Received: 9 July 2017 | Revised: 23 March 2018 | Accepted: 20 April 2018
DOI: 10.1002/ajp.22867

REVIEW ARTICLE

WILEY AMERICAN JOURNAL OF PRIMATOLOGY

The gut microbiome of nonhuman primates: Lessons in ecology and evolution

Jonathan B. Clayton[1,2,3] | Andres Gomez[3,4] | Katherine Amato[3,5] | Dan Knights[3,6,7] | Dominic A. Travis[3,8] | Ran Blekhman[3,9,10] | Rob Knight[3,11,12,13] | Steven Leigh[3,14,15] | Rebecca Stumpf[3,15,16] | Tiffany Wolf[3,8] | Kenneth E. Glander[3,17] | Francis Cabana[3,18] | Timothy J. Johnson[1,3,19]

[1] Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, Minnesota
[2] GreenViet Biodiversity Conservation Center, Son Tra District, Danang, Vietnam
[3] Primate Microbiome Project, Minneapolis, Minnesota
[4] Department of Animal Science, University of Minnesota, St Paul, Minnesota
[5] Department of Anthropology, Northwestern University, Evanston, Illinois
[6] Biotechnology Institute, University of Minnesota, Saint Paul, Minnesota
[7] Department of Computer Science and Engineering, University of Minnesota, Minneapolis, Minnesota
[8] Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, Minnesota
[9] Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota
[10] Department of Ecology, Evolution, and Behavior, University of Minnesota, Falcon Heights, Minnesota
[11] Department of Computer Science & Engineering, UC San Diego, La Jolla, California
[12] Department of Pediatrics, UC San Diego, La Jolla, California
[13] Center for Microbiome Innovation, UC San Diego, La Jolla, California
[14] Department of Anthropology, University of Colorado Boulder, Boulder, Colorado
[15] C.R. Woese Institute for Genomic Biology, University of Illinois, Urbana, Illinois
[16] Department of Anthropology, University of Illinois, Urbana, Illinois
[17] Department of Evolutionary Anthropology, Duke University, Durham, North Carolina
[18] Wildlife Nutrition Centre, Wildlife Reserves Singapore, Singapore
[19] University of Minnesota, Mid-Central Research and Outreach Center, Willmar, Minnesota

Correspondence
Timothy J. Johnson, Department of Veterinary and Biomedical Sciences, University of Minnesota, 1971 Commonwealth Avenue, 205 Veterinary Science, Saint Paul, MN 55108.
Email: tjj@umn.edu

Funding information
National Institute on Drug Abuse, Grant number: DA007097-32; National Science Foundation, Grant numbers: BCS 0935374, BCS 1441409

The mammalian gastrointestinal (GI) tract is home to trillions of bacteria that play a substantial role in host metabolism and immunity. While progress has been made in understanding the role that microbial communities play in human health and disease, much less attention has been given to host-associated microbiomes in nonhuman primates (NHPs). Here we review past and current research exploring the gut microbiome of NHPs. First, we summarize methods for characterization of the NHP gut microbiome. Then we discuss variation in gut microbiome composition and function across different NHP taxa. Finally, we highlight how studying the gut microbiome offers new insights into primate nutrition, physiology, and immune system function, as well as enhances our understanding of primate ecology and

Jonathan B. Clayton and Andres Gomez equally contributed to the manuscript.

evolution. Microbiome approaches are useful tools for studying relevant issues in primate ecology. Further study of the gut microbiome of NHPs will offer new insight into primate ecology and evolution as well as human health.

KEYWORDS
ecology, evolution, microbiome, nonhuman primate (NHP)

1 | INTRODUCTION

All animals possess a microbiome, often defined as the collection of viruses, bacteria, archaea, fungi, and protists colonizing the body, and their genetic material. The relationship between animals and their microbiomes likely started from the moment pluricellular systems evolved in a biosphere where microbes, primarily bacteria, had dominated for at least 2.5 billion years (Hooper & Gordon, 2001; Ley et al., 2008). Thus, microbial colonization of multicellular organisms may have been inevitable, as processes of evolutionary diversification shaped the tree of life, including the adaptive radiation of primates around 55 million years ago.

Recent research indicates a complex relationship between hosts and their microbiomes. Although microbes inhabit multiple parts of the body including the oral cavity, the skin, and the urogenital tract, most of what is known about the microbiome focuses on the gastrointestinal tract (referred to herein as the gastrointestinal microbiome). The number of microbes in the GI tract matches or exceeds the number of host somatic cells (Savage, 1977; Sender, Fuchs, & Milo, 2016) and the collective functions encoded by genes of the gastrointestinal microbiome greatly surpass those of the host. As a result, hosts benefit from complementing the functions encoded in their own genomes with those of their associated microbiomes (Backhed, Ley, Sonnenburg, Peterson, & Gordon, 2005; Hooper & Gordon, 2001; Ochman et al., 2010; Toft & Andersson, 2010).

To date, the main two methodological approaches used to study host-associated microbes are culture-dependent and culture-independent methods. Historically, culture-dependent methods were primarily used. Since the 1960's, culture-dependent methods have been used to study NHP-associated bacteria (Bauchop, 1971; Benno, Honjo, & Mitsuoka, 1987; Benno, Itoh, Miyao, & Mitsuoka, 1987; Bauchop & Martucci, 1968; Brinkley & Mott, 1978). In one of the first studies, Bauchop (1971) analyzed the rhesus macaque gut microbiome by culturing bacteria from multiple segments of the GI tract. In another early study, Brinkley and Mott (1978) identified the predominant genera present in baboon feces. However, microbial cultivation fails to identify most microbial taxa due to limitations associated with culture-based methods. Specifically, it is estimated that existing culture methods can reproduce viable conditions for only 20% of mammalian gut microbes (Eckburg et al., 2005; Savage, 1977; Zoetendal, Collier, Koike, Mackie, & Gaskins, 2004) and less than 5% of all existing bacterial species (Hugenholtz, Goebel, & Pace, 1998; Pace, 1997; Rappe & Giovannoni, 2003). Additionally, in vitro isolation of microbes does not necessarily reflect the complex interactions among the vast diversity of organisms in the gastrointestinal microbiome or their functional relevance. In the late 1970s, Carl Woese and George E. Fox pioneered the use of 16S rRNA in phylogenetics, and ultimately discovered a new domain of life, archaebacteria (Woese, 1987; Woese, Kandler, & Wheelis, 1990). This discovery lead to the survey of bacterial sequences directly from the environment (Lane et al., 1985). Since then, the use of culture-independent techniques to study bacteria has substantially increased our knowledge of both environmental and host-associated microbial communities.

The gastrointestinal microbiome has recently been shown to play key roles in many host physiological processes. For example, the gastrointestinal microbiome allows hosts to recover energy from otherwise indigestible foods. Mammals do not possess the glycoside hydrolases, polysaccharide lyases and carbohydrate esterases required to breakdown the β-1,4 glycosidic linkages in complex plant polysaccharides (Bayer, Lamed, White, & Flint, 2008). Instead, the gastrointestinal microbiome is entirely responsible for breaking down and fermenting structural polysaccharides in plants to yield energy-rich short chain fatty acids (SCFAs) (Hume, 1997). These SCFAs can be absorbed by the host and utilized as an energy source. This function is essential for host nutrition. Nonhuman primates (NHPs) depend on plant material as their main source of nutrients (Milton, 1987) and may obtain from 30% to 57% of their daily energy budget from SCFAs (Milton & McBee, 1983; Popovich et al., 1997). In terms of digestibility, Remis and Dierenfeld (2004) measured digestibility of two gorilla diets by comparing nutritional and chemical content of ingesta and fecal dry matter across two study phases. During phase 1, gorillas ate their regular diet, whereas during phase II the diet was altered by substituting a higher fiber, less digestible biscuit and reducing the amount of browse offered. Through their analyses, they determined that the phase II diet was less digestible than the original diet. Specifically, fiber digestibility was *ca.* 70% for NDF in 2000 and 45% in 2001, and *ca.* 0.03% for ADF in 2000 and 30% in 2001 (Remis & Dierenfeld, 2004). Edwards and Ullrey (1999) fed two test diets with varying acid detergent fiber (ADF) concentrations to adult hindgut- and foregut-fermenting NHPs. Their results showed a significant reduction in dry matter (DM) digestibility in hindgut fermenters fed diet 30ADF versus 15ADF, suggesting that hindgut fermenters are less

able to utilize a higher fiber food when compared to foregut fermenters (Edwards & Ullrey, 1999).

The gastrointestinal microbiome is also responsible for maintaining proper host innate and adaptive immune responses by establishing a close spatial and functional relationship with the host's gut epithelia and associated lymphoid tissues (Lee & Mazmanian, 2010; McFall-Ngai, 2007; Round et al., 2011). The absence of a balanced and healthy gastrointestinal microbiome, often referred to as dysbiosis, has been linked to susceptibility to infection, decreased lymphocyte and intestinal macrophage proliferation, and low serum immunoglobulin levels (particularly IgA) (Bäckhed et al., 2004; Dicksved et al., 2008; Larsen et al., 2010; Rautava & Isolauri, 2002; Round & Mazmanian, 2009). The gastrointestinal microbiome has been linked to a number of diseases, including obesity (Turnbaugh, Bäckhed, Fulton, & Gordon, 2008; Turnbaugh et al., 2006, 2009), diabetes (Boerner & Sarvetnick, 2011; Brown et al., 2011; Giongo et al., 2011), Crohn's (Gevers et al., 2014; Knights, Lassen, & Xavier, 2013), and Alzheimer's (Bhattacharjee & Lukiw, 2013), among others.

These studies exemplify the role the gastrointestinal microbiome plays in mammalian physiology and human health and disease. However, such associations have yet to be investigated in depth in NHPs. NHPs are the most biologically relevant research animal models for humans, and are unmatched in terms of their relevance compared to other animals used to study many human conditions (Chen, Niu, & Ji, 2012; Stone, Treichel, & VandeBerg, 1987). A better understanding of host-microbiome interactions in NHPs is also critical for advancing understanding of the role microbes have played in human evolution, including adaptation to novel diets based on gastrointestinal microbiome composition. In the context of animal biology, a better understanding of the composition and function of the NHP gastrointestinal microbiome would provide an opportunity to assess the influence of these microbial communities in NHP ecology and evolution. This review aims to: 1) summarize the methodology that provides the foundation for gastrointestinal microbiome research (Figures 1 and 2 and Table 1), 2) summarize the current state of knowledge about the gastrointestinal microbiome of NHPs across a wide range of relevant issues in primatological research,(Figure 3; Tables 1 and 2), and 3) explore how study of the gastrointestinal microbiome can offer new perspectives on primate nutrition and physiology. The intent is to motivate scientists involved in primatological research to use microbiome approaches to study relevant issues in primate ecology.

1.1 | Methods used in primate microbiome research

The recognition of the critical role the mammalian gastrointestinal microbiome plays in physiology necessitates characterization of its composition and diversity. While microbial cultivation fails to recover the majority of microbial species due to limitations imposed by culture methods, culture-independent methods mitigate these limitations. A breakthrough came from use of small subunit rRNA sequencing for phylogenetic studies (Woese, 1987; Woese et al., 1990), and the survey of bacterial mixed ribosomal sequences directly from the

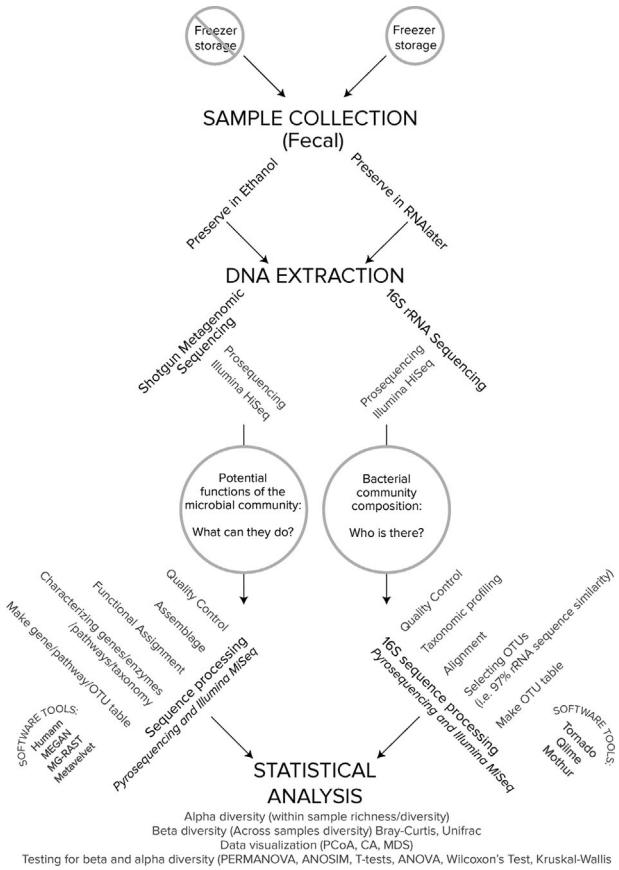

FIGURE 1 Overview of methods used in microbiome studies

environment (Lane et al., 1985). Since then, the use of culture-independent molecular techniques to study DNA sequences has enhanced our knowledge of microbial communities. Bacterial community composition is most commonly assessed using molecular methods that exploit the hypervariable regions of the "universal" 16S bacterial and archaeal ribosomal RNA gene sequence (16S rRNA) as a phylogenetic marker. The 16S rRNA gene includes nine hypervariable (V1–V9) regions, and sequence dissimilarity among microbes within these regions allows researchers to identify and differentiate organisms taxonomically (Pace, 1997). Of these nine regions, some are sequenced for phylogenetic analysis and taxonomic classification more commonly than others, such as V4–V6 (Yang, Wang, & Qian, 2016). For example, the Earth Microbiome Project, a very large collaborative project aimed at characterizing microbial life on planet earth, is based on the usage of V4 (Gilbert, Jansson, & Knight, 2014; Gilbert et al., 2010; Thompson et al., 2017). Nucleic acid-based methods for profiling microbial diversity have rapidly changed with evolving DNA sequencing technologies. High-throughput sequencing platforms, such as 454 pyrosequencing and Illumina, allowed researchers to analyze the bacterial community composition of hundreds to thousands of samples simultaneously, while recovering large numbers of 16S rRNA reads per sample. Most microbial ecology studies assume that the DNA sequences of two or more organisms sharing more than 97% 16S rRNA sequence identity belong to the same species-level taxon, and are known as an operational taxonomic

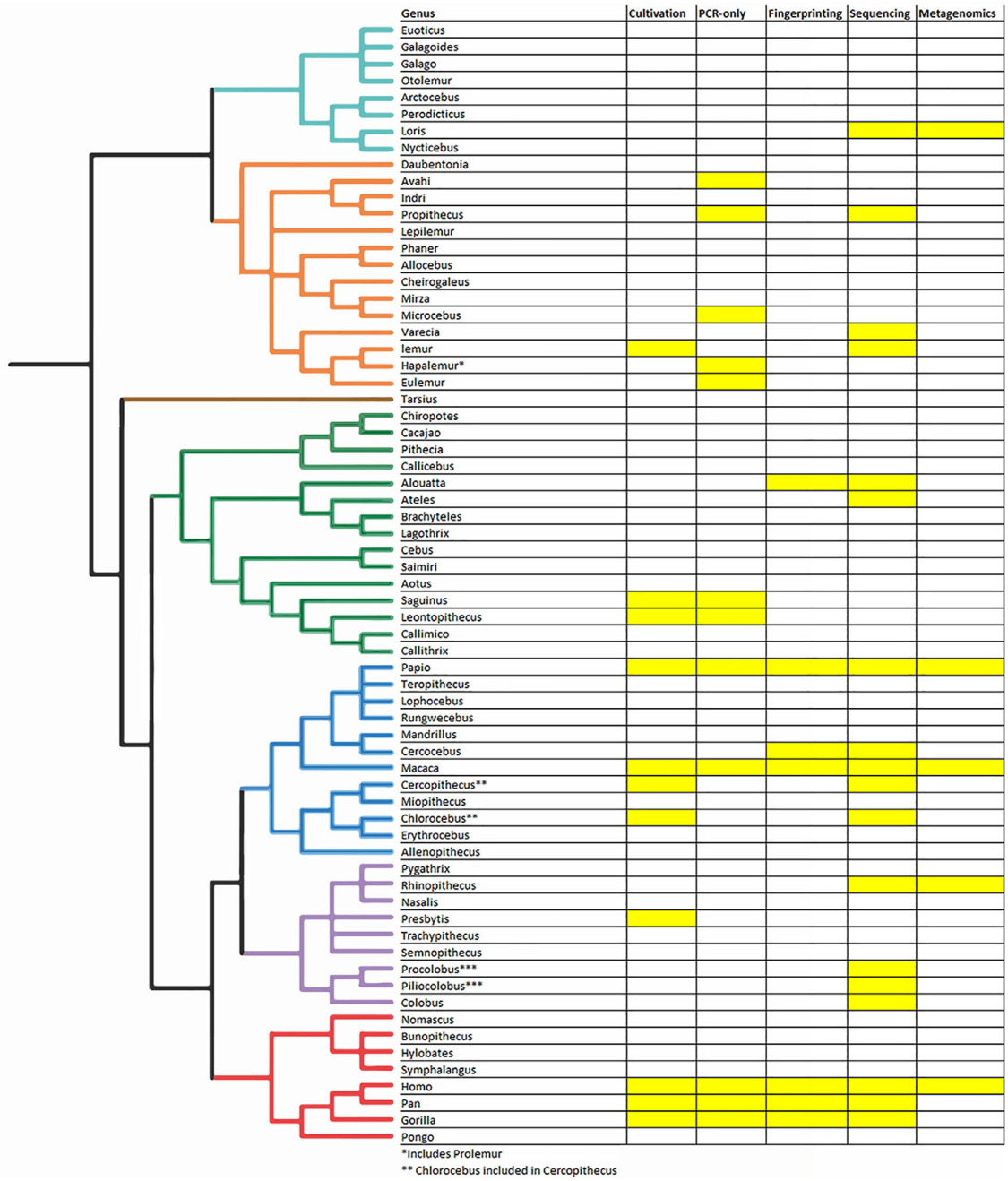

FIGURE 2 Studies examining microbial communities of NHPs listed by methodology used. * Includes Prolemur ** Chlorocebus included in Cercopithecus *** Piliocolobus included in Procolobus

unit (OTU). However, this delineation is arbitrary and may vary among studies adopting different thresholds for similarity (Forney, Zhou, & Brown, 2004). Different algorithms (Caporaso et al., 2010; Chen, Zhang, Cheng, Zhang, & Zhao, 2013; Schmidt, Matias Rodrigues, & von Mering, 2015) for calculating OTUs can have even more of an impact on which sequences are grouped into the same OTU than the chosen similarity threshold (Schloss & Handelsman, 2005) so attention to bioinformatics considerations is crucial for interpreting results. For example, open-reference OTU picking, closed-reference OTU picking, and de novo OTU picking all have their pros and cons (Caporaso et al., 2010). The aforementioned sequence approaches enable improved sensitivity and diversity coverage, and overcome many of the problems associated with previous cloning and fingerprinting techniques (Hamady & Knight, 2009; Robinson, Bohannan, & Young, 2010). Another method for analyzing amplicon sequencing data is DAD2, which uses produces tables of amplicon sequence variants (ASVs), as opposed to OTU tables (Callahan et al., 2016). The principle of high-throughput sequencing relies on gathering more 16S rRNA short-length sequences rather than the longer sequences obtained when analyzing the full-length 16S rRNA (Liu, Lozupone, Hamady, Bushman, & Knight, 2007; Petrosino, Highlander, Luna, Gibbs, & Versalovic, 2009; Ronaghi, 2001).

