Appendix S6: results after SWARM clustering. Supplementary Materials of "Finding fungi in a needle stack: high alpha and low beta-diversity of foliar endophytic Ascomycetes revealed by metabarcoding in Corsican pine forests".

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Abstract

Plant leaves host highly diverse communities of foliar endophytic fungi (FEF). Compared to the other compartments of the plant microbiome, FEF diversity is poorly known. We here document the communities of FEF associated with the endemic Corsican black pine *Pinus nigra* subsp. *laricio* at three sites across its natural range and examine the effect of tree age and light exposure on FEF composition. Metabarcoding using next-generation sequencing provided 8243608 Ascomycota ITS2 sequences clustered into 642 FEF operational taxonomic units (OTUs). Site is the main determinant to explain the diversity and composition of FEF communities. Tree age somewhat affects FEF community composition, whereas needle location (shade vs canopy) has no effect. Results are robust against the various options of the bioinformatic pipeline specifically developed. This study provides the first picture of FEF diversity in a Mediterranean island and underlines the complementarity of forest massifs for fungal conservation.

Key words: foliar endophyte; fungi; community ecology; metabarcoding; Cyclaneusma minus, Pinus nigra subsp. laricio, Mediterranean, endemism, environmental sequencing

To set the filter parameter, see directly section 'Choice of filter parameters' 2.1.
To read a summary of this appendix, see directly section 'Summary' 7.

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Contents

1   Introduction

This supplementary material presents the ecological analysis of endophytic fungal communities in *Pinus nigra* subsp. *laricio*, an endemic species of Corsica. The dataset analysed here was computed using SWARM clustering (see main article and Sup. Mat. 1 for more details).

1.1   R requirements

First, set the working directory. In this directory, there is data folder and a R script "functions_for_phyloseq.R".

```r
setwd("~/Nextcloud/GitHub/FEF_paper/")
```

Then, we may need to install packages.

```r
#  install.packages(c('ape', 'biom', 'optparse', 'RColorBrewer', 'randomForest',  'vegan',
#                      'VennDiagram', 'venneuler', 'xtable', 'schoRsch', 'ape',
#                      'ips', 'adegenet', 'mvabund', 'rCharts', 'networkD3', 'data.tree'))
#
# # Upgrade Bioconductor to the latest version available for this version of R
# source("http://bioconductor.org/biocLite.R")
# biocLite(c("multtest", "DECIPHER", "edgeR", "phyloseq", 'DESeq2', 'metagenomeSeq'))
#
# require(devtools)
# install_github('ramnathv/rCharts')
# install_github("timelyportfolio/d3treeR")
```

```r
## May be needed under windows
Sys.setenv(JAVA_HOME = "C:\\Program Files\\Java\\jdk1.8.0_73")

#Load the packages.
lapply(list("ggplot2", "phyloseq", "cluster", "plyr", "VennDiagram",
            "circlize", "xtable", "schoRsch", "DESeq2", "mvabund",
            "edgeR", "phangorn", "DECIPHER", "ips", "adegenet", "multtest",
            "networkD3", "treemap", "data.tree", "d3treeR", "venneuler",
            "gridExtra"), library,
       character.only = TRUE)
library(vegan)
```

1.2   System and session informations

This document was created with R version 3.4.2 (2017-09-28) on Linux the 2017-11-09 15:03:33. See below for more information.

```r
sessionInfo()

## R version 3.4.2 (2017-09-28)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.3 LTS
##
## Matrix products: default
## BLAS: /usr/lib/libblas/libblas.so.3.6.0
## LAPACK: /usr/lib/lapack/liblapack.so.3.6.0
##
```

```
## locale:
##  [1] LC_CTYPE=fr_FR.UTF-8        LC_NUMERIC=C
##  [3] LC_TIME=fr_FR.UTF-8         LC_COLLATE=fr_FR.UTF-8
##  [5] LC_MONETARY=fr_FR.UTF-8     LC_MESSAGES=fr_FR.UTF-8
##  [7] LC_PAPER=fr_FR.UTF-8        LC_NAME=fr_FR.UTF-8
##  [9] LC_ADDRESS=fr_FR.UTF-8      LC_TELEPHONE=fr_FR.UTF-8
## [11] LC_MEASUREMENT=fr_FR.UTF-8  LC_IDENTIFICATION=fr_FR.UTF-8
##
## attached base packages:
## [1] parallel  stats4    grid      stats     graphics  grDevices utils
## [8] datasets  methods   base
##
## other attached packages:
##  [1] vegan_2.4-4             lattice_0.20-35
##  [3] permute_0.9-4           gridExtra_2.2.1
##  [5] venneuler_1.1-0         rJava_0.9-8
##  [7] d3treeR_0.1             data.tree_0.7.0
##  [9] treemap_2.4-2           networkD3_0.4
## [11] multtest_2.32.0         adegenet_2.1.0
## [13] ade4_1.7-8              ips_0.0-7
## [15] XML_3.98-1.9            colorspace_1.3-2
## [17] DECIPHER_2.4.0          RSQLite_2.0
## [19] Biostrings_2.44.2       XVector_0.16.0
## [21] phangorn_2.2.0          ape_4.1
## [23] edgeR_3.18.1            limma_3.32.5
## [25] mvabund_3.12.3          DESeq2_1.16.1
## [27] SummarizedExperiment_1.6.3 DelayedArray_0.2.7
## [29] matrixStats_0.52.2      Biobase_2.36.2
## [31] GenomicRanges_1.28.4    GenomeInfoDb_1.12.2
## [33] IRanges_2.10.3          S4Vectors_0.14.3
## [35] BiocGenerics_0.22.0     schoRsch_1.4
## [37] xtable_1.8-2            circlize_0.4.1
## [39] VennDiagram_1.6.17      futile.logger_1.4.3
## [41] plyr_1.8.4              cluster_2.0.6
## [43] phyloseq_1.20.0         ggplot2_2.2.1
## [45] knitr_1.17
##
## loaded via a namespace (and not attached):
##   [1] backports_1.1.0        Hmisc_4.0-3
##   [3] fastmatch_1.1-0        igraph_1.1.2
##   [5] lazyeval_0.2.0         sp_1.2-5
##   [7] splines_3.4.2          BiocParallel_1.10.1
##   [9] gridBase_0.4-7         digest_0.6.12
##  [11] foreach_1.4.3          htmltools_0.3.6
##  [13] viridis_0.4.0          gdata_2.18.0
##  [15] magrittr_1.5           checkmate_1.8.3
##  [17] memoise_1.1.0          readr_1.1.1
##  [19] annotate_1.54.0        gmodels_2.16.2
##  [21] blob_1.1.0             dplyr_0.7.2
##  [23] RCurl_1.95-4.8         jsonlite_1.5
##  [25] genefilter_1.58.1      bindr_0.1
##  [27] brew_1.0-6             survival_2.41-3
##  [29] iterators_1.0.8        glue_1.1.1
##  [31] gtable_0.2.0           zlibbioc_1.22.0
##  [33] seqinr_3.4-5           Rook_1.1-1
##  [35] shape_1.4.3            scales_0.5.0
##  [37] futile.options_1.0.0   DBI_0.7
##  [39] Rcpp_0.12.12           viridisLite_0.2.0
##  [41] htmlTable_1.9          foreign_0.8-69
##  [43] bit_1.1-12             spdep_0.6-15
##  [45] Formula_1.2-2          tweedie_2.2.5
##  [47] htmlwidgets_0.9        DiagrammeR_0.9.1
##  [49] RColorBrewer_1.1-2     acepack_1.4.1
##  [51] pkgconfig_2.0.1        nnet_7.3-12
##  [53] deldir_0.1-14          locfit_1.5-9.1
##  [55] rlang_0.1.2            reshape2_1.4.2
##  [57] AnnotationDbi_1.38.2   visNetwork_2.0.1
##  [59] munsell_0.4.3          tools_3.4.2
##  [61] downloader_0.4         evaluate_0.10.1
##  [63] biomformat_1.4.0       stringr_1.2.0
##  [65] bit64_0.9-7            purrr_0.2.3
##  [67] bindrcpp_0.2           nlme_3.1-131
##  [69] mime_0.5               rstudioapi_0.6
##  [71] compiler_3.4.2         rgexf_0.15.3
##  [73] tibble_1.3.4           statmod_1.4.30
##  [75] geneplotter_1.54.0     stringi_1.1.5
##  [77] highr_0.6              Matrix_1.2-11
##  [79] LearnBayes_2.15        GlobalOptions_0.0.12
##  [81] data.table_1.10.4      bitops_1.0-6
##  [83] httpuv_1.3.5           R6_2.2.2
##  [85] latticeExtra_0.6-28    gridSVG_1.5-1
##  [87] codetools_0.2-15       lambda.r_1.1.9
##  [89] boot_1.3-20            MASS_7.3-47
##  [91] gtools_3.5.0           assertthat_0.2.0
##  [93] rhdf5_2.20.0           GenomeInfoDbData_0.99.0
##  [95] mgcv_1.8-22            expm_0.999-2
##  [97] hms_0.3                influenceR_0.1.0
##  [99] quadprog_1.5-5         rpart_4.1-11
## [101] tidyr_0.7.1            coda_0.19-1
## [103] shiny_1.0.5            base64enc_0.1-3
```

1.3 Some usefull functions

The function `as.binaryOtuTable` converts a phyloseq object into a phyloseq object with binary (*i.e.* 0/1) OTU table. It allows to suppress effect due to the number of sequences wich may be the result of a lot of molecular artefact (Lindhal et al., 2013).

`funky.color` and `transpa` allow to create nice color palette.

`accu_plot` allows to plot accumulation curves in fonction of a factor in samples data (`@sam_data` of phyloseq object).

`otu_circle` uses the package `circlize` to plot circle of OTUs/sequences distributions in samples. `sankey_phyloseq` is an alternative using Sankey plot.

`phyloseq_to_edgeR`, wrote by Paul J. McMurdie, converts phyloseq OTU count data into DGEList for edgeR package.

`plot_deseq2_phyloseq` and `plot_edgeR_phyloseq` plot the result of differential analysis of count data (using either the package DESeq2 or edgeR).

```r
source(file = "functions_for_phyloseq.R")
```

2 Data

2.1 Choice of filter parameters

```r
#Choose the dataset folder
data_folder <- "Swarm"

#Choose the minimum number of sequences by sample.
N_sam_min <- 20000

#Choose the minimum number of samples by OTU.
N_otu_sam_min <- 1

#Choose the minimum number of sequences by OTU.
N_seq_otu_min <- 5
```

2.2 Load and convert loading

2.2.1 Otu table

```r
#Import biom data
dataBiom   <- import_biom(paste("data/", data_folder, "/otu_table.biom", sep=""))
```

2.2.2 Taxonomy

```r
#Import taxonomy data
taxRDP_brut <- readLines(paste("data/", data_folder, "/tax_assignments.txt", sep=""))
taxRDP_brut <- gsub(";", "\t", taxRDP_brut)
taxRDP_brut <- gsub(")", "", taxRDP_brut)
taxRDP_brut <- gsub("\\(", "\t", taxRDP_brut)
taxRDP_brut <- gsub("*__", "\t", taxRDP_brut)
taxRDP_brut <- read.table(textConnection(taxRDP_brut), sep = "\t", fill = TRUE)


# Format taxonomy for phyloseq
taxRDP <- taxRDP_brut[match(taxa_names(dataBiom), taxRDP_brut[, 1]),
                      c(3, 5, 7, 9, 11, 13, 15)]
taxRDP <- tax_table(as.matrix(taxRDP))
taxa_names(taxRDP) <- taxa_names(dataBiom)
colnames(taxRDP) <- c("Domain", "Phylum", "Class", "Order", "Family",
                      "Genus", "Species")
```

2.2.3   Add FUNguild information to taxonomy Table

```r
taxRDP2 <- as.data.frame(taxRDP)
funguild <- read.delim(paste("data/", data_folder, "/FUNGUILD.guilds.txt", sep = ""))

match_interm <- match(rownames(taxRDP2), funguild$OTU_ID)

taxRDP2$Trophic_Mode <- NA
taxRDP2$Trophic_Mode <- as.character(funguild$Trophic.Mode)[match_interm]
taxRDP2$Guild <- NA
taxRDP2$Guild <- as.character(funguild$Guild)[match_interm]
taxRDP2$Confidence_Ranking <- NA
taxRDP2$Confidence_Ranking <- as.character(funguild$Confidence.Ranking)[match_interm]
taxRDP2$Growth_Morphology <- NA
taxRDP2$Growth_Morphology <- as.character(funguild$Growth.Morphology)[match_interm]
taxRDP2$Trait <- NA
taxRDP2$Trait <- as.character(funguild$Trait)[match_interm]


taxRDP2 <- tax_table(as.matrix(taxRDP2))
taxa_names(taxRDP2) <- taxa_names(dataBiom)
colnames(taxRDP2) <- c("Domain", "Phylum", "Class", "Order", "Family", "Genus", "Species",
                       "Trophic_Mode", "Guild", "Confidence_Ranking", "Growth_Morphology",
                       "Trait")
```

2.2.4   Representative sequences

```r
map_endo <-
  import_qiime(map = "data/map_qiimedata.txt")

## Processing map file...

map_endo <- map_endo[order(rownames(map_endo)),]
```

2.2.5 Samples information

```
repset <- import_qiime(refseqfilename = paste("data/", data_folder, "/seq.fasta",
                                               sep = ""))

## Processing Reference Sequences...
```

2.2.6 Create the phyloseq object

```
data_all <- merge_phyloseq(dataBiom, repset, taxRDP2)

sample_data(data_all) <- map_endo

data_all@tax_table[data_all@tax_table == ""] <- NA
```

2.2.7 Caracteristics of the phyloseq data

```
data_all

## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 15479 taxa and 80 samples ]
## sample_data() Sample Data:       [ 80 samples by 6 sample variables ]
## tax_table()   Taxonomy Table:    [ 15479 taxa by 12 taxonomic ranks ]
## refseq()      DNAStringSet:      [ 15479 reference sequences ]
```

The data are made of 8.419809×10^6 sequences representing 15479 OTUs allocate to 80 samples.

2.3 Filter sample by number of sequences

```
N_sam_min

## [1] 20000
```

If we discard samples with less than 2×10^4 sequences, we keep 72 on the 80 samples (90%).

```
barplot(sort(sample_sums(data_all)))
abline(h = N_sam_min)
data.f1 <- prune_samples(sample_sums(data_all) > N_sam_min, data_all)
data.f1 <- prune_taxa(taxa_sums(data.f1) >=  1, data.f1)
```

2.4 Filter OTUs by number of samples

First, we can visualize the number of OTUs in a given number of samples (Figure 2.2).
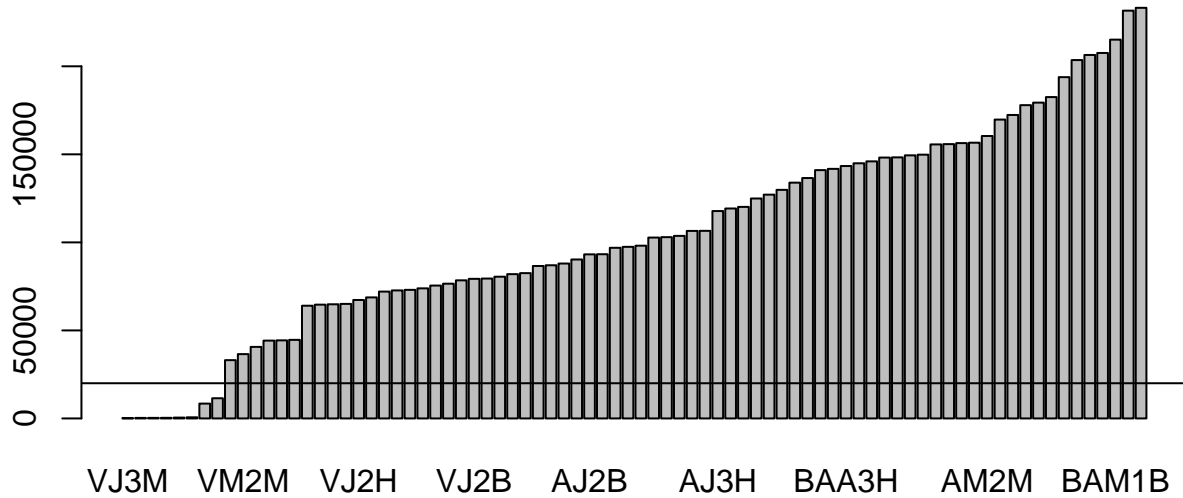
Figure 2.1: Number of sequences by sample. Horizontal line indicates the filtering parameter.

```
df_nbOtu_sample <- data.frame("Nb of OTUs" = table(rowSums(as.binaryOtuTable(
    data.f1)@otu_table))[table(rowSums(as.binaryOtuTable(data.f1)@otu_table)) > 1],
    "Nb samples" = as.numeric(names(table(rowSums(as.binaryOtuTable(data.f1)@otu_table))
                        [table(rowSums(as.binaryOtuTable(data.f1)@otu_table)) > 1])))

g <- ggplot(df_nbOtu_sample, aes(y = Nb.of.OTUs.Freq, x = Nb.samples))
g + geom_point(size = 4, col = rgb(0.1, 0.1, 0.1, 0.5)) +
    scale_y_continuous(trans = 'log10') +
    geom_smooth(size = 2, col = rgb(0.1, 0.1, 0.1, 0.5)) +
    geom_vline(xintercept= N_otu_sam_min)

## 'geom_smooth()' using method = 'loess'

summary(df_nbOtu_sample$Nb.samples)

##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##    1.00   18.75   36.50   36.50   54.25   72.00


N_otu_sam_min

## [1] 1


data.f2 <- prune_taxa(rowSums(as.binaryOtuTable(data.f1)@otu_table) >=
                        N_otu_sam_min, data.f1)
```

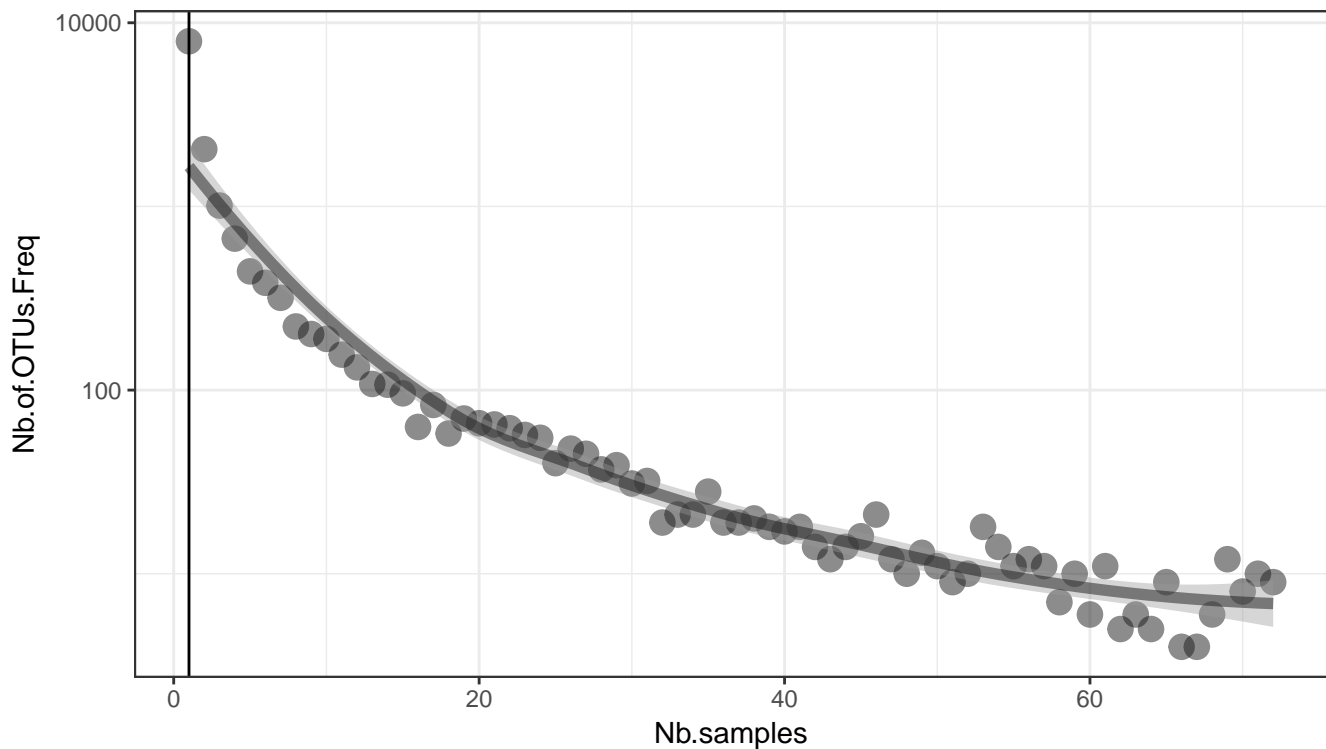If we discard OTUs present in less than 1 sample, we keep 15391 on the 15391 OTUs (100%).

