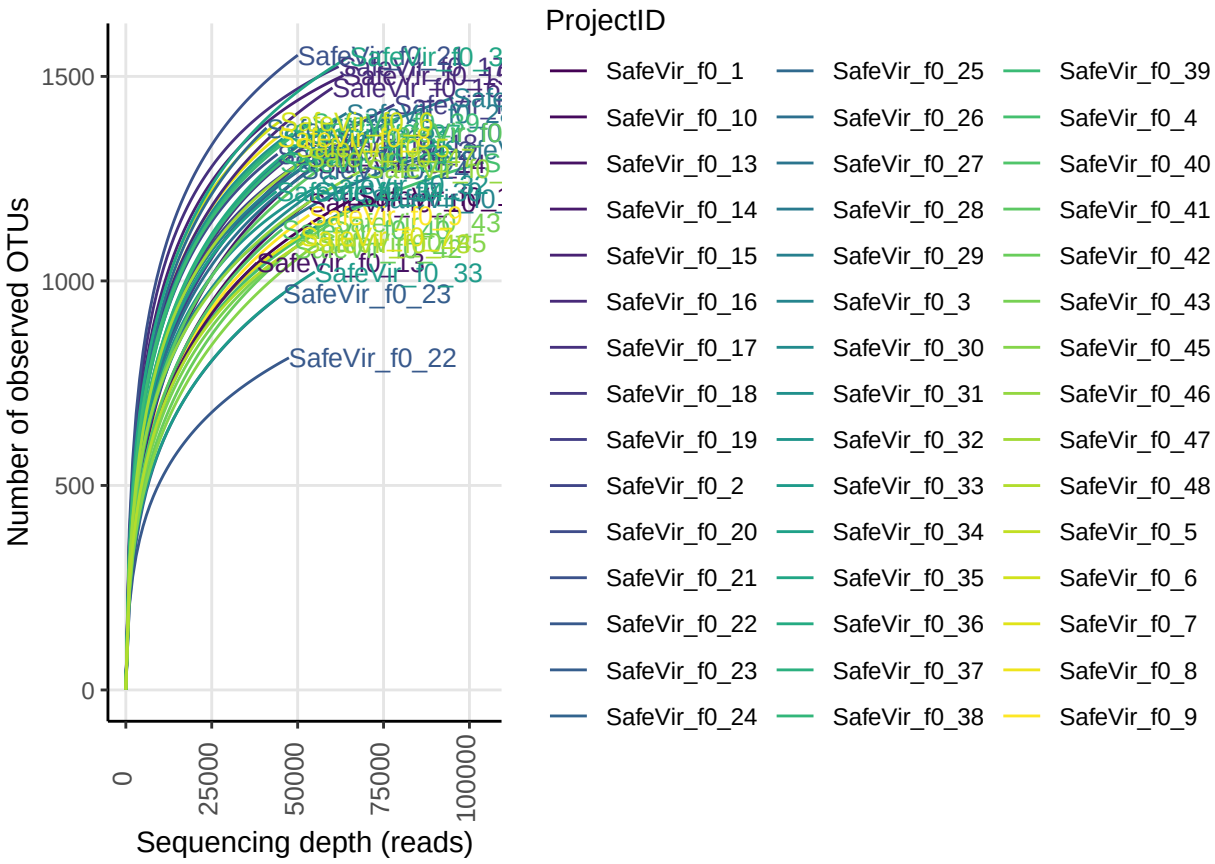
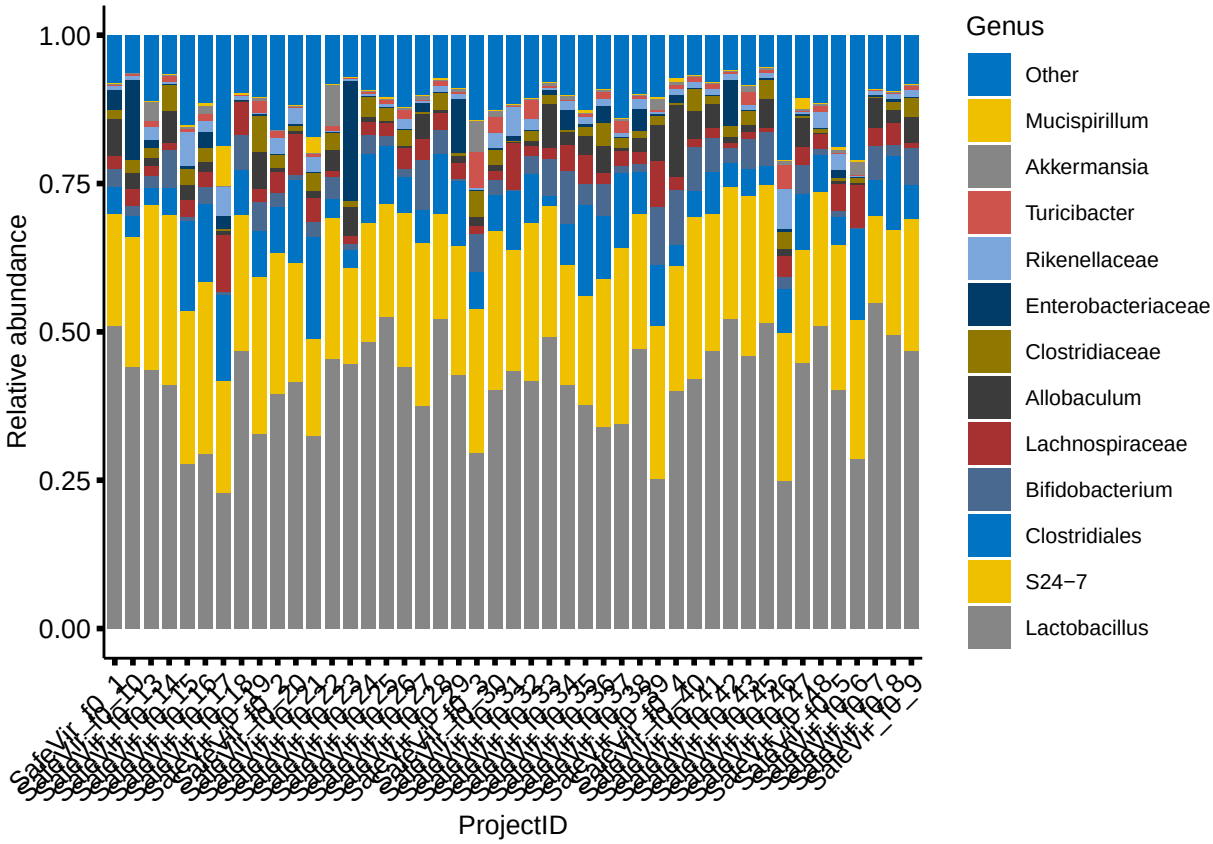
16S rRNA gene amplicon analysis - SafeVir - DIO - Before_1st_FVT

