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PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Phylogenetic and ecological factors impact the gut microbiota of two Neotropical primate species

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Abstract Recent studies suggest that variation in diet across time and space results in changes in the mammalian gut microbiota. This variation may ultimately impact host ecology by altering nutritional status and health. Wild animal populations provide an excellent opportunity for understanding these interactions. However, compared to clinical studies, microbial research targeting wild animals is currently limited, and many published studies focus only on a single population of a single host species. In this study we utilize fecal samples from two species of howler monkey (*Alouatta pigra* and *A. palliata*) collected at four sites to investigate factors influencing the gut microbiota at three scales: taxonomic (host species), ecosystemic (forest type), and local (habitat disturbance/season). The results demonstrate that the effect of host species on the gut microbiota is stronger than the effect of host forest type, which is stronger than the effect of habitat disturbance or seasonality. Nevertheless, within host species, gut microbiota composition differs in response to forest type, habitat disturbance, and season. Variations in the effect size of these factors are associated both with host species and environment. This information may be beneficial for understanding ecological and evolutionary questions associated with Mesoamerican howler monkeys, as well as determining conservation challenges facing each species. These mechanisms may also provide insight into the ecology of other species of howler monkeys, non-human primates, and mammals.

Keywords *Alouatta* · Microbiome · Habitat · Season · Disturbance

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Introduction

Through effects on host nutrition, health and behavior, the mammalian gut microbiota can impact host ecology strongly (Amato 2013; Foster and McVey Neufeld 2013; Kau et al. 2011; Ley et al. 2008). In addition, studies of humans and animal models suggest that changes in host diet can alter the composition of the gut microbial community, which in turn affects factors such as host digestive efficiency, immune response, and stress response (Bailey 2012; Bauer et al. 2006; David et al. 2014; Hooper et al. 2012; Hume and Warner 1980; Turnbaugh et al. 2009). Variation in these processes influences host reproductive potential and fitness (Altmann 1998; Dunbar 1980; Gogarten et al. 2012; Hamilton 1985), making the understanding of host-gut microbe interactions in natural, selective environments critical for studies of mammalian evolution.

Wild mammals often consume distinct diets across seasons or habitats in response to changes in food availability (Adamczewski et al. 1988; Andelt et al. 1987; Cerling and Viehl 2004; Chaves et al. 2011; Nakagawa 1997; Overdorff et al. 1997). Therefore, based on data from humans and model animal systems (Arumugam et al. 2011; David et al. 2014; Turnbaugh et al. 2009; Yatsunenko et al. 2012), we would expect wild mammals to experience spatial and temporal variation in gut microbial community composition and function as well. A handful of studies focused on non-human primates, pandas, and horses support this prediction (Amato et al. 2013, 2015; Barelli et al. 2015; Kobayashi et al. 2006; Williams et al. 2012). In particular, recent research with Mexican black howler monkeys (*Alouatta pigra*) demonstrated that the gut microbiota responds to changes in diet across time and space and may affect host nutrition and health (Amato et al. 2013, 2015). For example, in a primary continuous evergreen rainforest, howler monkey gut microbial composition and production of short-chain fatty acids (that can be utilized by hosts for energy) shifted with diet across season, suggesting that gut microbiota has a nutritional buffering effect (Amato et al. 2015). In contrast, howler monkeys inhabiting a fragmented, secondary evergreen rainforest had lower gut microbial diversity, reduced relative abundances of butyrate-producing bacteria (a short-chain fatty acid), and higher relative abundances of potentially toxic hydrogen-sulfide-producing bacteria that can negatively affect smooth muscle function and promote inflammation (Amato et al. 2013). These changes increase risks of host nutritional stress and disease susceptibility. Similar patterns in gut microbial diversity as well as shifts in gut microbiota composition and predicted function have been reported for the Udzungwa red colobus in disturbed forests (Barelli et al. 2015).

These findings have important implications for mammalian ecology, evolution, and conservation. In the case of howler monkeys, they suggest that the gut microbiota plays an important role in allowing hosts to endure seasonal variation in food availability and to occupy a wide range of habitats with comparatively strong seasonal patterns in plant phenology (Arroyo-Rodriguez and Dias 2009; Bicca-Marques 2003). These data also point to health risks associated with howler monkey habitat degradation, despite the well-documented ability of howler monkeys to persist in forest fragments (Arroyo-Rodriguez and Dias 2009; Arroyo-Rodriguez and Mandujano 2006; Bicca-Marques 2003; Cristobal-Azkarate and Arroyo-Rodriguez 2007; Dunn et al. 2009; Laurance et al. 2000; Malcolm 1994). A better comprehension of these patterns would complement current research and strengthen existing frameworks for understanding howler monkey biology.

However, it is difficult to draw broader ecological and evolutionary inferences about host-gut microbe interactions based on a single population of a single primate species. These kinds of analyses leave several questions unaddressed. First, ecological differences among distinct populations of the same host species could directly affect host-gut microbe interactions. While the gut microbiota appears to provide black howler monkeys with nutritional compensation during periods of low energy intake in primary, continuous evergreen rainforests, it is unknown whether or not the same mechanism functions for black howler monkeys in different forest types and habitats. Likewise, season and habitat disturbance may have distinct impacts on the howler monkey gut microbiota in different forest types since they alter plant species diversity in semi-deciduous or dry forests differently than in evergreen forests. Studies of the plant communities of tropical dry forests in Brazil report little to no impact of fragmentation on tree species diversity (Oliveira et al. 2013; Sampaio and Scariot 2011). Finally, there may be host species effects that produce differing host-gut microbe dynamics. Although all howler monkey species rely on leaves seasonally throughout Meso- and South America, the proportion of leaves that each howler species consume varies markedly (Di Fiore et al. 2011; Garber et al. 2015). These gross dietary differences, as well as differences in host species physiology and behavior, likely impact host-gut microbe dynamics.

To allow a more complete understanding of the *Alouatta* gut microbiota and which factors dictate its composition, here we evaluate variation in the *Alouatta* gut microbiota at three different scales: taxonomic (host species), ecosystemic (forest type), and local (habitat disturbance/season). We begin by comparing overall gut microbial community composition between two *Alouatta* species, *A. pigra* and

Table 1 Sampling scheme

Forest type	Sampling period	Habitat type	A. pigra (PNP)	A. pigra (ET)	A. palliata (LS)	A. palliata (OM)
Evergreen rainforest	High fruit	Secondary	x		x	
		Primary	x		x	
	Low fruit	Secondary	x			
		Primary	x			
Semi-deciduous forest	High fruit	Secondary		x		x
		Primary		x		x
	Low fruit	Secondary		x		x
		Primary		x		x

x samples collected

A. palliata. Next, we examine differences in the gut microbial community composition of *A. pigra* and *A. palliata* in two forest types: evergreen forest and tropical semi-deciduous forest. Finally, we investigate the effects of seasonal diet shifts (<50 % fruit in the diet vs. >50 % fruit) and habitat disturbance on the gut microbiota of *A. pigra* and *A. palliata* in both evergreen rainforest and tropical semi-deciduous forest (Table 1). Although *A. pigra* and *A. palliata* are closely associated both phylogenetically (monophyletic origin with an estimated split approximately 3 Ma; Cortes-Ortiz et al. 2003) and geographically, and consume similar diets (Di Fiore et al. 2011; Rylands et al. 2006), key ecological differences make comparing host-gut microbe interactions in these two species especially interesting (Di Fiore et al. 2011). In particular, *A. pigra* is an endemic species with small group sizes [3–9 individuals (Di Fiore et al. 2011)] found only in Mexico, Guatemala, and Belize (Di Fiore et al. 2011), while *A. palliata* lives in large groups [10–21 individuals (Di Fiore et al. 2011)] and is distributed from Mexico to Ecuador (Cuaron et al. 2008). As a result, it is believed that *A. palliata* has a greater ecological tolerance and superior dispersal abilities (Baumgarten and Williamson 2007; Ford 2006). However, while some researchers suggest *A. pigra* may be a habitat specialist, occupying evergreen rainforest at low elevations (Crockett 1998; Horwich and Johnson 1986), *A. pigra* occupies a variety of habitats (evergreen rainforest, deciduous forest, mangroves, lowland forest, etc.) (Baumgarten and Williamson 2007; Garber et al. 2015; Marsh et al. 2008), and evidence of physiological differences that limit *A. pigra*'s distribution has not been forthcoming.

