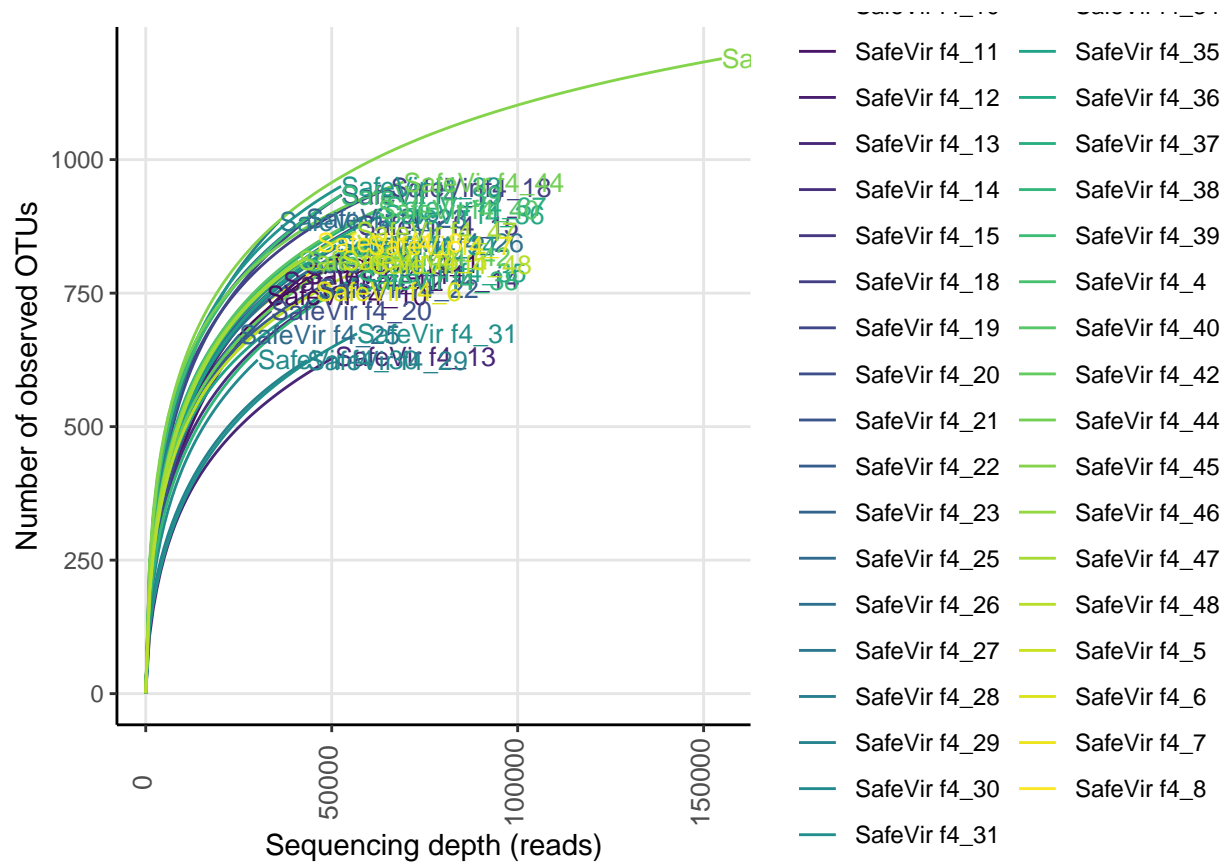
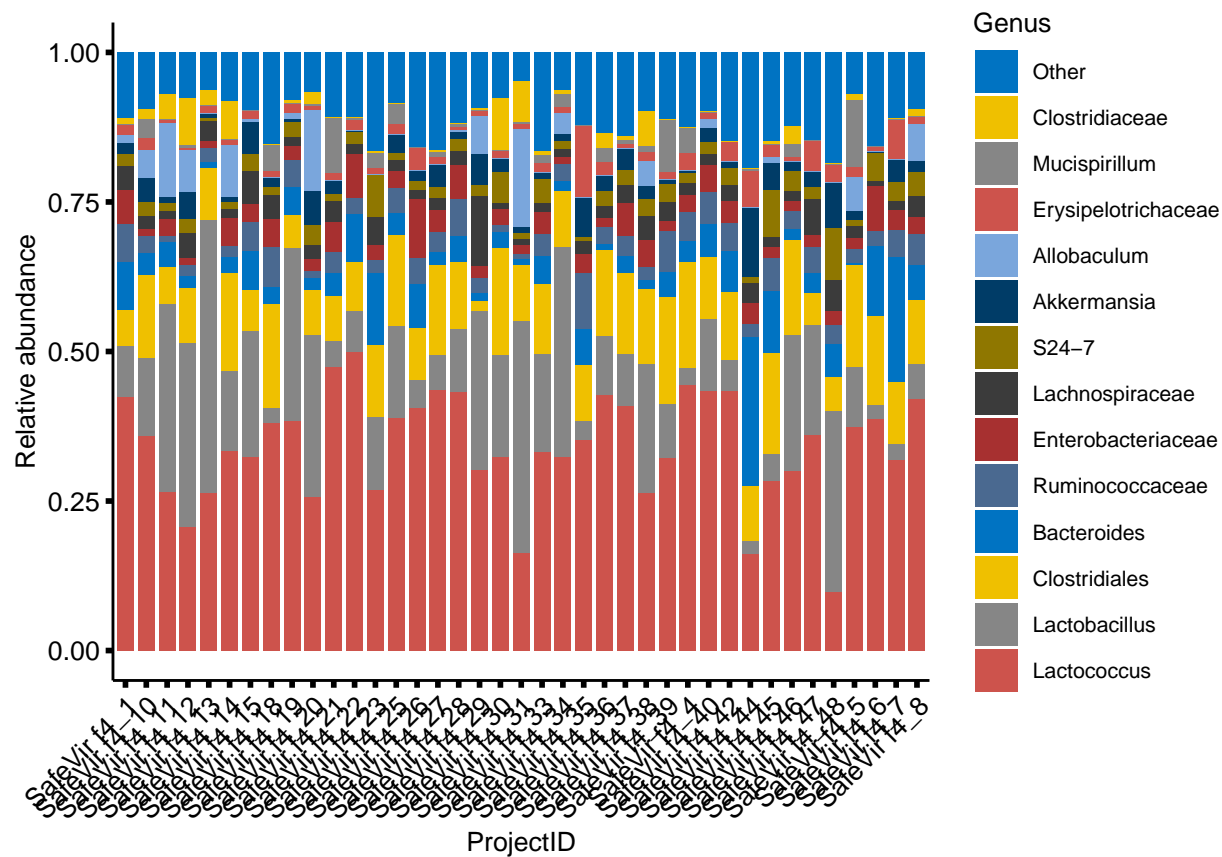