Figure 2.2: Number of OTU present in a given number of samples. Vertical bar illustrates the filtering parameter.

2.5 Filter OTUs by number of sequences

We can visualize the number of sequences by OTU (Figure 2.3).

```r
df_nbseq_Otu <- data.frame("Nb of sequences by OTUs" = rowSums(data.f2@otu_table))
g <- ggplot(df_nbseq_Otu, aes(x = Nb.of.sequences.by.OTUs))
g + geom_histogram(size = 2, col = rgb(0.8, 0.8, 0.8, 0.3)) +
  scale_x_continuous(trans = 'log10') +
  geom_vline(xintercept= N_seq_otu_min)
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```

```r
summary(df_nbseq_Otu[, 1])
```

```
##     Min.   1st Qu.    Median      Mean   3rd Qu.       Max.
##      1.0       1.0       2.0     545.6      14.0 1236989.0
```

```r
N_seq_otu_min
```

```
## [1] 5
```

If we discard OTUs with less than 1 sequences, we keep 6064 on the 15479 OTUs (39.18%).

```r
data.f3 <- prune_taxa(rowSums(data.f2@otu_table) >= N_seq_otu_min, data.f2)
```
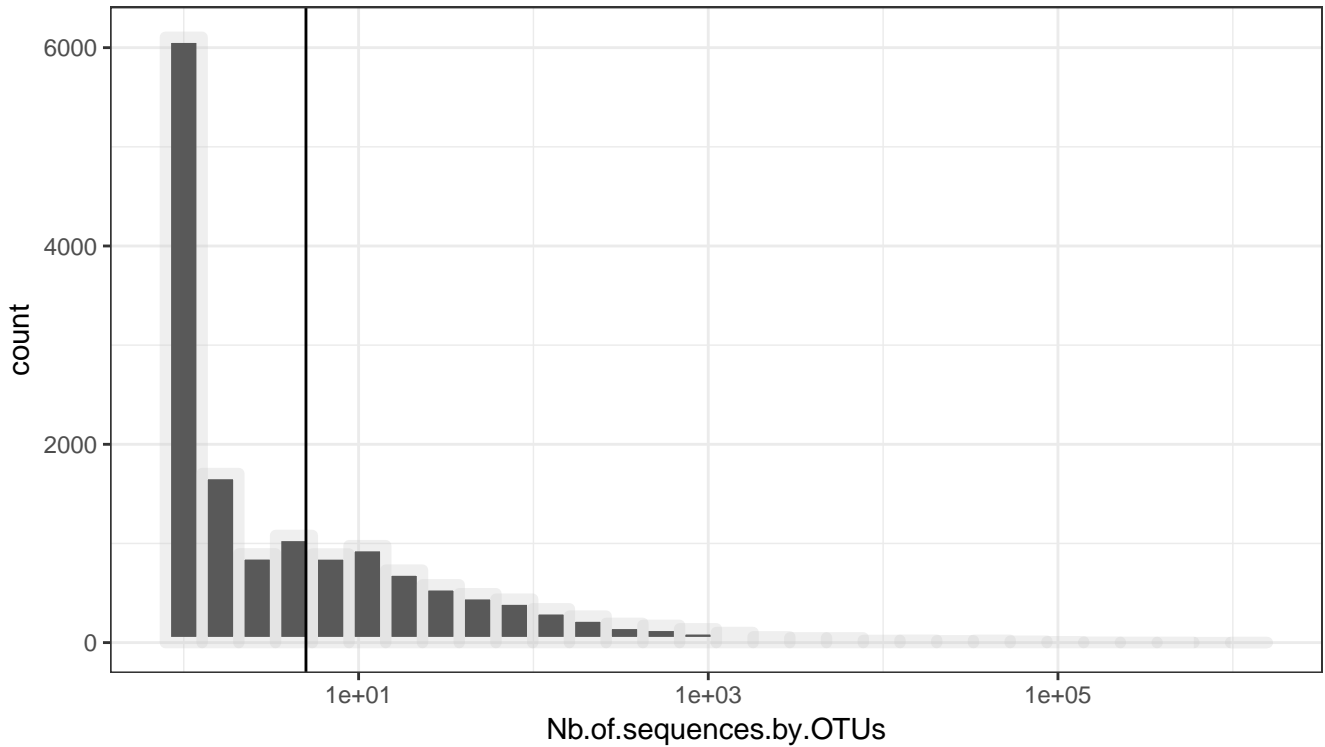
Figure 2.3: Number of sequences by OTU (log10 transformed). Horizontal bar illustrates the filtering parameter.

2.6 Summary of filtration workflow

The filtered data are made of 8.382948×10^6 sequences representing 6064 OTUs allocate to 72 samples.

	Nb.of.OTUs	Nb.of.samples	Nb.of.sequences
No filter	15479	80	8419809.00
Nb of sequences by sample >= 20000	15391	72	8397636.00
Nb of sample by OTUs >= 1	15391	72	8397636.00
Nb of sequences by OTUs >= 5	6064	72	8382948.00

Table 1: Number of OTUs, samples and sequences after filtering

3 Simple description of the dataset

3.1 Number of sequences and OTUs by samples

```
df_nbseq_nbotu <- data.frame("Nb of sequences by samples" = colSums(data.f3@otu_table),
                       "Nb of OTUs by samples" =
                             colSums(as.binaryOtuTable(data.f3)@otu_table))

g <- ggplot(df_nbseq_nbotu, aes(x = Nb.of.OTUs.by.samples,
                             y = Nb.of.sequences.by.samples))
g + geom_point(size = 3, col = rgb(0.1, 0.1, 0.1, 0.5)) +
  scale_y_continuous(trans = 'log10') +
```
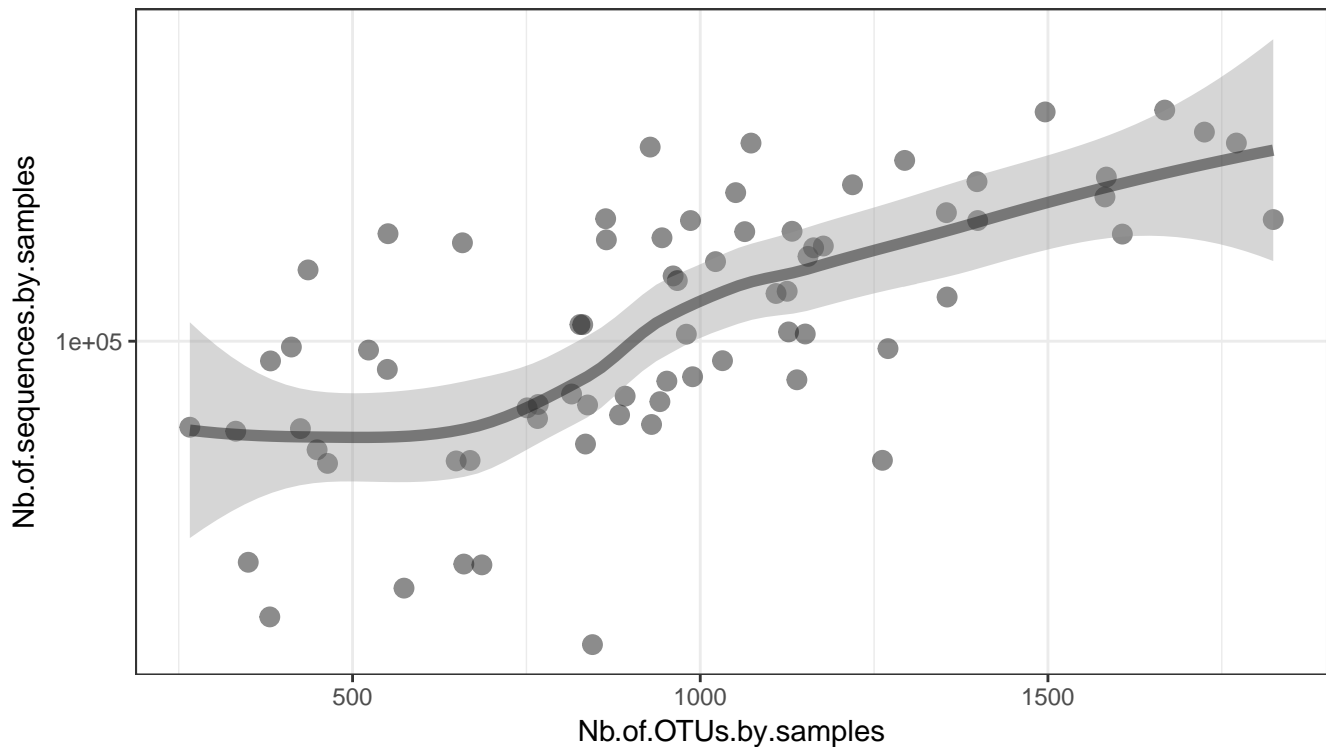
Figure 3.1: Number of OTUs by sample in fonction of the number of sequences by sample (log10 axe). The tendency is represented by the line obtained from loess (Local Polynomial Regression Fitting).

```
  geom_smooth(size = 2, col = rgb(0.1, 0.1, 0.1, 0.5))
```

```
## 'geom_smooth()' using method = 'loess'
```

```
ggplot(as.data.frame(data.f3@refseq@ranges), aes(x = width)) + geom_density() +
  ylab("Reference sequences length")
```

3.2   Number of sequences and samples for each OTUs

```
df_nbseq_nbsam <- data.frame("Nb of sequences by OTUs" = rowSums(data.f3@otu_table)
                             [rowSums(data.f3@otu_table) > 0],
                             "Nb of samples by OTUs" =
                               rowSums(as.binaryOtuTable(data.f3)@otu_table)
                             [rowSums(data.f3@otu_table) > 0])

g <- ggplot(df_nbseq_nbsam, aes(y = Nb.of.samples.by.OTUs,
                                x = Nb.of.sequences.by.OTUs))
g + geom_point(size = 3, col = rgb(0.1, 0.1, 0.1, 0.5)) +
  scale_x_continuous(trans = 'log10') +
  geom_smooth(size = 2, col = rgb(0.1, 0.1, 0.1, 0.5), method = "gam",
              formula  = y ~ s(x, bs = "cs"))
```
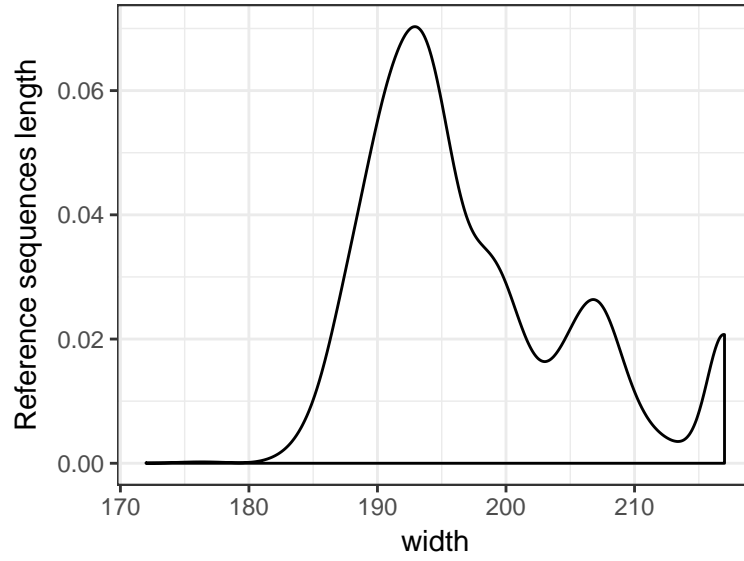
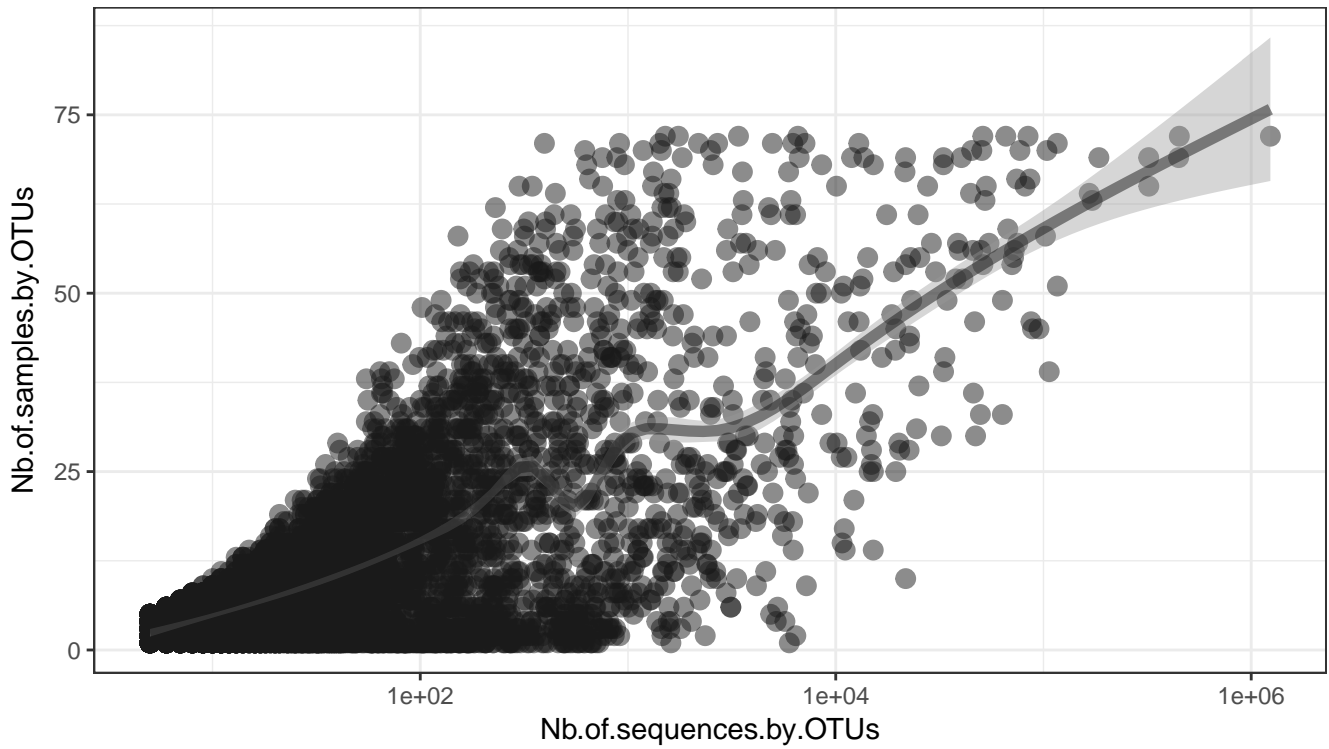**Figure 3.2:** Distribution of reference sequences length.

Figure 3.3: Number of sequences by OTUs (log10 axe) in fonction of the number of samples where OTUs were found. The tendency is represented by the line obtain from gam (Generalized additive models with integrated smoothness estimation).
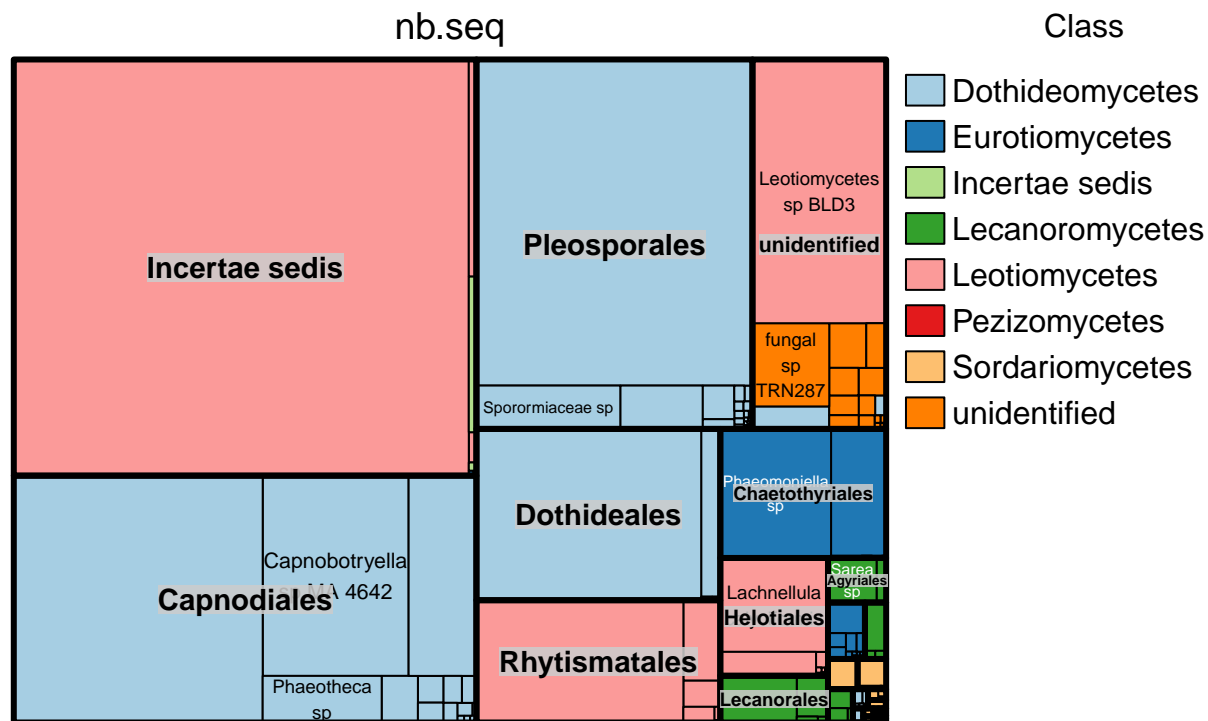
Figure 3.4: Distribution of the number of sequences in the Ascomycota taxonomy. Colors represent Class, bold lines delimit Order and thick line delimit species.

3.3 Distribution of sequences in the taxonomy

