Metagenomics Analysis on Fungal Communities in Stone Fruit Ecosystems in Canada

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Contents

0.1 Investigation of Fungal Diversity in Stone Fruit Samples in BC and ON

0.1.1 (1) Loading Packages

A comprehensive list of packages required to perform metagenomics analysis for *ION torrent* data

```
library("knitr")
library("qiime2R") # devtools::install_github("jbisanz/qiime2R")
library("phyloseq")
library("readxl")
library("tibble")
library("vegan")
library("DESeq2") #BiocManager::install("DESeq2")
library("speedyseq") # remotes::install_github("mikemc/speedyseq")
library("ape")
library("ggstar")
library("forcats")
library("patchwork")
library("ggpubr")
library("plotROC")
library("viridis")
library("cowplot")
library("ggplot2")
library("microbiome") # BiocManager::install("microbiome")
library("microbiomeutilities")

library("ggtree") # BiocManager::install("ggtree")
library("ggtreeExtra") #install.packages("ggExtra")
library('MicrobiotaProcess') # BiocManager::install("MicrobiotaProcess")
#library("tidytree")
library("file2meco")
library("microeco")

#install.packages('tinytex')
#tinytex::install_tinytex()  # install TinyTeX
```

0.1.2 (2) Read Depth Analysis

Image is loaded from Ch1_Setup.md , thus we have to remove a number of PS objects and retain only applicable PS Objects for this analysis

Observe read depth to make informed decisions about normalization or standardization of data

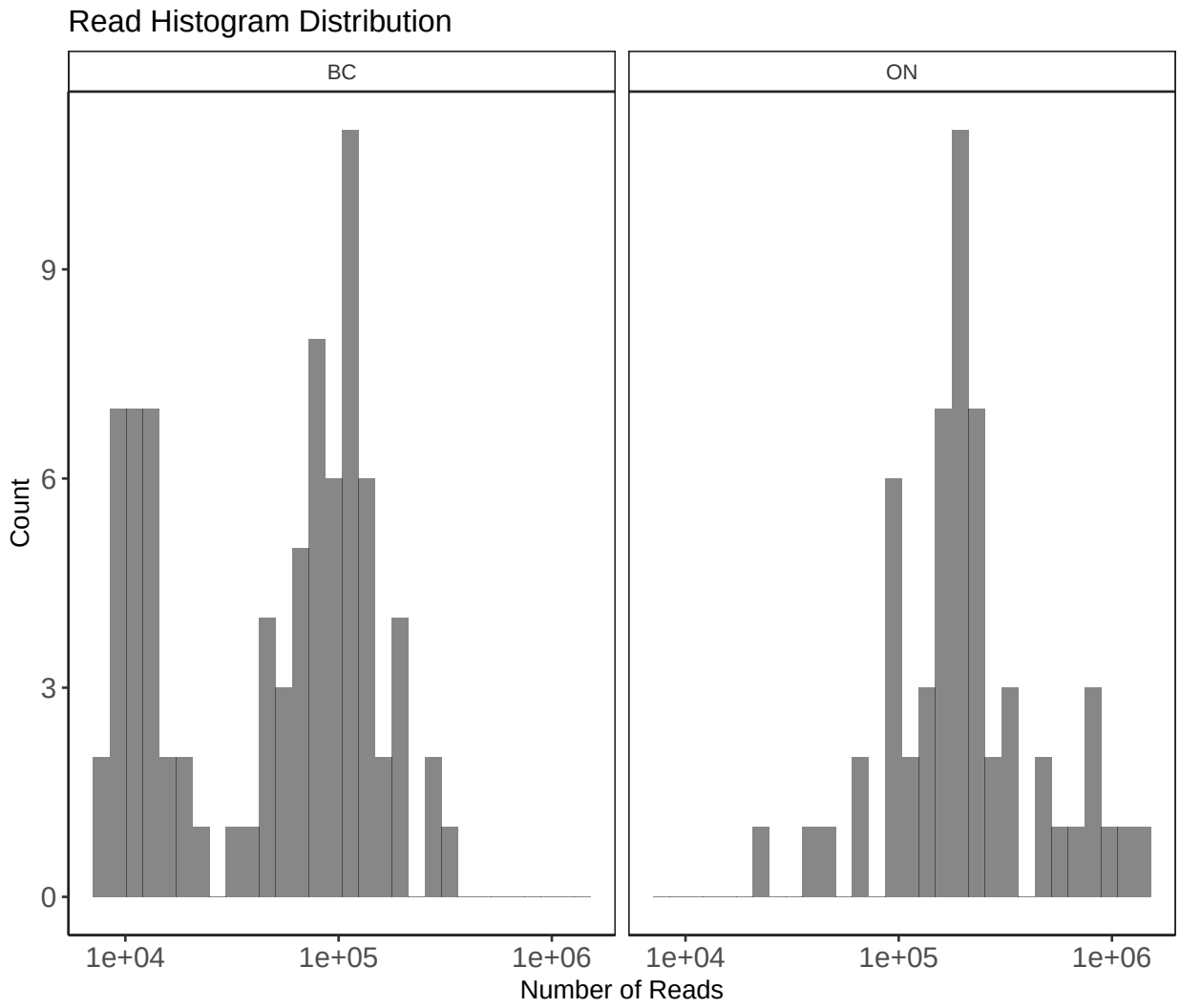
```
#load data from previous Ch1_Setup
load("D:\\Grad_School\\R_Projects\\BeeProject_Metagenomics\\Images\\Setup.RData")

#Remove unnessecary PSObjects
rm(PS_16S_BC_ON,PS_16S_BC_ON_BB,PS_16S_BC_ON_SF,PS_16S_Global)

#visualize the objects
PS_ITS1R_BC_ON_SF
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:          [ 11005 taxa and 138 samples ]:
## sample_data() Sample Data:        [ 138 samples by 15 sample variables ]:
## tax_table()   Taxonomy Table:     [ 11005 taxa by 7 taxonomic ranks ]:
## phy_tree()    Phylogenetic Tree:  [ 11005 tips and 10923 internal nodes ]:
## taxa are rows
```

```
#Visualize the metadatafile
head(meta(PS_ITS1R_BC_ON_SF))
```

```
## # A tibble: 6 x 15
##   barcode.seqeunce linker.primer.seqeunce    InputFileName Description RunNumber
##   <chr>            <chr>                     <chr>         <chr>       <chr>
## 1 CTAAGGTAA        CGATCTTGGTCATTTAGAGGAAGT~ A01_9_L001_R~ ITS2_A_B01  BCC_R1
## 2 TAAGGAGAA        CGATCTTGGTCATTTAGAGGAAGT~ A02_9_L001_R~ ITS2_A_B02  BCC_R1
## 3 AAGAGGATT        CGATCTTGGTCATTTAGAGGAAGT~ A03_9_L001_R~ ITS2_A_B03  BCC_R1
## 4 TACCAAGAT        CGATCTTGGTCATTTAGAGGAAGT~ A04_9_L001_R~ ITS2_A_B04  BCC_R1
## 5 CAGAAGGAA        CGATCTTGGTCATTTAGAGGAAGT~ A05_9_L001_R~ ITS2_A_B05  BCC_R1
## 6 CTGCAAGTT        CGATCTTGGTCATTTAGAGGAAGT~ A06_9_L001_R~ ITS2_A_B06  BCC_R1
## # i 10 more variables: SampleName <chr>, Number <int>, Province <chr>,
## #   Location <chr>, Plant <chr>, Sample.type <chr>, Site <chr>,
## #   DateCollected <chr>, Colony <chr>, DNANg <dbl>
```

```
#meta(PS_ITS1R_BC_ON_SF)

#Read depth analysis processing
Read_Depth_PS_ITS1R_BC_ON_SF<-plot_read_distribution(PS_ITS1R_BC_ON_SF, groups = "Province",
                           plot.type = "histogram")+
  theme_biome_utils()+
  scale_x_continuous(trans='log10')+
  scale_fill_manual(values=c("#111111"))+
  theme(legend.position="none")+
  labs(title = "Read Histogram Distribution",x = "Number of Reads", y = "Count")

#Visualize
Read_Depth_PS_ITS1R_BC_ON_SF
```



0.1.3 (3) Singleton Removal

A singleton is defined as a read that occurs uniquely in the dataset. Normally, we remove singletons as they are usually artifacts generated from sequencing error.

Here we identify 4563 singletons and we remove them to create a **Pruned** PS Object

```
#Summarize the PS Object to observe singletons
summarize_phyloseq(PS_ITS1R_BC_ON_SF)
```

```
## [[1]]
## [1] "1] Min. number of reads = 7904"
##
## [[2]]
## [1] "2] Max. number of reads = 1422317"
##
## [[3]]
## [1] "3] Total number of reads = 22291019"
##
## [[4]]
## [1] "4] Average number of reads = 161529.123188406"
```

```
##
## [[5]]
## [1] "5] Median number of reads = 108408.5"
##
## [[6]]
## [1] "7] Sparsity = 0.993258663716756"
##
## [[7]]
## [1] "6] Any OTU sum to 1 or less? YES"
##
## [[8]]
## [1] "8] Number of singletons = 4563"
##
## [[9]]
## [1] "9] Percent of OTUs that are singletons \n        (i.e. exactly one read detected across all sam
##
## [[10]]
## [1] "10] Number of sample variables are: 15"
##
## [[11]]
##  [1] "barcode.seqeunce"       "linker.primer.seqeunce" "InputFileName"
##  [4] "Description"            "RunNumber"              "SampleName"
##  [7] "Number"                 "Province"               "Location"
## [10] "Plant"                  "Sample.type"            "Site"
## [13] "DateCollected"          "Colony"                 "DNANg"
```

```
#Number of singletons = 4563

#We need to remove the so we remove any taxa sums that are less than 2 reads, so we use the prune_taxa
PS_ITS1R_BC_ON_SF_Pruned = prune_taxa(taxa_sums(PS_ITS1R_BC_ON_SF) > 02, PS_ITS1R_BC_ON_SF)

PS_ITS1R_BC_ON_SF_Pruned
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:          [ 6377 taxa and 138 samples ]:
## sample_data() Sample Data:        [ 138 samples by 15 sample variables ]:
## tax_table()   Taxonomy Table:     [ 6377 taxa by 7 taxonomic ranks ]:
## phy_tree()    Phylogenetic Tree:  [ 6377 tips and 6338 internal nodes ]:
## taxa are rows
```

```
summarize_phyloseq(PS_ITS1R_BC_ON_SF_Pruned)
```

```
## [[1]]
## [1] "1] Min. number of reads = 7904"
##
## [[2]]
## [1] "2] Max. number of reads = 1422313"
##
## [[3]]
## [1] "3] Total number of reads = 22290889"
##
## [[4]]
## [1] "4] Average number of reads = 161528.18115942"
##
## [[5]]
```