# 16S rRNA gene amplicon analysis - SafeVir - DIO - Termination

## Sequencing depth

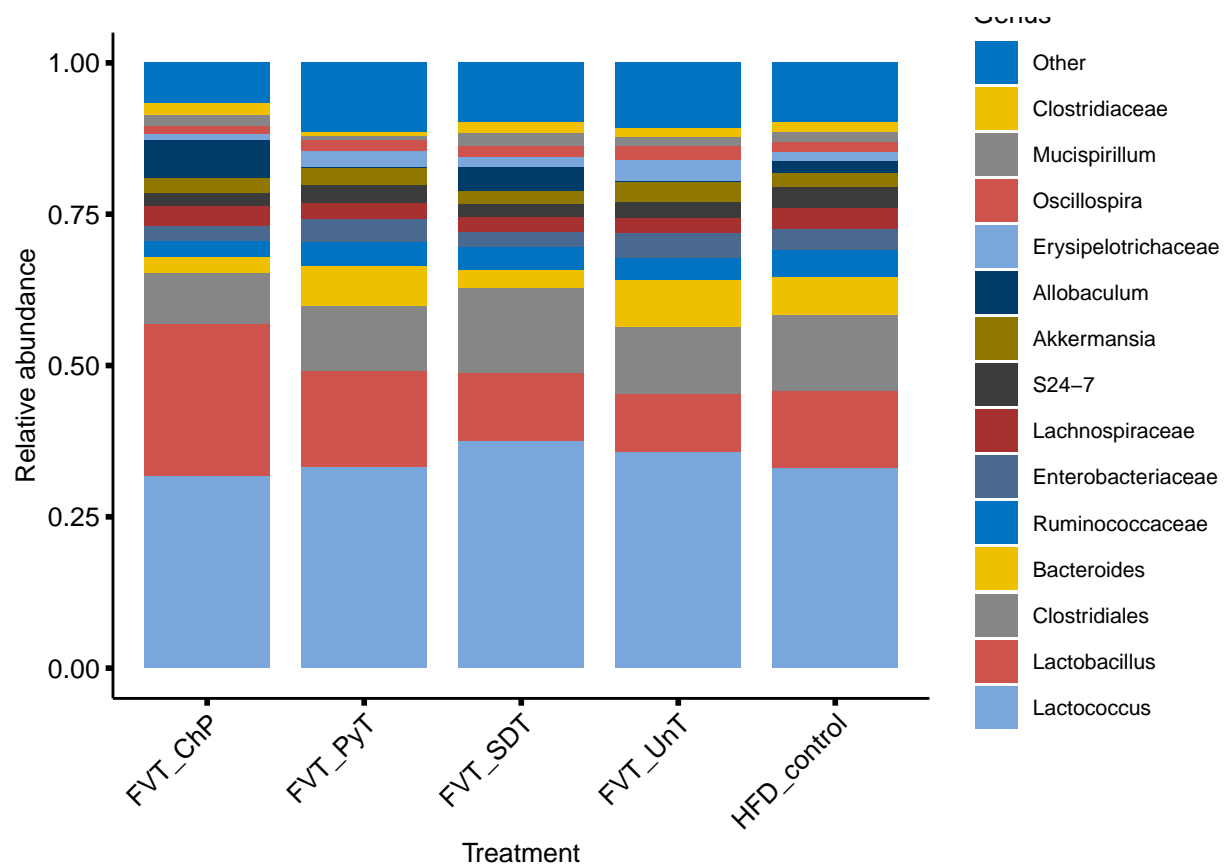


## Individual sample barplots.



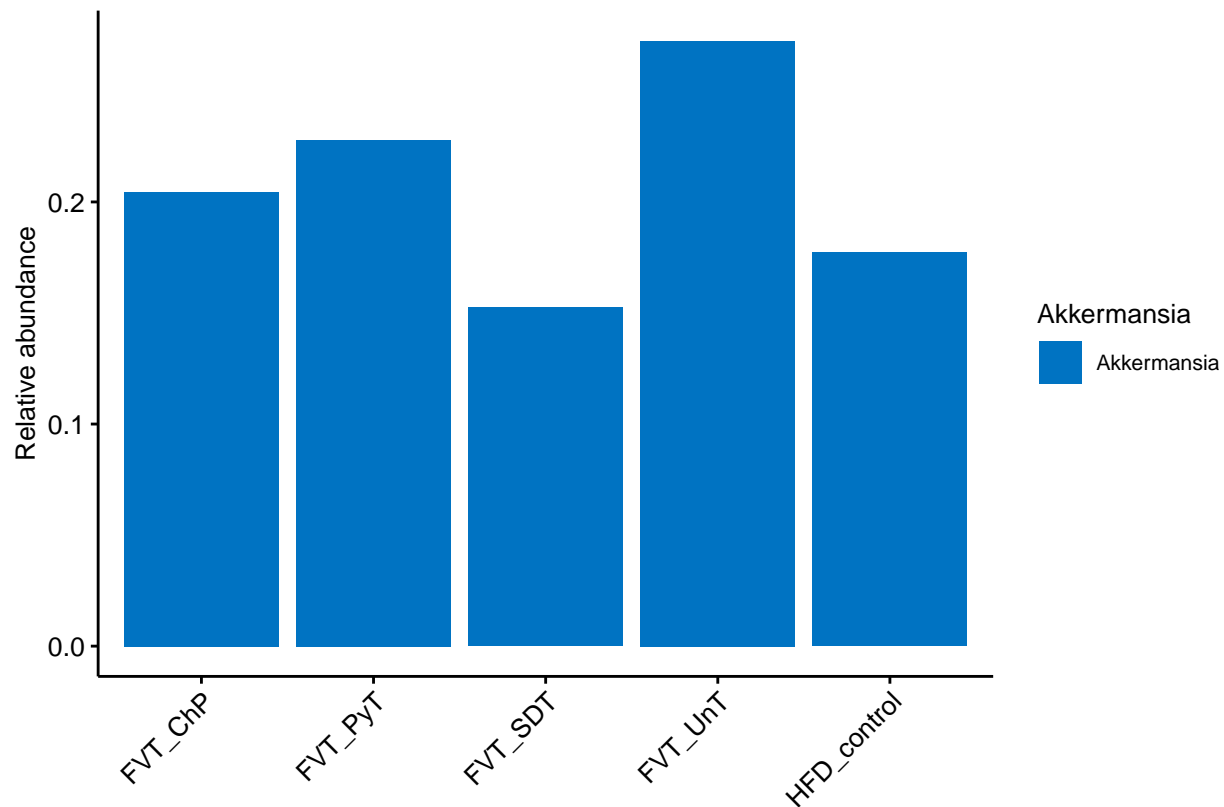
## Barplots based on treatments.

```
## # A tibble: 575 x 3
## # Groups:   Sample [5]
##   Sample      tax      Mean
##   <chr>      <chr>    <dbl>
## 1 FVT_SDT    Lactococcus 0.376
## 2 FVT_UnT    Lactococcus 0.358
## 3 HFD_control Lactococcus 0.332
## 4 FVT_PyT    Lactococcus 0.332
## 5 FVT_ChP    Lactococcus 0.318
## 6 FVT_ChP    Lactobacillus 0.251
## 7 FVT_PyT    Lactobacillus 0.160
## 8 FVT_SDT    Clostridiales 0.141
## 9 HFD_control Lactobacillus 0.127
## 10 HFD_control Clostridiales 0.124
## # ... with 565 more rows
```



```
## # A tibble: 39 x 3
## # Groups:   Sample [39]
##   Sample      tax      Mean
##   <chr>      <chr>    <dbl>
## 1 NXT075Mao276 Akkermansia 0.117
## 2 NXT075Mao280 Akkermansia 0.0757
## 3 NXT075Mao267 Akkermansia 0.0658
## 4 NXT075Mao178 Akkermansia 0.0564
## 5 NXT075Mao173 Akkermansia 0.0544
## 6 NXT075Mao187 Akkermansia 0.0525
## 7 NXT075Mao170 Akkermansia 0.0453
## 8 NXT075Mao277 Akkermansia 0.0447
## 9 NXT075Mao168 Akkermansia 0.0407
## 10 NXT075Mao165 Akkermansia 0.0386
## # ... with 29 more rows

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

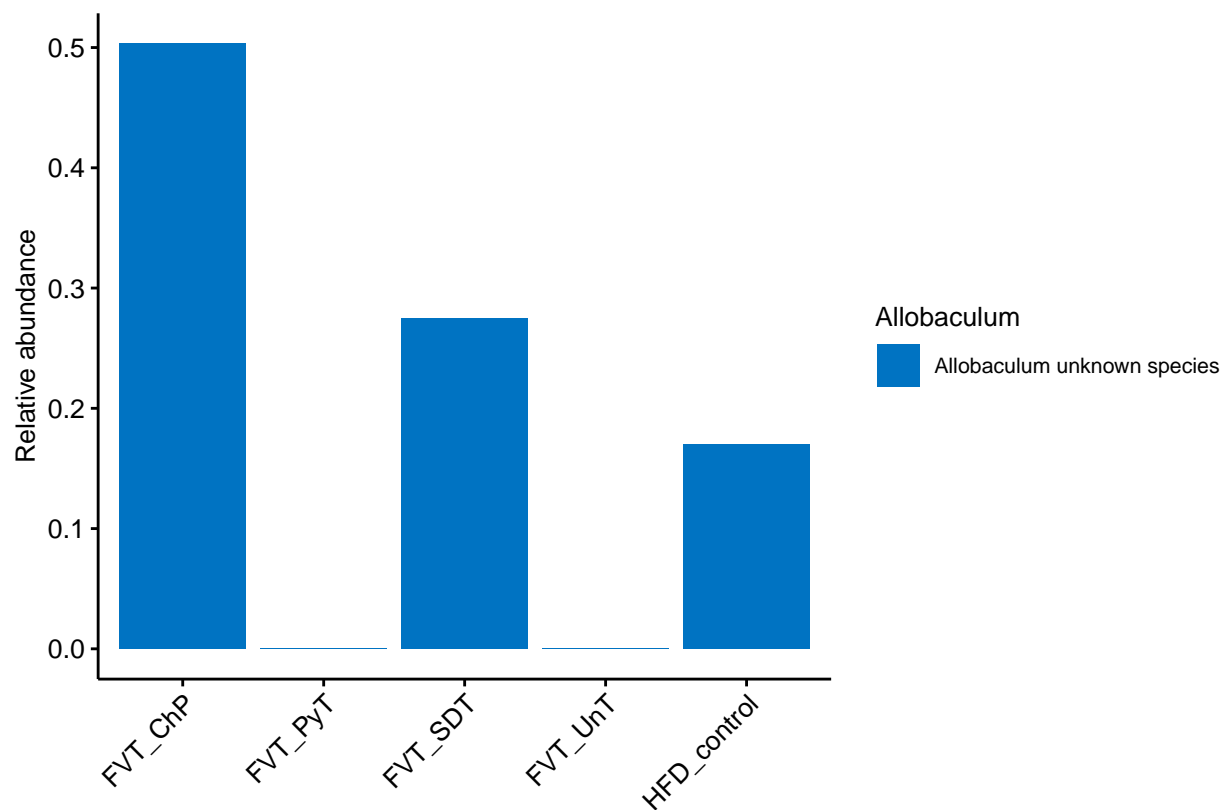


