Data-analyysi Raajärvi näytteet ITS1

R libraries

```r
library(tidyverse)
library(knitr)
library(ggthemes)
library(ggsci)
library(ggpubr)
library(kableExtra)
library(RColorBrewer)
library(mia)
library(miaViz)
library(scater)
library(ape)
library(pheatmap)
library(bluster)
library(cluster)
library(ALDEx2)
library(ANCOMBC)
library(Maaslin2)
```

Load data object

```r
# Load data from rds
tse <- readRDS("results/tse_its1.rds")
set.seed(123412)
```

Prevalent features on data set

Features that have minimun abundance of 1 % and prevalence of over 50 %

```r
# Agglomerate to genus
tse <- agglomerateByRank(tse, rank="Genus", na.rm=T)
tse <- tse[,1:23]
# Filter abundance
rowData(tse)$prevalence <- getPrevalence(tse, detection = 1/100,
                                         as.relative=T)
# Filter prevalence
filtered_prevalence <- tse[rowData(tse)$prevalence >= 0.5,]
# Plot
plot <- plotRowData(filtered_prevalence, "prevalence", colour_by="Genus")
plot$scales$scales <- list()
plot$layers[[2]]$aes_params$size <- 2.5
plot + theme_pubclean(base_size=8) + scale_color_lancet() +
    labs(color="Genus", caption="Prevalence plot")
```

Prevalence plot
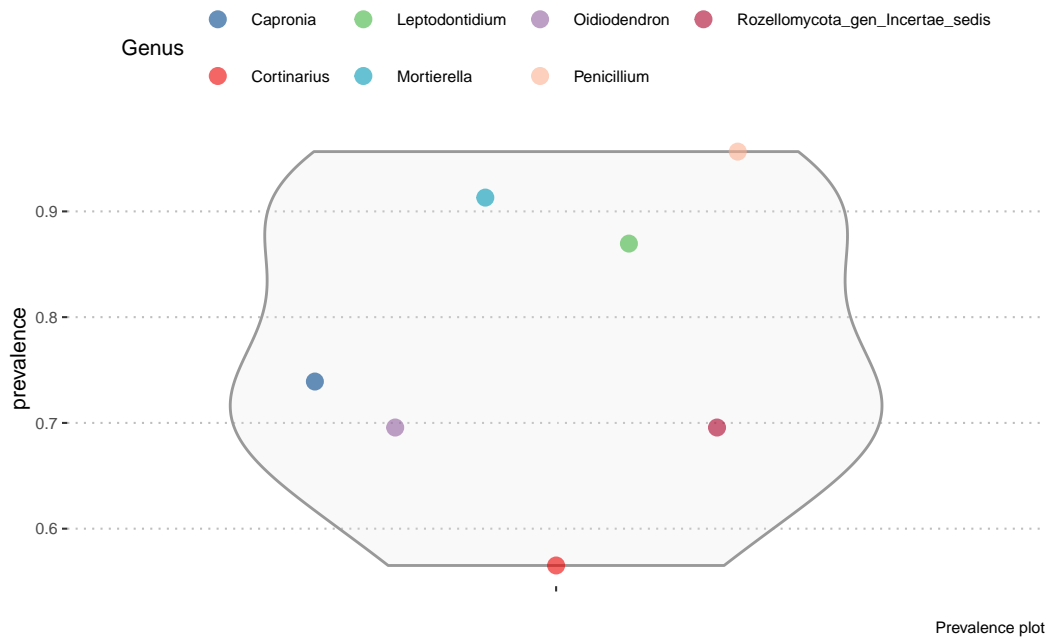
Community composition plots

Top ten features calculated by median relative abundance

```r
# Reload data
tse <- readRDS("results/tse_its1.rds")
tse <- tse[,1:23]
# Agglomerate to genus
tse <- agglomerateByRank(tse, rank="Genus", na.rm=T)
# Calculate relative abundance
tse <- transformAssay(tse, method="relabundance")
# Get top features
top_features <- getTop(tse, top=10, method="median",
                       assay.type="relabundance")
# Get data and filter using top features
df_genus <- data.frame(assay(tse, "relabundance"))
df_genus <- df_genus %>% rownames_to_column(var="Genus") %>%
    filter(Genus %in% top_features) %>%
    bind_rows(summarise(., Genus = "Others", across(where(is.numeric),
                                                    ~ 1 - sum(.))))
# Sample names from colData
coldata_df <- as.data.frame(colData(tse))
coldata_df <- rownames_to_column(coldata_df, var="Sample")
# Create a long table
genus_long <- pivot_longer(df_genus, names_to="Sample",
                           values_to="Abundance",
                           cols = starts_with("Barcode"))
genus_long <- merge(coldata_df, genus_long, by="Sample")
#barplot theme settings
bar_theme <- list(theme_pubclean(base_size=7),scale_fill_igv(), xlab("Sample"),
                  theme(axis.text.x = element_text(angle = 90,
                                                   vjust = 0.5,
                                                   hjust = 1)))
```

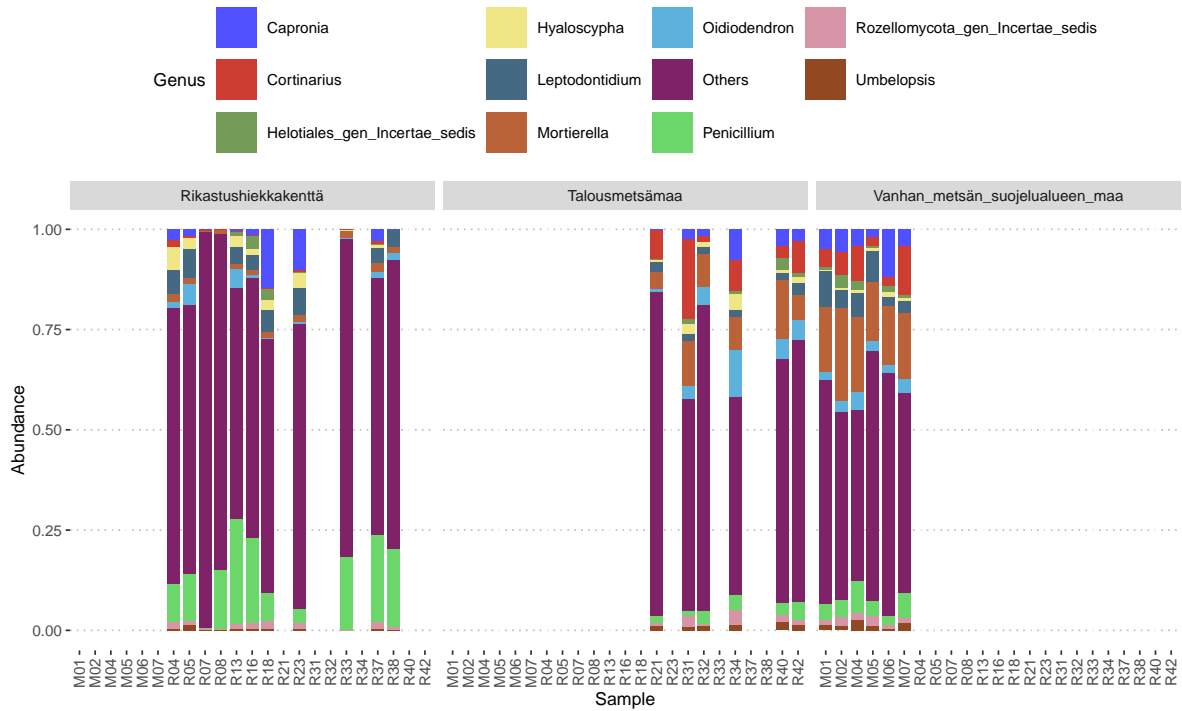# Barplot faceted by vegetation

```
barplot1 <- ggplot(genus_long, aes(x=Labnro, y=Abundance, fill=Genus)) +
    geom_bar(stat="identity", width=0.8) +
    facet_wrap(~ Kasvillisuus) + bar_theme
barplot1
```
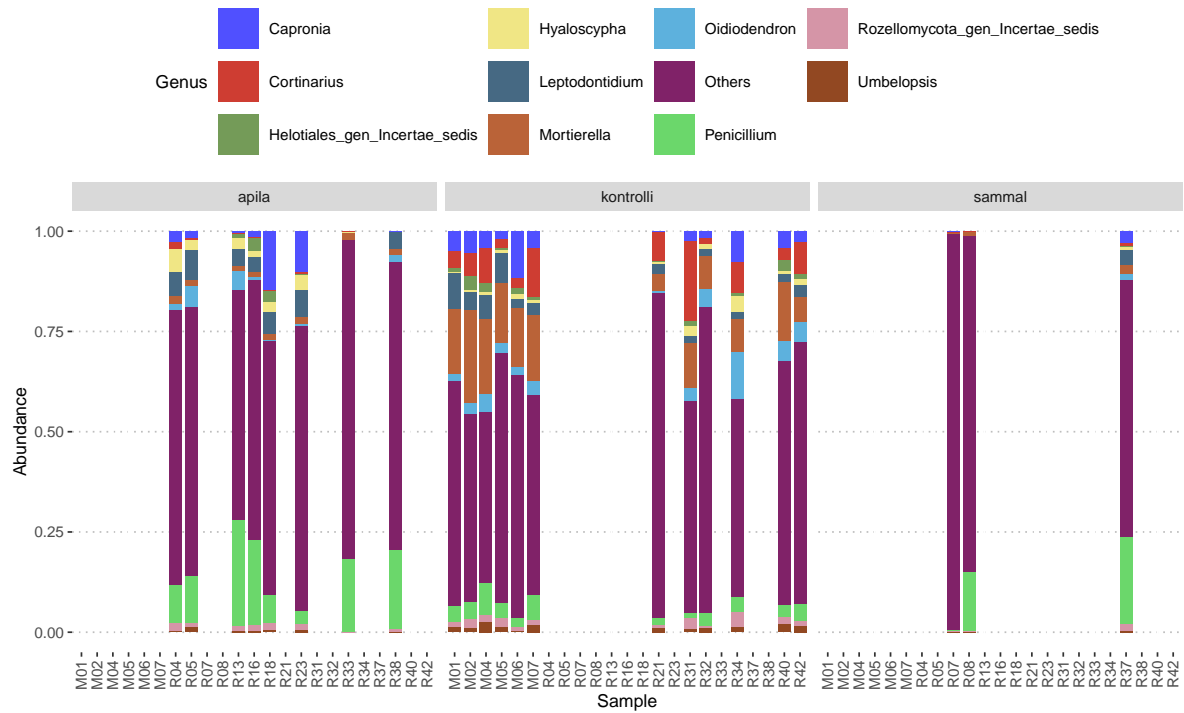
Barplot faceted by soil

```
barplot2 <- ggplot(genus_long, aes(x=Labnro, y=Abundance, fill=Genus)) +
    geom_bar(stat="identity", width=0.8) + facet_grid(~ Maanäyte) + bar_theme
barplot2
```
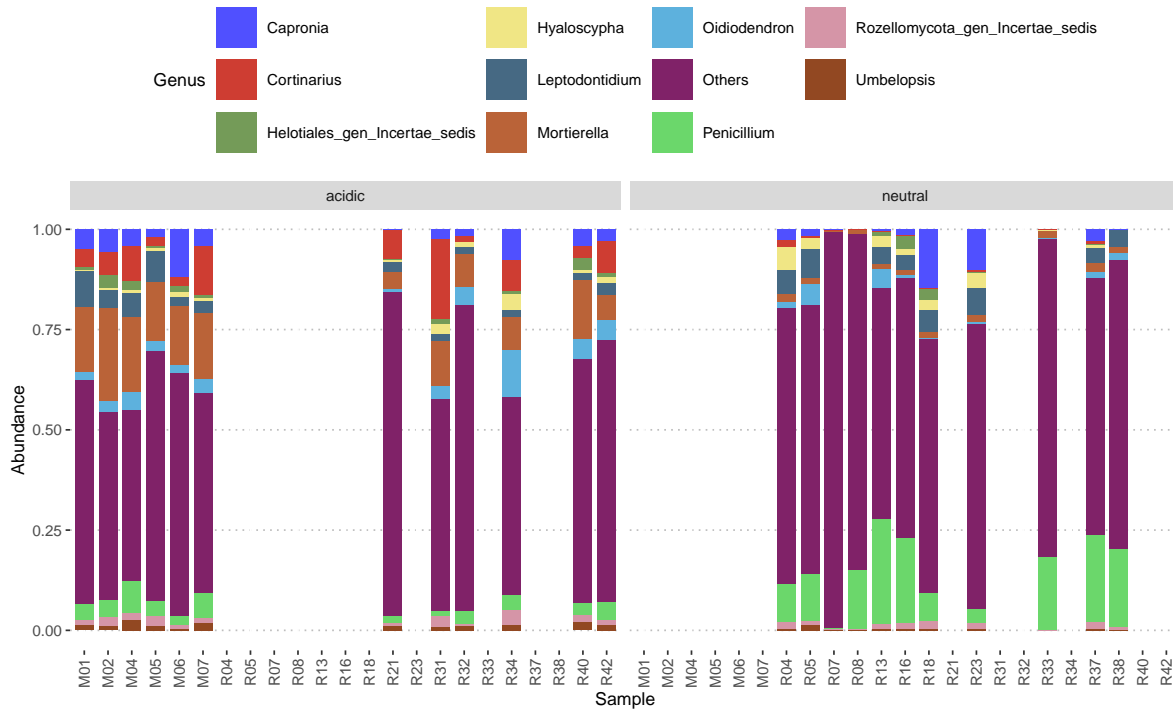
Barplot faceted by pH

```
barplot3 <- ggplot(genus_long, aes(x=Labnro, y=Abundance, fill=Genus)) +
    geom_bar(stat="identity", width=0.8) + facet_grid(~ pH) + bar_theme
barplot3
```