TABLE 1 Similarities and differences (culture-independent methods only) between the main bacterial taxa found in nonhuman primates using molecular methods. Higher to low abundance is seen from left to right when available.

					Main phyla detected (and class or genus when available)			
Primate taxonomy	**Study**	**Sequencing method**	**Captive or Wild**	**Species**	**1**	**2**	**3**	**4**
Prosimians								
Genus *Lemur* (ring-tailed lemur)	Fogel (2015)	Illumina HiSeq	Both	*Lemur catta*	Wild: Firmicutes Captive: Bacteroidetes	Wild: Bacteroidetes Captive: Spirochaetes	Wild: Proteobacteria Captive: Proteobacteria	Wild: Euryarchaeota Captive: Firmicutes
	Bennett et al. (2016)	Illumina MiSeq	Wild	*Lemur catta*	Firmicutes	Bacteroidetes	Proteobacteria	Tenericutes
	McKenney et al. (2015)	Illumina MiSeq	Captive	*Lemur catta*	Bacteroidetes	Firmicutes	Proteobacteria	Spirochaetes
Genus *Varecia* (ruffed lemurs)	McKenney et al. (2015)	Illumina MiSeq	Captive	*Varecia variegata*	Bacteroidetes	Firmicutes	Spirochaetes	Proteobacteria
Genus *Propithecus* (sifakas)	Fogel (2015)	Illumina HiSeq	Wild	*Propithecus verreauxi*	Firmicutes	Bacteroidetes	Proteobacteria	Actinobacteria
	McKenney et al. (2015)	Illumina MiSeq	Captive	*Propithecus coquereli*	Firmicutes	Bacteroidetes	Verrucomicrobia	Proteobacteria
Genus *Nycticebus* (slow lorises)	Bo et al. (2010)	Cloning; RFLP	Wild	*Nycticebus pygmaeus*	Firmicutes (*Bacillus-Clostridium-Eubacterium-Unclassified*)	Proteobacteria (*Pseudomonas-Acinetobacter-Psycrobacter-Enterobacter-Hafnia*)	Bacteroidetes (*Bacteroides*)	Actinobacteria (*Corynebacterium*)
	Xu et al. (2013)	Shotgun sequencing (Pyrosequencing)	Wild	*Nycticebus pygmaeus*	Bacteroidetes (*Bacteroides-Prevotella-Parabacteroides*)	Proteobacteria (*Pseudomonas*)	Actinobacteria	Firmicutes (Clostridia-Bacilli)
New World monkeys								
Genus *Alouatta* (howler monkeys)	Nakamura et al. (2011)	Cloning; DGGE	Both	*Alouatta pigra*	*This study was specific to hydrogenotrophic bacteria and did not discuss relative abundance of taxa.			
	Amato et al. (2016)	Illumina MiSeq	Wild	*Alouatta palliata*	Firmicutes	Bacteroidetes	Cyanobacteria	Tenericutes
	Amato et al. (2013, 2014, 2015, 2016)	Illumina MiSeq; Pyrosequencing; ARISA	Both	*Alouatta pigra*	Firmicutes	Bacteroidetes	Proteobacteria	Verrucomicrobia Actinobacteria
	Clayton et al. (2016)	Illumina MiSeq	Both	*Alouatta palliata*	Firmicutes	Tenericutes	Bacteroidetes Proteobacteria	Actinobacteria
Genus *Ateles* (spider monkeys)	Hale et al. (2015)	Illumina MiSeq	Captive	*Ateles geoffroyi*	Bacteroidetes	Firmicutes	Proteobacteria	Verrucomicrobia

(Continues)

TABLE 1 (Continued)

Primate taxonomy	Study	Sequencing method	Captive or Wild	Species	Main phyla detected (and class or genus when available) 1	2	3	4
Old World Monkeys								
Genus *Macaca* (macaques)	Wireman et al. (2006)	Slot blot hybridization; qPCR; flow cytometry microarray	Captive	*Macaca fascicularis*	*Lactobacillus*	*Clostridium-Eubacterium*	*Bacteroides*	*Enterococcus-Bifidobacterium-Escherichia coli*
	Wireman et al. (2006)	Slot blot hybridization; qPCR; flow cytometry microarray	Captive	*Macaca mulatta*	*Clostridium-Eubacterium*	*Lactobacillus*	*Bacteroides*	*Enterococcus-Bifidobacterium-Escherichia coli*
	McKenna et al. (2008)	Pyrosequencing	Captive	*Macaca mulatta*	Firmicutes (*Clostridia-Lactobacilli-Bacilli*)	Bacteroidetes (*Prevotella-Rikenella*)	Spirochetes (*Treponema*)	Tenericutes (*Mollicutes*) Proteobacteria (*Enterobacteriaceae, Helicobacter*) Actinobacteria Verrucomicrobia Fibrobacter
	Seekatz et al. (2013)	Pyrosequencing	Captive	*Macaca fascicularis*	Firmicutes (*Lactobacillus-Streptococcus-Clostridium-Enterococcus-Oscillospira-Bulleidia-Ruminococcus-Sarcina-Coprococcus-Blautia*)	Bacteroidetes (*Prevotella*)	Tenericutes	Proteobacteria Spirochaetes (*Treponema*)
	Ardeshir et al. (2014)	PhyloChip	Captive	*Macaca mulatta*	*Study compared DR and NR macaques. Due to this, all taxa abundance values are related to the differences in the two groups.			
	Ma et al. (2014)	Pyrosequencing	Captive	*Macaca fuscata*	Bacteroidetes (*Prevotella*)	Firmicutes (*Faecalibacterium-Roseburia-Ruminococcus-Sporobacter*)	Spirochaetes (*Treponema*)	Proteobacteria (*Helicobacter*)
	Klase et al. (2015)	Pyrosequencing	Captive	*Macaca nemestrina*	Bacteroidetes (*Bacteroidia*)	Firmicutes (Clostridia-Bacilli)	Spirochaetes	Proteobacteria (Alphaproteobacteria-Betaproteobacteria)
	Yasuda et al. (2015)	Illumina MiSeq	Captive	*Macaca mulatta*	Stool: Firmicutes Lumen: Firmicutes	Stool: Bacteroidetes Lumen: Bacteroidetes	Stool: Spirochaetes Lumen: Spirochaetes	Stool: Proteobacteria; Euryarchaeota Lumen: Proteobacteria; Euryarchaeota

(Continues)

TABLE 1 (Continued)

Primate taxonomy	Study	Sequencing method	Captive or Wild	Species	Main phyla detected (and class or genus when available)			
					1	2	3	4
Genus *Papio* (baboons)	Nakamura et al. (2009)	Cloning; DGGE	Captive	*Papio hamadryas*	*This study was specific to hydrogenotrophic bacteria and did not discuss relative abundance of taxa.			
	McKenney et al. (2014)	Cloning; *cpn60* gene sequencing	Captive	*Papio hamadryas*	Proteobacteria (Alphaproteobacteria-*Acidiphilium-Arcobacter-Sorangium*)	Firmicutes (Clostridia-Bacilli-*Ruminococcus-Lactobacillus*)	Bacteroidetes (Bacteroidia-Flavobacteria-*Prevotella-Flavobacterium*)	Actinobacteria (*Arthrobacter*) Chlorobi (*Chlorobium*) Verrucomicrobia (*Opitutus*)
	Tung et al. (2015)	Shotgun sequencing (Illumina HiSeq)	Wild	*Papio cynocephalus*	Firmicutes	Proteobacteria	Actinobacteria	Bacteroidetes
	Ren et al. (2015)	Pyrosequencing	Wild	*Papio cynocephalus*	Firmicutes	Actinobacteria	Bacteroidetes	Proteobacteria
Genus *Chlorocebus*	Amato et al. (2015)	Pyrosequencing	Both	*Chlorocebus aethiops*	Firmicutes	Proteobacteria	Bacteroidetes	Spirochaetes
Genus *Cercocebus* (white-eyelid mangabeys)	Nakamura et al. (2009)	Cloning; DGGE	Captive	*Cercocebus atys*	*This study was specific to hydrogenotrophic bacteria and did not discuss relative abundance of taxa.			
Genus *Cercopithecus* (guenons)	Yildirim et al. (2010)	Pyrosequencing	Wild	*Cercopithecus ascanius*	Firmicutes (*Oscillibacter-Faecalibacterium-Roseburia-Ruminococcus-Blautia-Butirycicoccus-Coprococcus-Coprobacillus-Subdunigranulum-Dorea-Lactobcillus*)	Bacteroidetes (*Prevotella*)	Spirochetes (*Treponema*) Verrucomicrobia Tenericutes (*Anaeroplasma*) Proteobacteria Actinobacteria	
	McCord et al. (2014)	ARISA	Wild	*Cercopithecus ascanius*	*Did not include taxonomic information			
Genus *Colobus* (black-and-white colobus monkeys)	Yildirim et al. (2010)	Pyrosequencing	Wild	*Colobus guereza*	Firmicutes (*Oscillibacter-Faecalibacterium-Roseburia-Ruminococcus-Anaerotruncus-Turibacter-Coprococccus-Lactobacillus-Dorea-Blautia*)	Bacteroidetes (*Bacteroides-Odoribacter*)	Tenericutes (*Anaeroplasma*) Verrucomicrobia TM7 Planctomycetes Actinobacteria Proteobacteria (*Parasuterella*) Fibrobacter	
	McCord et al. (2014)	ARISA	Wild	*Colobus guereza*	*Did not include taxonomic information			

(Continues)

TABLE 1 (Continued)

Primate taxonomy	Study	Sequencing method	Captive or Wild	Species	Main phyla detected (and class or genus when available)			
					1	2	3	4
Genus *Procolobus* (red colobus monkeys)	Yildirim et al. (2010)	Pyrosequencing	Wild	*Procolobus tephrosceles*	Firmicutes (*Oscillibacter-Roseburia-Ruminococcus-Coprococccus-Blautia-Dorea-Peptococcus*)	Bacteroidetes (*Odoribacter-Hallella-Paludibacter-Parabacteroides*)	Tenericutes (*Anaeroplasma*) Proteobacteria (Campylobacter) Spirochetes (*Treponema*) Verrucomicrobia (*Opitutus*)	
	Barelli et al. (2015)	Pyrosequencing	Wild	*Procolobus gordonorum*	Firmicutes	Bacteroidetes	Verrucomicrobia	Spirochaetes
	McCord et al. (2014)	ARISA	Wild	*Procolobus rufomitratus*	*Did not include taxonomic information			
Genus *Pygathrix* (doucs)	Clayton et al. (2016)	Illumina MiSeq	Both	*Pygathrix nemaeus*	Firmicutes	Bacteroidetes	Verrucomicrobia Tenericutes	Spirochaetes Actinobacteria
Genus *Rhinopithecus* (snub-nosed monkeys)	Zhou et al. (2014)	Illumina MiSeq	Captive	*Rhinopithecus roxellana*	Firmicutes (*Oscillibacter-Roseburia-Ruminococcus-Coprococccus-Blautia-Dorea-Lactobcillus-Bulleida*)	Proteobacteria (*Pseudomonas-Acinetobacter*)	Bacteroidetes (*Prevotella-Flavobacterium-Rikenella*)	Actinobacteria (*Bifidobacterium*)
	Xu et al. (2015)	Shotgun sequencing (Pyrosequencing)	Wild	*Rhinopithecus bieti*	Firmicutes (*Clostridia-Clostridium-Ruminococcus*)	Bacteroidetes (*Bacteroides-Flavobacterium*)	Proteobacteria (*Pseudomonas*)	Actinobacteria
Apes								
Genus *Gorilla* (gorillas)	Frey et al. (2006)	Cloning; T-RFLP	Wild	*Gorilla beringei*	Firmicutes (*Clostridia-Mollicutes-Bacilli-Bulleida extructa-Unidentified*)	Verrucomicrobia	Actinobacteria	Lentisphaerae, Bacteroidetes, Spirochetes, Planctomycetes
	Ochman et al. (2010)	Pyrosequencing	Wild	*Gorilla beringei*	Proteobacteria	Bacteroidetes	Firmicutes	Actinobacteria
	Ochman et al. (2010)	Pyrosequencing	Wild	*Gorilla gorilla*	Proteobacteria	Bacteroidetes	Firmicutes	Actinobacteria
	Vlčková, Mrázek, Kopečný, & Petrželková, (2012)	DGGE	Captive	*Gorilla gorilla*	Firmicutes (*clostridia*)	Actinobacteria (*Bifidobacteria*)		
	Moeller, Shilts, et al. (2013)	Pyrosequencing	Wild	*Gorilla gorilla gorilla*	*The study did not specify abundance of taxa			
	Moeller, Peeters, et al. (2013)	Pyrosequencing	Wild	*Gorilla beringei graueri*	*The study did not specify abundance of taxa			
	Bittar et al. (2014)	Pyrosequencing	Wild	*Gorilla gorilla*	Firmicutes	Actinobacteria		

(Continues)

TABLE 1 (Continued)

Primate taxonomy	Study	Sequencing method	Captive or Wild	Species	Main phyla detected (and class or genus when available)			
					1	2	3	4
	Moeller et al. (2014)	Illumina MiSeq	Wild	*Gorilla gorilla*	*Did not include taxonomic information (relative abundance) for each primate species examined			
	McKenney et al. (2014)	Cloning; *cpn60* gene sequencing	Captive	*Gorilla gorilla*	Firmicutes (*Clostridia-Bacilli-Lactobacillus-Ruminococcus-Acetohalobium*)	Proteobacteria (Alphaproteobacteria-*Acidiphilium-Enterobacter-Sorangium-Arcobacter-Acinetobacter-Methylobacterium*)	Bacteroidetes (Bacteroidia-*Bacteroides*)	Chloroflexi (*Chloroflexus*) Elusimicrobia (*Elusimicrobium*) Nitrospirae (*Thermodesulfovibrio*)
	Moeller et al. (2015)	Illumina MiSeq	Wild	*Gorilla gorilla*	Firmicutes	Bacteroidetes Proteobacteria	Actinobacteria	Spirochetes Euryarchaeota Verrucomicrobia
	Gomez et al. (2015, 2016)	Pyrosequencing	Wild	*Gorilla gorilla*	Firmicutes (Lactobacillus Lachnospiraceae 1) *Ruminococcus* 2) *Mogibacterium* Eubacteriaceae Unclassified)	Bacteroidetes (Prevotellaceae)	Chloroflexi (Anaerolinaceae)	Actinobacteria (*Gordonibacter* Coriobacteriaceae) Proteobacteria (Campylobacter Rhodocyclaceae Betaproteobacteria) Fusobacteria (Fusobacteriaceae) Tenericutes (*Anaeroplasma*)
Genus *Pan* (chimpanzees)	Uenishi et al. (2007)	Cloning; TGGE; ARDRA	Both	*Pan troglodytes*	Firmicutes (*Eubacterium-Clostridium-Ruminococcus-Lactobacillus*)	Actinobacteria (*Bifidobacterium*)	Bacteroidetes (*Prevotella-Bacteroides*)	Tenericutes (*Anaeroplasma*), Proteobacteria (*Succinimonas-Succinivibrio*) Spirochaetes (*Treponema*)
	Kisidayová et al. (2009)	Cloning; DGGE	Captive	*Pan troglodytes*	Euryarchaeota (*Picrophilus torridus* in LFD and *Methanobrevibacter woesei* in HFD) *Tetratrichomonas sp.*	Firmicutes (*Bulleida extructa* in LFD and *Eubacterium biforme* in HFD)		
	Szekely et al. (2010)	T-RFLP	Wild	*Pan troglodytes schweinfurthii*	Firmicutes (*Clostridia-Bacilli-Lactobacilli*)	Bacteroidetes Mollicutes	Actinobacteria	
	Ochman et al. (2010)	Pyrosequencing	Wild	*Pan troglodytes schweinfurthii*	Firmicutes	Bacteroidetes	Actinobacteria	Proteobacteria
	Ochman et al. (2010); Moeller, Peeters, et al. (2013)	Pyrosequencing;	Wild	*Pan troglodytes troglodytes*	Firmicutes	Bacteroidetes	Proteobacteria	Actinobacteria