At each of the three roughly hierarchical scales of gut microbial variation that we investigated, we tested distinct hypotheses. First, at the taxonomic scale, due to their close phylogenetic relationship, we predicted that *A. pigra* and *A. palliata* would exhibit generally similar gut microbial communities with differences in the relative abundances of a small number of microbial genera. However, because *A. palliata* is a widespread species with potentially superior

dispersal abilities, we hypothesized that on an ecosystemic scale its gut microbiota would react less strongly to differences in forest type than that of *A. pigra*. In particular, we expected *A. pigra* to exhibit stronger reductions in gut microbial diversity and shifts in gut microbial community composition than *A. palliata* when inhabiting a tropical semi-deciduous forest compared to an evergreen rainforest. Finally, at the local scale we hypothesized that gut microbial community diversity and composition would differ in response to season as well as habitat disturbance for both *Alouatta* species in both forest types. Generally, we predicted the following effects: 1) Microbial diversity and relative abundances of short-chain-fatty-acid-producing microbes would be lower both during periods of high fruit consumption and in secondary forests (Amato et al. 2013, 2015). 2) Assuming the diets of howler monkeys in primary and secondary forest are most distinct during periods of high fruit intake because primary forest howler monkeys are able to utilize more large, fruiting trees (Laurance et al. 2000; Malcolm 1994), the effects of habitat disturbance would be stronger during periods of high fruit intake. 3) Assuming secondary forest has fewer large fruiting trees (Laurance et al. 2000; Malcolm 1994) and therefore less dramatic seasonal shifts in fruit availability, the gut microbiota of howler monkeys in secondary forest would shift less markedly across seasons than those of howler monkeys in primary forest.

Additionally, we hypothesized that, although similar, the effects of season and habitat disturbance on the gut microbiota would differ depending on the host species and forest type. We used *A. pigra* inhabiting an evergreen rainforest as a baseline to which to compare the other *Alouatta* populations, since this population is the most extensively studied in the context of the gut microbiome (Amato et al. 2013, 2014, 2015; Nakamura et al. 2011). Specifically, because of its limited distribution and potentially reduced ecological flexibility, we expected *A. pigra* to exhibit more dramatic changes in microbial diversity and composition in response to season and habitat disturbance in both forest types

compared to *A. palliata*. Given extreme seasonal variation in climate and fruit phenology (Van Schaik and Brockman 2005), we expected the effect of season to be stronger in semi-deciduous forest than evergreen rainforest for both howler species. Also, since some studies suggest that the impact of habitat disturbance on plant species composition is smaller in dry forests (Oliveira et al. 2013; Sampaio and Scariot 2011), we expected the effect of habitat disturbance on the gut microbiota to be weaker in semi-deciduous forest.

Materials and methods

Sample collection

A. pigra, Palenque National Park, Mexico

Fecal samples were collected from six social groups of black howler monkeys ($N = 41$ individuals) in Palenque, State of Chiapas, Mexico by KRA (Table 1, S1). Three of the sampled groups inhabited primary evergreen rainforest in Palenque National Park (900 ha, Balam, Motiepa and Pakal groups). One group inhabited secondary evergreen rainforest (60 ha) on hotel property outside the national park (Chan-Kah group). One inhabited secondary evergreen rainforest (26 ha) adjacent to an airport and a housing development near the town of Palenque (CBTA group), and the last inhabited secondary evergreen rainforest (120 ha) adjacent to the main road outside the national park (Pan-Chan group). All social groups experience daily exposure to humans (i.e. humans are present on the forest floor but not directly interacting with the monkeys). Samples were collected during two seasons. The first represented a period of high rainfall and high fruit intake (October 2010). The second represented a period of low rainfall and low fruit intake (February 2011). These periods were determined to be either high-fruit or low-fruit intake based on detailed behavioral data collected from two of the howler groups in the primary forest (52 % fruit in the daily diet during Sept-Nov 2010 vs. 30 % fruit in the daily diet during Jan–March 2011) (Amato and Garber 2014; Amato et al. 2015). Samples were preserved in 96 % ethanol and kept at 4 °C until transport to the US, where they were kept at −20 °C until processing.

A. pigra, El Tormento Experimental Forest, Mexico

Fecal samples were collected from five social groups of black howler monkeys ($N = 34$ individuals) near the town of Escarcega, State of Campeche, Mexico by NR and RMM (Table 1, S1). Two of the sampled groups (M and J groups) inhabited a primary, 2100-ha semi-deciduous

forest (El Tormento Experimental Forest). Three inhabited secondary semi-deciduous forest (2–9 ha) in the area (PE, FE and CH groups). The home range of the two groups living in the primary forest was characterized by higher feeding-tree species diversity compared to the home range of black howlers from secondary forest (Shannon diversity index: 2.34 ± 0.05 vs. 1.85 ± 0.11). Also, feeding trees in the primary forest had a larger basal area than in secondary forest (50.7 ± 11.4 m²/ha vs. 24.6 ± 5.7 m²/ha), resulting in higher fruit production. All social groups in the secondary forest experienced daily exposure to humans. Samples were collected during two seasons: the first (August 2011) represented a period of high rainfall and high fruit intake; the second (February/March 2011) represented a period of low rainfall and lower fruit intake. The characterization of these periods was based on detailed behavioral and ecological data collected from the two howler groups in the primary forest (85 % $\pm$ 7 of fruit in the daily diet during August 2011 vs. 47 % $\pm$ 26 of fruit in the daily diet during February/March 2011) (Righini 2014). Samples were preserved in 96 % ethanol and kept at 4 °C until transport to the U.S., where they were kept at −20 °C until processing.

A. palliata, La Suerte, Costa Rica

Fecal samples were collected from two social groups of mantled howler monkeys ($N = 20$ individuals) inhabiting evergreen rainforest at La Suerte Biological Field Station in northeastern Costa Rica by FC (Table 1, S1). One group inhabited primary evergreen rainforest (20 ha) while the other inhabited secondary evergreen rainforest (250 ha) established in the 1970s. Samples were collected during a period of low rainfall and high fruit intake (July–September 2012) based on data collected in previous studies of other primates in the area (Urbani 2009). Samples were immediately frozen and preserved with 20 ml of N_2 until transport to the University of Puerto Rico, where they were kept at −80 °C until processing.

