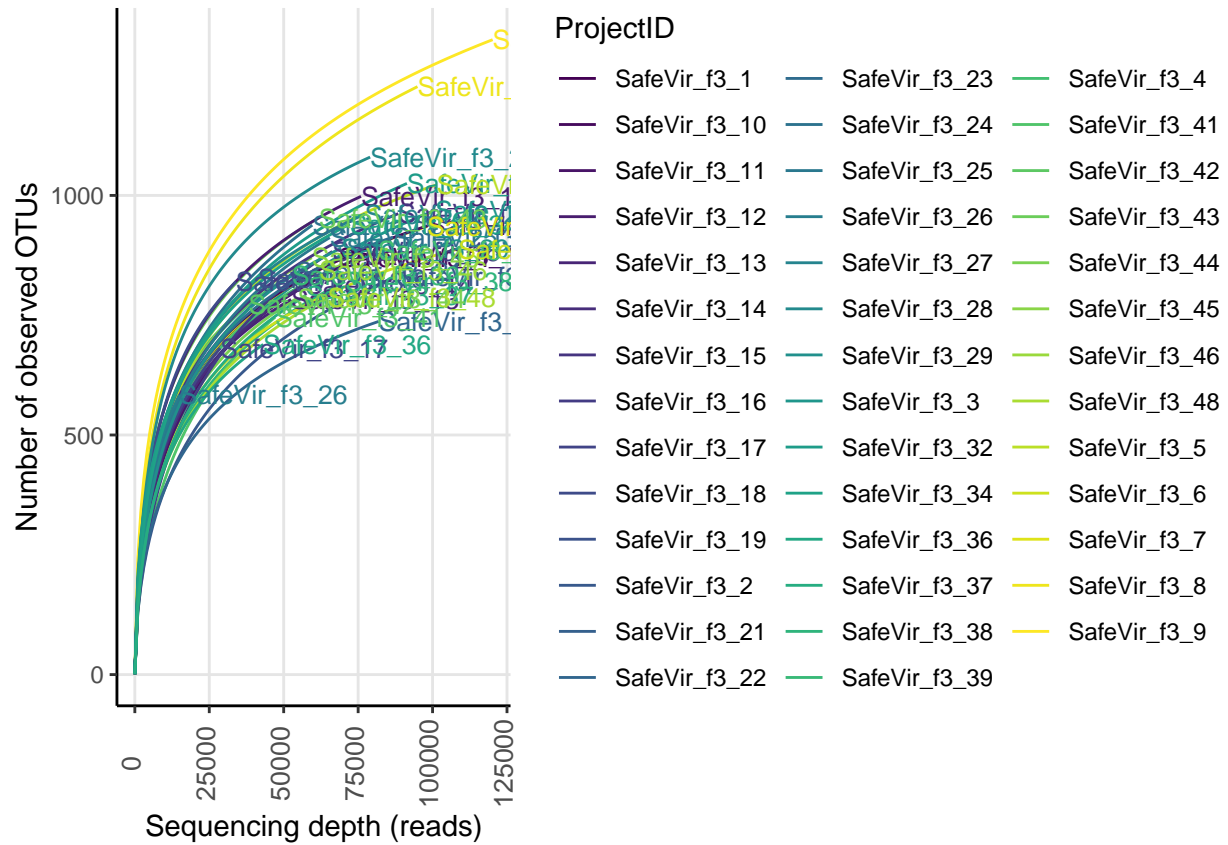
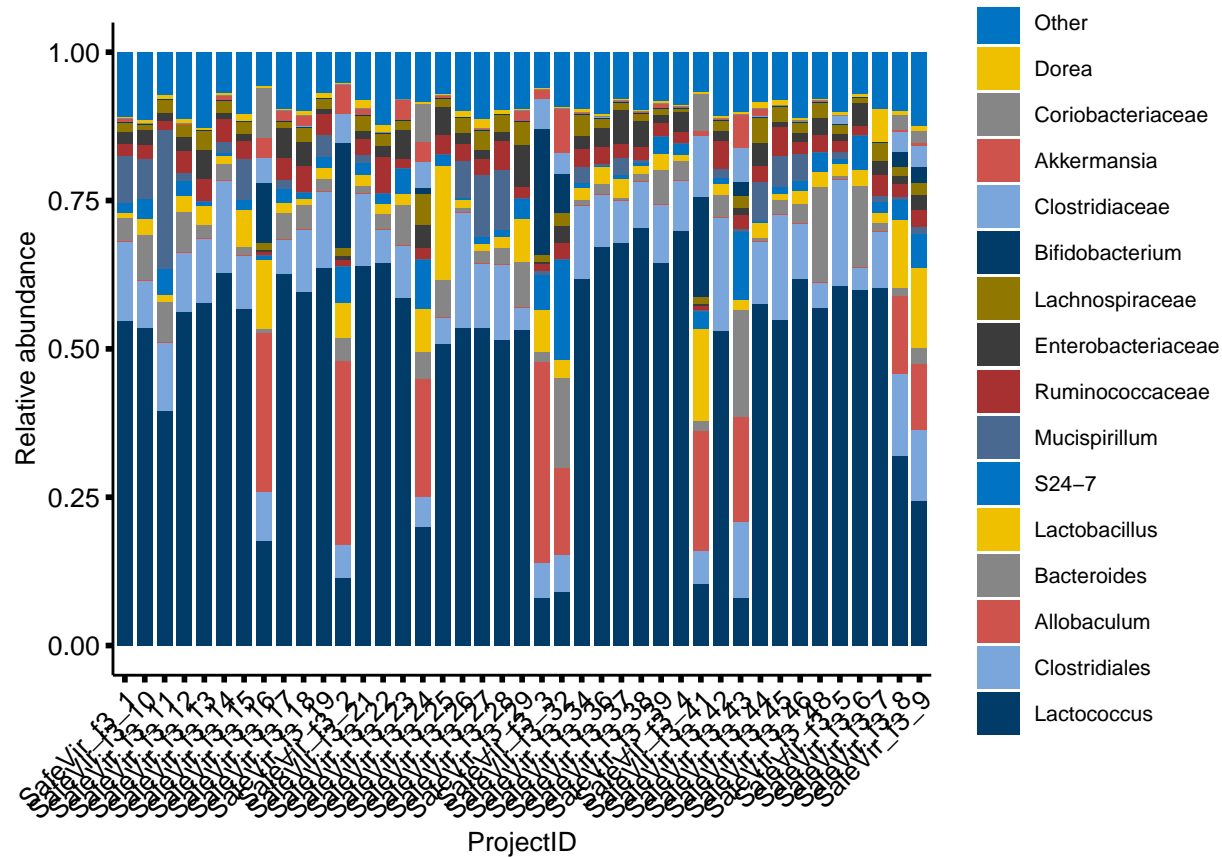