```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.033825 -0.015492 -0.008215  0.013281  0.082660
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.0221704  0.0088336   2.510   0.017 *
## TreatmentFVT_ChP  0.0033746  0.0124926   0.270   0.789
## TreatmentFVT_PyT  0.0062864  0.0124926   0.503   0.618
## TreatmentFVT_SDT -0.0003837  0.0129311  -0.030   0.976
## TreatmentFVT_UnT  0.0118951  0.0124926   0.952   0.348
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02499 on 34 degrees of freedom
## Multiple R-squared:  0.03638,    Adjusted R-squared:  -0.07699
## F-statistic: 0.3209 on 4 and 34 DF,  p-value: 0.862

## # A tibble: 39 x 3
## # Groups:   Sample [39]
##   Sample      tax      Mean
```

```
##      <chr>      <chr>      <dbl>
## 1 NXT075Mao189 Allobaculum unknown species 0.165
## 2 NXT075Mao178 Allobaculum unknown species 0.137
## 3 NXT075Mao169 Allobaculum unknown species 0.124
## 4 NXT075Mao172 Allobaculum unknown species 0.0867
## 5 NXT075Mao170 Allobaculum unknown species 0.0705
## 6 NXT075Mao187 Allobaculum unknown species 0.0647
## 7 NXT075Mao166 Allobaculum unknown species 0.0623
## 8 NXT075Mao163 Allobaculum unknown species 0.0580
## 9 NXT075Mao168 Allobaculum unknown species 0.0468
## 10 NXT075Mao270 Allobaculum unknown species 0.0422
## # ... with 29 more rows
```

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



```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.060967 -0.015020 -0.000006  0.000029  0.126094
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
```

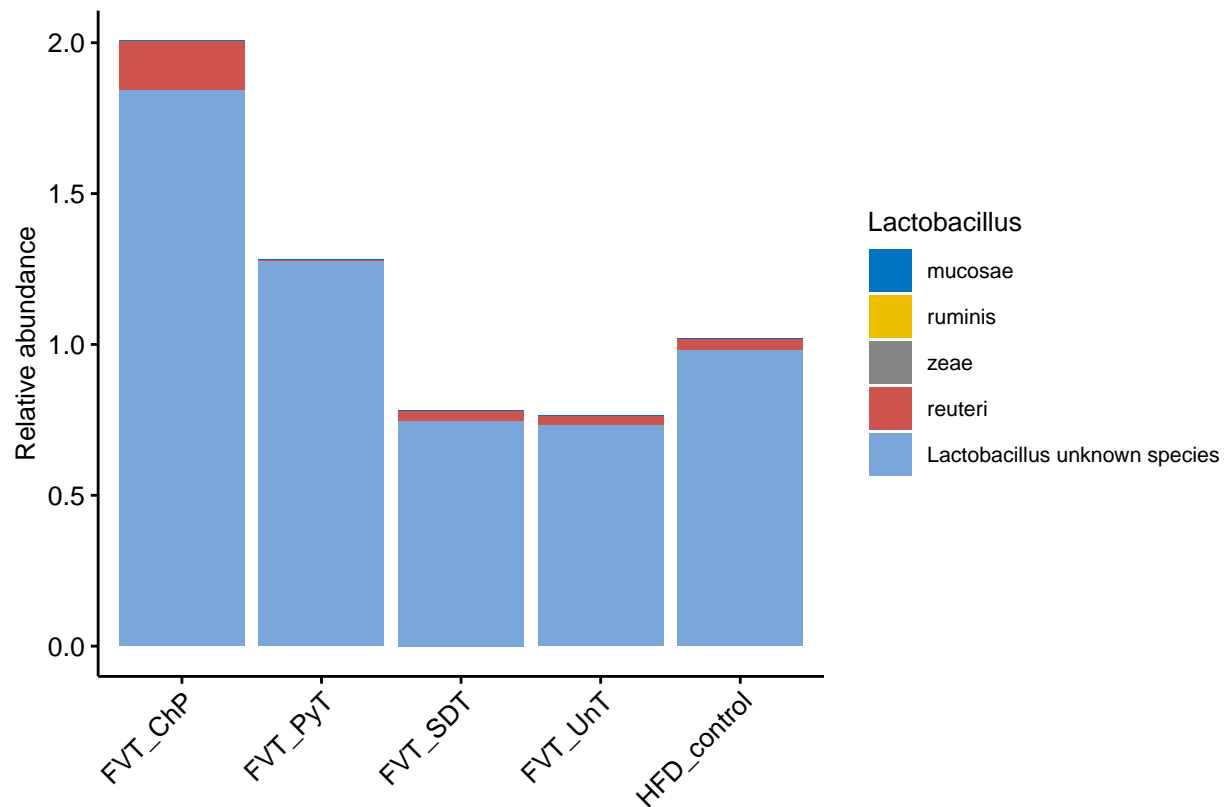
```

