

Diet affects the composition and diversity of the Mammalian gut microbiota

Grace Chen, Esther Fang, Stephy Mak, Lena Xiong

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY The mammalian gut microbiota is colonized by a complex and dynamic population of microbes, and has been shown to play a substantial role in mediating health and disease in individuals. Although the gut microbiome is subjected to a wide variety of host and environmental factors that influence microbial composition, diet is considered as one of the main drivers involved in shaping the gut microbiome structure. The composition of the mammalian gut microbiota has been shown to correlate with the secretion of specialized enzymes used to metabolize distinct substrates across the three main diet categories: carnivory, omnivory, and herbivory. Herbivores have been shown to have the greatest microbial diversity, driven by the need for microbial assemblages to break down recalcitrant plant fibres into usable energy. In this study, we analyzed alpha and beta diversity from a data set composed of 41 mammalian species spanning across 6 orders. We confirm previous results that mammals belonging to different diet types harbour distinctly different gut microbial communities. We also show that certain microbial families are associated with each of the three diet categories through indicator taxonomy analysis. Furthermore, the relationship between the proportion of plants in the diet and the gut microbial composition in omnivores is inconclusive. Collectively, these results allowed us to further our understanding into the effects of diet on the mammalian gut microbiota.

INTRODUCTION

Our understanding of the gut microbiome has extended far beyond its association with host metabolism and nutrient acquisition. Studies in recent years have demonstrated that the gut microbiome is also vital in providing a range of functions, including development of the immune system (1), modulation of gastrointestinal diseases (2), and prevention of pathogen invasion (3). Perturbations in the composition of the gut microbiome have also been shown to cause diseases (4, 5).

A variety of factors have been shown to affect microbial composition in the mammalian intestines, including host phylogeny, digestive tract morphology, living environment, and diet type (6, 7). Of these factors, diet type has been identified as the dominant contributor to shaping the gut microbiome (8). Studies that experimentally established short-term dietary interventions in humans have shown that the gut microbiome is dynamic, and subtle dietary perturbations in individuals can rapidly shift the composition of the gut microbiome (9). Varying the proportion of fat, protein, and carbohydrates in the diet have all shown to impact the composition and diversity of the gut microbial community (10).

In an attempt to further define the link between diet and the gut microbiota, we explored the variation in mammalian gut microbial composition for different diet types, which are herbivory, omnivory, and carnivory. Based on previous findings that long term dietary patterns influence the gut microbiome (11), and the large dietary difference between these diet types, we hypothesize that mammalian species with different diet types have different gut microbial composition. If so, this difference in gut microbial communities can be explained by microbial enzymes needed to metabolize different substrates in distinct diet types. For instance, the gut microbiome of herbivores secrete specialized enzymes that degrade plant matter (6), while the gut microbiome of carnivores secrete enzymes that break down proteins (8).

Subsequently, we explored the effects of diet composition on the gut microbiota in only

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Address correspondence to:
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omnivores. Previous research on the relationship between diet and the gut microbiome predominantly compared herbivores, carnivores, and omnivores as distinct experimental groups. To provide more insight regarding omnivores, we examined the omnivorous diet as a spectrum of different proportions of plants in the diet.

Herbivorous mammals are associated with certain microbial taxa in their gut microbiome, characterized by their dependence on these gut microbes to obtain energy from food, synthesize vitamins, and detoxify plant-derived compounds (12). This dependence leads to herbivores displaying the most diverse and complex gut microbial composition of the three diet types (13). This observed increase in gut microbial diversity in herbivores is due to the complex polysaccharides that make up the plant-based diet, which requires specialized enzymes secreted by an extensive microbial repertoire to break down into usable energy (14). Since the gut microbiome is needed for omnivores to digest plant matter, we hypothesize that omnivores with plants making up a higher percentage of their diet are associated with a different composition and an increase in diversity of the gut microbiome.

Here we examine the impact that dietary type and the proportion of plants in the omnivorous diet have on the composition and diversity of the mammalian gut microbiome. We analyzed a mammalian distal gut microbiome 16S rRNA dataset, composed of wild and captive animals that include 41 species spanning across 6 orders (15).

METHODS AND MATERIALS

All the scripts for QIIME2 and R are provided in the supplemental materials section (Supplemental Script S1&S2).

Sourcing gut microbiome sequencing data from a previous study. The mammalian gut microbiome sequencing data analyzed in this study were obtained from a study that examined the effects of captivity on the mammalian gut microbiome (15).

