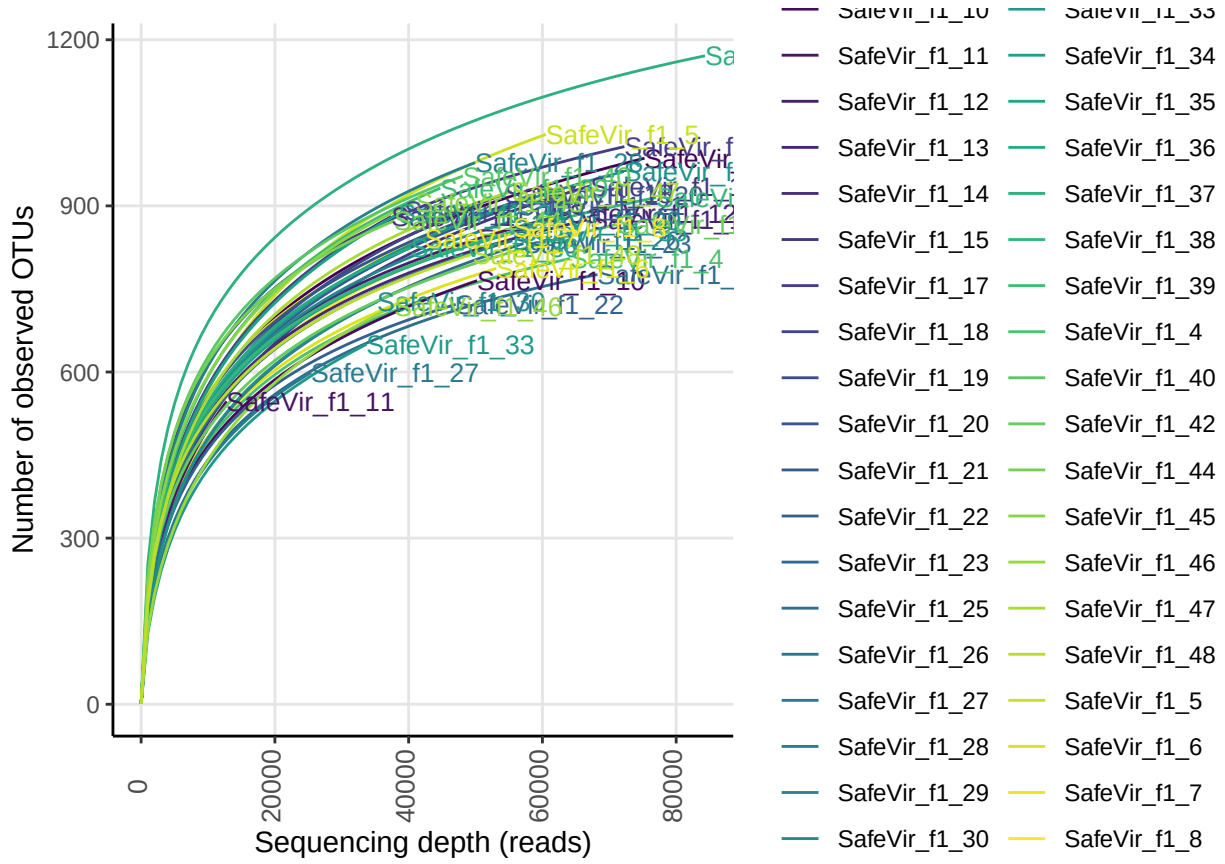
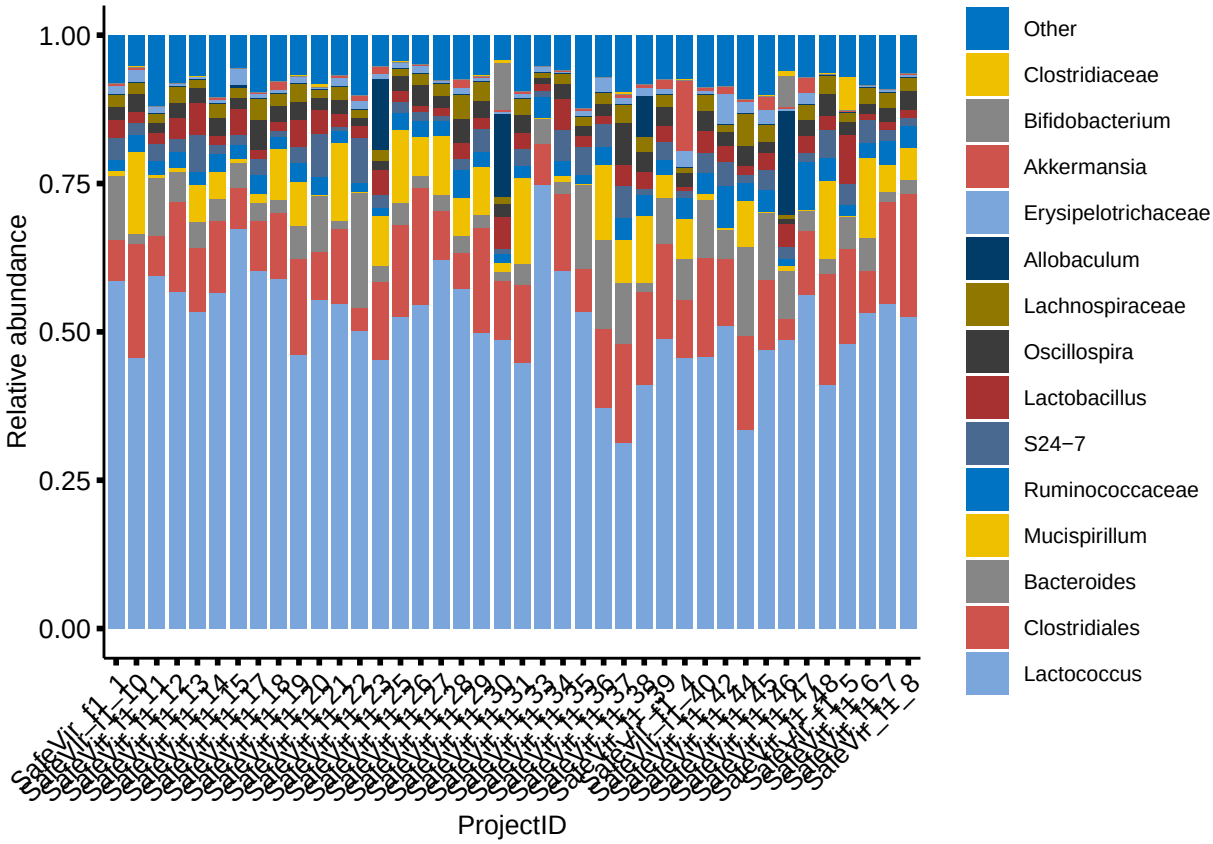
16S rRNA gene amplicon analysis - SafeVir - DIO - Before_1st_FVT

