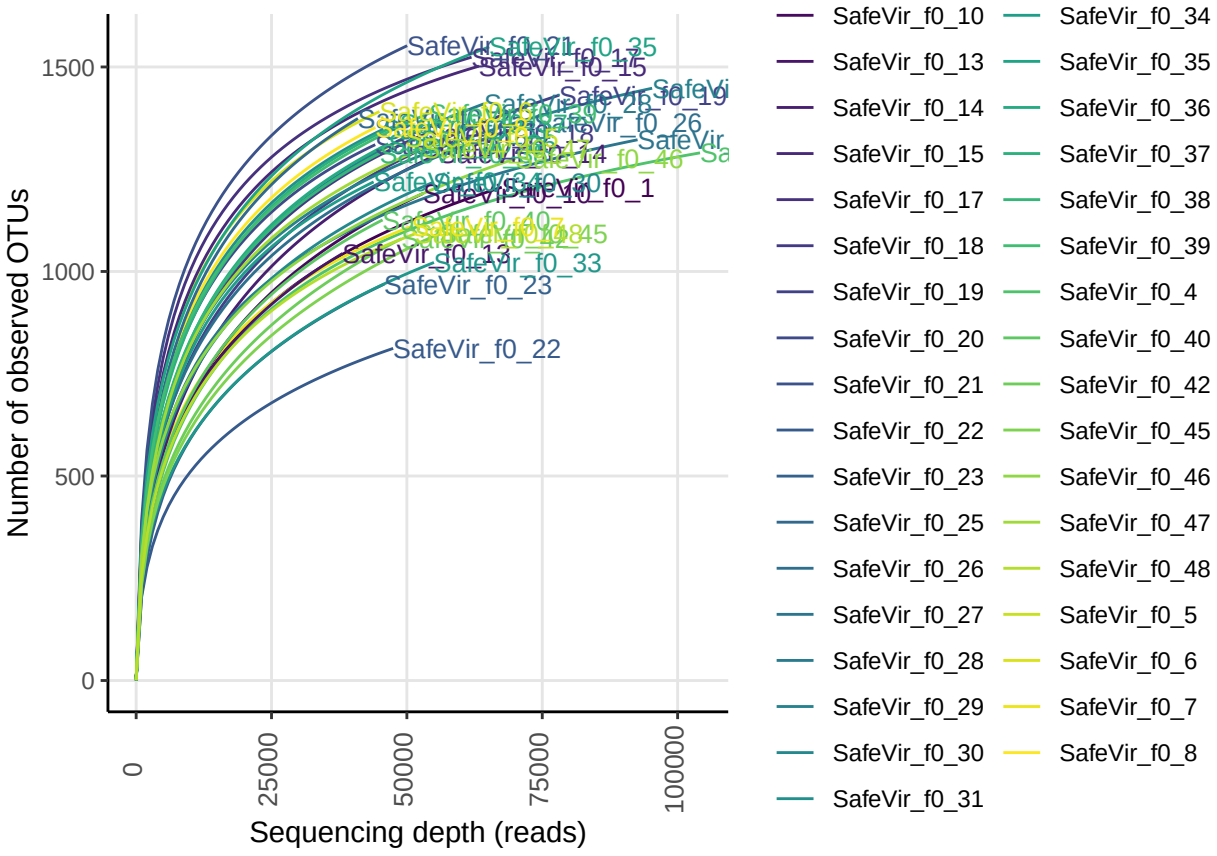
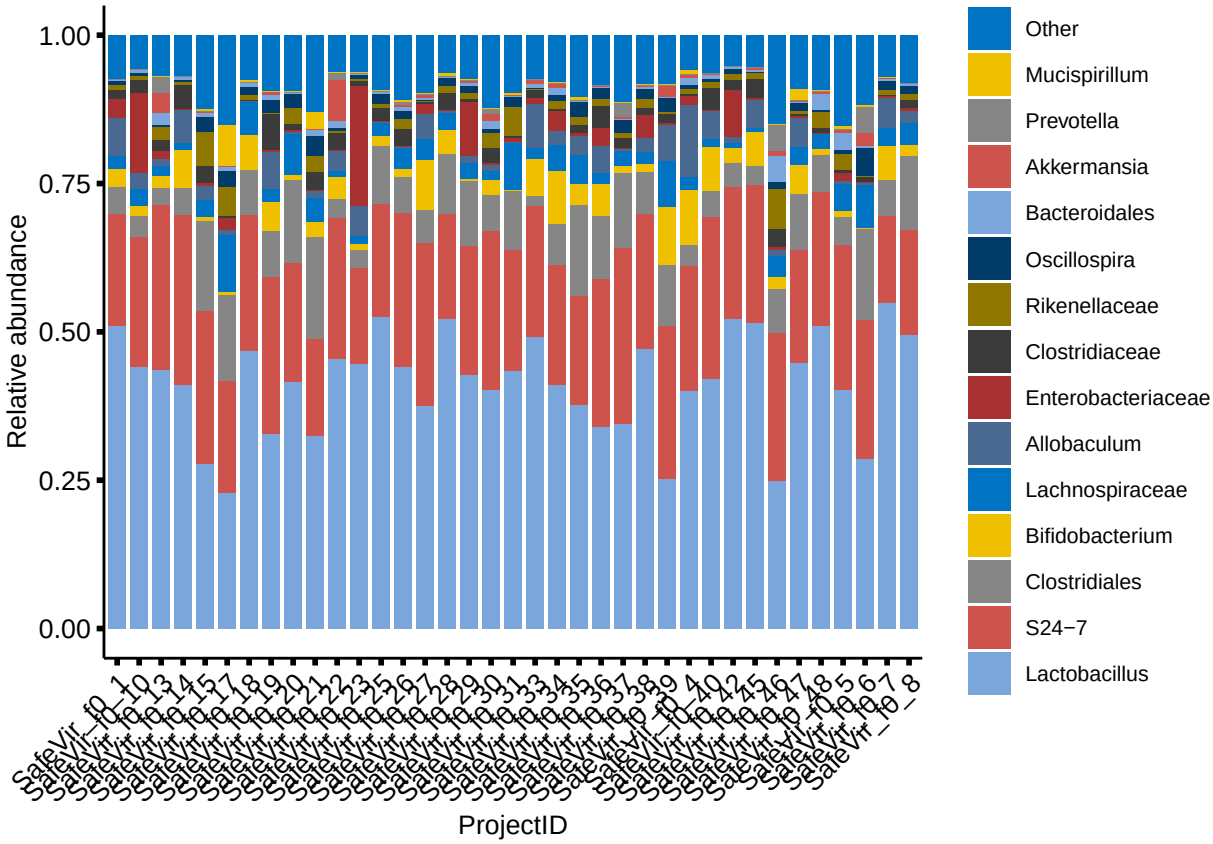
16S rRNA gene amplicon analysis - SafeVir - DIO - Arrival