Classifying mammals based on diet. In addition to the gut microbiome sequencing data, the original paper also provided metadata on various species traits sourced from public databases (15). Of these traits, diet type and diet composition were analyzed in this study to determine their association with the mammalian gut microbiome. Species were classified as carnivores, herbivores or omnivores (Table 1). Diet composition, which describes the proportion of the diet composed of distinct food types, including invertebrates, scavengers, endothermic vertebrates, unknown vertebrates, fruits, seeds, and other plant material (15), was also recorded for omnivores. The diet compositions provided were sourced from online databases and reflected estimated values only (15). To examine how an increased proportion of plant matter in the diet of omnivores impacts their gut microbiota, omnivores were grouped based on the percentage of their diet composed of plant-based foods (Table 2). Plant-based foods were identified as fruits, seeds, and other plant materials. The omnivores in this dataset had 50%, 70%, 80%, 90% or 100% plant-based foods in their diet. Omnivores with 100% plant-based foods were not classified as herbivores because they primarily consume plant-matter but are also capable of consuming animal matter, such as insects (16, 17).

Bioinformatics in QIIME2. Amplicon sequence data from 296 mammalian samples were demultiplexed and quality filtered using QIIME2 (18). Using DADA2 (19), chimeric and phiX reads were filtered out, and amplicon sequences were truncated at the 150th nucleotide position to remove regions with low quality scores. To study the gut microbial diversity of mammals based on diet type, all samples were rarefied to a depth of 12018 sequences, resulting in 288 samples. To study the gut microbial diversity of omnivores with different proportions of plant-based foods in their diets, herbivores and carnivores were filtered out from the analysis, and the remaining omnivore samples were rarefied to 17292 sequences, resulting in 104 samples.

TABLE 1 Mammalian host taxonomy and sample numbers grouped by diet type

Diet Type	Host Taxonomy			Total (n)		
	Order	Family	Genus species			
Carnivore	Carnivora	Canidae	<i>Canis lupus</i>	4		
		Canidae	<i>Lycan pictus</i>	5		
		Felidae	<i>Acinonyx jubatus</i>	3		
Herbivore	Pilosa	Myrmecophagidae	<i>Myrmecophaga tridactyla</i>	34		
	Tubulidentata	Orycteropodidae	<i>Orycteropus afer</i>	22		
	Cetartiodactyla	Bovidae	<i>Aepyceros melampus</i>	6		
		Bovidae	<i>Antidorcas marsupialis</i>	8		
		Bovidae	<i>Connochaetes gnou</i>	1		
		Bovidae	<i>Connochaetes taurinus</i>	7		
		Bovidae	<i>Hippotragus equinus</i>	1		
		Bovidae	<i>Hippotragus niger</i>	6		
		Giraffidae	<i>Giraffa camelopardalis</i>	6		
		Perissodactyla	Equidae	<i>Equus asinus</i>	4	
			Equidae	<i>Equus grevyi</i>	2	
			Equidae	<i>Equus hemionus</i>	3	
	Equidae		<i>Equus przewalskii</i>	4		
	Equidae		<i>Equus quagga</i>	6		
	Omnivore	Cetartiodactyla	Equidae	<i>Equus zebra</i>	5	
Rhinocerotidae			<i>Ceratotherium simum</i>	6		
Rhinocerotidae			<i>Diceros bicornis</i>	5		
Primates			Hominidae	<i>Gorilla gorilla</i>	19	
			Lemuridae	<i>Eulemur rubriventer</i>	12	
			Lemuridae	<i>Eulemur rufus</i>	2	
			Lemuridae	<i>Lemur catta</i>	13	
Cetartiodactyla			Suidae	<i>Phacochoerus africanus</i>	5	
			Primates	Atelidae	<i>Alouatta caraya</i>	12
				Atelidae	<i>Alouatta palliata</i>	12
	Atelidae	<i>Alouatta pigra</i>		15		
	Atelidae	<i>Alouatta seniculus</i>		10		
	Atelidae	<i>Ateles belzebuth</i>		5		
	Atelidae	<i>Ateles fusciceps</i>		2		
	Atelidae	<i>Ateles hybridus</i>		3		
	Cercopithecidae	<i>Cercopithecus ascanius</i>		9		
	Cercopithecidae	<i>Cercopithecus cephus</i>		2		
	Cercopithecidae	<i>Cercopithecus neglectus</i>		1		
	Cercopithecidae	<i>Cercopithecus wolffi</i>		1		
	Cercopithecidae	<i>Colobus angolensis</i>		2		
	Cercopithecidae	<i>Colobus guereza</i>		9		
	Cercopithecidae	<i>Papio anubis</i>		7		
Cercopithecidae	<i>Papio hamadryas</i>	7				
Cercopithecidae	<i>Papio ursinus</i>	2				

Alpha diversity analyses. To investigate the effects of diet on alpha diversity of the mammalian gut microbiome, observed microbial richness of each sample was calculated for each of the three diet types using QIIME2. Kruskal-Wallis tests were performed in QIIME2 to determine the statistical significance of the differences observed between the three diet groups. Correlation analysis was performed to determine the association between the percentage of plant-based foods in the omnivorous diet and gut microbial richness.