Sequencing depth

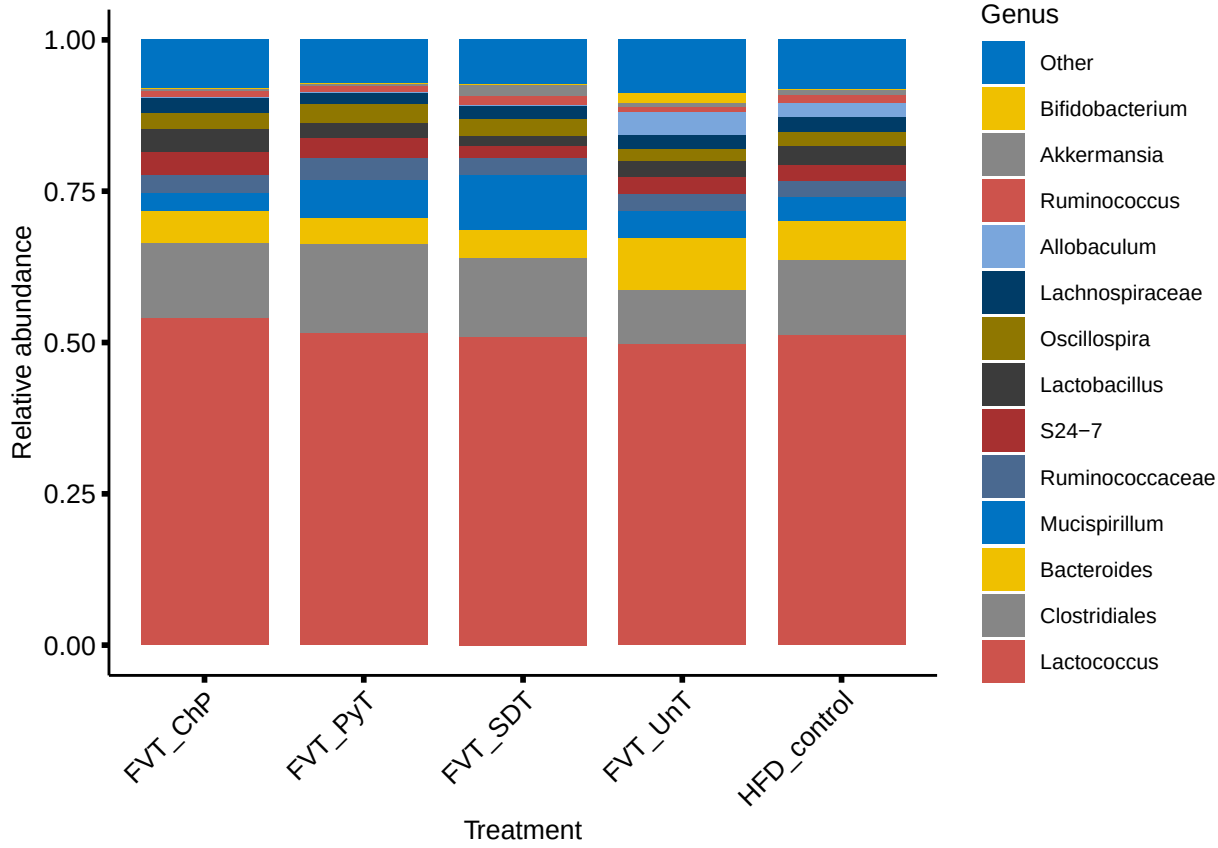


Individual sample barplots.

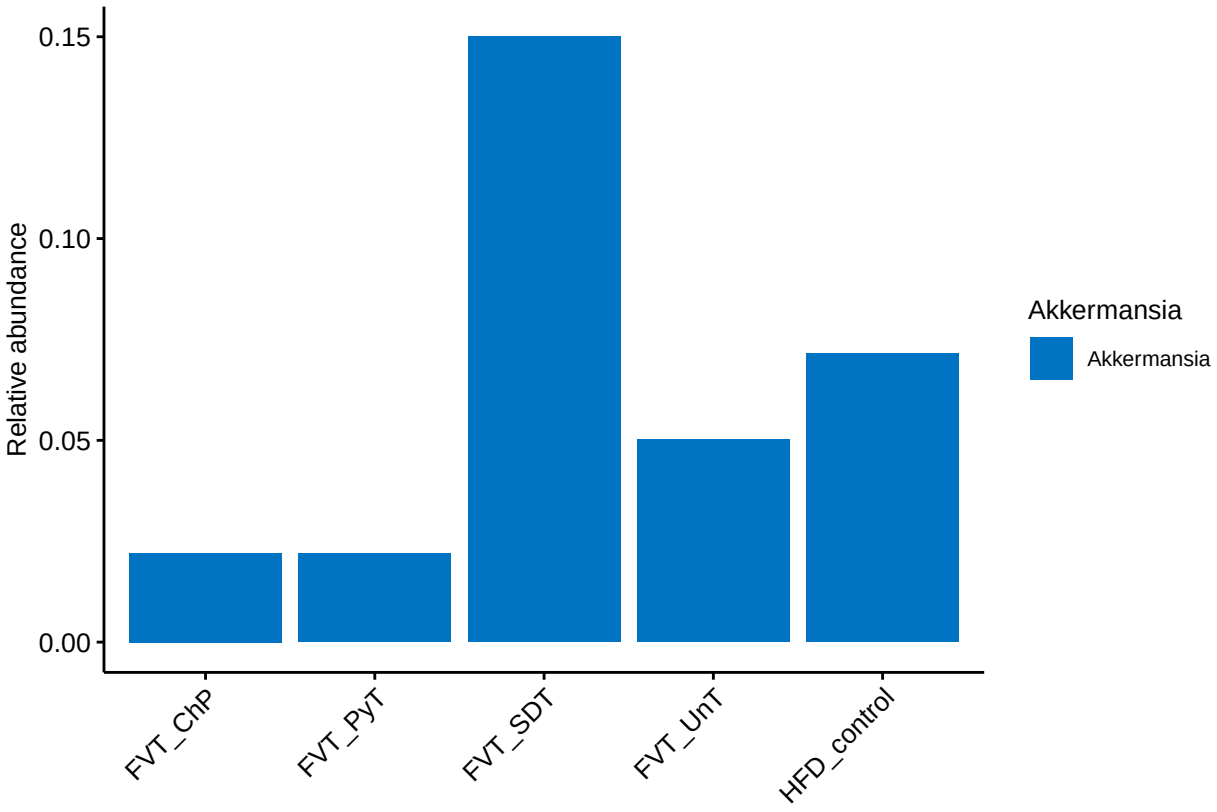


Barplots based on treatments.

```
## # A tibble: 575 x 3
## # Groups:   Sample [5]
##    Sample      tax             Mean
##    <chr>       <chr>          <dbl>
##  1 FVT_ChP     Lactococcus   0.541
##  2 FVT_PyT     Lactococcus   0.517
##  3 HFD_control Lactococcus   0.513
##  4 FVT_SDT     Lactococcus   0.509
##  5 FVT_UnT     Lactococcus   0.498
##  6 FVT_PyT     Clostridiales 0.146
##  7 FVT_SDT     Clostridiales 0.130
##  8 HFD_control Clostridiales 0.124
##  9 FVT_ChP     Clostridiales 0.124
## 10 FVT_SDT     Mucispirillum 0.0918
## # ... with 565 more rows
```



