

DIET COMPOSITION AND SPECIES RICHNESS IN THE GUT MICROBIOME

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ABSTRACT: The gut microbiome plays a very important role in the health of human beings by helping to control digestion and benefiting the immune system. Furthermore, the role of the human gut microbiome stretches its involvement in human metabolism, nutrition, and physiology. The role of diet in the human gut microbiome, in particular, has been linked to regulating the risk of several chronic diseases like obesity, type 2 diabetes, cardiovascular disease, and cancer. Researchers now know that diet plays a significant role in shaping the gut microbiome, with studies showing that dietary alterations can induce large, temporary microbial shifts within a short period of time. Monitoring how a change in diet can affect the overall diversity and stability of the human gut microbiome can help researchers understand the extent of microbiota plasticity. The purpose of this paper is to examine the microbiota richness in the human gut microbiome present in a plant-based diet versus a meat-based diet. In order to compare the results of the existing literature on diet and microbiome change, we conducted a meta-analysis of four studies using raw meta-data from the QIITA database, an open-source microbial study platform.

The data from studies previously conducted were extracted from the QIITA database and were analyzed by running alpha diversity significance and beta diversity significance tests in the QIIME2 server, a software for microbiology analysis. The results for the study that evaluated the microbiome richness and the total number of microbiomes in the gut in a high-fat versus a low-fat group showed that there was a statistically significant difference among the diets with clustering shown in the data. The analysis of a study that examined changes in the microbiome of the gut induced by the consumption of a meat-based versus a plant-based diet indicated a statistically significant decrease in species richness in those that consumed a meat-based diet. The results for the study that evaluated the microbiome richness in a high meat versus a high polysaccharide diet showed that there was a statistically significant difference among the diets, with clustering and dispersion shown in the data. Conversely, a study that evaluated the microbiomes of young children that ate a high versus low number of daily servings of animal protein showed insignificant differences between the two groups due to confounding variables. These findings can further contribute to existing data on how a change in diet can affect the types and abundance of microbiota present in the human gut microbiome.

Introduction

The human body has been the subject of intensive study by scientists all around the world. While a lot of discoveries regarding its anatomy and physiology have taken place, there are many components of the organism that continue to be experimentally investigated. One heavily studied aspect of the human body is the microbial community of the gut, and how its stability and species diversity are

impacted by various factors. There are many different factors that can induce changes in the bacterial communities of the gut, one of them being the composition and diversity of diet consumed by humans. Because the dense microbiome ecosystem found in the human gut is fundamental in maintaining healthy physiological function (Osman et al. 2018), disruptions in the stability of the microbial communities induced by stressors such as a change in diet composition may be correlated

with a predisposition of developing disease, although this aspect remains the topic of much research. Furthermore, microbiomes often show plasticity in response to diet, meaning that changes in diet can alter the composition of microbial communities and thus have the potential to shape the overall function of the community (Harris et al. 2019).

The impact of diet on the bacterial species that are in the gut can be exhibited in short-term experimental scenarios, such as studies that alter subjects' diets and then assess microbial shifts in feces the following day. There are also studies which indicate that changes in diet can induce large, yet temporary shifts in the microbiota of the gut within 24 hours (Singh et al. 2017). When tested in long-term scenarios, an examination of how the stressor of diet composition affects the microbiome stability can be useful in regulating the risk of various chronic illnesses, such as inflammatory bowel disease, obesity, type 2 diabetes, cardiovascular disease, and cancer (Singh et al. 2017). Therefore, there are various applications for analyzing the effects of a stressor on the human microbiome. One of the goals for this kind of research is to expand beyond a simple description of microbial species and genes that are present in a particular habitat and connect the composition and the functions of microbial communities to human biology and pathobiology (Turnbaugh et al. 2009). There are various ways to quantify bacterial species composition in response to a stressor, but common DNA-based methods in current use rely on identifying 16S rRNA, a gene that encodes for the 30S small subunit of the ribosome which is easy to amplify due to conserved regions, and is specific to each bacterial species due to regions of the gene that are highly variable (Sweeney and Morton 2015). The reason this specific gene is sequenced is because the 16S rRNA gene is an exclusive gene in prokaryotic organisms that can be used to determine microbial species within samples of the microbiome (Osman et al. 2018). The effect of diet composition can be studied in human gut

microbiomes across all age groups, and many studies use fecal samples from adult humans to examine any changes in the gut microbiome. In this study, we seek to examine how a meat-based diet versus a plant-based diet affects the richness of microbial communities in the guts of adult humans. We will do this by conducting a meta-analysis of four different existing studies that explored changes in microbial richness in response to plant- and meat-based diets. We expect that a meat-based diet will decrease microbiome richness because it will have a smaller range of species diversity than a plant-based diet.

