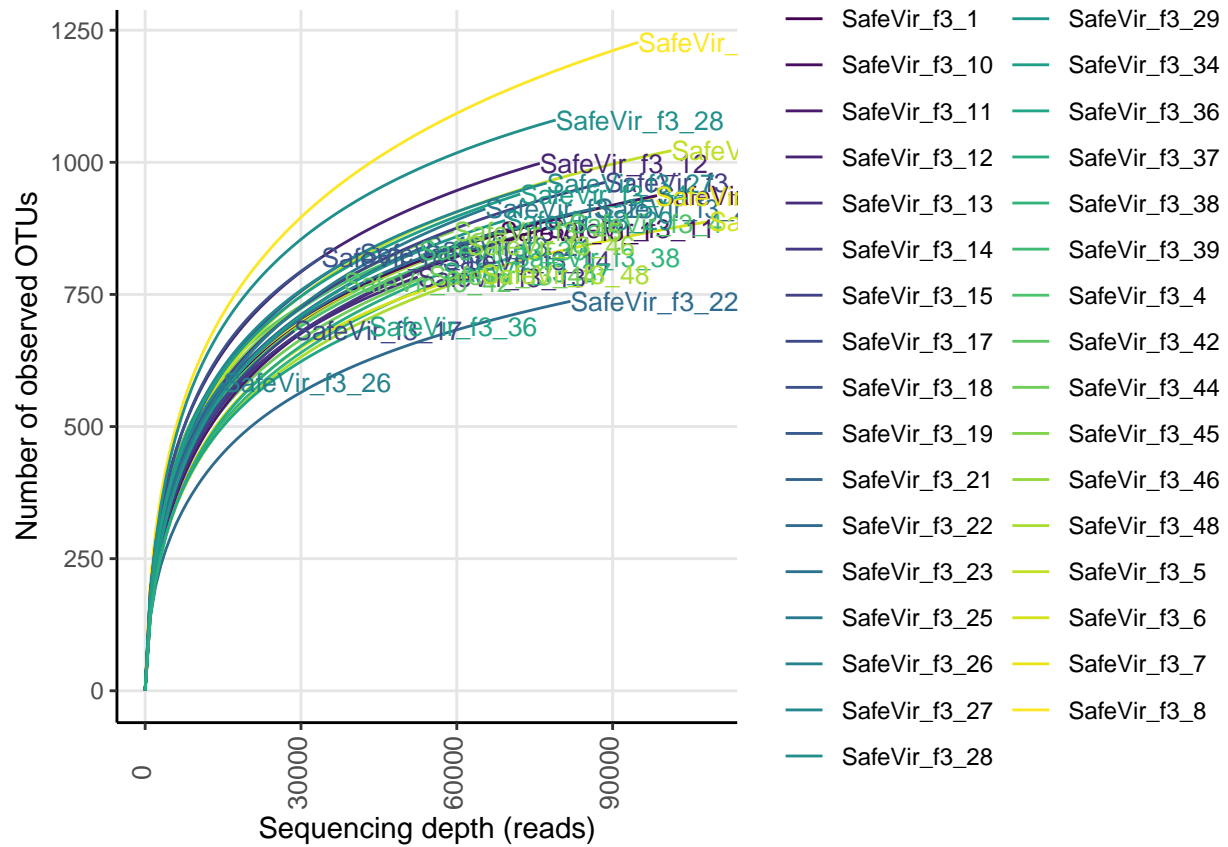
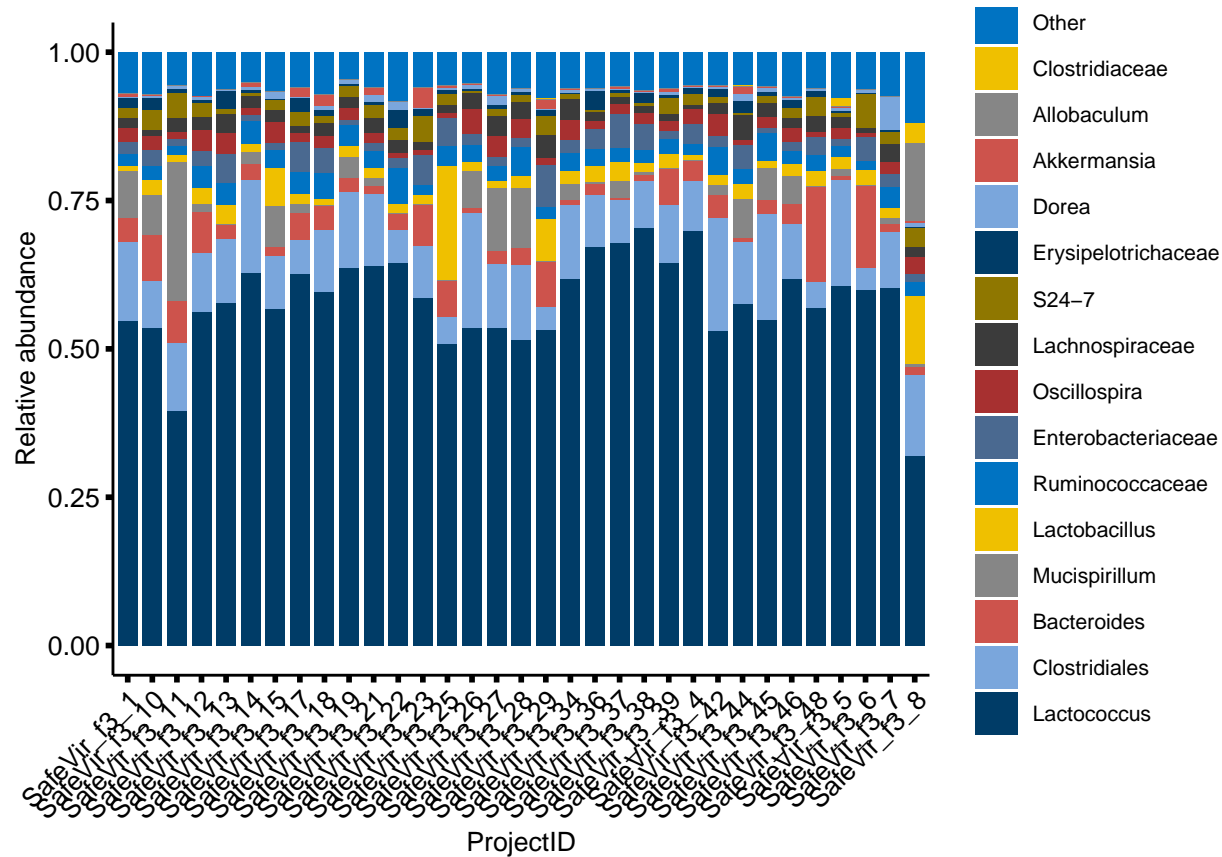


