

## RESEARCH ARTICLE

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# Influence of fruit and invertebrate consumption on the gut microbiota of wild white-faced capuchins (*Cebus capucinus*)

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## Abstract

**Objectives:** Invertebrate consumption is thought to be an integral part of early hominin diets, and many modern human populations regularly consume insects and other arthropods. This study examines the response of gut microbial community structure and function to changes in diet in wild white-faced capuchins (*Cebus capucinus*), a primate that incorporates a large proportion of invertebrates in its diet. The goal of the study is to better understand the role of both fruit and invertebrate prey consumption on shaping primate gut microbiomes.

**Materials and methods:** Fecal samples ( $n = 169$ ) and dietary data were collected over 12 months. The V3-V5 region of microbial 16S rRNA genes was amplified and sequenced. The IM-TORNADO pipeline was used to analyze sequences.

**Results:** White-faced capuchin gut bacterial communities were characterized primarily by Firmicutes (41.6%) and Proteobacteria (39.2%). There was a significant relationship between the invertebrate diet composition of individual capuchins and their gut microbiome composition. However, there was no relationship between the fruit diet composition of individual capuchins and their gut microbiome composition, even when examining multiple timescales.

**Discussion:** The results of our study indicate that there is a stronger relationship between gut microbial community structure and invertebrate diet composition than between gut microbial community structure and fruit consumption. As invertebrates and other animal prey play an important role in the diet of many primates, these results give important insight into the role of faunivory in shaping the evolution of host-microbe interactions in primates.

## KEYWORDS

gut microbiome, human evolution, insectivory, omnivory, white-faced capuchins

## 1 | INTRODUCTION

The gut microbiome plays an integral role in animal nutrition, digesting otherwise unavailable resources, providing substrates for nutrient metabolism in the gut, and increasing nutrient uptake and utilization in the gut (Cummings & Macfarlane, 1997; Hooper, Midtvedt, & Gordon, 2002). Many studies have shown that gut microbial community composition is related to differences in both the macronutrient composition of the diet and the major types of food consumed (e.g., fruit vs. leaves vs. animal prey), and that different gut microbial taxa can increase or decrease the amount of energy a host absorbs from a particular food (Claesson et al., 2012; De Filippo et al., 2010; Hildebrandt et al., 2009;

Nelson, Rogers, Carlini, & Brown, 2013; Wu et al., 2011). Despite a relatively robust understanding of how experimentally manipulated diet influences the gut microbiome in humans and some nonhuman primates, with individuals who consume more plant-based diets having increased relative abundances of Bacteroidetes and Actinobacteria and decreased relative abundance of Firmicutes and Proteobacteria (Duncan et al., 2007; Faith, McNulty, Rey, & Gordon, 2011; Kisidayová et al., 2009; Ley, Turnbaugh, Klein, & Gordon, 2006; Turnbaugh et al., 2009), the effect that natural variation in diet has on gut microbial community composition of wild populations is not well studied (Amato, 2013a; Amato et al., 2013, 2014, 2015a; Gomez, 2014; Gomez et al., 2015; Sun et al., 2016).

Dietary choices are driven by the spatial and temporal availability of particular foods types and plant species, social dominance and access to resources also sought by other group members, and individual nutrient requirements. As a result, an individual's diet can undergo major shifts both in which types of food items are eaten (e.g., fruits, leaves, arthropods), and in which taxa of those food types are eaten over the course of days, weeks, and months (Chapman, Chapman, Rode, Hauck, & McDowell, 2003). In addition, given that fruits and insects vary in the amount of lipids, protein, sugars, complex polysaccharides, and chitin they contain, both between and within taxa, nutrient mixing is required for hosts to obtain a nutritionally adequate diet (Bell, 1990; Chapman et al., 2003; Chivers, 1998; O'Driscoll Worman & Chapman, 2005; Raubenheimer & Rothman, 2013; Rothman, Dierenfeld, Hintz, & Pell, 2008). Recent studies, both in primates and other mammals, indicate that the gut microbiota buffers these dietary changes by increasing the bacterial production of short-chain fatty acids and other molecules that then can be used by the host for energy during periods of nutritional shortfalls (Amato, 2013b; Amato et al., 2015a; Gomez et al., 2015; Schnorr et al., 2014; Smits et al., 2017; Sun et al., 2016; Tremaroli & Bäckhed, 2012). Despite the fact that many primates species include invertebrates as an important dietary component, the majority of studies of the gut microbiota have focused on humans or species of wild primates that consume a primarily plant-based diet (Amato, 2013b). In order to better understand the evolution of host-microbe interactions across a broad range of taxa, a more comprehensive understanding of the gut microbial communities of primates with a diet that includes consumption of animal proteins and lipids is needed (Amato, 2013b).

Insectivory is essential component of the diet of both larger (humans, chimpanzees, gibbons, woolly monkeys, patas monkeys, mangabays) and smaller-bodied (capuchins, tamarins, squirrel monkeys, titi monkeys, galagos, lorises, mouse lemurs) primates (Bogart & Pruett, 2011; Gursky, 2000; Isbell, 1998; McGrew, 2014; Nickle & Heymann, 1996; O'Malley & McGrew, 2014; Raubenheimer & Rothman, 2013; Raubenheimer, Rothman, Pontzer, & Simpson, 2014). There is evidence that early hominins supplemented their diet with arthropods in addition to vertebrate prey (Backwell & D'Errico, 2001; McGrew, 2001, 2014), and regular consumption of insects and other invertebrates in many modern human populations provides a readily digestible source of proteins and lipids necessary for growing and maintaining neural tissue (Aiello & Wheeler, 1995; Gunst, Boinski, & Frigaszy, 2010; Raubenheimer & Rothman, 2013). Additionally, invertebrates eaten by humans and other primates may serve as a source of important vitamins and fatty acids that are unavailable from plant-based foods (Rothman, Raubenheimer, Bryer, Takahashi, & Gilbert, 2014). Given the important link that the gut microbiota creates between host diet and physiology, knowledge of the effects that temporal changes in diet, including switching from a fruit-rich to an invertebrate-rich consumption pattern, offers insight into the role of the gut microbiome in meeting the energetic needs of primates.