Methods

To analyze the effects of diet composition in the richness of microbial communities in the human gut, we first gathered multiple studies from the QIITA database. QIITA is an open-source platform used for microbial study and houses raw 16S rRNA gene sequencing meta-data collected by other researchers. Each member in our research group downloaded all mapping and BIOM files of one study from the QIITA database and uploaded them to the input file in QIIME using the CyberDuck application. We then proceeded to unzip the raw 16S rRNA meta-data from the procedure folder using specific computational commands, which can be found in the supplementary data section of this paper. This created 3 folders in QIIME: BIOM, mapping_files, and processed_data. To simplify our analysis, we copied the sequence and feature table data from what is the most recent version and put it into the top-level input directory. The next step was to import the BIOMfile and all.seqs.fa into a QIIME 2 FeatureTable[Frequency].qza file and QIIME respectively.

Before running alpha and beta diversity analyses, we rarified the feature table to an even number of reads because if one sample has 1,000 reads while another has 100,000 reads, it could affect how similar they appear even if

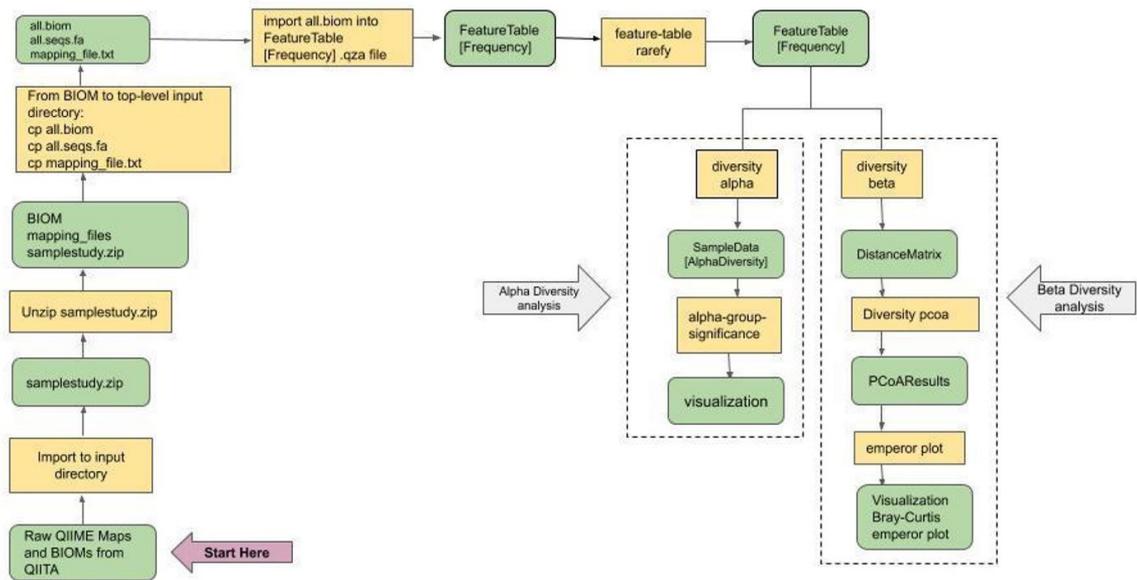


Figure 1. Workflow diagram of methods used for data analysis.

biologically they are the same. To test for beta diversity of our samples, we ran two statistical analysis tests, PERMANOVA and PERMDISP, using the `bray_curtis_distance_martrix.qza` parameter. The PERMANOVA statistical analysis test would tell us if we either have a clustering or dispersion effect by giving us a significant p-value, whereas PERMDISP would give us a significant p-value for dispersion. Next, we ran a test for alpha diversity using the `observed_features_vector` parameter. Finally, we combined the results of the beta and alpha group significance test to analyze our data.