Beta diversity analyses. To investigate the effects of diet on beta diversity of the mammalian gut microbiome, weighted UniFrac distances were calculated between samples of the three diet types. The weighted UniFrac metric was chosen because it accounts for both microbial abundance and phylogenetic relatedness (20). PERMANOVA tests were performed in QIIME2 to determine the statistical significance of the observed distances between the groups in each analysis. Principle coordinate analysis (PCoA) plots were generated using R to visualize beta diversity differences between samples.

Diet type indicator taxa. Taxonomic information was assigned to each amplicon sequence variant (ASV) using the Greengenes 16S rRNA reference database clustered at 99% sequence similarity (21) in QIIME2. The accuracy of the taxonomic identifications was

improved by training the Naives Bayes classifier on only the region of the 16S rRNA sequenced in this dataset. Indicator taxa were identified at the family level for each group using the indicpecies package (Table S1) (22).

TABLE 2 Diet composition of omnivorous species and the proportion of plant-based foods

Host Taxonomy	Plant-based foods (%)	Diet Composition						
		Fruit	Seeds	Plant-Others	Invertebrates	Scavengings	Endothermic Vertebrates	Unknown Vertebrates
<i>Alouatta caraya</i>	100	40	0	60	0	0	0	0
<i>Alouatta palliata</i>	100	40	0	60	0	0	0	0
<i>Alouatta pigra</i>	100	40	0	60	0	0	0	0
<i>Alouatta seniculus</i>	100	40	0	60	0	0	0	0
<i>Ateles belzebuth</i>	80	60	10	10	10	0	0	10
<i>Ateles fusciceps</i>	80	60	10	10	10	0	0	10
<i>Ateles hybridus</i>	80	60	10	10	10	0	0	10
<i>Cercopithecus ascanius</i>	70	50	10	10	20	0	0	10
<i>Cercopithecus cephus</i>	70	50	10	10	20	0	0	10
<i>Cercopithecus neglectus</i>	70	50	10	10	20	0	0	10
<i>Cercopithecus wolfi</i>	100	70	20	10	0	0	0	0
<i>Colobus angolensis</i>	100	30	0	70	0	0	0	0
<i>Colobus guereza</i>	100	30	0	70	0	0	0	0
<i>Papio anubis</i>	50	20	0	30	10	0	40	0
<i>Papio hamadryas</i>	80	0	0	80	10	0	0	10
<i>Papio ursinus</i>	70	40	20	10	20	0	0	10
<i>Phacochoerus africanus</i>	90	10	0	80	0	10	0	0

RESULTS

Carnivores, omnivores, and herbivores differ in gut microbial diversity. There was a clear difference in gut microbial richness for mammals of the three diet types (Figure 1, $p = 5.39e-27$). Richness decreased from herbivores (median = 958), omnivores (median = 416), to carnivores (median = 156) (Figure 1). These results confirmed the results from previous studies (6, 23) and supported our hypothesis.

Carnivores, omnivores, and herbivores differ in gut microbial composition. In a PCoA plot based on weighted UniFrac distances, the gut microbial samples clustered according to their diet types, indicating a difference in gut microbial composition between the three groups (Figure 2). The difference was statistically significant based on the group PERMANOVA ($p = 0.001$) (Figure 2). Note that despite the significant difference in composition, there were still some overlaps between the diet types, implying that their compositions were not completely distinct from one another.

Indicator taxa of carnivores, omnivores and herbivores. Since carnivores, omnivores, and herbivores differed in gut microbial composition, we performed indicator taxa analysis in R to determine taxa that were potentially significant to mammals from each diet type. A higher indicator taxa value indicates that more of a taxa is found in a specific diet type and not the others. We found that *Ruminococcaceae* was a significant indicator of carnivores; *Flavobacteriaceae* and *Microbacteriaceae* were significant indicators for herbivores; and *Lachnospiraceae*, *Prevotellaceae*, *Lactobacillaceae* and *Christensenellaceae* were significant indicators for omnivores.

