Data-analyysi Raajärvi näytteet ITS2

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_its2.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
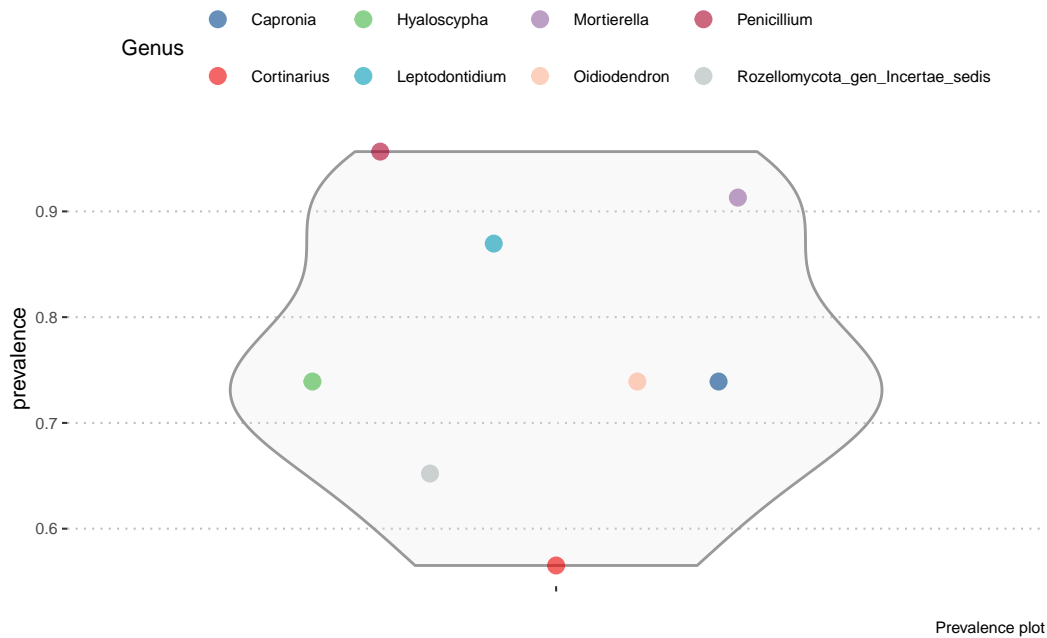
Community composition plots

Top ten features calculated by median relative abundance

```r
# Reload data
tse <- readRDS("results/tse_its2.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)))
```

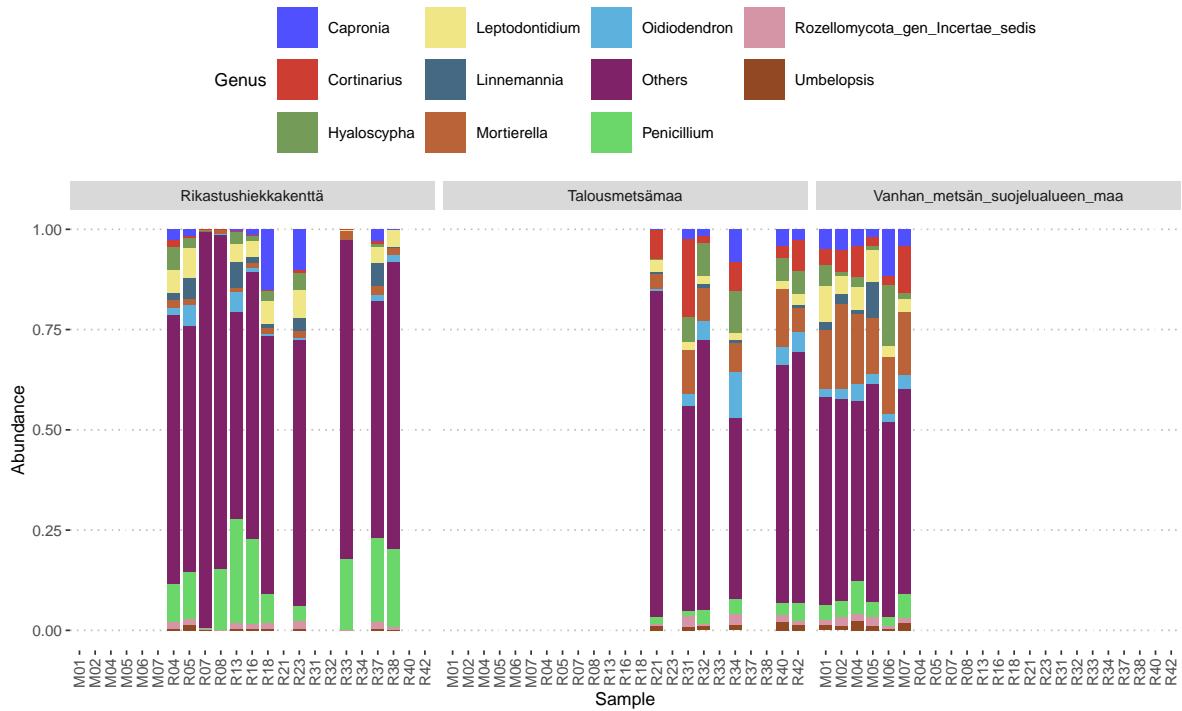# Barplot faceted by vegetation

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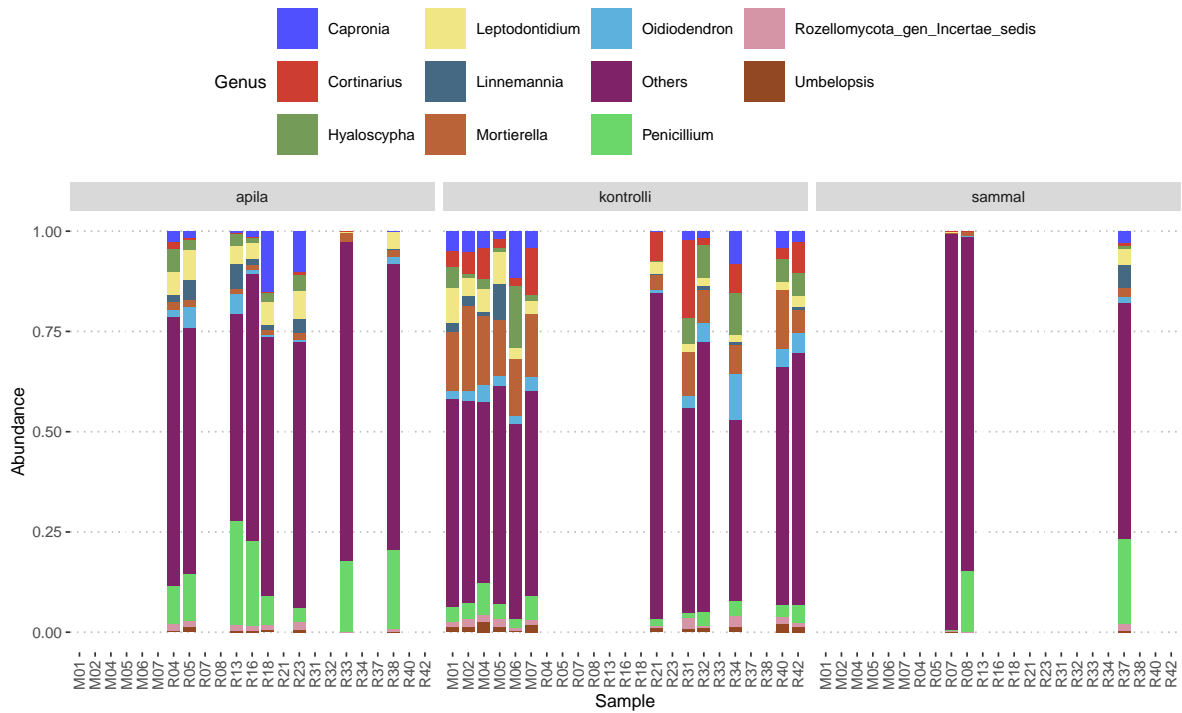
Barplot faceted by soil

