Data-analyysi Raajärvi näytteet ITS2

#### 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)

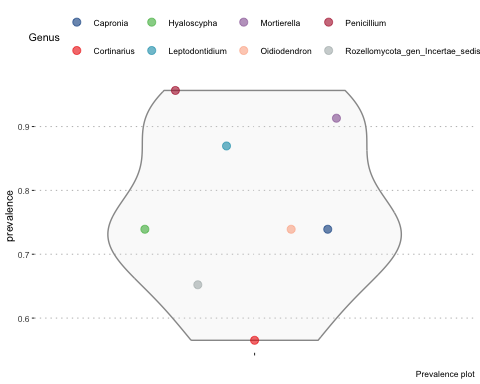
#### Load data object

# Load data from rds  
tse <- readRDS("results/tse\_its2.rds")  
set.seed(123412)

#### Prevalent features on data set

Features that have minimun 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")



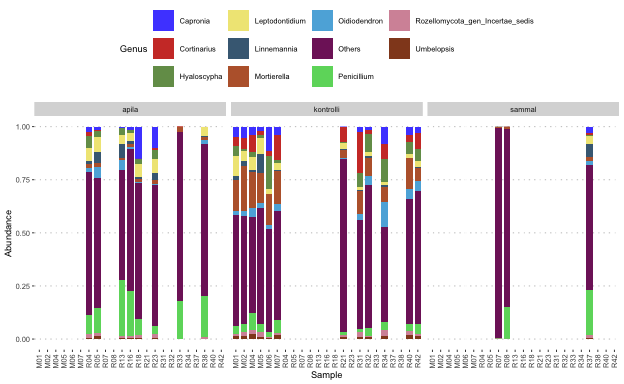
#### Community composition plots

Top ten features calculated by median relative abundance

# Reload data  
tse <- readRDS("results/tse\_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)))

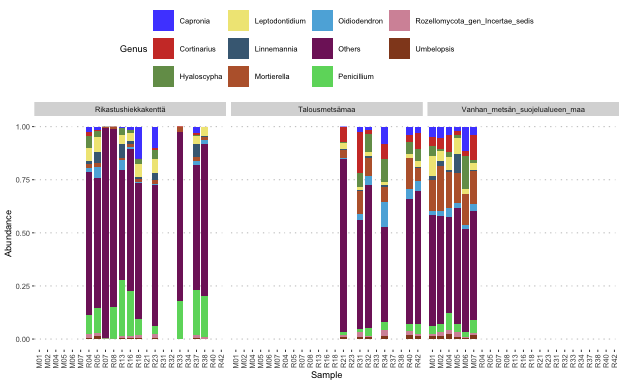
Barplot faceted by vegetation

barplot1 <- ggplot(genus\_long, aes(x=Labnro, y=Abundance, fill=Genus)) +  
 geom\_bar(stat="identity", width=0.8) +  
 facet\_wrap(~ Kasvillisuus) + bar\_theme  
barplot1



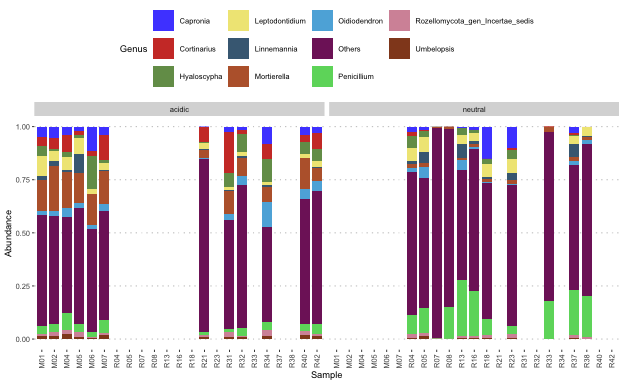
Barplot faceted by soil

barplot2 <- ggplot(genus\_long, aes(x=Labnro, y=Abundance, fill=Genus)) +  
 geom\_bar(stat="identity", width=0.8) + facet\_grid(~ Maanäyte) + bar\_theme  
barplot2



