Purcell Project Markdown

Dan Hudson and Xochitl Morgan

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Load phyloseq object

Phyloseq object was generated on the server using serverScript.R, following the running of this script it was downloaded to the local machine and used to make plots

```r
# load data
ps0  <- readRDS("../PrimaryData/phyloObject.rds")

# read metadata
meta <- read.csv("../PrimaryData/purcell_meta.csv")

# add tree
tree <- read_tree("../PrimaryData/purcellFastTree_edit.tre")

# load metadata and tree into phyloseq object
meta <- sample_data(meta)
meta$Individual <- as.factor(meta$Individual)
row.names(meta) <- meta$Sample_name
ps <- merge_phyloseq(ps0, meta, tree)


# unedited phyloseq object
psOG <- ps

# Assign DNA sequences to refseq slot and replace with simple names to improve readability
dna <- Biostrings::DNAStringSet(taxa_names(ps))
names(dna) <- taxa_names(ps)
ps <- merge_phyloseq(ps, dna)
taxa_names(ps) <- paste0("ASV", seq(ntaxa(ps)))
ps
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 4872 taxa and 60 samples ]
## sample_data() Sample Data:       [ 60 samples by 7 sample variables ]
## tax_table()   Taxonomy Table:    [ 4872 taxa by 6 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 4872 tips and 4870 internal nodes ]
## refseq()      DNAStringSet:      [ 4872 reference sequences ]
```

Custom Rarefaction Plot

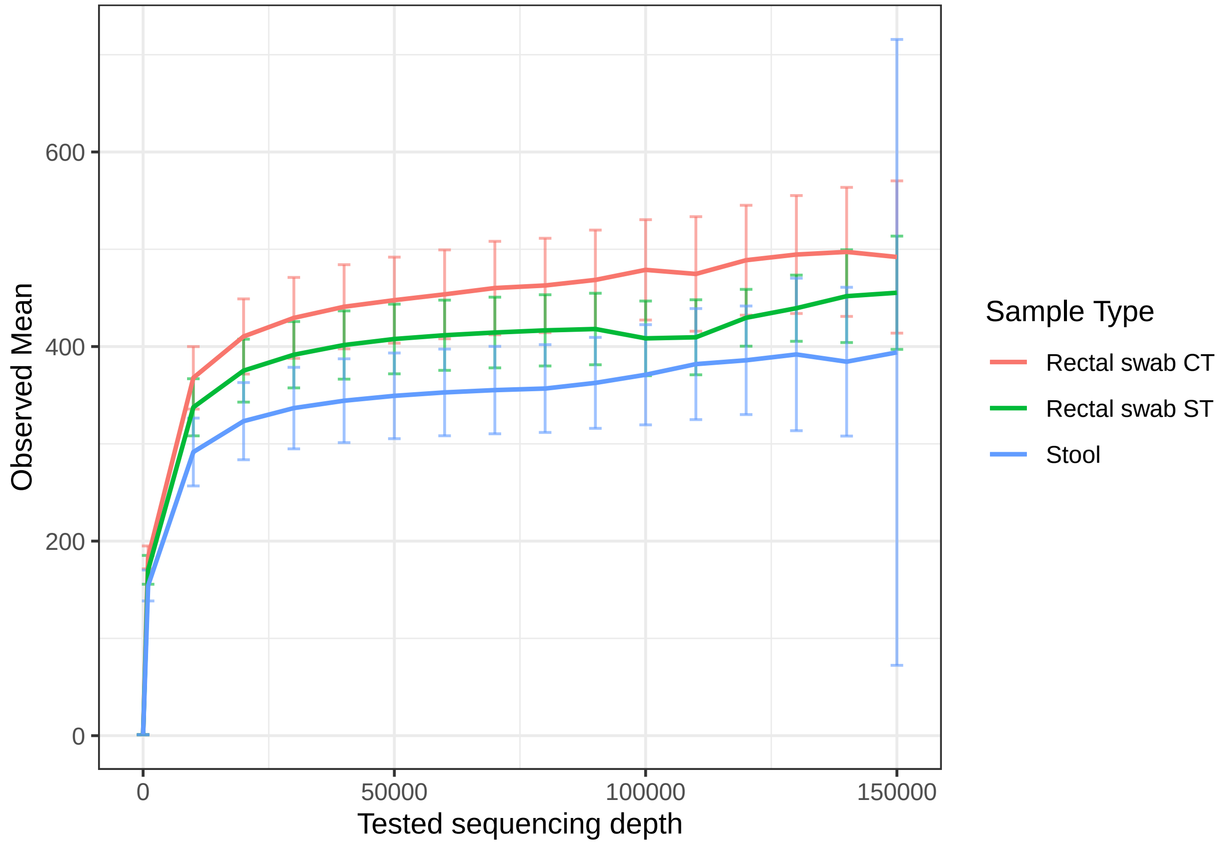
Not run in this Markdown

```r
# Data
psdata <- ps

# Loading required library and displaying core configuration
library('doParallel')
detectCores(all.tests = TRUE)

# Setting up and registering the cluster
cl <- makeCluster(detectCores(all.tests = TRUE)-1)
registerDoParallel(cl)

# Calculate alpha diversity
calculate_rarefaction_curves <- function(psdata, measures, depths, parallel = FALSE) {
  require('plyr') # ldply
  require('reshape2') # melt
  require('doParallel')

  # set parallel options if required
  if (parallel) {
    paropts <- list(.packages = c("phyloseq", "reshape2"))
  } else {
    paropts <- NULL
  }

  estimate_rarified_richness <- function(psdata, measures, depth) {
    if(max(sample_sums(psdata)) < depth) return()
    psdata <- prune_samples(sample_sums(psdata) >= depth, psdata)

    rarified_psdata <- rarefy_even_depth(psdata, depth, verbose = FALSE)

    alpha_diversity <- estimate_richness(rarified_psdata, measures = measures)

    # as.matrix forces the use of melt.array, which includes the Sample names (rownames)
    molten_alpha_diversity <- melt(as.matrix(alpha_diversity),
                                   varnames = c('Sample', 'Measure'),
                                   value.name = 'Alpha_diversity')

    molten_alpha_diversity
  }

  names(depths) <- depths # this enables automatic addition of the Depth to the output by ldply
  rarefaction_curve_data <- ldply(depths,
                                  estimate_rarified_richness,
                                  psdata = psdata,
                                  measures = measures,
                                  .id = 'Depth',
                                  .progress = ifelse(interactive() && ! parallel, 'text', 'none'),
                                  .parallel = parallel,
                                  .paropts = paropts)

  # convert Depth from factor to numeric
  rarefaction_curve_data$Depth <- as.numeric(levels(rarefaction_curve_data$Depth))[rarefaction_curve_da
  rarefaction_curve_data
}

rarefaction_curve_data <- calculate_rarefaction_curves(psdata, c('Observed'),
                                                       rep(c(1, 100, 1:150 * 1000),
                                                           each = 10))
summary(rarefaction_curve_data)
saveRDS(rarefaction_curve_data, file = "../PrimaryData/rare_object.rds")
```

```
# Data
psdata <- ps

# Load Rarefaction Curve Data Object
rarefaction_curve_data <- readRDS(file = "../PrimaryData/rare_object.rds")
summary(rarefaction_curve_data)
```

```
##      Depth            Sample          Measure      Alpha_diversity
##  Min.   :     1   X10B   : 1520   Observed:77740   Min.   :  1.0
##  1st Qu.: 31000   X12B   : 1520                    1st Qu.:321.0
##  Median : 63000   X12C   : 1520                    Median :403.0
##  Mean   : 65150   X13A   : 1520                    Mean   :391.3
##  3rd Qu.: 97000   X13B   : 1520                    3rd Qu.:464.0
##  Max.   :150000   X14A   : 1520                    Max.   :674.0
##                   (Other):68620
```

```
# Summarise alpha diversity
rarefaction_curve_data_summary <- ddply(rarefaction_curve_data,
                                        c('Depth', 'Sample', 'Measure'),
                                        summarise,
                                        Alpha_diversity_mean = mean(Alpha_diversity),
                                        Alpha_diversity_sd = sd(Alpha_diversity))

colnames(rarefaction_curve_data_summary) <- gsub("X","",
                                                 colnames(rarefaction_curve_data_summary))
rarefaction_curve_data_summary$Sample <- gsub("X","", rarefaction_curve_data_summary$Sample)

# Add sample data
rarefaction_curve_data_summary_verbose <- merge(rarefaction_curve_data_summary,
                                                data.frame(sample_data(psdata)),
                                                by.x = 'Sample',
                                                by.y = 'row.names')

# Produce summary df of rarefaction data
df_mod <- summarySE(rarefaction_curve_data_summary_verbose,
                   measurevar = "Alpha_diversity_mean",
                   groupvars = c("Depth", "Sample_type"))
df_mod <- df_mod %>%
  subset(Depth == 1 | Depth == 1000 | Depth == 10000 | Depth == 20000 | Depth == 30000 | Depth == 40000

ggplot(df_mod, aes(x = Depth,
                   y = Alpha_diversity_mean,
                   ymin = Alpha_diversity_mean - ci,
                   ymax = Alpha_diversity_mean + ci,
                   colour = Sample_type)) +
  geom_errorbar(size = 0.5, width = 2500, alpha = 0.6) +
  geom_line(size = 0.8) +
  labs(x = "Tested sequencing depth", y = "Observed Mean", color = "Sample Type")
```



```
ggsave("../Results/S1)Rarefaction_Curve.pdf", width = 11, height = 8)
```

Rarefy

```
# Rarefy to even sequencing depth, 90% of minimum sample depth, seed for randomness is 1
ps_rare <- rarefy_even_depth(ps, rngseed = 1,
                             sample.size = 0.9 * min(sample_sums(ps)),
                             replace = FALSE)
```

```
## `set.seed(1)` was used to initialize repeatable random subsampling.

## Please record this for your records so others can reproduce.

## Try `set.seed(1); .Random.seed` for the full vector

## ...

## 243OTUs were removed because they are no longer
## present in any sample after random subsampling

## ...
```

```
sample_sums(ps)
```

```
##     10A     10B     10C     11A     11B     11C     12A     12B     12C     13A     13B
##  97672 152224 136830 107226  92295 142349  63696 151049 153224 170086 154765
##    13C    14A    14B    14C    15A    15B    15C    16A    16B    16C    17A
## 146933 160605 171722 140943 175324 114245 168613 120816 131462 141789 153959
##    17B    17C    18A    18B    18C    19A    19B    19C     1A     1B     1C
## 127615  94965 160212 126836 159814 161407 153370 121330 165497  96844 113268
##    20A    20B    20C     2A     2B     2C     3A     3B     3C     4A     4B
## 195853 115506 127239 110007 118680 110327 146390 136636 106307 104581 125868
##     4C     5A     5B     5C     6A     6B     6C     7A     7B     7C     8A
## 131775 160742 121440  88650 140459 164106  92481 137767 138331 120381 140622
##     8B     8C     9A     9B     9C
##  97857 112182  84876 143122 108117
```

```r
sample_sums(ps_rare)
```

```
##   10A   10B   10C   11A   11B   11C   12A   12B   12C   13A   13B   13C   14A
## 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326
##   14B   14C   15A   15B   15C   16A   16B   16C   17A   17B   17C   18A   18B
## 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326
##   18C   19A   19B   19C    1A    1B    1C   20A   20B   20C    2A    2B    2C
## 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326
##    3A    3B    3C    4A    4B    4C    5A    5B    5C    6A    6B    6C    7A
## 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326 57326
##    7B    7C    8A    8B    8C    9A    9B    9C
## 57326 57326 57326 57326 57326 57326 57326 57326
```

Alpha Diversity

```r
# Calculate alpha diversity, using Richness and Shannon
alpha_summary <- estimate_richness(ps_rare, measures = c("Observed", "Shannon"))
shapiro.test(alpha_summary$Observed)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  alpha_summary$Observed
## W = 0.99288, p-value = 0.9797
```

```r
shapiro.test(alpha_summary$Shannon)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  alpha_summary$Shannon
## W = 0.97844, p-value = 0.3662
```

```r
# Blocking Test
rO <- alpha_summary$Observed
rS <- alpha_summary$Shannon

f <- c("Clinician", "Self", "Stool") # treatment levels
k <- 3 # number of treatment levels
n <- 20 # number of control blocks

tm <- gl(k, 1, n*k, factor(f)) # matching treatment
```

```
blk <- gl(n, k, k*n) # blocking factor

av0 <- aov(r0 ~ tm + blk)
summary(av0)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)    
## tm           2 106186   53093  14.357 2.27e-05 ***
## blk         19 332244   17487   4.729 2.33e-05 ***
## Residuals   38 140525    3698                     
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
avS <- aov(rS ~ tm + blk)
summary(avS)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)    
## tm           2  0.849  0.4247   6.675 0.003278 ** 
## blk         19  4.871  0.2564   4.030 0.000126 ***
## Residuals   38  2.418  0.0636                     
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Test whether the observed number of OTUs differs significantly between samples
# p adjustment using Benjamini and Hochberg
pairwise.t.test(alpha_summary$Observed, sample_data(ps_rare)$Sample_type, p.adjust = "BH")
```

```
## 
##  Pairwise comparisons using t tests with pooled SD 
## 
## data:  alpha_summary$Observed and sample_data(ps_rare)$Sample_type 
## 
##                Rectal swab CT Rectal swab ST
## Rectal swab ST 0.1409         -             
## Stool          0.0023         0.0646        
## 
## P value adjustment method: BH
```

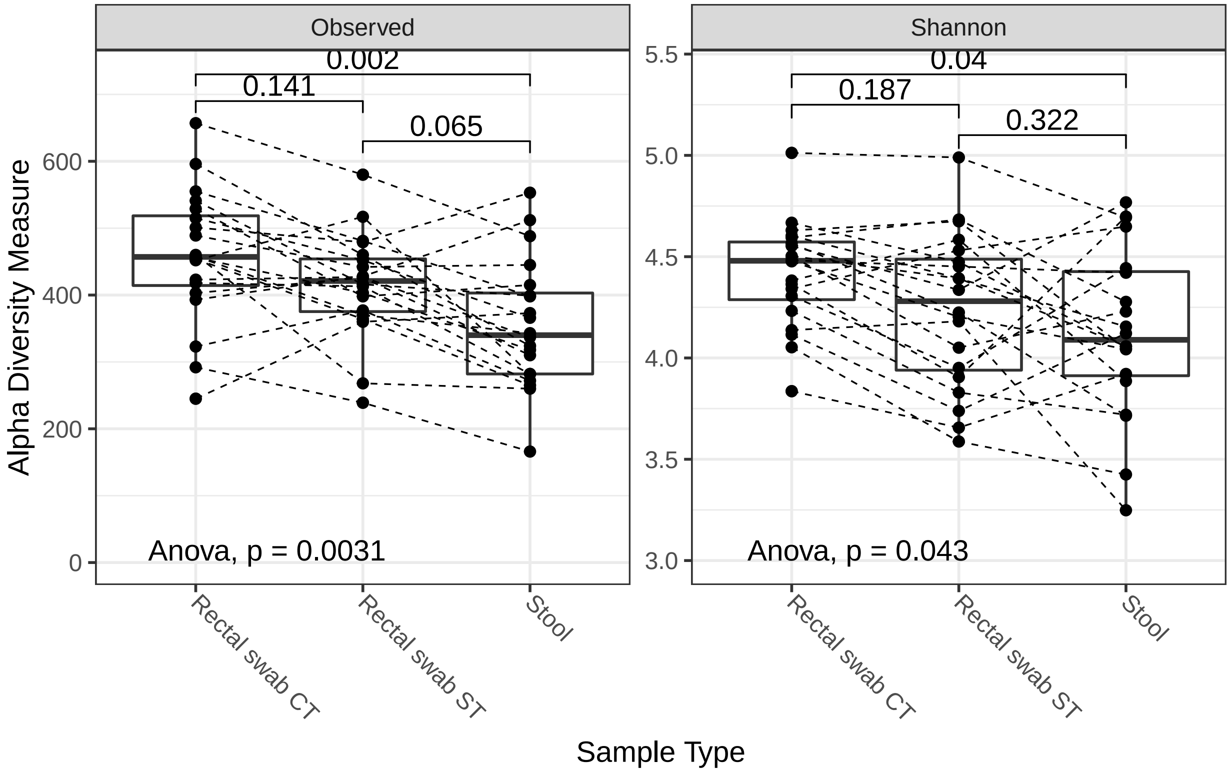
```
pairwise.t.test(alpha_summary$Shannon, sample_data(ps_rare)$Sample_type, p.adjust = "BH")
```

```
## 
##  Pairwise comparisons using t tests with pooled SD 
## 
## data:  alpha_summary$Shannon and sample_data(ps_rare)$Sample_type 
## 
##                Rectal swab CT Rectal swab ST
## Rectal swab ST 0.19           -             
## Stool          0.04           0.32          
## 
## P value adjustment method: BH
```

```
# Make adjusted p value dataframe
pObs <- pairwise.t.test(alpha_summary$Observed, sample_data(ps_rare)$Sample_type, p.adjust = "BH")
pSha <- pairwise.t.test(alpha_summary$Shannon, sample_data(ps_rare)$Sample_type, p.adjust = "BH")

variable <- c("Observed", "Observed", "Observed", "Shannon", "Shannon", "Shannon")
group1 <- c("Rectal swab CT", "Rectal swab ST", "Rectal swab CT",
            "Rectal swab CT", "Rectal swab ST", "Rectal swab CT")
group2 <- c("Stool", "Stool", "Rectal swab ST", "Stool", "Stool", "Rectal swab ST")
pVal <- c(round(pObs$p.value[2,1], 3), round(pObs$p.value[2,2], 3), round(pObs$p.value[1,1], 3),
       round(pSha$p.value[2,1], 3), round(pSha$p.value[2,2], 3), round(pSha$p.value[1,1], 3))
y.position <- c(730, 630, 690, 5.4, 5.1, 5.25)

pAdjusted <- bind_cols(variable, group1, group2, pVal, y.position)
```

```
## New names:
## * NA -> ...1
## * NA -> ...2
## * NA -> ...3
## * NA -> ...4
## * NA -> ...5
```

```
colnames(pAdjusted) <- c("variable", "group1", "group2", "p", "y.position")

# Plot Observed richness, Shannon, and Simpson diversity values
p <- plot_richness(ps_rare, x = "Sample_type",
                   measures = c("Observed", "Shannon"))

# Add boxplot, individual data points, and linked lines using geom layers
p$layers <- p$layers[-1]
p + geom_boxplot() + geom_point() + xlab("Sample Type") +
  geom_line(aes(group = Individual), size = 0.3, linetype = "dashed") +
  theme(axis.text.x = element_text(angle = 315, hjust = 0),
        aspect.ratio = 1, legend.position = "none") +
  stat_pvalue_manual(pAdjusted) +
  stat_compare_means(method = "anova", label.y = 3)
```



```r
ggsave("../Results/1)Alpha_Diversity.pdf", width = 7, height = 4.5)
```

Beta Diversity - Bray-Curtis

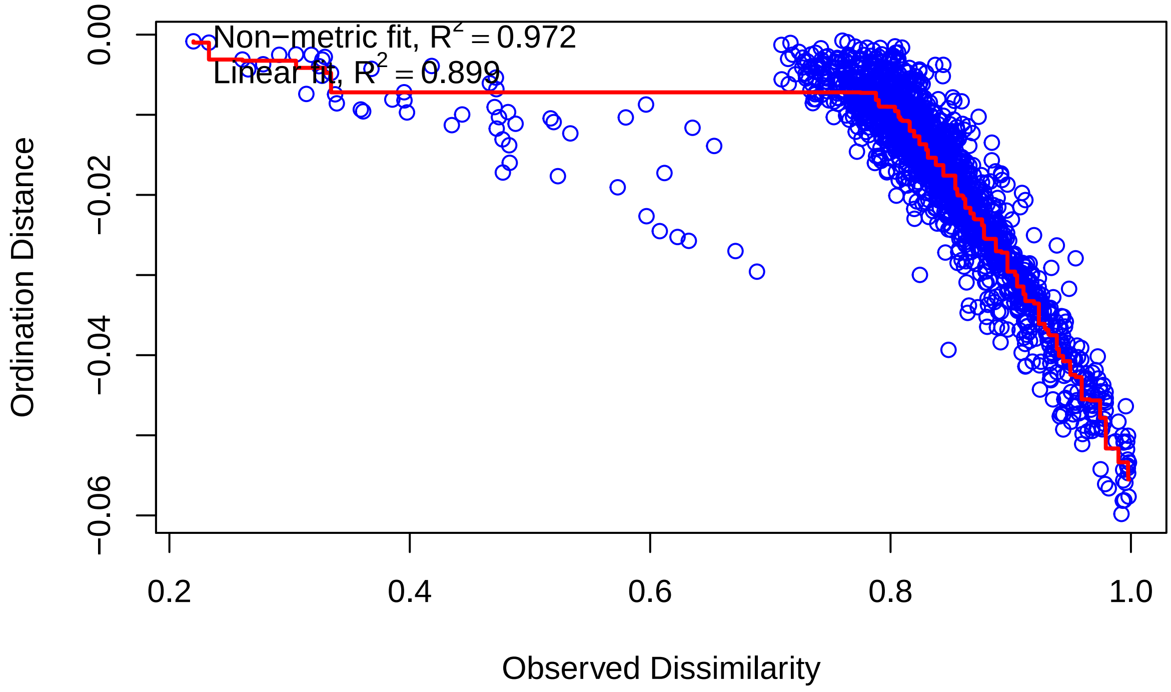
```r
# Ordinate data using Non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarity (distance
bray_dist <- phyloseq::distance(ps_rare, method = "bray")
ord.nmds.bray <- ordinate(ps_rare, "NMDS", "bray")
```