# 16S rRNA gene amplicon analysis - SafeVir - DIO - 1w\_after\_FVT

## Sequencing depth

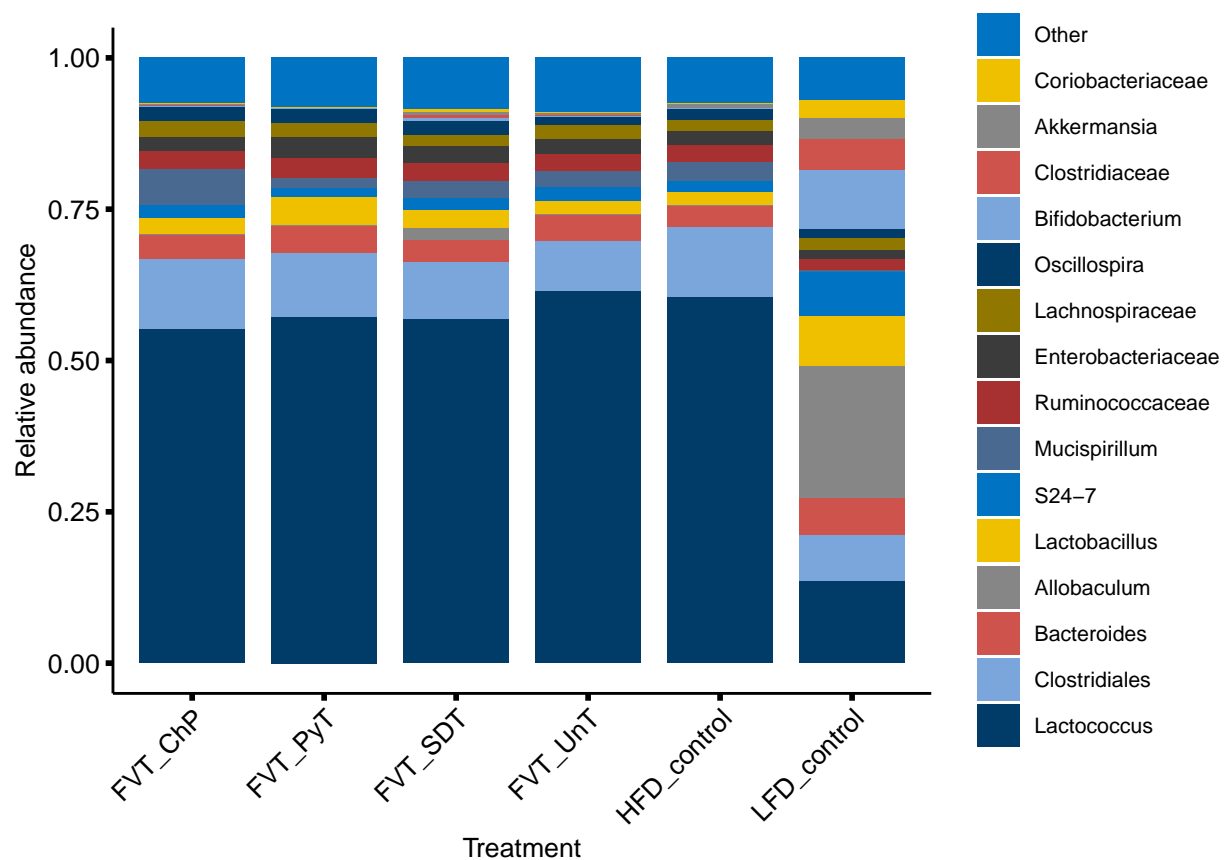


## Individual sample barplots.



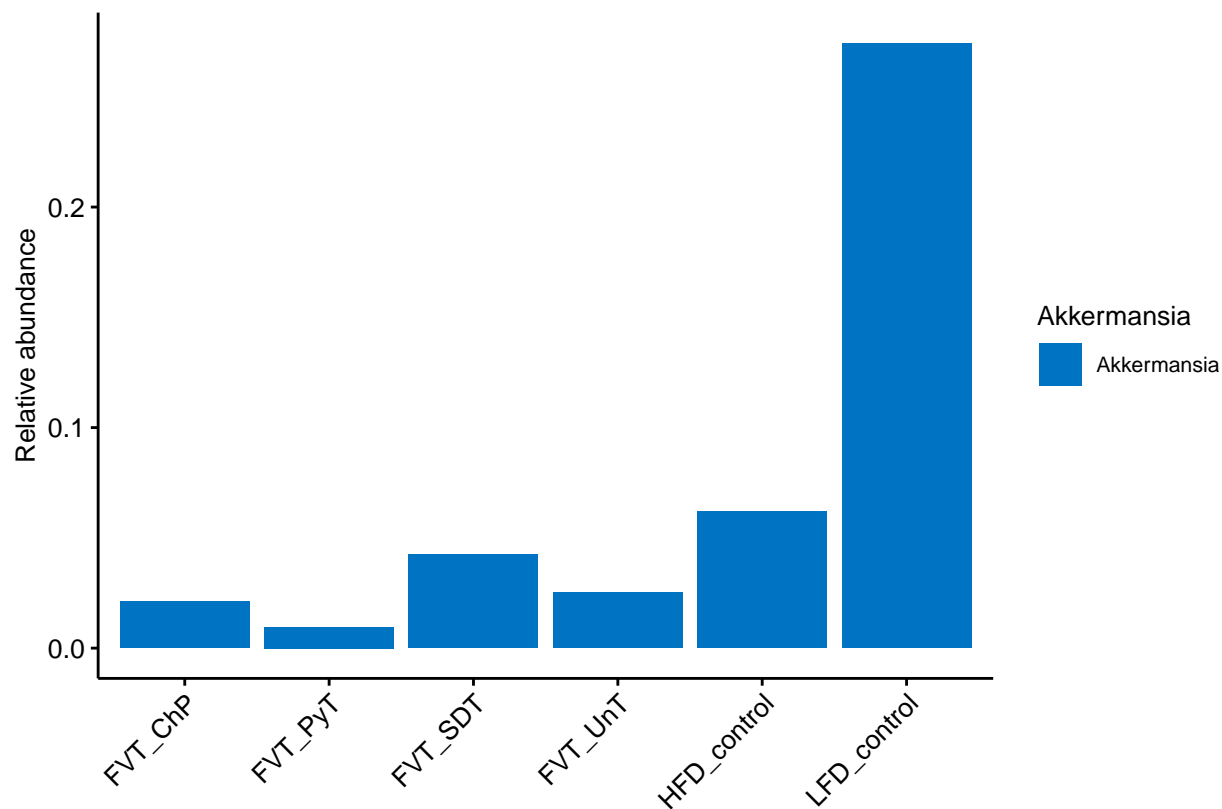
## Barplots based on treatments.

```
## # A tibble: 690 x 3
## # Groups:   Sample [6]
##   Sample      tax      Mean
##   <chr>      <chr>    <dbl>
## 1 FVT_UnT    Lactococcus 0.616
## 2 HFD_control Lactococcus 0.604
## 3 FVT_PyT    Lactococcus 0.572
## 4 FVT_SDT    Lactococcus 0.569
## 5 FVT_ChP    Lactococcus 0.553
## 6 LFD_control Allobaculum 0.219
## 7 LFD_control Lactococcus 0.136
## 8 HFD_control Clostridiales 0.117
## 9 FVT_ChP    Clostridiales 0.115
## 10 FVT_PyT    Clostridiales 0.107
## # ... with 680 more rows
```



```
## # A tibble: 41 x 3
## # Groups:   Sample [41]
##   Sample      tax      Mean
##   <chr>      <chr>    <dbl>
## 1 NXT075Mao94 Akkermansia 0.0761
## 2 NXT075Mao104 Akkermansia 0.0558
## 3 NXT075Mao66 Akkermansia 0.0505
## 4 NXT075Mao86 Akkermansia 0.0347
## 5 NXT075Mao80 Akkermansia 0.0328
## 6 NXT075Mao87 Akkermansia 0.0323
## 7 NXT075Mao82 Akkermansia 0.0185
## 8 NXT075Mao92 Akkermansia 0.0175
## 9 NXT075Mao81 Akkermansia 0.0164
## 10 NXT075Mao67 Akkermansia 0.0145
## # ... with 31 more rows

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

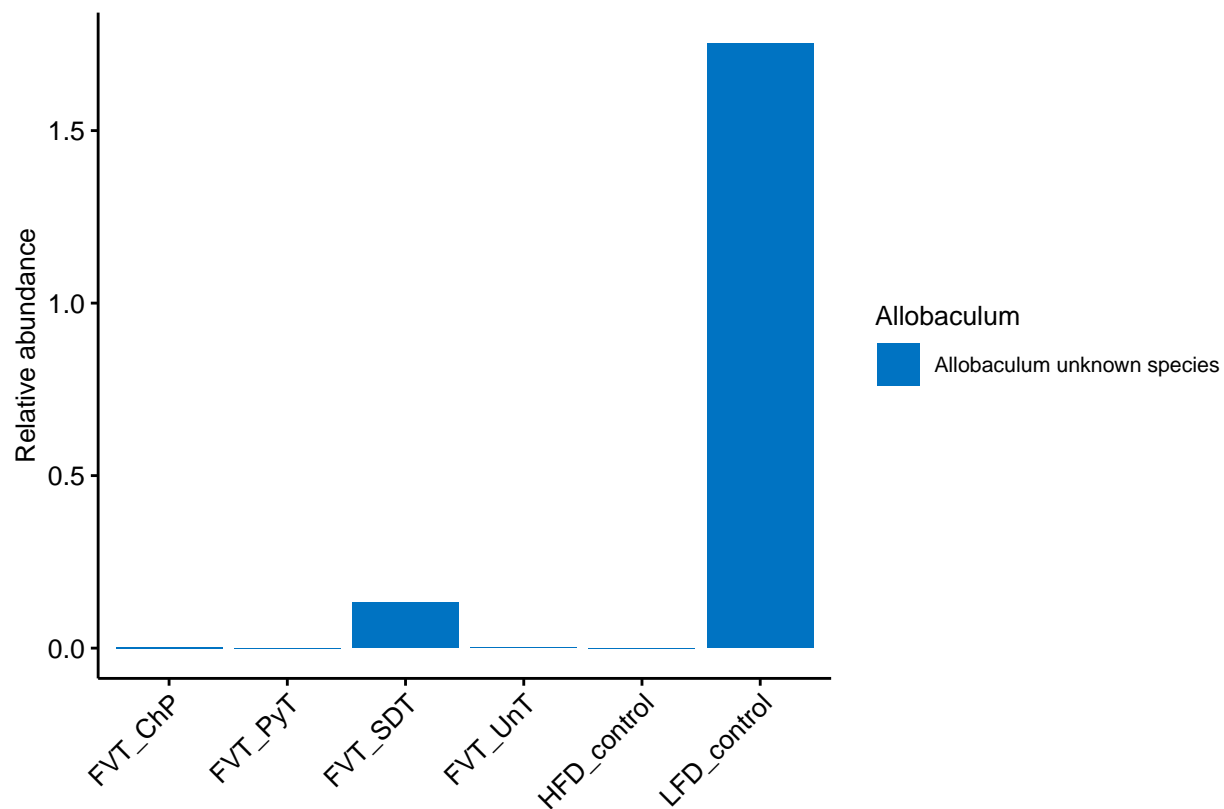