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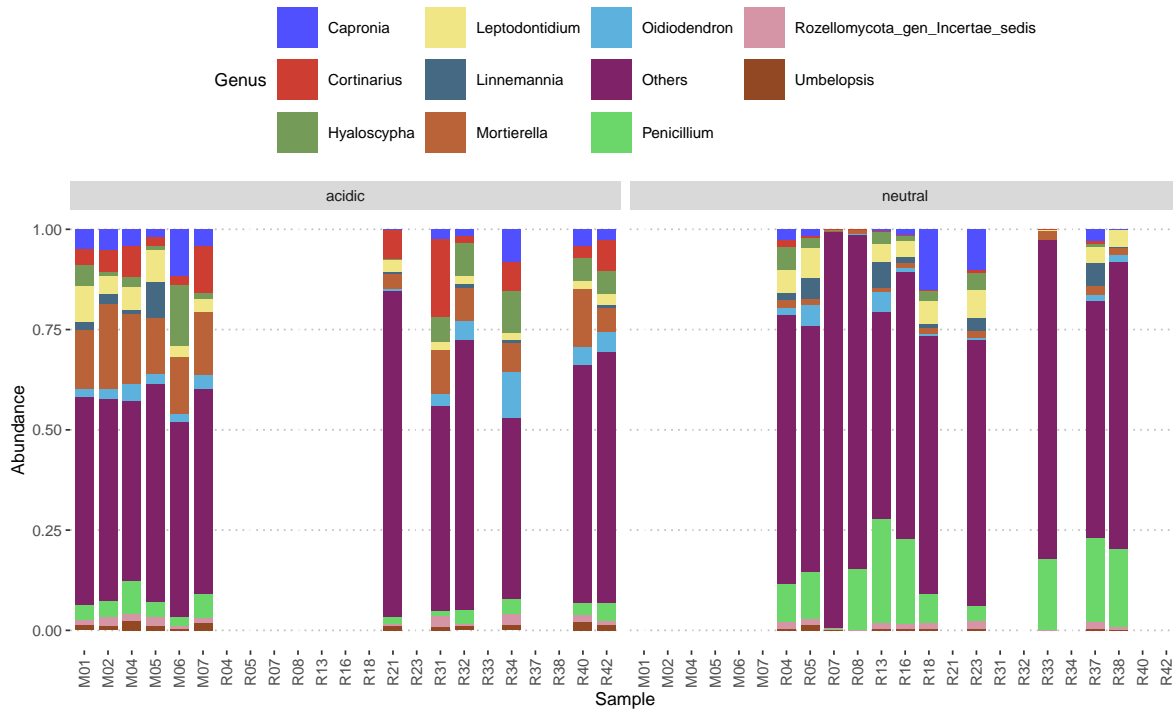
Barplot faceted by pH

```
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_its2.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
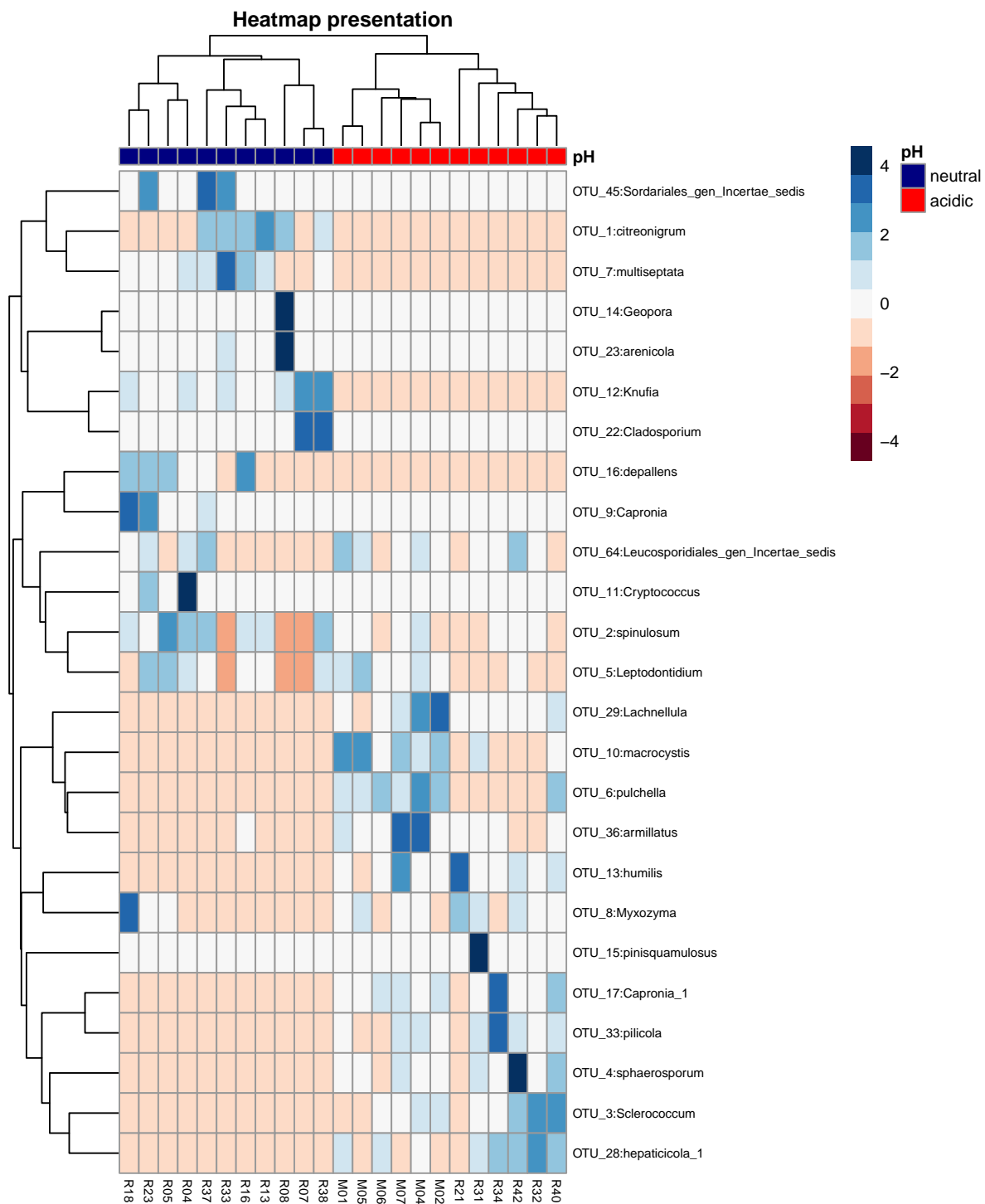
Taxonomy tree plot objects for soil pH groups

```r
# Reload data
tse <- readRDS("results/tse_its2.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

edge_colour_by
— Ascomycota
— Basidiomycota
— Mortierellomycota
— Mucoromycota
— Rozellomycota

prevalence
1.00
0.75
0.50
0.25
0.00

Soil = acidic

edge_colour_by
— Ascomycota
— Basidiomycota
— Mortierellomycota
— Mucoromycota
— Rozellomycota

prevalence
1.00
0.75
0.50
0.25
0.00
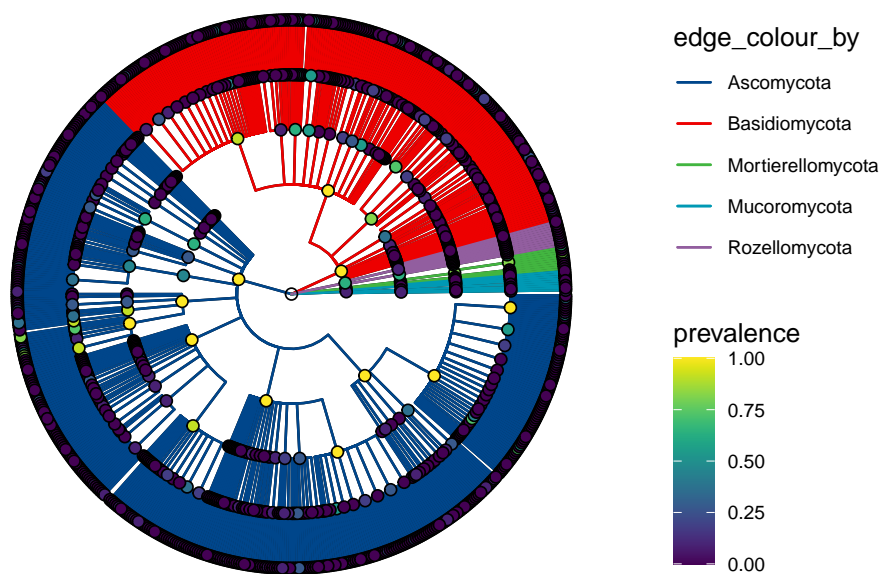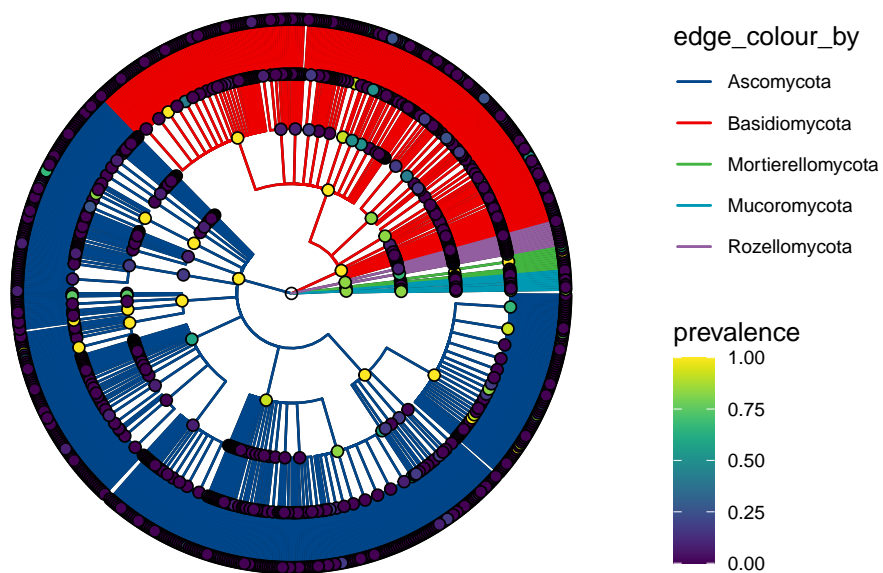
Alpha diversity

