes fluctuate seasonally in response to diet. Nat Commun. 2018;9:1786.
13. Baniel A, Amato KR, Beehner JC, Bergman TJ, Mercer A, Perlman RF, et al. Seasonal shifts in the gut microbiome indicate plastic responses to diet in wild geladas. Microbiome. 2021;9:1–20.
14. Ren T, Grieneisen LE, Alberts SC, Archie EA, Wu M. Development, diet and dynamism: longitudinal and cross-sectional predictors of gut microbial communities in wild baboons. Environ Microbiol. 2016;18:1312–25.
15. Větrovský T, Baldrian P. The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. PLoS One. 2013;8:e57923.
16. De Vrieze J, Pinto AJ, Sloan WT, Ijaz UZ. The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool. Microbiome. 2018;6:63.
17. Kappeler PM, Fichtel C. A 15-year perspective on the social organization and life history of sifaka in Kirindy Forest. In: Long-term field studies of primates. Springer Berlin Heidelberg; 2012. p. 101–21.
18. Peckre LR, Defolie C, Kappeler PM, Fichtel C. Potential self-medication using millipede secretions in red-fronted lemurs: combining anointment and ingestion for a joint action against gastrointestinal parasites? Primates. 2018;59:483–94.
19. Ostner J. Social thermoregulation in redfronted lemurs (Eulemur fulvus rufus). Folia Primatol. 2002;73:175–80.

20. Amoroso CR, Kappeler PM, Fichtel C, Nunn CL. Water availability impacts habitat use by red-fronted lemurs (Eulemur rufifrons): An experimental and observational study. Int J Primatol. 2020;41:61–80.
21. Koch F, Ganzhorn JU, Rothman JM, Chapman CA, Fichtel C. Sex and seasonal differences in diet and nutrient intake in Verreaux's sifakas (Propithecus verreauxi). Am J Primatol. 2017;79:1–10.
22. Clough D. Gastro-intestinal parasites of red-fronted lemurs in Kirindy Forest, western Madagascar. J Parasitol. 2010;96:245–51.
23. Gogarten JF, Calvignac-Spencer S, Nunn CL, Ulrich M, Saiepour N, Nielsen HV, et al. Metabarcoding of eukaryotic parasite communities describes diverse parasite assemblages spanning the primate phylogeny. Mol Ecol Resour. 2020;20:204–15.
24. Lai GC, Tan TG, Pavelka N. The mammalian mycobiome: A complex system in a dynamic relationship with the host. Wiley Interdiscip Rev Syst Biol Med. 2019;11:e1438.
25. Mann AE, Mazel F, Lemay MA, Morien E, Billy V, Kowalewski M, et al. Biodiversity of protists and nematodes in the wild nonhuman primate gut. ISME J. 2020;14:609–22.
26. Grieneisen LE, Livermore J, Alberts S, Tung J, Archie EA. Group living and male dispersal predict the core gut microbiome in wild baboons. Integr Comp Biol. 2017;57:770–85.
27. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013;41:1–11.
28. Toju H, Tanabe AS, Yamamoto S, Sato H. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS One. 2012;7:e40863.
29. Porat I, Vishnivetskaya TA, Mosher JJ, Brandt CC, Yang ZK, Brooks SC, et al. Characterization of archaeal community in contaminated and uncontaminated surface stream sediments. Microb Ecol. 2010;60:784–95.
30. Gantner S, Andersson AF, Alonso-Sáez L, Bertilsson S. Novel primers for 16S rRNA-based archaeal community analyses in environmental samples. J Microbiol Methods. 2011;84:12–8.
31. Stoeck T, Bass D, Nebel M, Christen R, Jones MDM, Breiner H-W, et al. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol. 2010;19:21–31.
32. Bahram M, Anslan S, Hildebrand F, Bork P, Tedersoo L. Newly designed 16S rRNA metabarcoding primers amplify diverse and novel archaeal taxa from the environment. Environ Microbiol Rep. 2019;11:487–94.
33. Berkelmann D, Schneider D, Hennings N, Meryandini A, Daniel R. Soil bacterial community structures in relation to different oil palm management practices. Sci Data. 2020;7:1–7.
34. Chen S, Zhou Y, Chen Y, Gu J. Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34:i884–90.
35. Zhang J, Kobert K, Flouri T, Stamatakis A. PEAR: A fast and accurate Illumina paired-end read mergeR. Bioinformatics. 2014;30:614–20.
36. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 2011;17:10–2.
37. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: A versatile open source tool for metagenomics. PeerJ. 2016;2016:1–22.
38. Edgar RC UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv. 2016;081257.
39. Edgar RC UCHIME2: improved chimera prediction for amplicon sequencing. bioRxiv. 2016;074252.
40. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10.
41. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 2013;41:590–6.
42. Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, et al. The protist ribosomal reference database (PR2): A catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 2013;41:597–604.
43. Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, et al. The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 2019;47:D259–64.
44. Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, et al. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat Rev Microbiol. 2014;12:635–45.
45. Louca S, Parfrey LW, Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. Science. 2016;353:1272–7.
46. Team Rc. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.r-project.org.
47. Team Rs. RStudio: Integrated Development for R. Boston, MA: RStudio; 2019.
48. Andersen KS, Kirkegaard RH, Albertsen M ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. bioRxiv. 2018;10–1.
49. Paradis E, Claude J, Strimmer K. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics. 2004;20:289–90.
50. Wickham H, Rstudio. stringr: Simple, consistent wrappers for common string operations. 2019. Available from: https://cran.r-project.org/package=stringr.
51. Wickham H. Reshaping data with the reshape package. J Stat Softw. 2007;21:1–20.
52. Dowle M, Srinivasan A, Gorecki J, Chirico M, Stetsenko P, Short T, et al. data.table: Extension of "data.frame". 2019. Available from: https://cran.r-project.org/package=data.table.
53. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome to the Tidyverse. J Open Source Softw. 2019;4:1686.
54. Wickham H ggplot2: Elegant graphics for data analysis. New York: Spinger-Verlag New York; 2016.
55. Chen L, Reeve J, Zhang L, Huang S, Wang X, Chen J. GMPR: A robust normalization method for zero-inflated count data with application to microbiome sequencing data. PeerJ. 2018;2018:1–20.
56. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol. 2013;30:772–80.
57. Price MN, Dehal PS, Arkin AP. FastTree 2—Approximately maximum-likelihood trees for large alignments. PLoS One. 2010;5:e9490.
58. Rambaut A. FigTree—tree figure drawing tool. Institute of Evolutionary Biology, University of Edinburg; 2020. Available from: http://tree.bio.ed.ac.uk/software/figtree.
59. Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, et al. Picante: R tools for integrating phylogenies and ecology. Bioinformatics. 2010;26:1463–4.
60. Marzano V, Mancinelli L, Bracaglia G, Del Chierico F, Vernocchi P, Di Girolamo F, et al. "Omic" investigations of protozoa and worms for a deeper understanding of the human gut "parasitome.". PLoS Negl Trop Dis. 2017;11:e0005916.
61. Mandal S, Van Treuren W, White RA, Eggesbø M, Knight R, Peddada SD. Analysis of composition of microbiomes: a novel method for studying microbial composition. Microb Ecol Heal Dis. 2015;26:27663.
62. Hothorn T, Hornik K exactRankTests: exact distributions for rank and permutation tests. 2019. Available from: https://cran.r-project.org/package=exactRankTests.
63. Pinheiro J, Bates D, DebRoy S, Sarkar D, Core Team R nlme: Linear and nonlinear mixed effects models. 2020. Available from: https://cran.r-project.org/package=nlme.
64. van den Boogaart G, Tolosana R compositions: an R package for compositional data analysis. 2020. Available from: http://www.stat.boogaart.de/compositions/.
65. Wickham H, Francois R, Core Team R, Rstudio, Jylänki J, Jorgensen M readr: Read rectangular text data. 2018. Available from: http://readr.tidyverse.org.
66. Oksanen J, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community ecology package. 2019. Available from: https://cran.r-project.org/package=vegan.
67. Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. UniFrac: An effective distance metric for microbial community comparison. ISME J. 2011;5:169–72.
68. Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67:1–48.
69. Reitmeier S, Hitch TCA, Treichel N, Fikas N, Hausmann B, Ramer-Tait AE, et al. Handling of spurious sequences affects the outcome of high-throughput 16S rRNA gene amplicon profiling. ISME COMMUN. 2021;1:31.
70. Sweeny AR, Lemon H, Ibrahim A, Nussey DH, Free A, McNally L. A mixed model approach for estimating drivers of microbiota community composition and differential taxonomic abundance (preprint). bioRxiv. 2020; https://doi.org/10.1101/2020.11.24.395715.
71. Barr DJ, Levy R, Scheepers C, Tily HJ. Random effects structure for confirmatory hypothesis testing: Keep it maximal. J Mem Lang. 2013;68:255–78.
72. Matuschek H, Kliegl R, Vasishth S, Baayen H, Bates D. Balancing type I error and power in linear mixed models. J Mem Lang. 2017;94:305–15.
73. Fox J, Weisberg S. An R companion to applied regression. 3rd ed. Thousand Oaks, California: Sage Publishing; 2019.
74. Bolker BM. Ecological models and data in R. New Jersey, USA: Princeton University Press; 2008.
75. Adams DC, Anthony CD. Using randomization techniques to analyse behavioural data. Anim Behav. 1996;51:733–8.
76. Baayen RH. Analyzing linguistic data. Cambridge: Cambridge University Press; 2008.
77. Bartón K MuMIn: Multi-Model Inference. 2020. Available from: https://cran.r-project.org/package=MuMIn.
78. Lüdecke D. sjPlot: Data Visualization for Statistics in Social Science. 2021. Available from: https://cran.r-project.org/package=sjPlot.
79. Edgar RC. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32:1792–7.
80. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35:1547–9.
81. NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2018;46:D8–13.

82. Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol. 2019;17:95–109. 15.
83. Koskinen K, Pausan MR, Perras AK, Beck M, Bang C, Mora M, et al. First insights into the diverse human archaeome: specific detection of archaea in the gastrointestinal tract, lung, and nose and on skin. MBio. 2017;8:1–17.
84. Raymann K, Moeller AH, Goodman AL, Ochman H. Unexplored archaeal diversity in the great ape gut microbiome. mSphere. 2017;2:1–12.
85. Ley RE. Prevotella in the gut: choose carefully. Nat Rev Gastroenterol Hepatol. 2016;13:69–70.
86. Manara S, Asnicar F, Beghini F, Bazzani D, Cumbo F, Zolfo M, et al. Microbial genomes from non-human primate gut metagenomes expand the primate-associated bacterial tree of life with over 1000 novel species. Genome Biol. 2019;20:299.
87. Umanets A, de Winter I, IJdema F, Ramiro-Garcia J, van Hooft P, Heitkönig IMA, et al. Occupancy strongly influences faecal microbial composition of wild lemurs. FEMS Microbiol Ecol. 2018;94:1–13.
88. Greene LK, Clayton JB, Rothman RS, Semel BP, Semel MA, Gillespie TR, et al. Local habitat, not phylogenetic relatedness, predicts gut microbiota better within folivorous than frugivorous lemur lineages. Biol Lett. 2019;15:5–11.
89. Daniel H, Gholami AM, Berry D, Desmarchelier C, Hahne H, Loh G, et al. High-fat diet alters gut microbiota physiology in mice. ISME J. 2014;8:295–308.
90. Hiippala K, Kainulainen V, Kalliomäki M, Arkkila P, Satokari R. Mucosal prevalence and interactions with the epithelium indicate commensalism of Sutterella spp. Front Microbiol. 2016;7:1–13.
91. Heintz-Buschart A, Wilmes P. Human gut microbiome: Function matters. Trends Microbiol. 2018;26:563–74.
92. Ricaboni D, Mailhe M, Cadoret F, Vitton V, Fournier PE, Raoult D. 'Colidextribacter massiliensis' gen. nov., sp. nov., isolated from human right colon. New Microbes New Infect. 2017;17:27–9.
93. Qin P, Zou Y, Dai Y, Luo G, Zhang X, Xiao L. Characterization a novel butyric acid-producing bacterium Collinsella aerofaciens subsp. shenzhenensis subsp. nov. Microorganisms. 2019;7:78.
94. Sizova MV, Muller PA, Stancyk D, Panikov NS, Mandalakis M, Hazen A, et al. Oribacterium parvum sp. nov. and Oribacterium asaccharolyticum sp. nov., obligately anaerobic bacteria from the human oral cavity, and emended description of the genus Oribacterium. Int J Syst Evol Microbiol. 2014;64:2642–9.
95. Amato KR, Garber PA. Nutrition and foraging strategies of the black howler monkey (Alouatta pigra) in Palenque National Park, Mexico. Am J Primatol. 2014;76:774–87.
96. White TCR. The significance of unripe seeds and animal tissues in the protein nutrition of herbivores. Biol Rev. 2011;86:217–24.
97. Ortmann S, Bradley BJ, Stolter C, Ganzhorn JU. Estimating the quality and composition of wild animal diets—a critical survey of methods. In: Hohmann G, Robbins M, Boesch C, editors. Feeding ecology in apes and other primates ecological, physical, and behavioral aspects. Cambridge: Cambridge University Press; 2006. p. 395–418.
98. Hippe H, Hagelstein A, Kramer I, Swiderski J, Stackebrandt E. Phylogenetic analysis of Formivibrio citricus, Propionivibrio dicarboxylicus, Anaerobiospirillum thomasii, Succinirnonas amylolytica and Succinivibrio dextrinosolvens and proposal of Succinivibrionaceae fam. nov. Int J Syst Evol Microbiol. 1999;49:779–82.
99. Privé F, Kaderbhai NN, Girdwood S, Worgan HJ, Pinloche E, Scollan ND, et al. Identification and characterization of three novel lipases belonging to families II and V from Anaerovibrio lipolyticus 5ST. PLoS One. 2013;8:e69076.
100. Flaiz M, Baur T, Brahner S, Poehlein A, Daniel R, Bengelsdorf FR. Caproicibacter fermentans gen. nov., sp. nov., a new caproate-producing bacterium and emended description of the genus Caproiciproducens. Int J Syst Evol Microbiol. 2020;70:4269–79.
101. Watanabe Y, Nagai F, Morotomi M. Characterization of Phascolarctobacterium succinatutens sp. Nov., an asaccharolytic, succinate-utilizing bacterium isolated from human feces. Appl Environ Microbiol. 2012;78:511–8.
102. Clough D, Heistermann M, Kappeler PM. Host intrinsic determinants and potential consequences of parasite infection in free-ranging red-fronted lemurs (Eulemur fulvus rufus). Am J Phys Anthropol. 2010;142:441–52.
103. Sarkar A, Harty S, Johnson KVA, Moeller AH, Archie EA, Schell LD, et al. Microbial transmission in animal social networks and the social microbiome. Nat Ecol Evol. 2020;4:1020–35.
104. van Vliet DM, Lin Y, Bale NJ, Koenen M, Villanueva L, Stams AJM, et al. Pontiella desulfatans gen. nov., sp. nov., and Pontiella sulfatireligans sp. nov., two marine anaerobes of the Pontiellaceae fam. nov. producing sulfated glycosaminoglycan-like exopolymers. Microorganisms. 2020;8:920. 18.

ACKNOWLEDGEMENTS

This project was funded by the Deutsche Forschungsgemeinschaft (FI 929/7-2, DA 374/13-2) and is embedded in the research unit "Sociality and Health in Primates" (SoHaPi). Research permits were granted by the Malagasy Ministère de l'Environnement et des Eaux et Forêts, the University of Antananarivo, and the Centre National de Formation, d'Etudes et de Recherche en Environnement et Foresterie. For support in sample and data collection, we thank Jean-Pierre Tolojanahary, Mamy Razafindrasamba, Tianasoa Andrianjanahary, Judit Stolla, Luise Waldow, and Alexandra Michiels. We thank Dr. Anja Poehlein, Melanie Heinemann, and Sarah Schüßler from the Göttingen Genomics Laboratory for amplicon sequencing. For discussion and statistical advice, we thank Dr. Roger Mundry and Dr. Louise Peckre.

AUTHOR CONTRIBUTIONS

CF and RD obtained the funding and designed the study. TM conducted the laboratory work and field sampling and wrote the first draft of the manuscript. TM and DS analyzed and visualized the data. All authors interpreted the results, and edited, reviewed and revised the manuscript.

FUNDING

Open Access funding enabled and organized by Projekt DEAL.

COMPETING INTERESTS

The authors declare no competing interests.

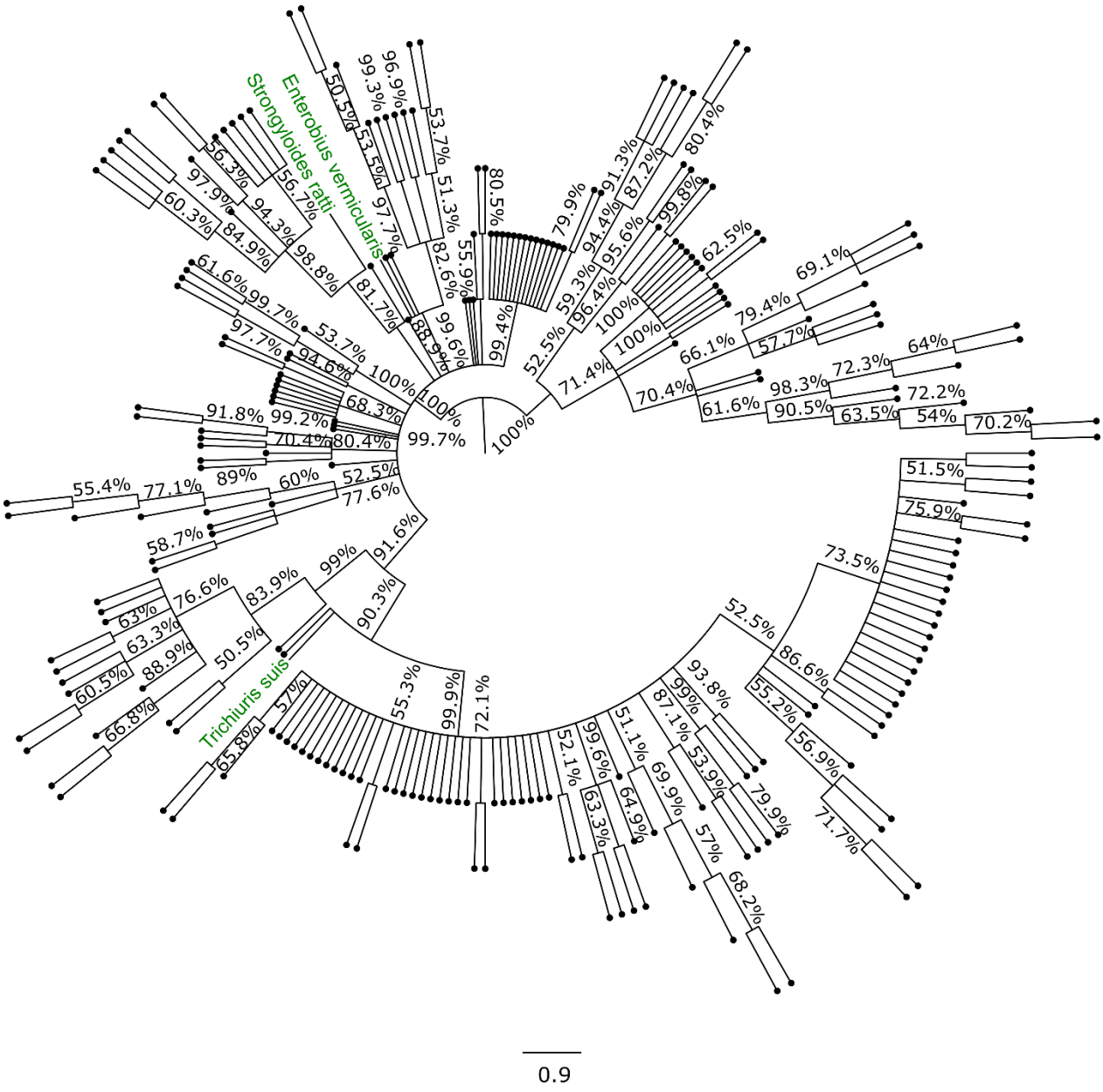
ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43705-021-00086-0.

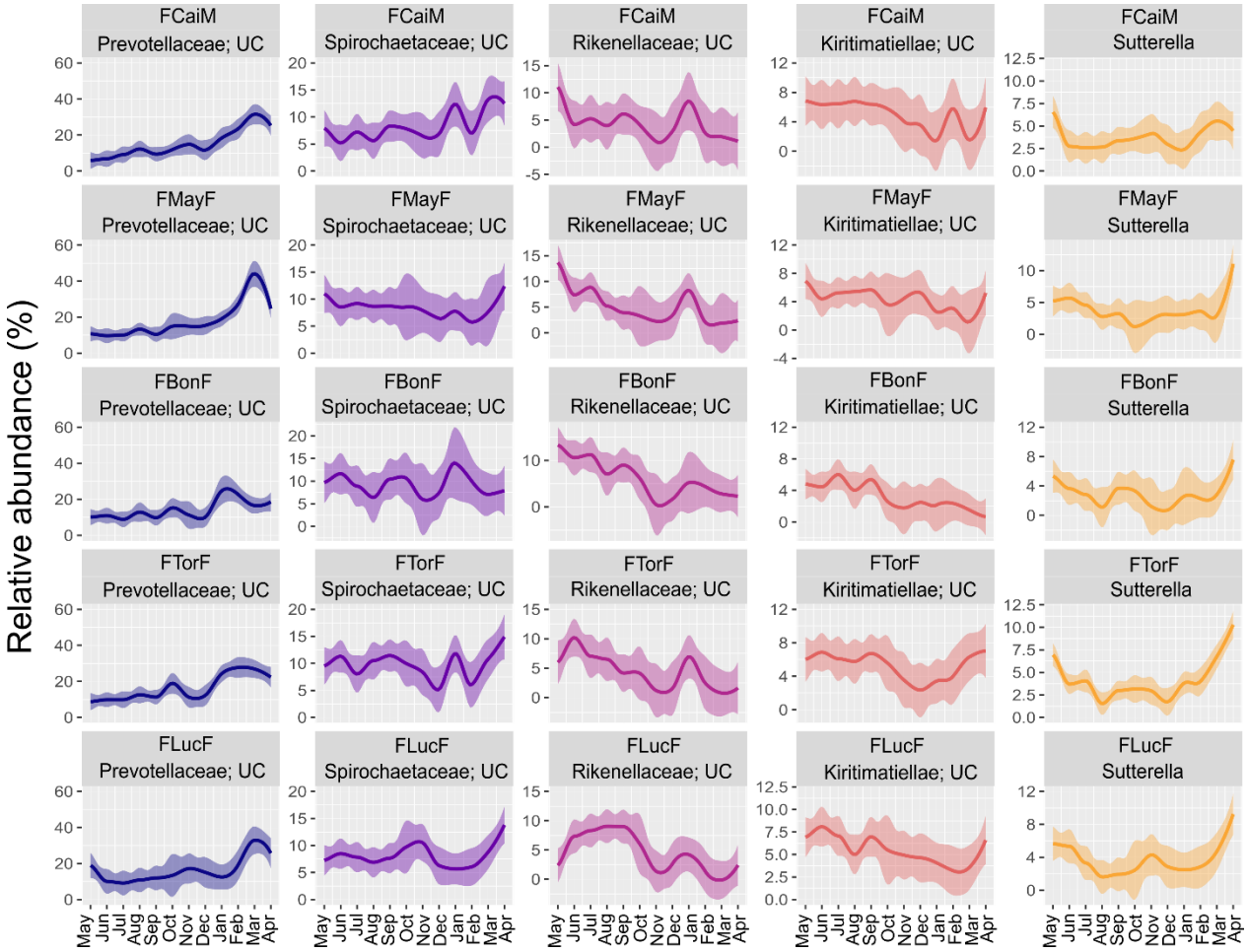
Correspondence and requests for materials should be addressed to Rolf Daniel.

Reprints and permission information is available at http://www.nature.com/reprints

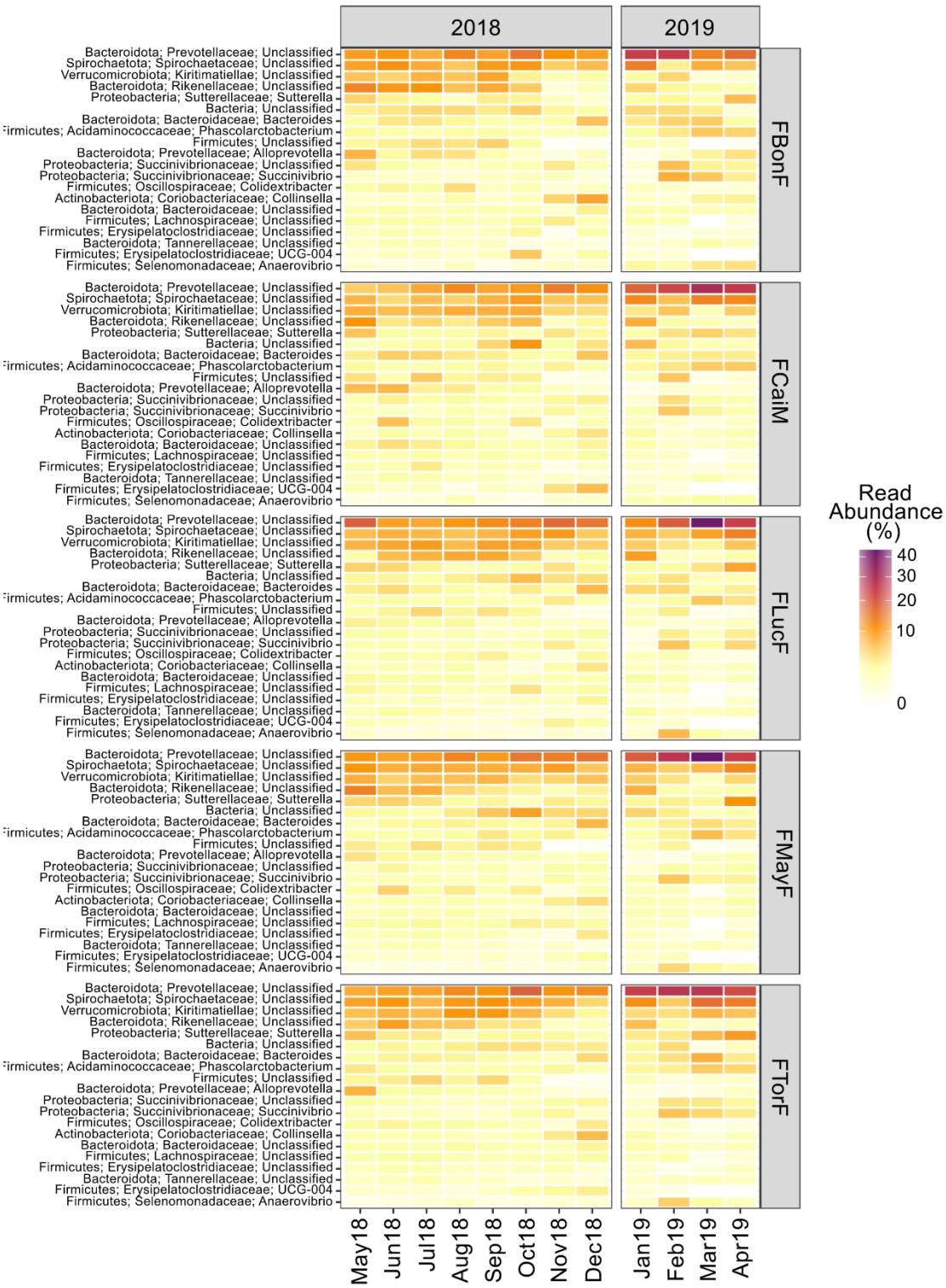
Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



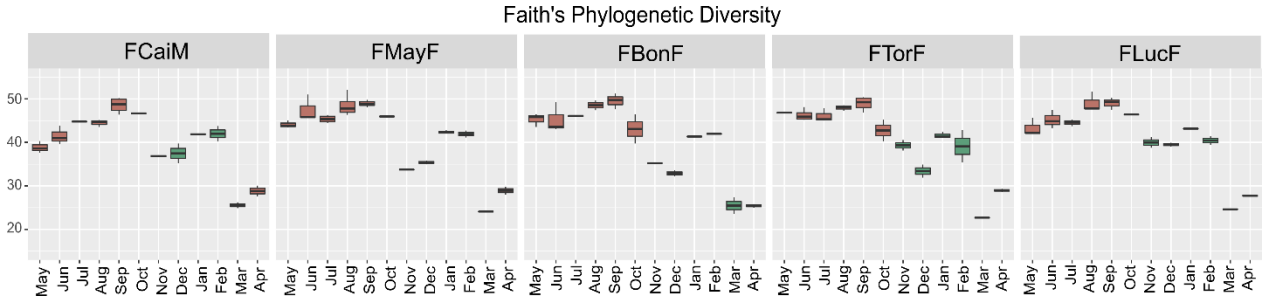
Supplemental Figure S1. Maximum likelihood phylogenetic tree of the ASVs classified as *Chromadorea* including representative parasitic nematodes of humans and animals. Sequences were aligned with MUSCLE with UPGMA and default settings, and the phylogenetic tree was generated in MEGA X with the Tamura-Nei model and 1 000 bootstrap. 18S rRNA gene sequences from representative parasitic nematodes of humans and animals were retrieved from the NCBI database.



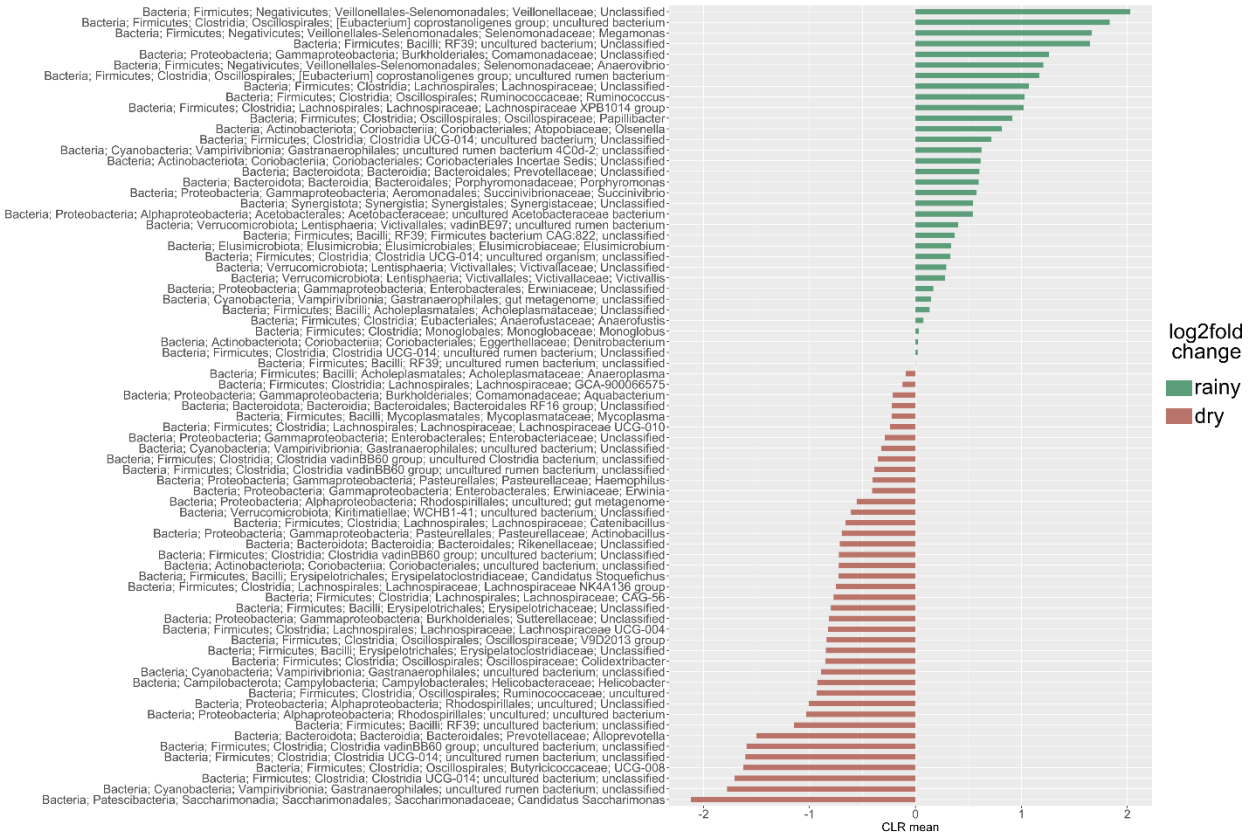
Supplemental Figure S2. Monthly fluctuations of the five most abundant bacterial genera in each individual. Linecharts depict relative abundances of bacterial genera from normalized counts.



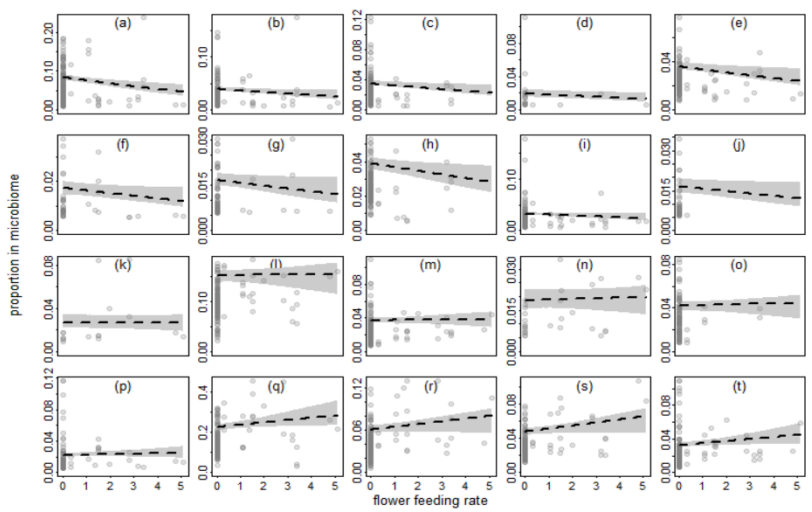
Supplemental Figure S3. Heatmap of the 20 most abundant bacterial genera averaged per month for each individual during the study period. Relative abundances were estimated from normalized counts.



Supplemental Figure S4. Monthly individual fluctuations in alpha diversity measured as Faith's Phylogenetic diversity index.

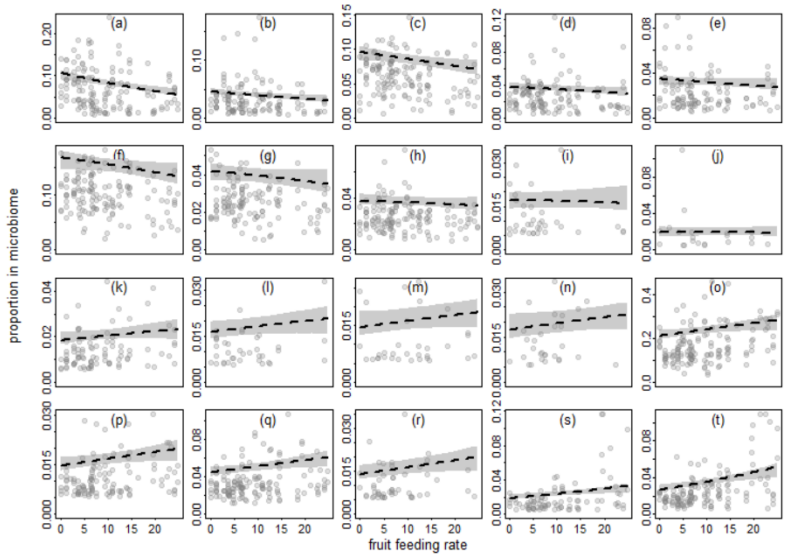


Supplemental Figure S5. Log2fold changes of mean abundances of bacterial genera between dry and rainy season as detected with ANCOM 2.1.



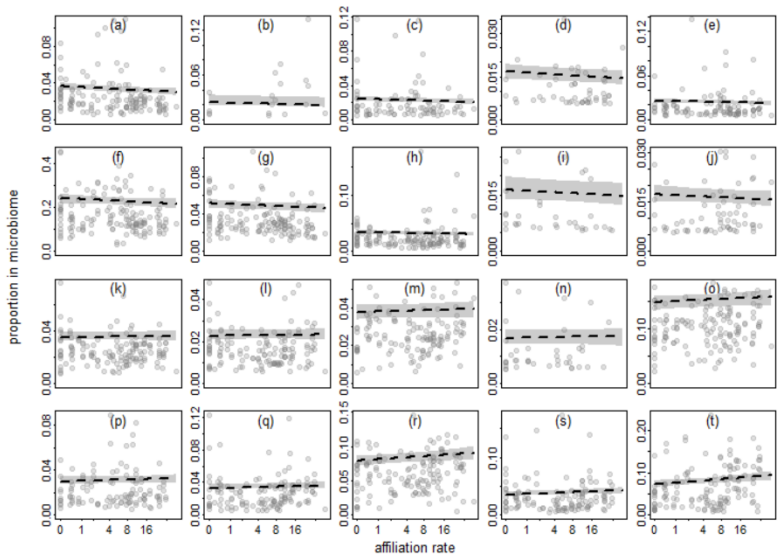
Subplot	Taxon
a	Rikenellaceae:Unclassified
b	Prevotellaceae:Alloprevotella
c	Oscillospiraceae:Colidextribacter
d	Veillonellaceae:Unclassified
e	Bacteroidaceae:Unclassified
f	Eggerthellaceae:uncultured
g	Oscillospiraceae:UCG-005
h	Lachnospiraceae:uncultured
i	Coriobacteriaceae:Collinsella
j	Prevotellaceae:UCG-004
k	Coriobacteriaceae:Coriobacterium
l	Spirochaetaceae:Unclassified
m	Succinivibrionaceae:uncultured
n	Akkermansiaceae:Akkermansia
o	uncultured:Unclassified
p	Selenomonadaceae:Anaerovibrio
q	Prevotellaceae:Unclassified
r	Sutterellaceae:Sutterella
s	Acidaminococcaceae:Phascolarctobacterium
t	Succinivibrionaceae:Succinivibrio

Supplemental Figure S6. Effects of flower consumption rates on the 20 bacterial taxa for which the taxon-specific effect differed most from the average effect across all taxa. LMMs were calculated including the random intercept of individual, taxon, sample, and taxon nested within individual (taxon-individual) and the random slope and fixed effect of monthly rates of flower feeding. A significant effect of flower feeding on community composition was determined by comparing the log likelihoods of the full model to one lacking the random slopes of all test predictors within taxon, and another one without flower feeding rates within taxon. Additionally, 1 000 permutations were performed by shuffling the labels of taxa within sample to detect a specific effect of flower feeding in a bacterial taxon. Significance was determined as the proportion of permutations where the test statistic was at least as large as the original data.



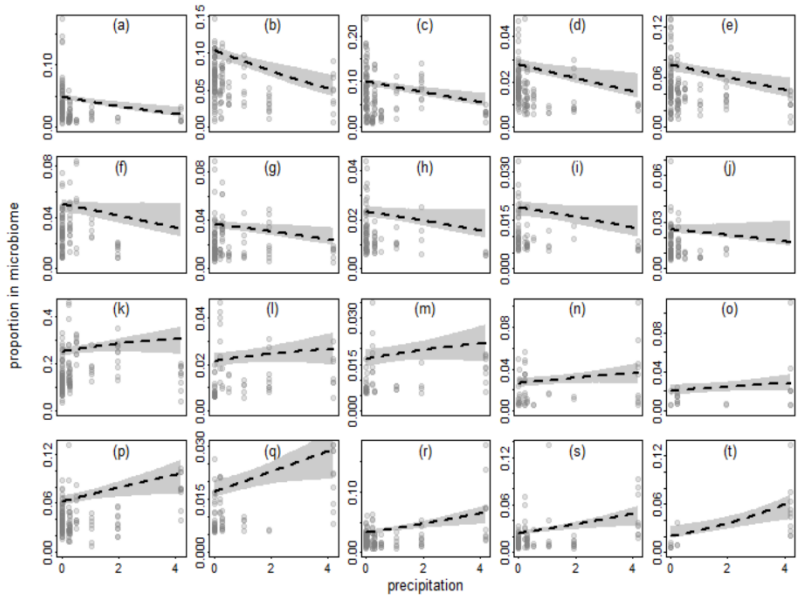
Subplot	Taxon
a	Rikenellaceae:Unclassified
b	Prevotellaceae:Alloprevotella
c	Oscillospiraceae:Colidextribacter
d	Veillonellaceae:Unclassified
e	Bacteroidaceae:Unclassified
f	Eggerthellaceae:uncultured
g	Oscillospiraceae:UCG-005
h	Lachnospiraceae:uncultured
i	Coriobacteriaceae:Collinsella
j	Prevotellaceae:UCG-004
k	Coriobacteriaceae:Coriobacterium
l	Spirochaetaceae:Unclassified
m	Succinivibrionaceae:uncultured
n	Akkermansiaceae:Akkermansia
o	uncultured:Unclassified
p	Selenomonadaceae:Anaerovibrio
q	Prevotellaceae:Unclassified
r	Sutterellaceae:Sutterella
s	Acidaminococcaceae:Phascolarctobacterium
t	Succinivibrionaceae:Succinivibrio

Supplemental Figure S7. Effects of fruit consumption rates on the 20 bacterial taxa for which the taxon-specific effect differed most from the average effect across all taxa. LMMs were calculated including the random intercept of individual, taxon, sample, and taxon nested within individual (taxon-individual) and the random slope and fixed effect of monthly rates of fruit feeding. A significant effect of fruit feeding on community composition was determined by comparing the log likelihoods of the full model to one lacking the random slopes of all test predictors within taxon, and another one without fruit feeding rates within taxon. Additionally, 1 000 permutations were performed by shuffling the labels of taxa within sample to detect a specific effect of fruit feeding in a bacterial taxon. Significance was determined as the proportion of permutations where the test statistic was at least as large as the original data.



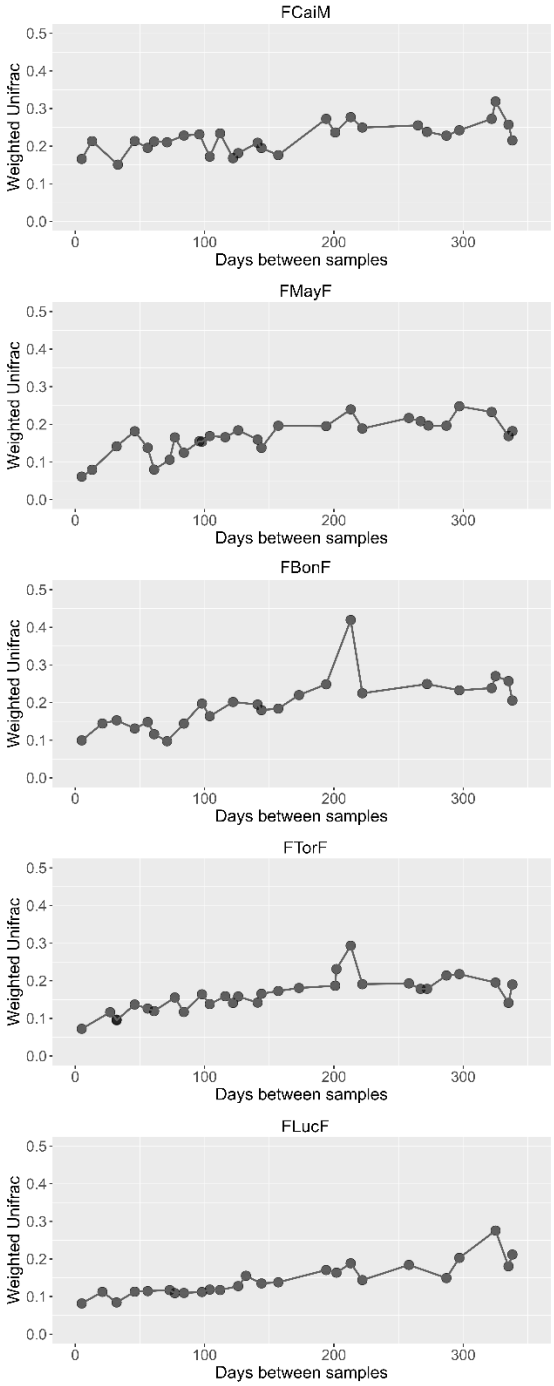
Subplot	Taxon
a	Rikenellaceae:Unclassified
b	Prevotellaceae:Alloprevotella
c	Oscillospiraceae:Colidextribacter
d	Veillonellaceae:Unclassified
e	Bacteroidaceae:Unclassified
f	Eggerthellaceae:uncultured
g	Oscillospiraceae:UCG-005
h	Lachnospiraceae:uncultured
i	Coriobacteriaceae:Collinsella
j	Prevotellaceae:UCG-004
k	Coriobacteriaceae:Coriobacterium
l	Spirochaetaceae:Unclassified
m	Succinivibrionaceae:uncultured
n	Akkermansiaceae:Akkermansia
o	uncultured:Unclassified
p	Selenomonadaceae:Anaerovibrio
q	Prevotellaceae:Unclassified
r	Sutterellaceae:Sutterella
s	Acidaminococcaceae:Phascolarctobacterium
t	Succinivibrionaceae:Succinivibrio

Supplemental Figure S8. Effects of affiliation rates on the 20 bacterial taxa for which the taxon-specific effect differed most from the average effect across all taxa. LMMs were calculated including the random intercept of individual, taxon, sample, and taxon nested within individual (taxon-individual) and the random slope and fixed effect of monthly affiliation rates. A significant effect of affiliation rates on community composition was determined by comparing the log likelihoods of the full model to one lacking the random slopes of all test predictors within, and another one without affiliation rates within taxon. Additionally, 1 000 permutations were performed by shuffling the labels of taxa within sample to detect a specific effect of affiliation rates in a bacterial taxon. Significance was determined as the proportion of permutations where the test statistic was at least as large as the original data.

