

Data-analyysi Raajärvi näytteet Full-ITS

R libraries

```
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)
```

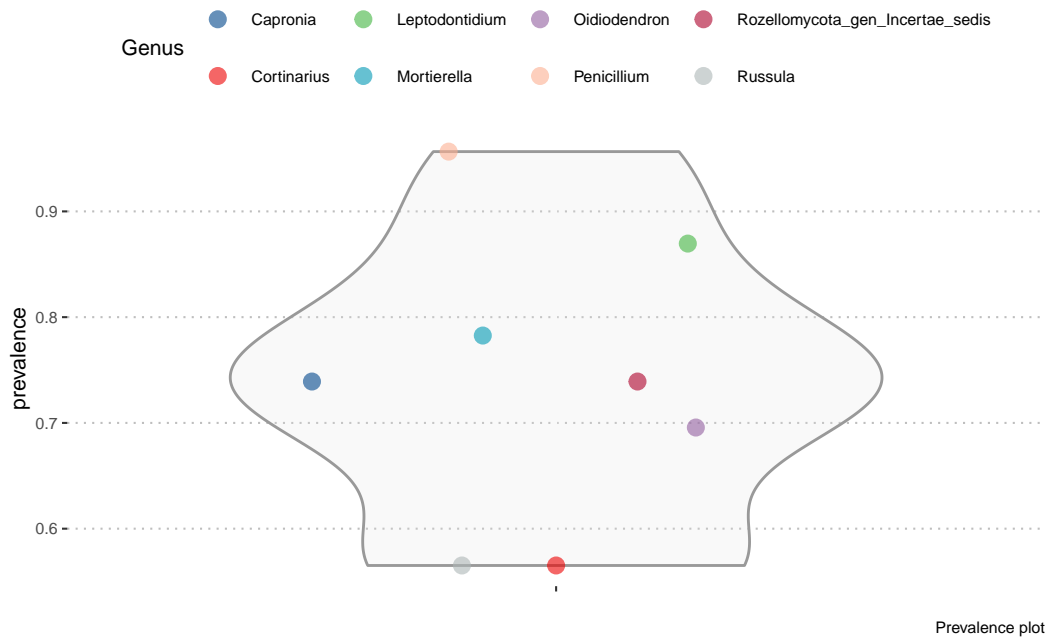
Load data object

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

Prevalent features on data set

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

```
# 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")
```