(Continues)

TABLE 1 (Continued)

					Main phyla detected (and class or genus when available)			
Primate taxonomy	**Study**	**Sequencing method**	**Captive or Wild**	**Species**	**1**	**2**	**3**	**4**
	Ochman et al. (2010); Moeller, Peeters, et al. (2013)	Pyrosequencing	Wild	*Pan troglodytes ellioti*	Proteobacteria	Firmicutes	Bacteroidetes	Actinobacteria
	Ochman et al. (2010); Moeller, Peeters, et al. (2013)	Pyrosequencing	Wild	*Pan paniscus*	Firmicutes	Bacteroidetes	Actinobacteria	Proteobacteria
	Degnan et al. (2012); Moeller et al. (2012)	Pyrosequencing (16S rDNA pyrotag); Illumina (iTag sequencing)	Wild	*Pan troglodytes schweinfurthii*	Firmicutes	Bacteroidetes	Actinobacteria	Tenericutes
	Moeller, Peeters, et al. (2013)	Pyrosequencing	Wild	*Pan troglodytes schweinfurthii*	*Same data as Ochman et al. (2010); Degnan et al. (2012)			
	Moeller, Shilts, et al. (2013)	Pyrosequencing (16S rDNA pyrotag)	Wild	*Pan troglodytes schweinfurthii*	Firmicutes	Actinobacteria		Verrucomicrobia Proteobacteria Tenericutes
	McKenney et al. (2014)	Cloning; cpn60 gene sequencing	Captive	*Pan troglodytes*	Proteobacteria (*Alphaproteobacteria-Acidiphilium-Ochrobactrum*)	Firmicutes (*Clostridia-Bacilli-Ruminococcus-Streptococcus*)		Elusimicrobia (*Elusimicrobium*)
	Moeller et al. (2014)	Illumina MiSeq	Wild	*Pan troglodytes schweinfurthii*	*Did not include taxonomic information (relative abundance) for each primate species examined			
	Moeller et al. (2014)	Illumina MiSeq	Wild	*Pan paniscus*	*Did not include taxonomic information (relative abundance) for each primate species examined			
	Moeller et al. (2016)	Illumina MiSeq	Wild	*Pan troglodytes schweinfurthii*	*Did not include taxonomic information (relative abundance) of major taxa overall			

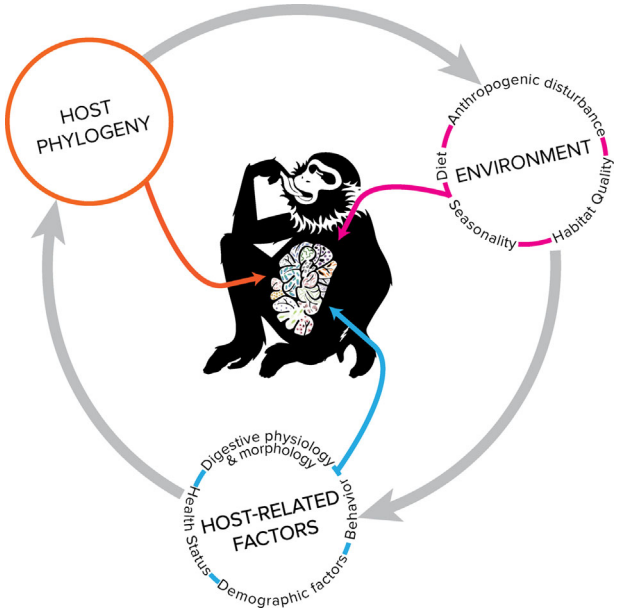

FIGURE 3 Dynamism of NHP microbiome profiles. NHP microbiomes originate from early evolutionary traits of their host-microbial system ancestors, but then are further shaped by host-related and environmental factors. In this way microbiomes conserve specific bacterial lineages from their ancestors but other bacterial groups can then be expanded or contracted according to constraints found in their surroundings

For both amplicon sequencing and fingerprinting techniques, challenges exist. Sample collection and storage can influence DNA quality, and ultimately results (Hale, Tan, Knight, & Amato, 2015). Nucleic acid extraction methods can influence results through biases toward or against certain microbes (Yuan, Cohen, Ravel, Abdo, & Forney, 2012), and similar biases are introduced via the utilization of primers during the polymerase chain reactions (PCR) necessary for generating amplicons (Hamady & Knight, 2009; Soergel, Dey, Knight, & Brenner, 2012). Other sequencing-based techniques avoiding these specific biases, such as shotgun metagenomics, have recently grown in popularity. However, these techniques also have their own respective biases. Metagenomics is the sequence-based analysis of all DNA obtained directly from a sample. It is not dependent on a PCR reaction, and instead sequences all of the genetic material that is present in a sample (Meyer et al., 2008; Riesenfeld, Schloss, & Handelsman, 2004; Tringe et al., 2005). Metagenomics has been used to characterize the functional landscape of different microbial communities, including that of the mammalian gastrointestinal microbiome (Abubucker et al., 2012; Mongodin, Emerson, & Nelson, 2005; Petrosino et al., 2009; Xu et al., 2013), but its higher costs compared to 16S rRNA profiling are still prohibitive for many projects. When analyzing data, multiple confounding variables must be considered, including diet, age, sex, health, geography, among others (Kim et al., 2017). Statistical tests can control for multiple confounding variables, including Bonferroni correction (Dunn, 1961), which is very conservative, and the Benjamini and Hochberg method (Benjamini & Hochberg, 1995), which is more popular currently.

While not the sole source of biological material used to study gastrointestinal microbiome, feces are by far the most common (Gu et al., 2013; Stearns et al., 2011; Yasuda et al., 2015). One area of research that warrants further investigation is what sections of the gastrointestinal tract do feces accurately represent. While it is generally accepted that feces are largely representative of the colonic bacteria, more information is needed to determine if feces are representative of the microbial communities in the small bowel, and if so, what sections. A number of studies in animals have shown regional variation in microbiota composition in the gastrointestinal tract (Dougal et al., 2012; Ericsson, Johnson, Lopes, Perry, & Lanter, 2016; Godoy-Vitorino et al., 2012; He et al., 2018; Li et al., 2017). For example, Dougal et al. (2012) used 16S rRNA gene sequencing to show that the caecum microbiome clusters separately from the other gut regions in horses and ponies. Godoy-Vitorino et al. (2012) showed that in hoatzins and cows microbiota composition clusters by gut environment (i.e., gut regions). In terms of studies with NHPs, Yasuda et al. (2015) examined the microbiome of ten different sites along the intestine in rhesus macaques, and then compared the results to fecal microbiome. Based on this study, feces is highly representative of the colonic lumen and mucosal microbiome. However, this and other studies have demonstrated that feces is not representative of the small intestine, especially with respect to *Proteobacteria* since *Proteobacteria* are under-detected in feces (Yasuda et al., 2015). Considering that feces is the most commonly used biological material for microbiome studies, the findings of this study highlight that the fecal microbiome does not represent the microbiome of the entire gastrointestinal tract.

1.2 | Gastrointestinal microbiome patterns in NHPs in the context of primatological research

While the microbiome field has made substantial progress in understanding the role microbial communities play in human health and disease, much less attention has been given to the microbiomes of NHPs. Since the 1960's, starting with the pioneering work of Bauchop and Martucci on bacteria inhabiting the forestomach of colobines (Bauchop, 1971; Bauchop & Martucci, 1968), a number of studies have attempted to characterize the diversity of the bacterial communities associated with the gastrointestinal tract of NHPs, from evolutionary, clinical and ecological perspectives. Here, we summarize those findings, providing evidence on the state of knowledge on microbiome research in nonhuman primate ecology and evolution.

1.3 | The microbiome of NHPs co-varies along with host phylogeny

The existence of interspecies differences in microbiome patterns across NHPs has been shown by a number of studies (McCord et al., 2014; Ochman et al., 2010; Yildirim et al., 2010). One of the first applications of high-throughput sequencing techniques in nonhuman primate microbiome research focused on assessing gut (fecal) microbiome patterns across different primate species. Yildirim et al. (2010) used 454 pyrosequencing to study bacterial diversity in fecal samples of colobines,

TABLE 2 Summary of studies investigating the impact of biological factors on the nonhuman primate microbiome

Factor	Host	Captive or wild	Study
Age differences	*Pan troglodytes*	Wild	Degnan et al. (2012)
	Lemur catta	Wild	Bennett et al. (2016)
	Varecia variegata	Captive	McKenney et al. (2015)
	Lemur catta	Captive	McKenney et al. (2015)
	Propithecus coquereli	Captive	McKenney et al. (2015)
	Papio cynocephalus	Wild	Ren et al. (2015)
	Papio cynocephalus	Wild	Tung et al. (2015)
	Alouatta pigra	Wild	Amato et al. (2014)
Sex differences	*Lemur catta*	Wild	Bennett et al. (2016)
	Alouatta pigra	Wild	Amato et al. (2014)
	Papio cynocephalus	Wild	Ren et al. (2015)
	Papio cynocephalus	Wild	Tung et al. (2015)
	Pan troglodytes	Wild	Degnan et al. (2012)
Effect of environmental variation	*Cercopithecus ascanius*	Wild	Goldberg et al. (2008)
	Alouatta pigra	Wild	Amato et al. (2014)
	Alouatta palliata	Wile	Amato et al. (2016)
	Avahi laniger	Wild	Bublitz et al. (2015)
	Eulemur rubiventer	Wild	Bublitz et al. (2015)
	Hapalemur aureus	Wild	Bublitz et al. (2015)
	Microcebus rufus	Wild	Bublitz et al. (2015)
	Propithecus edwardsi	Wild	Bublitz et al. (2015)
	Prolemur simus	Wild	Bublitz et al. (2015)
	Procolobus rufomitratus	Wild	McCord et al. (2014)
	Procolobus gordonorum	Wild	Barelli et al. (2015)
	Colobus guereza	Wild	McCord et al. (2014)
	Cercopithecus ascanius	Wild	McCord et al. (2014)
	Lemur catta	Wild	Fogel (2015)
	Lemur catta	Wild	Bennett et al. (2016)
	Gorilla gorilla	Wild	Gomez et al. (2015)
	Propithecus verreauxi	Wild	Fogel (2015)
Biogeographical drivers	*Pan troglodytes*	Wild	Moeller, Shilts, et al. (2013)
Host health status	*Macaca mulatta*	Captive	McKenna et al. (2008)
	Macaca fascicularis	Captive	Seekatz et al. (2013)
	Macaca nemestrina	Captive	Klase et al. (2015)
	Macaca mulatta	Captive	Klase et al. (2015)
	Pan troglodytes	Wild	Moeller et al. (2015)
	Gorilla gorilla gorilla	Wild	Moeller et al. (2015)
Diet	*Pan troglodytes*	Captive	Kisidayová et al. (2009)
	Macaca fuscata	Both	Benno, Itoh, Miyao, and Mitsuoka (1987)
	Macaca fuscata	Captive	Ma et al. (2014)
	Chlorocebus aethiops	Wild	Bruorton et al. (1991)
	Chlorocebus aethiops	Both	Amato et al. (2015)
	Cercopithecus mitis	Wild	Bruorton et al. (1991)
	Alouatta pigra	Wild	Amato et al. (2015)

(Continues)

TABLE 2 (Continued)

Factor	Host	Captive or wild	Study
	Macaca mulatta	Captive	Ardeshir et al. (2014)
	Papio cynocephalus	Wild	Tung et al. (2015)
	Papio cynocephalus	Wild	Ren et al. (2015)
Sociality/social group	*Pan troglodytes*	Wild	Degnan et al. (2012)
	Colobus guereza	Wild	McCord et al. (2014)
	Cercopithecus ascanius	Wild	McCord et al. (2014)
	Alouatta pigra	Wild	Amato et al. (2014)
	Lemur catta	Wild	Bennett et al. (2016)
	Procolobus rufomitratus	Wild	McCord et al. (2014)
	Papio cynocephalus	Wild	Ren et al. (2015)
	Papio cynocephalus	Wild	Tung et al. (2015)
Kinship	*Pan troglodytes*	Wild	Degnan et al. (2012)
Effects of captivity	*Pan troglodytes*	Both	Uenishi et al. (2007)
	Lemur catta	Captive	Villers et al. (2008)
	Lemur catta	Wild	Fogel (2015)
	Leontopithecus chrysopygus	Both	Carvalho et al. (2014)
	Alouatta pigra	Both	Amato et al. (2013)
	Rhinopithecus bieti	Wild	Xu et al. (2015)
	Alouatta palliata	Both	Clayton et al. (2016)
	Pygathrix nemaeus	Both	Clayton et al. (2016)

including black-and-white colobus monkeys (*Colobus guereza*), red colobus monkeys (*Procolobus tephrosceles*), and red-tailed guenons (*Cercopithecus ascanius*). Their results show that fecal microbiome of these three primate species largely reflect the host phylogenetic background, including the addition of humans in the comparisons. Ochman et al. (2010), also using 454 pyrosequencing, showed that the fecal microbiomes of *G. g. gorilla* and *G. b. beringei*, *P. paniscus*, *P. t. troglodytes*, *P. t. schweinfurthii*, and *P. t. ellioti* are also primarily driven by host phylogeny. Efforts to capture variation in interespecies microbiome composition driven by dietary factors, based on chloroplast sequence analyses from feces were unsuccessful in this study, leading the authors to conclude that host phylogenetic background superseeds dietary forces in shaping the primate gut microbiome. However, as genetic analyses based on chloroplasts sequences do not account for nutritional quality of plants consumed, thus, it cannot be assumed that different diets across species did not influence the species-specific microbiome arrangements observed. More recently, McCord et al. (2014) used Automated Ribosomal Intergenic Spacer Analysis (ARISA) to analyze the fecal microbiomes of the red-tailed guenon (*C. ascanius*), the red colobus (*Procolobus rufomitratus*) and black-and-white colobus (*Colobus guereza*). As expected, and replicating the analyses conducted by Yildirim et al. (2010), fecal microbiomes were host species-specific, with differences persisting in the face of habitat degradation.

In another study, Nakamura, Leigh, Mackie, and Gaskins (2009) aimed to better understand how methanogenic status is regulated in primates by using denaturing gradient gel electrophoresis (DGGE) applied to rectal swab samples to identify hydrogenotrophic microbial community profiles of hamadryas baboons and sooty mangabeys. The results of this study revealed that intestinal Archaea and sulfate-reducing bacteria (SRB) are present simultaneously in baboons and mangabeys, and that the hydrogenotrophic microbial community profiles of these NHP species differ. While the influence of environmental factors cannot be ruled out completely, the methanogenic gut microbiomes of these two NHP species were highly host species-specific, thus agreeing with previous findings (McCord et al., 2014; Ochman et al., 2010; Yildirim et al., 2010).

One method used to study the influence of host genetics on gut microbiome composition is to keep environmental factors, such as diet, constant across all groups/individuals included in a a study. One such study by Wireman et al. (2006) conducted a study where environmental factors, including diet was kept uniform, thus allowing for an examination, at least partially, of the effects of host genetics on gut microbiome composition. Noteworthy is the fact that host genetic effects are only partially responsible for producing a phylogenetic signal in microbiomes. Wireman et al. (2006) used culture-independent methods to examine gut microbial communities in four male macaques (*M. fascicularis* and *M. mulatta*) for a period of eight months. The major findings of this study were that the macaque gastrointestinal microbiome is dynamic, exhibits positive and negative correlations among certain bacterial taxa, and is dominated by the *Clostridium-Eubacterium*, *Lactobacillus*, and *Bacteroides* groups. Given that the diet consumed by the four macaques included in this study was kept uniform, the inter-individual differences in gut microbiota composition observed were likely due to factors other than diet.

1.4 | Diet as a driver of NHP gut microbiome composition

Although, host phylogenetic background has been shown to be a very important driver of gut microbiome composition across different NHP species, the primate gut microbiome also shows significant plasticity in response to dietary changes. Diet-driven patterns of microbiome composition in NHPs have been extensively investigated, in the context of habitat heterogeneity, social group affiliation, and seasonal variation in food availability. For instance, the gastrointestinal microbiomes of *P. t. schweinfurthii* in Tanzania are reported to reflect the biogeographical and community affiliation patterns of the host (Degnan et al., 2012). These patterns could be hypothesized to arise from shared ecological factors (i.e., diet, social contact). Additionally, this biogeographical signal was found to persist over a long period (nearly a decade), even after dispersal of individuals to other communities or ranges. The use of high-throughput sequencing techniques to explore the gastrointestinal microbiome of chimpanzees has also led to reports that their gastrointestinal microbiome assorts into enterotypes, analogous to those reported in humans using similar methods (Arumugam et al., 2011; Moeller et al., 2012). However, despite increased abundance of *Prevotella* (a taxon usually linked to fiber and starch degradation) in all chimpanzees compared to humans, no associations between enterotypes and dietary factors in the chimpanzee gastrointestinal microbiome were reported.