```
df3 <- data.frame(as.data.frame(data.f3@tax_table), nb.seq = rowSums(data.f3@otu_table))
tm <- treemap(df3, index = c("Order", "Species"), vSize = "nb.seq", vColor = "Class",
        type = "categorical", palette = "Paired")
# For an interactive version in html
# d3tree(tm)
```

3.4 Focus on the 30 more abundant OTUs (number of sequences)

```
the30mostfrequents <- sort(decreasing = T, rowSums(data.f3@otu_table))[1:30]
barplot(the30mostfrequents, horiz = T, cex.names = 0.4, las = 2)
```

```r
print(xtable(df_the30mostfrequents[, c(1:8, 12)], auto = T,
            caption = "Taxonomie of the 30 more
            abundant OTUs (number of sequences)"),
      size = "\\tiny", include.rownames = FALSE)
```

Phylum	Class	Order	Family	Genus	Species	Trophic_Mode	Guild	Nb.sequences
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	1236989
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	450861
Ascomycota	Dothideomycetes	Pleosporales				-	-	447439
Ascomycota	Dothideomycetes	Pleosporales				-	-	321439
Ascomycota	Leotiomycetes	unidentified	unidentified	unidentified	Leotiomycetes sp BLD3	-	-	321041
Ascomycota	Dothideomycetes	Capnodiales				-	-	184871
Ascomycota	Dothideomycetes	Capnodiales	Incertae sedis	Capnobotryella	Capnobotryella sp MA 4642	Saprotroph	Undefined Saprotroph	171529
Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	unidentified	Dothioraceae sp	-	-	165977
Ascomycota	Dothideomycetes	Pleosporales				-	-	116606
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	116577
Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	unidentified	Dothioraceae sp	-	-	106621
						-	-	103925
						-	-	102289
Ascomycota	Dothideomycetes	Pleosporales				-	-	95146
Ascomycota	Dothideomycetes	Capnodiales	Incertae sedis	Capnobotryella	Capnobotryella sp MA 4642	Saprotroph	Undefined Saprotroph	88575
						-	-	87076
Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Lophodermium		Pathotroph	Plant Pathogen	86099
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	84315
Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	unidentified	Dothioraceae sp	-	-	81555
Ascomycota	Dothideomycetes	Capnodiales				-	-	77060
Ascomycota	Leotiomycetes	unidentified	unidentified	unidentified	Leotiomycetes sp BLD3	-	-	75144
Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Lophodermium		Pathotroph	Plant Pathogen	74295
						-	-	72400
						-	-	71797
Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Phaeothecoidea	Phaeothecoidea sp	Saprotroph	Undefined Saprotroph	70457
Ascomycota	Dothideomycetes	Capnodiales	Incertae sedis	Capnobotryella	Capnobotryella sp MA 4642	Saprotroph	Undefined Saprotroph	67353
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	65868
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Lachnellula	Lachnellula calyciformis	Saprotroph	Undefined Saprotroph	63453
Ascomycota						-	-	63406

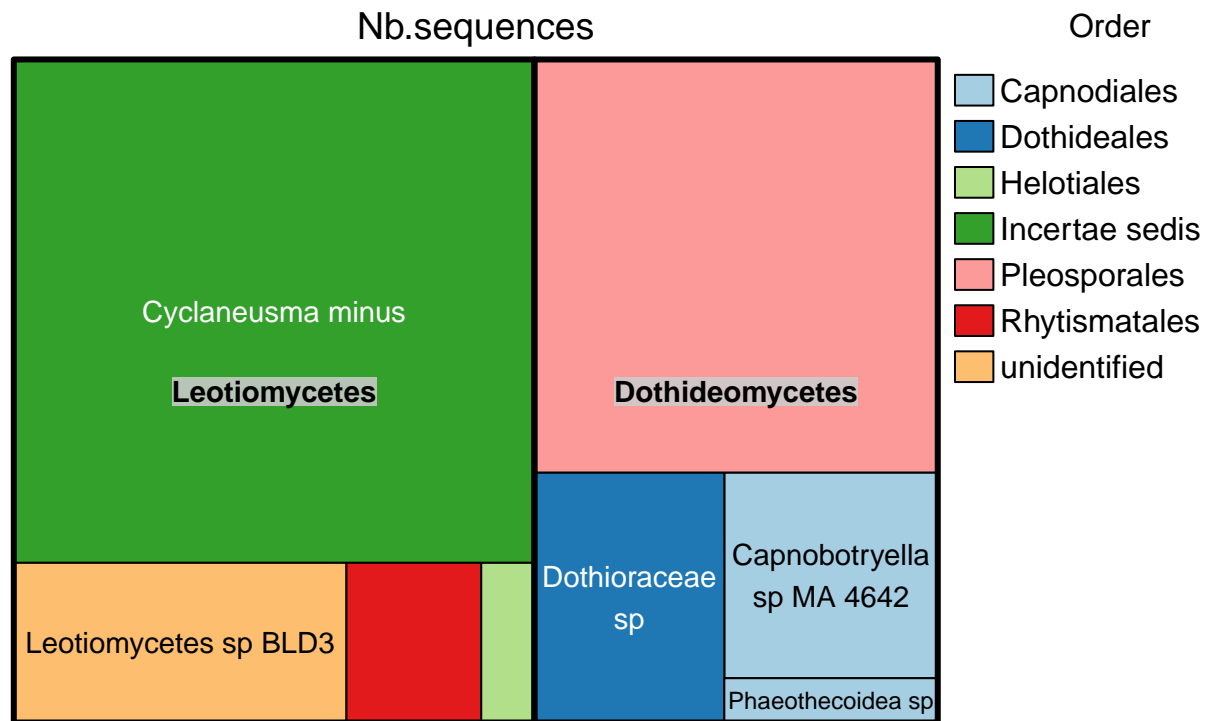**Table 2:** Taxonomie of the 30 more abundant OTUs (number of sequences)

Figure 3.5: Number of sequences of the 30 most abundant OTUs (number of sequences). Colors indicate Order, bold lines delimit Class and thick lines delimit species.

```
treemap(df_the30mostfrequents, index = c("Class", "Species"), vSize = "Nb.sequences",
        vColor = "Order", type = "categorical", palette = "Paired")
```

3.5 Focus on the 30 more frequent OTUs (number of samples)

```
the30mostfrequents_samp <- sort(decreasing = T,
                                rowSums(as.binaryOtuTable(data.f3)@otu_table))[1:30]
barplot(the30mostfrequents_samp, horiz = T, cex.names = 0.4, las = 2)
```

```r
print(xtable(df_the30mostfrequents_samp[, c(1:8, 12)], auto = T,
      caption = "Taxonomie of the 30 more frequent OTUs (number of samples)"),
      size = "\\tiny", include.rownames = FALSE)
```

Phylum	Class	Order	Family	Genus	Species	Trophic_Mode	Guild	Nb.samples
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	72
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	72
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	72
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	72
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	72
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	72
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	72
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	72
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	72
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	71
						-	-	70
Ascomycota	Dothideomycetes	Capnodiales				-	-	70
Ascomycota	Dothideomycetes	Capnodiales				-	-	70
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	70
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	70
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	70
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	70
Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Cyclaneusma	Cyclaneusma minus	-	-	70

Table 3: Taxonomie of the 30 more frequent OTUs (number of samples)
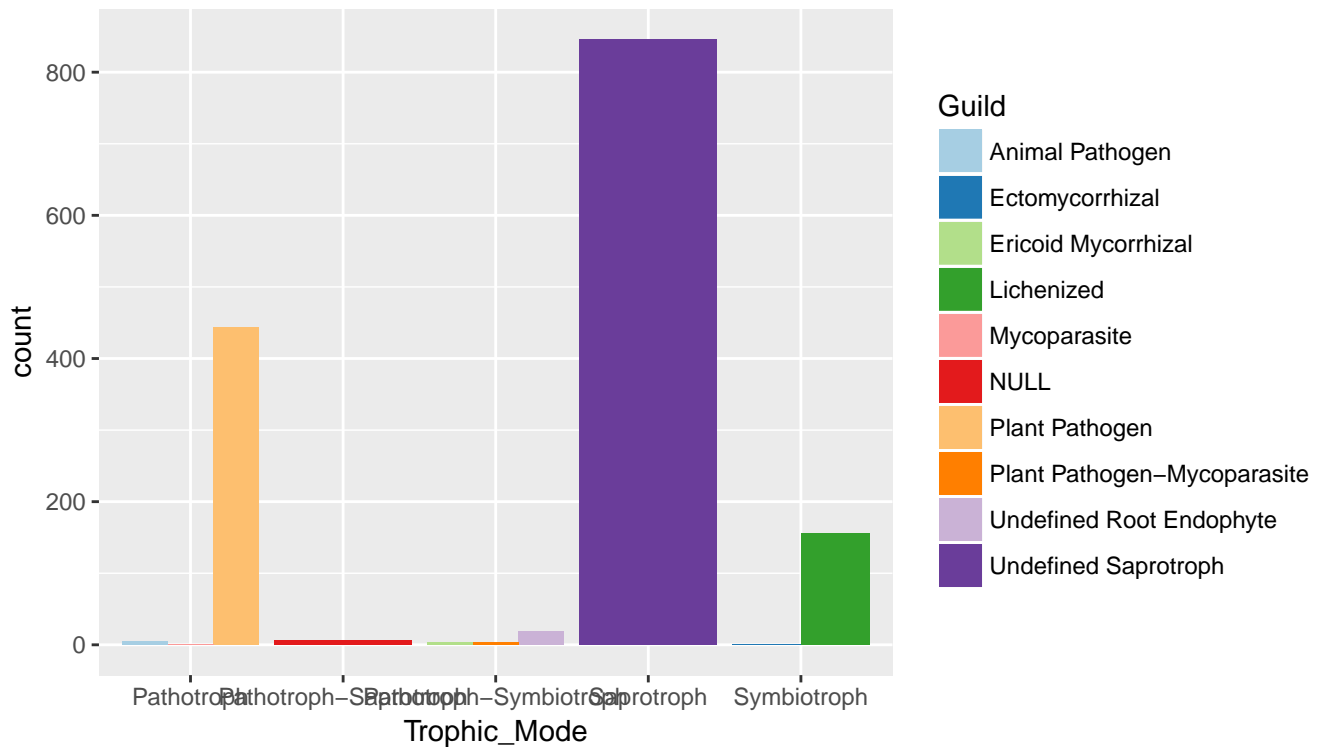
Figure 4.1: Distribution of OTUs into functional Guild.

4 Number of sequences and OTUs in function of putative ecology (using FUNGuild software; Nguyen et al, 2015)

```
tabPutativeEcology <- apply(data.f3@tax_table, 2, function(x) table(x))
tabPutativeEcology_percent <- apply(data.f3@tax_table, 2, function(x)
  round(table(x)/dim(data.f3@tax_table)[1]*100, 3))
sum(data.f3@otu_table[data.f3@tax_table[,"Trophic_Mode"] == "-"]) /
  sum(data.f3@otu_table)*100
```

```
## [1] 82.06287
```

```
tmdata <- as.data.frame(data.f3@tax_table[data.f3@tax_table[,"Trophic_Mode"] != "-"])
tmdata$Nb.sequences <- rowSums(data.f3@otu_table[data.f3@tax_table[,"Trophic_Mode"] != "-"])
tmdata$Nb.OTU <- rep(1, length(tmdata$Nb.sequences))
```

```
ggplot(tmdata) + geom_bar(aes(x = Trophic_Mode, fill=Guild), position = "dodge") +
  scale_fill_discrete("Paired") + theme_grey()
```

```
ggplot(tmdata, stat = "identity") +
  geom_bar(aes(x = Trophic_Mode, weight = Nb.sequences,  fill = Guild), position = "dodge") +
  scale_fill_discrete("Paired") + scale_y_log10() + theme_grey()
```
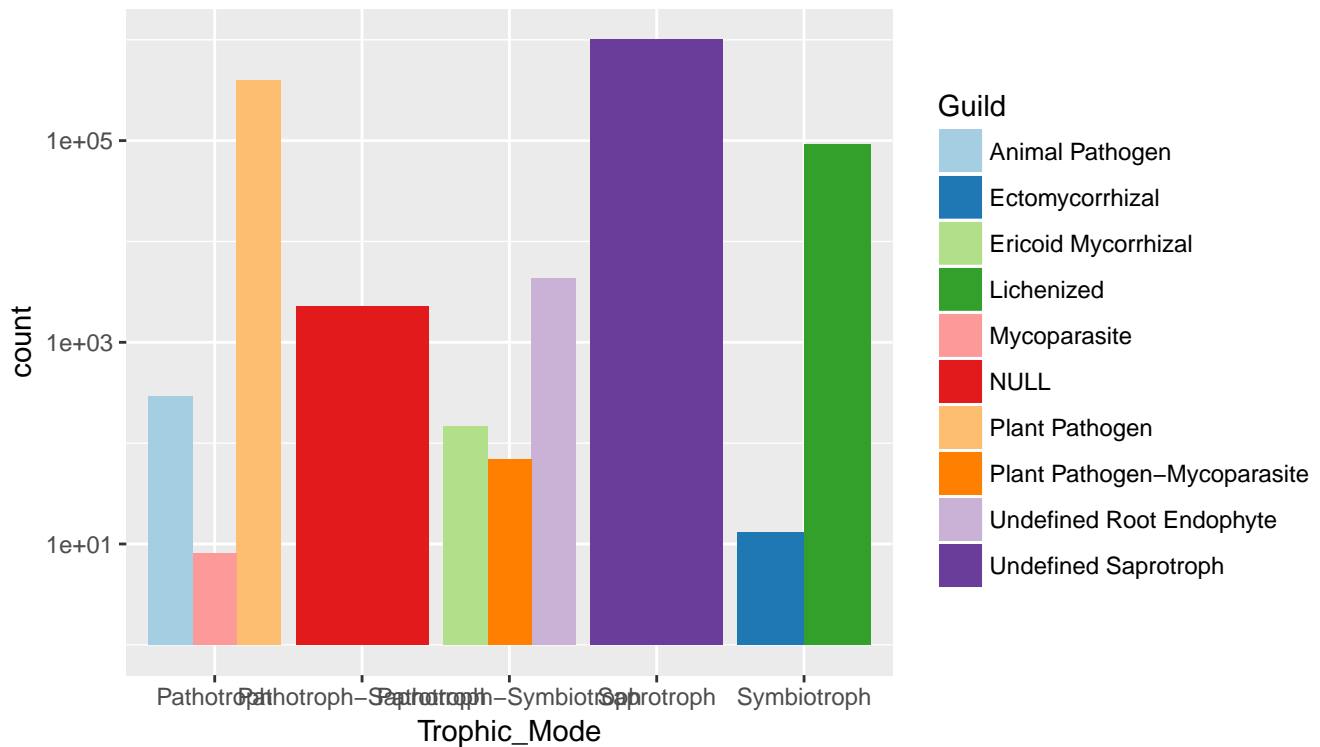
Figure 4.2: Distribution of sequences (log10 transformed) into functional Guild.

5 Distribution of fungal endophytic alpha-biodiversity

5.1 Local diversity = Diversity by sites

```
accu_plot(data.f3, "Sites", nbSeq = FALSE)
```

```
accu_plot(data.f3, "Sites", step = 5000)
```

```
measures_index <- c("Observed", "Chao1", "Shannon", "Simpson")
p <- plot_richness(data.f3, x = "Sites", color = "Sites", measures = measures_index)
p + geom_boxplot(data = p$data, alpha = 0.5)
```

5.2 Diversity by age of tree

```
accu_plot(data.f3, "Age", nbSeq = FALSE)
```

```
measures_index <- c("Observed", "Chao1", "Shannon", "Simpson")
p <- plot_richness(data.f3, x = "Age", color = "Sites", measures = measures_index)
p + geom_boxplot(data = p$data, aes(x = p$data$Age, y = value, color = NULL),
                 alpha = 0.5)
```
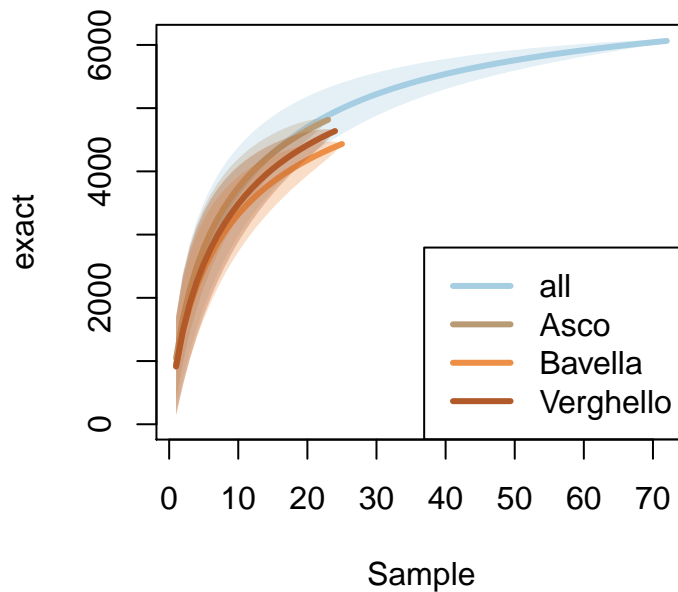
Figure 5.1: Rarefaction curves for each site. Note that if singletons were removed, these curves are biaised.

5.3 Diversity by elevation of the sample

```
accu_plot(data.f3, "Elevation", nbSeq = FALSE)
```

```
measures_index <- c("Observed", "Chao1", "Shannon", "Simpson")
p <- plot_richness(data.f3, x = "Elevation", color = "Sites", measures = measures_index)
p + geom_boxplot(data = p$data, aes(x = p$data$Elevation, y = value, color = NULL),
                 alpha = 0.5)
```

5.4 Which factor affect diversity?