```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.029997 -0.004345 -0.001500  0.001307  0.041799
##
## Coefficients:
##              Estimate Std. Error t value    Pr(>|t|)
## (Intercept)    0.034307   0.004661   7.360 0.0000000132 ***
## TreatmentFVT_ChP -0.031266   0.006823  -4.582 0.0000562499 ***
## TreatmentFVT_PyT -0.032972   0.006823  -4.832 0.0000265902 ***
## TreatmentFVT_SDT -0.028212   0.006823  -4.135   0.000211 ***
## TreatmentFVT_UnT -0.029249   0.007516  -3.892   0.000427 ***
## TreatmentHFD_control -0.025476   0.006823  -3.734   0.000669 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01318 on 35 degrees of freedom
## Multiple R-squared:  0.4887, Adjusted R-squared:  0.4157
## F-statistic: 6.691 on 5 and 35 DF, p-value: 0.0001808
##
##
## Call:
```

```
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.029997 -0.004345 -0.001500  0.001307  0.041799
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.008831   0.004983   1.772 0.085061 .
## TreatmentLFD_control 0.025476   0.006823   3.734 0.000669 ***
## TreatmentFVT_ChP    -0.005790   0.007047  -0.822 0.416843
## TreatmentFVT_PyT    -0.007496   0.007047  -1.064 0.294742
## TreatmentFVT_SDT    -0.002736   0.007047  -0.388 0.700228
## TreatmentFVT_UnT    -0.003773   0.007719  -0.489 0.628090
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01318 on 35 degrees of freedom
## Multiple R-squared:  0.4887, Adjusted R-squared:  0.4157
## F-statistic: 6.691 on 5 and 35 DF,  p-value: 0.0001808

## # A tibble: 41 x 3
## # Groups:   Sample [41]
##   Sample      tax      Mean
##   <chr>      <chr>    <dbl>
## 1 NXT075Mao67 Allobaculum unknown species 0.339
## 2 NXT075Mao66 Allobaculum unknown species 0.312
## 3 NXT075Mao80 Allobaculum unknown species 0.267
## 4 NXT075Mao102 Allobaculum unknown species 0.203
## 5 NXT075Mao87 Allobaculum unknown species 0.200
## 6 NXT075Mao104 Allobaculum unknown species 0.178
## 7 NXT075Mao94 Allobaculum unknown species 0.145
## 8 NXT075Mao72 Allobaculum unknown species 0.133
## 9 NXT075Mao73 Allobaculum unknown species 0.111
## 10 NXT075Mao105 Allobaculum unknown species 0.00146
## # ... with 31 more rows