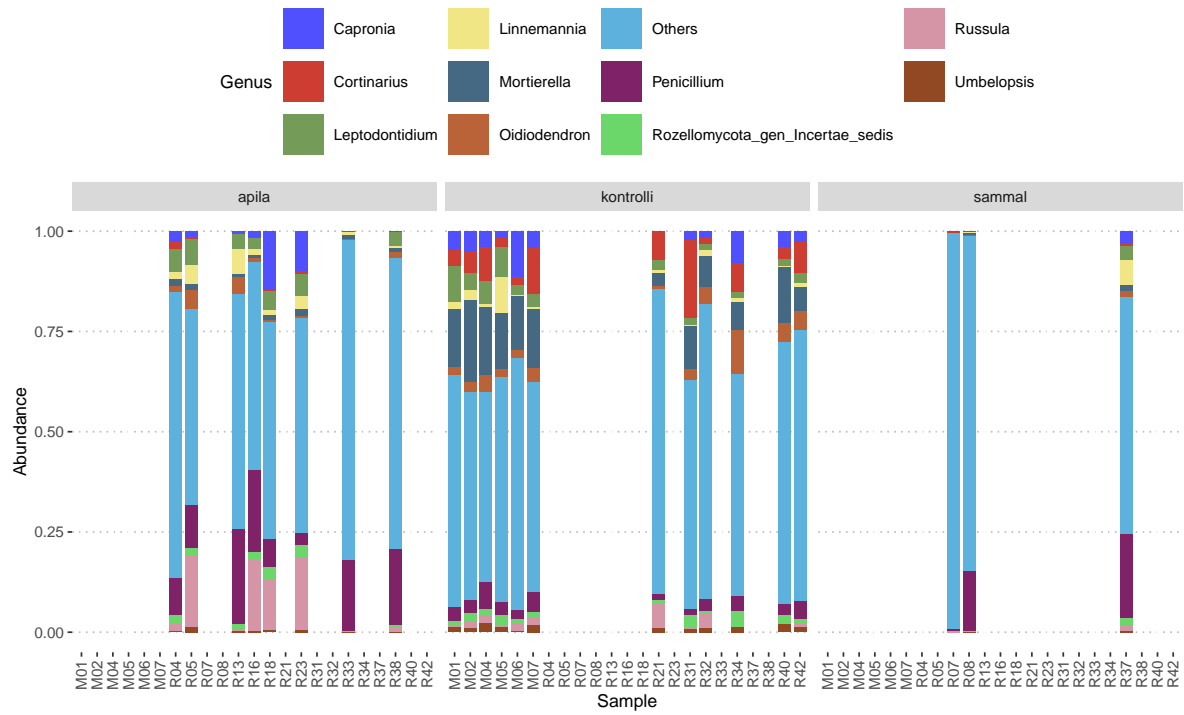
Community composition plots

Top ten features calculated by median relative abundance

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

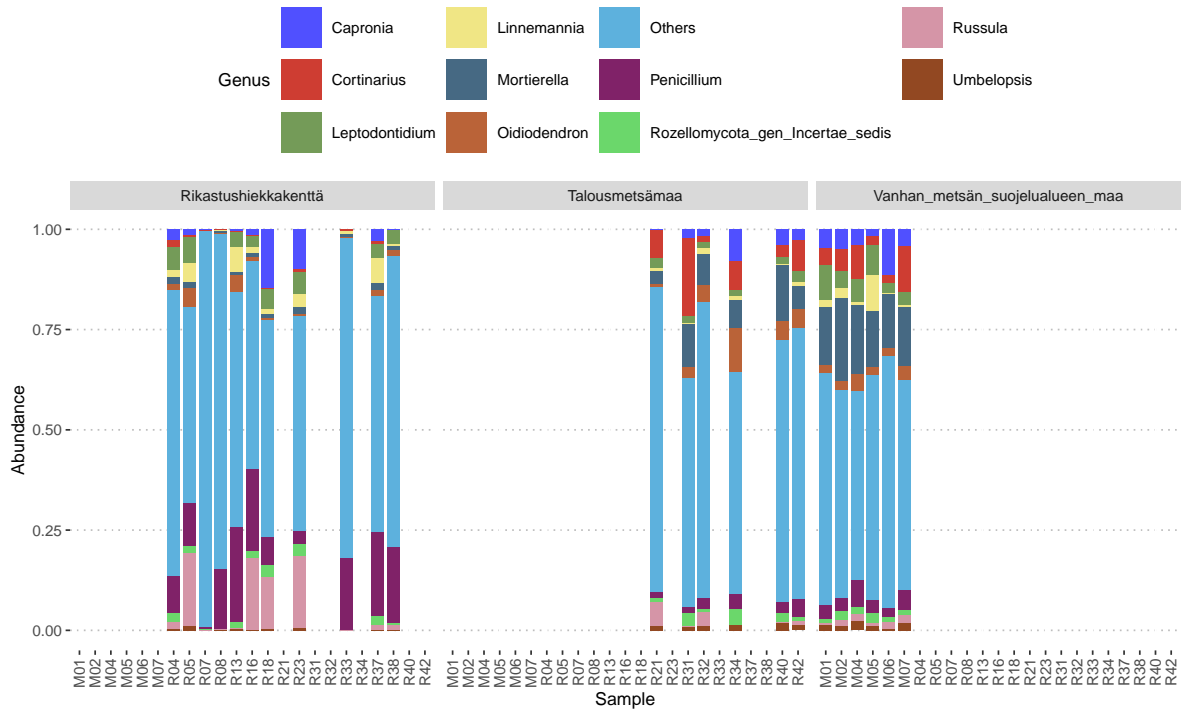
Barplot faceted by vegetation

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



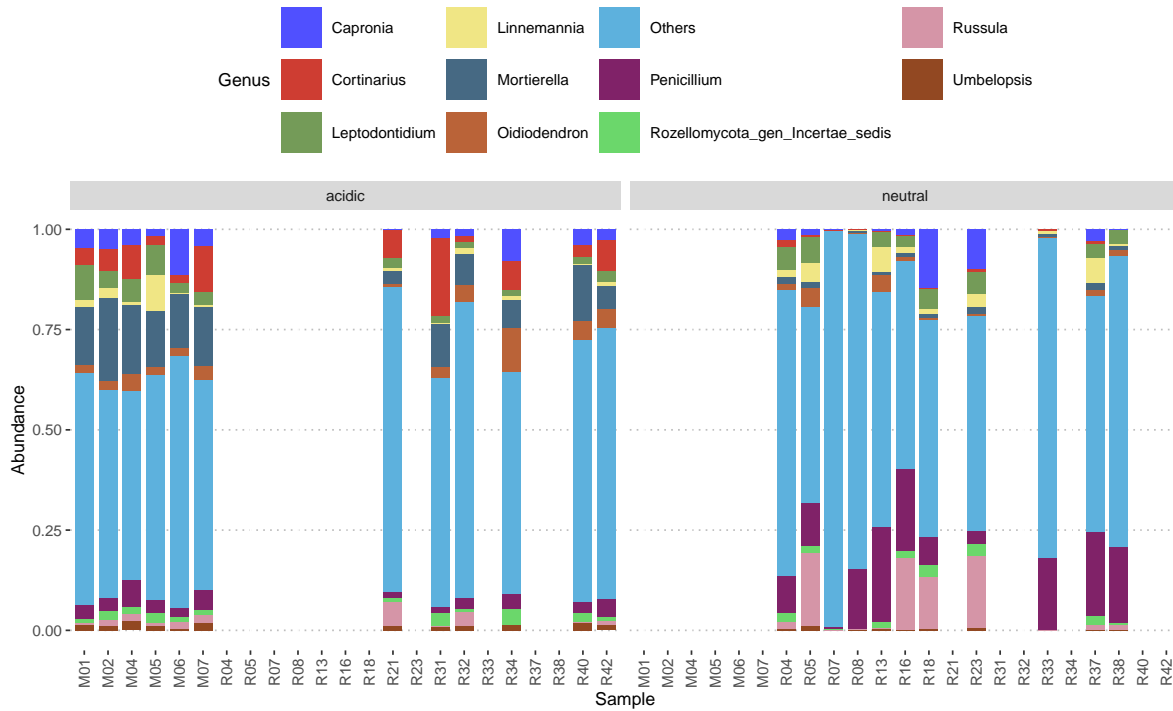
Barplot faceted by soil

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



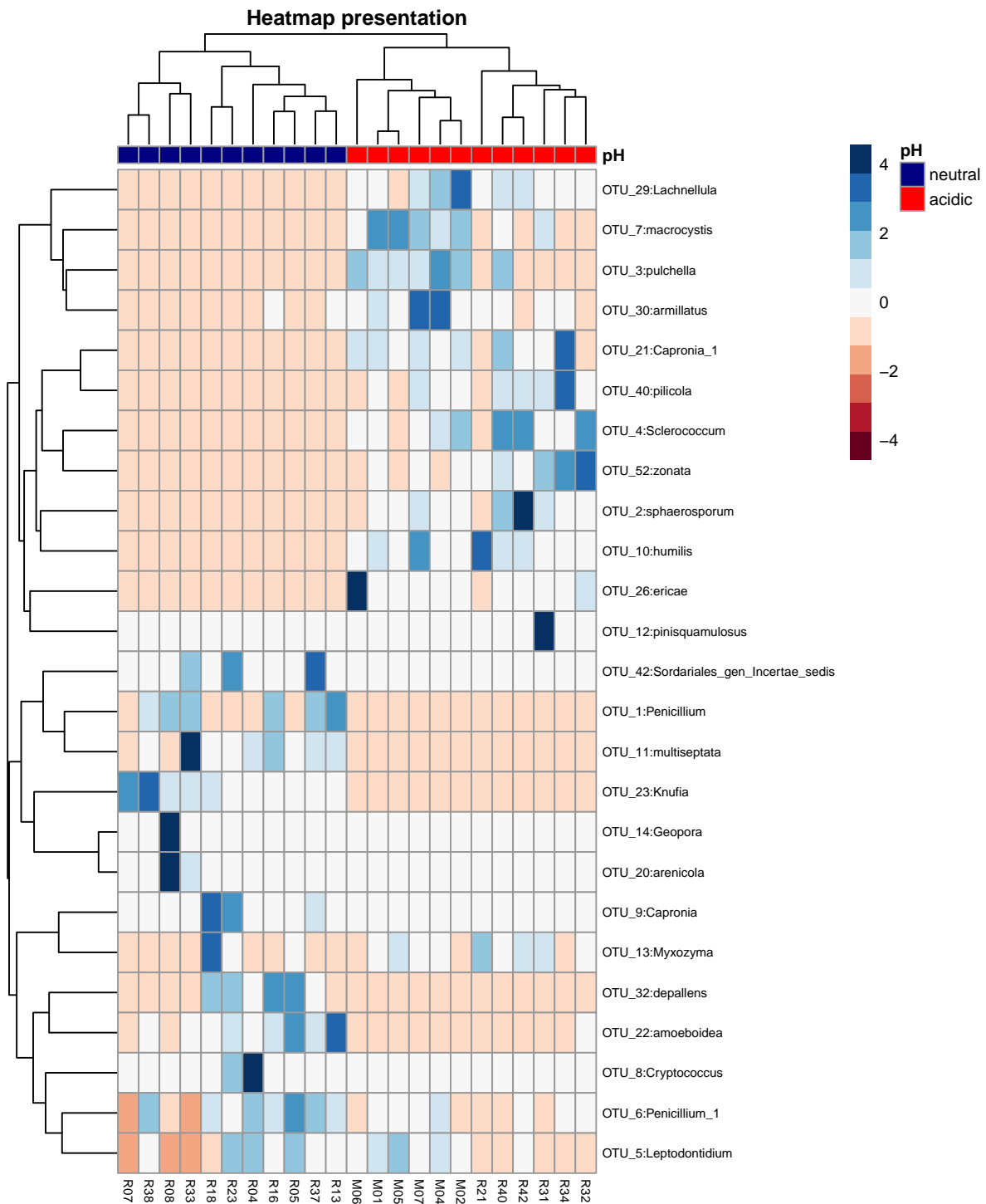
Barplot faceted by pH

```
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

```
# Reload object and count relabundance
tse <- readRDS("results/tse_itsall.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)
```



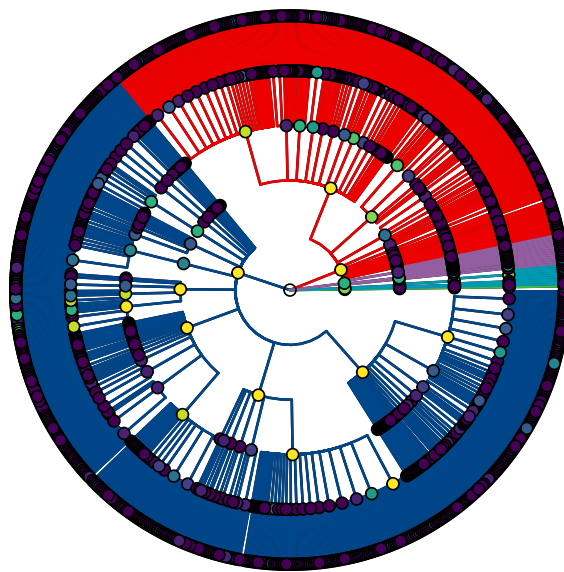
Taxonomy tree plot objects for soil pH groups

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

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

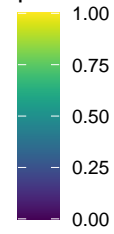

Soil = neutral



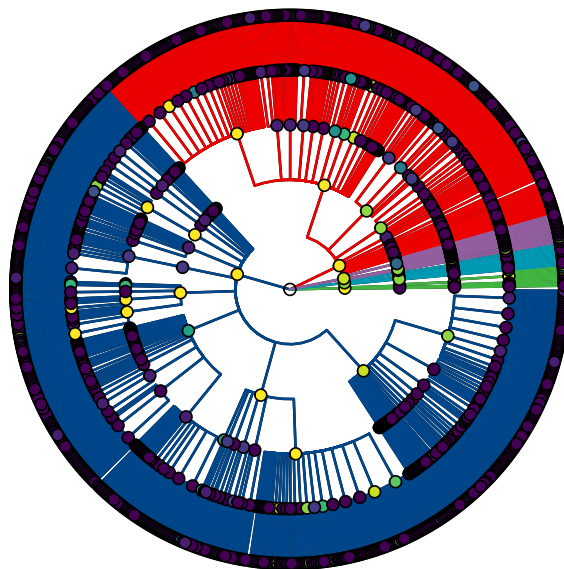
edge_colour_by

- Ascomycota
- Basidiomycota
- Fungi_pty_Incertae_sedis
- Mortierellomycota
- Rozellomycota

prevalence



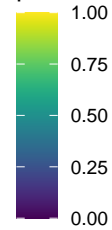
Soil = acidic



edge_colour_by

- Ascomycota
- Basidiomycota
- Mortierellomycota
- Mucoromycota
- Rozellomycota

prevalence



Alpha diversity

Calculation of alpha diversity indexes

```
tse <- readRDS("results/tse_itsall.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	3.35	0.89	0.50	858
R16	3.92	0.94	0.55	1254
R07	4.43	0.97	0.64	1036
R37	3.81	0.95	0.56	859
R33	3.40	0.91	0.49	1087
R13	4.21	0.95	0.57	1581
M06	4.56	0.97	0.62	1655
R38	4.19	0.96	0.59	1240
R05	4.30	0.96	0.59	1566
R34	4.75	0.98	0.63	1805
R32	4.75	0.97	0.63	1832
R04	4.34	0.96	0.59	1606
M04	4.70	0.98	0.62	2061
M07	4.74	0.98	0.62	1999
M01	4.92	0.98	0.64	2043
R21	4.74	0.97	0.64	1660
R18	4.05	0.95	0.56	1313
R40	4.75	0.98	0.64	1636
R42	4.35	0.95	0.58	1874
M05	4.65	0.97	0.62	1777
M02	4.69	0.98	0.62	2057
R31	4.45	0.96	0.60	1676
R23	4.38	0.96	0.58	1854

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")