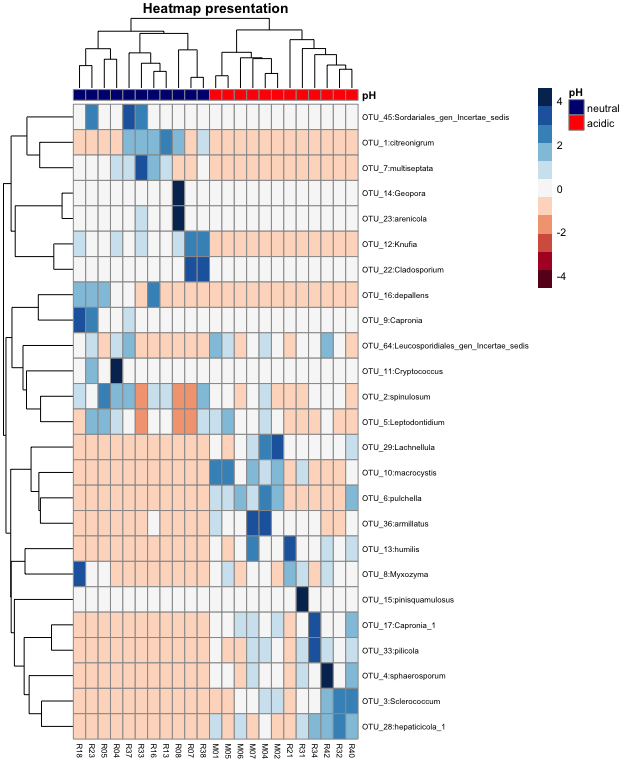
Barplot faceted by pH

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

# 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)

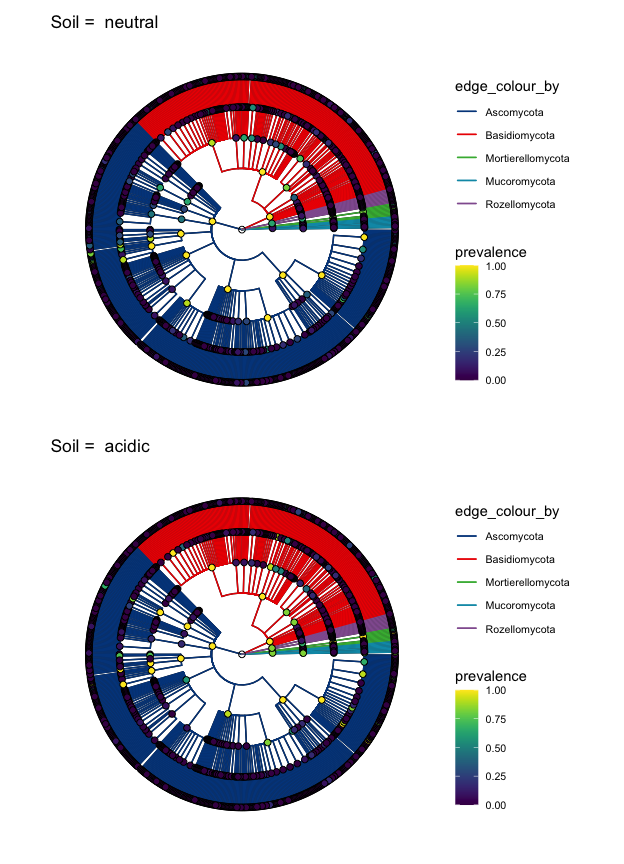


Taxonomy tree plot objects for soil pH groups

# 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

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



#### 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")

Diversity indexes

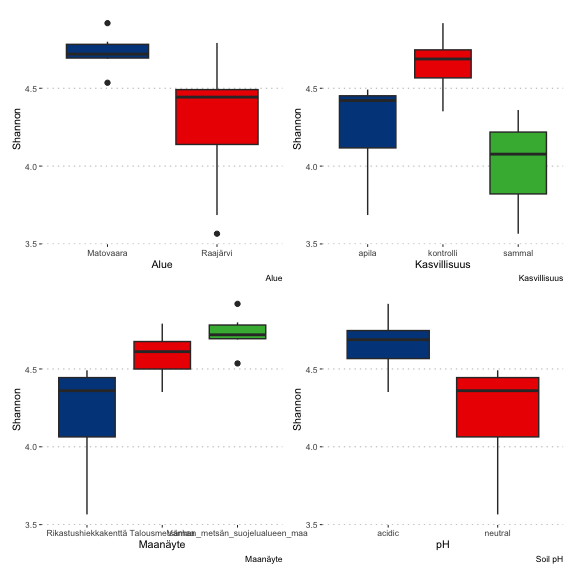
| Sample | Shannon | Gini\_Simpson | Pielou | Observed\_features |
| --- | --- | --- | --- | --- |
| R08 | 3.57 | 0.90 | 0.49 | 1481 |
| R16 | 4.05 | 0.94 | 0.52 | 2272 |
| R07 | 4.36 | 0.97 | 0.62 | 1191 |
| R37 | 4.08 | 0.95 | 0.55 | 1706 |
| R33 | 3.69 | 0.92 | 0.49 | 1724 |
| R13 | 4.49 | 0.96 | 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

# 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 = 13.809, df = 2, p-value = 0.001003

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

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

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

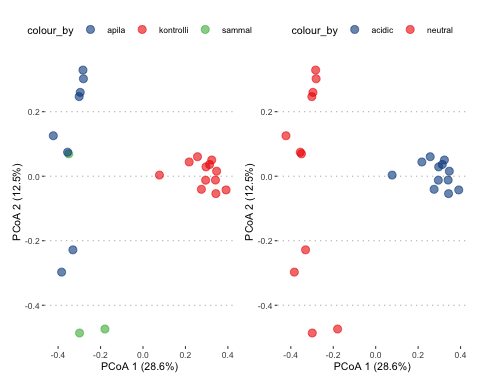
#### Betadiversity

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()



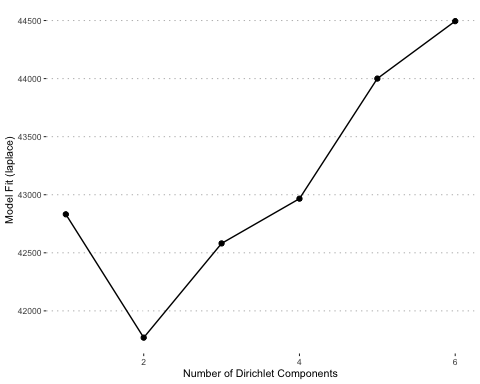
#### Dirichlet-Multinomial Mixture model

Cluster calculation

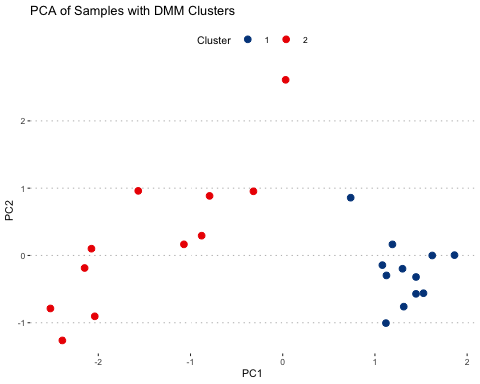
# Load the tse object  
tse <- readRDS("results/tse\_its2.rds")  
tse <- tse[,1:23]  
tse <- transformAssay(tse, method="hellinger")  
# Prevalence is min. 2 samples and rank agglomerated to genus  
tse <- agglomerateByPrevalence(tse, rank="Genus", prevalence=0.05, detection=1/1000)  
# Add DMM clusters  
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")