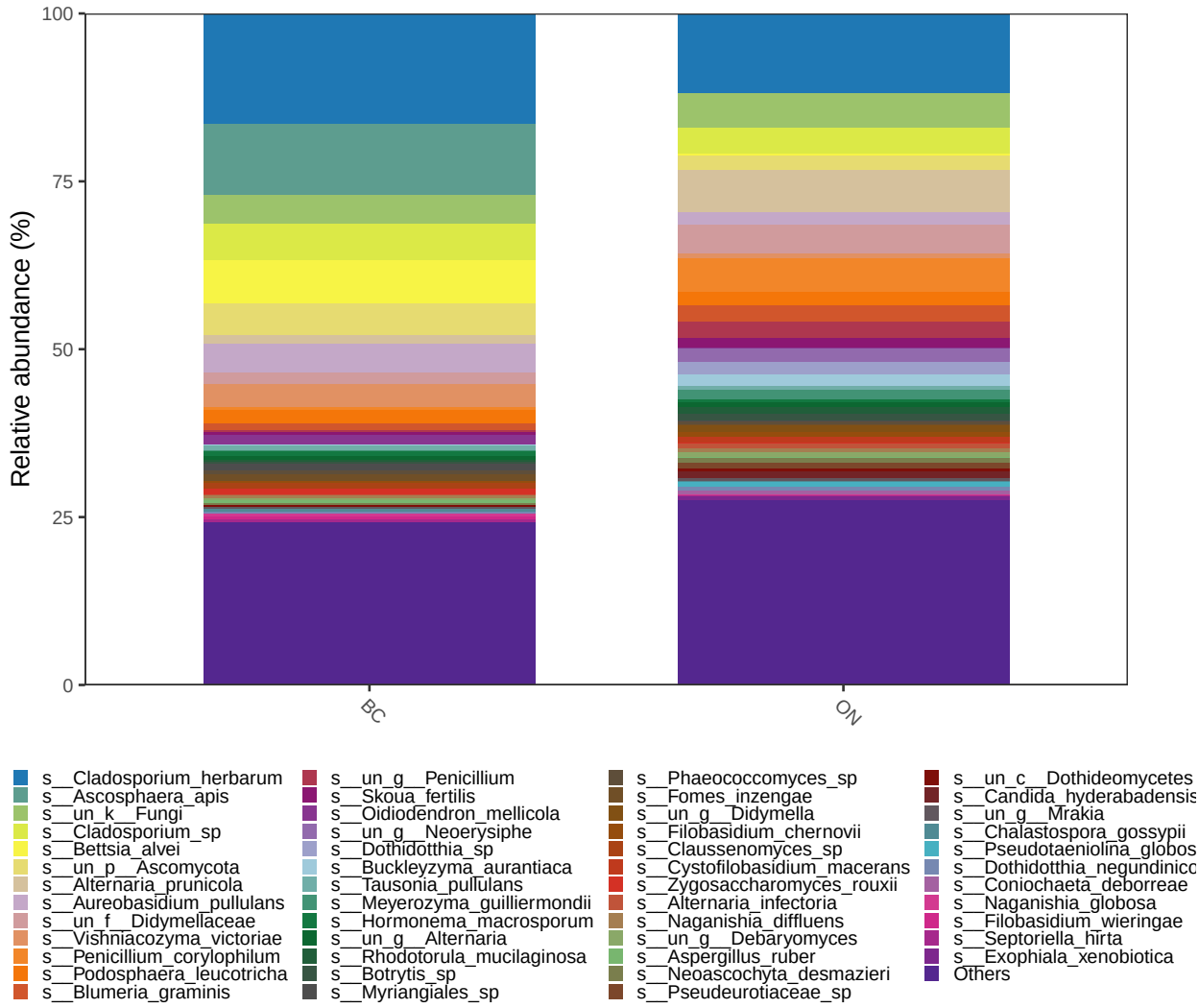
```
## [1] "5] Median number of reads = 108408.5"
##
## [[6]]
## [1] "7] Sparsity = 0.988440114269351"
##
## [[7]]
## [1] "6] Any OTU sum to 1 or less? NO"
##
## [[8]]
## [1] "8] Number of singletons = 0"
##
## [[9]]
## [1] "9] Percent of OTUs that are singletons \n        (i.e. exactly one read detected across all sam
##
## [[10]]
## [1] "10] Number of sample variables are: 15"
##
## [[11]]
##  [1] "barcode.seqeunce"       "linker.primer.seqeunce" "InputFileName"
##  [4] "Description"            "RunNumber"              "SampleName"
##  [7] "Number"                 "Province"               "Location"
## [10] "Plant"                  "Sample.type"            "Site"
## [13] "DateCollected"          "Colony"                 "DNANg"
```

Singletons were successfully removed as singleton count = 0

0.1.4 (4) Observation of Ubiqitous species

Now I generated a **relative abundance** profile for all fungal hits in the pruned object. We observe a number of species that are considered **ubiquitous** and not necessary for the goals of the project.

```
#top_taxa(PS_ITS1R_BC_ON_SF_Pruned)

#Utilzation of taxalevel 7 to obtain species level detections
classtaxa_ITS1R_BC_ON_SF_Pruned <- get_taxadf(obj=PS_ITS1R_BC_ON_SF_Pruned, taxlevel=7)
# The 50 most abundant taxonomy will be visualized by default (parameter `topn=50`).
TopTaxa_ITS1R_BC_ON_SF_Pruned<- ggbartax(obj=classtaxa_ITS1R_BC_ON_SF_Pruned,
                   facetNames="Province",
                   plotgroup=TRUE,
                   topn=50) +
  xlab(NULL) +
  ylab("Relative abundance (%)") +
  guides(fill= guide_legend(keywidth = 0.5, keyheight = 0.5, ncol=4))

TopTaxa_ITS1R_BC_ON_SF_Pruned
```




```
#Take a closer look at the species we can remove by creating a taxa table
Taxa_Table_ITS1R_BC_ON_SF_Pruned<-as.data.frame(phyloseq::tax_table(PS_ITS1R_BC_ON_SF_Pruned))

#Taxa_Table_ITS1R_BC_ON_SF_Pruned

head(meta(Taxa_Table_ITS1R_BC_ON_SF_Pruned))
```

```
## # A tibble: 6 x 7
##   Kingdom Phylum     Class           Order           Family      Genus Species
##   <chr>   <chr>      <chr>           <chr>           <chr>       <chr> <chr>
## 1 Fungi   Ascomycota Saccharomycetes Saccharomycetales Phaffomyce~ Cybe~ Cyberl~
## 2 Fungi   Ascomycota Saccharomycetes Saccharomycetales Phaffomyce~ Cybe~ Cyberl~
## 3 Fungi   Ascomycota Saccharomycetes Saccharomycetales Phaffomyce~ Cybe~ Cyberl~
## 4 Fungi   Ascomycota Saccharomycetes Saccharomycetales Phaffomyce~ Cybe~ Cyberl~
## 5 Fungi   Ascomycota Saccharomycetes Saccharomycetales Phaffomyce~ Cybe~ Cyberl~
## 6 Fungi   Ascomycota Saccharomycetes Saccharomycetales Phaffomyce~ Cybe~ Cyberl~
```

Based on the above relative abundance profile, we observe high abundance of *Cladosporium herbarum* and through taxa_tables we identify high levels of *Saccharomycetes* and *N/A* hits.

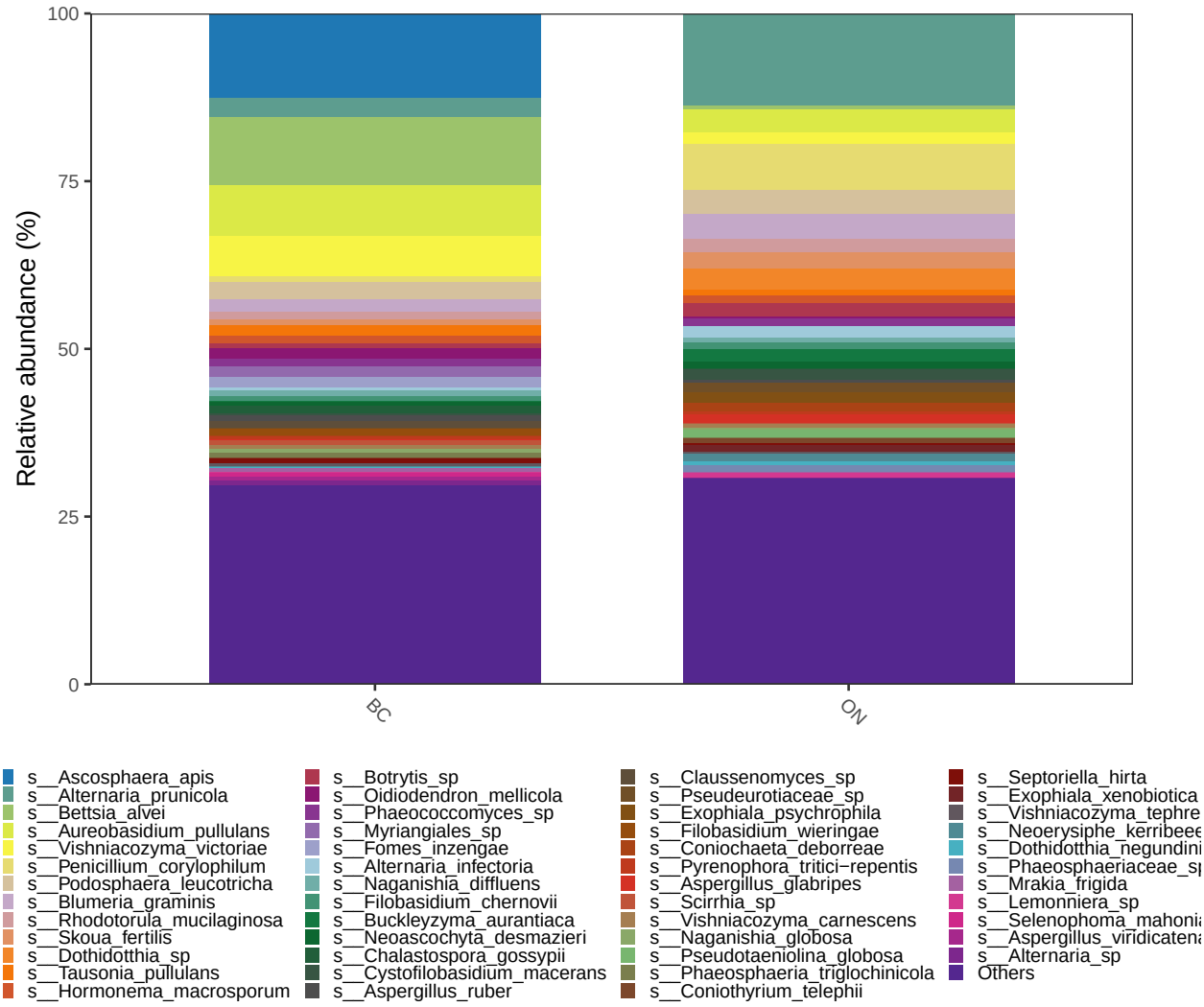
We aim to remove these in the next code chunk We remove them using a subsetting strategy, where we create

subsets of the pruned object and keep removing specific organisms based on phylum.

```
# Removal of ubiqitous species and unknown phylums
# We generate a new Filtered object by continiously removing certain species and phylum
PS_ITS1R_BC_ON_SF_Filtered <- subset_taxa(PS_ITS1R_BC_ON_SF_Pruned, Species !="Cladosporium_herbarum")
PS_ITS1R_BC_ON_SF_Filtered <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Species !="Cladosporium_sp")
PS_ITS1R_BC_ON_SF_Filtered <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Class!="Saccharomycetes")
PS_ITS1R_BC_ON_SF_Filtered <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Phylum!="N/A")

PS_ITS1R_BC_ON_SF_Filtered
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:          [ 3916 taxa and 138 samples ]:
## sample_data() Sample Data:        [ 138 samples by 15 sample variables ]:
## tax_table()   Taxonomy Table:     [ 3916 taxa by 7 taxonomic ranks ]:
## phy_tree()    Phylogenetic Tree:  [ 3916 tips and 3896 internal nodes ]:
## taxa are rows
```

```
#Re-checking the Filtered object to check if filtering via subsetting worked?

classtaxa_ITS1R_BC_ON_SF_Filtered <- get_taxadf(obj=PS_ITS1R_BC_ON_SF_Filtered, taxlevel=7)
# The 30 most abundant taxonomy will be visualized by default (parameter `topn=30`).
TopTaxa_ITS1R_BC_ON_SF_Filtered<- ggbartax(obj=classtaxa_ITS1R_BC_ON_SF_Filtered,
                   facetNames="Province",
                   plotgroup=TRUE,
                   topn=50) +
  xlab(NULL) +
  ylab("Relative abundance (%)") +
  guides(fill= guide_legend(keywidth = 0.5, keyheight = 0.5, ncol=4))

TopTaxa_ITS1R_BC_ON_SF_Filtered
```