```
## Uneven sequencing depth may have an impact
readNumbers = apply(t(data.f3@otu_table), 1, sum)

otuHill <- renyi(t(data.f3@otu_table), scale = c(0, 1, 2), hill = T)

hill.1 = otuHill$"0"
hill.2 = otuHill$"1"
hill.3 = otuHill$"2"

hill.1.m1 = lm(hill.1 ~ sqrt(readNumbers) + data.f3@sam_data$Sites +
               data.f3@sam_data$Age + data.f3@sam_data$Elevation)
```
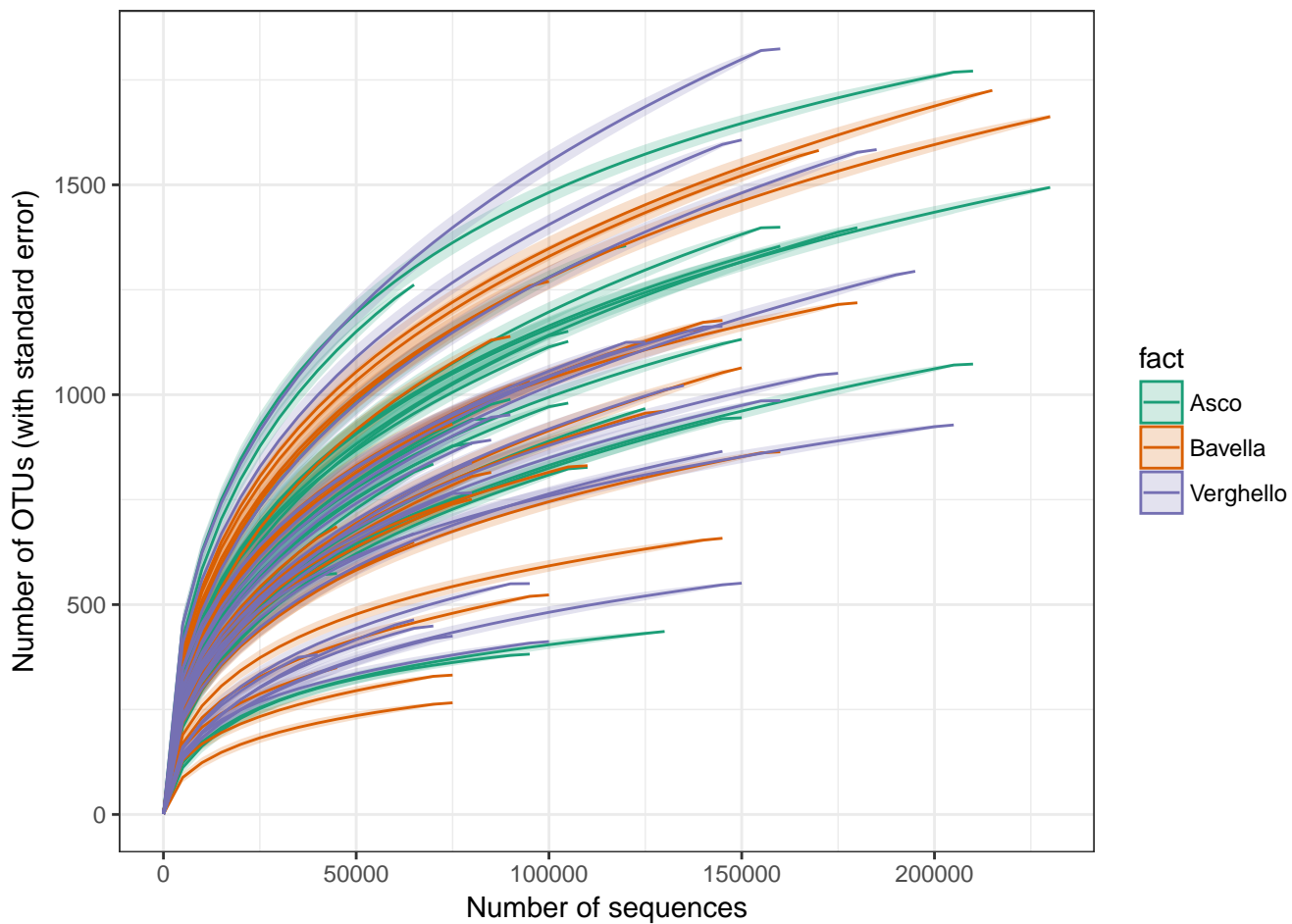
Figure 5.2: Rarefaction curves for each sample using sequences number on x-axes. Note that if singletons were removed, these curves are biaised.
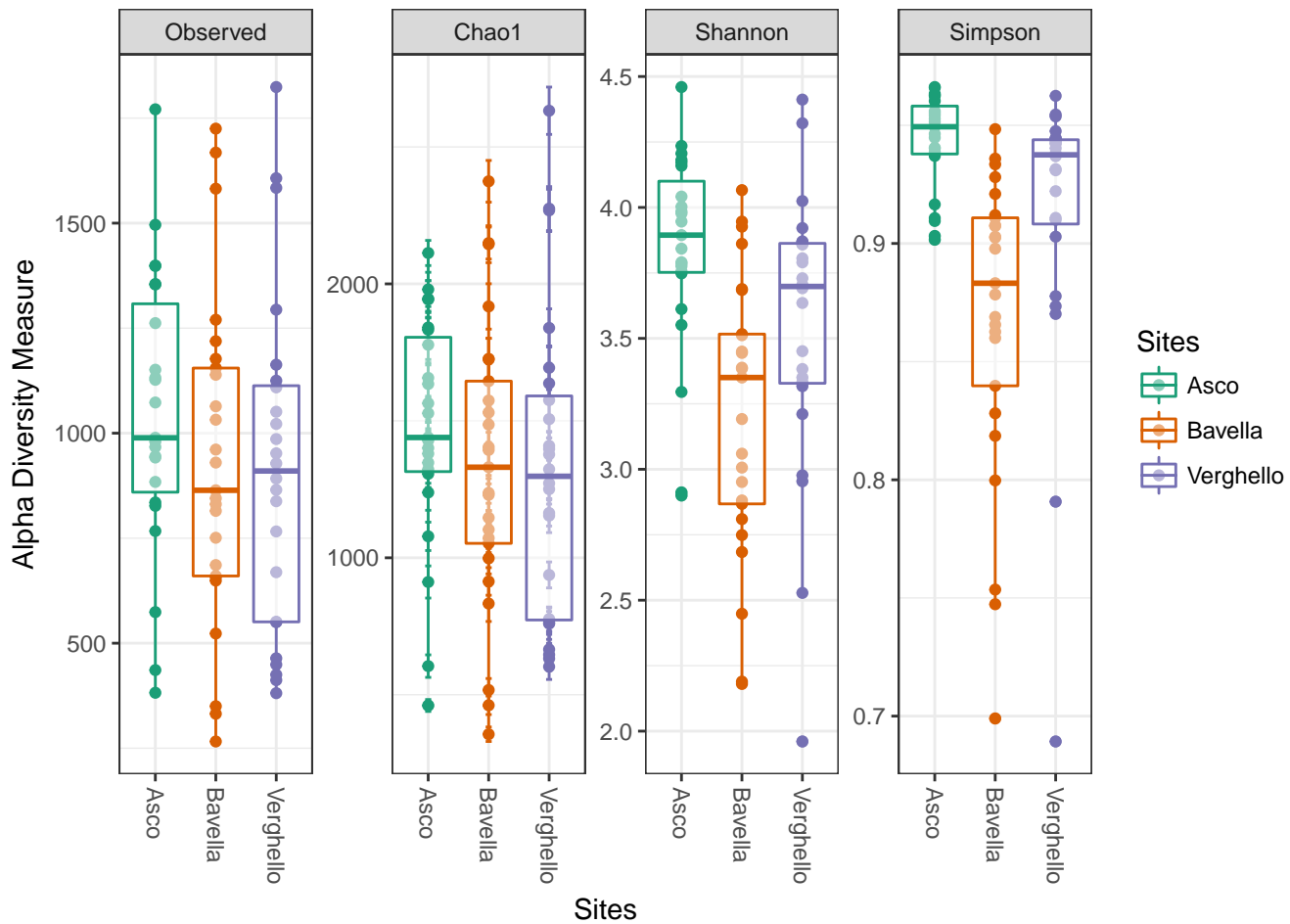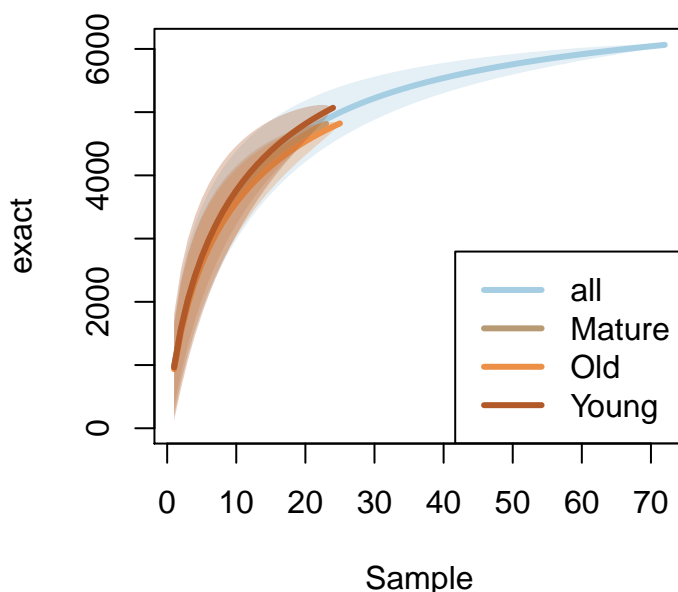
Figure 5.3: Diversity of each sites

Figure 5.4: Rarefaction curves for each host age. Note that if singletons were removed, these curves are biaised.

```
hill.2.m1 = lm(hill.2 ~ sqrt(readNumbers) + data.f3@sam_data$Sites +
            data.f3@sam_data$Age + data.f3@sam_data$Elevation)
hill.3.m1 = lm(hill.3 ~ sqrt(readNumbers) + data.f3@sam_data$Sites +
            data.f3@sam_data$Age + data.f3@sam_data$Elevation)
```

	Estimate	Std. Error	t value	Pr($>$|t|)
(Intercept)	-169.7099523	180.4367338	-0.9405510	0.3504716
sqrt(readNumbers)	3.6352080	0.4587897	7.9234730	0.0000000
data.f3@sam_data$SitesBavella	-49.6428188	80.4392050	-0.6171471	0.5393272
data.f3@sam_data$SitesVerghello	-114.7273185	80.5393450	-1.4244879	0.1591634
data.f3@sam_data$AgeOld	-14.6366862	80.0911628	-0.1827503	0.8555716
data.f3@sam_data$AgeYoung	-120.4101491	82.0227984	-1.4680083	0.1469997
data.f3@sam_data$ElevationMiddle	70.9260851	81.1127439	0.8744136	0.3851626
data.f3@sam_data$ElevationTop	-14.1869138	80.0164374	-0.1773000	0.8598327

Table 4: Summary of the linear model of species richness (Hill number with q = 0)

Post-hoc Tukey tests among the three experimental treatments with partial residuals, after accounting for differential sequencing success.

```
tuk1 <- TukeyHSD(aov(lm(hill.1 ~ sqrt(readNumbers))$residuals ~ data.f3@sam_data$Site))
tuk2 <- TukeyHSD(aov(lm(hill.2 ~ sqrt(readNumbers))$residuals ~ data.f3@sam_data$Site))
tuk3 <- TukeyHSD(aov(lm(hill.3 ~ sqrt(readNumbers))$residuals ~ data.f3@sam_data$Site))
```
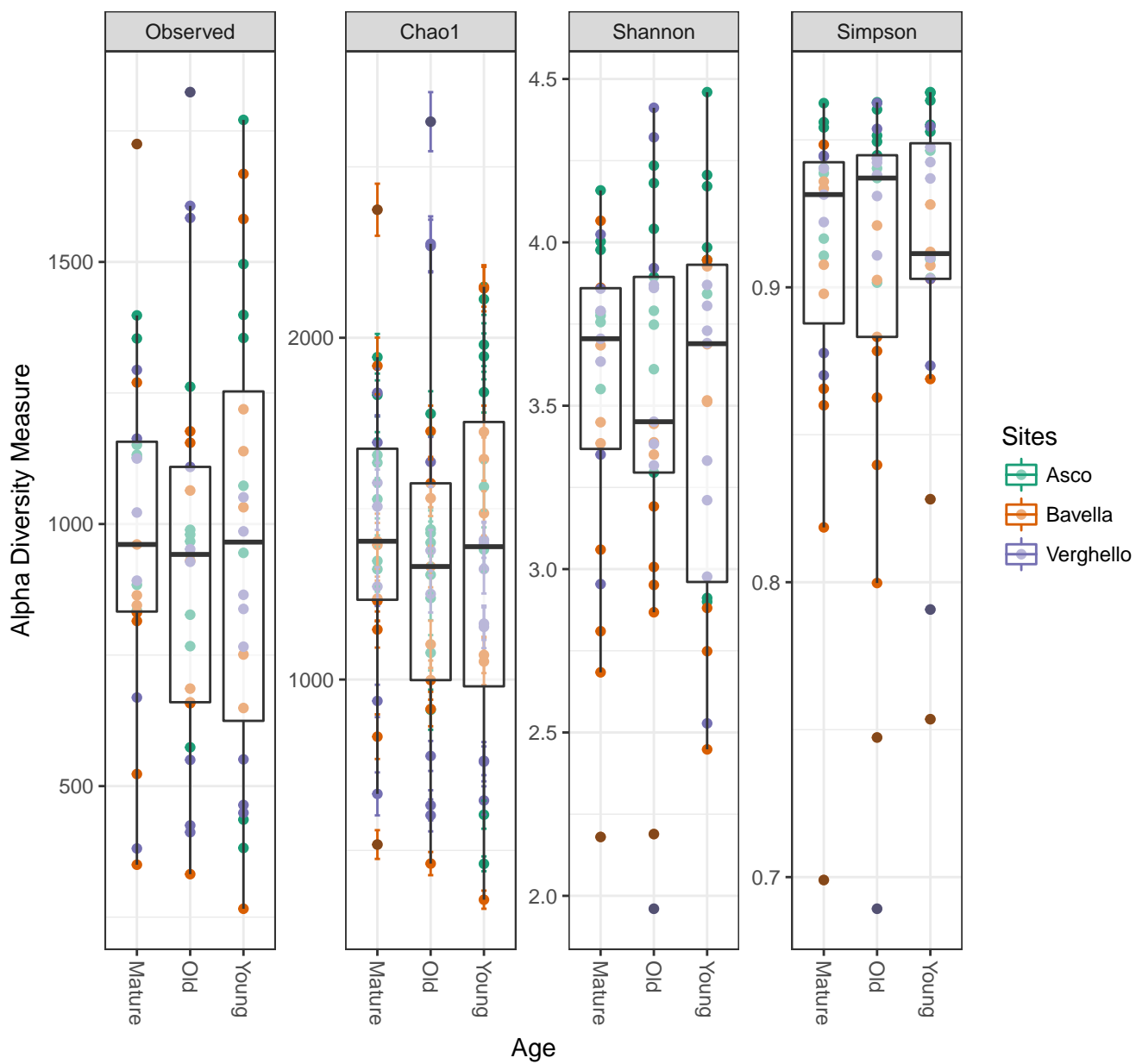
Figure 5.5: Diversity in function of tree age. Color represent sites.
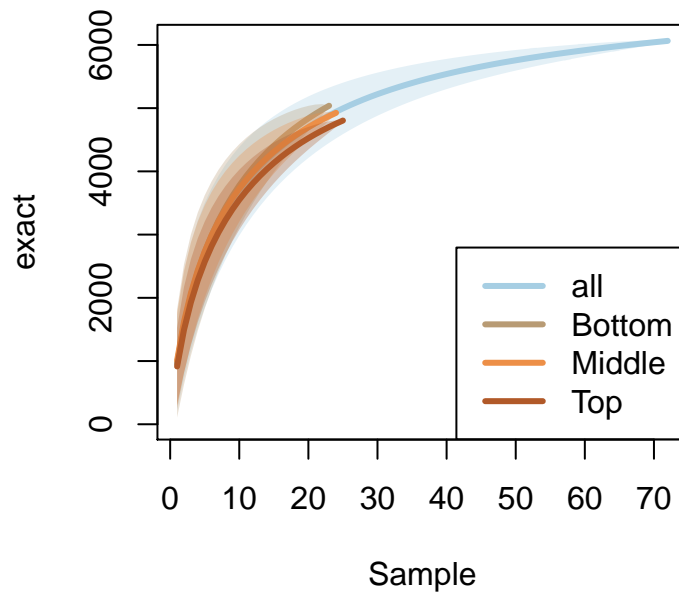
Figure 5.6: Rarefaction curves for each elevation. Notes that if singletons were removed, these curves are biaised.

