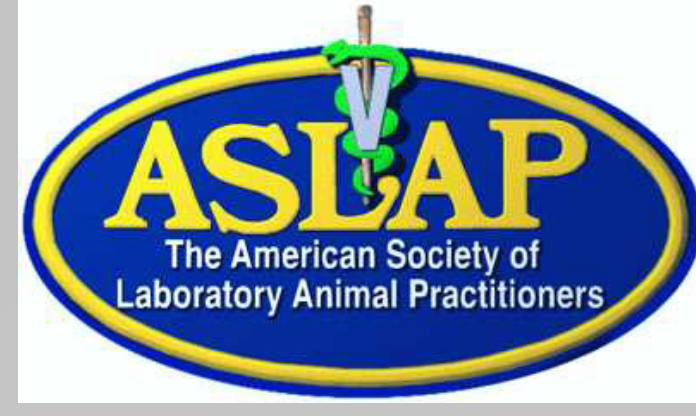


Effects of retaining feed on hopper pellet mycobiome and microbiome



Authors: Shaina Furman¹, Aaron Ericsson², Craig Franklin², Samantha Gerb²
 Affiliations: Michigan State University, East Lansing, MI¹; University of Missouri, Columbia, MO²

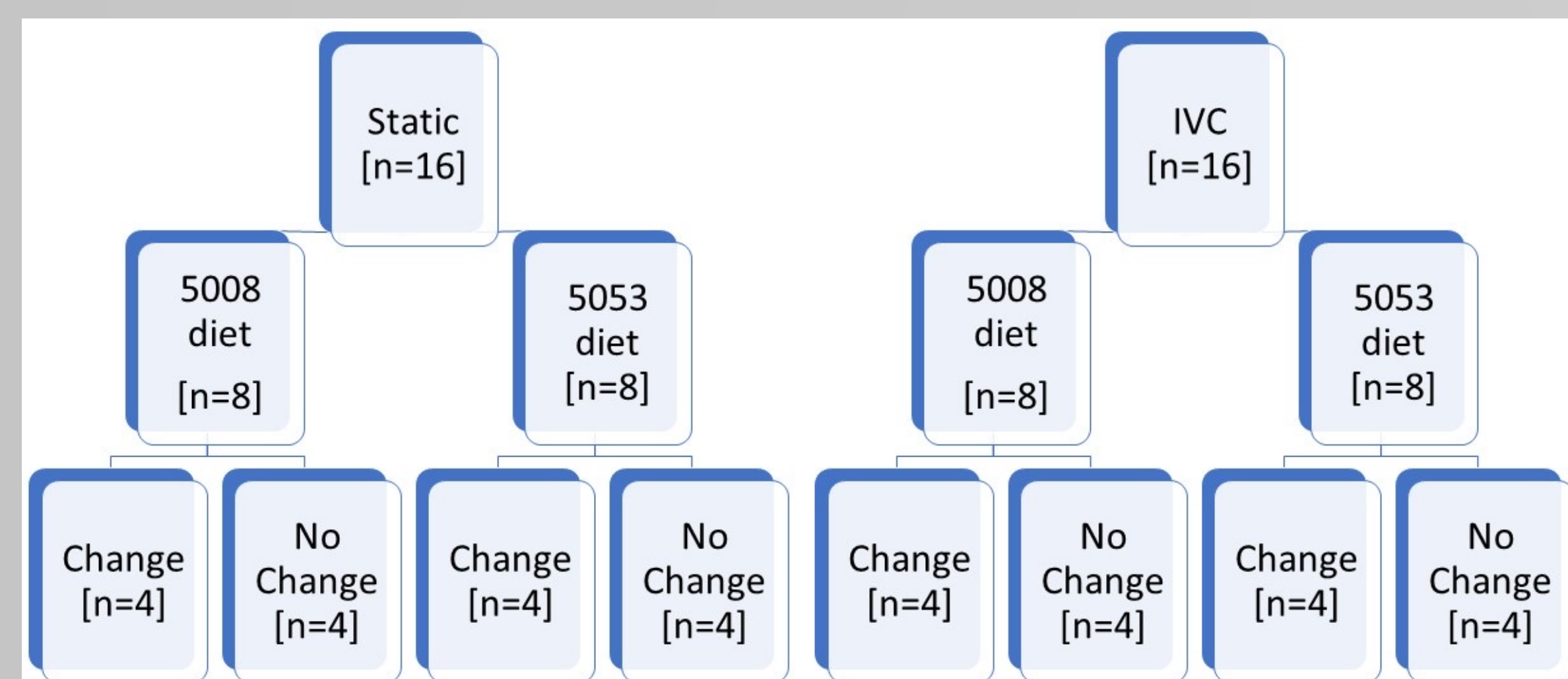


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Significance/Rationale

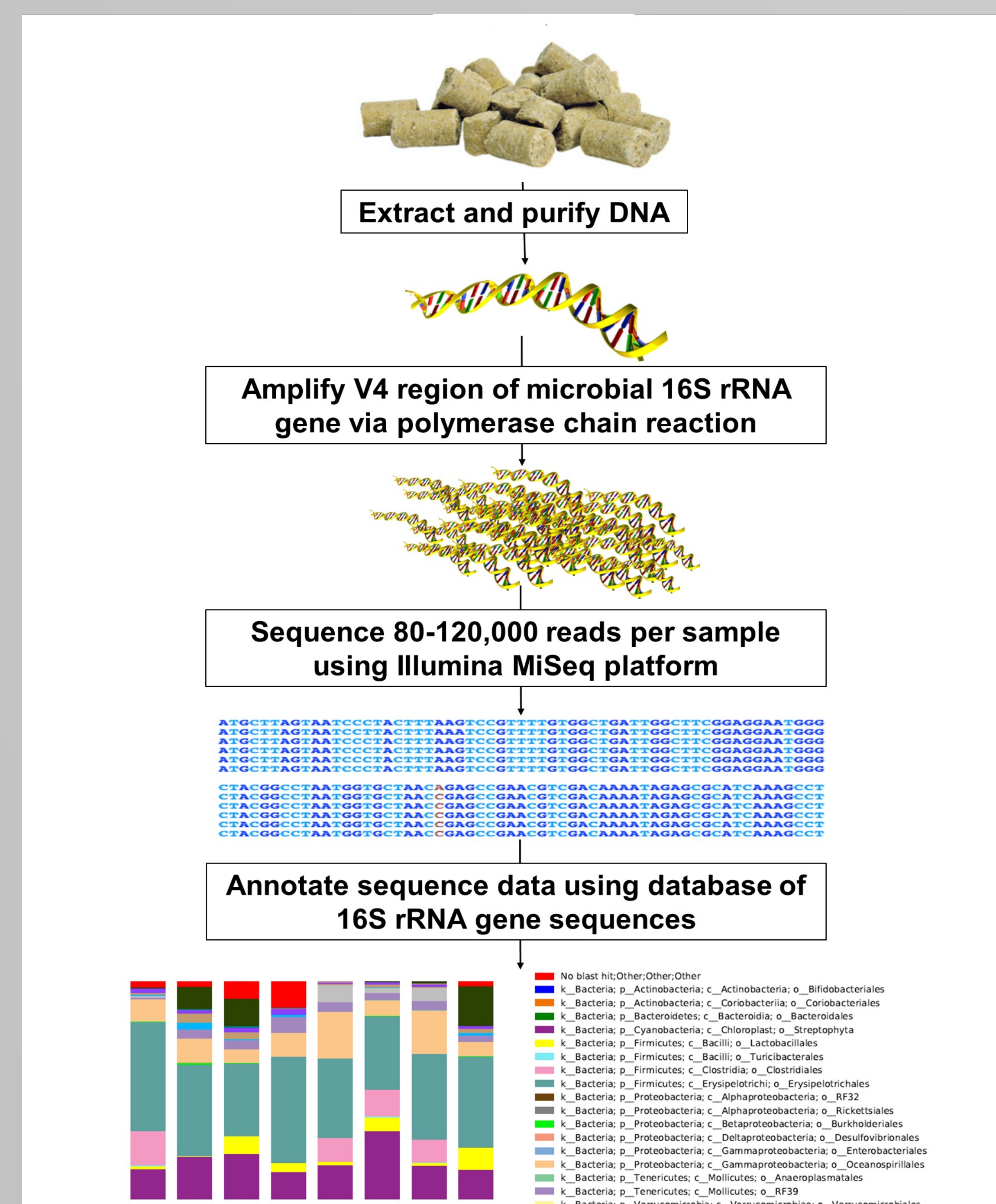
Mice used in research are fed commercial diets that are commonly autoclaved and/or irradiated prior to use to ensure minimal contaminants are introduced into mouse cages. During cage changes, some institutions will dump the food out of concerns of spoilage, while other institutions have foregone this step as a cost saving measure. As an indicator of feed quality, we are assessing whether the mycobiome and microbiome differs in feed that has been retained in hoppers vs feed that is regularly changed. Our hypothesis is that no differences will be seen between these groups, which would build upon a rationale for not dumping food at cage change.