Calculation of alpha diversity indexes

```
tse <- readRDS("results/tse_its2.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	3.57	0.90	0.49	1481
R16	4.05	0.94	0.52	2272
R07	4.36	0.97	0.62	1191
R37	4.08	0.95	0.55	1706
R33	3.69	0.92	0.49	1724
R13	4.49	0.96	0.57	2671
M06	4.54	0.97	0.60	2008
R38	4.40	0.97	0.57	2185
R05	4.47	0.96	0.57	2573
R34	4.69	0.98	0.61	2285
R32	4.58	0.97	0.60	2178
R04	4.44	0.96	0.57	2286
M04	4.71	0.98	0.60	2656
M07	4.80	0.98	0.61	2567
M01	4.92	0.98	0.63	2591
R21	4.79	0.97	0.63	2105
R18	4.14	0.95	0.54	2024
R40	4.65	0.97	0.61	2104
R42	4.35	0.96	0.56	2481
M05	4.73	0.98	0.61	2263
M02	4.69	0.98	0.60	2450
R31	4.47	0.96	0.58	2108
R23	4.45	0.96	0.57	2456

Group comparison objects

```r
# 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

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

Shannon — Alue: Matovaara, Raajärvi

Shannon — Kasvillisuus: apila, kontrolli, sammal

Shannon — Maanäyte: Rikastushiekkakenttä, Talousmetsän maa, Luonnon_metsän_suojelualueen_maa

Shannon — pH: acidic, neutral

Soil pH
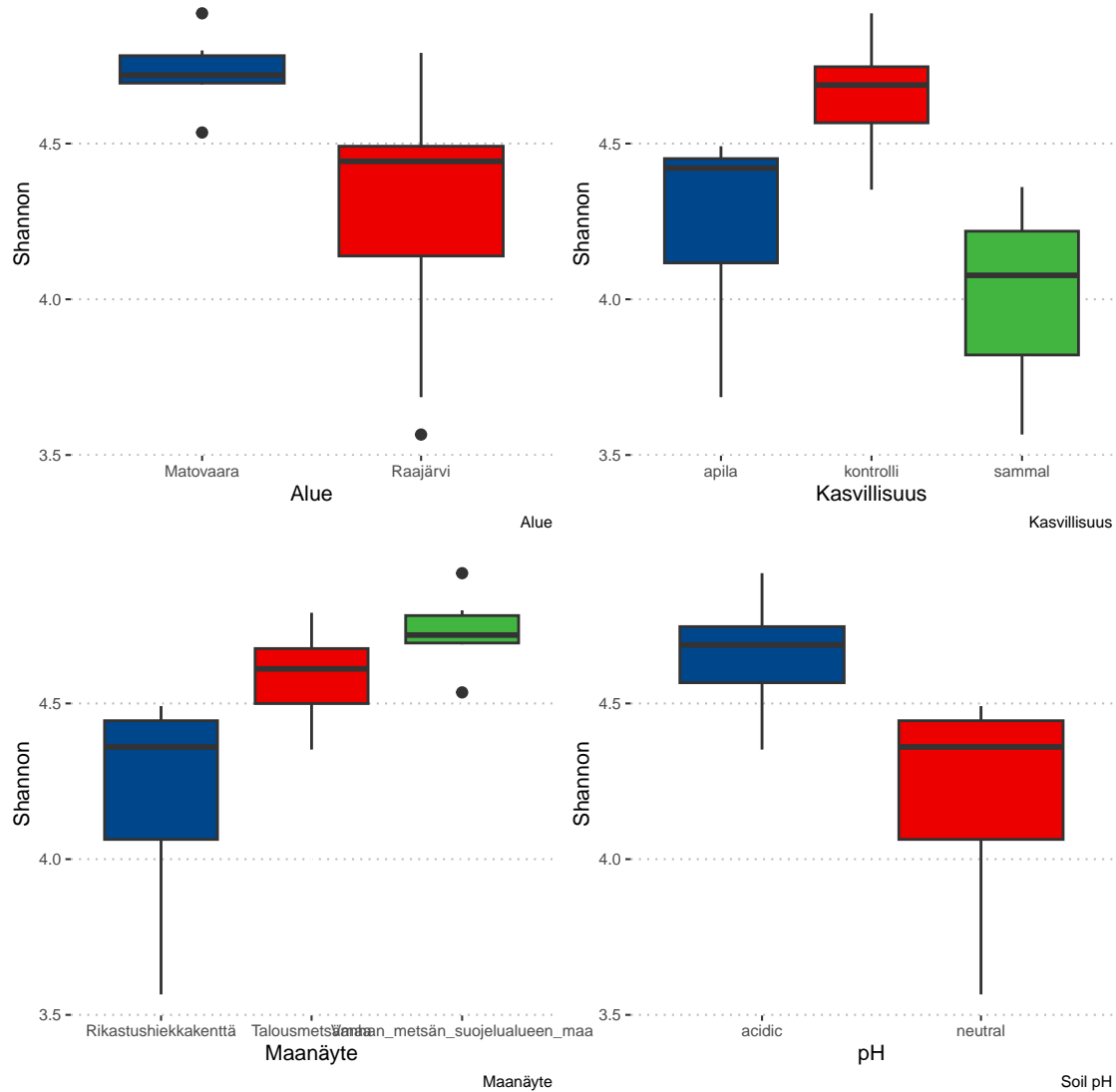
Kruskal-Wallis tests for variables

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

```
    Kruskal-Wallis rank sum test

data:  Shannon by Kasvillisuus
Kruskal-Wallis chi-squared = 13.809, df = 2, p-value = 0.001003
```

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

```
    Kruskal-Wallis rank sum test

data:  Shannon by Alue
Kruskal-Wallis chi-squared = 9.4902, df = 1, p-value = 0.002066
```

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

```
    Kruskal-Wallis rank sum test

data:  Shannon by Maanäyte
Kruskal-Wallis chi-squared = 14.709, df = 2, p-value = 0.0006397
```

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

```
    Kruskal-Wallis rank sum test

data:  Shannon by pH
Kruskal-Wallis chi-squared = 13.186, df = 1, p-value = 0.0002821
```

Betadiversity

Bray-Curtis distances ja PCoA ordination plots

```
# Reload object
tse <- readRDS("results/tse_its2.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()
```
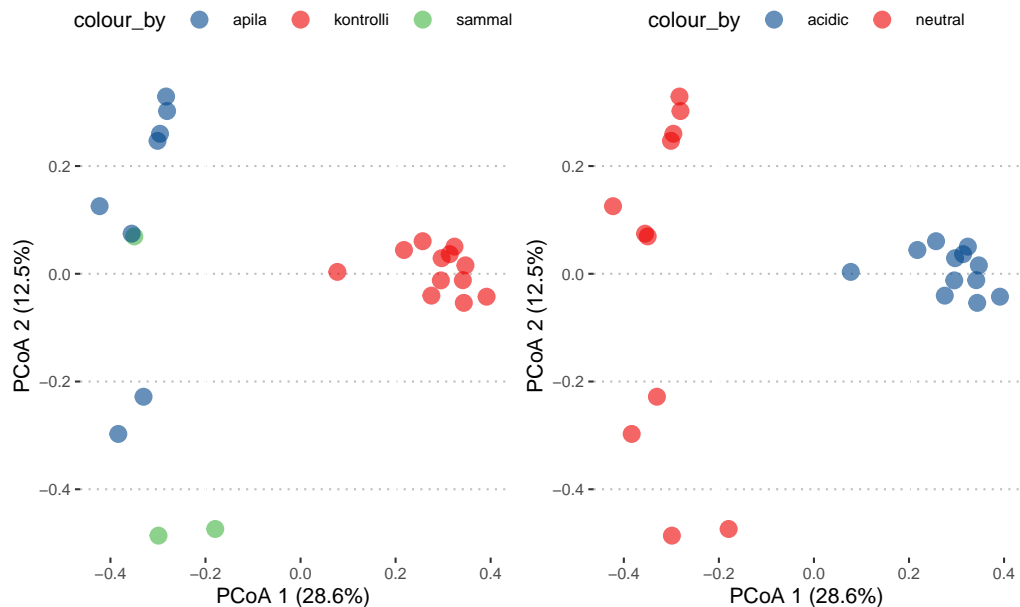
Dirichlet-Multinomial Mixture model