# 16S rRNA gene amplicon analysis - SafeVir - DIO - 1w\_after\_FVT

## Sequencing depth

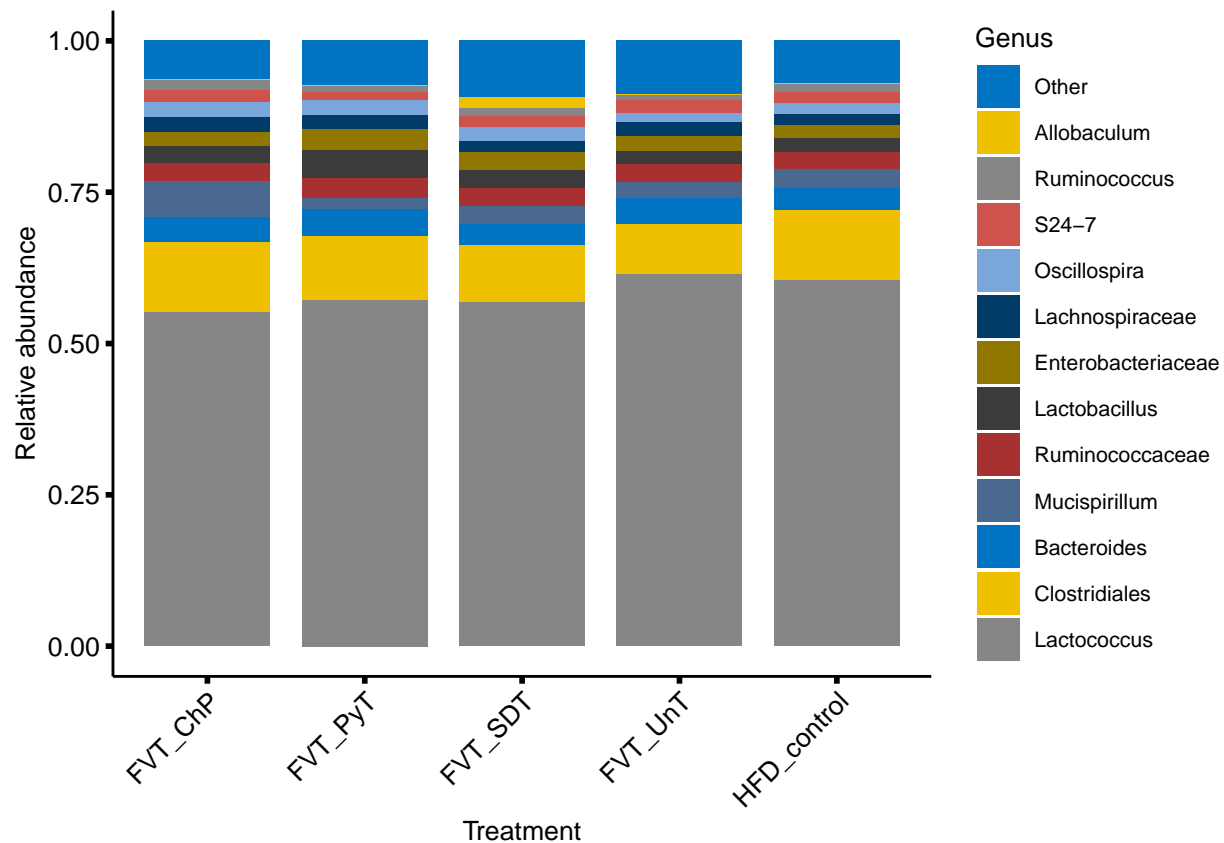


## Individual sample barplots.



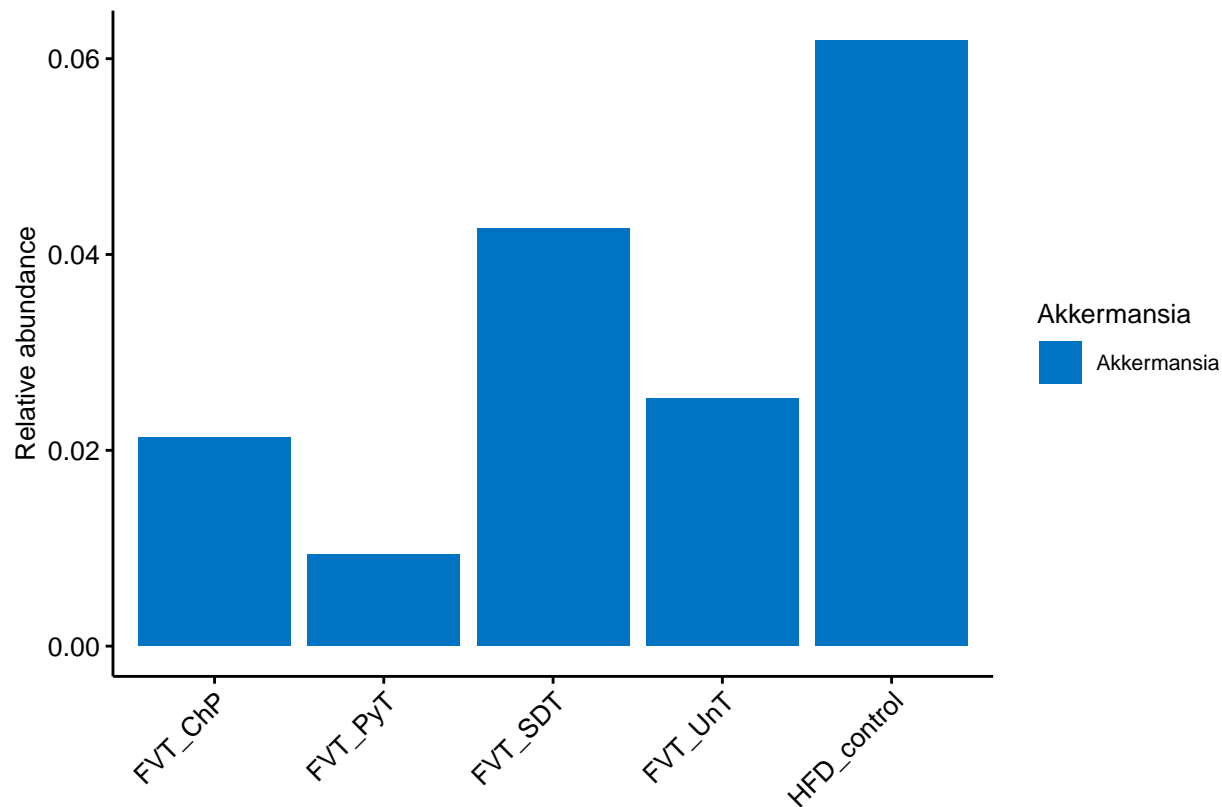
## Barplots based on treatments.

```
## # A tibble: 575 x 3
## # Groups:   Sample [5]
##   Sample      tax      Mean
##   <chr>      <chr>    <dbl>
## 1 FVT_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 HFD_control Clostridiales 0.117
## 7 FVT_ChP    Clostridiales 0.115
## 8 FVT_PyT    Clostridiales 0.107
## 9 FVT_SDT    Clostridiales 0.0940
## 10 FVT_UnT    Clostridiales 0.0822
## # ... with 565 more rows
```



```
## # A tibble: 33 x 3
## # Groups:   Sample [33]
##   Sample      tax      Mean
##   <chr>      <chr>    <dbl>
## 1 NXT075Mao86 Akkermansia 0.0347
## 2 NXT075Mao82 Akkermansia 0.0185
## 3 NXT075Mao92 Akkermansia 0.0175
## 4 NXT075Mao81 Akkermansia 0.0164
## 5 NXT075Mao105 Akkermansia 0.0122
## 6 NXT075Mao84 Akkermansia 0.0117
## 7 NXT075Mao78 Akkermansia 0.00814
## 8 NXT075Mao65 Akkermansia 0.00753
## 9 NXT075Mao101 Akkermansia 0.00741
## 10 NXT075Mao88 Akkermansia 0.00415
## # ... with 23 more rows

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

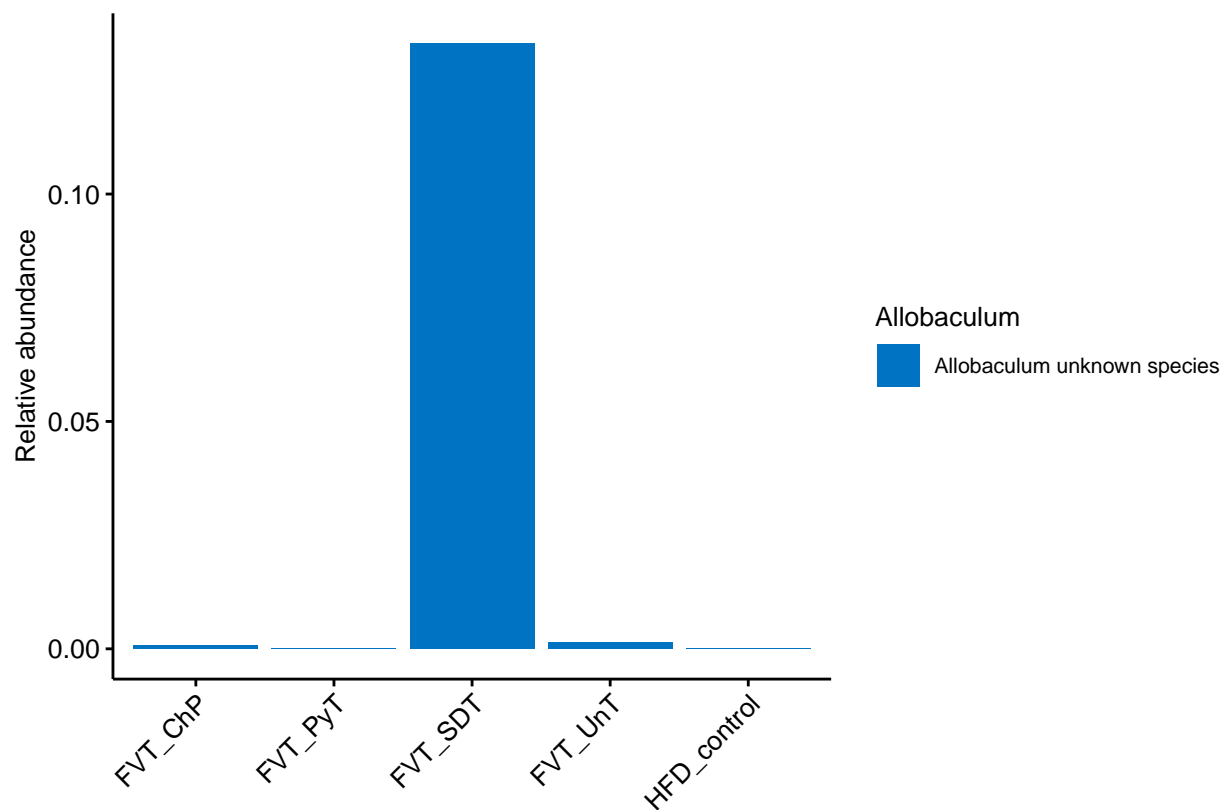


```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.0088083 -0.0043157 -0.0014161  0.0005468  0.0258817
