

The effects of hypoxia on human gut microbiota in individuals with T2DM

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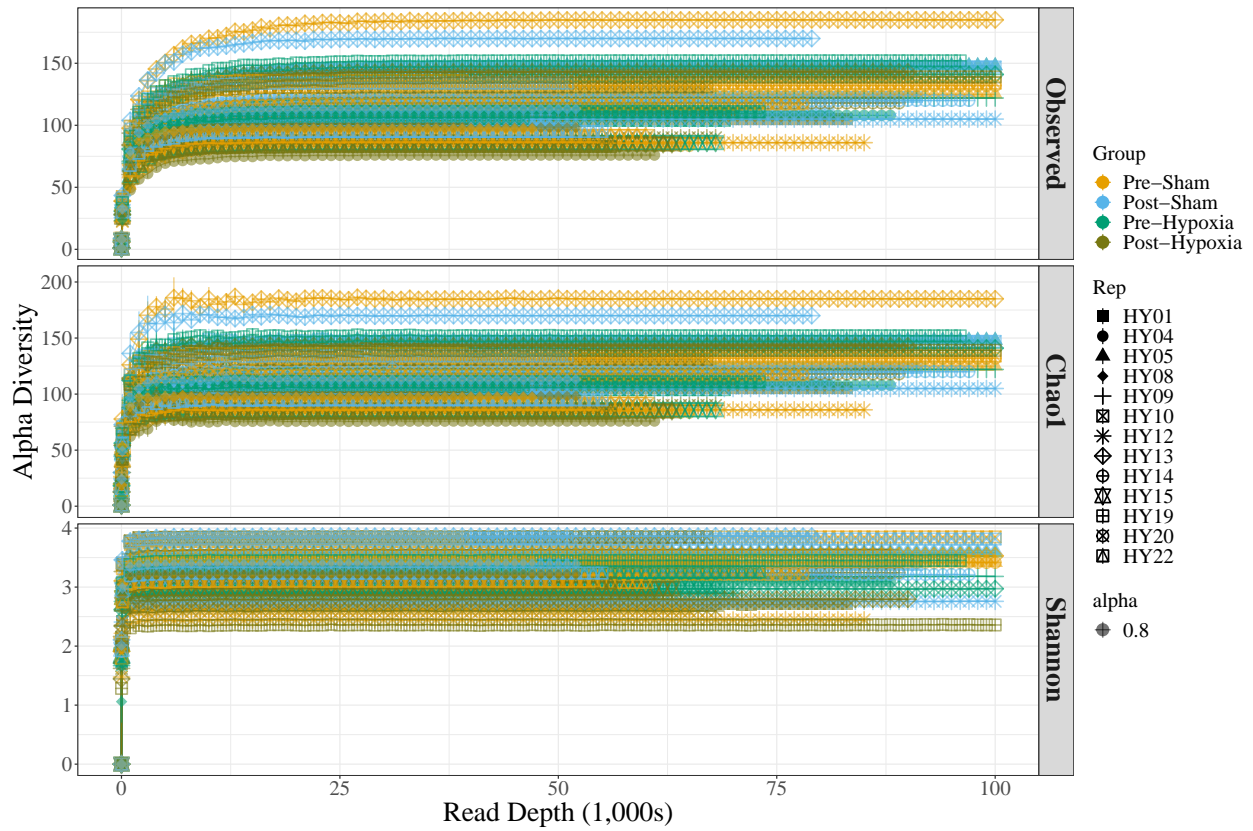
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Fix for <code>stat_compare_means()</code> function to allow Times New Roman font.	
Thank you	

Section 1: Quality Control - all data

Rarefaction curve

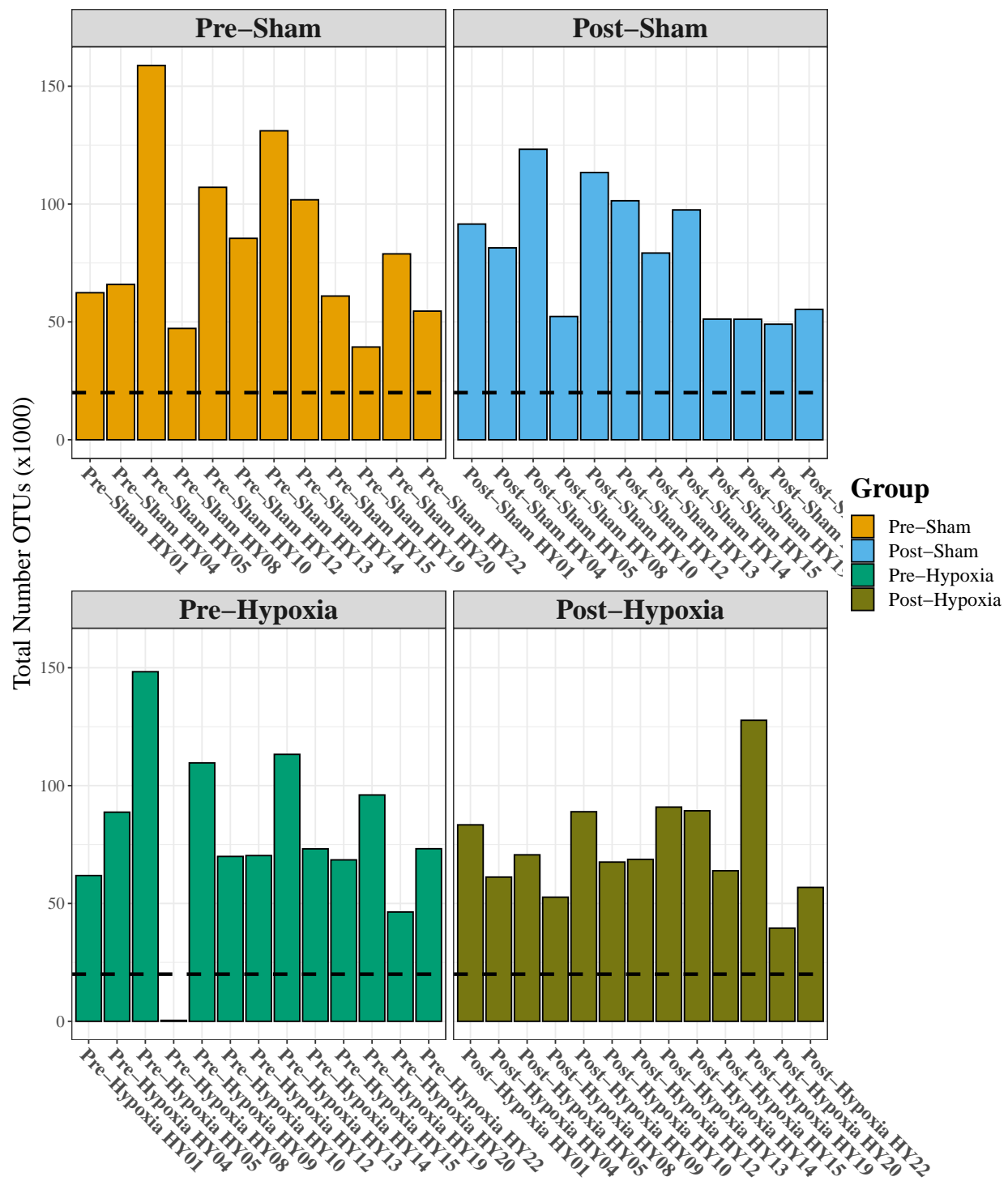
We can check our coverage to be sure that we have ample coverage to allow us to detect all species present in these samples. We estimate this by using rarefaction curves, which show the diversity when looking at random subsets of the data. The idea here is that if we have saturated the sample, we should see the same diversity if we only look at a subset of the sample. By looking at the curve of successive sub-populations, we can see at what coverage we see saturation. This will appear as the point at which the curve becomes asymptotic:

Plot



Taxa count per sample - All data

The following plot shows the total number of observed taxa (OTUs or ASVs) per sample, with the minimum number of taxa specified above (min_otus) highlighted with a dashed line. Samples lower than this will be removed below in Section 2.



Section 2: Quality Control - Rarefied and filtered data

Sample filtering and rarefaction

In this section, the data will be filtered to remove all samples with fewer than 2×10^4 OTUs in the data set. The remaining data will be rarefied to reduce all samples to the same depth, which will be the lowest depth from the remaining samples following filtering.

```
## Check sample sums
sample_sums_values <- sample_sums(phyloseq_dat)

## Find samples with sums below 20,000
samples_below_min_otus <- names(sample_sums_values[sample_sums_values <= min_otus])

## Print
print(samples_below_min_otus)

## [1] "HF_EXP001_EXP002_0051"

## Now identify UIN number to remove
filter_UIN_to_remove <- sample_data(phyloseq_dat)$Rep[sample_data(phyloseq_dat)$sample_code %in% samples_below_min_otus]

## Print
print(filter_UIN_to_remove)

## [1] "HY08"

## Remove HY09 from analysis as it did not provide samples for Sham condition
filter_UIN_to_remove <- c(filter_UIN_to_remove, "HY09")

## Print
print(filter_UIN_to_remove)

## [1] "HY08" "HY09"

## Filter to remove UINs identified above
phyloseq_dat_filter <- prune_samples(!(sample_data(phyloseq_dat)$Rep %in% filter_UIN_to_remove), phyloseq_dat)

## tax_glom to species level
phyloseq_dat_filter <- tax_glom(phyloseq_dat_filter, taxrank = "Species")

## Create a non rarefied phyloseq object with filtered samples for DA analysis
phyloseq_dat_deseq <- phyloseq_dat_filter

## Rarefy the data to the minimum otu depth
rarefy_otu_depth <- min(sample_sums(phyloseq_dat_filter))
cat("Rarefication of data to", rarefy_otu_depth, "OTUs:\n")

## Rarefication of data to 37361 OTUs:
```

```
phyloseq_dat_filter <- rarefy_even_depth(phyloseq_dat_filter, rarefy_otu_depth, verbose = FALSE)
```

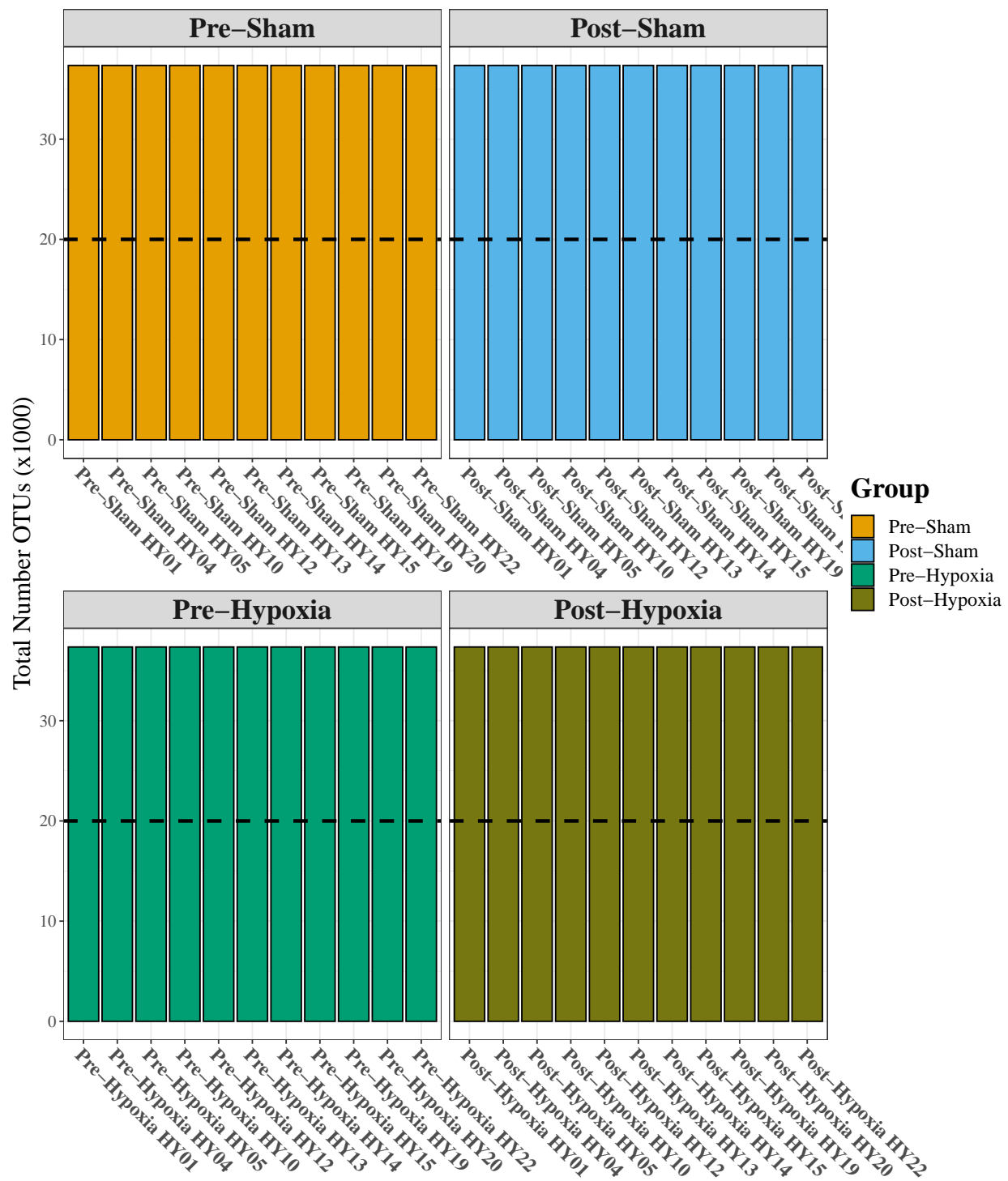
Summary N for Analysis

Below, a summary of the n taken forward for analysis is provided:

```
## group_data
##      Pre-Sham      Post-Sham  Pre-Hypoxia  Post-Hypoxia
##           11             11           11           11
```

Taxa count per sample - Rarefied and filtered data

The following plot shows the total number of observed taxa (OTUs or ASVs) per sample after rarefaction and filtering of samples to remove low coverage samples, with the minimum number of taxa specified above (min_otus) highlighted with a dashed line. Samples lower than this will be removed below in Section 2. All samples should have the same depth. If this is not the case, then please debug the code.



Alpha diversity - Rarefied and filtered data

Setup

Calculate Delta

Normality

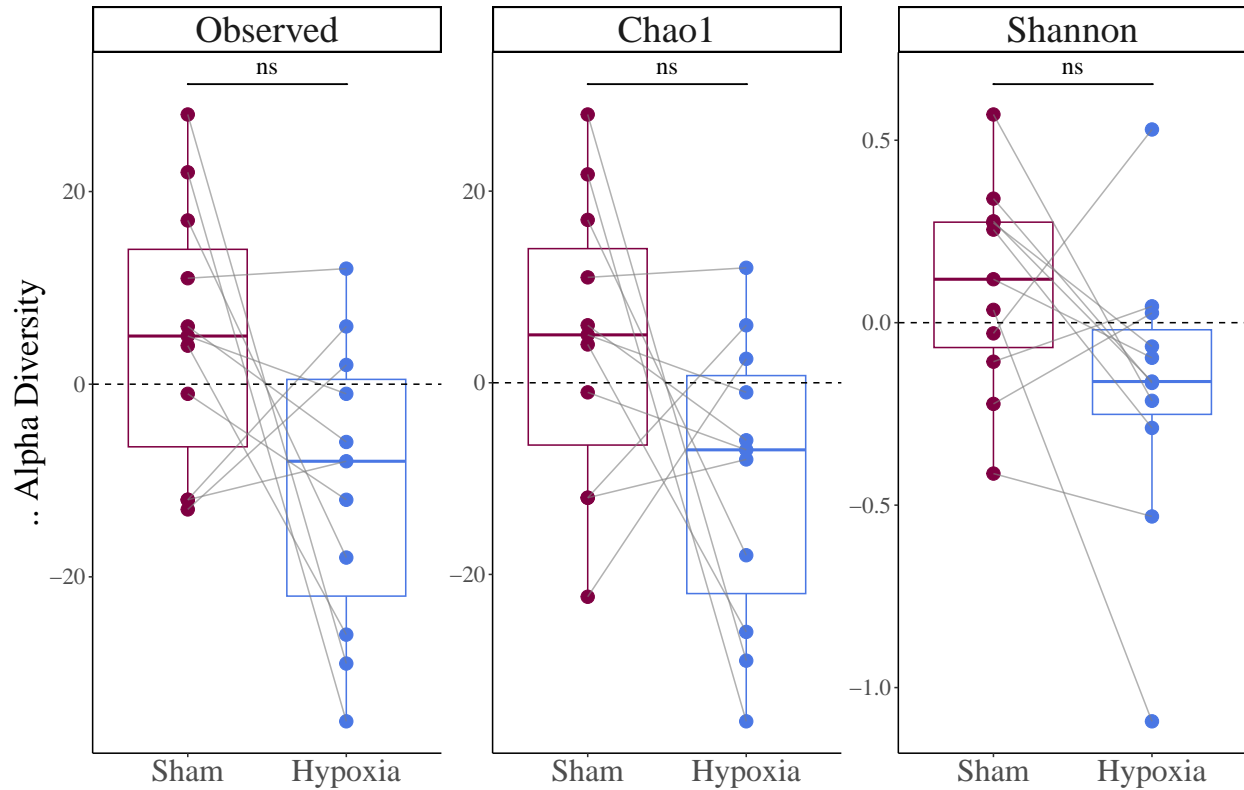
```
##
## DESCRIPTIVES
##
## Descriptives
##
##          Group      Observed      Chao1      Shannon
##
## N          Sham          11          11          11
##          Hypoxia         11          11          11
## Missing    Sham           0           0           0
##          Hypoxia          0           0           0
## Mean       Sham       5.000000      4.128788      0.09985707
##          Hypoxia     -10.45455     -9.984848     -0.1830139
## Median     Sham       5.000000      5.000000      0.1190718
##          Hypoxia     -8.000000     -7.000000     -0.1612773
## Standard deviation Sham      13.92121      15.31119      0.2822141
##          Hypoxia      15.14175      15.26114      0.3982451
## IQR        Sham      20.50000      20.50000      0.3435639
##          Hypoxia      22.50000      22.75000      0.2318430
## Minimum    Sham     -13.00000     -22.33333     -0.4135012
##          Hypoxia     -35.00000     -35.33333     -1.092484
## Maximum    Sham      28.00000      28.00000      0.5705666
##          Hypoxia      12.00000      12.00000      0.5294839
## Skewness    Sham       0.1427020     -0.1878951     -0.2574851
##          Hypoxia     -0.2358660     -0.3543918     -0.7944972
## Std. error skewness Sham       0.6606875      0.6606875      0.6606875
##          Hypoxia       0.6606875      0.6606875      0.6606875
## Kurtosis    Sham     -0.9513175     -0.5733236     -0.1990667
##          Hypoxia     -1.017531     -0.9775631      2.841489
## Std. error kurtosis Sham       1.279416      1.279416      1.279416
##          Hypoxia       1.279416      1.279416      1.279416
## Shapiro-Wilk W Sham       0.9384028      0.9741925      0.9793873
##          Hypoxia       0.9678520      0.9536025      0.9027493
## Shapiro-Wilk p Sham       0.5019076      0.9254309      0.9626598
##          Hypoxia       0.8640588      0.6902051      0.1996538
##
```

t-test and effect size

```
##
## PAIRED SAMPLES T-TEST
##
## Paired Samples T-Test
##
##                                     statistic      df      p
```