Cluster calculation

```
# Load the tse object
tse <- readRDS("results/tse_its2.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)
```
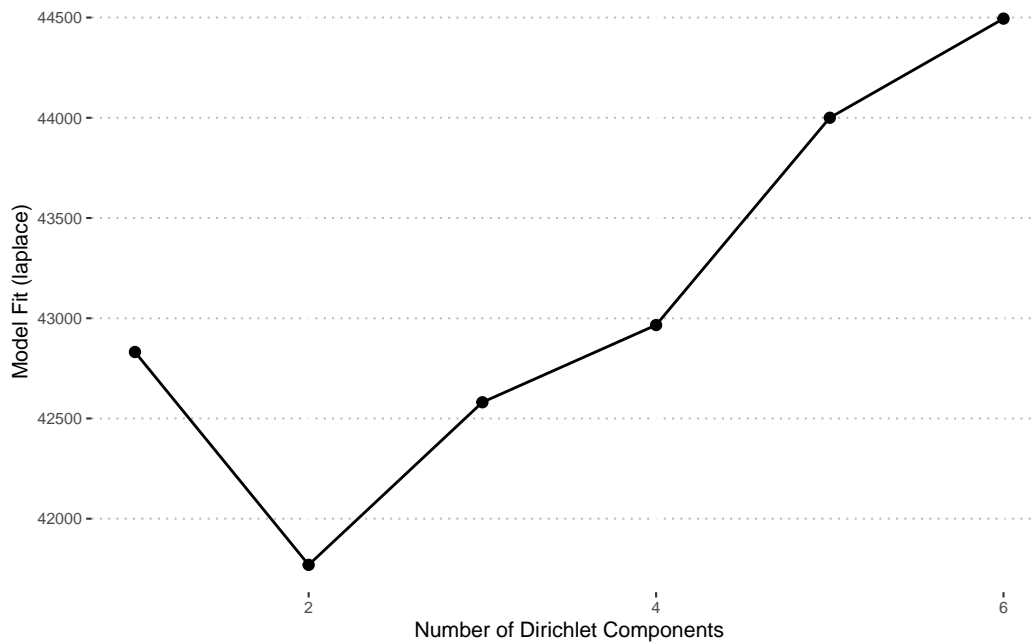
```
# Extract transformed data for PCA
data <- assay(altExp(tse, "prevalent"), "hellinger")
data <- as.matrix(data)
# Run PCA
pca_results <- prcomp(t(data))  # Transpose to have samples as rows
# Extract DMM clusters from metadata
clusters <- colData(altExp(tse, "prevalent"))$dmmclust
# Add PCA results to a data frame and include DMM clusters
pca_df <- as.data.frame(pca_results$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
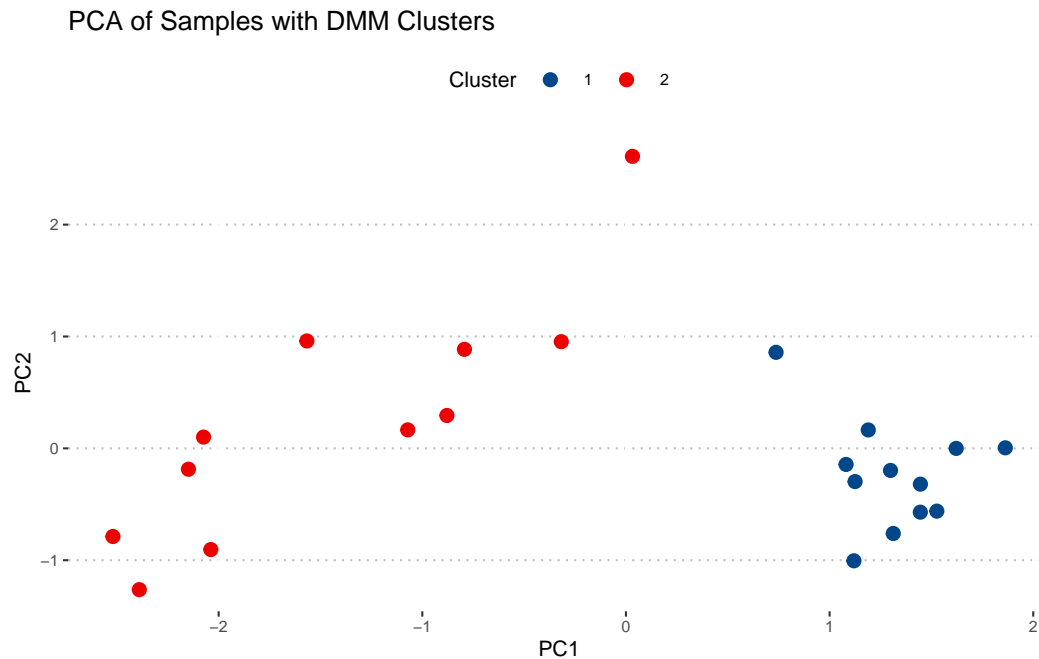
Eigen values for PCA plot

```
# 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	46.95	46.95
PC2	14.35	61.30

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

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

1 : 12 | 0.50

2 : 11 | 0.19

Silhouette width s_i

Average silhouette width : 0.35
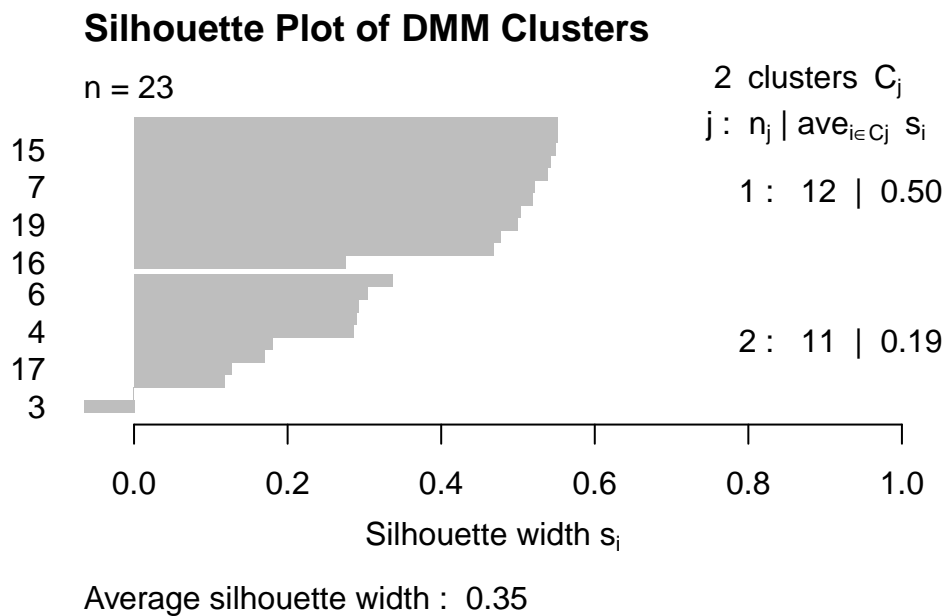
Cluster loadings - ten most important features driving samples into clusters 1 & 2

```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"))
```
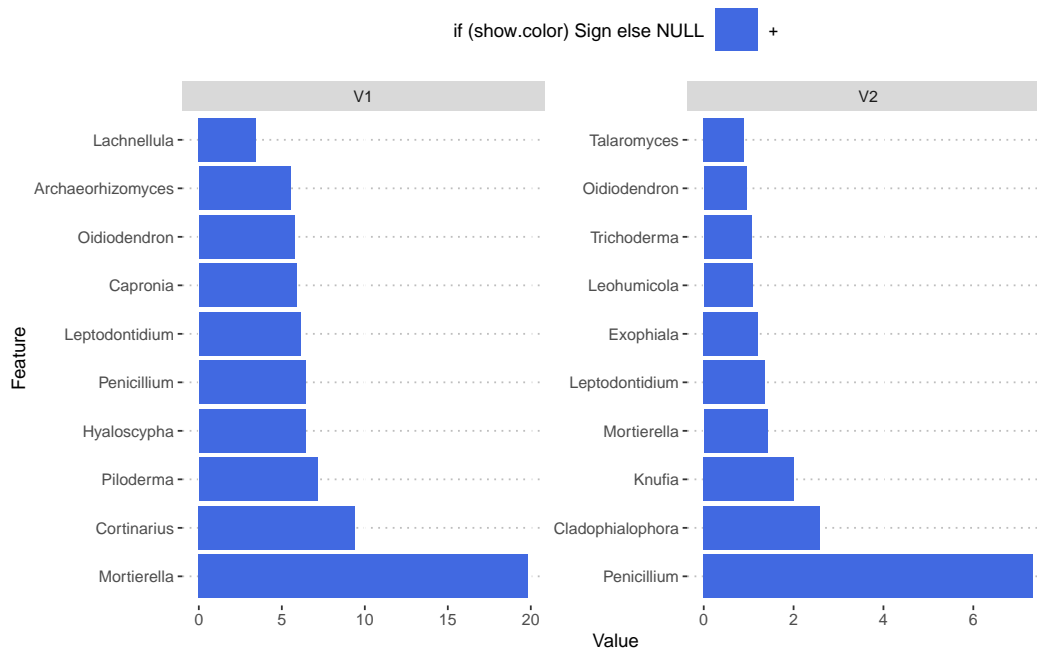
DAA analysis - pH variable