Sequencing depth

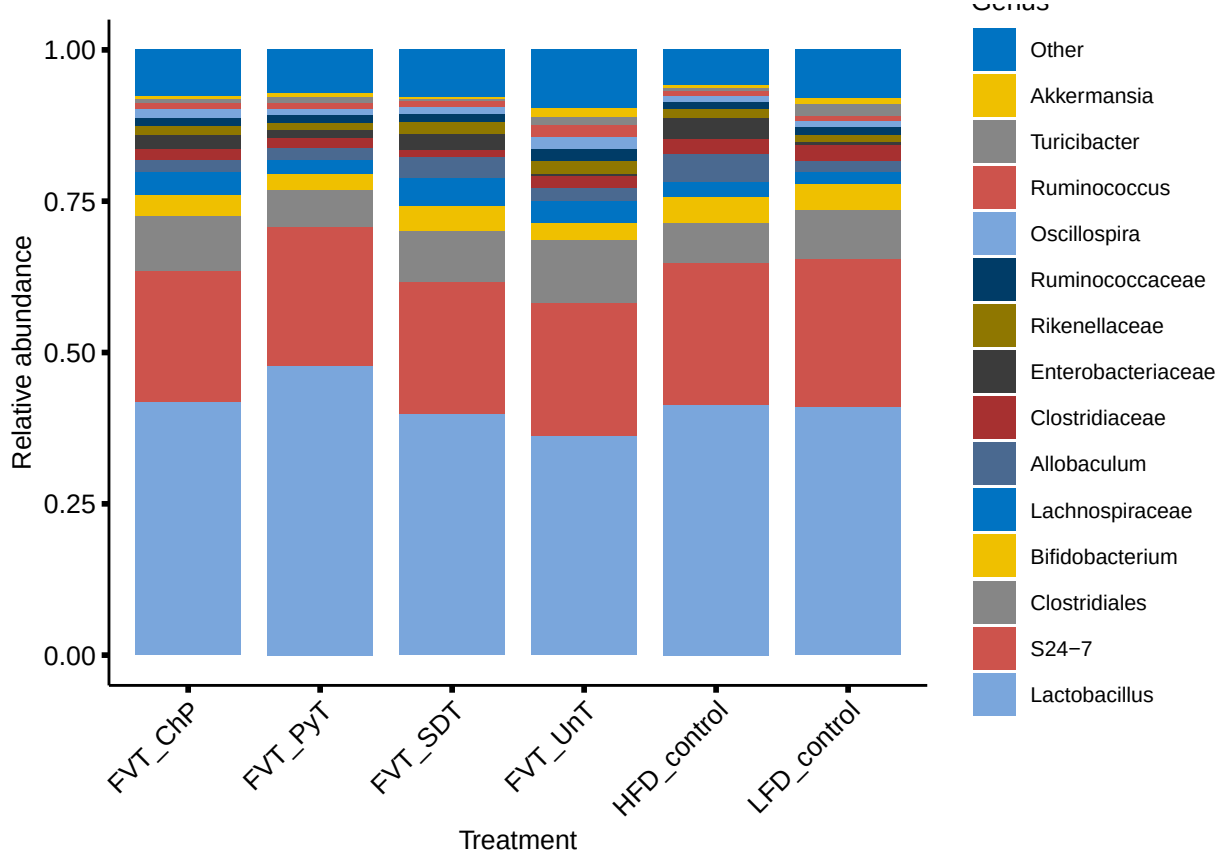


Individual sample barplots.



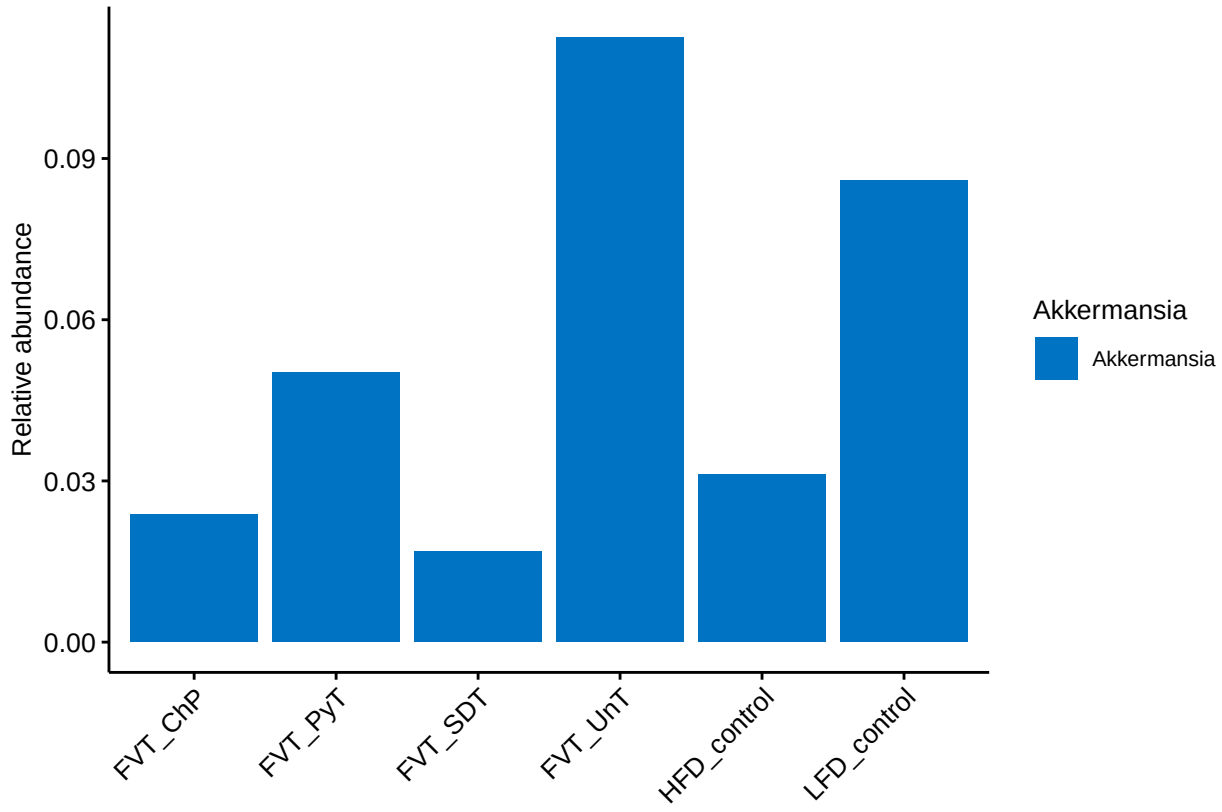
Barplots based on treatments.

```
## # A tibble: 690 x 3
## # Groups:   Sample [6]
##    Sample      tax            Mean
##    <chr>       <chr>         <dbl>
##  1 FVT_PyT     Lactobacillus 0.477
##  2 FVT_ChP     Lactobacillus 0.418
##  3 HFD_control Lactobacillus 0.413
##  4 LFD_control Lactobacillus 0.411
##  5 FVT_SDT     Lactobacillus 0.398
##  6 FVT_UnT     Lactobacillus 0.363
##  7 LFD_control S24-7         0.245
##  8 HFD_control S24-7         0.235
##  9 FVT_PyT     S24-7         0.230
## 10 FVT_SDT     S24-7         0.219
## # ... with 680 more rows
```




```
## # A tibble: 45 x 3
## # Groups:   Sample [45]
##    Sample       tax            Mean
##    <chr>        <chr>         <dbl>
##  1 NXT075Mao214 Akkermansia 0.0699
##  2 NXT075Mao195 Akkermansia 0.0532
##  3 NXT075Mao205 Akkermansia 0.0334
##  4 NXT075Mao200 Akkermansia 0.0225
##  5 NXT075Mao231 Akkermansia 0.0196
##  6 NXT075Mao208 Akkermansia 0.0138
##  7 NXT075Mao235 Akkermansia 0.0115
##  8 NXT075Mao222 Akkermansia 0.0105
##  9 NXT075Mao226 Akkermansia 0.00778
## 10 NXT075Mao219 Akkermansia 0.00771
## # ... with 35 more rows

## Warning: Unknown levels in ‘f‘: Other
```



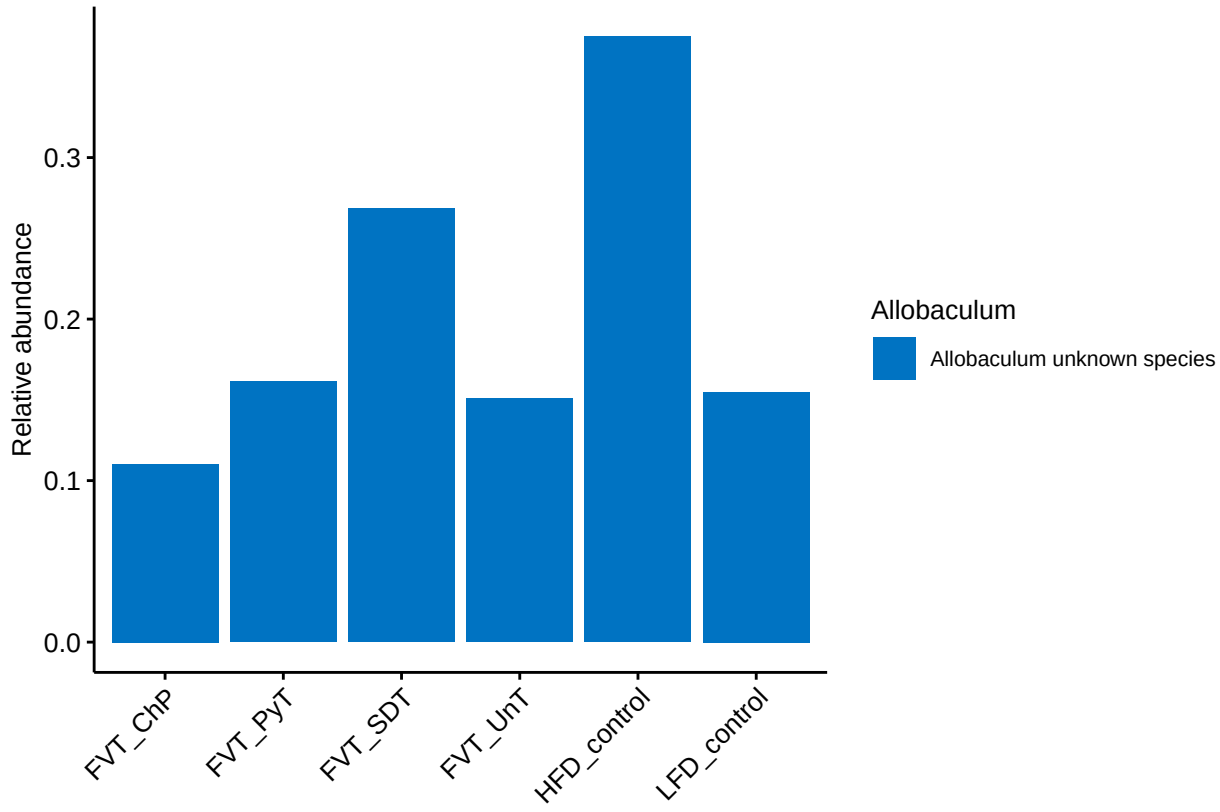

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.015911 -0.005552 -0.002186  0.000723  0.053834
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           0.010739   0.004828   2.224    0.032 *
## TreatmentFVT_ChP     -0.006775   0.007375  -0.919    0.364
## TreatmentFVT_PyT     -0.004456   0.006828  -0.653    0.518
## TreatmentFVT_SDT     -0.008620   0.006828  -1.262    0.214
## TreatmentFVT_UnT      0.005352   0.007067   0.757    0.453
## TreatmentHFD_control -0.006830   0.006828  -1.000    0.323
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01366 on 39 degrees of freedom
## Multiple R-squared:  0.1226, Adjusted R-squared:  0.0101
## F-statistic:  1.09 on 5 and 39 DF,  p-value: 0.3814