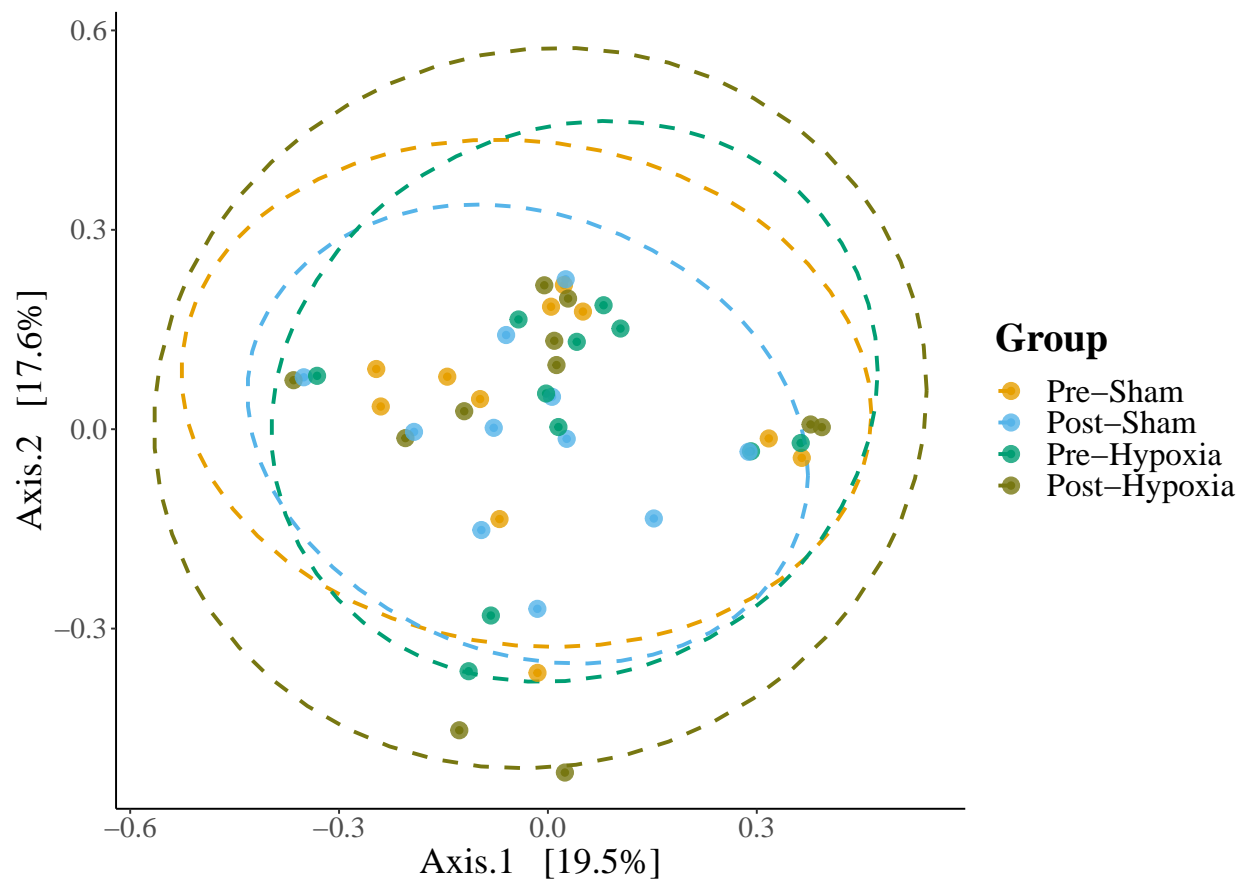
```
##
## Observed_Sham Observed_Hypoxia Student's t 1.956610 10.00000 0.0788788 Cohen's
## Chao1_Sham Chao1_Hypoxia Student's t 1.696284 10.00000 0.1206870 Cohen's
## Shannon_Sham Shannon_Hypoxia Student's t 1.946815 10.00000 0.0801706 Cohen's
##
## Note. H <sub>Measure 1 - Measure 2</sub> 0
```

Plot



Beta diversity - Rarefied and filtered data

The following principal coordinate analysis (PCoA) plot shows the beta diversity following rarefaction and filtering of samples, based on the Bray Curtis distance between samples.



PERMANOVA

This section will use permutational MANOVA (PERMANOVA) to look for significant effects of the Group variable on the abundance. Dissimilarity is again based on the Bray Curtis difference.

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = OTU_dist ~ Group, data = ind_dat, permutations = 9999, method = "bray")
##      Df SumOfSqs      R2      F Pr(>F)
## Group   3   0.1399 0.02609 0.3572     1
## Residual 40   5.2222 0.97391
## Total   43   5.3620 1.00000
```

Section 3: Microbial Abundance

Normalise

Here we normalise the taxa counts to represent the percentage of all taxa.

Summarise

Here we summarise the unique taxa at each taxonomic level on the non-rarefied, count data.

```
## [1] "Firmicutes"          "Bacteroidota"        "Verrucomicrobiota"
## [4] "Actinobacteriota"    "Proteobacteria"      "Desulfobacterota"
## [7] "Fusobacteriota"      "Campylobacterota"

## [1] "Bacilli"             "Bacteroidia"         "Clostridia"
## [4] "Verrucomicrobiae"    "Negativicutes"       "Coriobacteriia"
## [7] "Gammaproteobacteria" "Actinobacteria"      "Desulfovibrionia"
## [10] "Fusobacteriia"       "Campylobacteria"

## [1] "Lactobacillales"          "Bacteroidales"
## [3] "Lachnospirales"          "Oscillospirales"
## [5] "Verrucomicrobiales"      "Erysipelotrichales"
## [7] "Veillonellales-Selenomonadales" "Clostridiales"
## [9] "Coriobacteriales"        "Christensenellales"
## [11] "Enterobacterales"        "Peptostreptococcales-Tissierellales"
## [13] "Peptococcales"           "Actinomycetales"
## [15] "Monoglobales"            "Acidaminococcales"
## [17] "Corynebacteriales"       "Desulfovibrionales"
## [19] "Micrococcales"           "Burkholderiales"
## [21] "Eubacteriales"           "Bifidobacteriales"
## [23] "Fusobacteriales"         "Pseudomonadales"
## [25] "Xanthomonadales"         "Campylobacterales"
## [27] "Bacillales"              "Staphylococcales"
## [29] "Exiguobacterales"

## [1] "Lactobacillaceae"          "Prevotellaceae"
## [3] "Lachnospiraceae"          "Ruminococcaceae"
## [5] "Akermansiaceae"           "Erysipelotrichaceae"
## [7] "Erysipelatoclostridiaceae" "Streptococcaceae"
## [9] "Bacteroidaceae"           "Enterococcaceae"
## [11] "Selenomonadaceae"         "Clostridiaceae"
## [13] "Coriobacteriaceae"        "Christensenellaceae"
## [15] "Enterobacteriaceae"       "Peptostreptococcaceae"
## [17] "Veillonellaceae"          "Atopobiaceae"
## [19] "Tannerellaceae"           "Oscillospiraceae"
## [21] "Butyrericoccaceae"        "Peptococcaceae"
## [23] "Rikenellaceae"            "Eggerthellaceae"
## [25] "Barnesiellaceae"          "Actinomycetaceae"
## [27] "Monoglobaceae"            "Acidaminococcaceae"
## [29] "Anaerovoracaceae"         "Corynebacteriaceae"
## [31] "Desulfovibrionaceae"       "Micrococcaceae"
## [33] "Sutterellaceae"           "Marinifilaceae"
## [35] "Eubacteriaceae"           "Pasteurellaceae"
## [37] "Bifidobacteriaceae"       "Family XI"
## [39] "Morganellaceae"           "Fusobacteriaceae"
## [41] "Carnobacteriaceae"        "Porphyromonadaceae"
## [43] "Moraxellaceae"            "Defluviitaleaceae"
## [45] "Oxalobacteraceae"         "Anaerofustaceae"
## [47] "Xanthomonadaceae"         "Leptotrichiaceae"
```

## [49] "Succinivibrionaceae"	"Coriobacteriales Incertae Sedis"
## [51] "Campylobacteraceae"	"Bacillaceae"
## [53] "Listeriaceae"	"Staphylococcaceae"
## [55] "Hafniaceae"	"Pseudomonadaceae"
## [57] "Vibrionaceae"	"Exiguobacteraceae"
## [1] "Limosilactobacillus"	"Prevotella_9"
## [3] "Blautia"	"Ruminococcus"
## [5] "Akkermansia"	"Agathobacter"
## [7] "Holdemanella"	"Dorea"
## [9] "[Eubacterium] hallii group"	"Erysipelotrichaceae UCG-003"
## [11] "Streptococcus"	"Faecalibacterium"
## [13] "Bacteroides"	"Enterococcus"
## [15] "Lachnoclostridium"	"Fusicatenibacter"
## [17] "[Ruminococcus] torques group"	"Megamonas"
## [19] "Clostridium sensu stricto 1"	"Subdoligranulum"
## [21] "Collinsella"	"Incertae Sedis"
## [23] "[Ruminococcus] gauvreauui group"	"Coprococcus"
## [25] "Anaerostipes"	"Christensenellaceae R-7 group"
## [27] "Klebsiella"	"Lachnospiraceae UCG-008"
## [29] "Tyzzerella"	"Prevotella_7"
## [31] "Escherichia-Shigella"	"Roseburia"
## [33] "Alloprevotella"	"Lactobacillus"
## [35] "Faecalitalea"	"Lachnospiraceae ND3007 group"
## [37] "CAG-352"	"Ligilactobacillus"
## [39] "Romboutsia"	"Dialister"
## [41] "Prevotellaceae UCG-001"	"[Eubacterium] ventriosum group"
## [43] "Olsenella"	"Megasphaera"
## [45] "Frisingicoccus"	"Catenibacterium"
## [47] "Parabacteroides"	"Marvinbryantia"
## [49] "UCG-002"	"Erysipelatoclostridium"
## [51] "Butyricicoccus"	"Terrisporobacter"
## [53] "[Ruminococcus] gnavus group"	"Lachnospiraceae FCS020 group"
## [55] "Lachnospiraceae NC2004 group"	"Peptococcus"
## [57] "Lachnospiraceae NK4A136 group"	"Alistipes"
## [59] "CAG-56"	"HT002"
## [61] "Eggerthella"	"Lachnospira"
## [63] "Veillonella"	"UCG-005"
## [65] "NK4A214 group"	"Barnesiella"
## [67] "Actinomyces"	"[Clostridium] innocuum group"
## [69] "Lachnospiraceae UCG-004"	"Monoglobus"
## [71] "Lacticaseibacillus"	"Senegalimassilia"
## [73] "Intestinibacter"	"[Eubacterium] ruminantium group"
## [75] "Rikenellaceae RC9 gut group"	"Phascolarctobacterium"
## [77] "UCG-003"	"Eisenbergiella"
## [79] "Family XIII AD3011 group"	"[Eubacterium] siraeum group"
## [81] "Corynebacterium"	"Slackia"
## [83] "Negativibacillus"	"UBA1819"
## [85] "[Eubacterium] eligens group"	"[Eubacterium] xylanophilum group"
## [87] "Turicibacter"	"Coprobacillus"
## [89] "Dielma"	"Colidextribacter"
## [91] "Desulfovibrio"	"Rothia"
## [93] "Lactiplantibacillus"	"Acidaminococcus"
## [95] "Moryella"	"Enterorhabdus"

## [97]	"[Eubacterium] oxidoreducens group"	"Paludicola"
## [99]	"Sutterella"	"Howardella"
## [101]	"Prevotella"	"Odoribacter"
## [103]	"Enterobacter"	"Hungatella"
## [105]	"Lactococcus"	"Enorma"
## [107]	"Lachnospiraceae UCG-001"	"Paraprevotella"
## [109]	"[Eubacterium] brachy group"	"Eubacterium"
## [111]	"Family XIII UCG-001"	"Haemophilus"
## [113]	"Weissella"	"Libanicoccus"
## [115]	"Sellimonas"	"DTU089"
## [117]	"Catenibacillus"	"Bifidobacterium"
## [119]	"Adlercreutzia"	"Lachnospiraceae UCG-006"
## [121]	"Oscillibacter"	"Latilactobacillus"
## [123]	"Flavonifractor"	"Bilophila"
## [125]	"Merdibacter"	"Butyricimonas"
## [127]	"[Eubacterium] nodatum group"	"Butyrivibrio"
## [129]	"Tuzzerella"	"Ezakiella"
## [131]	"Citrobacter"	"Shuttleworthia"
## [133]	"Allisonella"	"Morganella"
## [135]	"Parvimonas"	"Fusobacterium"
## [137]	"UCG-009"	"Coriobacteriaceae UCG-003"
## [139]	"GCA-900066575"	"Clostridium sensu stricto 13"
## [141]	"Granulicatella"	"Candidatus Soleaferrea"
## [143]	"UCG-008"	"Parasutterella"
## [145]	"Coproacter"	"Porphyromonas"
## [147]	"Prevotellaceae NK3B31 group"	"Intestinimonas"
## [149]	"Christensenella"	"Lachnospiraceae UCG-010"
## [151]	"Anaerococcus"	"[Eubacterium] fissicatena group"
## [153]	"Holdemania"	"Fournierella"
## [155]	"Finegoldia"	"Acinetobacter"
## [157]	"Enteroscipio"	"Peptoniphilus"
## [159]	"Phoceia"	"Kluyvera"
## [161]	"Amylolactobacillus"	"Peptoclostridium"
## [163]	"Salmonella"	"Anaerotruncus"
## [165]	"Gordonibacter"	"Defluviitaleaceae UCG-011"
## [167]	"Oxalobacter"	"Oribacterium"
## [169]	"Anaerofustis"	"Paeniclostridium"
## [171]	"Stenotrophomonas"	"UC5-1-2E3"
## [173]	"Lentilactobacillus"	"Oscillospira"
## [175]	"Pseudocitrobacter"	"Murdochiella"
## [177]	"Leuconostoc"	"Leptotrichia"
## [179]	"Catonella"	"Fenollaria"
## [181]	"Epulopiscium"	"Mogibacterium"
## [183]	"Papillibacter"	"Candidatus Stoquefichus"
## [185]	"Lactonifactor"	"Levilactobacillus"
## [187]	"Succinivibrio"	"Alloscardovia"
## [189]	"Raoultibacter"	"Faecalibaculum"
## [191]	"Campylobacter"	"Bacillus"
## [193]	"Listeria"	"Staphylococcus"
## [195]	"Hafnia-Obesumbacterium"	"Pseudomonas"
## [197]	"Vibrio"	"Exiguobacterium"

A tibble: 4 x 7

##	Group	Unique_Phylum	Unique_Class	Unique_Order	Unique_Family	Unique_Genus
----	-------	---------------	--------------	--------------	---------------	--------------

```
##   <fct>           <int>         <int>         <int>         <int>         <int>
## 1 Pre-Sham           7           10           24           44           170
## 2 Post-Sham          8           11           24           45           165
## 3 Pre-Hypoxia        7           10           24           46           164
## 4 Post-Hypox~        7           10           22           41           146
## # i 1 more variable: Unique_OTU <int>
```

Comparison Function

Phylum

```
## # A tibble: 8 x 3
##   Taxa           `Pre-Sham` `Post-Sham`
##   <chr>         <lgl>    <lgl>
## 1 Firmicutes   TRUE     TRUE
## 2 Verrucomicrobiota TRUE     TRUE
## 3 Bacteroidota TRUE     TRUE
## 4 Proteobacteria TRUE     TRUE
## 5 Actinobacteriota TRUE     TRUE
## 6 Desulfobacterota TRUE     TRUE
## 7 Fusobacteriota TRUE     TRUE
## 8 Campylobacterota FALSE    TRUE

## # A tibble: 7 x 3
##   Taxa           `Pre-Hypoxia` `Post-Hypoxia`
##   <chr>         <lgl>    <lgl>
## 1 Firmicutes   TRUE     TRUE
## 2 Bacteroidota TRUE     TRUE
## 3 Actinobacteriota TRUE     TRUE
## 4 Proteobacteria TRUE     TRUE
## 5 Verrucomicrobiota TRUE     TRUE
## 6 Desulfobacterota TRUE     TRUE
## 7 Fusobacteriota TRUE     TRUE
```

```
## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##   <int>                 <int>         <int>
## 1           7           0           1
```

```
## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##   <int>                 <int>         <int>
## 1           7           0           0
```

Class

```
## # A tibble: 11 x 3
##   Taxa           `Pre-Sham` `Post-Sham`
##   <chr>         <lgl>    <lgl>
## 1 Bacilli      TRUE     TRUE
## 2 Clostridia   TRUE     TRUE
## 3 Verrucomicrobiae TRUE     TRUE
```

```
## 4 Bacteroidia TRUE TRUE
## 5 Negativicutes TRUE TRUE
## 6 Gammaproteobacteria TRUE TRUE
## 7 Coriobacteriia TRUE TRUE
## 8 Actinobacteria TRUE TRUE
## 9 Desulfovibrionia TRUE TRUE
## 10 Fusobacteriia TRUE TRUE
## 11 Campylobacteria FALSE TRUE
```

```
## # A tibble: 10 x 3
##   Taxa `Pre-Hypoxia` `Post-Hypoxia`
##   <chr> <lgl> <lgl>
## 1 Bacilli TRUE TRUE
## 2 Clostridia TRUE TRUE
## 3 Bacteroidia TRUE TRUE
## 4 Negativicutes TRUE TRUE
## 5 Coriobacteriia TRUE TRUE
## 6 Gammaproteobacteria TRUE TRUE
## 7 Verrucomicrobiae TRUE TRUE
## 8 Actinobacteria TRUE TRUE
## 9 Desulfovibrionia TRUE TRUE
## 10 Fusobacteriia TRUE TRUE
```

```
## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##   <int> <int> <int>
## 1 10 0 1
```

```
## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##   <int> <int> <int>
## 1 10 0 0
```

Order

```
## # A tibble: 25 x 3
##   Taxa `Pre-Sham` `Post-Sham`
##   <chr> <lgl> <lgl>
## 1 Lactobacillales TRUE TRUE
## 2 Lachnospirales TRUE TRUE
## 3 Verrucomicrobiales TRUE TRUE
## 4 Oscillospirales TRUE TRUE
## 5 Erysipelotrichales TRUE TRUE
## 6 Bacteroidales TRUE TRUE
## 7 Clostridiales TRUE TRUE
## 8 Veillonellales-Selenomonadales TRUE TRUE
## 9 Enterobacterales TRUE TRUE
## 10 Coriobacteriales TRUE TRUE
## # i 15 more rows
```

```
## # A tibble: 24 x 3
##   Taxa `Pre-Hypoxia` `Post-Hypoxia`
```



```
##      <chr>                                <lgl>          <lgl>
## 1 Lactobacillales                        TRUE           TRUE
## 2 Lachnospirales                        TRUE           TRUE
## 3 Oscillospirales                      TRUE           TRUE
## 4 Bacteroidales                        TRUE           TRUE
## 5 Erysipelotrichales                  TRUE           TRUE
## 6 Veillonellales-Selenomonadales     TRUE           TRUE
## 7 Coriobacteriales                    TRUE           TRUE
## 8 Enterobacterales                    TRUE           TRUE
## 9 Clostridiales                      TRUE           TRUE
## 10 Verrucomicrobiales                 TRUE           TRUE
## # i 14 more rows

## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##   <int>                <int>          <int>
## 1             23             1             1

## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##   <int>                <int>          <int>
## 1             22             2             0
```

Family

```
## # A tibble: 47 x 3
##   Taxa                                `Pre-Sham` `Post-Sham`
##   <chr>                            <lgl>     <lgl>
## 1 Lactobacillaceae                TRUE      TRUE
## 2 Lachnospiraceae                TRUE      TRUE
## 3 Akkermansiaceae                TRUE      TRUE
## 4 Ruminococcaceae                TRUE      TRUE
## 5 Erysipelotrichaceae            TRUE      TRUE
## 6 Streptococcaceae              TRUE      TRUE
## 7 Bacteroidaceae                 TRUE      TRUE
## 8 Erysipelatoclostridiaceae      TRUE      TRUE
## 9 Clostridiaceae                 TRUE      TRUE
## 10 Prevotellaceae                TRUE      TRUE
## # i 37 more rows