In this study, we examine how changes in fruit and invertebrate consumption over a 12-month period influence both gut microbial community structure and function in wild white-faced capuchins (*Cebus*

*capucinus*). White-faced capuchins are an excellent model for studying how variations in diet influence the gut microbiome. Based on several long-term studies, on average white-faced capuchins devote 44.4% of feeding and foraging time to fruit, 3.4% to flowers, 2.7% to leaves, 9.5% to nuts and seeds, 38.0% to invertebrate prey, and 1.2% to vertebrate prey (Baker, 1998; Buckley, 1983; McKinney, 2010; Rose, 1994; Urbani, 2009). Given that white-faced capuchins exploit a more insectivorous diet than expected for their body mass (2.61–3.97 kg) (Ford & Davis, 1992; Kay & Simons, 1980), interindividual, season, and habitat differences in invertebrate prey choice play an important role in shaping white-faced capuchin nutritional strategies (Melin, Young, Mosdossy, & Fedigan, 2014; Mosdossy, Melin, & Fedigan, 2015). Capuchins also are unusual among non-ape primates in that they are characterized by a large brain volume to body size ratio (72.95 cc/3.29 kg,  $n = 23$ ; Isler et al. 2008) and a set of delayed life history traits, including a long life span and extended juvenile period (Fedigan & Rose, 1995; Jack & Fedigan, 2004; Muniz et al., 2010; Perry, Manson, Muniz, Gros-Louis, & Vigilant, 2008). This makes them an excellent model for understanding interactions between human and ape diets, physiology, and gut microbiota (Fragaszy & Bard, 1997; Ross, 1991).

We combine observational data on foraging behavior and diet with DNA metabarcoding of invertebrates in the diet and microbial analyses of fecal samples collected during a 12-month study of wild white-faced capuchins to address four predictions. As studies of captive and wild primates indicate that the gut microbial phylum Bacteroidetes is associated with high-fiber, low-protein, low-fat diets and Firmicutes and Proteobacteria are associated with low-fiber, high-protein, high-fat diets (Amato et al., 2015a; De Filippo et al., 2010; Gomez et al., 2015; Turnbaugh et al., 2009), and gut microbial community structure rapidly responds to changes in macronutrient consumption (David et al., 2014), we expect that: (1) As the percentage of feeding and foraging time spent on fruits increases (using percentage of feeding and foraging time as a proxy for amount consumed), the prevalence of Firmicutes and Proteobacteria taxa will decrease, while the prevalence of Bacteroidetes taxa will increase. (2) As the percentage of feeding and foraging time spent on arthropods increases, the prevalence of Firmicutes and Proteobacteria taxa will increase and the prevalence of Bacteroidetes taxa will decrease. (3) As changes in gut microbial community composition in response to diet are related to changes in gut microbial function, fruit consumption will be positively correlated with the number of operational taxonomic units (OTUs) related to glycan and carbohydrate metabolism, which function in breaking down complex plant polysaccharides, while invertebrate consumption will be positively correlated with the number of OTUs related to amino acid and lipid metabolism due to their role in digesting animal prey.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site and population

Research was conducted from January 2013 through January 2014 at La Suerte Biological Field Station (LSBFS) in northeastern Costa Rica

(10.445N, 83.784W). LSBFS contains approximately 300 ha of aseasonal wet, lowland tropical forest, including 170 ha of advanced secondary forest and 130 ha of early secondary growth and regenerating pasture (Garber & Paciulli, 1997). Four groups of white-faced capuchins are present, one of which is habituated. The group of habituated, individually recognizable white-faced capuchins that were used for the study contained 21–22 individuals (four adult males, five adult females, eight to nine juvenile males, two to three juvenile females, and zero to two infants) during the study period. All data collection methods were approved by the University of Illinois IACUC, LSBFS, MINAET, SINAC, and CONAGEBIO. Appropriate import permits were obtained from the CDC and USDA.

## 2.2 | Observational data

One-hour instantaneous focal animal samples (2-min interval) of individually recognizable adult males, adult females, juvenile males, and juvenile females were conducted five days a week (20 days/month) for 12 months. In total, 841 hr of behavioral data and 575 hr of dietary data (portion of behavioral data spent feeding and foraging) during 1341 hr of observation on 237 days were collected. Information on activity budget (feeding, foraging, traveling, resting, social, other), diet (ripe fruit, unripe fruit, invertebrates, vertebrates, flowers, leaves, seeds, other), and social interactions (including affiliative, aggressive, and submissive behaviors) was collected. When possible, food items consumed were identified to species (for fruit) or order (for invertebrates). Not all juveniles were individually recognizable during the early months of the study; therefore, some juvenile focal follows were of individuals of unknown identity and sex. While data from juveniles of unknown identity were used in group-level analyses of diet and gut microbial composition where diet was calculated using foraging records for all individuals, these data were excluded from other analyses. The group's dietary composition was calculated as a percentage of total feeding and foraging time exploiting fruits or invertebrates across a two-week period. In this study group, there are no age- or sex-based differences in the percentage of total feeding and foraging time spent on fruit or invertebrates within the same two-week period (Mallott, Garber, & Malhi, 2017). Based on behavioral observations, plant-based diet at the species level was calculated over a two-week period as the minutes per hour spent consuming a specific species of fruit (calculated as

$$\left( \frac{\text{number of two-minute intervals individual was recorded consuming a fruit species}}{\text{number of two-minute intervals individual was observed}} * 2 \right)$$

using foraging records for all individuals. Given that fruits and insects accounted for at least 92.7% of feeding and foraging time across any two-week period, we choose to not include other food categories in assessing the impact of diet on the capuchin gut microbiome. We assumed that time spent feeding and foraging on a particular food type or fruit species represents a reasonable estimate of the amount of that food type or item ingested.

