

Experiment 1:

Group1: Nrf2^{-/-}, AIN93M (N=9)

Group2: Nrf2^{-/-}, 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^{-/-}, AIN93M (N=5)

Group10: Nrf2^{-/-}, AIN93M, DSS (N=5)

Group11: Nrf2^{-/-}, Cranberry, DSS (N=5)

Group12: Nrf2^{-/-}, PEITC, DSS (N=5)

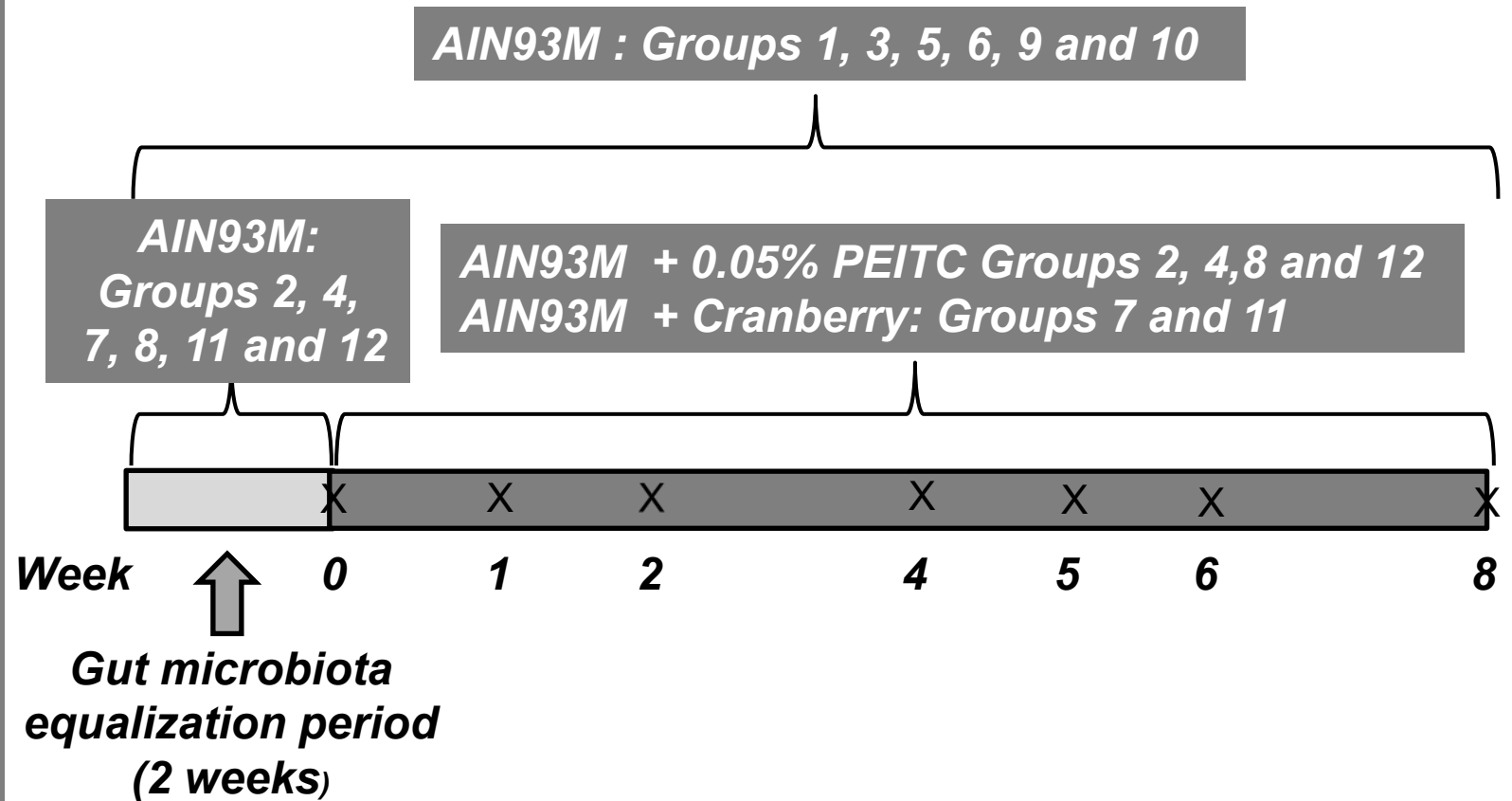
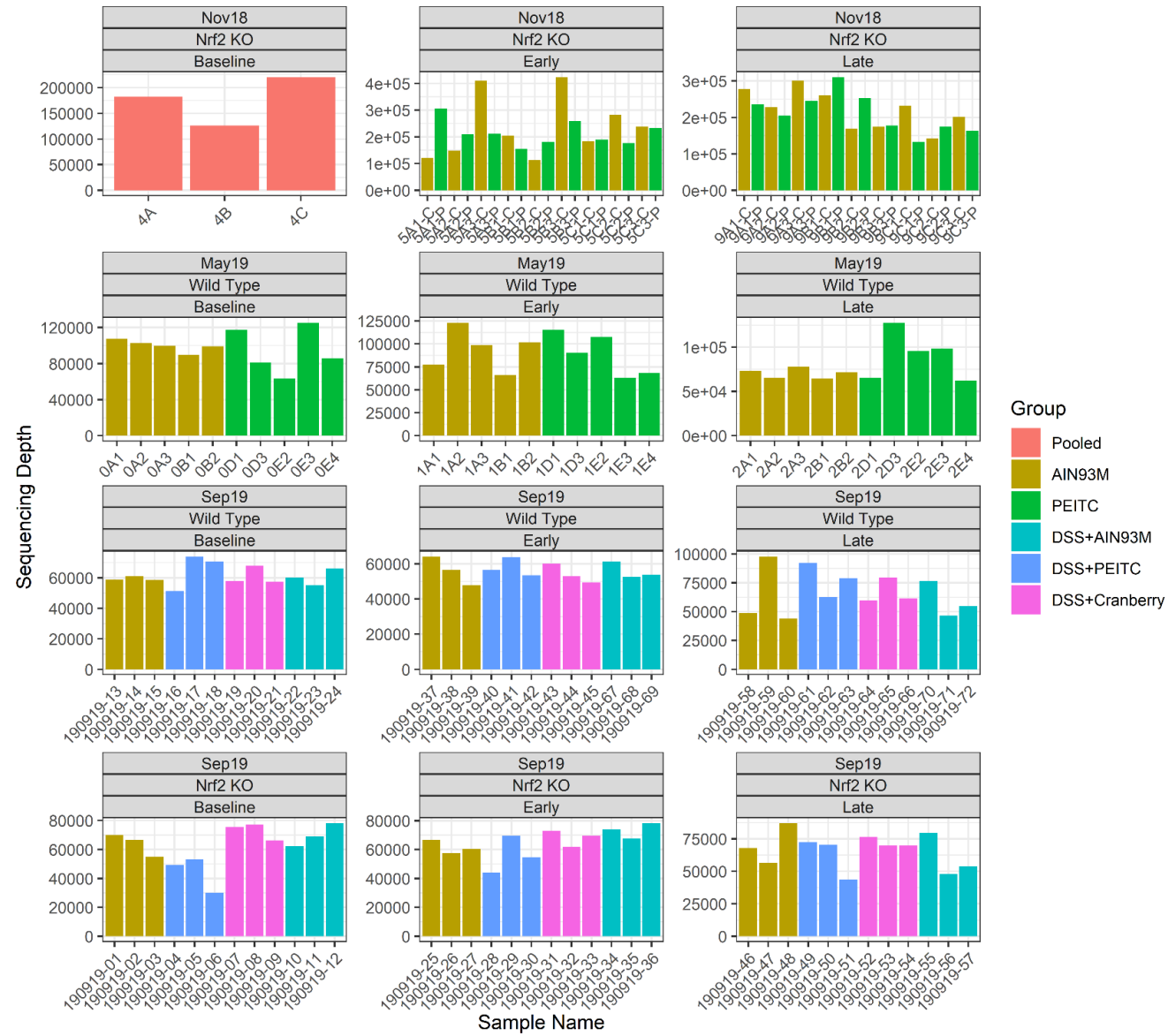