Sequencing depth

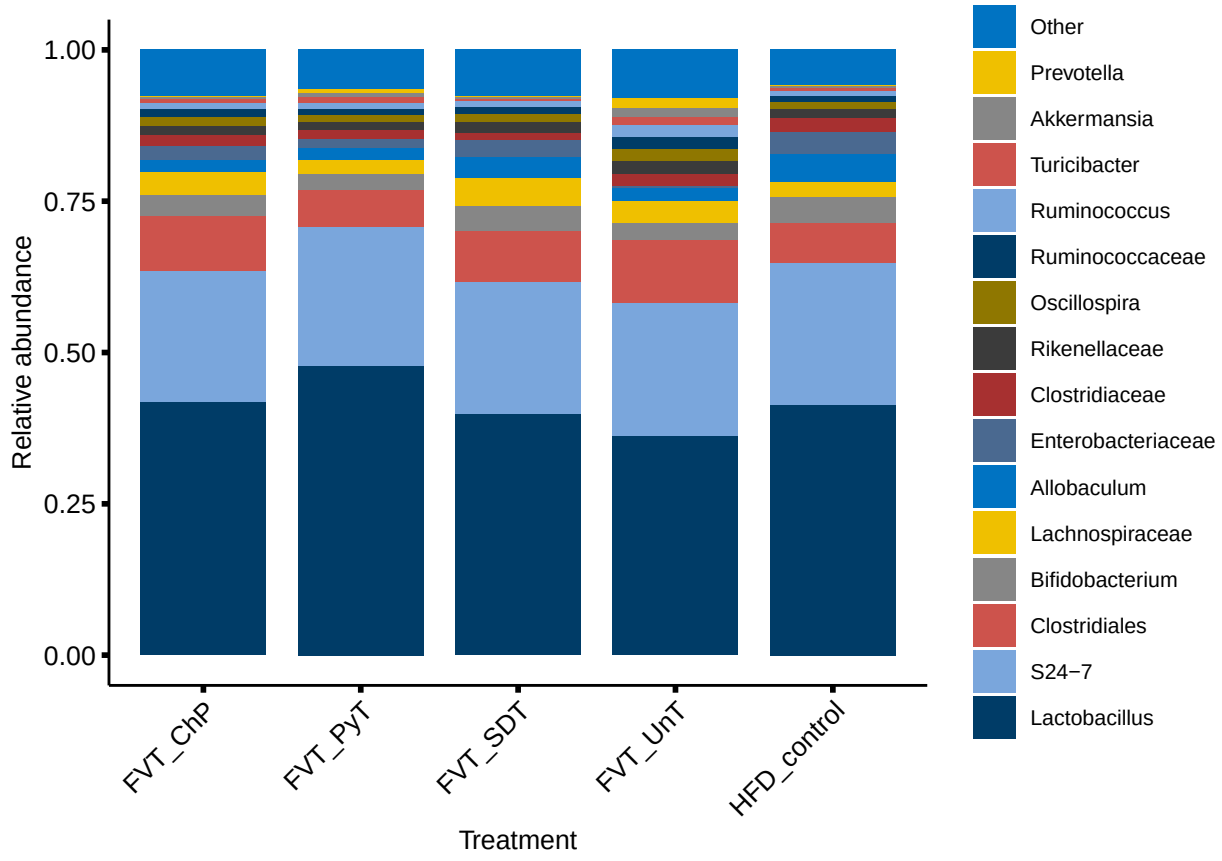


Individual sample barplots.

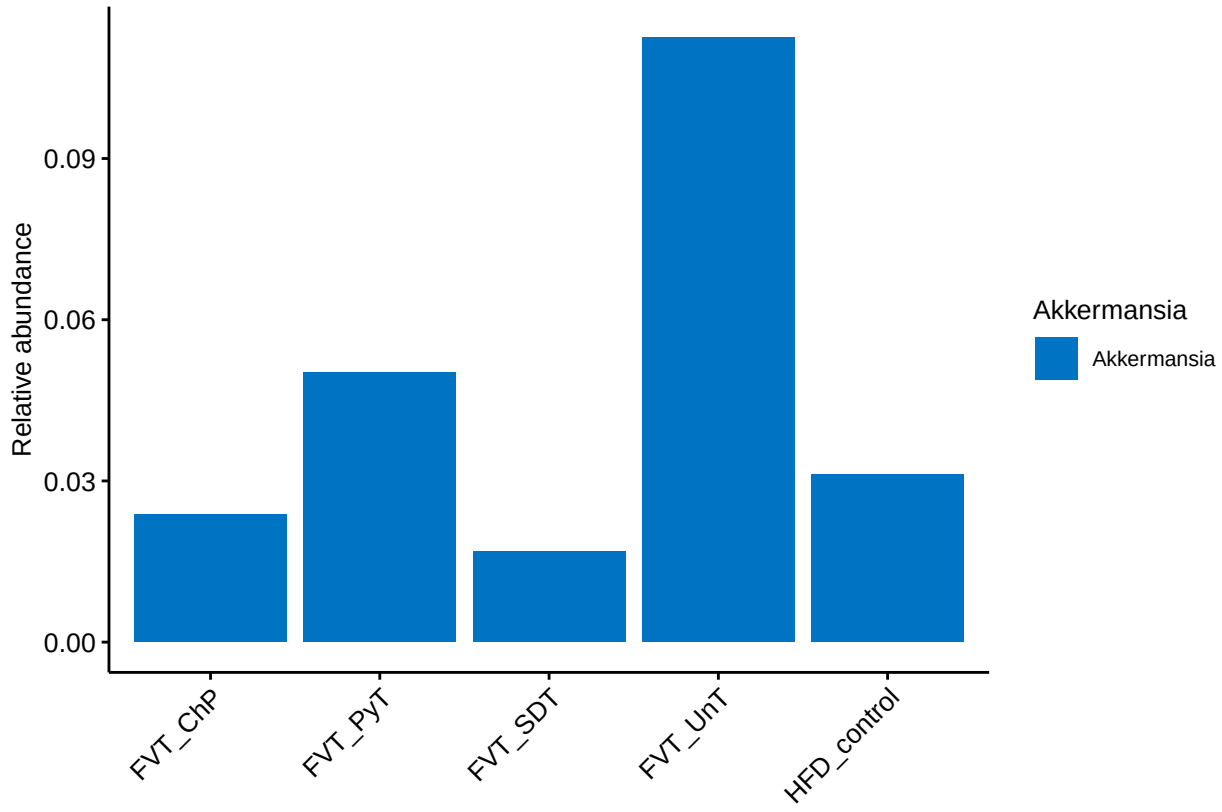


Barplots based on treatments.

```
## # A tibble: 575 x 3
## # Groups:   Sample [5]
##    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 FVT_SDT     Lactobacillus 0.398
##  5 FVT_UnT     Lactobacillus 0.363
##  6 HFD_control S24-7         0.235
##  7 FVT_PyT     S24-7         0.230
##  8 FVT_SDT     S24-7         0.219
##  9 FVT_UnT     S24-7         0.218
## 10 FVT_ChP     S24-7         0.218
## # ... with 565 more rows
```