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.008831   0.002884   3.062  0.00482 **
## TreatmentFVT_ChP -0.005790   0.004079  -1.420  0.16677
## TreatmentFVT_PyT -0.007496   0.004079  -1.838  0.07672 .
## TreatmentFVT_SDT -0.002736   0.004079  -0.671  0.50792
## TreatmentFVT_UnT -0.003773   0.004468  -0.844  0.40562
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.00763 on 28 degrees of freedom
## Multiple R-squared:  0.1243, Adjusted R-squared:  -0.0008064
## F-statistic: 0.9936 on 4 and 28 DF, p-value: 0.4273

## # A tibble: 33 x 3
## # Groups:   Sample [33]
##   Sample      tax      Mean
```

```
##      <chr>      <chr>      <dbl>
## 1 NXT075Mao72 Allobaculum unknown species 0.133
## 2 NXT075Mao105 Allobaculum unknown species 0.00146
## 3 NXT075Mao69 Allobaculum unknown species 0.000681
## 4 NXT075Mao86 Allobaculum unknown species 0.0000576
## 5 NXT075Mao82 Allobaculum unknown species 0.0000295
## 6 NXT075Mao83 Allobaculum unknown species 0.0000242
## 7 NXT075Mao107 Allobaculum unknown species 0.0000161
## 8 NXT075Mao100 Allobaculum unknown species 0
## 9 NXT075Mao101 Allobaculum unknown species 0
## 10 NXT075Mao103 Allobaculum unknown species 0
## # ... with 23 more rows
```

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

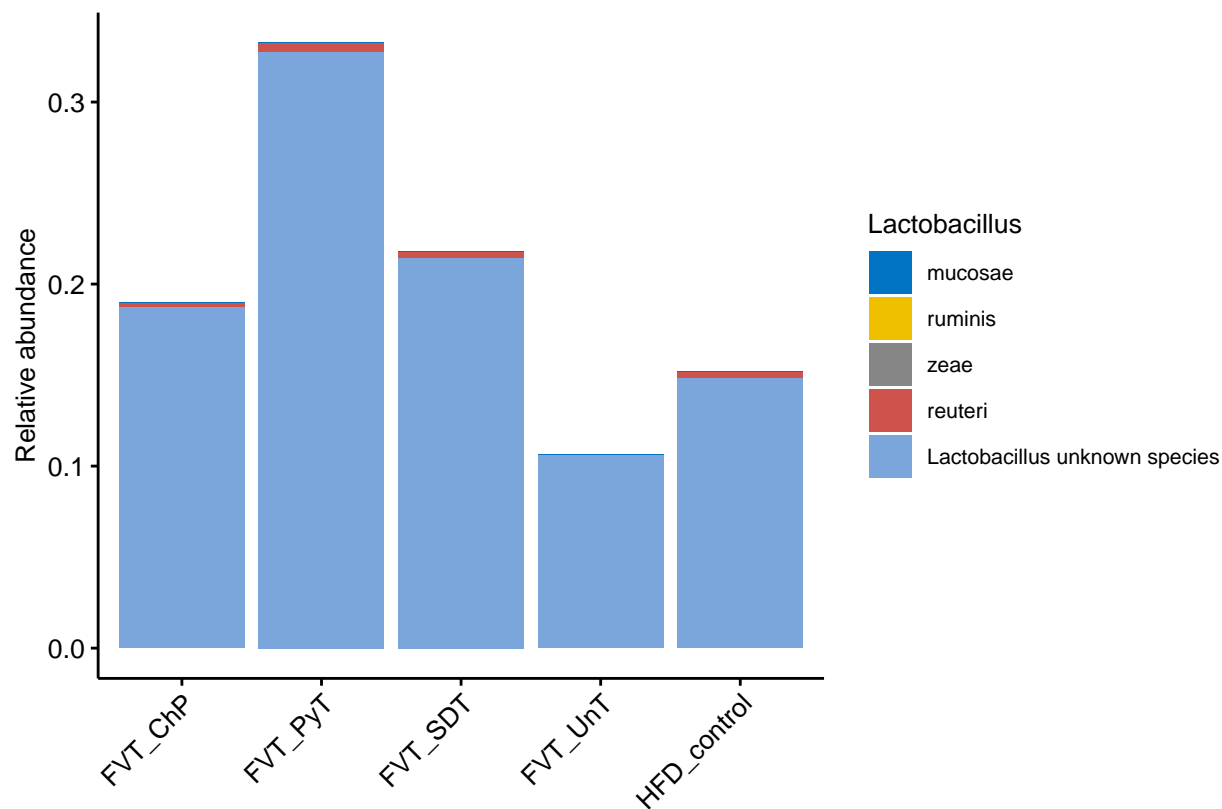