Clustered heatmap with soil pH groups

```r
# Reload object and count relabundance
tse <- readRDS("results/tse_its1.rds")
tse <- tse[,1:23]
tse <- transformAssay(tse, method="relabundance")
# Get assay data
assay_data <- assay(tse, "relabundance")
# Get taxa labels and merge with feature id
taxa <- data.frame(id = rownames(tse), label = getTaxonomyLabels(tse))
taxa$cleaned <- sub(".*:", "", taxa$label)
taxa$merged <- paste(taxa$id, taxa$cleaned, sep = ":")
# Replace rownames with new label
rownames(assay_data) <- taxa$merged
# Calculate rowsums
abundance <- rowSums(assay_data)
abundance <- order(abundance, decreasing = TRUE)[1:25]
# Filter assay data with top25 abundance
top25 <- assay_data[abundance, ]
# Extract sample names and material types from colData
sample_names <- colData(tse)$Labnro
material_types <- colData(tse)$pH
# Assign the sample names to the top25 matrix
colnames(top25) <- sample_names
# Create a dataframe for annotations
annotation_data <- data.frame(pH = material_types)
rownames(annotation_data) <- sample_names
# Set color for annotations
annotation_colors <- list(pH = c("neutral" = "navy", "acidic" = "red"))
# Plot the heatmap with annotations
pheatmap(
  top25,
  cluster_rows = TRUE,
  cluster_cols = TRUE,
  clustering_distance_cols = "correlation",
  main = "Heatmap presentation",
  scale = "row",
  color = brewer.pal(11, "RdBu"),
  fontsize = 8,
  fontsize_row = 6,
  fontsize_col = 6,
  annotation_col = annotation_data,
  annotation_colors = annotation_colors)
```

Heatmap presentation
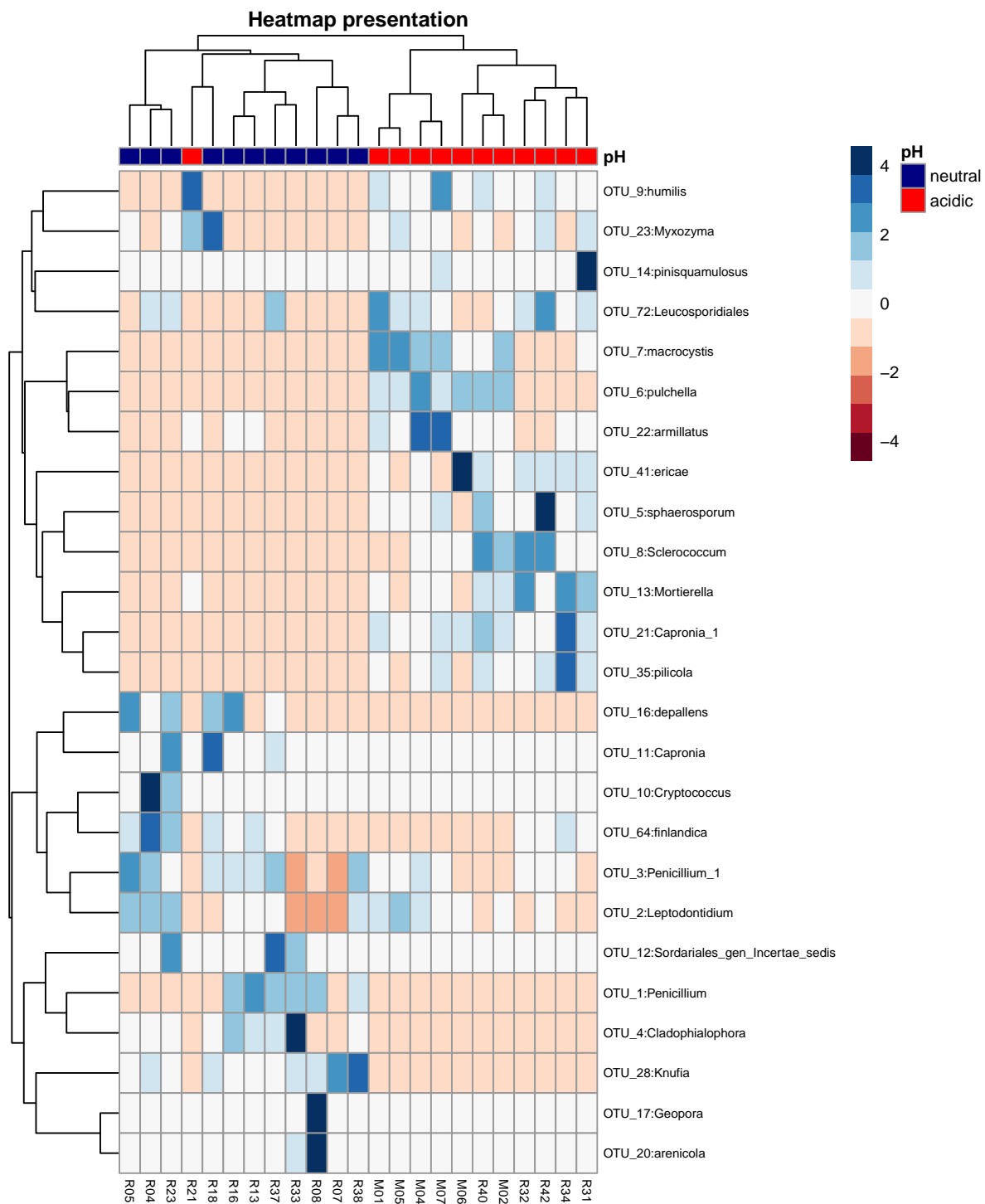
Taxonomy tree plot objects for soil pH groups

```r
# Reload data
tse <- readRDS("results/tse_its1.rds")
tse <- tse[,1:23]
plots <- list()
materials <- unique(tse$pH)
# For loop for different groups
for (i in seq_along(materials)) {
material <- materials[i]
# Subset materials
tse_sub <- tse[, tse$pH == material]
# Agglomerate omitting empty species column
tse_sub <- agglomerateByRanks(tse_sub, ranks = taxonomyRanks(tse_sub)[1:6])
# Calculate prevalence values
altExps(tse_sub) <- lapply(
    altExps(tse_sub), function(y){
    rowData(y)$prevalence <- getPrevalence(y, detection = 1/100,
                                        sort = F, as.relative = T,
                                        assay.type = "counts")
return(y)})
# Define top5 phyla
top_phyla <- getTop(altExp(tse_sub,"Phylum"),
                    method="mean",
                    top=5,
                    assay.type="counts")
# Unsplit agglomeration
taxas <- unsplitByRanks(tse_sub, ranks = taxonomyRanks(tse_sub)[1:6])
# Add hierarchy
taxas <- addHierarchyTree(taxas)
# Plot rowtree
p <- plotRowTree(
taxas[rowData(taxas)$Phylum %in% top_phyla,],
edge.colour.by = "Phylum",
tip.colour.by = "prevalence",
node.colour.by = "prevalence")
plots[[i]] <- p + ggtitle(paste("Soil = ",material)) + scale_color_lancet()
}
```