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.1711869
## Run 1 stress 0.1684076
## ... New best solution
## ... Procrustes: rmse 0.04403871  max resid 0.1986753
## Run 2 stress 0.1712507
## Run 3 stress 0.1712864
## Run 4 stress 0.1712903
## Run 5 stress 0.1684074
## ... New best solution
## ... Procrustes: rmse 0.0003485566  max resid 0.002061949
## ... Similar to previous best
## Run 6 stress 0.1684197
## ... Procrustes: rmse 0.001465667  max resid 0.006957013
## ... Similar to previous best
## Run 7 stress 0.1711862
## Run 8 stress 0.1712879
## Run 9 stress 0.1711864
## Run 10 stress 0.1684183
## ... Procrustes: rmse 0.001257738  max resid 0.006842475
## ... Similar to previous best
## Run 11 stress 0.168407
## ... New best solution
## ... Procrustes: rmse 0.0001416034  max resid 0.0008423535
## ... Similar to previous best
## Run 12 stress 0.2132788
## Run 13 stress 0.2033467
## Run 14 stress 0.1684073
## ... Procrustes: rmse 0.0001969173  max resid 0.001092735
## ... Similar to previous best
## Run 15 stress 0.2049835
## Run 16 stress 0.1684107
## ... Procrustes: rmse 0.0006893125  max resid 0.004159413
## ... Similar to previous best
## Run 17 stress 0.1684071
## ... Procrustes: rmse 0.0001272377  max resid 0.0008562442
## ... Similar to previous best
## Run 18 stress 0.1712444
## Run 19 stress 0.1684073
## ... Procrustes: rmse 0.0002210374  max resid 0.00137581
## ... Similar to previous best
## Run 20 stress 0.1712802
## *** Solution reached
```

```
# Call newly created file to get the stress value of the plot
ord.nmds.bray
```

```
##
## Call:
## metaMDS(comm = veganifyOTU(physeq), distance = distance)
##
## global Multidimensional Scaling using monoMDS
##
## Data:     wisconsin(sqrt(veganifyOTU(physeq)))
## Distance: bray
##
## Dimensions: 2
## Stress:     0.168407
## Stress type 1, weak ties
## Two convergent solutions found after 20 tries
## Scaling: centring, PC rotation, halfchange scaling
## Species: expanded scores based on 'wisconsin(sqrt(veganifyOTU(physeq)))'
```

```
# Stress plot
stressplot(ord.nmds.bray)
```



```
# Stats
# Test whether the sample types differ significantly from each other using PERMANOVA
adonis(bray_dist ~ sample_data(ps_rare)$Sample_type)
```

```
##
## Call:
## adonis(formula = bray_dist ~ sample_data(ps_rare)$Sample_type)
##
## Permutation: free
## Number of permutations: 999
```

```
##
## Terms added sequentially (first to last)
##
##                                  Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## sample_data(ps_rare)$Sample_type  2    1.3629 0.68145  2.0786 0.06798  0.001
## Residuals                        57   18.6866 0.32784         0.93202
## Total                            59   20.0495                 1.00000
##
## sample_data(ps_rare)$Sample_type ***
## Residuals
## Total
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis(formula = bray_dist ~ sample_data(ps_rare)$Individual)
```

```
##
## Call:
## adonis(formula = bray_dist ~ sample_data(ps_rare)$Individual)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                                 Df SumsOfSqs MeanSqs F.Model     R2 Pr(>F)
## sample_data(ps_rare)$Individual 19   14.9269 0.78563  6.1346 0.7445  0.001 ***
## Residuals                       40    5.1226 0.12807         0.2555
## Total                           59   20.0495                 1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis(bray_dist ~ sample_data(ps_rare)$Sample_type*sample_data(ps_rare)$Individual)
```

```
##
## Call:
## adonis(formula = bray_dist ~ sample_data(ps_rare)$Sample_type *      sample_data(ps_rare)$Individual
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                                                                  Df SumsOfSqs
## sample_data(ps_rare)$Sample_type                                  2    1.3629
## sample_data(ps_rare)$Individual                                  19   14.9269
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual 38    3.7597
## Residuals                                                         0    0.0000
## Total                                                            59   20.0495
##                                                                  MeanSqs
## sample_data(ps_rare)$Sample_type                                       1
## sample_data(ps_rare)$Individual                                        1
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual       0
## Residuals                                                            Inf
## Total
##                                                                   F.Model
## sample_data(ps_rare)$Sample_type                                        0
## sample_data(ps_rare)$Individual                                         0
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual       0
## Residuals
## Total
##                                                                        R2 Pr(>F)
## sample_data(ps_rare)$Sample_type                                 0.06798      1
## sample_data(ps_rare)$Individual                                  0.74450      1
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual 0.18752      1
## Residuals                                                        0.00000
## Total                                                            1.00000
```

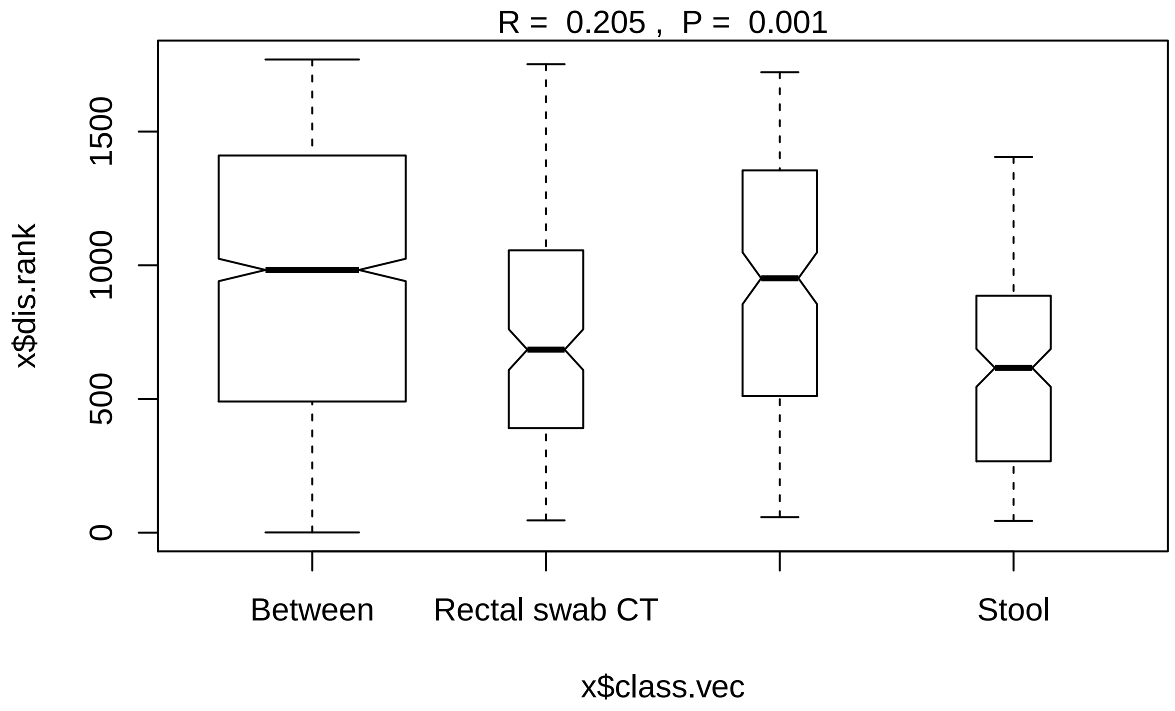
```
anosim(bray_dist, sample_data(ps_rare)$Sample_type)
```

```
##
## Call:
## anosim(x = bray_dist, grouping = sample_data(ps_rare)$Sample_type)
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.2048
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
```

```
BCanoSamp <- (anosim(bray_dist, sample_data(ps_rare)$Sample_type))
summary(BCanoSamp)
```

```
##
## Call:
## anosim(x = bray_dist, grouping = sample_data(ps_rare)$Sample_type)
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.2048
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
##
## Upper quantiles of permutations (null model):
##    90%    95%  97.5%    99%
## 0.0296 0.0435 0.0568 0.0732
##
## Dissimilarity ranks between and within classes:
##                 0%     25%    50%     75%   100%    N
## Between          1 491.250 982.50 1410.50 1769.5 1200
## Rectal swab CT 46 392.750 684.50 1053.25 1752.0  190
## Rectal swab ST 58 513.125 951.50 1353.25 1722.0  190
## Stool          44 267.750 616.25  885.75 1405.0  190
```

```
plot(BCanoSamp)
```



```
anosim(bray_dist, sample_data(ps_rare)$Individual)
```

```
##
## Call:
## anosim(x = bray_dist, grouping = sample_data(ps_rare)$Individual)
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.789
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
```

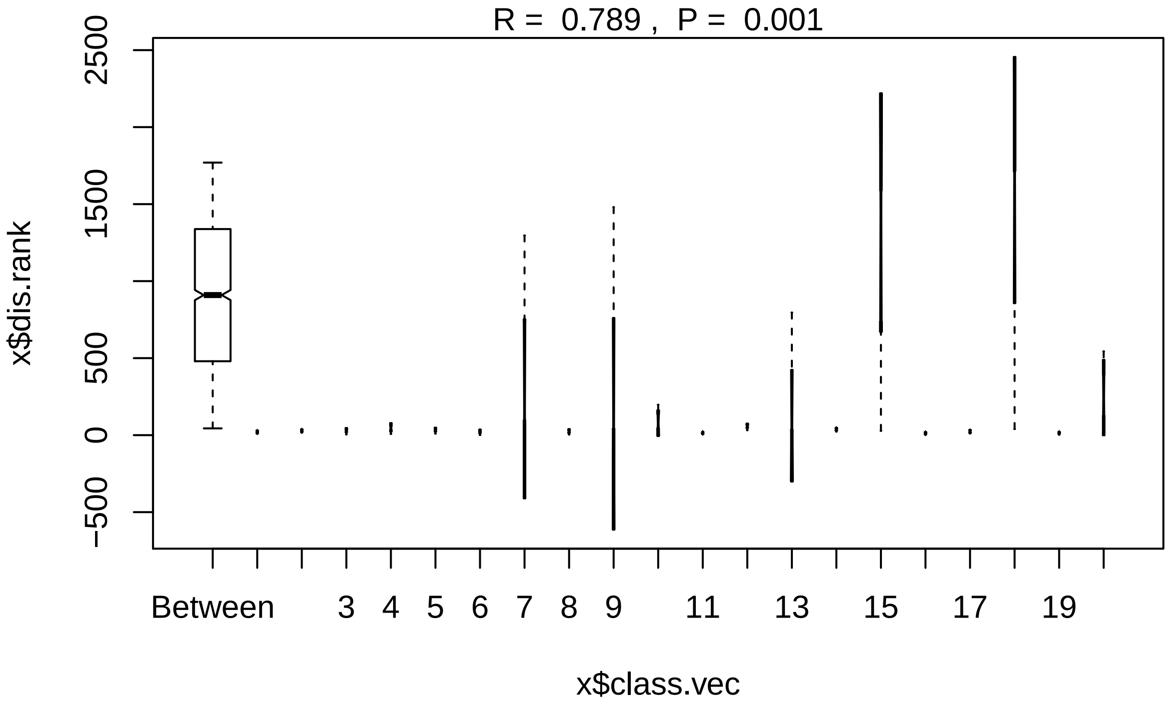
```
BCanoInd <- anosim(bray_dist, sample_data(ps_rare)$Individual)
summary(BCanoInd)
```

```
##
## Call:
## anosim(x = bray_dist, grouping = sample_data(ps_rare)$Individual)
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.789
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
##
## Upper quantiles of permutations (null model):
##    90%    95%  97.5%    99%
## 0.0732 0.1019 0.1270 0.1512
##
## Dissimilarity ranks between and within classes:
```

```
##          0%    25%    50%      75%   100%    N
## Between 44 480.25  909.5 1337.625 1769.5 1710
## 1        9  15.00   21.0   25.500   30.0    3
## 2       16  22.50   29.0   33.000   37.0    3
## 3        5  19.00   33.0   34.000   35.0    3
## 4        8  29.50   51.0   60.000   69.0    3
## 5       10  23.00   36.0   37.000   38.0    3
## 6        1  13.00   25.0   25.500   26.0    3
## 7       20  95.50  171.0  733.750 1296.5    3
## 8        4  15.50   27.0   29.000   31.0    3
## 9       41  41.50   42.0  761.000 1480.0    3
## 10      12  45.00   78.0  137.500  197.0    3
## 11       7  10.50   14.0   18.000   22.0    3
## 12      32  46.50   61.0   62.500   64.0    3
## 13      19  35.50   52.0  423.750  795.5    3
## 14      23  31.00   39.0   41.000   43.0    3
## 15      28 736.50 1445.0 1587.000 1729.0    3
## 16       2   7.50   13.0   15.500   18.0    3
## 17      11  17.50   24.0   29.000   34.0    3
## 18      40 857.00 1674.0 1714.500 1755.0    3
## 19       3   9.00   15.0   16.000   17.0    3
## 20       6 125.00  244.0  394.000  544.0    3
```

```
plot(BCanoInd)
```

```
## Warning in bxp(list(stats = structure(c(44, 480, 909.5, 1338, 1769.5, 9, : some
## notches went outside hinges ('box'): maybe set notch=FALSE
```



```
BCps.disper <- betadisper(bray_dist, sample_data(ps_rare)$Sample_type)
anova(BCps.disper)
```

```
## Analysis of Variance Table
##
```

```
## Response: Distances
##           Df   Sum Sq   Mean Sq F value  Pr(>F)
## Groups     2 0.010371 0.0051855  2.6804 0.07717 .
## Residuals 57 0.110273 0.0019346
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
permutest(BCps.disper)
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df   Sum Sq   Mean Sq      F N.Perm Pr(>F)
## Groups     2 0.010371 0.0051855 2.6804    999  0.087 .
## Residuals 57 0.110273 0.0019346
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
permutest(BCps.disper, pairwise = TRUE)
```

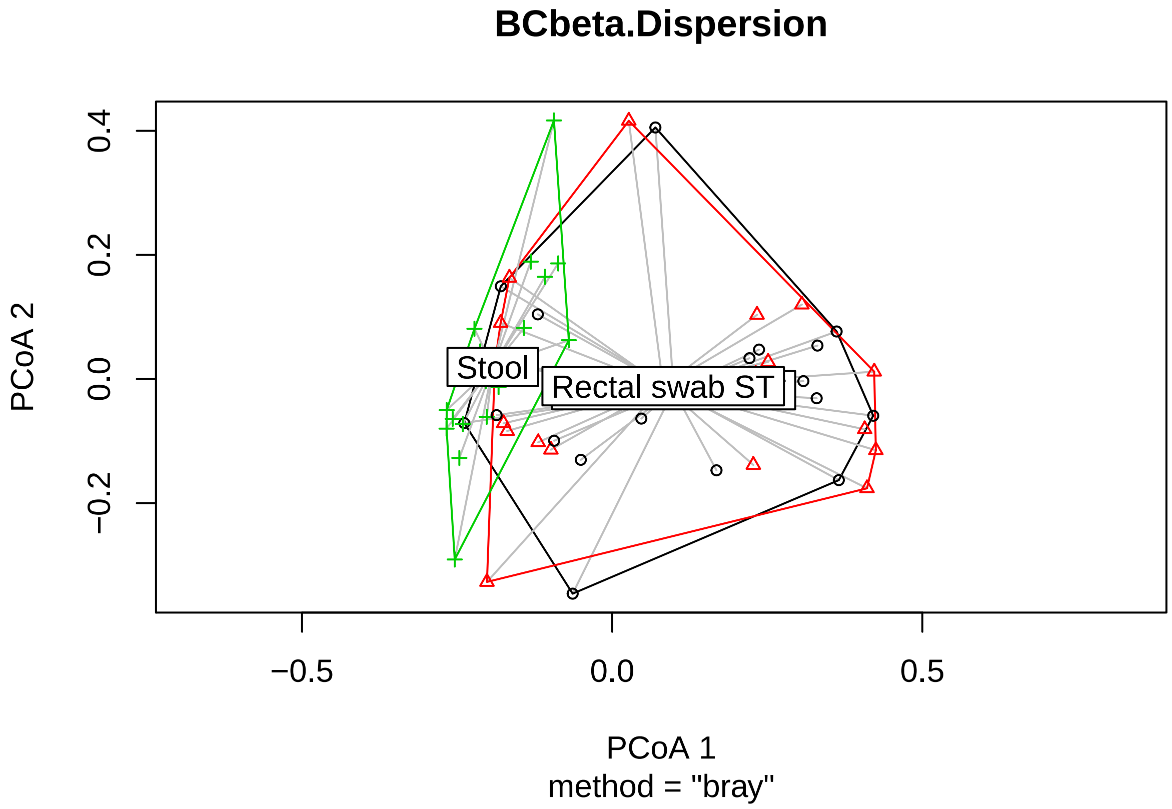
```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df   Sum Sq   Mean Sq      F N.Perm Pr(>F)
## Groups     2 0.010371 0.0051855 2.6804    999  0.087 .
## Residuals 57 0.110273 0.0019346
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##                 Rectal swab CT Rectal swab ST Stool
## Rectal swab CT                      0.152000 0.402
## Rectal swab ST       0.156123                0.034
## Stool                0.395585       0.028482
```

```
TukeyHSD(BCps.disper)
```

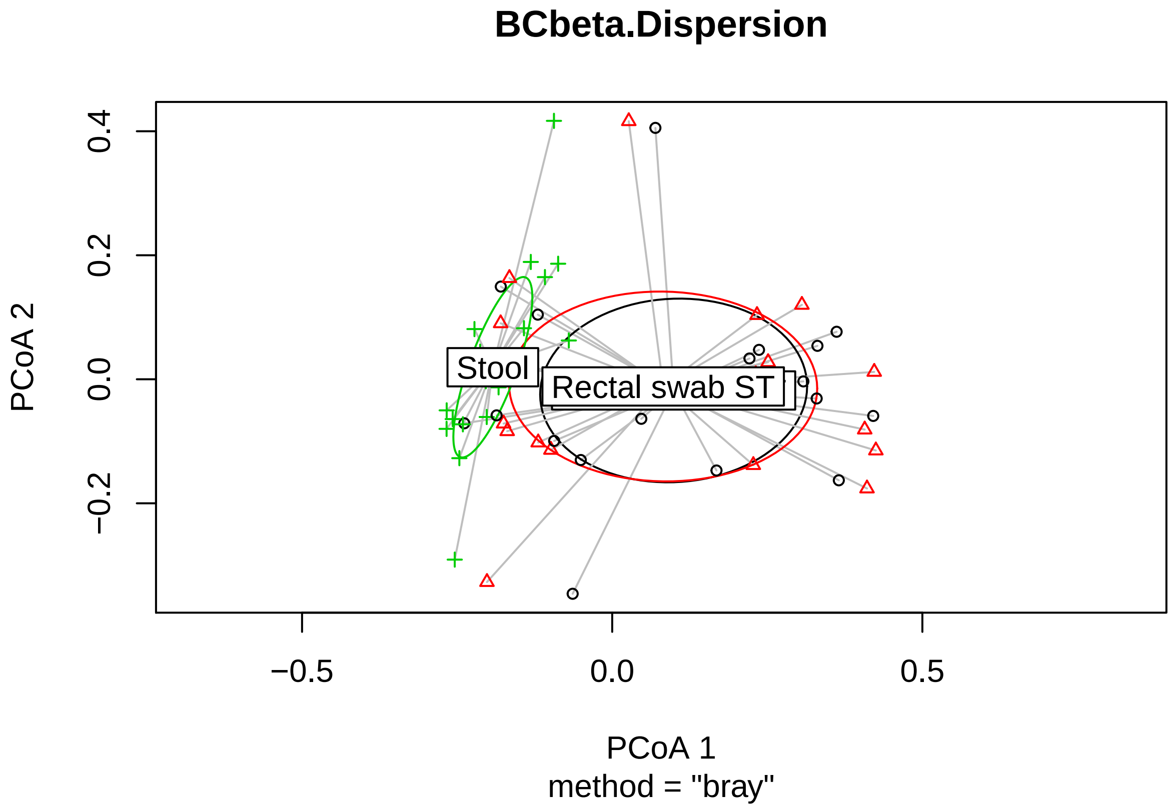
```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = distances ~ group, data = df)
##
## $group
##                                       diff         lwr        upr     p adj
## Rectal swab ST-Rectal swab CT  0.01985443 -0.01361660 0.05332546 0.3337714
## Stool-Rectal swab CT          -0.01203124 -0.04550227 0.02143979 0.6643777
## Stool-Rectal swab ST          -0.03188567 -0.06535670 0.00158536 0.0649792
```