Microbial diversity is weakly and negatively correlated with the proportion of plant-based food in the omnivorous diet. A weak, negative correlation was found between gut microbial richness in omnivores and the proportion of plant matter in their diet (Figure 3, $p = 0.03$; Pearson's correlation = -0.21), which did not support our hypothesis.

No discernible trend between gut microbial composition and the proportion of plant-based diet in omnivores. In a PCoA plot based on weighted UniFrac distances, the gut microbial samples of omnivores with 50%, 70%, and 80% plant-based diets greatly overlapped with each other (Figure 4). However, it was unclear if they are different in gut

microbial composition since the PCoA plot represented only 35.8% of the variance and as a result, there may have been distinct clusterings that were not visualized. Furthermore, we observed that the samples with 90% and 100% plant matter in the diet clustered separately from each other, indicating that they have significantly different microbial communities (Figure 4, $p_{100\% \text{ to } 90\%} = 0.001$). In addition, the 90% and 100% groups both overlapped with the 50%, 70%, and 80% groups (Figure 4, $p_{90\% \text{ to } 80\%/70\%} = 0.001$; $p_{90\% \text{ to } 50\%} = 0.002$; $p_{100\% \text{ to } 80\%/70\%/50\%} = 0.001$). Yet, due to the partially explained variance by the PCoA plot, it was unclear if the 90% or 100% group had a different microbial community than the 50%, 70%, or 80% groups (Figure 4). These results suggest no clear trend between gut microbial composition and the proportion of plant-based diet. It is thus inconclusive if omnivores with similar proportions of plant matter in their diets have more similar microbial compositions.

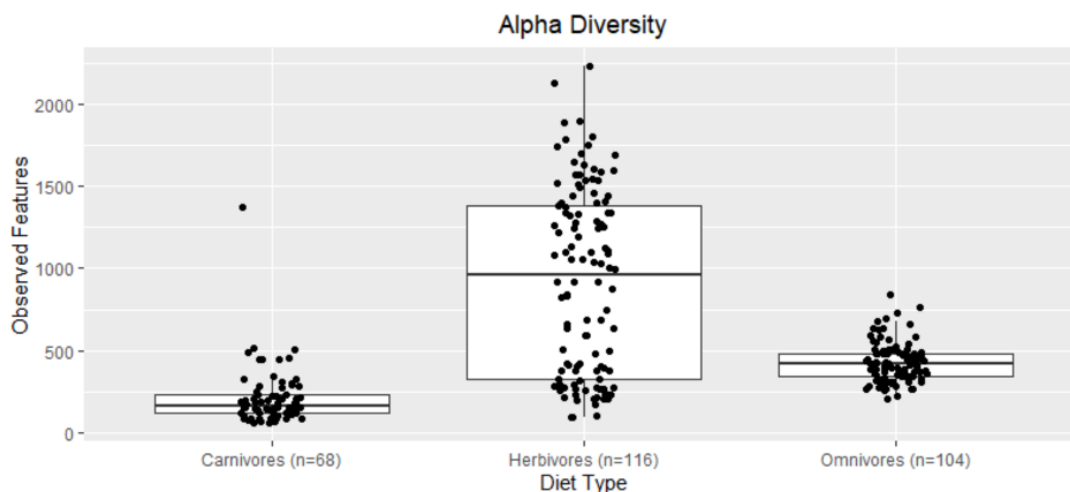


FIG. 1 Carnivores, omnivores, and herbivores differ in gut microbial diversity. A box-and-whiskers plot depicting alpha diversity based on observed features for the three diet types: carnivores, omnivores, and herbivores. Each point represents a gut microbial sample of mammals from their respective diet type. The box represents the interquartile range (IQR) and the line inside the box represents the median. The whiskers represent 1.5 times the IQR. $p = 5.39\text{e-}27$ (group Kruskal-Wallis test). $p_{\text{carnivore to herbivore}} = 1.84\text{e-}20$; $p_{\text{carnivore to omnivore}} = 1.01\text{e-}20$; $p_{\text{herbivore to omnivore}} = 7.28\text{e-}07$ (pairwise Kruskal-Wallis test).

DISCUSSION

Gut microbial diversity and composition are different in carnivores, omnivores, and herbivores. We found that the gut microbial community of mammals with different diet types differ in both richness and composition, which supports our hypothesis and validates previous studies analyzing the effects of diet on the gut microbiome (6, 23). A study conducted on 60 mammalian species showed clustering of gut microbial samples by diet type (carnivores, omnivores, and herbivores) based on PCoA plot of unweighted uniFrac distances (6). The same study also observed that herbivores had the greatest richness of gut microbes at the phylum and genus level, followed by omnivores and carnivores (6). We found a similar increase in ASV richness from carnivores, omnivores to herbivores.