##
## Call:
```

```
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.015911 -0.005552 -0.002186  0.000723  0.053834
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)            0.0039083  0.0048280   0.809   0.4231
## TreatmentLFD_control   0.0068305  0.0068278   1.000   0.3233
## TreatmentFVT_ChP       0.0000557  0.0073749   0.008   0.9940
## TreatmentFVT_PyT       0.0023740  0.0068278   0.348   0.7299
## TreatmentFVT_SDT      -0.0017891  0.0068278  -0.262   0.7947
## TreatmentFVT_UnT       0.0121827  0.0070675   1.724   0.0927 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01366 on 39 degrees of freedom
## Multiple R-squared:  0.1226, Adjusted R-squared:  0.0101
## F-statistic:  1.09 on 5 and 39 DF,  p-value: 0.3814
```

```
## # A tibble: 45 x 3
## # Groups:   Sample [45]
##    Sample       tax                           Mean
##    <chr>        <chr>                        <dbl>
##  1 NXT075Mao196 Allobaculum unknown species 0.123
##  2 NXT075Mao225 Allobaculum unknown species 0.0761
##  3 NXT075Mao193 Allobaculum unknown species 0.0635
##  4 NXT075Mao211 Allobaculum unknown species 0.0627
##  5 NXT075Mao231 Allobaculum unknown species 0.0601
##  6 NXT075Mao206 Allobaculum unknown species 0.0555
##  7 NXT075Mao201 Allobaculum unknown species 0.0505
##  8 NXT075Mao215 Allobaculum unknown species 0.0498
##  9 NXT075Mao264 Allobaculum unknown species 0.0488
## 10 NXT075Mao237 Allobaculum unknown species 0.0488
## # ... with 35 more rows
```

```
## Warning: Unknown levels in `f`: Other
```



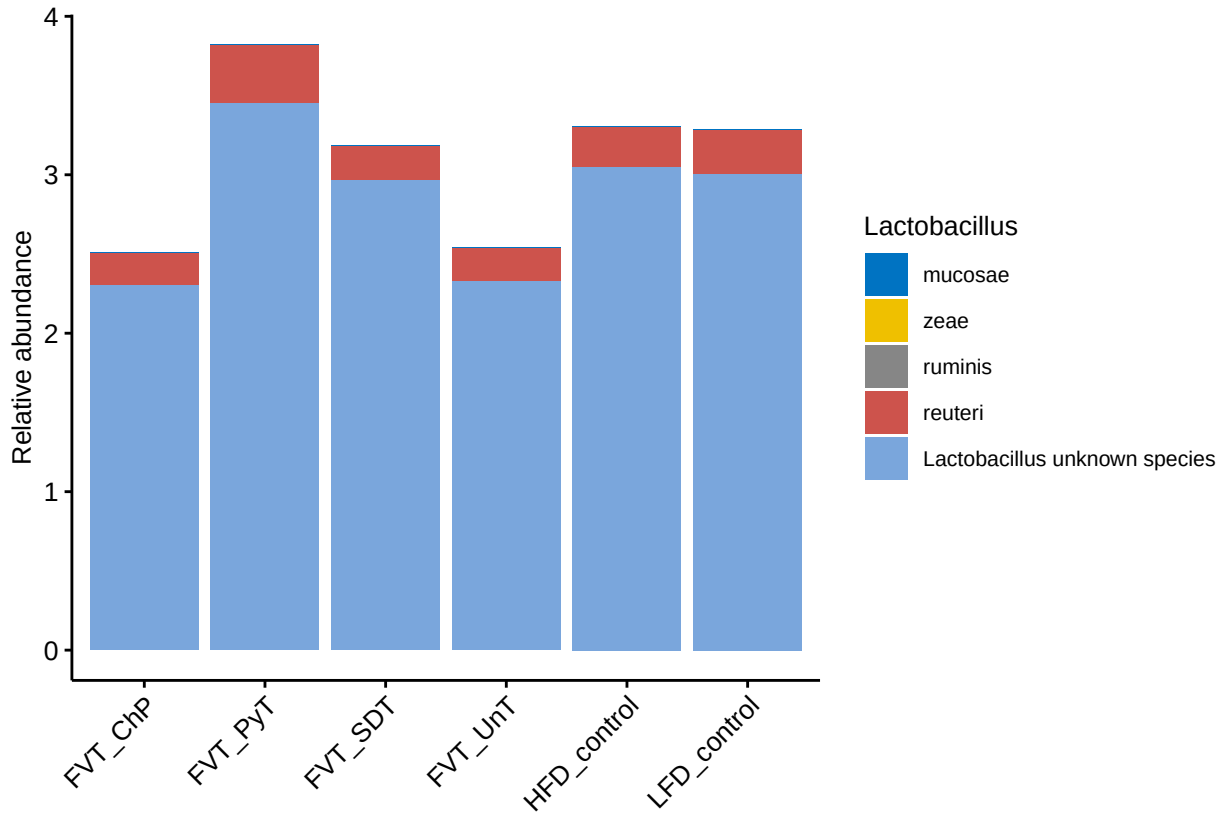

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.033190 -0.014390 -0.007134  0.010010  0.089195
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)             0.0193270  0.0088318   2.188   0.0347 *
## TreatmentFVT_ChP       -0.0009962  0.0134907  -0.074   0.9415
## TreatmentFVT_PyT        0.0009096  0.0124900   0.073   0.9423
## TreatmentFVT_SDT        0.0142922  0.0124900   1.144   0.2595
## TreatmentFVT_UnT        0.0021821  0.0129284   0.169   0.8668
## TreatmentHFD_control    0.0275142  0.0124900   2.203   0.0336 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02498 on 39 degrees of freedom
## Multiple R-squared:  0.1704, Adjusted R-squared:  0.06402
## F-statistic: 1.602 on 5 and 39 DF,  p-value: 0.1824

##
## Call:
```

```
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.033190 -0.014390 -0.007134  0.010010  0.089195
##
## Coefficients:
##                       Estimate Std. Error t value   Pr(>|t|)
## (Intercept)           0.046841   0.008832   5.304 0.00000478 ***
## TreatmentLFD_control -0.027514   0.012490  -2.203     0.0336 *
## TreatmentFVT_ChP     -0.028510   0.013491  -2.113     0.0410 *
## TreatmentFVT_PyT     -0.026605   0.012490  -2.130     0.0395 *
## TreatmentFVT_SDT     -0.013222   0.012490  -1.059     0.2963
## TreatmentFVT_UnT     -0.025332   0.012928  -1.959     0.0572 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02498 on 39 degrees of freedom
## Multiple R-squared:  0.1704, Adjusted R-squared:  0.06402
## F-statistic: 1.602 on 5 and 39 DF,  p-value: 0.1824
```

```
## # A tibble: 225 x 3
## # Groups:   Sample [45]
##    Sample       tax                            Mean
##    <chr>        <chr>                         <dbl>
##  1 NXT075Mao201 Lactobacillus unknown species 0.495
##  2 NXT075Mao217 Lactobacillus unknown species 0.489
##  3 NXT075Mao220 Lactobacillus unknown species 0.481
##  4 NXT075Mao237 Lactobacillus unknown species 0.481
##  5 NXT075Mao234 Lactobacillus unknown species 0.476
##  6 NXT075Mao193 Lactobacillus unknown species 0.459
##  7 NXT075Mao202 Lactobacillus unknown species 0.453
##  8 NXT075Mao265 Lactobacillus unknown species 0.450
##  9 NXT075Mao225 Lactobacillus unknown species 0.444
## 10 NXT075Mao233 Lactobacillus unknown species 0.442
## # ... with 215 more rows
```

```
## Warning: Unknown levels in `f`: Other
```