```
# Beta Dispersion Plots
BCbeta.Dispersion <- BCps.disper
```

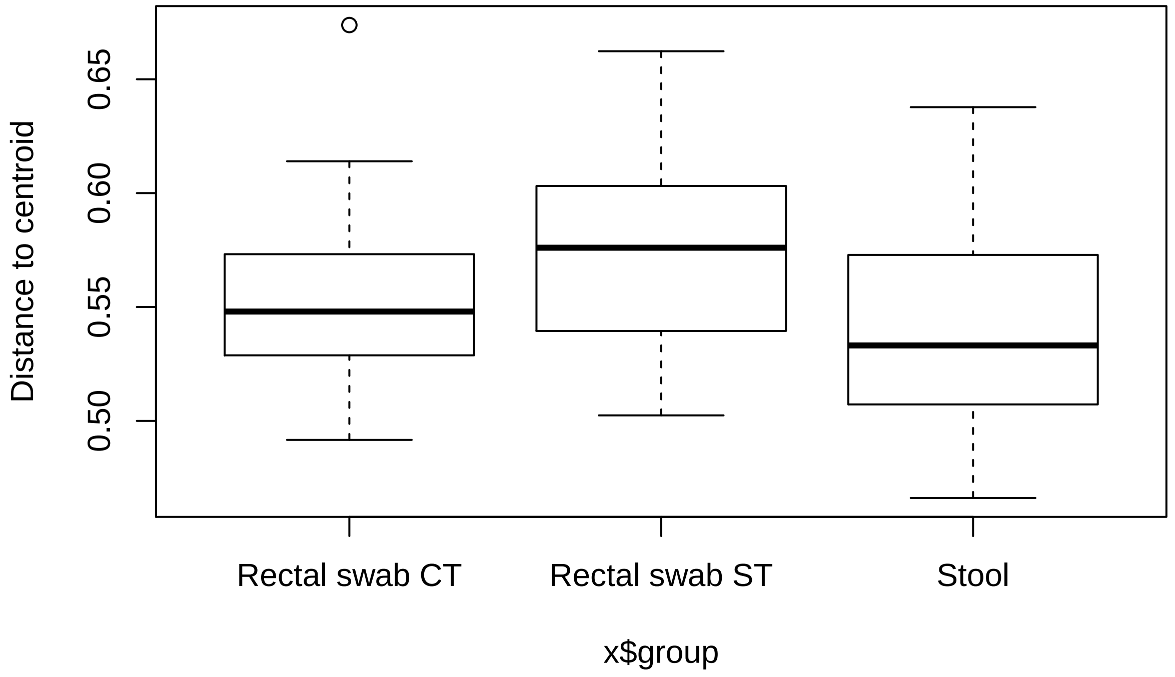
```
plot(BCbeta.Dispersion)
```



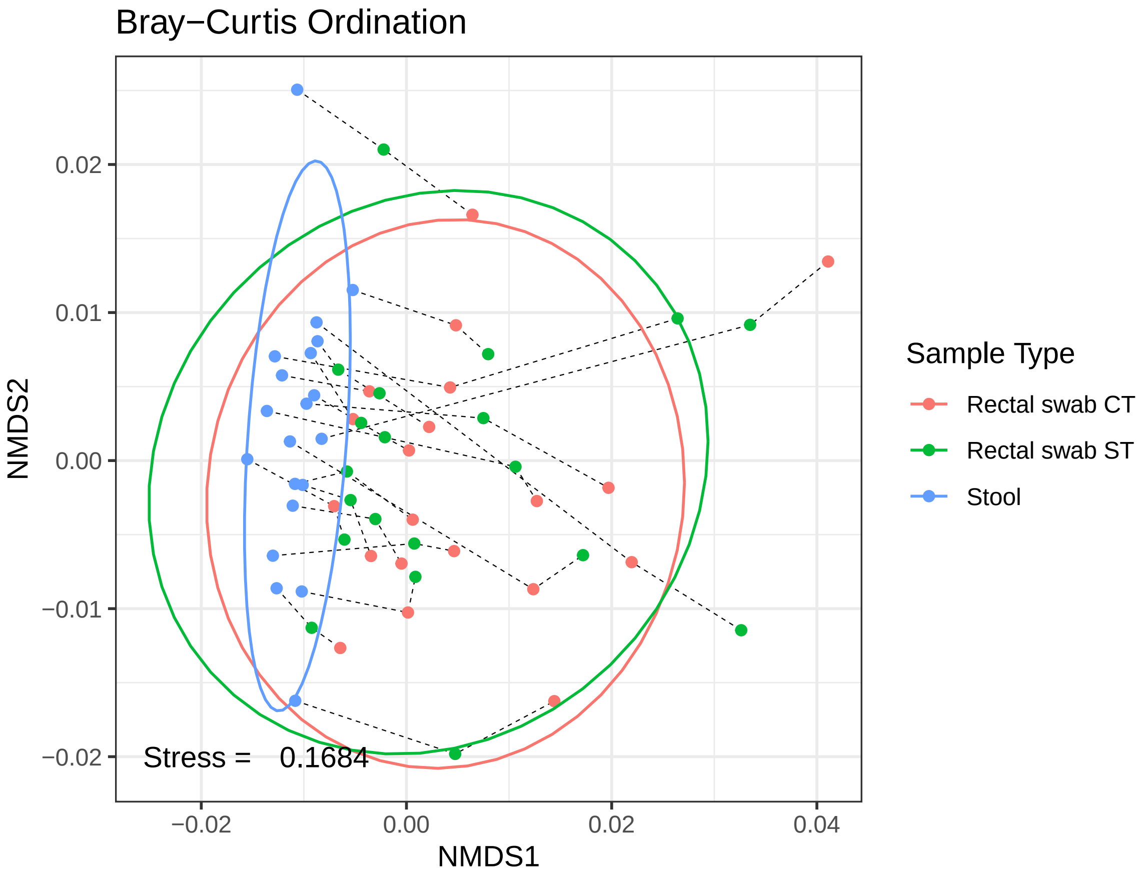
```
plot(BCbeta.Dispersion, hull = FALSE, ellipse = TRUE)
```



```r
boxplot(BCbeta.Dispersion)
```



```r
# Bray-Curtis NMDS plot
bcdf <- plot_ordination(ps_rare, ord.nmds.bray, justDF = TRUE)

BC_plot <- ggplot(bcdf, aes(x = NMDS1, y = NMDS2)) +
  geom_line(aes(group = Individual), size = 0.2, linetype = "dashed") +
  geom_point(aes(color = Sample_type)) +
  stat_ellipse(aes(color = Sample_type)) +
  annotate("text", x = -0.02, y = -0.02, label = "Stress = ") +
  annotate("text", x = -0.008, y = -0.02, label = round(ord.nmds.bray$stress, 4)) +
  ggtitle("Bray-Curtis Ordination") + labs(color = "Sample Type") +
  theme(aspect.ratio = 1, plot.margin = unit(c(0, 1, 0, 0), "pt"))

BC_plot
```



```
ggsave("../Results/2A)Beta_Diversity_BC.pdf", width = 6, height = 4.5)
```

Beta Diversity - Weighted UniFrac

```
# Ordinate data using Non-metric multidimensional scaling (NMDS) on Weighted Unifrac dissimilarity (dis
uni_dist <- phyloseq::distance(ps_rare, method = "wunifrac")
```

```
## Warning in UniFrac(physeq, weighted = TRUE, ...): Randomly assigning root as --
## ASV109 -- in the phylogenetic tree in the data you provided.
```

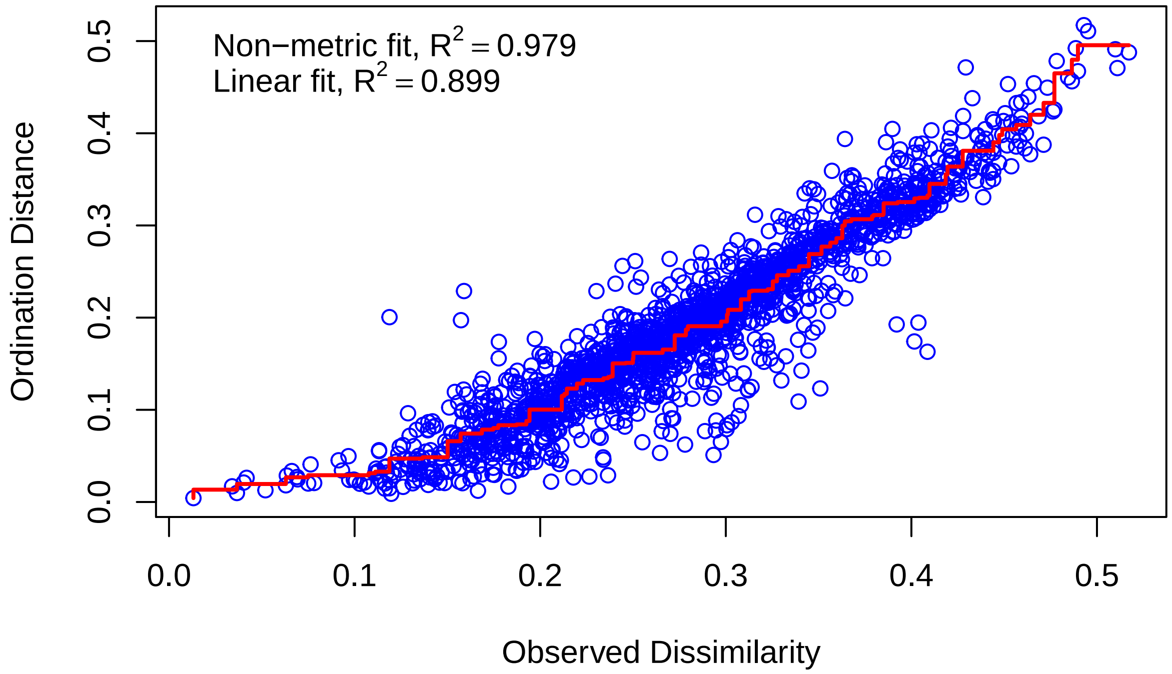
```
ord.nmds.uni <- ordinate(ps_rare, "NMDS", "wunifrac")
```

```
## Warning in UniFrac(physeq, weighted = TRUE, ...): Randomly assigning root as --
## ASV3449 -- in the phylogenetic tree in the data you provided.

## Run 0 stress 0.1443313
## Run 1 stress 0.1443314
## ... Procrustes: rmse 2.53073e-05  max resid 0.0001601472
## ... Similar to previous best
## Run 2 stress 0.2084974
## Run 3 stress 0.2200537
## Run 4 stress 0.1483259
## Run 5 stress 0.2072398
## Run 6 stress 0.1773185
## Run 7 stress 0.1799837
## Run 8 stress 0.1768409
## Run 9 stress 0.1470084
## Run 10 stress 0.2054333
## Run 11 stress 0.1483256
## Run 12 stress 0.1475256
## Run 13 stress 0.1497001
## Run 14 stress 0.1836523
## Run 15 stress 0.1493966
## Run 16 stress 0.1463929
## Run 17 stress 0.1494815
## Run 18 stress 0.206636
## Run 19 stress 0.1475256
## Run 20 stress 0.1475256
## *** Solution reached
```

```
# Call newly created file to get the stress value of the plot
ord.nmds.uni
```

```
##
## Call:
## metaMDS(comm = ps.dist)
##
## global Multidimensional Scaling using monoMDS
##
## Data:     ps.dist
## Distance: user supplied
##
## Dimensions: 2
## Stress:     0.1443313
## Stress type 1, weak ties
## Two convergent solutions found after 20 tries
## Scaling: centring, PC rotation
## Species: scores missing
```

```
# Stress plot
stressplot(ord.nmds.uni)
```



```
# Stats
# Test whether the sample types differ significantly from each other using PERMANOVA
adonis(uni_dist ~ sample_data(ps_rare)$Sample_type)
```

```
##
## Call:
## adonis(formula = uni_dist ~ sample_data(ps_rare)$Sample_type)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                                  Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## sample_data(ps_rare)$Sample_type  2   0.47754 0.238770   5.256 0.15571  0.001
## Residuals                        57   2.58939 0.045428         0.84429
## Total                            59   3.06693                  1.00000
##
## sample_data(ps_rare)$Sample_type ***
## Residuals
## Total
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis(formula = uni_dist ~ sample_data(ps_rare)$Individual)
```

```
##
## Call:
## adonis(formula = uni_dist ~ sample_data(ps_rare)$Individual)
##
## Permutation: free
## Number of permutations: 999
##
```

```
## Terms added sequentially (first to last)
##
##                                 Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## sample_data(ps_rare)$Individual 19    1.9315 0.101659  3.5814 0.62979  0.001
## Residuals                       40    1.1354 0.028385         0.37021
## Total                           59    3.0669                  1.00000
##
## sample_data(ps_rare)$Individual ***
## Residuals
## Total
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
adonis(uni_dist ~ sample_data(ps_rare)$Sample_type*sample_data(ps_rare)$Individual)
```

```
##
## Call:
## adonis(formula = uni_dist ~ sample_data(ps_rare)$Sample_type *      sample_data(ps_rare)$Individual)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                                                                   Df SumsOfSqs
## sample_data(ps_rare)$Sample_type                                   2   0.47754
## sample_data(ps_rare)$Individual                                   19   1.93153
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual 38   0.65786
## Residuals                                                          0   0.00000
## Total                                                             59   3.06693
##                                                                   MeanSqs
## sample_data(ps_rare)$Sample_type                                        0
## sample_data(ps_rare)$Individual                                         0
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual       0
## Residuals                                                            -Inf
## Total
##                                                                   F.Model
## sample_data(ps_rare)$Sample_type                                        0
## sample_data(ps_rare)$Individual                                         0
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual       0
## Residuals
## Total
##                                                                        R2 Pr(>F)
## sample_data(ps_rare)$Sample_type                                  0.15571      1
## sample_data(ps_rare)$Individual                                   0.62979      1
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual 0.21450      1
## Residuals                                                         0.00000
## Total                                                             1.00000
```

```r
anosim(uni_dist, sample_data(ps_rare)$Sample_type)
```

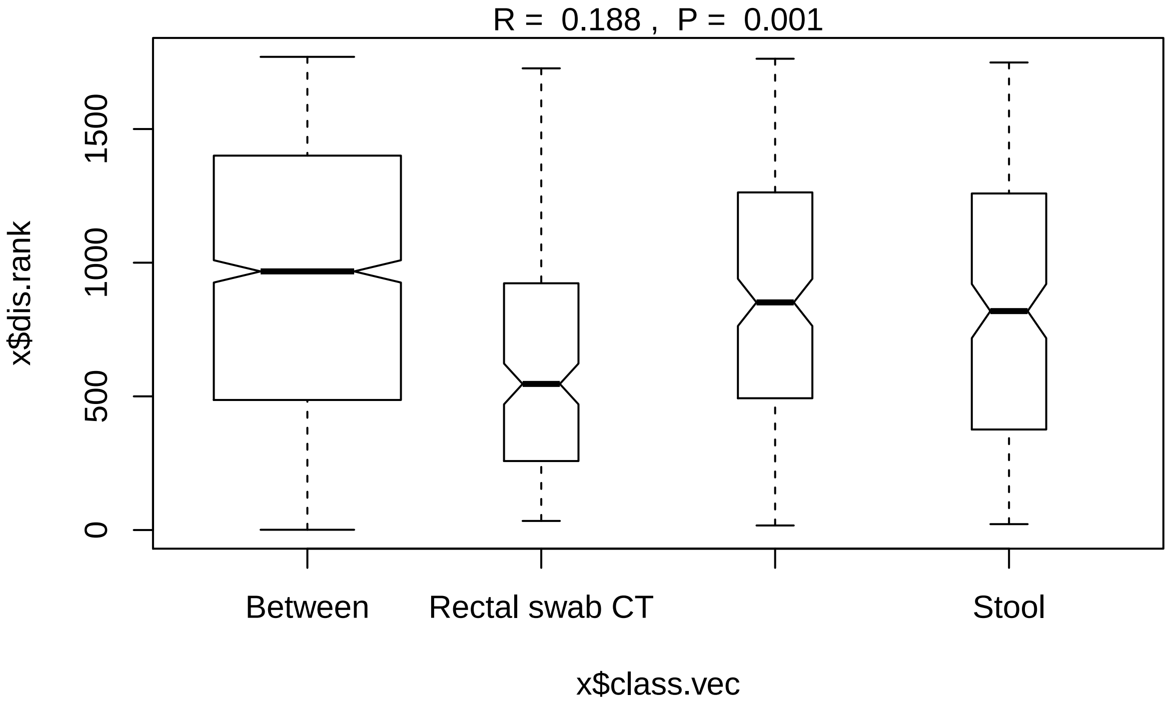
```
##
## Call:
## anosim(x = uni_dist, grouping = sample_data(ps_rare)$Sample_type)
## Dissimilarity:
```

```
##
## ANOSIM statistic R: 0.1875
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
```

```
UWFanoSamp <- (anosim(uni_dist, sample_data(ps_rare)$Sample_type))
summary(UWFanoSamp)
```

```
##
## Call:
## anosim(x = uni_dist, grouping = sample_data(ps_rare)$Sample_type)
## Dissimilarity:
##
## ANOSIM statistic R: 0.1875
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
##
## Upper quantiles of permutations (null model):
##    90%    95%  97.5%    99%
## 0.0325 0.0478 0.0615 0.0768
##
## Dissimilarity ranks between and within classes:
##                0%    25%   50%     75% 100%    N
## Between         1 486.75 967.5 1400.25 1770 1200
## Rectal swab CT 34 258.75 546.5  921.00 1727  190
## Rectal swab ST 17 493.25 851.5 1262.50 1763  190
## Stool          22 379.50 819.0 1258.50 1749  190
```

```
plot(UWFanoSamp)
```



```r
anosim(uni_dist, sample_data(ps_rare)$Individual)
```

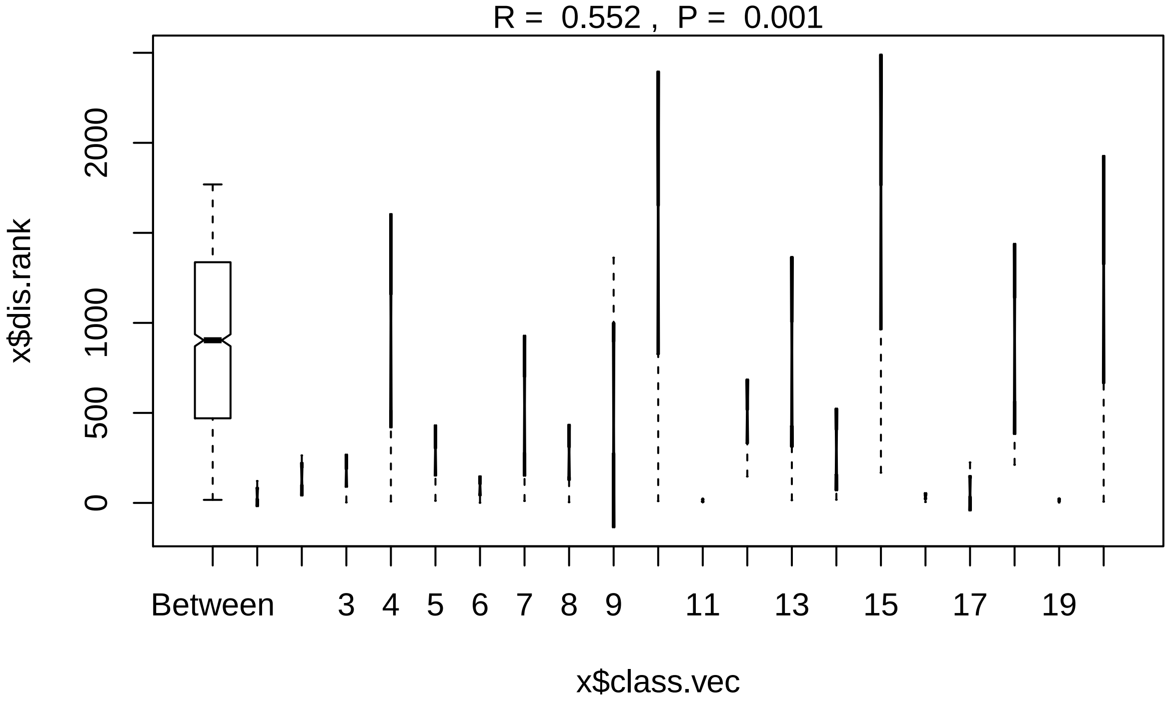
```
##
## Call:
## anosim(x = uni_dist, grouping = sample_data(ps_rare)$Individual)
## Dissimilarity:
##
## ANOSIM statistic R: 0.5524
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
```

```r
UWFanoInd <- anosim(uni_dist, sample_data(ps_rare)$Individual)
summary(UWFanoInd)
```

```
##
## Call:
## anosim(x = uni_dist, grouping = sample_data(ps_rare)$Individual)
## Dissimilarity:
##
## ANOSIM statistic R: 0.5524
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
##
## Upper quantiles of permutations (null model):
##    90%    95%  97.5%    99%
## 0.0848 0.1119 0.1333 0.1535
##
## Dissimilarity ranks between and within classes:
##          0%    25%    50%     75% 100%    N
## Between  17 470.25  903.5 1336.75 1769 1710
## 1         9  21.00   33.0   77.50  122    3
## 2        64  98.00  132.0  198.00  264    3
## 3         3  91.00  179.0  189.50  200    3
## 4         8 510.00 1012.0 1160.00 1308    3
## 5        12 152.00  292.0  305.00  318    3
## 6         1  48.00   95.0  106.50  118    3
## 7        11 275.50  540.0  702.50  865    3
## 8         4 142.50  281.0  311.00  341    3
## 9       117 274.50  432.0  897.00 1362    3
## 10       10 826.50 1643.0 1652.50 1662    3
## 11        6   9.50   13.0   18.50   24    3
## 12      147 331.50  516.0  518.00  520    3
## 13       15 427.00  839.0 1005.50 1172    3
## 14       18 157.50  297.0  407.00  517    3
## 15      168 964.00 1760.0 1765.00 1770    3
## 16        5  22.00   39.0   39.50   40    3
## 17       14  34.00   54.0  139.50  225    3
## 18      212 561.50  911.0 1141.00 1371    3
## 19        2   9.00   16.0   18.00   20    3
## 20        7 666.00 1325.0 1327.00 1329    3
```

```
plot(UWFanoInd)
```

```
## Warning in bxp(list(stats = structure(c(17, 470, 903.5, 1337, 1769, 9, 21, :
## some notches went outside hinges ('box'): maybe set notch=FALSE
```



```
UWFps.disper <- betadisper(uni_dist, sample_data(ps_rare)$Sample_type)
anova(UWFps.disper)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value Pr(>F)
## Groups     2 0.010549 0.0052747  1.9298 0.1545
## Residuals 57 0.155798 0.0027333
```

```
permutest(UWFps.disper)
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df   Sum Sq   Mean Sq      F N.Perm Pr(>F)
## Groups     2 0.010549 0.0052747 1.9298    999  0.172
## Residuals 57 0.155798 0.0027333
```