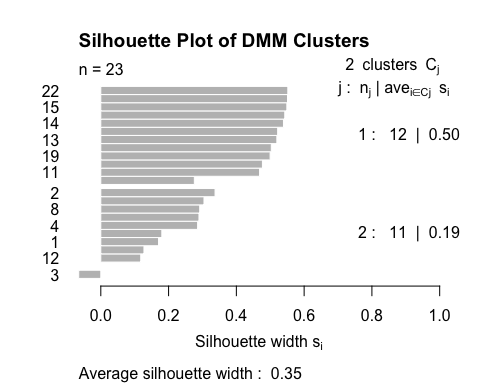
Eigen values for PCA plot

# 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 |

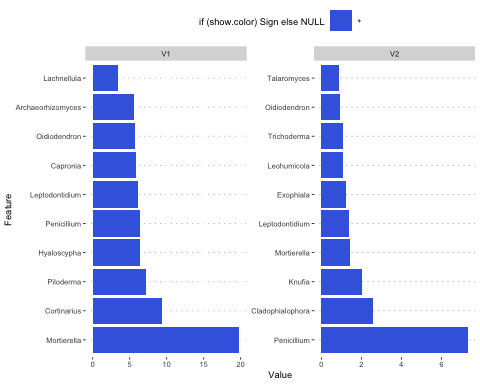
Silhoutte plot of clustered samples. Higher the values, tighter the cluster.

# 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")



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

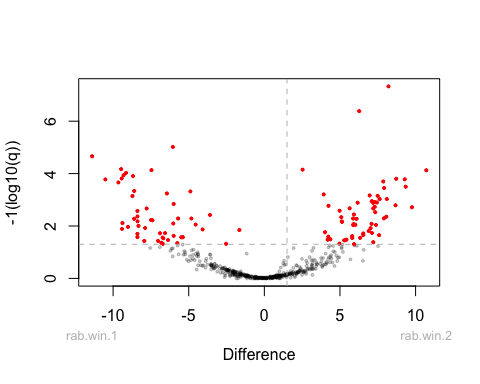
# Load tse  
tse <- readRDS("results/tse\_its2.rds")  
tse <- tse[,1:23]  
# Agglomerate by genus and subset by prevalence  
tse <- subsetByPrevalent(tse, rank = "Genus", prevalence = 10/100, detection=5/1000)  
# Transform assay to relative abundances  
tse <- transformAssay(tse, assay.type = "counts", method = "relabundance")  
# Factor pH category  
tse$pH <- factor(tse$pH, levels = c("neutral", "acidic"))

ALDEx2 analysis

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

Volcano plot

aldex.plot(aldex\_out, type="volcano", test="welch", xlab="Difference",  
 ylab="-1(log10(q))", Main ='Volcano plot')



Genera with significantly different abundance

aldex\_summary <- aldex\_out %>%  
 rownames\_to\_column(var = "Genus") %>%  
 filter(wi.eBH <= 0.05) %>%  
 dplyr::select(Genus, effect, overlap, we.eBH, wi.eBH) %>%  
 arrange(desc(effect))  
# Summary table  
kable(aldex\_summary, digits=2, longtable=T, booktabs=T) %>%  
 kable\_styling(latex\_options = c("HOLD\_position", "striped", "repeat\_header"),  
 font\_size = 7) %>% row\_spec(0, color = "white",  
 background = "teal")