## # A tibble: 48 x 3
##   Taxa                                `Pre-Hypoxia` `Post-Hypoxia`
##   <chr>                            <lgl>         <lgl>
## 1 Lactobacillaceae                TRUE          TRUE
## 2 Lachnospiraceae                TRUE          TRUE
## 3 Ruminococcaceae                TRUE          TRUE
## 4 Prevotellaceae                 TRUE          TRUE
## 5 Erysipelotrichaceae            TRUE          TRUE
## 6 Bacteroidaceae                 TRUE          TRUE
## 7 Erysipelatoclostridiaceae      TRUE          TRUE
## 8 Selenomonadaceae              TRUE          TRUE
## 9 Streptococcaceae              TRUE          TRUE
## 10 Enterococcaceae               TRUE          TRUE
## # i 38 more rows
```

```
## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##           <int>           <int>           <int>
## 1             42             2             3
```

```
## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##           <int>           <int>           <int>
## 1             39             7             2
```

Genus

```
## # A tibble: 183 x 3
##   Taxa           `Pre-Sham` `Post-Sham`
##   <chr>         <lgl>    <lgl>
## 1 Limosilactobacillus TRUE     TRUE
## 2 Blautia       TRUE     TRUE
## 3 Akkermansia   TRUE     TRUE
## 4 Ruminococcus  TRUE     TRUE
## 5 Holdemanella TRUE     TRUE
## 6 Dorea         TRUE     TRUE
## 7 Streptococcus TRUE     TRUE
## 8 Faecalibacterium TRUE     TRUE
## 9 Bacteroides   TRUE     TRUE
## 10 Agathobacter TRUE     TRUE
## # i 173 more rows
```

```
## # A tibble: 172 x 3
##   Taxa           `Pre-Hypoxia` `Post-Hypoxia`
##   <chr>         <lgl>    <lgl>
## 1 Limosilactobacillus TRUE     TRUE
## 2 Blautia       TRUE     TRUE
## 3 Ruminococcus  TRUE     TRUE
## 4 Prevotella_9  TRUE     TRUE
## 5 Agathobacter  TRUE     TRUE
## 6 Holdemanella  TRUE     TRUE
## 7 [Ruminococcus] torques group TRUE     TRUE
## 8 Bacteroides   TRUE     TRUE
## 9 Erysipelotrichaceae UCG-003 TRUE     TRUE
## 10 Faecalibacterium TRUE     TRUE
## # i 162 more rows
```

```
## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##           <int>           <int>           <int>
## 1             152             18             13
```

```
## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##           <int>           <int>           <int>
## 1             138             26             8
```

OTU

```
## # A tibble: 348 x 3
##   Taxa   `Pre-Sham` `Post-Sham`
##   <chr> <lgl>      <lgl>
## 1 485    TRUE        TRUE
## 2 21     TRUE        TRUE
## 3 6637   TRUE        TRUE
## 4 4292   TRUE        TRUE
## 5 7087   TRUE        TRUE
## 6 3972   TRUE        TRUE
## 7 106    TRUE        TRUE
## 8 5317   TRUE        TRUE
## 9 74     TRUE        TRUE
## 10 9993  TRUE        TRUE
## # i 338 more rows

## # A tibble: 333 x 3
##   Taxa   `Pre-Hypoxia` `Post-Hypoxia`
##   <chr> <lgl>      <lgl>
## 1 485    TRUE        TRUE
## 2 21     TRUE        TRUE
## 3 4292   TRUE        TRUE
## 4 394    TRUE        TRUE
## 5 6756   TRUE        TRUE
## 6 7087   TRUE        TRUE
## 7 3327   TRUE        TRUE
## 8 74     TRUE        TRUE
## 9 3075   TRUE        TRUE
## 10 5317  TRUE        TRUE
## # i 323 more rows

## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##   <int>                <int>                <int>
## 1          276              39              33

## # A tibble: 1 x 3
##   `Shared between groups` `Unique to Pre` `Unique to Post`
##   <int>                <int>                <int>
## 1          245              65              23
```

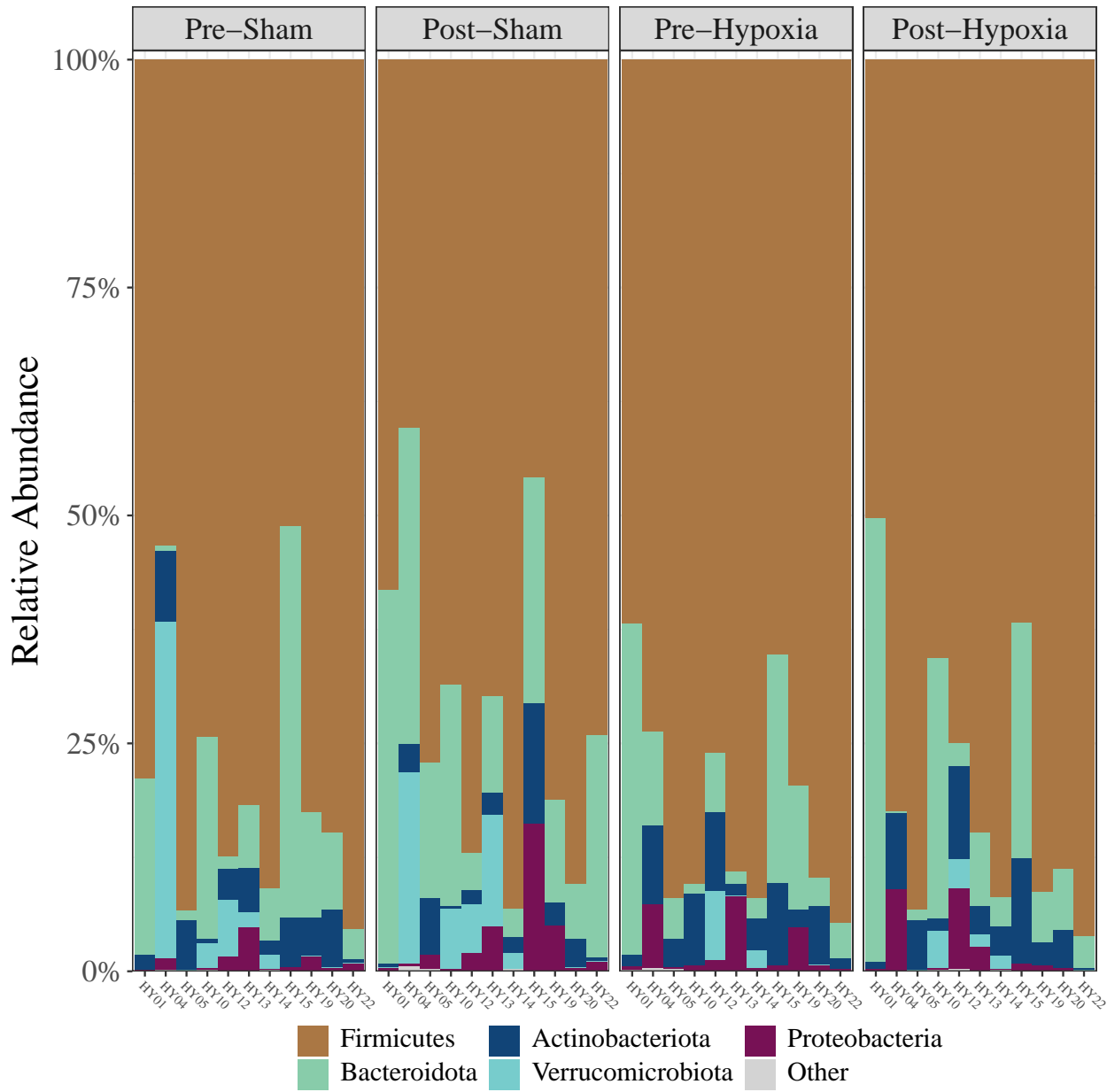
Individual Barplots

The following functions will plot a barplot showing the normalised abundance across all samples.

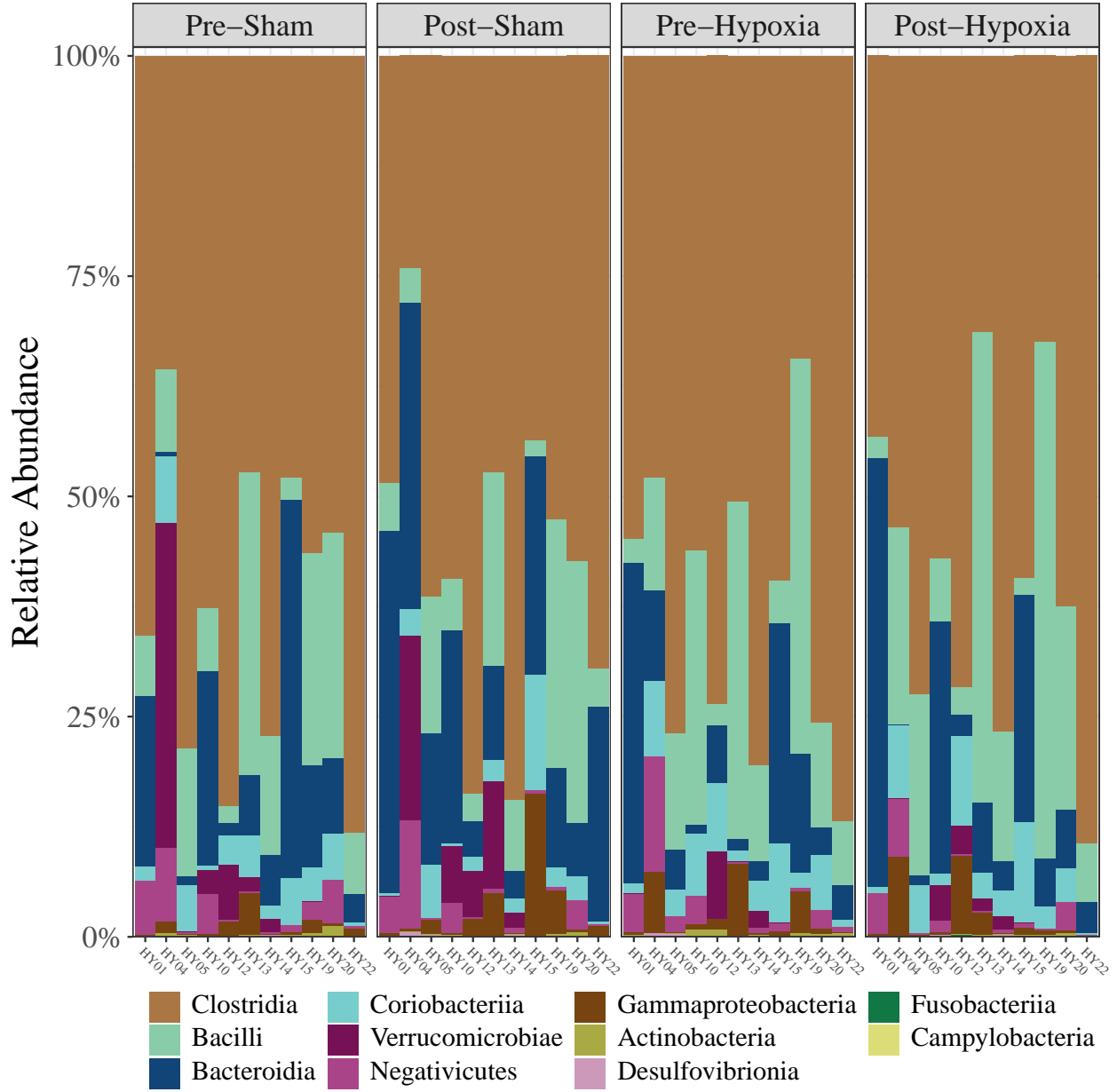
Barplot function: Normal text

Barplot function: *Italic text*

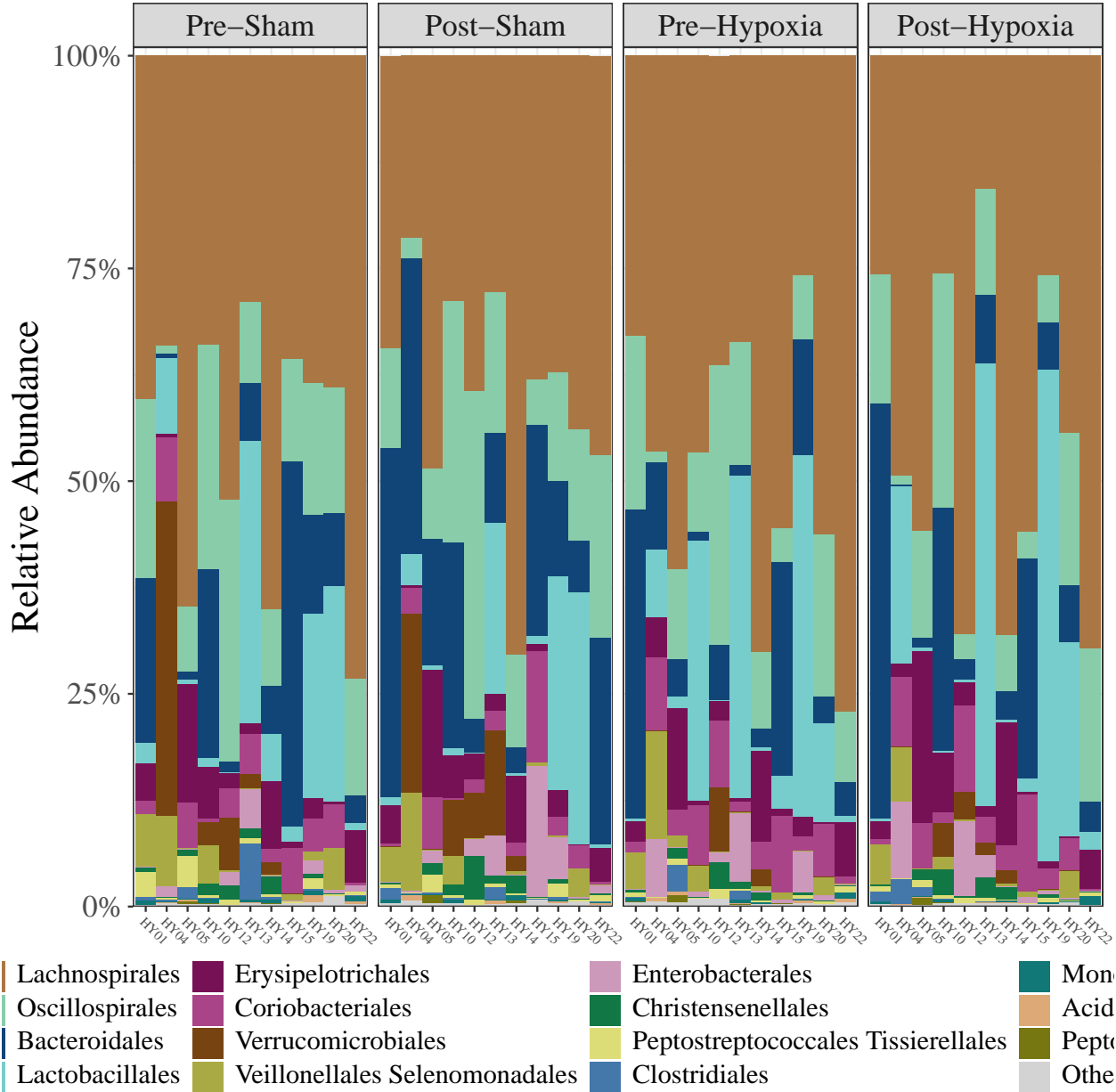
Phylum



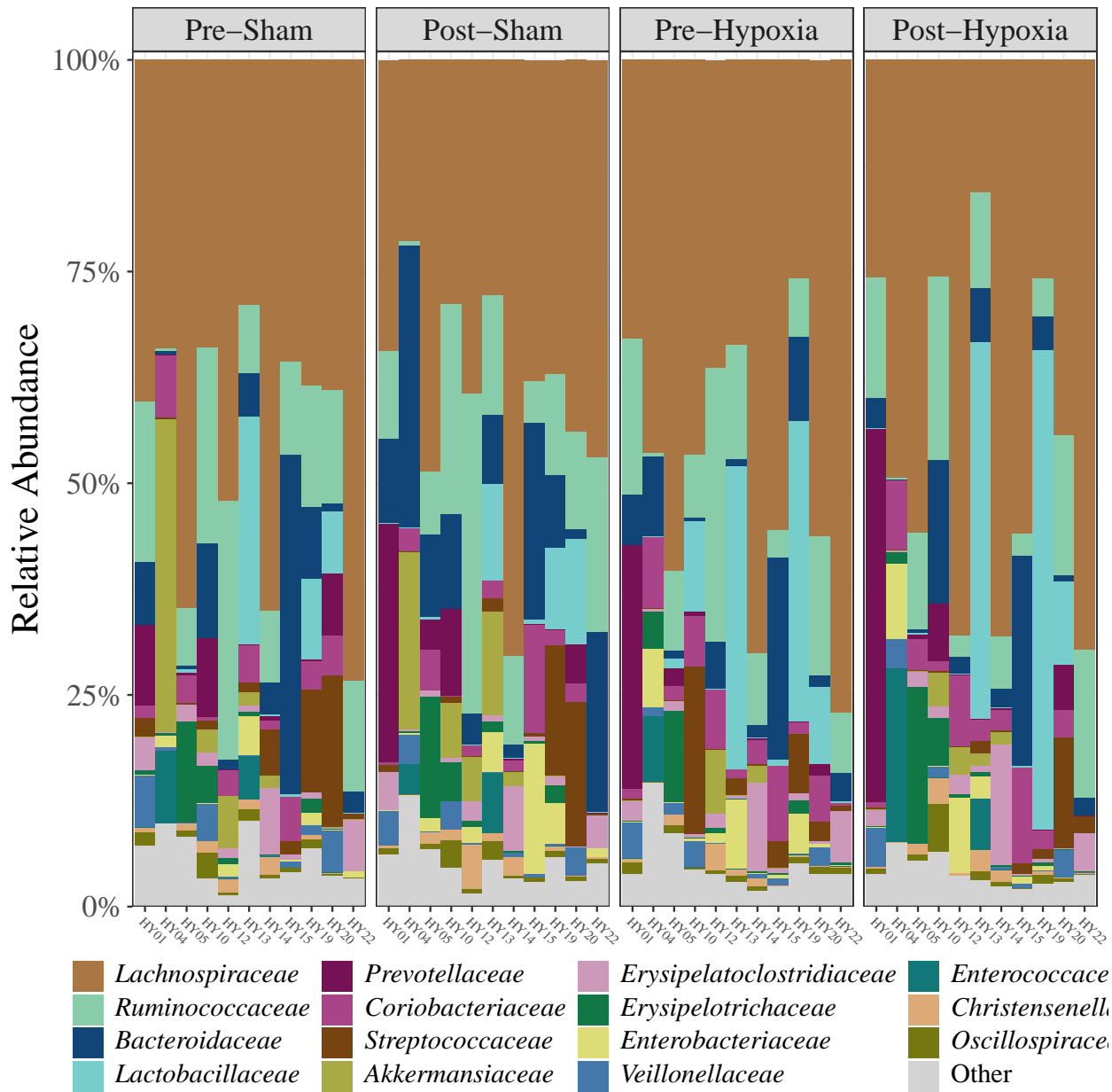
Class



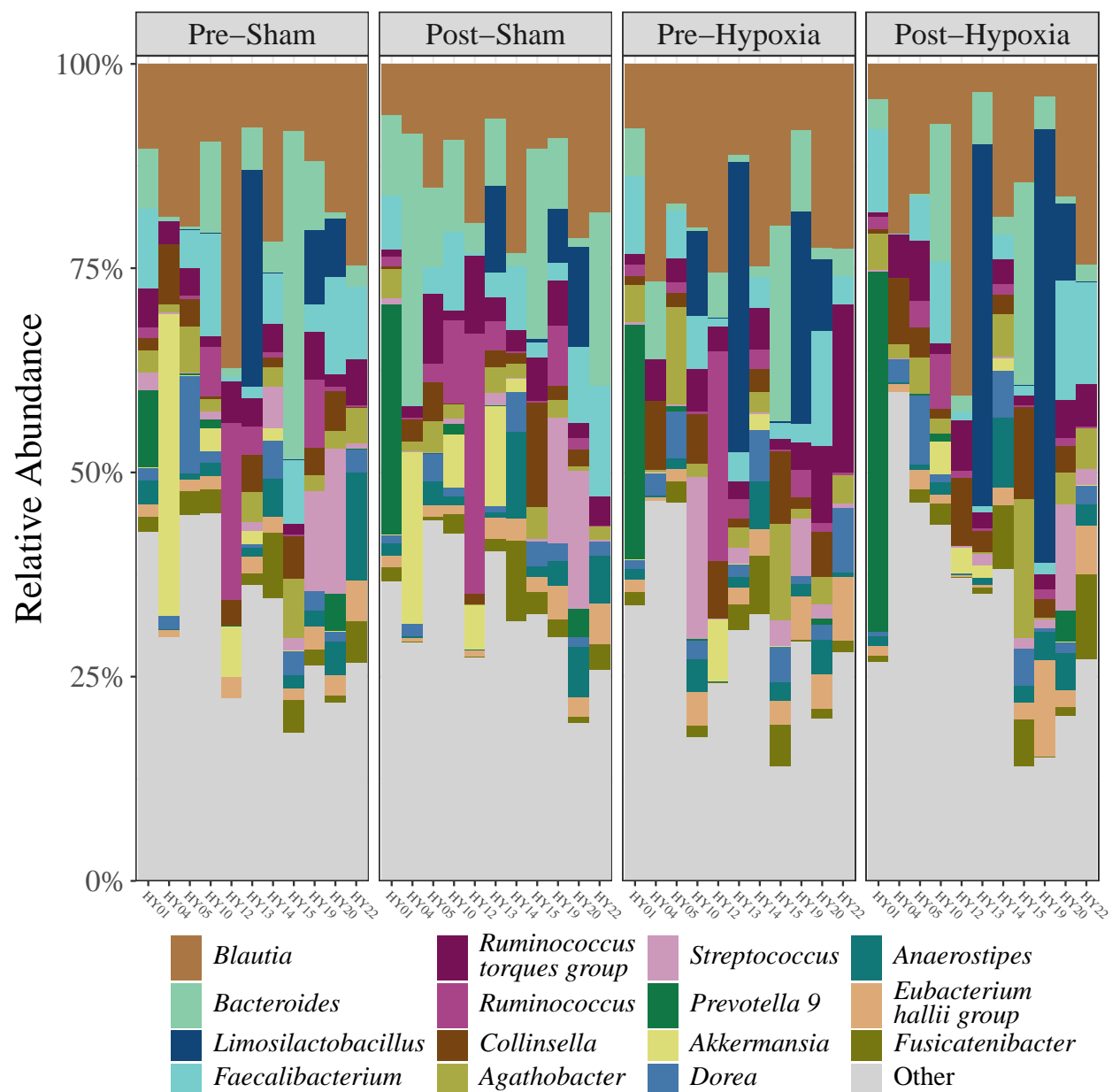
Order



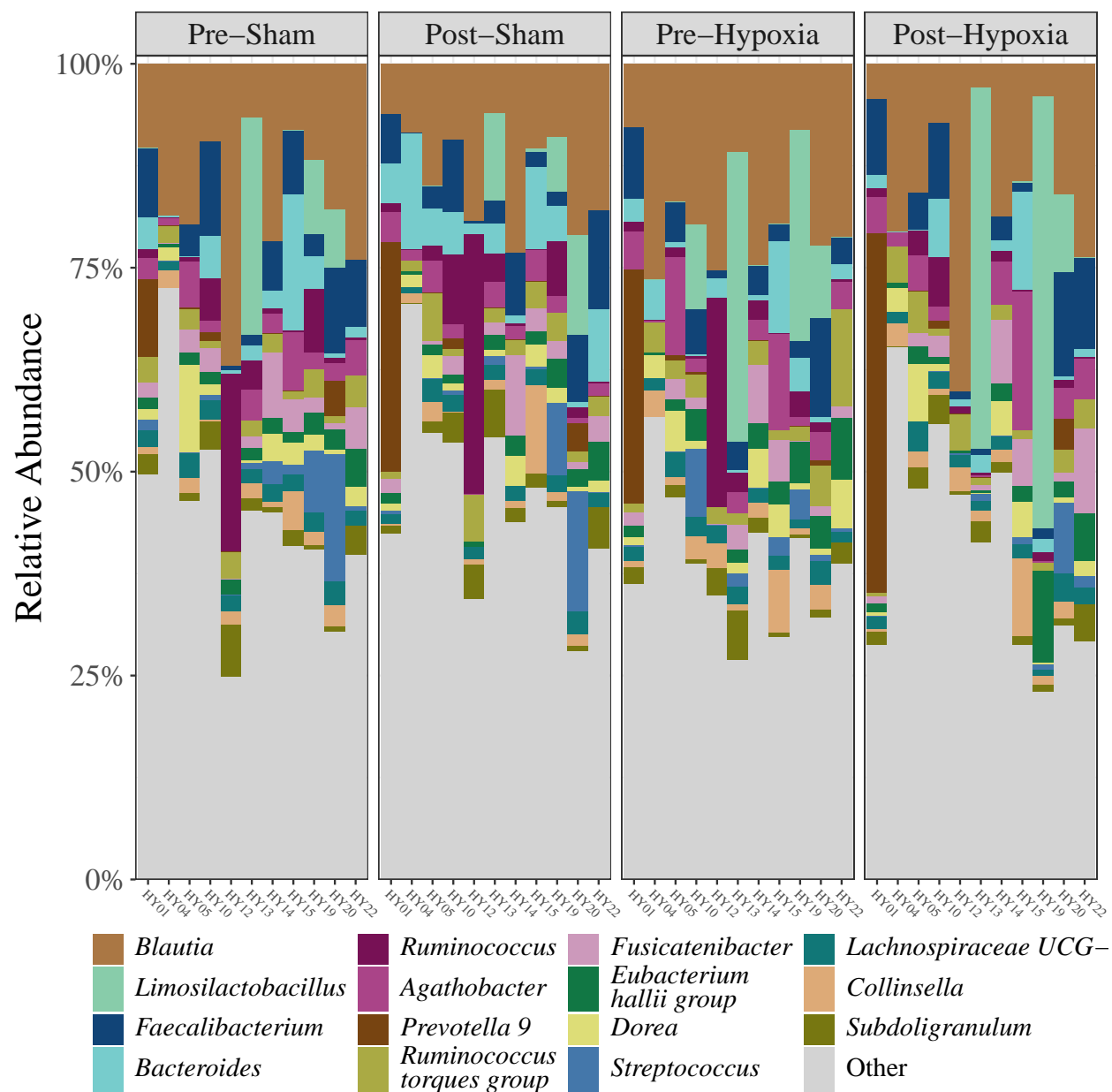
Family



Genus



Species



Firmicute/Bacteroidota ratio

This section will calculate the Firmicute/Bacteroidota (FB) ratio.

