

Data-analysis ITS amplicons

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)
```

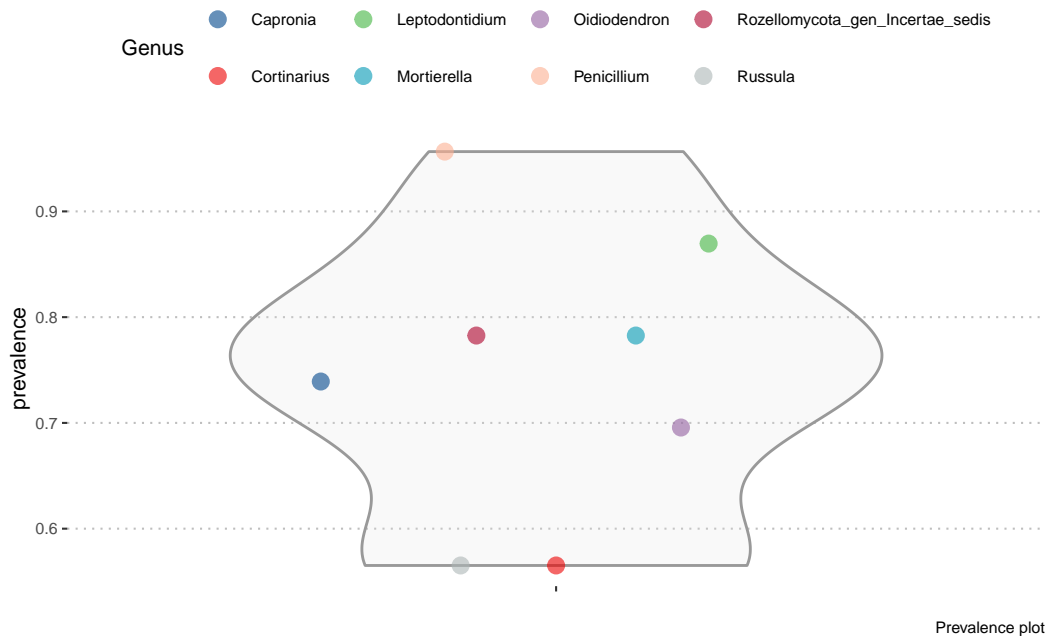
Load data object

```
# Load data from rds and set random seed number
tse <- readRDS("results/TSE.rds")
set.seed(123412)
# Create pH group
```

Prevalent features on data

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")
```