```
ggplot(data = df) + geom_linerange(aes(ymax = xSup, ymin = xInf, x = y), size = 2) +
  geom_point(aes(x=y, y=x), size=4, shape=21, fill="white") +
  coord_flip() + theme_gray() + geom_hline(yintercept = 0) +
  ylab("Differences in mean levels") + xlab("")
```
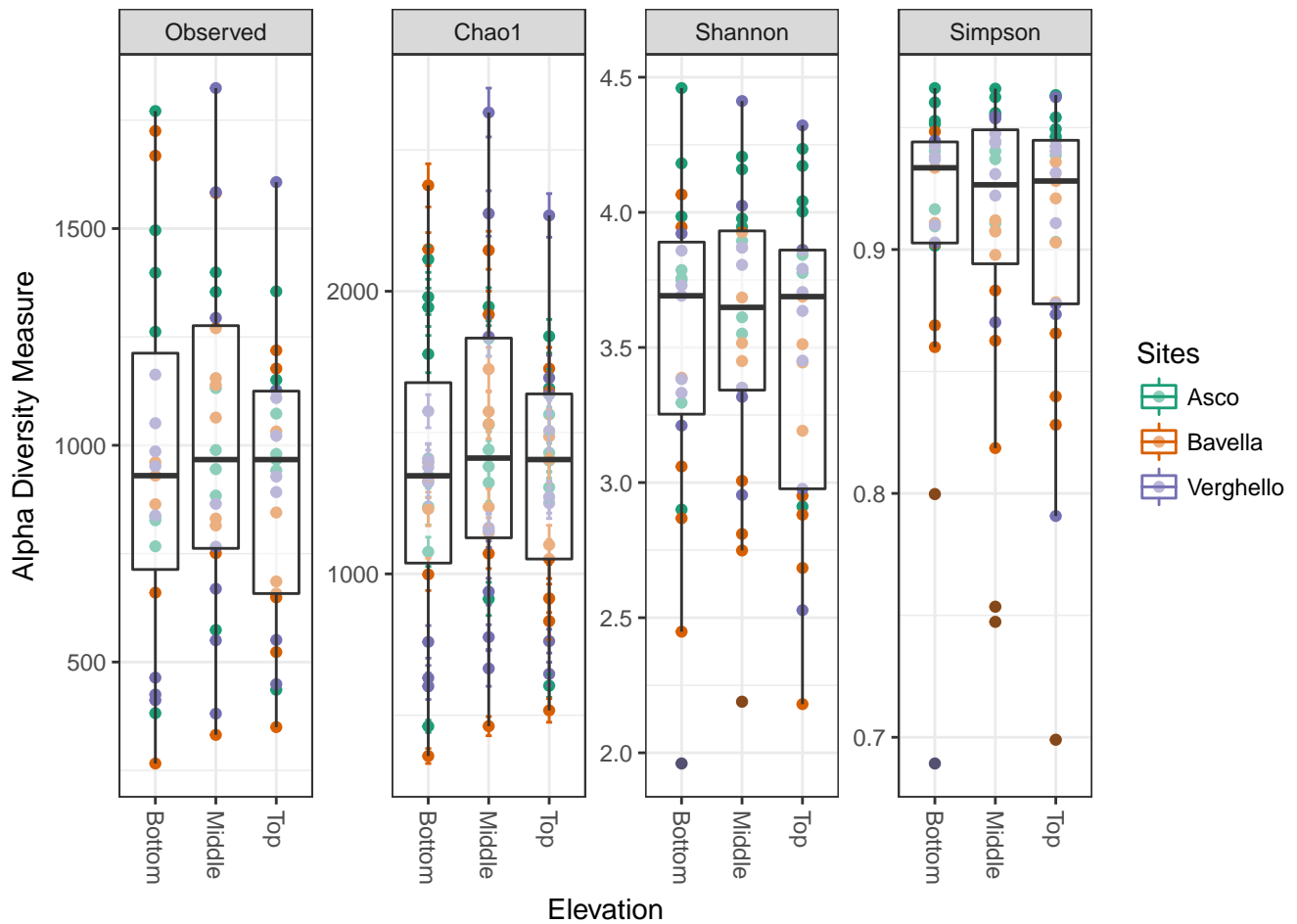
Figure 5.7: Diversity in function of elevation. Color represent sites.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	12.9703294	9.5718525	1.3550490	0.1801656
sqrt(readNumbers)	0.1035362	0.0243380	4.2540991	0.0000698
data.f3@sam_data$SitesBavella	-19.4074863	4.2671589	-4.5481049	0.0000247
data.f3@sam_data$SitesVerghello	-10.0679214	4.2724711	-2.3564633	0.0215228
data.f3@sam_data$AgeOld	0.7542946	4.2486958	0.1775356	0.8596484
data.f3@sam_data$AgeYoung	-3.3431380	4.3511657	-0.7683316	0.4451161
data.f3@sam_data$ElevationMiddle	4.0435791	4.3028889	0.9397359	0.3508864
data.f3@sam_data$ElevationTop	1.7596500	4.2447318	0.4145492	0.6798582

Table 5: Summary of the linear model of the exponential of Shannon's entropy index (Hill number with q = 1)

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	10.2095670	3.3737936	3.0261386	0.0035652
sqrt(readNumbers)	0.0258823	0.0085784	3.0171465	0.0036589
data.f3@sam_data$SitesBavella	-9.8360012	1.5040467	-6.5396913	0.0000000
data.f3@sam_data$SitesVerghello	-4.8631943	1.5059191	-3.2293861	0.0019593
data.f3@sam_data$AgeOld	0.4809093	1.4975390	0.3211331	0.7491559
data.f3@sam_data$AgeYoung	-0.1876484	1.5336566	-0.1223536	0.9030024
data.f3@sam_data$ElevationMiddle	1.0405173	1.5166405	0.6860672	0.4951487
data.f3@sam_data$ElevationTop	0.2540789	1.4961418	0.1698227	0.8656852

Table 6: Summary of the linear model of inverse of Simpson's concentration index (Hill number with q = 2)
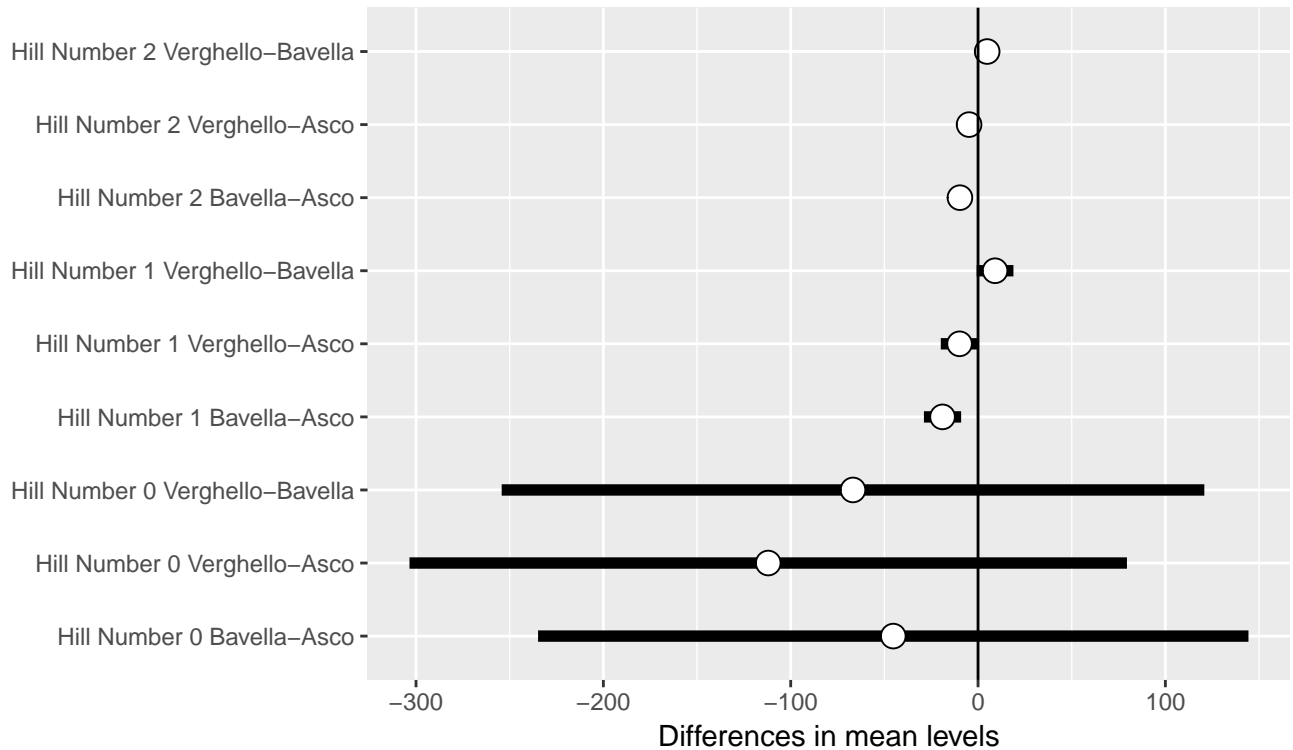

Figure 5.8: Results of the Tuckey HSD testing for differences in mean Hill numbers among pairs of modalities
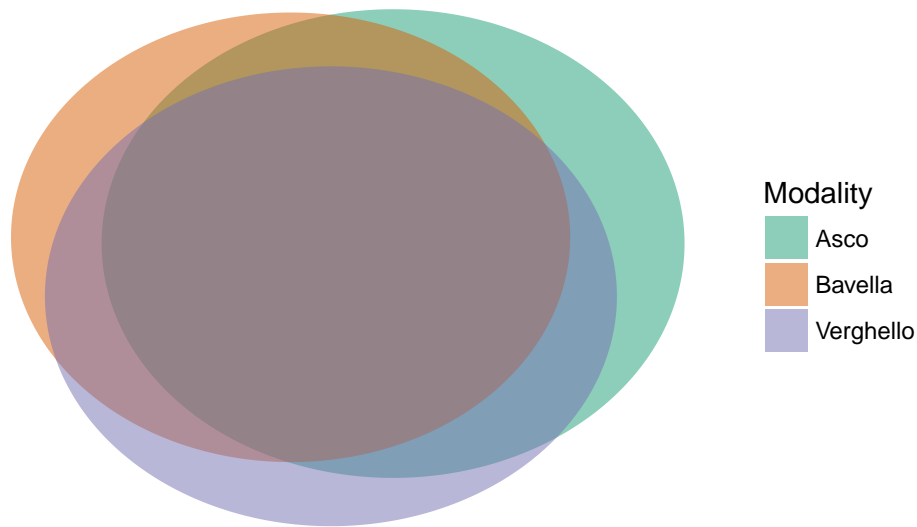
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Figure 6.1: Venn diagramm of the distribution of OTUs among Sites

6 Effect of site, age and elevation on fungal endophytic beta-diversity

6.1 Venn diagramm

```
venn_phyloseq(data.f3, "Sites", printValues = F)
```

```
venn_phyloseq(data.f3, "Age", printValues = F)
```

```
venn_phyloseq(data.f3, "Elevation", printValues = F)
```

6.2 Venn diagramm for OTUs present in at least 3 samples

```
data.f3_3samp <- subset_taxa(data.f3, rowSums(data.f3@otu_table>0)>2)
venn_phyloseq(data.f3_3samp, "Sites", printValues = F)
```

```
venn_phyloseq(data.f3_3samp, "Age", printValues = F)
```

```
venn_phyloseq(data.f3_3samp, "Elevation", printValues = F)
```

6.3 Ordination

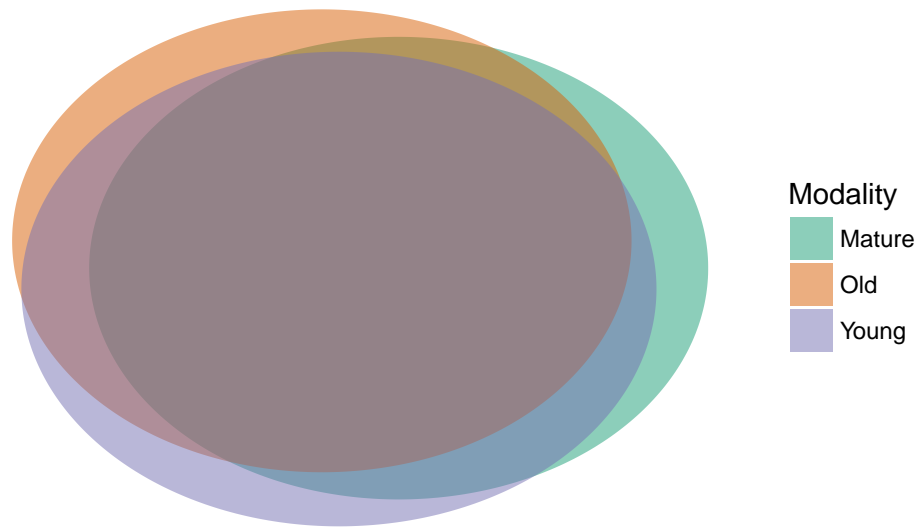Ordination of the OTUs table using NMDS (Non-metric MultiDimenstional Scaling).

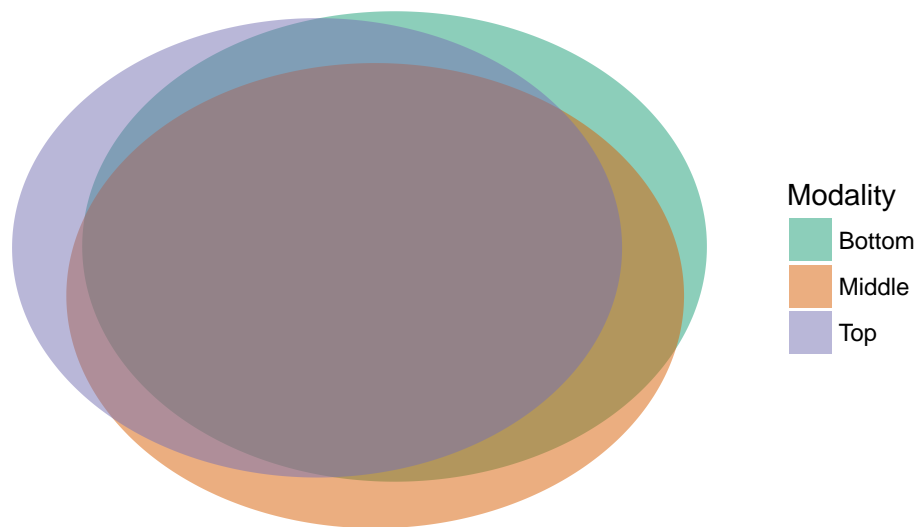**Figure 6.2:** Venn diagramm of the distribution of OTUs among host age

Figure 6.3: Venn diagramm of the distribution of OTUs among elevation of samples
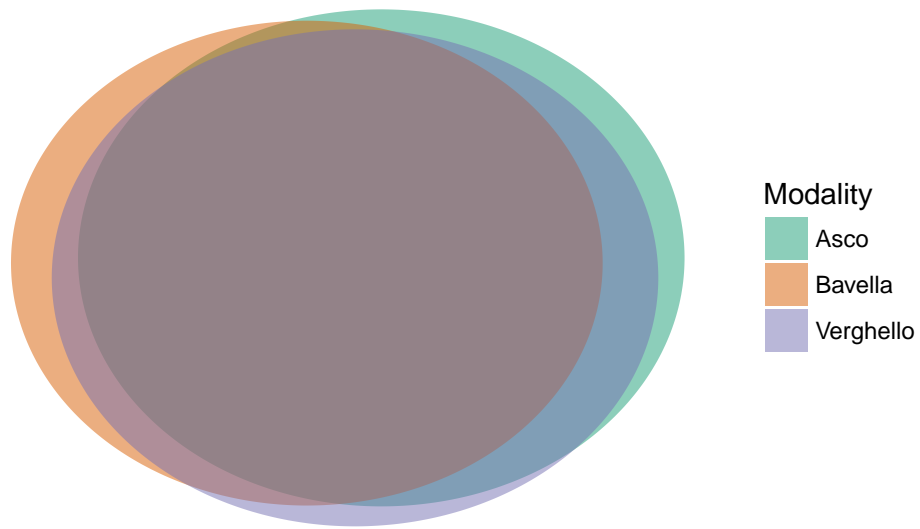
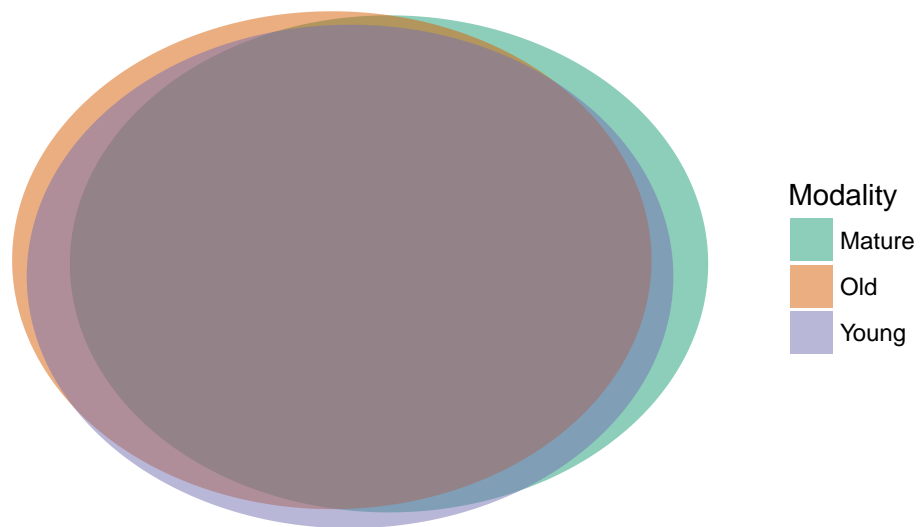**Figure 6.4:** Venn diagramm of the distribution of OTUs among Sites

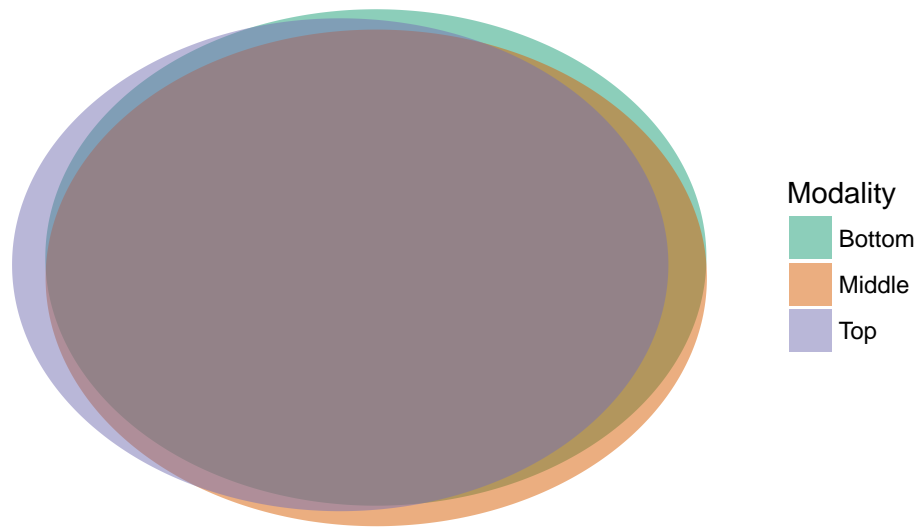**Figure 6.5:** Venn diagramm of the distribution of OTUs among host age

Figure 6.6: Venn diagramm of the distribution of OTUs among elevation of samples whitin the tree

```r
my.ord.nmds <- ordinate(data.f3, method = "NMDS")
my.ord.nmds$stress
```

```r
stressplot(my.ord.nmds)
```

```r
p <- plot_ordination(data.f3, my.ord.nmds, color = "Sites", shape = "Age")
p + geom_point(size = 0) +
  geom_text(aes(x = p$data$NMDS1, y = p$data$NMDS2,
                label = as.character(as.vector(data.f3@sam_data[, "CODE"]$CODE))))
```

```r
my.ord.nmds_gower <- ordinate(data.f3, distance = "gower",  method = "NMDS")

## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.1900518
## Run 1 stress 0.2173177
## Run 2 stress 0.1901357
## ... Procrustes: rmse 0.02115491  max resid 0.1447914
## Run 3 stress 0.1900932
## ... Procrustes: rmse 0.01468843  max resid 0.05968772
## Run 4 stress 0.1907819
## Run 5 stress 0.1901122
## ... Procrustes: rmse 0.0209189  max resid 0.1432302
## Run 6 stress 0.1896506
## ... New best solution
## ... Procrustes: rmse 0.01018155  max resid 0.05280492
## Run 7 stress 0.190831
## Run 8 stress 0.1906432
## Run 9 stress 0.4086769
```

Figure 6.7: Stress plot of the NMDS

Figure 6.8: NMDS of OTU table. Colors represent sites and shape the age of tree.