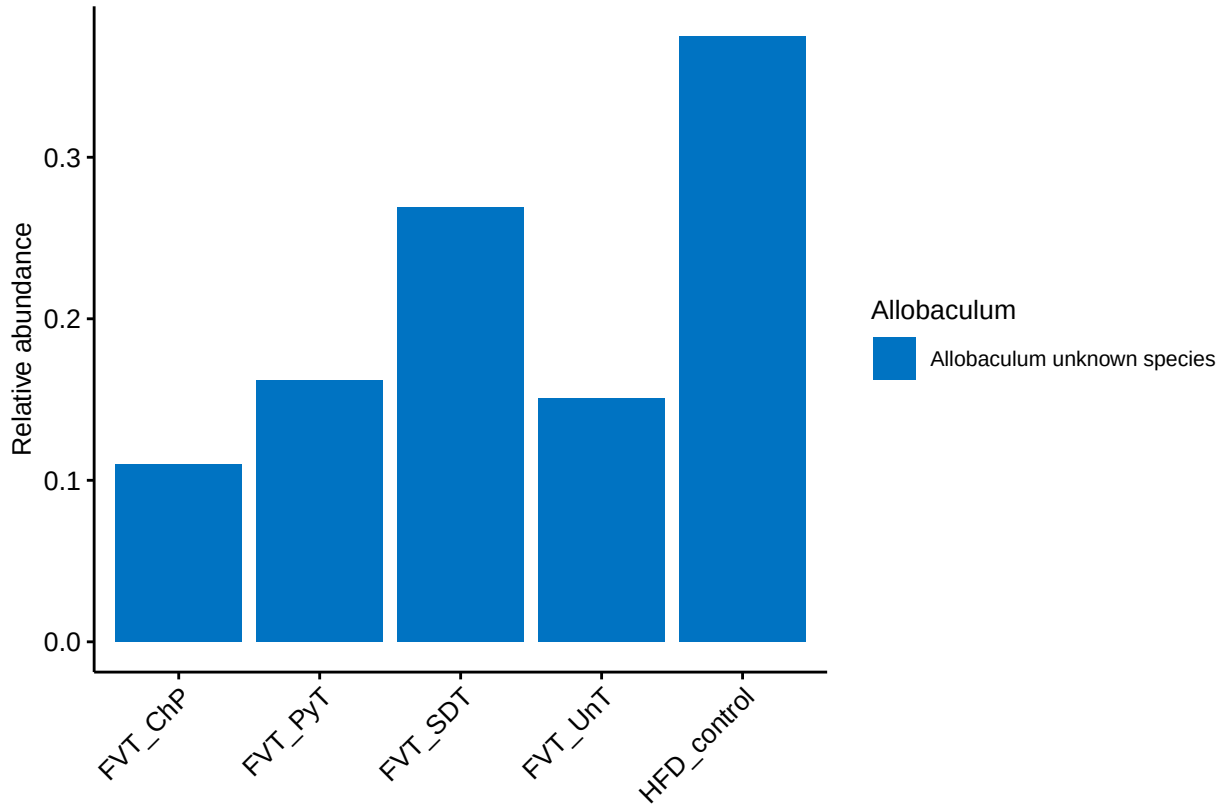

```
## # A tibble: 37 x 3
## # Groups:   Sample [37]
##    Sample       tax            Mean
##    <chr>        <chr>         <dbl>
##  1 NXT075Mao214 Akkermansia 0.0699
##  2 NXT075Mao205 Akkermansia 0.0334
##  3 NXT075Mao200 Akkermansia 0.0225
##  4 NXT075Mao231 Akkermansia 0.0196
##  5 NXT075Mao222 Akkermansia 0.0105
##  6 NXT075Mao226 Akkermansia 0.00778
##  7 NXT075Mao219 Akkermansia 0.00771
##  8 NXT075Mao238 Akkermansia 0.00681
##  9 NXT075Mao225 Akkermansia 0.00665
## 10 NXT075Mao199 Akkermansia 0.00622
## # ... with 27 more rows

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




```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.015911 -0.003828 -0.002119  0.000373  0.053834
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)       0.0039083  0.0044310   0.882   0.3843
## TreatmentFVT_ChP  0.0000557  0.0067685   0.008   0.9935
## TreatmentFVT_PyT  0.0023740  0.0062665   0.379   0.7073
## TreatmentFVT_SDT -0.0017891  0.0062665  -0.286   0.7771
## TreatmentFVT_UnT  0.0121827  0.0064864   1.878   0.0695 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01253 on 32 degrees of freedom
## Multiple R-squared:  0.1503, Adjusted R-squared:  0.04412
## F-statistic: 1.415 on 4 and 32 DF,  p-value: 0.2513

## # A tibble: 37 x 3
## # Groups:   Sample [37]
##    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 27 more rows
```

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

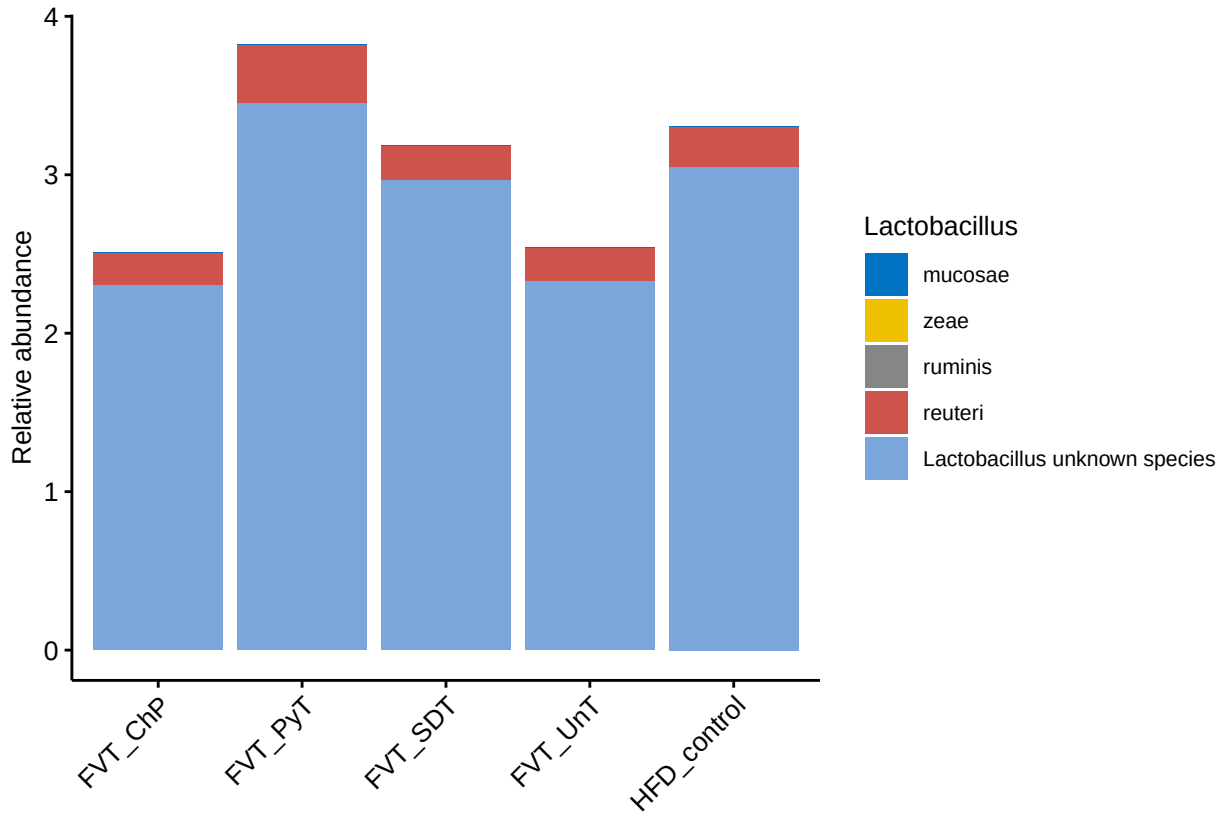



```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.033190 -0.015964 -0.007151  0.010010  0.089195
##
## Coefficients:
##                   Estimate Std. Error t value  Pr(>|t|)
```