A. palliata, Ometepe Island, Nicaragua

Fecal samples were collected from five social groups of mantled howler monkeys ($N = 37$ individuals) on the southeastern side of Ometepe Island, Nicaragua by KRA and MRS (Table 1, S1). Two of these groups inhabited primary semi-deciduous tropical forest at the base of the Maderas volcano (approximately 3500 ha, Arriba and Cascada groups). Three inhabited protected secondary semi-deciduous forest (approximately 3500 ha) near farmland in the area of San Ramón, Ometepe (Coffee Forest, Hacienda Mystica, and White Tree Forest groups). The average tree density, height, and diameter at breast height were higher in the primary forest compared to the secondary forest (0.17 ± 0.04

vs. 0.10 ± 0.02 trees/m²; 22 m ± 23 m vs. 11 m ± 10 m; 24 cm ± 61 cm vs. 19 cm ± 21 cm, respectively). All social groups experience daily exposure to humans. Samples were collected during a period of low rainfall and high leaf intake (January 2014) as well as a period of high rainfall and high fruit intake (August 2014) based on qualitative observations during collection as well as previously described seasonal feeding patterns of two howler groups (feeding time devoted to fruit: dry season 21 %, rainy season 42 %) (Raguet-Schofield 2010). Samples were preserved in RNAlater and kept at room temperature until transport to the U.S., where they were kept at −20 °C until processing.

Sampling of *A. pigra* and *A. palliata* was approved by the University of Illinois Institutional Animal Care and Use Committee (IACUCs #08044, #11046, #10051, and #10054) as well as the University of Colorado Boulder IACUC (#1311.01). Permits to collect and export fecal samples were obtained through the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT), the Comisión Nacional de Áreas Naturales Protegidas (CONANP), and the Secretaría de Agricultura, Ganadería, Desarollo Rural, Pesca y Alimentación (SAGARPA) in Mexico. In Nicaragua, they were obtained through the Ministerio del Ambiente y los Recursos Naturales (MARENA) and the Comité de Manejo Colaborativo del Parque Nacional Volcán Maderas. In Costa Rica, permits to collect and export fecal samples were obtained through the Minesterio de Salud (2012-07-03). Permits to import samples to the United States were obtained through the Center for Disease Control and Prevention (CDC).

Sample processing

We extracted microbial DNA from all samples using the MOBio PowerSoil DNA extraction kit. PCR targeting the V4 region of the 16S rRNA bacterial was performed with the 515F/806R primers, utilizing the protocol described in Caporaso et al. (2012). Amplicons were barcoded and pooled in equal concentrations for sequencing. The amplicon pool was purified with the MoBio UltraClean PCR Clean-up kit and sequenced on the Illumina MiSeq sequencing platform (MiSeq Control Software 2.0.5 and Real-Time Analysis software 1.16.18) at the BioFrontiers Institute Next-Generation Genomics Facility at University of Colorado, Boulder, USA. The same procedure was applied to samples from La Suerte, Costa Rica at New York University, New York, USA.

The single-end sequencing reads from the 515f primer were quality-checked using the default settings for the split_libraries_fastq.py function in QIIME v1.9.0. Sequences were clustered into representative bacterial operational taxonomic units (OTUs) using the sortmerna/sumaclust implementation of open-reference OTU-picking at 97 % sequence similarity. Taxonomy was assigned using the RDP classifier and the Green Genes 13_8 database. Any

OTUs representing less than 0.00005 % of the total dataset were filtered out as recommended for Illumina-generated sequencing data (Bokulich et al. 2013). Data were also rarefied to 5,018 reads per sample before analysis.

Statistical analysis

Chao1 indices of alpha diversity (Chao et al. 2005) were generated using QIIME (alpha_rarefaction.py). Beta diversity distance matrices were generated using QIIME (beta_diversity_through_plots.py), and clustering patterns among samples were visualized using non-metric multidimensional scaling (NMDS, R software, version 3.0.2). Pairwise distances between samples were calculated using Bray-Curtis similarity indices. However, patterns were similar for both unweighted and weighted UniFrac distances. We tested for significant differences in sample clustering patterns and microbial community composition across social groups and habitat types for each species using permutational analysis of variance (PERMANOVA, adonis package, R software, version 3.0.2). For overall comparisons between *A. palliata* and *A. pigra*, only samples collected from primary evergreen rainforest during periods of high fruit intake were utilized. For comparisons of gut microbiota across forest types for each *Alouatta* species, only samples collected from primary forest during periods of high fruit intake were utilized. Significant changes in the relative abundances of individual bacterial taxa were detected across species, forest types, habitats, and seasons using a series of Kruskal–Wallis tests (R software), and *p* values were corrected using family-wide error rates (FDR, R software). The percent of OTUs shared by *A. pigra* and *A. palliata,* shared by populations of each species in each forest type, and shared by populations of each species in primary and secondary forest in each forest type were calculated using QIIME (shared_phylotypes.py).

Although the use of different storage techniques is not ideal, studies suggest that the effects of storage on the sequence-based estimates of gut microbial community composition are relatively small and tend to differentially affect only a small subset of OTUs (Hale et al. 2015). Therefore, we believe that these sample sets are comparable for the questions addressed in this study.

Results

Impact of host species on the gut microbiota of *A. pigra* and *A. palliata*

When we examined the gut microbiota of *A. pigra* and *A. palliata* inhabiting primary evergreen rainforest and consuming fruit-heavy diets, we found several patterns. Although the
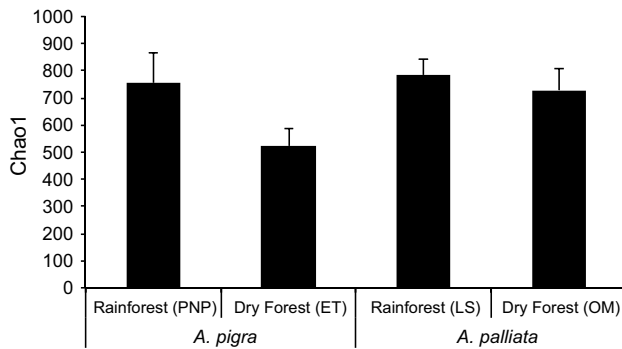
Fig. 1 Chao1 estimates for microbial diversity of *A. pigra* and *A. palliata* gut microbiota in different forest types. Samples represent individuals inhabiting continuous forest during periods of high fruit intake. *Error bars* represent the standard deviation of the mean. Data were rarefied to 5018 reads per sample. The only significant differences ($p < 0.05$) detected were across forest types for *A. pigra*

two host species had similar gut microbial diversity (Fig. 1), there were significant differences in gut microbial community composition ($F_{1,22} = 9.65$, $r^2 = 0.31$, $p < 0.01$; Fig. 2). Overall, only 51 % of the OTUs detected in *A. pigra* were also detected in *A. palliata*. Also, *A. palliata* exhibited higher relative abundances of Lentisphaerae, Euryarchaeota, Victivallaceae, *Roseburia,* and *Bifidobacterium*, and *A. pigra* exhibited higher relative abundances of Firmicutes, Actinobacteria, Erysipelotrichaceae, Ruminococcaceae, *Dialister, Faecalibacterium,* and *Clostridium* (Table S2).

Impact of forest type on the gut microbiota of *A. pigra* and *A. palliata*

Data from *A. pigra* and *A. palliata* consuming fruit-heavy diets but inhabiting either primary evergreen rainforest or primary semi-deciduous forest demonstrated that differences in the gut microbiota in response to host species were maintained regardless of the forest type a given species inhabited (Fig. 2). However, for both host species, forest type impacted the gut microbiota. Both *A. pigra* and *A. palliata* exhibited higher relative abundances of *Bacteroides* and *Bifidobacterium,* and lower relative abundances of Tenericutes, Opitutae, and *Bulleidia* in primary semi-deciduous forests compared to primary evergreen rainforests (Table S3).