Experimental Design



- 3-5 mice per cage
- Independent variables include change/no change, sex (4 males and 4 females per group), diet and housing type (static vs IVC)
- Food pellets closest to mice were collected each week for 4 weeks

Sample Analysis



Results

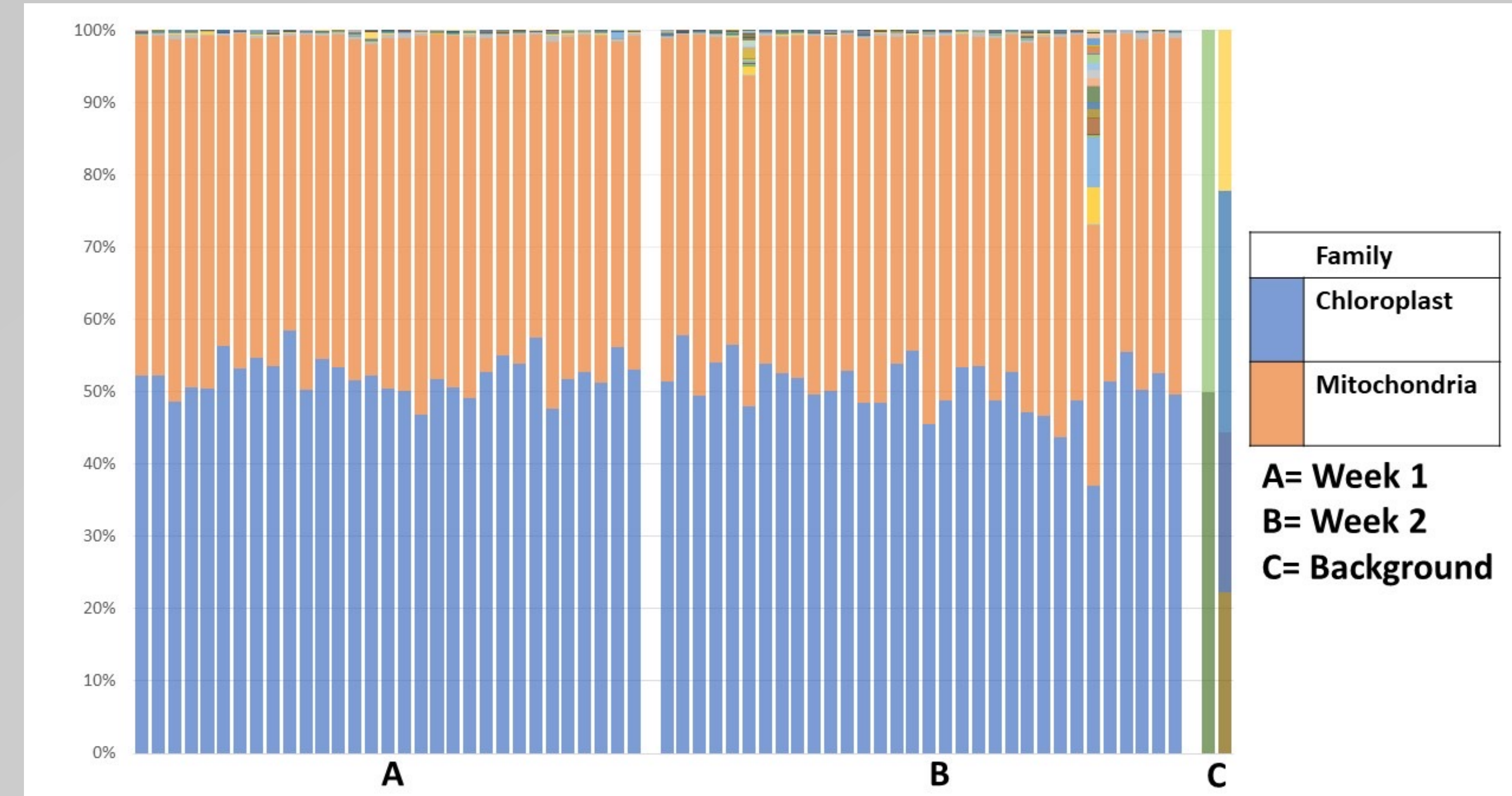


Figure 1. Family level analysis of microbiome in feed samples. The majority of the composition is from mitochondria and chloroplasts, likely from plant material composing food. Other minor species present may represent oral microbiome contaminants from mice.