## 2.3 | Fecal samples

During the observational study, 225 fecal samples were collected from individually recognizable adult females ( $n = 64$ ), adult males ( $n = 88$ ),

juvenile females ( $n = 15$ ), and juvenile males ( $n = 47$ ), as well as samples from juveniles of unknown sex ( $n = 11$ ). Fecal sample collection occurred throughout the day, but collection was biased toward the morning (145 morning samples vs. 80 afternoon samples; morning = 500–1,100 and afternoon = 1,100–1,700). While we attempted to collect one sample from each of 21–22 individuals within the same two-week period, on average we collected a total of 9 fecal samples per two-week period (range = 4–13). Samples were collected in a manner to minimize contamination from exogenous sources, with care taken to avoid collecting material in contact with the forest floor or leaf litter. Samples were stored in 90% ethanol at  $-20^{\circ}\text{C}$  prior to being shipped to the Molecular Anthropology Laboratory at the University of Illinois at Urbana-Champaign.

## 2.4 | DNA extraction

DNA was extracted from the fecal samples using a QIAamp DNA Stool Mini Kit following the provided protocol for "Isolation of DNA from Stool for Human DNA Analysis." Samples were homogenized using a vortexer prior to extraction. Modifications used by Pickett et al. (2012) were followed (see Mallott, Malhi, & Garber, 2015 for detailed methods).

## 2.5 | PCR amplification of arthropods

DNA metabarcoding of the fecal samples was used in order to determine the invertebrates consumed by white-faced capuchins. Two overlapping  $\sim 300$  bp fragments within the *COI* mitochondrial gene were amplified from 56 of the 225 samples with universal arthropod primers using a two-step polymerase chain reaction (PCR amplification, following published protocols; Mallott et al., 2015, 2017). The remaining 169 samples were amplified by PCR on a Fluidigm Access Array at the Roy J. Carver Biotechnology Center at UIUC (see Mallott et al., 2017 for detailed methods) using the above primer pair for invertebrate *COI* mitochondrial genes. All samples were quantitated on a Qubit, were run on a Fragment Analyzer (Advanced Analytics, Ames, IA), and amplicon regions and expected sizes were confirmed. Samples were then pooled based on product concentration, and were gel purified from a 2% agarose E-gel using a Qiagen gel extraction kit. All individually barcoded samples were sequenced on the Illumina MiSeq V2 platform.

## 2.6 | Arthropod sequence processing and analysis

Raw sequence reads were paired, demultiplexed, and trimmed in QIIME (Caporaso et al., 2010). Reads were de-replicated, clustered at a 1% sequence divergence threshold, and chimeric sequences were detected *denovo* and removed in USEARCH (Edgar, 2010; Edgar, Haas, Clemente, Quince, & Knight, 2011). Sequence reads were clustered into OTUs using a 3% sequence divergence criteria in USEARCH. OTUs were compared to the GenBank nt nucleotide sequence database and the Barcode of Life Database ([www.boldsystems.org](http://www.boldsystems.org)) using BLAST ([ncbi.nlm.nih.gov](http://ncbi.nlm.nih.gov)). OTUs were assigned to taxa in MEGAN5 using the standard settings (Huson, Mitra, & Ruscheweyh, 2011). Invertebrate diet was calculated as the percentage of fecal samples that contained sequences assigned to a

family of invertebrates within a two-week period. Individual invertebrate diet was calculated using the relative abundance of reads assigned to a family of invertebrates within each sample.

## 2.7 | PCR amplification of microbes

The V3-V5 region of microbial 16S rRNA genes (forward primer: 5'-CCTACGGGAGGCAGCAG-3'; reverse primer: 5'-CCGTCAATTCMTT-TRAGT-3') was amplified from 169 samples by PCR on a Fluidigm Access Array at the Roy J. Carver Biotechnology Center at UIUC (see Mallott et al., 2017 for specific methods). Individually barcoded samples were then sequenced on the Illumina MiSeq V2 platform.

## 2.8 | Microbial sequence processing and analysis

The IM-TORNADO pipeline (Sipos et al. 2010, version 2.0.3.1) was used to filter sequences, detect chimeras, trim and dereplicate sequences, and cluster sequences into OTUs at a 3% sequence divergence threshold. OTUs were assigned to taxonomy using the RDP database in IM-TORNADO. Functional predictions for each sample were made using PICRUST (Langille et al., 2013). The average weighted NSTI score across all 169 samples was  $0.066 \pm 0.053$  (range: 0.006–0.471).

## 2.9 | Statistics

Prior to further statistical analyses, we tested for multivariate homogeneity of group dispersions between different two-week periods to

assess the impact of uneven sampling on differences in within-group variance (betadisper function, *vegan* package, r-project.org). There was no significant effect of two-week sampling period on dispersion ( $F_{24,144} = 1.45$ ,  $p = .093$ ). Permutational multivariate analysis of variance (PERMANOVA) was used to examine the influence of the percentage of feeding and foraging time devoted to fruit and the percentage of feeding and foraging time devoted to invertebrates on gut microbial community composition, two-week period, and date collected based on a Bray-Curtis dissimilarity matrix, including individual as a random effect to control for pseudoreplication (Adonis function, *vegan* package, r-project.org). Spearman correlations were used to assess the relationship of percentage of feeding and foraging time devoted to fruit, percentage of feeding and foraging time devoted to invertebrates, minutes per hour spent consuming individual fruit species across a two-week period (10 most frequently consumed species from observational diet), and frequency of occurrence of invertebrate families in fecal samples collected during two-week periods (10 most frequently detected families) on the relative abundance of microbial genera and the relative abundance of OTUs assigned to a particular KEGG pathway.

Nonparametric Mantel tests were used to compare Bray-Curtis dissimilarity matrices of individual diet records and gut microbiome composition. Only samples for which we have >10 min of observed fruit foraging records for the same individual in the same period as the fecal sample was collected were used for comparisons of fruit consumption and gut microbiota. As molecular invertebrate diet were assessed for all fecal samples, invertebrate diet and gut microbiome

**TABLE 1** Fruit species comprising >0.5% of white-faced capuchin fruit feeding and foraging time ( $n = 17254$  feeding and foraging records)