```
## # A tibble: 6 x 3
##   Rep Group FBratio
##   <chr> <fct>   <dbl>
## 1 HY01 Sham    -2.65
## 2 HY01 Hypoxia -0.673
## 3 HY04 Sham   -92.5
## 4 HY04 Hypoxia 512.
```

```
## 5 HY05 Sham -87.6
## 6 HY05 Hypoxia 61.9
```

Normality

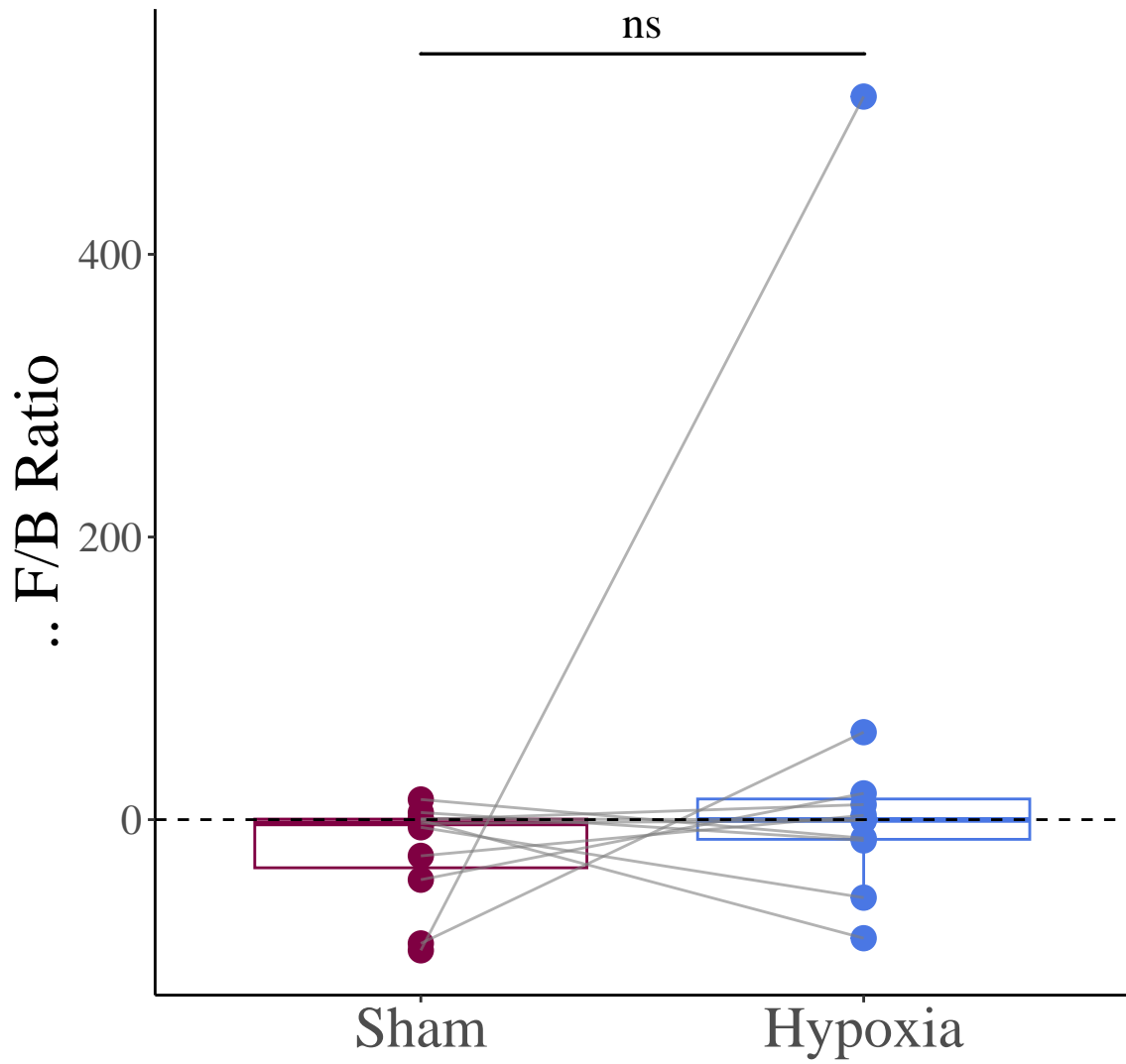
```
##
## DESCRIPTIVES
##
## Descriptives
##
##          Group      FBratio
##
## N          Sham      11
##          Hypoxia     11
## Missing    Sham      0
##          Hypoxia     0
## Mean       Sham     -21.52304
##          Hypoxia     39.81208
## Median     Sham     -2.651260
##          Hypoxia    -0.2160429
## Standard deviation Sham      37.22230
##          Hypoxia     160.9680
## IQR        Sham      34.45295
##          Hypoxia     28.55269
## Minimum    Sham     -92.46671
##          Hypoxia    -83.89692
## Maximum    Sham      14.25865
##          Hypoxia     511.6353
## Skewness   Sham     -1.300878
##          Hypoxia     2.982316
## Std. error skewness Sham      0.6606875
##          Hypoxia     0.6606875
## Kurtosis   Sham      0.3746956
##          Hypoxia     9.459569
## Std. error kurtosis Sham      1.279416
##          Hypoxia     1.279416
## Shapiro-Wilk W Sham      0.7845004
##          Hypoxia     0.5676667
## Shapiro-Wilk p Sham      0.0058833
##          Hypoxia     0.0000101
##
```

t-test and effect size

```
##
## PAIRED SAMPLES T-TEST
##
## Paired Samples T-Test
##
##          Statistic      p      Effect Size
##
## Sham Hypoxia Wilcoxon W 26.00000 0.5771484 Rank biserial correlation -0.21212
##
```

Note. H _{Measure 1 - Measure 2} 0

Plot



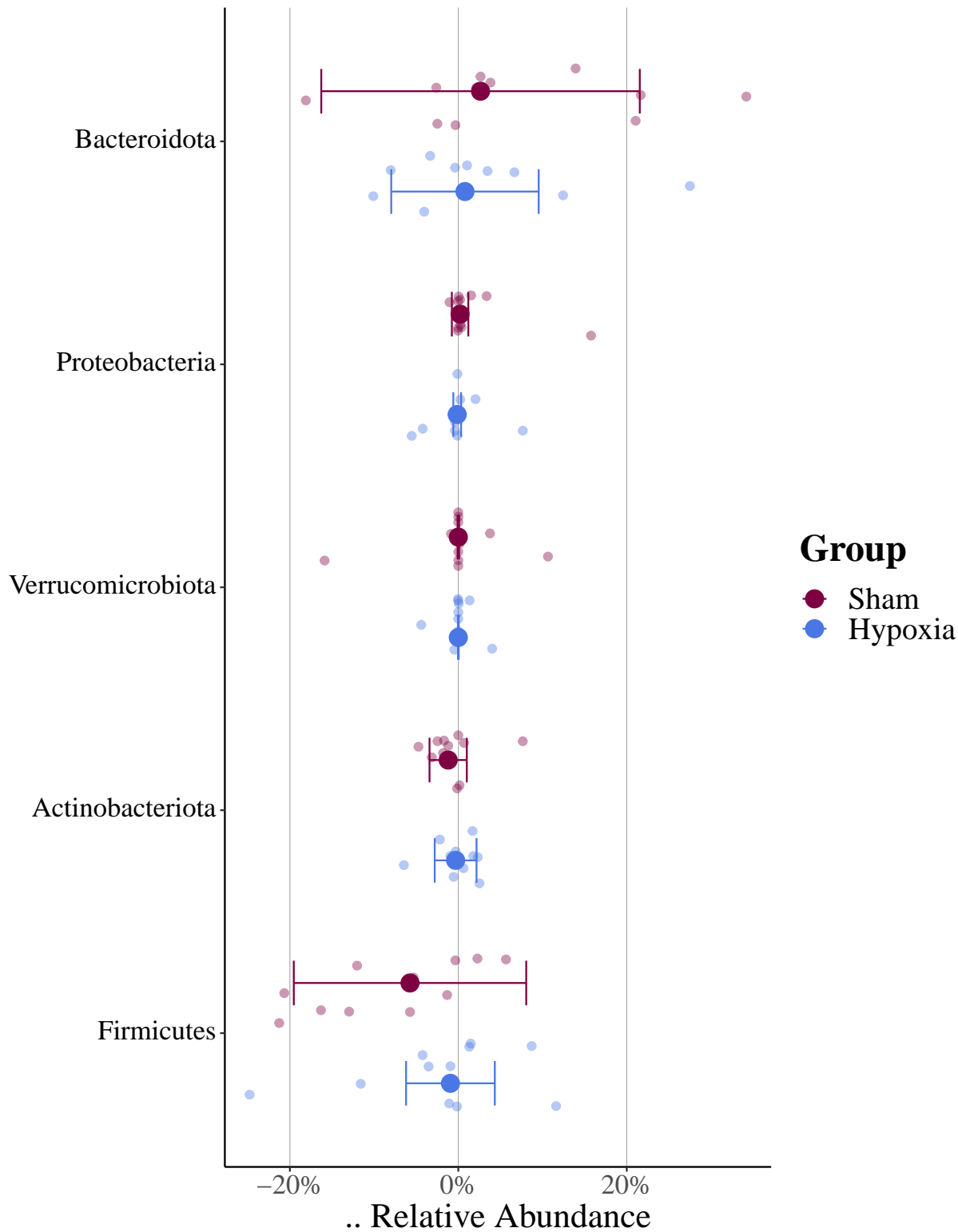
Relative Abundance Comparison Function

Below, we will compare the delta relative abundances of taxa at given taxonomic levels.

Plot function: Normal text

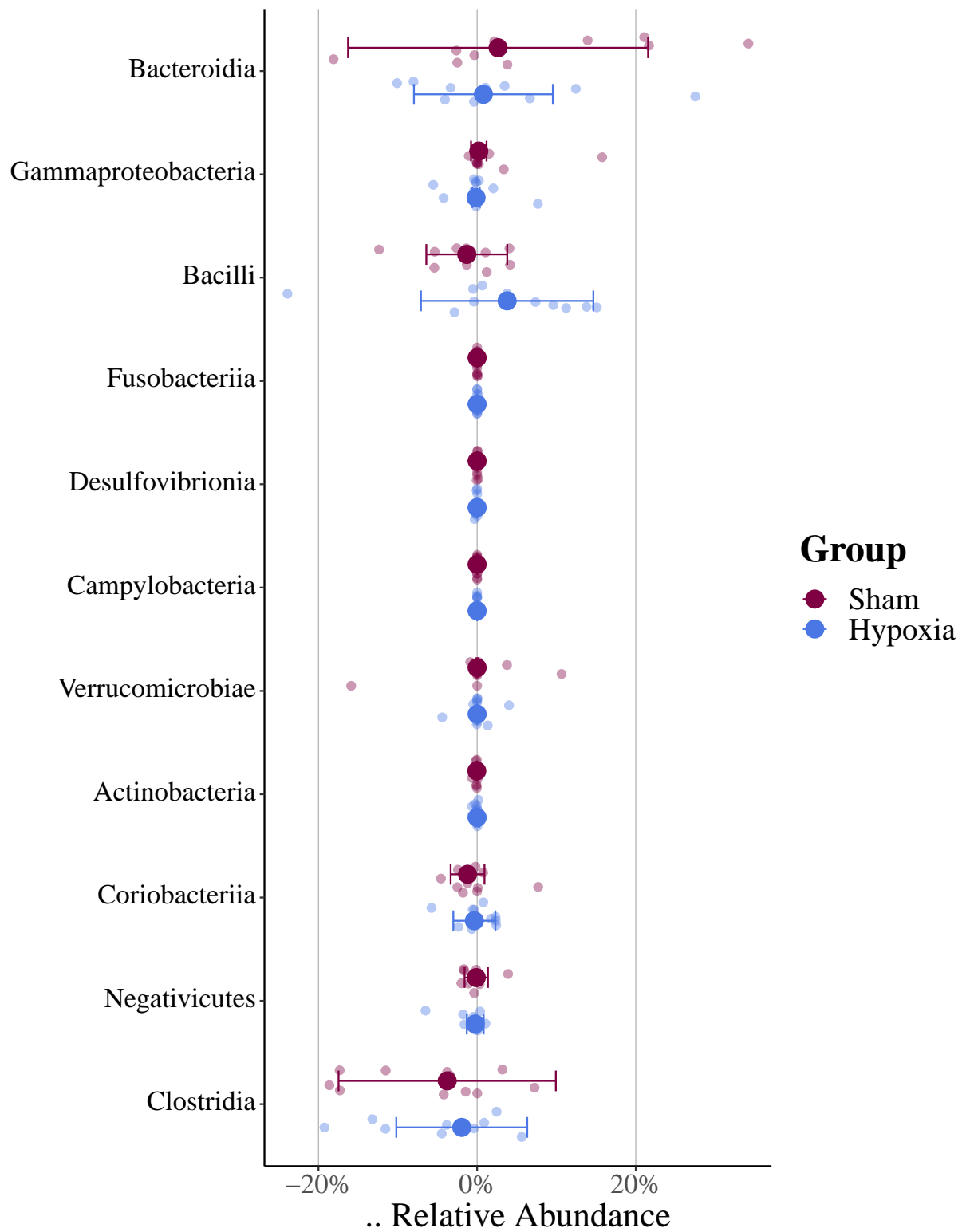
Plot function: *Italic text*

Phylum