## (Intercept)      0.02127    0.01288    1.651    0.1080
## TreatmentFVT_ChP 0.04165    0.01822    2.286    0.0286 *
## TreatmentFVT_PyT -0.02126    0.01822   -1.167    0.2513
## TreatmentFVT_SDT 0.01793    0.01886    0.951    0.3483
## TreatmentFVT_UnT -0.02126    0.01822   -1.167    0.2514
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.03644 on 34 degrees of freedom
## Multiple R-squared:  0.3375, Adjusted R-squared:  0.2596
## F-statistic:  4.33 on 4 and 34 DF,  p-value: 0.006145

## # A tibble: 195 x 3
## # Groups:   Sample [39]
##   Sample      tax      Mean
##   <chr>      <chr>    <dbl>
## 1 NXT075Mao171 Lactobacillus unknown species 0.456
## 2 NXT075Mao189 Lactobacillus unknown species 0.362
## 3 NXT075Mao266 Lactobacillus unknown species 0.330
## 4 NXT075Mao280 Lactobacillus unknown species 0.302
## 5 NXT075Mao169 Lactobacillus unknown species 0.282
## 6 NXT075Mao170 Lactobacillus unknown species 0.267
## 7 NXT075Mao177 Lactobacillus unknown species 0.264
## 8 NXT075Mao178 Lactobacillus unknown species 0.255
## 9 NXT075Mao187 Lactobacillus unknown species 0.249
## 10 NXT075Mao278 Lactobacillus unknown species 0.222
## # ... with 185 more rows

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

```

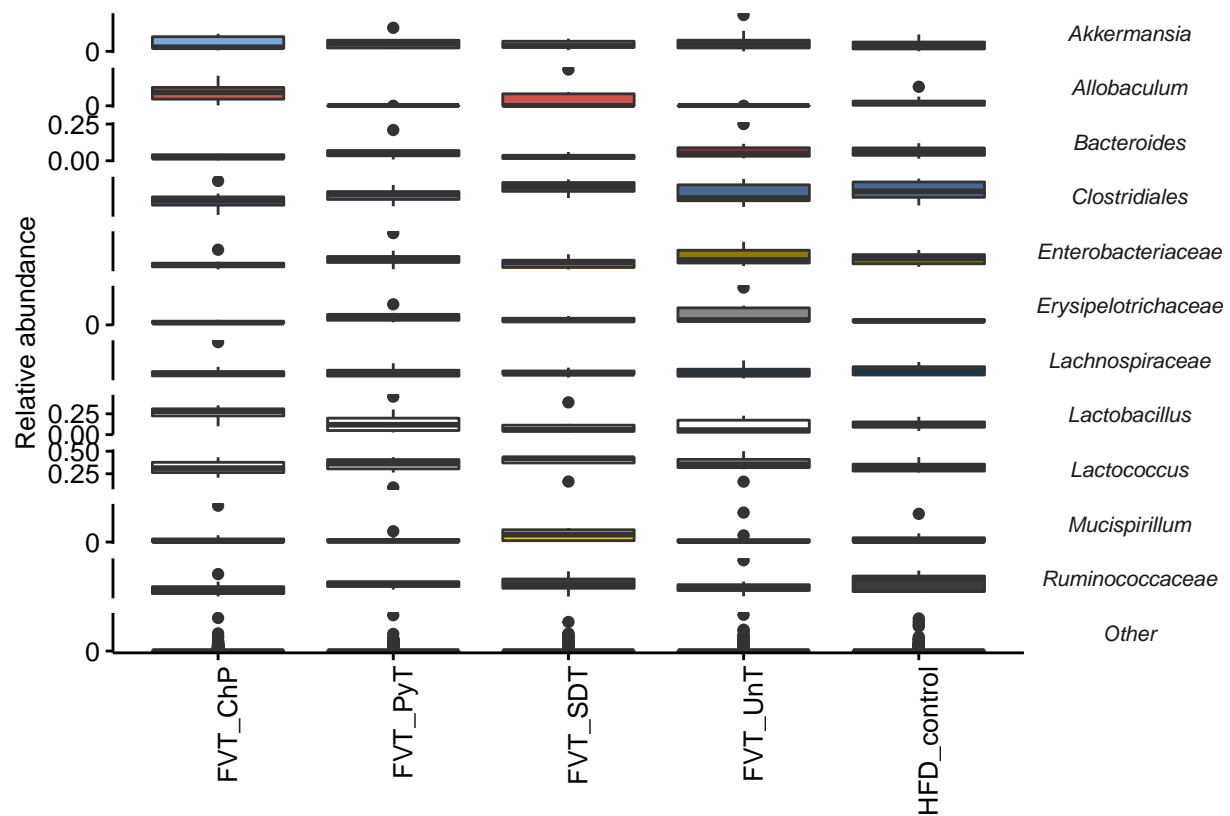


```
##
## Call:
## lm(formula = Abundance ~ Treatment, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.05016 -0.03205 -0.02227 -0.01863  0.42405
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.025453   0.011815   2.154  0.0325 *
## TreatmentFVT_ChP  0.024702   0.016708   1.478  0.1409
## TreatmentFVT_PyT  0.006592   0.016708   0.395  0.6936
## TreatmentFVT_SDT -0.003182   0.017295  -0.184  0.8542
## TreatmentFVT_UnT -0.006302   0.016708  -0.377  0.7065
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.07472 on 190 degrees of freedom
## Multiple R-squared:  0.02216,    Adjusted R-squared:  0.001575
## F-statistic: 1.077 on 4 and 190 DF,  p-value: 0.3694

