

### **Take-Away Points**

- There is little difference between High Fat, Saccharin, and Stevia samples.
- The majority of differences are between Low Fat and the other 3 treatments (all of which include a high fat diet)
- Differences in sex were detected, which highlights the different biases that may be present when working with animal models

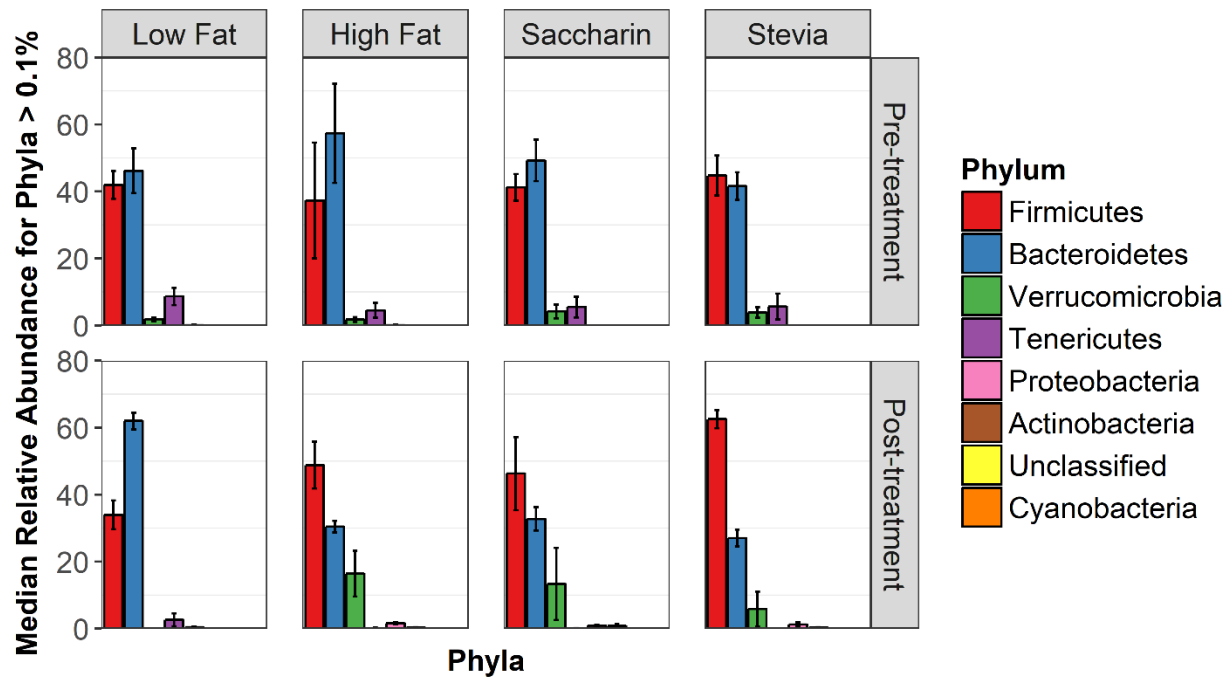
### **Description**

- This is an updated summary of the follow-up analyses to Sarah's thesis.
- I consolidated and polished up some of the figures, with the idea that some of these may be used in the publication. Looking forward to hear your thoughts on what plots to include and how they should be modified.
- A few differences between Sarah's analysis and my analysis:
  - I perform my analysis at the OTU-level (97% sequence similarity, akin to species-level)
  - I included only individuals with both pre-treatment and post-treatment samples because I run a few paired tests and want to keep the samples consistent throughout all analyses. I removed 4 individuals, resulting in the following final sample count:

Treatment	N	Female	Male
High Fat	9	4	5
Low Fat	10	5	5
Saccharin	9	5	4
Stevia	8	4	4

