

### Experiment 1:

**Group1: Nrf2<sup>-/-</sup>, AIN93M (N=9)**

**Group2: Nrf2<sup>-/-</sup>, PEITC (N=9)**

### Experiment 2:

**Group3: WT, AIN93M (N=5)**

**Group4: WT, PEITC (N=5)**

### Experiment 3:

**Group5: WT, AIN93M (N=5)**

**Group6: WT, AIN93M, DSS (N=5)**

**Group7: WT, Cranberry, DSS (N=5)**

**Group8: WT, PEITC, DSS (N=5)**

**Group9: Nrf2<sup>-/-</sup>, AIN93M (N=5)**

**Group10: Nrf2<sup>-/-</sup>, AIN93M, DSS (N=5)**

**Group11: Nrf2<sup>-/-</sup>, Cranberry, DSS (N=5)**

**Group12: Nrf2<sup>-/-</sup>, PEITC, DSS (N=5)**

**AIN93M : Groups 1, 3, 5, 6, 9 and 10**

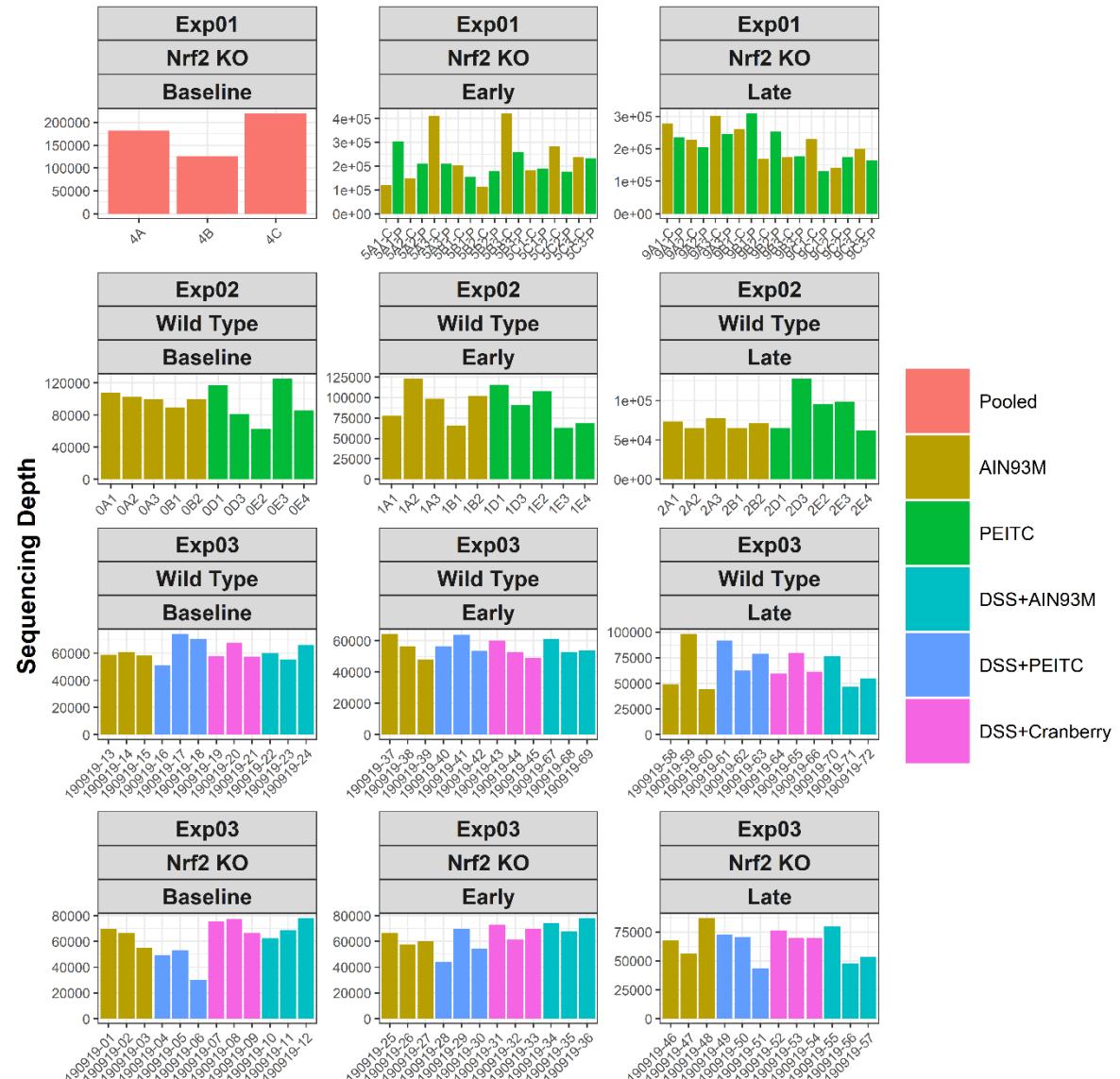
**AIN93M:  
Groups 2, 4,  
7, 8, 11 and 12**

**AIN93M + 0.05% PEITC Groups 2, 4, 8 and 12  
AIN93M + Cranberry: Groups 7 and 11**

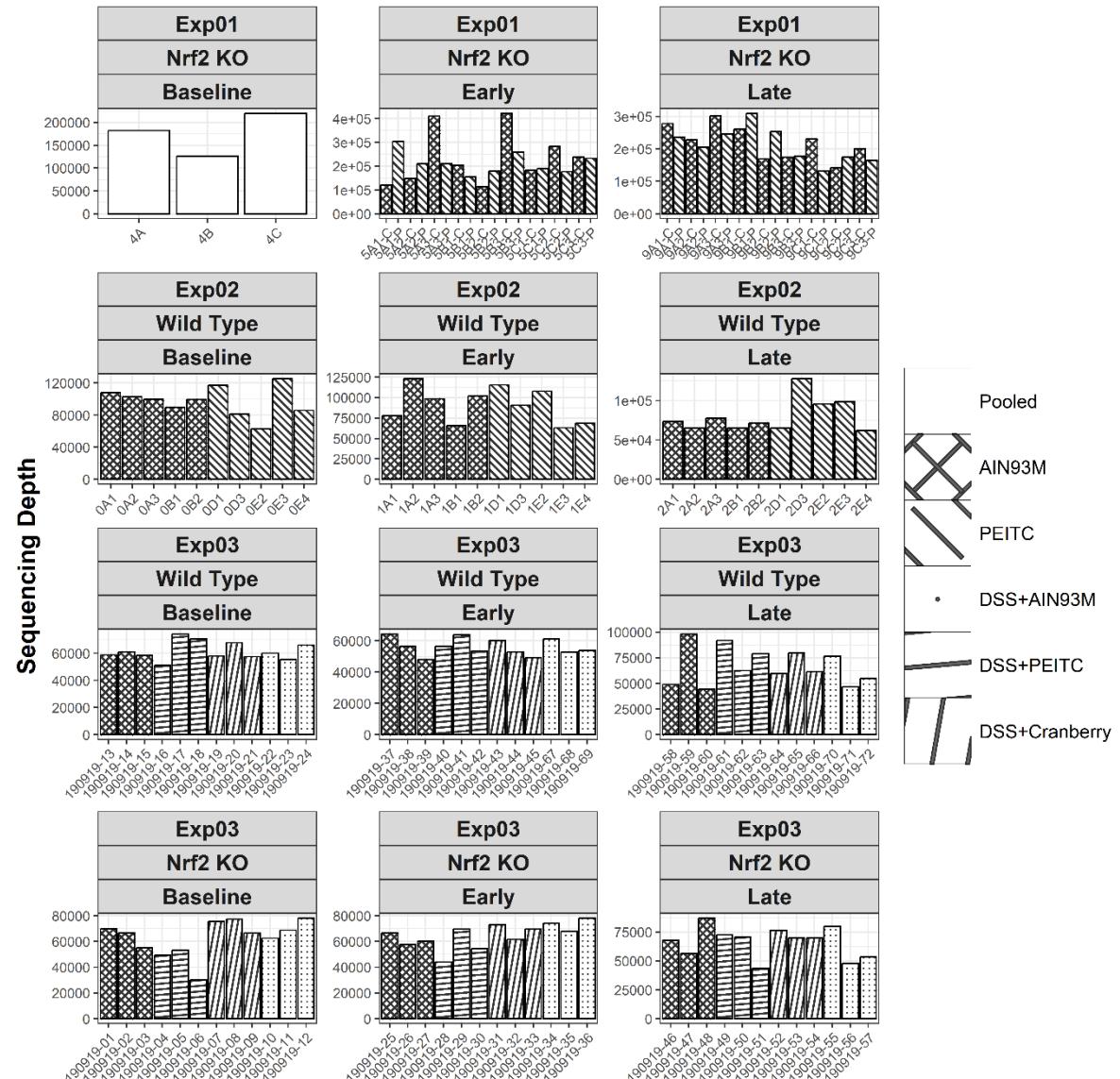


**Gut microbiota  
equalization period  
(2 weeks)**

Figure 1: Experimental design. Mice fecal samples for the 16S sequencing were collected individually at 3 timepoints – at the end of the 2-week equalization period (Week 0), at an early timepoint (Week 1) and at a late timepoint (Week 4 or Week 8). Samples used for metabolite analysis were collected at an early and a late timepoints (Weeks 2 and Week 6 respectively).

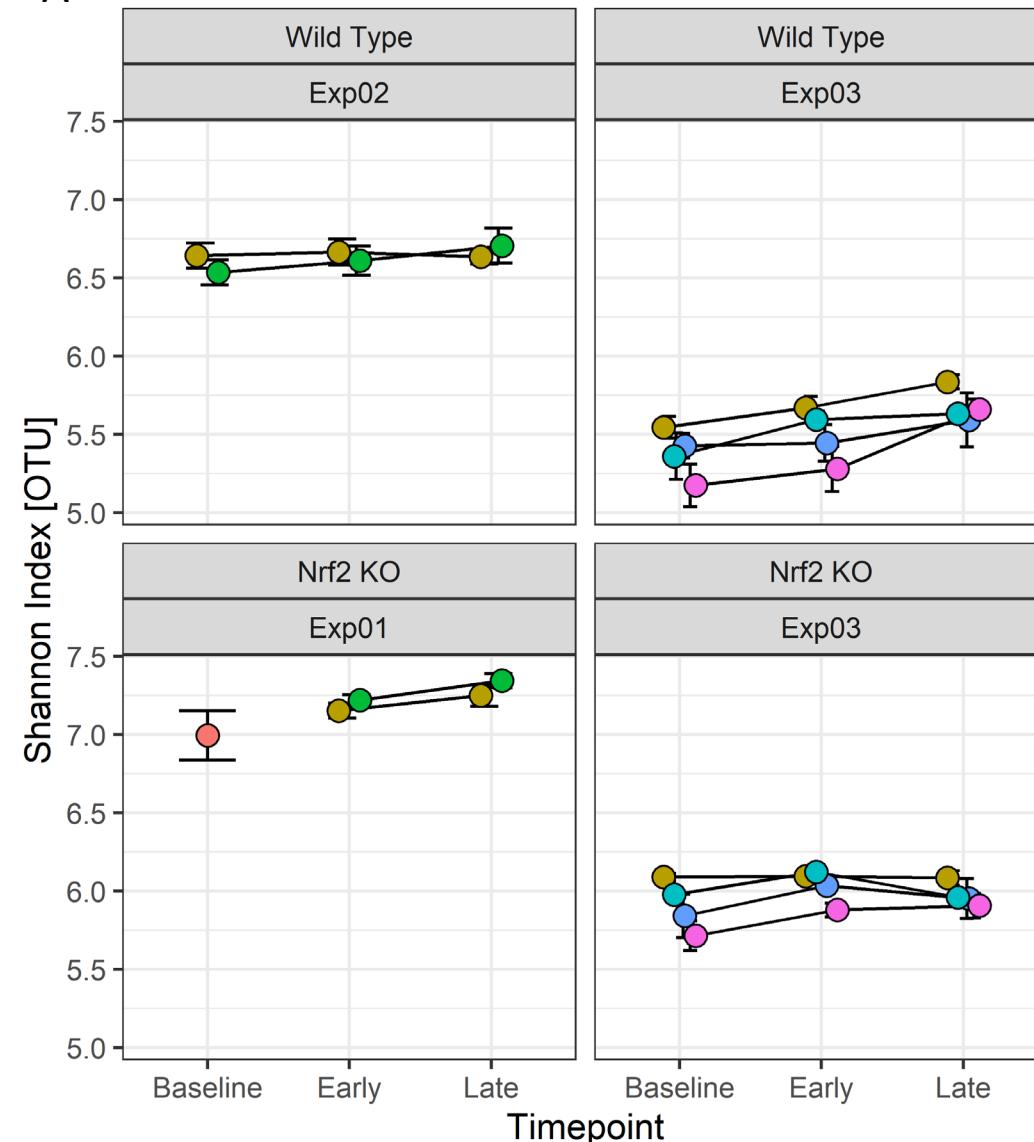


Supplemental Figure 1: 16S sequencing depth (total number of hits per sample).