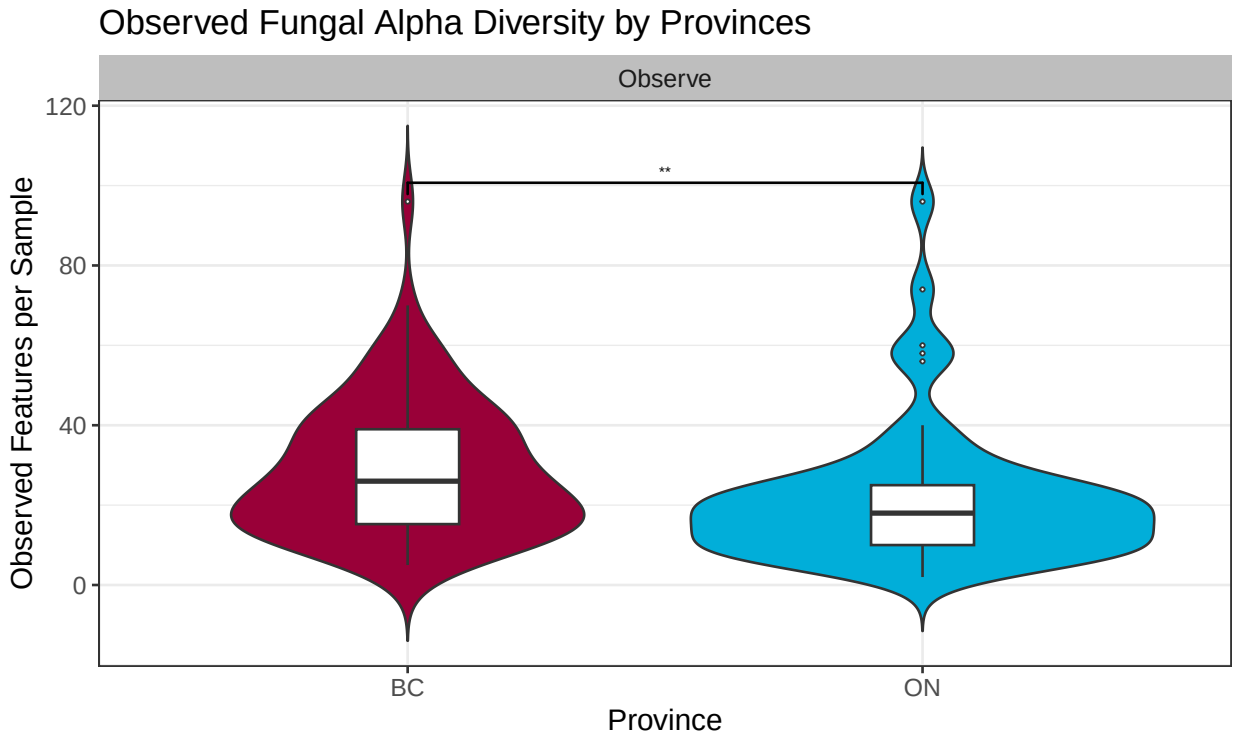


Observing the relative abundance charts we can see that *Cladosporium herbarum* and other phylum not required are removed successfully.

0.1.5 (5) Alpha Diversity Analysis

```
#utilize get_alphaindex to obtain alpha indexes for the filtered PS object
alphaobj_Stonefruit<- get_alphaindex(PS_ITS1R_BC_ON_SF_Filtered)

#Observe Analysis
Observe_status <- ggbox(alphaobj_Stonefruit,
                        geom="violin",
                        factorNames="Province",
                        compare = TRUE,
                        testmethod = "wilcox.test",
                        signifmap = TRUE,
                        indexNames="Observe")+
  labs(title = "Observed Fungal Alpha Diversity by Provinces") + ylab("Observed Features per Sample") +
  theme(aspect.ratio = 0.5)+
  theme(text = element_text(size = 12))+
  theme( legend.position="none")+
```

```
  theme(strip.background = element_rect(colour=NA, fill="grey"))+
  scale_fill_manual(values=c("#990038",
                             "#01AED9",
                             "#000000",
                             "#1B9E77",
                             "#000033",
                             "#FD9347"))


Observe_status
```



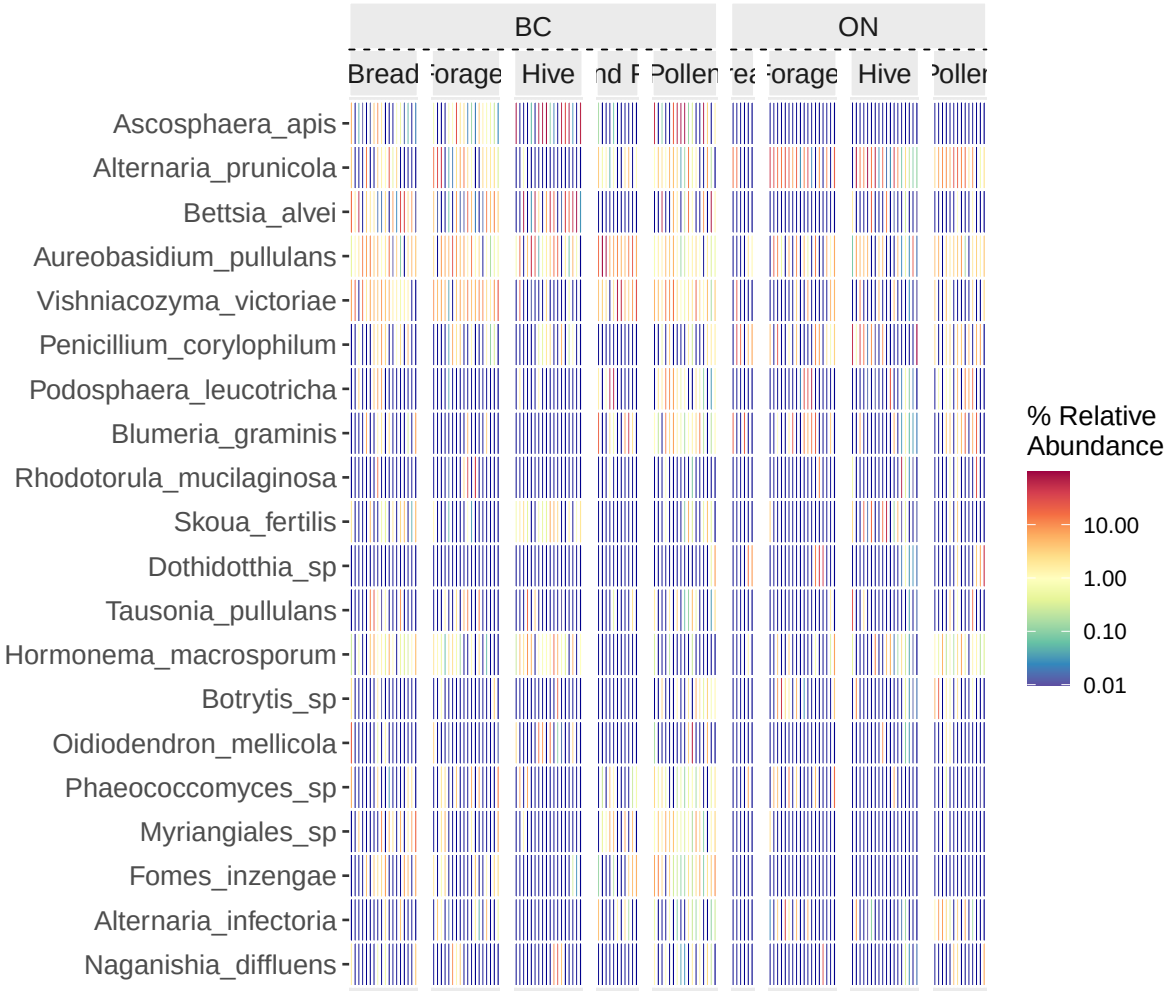
0.1.6 (6) Heatmap of Relative Abundance of Fungal Detections

We use functions to convert the PS object to a Meco object and represent this object using ***microeco*** package

Heatmaps offer a visual representation of taxonomic detections based on experiment parameters

```
#Object Conversion
Meco_SF <- phyloseq2meco(PS_ITS1R_BC_ON_SF_Filtered)
```

```
Heatmap_SF <- trans_abund$new(dataset = Meco_SF,

                        taxrank = "Species",

                        ntaxa = 20)

#this heatmap is plotted based on Province and Sample Type
Heatmap_SF$plot_heatmap(facet = c("Province","Sample.type"),
                  color_values = rev(RColorBrewer::brewer.pal(n = 11,
                                                              name = "Spectral")),
                  xtext_keep = FALSE,
                  withmargin = TRUE)
```



Based on the above heatmap we can identify some pathogenic species to **bees**: - *Ascospahera apis* - *Bettsia alvei*