Prepare data

```
# Load tse
tse <- readRDS("results/tse_its2.rds")
tse <- tse[,1:23]
# Agglomerate by genus and subset by prevalence
tse <- subsetByPrevalent(tse, rank = "Genus", prevalence = 10/100, detection=5/1000)
# 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')
```
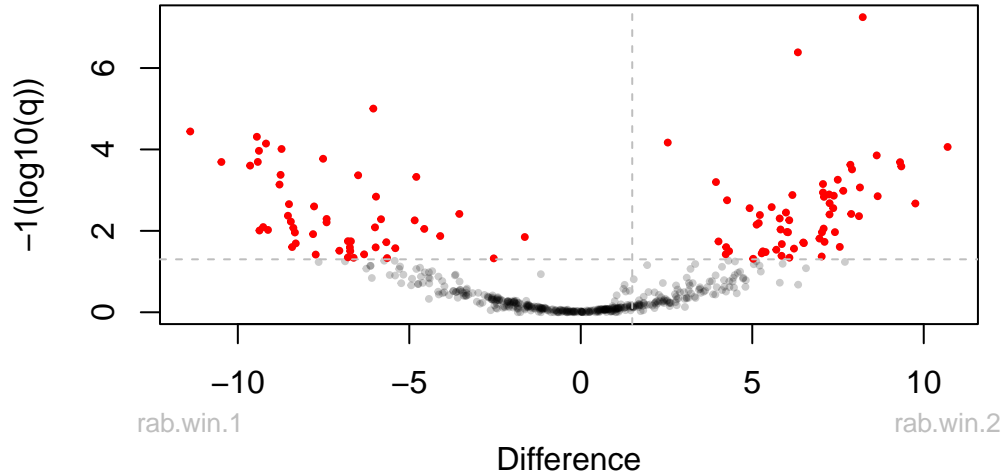
Genera with significantly different abundance

```
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	3.43	0.00	0.00	0.00
Piloderma	3.36	0.00	0.00	0.00
Leotiomycetes_gen_Incertae_sedis	3.22	0.08	0.00	0.00
Podila	3.09	0.00	0.00	0.00
Meliniomyces	3.06	0.00	0.00	0.00
Archaeorhizomyces	2.86	0.00	0.00	0.00
GS21_gen_Incertae_sedis	2.62	0.00	0.00	0.00
Rhizidium	2.55	0.00	0.00	0.00
Chaetothyriales_gen_Incertae_sedis	2.37	0.00	0.00	0.00
Mortierella	2.15	0.03	0.00	0.00
Scleropezicula	2.08	0.05	0.00	0.00
Sclerococcum	2.07	0.09	0.00	0.00
Botryobasidium	2.06	0.05	0.00	0.00
Cephalothecaceae_gen_Incertae_sedis	2.02	0.00	0.00	0.00
Lecanicillium	2.01	0.01	0.00	0.00
Crocicreas	1.84	0.00	0.00	0.00
Sugiyamaella	1.84	0.01	0.00	0.00
Helotiales_gen_Incertae_sedis	1.76	0.01	0.00	0.00
Humicolopsis	1.73	0.02	0.00	0.00
Brahmaculus	1.73	0.02	0.00	0.00
Serendipitaceae_gen_Incertae_sedis	1.68	0.01	0.00	0.00
Phacidium	1.67	0.01	0.00	0.00
Cortinarius	1.66	0.02	0.00	0.00
Cenococcum	1.64	0.04	0.00	0.00
Lachnellula	1.59	0.03	0.00	0.00
Acarosporales_gen_Incertae_sedis	1.58	0.06	0.01	0.00
Pseudoplectania	1.57	0.06	0.00	0.00
Hyphodiscus	1.55	0.04	0.00	0.00
Scytalidium	1.53	0.06	0.01	0.00
Mycena	1.51	0.04	0.00	0.00
Leptobacillium	1.50	0.06	0.01	0.00
Leccinum	1.48	0.04	0.00	0.00
Phialocephala	1.46	0.05	0.00	0.00
Leucosporidium	1.40	0.03	0.00	0.00
Hyaloscyphaceae_gen_Incertae_sedis	1.39	0.10	0.01	0.01
Pezizomycotina_gen_Incertae_sedis	1.31	0.11	0.01	0.01
Trechispora	1.30	0.06	0.01	0.00
GS22_gen_Incertae_sedis	1.30	0.11	0.02	0.01
Clavaria	1.29	0.02	0.00	0.00
Occultifur	1.24	0.10	0.02	0.01
Pezoloma	1.23	0.07	0.01	0.00
Tympanidaceae_gen_Incertae_sedis	1.20	0.11	0.02	0.01
Tremella	1.17	0.13	0.02	0.01
Hymenochaetales_gen_Incertae_sedis	1.15	0.12	0.02	0.02

Genus	effect	overlap	we.eBH	wi.eBH
Galerina	1.15	0.10	0.02	0.00
Luellia	1.14	0.12	0.03	0.01
Gloeopycnis	1.12	0.08	0.04	0.01
Apiotrichum	1.11	0.13	0.01	0.01
GS04_gen_Incertae_sedis	1.10	0.15	0.05	0.03
Pochonia	1.10	0.10	0.06	0.01
Tylospora	1.09	0.13	0.01	0.02
Pseudohyphozyma	1.08	0.10	0.03	0.01
Acarospora	1.06	0.12	0.02	0.01
Tolypocladium	1.06	0.13	0.03	0.01
Chloridium	1.04	0.12	0.03	0.01
Entoloma	1.02	0.12	0.10	0.01
Chytridiales_gen_Incertae_sedis	1.01	0.13	0.03	0.02
Cenangiaceae_gen_Incertae_sedis	1.00	0.16	0.06	0.04
Sorocybe	0.99	0.13	0.03	0.01
Filobasidiales_gen_Incertae_sedis	0.98	0.13	0.06	0.01
Auriculariales_gen_Incertae_sedis	0.97	0.14	0.04	0.03
Cystoderma	0.93	0.14	0.05	0.04
Babjeviella	0.93	0.15	0.07	0.04
Thaxterogaster	0.92	0.15	0.07	0.03
Dissophora	0.87	0.14	0.08	0.02
Syzygospora	0.86	0.17	0.05	0.03
Umbelopsis	0.85	0.11	0.07	0.01
Pseudogymnoascus	0.76	0.17	0.07	0.05
Geopora	-0.92	0.13	0.03	0.02
Ceratobasidium	-1.00	0.13	0.04	0.02
Lentitheciaceae_gen_Incertae_sedis	-1.00	0.14	0.05	0.05
Mallocybe	-1.03	0.13	0.04	0.03
Nectriella	-1.04	0.10	0.01	0.01
Cryptococcus	-1.04	0.16	0.07	0.04
Schizothecium	-1.05	0.14	0.05	0.04
Trichoderma	-1.06	0.14	0.01	0.02
Laetinaevia	-1.07	0.07	0.02	0.00
Spermospora	-1.07	0.13	0.03	0.03
Trichosporiella	-1.10	0.12	0.05	0.03
Sordariales_gen_Incertae_sedis	-1.11	0.07	0.01	0.00
Mycosphaerellales_gen_Incertae_sedis	-1.15	0.12	0.03	0.03
Oliveonia	-1.16	0.10	0.02	0.01
Nothodactylaria	-1.18	0.12	0.04	0.03
Caloplaca	-1.18	0.08	0.03	0.01
Entimomentora	-1.18	0.12	0.02	0.03
Rhizopogon	-1.19	0.13	0.01	0.01
Penicillium	-1.22	0.13	0.05	0.02
Inocybe	-1.22	0.09	0.01	0.01
Pulvinula	-1.22	0.12	0.02	0.03
Melanodiplodia	-1.28	0.09	0.02	0.01
Triscelophorus	-1.28	0.11	0.03	0.02
Vishniacozyma	-1.29	0.05	0.01	0.00
Rhexocercosporidium	-1.29	0.10	0.01	0.01
Keissleriella	-1.30	0.08	0.01	0.01
Cladophialophora	-1.33	0.06	0.00	0.00
Pseudocoleophoma	-1.40	0.08	0.01	0.01
Neonectria	-1.43	0.07	0.00	0.00
Helvella	-1.44	0.07	0.01	0.00
Verrucoccum	-1.44	0.09	0.01	0.01

Genus	effect	overlap	we.eBH	wi.eBH
Kurtzmanomyces	-1.46	0.06	0.01	0.01
Phomatospora	-1.51	0.08	0.01	0.01
Cosmospora	-1.51	0.06	0.00	0.00
Plectosphaerella	-1.62	0.09	0.01	0.01
Sclerostagonospora	-1.64	0.07	0.01	0.00
Lobulomycetales_gen_Incertae_sedis	-1.76	0.05	0.00	0.00
Pezizellaster	-1.76	0.04	0.00	0.00
Cladosporium	-1.77	0.01	0.00	0.00
Preussia	-1.80	0.04	0.00	0.00
Tetracladium	-1.87	0.02	0.00	0.00
Hamatocanthoscypha	-1.95	0.02	0.00	0.00
Paraphoma	-2.07	0.00	0.00	0.00
Fusarium	-2.08	0.00	0.00	0.00
Psoroglaena	-2.13	0.00	0.00	0.00
Kodamaea	-2.18	0.00	0.00	0.00
Pleotrichocladium	-2.25	0.02	0.00	0.00
Truncatella	-2.51	0.01	0.00	0.00
Exophiala	-2.56	0.01	0.00	0.00
Talaromyces	-3.09	0.09	0.00	0.01
Leohumicola	-3.79	0.00	0.00	0.00
Knufia	-3.97	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 = "counts",
    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
Leotiomycetes_gen_Incertae_sedis	6.94	0.43	0.00	TRUE
Archaeorhizomyces	6.90	0.60	0.00	TRUE
Meliniomyces	6.04	0.54	0.00	TRUE
Piloderma	5.90	0.52	0.00	TRUE
GS12_gen_Incertae_sedis	5.80	0.62	0.00	TRUE
Chaetothyriales_gen_Incertae_sedis	5.17	0.55	0.00	TRUE
Cephalothecaceae_gen_Incertae_sedis	5.11	0.62	0.00	TRUE
Rhizidium	4.82	0.44	0.00	TRUE
GS21_gen_Incertae_sedis	4.67	0.42	0.00	TRUE
Podila	4.65	0.47	0.00	TRUE
Sclerococcum	4.64	0.80	0.00	TRUE
Pseudoplectania	4.54	0.58	0.00	TRUE
Tympanidaceae_gen_Incertae_sedis	4.44	0.63	0.00	TRUE
Helotiales_gen_Incertae_sedis	4.44	0.61	0.00	TRUE
Herpotrichiellaceae_gen_Incertae_sedis	4.26	1.01	0.00	TRUE
Clavaria	4.09	0.81	0.00	TRUE
Crocicreas	4.03	0.51	0.00	TRUE
Humicolopsis	3.92	0.56	0.00	TRUE
Tylospora	3.83	0.89	0.00	TRUE
Brahmaculus	3.75	0.59	0.00	TRUE
Hyphodiscus	3.72	0.65	0.00	TRUE
Tolypocladium	3.66	0.74	0.00	TRUE
Serendipitaceae_gen_Incertae_sedis	3.62	0.51	0.00	TRUE
Leucosporidium	3.60	0.66	0.00	TRUE
Mycena	3.55	0.59	0.00	TRUE
Phialocephala	3.50	0.66	0.00	TRUE
Cenococcum	3.48	0.70	0.00	TRUE
Glutinomyces	3.46	0.61	0.00	TRUE
Lachnellula	3.43	0.63	0.00	TRUE
Hyaloscyphaceae_gen_Incertae_sedis	3.43	0.48	0.00	TRUE
Thaxterogaster	3.28	0.60	0.00	TRUE
Volutella	3.23	0.52	0.00	TRUE
Sugiyamaella	3.21	0.54	0.00	TRUE
Scytalidium	3.19	0.38	0.00	TRUE
Cortinarius	3.14	0.56	0.00	TRUE
Filobasidiales_gen_Incertae_sedis	3.08	0.54	0.00	TRUE
Pezoloma	3.06	0.66	0.00	TRUE
Myxozyma	2.97	0.96	0.01	TRUE
Apiotrichum	2.65	0.77	0.01	TRUE
Derxomyces	2.64	0.50	0.00	TRUE
Occultifur	2.57	0.70	0.01	TRUE
Umbelopsis	2.54	0.77	0.01	TRUE
Trechispora	2.52	0.55	0.00	TRUE
Phacidium	2.49	0.45	0.00	TRUE
Chloridium	2.48	0.55	0.00	TRUE

Genus	lfc	se	q	diff
Syzygospora	2.40	0.77	0.01	TRUE
Sistotrema	2.37	0.81	0.02	TRUE
Chytridiales_gen_Incertae_sedis	2.03	0.59	0.01	TRUE
Dissophora	2.03	0.51	0.00	TRUE
Mortierella	1.99	0.39	0.00	TRUE
Babjevia	1.98	0.72	0.02	TRUE
Claussenomyces	1.96	0.60	0.01	TRUE
Genolevuria	1.80	0.54	0.01	TRUE
Galerina	1.79	0.47	0.00	TRUE
Tyrannosorus	1.62	0.60	0.03	TRUE
Oidiodendron	1.31	0.54	0.05	TRUE
Coleophoma	-1.16	0.41	0.03	TRUE
Lophium	-1.18	0.38	0.02	TRUE
Mucorales_gen_Incertae_sedis	-1.27	0.34	0.02	TRUE
Cystobasidiomycetes_gen_Incertae_sedis	-1.28	0.48	0.04	TRUE
Bryochiton	-1.35	0.46	0.04	TRUE
Zoopagales_gen_Incertae_sedis	-1.50	0.37	0.01	TRUE
Lycoperdon	-1.70	0.69	0.05	TRUE
Keissleriella	-1.75	0.57	0.02	TRUE
Camptobasidiaceae_gen_Incertae_sedis	-1.77	0.38	0.01	TRUE
Hypomyces	-1.78	0.65	0.03	TRUE
Kurtzmaniella	-1.93	0.55	0.01	TRUE
Malassezia	-1.96	0.68	0.02	TRUE
Microbotryomycetes_gen_Incertae_sedis	-1.98	0.45	0.01	TRUE
Mytilinidiales_gen_Incertae_sedis	-2.00	0.43	0.02	TRUE
Sepedonium	-2.10	0.62	0.02	TRUE
Drechmeria	-2.16	0.49	0.00	TRUE
Fagicola	-2.20	0.38	0.00	TRUE
Sporormiella	-2.22	0.84	0.03	TRUE
Septoria	-2.25	0.49	0.00	TRUE
Cladophialophora	-2.26	0.55	0.00	TRUE
Thelebolus	-2.34	0.72	0.01	TRUE
Cistella	-2.37	0.81	0.02	TRUE
Taphrina	-2.39	0.54	0.01	TRUE
Agaricales_gen_Incertae_sedis	-2.44	0.40	0.00	TRUE
Laetinaevia	-2.62	0.69	0.01	TRUE
Hormonema	-2.68	0.39	0.00	TRUE
Mrakia	-2.70	0.41	0.00	TRUE
Ceratobasidium	-2.75	0.54	0.00	TRUE
Lapidomyces	-2.78	0.40	0.00	TRUE
Pseudeurotium	-2.80	0.68	0.00	TRUE
Vishniacozyma	-2.81	0.68	0.00	TRUE
Rhizopogon	-2.83	0.80	0.01	TRUE
Nectriella	-2.90	0.82	0.01	TRUE
Sakaguchia	-2.93	0.35	0.00	TRUE
Tomentella	-2.95	0.89	0.01	TRUE
Alternaria	-3.09	0.74	0.01	TRUE
Pustularia	-3.10	0.61	0.00	TRUE
Geopora	-3.15	1.06	0.02	TRUE
Neonectria	-3.20	0.86	0.01	TRUE
Ceratobasidiaceae_gen_Incertae_sedis	-3.34	0.46	0.00	TRUE
Preussia	-3.45	0.67	0.00	TRUE
Cladosporium	-3.48	0.64	0.00	TRUE
Talaromyces	-3.59	0.68	0.00	TRUE
Sordariales_gen_Incertae_sedis	-3.60	0.95	0.00	TRUE

(continued)

Genus	lfc	se	q	diff
Pezizellaster	-3.64	0.73	0.00	TRUE
Exophiala	-3.80	0.54	0.00	TRUE
Inocybe	-3.86	0.97	0.00	TRUE
Cryptococcus	-3.86	0.86	0.00	TRUE
Lecythophora	-3.97	0.74	0.00	TRUE
Paraphoma	-3.98	0.55	0.00	TRUE
Sclerostagonospora	-4.19	0.57	0.00	TRUE
Hamatocanthoscypha	-4.26	0.78	0.00	TRUE
Pleotrichocladium	-4.36	0.59	0.00	TRUE
Rhexocercosporidium	-4.41	0.87	0.00	TRUE
Fusarium	-4.72	0.67	0.00	TRUE
Tetracladium	-4.86	0.84	0.00	TRUE
Mallocybe	-5.27	0.86	0.00	TRUE
Kodamaea	-5.30	0.67	0.00	TRUE
Plectosphaerella	-5.45	0.49	0.00	TRUE
Knufia	-5.95	0.46	0.00	TRUE
Leohumicola	-6.64	0.45	0.00	TRUE

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

Maaslin2 analysis