```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.019013 -0.000296 -0.000008  0.000000  0.114047
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
```

```
## (Intercept)      0.000008235  0.008799508  0.001  0.999
## TreatmentFVT_ChP 0.000092533  0.012444383  0.007  0.994
## TreatmentFVT_PyT -0.000008235  0.012444383 -0.001  0.999
## TreatmentFVT_SDT 0.019004471  0.012444383  1.527  0.138
## TreatmentFVT_UnT 0.000287814  0.013632139  0.021  0.983
##
## Residual standard error: 0.02328 on 28 degrees of freedom
## Multiple R-squared:  0.1152, Adjusted R-squared:  -0.0112
## F-statistic: 0.9114 on 4 and 28 DF,  p-value: 0.4709

## # A tibble: 165 x 3
## # Groups:   Sample [33]
##   Sample      tax      Mean
##   <chr>      <chr>      <dbl>
## 1 NXT075Mao88 Lactobacillus unknown species 0.190
## 2 NXT075Mao72 Lactobacillus unknown species 0.113
## 3 NXT075Mao92 Lactobacillus unknown species 0.0713
## 4 NXT075Mao79 Lactobacillus unknown species 0.0592
## 5 NXT075Mao99 Lactobacillus unknown species 0.0340
## 6 NXT075Mao77 Lactobacillus unknown species 0.0318
## 7 NXT075Mao98 Lactobacillus unknown species 0.0289
## 8 NXT075Mao76 Lactobacillus unknown species 0.0263
## 9 NXT075Mao70 Lactobacillus unknown species 0.0262
## 10 NXT075Mao74 Lactobacillus unknown species 0.0262
## # ... with 155 more rows

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

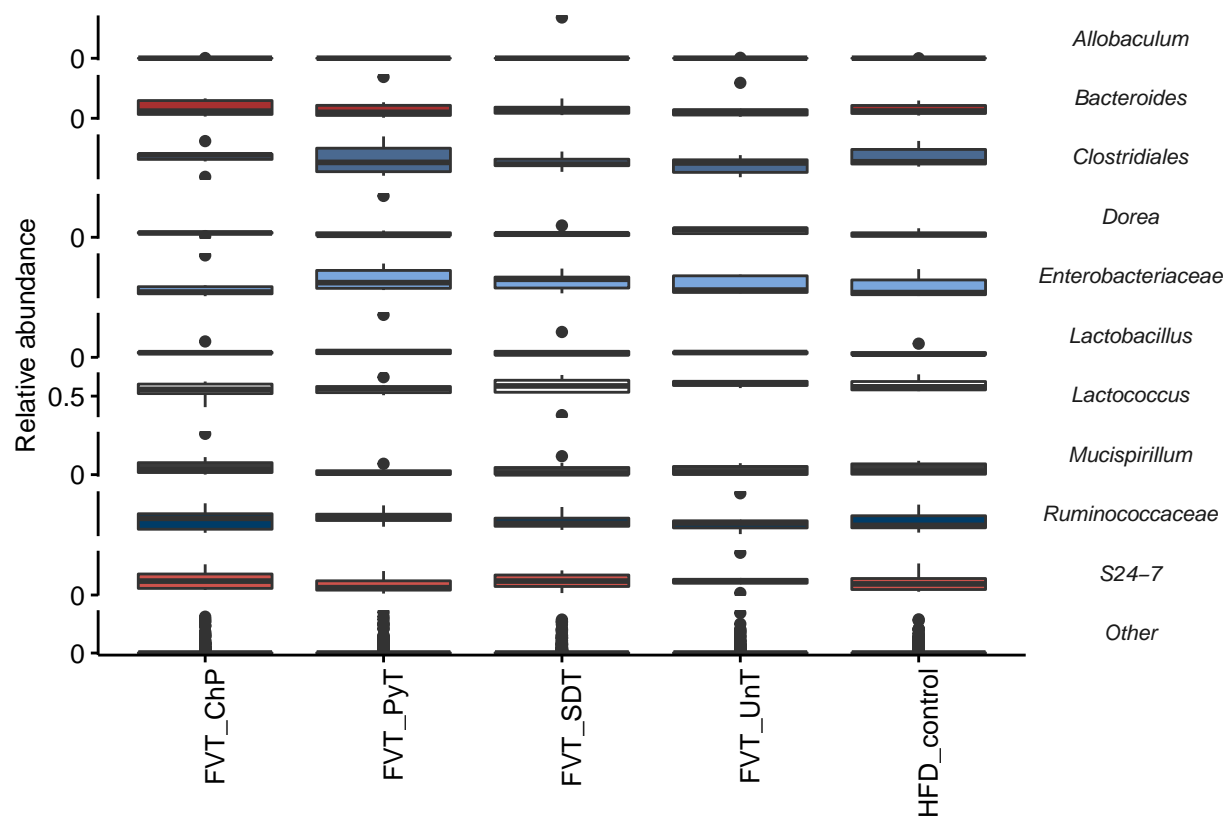


```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.009500 -0.006221 -0.004346 -0.004254  0.180160
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.00434643  0.00334088   1.301   0.195
## TreatmentFVT_ChP  0.00107815  0.00472472   0.228   0.820
## TreatmentFVT_PyT  0.00515368  0.00472472   1.091   0.277
## TreatmentFVT_SDT  0.00187430  0.00472472   0.397   0.692
## TreatmentFVT_UnT -0.00009223  0.00517568  -0.018   0.986
##
## Residual standard error: 0.01976 on 160 degrees of freedom
## Multiple R-squared:  0.00972,    Adjusted R-squared:  -0.01504
## F-statistic: 0.3926 on 4 and 160 DF,  p-value: 0.8137

## # A tibble: 3,795 x 3
## # Groups:   Sample [33]
##   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 3,785 more rows
```