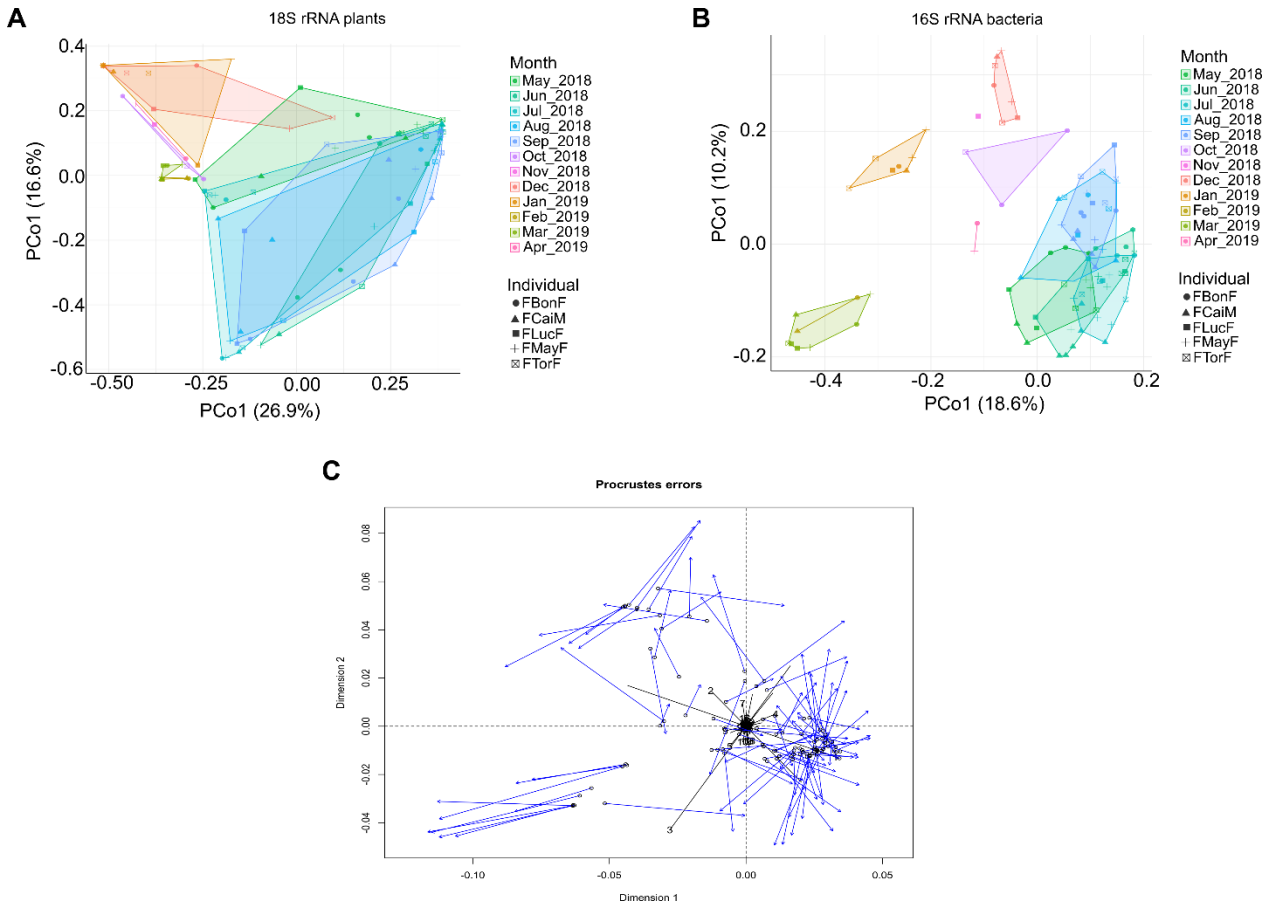


Subplot	Taxon
a	Prevotellaceae:Alloprevotella
b	Kiritimatiellae:WCHB1 -41
c	Rikenellaceae:Unclassified
d	Tannerellaceae:Unclassified
e	Sutterellaceae:Sutterella
f	Clostridia:UCG -14
g	Kiritimatiellae:WCHB1 -41
h	Erysipelotrichaceae:Unclassified
i	Lachnospiraceae:Syntrophococcus
j	Clostridia:UCG -14
k	Prevotellaceae:Unclassified
l	Lachnospiraceae:Unclassified
m	Oscillospiraceae:Unclassified
n	Selenomonadaceae:Megamonas
o	Veillonellaceae:Unclassified
p	Bacteroidaceae:Bacteroides
q	Oscillospiraceae:UCG -5
r	Coriobacteriaceae:Collinsella
s	Erysipelatoclostridiaceae:UCG -4
t	Atopobiaceae:Olsenella

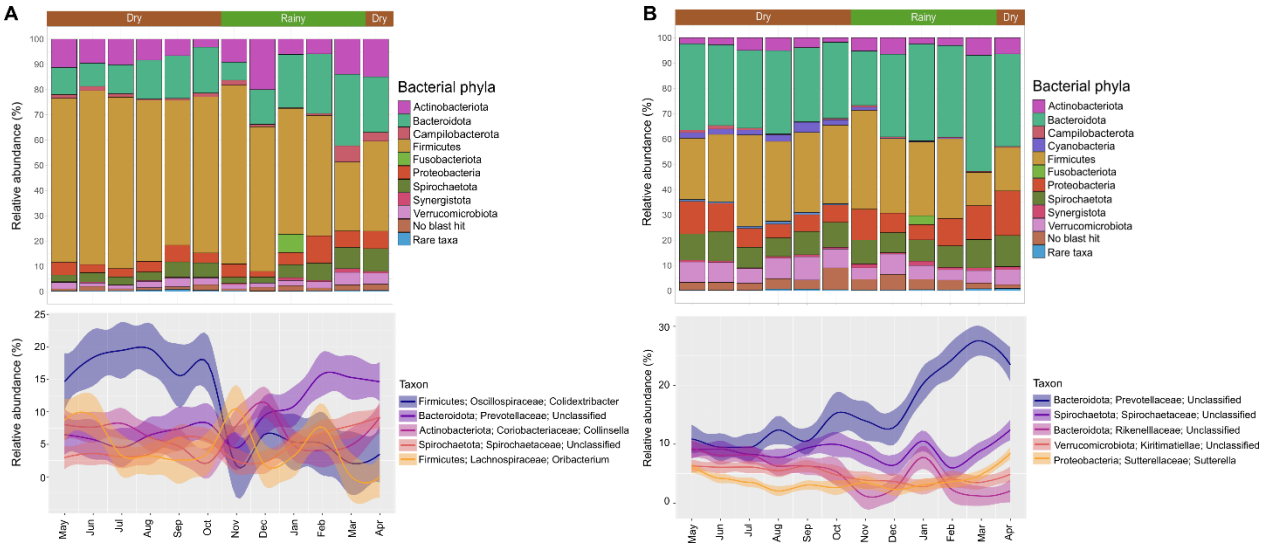
Supplemental Figure S9. Effects of mean precipitation on the 20 bacterial taxa for which the taxon-specific effect differed most from the average effect across all taxa. LMMs were calculated including the random intercept of individual, taxon, sample, and taxon nested within individual (taxon-individual) and the random slope and fixed effects of the test predictors: feeding rates of flowers, fruits and leaves, and affiliation rates. Precipitation was included as a control predictor, thus taxon-specific were not tested for significance.



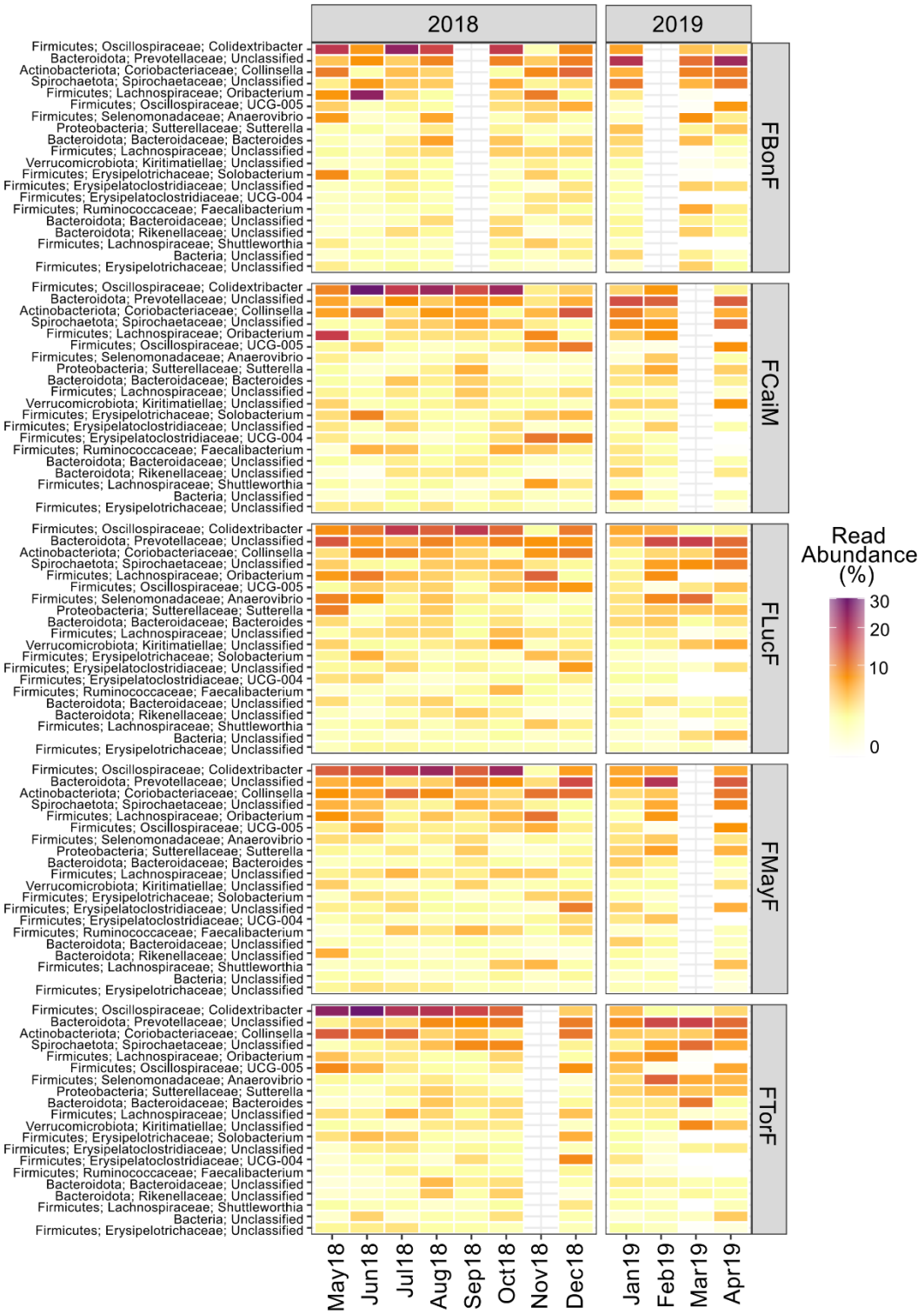
Supplemental Figure S10. Time decay plots of β-dissimilarities against the time span between sample collection for each individual. Wunifrac distance matrices for each individual were calculated and compared to matrices accounting for the days between sample collection. Mantel tests were used to determine correlations between sample dissimilarities and sampling interval.



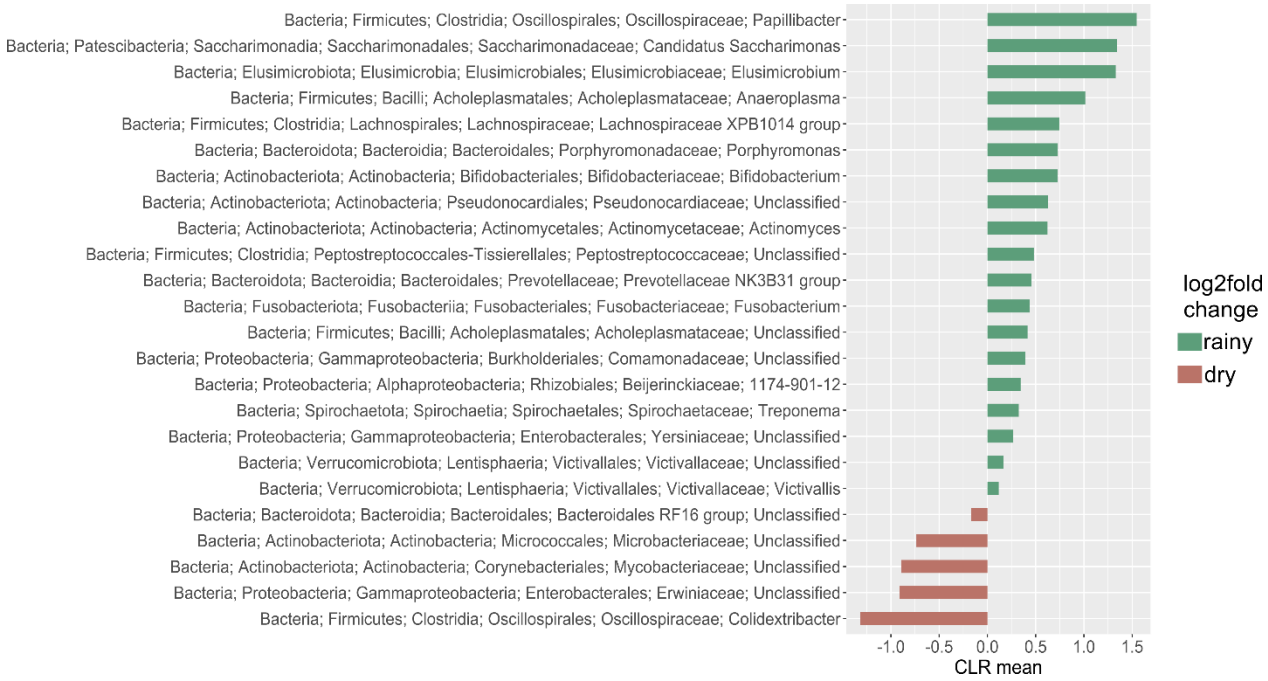
Supplemental Figure S11. Comparison between the monthly variations in gut microbiome composition and dietary items. **A.** PCoA from Bray Curtis dissimilarity distance of the dietary items detected from the 18S rRNA amplicon sequencing. **B.** PCoA from Bray Curtis dissimilarity distances of the entire bacterial community and its temporal variations. **C.** Procrustes comparison of the ordination analysis for the bacteria and dietary items identified.



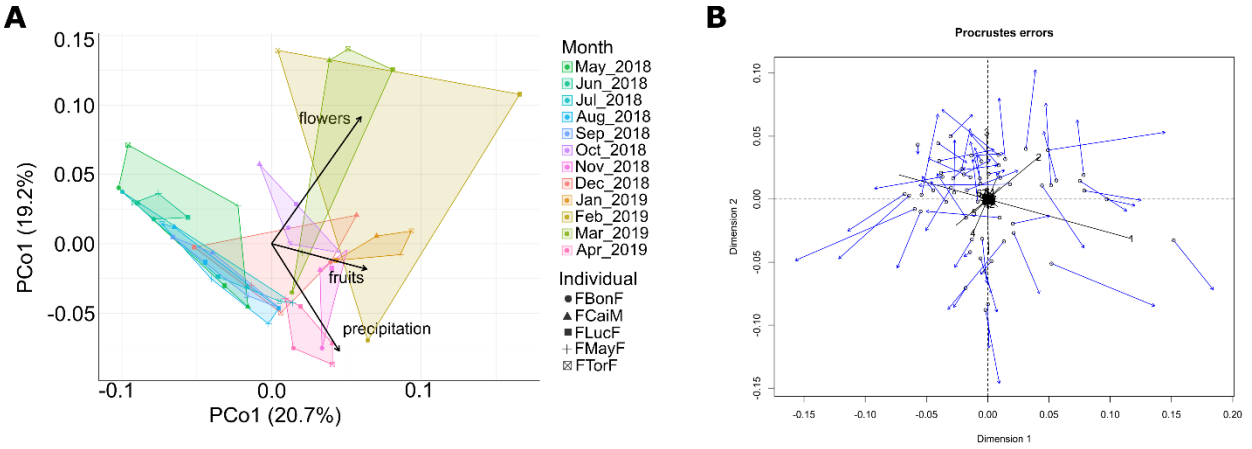
Supplemental Figure S12. Comparison of the bacterial composition to phyla level and the five most abundant genera for the **A.** active community and **B**. entire community and their monthly fluctuations from May 2018 to April 2019. Barcharts to phylum level and linecharts to genus level display relative abundances from normalized counts.



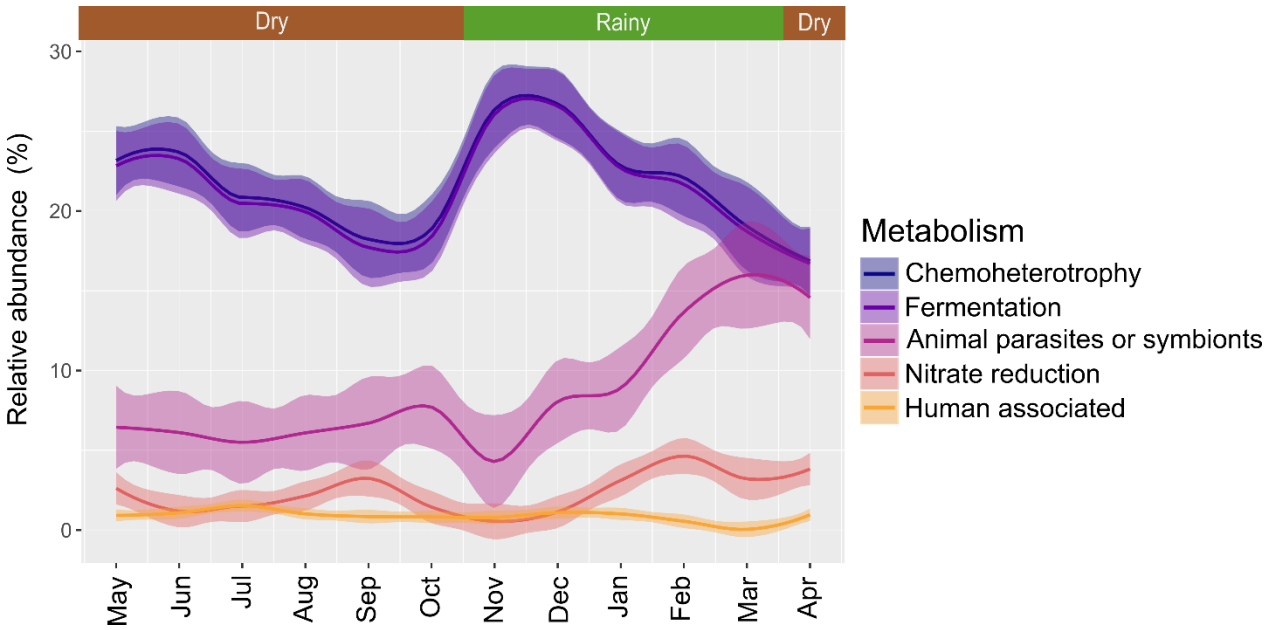
Supplemental Figure S13. Heatmap of the 20 most abundant bacterial genera in the potential active community and their monthly fluctuations in their relative abundances in each individual. Relative abundances were estimated from normalized counts.



Supplemental Figure S14. Log2fold changes in the mean abundances of bacterial genera between dry and rainy season in the potential active community as detected with ANCOM 2.1.



Supplemental Figure S15. Comparison between the entire and the active bacterial community. **A.** PCoA from WUnifrac of the reduced entire community and the dietary and environmental fit analysis depicting significant correlations between temporal fluctuations and the environmental, diet and social factors investigated. **B**. Results from the Procrustes comparison of the PCoAs from Wunifrac for the bacteria potential active and entire community.



Supplemental Figure S16. Functional predictions for the active bacterial community using Faprotax.

Supplementary Tables

Supplementary Table S1. Fecal sample list with metadata and respective marker genes analyzed. The table is deposited on the enclosed CD under \Chapter2_Supplementary_material\

Supplementary Table S2. Primers and PCR protocols for all the studied taxonomical marker genes. The table is deposited on the enclosed CD under \Chapter2_Supplementary_material\

Supplementary Table S3. Sequencing statistics for all taxonomical marker genes.

Taxonomical marker gene	Number of samples	Reads after quality filtering	Number of ASVs	Mean amplicon length (bp)	Number of unclassified reads	Reads for rarefaction
Bacteria 16S rRNA	141	7 068 908	2 675	417	3.5 % ± 1.5	18 794
Bacteria 16S rRNA (active)	56	1 285 188	2 437	417	1.5 % ± 0.6	9 032
Bacteria 16S rRNA (entire)	56	3 086 712	2 583	417	4.0 % ± 1.8	18 794
Eukaryota 18S rRNA	115	5 790 790	3 275	380	3.1 % ± 2.9	NA
Plants 18S rRNA	113	1 072 667	269	379	NA	1 000
Fungi ITS2	125	6 145 213	6 637	320	71.0 % ± 16.8	NA
Archaea 16S rRNA (Porat & Gantner)	5	238 045	60	408	0.7 % ± 0.4	NA
Archaea 16S rRNA (Bahram)	5	288 572	72	403	0.4 % ± 0.5	NA

Supplementary Table S4. *p*-values and correlation coefficients from statistical tests.

	p-value	r2
Permanova individual differences entire community	0.002	0.055
Environmental fit from PCoA of Wunifrac entire community		
Season	0.001	0.281
Mean precipitation	0.001	0.266
Rates of fruit feeding	0.003	0.112
Rates of feeding on leaves	0.044	0.045
Rates of flower feeding	0.001	0.266
Rates of affiliative interactions	0.006	0.099
Protest test from Procrustes comparison of entire and plant diet from 18S data	0.001	0.654
Environmental fit from PCoA from Wunifrac active community		
Season	0.007	0.095
Precipitation	0.013	0.149
Feeding fruits	0.102	0.080
Feeding leaves	0.230	0.052
Feeding flowers	0.003	0.220
Affiliation	0.507	0.026
Environmental fit from PCoA from Wunifrac reduced entire community		
Precipitation	0.003	0.200
Feeding fruits	0.056	0.109
Feeding leaves	0.830	0.083
Feeding flowers	0.002	0.299
Affiliation	0.200	0.055
Protest test from Procrustes comparison of entire and active community	0.001	0.769

Supplementary Table S5. File from the ASV table of the bacterial 16S rRNA gene and transcript data. The table is deposited on the enclosed CD under \Chapter2_Supplementary_material\

Supplementary Table S6. File from the raw ASV table of eukaryotic 18S rRNA data. The table is deposited on the enclosed CD under \Chapter2_Supplementary_material\

Supplementary Table S7. File from the raw ASV table of eukaryotic ITS2 data. The table is deposited on the enclosed CD under \Chapter2_Supplementary_material\

Supplementary Table S8. File from the raw ASV table of the *Archaea* 16S rRNA data analyzed with the primer set from Porat *et al*., 2010 and Gantner *et al*, 2011. The table is deposited on the enclosed CD under \Chapter2_Supplementary_material\

Supplementary Table S9. File from the raw ASV table of the *Archaea* 16S rRNA data analyzed with the primer set from Bahram *et al*., 2019. The table is deposited on the enclosed CD under \Chapter2_Supplementary_material\

Supplementary Table S10. Estimates for the linear mixed model of the feeding and social behaviors affecting the overall composition of the entire bacterial community. The table is deposited on the enclosed CD under \Chapter2_Supplementary_material\

Supplementary Table S11. Mantel correlation tests of the time series analysis for the entire bacterial community.

Individual	**Mantel *r* statistics**	***p* value**
Bonacca	0.627	0.001
Caicos	0.567	0.001
Lucia	0.611	0.001
Mayaguana	0.603	0.001
Tortuga	0.537	0.001

Supplementary Table S12. Estimates for the linear mixed model of the changes in alpha diversity in the entire bacterial community.

Model test	Value obtained
Full-null model comparison	p-value= 0.003
AIC	898.500
R^2m	0.360
R^2C	0.480

Term	Estimate	SE	Lower CI	Upper CI	x2	df	p	min	max
Intercept	41.679	0.655	40.404	43				41.38	42.453
Precipitation	-1.616	0.603	-2.83	-0.468	4.277	1	0.039	-2.255	-1.367
Feeding fruit	-0.867	0.833	-2.484	0.728	1.013	1	0.314	-1.776	-0.656
Feeding leaves	1.338	0.499	0.254	2.319	3.685	1	0.055	0.29	1.621
Feeding flowers	-2.836	0.484	-3.798	-1.826	9.271	1	0.002	-4.54	-2.51
Affiliative interactions	1.454	0.841	-0.169	3.298	2.152	1	0.142	0.572	2.231

Supplementary Table S13. Mantel correlation tests of the time series analysis for the active bacterial community.

Individual	Mantel *r* statistic	*p* value
Bonacca	0.463	0.002
Caicos	0.261	0.041
Lucia	0.262	0.025
Mayaguana	0.421	0.001
Tortuga	0.652	0.001

Supplementary Table S14. Estimates for the linear mixed model of the changes in alpha diversity in the active bacterial community.

Model test	Value obtained
Full-null model comparison	p-value= 0.012
AIC	35.510
R2m	0.320
R2C	0.380

Term	Estimate	SE	Lower CI	Upper CI	x2	df	p	min	max
Intercept	3.576	0.018	3.542	3.615				3.569	3.599
Precipitation	-0.022	0.018	-0.061	0.011	-45.521	1	0.221	-0.028	-0.013
Feeding fruit	-0.006	0.019	-0.044	0.033	-46.919	1	0.755	-0.02	0.005
Feeding leaves	0.031	0.018	-0.004	0.066	-44.975	1	0.153	0.015	0.043
Feeding flowers	-0.073	0.017	-0.105	-0.037	-37.079	1	0.002	-0.083	-0.068
Affiliative interactions	0.017	0.025	-0.032	0.064	-46.614	1	0.525	0.002	0.05

3 Multiscale study of temporal drivers of gut microbiome composition in wild redfronted lemurs

Tatiana Murillo[a,b], Dominik Schneider[b], Michael Heistermann[c], Rolf Daniel[b], and Claudia Fichtel[a]

[a]Behavioral Ecology and Sociobiology Unit, German Primate Center, Göttingen, Germany

[b]Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, Georg-August-University of Göttingen, Göttingen, Germany

[c] Endocrinology Laboratory, German Primate Center, Göttingen, Germany

Rolf Daniel and Claudia Fichtel contributed equally to this article

Author contributions:

CF and RD designed and conceived the study.

TM collected data and samples.

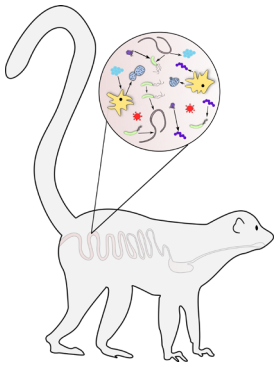
TM processed gut microbiome samples

MH conducted glucocorticoid analysis.

TM and DS prepared and analyzed the data.

All authors revised and approved the manuscript

Citation: Murillo T., Schneider D., Heistermann M., Daniel R. & Fichtel C. Multiscale study of temporal drivers of gut microbiome composition in wild redfronted lemurs. PREPRINT available at Research Square. https://doi.org/10.21203/rs.3.rs-1357320/v1.



Title: Multiscale study of temporal drivers of gut microbiome composition in wild redfronted lemurs

Tatiana Murillo[a,b], Dominik Schneider[b], Michael Heistermann[c], Rolf Daniel[b], and Claudia Fichtel[a]

[a] Behavioral Ecology and Sociobiology Unit, German Primate Center, Göttingen, Germany

[b] Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, Georg-August-University of Göttingen, Göttingen, Germany

[c] Endocrinology Laboratory, German Primate Center, Göttingen, Germany

Rolf Daniel and Claudia Fichtel contributed equally to this article

Abstract

Background

The gut microbiome influences host's immunity, development, and metabolism and participates in the gut-brain axis, thus impacting the health of the host. It is a dynamic community varying between individuals and within individuals at different time points. Hence, determining the factors causing this variability may elucidate their impact on host's health. However, understanding the drivers of variation has proven difficult particularly as multiple interactions occur simultaneously in the gut microbiome.

Results

We performed a longitudinal study to determine the temporal drivers of the gut microbiome in a wild primate, the redfronted lemur. Focal behavioral data and fecal samples were collected for one year in four groups of redfronted lemurs to determine individual social and feeding behaviors. We assessed bacteria, protozoa, and helminths through marker gene analysis. In addition, we measured fecal glucocorticoid metabolites (fGCM) concentrations, to investigate the impact of physiological stress. Higher consumption of leaves and fGCM concentrations correlated with higher alpha diversity, which also differed among groups. The major drivers of variation in beta diversity were group membership, precipitation and fGCM concentrations. We found positive and negative associations between bacterial genera and almost all studied

factors. Correlations between bacterial indicator networks and social networks indicate transmission of bacteria through social interactions.

Conclusions

Processes occurring inside and outside the host drive the temporal fluctuations of the gut microbiome of redfronted lemurs. Activation of the host's HPA axis, dietary changes, fluctuations in water availability and prokaryotic-eukaryotic interactions altering the gut environment impacted the gut bacterial communities. Coupled to dispersal processes of bacteria between hosts through social interactions, and the acquisition of microorganisms from environmental water sources.

Keywords: gut microbiome, lemur, wild primate, HPA axis, social relationships, parasites, metacommunity

Background

The gut microbiome are the prokaryotic and eukaryotic communities inhabiting the host's gastrointestinal tract which play a pivotal role in the health of the host (1–3). This community is dynamic, varying between individuals, and within an individual at different time points (4, 5). Hence, identifying the drivers of gut microbiome variability will help to understand how its fluctuations may associate with health outcomes (5, 6). However, detecting these drivers has proven difficult as few studies recognize the gut microbiome as an ecological system (7). Furthermore, longitudinal studies capturing the temporal dynamics of the gut microbiome are rare or used few individuals resulting in limited data (5). The metacommunity concept recognizes the gut microbiome as an ecological system in which multiple interactions occur simultaneously, thereby providing a framework for determining the drivers of the gut microbiome (6, 7). The metacommunity concept states that the local community assemblage is shaped by the dispersal of species between spaces, genetic diversification of its members, environmental selection by the niche, and ecological drift (6, 8, 9). Here, we investigated the temporal drivers of the gut microbiome in a wild primate applying the metacommunity concept focusing on dispersal mechanisms of bacteria and environmental selection in the gut.

In gut microbiome research, dispersal processes of the microorganisms between hosts and the environment can be assessed through social interactions and habitat sharing (5, 10). Group membership in wild non-human primates and cohabitation in humans

are predictors of gut microbiome similarity (11–16). Furthermore, the host's social behaviors can also predict gut microbiome similarity (17–20). Environmental selection for gut communities occurs in the intestinal niche through feedbacks between the host and the microorganisms and amongst microorganisms (6, 7). Host-associated factors such as, age, sex, and physiological stress, i.e., hypothalamic-pituitary-adrenal (HPA) axis activation, may influence immunity and intestinal physiology altering the gut microbiome (2, 3, 21). Furthermore, shifts in the host's diet impact gut bacterial communities as they alter nutrient availability (22–25). Gut inhabitants interact between themselves through trophic chains, predation and competition for resources (1, 26). For instance, in non-human primates, higher bacterial alpha diversity correlates to higher eukaryotic diversity (27). Therefore, the presence of helminths and/or protozoa may impact the abundances of bacterial taxa (28–30). Despite being challenging, research on wild animals provide an exceptional possibility to apply metacommunity concepts for investigating the temporal drivers of the gut microbiome in undisturbed scenarios (5, 9).

We examined the temporal drivers of the gut microbiome applying metacommunity concepts in a longitudinal setup in wild redfronted lemurs in Kirindy Forest, Madagascar. These lemurs live in small multifemale-multimale groups consisting of individuals of different ages allowing to estimate the potential impact of sex and age (31, 32). Kirindy Forest is a highly seasonal environment with a cold dry season with almost no precipitation (April-October) and a short warm rainy season (November-March) (33). These seasonal changes affect food availability, meaning redfronted lemurs must shift their diets (25, 34). Moreover, fluctuations in precipitation reduce the availability of drinking water (35, 36). HPA axis activation due to exposure to stressors has been previously investigated in these redfronted lemurs through standardized measurement of fecal glucocorticoid metabolites (fGCM) (37–39). For instance, during the dry season and in periods of social instability such as the mating (May-June) and the birth (September-October) season they have higher fGCM concentrations indicating an activation of their HPA axis (38, 40, 41). Furthermore, these lemurs harbor diverse protozoa and helminths in their guts, which can be assessed through marker gene analysis to investigate microbe-microbe interactions (25, 42, 43). Finally, behavioral observations of wild primates provide the opportunity to estimate effects of direct and indirect social contacts in dispersal processes of microbes within a group (10). Particularly, in redfronted lemurs that perform auto- and allogrooming with a

buccal structure, i.e., the toothcomb (44). Oral grooming may increase the possibility of up taking microorganisms from their own fur and the fur from other individuals in comparison to manual grooming which is exhibited in anthropoid primates (37). Altogether, these lemurs provide a unique possibility to study the drivers of the gut microbiome at multiple scales in a wildlife setting.

We investigated the temporal drivers of the gut microbiome from redfronted lemurs at the scales of a) the interactions between the host and the microorganisms, b) the interplay between gut prokaryotes and eukaryotes and c) dispersal processes of bacteria within and between groups in a longitudinal study using a dense sampling regime. Focal behavioral data and monthly fecal samples (N=799) were collected during one year from all individuals (N=35) belonging to four groups. Bacteria, protozoa, and helminths were identified with marker gene analysis and fGCM measurements were performed to determine HPA axis activation. Furthermore, precipitation was measured as a proxy for changes in available water sources. We hypothesized that 1) host intrinsic factors such as sex, age, and fGCM concentrations as well as extrinsic factors such as precipitation, and diet impact gut microbiome composition and diversity, 2) protist and helminth richness correlate with changes in bacterial diversity and composition, 3) group membership influences bacterial diversity and composition and 4) bacterial indicator networks of amplicon sequence variants (ASVs) correlate to social networks indicating bacterial transmission through social interactions.

Methods

Sample, behavioral, and environmental data collection

This study was conducted at the research station of the German Primate Center in Kirindy Forest, Western Madagascar (44° 39′ E, 20° 03′ S) from May 2018 to April 2019 (40). Samples and data were collected over one year from 35 redfronted lemurs belonging to four groups (A, B, F and J) (Supplementary Table S1). 799 fecal samples were collected in RNAlater (Thermofisher Scientific, Massachusetts, USA) from the forest floor immediately after defecation between 7:30 and 11:00, stored at -20°C in the field station and later at -80°C in Germany (Supplementary Table S1). 641 of these samples were splitted and feces were placed in 5mL of 80% ethanol for measuring fGCM concentrations using validated methodologies (see below). Behavioral data was collected by continuous focal observations for 30 minutes in the morning (7:30-11:00)

and afternoon (14:00-17:00). Feeding behaviors were recorded by protocolling the duration and the ingested food item (leaves, flowers, or fruits). For social interactions, we protocolled the duration of grooming and body contact, and the interacting partners. Precipitation was collected with a Tropos data logger (Lambrecht meteo, Göttingen, Germany) and we calculated the mean precipitation 30 days prior to sample collection according to previous publications (22).

Behavioral data analysis

For each fecal sample we estimated the following behaviors 30 days prior to collection (17): a) the proportion of time the individual spent feeding on fruits, flowers and/or leaves, and b) a social interaction diversity index: $(Shannon\ diversity\ of\ social\ interactions * Average\ interaction\ per\ dyad)$ for each individual, accounting for the number of interacting partners and duration of these interactions. This index increases with the average dyadic interaction time and when the interactions are more evenly distributed among dyads.

DNA extraction and amplification of taxonomic marker genes

DNA extractions were performed from 150 mg fecal sample following the manufacturer´s instructions but including a bead beating step of 6.5m/s and 24x2 for 20s using FastPrep-24^{TM}5G (MP Biomedicals, California, USA) with the PowerSoil DNA isolation kit (Qiagen, Hilden, Germany). For amplification of the 16S rRNA gene (Supplementary Table S1), each sample was amplified separately, whereas for the 18S rRNA gene monthly samples were pooled together before amplification (Supplementary Table S2). PCR reactions for both taxonomical marker genes were performed in triplicates with the primers and thermocycling protocols listed in the Supplementary Table S3 and included a negative control without DNA template and a positive control (45, 46). Triplicates per sample were pooled equimolar, purified, and sequenced as in (47).

Bioinformatic processing of amplicon data

Paired-end reads were quality-filtered with fastp v0.20.0 using default settings plus an increased per base phred score of 20, base pair corrections by overlap (-c), as well as 5′- and 3′-end read-trimming with a sliding window of 4, a mean quality of 20 and minimum sequence length of 50 bp. Quality-controlled reads were merged with PEAR v0.9.11 and primer-clipping was performed with cutadapt v2.5 with default settings.

VSEARCH 2.14.1 was used for size-sorting, size-filtering (16S rRNA ≥300bp; 18S rRNA ≥250bp) and dereplication. The sequences were denoised with UNOISE3 using default settings and chimeras were removed with UCHIME3 (*de novo* followed by reference-based) leading to the final set of amplicon sequence variants (ASVs). 16S rRNA were mapped against the ASVs and taxonomy was assigned with a minimum identity of 70% using BLAST 2.9.0+ against the SILVA SSU 138.1 NR (48). Best hits were only accepted if coverage ≥90 and blastn hit identities were corrected to unclassified according to the thresholds proposed by (49). 18S RNA reads were assigned using BLAST 2.9.0+ against the PR2 database (50) and taxonomy was determined with the Bayesian LCA-based Taxonomic Classification Method (BLCA) using a confidence score threshold of 0.80 (51). To control for spurious reads and index hopping, ASVs with <0.25% reads were removed before analysis (52). All sequencing statistics are in Supplementary Table S4.

Measurement of fecal glucocorticoid metabolites

Glucocorticoid metabolites (fGCM) were extracted from the fecal samples directly at the field site using a validated method (53) previously used for lemurs (54, 55). Extracts were stored in the field at ambient temperature in the dark and at -20°C in Germany. FGCM concentrations were determined using an enzyme immunoassay (EIA) for the analysis of immunoreactive 11-oxoetiocholanolone, a group-specific measurement of cortisol metabolites in primates (39). The EIA, carried out as described in (38), has been validated for tracking HPA axis activity in redfronted lemurs (37, 38). Inter- and intra-assay coefficients of variations (CVs) of high- and low-value quality controls were 10.9% (high, n=52) and 9.7% (low, n=52) and 6.8% (high, n=17) and 8.2% (low, n=17), respectively. FGCM values are expressed as mass per gram of wet fecal weight (ng/g).

Data analysis and statistics

Data visualization and statistical analysis were performed using R v4.1.0 and RStudio v1.4.1717 with ampvis2, ape, stringr, reshape2, viridis, data.table, tidyverse, and ggplot2. All data for alpha and beta diversity analysis was rarefied to the lowest read counts whereas for barcharts, linecharts, and network estimation it was normalized using GMPR (Supplementary Table S4). Bacterial alpha diversity was calculated as Faith´s phylogenetic diversity (PD) with picante using a phylogenetic tree generated by aligning all sequences with MAFFT v7.407-1 at 100 iterations, calculated using FastTreeMP v2.1.7 and midpoint-rooted using FigTreev 1.4.4.

Analysis of gut protozoa and helminth

ASVs from previously reported gut protozoa and helminth were extracted from the 18S rRNA gene data to remove environmental contaminants. The analyzed taxa were *Trichostomatia*, *Nematoda*, *Metamonada*, *Coccidiomorphea*, and *Cestoda* (27, 42, 56). Samples were merged per individual per month and parasite richness was estimated as the number of observed ASVs. A Jaccard matrix was calculated to investigate changes in parasite beta diversity and visualized with a Principal Coordinate Analysis (PCoA) in ampvis2. A PERMANOVA test to estimate beta diversity variation due to group, sex, age, and season was calculated with the adonis function from the vegan package using individual as strata to account for repeated sampling, 10,000 permutations and Benjamini-Hochberg FDR correction.

Testing the factors affecting bacterial alpha diversity

The effects of group, sex, age, social interactions, parasite richness, feeding on fruits, flower or leaves, and precipitation on PD were tested by fitting a Linear Mixed Model (LMM) with lme4. To ease model converged, PD was Box-Cox transformed. Test predictors were group, sex, age, social interactions, and parasite richness, whereas diet, and precipitation were control predictors. Age was log-transformed to achieve a more symmetrical distribution and avoid influential cases, and all predictors were z-transformed to facilitate model convergence. Individual identity was included as random intercept effect and the random slopes for all fixed effects (except for group and sex) into individual identity were included to keep the type I error at the nominal level of 5% (57). Correlations between random intercepts and random slopes were included. The significance of the test predictors was determined by calculating a null model excluding all test predictors and comparing it to the full model using a likelihood ratio test. The effects of single fixed effects were determined with the package lmerTest. Homogeneous and assumptions of normally distribution of residuals were checked visually with QQ-plots of residuals and plotted against fitted values revealing no obvious deviations. Calculation of Variance Inflation Factors using car was done on a model lacking all random effects and no issues of collinearity were detected (maximum:1.433). Model stability was determined by dropping predictors one at a time, fitting a full model from each of the subsets and comparing the estimates of these models to those obtained for the initial full model revealing it was acceptable. The same model was calculated for those samples having fGCM measurements by adding log-

transformed fGCM values as a test predictor. No collinearity was detected (maximum:1.404) and model stability was also acceptable.

Drivers of bacterial beta diversity dissimilarities

Weighted UniFrac matrices (WUnifrac) were calculated in ampvis2 and visualized with PCoA. To estimate the drivers of beta diversity variation, PERMANOVA tests were calculated from WUnifrac as discussed before. Three different datasets were tested: a) diet and social interactions (n=773), b) parasite richness (n=682) and c) fGCM levels (n=547) as for some samples either behavioral or parasite data was missing and PERMANOVA cannot be calculated in samples with missing data points. Group, sex, age, and precipitation were tested in all datasets.

Associations between bacterial genera and all covariates

Associations of group, sex, age, social interactions, diet, precipitation and fGCM concentrations to bacterial genera were determined using the package MaAsLin2. Two models with the random effect of individual were calculated: a) all factors without fGCM levels (n=799) and b) all factors including fGCM concentrations (n=641). ASV counts were centered-log ratio transformed.

Bacterial indicator and social network analysis

Bacterial indicator networks were calculated with indicspecies to identify correlations between ASVs abundances and individuals (58). multipatt was used to determine the phi coefficient of association and the association strength between an ASV and an individual using 999 permutations. Networks were visualized in Cytoscape v3.8.2 using the individuals and their associated bacterial taxa as nodes, whereas edges correlation coefficients $p<0.05$ between nodes. The networks had an edge-weighted spring embedded layout, taxon node size was adjusted according to taxa abundance, edge width represents association strength to target, and all nodes and edges were bundled. Undirected weighted social networks for each group were calculated using the Dyadic Sociality Index (DSI) including proportion of grooming, and body contact behaviors during the whole study, and visualized with igraph (59). Previously, correlations between both behaviors were determined with Mantel correlations tests. For group F and J, no correlations were detected, but for uniformity the DSI was also used. Correlations of the number of shared indicative ASV and the DSI between individuals were estimated with Mantel tests.

Availability of data and material

Raw reads were deposited in the NCBI Sequence Read Archive under the Bioproject PRJNA694983 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA694983) (Supplementary Table S1 and Supplementary Table S2). The datasets generated and analyzed during the current study are available in figshare: https://figshare.com/projects/Multiscale_study_of_temporal_drivers_of_gut_microbiome_composition_in_wild_redfronted_lemurs/126316. All R scripts can be found in https://github.com/tmurillocorrales/Redfrontedlemurs_gutmicrobiome.

Results

Bacterial, protozoan, and helminthic communities of redfronted lemurs

The five most abundant bacterial phyla showed consistent relative abundances in all four groups: *Bacteroidota* (35.49% ± 3.24), *Firmicutes* (30.01% ± 4.60), *Proteobacteria* (9.83% ± 3.00), *Spirochaetota* (9.41% ± 1.43) and *Verrucomicrobiota* (7.02% ± 1.01) (Fig.1A, Supplementary Table S5). On genus level the five most abundant bacteria were also consistent among all groups with variations in their abundances during the sampling period (Fig.1B). Although four genera could not be classified at genus level, they belong to the families *Prevotellaceae* (16.26% ± 5.75), *Spirochaetaceae* (9.33% ± 3.20), *Rikenellaceae* (6.62% ± 3.53) and *Kiritimatiellae* (5.44% ± 2.66) while the fifth most abundant genus was *Sutterella* (3.64% ± 2.62). Bacterial alpha diversity calculated as Faith's Phylogenetic diversity index (PD) had similar monthly trends in all groups (Fig.1C). Lower PD was detected in April for all groups towards the transition between rainy and dry season (A: 42.14 ± 5.67; B: 43.31 ± 4.44; F: 30.19 ± 7.33; J: 40.92 ± 8.24) whereas higher PD was observed in October in the transition from dry to rainy season (A: 50.40 ± 0.91; B: 50.49 ± 0.93; F: 48.96 ± 0.72; J: 50.18 ± 1.63).

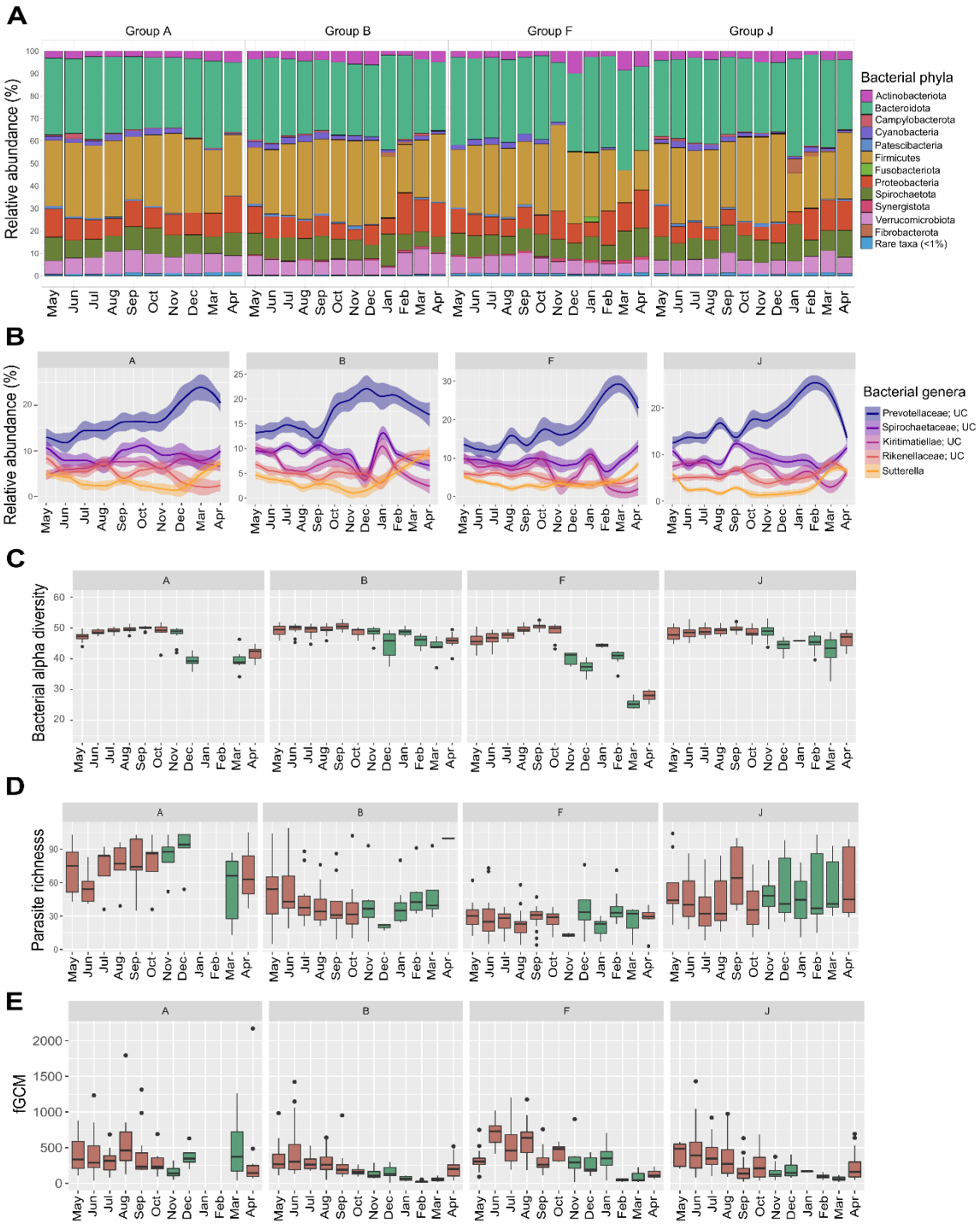


Figure 1. Overview of the temporal fluctuations of bacterial communities, bacterial alpha diversity, eukaryote parasite richness and fGCM concentrations for each lemur group. Box plots are color coded to indicate the dry (brown) and rainy (green) season. **A**. Monthly averaged relative abundances of bacterial phyla per lemur group. **B.** Top 5 most abundant bacterial genera and their monthly changes. **C.** Monthly variations in alpha diversity measured as Faith's Phylogenetic Diversity Index. **D.** Monthly changes

in parasite richness. **E.** Concentrations of fGCM measured as ng/g of wet feces aggregated per month.