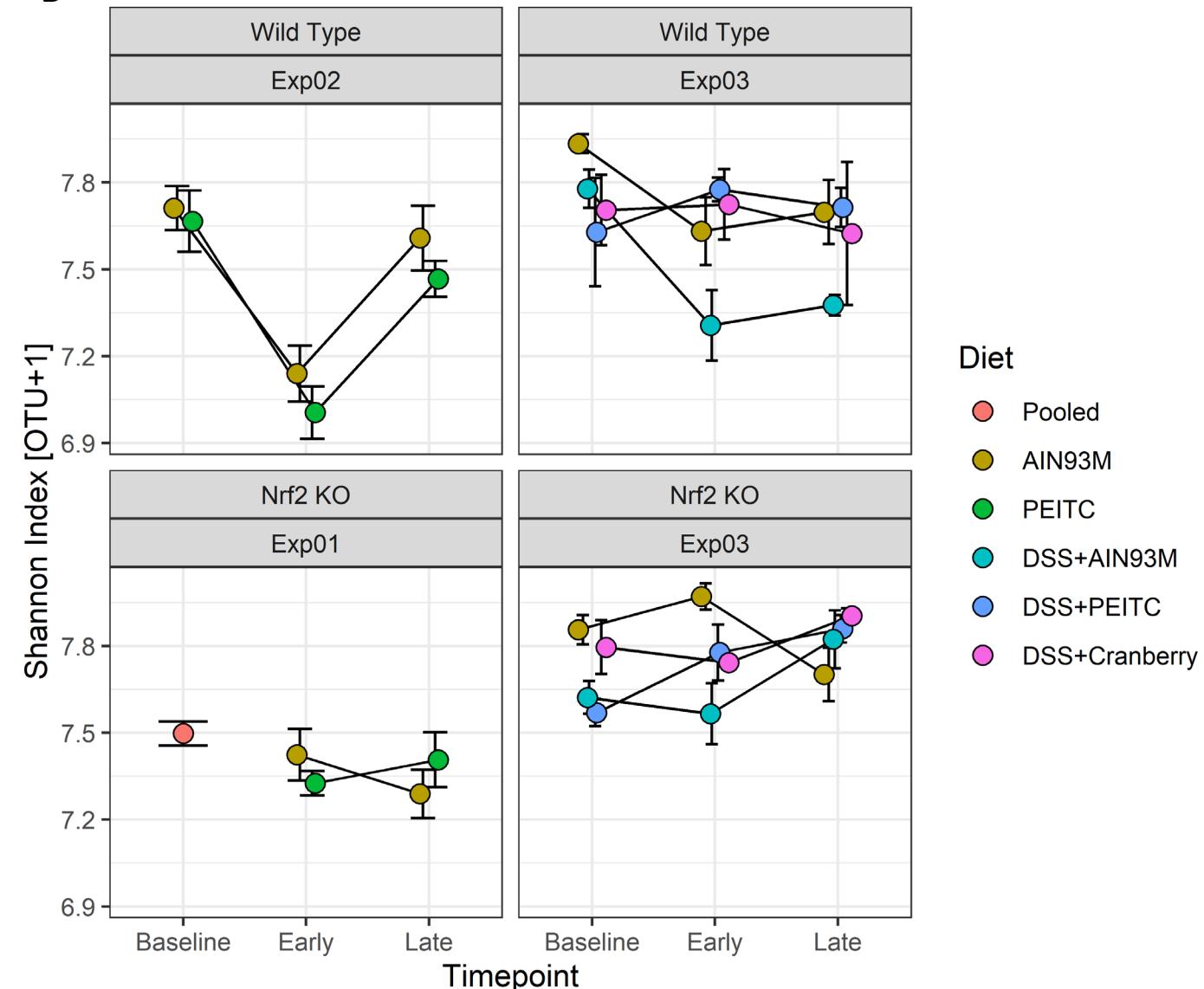


Supplemental Figure 1: 16S sequencing depth (total number of hits per sample).

A



B

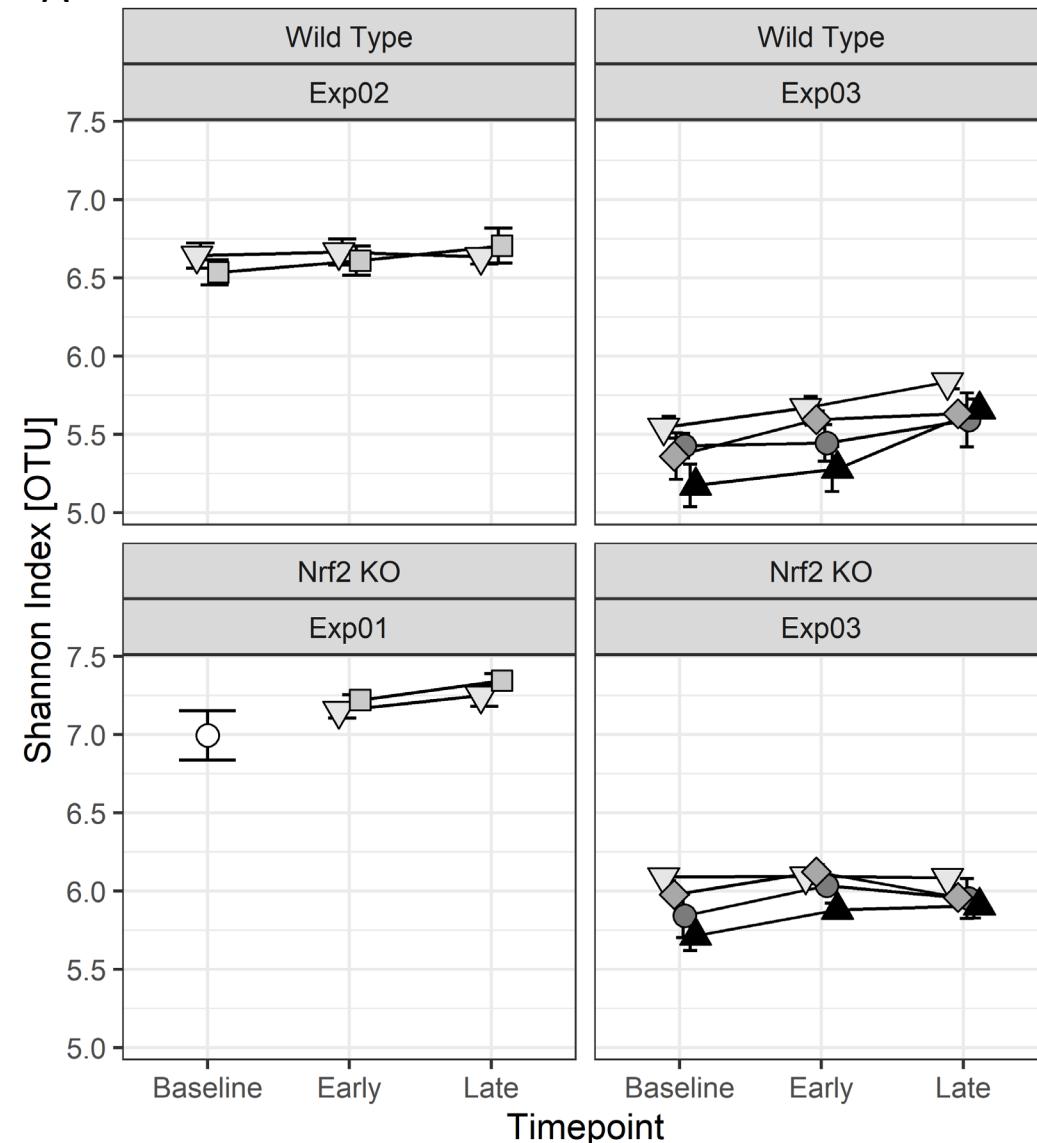


Diet

- Pooled
- AIN93M
- PEITC
- DSS+AIN93M
- DSS+PEITC
- DSS+Cranberry

Figure 2: Alpha diversity measured by Shannon index. (A) Averages of Shannon indices calculated on raw OTU numbers and (B) on corrected OTU numbers (OTU+1).