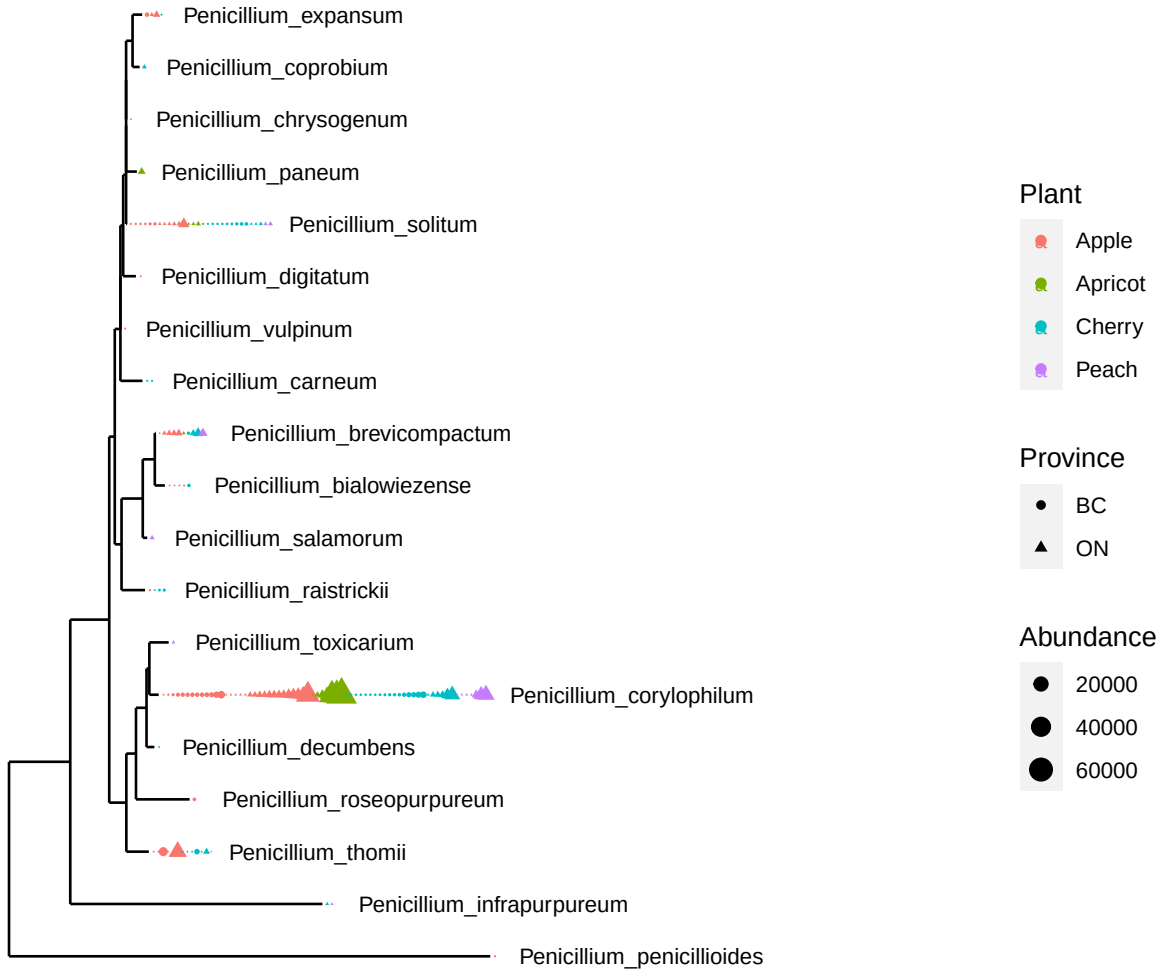
Based on the above heatmap we can identify some pathogenic species and genus to **plants**: - *Alternaria prunicola* - *Podosphaera leuctoricha* - *Blumeria graminis* - *Botrytis_sp*

0.1.7 (7) Specific Phylogenetic Subsets

Based on knowledge of plant pathogens , we can create sub objects of the filtered PS object to consist of a specific genus and attempt to target certain pathogenic species

(1) *Penicillium Expansum* - blue mold of apples

```
PS_ITS1R_Penicillium <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Genus == "Penicillium")

plot_tree(tax_glom(PS_ITS1R_Penicillium,
                   taxrank="Species"),
          method = "sampledodge",
          ladderize="left",
          nodelabf=nodeplotblank,
          color="Plant",
          label.tips="Species",
          text.size=3,
          base.spacing=0.01,
          justify="jagged",
          shape="Province",
          size="abundance",
          plot.margin =0.9)+
  scale_size_continuous(range = c(0.0001, 4))
```



(2) *Alternaria prunicola* - Cherry leaf spot

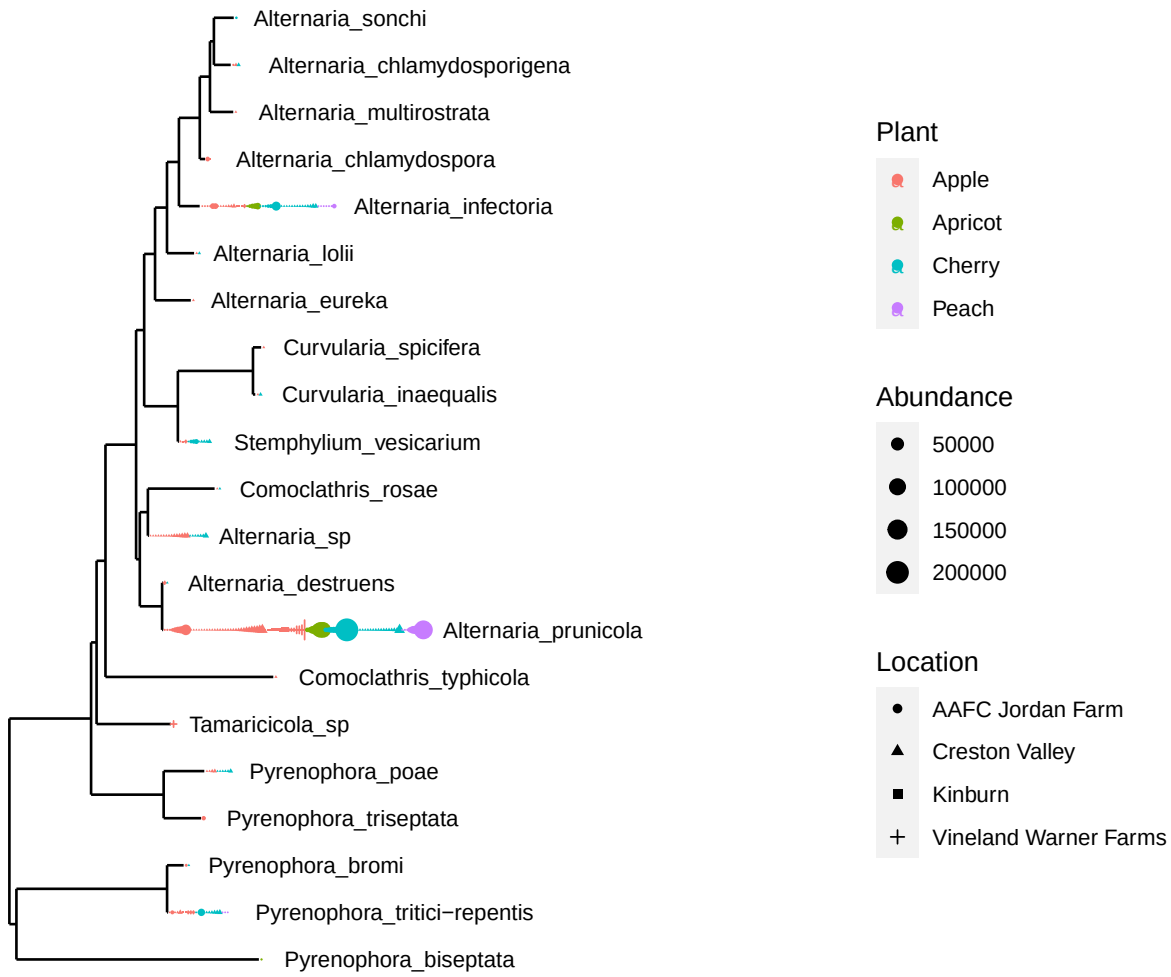
For a more complete phylogenetic tree we subset based on the family *Pleosporaceae*

```
PS_ITS1R_BC_ON_SF_Pleosporaceae <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Family =="Pleosporaceae")

PS_ITS1R_BC_ON_SF_Pleosporaceae
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:          [ 134 taxa and 138 samples ]:
## sample_data() Sample Data:        [ 138 samples by 15 sample variables ]:
## tax_table()   Taxonomy Table:     [ 134 taxa by 7 taxonomic ranks ]:
## phy_tree()    Phylogenetic Tree:  [ 134 tips and 130 internal nodes ]:
## taxa are rows
```

```
plot_tree(tax_glom(PS_ITS1R_BC_ON_SF_Pleosporaceae,
                   taxrank="Species"),
          method = "sampledodge",
          ladderize="left",
          nodelabf=nodeplotblank,
          color="Plant",
```

```
          label.tips="Species",
          text.size=3,
          base.spacing=0.01,
          justify="jagged",
          shape="Location",
          size="abundance",
          plot.margin =0.9)+
  scale_size_continuous(range = c(0.0001, 4))
```

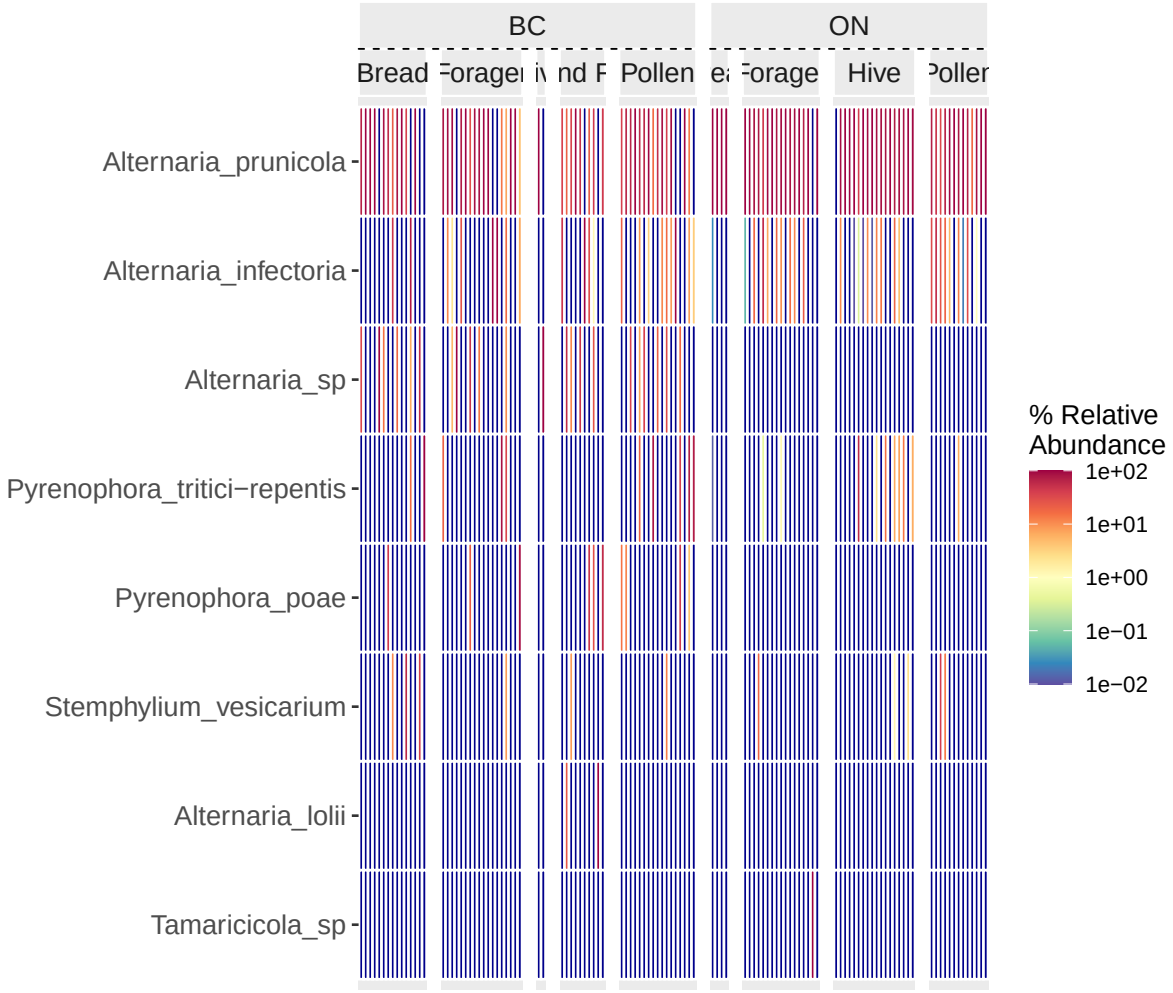



```
# Heatmap analysis for alternative view of detections based on sample type
meco_PS_ITS1R_BC_ON_SF_Pleosporaceae<- phyloseq2meco(PS_ITS1R_BC_ON_SF_Pleosporaceae)


b1_PS_ITS1R_BC_ON_SF_Pleosporaceae <- trans_abund$new(dataset = meco_PS_ITS1R_BC_ON_SF_Pleosporaceae,

                        taxrank = "Species",

                        ntaxa = 8)
```

```
## microtable-class object:
```

```
## sample_table have 114 rows and 15 columns
## otu_table have 134 rows and 114 columns
## tax_table have 134 rows and 7 columns
## phylo_tree have 134 tips
```

```
b1_PS_ITS1R_BC_ON_SF_Pleosporaceae$plot_heatmap(facet = c("Province","Sample.type"),
                color_values = rev(RColorBrewer::brewer.pal(n = 11,
                                                            name = "Spectral")),
                xtext_keep = FALSE,
                withmargin = TRUE)
```