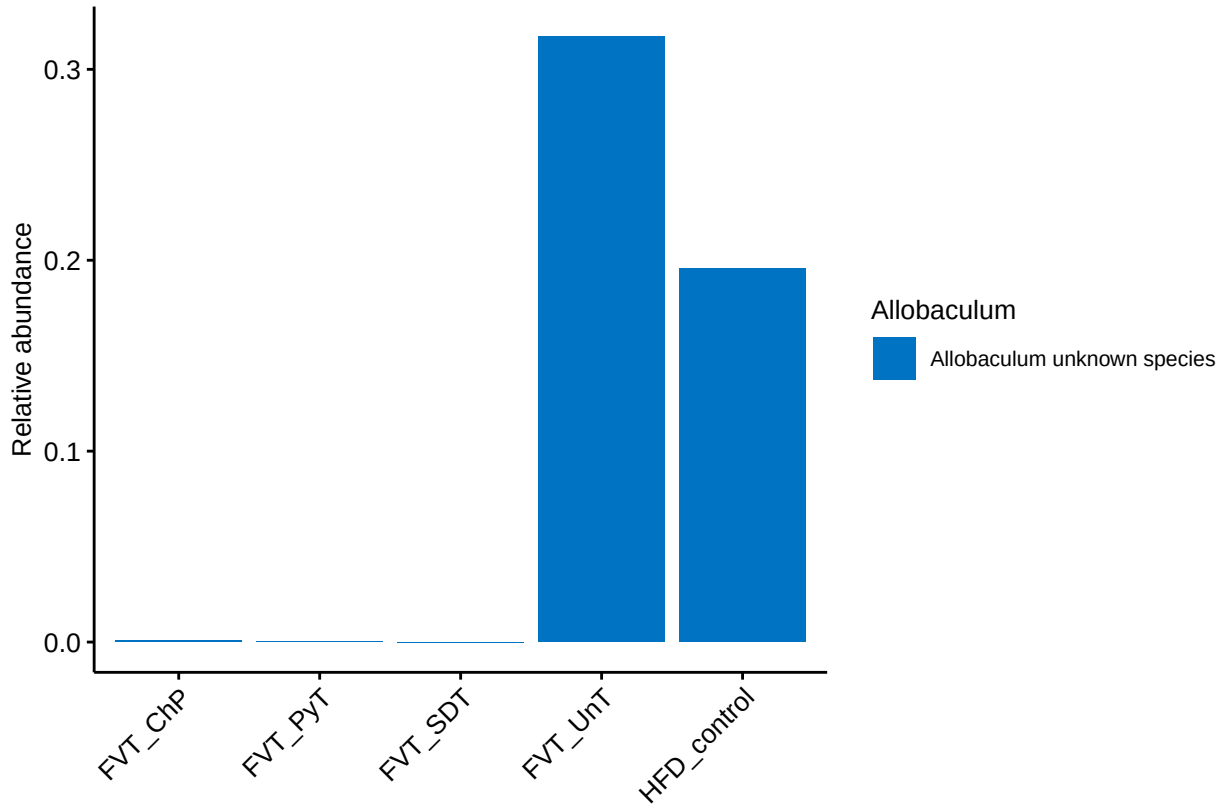

```
## # A tibble: 40 x 3
## # Groups:   Sample [40]
##    Sample       tax            Mean
##    <chr>        <chr>         <dbl>
##  1 NXT075Mao112 Akkermansia 0.120
##  2 NXT075Mao157 Akkermansia 0.0262
##  3 NXT075Mao155 Akkermansia 0.0230
##  4 NXT075Mao149 Akkermansia 0.0170
##  5 NXT075Mao126 Akkermansia 0.0159
##  6 NXT075Mao136 Akkermansia 0.0142
##  7 NXT075Mao152 Akkermansia 0.0128
##  8 NXT075Mao131 Akkermansia 0.0118
##  9 NXT075Mao130 Akkermansia 0.00965
## 10 NXT075Mao148 Akkermansia 0.00586
## # ... with 30 more rows

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




```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.018201 -0.004405 -0.002325 -0.000878  0.100911
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)       0.008945   0.006816   1.312    0.198
## TreatmentFVT_ChP -0.006185   0.009639  -0.642    0.525
## TreatmentFVT_PyT -0.006179   0.009639  -0.641    0.526
## TreatmentFVT_SDT  0.009802   0.009639   1.017    0.316
## TreatmentFVT_UnT -0.002672   0.009639  -0.277    0.783
##
## Residual standard error: 0.01928 on 35 degrees of freedom
## Multiple R-squared:  0.09677,    Adjusted R-squared:  -0.006462
## F-statistic: 0.9374 on 4 and 35 DF,  p-value: 0.4537

## # A tibble: 40 x 3
## # Groups:   Sample [40]
##    Sample       tax                              Mean
##    <chr>        <chr>                           <dbl>
##  1 NXT075Mao156 Allobaculum unknown species 0.176
```

```
##  2 NXT075Mao138 Allobaculum unknown species 0.141
##  3 NXT075Mao131 Allobaculum unknown species 0.121
##  4 NXT075Mao148 Allobaculum unknown species 0.0699
##  5 NXT075Mao123 Allobaculum unknown species 0.00461
##  6 NXT075Mao136 Allobaculum unknown species 0.000821
##  7 NXT075Mao121 Allobaculum unknown species 0.000387
##  8 NXT075Mao109 Allobaculum unknown species 0.000226
##  9 NXT075Mao113 Allobaculum unknown species 0.000116
## 10 NXT075Mao158 Allobaculum unknown species 0.0000605
## # ... with 30 more rows
```

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

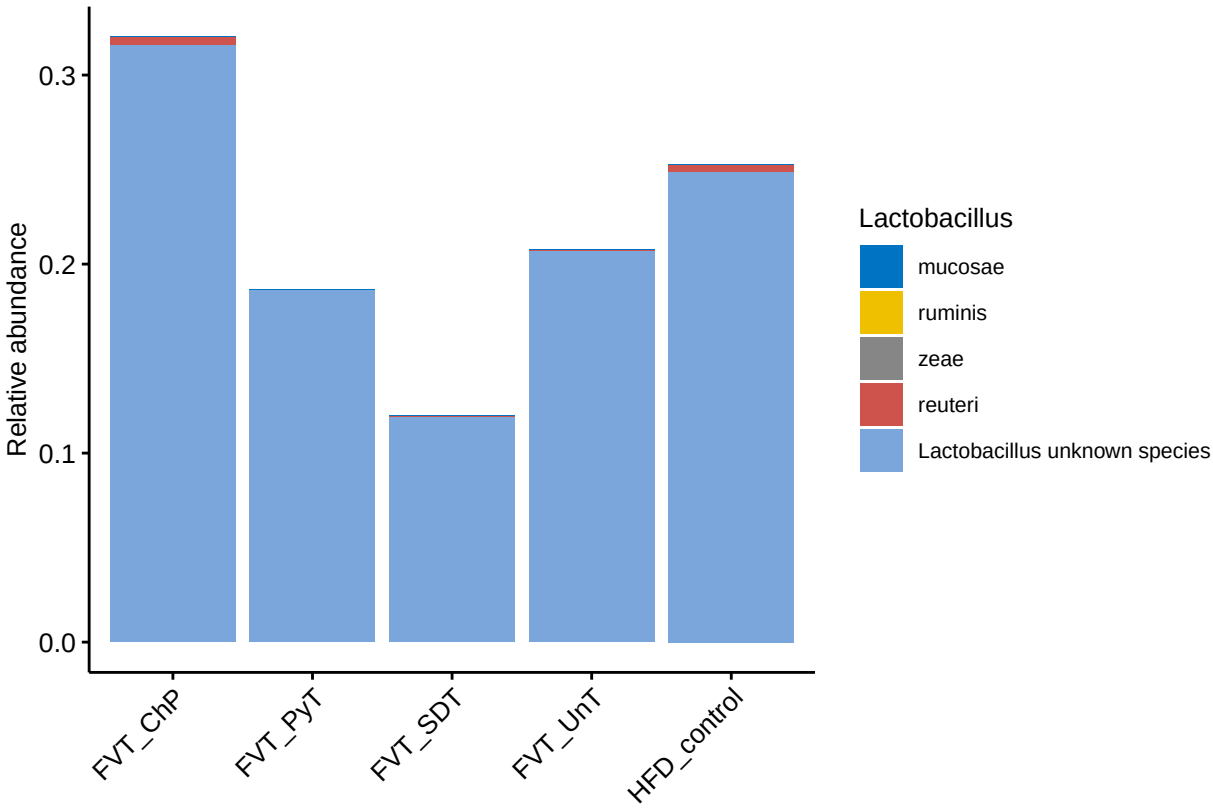