Despite these similarities, forest type had distinct effects on the gut microbiota of *A. pigra* and *A. palliata*. We observed reduced gut microbial diversity in *A. pigra* in semi-deciduous forest compared to evergreen rainforest ($\chi^2 = 18.3$, $df = 1$, $p < 0.01$) while the gut microbial diversity of *A. palliata* was the same no matter what forest type was being inhabited (Fig. 1). Additionally, 57 % of the OTUs observed in all *A. pigra* individuals in evergreen rainforest were also observed in semi-deciduous forest, while 75 % of the OTUs observed in *A. palliata* in evergreen rainforest
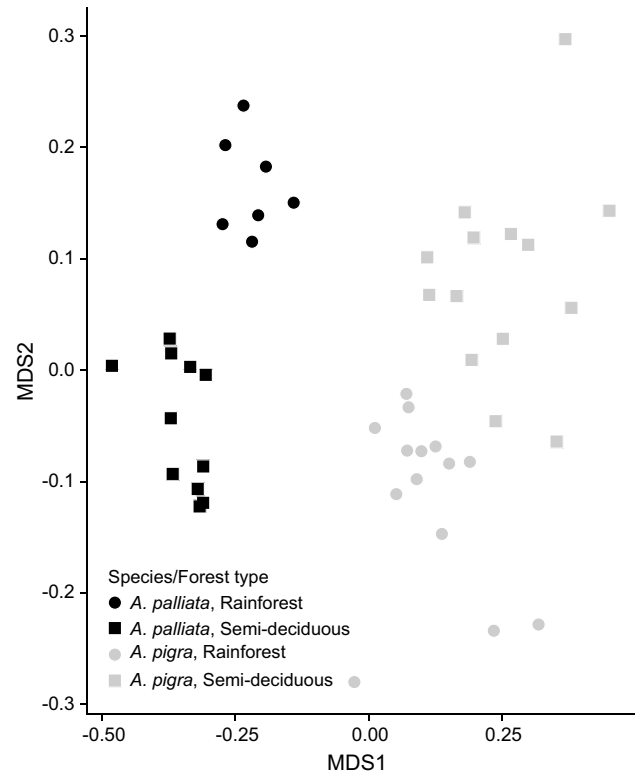
Fig. 2 NMDS plot demonstrating clustering of gut microbiomes by host species and forest type. Each point represents the gut microbiota of a single primate, and only samples collected from individuals inhabiting continuous forest during periods of high fruit intake were included. The plot was generated using Bray-Curtis distances. Data were rarefied to 5018 reads per sample. The effects of forest type are significant for both species ($p < 0.05$) as determined by PERMANOVA

occurred in semi-deciduous forest animals. In contrast, forest type had a stronger effect on the relative abundances of *A. palliata* gut microbes ($F_{1,17} = 7.36$, $r^2 = 0.32$, $p < 0.01$) compared to *A. pigra* ($F_{1,29} = 4.96$, $r^2 = 0.15$, $p < 0.01$, Fig. 2). In addition to the differences already listed for both species across forest types, *A. pigra* had higher relative abundances of Enterobacteraceae and *Megasphaera* and lower relative abundances of Alphaproteobacteria, *Lachnospira, Clostridium,* and *Coprococcus* in the semi-deciduous forest compared the evergreen rainforest (Table S3). *A. palliata* had higher relative abundances of Euryarchaeota, Bacteroidetes, Lentisphaerae, Rickenellaceae, *Escherichia*, *Lachnospira,* and *Dialister* in the semi-deciduous forest and lower relative abundances of *Roseburia* (Table S3).

Impact of season and habitat type on the gut microbiota of *A. pigra* and *A. palliata*

To look at local impacts on the gut microbiota, we examined the effect of both habitat disturbance and season on populations of both host species in both forest types.

Table 2 Alpha diversity statistics

Effect tested	Season/habitat	*A. pigra* (PNP)	*A. pigra* (ET)	*A. palliata* (LS)	*A. palliata* (OM)
Habitat	Fruit	$\chi^2 = 8.12, df = 1,$ $p < 0.01$	$\chi^2 = 0.004, df = 1,$ $p = 0.95$	$\chi^2 = 2.23, df = 1,$ $p = 0.13$	$\chi^2 = 0.12, df = 1, p = 0.73$
	Leaf	$\chi^2 = 12.1, df = 1,$ $p < 0.01$	$\chi^2 = 4.17, df = 1,$ $p = 0.04$	na	$\chi^2 = 0.21, df = 1, p = 0.65$
Season	Primary	$\chi^2 = 0.21, df = 1,$ $p = 0.65$	$\chi^2 = 0.01, df = 1,$ $p = 0.92$	na	$\chi^2 = 1.16, df = 1, p = 0.28$
	Secondary	$\chi^2 = 0.062, df = 1,$ $p = 0.80$	$\chi^2 = 0.72, df = 1,$ $p = 0.40$	na	$\chi^2 = 1.28, df = 1, p = 0.26$

Cells that are bold italic contain significant results
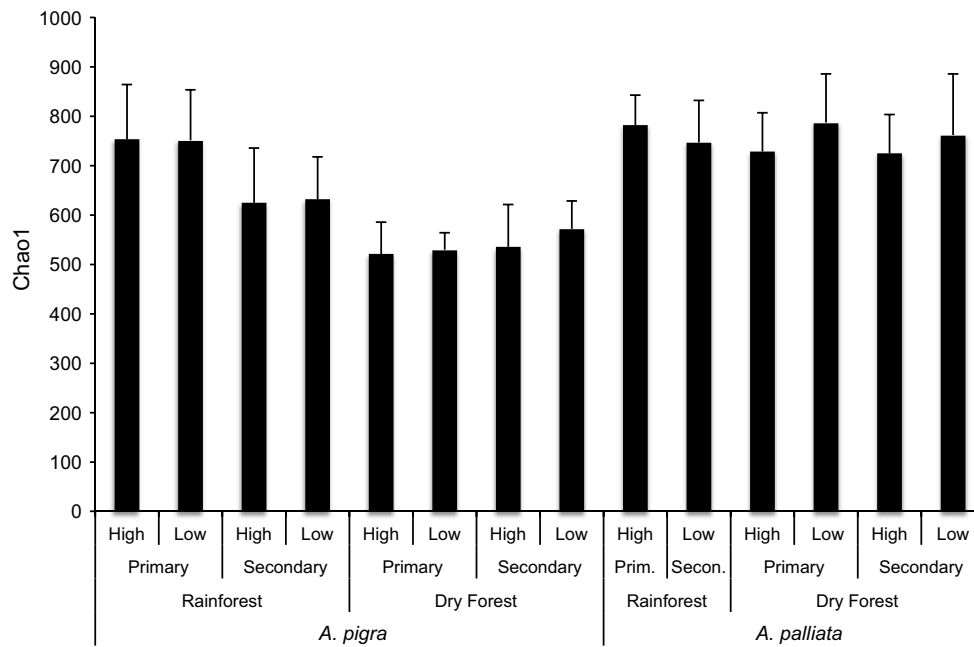
Fig. 3 Chao1 estimates for gut microbial diversity of *A. pigra* and *A. palliata* across seasons (high and low fruit intake) and habitats (primary and secondary forest). *Error bars* represent the standard deviation of the mean. Data were rarefied to 5018 reads per sample. The only significant differences ($p < 0.05$) detected were across habitats for *A. pigra* in the evergreen rainforest during both seasons and in the semi-deciduous forest during reduced fruit consumption

Because the gut microbiota of *A. pigra* inhabiting an evergreen rainforest has been previously well-described, we used this population as a baseline to which to compare the others. We detected a number of interesting patterns.

