

SH_Graph

Importing data and pre-processing

```
library(phyloseq)
library(ggplot2)
library(tidyverse)
library(RColorBrewer)
library(cowplot)
library(metacoder)
SH_ITS_data0 <- import_biom(BIOMfilename = "otu_table.biom")
SH_ITS_data0
```

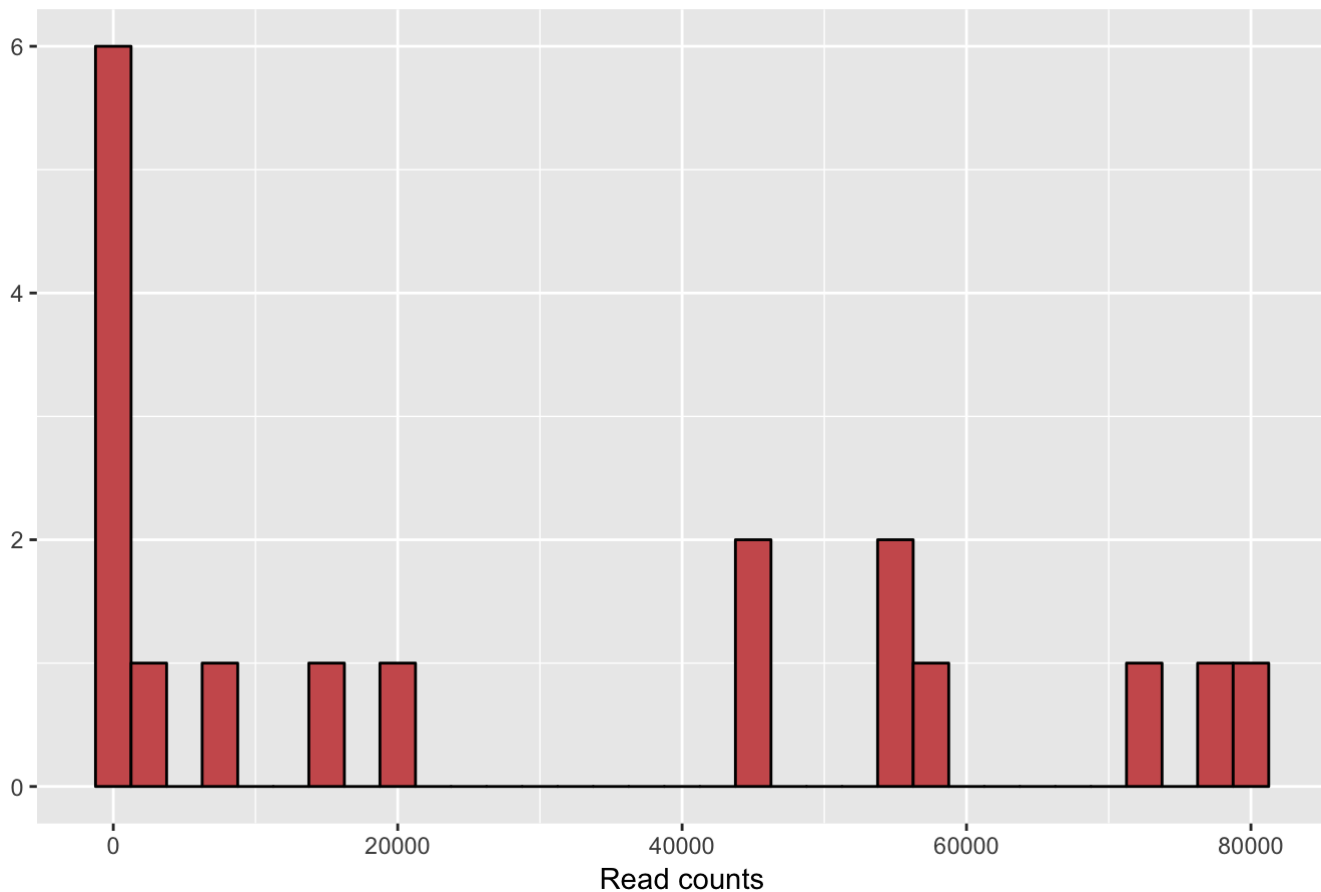
```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 59629 taxa and 18 samples ]
## tax_table()   Taxonomy Table:    [ 59629 taxa by 7 taxonomic ranks ]
```

```
sam.data <- read.csv (file = "mapping_file_ITS_R1_corrected.csv", row.names = 1, header
= TRUE)
sample_data (SH_ITS_data0) <- sam.data
```