Helminthic and protozoan gut communities were studied by amplifying the V4 region from the 18S rRNA gene. All amplified taxa were *Metazoa* including *Nematoda* (48.40% ± 10.69), *Craniata* (5.53% ± 4.22) and *Arthropoda* (2.98% ± 2.63), *Streptophyta: Embryophyceae* (21.86% ± 6.25), *Fungi: Ascomycota* (1.44% ± 1.07) and *Basidiomycota* (3.85% ± 6.59), *Ciliophora: Litostomatea* (9.50% ± 6.44) and *Metamonada: Trichomonadea* (1.24% ± 0.72) with a total of 4.04% ± 2.40 unclassified reads (Supplemental Figure S1A, Supplementary Table S6). Further on, only eukaryote orders formerly reported as inhabitants of the gut of humans or animals were analyzed. The orders detected were *Chromadorea*; *Nematoda* (A: 79.38% ± 17.04; B: 78.02% ± 22.00; F: 73.55% ± 28.44; J: 79.22% ± 21.37), *Trichostomatia*; *Litostomatea* (A: 19.49% ± 15.92; B: 15.95% ± 17.63; F: 16.82% ± 21.87; J: 18.59% ± 20.68), and *Trichomonadida*; *Trichomonadea* (A: 1.13% ± 2.80; B: 6.02% ± 16.28; F: 9.61% ± 21.33; J: 2.17% ± 4.43) present in all individuals (Supplemental Figure S1B). Except for *Litostomatea*, which was not detected in one individual from August until October. Subsequently, we determined the number of observed ASVs for the same taxa as a measure of parasite richness. Parasite richness showed variations between groups, individuals, and months (mean ± SD number of ASVs: group A: 71.44 ± 24.95; group B: 45.43 ± 26.27; group F: 27.44 ± 13.14; group J: 49.51 ± 26.44) (Fig.1D). A PERMANOVA based on Jaccard matrix showed that most of the variance on parasite richness was explained by season (r^2= 0.011, p=0.001) (Supplemental Figure S1C & Supplementary table S7). Parasite richness differed between groups and season.

The highest concentrations of fGCM were detected in August for group A (571.9ng/g ± 412.65), and in June in all other groups (B: 447.00ng/g ± 373.13; F: 706.33ng/g ± 177.87; J: 463.23ng/g ± 337.29) (Fig. 1E). Consumption of leaves, fruits and flowers varied across months and between groups (Supplementary figure S2A). December and January were the months with highest precipitation (Supplementary figure S2B).

Factors driving changes of bacterial alpha diversity

We analyzed the effects of sex, age, group membership, social interactions, parasite richness, dietary changes, and precipitation on alpha diversity measured as PD. The model (full-null model comparison: p=0.008, Supplementary table S8) detected an effect of group membership, with group F having a lower alpha diversity compared to

the other groups (p=0.009, Fig.2A). Additionally, feeding on leaves correlated positively with alpha diversity (p=0.000, Fig.2B). The second model for alpha diversity used a reduced dataset (see methods) including fGCM concentrations. Similarly, an effect of group membership for group F and feeding on leaves was detected (full-null model comparison: p=0.038; Supplementary table S9). FGCM concentrations correlated positively with alpha diversity (p=0.027, Fig.2C) with higher fGCM concentration resulting in a higher alpha diversity. No effects of sex, age, social interaction diversity index, or parasite richness were detected.

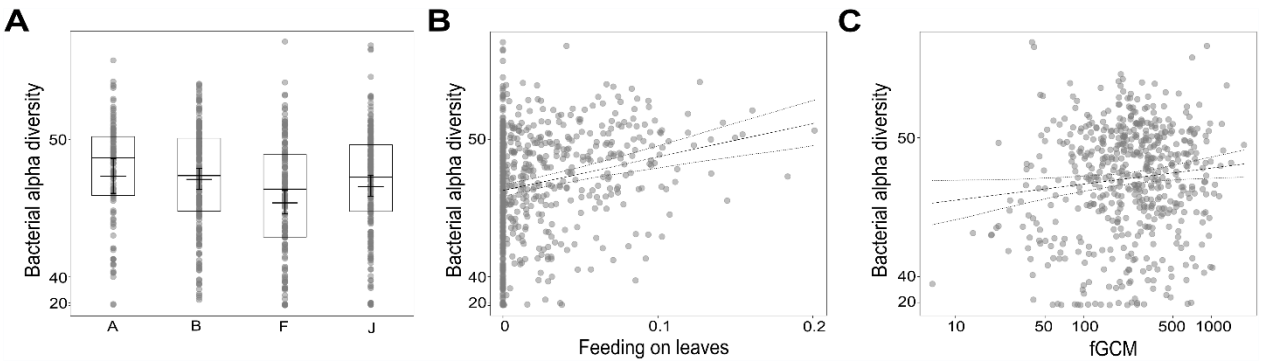


Figure 2. Effects of group membership, consumption of leaves and concentrations of fGCM on bacterial alpha diversity measured as PD. **A.** Group membership. **B.** Proportion of time feeding on leaves 30 days prior to sampling. **C.** Log-transformed fGCM concentrations given in ng/g feces.

Factors leading to dissimilarities between gut bacterial communities

To estimate the drivers of variance on beta diversity, PERMANOVA based on WUnifrac matrices on three different datasets were calculated due to missing data points (see methods). The factors tested in the first dataset explained 8.9% of the variance (Fig.3A-B), with group (r^2=0.035, p<0.000) and precipitation (r^2=0.021, p<0.000) being the strongest predictors (Supplementary table S10). In the second dataset (Supplementary Table S11) including the parasite data, the total variance explained was 10.4% with group (r^2=0.041, p<0.000) and precipitation (r^2=0.024, p<0.000) as strongest predictors. Finally in the dataset including fGCM concentrations (Supplementary Table S12) 14.5% of the variance was explained with fGCM (r^2=0.028, p<0.000), group (r^2=0.052, p<0.000) and precipitation (r^2=0.022, p<0.000) as strongest predictors.

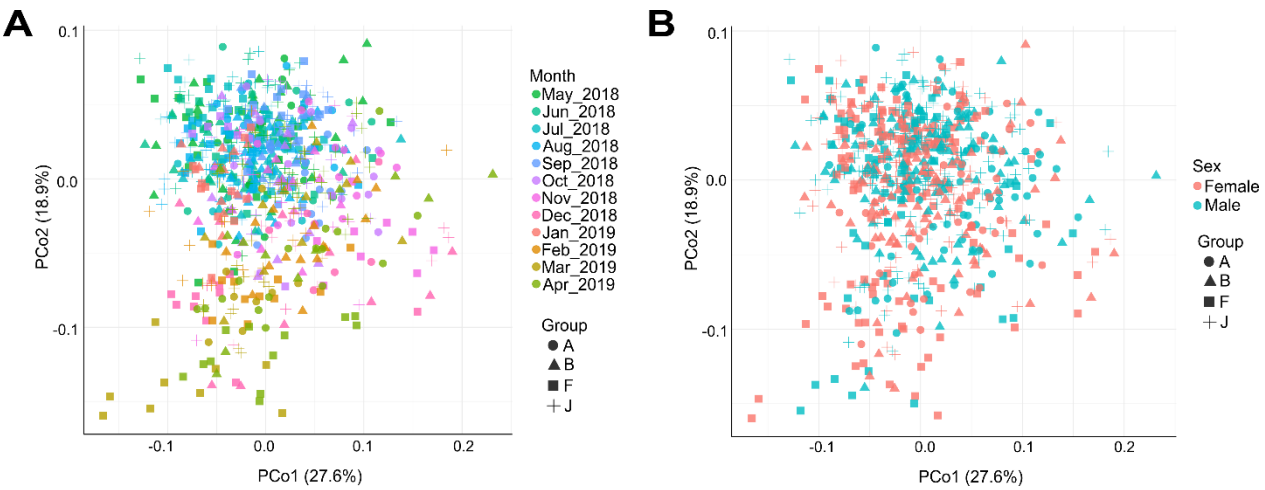


Figure 3. PCoA from Weighted Unifrac matrices WUnifrac of the bacterial community denoting beta diversity changes. **A.** Data points color coded to the different study months to depict monthly changes in beta diversity. **B.** Data points color coded to sex. Groups are depicted in A and B by symbols.

Associations of social interactions, parasite richness, fGCM concentrations, diet, and precipitation to bacterial genera composition

A total of 50 bacterial genera associated with group, social interaction diversity index, feeding on flowers, leaves or fruits, parasite richness, age, and precipitation in the full dataset (Fig.4A & Supplementary table S13). Precipitation and diet had the most associated taxa, with 33 and 36 genera, respectively. Dispersal processes attributed to group membership and social interactions had 27 and 2 associated taxa, respectively. Parasite richness exhibited 12 associated taxa. In the subsetted dataset including fGCM concentrations, 50 genera associated with at least one of the studied covariates (Fig.4B & Supplementary table S14). Twenty taxa associated with fGCM levels, whereas slight variations were detected for the other covariates: precipitation (26), diet (24), group (28), social interactions (2), and parasite richness (5). In both datasets, no genus associations with sex and age were detected.

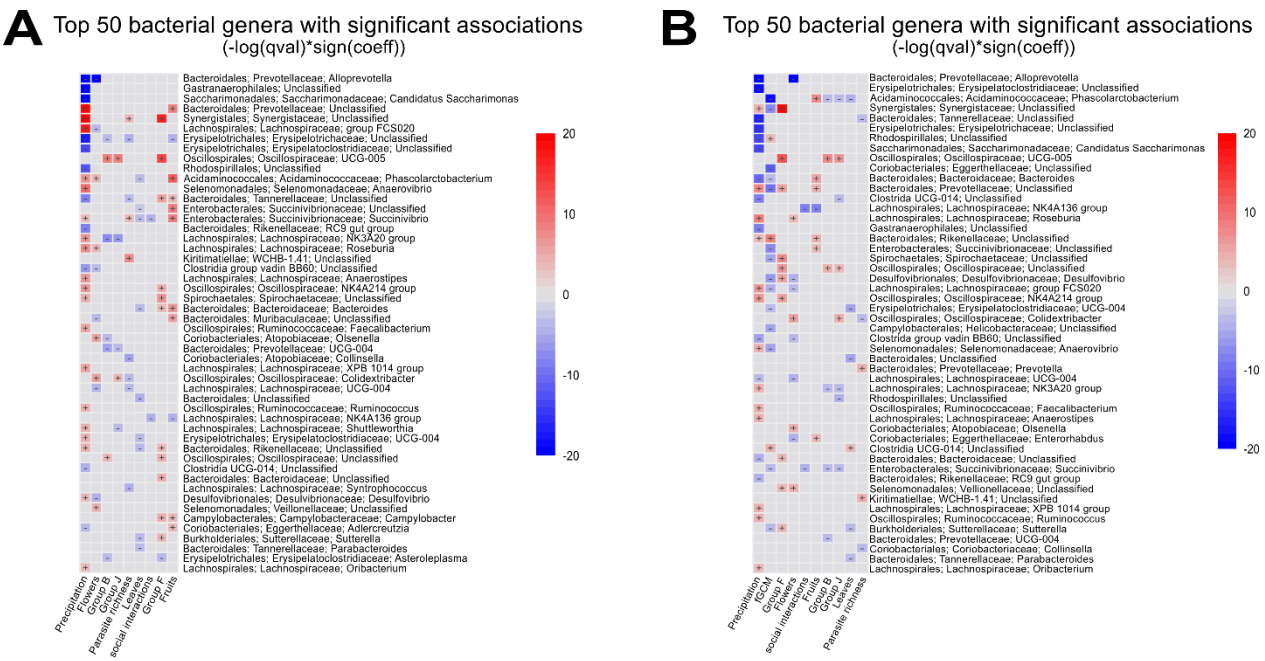


Figure 4. Top 50 most abundant bacterial genera associated with group, social interactions, age, sex, parasite richness, diet, and precipitation. Association directions are color coded positive (red) and negative (blue). **A.** Full dataset. **B.** Reduced dataset including fGCM concentrations. Group A was the reference category for group comparisons.

Correlation between social networks and bacteria indicator networks

To determine if sharing of bacterial ASVs between individuals correlates to an individual's social network, bacterial indicator networks were calculated. These networks were determined based on ASVs to identify bacterial ASVs whose relative abundance significantly correlate within and between individuals. Thus, suggesting that sharing of ASVs indicates microbe dispersal through social interactions. Correlations between bacterial indicator ASVs and social networks were detected for group A (r^2=0.536, p=0.002, Supplementary table S15 & S16), and B (r^2=0.399, p=0.013, Supplementary table S17 & S18), but not group F (r^2=0.502, p=0.089, Supplementary table S19 & S20) and J (r^2=0.235, p=0.060, Supplementary table S21 & S22) (Fig.5G & Fig.5H). Furthermore, individuals who emigrated from groups: A (AAmoM; Fig.5A & Fig.5B), B (BTilM; Fig.5C & Fig.5D), and F (FGozM; Fig.5E & Fig.5F) had less strong social relationships and a more differentiated bacterial indicator network profile than individuals that remained in the groups. One individual, BTilM, immigrated to group A, thus showing fewer connections in the social network, and sharing less ASVs with other group members.

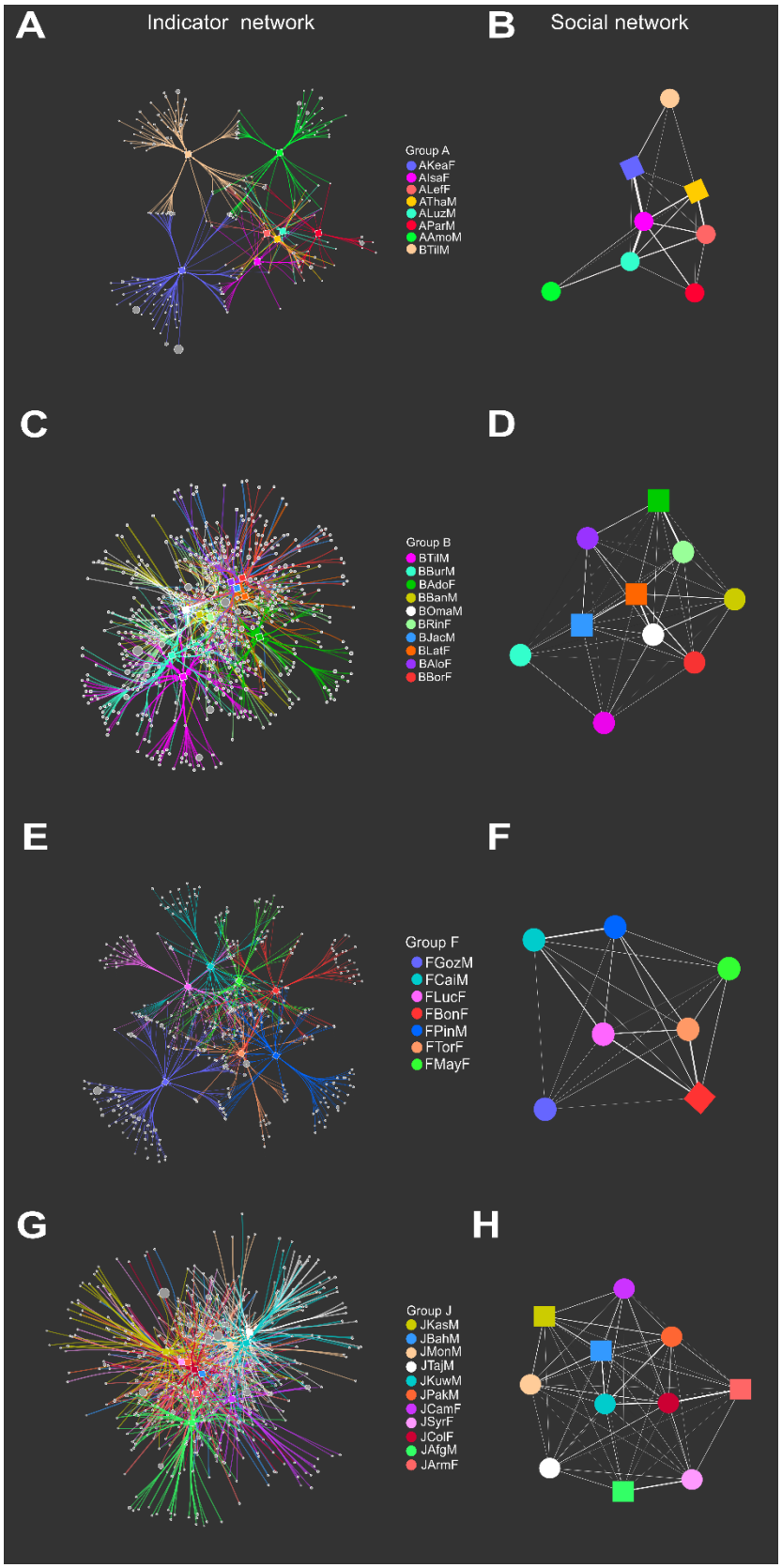


Figure 5. Indicative networks and social networks for the individuals of each group based on ASVs. Networks were colored by individual; nodes are shaped in the social network according to adult (circle) or juvenile/infant (square). Bacterial indicator ASV

network and social network of group A (A and B), group B (C and D), group F (E and F) and group J (G and H).

Discussion

Our longitudinal study revealed that host-microbe interactions, the interplay between bacteria and parasite richness, and dispersal processes of bacteria through social relationships impact temporal fluctuations of the gut microbiome. From the investigated host-associated factors, the HPA axis measured through fGCM concentrations revealed the strongest impact. Higher fGCM levels correlated to higher alpha diversity and associated with changes in bacterial abundances. Conversely, no impact of age and sex was identified. Interactions between eukaryotes and bacteria were detected as parasite richness explained a small amount of variance in beta diversity and impacted both, positively but also negatively, the abundances of specific bacterial genera. Dispersal processes of bacteria between hosts were estimated from social interactions and group membership. Group explained 3-5% of the variance in beta diversity, groups had different alpha diversity, and each group had its own associated bacterial genera. Diversity of social interactions explained only low variance in beta diversity but impacted the abundances of certain bacteria. An individual's social network correlated to its sharing of significantly associated bacterial ASVs with other individuals in two of the four groups, suggesting transmission of taxa through social interactions.

The HPA axis is an important driver of gut microbiome variation in wild lemurs

Higher fGCM concentrations, indicating HPA axis activation, correlated to increased bacterial alpha diversity. The highest mean fGCMs values for three of the four groups were detected during June indicating an influence of the mating season on HPA axis activity (40, 60). However, for one group the highest fGCM values were in August, a period when redfronted lemurs are exposed to environmental stressors due to reduced food and water available (33, 34, 38). Even though environmental stressors could have increase fGCM levels, we suspect that social stressors had a greater influence, as reported before in these lemurs (38). Our longitudinal approach aimed to capture these periods when redfronted lemurs experience social and environmental challenges made it possible to detect this impact (33, 34, 38). Previous studies detected no correlations or negative correlations between glucocorticoids and alpha diversity (61–64). We speculate that higher fGCM levels leading to higher bacterial alpha diversity might be

due to the down regulation of the immune response controlling the gut microbiome by glucocorticoids, thus allowing the colonization by other taxa (65, 66). Consumption of leaves during the dry season also correlated with higher alpha diversity which may contribute to a certain degree to the positive correlation between fGCM concentrations and alpha diversity. However, redfronted lemurs fed more on leaves in September/October, whereas fGCM concentrations peaked in June/August, indicating that feeding on leaves and fGCMs influence separately alpha diversity. fGCM concentrations was one of the covariates explaining most variation in beta diversity, indicating that fGCM concentrations drive differences in beta diversity. Positive associations were detected only to three genera from *Rikenellaceae*, *Rhodospirillales* and *Clostridia*. Higher abundances in genera from *Clostridia* have been reported in mice exposed to social stressors and western lowland gorillas with high fGCM measurements (62, 64). Fourteen genera were impacted negatively by fGCM, including genera from *Prevotellaceae*, *Spirochaetaceae* and *Sutterella*, some of the most abundant taxa detected in redfronted lemurs (25). A negative association to a genus from *Helicobacteraceae*, a potential pathogen, was also detected in yellow-legged gull (67). The activation of the HPA axis and its production of glucocorticoids can influence the gut microbiome through the increase of gut permeability allowing the translocation of bacteria from the lumen to other tissues (61). Also, HPA axis activation can reduce immune activation and increase susceptibility to infections by pathogens (61, 68, 69). Our results indicate that social stressors from the mating season like reproductive competition and female evictions can activate the HPA axis impacting the gut microbiome (40, 70).

Diversity of gut protozoa and helminth impact the bacterial community

Helminths and protozoa were prevalent all year in almost all individuals, and the orders detected coincide with our previous study (25). Variations in eukaryotic communities between samples were explained by season. Our results support previous reports from redfronted lemurs that detected seasonal differences in the abundances of *Chromadorea*, and protozoa diversity (71). Parasite richness only explained very low variation in bacterial beta diversity but associated positively but also negatively with certain bacterial taxa, supporting other studies from non-human primates (28–30). Positive associations with *Succinivibrio* and *Verrucomicrobiota* have been reported in humans as well (72, 73). Also, negative associations of genera from *Lachnospiraceae*

such as *Syntrophococcus* and XPB-1014 group, have been detected in humans with helminthic and helminthic-protozoan infections (72–74). Other negatively associated taxa like *Collinsella*, *Colidextribacter*, *Tannerellaceae* and *Erysipelotrichaceae* are gut bacteria with no association to parasites reported so far (75–77). Each parasite can have specific effects on the gut niche, thus explaining that parasite richness explains only a low amount of beta diversity since all parasites were investigated together (1, 78, 79). Also, it was not possible to compare infected vs. uninfected individuals, as parasites were prevalent in almost all individuals all year. We investigated only presence and absence of parasites, as abundance estimations from 18S RNA should be taken cautiously (43). Parasites can impact bacteria positively or negatively through trophic chains, predation, competition, and immunomodulation (1, 26, 78). These are all processes that could be occurring in redfronted lemurs due to their diverse eukaryotic communities.

Dispersal processes between hosts are drivers of gut microbiome community composition

Group membership, diversity of social interactions and social networks were used to estimate bacterial dispersal through social behaviors. Group membership was one of the covariates explaining the highest variance in beta diversity and having the most associated taxa, indicating that each group has a specific bacterial community despite temporal fluctuations of the microbiome. Group differences in the gut microbiome can be due to sharing of microorganisms through social interactions between group members, as it has been proposed previously (12–16, 80). Differences in bacterial communities can also be explained by habitat use, but all studied groups have overlapping home ranges with at least one group (81) However, group F, occupies a home range more distant to a river traversing the study area, which may affect the habitat quality and could explain the differences in alpha diversity (33, 35, 82). Kinship may also influence group differences but not all group members were related thus, we suppose that it may have a lower impact (17, 40). Diversity of social interactions only explained very low variance in beta diversity, but it had negatively associated taxa. *Succinivibrio*, a starch degrader, was impacted negatively indicating that social interactions can impact genera carrying out relevant metabolic functions (83). Correlations between bacterial indicator ASVs and social networks indicate that at least some of these indicator taxa are shared through affiliative interactions. Hence,

individuals exhibiting strong social relationships, share bacterial ASVs through their affiliative behaviors influencing bacterial presence and abundances. The fact that no correlations were detected for groups F and J indicate that this signal is harder to detect in groups with less differentiated social relationships. Less ASVs were shared by group members that emigrated or immigrated the groups possibly due to their short residency in the group as reported baboons (80, 84). Correlations between social networks and gut microbiome similarity have been reported in other wild primates (17, 19), but this study is the first to analyze the impact of social networks on bacterial taxon level.

Ecological determinants of variations in gut bacterial communities

Feeding on flowers, fruits, or leaves, and precipitation correlated to changes in beta diversity and had positive and negative associated taxa with each of them. Consistent with a previous study from these lemurs and other research, feeding on leaves correlated with a higher alpha diversity (22, 23, 25) Changes in precipitation had the most associated taxa. Precipitation affects the availability of water sources in the habitat of redfronted lemurs between dry and rainy season (33). During the rainy season redfronted lemurs drink water from temporal puddles, tree holes or the river, whereas during dry season only water ponds in the river remain (35, 85). We speculate that the changes in water intake due to reduced water availability may impact gut microbiome by influencing gut transit times, thus affecting clearance of microorganisms during excretion, and determining the availability of nutrients and water in the gut habitat (35, 86). In humans stool consistency is the strongest predictor of gut microbiome composition and it is relevant as it indicates differences in water availability and activity in the colon influencing the gut niche (86, 87). However, it is also possible that the lemurs ingest bacteria from water sources, and this uptake fluctuates according to the water sources available (22, 85). The type of food item consumed is another important driver of bacterial community composition as they are also their main energy source (88, 89). The capacity of the gut microbiome to adapt to dietary changes is essential for the acquisition of nutrients from food by the host (9). This effect was detected when shifting from a diet based on leaves, which is composed of complex polysaccharides, to a diet based on flowers and/or fruits, which is rich in mono- and disaccharides (25, 90).

Conclusion

The gut microbiome of wild redfronted lemurs is shaped by group membership, social interactions, fGCM levels, diet, precipitation, and parasite richness at different intensities. Thus, bacterial dispersal processes between hosts and the environment, plus selection by the gut niche through prokaryotic-eukaryotic interactions, changes in water availability, diet fluctuations, and the host's HPA axis activation impact the gut microbiome. Our results demonstrate the importance of longitudinal studies with dense sampling regimes to capture the drivers of gut microbiome variation within populations. This setup enabled to detect the time periods when each of the factors impacted the gut microbiome asserting that both processes outside and inside the host influence its temporal dynamics.

List of abbreviations

ASVs: amplicon sequence variants

fCGM: fecal glucocorticoid metabolites

PCoA: principal coordinate analysis

PD: Faith's Phylogenetic Diversity index

LMM: linear mixed model

WUnifrac: weighted unifrac

DSI: dyadic sociality index

Declarations

Ethical Approval and Consent to participate

Research permits were granted by the Malagasy Ministère de l'Environnement et des Eaux et Forêts, the University of Antananarivo, and the Centre National de Formation, d'Etudes et de Recherche en Environnement et Foresterie.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

Availability of data and materials

Raw reads were deposited in the NCBI Sequence Read Archive under the Bioproject PRJNA694983 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA694983). The datasets generated and analyzed during the current study are available in figshare: https://figshare.com/projects/Multiscale_study_of_temporal_drivers_of_gut_microbiome_composition_in_wild_redfronted_lemurs/126316. All R scripts can be found in https://github.com/tmurillocorrales/Redfrontedlemurs_gutmicrobiome.

Funding

This project was funded by the Deutsche Forschungsgemeinschaft (FI 929/7-2, DA 374/13-2) from the research unit “Sociality and Health in Primates” (SoHaPi).

Authors’ contributions

C.F. and R.D. designed the study and obtained the funding. TM conducted the sample and data collection in the field and the laboratory work on the gut microbiome. M.H. conducted glucocorticoid analysis. T.M. and D.S. analyzed and visualized the data. TM wrote the first draft of the manuscript. All authors interpreted the results, reviewed, and revised the manuscript.

Acknowledgements

For support in sample and data collection we thank Jean-Pierre Tolojanahary, Mamy Razafindrasamba, Tianasoa Andrianjanahary, Judit Stolla, Luise Waldow, and Alexandra Michiels. We thank Dr. Anja Poehlein, Melanie Heinemann, and Sarah Schüßler for amplicon sequencing. We are grateful to Christina Glaschke for helping with hormone measurements. For scientific discussion and statistical advice, we thank Dr. Roger Mundry, Dr. Louise Peckre, Dr. Dirk Berkelmann and Lea Prox.

Author’s information

Not applicable

References

1. Parfrey LW, Walters WA, Knight R. 2011. Microbial eukaryotes in the human microbiome: Ecology, evolution, and future directions. Front Microbiol 2:1–6.
2. Clemente JC, Ursell LK, Parfrey LW, Knight R. 2012. The impact of the gut microbiota on human health: An integrative view. Cell 148:1258–1270.

3. Cryan JF, O'riordan KJ, Cowan CSM, Sandhu K V., Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva A V., Guzzetta KE, Jaggar M, Long-Smith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E, O'connor R, Cruz-Pereira JS, Peterson VL, Rea K, Ritz NL, Sherwin E, Spichak S, Teichman EM, van de Wouw M, Ventura-Silva AP, Wallace-Fitzsimons SE, Hyland N, Clarke G, Dinan TG. 2019. The microbiota-gut-brain axis. Physiol Rev 99:1877–2013.

4. Caporaso JG, Lauber CL, Costello EK, Berg-Lyons D, Gonzalez A, Stombaugh J, Knights D, Gajer P, Ravel J, Fierer N, Gordon JI, Knight R. 2011. Moving pictures of the human microbiome. Genome Biol 12:R50

5. Björk JR, Dasari M, Grieneisen L, Archie EA. 2019. Primate microbiomes over time: Longitudinal answers to standing questions in microbiome research. Am J Primatol 81:1–23.

6. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. 2012. The application of ecological theory toward an understanding of the human microbiome. Science 336:1255–1262.

7. Miller ET, Svanbäck R, Bohannan BJM. 2018. Microbiomes as metacommunities: understanding host-associated microbes through metacommunity ecology. Trends Ecol Evol 33:926–935.

8. Koskella B, Hall LJ, Metcalf CJE. 2017. The microbiome beyond the horizon of ecological and evolutionary theory. Nat Ecol Evol 1:1606–1615.

9. McKenney EA, Koelle K, Dunn RR, Yoder AD. 2018. The ecosystem services of animal microbiomes. Mol Ecol 27:2164–2172.

10. Sarkar A, Harty S, Johnson KVA, Moeller AH, Archie EA, Schell LD, Carmody RN, Clutton-Brock TH, Dunbar RIM, Burnet PWJ. 2020. Microbial transmission in animal social networks and the social microbiome. Nat Ecol Evol 4:1020–1035.

11. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, Shilo S, Lador D, Vila AV, Zmora N, Pevsner-Fischer M, Israeli D, Kosower N, Malka G, Wolf BC, Avnit-Sagi T, Lotan-Pompan M, Weinberger A, Halpern Z, Carmi S, Fu J, Wijmenga C, Zhernakova A, Elinav E, Segal E. 2018. Environment dominates over host genetics in shaping human gut microbiota. Nature 555:210–215.

12. Degnan PH, Pusey AE, Lonsdorf E V., 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.

13. 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). Am J Primatol 78:883–892.

14. 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.

15. Raulo A, Ruokolainen L, Lane A, Amato K, Knight R, Leigh S, Stumpf R, White B, Nelson KE, Baden AL, Tecot SR. 2017. Social behaviour and gut microbiota in red-bellied lemurs (Eulemur rubriventer): In search of the role of immunity in the evolution of sociality. J Anim Ecol 388–399.
16. Springer A, Fichtel C, Al-Ghalith GA, Koch F, Amato KR, Clayton JB, Knights D, Kappeler PM. 2017. Patterns of seasonality and group membership characterize the gut microbiota in a longitudinal study of wild Verreaux's sifakas (Propithecus verreauxi). Ecol Evol 7:5732–5745.
17. Tung J, Barreiro LB, Burns MB, Grenier JC, Lynch J, Grieneisen LE, Altmann J, Alberts SC, Blekhman R, Archie EA. 2015. Social networks predict gut microbiome composition in wild baboons. Elife 2015:1–18.
18. Moeller AH, Foerster S, Wilson ML, Pusey AE, Hahn BH, Ochman H. 2016. Social behavior shapes the chimpanzee pan-microbiome. Sci Adv 2.
19. Perofsky AC, Lewis RJ, Abondano LA, Di Fiore A, Meyers LA. 2017. Hierarchical social networks shape gut microbial composition in wild Verreaux's sifaka. Proc R Soc B Biol Sci 284:20172274.
20. Raulo A, Allen BE, Troitsky T, Husby A, Firth JA, Coulson T, Knowles SCL. 2021. Social networks strongly predict the gut microbiota of wild mice. ISME J https://doi.org/10.1038/s41396-021-00949-3.
21. Arrieta MC, Stiemsma LT, Amenyogbe N, Brown E, Finlay B. 2014. The intestinal microbiome in early life: Health and disease. Front Immunol 5:1–18.
22. Ren T, Grieneisen LE, Alberts SC, Archie EA, Wu M. 2016. Development, diet and dynamism: longitudinal and cross-sectional predictors of gut microbial communities in wild baboons. Environ Microbiol 18:1312–1325.
23. Jagsi R, Jiang J, Momoh AO, Alderman A, Giordano SH, Buchholz TA, Pierce LJ, Kronowitz SJ, Smith BD. 2017. Seasonal cycling in the gut microbiome of the Hadza Hunter-Gatherers of Tanzania. Science 357:802–806.
24. 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.
25. Murillo T, Schneider D, Fichtel C, Daniel R. 2022. Dietary shifts and social interactions drive temporal fluctuations of the gut microbiome from wild redfronted lemurs. ISME Commun 2:3.
26. Laforest-Lapointe I, Arrieta M-C. 2018. Microbial eukaryotes: a missing link in gut microbiome studies. mSystems 3.
27. Mann AE, Mazel F, Lemay MA, Morien E, Billy V, Kowalewski M, Di Fiore A, Link A, Goldberg TL, Tecot S, Baden AL, Gomez A, Sauther ML, Cuozzo FP, Rice GAO, Dominy NJ, Stumpf R, Lewis RJ, Swedell L, Amato K, Wegener Parfrey L. 2020. Biodiversity of protists and nematodes in the wild nonhuman primate gut. ISME J 14:609–622.

28. Vlčková K, Pafčo B, Petrželková KJ, Modrý D, Todd A, Yeoman CJ, Torralba M, Wilson BA, Stumpf RM, White BA, Nelson KE, Leigh SR, Gomez A. 2018. Relationships between gastrointestinal parasite infections and the fecal microbiome in free-ranging western lowland gorillas. Front Microbiol 9:1–12.

29. Renelies-Hamilton J, Noguera-Julian M, Parera M, Paredes R, Pacheco L, Dacal E, Saugar JM, Rubio JM, Poulsen M, Köster PC, Carmena D. 2019. Exploring interactions between Blastocystis sp., Strongyloides spp. and the gut microbiomes of wild chimpanzees in Senegal. Infect Genet Evol 74:104010.

30. Martínez-Mota R, Righini N, Mallott EK, Gillespie TR, Amato KR. 2021. The relationship between pinworm (Trypanoxyuris) infection and gut bacteria in wild black howler monkeys (Alouatta pigra). Am J Primatol 83.

31. Pereira ME, Kaufman R, Kappeler PM, Overdoff DJ. 1990. Female dominance does not characterize all of the lemuridae. Folia Primatol 55:96–103.

32. Ostner J, Kappeler PM. 1999. Central males instead of multiple pairs in redfronted lemurs, Eulemur fulvus rufus (Primates, Lemuridae)? Anim Behav 58:1069–1078.

33. Kappeler PM, Fichtel C. 2012. A 15-year perspective on the social organization and life history of sifaka in Kirindy Forest, p. 101–121. *In* Long-Term Field Studies of Primates. Springer Berlin Heidelberg, Berlin, Heidelberg.

34. Koch F, Ganzhorn JU, Rothman JM, Chapman CA, Fichtel C. 2017. Sex and seasonal differences in diet and nutrient intake in Verreaux's sifakas (Propithecus verreauxi). Am J Primatol 79:1–10.

35. Scholz F, Kappeler PM. 2004. Effects of seasonal water scarcity on the ranging behavior of Eulemur fulvus rufus. Int J Primatol 25:599–613.

36. Amoroso CR, Kappeler PM, Fichtel C, Nunn CL. 2020. Water availability impacts habitat use by red-fronted lemurs (Eulemur rufifrons): An experimental and observational study. Int J Primatol 41:61–80.

37. Clough D, Heistermann M, Kappeler PM. 2010. Host intrinsic determinants and potential consequences of parasite infection in free-ranging red-fronted lemurs (Eulemur fulvus rufus). Am J Phys Anthropol 142:441–452.

38. Ostner J, Kappeler P, Heistermann M. 2008. Androgen and glucocorticoid levels reflect seasonally occurring social challenges in male redfronted lemurs (Eulemur fulvus rufus). Behav Ecol Sociobiol 62:627–638.

39. Heistermann M, Palme R, Ganswindt A. 2006. Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis. Am J Primatol 68:257–273.

40. Kappeler PM, Fichtel C. 2012. Female reproductive competition in Eulemur rufifrons: Eviction and reproductive restraint in a plurally breeding Malagasy primate. Mol Ecol 21:685–698.

41. Ostner J, Kappeler PM, Heistermann M. 2002. Seasonal variation and social

correlates of androgen excretion in male redfronted lemurs (Eulemur fulvus rufus). Behav Ecol Sociobiol 52:485–495.

42. Clough D. 2010. Gastro-intestinal parasites of red-fronted lemurs in Kirindy Forest, western Madagascar. J Parasitol 96:245–251.

43. Gogarten JF, Calvignac-Spencer S, Nunn CL, Ulrich M, Saiepour N, Nielsen HV, Deschner T, Fichtel C, Kappeler PM, Knauf S, Müller-Klein N, Ostner J, Robbins MM, Sangmaneedet S, Schülke O, Surbeck M, Wittig RM, Sliwa A, Strube C, Leendertz FH, Roos C, Noll A. 2020. Metabarcoding of eukaryotic parasite communities describes diverse parasite assemblages spanning the primate phylogeny. Mol Ecol Resour 20:204–215.

44. Barton RA. 1987. Allogrooming as mutualism in diurnal lemurs. Primates 28:539–542.

45. Stoeck T, Bass D, Nebel M, Christen R, Jones MDM, Breiner H-W, Richards TA. 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol 19:21–31.

46. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 41:1–11.

47. Berkelmann D, Schneider D, Hennings N, Meryandini A, Daniel R. 2020. Soil bacterial community structures in relation to different oil palm management practices. Sci Data 7:1–7.

48. 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 41:590–596.

49. Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, Whitman WB, Euzéby J, Amann R, Rosselló-Móra R. 2014. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat Rev Microbiol 12:635–645.

50. Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, Boutte C, Burgaud G, De Vargas C, Decelle J, Del Campo J, Dolan JR, Dunthorn M, Edvardsen B, Holzmann M, Kooistra WHCF, Lara E, Le Bescot N, Logares R, Mahé F, Massana R, Montresor M, Morard R, Not F, Pawlowski J, Probert I, Sauvadet AL, Siano R, Stoeck T, Vaulot D, Zimmermann P, Christen R. 2013. The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41:597–604.

51. Gao X, Lin H, Revanna K, Dong Q. 2017. A Bayesian taxonomic classification method for 16S rRNA gene sequences with improved species-level accuracy. BMC Bioinformatics 18:1–10.

52. Reitmeier S, Hitch TCA, Treichel N, Fikas N, Hausmann B, Ramer-Tait AE, Neuhaus K, Berry D, Haller D, Lagkouvardos I, Clavel T. 2021. Handling of

spurious sequences affects the outcome of high-throughput 16S rRNA gene amplicon profiling. ISME Commun 1.

53. Shutt K, Setchell JM, Heistermann M. 2012. Non-invasive monitoring of physiological stress in the Western lowland gorilla (Gorilla gorilla gorilla): Validation of a fecal glucocorticoid assay and methods for practical application in the field. Gen Comp Endocrinol 179:167–177.

54. Hämäläinen A, Heistermann M, Fenosoa ZSE, Kraus C. 2014. Evaluating capture stress in wild gray mouse lemurs via repeated fecal sampling: Method validation and the influence of prior experience and handling protocols on stress responses. Gen Comp Endocrinol 195:68–79.

55. Rudolph K, Fichtel C, Heistermann M, Kappeler PM. 2020. Dynamics and determinants of glucocorticoid metabolite concentrations in wild Verreaux's sifakas. Horm Behav 124:104760.

56. Heitlinger E, Ferreira SCM, Thierer D, Hofer H, East ML. 2017. The intestinal eukaryotic and bacterial biome of spotted hyenas: the impact of social status and age on diversity and composition. Front Cell Infect Microbiol 7.

57. Barr DJ, Levy R, Scheepers C, Tily HJ. 2013. Random effects structure for confirmatory hypothesis testing: Keep it maximal. J Mem Lang 68:255–278.

58. De Cáceres M, Legendre P, Moretti M. 2010. Improving indicator species analysis by combining groups of sites. Oikos 119:1674–1684.

59. Silk J, Cheney D, Seyfarth R. 2013. A practical guide to the study of social relationships. Evol Anthropol 22:213–225.

60. Ostner J, Nunn CL, Schülke O. 2008. Female reproductive synchrony predicts skewed paternity across primates. Behav Ecol 19:1150–1158.

61. Bailey MT, Dowd SE, Parry NMA, Galley JD, Schauer DB, Lyte M. 2010. Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium. Infect Immun 78:1509–1519.

62. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. 2011. Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. Brain Behav Immun 25:397–407.

63. Stothart MR, Bobbie CB, Schulte-Hostedde AI, Boonstra R, Palme R, Mykytczuk NCS, Newman AEM. 2016. Stress and the microbiome: Linking glucocorticoids to bacterial community dynamics in wild red squirrels. Biol Lett 12.

64. Vlčková K, Shutt-Phillip K, Heisterman M, Pafčo B, Petrželkov KJ, Todd A, Modrý D, Nelson KE, Wilson BA, Stumpf RM, White BA, Leigh SR, Gomez A. 2018. Impact of stress on the gut microbiome of free-ranging western lowland gorillas. Microbiol (United Kingdom) 164:40–44.

65. Chu H, Mazmanian SK. 2013. Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nat Immunol 14:668–675.

66. Zheng D, Liwinski T, Elinav E. 2020. Interaction between microbiota and

immunity in health and disease. Cell Res 30:492–506.

67. Noguera JC, Aira M, Pérez-Losada M, Domínguez J, Velando A. 2018. Glucocorticoids modulate gastrointestinal microbiome in a wild bird. R Soc Open Sci 5.

68. Round JL, Mazmanian SK. 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323.

69. Maltz RM, Keirsey J, Kim SC, Mackos AR, Gharaibeh RZ, Moore CC, Xu J, Bakthavatchalu V, Somogyi A, Bailey MT. 2018. Prolonged restraint stressor exposure in outbred CD-1 mice impacts microbiota, colonic inflammation, and short chain fatty acids. PLoS One 13:1–19.

70. Ostner J, Heistermann M. 2003. Endocrine characterization of female reproductive status in wild redfronted lemurs (Eulemur fulvus rufus). Gen Comp Endocrinol 131:274–283.

71. Peckre LR, Defolie C, Kappeler PM, Fichtel C. 2018. Potential self-medication using millipede secretions in red-fronted lemurs: combining anointment and ingestion for a joint action against gastrointestinal parasites? Primates 59:483–494.

72. Jenkins TP, Rathnayaka Y, Perera PK, Peachey LE, Nolan MJ, Krause L, Rajakaruna RS, Cantacessi C. 2017. Infections by human gastrointestinal helminths are associated with changes in faecal microbiota diversity and composition. PLoS One 12:1–18.

73. Rosa BA, Supali T, Gankpala L, Djuardi Y, Sartono E, Zhou Y, Fischer K, Martin J, Tyagi R, Bolay FK, Fischer PU, Yazdanbakhsh M, Mitreva M. 2018. Differential human gut microbiome assemblages during soil-transmitted helminth infections in Indonesia and Liberia. Microbiome 6:1–19.

74. Toro-Londono MA, Bedoya-Urrego K, Garcia-Montoya GM, Galvan-Diaz AL, Alzate JF. 2019. Intestinal parasitic infection alters bacterial gut microbiota in children. PeerJ 2019:1–24.

75. Kaakoush NO. 2015. Insights into the role of Erysipelotrichaceae in the human host. Front Cell Infect Microbiol 5:1–4.

76. Ricaboni D, Mailhe M, Cadoret F, Vitton V, Fournier PE, Raoult D. 2017. ‘Colidextribacter massiliensis’ gen. nov., sp. nov., isolated from human right colon. New Microbes New Infect 17:27–29.

77. Qin P, Zou Y, Dai Y, Luo G, Zhang X, Xiao L. 2019. Characterization a novel butyric acid-producing bacterium Collinsella aerofaciens subsp. shenzhenensis subsp. nov. Microorganisms 7.

78. Reynolds LA, Finlay BB, Maizels RM. 2015. Cohabitation in the intestine: interactions among helminth parasites, bacterial microbiota, and host immunity. J Immunol 195:4059–4066.

79. Wei Y, Gao J, Kou Y, Meng L, Zheng X, Liang M, Sun H, Liu Z, Wanga Y. 2020. Commensal bacteria impact a protozoan’s integration into the murine gut microbiota in a dietary nutrient-dependent manner. Appl Environ Microbiol 86.

80. Perofsky AC, Ancel Meyers L, Abondano LA, Di Fiore A, Lewis RJ. 2021. Social groups constrain the spatiotemporal dynamics of wild sifaka gut microbiomes. Mol Ecol 30:6759–6775.

81. Pyritz L, Kappeler PM, Fichtel C. 2011. Coordination of group movements in wild red-fronted lemurs (Eulemur rufifrons): Processes and influence of ecological and reproductive seasonality. Int J Primatol 32:1325–1347.

82. Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, Rex Gaskins H, Stumpf RM, Yildirim S, Torralba M, Gillis M, Wilson BA, Nelson KE, White BA, Leigh SR. 2013. Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes. ISME J 7:1344–1353.

83. Hippe H, Hagelstein A, Kramer I, Swiderski J, Stackebrandt E. 1999. Phylogenetic analysis of Formivibrio citricus, Propionivibrio dicarboxylicus, Anaerobiospirillum thomasii, Succinirnonas amylolytica and Succinivibrio dextrinosolvens and proposal of Succinivibrionaceae fam. nov. Int J Syst Evol Microbiol 49:779–782.

84. Grieneisen LE, Livermore J, Alberts S, Tung J, Archie EA. 2017. Group living and male dispersal predict the core gut microbiome in wild baboons. Integr Comp Biol 57:770–785.

85. Amoroso CR, Kappeler PM, Fichtel C, Nunn CL. 2019. Fecal contamination, parasite risk, and waterhole use by wild animals in a dry deciduous forest. Behav Ecol Sociobiol 73.

86. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J. 2016. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. Gut 65:57–62.

87. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, Kurilshikov A, Bonder MJ, Valles-Colomer M, Vandeputte D, Tito RY, Chaffron S, Rymenans L, Verspecht C, Sutter L De, Lima-Mendez G, D'hoe K, Jonckheere K, Homola D, Garcia R, Tigchelaar EF, Eeckhaudt L, Fu J, Henckaerts L, Zhernakova A, Wijmenga C, Raes J. 2016. Population-level analysis of gut microbiome variation. Science 352:560–564.