## # A tibble: 4,485 x 3
## # Groups:   Sample [39]
##   Sample      tax      Mean
```

```
##      <chr>      <chr>      <dbl>
## 1 NXT075Mao180 Lactococcus 0.500
## 2 NXT075Mao179 Lactococcus 0.475
## 3 NXT075Mao171 Lactobacillus 0.456
## 4 NXT075Mao162 Lactococcus 0.445
## 5 NXT075Mao185 Lactococcus 0.435
## 6 NXT075Mao272 Lactococcus 0.434
## 7 NXT075Mao274 Lactococcus 0.434
## 8 NXT075Mao186 Lactococcus 0.433
## 9 NXT075Mao268 Lactococcus 0.429
## 10 NXT075Mao159 Lactococcus 0.425
## # ... with 4,475 more rows
```

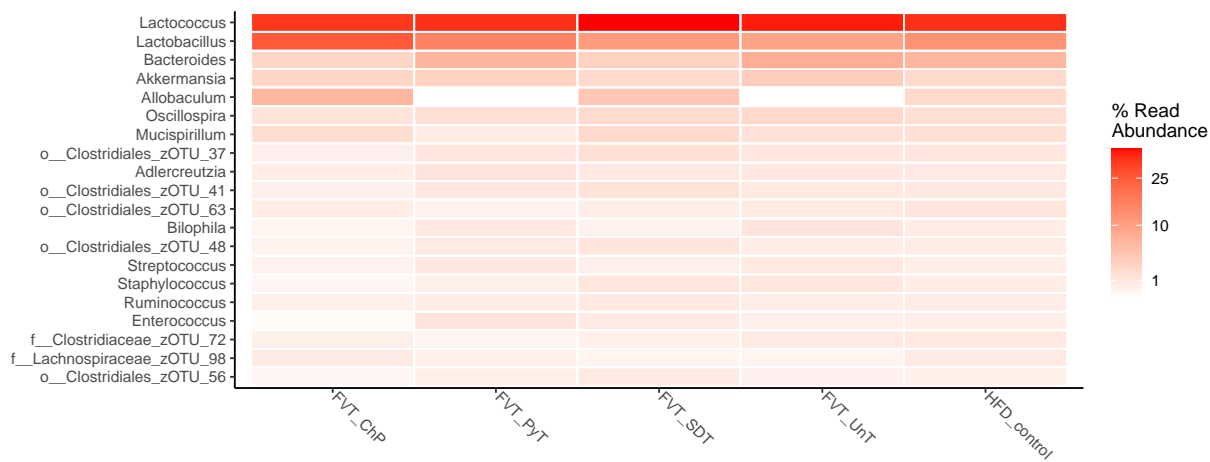
```
## Warning: This manual palette can handle a maximum of 10 values. You have
## supplied 12.
```





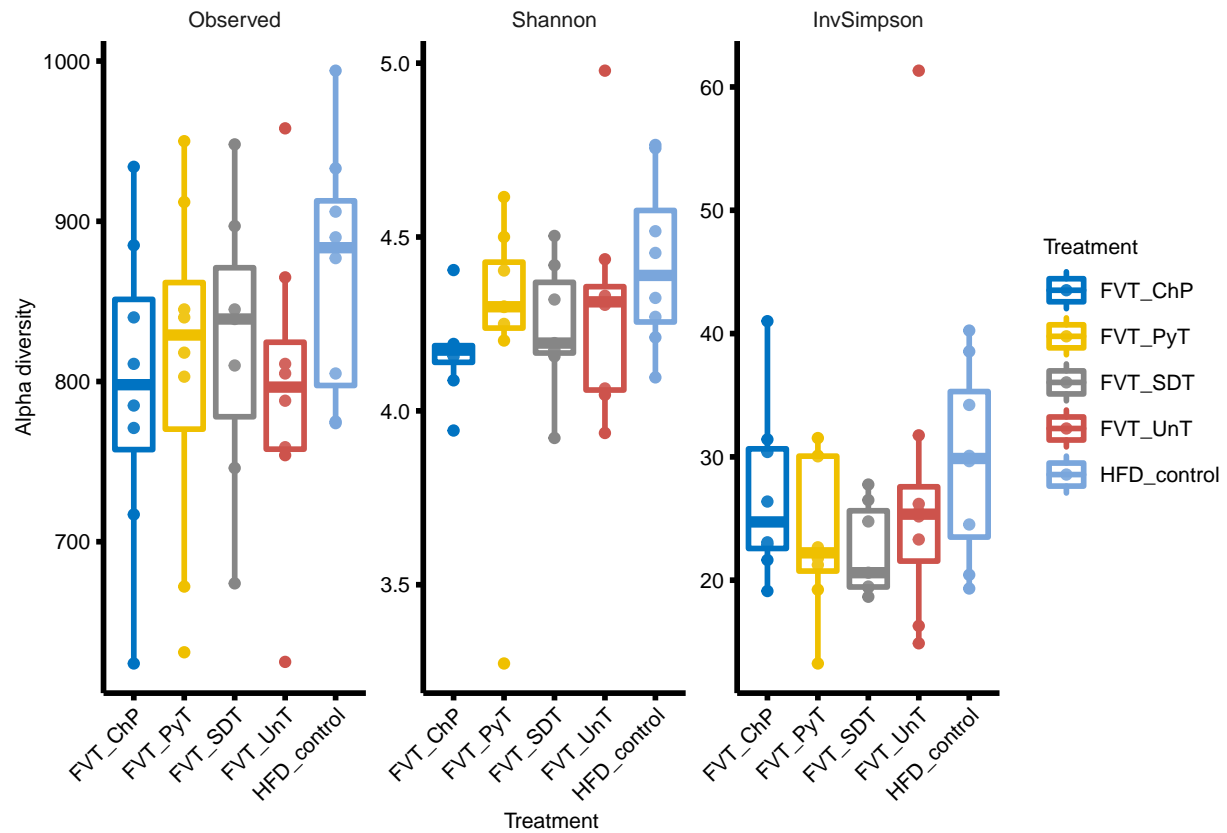
# Abundance heatmaps

## 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	0.06534750	-0.3379103	0.4686053	0.9898334
FVT_SDT-FVT_ChP	0.07662496	-0.3407865	0.4940364	0.9837733
FVT_UnT-FVT_ChP	0.13735881	-0.2658990	0.5406166	0.8619903
HFD_control-FVT_ChP	0.25916483	-0.1440929	0.6624226	0.3625926
FVT_SDT-FVT_PyT	0.01127747	-0.4061340	0.4286889	0.9999912
FVT_UnT-FVT_PyT	0.07201131	-0.3312465	0.4752691	0.9853589
HFD_control-FVT_PyT	0.19381734	-0.2094404	0.5970751	0.6417513
FVT_UnT-FVT_SDT	0.06073385	-0.3566776	0.4781453	0.9932485
HFD_control-FVT_SDT	0.18253987	-0.2348716	0.5999513	0.7171746
HFD_control-FVT_UnT	0.12180602	-0.2814518	0.5250638	0.9059377

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