```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.039630 -0.024256 -0.000070 -0.000002  0.136199
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        0.02443    0.01376   1.776   0.0844 .
## TreatmentFVT_ChP -0.02431    0.01945  -1.249   0.2198
```

```
## TreatmentFVT_PyT -0.02436    0.01945  -1.252   0.2188
## TreatmentFVT_SDT -0.02443    0.01945  -1.256   0.2175
## TreatmentFVT_UnT  0.01520    0.01945   0.781   0.4399
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.03891 on 35 degrees of freedom
## Multiple R-squared:  0.1685, Adjusted R-squared:  0.07342
## F-statistic: 1.773 on 4 and 35 DF,  p-value: 0.1565
```

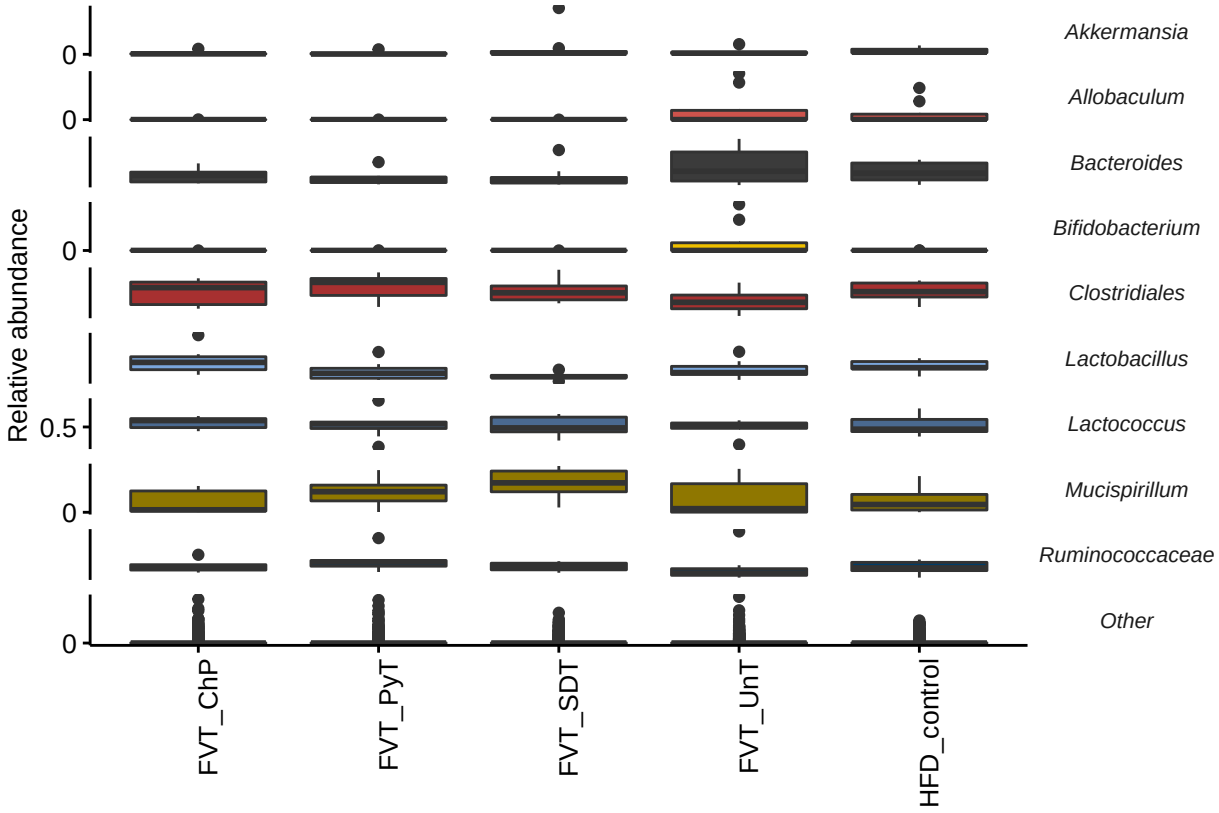
```
## # A tibble: 200 x 3
## # Groups:   Sample [40]
##    Sample       tax                             Mean
##    <chr>        <chr>                          <dbl>
##  1 NXT075Mao113 Lactobacillus unknown species 0.0830
##  2 NXT075Mao121 Lactobacillus unknown species 0.0552
##  3 NXT075Mao138 Lactobacillus unknown species 0.0552
##  4 NXT075Mao142 Lactobacillus unknown species 0.0510
##  5 NXT075Mao127 Lactobacillus unknown species 0.0444
##  6 NXT075Mao123 Lactobacillus unknown species 0.0439
##  7 NXT075Mao131 Lactobacillus unknown species 0.0417
##  8 NXT075Mao156 Lactobacillus unknown species 0.0399
##  9 NXT075Mao128 Lactobacillus unknown species 0.0397
## 10 NXT075Mao150 Lactobacillus unknown species 0.0373
## # ... with 190 more rows
```

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



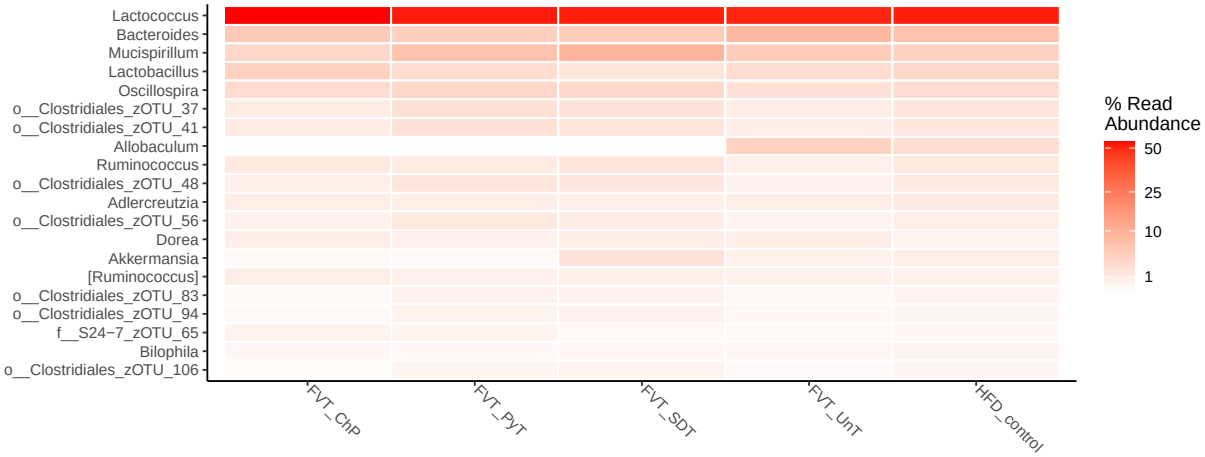

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.008005 -0.006320 -0.004671 -0.003002  0.075021
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)       0.006320   0.002044   3.092  0.00228 **
## TreatmentFVT_ChP  0.001685   0.002891   0.583  0.56070
## TreatmentFVT_PyT -0.001650   0.002891  -0.571  0.56888
## TreatmentFVT_SDT -0.003318   0.002891  -1.148  0.25248
## TreatmentFVT_UnT -0.001133   0.002891  -0.392  0.69546
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01293 on 195 degrees of freedom
## Multiple R-squared:  0.01684,    Adjusted R-squared:  -0.003332
## F-statistic: 0.8348 on 4 and 195 DF,  p-value: 0.5045

## # A tibble: 4,600 x 3
## # Groups:   Sample [40]
##    Sample       tax          Mean
```

```
##    <chr>        <chr>       <dbl>
##  1 NXT075Mao141 Lactococcus 0.748
##  2 NXT075Mao123 Lactococcus 0.674
##  3 NXT075Mao135 Lactococcus 0.622
##  4 NXT075Mao125 Lactococcus 0.603
##  5 NXT075Mao142 Lactococcus 0.603
##  6 NXT075Mao119 Lactococcus 0.594
##  7 NXT075Mao126 Lactococcus 0.589
##  8 NXT075Mao109 Lactococcus 0.585
##  9 NXT075Mao136 Lactococcus 0.572
## 10 NXT075Mao120 Lactococcus 0.568
## # ... with 4,590 more rows
```