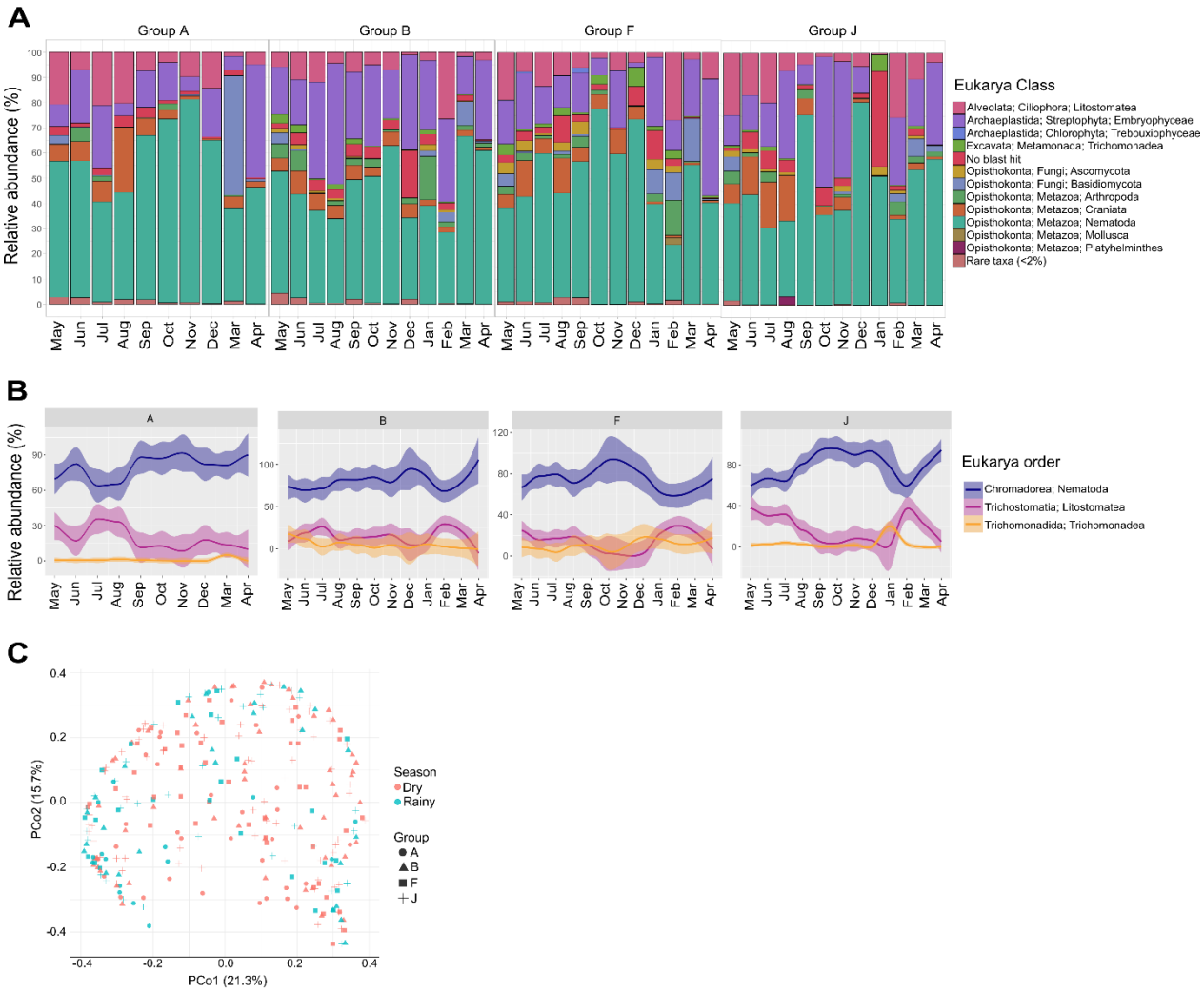
88. Sonnenburg JL, Bäckhed F. 2016. Diet-microbiota interactions as moderators of human metabolism. Nature 535:56–64.

89. Zmora N, Suez J, Elinav E. 2018. You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol 16.

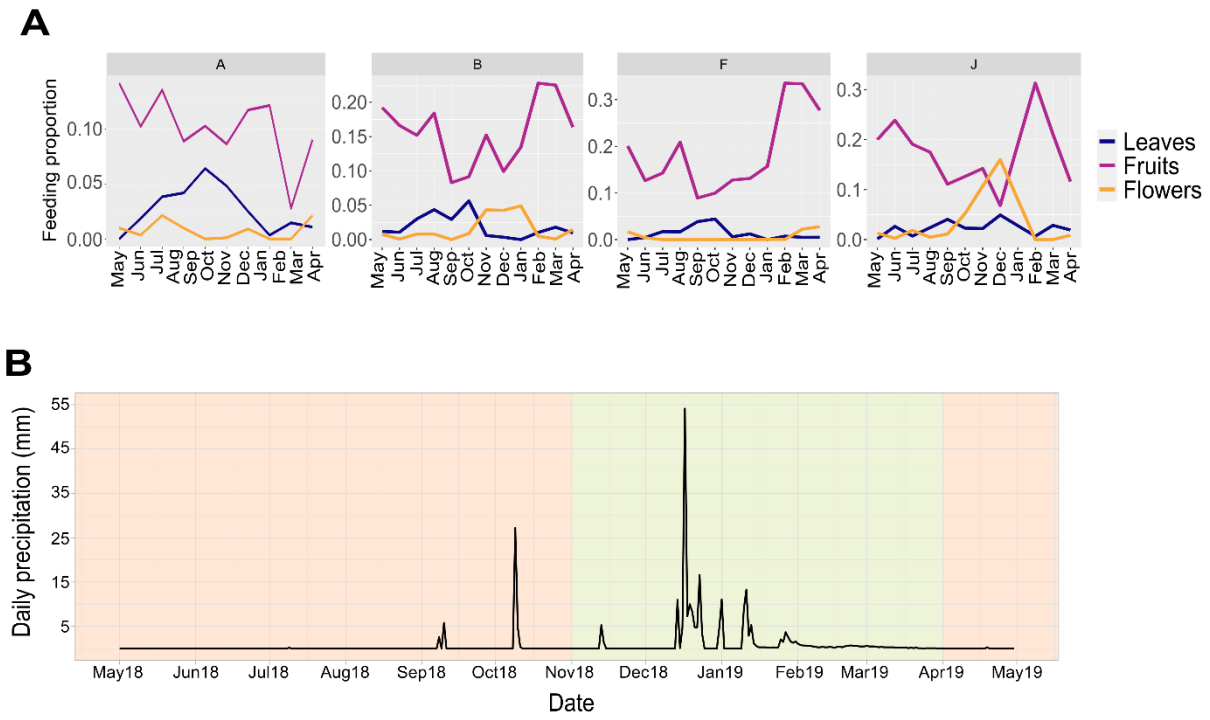
90. Ortmann S, Bradley BJ, Stolter C, Ganzhorn JU. 2006. Estimating the quality and composition of wild animal diets – a critical survey of methods, p. 395–418. *In* Hohmann, G, Robbins, M, Boesch, C (eds.), Feeding ecology in apes and other primates. ecological, physical, and behavioral aspects. Cambridge University Press, Cambridge.

SUPPLEMENTAL MATERIAL

FIGURES



Supplemental Figure S1. Eukaryotic organisms detected in redfronted lemurs fecal samples by using 18S rRNA gene sequencing. **A.** Monthly relative abundances of the eukaryotic organisms detected per lemur group. **B.** Monthly fluctuations in the relative abundances of the previously reported parasites of redfronted lemurs: *Chromadorea*, *Trichostomatia* and *Trichomonadida*. **C.** PCoA based on Jaccard distance matrix of eukaryotic endoparasites detected in redfronted lemurs with data points color coded to season. **D.** PCoA based on Jaccard distance matrix of eukaryotic endoparasites detected in redfronted lemurs with data points color coded to group.



Supplemental Figure S2. Food items consumed by redfronted lemurs and daily precipitation in Kirindy Forest recorded during the study period from May 2018 until April 2019. **A.** Monthly feeding proportions on fruits, leaves and flowers 30 days prior to sampling for each group. **B.** Daily precipitation measured in mm during the study period. Color coded panels indicate the dry (brown) and rainy (green) season.

TABLES

Supplementary Table 1. Fecal sample list for 16S rRNA analysis with metadata. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 2. Fecal sample list for 18S rRNA analysis. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 3. Primers and PCR protocols for the studied taxonomical marker genes.

Taxonomical marker gene	**Bacteria 16S rRNA**	**Eukaryota 18S rRNA**
Name - Primer Forward	S-D-Bact-0341-b-S-17	Reuk454FWD1
Sequence - Primer Forward	5´-CCTACGGGNGGCWGCAG-3´	5´-CCAGCASCYGCGGTAATTCC-3´
Miseq Adapter - Forward	5´-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3´	5´-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3´
Name - Primer Reverse	S-D-Bact-0785-a-A-21	TAReukREV3
Sequence - Primer reverse	5´-GACTACHVGGGTATCTAATCC-3´	5´-ACTTTCGTTCTTGATYRA-3´
Miseq Adapter - Reverse	5´-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3´	5´-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3´
PCR protocol	Final volume of 50 µl containing 10 µl of 5x GC Buffer (Thermo Scientific, Waltham, MA, USA), 5% DMSO, 0.2 mM of forward and reverse primer, 200 µM dNTPs, 0.2 mM MgCl2, 1 U Phusion High-Fidelity DNA polymerase (Thermo Scientific, Waltham, MA, USA) and 20–25 ng template DNA	Final volume of 50 µl containing 10 µl of 5x GC Buffer (Thermo Scientific, Waltham, MA, USA), 5% DMSO, 0.2 mM of forward and reverse primer, 200 µM dNTPs, 0.2 mM MgCl2, 1 U Phusion High-Fidelity DNA polymerase (Thermo Scientific, Waltham, MA, USA) and 50 ng template DNA
Thermocycling program	Denaturation 1 min at 98 °C, 25 cycles at 98 °C for 45 s, 45 s at 55 °C, and 30 s at 72 °C, and final extension at 72 °C for 5 min.	Denaturation 1 min at 98 °C, 25 cycles at 98 °C for 45 s, 45 s at 47 °C, and 30 s at 72 °C, and final extension at 72 °C for 5 min.
References	Klindworth et al., 2013	Stoeck et al., 2010
Positive control	*Escherichia coli*	*Aspergillus nidulans*

Supplementary Table 4. Sequencing statistics for 16S rRNA and 18S rRNA.

Taxonomical marker gene	Bacteria 16S rRNA	Eukaryota 18S rRNA
Number of samples	799	380
Reads after quality filtering	35 801 327	21 949 694
Number of ASVs	7 213	6 245
Mean amplicon length (bp)	416.44	380.81
Reads after 0.25% filtering	32 343 875	21 430 144
Number of ASVs after 0.25% filtering	1 028	783
Unclassified reads after 0.25% filtering	0.03% ± 0.4	3.22% ± 2.10
Reads for rarefaction	8 236	6 222

Supplementary Table 5. ASVs obtained for the 16S rRNA amplicon sequencing. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 6. ASVs obtained for the 18S rRNA amplicon sequencing. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 7. Results PERMANOVA test of Jaccard distance matrix for parasites.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	BH
group	3	3.905	1.302	4.025	0.038	0.001	0.002
sex	1	0.289	0.289	0.893	0.003	0.621	0.621
age_months	1	0.663	0.663	2.051	0.007	0.004	0.005
season	1	2.757	2.757	8.525	0.027	0.001	0.002
Residuals	291	94.104	0.323	NA	0.925	NA	NA
Total	297	101.718	NA	NA	1	NA	NA

Supplementary Table 8. Estimates LMM alpha diversity of the full dataset.

Model comparison	AIC	logLik	Chisq	Df	p value
Null	5188.386	2552.193	NA	NA	NA
Full	5183.373	-2542.69	19.0134	7	0.008145

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	26.017	1.760	32.650	14.783	0.000
sexmale	1.184	0.926	98.538	1.279	0.204
groupB	-0.650	1.861	32.334	-0.349	0.729
groupF	-5.358	1.960	46.604	-2.735	0.009
groupJ	-2.122	1.809	32.484	-1.173	0.249
z.log.age_months	-0.055	0.466	160.782	-0.117	0.907
z.soc.int	0.869	0.487	11.429	1.786	0.101
ffr.prop	-5.335	4.528	29.254	-1.178	0.248
fle.prop	67.495	12.385	65.573	5.450	0.000
rain	-1.760	0.982	6.645	-1.793	0.118
ffl.prop	-4.492	16.226	11.323	-0.277	0.787
z.richness.para	-1.017	0.647	23.165	-1.573	0.129

Supplementary Table 9. Estimates LMM alpha diversity of the dataset including fGCM values.

Model comparison	AIC	logLik	Chisq	Df	p value
Null	4201.814	-2049.907	NA	NA	NA
Full	4201.562	-2041.781	16.252	8	0.039

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	26.786	1.506	31.021	17.782	0.000
sexmale	0.801	0.997	197.154	0.804	0.423
groupB	0.074	1.728	31.751	0.043	0.966
groupF	-5.210	1.907	53.987	-2.733	0.008
groupJ	-1.777	1.711	34.705	-1.038	0.306
z.log.age_months	-0.001	0.510	204.977	-0.002	0.999
z.soc.int	0.613	0.618	8.713	0.992	0.348
z.rain	0.751	0.720	19.765	1.043	0.310
z.fle.prop	2.205	0.556	28.125	3.969	0.000
z.ffr.prop	-0.440	0.580	27.561	-0.757	0.455
z.ffl.prop	-0.700	0.560	6.979	-1.250	0.252
z.log.fgc	1.216	0.532	45.259	2.285	0.027
z.richness.para	-0.871	0.653	28.834	-1.334	0.193

Supplementary Table 10. Results PERMANOVA test of Wunifrac matrix for dataset with feeding and social interaction data.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	BH
group	3	0.305	0.102	9.641	0.035	0.000	0.000
soc_int	1	0.037	0.037	3.553	0.004	0.012	0.014
sex	1	0.047	0.047	4.442	0.005	0.000	0.000
age_months	1	0.017	0.017	1.576	0.002	0.074	0.074
rain	1	0.186	0.186	17.695	0.021	0.000	0.000
fle_prop	1	0.041	0.041	3.866	0.005	0.002	0.003
ffl_prop	1	0.073	0.073	6.934	0.008	0.000	0.000
ffr_prop	1	0.076	0.076	7.192	0.009	0.000	0.000
Residuals	762	8.025	0.011	NA	0.911	NA	NA
Total	772	8.806	NA	NA	1	NA	NA

Supplementary Table 11. Results PERMANOVA test of Wunifrac matrix for dataset including parasite richness.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	BH
group	3	0.295	0.098	10.161	0.041	0.000	0.000
soc_int	1	0.040	0.040	4.152	0.006	0.004	0.004
sex	1	0.036	0.036	3.754	0.005	0.000	0.000
age_months	1	0.014	0.014	1.438	0.002	0.004	0.004
richness_para	1	0.046	0.046	4.715	0.006	0.000	0.000
rain	1	0.177	0.177	18.306	0.024	0.000	0.000
fle_prop	1	0.037	0.037	3.790	0.005	0.002	0.002
ffl_prop	1	0.040	0.040	4.123	0.006	0.000	0.000
ffr_prop	1	0.066	0.066	6.824	0.009	0.000	0.000
Residuals	670	6.483	0.010	NA	0.896	NA	NA
Total	681	7.234	NA	NA	1	NA	NA

Supplementary Table 12. Results PERMANOVA test of Wunifrac matrix for dataset including fGCM values.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	BH
group	3	0.310	0.103	10.853	0.052	0.000	0.000
soc_int	1	0.031	0.031	3.238	0.005	0.021	0.021
sex	1	0.040	0.040	4.199	0.007	0.000	0.000
age_months	1	0.018	0.018	1.876	0.003	0.001	0.001
n11oxo_CM_wet_feces	1	0.167	0.167	17.513	0.028	0.000	0.000
rain	1	0.134	0.134	14.044	0.022	0.000	0.000
richness_para	1	0.033	0.033	3.449	0.006	0.000	0.000
fle_prop	1	0.024	0.024	2.494	0.004	0.012	0.013
ffl_prop	1	0.036	0.036	3.762	0.006	0.001	0.001
ffr_prop	1	0.072	0.072	7.558	0.012	0.000	0.000
Residuals	534	5.078	0.010	NA	0.855	NA	NA
Total	546	5.941	NA	NA	1	NA	NA

Supplementary Table 13. Results from MaAsLin2 analysis detecting associations between bacterial genera and group membership, social interactions, parasite richness, sex, age, diet, and precipitation. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 14. Results from MaAsLin2 analysis detecting associations between bacterial genera and group membership, social interactions, parasite richness, sex, age, fGCM levels, diet, and precipitation. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 15. Results indicative species analysis for the individuals of group A. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 16. DSI for the individuals of group A.

	Amorgos	**Isabella**	**Kea**	**Lefkada**	**Luzon**	**Paros**	**Thassos**	**Tilos**
Amorgos	NA	0.2010	0.0000	0.0176	0.4132	0.0000	0.0470	0.0000
Isabella	0.2010	NA	1.0000	0.4242	0.9361	0.4426	0.5532	0.0979
Kea	0.0000	1.0000	NA	0.1232	0.1194	0.0000	0.1110	0.3244
Lefkada	0.0176	0.4242	0.1232	NA	0.4757	0.2087	0.6823	0.0079
Luzon	0.4132	0.9361	0.1194	0.4757	NA	0.1571	0.3201	0.0014
Paros	0.0000	0.4426	0.0000	0.2087	0.1571	NA	0.0775	0.0000
Thassos	0.0470	0.5532	0.1110	0.6823	0.3201	0.0775	NA	0.1547
Tilos	0.0000	0.0979	0.3244	0.0079	0.0014	0.0000	0.1547	NA

Supplementary Table 17. Results indicative species analysis for the individuals of group B. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 18. DSI for the individuals of group B.

	Adonara	Aloha	Bangladesh	Bora	Buru	Jaco	Latalata	Oman	Rinca	Tilos
Adonara	NA	0.4048	0.1202	0.1436	0.0564	0.0402	0.2427	0.2386	1.0000	0.0353
Aloha	0.4048	NA	0.1470	0.1322	0.0054	0.3834	0.4534	0.3077	0.0489	0.0579
Bangladesh	0.1202	0.1470	NA	0.2953	0.0169	0.1352	0.4607	0.5225	0.2010	0.0417
Bora	0.1436	0.1322	0.2953	NA	0.0626	0.2230	0.6261	0.5161	0.2155	0.1856
Buru	0.0564	0.0054	0.0169	0.0626	NA	0.0331	0.2438	0.1426	0.1304	0.2499
Jaco	0.0402	0.3834	0.1352	0.2230	0.0331	NA	0.5213	0.2382	0.1793	0.2037
Latalata	0.2427	0.4534	0.4607	0.6261	0.2438	0.5213	NA	0.9415	0.4400	0.1001
Oman	0.2386	0.3077	0.5225	0.5161	0.1426	0.2382	0.9415	NA	0.3416	0.3332
Rinca	1.0000	0.0489	0.2010	0.2155	0.1304	0.1793	0.4400	0.3416	NA	0.0810
Tilos	0.0353	0.0579	0.0417	0.1856	0.2499	0.2037	0.1001	0.3332	0.0810	NA

Supplementary Table 19. Results indicative species analysis for the individuals of group F. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 20. DSI for the individuals of group F.

	Bonacca	**Caicos**	**Gozo**	**Lucia**	**Mayaguana**	**Pinos**	**Tortuga**
Bonacca	NA	0.1999	0.1165	0.8183	0.3858	0.4174	1.0000
Caicos	0.1999	NA	0.1373	0.6796	0.2393	0.8149	0.2270
Gozo	0.1165	0.1373	NA	0.1745	0.1257	0.1583	0.2246
Lucia	0.8183	0.6796	0.1745	NA	0.3382	0.3439	0.6008
Mayaguana	0.3858	0.2393	0.1257	0.3382	NA	0.2460	0.3943
Pinos	0.4174	0.8149	0.1583	0.3439	0.2460	NA	0.4919
Tortuga	1.0000	0.2270	0.2246	0.6008	0.3943	0.4919	NA

Supplementary Table 21. Results indicative species analysis for the individuals of group J. The table is deposited on the enclosed CD under \Chapter3_Supplementary_material\.

Supplementary Table 22. DSI for the individuals of group J.

	Afganistan	Armenia	Bahrain	Cambodia	Colanta	Kasachstan	Kuwait	Mongolei	Pakistan	Syria	Taji
Afganistan	NA	0.0925	0.0735	0.1543	0.0868	0.1617	0.2804	0.0809	0.1722	0.7911	0.2681
Armenia	0.0925	NA	0.2752	0.0696	1.0000	0.1305	0.0917	0.0832	0.2946	0.3067	0.0975
Bahrain	0.0735	0.2752	NA	0.4200	0.2820	0.5363	0.7211	0.5393	0.3814	0.1897	0.2137
Cambodia	0.1543	0.0696	0.4200	NA	0.3508	0.3440	0.3376	0.2530	0.1743	0.0977	0.0520
Colanta	0.0868	1.0000	0.2820	0.3508	NA	0.1211	0.6606	0.4099	0.4449	0.3987	0.3518
Kasachstan	0.1617	0.1305	0.5363	0.3440	0.1211	NA	0.2490	0.3939	0.4639	0.0077	0.2036
Kuwait	0.2804	0.0917	0.7211	0.3376	0.6606	0.2490	NA	0.2811	0.6428	0.1841	0.2314
Mongolei	0.0809	0.0832	0.5393	0.2530	0.4099	0.3939	0.2811	NA	0.2257	0.1461	0.1788
Pakistan	0.1722	0.2946	0.3814	0.1743	0.4449	0.4639	0.6428	0.2257	NA	0.1220	0.0870
Syria	0.7911	0.3067	0.1897	0.0977	0.3987	0.0077	0.1841	0.1461	0.1220	NA	0.2662
Taji	0.2681	0.0975	0.2137	0.0520	0.3518	0.2036	0.2314	0.1788	0.0870	0.2662	NA

4 Parasites in a social world - Lessons from primates

Baptiste Sadoughi[a,b,c], Simone Anzà[a,b], Charlotte Defolie[d,e], Virgile Manin[f,g], Nadine Müller-Klein[h], **Tatiana Murillo**[e,i], Markus Ulrich[j] & Doris Wu[j]

[a] Department of Behavioral Ecology, Johann-Friedrich-Blumenbach Institute for Zoology & Anthropology, University of Göttingen, Göttingen, Germany

[b] Research Group Primate Social Evolution, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

[c] Leibniz ScienceCampus Primate Cognition, German Primate Center, Göttingen, Germany

[d] Department for Sociobiology/Anthropology, Johann-Friedrich-Blumenbach Institute for Zoology & Anthropology, University of Göttingen, Göttingen, Germany

[e] Behavioral Ecology and Sociobiology Unit, German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany

[f] Department of Human Behavior, Ecology and Culture, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

[g] Tai Chimpanzee Project, Centre suisse de recherche Scientifique, Abidjan, Cote d'Ivoire

[h] Ulm University, Institute for Evolutionary Ecology and Conservation Genomics, Conservation Genomics and EcoHealth, Ulm, Germany

[i] Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, University of Göttingen, Göttingen, Germany

[j] Epidemiology of Highly Pathogenic Organisms, Robert Koch Institute, Berlin, Germany

Author contributions:

All authors conceived the study, reviewed literature, wrote, and revised the manuscript.

BS coordinated and contacted the editors.

Citation: Chapter 3. Sadoughi B, Anzà S, Defolie C, Manin V, Müller-Klein N, Murillo T, Ulrich M, Wu D. Parasites in a social world: lessons from primates. In: Ezenwa VO, Altizer S, Hall RJ, editors. Animal Behavior and Parasitism. Oxford University Press, 2022 (in press). DOI: 10.1093/oso/9780192895561.003.0003.

Abstract

Social behavior and parasitism interconnect at all levels of sociality—from the community to the population and from the group down to the individual. This chapter explores key findings on the parasite-related costs and benefits of sociality, focusing on primates. The research spans across social networks, dominance and affiliative relationships, and individual behavior and physiology—highlighting established links between primate sociality and parasitism and identifying important gaps for future research. Given the use of nuanced conceptual frameworks and new analytical methods, combined with experimental studies and growing empirical data from long-term field projects, primates are a particularly exciting and helpful taxon for studying sociality-parasite interactions.

Keywords: social behavior, disease ecology, social structure, anti-parasite behavior, social network, glucocorticoids, exposure, susceptibility, transmission, health

4.1 Introduction

Across the animal kingdom, species display marked variation in **sociality** (see Box 1 for glossary). Some **solitary** animals rarely interact with conspecifics (except during reproduction), while other animals are social—from facultative, short-term associations and loose aggregations based on shared needs, to living in permanent groups with differentiated social relationships[1,2]. The transition from solitary to social living[3] has resulted in far-reaching consequences—both costly and beneficial—for hosts and their parasites.

The repeated emergence of sociality suggests that the benefits of group living (e.g., increased vigilance and protection against out-group threats and predators) outweigh the costs (e.g., intragroup competition, infanticide). However, the risk of exposure to parasites or pathogens (these terms are used interchangeably to mean all disease-causing organisms) is considered a looming threat for group members, limiting close or frequent contact among individuals[4,5]. While a positive correlation between group size and infection risk was initially postulated as a major cost of group living several

decades ago[6,7], a growing body of research suggests that links between sociality and infectious diseases are more complex, with mixed outcomes for the transmission of and susceptibility to pathogens[8,9]. Consequently, studying the parasite-related costs and benefits of sociality is an increasingly active focal area in behavioral and evolutionary ecology.

Box 1. Glossary

Despotic: opposite of tolerant. Despotic dominance translates into higher aggression and lower rates of affiliation compared to more tolerant relationships, with low (sometimes absent) rates of peaceful post-conflict interactions. Dominance is clearly established.

Grooming: behavior by which animals clean or maintain their bodies or appearance. Allogrooming is the cleaning of a conspecific partner's skin or fur. In primates, allogrooming serves both hygienic (ectoparasite removal) and social (strengthens bonds between partners) functions.

Modular social network: a network divided into subgroups interacting more within than between subgroups. Network modularity increases as more highly cohesive subgroups are formed.

Multilevel societies: subgroups of animals from the same species formed at three or four levels. The first level of organization is the one-male-unit (semi-permanent reproductive units consisting of one leader male, sometimes a follower male, multiple females, and their offspring). One-male-units can associate in teams (second level), with sometimes solitary males or all-male-units (units composed exclusively of males). Teams associate in bands (third level) which associate in herds (fourth level). Also see Box 2.

Multi-male-multi-female groups: groups composed of multiple adult males and adult females, and their offspring.

One-male-multi-female groups: groups composed of one adult male, multiple adult females, and their offspring.

One-female-multi-male groups: groups composed of one adult female, multiple adult males, and their offspring.

Pair-living: groups composed of one adult male and female and their offspring.

Self-medication: utilization of plant or animal parts containing secondary compounds or other non-nutritional substances to prevent, combat, or control disease.

Social buffering: refers to availability of social support, assumed to mediate the negative relationship between perceived stress and health. Candidate physiological systems underlying social buffering effects include neural (e.g., prefrontal cortex, limbic system), endocrine (e.g., hypothalamic-pituitary-adrenal axis, oxytocin), and immune functions.

Social immunization: transfer of low doses of an infectious agent or pathogen during social interactions which activates the immune system, decreases susceptibility, and limits reinfection risk. Behavioral or chemical cues of sickness emitted by infected conspecifics may also elicit preventive activation of the immune system.

Social network: a social structure described by nodes (i.e., individuals) and the ties (i.e., connections) between these nodes. In primate studies, connections are often based on interactions (e.g., proximity, mating, grooming, or aggressive interactions). In an epidemiological network, parasites can spread along ties between nodes, representing individuals, groups, or even communities.

Sociality: tendency of groups and individuals to develop social bonds and live in communities.

Solitary vs group living: solitary animals spend a majority of their lives alone, with possible exceptions for mating and raising young. Conversely, group living is defined as individuals of the same species (conspecifics) maintaining spatial proximity to one another over time via social mechanisms.

Spillover: an event characterized by a pathogen spreading from a reservoir population with high pathogen prevalence to a novel recipient host population.

Tolerant: opposite of despotic. Tolerant dominance translates into more symmetrical relationships among dyads, less severe aggressive interactions, and higher rates of affiliation and post-conflict interactions.

Sociality impacts parasite transmission at multiple levels of interaction—from inter-specific communities, to the group level, and down to the individual—via two distinct mechanisms: exposure and susceptibility[10]. While exposure, especially to directly or environmentally transmitted pathogens, depends on direct contact, shared space, or resource use[4,10,11], susceptibility depends on individual genetics, physiology, and immuno-competence. Although environmental factors (e.g., rainfall, humidity) and individual immune and genetic profiles affect parasite transmission and susceptibility, this chapter focuses primarily on the sociality-parasite link. Non-human primates (hereafter primates) provide excellent study systems to investigate links between sociality and parasites given their exceptional diversity and remarkable within species and inter-individual variation in social systems and social behaviors, respectively (Box 2). The positive link between sociality and fitness in wild primates[12] also provides real-world study systems to explore these mechanisms. Additionally, wild primates are well-studied, with detailed data on individual behaviors and life histories across a range of long-term study populations worldwide (Figure 1). Primates also harbor diverse parasite communities (i.e., micro and macro-parasites: bacteria, viruses, protozoa, fungi, helminths, and arthropods) with various transmission routes[13]. Finally, given a shared evolutionary history with humans, primate species are a key model for establishing bridges between field research and biomedical research on physiology and disease, evolutionary medicine, and public health policies.

This chapter highlights significant contributions from primate research in advancing our understanding of the links between sociality and parasitism. First, we address mechanisms at the group level, which primarily influence exposure to parasites. Next, we move to a finer scale of sociality by discussing how positive or negative interactions

alter susceptibility. We also explore how individual behaviors may further mitigate parasite risk. Finally, we identify promising research tools and questions that can help advance our ability to manage the parasite-related costs and benefits of sociality in primates.

Figure 1. Several representatives of the Order Primate. From left to right, top to bottom. Red-fronted lemur (*Eulemur rufifrons*), credit Tatiana Murillo; sifakas (*Propithecus verreauxi*), credit Hasina Malalaharivony; chimpanzees (*Pan troglodytes*), credit Liran Samuni, Taï chimpanzee project; grey mouse lemurs (*Microcebus murinus*), credit Johanna Henke-von der Malsburg; coppery titi-monkeys (*Plecturocebus cupreus*), credit Sofya Dolotovskaya; Assamese macaques (*Macaca assamensis*), credit Simone Anzà; baboons (*Papio anubis*), credit Doris Wu.

Box 2. Diversity of primate social systems.

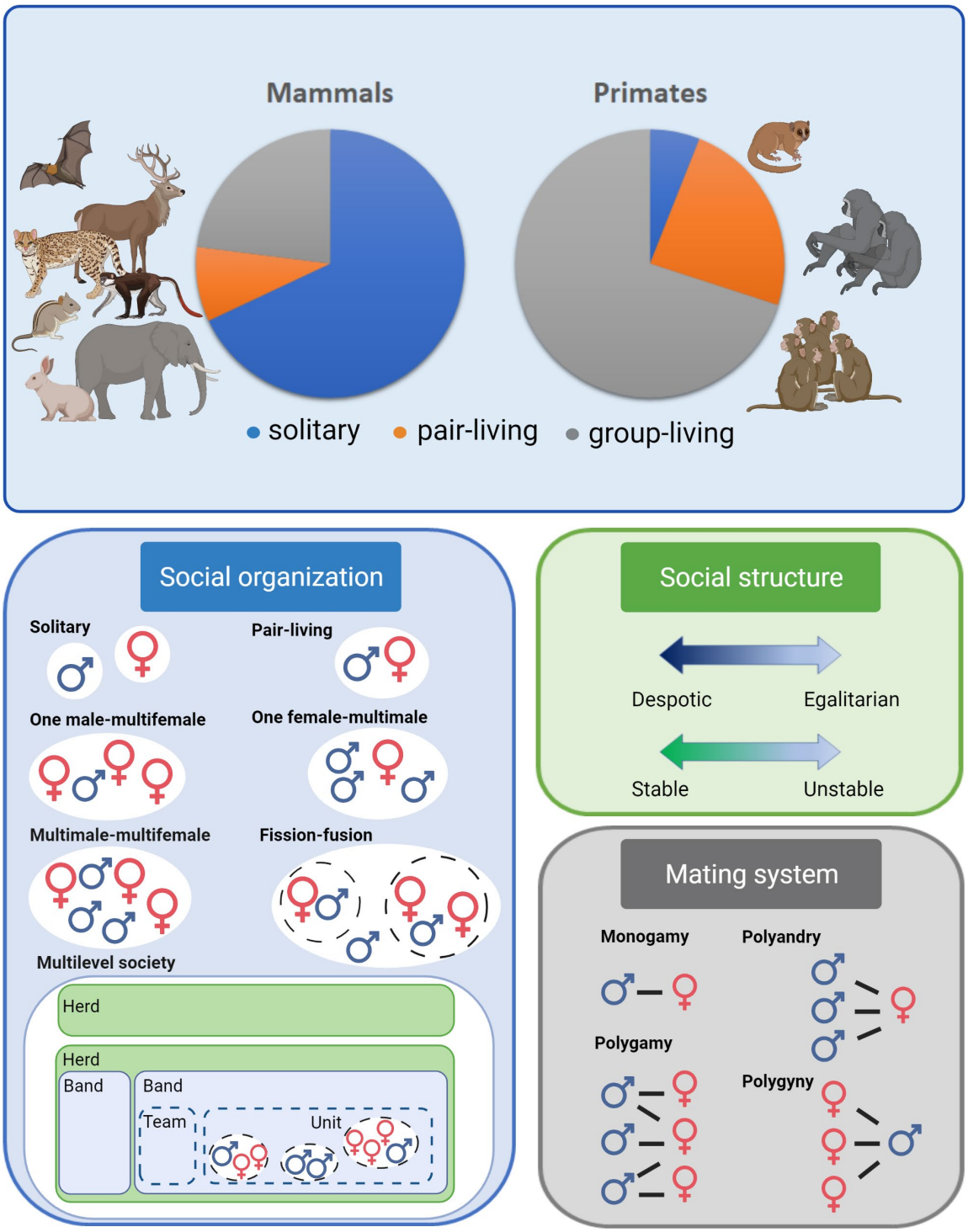


Figure 1. The components and diversity of social systems in primates. Figure created using BioRender.

In mammals, only 23% of all species live in groups, with 9% being **pair-living**[14]. Primates deviate from this general pattern, with ~66% of genera living in permanent mixed-sex groups[1], and 29% of species being pair-living[14]. Primate social systems are characterized by three complementary, but distinct components found in multiple combinations: social organization, social structure, and mating system[1] (Figure 1).

Social organization refers to the number and composition, as well as cohesion, of a social unit. Five types of social units have been described in primates, with varied sex-age composition and levels of relatedness. There are **solitary** species (e.g., Bornean orangutans *Pongo pygmaeus*), pair-living species (e.g., coppery titi monkeys *Plecturocebus cupreus*); **one-male-multi-female groups** (e.g., patas monkeys *Erythrocebus patas*); **one-female-multi-male groups** (e.g., facultative in many *Callitrichidae*); and **multi-male-multi-female groups** (e.g., Assamese macaques *Macaca assamensis*, red-fronted lemurs *Eulemur rufifrons*). These units can remain cohesive (most social primates), split into subgroups with changing size and composition over time (fission-fusion societies, e.g., chimpanzees *Pan troglodytes*), or be organized around nested levels forming **multilevel societies** (e.g., geladas *Theropithecus gelada*).

Social structure refers to the distribution, quality, and dynamics of relationships between group members. Social relationships may form preferentially between certain partners, with dominance interactions that vary from **despotic** (e.g., chimpanzees, rhesus macaques *M. mulatta*) to **tolerant** (e.g., red-fronted lemurs, tonkean macaques *M. tonkeana*); that are stable or unstable; and inherited or contested. Primates exhibit marked flexibility in social structure, showing variation both within and between species.

Mating system refers to sexual interactions. In primates, these interactions range from monogamous (e.g., coppery titi monkey) to polygynous (e.g., geladas), polyandrous (e.g., many *Callitrichidae*), and polygamous (e.g., chimpanzees).

4.2 Group level effects of sociality on parasitism

Socio-ecological models of primate sociality provide an integrated picture of the influence of food distribution, predation pressure, female-female tolerance, and male competition for monopolization and access to fertile females in shaping group living[1]. These spatiotemporal associations and interactions between individuals, in turn, impact parasite transmission[15]. At the group level, parasite-related costs and benefits of sociality are mediated by two important demographic features: group size and **social network** organization[15–18].

4.2.1 Group size and parasitism

The first readily and easily accessible demographic parameter of any study population is group size. In mammals, strong evidence indicates that the intensity of infection with directly and environmentally transmitted parasites (e.g., fleas, helminths) increases with host group size[16,19,20]. This is attributed to more frequent direct (body-to-body) and indirect (via environmental contamination with feces) contacts in larger groups promoting the transmission of infectious organisms. In primates, both empirical data and computer simulations lend support for a positive association between parasitism—measured either as parasite load or prevalence—and host group size[4,11]. For example, a long-term study of yellow baboons (*Papio cynocephalus*) found that larger groups had higher counts of helminth eggs[21]. A comparative study of two sympatric mouse lemur species *(Microcebus* spp.), a taxon displaying high variation in social organization with social units ranging from solitary to **multi-male-multi-female** sleeping associations, found that species with larger nest associations had increased lice prevalence[22]. With lice utilizing host-body contact for transmission, temporary associations at nests created parasite-related costs for larger sleeping groups.

Nevertheless, increasing group size has not been invariably linked to increased parasitism, especially in primate studies[15,23]. First, group size can actually reduce parasitism through an encounter-dilution effect[24] for mobile parasites exhibiting a constant attack rate or targeting one host at a time. As the same number of parasites is distributed among more available hosts, greater host densities drive a lower per-

individual infection risk[24]. For example, in chimpanzees (*Pan troglodytes*), sleeping in groups may minimize individual exposure to biting insects[25]. Second, group size interacts with other ecological variables (specifically predation pressure and food distribution) that may alter the host's ability to cope with parasitism. Helminth infections in a population of wild ungulates resulted in anorexia, but only for parasitized individuals living in smaller groups, suggesting that individuals living in larger groups cope better with infection[26]. Larger social groups may secure access to higher quality food patches and reduce time invested in anti-predator vigilance per individual, allowing members to allocate more energy towards reproduction and immune function. It would be compelling to investigate to what extent the benefits of a larger group size could offset competition within groups[1]—particularly as primates are exposed to seasonal changes in food availability and exhibit diverse feeding regimes from grazing to fruit-based diets with meat supplementation. Finally, although early case studies found that larger groups may harbor more diverse parasite communities[7], several meta-analyses in primates and other vertebrates showed no consistent relationship between host group size and parasite richness[13,19,23]. This suggests that species-specific movement patterns, contact rates[21], habitat use[27], or density[13] can prevail over group size in predicting parasite richness.

Although group size is a simple measurement, and thus the most extensively studied aspect of sociality[23], large groups of rarely interacting individuals can be wrongly considered highly social, while small cohesive groups can be erroneously seen as weakly social. Additionally, including group size as the sole proxy of sociality assumes that individuals in groups interact with each other at a constant rate, disregarding social dynamics and finer scale social interactions (i.e., the social structure of a group, see Box 2). Therefore, group size is often insufficient to fully explain the link between sociality and parasite infections[23,28].

4.2.2 Social network properties and parasitism

Parasite transmission is also influenced by substructure within and between social groups. For instance, the fragmentation of a population into subpopulations with limited movement of individuals among them create a natural barrier against the spread of

pathogens[29]. Within groups, animal networks are often **modular**, with interactions unevenly distributed across group members and tending to occur preferentially within subgroups[23,28,30]. Modularity has a non-linear effect on disease transmission[31,32]. The cost of increased disease transmission within subgroups first offsets the benefit of decreased transmission between subgroups. Above a certain threshold, highly modular social contact networks with cohesive subgroups effectively restrict infection to a few subgroups[31] (Figure 2A). Parasites are 'trapped' within subgroups resulting in smaller, or at least delayed, disease outbreaks depending on parasite transmissibility[15,30,33,34]. Interestingly, comparative studies based on empirical data suggest that modularity increases with group size in primates[15,30,31] (but see[32]). The positive relationship between group size and parasitism, group size and modularity, and negative relationship between modularity and parasitism suggest a possible inverted-U relationship between group size and parasitism in real world networks[31], with parasite spread being highest at intermediate group sizes. These results highlight the necessity of considering social structure (see Box 2) to better understand and predict parasite transmission dynamics in social species.

Individuals are exposed to heterogeneous parasite risks depending on network structure and their respective positions in groups[9,18] (see also Mistrick *et al.*[35] in this volume for a extensive review of animal networks and pathogen transmission). In primates, higher infection risk of helminth, protozoan, and possibly also viral[36] parasites, is associated with shared habitat use[11,37], increasing body contact, higher numbers of **grooming** partners, and centrality in grooming networks[28,37–39]. Consistent with patterns described in primates, a recent meta-analysis found that more central individuals in a network face higher parasitism[18] although, results also indicate wide heterogeneity in the strength and direction of associations. These differences may be expected based on parasite transmission modes and social behaviors measured[38]. Indeed, hygienic behaviors bringing individuals into close contact may help fight certain infectious agents, while also increasing exposure to others. For example, being central in a grooming network could lower lice infestation via removal from the partners' fur[40], while simultaneously increasing the risk of gastro-intestinal parasites as nematode eggs are ingested during grooming bouts[37,38]. To date, support for such theoretical predictions is ambiguous[38,40,41] stressing the necessity to incorporate other factors,

such as parasite life cycle relative to how exposure is measured, when applying network analyses to empirical systems. Furthermore, predictions about individual parasitism likely depend on interactions between group-level organization, an individual's position within the network, and other traits. To illustrate, a study on captive rhesus macaques (*Macaca mulatta*) found links between individual network position and *Shigella* infection in two groups, but not in a third, due to differences in sex-age composition[41]. The spread of directly transmitted parasites may also be hampered when the progressive acquisition of resistance among group members increases the chance that transmission chains will be broken before reaching susceptible individuals. Central individuals in the group, often considered to be “superspreaders,” may conversely be exceptionally efficient at slowing disease spread once they have acquired immunity[9,42] (Figure 2C).

In summary, group-level costs and benefits of sociality on parasitism are better understood in the context of multivariate factors[5,27]. Since group size oversimplifies sociodynamics, focusing on group structure and dynamics, rather than group size alone, can offer a more nuanced approach for understanding the vulnerability of social groups to parasites[17,23,38] and the costs and benefits of group living[15,33]. Nevertheless, the evidence presented so far in this chapter focuses on the impact of *quantitative* social measures, derived from the number of partners or the number or frequency of contacts, on parasitism. As shown in the next section, the *quality* of social interactions (whether positive or negative) also influences exposure and susceptibility to parasites.

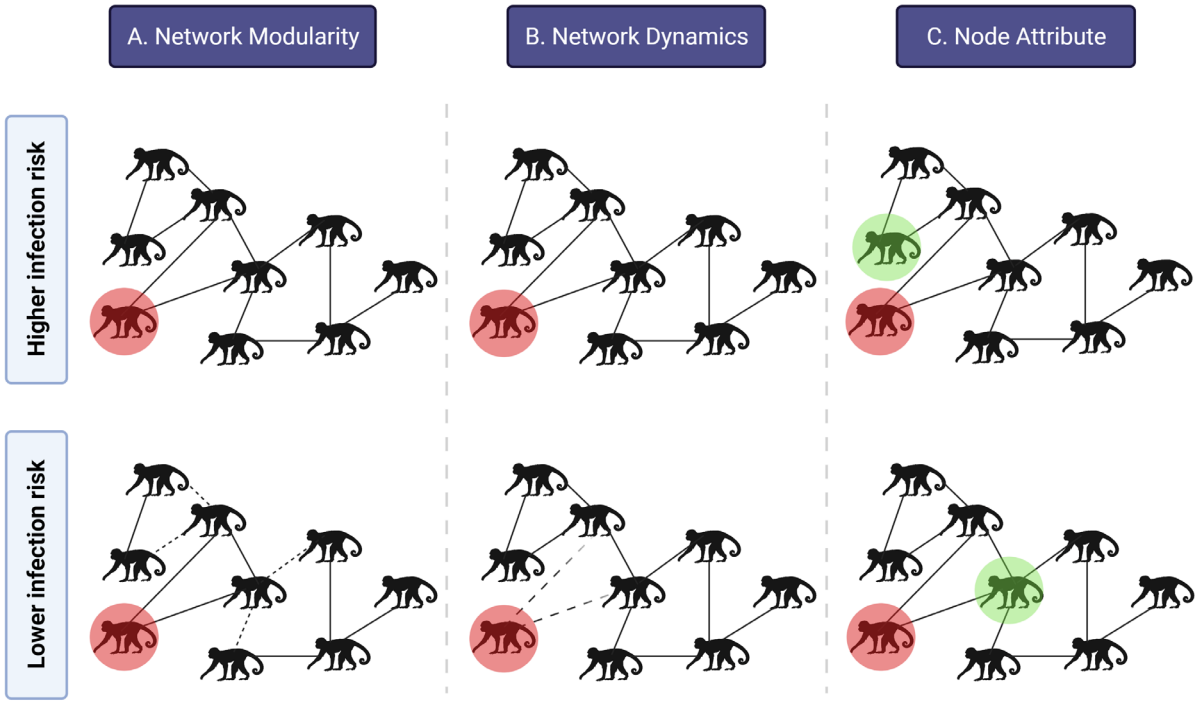


Figure 2. Social network structure influences infection risk in primates. All networks shown have the same number of nodes (individual primates) and edges (connection between the nodes). The spread of infectious diseases from an infected node (red circle) will depend on network properties, such as (A) modularity, which is the extent to which a network is divided into subgroups that interact more within than between subgroups. The lower network has both strong (full link) and weak (dashed link) connections, resulting in higher modularity than in the corresponding upper network. If infection leads to withdrawal from the group or avoidance of group members (B), network dynamics will further decrease infection risk (lower network, dashed lines vs. upper network full lines). Finally, the resistance of a network to disease spread may result from the combined effect of (C) a node's position and attributes. Immune nodes (green circle) with a central position (lower network) may be more efficient at slowing the spread of diseases than more peripheral ones (upper network). Figure created using BioRender.

4.3 Social interactions, susceptibility, and exposure to parasites

Social interactions inside a group are not random but tend to follow a set of rules with preferential interactions defining a social structure. Competitive and affiliative interactions are part of social living and influence susceptibility and exposure to parasites.

4.3.1 Competition and dominance

As individuals compete for access to food and mating partners, both within and between social groups, success depends on competitive abilities and/or alliances. Depending on the degree of tolerance, individuals face different social and environmental adversities. However, both social and environmental stressors activate the hypothalamic-pituitary-adrenal (HPA) axis, triggering a neuroendocrine cascade producing glucocorticoids. Glucocorticoids have immunomodulatory effects, especially if chronically elevated, and can result in reduced immunocompetence and increased parasite susceptibility[43–45]. For example, in wild olive baboons (*P. anubis)*, females harassed more often by aggressive males were also more immunocompromised[46]. The effects of such adversity on health and parasitism may vary according to the intensity of competition and inequality between group members. Inequality within groups is often studied through the lens of dominance hierarchies and individual rank[5,44].

In **despotic** hierarchies, dominant positions are obtained and maintained through physical aggression or threats, leading to more skewed access to resources. Several studies on wild and captive primates show a relationship between dominance rank and specific physiological profiles related to stress and immune function[44,47–49]. For example, being low-ranking in a despotic hierarchy can be associated with higher glucocorticoids levels[44], fewer circulating lymphocytes[44,46] and higher susceptibility to viral infections[43]. Moreover, there is increasing evidence of rank-related effects on immune function directly translating into increased susceptibility to specific pathogens. Using experimental manipulation of social rank in female rhesus macaques, Snyder-Mackler *et al.* (2016)[48] found various immune-gene expression patterns causally linked

to dominance rank, with high-ranking individuals expressing a more antiviral immune phenotype, and low-ranking individuals showing a pro-inflammatory immune profile more reactive against bacterial infections.