Indicator taxa of the diet types. Having established earlier that the three diet types differ in gut microbial composition, we performed indicator taxa analysis for each diet type to determine if any particular taxa could help explain the differences in the communities. Our results showed that *Ruminococcaceae* is a significant indicator of carnivores, which was previously shown to be abundant in the gut microbiome of rats fed with a protein-based diet consisting of pork, beef, fish and chicken (24). However, the role of *Ruminococcaceae* in the gut microbiome of carnivores has yet to be identified in the literature. As for herbivores, they depend on specialized microbes to digest plant carbohydrates, such as cellulose, and metabolize them into nutrients that can be absorbed by the host (23). Therefore, it is unsurprising that *Flavobacteriaceae* and *Microbacteriaceae*, which are significant indicators for herbivores, encode glycoside hydrolases that are important in breaking glycosidic bonds of complex sugars found in plant materials (25, 26). Finally, *Lachnospiraceae*, *Prevotellaceae*, *Lactobacillaceae* and *Christensenellaceae* were found to be significant indicator taxa for omnivores. *Lachnospiraceae* and *Prevotellaceae* are families of microbes involved in the degradation of carbohydrates from plants. *Prevotellaceae* can ferment plant fibres into short-chain fatty acids (SCFAs) (27), while

Lachnospiraceae can degrade complex carbohydrates into butyrate and SCFAs which can be absorbed as nutrients by mammals (28). *Lactobacillaceae*, on the other hand, were prevalent in rats fed with a protein-based diet (24). Interestingly, humans with an omnivorous diet have a higher abundance of *Christensenellaceae* compared to those with a vegetarian diet (29). In addition, *Christensenellaceae* is suspected to play a role in the fermentation of both protein and fibre (29), which may explain why *Christensenellaceae* was found to be an indicator of omnivores in our study. Since omnivores consume both plants and proteins in their diets, it is unsurprising that our indicator taxa analysis for omnivores found microbes involved in both the degradation of plant fibres and proteins.