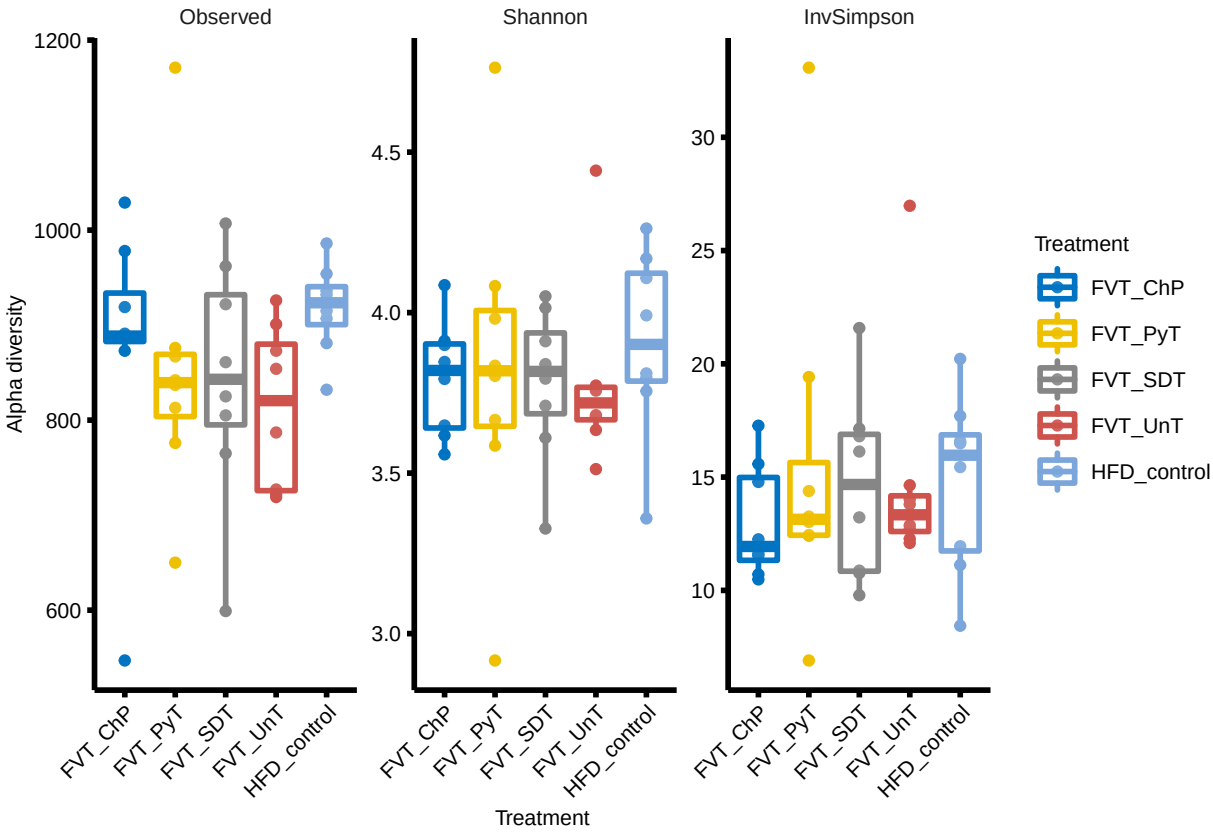
Abundance heatmaps

Bacteria - Treatment



Alpha diversity

By Treatment



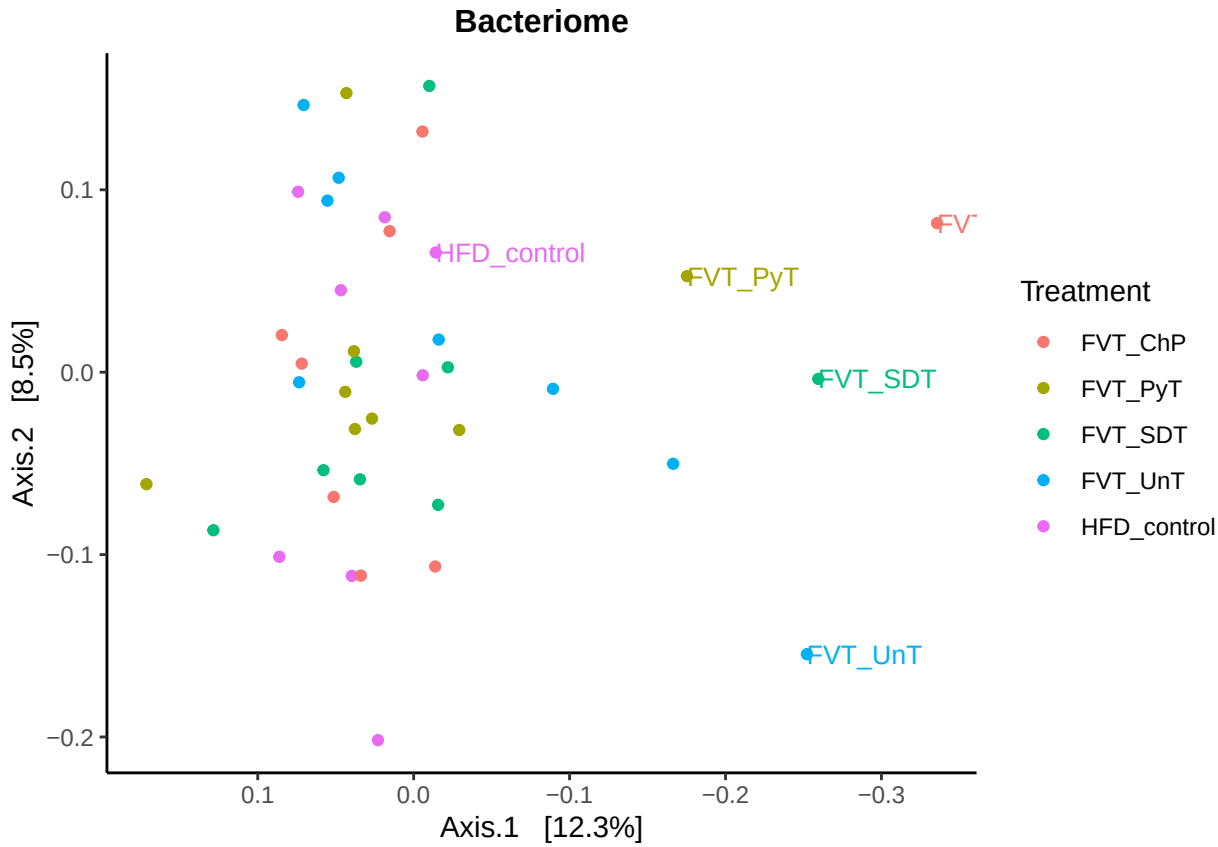
Anova and linear regression analysis

```
##   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      0.03398286 -0.4288751 0.4968408 0.9995354
## FVT_SDT-FVT_ChP     -0.01287049 -0.4757285 0.4499875 0.9999902
## FVT_UnT-FVT_ChP     -0.01487143 -0.4777294 0.4479865 0.9999826
## HFD_control-FVT_ChP  0.11133212 -0.3515259 0.5741901 0.9570024
## FVT_SDT-FVT_PyT     -0.04685336 -0.5097113 0.4160046 0.9983555
## FVT_UnT-FVT_PyT     -0.04885430 -0.5117123 0.4140037 0.9980634
## HFD_control-FVT_PyT  0.07734925 -0.3855087 0.5402072 0.9886635
## FVT_UnT-FVT_SDT     -0.00200094 -0.4648589 0.4608570 1.0000000
## HFD_control-FVT_SDT  0.12420261 -0.3386554 0.5870606 0.9371056
## HFD_control-FVT_UnT  0.12620355 -0.3366544 0.5890615 0.9335805

##
## Call:
```