Besides providing evidence of a link between low rank and pro-inflammatory responses known to be associated with several diseases[43,50], the work from macaques suggests that dominance rank may prime the immune system towards specific types of parasites, possibly as a result of dominance-associated biases in exposure. In partial support of this theory, results from a recent meta-analysis on the relationship between dominance rank and parasitism across several vertebrate clades showed that high-ranking males display greater parasitism than lower-ranking males, and revealed a similar, although non-significant, trend in females (restricted to primates only)[51]. The two best supporting explanations for this pattern are that high rank increases parasite exposure via priority access to food and trade-offs between reproduction and immune investment. However, drawing further conclusions about how infection risk for different parasites—and more generally, how parasite exposure and susceptibility—differ by rank, requires studying more diverse social and mating systems[51]. For example, in primates, the relationship between dominance, health, and parasitism has focused on strongly despotic systems, with a dearth of data on more **tolerant** dominance styles. In the latter, networks of interactions tend to be less modular; individuals' positions in the dominance hierarchy do not necessarily predict access to food resources, interactions with social partners, or risk of injuries, and offspring may be handled by both relatives and non-relatives, possibly increasing exposure. Conversely, individuals in a despotic system exposed to fierce competition may be more inclined to conceal symptoms, which may exacerbate pathogen spread. Finally, social instability in a group can further complicate the picture: during socially unstable times, high-ranking individuals can temporarily experience higher psychological and physical stress, display impaired HPA-axis activity[44], and develop stress-related diseases faster than low-ranking individuals[44]. When hierarchies stabilize, being low-ranking is once again associated with greater physiological indices of stress[44].

4.3.2 Social support and social learning

Relationships within groups also include a range of positive interactions between group members. Close preferential relationships provide individuals with social support (e.g., tolerance at feeding sites, coalition partners during agonistic interactions) that may mitigate infection through several mechanisms. First, social support reduces susceptibility to pathogens on a physiological level by buffering social stress. Past experimental work found that social support enhances immune function in captive primates[52], decreases the probability of acquiring influenza in humans[53], and reduces glucocorticoids levels associated with negative social experiences in wild primates[54,55]. All these mechanisms could also explain the relationship between social support and lower mortality risk in humans[56]. The protective effect of social support against infection may be particularly valuable to those with heightened stress. Both rhesus macaques with uncertain status in their group and human patients reporting high levels of social tension in their daily lives showed a substantial decrease in infection risk attributed to social support[41,53]. As the definition of social support varies between studies[12], the specific components of sociality relevant to the buffering effect against disease need further characterization[52]. For example in Barbary macaques (*M. sylvanus*), strong opposite-sex bonds but not same-sex bonds were found to have a protective effect against contracting gastro-intestinal nematodes[37], although the mechanisms explaining this difference remain unclear. Refining the concept of close friends in primatology to include reciprocity, predictability, and stability of interactions over time will help disentangle the different aspects of social support that contribute to **social buffering** effects.

Another form of social support occurs through (social) learning of anti-parasite behaviors. Some primates actively fight parasitism using natural resources—a phenomenon called **self-medication**[57]. First observed in wild African great apes, growing evidence has revealed that self-medication is widespread across the primate order[57]. A well-established example of self-medication in great apes is leaf-swallowing to fight internal parasites through properties of anthelmintic phytochemicals and trapping worms in leaf folds[57]. To fight external parasites, wedge-capped capuchins (*Cebus olivaceus*[58]) and red-fronted lemurs (*Eulemur rufifrons*[59]) perform fur rubbing,

using toxic secretions of millipedes as a repellant and even sharing millipedes with social partners. In these situations, knowledgeable group members may serve as role models for social learning. For example, young chimpanzees acquire knowledge of the curative properties of toxic plants by watching adults[60]. In contrast, when adults do not tolerate close proximity during feeding time, as in western lowland gorillas (*Gorilla gorilla gorilla*), young individuals learn curative behaviors by observing kin of similar age[60].

In summary, sociality is best understood if we go beyond group size and organization and also acknowledge the diversity of inter-individual relationships between group members. Primates navigate complex social worlds by mitigating the costs of competition and forming social bonds that in turn, have far-reaching consequences for parasitism and health.

4.4 Individual behaviors and parasite risk

Prior sections of this chapter describe how group composition and structure shape exposure and susceptibility to parasites at higher levels of sociality. The focus was on understanding how social dynamics within and between groups, as well as competitive and affiliative interactions, may enhance or impair exposure and susceptibility to parasites in primates. However, despite the overarching effects of such constraints at the species or group level, individuals can still adjust their behaviors in response to parasitism. Thus, to fully capture how sociality interacts with parasitism we must also consider social behaviors that flexibly respond to parasitism (e.g. avoidance behaviors, social immunization; Figure 3) and how larger scales of social organization shape these behaviors themselves.

4.4.1 Avoidance behavior

Depending on the mating system, risk of parasite exposure can be heightened in one sex (e.g., polygyny and polyandry) or equally distributed among males and females (e.g., polygamy and monogamy)[61]. In primates, there is evidence of a link between copulation rate and prevalence of sexually transmitted diseases[61]: in a group of olive

baboons (polygamous), females were found to avoid mating with males showing signs of infection with a sexually transmitted disease similar to syphilis (*Treponema pallidum pertenue*)[62]. Infected females also accepted fewer copulations, and all females mated with fewer partners (compared to olive baboons from uninfected populations), despite the large pool of males available[62]. Thus, when faced with a heightened risk of infection, sexual behaviors were modified, lowering exposure (Figure 3), and reducing mating success for infected individuals, with major implications for disease transmission. Sexual behaviors, which are rarely considered in primate social network studies, could be used to investigate how flexibility of sexual networks may limit pathogen spread.

Beyond sexual interactions, individuals appear to modulate their overall social connectedness in response to disease outbreaks, or in the presence of infected individuals, by adjusting their interactions to minimize risk[36,37,63–65] (Figure 2B). Evidence of social distancing in species as phylogenetically distant as arthropods and primates, points towards multiple independent emergence of a similar behavioral strategy[29]. Although sick individuals may decrease their social interactions as a result of lethargy and fever resulting from infection, true social distancing involves the expression of specific behaviors that reduce the transmission of pathogens by increasing spatial distance among conspecifics[29]. For example, mandrills (*Mandrillus sphinx*) interact with healthy social partners but avoid group members infected with fecally transmitted gastro-intestinal parasites[63]. However, avoidance mechanisms may not always be effective if, for example, individuals disregard cues of infection or try to conceal sickness. Mandrills also do not avoid sick kin, suggesting that kinship interferes with avoidance[66]. However, testing whether social distancing is effective in the wild remains challenging since conducting controlled experiments is nearly impossible. Sanctuaries with semi-free ranging populations may provide a semi-natural setting where such studies can be done.

4.4.2 Social immunization and the gut microbiome

While the benefits associated with avoiding infected individuals are well-known, the potential benefits resulting from gradual exposure to parasites in building immunity

remain mostly untested[8]. A lack of immune challenges and acquired immunity during early life can significantly affect resilience to infection with aging[67]. Therefore, the possibility that sharing parasitic agents (e.g., from parents to offspring or between individuals during play) may contribute to acquired immunity and partially offset certain future costs associated with socially transmitted parasites needs to be considered. This phenomenon of **social immunization** (Figure 3) through low dose exposure[68] has not yet been investigated in primates, although primate-helminth systems, characterized by high species-specificity[69], offer an exciting potential model. In addition to providing gradual exposure to parasites, social interactions through physical contact, close proximity and movement between groups also facilitates the acquisition of beneficial microbes composing the gut microbiome[70]. This community of bacteria, eukaryotes, archaea, fungi, and viruses inhabiting the gastro-intestinal tract of primates plays an essential role in food digestion and the production of metabolites and vitamins, prevents colonization by opportunistic pathogens, participates in the development of the hosts' immune response, and the metabolism of toxic compounds—all of which help reduce host susceptibility to infection[70,71]. Recent evidence suggests that the gut microbiome may affect host social behavior to promote its own transmission by affecting an individual's olfactory signaling and influencing conspecific recognition and bonding or group-specific scent marks[71]. These findings raise new questions about how microbial commensals influence social networks and the evolution of sociality[70,71]. Finally, evidence that early-life microbiome composition influences parasite susceptibility in adult frogs[72] provides a starting point for testing how an individual's past and present social life interact to shape its infection status (Figure 3).

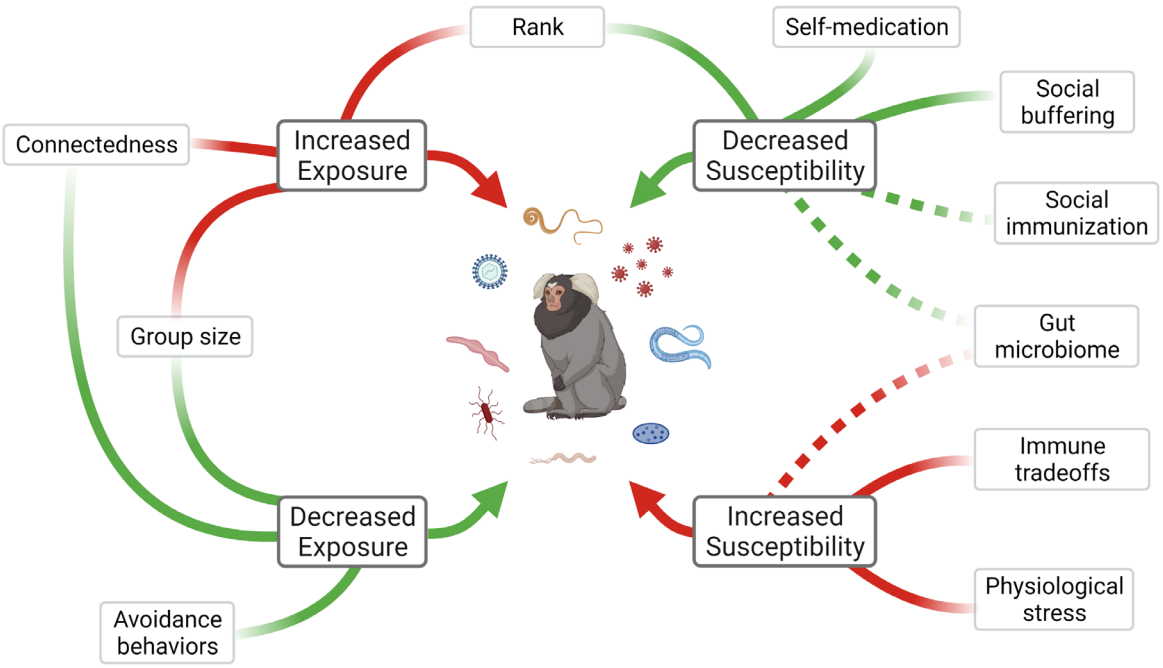


Figure 3. Primate sociality: parasite-related costs and benefits. Illustrated summary of the evidence linking sociality and parasitism in primates. Correlates of sociality increase (red arrows) or decrease (green arrows) parasitism by modulating exposure and susceptibility to parasites. Correlates of sociality with established links to parasitism in primates are depicted with full arrows. Key social drivers of parasitism in other taxa of special interest for primates are depicted with dashed arrows. Evidence gathered from primates highlight that exposure and susceptibility are not determined at one level of sociality, but by the interplay of group and individual attributes. Individuals modulate parasitic risks exerted by *group size* and *connectedness* with *avoidance behaviors*. Understanding the consequence of social attributes such as *rank* on infection risk, requires accounting for increased exposure, engagement in social interactions inducing *physiological stress*, and access to support from close partners providing *social buffering*. Ultimately, the consequences of infection depend on individual susceptibility, shaped by *immune tradeoffs*, protection acquired from *social immunization* or modulated by physiological systems such as the *gut microbiome* or the use of *self-medication*. Figure created using BioRender.

Freeland first depicted primate groups as homogeneous biological islands[7] whose parasite diversity is mainly governed by group size and ecology (e.g., diet, habitat use). However, building on these concepts has made room for more fluid and fine-tuned connections between the different levels of sociality. Importantly, the original emphasis on the costs of parasite transmission shaping sociality has been enriched by a better understanding of within-group drivers of parasitism. By including more complex levels of sociality, we expand our understanding on how social networks and individual behaviors are linked to overall health (Figure 3). A growing body of research has also allowed us to draw a more nuanced picture of assumed benefits of hygienic behaviors (e.g., grooming), while documenting more cases of rare behaviors (e.g., self-medication). Several bold concepts, including social immunization described in other animal phyla and postulated in primates, remain largely untested; while a recent focus on microbiomes has added a new dimension in understanding links between sociality and parasitism.

4.5 Future directions and conclusions

4.5.1 Perspectives: important directions for future research on sociality and parasitism in primates

Social network analysis has greatly contributed towards our understanding of how social structure relates to parasite risk. However, most studies to date rely on networks in which all connections between individuals represent the same type of interaction (using a single or combining multiple behaviors to produce a single aggregate measure). Such static networks conceal important social dynamics; in contrast, multilayer networks can incorporate multiple sets of relationships, with each layer representing a distinct form of connection (e.g., aggression vs affiliation)[73]. Static networks also fail to capture spatiotemporally dynamic environments that can destabilize or alter network ties (Box 3). Furthermore, given the interdependencies between hosts and parasites, multilayer networks allow researchers to address questions related to temporally dynamic factors, such as testing the resilience of networks to novel pathogens in comparison to endemic pathogens[5,9,74]. In addition, multilayer networks can be used not to only compare similar sets of individuals (within

species), but also interconnected systems. For example, Gomez *et al.* (2016)[20] modelled pathogen transmission on networks where nodes represented species rather than individuals, and found that primate species infected with parasites infecting many other primate species were also more likely to harbor pathogens similar to those identified as emerging diseases in humans.

At the individual level, physiological and social mediators of susceptibility to infection are still poorly understood. Although the role of glucocorticoids in response to stress, social isolation, and social buffering[50] makes them ideal candidates linking sociality and parasitism, recent meta-analyses suggest that parasitism itself causes an elevation in glucocorticoids, rather than vice versa[45,75]. The search for physiological mediators between social adversity and susceptibility to parasitism continues. Other possible suspects tying sociality and parasite transmission also require more exploration. This could include flexible behavioral defenses to avoid parasite infections, differences in cognitive capacities to learn anti-parasitic behaviors, and the importance of social interactions in the acquisition of beneficial microorganisms to build up the immune response and compete against pathogens.

Box 3. Case study: Dynamic vs. static social networks in models of parasite transmission: predicting *Cryptosporidium* spread in wild lemurs

Social networks are used to assess how pathogens spread among a population, group, or between individuals. However, most network models are static and discount temporal fluctuations, such as seasonal changes. This tends to result in networks with high density, as ties accumulate over time, but in which tie strength, averaged over the study period, likely underestimates the maximal strength between nodes. Springer *et al.* (2017)[34] predicted that short-term changes in the distribution of network ties would influence pathogen spread and outbreak size, which could only be successfully modeled by dynamic networks. They created an epidemiological model in which individuals were either susceptible, exposed, infected, recovered, or deceased to test how the pathogen *Cryptosporidium* spread between adjacent groups of wild Verreaux's sifakas (*Propithecus verreauxi*) in two three-month seasons. In their model, individuals

could become infected by body contact with an infectious conspecific (direct contact transmission) or by ranging over a contaminated area (environmental transmission). The probability of becoming infected in the modeled network was estimated using empirical data from behavioral observations and GPS tracking. The study explored three different transmission routes of the parasite: 1) both environmental and direct contact, 2) direct contact only, and 3) environmental only. For each season, static versions of networks for intergroup body contact and ranging overlap were created. Additionally, dynamic versions of the model were developed by updating networks of intergroup body contacts and ranging overlap every two weeks (Figure 1).

The dynamic and static models converged in predicting larger outbreaks when taking into account both social and environmental transmission. However, the static network model predicted a smaller outbreak size than the dynamic model in the dry season, due to the rapid increase in intergroup range overlap at the end of the dry season that was only adequately captured by the dynamic model. As a consequence, conclusions about the influence of seasonality on outbreak size (whether larger in the wet or dry season) entirely depended on the choice of network (i.e., dynamic vs. static). By comparing outbreak sizes across seasons in both dynamic and static networks, it becomes apparent that static models may simplify and limit certain predictions regarding disease dynamics.

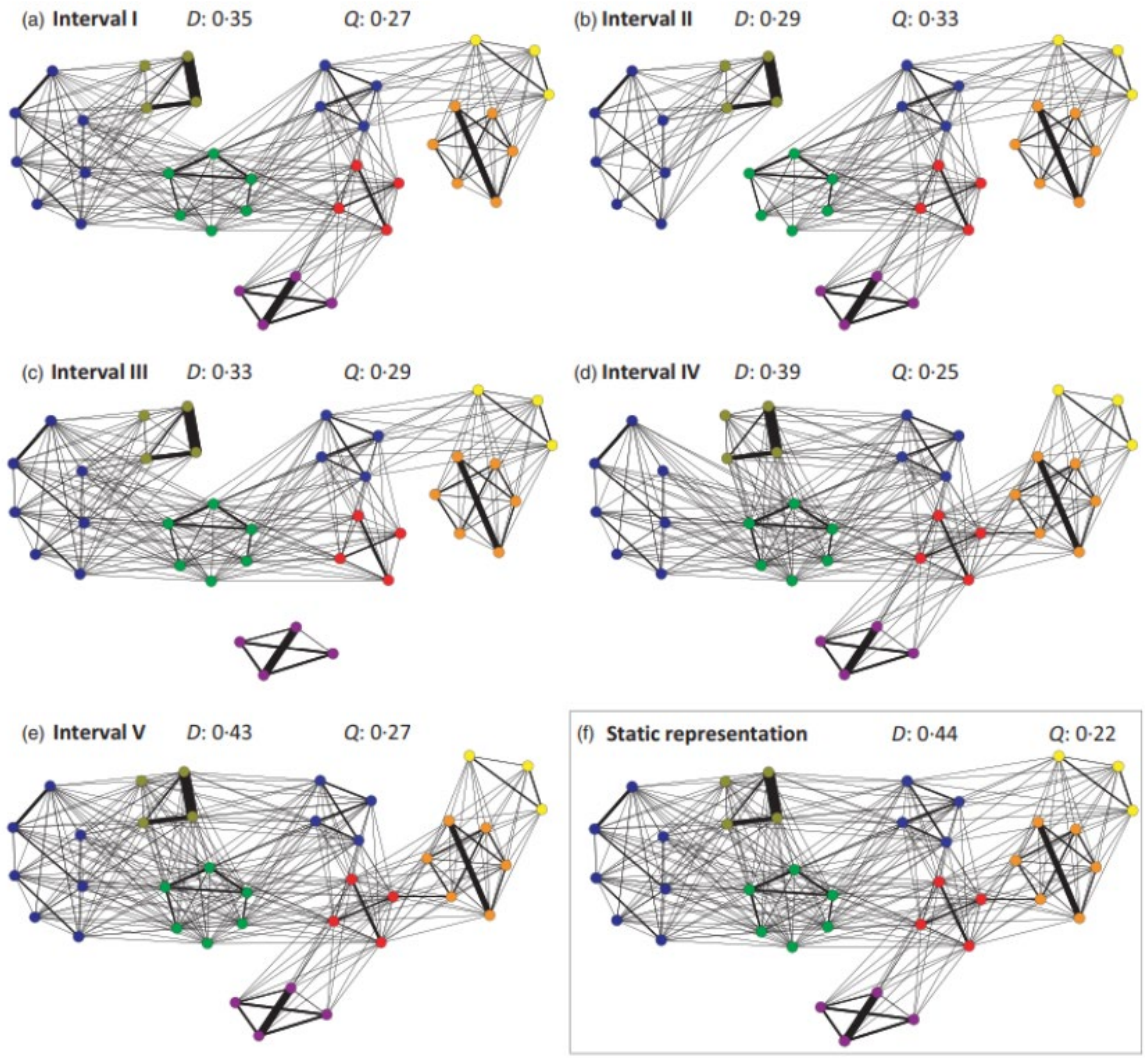


Figure 1. Dynamic (a-e) and static (f) networks calculated from behavioral observations of 8 groups of Verreaux's sifakas during the dry season. Tie strength is proportional to body contact rates calculated over bi-weekly intervals for the dynamic model, or the entire dry season for the static model. D = density, Q = modularity. Modified with permission from Springer *et al.* (2017)[34].

To further understand which aspects of network dynamics may influence outbreak size, the authors generated random networks on four social groups with different patterns of intergroup connections. Model predictions on pathogen spread were compared between a set of dynamic networks (updated every two weeks) and the corresponding static networks (generated over the cumulated period considered), for varying probability of infection and recovery. Dynamic networks with a low probability of

infection and slow recovery produced larger mean outbreak sizes, especially when the strength of interactions between nodes varied greatly over time. Thus, short-term strong connections detected by dynamic models have a larger influence on outbreak size than averaged connections from static networks—particularly in cases where transmission is low and with long recovery periods.

The importance of short-term bond formation in facilitating parasite transmission raises the possibility that the disappearance of short-term bonds might, conversely, be a major mechanism reducing transmission in social groups. Low probability of infection may provide social individuals with an opportunity to adapt their behavior to reduce infection risk before the pathogens have spread through most of the network. Considering these types of host feedback mechanisms between behavior and parasite transmission is critical for accurately predicting pathogen spread[76]. For example, a decrease in ranging behavior during the clinical phase of the infection, or transient social distancing between two closely bonded partners, may not be captured by static networks averaged over months of observation—and yet these factors strongly influence disease spread. Implications of these results go beyond the study of protozoan parasites in primates and influence predictions about the spread of other diseases (e.g. bacteria, viruses) with low transmissibility, such as tuberculosis or latent viral infections.

4.5.2 Conclusions: the relevance of studying host sociality and parasitism in primates

Over the past few decades, the field of primate disease ecology has made great strides towards understanding how group size and composition, social structure, and mating systems relate to parasitism. The level of detail gathered on the interactions and relationships between individually identified primates and their parasites has allowed the field to go beyond the analysis of ecological parameters influencing exposure and susceptibility to identify specific aspects of social life linked to parasitism. In addition, results from empirical studies have contributed to a broader effort of revisiting the links between group living and parasitism by documenting the benefits of sociality against parasitism[8].

Although there is growing understanding of the influence of social behavior on parasite risk, it remains unclear how, and to which degree, parasite infections alter the social behaviors of both infected hosts and their uninfected conspecifics. A perfect example is the recent global social distancing by humans in response to COVID-19[76,77], which illustrates how host social behavior can both respond to parasitism and influence it. Such bidirectional relationships between host social behavior and parasites have important implications for epidemiological dynamics and the evolution of sociality[76,77]. Understanding these types of interactions will contribute answering essential questions around the costs and benefits of animal sociality, and illuminate how these behaviors may contribute to pathogen emergence, spread, and evolution. Importantly, wild primates, with long-term data collection ongoing in several species worldwide, offer the possibility to explore questions that incorporate the complexities of host social behavior and the role it plays in the transmission of infectious diseases. Such studies in primates have increasing relevance for both public health and conservation.

In terms of public health, studies linking socio-ecological mechanisms involved in parasite transmission in primates may provide information on 'best pathogen candidates' for transmission and dissemination in humans[78]. Primates are the source of two of the deadliest modern day epidemics in human, HIV-1 (the virus responsible for AIDS) stemming from chimpanzees lentivirus[79], and *Plasmodium falciparum* (which causes malaria) from a strain infecting gorillas[80]. Yet, much uncertainty remains around the social interactions that facilitated zoonotic transmission[81]. Primates also represent sentinels for monitoring diseases like anthrax[82] and Ebola virus outbreaks[83] that threaten both wildlife and humans. Although **spillover events** are rare, episodic transmission of extremely severe infections (e.g. Ebola[83], cercopithecine herpesvirus B[78]) is of serious concern at the growing human-primate interface[78,84].

Likewise, a better understanding of species' social structure and response to infections can help inform conservation measures[20,42]. An illustration is given by efforts to control tuberculosis in badger populations in the UK. Culling interventions targeting males disturbed territorial defense behaviors and increased migrations between groups, resulting in greater disease spread[85,86]. Similarly, detailed understanding of the influence of sex, rank, or age on social position gathered from behavioral studies will be critical to implement conservation strategies in primates[42] as 60% of primates

species are threatened with extinction[84]. The increase in infectious diseases in primates is considered a consequence of deforestation, agriculture expansion, habitat fragmentation, mining, hunting, and climate change, all of which are associated with declines among primate populations[84,85]. Understanding the anthropogenic impact on the socio-ecological systems of primates and the consequences in those affected populations can contribute to an inclusive one health approach by reducing the infection risk for all primate species, including humans[78,85]. Only through long-term monitoring, health surveillance systems for researchers, their study species, as well as sentinel populations of neighboring primates can we detect emerging diseases and study the impact of pathogens on populations.[78,79]

Acknowledgments

This contribution is the result of collaborative work between past and current PhD students of the DFG-funded Sociality and Health in Primates (SoHaPi) Research Group (DFG FOR 2136) with the encouragement of Prof. Peter Kappeler. All authors contributed equally to this work. We would like to thank Prof. Peter Kappeler, Dr. Oliver Schülke, Dr. Jan Gogarten, Dr. Claudia Fichtel, and Prof. Julia Ostner for their insightful feedback on the manuscript, as well as all members of the SoHaPi Research Group for their support, and the German research foundation (DFG) for funding (grants LE 1813/10-1; OS 201/6-1; FI 929/7-1, KR 3834/5-1; FI 929/7-2, DA 374/312; SCHU 1554/6-1; OS 201/6-2). Finally, we are grateful to the editors, Prof. Vanessa Ezenwa, Prof. Sonia Altizer, and Dr. Richard Hall for their invitation to contribute to this volume, and to Prof. Ezenwa and three anonymous reviewers for their insightful feedback on earlier versions of the manuscript.

1. Silk, J. B. & Kappeler, P. M. Sociality in Primates. in *Comparative Social Evolution* (eds. Rubenstein, D. R. & Abbot, P.) 253–283 (Cambridge University Press, 2017).
2. Smith, J. E., Lacey, E. A. & Hayes, L. D. Sociality in non-primate mammals. in *Comparative Social Evolution* (eds. Rubenstein, D. R. & Abbot, P.) 284–319 (Cambridge University Press, 2017).

3. Krause, J. & Ruxton, G. D. *Living in Groups*. (Oxford University Press, 2002).

4. Altizer, S. *et al.* Social Organization and Parasite Risk in Mammals: Integrating Theory and Empirical Studies. *Annu. Rev. Ecol. Evol. Syst.* **34**, 517–547 (2003).

5. Kappeler, P. M., Cremer, S. & Nunn, C. L. Sociality and health: impacts of sociality on disease susceptibility and transmission in animal and human societies. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, (2015).

6. Alexander, R. D. The Evolution of Social Behavior. *Annu. Rev. Ecol. Syst.* **5**, 325–383 (1974).

7. Freeland, W. J. Primate Social Groups as Biological Islands. *Ecology* **60**, 719–728 (1979).

8. Ezenwa, V., Ghai, R. R., McKay, A. F. & Williams, A. E. Group living and pathogen infection revisited. *Curr. Opin. Behav. Sci.* **12**, 66–72 (2016).

9. Rushmore, J., Bisanzio, D. & Gillespie, T. R. Making New Connections: Insights from Primate–Parasite Networks. *Trends Parasitol.* **33**, 547–560 (2017).

10. Hawley, D. M., Etienne, R. S., Ezenwa, V. & Jolles, A. E. Does Animal Behavior Underlie Covariation Between Hosts' Exposure to Infectious Agents and Susceptibility to Infection? Implications for Disease Dynamics. *Integr. Comp. Biol.* **51**, 528–539 (2011).

11. Nunn, C. L., Thrall, P. H., Leendertz, F. H. & Boesch, C. The Spread of Fecally Transmitted Parasites in Socially-Structured Populations. *PLoS ONE* **6**, (2011).

12. Ostner, J. & Schülke, O. Chapter Four - Linking Sociality to Fitness in Primates: A Call for Mechanisms. in *Advances in the Study of Behavior* (eds. Naguib, M. et al.) vol. 50 127–175 (Academic Press, 2018).

13. Nunn, C. L., Altizer, S., Jones, K. E. & Sechrest, W. Comparative Tests of Parasite Species Richness in Primates. *Am. Nat.* **162**, 597–614 (2003).

14. Lukas, D. & Clutton-Brock, T. H. The Evolution of Social Monogamy in Mammals. *Science* **341**, 526–530 (2013).

15. Griffin, R. H. & Nunn, C. L. Community structure and the spread of infectious disease in primate social networks. *Evol. Ecol.* **26**, 779–800 (2012).

16. Côté, I. M. & Poulin, R. Parasitism and group size in social animals: a meta-analysis. *Behav. Ecol.* **6**, 159–165 (1995).

17. Pastor-Satorras, R., Castellano, C., Van Mieghem, P. & Vespignani, A. Epidemic processes in complex networks. *Rev. Mod. Phys.* **87**, 925–979 (2015).

18. Briard, L. & Ezenwa, V. O. Parasitism and host social behaviour: a meta-analysis of insights derived from social network analysis. *Anim. Behav.* **172**, 171–182 (2021).

19. Patterson, J. E. H. & Ruckstuhl, K. E. Parasite infection and host group size: a meta-analytical review. *Parasitology* **140**, 803–813 (2013).

20. Gómez, J. M., Nunn, C. L. & Verdú, M. Centrality in primate–parasite networks reveals the potential for the transmission of emerging infectious diseases to humans. *Proc. Natl. Acad. Sci.* **110**, 7738–7741 (2013).

21. Habig, B. *et al.* Multi-scale predictors of parasite risk in wild male savanna baboons (*Papio cynocephalus*). *Behav. Ecol. Sociobiol.* **73**, 134 (2019).

22. Klein, A. *et al.* Ectoparasite communities of small-bodied Malagasy primates: seasonal and socioecological influences on tick, mite and lice infestation of *Microcebus murinus* and *M. ravelobensis* in northwestern Madagascar. *Parasit. Vectors* **11**, 459 (2018).

23. Rifkin, J. L., Nunn, C. L. & Garamszegi, L. Z. Do Animals Living in Larger Groups Experience Greater Parasitism? A Meta-Analysis. *Am. Nat.* **180**, 70–82 (2012).

24. Buck, J. C. & Lutterschmidt, W. I. Parasite abundance decreases with host density: evidence of the encounter-dilution effect for a parasite with a complex life cycle. *Hydrobiologia* **784**, 201–210 (2017).

25. Samson, D. R. *et al.* Chimpanzee (*Pan troglodytes schweinfurthii*) Group Sleep and Pathogen-Vector Avoidance: Experimental Support for the Encounter-Dilution Effect. *Int. J. Primatol.* **40**, 647–659 (2019).

26. Ezenwa, V. & Worsley-Tonks, K. E. L. Social living simultaneously increases infection risk and decreases the cost of infection. *Proc. R. Soc. B Biol. Sci.* **285**, 20182142 (2018).

27. Ezenwa, V. Host social behavior and parasitic infection: a multifactorial approach. *Behav. Ecol.* **15**, 446–454 (2004).

28. Rimbach, R. *et al.* Brown spider monkeys (*Ateles hybridus*): a model for differentiating the role of social networks and physical contact on parasite transmission dynamics. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140110 (2015).

29. Butler, M. J., IV & Behringer, D. C. Behavioral Immunity and Social Distancing in the Wild: The Same as in Humans? *BioScience* (2021) doi:10.1093/biosci/biaa176.

30. Nunn, C. L., Jordán, F., McCabe, C. M., Verdolin, J. L. & Fewell, J. H. Infectious disease and group size: more than just a numbers game. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140111 (2015).

31. Romano, V., Shen, M., Pansanel, J., MacIntosh, A. J. J. & Sueur, C. Social transmission in networks: global efficiency peaks with intermediate levels of modularity. *Behav. Ecol. Sociobiol.* **72**, 154 (2018).

32. Sah, P., Leu, S. T., Cross, P. C., Hudson, P. J. & Bansal, S. Unraveling the disease consequences and mechanisms of modular structure in animal social networks. *Proc. Natl. Acad. Sci.* **114**, 4165–4170 (2017).

33. Snaith, T. V., Chapman, C. A., Rothman, J. M. & Wasserman, M. D. Bigger groups have fewer parasites and similar cortisol levels: a multi-group analysis in red colobus monkeys. *Am. J. Primatol.* **70**, 1072–1080 (2008).

34. Springer, A., Kappeler, P. M. & Nunn, C. L. Dynamic vs. static social networks in models of parasite transmission: predicting *Cryptosporidium* spread in wild lemurs. *J. Anim. Ecol.* **86**, 419–433 (2017).

35. Mistrick, J., Gilbertson, M., White, L. & Craft, M. Constructing animal networks for parasite transmission inference. in *Animal Behavior and Parasitism* (Oxford University Press, 2022).

36. Patrono, L. V. *et al.* Monkeypox virus emergence in wild chimpanzees reveals distinct clinical outcomes and viral diversity. *Nat. Microbiol.* **5**, 955–965 (2020).

37. Müller-Klein, N. *et al.* Exposure and susceptibility drive reinfection with gastrointestinal parasites in a social primate. *Funct. Ecol.* **33**, 1088–1098 (2019).

38. MacIntosh, A. J. J. *et al.* Monkeys in the Middle: Parasite Transmission through the Social Network of a Wild Primate. *PLOS ONE* **7**, e51144 (2012).

39. Friant, S., Ziegler, T. E. & Goldberg, T. L. Primate reinfection with gastrointestinal parasites: behavioural and physiological predictors of parasite acquisition. *Anim. Behav.* **117**, 105–113 (2016).

40. Duboscq, J., Romano, V., Sueur, C. & MacIntosh, A. J. J. Network centrality and seasonality interact to predict lice load in a social primate. *Sci. Rep.* **6**, 22095 (2016).

41. Balasubramaniam, K., Beisner, B., Vandeleest, J., Atwill, E. & McCowan, B. Social buffering and contact transmission: network connections have beneficial and detrimental effects on *Shigella* infection risk among captive rhesus macaques. *PeerJ* **4**, e2630 (2016).

42. Rushmore, J. *et al.* Network-based vaccination improves prospects for disease control in wild chimpanzees. *J. R. Soc. Interface* **11**, (2014).

43. Cohen, S. *et al.* Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proc. Natl. Acad. Sci.* **109**, 5995–5999 (2012).

44. Sapolsky, R. M. The Influence of Social Hierarchy on Primate Health. *Science* **308**, 648–652 (2005).

45. Defolie, C., Merkling, T. & Fichtel, C. Patterns and variation in the mammal parasite–glucocorticoid relationship. *Biol. Rev.* **95**, 74–93 (2020).

46. Sapolsky, R. M. Endocrinology alfresco: psychoendocrine studies of wild baboons. *Recent Prog. Horm. Res.* **48**, 437–468 (1993).

47. Capitanio, J. P. & Cole, S. W. Social instability and immunity in rhesus monkeys: the role of the sympathetic nervous system. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, (2015).

48. Snyder-Mackler, N. *et al.* Social status drives social relationships in groups of unrelated female rhesus macaques. *Anim. Behav.* **111**, 307–317 (2016).

49. Snyder-Mackler, N. *et al.* Social status alters chromatin accessibility and the gene regulatory response to glucocorticoid stimulation in rhesus macaques. *Proc. Natl. Acad. Sci.* **116**, 1219–1228 (2019).

50. Hostinar, C. E., Sullivan, R. M. & Gunnar, M. R. Psychobiological Mechanisms Underlying the Social Buffering of the HPA Axis: A Review of Animal Models and Human Studies across Development. *Psychol. Bull.* **140**, (2014).

51. Habig, B., Doellman, M. M., Woods, K., Olansen, J. & Archie, E. A. Social status and parasitism in male and female vertebrates: a meta-analysis. *Sci. Rep.* **8**, 3629 (2018).

52. Cohen, S., Kaplan, J. R., Cunnick, J. E., Manuck, S. B. & Rabin, B. S. Chronic Social Stress, Affiliation, and Cellular Immune Response in Nonhuman Primates. *Psychol. Sci.* **3**, 301–305 (1992).

53. Cohen, S., Janicki-Deverts, D., Turner, R. B. & Doyle, W. J. Does Hugging Provide Stress-Buffering Social Support? A Study of Susceptibility to Upper Respiratory Infection and Illness. *Psychol. Sci.* **26**, 135–147 (2015).

54. Young, C., Majolo, B., Heistermann, M., Schülke, O. & Ostner, J. Responses to social and environmental stress are attenuated by strong male bonds in wild macaques. *Proc. Natl. Acad. Sci.* **111**, 18195–18200 (2014).

55. Wittig, R. M. *et al.* Social support reduces stress hormone levels in wild chimpanzees across stressful events and everyday affiliations. *Nat. Commun.* **7**, 13361 (2016).

56. Holt-Lunstad, J., Smith, T. B., Baker, M., Harris, T. & Stephenson, D. Loneliness and social isolation as risk factors for mortality: a meta-analytic review. *Perspect. Psychol. Sci. J. Assoc. Psychol. Sci.* **10**, 227–237 (2015).

57. Huffman, M. A. Chimpanzee self-medication: a historical perspective of the key findings. in *Mahale Chimpanzees: 50 Years of Research* (eds. Hosaka, K., Zamma, K., Nakamura, M. & Itoh, N.) 340–353 (Cambridge University Press, 2015).

58. Valderrama, X., Robinson, J. G., Attygalle, A. B. & Eisner, T. Seasonal Anointment with Millipedes in a Wild Primate: A Chemical Defense Against Insects? *J. Chem. Ecol.* **26**, 2781–2790 (2000).

59. Peckre, L. R., Defolie, C., Kappeler, P. M. & Fichtel, C. Potential self-medication using millipede secretions in red-fronted lemurs: combining anointment and ingestion for a joint action against gastrointestinal parasites? *Primates J. Primatol.* **59**, 483–494 (2018).

60. Masi, S. *et al.* Unusual feeding behavior in wild great apes, a window to understand origins of self-medication in humans: Role of sociality and physiology on learning process. *Physiol. Behav.* **105**, 337–349 (2012).

61. Nunn, C. L. *et al.* Mating Competition, Promiscuity, and Life History Traits as Predictors of Sexually Transmitted Disease Risk in Primates. *Int. J. Primatol.* **35**, 764–786 (2014).

62. Paciência, F. M. D. *et al.* Mating avoidance in female olive baboons (*Papio anubis*) infected by Treponema pallidum. *Sci. Adv.* **5**, eaaw9724 (2019).

63. Poirotte, C. *et al.* Mandrills use olfaction to socially avoid parasitized conspecifics. *Sci. Adv.* **3**, e1601721 (2017).

64. Müller-Klein, N. *et al.* Physiological and social consequences of gastrointestinal nematode infection in a nonhuman primate. *Behav. Ecol.* **30**, 322–335 (2019).

65. Poirotte, C. & Charpentier, M. J. E. Inter-individual variation in parasite avoidance behaviors and its epidemiological, ecological, and evolutionary consequences. in *Animal Behavior and Parasitism* (Oxford University Press, 2022).

66. Poirotte, C. & Charpentier, M. J. E. Unconditional care from close maternal kin in the face of parasites. *Biol. Lett.* **16**, 20190869 (2020).

67. De Nys, H. M., Löhrich, T., Wu, D., Calvignac-Spencer, S. & Leendertz, F. H. Wild African great apes as natural hosts of malaria parasites: current knowledge and research perspectives. *Primate Biol.* **4**, 47–59 (2017).

68. Konrad, M. *et al.* Social Transfer of Pathogenic Fungus Promotes Active Immunisation in Ant Colonies. *PLOS Biol.* **10**, e1001300 (2012).

69. Pedersen, A. B., Altizer, S., Poss, M., Cunningham, A. A. & Nunn, C. L. Patterns of host specificity and transmission among parasites of wild primates. *Int. J. Parasitol.* **35**, 647–657 (2005).

70. Sarkar, A. *et al.* Microbial transmission in animal social networks and the social microbiome. *Nat. Ecol. Evol.* **4**, 1020–1035 (2020).

71. Sherwin, E., Bordenstein, S. R., Quinn, J. L., Dinan, T. G. & Cryan, J. F. Microbiota and the social brain. *Science* **366**, (2019).

72. Knutie, S. A., Wilkinson, C. L., Kohl, K. D. & Rohr, J. R. Early-life disruption of amphibian microbiota decreases later-life resistance to parasites. *Nat. Commun.* **8**, 86 (2017).

73. Finn, K. R., Silk, M. J., Porter, M. A. & Pinter-Wollman, N. The use of multilayer network analysis in animal behaviour. *Anim. Behav.* **149**, 7–22 (2019).

74. Wilson, S. N. *et al.* How Emergent Social Patterns in Allogrooming Combat Parasitic Infections. *Front. Ecol. Evol.* **8**, (2020).

75. O'Dwyer, K., Dargent, F., Forbes, M. R. & Koprivnikar, J. Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: A meta-analysis. *J. Anim. Ecol.* **89**, 519–529 (2020).

76. Ezenwa, V. *et al.* Host behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. *Proc. R. Soc. B Biol. Sci.* **283**, 20153078 (2016).

77. Hawley, D. M. & Ezenwa, V. O. Parasites, host behavior and their feedbacks. in *Animal Behavior and Parasitism* (Oxford University Press, 2022).

78. Devaux, C. A., Mediannikov, O., Medkour, H. & Raoult, D. Infectious Disease Risk Across the Growing Human-Non Human Primate Interface: A Review of the Evidence. *Front. Public Health* **7**, 305 (2019).

79. Sharp, P. M., Rayner, J. C. & Hahn, B. H. Great Apes and Zoonoses. *Science* **340**, 284–286 (2013).

80. Liu, W. *et al.* Origin of the human malaria parasite Plasmodium falciparum in gorillas. *Nature* **467**, 420–425 (2010).

81. Rupp, S., Ambata, P., Narat, V. & Giles-Vernick, T. Beyond the Cut Hunter: A Historical Epidemiology of HIV Beginnings in Central Africa. *EcoHealth* **13**, 661–671 (2016).

82. Hoffmann, C. *et al.* Persistent anthrax as a major driver of wildlife mortality in a tropical rainforest. *Nature* **548**, 82–86 (2017).

83. Leroy, E. M. *et al.* Multiple Ebola Virus Transmission Events and Rapid Decline of Central African Wildlife. *Science* **303**, 387–390 (2004).

84. Estrada, A. *et al.* Impending extinction crisis of the world's primates: Why primates matter. *Sci. Adv.* **3**, e1600946 (2017).

85. Herrera, J. & Nunn, C. L. Behavioural ecology and infectious disease: implications for conservation of biodiversity. *Philos. Trans. R. Soc. B Biol. Sci.* **374**, 20180054 (2019).

86. McDonald, R. A., Delahay, R. J., Carter, S. P., Smith, G. C. & Cheeseman, C. L. Perturbing implications of wildlife ecology for disease control. *Trends Ecol. Evol.* **23**, 53–56 (2008).

5 General discussion

In this study, the temporal fluctuations of the gut microbiome from wild redfronted lemurs were investigated and the factors shaping the entire and potential active bacterial communities determined. We aimed at understanding the impact of social relationships on gut microbiome composition and diversity. Furthermore, the influence of sociality on parasite transmission was investigated as the gut microbiome can be transmitted and affected by similar mechanisms in group-living individuals.

5.1 Gut microbial communities from wild redfronted lemurs and their temporal dynamics

5.1.1 Entire and potential active bacterial community differ in most abundant organisms but not in overall composition

The entire and active bacterial community were investigated and compared in chapter 2. The most abundant phyla in the entire bacterial community identified were *Bacteroidota*, *Firmicutes*, *Proteobacteriota*, *Spirochaetota*, *Verrucomicrobiota*, and *Actinobacteriota*. The same taxa have been detected in humans (Pasolli *et al.*, 2019) and non-human primates, such as lemurs (Springer *et al.*, 2017; Greene *et al.*, 2020), great apes (Degnan *et al.*, 2012; Gogarten *et al.*, 2018; Hicks *et al.*, 2018), geladas (Baniel *et al.*, 2021), free-ranging Rhesus macaques (Janiak *et al.*, 2021), white-faced capuchins (Orkin *et al.*, 2019) and colobus monkeys (Gogarten *et al.*, 2018). These results were consistent in the four studied groups, but their abundances varied slightly during the study, *Firmicutes* and *Bacteroidota* were the most abundant. These two phyla are reported as the most abundant in the gut microbiome of several non-human primates with the exception of white-faced capuchins (Orkin *et al.*, 2019), and yellow baboons (Ren *et al.*, 2016).

The most abundant bacterial genera found are undescribed organisms belonging to the families *Prevotellaceae*, *Spirochaetaceae*, and *Rikenellaceae*, and the order *Kiritimatiellae*>WCHB1-41. This supports the notion that the gut microbiome of non-human primates harbors many unexplored bacterial species (Manara *et al.*, 2019). In humans, *Prevotellaceae* and *Spirochaetaceae* have been linked to plant-rich diets, and

are more abundant in people living in rural settings with traditional lifestyles (Jagsi *et al.*, 2017). *Prevotella* members degrade plant polysaccharides (Accetto and Avguštin, 2015; Ley, 2016), which is possibly the function of the undescribed genus detected in the gut of redfronted lemurs. However, strains from *Prevotella* exhibit a high level of genomic diversity making it difficult to predict its function and thus highlighting the importance of characterizing these undescribed bacteria to assess their activity in the gut (Ley, 2016). Furthermore, different ASVs were classified as undescribed *Prevotellaceae* suggesting that different strains thrive in the gut of lemurs. High abundances of *Prevotella* or an unclassified genus from *Prevotellaceae* have been also reported in lemurs (Springer *et al.*, 2017; Manara *et al.*, 2019; Greene *et al.*, 2020) and great apes (Degnan *et al.*, 2012; Hicks *et al.*, 2018). Although an apparent increase of *Prevotellaceae* during the rainy season was observed for all groups, no significant differences in its abundances between seasons were detected. We could not classify the most abundant *Spirochaetaceae* to genus level, but our results coincide with reports of *Treponema* being highly prevalent in non-human primates (Manara *et al.*, 2019), and in humans from non-industrialized countries (Jagsi *et al.*, 2017). A comparative metagenomics study from non-human primates showed that these treponemes are host-specific and provide the host with pathways for the metabolism of sucrose, glycerolipid, glycerophospholipid, sulfur and methane, and the biosynthesis of amino acids (Manara *et al.*, 2019). From the undescribed genera, *Kiritimatiellae* WCHB1-41 belonging to the *Verrucomicrobiota* is the least described. The member of the class *Kiritimatiellae* or the phylum *Verrucomicrobiota* have been identified in the gut microbiota of baboons (Ren *et al.*, 2016), geladas (Baniel *et al.*, 2021) and lemurs (Greene *et al.*, 2020). *Verrucomicrobiota* were detected in humans from industrialized countries where the members have been identified as a mucin-utilizing (Jagsi *et al.*, 2017). *Rikenellaceae* has been reported in lemurs (Greene *et al.*, 2020), Rhesus macaques (Janiak *et al.*, 2021), geladas (Baniel *et al.*, 2021), yellow baboons (Ren *et al.*, 2016) and humans (Schnorr *et al.*, 2014). Genera from *Rikenellaceae* can ferment carbohydrates or proteins and have been associated to high-fat diets in mice (Daniel *et al.*, 2014; Su *et al.*, 2014). Thus, the most abundant genera detected are important for the digestion of a plant-based diet as the one from redfronted lemurs.