A. pigra *in evergreen rainforest:* Black howler monkeys in Palenque, Mexico exhibited shifts in their gut microbiota across both habitats and seasons. On average, gut microbial diversity was lower for individuals in the secondary evergreen rainforest during both the period of high fruit consumption and the period of high leaf consumption (Table 2; Fig. 3). However, 80 % of the OTUs found in all individuals in primary forest were also observed in the secondary forest population. Gut microbial community composition

was also distinct across habitats during both sampling periods (Table 3; Fig. 4a). During the fruit season, howler monkeys in the secondary evergreen rainforest had a lower relative abundance of Firmicutes, Ruminococcaceae, and *Faecalibacterium* and a higher relative abundance of Euryarchaeota and Verrucomicrobia (Table S4). During the leaf season, howler monkeys in the secondary evergreen rainforest had higher relative abundances of Helicobacteraceae (Table S4).

We detected no significant difference in gut microbial diversity across seasons in either habitat sampled (Table 2; Fig. 3). However, gut microbial community composition shifted with season in the primary evergreen rainforest

Table 3 Beta diversity statistics (Bray Curtis)

Effect tested	Season/habitat	*A. pigra* (PNP)	*A. pigra* (ET)	*A. palliata* (LS)	*A. palliata* (OM)
Habitat	Fruit	$F_{1,36} = 4.30, r^2 = 0.11, p < 0.01$	$F_{1,22} = 2.24, r^2 = 0.10, p < 0.01$	$F_{1,27} = 1.68, r^2 = 0.095, p = 0.04$	$F_{1,27} = 2.12, r^2 = 0.08, p < 0.01$
	Leaf	$F_{1,37} = 3.62, r^2 = 0.10, p < 0.01$	$F_{1,27} = 4.86, r^2 = 0.16, p < 0.01$	na	$F_{1,25} = 2.03, r^2 = 0.08, p = 0.01$
Season	Primary	$F_{1,36} = 2.24, r^2 = 0.06, p < 0.01$	$F_{1,25} = 4.12, r^2 = 0.15, p < 0.01$	na	$F_{1,15} = 2.42, r^2 = 0.15, p = 0.01$
	Secondary	$F_{1,37} = 1.15, r^2 = 0.03, p = 0.24$	$F_{1,24} = 2.03, r^2 = 0.08, p < 0.01$	na	$F_{1,37} = 2.92, r^2 = 0.08, p < 0.01$

Cells that are bold italic contain significant results

(Table 3; Fig. 4a). In particular, the howler monkeys exhibited a lower relative abundance of Ruminococcaceae and Erysipelotrichaceae during the period of low fruit consumption (Table S4). A similar shift in gut microbial community composition was not observed in the secondary evergreen rainforest.

A. pigra in semi-deciduous forest: We observed slightly different changes in the gut microbiota of black howler monkeys in the semi-deciduous forest of El Tormento, Mexico across habitats and seasons compared to the black howlers in Palenque. First, gut microbial diversity was higher in the secondary semi-deciduous forest only during the low-fruit intake season (Table 2; Fig. 3). Sixty-eight percent of the OTUs found in all individuals in primary forest were also observed in the secondary forest population. However, like the black howlers in Palenque, we detected shifts in gut microbial composition across habitats during both seasons (Table 3; Fig. 4b). During the high fruit intake season, these changes were not driven by any specific microbial taxa while during the months of lower fruit intake, relative abundances of *Tanerella* were lower in the secondary forest than in the primary forest (Table S5).

Similar to black howler monkeys in Palenque, we did not observe any significant changes in microbial diversity across seasons in either primary or secondary semi-deciduous forest at El Tormento (Table 2; Fig. 3). However, unlike Palenque, in El Tormento, gut microbial community composition shifted with season in both habitats (Table 3; Fig. 4b). The effect of season was larger in the primary forest. In the primary forest, there were higher relative abundances of Actinobacteria during the low fruit intake season. In the secondary semi-decidous forest, no one microbe drove seasonal shifts in the gut microbiota.

A. palliata in evergreen rainforest: Samples were collected during a single season for mantled howler monkeys in La Suerte, Costa Rica so only the effect of habitat during high fruit intake could be explored. Unlike black howler monkeys, mantled howler monkeys did not show a difference in gut microbial diversity in response to habitat (Table 2; Fig. 3) in the evergreen rainforest. Additionally,

86 % of the OTUs found in all individuals in primary forest were also observed in the secondary forest population. However, there was an effect of habitat on gut microbial community composition (Table 3; Fig. 4c). This effect was not the result of shifts in any particular gut microbes.

A. palliata in semi-deciduous forest: Like mantled howler monkeys at La Suerte, mantled howler monkeys in semi-deciduous forest at Ometepe exhibited no shifts in gut microbial diversity across habitats (Table 2; Fig. 3). This pattern was the same across seasons, and 83 % of the OTUs found in all individuals in primary forest were also observed in the secondary forest population. Gut microbial community composition, however, differed across primary and secondary forest (Table 3; Fig. 4d). As at La Suerte, these changes were not driven by any specific microbial taxa.

Across seasons, there were no significant changes in microbial diversity in either habitat (Table 2; Fig. 3), but gut microbial community composition shifted with season in both habitats (Table 3; Fig. 4d). As in the black howler monkeys, the effect of season was larger in the primary forest than the secondary forest. These differences were not driven by changes in any single microbial taxon in the primary forest. In the secondary forest, there was a higher relative abundance of Verrucomicrobia, Enterobacteraceae, and *Bifidobacterium* and a lower relative abundance of Firmicutes and Lachnospiraceae during the high fruit intake season (Table S6).

