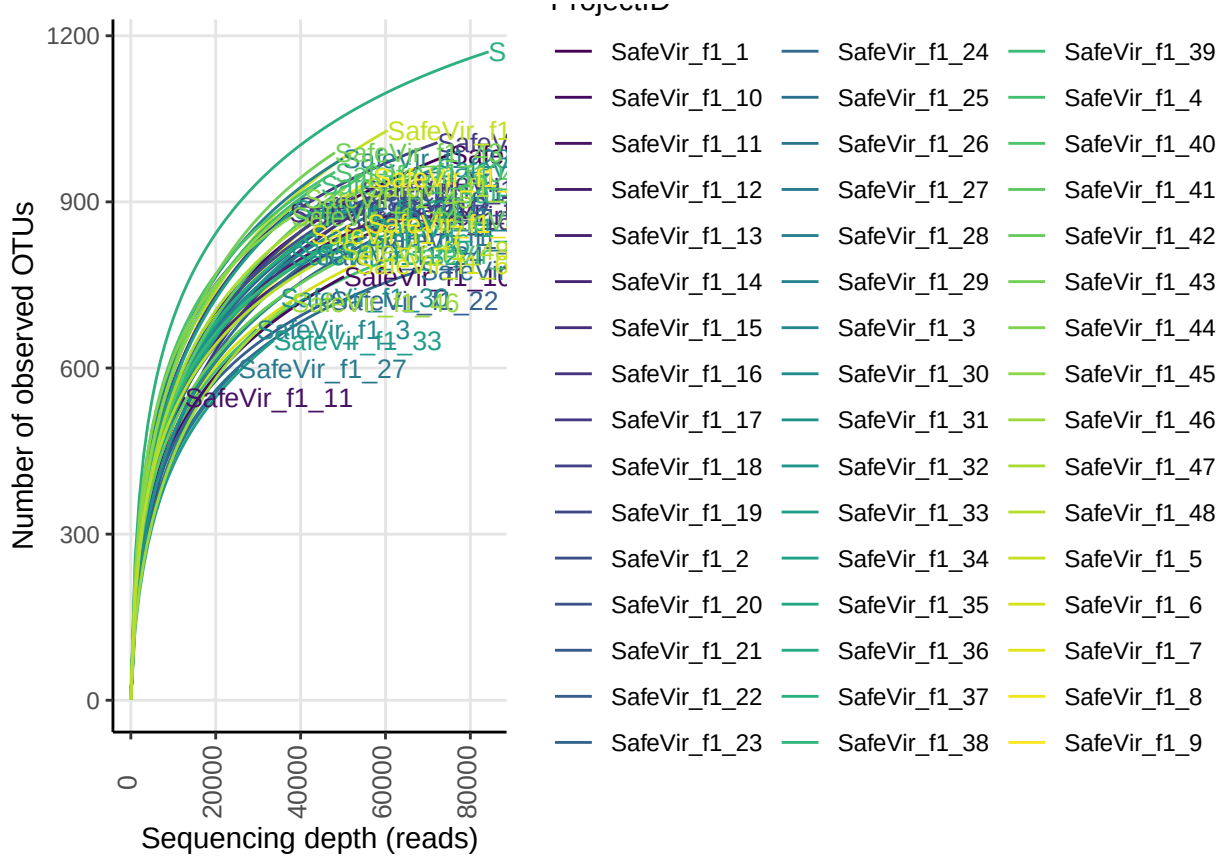
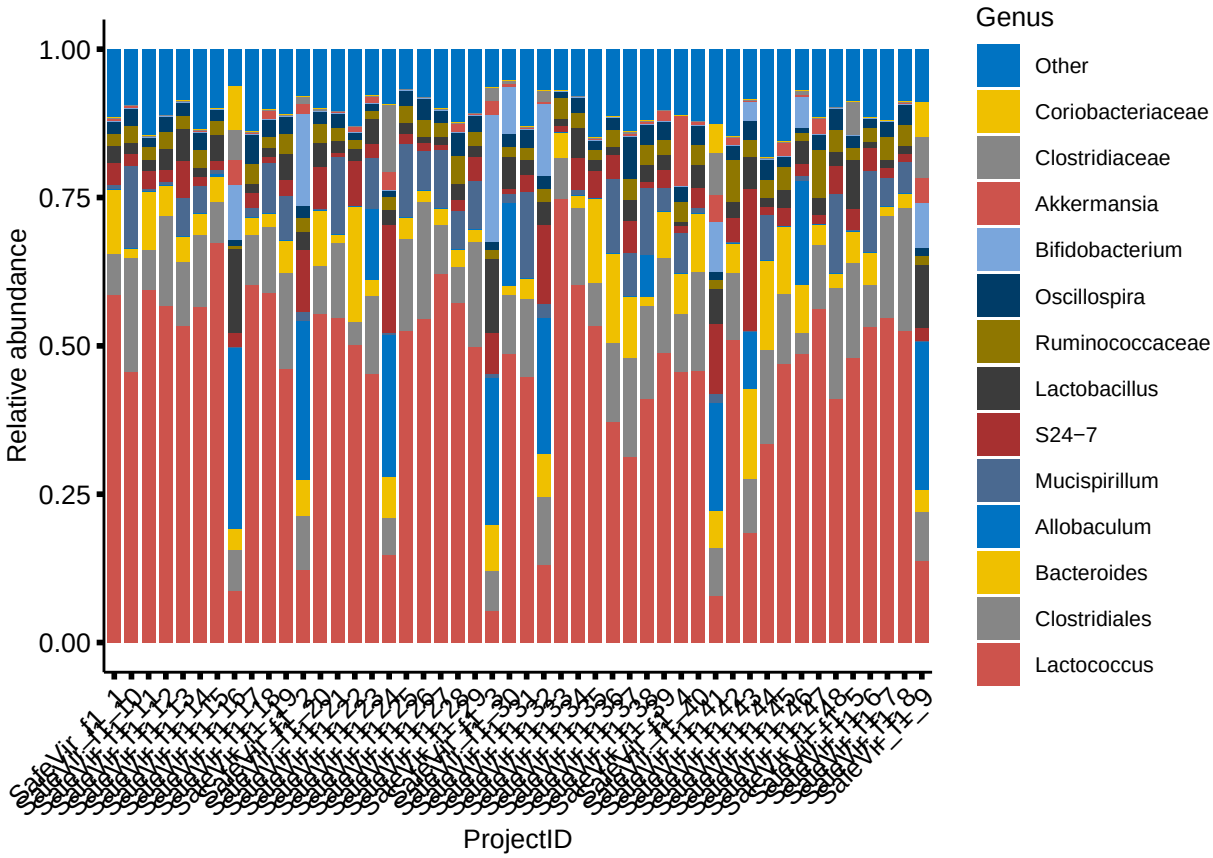
16S rRNA gene amplicon analysis - SafeVir - DIO - Before_1st_FVT

