

Introduction

- The gut microbiota is defined as an array of commensal, symbiotic microorganisms that inhabit the intestinal tracts of animal species. There is emerging evidence that differences in gut microbiota influence the phenotype of several animal models.
- Previous research in our laboratory has shown that factors including vender, age, and strain affect the gut microbiota of mice and rats.
- In continuation of these studies, this project is determining if factors such as housing, bedding, and diet will alter the gut microbiota of CrI:CD1 (ICR) mice.
- Understanding which factors influence that gut microbiota will help researchers optimize their experiments and control for these variables.

Hypotheses

- The gut microbiota of the mice living in ventilated racks will differ from the microbiota of mice living in static microisolators.
- The gut microbiota will differ in mice fed differing diets.
- Different bedding types (paper chip versus aspen) will not affect the gut microbiota.

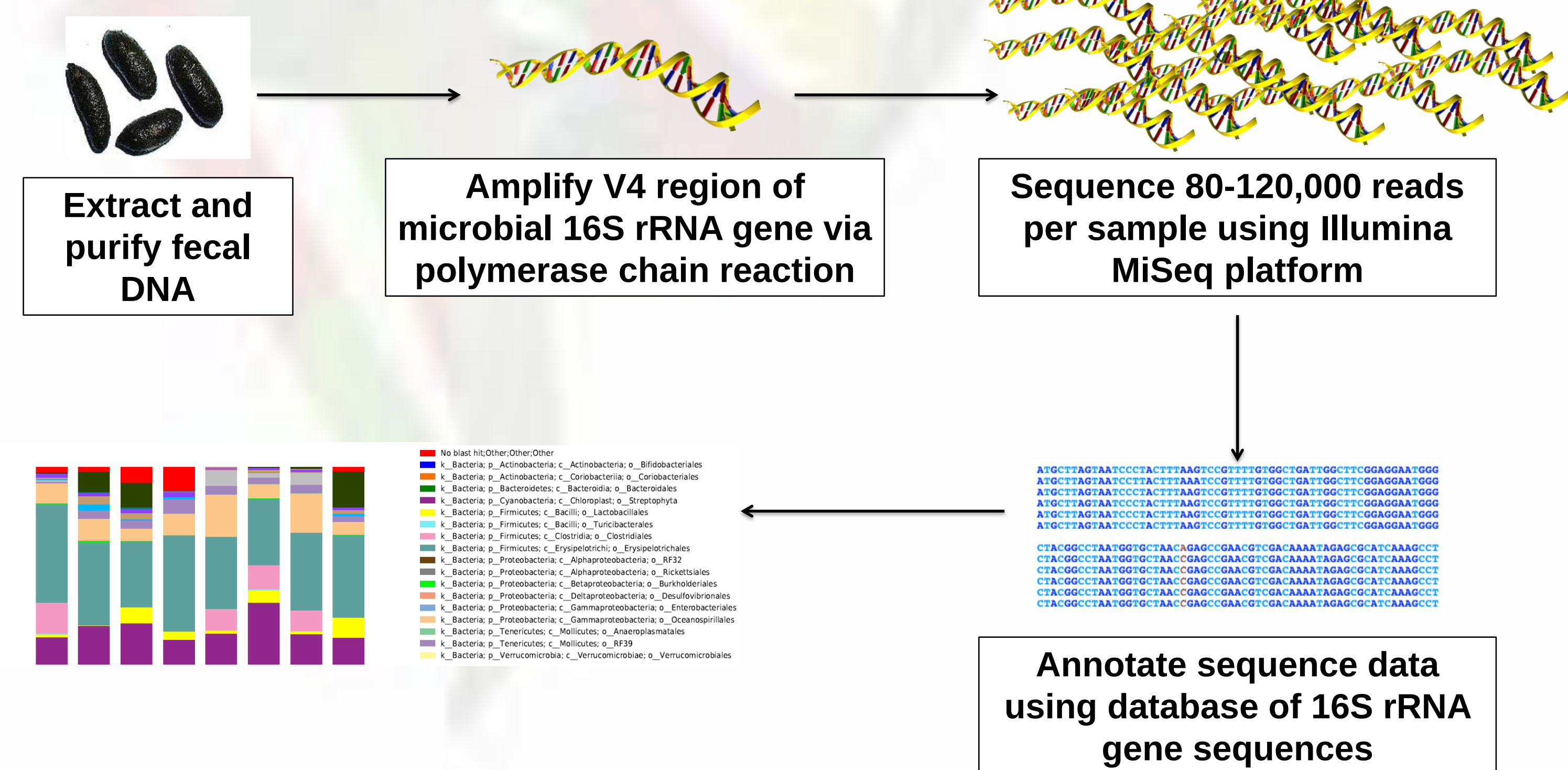
Methods

- There were a total of twelve groups of mice with three variables assessed: diet, bedding, and housing.
- For each group, there were three cages, each containing four mice. The four mice in each cage were identified as animal one, two, three, or four using ear notches. Overall, there were 144 mice in 36 cages.
- To characterize the microbial communities, DNA was extracted from fecal samples collected weekly beginning at weaning and ending at 12 weeks of age. Next-generation sequencing was performed and results analyzed using Quantitative Insights Into Microbial Ecology (QIIME) software.

Variables Assessed	Groups											
	1	2	3	4	5	6	7	8	9	10	11	12
Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Bedding	Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
Housing	Static	Static	Static	Static	Static	Static	VR	VR	VR	VR	VR	VR