| Genus | effect | overlap | we.eBH | wi.eBH |
| --- | --- | --- | --- | --- |
| Entomortierella | 3.47 | 0.00 | 0.00 | 0.00 |
| Piloderma | 3.33 | 0.00 | 0.00 | 0.00 |
| Leotiomycetes\_gen\_Incertae\_sedis | 3.27 | 0.07 | 0.00 | 0.00 |
| Podila | 3.17 | 0.00 | 0.00 | 0.00 |
| Meliniomyces | 3.04 | 0.00 | 0.00 | 0.00 |
| Archaeorhizomyces | 2.86 | 0.00 | 0.00 | 0.00 |
| GS21\_gen\_Incertae\_sedis | 2.59 | 0.00 | 0.00 | 0.00 |
| Rhizidium | 2.55 | 0.00 | 0.00 | 0.00 |
| Chaetothyriales\_gen\_Incertae\_sedis | 2.31 | 0.01 | 0.00 | 0.00 |
| Sclerococcum | 2.14 | 0.09 | 0.00 | 0.00 |
| Mortierella | 2.10 | 0.03 | 0.00 | 0.00 |
| Scleropezicula | 2.08 | 0.05 | 0.00 | 0.00 |
| Botryobasidium | 2.08 | 0.05 | 0.00 | 0.00 |
| Cephalothecaceae\_gen\_Incertae\_sedis | 2.06 | 0.00 | 0.00 | 0.00 |
| Lecanicillium | 1.96 | 0.02 | 0.00 | 0.00 |
| Crocicreas | 1.88 | 0.00 | 0.00 | 0.00 |
| Sugiyamaella | 1.82 | 0.02 | 0.00 | 0.00 |
| Helotiales\_gen\_Incertae\_sedis | 1.80 | 0.01 | 0.00 | 0.00 |
| Brahmaculus | 1.76 | 0.01 | 0.00 | 0.00 |
| Humicolopsis | 1.73 | 0.02 | 0.00 | 0.00 |
| Serendipitaceae\_gen\_Incertae\_sedis | 1.69 | 0.01 | 0.00 | 0.00 |
| Phacidium | 1.66 | 0.01 | 0.00 | 0.00 |
| Acarosporales\_gen\_Incertae\_sedis | 1.64 | 0.06 | 0.01 | 0.00 |
| Cenococcum | 1.63 | 0.05 | 0.00 | 0.00 |
| Lachnellula | 1.63 | 0.03 | 0.00 | 0.00 |
| Cortinarius | 1.62 | 0.01 | 0.00 | 0.00 |
| Pseudoplectania | 1.61 | 0.06 | 0.01 | 0.00 |
| Scytalidium | 1.54 | 0.06 | 0.01 | 0.00 |
| Hyphodiscus | 1.54 | 0.04 | 0.00 | 0.00 |
| Leptobacillium | 1.51 | 0.06 | 0.01 | 0.00 |
| Mycena | 1.50 | 0.04 | 0.00 | 0.00 |
| Phialocephala | 1.46 | 0.05 | 0.00 | 0.00 |
| Leucosporidium | 1.44 | 0.03 | 0.01 | 0.00 |
| Leccinum | 1.44 | 0.04 | 0.00 | 0.00 |
| Hyaloscyphaceae\_gen\_Incertae\_sedis | 1.37 | 0.10 | 0.01 | 0.01 |
| Clavaria | 1.32 | 0.03 | 0.00 | 0.00 |
| Trechispora | 1.32 | 0.06 | 0.01 | 0.00 |
| GS22\_gen\_Incertae\_sedis | 1.31 | 0.11 | 0.02 | 0.01 |
| Pezizomycotina\_gen\_Incertae\_sedis | 1.27 | 0.11 | 0.01 | 0.01 |
| Occultifur | 1.23 | 0.11 | 0.02 | 0.01 |
| Pezoloma | 1.21 | 0.07 | 0.01 | 0.00 |
| Tympanidaceae\_gen\_Incertae\_sedis | 1.18 | 0.11 | 0.02 | 0.01 |
| Hymenochaetales\_gen\_Incertae\_sedis | 1.17 | 0.13 | 0.02 | 0.02 |
| Luellia | 1.16 | 0.11 | 0.03 | 0.01 |
| Gloeopycnis | 1.13 | 0.08 | 0.03 | 0.01 |
| Tremella | 1.12 | 0.14 | 0.02 | 0.02 |
| Galerina | 1.12 | 0.09 | 0.02 | 0.00 |
| Apiotrichum | 1.12 | 0.13 | 0.01 | 0.01 |
| Pseudohyphozyma | 1.10 | 0.09 | 0.03 | 0.01 |
| Tylospora | 1.10 | 0.13 | 0.01 | 0.02 |
| Pochonia | 1.08 | 0.11 | 0.06 | 0.01 |
| GS04\_gen\_Incertae\_sedis | 1.07 | 0.15 | 0.06 | 0.03 |
| Acarospora | 1.06 | 0.12 | 0.02 | 0.01 |
| Tolypocladium | 1.06 | 0.13 | 0.03 | 0.01 |
| Chytridiales\_gen\_Incertae\_sedis | 1.04 | 0.13 | 0.03 | 0.02 |
| Cenangiaceae\_gen\_Incertae\_sedis | 1.02 | 0.15 | 0.06 | 0.04 |
| Auriculariales\_gen\_Incertae\_sedis | 1.01 | 0.14 | 0.05 | 0.03 |
| Entoloma | 1.00 | 0.13 | 0.10 | 0.01 |