One notable examination of diet-microbiome relationships was work by Amato et al. (2015), which provided evidence that wild black howler gastrointestinal microbiomes vary with seasonal shifts in diet. In this study, the authors concluded that microbial shifts may help howlers meet their nutritional demands during times when the consumption of a less energetically favorable diet is the norm (Amato et al., 2015). During periods when howler energy intake was lowest, relative abundances of Ruminococcaeceae were highest and relative abundances of Lachnospiraceae were lowest. Also, *Butyricicoccus* was most abundant when the howler diet was dominated by young leaves and unripe fruit. Not only did the gastrointestinal microbiome shift in composition, but when energy intake was reduced, howlers showed increased fecal VFA concentrations, indicating increased microbial energy production. Because howlers also showed little variation in their activity levels over the 10-month study period despite variation in diet, it appears that shifts in the gastrointestinal microbiome help compensate for seasonal reductions in howler energy intake.

Other studies have investigated the relationship between diet and gut microbiome composition, including a few comparing the gastrointestinal microbiome between lemur genera (Fogel, 2015; Ley et al., 2008; McKenney, Rodrigo, & Yoder, 2015). McKenney et al. (2015) examined the microbiome of captive frugivorous black-and-white ruffed lemurs, generalist ring-tailed lemurs, and folivorous Coquerel's sifakas using high-throughput sequencing. The authors found that the gastrointestinal microbiome composition was host species-specific, potentially due to the high-fiber diet consumed by the sifaka. Specifically, the sifaka harbored the greatest microbial diversity and possessed four genera of cellulose degraders (Ruminococcaceae). Ring-tailed lemurs and black-and-white ruffed lemurs consumed similar diets and exhibited similar microbial diversity. However, their gastrointestinal microbiomes could be distinguished based on several bacterial lineages, and distinct microbiome compositions were observed across life stages in each of the three lemur species studied (McKenney et al., 2015). Based on these findings, it is hard to determine whether the differences in gut microbiome observed between lemur species was due to host genetics or environmental factors, such as diet. In a similar way, Fogel (2015) examined the microbiome of wild ring-tailed lemurs and Verreaux's sifakas. Although the abundance of microbes differed between lemur species, gastrointestinal microbiome composition did not. Given two different lemur species were examined, which consume substantially different diets, it is perplexing that host species-specific differences in gut microbiome composition were not observed. Despite this, inter-individual and seasonal (wet vs. dry season) variation in gastrointestinal microbiome composition within each species was high, which supports the notion that environmental factors, at least partially, shape the gut microbiome of NHPs.

In addition to lemurs, studies have described the microbiome of lorises (Bo et al., 2010; Xu et al., 2013, 2014). Bo et al. (2010) demonstrated through clone libraries that the phylum Proteobacteria accounted for the second highest percentage (36%) of bacteria in the fecal microbiome of the wild pygmy loris, and within the phylum Proteobacteria, *Pseudomonas* (13.79% of clone sequences) was the predominant genus. Because the authors found sequences closely related to *P. putida*, which are well known hydrocarbon-degrading bacteria (Gomez, Yannarell, Sims, Cadavid-Restrepo, & Moreno Herrera, 2011; Rentz, Alvarez, & Schnoor, 2004), it seems that *Pseudomonas* plays a vital role in the digestion of plant materials. Other microbial taxa that might play a role in breaking down plant exudates such as *Acinetobacter*, *Alkalibacterium* (Phylum Proteobacteria), *Corynebacterium* (Phylum Actinobacteria), *Clostridium*, *Eubacterium* and *Bacillus* were also detected. Similar to Bo et al. (2010), Xu et al. (2013) used high-throughput sequencing to study the pygmy loris gut microbiome, and discovered that the major genus represented in the phylum Proteobacteria was *Pseudomonas*. Additionally, Xu et al. found that sequences involved in aromatic compound metabolism were overrepresented, specifically sequences in the benzoate degradation pathway. Finally, Xu et al. (2013) identified a novel microbial gene (*amyPL*) coding for α-amylase, which is important for the breakdown of α-linked polysaccharides, such as starch and glycogen in the pygmy loris diet (Buisson, Duee, Payan, & Haser, 1987; Nekaris, Starr, Collins, & Wilson, 2010). Taken together, it appears that the gut microbiota of the pygmy loris is adapted to ferment sugars and degrade complex aromatic compounds. Both of these processes are likely important for the fermentation of the soluble fiber component of plant exudates, which make up the majority of their diet (Starr & Nekaris, 2013).

Links between diet and microbiome have been examined in vivo using macaques. In order to examine the effects of diet on gut microbiome composition, Ma et al. (2014) exposed Japanese macaques to both high-fat and low-fat diets and analyzed their fecal microbiomes

over an extended period. Additionally, due to the existence of a previously established link between the gastrointestinal microbiome and obesity in mammals (Turnbaugh et al., 2006, 2009), Ma et al. wanted to determine if a diet-microbiome-obesity link was present in captive macaques (Ma et al., 2014). Through this study, Ma et al. (2014) determined that diet shapes the maternal gastrointestinal microbiome, and maternal diet during gestation and lactation shapes the offsprings microbiome. Specifically, the offspring's gastrointestinal microbiome was negatively altered when the dam was fed a high-fat diet during pregnancy or lactation. Specifically, non-pathogenic *Campylobacter* was far less abundant in offspring exposed to a high-fat diet early in life compared to those exposed to a low-fat diet. Additionally, the consumption of a low-fat diet by the offspring post-weaning only partially corrected the dysbiosis. Similar to Ma et al. (2014), Ardeshir et al. (2014) examined the link between diet and gut microbiota in macaques. Specifically, breast-fed and bottle-fed rhesus macaques were studied to see how these two different nursing practices influence gastrointestinal microbiome composition, and the resulting effects on immune system development. Compared to bottle-fed macaques, breast-fed macaques had increased abundances of *Prevotella* and *Ruminococcus* and a decreased abundance of *Clostridium* (Ardeshir et al., 2014). In addition to major differences in gut microbiota, breast-fed and bottle-fed macaques had vast differences in the immune systems, including the development of robust T_H17 cell populations exclusively in breast-fed macaques. Collectively, the results of these studies highlight both that diet strongly influences gastrointestinal microbiome composition and that gut microbial communities strongly influence offspring immune system development and metabolism.

Aside from Ma et al. (2014) and Ardeshir et al. (2014) another study tested the specific effects of diet on gut microbiome composition in vivo using vervet monkeys (*C. aethiops*). In this study, a high-fat, low-fiber diet on the gut microbiota of vervet monkeys by comparing samples from captive individuals on a 6-month diet challenge and wild individuals from St. Kitts consuming a high-fiber, low-fat diet composed primarily of fruits and leaves (Amato et al., 2015). Not surprisingly the two groups of monkeys had markedly distinct gastrointestinal microbiomes. However, when compared to humans, the vervets exhibited the opposite microbial responses to similar diets. While humans generally possess increased relative abundances of Firmicutes and *Bacteroides* and decreased relative abundances of Bacteroidetes and *Prevotella* when consuming a high-fat, low-fiber diet, the vervets had higher relative abundances of Bacteroidetes and *Prevotella* and lower relative abundances of Firmicutes and *Bacteroides*. The authors suggest that these results may indicate a unique relationship between diet, physiology, and the gut microbiota in humans compared to other primates.

Prior to the use of in vivo models to examine links between environmental factors and gut bacteria, in vitro models were used. For example, Costa, Mehta, and Males (1989) used a continuous culture system to compare bacteria present in vervet monkey feces and colonic contents. In this study, they tested the effect of feeding continuous cultures different sources of dietary fiber, including psyllium husk, which is fermentable, and cellulose, which is less fermentable, and found that the ratio of anaerobes to aerobes was lower in cultures fed psyllium husk compared to cultures fed cellulose. However, interestingly, they found that both inoculum source

s (feces and colonic contents) produced similar results for characteristics related to microbial metabolism measured in this study, including total viable counts of anaerobes and aerobes, microbial β-glucuronidase activity, volatile fatty acid (VFA) and ammonia nitrogen concentrations, dry matter, pH and oxidation-reduction potential. Based on their results, Costa et al. (1989) concluded that feces can serve as an inoculum source for in vitro studies examining changes in colonic microbial metabolism as a result of diet. Given that feces are the most common source of biological material used to represent the distal gastrointestinal microbiome (i.e., colonic microbiota) in culture-independent studies, the findings of this study are highly valuable, as they again provide evidence that the fecal microbiome is representative of the colonic microbiome.

Aside from in vitro and in vivo methods, NHP microbiomes and their relationships with diet have been studied using cultivation techniques and electron microscopy. For example, Bruorton, Davis, and Perrin (1991) relied on these methods to identify the gastrointestinal microbiome of the blue monkey or samango (*Cercopithecus mitis*). The authors identified a characteristic abundant population of rod-shaped bacteria in the stomach of the samango through light microscopy, although taxonomic tests were not performed to confirm the identity to genus- or species-level. The study also identified a number of isolates capable of metabolizing cellulose, thus suggesting the presence a diet-microbiome relationship.

Even studies that have shown gut microbiome composition to be host species-specific, have shown links between diet and gut microbiota within host species. One such example is the work of Yildirim et al. (2010), which examined the species-specific differences in fecal microbiomes of three African NHPs, including the red-tailed guenon (*Cercopithecus ascanius*), black-and-white colobus monkey (*Colobus guereza*), and red colobus monkey (*Procolobus tephrosceles*). Except for exhibiting lower levels of *Prevotella* (Phylum Bacteroidetes), the microbiomes of the two colobine species examined by Yildirim et al. (2010) were highly similar to that found in guenons. *Prevotella*, usually isolated from human oral cavities and feces and the rumen ecosystem, are proteolytic and saccharolytic, and have the capacity to ferment sugars, including glucose, lactose, maltose, mannose, raffinose and sucrose (Alauzet et al., 2007; Downes, Liu, Kononen, & Wade, 2009; Hardham et al., 2008; Sakamoto & Benno, 2006; Ueki, Akasaka, Suzuki, & Ueki, 2006). This is consistent with the frugivorous profile of guenons (Lambert, 2001, 2002) in which energy may be derived from lipid, sugar and protein calories.

Oscillibacter (Phylum Firmicutes, class Clostridia), was the most abundant genus found in all three NHP species's fecal samples examined by Yildirim et al. (2010). Walker et al. (2011) report that *Oscillibacter* and *Subdunigranulum* were enriched in the fecal samples of individuals under diets rich in resistant starch and non-starch polysaccharides. Mondot et al. (Daniel et al., 2014) report that *Oscillibacter* and *Fecaelibacterium* are associated with healthy

individuals under diets low in sugar and fat calories (Sokol et al., 2008). Other genera from the Firmicutes phylum found in the fecal samples of the three NHP species examined, such as *Roseburia* and *Ruminococcus*, are associated with fermentation and production of H2, CO2 and VFAs (e.g., butyrate) (Kim, Morrison, & Yu, 2011; Nakamura et al., 2011). For the guenons, this could mean that despite being mostly frugivorous, the microbiota of guenons can also adapt to a diet high in fiber, or that fruit consumed by guenons are fibrous (Lambert, 2002). Similarly, the ability of the microbiota to process fiber structural polysaccharides also represents an advantage when breaking down the exoskeleton of arthropods, which *C. ascanius* regularly consumes. For the two colobine species examined, all Firmicutes bacteria found in the fecal samples are known fiber fermenters. This mainly fibrolytic microbiota may support the highly folivorous diet of colobines (Kay & Davies, 1994). However, the extent to which the fecal bacterial profiles in foregut-fermenting colobines faithfully reflect the population of fibrolytic and fermenting bacteria in the foregut is not clear. The microbiota profiles detected in colobines by Yildirim et al. (2010) may represent bacterial communities associated with late colonic fermentation of plant residues that could not be fermented in the saccus gastricus (Yildirim et al., 2010). This may also explain why the fermentative microbiota of the caeco-colic fermenting guenon was qualitatively similar to that found in colobines, and could mean that both species share bacterial lineages of colonic bacteria with similar ancestors that expanded or contracted to adapt to ecological constraints imposed by diet.

As evidenced by Yildirim et al. (2010), members of the subfamily Colobinae represent good models to study the relationship between diet and gut microbiome composition (Yildirim et al., 2010). In fact, since the 1960s there has been increasing interest in exploring the gut microbiology of foregut fermenting colobines. Colobines are the only monkeys capable of foregut fermentation, facilitated by their enlarged and multi-chambered stomach (Bauchop, 1971; Caton, 1999; Chivers & Hladik, 1980; Kay & Davies, 1994; Lambert, 1998). Microbial fermentation and absorption of VFAs occur in the specialized saccus gastricus of colobines, where digesta then passes to the tubus gastricus (Kay & Davies, 1994). This adaptation allows them to ferment plant material, absorb VFAs and ammonia and transform plant secondary metabolites present in leaves by increasing retention time in the stomach. As is the case with ruminants, microbes provide the majority of both energy and protein for these specialized primates (Henderson et al., 2015; McKenzie et al., 2017). Compared to their voluminous stomach, colobines have a relatively small midgut, which allows them to carry on extended fermentation of fibrous material, and thus more efficient absorption of VFAs compared to caeco-colic fermenters (Chivers, 1994; Kay & Davies, 1994). Because it is in the pancreas and liver where hydrolysis and digestion of protein ultimately takes place (Kay & Davies, 1994), part of the foregut microbiota can be also digested, unlike the colonic microbiota of other primates (Lambert, 1998; Ley et al., 2008).

One of the first attempts to show the bacterial communities in foregut contents examined two NHP species, *Presbytis entellus* and *Presbytis cristatus* (Bauchop & Martucci, 1968). They noted that bacterial fermentation occurs in the langur stomach, which is diverticular in form. Additionally, Bauchop and Martucci (1968) noted that bacterial fermentation of the leafy diet consumed by colobines results in the production of vital nutrients, such as volatile short-chain fatty acids required for these primates survival. It has since been discovered that this microbial community also produces essential amino acids, water soluble vitamins and plant secondary metabolite neutralizing compounds (Kay & Davies, 1994).

Since the work of Bauchop and Martucci (1968), minimal research on the foregut microbiota has been conducted. This is likely due to a number of reasons, one of which being the invasive nature of sample collection required to study this specific microbial environment. However, a recent study examining both host genetics and the microbiome of snub-nosed monkeys focused on the stomach microbiota (Zhou et al., 2014). Zhou et al. (2014) detected similarities between the stomach microbiomes of *R. roxellana* and the stomach microbiome of both humans and cattle. Specifically, the stomach microbiome of *R. roxellana* was more similar to that of the cattle rumen. Zhou et al. (2014) also analysed microbial function, and identified genes involved in the digestion of cellulose, including 27 cellulose genes, 17 1,4-β-cellobiosidase genes and 179 β-glucosidase genes. Similar to Zhou et al. (2014), Xu et al. (2015) found that the fecal microbiome of *Rhinopithecus bieti* was closely related to that of cattle (Xu et al., 2015). Specifically, Xu et al. (2015) found that the glycoside hydrolase profile of the *R. bieti* fecal microbiome was most closely related to that of the cow rumen. Glycoside hydrolases are enzymes responsible for the degradation of cellulose, hemicellulose, and starch (Langston et al., 2011), which are main components of the *R. bieti* diet. Collectively, these results suggest the presence of a strong diet-microbiome link in the stomach of *R. roxellana*, and in the colon of *R. bieti*.

Another useful model for studying the relationship between dietary composition and gut microbial communities is the Gorilla (*Gorilla* spp.). Despite this, few studies have explored bacterial diversity and influence of the gastrointestinal microbiome in the feeding ecology of *Gorilla* spp. The first study to explore gorilla gastrointestinal microbiomes using molecular techniques (Frey et al., 2006) showed that mountain gorillas at Bwindi Impenetrable Forest (Uganda) (*G. b. beringei*, *n* = 1, a silverback) harbor microbial taxa potentially associated with fiber processing and fermentation (*Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Bulleidia extructa*), as well as degradation of condensed tannins *(Eubacterium oxidorreducens*). More recent reports have illustrated the importance of foraging constraints on the gastrointestinal microbiome of gorillas. For instance, high-throughput sequences of 34 western lowland gorillas revealed significantly different gut bacterial communities and metabolomic profiles among groups with non-overlapping home ranges and evident distinctions in diet (Gomez et al., 2015). For example, there was an increased abundance of *Prevotella*, *Anaeroplasma* and metabolites associated with fiber and phenolic processing in gorillas consuming more leaves and herbaceous vegetation. In contrast, gorillas consuming more fruit showed increased abundance of *Lactobacillus*, taxa related to the Lachnospiraceae, Erysipelotrichaceae, and metabolites involved in

lipid processing and fermentation of more soluble sugars. Further support for the influence of ecological and dietary factors on shaping gorilla gastrointestinal microbiomes points to increased patterns of shared taxa between sympatric chimpanzees and western lowland gorillas compared to allopatric individuals (Moeller, Peeters, et al., 2013). These convergent microbiome patterns in sympatric gorillas and chimpanzees were hypothesized to be caused by shared diets. Indeed, convergent microbiome patterns driven by dietary behaviors in two different ape species were also reported by Gomez et al. (2016), showing that the gut microbiomes of mountain gorillas (*G. b. beringei*) western lowland gorillas (*G. g. gorilla*) converge when the latter emphasize more fiber in their diets (Gomez et al., 2016). However, Gomez et al. (2016) also report there a significant fraction of previously uncharacterized diversity in the gut microbiome of Gorilla species. In this regard, recent culture-based studies suggest novel diversity and prevalence of bacteria usually associated with disease in humans in the gastrointestinal microbiome of western lowland gorillas (Bittar et al., 2014). These observations imply strong evolutionary and ecological drivers on the gastrointestinal microbiomes of apes that are yet to be fully explored.