Literature Review

Previous research has examined the impact of various diets on the balance and composition of the gut microbiome, ranging from strictly vegan diets to entirely carnivorous diets. For the purposes of our research, we focused on investigating a plant-based diet and a meat-based diet, where a plant-based diet consists of no animal product consumption and can best be described as a vegetarian diet, in which nutrients

are mainly derived from fruits, vegetables, grains, and legumes (David et al. 2013). A meat-based diet is that in which nutrients are largely derived from animal products and is relatively higher in fat than a plant-based diet (David et al. 2013). Studies have indicated that consumption of a plant-based diet has been associated with an increase in the stability of the gut microbiome, as a higher abundance of microbial species responsible for cell mobility, hydrolyzing enzymes, transport systems, and the synthesis of vital amino acids and vitamins has been observed (De Angelis et al. 2020). In contrast, consumption of a meat-based diet is seen to be correlated with a decrease in the beta diversity of microbial species of the gut, while little difference in alpha diversity between subjects that consumed either a plant-based diet or a meat-based diet has been observed (David et al. 2013). Recall that alpha (α) diversity can be described as the quantification of microbial species diversity and abundance in the gut at the point in time when samples were analyzed, and beta (β) diversity is a measure of the difference

between the baseline microbial composition of the gut and microbial composition of the gut after exposure to the diet being studied (David et al. 2013). In previously published literature that we examined as part of this research project as well as in our data analysis, it was observed that similar phylogenetic groups of microbiotas tend to cluster together in graphical analysis plots of beta diversity like the Bray-Curtis emperor plot, the statistical significance of which is measured by the PERMDISP and PERMANOVA statistical tests. For example, in a study conducted by David et al. in 2013, the analysis of 16S rRNA gene sequencing data indicated that the consumption of a meat-based diet had a larger impact on the composition of the gut microbiome than a plant-based diet, as 22 clusters of microbial species showed a significant change in abundance. However, when participants were exposed to a plant-based diet, only 3 clusters showed statistically significant change in abundance.

We did our own analysis of raw 16S rRNA metadata collected from subjects in four different studies where participants consumed either a predominantly plant-based or meat-based diet, the original results of which we will now describe. The first study we analyzed was conducted by Wu et al. in 2011, and his research group observed the relationship between different diets and their impact on gut microbiota. Specifically, his research team explored how high fat (meat-based) and low fat (plant-based) diet groups changed the composition of gut microbiota. They found that over the 10 days in which they exposed 10 subjects to either diet group (low fat/plant-based or high fat/meat based), changes in enterotypes were significant and rapid. However, the changes were not sufficient enough to categorize individuals into enterotype clusters. They also found that dietary associations to gut microbiomes paralleled a European study that tested diet with high protein and fat versus high carbohydrate and low-fat diet (Wu et al. 2011). The second study we analyzed was originally conducted by David et al. in 2014,

and his research team investigated the effects of a meat-based diet versus a plant-based diet on the composition of the gut microbiome. As described previously in this section, the results of this study indicated that consumption of an animal-based diet was correlated with an increase in beta-diversity in the gut, as the abundance of 22 clusters of bacterial species significantly changed (David et al. 2011). It was observed that adherence to a plant-based diet resulted in only 3 clusters of bacterial species showing changes in abundance over the experimental period (David et al. 2011). They also found that clusters of bacteria seen most prominently in the gut after consumption of an animal-based diet were resistant to bile, which is consistent with the observation that a high fat intake (seen in a meat-based diet) causes higher levels of bile to be secreted (David et al. 2011). A third study by Ruggles et al. focused on examining the human gut microbiome richness between a high meat-based diet (associated with an urban environment) versus a high polysaccharide-based (a plant-based diet associated with a traditional rainforest environment) diet in adults and children. They observed that beta diversity and alpha diversity decreased in individuals that consumed a plant-based diet, while alpha diversity increased in children consuming the same diet (Ruggles et al. 2018). Their overall results indicated that there is greater stability in the adult microbiota, with less resilient bacterial species in children's microbiota that changed most prominently in response to dietary changes made during the experimental period (Ruggles et al. 2018). The last study we analyzed was conducted by Smith-Brown et al. and explored changes in the gut microbiome of children between the ages of 2 and 3 that consumed either a high or low number of daily servings of animal proteins, with a median of 1.88 servings. Their results indicated that consumption of animal protein was associated with a decrease in species richness and diversity in the gut (Smith-Brown et al. 2016). Additionally, they observed that greater consumption of animal protein led

Results

to a decrease in abundance of bacterial species in the Bacteroides phylum, while consumption of vegetable protein (plant-based diet) was associated with a decrease in abundance of bacterial species of the Firmicutes phylum (Smith-Brown et al. 2016). Using the raw metadata from these studies, accessed through QIITA, we generated Bray-Curtis emperor plots that describe beta diversity and box plots that describe alpha diversity, the results of which are described in the following section.