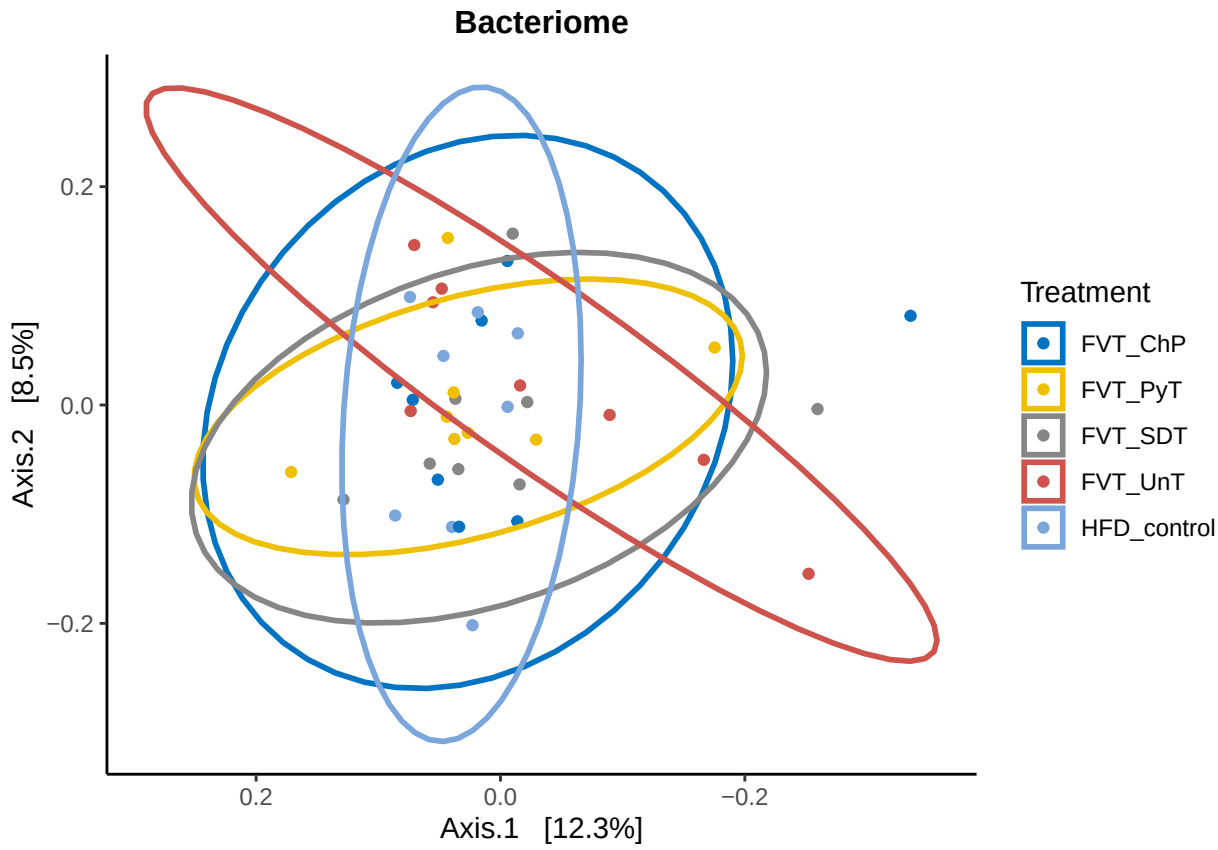
```
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.91287 -0.14780 -0.01103  0.13452  0.93415
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)      3.90645    0.11384  34.316   <2e-16 ***
## variableFVT_ChP -0.11133    0.16099  -0.692    0.494
## variableFVT_PyT -0.07735    0.16099  -0.480    0.634
## variableFVT_SDT -0.12420    0.16099  -0.771    0.446
## variableFVT_UnT -0.12620    0.16099  -0.784    0.438
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.322 on 35 degrees of freedom
## Multiple R-squared:  0.02404,    Adjusted R-squared:  -0.0875
## F-statistic: 0.2155 on 4 and 35 DF,  p-value: 0.928


##
## Call:
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.91287 -0.14780 -0.01103  0.13452  0.93415
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         3.780245   0.113838  33.207   <2e-16 ***
## variableHFD_control 0.126204   0.160991   0.784    0.438
## variableFVT_ChP     0.014871   0.160991   0.092    0.927
## variableFVT_PyT     0.048854   0.160991   0.303    0.763
## variableFVT_SDT     0.002001   0.160991   0.012    0.990
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.322 on 35 degrees of freedom
## Multiple R-squared:  0.02404,    Adjusted R-squared:  -0.0875
## F-statistic: 0.2155 on 4 and 35 DF,  p-value: 0.928
```

Beta diversity

basically the mouse ID.