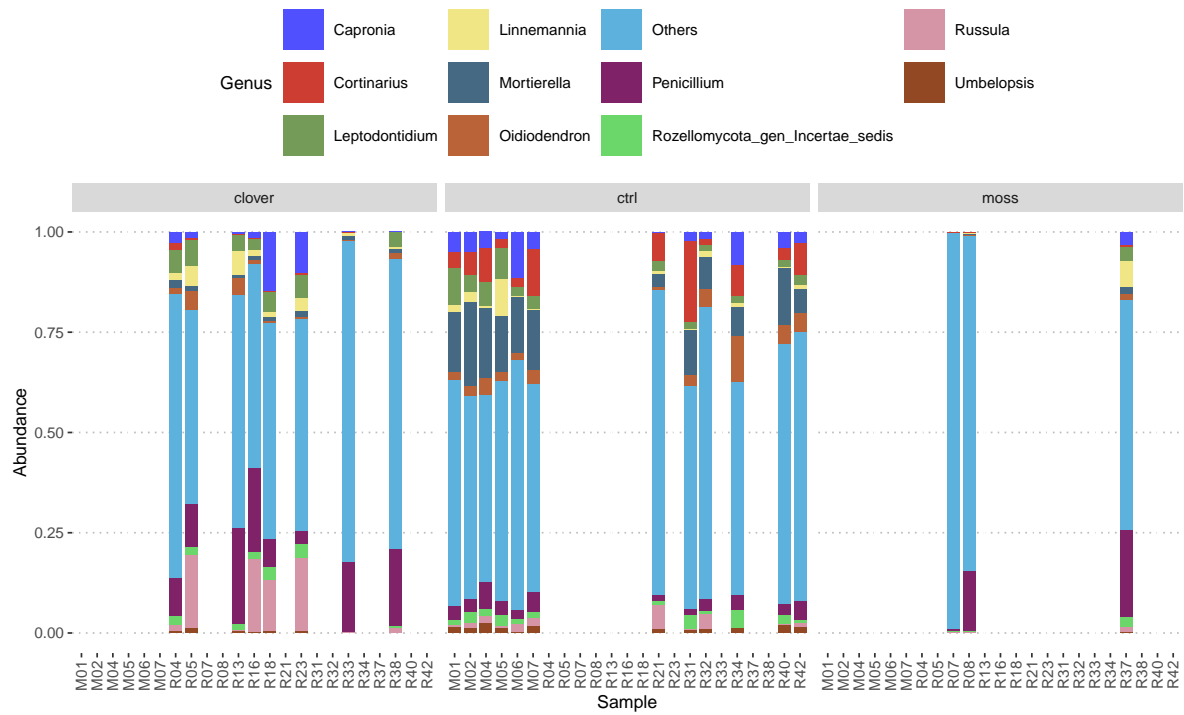
Community composition plots

Top ten features calculated by median relative abundance

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

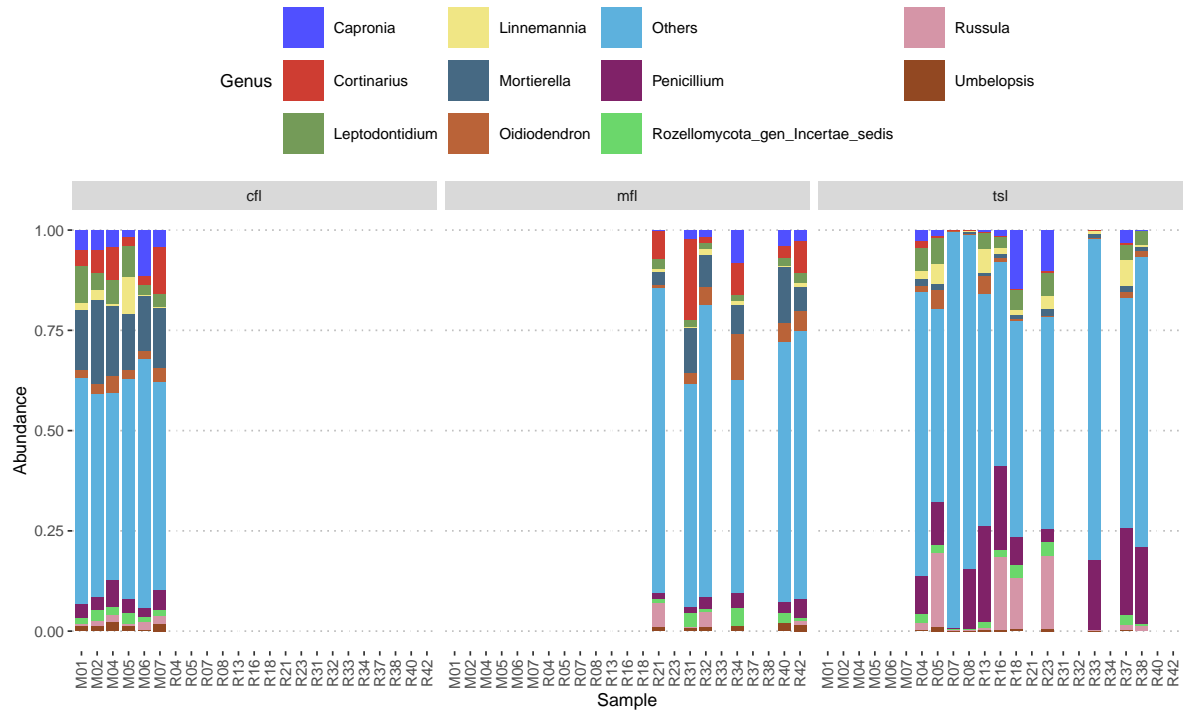
Barplot faceted by vegetation

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



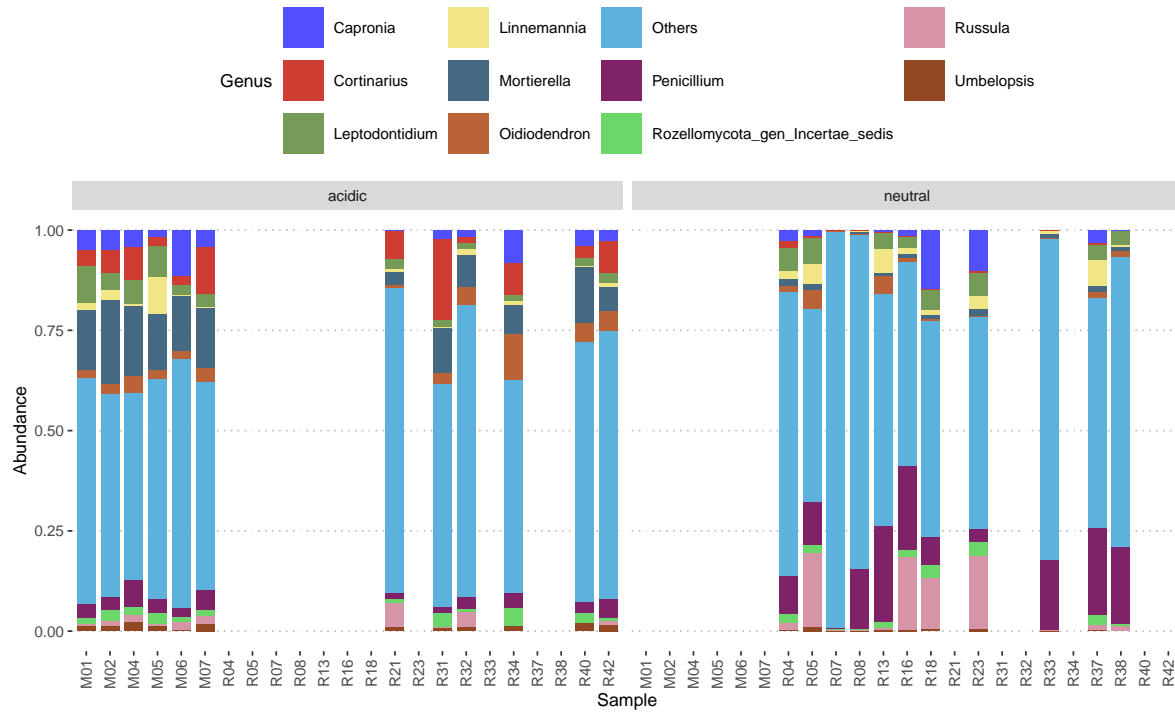
Barplot faceted by type

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



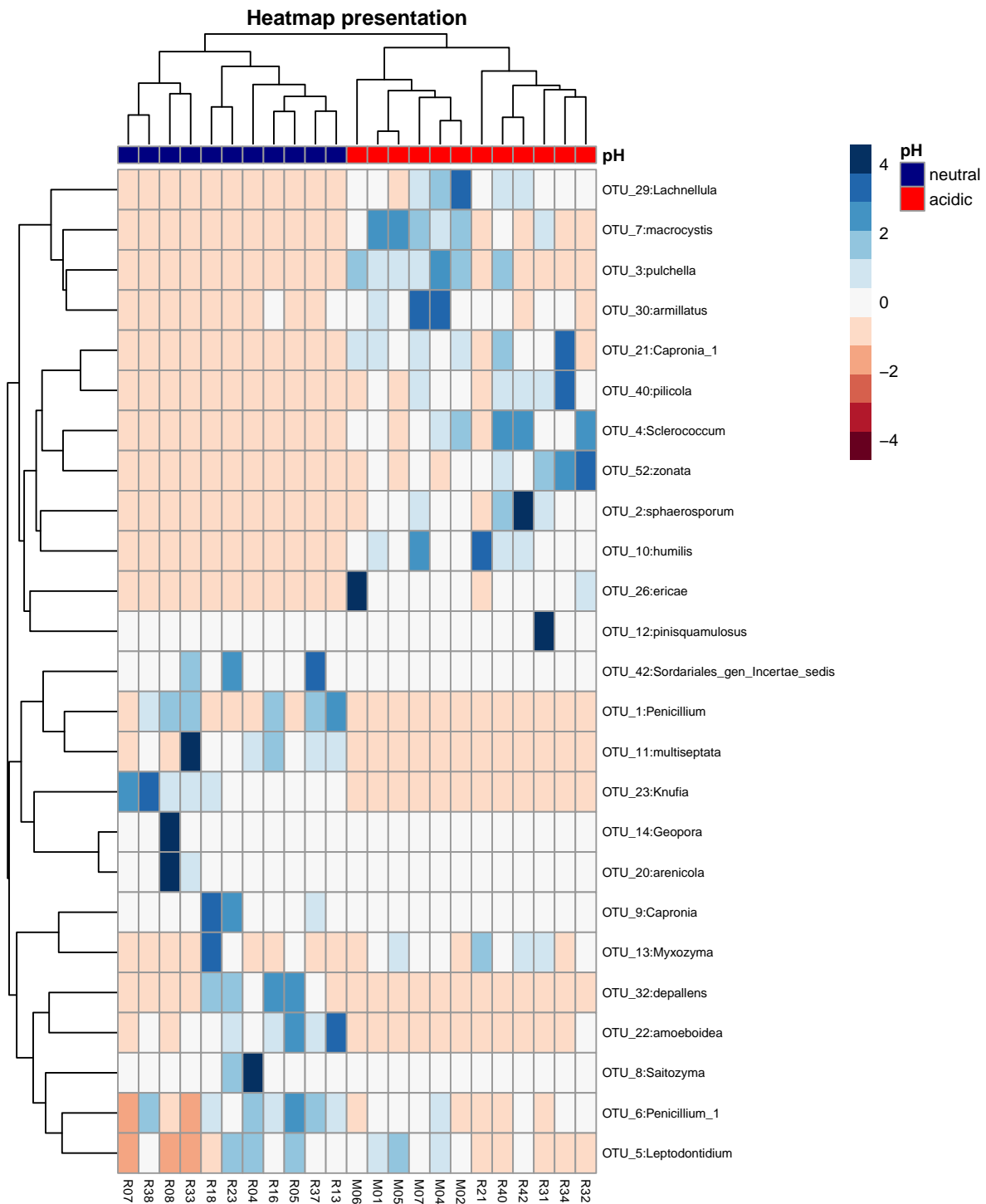
Barplot faceted by pH

```
barplot3 <- ggplot(genus_long, aes(x=Labc, 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.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)$Labc
material_types <- colData(tse)$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)
```



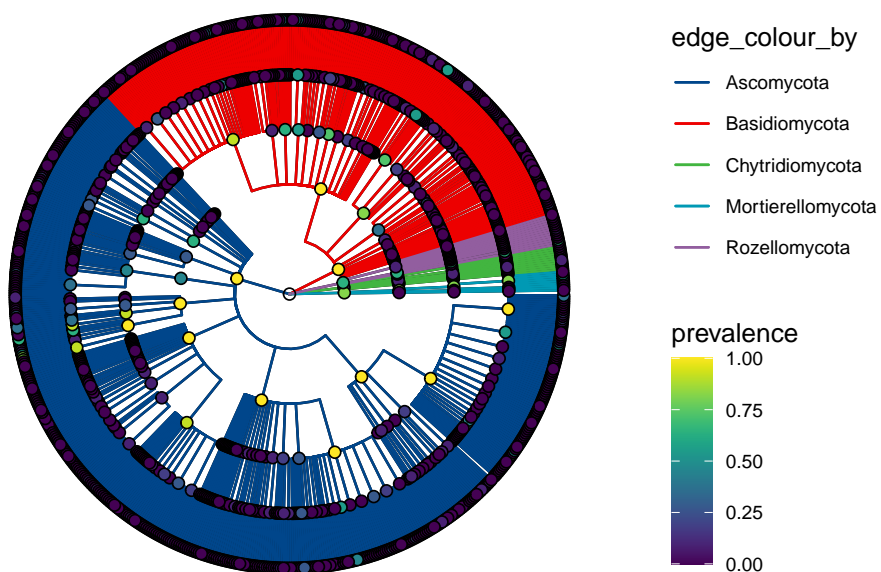
Taxonomy tree plot objects for soil pH groups