| Chloridium | 1.00 | 0.13 | 0.03 | 0.01 |
| Sorocybe | 0.99 | 0.12 | 0.03 | 0.01 |
| Filobasidiales\_gen\_Incertae\_sedis | 0.97 | 0.13 | 0.05 | 0.01 |
| Cystoderma | 0.93 | 0.14 | 0.06 | 0.04 |
| Babjeviella | 0.93 | 0.16 | 0.07 | 0.05 |
| Thaxterogaster | 0.92 | 0.15 | 0.06 | 0.02 |
| Umbelopsis | 0.90 | 0.11 | 0.07 | 0.01 |
| Dissophora | 0.89 | 0.13 | 0.07 | 0.02 |
| Syzygospora | 0.89 | 0.17 | 0.05 | 0.03 |
| Cyphellophoraceae\_gen\_Incertae\_sedis | -0.88 | 0.16 | 0.05 | 0.05 |
| Geopora | -0.92 | 0.12 | 0.03 | 0.02 |
| Ceratobasidium | -0.99 | 0.12 | 0.04 | 0.02 |
| Lentitheciaceae\_gen\_Incertae\_sedis | -1.01 | 0.13 | 0.05 | 0.05 |
| Cryptococcus | -1.01 | 0.17 | 0.07 | 0.04 |
| Spermospora | -1.04 | 0.13 | 0.03 | 0.03 |
| Laetinaevia | -1.05 | 0.07 | 0.03 | 0.01 |
| Schizothecium | -1.06 | 0.13 | 0.06 | 0.04 |
| Nectriella | -1.06 | 0.10 | 0.01 | 0.01 |
| Mallocybe | -1.06 | 0.13 | 0.04 | 0.03 |
| Trichoderma | -1.06 | 0.13 | 0.01 | 0.02 |
| Trichosporiella | -1.09 | 0.11 | 0.05 | 0.03 |
| Sordariales\_gen\_Incertae\_sedis | -1.14 | 0.07 | 0.01 | 0.00 |
| Mycosphaerellales\_gen\_Incertae\_sedis | -1.15 | 0.12 | 0.04 | 0.03 |
| Oliveonia | -1.15 | 0.11 | 0.02 | 0.01 |
| Caloplaca | -1.16 | 0.08 | 0.02 | 0.01 |
| Rhizopogon | -1.16 | 0.13 | 0.01 | 0.01 |
| Nothodactylaria | -1.21 | 0.11 | 0.03 | 0.03 |
| Penicillium | -1.22 | 0.13 | 0.05 | 0.02 |
| Entimomentora | -1.22 | 0.13 | 0.02 | 0.03 |
| Pulvinula | -1.23 | 0.12 | 0.03 | 0.03 |
| Inocybe | -1.24 | 0.09 | 0.01 | 0.01 |
| Melanodiplodia | -1.25 | 0.09 | 0.02 | 0.01 |
| Rhexocercosporidium | -1.27 | 0.10 | 0.01 | 0.01 |
| Triscelophorus | -1.28 | 0.10 | 0.03 | 0.02 |
| Vishniacozyma | -1.29 | 0.05 | 0.01 | 0.00 |
| Keissleriella | -1.31 | 0.08 | 0.01 | 0.01 |
| Cladophialophora | -1.32 | 0.07 | 0.00 | 0.00 |
| Pseudocoleophoma | -1.38 | 0.09 | 0.01 | 0.01 |
| Neonectria | -1.38 | 0.07 | 0.00 | 0.00 |
| Helvella | -1.46 | 0.07 | 0.01 | 0.00 |
| Kurtzmanomyces | -1.46 | 0.07 | 0.01 | 0.01 |
| Verrucoccum | -1.46 | 0.09 | 0.01 | 0.01 |
| Phomatospora | -1.47 | 0.08 | 0.01 | 0.01 |
| Cosmospora | -1.48 | 0.06 | 0.00 | 0.00 |
| Sclerostagonospora | -1.63 | 0.07 | 0.01 | 0.00 |
| Plectosphaerella | -1.66 | 0.09 | 0.01 | 0.01 |
| Pezizellaster | -1.75 | 0.04 | 0.00 | 0.00 |
| Lobulomycetales\_gen\_Incertae\_sedis | -1.77 | 0.05 | 0.00 | 0.00 |
| Preussia | -1.78 | 0.04 | 0.00 | 0.00 |
| Cladosporium | -1.80 | 0.01 | 0.00 | 0.00 |
| Tetracladium | -1.87 | 0.02 | 0.00 | 0.00 |
| Hamatocanthoscypha | -1.94 | 0.03 | 0.00 | 0.00 |
| Paraphoma | -2.07 | 0.00 | 0.00 | 0.00 |
| Psoroglaena | -2.10 | 0.00 | 0.00 | 0.00 |
| Fusarium | -2.11 | 0.00 | 0.00 | 0.00 |
| Kodamaea | -2.17 | 0.00 | 0.00 | 0.00 |
| Pleotrichocladium | -2.26 | 0.02 | 0.00 | 0.00 |
| Truncatella | -2.48 | 0.01 | 0.00 | 0.00 |
| Exophiala | -2.51 | 0.01 | 0.00 | 0.00 |
| Talaromyces | -3.03 | 0.09 | 0.00 | 0.01 |
| Leohumicola | -3.88 | 0.00 | 0.00 | 0.00 |
| Knufia | -3.99 | 0.00 | 0.00 | 0.00 |