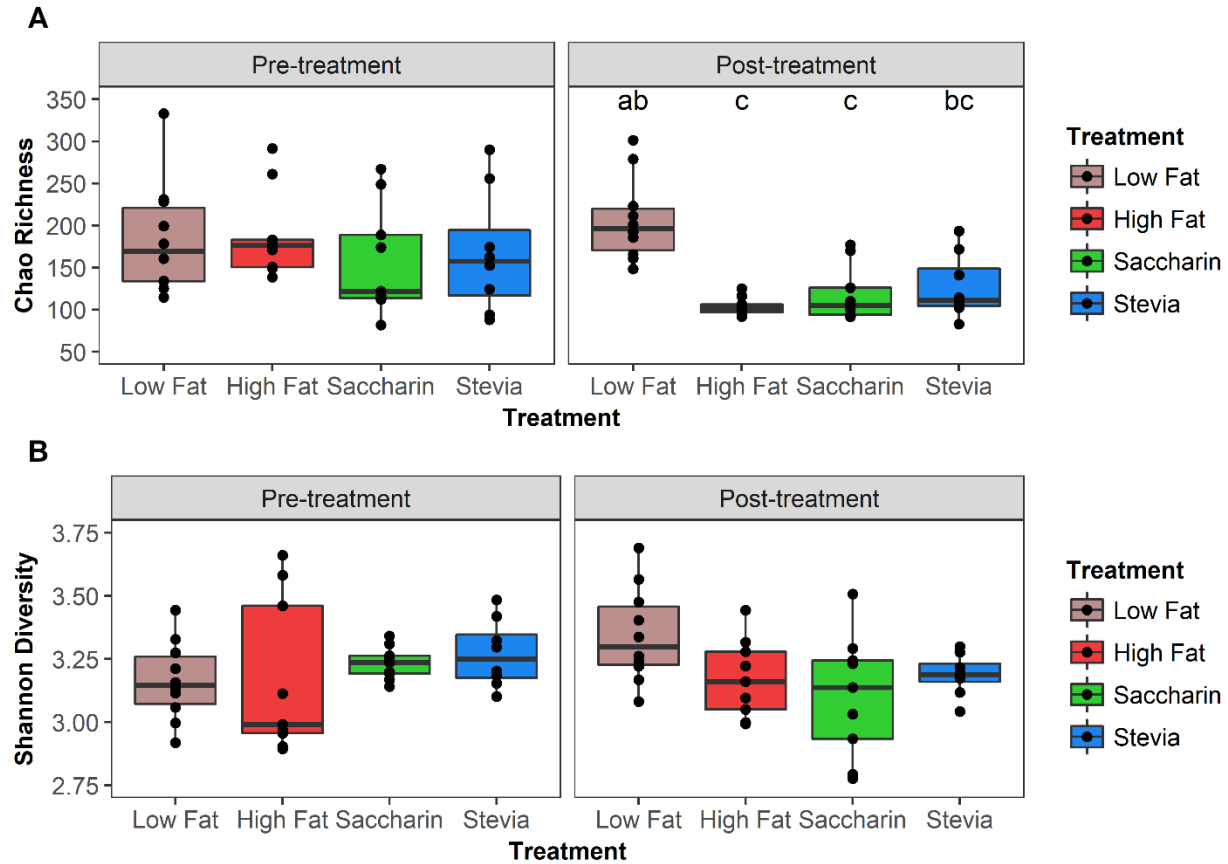
### Phylum-Level Composition

- The phyla are listed in order of highest overall relative abundance to lowest overall relative abundance.
- Phyla with a total relative abundance of < 0.1% were removed.
- Error bars represent the interquartile range.



- Dominant phyla include Firmicutes and Bacteroidetes, with lesser contributions by Verrucomicrobia and Tenericutes.
- Minor contributions are by Proteobacteria, Actinobacteria, unclassified bacteria, and Cyanobacteria.

### Within-Sample Diversity (Alpha)



- Shared letter (or no letters in a panel) = groups are not significantly different

- No shared letter = groups are significantly different

Chao is an estimate of richness (# unique OTUs exist in a particular sample).