1.5 | Geographical and forest fragmentation patterns as microbiome driving factors

Nonhuman primates, such as howler monkeys (*Alouatta* spp.) can act as sentinels for unhealthy shifts in their habitat ecosystems. Amato et al. (2013) used high-throughput sequencing showed that a relationship exists between habitat quality and black howler monkey gastrointestinal microbiome composition. Specifically, gut microbial diversity and richness is highest in howler monkeys inhabiting continuous, evergreen rainforest compared to fragmented rainforest and captivity. These patterns in microbial diversity appeared to match patterns in diet diversity (Amato et al., 2013). A more recent examination of the howler monkey gut microbiota compared the effect of forest type, season, and habitat disturbance on the gut microbiota of black howler monkeys (*Alouatta pigra*) and mantled howler monkeys (*Alouatta palliata*) (Amato et al., 2016). All three factors affected the composition of the howler monkey gut microbiota, but black howler monkeys were more sensitive to changes in forest type and habitat disturbance than mantled howler monkeys. Amato et al. (2016) speculated that the increased plasticity of the mantled howler monkey gut microbiota may contribute to its wide distribution, in contrast to black howler monkeys which appear to have a less flexible microbial community and is endemic to southeastern Mexico, Belize and Guatemala. However, it is currently unknown whether shifts in their gut microbiota accompany increased stress or other health issues related to habitat encroachment, and thus a better understanding of the cause-and-effect relationship is critical if we are to utilize microbiome-related information to aid in the conservation of wild primates and their associated habitats.

In addition to howler monkeys, other NHP species have been used to study the influence of geography and fragmentation on gut microbiome composition. A recent study using high-throughput sequencing determined that the ring-tailed lemur gastrointestinal microbiome varies in response to several factors, including habitat (Bennett et al., 2016). While microbial diversity is similar, lemurs inhabiting areas around human dwellings and in nearby marginal habitats have distinct gastrointestinal microbiome composition compared to lemurs in the Beza Mahafaly Reserve, Madagascar (Bennett et al., 2016).

Along with culture-independent investigations of microbiome composition, culture-dependent methods have been used to examine how gut bacteria found in different mammalian species occupying a given geographic area are related (Goldberg, Gillespie, Rwego, Estoff, & Chapman, 2008). In a study using *E. coli*, a readily culturable bacterial species and known inhabitant of the mammalian colon, Goldberg et al. (2008) used red-tailed guenons (*Cercopithecus ascanius*), as well as two other African species of NHP, black-and-white colobus monkeys (*Colobus guereza*) and red colobus monkeys (*Procolobus tephrosceles*), to investigate how anthropogenic change, such as forest fragmentation, affects bacterial transmission among NHPs, humans, and livestock (Goldberg et al., 2008). They determined that deforestation and agricultural land use increased interspecific bacterial transmission, as these disruptions in the ecosystem lead to an increase in ecologic overlap between wild primate populations, humans, and domestic livestock. Their results suggest that environmental contamination is the most likely source of interspecific bacterial transmission. Information gleaned from this and similar studies allows for the calculation of risk factors associated with human encroachment, notably habitat disturbance, on wild NHP populations, and help with the establishment of conservation strategies aimed at protecting threatened NHPs.

In addition to Goldberg et al. (2008), Bublitz et al. (2015) used culture-based methods to examine how anthropogenic activities affect lemur exposure to bacterial pathogens in Madagascar (Bublitz et al., 2015). Specifically, Bublitz et al. (2015) tested six species of wild lemurs in Ranomafana National Park for the presence of enteric bacterial pathogens, including Enterotoxigenic *Escherichia coli*, *Shigella* spp., *Salmonella enterica*, *Vibrio cholerae*, and *Yersinia* spp. (*enterocolitica* and *pseudotuberculosis*), which are all commonly associated with diarrheal disease in human populations in Madagascar. Bublitz et al. (2015) found that lemurs inhabiting disturbed areas of habitat tested positive for these bacterial pathogens while lemurs found in intact forests tested negative.

1.6 | Captivity alters the NHP gut microbiome

The relationship between lifestyle, notably captivity, and gut microbiome in NHPs has been studied to a limited extent. In one of the first reports exploring the gastrointestinal microbiome of wild and captive NHPs, Uenishi et al. (2007) used molecular fingerprint methods to show that captive and wild chimpanzees (*Pan troglodytes*) harbored significantly different gut microbial communities (Uenishi et al., 2007). Using complementary cloning techniques, the study also identified several taxa affiliated with the *Eubacterium*, *Clostridium*, *Ruminococcus Lactobacillus*, *Bifidobacterium* and *Prevotella*, in both captive and wild individuals. These are all taxa that could potentially have saccharolytic,

fibrolytic and fermentative roles in the colonic ecosystem. Additional work in wild *P. t. schweinfurthii* from Tanzania, employing molecular fingerprinting techniques (T-RFLP), confirms most of these taxonomic patterns and reports marked interindividual differences in the community profiles detected, just as in humans (Szekely et al., 2010). Analyses of the fiber digesting capabilities of captive *P. t. troglodytes*, coupled with molecular fingerprinting of their gut bacterial communities (DGGE) showed a shift in microbiome composition between diets high in fiber (26% neutral detergent fiber, 15% cellulose) and low in fiber (14% neutral detergent fiber, 5% cellulose) (Kisidayová et al., 2009). This study reports blooms of *Eubacterium biforme* and increased production of short chain fatty acids from fecal innocula under high fiber diets. However, it also shows that digestibility of high cellulose substrates in the gut of captive *Pan* is limited.

Howler monkeys are believed to rely primarily on microbial fermentation to break down the structural components of leaves and possibly plant secondary compounds (Milton, 1998) in the cecum and colon (Edwards & Ullrey, 1999; Milton & McBee, 1983; Ullrey, 1986). A 2011 study examining gut microbial communities of wild and captive black howler monkeys (*Alouatta pigra*) with denaturing gradient gel electrophoresis (DGGE) found clear differences in sulfate reducing and other hydrogenotrophic bacteria and archaea (methanogens) between captive and wild populations (Nakamura et al., 2011). The study showed that captive howlers had reduced diversity of hydrogenotrophic bacteria compared to their wild counterparts; whereas, fecal samples of wild howlers showed greater diversity of sulfate reducing bacteria. Additionally, captive individuals had very similar hydrogenotrophic microbial profiles, which were dominated by a pectin degrader, *Lachnospiraceae pectinoschiza* (phylum Firmicutes), despite being rescued from different geographic locations (Cornick, Jensen, Stahl, Hartman, & Allison, 1994). This pattern suggests a strong role of a captive diet, rich in domesticated fruits, in shaping the howler gastrointestinal microbiome over periods of months and years. Amato et al. (2013) also demonstrated that captive black howler monkeys harbored higher relative abundances of *Prevotella* than wild individuals, which is likely related to higher levels of simple carbohydrates in the captive diet (fruits, cereal, and primate pellets) (Amato et al., 2013).

The importance of studying the effects of lifestyle disruption, such as captivity, on gut microbiome composition are many. Of these, health-related factors is arguably the most critical. Some endangered primate species fail to thrive in captivity due to gastrointestinal disease; comparison of wild and captive animals of the same species may shed light on whether shifts in gut microbiota are linked with gastrointestinal health in captivity (Clayton et al., 2016; Gohl et al., 2016). In a 2016 study examining the differences in microbiome composition between captive and wild red-shanked doucs (*Pygathrix nemaeus*) and mantled howler monkeys (*Alouatta palliata*), Clayton et al. (2016) measured gut microbial communities and diet of wild, semi-captive, and captive individuals (Clayton et al., 2016). The major finding in this study was that captivity and loss of dietary fiber in captive primates are associated with loss of native gut microbial taxa. Additionally, they found that captive individuals harbor microbial taxa that dominate the modern human microbiome, including *Bacteroidetes* and *Prevotella*. Another recent study of captive Asian and African colobines (*Pygathrix*, *Trachypithecus*, *Colobus*) from the San Diego Zoo demonstrates that even captive colobine genera have distinct gut microbiota (bacteria, archaea, and eukaryotes) and that individuals suffering from gastrointestinal disease have distinct gut microbial characteristics compared to healthy individuals (Amato et al., 2016). GI-unhealthy individuals were enriched for *Succinovibrio*, *Bulleidia*, *Pastuerella*, *Eubacterium*, *Campylobacter*, *Megasphaera*, *Succiniclasticum*, *Selenomonas*, *Streptococcus*, *Acidaminococcus*, and *Phascolarctobacterium*. The study also compared wild and captive Asian colobines (*Pygathrix*, *Rhinopithecus*) and detected higher relative abundances of *Dehalobacterium*, *Oscillospira*, *Atopobium*, *Blautia*, *Coprobacillus*, *Desulfotomaculum*, *Clostridium*, and *Ruminococcus* and lower relative abundances of *Parabacteroides*, *Prevotella*, *Epulopiscium*, *Bacteroides*, *Desulfovibrio*, *Butyricimonas*, *Methanobrevibacter*, *Phascolarctobacterium*, and *Dialister* in wild individuals. Fogel (2015) also detected differences in the microbiome of wild and captive NHPs, specifically *L. catta*, using high-throughput sequencing. The gastrointestinal microbiome of wild individuals contained an increased relative abundance of *Firmicutes*, *Actinobacteria* and *Euryarchaeota* and a decreased relative abundance of *Bacteroidetes* and *Spirochaetes* compared to captive individuals (Fogel, 2015). Similar to Fogel (2015), Ley et al. (2008) found an enrichment of Spirochaetes in a hamadryas baboon. Conversely, McKenney, Ashwell, Lambert, and Fellner (2014) did not report finding any Spirochaetes in their examination of microbial communities in captive hamadryas baboons (McKenney et al., 2014). The distinctions highlighted by Fogel (2015) appear to be a result of diet and individual host identities differing between environments.

In addition to the culture-independent studies focused on the relationship between captivity and gut microbial community structure in NHPs, a number of culture-dependent studies examining this relationship have been conducted (Benno, Itoh, Miyao, & Mitsuoka, 1987; Carvalho et al., 2014; Villers, Jang, Lent, Lewin-Koh, & Norosoarinaivo, 2008). In an early study examining gut bacteria in wild and captive Japanese macaques, wild and captive individuals differed in their microbial compositions, likely as a result of diet. The wild group mainly fed on tree bark, while the captive group was fed a commercial diet (Benno, Honjo, & Mitsuoka, 1987). The authors observed significantly higher total bacterial counts in captive macaques. Despite this, the ratio of anaerobic bacteria to aerobic bacteria was much higher in wild macaques. Most interestingly, a significant reduction of *Bacteroides* spp. was observed in wild macaques. Villers et al. (2008) examined captive and wild populations of ring-tailed lemurs (*Lemur catta*) using culture-based methods in an effort to identify the major intestinal species of aerobic bacteria. Interestingly, more bacterial species were shared among wild populations than were shared between captive and wild populations, suggesting that differences in gut bacterial composition between captive and wild individuals are greater than those between wild individuals located at different field sites (Villers et al., 2008). Carvalho et al. (2014) screened for potentially pathogenic bacteria and fungi from the rectum, as well as the nasal and oral cavities, of both free-ranging and captive black lion tamarins (*Leontopithecus chrysopygus*)

using a sterile swabbing technique followed by culture for microbial identification. In this study, there were no statistically significant differences in the proportion of bacterial groups between isolates from free-ranging and captive individuals (Carvalho et al., 2014). Overall, Carvalho et al. (2014) found Gram negative bacteria to be more frequent than Gram positive bacteria in the *L. chrysopygus* rectum, particularly, increased abundances of cultured, *E. coli* and *Serratia* spp.

1.7 | Age and sex as microbiome determinants

To date, only a limited number of studies have focused specifically on better understanding the relationship between age and sex and gut microbiome composition. One of those studies was conducted by Amato et al. (2014), which longitudinally tracked black howler gastrointestinal microbiome composition in individuals over 10 months. Amato et al. (2014) determined that adult males, adult females, and juveniles have distinct microbiome compositions, and that juvenile and adult female howlers may derive nutritional benefits from the gastrointestinal microbiome that compensate for the demands of growth and reproduction. Specifically, the microbiome of juvenile howlers was dominated by the phylum Firmicutes, including *Roseburia* and *Ruminococcus* while adult females had a higher than expected Firmicutes to Bacteroidetes ratio and were characterized by *Lactococcus* (Amato et al., 2014). Additionally, juvenile howlers exhibited high fecal volatile fatty acid (VFA) content relative to body size, suggesting that microbes greatly contribute to host energy balance. These results indicate the potential for juvenile and adult female gastrointestinal microbiome's to produce additional energy and vitamins for their hosts. Although diets varied across howler age and sex classes as well, patterns in gastrointestinal microbiome composition were not correlated with patterns in diet. As a result, other mechanisms such as hormone shifts likely influence gastrointestinal microbiome differences.

Similar to Amato et al. (2014), Ren et al. (2015) attempted to determine predictors of gastrointestinal microbiome composition in wild NHPs, including host-specific factors, such as identity, age and sex, as well as other factors, such as rainfall, natal social group, current social group and group size. Using fecal samples collected over a 13-year period, Ren et al. (2015) observed that wild yellow baboons (*Papio cynocephalus*) possess two specific microbiome configurations; one dominated by *Bifidobacterium*, *Butyrivibrio*, *Megasphaera* and another dominated by *Oscillibacter* and *Ruminococcus*. Of greatest interest, Ren et al. (2015) determined that host age, as well as diet and rainfall, were largely responsible for variation in the gastrointestinal microbiome (Ren, Grieneisen, Alberts, Archie, & Wu, 2015).

1.8 | Gut microbiome patterns follow social group affiliation

The relationship between social networks and microbiome composition has drawn great interest recently in the field of microbiome research (Archie & Tung, 2015). Despite interest among primatologists, little work in this area of research has been done to date. Of the work that has been done, the most notable is a study by Tung et al. (2015), which examined the relationship between social networks and gastrointestinal microbiome composition in wild yellow baboons. Tung et al. (2015) found that contact rates directly explained the observed variation in gastrointestinal microbiome composition among wild baboons, as other potential confounding factors were controlled for, including diet, kinship, and overlapping geographic space. These results suggest that gastrointestinal microbiome composition in wild baboons is strongly influenced by social relationships (Tung et al., 2015). Following this study, Moeller et al. (2016) and Perofsky, Lewis, Abondano, Di Fiore, and Meyers (2017) also demonstrated that NHP gut microbiomes are influenced by host social interactions. Individuals have more similar gut microbiota during seasons when social contact is increased. Additionally, juveniles appear to inherit many gut microbial taxa from previous generations, highlighting the importance of vertical transmission of these communities (Yang et al., 2013).

1.9 | The microbiome as an indicator of host-health status

In humans, the link between health and microbiome is of great interest, as is an area of intensive investigation by researchers globally (Cryan & Dinan, 2012; De Palma et al., 2015; Kelly et al., 2015; Ley et al., 2005; Morgan et al., 2012; O'Mahony et al., 2009; Petersen & Round, 2014; Turnbaugh et al., 2006; Wang & Wu, 2005). While some studies focused on links between NHP health and microbiome composition have been conducted, substantially less information is available when compared to what we know about the role the microbiome plays in the maintenance of human health. Early studies focused on links between host-health status and microbiome used culture-dependent techniques. In one of the first studies, Bauchop (1971) analyzed the rhesus macaque gut-associated microbiota using culture-based isolation of bacteria from intestinal and stomach contents of sacrificed animals. *Lactobacillus* and *Clostridium* spp. (Firmicutes phylum) were the most abundant genera (Bauchop, 1971). Other culture-dependent examinations of macaque gut microbial communities have reported the presence of *Lactobacillus* sp., especially in infant and young Japanese macaques (*Macaca fuscata*) and long-tailed macaques (*Macaca fascicularis*) (Bailey & Coe, 1999; Benno, Itoh, Miyao, & Mitsuoka, 1987). Bacterial species belonging to the genus *Lactobacillus* are most known for their health-promoting properties. Their positive association with health has led to the selection of a number of species being used as probiotics, which are live microorganisms that can offer health benefits to the host (Walter, 2008). In a study examining the response of the long-tailed macaque gastrointestinal microbiome to *Shigella* infection, Seekatz et al. (2013) identified *Lactobacillus* as a dominant member of the gastrointestinal microbiome. In fact, based on relative abundance calculations performed, *Lactobacillus* was the most abundant of all genera observed (Seekatz et al., 2013). In a landmark study of baboon gut bacteria, Brinkley and Mott (1978) found the presence of Fusobacteria, which have also been found in humans (Eckburg et al., 2005). Using culture-based methods (anaerobic

culture), they identified the predominant genera present in baboon feces, which were *Lactobacillus*, *Eubacterium*, *Streptococcus*, and *Bacteroides* (Brinkley & Mott, 1978). In another study focused on baboon gastrointestinal microbiome, Brinkley et al. (1982) used culture-dependent methods to isolate and characterize nine novel bacterial strains from feces and intestinal contents, all of which were cholesterol-reducing bacteria (Brinkley, Gottesman, & Mott, 1982). Finally, Modesto et al. (2015) discovered a novel bacterial species within the genus *Bifidobacterium* in ring-tailed lemurs using culture-dependent methods (Modesto et al., 2015). Bifidobacteria are normal inhabitants of the mammalian colon and thought to contribute to intestinal health in a number of ways, including pathogen inhibition, production of vitamins, and immune system modulation (Mayo & van Sinderen, 2010).