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

Ancombc2 analysis

# Run ANCOM-BC at the genus level and only including the prevalent genera  
ancombc2\_out <- ancombc2(  
 data = tse,  
 assay.type = "relabundance",  
 fix\_formula = "pH",  
 p\_adj\_method = "fdr",  
 prv\_cut = 0,  
 group = "pH",  
 struc\_zero = TRUE,  
 neg\_lb = TRUE,  
 global = FALSE,  
 verbose = FALSE  
 )

Genera with significantly different abundance

ancom\_summary <- ancombc2\_out$res %>%  
 dplyr::select(Genus = taxon, lfc = lfc\_pHacidic, se = se\_pHacidic, q = q\_pHacidic, diff = diff\_pHacidic) %>%  
 filter(diff == TRUE) %>% arrange(desc(lfc))  
# Summary table  
kable(ancom\_summary, digits=2, longtable=T, booktabs=T) %>%  
 kable\_styling(latex\_options = c("HOLD\_position", "striped", "repeat\_header"),  
 font\_size = 7) %>% row\_spec(0, color = "white",  
 background = "teal")

| Genus | lfc | se | q | diff |
| --- | --- | --- | --- | --- |
| Leotiomycetes\_gen\_Incertae\_sedis | 6.87 | 0.43 | 0.00 | TRUE |
| Archaeorhizomyces | 6.83 | 0.60 | 0.00 | TRUE |
| Meliniomyces | 5.97 | 0.54 | 0.00 | TRUE |
| Piloderma | 5.83 | 0.51 | 0.00 | TRUE |
| GS12\_gen\_Incertae\_sedis | 5.73 | 0.62 | 0.00 | TRUE |
| Chaetothyriales\_gen\_Incertae\_sedis | 5.11 | 0.55 | 0.00 | TRUE |
| Cephalothecaceae\_gen\_Incertae\_sedis | 5.04 | 0.62 | 0.00 | TRUE |
| Rhizidium | 4.75 | 0.44 | 0.00 | TRUE |
| GS21\_gen\_Incertae\_sedis | 4.60 | 0.42 | 0.00 | TRUE |
| Podila | 4.58 | 0.47 | 0.00 | TRUE |
| Sclerococcum | 4.57 | 0.80 | 0.00 | TRUE |
| Pseudoplectania | 4.48 | 0.58 | 0.00 | TRUE |
| Tympanidaceae\_gen\_Incertae\_sedis | 4.37 | 0.63 | 0.00 | TRUE |
| Helotiales\_gen\_Incertae\_sedis | 4.37 | 0.61 | 0.00 | TRUE |
| Herpotrichiellaceae\_gen\_Incertae\_sedis | 4.19 | 1.01 | 0.00 | TRUE |
| Clavaria | 4.02 | 0.80 | 0.00 | TRUE |
| Crocicreas | 3.96 | 0.51 | 0.00 | TRUE |
| Humicolopsis | 3.85 | 0.56 | 0.00 | TRUE |
| Tylospora | 3.76 | 0.89 | 0.00 | TRUE |
| Brahmaculus | 3.68 | 0.59 | 0.00 | TRUE |
| Hyphodiscus | 3.65 | 0.65 | 0.00 | TRUE |
| Tolypocladium | 3.59 | 0.74 | 0.00 | TRUE |
| Serendipitaceae\_gen\_Incertae\_sedis | 3.55 | 0.51 | 0.00 | TRUE |
| Leucosporidium | 3.53 | 0.66 | 0.00 | TRUE |
| Mycena | 3.48 | 0.59 | 0.00 | TRUE |
| Phialocephala | 3.43 | 0.66 | 0.00 | TRUE |
| Cenococcum | 3.41 | 0.70 | 0.00 | TRUE |
| Glutinomyces | 3.40 | 0.61 | 0.00 | TRUE |
| Lachnellula | 3.36 | 0.63 | 0.00 | TRUE |
| Hyaloscyphaceae\_gen\_Incertae\_sedis | 3.35 | 0.48 | 0.00 | TRUE |
| Thaxterogaster | 3.21 | 0.60 | 0.00 | TRUE |
| Volutella | 3.15 | 0.52 | 0.00 | TRUE |
| Sugiyamaella | 3.14 | 0.54 | 0.00 | TRUE |
| Scytalidium | 3.12 | 0.38 | 0.00 | TRUE |
| Cortinarius | 3.07 | 0.56 | 0.00 | TRUE |
| Filobasidiales\_gen\_Incertae\_sedis | 3.01 | 0.54 | 0.00 | TRUE |
| Pezoloma | 2.99 | 0.66 | 0.00 | TRUE |
| Myxozyma | 2.90 | 0.96 | 0.02 | TRUE |
| Apiotrichum | 2.58 | 0.77 | 0.01 | TRUE |
| Derxomyces | 2.56 | 0.50 | 0.00 | TRUE |
| Occultifur | 2.50 | 0.70 | 0.01 | TRUE |
| Umbelopsis | 2.48 | 0.77 | 0.01 | TRUE |
| Trechispora | 2.46 | 0.55 | 0.00 | TRUE |
| Phacidium | 2.43 | 0.45 | 0.00 | TRUE |
| Chloridium | 2.41 | 0.56 | 0.00 | TRUE |
| Syzygospora | 2.33 | 0.77 | 0.02 | TRUE |
| Sistotrema | 2.30 | 0.81 | 0.03 | TRUE |
| Chytridiales\_gen\_Incertae\_sedis | 1.96 | 0.59 | 0.01 | TRUE |
| Dissophora | 1.96 | 0.51 | 0.00 | TRUE |
| Mortierella | 1.92 | 0.39 | 0.00 | TRUE |
| Babjevia | 1.91 | 0.72 | 0.03 | TRUE |
| Claussenomyces | 1.89 | 0.60 | 0.02 | TRUE |
| Genolevuria | 1.73 | 0.54 | 0.02 | TRUE |
| Galerina | 1.72 | 0.47 | 0.01 | TRUE |
| Tyrannosorus | 1.55 | 0.60 | 0.04 | TRUE |
| Hamamotoa | -1.00 | 0.37 | 0.05 | TRUE |
| Coleophoma | -1.23 | 0.41 | 0.02 | TRUE |
| Penicillium | -1.23 | 0.52 | 0.05 | TRUE |
| Lophium | -1.25 | 0.38 | 0.02 | TRUE |
| Mucorales\_gen\_Incertae\_sedis | -1.34 | 0.34 | 0.01 | TRUE |
| Cystobasidiomycetes\_gen\_Incertae\_sedis | -1.34 | 0.48 | 0.03 | TRUE |
| Bryochiton | -1.42 | 0.46 | 0.04 | TRUE |
| Zoopagales\_gen\_Incertae\_sedis | -1.58 | 0.37 | 0.01 | TRUE |
| Lycoperdon | -1.77 | 0.69 | 0.04 | TRUE |
| Keissleriella | -1.82 | 0.57 | 0.01 | TRUE |
| Camptobasidiaceae\_gen\_Incertae\_sedis | -1.83 | 0.38 | 0.01 | TRUE |
| Hypomyces | -1.85 | 0.65 | 0.03 | TRUE |
| Kurtzmaniella | -2.00 | 0.55 | 0.01 | TRUE |
| Malassezia | -2.03 | 0.68 | 0.02 | TRUE |
| Microbotryomycetes\_gen\_Incertae\_sedis | -2.06 | 0.45 | 0.00 | TRUE |
| Mytilinidiales\_gen\_Incertae\_sedis | -2.06 | 0.43 | 0.02 | TRUE |
| Sepedonium | -2.17 | 0.62 | 0.01 | TRUE |
| Drechmeria | -2.22 | 0.49 | 0.00 | TRUE |
| Fagicola | -2.27 | 0.38 | 0.00 | TRUE |
| Sporormiella | -2.28 | 0.84 | 0.03 | TRUE |
| Septoria | -2.32 | 0.49 | 0.00 | TRUE |