```

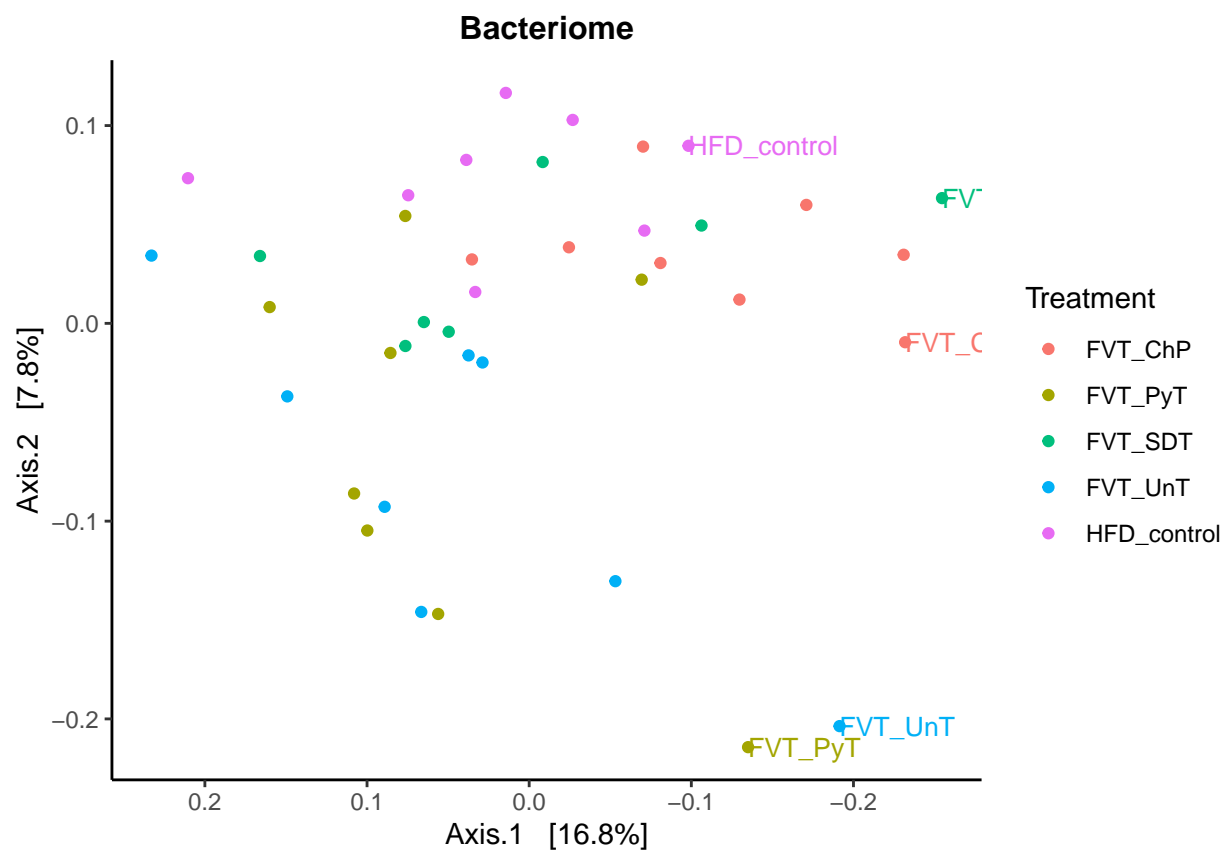
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.95699 -0.09204  0.01961  0.11299  0.67599
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    4.42424    0.09902  44.678  <2e-16 ***
## variableFVT_ChP -0.25916    0.14004  -1.851   0.0729 .
## variableFVT_PyT -0.19382    0.14004  -1.384   0.1754
## variableFVT_SDT -0.18254    0.14496  -1.259   0.2165
## variableFVT_UnT -0.12181    0.14004  -0.870   0.3905
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2801 on 34 degrees of freedom
## Multiple R-squared:  0.1025, Adjusted R-squared:  -0.003143
## F-statistic: 0.9702 on 4 and 34 DF,  p-value: 0.4365

##
## Call:
## lm(formula = Shannon ~ variable, data = rich)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.95699 -0.09204  0.01961  0.11299  0.67599
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    4.30244    0.09902  43.448  <2e-16 ***
## variableHFD_control  0.12181    0.14004   0.870   0.391
## variableFVT_ChP    -0.13736    0.14004  -0.981   0.334
## variableFVT_PyT    -0.07201    0.14004  -0.514   0.610
## variableFVT_SDT    -0.06073    0.14496  -0.419   0.678
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2801 on 34 degrees of freedom
## Multiple R-squared:  0.1025, Adjusted R-squared:  -0.003143
## F-statistic: 0.9702 on 4 and 34 DF,  p-value: 0.4365

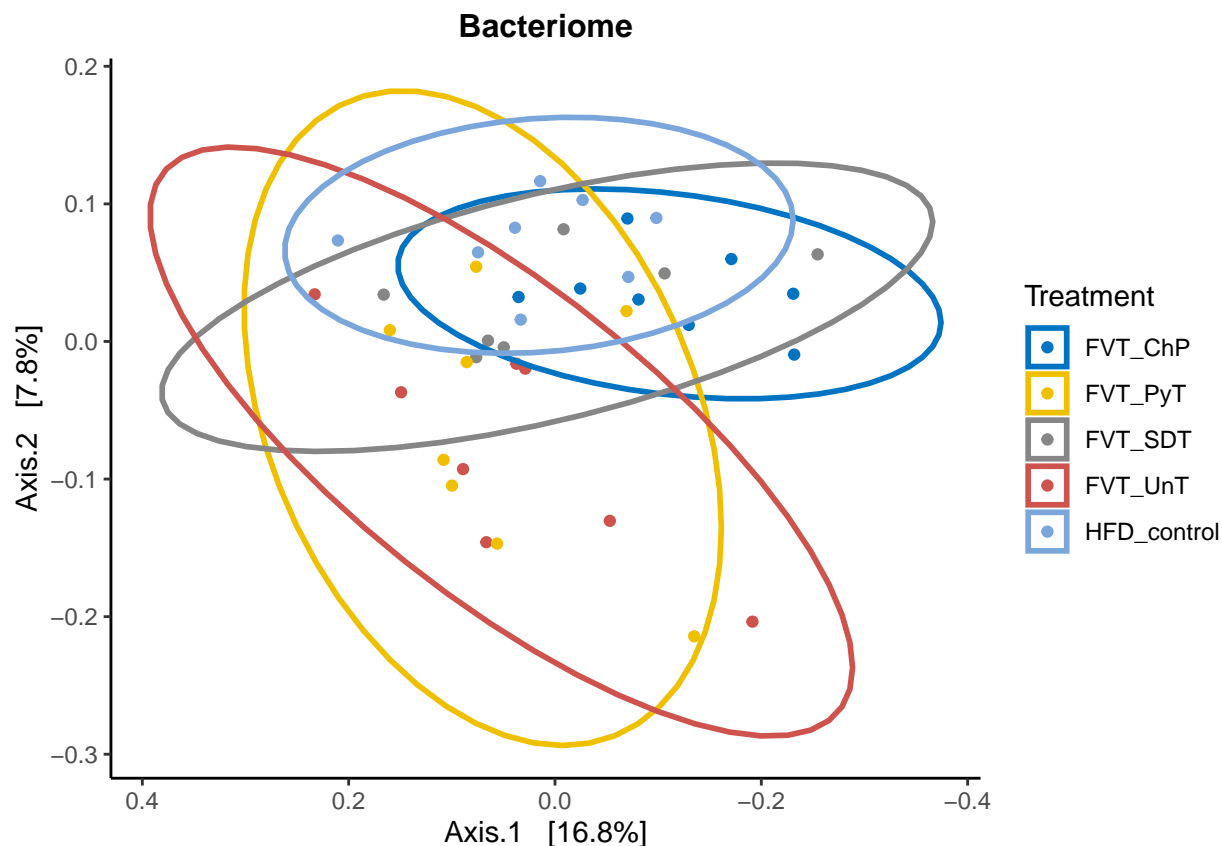
```

# Beta diversity

basically the mouse ID.



## Treatment



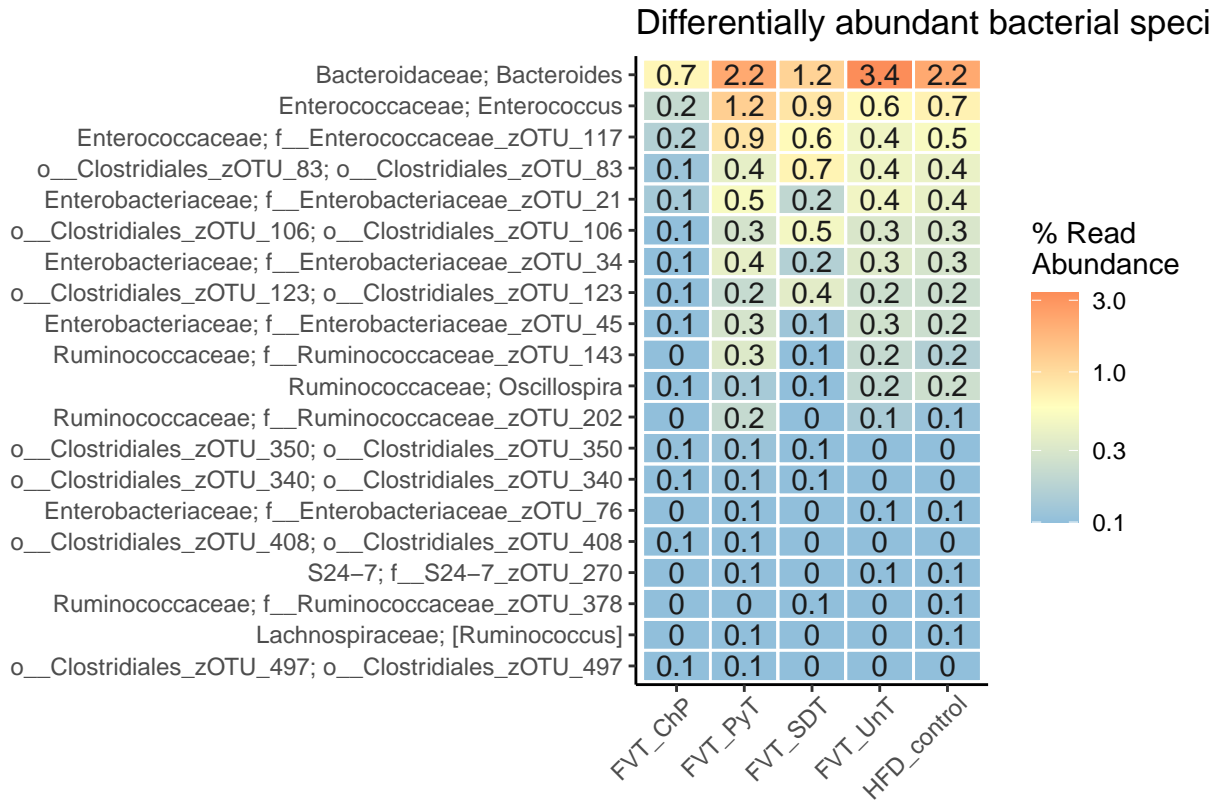
Adonis and pairwise permanova analysis - Treatment