```
# 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 = "ITS2",
    transform = "AST",
    fixed_effects = "pH",
    reference = "pH,neutral",
    normalization = "TSS",
    standardize = FALSE,
    min_prevalence = 0)
saveRDS(maaslin2_out, "results/its2_maaslin2.rds")
```

Genera with significantly different abundance

```
maaslin2_out <- readRDS("results/its2_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	0.23	0.03	0.00	23
Piloderma	0.21	0.04	0.00	23
Archaeorhizomyces	0.19	0.03	0.00	21
Cortinarius	0.19	0.03	0.00	23
Sclerococcum	0.15	0.03	0.00	23
Podila	0.13	0.02	0.00	23
Meliniomyces	0.13	0.02	0.00	22
Lachnellula	0.11	0.02	0.00	23

Genus	coef	stderr	qval	N.not.zero
Leotiomycetes_gen_Incertae_sedis	0.10	0.02	0.00	20
Tylospora	0.10	0.03	0.03	21
Rhizidium	0.08	0.01	0.00	20
Oidiodendron	0.08	0.03	0.04	23
Helotiales_gen_Incertae_sedis	0.07	0.01	0.00	19
Cephalothecaceae_gen_Incertae_sedis	0.07	0.02	0.00	20
Chaetothyriales_gen_Incertae_sedis	0.07	0.01	0.00	17
Umbelopsis	0.07	0.01	0.00	23
Serendipitaceae_gen_Incertae_sedis	0.07	0.01	0.00	18
Herpotrichiellaceae_gen_Incertae_sedis	0.06	0.02	0.04	20
Leucosporidium	0.06	0.01	0.00	20
Phialocephala	0.06	0.01	0.00	18
Apiotrichum	0.05	0.01	0.01	18
Cenococcum	0.04	0.01	0.00	16
Entomortierella	0.04	0.00	0.00	14
Pseudoplectania	0.04	0.01	0.04	14
Mycena	0.04	0.01	0.00	20
Tympanidaceae_gen_Incertae_sedis	0.04	0.01	0.04	13
Pezoloma	0.04	0.01	0.02	20
Brahmaculus	0.03	0.01	0.00	17
Sugiyamaella	0.03	0.01	0.00	15
Humicolopsis	0.03	0.01	0.00	20
GS12_gen_Incertae_sedis	0.03	0.01	0.04	10
Hyphodiscus	0.03	0.01	0.01	17
Syzygospora	0.03	0.01	0.02	20
Tolypocladium	0.03	0.01	0.02	18
Diademospora	0.03	0.01	0.02	11
Babjevia	0.03	0.01	0.03	23
Hyaloscyphaceae_gen_Incertae_sedis	0.03	0.00	0.00	15
GS21_gen_Incertae_sedis	0.03	0.00	0.00	15
Hymenochaetales_gen_Incertae_sedis	0.02	0.01	0.01	11
Crocicreas	0.02	0.01	0.00	16
Venturia	0.02	0.01	0.05	23
Trechispora	0.02	0.01	0.01	18
Lecanicillium	0.02	0.00	0.00	13
Chytridiales_gen_Incertae_sedis	0.02	0.00	0.00	15
Nadsonia	0.02	0.01	0.03	9
Pezizomycotina_gen_Incertae_sedis	0.02	0.01	0.04	14
Scleropezicula	0.02	0.00	0.00	11
Luellia	0.02	0.00	0.02	11
Phacidium	0.02	0.01	0.05	15
Botryobasidium	0.02	0.00	0.00	11
Auriculariales_gen_Incertae_sedis	0.02	0.01	0.04	11
GS22_gen_Incertae_sedis	0.01	0.00	0.01	10
Scytalidium	0.01	0.00	0.00	14
Chaetosphaeria	0.01	0.00	0.04	11
Leptobacillium	0.01	0.00	0.00	14
Cenangiaceae_gen_Incertae_sedis	0.01	0.00	0.01	10
Acarosporales_gen_Incertae_sedis	0.01	0.00	0.00	13
Galerina	0.01	0.00	0.00	18
Dissophora	0.01	0.00	0.02	16
Genolevuria	0.01	0.00	0.04	14
GS04_gen_Incertae_sedis	0.01	0.00	0.00	9
Synnemapestaloides	0.01	0.00	0.04	11
Tyrannosorus	0.01	0.00	0.04	17