Plot the figure

```r
plots[[1]]/plots[[2]]
```

Soil = neutral
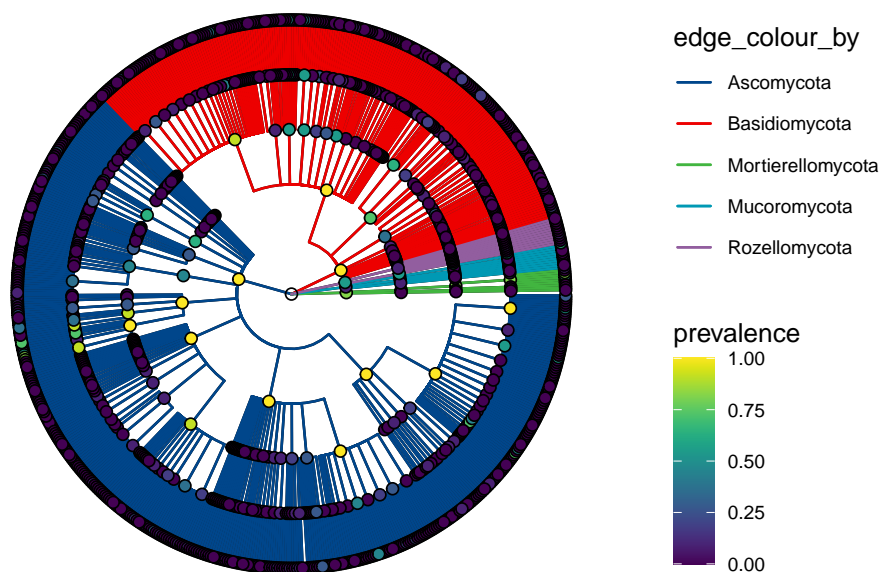
Soil = acidic

Alpha diversity

Calculation of alpha diversity indexes
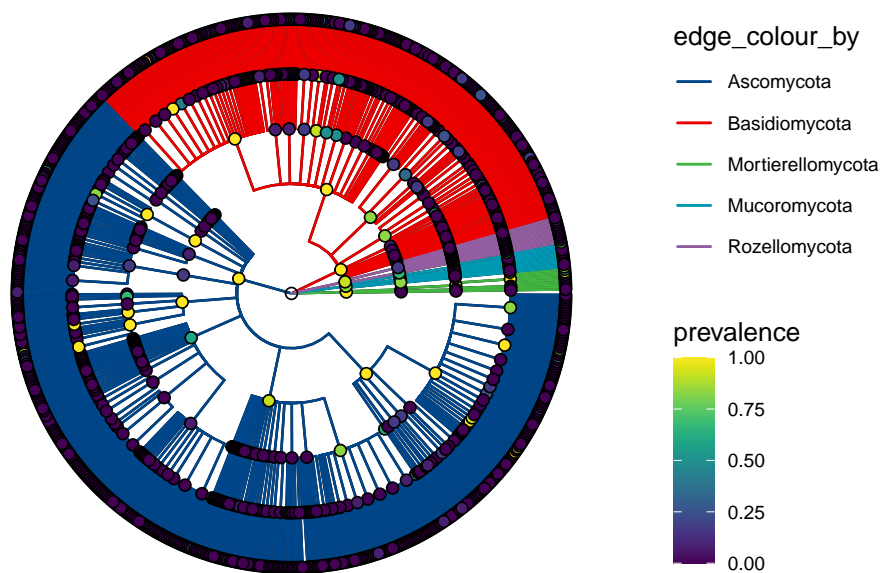
```
tse <- readRDS("results/tse_its1.rds")
tse <- tse[,1:23]
# Calculate alpha and create df
tse <- addAlpha(tse, assay.type="counts",
                index=c("shannon_diversity", "gini_simpson_diversity",
                        "pielou_evenness", "observed_richness"))
alpha_table <- data.frame(Sample=colData(tse)$Labnro,
                          Shannon = colData(tse)$shannon_diversity,
                          Gini_Simpson = colData(tse)$gini_simpson_diversity,
                          Pielou = colData(tse)$pielou_evenness,
                          Observed_features = colData(tse)$observed_richness)
common_theme <- list(theme_pubclean(base_size=8), scale_fill_lancet(),
                     theme(legend.position="none"))
```

Alpha diversity index table

```
kable(alpha_table, digits = 2, caption = "Diversity indexes") %>%
kable_styling(latex_options = c("HOLD_position", "striped"),
font_size = 10) %>% row_spec(0, background = "teal", color = "white")
```

Table 1: Diversity indexes

Sample	Shannon	Gini_Simpson	Pielou	Observed_features
R08	3.44	0.89	0.48	1248
R16	4.01	0.94	0.53	2038
R07	4.41	0.97	0.62	1195
R37	3.90	0.95	0.53	1476
R33	3.48	0.92	0.48	1425
R13	4.30	0.95	0.55	2316
M06	4.57	0.97	0.60	2032
R38	4.28	0.96	0.57	1868
R05	4.45	0.97	0.57	2489
R34	4.72	0.98	0.62	2109
R32	4.68	0.97	0.61	2134
R04	4.36	0.96	0.57	2169
M04	4.72	0.98	0.60	2602
M07	4.78	0.98	0.61	2531
M01	4.87	0.98	0.62	2525
R21	4.79	0.98	0.63	2038
R18	4.14	0.95	0.55	1957
R40	4.73	0.98	0.62	2056
R42	4.32	0.95	0.56	2300
M05	4.69	0.97	0.61	2226
M02	4.71	0.98	0.60	2434
R31	4.42	0.96	0.58	1959
R23	4.36	0.96	0.56	2450

Group comparison objects

```
# Data frame for shannon boxplots
alphadf <- data.frame(colData(tse)) %>%
    dplyr::select(Labnro, Alue, Kasvillisuus, Maanäyte, pH,
                  Shannon=shannon_diversity)
# Vegetation
plot_kasvillisuus <- ggplot(alphadf, aes(x=Kasvillisuus, y=Shannon,
                                    fill=Kasvillisuus)) + geom_boxplot() +
    common_theme + labs(caption="Kasvillisuus")
# Area
plot_alue <- ggplot(alphadf, aes(x=Alue, y=Shannon,
                              fill=Alue)) + geom_boxplot() +
    common_theme + labs(caption="Alue")
# Soil_type
plot_Maanäyte <- ggplot(alphadf, aes(x=Maanäyte, y=Shannon,
                                 fill=Maanäyte)) +
    geom_boxplot() + common_theme + labs(caption="Maanäyte")
# pH
plot_pH <- ggplot(alphadf, aes(x=pH, y=Shannon,
                            fill=pH)) +
    geom_boxplot() + common_theme + labs(caption="Soil pH")
```

Plots

```
plot_alue + plot_kasvillisuus + plot_Maanäyte + plot_pH
```

Shannon

4.5
4.0
3.5

Matovaara Raajärvi

Alue

Alue

Shannon

4.5
4.0
3.5

apila kontrolli sammal

Kasvillisuus

Kasvillisuus

Shannon

4.5
4.0
3.5

Rikastushiekkakenttä Talousmetsämaa Vanhan_metsän_suojelualueen_maa

Maanäyte

Maanäyte

Shannon

4.5
4.0
3.5

acidic neutral

pH

Soil pH
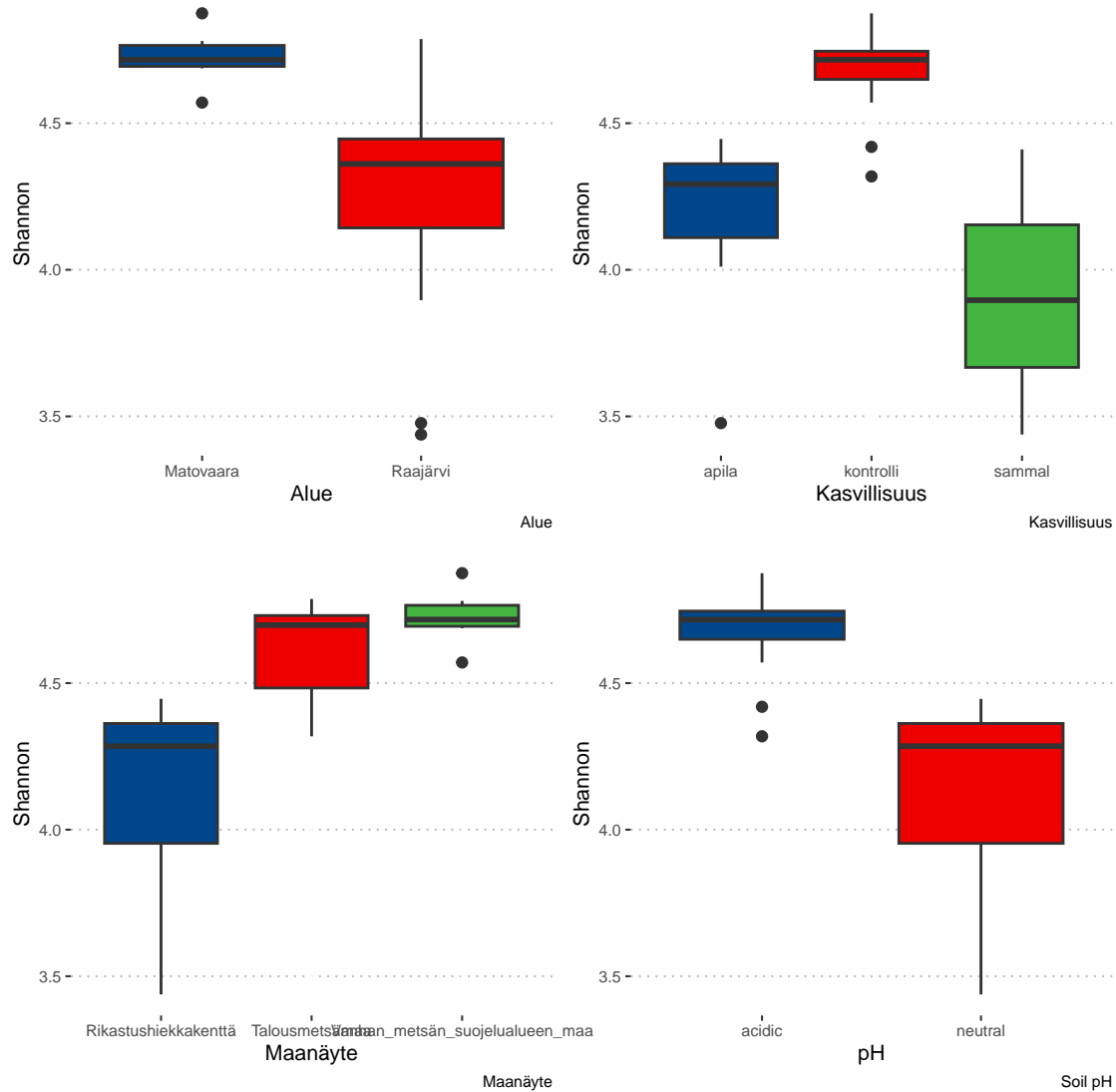
Kruskal-Wallis tests for variables

```
kruskal.test(Shannon ~ Kasvillisuus, data=alphadf)
```

```
	Kruskal-Wallis rank sum test

data:  Shannon by Kasvillisuus
Kruskal-Wallis chi-squared = 14.284, df = 2, p-value = 0.000791
```

```
kruskal.test(Shannon ~ Alue, data=alphadf)
```

```
    Kruskal-Wallis rank sum test

data:  Shannon by Alue
Kruskal-Wallis chi-squared = 7.0784, df = 1, p-value = 0.007802
```

```
kruskal.test(Shannon ~ Maanäyte, data=alphadf)
```

```
    Kruskal-Wallis rank sum test

data:  Shannon by Maanäyte
Kruskal-Wallis chi-squared = 14.502, df = 2, p-value = 0.0007094
```

```
kruskal.test(Shannon ~ pH, data=alphadf)
```

```
    Kruskal-Wallis rank sum test

data:  Shannon by pH
Kruskal-Wallis chi-squared = 14.095, df = 1, p-value = 0.0001738
```

Betadiversity

Bray-Curtis distances ja PCoA ordination plots

```
# Reload object
tse <- readRDS("results/tse_its1.rds")
tse <- tse[,1:23]
tse <- transformAssay(
    tse, assay.type = "counts", method = "relabundance")
# Run PCoA on relabundance assay with Bray-Curtis distances
tse <- runMDS(tse, FUN = getDissimilarity,
              method = "bray", assay.type = "relabundance",
              name = "MDS_bray", sample=130000)
# Create ggplot object
p1 <- plotReducedDim(tse, "MDS_bray", colour_by = "Kasvillisuus")
# Calculate explained variance
e <- attr(reducedDim(tse, "MDS_bray"), "eig")
rel_eig <- e / sum(e[e > 0])
p1 <- p1 + labs(
    x = paste("PCoA 1 (", round(100 * rel_eig[[1]], 1), "%", ")", sep = ""),
    y = paste("PCoA 2 (", round(100 * rel_eig[[2]], 1), "%", ")", sep = "")
    ) + theme_pubclean(base_size=8)
p1$scales$scales <- list()
p1$layers[[1]]$aes_params$size <- 2.5
# pH plot
p2 <- plotReducedDim(tse, "MDS_bray", colour_by = "pH")
# Calculate explained variance
e <- attr(reducedDim(tse, "MDS_bray"), "eig")
rel_eig <- e / sum(e[e > 0])
p2 <- p2 + labs(
    x = paste("PCoA 1 (", round(100 * rel_eig[[1]], 1), "%", ")", sep = ""),
    y = paste("PCoA 2 (", round(100 * rel_eig[[2]], 1), "%", ")", sep = "")
    ) + theme_pubclean(base_size=8)
p2$scales$scales <- list()
p2$layers[[1]]$aes_params$size <- 2.5
```