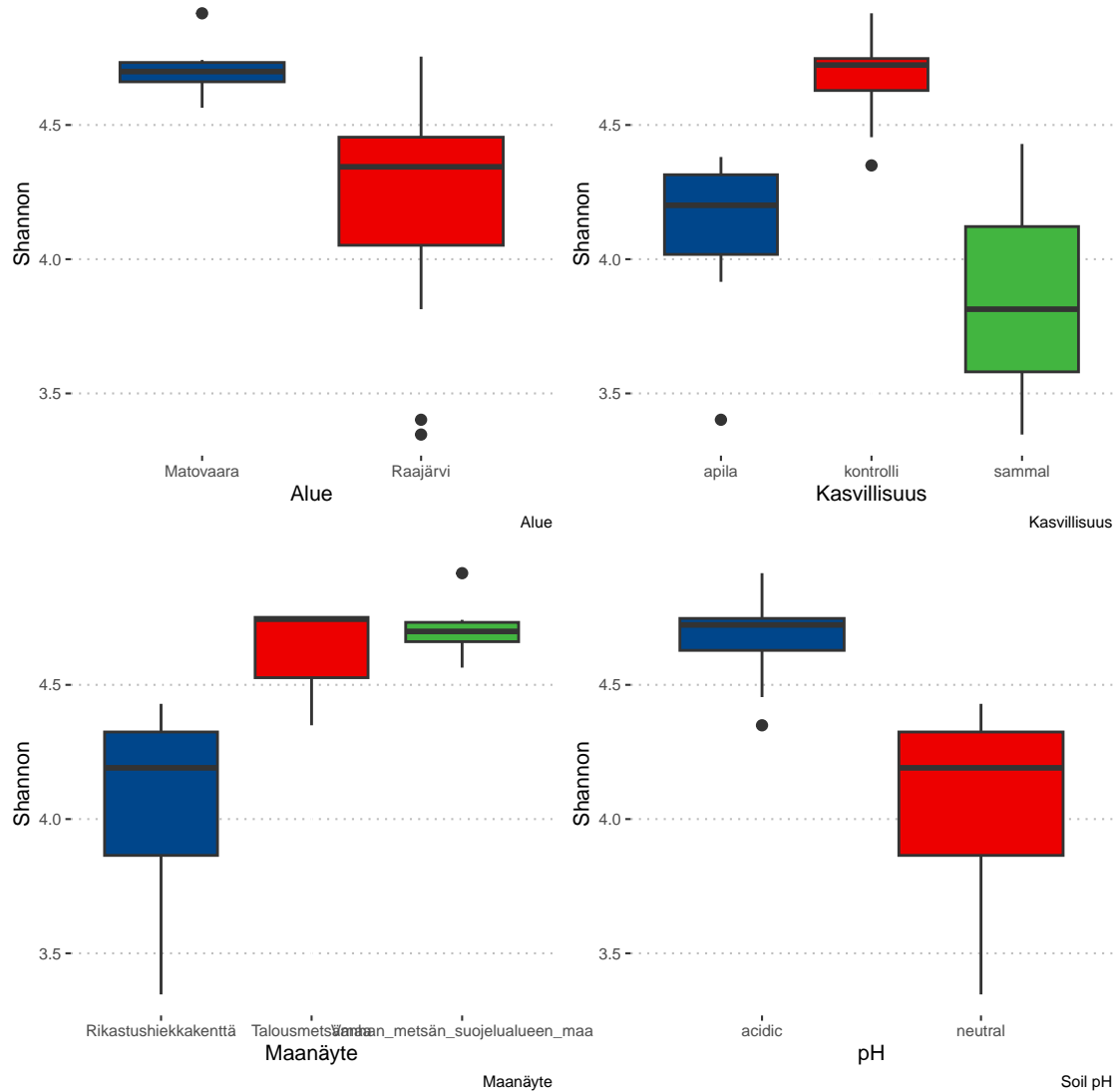
# Area
plot_alue <- ggplot(alphadf, aes(x=Alue, y=Shannon,
                                fill=Alue)) + geom_boxplot() +
  common_theme + labs(caption="Alue")

# 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
```



Kruskal-Wallis tests for variables

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

Kruskal-Wallis rank sum test

data: Shannon by Kasvillisuus
Kruskal-Wallis chi-squared = 15.58, df = 2, p-value = 0.0004139

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

Kruskal-Wallis rank sum test

data: Shannon by Alue

Kruskal-Wallis chi-squared = 4.7108, df = 1, p-value = 0.02997

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

Kruskal-Wallis rank sum test

data: Shannon by Maanäyte

Kruskal-Wallis chi-squared = 15.522, df = 2, p-value = 0.0004259

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

Kruskal-Wallis rank sum test

data: Shannon by pH

Kruskal-Wallis chi-squared = 15.515, df = 1, p-value = 8.185e-05

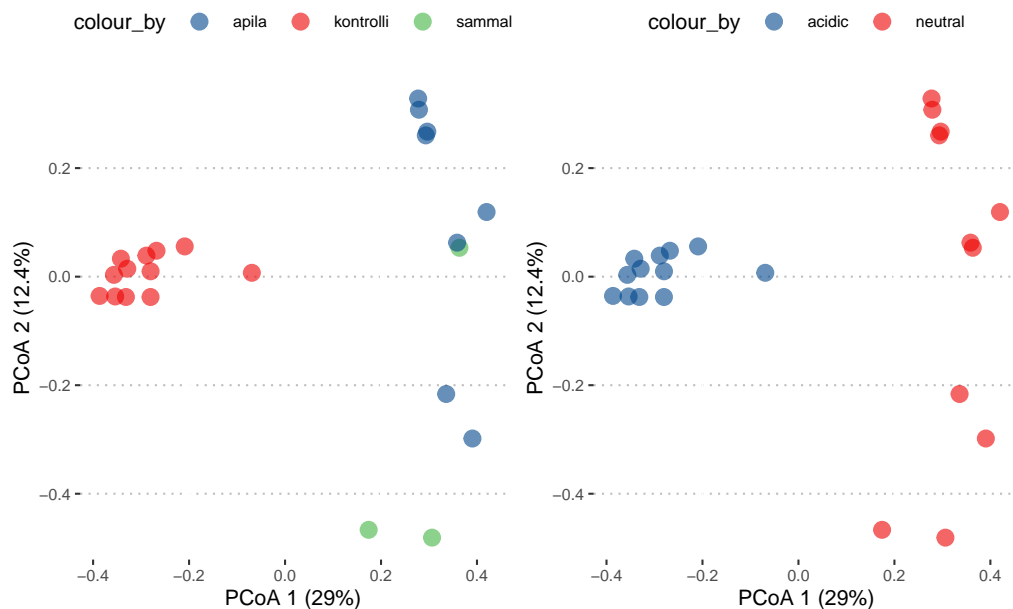
Betadiversity

Bray-Curtis distances ja PCoA ordination plots

```
# Reload object
tse <- readRDS("results/tse_itsall.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()
```



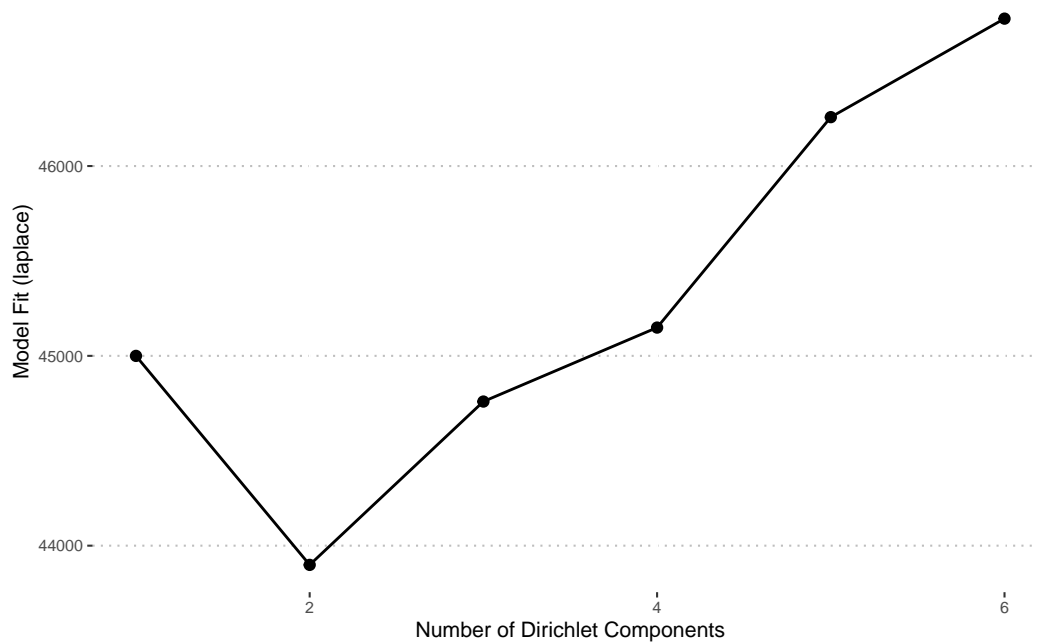
Dirichlet-Multinomial Mixture model

Cluster calculation

```
# Load the tse object
tse <- readRDS("results/tse_itsall.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)