Sequencing depth

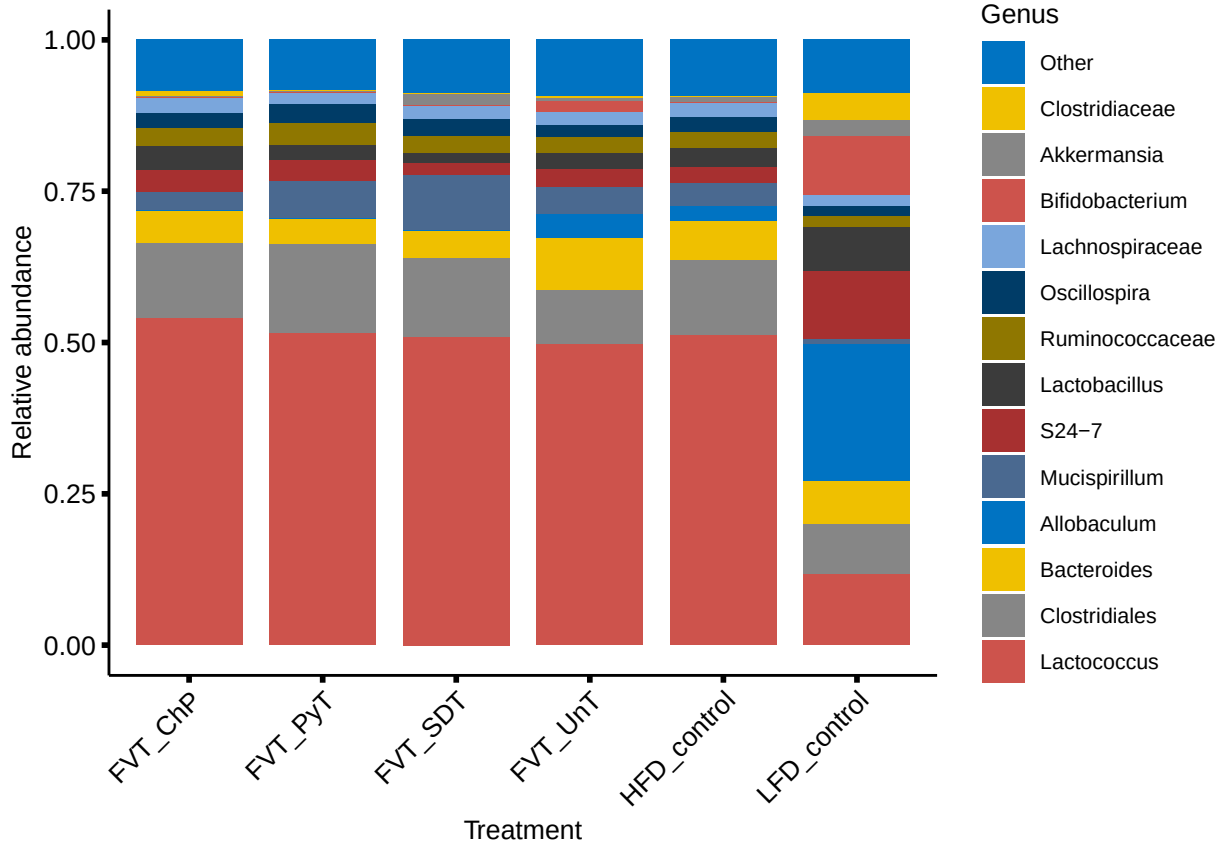


Individual sample barplots.



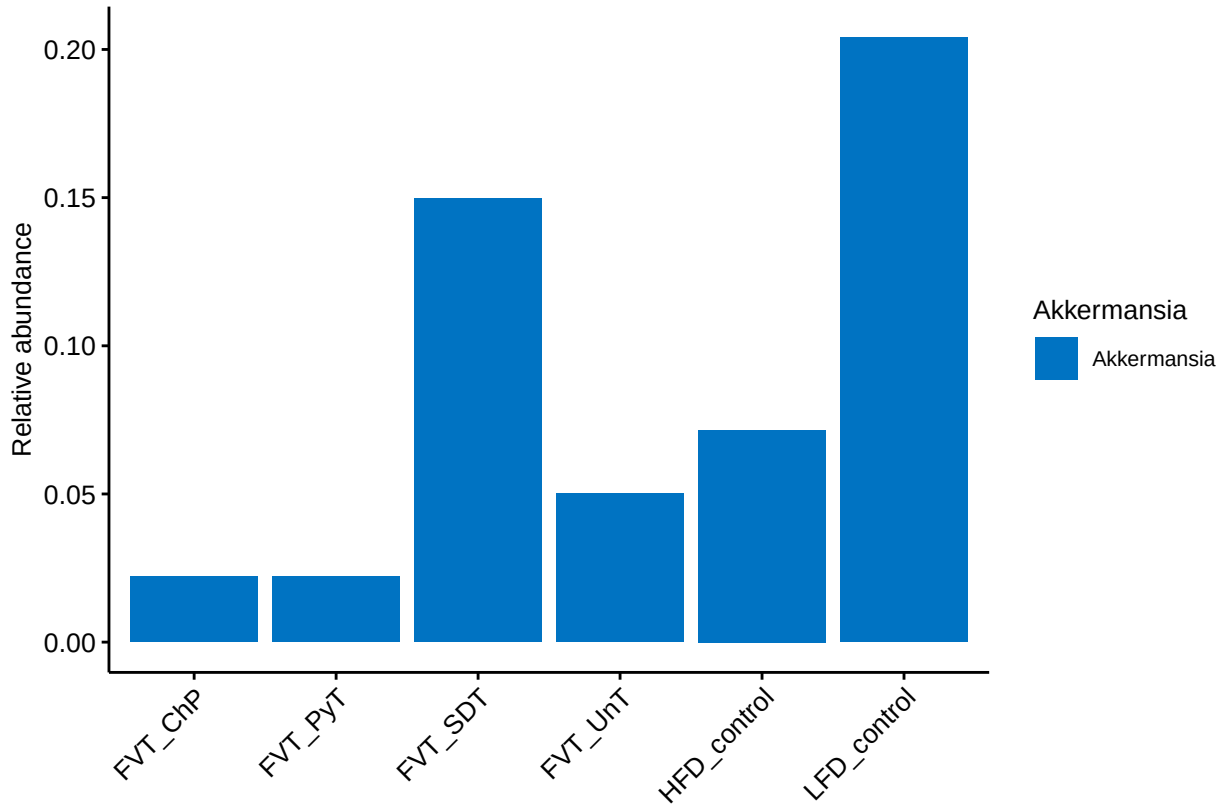
Barplots based on treatments.

```
## # A tibble: 690 x 3
## # Groups:   Sample [6]
##    Sample       tax            Mean
##    <chr>        <chr>         <dbl>
##  1 FVT_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 LFD_control Allobaculum   0.228
##  7 FVT_PyT      Clostridiales 0.146
##  8 FVT_SDT      Clostridiales 0.130
##  9 HFD_control Clostridiales 0.124
## 10 FVT_ChP      Clostridiales 0.124
## # ... with 680 more rows
```




```
## # A tibble: 48 x 3
## # Groups:   Sample [48]
##    Sample       tax           Mean
##    <chr>        <chr>        <dbl>
##  1 NXT075Mao112 Akkermansia 0.120
##  2 NXT075Mao151 Akkermansia 0.0442
##  3 NXT075Mao117 Akkermansia 0.0415
##  4 NXT075Mao124 Akkermansia 0.0412
##  5 NXT075Mao132 Akkermansia 0.0310
##  6 NXT075Mao157 Akkermansia 0.0262
##  7 NXT075Mao111 Akkermansia 0.0245
##  8 NXT075Mao155 Akkermansia 0.0230
##  9 NXT075Mao110 Akkermansia 0.0173
## 10 NXT075Mao149 Akkermansia 0.0170
## # ... with 38 more rows

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



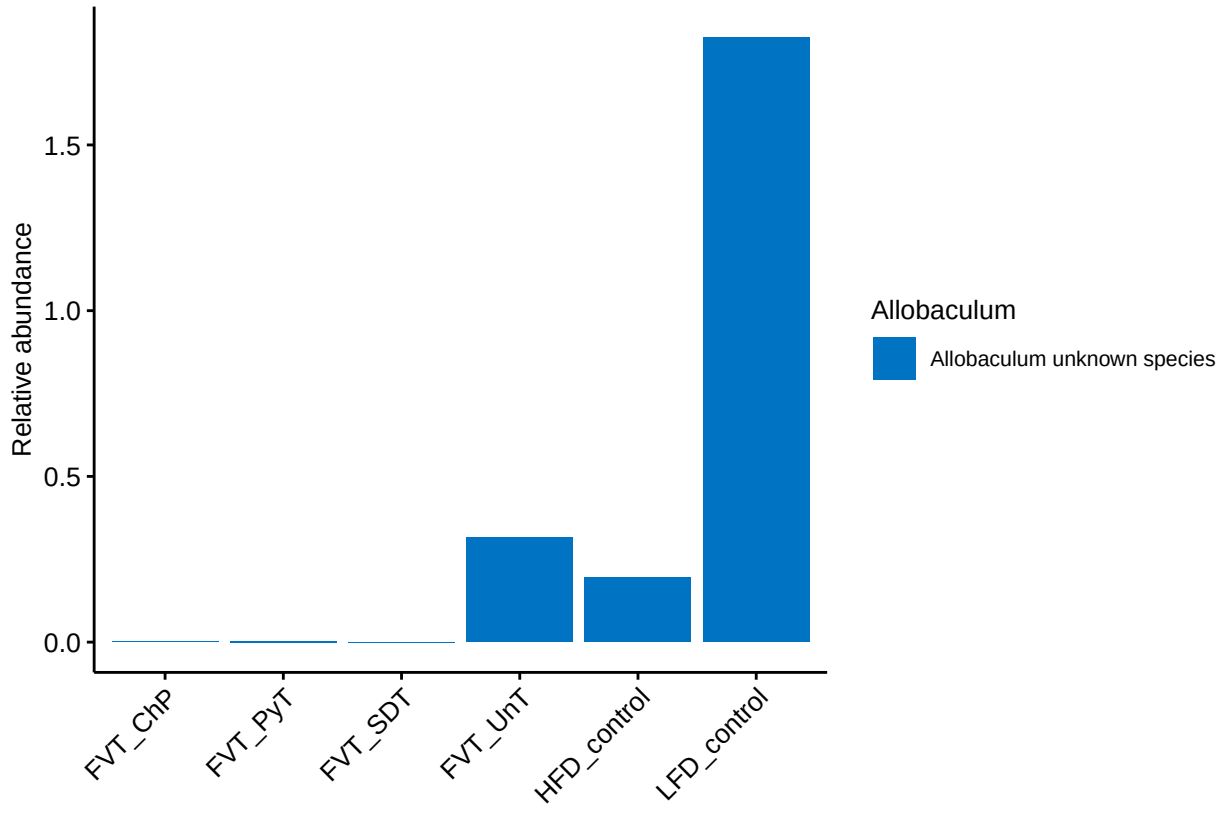

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.023543 -0.004655 -0.002310  0.002449  0.100911
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)           0.025531   0.006689   3.817 0.000438 ***
## TreatmentFVT_ChP     -0.022771   0.009460  -2.407 0.020550 *
## TreatmentFVT_PyT     -0.022766   0.009460  -2.407 0.020580 *
## TreatmentFVT_SDT     -0.006784   0.009460  -0.717 0.477240
## TreatmentFVT_UnT     -0.019259   0.009460  -2.036 0.048106 *
## TreatmentHFD_control -0.016586   0.009460  -1.753 0.086836 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01892 on 42 degrees of freedom
## Multiple R-squared:  0.1874, Adjusted R-squared:  0.09062
## F-statistic: 1.937 on 5 and 42 DF,  p-value: 0.1084

##
## Call:
```

```
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.023543 -0.004655 -0.002310  0.002449  0.100911
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           0.008945   0.006689   1.337   0.1884
## TreatmentLFD_control  0.016586   0.009460   1.753   0.0868 .
## TreatmentFVT_ChP     -0.006185   0.009460  -0.654   0.5168
## TreatmentFVT_PyT     -0.006179   0.009460  -0.653   0.5172
## TreatmentFVT_SDT      0.009802   0.009460   1.036   0.3060
## TreatmentFVT_UnT     -0.002672   0.009460  -0.282   0.7790
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01892 on 42 degrees of freedom
## Multiple R-squared:  0.1874, Adjusted R-squared:  0.09062
## F-statistic: 1.937 on 5 and 42 DF,  p-value: 0.1084
```

```
## # A tibble: 48 x 3
## # Groups:   Sample [48]
##    Sample       tax                          Mean
##    <chr>        <chr>                       <dbl>
##  1 NXT075Mao124 Allobaculum unknown species 0.307
##  2 NXT075Mao110 Allobaculum unknown species 0.268
##  3 NXT075Mao117 Allobaculum unknown species 0.252
##  4 NXT075Mao111 Allobaculum unknown species 0.248
##  5 NXT075Mao132 Allobaculum unknown species 0.240
##  6 NXT075Mao140 Allobaculum unknown species 0.231
##  7 NXT075Mao151 Allobaculum unknown species 0.183
##  8 NXT075Mao156 Allobaculum unknown species 0.176
##  9 NXT075Mao138 Allobaculum unknown species 0.141
## 10 NXT075Mao131 Allobaculum unknown species 0.121
## # ... with 38 more rows
```