```
## # A tibble: 10 x 8
## # Groups:   Group [2]
##   Group TaxName      average variability    pval pval_adj pval_adj_star .group
##   <fct> <fct>          <dbl>         <dbl> <dbl>   <dbl> <chr>         <int>
## 1 Sham  Firmicutes    -5.74         13.8    NA      NA      ""             1
## 2 Sham  Bacteroidota  2.64         18.9    NA      NA      ""             1
## 3 Sham  Actinobacte~ -1.21         2.22    NA      NA      ""             1
## 4 Sham  Verrucomicr~ 0           0.0861    NA      NA      ""             1
## 5 Sham  Proteobacte~ 0.209        0.978    NA      NA      ""             1
## 6 Hypoxia Firmicutes    -0.935        5.27    0.175    0.437 ""             2
## 7 Hypoxia Bacteroidota  0.790        8.75    0.465    0.649 ""             2
## 8 Hypoxia Actinobacte~ -0.321        2.48    0.520    0.649 ""             2
## 9 Hypoxia Verrucomicr~ 0           0.0437    0.800    0.800 ""             2
## 10 Hypoxia Proteobacte~ -0.132        0.462    0.147    0.437 ""             2
```

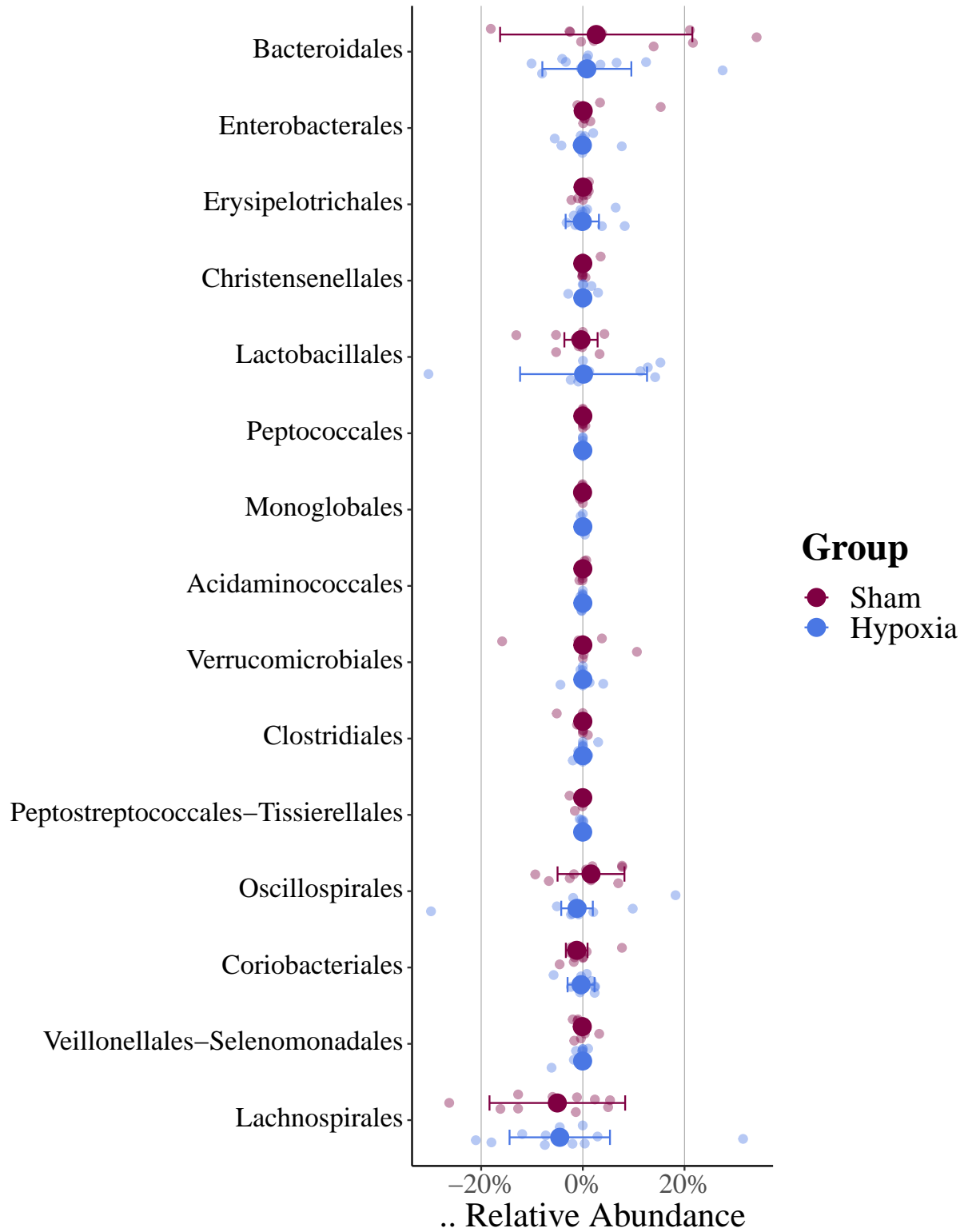
Class



```
## # A tibble: 22 x 8
## # Groups:   Group [2]
```

##	Group	TaxName	average	variability	pval	pval_adj	pval_adj_star	.group
##	<fct>	<fct>	<dbl>	<dbl>	<dbl>	<dbl>	<chr>	<int>
##	1 Sham	Clostridia	-3.77	13.7	NA	NA	""	1
##	2 Sham	Bacilli	-1.31	5.10	NA	NA	""	1
##	3 Sham	Bacteroidia	2.64	18.9	NA	NA	""	1
##	4 Sham	Coriobacter~	-1.21	2.13	NA	NA	""	1
##	5 Sham	Verrucomicrob~	0	0.0861	NA	NA	""	1
##	6 Sham	Negativicutes	-0.109	1.47	NA	NA	""	1
##	7 Sham	Gammaproteobacteria	0.209	0.978	NA	NA	""	1
##	8 Sham	Actinobacteriota	-0.0424	0.0808	NA	NA	""	1
##	9 Sham	Desulfovibrionia	0	0.0331	NA	NA	""	1
##	10 Sham	Fusobacteriota	0	0	NA	NA	""	1
##	11 Sham	Campylobacteriota	0	0	NA	NA	""	1
##	12 Hypoxia	Clostridia	-1.94	8.25	0.520	0.978	""	2
##	13 Hypoxia	Bacilli	3.78	10.9	0.123	0.811	""	2
##	14 Hypoxia	Bacteroidia	0.790	8.75	0.465	0.978	""	2
##	15 Hypoxia	Coriobacter~	-0.350	2.65	0.638	0.978	""	2
##	16 Hypoxia	Verrucomicrob~	0	0.0437	0.800	0.978	""	2
##	17 Hypoxia	Negativicutes	-0.241	1.06	0.765	0.978	""	2
##	18 Hypoxia	Gammaproteobacteria	-0.132	0.462	0.147	0.811	""	2
##	19 Hypoxia	Actinobacteriota	0	0.244	0.919	1	""	2
##	20 Hypoxia	Desulfovibrionia	0	0.0715	0.636	0.978	""	2
##	21 Hypoxia	Fusobacteriota	0	0	0.789	0.978	""	2
##	22 Hypoxia	Campylobacteriota	0	0	1	1	""	2

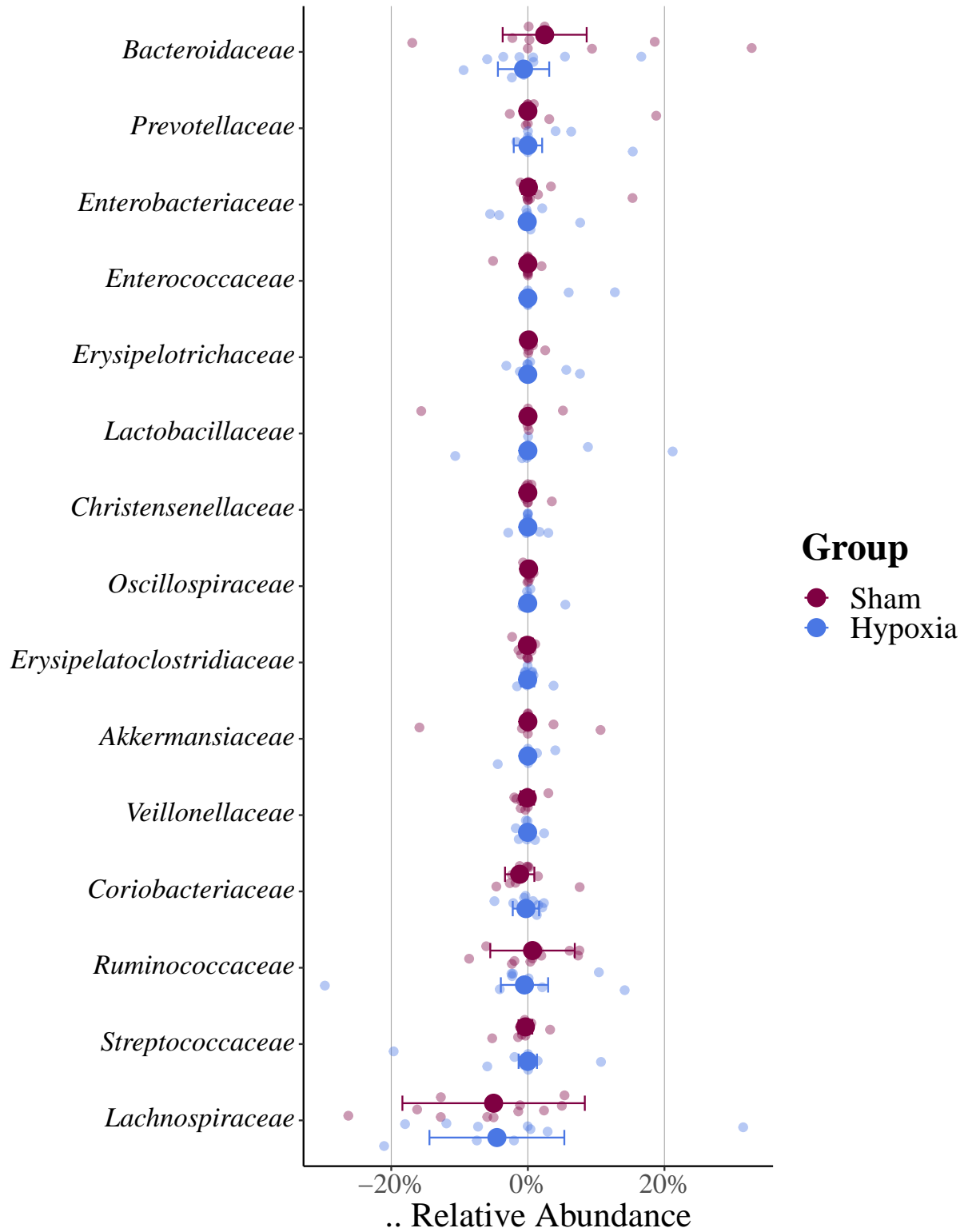
Order



```
## # A tibble: 30 x 8
## # Groups:   Group [2]
```


##	Group	TaxName	average	variability	pval	pval_adj	pval_adj_star	.group
##	<fct>	<fct>	<dbl>	<dbl>	<dbl>	<dbl>	<chr>	<int>
##	1 Sham	Lachnospir~	-5.02	13.3	NA	NA	""	1
##	2 Sham	Oscillospi~	1.61	6.58	NA	NA	""	1
##	3 Sham	Bacteroida~	2.64	18.9	NA	NA	""	1
##	4 Sham	Lactobacil~	-0.360	3.27	NA	NA	""	1
##	5 Sham	Erysipelot~	0.0424	0.868	NA	NA	""	1
##	6 Sham	Coriobacte~	-1.21	2.13	NA	NA	""	1
##	7 Sham	Verrucomi~	0	0.0861	NA	NA	""	1
##	8 Sham	Veillonell~	-0.109	0.995	NA	NA	""	1
##	9 Sham	Enterobact~	0.0715	0.930	NA	NA	""	1
##	10 Sham	Christense~	-0.00795	0.208	NA	NA	""	1
##	11 Sham	Peptostrep~	-0.0159	0.175	NA	NA	""	1
##	12 Sham	Clostridia~	0	0.401	NA	NA	""	1
##	13 Sham	Monoglobal~	-0.0477	0.152	NA	NA	""	1
##	14 Sham	Acidaminoc~	0	0.0490	NA	NA	""	1
##	15 Sham	Peptococca~	0	0	NA	NA	""	1
##	16 Hypoxia	Lachnospir~	-4.54	9.88	0.966	1	""	2
##	17 Hypoxia	Oscillospi~	-1.14	3.11	0.831	0.959	""	2
##	18 Hypoxia	Bacteroida~	0.790	8.75	0.465	0.959	""	2
##	19 Hypoxia	Lactobacil~	0.143	12.5	0.175	0.959	""	2
##	20 Hypoxia	Erysipelot~	-0.103	3.27	0.831	0.959	""	2
##	21 Hypoxia	Coriobacte~	-0.350	2.65	0.638	0.959	""	2
##	22 Hypoxia	Verrucomi~	0	0.0437	0.800	0.959	""	2
##	23 Hypoxia	Veillonell~	-0.0397	0.874	1	1	""	2
##	24 Hypoxia	Enterobact~	-0.0927	0.662	0.185	0.959	""	2
##	25 Hypoxia	Christense~	0	0.348	0.721	0.959	""	2
##	26 Hypoxia	Peptostrep~	-0.0291	0.143	0.638	0.959	""	2
##	27 Hypoxia	Clostridia~	0	0.00265	0.624	0.959	""	2
##	28 Hypoxia	Monoglobal~	0	0.0159	0.441	0.959	""	2
##	29 Hypoxia	Acidaminoc~	0	0.235	0.447	0.959	""	2
##	30 Hypoxia	Peptococca~	0	0	0.371	0.959	""	2

Family

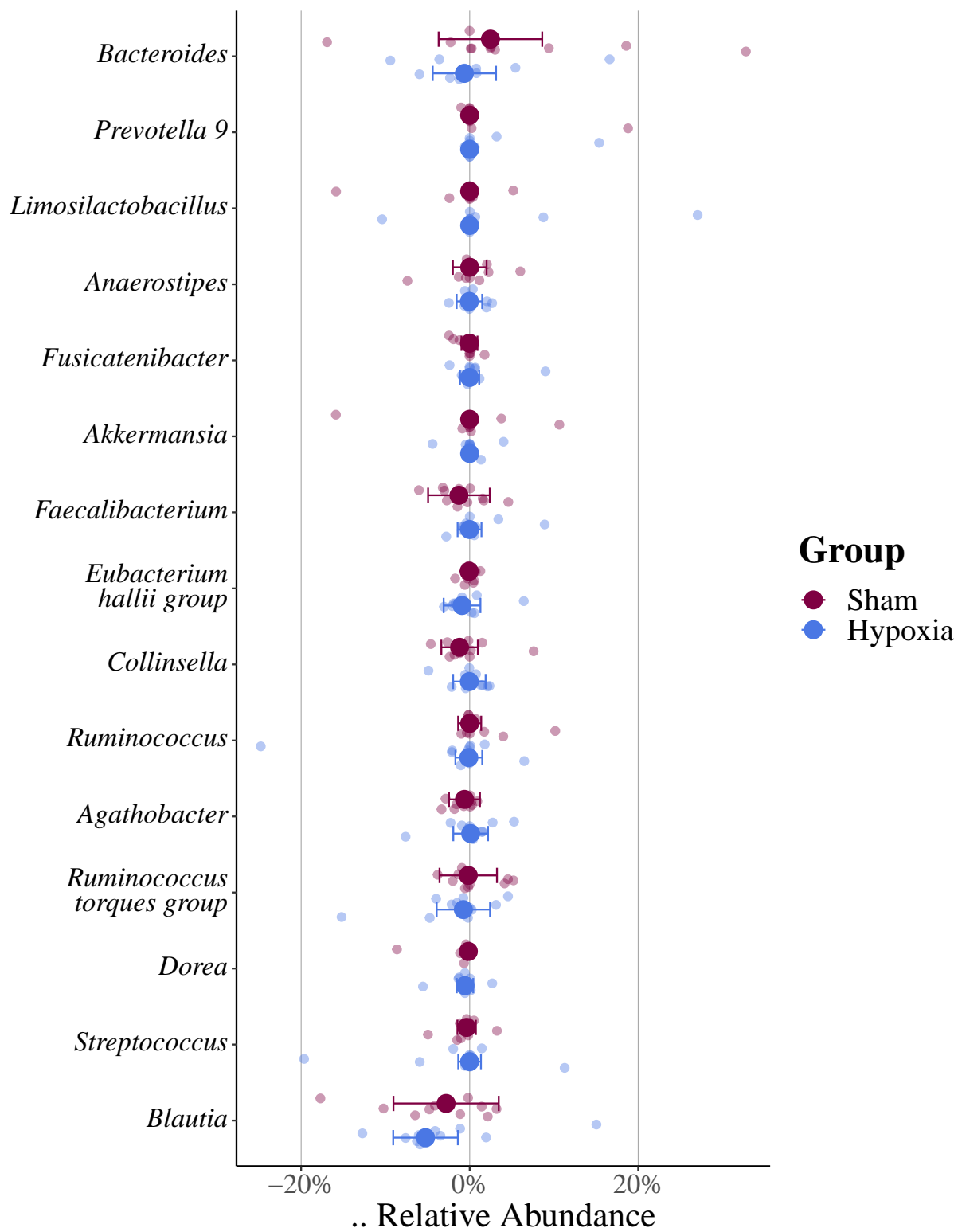


```
## # A tibble: 30 x 9
## # Groups:   Group [2]
```

##	Group	TaxName	average	variability	pval	pval_adj	pval_adj_star
##	<fct>	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<chr>
##	1 Sham	Lachnospiraceae	-5.02	13.4	NA	NA	""
##	2 Sham	Ruminococcaceae	0.681	6.19	NA	NA	""
##	3 Sham	Bacteroidaceae	2.46	6.15	NA	NA	""
##	4 Sham	Lactobacillaceae	0.0159	0.107	NA	NA	""
##	5 Sham	Prevotellaceae	0	0.448	NA	NA	""
##	6 Sham	Coriobacteriaceae	-1.20	2.15	NA	NA	""
##	7 Sham	Streptococcaceae	-0.360	1.01	NA	NA	""
##	8 Sham	Akkermansiaceae	0	0.0861	NA	NA	""
##	9 Sham	Erysipelatoclostr~	-0.0715	0.828	NA	NA	""
##	10 Sham	Erysipelotrichace~	0.0795	0.257	NA	NA	""
##	11 Sham	Enterobacteriaceae	0.0715	0.930	NA	NA	""
##	12 Sham	Veillonellaceae	-0.0821	0.995	NA	NA	""
##	13 Sham	Enterococcaceae	0	0	NA	NA	""
##	14 Sham	Christensenellace~	-0.00795	0.208	NA	NA	""
##	15 Sham	Oscillospiraceae	0.103	0.359	NA	NA	""
##	16 Hypoxia	Lachnospiraceae	-4.53	9.88	0.966	0.966	""
##	17 Hypoxia	Ruminococcaceae	-0.496	3.46	0.765	0.898	""
##	18 Hypoxia	Bacteroidaceae	-0.631	3.76	0.365	0.898	""
##	19 Hypoxia	Lactobacillaceae	0	0.605	0.610	0.898	""
##	20 Hypoxia	Prevotellaceae	0.0132	2.08	0.447	0.898	""
##	21 Hypoxia	Coriobacteriaceae	-0.284	1.93	0.520	0.898	""
##	22 Hypoxia	Streptococcaceae	-0.00795	1.34	0.831	0.898	""
##	23 Hypoxia	Akkermansiaceae	0	0.0437	0.800	0.898	""
##	24 Hypoxia	Erysipelatoclostr~	-0.0371	0.975	0.465	0.898	""
##	25 Hypoxia	Erysipelotrichace~	-0.0185	0.335	0.838	0.898	""
##	26 Hypoxia	Enterobacteriaceae	-0.0927	0.513	0.185	0.898	""
##	27 Hypoxia	Veillonellaceae	-0.0397	0.401	0.722	0.898	""
##	28 Hypoxia	Enterococcaceae	0	0	0.584	0.898	""
##	29 Hypoxia	Christensenellace~	0	0.348	0.721	0.898	""
##	30 Hypoxia	Oscillospiraceae	-0.0238	0.547	0.320	0.898	""

i 2 more variables: FormattedTaxName <chr>, .group <int>

Genus