Species	Family	% of fruit diet	% of diet	Months eaten
<i>Psidium guajava</i>	Myrtaceae	19.0	8.6	All
<i>Hampea appendiculata</i>	Malvaceae	8.7	3.9	Jan, Feb, Nov, Dec
<i>Dipteryx panamensis</i>	Fabaceae	7.6	3.4	Jan–Jun
<i>Inga spectabilis</i>	Fabaceae	6.1	2.7	Feb–May, Oct
<i>Urera baccifera</i>	Urticaceae	5.8	2.6	Oct–Dec
<i>Inga thibaudiana</i>	Fabaceae	5.7	2.6	Jan, Mar–May, Jul–Dec
<i>Ficus colubrinae</i>	Moraceae	3.7	1.7	Feb, Mar, May–Aug, Oct–Dec
<i>Ficus trigonata</i>	Moraceae	3.3	1.5	Apr, May, Oct, Nov
<i>Conostegia xalapensis</i>	Melastomataceae	3.1	1.4	Jan, Apr–Jun, Aug, Sep, Nov, Dec
<i>Sapium grandulosum</i>	Euphorbiaceae	2.5	1.1	Jul, Aug, Oct, Nov
<i>Ficus schippii</i>	Moraceae	2.0	0.9	May, Jun, Nov
<i>Inga marginata</i>	Fabaceae	1.9	0.9	Jan, Feb, Jun, Aug, Dec
<i>Ficus tonduzii</i>	Moraceae	1.6	0.7	May, Oct
<i>Nephelium lappaceum</i>	Sapindaceae	1.5	0.7	Jun–Oct
<i>Cestrum megalophyllum</i>	Solanaceae	1.5	0.7	Dec
<i>Dendropanax arboreus</i>	Araliaceae	1.4	0.6	Sep, Oct
<i>Piper spp.</i>	Piperaceae	1.2	0.5	Feb, Apr, Nov, Dec
<i>Miconia affinis</i>	Melastomataceae	1.1	0.5	Apr–Jun, Aug, Sep
<i>Casearia arborea</i>	Salicaceae	1.0	0.5	Jun, Oct, Nov



composition were compared by treating each sample as an individual record. To compare molecular arthropod diet over a two-week period with gut microbial community composition, both invertebrate diet and microbial OTU relative abundances were averaged for each individual across the time-scale of interest. While proportion of fecal samples containing a DNA from a specific taxa of animals or plants is more commonly used as a measure of the rate of consumption of food items, the relative abundance of sequences is a better proxy for observed diet in this population (Mallott, Garber, & Malhi, in review).

### 3 | RESULTS

#### 3.1 | Diet

Over the course of the 12-month study, 47.8% of feeding and foraging time was spent on fruit, 49.8% on invertebrates, 1.2% on flowers, 0.5% on leaves, 0.01% on vertebrates, and 0.6% on other plant foods. All subsequent analyses focused only on the invertebrate and fruit component of the capuchin diet. The group was observed eating 59 species of fruit during the study period, with 19 species being consumed in >0.5% of their fruit feeding and foraging records (Table 1). *Psidium guajava* (8.6% of total time spent feeding and foraging), *Hampea appendiculata* (3.9%), *Dipteryx panamensis* (3.4%), and *Inga spectabilis* (2.7%) dominated the plant component of the diet. With the exception of *P. guajava*, which is a domesticated plant, all other fruit species were highly seasonal in their availability (Table 1). Molecular results indicate that 29 orders and 90 families of arthropods were present in white-faced capuchin fecal samples during the study period, with 23 orders and 50 families being identified in >1% of fecal samples and nine orders and nine families being identified in >5% of fecal samples (Supporting Information 1). Families of invertebrates that were commonly found in the feces of white-faced capuchins included Tephritidae (11.6%), Formicidae (11.1%), Curculionidae (11.1%), Acrididae (10.7%), and Cercopidae (9.8%) (Supporting Information 1). These values are based on the frequency of occurrence of each Order in the fecal samples from the molecular analysis and do not represent the proportion of insects consumed.

#### 3.2 | Gut microbial community structure

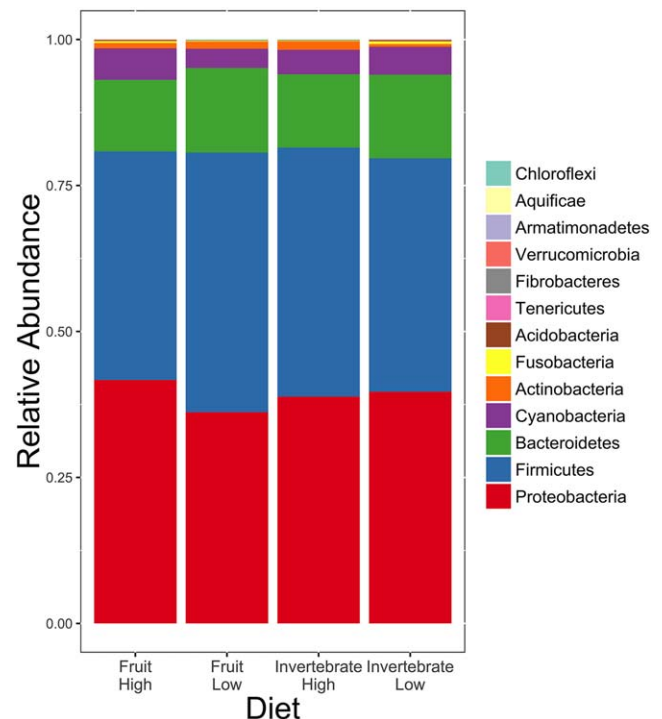
The white-faced capuchin gut microbiome was characterized principally by Firmicutes (41.6%), Proteobacteria (39.2%), and Bacteroidetes (13.3%). The relative abundance of major genera across all samples included *Clostridium XIVa* (Firmicutes) (16.4%), *Streptococcus* (Firmicutes) (13.2%), *Xylanibacter* (Bacteroidetes) (10.4%), *Actinobacillus* (Proteobacteria) (7.5%), *Sutterella* (Proteobacteria) (6.1%), *Megamonas* (Firmicutes) (5.6%), *Streptophyta* (Cyanobacteria) (4.4%), *Escherichia shigella* (Proteobacteria) (4.1%), *Xanthomonas* (Proteobacteria) (2.9%), and *Pseudomonas* (Proteobacteria) (2.4%) (Supporting Information 2).