Figure 1: Experimental design. Mice fecal samples for the 16S sequencing were collected individually at 3 timepoints – at the end of the 2-week equalization period (Week 0), at an early timepoint (Week 1) and at a late timepoint (Week 4 or Week 8). Samples used for metabolite analysis were collected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



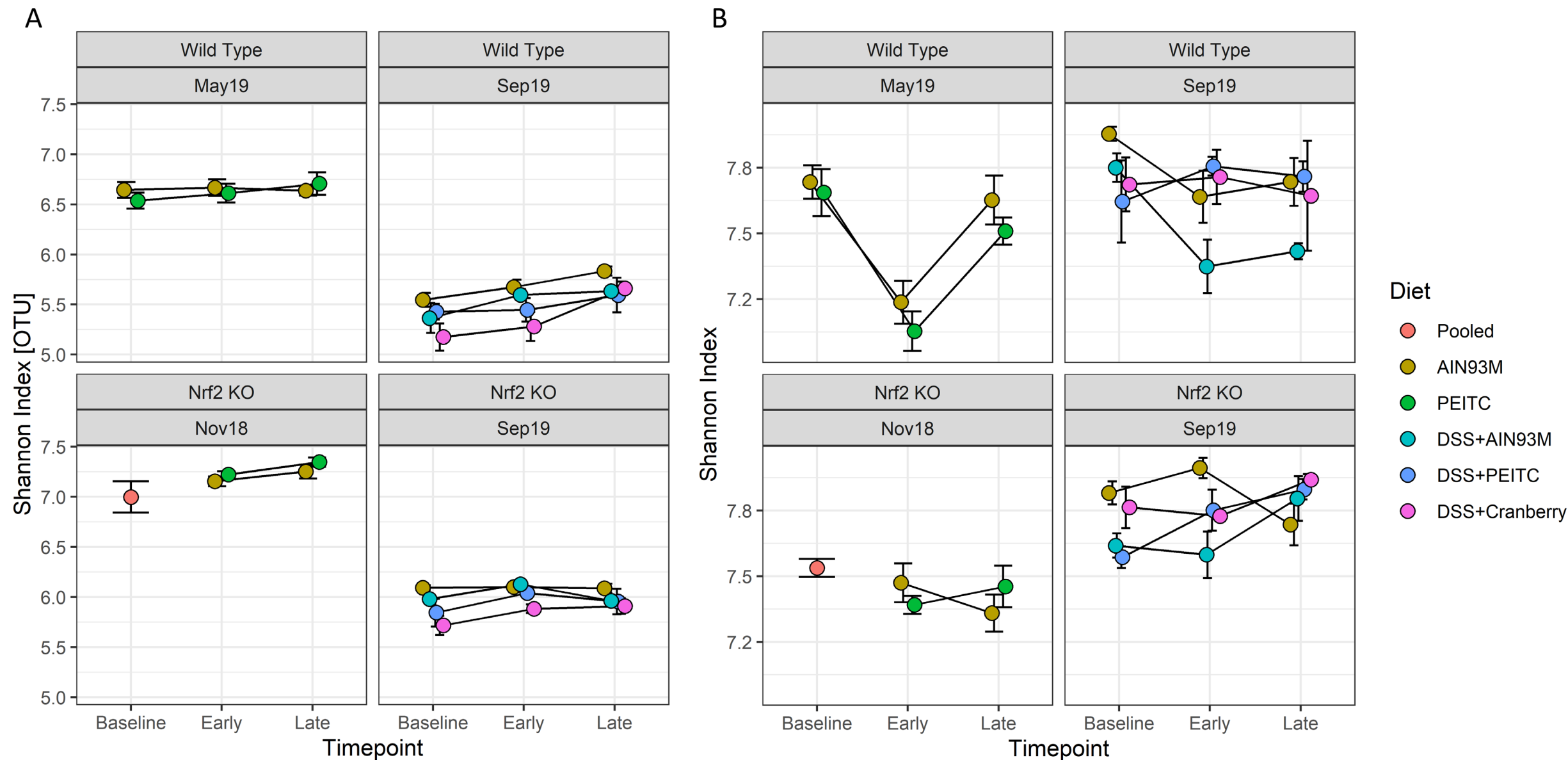
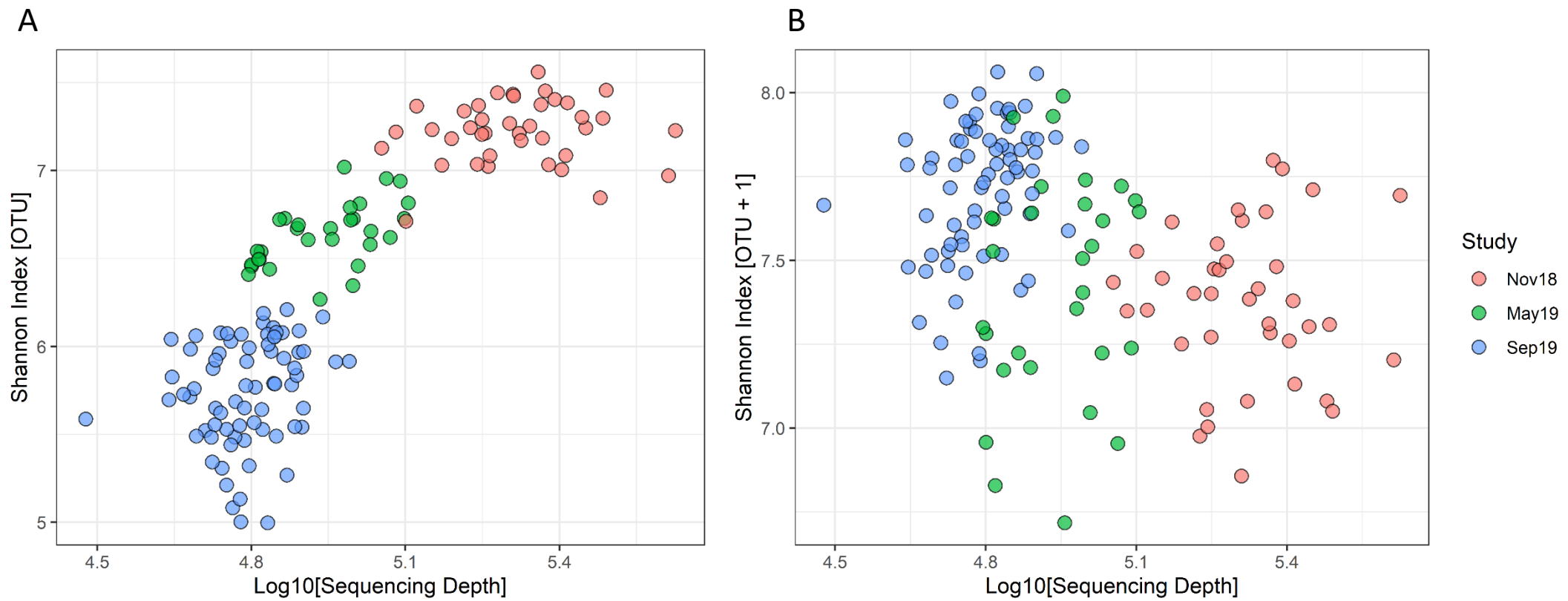
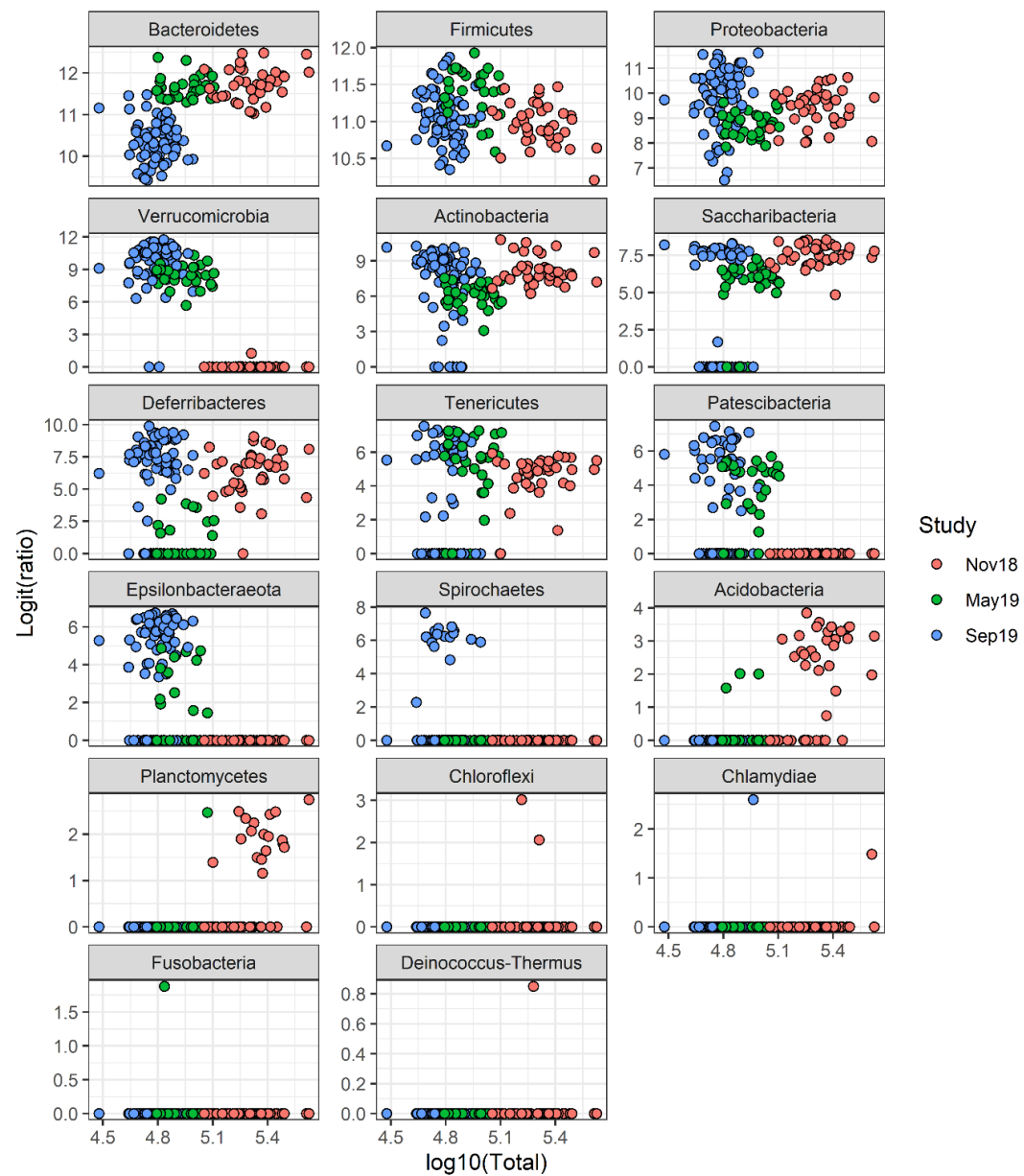