```
## (Intercept)       0.046841   0.009408   4.979 0.0000211 ***
## TreatmentFVT_ChP -0.028510   0.014370  -1.984    0.0559 .
## TreatmentFVT_PyT -0.026605   0.013304  -2.000    0.0541 .
## TreatmentFVT_SDT -0.013222   0.013304  -0.994    0.3278
## TreatmentFVT_UnT -0.025332   0.013771  -1.839    0.0751 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02661 on 32 degrees of freedom
## Multiple R-squared:  0.1628, Adjusted R-squared:  0.05816
## F-statistic: 1.556 on 4 and 32 DF,  p-value: 0.2099
```

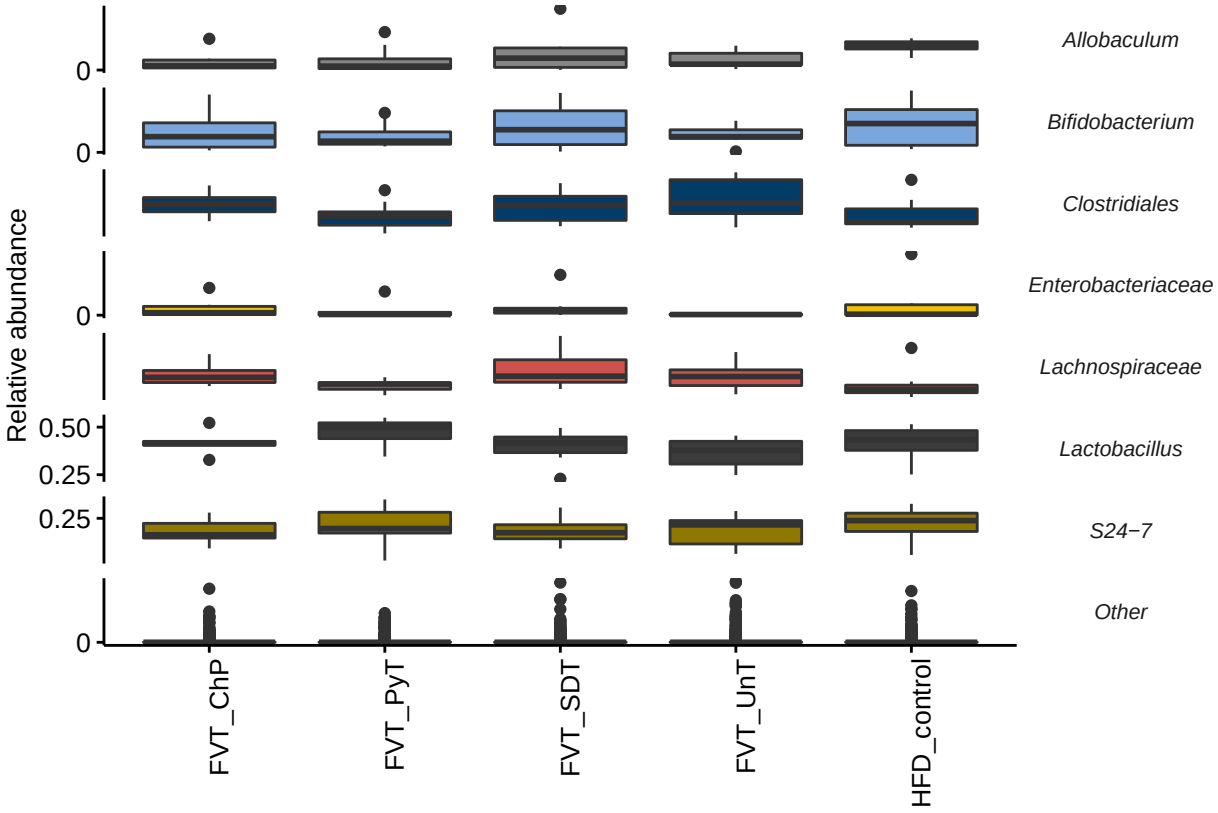
```
## # A tibble: 185 x 3
## # Groups:   Sample [37]
##    Sample       tax                             Mean
##    <chr>        <chr>                          <dbl>
##  1 NXT075Mao201 Lactobacillus unknown species 0.495
##  2 NXT075Mao217 Lactobacillus unknown species 0.489
##  3 NXT075Mao220 Lactobacillus 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 NXT075Mao210 Lactobacillus unknown species 0.436
## # ... with 175 more rows
```

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



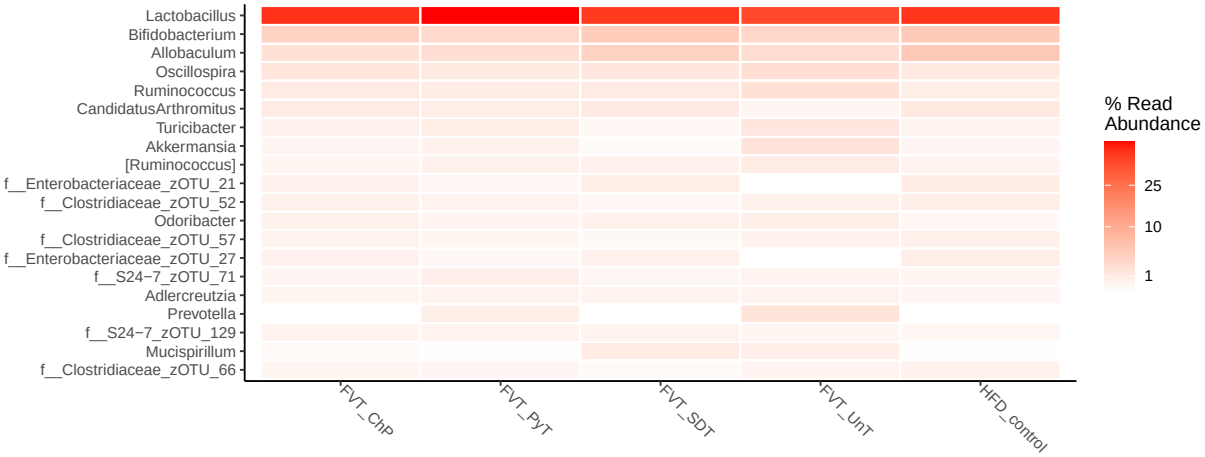

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.09550 -0.08267 -0.07267 -0.03918  0.39976
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)       0.0826717  0.0245744   3.364 0.000938 ***
## TreatmentFVT_ChP  0.0008948  0.0375380   0.024 0.981009
## TreatmentFVT_PyT  0.0128240  0.0347535   0.369 0.712562
## TreatmentFVT_SDT -0.0030411  0.0347535  -0.088 0.930367
## TreatmentFVT_UnT -0.0099978  0.0359733  -0.278 0.781391
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
##
## Residual standard error: 0.1554 on 180 degrees of freedom
## Multiple R-squared:  0.002397,   Adjusted R-squared:  -0.01977
## F-statistic: 0.1081 on 4 and 180 DF,  p-value: 0.9796

## # A tibble: 4,255 x 3
## # Groups:   Sample [37]
##    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 NXT075Mao230 Lactobacillus 0.472
## # ... with 4,245 more rows
```