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

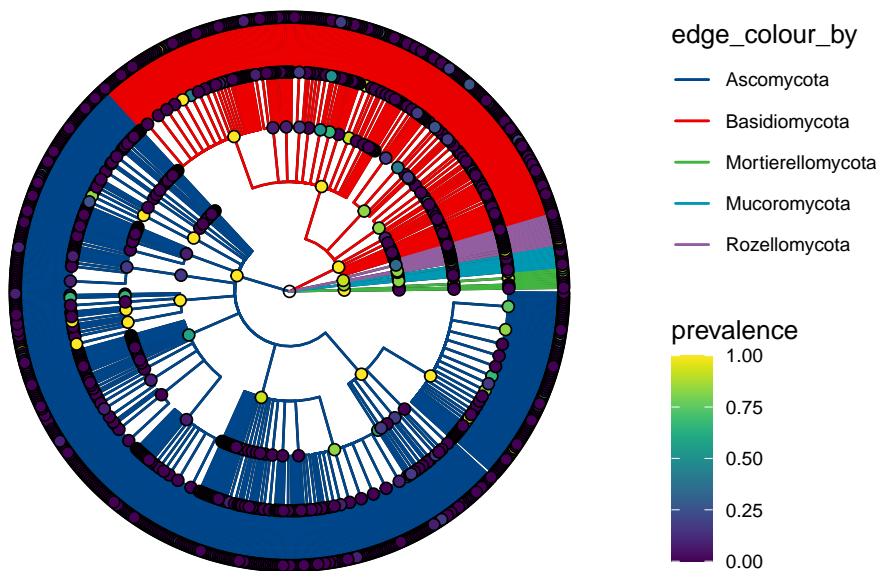
Plot the figure

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


Soil = neutral



Soil = acidic



Alpha diversity

Calculation of alpha diversity indexes

```
tse <- readRDS("results/TSE.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)$Labc,
                        Shannon = colData(tse)$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(Labc, Area, Veg, Type, pH,
                Shannon=shannon_diversity)

# Vegetation
plot_vegetation <- ggplot(alphadf, aes(x=Veg, y=Shannon,
                                       fill=Veg)) + geom_boxplot() +
  common_theme + labs(caption="Vegetation")

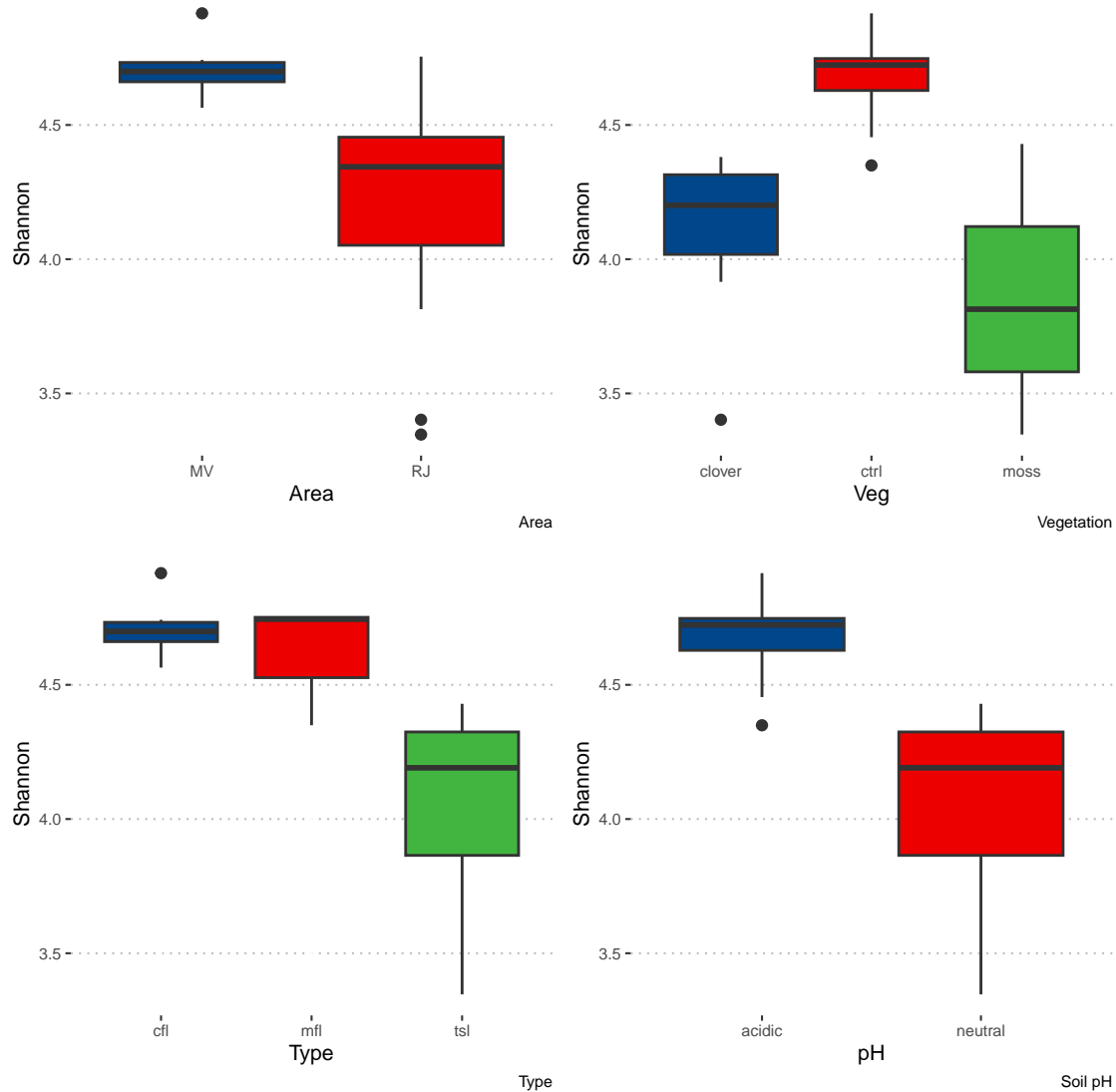
# Area
plot_area <- ggplot(alphadf, aes(x=Area, y=Shannon,
                                fill=Area)) + geom_boxplot() +
  common_theme + labs(caption="Area")

# Soil_type
plot_type <- ggplot(alphadf, aes(x=Type, y=Shannon,
                                fill=Type)) +
  geom_boxplot() + common_theme + labs(caption="Type")

# pH
plot_pH <- ggplot(alphadf, aes(x=pH, y=Shannon,
                              fill=pH)) +
  geom_boxplot() + common_theme + labs(caption="Soil pH")
```

Plots

```
plot_area + plot_vegetation + plot_type + plot_pH
```



Kruskal-Wallis tests for variables

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

Kruskal-Wallis rank sum test

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

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

```
Kruskal-Wallis rank sum test

data:  Shannon by Area
Kruskal-Wallis chi-squared = 4.7108, df = 1, p-value = 0.02997
```

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

```
Kruskal-Wallis rank sum test

data:  Shannon by Type
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
```

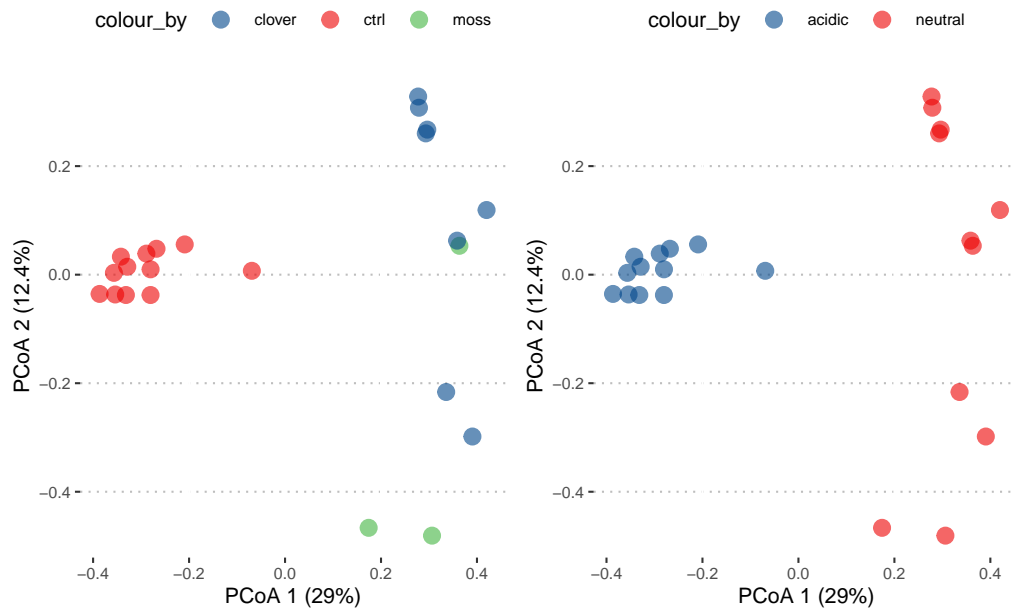
Betadiversity

Bray-Curtis distances ja PCoA ordination plots

```
# Reload object
tse <- readRDS("results/TSE.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 = "Veg")
# 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()
```