Figure 2: Alpha diversity measured by Shannon index. (A) Averages of Shannon indices calculated on raw OTU numbers and (B) on corrected OTU numbers (OTU+1).



Supplemental Figure 2: Shannon index vs. sequencing depth.



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

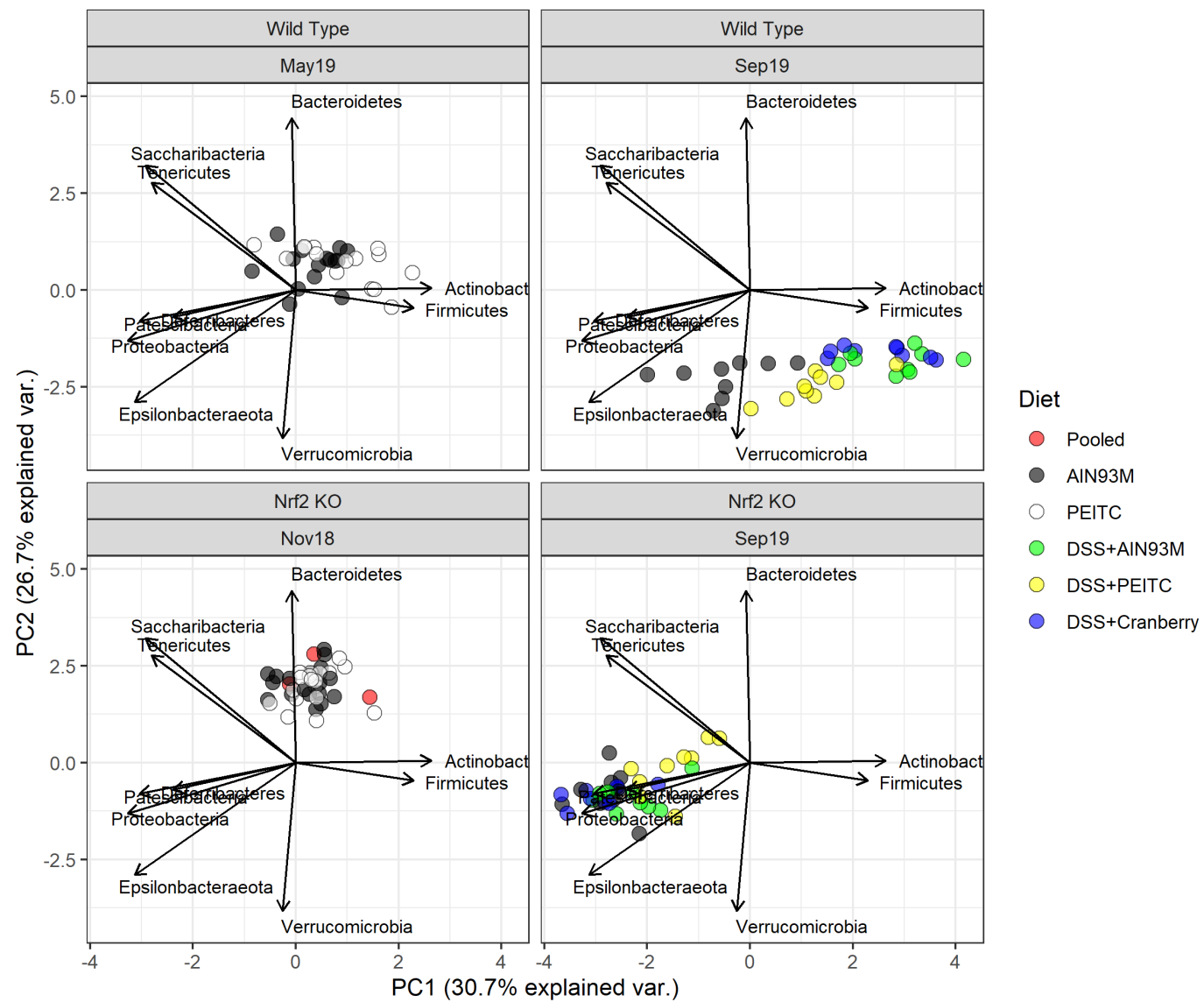


Figure 4: Biplot of logit relative abundance of Phylum

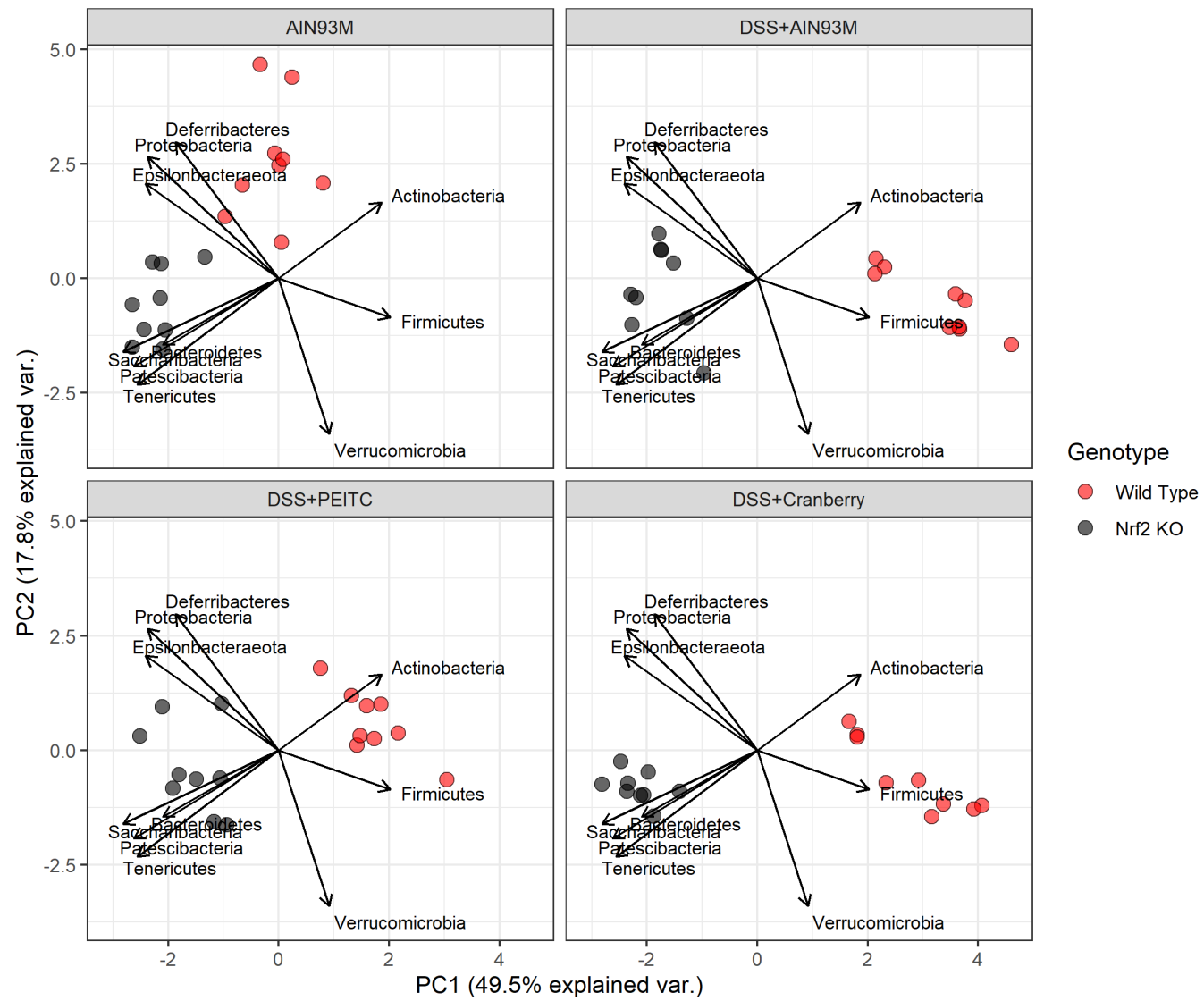


Figure 5: biplot of logit relative abundance of Phylum in Exp03 only

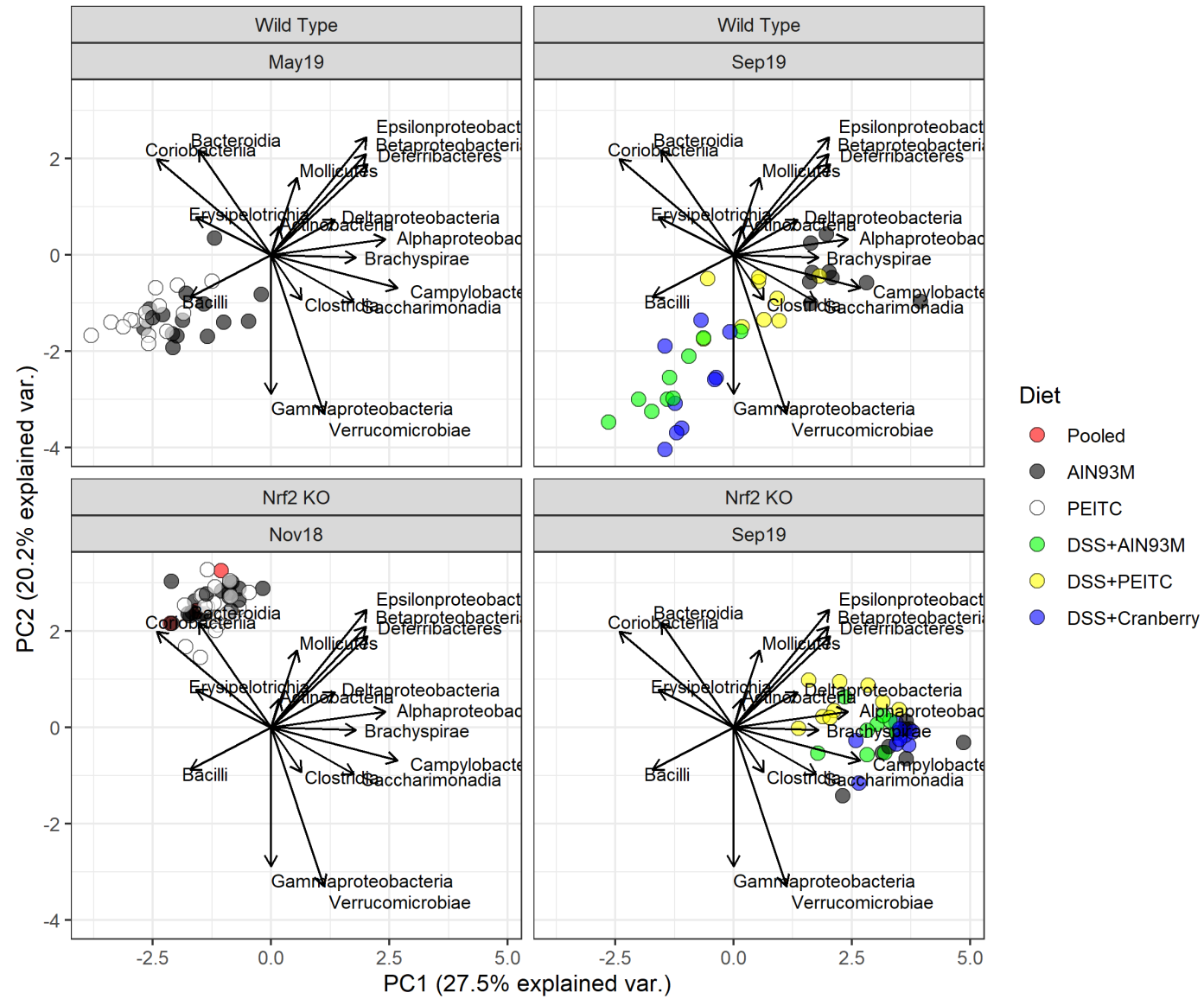


Figure 6: Biplot of logit relative abundance of bacterial classes