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



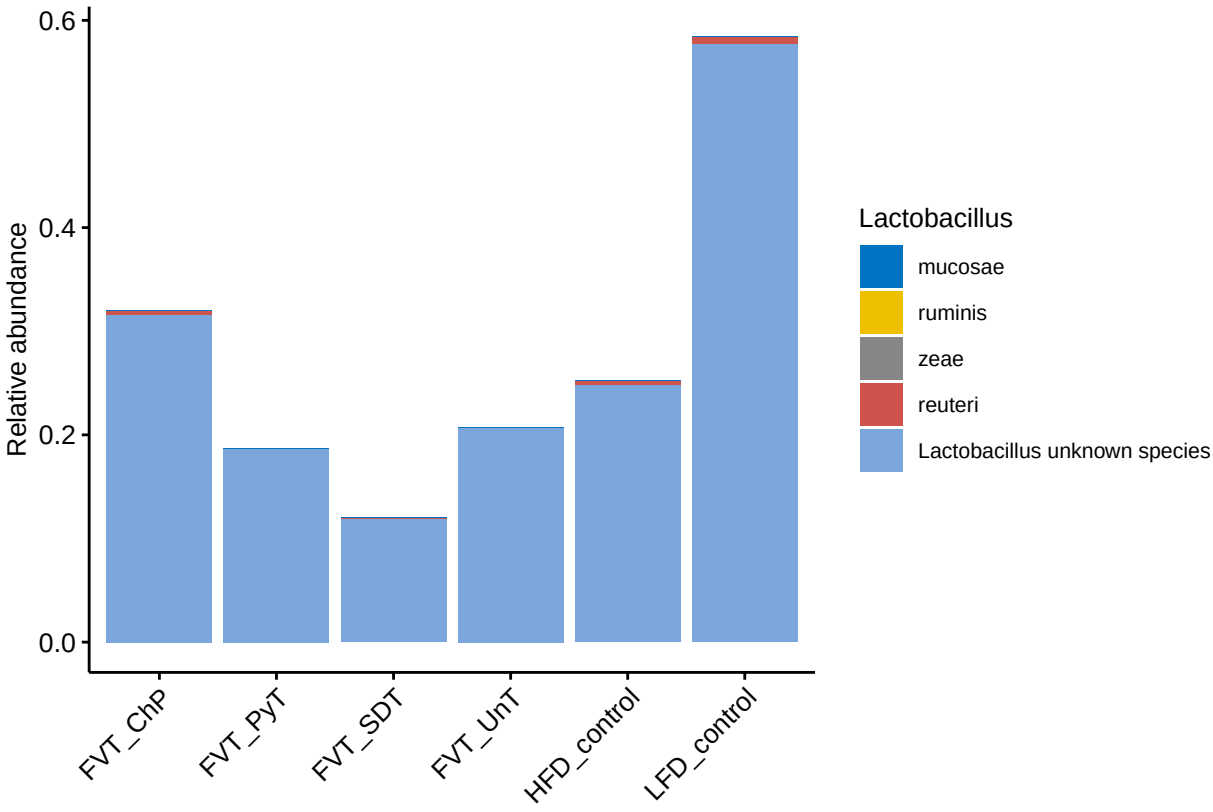

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.132232 -0.024256 -0.000042  0.000089  0.136199
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)           0.22825    0.01557  14.662  < 2e-16 ***
## TreatmentFVT_ChP     -0.22813    0.02202 -10.362 3.84e-13 ***
## TreatmentFVT_PyT     -0.22818    0.02202 -10.365 3.81e-13 ***
## TreatmentFVT_SDT     -0.22825    0.02202 -10.368 3.77e-13 ***
## TreatmentFVT_UnT     -0.18862    0.02202  -8.568 9.16e-11 ***
## TreatmentHFD_control -0.20382    0.02202  -9.258 1.06e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04403 on 42 degrees of freedom
## Multiple R-squared:  0.7972, Adjusted R-squared:  0.773
## F-statistic: 33.02 on 5 and 42 DF,  p-value: 1.608e-13

##
## Call:
```

```
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.132232 -0.024256 -0.000042  0.000089  0.136199
##
## Coefficients:
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)           0.02443    0.01557   1.569    0.124
## TreatmentLFD_control  0.20382    0.02202   9.258 1.06e-11 ***
## TreatmentFVT_ChP     -0.02431    0.02202  -1.104    0.276
## TreatmentFVT_PyT     -0.02436    0.02202  -1.107    0.275
## TreatmentFVT_SDT     -0.02443    0.02202  -1.110    0.273
## TreatmentFVT_UnT      0.01520    0.02202   0.690    0.494
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04403 on 42 degrees of freedom
## Multiple R-squared:  0.7972, Adjusted R-squared:  0.773
## F-statistic: 33.02 on 5 and 42 DF,  p-value: 1.608e-13
```