Checking sequencing depth

```
# Make a data frame with a column for the read counts of each sample
sample_sum_df <- data.frame(sum = sample_sums(SH_ITS_data0))
# Histogram of sample read counts
ggplot(sample_sum_df, aes(x = sum)) +
  geom_histogram(color = "black", fill = "indianred", binwidth = 2500) +
  ggtitle("Distribution of sample sequencing depth") +
  xlab("Read counts") +
  theme_gray() + theme(axis.title.y = element_blank())
```

Distribution of sample sequencing depth



Standardizing by sequencing depth

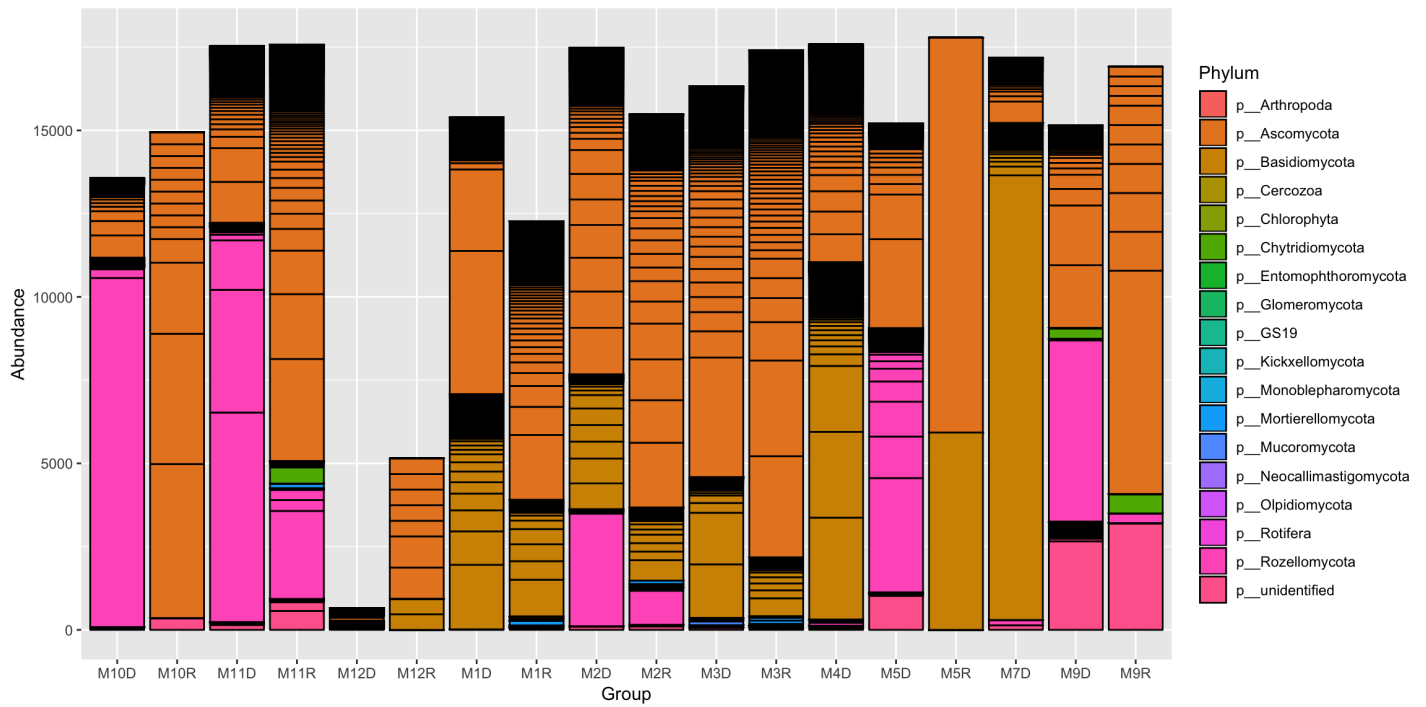
```
#Standardize abundances to the median sequencing depth
total <- median(sample_sums(SH_ITS_data0))
standf <- function(x, t=total) round(t * (x/sum(x)))
SH_ITS_data0.std <- transform_sample_counts(SH_ITS_data0, standf)
```