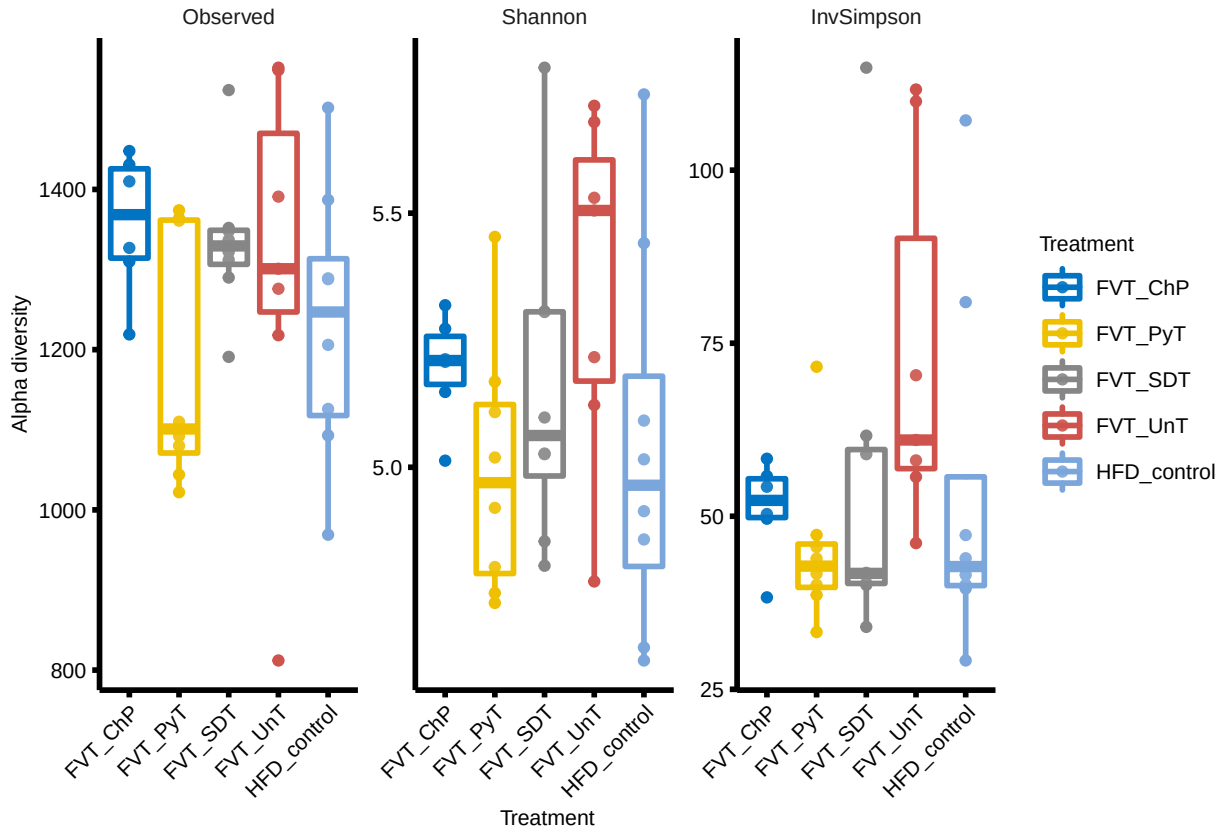
Abundance heatmaps

Bacteria - Treatment



Alpha diversity

By Treatment



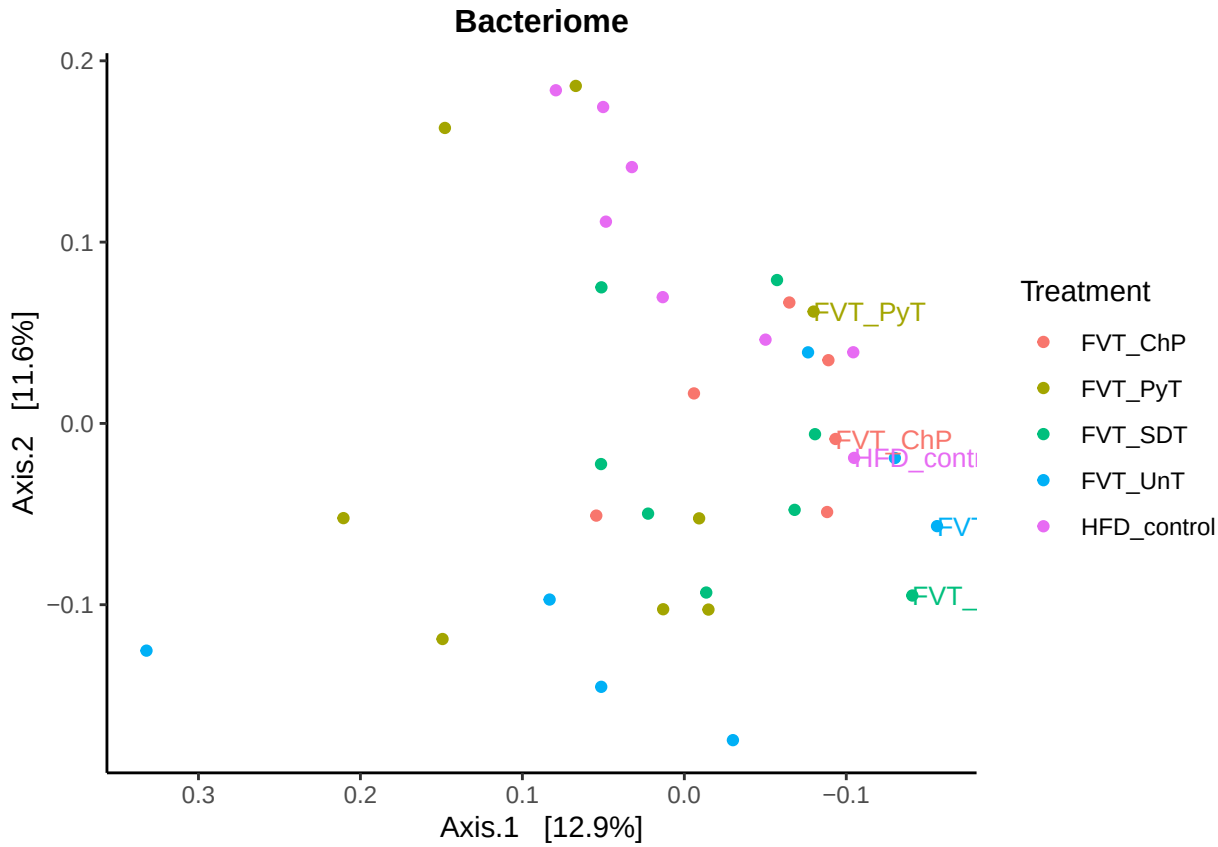
Anova and linear regression analysis

```
##   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     -0.20056973 -0.66987981 0.2687403 0.7314455
## FVT_SDT-FVT_ChP     -0.04420038 -0.51351045 0.4251097 0.9987300
## FVT_UnT-FVT_ChP      0.16753899 -0.31592431 0.6510023 0.8528112
## HFD_control-FVT_ChP -0.15557846 -0.62488853 0.3137316 0.8717073
## FVT_SDT-FVT_PyT      0.15636936 -0.27812734 0.5908661 0.8349554
## FVT_UnT-FVT_PyT      0.36810873 -0.08163809 0.8178555 0.1513399
## HFD_control-FVT_PyT  0.04499128 -0.38950542 0.4794880 0.9981592
## FVT_UnT-FVT_SDT      0.21173937 -0.23800744 0.6614862 0.6565295
## HFD_control-FVT_SDT -0.11137808 -0.54587478 0.3231186 0.9451972
## HFD_control-FVT_UnT -0.32311745 -0.77286426 0.1266294 0.2550711

##
## Call:
```