Plot vegetation and pH groups

```
p1 + scale_color_lancet() + p2 + scale_color_lancet()
```
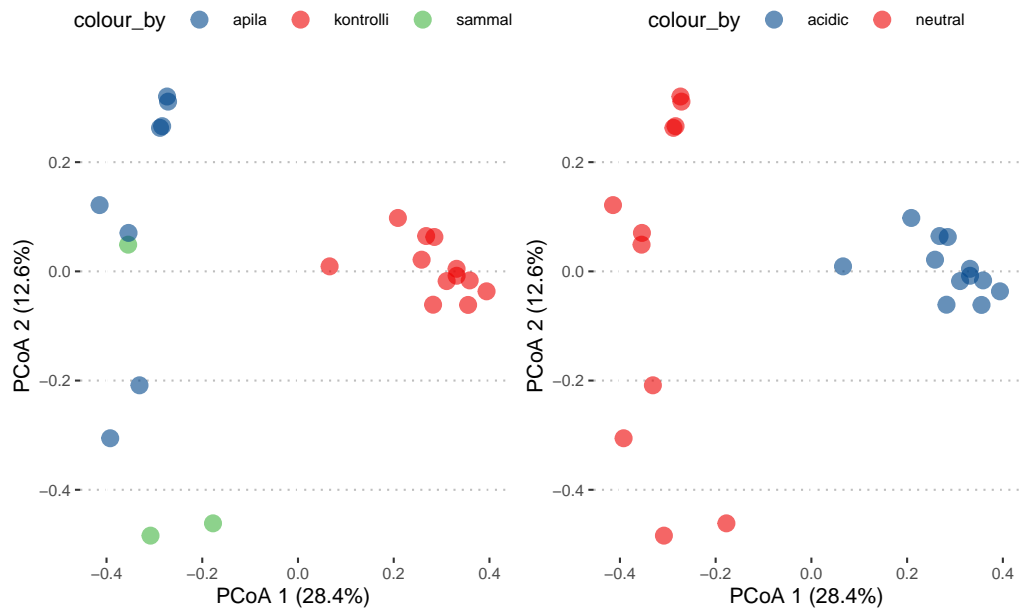
Dirichlet-Multinomial Mixture model

Cluster calculation

```
# Load the tse object
tse <- readRDS("results/tse_its1.rds")
tse <- tse[,1:23]
tse <- transformAssay(tse, method="hellinger")
# Prevalence is min. 2 samples and rank agglomerated to genus
tse <- agglomerateByPrevalence(tse, rank="Genus", prevalence=0.05, detection=1/1000)
# Add DMM clusters
altExp(tse, "prevalent") <- addCluster(
    tse,
    assay.type = "counts",
    name = "DMM",
    DmmParam(k = 1:6, type = "laplace"),
    MARGIN = "samples",
    full = TRUE,
    clust.col = "dmmclust")
```

Laplace approximation tells us optimal number of clusters in data set (2)

```
# Plot Laplace approximation results
p <- plotDMNFit(altExp(tse, "prevalent"), type = "laplace", name = "DMM")
p + theme_pubclean(base_size = 8)
```
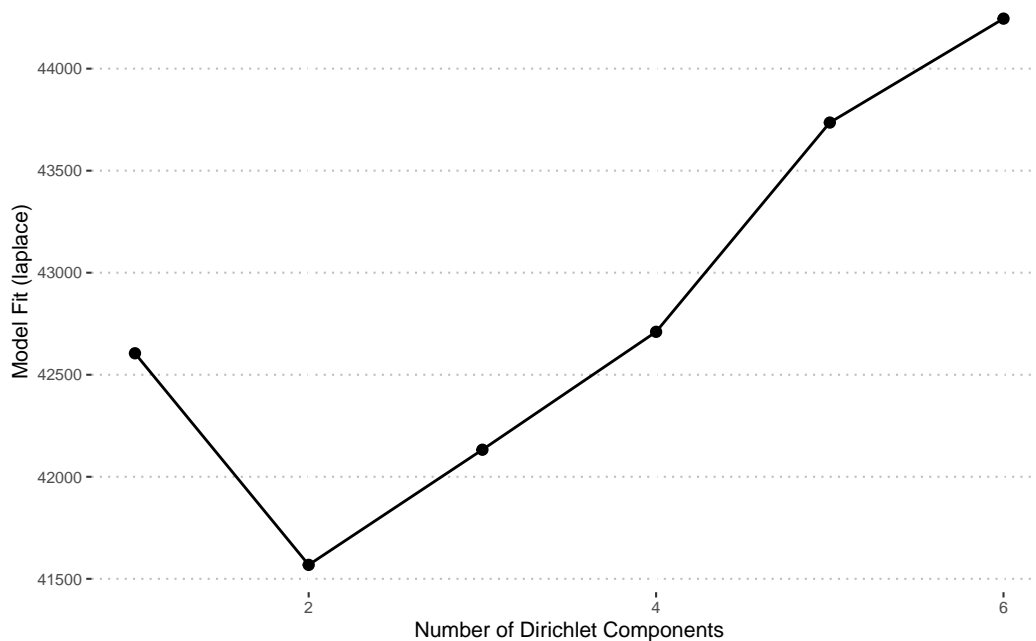
```
# Extract transformed data for PCA
data <- assay(altExp(tse, "prevalent"), "hellinger")
data <- as.matrix(data)
# Run PCA
pca_results <- prcomp(t(data))  # Transpose to have samples as rows
# Extract DMM clusters from metadata
clusters <- colData(altExp(tse, "prevalent"))$dmmclust
# Add PCA results to a data frame and include DMM clusters
pca_df <- as.data.frame(pca_results$x)
pca_df$Cluster <- as.factor(clusters)
# Plot PCA
ggplot(pca_df, aes(x = PC1, y = PC2, color = Cluster)) +
  geom_point(size = 2) +
  theme_pubclean(base_size = 8) +
  scale_color_lancet() +
  labs(title = "PCA of Samples with DMM Clusters", color = "Cluster")
```


PCA of Samples with DMM Clusters
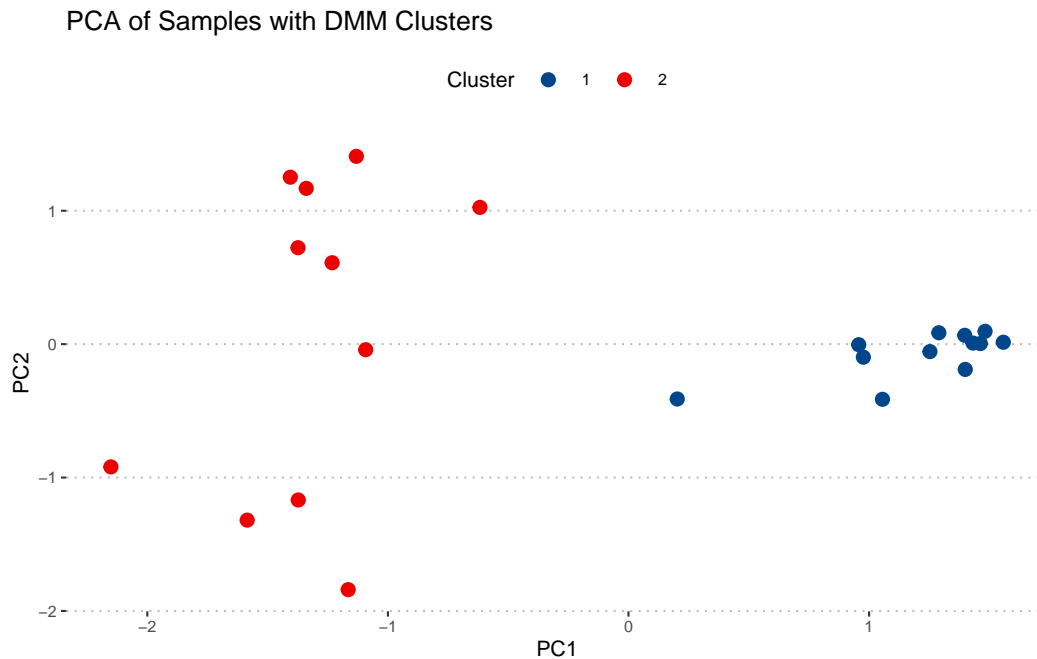
Eigen values for PCA plot

```
# Calculate eigenvalues and variance explained
eigenvalues <- pca_results$sdev^2
variance_explained <- eigenvalues / sum(eigenvalues) * 100
cumulative_variance <- data.frame(
  Principal_Component = paste0("PC", 1:length(variance_explained)),
  Variance_Explained = variance_explained,
  Cumulative_Variance = cumsum(variance_explained))
# Display eigenvalues in a table
kable(cumulative_variance[1:2,], digits = 2,
      col.names = c("Principal Component", "Variance Explained (%)",
                    "Cumulative Variance (%)")) %>%
kable_styling(latex_options = c("HOLD_position"),
font_size = 11) %>% row_spec(0, background = "teal", color = "white")
```

Principal Component	Variance Explained (%)	Cumulative Variance (%)
PC1	42.74	42.74
PC2	15.85	58.60

Silhoutte plot of clustered samples. Higher the values, tighter the cluster.

```
# Convert clusters to numeric
numeric_clusters <- as.numeric(as.character(clusters))
# Compute silhouette scores
sil <- silhouette(numeric_clusters, dist(t(data)))
# Plot silhouette
plot(sil, main = "Silhouette Plot of DMM Clusters")
```