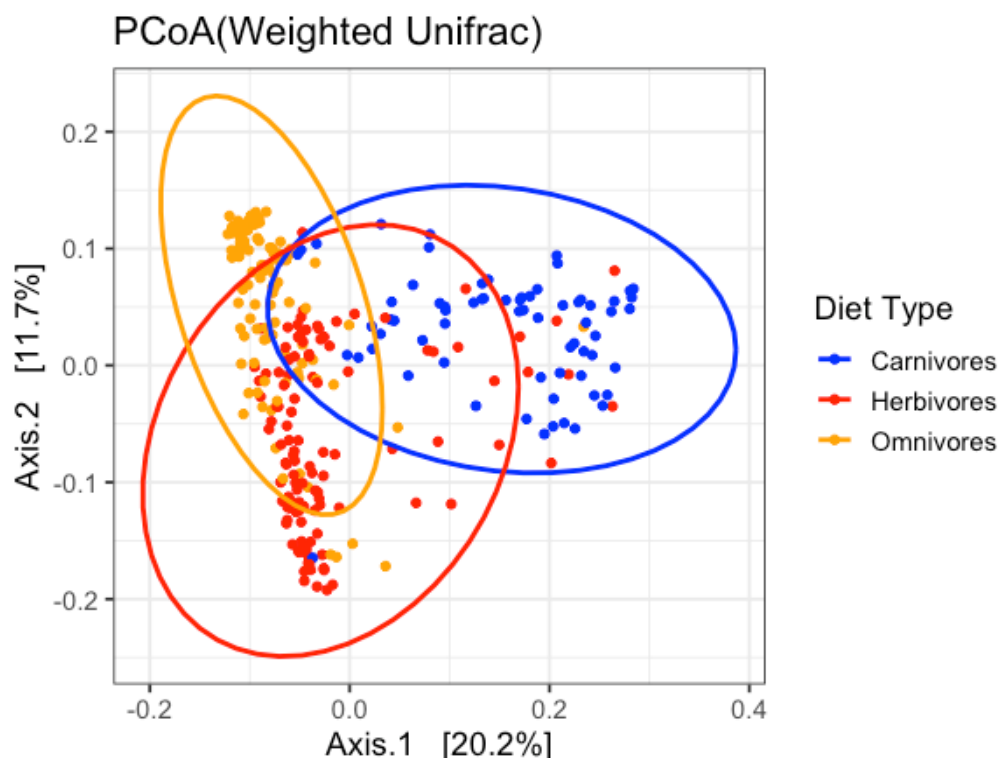


FIG. 2 Carnivores, omnivores, and herbivores differ in gut microbial composition. A principal coordinate analysis (PCoA) of weighted UniFrac distances depicting the clustering of the three diet types. The two axes of the PCoA plot explain 31.9% of the variance of the data. Each point represents a gut microbial sample of an individual mammal from their respective diet type (blue = carnivores, red = herbivores, yellow = omnivores). The ellipses, representing the estimated 95% confidence intervals of the samples, are added to better visualize the clustering between diet types. $p = 0.001$ (group PERMANOVA test). $p_{\text{carnivore to herbivore}} = 0.001$; $p_{\text{carnivore to omnivore}} = 0.001$; $p_{\text{herbivore to omnivore}} = 0.001$ (pairwise PERMANOVA test).

The correlation between gut microbial diversity of omnivores and the proportion of plants in their diet is inconclusive. We found a weak, negative correlation between gut microbial diversity of omnivores and the proportion of plants in their diet (Fig. 3, $p = 0.03$; Pearson's correlation = -0.21), which is opposite to what we hypothesized. Despite the correlation observed, we have identified variables in the dataset that likely confounded the analysis. As a result, the correlation between these two variables is inconclusive from this study.

One confounding variable is the difference in dietary breadth, which is the diversity of foods consumed by omnivores. A controlled study conducted on cattle showed that cattle provided with an omnivorous diet have a greater gut microbial diversity compared to herbivorous cattle, which may be explained by a more diverse diet in the omnivorous cattle (30). This result is consistent with a study conducted on humans, which concluded that gut microbial diversity is enhanced by dietary breadth (31). In the dataset we use here, the specific plant materials were grouped into a single metric, known as "plant-based foods". We suspect that the exact proportions of specific plant-based food types, such as seeds, leaves, and fruits, may affect gut microbial diversity in mammals to a greater extent than the collective proportion of plant matter in their diets. Although these food types share a plant-based origin, they still vary greatly in their overall nutritional composition, such as the amount of sugar, fat, and fibre, and will therefore be metabolized differently.

We found that previous studies have shown conflicting results regarding whether herbivores or omnivores have greater gut microbial diversity. The study conducted on cattle observed a greater microbial diversity in omnivorous cattle compared to herbivorous cattle (30), but a study of 60 mammalian species found greater diversity in herbivores than omnivores (1), the latter of which we based our hypothesis on. Nevertheless, the two studies do not necessarily contradict each other, since the cattle study is an intraspecies comparison (30), while the mammalian study is an interspecies comparison (1). We speculate that the influence of variation in dietary breadth on the gut microbial community may only be clear when we look specifically into a single mammalian species without the influence of species-level differences. We suspect that interspecies variation is much greater than intraspecies variation, hence the species-level differences likely overshadowed any within species variation. It is still necessary to investigate whether or not a true correlation exists once confounding variables are accounted for.