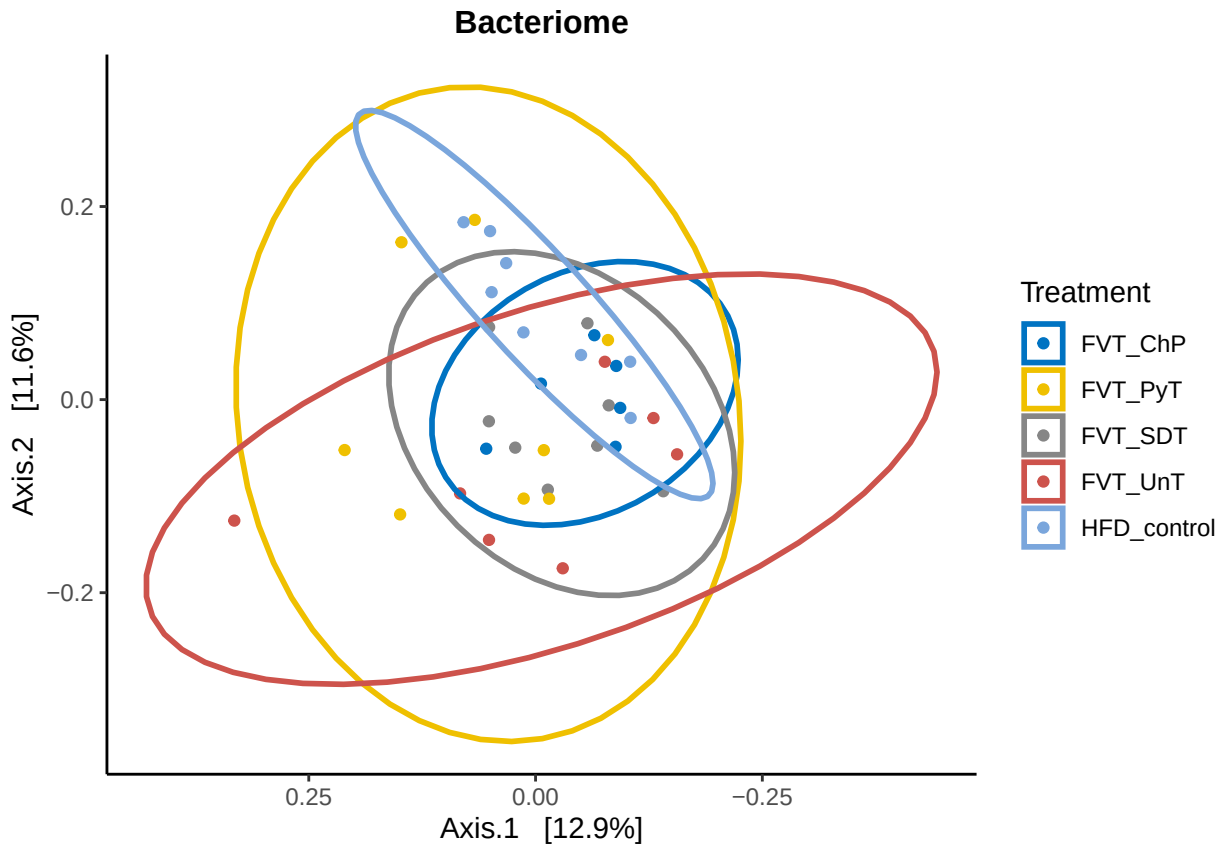
```
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.58771 -0.18245 -0.02426  0.15429  0.69388
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)      5.03986    0.10633  47.397   <2e-16 ***
## variableFVT_ChP  0.15558    0.16243   0.958    0.345
## variableFVT_PyT -0.04499    0.15038  -0.299    0.767
## variableFVT_SDT  0.11138    0.15038   0.741    0.464
## variableFVT_UnT  0.32312    0.15565   2.076    0.046 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3008 on 32 degrees of freedom
## Multiple R-squared:  0.1755, Adjusted R-squared:  0.07246
## F-statistic: 1.703 on 4 and 32 DF,  p-value: 0.1736


##
## Call:
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.58771 -0.18245 -0.02426  0.15429  0.69388
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)           5.3630     0.1137  47.179   <2e-16 ***
## variableHFD_control  -0.3231     0.1557  -2.076   0.0460 *
## variableFVT_ChP      -0.1675     0.1673  -1.001   0.3242
## variableFVT_PyT      -0.3681     0.1557  -2.365   0.0243 *
## variableFVT_SDT      -0.2117     0.1557  -1.360   0.1832
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3008 on 32 degrees of freedom
## Multiple R-squared:  0.1755, Adjusted R-squared:  0.07246
## F-statistic: 1.703 on 4 and 32 DF,  p-value: 0.1736
```

Beta diversity

basically the mouse ID.