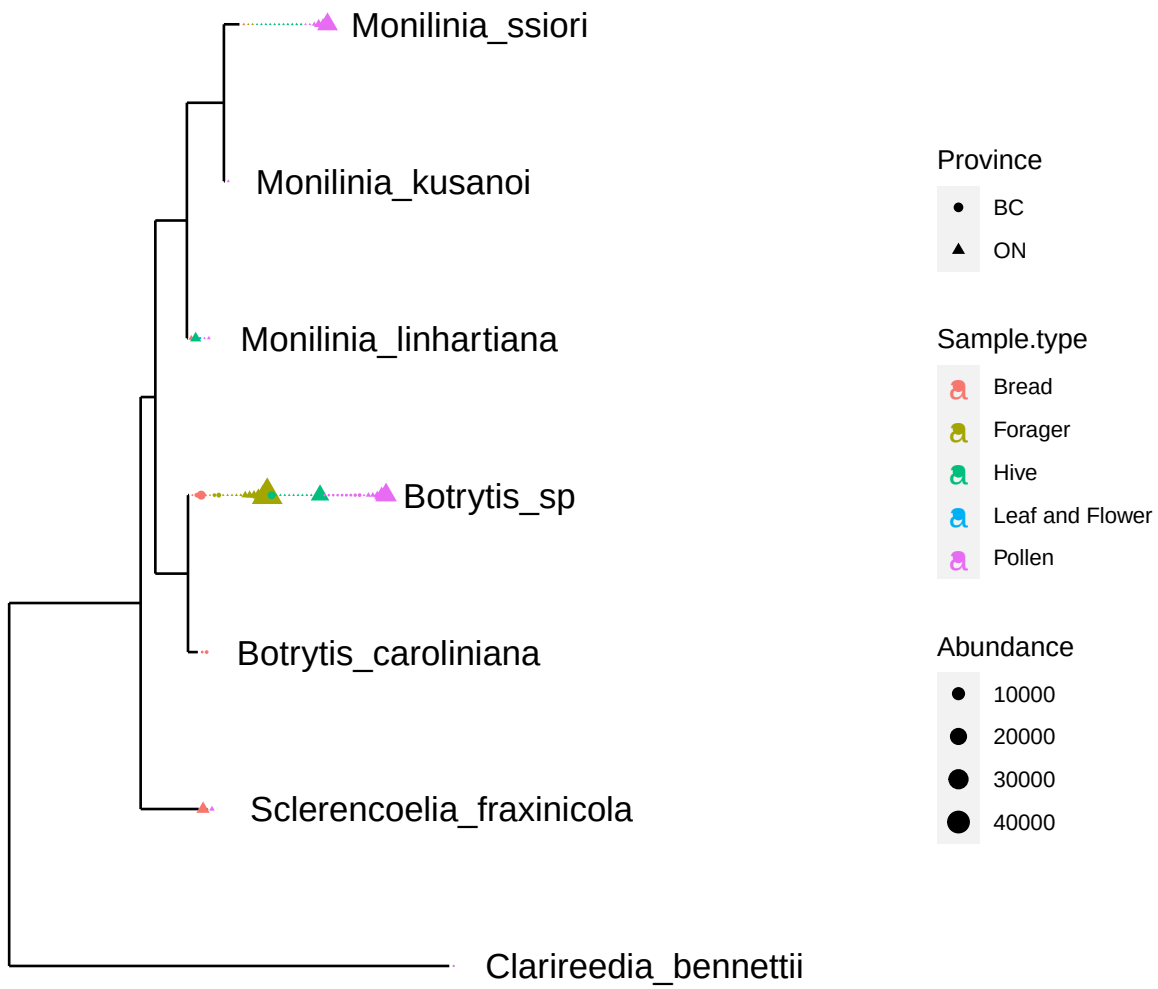
NOTE : The heatmaps may look difficult to visualize here hwoever when run in Rstudio environment, it is possible to export to PNG and have a clearer output

(2) *Monilinia_sp* - Brown Rot

For a more complete phylogenetic tree we subset based on the family *Sclerotiniaceae*

```
PS_ITS1R_Sclero <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Family =="Sclerotiniaceae")

plot_tree(tax_glom(PS_ITS1R_Sclero,
                   taxrank="Species"),
```

```
          method = "sampledodge",
          ladderize="left",
          nodelabf=nodeplotblank,
          color="Sample.type",
          label.tips="Species",
          text.size=5,
          base.spacing=0.01,
          justify="jagged",
          shape="Province",
          size="abundance",
          plot.margin =0.9)+
  scale_size_continuous(range = c(0.0001, 4))
```

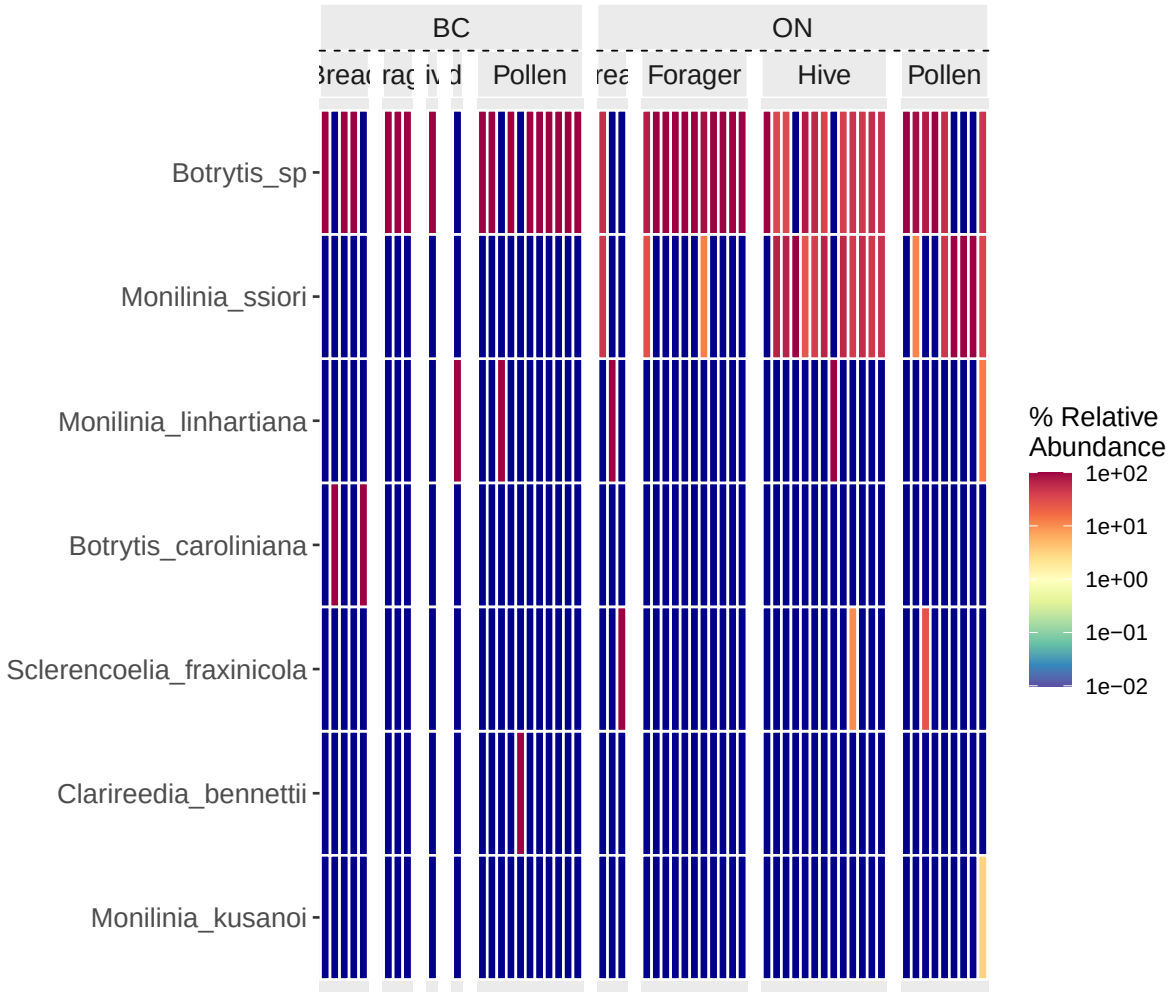



```
meco_ITS2_BC_ON_SF_Sclerotiniaceae<- phyloseq2meco(PS_ITS1R_Sclero)


ITS2_BC_ON_SF_Sclerotiniaceae <- trans_abund$new(dataset = meco_ITS2_BC_ON_SF_Sclerotiniaceae,
                        taxrank = "Species",
```

```
                      ntaxa = 15)
```

```
## microtable-class object:
## sample_table have 57 rows and 15 columns
## otu_table have 50 rows and 57 columns
## tax_table have 50 rows and 7 columns
## phylo_tree have 50 tips
```

```
ITS2_BC_ON_SF_Sclerotiniaceae$plot_heatmap(facet = c("Province","Sample.type"),
                color_values = rev(RColorBrewer::brewer.pal(n = 11,
                                                            name = "Spectral")),
                xtext_keep = FALSE,
                withmargin = TRUE)
```