Silhouette Plot of DMM Clusters

n = 23

2 clusters C_j

$j : n_j \mid ave_{i \in C_j} \; s_i$

1 : 12 | 0.43

2 : 11 | 0.24

Silhouette width s_i

Average silhouette width : 0.34
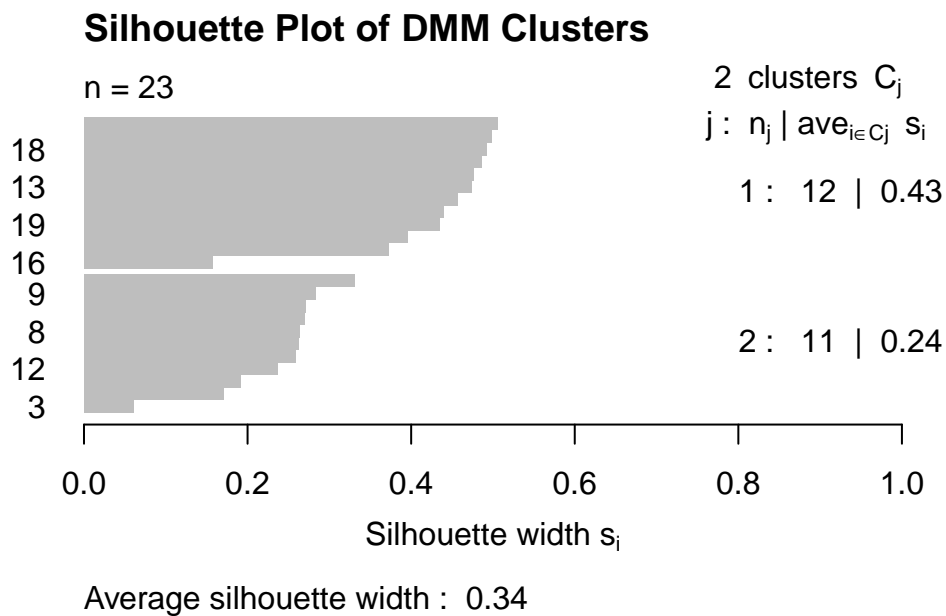
Cluster loadings - ten most important features driving samples into clusters 1 & 2

```r
# Extract best DMM model
best_model <- metadata(altExp(tse, "prevalent"))$DMM$dmm[2]
# Extract cluster loadings
drivers <- as.data.frame(best_model[[1]]@fit$Estimate)
# Plot cluster loadings
plotLoadings(as.matrix(drivers), ncomponents = 2) +
  theme_pubclean(base_size = 7) +
  scale_fill_manual(values = c("royalblue", "coral"))
```
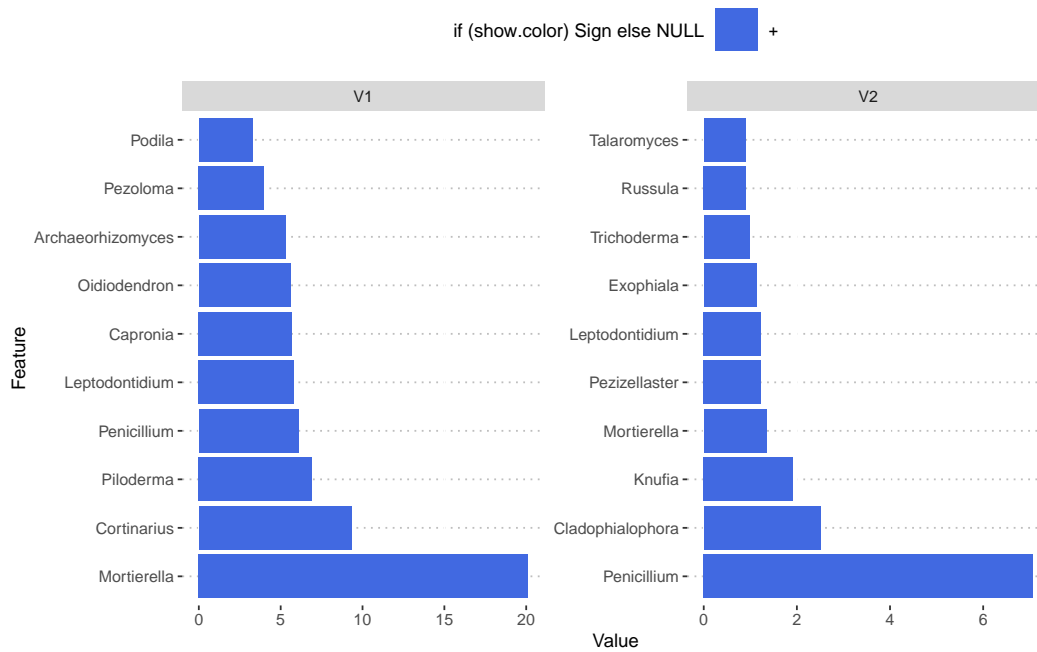
DAA analysis - pH variable

Prepare data

```
# Load tse
tse <- readRDS("results/tse_its1.rds")
tse <- tse[,1:23]
# Agglomerate by genus and subset by prevalence
tse <- subsetByPrevalent(tse, rank = "Genus", prevalence = 10/100, detection=5/1000)
# Transform assay to relative abundances
tse <- transformAssay(tse, assay.type = "counts", method = "relabundance")
# Factor pH category
tse$pH <- factor(tse$pH, levels = c("neutral", "acidic"))
```

ALDEx2 analysis

```
conds <- ifelse(tse$pH == "neutral", 1, 2)
# Generate Monte Carlo samples of the Dirichlet distribution for each sample.
x <- aldex.clr(assay(tse), conds, mc.samples = 500)
x_tt <- aldex.ttest(x, paired.test = FALSE, verbose = FALSE)
x_effect <- aldex.effect(x, CI = TRUE, verbose = FALSE)
# combine outputs
aldex_out <- data.frame(x_tt, x_effect)
```

Volcano plot

```
aldex.plot(aldex_out, type="volcano", test="welch", xlab="Difference",
    ylab="-1(log10(q))", Main ='Volcano plot')
```
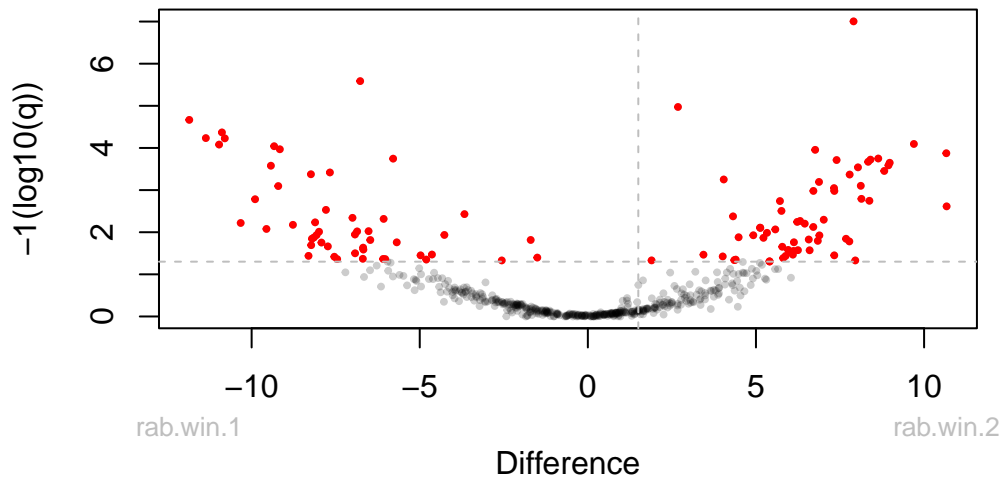
Genera with significantly different abundance

```
aldex_summary <- aldex_out %>%
  rownames_to_column(var = "Genus") %>%
  filter(wi.eBH <= 0.05)  %>%
  dplyr::select(Genus, effect, overlap, we.eBH, wi.eBH) %>%
  arrange(desc(effect))
# Summary table
kable(aldex_summary, digits=2, longtable=T, booktabs=T) %>%
    kable_styling(latex_options = c("HOLD_position", "striped", "repeat_header"),
                  font_size = 7) %>% row_spec(0, color = "white",
                                     background = "teal")
```

Genus	effect	overlap	we.eBH	wi.eBH
Entomortierella	3.28	0.00	0.00	0.00
Piloderma	3.15	0.00	0.00	0.00
Rhizidium	3.12	0.00	0.00	0.00
Podila	2.92	0.00	0.00	0.00
Chaetothyriales_gen_Incertae_sedis	2.81	0.00	0.00	0.00
Archaeorhizomyces	2.75	0.00	0.00	0.00
Meliniomyces	2.72	0.00	0.00	0.00
Mortierella	2.55	0.02	0.00	0.00
Botryobasidium	2.52	0.00	0.00	0.00
Serendipitaceae_gen_Incertae_sedis	2.47	0.00	0.00	0.00
Hyphodiscus	2.47	0.00	0.00	0.00
GS21_gen_Incertae_sedis	2.45	0.00	0.00	0.00
Humicolopsis	2.07	0.01	0.00	0.00
Cephalothecaceae_gen_Incertae_sedis	2.07	0.00	0.00	0.00
Lecanicillium	1.99	0.02	0.00	0.00
Sugiyamaella	1.87	0.02	0.00	0.00
Pezoloma	1.86	0.03	0.00	0.00
Blastocladiomycota_gen_Incertae_sedis	1.81	0.02	0.00	0.00
Sclerococcum	1.78	0.07	0.00	0.00
Hyaloscyphaceae_gen_Incertae_sedis	1.78	0.05	0.00	0.00
Cyathicula	1.71	0.00	0.00	0.00
Gloeopycnis	1.66	0.03	0.00	0.00
Acarosporales_gen_Incertae_sedis	1.66	0.06	0.01	0.00
Leucosporidium	1.65	0.03	0.00	0.00
Dermateaceae_gen_Incertae_sedis	1.63	0.06	0.01	0.00
Cortinarius	1.63	0.02	0.00	0.00
GS15_gen_Incertae_sedis	1.59	0.05	0.01	0.00
Cenococcum	1.56	0.05	0.00	0.00
Leptobacillium	1.54	0.07	0.01	0.00
Leotiomycetes_gen_Incertae_sedis	1.44	0.10	0.02	0.01
Epibryon	1.37	0.05	0.01	0.00
Pseudoplectania	1.34	0.09	0.01	0.00
Trechispora	1.33	0.06	0.00	0.00
Mycena	1.33	0.04	0.01	0.00
GS22_gen_Incertae_sedis	1.30	0.11	0.01	0.01
Syncephalis	1.29	0.06	0.01	0.00
Leccinum	1.28	0.07	0.01	0.00
Phialocephala	1.28	0.07	0.01	0.00
Occultifur	1.28	0.11	0.01	0.01
Tremella	1.19	0.13	0.02	0.01
Galerina	1.18	0.09	0.01	0.00
Luellia	1.16	0.12	0.03	0.01
GS04_gen_Incertae_sedis	1.16	0.13	0.03	0.02
Apiotrichum	1.16	0.13	0.01	0.01

Genus	effect	overlap	we.eBH	wi.eBH
Hymenochaetales_gen_Incertae_sedis	1.15	0.11	0.02	0.01
Cenangiaceae_gen_Incertae_sedis	1.14	0.13	0.03	0.01
Thaxterogaster	1.10	0.09	0.01	0.00
Pochonia	1.10	0.10	0.05	0.01
Chloridium	1.06	0.12	0.03	0.01
Flagellospora	1.05	0.14	0.04	0.03
Acarospora	1.03	0.13	0.03	0.02
Myxozyma	1.03	0.19	0.04	0.05
Clavaria	1.02	0.11	0.02	0.01
Tolypocladium	1.02	0.15	0.03	0.03
Branch01_gen_Incertae_sedis	0.99	0.16	0.04	0.04
Filobasidiales_gen_Incertae_sedis	0.99	0.13	0.05	0.02
Tympanidaceae_gen_Incertae_sedis	0.96	0.13	0.05	0.01
Scleropezicula	0.95	0.15	0.06	0.03
Cystoderma	0.95	0.13	0.04	0.02
Chytridiales_gen_Incertae_sedis	0.94	0.15	0.08	0.03
Pseudogymnoascus	0.94	0.13	0.03	0.02
Entoloma	0.92	0.13	0.13	0.01
Pezicula	0.92	0.13	0.09	0.01
Sorocybe	0.92	0.15	0.04	0.02
Umbelopsis	0.91	0.12	0.05	0.01
Fayodia	0.91	0.15	0.09	0.04
Sympoventuriaceae_gen_Incertae_sedis	0.90	0.14	0.07	0.03
Genolevuria	0.90	0.18	0.08	0.05
Pseudohyphozyma	0.90	0.15	0.09	0.03
Syzygospora	0.83	0.18	0.05	0.04
Sordariales_gen_Incertae_sedis	-0.90	0.14	0.04	0.03
Cyphellophoraceae_gen_Incertae_sedis	-1.01	0.13	0.04	0.04
Mortierellales_gen_Incertae_sedis	-1.03	0.13	0.05	0.04
Mallocybe	-1.04	0.14	0.04	0.03
Sebacinales_gen_Incertae_sedis	-1.06	0.11	0.03	0.02
Hamatocanthoscypha	-1.08	0.12	0.04	0.03
Schizothecium	-1.09	0.14	0.04	0.04
Fungi_gen_Incertae_sedis	-1.09	0.14	0.02	0.02
Entimomentora	-1.10	0.15	0.04	0.05
Nothodactylaria	-1.10	0.14	0.05	0.04
Laetinaevia	-1.10	0.06	0.02	0.00
Vishniacozyma	-1.10	0.10	0.04	0.01
Rhizopogon	-1.12	0.13	0.01	0.02
Mycosphaerellales_gen_Incertae_sedis	-1.13	0.11	0.03	0.03
Inocybe	-1.15	0.10	0.01	0.01
Verrucoccum	-1.15	0.14	0.04	0.05
Microdochium	-1.17	0.11	0.02	0.02
Cryptococcus	-1.18	0.13	0.06	0.02
Oliveonia	-1.20	0.09	0.01	0.01
Penicillium	-1.20	0.14	0.05	0.03
Triscelophorus	-1.23	0.11	0.02	0.02
Kurtzmanomyces	-1.24	0.11	0.02	0.02
Neonectria	-1.25	0.12	0.02	0.02
Geopora	-1.26	0.06	0.00	0.00
Ceratellopsis	-1.29	0.08	0.02	0.01
Herpotrichia	-1.31	0.07	0.01	0.01
Preussia	-1.31	0.06	0.00	0.00
Cladophialophora	-1.32	0.06	0.00	0.00
Bionectriaceae_gen_Incertae_sedis	-1.35	0.09	0.01	0.01