#### 3.3 | Gut microbiome composition and diet

When examining the relationship between diet and the gut microbiota of the entire study group, there is evidence that the specific taxa of

fruit and invertebrates consumed shape the relative abundance of individual gut microbial genera, but that overall foraging behavior, defined as percentage of feeding and foraging time spent on fruit or invertebrates, does not. There was no significant effect of percentage of feeding and foraging time spent on fruits or invertebrates over a two-week period on gut microbe community composition ( $F_{1,142} = 0.533$ ,  $R^2 = 0.003$ ,  $p = .950$  and  $F_{1,142} = 0.814$ ,  $R^2 = 0.005$ ,  $p = .666$ ) (Figure 1). Minutes per hour spent consuming specific taxa of fruit were significantly correlated (both positively and negatively) with the relative abundance of OTUs assigned to specific genera of gut bacteria (Table 2 and Figure 2). Overall, these results suggest that frequently consumed fruits such as *Psidium guajava*, *Dipteryx panamensis*, and *Hampea appendiculata* are significantly influencing gut microbial community structure.

In the case of invertebrates, the frequency of occurrence in fecal samples over a two-week period of Tephritidae was significantly positively correlated with *Escherichia Shigella* (Proteobacteria) ( $\rho = .403$ ,  $p = .046$ ), and the frequency of occurrence of Cercopidae in fecal samples was significantly negatively correlated with *Clostridium XIVa* (Firmicutes) ( $\rho = -.428$ ,  $p = .0033$ ) (Table 3). Thus, contrary to our predictions, the relative abundance of Firmicutes decreased as the frequency of consumption of one family of arthropods increased (Figure 2). However, the relative abundance of Proteobacteria increased as the frequency of consumption of one family of arthropods increased (Figure 2).



**FIGURE 1** White-faced capuchin gut microbial community composition averaged across all individuals in periods of higher percentage of feeding and foraging time spent on both fruit (48.1–64.1%) and invertebrates (49.2–67.9%) and lower percentage of feeding and foraging time spent on both fruit (30.6–47.6%) and invertebrates (34.3–47.5%)

TABLE 2 Spearman correlation coefficients between the 10 most abundant microbial genera and 5 most frequently consumed fruit species

	Clostridium XIVa 83.6 (Firmicutes)	Streptococcus 100.2 (Firmicutes)	Xylanibacter 79 (Bacteroidetes)	Actinobacillus 46.3 (Proteobacteria)	Sutterella 98.9 (Proteobacteria)	Megamonas 100.2 (Firmicutes)	Streptophyta 100.1 (Cyanobacteria)	Xanthomonas 93.3 (Proteobacteria)	Escherichia Shigella 98.7 (Proteobacteria)	Clostridium XIVa 58.3 (Firmicutes)
<i>Psidium guajava</i>	−0.111	0.102	−0.202**	−0.117	−0.139	−0.058	0.057	−0.149*	0.001	−0.216***
<i>Hampea appendiculata</i>	0.001	−0.059	0.207**	0.106	−0.007	0.064	0.009	−0.103	−0.079	−0.206**
<i>Dipteryx panamensis</i>	−0.101	0.086	−0.029	−0.029	−0.133	−0.032	−0.039	−0.093	0.044	−0.287***
<i>Inga thibaudiana</i>	0.003	−0.050	−0.028	−0.088	0.033	0.155*	−0.051	−0.005	−0.131	0.062
<i>Inga spectabilis</i>	−0.055	0.013	−0.139	−0.106	−0.047	−0.074	−0.003	0.026	0.128	−0.050

Note. Starred results are significant correlations.

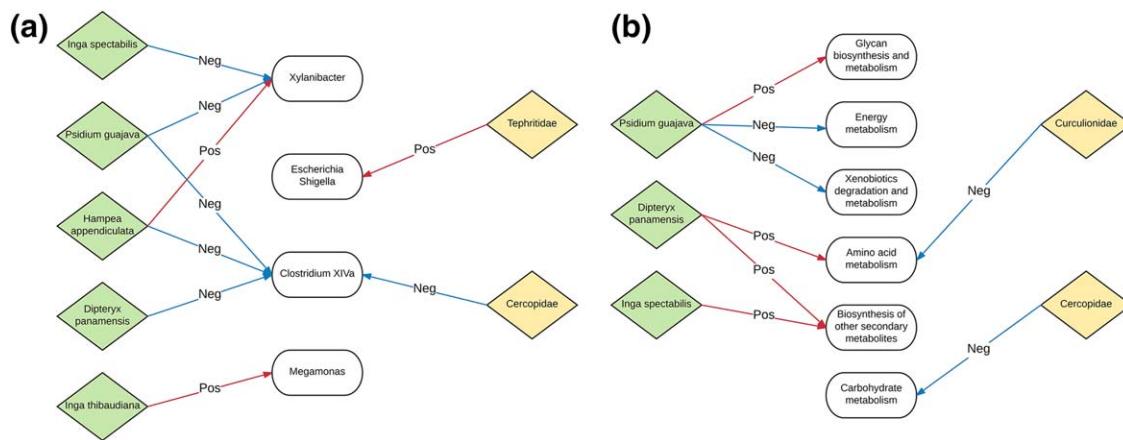
\* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ 

### 3.4 | Individual-level gut microbiome composition and diet variation

When examining the relationship between diet and the gut microbiota at an individual level, it appears that the invertebrate genera consumed shape gut microbial community composition, but that the fruit species consumed do not. Using invertebrate diet and gut microbial community composition from the same fecal samples, there was a significant positive correlation between the relative abundance of invertebrate genera and gut microbial community composition ( $p = .127$ ,  $p = .030$ ), indicating that individuals with more similar invertebrate dietary composition had more similar gut microbiomes. This result should be interpreted cautiously, however, as the relatedness of individuals in this population is not known and, thus, we cannot control for similarities in gut microbial community composition between individuals due to kinship. Additionally, when averaging both the relative abundance of invertebrate genera and gut microbial community composition over a two-week period, there was no relationship between the two ( $p = .032$ ,  $p = .145$ ). When examining the fruit dietary data, there was no correlation between the observed species of fruits consumed and gut microbial community composition for a given individual over a two-week period ( $p = -.0753$ ,  $p = .934$ ), indicating that the species of fruit individuals consumed had no systematic effect on their gut microbial communities.