A



B

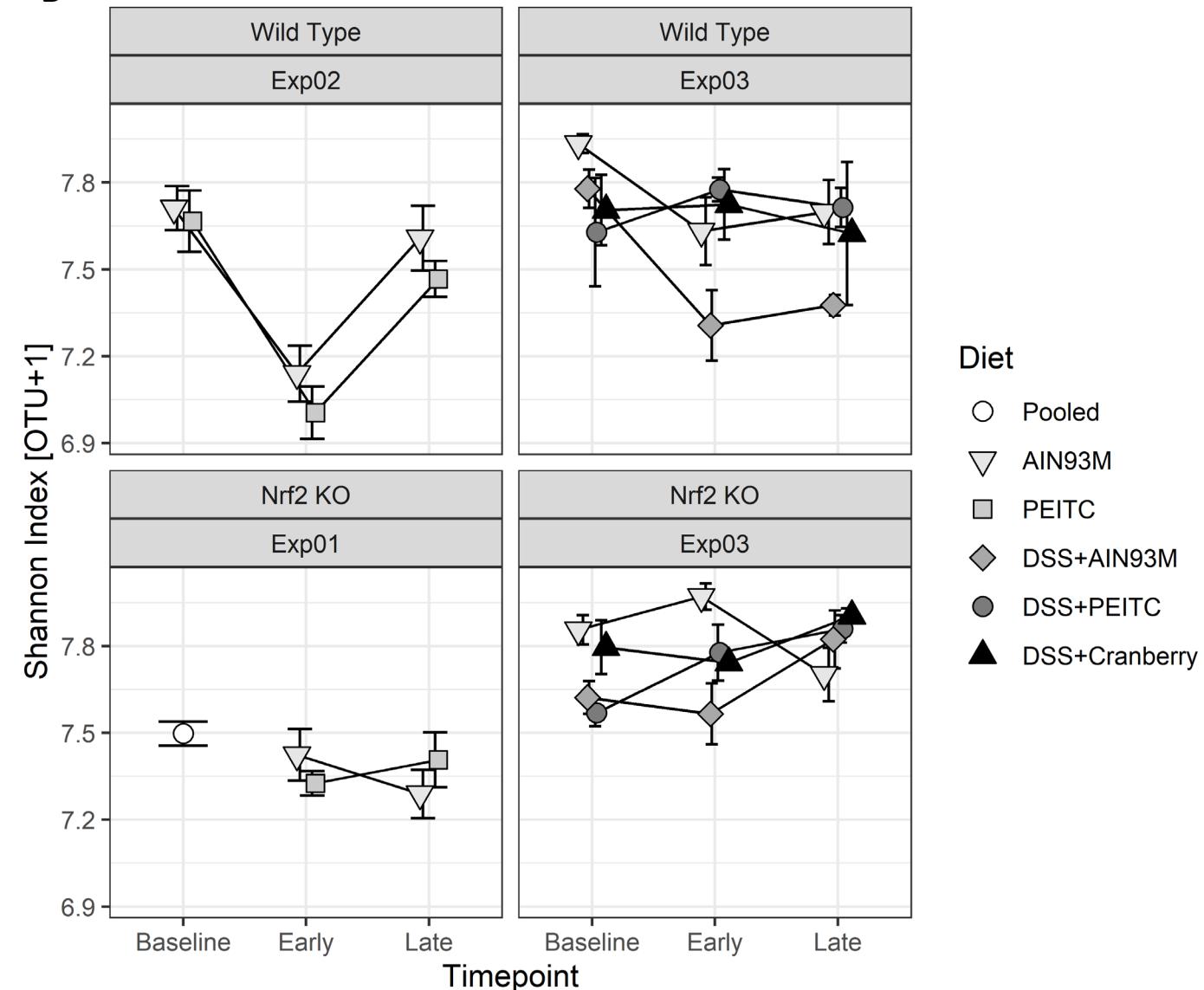
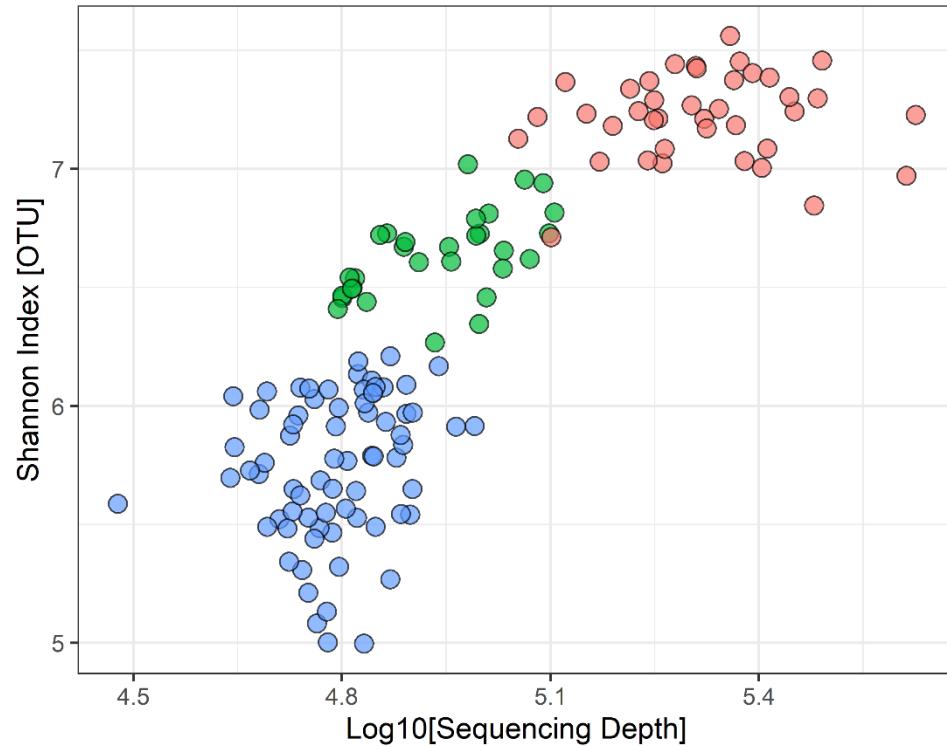
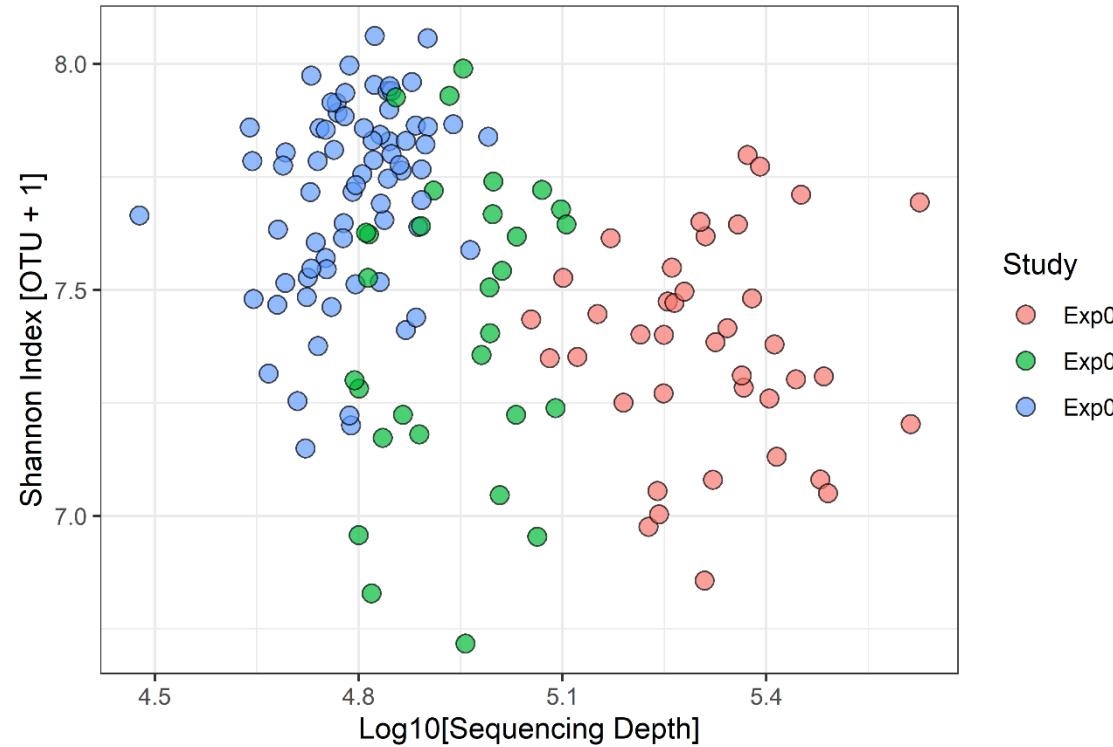
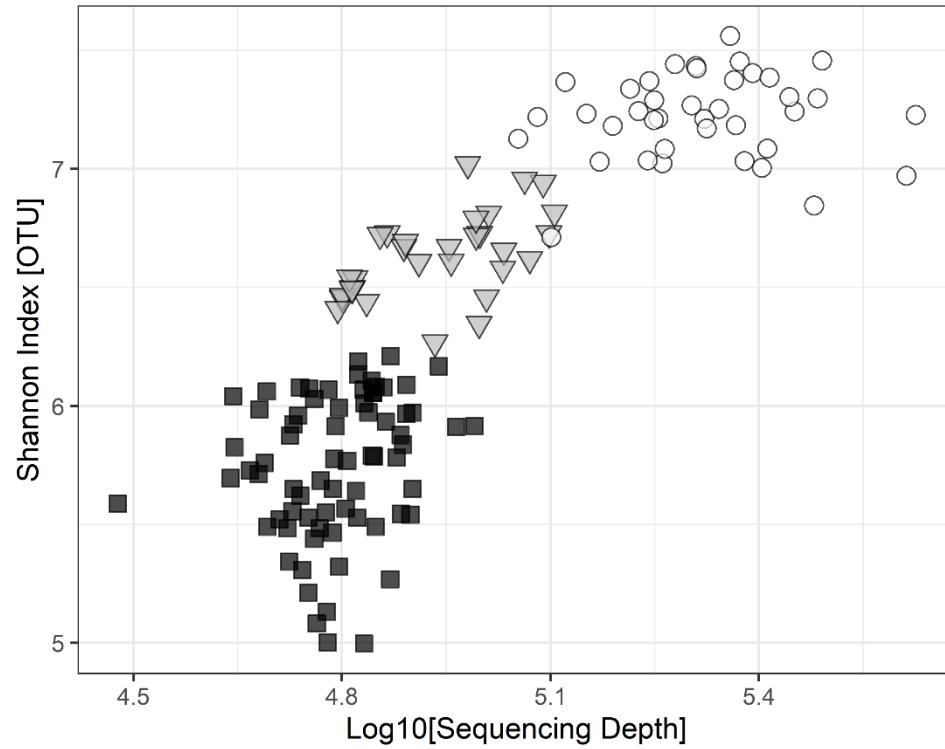
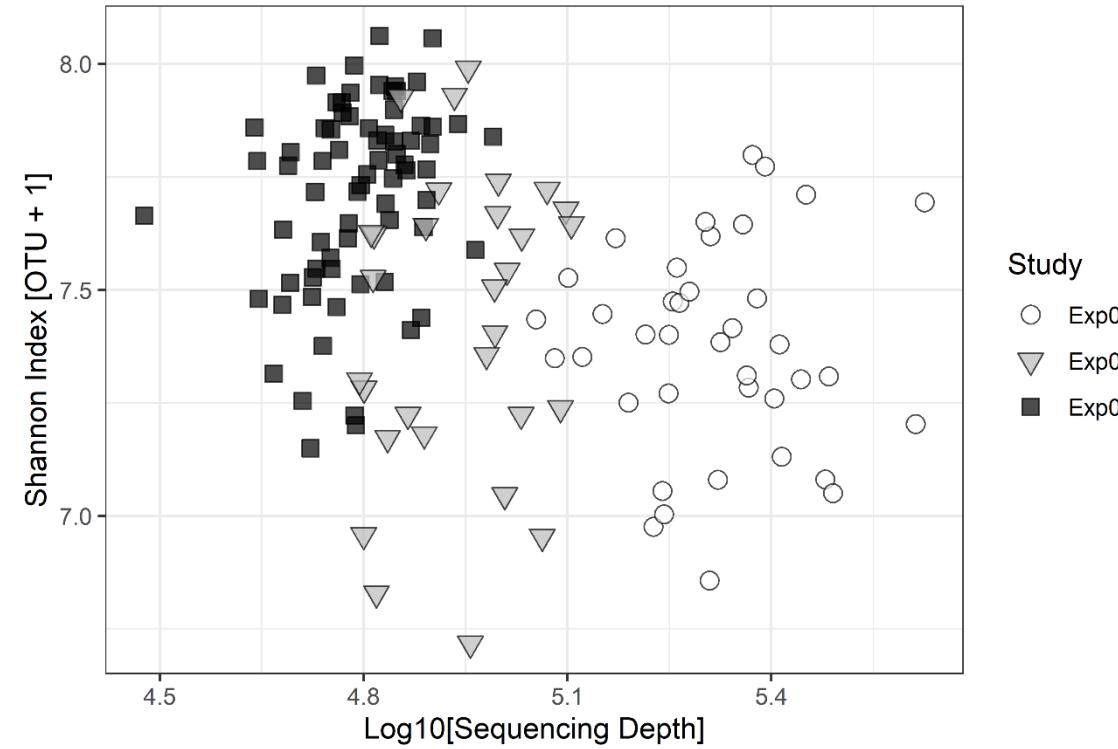


Figure 2: Alpha diversity measured by Shannon index. (A) Averages of Shannon indices calculated on raw OTU numbers and (B) on corrected OTU numbers (OTU+1).

**A****B****Study**

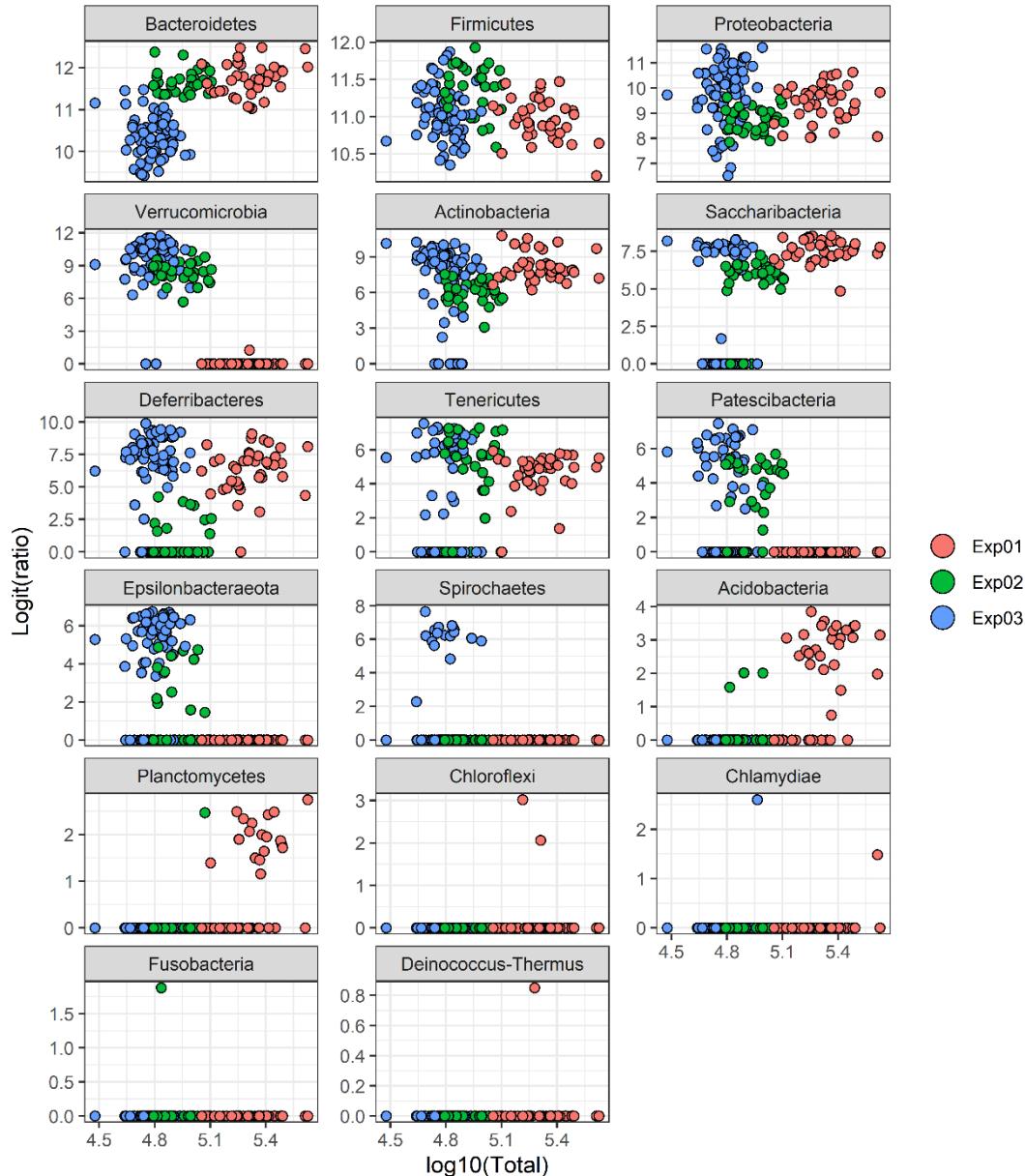
- Exp01
- Exp02
- Exp03

Supplemental Figure 2: Shannon index vs. sequencing depth.