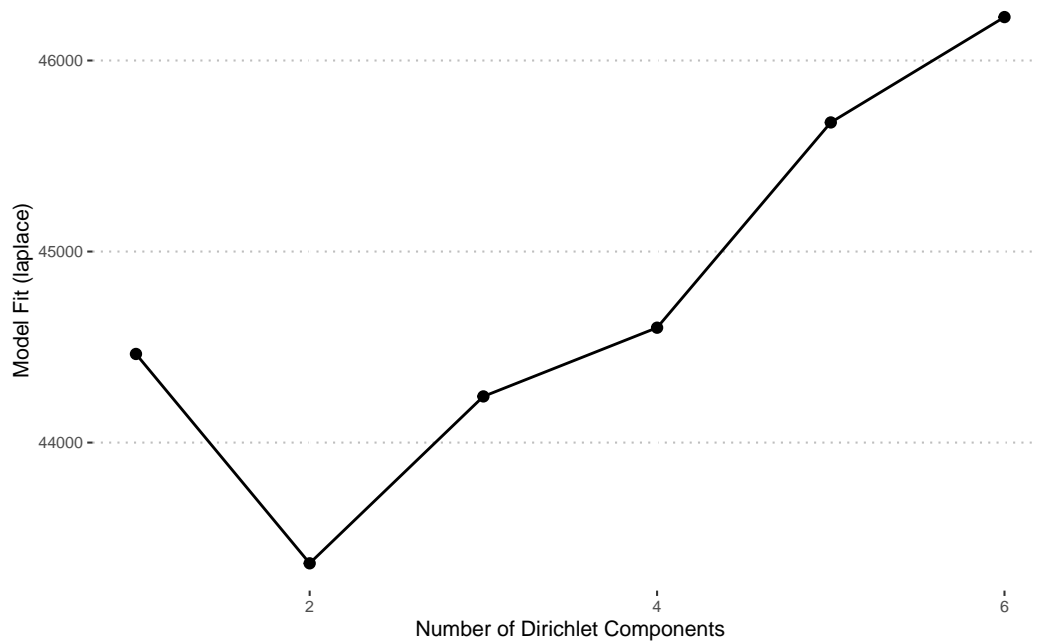
Dirichlet-Multinomial Mixture model

Cluster calculation

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

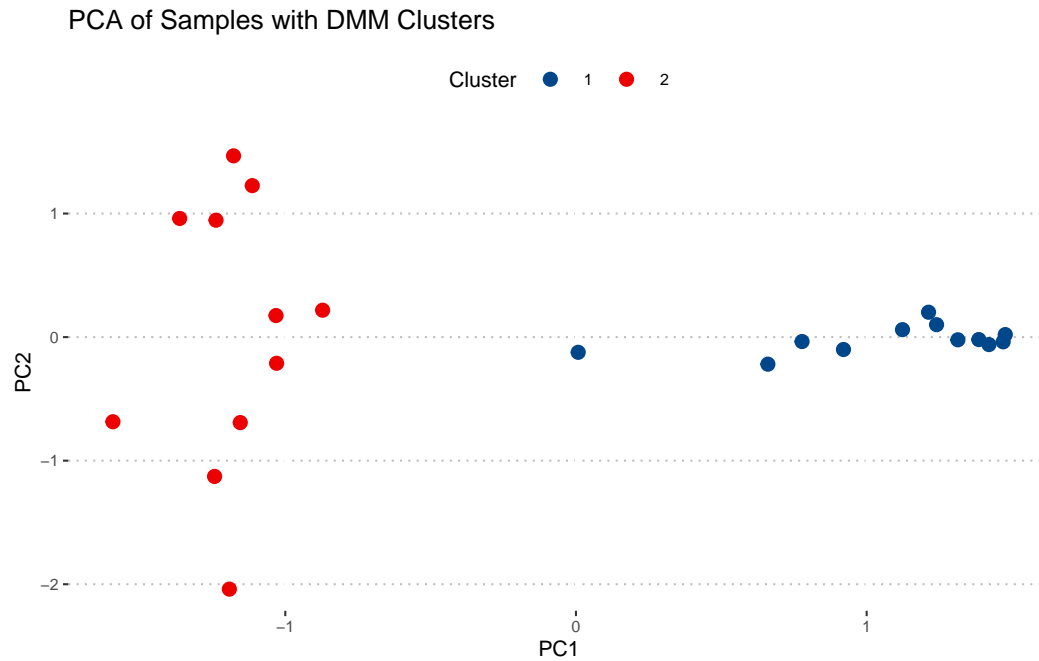
```
# Plot Laplace approximation results
p <- plotDMMFit(altExp(tse, "prevalent"), type = "laplace", name = "DMM")
p + theme_pubclean(base_size = 8)
```



```

# 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")

```



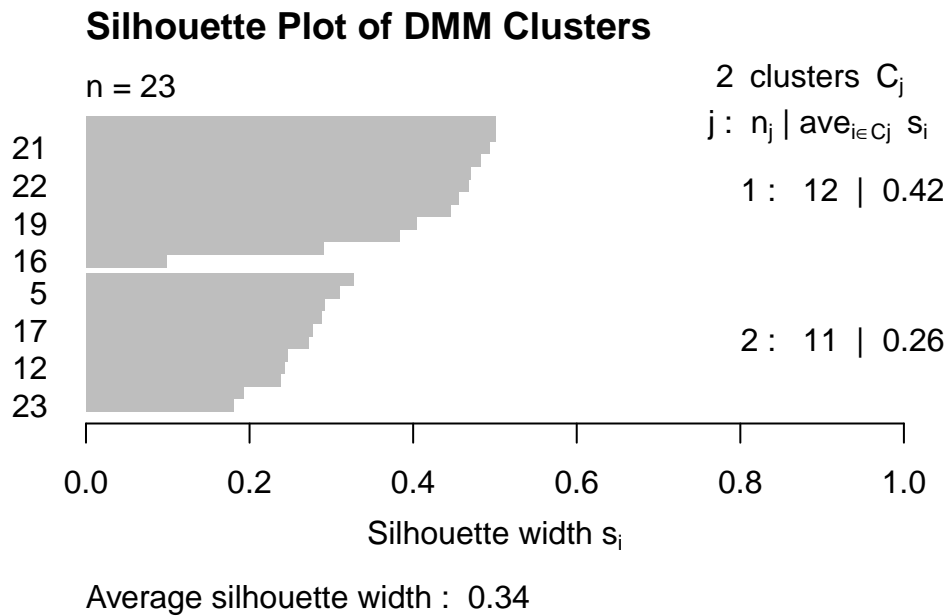
Eigen values for PCA plot

```
# 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	44.17	44.17
PC2	16.76	60.93

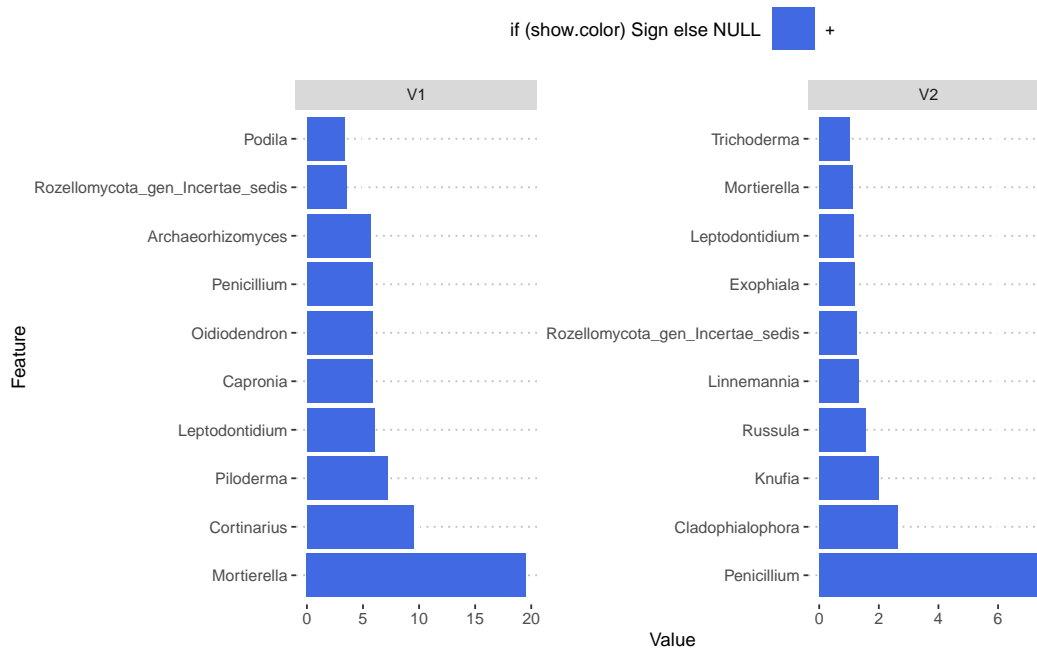
Silhouette plot of clustered samples. Higher the values, tighter the cluster.