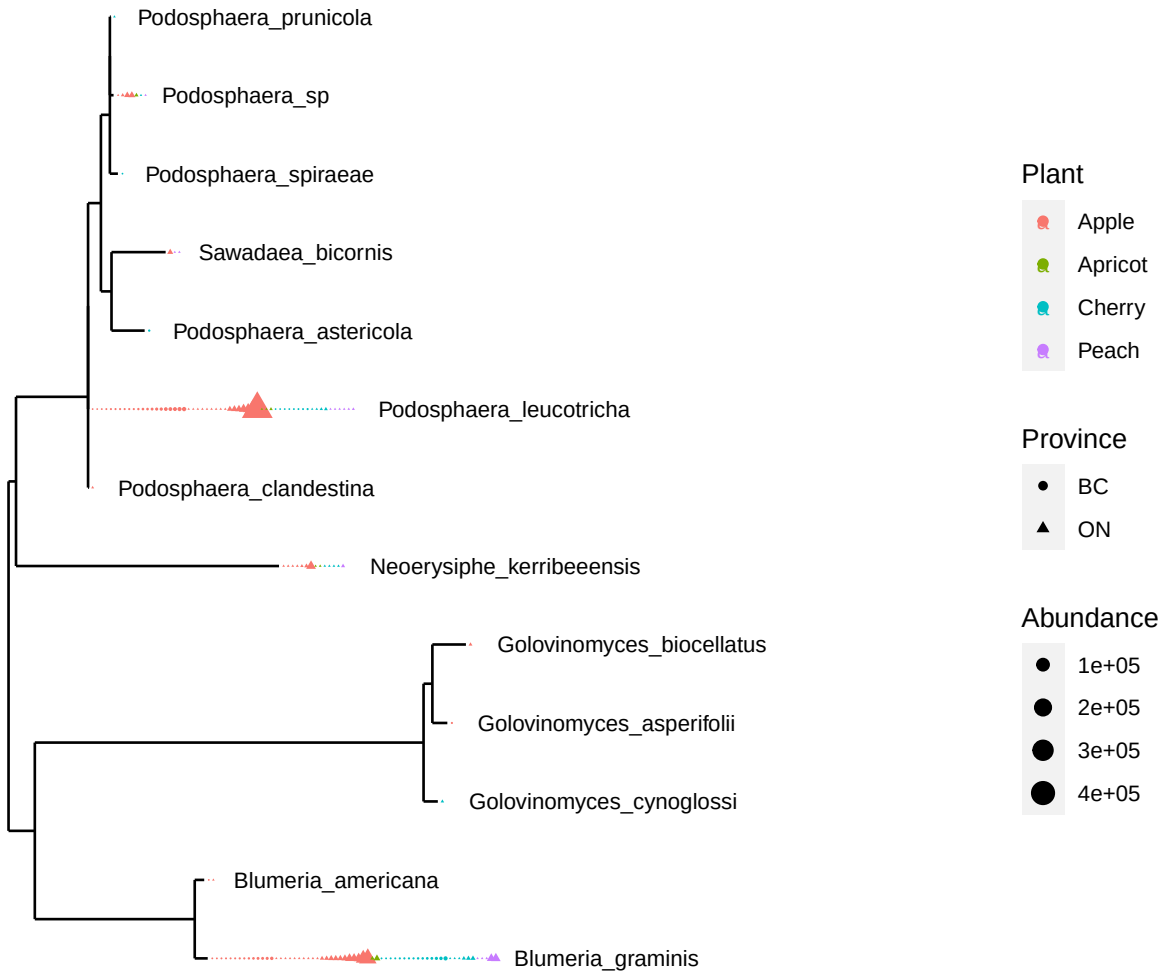


(3) *Podosphaera Leucotricha* - Powdery Mildew

For a more complete phylogenetic tree we subset based on the family *Erysiphaceae*

```
PS_ITS2_BC_ON_SF_Erysiphaceae <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Family == "Erysiphaceae")

plot_tree(tax_glom(PS_ITS2_BC_ON_SF_Erysiphaceae,
```

```
                    taxrank="Species"),
          method = "sampledodge",
          ladderize="left",
          nodelabf=nodeplotblank,
          color="Plant",
          label.tips="Species",
          text.size=3,
          base.spacing=0.01,
          justify="jagged",
          shape="Province",
          size="abundance",
          plot.margin =0.9)+
  scale_size_continuous(range = c(0.0001, 4))
```

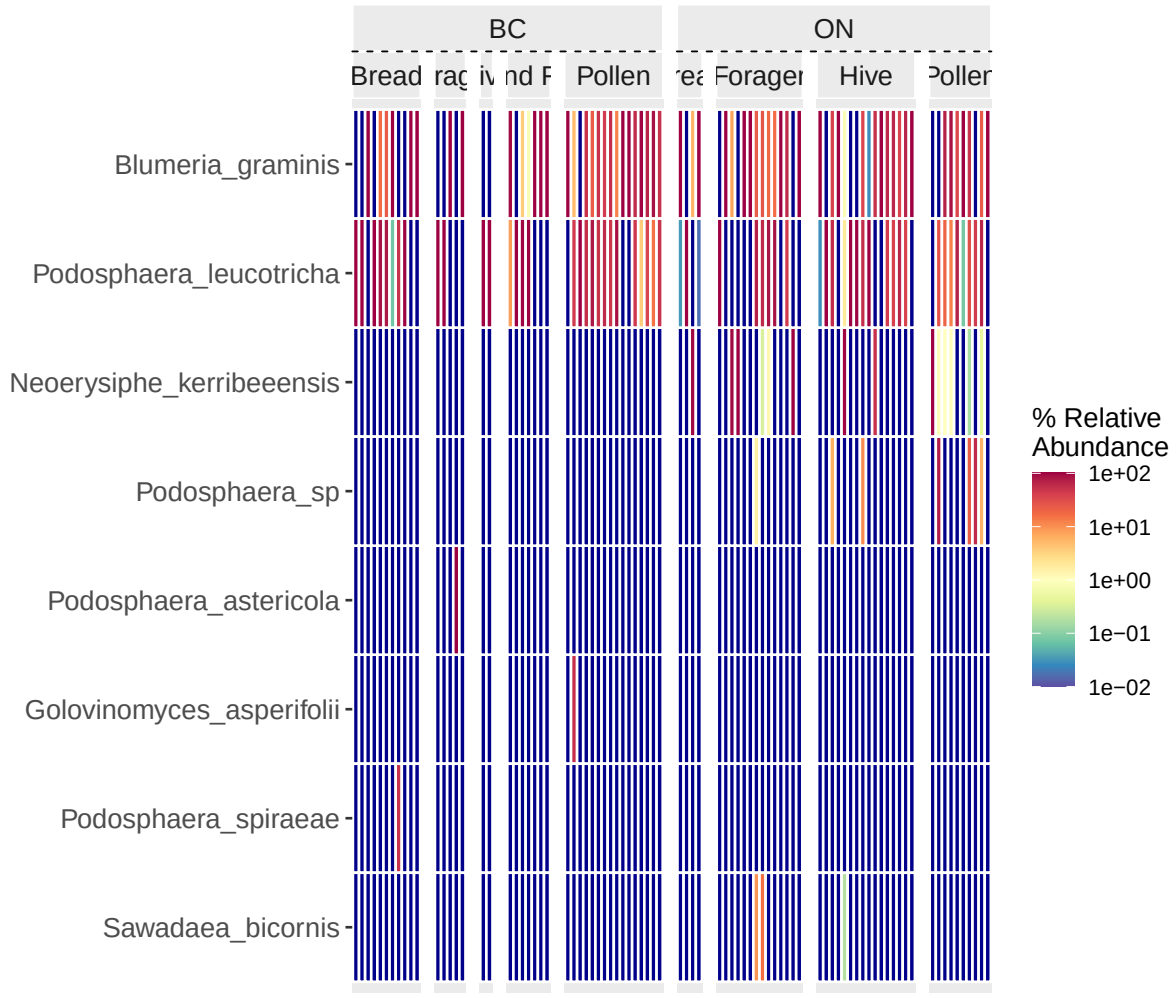



```
##HEATMAP
meco_ITS2_BC_ON_SF_Erysiphaceae<- phyloseq2meco(PS_ITS2_BC_ON_SF_Erysiphaceae)


b1_ITS2_BC_ON_SF_Erysiphaceae <- trans_abund$new(dataset = meco_ITS2_BC_ON_SF_Erysiphaceae,
```

```
                    taxrank = "Species",

                    ntaxa = 8)
```

```
## microtable-class object:
## sample_table have 85 rows and 15 columns
## otu_table have 144 rows and 85 columns
## tax_table have 144 rows and 7 columns
## phylo_tree have 144 tips
```

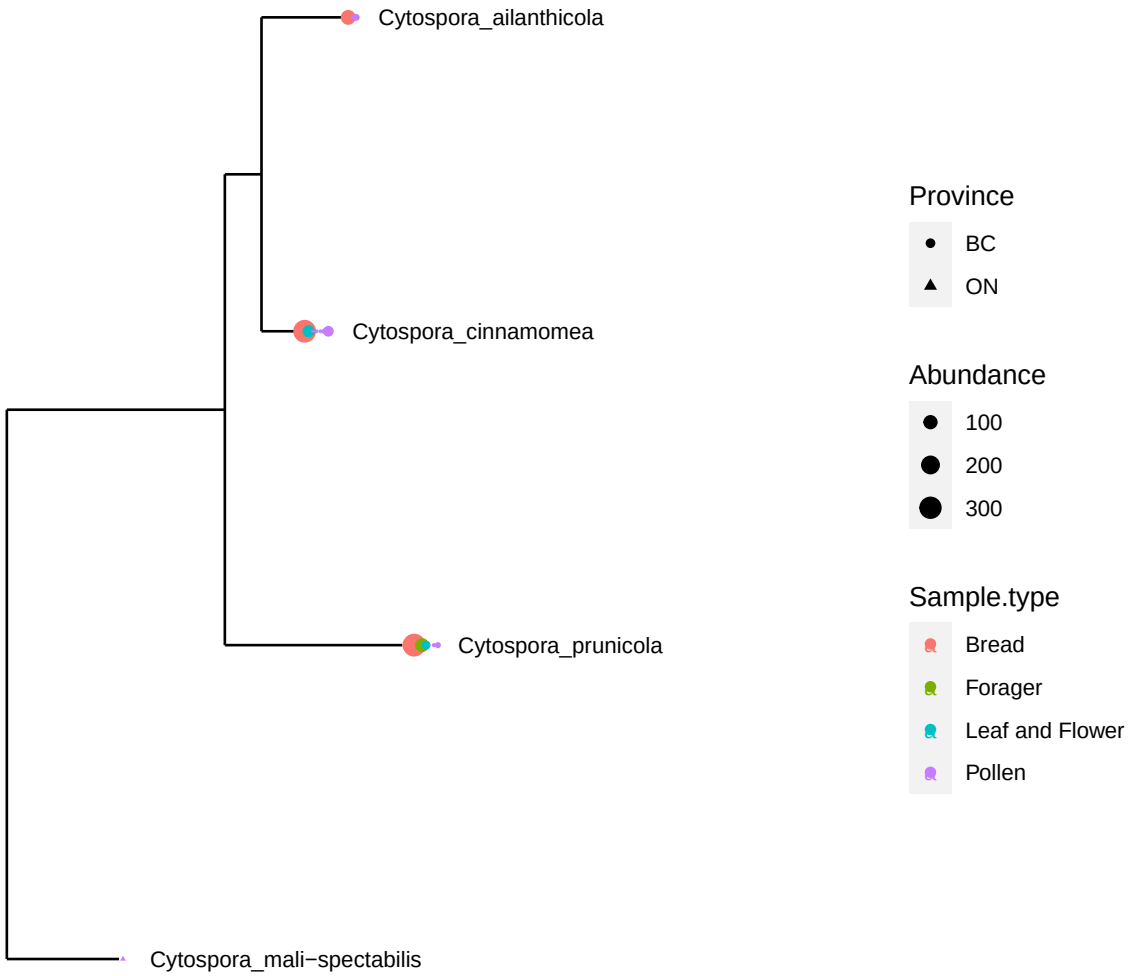
```
b1_ITS2_BC_ON_SF_Erysiphaceae$plot_heatmap(facet = c("Province","Sample.type"),
                color_values = rev(RColorBrewer::brewer.pal(n = 11,
                                                            name = "Spectral")),
                xtext_keep = FALSE,
                withmargin = TRUE)
```



(4) *Cytospora Cinnamomea* - Cherry Canker Disease

For a more complete phylogenetic tree we subset based on the genus *Cytospora*

```
PS_ITS1R_Cytospora <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Genus == "Cytospora")

plot_tree(tax_glom(PS_ITS1R_Cytospora,
                   taxrank="Species"),
          method = "sampledodge",
          ladderize="left",
          nodelabf=nodeplotblank,
          color="Sample.type",
          label.tips="Species",
          text.size=3,
          base.spacing=0.01,
          justify="jagged",
          shape="Province",
          size="abundance",
          plot.margin =0.9)+
  scale_size_continuous(range = c(0.0001, 4))
```