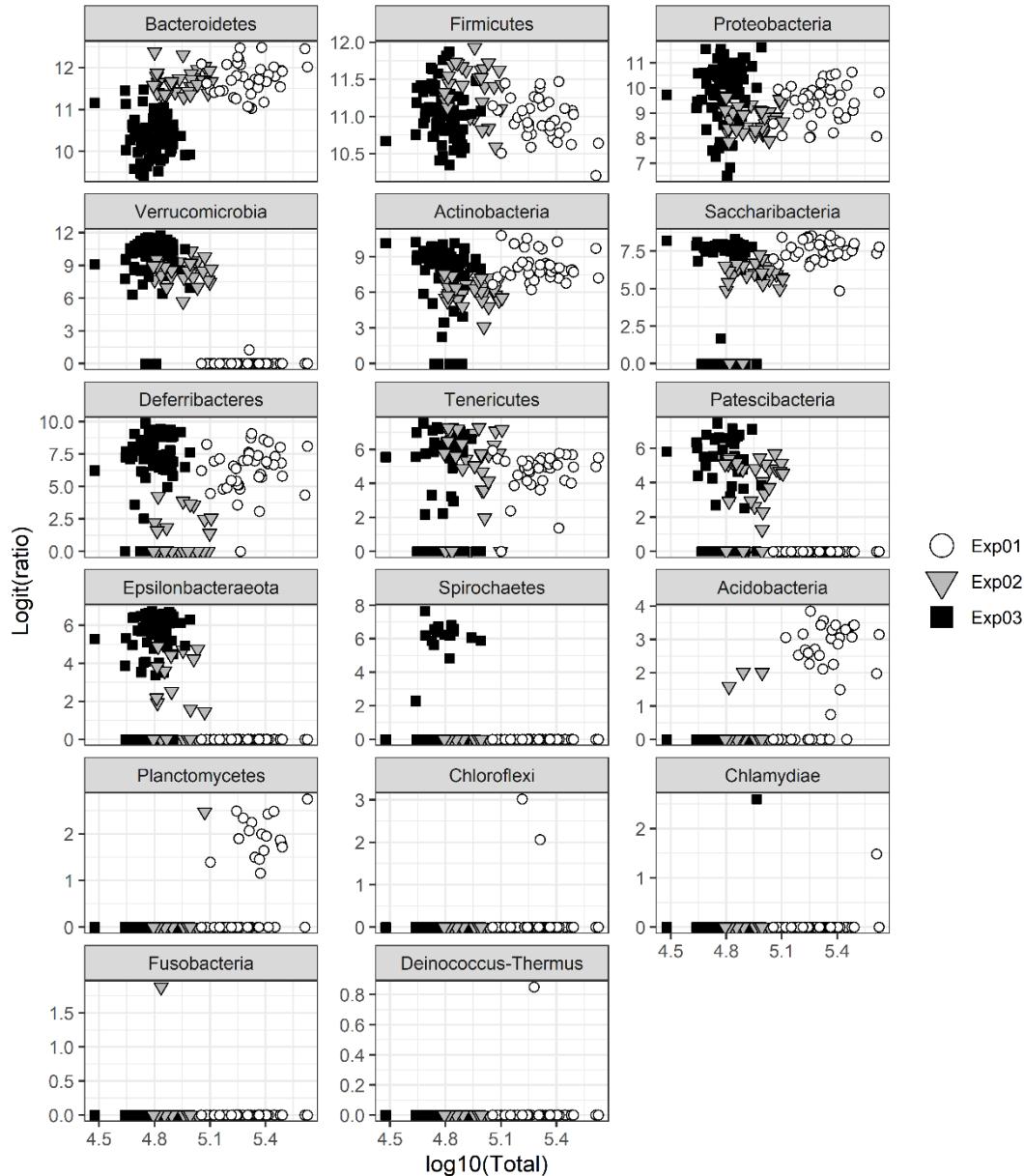
**A****B****Study**

- Exp01
- ▽ Exp02
- Exp03

Supplemental Figure 2: Shannon index vs. sequencing depth.



Supplemental Figure 3: logit of the relative abundance of Phylum vs. sequencing depth.



Supplemental Figure 3: logit of the relative abundance of Phylum vs. sequencing depth.