```
# 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

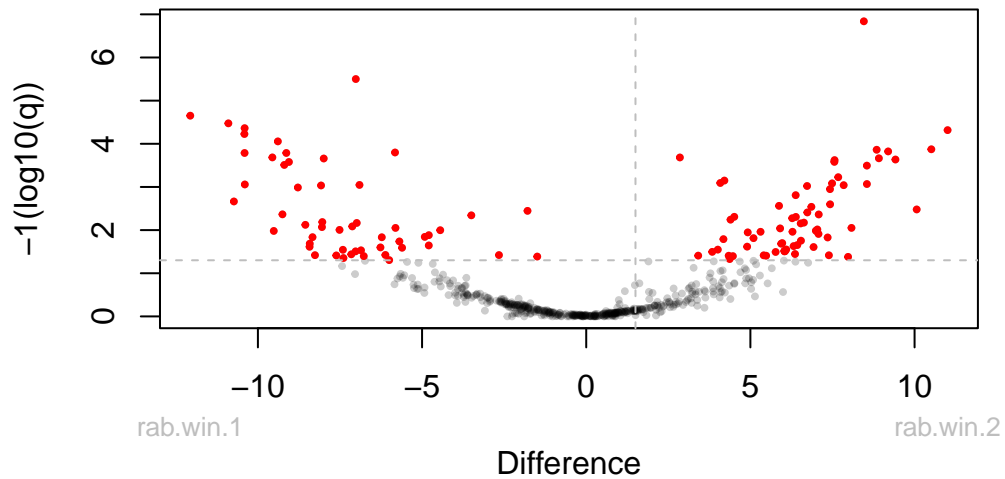
```
# Load tse
tse <- readRDS("results/TSE.rds")
tse <- tse[,1:23]
# Agglomerate by genus and subset by prevalence
tse <- subsetByPrevalent(tse, rank = "Genus", prevalence = 10/100, detection=5/1000)
# Factor pH category
tse$pH <- factor(tse$pH, levels = c("neutral", "acidic"))
```

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	3.24	0.00	0.00	0.00
Piloderma	3.22	0.00	0.00	0.00
Archaeorhizomyces	3.01	0.00	0.00	0.00
Meliniomyces	2.96	0.00	0.00	0.00
Podila	2.76	0.00	0.00	0.00
Leotiomycetes_gen_Incertae_sedis	2.69	0.02	0.00	0.00
Hyphodiscus	2.56	0.00	0.00	0.00
Mortierella	2.52	0.01	0.00	0.00
Rhizidium	2.40	0.00	0.00	0.00
GS21_gen_Incertae_sedis	2.35	0.00	0.00	0.00
Pezoloma	2.23	0.08	0.02	0.00
Lecanicillium	2.05	0.01	0.00	0.00
Cephalothecaceae_gen_Incertae_sedis	2.05	0.00	0.00	0.00
Cenococcum	2.03	0.01	0.00	0.00
Botryobasidium	2.00	0.06	0.00	0.00
Blastocladiomycota_gen_Incertae_sedis	1.97	0.01	0.00	0.00
Humicolopsis	1.93	0.01	0.00	0.00
Sugiyamaella	1.90	0.02	0.00	0.00
Sclerococcum	1.90	0.08	0.00	0.00
Acarosporales_gen_Incertae_sedis	1.74	0.06	0.01	0.00
Crocicreas	1.73	0.01	0.00	0.00
Mycena	1.73	0.03	0.00	0.00
Lachnellula	1.72	0.04	0.00	0.00
Dermateaceae_gen_Incertae_sedis	1.64	0.06	0.00	0.00
Cortinarius	1.62	0.02	0.00	0.00
Leucosporidium	1.59	0.03	0.00	0.00
Serendipitaceae_gen_Incertae_sedis	1.55	0.02	0.00	0.00
Leptobacillium	1.53	0.06	0.01	0.00
Tyrannosorus	1.52	0.03	0.00	0.00
GS15_gen_Incertae_sedis	1.48	0.06	0.00	0.00
Pseudoplectania	1.42	0.08	0.01	0.00
Phacidium	1.40	0.02	0.00	0.00
Branch01_gen_Incertae_sedis	1.36	0.10	0.01	0.01
Phialocephala	1.36	0.06	0.01	0.00
Leccinum	1.33	0.06	0.01	0.00
Trechispora	1.32	0.06	0.01	0.00
Syncephalis	1.32	0.06	0.01	0.00
Brahmaculus	1.31	0.06	0.01	0.00
GS22_gen_Incertae_sedis	1.28	0.10	0.02	0.01
Chaetothyriales_gen_Incertae_sedis	1.26	0.10	0.01	0.01
Occultifur	1.23	0.10	0.01	0.01
Hymenochaetales_gen_Incertae_sedis	1.21	0.11	0.01	0.01
Haptocillium	1.19	0.12	0.02	0.01
Luellia	1.19	0.11	0.02	0.01

(continued)

Genus	effect	overlap	we.eBH	wi.eBH
Tremella	1.15	0.14	0.02	0.01
GS04_gen_Incertae_sedis	1.15	0.13	0.03	0.02
Cenangiaceae_gen_Incertae_sedis	1.14	0.12	0.03	0.01
Apiotrichum	1.13	0.12	0.01	0.01
Galerina	1.13	0.09	0.02	0.00
Pochonia	1.11	0.10	0.04	0.01
Agaricostilbales_gen_Incertae_sedis	1.11	0.13	0.04	0.02
Scleropezicula	1.11	0.13	0.03	0.02
Chloridium	1.06	0.12	0.03	0.01
Clavaria	1.05	0.10	0.02	0.01
Tolypocladium	1.05	0.14	0.03	0.02
Diademospora	1.04	0.17	0.04	0.05
Entoloma	1.03	0.13	0.09	0.01
Tympanidaceae_gen_Incertae_sedis	1.03	0.12	0.04	0.01
Auriculariales_gen_Incertae_sedis	1.03	0.13	0.05	0.03
Acarospora	1.02	0.14	0.02	0.02
Filobasidiales_gen_Incertae_sedis	1.01	0.12	0.04	0.01
Sorocybe	1.01	0.12	0.03	0.01
Allantophomopsiella	1.00	0.12	0.03	0.02
Endophragmiella	1.00	0.17	0.05	0.05
Myxozyma	0.99	0.18	0.04	0.04
Fayodia	0.98	0.15	0.08	0.03
Pezizomycotina_gen_Incertae_sedis	0.96	0.14	0.04	0.02
Babjeviella	0.94	0.16	0.06	0.04
Pezicula	0.94	0.12	0.09	0.01
Cystoderma	0.93	0.13	0.05	0.03
Pseudohyphozyma	0.92	0.13	0.09	0.03
Thaxterogaster	0.92	0.12	0.02	0.01
Aspicilia	0.91	0.16	0.08	0.05
Dothiorella	0.90	0.14	0.05	0.03
Tylospora	0.90	0.17	0.05	0.04
Pseudogymnoascus	0.89	0.14	0.04	0.02
Umbelopsis	0.87	0.11	0.05	0.01
Syzygospora	0.85	0.18	0.05	0.04
Ceratobasidium	-0.90	0.13	0.05	0.03
Kurtzmaniella	-0.97	0.14	0.06	0.04
Vishniacozyma	-1.01	0.12	0.06	0.02
Sebacinales_gen_Incertae_sedis	-1.02	0.13	0.05	0.04
Thelephora	-1.02	0.14	0.02	0.03
Mallocybe	-1.02	0.14	0.04	0.03
Schizothecium	-1.04	0.14	0.05	0.05
Preussia	-1.06	0.12	0.01	0.01
Hypomyces	-1.06	0.11	0.03	0.01
Sporormiella	-1.08	0.15	0.03	0.04
Hamatocanthoscypha	-1.11	0.11	0.04	0.03
Laetinaevia	-1.11	0.06	0.02	0.00
Rhizopogon	-1.12	0.12	0.01	0.02
Mycosphaerellales_gen_Incertae_sedis	-1.12	0.11	0.03	0.03
Sordariales_gen_Incertae_sedis	-1.13	0.07	0.01	0.00
Nothodactylaria	-1.16	0.13	0.04	0.03
Verrucocccum	-1.17	0.13	0.04	0.04
Cadophora	-1.17	0.08	0.01	0.00
Inocybe	-1.19	0.09	0.01	0.01
Cyphellophoraceae_gen_Incertae_sedis	-1.20	0.06	0.01	0.01
Neonectria	-1.21	0.13	0.02	0.02