Discussion

In this study we investigated patterns in the gut microbiota of the *Alouatta* genus at a variety of scales: taxonomic, ecosystemic, and local. We hypothesized that *A. pigra* and *A. palliata* would have similar gut microbiota overall, but that *A. pigra* would exhibit more marked gut microbiota responses to changes in forest type. We also hypothesized that gut microbiota diversity and composition would differ in response to seasonal shifts in diet
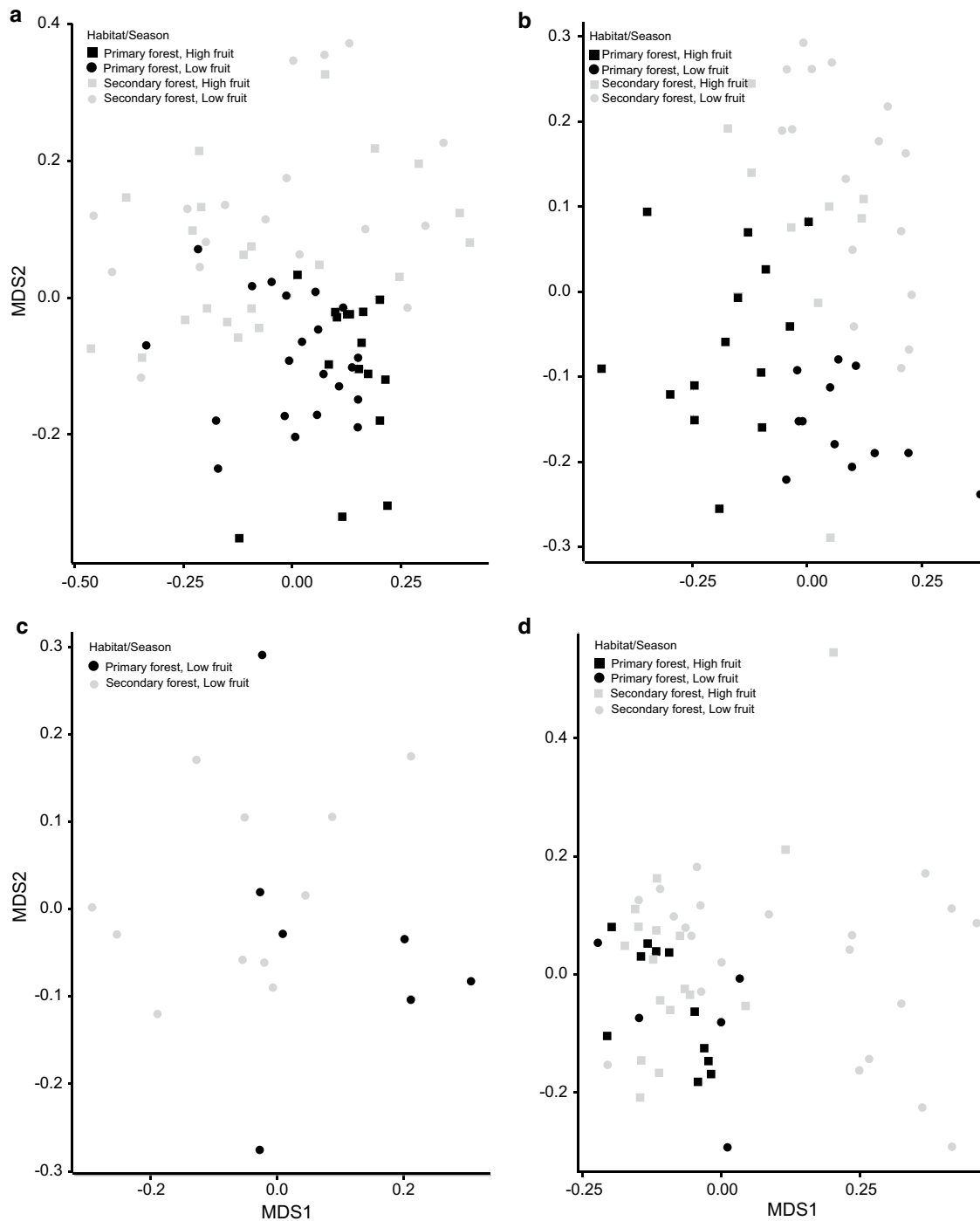
Fig. 4 NMDS plot demonstrating clustering of **a** *A. pigra* rainforest gut microbiomes, **b** *A. pigra* semi-deciduous forest microbiomes, **c** *A. palliata* rainforest microbiomes, and **d** *A. palliata* semi-deciduous forest microbiomes by habitat type and by seasonal diet composition. Each point represents the gut microbiota of a single howler monkey.

Data were rarefied to 5018 reads per sample. The plot was generated using Bray Curtis distances. The effects of both habitat and season are significant ($p < 0.05$) for each population as determined by PER-MANOVA

as well as habitat disturbance for both *A. pigra* and *A. palliata* regardless of forest type, with variation in the size and types of differences observed between species and between forest types. Our data provide support for

most of these hypotheses (Table 4). They also indicate that the effect of host taxonomy on the gut microbiota of *Alouatta* is stronger than the effect of host forest type, which is stronger than the effect of habitat disturbance or

Table 4 Summary of main findings

Effect	Diversity	Composition
Host species	No effect	*A. pigra*: Reduced Lentisphaerae, Euryarchaeota, Victivallaceae, *Roseburia, Bifidobacterium*; elevated Firmicutes, Actinobacteria, Erysipelotrichaceae, Ruminococcaceae, *Dialister, Faecalibacterium, Clostridium*
Forest type		
A. pigra	Reduced in semi-deciduous	Semi-deciduous: Reduced Alphaproteobacteria, *Lachnospira, Clostridium, Coprococcus*; elevated Enterobacteraceae, *Bacteroides, Bifidobacterium, Megasphaera*
A. palliata	No effect	Semi-deciduous: Reduced *Roseburia*; elevated Euryarchaeota, Bacteroidetes, Lentisphaerae, Rickenellaceae, *Bacteroides, Bifidobacterium, Escherichia, Lachnospira, Dialister*
Disturbance		
A. pigra (PNP)		
High fruit	Reduced in secondary forest	Secondary forest: Reduced Firmicutes, Ruminococcaceae; elevated Verrucomicrobia
Low fruit	Reduced in secondary forest	Secondary forest: Elevated Helicobacteraceae
A. pigra (ET)		
High fruit	No effect	Effect but no individual microbes responsible
Low fruit	Elevated in secondary forest	Secondary forest: Reduced *Tanerella*
A. palliata (LS)		
High fruit	No effect	Effect but no individual microbes responsible
Low fruit	na	na
A. palliata (OM)		
High fruit	No effect	Effect but no individual microbes responsible
Low fruit	No effect	Effect but no individual microbes responsible
Season		
A. pigra (PNP)		
Primary forest	No effect	Low fruit: Reduced Ruminococcaceae, Erysipeltrichaceae
Secondary forest	No effect	No effect
A. pigra (ET)		
Primary forest	No effect	Low fruit: Reduced Actinobacteria
Secondary forest	No effect	Effect but no individual microbes responsible
A. palliata (LS)		
Primary forest	na	na
Secondary forest	na	na
A. palliata (OM)		
Primary forest	No effect	Effect but no individual microbes responsible
Secondary forest	No effect	Low fruit: Reduced Verrucomicrobia, Enterobacteraceae, *Bifidobacterium*; elevated Firmicutes, Lachnospiraceae

seasonality. Nevertheless, within host species, gut microbial diversity remains relatively stable, while gut microbiota composition responds to changes in host environment and presumably diet (Table 4). Exceptions to this pattern appear to be associated with host endemism and anthropogenic habitat disturbance. Taken as a whole, these results have important implications for Mesoamerican howler monkey ecology, evolution, and conservation that may be relevant for other Neotropical primates and mammals.

The impact of host species on the gut microbiota of *A. pigra* and *A. palliata*

Although *A. palliata* and *A. pigra* are closely related howler monkey species (Cortes-Ortiz et al. 2003) that occupy similar habitats in Mesoamerica and consume similar diets (Di Fiore et al. 2011), we detected differences in the gut microbial composition of each species. In fact, as shown in studies of the gut microbiota of other primates (McCord et al. 2013; Moeller et al. 2013, 2014), host taxonomy had

the largest effect on the gut microbiota compared to the other factors investigated in this study. Only about half of the OTUs detected in the population of *A. pigra* were also detected in the population of *A. palliata.* These data suggest that small differences in host genetics may play a role in differentiating the gut microbiota across species. Additionally, traits that vary between host species such as group size (*A. pigra*: 6–9 individuals vs. *A. palliata:* 10–21 individuals), body size (*A. pigra* males: 7.6 kg vs. *A. palliata* males: 5.8 kg), interbirth intervals (*A. pigra*: 15.5 mos vs. *A. palliata:* 19.9–22.5 mos) (Chapman and Pavelka 2005; Di Fiore et al. 2011; Dias et al. 2011; Fedigan and Rose 1995; Kelaita et al. 2011) or other subtle physiological/anatomical differences (Table S7) may influence host physiology and host-gut microbe dynamics.