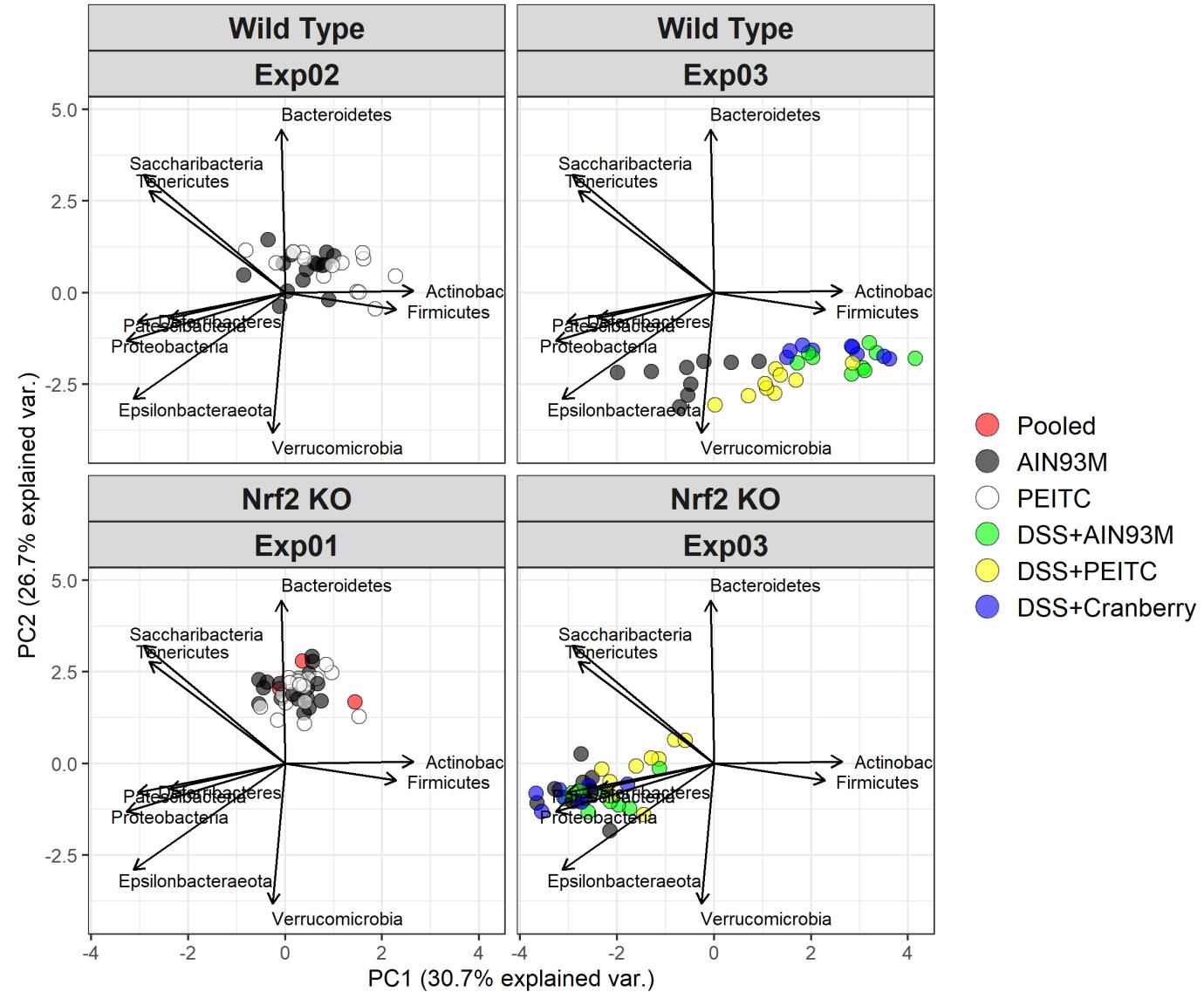


Figure 4: Biplot of logit relative abundance of Phylum

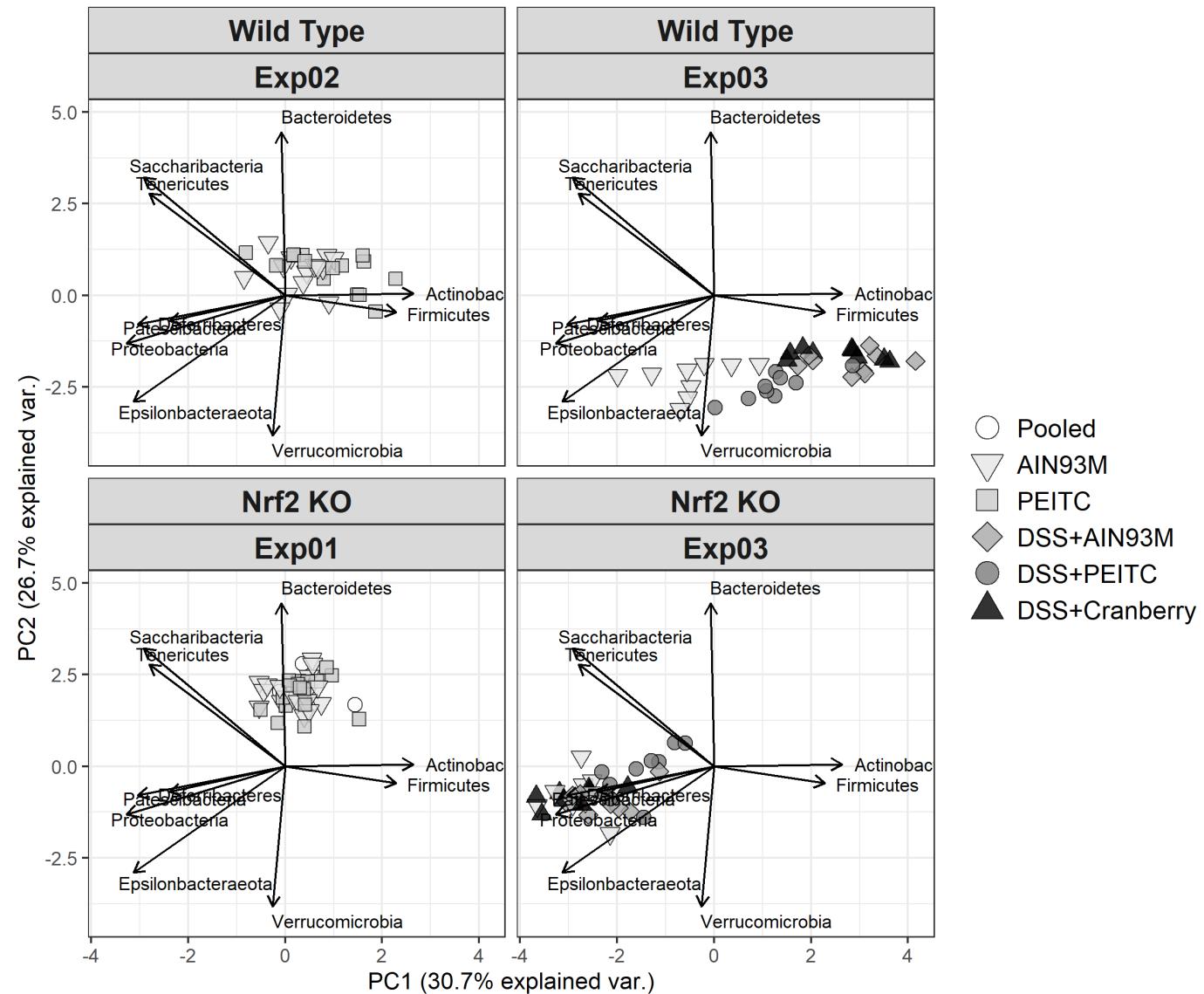


Figure 4: Biplot of logit relative abundance of Phylum

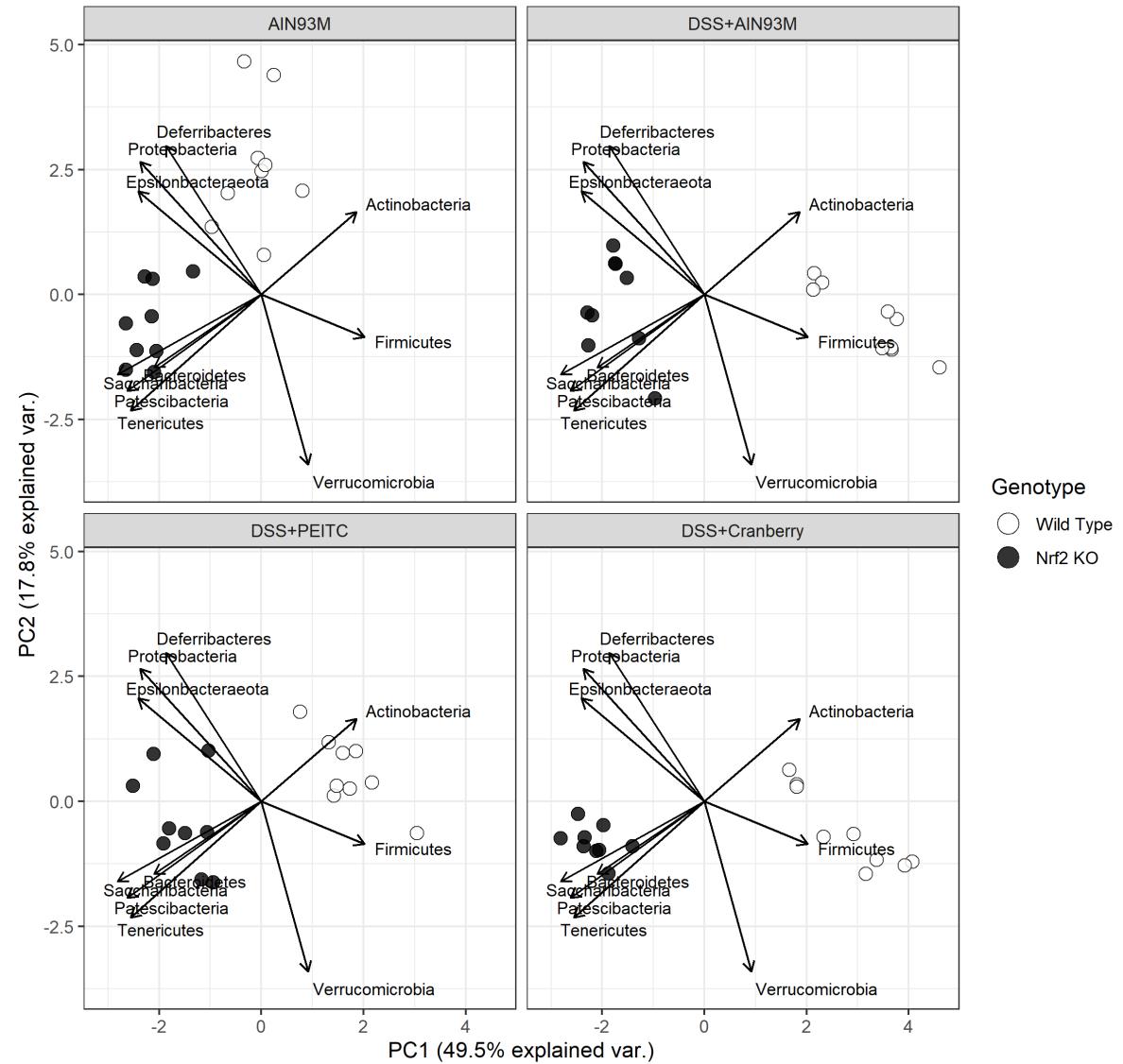


Figure 5: biplot of logit relative abundance of Phylum in Exp03 only

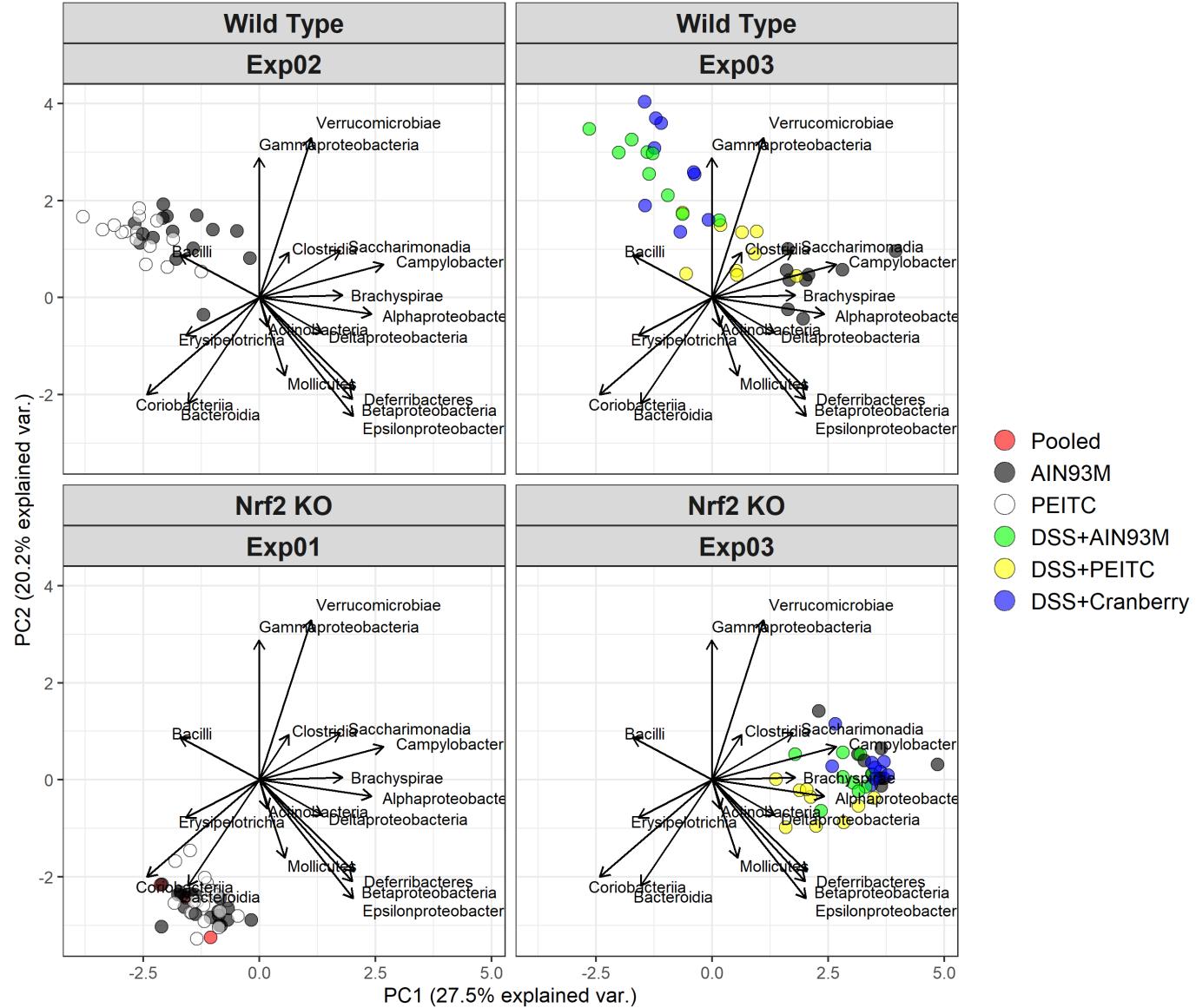


Figure 6: Biplot of logit relative abundance of bacterial classes

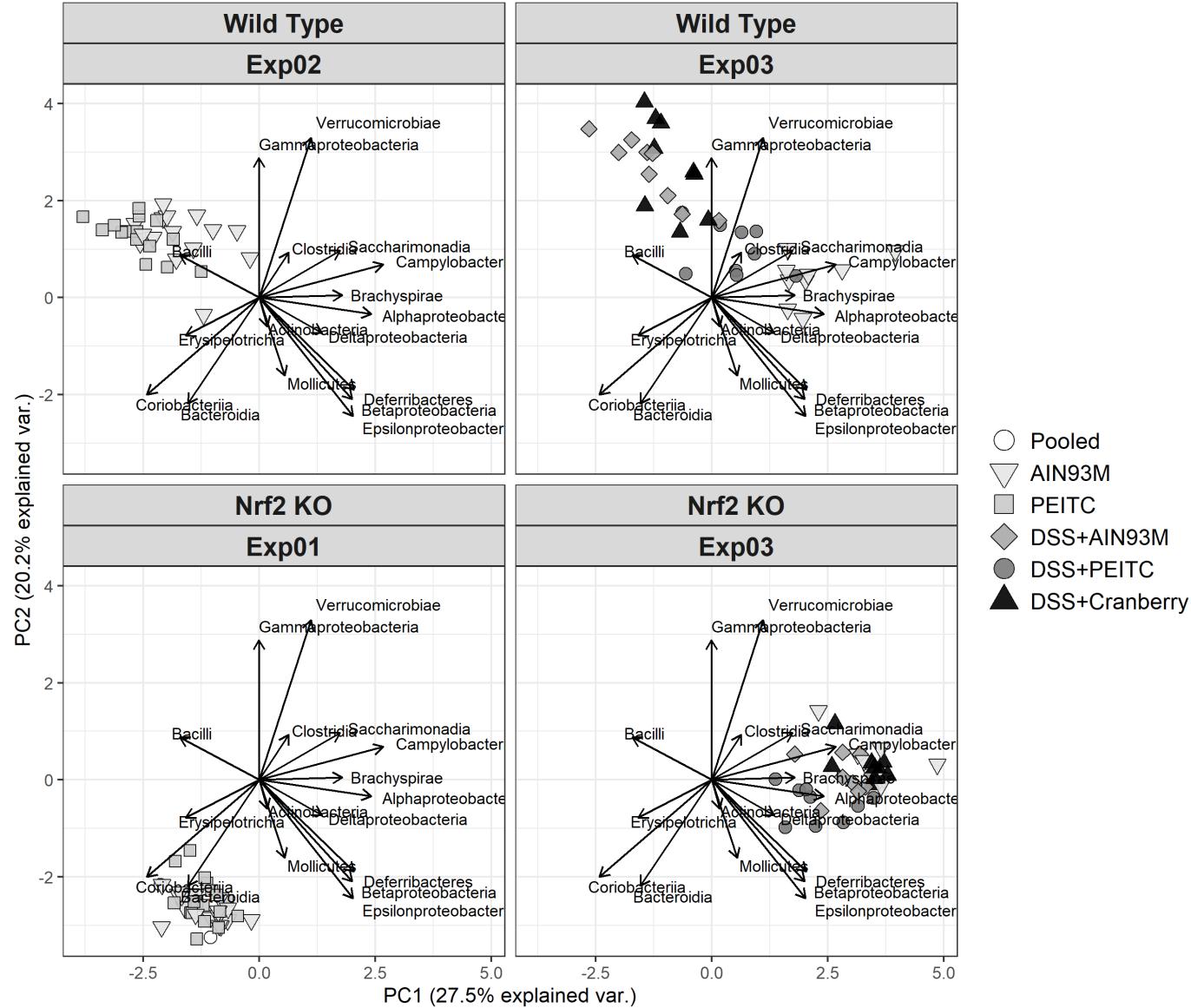


Figure 6: Biplot of logit relative abundance of bacterial classes

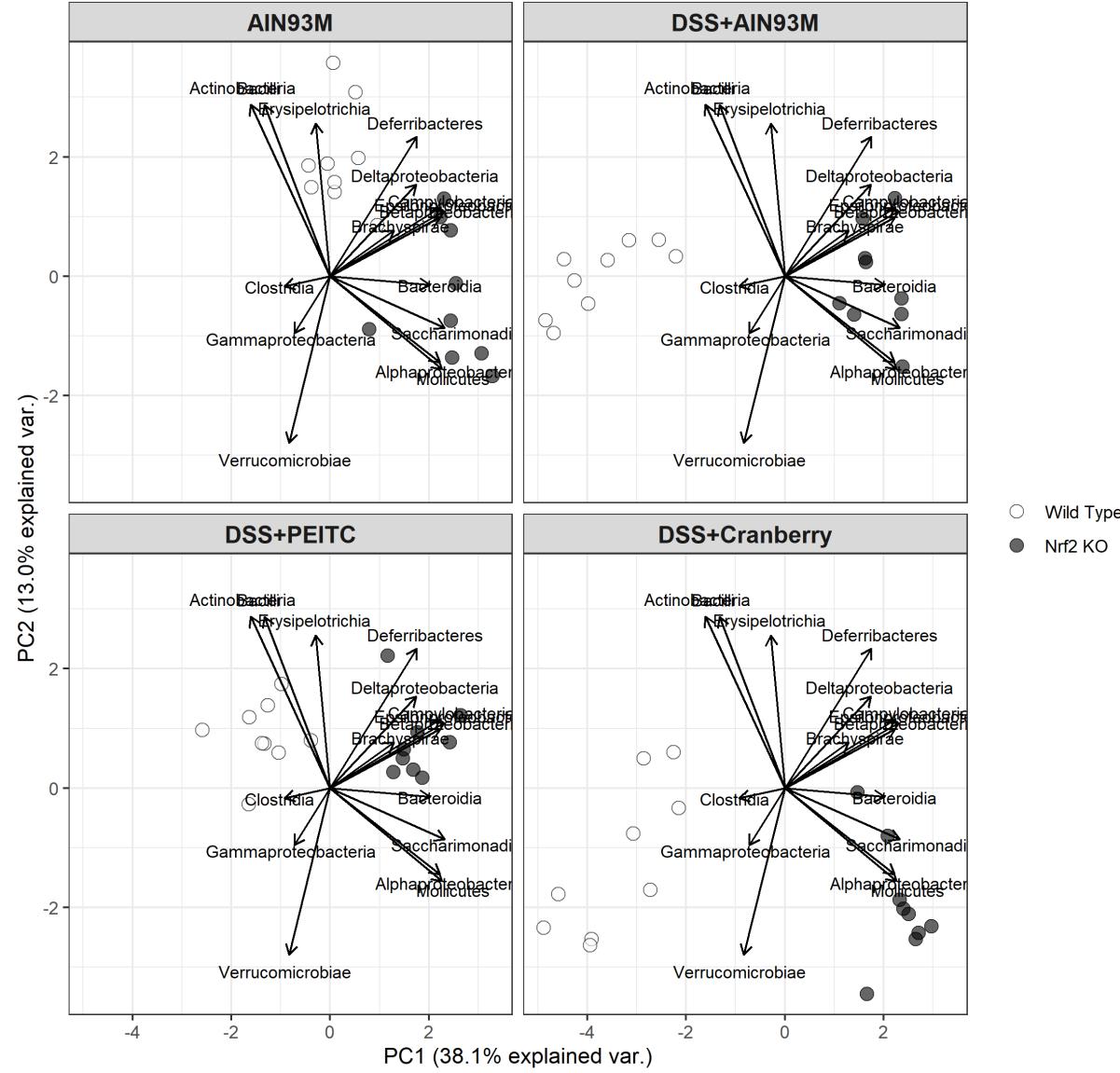