Genus	coef	stderr	qval	N.not.zero
Leccinum	0.01	0.00	0.00	14
Tremella	0.01	0.00	0.02	14
Babjeviella	0.01	0.00	0.01	11
Pseudogymnoascus	0.01	0.00	0.03	18
Coccomyces	0.01	0.00	0.05	8
Sorocybe	0.01	0.00	0.00	15
Sporidiobolales_gen_Incertae_sedis	0.01	0.00	0.04	8
Endogonomycetes_gen_Incertae_sedis	0.01	0.00	0.02	10
Cystoderma	0.01	0.00	0.01	12
Fayodia	0.01	0.00	0.02	9
Zoopagomycota_gen_Incertae_sedis	0.01	0.00	0.02	8
Scolecolachnum	0.01	0.00	0.03	9
Sympoventuriaceae_gen_Incertae_sedis	0.01	0.00	0.03	9
Pochonia	0.01	0.00	0.00	10
Pseudohyphozyma	0.01	0.00	0.00	11
Mortierellomycetes_gen_Incertae_sedis	0.01	0.00	0.04	8
Dothiorella	0.01	0.00	0.02	15
Gloeopycnis	0.01	0.00	0.00	12
Haplographium	0.01	0.00	0.05	8
Flammula	0.01	0.00	0.01	16
Micarea	0.01	0.00	0.05	7
Dermea	0.01	0.00	0.02	8
Ophiocordycipitaceae_gen_Incertae_sedis	0.01	0.00	0.04	9
Cladoriellaceae_gen_Incertae_sedis	0.01	0.00	0.01	9
Bicornispora	0.00	0.00	0.03	15
Phaeotremella	0.00	0.00	0.03	12
Endogonales_gen_Incertae_sedis	0.00	0.00	0.04	6
Calycellina	0.00	0.00	0.03	6
Gymnopilus	0.00	0.00	0.02	8
Krasilnikovozyma	0.00	0.00	0.02	8
Micropeltidaceae_gen_Incertae_sedis	0.00	0.00	0.04	8
Spiculogloeomycetes_gen_Incertae_sedis	0.00	0.00	0.00	10
Gelasinospora	0.00	0.00	0.01	10
Dothiora	0.00	0.00	0.01	10
Dermateaceae_gen_Incertae_sedis	0.00	0.00	0.04	6
Chlorencoelia	0.00	0.00	0.03	6
Yamadamyces	0.00	0.00	0.03	9
Hypocreaceae_gen_Incertae_sedis	0.00	0.00	0.03	7
Branch01_gen_Incertae_sedis	0.00	0.00	0.04	6
Aphanocladium	0.00	0.00	0.05	7
Scleroconidioma	0.00	0.00	0.03	6
Slooffia	0.00	0.00	0.04	6
Isthmomyces	0.00	0.00	0.02	7
Lyomyces	0.00	0.00	0.03	6
Acrophialophora	-0.01	0.00	0.03	6
Achroceratosphaeria	-0.01	0.00	0.04	5
Rhizophagus	-0.01	0.00	0.03	7
Trichosporiella	-0.01	0.00	0.03	8
Nothodactylaria	-0.01	0.00	0.00	8
Kurtzmaniella	-0.01	0.00	0.04	15
Sporormiaceae_gen_Incertae_sedis	-0.01	0.00	0.05	6
Schizothecium	-0.01	0.00	0.02	10
Keissleriella	-0.01	0.00	0.00	16
Triscelophorus	-0.01	0.00	0.00	9
Melanodiplodia	-0.01	0.00	0.01	9

(continued)

Genus	coef	stderr	qval	N.not.zero
Verrucoccum	-0.01	0.00	0.00	9
Spermospora	-0.01	0.01	0.05	12
Lobulomycetales_gen_Incertae_sedis	-0.01	0.00	0.00	11
Cistella	-0.02	0.01	0.04	17
Mycosphaerellales_gen_Incertae_sedis	-0.02	0.01	0.03	9
Ceratobasidiaceae_gen_Incertae_sedis	-0.02	0.01	0.04	15
Ochroconis	-0.02	0.01	0.04	8
Oliveonia	-0.02	0.01	0.04	12
Cosmospora	-0.02	0.00	0.00	11
Phomatospora	-0.02	0.01	0.02	9
Entimomentora	-0.02	0.01	0.03	11
Kurtzmanomyces	-0.02	0.01	0.02	9
Pseudocoleophoma	-0.02	0.01	0.00	10
Pulvinula	-0.02	0.01	0.01	9
Psoroglaena	-0.02	0.01	0.04	11
Truncatella	-0.03	0.01	0.00	12
Sclerostagonospora	-0.03	0.01	0.02	16
Ceratobasidium	-0.03	0.01	0.04	14
Helvella	-0.03	0.01	0.02	12
Pezizellaster	-0.03	0.01	0.00	16
Preussia	-0.03	0.01	0.03	15
Neonectria	-0.03	0.01	0.01	16
Pleotrichocladium	-0.04	0.01	0.00	15
Plectosphaerella	-0.04	0.01	0.02	12
Sporormiella	-0.04	0.01	0.02	23
Lecythophora	-0.04	0.01	0.03	16
Paraphoma	-0.04	0.01	0.00	21
Hamatocanthoscypha	-0.04	0.01	0.00	14
Rhexocercosporidium	-0.05	0.01	0.00	16
Rhizopogon	-0.06	0.02	0.02	23
Tomentella	-0.06	0.02	0.04	21
Fusarium	-0.07	0.02	0.01	22
Kodamaea	-0.08	0.02	0.01	19
Cladosporium	-0.08	0.03	0.04	23
Tetracladium	-0.09	0.03	0.01	16
Talaromyces	-0.09	0.02	0.00	23
Leohumicola	-0.10	0.01	0.00	18
Cryptococcus	-0.10	0.04	0.04	21
Exophiala	-0.10	0.02	0.00	23
Cladophialophora	-0.15	0.04	0.01	23
Knufia	-0.16	0.02	0.00	21
Penicillium	-0.17	0.04	0.01	23

In maaslin2 analysis, control is neutral, effect is acidic. Changes are calculated with formula log(q)*sign(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	FALSE	TRUE
Piloderma	TRUE	TRUE	TRUE
Leotiomycetes_gen_Incertae_sedis	TRUE	TRUE	TRUE
Podila	TRUE	TRUE	TRUE
Meliniomyces	TRUE	TRUE	TRUE
Archaeorhizomyces	TRUE	TRUE	TRUE
GS21_gen_Incertae_sedis	TRUE	TRUE	TRUE
Rhizidium	TRUE	TRUE	TRUE
Chaetothyriales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Mortierella	TRUE	TRUE	TRUE
Scleropezicula	TRUE	FALSE	TRUE
Sclerococcum	TRUE	TRUE	TRUE
Botryobasidium	TRUE	FALSE	TRUE
Cephalothecaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Lecanicillium	TRUE	FALSE	TRUE
Crocicreas	TRUE	TRUE	TRUE
Sugiyamaella	TRUE	TRUE	TRUE
Helotiales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Humicolopsis	TRUE	TRUE	TRUE
Brahmaculus	TRUE	TRUE	TRUE
Serendipitaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Phacidium	TRUE	TRUE	TRUE
Cortinarius	TRUE	TRUE	TRUE
Cenococcum	TRUE	TRUE	TRUE
Lachnellula	TRUE	TRUE	TRUE
Acarosporales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Pseudoplectania	TRUE	TRUE	TRUE
Hyphodiscus	TRUE	TRUE	TRUE
Scytalidium	TRUE	TRUE	TRUE
Mycena	TRUE	TRUE	TRUE
Leptobacillium	TRUE	FALSE	TRUE
Leccinum	TRUE	FALSE	TRUE
Phialocephala	TRUE	TRUE	TRUE
Leucosporidium	TRUE	TRUE	TRUE
Hyaloscyphaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Pezizomycotina_gen_Incertae_sedis	TRUE	FALSE	TRUE
Trechispora	TRUE	TRUE	TRUE
GS22_gen_Incertae_sedis	TRUE	FALSE	TRUE
Clavaria	TRUE	TRUE	FALSE
Occultifur	TRUE	TRUE	FALSE
Pezoloma	TRUE	TRUE	TRUE
Tympanidaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Tremella	TRUE	FALSE	TRUE
Hymenochaetales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Galerina	TRUE	TRUE	TRUE
Luellia	TRUE	FALSE	TRUE
Gloeopycnis	TRUE	FALSE	TRUE
Apiotrichum	TRUE	TRUE	TRUE