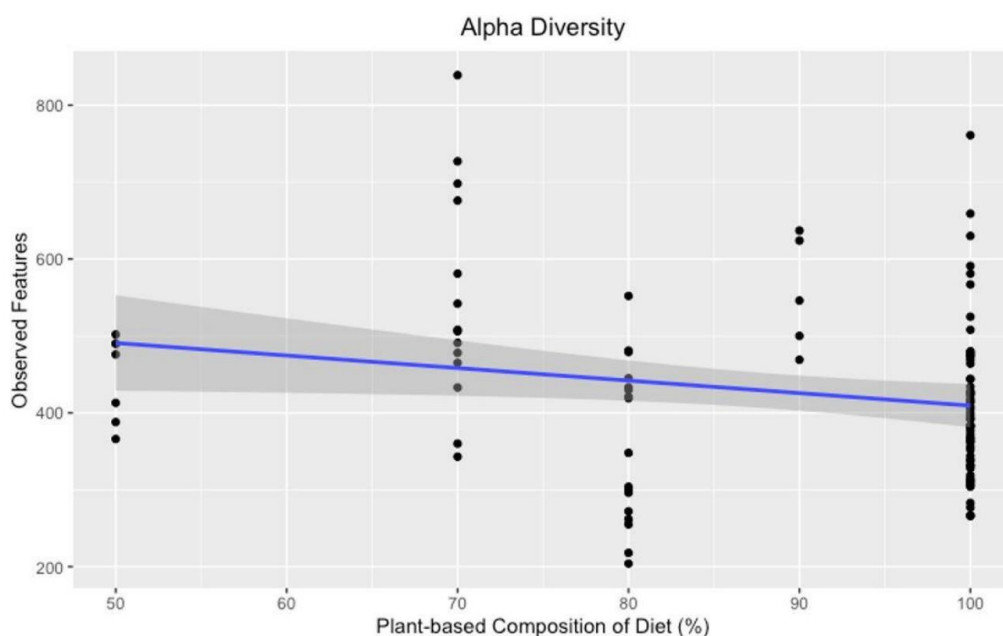


FIG. 3 Microbial diversity was weakly and negatively correlated with the proportion of plants in omnivores' diets. A scatter plot showing gut microbial diversity based on observed features for omnivores with 50%, 70%, 80%, 90% and 100% plants in their diet. Each point represents a gut microbial sample from an omnivore. The straight line represents the line of best fit to the data points, and the grey, shaded area around the line represents the 95% confidence interval. $p = 0.03$. Pearson's correlation = -0.21.

No discernible trend between gut microbial composition of omnivores and the proportion of plant-based foods in their diet. We found that omnivores with a 100% plant-based diet have a different gut microbial composition compared to the 90% group (Figure 4). However, the 90% plant-based diet group consists only of the species *Phacochoerus africanus* (Warthog), while the 100% plant-based diet group consisted of 7 different species of primates. As a result, the clustering of gut microbial samples in the PCoA plot (Figure 4) may be due to phylogenetic relatedness rather than the proportion of plant matter in the diet. We speculate that the taxonomic-level differences likely outweighed the diet-based differences, making it hard to draw conclusions from the data we have. In summary, we do not observe a clear monotonic trend between microbial composition and the proportion of plant matter in the diet.

However, as we originally hypothesized, it is probable that omnivores with similar diet compositions have similar microbial compositions too. A study conducted on cattle showed that omnivorous cattle have different gut microbial compositions due to changes in food digestion and energy production (30). This change in the diet may lead to a gain or loss of substrates that are required for the survival of certain microbes (30), leading to a change in gut microbial communities. However, this is a within species trend and our study is investigating between species trends. Further research could focus on studying single mammalian species to better understand the proportion of plants in the diet and its influence on gut microbial composition.