(continued)

Genus	effect	overlap	we.eBH	wi.eBH
Oliveonia	-1.24	0.08	0.01	0.01
Pulvinula	-1.26	0.12	0.02	0.03
Pleosporales_gen_Incertae_sedis	-1.27	0.11	0.03	0.03
Triscelophorus	-1.32	0.11	0.03	0.02
Cladophialophora	-1.32	0.07	0.00	0.00
Penicillium	-1.33	0.13	0.04	0.02
Fungi_gen_Incertae_sedis	-1.34	0.09	0.00	0.01
Cosmospora	-1.43	0.07	0.01	0.01
Psoroglaena	-1.43	0.09	0.01	0.01
Phomatospora	-1.45	0.08	0.01	0.01
Lentitheciaceae_gen_Incertae_sedis	-1.45	0.07	0.01	0.01
Kurtzmanomyces	-1.55	0.06	0.01	0.01
Pseudocoleophoma	-1.57	0.06	0.00	0.00
Plectosphaerella	-1.58	0.09	0.01	0.01
Helvella	-1.58	0.04	0.00	0.00
Leohumicola	-1.61	0.05	0.00	0.00
Geopora	-1.71	0.05	0.00	0.00
Alpinaria	-1.77	0.04	0.00	0.00
Nectriella	-1.84	0.01	0.00	0.00
Cladosporium	-1.85	0.02	0.00	0.00
Tetracladium	-1.88	0.02	0.00	0.00
Lobulomycetales_gen_Incertae_sedis	-1.92	0.01	0.00	0.00
Talaromyces	-1.96	0.05	0.00	0.00
Pleotrichocladium	-1.96	0.03	0.00	0.00
Kodamaea	-2.15	0.00	0.00	0.00
Sclerostagonospora	-2.23	0.01	0.00	0.00
Truncatella	-2.34	0.01	0.00	0.00
Fusarium	-2.51	0.00	0.00	0.00
Paraphoma	-2.86	0.00	0.00	0.00
Exophiala	-3.19	0.00	0.00	0.00
Pezizellaster	-3.31	0.00	0.00	0.00
Knufia	-3.41	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	7.32	0.73	0.00	TRUE
Sclerococcum	6.56	0.71	0.00	TRUE
Leotiomycetes_gen_Incertae_sedis	6.27	0.71	0.00	TRUE
Meliniomyces	6.10	0.63	0.00	TRUE
Piloderma	6.01	0.62	0.00	TRUE
Hyphodiscus	5.61	0.62	0.00	TRUE
Podila	5.33	0.61	0.00	TRUE
Pezoloma	5.33	0.63	0.00	TRUE
Rhizidium	5.27	0.68	0.00	TRUE
GS12_gen_Incertae_sedis	5.22	0.73	0.00	TRUE
Entomortierella	5.13	0.42	0.00	TRUE
Cephalothecaceae_gen_Incertae_sedis	5.13	0.70	0.00	TRUE
Branch01_gen_Incertae_sedis	4.88	0.79	0.00	TRUE
Pseudoplectania	4.75	0.68	0.00	TRUE
Herpotrichiellaceae_gen_Incertae_sedis	4.47	1.05	0.00	TRUE
GS21_gen_Incertae_sedis	4.23	0.52	0.00	TRUE
Humicolopsis	4.02	0.59	0.00	TRUE
Tympanidaceae_gen_Incertae_sedis	4.00	0.72	0.00	TRUE
Clavaria	3.97	0.89	0.00	TRUE
Mycena	3.91	0.66	0.00	TRUE
Leucosporidium	3.88	0.75	0.00	TRUE
Chaetothyriales_gen_Incertae_sedis	3.74	0.89	0.00	TRUE
Phialocephala	3.67	0.74	0.00	TRUE
Serendipitaceae_gen_Incertae_sedis	3.67	0.66	0.00	TRUE
Tolypocladium	3.65	0.86	0.00	TRUE
Crocicreas	3.47	0.58	0.00	TRUE
Tyrannosorus	3.35	0.66	0.00	TRUE
Tylospora	3.29	1.09	0.02	TRUE
Cortinarius	3.23	0.65	0.00	TRUE
Lachnellula	3.21	0.63	0.00	TRUE
Myxozyma	3.14	1.08	0.02	TRUE
Hymenochaetales_gen_Incertae_sedis	2.99	0.74	0.01	TRUE
Trechispora	2.96	0.67	0.00	TRUE
Glutiniomyces	2.83	0.66	0.01	TRUE
Mycosymbiocytes	2.75	1.01	0.03	TRUE
Apiotrichum	2.63	0.84	0.02	TRUE
Syncephalis	2.60	0.57	0.00	TRUE
Sistotrema	2.31	0.86	0.04	TRUE
Mortierella	2.31	0.51	0.00	TRUE
Volutella	2.30	0.72	0.03	TRUE
Phacidium	2.29	0.55	0.00	TRUE
Umbelopsis	2.29	0.74	0.02	TRUE
Dissophora	2.12	0.56	0.02	TRUE
Occultifur	1.96	0.71	0.03	TRUE
Chytridiales_gen_Incertae_sedis	1.90	0.74	0.04	TRUE

(continued)

Genus	lfc	se	q	diff
Galerina	1.80	0.56	0.01	TRUE
Genolevuria	1.79	0.62	0.03	TRUE
Chloridium	1.47	0.56	0.05	TRUE
Lophium	-1.25	0.46	0.04	TRUE
Spirographa	-1.40	0.45	0.03	TRUE
Didymella	-1.55	0.53	0.04	TRUE
Campitobasidiaceae_gen_Incertae_sedis	-1.67	0.45	0.03	TRUE
Sanchytrium	-1.86	0.65	0.03	TRUE
Lycoperdon	-1.94	0.72	0.04	TRUE
Rhizophydiales_gen_Incertae_sedis	-1.96	0.69	0.03	TRUE
Agaricostilbomycetes_gen_Incertae_sedis	-1.98	0.42	0.01	TRUE
Kurtzmaniella	-2.01	0.62	0.02	TRUE
Malassezia	-2.12	0.72	0.03	TRUE
Sakaguchia	-2.25	0.54	0.01	TRUE
Cladophialophora	-2.26	0.61	0.01	TRUE
Taphrina	-2.35	0.63	0.03	TRUE
Cadophora	-2.47	0.69	0.01	TRUE
Pseudeurotium	-2.48	0.89	0.03	TRUE
Thelebolus	-2.51	0.80	0.02	TRUE
Drechmeria	-2.51	0.71	0.01	TRUE
Pyrenopeziza	-2.53	0.65	0.01	TRUE
Agaricales_gen_Incertae_sedis	-2.62	0.48	0.01	TRUE
Dothideales_gen_Incertae_sedis	-2.62	0.48	0.01	TRUE
Podospora	-2.66	0.46	0.01	TRUE
Ceratobasidium	-2.67	0.82	0.01	TRUE
Mrakia	-2.72	0.48	0.01	TRUE
Alpinaria	-2.72	0.67	0.01	TRUE
Laetinaevia	-2.74	0.74	0.01	TRUE
Hypomyces	-2.76	0.75	0.01	TRUE
Hormonema	-2.80	0.48	0.00	TRUE
Sporormiella	-2.82	0.95	0.02	TRUE
Preussia	-2.82	0.87	0.01	TRUE
Rhizopogon	-2.88	0.88	0.01	TRUE
Lapidomyces	-2.95	0.49	0.00	TRUE
Vishniacozyma	-3.05	0.76	0.00	TRUE
Thelephora	-3.11	0.95	0.01	TRUE
Lobulomycetales_gen_Incertae_sedis	-3.13	0.53	0.00	TRUE
Neonectria	-3.42	0.57	0.00	TRUE
Cosmospora	-3.50	0.69	0.00	TRUE
Helminthosphaeriaceae_gen_Incertae_sedis	-3.58	0.58	0.00	TRUE
Ceratobasidiaceae_gen_Incertae_sedis	-3.81	0.49	0.00	TRUE
Sordariales_gen_Incertae_sedis	-3.85	1.04	0.01	TRUE
Pleotrichocladium	-3.94	0.75	0.00	TRUE
Inocybe	-4.01	1.07	0.01	TRUE
Cladosporium	-4.01	0.76	0.00	TRUE
Sclerostagonospora	-4.25	0.64	0.00	TRUE
Alternaria	-4.32	0.71	0.01	TRUE
Lecythophora	-4.47	0.71	0.00	TRUE
Leohumicola	-4.47	1.01	0.00	TRUE
Exophiala	-4.55	0.60	0.00	TRUE
Tetracladium	-4.78	0.90	0.00	TRUE
Talaromyces	-4.96	0.85	0.00	TRUE
Fusarium	-5.41	0.67	0.00	TRUE
Kodamaea	-5.45	0.75	0.00	TRUE
Mallocybe	-5.46	0.91	0.00	TRUE