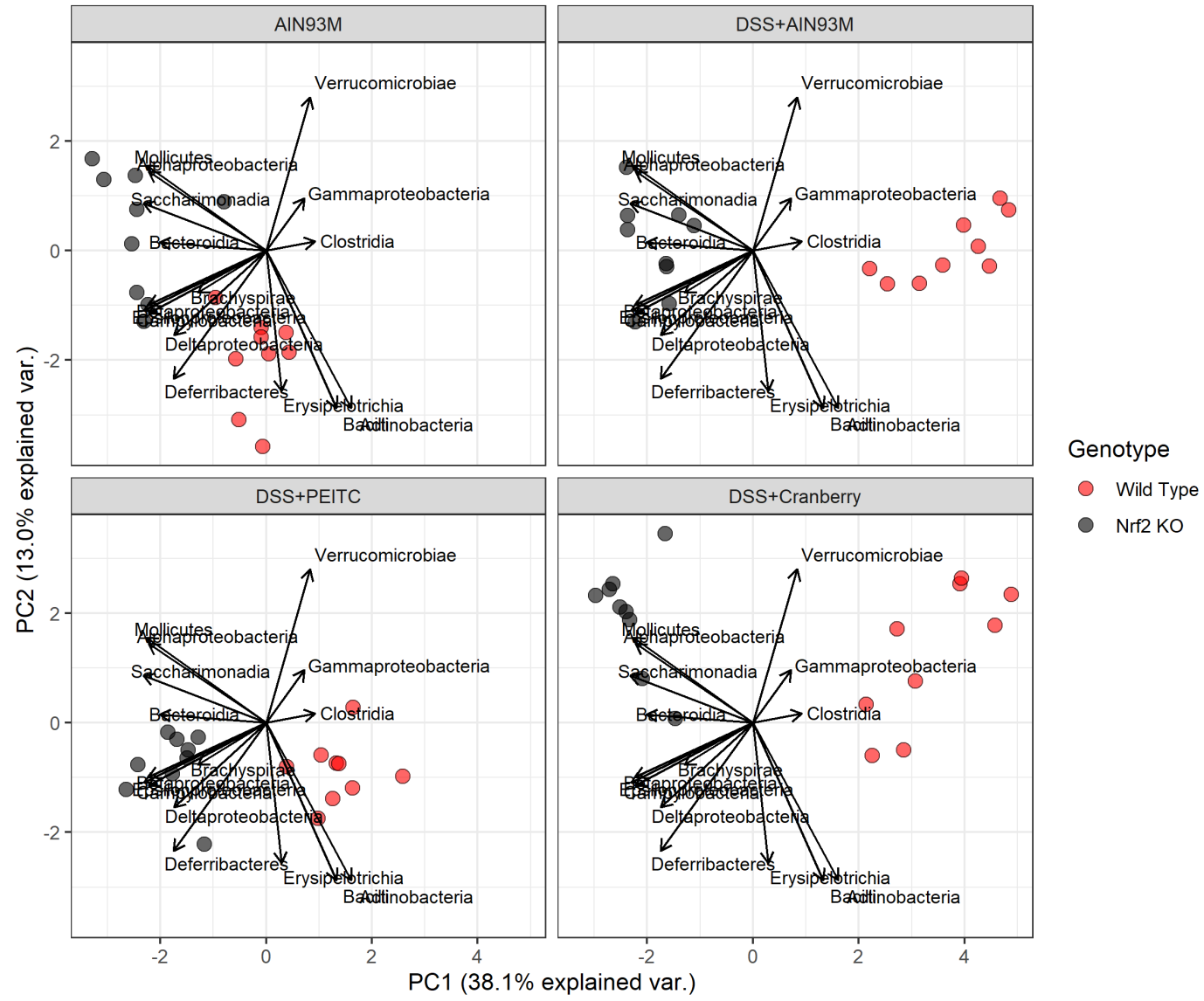


Figure 7: biplot of logit relative abundance of bacterial classes in Exp03 only

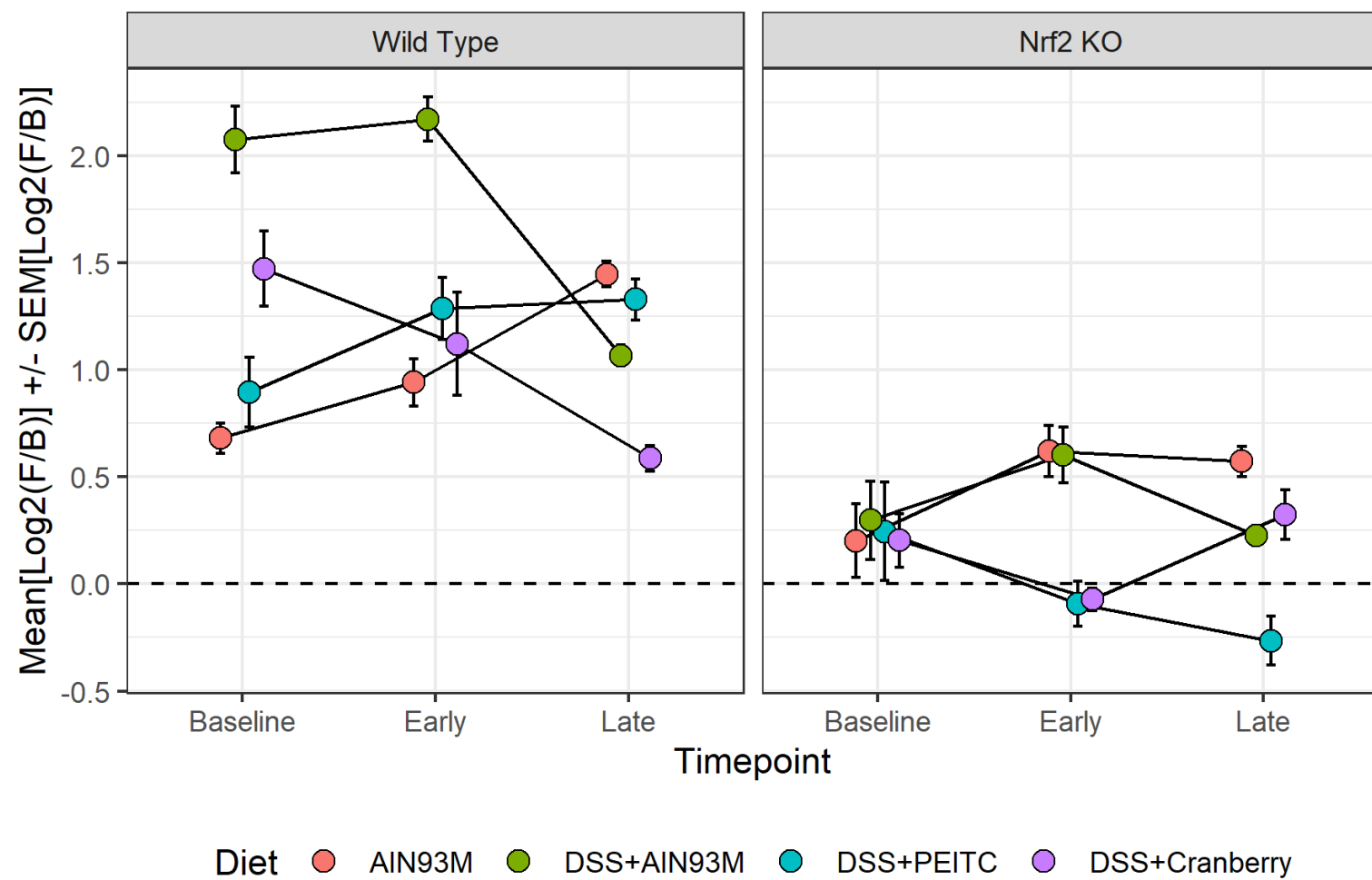


Figure 8: Means of log2 F/B ratios by genotype and diet over time. The bars represent standard errors of log2(F/B) ratios.