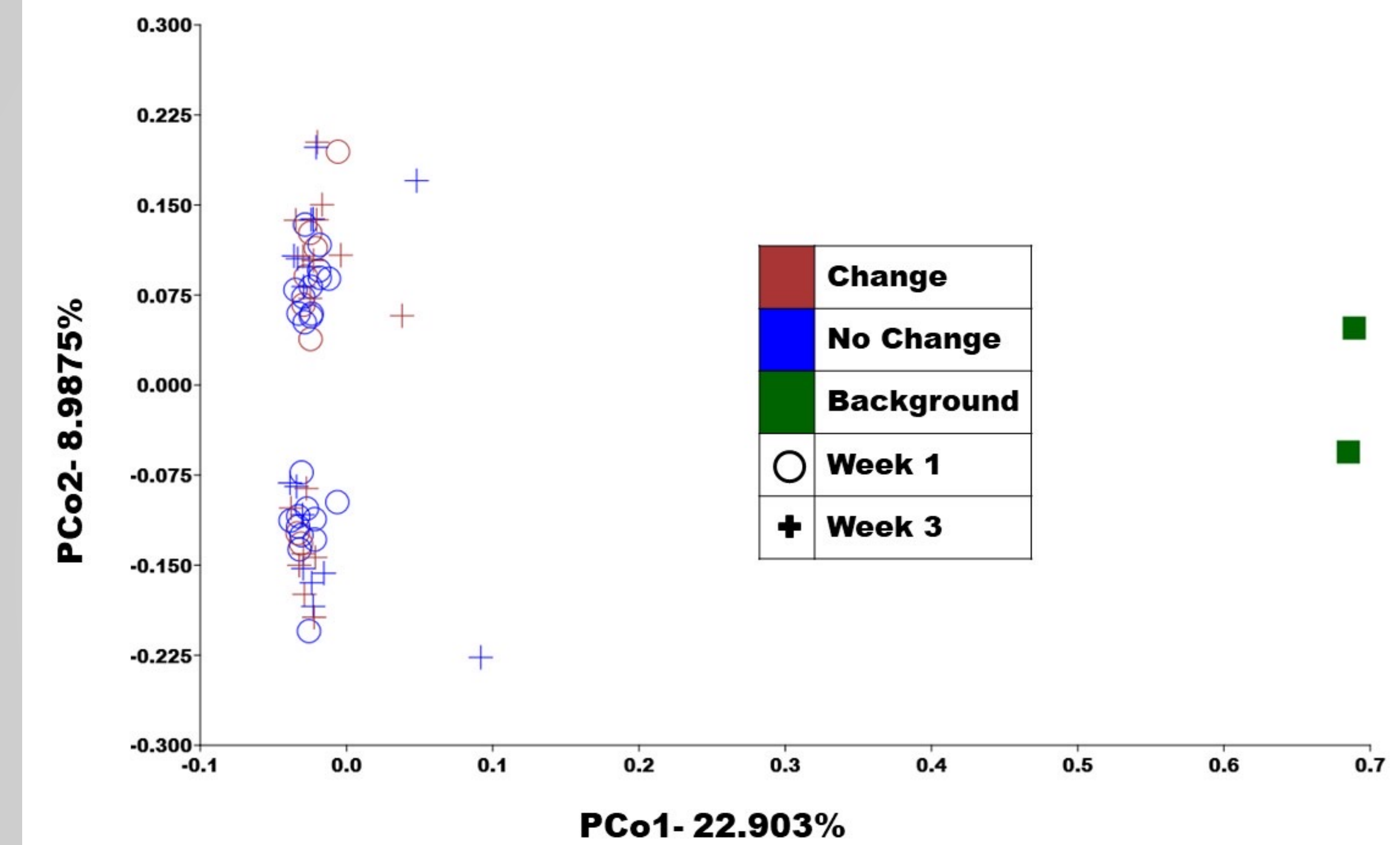


Figure 2. PCoA using Bray-Curtis similarity index. Background samples are significantly different than other samples tested. While no differences are apparent between the change and no change groups, a bimodal distribution of data is apparent in collected food samples.