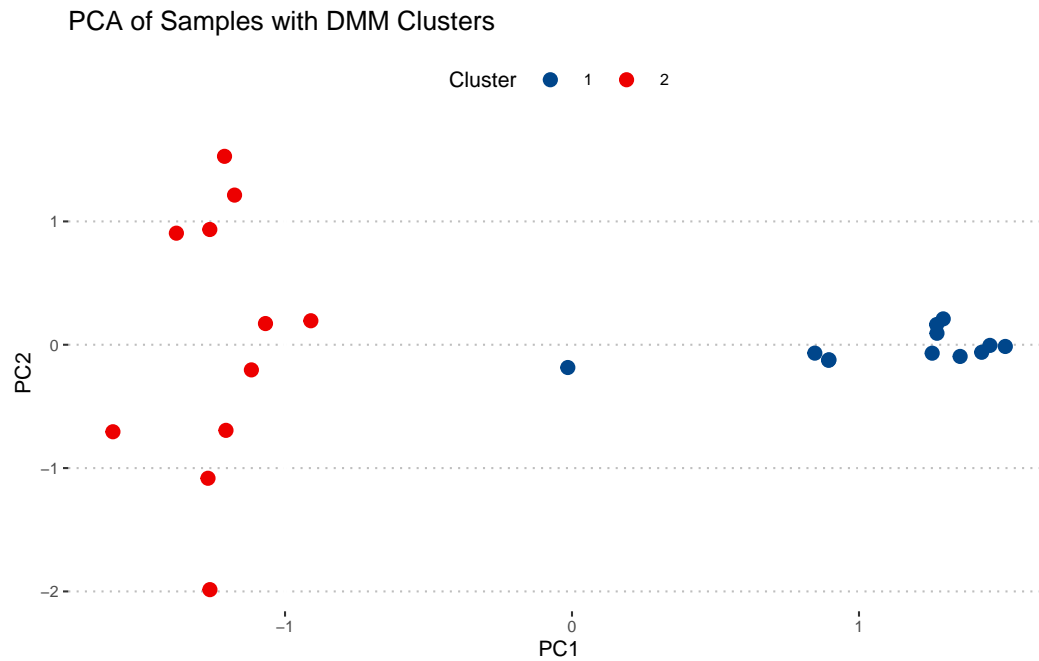
```
# Plot Laplace approximation results
p <- plotDMMFit(altExp(tse, "prevalent"), type = "laplace", name = "DMM")
p + theme_pubclean(base_size = 8)
```



```

# 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")

```



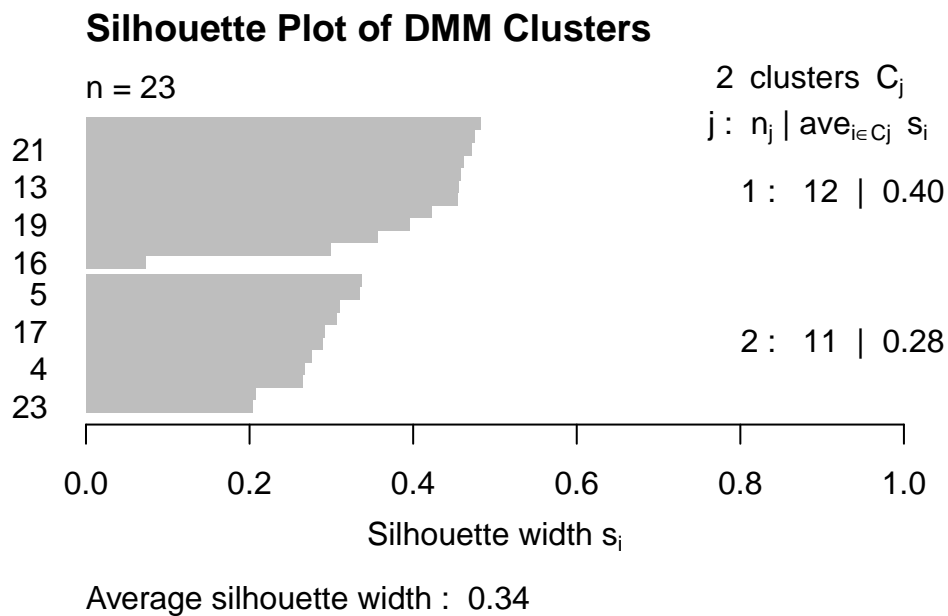
Eigen values for PCA plot

```
# 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	44.27	44.27
PC2	15.53	59.80

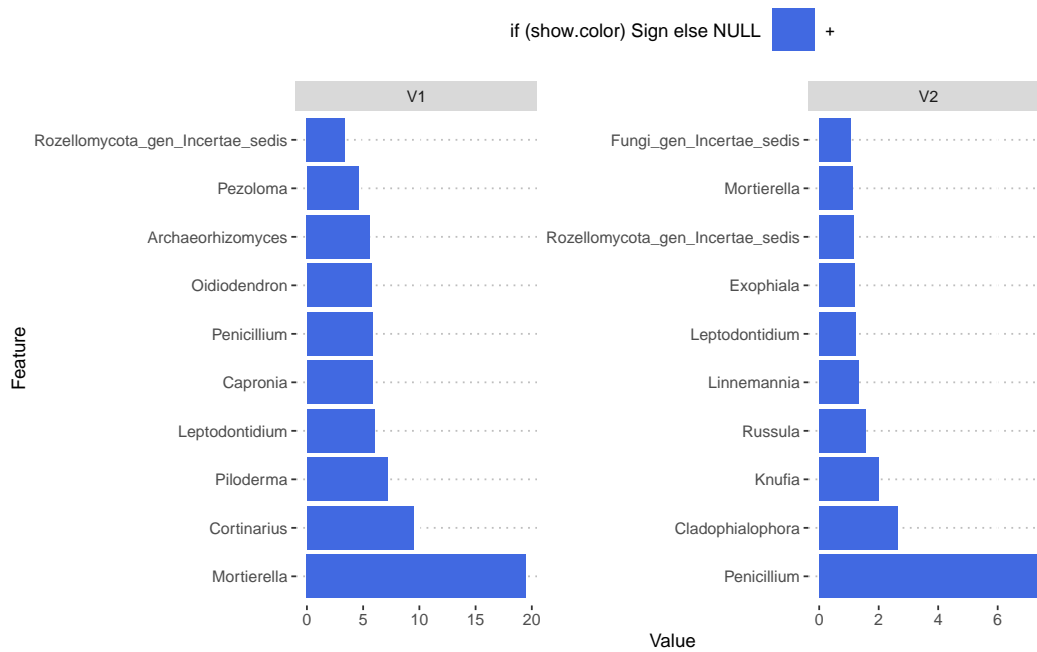
Silhouette plot of clustered samples. Higher the values, tighter the cluster.

```
# 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")
```



Cluster loadings - ten most important features driving samples into clusters 1 & 2

```
# 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

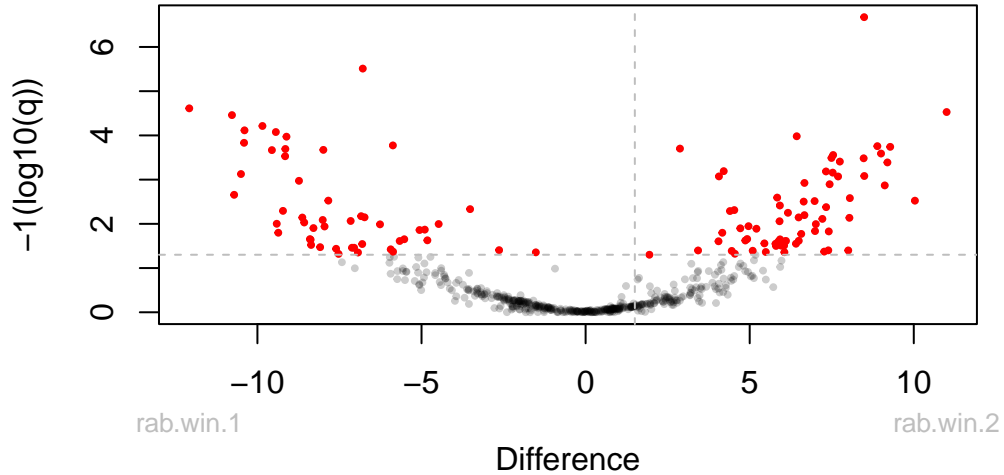
```
# Load tse
tse <- readRDS("results/tse_itsall.rds")
tse <- tse[,1:23]
# Agglomerate by genus and subset by prevalence
tse <- subsetByPrevalent(tse, rank = "Genus", prevalence = 10/100, detection=5/1000)
# Factor pH category
tse$pH <- factor(tse$pH, levels = c("neutral", "acidic"))
```