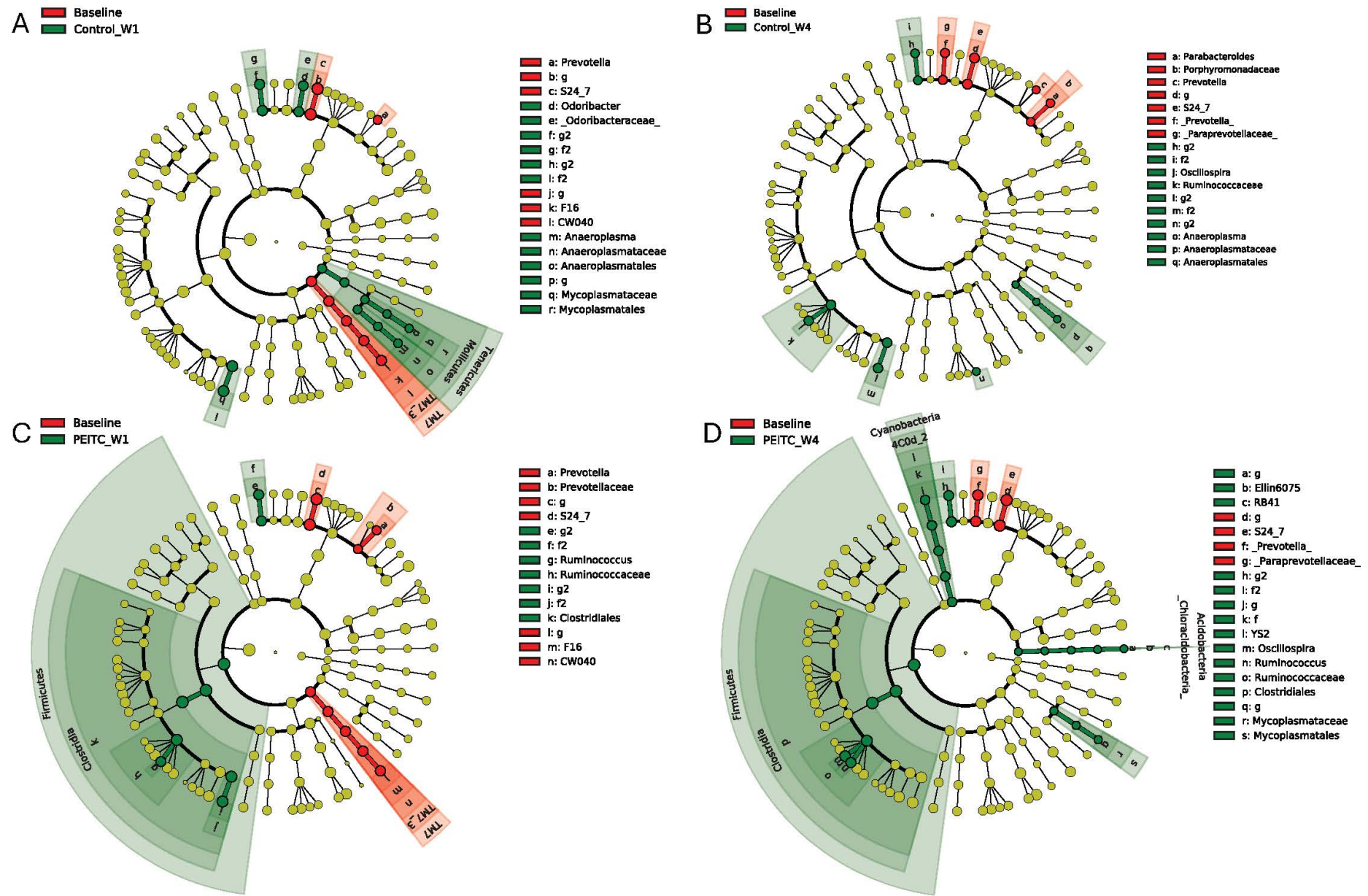
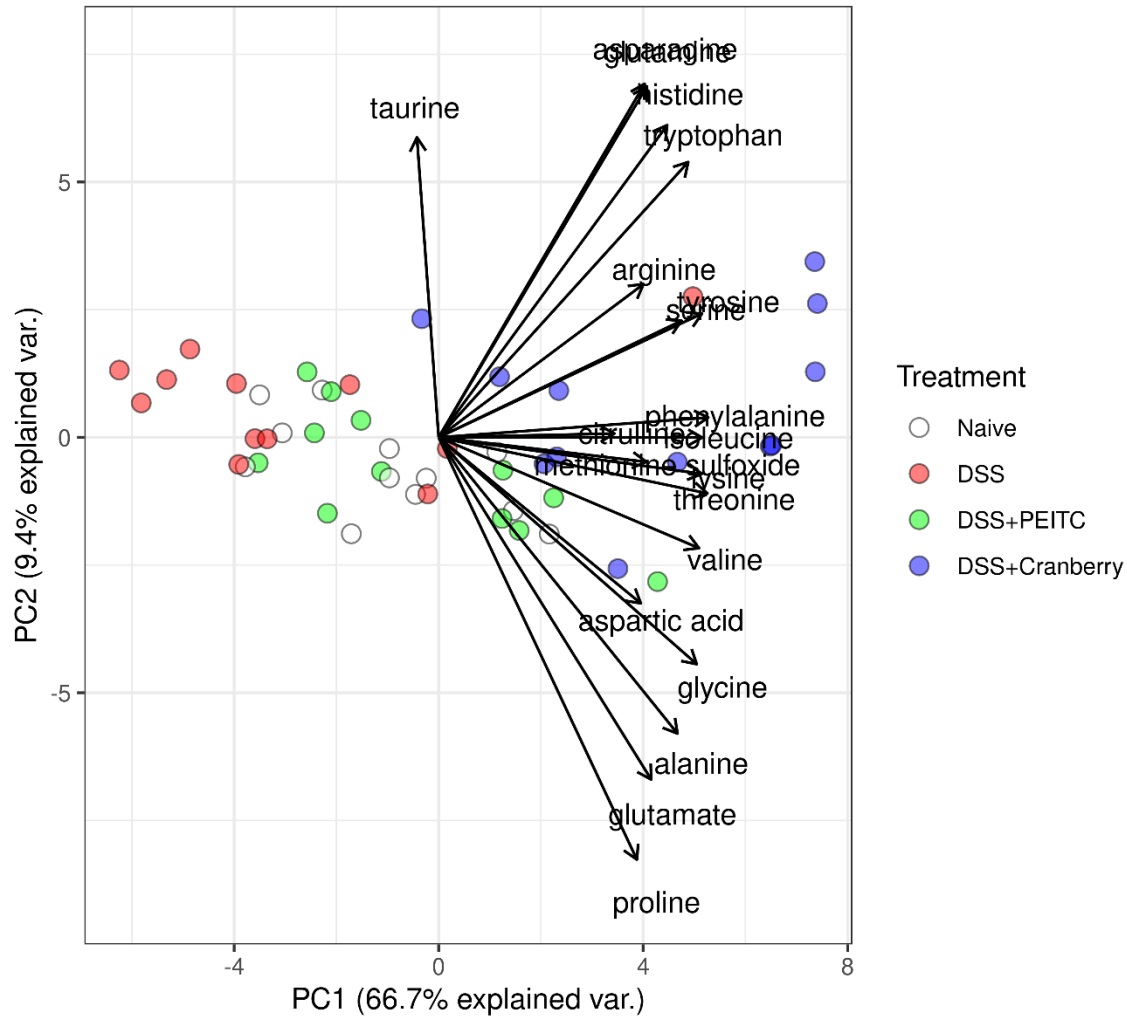


Figure 9: Linear discriminant analysis Effect Size (LEfSe) analysis of aging and PEITC dietary additives effect.