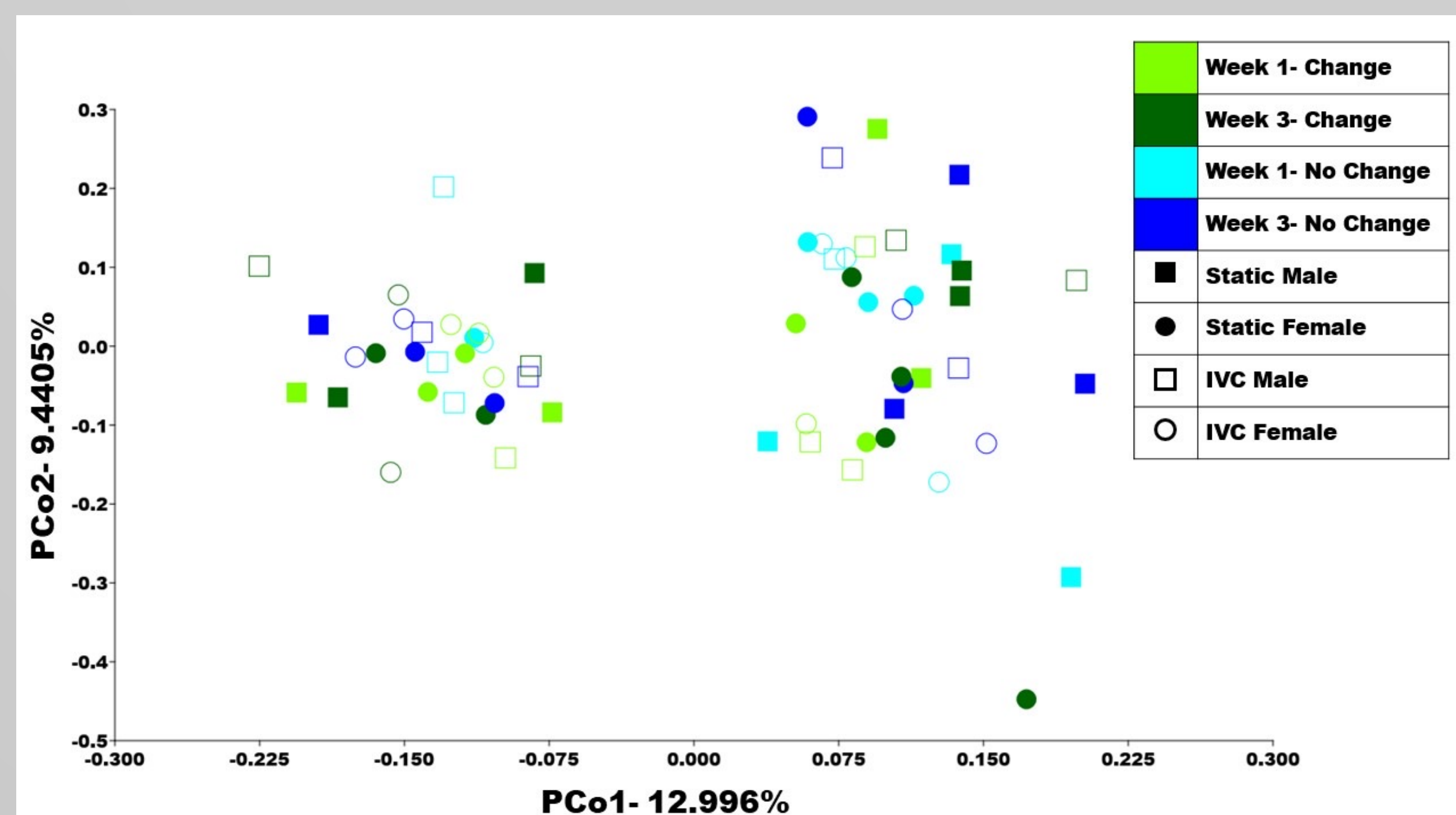


Figure 3. PCoA using Bray-Curtis similarity index. Background samples were removed for simplicity. No differences were readily apparent in the variables change/no change, sex and week.