```
permutest(UWFps.disper, pairwise = TRUE)
```

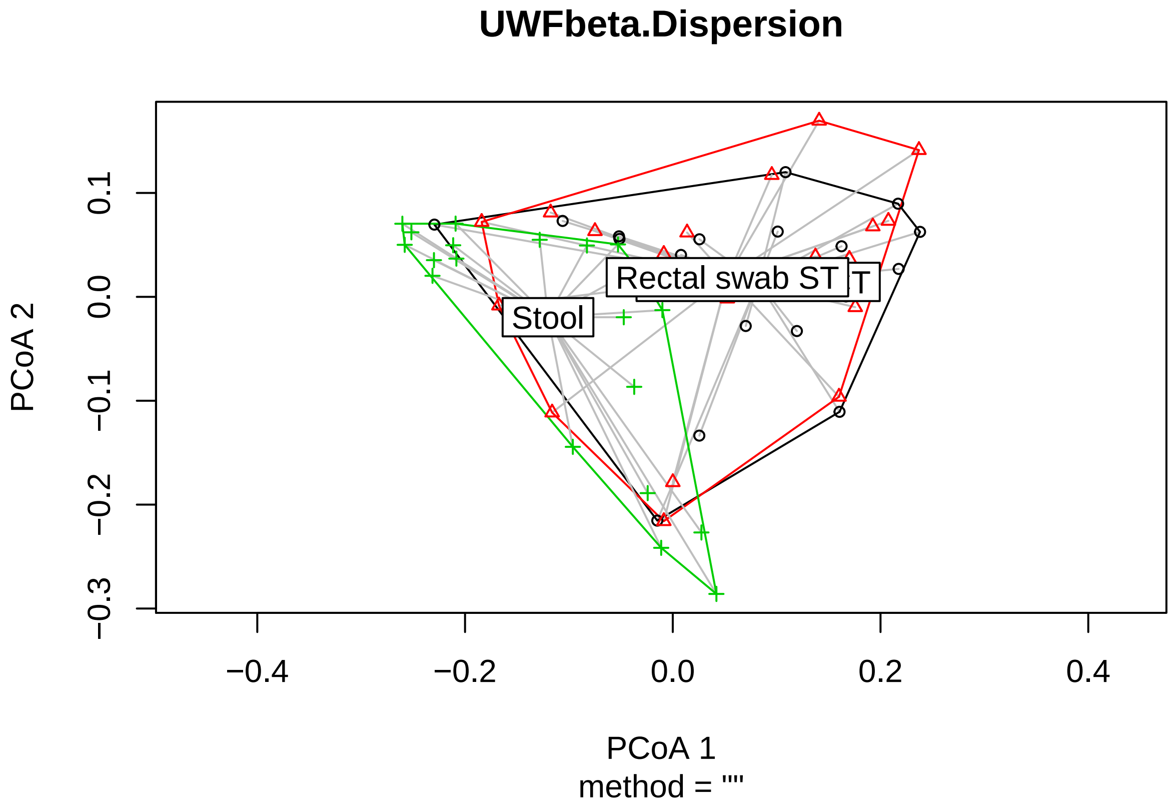
```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
```

```
##
## Response: Distances
##           Df   Sum Sq   Mean Sq      F N.Perm Pr(>F)
## Groups     2 0.010549 0.0052747 1.9298    999  0.151
## Residuals 57 0.155798 0.0027333
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##                 Rectal swab CT Rectal swab ST Stool
## Rectal swab CT                       0.091000 0.132
## Rectal swab ST        0.081515                0.818
## Stool                 0.128939       0.824097
```

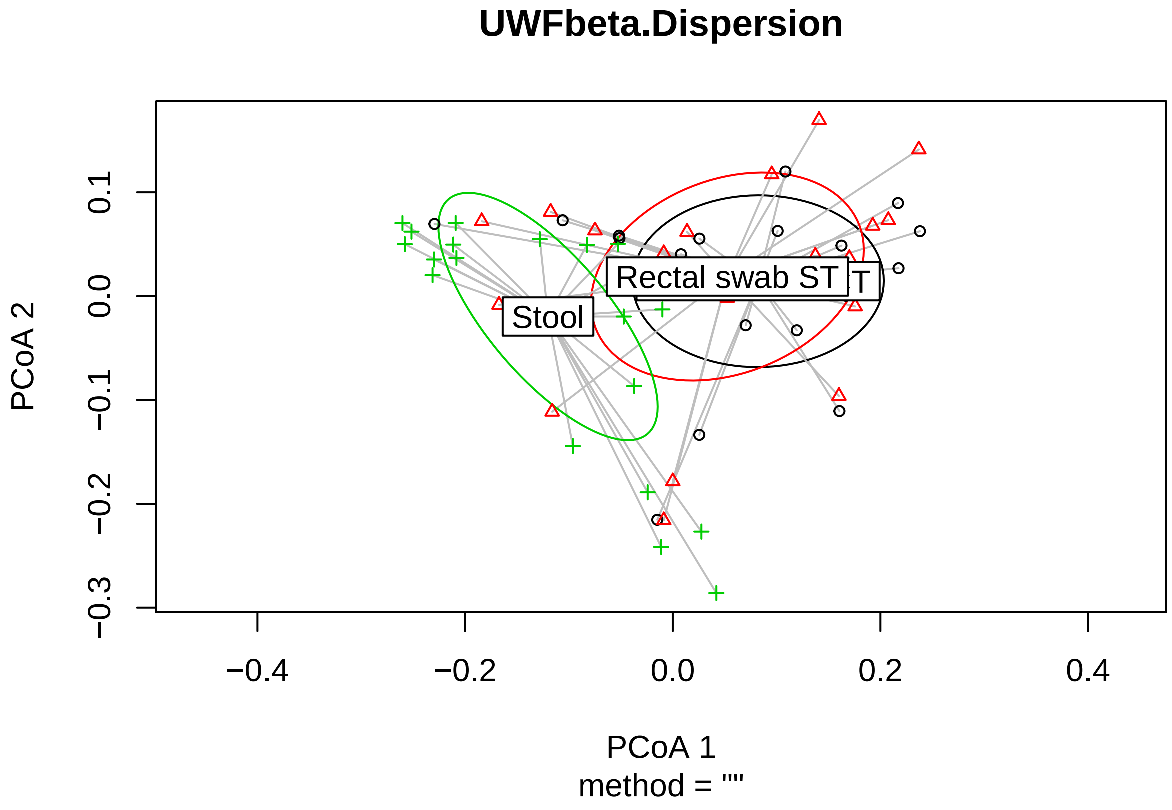
```
TukeyHSD(UWFps.disper)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = distances ~ group, data = df)
##
## $group
##                                       diff         lwr        upr     p adj
## Rectal swab ST-Rectal swab CT  0.029757314 -0.01002727 0.06954190 0.1787741
## Stool-Rectal swab CT           0.026151485 -0.01363310 0.06593607 0.2617186
## Stool-Rectal swab ST          -0.003605828 -0.04339042 0.03617876 0.9741292
```

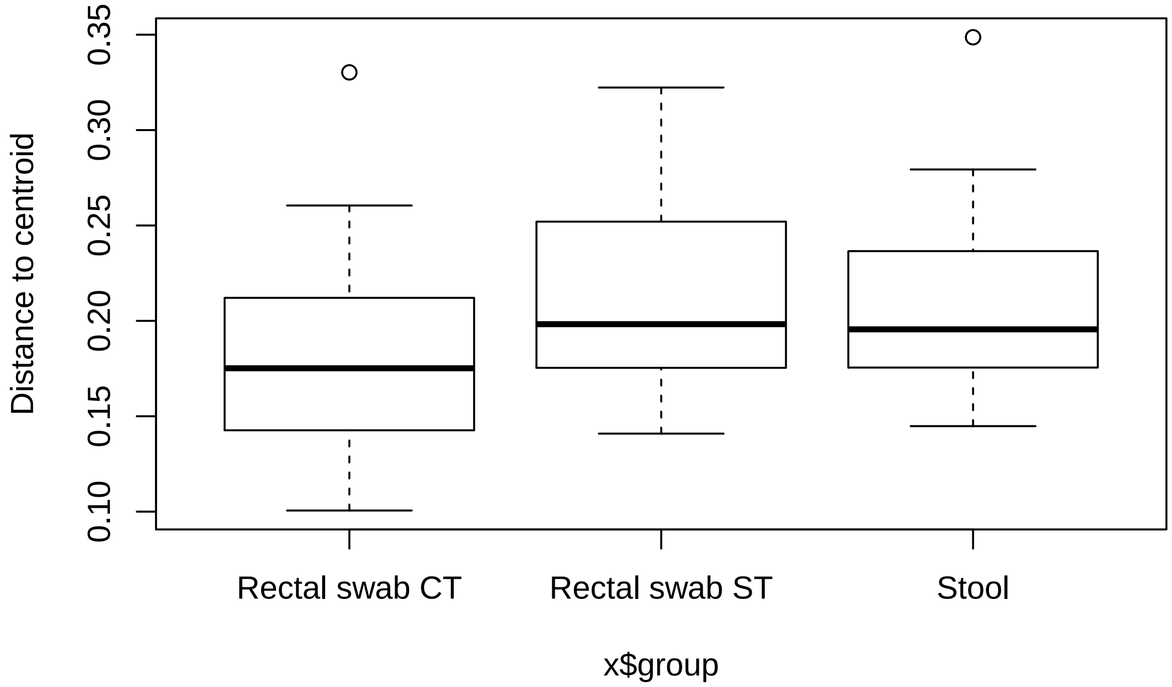
```
# Beta Dispersion Plots
UWFbeta.Dispersion <- UWFps.disper
plot(UWFbeta.Dispersion)
```



```
plot(UWFbeta.Dispersion, hull = FALSE, ellipse = TRUE)
```

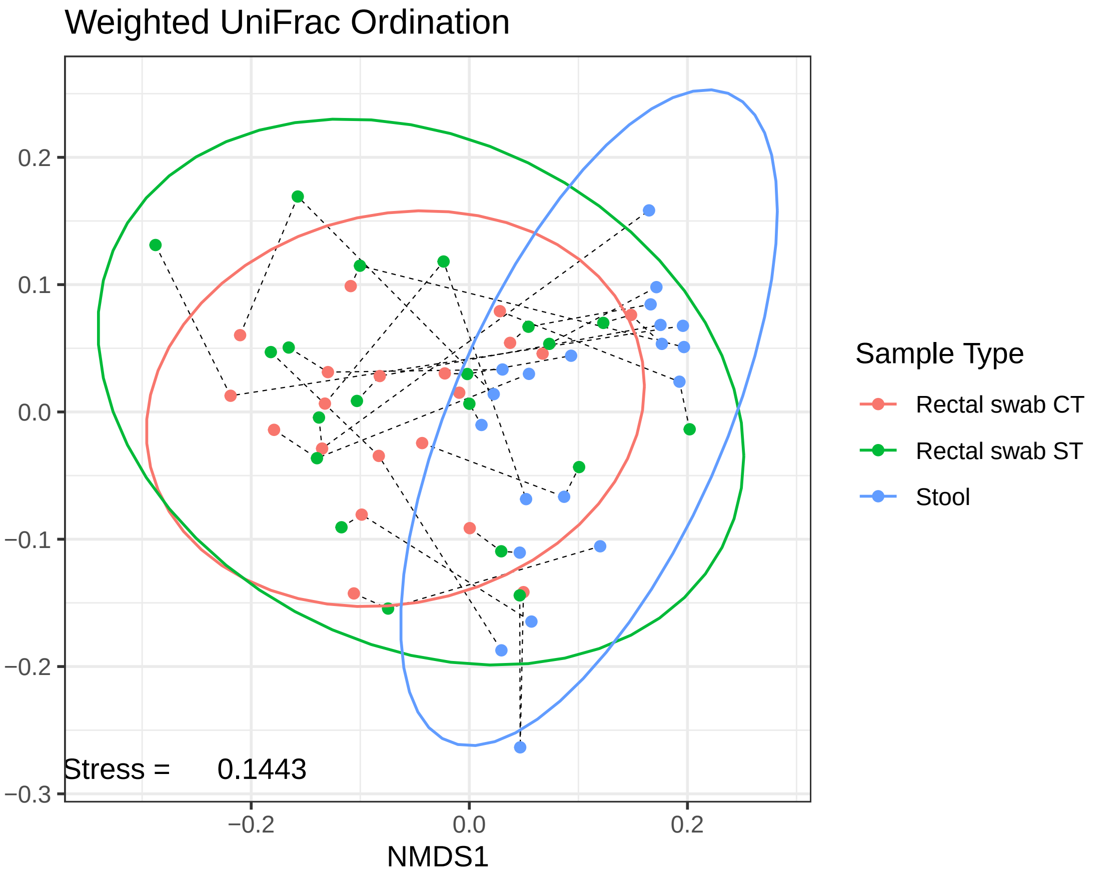


```
boxplot(UWFbeta.Dispersion)
```



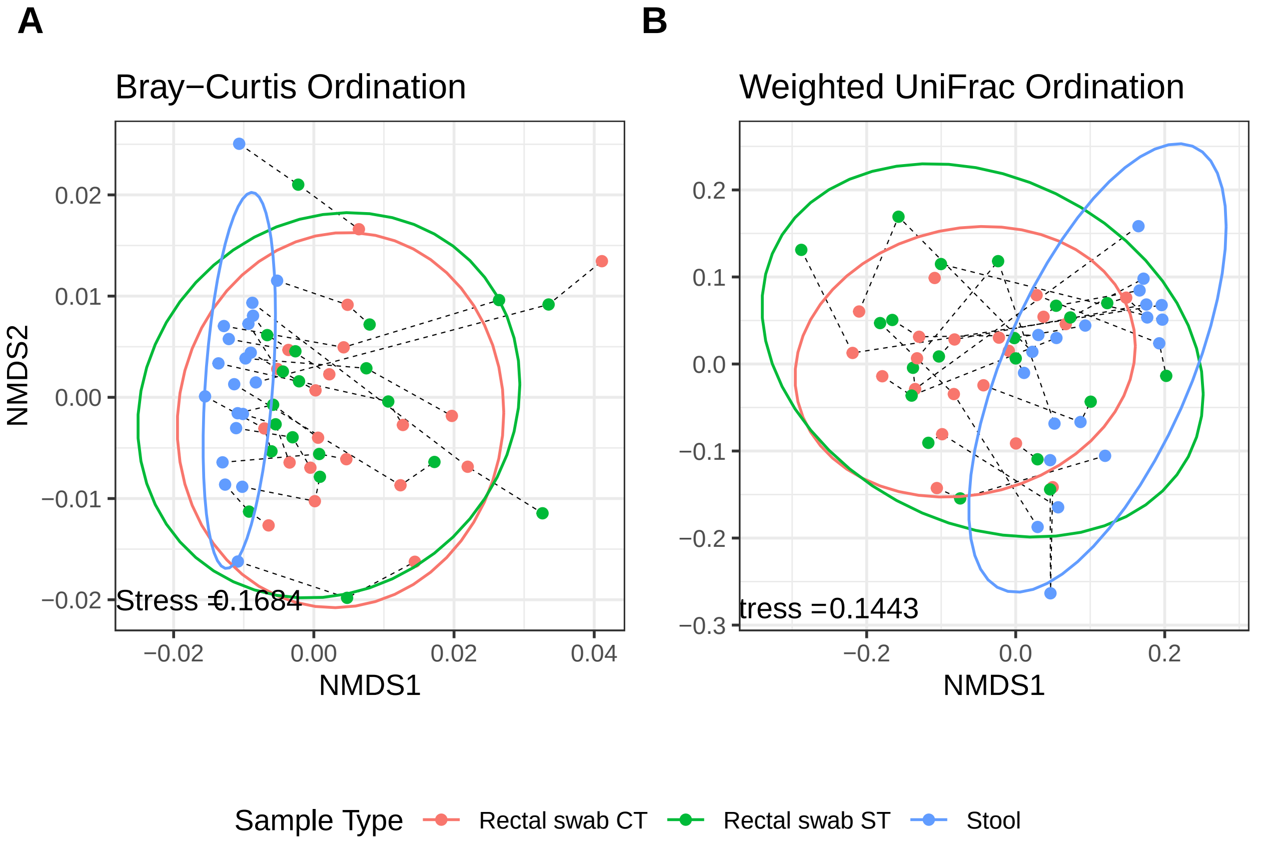
```
# UniFrac NMDS Plot
wuni <- plot_ordination(ps_rare, ord.nmds.uni, justDF = TRUE)
```

```
UWF_plot <- ggplot(wuni, aes(x = NMDS1, y = NMDS2)) +
  geom_line(aes(group = Individual), size = 0.2, linetype = "dashed") +
  geom_point(aes(color = Sample_type)) +
  stat_ellipse(aes(color = Sample_type)) +
  annotate("text", x = -0.32, y = -0.28, label = "Stress = ") +
  annotate("text", x = -0.19, y = -0.28, label = round(ord.nmds.uni$stress, 4)) +
  ggtitle("Weighted UniFrac Ordination") + labs(color = "Sample Type") +
  theme(aspect.ratio = 1, plot.margin = unit(c(0, 0, 0, 1), "pt"),
        axis.title.y = element_blank())

UWF_plot
```



```
ggsave("../Results/2B)Beta_Diversity_wUni.pdf", width = 6, height = 4.5)
```

```
ggarrange(BC_plot, UWF_plot, common.legend = TRUE, legend = c("bottom"),
          align = ("hv"), labels = "AUTO")
```

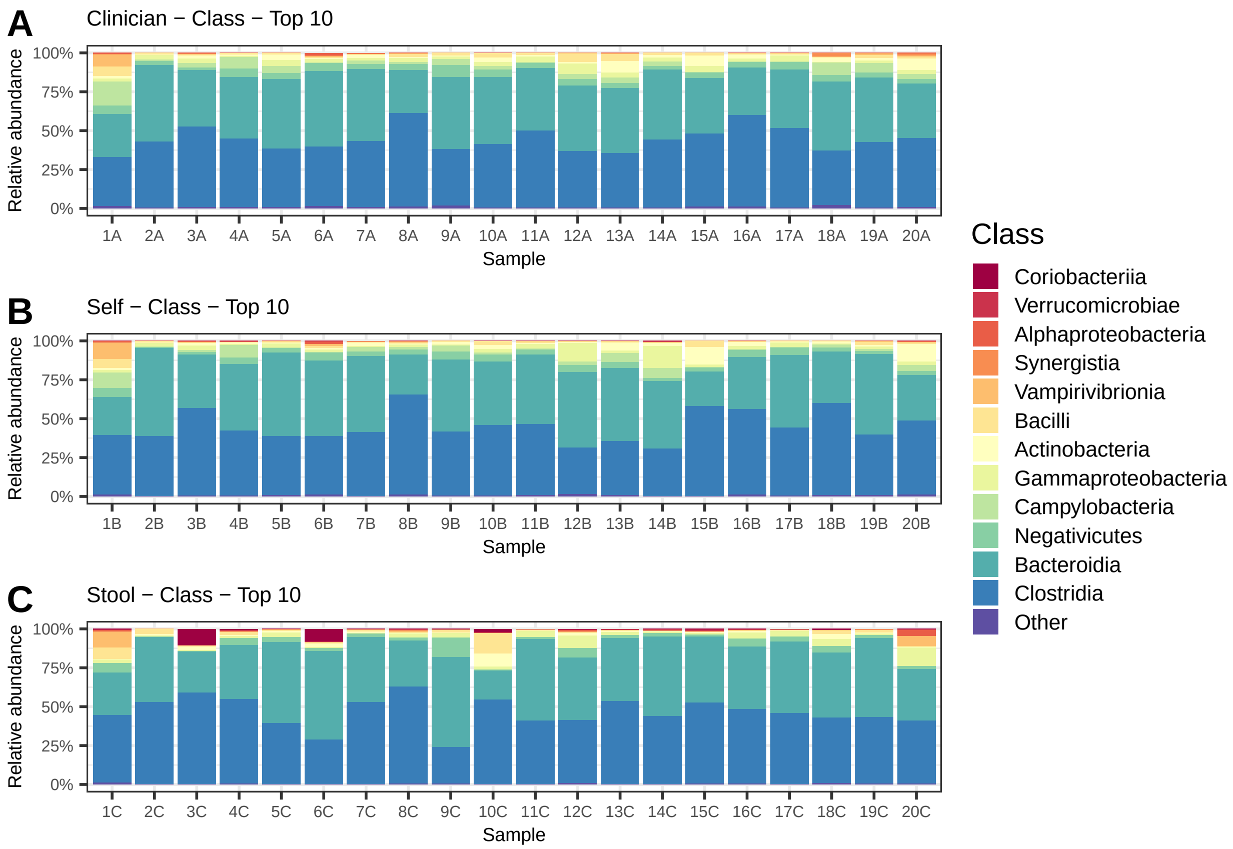


```
ggsave("../Results/2)Beta_Diversity.pdf", width = 8.5, height = 4.5)
```

RELATIVE ABUNDANCE - Using Taxonomic Level Class

```
# Subset Phyloseq Objects
ps_class <- subset_taxa(ps_rare, Class != "NA")

sample_clin <- subset_samples(ps_class, Sample_type == "Rectal swab CT")
sample_self <- subset_samples(ps_class, Sample_type == "Rectal swab ST")
sample_stool <- subset_samples(ps_class, Sample_type == "Stool")

# Relative Abundance - Clinician Taken Swab
clin_class <- tax_glom(sample_clin, taxrank = "Class") # agglomerate taxa
clin_class <- transform_sample_counts(clin_class, function(x) x/sum(x)) #get abundance in %
clin_melt <- psmelt(clin_class) # create dataframe from phyloseq object
clin_melt$Class <- as.character(clin_melt$Class) #convert to character
clin_melt <- clin_melt[order(-clin_melt$Abundance),]
clin_melt[!clin_melt$Class %in% c(unique(clin_melt$Class)[1:10]), "Class"] <- "Other"

# Relative Abundance - Self Taken Swab
self_class <- tax_glom(sample_self, taxrank = "Class") # agglomerate taxa
self_class <- transform_sample_counts(self_class, function(x) x/sum(x)) #get abundance in %
self_melt <- psmelt(self_class) # create dataframe from phyloseq object
self_melt$Class <- as.character(self_melt$Class) #convert to character
self_melt <- self_melt[order(-self_melt$Abundance),]
```

```
self_melt[!self_melt$Class %in% c(unique(self_melt$Class)[1:10]), "Class"] <- "Other"

# Relative Abundance - Stool Sample
stool_class <- tax_glom(sample_stool, taxrank = "Class") # agglomerate taxa
stool_class <- transform_sample_counts(stool_class, function(x) x/sum(x)) #get abundance in %
stool_melt <- psmelt(stool_class) # create dataframe from phyloseq object
stool_melt$Class <- as.character(stool_melt$Class) #convert to character
stool_melt <- stool_melt[order(-stool_melt$Abundance),]
stool_melt[!stool_melt$Class %in% c(unique(stool_melt$Class)[1:10]), "Class"] <- "Other"