One of the first studies using culture-independent techniques to studies links between the microbiome and health and disease was conducted by McKenna et al. (2008), which used pyrosequencing to examine captive enterocolitis-affected rhesus macaques (McKenna et al., 2008). McKenna et al. (2008) reported differences in the numbers of taxa from the Bacteroides and Firmicutes phyla. Specifically, they found a higher prevalence of *Campylobacter* in the symptomatic animals than in healthy animals. McKenna et al. (2008) also reported the abundance of *Treponema* and *Helicobacter* in *M. mulatta* from feces and tissue taken from different sites along the gastrointestinal tract (jejunum and colon). No diet data were collected, but it is possible that captive diets are low in fiber and high in calories from lipids and sugars, which influenced gastrointestinal microbiome composition.

Following the previous investigation of the link between gastrointestinal microbiome and host health, Klase et al. (2015) examined how simian immunodeficiency virus (SIV) infection status, and the associated immunological effects, influences bacterial translocation in a macaque model. Previous investigations in both humans and NHPs have confirmed that bacterial translocation occurs with both acute human immunodeficiency virus (HIV) and SIV infection. However, these studies failed to determine the identity of translocating bacteria (Klase et al., 2015). Klase et al. (2015) found that while differences in gastrointestinal microbiome composition of healthy versus SIV-infected macaques due to infection alone were unremarkable, differences in gastrointestinal microbiome composition after the administration of antiretroviral therapy were substantial. A key finding in this study was the increased abundance of Proteobacteria observed in the tissues of SIV-infected macaques, which suggested Proteobacterial species preferentially translocate. Similarly, Moeller, Peeters, et al., 2013 reported on the effect of SIV infection on the gastrointestinal microbiome of chimpanzees, and showed that infection can trigger increased abundance of potentially pathogenic taxa in the chimpanzee gut (i.e., *Staphylococcus*) (Moeller, Shilts, et al., 2013). No associations with environmental factors on the gastrointestinal microbiome of infected and uninfected chimpanzees were examined. However, a recent study showed that significant compositional changes in fecal bacterial communities are present only in individuals with end-stage SIVcpz infection (Barbian et al., 2018).

1.10 | The use of microbiome research for the field of primatology

Understanding host-microbiome interactions of primates covering the entire tree of primate evolution is of great importance to the field of primatology. Gastrointestinal microbiome research can be especially beneficial for understanding primate health, evolution, behavior, and conservation. Some expected impacts in these four areas of primatology are as follows.

1.10.1 | Health

The gastrointestinal microbiome is so intimately related to animal health that it is often referred to as an additional organ of the body. These bacteria are critical players in primate health and development: They protect the host from infection, aid digestion, produce vitamins from the diet, and influence immune system development (Ley et al., 2005; Morgan et al., 2012; Petersen & Round, 2014; Turnbaugh et al., 2006). Recent advances in human and primate microbiome research have fundamentally changed our understanding of primate immune and metabolic health (Knights et al., 2013; Muegge et al., 2011; Ridaura et al., 2013). Despite these studies, much more work is still needed to fully understand how the microbial communities impact primate health.

Health and pathogen resistance in NHPs have direct links to human health, for example in the case of SIV. It is well established that HIV, the causative agent of AIDS, originated from related viruses of chimpanzees (*Pan troglodytes*) and sooty mangabeys (*Cercocebus atys*) (Hahn, Shaw, De Cock, & Sharp, 2000; LeBreton et al., 2007; Wolfe, Daszak, Kilpatrick, & Burke, 2005). Other notable examples of diseases shared by humans and NHPs include, herpes B virus, monkeypox, polio virus, ebola virus, tuberculosis, malaria, and yellow fever, just to name a few (Chapman, Gillespie, & Goldberg, 2005). Many of these diseases are highly pathogenic, such as ebola virus, which was likely responsible for an estimated 5,000 gorilla deaths in northwest Republic of Congo between 2002 and 2003 (Bermejo et al., 2006). The increasing occurrence of emerging infectious diseases plaguing human and NHPs raises awareness of the importance of understanding infectious disease ecology and how to establish mechanisms for protecting both human and NHP populations (Chapman et al., 2005). A better understanding of the role microbial communities play in the maintenance of health will help with determining these mechanisms.

Given that microbes can act as indicators for health of the host, broad primate microbiome surveys could also aid in the development of predictive biomarkers for certain diseases that affect both humans and NHPs alike. NHPs are the closest animal models to humans, and understanding what drives the structure and variation of their microbiota will help us understand our own.

1.10.2 | Evolution

NHPs are of interest because of their importance to understanding human evolution, underscored by the fact that humans and

chimpanzees share 98.77% nucleotide and 99% amino acid identity across their genomes. While human and NHPs are closely related, there are substantial differences in appearance and behavior from species to species, including stark differences in diet. Current understanding of how adaptations to diet have shaped primate evolution is based primarily on ecological, morphological, and behavioral data. However, much remains to be gained by understanding the relationship between the host and one of the most important factors in digestive health and energy acquisition, gut microbial composition (Ochman et al., 2010; Yildirim et al., 2010). The symbiotic relationship between host and gut microbes is a likely a major determinant of feeding ecology and the adaptation to a specific diet since gut microbiota provide the host with critical metabolic specializations, including digestive enzymes.

In the gastrointestinal tract, characterization of these populations will help to explain divergent adaptations in closely related species. The emerging field of metagenomics allows direct, unbiased interrogation of microbial populations (i.e., microbiomes), thus enabling the investigation of unique dietary differences in primate species that may reveal the role of microbial communities in primate evolutionary history (Ochman et al., 2010; Yildirim et al., 2010). It is likely that these changes played a major role in the ability of the hominin ancestor to move from a forest (woody plant) diet to a savanna (grass) diet, as the gut microbiota largely govern what types of foods can be digested by the host. In summary, the gut microbiota likely played an important role in primate specialization of diet and gut physiology, however this role has yet to be elucidated.

1.10.3 | Behavior

Evidence exists suggesting that there is a relationship between gut microbiota and nervous system function (Cryan & Dinan, 2012). Some early studies identified changes in the gut microbiota associated with stress, and a role for the microbiota in modulating stress and stress-related behavior. A list of potential mechanisms by which the microbiota can affect CNS function has been generated, mainly in rodent models, and includes immune system activation, vagus nerve activation, generation of metabolites with neuroactive properties, and production of cell wall sugars that impact function of primary afferent axons (Cryan & Dinan, 2012). Chronic inflammation due to bacterial infection has also been shown to exhibit a dramatic effect on nervous system function.

The microbiota-gut-brain axis is an emerging concept that suggests an intricate role for our microbes in the establishment and treatment of nervous system disorders, including stress-related psychiatric disorders (Cryan & Dinan, 2012; Kelly et al., 2015). For example, Park et al. (2013) used a mouse model of depression to show a relationship exists between elevated central CRH expression and alterations in the gut microbiota (Park et al., 2013). Other studies have shown an intimate relationship between perturbations in the gut microbiota and the presence of stress (Bailey & Coe, 1999; De Palma et al., 2015; O'Mahony et al., 2009; Wang & Wu, 2005). While some work has been done to date, the microbiota-gut-brain axis remains an emerging area that has been studied in a very limited fashion. With the availability of novel technologies to study the microbiota in a host, it is now possible to identify microbiome modulations that impact nervous system function and behavior.

Although gut-brain communication is well established in rodent models, little is known about this form of communication in NHPs, despite the fact that NHPs are ideal models by which to study the microbe-gut-brain relationship in a natural context. By collecting longitudinal and cross-sectional gastrointestinal microbiome samples while tracking feeding and social behavior of individual animals, a better understanding of how microbes influence primate behavior can be established.

1.10.4 | Conservation

The United States Census Bureau currently estimates the world population to number slightly over 7 billion. Human population expansion has and continues to take a massive toll on the environment (McNeill, 2001). Rapid human population expansion has led to the need for more resources, many of which are taken from the Earth's remaining forests. For example, forests provide mankind with timber, medicine, and food (Costanza et al., 1998). Deforestation poses a major threat to the world's remaining wildlife populations, especially NHPs, and is a primary concern of conservationists (Thomas et al., 2004). Forest fragmentation presents major hurdles for primate populations, as fragmentation disrupts natural ranging patterns, which are necessary for primates to locate enough food to meet their daily energetic demands (Arroyo-Rodriguez & Dias, 2010).

The spread of infectious diseases represents a major threat to wildlife populations and humans alike (LeBreton et al., 2007; Wolfe, Dunavan, & Diamond, 2007; Wolfe et al., 2005). As primates struggle to move through their fragmented home ranges, NHP interactions with humans and domestic livestock increase dramatically (Chapman et al., 2005; Goldberg et al., 2007, 2008). These interactions are problematic because humans and NHPs are susceptible to similar pathogens, which may have high morbidity or mortality in populations that are immunologically naive (e.g., herpes B virus in humans, human metapneumovirus in apes). As such, the potential for zoonotic transfer is drastically elevated with human-primate interactions compared to human contact with other non-primate species, and the consequences potentially quite high (Chapman et al., 2005).

Rescue centers are numerous throughout biodiversity hotspots, with most aiming to rehabilitate and release animals which were victims of poaching and habitat loss. Typical captive diets include large amounts of fruits and rice with varying quantities of concentrate feeds, browse, vegetables, insects, eggs, etc. It is already clear from past research how the NHP gastrointestinal microbiome responds to a long term soluble carbohydrate and low fiber diet. Although the individual species of microbes vary per NHP species, the overall results of wild versus captive microbiome comparisons can be described as similar. A general decrease in gastrointestinal microbiome diversity in captive individuals has been found, including a reduction in species known to produce a protective effect (Kisidayová et al., 2009; Nakamura et al.,

2011; Villers et al., 2008). Consequently, species that could be pathogenic and/or are associated with poor health are found in greater abundance (Benno, Itoh, Miyao, & Mitsuoka, 1987; Kisidayová et al., 2009; Ley et al., 2008; McKenna et al., 2008). While the microbiome can be influenced by abiotic environmental factors, diet is considered one of the major driving forces to the microbiome changes previously mentioned in captivity. Dysbiotic animals are then released, with a gastrointestinal microbiome that is adapted to a diet far different than their wild type. This may lead to a compromised immune system, energy and nutrient malabsorption or pathology, all of which are detrimental to the success of any rehabilitation and release program. Studies of the gastrointestinal microbiome can help identify ideal captive diets for rescue centers and zoos alike, enhancing both animal health and welfare within captivity and during rehabilitation as well as the success of release back into the wild.

2 | CONCLUSIONS

Although the gastrointestinal microbiomes of dozens of NHP taxa have been characterized, most studies are based on small sample sizes and primarily report a taxonomic account of the bacterial diversity in the primate gut. In order to further advance our understanding of how gut microbes impact primate health and ecology, large-scale studies that include both cross-sectional and longitudinal samples are necessary. This approach provides an understanding of how stable the primate microbiome is in multiple ecological states and offers clues to determine whether there is a core microbiome across a wide range of species useful to establish biomarkers of health and disease. Along these lines, taxonomic accounts of the primate microbiome should also include functional assessment of the microbial communities (metabolomics, metagenomics, metatranscriptomics) and immunological profiling of the host. This is a step that very few reports have taken and it is critical to reconcile descriptive views on the primate microbiome with how microbes actually impact primate physiology and health. Finally, collaboration among groups conducting primate microbiome research will provide grounds for establishing standard operating procedures and will allow researchers to share comparable datasets covering multiple species and ecological niches. This is one of the main objectives of the Primate Microbiome Project (http://www.primatemicrobiome.org/).

ACKNOWLEDGMENTS

We thank Dr. Michael Murtaugh and Dr. Herbert Covert for critically reading the manuscript and providing feedback; the National Institutes of Health through a PharmacoNeuroImmunology Fellowship (NIH/National Institute on Drug Abuse T32 DA007097-32) awarded to JBC; and NSF BCS 0935374 and NSF BCS 1441409 awarded to RMS.

ORCID

Jonathan B. Clayton http://orcid.org/0000-0003-3709-3362
Dominic A. Travis http://orcid.org/0000-0003-1542-3375
Timothy J. Johnson http://orcid.org/0000-0001-7556-9347

REFERENCES

Abubucker, S., Segata, N., Goll, J., Schubert, A. M., Izard, J., Cantarel, B. L., ... Huttenhower, C. (2012). Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS Computational Biology*, *8*(6), e1002358.

Alauzet, C., Mory, F., Carlier, J. P., Marchandin, H., Jumas-Bilak, E., & Lozniewski, A. (2007). *Prevotella nanceiensis* sp. nov., isolated from human clinical samples. *International Journal of Systematic and Evolutionary Microbiology*, *57*(Pt 10), 2216–2220.

Amato, K. R., Leigh, S. R., Kent, A., Mackie, R. I., Yeoman, C. J., Stumpf, R. M., ... Garber, P. A. (2014). The role of gut microbes in satisfying the nutritional demands of adult and juvenile wild, black howler monkeys (*Alouatta pigra*). *American Journal of Physical Anthropology*, *155*(4), 652–664.

Amato, K. R., Leigh, S. R., Kent, A., Mackie, R. I., Yeoman, C. J., Stumpf, R. M., ... Garber, P. A. (2015). The gut microbiota appears to compensate for seasonal diet variation in the wild black howler monkey (*Alouatta pigra*). *Microbial Ecology*, *69*(2), 434–443.

Amato, K. R., Metcalf, J. L., Song, S. J., Hale, V. L., Clayton, J., Ackermann, G., ... Braun, J. (2016). Using the gut microbiota as a novel tool for examining colobine primate GI health. *Global Ecology and Conservation*, *7*, 225–237. https://doi.org/10.1016/j.gecco.2016.06.004

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., ... Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *ISME Journal*, *7*(7), 1344–1353.

Archie, E. A., & Tung, J. (2015). Social behavior and the microbiome. *Current Opinion in Behavioral Sciences*, https://doi.org/10.1016/j.cobeha.2015.07.008.

Ardeshir, A., Narayan, N. R., Méndez-Lagares, G., Lu, D., Rauch, M., Huang, Y., ... Hartigan-O'Connor, D. J. (2014). Breast-fed and bottle-fed infant rhesus macaques develop distinct gut microbiotas and immune systems. *Science Translational Medicine*, *6*(252), 252ra120.

Arroyo-Rodriguez, V., & Dias, P. A. D. (2010). Effects of habitat fragmentation and disturbance on howler monkeys: A review. *American Journal of Primatology*, *72*(1), 1–16.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., ... Bork, P. (2011). Enterotypes of the human gut microbiome. *Nature*, *473*(7346), 174–180.

Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., ... Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(44), 15718–15723.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science*, *307*, 1915–1920.

Bailey, M. T., & Coe, C. L. (1999). Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Developmental Psychobiology*, *35*(2), 146–155.

Barbian, H. J., Li, Y., Ramirez, M., Klase, Z., Lipende, I., Mjungu, D., ... Bushman, F. D. (2018). Destabilization of the gut microbiome marks the end-stage of simian immunodeficiency virus infection in wild chimpanzees. *American Journal of Primatology*, *80*(1), e22515.

Barelli, C., Albanese, D., Donati, C., Pindo, M., Dallago, C., Rovero, F., ... De Filippo, C. (2015). Habitat fragmentation is associated to gut microbiota diversity of an endangered primate: Implications for conservation. *Scientific Reports*, *5*, 14862.

Bauchop, T. (1971). Stomach microbiology of primates. *Annual Review of Microbiology*, *25*, 429–436.

Bauchop, T., & Martucci, R. W. (1968). {Ruminant-Like} digestion of the langur monkey. *Science*, *161*(3842), 698–700.

Bayer, E. A., Lamed, R., White, B. A., & Flint, H. J. (2008). From cellulosomes to cellulosomics. *Chemical Record*, *8*(6), 364–377.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*, 289–300.

Bennett, G., Malone, M., Sauther, M. L., Cuozzo, F. P., White, B., Nelson, K. E., … Amato, K. R. (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), 883–892. https://doi.org/10.1002/ajp.22555

Benno, Y., Honjo, S., & Mitsuoka, T. (1987). Effect of the {Two-Year} {Milk-Feeding} on the gastrointestinal microflora of the cynomolgus monkey (*Macaca fascicularis*). *Microbiology and Immunology*, *31*(9), 943–947.

Benno, Y., Itoh, K., Miyao, Y., & Mitsuoka, T. (1987). Comparison of fecal microflora between wild Japanese monkeys in a snowy area and laboratory-reared Japanese monkeys. *Nippon Juigaku Zasshi - Japanese Journal of Veterinary Science*, *49*(6), 1059–1064.

Bermejo, M., Rodríguez-Teijeiro, J. D., Illera, G., Barroso, A., Vilà, C., & Walsh, P. D. (2006). Ebola outbreak killed 5000 gorillas. *Science*, *314*(5805), 1564.

Bhattacharjee, S., & Lukiw, W. J. (2013). Alzheimer's disease and the microbiome. *Frontiers in Cellular Neuroscience*, *7*, 153.

Bittar, F., Keita, M. B., Lagier, J.-C., Peeters, M., Delaporte, E., & Raoult, D. (2014). *Gorilla gorilla* gorilla gut: A potential reservoir of pathogenic bacteria as revealed using culturomics and molecular tools. *Scientific Reports*, *4*, 7174.

Bo, X., Zun-xi, H., Xiao-yan, W., Run-chi, G., Xiang-hua, T., Yue-lin, M., … Li-da, Z. (2010). Phylogenetic analysis of the fecal flora of the wild pygmy loris. *American Journal of Primatology*, *72*(8), 699–706.

Boerner, B. P., & Sarvetnick, N. E. (2011). Type 1 diabetes: Role of intestinal microbiome in humans and mice. *Annals of the New York Academy of Sciences*, *1243*, 103–118.

Brinkley, A. W., Gottesman, A. R., & Mott, G. E. (1982). Isolation and characterization of new strains of cholesterol-reducing bacteria from baboons. *Applied and Environmental Microbiology*, *43*(1), 86–89.