(continued)

Genus	lfc	se	q	diff
Plectosphaerella	-5.62	0.59	0.00	TRUE
Pezizellaster	-6.04	0.61	0.00	TRUE
Knufia	-6.67	0.55	0.00	TRUE

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

Maaslin3 analysis

```
# specifying a ref is especially important if you have more than 2 levels
maaslin3_out <- maaslin3(
  input_data = as.data.frame(t(assay(tse))),
  input_metadata = as.data.frame(colData(tse)),
  output = "ITS_m3",
  transform = "LOG",
  fixed_effects = "pH",
  reference = "pH,neutral",
  normalization = "TSS",
  standardize = FALSE,
  min_prevalence = 0)
saveRDS(maaslin3_out, "results/maaslin3.rds")
```

Genera with significantly different abundance

```
maaslin3_out <- readRDS("results/maaslin3.rds")
maaslin_summary <- maaslin3_out$fit_data_abundance$results %>% dplyr::select(Genus = feature, coef, stderr, qval_joint) %>%
  filter(qval_joint < 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_joint
Archaeorhizomyces	10.76	0.87	0.00
Sclerococcum	9.61	1.03	0.00
Hygrophorus	9.37	0.22	0.01
Leotiomyces_gen_Incertae_sedis	9.08	1.08	0.00
Meliniomyces	9.06	0.73	0.00
Piloderma	8.89	0.66	0.00
Hyphodiscus	8.18	1.24	0.00
Podila	7.85	0.72	0.00
Pezoloma	7.82	0.77	0.00
Rhizidium	7.79	0.83	0.00
GS12_gen_Incertae_sedis	7.79	2.04	0.03
Cephalothecaceae_gen_Incertae_sedis	7.58	1.08	0.00
Entomortierella	7.56	0.60	0.00
Branch01_gen_Incertae_sedis	7.15	1.83	0.02
Pseudoplectania	6.95	1.13	0.00
Herpotrichiellaceae_gen_Incertae_sedis	6.53	1.69	0.01
GS21_gen_Incertae_sedis	6.23	0.99	0.00
Tympanidaceae_gen_Incertae_sedis	6.09	1.44	0.01
Humicolopsis	5.98	0.91	0.00
Botryobasidium	5.95	0.90	0.00
Mycena	5.79	0.95	0.00
Clavaria	5.78	1.65	0.02

(continued)

Genus	coef	stderr	qval_joint
Leucosporidium	5.77	0.92	0.00
Chaetothyriales_gen_Incertae_sedis	5.61	1.19	0.00
Brahmaculus	5.45	2.72	0.03
Phialocephala	5.43	1.06	0.00
Tolypocladium	5.41	1.40	0.01
Serendipitaceae_gen_Incertae_sedis	5.40	0.92	0.00
Acarosporales_gen_Incertae_sedis	5.36	1.06	0.00
Crocicreas	5.06	1.10	0.00
GS04_gen_Incertae_sedis	5.04	2.11	0.04
Tylospora	4.96	1.48	0.02
Tyrannosorus	4.95	1.14	0.00
Cortinarius	4.88	0.72	0.00
Lachnellula	4.86	0.67	0.00
Myxozyma	4.80	1.68	0.04
Auriculariales_gen_Incertae_sedis	4.75	3.10	0.04
Trechispora	4.46	0.89	0.00
Cenangiaceae_gen_Incertae_sedis	4.36	2.18	0.04
Babjeviella	4.11	2.61	0.04
Leccinum	4.11	1.82	0.03
Mycosymbiaces	4.10	1.41	0.04
Syncephalis	3.93	0.74	0.00
Luellia	3.77	2.46	0.04
Phacidium	3.64	1.07	0.04
Mortierella	3.55	0.41	0.00
Umbelopsis	3.52	0.89	0.00
Babjevia	3.15	1.15	0.04
Cystoderma	2.80	2.60	0.03
Galerina	2.79	0.85	0.04
Oidiodendron	2.57	0.85	0.03
Acarospora	2.51	2.73	0.04
Dothiorella	1.36	1.50	0.04
Cyphellophoraceae_gen_Incertae_sedis	-2.06	3.74	0.03
Cladophialophora	-3.04	0.67	0.02
Rhizopogon	-3.93	1.13	0.05
Hormonema	-4.03	0.84	0.04
Lobulomycetales_gen_Incertae_sedis	-4.14	0.82	0.02
Vishniacozyma	-4.16	1.08	0.03
Epithamnolia	-4.28	0.25	0.03
Helminthosphaeriaceae_gen_Incertae_sedis	-4.99	0.94	0.01
Ceratobasidiaceae_gen_Incertae_sedis	-5.03	1.16	0.05
Sordariales_gen_Incertae_sedis	-5.34	1.45	0.03
Inocybe	-5.56	1.56	0.04
Cladosporium	-5.57	0.97	0.00
Triscelophorus	-5.59	1.09	0.03
Phomatospora	-5.70	3.07	0.04
Sclerostagonospora	-5.90	0.99	0.00
Pseudocoleophoma	-5.95	3.75	0.03
Lecythophora	-6.07	1.42	0.02
Geopora	-6.28	4.33	0.03
Exophiala	-6.35	0.62	0.00
Tetracladium	-6.63	1.83	0.04
Talaromyces	-6.89	1.27	0.00
Fusarium	-7.40	1.28	0.00
Kodamaea	-7.52	1.11	0.00
Mallocybe	-7.61	1.69	0.01

(continued)

Genus	coef	stderr	qval_joint
Plectosphaerella	-7.86	1.50	0.01
Pezizellaster	-8.41	0.94	0.00
Knufia	-9.29	0.79	0.00

In Maaslin3 analysis, control is neutral, effect is acidic. Changes are calculated with formula $\log(q) \cdot \text{sign}(\text{coeff})$. Compared to Maaslin2, analysis provides more stringent filtering (80 positives vs 156 positives).

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
Archaeorhizomyces	TRUE	TRUE	TRUE
Meliniomyces	TRUE	TRUE	TRUE
Podila	TRUE	TRUE	TRUE
Leotiomycetes_gen_Incertae_sedis	TRUE	TRUE	TRUE
Hyphodiscus	TRUE	TRUE	TRUE
Mortierella	TRUE	TRUE	TRUE
Rhizidium	TRUE	TRUE	TRUE
GS21_gen_Incertae_sedis	TRUE	TRUE	TRUE
Pezoloma	TRUE	TRUE	TRUE
Lecanicillium	TRUE	FALSE	FALSE
Cephalothecaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Cenococcum	TRUE	FALSE	FALSE
Botryobasidium	TRUE	FALSE	TRUE
Blastocladiomycota_gen_Incertae_sedis	TRUE	FALSE	FALSE
Humicolopsis	TRUE	TRUE	TRUE
Sugiyamaella	TRUE	FALSE	FALSE
Sclerococcum	TRUE	TRUE	TRUE
Acarosporales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Crocicreas	TRUE	TRUE	TRUE
Mycena	TRUE	TRUE	TRUE
Lachnellula	TRUE	TRUE	TRUE
Dermateaceae_gen_Incertae_sedis	TRUE	FALSE	FALSE
Cortinarius	TRUE	TRUE	TRUE
Leucosporidium	TRUE	TRUE	TRUE
Serendipitaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Leptobacillium	TRUE	FALSE	FALSE