```
## # A tibble: 240 x 3
## # Groups:   Sample [48]
##    Sample       tax                             Mean
##    <chr>        <chr>                          <dbl>
##  1 NXT075Mao124 Lactobacillus unknown species 0.141
##  2 NXT075Mao111 Lactobacillus unknown species 0.124
##  3 NXT075Mao117 Lactobacillus unknown species 0.105
##  4 NXT075Mao113 Lactobacillus unknown species 0.0830
##  5 NXT075Mao151 Lactobacillus unknown species 0.0581
##  6 NXT075Mao121 Lactobacillus unknown species 0.0552
##  7 NXT075Mao138 Lactobacillus unknown species 0.0552
##  8 NXT075Mao153 Lactobacillus unknown species 0.0542
##  9 NXT075Mao142 Lactobacillus unknown species 0.0510
## 10 NXT075Mao127 Lactobacillus unknown species 0.0444
## # ... with 230 more rows
```

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



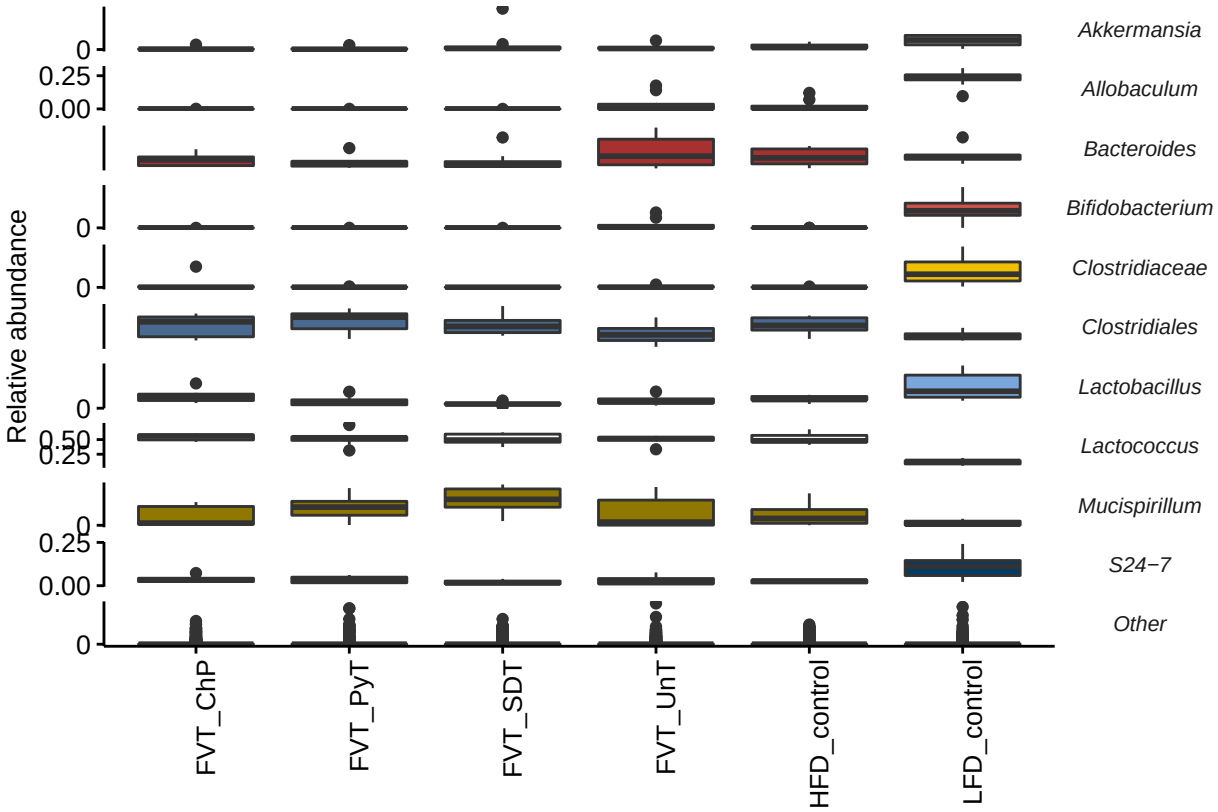

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.014601 -0.007523 -0.005187 -0.003002  0.126192
##
## Coefficients:
##                       Estimate Std. Error t value   Pr(>|t|)
## (Intercept)           0.014601   0.002919   5.002 0.00000111 ***
## TreatmentFVT_ChP     -0.006596   0.004128  -1.598    0.11145
## TreatmentFVT_PyT     -0.009931   0.004128  -2.405    0.01693 *
## TreatmentFVT_SDT     -0.011599   0.004128  -2.810    0.00538 **
## TreatmentFVT_UnT     -0.009414   0.004128  -2.280    0.02348 *
## TreatmentHFD_control -0.008281   0.004128  -2.006    0.04602 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01846 on 234 degrees of freedom
## Multiple R-squared:  0.04039,    Adjusted R-squared:  0.01989
## F-statistic:  1.97 on 5 and 234 DF,  p-value: 0.08389

##
## Call:
```

```
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.014601 -0.007523 -0.005187 -0.003002  0.126192
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           0.006320   0.002919   2.165   0.0314 *
## TreatmentLFD_control  0.008281   0.004128   2.006   0.0460 *
## TreatmentFVT_ChP      0.001685   0.004128   0.408   0.6836
## TreatmentFVT_PyT     -0.001650   0.004128  -0.400   0.6898
## TreatmentFVT_SDT     -0.003318   0.004128  -0.804   0.4224
## TreatmentFVT_UnT     -0.001133   0.004128  -0.275   0.7839
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01846 on 234 degrees of freedom
## Multiple R-squared:  0.04039,    Adjusted R-squared:  0.01989
## F-statistic:  1.97 on 5 and 234 DF,  p-value: 0.08389
```

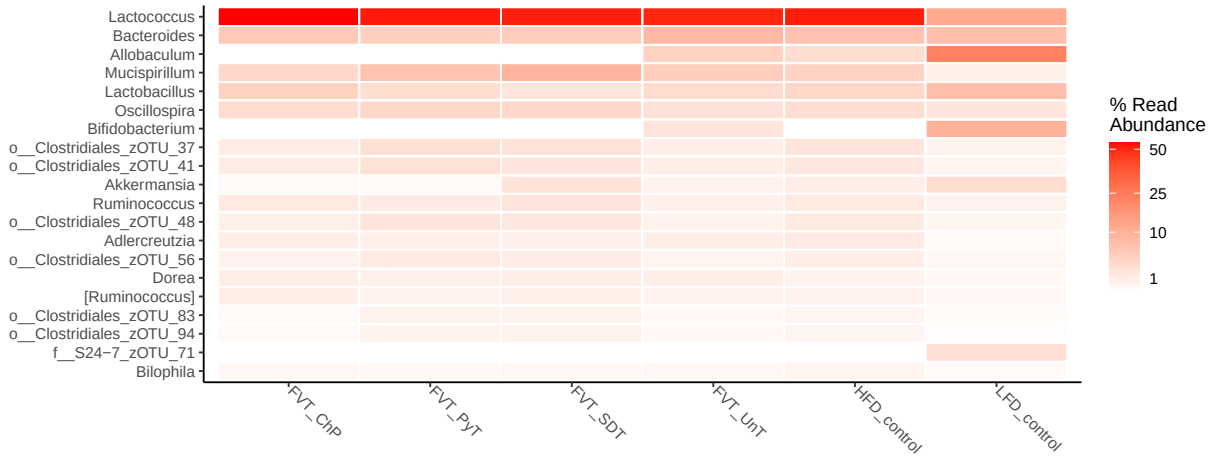
```
## # A tibble: 5,520 x 3
## # Groups:   Sample [48]
##    Sample       tax          Mean
##    <chr>        <chr>       <dbl>
##  1 NXT075Mao141 Lactococcus 0.748
##  2 NXT075Mao123 Lactococcus 0.674
##  3 NXT075Mao135 Lactococcus 0.622
##  4 NXT075Mao125 Lactococcus 0.603
##  5 NXT075Mao142 Lactococcus 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 5,510 more rows
```

```
## Warning: This manual palette can handle a maximum of 10 values. You have
## supplied 11.
```