# Set order of bars and get colours
sort.clin <- clin_melt %>%
  plyr::count("Class", wt = "Abundance") %>%
  arrange(desc(freq)) %>%
  pull(Class)
sort.clin <- sort.clin[!sort.clin %in% "Other"]
sort.clin <- append("Other", sort.clin)

sort.self <- self_melt %>%
  plyr::count("Class", wt = "Abundance") %>%
  arrange(desc(freq)) %>%
  pull(Class)
sort.self <- sort.self[!sort.self %in% "Other"]
sort.self <- append("Other", sort.self)

sort.stool <- stool_melt %>%
  plyr::count("Class", wt = "Abundance") %>%
  arrange(desc(freq)) %>%
  pull(Class)
sort.stool <- sort.stool[!sort.stool %in% "Other"]
sort.stool <- append("Other", sort.stool)

barOrder <- unique(c(sort.clin, sort.self, sort.stool))

# Get Colours and Assign to Bacteria
spectralExtra <- colorRampPalette(brewer.pal(11, "Spectral"))(length(barOrder))
cols <- setNames(c(spectralExtra), c(rev(barOrder)))

# Create Custom Legend
dummy_df <- data.frame(
  Class = as.factor(barOrder) ,
  value = c(1,2,3,4,5,6,7,8,9,10,11,12,13))
dummy_df <- mutate(dummy_df, Class = factor(Class, levels = rev(barOrder)))

rel_legend <- get_legend(ggplot(dummy_df, aes(x = Class, y = value)) +
    geom_bar(stat = "identity", aes(fill = Class)) +
    scale_fill_manual(values = cols) +
    theme(legend.text = element_text(size = 8), legend.key.size = unit(0.75, "line")))


# Plot - Relative Abundance - Clinician Taken Swab
t1_class <- clin_melt %>%
  mutate(Sample = factor(Sample, levels = c("1A", "2A", "3A", "4A", "5A",
                                            "6A", "7A", "8A", '9A', "10A",
                                            "11A", "12A", "13A", "14A", "15A",
                                            "16A", "17A", "18A", "19A", "20A"))) %>%
  mutate(Class = factor(Class, levels = rev(barOrder))) %>%
  ggplot(aes(x = Sample, y = Abundance, fill = Class)) +
  geom_bar(stat = "identity", position = "fill") +
  scale_y_continuous(labels = scales::percent_format()) +
  theme(text = element_text(size = 7)) +
  ggtitle("Clinician - Class - Top 10") +
  ylab("Relative abundance") +
  scale_fill_manual(values = cols) + theme(legend.position = "none")

# Plot - Relative Abundance - Self Taken Swab
t2_class <- self_melt %>%
  mutate(Sample = factor(Sample, levels = c("1B", "2B", "3B", "4B", "5B",
                                            "6B", "7B", "8B", "9B", "10B",
                                            "11B", "12B", "13B", "14B", "15B",
                                            "16B", "17B", "18B", "19B", "20B"))) %>%
  mutate(Class = factor(Class, levels = rev(barOrder))) %>%
  ggplot(aes(x = Sample, y = Abundance, fill = Class)) +
  geom_bar(stat = "identity", position = "fill") +
  scale_y_continuous(labels = scales::percent_format()) +
  theme(text = element_text(size = 7)) +
  ggtitle("Self - Class - Top 10") +
  ylab("Relative abundance") +
  scale_fill_manual(values = cols) + theme(legend.position = "none")

# Plot - Relative Abundance - Stool Sample
t3_class <- stool_melt %>%
  mutate(Sample = factor(Sample, levels = c("1C", "2C", "3C", "4C", "5C",
                                            "6C", "7C", "8C", "9C", "10C",
                                            "11C", "12C", "13C", "14C", "15C",
                                            "16C", "17C", "18C", "19C", "20C"))) %>%
  mutate(Class = factor(Class, levels = rev(barOrder))) %>%
  ggplot(aes(x = Sample, y = Abundance, fill = Class)) +
  geom_bar(stat = "identity", position = "fill") +
  scale_y_continuous(labels = scales::percent_format()) +
  theme(text = element_text(size = 7)) +
  ggtitle("Stool - Class - Top 10") +
  ylab("Relative abundance") +
  scale_fill_manual(values = cols) + theme(legend.position = "none")

plots <- ggarrange(t1_class, t2_class, t3_class, nrow = 3, labels = "AUTO")
ggarrange(plots, legend.grob = rel_legend, legend = "right")
```



```
ggsave("../Results/3)Relative_Abundance.pdf", width = 7, height = 8)
```

OTU differential abundance testing with DESeq2

```
ps_deseq <- ps %>%
  tax_glom(taxrank = "Genus")

sample_data(ps_deseq)$Sample_type <- gsub(" ", "_", sample_data(ps_deseq)$Sample_type)
sample_data(ps_deseq)$Sample_type <- as.factor(sample_data(ps_deseq)$Sample_type)

# Convert the phyloseq object to a DESeqDataSet
ds <- phyloseq_to_deseq2(ps_deseq, ~ Sample_type)
```

```
## converting counts to integer mode
```

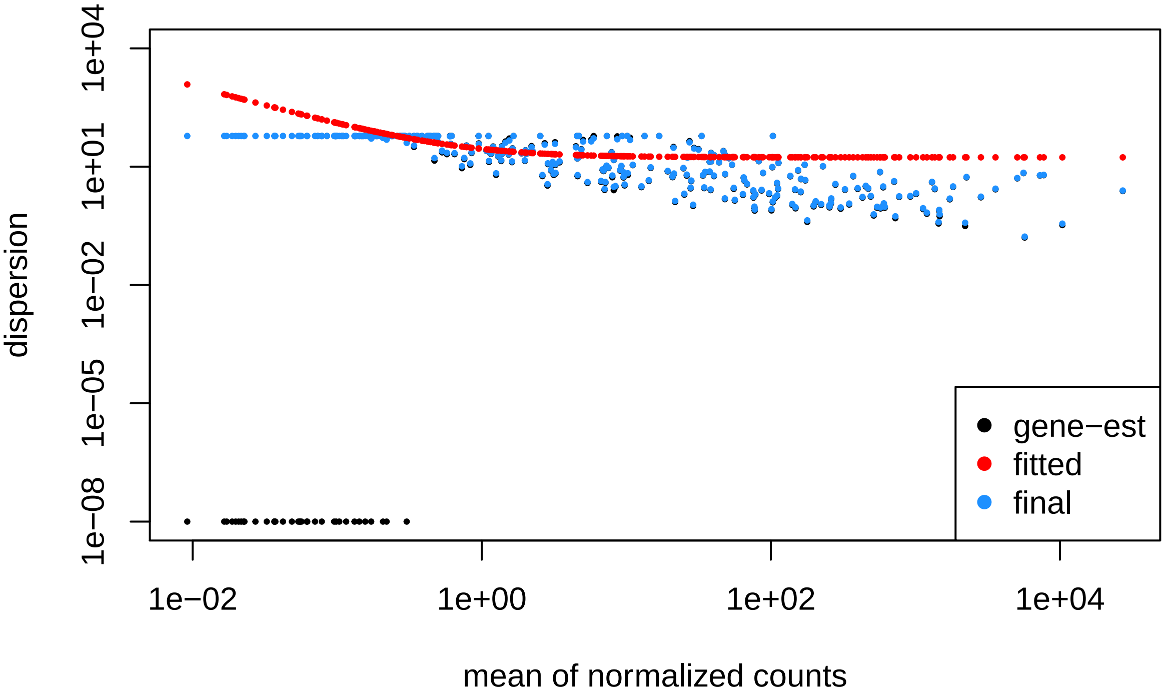
```
ds <- DESeq(ds)
```

```
## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing
## -- replacing outliers and refitting for 151 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

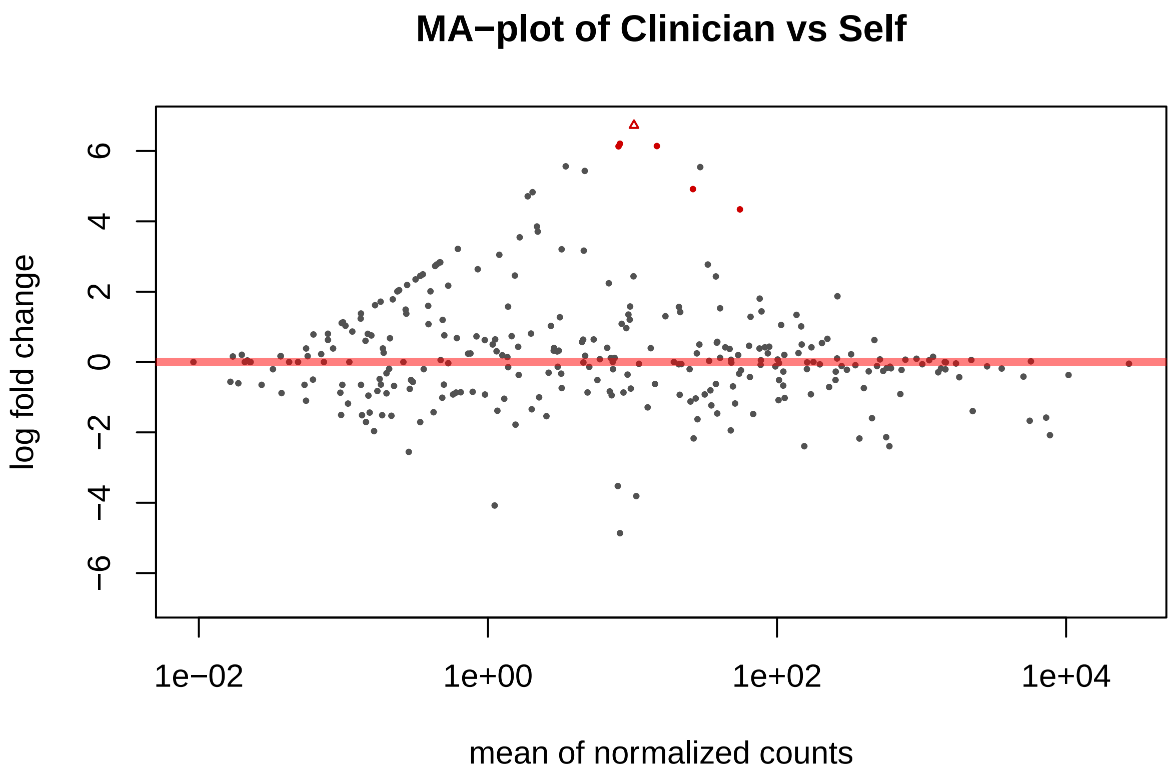
```
## estimating dispersions
```

```
## fitting model and testing
```

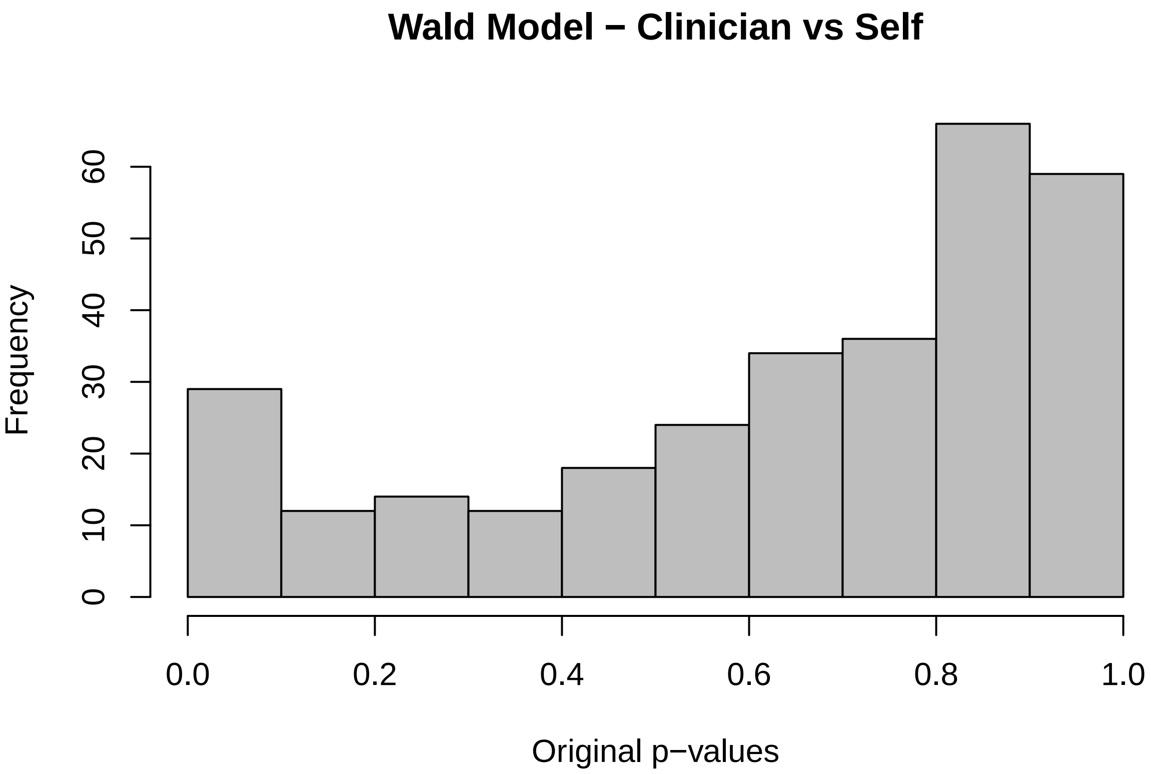
```
# Plot of Dispersion Estimates
plotDispEsts(ds, ylim = c(1e-8, 1e4))
```



```
# Extract the result table from the ds object usind the DESeq2 function results and filter the OTUs usi
alpha <- 0.01

# Swab CT vs Swab ST
resCTST <- results(ds, contrast = c("Sample_type", "Rectal_swab_CT", "Rectal_swab_ST"),
                   alpha = alpha)
resCTST <- resCTST[order(resCTST$padj, na.last = NA), ]
plotMA(resCTST, alpha = 0.01, main = "MA-plot of Clinician vs Self")
```



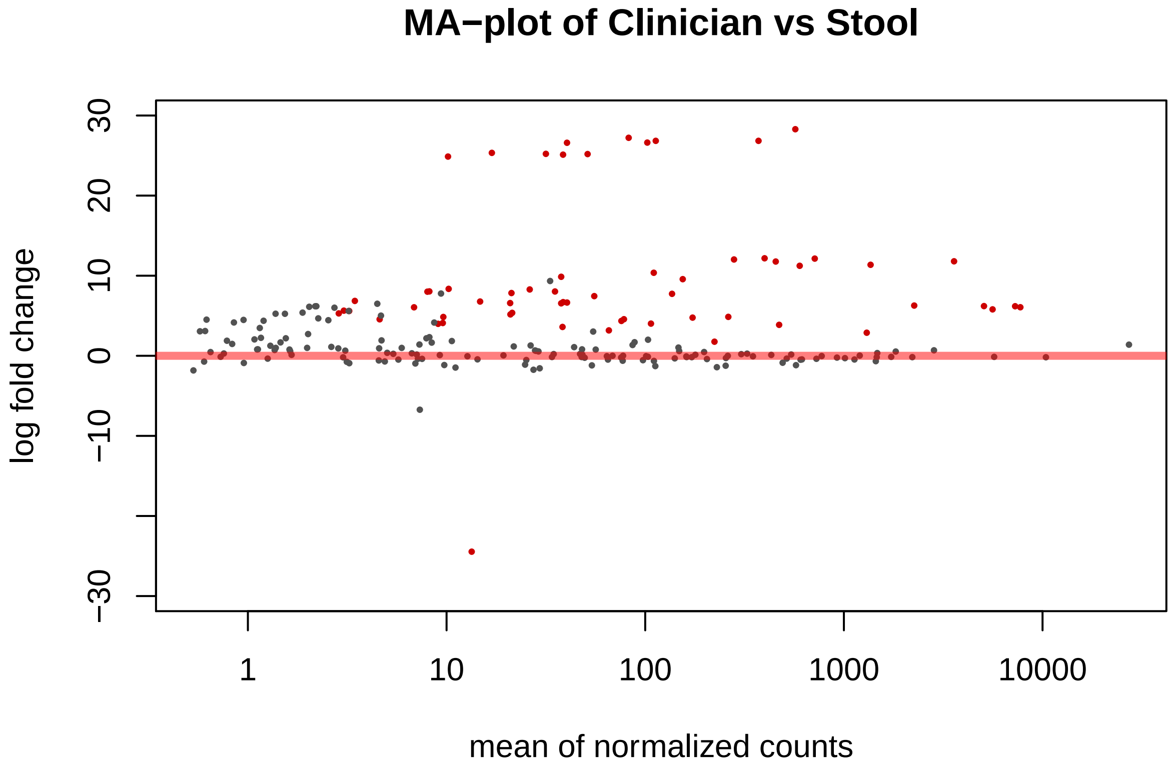
```r
hist(resCTST$pvalue, col = "gray", main = "Wald Model - Clinician vs Self", xlab = "Original p-values")
```



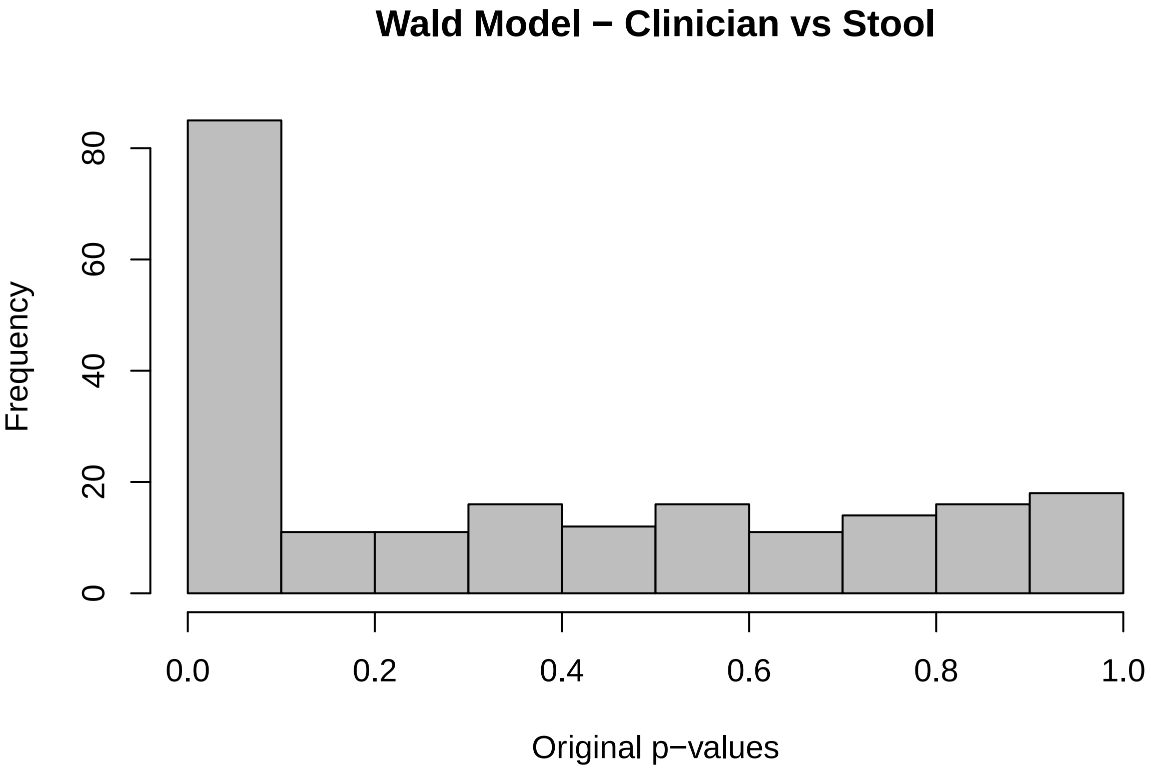
```r
resCTST_sig <- resCTST[(resCTST$padj < alpha), ]
resCTST_sig <- cbind(as(resCTST_sig, "data.frame"), as(tax_table(ps)[rownames(resCTST_sig), ], "matrix"
head(resCTST_sig)
```

```
##          baseMean log2FoldChange     lfcSE     stat       pvalue         padj
## ASV1580  8.191810       6.205783 0.9102231 6.817871 9.239974e-12 2.808952e-09
## ASV3522 10.246362       7.072852 1.2769844 5.538714 3.046999e-08 4.631439e-06
## ASV1634 55.398735       4.339785 0.7977984 5.439702 5.336990e-08 5.408149e-06
## ASV3554  8.010744       6.131689 1.1953911 5.129441 2.906035e-07 2.208587e-05
## ASV1485 26.218338       4.915912 1.1726628 4.192093 2.763921e-05 1.473097e-03
## ASV1582 14.753210       6.139537 1.4685785 4.180598 2.907429e-05 1.473097e-03
##          Kingdom         Phylum               Class             Order
## ASV1580 Bacteria Proteobacteria Gammaproteobacteria Enterobacterales
## ASV3522 Bacteria     Firmicutes          Clostridia    Clostridiales
## ASV1634 Bacteria Proteobacteria Gammaproteobacteria  Pseudomonadales
## ASV3554 Bacteria     Firmicutes          Clostridia    Clostridiales
## ASV1485 Bacteria Proteobacteria Gammaproteobacteria    Aeromonadales
## ASV1582 Bacteria Proteobacteria Gammaproteobacteria Enterobacterales
##                   Family                        Genus
## ASV1580     Yersiniaceae                     Yersinia
## ASV3522   Clostridiaceae  Clostridium_sensu_stricto_5
## ASV1634 Pseudomonadaceae                  Pseudomonas
## ASV3554   Clostridiaceae Clostridium_sensu_stricto_13
## ASV1485   Aeromonadaceae                    Aeromonas
## ASV1582      Hafniaceae       Hafnia-Obesumbacterium
```

```
# Swab CT vs Stool
resCTS <- results(ds, contrast = c("Sample_type", "Rectal_swab_CT", "Stool"),
                  alpha = alpha)
resCTS <- resCTS[order(resCTS$padj, na.last = NA), ]
plotMA(resCTS, alpha = 0.01, main = "MA-plot of Clinician vs Stool")
```