Figure 7: biplot of logit relative abundance of bacterial classes in Exp03 only

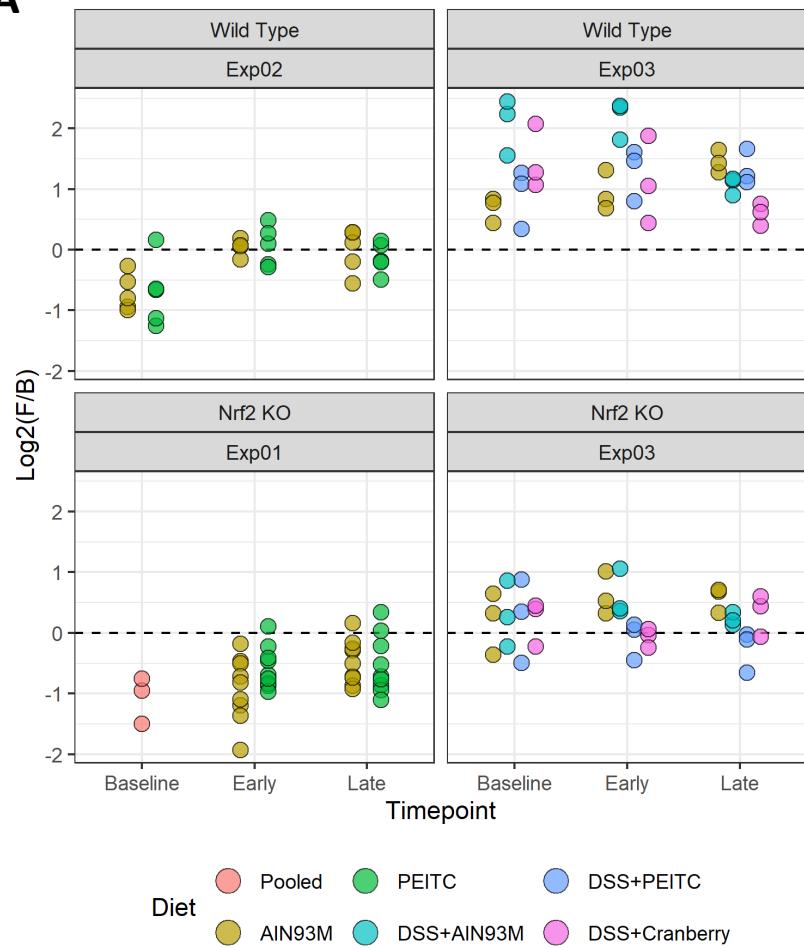
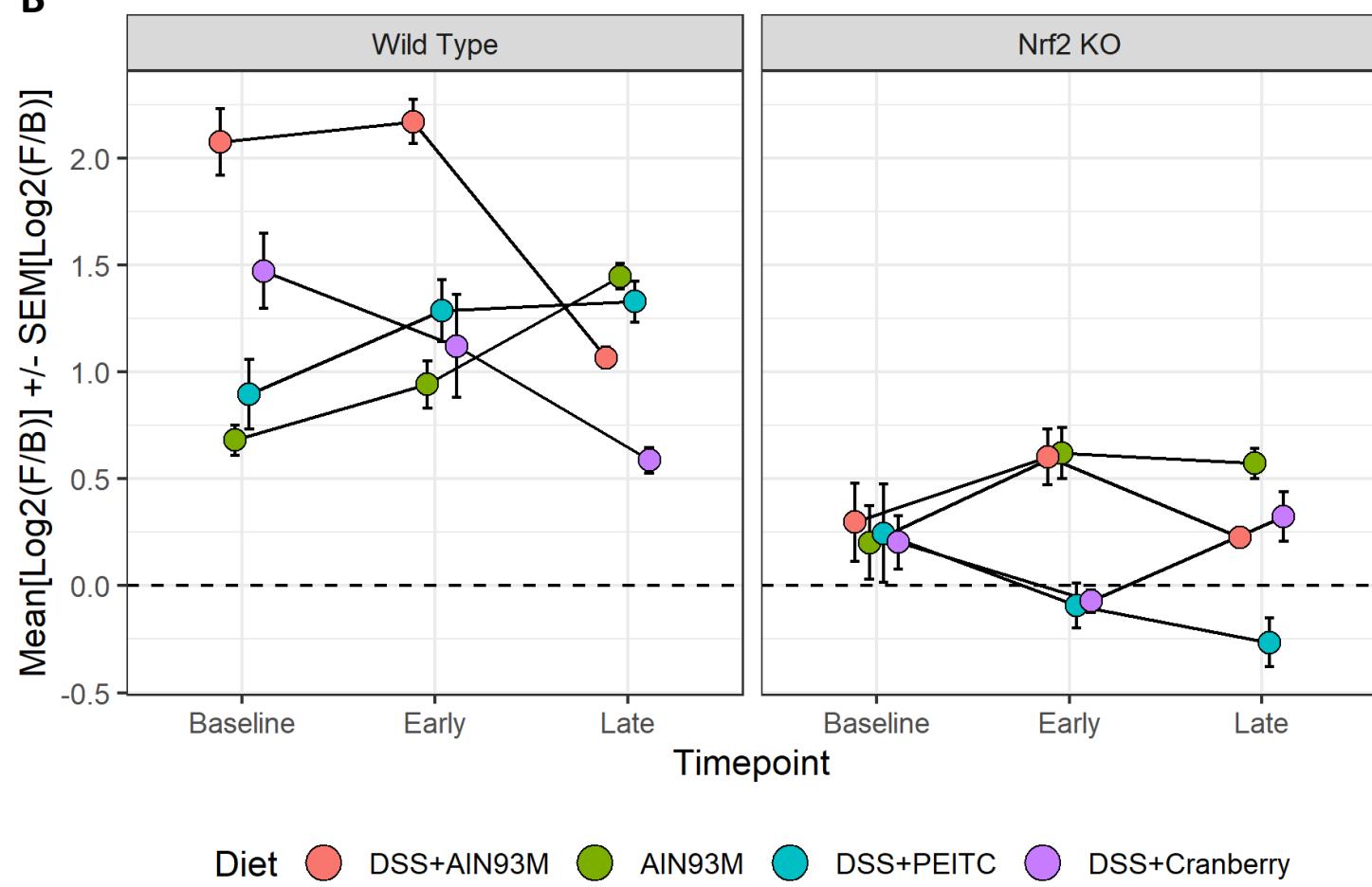
**A****B**

Figure 8: Means of log<sub>2</sub> F/B ratios by genotype and diet over time in Exp03. The bars represent standard errors of log<sub>2</sub>(F/B) ratios.

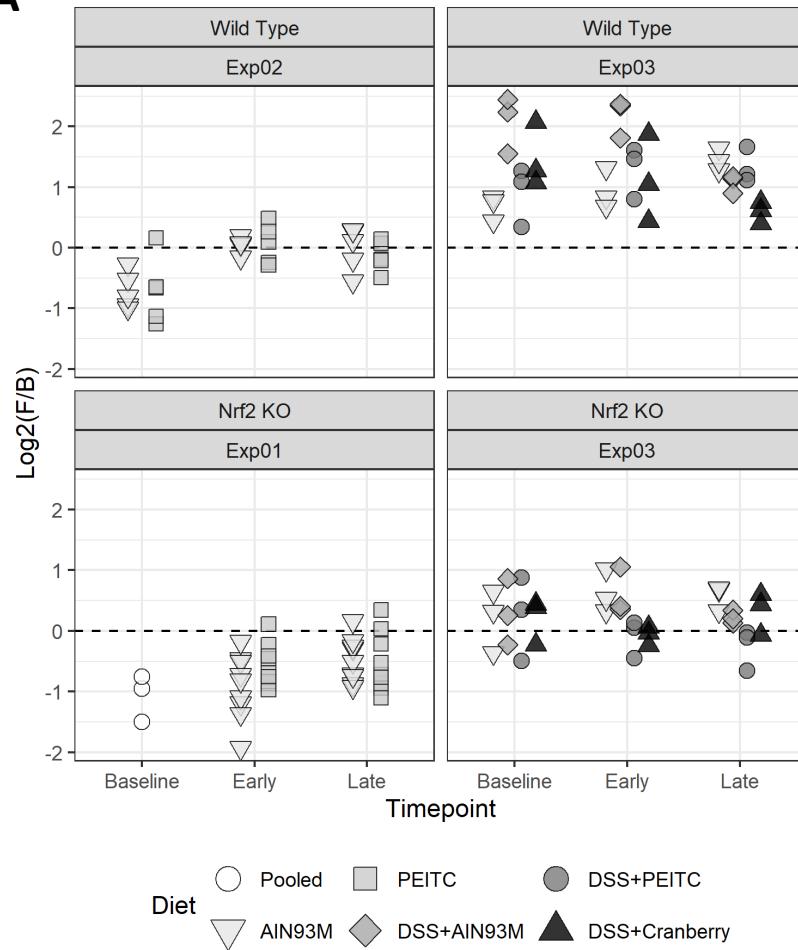
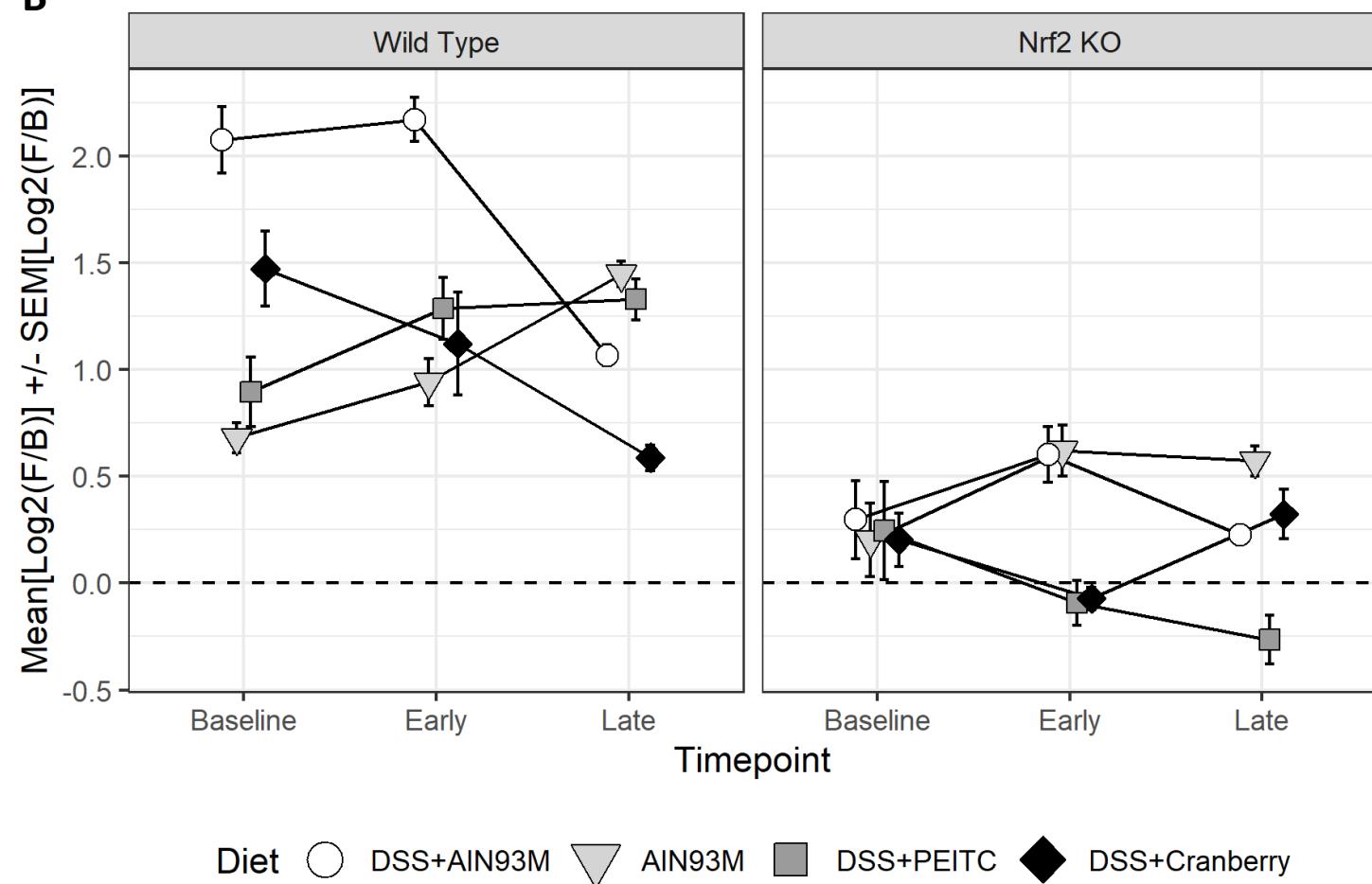
**A****B**

Figure 8: Means of  $\text{log2 F/B}$  ratios by genotype and diet over time in Exp03. The bars represent standard errors of  $\text{log2}(F/B)$  ratios.