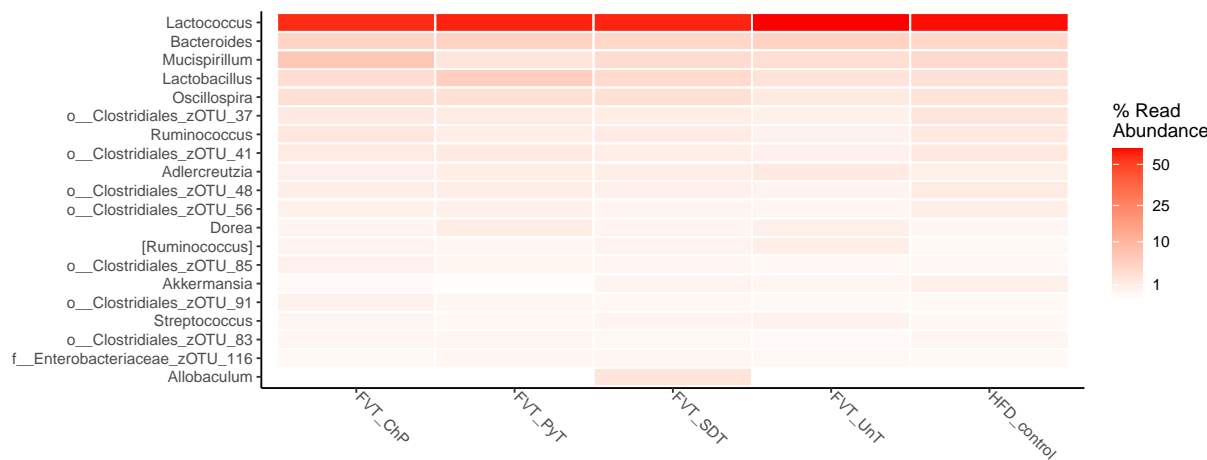
```
## Warning: This manual palette can handle a maximum of 10 values. You have
## supplied 11.
```