| Cladophialophora | -2.32 | 0.56 | 0.00 | TRUE |
| Thelebolus | -2.41 | 0.72 | 0.01 | TRUE |
| Cistella | -2.44 | 0.81 | 0.02 | TRUE |
| Taphrina | -2.46 | 0.54 | 0.01 | TRUE |
| Agaricales\_gen\_Incertae\_sedis | -2.51 | 0.40 | 0.00 | TRUE |
| Laetinaevia | -2.68 | 0.69 | 0.00 | TRUE |
| Hormonema | -2.75 | 0.39 | 0.00 | TRUE |
| Mrakia | -2.75 | 0.41 | 0.00 | TRUE |
| Ceratobasidium | -2.81 | 0.54 | 0.00 | TRUE |
| Lapidomyces | -2.83 | 0.40 | 0.00 | TRUE |
| Pseudeurotium | -2.86 | 0.68 | 0.00 | TRUE |
| Vishniacozyma | -2.88 | 0.68 | 0.00 | TRUE |
| Rhizopogon | -2.89 | 0.80 | 0.00 | TRUE |
| Nectriella | -2.97 | 0.82 | 0.00 | TRUE |
| Sakaguchia | -3.00 | 0.35 | 0.00 | TRUE |
| Tomentella | -3.02 | 0.89 | 0.01 | TRUE |
| Alternaria | -3.15 | 0.74 | 0.01 | TRUE |
| Pustularia | -3.18 | 0.61 | 0.00 | TRUE |
| Geopora | -3.22 | 1.06 | 0.01 | TRUE |
| Neonectria | -3.27 | 0.86 | 0.01 | TRUE |
| Ceratobasidiaceae\_gen\_Incertae\_sedis | -3.41 | 0.46 | 0.00 | TRUE |
| Preussia | -3.51 | 0.67 | 0.00 | TRUE |
| Cladosporium | -3.54 | 0.64 | 0.00 | TRUE |
| Talaromyces | -3.66 | 0.68 | 0.00 | TRUE |
| Sordariales\_gen\_Incertae\_sedis | -3.67 | 0.95 | 0.00 | TRUE |
| Pezizellaster | -3.70 | 0.73 | 0.00 | TRUE |
| Exophiala | -3.87 | 0.54 | 0.00 | TRUE |
| Inocybe | -3.92 | 0.97 | 0.00 | TRUE |
| Cryptococcus | -3.93 | 0.86 | 0.00 | TRUE |
| Lecythophora | -4.04 | 0.74 | 0.00 | TRUE |
| Paraphoma | -4.05 | 0.55 | 0.00 | TRUE |
| Sclerostagonospora | -4.25 | 0.57 | 0.00 | TRUE |
| Hamatocanthoscypha | -4.31 | 0.78 | 0.00 | TRUE |
| Pleotrichocladium | -4.42 | 0.59 | 0.00 | TRUE |
| Rhexocercosporidium | -4.48 | 0.87 | 0.00 | TRUE |
| Fusarium | -4.79 | 0.67 | 0.00 | TRUE |
| Tetracladium | -4.93 | 0.84 | 0.00 | TRUE |
| Mallocybe | -5.34 | 0.86 | 0.00 | TRUE |
| Kodamaea | -5.36 | 0.67 | 0.00 | TRUE |
| Plectosphaerella | -5.51 | 0.49 | 0.00 | TRUE |
| Knufia | -6.02 | 0.45 | 0.00 | TRUE |
| Leohumicola | -6.71 | 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 | 0.24 | 0.03 | 0.00 | 23 |
| Piloderma | 0.21 | 0.04 | 0.00 | 23 |
| Cortinarius | 0.19 | 0.03 | 0.00 | 23 |
| 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 |
| 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 |
| Gyoerffyella | 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 |
| 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 |
| 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 |
| 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 |
| Cylindrosympodium | -0.01 | 0.00 | 0.04 | 12 |
| Helminthosphaeriaceae\_gen\_Incertae\_sedis | -0.01 | 0.00 | 0.04 | 15 |
| Verrucoccum | -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 |
| Phomatospora | -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)\*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 |
| Sclerococcum | TRUE | TRUE | TRUE |
| Mortierella | TRUE | TRUE | TRUE |
| Scleropezicula | TRUE | FALSE | 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 | FALSE |
| Brahmaculus | TRUE | TRUE | TRUE |
| Humicolopsis | TRUE | TRUE | TRUE |
| Serendipitaceae\_gen\_Incertae\_sedis | TRUE | TRUE | TRUE |
| Phacidium | TRUE | TRUE | FALSE |
| Acarosporales\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Cenococcum | TRUE | TRUE | TRUE |
| Lachnellula | TRUE | TRUE | TRUE |
| Cortinarius | TRUE | TRUE | TRUE |
| Pseudoplectania | TRUE | TRUE | TRUE |
| Scytalidium | TRUE | TRUE | FALSE |
| Hyphodiscus | TRUE | TRUE | TRUE |
| Leptobacillium | TRUE | FALSE | TRUE |
| Mycena | TRUE | TRUE | TRUE |
| Phialocephala | TRUE | TRUE | TRUE |
| Leucosporidium | TRUE | TRUE | TRUE |
| Leccinum | TRUE | FALSE | TRUE |
| Hyaloscyphaceae\_gen\_Incertae\_sedis | TRUE | TRUE | FALSE |
| Clavaria | TRUE | TRUE | FALSE |
| Trechispora | TRUE | TRUE | TRUE |
| GS22\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Pezizomycotina\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Occultifur | TRUE | TRUE | FALSE |
| Pezoloma | TRUE | TRUE | TRUE |
| Tympanidaceae\_gen\_Incertae\_sedis | TRUE | TRUE | FALSE |
| Hymenochaetales\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Luellia | TRUE | FALSE | TRUE |
| Gloeopycnis | TRUE | FALSE | FALSE |
| Tremella | TRUE | FALSE | TRUE |
| Galerina | TRUE | TRUE | TRUE |
| Apiotrichum | TRUE | TRUE | TRUE |
| Pseudohyphozyma | TRUE | FALSE | TRUE |
| Tylospora | TRUE | TRUE | TRUE |
| Pochonia | TRUE | FALSE | TRUE |
| GS04\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Acarospora | TRUE | FALSE | FALSE |
| Tolypocladium | TRUE | TRUE | TRUE |
| Chytridiales\_gen\_Incertae\_sedis | TRUE | TRUE | TRUE |
| Cenangiaceae\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Auriculariales\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Entoloma | TRUE | FALSE | FALSE |