- Post-treatment groups are significantly different (adj p-val = 0.004). Specifically:
  - High Fat vs. Low Fat
  - Low Fat vs. Saccharin
- High Fat Pre-treatment and Post-treatment are significantly different (adj p-val = 0.018)

Shannon's Index is an estimate of diversity, taking into account both the presence/absence and abundance of OTUs.

- No significant differences.
- This suggests that differences in within-sample diversity are driven by presence/absence of OTUs rather than their relative abundances.

### **Between-Sample Diversity (Beta)**

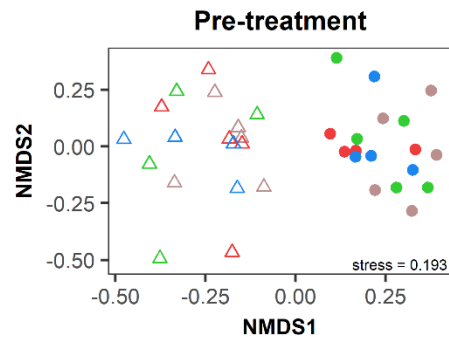
I used the Bray-Curtis dissimilarity metric, which considers both OTU presence/absence and weight (abundance).

In the ordination, each point is 1 sample. The distance between points is inversely related to how similar the samples are.

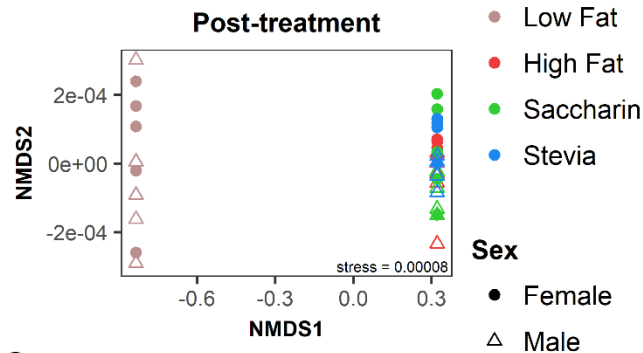
Closer points = more similar.

Farther points = less similar.

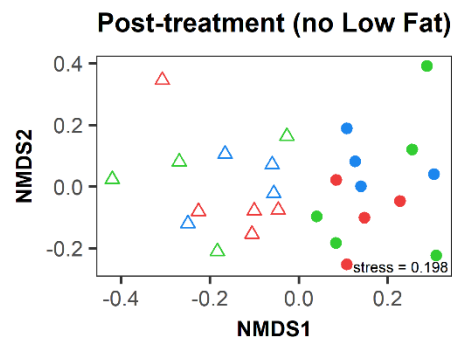
**A**



**B**



**C**



- Within each treatment, Pre vs. Post are significantly different (saccharin adj pval = 0.002, all other adj pvals = 0.001)

To determine to what extent the categorical factors contribute to between-sample differences, I used PERMANOVA with interaction terms:

Factor	P-value	R2
Time	0.001	0.25838
Treatment	0.001	0.12309
Sex	0.001	0.07370
Time x Treatment	0.001	0.09180
Time x Sex	0.001	0.05753
Treatment x Sex	0.259	0.02191
Time x Treatment x Sex	0.298	0.02160
Residuals	0.352	0.35198

- Time = Pre-treatment, Post-treatment
- Treatment = Low Fat, High Fat, Saccharin, Stevia
- Sex = Female, Male

- Time explained the most variability in between-sample diversity, followed by treatment
- Sex, the interaction between time and treatment, and the interaction between time and sex were also significant

**Pre-treatment:**

- There is no significance between treatments (adj pval = 0.755,  $R^2 = 0.065$ )
- There *is* a significant difference between sex (adj pval = 0.002,  $R^2 = 0.344$ ).