Firmicutes were more abundant and the predominant phyla in the potential active community compared to the entire community. Hence, indicating that despite

Bacteroidota and *Firmicutes* having similar relative abundances at the entire community level, *Firmicutes* are more actively replicating and possibly carrying out more functions. The relative abundances of *Actinobacteriota* are also higher in the active community compared to the entire community. Furthermore, some of the most abundant genera differ as well. *Colidextribacter* and *Collinsella*, genera belonging to *Firmicutes* and *Actinobacteriota.* respectively, are highly abundant in the active community. Even though a species from *Colidextribacter* was isolated from a human, it has not been genomically or metabolically characterized (Ricaboni *et al.*, 2017). Furthermore, this genus was significantly more abundant during the dry season. The second most abundant genus, *Collinsella,* can metabolize different types of polysaccharides producing different types of acids, such as acetic, formic, lactic and butyric acid (Kageyama and Benno, 2000; Qin *et al.*, 2019). *Collinsella* has been reported in several non-human primates including white-faced capuchins (Orkin *et al.*, 2019), western lowland gorillas (Gomez *et al.*, 2015), mantled howler monkeys (Clayton *et al.*, 2016), Rhesus macaques (Janiak *et al.*, 2021), yellow baboons (Grieneisen *et al.*, 2021), captive marmosets (Zhu *et al.*, 2020) and black-and-white ruffed lemur (*Varecia variegata*) (McKenney, O'Connell, *et al.*, 2018). In humans, this genus correlates negatively to fiber intake and associates to several pathologies (Gomez-Arango *et al.*, 2018; Astbury *et al.*, 2020; Zheng, Liwinski and Elinav, 2020). In contrast to humans, this bacterial genus in non-human primates should associate to plant-based diets, as these are their main dietary items. Therefore, these bacteria found in non-human primates should be further investigated to determine their function as it might differ from what has been described so far in humans or laboratory animals.

Despite the disparities between the most abundant taxa in the entire and the active communities, their composition was not significantly different. Hence, studying the entire community does provide insights into the gut microbiome, which is satisfactory as DNA-based marker gene analysis is more widely use thus allowing comparative studies (Knight *et al.*, 2018). Although, in chapter 2 we showed it is possible to perform RNA-based marker gene analysis in field research. However, the differences detected in bacterial relative abundances do highlight the importance of studying the functional counterpart of the gut microbiome to understand the activities and roles in this ecosystem (Heintz-Buschart and Wilmes, 2018). Furthermore, DNA-based marker gene analysis may be biased by the amplification of nucleic acids from dormant cells,

not functionally active bacteria, or dead cells (De Vrieze *et al.*, 2018). Nonetheless, is also important to consider that marker gene analysis is impacted by 16S rRNA gene copy numbers, particularly RNA-based marker gene analysis (Louca, Doebeli and Parfrey, 2018). For instance, the genomes from *Firmicutes* can have 5.8 ± 2.8 copies, a number varying within the phylum (Větrovský and Baldrian, 2013). Therefore, a higher number of 16S rRNA copy number could inflate the relative abundances of *Firmicutes*, as detected in the potential active community. To conclude, investigating the entire bacterial community does portrait the composition of the gut microbiome but to determine the major functional bacteria RNA methods should be attempted.

Bacterial alpha diversity presented monthly fluctuations in all groups in chapter 3, and in both the entire and the active community in chapter 2. The highest values were detected at the end of the dry season (September 2018-October 2018) and the beginning of the next rainy season (November 2018), as detected in previous longitudinal studies in humans (Jagsi *et al.*, 2017) and yellow baboons (Ren *et al.*, 2016), and Verreaux's sifakas (Springer *et al.*, 2017) living in the same forest. Furthermore, during the rainy season (December 2018-March 2019) and the transition to the next dry season (April 2019), alpha diversity measurements varied more compared to the dry season. The potential active community had a lower alpha diversity than at the entire community level.This indicates metabolic redundancy in the bacterial community meaning that several members can perform the same metabolic function, thus not all of them are all actively replicating at the same time or enter dormant states (De Vrieze *et al.*, 2018; Heintz-Buschart and Wilmes, 2018). Overall, these results imply that there is resilience in the gut microbiome because if there are perturbations in the taxonomic structure of the community other members can perform the same function thereby maintaining stability of the system (Heintz-Buschart and Wilmes, 2018).

In chapter 2 the temporal dynamics of the entire and active bacterial communities were detected in a time series analysis because the gut microbiome composition became more different the longer the timespan between samples. Similar observations were obtained in humans (Caporaso *et al.*, 2011; Jagsi *et al.*, 2017) and yellow baboons (Ren *et al.*, 2016). A longer-term study could help determine if samples from the same season but from different years are more similar between them, thus showing a cycling

of the gut microbiome. Similarly, to reports from the Hadza hunter-gatherers (Jagsi *et al.*, 2017), a rural tribe whose diet varies according to seasonality, and in wild mice (Maurice *et al.*, 2015). Additionally, the PCoA analysis (Fig.3B, chapter 2) showed that samples from the dry season clustered together, whereas sample from the rainy season did not. Thus, the bacterial community fluctuates more during the rainy season, which could be due to dietary changes or higher availability of water sources, which will be discussed in the section 5.2. Similar patterns were reported in Tibetan macaques (Sun *et al.*, 2016).

5.1.2 Only one archaeon family is part of the gut microbiome

The archaeal community was assessed with two different sets of primers designed for archaeal aiming to recover sequences of different lineages. The first set of primers was a combination of those published by Porat *et al.*, 2010 and Gantner *et al.*, 2011 (Porat *et al.*, 2010; Gantner *et al.*, 2011), and the second were proposed by Bahram *et al.*, 2019 (Bahram *et al.*, 2019). However, only the family *Methanomethylophilaceae* was detected. Similar to humans, the archaeal community of the gut of redfronted lemurs has a low diversity (Koskinen *et al.*, 2017; Nkamga, Henrissat and Drancourt, 2017). *Methanomethylophilaceae* has been found in the gut microbiome of humans, and the order *Methanomassilicoccales* has been detected in great apes (Koskinen *et al.*, 2017; Raymann *et al.*, 2017). The role of these methanogens in the human gut is to transform the excess H_2 to methane improving digestion and conversion of toxic metals and metalloids (Koskinen *et al.*, 2017; Nkamga, Henrissat and Drancourt, 2017).

5.1.3 Diverse helminths and protists from the gut of redfronted lemurs

This research supports previous morphological studies from redfronted lemurs in which high diversity of protist and helminths was detected (Clough, 2010; Clough, Heistermann and Kappeler, 2010). Helminths were more abundant and more diverse than protists and had higher abundances at the end of the dry season (October2018). However, one major difficulty was the lack of information in databases which did not allow to classify with a taxonomic resolution higher than order level. Many of the sequences in the databases derive from eukaryotic organisms parasitizing humans or laboratory animals or model organisms (e.g., *Caenorhabditis elegans*), thus providing a hint but these classifications should be taken cautiously (Marzano *et al.*, 2017;

Coghlan *et al.*, 2019; McVeigh, 2020). For example, most of the ASVs from *Oxyuridae* were classified initially as the pinworm *Enterobius vermicularis*, a human parasite (Coghlan *et al.*, 2019; Taghipour *et al.*, 2020). However, the pinworms reported in redfronted lemurs are *Lemuricola vauceli* and *Callistoura sp*. (Clough, 2010). In the NCBI database there are no entries in the databases for *Callistoura sp.* while for *L. vauceli* there is one entry for the 28S rRNA gene and none for the 18S rRNA gene, the marker gene used in this study (Stoeck *et al.*, 2010; NCBI Resource Coordinators, 2018; Frias *et al.*, 2019). A misclassification as *E. vermicularis* may suggest the risk of zoonosis or transmission from humans to animals as transmission of *E. vermicularis* is relatively simple through ingestion or inhalation of the eggs, which could occur by fecal contamination (Taghipour *et al.*, 2020). Due to the difficulties to identify the helminths at higher taxonomic levels, another marker gene was investigated, the cytochrome C oxidase (*cox1*/COI) (Folmer *et al.*, 1994; Derycke *et al.*, 2010). This project was performed as part of a bachelor thesis where the student standardized the PCR protocol base from a study of pinworms parasitizing orangutans (Foitová *et al.*, 2014; Wiegräbe, 2020). This alternative protocol made it possible to identify the ASVs as *Lemuricola sp.* but only in few samples while many ASVs remained unclassified and the positive control, *Plectus velox*, was misclassified (Figure 1) (Wiegräbe, 2020). Thus, it was only possible to improve the classification of few ASVs. Another reported disadvantage from using of *cox1*/COI as marker gene is that the binding sites for primers are not highly conserved as in the 18S gene (Deagle *et al.*, 2014). Therefore, 18S rRNA marker gene analysis continues to be the better approach to investigate the eukaryotic community.

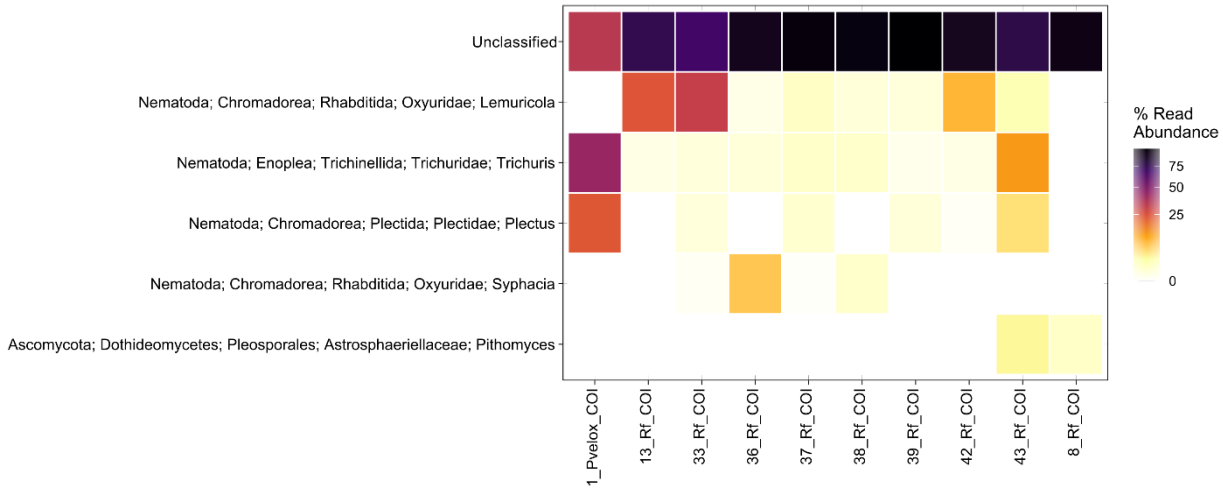


Figure 1. Heatmap showing the taxonomical classification to genus levels of ASVs obtained through cox1/COI marker gene analysis. *P. velox* was used as a positive control while the other samples are from redfronted lemurs. Modified from Wiegräbe, 2020 (Wiegräbe, 2020).

Similarly, to the helminths, the ASVs belonging to protists were classified only to order level. The most abundant protists were from *Trichostomatia*, which increased during the months of January and February. Accordingly, to previous morphological reports, the detect protist might be *Balantidium coli* (Clough, 2010). However, it might also be possible that they belong to a new undescribed protist. The second order of protist detected was *Trichomonadida*, which has been reported in these lemurs from marker gene analysis but not morphological studies previously (Clough, 2010; Gogarten *et al.*, 2020). Trichomonads are frequent inhabitants of the gut of animals, but they are difficult to detect via microscopical analysis (Li *et al.*, 2015, 2020). These results highlight the importance of molecular or metagenomic methods for increasing the detection capacity of microeukaryotes present in the gut (Tanaka *et al.*, 2014; Marzano *et al.*, 2017).

The number of ASVs obtained for helminths and protists were used to determine monthly parasite richness for each individual, however it is important to note that it is not possible to determine if the detected eukaryotes are pathogens or endosymbionts (Clough, Heistermann and Kappeler, 2010; Tanaka *et al.*, 2014). Additionally, in some cases where number of parasite richness was very high, it should be considered that helminths and protozoa may have multiple copies of the 18S gene and these copies

may vary between them, thus increasing diversity estimates (Větrovský and Baldrian, 2013; Coghlan *et al.*, 2019).

Studies including repeated sampling of individuals, such as this one, increase the detection capacity of parasites, particularly of helminths, whose release of eggs is not constant thus limiting the sensitivity of any analysis (Gillespie, 2006; Clough, Heistermann and Kappeler, 2010). Further research of the protist and helminths of redfronted lemurs should attempt their morphological and genomic characterization to provide more insights into their impact on the health of the host. As these lemurs provide an interesting study system to determine how the carriage of diverse eukaryotes impact health.

5.1.4 The unexplored gut fungi from redfronted lemurs

Although fungal organisms were only detected in low abundances, there is a gut fungal community that needs to be explored further. This study showed that there are many fungal organisms from Madagascar that remain to be characterized (Chapter 2). Second, some researchers sustained it is difficult to determine if the fungi detected in fecal samples originated from diet or the outer environment, for example coming from the inhalation of spores or as pathogens on food items (Lai, Tan and Pavelka, 2019; Nilsson *et al.*, 2019). A way to solve this issue is to focus on ASVs from previous identified gut fungi, as in this project. Another possibility is to perform a metagenomic investigation of the environment or diet jointly with the analysis of fecal samples, as done for lemurs who feed on soil (geophagic) (Borruso *et al.*, 2021) and mice (Iliev *et al.*, 2012). Additionally, other researchers recommend cultivation of the organisms from the fecal samples as an indication for gut symbionts (Hamad *et al.*, 2014; Auchtung *et al.*, 2018). Third, previous reports from humans state fungi abundances in the gut are relatively low compared to bacteria, hence possibly environmental contaminants will be most of the amplified and sequenced material (Auchtung *et al.*, 2018; Laforest-Lapointe and Arrieta, 2018; Nilsson *et al.*, 2019). Perhaps, including a lyticase treatment to degrade the fungal cell wall during DNA extraction can increase the amount of material without altering the bacterial community (Pierre *et al.*, 2021). *Fusarium*, *Penicillium, Cladosporium* and *Aspergillus*, fungi detected in abundances >1% in this study, have been identified in the feces of indri (*Indri indri*) lemurs (Borruso

et al., 2021), Tibetan macaques, humans and mice (Li *et al.*, 2018; Sun *et al.*, 2018; Borruso *et al.*, 2021). These fungi have enzymes for the degradation of plant polysaccharides, thus aiding in the processing of the host's diet (Liao *et al.*, 2014). Yeasts previously reported as gut symbionts from humans and mice such as *Malassezia*, *Saccharomyces*, *Candida and Cryptococcus* were detected in very low abundances but these could portray their low abundances in the gut of lemurs (Nash *et al.*, 2017; Li *et al.*, 2018). However, in the case of *Candida* it has been proposed that they are members of the oral microbiota and are therefore detected in fecal samples, which should be taken into consideration (Auchtung *et al.*, 2018). Nevertheless, this study supports that there are gut fungi in non-human primates despite a previous report suggesting the opposite, which used the 18S rRNA gene instead of the recommended ITS region (Mann *et al.*, 2020).

5.2 Drivers of gut microbiome composition and diversity in wild redfronted lemurs

This study demonstrated that social relationships, HPA axis activation, diet, precipitation, and parasite richness impact the gut microbiome of wild redfronted lemurs, whereas no influence of sex and age were detected. This was investigated by a thorough study design including the time series collection of fecal samples coupled with behavioral and environmental data to investigate gut microbiome community structure and temporal variability of community composition and the drivers of alteration.

5.2.1 Social relationships and their impact on the gut microbiome

The influence of social interactions on gut microbiome composition was assessed as monthly rates of affiliative interactions (chapter 2) and as diversity of social interactions and correlations between social networks and bacterial indicator networks (chapter 3). In chapter 2, monthly rates of affiliative interactions were used to determine how temporal changes in social behaviors can impact gut microbiome structure and diversity. Affiliative interactions correlated to changes in beta diversity during the months of the dry season (June-August, figure2). This time coincides with the lowest temperatures during the study period which increases behaviors of social

thermoregulations such as huddling (Ostner, 2002). Furthermore, the rate of affiliative interactions influenced microbiome composition and taxon-specific effects (chapter 2). Thus, monthly changes in social behaviors influenced temporal shifts in gut microbiome diversity and composition.

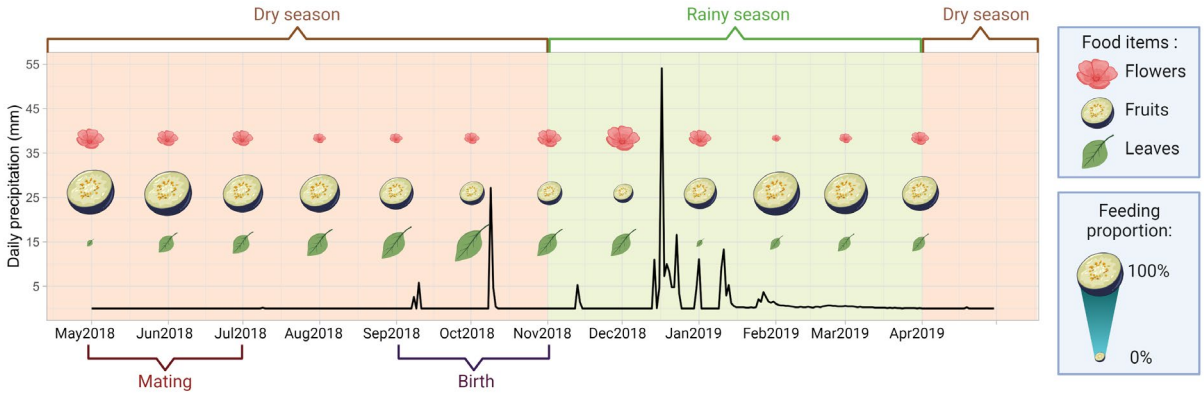


Figure 2. Alteration of preferred food items of redfronted lemurs and precipitation in Kirindy Forest from May 2018 until April 2019, and time delimitation of the environmental (dry - rainy) and behavioral (mating - breeding) seasons as defined by previous studies. Created with BioRender.com

Previous studies have used group size as a proxy for determining correlations between higher number of interacting partners and higher bacterial alpha diversity (Grieneisen *et al.*, 2017). As determined in this study (chapter 4) investigating only group size might provide information about the pool of available microorganisms within a population but it disregards the social dynamics within a group and assumes constant interactions rates between individuals (Patterson and Ruckstuhl, 2013; Briard and Ezenwa, 2021). Furthermore, it does not consider the different transmission routes of microorganisms (Briard and Ezenwa, 2021). Therefore, to investigate if a higher number of partners or interactions influenced bacterial alpha diversity a social interaction diversity index was calculated in this study (chapter 3). This index estimated the number of interacting partners of an individual, the duration and how distributed were these interactions between the dyad. To determine if a higher number of interacting partners and longer interactions increased bacterial alpha diversity, however no effects were detected. Diversity of social interactions had a low impact on beta diversity and a small number of bacterial taxa associated negatively to this factor. The smaller impact detected for diversity of social interactions could be caused by the temporal distribution of fecal

samples or variations in behaviors between groups reducing the capacity to detect stronger effects (Ren *et al.*, 2016).

In this study (chapter 3), social networks were used to predict sharing of bacterial ASVs between individuals. Social network analysis can be applied to understand the transmission of the gut microbiome through social interactions, as it has been done for parasites (chapter 4). Research from yellow baboons (Tung *et al.*, 2015), Verreaux's sifakas (Perofsky *et al.*, 2017) and wild mice (Raulo *et al.*, 2021) have shown that social networks predict gut microbiome similarity. These previous reports compared social networks with microbiome dissimilarity matrices (Bray-Curtis or Weighted Unifrac) (Tung *et al.*, 2015; Perofsky *et al.*, 2017; Raulo *et al.*, 2021). In contrast, a novel approach was used in this study because social networks were compared to bacterial indicator networks to ASV level. Correlations between indicator ASVs and social networks were detected in three of the four groups, thus suggesting transmission of bacteria through social interactions (Fig.5, chapter 3). Previous longitudinal studies have not achieved to detect these effects due to the temporal distribution of samples obscuring them (Ren *et al.*, 2016).

Group membership was one of the strongest predictors of gut microbiome diversity and composition, indicating transmission of microorganisms between group members and showing that each group had a distinct gut microbiome (Degnan *et al.*, 2012; Bennett *et al.*, 2016; Amato *et al.*, 2017; Grieneisen *et al.*, 2017; Raulo *et al.*, 2017; Springer *et al.*, 2017; Gogarten *et al.*, 2018). However, kin relationships or distinctive home ranges could also influence group differences in gut microbiome composition and diversity, as previously reported (Amato *et al.*, 2013; Tung *et al.*, 2015; Springer *et al.*, 2017; Grieneisen *et al.*, 2021). Nonetheless, an overall impact of social interactions through diverse social measurements was detected.

As investigated in the third study (chapter 4), social interactions may also be important to potentiate social immunity through the exposure to low doses of a pathogen or sharing beneficial microorganisms (Kappeler, Cremer and Nunn, 2015; Ezenwa *et al.*, 2016). For instance, the gut microbiome is essential for the proper development of the immune response thus certain essential microorganisms could be acquired at a young age through social relationships (Round and Mazmanian, 2009; Clemente *et al.*, 2012;

Laforest-Lapointe and Arrieta, 2017). Furthermore, acquisition of gut microbiome through social interactions can protect against pathogens through colonization resistance (Bansal *et al.*, 2010; Estrela, Whiteley and Brown, 2015; McKenney, Koelle, *et al.*, 2018). For example, social interactions in bumble bees (*Bombus terrestris*) protect them against the parasite *Crithidia bombi* (Koch and Schmid-Hempel, 2011).

Group-living can provide other advantages for acquisition of the gut microbiome (chapter 4). Social learning of behaviors such as, coprophagy (feeding on feces), or consumption of specific food items, and social support through food-sharing might be important for the development of the gut microbiome (Sarkar *et al.*, 2020). Coprophagy has been observed in mammals as a mechanism for acquiring nutrients, however microorganisms from the fecal sample are also ingested (Overdorff, 1993; McKenney, Koelle, *et al.*, 2018; Caruso *et al.*, 2019; Raulo *et al.*, 2021). For instance, cohousing laboratory mice is used as a mean for normalization of the microbial communities through coprophagy (Caruso *et al.*, 2019). Furthermore, in Brandt's voles (*Microtus brandti*), prevention of coprophagy produced changes in the composition and diversity of the gut microbiome (Bo *et al.*, 2020). As an example of social support, koala (*Phascolarctos cinereus*) mothers provide their juveniles during weaning with a pap, a special form of their feces. In this way, the bacteria necessary for the digestion of eucalyptus are transferred (Osawa, Blanshard and Callaghan, 1993).

5.2.2 Short-term dietary changes impact the gut microbiome

It is well established that diet impacts the gut microbiome, but most studies in wild animals determined these effects indirectly through detecting seasonal changes or measuring fluctuations in food availability (David *et al.*, 2014; Sun *et al.*, 2016; Hicks *et al.*, 2018; Baniel *et al.*, 2021). The few studies that performed behavioral observations of feeding behaviors were cross-sectional, thus missing some dietary changes or are based on data from group scans (Amato *et al.*, 2014; Ren *et al.*, 2016; Springer *et al.*, 2017; Orkin *et al.*, 2019). In this project, focal feeding behaviors were recorded and their monthly fluctuations were determined as shown in figure 2. A direct influence of consumption of leaves, fruits, and flowers on the gut microbiome through coupling focal data with fecal samples was detected. We detected a stronger impact of feeding behaviors on alpha and beta diversity because the study in chapter 2

focused on one group thereby removing the confounding factor of group membership. In addition, this group was sampled more regularly, thus increasing thereby the statistical robustness of the data (Degnan *et al.*, 2012; Grieneisen *et al.*, 2017; Björk *et al.*, 2019). Nevertheless, feeding on leaves correlated with lower bacterial alpha diversity in both studies. Furthermore, some of the bacterial genera positively associated to changes in fruit feeding such as *Succinivibrio*, *Phascolarctobacterium*, S*uccinivibrionaceae* and *Prevotellaceae* coincide in both studies. However, some other positively associated taxa differ between studies, such as *Anaerovibrio* and *Bacteroides*. Also, in the second study (chapter 3) taxa positively and negatively associated to feeding on leaves were detected that were not identified in the first one. Discrepancies between the associated taxa for each food item can occur due to the use of different statistical methods for the determination of taxon-specific effects. In the first study (chapter 2), a linear mixed model which tested the effects of the covariates in the random effect of taxon was generated (Sweeny *et al.*, 2020). This model could not be used for the second study (chapter 3) with more data points due to computational limitations associated to the complexity and heterogeneity of metagenomic datasets, so the linear mixed models from MaAsLin2 (Microbiome Multivariable Associations with Linear Models) were used (Mallick *et al.*, 2021). MaAsLin2 is the improved version from MaAsLin for longitudinal metagenomic studies with the possibility to control for repeated sampling (Mallick *et al.*, 2021). MaAsLin calculates multivariate linear models associating the covariates with each taxon independently, and any covariate selected in at least 1% of the iterations is tested for significance in a standardized generalized linear mixed model (Morgan *et al.*, 2012, 2015). Furthermore, discrepancies between the differentially abundant taxa might be due to individual differences in feeding behaviors, which may have a greater impact in the second study including more individuals. Thus, also showing that extrapolating the dietary changes of the whole group from individual feeding behaviors is not precise enough. Also, monthly group differences in preferred food items were detected in the chapter 3 showing that alterations in food availability do not necessary represent what the animals are feeding on. All data considered, we detected bacteria relevant for the digestion of specific food items, and short-term dietary changes such as, consumption of fruits and flowers impact gut microbiome composition and diversity. Further functional investigations from the differentially abundant bacteria using other omics

methods will provide more information on their role in the gastrointestinal metabolism (Heintz-Buschart and Wilmes, 2018).

5.2.3 HPA axis activation influences diversity and composition of the gut microbiome

In the second study (chapter 3) it was determined that social stressors increase bacterial alpha diversity, explain variation in beta diversity and associate to differentially abundant taxa. The mean highest fGCM levels were detected in June during the mating season (figure 2) for three of the four groups suggesting that social stressors associated to an increase in bacterial alpha diversity during this period. Accordingly, previous research from males of this population identified a significant increase in fGCM levels during the mating season (Ostner, Kappeler and Heistermann, 2008). Reported behaviors such as mate-guarding and male-male aggressions during the mating season could increase fGCM levels (Ostner and Heistermann, 2003). Females are also expose to stressful situations, which might increase fGCM levels during this time, since there is reproductive competition and the risk of eviction by other females (Kappeler and Fichtel, 2012b). Although in this previous study the higher fGCM levels detected in males redfronted lemurs were not explained by rank differences, incremented aggressions between group members or a surge in intergroup encounters (Ostner, Kappeler and Heistermann, 2008). Significant differences in fGCM levels between the dominant and the subordinate males of the same group were identified suggesting that either individual or subtle factors not related to rank influence fGCM levels (Ostner, Kappeler and Heistermann, 2008). This previous study and the results from chapters 3 and 4 indicate that the mating season is a period of social unrest possibly for all group members influencing HPA axis activation and increasing bacterial alpha diversity. Furthermore, fGCM levels were strong predictors of variance in beta diversity and 17 bacterial genera associated positively or negatively to them (discussed in detail in chapter 3). Most of the research on the microbiome-gut-brain axis focuses on the impact of the microorganisms on the brain and metabolism, and has been associated to diverse pathologies such as, psychiatric disorders, obesity, diabetes, and inflammatory bowel syndrome (Rogers *et al.*, 2016; Gérard and Vidal, 2019; Martin *et al.*, 2019). However, less is known on how host mechanisms like HPA axis impact the gut microbiome. Immunomodulation by glucocorticoids could alter the homeostatic control of the gut microbiome by the

immune response (Chu and Mazmanian, 2013). Furthermore, activation of gluconeogenesis by glucocorticoids could also be implicated, as SCFAs produced by intestinal bacteria are substrates for this mechanism (Gérard and Vidal, 2019; Martin *et al.*, 2019). Investigating the direct links of these two processes might provide clearer explanations of how the HPA axis influences the gut microbiome.

5.2.4 Environmental changes due to precipitation explain most of the variance in diversity and composition

The impact of fluctuations in precipitation on the gut microbiome of wild primates has been widely studied, and its influence has been frequently associated to changes in food availability given that diet is such a strong predictor of gut microbiome composition and diversity (Ren *et al.*, 2016; Hicks *et al.*, 2018; Baniel *et al.*, 2021). However, more direct links between environmental precipitation and gut microbiome have not been investigated. In this project daily precipitation was recorded (figure 2) and investigated separately from diet or seasonal effects. In both studies (chapters 2 and 3) higher precipitation correlated to a decrease on bacterial alpha diversity. Precipitation changes also explained variation in beta diversity and had the highest number of negatively and positively associated taxa. Although, there are differences of the associated bacterial genera to precipitation between studies, as mentioned before this could be due to the use of two different statistical tools for the identification of these relationships (Sweeny *et al.*, 2020; Mallick *et al.*, 2021). Also, the second study (chapter 3) had a higher number of samples and included more individuals, thus accounting for individual or group differences regarding its impact. Nevertheless, associated genera between studies were shared. For example, negative associations to *Alloprevotella*, *Tannerallaceae*, *Syntrophococcus*, and *Erysipelotrichaceae* were detected, whereas *Prevotellaceae* and *Synergistaceae* were positively associated. Precipitation can influence the gut microbiome by changing water availability, reducing water intake, decreasing gut transit time and impacting the gut-niche (Vandeputte *et al.*, 2016). Higher precipitation translates into more available water drinking sources and is indicative of lower stool consistency and higher gut transit times (Vandeputte *et al.*, 2016). Coincidentally, in the first study (chapter 2) higher precipitation correlated to lower alpha diversity possible due to an increase in water intake which influences gut transit times. Moreover, animals could uptake microorganisms from water sources and

if these sources change due to precipitation, then the ingestion and availability of these microorganisms in the gut will also be affected (Ren *et al.*, 2016; Browne *et al.*, 2017). Further clarification of the direct links between precipitation and the gut microbiome could be achieved by 1) investigating drinking behaviors and stool consistency and 2) determining the microbial communities from these water sources to investigate correlations indicating transmission of microorganisms from water.

5.2.5 Assessment of transkingdom interactions between bacteria and eukaryotic parasites

Parasite richness had a small impact on the variability in beta diversity and did not influence bacterial alpha diversity (Chapter 3). Previous studies have found that the impact of eukaryotic parasites on bacterial alpha diversity varies according to the parasite species (Reynolds, Finlay and Maizels, 2015; Yang *et al.*, 2017; Wei *et al.*, 2020). Thus, it is possible that no correlations between parasite richness and bacterial alpha diversity were detected as all detected protozoa and helminths were analyzed together. Furthermore, it was not feasible to determine differences in bacterial alpha diversity associated to presence/absence of parasites between or within individuals, as all identified parasites were prevalent over the entire year. Thus, it was not feasible to compare infected vs. uninfected individuals as done in other studies. Moreover, no investigations between fluctuations in the abundances of a particular parasite and the gut microbiome were performed, as values from 18S rRNA do not portray reliably the abundances of each parasite in the gut (Gogarten *et al.*, 2020). Likewise, parasite richness only explained a low amount of variance in beta diversity possibly due to the same reasons as for alpha diversity. However, positive, and negative associations were detected to specific bacterial genera. Positive associations could indicate mutualistic or symbiotic interactions between parasite and bacteria, such as trophic chains or predation of stronger bacterial competitors by parasites allowing weaker competitors to thrive (Laforest-Lapointe and Arrieta, 2018). The negatively associated bacteria could be affected by competition for resources, predation or the secretion of antibacterial molecules by parasites (Cotton *et al.*, 2012; Laforest-Lapointe and Arrieta, 2018; Coghlan *et al.*, 2019). For example, *Tritrichomonas musculis*, a trichomonad symbiont of mice, competes for dietary fiber with gut bacteria (Wei *et al.*, 2020). Protozoa and helminths can also influence gut bacteria indirectly through their

interactions with the immune response or by changing the gut niche (Reynolds, Finlay and Maizels, 2015). For instance, parasitic nematodes use excretory-secretory products to immunomodulate inducing regulatory T cells, blocking pro-inflammatory responses, and activating Th2 immunity (Harnett, 2014; Afrin *et al.*, 2019). Infections with *Enterobius vermicularis*, a nematode parasitizing humans, are associated with decreased levels of secretory immunoglobulin A (SIgA) essential for controlling the gut bacteria and protect against bacterial pathogens (Macpherson, Geuking and McCoy, 2005; Palm *et al.*, 2014; Taghipour *et al.*, 2020). Conversely, they can also increase antibacterial defenses, as reported in *T. musculis*, which activates the immune response of intestinal epithelial cells (Chudnovskiy *et al.*, 2016). Parasites can also damage intestinal epithelial junctions allowing translocation of bacteria from the lumen to other tissues increasing the risk of sepsis (Afrin *et al.*, 2019). Additionally, they stimulate mucus production that can promote mucin-utilizing bacteria (Reynolds, Finlay and Maizels, 2015). Mucin production from the activation of Th2 immune responses during mice infections with the nematode *Trichuris muris* favor the growth of *Clostridiales* inhibiting colonization by *Bacteroides vulgatus*, a proinflammatory species (Ramanan *et al.*, 2016). Likewise, increase mucus production in *Rhesus macaques* infected with the nematode *Trichurus trichiura* decrease the attachment of pro-inflammatory bacteria decreasing gut inflammation (Broadhurst *et al.*, 2012). In conclusion, it is important to note that eukaryotic-prokaryotic interactions are microorganism-specific as parasites have wide impacts in the gut niche. They are also parasite – bacteria species specific as seen in *T. musculis* for which competitive, and cooperative interactions to different species of *Bifidobacterium* were detected (Wei *et al.*, 2020). In this study, some of the bacterial genera impacted by the parasitic consortium were identified. The next steps should attempt to identified parasite species specific interactions with bacteria and the underlying mechanisms. This could be performed by combining 16S and 18S rRNA marker gene analysis with parasite morphological studies and non-invasive immunological markers (Reynolds, Finlay and Maizels, 2015; Heitlinger *et al.*, 2017).

5.2.6 Small influence of host age and sex on the gut microbiome

Age only explained a small amount of variance on beta diversity and one bacteria genus associated to this variable (chapter 3). Age differences in gut microbiome

composition and diversity are mostly detected during infancy (Arrieta *et al.*, 2014). It is feasible that no large effects of age were identified as the infants were sampled relatively late, starting from six months of age, and only during their lasts six months of infancy. With twelve months they are already juveniles (Kappeler and Fichtel, 2012b). Furthermore, there were only six infants during the study period reducing the statistical power (Björk *et al.*, 2019). Despite the low number of individuals investigated, this study suggests age-related differences in gut microbiome occur during beginning of infancy (<6 months of age) in redfronted lemurs. Moreover, it indicates that the gut microbiome of redfronted lemur juveniles does not differ from the one of adults, as seen in Rhesus macaques (Rhoades *et al.*, 2019; Janiak *et al.*, 2021). Nonetheless, *Roseburia* was negatively associated to age. This genus degrades β-mannans producing butyrate, which is the main energy source for colonic cells, has anti-inflammatory effects and possess barrier protective properties thus, it relates to gut health (La Rosa *et al.*, 2019). In addition, apparent effects of older age on the gut microbiome were not detected. Possibly, this was because these lemurs in the wild do not reach an age where this effect can be identified due to predation and other pressures (Fichtel and Kappeler, 2002). For instance, in humans this impact is distinguished in elder individuals (>60 years) (Claesson *et al.*, 2011; Jackson *et al.*, 2016). So far, an effect of age on the gut microbiome of wild animals, has been difficult to detect, or is only identified when investigating age categories and not age as a continuous variable, as done in this study (Degnan *et al.*, 2012; Bennett *et al.*, 2016; Heitlinger *et al.*, 2017; Raulo *et al.*, 2017; Pafčo *et al.*, 2019; Janiak *et al.*, 2021). In the second study (chapter 3), host sex only explained a small amount of variance on beta diversity. Host sex differences in gut microbiome composition and diversity have been difficult to detect in previous studies, although they should be expected as sexual dimorphic immunity could impact the interaction between the immune response with gut members (Elderman, de Vos and Faas, 2018). These difficulties suggest that the effect is not strong and therefore hard to detect with other confounding factors. For example, in gorillas host's sex effects on the gut microbiome were identified only during a particular season (Pafčo *et al.*, 2019), and the impact detected in chimpanzees could not be distinguished from dietary effects (Degnan *et al.*, 2012). However, an effect due to the gonadal hormones has been seen in interventional studies in laboratory animals and rufous mouse lemurs (Yurkovetskiy *et al.*, 2013; Moreno-Indias *et al.*, 2016; Aivelo and Norberg, 2017). Perhaps future

attempts to identify sex differences in wild animals should be cross-sectional and focus on times of elevated estrogen, progesterone or androgen production, such as the mating or birth season in redfronted lemurs, to detect their impact on the gut microbiota (Ostner, Kappeler and Heistermann, 2002; Ostner and Heistermann, 2003).

5.3 Future directions: does the gut microbiome influences social behaviors?

Due to the wide known effects of the gut microbiome on the health of the host a new avenue of research is to determine if microbial mechanisms affect or even manipulate the host's social behaviors for the sake of their own transmission and survival (Sherwin *et al.*, 2019). Some studies already indicated that they do impact social behaviors (Wu *et al.*, 2021). For instance, esters and volatile fatty acids (VFA) for olfactory communication in hyenas and meerkats (*Suricata suricata*) are produced as bacterial metabolites (Theis *et al.*, 2013; Leclaire *et al.*, 2017). The paste used in olfactory signaling by hyenas is more variable between individuals and has a higher bacterial richness in the highly sociable spotted hyenas (*Crocuta crocuta*) compared to the more solitary striped hyena (*Hyena hyena*), indicating that a more complex signaling is necessary in contexts with more intricate social interactions (Theis *et al.*, 2013). In wild meerkats, the chemical composition of the anal pouch covaried with its bacterial communities, and the bacterial communities differed between dominant and subordinate males indicating a participation of bacteria on communicating rank information (Leclaire *et al.*, 2017). Also, in invertebrates presence of specific bacterial genera or pheromones produced by gut bacteria promote the aggregation of conspecifics (Wada-Katsumata *et al.*, 2015; Sherwin *et al.*, 2019). Human neuropsychiatric disorders associated with deficits in social behaviors like autism spectrum disorder, social anxiety, depression, and schizophrenia can associate to perturbations in the gut microbiome (Cryan *et al.*, 2019). These changes include lower bacterial diversity, absence of beneficial taxa and presence of inflammation inducing bacteria (Rogers *et al.*, 2016). These results suggest that the gut microbiome could impact social behaviors in humans, although diet, and genotype are confounding factors limiting the explanatory power (Sherwin *et al.*, 2019). Furthermore, the gut microbiome impacts the availability of serotonin, which can also influence social

behaviors (Yano *et al.*, 2015; Martin *et al.*, 2019; Sherwin *et al.*, 2019). Laboratory animals with impaired function of the microglia present aberrant social behaviors which could be link to changes in the gut microbiome as it influences the proper development of the immune function of the microglia (Colonna and Butovsky, 2017; Sherwin *et al.*, 2019). Germ-free and antibiotic treated mice present aberrant social behaviors mediated by HPA axis activation, which can be restored by colonization of *Enterococcus faecalis* (Wu *et al.*, 2021). Thus, a complex gut microbiome and specific bacterial species dampens HPA axis activation influencing social behaviors (Wu *et al.*, 2021). Moreover, it has been speculated that social relationships could be particularly important for acquiring the microorganisms necessary for herbivorous diets, which require, compared to carnivorous diets, more diverse bacterial communities capable to degrade complex polysaccharides (Ley *et al.*, 2008; Nishida and Ochman, 2018; Sherwin *et al.*, 2019).

Hence, previous research indicate that the gut microbiome impacts sociality. It would be interesting to compare differences in composition and diversity between different types of social organization structures. In this regard, non-human primates provide unique study subjects to compare between social organization having solitary to multi-level societies and with a variety of dominant interactions, from tolerant to despotic. Perhaps even providing information regarding the influence of the gut microbiome on the evolution of sociality (Biedermann *et al.*, 2021). Most research on this topic has focused so far in hymenopteran and isopteran insects limiting the understanding of the evolution of sociality in other animal taxa (Biedermann *et al.*, 2021). Therefore, comparative studies from wild non-human primates may provide a distinct answer. Future research should aim to translate results obtained from laboratory animals and invertebrates into wild primates. For example, searching for microbial molecules or genes associated to social behaviors in other study systems in non-human primate gut microbiomes could be investigated.

5.4 Conclusion

Social relationships impact the temporal fluctuations of the gut microbiome from wild redfronted lemurs. Social relationships influence the diversity and composition of the gut microbiome directly through affiliative behaviors, which promote the transmission

of bacteria between individuals. These results indicate that there is a pool of gut bacteria that is shared within a group explaining at least partially the strong impact of group membership on gut microbiome composition and diversity. Social relationships also affect the gut microbiome indirectly through the activation of the HPA axis by social stressors inducing the release of glucocorticoids and provoking changes in the gut physiology and immunity. However, precipitation and dietary changes are the strongest drivers of temporal fluctuations in the gut microbiome. The members of the gut of redfronted lemurs include a great diversity of protozoa and helminths, fungi in low abundances and bacterial phyla, i.e. *Firmicutes*, *Bacteroidota* and *Spirochaetota*. Comparisons between the entire and potential active bacterial community indicate that to further understand the impact of these drivers, the functional pathways of these gut microorganisms should be investigated.

5.5 References general introduction and discussion

Aagaard, K. *et al.* (2014) 'The placenta harbors a unique microbiome', *Science Translational Medicine*, 6(237), pp. 237ra65-237ra65. doi: 10.1126/scitranslmed.3008599.

Abrams, G., Bauer, H. and Sprinz, H. (1963) 'Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice.', *Laboratory Investigation*, 12, pp. 355–365.

Accetto, T. and Avguštin, G. (2015) 'Polysaccharide utilization locus and CAZYme genome repertoires reveal diverse ecological adaptation of Prevotella species', *Systematic and Applied Microbiology*, 38(7), pp. 453–461. doi: 10.1016/j.syapm.2015.07.007.

Afrin, T. *et al.* (2019) 'Sequential changes in the host gut microbiota during infection with the intestinal parasitic nematode Strongyloides venezuelensis', *Frontiers in Cellular and Infection Microbiology*, 9, pp. 1–11. doi: 10.3389/fcimb.2019.00217.

Ahuja, M. *et al.* (2017) 'Orai1-mediated antimicrobial secretion from pancreatic acini shapes the gut microbiome and regulates gut innate immunity', *Cell Metabolism*. Elsevier Inc., 25(3), pp. 635–646. doi: 10.1016/j.cmet.2017.02.007.

Aivelo, T., Laakkonen, J. and Jernvall, J. (2016) 'Population-and individual-level dynamics of the intestinal microbiota of a small primate', *Applied and Environmental Microbiology*, 82(12), pp. 3537–3545. doi: 10.1128/AEM.00559-16.

Aivelo, T. and Norberg, A. (2017) 'Parasite-microbiota interactions potentially affect intestinal communities in wild mammals', *Journal of Animal Ecology*, pp. 438–447. doi: 10.1111/1365-2656.12708.

Amaral, F. A. *et al.* (2008) 'Commensal microbiota is fundamental for the development of inflammatory pain', *Proceedings of the National Academy of Sciences of the United States of America*, 105(6), pp. 2193–2197. doi: 10.1073/pnas.0711891105.

Amato, K. R. *et al.* (2013) 'Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes', *ISME Journal*. Nature Publishing Group, 7(7), pp. 1344–1353. doi: 10.1038/ismej.2013.16.

Amato, K. R. *et al.* (2014) 'The gut microbiota appears to compensate for seasonal diet variation in the wild black howler monkey (Alouatta pigra)', *Microbial Ecology*, 69(2), pp. 434–443. doi: 10.1007/s00248-014-0554-7.

Amato, K. R. *et al.* (2017) 'Patterns in gut microbiota similarity associated with degree of sociality among sex classes of a neotropical primate', *Microbial Ecology*. Microbial Ecology, 74(1), pp. 250–258. doi: 10.1007/s00248-017-0938-6.

Amato, K. R. *et al.* (2019) 'Evolutionary trends in host physiology outweigh dietary niche in structuring primate gut microbiomes', *ISME Journal*. Springer US, 13(3), pp. 576–587. doi: 10.1038/s41396-018-0175-0.

Antwis, R. E. *et al.* (2018) 'Gut microbiome composition is associated with spatial structuring and social interactions in semi-feral Welsh Mountain ponies', *Microbiome*. Microbiome, 6(1), p. 207. doi: 10.1186/s40168-018-0593-2.

Arrieta, M. C. *et al.* (2014) 'The intestinal microbiome in early life: Health and disease', *Frontiers in Immunology*, 5, pp. 1–18. doi: 10.3389/fimmu.2014.00427.

Astbury, S. *et al.* (2020) 'Lower gut microbiome diversity and higher abundance of proinflammatory genus Collinsella are associated with biopsy-proven nonalcoholic steatohepatitis', *Gut Microbes*. 11(3), pp. 569–580. doi: 10.1080/19490976.2019.1681861.

Auchtung, T. A. *et al.* (2018) 'Investigating colonization of the healthy adult gastrointestinal tract by fungi', *mSphere*, 3(2). doi: 10.1128/msphere.00092-18.

Audebert, C. *et al.* (2016) 'Colonization with the enteric protozoa Blastocystis is associated with increased diversity of human gut bacterial microbiota', *Scientific Reports*. 6, pp. 1–11. doi: 10.1038/srep25255.

Bäckhed, F. *et al.* (2005) 'Host-bacterial mutualism in the human intestine', *Science*, 307(5717), pp. 1915–1920. doi: 10.1126/science.1104816.

Bahram, M. *et al.* (2019) 'Newly designed 16S rRNA metabarcoding primers amplify diverse and novel archaeal taxa from the environment', *Environmental Microbiology Reports*, 11(4), pp. 487–494. doi: 10.1111/1758-2229.12684.

Bailey, M. T. *et al.* (2010) 'Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium', *Infection and Immunity*, 78(4), pp. 1509–1519. doi: 10.1128/IAI.00862-09.

Bailey, M. T. *et al.* (2011) 'Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation', *Brain, Behavior, and Immunity*, 25(3), pp. 397–407. doi: 10.1016/j.bbi.2010.10.023.

Baniel, A. *et al.* (2021) 'Seasonal shifts in the gut microbiome indicate plastic responses to diet in wild geladas', *Microbiome*. Microbiome, 9(1), pp. 1–20. doi: 10.1186/s40168-020-00977-9.

Bansal, T. *et al.* (2010) 'The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation', *Proceedings of the National Academy of Sciences of the United States of America*, 107(1), pp. 228–233. doi: 10.1073/pnas.0906112107.

Barbosa, A. *et al.* (2016) 'Age-related differences in the gastrointestinal microbiota of chinstrap penguins (pygoscelis antarctica)', *PLoS ONE*, 11(4), pp. 1–14. doi: 10.1371/journal.pone.0153215.

Barr, J. J. *et al.* (2013) 'Bacteriophage adhering to mucus provide a non-host-derived immunity', *Proceedings of the National Academy of Sciences of the United States of America*, 110(26), pp. 10771–10776. doi: 10.1073/pnas.1305923110.

Barrett, E. *et al.* (2012) 'γ-Aminobutyric acid production by culturable bacteria from the human intestine', *Journal of Applied Microbiology*, 113(2), pp. 411–417. doi: 10.1111/j.1365-2672.2012.05344.x.