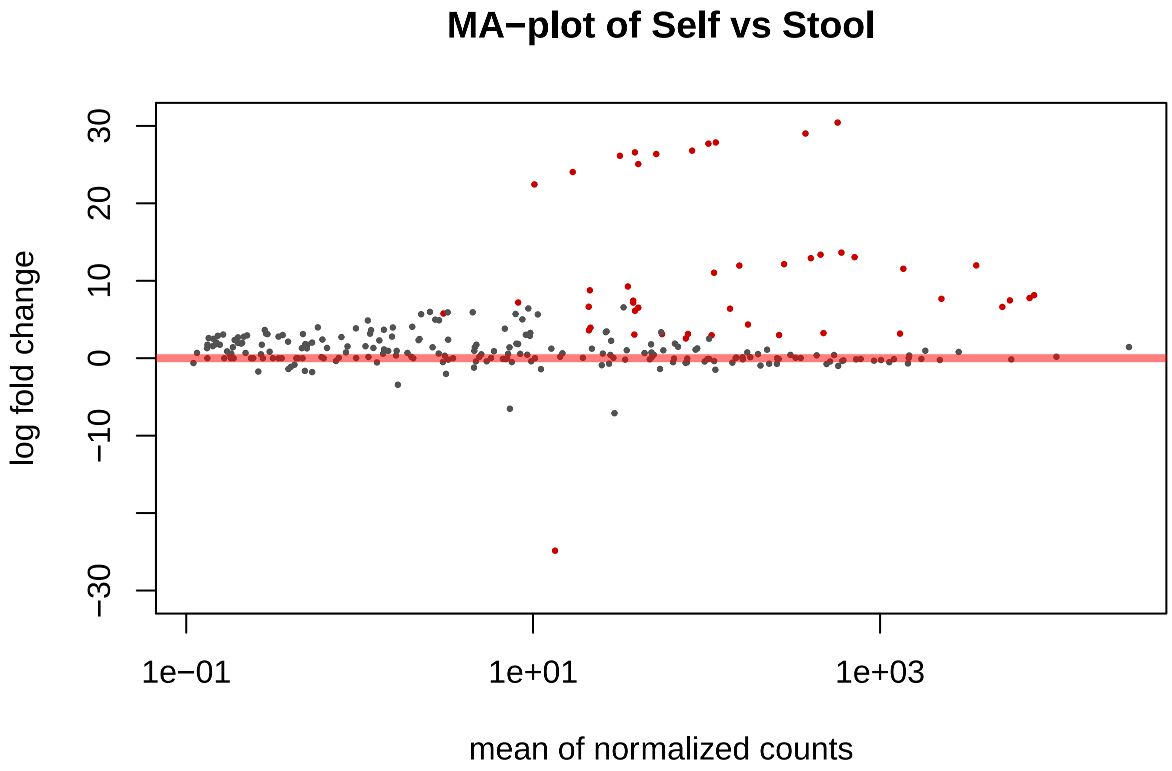
```
hist(resCTS$pvalue, col = "gray", main = "Wald Model - Clinician vs Stool", xlab = "Original p-values")
```



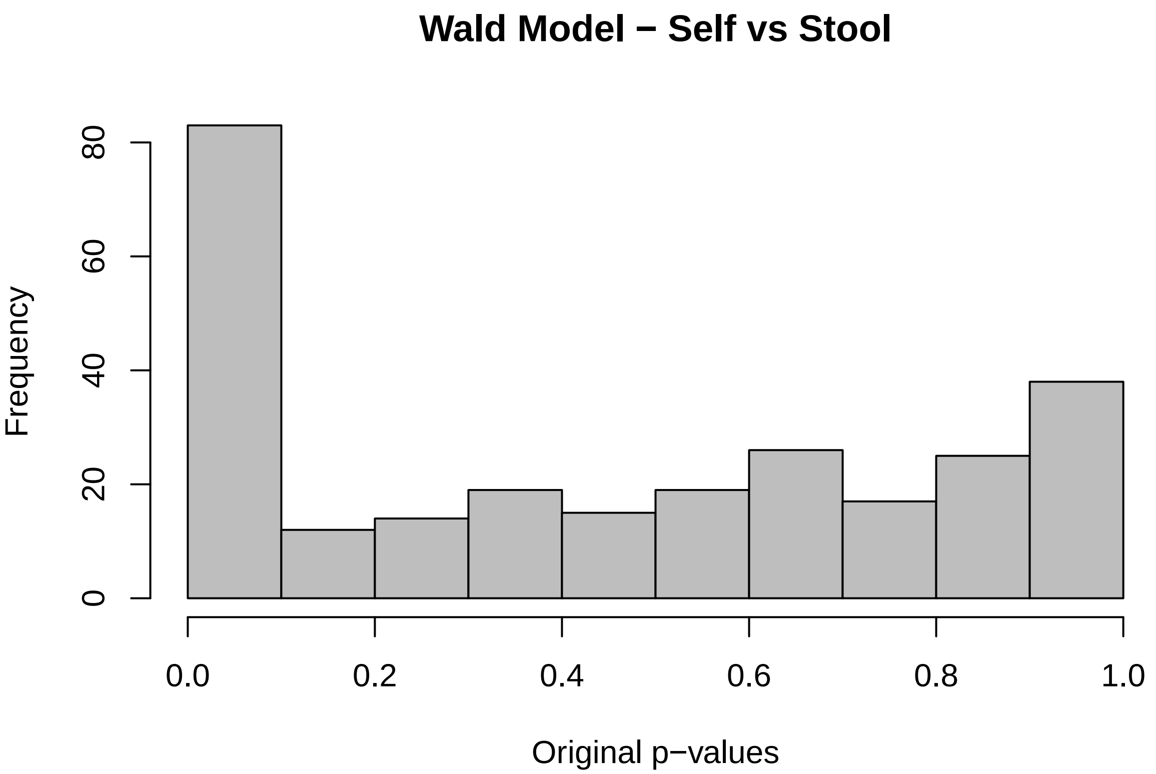
```
resCTS_sig <- resCTS[(resCTS$padj < alpha), ]
resCTS_sig <- cbind(as(resCTS_sig, "data.frame"), as(tax_table(ps)[rownames(resCTS_sig), ], "matrix"))
head(resCTS_sig)
```

```
##           baseMean log2FoldChange     lfcSE     stat        pvalue
## ASV3460  371.68353       26.83913 1.1950890 22.45785 1.072464e-111
## ASV3599  569.52608       28.29018 1.3237180 21.37176 2.447353e-101
## ASV940   102.45995       26.62138 1.5030199 17.71193  3.392565e-70
## ASV3146  112.98959       26.83745 1.6813612 15.96174  2.360599e-57
## ASV1183   82.54958       27.21862 1.7630615 15.43827  9.049272e-54
## ASV1254 3585.31720       11.79910 0.7864814 15.00239  7.082408e-51
##                  padj  Kingdom           Phylum           Class
## ASV3460 2.252174e-109 Bacteria       Firmicutes   Negativicutes
## ASV3599  2.569720e-99 Bacteria       Firmicutes      Clostridia
## ASV940   2.374796e-68 Bacteria       Firmicutes      Clostridia
## ASV3146  1.239315e-55 Bacteria       Firmicutes         Bacilli
## ASV1183  3.800694e-52 Bacteria     Synergistota     Synergistia
## ASV1254  2.478843e-49 Bacteria Campilobacterota Campylobacteria
##                                       Order
## ASV3460      Veillonellales-Selenomonadales
## ASV3599                       Clostridia_or
## ASV940  Peptostreptococcales-Tissierellales
## ASV3146                     Lactobacillales
## ASV1183                       Synergistales
## ASV1254                   Campylobacterales
##                                         Family          Genus
## ASV3460                        Veillonellaceae Negativicoccus
## ASV3599                 Hungateiclostridiaceae Fastidiosipila
## ASV940  Peptostreptococcales-Tissierellales_fa      Gallicola
## ASV3146                          Aerococcaceae      Facklamia
## ASV1183                         Synergistaceae Pyramidobacter
## ASV1254                     Campylobacteraceae  Campylobacter
```

```
# Swab ST vs Stool
resSTS <- results(ds, contrast = c("Sample_type", "Rectal_swab_ST", "Stool"),
                  alpha = alpha)
resSTS <- resSTS[order(resSTS$padj, na.last = NA), ]
plotMA(resSTS, alpha = 0.01, main = "MA-plot of Self vs Stool")
```



```
hist(resSTS$pvalue, col = "gray", main = "Wald Model - Self vs Stool", xlab = "Original p-values")
```



```
resSTS_sig <- resSTS[(resSTS$padj < alpha), ]
resSTS_sig <- cbind(as(resSTS_sig, "data.frame"), as(tax_table(ps)[rownames(resSTS_sig), ], "matrix"))
head(resSTS_sig)
```

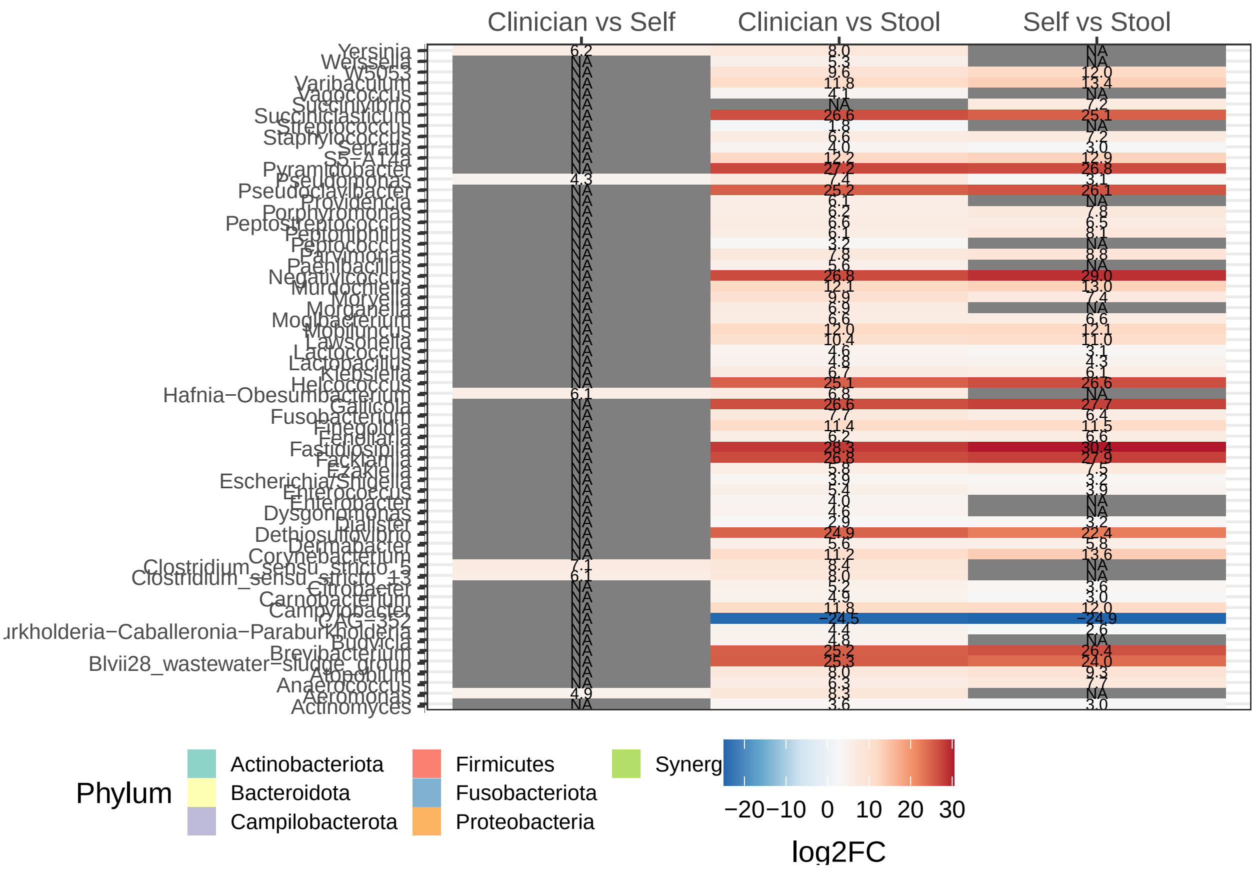
```
##            baseMean log2FoldChange     lfcSE     stat        pvalue
## ASV3460  371.68353       29.01258 1.1939331 24.30001 1.960067e-130
## ASV3599  569.52608       30.42730 1.3230276 22.99823 4.854894e-117
## ASV940   102.45995       27.70469 1.5015082 18.45124  5.095997e-76
## ASV3146  112.98959       27.85901 1.6801511 16.58125  9.522523e-62
## ASV1254 3585.31720       11.98355 0.7864397 15.23772  1.986922e-52
## ASV1183   82.54958       26.79915 1.7628105 15.20251  3.403086e-52
##                  padj  Kingdom           Phylum          Class
## ASV3460 5.252978e-128 Bacteria       Firmicutes  Negativicutes
## ASV3599 6.505558e-115 Bacteria       Firmicutes     Clostridia
## ASV940   4.552424e-74 Bacteria       Firmicutes     Clostridia
## ASV3146  6.380090e-60 Bacteria       Firmicutes        Bacilli
## ASV1254  1.064990e-50 Bacteria Campilobacterota Campylobacteria
## ASV1183  1.520045e-50 Bacteria     Synergistota     Synergistia
##                                    Order
## ASV3460     Veillonellales-Selenomonadales
## ASV3599                    Clostridia_or
## ASV940  Peptostreptococcales-Tissierellales
## ASV3146                  Lactobacillales
## ASV1254                Campylobacterales
## ASV1183                    Synergistales
##                                       Family          Genus
## ASV3460                        Veillonellaceae Negativicoccus
## ASV3599                  Hungateiclostridiaceae Fastidiosipila
## ASV940  Peptostreptococcales-Tissierellales_fa      Gallicola
## ASV3146                          Aerococcaceae      Facklamia
## ASV1254                     Campylobacteraceae  Campylobacter
## ASV1183                         Synergistaceae Pyramidobacter
```

```
# Save .csv of significant fold change results
resCTST_sig$Comparison <- "Clinician Taken Swab vs Self Taken Swab"
resCTS_sig$Comparison <- "Clinician Taken Swab vs Stool"
resSTS_sig$Comparison <- "Self Taken Swab vs Stool"

SignificantResults <- rbind(resCTST_sig, resCTS_sig, resSTS_sig)
write.csv(SignificantResults, file = "../Results/SignificantFoldChangeResults.csv")
```

Differential Abundance - ggplot Heatmap

```
diffCTST <- resCTST_sig %>%
  select(log2FoldChange, Phylum, Genus)
colnames(diffCTST)[1] <- "CTST_log2FoldChange"

diffCTS <- resCTS_sig %>%
  select(log2FoldChange, Phylum, Genus)
colnames(diffCTS)[1] <- "CTS_log2FoldChange"

diffSTS <- resSTS_sig %>%
  select(log2FoldChange, Phylum, Genus)
colnames(diffSTS)[1] <- "STS_log2FoldChange"

heat <- rbind.fill(as.data.frame(t(diffCTS)), as.data.frame(t(diffSTS)))
heat <- rbind.fill(as.data.frame(heat), as.data.frame(t(diffCTST)))
heat <- t(heat)
heat <- as.data.frame(heat)
colnames(heat) <- c("CTS", "CTS_phylum", "CTS_genus",
                    "STS", "STS_phylum", "STS_genus",
                    "CTST", "CTST_phylum", "CTST_genus")

heat$sigPhylum <- as.character(heat$CTS_phylum)
heat$sigPhylum[nrow(heat)] <- as.character(heat$STS_phylum[nrow(heat)])

heat$sigGenus <- as.character(heat$CTS_genus)
heat$sigGenus[nrow(heat)] <- as.character(heat$STS_genus[nrow(heat)])

heat <- select(heat, -CTS_genus, -STS_genus, -CTST_genus, -CTS_phylum, -STS_phylum, -CTST_phylum)

# file for ggplot based heatmap
SamplingComparison <- c(1:(nrow(heat)*3))
SamplingComparison[1:nrow(heat)] <- "CTS"
SamplingComparison[(nrow(heat)+1):(nrow(heat)*2)] <- "STS"
SamplingComparison[((nrow(heat)*2)+1):(nrow(heat)*3)] <- "CTST"
log2FC <- c(1:(nrow(heat)*3))
log2FC[1:nrow(heat)] <- as.numeric(as.character(heat$CTS))
log2FC[(nrow(heat)+1):(nrow(heat)*2)] <- as.numeric(as.character(heat$STS))
log2FC[((nrow(heat)*2)+1):(nrow(heat)*3)] <- as.numeric(as.character(heat$CTST))
Phylum <- c(1:(nrow(heat)*3))
Phylum[1:nrow(heat)] <- heat$sigPhylum
```

```r
Phylum[(nrow(heat)+1):(nrow(heat)*2)] <- heat$sigPhylum
Phylum[((nrow(heat)*2)+1):(nrow(heat)*3)] <- heat$sigPhylum
Genus <- c(1:(nrow(heat)*3))
Genus[1:nrow(heat)] <- heat$sigGenus
Genus[(nrow(heat)+1):(nrow(heat)*2)] <- heat$sigGenus
Genus[((nrow(heat)*2)+1):(nrow(heat)*3)] <- heat$sigGenus
ftp <- as.data.frame(cbind(SamplingComparison, log2FC, Phylum, Genus))

ftp$log2FC <- as.numeric(as.character(ftp$log2FC))
ftp$SamplingComparison <- factor(ftp$SamplingComparison, levels = c("CTST", "CTS", "STS"))

heatLog <- ggplot(ftp, aes(SamplingComparison, Genus, fill = log2FC)) + geom_tile() +
  geom_text(aes(label = sprintf("%2.1f", log2FC)), size = 2) +
  theme(axis.title = element_blank(), legend.position = "bottom",
        axis.text.y = element_blank(),
        axis.text.x = element_text(family = "Helvetica", size = 10, face = "plain"),
        plot.background = element_blank(),
        plot.margin = margin(t = 2, r = 0, b = 0, l = 0, unit = "pt"),
        legend.margin = margin(t = 0, r = 0, b = 0, l = 0, unit = "pt")) +
  guides(fill = guide_colourbar(title.position = "bottom", title.hjust = 0.5)) +
  scale_fill_distiller(palette = "RdBu") +
  scale_x_discrete(position = "top", labels = (c("Clinician vs Self",
                                                 "Clinician vs Stool",
                                                 "Self vs Stool")))

heatPhylum <- ggplot(ftp, aes(SamplingComparison, Genus, fill = Phylum)) + geom_tile() +
  theme(axis.title = element_blank(), legend.position = "bottom",
        axis.text.y = element_text(size = 8),
        axis.text.x = element_blank(), axis.ticks.x = element_blank(),
        plot.margin = margin(t = 16.5, r = 5, b = 11, l = 0, unit = "pt"),
        legend.margin = margin(t = 0, r = 0, b = 0, l = 0, unit = "pt"),
        legend.text = element_text(size = 8), legend.key.size = unit(0.75, "line")) +
  scale_fill_brewer(palette = "Set3", guide = guide_legend(ncol = 3))

heatChanges <- ggarrange(heatPhylum, heatLog, widths = c(1, 2))
heatChanges
```



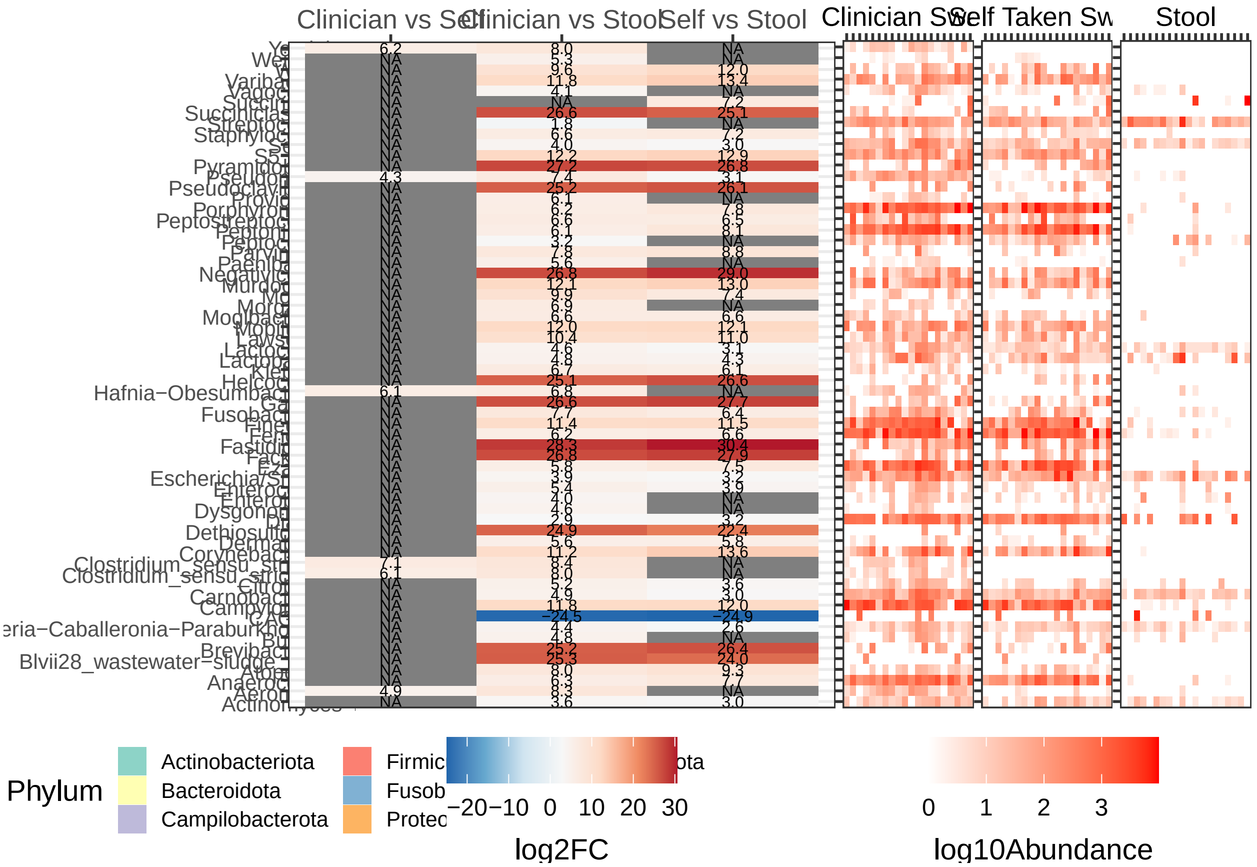
```r
ggsave("../Results/4)Differential_Abundance_heatmap.pdf", width = 8, height = 8)
```