Genus	Aldex2	Ancombc	Maaslin2
GS04_gen_Incertae_sedis	TRUE	FALSE	TRUE
Pochonia	TRUE	FALSE	TRUE
Tylospora	TRUE	TRUE	TRUE
Pseudohyphozyma	TRUE	FALSE	TRUE
Acarospora	TRUE	FALSE	FALSE
Tolypocladium	TRUE	TRUE	TRUE
Chloridium	TRUE	TRUE	FALSE
Entoloma	TRUE	FALSE	FALSE
Chytridiales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Cenangiaceae_gen_Incertae_sedis	TRUE	FALSE	TRUE
Sorocybe	TRUE	FALSE	TRUE
Filobasidiales_gen_Incertae_sedis	TRUE	TRUE	FALSE
Auriculariales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Cystoderma	TRUE	FALSE	TRUE
Babjeviella	TRUE	FALSE	TRUE
Thaxterogaster	TRUE	TRUE	FALSE
Dissophora	TRUE	TRUE	TRUE
Syzygospora	TRUE	TRUE	TRUE
Umbelopsis	TRUE	TRUE	TRUE
Pseudogymnoascus	TRUE	FALSE	TRUE
Geopora	TRUE	TRUE	FALSE
Ceratobasidium	TRUE	TRUE	TRUE
Lentitheciaceae_gen_Incertae_sedis	TRUE	FALSE	FALSE
Mallocybe	TRUE	TRUE	FALSE
Nectriella	TRUE	TRUE	FALSE
Cryptococcus	TRUE	TRUE	TRUE
Schizothecium	TRUE	FALSE	TRUE
Trichoderma	TRUE	FALSE	FALSE
Laetinaevia	TRUE	TRUE	FALSE
Spermospora	TRUE	FALSE	TRUE
Trichosporiella	TRUE	FALSE	TRUE
Sordariales_gen_Incertae_sedis	TRUE	TRUE	FALSE
Mycosphaerellales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Oliveonia	TRUE	FALSE	TRUE
Nothodactylaria	TRUE	FALSE	TRUE
Caloplaca	TRUE	FALSE	FALSE
Entimomentora	TRUE	FALSE	TRUE
Rhizopogon	TRUE	TRUE	TRUE
Penicillium	TRUE	FALSE	TRUE
Inocybe	TRUE	TRUE	FALSE
Pulvinula	TRUE	FALSE	TRUE
Melanodiplodia	TRUE	FALSE	TRUE
Triscelophorus	TRUE	FALSE	TRUE
Vishniacozyma	TRUE	TRUE	FALSE
Rhexocercosporidium	TRUE	TRUE	TRUE
Keissleriella	TRUE	TRUE	TRUE
Cladophialophora	TRUE	TRUE	TRUE
Pseudocoleophoma	TRUE	FALSE	TRUE
Neonectria	TRUE	TRUE	TRUE
Helvella	TRUE	FALSE	TRUE
Verrucoccum	TRUE	FALSE	TRUE
Kurtzmanomyces	TRUE	FALSE	TRUE
Phomatospora	TRUE	FALSE	TRUE
Cosmospora	TRUE	FALSE	TRUE
Plectosphaerella	TRUE	TRUE	TRUE

Genus	Aldex2	Ancombc	Maaslin2
Sclerostagonospora	TRUE	TRUE	TRUE
Lobulomycetales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Pezizellaster	TRUE	TRUE	TRUE
Cladosporium	TRUE	TRUE	TRUE
Preussia	TRUE	TRUE	TRUE
Tetracladium	TRUE	TRUE	TRUE
Hamatocanthoscypha	TRUE	TRUE	TRUE
Paraphoma	TRUE	TRUE	TRUE
Fusarium	TRUE	TRUE	TRUE
Psoroglaena	TRUE	FALSE	TRUE
Kodamaea	TRUE	TRUE	TRUE
Pleotrichocladium	TRUE	TRUE	TRUE
Truncatella	TRUE	FALSE	TRUE
Exophiala	TRUE	TRUE	TRUE
Talaromyces	TRUE	TRUE	TRUE
Leohumicola	TRUE	TRUE	TRUE
Knufia	TRUE	TRUE	TRUE
GS12_gen_Incertae_sedis	FALSE	TRUE	TRUE
Herpotrichiellaceae_gen_Incertae_sedis	FALSE	TRUE	TRUE
Glutinomyces	FALSE	TRUE	FALSE
Volutella	FALSE	TRUE	FALSE
Myxozyma	FALSE	TRUE	FALSE
Derxomyces	FALSE	TRUE	FALSE
Sistotrema	FALSE	TRUE	FALSE
Babjevia	FALSE	TRUE	TRUE
Claussenomyces	FALSE	TRUE	FALSE
Genolevuria	FALSE	TRUE	TRUE
Tyrannosorus	FALSE	TRUE	TRUE
Oidiodendron	FALSE	TRUE	TRUE
Coleophoma	FALSE	TRUE	FALSE
Lophium	FALSE	TRUE	FALSE
Mucorales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Cystobasidiomycetes_gen_Incertae_sedis	FALSE	TRUE	FALSE
Bryochiton	FALSE	TRUE	FALSE
Zoopagales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Lycoperdon	FALSE	TRUE	FALSE
Camptobasidiaceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Hypomyces	FALSE	TRUE	FALSE
Kurtzmaniella	FALSE	TRUE	TRUE
Malassezia	FALSE	TRUE	FALSE
Microbotryomycetes_gen_Incertae_sedis	FALSE	TRUE	FALSE
Mytilinidiales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Sepedonium	FALSE	TRUE	FALSE
Drechmeria	FALSE	TRUE	FALSE
Fagicola	FALSE	TRUE	FALSE
Sporormiella	FALSE	TRUE	TRUE
Septoria	FALSE	TRUE	FALSE
Thelebolus	FALSE	TRUE	FALSE
Cistella	FALSE	TRUE	TRUE
Taphrina	FALSE	TRUE	FALSE
Agaricales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Hormonema	FALSE	TRUE	FALSE
Mrakia	FALSE	TRUE	FALSE
Lapidomyces	FALSE	TRUE	FALSE
Pseudeurotium	FALSE	TRUE	FALSE

(continued)

Genus	Aldex2	Ancombc	Maaslin2
Sakaguchia	FALSE	TRUE	FALSE
Tomentella	FALSE	TRUE	TRUE
Alternaria	FALSE	TRUE	FALSE
Pustularia	FALSE	TRUE	FALSE
Ceratobasidiaceae_gen_Incertae_sedis	FALSE	TRUE	TRUE
Lecythophora	FALSE	TRUE	TRUE
Diademospora	FALSE	FALSE	TRUE
Venturia	FALSE	FALSE	TRUE
Nadsonia	FALSE	FALSE	TRUE
Chaetosphaeria	FALSE	FALSE	TRUE
Synnemapestaloides	FALSE	FALSE	TRUE
Coccomyces	FALSE	FALSE	TRUE
Sporidiobolales_gen_Incertae_sedis	FALSE	FALSE	TRUE
Endogonomycetes_gen_Incertae_sedis	FALSE	FALSE	TRUE
Fayodia	FALSE	FALSE	TRUE
Zoopagomycota_gen_Incertae_sedis	FALSE	FALSE	TRUE
Scolecolachnum	FALSE	FALSE	TRUE
Sympoventuriaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Mortierellomycetes_gen_Incertae_sedis	FALSE	FALSE	TRUE
Dothiorella	FALSE	FALSE	TRUE
Haplographium	FALSE	FALSE	TRUE
Flammula	FALSE	FALSE	TRUE
Micarea	FALSE	FALSE	TRUE
Dermea	FALSE	FALSE	TRUE
Ophiocordycipitaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Cladoriellaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Bicornispora	FALSE	FALSE	TRUE
Phaeotremella	FALSE	FALSE	TRUE
Endogonales_gen_Incertae_sedis	FALSE	FALSE	TRUE
Calycellina	FALSE	FALSE	TRUE
Gymnopilus	FALSE	FALSE	TRUE
Krasilnikovozyma	FALSE	FALSE	TRUE
Micropeltidaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Spiculogloeomycetes_gen_Incertae_sedis	FALSE	FALSE	TRUE
Gelasinospora	FALSE	FALSE	TRUE
Dothiora	FALSE	FALSE	TRUE
Dermateaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Chlorencoelia	FALSE	FALSE	TRUE
Yamadamyces	FALSE	FALSE	TRUE
Hypocreaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Branch01_gen_Incertae_sedis	FALSE	FALSE	TRUE
Aphanocladium	FALSE	FALSE	TRUE
Scleroconidioma	FALSE	FALSE	TRUE
Slooffia	FALSE	FALSE	TRUE
Isthmomyces	FALSE	FALSE	TRUE
Lyomyces	FALSE	FALSE	TRUE
Acrophialophora	FALSE	FALSE	TRUE
Achroceratosphaeria	FALSE	FALSE	TRUE
Rhizophagus	FALSE	FALSE	TRUE
Sporormiaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Ochroconis	FALSE	FALSE	TRUE