Treatment



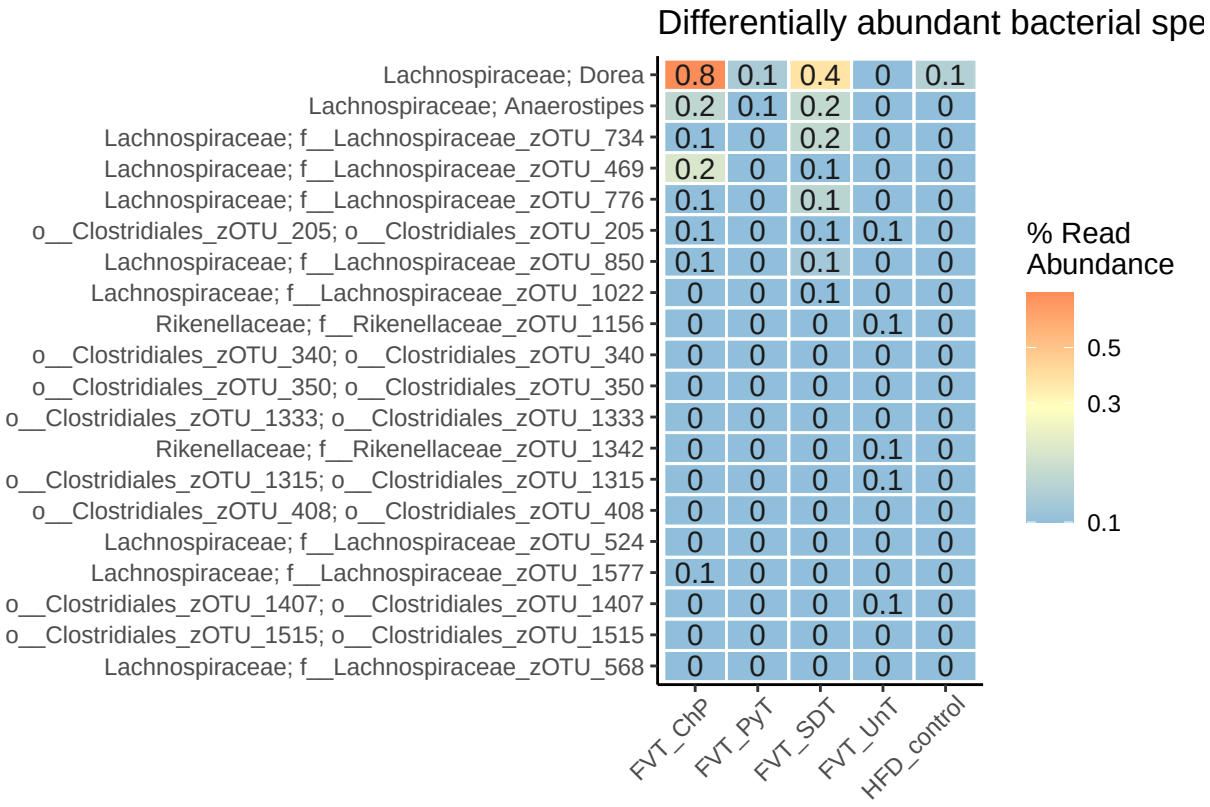
Adonis and pairwise permanova analysis - Treatment

```
## 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  4  0.42053 0.14382 1.3438  0.006 **
## Residual  32  2.50347 0.85618
## Total     36  2.92401 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

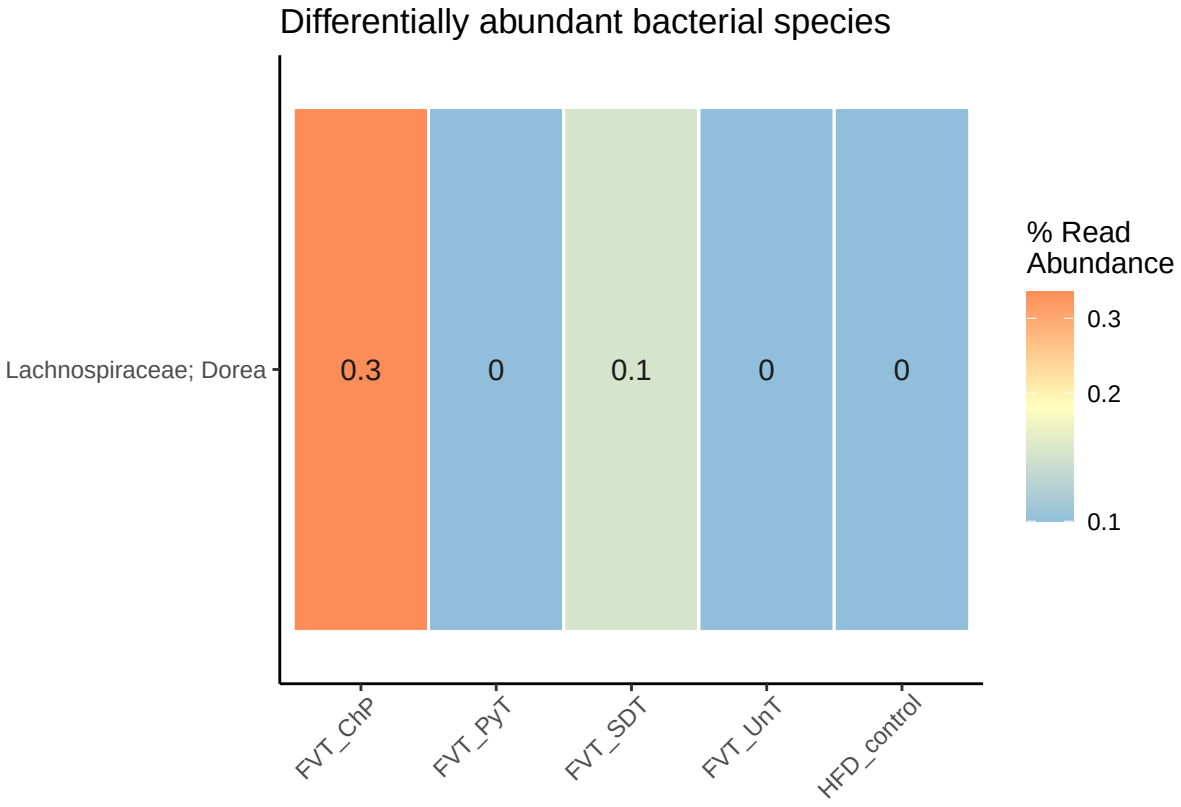
```
##         X1          X2         R2  pval pvalBon pvalFDR
## 1  FVT_ChP     FVT_PyT 0.09324268 0.137    1.37   0.171
## 2  FVT_ChP     FVT_SDT 0.06490570 0.864    8.64   0.864
## 3  FVT_ChP     FVT_UnT 0.11189007 0.070    0.70   0.140
## 4  FVT_ChP HFD_control 0.09160682 0.136    1.36   0.194
## 5  FVT_PyT     FVT_SDT 0.08845132 0.063    0.63   0.158
## 6  FVT_PyT     FVT_UnT 0.07075978 0.440    4.40   0.489
## 7  FVT_PyT HFD_control 0.08700653 0.084    0.84   0.140
## 8  FVT_SDT     FVT_UnT 0.09710813 0.056    0.56   0.187
```