Species Abundance ggplot Heatmap

```r
# Make figure with individual abundance to go next to heatmap
heat_ps <- subset_taxa(ps_rare, Genus %in% heat$sigGenus)
heat_ps <- heat_ps %>%
  tax_glom(taxrank = "Genus")

# Clinician Swab
heat_clin <- subset_samples(heat_ps, Sample_type == "Rectal swab CT")
melted_clin <- psmelt(heat_clin)
melted_clin <- select(melted_clin, Individual, Genus, Abundance)
melted_clin$Abundance[melted_clin$Abundance == 0] <- 1
melted_clin$log2Abundance <- log2(melted_clin$Abundance)
melted_clin$log10Abundance <- log10(melted_clin$Abundance)

heatCS <- ggplot(melted_clin, aes(Individual, Genus, fill = log10Abundance)) + geom_tile() +
  scale_x_discrete(position = "top") + xlab("Clinician Swab") +
  theme(axis.title.x = element_text(family = "Helvetica", size = 10, face = "plain"),
        axis.title.y = element_blank(),
        axis.text = element_blank(), legend.position = "bottom", legend.background = element_blank(),
        plot.margin = margin(t = 1, r = 0, b = 0, l = 0, unit = "pt"),
        legend.margin = margin(t = 11, r = 0, b = 0, l = 0, unit = "pt")) +
  scale_fill_gradient(low = "white", high = "red") +
  guides(fill = guide_colourbar(title.position = "bottom", title.hjust = 0.5))

# Self Swab
heat_self <- subset_samples(heat_ps, Sample_type == "Rectal swab ST")
melted_self <- psmelt(heat_self)
melted_self <- select(melted_self, Individual, Genus, Abundance)
melted_self$Abundance[melted_self$Abundance == 0] <- 1
melted_self$log2Abundance <- log2(melted_self$Abundance)
melted_self$log10Abundance <- log10(melted_self$Abundance)

heatSS <- ggplot(melted_self, aes(Individual, Genus, fill = log10Abundance)) + geom_tile() +
  scale_x_discrete(position = "top") + xlab("Self Taken Swab") +
  theme(axis.title.x = element_text(family = "Helvetica", size = 10, face = "plain"),
        axis.title.y = element_blank(),
        axis.text = element_blank(), legend.position = "bottom", legend.background = element_blank(),
        plot.margin = margin(t = 1, r = 0, b = 0, l = 0, unit = "pt"),
        legend.margin = margin(t = 11, r = 0, b = 0, l = 0, unit = "pt")) +
  scale_fill_gradient(low = "white", high = "red") +
  guides(fill = guide_colourbar(title.position = "bottom", title.hjust = 0.5))

# Stool
heat_stool <- subset_samples(heat_ps, Sample_type == "Stool")
melted_stool <- psmelt(heat_stool)
melted_stool <- select(melted_stool, Individual, Genus, Abundance)
melted_stool$Abundance[melted_stool$Abundance == 0] <- 1
melted_stool$log2Abundance <- log2(melted_stool$Abundance)
melted_stool$log10Abundance <- log10(melted_stool$Abundance)

heatSt <- ggplot(melted_stool, aes(Individual, Genus, fill = log10Abundance)) + geom_tile() +
  scale_x_discrete(position = "top") + xlab("Stool") +
  theme(axis.title.x = element_text(family = "Helvetica", size = 10, face = "plain"),
        axis.title.y = element_blank(),
        axis.text = element_blank(), legend.position = "bottom", legend.background = element_blank(),
        plot.margin = margin(t = 1, r = 0, b = 0, l = 0, unit = "pt"),
        legend.margin = margin(t = 11, r = 0, b = 0, l = 0, unit = "pt")) +
  scale_fill_gradient(low = "white", high = "red") +
  guides(fill = guide_colourbar(title.position = "bottom", title.hjust = 0.5))


heatAbundance <- ggarrange(heatCS, heatSS, heatSt, ncol = 3, common.legend = TRUE, legend = c("bottom")
```

Combined Heatmaps

```r
ggarrange(heatChanges, heatAbundance, widths = c(2, 1), legend = c("bottom"))
```



```
ggsave("../Results/4)Differential_Abundance_heatmap_extra.pdf",width = 11, height = 8)
```

Supplementary 2 - Calculate some stats re deseq2 enrichment

```
mytax <- data.frame(tax_table(ps_deseq))
mytable <- mytax %>%
  group_by(Phylum) %>%
  summarize(Phycount = n_distinct(Genus))
```

```
## `summarise()` ungrouping output (override with `.groups` argument)
```

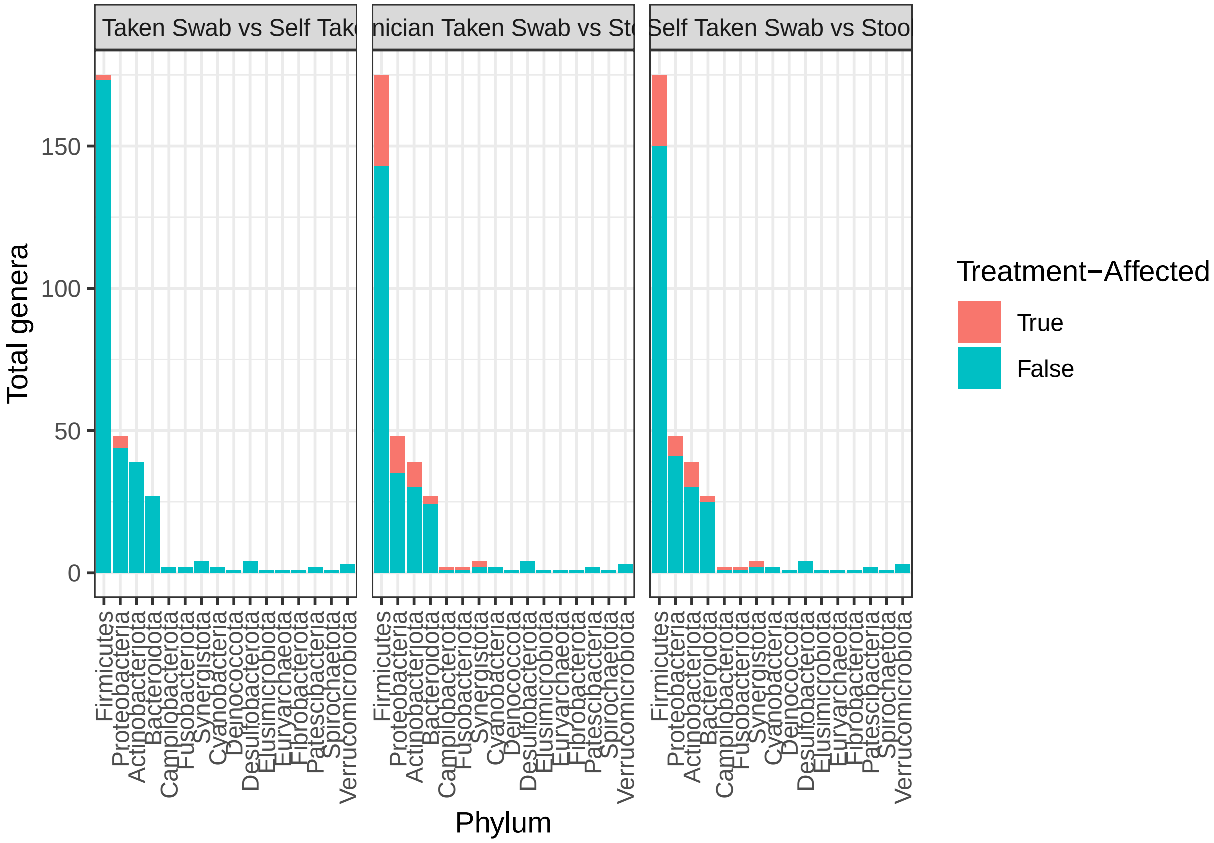
```
sigdat <- SignificantResults %>%
  group_by(Comparison, Phylum) %>%
  summarize(count = n_distinct(Genus))
```

```
## `summarise()` regrouping output by 'Comparison' (override with `.groups` argument)
```

```
mysum <- sum(mytable$Phycount)
#mytable$PhyRatio = mytable$Phycount /mysum
mytable$ConditionA = "Clinician Taken Swab vs Stool"
mytable$ConditionB = "Self Taken Swab vs Stool"
mytable$ConditionC = "Clinician Taken Swab vs Self Taken Swab"

data_long <- tidyr::gather(mytable, condition, Comparison, ConditionA:ConditionC, factor_key = TRUE)
joined2 <- sigdat %>%
  full_join(data_long)
```

```
## Joining, by = c("Comparison", "Phylum")
```

```
joined2<-joined2 %>%
  mutate_at(c(3), ~replace(., is.na(.), 0))
joined2$Phycount = joined2$Phycount - joined2$count

dat_long <- joined2 %>%
    tidyr::gather("count", "measurement", count, Phycount)
dat_long$measurement = as.numeric(as.character(dat_long$measurement))

ggplot(dat_long) +
  geom_col(aes(x = Phylum, y = measurement, fill = count)) +
  facet_grid(~Comparison) +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1)) +
  ylab("Total genera") +
  scale_fill_discrete(name = "Treatment-Affected", labels = c("True", "False"))
```



```
ggsave("../Results/S2)TreatmentAffectedGenera.pdf", width = 10, height = 6)


joined2$total = joined2$count + joined2$Phycount
joined2$condition = gsub("ConditionC", 0.019, joined2$condition)
joined2$condition = gsub("ConditionB", 0.15, joined2$condition)
joined2$condition = gsub("ConditionA", 0.195, joined2$condition)
joined2$condition = as.numeric(as.character(joined2$condition))
```

```r
f<-NULL;
for(i in c(1:48))
{
#print(joined2$total[i])
r<-binom.test(joined2$count[i], joined2$total[i], joined2$condition[i])
#print(r)
f<-rbind(f, c(r$statistic, r$parameter, r$null.value, r$p.value))
++i
}
colnames(f)[4] = "p.val"
# Not significant after FDR correction
p.adjust(f[,4], method = "BH")
```

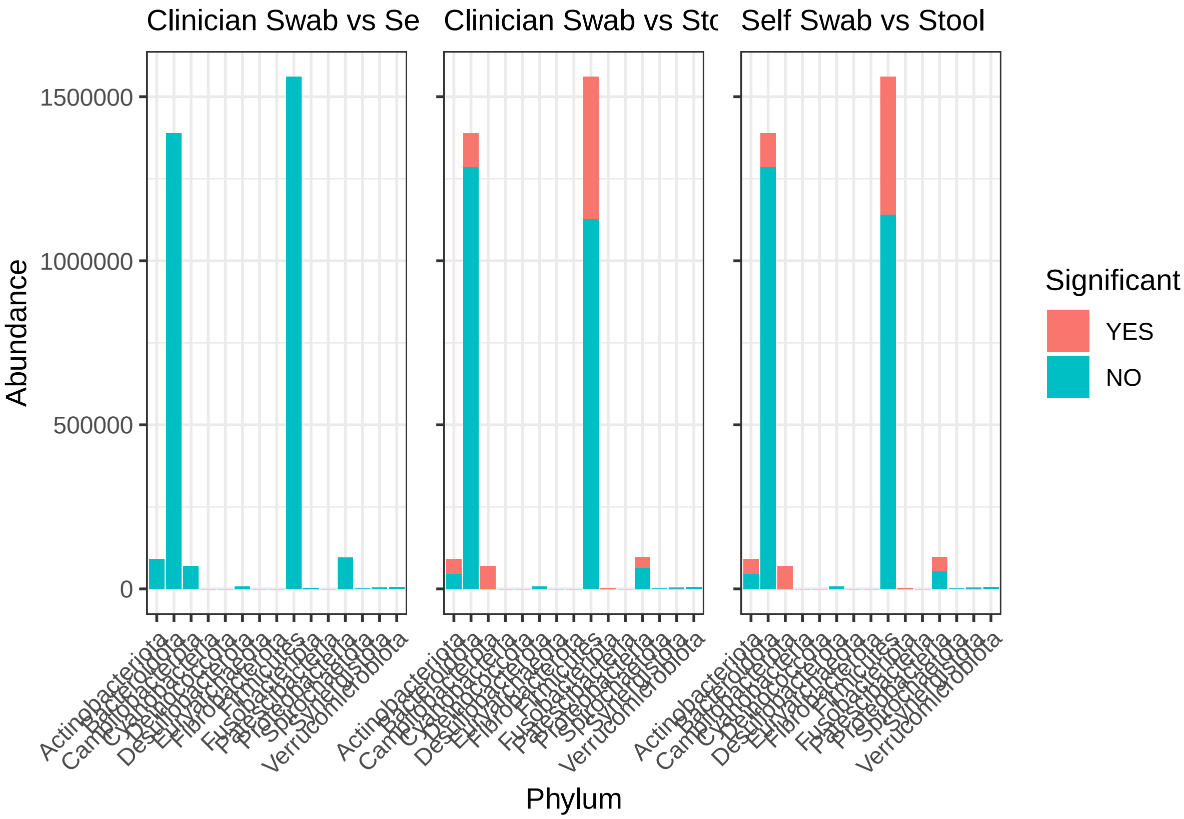
```
##  [1] 1.000000 0.626522 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000
##  [9] 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000
## [17] 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000
## [25] 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000
## [33] 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000
## [41] 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000 1.000000
```

```r
joined2[7:10] = f[,1:4]
colnames(joined2)[7:10] = c("successes", "trials", "pSuccess", "pval")
joined2$p.adjust = p.adjust(f[,4], method = "BH")
```

Supplementary 3 - DESeq2 Significance by Abundance

```r
library(patchwork)

sup_ps <- ps_rare %>%
  tax_glom(taxrank = "Genus")

sup_bugs <- as.character(unique(SignificantResults$Genus))
sup_bugsCTST <- as.character(resCTST_sig$Genus)
sup_bugsCTS <- as.character(resCTS_sig$Genus)
sup_bugsSTS <- as.character(resSTS_sig$Genus)

sup_melt <- psmelt(sup_ps)
sup_melt$Phylum <- as.character(sup_melt$Phylum)
sup_melt$Genus <- as.character(sup_melt$Genus)

sup_melt$Significant <- ifelse(sup_melt$Genus %in% sup_bugs, "YES", "NO")
sup_melt$Significant <- factor(sup_melt$Significant, levels = c("YES", "NO"))

sup_melt$CTST <- ifelse(sup_melt$Genus %in% sup_bugsCTST, "YES", "NO")
sup_melt$CTST <- factor(sup_melt$CTST, levels = c("YES", "NO"))

sup_melt$CTS <- ifelse(sup_melt$Genus %in% sup_bugsCTS, "YES", "NO")
sup_melt$CTS <- factor(sup_melt$CTS, levels = c("YES", "NO"))

sup_melt$STS <- ifelse(sup_melt$Genus %in% sup_bugsSTS, "YES", "NO")
sup_melt$STS <- factor(sup_melt$STS, levels = c("YES", "NO"))
```

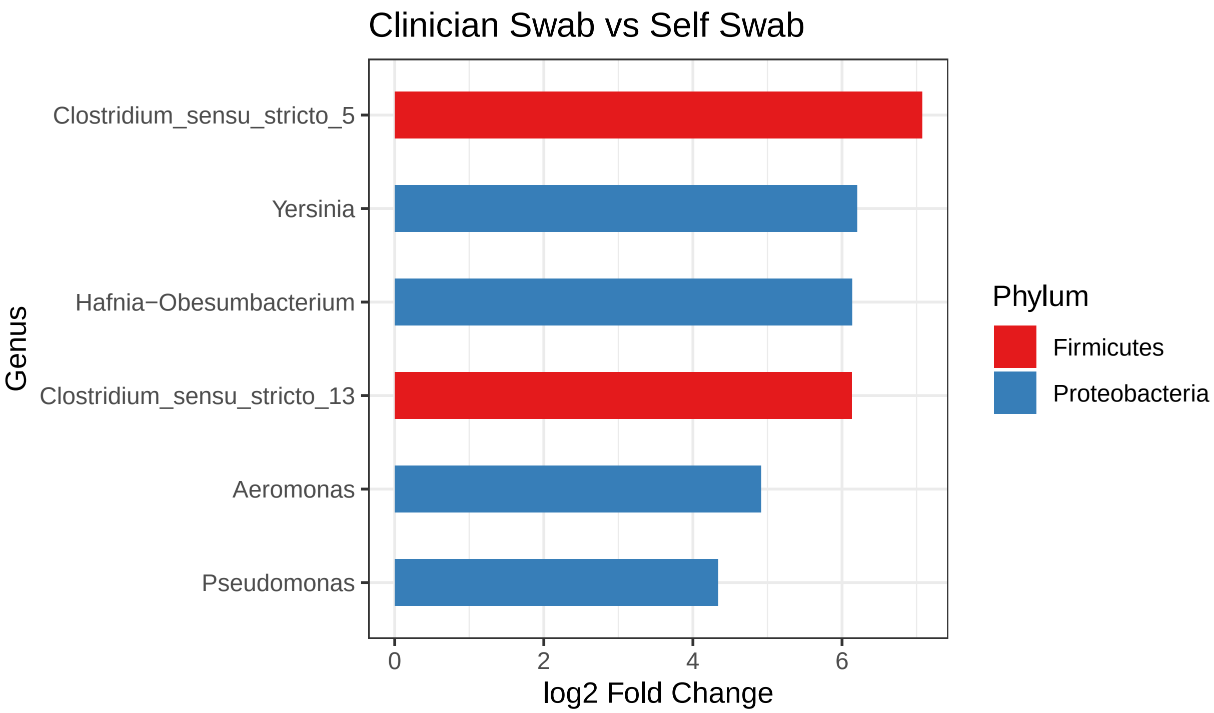
```
sup_CTST <- ggplot(sup_melt, aes(x = Phylum, y = Abundance, fill = CTST)) +
  geom_col() + labs(subtitle = "Clinician Swab vs Self Swab", fill = "Significant") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1),
        axis.title.x = element_blank(), legend.position = "none")

sup_CTS <- ggplot(sup_melt, aes(x = Phylum, y = Abundance, fill = CTS)) +
  geom_col() + labs(subtitle = "Clinician Swab vs Stool", fill = "Significant") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1),
        axis.title.y = element_blank(), axis.text.y = element_blank(),
        legend.position = "none")

sup_STS <- ggplot(sup_melt, aes(x = Phylum, y = Abundance, fill = STS)) +
  geom_col() + labs(subtitle = "Self Swab vs Stool", fill = "Significant") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1), axis.title.x = element_blank(),
        axis.title.y = element_blank(), axis.text.y = element_blank())

sup_CTST + sup_CTS + sup_STS + plot_layout(ncol = 3)
```



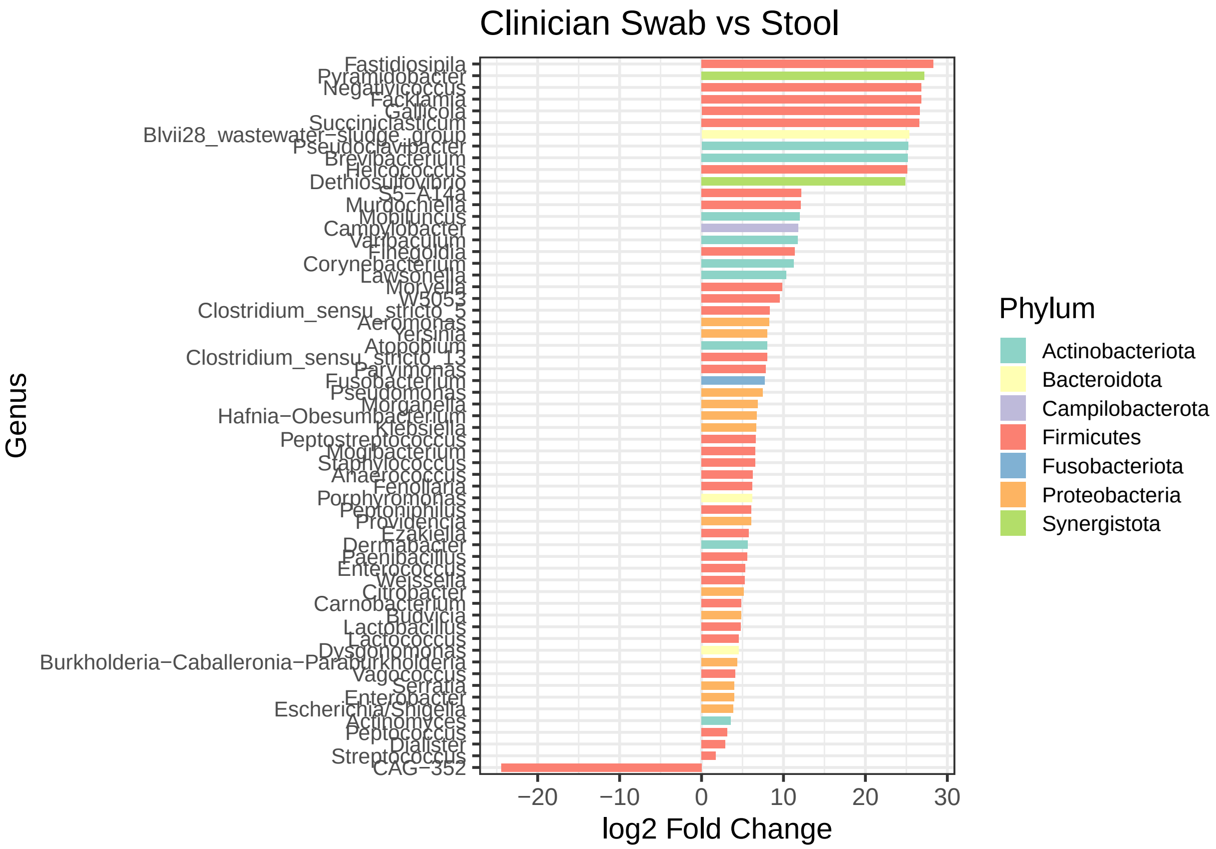
```
ggsave("../Results/S3)SignificanceByAbundance.pdf", width = 10, height = 6)
```