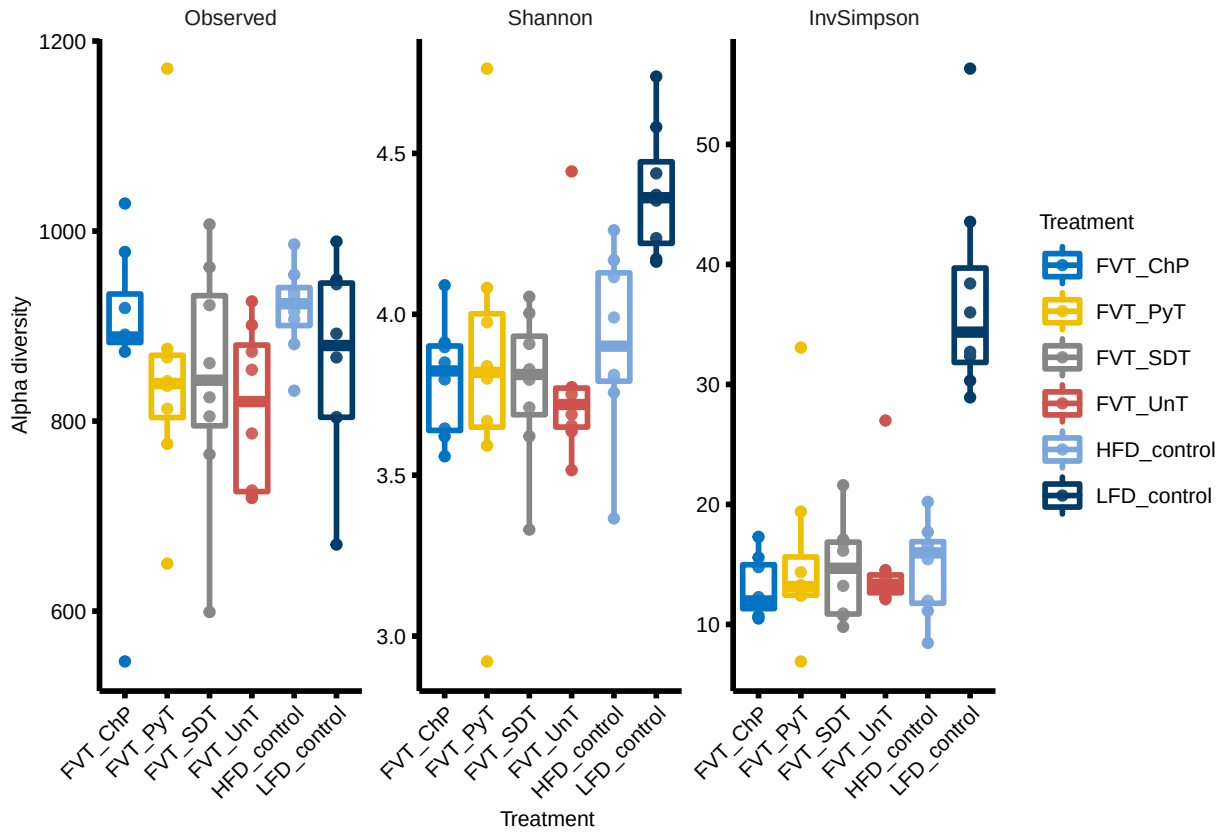
Abundance heatmaps

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          0.033543866 -0.42046791 0.4875556 0.9999227
## FVT_SDT-FVT_ChP         -0.014909404 -0.46892118 0.4391024 0.9999986
## FVT_UnT-FVT_ChP         -0.017605298 -0.47161708 0.4364065 0.9999969
## HFD_control-FVT_ChP      0.112513202 -0.34149857 0.5665250 0.9756862
## LFD_control-FVT_ChP      0.585244168  0.13123239 1.0392559 0.0050140
## FVT_SDT-FVT_PyT         -0.048453270 -0.50246505 0.4055585 0.9995293
## FVT_UnT-FVT_PyT         -0.051149165 -0.50516094 0.4028626 0.9993874
## HFD_control-FVT_PyT      0.078969336 -0.37504244 0.5329811 0.9951145
## LFD_control-FVT_PyT      0.551700302  0.09768852 1.0057121 0.0093630
## FVT_UnT-FVT_SDT         -0.002695894 -0.45670767 0.4513159 1.0000000
## HFD_control-FVT_SDT      0.127422606 -0.32658917 0.5814344 0.9586002
## LFD_control-FVT_SDT      0.600153572  0.14614180 1.0541653 0.0037737
## HFD_control-FVT_UnT      0.130118500 -0.32389328 0.5841303 0.9548193
```

```
## LFD_control-FVT_UnT      0.602849466  0.14883769 1.0568612 0.0035833
## LFD_control-HFD_control  0.472730966  0.01871919 0.9267427 0.0369727
```

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.014601 -0.007523 -0.005187 -0.003002  0.126192
##
## Coefficients:
##                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)           0.006320   0.002919   2.165   0.0314 *
## TreatmentLFD_control  0.008281   0.004128   2.006   0.0460 *
## TreatmentFVT_ChP      0.001685   0.004128   0.408   0.6836
## TreatmentFVT_PyT     -0.001650   0.004128  -0.400   0.6898
## TreatmentFVT_SDT     -0.003318   0.004128  -0.804   0.4224
## TreatmentFVT_UnT     -0.001133   0.004128  -0.275   0.7839
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01846 on 234 degrees of freedom
## Multiple R-squared:  0.04039,    Adjusted R-squared:  0.01989
## F-statistic:  1.97 on 5 and 234 DF,  p-value: 0.08389
```

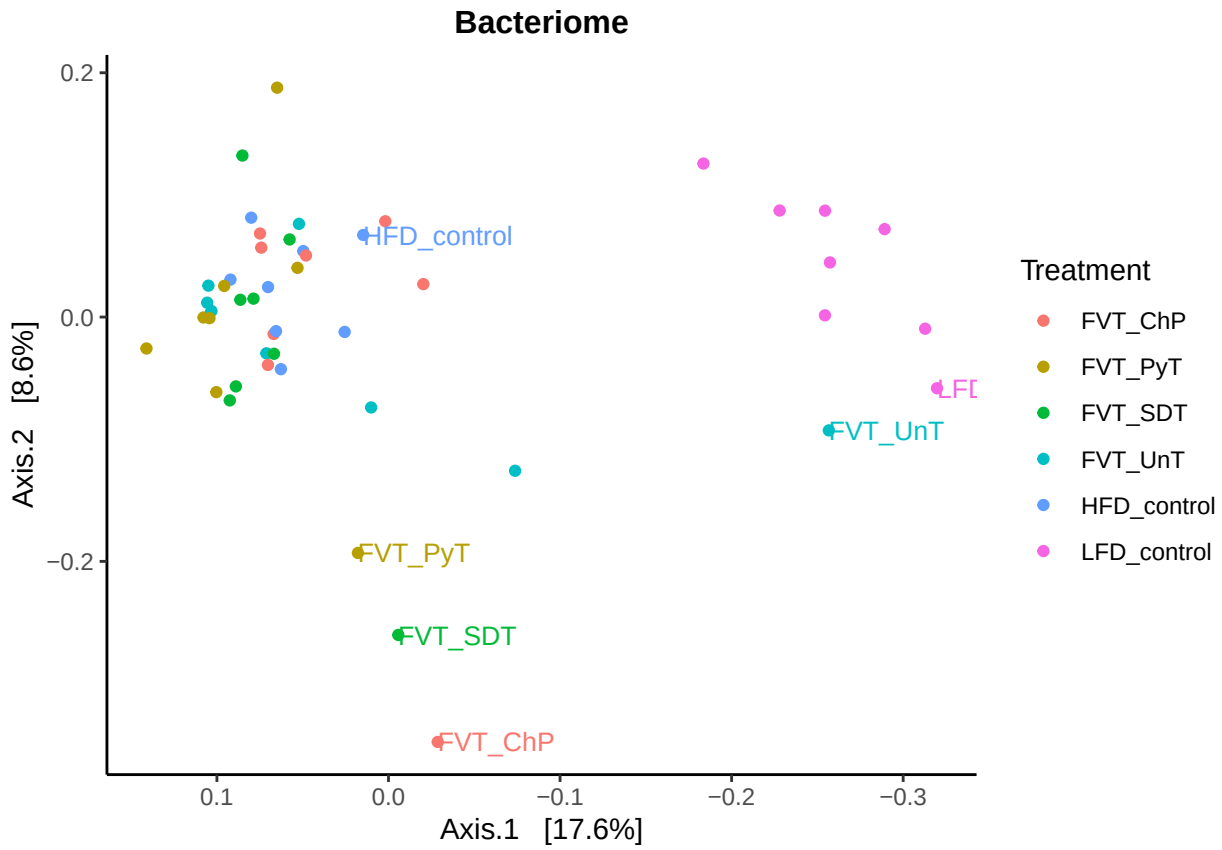
```
##
## Call:
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.90892 -0.15170 -0.00999  0.13088  0.93332
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)          3.90913    0.10754  36.350  < 2e-16 ***
## variableLFD_control  0.47273    0.15209   3.108  0.00337 **
## variableFVT_ChP     -0.11251    0.15209  -0.740  0.46353
## variableFVT_PyT     -0.07897    0.15209  -0.519  0.60632
## variableFVT_SDT     -0.12742    0.15209  -0.838  0.40686
## variableFVT_UnT     -0.13012    0.15209  -0.856  0.39709
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3042 on 42 degrees of freedom
## Multiple R-squared:  0.3619, Adjusted R-squared:  0.2859
## F-statistic: 4.763 on 5 and 42 DF,  p-value: 0.001546
```