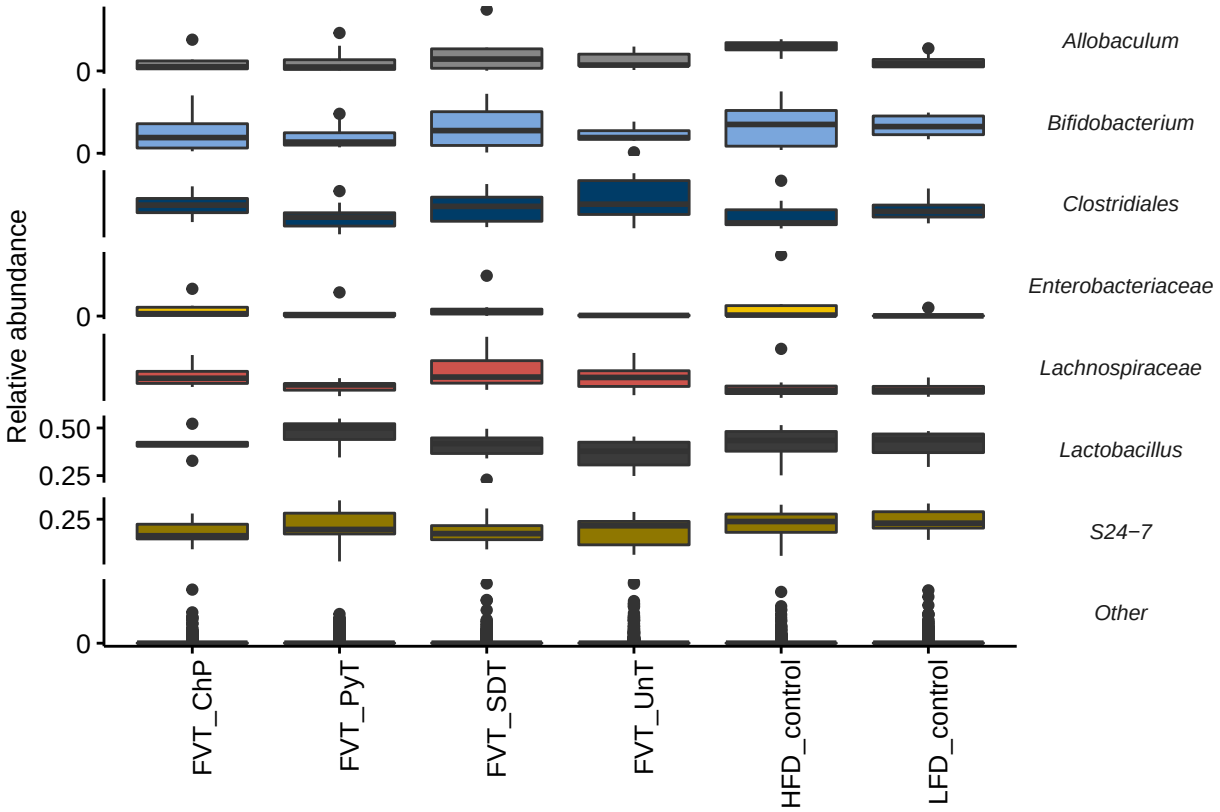



```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.09550 -0.08267 -0.07963 -0.03918  0.39976
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)            0.0821163  0.0244882   3.353 0.000941 ***
## TreatmentFVT_ChP       0.0014502  0.0374063   0.039 0.969110
## TreatmentFVT_PyT       0.0133794  0.0346315   0.386 0.699623
## TreatmentFVT_SDT      -0.0024857  0.0346315  -0.072 0.942845
## TreatmentFVT_UnT      -0.0094424  0.0358470  -0.263 0.792484
## TreatmentHFD_control   0.0005554  0.0346315   0.016 0.987219
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1549 on 219 degrees of freedom
## Multiple R-squared:  0.00199,    Adjusted R-squared:  -0.0208
## F-statistic: 0.08733 on 5 and 219 DF,  p-value: 0.9942

##
## Call:
```

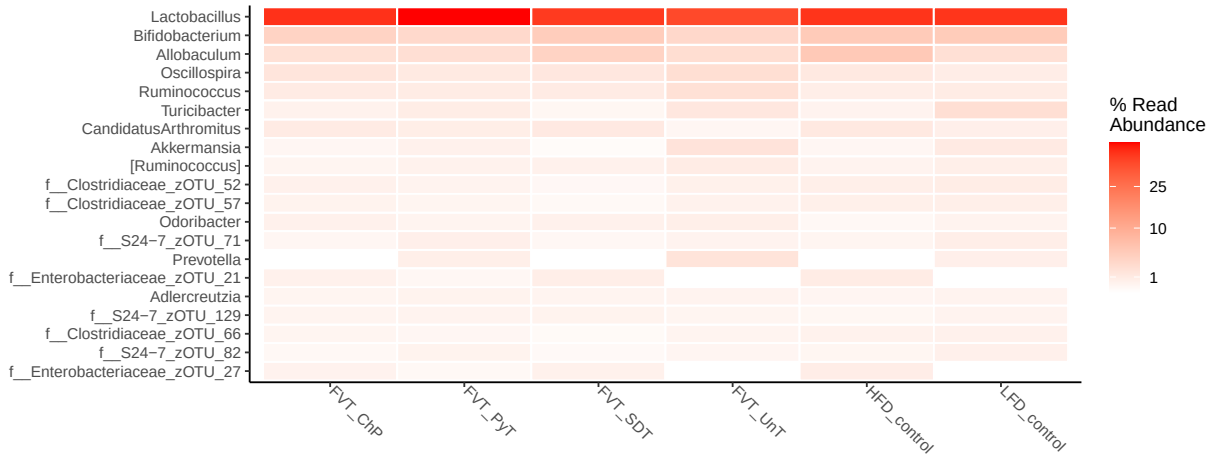
```
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.09550 -0.08267 -0.07963 -0.03918  0.39976
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)            0.0826717  0.0244882   3.376  0.00087 ***
## TreatmentLFD_control -0.0005554  0.0346315  -0.016  0.98722
## TreatmentFVT_ChP       0.0008948  0.0374063   0.024  0.98094
## TreatmentFVT_PyT       0.0128240  0.0346315   0.370  0.71152
## TreatmentFVT_SDT      -0.0030411  0.0346315  -0.088  0.93010
## TreatmentFVT_UnT      -0.0099978  0.0358470  -0.279  0.78058
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1549 on 219 degrees of freedom
## Multiple R-squared:  0.00199,    Adjusted R-squared:  -0.0208
## F-statistic: 0.08733 on 5 and 219 DF,  p-value: 0.9942
```

```
## # A tibble: 5,175 x 3
## # Groups:   Sample [45]
##    Sample       tax            Mean
##    <chr>        <chr>         <dbl>
##  1 NXT075Mao201 Lactobacillus 0.549
##  2 NXT075Mao217 Lactobacillus 0.525
##  3 NXT075Mao220 Lactobacillus 0.522
##  4 NXT075Mao234 Lactobacillus 0.521
##  5 NXT075Mao237 Lactobacillus 0.515
##  6 NXT075Mao265 Lactobacillus 0.510
##  7 NXT075Mao193 Lactobacillus 0.510
##  8 NXT075Mao202 Lactobacillus 0.496
##  9 NXT075Mao225 Lactobacillus 0.491
## 10 NXT075Mao216 Lactobacillus 0.484
## # ... with 5,165 more rows
```