Genus	effect	overlap	we.eBH	wi.eBH
Leptodophora	-1.35	0.08	0.01	0.01
Pulvinula	-1.36	0.10	0.01	0.01
Psoroglaena	-1.38	0.09	0.01	0.01
Cosmospora	-1.39	0.08	0.01	0.01
Phomatospora	-1.41	0.08	0.01	0.01
Helvella	-1.44	0.07	0.01	0.00
Plectosphaerella	-1.48	0.09	0.01	0.01
Pseudocoleophoma	-1.66	0.03	0.00	0.00
Lobulomycetales_gen_Incertae_sedis	-1.79	0.05	0.00	0.00
Pleotrichocladium	-1.84	0.06	0.00	0.00
Nectriella	-1.86	0.02	0.00	0.00
Talaromyces	-1.91	0.04	0.00	0.00
Cladosporium	-1.92	0.02	0.00	0.00
Sclerostagonospora	-2.04	0.03	0.00	0.00
Tetracladium	-2.06	0.01	0.00	0.00
Kodamaea	-2.22	0.00	0.00	0.00
Truncatella	-2.39	0.01	0.00	0.00
Fusarium	-2.55	0.00	0.00	0.00
Paraphoma	-2.88	0.00	0.00	0.00
Exophiala	-2.96	0.00	0.00	0.00
Pezizellaster	-3.43	0.00	0.00	0.00
Knufia	-3.59	0.00	0.00	0.00

Effect positive, genus more abundant in acidic pH (cond2, effect group). Effect negative, genus more abundant in neutral pH (cond1, control). Scale log2.

Ancombc2 analysis

```
# Run ANCOM-BC at the genus level and only including the prevalent genera
ancombc2_out <- ancombc2(
    data = tse,
    assay.type = "relabundance",
    fix_formula = "pH",
    p_adj_method = "fdr",
    prv_cut = 0,
    group = "pH",
    struc_zero = TRUE,
    neg_lb = TRUE,
    global = FALSE,
    verbose = FALSE
    )
```

Genera with significantly different abundance

```
ancom_summary <- ancombc2_out$res %>%
  dplyr::select(Genus = taxon, lfc = lfc_pHacidic, se = se_pHacidic, q = q_pHacidic, diff = diff_pHacidic) %>%
  filter(diff == TRUE) %>% arrange(desc(lfc))
# Summary table
kable(ancom_summary, digits=2, longtable=T, booktabs=T) %>%
    kable_styling(latex_options = c("HOLD_position", "striped", "repeat_header"),
                  font_size = 7) %>% row_spec(0, color = "white",
                                              background = "teal")
```

Genus	lfc	se	q	diff
Archaeorhizomyces	7.42	0.61	0.00	TRUE
Meliniomyces	6.54	0.55	0.00	TRUE
Sclerococcum	6.48	0.61	0.00	TRUE
Rhizidium	6.45	0.48	0.00	TRUE
Chaetothyriales_gen_Incertae_sedis	6.32	0.53	0.00	TRUE
Piloderma	6.21	0.52	0.00	TRUE
Serendipitaceae_gen_Incertae_sedis	5.89	0.55	0.00	TRUE
Entomortierella	5.82	0.36	0.00	TRUE
Podila	5.77	0.52	0.00	TRUE
Leotiomycetes_gen_Incertae_sedis	5.66	0.68	0.00	TRUE
Cephalothecaceae_gen_Incertae_sedis	5.60	0.63	0.00	TRUE
Pezoloma	5.44	0.76	0.00	TRUE
Humicolopsis	4.86	0.53	0.00	TRUE
Pseudoplectania	4.76	0.60	0.00	TRUE
Clavaria	4.45	0.80	0.00	TRUE
Cenococcum	4.38	0.72	0.00	TRUE
Herpotrichiellaceae_gen_Incertae_sedis	4.36	1.03	0.00	TRUE
GS12_gen_Incertae_sedis	4.30	0.74	0.00	TRUE
Leucosporidium	4.29	0.64	0.00	TRUE
Cyathicula	4.22	0.52	0.00	TRUE
Nadsonia	4.13	0.65	0.00	TRUE
Tympanidaceae_gen_Incertae_sedis	4.10	0.72	0.00	TRUE
Glutinomyces	3.98	0.71	0.00	TRUE
Myxozyma	3.91	1.00	0.00	TRUE
Blastocladiomycota_gen_Incertae_sedis	3.86	0.53	0.00	TRUE
Sugiyamaella	3.84	0.57	0.00	TRUE
Tolypocladium	3.83	0.81	0.00	TRUE
Ramariopsis	3.80	0.82	0.00	TRUE
Phialocephala	3.77	0.64	0.00	TRUE
Apiotrichum	3.73	0.81	0.00	TRUE
Cortinarius	3.71	0.57	0.00	TRUE
Tylospora	3.70	0.92	0.00	TRUE
Chytridiales_gen_Incertae_sedis	3.60	0.53	0.00	TRUE
Hyaloscyphaceae_gen_Incertae_sedis	3.52	0.59	0.00	TRUE
Trechispora	3.48	0.59	0.00	TRUE
Hymenochaetales_gen_Incertae_sedis	3.34	0.69	0.00	TRUE
Mycena	3.34	0.53	0.00	TRUE
Helotiales_gen_Incertae_sedis	3.12	0.91	0.01	TRUE
Syncephalis	3.12	0.50	0.00	TRUE
Suillus	3.01	0.88	0.01	TRUE
Pyronemataceae_gen_Incertae_sedis	2.92	0.94	0.02	TRUE
Babjevia	2.86	0.83	0.01	TRUE
Mycosymbioces	2.76	0.88	0.01	TRUE
Umbelopsis	2.73	0.65	0.00	TRUE
Genolevuria	2.65	0.52	0.00	TRUE

Genus	lfc	se	q	diff
Occultifur	2.62	0.68	0.01	TRUE
Lachnellula	2.60	0.71	0.00	TRUE
Mortierella	2.58	0.39	0.00	TRUE
Capronia	2.29	0.93	0.05	TRUE
Galerina	2.28	0.48	0.00	TRUE
Thaxterogaster	2.27	0.76	0.02	TRUE
Sistotrema	2.21	0.88	0.05	TRUE
Claussenomyces	2.19	0.64	0.01	TRUE
Oidiodendron	2.16	0.66	0.01	TRUE
Pseudogymnoascus	2.02	0.49	0.00	TRUE
Saccharomycetales_gen_Incertae_sedis	1.97	0.72	0.03	TRUE
Entoloma	1.97	0.73	0.03	TRUE
Glomeromycota_gen_Incertae_sedis	1.96	0.57	0.01	TRUE
Tyrannosorus	1.95	0.69	0.03	TRUE
Chloridium	1.92	0.49	0.01	TRUE
Epibryon	1.92	0.41	0.00	TRUE
Syzygospora	1.73	0.60	0.03	TRUE
Derxomyces	1.68	0.44	0.02	TRUE
Lachnum	1.65	0.53	0.02	TRUE
GS05_gen_Incertae_sedis	1.48	0.47	0.04	TRUE
Mollisia	1.43	0.55	0.04	TRUE
Venturia	1.41	0.56	0.04	TRUE
Melanommataceae_gen_Incertae_sedis	1.15	0.36	0.04	TRUE
Cystobasidiomycetes_gen_Incertae_sedis	-1.37	0.49	0.04	TRUE
Camptobasidiaceae_gen_Incertae_sedis	-1.38	0.39	0.04	TRUE
Podospora	-1.38	0.50	0.05	TRUE
Drechmeria	-1.55	0.50	0.03	TRUE
Kurtzmaniella	-1.70	0.57	0.03	TRUE
Cladophialophora	-1.80	0.57	0.01	TRUE
Pseudeurotium	-1.89	0.77	0.05	TRUE
Herpotrichia	-1.92	0.67	0.03	TRUE
Sporormiella	-1.96	0.64	0.02	TRUE
Rhizopogon	-2.04	0.76	0.03	TRUE
Laetinaevia	-2.15	0.68	0.02	TRUE
Sakaguchia	-2.20	0.35	0.00	TRUE
Ceratobasidium	-2.21	0.52	0.00	TRUE
Mrakia	-2.24	0.43	0.00	TRUE
Hormonema	-2.27	0.40	0.00	TRUE
Thelephora	-2.38	0.91	0.04	TRUE
Sordariales_gen_Incertae_sedis	-2.48	0.99	0.04	TRUE
Phaeothecaceae_gen_Incertae_sedis	-2.50	0.41	0.01	TRUE
Vishniacozyma	-2.62	0.64	0.00	TRUE
Taphrina	-2.69	0.47	0.01	TRUE
Sebacinales_gen_Incertae_sedis	-2.72	0.93	0.03	TRUE
Preussia	-2.79	0.80	0.01	TRUE
Ochroconis	-3.10	0.67	0.00	TRUE
Inocybe	-3.32	1.02	0.01	TRUE
Calycina	-3.42	0.52	0.00	TRUE
Sclerostagonospora	-3.45	0.63	0.00	TRUE
Cladosporium	-3.46	0.69	0.00	TRUE
Neonectria	-3.58	0.53	0.00	TRUE
Cryptococcus	-3.72	0.83	0.00	TRUE
Geopora	-3.88	1.03	0.00	TRUE
Exophiala	-3.93	0.56	0.00	TRUE
Lecythophora	-3.96	0.67	0.00	TRUE

(continued)

Genus	lfc	se	q	diff
Talaromyces	-4.14	0.73	0.00	TRUE
Ceratobasidiaceae_gen_Incertae_sedis	-4.36	0.45	0.00	TRUE
Fusarium	-4.61	0.59	0.00	TRUE
Tetracladium	-4.63	0.82	0.00	TRUE
Mallocybe	-4.83	0.87	0.00	TRUE
Kodamaea	-4.91	0.68	0.00	TRUE
Pezizellaster	-5.96	0.47	0.00	TRUE
Knufia	-6.55	0.52	0.00	TRUE

In ancombc analysis control is neutral, effect is acidic. Log fold change is log2 scale.