Brinkley, A. W., & Mott, G. E. (1978). Anaerobic fecal bacteria of the baboon. *Applied and Environmental Microbiology*, *36*(3), 530–532.

Brown, C. T., Davis-Richardson, A. G., Giongo, A., Gano, K. A., Crabb, D. B., Mukherjee, N., … Triplett, E. W. (2011). Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS ONE*, *6*(10), e25792.

Bruorton, M. R., Davis, C. L., & Perrin, M. R. (1991). Gut microflora of vervet and samango monkeys in relation to diet. *Applied and Environmental Microbiology*, *57*(2), 573–578.

Bublitz, D. C., Wright, P. C., Rasambainarivo, F. T., Arrigo-Nelson, S. J., Bodager, J. R., & Gillespie, T. R. (2015). Pathogenic enterobacteria in lemurs associated with anthropogenic disturbance. *American Journal of Primatology*, *77*(3), 330–337.

Buisson, G., Duee, E., Payan, F., & Haser, R. (1987). Alpha-amylase tertiary structures and their interactions with polysaccharides. *Food Hydrocolloids*, *1*(5–6), 399–406.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). Correspondence QIIME allows analysis of high-throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. *Nature Publishing Group*, *7*(5), 335–336. https://doi.org/10.1038/nmeth0510-335

Carvalho, V. M., Vanstreels, R. E. T., Paula, C. D., Kolesnikovas, C. K. M., Ramos, M. C. C., Coutinho, S. D., … Catão-Dias, J. L. (2014). Nasal, oral and rectal microbiota of Black lion tamarins (*Leontopithecus chrysopygus*). *Brazilian Journal of Microbiology*, *45*(4), 1531–1539.

Caton, J. M. (1999). Digestive strategy of the Asian colobine genus *Trachypithecus*. *Primates*, *40*(2), 311–325.

Chapman, C. A., Gillespie, T. R., & Goldberg, T. L. (2005). Primates and the ecology of their infectious diseases: How will anthropogenic change affect host-parasite interactions? *Evolutionary Anthropology: Issues, News, and Reviews*, *14*(4), 134–144.

Chen, W., Zhang, C. K., Cheng, Y., Zhang, S., & Zhao, H. (2013). A comparison of methods for clustering 16S rRNA sequences into OTUs. *PLoS ONE*, *8*(8), https://doi.org/10.1371/journal.pone.0070837

Chen, Y., Niu, Y., & Ji, W. (2012). Transgenic nonhuman primate models for human diseases: Approaches and contributing factors. *Journal of Genetics and Genomics*, *39*(6), 247–251.

Chivers, D. J. (1994). Functional anatomy of the gastrointestinal tract. *Colobine monkeys: Their ecology, behaviour and evolution* (pp. 205–227). Cambridge: Cambridge University Press.

Chivers, D. J., & Hladik, C. M. (1980). Morphology of the {Gastrointestinal-Tract} in primates—Comparisons with other mammals in relation to diet. *Journal of Morphology*, *166*(3), 337–386.

Clayton, J. B., Vangay, P., Huang, H., Ward, T., Hillmann, B. M., Al-Ghalith, G. A., … Knights, D. (2016). Captivity humanizes the primate microbiome. *Proceedings of the National Academy of Sciences*, *113*(37), 10376–10381. https://doi.org/10.1073/pnas.1521835113

Cornick, N. A., Jensen, N. S., Stahl, D. A., Hartman, P. A., & Allison, M. J. (1994). *Lachnospira pectinoschiza* sp. nov., an anaerobic pectinophile from the pig intestine. *International Journal of Systematic Bacteriology*, *44*(1), 87–93.

Costa, M. A., Mehta, T., & Males, J. R. (1989). Effects of dietary cellulose and psyllium husk on monkey colonic microbial metabolism in continuous culture. *The Journal of Nutrition*, *119*(7), 979–985.

Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., & Hannon, B., et al. (1998). The value of the world's ecosystem services and natural capital. *Ecological Economics*, *1*(25), 3–15.

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, *13*(10), 701–712.

Daniel, H., Moghaddas Gholami, A., Berry, D., Desmarchelier, C., Hahne, H., Loh, G., … Clavel, T. (2014). High-fat diet alters gut microbiota physiology in mice. *ISME Journal*, *8*(2), 295–308.

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A. J., Green, W., … Bercik, P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature Communications*, *6*, 7735. https://doi.org/10.1038/ncomms8735

Degnan, P. H., Pusey, A. E., Lonsdorf, E. V., Goodall, J., Wroblewski, E. E., Wilson, M. L., … Ochman, H. (2012). Factors associated with the diversification of the gut microbial communities within chimpanzees from Gombe National Park. *Proceedings of the National Academy of Sciences*, *109*(32), 13034–13039.

Dicksved, J., Halfvarson, J., Rosenquist, M., Jarnerot, G., Tysk, C., Apajalahti, J., … Jansson, J. K. (2008). Molecular analysis of the gut microbiota of identical twins with Crohn's disease. *ISME Journal*, *2*(7), 716–727.

Dougal, K., Harris, P. A., Edwards, A., Pachebat, J. A., Blackmore, T. M., Worgan, H. J., & Newbold, C. J. (2012). A comparison of the microbiome and the metabolome of different regions of the equine hindgut. *FEMS Microbiology Ecology*, *82*(3), 642–652.

Downes, J., Liu, M., Kononen, E., & Wade, W. G. (2009). *Prevotella micans* sp. nov., isolated from the human oral cavity. *International Journal of Systematic and Evolutionary Microbiology*, *59*(Pt 4), 771–774.

Dunn, O. J. (1961). Multiple comparisons among means. *Journal of the American Statistical Association*, *56*(293), 52–64.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., … Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*(5728), 1635–1638.

Edwards, M. S., & Ullrey, D. E. (1999). Effect of dietary fiber concentration on apparent digestibility and digesta passage in non-human primates. {II}. Hindgut-and foregut-fermenting folivores. *Zoo Biology*, *18*, 537–549.

Ericsson, A. C., Johnson, P. J., Lopes, M. A., Perry, S. C., & Lanter, H. R. (2016). A microbiological map of the healthy equine gastrointestinal tract. *PLoS ONE*, *11*(11), e0166523.

Fogel, A. T. (2015). The gut microbiome of wild lemurs: A comparison of sympatric lemur catta and propithecus verreauxi. *Folia Primatologica; International Journal of Primatology*, *86*(1-2), 85–95.

Forney, L., Zhou, X., & Brown, C. (2004). Molecular microbial ecology: Land of the one-eyed king. *Current Opinion in Microbiology*, *7*(3), 210–220.

Frey, J. C., Rothman, J. M., Pell, A. N., Nizeyi, J. B., Cranfield, M. R., & Angert, E. R. (2006). Fecal bacterial diversity in a wild gorilla. *Applied and Environmental Microbiology*, *72*(5), 3788–3792.

Gevers, D., Kugathasan, S., Denson, L. A., Vázquez-Baeza, Y., Van Treuren, W., Ren, B., ... Xavier, R. J. (2014). The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host & Microbe*, *15*(3), 382–392.

Gilbert, J. A., Jansson, J. K., & Knight, R. (2014). The earth microbiome project: Successes and aspirations. *BMC Biology*, *12*(1), 69.

Gilbert, J. A., Meyer, F., Antonopoulos, D., Balaji, P., Brown, C. T., Brown, C. T., ... Stevens, R. (2010). Meeting report: The terabase metagenomics workshop and the vision of an earth microbiome project. *Standards in Genomic Sciences*, *3*(3), 243–248.

Giongo, A., Gano, K. A., Crabb, D. B., Mukherjee, N., Novelo, L. L., Casella, G., ... Triplett, E. W. (2011). Toward defining the autoimmune microbiome for type 1 diabetes. *ISME Journal*, *5*(1), 82–91.

Godoy-Vitorino, F., Goldfarb, K. C., Karaoz, U., Leal, S., Garcia-Amado, M. A., Hugenholtz, P., ... Dominguez-Bello, M. G. (2012). Comparative analyses of foregut and hindgut bacterial communities in hoatzins and cows. *The ISME Journal*, *6*(3), 531.

Gohl, D. M., Vangay, P., Garbe, J., MacLean, A., Hauge, A., Becker, A., ... Beckman, K. B. (2016). Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. *Nature Biotechnology*, *34*(November 2015), 1–11. https://doi.org/10.1038/nbt.3601

Goldberg, T. L., Gillespie, T. R., Rwego, I. B., Estoff, E. L., & Chapman, C. A. (2008). Forest fragmentation as cause of bacterial transmission among nonhuman primates, humans, and livestock, Uganda. *Emerging Infectious Diseases*, *14*(9), 1375–1382.

Goldberg, T. L., Gillespie, T. R., Rwego, I. B., Wheeler, E., Estoff, E. L., & Chapman, C. A. (2007). Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. *Biological Conservation*, *135*(4), 511–517.

Gomez, A. M., Yannarell, A. C., Sims, G. K., Cadavid-Restrepo, G., & Moreno Herrera, C. X. (2011). Characterization of bacterial diversity at different depths in the Moravia Hill landfill site at Medellin, Colombia {RID} {A-2500-2008}. *Soil Biology & Biochemistry*, *43*(6), 1275–1284.

Gomez, A., Petrzelkova, K., Yeoman, C. J., Vlckova, K., Mrázek, J., Koppova, I., ... Leigh, S. R. (2015). Gut microbiome composition and metabolomic profiles of wild western lowland gorillas (*Gorilla gorilla gorilla*) reflect host ecology. *Molecular Ecology*, *24*(10), 2551–2565.

Gomez, A., Rothman, J. M., Petrzelkova, K., Yeoman, C. J., Vlckova, K., Umaña, J. D., ... Leigh, S. R. (2016). Temporal variation selects for diet-microbe co-metabolic traits in the gut of *Gorilla* spp. *ISME Journal*, *10*(2), 532.

Gu, S., Chen, D., Zhang, J.-N., Lv, X., Wang, K., Duan, L.-P., ... Wu, X.-L. (2013). Bacterial community mapping of the mouse gastrointestinal tract. *PLoS ONE*, *8*(10), e74957. https://doi.org/10.1371/journal.pone.0074957

Hahn, B. H., Shaw, G. M., De Cock, K. M., & Sharp, P. M. (2000). {AIDS} as a zoonosis: Scientific and public health implications. *Science*, *287*(5453), 607–614.

Hale, V. L., Tan, C. L., Knight, R., & Amato, K. R. (2015). Effect of preservation method on spider monkey (*Ateles geoffroyi*) fecal microbiota over 8weeks. *Journal of Microbiological Methods*, *113*, 16–26.

Hamady, M., & Knight, R. (2009). Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Research*, *19*(7), 1141–1152.

Hardham, J. M., King, K. W., Dreier, K., Wong, J., Strietzel, C., Eversole, R. R., ... Evans, R. T. (2008). Transfer of *Bacteroides splanchnicus* to *Odoribacter* gen. nov. as *Odoribacter splanchnicus* comb. nov., and description of *Odoribacter denticanis* sp. nov., isolated from the crevicular spaces of canine periodontitis patients. *International Journal of Systematic and Evolutionary Microbiology*, *58*(Pt 1), 103–109.

He, J., Yi, L., Hai, L., Ming, L., Gao, W., & Ji, R. (2018). Characterizing the bacterial microbiota in different gastrointestinal tract segments of the Bactrian camel. *Scientific Reports*, *8*(1), 654.

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., Janssen, P. H., ... Zunino, P. (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*, *5*, 14567. https://doi.org/10.1038/srep14567

Hooper, L. V., & Gordon, J. I. (2001). Commensal host-bacterial relationships in the gut. *Science*, *292*(5519), 1115–1118.

Hugenholtz, P., Goebel, B. M., & Pace, N. R. (1998). Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology*, *180*(18), 4765–4774.

Hume, I. D., (1997). Fermentation in the hindgut of mammals. In R. I. Mackie, & B. A. White, (Eds.), *Gastrointestinal microbiology*. Volumne 1. Gastrointesinal Ecosystems and Fermentations (pp. 84–115). New York, NY: Chapman & Hall.

Kay, R. N. B., & Davies, A. G. (1994). Digestive physiology. *Colobine Monkeys: Their Ecology, Behaviour and Evolution*, 229–249.

Kelly, J. R., Kennedy, P. J., Cryan, J. F., Dinan, T. G., Clarke, G., & Hyland, N. P. (2015). Breaking down the barriers: The gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Frontiers in Cellular Neuroscience*, *9*, 392. https://doi.org/10.3389/fncel.2015.00392

Kim, D., Hofstaedter, C. E., Zhao, C., Mattei, L., Tanes, C., Clarke, E., ... Conrad, M. (2017). Optimizing methods and dodging pitfalls in microbiome research. *Microbiome*, *5*(1), 52.

Kim, M., Morrison, M., & Yu, Z. (2011). Status of the phylogenetic diversity census of ruminal microbiomes. *FEMS Microbiology Ecology*, *76*(1), 49–63.

Kisidayová, S., Váradyová, Z., Pristas, P., Piknová, M., Nigutová, K., Petrzelková, K. J., ... Modrý, D. (2009). Effects of high- and low-fiber diets on fecal fermentation and fecal microbial populations of captive chimpanzees. *American Journal of Primatology*, *71*(7), 548–557.

Klase, Z., Ortiz, A., Deleage, C., Mudd, J. C., Quiñones, M., Schwartzman, E., ... Brenchley, J. M. (2015). Dysbiotic bacteria translocate in progressive {SIV} infection. *Mucosal Immunol*, *8*(5), 1009–1020.

Knights, D., Lassen, K. G., & Xavier, R. J. (2013). Advances in inflammatory bowel disease pathogenesis: Linking host genetics and the microbiome. *Gut*, *62*(10), 1505–1510.

Lambert, J. E. (1998). Primate digestion: Interactions among anatomy, physiology, and feeding ecology. *Evolutionary Anthropology*, *7*(1), 8–20.

Lambert, J. E. (2001). Red-tailed guenons (*Cercopithecus ascanius*) and *Strychnos mitis*: Evidence for plant benefits beyond seed dispersal. *International Journal of Primatology*, *22*(2), 189–201.

Lambert, J. E. (2002). Digestive retention times in forest guenons (*Cercopithecus* spp.) with reference to chimpanzees (*Pan troglodytes*). *International Journal of Primatology*, *23*(6), 1169–1185.

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L., & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(20), 6955–6959. https://doi.org/10.1073/pnas.82.20.6955

Langston, J. A., Shaghasi, T., Abbate, E., Xu, F., Vlasenko, E., & Sweeney, M. D. (2011). Oxidoreductive cellulose depolymerization by the enzymes cellobiose dehydrogenase and glycoside hydrolase 61. *Applied and Environmental Microbiology*, *77*(19), 7007–7015. https://doi.org/10.1128/AEM.05815-11

Larsen, N., Vogensen, F. K., van den Berg, F. W., Nielsen, D. S., Andreasen, A. S., Pedersen, B. K., ... Jakobsen, M. (2010). Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE*, *5*(2), e9085.

LeBreton, M., Yang, O., Tamoufe, U., Mpoudi-Ngole, E., Torimiro, J. N., Djoko, C. F., ... Wolfe, N. D. (2007). Exposure to wild primates among

{HIV-infected} persons. *Emerging Infectious Diseases*, *13*(10), 1579–1582.

Lee, Y. K., & Mazmanian, S. K. (2010). Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science*, *330*(6012), 1768–1773.

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(31), 11070–11075.

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., … Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science*, *320*, 1647–1651.

Li, D., Chen, H., Mao, B., Yang, Q., Zhao, J., Gu, Z., … Chen, W. (2017). Microbial biogeography and core microbiota of the rat digestive tract. *Scientific Reports*, *7*, 45840.

Liu, Z., Lozupone, C., Hamady, M., Bushman, F. D., & Knight, R. (2007). Short pyrosequencing reads suffice for accurate microbial community analysis. *Nucleic Acids Research*, *35*(18), e120.

Ma, J., Prince, A. L., Bader, D., Hu, M., Ganu, R., Baquero, K., … Aagaard, K. M. (2014). High-fat maternal diet during pregnancy persistently alters the offspring microbiome in a primate model. *Nature Communications*, *5*, 3889.

Mayo, B., & van Sinderen, D. (2010). *Bifidobacteria: Genomics and molecular aspects*. Caister Academic Press, Norfolk, UK.

McCord, A. I., Chapman, C. A., Weny, G., Tumukunde, A., Hyeroba, D., Klotz, K., … Goldberg, T. L. (2014). Fecal microbiomes of non-human primates in Western Uganda reveal species-specific communities largely resistant to habitat perturbation. *American Journal of Primatology*, *76*(4), 347–354.

McFall-Ngai, M. (2007). Adaptive immunity—Care for the community. *Nature*, *445*(7124), 153.

McKenna, P., Hoffmann, C., Minkah, N., Aye, P. P., Lackner, A., Liu, Z., … Bushman, F. D. (2008). The macaque gut microbiome in health, lentiviral infection, and chronic enterocolitis. *PLoS Pathogens*, *4*(2), e20.

McKenney, E. A., Ashwell, M., Lambert, J. E., & Fellner, V. (2014). Fecal microbial diversity and putative function in captive western lowland gorillas (*Gorilla gorilla* gorilla), common chimpanzees (*Pan troglodytes*), Hamadryas baboons (*Papio hamadryas*) and binturongs (*Arctictis binturong*). *Integrative Zoology*, *9*(5), 557–569.

McKenney, E. A., Rodrigo, A., & Yoder, A. D. (2015). Patterns of gut bacterial colonization in three primate species. *PLoS ONE*, *10*(5), e0124618.