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

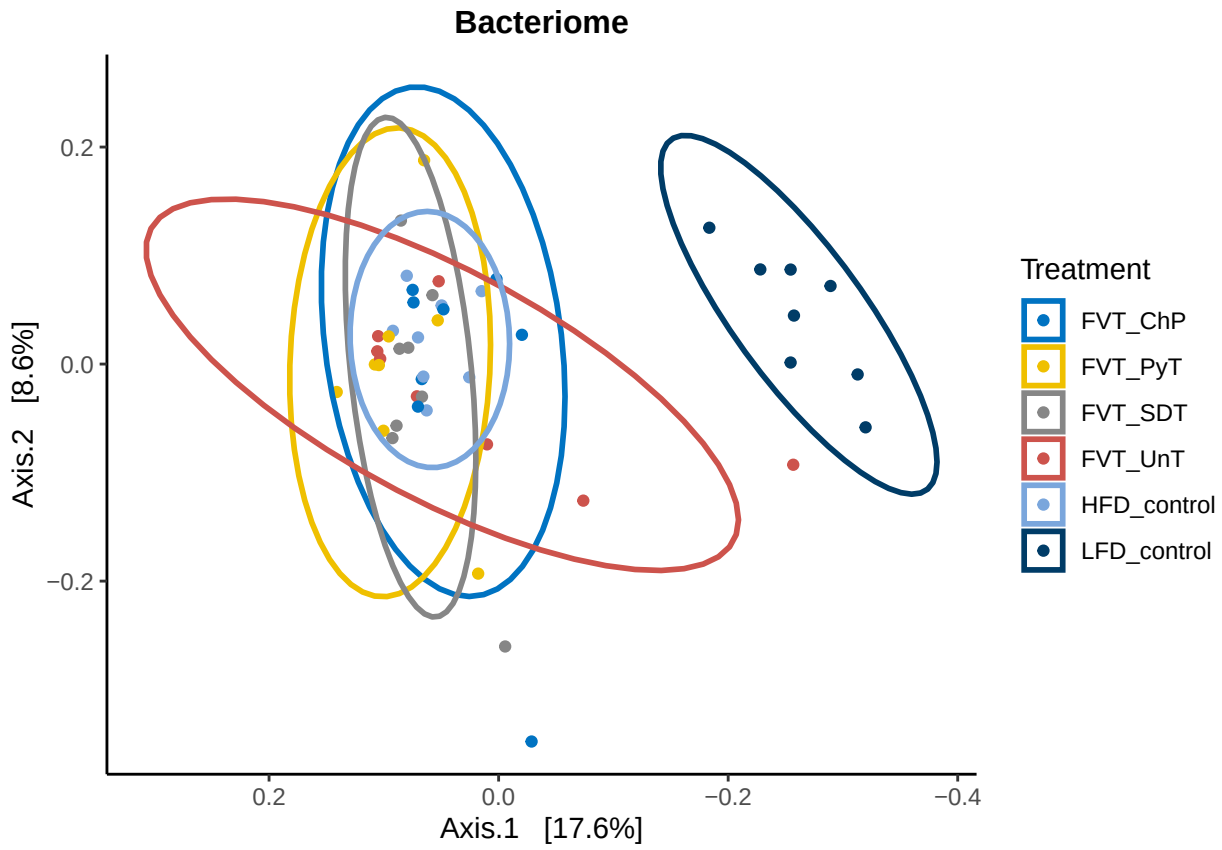
```
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.90892 -0.15170 -0.00999  0.13088  0.93332
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         3.779015   0.107540  35.140  < 2e-16 ***
## variableHFD_control 0.130119   0.152085   0.856 0.397094
## variableLFD_control 0.602849   0.152085   3.964 0.000281 ***
## variableFVT_ChP     0.017605   0.152085   0.116 0.908395
## variableFVT_PyT     0.051149   0.152085   0.336 0.738305
## variableFVT_SDT     0.002696   0.152085   0.018 0.985941
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3042 on 42 degrees of freedom
## Multiple R-squared:  0.3619, Adjusted R-squared:  0.2859
## F-statistic: 4.763 on 5 and 42 DF,  p-value: 0.001546
```

Beta diversity

Abbreviation - basically the mouse ID.



Treatment



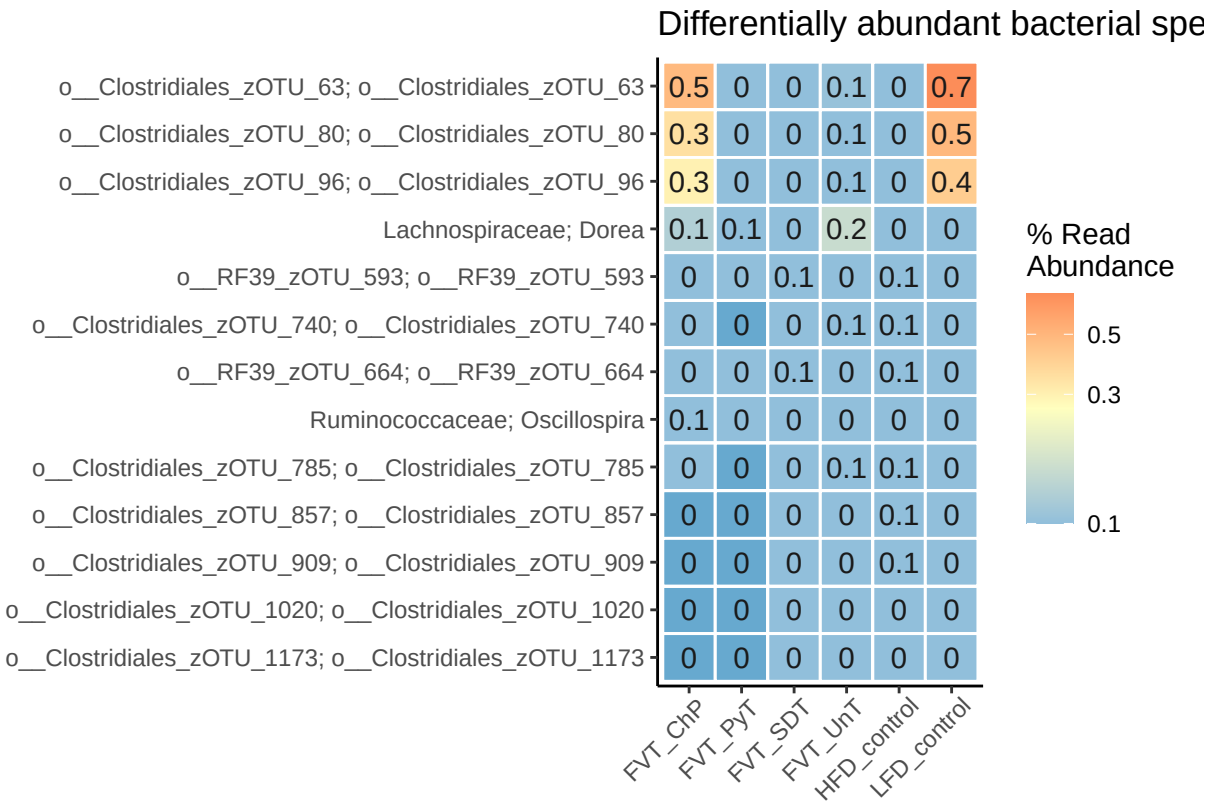
Adonis and pairwise permanova analysis - Treatment

```
## 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   1.2014 0.24942 2.7913  0.001 ***
## Residual  42   3.6155 0.75058
## Total     47   4.8169 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

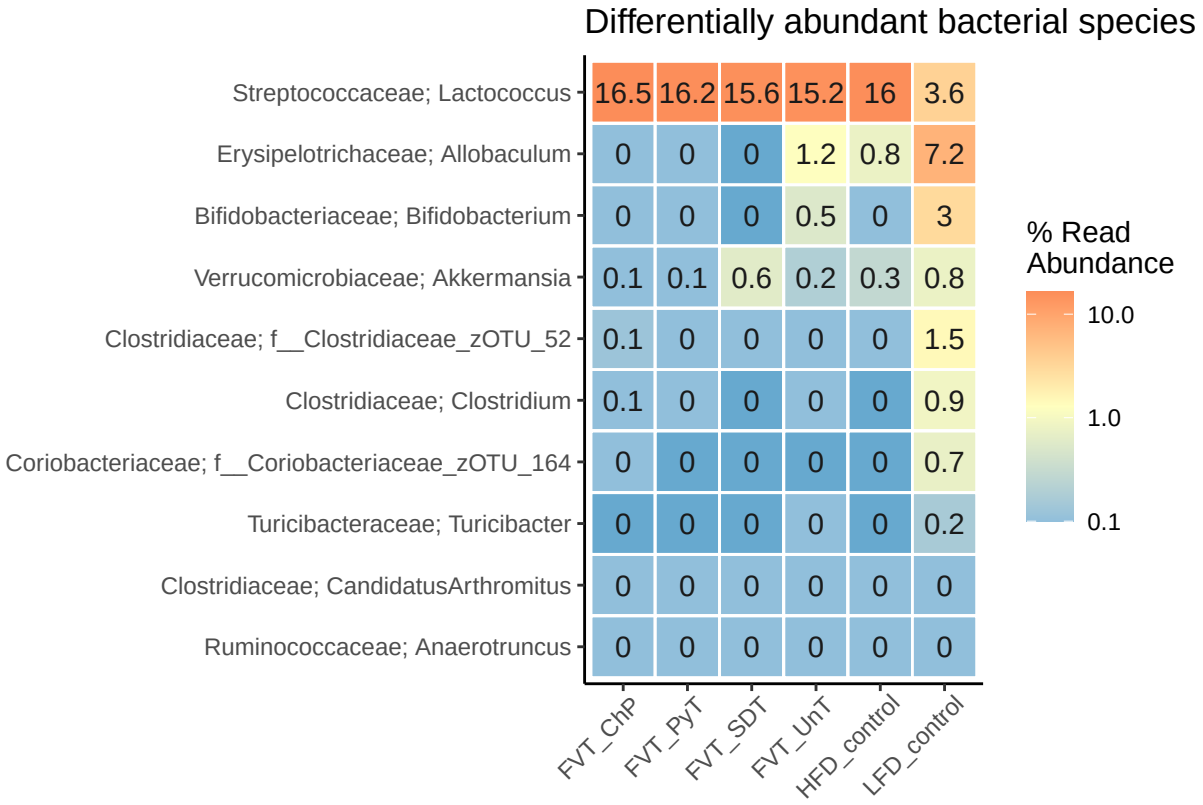
```
##             X1          X2         R2  pval pvalBon pvalFDR
## 1      FVT_ChP     FVT_PyT 0.10441535 0.017   0.255   0.026
## 2      FVT_ChP     FVT_SDT 0.08904713 0.098   1.470   0.105
## 3      FVT_ChP     FVT_UnT 0.08792072 0.078   1.170   0.098
## 4      FVT_ChP HFD_control 0.08677849 0.055   0.825   0.075
## 5      FVT_ChP LFD_control 0.24975438 0.001   0.015   0.005
## 6      FVT_PyT     FVT_SDT 0.07982791 0.155   2.325   0.155
## 7      FVT_PyT     FVT_UnT 0.10039254 0.015   0.225   0.025
## 8      FVT_PyT HFD_control 0.10543039 0.012   0.180   0.026
```