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

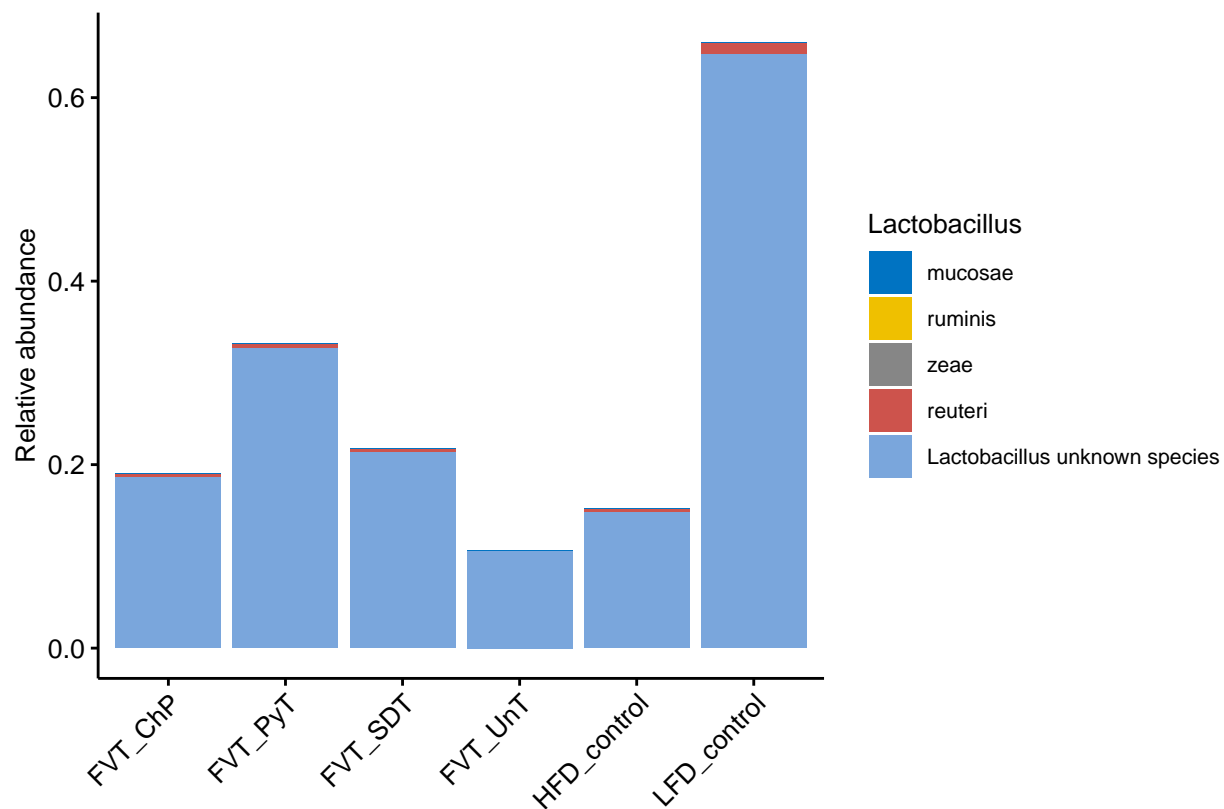


```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.108285 -0.016308 -0.000077  0.000000  0.119296
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.21929    0.01461   15.008 < 2e-16 ***
## TreatmentFVT_ChP -0.21919    0.02139  -10.248 4.45e-12 ***
## TreatmentFVT_PyT -0.21929    0.02139  -10.252 4.40e-12 ***
## TreatmentFVT_SDT -0.20028    0.02139   -9.363 4.61e-11 ***
## TreatmentFVT_UnT -0.21900    0.02356   -9.295 5.54e-11 ***
## TreatmentHFD_control -0.21928    0.02139  -10.252 4.40e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04133 on 35 degrees of freedom
## Multiple R-squared:  0.8339, Adjusted R-squared:  0.8102
## F-statistic: 35.14 on 5 and 35 DF,  p-value: 1.073e-12
##
##
## Call:
```

```
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.108285 -0.016308 -0.000077  0.000000  0.119296
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.000008235  0.015620864   0.001   1.000
## TreatmentLFD_control  0.219284420  0.021389749  10.252 4.4e-12 ***
## TreatmentFVT_ChP    0.000092533  0.022091238   0.004   0.997
## TreatmentFVT_PyT   -0.000008235  0.022091238   0.000   1.000
## TreatmentFVT_SDT    0.019004471  0.022091238   0.860   0.395
## TreatmentFVT_UnT    0.000287814  0.024199739   0.012   0.991
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04133 on 35 degrees of freedom
## Multiple R-squared:  0.8339, Adjusted R-squared:  0.8102
## F-statistic: 35.14 on 5 and 35 DF,  p-value: 1.073e-12

## # A tibble: 205 x 3
## # Groups:   Sample [41]
##   Sample      tax      Mean
##   <chr>      <chr>      <dbl>
## 1 NXT075Mao88 Lactobacillus unknown species 0.190
## 2 NXT075Mao102 Lactobacillus unknown species 0.154
## 3 NXT075Mao73 Lactobacillus unknown species 0.131
## 4 NXT075Mao80 Lactobacillus unknown species 0.115
## 5 NXT075Mao72 Lactobacillus unknown species 0.113
## 6 NXT075Mao87 Lactobacillus unknown species 0.0715
## 7 NXT075Mao92 Lactobacillus unknown species 0.0713
## 8 NXT075Mao67 Lactobacillus unknown species 0.0711
## 9 NXT075Mao79 Lactobacillus unknown species 0.0592
## 10 NXT075Mao66 Lactobacillus unknown species 0.0591
## # ... with 195 more rows

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



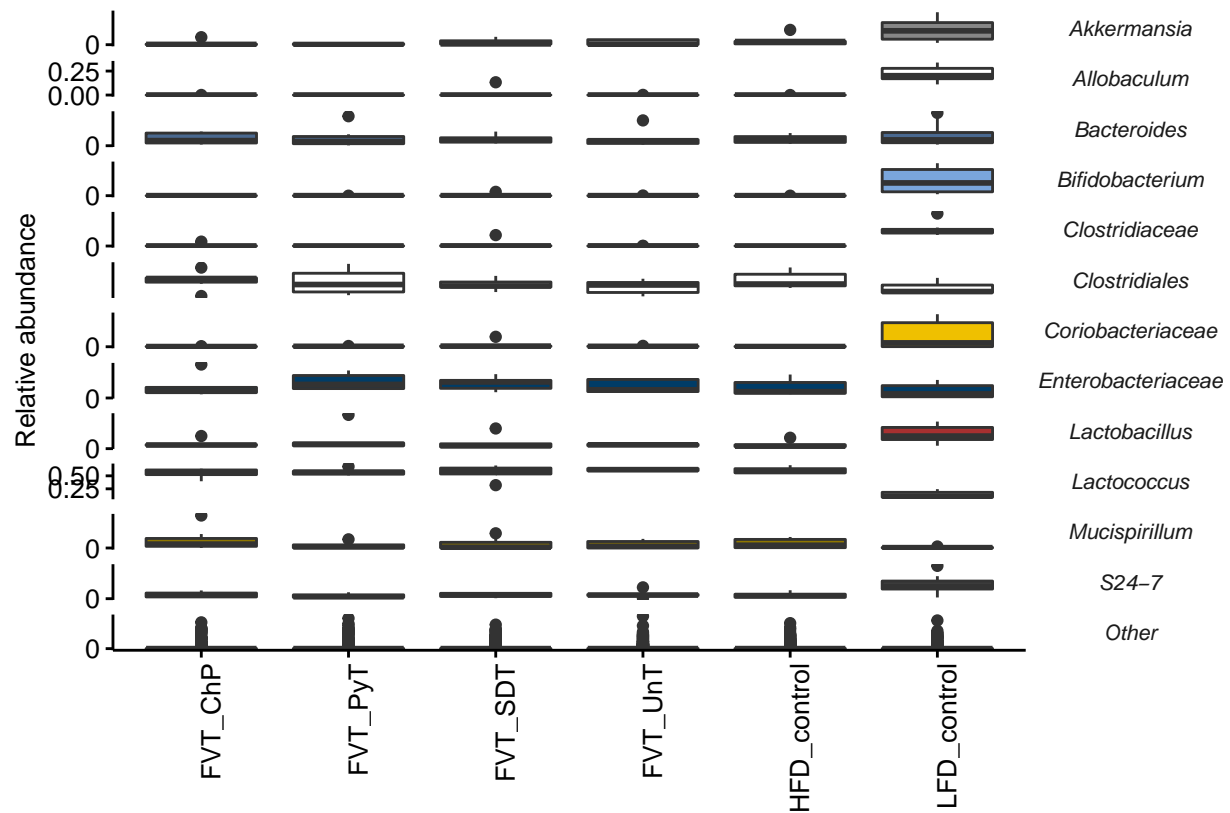
```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.016492 -0.009500 -0.005425 -0.004254  0.180160
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.016492   0.003893   4.236 0.0000347 ***
## TreatmentFVT_ChP -0.011068   0.005699  -1.942   0.0535 .
## TreatmentFVT_PyT -0.006992   0.005699  -1.227   0.2213
## TreatmentFVT_SDT -0.010272   0.005699  -1.802   0.0730 .
## TreatmentFVT_UnT -0.012238   0.006277  -1.950   0.0526 .
## TreatmentHFD_control -0.012146   0.005699  -2.131   0.0343 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02462 on 199 degrees of freedom
## Multiple R-squared:  0.03305,    Adjusted R-squared:  0.008752
## F-statistic: 1.36 on 5 and 199 DF,  p-value: 0.2409
##
##
## Call:
```



```
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.016492 -0.009500 -0.005425 -0.004254  0.180160
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.00434643  0.00416173   1.044   0.2976
## TreatmentLFD_control 0.01214600  0.00569868   2.131   0.0343 *
## TreatmentFVT_ChP    0.00107815  0.00588557   0.183   0.8548
## TreatmentFVT_PyT    0.00515368  0.00588557   0.876   0.3823
## TreatmentFVT_SDT    0.00187430  0.00588557   0.318   0.7505
## TreatmentFVT_UnT    -0.00009223  0.00644732  -0.014   0.9886
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02462 on 199 degrees of freedom
## Multiple R-squared:  0.03305,    Adjusted R-squared:  0.008752
## F-statistic:  1.36 on 5 and 199 DF,  p-value: 0.2409

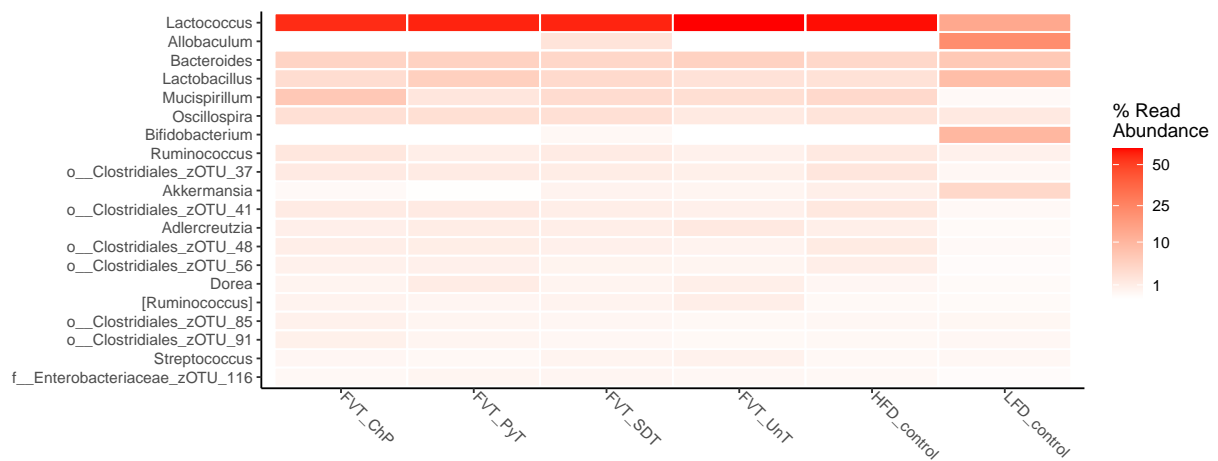
## # A tibble: 4,715 x 3
## # Groups:   Sample [41]
##   Sample      tax      Mean
##   <chr>      <chr>    <dbl>
## 1 NXT075Mao100 Lactococcus 0.705
## 2 NXT075Mao68  Lactococcus 0.699
## 3 NXT075Mao99  Lactococcus 0.678
## 4 NXT075Mao98  Lactococcus 0.672
## 5 NXT075Mao101 Lactococcus 0.646
## 6 NXT075Mao85  Lactococcus 0.645
## 7 NXT075Mao84  Lactococcus 0.639
## 8 NXT075Mao83  Lactococcus 0.637
## 9 NXT075Mao78  Lactococcus 0.629
## 10 NXT075Mao81 Lactococcus 0.626
## # ... with 4,705 more rows