Our beta diversity meta-analysis of the raw metadata from Wu et al. 2011 is depicted in the Bray Curtis emperor plot (Figure 2) and shows statistically significant clustering (PERMANOVA $p = 0.001$) in the high fat and low-fat diet groups. The PERMDISP test of beta diversity came back negative, indicating no significant dispersion of metadata. One interesting thing about these results is that there was another group of clusters at the far-left

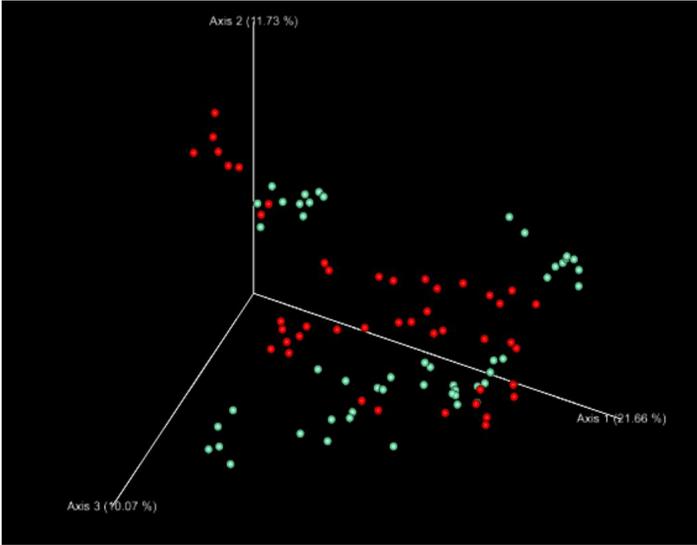


Figure 2. Bray-Curtis emperor plot of low fat (green) vs. high fat (red) groups show a statistically significant beta diversity (PERMANOVA $p = 0.001$, PERMDISP $p =$ negative)

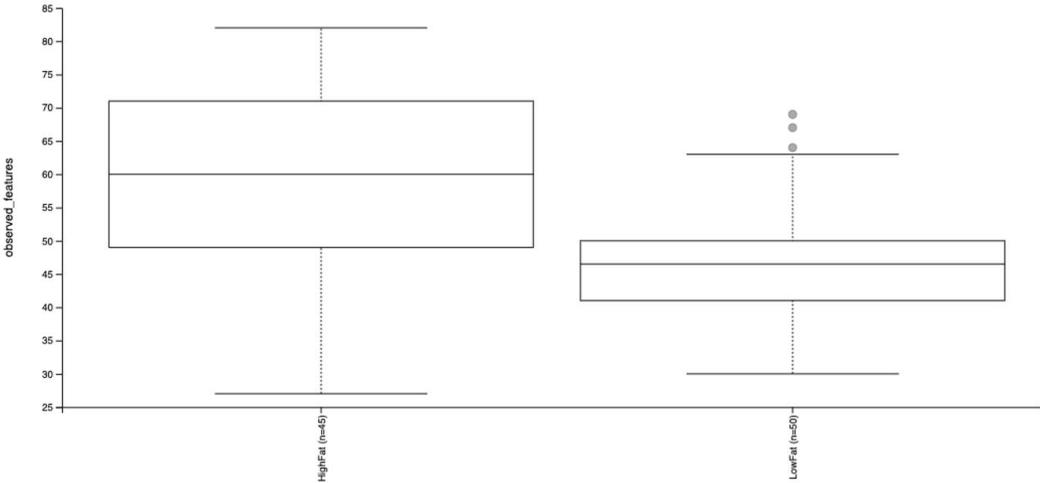


Figure 3. Observed features vector box plot of low fat (green) vs. high fat (red) groups show a statistically significant alpha diversity ($p = 0.000$)

region of the Bray-Curtis emperor plot (Figure 2). We investigated different categories in the metadata to see what drove this result and found that gender seemed to be the driving factor as the left clustering corresponded to samples of male participant data. Next, we ran an alpha diversity test on the raw metadata and found that a greater consumption of meat increased the alpha diversity of participants' microbiomes, as seen in the Observed Features Vector box plot (Figure 3; $p = 0.000$).