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
Piloderma	3.25	0.00	0.00	0.00
Entomortierella	3.22	0.00	0.00	0.00
Archaeorhizomyces	2.99	0.00	0.00	0.00
Meliniomyces	2.90	0.00	0.00	0.00
Podila	2.79	0.00	0.00	0.00
Hyphodiscus	2.63	0.00	0.00	0.00
Botryobasidium	2.58	0.00	0.00	0.00
Mortierella	2.49	0.01	0.00	0.00
Rhizidium	2.42	0.00	0.00	0.00
GS21_gen_Incertae_sedis	2.38	0.00	0.00	0.00
Chaetothyriales_gen_Incertae_sedis	2.23	0.01	0.00	0.00
Cephalothecaceae_gen_Incertae_sedis	2.01	0.00	0.00	0.00
Lecanicillium	1.97	0.01	0.00	0.00
Pezoloma	1.94	0.02	0.00	0.00
Humicolopsis	1.93	0.01	0.00	0.00
Sugiyamaella	1.92	0.02	0.00	0.00
Sclerococcum	1.89	0.08	0.00	0.00
Blastocladiomycota_gen_Incertae_sedis	1.86	0.02	0.00	0.00
Lachnellula	1.75	0.03	0.00	0.00
Acarosporales_gen_Incertae_sedis	1.72	0.05	0.01	0.00
Crocicreas	1.72	0.00	0.00	0.00
Cenococcum	1.65	0.05	0.00	0.00
Cortinarius	1.63	0.02	0.00	0.00
Leptobacillium	1.54	0.06	0.01	0.00
Leotiomycetes_gen_Incertae_sedis	1.54	0.06	0.00	0.00
Leucosporidium	1.53	0.03	0.00	0.00
Serendipitaceae_gen_Incertae_sedis	1.52	0.02	0.00	0.00
GS15_gen_Incertae_sedis	1.51	0.06	0.00	0.00
Mycena	1.45	0.04	0.00	0.00
Phacidium	1.43	0.02	0.00	0.00
Tyrannosorus	1.42	0.06	0.00	0.00
Pseudoplectania	1.41	0.08	0.01	0.00
Branch01_gen_Incertae_sedis	1.41	0.10	0.01	0.01
Phialocephala	1.39	0.06	0.01	0.00
Leccinum	1.38	0.07	0.01	0.00
Trechispora	1.34	0.05	0.01	0.00
GS22_gen_Incertae_sedis	1.30	0.10	0.02	0.01
Synccephalis	1.26	0.06	0.01	0.00
Occultifur	1.22	0.10	0.01	0.01
Hymenochaetales_gen_Incertae_sedis	1.21	0.11	0.01	0.01
Luellia	1.21	0.11	0.03	0.01
Galerina	1.19	0.10	0.02	0.00
Tremella	1.18	0.14	0.02	0.02
Sorocybe	1.17	0.08	0.01	0.00

(continued)

Genus	effect	overlap	we.eBH	wi.eBH
Scleropezicula	1.17	0.12	0.03	0.02
Pochonia	1.15	0.10	0.05	0.01
Cenangiaceae_gen_Incertae_sedis	1.14	0.11	0.02	0.01
Apiotrichum	1.14	0.13	0.01	0.01
Tylospora	1.14	0.13	0.01	0.01
Pezizomycotina_gen_Incertae_sedis	1.14	0.12	0.02	0.01
GS04_gen_Incertae_sedis	1.12	0.13	0.03	0.02
Claussenomyces	1.08	0.13	0.03	0.01
Dermateaceae_gen_Incertae_sedis	1.08	0.10	0.03	0.01
Chloridium	1.07	0.12	0.02	0.01
Diademospora	1.06	0.16	0.04	0.05
Filobasidiales_gen_Incertae_sedis	1.06	0.12	0.04	0.01
Clavaria	1.05	0.11	0.03	0.01
Allantophomopsiella	1.03	0.11	0.02	0.01
Tolypocladium	1.03	0.15	0.03	0.02
Chytridiales_gen_Incertae_sedis	1.02	0.14	0.04	0.02
Acarospora	1.02	0.14	0.02	0.02
Auriculariales_gen_Incertae_sedis	1.00	0.14	0.04	0.02
Entoloma	1.00	0.12	0.09	0.01
Myxozyma	0.97	0.18	0.04	0.04
Cystoderma	0.96	0.13	0.04	0.03
Pseudohyphozyma	0.95	0.13	0.07	0.03
Pezicula	0.95	0.12	0.09	0.01
Thaxterogaster	0.93	0.12	0.02	0.01
Aspicilia	0.93	0.16	0.09	0.04
Fayodia	0.92	0.15	0.08	0.04
Umbelopsis	0.91	0.11	0.05	0.01
Babjeviella	0.91	0.15	0.07	0.05
Pseudogymnoascus	0.88	0.16	0.04	0.03
Nagrajchalara	0.86	0.16	0.12	0.04
Syzygospora	0.86	0.17	0.05	0.03
Ceratobasidium	-0.94	0.13	0.04	0.02
Kurtzmaniella	-0.96	0.14	0.06	0.04
Thelephora	-1.00	0.15	0.02	0.03
Vishniacozyma	-1.00	0.13	0.06	0.02
Cylindrosyndrium	-1.03	0.16	0.04	0.05
Mallocybe	-1.04	0.13	0.05	0.03
Schizothecium	-1.05	0.13	0.06	0.05
Cryptococcus	-1.08	0.16	0.08	0.04
Preussia	-1.09	0.10	0.01	0.01
Sporormiella	-1.09	0.15	0.02	0.04
Laetinaevia	-1.09	0.07	0.02	0.00
Hamatocanthoscypha	-1.10	0.12	0.03	0.03
Nothodactylaria	-1.10	0.13	0.06	0.04
Herpotrichia	-1.11	0.13	0.04	0.03
Verrucocum	-1.13	0.14	0.04	0.04
Rhizopogon	-1.14	0.13	0.01	0.02
Mycosphaerellales_gen_Incertae_sedis	-1.15	0.12	0.03	0.03
Cadophora	-1.18	0.08	0.01	0.01
Oliveonia	-1.19	0.07	0.01	0.01
Neonectria	-1.20	0.12	0.02	0.02
Inocybe	-1.20	0.09	0.01	0.01
Cyphellophoraceae_gen_Incertae_sedis	-1.21	0.06	0.01	0.00
Polyphilus	-1.22	0.14	0.03	0.04
Leptodophora	-1.24	0.10	0.02	0.01

(continued)

Genus	effect	overlap	we.eBH	wi.eBH
Pulvinula	-1.24	0.12	0.02	0.03
Penicillium	-1.30	0.12	0.04	0.02
Cladophialophora	-1.31	0.07	0.00	0.00
Triscelophorus	-1.32	0.11	0.03	0.03
Psoroglaena	-1.37	0.09	0.01	0.01
Cosmospora	-1.43	0.07	0.01	0.00
Ascochyta	-1.43	0.04	0.01	0.00
Phomatospora	-1.44	0.08	0.01	0.01
Plectosphaerella	-1.52	0.09	0.01	0.01
Kurtzmanomyces	-1.52	0.06	0.01	0.01
Helvella	-1.55	0.04	0.00	0.00
Pseudocoleophoma	-1.57	0.06	0.01	0.00
Geopora	-1.64	0.05	0.00	0.00
Leohumicola	-1.66	0.05	0.00	0.00
Lentitheciaceae_gen_Incertae_sedis	-1.67	0.07	0.01	0.01
Lobulomycetales_gen_Incertae_sedis	-1.76	0.05	0.00	0.00
Cladosporium	-1.79	0.02	0.00	0.00
Nectriella	-1.85	0.01	0.00	0.00
Tetracladium	-1.88	0.02	0.00	0.00
Talaromyces	-1.98	0.04	0.00	0.00
Pleotrichocladium	-2.00	0.03	0.00	0.00
Kodamaea	-2.16	0.00	0.00	0.00
Sclerostagonospora	-2.28	0.01	0.00	0.00
Truncatella	-2.37	0.01	0.00	0.00
Fusarium	-2.55	0.00	0.00	0.00
Paraphoma	-2.89	0.00	0.00	0.00
Exophiala	-2.99	0.00	0.00	0.00
Pezizellaster	-3.21	0.00	0.00	0.00
Knufia	-3.53	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
Archaeorhizomyces	7.79	0.65	0.00	TRUE
Sclerococcum	7.00	0.64	0.00	TRUE
Meliniomyces	6.58	0.55	0.00	TRUE
Piloderma	6.48	0.55	0.00	TRUE
Hyphodiscus	6.08	0.55	0.00	TRUE
Podila	5.82	0.54	0.00	TRUE
Rhizidium	5.74	0.61	0.00	TRUE
Entomortierella	5.66	0.35	0.00	TRUE
GS12_gen_Incertae_sedis	5.62	0.66	0.00	TRUE
Cephalothecaceae_gen_Incertae_sedis	5.60	0.63	0.00	TRUE
Branch01_gen_Incertae_sedis	5.33	0.72	0.00	TRUE
Chaetothyriales_gen_Incertae_sedis	5.26	0.54	0.00	TRUE
Pseudoplectania	5.22	0.61	0.00	TRUE
Pezoloma	5.20	0.70	0.00	TRUE
Leotiomycetes_gen_Incertae_sedis	4.92	0.79	0.00	TRUE
GS21_gen_Incertae_sedis	4.71	0.44	0.00	TRUE
Humicolopsis	4.51	0.52	0.00	TRUE
Clavaria	4.44	0.82	0.00	TRUE
Tylospora	4.33	0.90	0.00	TRUE
Leucosporidium	4.31	0.69	0.00	TRUE
Herpotrichiellaceae_gen_Incertae_sedis	4.25	0.95	0.00	TRUE
Phialocephala	4.16	0.67	0.00	TRUE
Tolypocladium	4.12	0.79	0.00	TRUE
Serendipitaceae_gen_Incertae_sedis	4.12	0.59	0.00	TRUE
Crocicreas	3.99	0.51	0.00	TRUE
Mycena	3.98	0.61	0.00	TRUE
Cortinarius	3.70	0.58	0.00	TRUE
Lachnellula	3.69	0.56	0.00	TRUE
Myxozyma	3.60	1.02	0.01	TRUE
Tyrannosorus	3.48	0.61	0.00	TRUE
Trechispora	3.44	0.60	0.00	TRUE
Hymenochaetales_gen_Incertae_sedis	3.39	0.67	0.00	TRUE
Glutinomyces	3.34	0.59	0.00	TRUE
Cenococcum	3.32	0.68	0.00	TRUE
Mycosymbiocytes	3.22	0.95	0.01	TRUE
Chytridiales_gen_Incertae_sedis	3.13	0.63	0.00	TRUE
Apiotrichum	3.13	0.78	0.00	TRUE
Syncephalis	3.09	0.49	0.00	TRUE
Claussenomyces	2.98	0.60	0.00	TRUE
Volutella	2.79	0.65	0.01	TRUE
Helotiales_gen_Incertae_sedis	2.78	0.92	0.02	TRUE
Mortierella	2.78	0.44	0.00	TRUE
Sistotrema	2.77	0.79	0.01	TRUE
Umbelopsis	2.76	0.67	0.00	TRUE
Phacidium	2.76	0.48	0.00	TRUE