Experimental Design



Results

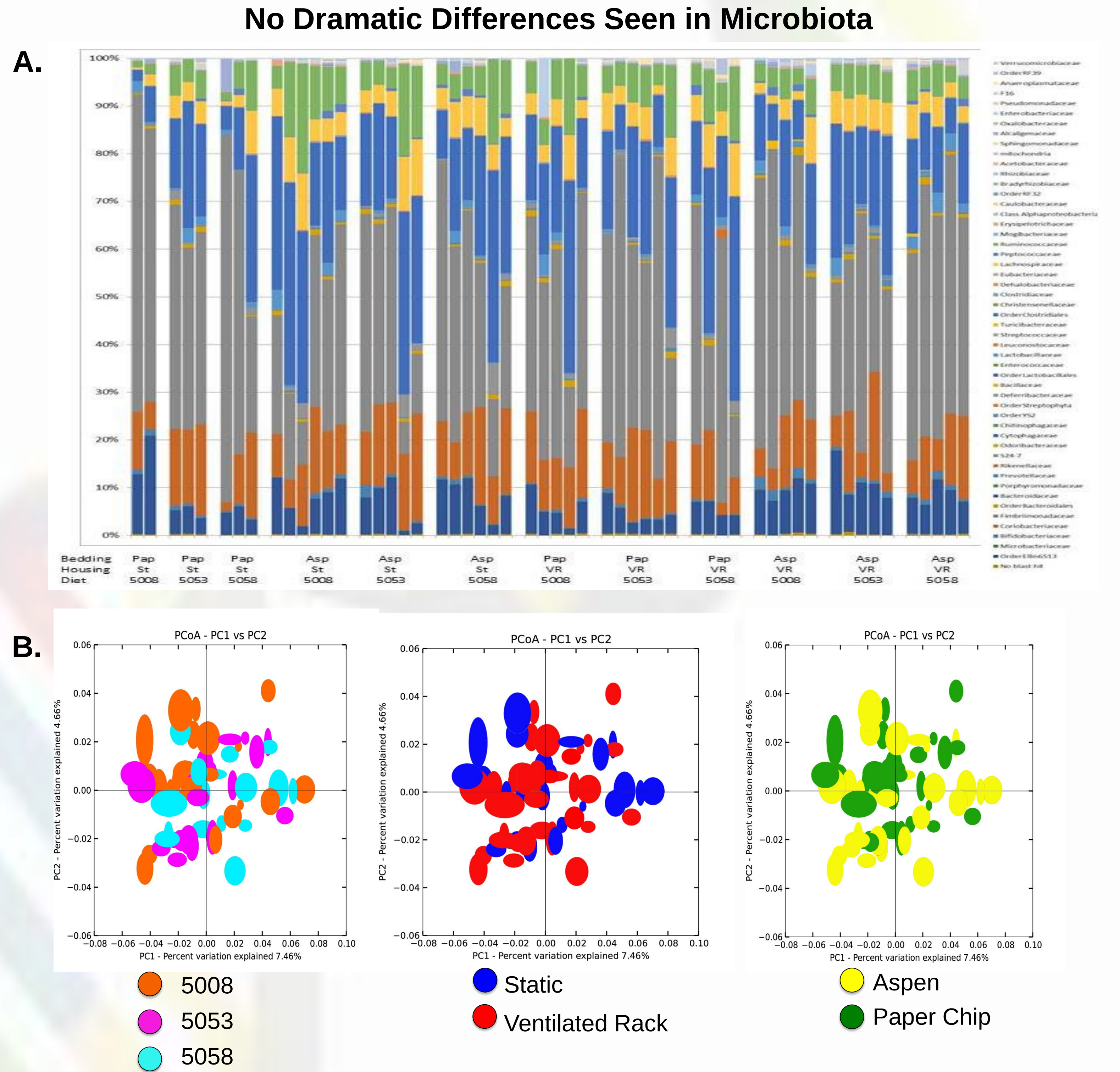


Figure 1. Bar charts displaying the gut microbiota of each group of CrI:CD1 (ICR) mice (A) ; No clear grouping established with principal component analysis of diet, housing, and bedding (B)

A. Subtle Differences Identified in Select Bacterial Families

Family	Main Effects			Interactions		
	Diet	Housing	Bedding	Diet vs. Housing	Diet vs. Bedding	Housing vs. Bedding
Coriobacteriaceae	NS	NS	NS	0.048	NS	NS
Bacteroidaceae	NS	NS	0.02 ^b	NS	NS	0.034
Prevotellaceae	NS	NS	0.023 ^b	NS	NS	0.006
S24-7	NS	NS	NS	NS	NS	0.003
Odoribacteraceae	NS	NS	NS	0.048	NS	NS
Cytophagaceae	NS	0.025 ^a	NS	NS	NS	NS
Order YS2 ^d	NS	0.046 ^a	NS	NS	NS	NS
Order Lactobacillales ^d	0.020 ^a	<0.001 ^a	NS	NS	NS	NS
Streptococcaceae	0.033 ^b	0.014 ^a	0.013 ^b	NS	NS	NS
Order Clostridiales ^d	NS	NS	NS	NS	NS	0.003
Dehalobacteriaceae	0.045 ^a	NS	NS	NS	NS	NS
Eubacteriaceae	NS	NS	0.015 ^a	NS	NS	NS
Lachnospiraceae	NS	NS	NS	NS	NS	0.008
Peptococcaceae	0.013 ^b	NS	NS	NS	NS	NS
Ruminococcaceae	NS	NS	NS	NS	NS	0.001
Mogibacteriaceae	NS	<0.001 ^a	NS	NS	NS	0.039
Erysipelotrichaceae	NS	NS	0.020 ^a	NS	NS	NS
Order RF32 ^d	NS	NS	0.034 ^b	NS	NS	NS

^a2 way AVOVA Diet vs. Housing
^b2 way AVOVA Diet vs. Bedding
^c2 way AVOVA Housing vs. Bedding
^dclassification achieved only to order level

Figure 2. Table illustrating differences in relative abundance of bacterial families (and interactions) due to diet, housing, and bedding. p -values ≤ 0.05 highlighted.

Summary and Acknowledgements

- None of the variables resulted in global shifts in the composition of the gut microbiota
- Diet, housing, and bedding all had significant effects on the relative abundance of specific microbial families
 - Families *Bacteroidaceae* and *Prevotellaceae* affected by bedding
 - Lactic-acid producing families (*Lactobacillales* and *Streptococcaceae*) susceptible to all variables
- The interaction between the aforementioned factors is complex
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