We next analyzed the raw meta-data from the David et al. study. Our meta-analysis of the beta diversity of the individuals that consumed either of the two dietary groups indicated a statistically significant difference in the species richness of the gut microbiome. The Bray-Curtis emperor plot (Figure 4) showed slight clustering (PERMANOVA $p = 0.001$) and obvious dispersion (PERMDISP $p = 0.001$). As can be

seen in Figure 4, there is no obvious clustering in the meat-based and plant-based metadata points, but there is some dispersion in the data points that corresponds to individuals that consumed a meat-based diet. This dispersion indicates that there is more variability in the gut microbiome of all the individuals that consumed a meat-based diet, meaning that their microbiomes were more unstable in terms of species richness than individuals that consumed a plant-based diet. Something to note in the results of our beta diversity meta-analysis is that while both PERMANOVA and PERMDISP tests were statistically significant, either clustering or dispersion of data could have occurred. However, the test cannot distinguish between the two. Overall, the Bray-Curtis emperor plot more obviously indicated dispersion of data points, and, therefore, we can say that there is a statistically significant difference in the beta diversity of individuals that consumed

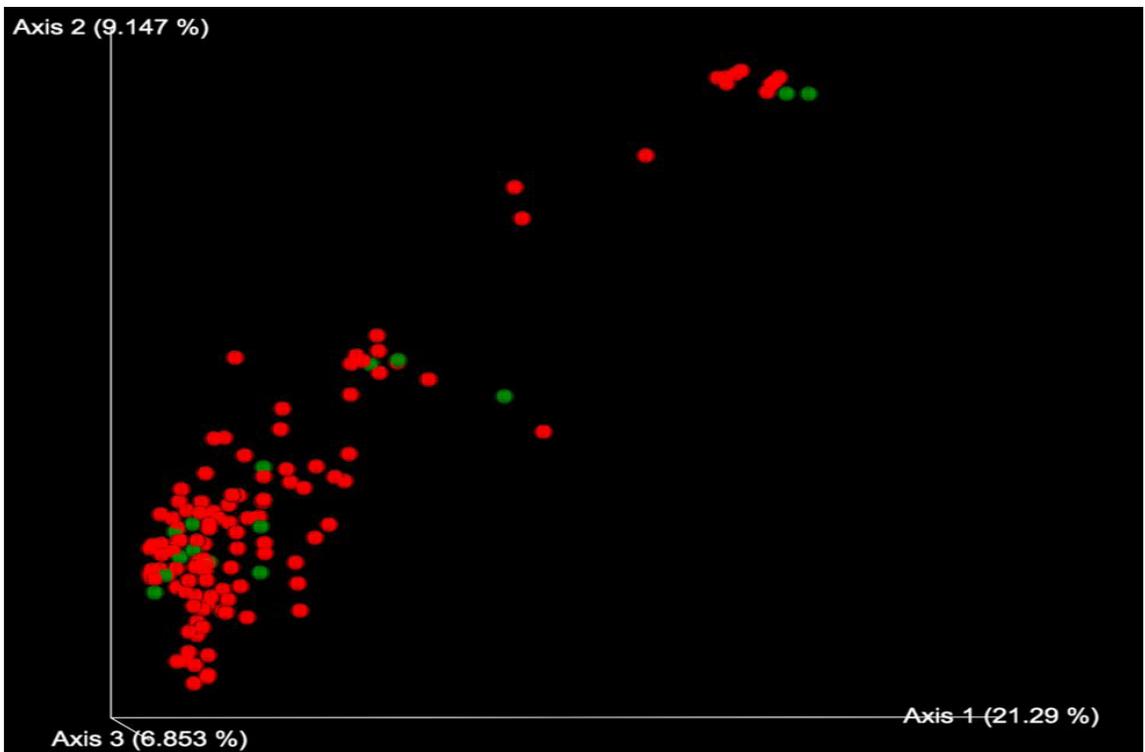


Figure 4. Bray-Curtis emperor plot of plant-based (green) vs. meat-based (red) groups show a statistically significant beta diversity (PERMANOVA $p = 0.001$, PERMDISP $p = 0.001$)

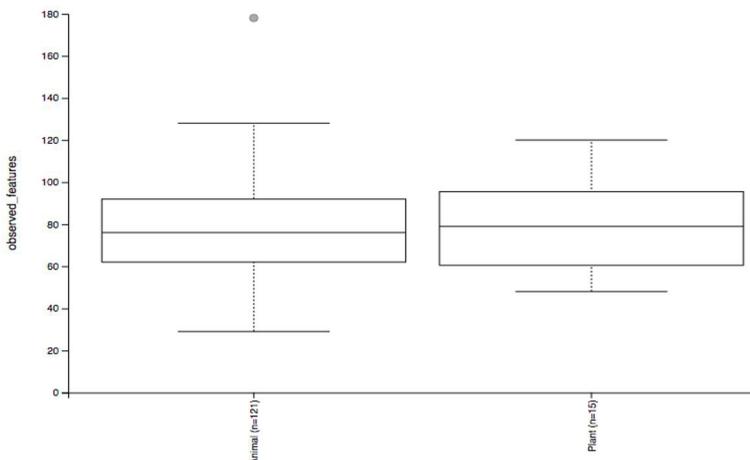


Figure 5. Observed features vector box plot of plant-based (green) vs. meat-based (red) groups show a statistically significantly alpha diversity ($p = 9.33 \times 10^{-13}$)