While additional research and more extensive sampling of each host species across its range are necessary to understand the mechanisms driving the host taxonomic differences we observed in this study, these data suggest that host taxonomy, and therefore host physiology and behavior, have a strong effect on the *Alouatta* gut microbiota, even at the host species level. Historically, defining species-specific traits has been a challenge within the *Alouatta* genus, since genetic, morphological, and behavioral characteristics suggest distinct phylogenetic relationships among species (de Oliveira et al. 2002; Rosenberger and Strier 1989; Villalobos et al. 2004). The patterns we detected in this study indicate that the gut microbiota may be used as an additional trait for evaluating *Alouatta* phylogenetic relationships. As microbial data from additional species are collected, a careful examination of correlations between species-specific traits is likely to be an important contribution to the current debate.

Furthermore, the connection between host physiology and the gut microbiota may improve our understanding of the general ecological flexibility of the *Alouatta* genus and its far-reaching distribution (Garber et al. 2015) despite more specialized diets, smaller home ranges and reduced cognitive abilities compared to other widespread primates such as *Papio* or *Macaca* (Campbell et al. 2011). Determining whether all widespread mammalian taxa share gut microbial taxa or whether host-gut microbe relationships are distinct in those with more specialized diet will provide insight into the role of the gut microbiota in determining host ecology and behavior.

Impact of forest type on the gut microbiota of *A. pigra* and *A. palliata*

Our results indicated that the type of forest a host population inhabits impacts the gut microbiota for both *A. pigra* and *A. palliata*. These data imply that the gut microbiota is plastic within the constraints of host physiology and behavior. This relationship has been observed in other systems as well (David et al. 2014; Delsuc et al. 2014; Kobayashi et al. 2006; Moeller et al. 2013; Williams et al. 2012) and is likely related to the ability of the gut microbiota to adapt to temporal shifts in host diet and physiology.

We observed some common patterns in the gut microbial responses of *A. pigra* and *A. palliata* to forest type (Table 4). These results suggest that regardless of host-specific or site-specific characteristics of the gut microbiota, the close phylogenetic relationship and similar diet of the howler monkeys sampled (Cortes-Ortiz et al. 2003; Di Fiore et al. 2011) lead to a gut microbiota that reacts to its host and the host environment similarly. There appear to be some microbial shifts that are necessary for both howler species to persist successfully in semi-deciduous forests. This may not be surprising given that many mammals must adjust to distinct patterns in forest composition and structure in semi-deciduous forests (van Schaik et al. 1993). Comparisons of howler monkeys and sympatric mammals will allow us to understand how generalizable these changes in the gut microbiota are.

Despite the similarities described, we did detect differences in the effect of forest type on the gut microbiota of the two howler species (Table 4). While the relative abundances of microbes differed more markedly for *A. palliata* than *A. pigra* in semi-deciduous forest compared to evergreen rainforest, *A. pigra* exhibited a significant loss of microbial diversity in the semi-deciduous forest, and the proportion of OTUs detected in both habitats was much lower for *A. pigra* than *A. palliata*. These patterns suggest that, in terms of the gut microbiota, *A. palliata* is able to occupy distinct forest types with fewer consequences than *A. pigra.* This result may be related to *A. palliata*'s widespread distribution and potentially superior dispersal abilities (Cuaron et al. 2008).

Impact of season and habitat disturbance on the gut microbiota of *A. pigra* and *A. palliata*

At the local scale, we found variation in the gut microbiota in response to both habitat disturbance and season. However, these effects were smaller than both the effects of host taxonomy and host forest type. There was also variation in the magnitude of these effects in response to host species and forest type. We discuss each of these factors independently below.

Habitat overall

Despite seasonal variation in the composition of the gut microbiota of all howler monkeys (except black howlers inhabiting secondary evergreen rainforest), we detected significant differences in the gut microbiota of howler

monkeys in each population across habitats during both periods of higher and lower fruit intake (Table 4). Although the microbial taxa driving these patterns differed according to howler monkey population, our data indicate that habitat disturbance is likely to affect the gut microbiota of all Mesoamerican howler monkey populations.

Previous research suggests that differences in diet across habitat are associated with differences in the gut microbiota (Amato et al. 2013), and it is likely that differences in the diversity and composition of the plant communities among primary and secondary forests lead to permanent differences in diet across habitats regardless of seasonal patterns in plant phenology (Benitez-Malvido and Martinez-Ramos 2003; Fahrig 2003; Laurance et al. 2000; Malcolm 1994; Rey-Benayas et al. 2007). For example, it has been suggested that large fig trees such as *Ficus* spp. are an important food resource for many species of primates (Conklin and Wrangham 1994; Estrada and Coates-Estrada 1984; Felton et al. 2008; O'Brien et al. 1998; Parr et al. 2011), and few of the howler monkeys in secondary forest were observed to have access to these tree species. It is possible that the lack of key food resources such as these affects howler monkey nutrition in disturbed forest both directly and indirectly via impacts on the gut microbiota. Because a number of mammals have been observed to utilize *Ficus* trees heavily in the Neotropics (Estrada and Coates-Estrada 1985), these patterns should be investigated in the gut microbiota of other host taxa inhabiting disturbed habitats as well.

Alternatively, habitat disturbance has been shown to have a negative impact on the diversity of a variety of organisms (Fahrig 2003; Fenoglio et al. 2012; Turner 1996). For example, the diversity of gastrointestinal parasites associated with howler monkeys in secondary forest in El Tormento, Mexico is lower than in primary forest (Martinez-Mota 2015). This pattern has also been found in parasites associated with birds, rodents, and marsupials (Bush et al. 2013; Chasar et al. 2009; Puttker et al. 2008). Therefore, it is possible that reductions in *Alouatta* gut microbial diversity in response to habitat disturbance reflect a general pattern of habitat degradation and reduced diversity in the ecological pool of microbial taxa available to colonize hosts.

Additional data are necessary to determine if the gut microbial shifts we detected in secondary forests are detrimental to host fitness. However, for the black howler monkeys occupying the evergreen rainforest, depending on the season, Verrucomicrobia and Helicobacteraceae were detected in higher abundances in the secondary forest than the primary forest, while *Faecalibacterium* (and Firmicutes and Ruminococcaceae more generally) was detected in lower abundances (Table S4). These results agree with previously reported patterns in black howler monkey gut microbiota across habitats in that they indicate a potential reduction in fermentation of structural carbohydrates and dysbiosis in the guts of black howler monkeys in fragmented, secondary forest (Amato et al. 2013). For example, Helicobacteraceae contain several pathogenic genera and are often associated with disease in humans (Chichlowski et al. 2008; Fox et al. 2001; Vandamme and De Ley 1991), while *Faecalibacterium* breaks down fiber to produce energy that can be absorbed by hosts and may reduce inflammation in the gut (Miguel et al. 2013; Sokol et al. 2008). *Akkermansia*, a genus from the Verrucomicrobia phylum, is a mucin-degrader (Derrien et al. 2004) that may be associated with host nutritional stress, and relative abundances of Verrucomicrobia tend to increase in relative abundance in captive leaf-eating primates that have severely altered diets (Amato et al. 2013). Therefore, the shifts in gut microbial community composition that we detected in howler monkeys in secondary forest may be detrimental. No published study has addressed the impact of habitat disturbance on the gut microbiota of another mammal, but we would expect to observe similar patterns, especially in those taxa with relatively specialized diets.

Finally, contrary to predictions, the effect of habitat on the gut microbiota did not vary with season in any of the howler populations we sampled (Table 4). These data suggest that the impact of habitat degradation on the howler monkey gut microbiota is independent of seasonal changes in diet and likely long-lasting. The only exception was represented by the black howler monkeys inhabiting the semideciduous forest. For this population, habitat differences were stronger during the period of lower fruit consumption. It is unclear what factors may have caused this difference, but it is likely to be a seasonal change in diet or host physiology particular to this site.