```
## 9      FVT_PyT LFD_control 0.29998741 0.001   0.015   0.005
## 10     FVT_SDT     FVT_UnT 0.10495964 0.014   0.210   0.026
## 11     FVT_SDT HFD_control 0.08683750 0.090   1.350   0.104
## 12     FVT_SDT LFD_control 0.29211684 0.001   0.015   0.005
## 13     FVT_UnT HFD_control 0.11127368 0.005   0.075   0.013
## 14     FVT_UnT LFD_control 0.22943373 0.001   0.015   0.005
## 15 HFD_control LFD_control 0.28610080 0.001   0.015   0.005
```

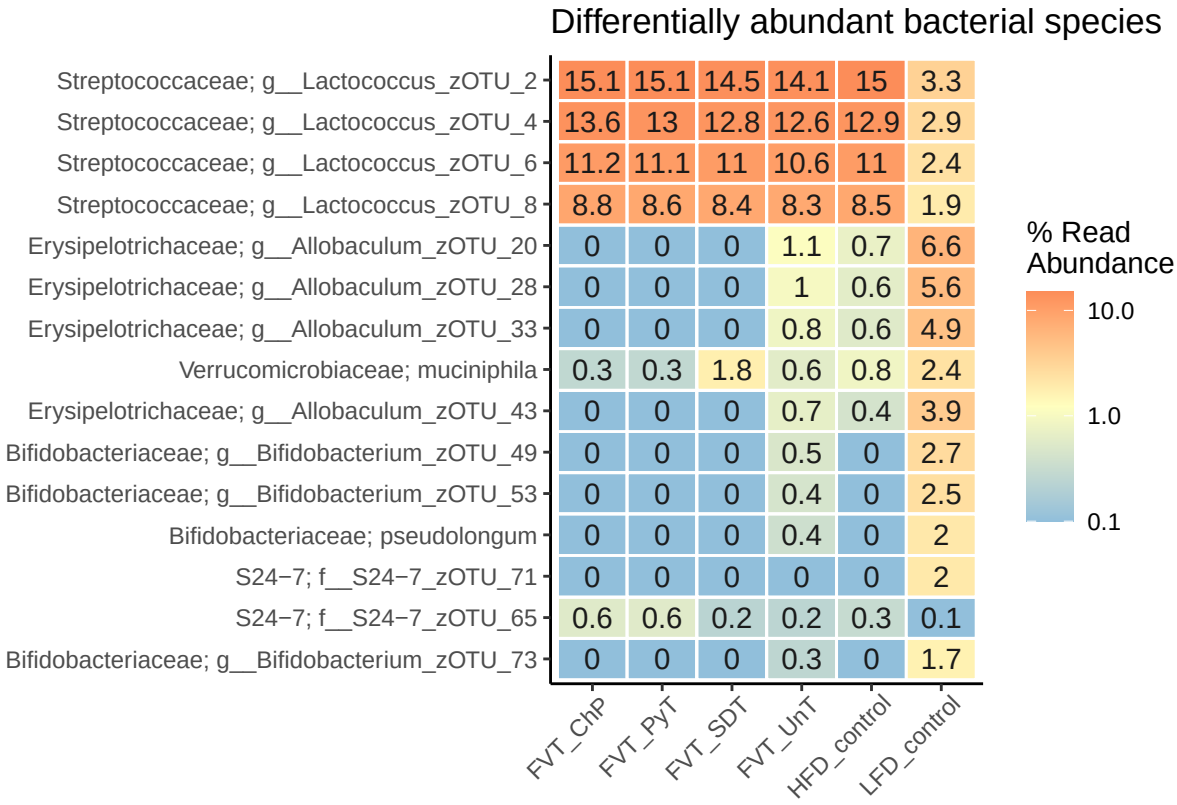
Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level - Comparing ChP vs HFD

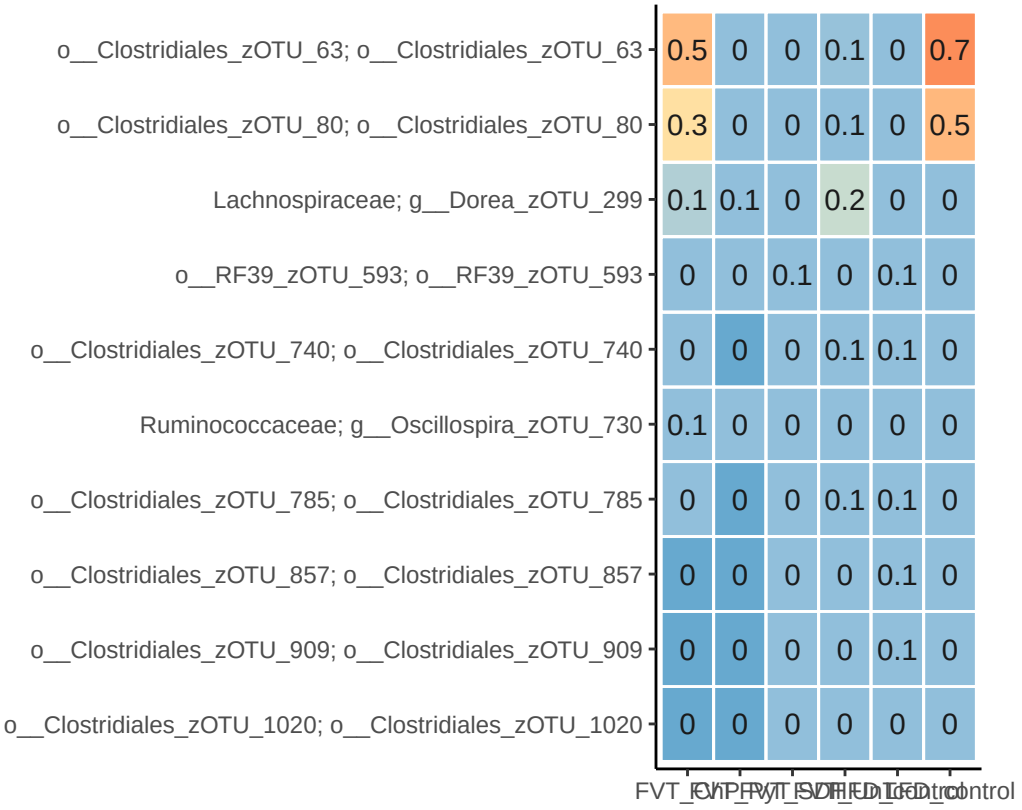


Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level