the plant-based diet and meat-based diet. We also did an alpha diversity analysis of the raw metadata and found that there was a statistically significant difference (Figure 4; $p = 9.33 \times 10^{-13}$) in the observed species richness in the gut microbiomes of individuals that consumed a meat-based diet versus a plant-based diet. Specifically, the consumption of a plant-based diet resulted in higher species richness than a meat-based diet, as indicated by the median of Figure 4. The effect size of these results is small, as the difference between the median of observed species in these two diet groups examined is small.

Our meta-analysis of beta diversity using the raw meta-data from the Ruggles et al. study resulted in statistically significant results, as seen in the Bray-Curtis emperor plot (Figure 6), which showed some subtle clustering (PERMANOVA $p = 0.001$) of the high meat and high polysaccharide diets and obvious dispersion of the data (PERMDISP $p = 0.009$), indicating dispersion among the high meat and high polysaccharide diets. Overall, while there is a difference in beta-diversity of the data examined, we cannot formally say that there is clustering or dispersion, though the Bray-Curtis emperor plot indicated dispersion of metadata

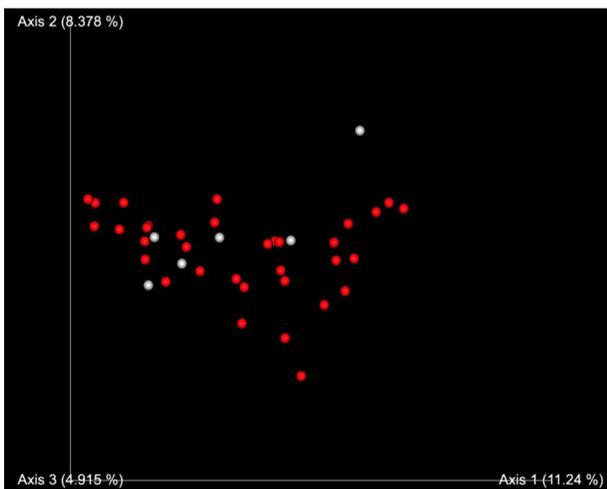


Figure 6. Bray-Curtis emperor plot of high meat (red) vs. high polysaccharide (white) groups show a statistically significant beta diversity (PERMANOVA $p = 0.001$, PERMDISP $p = 0.009$)

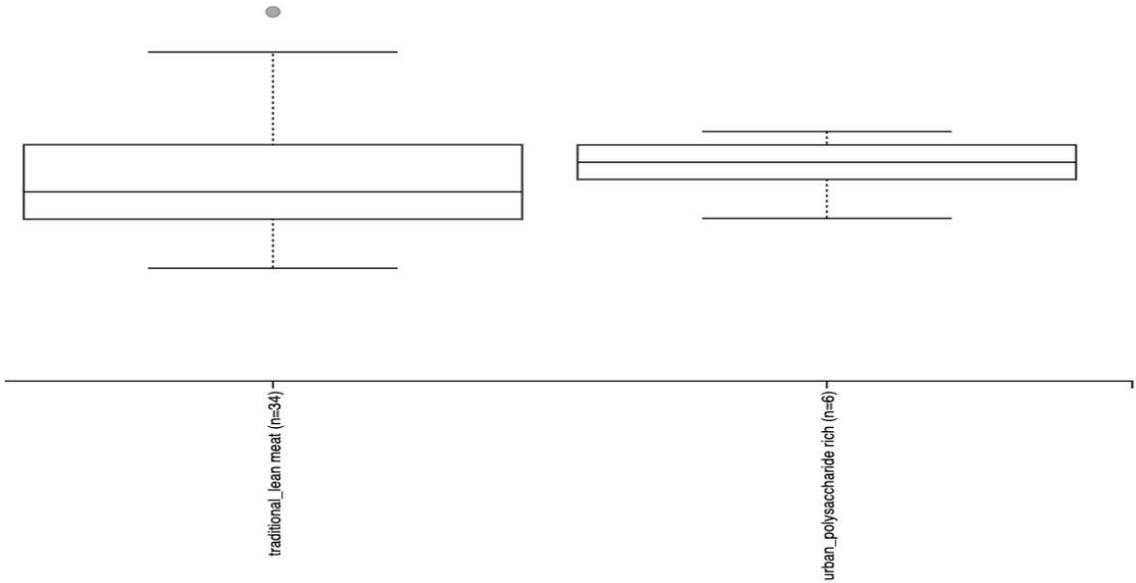


Figure 7. Observed features vector box plot of high meat (red) vs. high polysaccharide (white) groups show a statistically significantly alpha diversity ($p = 0.0004$)