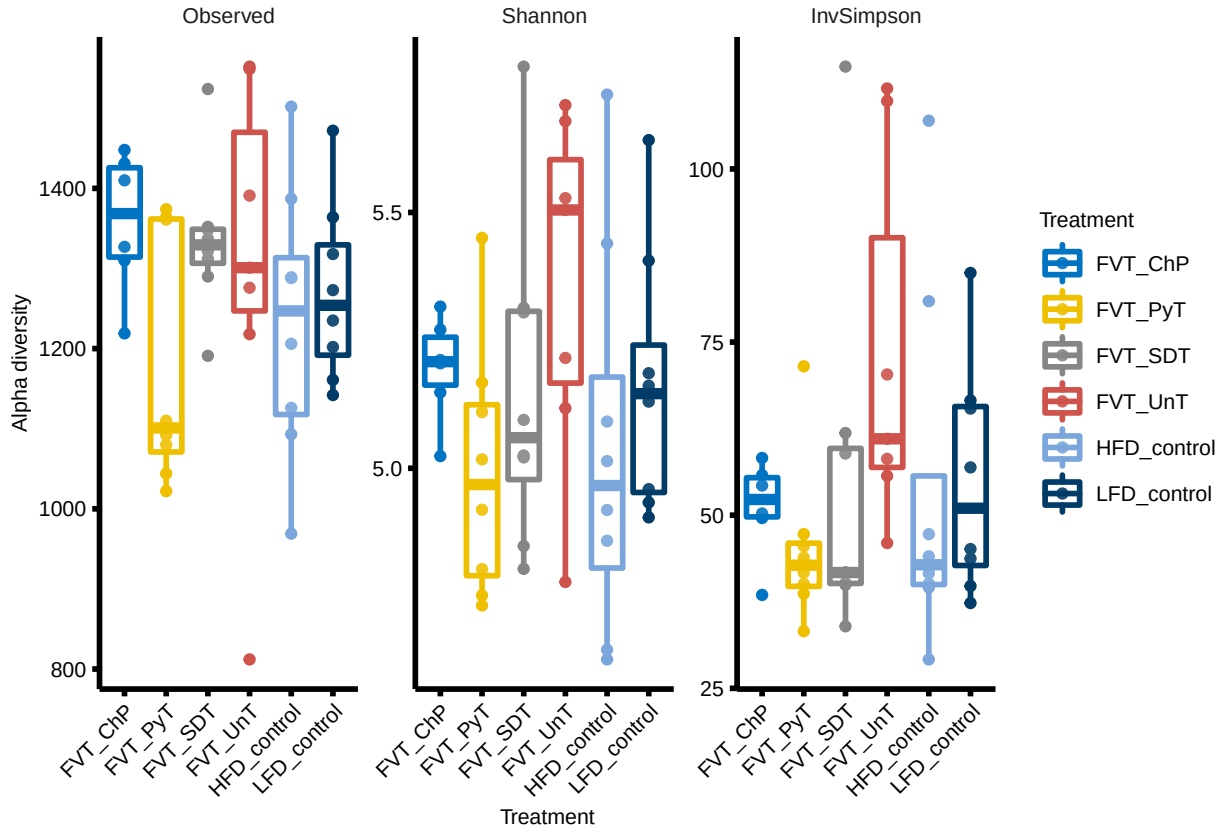
Abundance heatmaps

Bacteria - Treatment



Alpha diversity

By Treatment



Anova and linear regression analysis

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Shannon ~ variable, data = rich)
##
## $variable
##                                diff         lwr       upr     p adj
## FVT_PyT-FVT_ChP         -0.20220890 -0.67511026 0.2706925 0.7933263
## FVT_SDT-FVT_ChP         -0.04654655 -0.51944791 0.4263548 0.9996754
## FVT_UnT-FVT_ChP          0.16577982 -0.32138307 0.6529427 0.9085643
## HFD_control-FVT_ChP     -0.15551722 -0.62841859 0.3173841 0.9198997
## LFD_control-FVT_ChP     -0.03080578 -0.50370714 0.4420956 0.9999575
## FVT_SDT-FVT_PyT          0.15566235 -0.28215923 0.5934839 0.8920475
## FVT_UnT-FVT_PyT          0.36798872 -0.08519968 0.8211771 0.1701997
## HFD_control-FVT_PyT      0.04669168 -0.39112991 0.4845133 0.9995199
## LFD_control-FVT_PyT      0.17140312 -0.26641847 0.6092247 0.8468450
## FVT_UnT-FVT_SDT          0.21232636 -0.24086203 0.6655148 0.7245871
## HFD_control-FVT_SDT     -0.10897068 -0.54679226 0.3288509 0.9747345
## LFD_control-FVT_SDT      0.01574076 -0.42208082 0.4535623 0.9999978
## HFD_control-FVT_UnT     -0.32129704 -0.77448544 0.1318914 0.2965368
```

```
## LFD_control-FVT_UnT     -0.19658560 -0.64977399 0.2566028 0.7834229
## LFD_control-HFD_control  0.12471144 -0.31311014 0.5625330 0.9551459
```

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.09550 -0.08267 -0.07963 -0.03918  0.39976
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)            0.0826717  0.0244882   3.376  0.00087 ***
## TreatmentLFD_control -0.0005554  0.0346315  -0.016  0.98722
## TreatmentFVT_ChP       0.0008948  0.0374063   0.024  0.98094
## TreatmentFVT_PyT       0.0128240  0.0346315   0.370  0.71152
## TreatmentFVT_SDT      -0.0030411  0.0346315  -0.088  0.93010
## TreatmentFVT_UnT      -0.0099978  0.0358470  -0.279  0.78058
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1549 on 219 degrees of freedom
## Multiple R-squared:  0.00199,    Adjusted R-squared:  -0.0208
## F-statistic: 0.08733 on 5 and 219 DF,  p-value: 0.9942
```

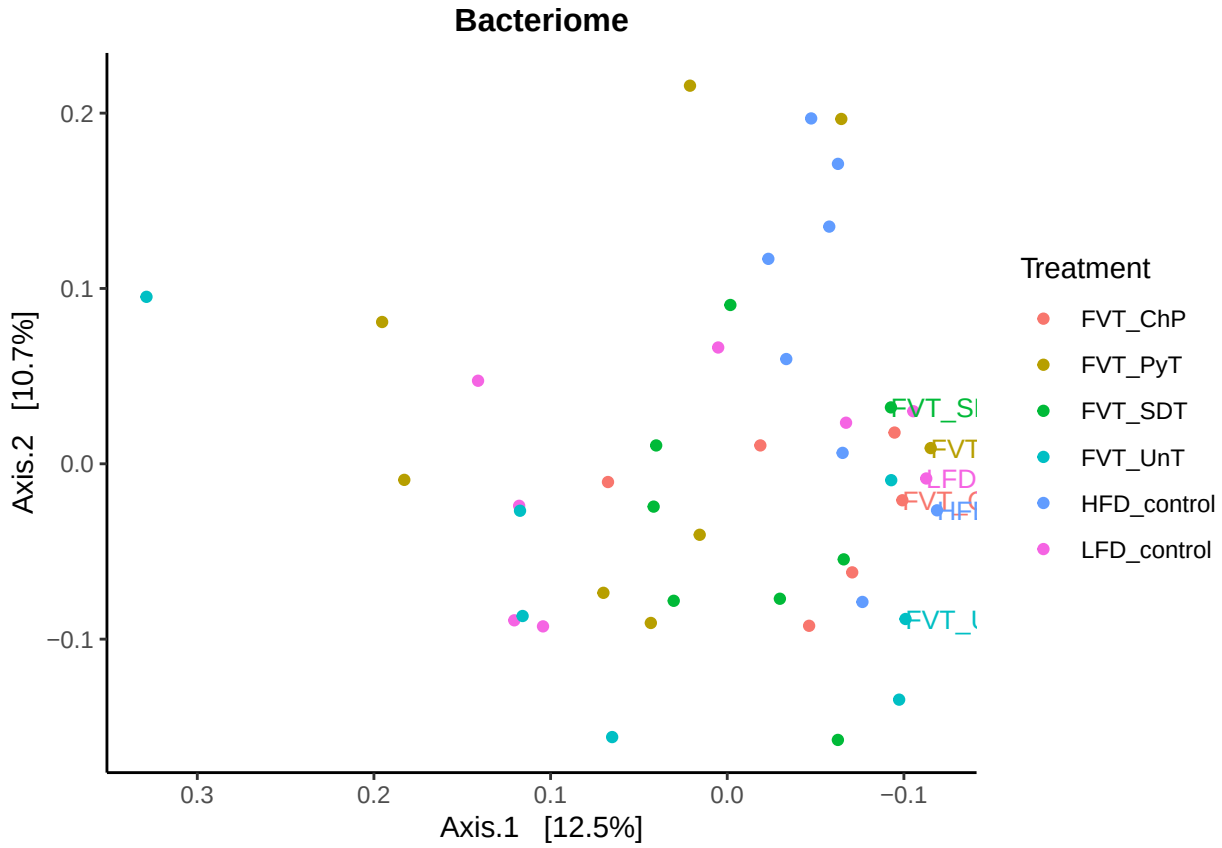
```
##
## Call:
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.58392 -0.19089 -0.02647  0.15522  0.68998
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)          5.04042    0.10333  48.778   <2e-16 ***
## variableLFD_control  0.12471    0.14614   0.853   0.3987
## variableFVT_ChP      0.15552    0.15784   0.985   0.3306
## variableFVT_PyT     -0.04669    0.14614  -0.320   0.7510
## variableFVT_SDT      0.10897    0.14614   0.746   0.4603
## variableFVT_UnT      0.32130    0.15127   2.124   0.0401 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2923 on 39 degrees of freedom
## Multiple R-squared:  0.1565, Adjusted R-squared:  0.0484
## F-statistic: 1.448 on 5 and 39 DF,  p-value: 0.2292
```

```
##
## Call:
## lm(formula = Shannon ~ variable, data = rich)
##
```