```
## 9  FVT_SDT HFD_control 0.10015725 0.037    0.37   0.185
## 10 FVT_UnT HFD_control 0.13738166 0.002    0.02   0.020
```

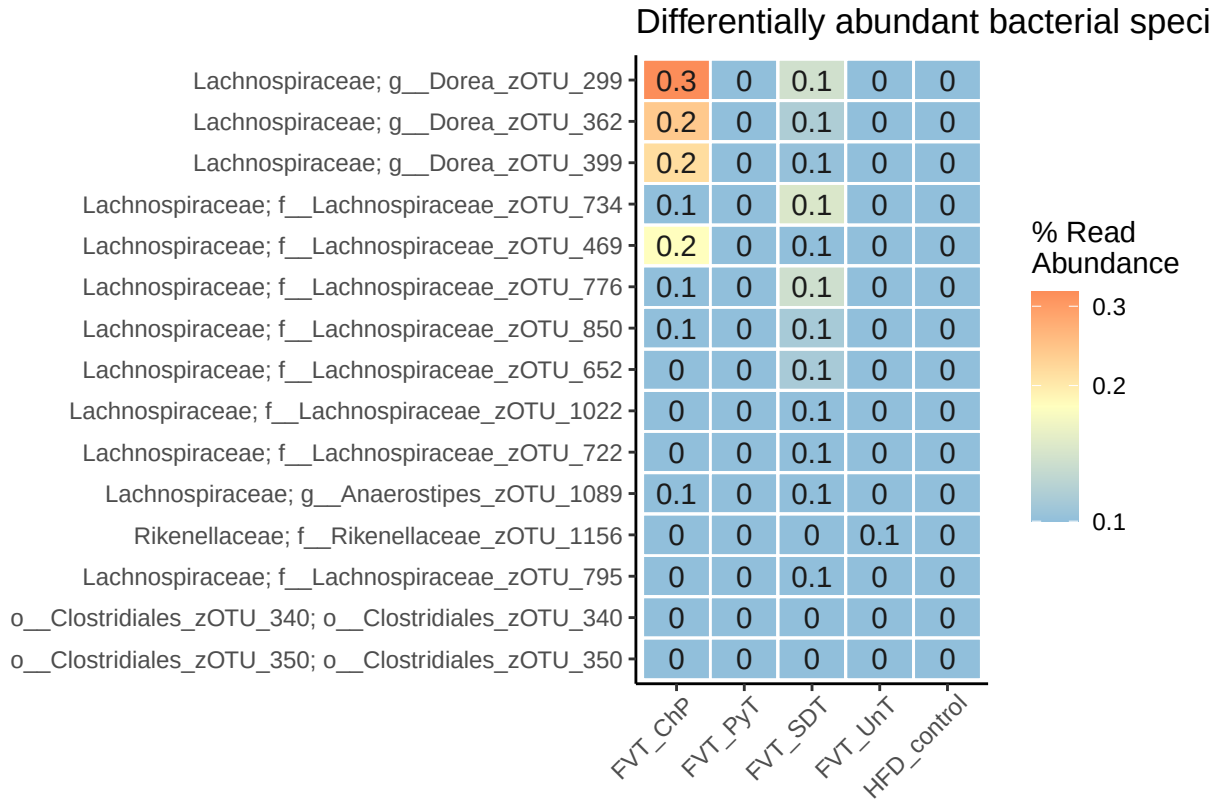
Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level - Comparing ChP vs HFD

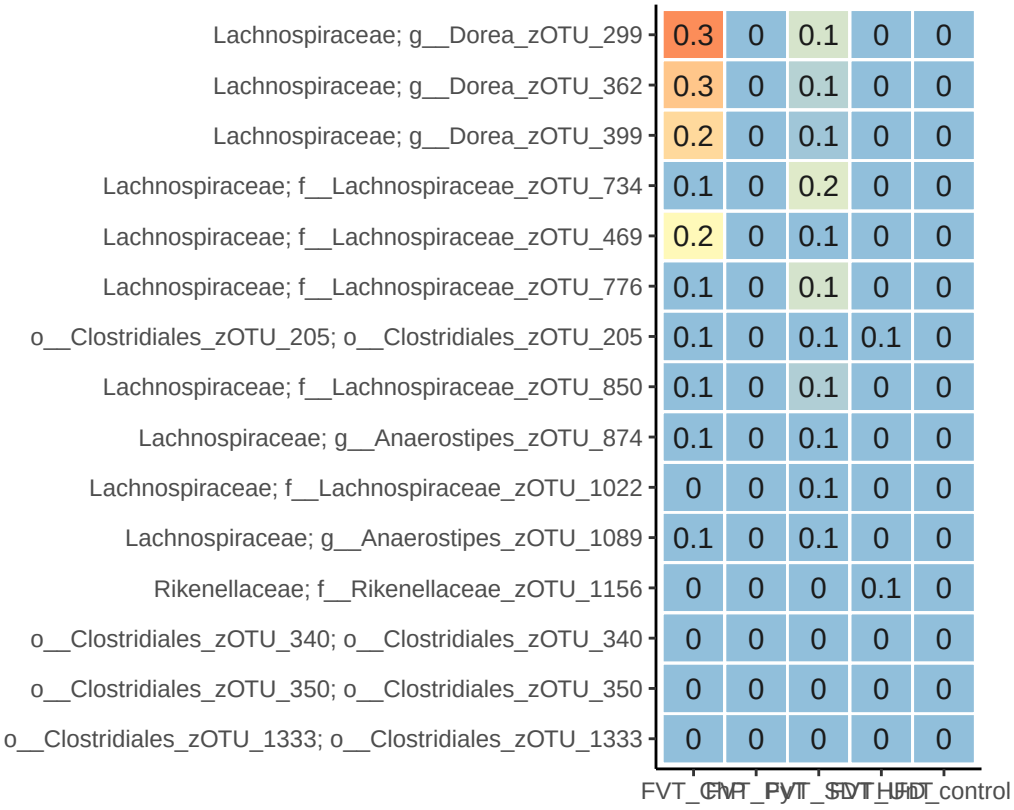


Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level



Bacteriome - Deseq2 - Treatment



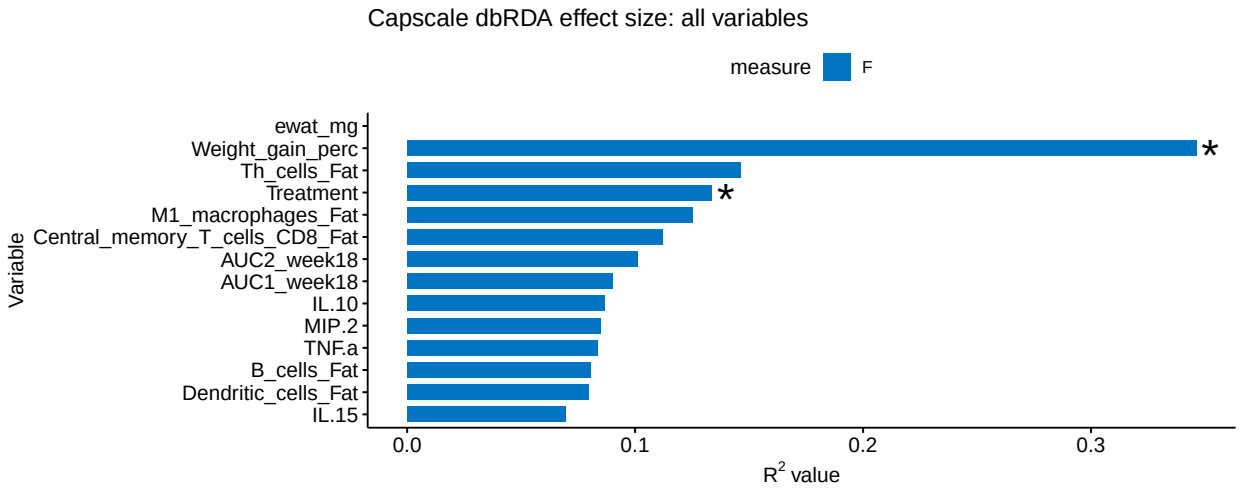


Deseq2 - defined comparison

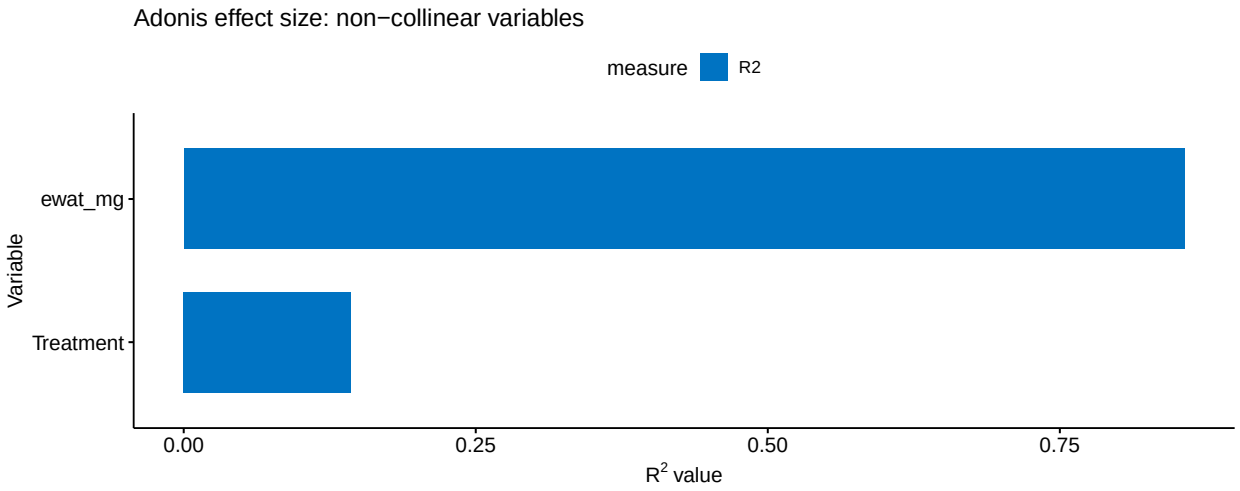
Bacteriome - Effect-size

Non-constrained

Capscale - independent effect sizes



ADONIS - decomposed to show individual contributions of factors


Adonis effect size: non−collinear variables
measure
R2
Variable
ewat_mg
Treatment
0.00
0.25
0.50
0.75
R2 value