Filtering “no hits”

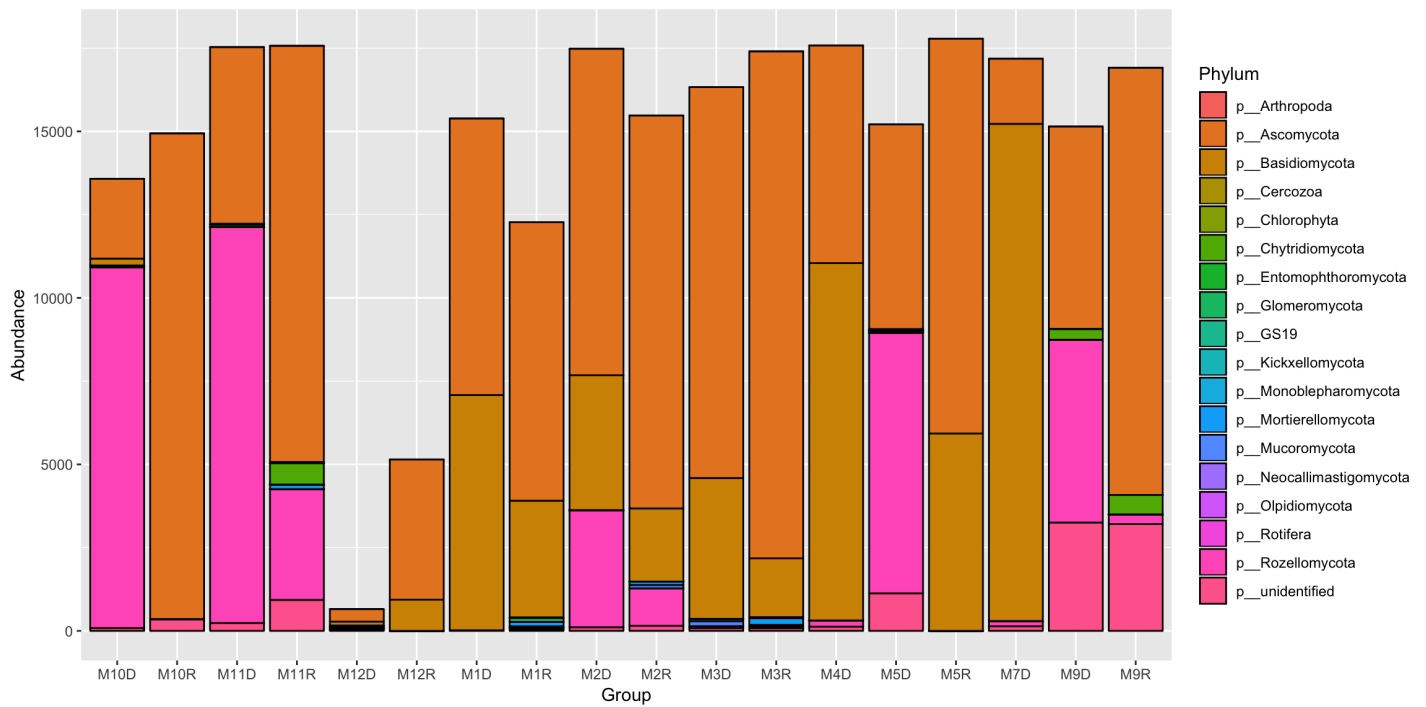
```
#Renaming taxonomy levels on tax_table
colnames(tax_table(SH_ITS_data0.std)) <- c("Kingdom", "Phylum", "Class", "Order", "Family", "Genus", "Species")
#Filtering no hit at Kingdom level
SH_ITS_data <- subset_taxa(SH_ITS_data0.std, Kingdom != "No blast hit")
```