Season

Seasonality had no effect on gut microbial diversity for howler monkeys in any habitat (Table 4). However, we did observe an effect of season on gut microbiota composition (Table 4). Because we sampled the same known individuals across seasons at both black howler monkey primary forest sites, it is unlikely that these patterns are the result of randomly sampling different individuals during each season. In addition, previous studies of black howler monkeys demonstrate that both intra-individual differences as well as inter-individual differences in gut microbiome composition during a given season are smaller than season differences (Amato et al. 2015). Therefore, our data imply that seasonal differences in the gut microbiota are driven by shifts in howler diet. Specifically, our data suggest that dietary diversity may be similar for howler monkeys in a given habitat across seasons but that the plant parts and plant

species being consumed change. Studies of howler monkey feeding ecology provide some support for this explanation (Dias and Rangel-Negrin 2015).

As predicted, the effects of seasonality on the howler monkey gut microbiota differed with habitat (Table 4). We observed strong seasonal shifts in the composition of the gut microbiota of howler monkeys in the primary forest while the gut microbiota of howler monkeys in the secondary forest remained more stable over time. This pattern was especially apparent in black howler monkeys inhabiting evergreen rainforest. Because previous research has associated seasonal changes in the composition of the howler monkey gut microbiota with seasonal changes in the howler monkey diet (Amato et al. 2015), it is likely that the disparity we detected across habitats is a direct result of differences in food availability and diet. Assuming the diversity of tree species available for consumption in secondary forest is reduced (Benitez-Malvido and Martinez-Ramos 2003; Fahrig 2003; Rey-Benayas et al. 2007), it is possible that seasonal variation in the availability of plant parts or plant species is also reduced, resulting in less temporal variation in the howler monkey diet in secondary forest. Studies of several howler monkey species provide evidence for this type of mechanism (Chiarello 1994; Palma et al. 2011; Pozo-Montuy and Serio-Silva 2006). A study of *A. seniculus* in an Andean forest fragment reports little variation in the time spent feeding on leaves from month to month (Palma et al. 2011), and a study of *A. pigra* in a forest fragment in Balancan, Mexico shows variation in the plant species but not the plant parts consumed across seasons (Pozo-Montuy and Serio-Silva 2006). Likewise, *A. guariba* in fragmented forest in Brazil exhibits a relatively consistent diet across months in terms of plant parts and plant species (Chiarello 1994).

However, it is important to note that plant species diversity and phenology differ in response to habitat disturbance depending on factors such as fragment size, distance to other fragments, and climate (Arroyo-Rodriguez and Dias 2009; Bicca-Marques 2003), and as a result, howler monkey diets may not always be less seasonally variable in secondary forest than primary forest. For example, studies of *A. palliata* in Los Tuxtlas, Mexico indicate marked seasonal variation in diet, which is exaggerated in smaller forest fragments with fewer fruiting trees (Dunn et al. 2010; Estrada et al. 1999). Therefore, while our microbial results suggest there may be reduced seasonal dietary variation for the howler monkeys sampled in secondary forest, our ability to detect seasonal shifts in the gut microbiota of some fragment-dwelling howler monkeys suggests that variation in the characteristics of secondary forest at different sites are important. Combining microbial analyses with quantitative diet data for howler monkeys, and other mammals, in

these habitats will be an important next step for verifying the relationship between diet and the gut microbiota.

The patterns in gut microbiota composition that we detected across seasons and habitats for the black howler monkeys in the evergreen rainforest also suggests that fruit may be a critical component of the howler monkey diet. We observed reductions in the relative abundance of Ruminococcaceae in black howler monkeys in primary forest with seasonally reduced fruit intake, as well as in howler monkeys in secondary forest with potentially chronic reduced fruit intake. Although detailed dietary data are necessary to verify this relationship, the implications for howler monkey ecology and conservation are strong, since these changes in the gut microbiota have the potential to reduce microbial energy-production and increase health risks (Carbonero et al. 2012; Donohoe et al. 2011; Medani et al. 2011). In this sense, fruit may not only represent an important source of energy and nutrients (Norconk et al. 2009), but also may promote a healthy gut microbiota with additional impacts on host nutrition and health. While the gut microbiota may compensate for seasonal reductions in fruit intake by producing more energy-rich short-chain fatty acids for hosts (Amato et al. 2015), chronic reductions in fruit intake may lead to dysbiosis. Other mammals such as coatis also rely on fruit seasonally (Alves-Costa et al. 2004) and are likely to experience similar consequences. However, data describing the gut microbiota and dietary patterns are necessary for these taxa.

Variation by host species and forest type

Despite similarities in how the gut microbiota of each howler monkey population reacted to host habitat and season, we observed two notable differences. To begin with, host habitat disturbance appears to affect gut microbial community composition in the mantled howler monkey less than it affects gut microbial composition in the black howler monkey. The effect size associated with habitat differences is somewhat smaller for the mantled howler monkey than the black howler monkey, and qualitatively, clustering patterns on ordination plots are more dispersed for the mantled howler monkey.

Overall, these data, like our data for forest type, suggest that the gut microbiota of the cosmopolitan mantled howler monkey is more resistant to differences in diet across habitats than that of the endemic black howler monkey. While many of the microbial genera observed in each howler species are the same, it is possible that, at a finer taxonomic level (i.e., operational taxonomic unit), the mantled howler monkey has co-evolved with gut microbes that have the ability to utilize a wider variety of substrates for metabolism and are therefore less strongly impacted by changes in host diet. Whether mantled howler monkey expansion

across a wide geographical range led to the evolution of a more resistant gut microbiota or a resistant gut microbiota allowed the mantled howler monkey to expand across a wide geographical range will be difficult to determine, but this relationship should be investigated in more endemic species.

Secondly, while the effect of habitat disturbance was similar between forest types, season appears to affect gut microbiota composition more in the semi-deciduous forest than the rainforest. The effect size of season is greater in the semi-deciduous forest. Semi-deciduous forests tend to have increased seasonality compared to rainforests both in terms of climate and plant phenology (Murphy 1986), which could lead to more marked seasonal changes in the gut microbiota. These dramatic climatic patterns can be observed in the data generated from the semi-deciduous forests where we collected samples (Table S1).

Conclusions

This study identifies factors that impact the gut microbiota of Mesoamerican howler monkeys. This information may be critical for understanding ecological and evolutionary questions, along with determining conservation challenges facing each species. For example, if the endemic black howler monkey has a microbiome that is more sensitive to environmental perturbation (and associated changes in diet) compared to the widely distributed mantled howler monkey, it may also face more challenges to nutrition and health across time and space and ultimately be more ecologically limited than the mantled howler monkey. Such a mechanism would provide insight regarding the distinct distributions of two ostensibly similar howler species.

These mechanisms may also provide insight into the ecology of other species of howler monkeys, primates, and mammals. Understanding the role of the gut microbiota in allowing mammalian hosts to meet nutritional demands across seasons and habitats is critical to our understanding of the mechanisms driving dietary plasticity, and the evolutionary trajectory of a wide range of mammals, including humans, has been heavily influenced by dietary plasticity (Teaford and Ungar 2000). However, due to the current paucity of data describing the gut microbiota of wild mammals, further studies of these host-microbe interactions in natural, selective environments will be crucial to determine if the observed patterns can be generalized.

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