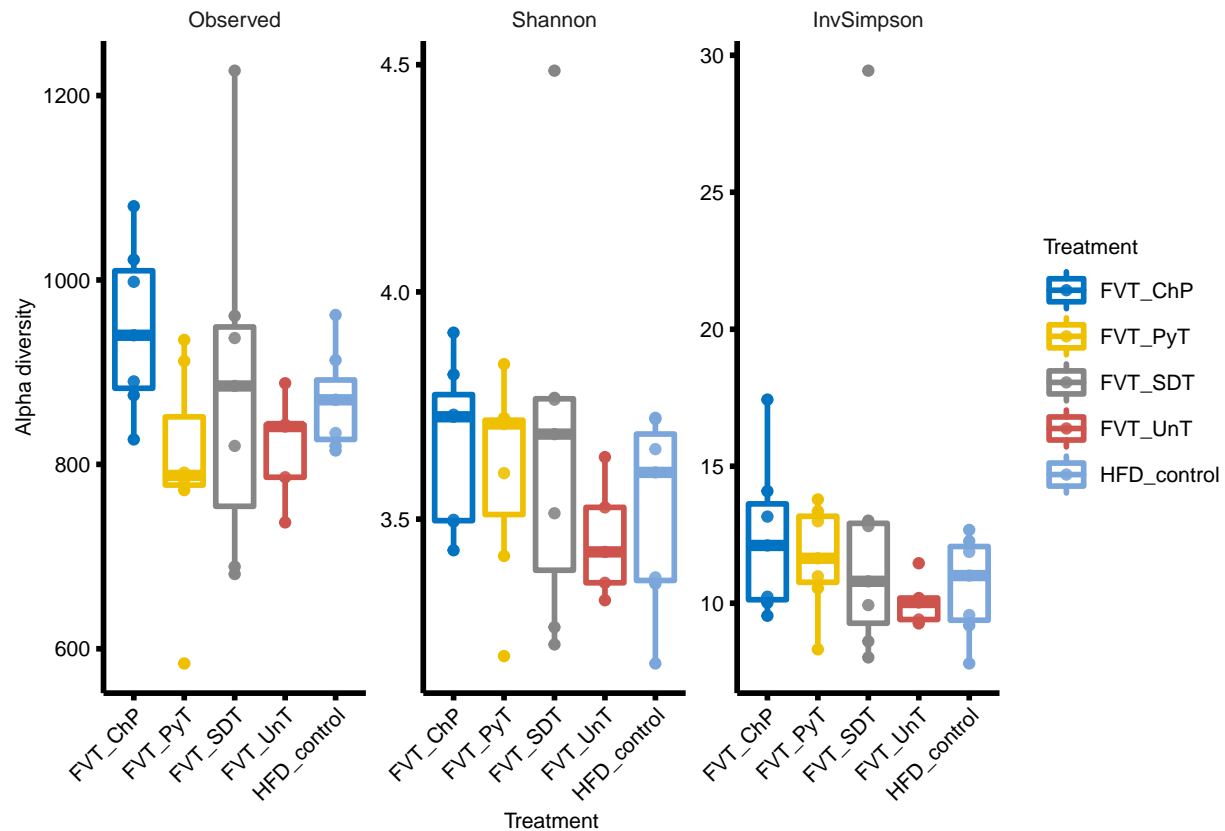
# Abundance heatmaps

## 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	-0.05769599	-0.4637163	0.3483243	0.9934851
FVT_SDT-FVT_ChP	0.01352473	-0.3924956	0.4195450	0.9999786
FVT_UnT-FVT_ChP	-0.20399863	-0.6487716	0.2407743	0.6715746
HFD_control-FVT_ChP	-0.14227439	-0.5482947	0.2637459	0.8435810
FVT_SDT-FVT_PyT	0.07122072	-0.3347996	0.4772410	0.9855624
FVT_UnT-FVT_PyT	-0.14630265	-0.5910756	0.2984703	0.8711982
HFD_control-FVT_PyT	-0.08457840	-0.4905987	0.3214419	0.9728219
FVT_UnT-FVT_SDT	-0.21752336	-0.6622963	0.2272496	0.6173890
HFD_control-FVT_SDT	-0.15579912	-0.5618194	0.2502212	0.7957624
HFD_control-FVT_UnT	0.06172424	-0.3830487	0.5064972	0.9940491

```
##
## Call:
```

```

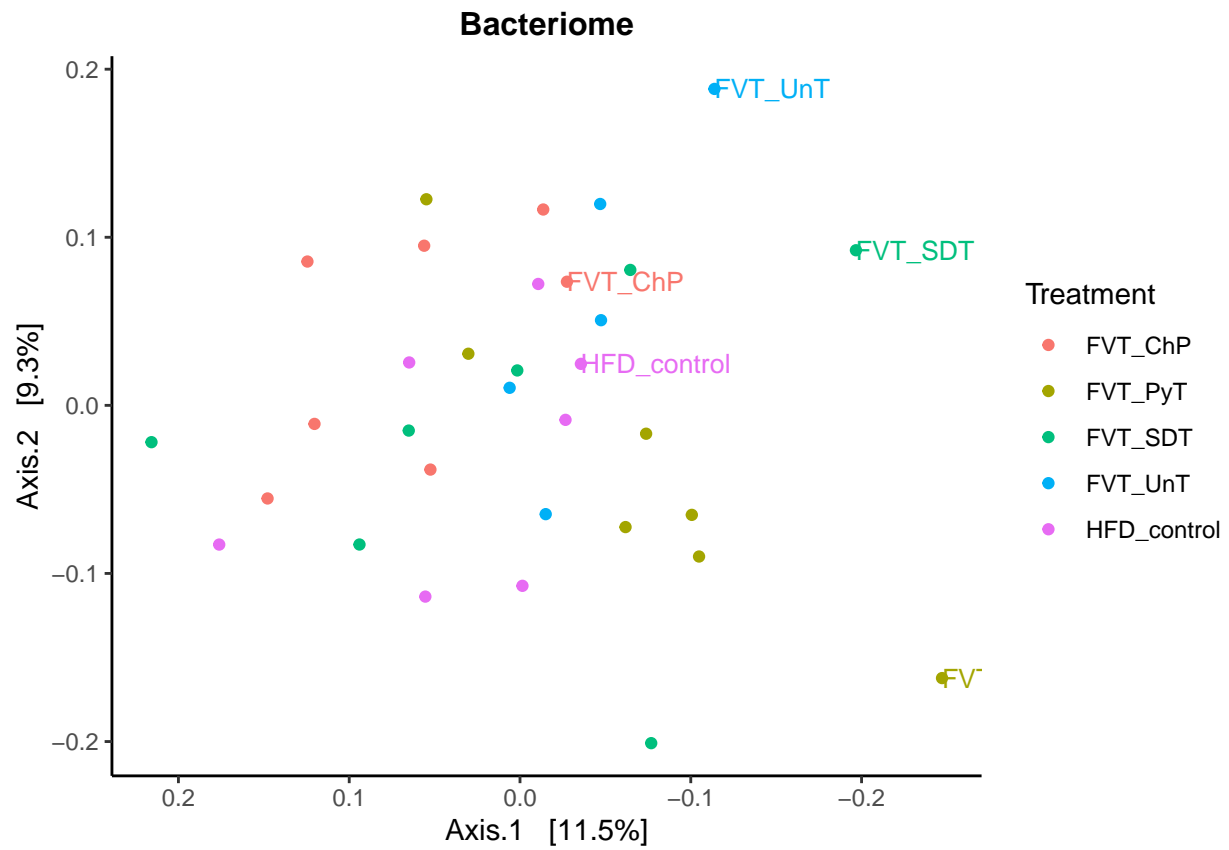
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.4476 -0.1590  0.0669  0.1218  0.8150
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    3.51605    0.09854  35.681  <2e-16 ***
## variableFVT_ChP  0.14227    0.13936   1.021   0.316
## variableFVT_PyT  0.08458    0.13936   0.607   0.549
## variableFVT_SDT  0.15580    0.13936   1.118   0.273
## variableFVT_UnT -0.06172    0.15266  -0.404   0.689
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2607 on 28 degrees of freedom
## Multiple R-squared:  0.0996, Adjusted R-squared:  -0.02903
## F-statistic: 0.7743 on 4 and 28 DF,  p-value: 0.5512

##
## Call:
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.4476 -0.1590  0.0669  0.1218  0.8150
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    3.45433    0.11660  29.627  <2e-16 ***
## variableHFD_control 0.06172    0.15266   0.404   0.689
## variableFVT_ChP    0.20400    0.15266   1.336   0.192
## variableFVT_PyT    0.14630    0.15266   0.958   0.346
## variableFVT_SDT    0.21752    0.15266   1.425   0.165
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2607 on 28 degrees of freedom
## Multiple R-squared:  0.0996, Adjusted R-squared:  -0.02903
## F-statistic: 0.7743 on 4 and 28 DF,  p-value: 0.5512

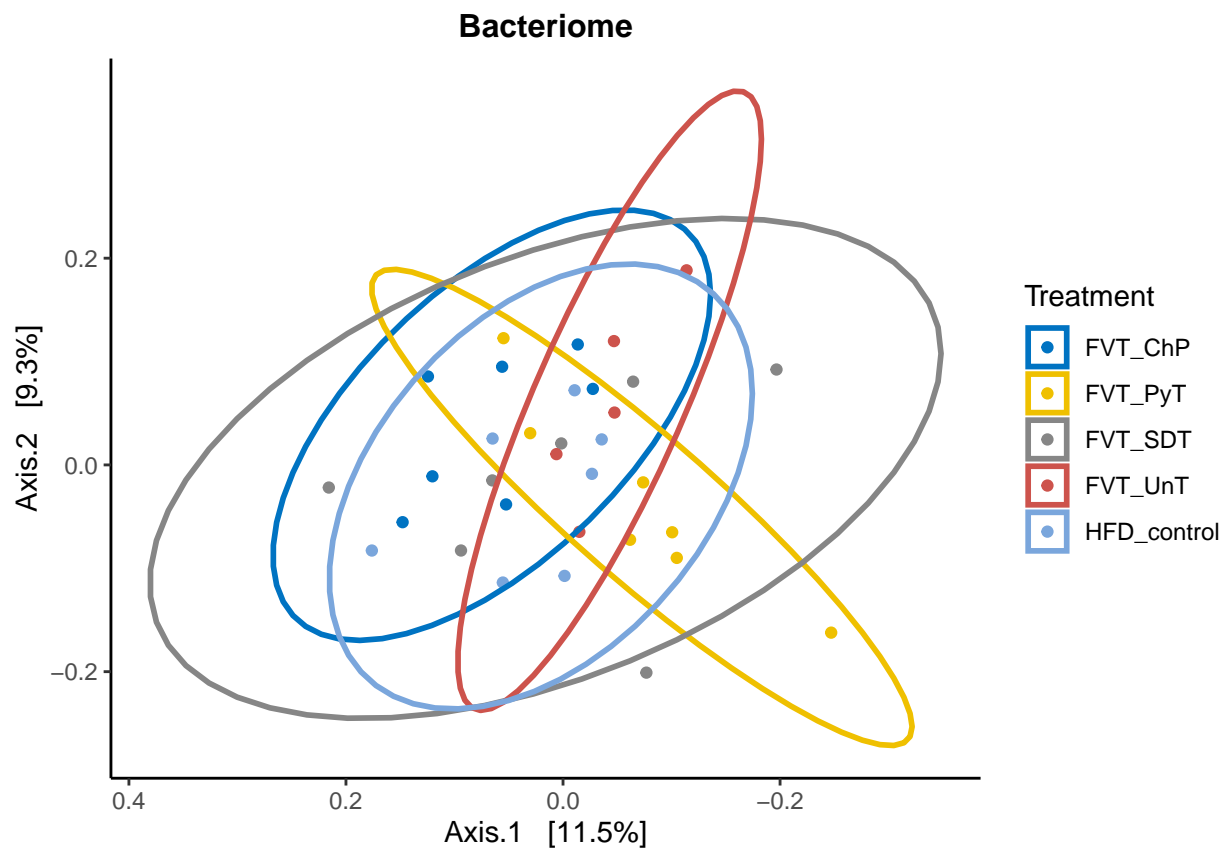
```

## Beta diversity

basically the mouse ID.