### 3.5 | Gut microbial community function and diet

The percentages of feeding and foraging time spent on fruit and invertebrates were not significantly correlated with the relative abundance of functional pathways (all  $p > .05$ ). Minutes per hour spent consuming *P. guajava* over a two-week period were positively correlated with the relative abundance of OTUs assigned to glycan biosynthesis and metabolism pathways ( $p = .168$ ,  $p = .029$ ), and negatively correlated with the number of OTUs assigned to energy metabolism pathways ( $p = -.172$ ,  $p = .025$ ) and xenobiotics degradation and metabolism pathways ( $p = -.222$ ,  $p = .004$ ), partially supporting prediction three (Figure 2). Time spent consuming *D. panamensis* was positively correlated with the relative abundance of OTUs assigned to amino acid metabolism pathways ( $p = .156$ ,  $p = .042$ ) and biosynthesis of other secondary metabolites pathways ( $p = 0.188$ ,  $p = .014$ ) (Figure 2). Minutes per hour spent consuming *I. spectabilis* was positively correlated with the relative abundance of OTUs assigned to biosynthesis of other secondary metabolites pathways ( $p = .206$ ,  $p = .007$ ) (Figure 2). The relationship between *D. panamensis* and *I. spectabilis* may indicate that these two Fabaceae species are high in tannins or other secondary metabolites, similar to other species of *Dipteryx* and *Inga* (Marin, Siqueira, & Arruda, 2009). The frequency of occurrence within a two-week period of Cercopidae in fecal samples was significantly negatively correlated with the relative abundance of OTUs assigned to carbohydrate metabolism pathways ( $p = -.447$ ,  $p = .025$ ) (Figure 2). The frequency of occurrence within a two-week period of Curculionidae in fecal samples was significantly negatively correlated with the relative abundance of OTUs assigned to metabolism of other amino acid pathways ( $p = -.447$ ,  $p = .025$ ) (Figure 2), contrary to our predictions that



**FIGURE 2** Significant positive (Pos—red) and negative (Neg—blue) correlations between rate of consumption of fruit (green) or invertebrates (yellow) and relative abundance of microbial genera (a) and predicted function (b)

the consumption of invertebrates would be positively correlated with the relative abundance of amino acid metabolism pathways.

#### 4 | DISCUSSION

This study examined the relationship between diet and the gut microbial community composition and function in a group of wild white-faced capuchins. Specifically, we tested three predictions: feeding and foraging time spent on fruit would be negatively correlated with the relative abundance of Firmicutes and Proteobacteria, but positively correlated with Bacteroidetes; feeding and foraging time spent on invertebrates would be positively correlated with the relative abundance of Firmicutes and Proteobacteria, but negatively correlated with Bacteroidetes; and fruit consumption would be positively correlated with glycan and carbohydrate metabolic pathways, while invertebrate consumption would be positively correlated with amino acid and lipid metabolizing pathways. Based on molecular dietary data, we found that the frequency of consumption of specific invertebrate families and individual invertebrate dietary choices influenced the relative abundance of specific genera of gut bacteria and overall gut microbial community composition. However, changes in time spent feeding and foraging for fruit measured at the group level, and fruit diet composition measured at the individual level, defined as minutes per hour an individual spent eating a fruit species in a given time period, did not correspond to changes in overall gut microbial community structure. We also did not find an effect of the percentage of feeding and foraging time spent consuming invertebrates measured at the group level on overall gut microbial community structure. This may be due to the fact that invertebrate prey eaten by capuchins do not generally contain indigestible compounds, other than chitin, and capuchins are known to have active endogenous chitinases (Janiak, 2016; Raubenheimer & Rothman, 2013; Stevens & Hume, 1995). It also may be attributed to fact that diet measured at such a broad scale (e.g., lumping all fruit species into a single category and all invertebrate taxa into a single category) does not predict changes in the gut microbiome, or that percent time spent

feeding and foraging is not a strong measure of amount of food consumed or the amount of specific nutrients ingested (Garber, Righini, & Kowalewski, 2015).

There was a significant relationship between invertebrate diet composition and gut microbiome composition. Individuals with more similar invertebrate diets had more similar gut microbiota. In line with prediction two, one arthropod family was positively correlated with a genera related to Proteobacteria. However, contrary to this prediction, a second arthropod family, Cercopidae, and *Clostridium XIVa* (Firmicutes) are negatively correlated. Our invertebrate results are only partially consistent with the available data indicating that Firmicutes and Proteobacteria are more abundant when individuals consume lipid and animal protein rich foods sources (Amato et al., 2015a; De Filippo et al., 2010; Gomez et al., 2015; Turnbaugh & Gordon, 2009; Williams et al., 2013). However, it appears from our data that Proteobacteria is a major component of white-faced capuchin gut microbial communities and may play a large role in lipid metabolism in their gut microbiome. The white-faced capuchin gut microbial community composition appears to be different than that of most primates studied to date, with a higher relative abundance of Proteobacteria and lower relative abundances of Bacteroidetes (Amato and Righini, 2015; Amato et al., 2015b; Barelli et al., 2015; Bennett et al., 2016; Degnan et al., 2012; Frey et al., 2006; Hale, Tan, Knight, & Amato, 2015; Moeller et al., 2013; Ochman et al., 2010; Ren, Grieneisen, Alberts, Archie, & Wu, 2016; Su et al., 2016; Sun et al., 2016; Tung et al., 2015), with the caveat that the variation in methods between published studies makes a direct comparison difficult (Table 4). There are, however, similarities in the gut microbiome between capuchins and pygmy lorises (Bo et al., 2010) (Table 4). Pygmy lorises, whose diet is ~30% invertebrates and ~35% fruit (based on feeding time) (Starr & Nekaris, 2013), had similarly high relative abundances of Proteobacteria (30.4%), and, at the level of genus, high relative abundances of *Pseudomonas* and *Bacteroides* (Xu et al., 2013). In white-faced capuchins, *Bacteroides* had the third highest relative abundances of genera related to Bacteroidetes, and *Pseudomonas* was the genus with the fifth highest relative abundance within Proteobacteria (Table S2). In other species of mammals

TABLE 3 Spearman correlation coefficients between the 10 most abundant microbial genera and 5 most frequently consumed invertebrate families