Barton, R. A. (1987) 'Allogrooming as mutualism in diurnal lemurs', *Primates*, 28(4), pp. 539–542. doi: 10.1007/BF02380868.

Beghini, F. *et al.* (2017) 'Large-scale comparative metagenomics of Blastocystis, a common member of the human gut microbiome', *ISME Journal*, 11(12), pp. 2848–2863. doi: 10.1038/ismej.2017.139.

Belkaid, Y. and Naik, S. (2013) 'Compartmentalized and systemic control of tissue immunity by commensals', *Nature Immunology*, 14(7), pp. 646–653. doi: 10.1038/ni.2604.

Bennett, G. *et al.* (2016) 'Host age, social group, and habitat type influence the gut microbiota of wild ring-tailed lemurs (Lemur catta)', *American journal of primatology*, 78(8), pp. 883–892. doi: 10.1002/ajp.22555.

Berkelmann, D. *et al.* (2018) 'How rainforest conversion to agricultural systems in Sumatra (Indonesia) affects active soil bacterial communities', *Frontiers in Microbiology*, 9(OCT), pp. 1–13. doi: 10.3389/fmicb.2018.02381.

Berkelmann, D. *et al.* (2020) 'Soil bacterial community structures in relation to different oil palm management practices', *Scientific Data*, 7(1), pp. 1–7. doi: 10.1038/s41597-020-00752-3.

Berry, A. S. F. *et al.* (2020) 'Natural infection with Giardia is associated with altered community structure of the human and canine gut microbiome', *mSphere*, 5(4). doi: 10.1128/msphere.00670-20.

Biedermann, P. H. W. *et al.* (2021) 'Editorial: Microbial Drivers of Sociality – From Multicellularity to Animal Societies', *Frontiers in Ecology and Evolution*, 9, pp. 1–4. doi: 10.3389/fevo.2021.752906.

Biragyn, A. *et al.* (2002) 'Toll-like receptor 4-dependent activation of dendritic cells by β-defensin 2', *Science*, 298(5595), pp. 1025–1029. doi: 10.1126/science.1075565.

Björk, J. R. *et al.* (2019) 'Primate microbiomes over time: Longitudinal answers to standing questions in microbiome research', *American Journal of Primatology*, 81(10–11), pp. 1–23. doi: 10.1002/ajp.22970.

Blanton, L. V. *et al.* (2016) 'Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children', *Science*, 351(6275), pp. aad3311–aad3311. doi: 10.1126/science.aad3311.

Blekhman, R. *et al.* (2015) 'Host genetic variation impacts microbiome composition across human body sites', *Genome Biology*. Genome Biology, 16(1), pp. 1–12. doi: 10.1186/s13059-015-0759-1.

Bo, T. B. *et al.* (2020) 'Coprophagy prevention alters microbiome, metabolism, neurochemistry, and cognitive behavior in a small mammal', *ISME Journal*, 14(10), pp. 2625–2645. doi: 10.1038/s41396-020-0711-6.

Bolnick, D. I. *et al.* (2014) 'Individual diet has sex-dependent effects on vertebrate gut microbiota', *Nature Communications*, 5. doi: 10.1038/ncomms5500.

Borruso, L. *et al.* (2021) 'I Like the Way You Eat It: Lemur (Indri indri) Gut Mycobiome and Geophagy', *Microbial Ecology*. Microbial Ecology. doi: 10.1007/s00248-020-01677-5.

Bouskra, D. *et al.* (2008) 'Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis', *Nature*, 456(7221), pp. 507–510. doi: 10.1038/nature07450.

Bradley, Ian M; Pinto, Ameet; Guest, J. S. (2016) 'Gene-specific primers for improved characterization of mixed phototrophic communities', *Applied and Environmental Microbiology*, 82(19), pp. 5878–5891. doi: 10.1128/AEM.01630-16.Editor.

Breitbart, M. *et al.* (2008) 'Viral diversity and dynamics in an infant gut', *Research in Microbiology*, 159(5), pp. 367–373. doi: 10.1016/j.resmic.2008.04.006.

Briard, L. and Ezenwa, V. O. (2021) 'Parasitism and host social behaviour: a meta-analysis of insights derived from social network analysis', *Animal Behaviour*, 172, pp. 171–182. doi: 10.1016/j.anbehav.2020.11.010.

Broadhurst, M. J. *et al.* (2012) 'Therapeutic helminth infection of macaques with idiopathic chronic diarrhea alters the inflammatory signature and mucosal microbiota of the colon', *PLoS Pathogens*, 8(11). doi: 10.1371/journal.ppat.1003000.

Brooks, A. W. *et al.* (2017) 'Phylosymbiosis: Relationships and functional effects of microbial communities across host evolutionary history', *PLOS Biology*, 15(1), p. e1002587. doi: 10.1371/journal.pbio.1002587.

Browne, H. P. *et al.* (2017) 'Transmission of the gut microbiota: Spreading of health', *Nature Reviews Microbiology*, pp. 531–543. doi: 10.1038/nrmicro.2017.50.

Callahan, B. J., McMurdie, P. J. and Holmes, S. P. (2017) 'Exact sequence variants should replace operational taxonomic units in marker-gene data analysis', *ISME Journal*, 11(12), pp. 2639–2643. doi: 10.1038/ismej.2017.119.

Caporaso, J. G. *et al.* (2011) 'Moving pictures of the human microbiome', *Genome Biology*, 12(5). doi: 10.1186/gb-2011-12-5-r50.

Caruso, R. *et al.* (2019) 'Dynamic and asymmetric changes of the microbial communities after cohousing in laboratory mice', *Cell Reports*, 27(11), pp. 3401-3412.e3. doi: 10.1016/j.celrep.2019.05.042.

Cavigelli, S. A. and Caruso, M. J. (2015) 'Sex, social status and physiological stress in primates: The importance of social and glucocorticoid dynamics', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1669). doi: 10.1098/rstb.2014.0103.

Ceacero, F. *et al.* (2012) 'Benefits for dominant red deer hinds under a competitive feeding system: Food access behavior, diet and nutrient selection', *PLoS ONE*, 7(3), pp. 1–9. doi: 10.1371/journal.pone.0032780.

Chabé, M., Lokmer, A. and Ségurel, L. (2017) 'Gut protozoa: friends or foes of the human gut microbiota?', *Trends in Parasitology*, 33(12), pp. 925–934. doi: 10.1016/j.pt.2017.08.005.

Cho, I. *et al.* (2012) 'Antibiotics in early life alter the murine colonic microbiome and adiposity', *Nature*, 488(7413), pp. 621–626. doi: 10.1038/nature11400.

Choi, J. and Park, J. S. (2020) 'Comparative analyses of the V4 and V9 regions of 18S rDNA for the extant eukaryotic community using the Illumina platform', *Scientific Reports*, 10(1), pp. 1–11. doi: 10.1038/s41598-020-63561-z.

Choudhuri, S. (2014) 'Phylogenetic Analysis', in *Bioinformatics for Beginners*. Elsevier, pp. 209–218. doi: 10.1016/B978-0-12-410471-6.00009-8.

Chu, H. and Mazmanian, S. K. (2013) 'Innate immune recognition of the microbiota promotes host-microbial symbiosis', *Nature Immunology*, 14(7), pp. 668–675. doi: 10.1038/ni.2635.

Chudnovskiy, A. *et al.* (2016) 'Host-protozoan interactions protect from mucosal infections through activation of the inflammasome', *Cell*, 167(2), pp. 444-456.e14. doi: 10.1016/j.cell.2016.08.076.

Claesson, M. J. *et al.* (2011) 'Composition, variability, and temporal stability of the intestinal microbiota of the elderly', *Proceedings of the National Academy of Sciences of the United States of America*, 108, pp. 4586–4591. doi: 10.1073/pnas.1000097107.

Clarke, G. *et al.* (2013) 'The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner', *Molecular Psychiatry*, 18(6), pp. 666–673. doi: 10.1038/mp.2012.77.

Clauss, M., Hume, I. D. and Hummel, J. (2010) 'Evolutionary adaptations of ruminants and their potential relevance for modern production systems', *Animal*. Elsevier, 4(7), pp. 979–992. doi: 10.1017/S1751731110000388.

Clayton, J. B. *et al.* (2016) 'Captivity humanizes the primate microbiome', *Proceedings of the National Academy of Sciences of the United States of America*, 113(37), pp. 10376–10381. doi: 10.1073/pnas.1521835113.

Clemente, J. C. *et al.* (2012) 'The impact of the gut microbiota on human health: An integrative view', *Cell*, 148(6), pp. 1258–1270. doi: 10.1016/j.cell.2012.01.035.

Clemente, J. C. *et al.* (2015) 'The microbiome of uncontacted Amerindians', *Science Advances*, 1(3). doi: 10.1126/sciadv.1500183.

Clough, D. (2010) 'Gastro-intestinal parasites of red-fronted lemurs in Kirindy Forest, western Madagascar', *Journal of Parasitology*, 96(2), pp. 245–251. doi: 10.1645/GE-2258.1.

Clough, D., Heistermann, M. and Kappeler, P. M. (2010) 'Host intrinsic determinants and potential consequences of parasite infection in free-ranging red-fronted lemurs (Eulemur fulvus rufus)', *American Journal of Physical Anthropology*, 142(3), pp. 441–452. doi: 10.1002/ajpa.21243.

Coghlan, A. *et al.* (2019) 'Comparative genomics of the major parasitic worms', *Nature Genetics*, 51(1), pp. 163–174. doi: 10.1038/s41588-018-0262-1.

Cohen, S. *et al.* (1992) 'Chronic social stress, affiliation, and cellular immune response in nonhuman primates', *Psychological Science*, 3(5), pp. 301–305. doi: 10.1111/j.1467-9280.1992.tb00677.x.

Colonna, M. and Butovsky, O. (2017) 'Microglia function in the central nervous system during health and neurodegeneration', *Annual Review of Immunology*, 35(1), pp. 441–468. doi: 10.1146/annurev-immunol-051116-052358.

Cortés, A. *et al.* (2019) 'Helminths and microbes within the vertebrate gut - Not all studies are created equal', *Parasitology*, 146(11), pp. 1371–1378. doi: 10.1017/S003118201900088X.

Cotton, S. *et al.* (2012) 'Defense peptides secreted by helminth pathogens: Antimicrobial and/or immunomodulator molecules?', *Frontiers in Immunology*, 3(AUG), pp. 1–7. doi: 10.3389/fimmu.2012.00269.

Coutinho, A. E. and Chapman, K. E. (2011) 'The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights', *Molecular and Cellular Endocrinology*, 335(1), pp. 2–13. doi: 10.1016/j.mce.2010.04.005.

Cryan, J. F. *et al.* (2019) 'The microbiota-gut-brain axis', *Physiological Reviews*, 99(4), pp. 1877–2013. doi: 10.1152/physrev.00018.2018.

Daniel, H. *et al.* (2014) 'High-fat diet alters gut microbiota physiology in mice', *ISME Journal*, 8(2), pp. 295–308. doi: 10.1038/ismej.2013.155.

David, L. A. *et al.* (2014) 'Diet rapidly and reproducibly alters the human gut microbiome', *Nature*, 505(7484), pp. 559–563. doi: 10.1038/nature12820.

Deagle, B. E. *et al.* (2014) 'DNA metabarcoding and the cytochrome c oxidase subunit I marker: Not a perfect match', *Biology Letters*, 10(9), pp. 2–5. doi: 10.1098/rsbl.2014.0562.

Degnan, P. H. *et al.* (2012) 'Factors associated with the diversification of the gut microbial communities within chimpanzees from Gombe National Park', *Proceedings of the National Academy of Sciences of the United States of America*, 109(32), pp. 13034–13039. doi: 10.1073/pnas.1110994109.

Demaude, J. *et al.* (2006) 'Phenotypic changes in colonocytes following acute stress or activation of mast cells in mice: Implications for delayed epithelial barrier dysfunction', *Gut*, 55(5), pp. 655–661. doi: 10.1136/gut.2005.078675.

Derycke, S. *et al.* (2010) 'Exploring the use of cytochrome oxidase c subunit 1 (COI) for DNA barcoding of free-living marine nematodes', *PLoS ONE*, 5(10). doi: 10.1371/journal.pone.0013716.

Desbonnet, L. *et al.* (2014) 'Microbiota is essential for social development in the mouse', *Molecular Psychiatry*, 19(2), pp. 146–148. doi: 10.1038/mp.2013.65.

Diaz Heijtz, R. *et al.* (2011) 'Normal gut microbiota modulates brain development and behavior', *Proceedings of the National Academy of Sciences of the United States of America*, 108(7), pp. 3047–3052. doi: 10.1073/pnas.1010529108.

Dominianni, C. *et al.* (2015) 'Sex, body mass index, and dietary fiber intake influence the human gut microbiome', *PLoS ONE*, 10(4), pp. 1–14. doi: 10.1371/journal.pone.0124599.

Donati, G. *et al.* (2001) 'Nocturnal activity in the cathemeral red-fronted lemur (Eulemur fulvus rufus), with observations during a lunar eclipse', *American Journal of Primatology*, 53(2), pp. 69–78.

Drudy, D. *et al.* (2004) 'Human antibody response to surface layer proteins in Clostridium difficile infection.', *FEMS immunology and medical microbiology*, 41(3), pp. 237–42. doi: 10.1016/j.femsim.2004.03.007.

Eckburg, P. B. *et al.* (2005) 'Diversity of the Human Intestinal Microbial Flora', *Science*, 308(5728), pp. 1635–1638. doi: 10.1126/science.1110591.

Economou, V. and Gousia, P. (2015) 'Agriculture and food animals as a source of antimicrobial-resistant bacteria', *Infection and Drug Resistance*, 8, pp. 49–61. doi: 10.2147/IDR.S55778.

Ege, M. J. *et al.* (2011) 'Exposure to Environmental Microorganisms and Childhood Asthma', *New England Journal of Medicine*, 364(8), pp. 701–709. doi: 10.1056/NEJMoa1007302.

Ehmann, D. *et al.* (2019) 'Paneth cell α-defensins HD-5 and HD-6 display differential degradation into active antimicrobial fragments', *Proceedings of the National Academy of Sciences of the United States of America*, 116(9), pp. 3746–3751. doi: 10.1073/pnas.1817376116.

Elderman, M., de Vos, P. and Faas, M. (2018) 'Role of microbiota in sexually dimorphic immunity', *Frontiers in Immunology*, 9, pp. 1–6. doi: 10.3389/fimmu.2018.01018.

Erny, D. *et al.* (2015) 'Host microbiota constantly control maturation and function of microglia in the CNS', *Nature Neuroscience*, 18(7), pp. 965–977. doi: 10.1038/nn.4030.

Erturk-Hasdemir, D. *et al.* (2019) 'Symbionts exploit complex signaling to educate the immune system', *Proceedings of the National Academy of Sciences of the United States of America*, 116(52), pp. 26157–26166. doi: 10.1073/pnas.1915978116.

Escallón, C., Belden, L. K. and Moore, I. T. (2019) 'The cloacal microbiome changes with the breeding season in a wild bird', *Integrative Organismal Biology*, 1(1), pp. 1–16. doi: 10.1093/iob/oby009.

Estrela, S., Whiteley, M. and Brown, S. P. (2015) 'The demographic determinants of human microbiome health', *Trends in Microbiology*, 23(3), pp. 134–141. doi: 10.1016/j.tim.2014.11.005.

Ezenwa, V. O. *et al.* (2016) 'Group living and pathogen infection revisited', *Current Opinion in Behavioral Sciences*, 12, pp. 66–72. doi: 10.1016/j.cobeha.2016.09.006.

Falk, P. G. *et al.* (1998) 'Creating and Maintaining the Gastrointestinal Ecosystem: What We Know and Need To Know from Gnotobiology', *Microbiology and Molecular Biology Reviews*, 62(4), pp. 1157–1170. doi: 10.1128/mmbr.62.4.1157-1170.1998.

Falony, G. *et al.* (2016) 'Population-level analysis of gut microbiome variation', *Science*, 352(6285), pp. 560–564. doi: 10.1126/science.aad3503.

Fichtel, C. and Kappeler, P. M. (2002) 'Anti-predator behavior of group-living Malagasy primates: Mixed evidence for a referential alarm call system', *Behavioral Ecology and Sociobiology*, 51(3), pp. 262–275. doi: 10.1007/s00265-001-0436-0.

Fichtel, C., Schnoell, A. V. and Kappeler, P. M. (2017) 'Measuring social tolerance: An experimental approach in two lemurid primates', *Ethology*, 124(1), pp. 65–73. doi: 10.1111/eth.12706.

De Filippo, C. *et al.* (2010) 'Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa', *Proceedings of the National Academy of Sciences of the United States of America*, 107(33), pp. 14691–14696. doi: 10.1073/pnas.1005963107.

Foitová, I. *et al.* (2014) 'Phylogenetic relationships between pinworms (Nematoda: Enterobiinae) parasitising the critically endangered orangutan, according to the characterisation of molecular genomic and mitochondrial markers', *Parasitology Research*, 113(7), pp. 2455–2466. doi: 10.1007/s00436-014-3892-y.

Folmer, O. *et al.* (1994) 'DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.', *Molecular marine biology and biotechnology*, 3(5), pp. 294–299. doi: 10.1071/ZO9660275.

Frankiensztajn, L. M., Elliott, E. and Koren, O. (2020) 'The microbiota and the hypothalamus-pituitary-adrenocortical (HPA) axis, implications for anxiety and stress disorders', *Current Opinion in Neurobiology*, 62, pp. 76–82. doi: 10.1016/j.conb.2019.12.003.

Frias, L. *et al.* (2019) 'A pinworm's tale: The evolutionary history of Lemuricola (Protenterobius) nycticebi', *International Journal for Parasitology: Parasites and Wildlife*. Elsevier, 8, pp. 25–32. doi: 10.1016/j.ijppaw.2018.11.009.

Fujimura, K. E. *et al.* (2016) 'Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation', *Nature Medicine*, 22(10), pp. 1187–1191. doi: 10.1038/nm.4176.

Fukuda, K. *et al.* (2016) 'Molecular approaches to studying microbial communities: Targeting the 16S ribosomal RNA gene', *Journal of UOEH*, 38(3), pp. 223–232. doi: 10.7888/juoeh.38.223.

Fukui, H. *et al.* (2018) 'Effect of probiotic Bifidobacterium bifidum G9-1 on the relationship between gut microbiota profile and stress sensitivity in maternally separated rats', *Scientific Reports*, pp. 1–10. doi: 10.1038/s41598-018-30943-3.

Fulde, M. *et al.* (2018) 'Neonatal selection by Toll-like receptor 5 influences long-term gut microbiota composition', *Nature*, 560(7719), pp. 489–493. doi: 10.1038/s41586-018-0395-5.

Gantner, S. *et al.* (2011) 'Novel primers for 16S rRNA-based archaeal community analyses in environmental samples', *Journal of Microbiological Methods*, 84(1), pp. 12–18. doi: 10.1016/j.mimet.2010.10.001.

Garber, P. A. *et al.* (2019) 'The gut microbiome and metabolome of saddleback tamarins (Leontocebus weddelli): Insights into the foraging ecology of a small-bodied primate', *American Journal of Primatology*, 81(10–11), pp. 1–13. doi: 10.1002/ajp.23003.

Gareau, M. G. *et al.* (2011) 'Bacterial infection causes stress-induced memory dysfunction in mice', *Gut*, 60(3), pp. 307–317. doi: 10.1136/gut.2009.202515.

Gehrig, J. L. *et al.* (2019) 'Effects of microbiota-directed foods in gnotobiotic animals and undernourished children', *Science*, 365(6449). doi: 10.1126/science.aau4732.

Gérard, C. and Vidal, H. (2019) 'Impact of gut microbiota on host glycemic control', *Frontiers in Endocrinology*, pp. 1–13. doi: 10.3389/fendo.2019.00029.

Gevers, D. *et al.* (2014) 'The treatment-naive microbiome in new-onset Crohn's disease', *Cell Host and Microbe*, 15(3), pp. 382–392. doi: 10.1016/j.chom.2014.02.005.

Gill, S. R. *et al.* (2006) 'Metagenomic analysis of the human distal gut microbiome', *Science*, 312(5778), pp. 1355–1359. doi: 10.1126/science.1124234.

Gillespie, T. R. (2006) 'Noninvasive Assessment of Gastrointestinal Parasite Infections in Free-Ranging Primates', *International Journal of Primatology*, 27(4), pp. 1129–1143. doi: 10.1007/s10764-006-9064-x.

Glendinning, L. *et al.* (2014) 'The microbiota and helminths: Sharing the same niche in the human host', *Parasitology*, 141(10), pp. 1255–1271. doi: 10.1017/S0031182014000699.

Gogarten, J. F. *et al.* (2018) 'Factors influencing bacterial microbiome composition in a wild non-human primate community in Taï National Park, Côte d'Ivoire', *ISME Journal*, 12(10), pp. 2559–2574. doi: 10.1038/s41396-018-0166-1.

Gogarten, J. F. *et al.* (2020) 'Metabarcoding of eukaryotic parasite communities describes diverse parasite assemblages spanning the primate phylogeny', *Molecular Ecology Resources*, 20(1), pp. 204–215. doi: 10.1111/1755-0998.13101.

Gogarten, J. F. *et al.* (2021) 'Primate phageomes are structured by superhost phylogeny and environment', *Proceedings of the National Academy of Sciences of the United States of America*, 118(15), pp. 1–9. doi: 10.1073/pnas.2013535118.

Gomez-Arango, L. F. *et al.* (2018) 'Low dietary fiber intake increases Collinsella abundance in the gut microbiota of overweight and obese pregnant women', *Gut Microbes*, 9(3), pp. 189–201. doi: 10.1080/19490976.2017.1406584.

Gomez, A. *et al.* (2015) 'Gut microbiome composition and metabolomic profiles of wild western lowland gorillas (Gorilla gorilla gorilla) reflect host ecology', *Molecular Ecology*, 24(10), pp. 2551–2565. doi: 10.1111/mec.13181.

Goodfellow, C. K. *et al.* (2019) 'Divergence in gut microbial communities mirrors a social group fission event in a black-and-white colobus monkey (Colobus vellerosus)', *American Journal of Primatology*, 81(10–11), pp. 1–9. doi: 10.1002/ajp.22966.

Goodrich, J. K. *et al.* (2014) 'Human genetics shape the gut microbiome', *Cell*. Elsevier Inc., 159(4), pp. 789–799. doi: 10.1016/j.cell.2014.09.053.

Graham-Rowe, D. (2011) 'When allergies go west', *Nature*, 479, pp. S2–S4. Available at: http://www.nature.com/doifinder/10.1038/479S2a.

Greene, L. K. *et al.* (2020) 'A role for gut microbiota in host niche differentiation', *ISME Journal*, 14(7), pp. 1675–1687. doi: 10.1038/s41396-020-0640-4.

Grieneisen, L. *et al.* (2021) 'Gut microbiome heritability is nearly universal but environmentally contingent', *Science*, 373(6551), pp. 181–186. doi: 10.1126/science.aba5483.

Grieneisen, L. E. *et al.* (2017) 'Group living and male dispersal predict the core gut microbiome in wild baboons', *Integrative and Comparative Biology*, 57(4), pp. 770–785. doi: 10.1093/icb/icx046.

Grieneisen, L. E. *et al.* (2019) 'Genes, geology and germs: Gut microbiota across a primate hybrid zone are explained by site soil properties, not host species', *Proceedings of the Royal Society B: Biological Sciences*, 286(1901). doi: 10.1098/rspb.2019.0431.

Groussin, M. *et al.* (2017) 'Unraveling the processes shaping mammalian gut microbiomes over evolutionary time', *Nature Communications*, 8. doi: 10.1038/ncomms14319.

Gruninger, R. J. *et al.* (2014) 'Anaerobic fungi (phylum Neocallimastigomycota): Advances in understanding their taxonomy, life cycle, ecology, role and biotechnological potential', *FEMS Microbiology Ecology*, 90(1), pp. 1–17. doi: 10.1111/1574-6941.12383.

Gu, S. *et al.* (2013) 'Bacterial Community Mapping of the Mouse Gastrointestinal Tract', *PLoS ONE*, 8(10). doi: 10.1371/journal.pone.0074957.

Guevarra, R. B. *et al.* (2019) 'Piglet gut microbial shifts early in life: Causes and effects', *Journal of Animal Science and Biotechnology*. Journal of Animal Science and Biotechnology, 10(1), pp. 1–10. doi: 10.1186/s40104-018-0308-3.

Hamad, I. *et al.* (2014) 'Pathogenic eukaryotes in gut microbiota of western lowland gorillas as revealed by molecular survey', *Scientific Reports*, 4, pp. 1–9. doi: 10.1038/srep06417.

Harnett, W. (2014) 'Secretory products of helminth parasites as immunomodulators.', *Molecular and biochemical parasitology*, 195(2), pp. 1–7. doi: 10.1016/j.molbiopara.2014.03.007.

Haro, C. *et al.* (2016) 'Intestinal microbiota is influenced by gender and body mass index', *PLoS ONE*, 11(5), pp. 1–16. doi: 10.1371/journal.pone.0154090.

He, G. *et al.* (1999) 'Noninvasive measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of living mice with spatial and spectral EPR imaging', *Proceedings of the National Academy of Sciences*, 96, pp. 4586–4591. doi: https://doi.org/10.1073/pnas.96.8.4586.

Heintz-Buschart, A. and Wilmes, P. (2018) 'Human gut microbiome: Function matters', *Trends in Microbiology*, 26(7), pp. 563–574. doi: 10.1016/j.tim.2017.11.002.

Heistermann, M., Palme, R. and Ganswindt, A. (2006) 'Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis', *American Journal of Primatology*, 68(3), pp. 257–273. doi: 10.1002/ajp.20222.

Heitlinger, E. *et al.* (2017) 'The Intestinal Eukaryotic and Bacterial Biome of Spotted Hyenas: The Impact of Social Status and Age on Diversity and Composition', *Frontiers in Cellular and Infection Microbiology*, 7. doi: 10.3389/fcimb.2017.00262.

Hicks, A. L. *et al.* (2018) 'Gut microbiomes of wild great apes fluctuate seasonally in response to diet', *Nature Communications*, 9(1), p. 1786. doi: 10.1038/s41467-018-04204-w.

Hoarau, G. *et al.* (2016) 'Bacteriome and mycobiome interactions underscore microbial dysbiosis in familial Crohn's disease', *mBio*, 7(5). doi: 10.1128/mBio.01250-16.

Hooks, K. B. and O'Malley, M. A. (2017) 'Dysbiosis and its discontents', *mBio*, 8(5), pp. 1–11. doi: 10.1128/mBio.01492-17.

Hoy, Y. E. *et al.* (2015) 'Variation in taxonomic composition of the fecal microbiota in an inbred mouse strain across individuals and time', *PLoS ONE*, 10(11), pp. 1–17. doi: 10.1371/journal.pone.0142825.

Hufeldt, M. R. *et al.* (2010) 'Variation in the gut microbiota of laboratory mice is related to both genetic and environmental factors', *Comparative Medicine*, 60(5), pp. 336–342.

Huttenhower, C. *et al.* (2012) 'Structure, function and diversity of the healthy human microbiome', *Nature*, 486(7402), pp. 207–214. doi: 10.1038/nature11234.

Iliev, I. D. *et al.* (2012) 'Interactions between commensal fungi and the C-type lectin receptor dectin-1 influence colitis', *Science*, 336(6086), pp. 1314–1317. doi: 10.1126/science.1221789.

Jackson, M. A. *et al.* (2016) 'Signatures of early frailty in the gut microbiota', *Genome Medicine*, pp. 1–11. doi: 10.1186/s13073-016-0262-7.

Jagsi, R. *et al.* (2017) 'Seasonal cycling in the gut microbiome of the Hadza Hunter-Gatherers of Tanzania', *Science*, 357(6353), pp. 802–806.

Jang, H. *et al.* (2018) 'Immobilization stress-induced Escherichia coli causes anxiety by inducing NF- κ B activation through gut microbiota disturbance', *Scientific Reports*, pp. 1–14. doi: 10.1038/s41598-018-31764-0.

Janiak, M. C. *et al.* (2021) 'Age and sex-associated variation in the multi-site microbiome of an entire social group of free-ranging rhesus macaques', *Microbiome*, 9(1), pp. 1–17. doi: 10.1186/s40168-021-01009-w.

Johnson, A. J. *et al.* (2019) 'Daily sampling reveals personalized diet-microbiome associations in humans', *Cell Host and Microbe*, 25(6), pp. 789-802.e5. doi: 10.1016/j.chom.2019.05.005.

Johnson, J. S. *et al.* (2019) 'Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis', *Nature Communications*, 10(1), pp. 1–11. doi: 10.1038/s41467-019-13036-1.

Johnson, S. *et al.* (2020) *Eulemur rufifrons*, *The IUCN Red List of Threatened Species 2020*. Available at: https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T136269A115581600.en.

Kageyama, A. and Benno, Y. (2000) 'Emendation of genus Collinsella and proposal of Collinsella stercoris sp. nov. and Collinsella intestinalis sp. nov.', *International Journal of Systematic and Evolutionary Microbiology*, 50(5), pp. 1767–1774. doi: 10.1099/00207713-50-5-1767.

Kappeler, P. M., Cremer, S. and Nunn, C. L. (2015) 'Sociality and health: impacts of sociality on disease susceptibility and transmission in animal and human societies', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1669), pp. 20140116–20140116. doi: 10.1098/rstb.2014.0116.

Kappeler, P. M. and Fichtel, C. (2012a) 'A 15-year perspective on the social organization and life history of sifaka in Kirindy Forest', in *Long-Term Field Studies of Primates*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 101–121. doi: 10.1007/978-3-642-22514-7_5.

Kappeler, P. M. and Fichtel, C. (2012b) 'Female reproductive competition in Eulemur rufifrons: Eviction and reproductive restraint in a plurally breeding Malagasy primate', *Molecular Ecology*, 21(3), pp. 685–698. doi: 10.1111/j.1365-294X.2011.05255.x.

Kappeler, P. M. and Port, M. (2008) 'Mutual tolerance or reproductive competition? Patterns of reproductive skew among male redfronted lemurs (Eulemur fulvus rufus)', *Behavioral Ecology and Sociobiology*, 62(9), pp. 1477–1488. doi: 10.1007/s00265-008-0577-5.

Kartzinel, T. R. *et al.* (2019) 'Covariation of diet and gut microbiome in African megafauna', *Proceedings of the National Academy of Sciences of the United States of America*, 116(47), pp. 23588–23593. doi: 10.1073/pnas.1905666116.

Kimura, I. *et al.* (2011) 'Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41)', *Proceedings of the National Academy of Sciences of the United States of America*, 108(19), pp. 8030–8035. doi: 10.1073/pnas.1016088108.

Klein, S. L. and Flanagan, K. L. (2016) 'Sex differences in immune responses', *Nature Reviews Immunology*, 16(10), pp. 626–638. doi: 10.1038/nri.2016.90.

Klindworth, A. *et al.* (2013) 'Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies', *Nucleic Acids Research*, 41(1), pp. 1–11. doi: 10.1093/nar/gks808.

Knight, R. (2016) 'Microbiome', in *Encyclopedia of Evolutionary Biology*. Elsevier, pp. 14–18. doi: 10.1016/B978-0-12-800049-6.00190-6.

Knight, R. *et al.* (2018) 'Best practices for analysing microbiomes', *Nature Reviews Microbiology*, 16(7), pp. 410–422. doi: 10.1038/s41579-018-0029-9.

Koch, F. *et al.* (2017) 'Sex and seasonal differences in diet and nutrient intake in Verreaux's sifakas (Propithecus verreauxi)', *American Journal of Primatology*, 79(4), pp. 1–10. doi: 10.1002/ajp.22595.

Koch, H. and Schmid-Hempel, P. (2011) 'Socially transmitted gut microbiota protect bumble bees against an intestinal parasite', *Proceedings of the National Academy of Sciences of the United States of America*, 108(48), pp. 19288–19292. doi: 10.1073/pnas.1110474108.

Koenig, J. E. *et al.* (2011) 'Succession of microbial consortia in the developing infant gut microbiome', *Proceedings of the National Academy of Sciences of the United States of America*, 108, pp. 4578–4585. doi: 10.1073/pnas.1000081107.

Koskinen, K. *et al.* (2017) 'First insights into the diverse human archaeome: specific detection of archaea in the gastrointestinal tract, lung, and nose and on skin', *mBio*, 8(6), pp. 1–17. doi: 10.1128/mBio.00824-17.

Kreisinger, J. *et al.* (2015) 'Interactions between multiple helminths and the gut microbiota in wild rodents', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1675). doi: 10.1098/rstb.2014.0295.

Kurilshikov, A. *et al.* (2021) 'Large-scale association analyses identify host factors influencing human gut microbiome composition', *Nature Genetics*, 53(2), pp. 156–165. doi: 10.1038/s41588-020-00763-1.

Laforest-Lapointe, I. and Arrieta, M.-C. (2018) 'Microbial eukaryotes: a missing link in gut microbiome studies', *mSystems*, 3(2). doi: 10.1128/msystems.00201-17.

Laforest-Lapointe, I. and Arrieta, M. C. (2017) 'Patterns of early-life gut microbial colonization during human immune development: An ecological perspective', *Frontiers in Immunology*, pp. 1–13. doi: 10.3389/fimmu.2017.00788.

Lai, G. C., Tan, T. G. and Pavelka, N. (2019) 'The mammalian mycobiome: A complex system in a dynamic relationship with the host', *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 11(1), p. e1438. doi: 10.1002/wsbm.1438.

Lathrop, S. K. *et al.* (2011) 'Peripheral education of the immune system by colonic

commensal microbiota', *Nature*, 478(7368), pp. 250–254. doi: 10.1038/nature10434.

Leclaire, S. *et al.* (2017) 'Social odours covary with bacterial community in the anal secretions of wild meerkats', *Scientific Reports*, 7(1), pp. 1–13. doi: 10.1038/s41598-017-03356-x.

Lee, S. M. *et al.* (2013) 'Bacterial colonization factors control specificity and stability of the gut microbiota', *Nature*, 501(7467), pp. 426–429. doi: 10.1038/nature12447.

Lee, Y. K. and Mazmanian, S. K. (2010) 'Has the microbiota played a critical role?', *Science*, 330(6012), pp. 1768–1773. doi: 10.1126/science.1195568.Has.

Levin, I. I. *et al.* (2016) 'Stress response, gut microbial diversity and sexual signals correlate with social interactions', *Biology Letters*, 12(6). doi: 10.1098/rsbl.2016.0352.

Ley, R. E. *et al.* (2008) 'Evolution of mammals and their gut microbes', *Science*, 320(5883), pp. 1647–1651. doi: 10.1126/science.1155725.

Ley, R. E. (2016) 'Prevotella in the gut: choose carefully', *Nature Reviews Gastroenterology & Hepatology*, 13(2), pp. 69–70. doi: 10.1038/nrgastro.2016.4.

Li, J. *et al.* (2018) 'Fungi in gastrointestinal tracts of human and mice: from community to functions', *Microbial Ecology*, 75(4), pp. 821–829. doi: 10.1007/s00248-017-1105-9.

Li, W. *et al.* (2020) 'Prevalence of Tetratrichomonas buttreyi and Pentatrichomonas hominis in yellow cattle, dairy cattle, and water buffalo in China', *Parasitology Research*, 119(2), pp. 637–647. doi: 10.1007/s00436-019-06550-0.

Li, Weizhi *et al.* (2015) 'The prevalence of intestinal trichomonads in Chinese pigs', *Veterinary Parasitology*, 211(1–2), pp. 12–15. doi: 10.1016/j.vetpar.2015.04.028.

Liao, H. *et al.* (2014) 'Insights into high-efficiency lignocellulolytic enzyme production by Penicillium oxalicum GZ-2 induced by a complex substrate', *Biotechnology for Biofuels*, 7(1), pp. 1–17. doi: 10.1186/s13068-014-0162-2.

Louca, S., Doebeli, M. and Parfrey, L. W. (2018) 'Correcting for 16S rRNA gene copy numbers in microbiome surveys remains an unsolved problem', *Microbiome*, 6(1), pp. 1–12. doi: 10.1186/s40168-018-0420-9.

Lu, A. *et al.* (2019) 'Developmental responses to early-life adversity: Evolutionary and mechanistic perspectives', *Evolutionary Anthropology*, 28(5), pp. 249–266. doi: 10.1002/evan.21791.

Lukeš, J. *et al.* (2015) 'Are human intestinal eukaryotes beneficial or commensals?', *PLoS Pathogens*, 11(8), pp. 7–12. doi: 10.1371/journal.ppat.1005039.

MacFabe, D. F. *et al.* (2007) 'Neurobiological effects of intraventricular propionic acid in rats: Possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders', *Behavioural Brain Research*, 176(1), pp. 149–169. doi: 10.1016/j.bbr.2006.07.025.

MacFabe, D. F. (2012) 'Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders', *Microbial Ecology in Health & Disease*, 23(0). doi: 10.3402/mehd.v23i0.19260.

Macfarlane, S. and Macfarlane, G. T. (2003) 'Regulation of short-chain fatty acid production', *Proceedings of the Nutrition Society*, 62(1), pp. 67–72. doi: 10.1079/pns2002207.

MacIntosh, A. J. J. *et al.* (2012) 'Monkeys in the middle: Parasite transmission through the social network of a wild primate', *PLoS ONE*, 7(12), pp. 15–21. doi: 10.1371/journal.pone.0051144.

Macpherson, A. J., Geuking, M. B. and McCoy, K. D. (2005) 'Immune responses that adapt the intestinal mucosa to commensal intestinal bacteria', *Immunology*, 115(2), pp. 153–162. doi: 10.1111/j.1365-2567.2005.02159.x.

Maier, B. R. and Hentges, D. J. (1972) 'Experimental Shigella Infections in Laboratory Animals', *Infection and Immunity*, 6(2), pp. 168–173.

Mallick, H. *et al.* (2021) 'Multivariable association discovery in population-scale meta-omics studies', *bioRxiv*, p. 2021.01.20.427420. Available at: https://doi.org/10.1101/2021.01.20.427420.

Malmuthuge, N. and Guan, L. L. (2017) 'Understanding the gut microbiome of dairy calves: Opportunities to improve early-life gut health', *Journal of Dairy Science*, 100(7), pp. 5996–6005. doi: 10.3168/jds.2016-12239.

Manara, S. *et al.* (2019) 'Microbial genomes from non-human primate gut metagenomes expand the primate-associated bacterial tree of life with over 1000 novel species', *Genome Biology*, 20(1), p. 299. doi: 10.1186/s13059-019-1923-9.

Mann, A. E. *et al.* (2020) 'Biodiversity of protists and nematodes in the wild nonhuman primate gut', *The ISME Journal*, 14(2), pp. 609–622. doi: 10.1038/s41396-019-0551-4.

Martin, A. M. *et al.* (2019) 'The influence of the gut microbiome on host metabolism through the regulation of gut hormone release', *Frontiers in Physiology*, pp. 1–11. doi: 10.3389/fphys.2019.00428.

Martínez, I. *et al.* (2015) 'The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes', *Cell Reports*, 11(4), pp. 527–538. doi: 10.1016/j.celrep.2015.03.049.

Martinson, Vi. G. *et al.* (2011) 'A simple and distinctive microbiota associated with honey bees and bumble bees', *Molecular Ecology*, 20(3), pp. 619–628. doi: 10.1111/j.1365-294X.2010.04959.x.

Marzano, V. *et al.* (2017) '"Omic" investigations of protozoa and worms for a deeper understanding of the human gut "parasitome"', *PLoS Neglected Tropical Diseases*, 11(11), p. e0005916. doi: 10.1371/journal.pntd.0005916.

Maurice, C. F. *et al.* (2015) 'Marked seasonal variation in the wild mouse gut microbiota', *ISME Journal*, 9(11), pp. 2423–2434. doi: 10.1038/ismej.2015.53.

Mayer, E. A. (2000) 'The neurobiology of stress and gastrointestinal disease', *Gut*, 47(6), pp. 861–869. doi: 10.1136/gut.47.6.861.

McKenney, E. A., O'Connell, T. M., *et al.* (2018) 'Feeding strategy shapes gut metagenomic enrichment and functional specialization in captive lemurs', *Gut Microbes*. Taylor & Francis, 9(3), pp. 202–217. doi: 10.1080/19490976.2017.1408762.

McKenney, E. A., Koelle, K., *et al.* (2018) 'The ecosystem services of animal microbiomes', *Molecular Ecology*, 27(8), pp. 2164–2172. doi: 10.1111/mec.14532.

McVeigh, P. (2020) 'Post-genomic progress in helminth parasitology', *Parasitology*, 147(8), pp. 835–840. doi: 10.1017/S0031182020000591.

Miller, E. T., Svanbäck, R. and Bohannan, B. J. M. (2018) 'Microbiomes as metacommunities: understanding host-associated microbes through metacommunity ecology', *Trends in Ecology and Evolution*, 33(12), pp. 926–935. doi: 10.1016/j.tree.2018.09.002.

Milligan, T. W. *et al.* (1978) 'Growth and amino acid requirements of various strains of group B streptococci', *Journal of Clinical Microbiology*, 7(1), pp. 28–33. doi: 10.1128/jcm.7.1.28-33.1978.

Moeller, A. H. *et al.* (2016) 'Social behavior shapes the chimpanzee pan-microbiome', *Science Advances*, 2(1). doi: 10.1126/sciadv.1500997.

Moreno-Indias, I. *et al.* (2016) 'Neonatal androgen exposure causes persistent gut microbiota dysbiosis related to metabolic disease in adult female rats', *Endocrinology*, 157(12), pp. 4888–4898. doi: 10.1210/en.2016-1317.

Morgan, X. C. *et al.* (2012) 'Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment', *Genome Biology*, 13(9), p. R79. doi: 10.1186/gb-2012-13-9-r79.

Morgan, X. C. *et al.* (2015) 'Associations between host gene expression, the mucosal microbiome, and clinical outcome in the pelvic pouch of patients with inflammatory bowel disease', *Genome Biology*, 16(1), pp. 1–15. doi: 10.1186/s13059-015-0637-x.

Muniesa, M. and Jofre, J. (2014) 'Identifying and analyzing bacteriophages in human fecal samples: what could we discover?', *Future Microbiology*, 9(7), pp. 879–86. doi: 10.2217/fmb.14.47.

Nash, A. K. *et al.* (2017) 'The gut mycobiome of the Human Microbiome Project healthy cohort', *Microbiome*, 5(1), p. 153. doi: 10.1186/s40168-017-0373-4.

NCBI Resource Coordinators (2018) 'Database resources of the National Center for Biotechnology Information', *Nucleic Acids Research*, 46(D1), pp. D8–D13. doi: 10.1093/nar/gkx1095.

Nearing, J. T. *et al.* (2018) 'Denoising the Denoisers: An independent evaluation of microbiome sequence error- correction approaches', *PeerJ*, 2018(8), pp. 1–22. doi: 10.7717/peerj.5364.

Nilsson, R. H. *et al.* (2019) 'Mycobiome diversity: high-throughput sequencing and identification of fungi', *Nature Reviews Microbiology*, 17(2), pp. 95–109. doi: 10.1038/s41579-018-0116-y.

Nishida, A. H. and Ochman, H. (2018) 'Rates of gut microbiome divergence in mammals', *Molecular Ecology*, 27(8), pp. 1884–1897. doi: 10.1111/mec.14473.

Nkamga, V. D., Henrissat, B. and Drancourt, M. (2017) 'Archaea: Essential inhabitants of the human digestive microbiota', *Human Microbiome Journal*, 3, pp. 1–8. doi: 10.1016/j.humic.2016.11.005.

Noguera, J. C. *et al.* (2018) 'Glucocorticoids modulate gastrointestinal microbiome in a wild bird', *Royal Society Open Science*, 5(4). doi: 10.1098/rsos.171743.

Noverr, M. C. and Huffnagle, G. B. (2004) 'Regulation of Candida albicans morphogenesis by fatty acid metabolites', *Infection and Immunity*, 72(11), pp. 6206–6210. doi: 10.1128/IAI.72.11.6206-6210.2004.

O'Hara, A. M. and Shanahan, F. (2006) 'The gut flora as a forgotten organ', *EMBO Reports*, 7(7), pp. 688–693. doi: 10.1038/sj.embor.7400731.

Olaniyi, K. S. *et al.* (2020) 'Placental microbial colonization and its association with pre-eclampsia', *Frontiers in Cellular and Infection Microbiology*, pp. 1–11. doi: 10.3389/fcimb.2020.00413.

Org, E. *et al.* (2016) 'Sex differences and hormonal effects on gut microbiota composition in mice', *Gut Microbes*, 7(4), pp. 313–322. doi: 10.1080/19490976.2016.1203502.

Orkin, J. D. *et al.* (2019) 'Seasonality of the gut microbiota of free-ranging white-faced capuchins in a tropical dry forest', *ISME Journal*. Springer US, 13(1), pp. 183–196. doi: 10.1038/s41396-018-0256-0.

Orkin, J. D., Webb, S. E. and Melin, A. D. (2019) 'Small to modest impact of social group on the gut microbiome of wild Costa Rican capuchins in a seasonal forest', *American Journal of Primatology*, 81(10–11), pp. 1–10. doi: 10.1002/ajp.22985.

Osawa, R., Blanshard, W. H. and Callaghan, P. G. O. (1993) 'Microbiological studies of the intestinal microflora maternal faeces consumed by juvenile koalas', *Australian Journal of Zoology*, 41(6), pp. 611–620.

Ostner, J. (2002) 'Social thermoregulation in redfronted lemurs (Eulemur fulvus rufus)', *Folia Primatologica*, 73(4), pp. 175–180. doi: 10.1159/000065425.

Ostner, J. and Heistermann, M. (2003) 'Endocrine characterization of female reproductive status in wild redfronted lemurs (Eulemur fulvus rufus)', *General and Comparative Endocrinology*, 131(3), pp. 274–283. doi: 10.1016/S0016-6480(03)00013-3.

Ostner, J., Kappeler, P. and Heistermann, M. (2008) 'Androgen and glucocorticoid levels reflect seasonally occurring social challenges in male redfronted lemurs (Eulemur fulvus rufus)', *Behavioral Ecology and Sociobiology*, 62(4), pp. 627–638. doi: 10.1007/s00265-007-0487-y.

Ostner, J. and Kappeler, P. M. (1999) 'Central males instead of multiple pairs in redfronted lemurs, Eulemur fulvus rufus (Primates, Lemuridae)?', *Animal Behaviour*, 58(5), pp. 1069–1078. doi: 10.1006/anbe.1999.1222.

Ostner, J., Kappeler, P. M. and Heistermann, M. (2002) 'Seasonal variation and social correlates of androgen excretion in male redfronted lemurs (Eulemur fulvus rufus)', *Behavioral Ecology and Sociobiology*, 52(6), pp. 485–495. doi: 10.1007/s00265-002-0532-9.

Overdorff, D. J. (1993) *Similarities, differences, and seasonal patterns in the diets of* Eulemur rubriventer *and* Eulemur rufifrons *in the Ranomafana National Park, Madagascar*, *International Journal of Primatology*. doi: 10.1007/BF02192188.

Pafčo, B. *et al.* (2019) 'Gut microbiome composition of wild western lowland gorillas is associated with individual age and sex factors', *American Journal of Physical Anthropology*, 169(3), pp. 575–585. doi: 10.1002/ajpa.23842.

Palm, N. W. *et al.* (2014) 'Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease', *Cell*, 158(5), pp. 1000–1010. doi: 10.1016/j.cell.2014.08.006.