(continued)

Genus	lfc	se	q	diff
Dissophora	2.60	0.49	0.01	TRUE
Babjevia	2.54	0.80	0.01	TRUE
Saccharomycetales_gen_Incertae_sedis	2.53	0.88	0.02	TRUE
Occultifur	2.41	0.64	0.01	TRUE
Syzygospora	2.41	0.72	0.01	TRUE
Ramariopsis	2.38	0.91	0.05	TRUE
Galerina	2.28	0.48	0.00	TRUE
Pyronemataceae_gen_Incertae_sedis	2.26	0.89	0.05	TRUE
Genolevuria	2.24	0.54	0.00	TRUE
Pezizomycotina_gen_Incertae_sedis	2.22	0.55	0.00	TRUE
Thaxterogaster	2.22	0.82	0.04	TRUE
Oidiodendron	2.10	0.64	0.01	TRUE
Gymnopus	2.05	0.70	0.04	TRUE
Basidiomycota_gen_Incertae_sedis	1.96	0.44	0.01	TRUE
Chloridium	1.94	0.50	0.01	TRUE
Pseudogymnoascus	1.82	0.49	0.01	TRUE
Entoloma	1.81	0.73	0.05	TRUE
Leucosporidiales_gen_Incertae_sedis	1.66	0.68	0.05	TRUE
Xenochalara	1.55	0.62	0.04	TRUE
Sorocybe	1.52	0.45	0.01	TRUE
Mollisia	1.36	0.55	0.05	TRUE
Dothideales_gen_Incertae_sedis	-1.12	0.34	0.04	TRUE
Camptobasidiaceae_gen_Incertae_sedis	-1.23	0.37	0.04	TRUE
Sanchytium	-1.41	0.58	0.05	TRUE
Podospora	-1.47	0.50	0.04	TRUE
Kurtzmaniella	-1.53	0.55	0.04	TRUE
Mytilinidiales_gen_Incertae_sedis	-1.60	0.43	0.04	TRUE
Epibryon	-1.62	0.43	0.04	TRUE
Sepedonium	-1.63	0.61	0.05	TRUE
Drechmeria	-1.64	0.49	0.02	TRUE
Malassezia	-1.65	0.66	0.05	TRUE
Agaricales_gen_Incertae_sedis	-1.70	0.39	0.01	TRUE
Cladophialophora	-1.78	0.54	0.01	TRUE
Sakaguchia	-1.80	0.46	0.01	TRUE
Leprocaulon	-1.81	0.42	0.01	TRUE
Chalara	-1.85	0.68	0.03	TRUE
Taphrina	-1.87	0.56	0.03	TRUE
Lycoperdon	-1.89	0.71	0.04	TRUE
Neocrodontium	-1.98	0.48	0.01	TRUE
Cadophora	-1.99	0.61	0.01	TRUE
Thelebolus	-2.04	0.74	0.03	TRUE
Ceratobasidium	-2.04	0.70	0.02	TRUE
Paramicrosporidium	-2.18	0.44	0.01	TRUE
Mrakia	-2.18	0.41	0.01	TRUE
Laetinaevia	-2.22	0.68	0.01	TRUE
Sporormiella	-2.34	0.89	0.03	TRUE
Hormonema	-2.34	0.40	0.00	TRUE
Preussia	-2.39	0.80	0.02	TRUE
Rhizopogon	-2.40	0.81	0.02	TRUE
Lapidomyces	-2.45	0.40	0.00	TRUE
Vishniacozyma	-2.58	0.69	0.00	TRUE
Thelephora	-2.64	0.89	0.02	TRUE
Neonectria	-2.94	0.50	0.00	TRUE
Cosmospora	-3.00	0.62	0.00	TRUE
Pseudeurotium	-3.04	0.67	0.00	TRUE

(continued)

Genus	lfc	se	q	diff
Ceratobasidiaceae_gen_Incertae_sedis	-3.31	0.47	0.00	TRUE
Helminthosphaeriaceae_gen_Incertae_sedis	-3.38	0.49	0.00	TRUE
Pleotrichocladium	-3.45	0.67	0.00	TRUE
Pleosporales_gen_Incertae_sedis	-3.51	0.35	0.00	TRUE
Inocybe	-3.53	1.01	0.01	TRUE
Cladosporium	-3.54	0.69	0.00	TRUE
Leptodophora	-3.68	0.90	0.00	TRUE
Cryptococcus	-3.70	0.87	0.00	TRUE
Sclerostagonospora	-3.79	0.56	0.00	TRUE
Alternaria	-3.84	0.64	0.01	TRUE
Exophiala	-3.98	0.54	0.00	TRUE
Lecythophora	-3.99	0.63	0.00	TRUE
Leohumicola	-4.00	0.95	0.00	TRUE
Tetracladium	-4.34	0.85	0.00	TRUE
Talaromyces	-4.48	0.79	0.00	TRUE
Lentitheciaceae_gen_Incertae_sedis	-4.82	0.48	0.00	TRUE
Kodamaea	-4.97	0.68	0.00	TRUE
Mallocybe	-4.98	0.86	0.00	TRUE
Plectosphaerella	-5.15	0.51	0.00	TRUE
Fusarium	-5.24	0.60	0.00	TRUE
Pezizellaster	-5.63	0.55	0.00	TRUE
Knufia	-6.20	0.48	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 = "ITSF",
  transform = "AST",
  fixed_effects = "pH",
  reference = "pH,neutral",
  normalization = "TSS",
  standardize = FALSE,
  min_prevalence = 0)
saveRDS(maaslin2_out, "results/itsfull_maaslin2.rds")
```

Genera with significantly different abundance

```
maaslin2_out <- readRDS("results/itsfull_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	0.24	0.03	0.00	23
Piloderma	0.21	0.04	0.00	23
Cortinarius	0.19	0.03	0.00	23

(continued)

Genus	coef	stderr	qval	N.not.zero
Archaeorhizomyces	0.19	0.03	0.00	22
Pezoloma	0.17	0.03	0.00	23
Sclerococcum	0.15	0.03	0.00	19
Podila	0.13	0.02	0.00	22
Meliniomyces	0.13	0.02	0.00	22
Lachnellula	0.10	0.02	0.00	23
Tylospora	0.09	0.03	0.03	21
Leotiomycetes_gen_Incertae_sedis	0.09	0.02	0.00	20
Oidiodendron	0.09	0.03	0.03	23
Rhizidium	0.08	0.01	0.00	22
Cephalothecaceae_gen_Incertae_sedis	0.07	0.02	0.00	19
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	0.05	0.01	0.00	19
Phialocephala	0.05	0.01	0.00	20
Apiotrichum	0.05	0.01	0.01	18
Leucosporidium	0.05	0.01	0.00	22
Cenococcum	0.04	0.01	0.00	15
Hyphodiscus	0.04	0.01	0.00	15
Entomortierella	0.04	0.00	0.00	15
Pseudoplectania	0.04	0.01	0.04	18
Branch01_gen_Incertae_sedis	0.04	0.01	0.02	15
Mycena	0.04	0.01	0.00	20
Humicolopsis	0.03	0.01	0.00	17
Sugiyamaella	0.03	0.01	0.00	14
GS12_gen_Incertae_sedis	0.03	0.01	0.04	11
Tyrannosorus	0.03	0.01	0.00	17
Syncephalis	0.03	0.01	0.00	19
Babjevia	0.03	0.01	0.03	22
Syzygospora	0.03	0.01	0.02	19
Tolypocladium	0.03	0.01	0.03	18
Diademospora	0.03	0.01	0.02	11
Blastocladiomycota_gen_Incertae_sedis	0.03	0.01	0.00	14
GS21_gen_Incertae_sedis	0.03	0.00	0.00	15
Hymenochaetales_gen_Incertae_sedis	0.02	0.01	0.01	14
Crocicreas	0.02	0.01	0.00	16
Trechispora	0.02	0.01	0.01	21
Claussenomyces	0.02	0.01	0.01	16
Lecanicillium	0.02	0.00	0.00	13
Pezizomycotina_gen_Incertae_sedis	0.02	0.01	0.02	18
Glomeromycota_gen_Incertae_sedis	0.02	0.00	0.01	9
Chytridiales_gen_Incertae_sedis	0.02	0.00	0.01	17
Botryobasidium	0.02	0.00	0.00	13
Luellia	0.02	0.00	0.02	11
GS15_gen_Incertae_sedis	0.02	0.00	0.00	13
Chaetosphaeria	0.02	0.01	0.03	10
Auriculariales_gen_Incertae_sedis	0.01	0.01	0.04	11
Nadsonia	0.01	0.01	0.05	7
GS22_gen_Incertae_sedis	0.01	0.00	0.01	10
Leptobacillium	0.01	0.00	0.00	14
Cenangiaceae_gen_Incertae_sedis	0.01	0.00	0.01	11
Acarosporales_gen_Incertae_sedis	0.01	0.00	0.00	12
Galerina	0.01	0.00	0.00	18
Genolevuria	0.01	0.00	0.04	14