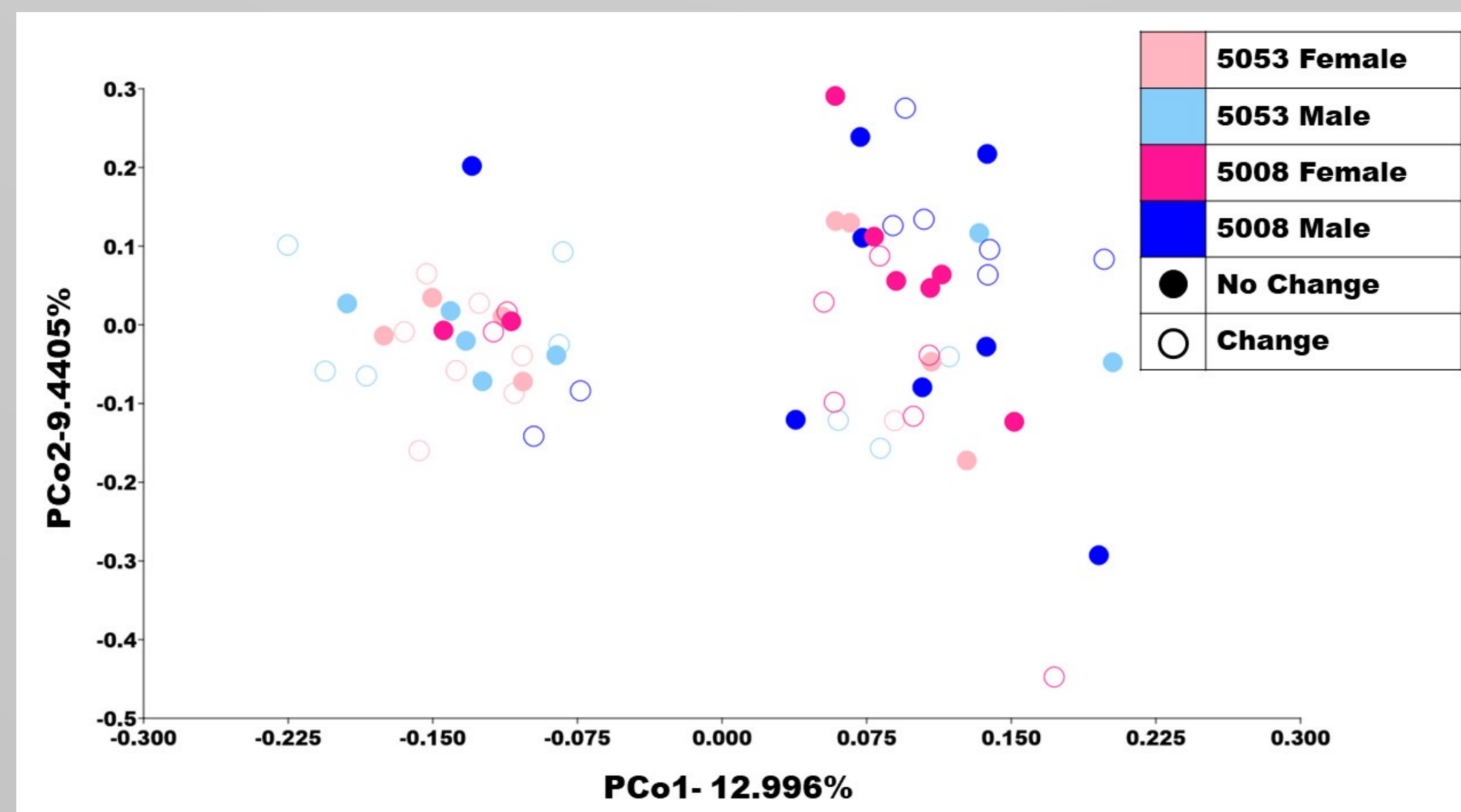


Figure 4. PCoA using Bray-Curtis similarity index. Background samples were removed for simplicity. No differences were readily apparent in the variables change/no change, sex and food type.