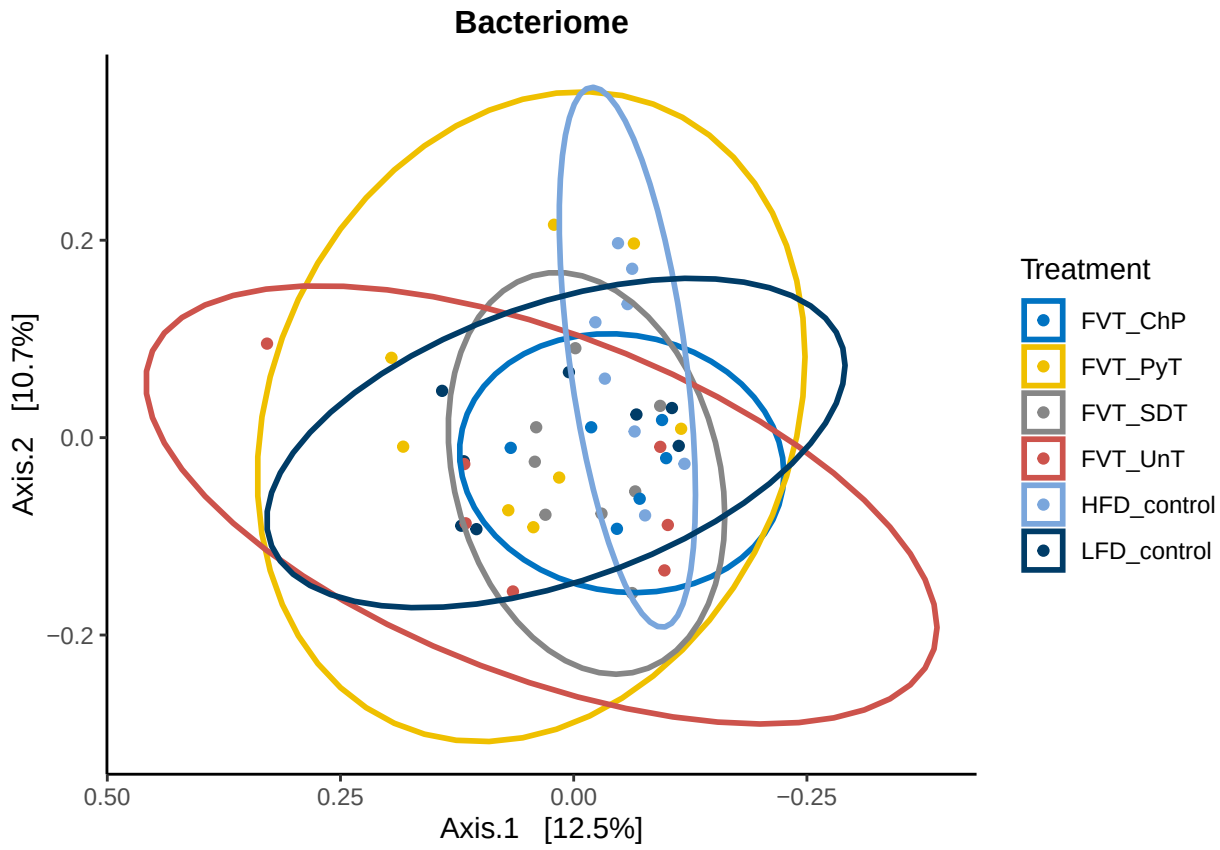
```
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.58392 -0.19089 -0.02647  0.15522  0.68998
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)           5.3617     0.1105  48.536   <2e-16 ***
## variableHFD_control  -0.3213     0.1513  -2.124   0.0401 *
## variableLFD_control  -0.1966     0.1513  -1.300   0.2014
## variableFVT_ChP      -0.1658     0.1626  -1.020   0.3142
## variableFVT_PyT      -0.3680     0.1513  -2.433   0.0197 *
## variableFVT_SDT      -0.2123     0.1513  -1.404   0.1683
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2923 on 39 degrees of freedom
## Multiple R-squared:  0.1565, Adjusted R-squared:  0.0484
## F-statistic: 1.448 on 5 and 39 DF,  p-value: 0.2292
```

Beta diversity

Abbreviation - basically the mouse ID.



Treatment



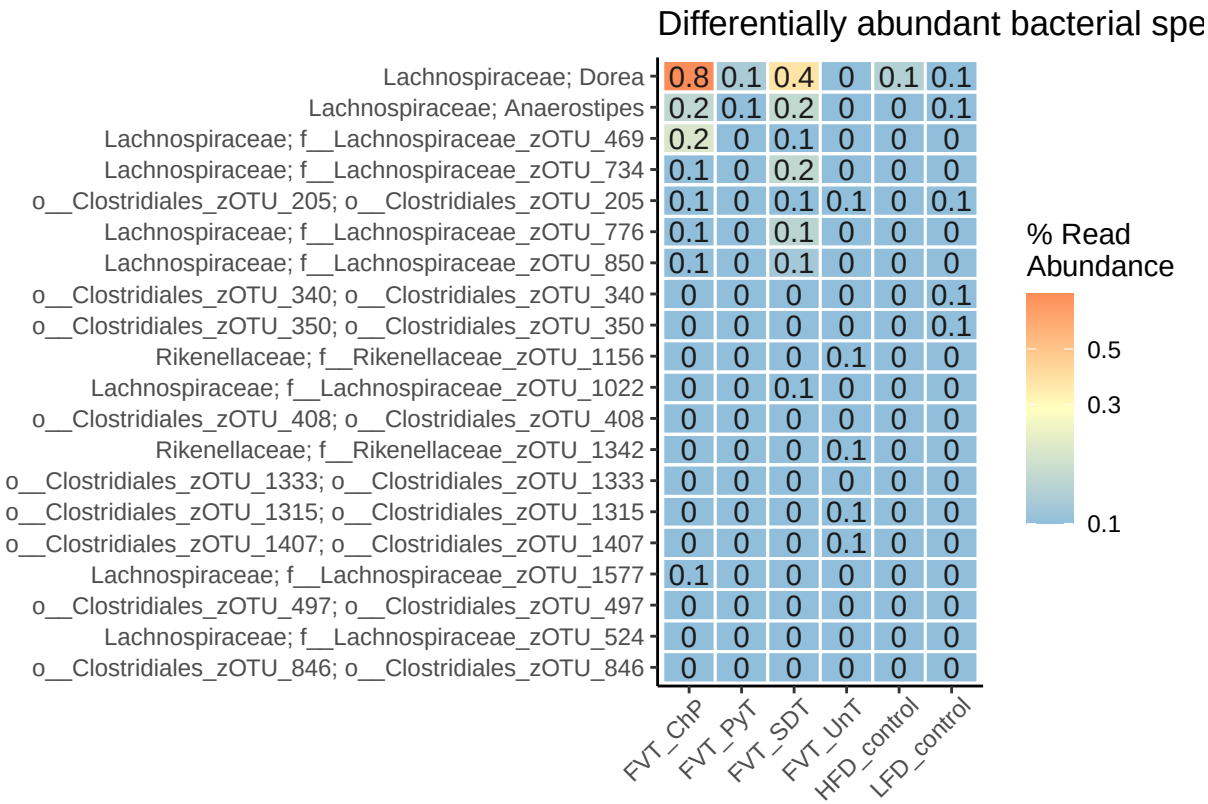
Adonis and pairwise permanova analysis - Treatment

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.PSB ~ Treatment, data = sampledf.PSB, permutations = 999, method = "bray")
##           Df SumOfSqs      R2      F Pr(>F)
## Treatment  5   0.4972 0.14178 1.2886  0.013 *
## Residual  39   3.0095 0.85822
## Total     44   3.5067 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