Maaslin2 analysis

```r
# specifying a ref is especially important if you have more than 2 levels
maaslin2_out <- Maaslin2(
    input_data = as.data.frame(t(assay(tse))),
    input_metadata = as.data.frame(colData(tse)),
    output = "ITS1",
    transform = "AST",
    fixed_effects = "pH",
    reference = "pH,neutral",
    normalization = "TSS",
    standardize = FALSE,
    min_prevalence = 0)
saveRDS(maaslin2_out, "results/its1_maaslin2.rds")
```

Genera with significantly different abundance

```r
maaslin2_out <- readRDS("results/its1_maaslin2.rds")
maaslin_summary <- maaslin2_out$results %>% dplyr::select(Genus = feature, coef, stderr, qval, N.not.zero) %>%
  filter(qval < 0.05) %>% arrange(desc(coef))
# Summary table
kable(maaslin_summary, digits=2, longtable=T) %>%
    kable_styling(latex_options = c("HOLD_position", "striped", "repeat_header"),
                font_size = 7) %>% row_spec(0, color = "white",
                                    background = "teal")
```

Genus	coef	stderr	qval	N.not.zero
Mortierella	0.25	0.03	0.00	23
Piloderma	0.21	0.04	0.00	23
Cortinarius	0.20	0.03	0.00	23
Archaeorhizomyces	0.19	0.03	0.00	21
Pezoloma	0.17	0.03	0.00	23
Sclerococcum	0.16	0.03	0.00	16
Podila	0.13	0.02	0.00	22
Meliniomyces	0.13	0.02	0.00	21
Leotiomycetes_gen_Incertae_sedis	0.09	0.02	0.00	20
Tylospora	0.09	0.03	0.04	23
Oidiodendron	0.09	0.03	0.03	23
Rhizidium	0.08	0.01	0.00	20
Cephalothecaceae_gen_Incertae_sedis	0.07	0.02	0.00	18
Chaetothyriales_gen_Incertae_sedis	0.07	0.01	0.00	17
Umbelopsis	0.07	0.01	0.00	23
Herpotrichiellaceae_gen_Incertae_sedis	0.06	0.02	0.04	20
Serendipitaceae_gen_Incertae_sedis	0.06	0.01	0.00	19

Genus	coef	stderr	qval	N.not.zero
Phialocephala	0.06	0.01	0.00	19
Leucosporidium	0.05	0.01	0.00	22
Apiotrichum	0.05	0.01	0.01	20
Lachnellula	0.05	0.01	0.02	23
Cenococcum	0.04	0.01	0.00	17
Entomortierella	0.04	0.00	0.00	17
Pseudoplectania	0.04	0.01	0.04	17
Mycena	0.04	0.01	0.00	19
Humicolopsis	0.04	0.01	0.00	17
Glutinomyces	0.03	0.01	0.04	17
Sugiyamaella	0.03	0.01	0.00	15
Babjevia	0.03	0.01	0.02	23
Hyphodiscus	0.03	0.01	0.00	14
Syncephalis	0.03	0.00	0.00	19
Syzygospora	0.03	0.01	0.02	18
Diademospora	0.03	0.01	0.02	11
Tolypocladium	0.03	0.01	0.02	17
Branch01_gen_Incertae_sedis	0.03	0.01	0.04	12
Hyaloscyphaceae_gen_Incertae_sedis	0.03	0.01	0.00	15
GS21_gen_Incertae_sedis	0.03	0.00	0.00	14
Blastocladiomycota_gen_Incertae_sedis	0.03	0.01	0.00	15
Hymenochaetales_gen_Incertae_sedis	0.02	0.01	0.01	14
Trechispora	0.02	0.01	0.01	21
Cyathicula	0.02	0.00	0.00	15
Lecanicillium	0.02	0.00	0.00	13
Chytridiales_gen_Incertae_sedis	0.02	0.00	0.01	16
Botryobasidium	0.02	0.00	0.00	14
GS15_gen_Incertae_sedis	0.02	0.00	0.00	11
Luellia	0.02	0.00	0.02	11
Chaetosphaeria	0.02	0.01	0.03	11
GS22_gen_Incertae_sedis	0.01	0.00	0.01	10
Leptobacillium	0.01	0.00	0.00	14
Galerina	0.01	0.00	0.00	17
Cenangiaceae_gen_Incertae_sedis	0.01	0.00	0.01	11
Flagellospora	0.01	0.00	0.01	10
Acarosporales_gen_Incertae_sedis	0.01	0.00	0.00	12
Genolevuria	0.01	0.00	0.02	13
Dermateaceae_gen_Incertae_sedis	0.01	0.00	0.00	11
Gloeopycnis	0.01	0.00	0.00	14
GS04_gen_Incertae_sedis	0.01	0.00	0.00	11
Epibryon	0.01	0.00	0.00	15
Synnemapestaloides	0.01	0.00	0.04	11
Tremella	0.01	0.00	0.01	14
Siepmannia	0.01	0.00	0.03	8
Crocicreas	0.01	0.00	0.04	12
Scleropezicula	0.01	0.00	0.01	11
Pseudogymnoascus	0.01	0.00	0.02	18
Leccinum	0.01	0.00	0.00	12
Babjeviella	0.01	0.00	0.01	12
Rhodotorula	0.01	0.00	0.03	10
Aspicilia	0.01	0.00	0.02	9
Endogonomycetes_gen_Incertae_sedis	0.01	0.00	0.03	7
Cystoderma	0.01	0.00	0.01	12
Sorocybe	0.01	0.00	0.01	15
Fayodia	0.01	0.00	0.01	9

Genus	coef	stderr	qval	N.not.zero
Sympoventuriaceae_gen_Incertae_sedis	0.01	0.00	0.02	10
Tremellomycetes_gen_Incertae_sedis	0.01	0.00	0.04	8
Mortierellomycetes_gen_Incertae_sedis	0.01	0.00	0.04	8
Pochonia	0.01	0.00	0.00	10
Haplographium	0.01	0.00	0.04	8
Flammula	0.01	0.00	0.01	16
Micarea	0.01	0.00	0.04	7
Dothiorella	0.01	0.00	0.03	14
Pseudohyphozyma	0.01	0.00	0.01	10
Microsporomycetaceae_gen_Incertae_sedis	0.01	0.00	0.02	10
Dermea	0.01	0.00	0.02	8
Ophiocordycipitaceae_gen_Incertae_sedis	0.01	0.00	0.03	8
Rhizosphaera	0.00	0.00	0.01	10
Cladoriellaceae_gen_Incertae_sedis	0.00	0.00	0.01	9
Endogonales_gen_Incertae_sedis	0.00	0.00	0.04	8
Hirsutella	0.00	0.00	0.03	8
Phaeotremella	0.00	0.00	0.04	11
Gymnopilus	0.00	0.00	0.01	8
Micropeltidaceae_gen_Incertae_sedis	0.00	0.00	0.04	8
Krasilnikovozyma	0.00	0.00	0.02	8
Vestigium	0.00	0.00	0.01	7
Dothiora	0.00	0.00	0.01	10
Dictyolus	0.00	0.00	0.03	7
Yamadamyces	0.00	0.00	0.03	9
Monoblepharomycota_gen_Incertae_sedis	0.00	0.00	0.04	6
Aphanocladium	0.00	0.00	0.04	8
Rhizophagus	-0.01	0.00	0.04	7
Achroceratosphaeria	-0.01	0.00	0.04	5
Lentitheciaceae_gen_Incertae_sedis	-0.01	0.00	0.05	7
Nothodactylaria	-0.01	0.00	0.01	9
Kurtzmaniella	-0.01	0.00	0.04	15
Sporormiaceae_gen_Incertae_sedis	-0.01	0.00	0.05	6
Triscelophorus	-0.01	0.00	0.00	9
Schizothecium	-0.01	0.00	0.02	10
Ceratellopsis	-0.01	0.00	0.01	10
Septoria	-0.01	0.00	0.05	9
Verrucoccum	-0.01	0.00	0.01	8
Lobulomycetales_gen_Incertae_sedis	-0.01	0.00	0.00	11
Herpotrichia	-0.01	0.00	0.03	13
Hamatocanthoscypha	-0.02	0.01	0.04	9
Mycosphaerellales_gen_Incertae_sedis	-0.02	0.01	0.03	9
Cosmospora	-0.02	0.00	0.00	12
Phomatospora	-0.02	0.01	0.02	10
Oliveonia	-0.02	0.01	0.03	12
Entimomentora	-0.02	0.01	0.03	10
Ceratobasidium	-0.02	0.01	0.02	18
Kurtzmanomyces	-0.02	0.01	0.04	10
Pulvinula	-0.02	0.01	0.01	11
Bionectriaceae_gen_Incertae_sedis	-0.02	0.01	0.02	11
Truncatella	-0.03	0.01	0.00	12
Pseudocoleophoma	-0.03	0.01	0.00	12
Helvella	-0.03	0.01	0.02	12
Sclerostagonospora	-0.03	0.01	0.01	16
Neonectria	-0.03	0.01	0.01	14
Pleotrichocladium	-0.03	0.01	0.00	12

Genus	coef	stderr	qval	N.not.zero
Plectosphaerella	-0.04	0.01	0.02	11
Lecythophora	-0.04	0.01	0.03	14
Preussia	-0.04	0.01	0.03	21
Paraphoma	-0.04	0.01	0.00	12
Thelephora	-0.04	0.02	0.04	23
Leptodophora	-0.05	0.01	0.00	12
Rhizopogon	-0.06	0.02	0.02	23
Fusarium	-0.07	0.02	0.01	15
Kodamaea	-0.08	0.02	0.01	19
Tetracladium	-0.08	0.02	0.01	15
Cladosporium	-0.08	0.03	0.04	23
Talaromyces	-0.09	0.02	0.00	21
Cryptococcus	-0.10	0.04	0.04	22
Exophiala	-0.10	0.02	0.00	23
Pezizellaster	-0.11	0.01	0.00	18
Cladophialophora	-0.15	0.04	0.01	23
Knufia	-0.16	0.02	0.00	19
Penicillium	-0.17	0.04	0.01	23

In maaslin2 analysis, control is neutral, effect is acidic. Changes are calculated with formula $\log(q)*\text{sign}(\text{coeff})$

DAA analysis summary table

```
summary <- aldex_summary %>% dplyr::select(Genus)
summary$Aldex2 <- TRUE
summary <- dplyr::full_join(summary, ancom_summary, by="Genus")
summary <- summary %>% dplyr::select(Genus, Aldex2, Ancombc = diff)
summary <- dplyr::full_join(summary, maaslin_summary, by="Genus")
summary <- summary %>% mutate(Maaslin2=ifelse(is.na(summary$coef) , FALSE, TRUE))
summary <- summary %>% dplyr::select(Genus, Aldex2, Ancombc, Maaslin2)
summary[is.na(summary)] <- FALSE
```

Comparison of DAA analysis results

```
# Summary table
kable(summary, digits=2, longtable=T, booktabs=T) %>%
    kable_styling(latex_options = c("HOLD_position", "striped", "repeat_header"),
                  font_size = 7) %>% row_spec(0, color = "white",
                                              background = "teal")
```