	C_08_R_1	NC_08_R_1	NC_08_IVC	C_08_IVC_1	C_53_R_1	NC_53_R_1	C_53_IVC_1	NC_53_IVC_1	C_08_R_3	NC_08_R_3	NC_08_IVC_3	C_08_IVC_3	C_53_R_3	NC_53_R_3	C_53_IVC_3	R_53	R_08
C_08_R_1		0.4531	0.0937	0.8448	0.7751	1	0.3403	0.9413	0.1433	0.1119	0.5356	0.188	0.5687	1	0.8003	0.478	0.1887
NC_08_R_1	0.4531		0.0582	0.2866	0.4567	0.6223	0.4249	0.4807	0.2208	0.057	0.7783	0.5122	0.0284	0.7972	0.0329	0.0277	0.1991
NC_08_IVC_1	0.0937	0.0582		0.1691	0.2835	0.8314	0.1427	0.3478	0.0397	0.0295	0.1125	0.0272	0.1487	0.5155	0.1485	0.1164	0.1896
C_08_IVC_1	0.8448	0.2866	0.1691		0.3125	0.3423	0.0877	0.2027	0.058	0.0543	0.0271	0.0288	0.1396	0.4789	0.3544	0.0554	0.1983
C_53_R_1	0.7751	0.4567	0.2835	0.3125		0.5704	0.55	0.6528	0.0911	0.1096	0.1365	0.0852	0.3917	0.7737	0.7136	0.3182	0.1956
NC_53_R_1	1	0.6223	0.8314	0.3422	0.5704		0.314	0.4729	0.2232	0.23	0.2249	0.4205	0.1189	0.6258	0.1121	0.0568	0.2454
C_53_IVC_1	0.3403	0.4249	0.1427	0.0877	0.55	0.314		0.084	0.0316	0.0275	0.0385	0.0288	0.0557	0.5484	0.1458	0.0879	0.2023
NC_53_IVC_1	0.9413	0.4807	0.3478	0.2027	0.6528	0.3729	0.084		0.049	0.0279	0.222	0.0839	0.2529	0.3655	0.0899	0.0929	0.1854
C_08_R_3	0.1433	0.2208	0.0397	0.058	0.0901	0.2232	0.0316	0.059		0.0555	0.2763	0.3054	0.0292	0.2585	0.8302	0.0271	0.2019
NC_08_R_3	0.1119	0.057	0.0295	0.0342	0.1096	0.23	0.0275	0.0279	0.0555		0.0291	0.029	0.03	0.169	0.0286	0.0284	0.2046
C_08_IVC_3	0.5356	0.7783	0.1125	0.0588	0.0852	0.4005	0.0288	0.0839	0.3994	0.029	0.2839		0.0285	0.3168	0.0256	0.026	0.2083
NC_08_IVC_3	0.188	0.5122	0.0272	0.0588	0.0852	0.4005	0.0288	0.0839	0.3994	0.029	0.2839		0.0285	0.3168	0.0256	0.026	0.2083
C_53_R_3	0.5687	0.0284	0.1487	0.1396	0.3917	0.1189	0.0557	0.2559	0.0292	0.03	0.0285	0.0285		0.2853	0.9161	0.6399	0.1896
NC_53_R_3	1	0.7972	0.5105	0.4285	0.7737	0.6208	0.5484	0.3655	0.2385	0.169	0.3166	0.2501	0.2863		0.8201	0.1171	0.2049