A

Biplot of Aminoacids



B

Biplot of Bile Acids

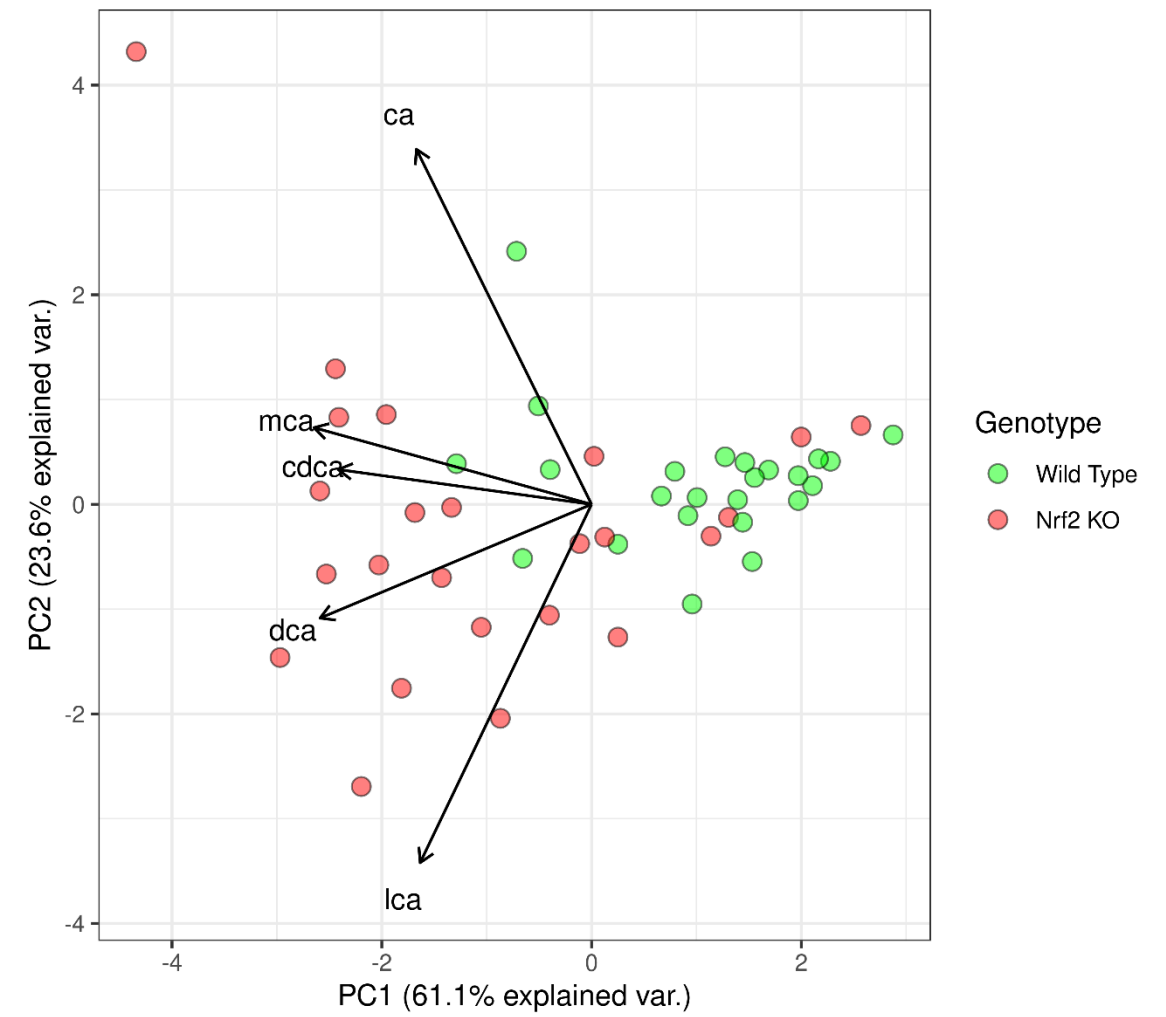


Figure 10: Biplots of amino acids by diet (A) and bile acids by genotype (B) .