most clearly. Next, we ran an alpha diversity analysis of the raw metadata and found that individuals who consumed a high meat-based diet had a statistically significant (Figure 7; $p = 0.0004$) lower species richness in their gut microbiome compared to those who consumed a high polysaccharide-based diet. This result can be visualized in the Observed Features Vector box plot (Figure 7), where the median is lower in the high meat diet group compared to that of the high polysaccharide group, which again indicates that the species richness of the gut decreased in individuals who consumed a high meat diet.

Lastly, we analyzed the raw-metadata from the Smith-Brown et al. study. Again, we conducted beta diversity and alpha diversity meta-analysis of the raw data collected by Smith-Brown et al., the results of which came back to be not statistically significant. The Bray-Curtis emperor plot (Figure 8) showed minimal clustering (PERMANOVA $p = 0.232$) and no significant

differences in dispersion (PERMDISP $p = 0.210$) between diet categories examined. The alpha diversity analysis of this data (Figure 9) indicated that individuals who consumed a high meat diet had lower species richness in their gut microbiome than those who consumed a low meat diet, but as mentioned previously, this data was not statistically significant.

Discussion

A meat-based diet was significantly associated with a decreased microbiome richness when compared to a plant-based diet. The beta group significance tests, ran for 3 out of the 4 studies analyzed, showed significant differences between the meat-based and plant-based groups based on the computed p-values for PERMANOVA and PERMDISP, and visualized by the Bray-Curtis emperor plots. The alpha diversity observed features vector box plots for 3 out of the 4 studies showed the meat-based group as having a lower median, or less

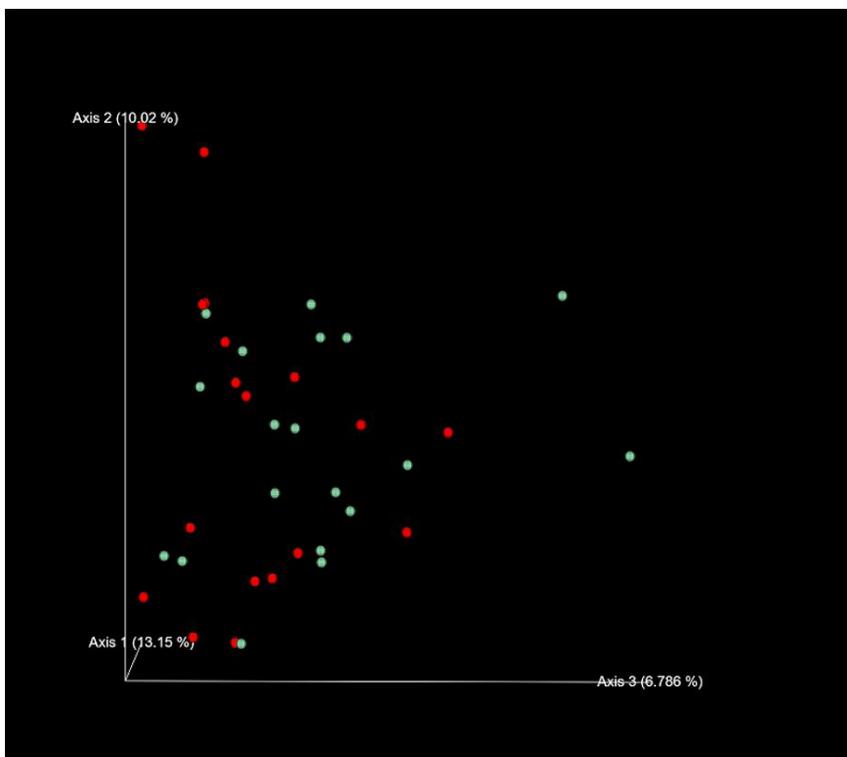


Figure 8. Bray-Curtis emperor plot of high (red) vs. low (green) daily servings of animal protein does not show a statistically significant beta diversity (PERMANOVA $p = 0.232$, PERMDISP $p = 0.210$)