McKenzie, V. J., Song, S. J., Delsuc, F., Prest, T. L., Oliverio, A. M., Korpita, T. M., … Knight, R. (2017). The effects of captivity on the mammalian gut microbiome. *Integrative and Comparative Biology*, *57*(4), 690–704. https://doi.org/10.1093/icb/icx090

McNeill, J. R. (2001). *Something new under the sun: An environmental history of the twentieth-century world (the global century series)*. WW Norton & Company, New York, NY.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., & Kubal, M., et al. (2008). The metagenomics {RAST} server—A public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics*, *9*(1), 386.

Milton, K. (1987). Primate diets and gut morphology: Implications for hominid evolution. *Food and Evolution: Toward a Theory of Human Food Habits*, 93–115.

Milton, K. (1998). Physiological ecology of howlers (*Alouatta*): energetic and digestive considerations and comparison with the Colobinae. *International Journal of Primatology*, *19*, 513–548.

Milton, K., & McBee, R. H. (1983). Rates of fermentative digestion in the howler monkey, Alouatta palliata (Primates: Ceboidea). *Comparative Biochemistry and Physiology*, *74*, 29–31.

Modesto, M., Michelini, S., Stefanini, I., Sandri, C., Spiezio, C., Pisi, A., … Mattarelli, P. (2015). *Bifidobacterium lemurum* sp. nov., from the faeces of the ring-tailed lemur (Lemur catta). *International Journal of Systematic and Evolutionary Microbiology*, *65*(Pt 6), 1726–1734.

Moeller, A. H., Degnan, P. H., Pusey, A. E., Wilson, M. L., Hahn, B. H., & Ochman, H. (2012). Chimpanzees and humans harbour compositionally similar gut enterotypes. *Nature Communications*, *3*, 1179.

Moeller, A. H., Foerster, S., Wilson, M. L., Pusey, A. E., Hahn, B. H., & Ochman, H. (2016). Social behavior shapes the chimpanzee pan-microbiome. *Science Advances*, *2*(1), e1500997.

Moeller, A. H., Li, Y., Mpoudi Ngole, E., Ahuka-Mundeke, S., Lonsdorf, E. V., Pusey A. E., … Ochman, H. (2014). Rapid changes in the gut microbiome during human evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 16431–16435.

Moeller, A. H., Peeters, M., Ayouba, A., Ngole, E. M., Esteban, A., Hahn, B. H., & Ochman, H. (2015). Stability of the gorilla microbiome despite simian immunodeficiency virus infection. *Molecular Ecology*, *24*, 690–697.

Moeller, A. H., Peeters, M., Ndjango, J. B., Li, Y., Hahn, B. H., & Ochman, H. (2013). Sympatric chimpanzees and gorillas harbor convergent gut microbial communities. *Genome*, *23*, 1715–1720.

Moeller, A. H., Shilts, M., Li, Y., Rudicell, R. S., Lonsdorf, E. V., Pusey, A. E., … Ochman, H. (2013). {SIV-induced} instability of the chimpanzee gut microbiome. *Cell Host & Microbe*, *14*(3), 340–345.

Mongodin, E. F., Emerson, J. B., & Nelson, K. E. (2005). Microbial metagenomics. *Genome Biology*, *6*(10), 347.

Morgan, X. C., Tickle, T. L., Sokol, H., Gevers, D., Devaney, K. L., Ward, D. V., … Huttenhower, C. (2012). Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biology*, *13*(9), R79.

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., González, A., Fontana, L., … Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science*, *332*(6032), 970–974.

Nakamura, N., Amato, K. R., Garber, P., Estrada, A., Mackie, R. I., & Gaskins, H. R. (2011). Analysis of the hydrogenotrophic microbiota of wild and captive black howler monkeys (*Alouatta pigra*) in palenque national park, Mexico. *American Journal of Primatology*, *73*(9), 909–919.

Nakamura, N., Leigh, S. R., Mackie, R. I., & Gaskins, H. R. (2009). Microbial community analysis of rectal methanogens and sulfate reducing bacteria in two non-human primate species. *Journal of Medical Primatology*, *38*(5), 360–370.

Nekaris, K., Starr, C. R., Collins, R. L., & Wilson, A. (2010). Comparative ecology of exudate feeding by lorises (*Nycticebus*, *loris*) and pottos (Perodicticus, arctocebus). *The evolution of exudativory in primates* (pp. 155–168). New York: Springer.

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M. M., … Dinan, T. G. (2009). Early life stress alters behavior, immunity, and microbiota in rats: Implications for irritable bowel syndrome and psychiatric illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

Ochman, H., Worobey, M., Kuo, C.-H., Ndjango, J.-B. N., Peeters, M., Hahn, B. H., & Hugenholtz, P. (2010). Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS Biology*, *8*(11), e1000546.

Pace, N. R. (1997). A molecular view of microbial diversity and the biosphere. *Science*, *276*(5313), 734–740.

Park, A. J., Collins, J., Blennerhassett, P. A., Ghia, J. E., Verdu, E. F., Bercik, P., & Collins, S. M. (2013). Altered colonic function and microbiota profile in a mouse model of chronic depression. *Neurogastroenterology and Motility*, *25*(9), 733–e575. https://doi.org/10.1111/nmo.12153

Perofsky, A. C., Lewis, R. J., Abondano, L. A., Di Fiore, A., & Meyers, L. A. (2017). Hierarchical social networks shape gut microbial composition in wild Verreaux's sifaka. *Proceedings of the Royal Society B*, *284*(1868), 20172274.

Petersen, C., & Round, J. L. (2014). Defining dysbiosis and its influence on host immunity and disease. *Cellular Microbiology*, *16*(7), 1024–1033.

Petrosino, J. F., Highlander, S., Luna, R. A., Gibbs, R. A., & Versalovic, J. (2009). Metagenomic pyrosequencing and microbial identification. *Clinical Chemistry*, *55*(5), 856–866.

Popovich, D. G., Jenkins, D., Kendall, C., Dierenfeld, E. S., Carroll, R. W., Tariq, N., & Vidgen, E. (1997). The western lowland gorilla diet has implications for the health of humans and other hominoids. *The Journal of Nutrition*, *127*(10), 2000–2005.

Rappe, M. S., & Giovannoni, S. J. (2003). The uncultured microbial majority. *Annual Review of Microbiology*, *57*, 369–394.

Rautava, S., & Isolauri, E. (2002). The development of gut immune responses and gut microbiota: Effects of probiotics in prevention and treatment of allergic disease. *Current Issues in Intestinal Microbiology*, *3*(1), 15–22.

Remis, M. J., & Dierenfeld, E. S. (2004). Digesta passage, digestibility and behavior in captive gorillas under two dietary regimens. *International Journal of Primatology*, *25*(4), 825–845.

Ren, T., Grieneisen, L. E., Alberts, S. C., Archie, E. A., & Wu, M. (2015). Development, diet and dynamism: Longitudinal and cross-sectional predictors of gut microbial communities in wild baboons. *Environmental Microbiology*, *18*(5), 1312–1325.

Rentz, J. A., Alvarez, P. J., & Schnoor, J. L. (2004). Repression of *Pseudomonas putida* phenanthrene-degrading activity by plant root extracts and exudates. *Environmental Microbiology*, *6*(6), 574–583.

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., & Gordon, J. I. . . .(2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science*, *341*(6150), 1241214.

Riesenfeld, C. S., Schloss, P. D., & Handelsman, J. (2004). Metagenomics: Genomic analysis of microbial communities. *Annual Review of Genetics*, *38*, 525–552.

Robinson, C. J., Bohannan, B. J., & Young, V. B. (2010). From structure to function: The ecology of host-associated microbial communities. *Microbiology and Molecular Biology Reviews*, *74*(3), 453–476.

Ronaghi, M. (2001). Pyrosequencing sheds light on {DNA} sequencing. *Genome Research*, *11*(1), 3–11.

Round, J. L., Lee, S. M., Li, J., Tran, G., Jabri, B., Chatila, T. A., & Mazmanian, S. K. (2011). The toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science*, *332*(6032), 974–977.

Round, J. L., & Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews. Immunology*, *9*(5), 313–323.

Sakamoto, M., & Benno, Y. (2006). Reclassification of *Bacteroides distasonis*, *Bacteroides goldsteinii* and *Bacteroides merdae* as *Parabacteroides distasonis* gen. nov., comb. nov., *Parabacteroides goldsteinii* comb. nov. and *Parabacteroides merdae* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *56*(7), 1599–1605.

Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual Reviews in Microbiology*, *31*, 107–133.

Schloss, P. D., & Handelsman, J. (2005). Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Applied and Environmental Microbiology*, *71*(3), 1501–1506. https://doi.org/10.1128/AEM.71.3.1501

Schmidt, T. S. B., Matias Rodrigues, J. F., & von Mering, C. (2015). Limits to robustness and reproducibility in the demarcation of operational taxonomic units. *Environmental Microbiology*, *17*(5), 1689–1706. https://doi.org/10.1111/1462-2920.12610

Seekatz, A. M., Panda, A., Rasko, D. A., Toapanta, F. R., Eloe-Fadrosh, E. A., Khan, A. Q., … Fraser, C. M. (2013). Differential response of the cynomolgus macaque gut microbiota to *Shigella* infection. *PLoS ONE*, *8*(6), e64212.

Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biology*, *14*(8), https://doi.org/10.1371/journal.pbio.1002533

Soergel, D. A. W., Dey, N., Knight, R., & Brenner, S. E. (2012). Selection of primers for optimal taxonomic classification of environmental 16S rRNA gene sequences. *ISME Journal*, *6*(7), 1440–1444. https://doi.org/10.1038/ismej.2011.208

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L. G., Gratadoux, J. J., . . . Langella, P. (2008). *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(43), 16731–16736.

Starr, C., & Nekaris, K. A. I. (2013). Obligate exudativory characterizes the diet of the pygmy slow loris *Nycticebus pygmaeus*. *American Journal of Primatology*, *75*(10), 1054–1061.

Stearns, J. C., Lynch, M. D. J., Senadheera, D. B., Tenenbaum, H. C., Goldberg, M. B., Cvitkovitch, D. G., . . . Neufeld, J. D. (2011). Bacterial biogeography of the human digestive tract. *Scientific Reports*, *1*, https://doi.org/10.1038/srep00170

Stone, W. H., Treichel, R. C., & VandeBerg, J. L. (1987). Genetic significance of some common primate models in biomedical research. *Progress in Clinical and Biological Research*, *229*, 73–93.

Szekely, B. A., Singh, J., Marsh, T. L., Hagedorn, C., Werre, S. R., & Kaur, T. (2010). Fecal bacterial diversity of human-habituated wild chimpanzees (*Pan troglodytes* schweinfurthii) at Mahale Mountains National Park, Western Tanzania. *American Journal of Primatology*, *72*(7), 566–574.

Thomas, J. A., Telfer, M. G., Roy, D. B., Preston, C. D., Greenwood, J. J. D., Asher, J., … Lawton, J. H. (2004). Comparative losses of British butterflies, birds, and plants and the global extinction crisis. *Science*, *303*(5665), 1879–1881.

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., . . . The Earth Microbiome Project Consortium. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, *551*, 457–463.

Toft, C., & Andersson, S. G. (2010). Evolutionary microbial genomics: Insights into bacterial host adaptation. *Nature Reviews Genetics*, *11*(7), 465–475.

Tringe, S. G., von Mering, C., Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., . . . Rubin, E. M. (2005). Comparative metagenomics of microbial communities. *Science*, *308*(5721), 554–557.

Tung, J., Barreiro, L. B., Burns, M. B., Grenier, J.-C., Lynch, J., Grieneisen, L. E., . . . Archie, E. A. (2015). Social networks predict gut microbiome composition in wild baboons. *eLife*, *4*, e05224.

Turnbaugh, P. J., Bäckhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host and Microbe*, *3*(4), 213–223.

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., . . . Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. *Nature*, *457*(7228), 480–484.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, *444*(7122), 1027–1031.

Ueki, A., Akasaka, H., Suzuki, D., & Ueki, K. (2006). *Paludibacter propionicigenes* gen. nov., sp. nov., a novel strictly anaerobic, Gram-negative, propionate-producing bacterium isolated from plant residue in irrigated rice-field soil in Japan. *International Journal of Systematic and Evolutionary Microbiology*, *56*(Pt 1), 39–44.

Uenishi, G., Fujita, S., Ohashi, G., Kato, A., Yamauchi, S., Matsuzawa, T., & Ushida, K. (2007). Molecular analyses of the intestinal microbiota of chimpanzees in the wild and in captivity. *American Journal of Primatology*, *69*(4), 367–376.

Ullrey, D. E., (1986). Nutrition of primates in captivity. In K. Benirschke, (Ed.), *Primates: The road to self-sustaining populations* (pp. 823–835). New York: Springer-Verlag.

Vlčková, K., Mrázek, J., Kopečný, J., & Petrželková, K. J. (2012). Evaluation of different storage methods to characterize the fecal bacterial communities of captive western lowland gorillas (*Gorilla gorilla gorilla*). *Journal of Microbiological Methods*, *91*(1), 45–51.

Villers, L. M., Jang, S. S., Lent, C. L., Lewin-Koh, S.-C., & Norosoarinaivo, J. A. (2008). Survey and comparison of major intestinal flora in captive and wild ring-tailed lemur (*Lemur catta*) populations. *American Journal of Primatology*, *70*(2), 175–184.

Walker, A. W., Ince, J., Duncan, S. H., Webster, L. M., Holtrop, G., Ze, X., . . . Flint, H. J. (2011). Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME Journal*, *5*(2), 220–230.

Walter, J. (2008). Ecological role of lactobacilli in the gastrointestinal tract: Implications for fundamental and biomedical research. *Applied and Environmental Microbiology*, *74*(16), 4985–4996.

Wang, S. X., & Wu, W. C. (2005). Effects of psychological stress on small intestinal motility and bacteria and mucosa in mice. *World Journal of Gastroenterology*, *11*(13), 2016–2021. https://doi.org/10.3748/wjg.v11.i13.2016

Wireman, J., Lowe, M., Spiro, A., Zhang, Y.-Z., Sornborger, A., & Summers, A. O. (2006). Quantitative, longitudinal profiling of the primate fecal microbiota reveals idiosyncratic, dynamic communities. *Environmental Microbiology*, *8*(3), 490–503.

Woese, C. R. (1987). Bacterial evolution. *Microbiological Reviews*, *51*(2), 221.

Woese, C. R., Kandler, O., & Wheelis, M. L. (1990). Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(12), 4576–4579.

Wolfe, N. D., Daszak, P., Kilpatrick, A. M., & Burke, D. S. (2005). Bushmeat hunting, deforestation, and prediction of zoonotic disease. *Emerging Infectious Disease Journal*, *11*(12), 1822.

Wolfe, N. D., Dunavan, C. P., & Diamond, J. (2007). Origins of major human infectious diseases. *Nature*, *447*(7142), 279–283.

Xu, B., Xu, W., Li, J., Dai, L., Xiong, C., & Tang, X., et al. (2015). Metagenomic analysis of the Rhinopithecus bieti fecal microbiome reveals a broad diversity of bacterial and glycoside hydrolase profiles related to lignocellulose degradation. *BMC Genomics*, *16*(1), 174.

Xu, B., Xu, W., Yang, F., Li, J., Yang, Y., Tang, X., … Huang, Z. (2013). Metagenomic analysis of the pygmy loris fecal microbiome reveals unique functional capacity related to metabolism of aromatic compounds. *PLoS ONE*, *8*(2), e56565.

Xu, B., Yang, F., Xiong, C., Li, J., Tang, X., Zhou, J., … Huang, Z. (2014). Cloning and characterization of a novel α-amylase from a fecal microbial metagenome. *Journal of Microbiology and Biotechnology*, *24*(4), 447–452.

Yang, B., Wang, Y., Qian, P.-Y., Fernandes, K. A., Kittelmann, S., Rogers, C. W., … Center for History and New Media, (2013). Social behavior shapes the chimpanzee pan-microbiome. *Nature*, *2*(1), e1500997. https://doi.org/10.1126/sciadv.1500997

Yang, B., Wang, Y., & Qian, P. Y. (2016). Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics*, *17*(1), 135.

Yasuda, K., Oh, K., Ren, B., Tickle, T. L., Franzosa, E. A., Wachtman, L. M., … Morgan, X. C. (2015). Biogeography of the intestinal mucosal and lumenal microbiome in the rhesus macaque. *Cell Host & Microbe*, *17*(3), 385–391.

Yildirim, S., Yeoman, C. J., Sipos, M., Torralba, M., Wilson, B. A., Goldberg, T. L., … Nelson, K. E. (2010). Characterization of the fecal microbiome from non-human wild primates reveals species specific microbial communities. *PLoS ONE*, *5*(11), e13963.

Yuan, S., Cohen, D. B., Ravel, J., Abdo, Z., & Forney, L. J. (2012). Evaluation of methods for the extraction and purification of {DNA} from the human microbiome. *PLoS ONE*, *7*(3), e33865.

Zhou, X., Wang, B., Pan, Q., Zhang, J., Kumar, S., Sun, X., … Li, M. (2014). Whole-genome sequencing of the snub-nosed monkey provides insights into folivory and evolutionary history. *Nature Genetics*, *46*(12), 1303–1310.

Zoetendal, E. G., Collier, C. T., Koike, S., Mackie, R. I., & Gaskins, H. R. (2004). Molecular ecological analysis of the gastrointestinal microbiota: A review. *The Journal of Nutrition*, *134*(2), 465–472.

How to cite this article: Clayton JB, Gomez A, Amato K, et al. The gut microbiome of nonhuman primates: Lessons in ecology and evolution. *Am J Primatol*. 2018;80:e22867. https://doi.org/10.1002/ajp.22867