Treatment



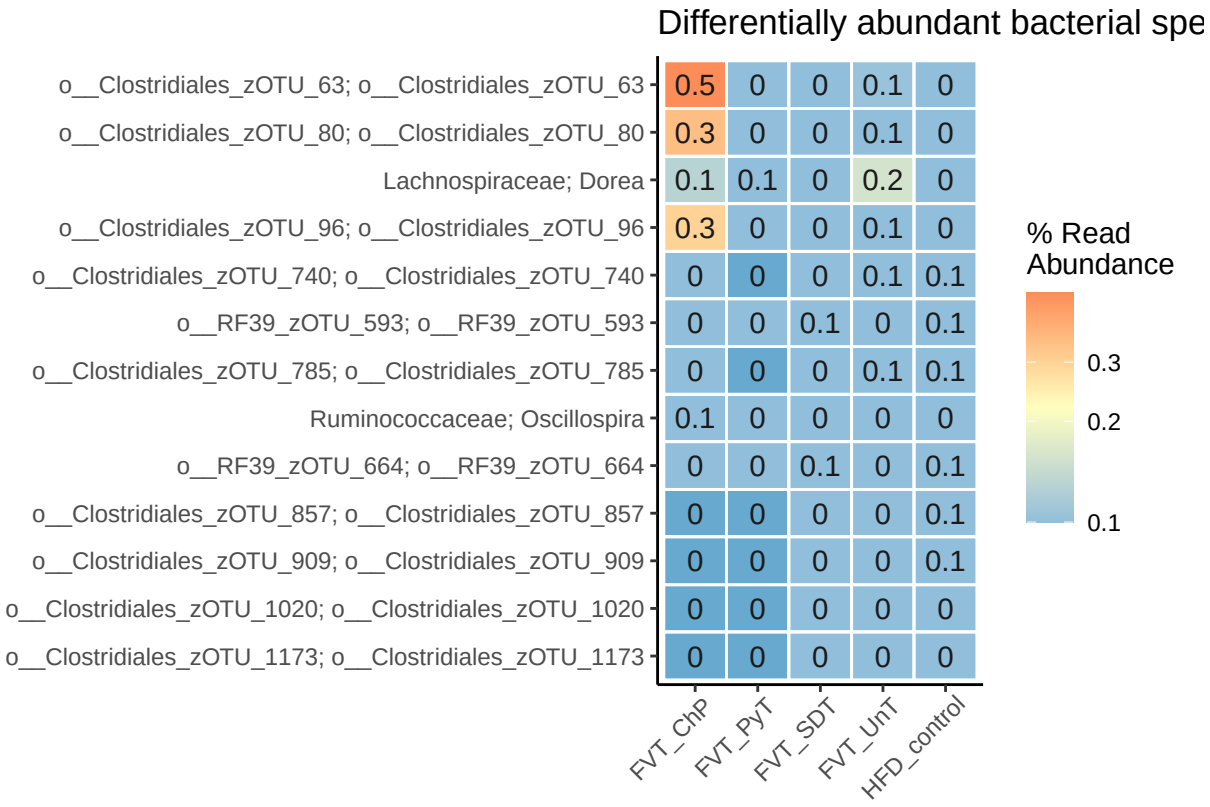
Adonis and pairwise permanova analysis - Treatment

```
## 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   0.4986 0.14517 1.4859  0.001 ***
## Residual  35   2.9358 0.85483
## Total     39   3.4343 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

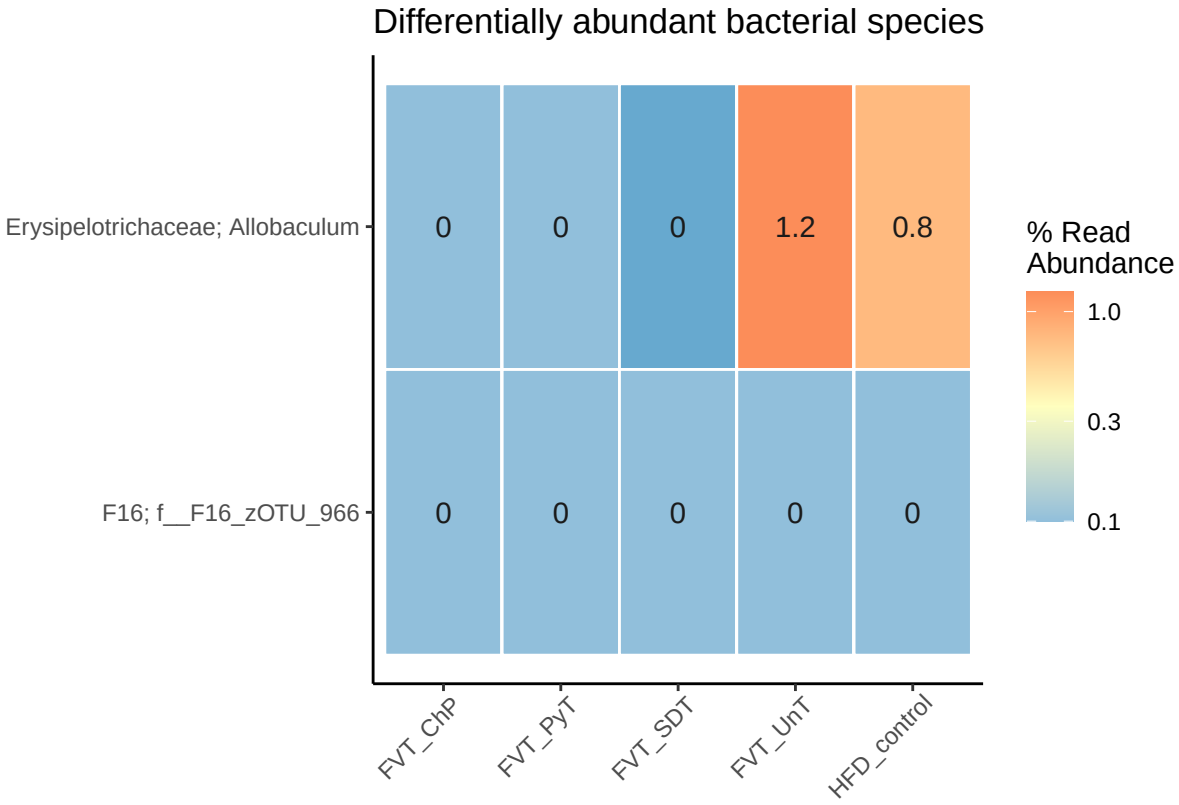
```
##         X1          X2         R2  pval pvalBon pvalFDR
## 1  FVT_ChP     FVT_PyT 0.10441535 0.013    0.13   0.043
## 2  FVT_ChP     FVT_SDT 0.08904713 0.083    0.83   0.104
## 3  FVT_ChP     FVT_UnT 0.08792072 0.086    0.86   0.096
## 4  FVT_ChP HFD_control 0.08677849 0.058    0.58   0.097
## 5  FVT_PyT     FVT_SDT 0.07982791 0.154    1.54   0.154
## 6  FVT_PyT     FVT_UnT 0.10039254 0.024    0.24   0.048
## 7  FVT_PyT HFD_control 0.10543039 0.011    0.11   0.055
## 8  FVT_SDT     FVT_UnT 0.10495964 0.016    0.16   0.040
```