## Warning: This manual palette can handle a maximum of 10 values. You have
## supplied 13.
```



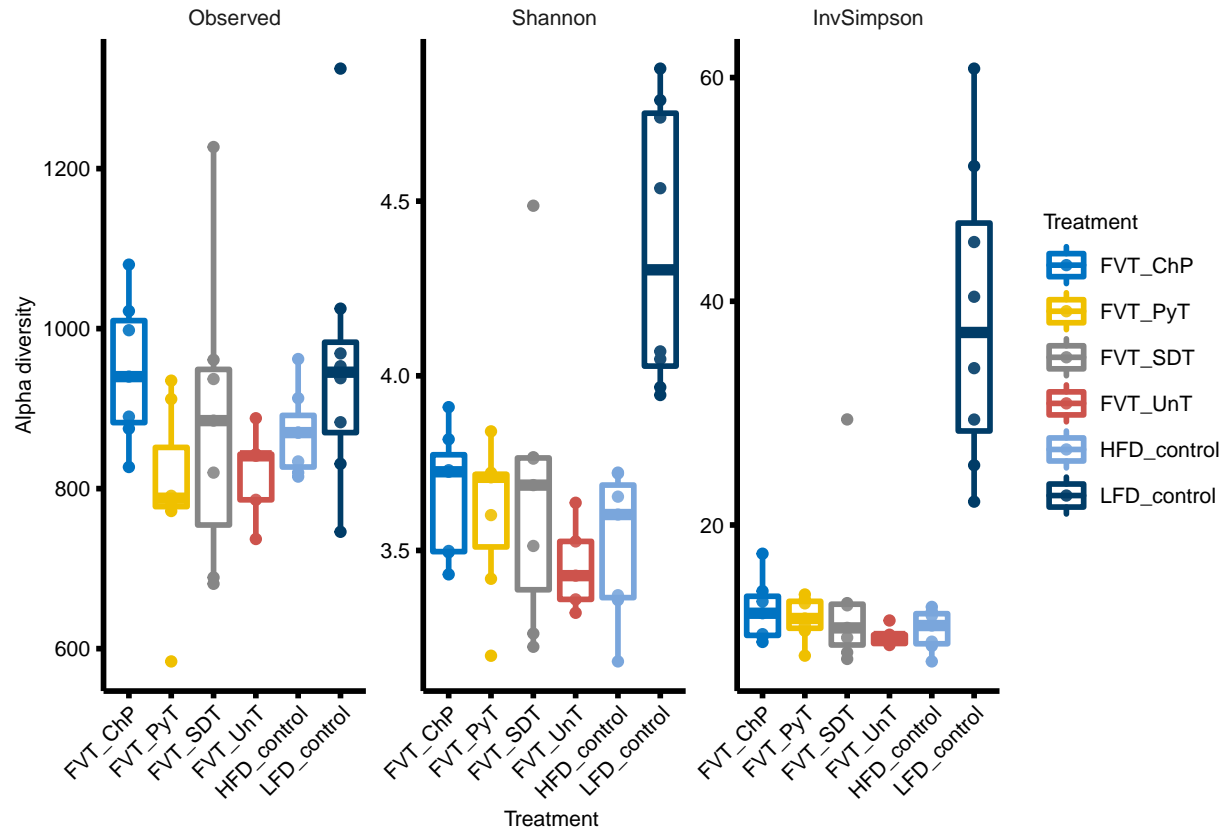
## Abundance heatmaps

### 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	-0.05769599	-0.5323215	0.4169295	0.9990592
FVT_SDT-FVT_ChP	0.01352473	-0.4611008	0.4881502	0.9999993
FVT_UnT-FVT_ChP	-0.20399863	-0.7239248	0.3159276	0.8421877
HFD_control-FVT_ChP	-0.14227439	-0.6168999	0.3323511	0.9430790
LFD_control-FVT_ChP	0.71354197	0.2539878	1.1730961	0.0005623
FVT_SDT-FVT_PyT	0.07122072	-0.4034048	0.5458462	0.9974190
FVT_UnT-FVT_PyT	-0.14630265	-0.6662288	0.3736236	0.9561015
HFD_control-FVT_PyT	-0.08457840	-0.5592039	0.3900471	0.9942114
LFD_control-FVT_PyT	0.77123796	0.3116838	1.2307921	0.0001842
FVT_UnT-FVT_SDT	-0.21752336	-0.7374496	0.3024028	0.8037269
HFD_control-FVT_SDT	-0.15579912	-0.6304246	0.3188264	0.9183455
LFD_control-FVT_SDT	0.70001724	0.2404631	1.1595714	0.0007283
HFD_control-FVT_UnT	0.06172424	-0.4582020	0.5816504	0.9991610

```
## LFD_control-FVT_UnT      0.91754060  0.4113352 1.4237460 0.0000550
## LFD_control-HFD_control  0.85581636  0.3962622 1.3153705 0.0000351
```