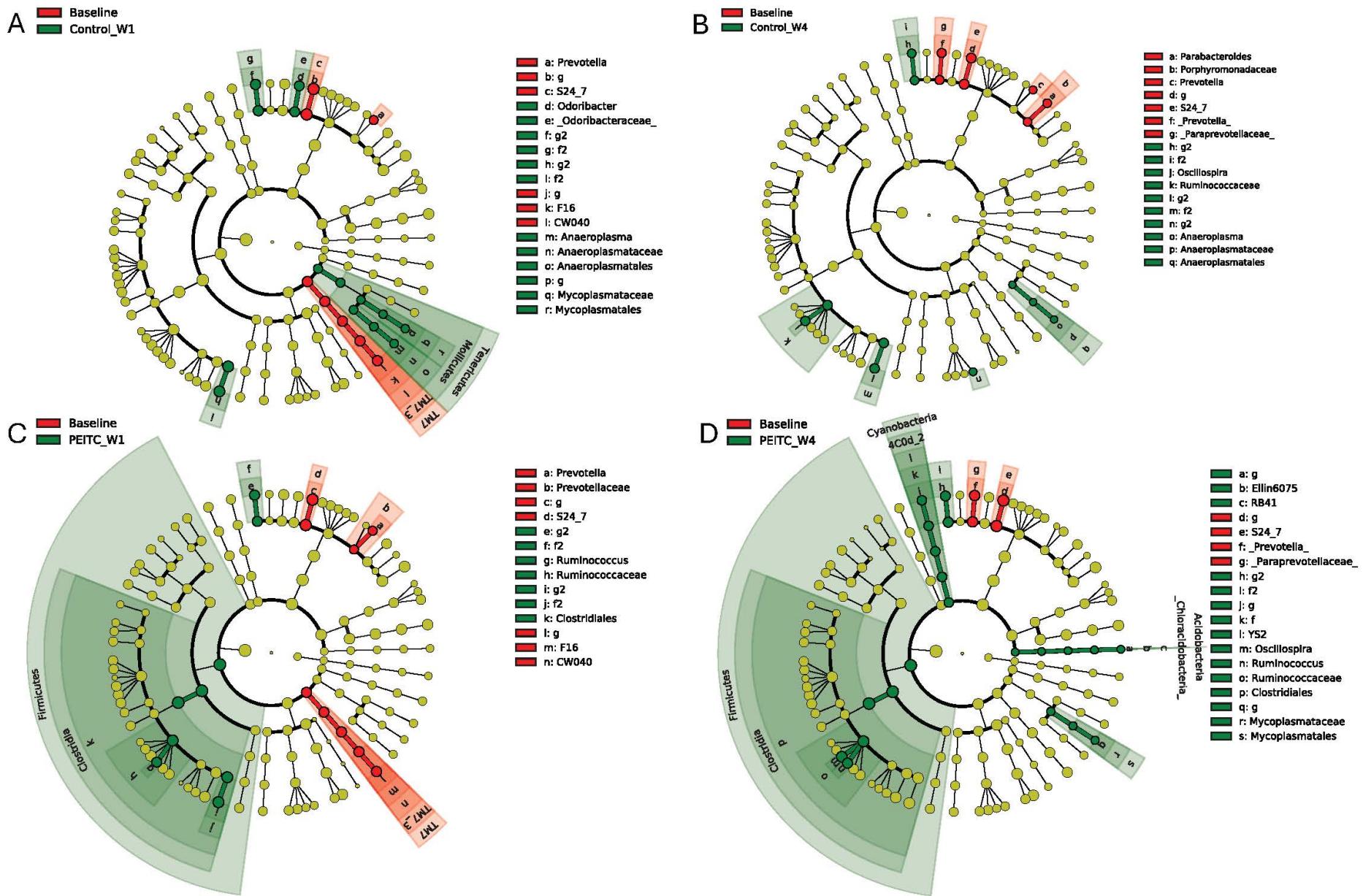


Figure 9: Linear discriminant analysis Effect Size (LEfSe) analysis of aging and PEITC dietary additives effect.

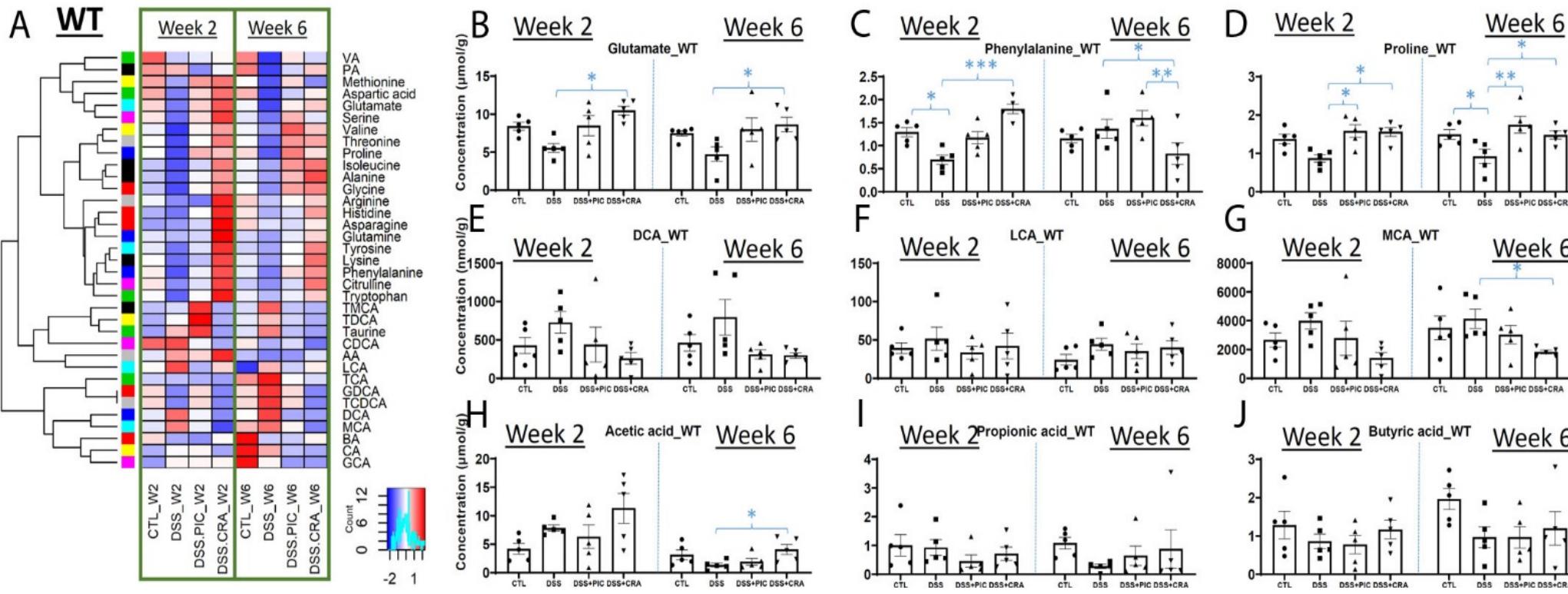


Figure 10: Effects of DSS, PEITC and cranberry cotreatments on fecal metabolome of WT mice. Fecal samples collected at week 2 and 6 of 4 treatments, including control (CTL), DSS, DSS+PEITC (DSS+PIC), and DSS+cranberry (DSS+CRA), were analyzed by 4 LC-MS methods (143). The concentrations of amino acids, bile acids, and SCFA were quantified. (A) A heatmap on the distribution of amino acids, bile acids and SCFA in fecal samples from 4 treatments. (B-D) Concentrations of major amino acids, including glutamate, phenylalanine, and proline. (E-G) Concentrations of major bile acids, including DCA, LCA, and MCA. (H-J) Concentrations of major SCFA, including acetic acid (AA), propionic acid (PA), and butyric acid (BA).

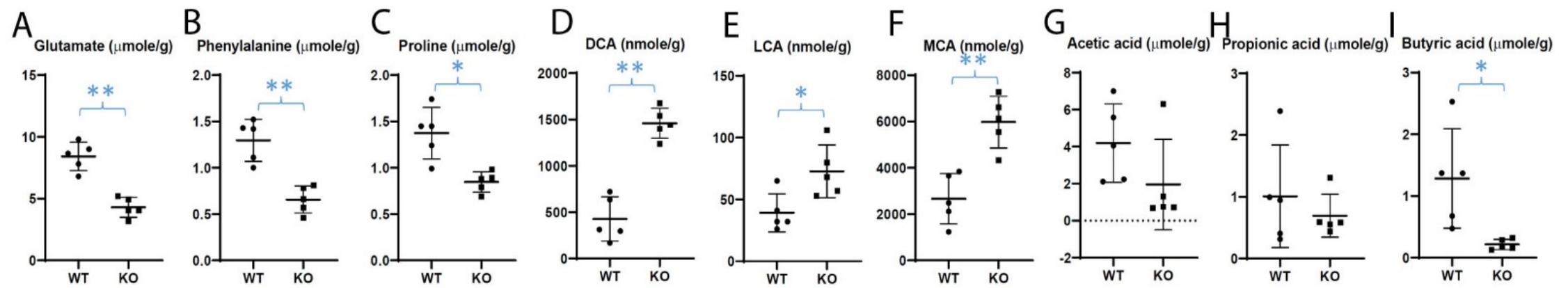


Figure 11: Differences in fecal metabolite profile between WT and Nrf2-null (KO) mice. The concentrations of amino acids, bile acids, and SCFA were quantified in the fecal samples from untreated WT and KO mice (143). (A-C) Concentrations of glutamate, phenylalanine, and proline. (D-F) Concentrations of major bile acids. (G-I) Concentrations of major SCFA.