(continued)

Genus	coef	stderr	qval	N.not.zero
GS04_gen_Incertae_sedis	0.01	0.00	0.00	11
Synnemapestaloides	0.01	0.00	0.04	11
Siepmannia	0.01	0.00	0.03	8
Scleropezicula	0.01	0.00	0.00	10
Dermateaceae_gen_Incertae_sedis	0.01	0.00	0.00	13
Tremella	0.01	0.00	0.02	14
Pseudogymnoascus	0.01	0.00	0.02	18
Leccinum	0.01	0.00	0.00	12
Babjeviella	0.01	0.00	0.01	11
Sorocybe	0.01	0.00	0.00	15
Rhodotorula	0.01	0.00	0.04	9
Coccomyces	0.01	0.00	0.05	7
Aspicilia	0.01	0.00	0.02	9
Endogonomycetes_gen_Incertae_sedis	0.01	0.00	0.03	9
Fayodia	0.01	0.00	0.01	9
Cystoderma	0.01	0.00	0.01	12
Sympoventuriaceae_gen_Incertae_sedis	0.01	0.00	0.03	9
Tremellomycetes_gen_Incertae_sedis	0.01	0.00	0.04	8
Pochonia	0.01	0.00	0.00	10
Mortierellomycetes_gen_Incertae_sedis	0.01	0.00	0.04	8
Dothiorella	0.01	0.00	0.03	14
Haplographium	0.01	0.00	0.05	8
Gyoeffya	0.01	0.00	0.03	7
Allantophomopsiella	0.01	0.00	0.00	13
Pseudohyphozyma	0.01	0.00	0.01	10
Flammula	0.01	0.00	0.01	16
Rhizosphaera	0.01	0.00	0.02	10
Microsporomycetaceae_gen_Incertae_sedis	0.01	0.00	0.02	10
Brahmaculus	0.01	0.00	0.03	11
Ophiocordycipitaceae_gen_Incertae_sedis	0.01	0.00	0.03	9
Nagrajchalara	0.01	0.00	0.01	9
Cladoriellaceae_gen_Incertae_sedis	0.01	0.00	0.01	9
Sanchytriaceae_gen_Incertae_sedis	0.01	0.00	0.05	7
Dermea	0.00	0.00	0.03	9
Phaeotremella	0.00	0.00	0.02	12
Endogonales_gen_Incertae_sedis	0.00	0.00	0.04	8
Gymnopilus	0.00	0.00	0.02	8
Krasilnikovozyrna	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
Dothiora	0.00	0.00	0.01	10
Yamadamyces	0.00	0.00	0.03	9
Monoblepharomycota_gen_Incertae_sedis	0.00	0.00	0.04	6
Vestigium	0.00	0.00	0.03	6
Aphanocladium	0.00	0.00	0.03	8
Geomyces	0.00	0.00	0.04	6
Scleroconidioma	0.00	0.00	0.04	6
Isthmomyces	0.00	0.00	0.03	7
Lyomyces	0.00	0.00	0.03	6
Trichosporiella	-0.01	0.00	0.05	8
Rhizophagus	-0.01	0.00	0.04	7
Achroceratosphaeria	-0.01	0.00	0.04	5
Nothodactylaria	-0.01	0.00	0.01	9
Herpotrichia	-0.01	0.00	0.03	10
Kurtzmaniella	-0.01	0.00	0.03	13

(continued)

Genus	coef	stderr	qval	N.not.zero
Sporormiaceae_gen_Incertae_sedis	-0.01	0.00	0.05	6
Schizothecium	-0.01	0.00	0.02	10
Triscelophorus	-0.01	0.00	0.00	9
Septoria	-0.01	0.00	0.04	7
Cylindrosyndrium	-0.01	0.00	0.04	12
Helminthosphaeriaceae_gen_Incertae_sedis	-0.01	0.00	0.04	15
Verrucococcum	-0.01	0.00	0.00	8
Lobulomycetales_gen_Incertae_sedis	-0.01	0.00	0.00	11
Ascochyta	-0.02	0.01	0.03	12
Mycosphaerellales_gen_Incertae_sedis	-0.02	0.01	0.03	9
Phomatosporea	-0.02	0.01	0.02	10
Oliveonia	-0.02	0.01	0.03	13
Hamatocanthoscypha	-0.02	0.01	0.03	10
Cosmospora	-0.02	0.00	0.00	13
Entimomentora	-0.02	0.01	0.03	10
Polyphilus	-0.02	0.01	0.01	10
Pulvinula	-0.02	0.01	0.01	9
Kurtzmanomyces	-0.02	0.01	0.02	9
Truncatella	-0.03	0.01	0.00	12
Pseudocoleophoma	-0.03	0.01	0.00	11
Helvella	-0.03	0.01	0.02	13
Lentitheciaceae_gen_Incertae_sedis	-0.03	0.01	0.03	12
Sclerostagonospora	-0.03	0.01	0.00	17
Cadophora	-0.03	0.01	0.01	21
Neonectria	-0.03	0.01	0.01	13
Pleotrichocladium	-0.03	0.01	0.00	14
Plectosphaerella	-0.04	0.01	0.02	12
Lecythophora	-0.04	0.01	0.03	14
Sporormiella	-0.04	0.01	0.01	22
Leptodophora	-0.04	0.01	0.01	15
Paraphoma	-0.04	0.01	0.00	12
Thelephora	-0.04	0.02	0.04	23
Leohumicola	-0.06	0.01	0.00	15
Rhizopogon	-0.06	0.02	0.02	23
Fusarium	-0.07	0.02	0.01	18
Kodamaea	-0.07	0.02	0.01	19
Pezizellaster	-0.08	0.01	0.00	19
Cladosporium	-0.09	0.03	0.05	23
Tetracladium	-0.09	0.03	0.02	16
Talaromyces	-0.10	0.02	0.00	19
Cryptococcus	-0.10	0.04	0.05	22
Exophiala	-0.10	0.01	0.00	23
Cladophialophora	-0.15	0.04	0.01	23
Knufia	-0.16	0.02	0.00	17
Penicillium	-0.18	0.04	0.00	23

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

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	Maasin2
Piloderma	TRUE	TRUE	TRUE
Entomortierella	TRUE	TRUE	TRUE
Archaeorhizomyces	TRUE	TRUE	TRUE
Meliniomyces	TRUE	TRUE	TRUE
Podila	TRUE	TRUE	TRUE
Hyphodiscus	TRUE	TRUE	TRUE
Botryobasidium	TRUE	FALSE	TRUE
Mortierella	TRUE	TRUE	TRUE
Rhizidium	TRUE	TRUE	TRUE
GS21_gen_Incertae_sedis	TRUE	TRUE	TRUE
Chaetothyriales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Cephalothecaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Lecanicillium	TRUE	FALSE	TRUE
Pezoloma	TRUE	TRUE	TRUE
Humicolopsis	TRUE	TRUE	TRUE
Sugiyamaella	TRUE	FALSE	TRUE
Sclerococcum	TRUE	TRUE	TRUE
Blastocladiomycota_gen_Incertae_sedis	TRUE	FALSE	TRUE
Lachnellula	TRUE	TRUE	TRUE
Acarosporales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Crocicreas	TRUE	TRUE	TRUE
Cenococcum	TRUE	TRUE	TRUE
Cortinarius	TRUE	TRUE	TRUE
Leptobacillium	TRUE	FALSE	TRUE
Leotiomyces_gen_Incertae_sedis	TRUE	TRUE	TRUE
Leucosporidium	TRUE	TRUE	TRUE
Serendipitaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
GS15_gen_Incertae_sedis	TRUE	FALSE	TRUE
Mycena	TRUE	TRUE	TRUE
Phacidium	TRUE	TRUE	FALSE
Tyrannosorus	TRUE	TRUE	TRUE
Pseudoplectania	TRUE	TRUE	TRUE
Branch01_gen_Incertae_sedis	TRUE	TRUE	TRUE
Phialocephala	TRUE	TRUE	TRUE
Leccinum	TRUE	FALSE	TRUE
Trechispora	TRUE	TRUE	TRUE
GS22_gen_Incertae_sedis	TRUE	FALSE	TRUE
Syncephalis	TRUE	TRUE	TRUE
Occultifur	TRUE	TRUE	FALSE
Hymenochaetales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Luellia	TRUE	FALSE	TRUE
Galerina	TRUE	TRUE	TRUE
Tremella	TRUE	FALSE	TRUE
Sorocybe	TRUE	TRUE	TRUE
Scleropezicula	TRUE	FALSE	TRUE
Pochonia	TRUE	FALSE	TRUE
Cenangiaceae_gen_Incertae_sedis	TRUE	FALSE	TRUE
Apiotrichum	TRUE	TRUE	TRUE