Bacteriome - Deseq2 - Treatment



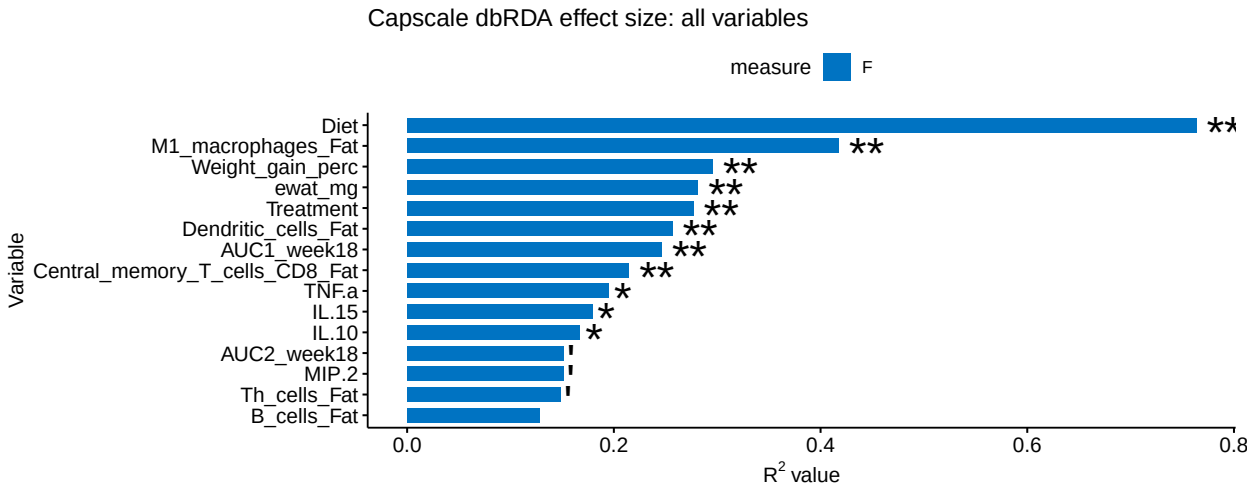


Deseq2 - defined comparison

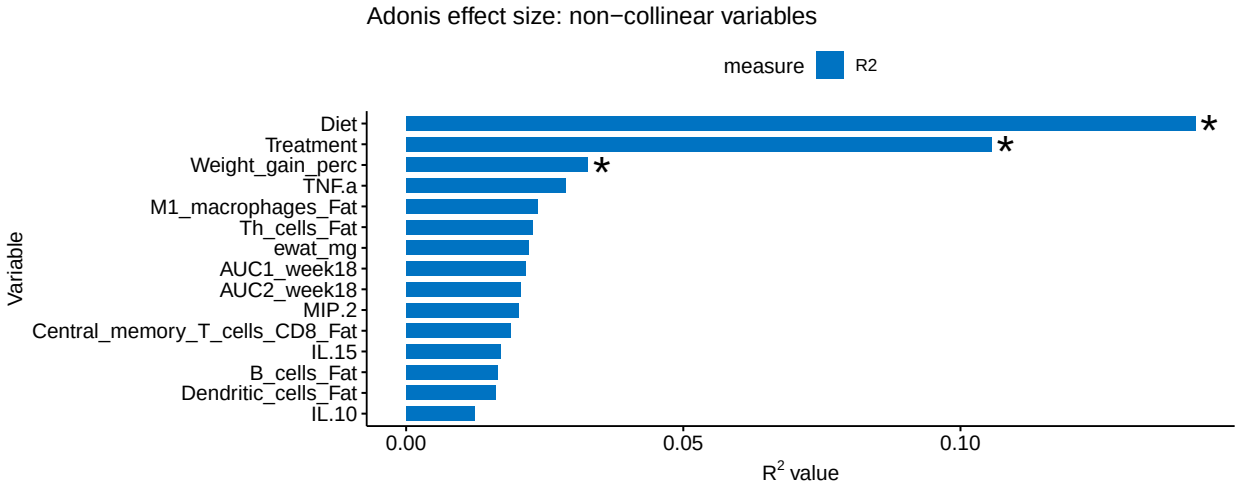
Bacteriome - Effect-size

Non-constrained

Capscale - independent effect sizes

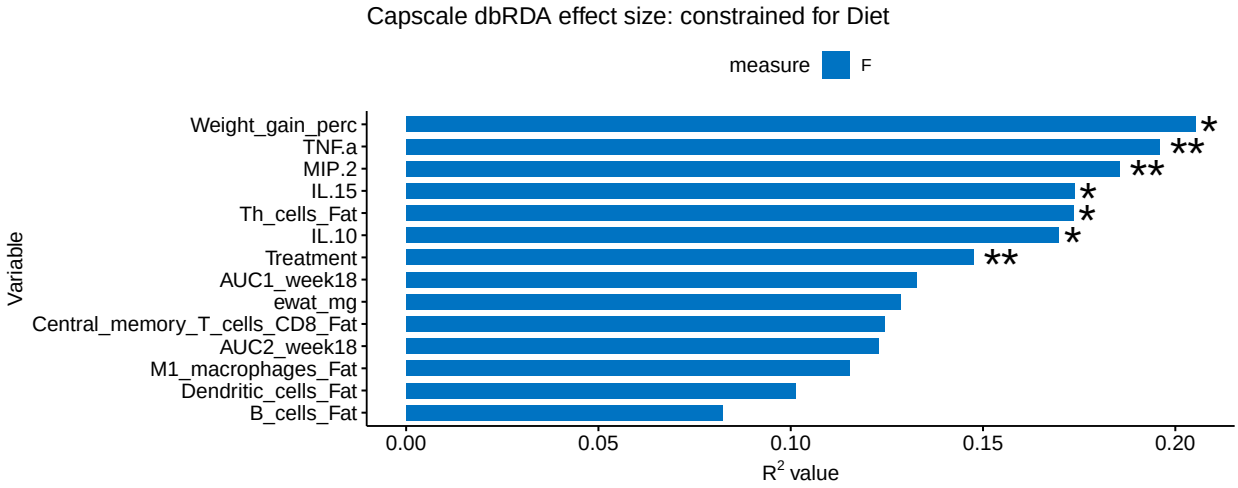


ADONIS - decomposed to show individual contributions of factors

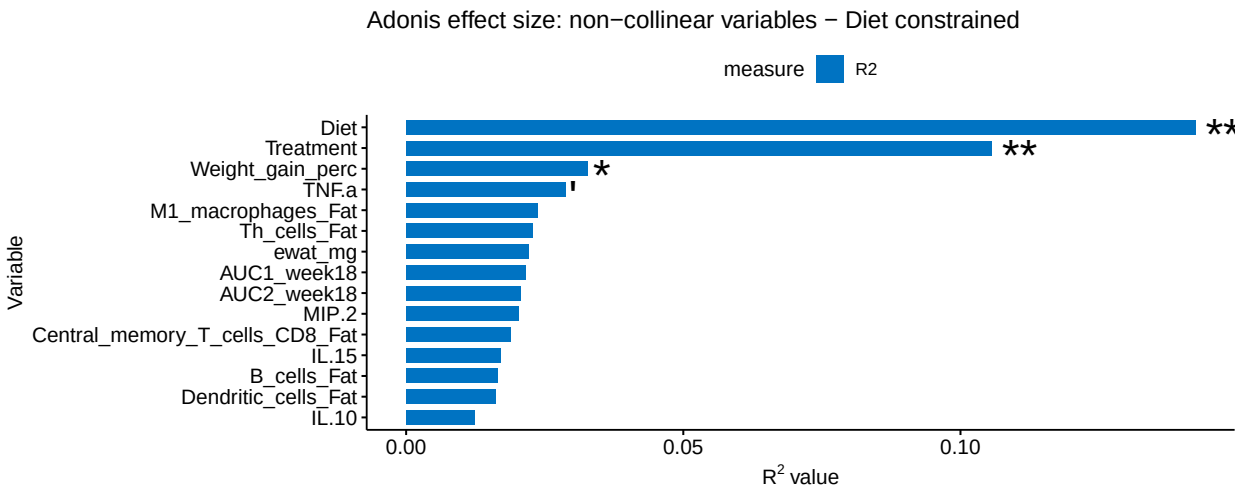


Constrained by Diet

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
Diet
Treatment
Weight_gain_perc
TNF.a
M1_macrophages_Fat
Th_cells_Fat
ewat_mg
AUC1_week18
AUC2_week18
MIP.2
Central_memory_T_cells_CD8_Fat
IL.15
B_cells_Fat
Dendritic_cells_Fat
IL.10
Variable
0.00
0.05
0.10
R2 value