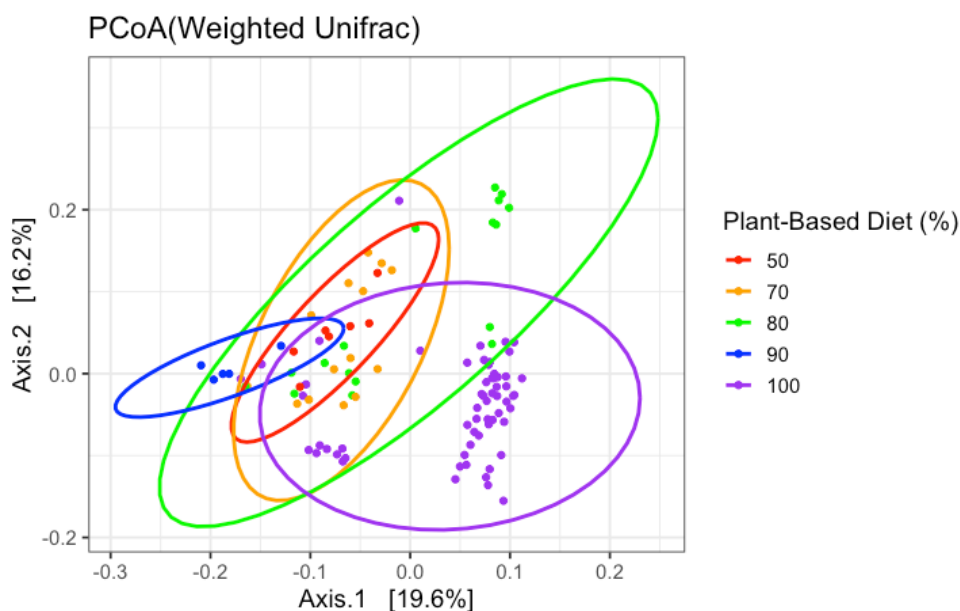


FIG. 4 No discernible trend between microbial composition and the proportion of plant-based diets. A principal coordinate analysis (PCoA) of weighted UniFrac distances, which shows the clustering of microbial communities in omnivores with different proportions of plants in their diets. The two axes of the PCoA plot explain 35.8% of the variance of the data. Each point represents a gut microbial sample from an omnivore. The rainbow spectrum represents samples with 50% - 100% plants in their diets along a gradient. The ellipses, representing the estimated 95% confidence intervals of the samples, are added to better visualize the clustering of samples with different proportions of plants. $p = 0.001$ (group PERMANOVA test). $p_{100\% \text{ to } 90\%/80\%/70\%/50\%} = 0.001$; $p_{90\% \text{ to } 80\%/70\%} = 0.001$; $p_{90\% \text{ to } 50\%} = 0.002$; $p_{80\% \text{ to } 70\%} = 0.012$; $p_{80\% \text{ to } 50\%} = 0.033$; $p_{70\% \text{ to } 50\%} = 0.01$ (pairwise PERMANOVA test).

Limitations One major constraint of this study is the limited breadth provided in the dataset. The 41 species of mammals examined in this study represent only 6 orders of the mammalian class, constituting a small subset of the 27 currently identified orders of mammals (the number is yet to be agreed upon) (32). Within each order, a smaller subset of families, genera and species were sampled, which further limits the generalizability of our conclusions to the entire mammalian class. It is also worth noting that the dataset does not consist of a random sample from each order, given that certain orders of mammals in this dataset have significantly more genera, species, or individuals than others. Since closely related taxa may have inherent similarities in the gut microbiota, this may have direct implications when comparing the gut microbiome of omnivores grouped by proportion of plants in their diets. For example, in our analysis of omnivores, some groups had a small number of samples and were composed of closely related mammals. In particular, groups with 50% and 90% plant-based foods consisted of only mammals from the same species.

It should also be noted that the diet proportions provided to us in the metadata were based on species-level estimates from the literature, and were not directly measured from observing individual mammals. Consequently, no individual-level trends were analyzed in our study. In the dataset we use, percentages of diet composition were estimated in increments of 10%, which may not represent accurate values. Although these values do not reflect the true diet of the sampled mammals, species-level trends in diet may be large enough to override individual-level ones. Therefore, estimated diet proportions can still provide broad and important insights on the effects of diet on the gut microbiota.

Conclusions Our study investigated the effects of diet on the mammalian gut microbiome. The results show that carnivores, omnivores, and herbivores differ in gut microbial diversity and composition, which supports our hypothesis. In addition, we found that richness increases from carnivores, omnivores, to herbivores. On the other hand, our findings are inconclusive for our initial hypothesis that omnivores with a higher proportion of plants in the diet would be associated with an increase in diversity of the gut microbiome.

Future Directions Future studies can build upon our current understanding of the mammalian gut microbiota by examining a more diverse and random assortment of mammals than what was included in this study. It is of interest to sample more taxonomic groups to better capture the diversity of the mammalian class, and to investigate if patterns

of dietary influence on the gut microbiota can be generalized across more branches of the mammalian tree.

In addition, future studies may aim to improve our understanding of how omnivores with different proportions of plant and animal matter in their diets vary in the diversity and composition of their gut microbiota. In this study, the metadata consisted of only omnivores with greater than 50% plant-based foods in their diet, which provided a limited scope of the full spectrum in which diet composition can vary upon. The diet composition of omnivores can be examined along a larger spectrum by sampling species with less than 50% of their diet composed of plant-based foods as well. This data will effectively provide a more complete overview of how the omnivorous diet varies between the two extremes of an herbivorous and carnivorous diet. With this information, it may also be worthwhile to determine if certain bacterial taxa change in abundance as the proportion of plant-based foods or animal-based foods increase, and how this may aid in the digestion of certain foods in the omnivorous diet.

Indicator bacterial taxa were also identified for each of the three diet types, but their functional role in host digestion and nutrient metabolism have yet to be fully elucidated. Since many of the identified indicator taxa have neither been thoroughly curated or described in the literature in terms of their role in the gut microbiota, future studies may seek to address this knowledge gap. Future studies may also strive to determine the digestive enzymes encoded and expressed by these bacteria, and understand how metabolism is distributed in the bacterial community to accomplish this goal.

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CONTRIBUTIONS

All members were involved in generating QIIME2 and R scripts for the workflow and analyses. Individually, Grace participated in doing background research and writing the introduction and the abstract. Lena participated in writing the materials and methods, study limitations and future directions of the paper. Stephy wrote the results and discussion for the diet type research question, as well as the conclusion. Esther wrote the results and discussion for the omnivores' plant-based diet research question.

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