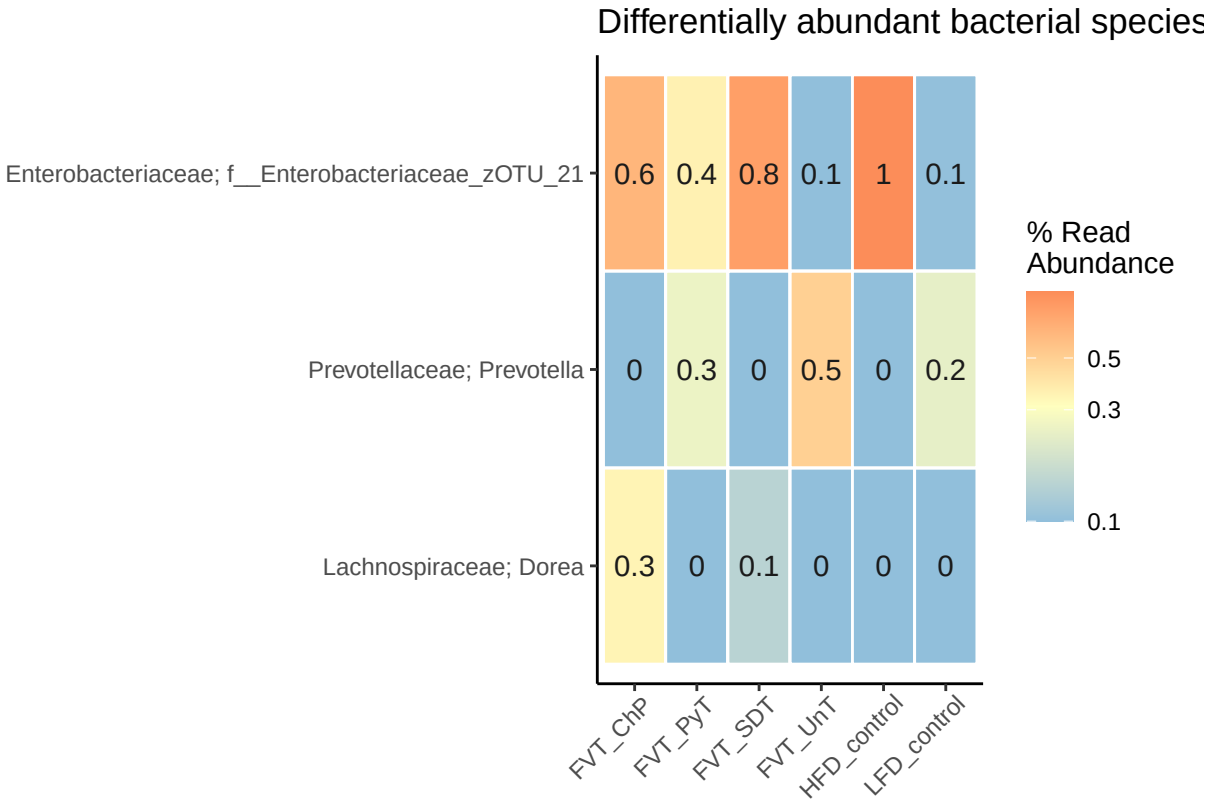
```
##             X1          X2         R2  pval pvalBon pvalFDR
## 1      FVT_ChP     FVT_PyT 0.09324268 0.127   1.905   0.190
## 2      FVT_ChP     FVT_SDT 0.06490570 0.845  12.675   0.905
## 3      FVT_ChP     FVT_UnT 0.11189007 0.075   1.125   0.141
## 4      FVT_ChP HFD_control 0.09160682 0.129   1.935   0.176
## 5      FVT_ChP LFD_control 0.09327235 0.095   1.425   0.158
## 6      FVT_PyT     FVT_SDT 0.08845132 0.061   0.915   0.229
## 7      FVT_PyT     FVT_UnT 0.07075978 0.403   6.045   0.504
## 8      FVT_PyT HFD_control 0.08700653 0.070   1.050   0.150
```

```
## 9      FVT_PyT LFD_control 0.05315001 0.882  13.230   0.882
## 10     FVT_SDT     FVT_UnT 0.09710813 0.069   1.035   0.173
## 11     FVT_SDT HFD_control 0.10015725 0.025   0.375   0.125
## 12     FVT_SDT LFD_control 0.08706438 0.068   1.020   0.204
## 13     FVT_UnT HFD_control 0.13738166 0.003   0.045   0.045
## 14     FVT_UnT LFD_control 0.06470972 0.561   8.415   0.647
## 15 HFD_control LFD_control 0.10771415 0.010   0.150   0.075
```

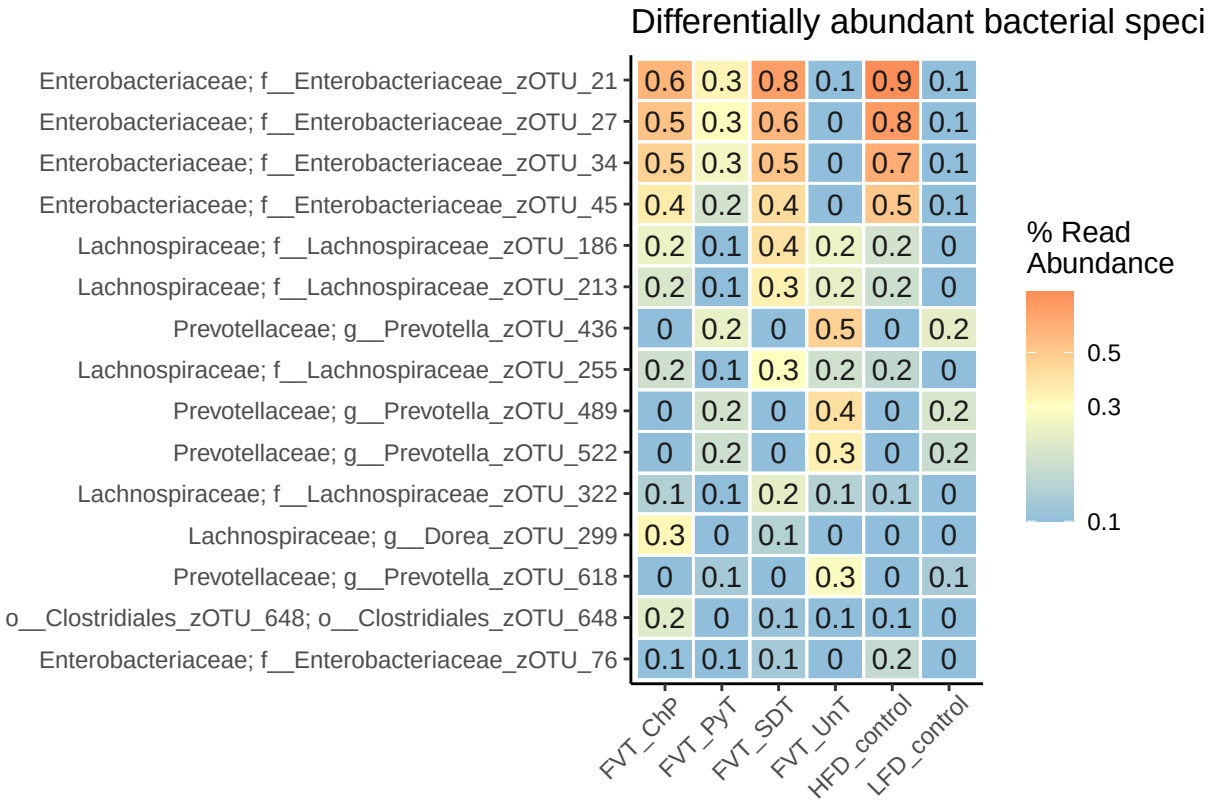
Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level - Comparing ChP vs HFD