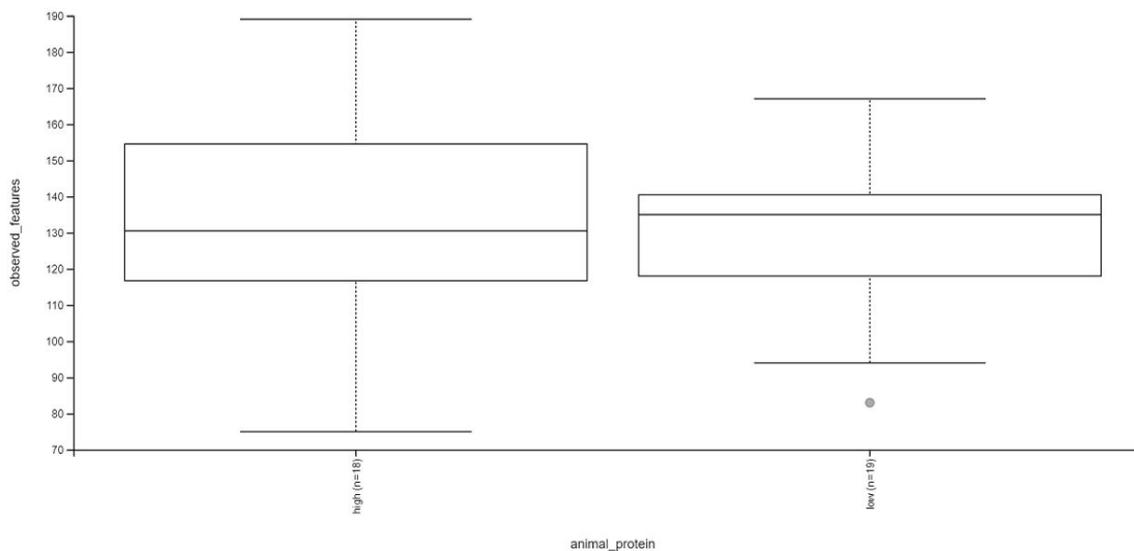


Figure 9. Observed features vector box plot of high (left) vs. low (right) daily servings of animal protein does not show a statistically significant alpha diversity ($p = 0.964$)

richness, than the plant-based diet group. In the high fat versus low fat analysis done, gender of participants played a role in driving clustering between the two clear clusters seen on the Bray-Curtis emperor plot. This was an interesting piece of information that was observed after sorting the data by a different category.

In regard to our results for the analysis of the raw meta-data from the Smith-Brown et al. study, there were confounding variables that were attributed to the results found for both beta diversity and alpha diversity. The participants in this study were between the ages of 2 and 3. This is of significance because during this time period, eating habits are being established that can stimulate or inhibit the growth of healthy gut flora (Smith-Brown et al. 2016). Additionally, the participants in this study did not adhere to a strict meat-based or plant-based diet; rather they recorded each individual’s daily servings of various food groups before examining their microbiome. In a future analysis, it would be helpful to conduct follow up studies on these participants as they age to see if there are any

significant changes in beta diversity based on continued high versus low daily servings of animal protein.

Our findings are consistent with what is already known about the important role diet plays in maintaining a healthy and diverse gut microbiome. Plant-based foods contain fibers that promote fermentation and increase diversity by stimulating the growth and regulation of healthy gut microbiota (Ferranti et al. 2014). In future studies, it would be interesting to see if the difference in beta and alpha diversity would be more significant between people who adhere to a strict carnivore diet versus a strict vegan diet.

In Table 1, the up and down arrows indicated in the alpha diversity column represent the high meat-based diet’s median compared to the plant-based group’s. Three out of the four studies showed a high meat-based diet as having a lower median, meaning less species richness. The negative sign in the high versus low serving’s box means that it was not statistically

Diet	Alpha Diversity	PERMANOVA	PERMDISP
High Fat (meat-based) vs. Low Fat (plant-based) Wu et al. 2011	↑	+	-
Meat-based vs. Plant-based David et al. 2014	↓	+	↑
High Meat vs. High Polysaccharide Ruggles et al. 2018	↓	+	↑
High vs. Low Number of Daily Servings of Animal Protein Smith-Brown et al. 2016	- ↓	-	-

Table 1. Summary of alpha and beta diversity analysis results for each study examined.

significant. The arrows indicated in the PERMDISP column mean that the high meat-based group had a higher significant difference than the plant-based diet group.

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Supplemental Information

Supplemental data sets from the scholarly articles the authors referenced are available on our website at: <https://www.uwbcrow.com/>

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