Parfrey, L. W., Walters, W. A. and Knight, R. (2011) 'Microbial eukaryotes in the human microbiome: Ecology, evolution, and future directions', *Frontiers in Microbiology*, pp. 1–6. doi: 10.3389/fmicb.2011.00153.

Pasolli, E. *et al.* (2019) 'Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle.', *Cell*. Elsevier Inc., 176(3), pp. 649-662.e20. doi: 10.1016/j.cell.2019.01.001.

Patterson, J. E. H. and Ruckstuhl, K. E. (2013) 'Parasite infection and host group size: A meta-analytical review', *Parasitology*, 140(7), pp. 803–813. doi: 10.1017/S0031182012002259.

Peckre, L. R. *et al.* (2018) 'Potential self-medication using millipede secretions in red-fronted lemurs: combining anointment and ingestion for a joint action against gastrointestinal parasites?', *Primates*, 59(5), pp. 483–494. doi: 10.1007/s10329-018-0674-7.

Pédron, T. *et al.* (2012) 'A crypt-specific core microbiota resides in the mouse colon', *mBio*, 3(3), pp. 1–7. doi: 10.1128/mBio.00116-12.

Pereira, F. C. and Berry, D. (2017) 'Microbial nutrient niches in the gut', *Environmental Microbiology*, 19(4), pp. 1366–1378. doi: 10.1111/1462-2920.13659.

Pereira, M. E. *et al.* (1990) 'Female dominance does not characterize all of the lemuridae', *Folia Primatologica*, 55(2), pp. 96–103. doi: 10.1159/000156505.

Perez-Carrasco, V. *et al.* (2021) 'Urinary Microbiome: Yin and Yang of the Urinary Tract', *Frontiers in Cellular and Infection Microbiology*, pp. 1–17. doi: 10.3389/fcimb.2021.617002.

Perofsky, A. C. *et al.* (2017) 'Hierarchical social networks shape gut microbial composition in wild Verreaux's sifaka', *Proceedings of the Royal Society B: Biological Sciences*, 284(1868), p. 20172274. doi: 10.1098/rspb.2017.2274.

Petrullo, L. *et al.* (2019) 'Composition and stability of the vervet monkey milk microbiome', *American Journal of Primatology*, 81(10–11), pp. 1–12. doi: 10.1002/ajp.22982.

Pierre, J. F. *et al.* (2021) 'Lyticase facilitates mycobiome resolution without disrupting microbiome fidelity in primates', *Journal of Surgical Research*, 267(267), pp. 336–341. doi: 10.1016/j.jss.2021.06.023.

Porat, I. *et al.* (2010) 'Characterization of archaeal community in contaminated and uncontaminated surface stream sediments', *Microbial Ecology*, 60(4), pp. 784–795. doi: 10.1007/s00248-010-9734-2.

Port, M., Clough, D. and Kappeler, P. M. (2009) 'Market effects offset the reciprocation of grooming in free-ranging redfronted lemurs, Eulemur fulvus rufus', *Animal Behaviour*. Elsevier Ltd, 77(1), pp. 29–36. doi: 10.1016/j.anbehav.2008.08.032.

Qin, J. *et al.* (2010) 'A human gut microbial gene catalog established by metagenomic sequencing', *Nature*, 464(7285), pp. 59–65. doi: 10.1038/nature08821.A.

Qin, P. *et al.* (2019) 'Characterization a novel butyric acid-producing bacterium Collinsella aerofaciens subsp. shenzhenensis subsp. nov.', *Microorganisms*, 7(28). doi: 10.3390/microorganisms7030078.

Quince, C. *et al.* (2017) 'Shotgun metagenomics, from sampling to analysis', *Nature Biotechnology*, 35(9), pp. 833–844. doi: 10.1038/nbt.3935.

Ramanan, D. *et al.* (2016) 'Helminth infection promotes colonization resistance via type 2 immunity', *Science*, 352(6285), pp. 608–612. doi: 10.1126/science.aaf3229.

Raulo, A. *et al.* (2017) 'Social behaviour and gut microbiota in red-bellied lemurs (Eulemur rubriventer): In search of the role of immunity in the evolution of sociality', *Journal of Animal Ecology*, pp. 388–399. doi: 10.1111/1365-2656.12781.

Raulo, A. *et al.* (2021) 'Social networks strongly predict the gut microbiota of wild mice', *ISME Journal*, 15(9), pp. 2601-2613. doi: 10.1038/s41396-021-00949-3.

Raymann, K. *et al.* (2017) 'Unexplored archaeal diversity in the great ape gut microbiome', *mSphere*, 2(1), pp. 1–12. doi: 10.1128/msphere.00026-17.

Ren, T. *et al.* (2016) 'Development, diet and dynamism: longitudinal and cross-sectional predictors of gut microbial communities in wild baboons', *Environmental Microbiology*, 18(5), pp. 1312–1325. doi: 10.1111/1462-2920.12852.

Renelies-Hamilton, J. *et al.* (2019) 'Exploring interactions between Blastocystis sp., Strongyloides spp. and the gut microbiomes of wild chimpanzees in Senegal', *Infection, Genetics and Evolution*, 74, p. 104010. doi: 10.1016/j.meegid.2019.104010.

Reynolds, L. A., Finlay, B. B. and Maizels, R. M. (2015) 'Cohabitation in the intestine: interactions among helminth parasites, bacterial microbiota, and host immunity', *The Journal of Immunology*, 195(9), pp. 4059–4066. doi: 10.4049/jimmunol.1501432.

Rhoades, N. *et al.* (2019) 'Maturation of the infant rhesus macaque gut microbiome and its role in the development of diarrheal disease', *Genome Biology*, 20(1), pp. 1–16. doi: 10.1186/s13059-019-1789-x.

Ricaboni, D. *et al.* (2017) '"Colidextribacter massiliensis" gen. nov., sp. nov., isolated from human right colon', *New Microbes and New Infections*, 17, pp. 27–29. doi: 10.1016/j.nmni.2016.11.023.

Rimbach, R. *et al.* (2015) 'Brown spider monkeys (Ateles hybridus): A model for differentiating the role of social networks and physical contact on parasite transmission dynamics', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1669). doi: 10.1098/rstb.2014.0110.

Rogers, G. B. *et al.* (2014) 'Functional divergence in gastrointestinal microbiota in physically-separated genetically identical mice', *Scientific Reports*, 4, pp. 1–5. doi: 10.1038/srep05437.

Rogers, G. B. *et al.* (2016) 'From gut dysbiosis to altered brain function and mental illness: Mechanisms and pathways', *Molecular Psychiatry*, 21(6), pp. 738–748. doi: 10.1038/mp.2016.50.

Rojas, C. A. *et al.* (2021) 'Host phylogeny and host ecology structure the mammalian gut microbiota at different taxonomic scales', *Animal Microbiome*, 3(1). doi: 10.1186/s42523-021-00094-4.

La Rosa, S. L. *et al.* (2019) 'The human gut Firmicute Roseburia intestinalis is a primary degrader of dietary β-mannans', *Nature Communications*, 10(1), pp. 1–14. doi: 10.1038/s41467-019-08812-y.

Rothschild, D. *et al.* (2018) 'Environment dominates over host genetics in shaping human gut microbiota', *Nature*, 555(7695), pp. 210–215. doi: 10.1038/nature25973.

Round, J. L. and Mazmanian, S. K. (2009) 'The gut microbiota shapes intestinal immune responses during health and disease', *Nature Reviews Immunology*, 9(5), pp. 313–323. doi: 10.1038/nri2515.

Rychlik, J. L. and May, T. (2000) 'The effect of a methanogen, Methanobrevibacter smithii, on the growth rate, organic acid production, and specific ATP activity of three predominant ruminal cellulolytic bacteria', *Current Microbiology*, 40(3), pp. 176–180. doi: 10.1007/s002849910035.

Sarkar, A. *et al.* (2020) 'Microbial transmission in animal social networks and the social microbiome', *Nature Ecology & Evolution*, 4(8), pp. 1020–1035. doi: 10.1038/s41559-020-1220-8.

Schéle, E. *et al.* (2013) 'The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (Gcg) and brain-derived neurotrophic factor (Bdnf) in the central nervous system', *Endocrinology*, 154(10), pp. 3643–3651. doi: 10.1210/en.2012-2151.

Schnoell, A. V. and Fichtel, C. (2013) 'A novel feeding behaviour in wild redfronted lemurs (Eulemur rufifrons): Depletion of spider nests', *Primates*, 54(4), pp. 371–375. doi: 10.1007/s10329-013-0356-4.

Schnorr, S. L. *et al.* (2014) 'Gut microbiome of the Hadza hunter-gatherers', *Nature Communications*, 5. doi: 10.1038/ncomms4654.

Schretter, C. E. *et al.* (2018) 'A gut microbial factor modulates locomotor behaviour in Drosophila', *Nature*, 563(7731), pp. 402–406. doi: 10.1038/s41586-018-0634-9.

Schwarz, R. S., Moran, N. A. and Evans, J. D. (2016) 'Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers', *Proceedings of the National Academy of Sciences of the United States of America*, 113(33), pp. 9345–9350. doi: 10.1073/pnas.1606631113.

Shan, M. *et al.* (2013) 'Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals', *Science*, 342(6157), pp. 447–453. doi: 10.1126/science.1237910.

Sheng, L. *et al.* (2017) 'Gender Differences in Bile Acids and Microbiota in Relationship with Gender Dissimilarity in Steatosis Induced by Diet and FXR Inactivation', *Scientific Reports*, 7(1), pp. 1–12. doi: 10.1038/s41598-017-01576-9.

Sherwin, E. *et al.* (2019) 'Microbiota and the social brain', *Science*, 366(6465), p. eaar2016. doi: 10.1126/science.aar2016.

Shkoporov, A. N. *et al.* (2019) 'The human gut virome is highly diverse, stable, and individual specific', *Cell Host and Microbe*, 26(4), pp. 527-541.e5. doi: 10.1016/j.chom.2019.09.009.

Smith, P. M. and Garrett, W. S. (2011) 'The gut microbiota and mucosal T cells', *Frontiers in Microbiology*, 2, pp. 1–6. doi: 10.3389/fmicb.2011.00111.

Snyder-Mackler, N. *et al.* (2016) 'Social status alters immune regulation and response to infection in macaques', *Science*, 354(6315), pp. 1041 LP – 1045.

Sokol, H. *et al.* (2017) 'Fungal microbiota dysbiosis in IBD', *Gut*, 66(6), pp. 1039–1048. doi: 10.1136/gutjnl-2015-310746.

Song, S. J. *et al.* (2020) 'Comparative analyses of vertebrate gut microbiomes reveal convergence between birds and bats', *mBio*, 11(1). doi: 10.1128/mBio.02901-19.

Spanogiannopoulos, P. *et al.* (2016) 'The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism', *Nature Reviews Microbiology*, 14(5), pp. 273–287. doi: 10.1038/nrmicro.2016.17.

Springer, A. *et al.* (2016) 'Social structure and *Escherichia coli* sharing in a group-living wild primate, Verreaux's sifaka', *BMC ecology*, 16(1), p. 6. doi: 10.1186/s12898-016-0059-y.

Springer, A. *et al.* (2017) 'Patterns of seasonality and group membership characterize the gut microbiota in a longitudinal study of wild Verreaux's sifakas (Propithecus verreauxi)', *Ecology and Evolution*, 7(15), pp. 5732–5745. doi: 10.1002/ece3.3148.

Stams, A. J. M. (1994) 'Metabolic interactions between anaerobic bacteria in methanogenic environments', *Antonie van Leeuwenhoek*, 66(1–3), pp. 271–294. doi: 10.1007/BF00871644.

Stephenson, M. and Rowatt, E. (1947) 'The production of acetylcholine by a strain of Lactobacillus plantarum', *Journal of general microbiology*, 1(3), pp. 279–298. doi: 10.1099/00221287-1-3-279.

Stoeck, T. *et al.* (2010) 'Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water', *Molecular Ecology*, 19, pp. 21–31. doi: 10.1111/j.1365-294X.2009.04480.x.

Stothart, M. R. *et al.* (2016) 'Stress and the microbiome: Linking glucocorticoids to bacterial community dynamics in wild red squirrels', *Biology Letters*, 12(1). doi: 10.1098/rsbl.2015.0875.

Stothart, M. R., Palme, R. and Newman, A. E. M. (2019) 'It's what's on the inside that counts: Stress physiology and the bacterial microbiome of a wild urban mammal', *Proceedings of the Royal Society B: Biological Sciences*, 286(1913). doi: 10.1098/rspb.2019.2111.

Su, X. L. *et al.* (2014) 'Acetobacteroides hydrogenigenes gen. nov., Sp. nov., An anaerobic hydrogen-producing bacterium in the family Rikenellaceae isolated from a reed swamp', *International Journal of Systematic and Evolutionary Microbiology*, 64, pp. 2986–2991. doi: 10.1099/ijs.0.063917-0.

Sudo, N. *et al.* (2004) 'Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice', *Journal of Physiology*, 558(1), pp. 263–275. doi: 10.1113/jphysiol.2004.063388.

Sudo, N. (2019) 'Biogenic Amines: Signals Between Commensal Microbiota and Gut Physiology', *Frontiers in Endocrinology*, 10(July), pp. 1–7. doi: 10.3389/fendo.2019.00504.

Sun, B. *et al.* (2016) 'Marked variation between winter and spring gut microbiota in free-ranging Tibetan Macaques (Macaca thibetana)', *Scientific Reports*, 6(April), pp. 1–8. doi: 10.1038/srep26035.

Sun, B. *et al.* (2018) 'Season, age, and sex affect the fecal mycobiota of free-ranging Tibetan macaques (Macaca thibetana)', *American Journal of Primatology*, 80(7). doi: 10.1002/ajp.22880.

Suzuki, K. *et al.* (2004) 'Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut', *Proceedings of the National Academy of Sciences of the United States of America*, 101(7), pp. 1981–1986. doi: 10.1073/pnas.0307317101.

Sweeny, A. R. *et al.* (2020) 'A mixed model approach for estimating drivers of microbiota community composition and differential taxonomic abundance', *bioRxiv*. doi: 10.1101/2020.11.24.395715.

Taghipour, A. *et al.* (2020) 'The neglected role of Enterobius vermicularis in appendicitis: A systematic review and metaanalysis', *PLoS ONE*, 15(4), pp. 1–15. doi: 10.1371/journal.pone.0232143.

Tanaka, R. *et al.* (2014) 'Assessment of helminth biodiversity in wild rats using 18S rDNA based metagenomics', *PLoS ONE*, 9(10), p. e110769. doi: 10.1371/journal.pone.0110769.

Tedersoo, L. *et al.* (2015) 'Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi', *MycoKeys*, 10, pp. 1–43. doi: 10.3897/mycokeys.10.4852.

Tetel, M. J. *et al.* (2018) 'Steroids, stress and the gut microbiome-brain axis', *Journal of Neuroendocrinology*, 30(2), p. e12548. doi: 10.1111/jne.12548.

Theis, K. R. *et al.* (2013) 'Symbiotic bacteria appear to mediate hyena social odors', *Proceedings of the National Academy of Sciences of the United States of America*, 110(49), pp. 19832–19837. doi: 10.1073/pnas.1306477110.

Thomas, C. M. *et al.* (2012) 'Histamine derived from probiotic Lactobacillus reuteri suppresses TNF via modulation of PKA and ERK signaling', *PLoS ONE*, 7(2). doi: 10.1371/journal.pone.0031951.

van Tilburg Bernardes, E. *et al.* (2020) 'Intestinal fungi are causally implicated in microbiome assembly and immune development in mice', *Nature Communications*, 11(1), p. 2577. doi: 10.1038/s41467-020-16431-1.

Toju, H. *et al.* (2012) 'High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples', *PLoS ONE*, 7(7). doi: 10.1371/journal.pone.0040863.

Topping, D. L. and Clifton, P. M. (2001) 'Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides', *Physiological Reviews*, 81(3), pp. 1031–1064. doi: 10.1152/physrev.2001.81.3.1031.

Tung, J. *et al.* (2015) 'Social networks predict gut microbiome composition in wild baboons', *eLife*, 2015(4), pp. 1–18. doi: 10.7554/eLife.05224.

Turpin, W. *et al.* (2016) 'Association of host genome with intestinal microbial composition in a large healthy cohort', *Nature Genetics*, 48(11), pp. 1413–1417. doi: 10.1038/ng.3693.

Uenishi, G. *et al.* (2007) 'Molecular analyses of the intestinal microbiota of chimpanzees in the wild and in captivity', *American Journal of Primatology*, 69(4), pp. 367–376. doi: 10.1002/ajp.20351.

Ugelvig, L. V. and Cremer, S. (2007) 'Social Prophylaxis: Group Interaction Promotes Collective Immunity in Ant Colonies', *Current Biology*, 17(22), pp. 1967–1971. doi: 10.1016/j.cub.2007.10.029.

Umanets, A. *et al.* (2018) 'Occupancy strongly influences faecal microbial composition of wild lemurs', *FEMS Microbiology Ecology*, 94(3), pp. 1–13. doi: 10.1093/femsec/fiy017.

Vandeputte, D. *et al.* (2016) 'Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates', *Gut*, 65(1), pp. 57–62. doi: 10.1136/gutjnl-2015-309618.

Větrovský, T. and Baldrian, P. (2013) 'The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses', *PLoS ONE*, 8(2), p. e57923. doi: 10.1371/journal.pone.0057923.

Vlčková, K. *et al.* (2018) 'Impact of stress on the gut microbiome of free-ranging western lowland gorillas', *Microbiology*, 164(1), pp. 40–44. doi: 10.1099/mic.0.000587.

De Vrieze, J. *et al.* (2018) 'The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool', *Microbiome*. Microbiome, 6(1), p. 63. doi: 10.1186/s40168-018-0449-9.

Wada-Katsumata, A. *et al.* (2015) 'Gut bacteria mediate aggregation in the German cockroach', *Proceedings of the National Academy of Sciences of the United States of America*, 112(51), pp. 15678–15683. doi: 10.1073/pnas.1504031112.

Wampach, L. *et al.* (2017) 'Colonization and succession within the human gut microbiome by archaea, bacteria, and microeukaryotes during the first year of life', *Frontiers in Microbiology*, 8, pp. 1–21. doi: 10.3389/fmicb.2017.00738.

Wang, Y. *et al.* (2019) 'Molecular dating of the emergence of anaerobic rumen fungi and the impact of laterally acquired genes', *mSystems*, 4(4). doi: 10.1128/msystems.00247-19.

Ward, T. L. *et al.* (2018) 'Development of the human mycobiome over the first month of life and across body sites', *mSystems*, 3(3). doi: 10.1128/msystems.00140-17.

Wei, S. *et al.* (2021) 'Determining Gut Microbial Dysbiosis: a Review of Applied Indexes for Assessment of Intestinal Microbiota Imbalances', *Applied and Environmental Microbiology*, 87(11), pp. 1–13. doi: 10.1128/AEM.00395-21.

Wei, Y. *et al.* (2020) 'Commensal bacteria impact a protozoan's integration into the murine gut microbiota in a dietary nutrient-dependent manner', *Applied and Environmental Microbiology*, 86(11). doi: 10.1128/AEM.00303-20.

De Weirdt, R. and Van De Wiele, T. (2015) 'Micromanagement in the gut: Microenvironmental factors govern colon mucosal biofilm structure and functionality', *npj Biofilms and Microbiomes*, 1(October), pp. 1–6. doi: 10.1038/npjbiofilms.2015.26.

Whitman, W. B., Coleman, D. C. and Wiebe, W. J. (1998) 'Perspective prokaryotes : The unseen majority', *Proceedings of the National Academy of Sciences*, 95, pp. 6578–6583.

Wiegräbe, N. (2020) 'Assessment of the gastrointestinal nematodes and the diet from redfronted lemurs', Bachelor thesis, University of Göttingen, Göttingen.

Wilmanski, T. *et al.* (2021) 'Gut microbiome pattern reflects healthy ageing and predicts survival in humans', *Nature Metabolism*, 3(2), pp. 274–286. doi: 10.1038/s42255-021-00348-0.

de Winter, I. I. *et al.* (2020) 'Effects of seasonality and previous logging on faecal helminth-microbiota associations in wild lemurs', *Scientific Reports*, 10(1), pp. 1–16. doi: 10.1038/s41598-020-73827-1.

Wittig, R. M. *et al.* (2016a) 'Social support reduces stress hormone levels in wild chimpanzees across stressful events and everyday affiliations', *Nature Communications*, 7, pp. 4–11. doi: 10.1038/ncomms13361.

Wittig, R. M. *et al.* (2016b) 'Social support reduces stress hormone levels in wild chimpanzees across stressful events and everyday affiliations', *Nature Communications*, 7, pp. 4–11. doi: 10.1038/ncomms13361.

Wren, A. M. and Bloom, S. R. (2007) 'Gut Hormones and Appetite Control', *Gastroenterology*, 132(6), pp. 2116–2130. doi: 10.1053/j.gastro.2007.03.048.

Wu, Q. *et al.* (2017) 'Seasonal variation in nutrient utilization shapes gut microbiome structure and function in wild giant pandas', *Proceedings of the Royal Society B: Biological Sciences*, 284(1862). doi: 10.1098/rspb.2017.0955.

Wu, W. L. *et al.* (2021) 'Microbiota regulate social behaviour via stress response neurons in the brain', *Nature*, 595(7867), pp. 409–414. doi: 10.1038/s41586-021-03669-y.

Xia, Y., Sun, J. and Chen, D.-G. (2018) *Statistical Analysis of Microbiome Data with R*. Edited by J. Chen and D.-C. Chen. Singapore: Springer Nature Singapore Pte Ltd. doi: 10.1007/978-981-13-1534-3.

Xiao, L. *et al.* (2015) 'A catalog of the mouse gut metagenome', *Nature Biotechnology*, 33(10), pp. 1103–1108. doi: 10.1038/nbt.3353.

Xie, H. *et al.* (2016) 'Shotgun Metagenomics of 250 Adult Twins Reveals Genetic and Environmental Impacts on the Gut Microbiome', *Cell Systems*, 3(6), pp. 572-584.e3. doi: 10.1016/j.cels.2016.10.004.

Yang, C. A. *et al.* (2017) 'Impact of Enterobius vermicularis infection and mebendazole treatment on intestinal microbiota and host immune response', *PLoS Neglected Tropical Diseases*, 11(9), pp. 1–16. doi: 10.1371/journal.pntd.0005963.

Yano, J. M. *et al.* (2015) 'Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis', *Cell*, 161(2), pp. 264–276. doi: 10.1016/j.cell.2015.02.047.

Yasuda, K. *et al.* (2015) 'Biogeography of the intestinal mucosal and lumenal microbiome in the rhesus macaque', *Cell Host and Microbe*, 17(3), pp. 385–391. doi: 10.1016/j.chom.2015.01.015.

Yatsunenko, T. *et al.* (2012) 'Human gut microbiome viewed across age and geography', *Nature*, 486(7402), pp. 222–227. doi: 10.1038/nature11053.

Young, C. *et al.* (2014) 'Responses to social and environmental stress are attenuated by strong male bonds in wild macaques', *Proceedings of the National Academy of Sciences*, 111(51), pp. 18195–18200. doi: 10.1073/pnas.1411450111.

Youngblut, N. D. *et al.* (2019) 'Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades', *Nature Communications*, 10(1), pp. 1–15. doi: 10.1038/s41467-019-10191-3.

Yurkovetskiy, L. *et al.* (2013) 'Gender bias in autoimmunity is influenced by microbiota', *Immunity*, 39(2), pp. 400–412. doi: 10.1016/j.immuni.2013.08.013.

Zachar, Z. and Savage, D. C. (1979) 'Microbial interference and colonization of the murine gastrointestinal tract by Listeria monocytogenes', *Infection and Immunity*, 23(1), pp. 168–174. doi: 10.1128/iai.23.1.168-174.1979.

Zheng, D., Liwinski, T. and Elinav, E. (2020) 'Interaction between microbiota and immunity in health and disease', *Cell Research*, 30(6), pp. 492–506. doi: 10.1038/s41422-020-0332-7.

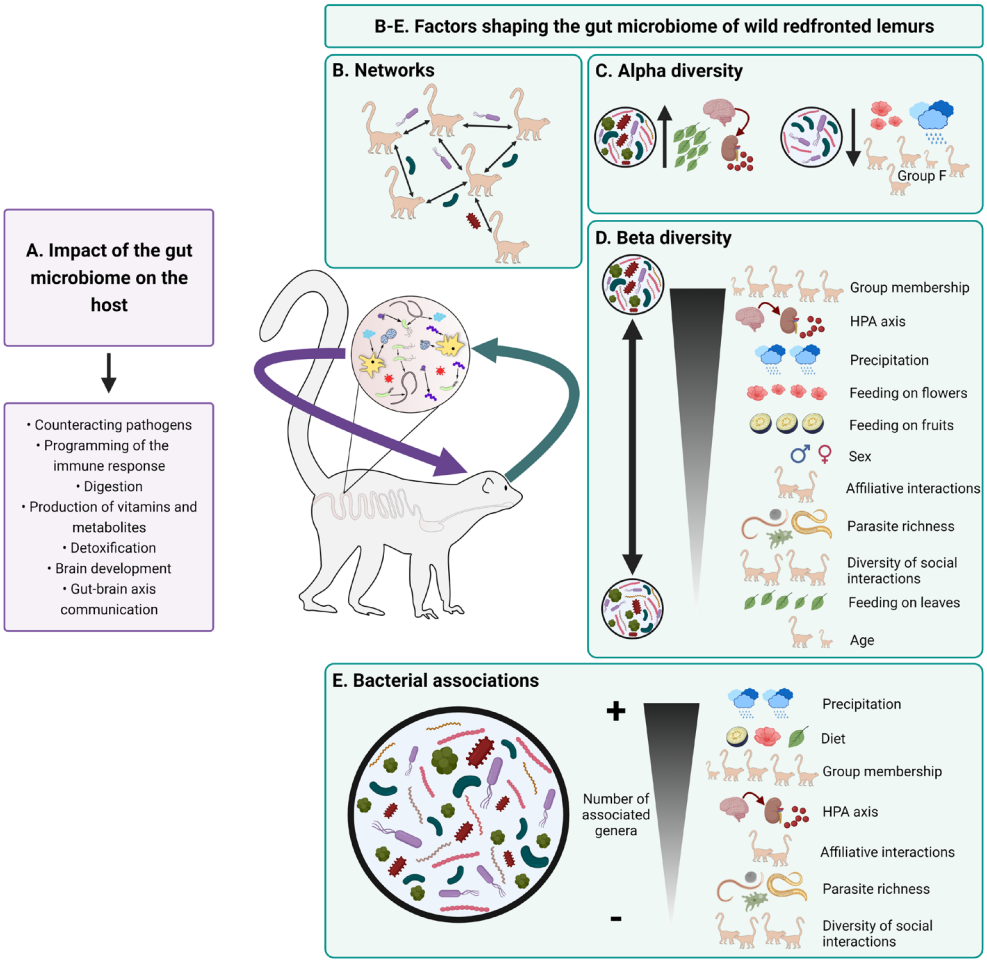
Zhu, L. *et al.* (2020) ‘Sex bias in gut microbiome transmission in newly paired marmosets (Callithrix jacchus)’, *mSystems*, 5(2). doi: 10.1128/msystems.00910-19.

Zmora, N., Suez, J. and Elinav, E. (2018) ‘You are what you eat: diet, health and the gut microbiota’, *Nature Reviews Gastroenterology and Hepatology*, 16. doi: 10.1038/s41575-018-0061-2.

Zoetendal, E. G. *et al.* (2012) ‘The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates’, *ISME Journal*, 6(7), pp. 1415–1426. doi: 10.1038/ismej.2011.212.

6 Appendix

6.1 Summary figure



Summary figure 1. Factors shaping the temporal dynamic of the gut microbiome from wild redfronted lemurs. The host and its gut microbiome are in constant feedback loops between each other. **A**. Research from humans and laboratory animals has determined that the gut microbiome impacts the health of the host. **(B-E)** Conversely, the host and its environment influence the composition and diversity of the gut microbiome. **B.** In wild redfronted lemurs, correlations between social networks and bacterial indicator taxa determined sharing of bacteria through social interactions. **C.** Bacterial alpha diversity increases when feeding on leaves and higher fecal glucocorticoid metabolites, whereas decreases when feeding on flowers, higher precipitation and was lowered in one of the studied groups. **D.** Variation in beta diversity is explained by several factors being the strongest ones group membership, fecal glucocorticoid metabolites and precipitation. **E.** Specific bacterial taxa are positively or negatively associated to all the studied factors in this thesis, but group membership, diet and precipitation had the highest number of associated taxa. The temporal dynamics of the gut microbiome from wild redfronted lemurs are shaped by processes taking place inside the host's intestinal tract and through interactions with microbial communities outside the host. Created with BioRender.com.

6.2 Summary

The gut microbiome consists of the prokaryotic and eukaryotic communities inhabiting the gastrointestinal tract of an animal and plays a pivotal role in the health of the host. This microbial community is highly dynamic and the factors driving these fluctuations remain to be determined. This thesis presents a longitudinal study investigating the factors that shape the gut microbiome of wild redfronted lemurs aiming to detect an impact from social relationships. Social relationships can influence the gut microbiome directly through transmission of microorganisms during social interactions, or indirectly through activation of the hypothalamic-pituitary-adrenal (HPA) axis due to social stressors.

In Chapter 2 the temporal variations in the diverse microbial communities of redfronted lemurs were analyzed. The study showed that fluctuations in diet, affiliative interactions, and precipitation impact the bacterial entire and active community. Temporal variations in bacterial diversity were driven by swift changes in the food items consumed (fruits, flowers, and leaves), affiliative interactions and precipitation. Feeding on leaves increased bacterial alpha diversity whereas feeding on flowers and higher precipitation decreased bacterial diversity. Feeding on flowers and fruits and affiliative interactions affected the gut microbiome composition. Specific bacterial genera associated to feeding on flowers and fruits, affiliative interactions, and precipitation were detected. Fermenters of polysaccharides and glycolipids like *Succinivibrio, Oscillospiraceae, Prevotellaceae*, and *Anaerovibrio* were positively affected with consumption of flowers and fruits. *Rikenellaceae, Alloprevotella, Kiritimatiellae*, and *Spirochaetaceae* were positively affected by affiliative interactions. Higher precipitation had a negative impact on *Kiritimatiellae*, suggesting that this order is acquired from other water sources only present during the dry season. Thus, showing that the investigated factors shape the longitudinal dynamics of the gut microbiome.

Chapter 3 focused on the investigation of environmental selection in the gut niche due to host's sex, age, HPA axis activation, parasite richness, diet and water intake. Additionally, dispersal processes of microorganisms between hosts through social interactions and with environmental water were examined. Bacterial alpha diversity

increased with higher fecal glucocorticoid metabolite (fGCM) measurements, consumption on leaves, while being significantly lower in one group. Group membership, fGCM levels and precipitation explained the highest amount of variation in beta diversity. Associations between bacterial genera and all studied factors were detected, excluding host's sex. For instance, *Tyzzerella* associated with higher fGCM concentrations whereas genera from *Helicobacteraceae* and *Mycoplasmataceae* presented negative associations. Parasite richness associated to changes in abundances of bacterial genera but had a small impact on bacterial beta diversity. As reported in humans, *Succinivibrio* and *Verrucomicrobiota* associated positively with parasite richness while genera from *Lachnospiraceae* had negative associations. Correlations between bacterial indicator taxa and social networks were detected, suggesting transmission of bacteria through social interactions. Thus, environmental selection at the gut niche and dispersal processes of microorganisms between hosts and the environment influenced the gut microbiome at different intensities.

Finally, Chapter 4 reviews the impact of social behaviors in primates and their influence on parasite transmission and susceptibility to disease. Group-living provides advantages for an individual but also increases exposure to parasites. The same processes associated to parasite transmission and susceptibility can be important drivers of the gut microbiome. Thus, this knowledge and methods used in these investigations can be applicable to the study of the gut microbiome.

In conclusion, this project demonstrates that social relationships impact the gut microbiome directly through social interactions, group membership and indirectly through HPA axis activation. Diet and precipitation are important drivers of the temporal variations in the gut microbiome. Parasite richness impacted the abundance of bacterial genera but not diversity, possibly because bacteria-parasite interactions are species specific. The detection of the temporal variations of the gut microbiome of wild redfronted lemurs and its drivers was possible due to the longitudinal setup with a dense sampling regime coupled with the collection of focal behavioral data and environmental records. Thus, the temporal dynamics of the gut microbiome of wild redfronted lemurs are shaped by factors inside and outside the hosts, including the dispersal processes of bacteria between hosts through social interactions.

6.3 Zusammenfassung

Das Darm Mikrobiom besteht aus den prokaryotischen und eukaryotischen Gemeinschaften, die den Magen-Darmtrakt eines Tieres bewohnen und spielen eine ausschlaggebende Rolle für die Gesundheit des Wirts. Diese mikrobielle Gemeinschaft ist hoch dynamisch und die Faktoren, die diese Fluktuationen beeinflussen, sind nicht vollständig erforscht. Diese Dissertation präsentiert eine Verlaufsstudie mit dem Ziel, die Faktoren, welche das Darm Mikrobiom von Rotstirnmakis formen, zu bestimmen und einen möglichen Einfluss sozialer Beziehungen zu untersuchen. Sozialbeziehungen können das Darm Mikrobiom durch direkte Transmission von Mikroorganismen durch soziale Interaktionen, oder indirekt durch Aktivierung der Hypothalamus-Hypophysen-Nebennierenrinden-Achse (HPA) beeinflussen.

In Kapitel 2 wurden die temporalen Variationen in den diversen mikrobiellen Gemeinschaften von Rotstirnmakis analysiert. Diese Studie zeigte, dass Fluktuationen der Ernährung, affiliative Interaktionen, sowie Niederschlag die gesamten und aktiven bakteriellen Gemeinschaften beeinflussen. Temporale Variationen der bakteriellen Diversität wurden durch Veränderungen der Nahrungsquelle (Früchte, Blüten oder Blätter), affiliative Interaktionen und Niederschlag beeinflusst. Eine auf Blättern basierte Ernährung erhöhte die bakterielle Diversität, während eine Blüten-basierte Ernährung und höherer Niederschlag die bakterielle Diversität verringerten. Der Verzehr von Blüten und Früchten, sowie affiliative Interaktionen beeinflussten ebenfalls die Komposition der mikrobiellen Gemeinschaft. Es wurden spezifische bakterielle Genera identifiziert, die mit diesen Faktoren, sowie Niederschlag assoziiert werden konnten. Polysaccharide und Glycolipide fermentierende Organismen wie *Succinivibrio*, *Oscillospiraceae*, *Prevotellaceae*, und *Anaerovibrio* korrelierten mit der Aufnahme von Blüten und Früchten. *Rikenellaceae*, *Alloprevotella*, *Kiritimatiellae*, und *Spirochaetaceae* wurden durch affiliative Interaktionen positiv beeinflusst. *Kiritimatiellae* zeigten eine negative Korrelation mit erhöhtem Niederschlag, was eine Aufnahme durch andere Wasserquellen während der Trockenzeit vermuten lässt. Dies zeigt, dass die analysierten Faktoren die longitudinale Dynamik des Magen-Darm Mikrobioms beeinflussen.

Kapitel 3 konzentrierte sich auf die Untersuchung von möglicher Selektion durch äußere Einflüsse wie das Geschlecht des Wirts, Alter, HPA Aktivierung, Parasitenvorkommen, Ernährung und Wasseraufnahme in Bezug auf die ökologische Nische im Magen-Darmtrakt. Zusätzlich wurde die Verbreitung von Mikroorganismen zwischen Wirten durch soziale Interaktionen und Wasserkontakt untersucht. Die bakterielle Alpha Diversität stieg mit höheren fäkal Glucocorticoid-Messungen (fGCM), sowie dem Verzehr von Blättern, während in einer der untersuchten Gruppen hingegen eine Verminderung beobachtet wurde. Gruppenzugehörigkeit, fGCM Werte und Niederschlag erklärten den größten Teil der Beta-Diversität Varianz. Es wurden Assoziationen zwischen bakteriellen Genera und allen miteinbezogenen Faktoren bis auf das Geschlecht des Wirts detektiert. *Tyzzerella* konnten mit höheren fGCM Konzentrationen assoziiert werden, während die Genera *Helicobacteraceae* und *Mycoplasmataceae* negative Assoziationen zeigten. Eine hohe Vielfalt an Parasiten konnte mit Abundanz-veränderungen bestimmter bakterieller Genera in Verbindung gebracht werden, zeigte aber nur geringen Einfluss auf bakterielle Beta-Diversität. Wie bereits in Menschen gezeigt, waren *Succinivibrio* and *Verrucomicrobiota* mit parasitärer Vielfalt assoziiert, während *Lachnospiraceae* eine negative Korrelation zeigten. Korrelationen zwischen bakterieller Indikator-Spezies und sozialen Netzwerken suggerieren eine Übertragung von Bakterien durch soziale Strukturen, dadurch einen selektiven Einfluss der Umwelt auf die ökologische Nische des Magen-Darmtrakts, Ausbreitungsprozesse von Mikroorganismen zwischen Wirt und der Umwelt und demonstrieren somit einen Einfluss der Umwelt auf das Mikrobiom des Magen-Darmtrakts.

Kapitel 4 diskutiert den Einfluss sozialen Verhaltens von Primaten auf Transmission von Parasiten. Das Leben in einer Gruppe bietet Vorteile für das Individuum, erhöht aber auch die Exposition zu Parasiten. Diese Prozesse können ebenfalls wichtige Faktoren für das Mikrobiom des Magen-Darmtrakts sein. Die Erkenntnisse und Methoden aus dieser Studie könne daher bei der weiteren Erforschung des Darm-Mikrobioms Verwendung finden.

Dieses Projekt zeigt, dass soziale Beziehungen und Gruppenzugehörigkeit einen direkten und HPA Aktivierung einen indirekten Einfluss auf das Mikrobiom des Magen-Darmtrakts ausüben. Ernährung und Niederschlag sind wichtige Faktoren der

temporalen Variationen des Mikrobioms. Parasitäre Vielfalt beeinflusste die Abundanz bakterieller Genera, nicht aber die bakterielle Diversität. Möglicherweise durch spezifische Interaktionen zwischen Bakterien und Parasiten. Die Detektion temporaler Veränderungen des Mikrobioms im Magen-Darmtrakt von Rotstirnmakis und dessen beeinflussende Faktoren wurden durch ein longitudinales Studiendesign mit engmaschiger Beprobung und der Erhebung verhaltensspezifischer und abiotischer Daten ermöglicht. Daher lässt sich sagen, dass die Dynamik des Mikrobioms der Rotstirnmakis durch verschiedene Faktoren in und außerhalb des Wirts beeinflusst wird und mit Übertragungsprozessen zwischen Wirten durch soziale Interaktionen in Verbindung steht.

6.4 Declaration of independent work

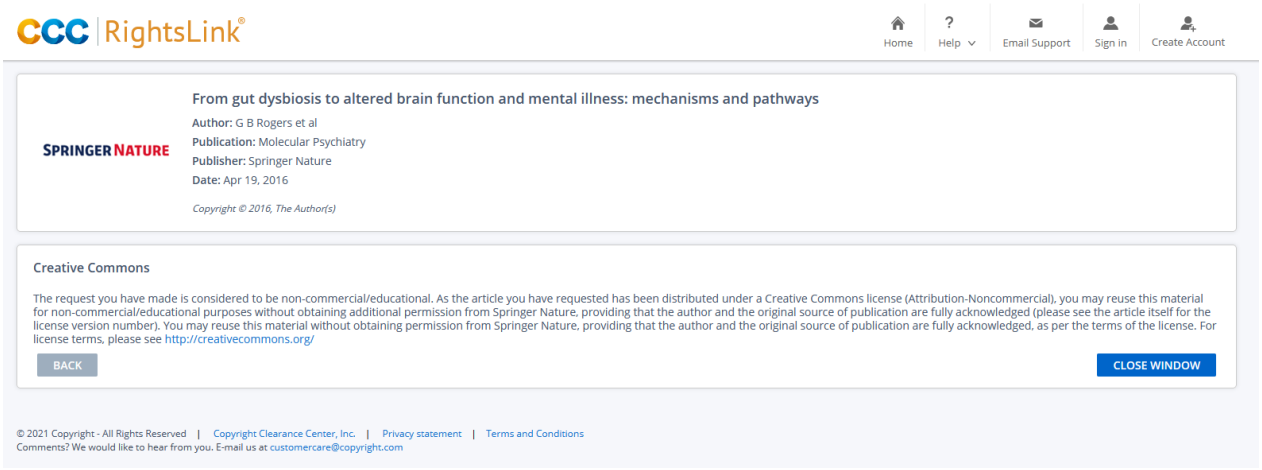
All parts of the dissertation were written by myself, assistance of third parties was only accepted when scientifically justifiable and acceptable in regards to the examination regulations and all used sources were quoted. Moreover, I have not submitted this thesis previously in any form for another degree at any other institution or university.

Sonia Tatiana Murillo Corrales

15.11.2021

Göttingen

6.5 Permission figure Rogers et al., 2016.



6.6 Acknowledgements

Thanks Claudia and Rolf for giving an opportunity to a persistent Costa Rican who wanted to become a researcher and learn more about bacterial genomics and bioinformatics but ended up with five times what I ever thought I could ever learn and experience.

Thanks, Rolf, for supporting me since the beginning, I cannot be grateful enough of the trust you placed in me. Thanks also for the jokes, the talks, the football and the wonderful parties you organized for the department. I am very happy I was able to be part of your team.

Thanks, Claudia, for giving me an opportunity to live and work in the lovely Kirindy forest and meet the gidros. Thank you also for your advice, the nice Kirindy nights and trusting a lab primate to do field work.

Thanks to Dominik for being such a supportive supervisor, keeping my spirits up, and guiding me through the path of bioinformatics. You are so incredibly knowledgeable and you're an awesome researcher and person. Forever Dominikan! Everything is awesome! #Science24

Thanks, Peter, for your supportive words and for an exciting Schalke game. Furthermore, I am kindly grateful because you are part of the existence of Kirindy Forest Research Station and aiding in the conservation of this small piece of paradise.

Thanks to my examination committee: Dr. Oliver Schülke, Prof. Dr. Stefanie Pöggeler, Dr. Verena Behringer, and Dr. Christian Roos for your time and helpful scientific discussion.

Thanks to Anja Poehlein who has been supportive from the beginning, patient and helpful.

Dr. Michael Heistermann, thank you for your help and guidance in the fecal glucocorticoid analysis.

To Roger Mundry for being such a cool person and captivating our minds through statistics. You have been a great companion this last year.

Major part of the success of this project was the support, help and company of the Malagasy team in the Kirindy Field Station: Jipa, Tina, Mamy, Pata, Adrien, Mama Suly, Elisa, Remi, Brunot, Alain, Rodin, Léon, and Gregoir. Misaotra betsaka tompoko!

Thanks to the bachelor and master students who helped me with field and laboratory work, with whom I shared fun experiences and who taught me a lot: Luise Waldow, Judit Stolla, Joe Büchner, Alexandra Michiels, Natascha Wiegraebe and JunHyeuk Shin.

I am very grateful to all the Posdocs (Jacky, Birgit, HeikoN, HeikoL, Bernd and Robert), colleagues (Miri, Tim, Inka), technical (Mechtild, Melanie and Sarah) and administrative staff (Petra, Nicole, and Christiane) from AG Daniel. Thank you for sharing your knowledge, your support, beautiful Christmas parties, jokes and dealing with essential organizational matters.

I am also very grateful to the SoBi team: Christina, Ulli, Henning, Eckhard, Lea, Leonie, Katja, Johanna and Gabriel. You have all contributed with scientific discussion, cheerful talks, and joyful lunch meetings.

Special thanks to Simon for his funny conversations, being a great listener and providing the perfect 10 (20?) min talk break.

Thanks to my lovely microbes, Stefi (& Martin), Amelie, Avi, Ines, Katrina, Kerrin, Alex, Randi, and Richard. You have been so supportive and made my days easier with advice, laughs, drinks, good music, and some dancing. You are all great researchers!

Thanks to Genis, from a conference dinner to becoming part of your family, you are partly responsible for me achieving my goal and I admire you a lot. To Sina, Luciano, and Mateo for becoming my family and making my life in Germany so lovely.

Thanks to the behavioral biologists who have been there to guide me into the world of animal behavior but also became my Göttingen family, Sofya, Clémence, Lotte, Simone, Lluis, Sonia, Hasina, Franzi and Charlotte. Extra thanks go to Louise, who was super patient with me until the founding of the Gidro team, we shared beautiful times in Kirindy and in Göttingen. Also, I am very grateful to Sofy for proofreading my thesis.

I am very grateful to the SoHaPi team for the experience with the book chapter Baptiste, Simone, Virgile, Charlotte, Nadine, Markus, and Doris. I am very glad we had the opportunity to build this project together.

Thanks to Steffi Reher, the bat lady, for sharing with me the beautiful Kirindy nights and long talks in Germany and Sevilla, it has been a pleasure to meet you.

Thanks to Lauren Seex, my first companionship through my field work, always supportive and an incredible source of information regarding animal behavior.

Thanks to Virgile Manin for being an incredible source of information and a great companion for SoHaPi beers.

Thanks, Hanitra and Matze for letting me be part of Chances for Nature and Little Rangers, which also became an achievement during my PhD times.

To the CRC team & associates Kristina, Pierre, Joyson, Greta, Florian, Clara, Isabelle and Dominik. We have spent very fun times together. Special thanks to Britta, Carina and Rahmi who became close friends and have been a great support this last year.

Thanks to the lovely people I was able to meet through Dirk who also became my dear friends, Marlene, Marina, Benno, Felix, Rosi, and Andi. Thank you for welcoming me to your lives.

To my friends in Costa Rica who have been my fiercest supporters and whom I missed very much during this adventure: Mariann, Adri, Lucy, Moni, Albin, Nacho, Ibra, Jose Molina, Diego, Juli, Anita, Claudio, María, Mau, Popi, Soto and Diana. Los quiero!!!

I am very grateful to the awesome researchers I met during my master studies, Diana, Pame, Jaz, and Gaby. I am so proud of all of us, we left our comfort zone and dived deep into research. Thanks for your relentless friendship and advice!

I extend my gratefulness to Dr. César Rodríguez, Dr. Eugenia Corrales-Aguilar, Dr. Carlos Chacón-Díaz and Dr. Bruno Lomonte for their constant support during this journey and for being excellent role models.

So Latin American families are big, and I need to thank all of them! Gracias a mis tías (Elsita, Antonieta, Sonia, Gloria y Marta), tíos (Martín, Mario e Ismael), primas (Luli, Jeanni, Gloriela, Clau y Karen) y primos (Pedro, Fabio y Juan Pablo), gracias por darme el impulso para realizar mis aventuras y por sus palabras de aliento. ¡Todo es más fácil cuando se tiene tantísimo apoyo!

Thanks to my brother, Pablo, you are so brave, and you have been a great support during this adventure while impressing me with achieving your own goals. I am so grateful to have you as my brother.

Dirk, our crazy adventure keeps going and I am very glad it does. Thanks for always being there to lift my spirits and reminding me to believe in myself. I am at awe of the many things you have achieved and what you've become. Love you.

Gracias mami y papi, sus historias de la Universidad de Costa Rica y la pasión de mami por la biología definitivamente guiaron mis pasos. Gracias por haberme instado a alcanzar mis metas de estudio y por su apoyo incondicional. Logré alcanzar un sueño compartido de estudiar en el extranjero y convertirme en la primera PhD de la familia. Los amo.

Caminante, son tus huellas
el camino y nada más;
Caminante, no hay camino,
se hace camino al andar.
Al andar se hace el camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.
Caminante no hay camino
sino estelas en la mar.

Antonio Machado