Taxa abundance at different rank levels

```
#summarizing by tax rank
SH_ITS.phylum <- tax_glom(SH_ITS_data, "Phylum")
plot_bar(SH_ITS_data, fill = "Phylum", x = "Group") + theme_gray()
```



```
plot_bar(SH_ITS.phylum, fill = "Phylum", x = "Group") + theme_gray()
```



```
#Plot by family
SH_ITS.fam <- tax_glom(SH_ITS_data, "Family")
plot_bar(SH_ITS.fam, fill = "Family", x = "Group") + theme_gray()
```

	f__Geoglossaceae	f__Hydnangiaceae	f__Lophiostomataceae	f__Myrangiaceae	f__Phaeosphaeriaceae
	f__Gigasporaceae	f__Hydnodontaceae	f__Lyophyllaceae	f__Myrmecridiaceae	f__Phaeotremellaceae
dis	f__Gloeophyllaceae	f__Hygrophoraceae	f__Magnaporthaceae	f__Myxotrichaceae	f__Phyllostictaceae
	f__Glomeraceae	f__Hymenochaetaceae	f__Maliasseziaceae	f__Nectriaceae	f__Physalacriaceae
	f__Glomerellaceae	f__Hymenochaetales_fam_Incertae_sedis	f__Marasmiaceae	f__Neocallimastigaceae	f__Physciaceae
	f__Gloniaceae	f__Hymenogastraceae	f__Massarinaceae	f__Neoelectaceae	f__Pichiaceae
	f__Gnomoniaceae	f__Hyphodermataceae	f__Megasporeaceae	f__Ochrolechiaceae	f__Piskurozymaceae
	f__Gonapodyaceae	f__Hypocreaceae	f__Melanconidaceae	f__Omphalotaceae	f__Pisolithaceae
	f__Graphidaceae	f__Hypocreales_fam_Incertae_sedis	f__Meruliaceae	f__Onygenales_fam_Incertae_sedis	f__Platyglaceae
	f__Grifolaceae	f__Hyponectriaceae	f__Micareaceae	f__Ophiocordycipitaceae	f__Plectosphaerella
	f__Haematommataceae	f__Inocybaceae	f__Microascaceae	f__Ophiostomataceae	f__Pleosporaceae
	f__Halomycetaceae	f__Kappamycetaceae	f__Microbotryomycetes_fam_Incertae_sedis	f__Orbiliaceae	f__Pleosporales_far
dis	f__Hebelomataceae	f__Kickxellaceae	f__Microdochiaceae	f__Parmeliaceae	f__Pleurostomataceae
	f__Helotiaceae	f__Lachnocladiaceae	f__Microsporomycetaceae	f__Peniophoraceae	f__Pleurotheciaceae
	f__Helotiales_fam_Incertae_sedis	f__Lasiosphaeriaceae	f__Morosphaeriaceae	f__Periconiaceae	f__Pluteaceae
	f__Herpotrichiellaceae	f__Lecanoraceae	f__Mortierellaceae	f__Pezizaceae	f__Polyporaceae
	f__Holtermanniales_fam_Incertae_sedis	f__Lecanoromycetes_fam_Incertae_sedis	f__Mrakiaceae	f__Phacidaceae	f__Polyporales_fam
	f__Hyaloriaceae	f__Leotiaceae	f__Mycenaceae	f__Phaeochoraceae	f__Porpidiaceae
	f__Hyaloscyphaeaceae	f__Leptosphaeriaceae	f__Mycocaliciaceae	f__Phaeococomycetaceae	f__Protomycetaceae
	f__Hydnaceae	f__Lichtheimiaceae	f__Mycosphaerellaceae	f__Phaeomoniellaceae	f__Psathyrellaceae

Relative abundance of genus and class in BGM soil and root samples

```
TopNOTUS <- function(sample,N) {
  names(sort(taxa_sums(sample), TRUE)[1:N])
}
#Creating data frame from phyloseq object
top.SH_ITS <- TopNOTUS(SH_ITS_data, 50)
SH_ITS.df <- prune_taxa(top.SH_ITS, SH_ITS_data) %>% psmelt()
#Sumarize data by site
#test <- BGM.df %>% group_by(Genus) %>% summarise(sum(Abundance))
#plot by relative abundance
#Plot
pal <- colorRampPalette((brewer.pal(10, "Paired")))
SH_ITS.genus <- ggplot(data = SH_ITS.df, aes(Group, Abundance, fill = Genus)) +
  geom_bar(stat = "identity", position = position_fill()) + coord_flip() +
  scale_fill_manual(values = (rev(pal(24)))) +
  guides(fill = guide_legend(reverse = TRUE, ncol = 1, keyheight = 0.8)) +
  theme(text = element_text(size = 15)) + theme_gray()

SH_ITS.genus
```