```
## Run 10 stress 0.2104146
## Run 11 stress 0.1895849
## ... New best solution
## ... Procrustes: rmse 0.006033086  max resid 0.03771183
## Run 12 stress 0.2067051
## Run 13 stress 0.2094959
## Run 14 stress 0.1895764
## ... New best solution
## ... Procrustes: rmse 0.002154834  max resid 0.01176039
## Run 15 stress 0.2148128
## Run 16 stress 0.2299369
## Run 17 stress 0.1906359
## Run 18 stress 0.2112671
## Run 19 stress 0.1904534
## Run 20 stress 0.1907523
## *** No convergence -- monoMDS stopping criteria:
##      2: no. of iterations >= maxit
##     18: stress ratio > sratmax

my.ord.PCoA <- ordinate(data.f3, method = "PCoA")
my.ord.PCoA_gower <- ordinate(data.f3, distance = "gower", method = "PCoA")
my.ord.DCA <- ordinate(data.f3, method = "DCA")
my.ord.DCA_gower <- ordinate(data.f3, distance = "gower", method = "DCA")


p_NMDS_BRAY <- plot_ordination(data.f3, my.ord.nmds, color = "Sites",
                               shape = "Age", label = "CODE") + geom_point(size = 5)
p_NMDS_GOWER <- plot_ordination(data.f3, my.ord.nmds_gower, color = "Sites",
                                shape = "Age", label = "CODE") + geom_point(size = 5)
p_PCoA_BRAY <- plot_ordination(data.f3, my.ord.PCoA, color = "Sites",
                               shape = "Age", label = "CODE") + geom_point(size = 5)
p_PCoA_GOWER <- plot_ordination(data.f3, my.ord.PCoA_gower, color = "Sites",
                                shape = "Age", label = "CODE") + geom_point(size = 5)
p_DCA_BRAY <- plot_ordination(data.f3, my.ord.DCA, color = "Sites",
                              shape = "Age", label = "CODE") + geom_point(size = 5)
p_DCA_GOWER <- plot_ordination(data.f3, my.ord.DCA_gower, color = "Sites",
                               shape = "Age", label = "CODE") + geom_point(size = 5)
```
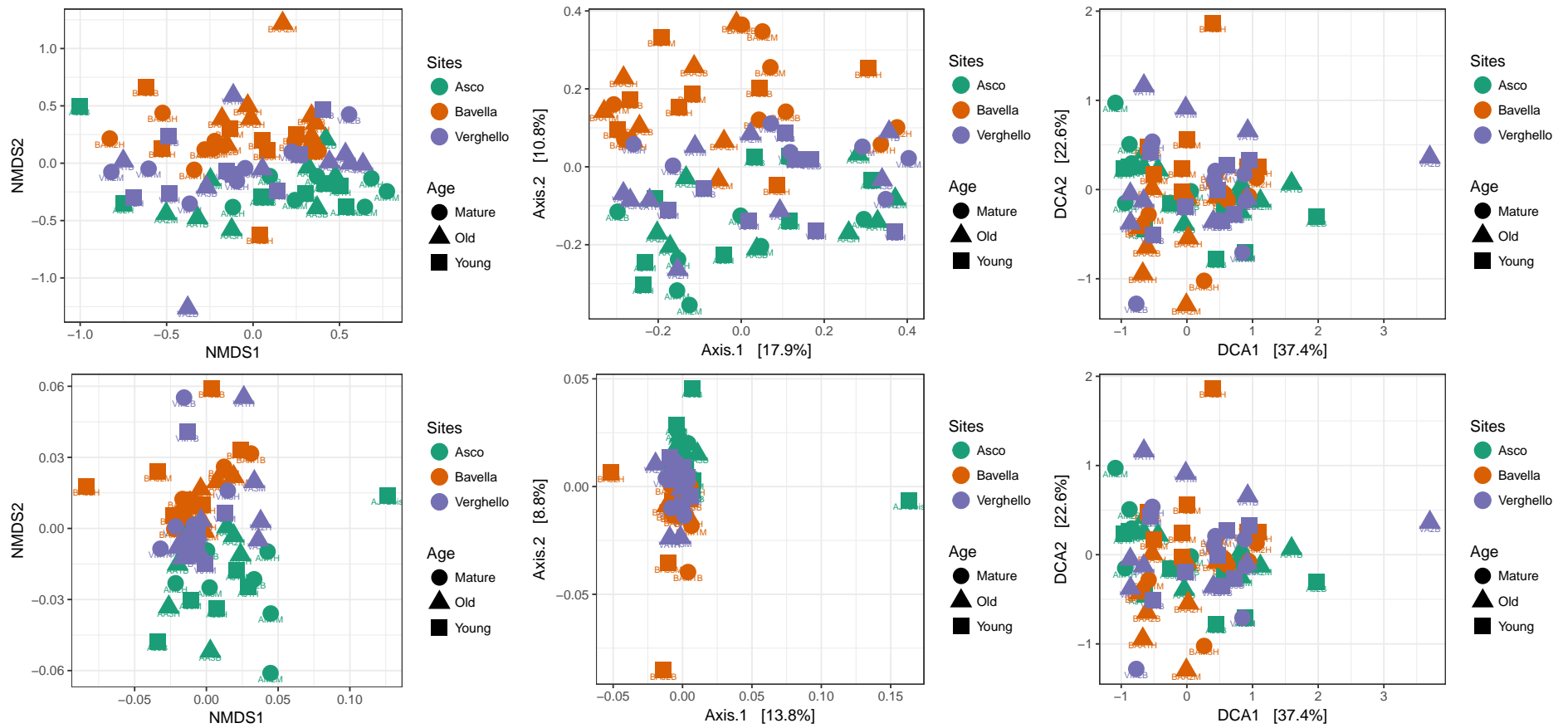
Figure 6.9: Comparison of different distances (bray (up) and gower (bottom)) and ordination methods (NMDS (left), PCoA (center) and DCA (right)).

```
multiplot(p_NMDS_BRAY, p_NMDS_GOWER, p_PCoA_BRAY, p_PCoA_GOWER, p_DCA_BRAY, p_DCA_GOWER,
          cols = 3)
```

6.4 Permanova on sites, host ages and elevation

```
sam_data <- as.data.frame(unclass(data.f3@sam_data))
sam_data$IndividualTree <- paste(sam_data$Sites, sam_data$Age, sam_data$Tree)
res.ado <- adonis(t(data.f3@otu_table) ~ Sites * Age * Elevation, sam_data,
                  permutation = 9999)
```

If we only keep the 583 OTUs present in more than 30 sample, the Permanova results is the following:

```
res.ado_sampMin30 <- adonis(t(data.f3@otu_table[rowSums(data.f3@otu_table>0)>=30,]) ~
                            Sites * Age * Elevation, sam_data, permutation = 9999)
```

```
data.f3_without_C_minus <- subset_taxa(data.f3, taxa_names(data.f3)!="OTU_1")
res.ado_without_C_minus <- adonis(t(data.f3_without_C_minus@otu_table) ~
                            Sites * Age * Elevation, sam_data, permutation = 9999)
```

```
xtable(res.ado$aov.tab, caption = "Result of the permanova on abundances
       (number of sequence).")
```

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Sites	2	1.91	0.96	4.19	0.10	0.0001
Age	2	0.67	0.34	1.47	0.04	0.0444
Elevation	2	0.54	0.27	1.18	0.03	0.2007
Sites:Age	4	1.55	0.39	1.69	0.08	0.0017
Sites:Elevation	4	0.91	0.23	0.99	0.05	0.4715
Age:Elevation	4	1.10	0.27	1.20	0.06	0.1231
Sites:Age:Elevation	8	1.85	0.23	1.01	0.10	0.4306
Residuals	45	10.27	0.23		0.55	
Total	71	18.79			1.00	

Table 7: Result of the permanova on abundances (number of sequence).

```
xtable(res.ado_sampMin30$aov.tab, caption = "Result of the permanova on abundances
       (number of sequence) using only OTUs present in more than 30 samples")
```

```
res.ado_bin <- adonis(t(as.binaryOtuTable(data.f3)@otu_table) ~ Sites * Age *
                      Elevation, sam_data, permutation = 9999)
```

```
xtable(res.ado_bin$aov.tab, caption = "Result of the permanova on OTUs
       (each OTU is representing by one sequence)).")
```

6.5 Permanova on sites, host ages and individual trees

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Sites	2	1.86	0.93	4.48	0.11	0.0001
Age	2	0.63	0.32	1.52	0.04	0.0427
Elevation	2	0.51	0.25	1.22	0.03	0.1883
Sites:Age	4	1.48	0.37	1.78	0.09	0.0007
Sites:Elevation	4	0.85	0.21	1.02	0.05	0.4256
Age:Elevation	4	1.04	0.26	1.24	0.06	0.1096
Sites:Age:Elevation	8	1.69	0.21	1.02	0.10	0.4228
Residuals	45	9.35	0.21		0.54	
Total	71	17.42			1.00	

Table 8: Result of the permanova on abundances (number of sequence) using only OTUs present in more than 30 samples

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Sites	2	1.28	0.64	3.40	0.09	0.0001
Age	2	0.52	0.26	1.38	0.04	0.0412
Elevation	2	0.40	0.20	1.06	0.03	0.3178
Sites:Age	4	1.09	0.27	1.44	0.07	0.0056
Sites:Elevation	4	0.64	0.16	0.85	0.04	0.8820
Age:Elevation	4	0.88	0.22	1.18	0.06	0.1080
Sites:Age:Elevation	8	1.51	0.19	1.01	0.10	0.4517
Residuals	45	8.45	0.19		0.57	
Total	71	14.76			1.00	

Table 9: Result of the permanova on OTUs (each OTU is representing by one sequence)).

```
res.ado_Tree <- adonis(t(data.f3@otu_table) ~   Sites*Age + Sites:Age:IndividualTree ,
                  sam_data, permutation = 9999)
res.ado_Tree_sampMin30 <- adonis(t(data.f3@otu_table[rowSums(data.f3@otu_table>0)>=30,]) ~
                          Sites*Age + Sites:Age:IndividualTree , sam_data,
                        permutation = 9999)
res.ado_Tree_bin <- adonis(t(as.binaryOtuTable(data.f3)@otu_table) ~
                      Sites*Age + Sites:Age:IndividualTree , sam_data,
                    permutation = 9999)
```

```
xtable(res.ado_Tree$aov.tab, caption = "Result of the permanova on abundances
      (number of sequence).")
```

```
xtable(res.ado_Tree_sampMin30$aov.tab, caption = "Result of the permanova on abundances
      (number of sequence) using only OTUs present in more than 30 samples")
```

```
xtable(res.ado_Tree_bin$aov.tab, caption = "Result of the permanova on OTUs
      (each OTU is representing by one sequence)).")
```

6.6   Differences in abundances and OTUs number by Order.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Sites	2	1.91	0.96	4.67	0.10	0.0001
Age	2	0.67	0.34	1.64	0.04	0.0158
Sites:Age	4	1.54	0.39	1.88	0.08	0.0006
Sites:Age:IndividualTree	18	5.45	0.30	1.48	0.29	0.0001
Residuals	45	9.22	0.20		0.49	
Total	71	18.79			1.00	

Table 10: Result of the permanova on abundances (number of sequence).

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Sites	2	1.86	0.93	5.00	0.11	0.0001
Age	2	0.63	0.32	1.70	0.04	0.0174
Sites:Age	4	1.48	0.37	1.98	0.08	0.0003
Sites:Age:IndividualTree	18	5.07	0.28	1.51	0.29	0.0002
Residuals	45	8.38	0.19		0.48	
Total	71	17.42			1.00	

Table 11: Result of the permanova on abundances (number of sequence) using only OTUs present in more than 30 samples

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Sites	2	1.28	0.64	3.73	0.09	0.0001
Age	2	0.52	0.26	1.52	0.04	0.0171
Sites:Age	4	1.10	0.28	1.61	0.07	0.0008
Sites:Age:IndividualTree	18	4.16	0.23	1.35	0.28	0.0001
Residuals	45	7.70	0.17		0.52	
Total	71	14.76			1.00	

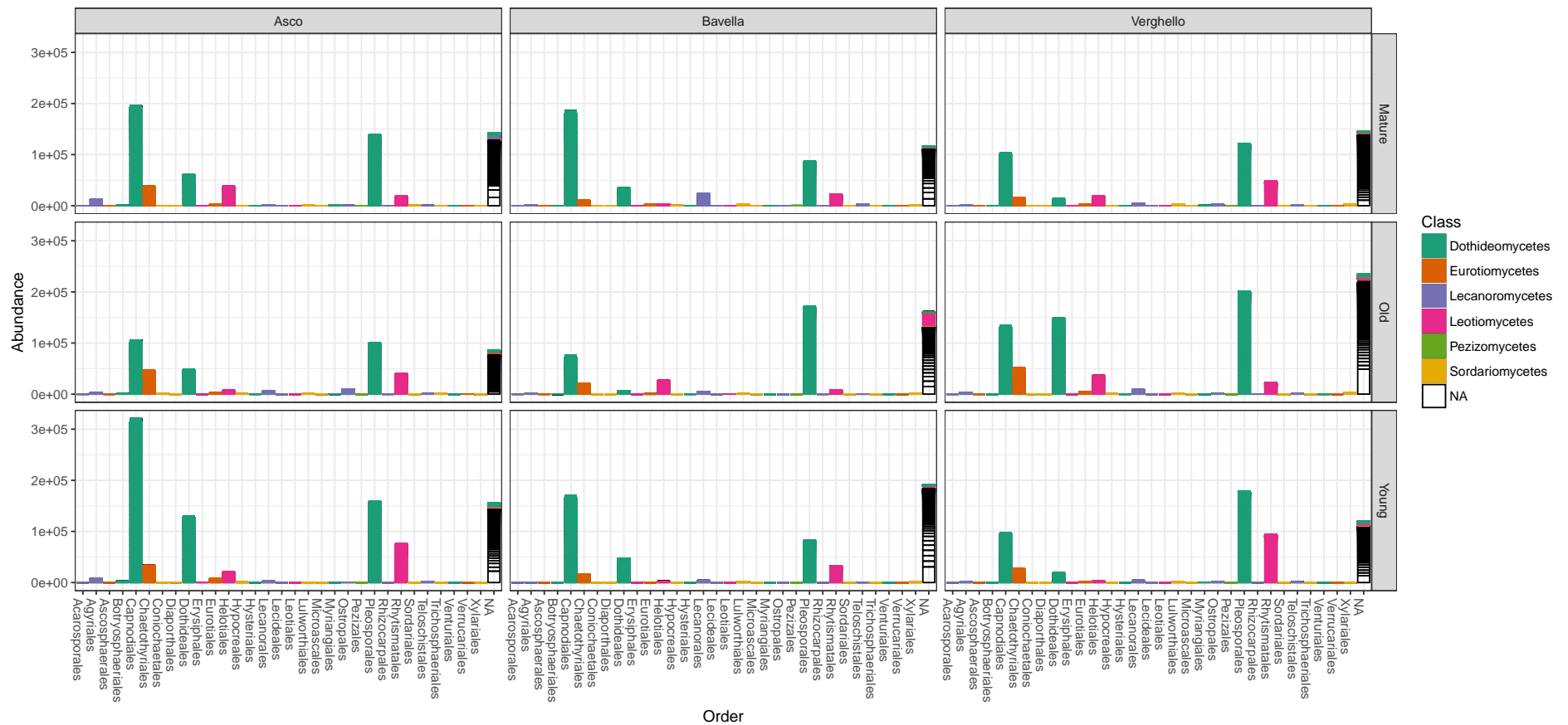**Table 12:** Result of the permanova on OTUs (each OTU is representing by one sequence)).

Figure 6.10: Taxonomic distribution of sequences in the different site * age combinaison.

```
data.f3_taxo_known <- prune_taxa(taxa_names(data.f3@tax_table)
        [!(data.f3@tax_table[, 4] %in% c("unidentified", "Incertae sedis"))], data.f3)
p <- plot_bar(data.f3_taxo_known, "Order", fill = "Class", facet_grid = Age ~ Sites)
p + geom_bar(aes(color = Class, fill = Class), stat = "identity", position = "stack")
```
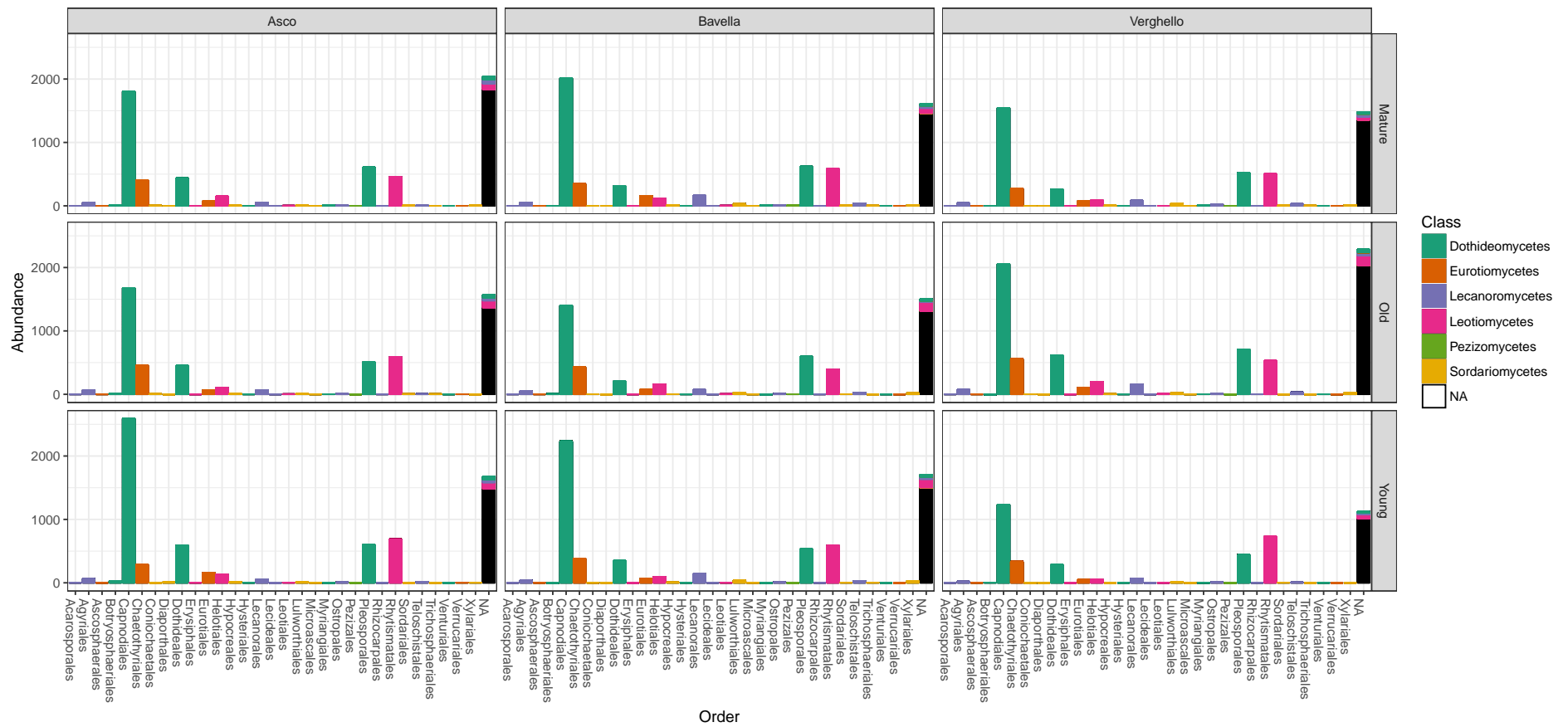
Figure 6.11: Taxonomic distribution of OTUs in the different site * age combinaison.