```
## 9  FVT_SDT HFD_control 0.08683750 0.080    0.80   0.114
## 10 FVT_UnT HFD_control 0.11127368 0.004    0.04   0.040
```

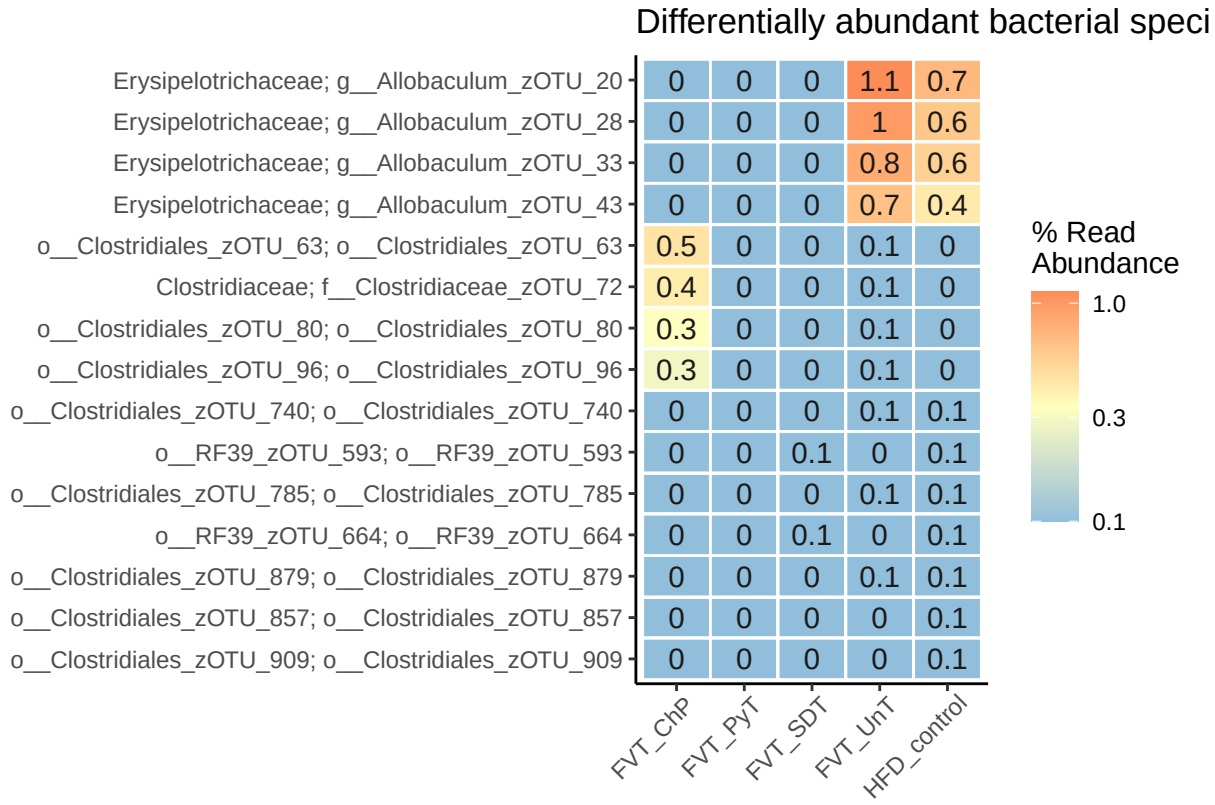
Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level - Comparing ChP vs HFD

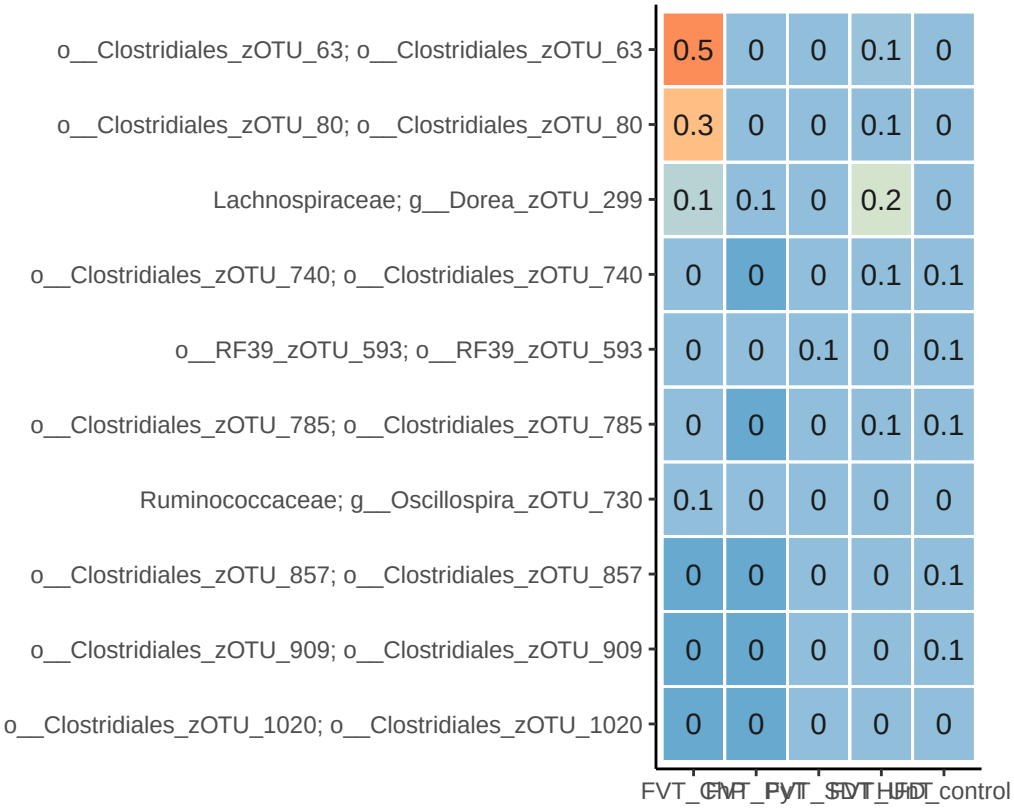


Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level



Bacteriome - Deseq2 - Treatment



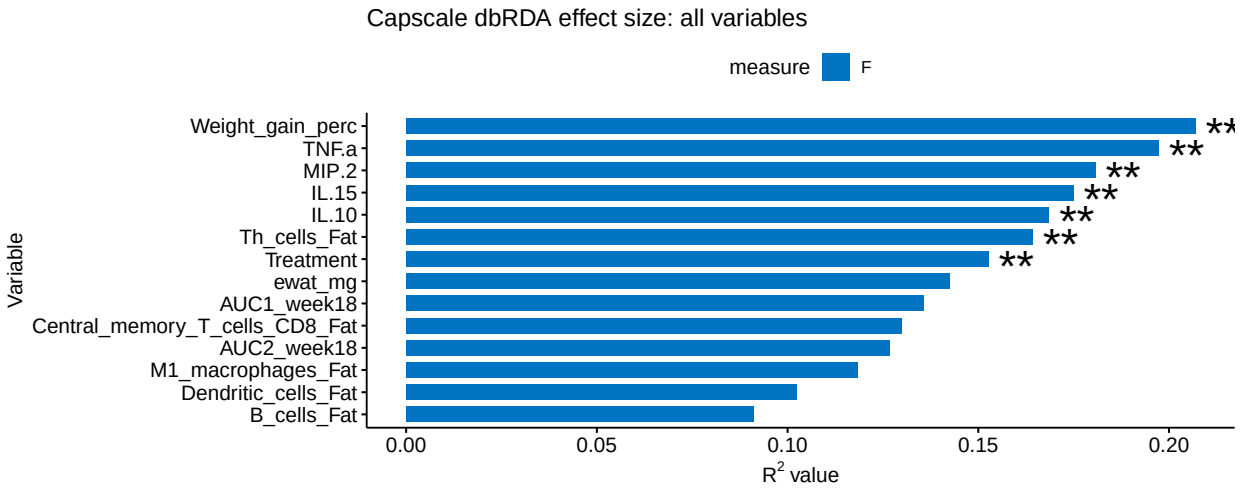


Deseq2 - defined comparison

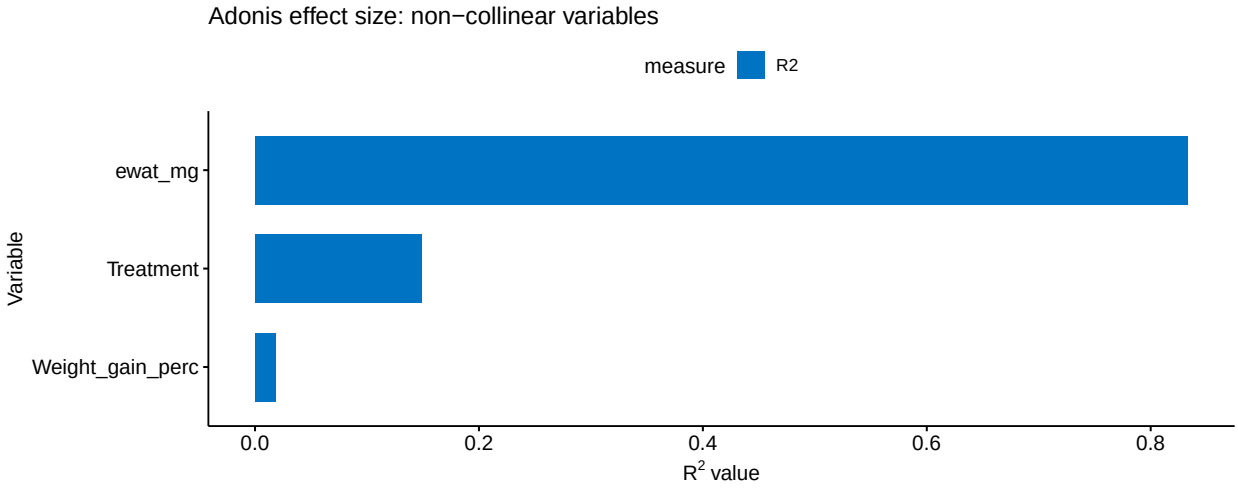
Bacteriome - Effect-size

Non-constrained

Capscale - independent effect sizes



ADONIS - decomposed to show individual contributions of factors


Adonis effect size: non−collinear variables
measure
R2
ewat_mg
Treatment
Weight_gain_perc
Variable
0.0
0.2
0.4
0.6
0.8
R2 value