## Treatment



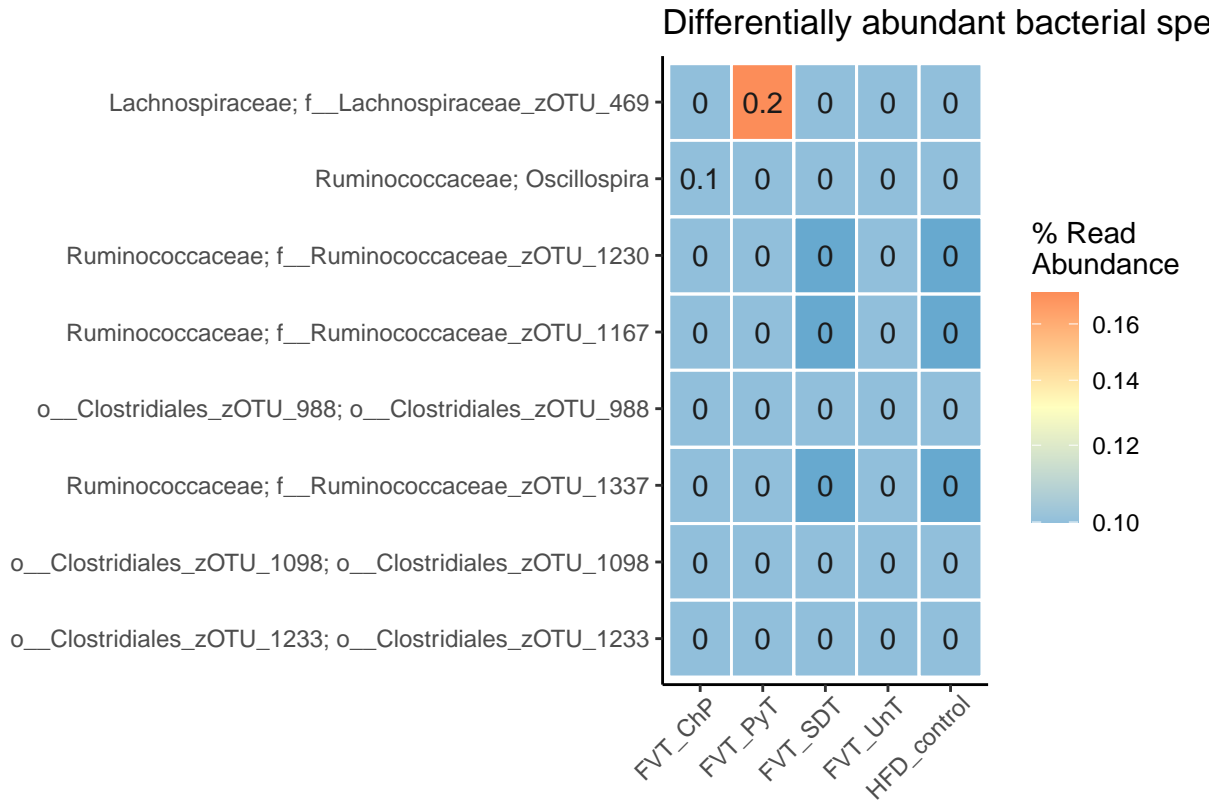
Adonis and pairwise permanova analysis - Treatment

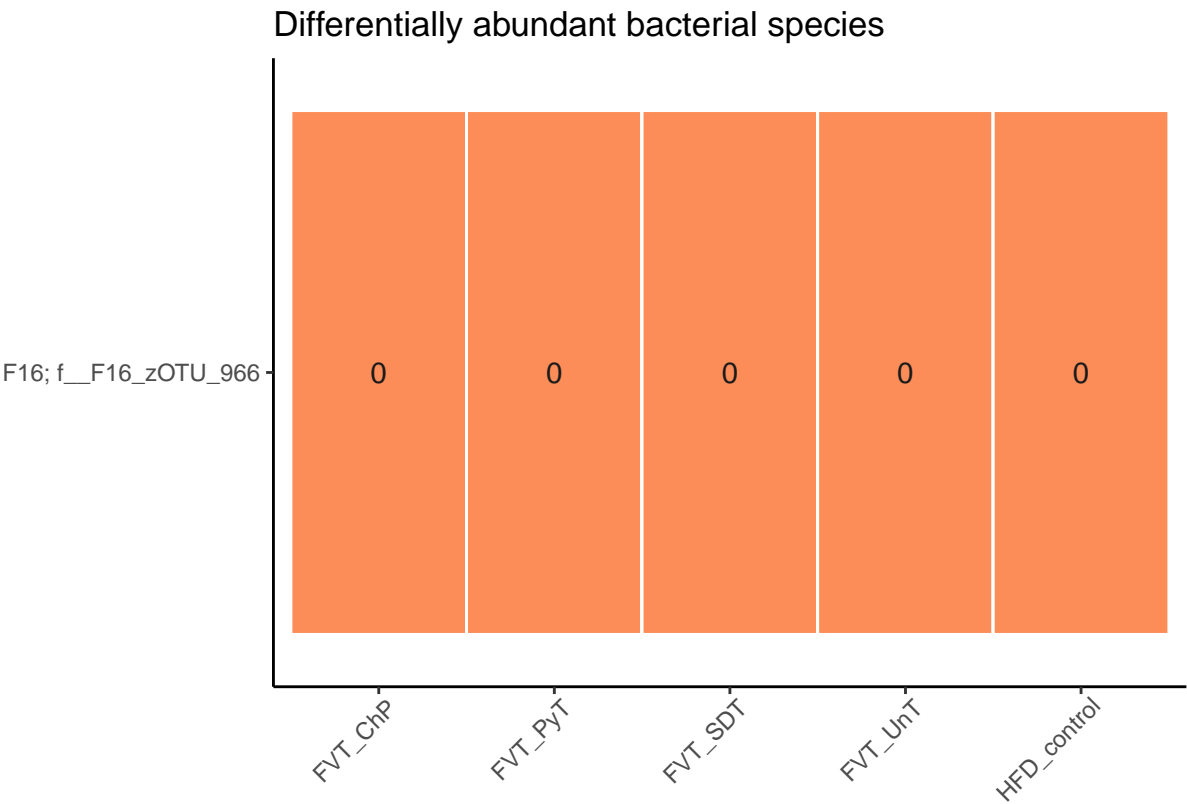
```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.PSB ~ Treatment, data = sampled.f.PSB, permutations = 999, method = "bray")
##      Df SumOfSqs    R2      F Pr(>F)
## Treatment  4  0.48673 0.1748 1.4828  0.001 ***
## Residual 28  2.29774 0.8252
## Total    32  2.78447 1.0000
## ---
## 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	0.11	0.027
## 2	FVT_ChP	FVT_SDT	0.09464042	0.130	1.30	0.162
## 3	FVT_ChP	FVT_UnT	0.14384829	0.009	0.09	0.030
## 4	FVT_ChP	HFD_control	0.10282876	0.048	0.48	0.080
## 5	FVT_PyT	FVT_SDT	0.09431398	0.091	0.91	0.130
## 6	FVT_PyT	FVT_UnT	0.10747890	0.157	1.57	0.174
## 7	FVT_PyT	HFD_control	0.12589985	0.006	0.06	0.030
## 8	FVT_SDT	FVT_UnT	0.13143246	0.040	0.40	0.080