(continued)

Genus	Aldex2	Ancombc	Maaslin2
Tylospora	TRUE	TRUE	TRUE
Pezizomycotina_gen_Incertae_sedis	TRUE	TRUE	TRUE
GS04_gen_Incertae_sedis	TRUE	FALSE	TRUE
Claussenomyces	TRUE	TRUE	TRUE
Dermateaceae_gen_Incertae_sedis	TRUE	FALSE	TRUE
Chloridium	TRUE	TRUE	FALSE
Diademospora	TRUE	FALSE	TRUE
Filobasidiales_gen_Incertae_sedis	TRUE	FALSE	FALSE
Clavaria	TRUE	TRUE	FALSE
Allantophomopsiella	TRUE	FALSE	TRUE
Tolypocladium	TRUE	TRUE	TRUE
Chytridiales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Acarospora	TRUE	FALSE	FALSE
Auriculariales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Entoloma	TRUE	TRUE	FALSE
Myxozyma	TRUE	TRUE	FALSE
Cystoderma	TRUE	FALSE	TRUE
Pseudohyphozyma	TRUE	FALSE	TRUE
Pezicula	TRUE	FALSE	FALSE
Thaxterogaster	TRUE	TRUE	FALSE
Aspicilia	TRUE	FALSE	TRUE
Fayodia	TRUE	FALSE	TRUE
Umbelopsis	TRUE	TRUE	TRUE
Babjeviella	TRUE	FALSE	TRUE
Pseudogymnoascus	TRUE	TRUE	TRUE
Nagrajchalara	TRUE	FALSE	TRUE
Syzygospora	TRUE	TRUE	TRUE
Ceratobasidium	TRUE	TRUE	FALSE
Kurtzmaniella	TRUE	TRUE	TRUE
Thelephora	TRUE	TRUE	TRUE
Vishniacozyma	TRUE	TRUE	FALSE
Cylindrosyndrium	TRUE	FALSE	TRUE
Mallocybe	TRUE	TRUE	FALSE
Schizothecium	TRUE	FALSE	TRUE
Cryptococcus	TRUE	TRUE	TRUE
Preussia	TRUE	TRUE	FALSE
Sporormiella	TRUE	TRUE	TRUE
Laetinaevia	TRUE	TRUE	FALSE
Hamatocanthoscypha	TRUE	FALSE	TRUE
Nothodactylaria	TRUE	FALSE	TRUE
Herpotrichia	TRUE	FALSE	TRUE
Verrucocum	TRUE	FALSE	TRUE
Rhizopogon	TRUE	TRUE	TRUE
Mycosphaerellales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Cadophora	TRUE	TRUE	TRUE
Oliveonia	TRUE	FALSE	TRUE
Neonectria	TRUE	TRUE	TRUE
Inocybe	TRUE	TRUE	FALSE
Cyphellophoraceae_gen_Incertae_sedis	TRUE	FALSE	FALSE
Polyphilus	TRUE	FALSE	TRUE
Leptodophora	TRUE	TRUE	TRUE
Pulvinula	TRUE	FALSE	TRUE
Penicillium	TRUE	FALSE	TRUE
Cladophialophora	TRUE	TRUE	TRUE
Triscelophorus	TRUE	FALSE	TRUE

(continued)

Genus	Aldex2	Ancombc	Maasin2
Psoroglaena	TRUE	FALSE	FALSE
Cosmospora	TRUE	TRUE	TRUE
Ascochyta	TRUE	FALSE	TRUE
Phomatospora	TRUE	FALSE	TRUE
Plectosphaerella	TRUE	TRUE	TRUE
Kurtzmanomyces	TRUE	FALSE	TRUE
Helvella	TRUE	FALSE	TRUE
Pseudocoleophoma	TRUE	FALSE	TRUE
Geopora	TRUE	FALSE	FALSE
Leohumicola	TRUE	TRUE	TRUE
Lentitheciaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Lobulomycetales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Cladosporium	TRUE	TRUE	TRUE
Nectriella	TRUE	FALSE	FALSE
Tetracladium	TRUE	TRUE	TRUE
Talaromyces	TRUE	TRUE	TRUE
Pleotrichocladium	TRUE	TRUE	TRUE
Kodamaea	TRUE	TRUE	TRUE
Sclerostagonospora	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
GS12_gen_Incertae_sedis	FALSE	TRUE	TRUE
Herpotrichiellaceae_gen_Incertae_sedis	FALSE	TRUE	TRUE
Glutinomyces	FALSE	TRUE	FALSE
Mycosymbiocytes	FALSE	TRUE	FALSE
Volutella	FALSE	TRUE	FALSE
Helotiales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Sistotrema	FALSE	TRUE	FALSE
Dissophora	FALSE	TRUE	FALSE
Babjevia	FALSE	TRUE	TRUE
Saccharomycetales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Ramariopsis	FALSE	TRUE	FALSE
Pyronemataceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Genolevuria	FALSE	TRUE	TRUE
Oidiodendron	FALSE	TRUE	TRUE
Gymnopus	FALSE	TRUE	FALSE
Basidiomycota_gen_Incertae_sedis	FALSE	TRUE	FALSE
Leucosporidiales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Xenochalara	FALSE	TRUE	FALSE
Mollisia	FALSE	TRUE	FALSE
Dothideales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Camptobasidiaceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Sanchytrium	FALSE	TRUE	FALSE
Podospora	FALSE	TRUE	FALSE
Mytiliniidiales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Epibryon	FALSE	TRUE	FALSE
Sepedonium	FALSE	TRUE	FALSE
Drechmeria	FALSE	TRUE	FALSE
Malassezia	FALSE	TRUE	FALSE
Agaricales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Sakaguchia	FALSE	TRUE	FALSE

(continued)

Genus	Aldex2	Ancombc	Maasin2
Leprocaulon	FALSE	TRUE	FALSE
Chalara	FALSE	TRUE	FALSE
Taphrina	FALSE	TRUE	FALSE
Lycoperdon	FALSE	TRUE	FALSE
Neoacrodontium	FALSE	TRUE	FALSE
Thelebolus	FALSE	TRUE	FALSE
Paramicrosporidium	FALSE	TRUE	FALSE
Mrakia	FALSE	TRUE	FALSE
Hormonema	FALSE	TRUE	FALSE
Lapidomyces	FALSE	TRUE	FALSE
Pseudeurotium	FALSE	TRUE	FALSE
Ceratobasidiaceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Helminthosphaeriaceae_gen_Incertae_sedis	FALSE	TRUE	TRUE
Pleosporales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Alternaria	FALSE	TRUE	FALSE
Lecythophora	FALSE	TRUE	TRUE
Glomeromycota_gen_Incertae_sedis	FALSE	FALSE	TRUE
Chaetosphaeria	FALSE	FALSE	TRUE
Nadsonia	FALSE	FALSE	TRUE
Synnemapestaloides	FALSE	FALSE	TRUE
Siepmannia	FALSE	FALSE	TRUE
Rhodotorula	FALSE	FALSE	TRUE
Coccomyces	FALSE	FALSE	TRUE
Endogonomycetes_gen_Incertae_sedis	FALSE	FALSE	TRUE
Sympoventuriaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Tremellomycetes_gen_Incertae_sedis	FALSE	FALSE	TRUE
Mortierellomycetes_gen_Incertae_sedis	FALSE	FALSE	TRUE
Dothiorella	FALSE	FALSE	TRUE
Haplographium	FALSE	FALSE	TRUE
Gyoerffyyella	FALSE	FALSE	TRUE
Flammula	FALSE	FALSE	TRUE
Rhizosphaera	FALSE	FALSE	TRUE
Microsporomycetaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Brahmaculus	FALSE	FALSE	TRUE
Ophiocordycipitaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Cladoriellaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Sanchytriaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Dermea	FALSE	FALSE	TRUE
Phaeotremella	FALSE	FALSE	TRUE
Endogonales_gen_Incertae_sedis	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
Dothiora	FALSE	FALSE	TRUE
Yamadamyces	FALSE	FALSE	TRUE
Monoblepharomycota_gen_Incertae_sedis	FALSE	FALSE	TRUE
Vestigium	FALSE	FALSE	TRUE
Aphanocladium	FALSE	FALSE	TRUE
Geomyces	FALSE	FALSE	TRUE
Scleroconidioma	FALSE	FALSE	TRUE
Isthmomyces	FALSE	FALSE	TRUE
Lyomyces	FALSE	FALSE	TRUE
Trichosporiella	FALSE	FALSE	TRUE
Rhizophagus	FALSE	FALSE	TRUE

(continued)

Genus	Aldex2	Ancombc	Maaslin2
Achroceratosphaeria	FALSE	FALSE	TRUE
Sporormiaceae_gen_Incertae_sedis	FALSE	FALSE	TRUE
Septoria	FALSE	FALSE	TRUE
Entimomentora	FALSE	FALSE	TRUE