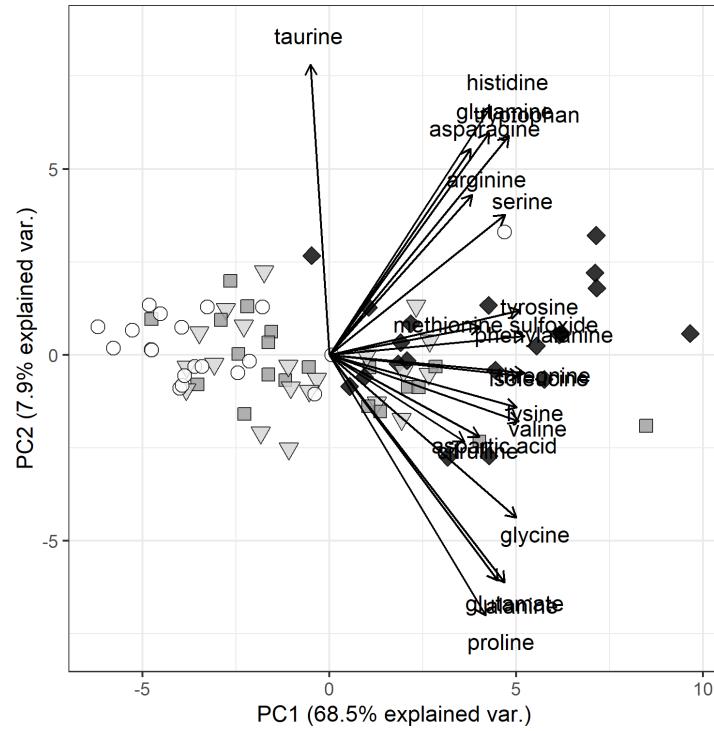
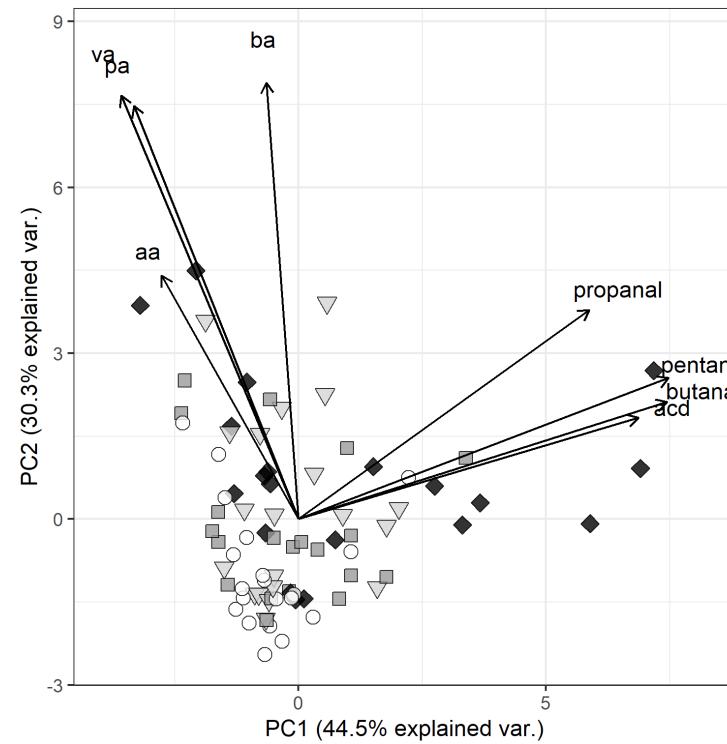
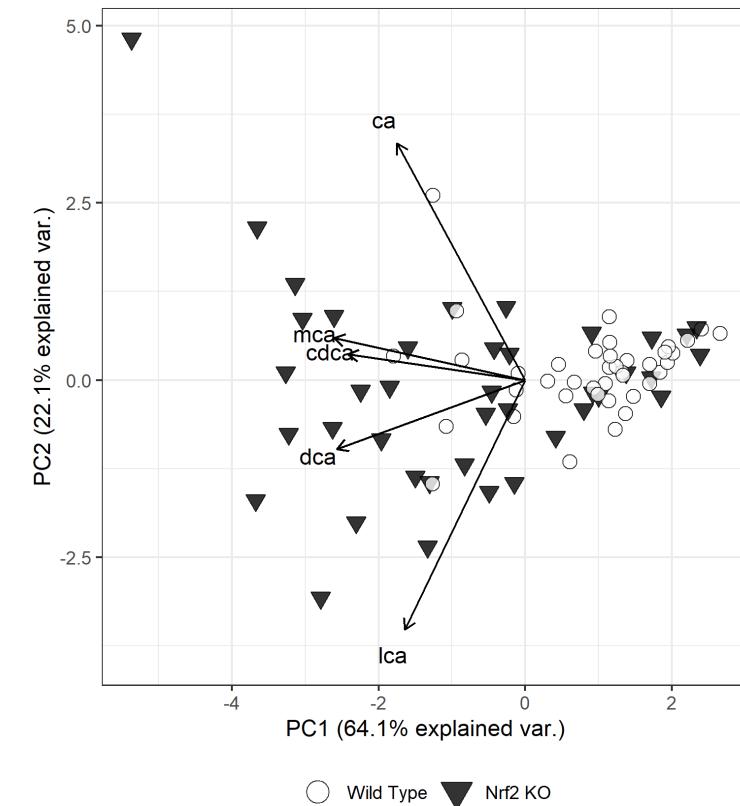
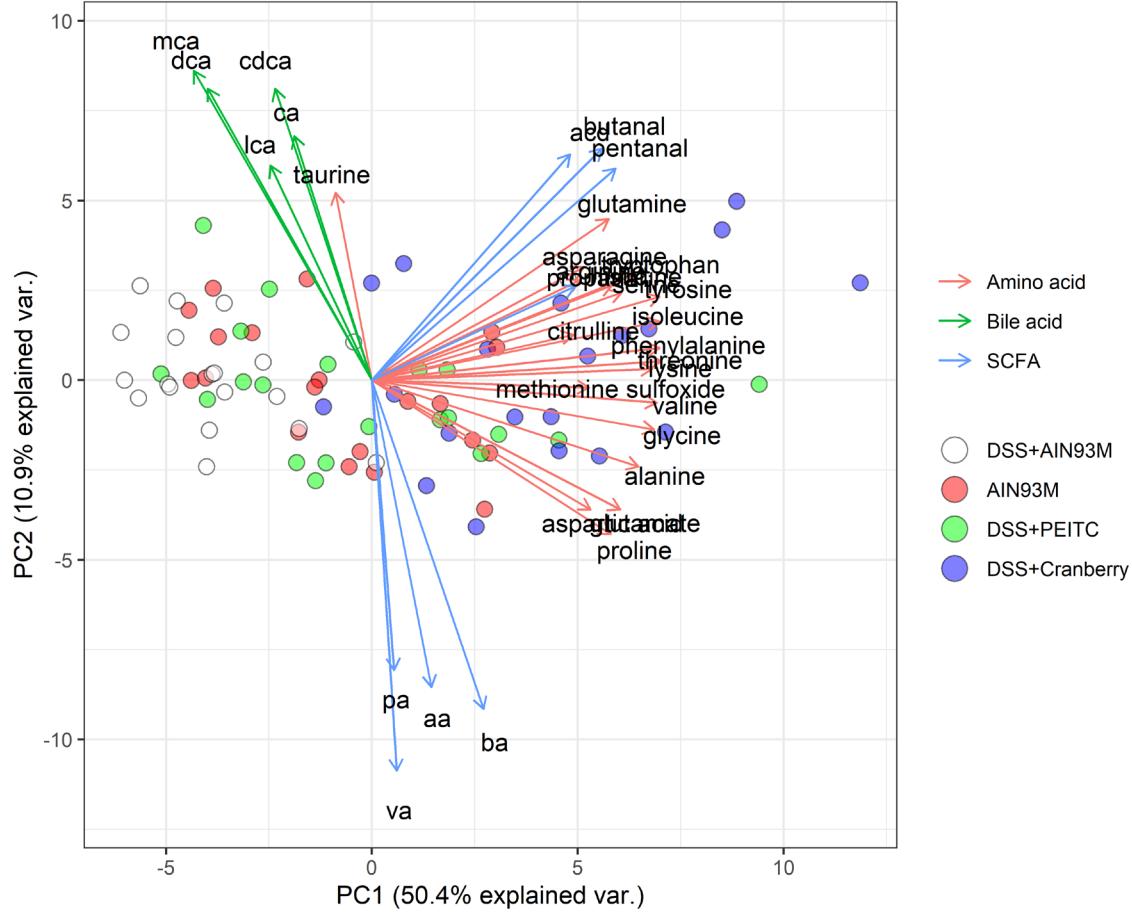
**A****Amino Acids****B****SCFA****C****Bile Acids**

Figure 12: Biplots of amino acids (A) and SCFA (B) by diet, and bile acids (C) by genotype.

A



B

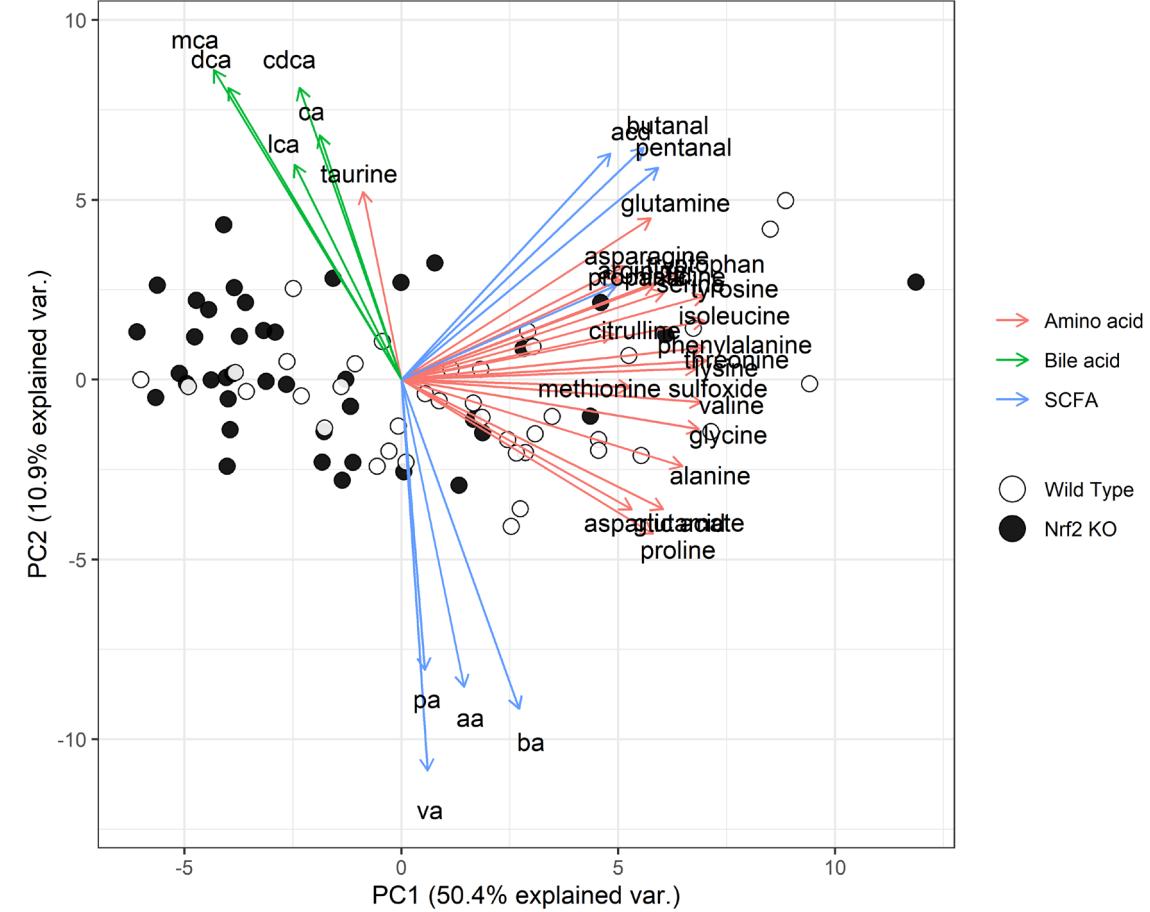


Figure 13: Biplots of bacterial metabolites based on the results of PCA on combined data with concentrations of amino acids, SCFA and bile acids by diet (A) and genotype (B).

| Forward Primer     | Reverse Primer       |
|--------------------|----------------------|
| 515F (Parada)      | 806R (Apprill)       |
| GTGYCAGCMGCCGCGTAA | GGACTACNVGGGTWTCTAAT |

Supplemental Table 1: V4 primer sequence used for 16s RNA sequencing library preparation

| Kingdom   | Experiment 1:<br>Nrf2 KO Mice | Experiment 2:<br>WT Mice | Experiment 3:<br>WT and Nrf2<br>KO | Combined        |
|-----------|-------------------------------|--------------------------|------------------------------------|-----------------|
| Bacteria  | 10,197 (94.78%)               | 7,994 (98.34%)           | 7,558 (96.07%)                     | 22,251 (95.73%) |
| Eukaryota | 472 (4.39%)                   | 116 (1.43%)              | 232 (2.95%)                        | 812 (3.49%)     |
| Archaea   | 4 (0.04%)                     | 0 (0%)                   | 2 (0.03%)                          | 6 (0.03%)       |
| Unknown   | 86 (0.80%)                    | 19 (0.23%)               | 75 (0.95%)                         | 175 (0.75%)     |

Table 1: OTU mapping to Kingdoms. Number of OTUs found in each experiment (% total).

| Predicted diet and DSS challenge<br>(PC1+PC2+PC3+PC4+PC5) | Observed diet and DSS challenge |               |           |                 |
|---|---------------------------------|---------------|-----------|-----------------|
|   | DSS+AIN93M                      | No DSS+AIN93M | DSS+PEITC | DSS + Cranberry |
| DSS + AIN93M  | 15                              | 3             | 5         | 0               |
| No DSS + AIN93M   | 2                               | 10            | 4         | 4               |
| DSS + PEITC   | 3                               | 5             | 9         | 2               |
| DSS + Cranberry   | 0                               | 2             | 2         | 14              |

Table 2: multinomial regression predictions of treatment groups by microbial metabolite PCA

| Predicted genotype<br>(PC1+PC2+PC3+PC4+PC5) | Observed genotype |         |
|---|-------------------|---------|
|   | Wild Type         | Nrf2 KO |
| Wild Type                                   | 33                | 12      |
| Nrf2 KO                                     | 7                 | 28      |

Table 3: multinomial regression predictions of genotype by microbial metabolite PCA