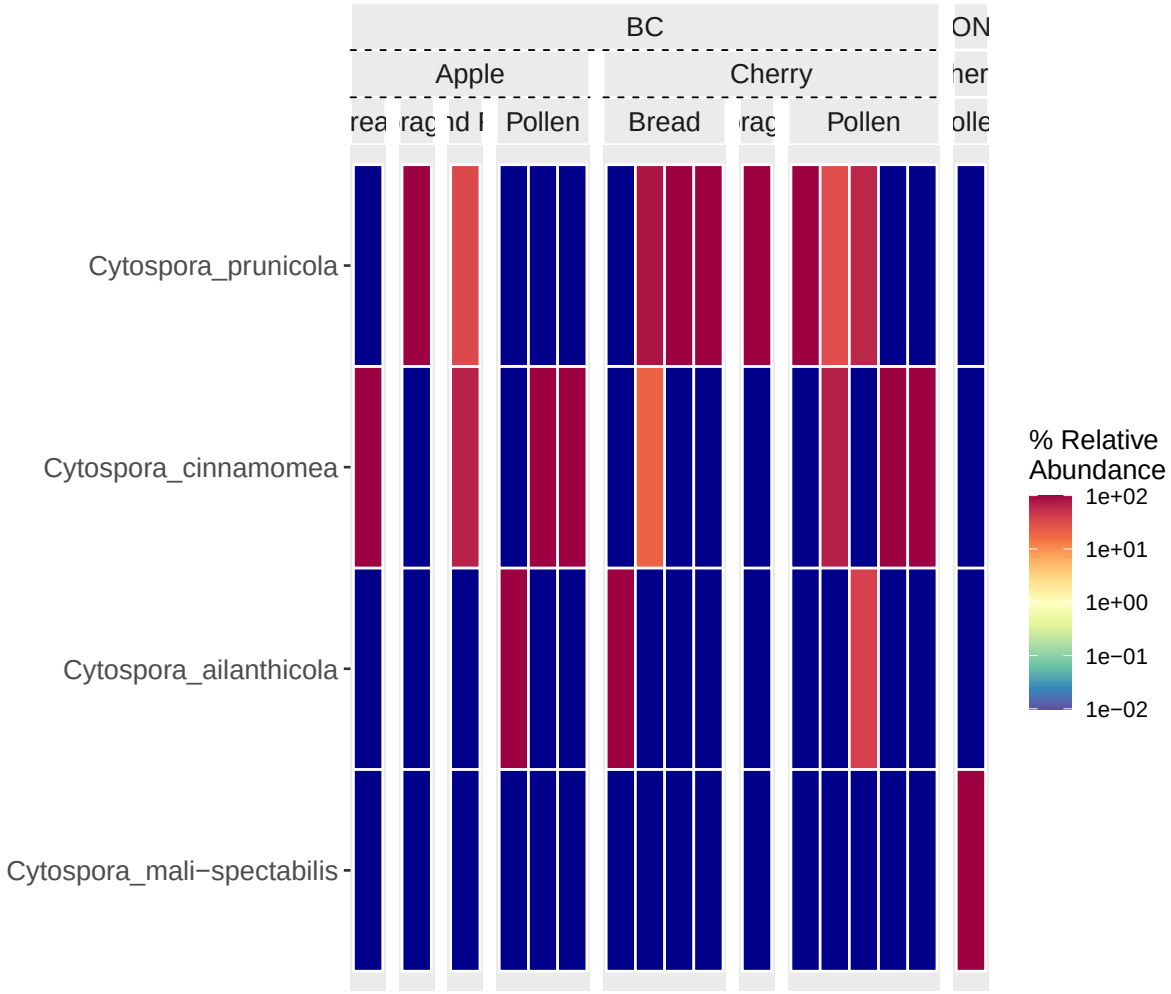



```
##HEATMAP
meco_ITS1R_Cytospora<- phyloseq2meco(PS_ITS1R_Cytospora)
```

```
b1_ITS1R_Cytospora <- trans_abund$new(dataset = meco_ITS1R_Cytospora,

                      taxrank = "Species",

                      ntaxa = 8)
```

```
## microtable-class object:
## sample_table have 17 rows and 15 columns
## otu_table have 15 rows and 17 columns
## tax_table have 15 rows and 7 columns
## phylo_tree have 15 tips
```

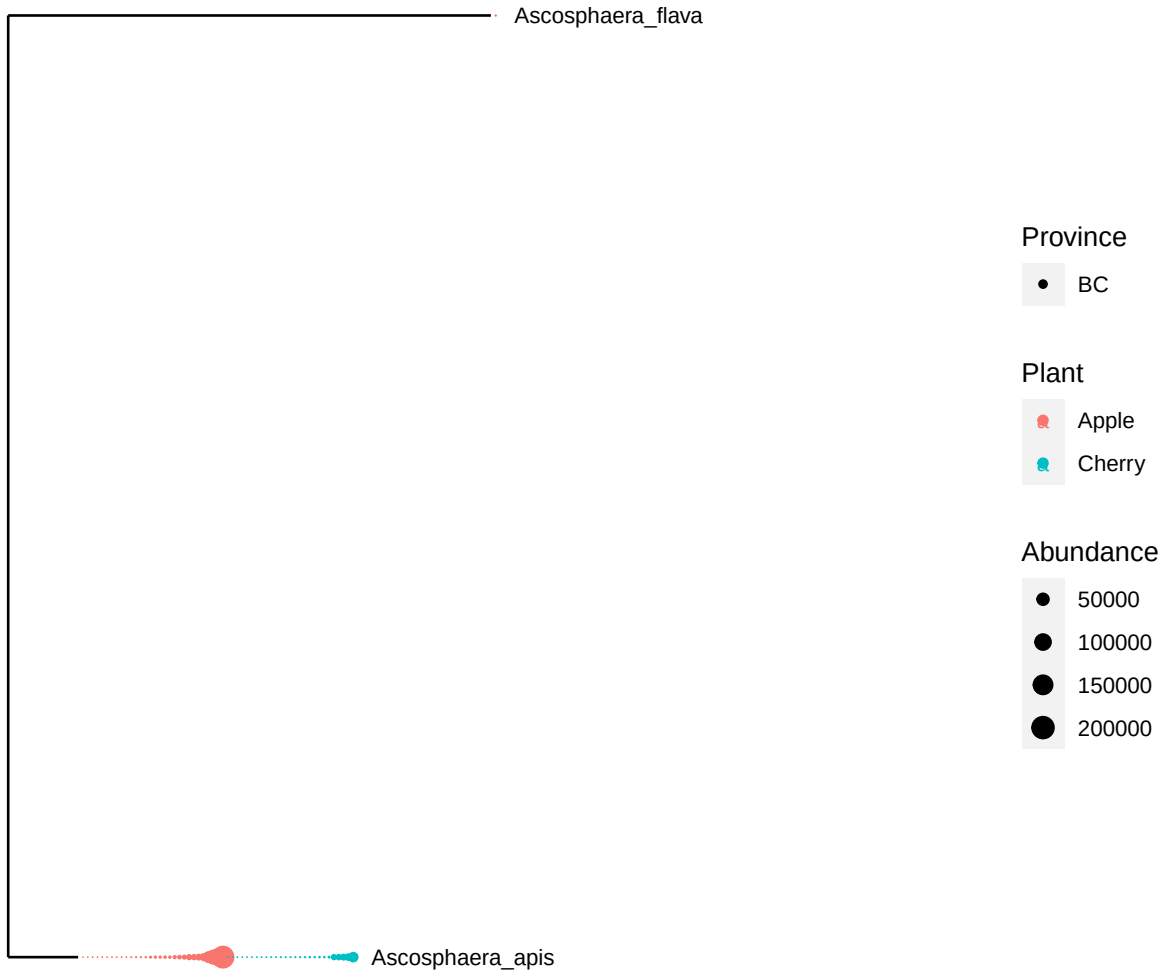
```
b1_ITS1R_Cytospora$plot_heatmap(facet = c("Province","Plant","Sample.type"),
                color_values = rev(RColorBrewer::brewer.pal(n = 11,
                                                            name = "Spectral")),
                xtext_keep = FALSE,
                withmargin = TRUE)
```



(5) *Ascosphaera Apis* - Chalkbrood Disease

For a more complete phylogenetic tree we subset based on the family *Ascosphaeraceae*

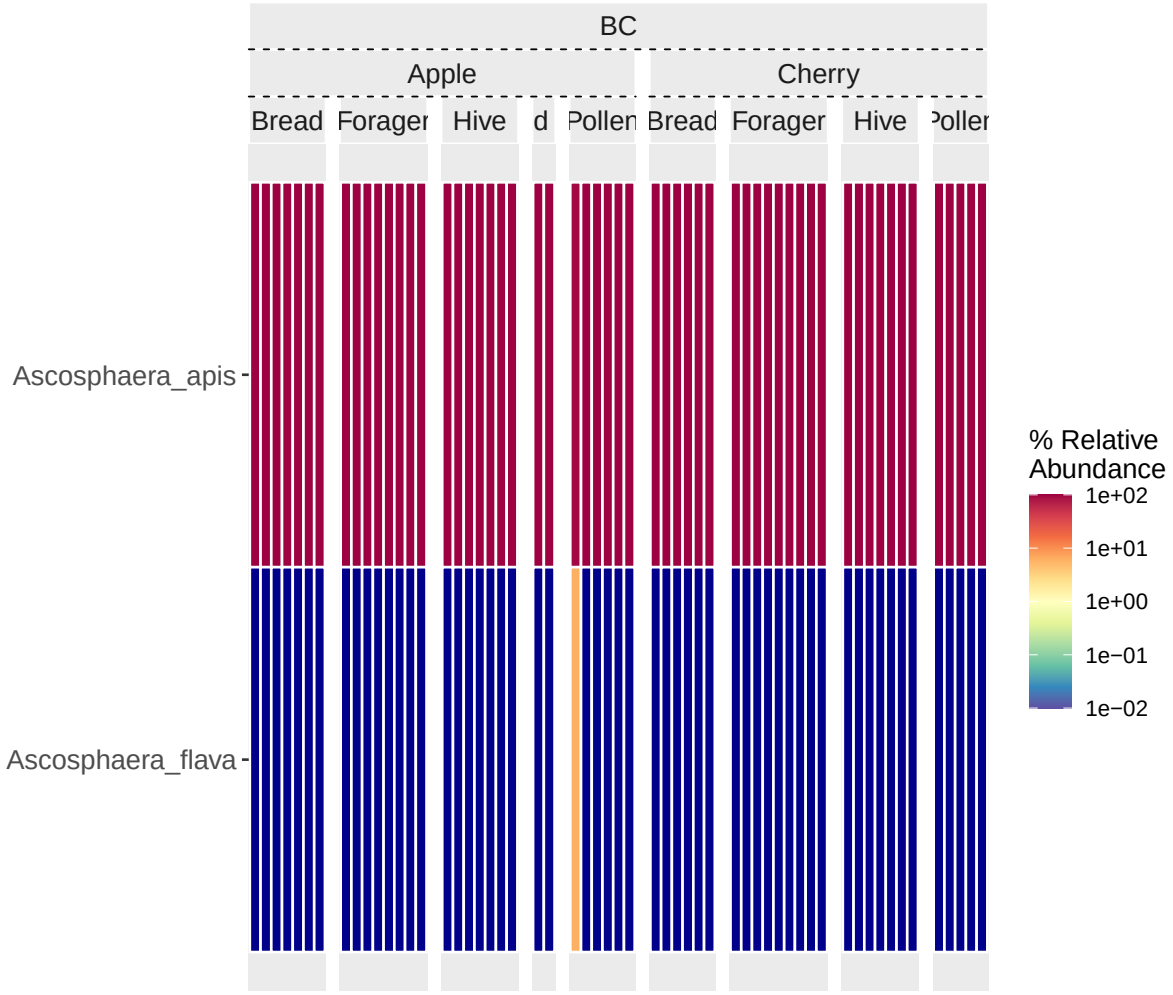
```
##BEE PATHOGENS

PS_ITS2_BC_ON_SF_Ascosphaeraceae <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Family == "Ascosphaeraceae")

plot_tree(tax_glom(PS_ITS2_BC_ON_SF_Ascosphaeraceae,
                   taxrank="Species"),
          method = "sampledodge",
          ladderize="left",
          nodelabf=nodeplotblank,
          color="Plant",
          label.tips="Species",
          text.size=3,
          base.spacing=0.01,
          justify="jagged",
          shape="Province",
          size="abundance",
          plot.margin =0.9)+
  scale_size_continuous(range = c(0.0001, 4))
```



```
##HEATMAP
meco_ITS2_BC_ON_SF_Ascosphaeraceae<- phyloseq2meco(PS_ITS2_BC_ON_SF_Ascosphaeraceae)


b1_ITS2_BC_ON_SF_Ascosphaeraceae <- trans_abund$new(dataset = meco_ITS2_BC_ON_SF_Ascosphaeraceae,

                      taxrank = "Species",

                      ntaxa = 10)
```

```
## microtable-class object:
## sample_table have 57 rows and 15 columns
## otu_table have 40 rows and 57 columns
## tax_table have 40 rows and 7 columns
## phylo_tree have 40 tips
```