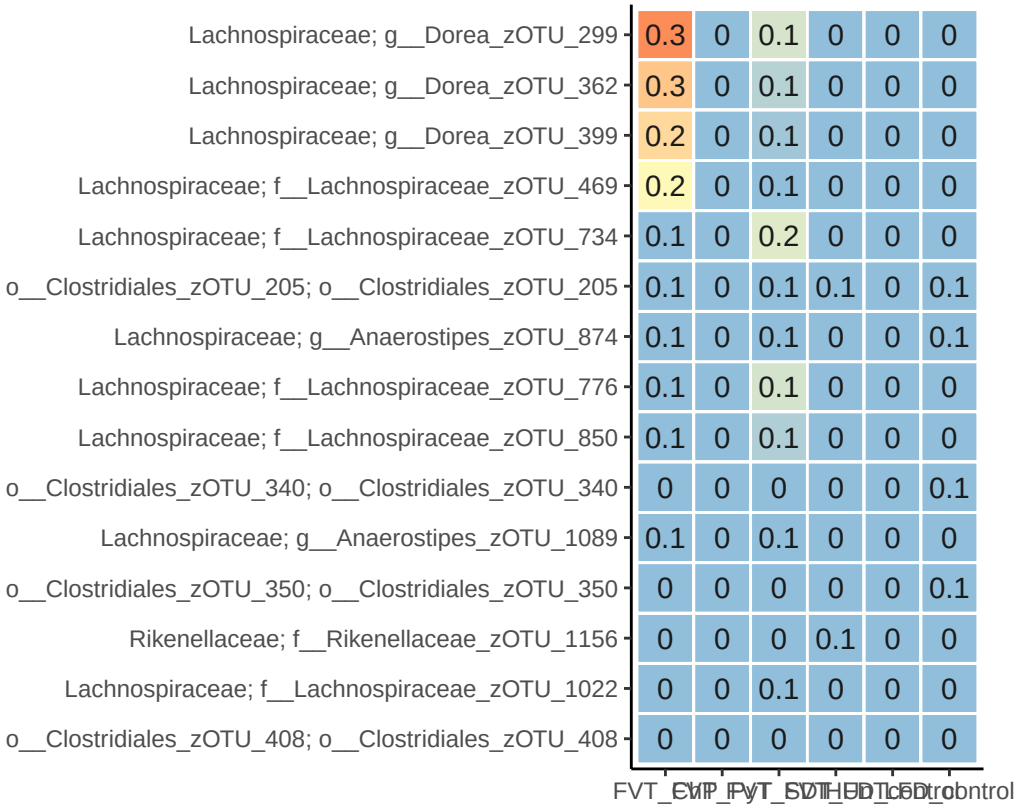


Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level



Bacteriome - Deseq2 - Treatment



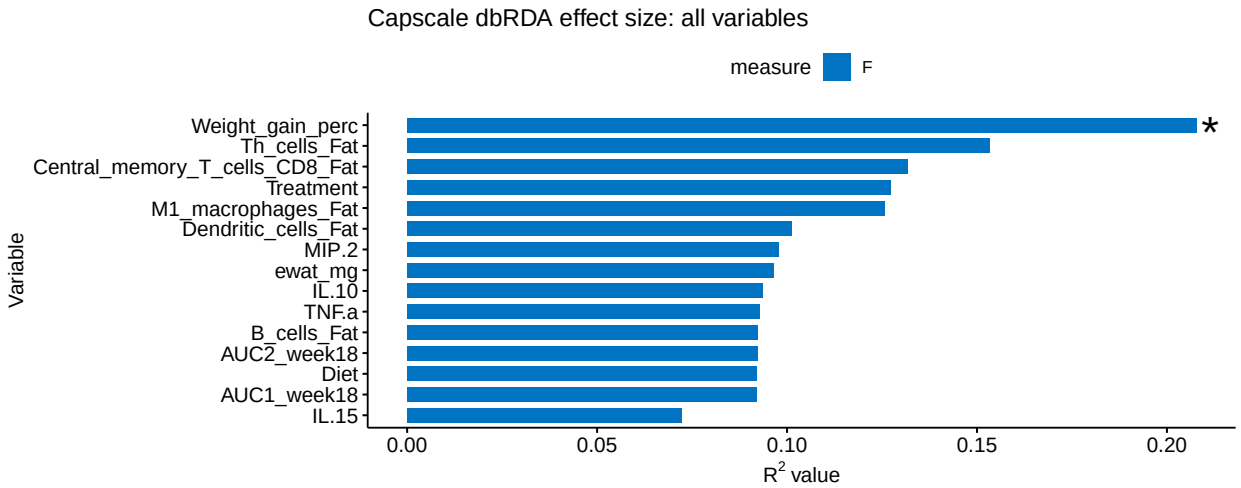


Deseq2 - defined comparison

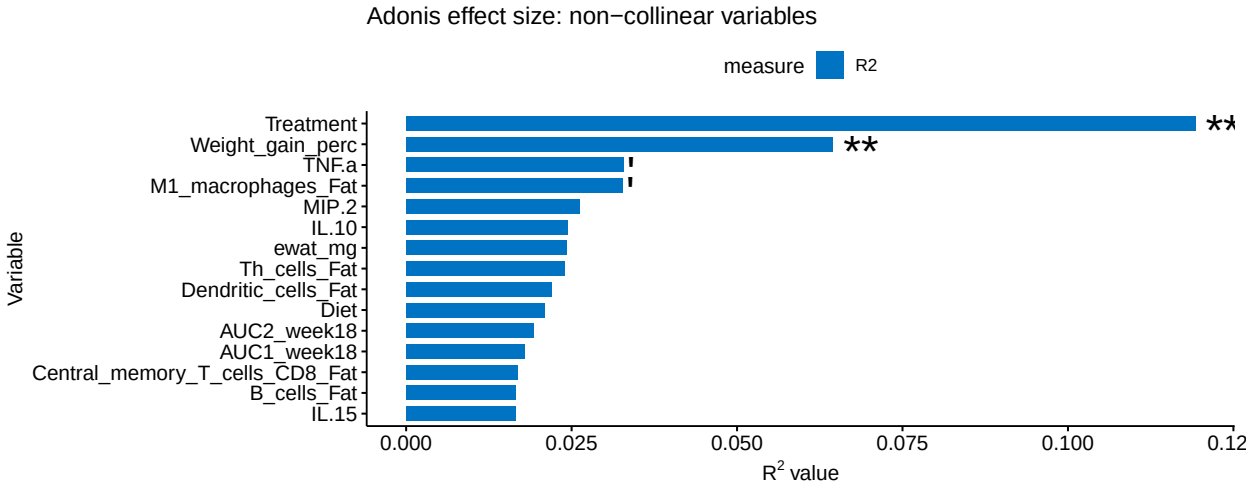
Bacteriome - Effect-size

Non-constrained

Capscale - independent effect sizes

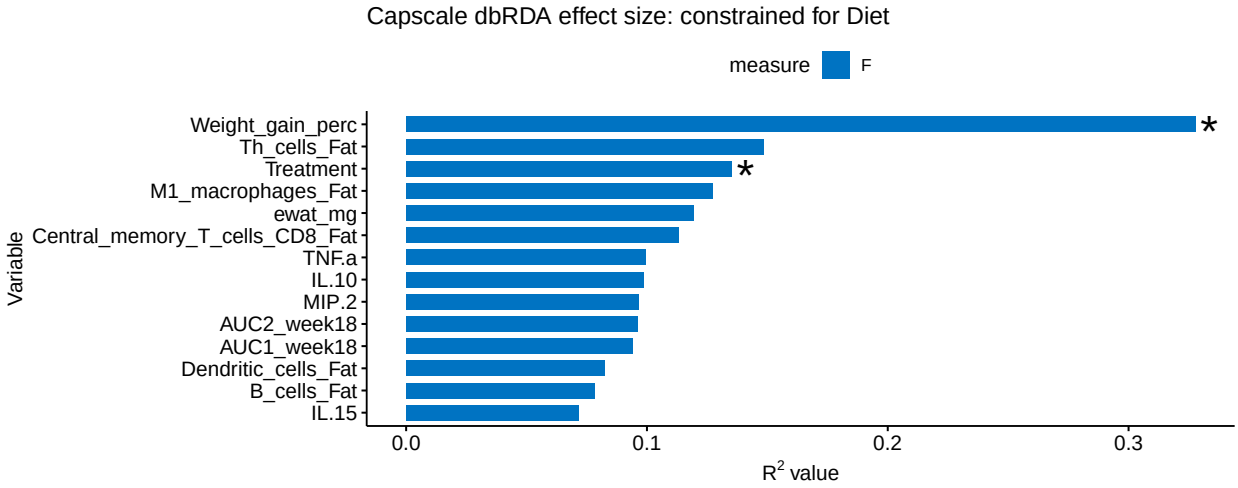


ADONIS - decomposed to show individual contributions of factors

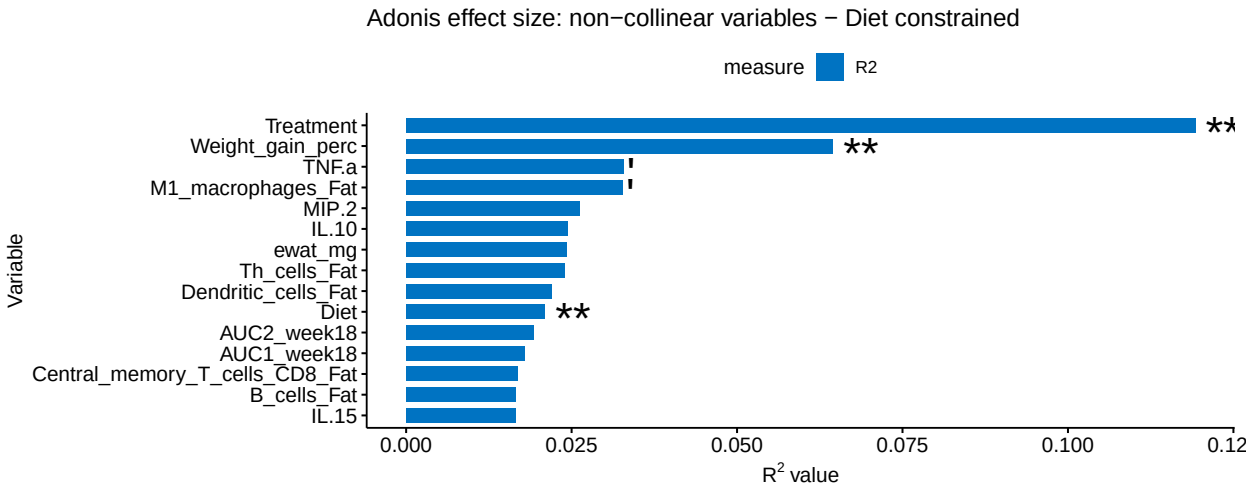


Constrained by Diet

Capscale - independent effect sizes



ADONIS - decomposed to show individual contributions of factors. Contrained for Diet


Adonis effect size: non−collinear variables − Diet constrained
measure
R2
Variable
Treatment
Weight_gain_perc
TNF.a
M1_macrophages_Fat
MIP.2
IL.10
ewat_mg
Th_cells_Fat
Dendritic_cells_Fat
Diet
AUC2_week18
AUC1_week18
Central_memory_T_cells_CD8_Fat
B_cells_Fat
IL.15
0.000
0.025
0.050
0.075
0.100
0.12
R2 value