C_53_IVC_3	0.6003	0.0329	0.1485	0.2544	0.7136	0.1121	0.1458	0.0899	0.0302	0.0296	0.0256	0.0269	0.5961	0.5201		0.1411	0.2051
NC_53_IVC_3	0.478	0.0277	0.1164	0.0554	0.3182	0.0568	0.0879	0.0829	0.0271	0.0284	0.026	0.0261	0.8399	0.1711	0.1411		0.1884
R_53	0.2053	0.1951	0.1958	0.1983	0.1956	0.2461	0.2023	0.1954	0.2019	0.2048	0.2083	0.2083	0.1986	0.2049	0.2051	0.1984	1
R_08	0.1987	0.2029	0.1956	0.2012	0.1943	0.2475	0.2085	0.2015	0.2032	0.2033	0.2096	0.2027	0.2001	0.196	0.2042	0.211	1

Table 1: PERMANOVA Main factor F=1.512 and p=0.0001. Highlighted values are significant with p<0.05. Most significant differences are associated with differences in food types. There are also differences noted between week one and week three in the 5008 diet.

Abbreviation	Change
C	Change
NC	No Change
08	5008 Food
53	5053 Food
S	Static
IVC	Individually Ventilated Cage
1	Week 1
3	Week 3

Conclusion

- The difference in the background samples versus the exposed samples suggest that once the feed enters the mouse cage, the microbiome will change.
- No consistent differences were seen between change and no change groups suggesting that the practice of dumping of food may be unnecessary.
- While not readily apparent in PCoA analysis, PERMANOVA analysis suggests there are differences in the microbiome of different feeds. There were also inconsistent differences when examining other variables.
- The discrepancy between PCoA and PERMANOVA analysis suggest that difference are subtle. These may be related to minor species, and the biological relevance of these changes awaits further studies.

Future Directions

- Perform mycobiome analysis
- Determine the cause of the bimodal distribution
- Assess minor bacterial species present in each group
- Quantitative analysis of bacterial load in samples
- Determine how long food stays in the cage before being completely consumed by mice

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