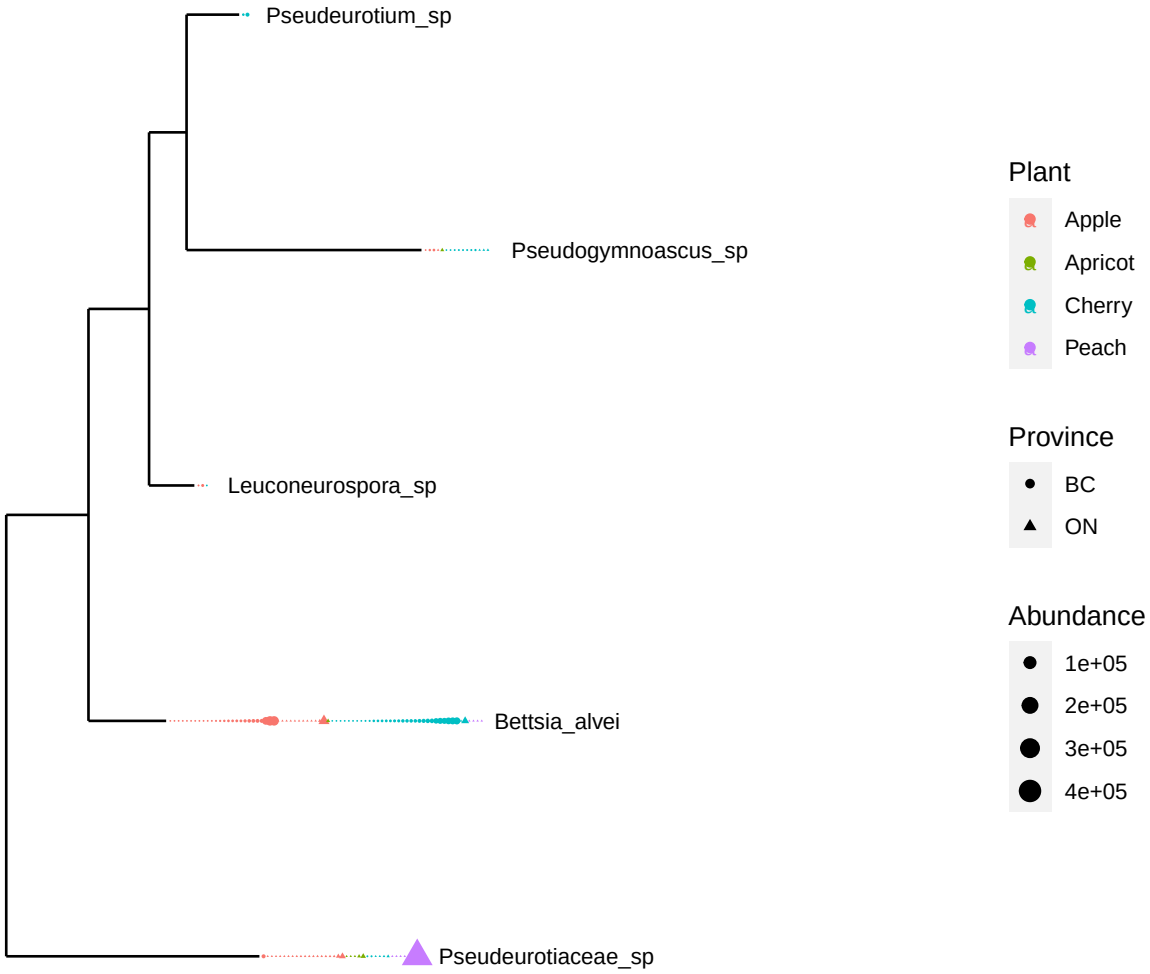
```
b1_ITS2_BC_ON_SF_Ascosphaeraceae$plot_heatmap(facet = c("Province","Plant","Sample.type"),
                color_values = rev(RColorBrewer::brewer.pal(n = 11,
                                                            name = "Spectral")),
                xtext_keep = FALSE,
                withmargin = TRUE)
```



(6) *Bettsia alvei* - Pollen Mould Disease

For a more complete phylogenetic tree we subset based on the family *Pseudeurotiaceae*

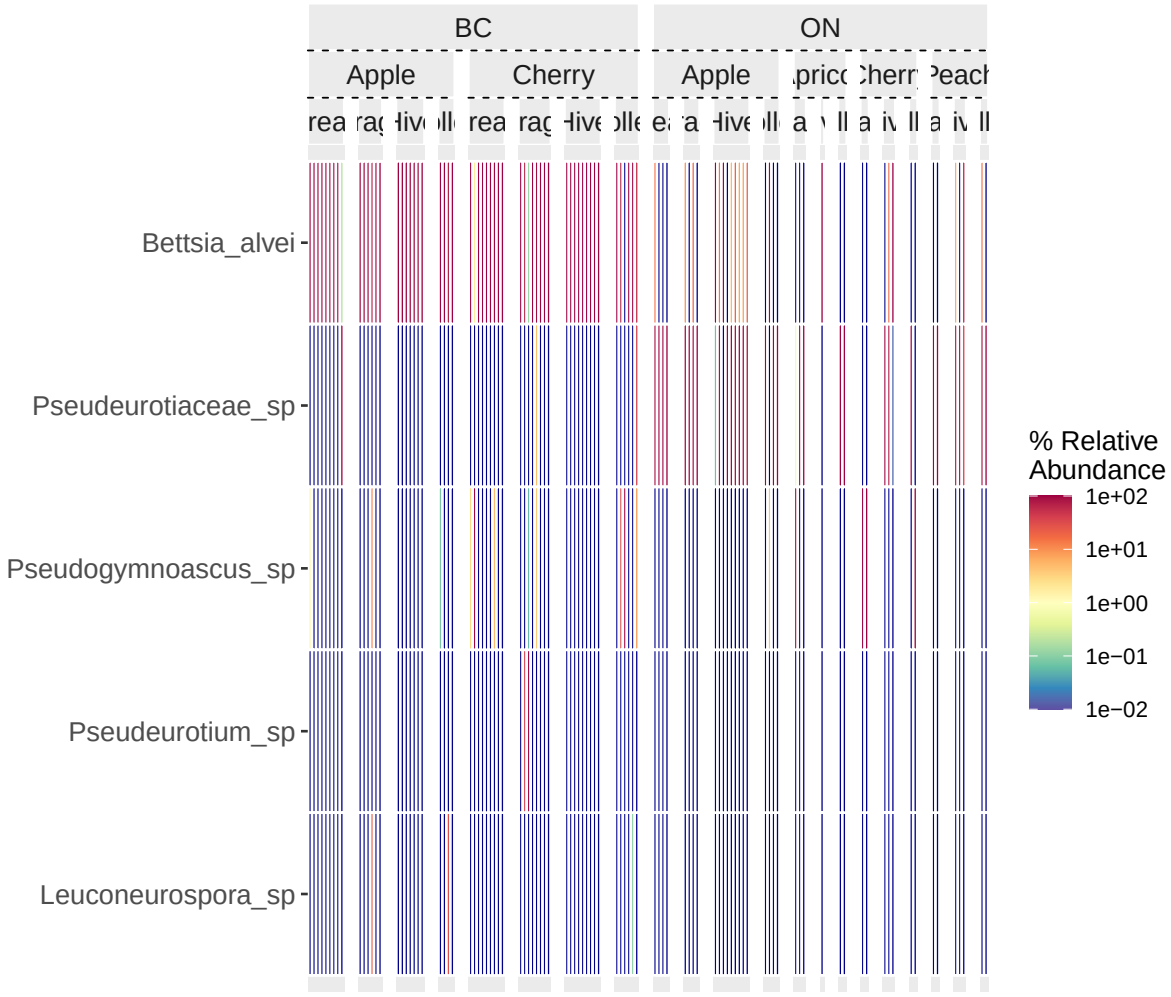
```
PS_ITS2_BC_ON_SF_Pseudeurotiaceae <- subset_taxa(PS_ITS1R_BC_ON_SF_Filtered, Family == "Pseudeurotiacea

plot_tree(tax_glom(PS_ITS2_BC_ON_SF_Pseudeurotiaceae,
                   taxrank="Species"),
          method = "sampledodge",
          ladderize="left",
          nodelabf=nodeplotblank,
          color="Plant",
          label.tips="Species",
          text.size=3,
          base.spacing=0.01,
          justify="jagged",
          shape="Province",
          size="abundance",
          plot.margin =0.9)+
  scale_size_continuous(range = c(0.0001, 4))
```




```
##HEATMAP
meco_ITS2_BC_ON_SF_Pseudeurotiaceae<- phyloseq2meco(PS_ITS2_BC_ON_SF_Pseudeurotiaceae)


b1_ITS2_BC_ON_SF_Pseudeurotiaceae<- trans_abund$new(dataset = meco_ITS2_BC_ON_SF_Pseudeurotiaceae,

                      taxrank = "Species",

                      ntaxa = 10)
```

```
## microtable-class object:
## sample_table have 99 rows and 15 columns
## otu_table have 78 rows and 99 columns
## tax_table have 78 rows and 7 columns
## phylo_tree have 78 tips
```

```
b1_ITS2_BC_ON_SF_Pseudeurotiaceae$plot_heatmap(facet = c("Province","Plant","Sample.type"),
                color_values = rev(RColorBrewer::brewer.pal(n = 11,
                                                            name = "Spectral")),
                xtext_keep = FALSE,
                withmargin = TRUE)
```


BC
ON
Apple
Cherry
Apple
Bettsia_alvei
Pseudeurotiaceae_sp
Pseudogymnoascus_sp
Pseudeurotium_sp
Leuconeurospora_sp
% Relative Abundance
1e+02
1e+01
1e+00
1e−01
1e−02