| Chloridium | TRUE | TRUE | FALSE |
| Sorocybe | TRUE | FALSE | TRUE |
| Filobasidiales\_gen\_Incertae\_sedis | TRUE | TRUE | FALSE |
| Cystoderma | TRUE | FALSE | TRUE |
| Babjeviella | TRUE | FALSE | TRUE |
| Thaxterogaster | TRUE | TRUE | FALSE |
| Umbelopsis | TRUE | TRUE | TRUE |
| Dissophora | TRUE | TRUE | FALSE |
| Syzygospora | TRUE | TRUE | TRUE |
| Cyphellophoraceae\_gen\_Incertae\_sedis | TRUE | FALSE | FALSE |
| Geopora | TRUE | TRUE | FALSE |
| Ceratobasidium | TRUE | TRUE | FALSE |
| Lentitheciaceae\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Cryptococcus | TRUE | TRUE | TRUE |
| Spermospora | TRUE | FALSE | FALSE |
| Laetinaevia | TRUE | TRUE | FALSE |
| Schizothecium | TRUE | FALSE | TRUE |
| Nectriella | TRUE | TRUE | FALSE |
| Mallocybe | TRUE | TRUE | FALSE |
| Trichoderma | TRUE | FALSE | FALSE |
| Trichosporiella | TRUE | FALSE | TRUE |
| Sordariales\_gen\_Incertae\_sedis | TRUE | TRUE | FALSE |
| Mycosphaerellales\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Oliveonia | TRUE | FALSE | TRUE |
| Caloplaca | TRUE | FALSE | FALSE |
| Rhizopogon | TRUE | TRUE | TRUE |
| Nothodactylaria | TRUE | FALSE | TRUE |
| Penicillium | TRUE | TRUE | TRUE |
| Entimomentora | TRUE | FALSE | TRUE |
| Pulvinula | TRUE | FALSE | TRUE |
| Inocybe | TRUE | TRUE | FALSE |
| Melanodiplodia | TRUE | FALSE | FALSE |
| Rhexocercosporidium | TRUE | TRUE | FALSE |
| Triscelophorus | TRUE | FALSE | TRUE |
| Vishniacozyma | TRUE | TRUE | FALSE |
| Keissleriella | TRUE | TRUE | FALSE |
| Cladophialophora | TRUE | TRUE | TRUE |
| Pseudocoleophoma | TRUE | FALSE | TRUE |
| Neonectria | TRUE | TRUE | TRUE |
| Helvella | TRUE | FALSE | TRUE |
| Kurtzmanomyces | TRUE | FALSE | TRUE |
| Verrucoccum | TRUE | FALSE | TRUE |
| Phomatospora | TRUE | FALSE | TRUE |
| Cosmospora | TRUE | FALSE | TRUE |
| Sclerostagonospora | TRUE | TRUE | TRUE |
| Plectosphaerella | TRUE | TRUE | TRUE |
| Pezizellaster | TRUE | TRUE | TRUE |
| Lobulomycetales\_gen\_Incertae\_sedis | TRUE | FALSE | TRUE |
| Preussia | TRUE | TRUE | FALSE |
| Cladosporium | TRUE | TRUE | TRUE |
| Tetracladium | TRUE | TRUE | TRUE |
| Hamatocanthoscypha | TRUE | TRUE | TRUE |
| Paraphoma | TRUE | TRUE | TRUE |
| Psoroglaena | TRUE | FALSE | FALSE |
| Fusarium | TRUE | TRUE | 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 | TRUE |
| Genolevuria | FALSE | TRUE | TRUE |
| Tyrannosorus | FALSE | TRUE | TRUE |
| Hamamotoa | FALSE | TRUE | FALSE |
| 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 | TRUE |
| Thelebolus | FALSE | TRUE | FALSE |
| Cistella | FALSE | TRUE | FALSE |
| 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 |
| Sakaguchia | FALSE | TRUE | FALSE |
| Tomentella | FALSE | TRUE | FALSE |
| Alternaria | FALSE | TRUE | FALSE |
| Pustularia | FALSE | TRUE | FALSE |
| Ceratobasidiaceae\_gen\_Incertae\_sedis | FALSE | TRUE | FALSE |
| Lecythophora | FALSE | TRUE | TRUE |
| Oidiodendron | FALSE | FALSE | TRUE |
| Branch01\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| Syncephalis | FALSE | FALSE | TRUE |
| Diademospora | FALSE | FALSE | TRUE |
| Blastocladiomycota\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| Glomeromycota\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| GS15\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| Chaetosphaeria | FALSE | FALSE | TRUE |
| Nadsonia | FALSE | FALSE | TRUE |
| Synnemapestaloides | FALSE | FALSE | TRUE |
| Siepmannia | FALSE | FALSE | TRUE |
| Dermateaceae\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| Pseudogymnoascus | FALSE | FALSE | TRUE |
| Rhodotorula | FALSE | FALSE | TRUE |
| Coccomyces | FALSE | FALSE | TRUE |
| Aspicilia | FALSE | FALSE | TRUE |
| Endogonomycetes\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| Fayodia | 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 |
| Gyoerffyella | FALSE | FALSE | TRUE |
| Allantophomopsiella | FALSE | FALSE | TRUE |
| Flammula | FALSE | FALSE | TRUE |
| Rhizosphaera | FALSE | FALSE | TRUE |
| Microsporomycetaceae\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| Ophiocordycipitaceae\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| Nagrajchalara | 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 |
| Rhizophagus | FALSE | FALSE | TRUE |
| Achroceratosphaeria | FALSE | FALSE | TRUE |
| Herpotrichia | FALSE | FALSE | TRUE |
| Sporormiaceae\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| Cylindrosympodium | FALSE | FALSE | TRUE |
| Helminthosphaeriaceae\_gen\_Incertae\_sedis | FALSE | FALSE | TRUE |
| Ascochyta | FALSE | FALSE | TRUE |
| Polyphilus | FALSE | FALSE | TRUE |
| Cadophora | FALSE | FALSE | TRUE |
| Leptodophora | FALSE | FALSE | TRUE |
| Thelephora | FALSE | FALSE | TRUE |