```
## # A tibble: 30 x 9
## # Groups:   Group [2]
```

```
##      Group  TaxName      average variability    pval pval_adj pval_adj_star
##      <fct>   <chr>         <dbl>         <dbl>    <dbl>    <dbl>    <chr>
##  1 Sham    Blautia        -2.80          6.25     NA        NA        ""
##  2 Sham    Bacteroides      2.46          6.15     NA        NA        ""
##  3 Sham    Limosilactobacill~  0            0.00795  NA        NA        ""
##  4 Sham    Faecalibacterium    -1.27          3.66     NA        NA        ""
##  5 Sham    Ruminococcus torq~ -0.172         3.41     NA        NA        ""
##  6 Sham    Ruminococcus         0            1.37     NA        NA        ""
##  7 Sham    Collinsella        -1.20          2.16     NA        NA        ""
##  8 Sham    Agathobacter       -0.617         1.84     NA        NA        ""
##  9 Sham    Streptococcus      -0.360         1.09     NA        NA        ""
## 10 Sham    Prevotella 9         0            0.00927  NA        NA        ""
## 11 Sham    Akkermansia         0            0.0861  NA        NA        ""
## 12 Sham    Dorea              -0.156         0.566    NA        NA        ""
## 13 Sham    Anaerostipes        0.00530        2.00     NA        NA        ""
## 14 Sham    Eubacterium halli~ -0.0662        0.706    NA        NA        ""
## 15 Sham    Fusicatenibacter   -0.0238        0.950    NA        NA        ""
## 16 Hypoxia Blautia        -5.24          3.84     0.638     0.955    ""
## 17 Hypoxia Bacteroides    -0.631         3.76     0.365     0.955    ""
## 18 Hypoxia Limosilactobacill~  0            0.346     0.944     1        ""
## 19 Hypoxia Faecalibacterium    -0.0159        1.41     0.123     0.923    ""
## 20 Hypoxia Ruminococcus torq~ -0.750         3.17     0.102     0.923    ""
## 21 Hypoxia Ruminococcus    -0.103         1.59     0.278     0.955    ""
## 22 Hypoxia Collinsella      -0.0371        1.93     0.520     0.955    ""
## 23 Hypoxia Agathobacter      0.117         2.06     0.577     0.955    ""
## 24 Hypoxia Streptococcus    -0.00795       1.34     0.831     0.959    ""
## 25 Hypoxia Prevotella 9      0            0.302     1         1        ""
## 26 Hypoxia Akkermansia      0            0.0437    0.800     0.959    ""
## 27 Hypoxia Dorea          -0.556         0.978     0.320     0.955    ""
## 28 Hypoxia Anaerostipes    -0.0318        1.52     0.577     0.955    ""
## 29 Hypoxia Eubacterium halli~ -0.906         2.18     0.413     0.955    ""
## 30 Hypoxia Fusicatenibacter  -0.0106        1.14     0.700     0.955    ""
## # i 2 more variables: FormattedTaxName <chr>, .group <int>
```

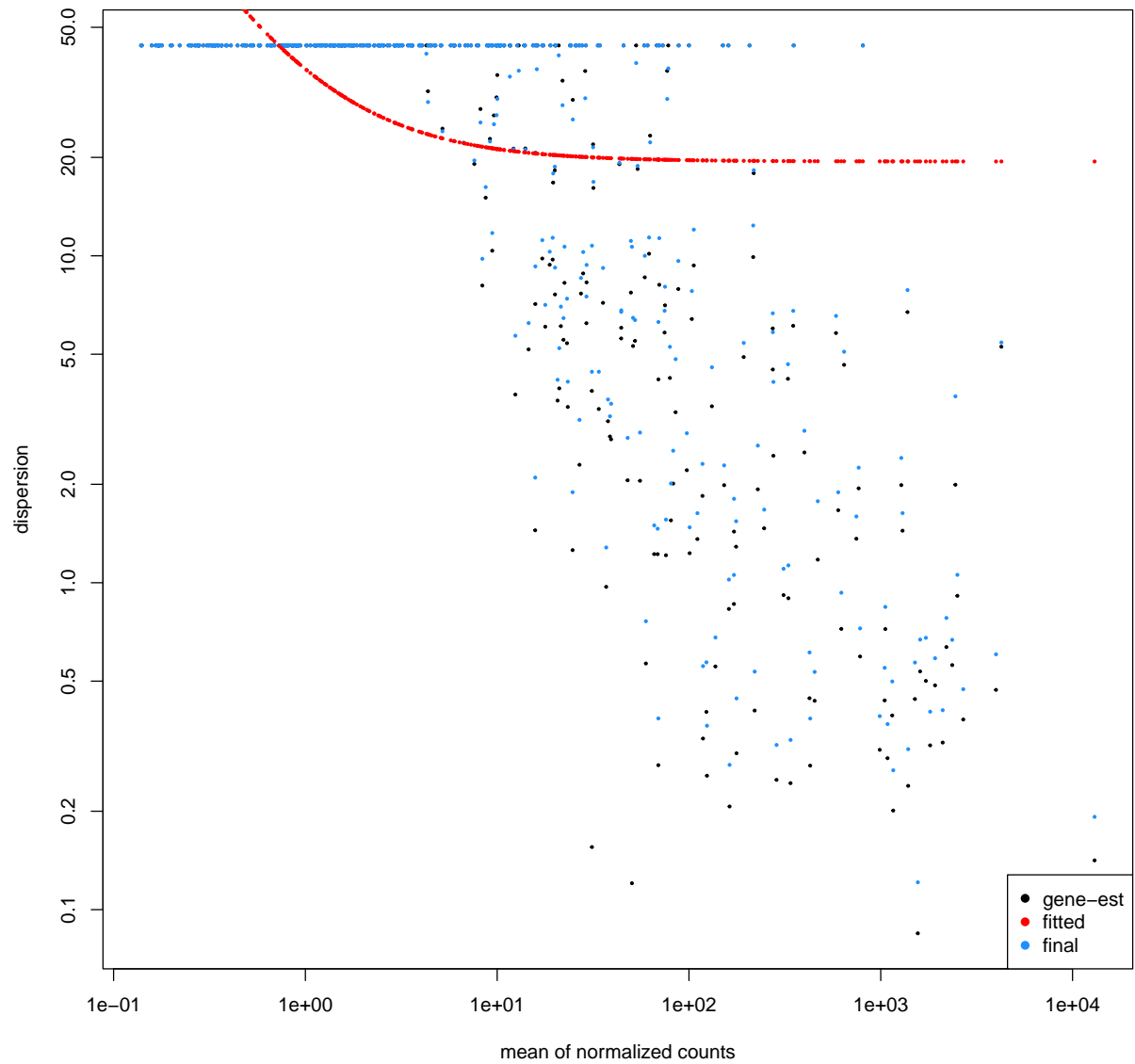
Section 4: Differential Abundance Analysis

DESeq2

In this section, we will use the DESeq2 and ALDEx2 package in R to identify differentially abundant taxa between the conditions. We will deem taxa as significantly expressed if both packages agree.

Setup

Model



```
## [1] "Intercept" "Rep_HY04_vs_HY01"
## [3] "Rep_HY05_vs_HY01" "Rep_HY10_vs_HY01"
## [5] "Rep_HY12_vs_HY01" "Rep_HY13_vs_HY01"
## [7] "Rep_HY14_vs_HY01" "Rep_HY15_vs_HY01"
## [9] "Rep_HY19_vs_HY01" "Rep_HY20_vs_HY01"
## [11] "Rep_HY22_vs_HY01" "Group_Post.Sham_vs_Pre.Sham"
## [13] "Group_Pre.Hypoxia_vs_Pre.Sham" "Group_Post.Hypoxia_vs_Pre.Sham"
```

Functions

Post-Sham vs Pre-Sham

The following results show the differentially abundant taxa.

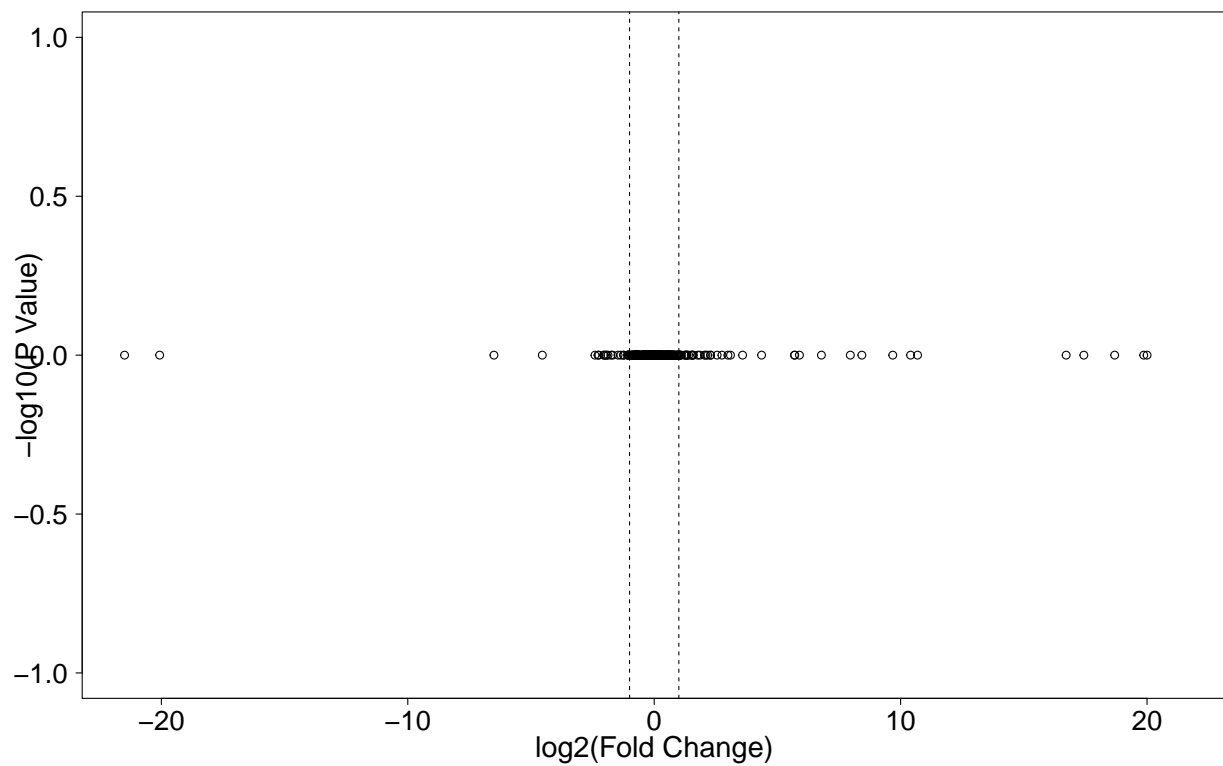
```
## Error: No significant differences identified for Post-Sham vs Pre-Sham
```

```
## Total number of differentially abundant OTUs: 0
```

```
## Upregulated number of differentially abundant OTUs: 0
```

```
## Downregulated number of differentially abundant OTUs: 0
```

Below is a volcano plot showing the overall differential abundance results for this comparison.



And here are the taxa showing the highest increase or decrease between the groups. (Null if no differences)

```
## NULL
```

Post-Hypoxia vs Pre-Hypoxia

The following results show the differentially abundant taxa.

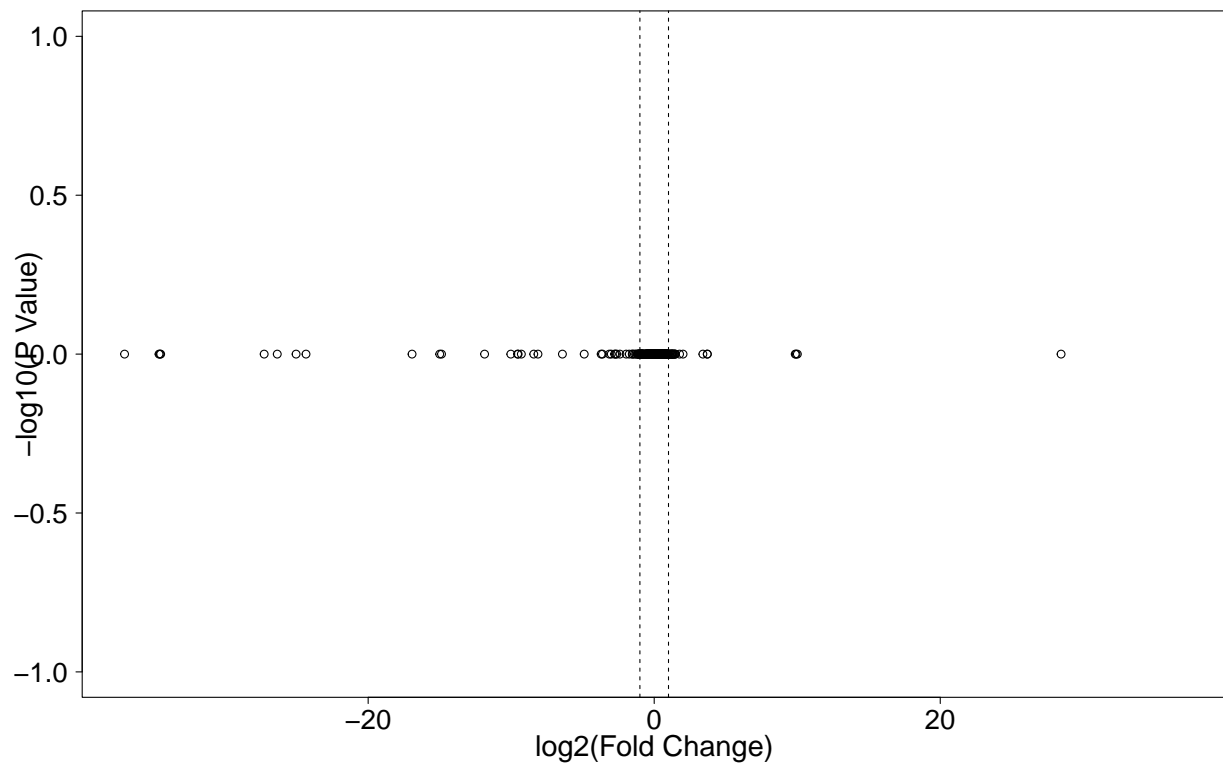
```
## Error: No significant differences identified for Post-Hypoxia vs Pre-Hypoxia
```

```
## Total number of differentially abundant OTUs: 0
```

```
## Upregulated number of differentially abundant OTUs: 0
```

```
## Downregulated number of differentially abundant OTUs: 0
```

Below is a volcano plot showing the overall differential abundance results for this comparison.



And here are the taxa showing the highest increase or decrease between the groups. (Null if no differences)

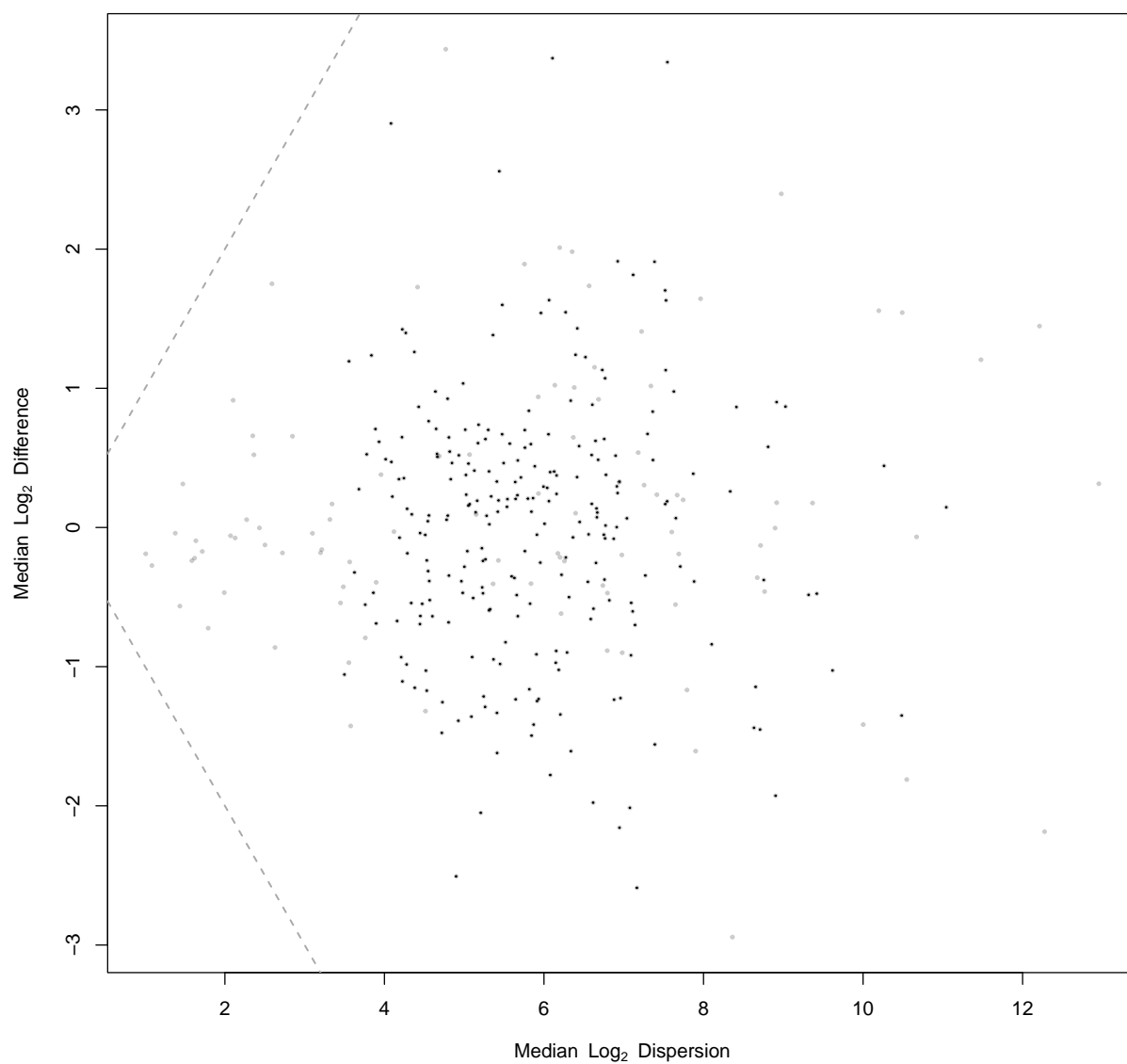
```
## NULL
```

ALDEx2

Setup

Post-Sham vs Pre-Sham