Tyrannosorus	TRUE	TRUE	TRUE
GS15_gen_Incertae_sedis	TRUE	FALSE	FALSE
Pseudoplectania	TRUE	TRUE	TRUE
Phacidium	TRUE	TRUE	TRUE
Branch01_gen_Incertae_sedis	TRUE	TRUE	TRUE
Phialocephala	TRUE	TRUE	TRUE
Leccinum	TRUE	FALSE	TRUE
Trechispora	TRUE	TRUE	TRUE
Syncephalis	TRUE	TRUE	TRUE
Brahmaculus	TRUE	FALSE	TRUE
GS22_gen_Incertae_sedis	TRUE	FALSE	FALSE
Chaetothyriales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Occultifur	TRUE	TRUE	FALSE
Hymenochaetales_gen_Incertae_sedis	TRUE	TRUE	FALSE
Haptocillium	TRUE	FALSE	FALSE
Luellia	TRUE	FALSE	TRUE
Tremella	TRUE	FALSE	FALSE
GS04_gen_Incertae_sedis	TRUE	FALSE	TRUE
Cenangiaceae_gen_Incertae_sedis	TRUE	FALSE	TRUE
Apiotrichum	TRUE	TRUE	FALSE

(continued)

Genus	Aldex2	Ancombc	Maasin2
Galerina	TRUE	TRUE	TRUE
Pochonia	TRUE	FALSE	FALSE
Agaricostilbales_gen_Incertae_sedis	TRUE	FALSE	FALSE
Scleropezicula	TRUE	FALSE	FALSE
Chloridium	TRUE	TRUE	FALSE
Clavaria	TRUE	TRUE	TRUE
Tolypocladium	TRUE	TRUE	TRUE
Diademospora	TRUE	FALSE	FALSE
Entoloma	TRUE	FALSE	FALSE
Tympanidaceae_gen_Incertae_sedis	TRUE	TRUE	TRUE
Auriculariales_gen_Incertae_sedis	TRUE	FALSE	TRUE
Acarospora	TRUE	FALSE	TRUE
Filobasidiales_gen_Incertae_sedis	TRUE	FALSE	FALSE
Sorocybe	TRUE	FALSE	FALSE
Allantophomopsiella	TRUE	FALSE	FALSE
Endophragmiella	TRUE	FALSE	FALSE
Myxozyma	TRUE	TRUE	TRUE
Fayodia	TRUE	FALSE	FALSE
Pezizomycotina_gen_Incertae_sedis	TRUE	FALSE	FALSE
Babjeviella	TRUE	FALSE	TRUE
Pezicula	TRUE	FALSE	FALSE
Cystoderma	TRUE	FALSE	TRUE
Pseudohyphozyma	TRUE	FALSE	FALSE
Thaxterogaster	TRUE	FALSE	FALSE
Aspicilia	TRUE	FALSE	FALSE
Dothiorella	TRUE	FALSE	TRUE
Tylospora	TRUE	TRUE	TRUE
Pseudogymnoascus	TRUE	FALSE	FALSE
Umbelopsis	TRUE	TRUE	TRUE
Syzygospora	TRUE	FALSE	FALSE
Ceratobasidium	TRUE	TRUE	FALSE
Kurtzmaniella	TRUE	TRUE	FALSE
Vishniacozyma	TRUE	TRUE	TRUE
Sebacinales_gen_Incertae_sedis	TRUE	FALSE	FALSE
Thelephora	TRUE	TRUE	FALSE
Mallocybe	TRUE	TRUE	TRUE
Schizothecium	TRUE	FALSE	FALSE
Preussia	TRUE	TRUE	FALSE
Hypomyces	TRUE	TRUE	FALSE
Sporormiella	TRUE	TRUE	FALSE
Hamatocanthoscypha	TRUE	FALSE	FALSE
Laetinaevia	TRUE	TRUE	FALSE
Rhizopogon	TRUE	TRUE	TRUE
Mycosphaerellales_gen_Incertae_sedis	TRUE	FALSE	FALSE
Sordariales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Nothodactylaria	TRUE	FALSE	FALSE
Verrucocccum	TRUE	FALSE	FALSE
Cadophora	TRUE	TRUE	FALSE
Inocybe	TRUE	TRUE	TRUE
Cyphellophoraceae_gen_Incertae_sedis	TRUE	FALSE	TRUE
Neonectria	TRUE	TRUE	FALSE
Oliveonia	TRUE	FALSE	FALSE
Pulvinula	TRUE	FALSE	FALSE
Pleosporales_gen_Incertae_sedis	TRUE	FALSE	FALSE
Triscelophorus	TRUE	FALSE	TRUE

(continued)

Genus	Aldex2	Ancombc	Maasin2
Cladophialophora	TRUE	TRUE	TRUE
Penicillium	TRUE	FALSE	FALSE
Fungi_gen_Incertae_sedis	TRUE	FALSE	FALSE
Cosmospora	TRUE	TRUE	FALSE
Psoroglaena	TRUE	FALSE	FALSE
Phomatospora	TRUE	FALSE	TRUE
Lentitheciaceae_gen_Incertae_sedis	TRUE	FALSE	FALSE
Kurtzmanomyces	TRUE	FALSE	FALSE
Pseudocoleophoma	TRUE	FALSE	TRUE
Plectosphaerella	TRUE	TRUE	TRUE
Helvella	TRUE	FALSE	FALSE
Leohumicola	TRUE	TRUE	FALSE
Geopora	TRUE	FALSE	TRUE
Alpinaria	TRUE	TRUE	FALSE
Nectriella	TRUE	FALSE	FALSE
Cladosporium	TRUE	TRUE	TRUE
Tetracladium	TRUE	TRUE	TRUE
Lobulomycetales_gen_Incertae_sedis	TRUE	TRUE	TRUE
Talaromyces	TRUE	TRUE	TRUE
Pleotrichocladium	TRUE	TRUE	FALSE
Kodamaea	TRUE	TRUE	TRUE
Sclerostagonospora	TRUE	TRUE	TRUE
Truncatella	TRUE	FALSE	FALSE
Fusarium	TRUE	TRUE	TRUE
Paraphoma	TRUE	FALSE	FALSE
Exophiala	TRUE	TRUE	TRUE
Pezizellaster	TRUE	TRUE	TRUE
Knufia	TRUE	TRUE	TRUE
GS12_gen_Incertae_sedis	FALSE	TRUE	TRUE
Herpotrichiaceae_gen_Incertae_sedis	FALSE	TRUE	TRUE
Glutinomyces	FALSE	TRUE	FALSE
Mycosymbiodes	FALSE	TRUE	TRUE
Sistotrema	FALSE	TRUE	FALSE
Volutella	FALSE	TRUE	FALSE
Dissophora	FALSE	TRUE	FALSE
Chytridiales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Genoleuria	FALSE	TRUE	FALSE
Lophium	FALSE	TRUE	FALSE
Spirographa	FALSE	TRUE	FALSE
Didymella	FALSE	TRUE	FALSE
Campobasidiaceae_gen_Incertae_sedis	FALSE	TRUE	FALSE
Sanchytrium	FALSE	TRUE	FALSE
Lycoperdon	FALSE	TRUE	FALSE
Rhizophydiales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Agaricostilbomycetes_gen_Incertae_sedis	FALSE	TRUE	FALSE
Malassezia	FALSE	TRUE	FALSE
Sakaguchia	FALSE	TRUE	FALSE
Taphrina	FALSE	TRUE	FALSE
Pseudeurotium	FALSE	TRUE	FALSE
Thelebolus	FALSE	TRUE	FALSE
Drechmeria	FALSE	TRUE	FALSE
Pyrenopeziza	FALSE	TRUE	FALSE
Agaricales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Dothideales_gen_Incertae_sedis	FALSE	TRUE	FALSE
Podospora	FALSE	TRUE	FALSE

(continued)

Genus	Aldex2	Ancombc	Maaslin2
Mrakia	FALSE	TRUE	FALSE
Hormonema	FALSE	TRUE	TRUE
Lapidomyces	FALSE	TRUE	FALSE
Helminthosphaeriaceae_gen_Incertae_sedis	FALSE	TRUE	TRUE
Ceratobasidiaceae_gen_Incertae_sedis	FALSE	TRUE	TRUE
Alternaria	FALSE	TRUE	FALSE
Lecythophora	FALSE	TRUE	TRUE
Hygrophorus	FALSE	FALSE	TRUE
Babjevia	FALSE	FALSE	TRUE
Oidiodendron	FALSE	FALSE	TRUE
Epithamnolia	FALSE	FALSE	TRUE