Genus	Aldex2	Ancombc	Maaslin2
Entomortierella	TRUE	TRUE	TRUE
Piloderma	TRUE	TRUE	TRUE
Rhizidium	TRUE	TRUE	TRUE
Podila	TRUE	TRUE	TRUE
Chaetothyriales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Archaeorhizomyces	TRUE	TRUE	TRUE
Meliniomyces	TRUE	TRUE	TRUE
Mortierella	TRUE	TRUE	TRUE
Botryobasidium	TRUE	FALSE	TRUE
Serendipitaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Hyphodiscus	TRUE	FALSE	TRUE

Genus	Aldex2	Ancombc	Maaslin2
GS21_gen_Incertae_sedis	TRUE	FALSE	TRUE
Humicolopsis	TRUE	TRUE	TRUE
Cephalothecaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Lecanicillium	TRUE	FALSE	TRUE
Sugiyamaella	TRUE	TRUE	TRUE
Pezoloma	TRUE	TRUE	TRUE
Blastocladiomycota_gen_Incertae_sedis	TRUE	TRUE	TRUE
Sclerococcum	TRUE	TRUE	TRUE
Hyaloscyphaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Cyathicula	TRUE	TRUE	TRUE
Gloeopycnis	TRUE	FALSE	TRUE
Acarosporales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Leucosporidium	TRUE	TRUE	TRUE
Dermateaceae_gen_Incertae_sedis	TRUE	FALSE	TRUE
Cortinarius	TRUE	TRUE	TRUE
GS15_gen_Incertae_sedis	TRUE	FALSE	TRUE
Cenococcum	TRUE	TRUE	TRUE
Leptobacillium	TRUE	FALSE	TRUE
Leotiomycetes_gen_Incertae_sedis	TRUE	TRUE	TRUE
Epibryon	TRUE	TRUE	TRUE
Pseudoplectania	TRUE	TRUE	TRUE
Trechispora	TRUE	TRUE	TRUE
Mycena	TRUE	TRUE	TRUE
GS22_gen_Incertae_sedis	TRUE	FALSE	TRUE
Syncephalis	TRUE	TRUE	TRUE
Leccinum	TRUE	FALSE	TRUE
Phialocephala	TRUE	TRUE	TRUE
Occultifur	TRUE	TRUE	FALSE
Tremella	TRUE	FALSE	TRUE
Galerina	TRUE	TRUE	TRUE
Luellia	TRUE	FALSE	TRUE
GS04_gen_Incertae_sedis	TRUE	FALSE	TRUE
Apiotrichum	TRUE	TRUE	TRUE
Hymenochaetales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Cenangiaceae_gen_Incertae_sedis	TRUE	FALSE	TRUE
Thaxterogaster	TRUE	TRUE	FALSE
Pochonia	TRUE	FALSE	TRUE
Chloridium	TRUE	TRUE	FALSE
Flagellospora	TRUE	FALSE	TRUE
Acarospora	TRUE	FALSE	FALSE
Myxozyma	TRUE	TRUE	FALSE
Clavaria	TRUE	TRUE	FALSE
Tolypocladium	TRUE	TRUE	TRUE
Branch01_gen_Incertae_sedis	TRUE	FALSE	TRUE
Filobasidiales_gen_Incertae_sedis	TRUE	FALSE	FALSE
Tympanidaceae_gen_Incertae_sedis	TRUE	TRUE	FALSE
Scleropezicula	TRUE	FALSE	TRUE
Cystoderma	TRUE	FALSE	TRUE
Chytridiales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Pseudogymnoascus	TRUE	TRUE	TRUE
Entoloma	TRUE	TRUE	FALSE
Pezicula	TRUE	FALSE	FALSE
Sorocybe	TRUE	FALSE	TRUE
Umbelopsis	TRUE	TRUE	TRUE
Fayodia	TRUE	FALSE	TRUE

Genus	Aldex2	Ancombc	Maaslin2
Sympoventuriaceae_gen_Incertae_sedis	TRUE	FALSE	TRUE
Genolevuria	TRUE	TRUE	TRUE
Pseudohyphozyma	TRUE	FALSE	TRUE
Syzygospora	TRUE	TRUE	TRUE
Sordariales_gen_Incertae_sedis	TRUE	TRUE	FALSE
Cyphellophoraceae_gen_Incertae_sedis	TRUE	FALSE	FALSE
Mortierellales_gen_Incertae_sedis	TRUE	FALSE	FALSE
Mallocybe	TRUE	TRUE	FALSE
Sebacinales_gen_Incertae_sedis	TRUE	TRUE	FALSE
Hamatocanthoscypha	TRUE	FALSE	TRUE
Schizothecium	TRUE	FALSE	TRUE
Fungi_gen_Incertae_sedis	TRUE	FALSE	FALSE
Entimomentora	TRUE	FALSE	TRUE
Nothodactylaria	TRUE	FALSE	TRUE
Laetinaevia	TRUE	TRUE	FALSE
Vishniacozyma	TRUE	TRUE	FALSE
Rhizopogon	TRUE	TRUE	TRUE
Mycosphaerellales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Inocybe	TRUE	TRUE	FALSE
Verrucoccum	TRUE	FALSE	TRUE
Microdochium	TRUE	FALSE	FALSE
Cryptococcus	TRUE	TRUE	TRUE
Oliveonia	TRUE	FALSE	TRUE
Penicillium	TRUE	FALSE	TRUE
Triscelophorus	TRUE	FALSE	TRUE
Kurtzmanomyces	TRUE	FALSE	TRUE
Neonectria	TRUE	TRUE	TRUE
Geopora	TRUE	TRUE	FALSE
Ceratellopsis	TRUE	FALSE	TRUE
Herpotrichia	TRUE	TRUE	TRUE
Preussia	TRUE	TRUE	TRUE
Cladophialophora	TRUE	TRUE	TRUE
Bionectriaceae_gen_Incertae_sedis	TRUE	FALSE	TRUE
Leptodophora	TRUE	FALSE	TRUE
Pulvinula	TRUE	FALSE	TRUE
Psoroglaena	TRUE	FALSE	FALSE
Cosmospora	TRUE	FALSE	TRUE
Phomatospora	TRUE	FALSE	TRUE
Helvella	TRUE	FALSE	TRUE
Plectosphaerella	TRUE	FALSE	TRUE
Pseudocoleophoma	TRUE	FALSE	TRUE
Lobulomycetales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Pleotrichocladium	TRUE	FALSE	TRUE
Nectriella	TRUE	FALSE	FALSE
Talaromyces	TRUE	TRUE	TRUE
Cladosporium	TRUE	TRUE	TRUE
Sclerostagonospora	TRUE	TRUE	TRUE
Tetracladium	TRUE	TRUE	TRUE
Kodamaea	TRUE	TRUE	TRUE
Truncatella	TRUE	FALSE	TRUE
Fusarium	TRUE	TRUE	TRUE
Paraphoma	TRUE	FALSE	TRUE
Exophiala	TRUE	TRUE	TRUE
Pezizellaster	TRUE	TRUE	TRUE
Knufia	TRUE	TRUE	TRUE

Genus	Aldex2	Ancombc	Maaslin2
Herpotrichiellaceae_gen_Incertae_sedis	FALSE	TRUE	TRUE
GS12_gen_Incertae_sedis	FALSE	TRUE	FALSE
Nadsonia	FALSE	TRUE	FALSE
Glutinomyces	FALSE	TRUE	TRUE
Ramariopsis	FALSE	TRUE	FALSE
Tylospora	FALSE	TRUE	TRUE
Helotiales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Suillus	FALSE	TRUE	FALSE
Pyronemataceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Babjevia	FALSE	TRUE	TRUE
Mycosymbioces	FALSE	TRUE	FALSE
Lachnellula	FALSE	TRUE	TRUE
Capronia	FALSE	TRUE	FALSE
Sistotrema	FALSE	TRUE	FALSE
Claussenomyces	FALSE	TRUE	FALSE
Oidiodendron	FALSE	TRUE	TRUE
Saccharomycetales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Glomeromycota_gen_Incertae_sedis	FALSE	TRUE	FALSE
Tyrannosorus	FALSE	TRUE	FALSE
Derxomyces	FALSE	TRUE	FALSE
Lachnum	FALSE	TRUE	FALSE
GS05_gen_Incertae_sedis	FALSE	TRUE	FALSE
Mollisia	FALSE	TRUE	FALSE
Venturia	FALSE	TRUE	FALSE
Melanommataceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Cystobasidiomycetes_gen_Incertae_sedis	FALSE	TRUE	FALSE
Camptobasidiaceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Podospora	FALSE	TRUE	FALSE
Drechmeria	FALSE	TRUE	FALSE
Kurtzmaniella	FALSE	TRUE	TRUE
Pseudeurotium	FALSE	TRUE	FALSE
Sporormiella	FALSE	TRUE	FALSE
Sakaguchia	FALSE	TRUE	FALSE
Ceratobasidium	FALSE	TRUE	TRUE
Mrakia	FALSE	TRUE	FALSE
Hormonema	FALSE	TRUE	FALSE
Thelephora	FALSE	TRUE	TRUE
Phaeothecaceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Taphrina	FALSE	TRUE	FALSE
Ochroconis	FALSE	TRUE	FALSE
Calycina	FALSE	TRUE	FALSE
Lecythophora	FALSE	TRUE	TRUE
Ceratobasidiaceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Diademospora	FALSE	FALSE	TRUE
Chaetosphaeria	FALSE	FALSE	TRUE
Synnemapestaloides	FALSE	FALSE	TRUE
Siepmannia	FALSE	FALSE	TRUE
Crocicreas	FALSE	FALSE	TRUE
Babjeviella	FALSE	FALSE	TRUE
Rhodotorula	FALSE	FALSE	TRUE
Aspicilia	FALSE	FALSE	TRUE
Endogonomycetes_gen_Incertae_sedis	FALSE	FALSE	TRUE
Tremellomycetes_gen_Incertae_sedis	FALSE	FALSE	TRUE
Mortierellomycetes_gen_Incertae_sedis	FALSE	FALSE	TRUE
Haplographium	FALSE	FALSE	TRUE

Genus	Aldex2	Ancombc	Maaslin2
Flammula	FALSE	FALSE	TRUE
Micarea	FALSE	FALSE	TRUE
Dothiorella	FALSE	FALSE	TRUE
Microsporomycetaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Dermea	FALSE	FALSE	TRUE
Ophiocordycipitaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Rhizosphaera	FALSE	FALSE	TRUE
Cladoriellaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Endogonales_gen_Incertae_sedis	FALSE	FALSE	TRUE
Hirsutella	FALSE	FALSE	TRUE
Phaeotremella	FALSE	FALSE	TRUE
Gymnopilus	FALSE	FALSE	TRUE
Micropeltidaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Krasilnikovozyma	FALSE	FALSE	TRUE
Vestigium	FALSE	FALSE	TRUE
Dothiora	FALSE	FALSE	TRUE
Dictyolus	FALSE	FALSE	TRUE
Yamadamyces	FALSE	FALSE	TRUE
Monoblepharomycota_gen_Incertae_sedis	FALSE	FALSE	TRUE
Aphanocladium	FALSE	FALSE	TRUE
Rhizophagus	FALSE	FALSE	TRUE
Achroceratosphaeria	FALSE	FALSE	TRUE
Lentitheciaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Sporormiaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Septoria	FALSE	FALSE	TRUE