```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.016492 -0.009500 -0.005425 -0.004254  0.180160
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.00434643  0.00416173   1.044   0.2976
## TreatmentLFD_control  0.01214600  0.00569868   2.131   0.0343 *
## TreatmentFVT_ChP    0.00107815  0.00588557   0.183   0.8548
## TreatmentFVT_PyT    0.00515368  0.00588557   0.876   0.3823
## TreatmentFVT_SDT    0.00187430  0.00588557   0.318   0.7505
## TreatmentFVT_UnT   -0.00009223  0.00644732  -0.014   0.9886
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02462 on 199 degrees of freedom
## Multiple R-squared:  0.03305, Adjusted R-squared:  0.008752
## F-statistic:  1.36 on 5 and 199 DF, p-value: 0.2409
```

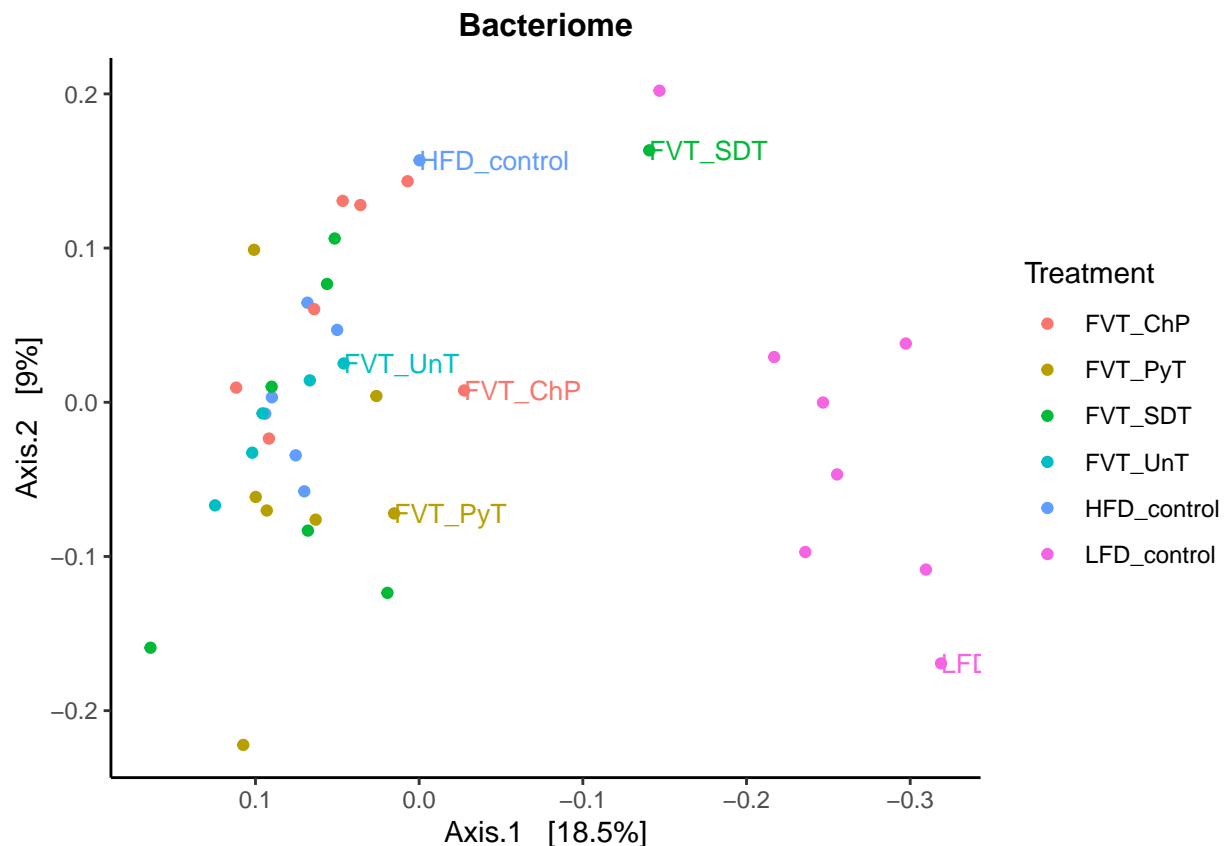
```
##
## Call:
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.4476 -0.1636  0.0669  0.1599  0.8150
##
## Coefficients:
##              Estimate Std. Error t value    Pr(>|t|)
## (Intercept)    3.51605    0.11138  31.569 < 2e-16 ***
## variableLFD_control  0.85582    0.15251   5.612 0.00000251 ***
## variableFVT_ChP    0.14227    0.15751   0.903    0.373
## variableFVT_PyT    0.08458    0.15751   0.537    0.595
## variableFVT_SDT    0.15580    0.15751   0.989    0.329
## variableFVT_UnT   -0.06172    0.17255  -0.358    0.723
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2947 on 35 degrees of freedom
## Multiple R-squared:  0.5783, Adjusted R-squared:  0.5181
## F-statistic: 9.601 on 5 and 35 DF, p-value: 0.000007789
```

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

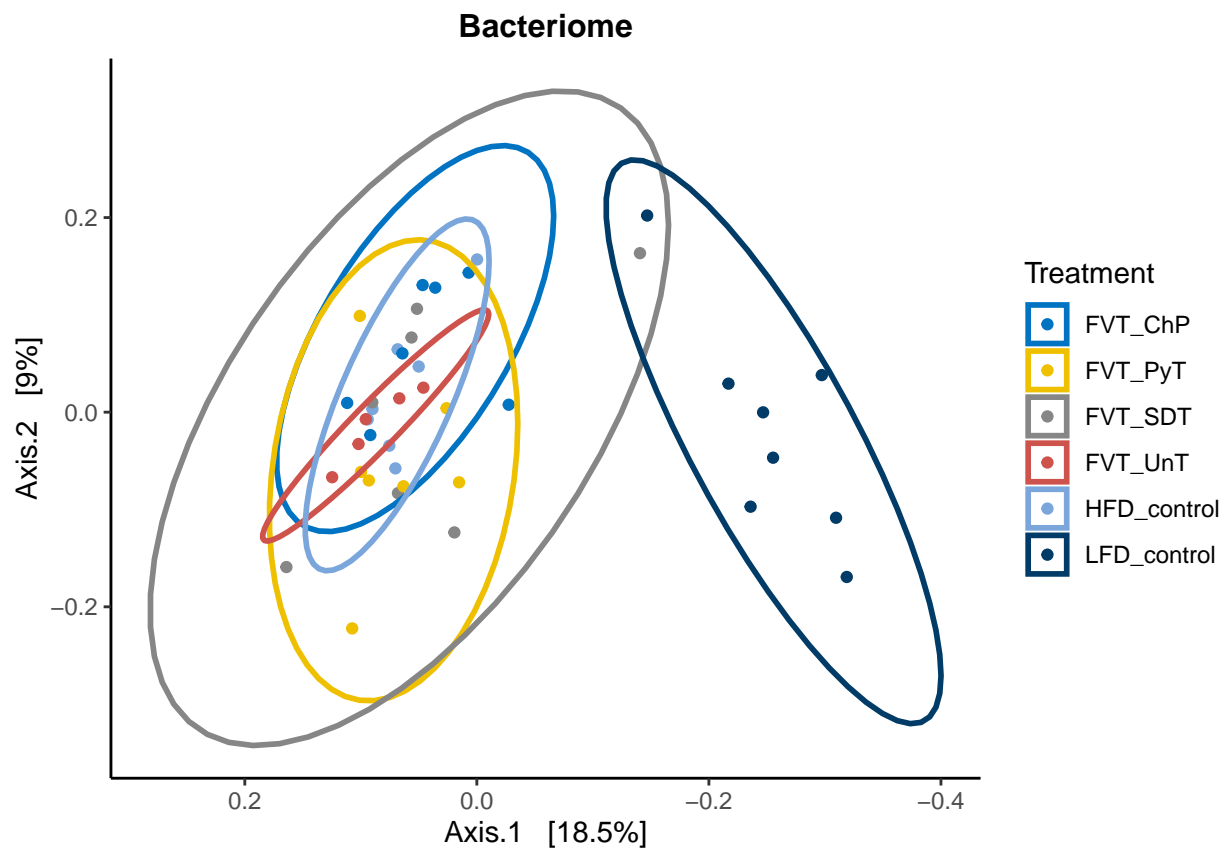
```
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.4476 -0.1636  0.0669  0.1599  0.8150
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    3.45433    0.13178  26.212  < 2e-16 ***
## variableHFD_control  0.06172    0.17255   0.358    0.723
## variableLFD_control  0.91754    0.16799   5.462 0.00000396 ***
## variableFVT_ChP      0.20400    0.17255   1.182    0.245
## variableFVT_PyT      0.14630    0.17255   0.848    0.402
## variableFVT_SDT      0.21752    0.17255   1.261    0.216
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2947 on 35 degrees of freedom
## Multiple R-squared:  0.5783, Adjusted R-squared:  0.5181
## F-statistic: 9.601 on 5 and 35 DF,  p-value: 0.000007789
```

## 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 = sampled.f.PSB, permutations = 999, method = "bray")
##      Df SumOfSqs      R2      F Pr(>F)
## Treatment  5   1.1387 0.27935 2.7135 0.001 ***
## Residual  35   2.9374 0.72065
## Total     40   4.0761 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

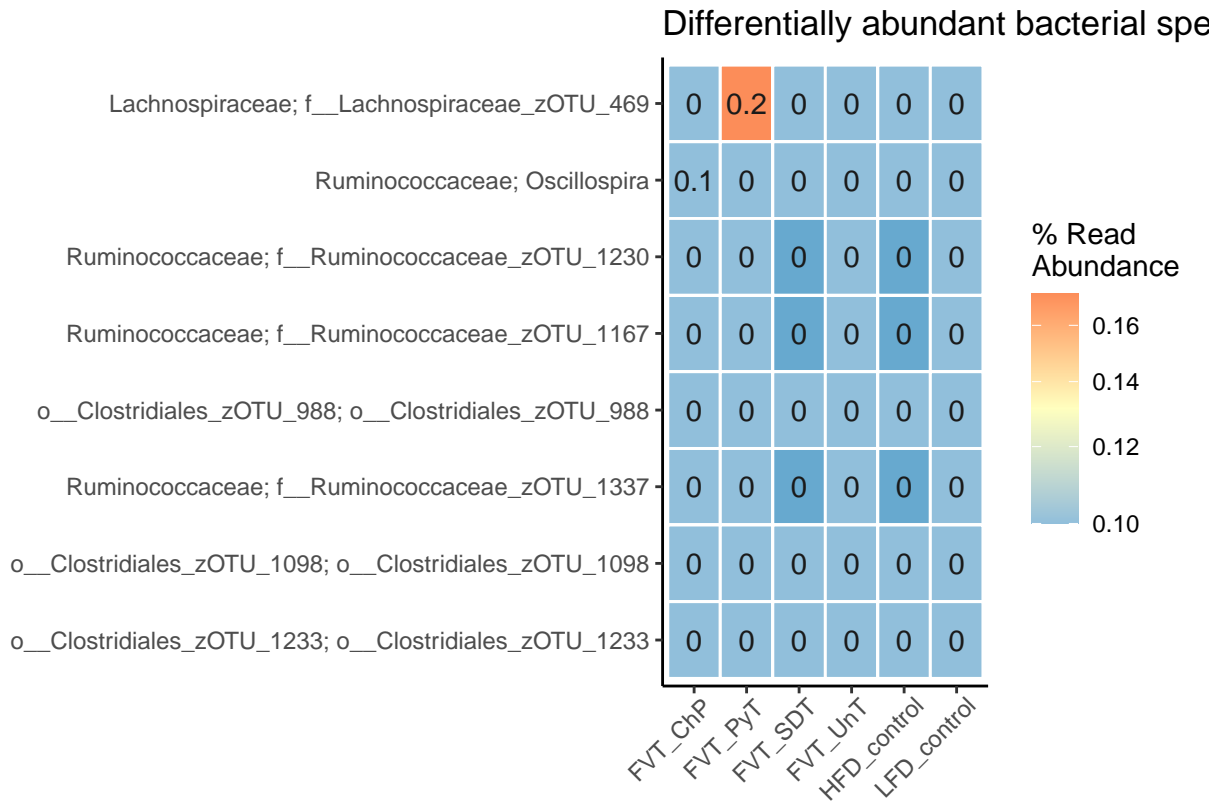
	X1	X2	R2	pval	pvalBon	pvalFDR
## 1	FVT_ChP	FVT_PyT	0.13056169	0.011	0.165	0.018
## 2	FVT_ChP	FVT_SDT	0.09464042	0.141	2.115	0.163
## 3	FVT_ChP	FVT_UnT	0.14384829	0.009	0.135	0.017
## 4	FVT_ChP	HFD_control	0.10282876	0.045	0.675	0.061
## 5	FVT_ChP	LFD_control	0.27103847	0.001	0.015	0.005
## 6	FVT_PyT	FVT_SDT	0.09431398	0.108	1.620	0.135
## 7	FVT_PyT	FVT_UnT	0.10747890	0.155	2.325	0.166
## 8	FVT_PyT	HFD_control	0.12589985	0.006	0.090	0.013