```
## 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   0.5902 0.17454 1.7973  0.001 ***
## Residual  34   2.7914 0.82546
## Total     38   3.3817 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

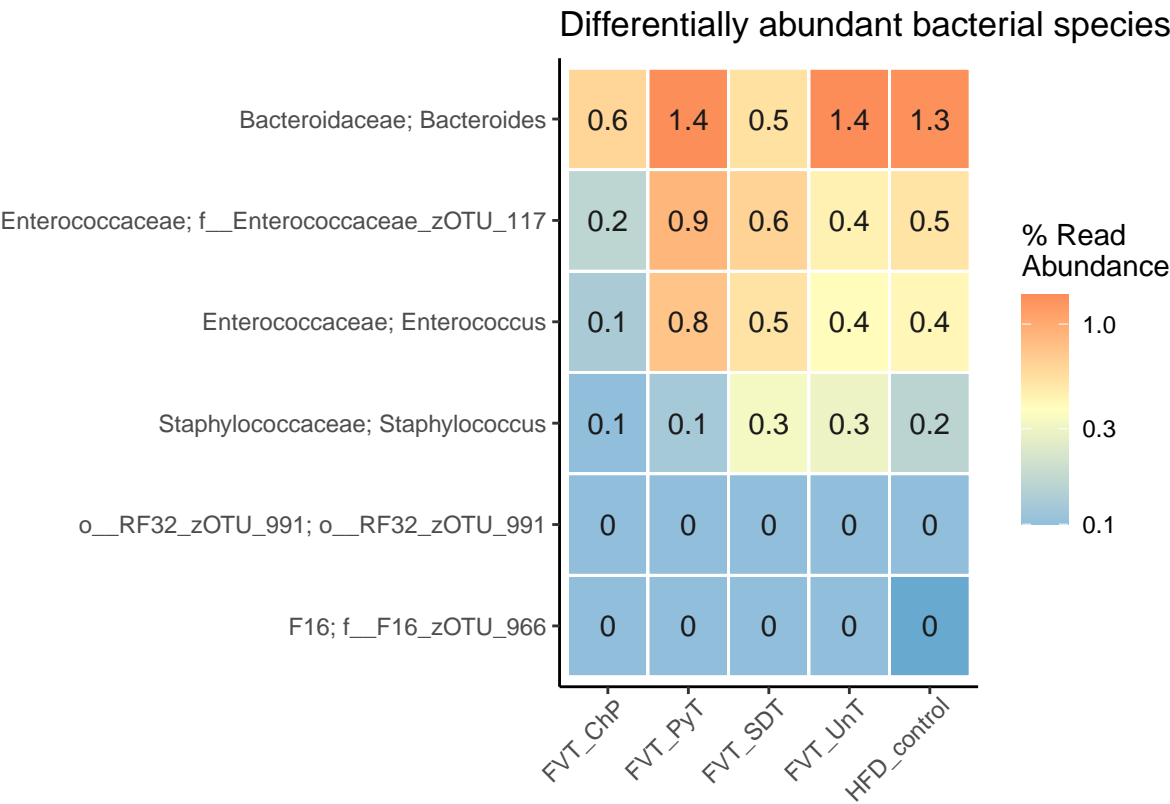
##	X1	X2	R2	pval	pvalBon	pvalFDR
## 1	FVT_ChP	FVT_PyT	0.15687400	0.001	0.01	0.010
## 2	FVT_ChP	FVT_SDT	0.11089517	0.049	0.49	0.070
## 3	FVT_ChP	FVT_UnT	0.14288308	0.004	0.04	0.009
## 4	FVT_ChP	HFD_control	0.12636392	0.004	0.04	0.009
## 5	FVT_PyT	FVT_SDT	0.10991761	0.034	0.34	0.057
## 6	FVT_PyT	FVT_UnT	0.07555661	0.239	2.39	0.239
## 7	FVT_PyT	HFD_control	0.11737362	0.002	0.02	0.010
## 8	FVT_SDT	FVT_UnT	0.10111868	0.065	0.65	0.081

```
## 9  FVT_SDT HFD_control 0.08368227 0.195 1.95 0.217
## 10 FVT_UnT HFD_control 0.12248504 0.003 0.03 0.010
```