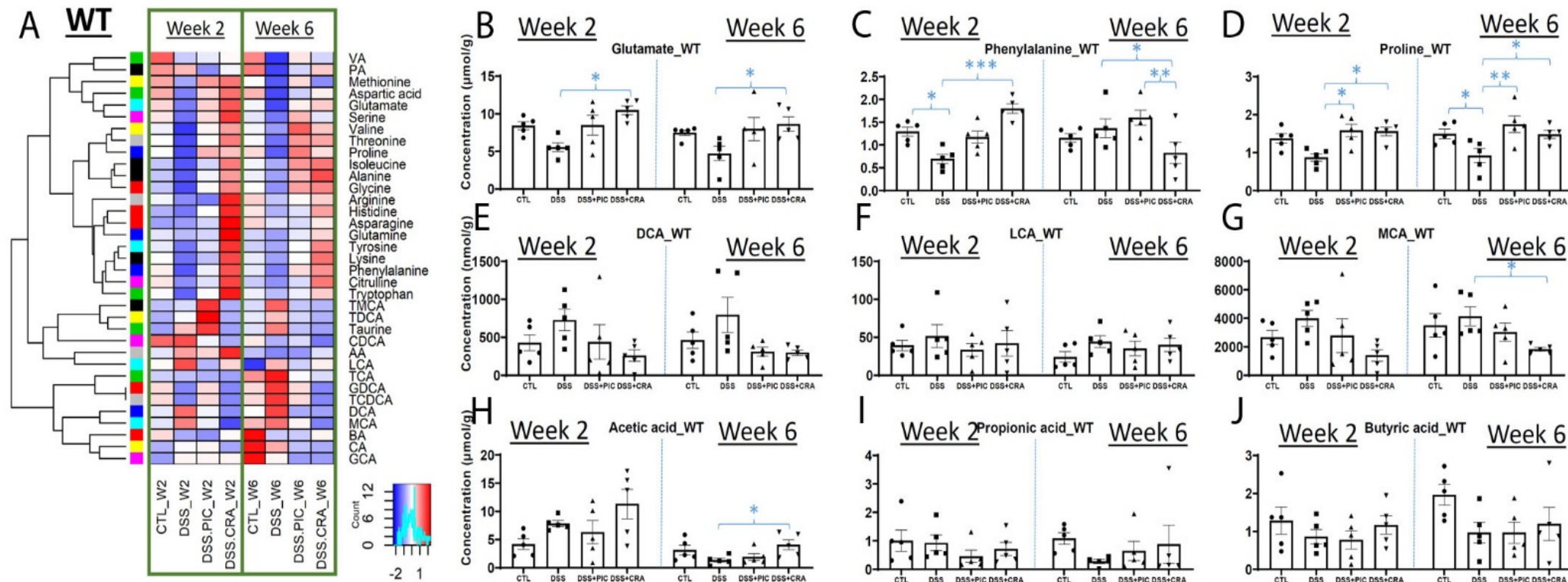


Figure 11: Effects of DSS, PEITC and cranberry cotreatments on fecal metabolome of WT mice. Fecal samples collected at week 2 and 6 of 4 treatments, including control (CTL), DSS, DSS+PEITC (DSS+PIC), and DSS+cranberry (DSS+CRA), were analyzed by 4 LC-MS methods (143). The concentrations of amino acids, 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).

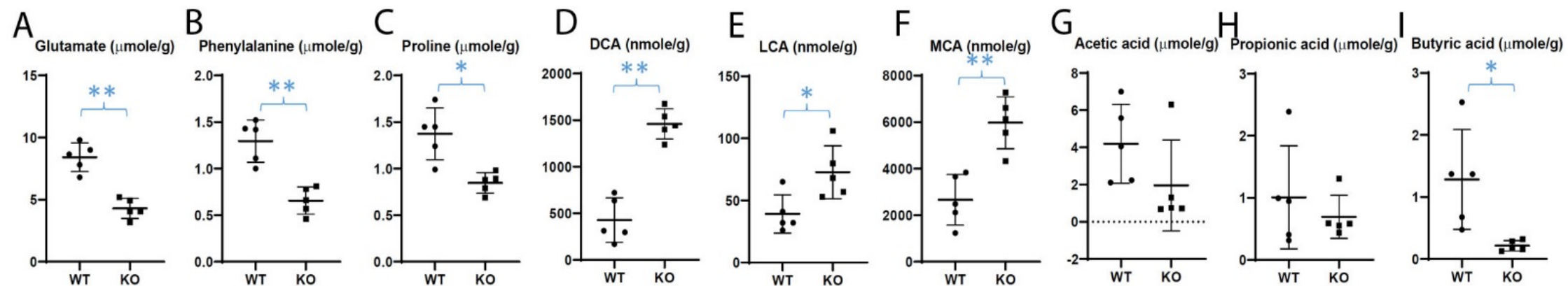


Figure 12: Differences in fecal metabolite profile between WT and Nrf2-null (KO) mice. The concentrations of amino acids, bile acids, and SCFA were quantified in the fecal samples from untreated WT and KO mice (143). (A-C) Concentrations of glutamate, phenylalanine, and proline. (D-F) Concentrations of major bile acids. (G-I) Concentrations of major SCFA.

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

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

| Kingdom | Experiment 1: Nrf2 KO Mice | Experiment 2: WT Mice | Experiment 3: WT and Nrf2 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 (PC1+PC2+PC3) | Observed diet and DSS challenge | | | |
|--|---------------------------------|------------|-----------|-----------------|
| | No DSS+AIN93M | DSS+AIN93M | DSS+PEITC | DSS + Cranberry |
| No DSS + AIN93M | 4 | 2 | 1 | 0 |
| DSS + AIN93M | 4 | 8 | 1 | 0 |
| DSS + PEITC | 3 | 1 | 6 | 1 |
| DSS + Cranberry | 1 | 1 | 1 | 11 |

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

| Predicted genotype (PC1) | Observed genotype | |
|--------------------------|-------------------|---------|
| | Wild Type | Nrf2 KO |
| Wild Type | 18 | 6 |
| Nrf2 KO | 8 | 16 |

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