```r
p <- plot_bar(as.binaryOtuTable(data.f3_taxo_known), "Order", fill = "Class",
              facet_grid = Age ~ Sites)
p + geom_bar(aes(color = Class, fill = Class), stat = "identity", position = "stack")
```
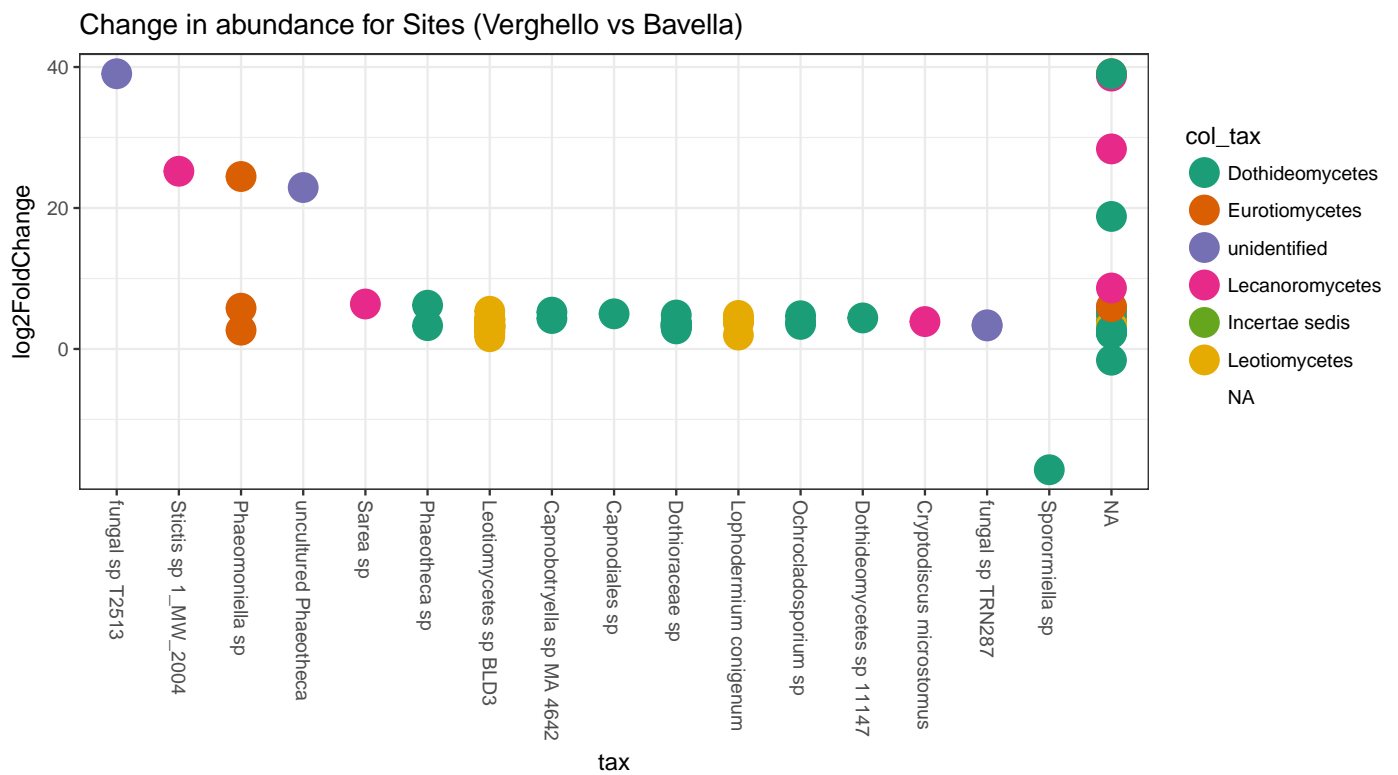
Figure 6.12: OTUs significantly different in terms of abundances between Verghello (positive values) and Asco (negative values)

6.7 Differences in abundances for each OTUs

6.7.1 Pairwise comparison of the OTUs composition by sites

```
library("DESeq2")
packageVersion("DESeq2")

## [1] '1.16.1'

data.f3_deseq2 <- phyloseq_to_deseq2(data.f3, ~ Sites)
data.f3_deseq2 <- DESeq(data.f3_deseq2, test = "Wald", fitType = "parametric")
res.f3_deseq2 <- results(data.f3_deseq2)
```

```
res_VA <- plot_deseq2_phyloseq(data.f3_deseq2, tax_table = data.f3@tax_table,
                               contrast = c("Sites", "Verghello", "Asco"),
                               taxa = "Species", color_tax = "Class")
res_VA
```

```
res_VB <- plot_deseq2_phyloseq(data.f3_deseq2, tax_table = data.f3@tax_table,
                               contrast = c("Sites", "Verghello", "Bavella"),
                               taxa = "Species", color_tax = "Class")
res_VB
```

Figure 6.13: OTUs significantly different in terms of abundances between Verghello (positive values) and Bavella (negative values)
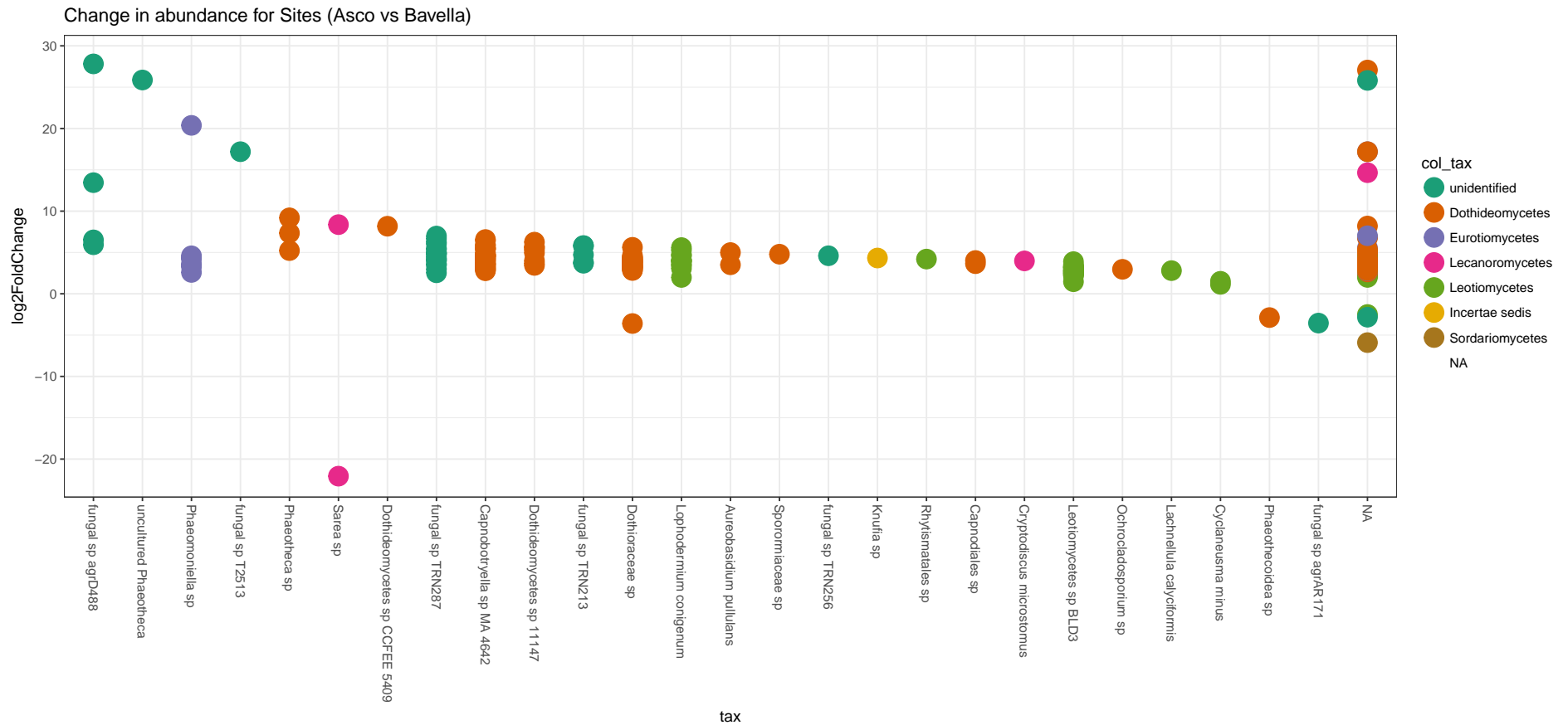
Figure 6.14: OTUs significantly different in terms of abundances between Asco (positive values) and Bavella (negative values)

```
res_AB <- plot_deseq2_phyloseq(data.f3_deseq2, tax_table = data.f3@tax_table,
                               contrast = c("Sites", "Asco", "Bavella"),
                               taxa = "Species", color_tax = "Class")
res_AB
```

6.7.2 Pairwise comparison of Order composition by sites

```
res_VA_o <- plot_deseq2_phyloseq(data.f3, contrast = c("Sites", "Verghello", "Asco"),
                                 taxDepth = "Order", color_tax = "Class")
res_VB_o <- plot_deseq2_phyloseq(data.f3, contrast = c("Sites", "Verghello", "Bavella"),
                                 taxDepth = "Order", color_tax = "Class")
res_AB_o <- plot_deseq2_phyloseq(data.f3, contrast = c("Sites", "Asco", "Bavella"),
                                 taxDepth = "Order", color_tax = "Class")
```

6.8 Distribution of OTUs abundance in the fungal phylogeny

```
library("cluster")
library("phytools")

## Loading required package:  maps
##
## Attaching package:  'maps'
## The following object is masked from 'package:plyr':
##
##     ozone
## The following object is masked from 'package:cluster':
##
##     votes.repub

data.f3_interm <- data.f3
data.f3_interm@otu_table <- otu_table(apply(data.f3@otu_table, 2, function(x) tapply(x, as.factor(da
data.f3_interm@tax_table <- tax_table(apply(data.f3@tax_table, 2, function(x) tapply(x, as.factor(da
data.f3_interm@refseq <- NULL

data.f3_interm <- subset_taxa(data.f3_interm, !grepl("uncultured", data.f3_interm@tax_table[,"Specie
data.f3_interm <- subset_taxa(data.f3_interm, !grepl("sp$", data.f3_interm@tax_table[,"Species"]))
data.f3_interm <- subset_taxa(data.f3_interm, !grepl("unidentified", data.f3_interm@tax_table[,"Fami
data.f3_interm <- subset_taxa(data.f3_interm, !grepl("unidentified", data.f3_interm@tax_table[,"Orde
data.f3_interm <- subset_taxa(data.f3_interm, !grepl("unidentified", data.f3_interm@tax_table[,"Clas
data.f3_interm <- subset_taxa(data.f3_interm, !grepl("Myxotrichaceae", data.f3_interm@tax_table[,"Sp
data.f3_interm <- subset_taxa(data.f3_interm, rowSums(data.f3_interm@otu_table)>100)


tree_tax_interm <- as.data.frame(unclass(data.f3_interm@tax_table))
tree_tax_interm$OTUs <- rownames(tree_tax_interm)

tree_tax_interm <- as.data.frame(replace(as.matrix(tree_tax_interm), which(is.na(tree_tax_interm)),

data.f3_interm@tax_table <- tax_table(as.matrix(tree_tax_interm))

tree_tax_interm$pathString <- paste("Fungi",
                            tree_tax_interm$Phylum,
                            tree_tax_interm$Class,
                            tree_tax_interm$Order,
```

```
                            tree_tax_interm$Family,
                            tree_tax_interm$Genus,
                            tree_tax_interm$OTUs,
                            sep = "/")

write(ToNewick(as.Node(tree_tax_interm, na.rm = TRUE)), file="tree.txt")
tree <- phytools::read.newick(file="tree.txt")
tree <- ape::collapse.singles(tree)

data.f3_interm@phy_tree <- tree
taxa_names(data.f3_interm@phy_tree) <- gsub("_", " ", taxa_names(data.f3_interm@phy_tree))
taxa_names(data.f3_interm@otu_table) <- gsub("_", " ", taxa_names(data.f3_interm@otu_table))
taxa_names(data.f3_interm@tax_table) <- gsub("_", " ", taxa_names(data.f3_interm@tax_table))
taxa_names(data.f3_interm@phy_tree) <- gsub(",", "", taxa_names(data.f3_interm@phy_tree))
taxa_names(data.f3_interm@otu_table) <- gsub(",", "", taxa_names(data.f3_interm@otu_table))
taxa_names(data.f3_interm@tax_table) <- gsub(",", "", taxa_names(data.f3_interm@tax_table))

ptree <- plot_tree(data.f3_interm, color = "Class", shape = "Sites", ladderize = "left", justify = '

cond <- gsub(",", "", rownames(data.f3_interm@otu_table)[rowSums(data.f3_interm@otu_table) >= 1])
df_cond <- as.data.frame(ptree$data)[ptree$data$OTU %in% cond,]
df_cond$Species <- data.f3_interm@tax_table[taxa_names(data.f3_interm) %in% cond,"OTUs"]


cond_Deseq <- levels(df$Species)
df_cond_Deseq <- as.data.frame(ptree$data)[ptree$data$OTU %in% cond_Deseq,]
df_cond_Deseq$Species <- data.f3_interm@tax_table[gsub("_", " ", taxa_names(data.f3_interm)) %in% co

ptree + geom_text(data = df_cond, aes(x = 215, y = y, label = OTU), hjust = "left") + scale_shape_ma

sum(!is.na(match(gsub("_", " ", data.f3@tax_table[,"Species"]), gsub("_", " ", tree$tip.label))))

## [1] 1066

sum(rowSums(data.f3@otu_table)[gsub("_", " ", data.f3@tax_table[,"Species"]) %in% gsub("_", " ", tre