	Clostridium XIVa 83.6	Streptococcus 100.2	Xylanibacter 79	Actinobacillus 46.3	Sutterella 98.9	Megamonas 100.2	Streptophyta 100.1	Xanthomonas 93.3	Escherichia Shigella 98.7	Clostridium XIVa 58.3
Tephritidae	0.083	0.151	0.017	0.04	-0.131	-0.067	-0.149	-0.184	0.403*	0.11
Cercopidae	-0.19	-0.046	-0.14	0.006	-0.293	-0.148	0.238	0.217	0.018	-0.428*
Formicidae	-0.377	-0.045	0.231	-0.194	0.130	-0.231	0.110	-0.323	-0.181	-0.238
Acrididae	-0.165	-0.313	0.335	0.191	0.007	-0.033	-0.43*	-0.03	0.148	0.096
Curculionidae	-0.149	-0.126	0.382	-0.065	0.043	0.236	-0.240	-0.216	-0.370	-0.316

Note. Starred results are significant correlations.

\* $p < .05$ .

that regularly consume invertebrates, Proteobacteria also seem to figure prominently in their gut microbial communities; the gut microbiomes of myrmecophagous mammals are enriched for some taxa of Proteobacteria, including *Klebsiella*, compared with closely related non-myrmecophagous species (Delsuc et al., 2014). There is little data, however, on how Proteobacteria are involved in nutrient metabolism, or, more specifically, involved in the digestion of invertebrate prey.

Proteobacteria may increase nutrient uptake, particularly from lipid-rich dietary resources such as invertebrates, during periods of energetic shortfalls (Sun et al., 2016). This may be an alternative strategy to increasing the ratio of Firmicutes to Bacteroidetes in order to increase energy uptake in the gut. Species that have relatively high rates of insectivory or faunivory and high energetic demands relative to their body size due to increases in relative brain size, decreased interbirth intervals, or increased life spans may have higher relative abundances of Proteobacteria compared with closely related taxa (Delsuc et al., 2014). It is unclear, however, whether these strategies are part of a larger evolutionary pattern and whether host taxa are constrained in their ability to make use of either strategy by ancestral host-microbe co-evolutionary relationships.

Even though there was no relationship between fruit diet composition and gut microbiome composition at the individual level, we did find some effects of fruit consumption and fruit choices on the gut microbiome. Previous studies suggest that the relative abundance of genera related to Firmicutes and Proteobacteria in black howler monkeys (*Alouatta pigra*), a primate species that consumes a leaf and fruit heavy diet, are negatively correlated with the time spent consuming individual fruit species (Amato et al., 2015a). Our results partially support this, with consumption of several fruit species and families (leaves accounted for only 0.5% of feeding and foraging time in our white-faced capuchin study group) being negatively correlated with both genera related to Firmicutes and Proteobacteria. This was consistent when examining both the observational dietary data and the molecular plant dietary data. The exception to this is the positive correlation between *I. thibaudiana* and *Megamonas* (Firmicutes). The relationships between taxa related to Bacteroidetes and fruit consumption also is not clear, as there were negative correlations with two fruit species (*P. guajava* and *I. spectabilis*), but a positive correlation with a third (*H. appendiculata*).

We found that *P. guajava* consumption is positively correlated with the predicted abundance of glycan metabolizing pathways, which is consistent with the published nutrient content data of *P. guajava* that shows it has a relatively high fiber and cellulose content compared with other fruit commonly eaten by white-faced capuchins (Eadie, 2012). Glycan metabolizing bacteria would allow white-faced capuchins to increase their digestive efficiency when relying heavily on *P. guajava*. Additionally, *P. guajava* has relatively less energy compared with other commonly consumed foods, with a caloric content of 178 kCal/100g, whereas average caloric content of all fruits eaten by white-faced capuchins sampled in a wet tropical forest at the Pacuare Nature Reserve (also in northeastern Costa Rica) was 365.6 kCal/100 g (range = 178–632 kCal/100 g) (Eadie, 2012). If these values are similar to the values at our field site, we expect to see that energy metabolizing pathways would be negatively correlated with the consumption of



**TABLE 4** Published data on the average relative abundance of Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria in the gut microbial communities of wild primates