```

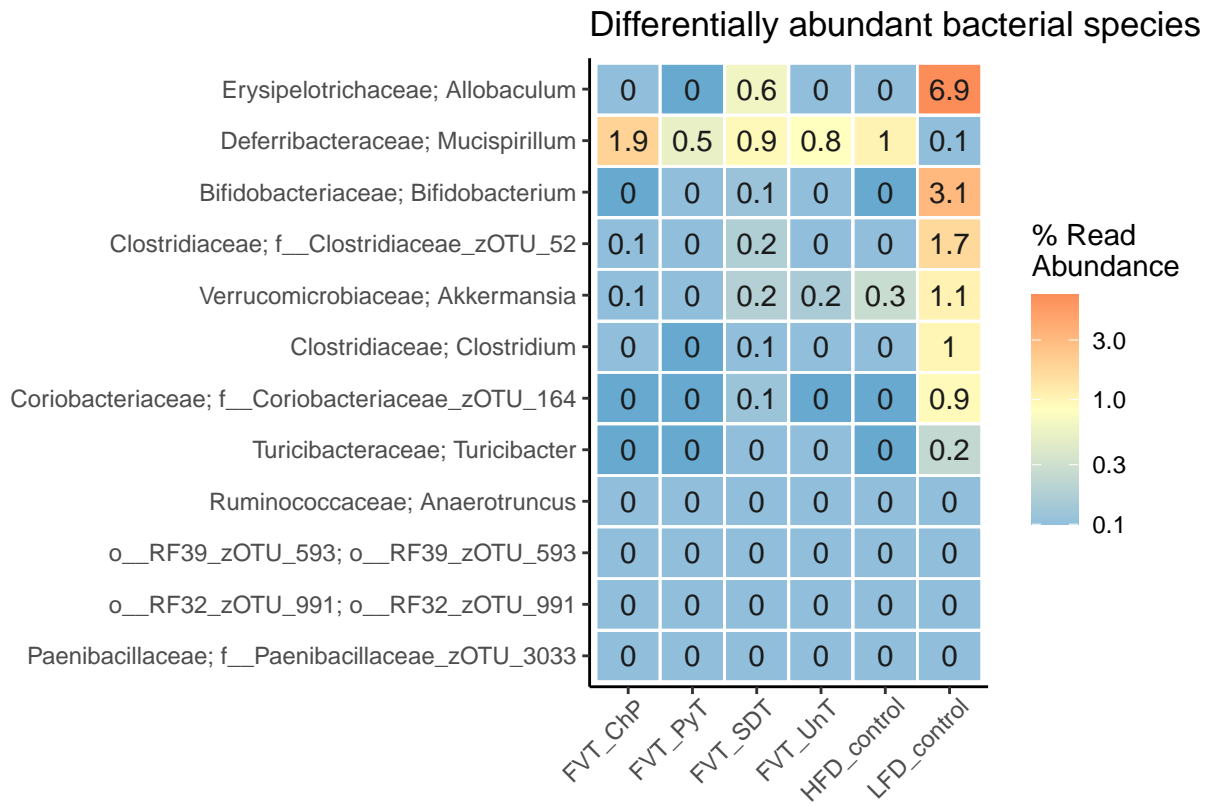
## 9      FVT_PyT LFD_control 0.27578404 0.001 0.015 0.005
## 10     FVT_SDT      FVT_UnT 0.13143246 0.029 0.435 0.044
## 11     FVT_SDT HFD_control 0.08403615 0.241 3.615 0.241
## 12     FVT_SDT LFD_control 0.24172331 0.001 0.015 0.005
## 13     FVT_UnT HFD_control 0.17195360 0.001 0.015 0.005
## 14     FVT_UnT LFD_control 0.30010042 0.004 0.060 0.010
## 15 HFD_control LFD_control 0.29578952 0.001 0.015 0.005

```

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

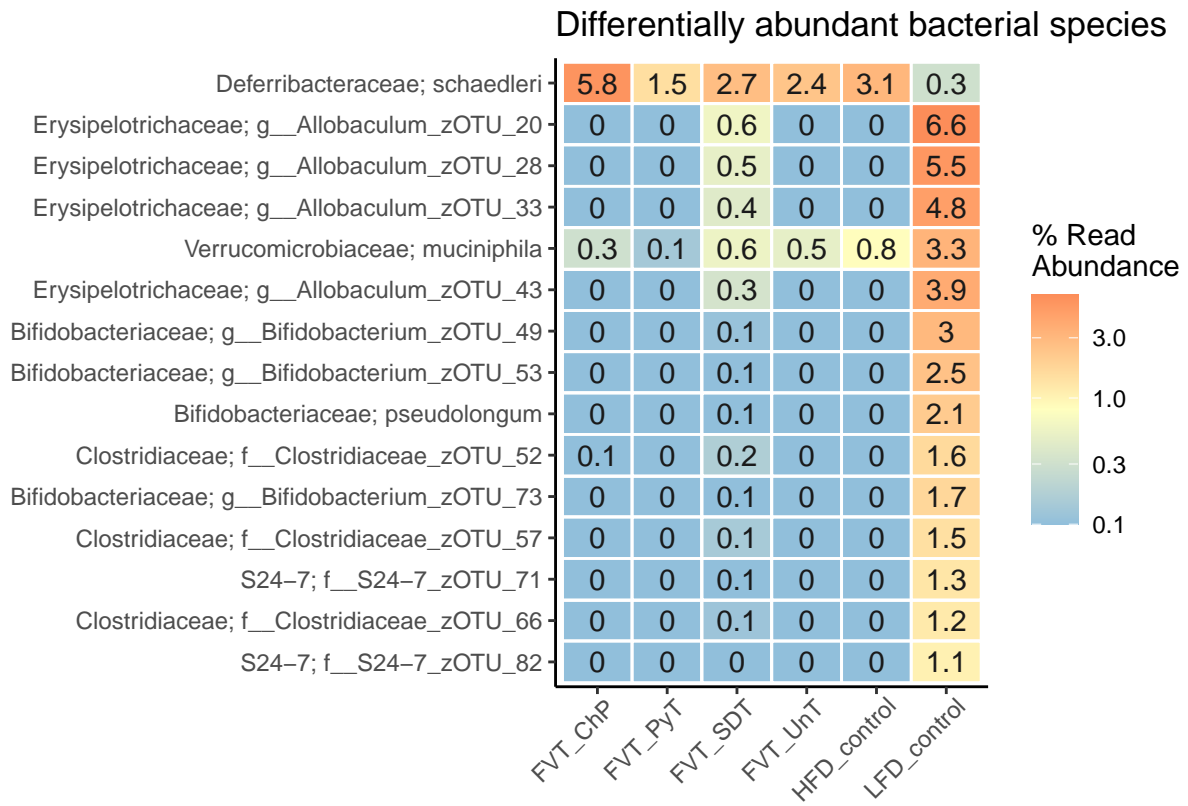


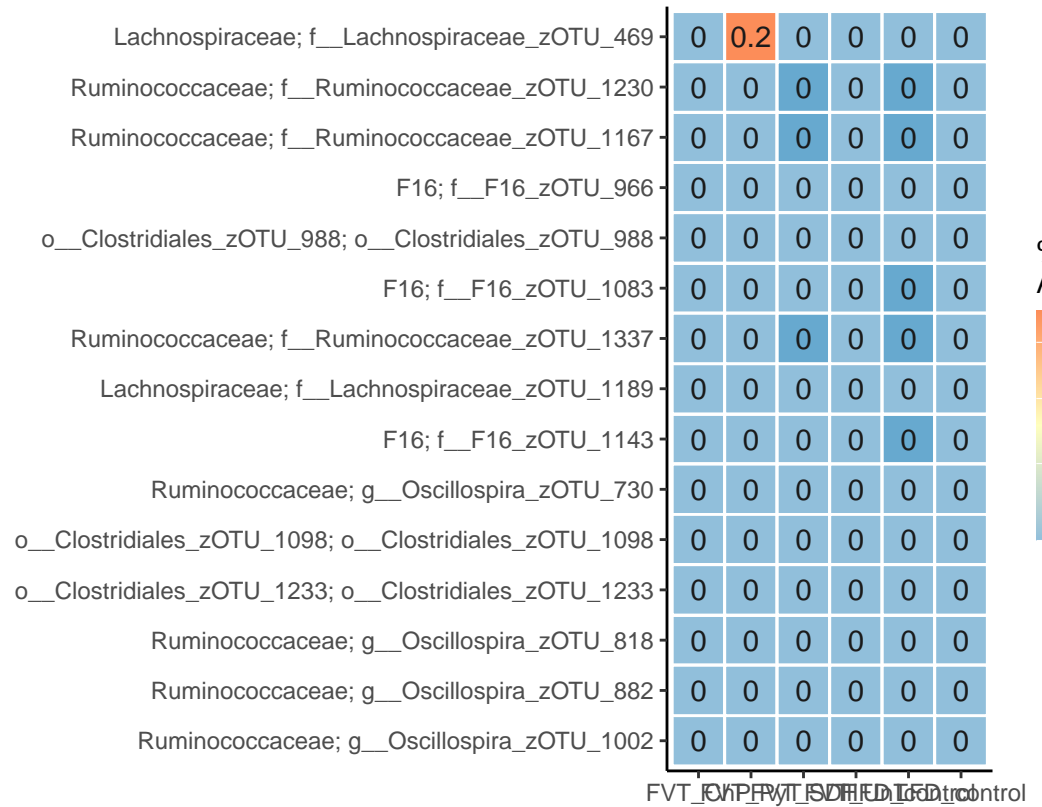
Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level





## Bacteriome - Deseq2 - Treatment



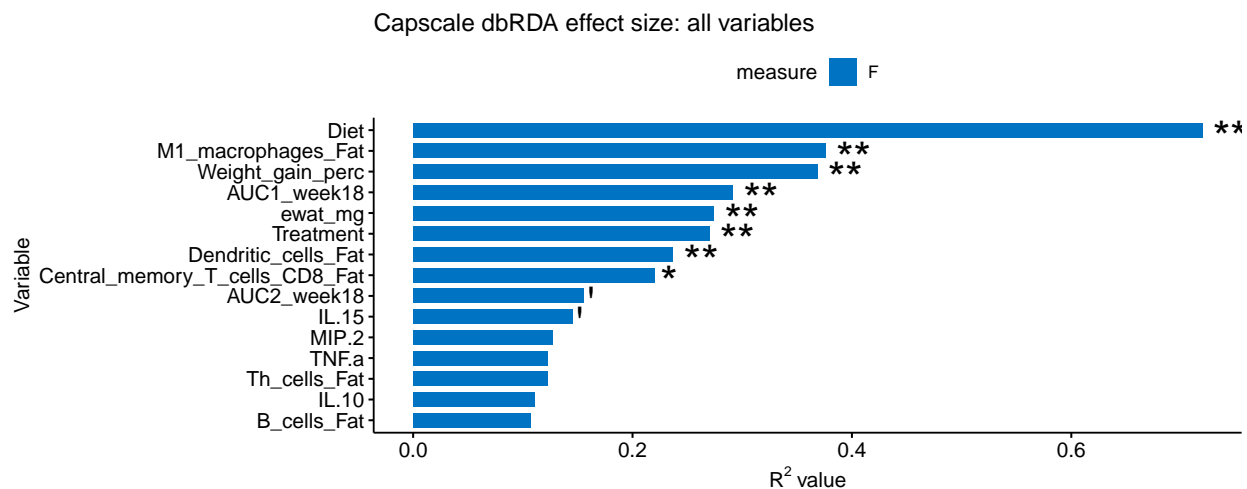


Deseq2 - defined comparison

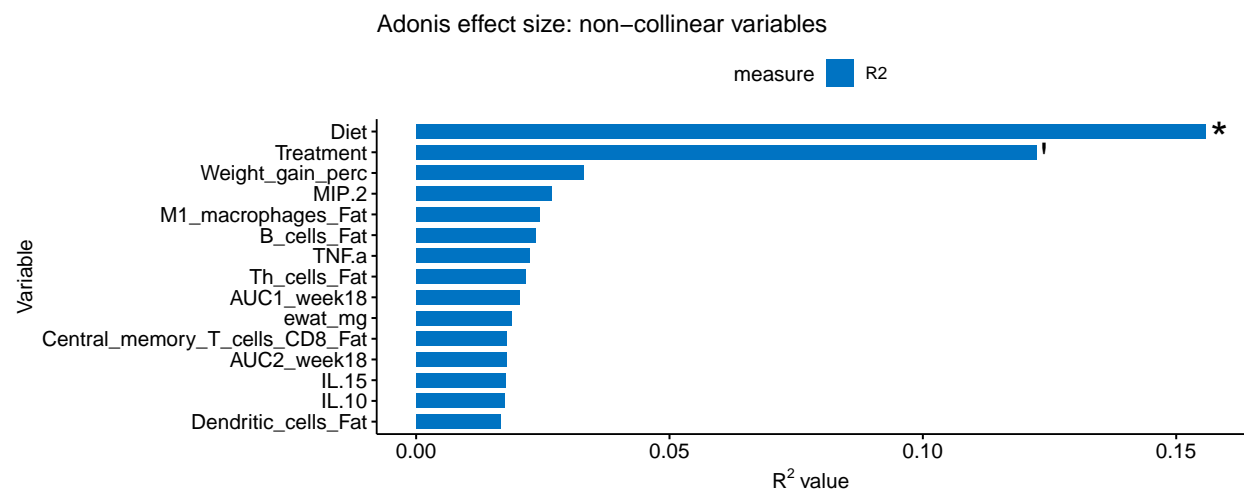
## Bacteriome - Effect-size

### Non-constrained

#### Capscale - independent effect sizes

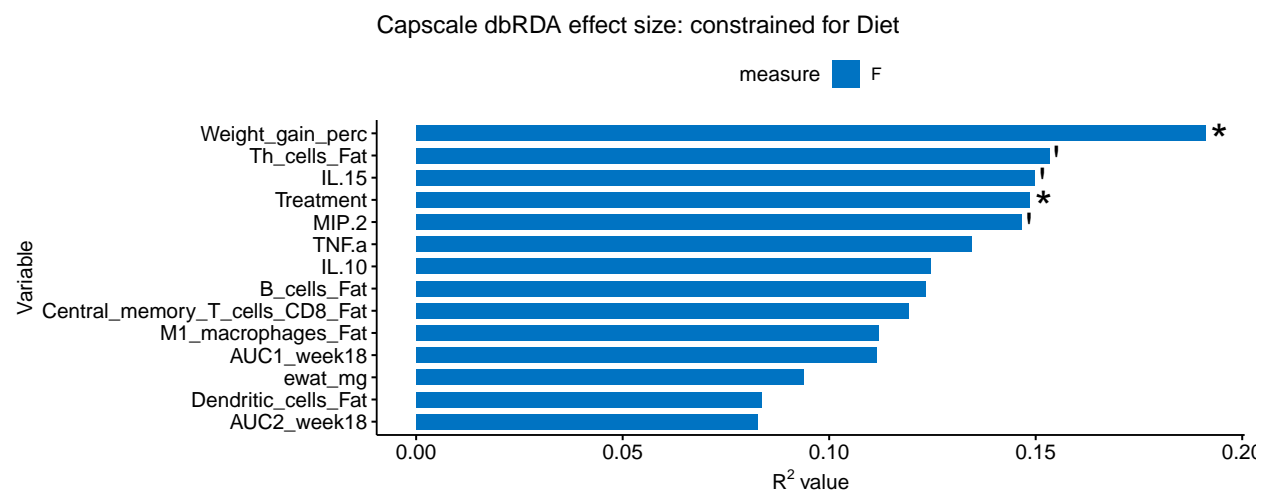


#### ADONIS - decomposed to show individual contributions of factors



Constrained by Diet

Capscale - independent effect sizes



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

Adonis effect size: non-collinear variables – Diet constrained