```
## |----- (25%) ----- (50%) ----- (75%) ----- |
```

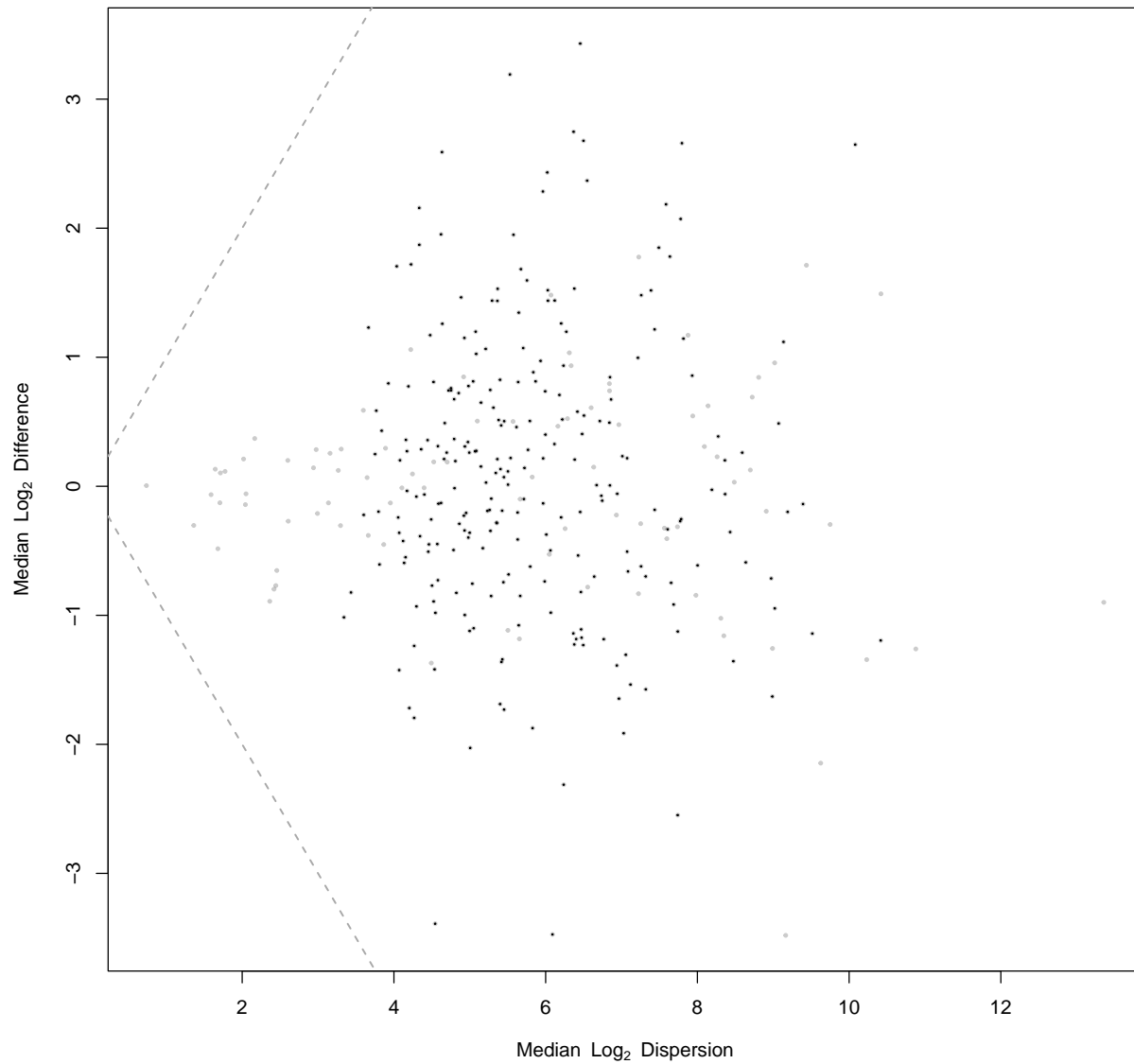



```
## [1] GroupPost-Sham:pval.padj
## <0 rows> (or 0-length row.names)
```

```
## [1] 1.0000000 0.8227373 0.8780870
```

Post-Hypoxia vs Pre-Hypoxia

```
## |------(25%)------(50%)------(75%)-----|
```



```
## [1] GroupPost-Hypoxia:pval.padj
## <0 rows> (or 0-length row.names)
```

```
## [1] 1.0000000 0.9759411 0.8264517 0.9863736 0.9751717 0.9578362 0.9296805
```

Section 5: Positive Control Analysis

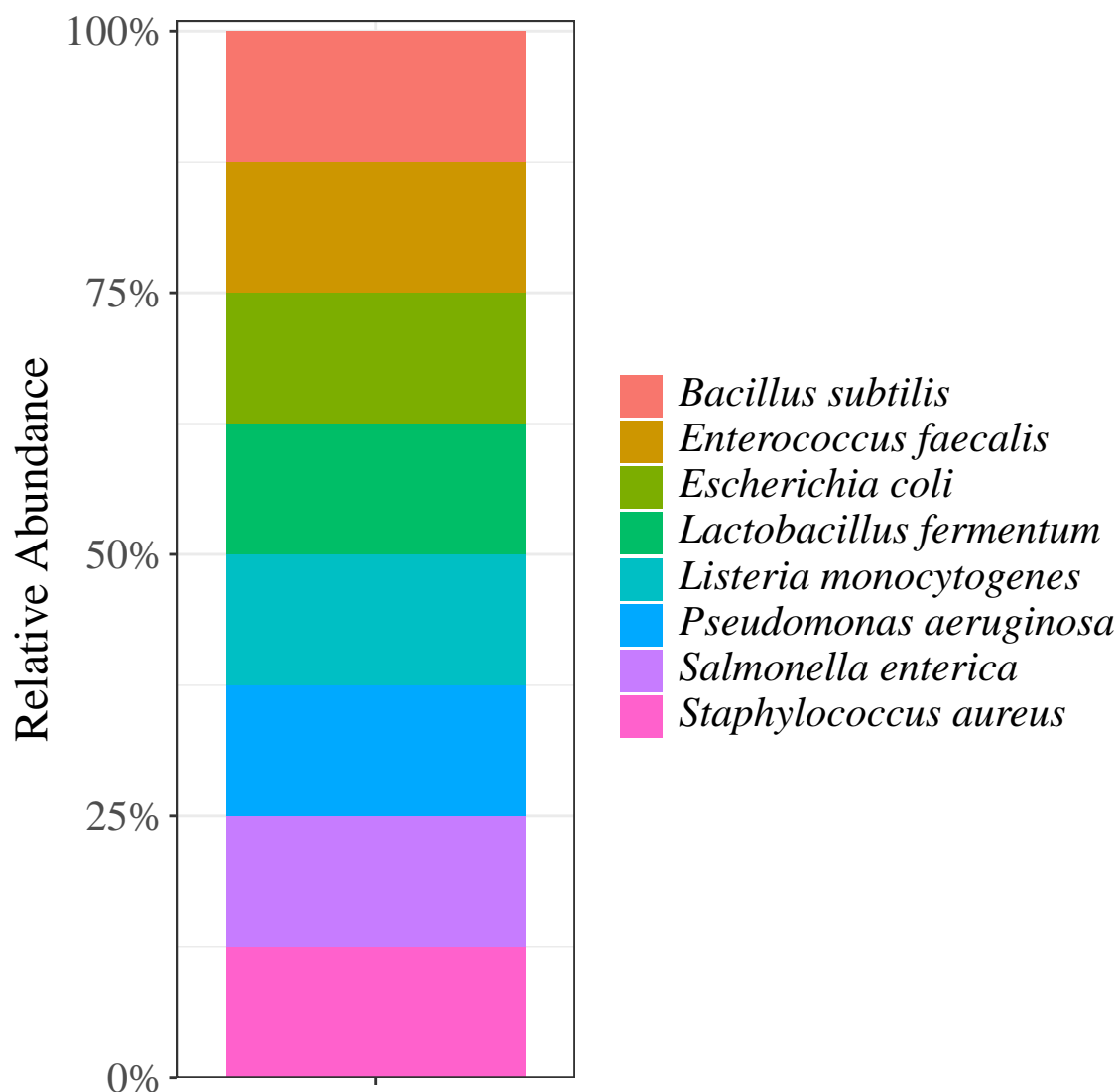
This data set contained samples generated from the Zymobiomics Microbial Community Standard. This is used to assess bias and errors in the extraction methods and identify potential contamination across the data set.

Theoretical distribution

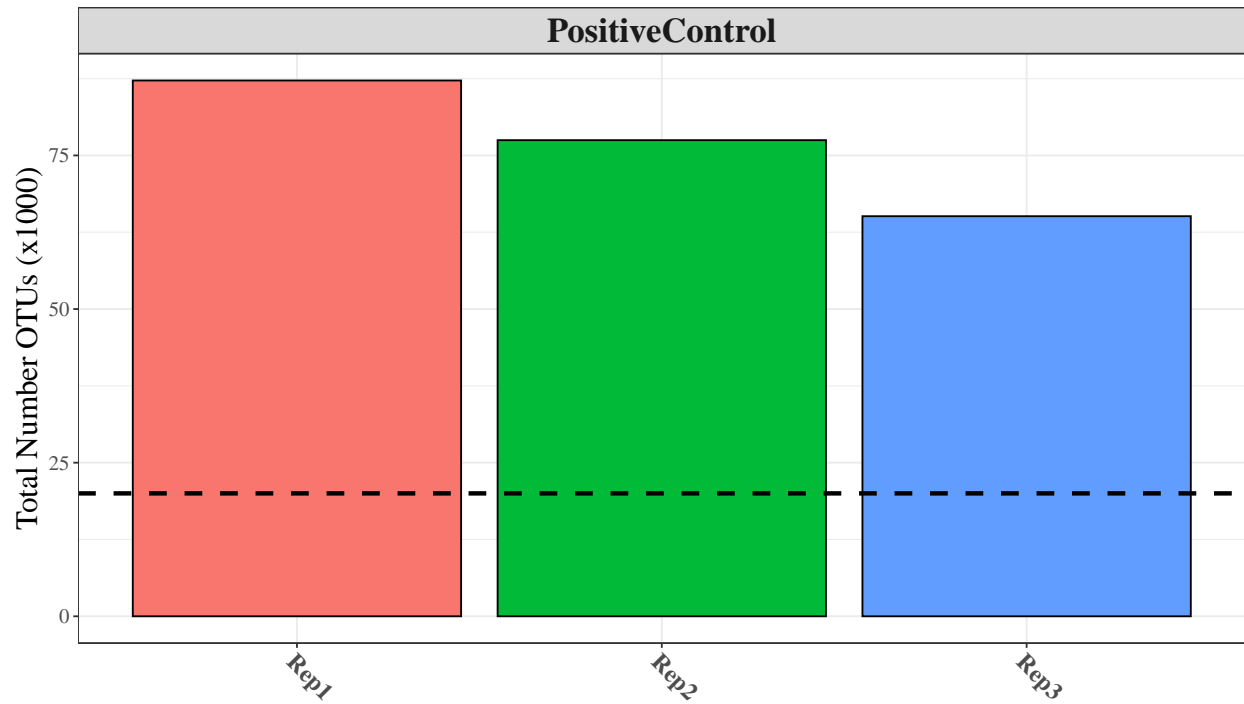
These samples have a theoretical Composition Based on Genomic DNA which should be represented in the output distributions following data processing alongside the other samples in the data set. This distribution is as follows:

Listeria monocytogenes - 12%, *Pseudomonas aeruginosa* - 12%, *Bacillus subtilis* - 12%, *Escherichia coli* - 12%, *Salmonella enterica* - 12%, *Lactobacillus fermentum* - 12%, *Enterococcus faecalis* - 12%, *Staphylococcus aureus* - 12%, *Saccharomyces cerevisiae* - 2%, and *Cryptococcus neoformans* - 2%

Note that only the bacterial species (all excluding *Saccharomyces cerevisiae* and *Cryptococcus neoformans*) will be identified through 16S rRNA amplicon based sequencing. The theoretical distribution for only the bacterial species is shown below:



The total number of taxa (in thousands) for the PC samples is shown below.



Then we filter to remove the samples with too few taxa, rarefy to the depth of the lowest sample, and normalise to percentage abundance as we did above.