Supplementary 4 - Differential Abundance Figure

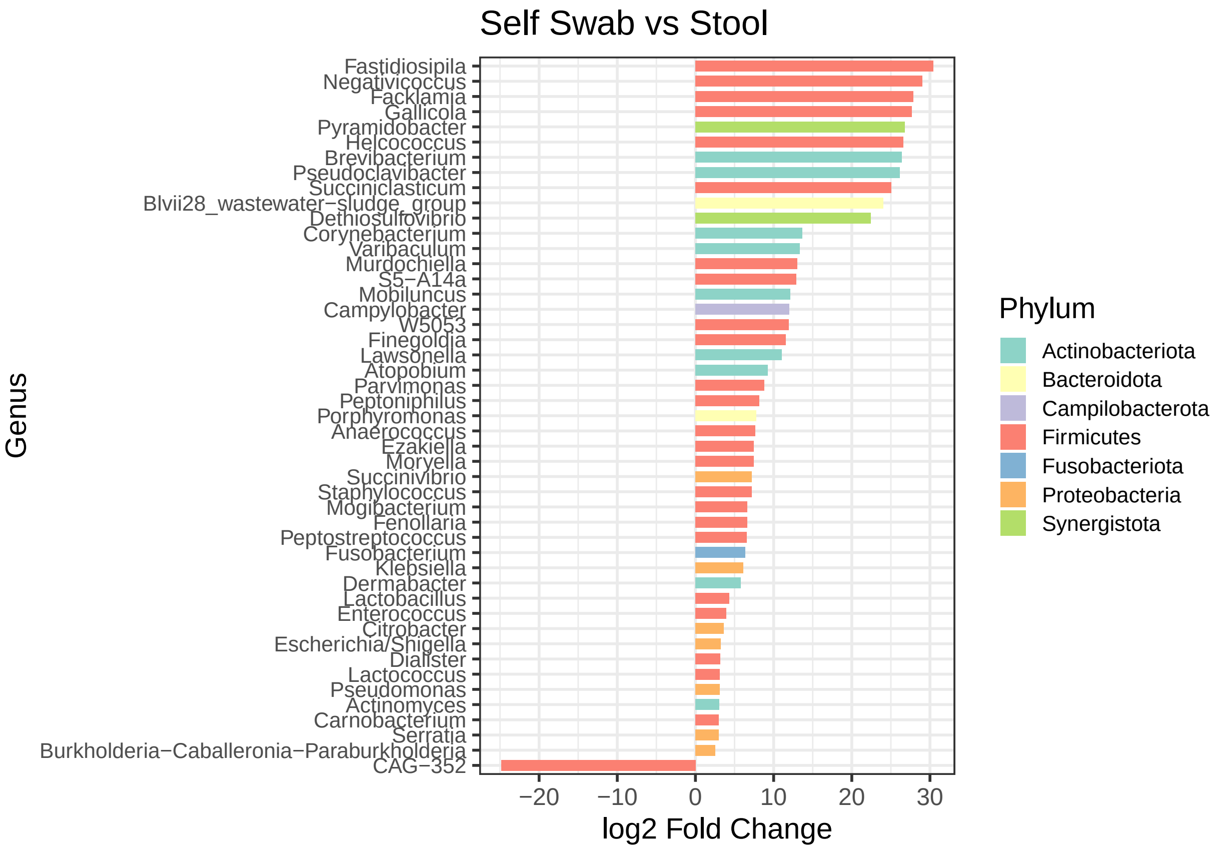
```
ggplot(resCTST_sig, aes(x = log2FoldChange, y = reorder(Genus, log2FoldChange), fill= Phylum)) +
  geom_bar(stat = "identity", position = "identity", width = 0.5) +
  labs(title = "Clinician Swab vs Self Swab", y = "Genus", x = "log2 Fold Change") +
  theme(aspect.ratio = 1) +
  scale_fill_brewer(palette = "Set1")
```



```r
ggsave("../Results/S4)Differential_Abundance_clinVSself.pdf", width = 7, height = 4)

clinVSstool <- ggplot(resCTS_sig, aes(x = log2FoldChange,
                                       y = reorder(Genus, log2FoldChange),
                                       fill= Phylum)) +
  geom_bar(stat = "identity", position = "identity", width = 0.7) +
  labs(title = "Clinician Swab vs Stool", y = "Genus", x = "log2 Fold Change") +
  scale_fill_brewer(palette = "Set3") +
  theme(axis.text.y = element_text(size = 8),
        legend.text = element_text(size = 8), legend.key.size = unit(0.75, "line"))
clinVSstool
```



```
selfVSstool <- ggplot(resSTS_sig, aes(x = log2FoldChange,
                                      y = reorder(Genus, log2FoldChange),
                                      fill= Phylum)) +
  geom_bar(stat = "identity", position = "identity", width = 0.7) +
  labs(title = "Self Swab vs Stool", y = "Genus", x = "log2 Fold Change") +
  scale_fill_brewer(palette = "Set3") +
  theme(axis.text.y = element_text(size = 8),
        legend.text = element_text(size = 8), legend.key.size = unit(0.75, "line"))
selfVSstool
```



```r
ggarrange(clinVSstool, selfVSstool, ncol = 2, common.legend = TRUE, legend = "right", labels = "AUTO")
```

A
C
B
Self Swab vs Stc
Genus
Fastidiosipila
Pyramidobacter
Negativicoccus
Facklamia
Gallicola
Succiniclasticum
Blvii28_wastewater−sludge_group
Pseudoclavibacter
Brevibacterium
Helcococcus
Dethiosulfovibrio
S5−A14a
Murdochiella
Mobiluncus
Campylobacter
Varibaculum
Finegoldia
Corynebacterium
Lawsonella
Moryella
W5053
Clostridium_sensu_stricto_5
Aeromonas
Yersinia
Atopobium
Clostridium_sensu_stricto_13
Parvimonas
Fusobacterium
Pseudomonas
Morganella
Hafnia−Obesumbacterium
Klebsiella
Peptostreptococcus
Mogibacterium
Staphylococcus
Anaerococcus
Fenollaria
Porphyromonas
Peptoniphilus
Providencia
Ezakiella
Dermabacter
Paenibacillus
Enterococcus
Weissella
Citrobacter
Carnobacterium
Budvicia
Lactobacillus
Lactococcus
Dysgonomonas
Burkholderia−Caballeronia−Paraburkholderia
Vagococcus
Serratia
Enterobacter
Escherichia/Shigella
Actinomyces
Peptococcus
Dialister
Streptococcus
CAG−352
log2 Fold
Genus
Fastidiosipila
Negativicoccus
Facklamia
Gallicola
Pyramidobacter
Helcococcus
Brevibacterium
Pseudoclavibacter
Succiniclasticum
Blvii28_wastewater−sludge_group
Dethiosulfovibrio
Corynebacterium
Varibaculum
Murdochiella
S5−A14a
Mobiluncus
Campylobacter
W5053
Finegoldia
Lawsonella
Atopobium
Parvimonas
Peptoniphilus
Porphyromonas
Anaerococcus
Ezakiella
Moryella
Succinivibrio
Staphylococcus
Mogibacterium
Fenollaria
Peptostreptococcus
Fusobacterium
Klebsiella
Dermabacter
Lactobacillus
Enterococcus
Citrobacter
Escherichia/Shigella
Dialister
Lactococcus
Pseudomonas
Actinomyces
Carnobacterium
Serratia
Burkholderia−Caballeronia−Paraburkholderia
CAG−352
log2 Fold Change
Phylum
🟩 Actinobacteriota
🟨 Bacteroidota
🟪 Campilobacterota
🟥 Firmicutes
🟦 Fusobacteriota
🟧 Proteobacteria
🟩 Synergistota

```r
ggsave("../Results/S4)Differential_Abundance_swabsVSstool.pdf", width = 12, height = 8)
```

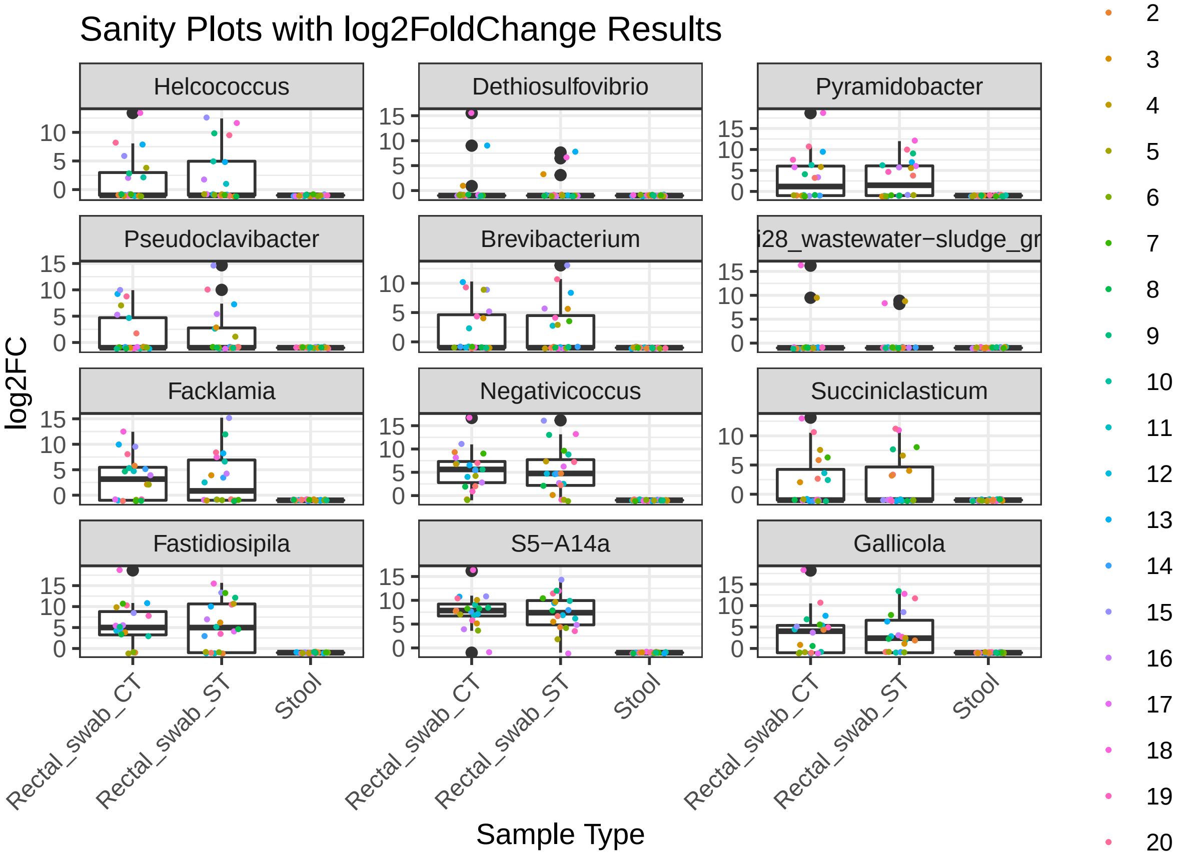
Supplementary 5 - Boxplot Sanity Checks

```r
resCTS_sig <- resCTS_sig[order(-resCTS_sig$log2FoldChange),]

int <- row.names(resCTS_sig)[1:12]
ASVlabs <- tax_table(ps)[int, 6]
names(ASVlabs) <- int
ASVlabs <- as.list(ASVlabs)

ASV_labeller <- function(variable,value){
  return(ASVlabs[value])
}

# Sanity Plots with Fold Change
tcounts <- t(log2((counts(ds[int, ], normalized = TRUE, replaced = FALSE) + .5))) %>%
  merge(colData(ds), ., by = "row.names") %>%
  tidyr::gather(ASV, log2FC, (ncol(.)-length(int) + 1):ncol(.))

tcounts %>%
  select(Row.names, Sample_type, Individual, ASV, log2FC) %>%
  head %>%
  knitr::kable()
```

Row.names	Sample_type	Individual	ASV	log2FC
10A	Rectal_swab_CT	10	ASV3599	3.017179
10B	Rectal_swab_ST	10	ASV3599	5.359164
10C	Stool	10	ASV3599	-1.000000
11A	Rectal_swab_CT	11	ASV3599	4.888552
11B	Rectal_swab_ST	11	ASV3599	-1.000000
11C	Stool	11	ASV3599	-1.000000

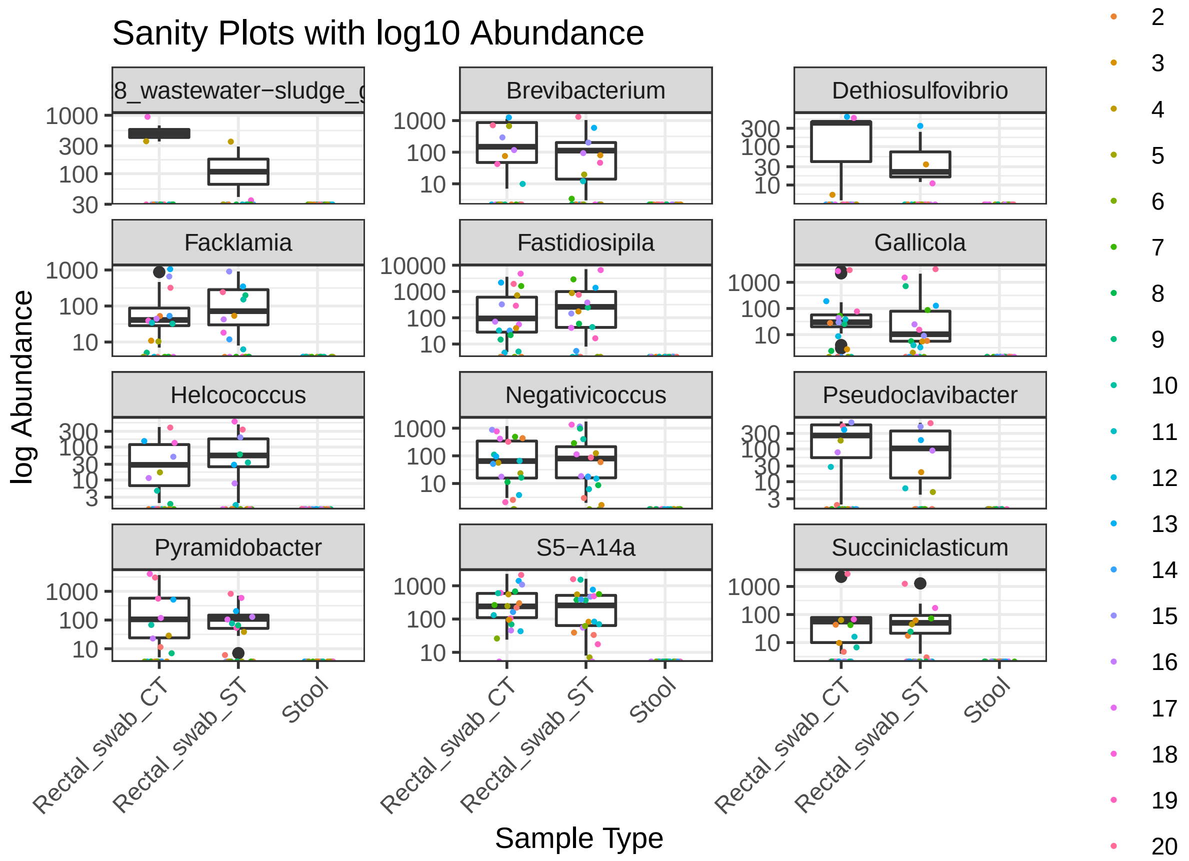
```r
ggplot(tcounts, aes(Sample_type, log2FC)) +
  geom_boxplot() + geom_jitter(width = 0.2, height = 0.2, size = 0.4, aes(color = Individual)) +
  facet_wrap(~ASV, scales = "free_y", labeller = ASV_labeller, nrow = 4) +
  labs(x = "Sample Type",
       y = "log2FC",
       title = "Sanity Plots with log2FoldChange Results") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```

```
## Warning: The labeller API has been updated. Labellers taking `variable` and
## `value` arguments are now deprecated. See labellers documentation.
```



```r
ggsave("../Results/S5)Sanity_FoldChange_plots.pdf", width = 7, height = 8)

# Sanity Plots with Abundance
sanity_ps <- subset_taxa(ps_deseq, taxa_names(ps_deseq) %in% int)
sanity <- psmelt(sanity_ps)

ggplot(sanity, aes(Sample_type, Abundance)) +
  geom_boxplot() + geom_jitter(width = 0.2, height = 0.2, size = 0.4, aes(color = Individual)) +
  facet_wrap(~Genus, scales = "free_y", nrow = 4) +
  scale_y_log10() +
  labs(x = "Sample Type",
       y = "log Abundance",
       title = "Sanity Plots with log10 Abundance") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```

```
## Warning: Transformation introduced infinite values in continuous y-axis

## Warning: Transformation introduced infinite values in continuous y-axis

## Warning: Removed 474 rows containing non-finite values (stat_boxplot).
```



```
ggsave("../Results/S5)Sanity_logAbundance_plots.pdf", width = 7, height = 8)
```

```
## Warning: Transformation introduced infinite values in continuous y-axis

## Warning: Transformation introduced infinite values in continuous y-axis

## Warning: Removed 474 rows containing non-finite values (stat_boxplot).
```

Session Info

```
sessionInfo()
```

```
## R version 3.6.3 (2020-02-29)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Sierra 10.12.6
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_NZ.UTF-8/en_NZ.UTF-8/en_NZ.UTF-8/C/en_NZ.UTF-8/en_NZ.UTF-8
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] patchwork_1.0.1             DESeq2_1.26.0
##  [3] SummarizedExperiment_1.16.1 DelayedArray_0.12.3
##  [5] BiocParallel_1.20.1         matrixStats_0.56.0
##  [7] Biobase_2.46.0              GenomicRanges_1.38.0
##  [9] GenomeInfoDb_1.22.1         IRanges_2.20.2
## [11] S4Vectors_0.24.4            BiocGenerics_0.32.0
## [13] ggpubr_0.4.0                ggplot2_3.3.2
## [15] phyloseq_1.30.0             dplyr_1.0.2
## [17] vegan_2.5-6                 permute_0.9-5
## [19] Rmisc_1.5                   plyr_1.8.6
## [21] lattice_0.20-41             RColorBrewer_1.1-2
##
## loaded via a namespace (and not attached):
##   [1] colorspace_1.4-1       ggsignif_0.6.0         ellipsis_0.3.1
##   [4] rio_0.5.16             htmlTable_2.1.0        XVector_0.26.0
##   [7] base64enc_0.1-3        rstudioapi_0.11        farver_2.0.3
##  [10] bit64_4.0.5            AnnotationDbi_1.48.0   codetools_0.2-16
##  [13] splines_3.6.3          geneplotter_1.64.0     knitr_1.30
##  [16] ade4_1.7-15            Formula_1.2-3          jsonlite_1.7.1
##  [19] broom_0.7.0            annotate_1.64.0        cluster_2.1.0
##  [22] png_0.1-7              compiler_3.6.3         backports_1.1.10
##  [25] Matrix_1.2-18          htmltools_0.5.0        tools_3.6.3
##  [28] igraph_1.2.5           gtable_0.3.0           glue_1.4.2
##  [31] GenomeInfoDbData_1.2.2 reshape2_1.4.4         Rcpp_1.0.5
##  [34] carData_3.0-4          cellranger_1.1.0       vctrs_0.3.4
##  [37] Biostrings_2.54.0      multtest_2.42.0        ape_5.4-1
##  [40] nlme_3.1-149           iterators_1.0.13       xfun_0.19
##  [43] stringr_1.4.0          openxlsx_4.2.2         lifecycle_0.2.0
##  [46] XML_3.99-0.3           rstatix_0.6.0          zlibbioc_1.32.0
##  [49] MASS_7.3-53            scales_1.1.1           hms_0.5.3
##  [52] biomformat_1.14.0      rhdf5_2.30.1           yaml_2.2.1
##  [55] curl_4.3               memoise_1.1.0          gridExtra_2.3
##  [58] rpart_4.1-15           RSQLite_2.2.0          latticeExtra_0.6-29
##  [61] stringi_1.5.3          highr_0.8              genefilter_1.68.0
##  [64] foreach_1.5.1          checkmate_2.0.0        zip_2.1.1
##  [67] rlang_0.4.8            pkgconfig_2.0.3        bitops_1.0-6
##  [70] evaluate_0.14          purrr_0.3.4            Rhdf5lib_1.8.0
##  [73] labeling_0.4.2         htmlwidgets_1.5.2      cowplot_1.1.0
##  [76] bit_4.0.4              tidyselect_1.1.0       magrittr_1.5
##  [79] R6_2.5.0               generics_0.1.0         Hmisc_4.4-1
##  [82] DBI_1.1.0              pillar_1.4.6           haven_2.3.1
##  [85] foreign_0.8-76         withr_2.3.0            mgcv_1.8-33
##  [88] survival_3.2-3         abind_1.4-5            RCurl_1.98-1.2
##  [91] nnet_7.3-14            tibble_3.0.4           crayon_1.3.4
##  [94] car_3.0-9              rmarkdown_2.3          jpeg_0.1-8.1
##  [97] locfit_1.5-9.4         grid_3.6.3             readxl_1.3.1
## [100] data.table_1.13.2      blob_1.2.1             forcats_0.5.0
## [103] digest_0.6.27          xtable_1.8-4           tidyr_1.1.2
## [106] munsell_0.5.0
```