```
## 9  FVT_SDT HFD_control 0.08403615 0.267 2.67 0.267
## 10 FVT_UnT HFD_control 0.17195360 0.001 0.01 0.010
```

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



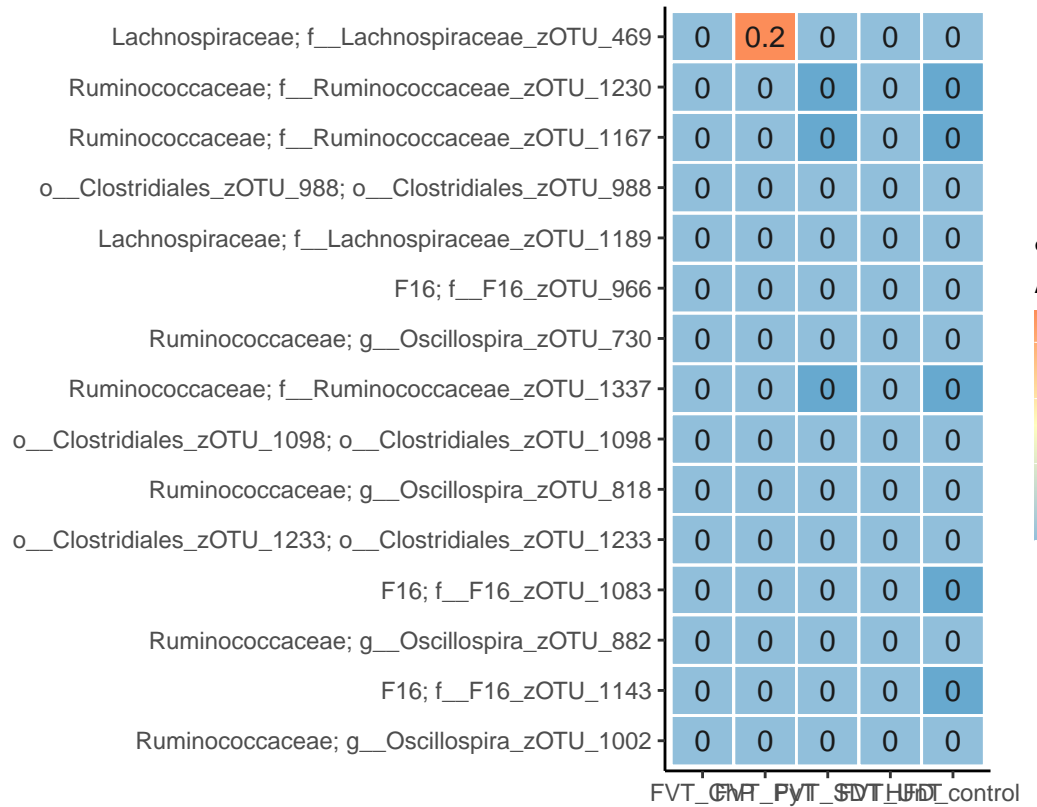


## Bacteriome - Deseq2 - Treatment

### Differentially abundant bacterial speci

Lactobacillaceae; reuteri	0	0	0	0	0
Ruminococcaceae; f__Ruminococcaceae_zOTU_1230	0	0	0	0	0
Ruminococcaceae; f__Ruminococcaceae_zOTU_1167	0	0	0	0	0
o__Clostridiales_zOTU_988; o__Clostridiales_zOTU_988	0	0	0	0	0
Ruminococcaceae; g__Oscillospira_zOTU_730	0	0	0	0	0
Ruminococcaceae; f__Ruminococcaceae_zOTU_1337	0	0	0	0	0
Ruminococcaceae; g__Oscillospira_zOTU_818	0	0	0	0	0
F16; f__F16_zOTU_1083	0	0	0	0	0
Ruminococcaceae; g__Oscillospira_zOTU_882	0	0	0	0	0
F16; f__F16_zOTU_1143	0	0	0	0	0
Ruminococcaceae; g__Oscillospira_zOTU_1002	0	0	0	0	0
Ruminococcaceae; g__Ruminococcus_zOTU_2927	0	0	0	0	0
	FVT_ChIP	FVT_PyT	FVT_SDT	FVT_UnT	HFD_control



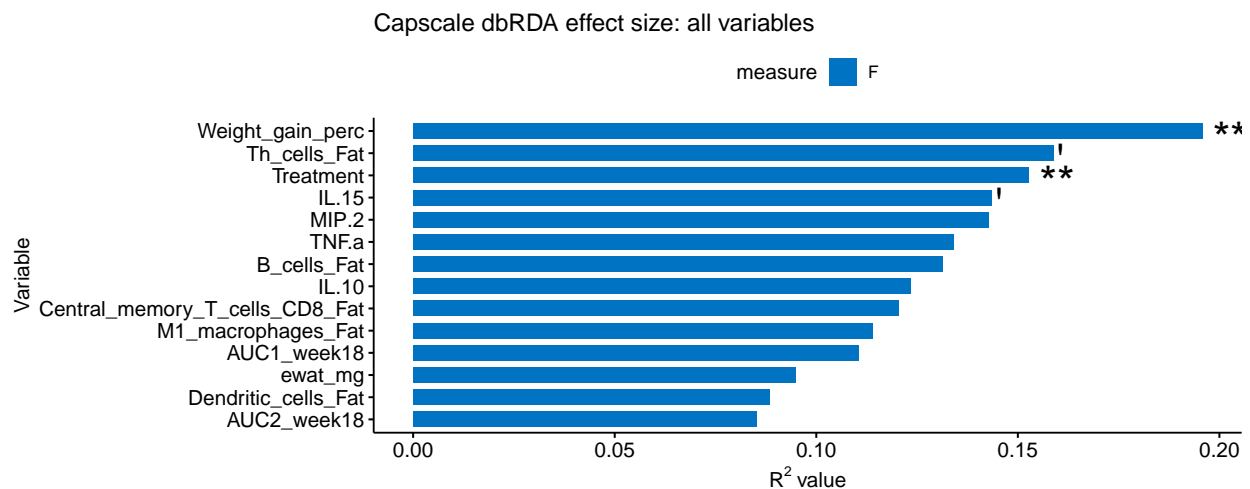


Deseq2 - defined comparison

## Bacteriome - Effect-size

### Non-constrained

Capscale - independent effect sizes



ADONIS - decomposed to show individual contributions of factors