### Post-treatment:

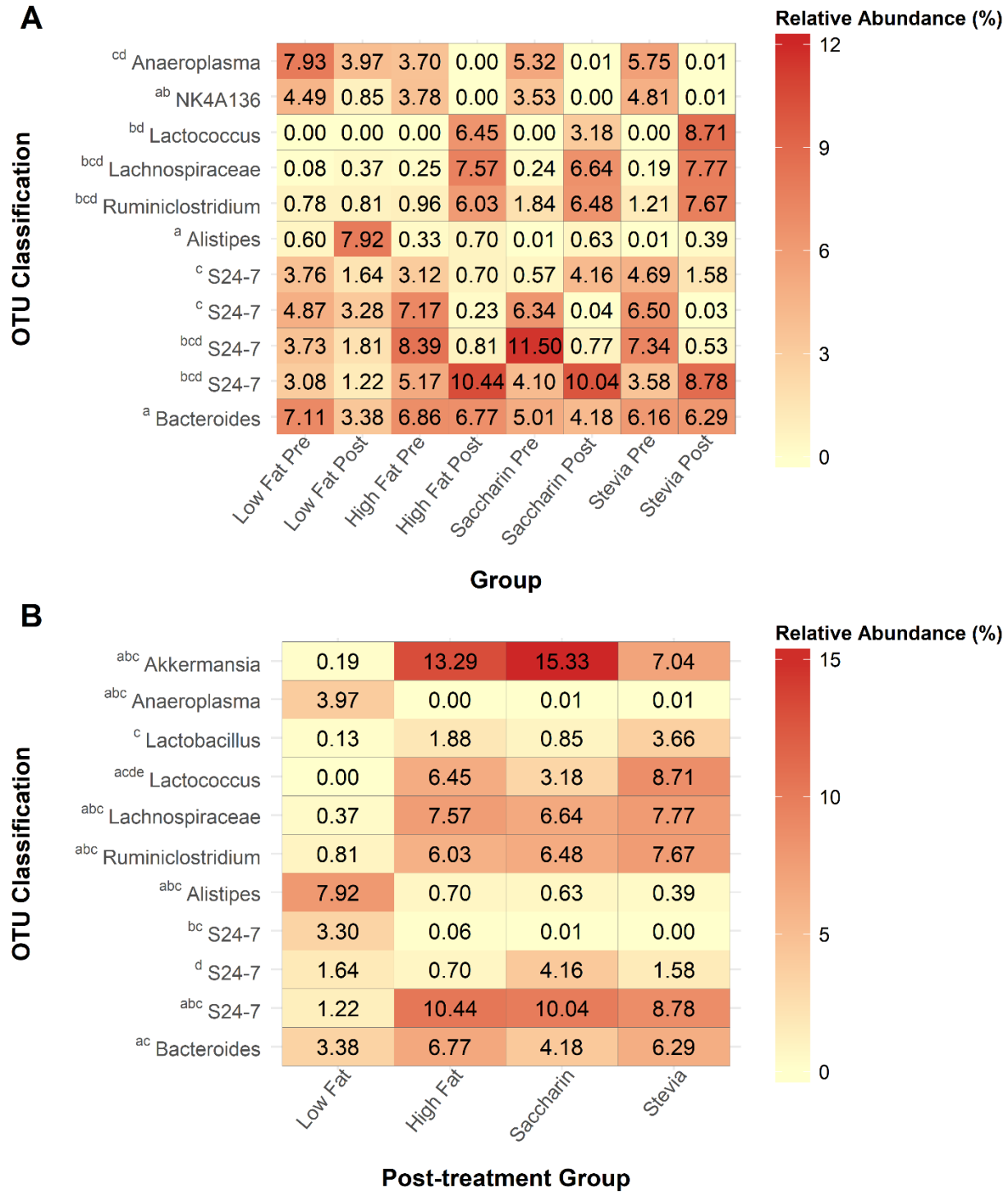
- Treatment explains almost half the variability in between-sample diversity
- Sex explains a smaller amount
  - Note: When testing Sex alone, there was no significance. However, there was a significance after accounting for differences in Treatment