```
## Rarefication of data to 65109 OTUs:
```

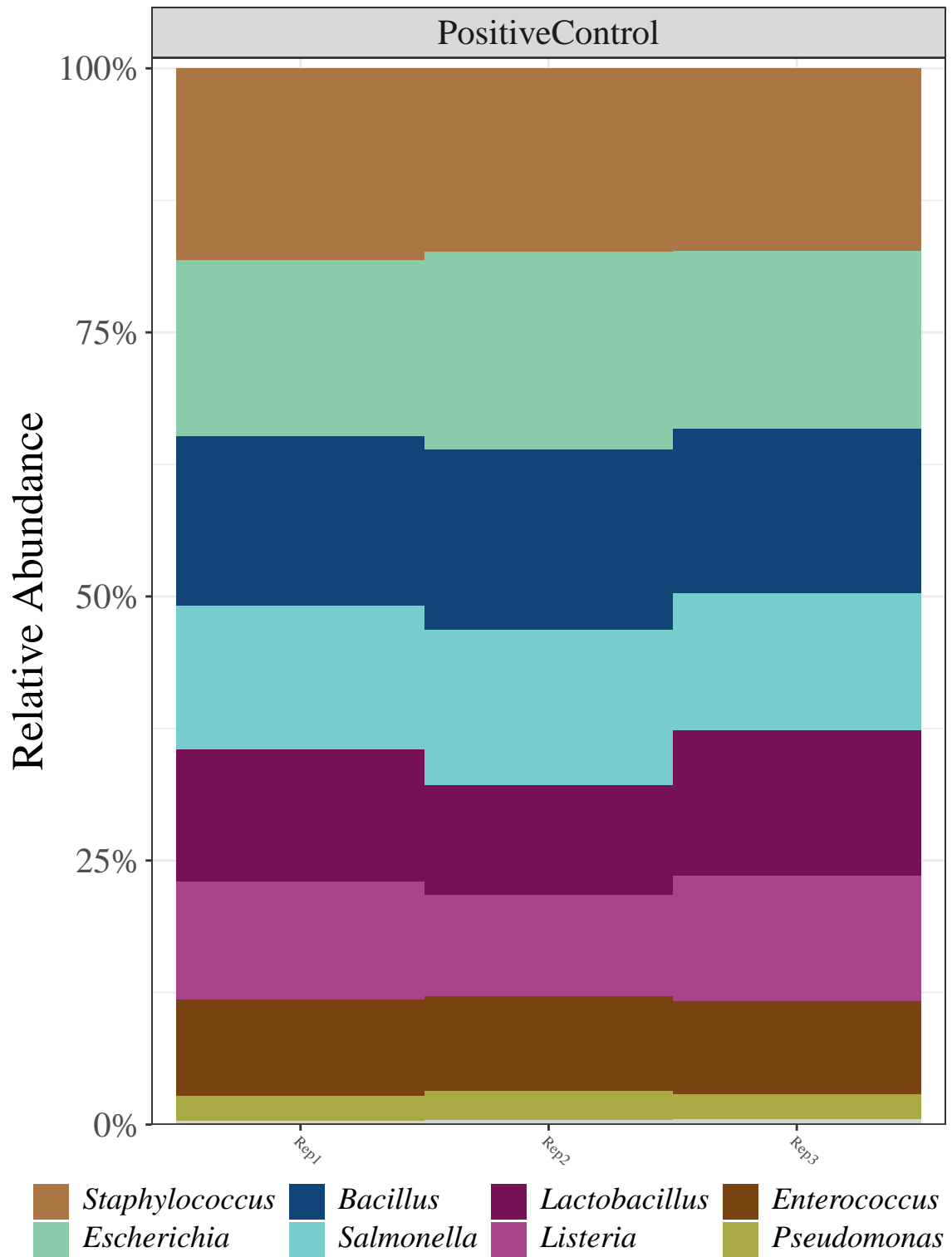
Chi-squared

Below is a chi-squared based comparison between the PC replicates against the expected distribution:

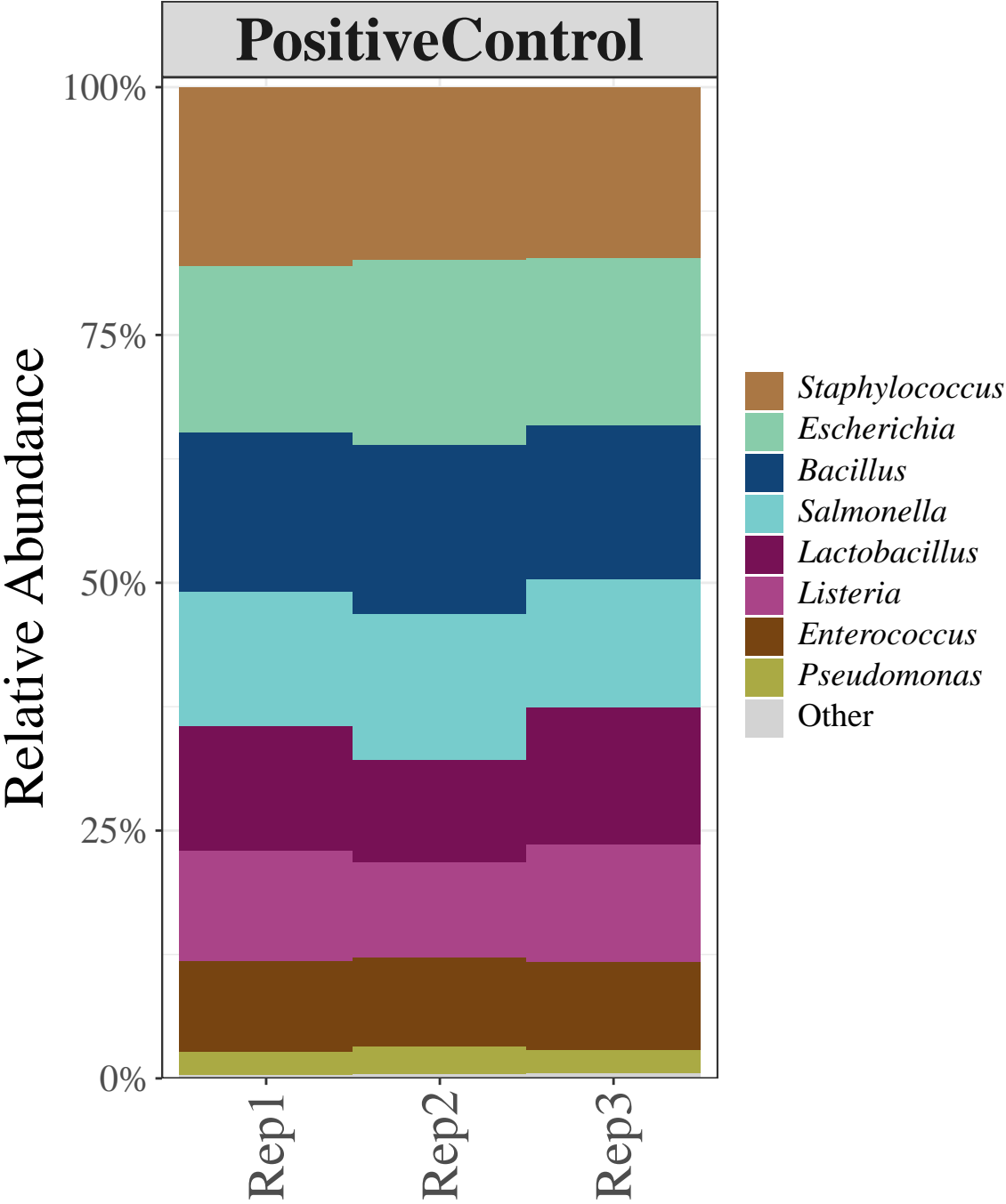
```
##
## Pearson's Chi-squared test
##
## data: PC_abun_merge[, -1]
## X-squared = 20.407, df = 21, p-value = 0.4957
```

Plot

The distribution of taxa at the Genus level is shown below for the PC samples:



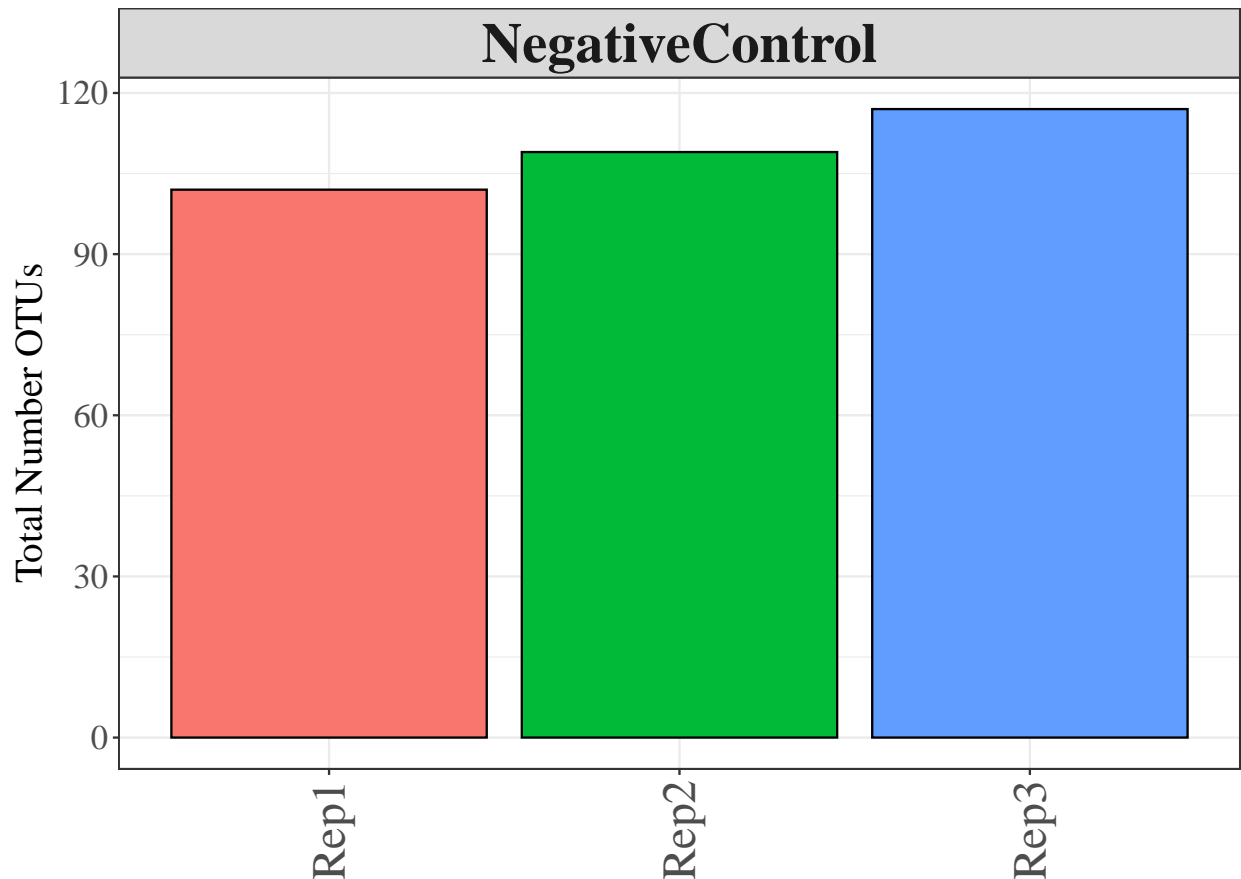
Chi-Squared: $X^2_{(21)} = 20.407, p = 0.496$



Section 6: Negative Control Analysis

Taxa count

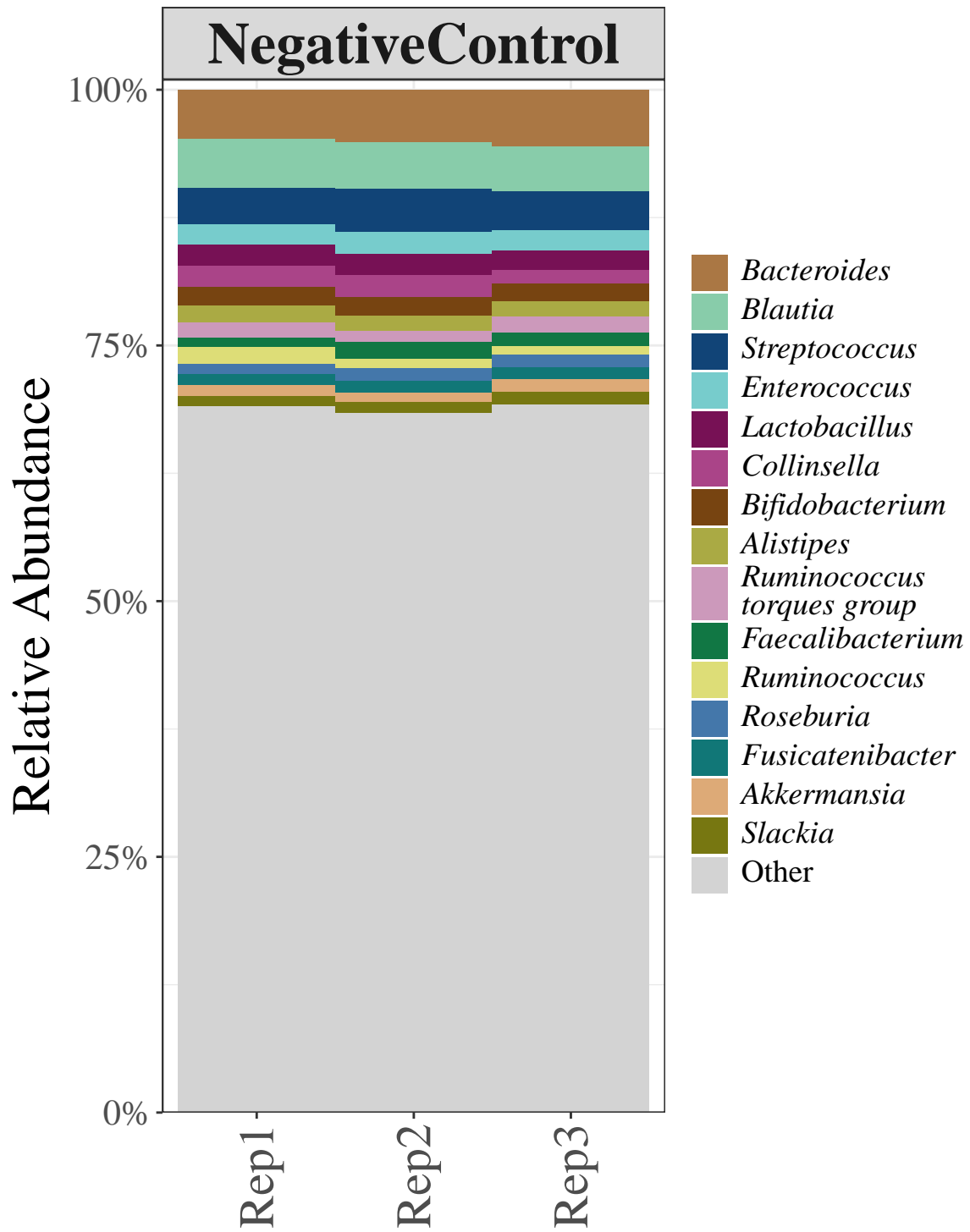
The total number of taxa for the NFW samples is shown below. This should be as low as possible for all replicates



Then we filter normalise to percentage abundance as we did above (note that we are not filtering or rarefying these sample, as we want to observe the negative influences on the data).

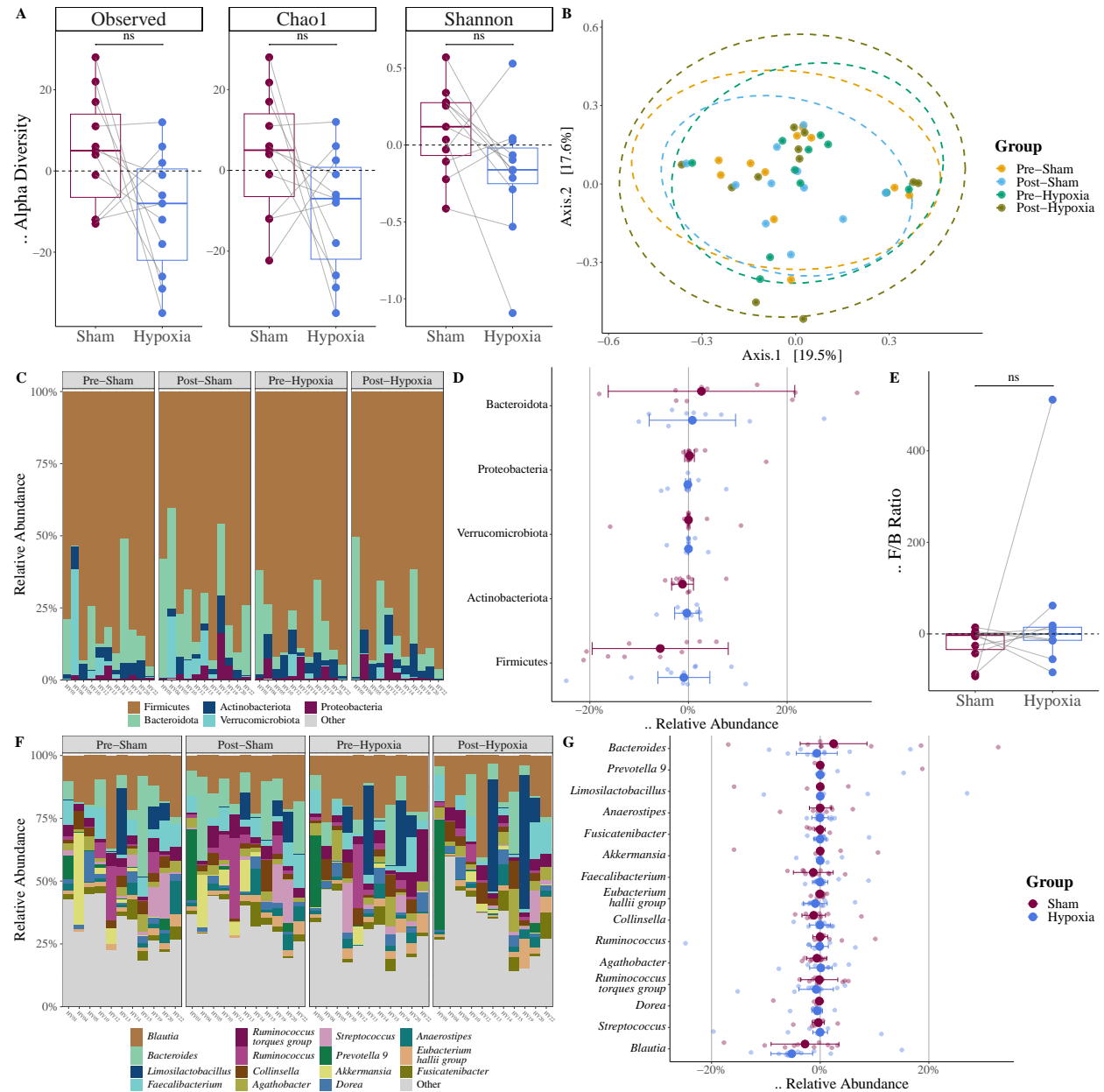
Plot

The distribution of taxa at the Genus level is shown below for the NFW samples (note that these data are NOT filtered, as this would remove all samples - so this is based on very low levels of reads). Note that this plot may be influenced by the fact that all taxa had one added to them when normalising to avoid divide by zero errors, so if the read count is low then this plot can be ignored.

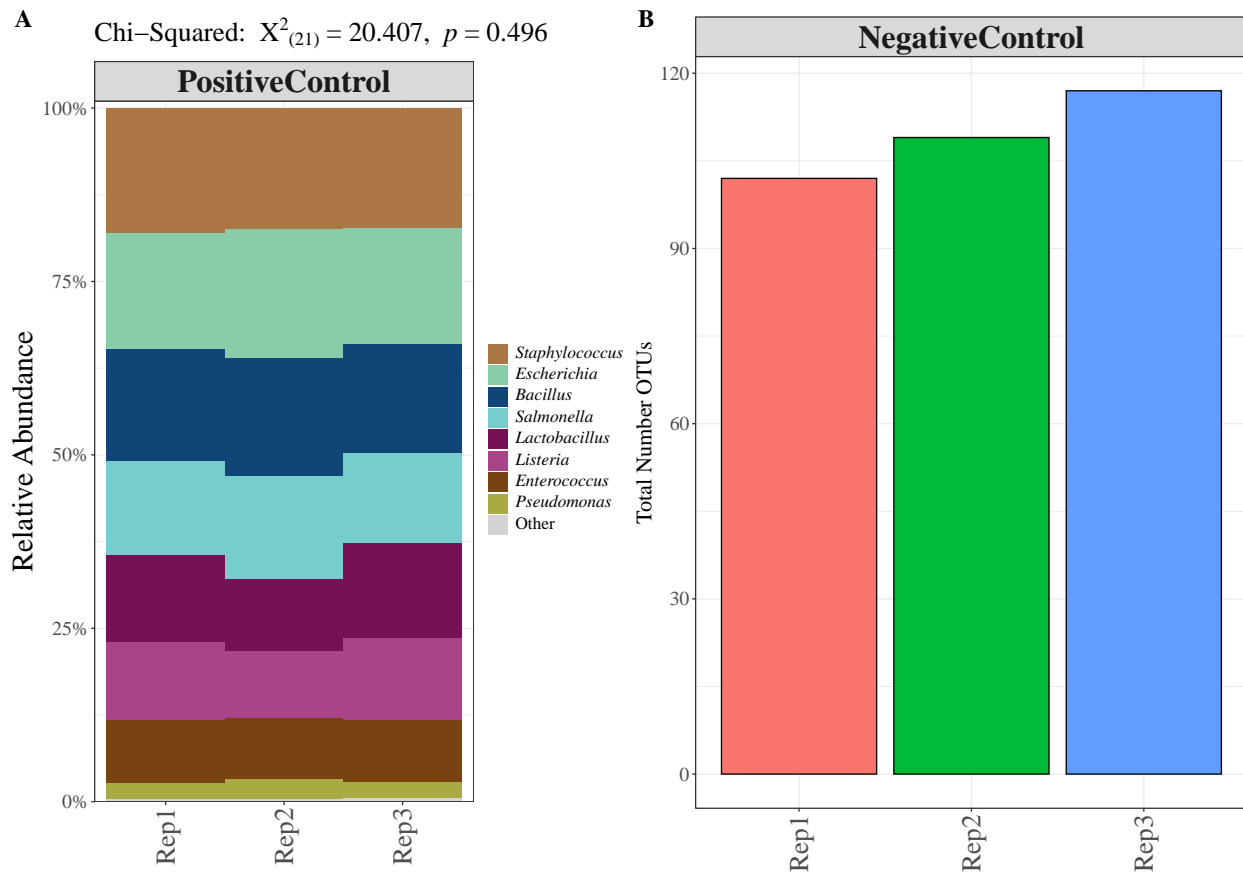


Section 7: Figures

Diveristy and Abundance Panel



Controls Panel



Session Info

```
## R version 4.3.1 (2023-06-16 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19045)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United Kingdom.utf8
## [2] LC_CTYPE=English_United Kingdom.utf8
## [3] LC_MONETARY=English_United Kingdom.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United Kingdom.utf8
##
## time zone: Europe/London
## tzcode source: internal
##
## attached base packages:
```

```

## [1] stats4      stats      graphics  grDevices utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] rstatix_0.7.2          ggtext_0.1.2
## [3] scales_1.3.0          ALDEx2_1.34.0
## [5] latticeExtra_0.6-30    zCompositions_1.5.0-1
## [7] truncnorm_1.0-9        NADA_1.6-1.1
## [9] survival_3.5-7         MASS_7.3-60.0.1
## [11] DESeq2_1.42.0          SummarizedExperiment_1.32.0
## [13] Biobase_2.62.0         MatrixGenerics_1.14.0
## [15] matrixStats_1.2.0      GenomicRanges_1.54.1
## [17] GenomeInfoDb_1.38.5    IRanges_2.36.0
## [19] S4Vectors_0.40.2       BiocGenerics_0.48.1
## [21] reshape2_1.4.4         plyr_1.8.9
## [23] vegan_2.6-4            lattice_0.22-5
## [25] permute_0.9-7          ggpubr_0.6.0
## [27] jmv_2.4.11             qiime2R_0.99.6
## [29] xlsx_0.6.5             lubridate_1.9.3
## [31] forcats_1.0.0          stringr_1.5.1
## [33] dplyr_1.1.4            purrr_1.0.2
## [35] readr_2.1.5            tidyr_1.3.1
## [37] tibble_3.2.1           ggplot2_3.4.4
## [39] tidyverse_2.0.0        phyloseq_1.46.0
## [41] RMySQL_0.10.27         DBI_1.2.1
##
## loaded via a namespace (and not attached):
## [1] RColorBrewer_1.1-3      rstudioapi_0.15.0      jsonlite_1.8.8
## [4] magrittr_2.0.3          farver_2.1.1           rmarkdown_2.25
## [7] zlibbioc_1.48.0         vctrs_0.6.5            multtest_2.58.0
## [10] RCurl_1.98-1.14         base64enc_0.1-3        htmltools_0.5.7
## [13] S4Arrays_1.2.0          broom_1.0.5            Rhdf5lib_1.24.1
## [16] SparseArray_1.2.3       Formula_1.2-5          rhdf5_2.46.1
## [19] htmlwidgets_1.6.4       commonmark_1.9.0       igraph_1.6.0
## [22] lifecycle_1.0.4         iterators_1.0.14       pkgconfig_2.0.3
## [25] Matrix_1.6-5            R6_2.5.1               fastmap_1.1.1
## [28] GenomeInfoDbData_1.2.11 digest_0.6.34           colorspace_2.1-0
## [31] Hmisc_5.1-1             labeling_0.4.3          RcppZigurat_0.1.6
## [34] fansi_1.0.6             timechange_0.3.0       abind_1.4-5
## [37] mgcv_1.9-1              compiler_4.3.1         withr_3.0.0
## [40] htmlTable_2.4.2         backports_1.4.1        BiocParallel_1.36.0
## [43] carData_3.0-5           psych_2.4.1            ggsignif_0.6.4
## [46] DelayedArray_0.28.0     biomformat_1.30.0      tools_4.3.1
## [49] foreign_0.8-86          ape_5.7-1              nnet_7.3-19
## [52] quadprog_1.5-8          glue_1.7.0             nlme_3.1-164
## [55] rhdf5filters_1.14.1     gridtext_0.1.5         grid_4.3.1
## [58] checkmate_2.3.1         cluster_2.1.6          ade4_1.7-22
## [61] generics_0.1.3          gtable_0.3.4           tzdb_0.4.0
## [64] data.table_1.14.10      hms_1.1.3              xml2_1.3.6
## [67] car_3.1-2              utf8_1.2.4             XVector_0.42.0
## [70] markdown_1.12           foreach_1.5.2           pillar_1.9.0
## [73] rJava_1.0-10            splines_4.3.1          deldir_2.0-2
## [76] directlabels_2024.1.21 tidyselect_1.2.0       locfit_1.5-9.8
## [79] Biostrings_2.70.1       knitr_1.45             gridExtra_2.3

```

## [82] xfun_0.41	jmvcore_2.4.7	DT_0.31
## [85] stringi_1.8.3	yaml_2.3.8	evaluate_0.23
## [88] codetools_0.2-19	xlsxjars_0.6.1	interp_1.1-6
## [91] cli_3.6.2	RcppParallel_5.1.7	rpart_4.1.23
## [94] munsell_0.5.0	Rcpp_1.0.12	png_0.1-8
## [97] Rfast_2.1.0	parallel_4.3.1	jpeg_0.1-10
## [100] bitops_1.0-7	crayon_1.5.2	rlang_1.1.3
## [103] cowplot_1.1.3	mnormt_2.1.1	