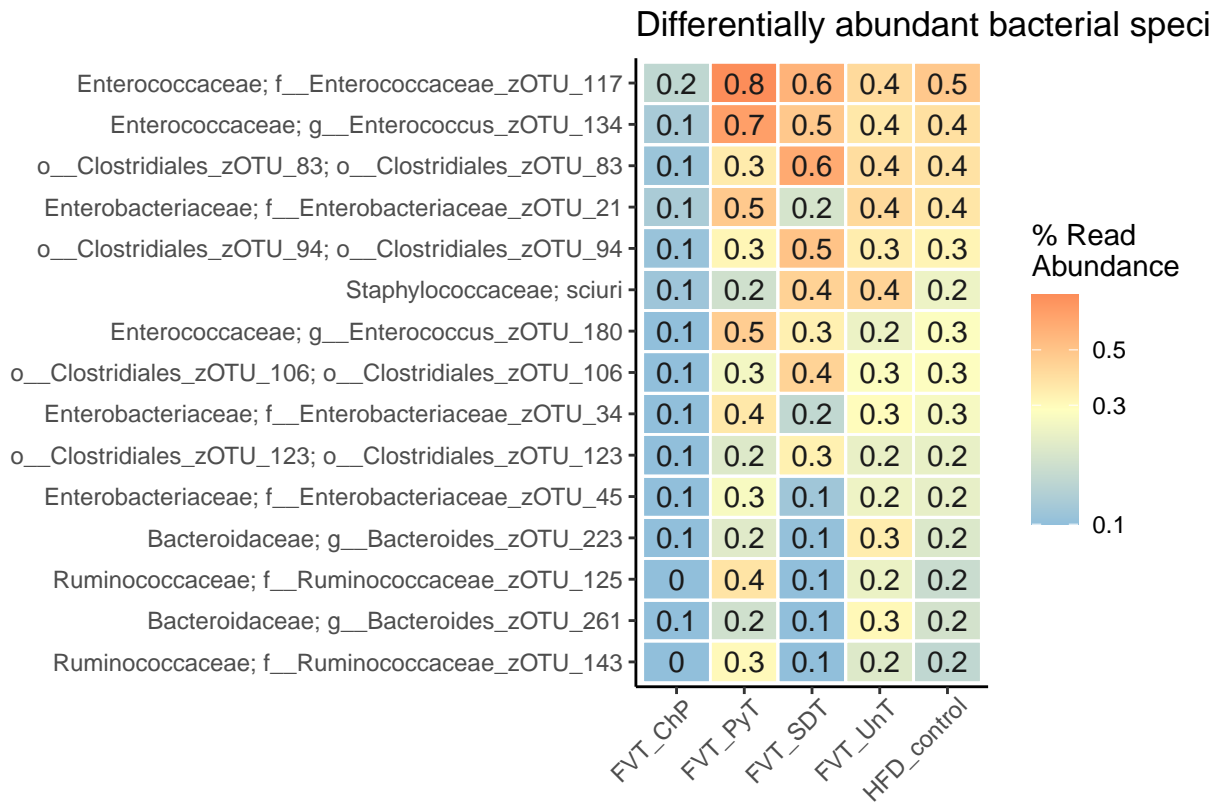
# Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level - Comparing ChP vs HFD



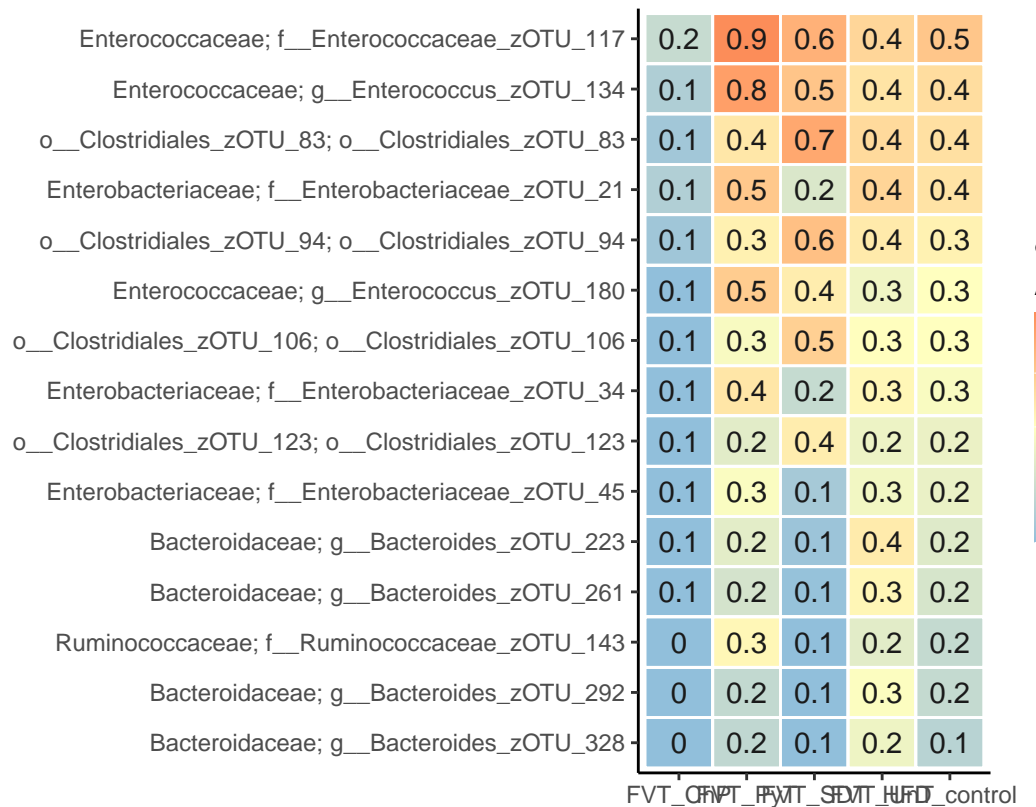
Bacteriome - Deseq2 - Treatment - Collapsed on Genus-level



## Bacteriome - Deseq2 - Treatment





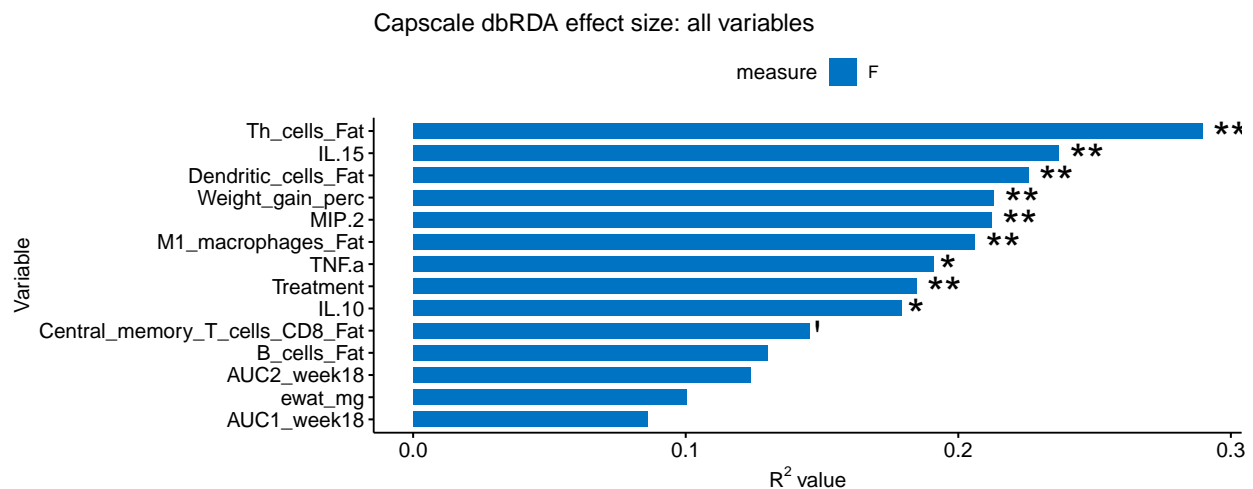


Deseq2 - defined comparison

## Bacteriome - Effect-size

### Non-constrained

#### Capscale - independent effect sizes



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