Factor	P-value	R <sup>2</sup>
Treatment	0.001	0.474
Sex	0.036	0.042

- Pairwise comparisons between treatments:
  - Low Fat is significantly different from all other treatments (all adj pvals = 0.002)
  - Saccharin and Stevia are significantly different (adj pval = 0.047)
- Sex comparisons within each treatment:
  - Rationale:
    - Given that Sex was significant only after accounting for differences in Treatment, I wanted to see if the differences identified in my previous bullet point held true for females and males in each treatment group
  - Females:
    - Low fat is significantly different from all other treatments (all adj pvals = 0.018)
    - Saccharin and Stevia trend towards a difference (adj pval = 0.053)
  - Males:
    - Low fat is significantly different from all other treatments (all adj pvals = 0.018)
    - Saccharin and Stevia are *not* different (adj pval = 0.441)

### Important OTUs

- I identified the OTUs that contribute the most to variation in between-sample diversity using a similarity percentages (SIMPER) analysis and pairwise comparisons.
- These OTUs are the most abundant and/or variable in the samples.
- Below are 2 heatmaps that show all significant OTUs for:
  - A) Each treatment comparing Pre- and Post-treatment
  - B) All Post-treatment samples
- OTU classification is shown for the most resolved taxonomic level that was classified (ranges from Family – Genus)
- Letters represent the comparison for which the OTU was significant (see text following heatmap)



Heatmap A:

- a = Different between Low Fat Pre vs. Post
  - 3 OTUs explain 12.03% of the difference in between-sample diversity
- b = Different between High Fat Pre vs. Post
  - 6 OTUs explain 22.89% of the difference in between-sample diversity

- c = Different between Saccharin Pre vs. Post
  - 7 OTUs explain 29.20% of the difference in between-sample diversity
- d = Different between Stevia Pre vs. Post
  - 6 OTUs explain 26.17% of the difference in between-sample diversity

## Heatmap B:

- a = Different between Low Fat and High Fat
  - 8 OTUs explain 35.35% of the difference in between-sample diversity
- b = Different between Low Fat and Saccharin
  - 7 OTUs explain 32.22% of the difference in between-sample diversity
- c = Different between Low Fat and Stevia
  - 10 OTUs explain 37.13% of the difference in between-sample diversity
- d = Different between High Fat and Saccharin
  - 2 OTUs explain 8.72% of the difference in between-sample diversity
- e = Different between Saccharin and Stevia
  - 1 OTU explains 6.19% of the difference in between-sample diversity

## Male vs. Female

- These results are for Pre-treatment only.
- No OTUs were detected within Post-treatment samples overall.

Variation Explained (%)	Adj Pval	Phylum	Class	Order	Family	Genus	Female Rel Abund (%)	Male Rel Abund (%)
9.74	7.17e-07	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7	Unclassified	0.123	8.74e-05
7.97	3.39e-06	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified	0.002	0.102
5.58	1.04e-05	Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	Anaeroplasma	0.092	0.023
5.85	1.61e-05	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7	Unclassified	0.081	0.014
7.06	1.66e-3	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7	Unclassified	0.039	0.114

- 5 OTUs explain 36.21% of the difference in between-sample diversity
- Do you prefer a heatmap or table for displaying these results?

## Post-treatment correlation with physiology

- The tests described here are exploratory and meant for food for thought on whether we might want to pursue a story that's more weighted towards the Low Fat vs. High Fat differences (since those are the main differences we see).
- There is no difference in post-treatment body weight and blood glucose for High Fat, Saccharin, and Stevia.
- However, I was curious if the variation in post-treatment body weight and blood glucose for only the 3 high fat diets was correlated with certain taxa (using miLineage package)



- No correlations between taxa and body weight or blood glucose were detected
- Depending on the story we choose, it may or may not be of interest to look for correlations between all post-treatment groups
- In this comparison, I do see significant taxa that are correlated with body weight or blood glucose.