Species	Proteobacteria	Firmicutes	Bacteroidetes	Actinobacteria	Study
<i>Pan troglodytes ellioti</i>	52.5 ± 7.8%	27.0 ± 7.1%	19.0 ± 0%	0.8 ± 0.3%	Ochman et al. (2010) <sup>a</sup>
<i>Gorilla beringei</i>	52.0 ± 2.8%	9.9 ± 1.6%	35.0 ± 2.8%	3.0 ± 2.3%	Ochman et al. (2010) <sup>a</sup>
<i>Gorilla gorilla</i>	48.0 ± 7.1%	5.0 ± 2.4%	45.0 ± 9.9%	2.0 ± 0.8%	Ochman et al. (2010) <sup>a</sup>
<i>Pan troglodytes ellioti</i>	40.3 ± 30.0%	39.7 ± 22.5%	17.3 ± 6.8%	0.7 ± 0.3%	Moeller et al. (2013) <sup>a</sup>
<i>Gorilla beringei grauer</i>	39.5 ± 13.6%	18.7 ± 13.5%	35.3 ± 1.6%	1.7 ± 2.2%	Moeller et al. (2013) <sup>a</sup>
<i>Cebus capucinus</i>	39.2 ± 24.6%	41.6 ± 23.8%	13.3 ± 17.3%	1.1 ± 4.3%	This study
<i>Nycticebus pygmaeus</i>	34.5%	43.1%	17.2%	5.2%	Bo et al. (2010)
<i>Gorilla gorilla gorilla</i>	29.2 ± 23.6%	24.9 ± 21.8%	36.9 ± 12.3%	1.5 ± 1.3%	Moeller et al. (2013) <sup>a</sup>
<i>Chlorocebus aethiops sabaues</i>	17.2 ± 14.9%	59.0 ± 16.4%	10.3 ± 11.3%	1.6 ± 2.1%	Amato et al. (2015b)
<i>Macaca thibetana</i>	14.7 ± 8.8%	43.4 ± 8.5%	35.2 ± 6.4%	0.5 ± 0.6%	Sun et al. (2016)
<i>Pan troglodytes troglodytes</i>	13.9 ± 14.8%	52.1 ± 20.1%	27.9 ± 6.8%	4.1 ± 4.5%	Ochman et al. (2010) <sup>a</sup>
<i>Papio cynocephalus</i>	13.0 ± 2.8%	42.2 ± 8.4%	7.3 ± 2.4%	9.4 ± 4.6%	Tung et al. (2015)
<i>Pan troglodytes troglodytes</i>	12.4 ± 12.7%	55.8 ± 17.7%	20.1 ± 8.1%	3.0 ± 2.7%	Moeller et al. (2013) <sup>a</sup>
<i>Pan paniscus</i>	12.1 ± 20.4%	57.3 ± 30.6%	20.2 ± 14.9%	2.8 ± 1.5%	Moeller et al. (2013) <sup>a</sup>
<i>Lemur catta</i>	5.2%	51.6%	15.8%	nr	Bennett et al. (2016)
<i>Papio cynocephalus</i>	4.1%	48.8%	7.2%	17.2%	Ren et al. (2016)
<i>Pan troglodytes schweinfurthii</i>	2.9 ± 3.1%	61.6 ± 14.9%	28.6 ± 11.8%	4.3 ± 0.7%	Ochman et al. (2010) <sup>a</sup>
<i>Pan troglodytes schweinfurthii</i>	1.2 ± 0.9%	50.1 ± 9.5%	24.3 ± 8.3%	2.6 ± 1.8%	Moeller et al. (2013) <sup>a</sup>
<i>Pan paniscus</i>	1.0 ± 1.0%	71.0 ± 6.1%	19.2 ± 3.6%	6.9 ± 4.3%	Ochman et al. (2010) <sup>a</sup>
<i>Alouatta pigra</i>	0.9%	68.4%	13.3%	nr	Amato and Righini (2015)
<i>Procolobus gordonorum</i>	0.4%	75.4%	12.1%	0.0%	Barelli et al. (2015)
<i>Gorilla beringei</i>	nr	71.0%	1.1%	5.3%	Frey et al. (2006) <sup>a</sup>
<i>Pan troglodytes schweinfurthii</i>	nr	59.8%	17.9%	nr	Degnan et al. (2012)
<i>Gorilla gorilla gorilla</i>	Nr	44.8 ± 7.6%	20.1 ± 6.8%	12.2 ± 5.6%	Gomez et al. (2015)
<i>Rhinopithecus roxellana</i>	Nr	32.4%	14.7%	4.4%	Su et al. (2016)

Note. nr = not reported.

<sup>a</sup>Studies where not all fecal samples collected were fresh.

this particular fruit, assuming other foods eaten also have a low energy content. *P. guajava* is an introduced domesticated fruit and was one of the top three most commonly consumed fruit species consumed by capuchins in our study group during ten months of the year. We hypothesize that it plays a role in shaping gut microbial community composition and function in this population of white-faced capuchins. However, the functional data in this study should be interpreted cautiously, as we do not have data on digestive metabolites to corroborate the predicted functional pathways.

It is also likely that other factors, such as the nutrients being consumed or the nutritional requirements of individuals, are playing a greater role in shaping gut microbial communities than the species of fruit being consumed. This is supported by a study of black howler monkeys, which indicated that energy intake was significantly related to the relative abundance of gut microbial genera (Amato et al., 2015a).

In contrast, in the same population, while there were age- and sex-based differences in both macronutrient consumption and gut microbial community composition, there was little relationship between the amount of individual dietary components consumed or plant metabolite concentration and gut microbial community composition (Amato et al., 2014). Thus, the relationship between nutrient requirements, the food items consumed, macronutrient consumption, and gut microbial community composition and function is still unclear. There is limited available data on how both nutrient requirements and nutrient consumption influence gut microbial composition and function within a single population of animals. Although we did not measure actual nutrient consumption and there are no published data for other foods consumed by individuals in our population, many authors have reported significant differences in the lipids, protein, sugars, complex polysaccharides, and chitin content of invertebrates and fruits eaten by primates,

both between and within taxa (Bell, 1990; Chapman et al., 2003; Chivers, 1998; Houle, Chapman, & Vickery, 2007; O'Driscoll Worman & Chapman, 2005; Raubenheimer & Rothman, 2013; Rothman et al., 2008). Even though we found relationships between the nutrient content of some fruits and gut microbial function, this does not represent a complete picture of how variation in macronutrient consumption influences white-faced capuchin gut microbial community structure and function. Although we predicted that all species of a particular food type would similarly influence gut microbial community structure and function, it is not unexpected that specific plant species or invertebrate families differ in their influence on the gut microbiome in terms of what specific microbial genera and functional pathways are influenced by changes in fruit species or invertebrate family consumption. It is likely that broad dietary categories, such as fruit, leaves, or invertebrates, are not as important as the specific taxa of foods, types of plant tissues, and nutrients consumed in influencing the gut microbiome (Amato et al., 2015a).

In particular, the strength of the relationship between gut microbial community structure and invertebrate dietary choices bears special note given the importance of invertebrates in the diets of both human and nonhuman primates (Backwell & D'Errico, 2001; McGrew, 2001, 2014; Raubenheimer & Rothman, 2013; Raubenheimer et al., 2014). From this study, it appears that invertebrates are playing a role in shaping the gut microbiome of white-faced capuchins, based on the correlation between individual diet and gut microbial community composition and the relationship between rates of consumption of invertebrates from specific families and the relative abundance of microbial genera and predicted microbial functions. The results of our study emphasize the necessity of sampling the gut microbiome of the same species across a range of diets and environments in addition to across a diversity of taxa, allowing us to gain a more complete understanding of how environmental factors, including diet, influence the gut microbiome, informing studies of the co-evolution of gut microbes and host species (Amato, 2013b). Avenues for future research include sampling additional species of primates whose diet contains a large proportion of invertebrate and vertebrate prey, sampling populations from the same species in different environments to control for host phylogenetic effects, assessing dietary change over shorter timescales, and the addition of data on the nutrient content of the foods being consumed and metabolomic data to provide a richer understanding of the complex relationship between environmental factors, host species behavior, and gut microbial community composition and function.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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