## [1] 36.11315
```
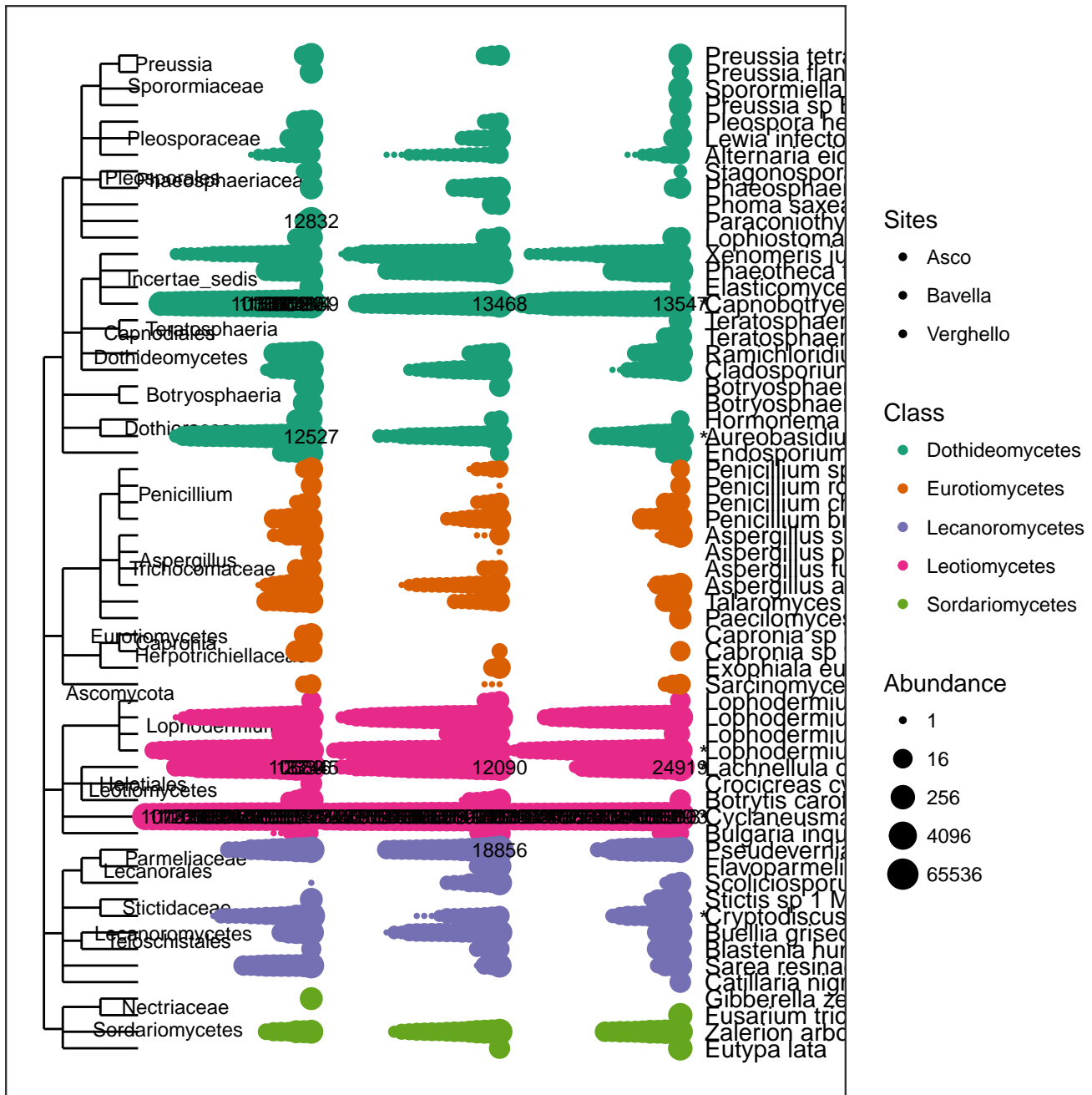
```
ptree + geom_text(data = df_cond, aes(x = 585, y = y, label = OTU), hjust = "left") + scale_shape_ma
ggsave("phylo_map.pdf",  width = 20, height = 15)
```

7 Summary

7.1 Filtering summary

The raw data are made of 8.419809×10^6 sequences representing 15479 OTUs allocated to 80 samples.

After filtering, the dataset includes 8.382948×10^6 sequences representing 6064 OTUs allocated to 72 samples.

7.2 Alpha diversity

Host age and elevation within tree do not impact any aspect of fungal local diversity. Despite similar OTUs richness, Asco is a site more diverse than Verghello and Bavella.

7.3 Beta diversity

Site ($R^2 = 0.102$), age ($R^2 = 0.036$) and interaction age*site ($R^2 = 0.082$) statistically structured the fungal endophytic beta-diversity.

	Comparison	OTU_names	Species	Class	log2FoldChange (negative = m...
1	Verghello vs Asco	2129fb2ad89bb9ecd9cc6bb0cda13444b821f154_81752	Capnobotryella sp MA 4642	Dothideomycetes	-2.93114820365422
2	Verghello vs Asco	5ab69a49181f1fa7edeb755d9379914bd37e476b_67	Stictis sp 1_MW_2004	Lecanoromycetes	22.3685008769311
3	Verghello vs Asco	95aba3eb0bb4faae823c18bc459a2bf6c8a87457_1614			-5.84097733807965
4	Verghello vs Asco	4f72dab9d420ac7a5e9a4e6c542ed94ffd61c95b_1141		Dothideomycetes	-2.58677613505865
5	Verghello vs Asco	85ed55d0ae94db1f366bb5f6e5ae40787d4a4f17_1		Dothideomycetes	-4.25857698788655
6	Verghello vs Asco	0b2b99a97f9103f9ba9cdfb6e60568bb150a577f_3	Capnobotryella sp MA 4642	Dothideomycetes	-2.87423257789545
7	Verghello vs Asco	8dfc55e0659d2a2eecc08d015f9650b399407f32_476		Dothideomycetes	-4.48215425478388
8	Verghello vs Asco	fbbd84ea1fd0cfee335230b0d21453462bfa3ab7d_55	Phaeomoniella sp	Eurotiomycetes	-3.30593414737481
9	Verghello vs Asco	1ea239897ec572eb01eaaf1b4a2fd74a5bb1ddcf_132		Dothideomycetes	-3.82031913457165
10	Verghello vs Asco	5d1644228ec3b5d52480c261aaffd01437311e75_120			-2.62150219323512
11	Verghello vs Asco	14bbcfaef0ec60345e805b41b12c8eaf4dc63027_3	fungal sp TRN287	unidentified	-3.45698293143302
12	Verghello vs Asco	90f76bb914ca347249c71a259a3b08c0c1ade366_350		Dothideomycetes	-3.9637947670544
13	Verghello vs Asco	ef43680f3d2b235c0211f05991b798d7dc781793_67	Capnobotryella sp MA 4642	Dothideomycetes	-2.7159309102354
14	Verghello vs Asco	13bcb1e7e6715b0b196acc58d4bead0a5ca9f3ca_4	fungal sp TRN256	unidentified	-5.17884399120278
15	Verghello vs Asco	6db67c487123e197f5d0c040168a7b442f4c6df1_91		Dothideomycetes	-3.12356301062611
16	Verghello vs Asco	3234c07e4d0375036a68bac59a46a07831bfc8db_12631		Dothideomycetes	-5.4060113758744
17	Verghello vs Asco	c29fce62a47af00efa6caf6272cff91ddd9e1025_3		Dothideomycetes	-8.27092379569942
18	Verghello vs Asco	03206ca39779c903b8d12013d31df11c5c1686df_1		Dothideomycetes	-2.93312234199654
19	Verghello vs Asco	7d364ad429d4be24e692f6847bd4e27931260139_591		Leotiomycetes	-2.03892751418503
20	Verghello vs Asco	381bedacc1f1791bf0e34583cac7ed332f1172ae_7102	Dothioraceae sp	Dothideomycetes	4.014939530706
21	Verghello vs Asco	621b7a08c54cef0ad6074f201abd82958d4f24ca_1	Capnobotryella sp MA 4642	Dothideomycetes	-3.2601286252703
22	Verghello vs Asco	4022c06f1a6e238a46d4fbb544d9c22ef53645d3_97		Dothideomycetes	-3.63069804913526
23	Verghello vs Asco	466dc0940aa9251ae68eb93c4cbcf1ab566b3c93_87	Capnobotryella sp MA 4642	Dothideomycetes	-3.81935199163165
24	Verghello vs Asco	51c20c6414a0bf55d51ad79ebee0f35b0c546f2b_1076	Rhytismatales sp	Leotiomycetes	-6.95889638970096
25	Verghello vs Asco	c5756692597cfc490e3cafe28728c64bdf8b270d_93	fungal sp TRN287	unidentified	-4.17465810320262
26	Verghello vs Asco	befc0eee9082cdb4f1f9cb5ef73d48cd66aed4c1_6919	fungal sp agrD488	unidentified	-27.1201718921273
27	Verghello vs Asco	b439655309822c91bd7c1fde4b9dca85c0c90444_4134	Rhytismatales sp	Leotiomycetes	-4.48542041655923
28	Verghello vs Asco	b6d9befbf378e96475bfe0b8fefdc2886e27c80f_9234	fungal sp agrD488	unidentified	-9.7849603590504
29	Verghello vs Asco	ebf284d7e6c56983ff36773b7a7972bec7cbac7b_31	fungal sp TRN287	unidentified	-4.13942183448567
30	Verghello vs Asco	2144541a5ecd7cb520ca68a6912a5fc4b4eb086d_115			-5.45200967866985
31	Verghello vs Asco	46916e5039bffebd1989535cb6eac49505e5e705_5			-3.36681406736109
32	Verghello vs Asco	116e7af14dface1be67ea1f800245b925846cc94_46			-2.99250784454208
33	Verghello vs Asco	40623c2877f958f10d0d4aa385bc5bfb4e7c5f7_1	Capnobotryella sp MA 4642	Dothideomycetes	-4.01239711127149
34	Verghello vs Asco	4d958cc9c807dc047087f2865d62398d5d545d49_1	Cyclaneusma minus	Leotiomycetes	-1.80474289792475
35	Verghello vs Asco	d7fc3882fe8ba96cb73bf59d3c4ee6e05e96b827_80		unidentified	-4.21782974931364
36	Verghello vs Asco	9cb34be49dd721930e6eec6f12c4f3371152daa8_4896			-28.0181191651132
37	Verghello vs Asco	4c0206c663267f9aa5d260caef9cb46ad0b6f06d_36		Dothideomycetes	-2.80070904694851
38	Verghello vs Asco	dc07788c070e0f4f57d8233bc419ab6ee441a007_91		Dothideomycetes	-3.14596409444666
39	Verghello vs Asco	99d208ffccb844b7ba22cd50554feae679bf3d21_132			-6.78688333280114
40	Verghello vs Asco	9a84deda5a9f9b6aec75e2462948151248b01cca_2333	Sporormiella sp	Dothideomycetes	-25.0042260696885
41	Verghello vs Asco	172baa366762ff89576646619adcbc719a865e16_5	Sarea sp	Lecanoromycetes	22.5395100864668
42	Verghello vs Asco	1a03c65fd2b4316c6385edbf31db362659ceb7b_30694	Sporormiaceae sp	Dothideomycetes	-6.19162005871566
43	Verghello vs Asco	a94921efa4a8f861af50a9bea9010a3cafdde656_9		unidentified	-25.0526484192102
44	Verghello vs Asco	4b7944e582835381c446a61dff2a12f3ca0fa57f_771			-8.20648260004555
45	Verghello vs Asco	b1028640f0591f1d2d869eb43929e018902d030_150	Sporormiaceae sp	Dothideomycetes	-5.70653083752731
46	Verghello vs Asco	1fec2b70134ab6a98c237dd7b1493aacd6c93024_198			-24.0959815798234
47	Verghello vs Asco	202a8ed543d9bb639a346b26c6529d72f72ea83f_3000			-26.3191580209506
48	Verghello vs Asco	0be386c31e50dbb19f1283396b8f35d6b8887d9a_728			21.8459176117285
49	Verghello vs Asco	4688c6bb5f112d43356a680e5a20f7c2b4cb9923_11		Eurotiomycetes	21.8569083821111
50	Verghello vs Asco	e52b5868f08f9b10b7929de0be0e47346d788f30_312		Lecanoromycetes	24.0607760960432
51	Verghello vs Asco	1dd0ba94ebbb323d30a6979e8c5d33f947293e97b_879		Lecanoromycetes	24.6301227542991
52	Verghello vs Asco	f5f08802062cfa41d651f0b204d214acbb1b4301_12		Dothideomycetes	21.8569083821111
53	Verghello vs Asco	c77c28a3b4952ab9870072e73fc27baee9ee10fc_12			21.8569083821111
54	Verghello vs Asco	e126aa1418a1e41179d5d045973a9115f19c95b6_12	fungal sp T2513	unidentified	21.8569083821111
55	Verghello vs Bavella	4dd30fcedfbab3c28d9baf94a363b945e33f3b58_52236	Phaeomoniella sp	Eurotiomycetes	5.78672813762305
56	Verghello vs Bavella	4d15dd7db92d1f1d8ac80e6177ee0e15c17f97c4_1		Leotiomycetes	4.01332409347105
57	Verghello vs Bavella	a959b6e4abca32ab54e6419a12f6bdd45a7926dc_14125	fungal sp TRN287	unidentified	3.42949079800536
58	Verghello vs Bavella	b15583cc2881bbe34420ea9770606960d1632f6e_1	Capnobotryella sp MA 4642	Dothideomycetes	4.31883338220664
59	Verghello vs Bavella	234faa41c01668951fad40aab26275d2e6bb9ea3_792		Dothideomycetes	2.68278753635605
60	Verghello vs Bavella	099296f239ebbd73b6dc0053259a1c1bb2d76196_18178	Cryptodiscus microstomus	Lecanoromycetes	3.88600637060885
61	Verghello vs Bavella	dbb30889e95069d373cb657d40c0a65c0a399670_1		Dothideomycetes	4.26388017017067
62	Verghello vs Bavella	5608683099b4de4c36138df70c7ea522653429d6_530	Leotiomycetes sp BLD3	Leotiomycetes	3.43302618060627
63	Verghello vs Bavella	25ec544e83cad160b3751c905cab409a66d68425_55573		Incertae sedis	5.93108047853807
64	Verghello vs Bavella	7cac233e0e644664066853e91c903d44a0557e9e_29		Dothideomycetes	3.66541148817684
65	Verghello vs Bavella	bc8a21206e4a1b909f6790ae1a19cd45eca1990b_1	Capnobotryella sp MA 4642	Dothideomycetes	5.22495694473858
66	Verghello vs Bavella	13738155c6dcd25e7f4685b1e2d08d7320d434135_792	Dothioraceae sp	Dothideomycetes	3.62082434135792
67	Verghello vs Bavella	b40a7185954b06f920b2dcda283f6ceddf081815_1		Leotiomycetes	3.95549418036625
68	Verghello vs Bavella	1102cc9da608900e057c8410fb413f25ea96b997_1		Leotiomycetes	4.48497662689627
69	Verghello vs Bavella	a96597ad7bfc2fbada9ae439cc7d22266ad645dd_20703	Phaeotheca sp	Dothideomycetes	6.2091329851208
70	Verghello vs Bavella	42122073ae9cc03ee0d6dfc5c02337fb36c11281_40406			5.77093401015807
71	Verghello vs Bavella	856b266be4f4b8e68c9e3766feb889d6b6fdd9ae_4		Dothideomycetes	2.64259975081644
72	Verghello vs Bavella	eb69d15623e27732be2a049452453941082d3_146924		Leotiomycetes	3.10557412028489
73	Verghello vs Bavella	19e9f538c69ae04314cd541eb4ad3be5243725a3_9358	Capnodiales sp	Dothideomycetes	4.98614177753062
74	Verghello vs Bavella	5ab69a49181f1fa7edeb755d9379914bd37e476b_67	Stictis sp 1_MW_2004	Lecanoromycetes	25.2128942693194
75	Verghello vs Bavella	d5b42a733721b90b0e9e89050643a004e85d790a_12664	Leotiomycetes sp BLD3	Leotiomycetes	1.73997788992562
76	Verghello vs Bavella	eba06395bfd92916a1c78548a8d961bfc3b1043e_366	fungal sp TRN287	unidentified	3.26950052589055
77	Verghello vs Bavella	63c2a2c8647ff56829e56b7e20e84cad1acf3be2_26997		Eurotiomycetes	4.43323775979294
78	Verghello vs Bavella	69638056c08d5fa5a610fde3f7f388c9cb032975_36	Ochrocladosporium sp	Dothideomycetes	4.71256822280818
79	Verghello vs Bavella	4fa6fb77b76eda0b865a01d11ad86c6f38cdf97d_22	Lophodermium conigenum	Leotiomycetes	4.76069433149405
80	Verghello vs Bavella	f3792f5540e5316a15f7e569d12ae304f7780015_6102			3.46029790137771
81	Verghello vs Bavella	964184d85d581575f8a6cf719e92b466fa6855eb_66			5.56504794959214
82	Verghello vs Bavella	95eea59c8f1e062a86caf86b96de7e86247216f6_78835			4.09288211668666
83	Verghello vs Bavella	e3b16eabc478dd436c9f47bb7c27074b1ae6dc3f_10696		Dothideomycetes	-1.59398019887299
84	Verghello vs Bavella	e234ed0fcf2707d707d65c9d253b6031edbdcb3f_596	Ochrocladosporium sp	Dothideomycetes	3.84996914288871
85	Verghello vs Bavella	4c6f9ac4fdc4d461f5881019b629ef9e7111c26a_41			4.04298222600048
86	Verghello vs Bavella	d96b42e41a11b76e61a7da9e5d5b950d95088afb_748		Leotiomycetes	2.64152393767329
87	Verghello vs Bavella	c937116b0ba8c96272c82250c7401c23e3027a67_1		Dothideomycetes	3.09518077437343
88	Verghello vs Bavella	13ade7543082ddf80b87bcf07ee2b68a7942cfb7_942	Lophodermium conigenum	Leotiomycetes	1.97101710800663
89	Verghello vs Bavella	18b880d8ef8e75bc9226aa7019d31409ad858f79_1		Dothideomycetes	3.10862874563196
90	Verghello vs Bavella	d524f6695383c80cfc18937c1bd318fbdf28d719_168		Dothideomycetes	4.11663613083704
91	Verghello vs Bavella	9cb8390ee4533dc541bc593fa443effa036501fa_1	Leotiomycetes sp BLD3	Leotiomycetes	3.49728913576814
92	Verghello vs Bavella	eed2f73eda1c60d6d7188d7a237f48eb01af06a5_1			24.6306260836598
93	Verghello vs Bavella	9d49521660fa6eb0c11c5226e1d842693da332d6_6044			8.52932323867265
94	Verghello vs Bavella	5784dee0b4e2fcaaa6b46414797230723 1cc22e6_1	Leotiomycetes sp BLD3	Leotiomycetes	3.36282441931893
95	Verghello vs Bavella	300fe1f394e0556d97b5877aab6125908d6065ed_1	Dothioraceae sp	Dothideomycetes	3.28545721365983
96	Verghello vs Bavella	f2404b634f814b0e01170c84e1256da9c051a946_165		Leotiomycetes	3.08047242959055
97	Verghello vs Bavella	5d1644228ec3b5d52480c261aaffd01437311e75_120			4.26604433939836
98	Verghello vs Bavella	37878801c5eddab03c0eb619b0e2af82841551ba_1		Dothideomycetes	2.90316282094164
99	Verghello vs Bavella	98aae36a95000090b0e50955da68ec9bfc20a326_423	Leotiomycetes sp BLD3	Leotiomycetes	3.15422999323021
100	Verghello vs Bavella	27231e0e49b69c74b5ff95c2ff79bcdb83758661_331	Leotiomycetes sp BLD3	Leotiomycetes	3.0126444882346
101	Verghello vs Bavella	0a0a52b1eeba44141a974273789b654a42033c94_1			3.32041444693928
102	Verghello vs Bavella	85e755847d3435620c3b906b582418f7c829b765_234	Phaeomoniella sp	Eurotiomycetes	2.66606625028166
103	Verghello vs Bavella	4b803d39fa1e2082339eb0aafbf62f71db711552_307		Dothideomycetes	2.34789728414598
104	Verghello vs Bavella	1c312f5e901cfd7e11a329ef2ad4bf1a273e60b3_1		Leotiomycetes	3.47077541485874
105	Verghello vs Bavella	1c5321e2dc523f878d2696fae7c4e8b27c30ee2_2318	Leotiomycetes sp BLD3	Leotiomycetes	2.55422273719836
106	Verghello vs Bavella	6a22a2d035be9bdb18801ee57db8286e2e4ddf8f_260	Dothioraceae sp	Dothideomycetes	3.24788520147175
107	Verghello vs Bavella	b0c7ddb855124715553? b4288c84b235d5d3d7d_1			4.396394572237?

	Comparison	Order	Class	log2FoldChange (negative = more on second levels)
1	Verghello vs Asco	Xylariales	Sordariomycetes	4.94170312130346
2	Verghello vs Bavella	Capnodiales	Dothideomycetes	-0.83630320304439
3	Verghello vs Bavella	Incertae sedis	Leotiomycetes	-1.24689914032906
4	Verghello vs Bavella	Ostropales	Lecanoromycetes	3.8455899279882
5	Verghello vs Bavella	unidentified	unidentified	1.55993729587718
6	Asco vs Bavella	Botryosphaeriales	Dothideomycetes	7.35244451656061
7	Asco vs Bavella	Eurotiales	Eurotiomycetes	1.84728065403879
8	Asco vs Bavella	Incertae sedis	Leotiomycetes	-1.66769964844934
9	Asco vs Bavella	unidentified	unidentified	1.48006941015658
10	Asco vs Bavella	Xylariales	Sordariomycetes	-4.86052036037445

Table 14: Order showing differential abundances in the different sites.

	Nb.of.OTUs	Nb.of.samples	Nb.of.sequences
No filter	15479	80	8419809.00
Nb of sequences by sample >= 20000	15391	72	8397636.00
Nb of sample by OTUs >= 1	15391	72	8397636.00
Nb of sequences by OTUs >= 5	6064	72	8382948.00

Table 15: Number of OTUs, samples and sequences after filtering

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