Shifts in root-associated fungal communities under drought conditions in Ricinus communis L.

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31 - 05 - 2022

Qiime2 Scripts

Step 1: EXTRACT BARCODES

For this step, It will be used the 'extract_barcodes.py' script used in qiime1.

```
#I'll use one library called "Ste1" with Ste1_1.fastq and Ste2_1.fastq

extract_barcodes.py -f Ste1_1.fastq -r Ste1_2.fastq -c barcode_paired_end \
--bc1_len 8 --bc2_len 8 -o extract_barcode_ste1
```

-f : forward reads

-r : reverse reads

-c: input type [default: barcode_single_end]

-bc1_len and –bc2_len : Specify the length, in base pairs, of barcodes

-o : output

Step 2: IMPORT TO QIIME AND DEMULTIPLEX SEQUENCES

For this step, we need to create a directory with the three files output from the previous step, containing:

1. forward.fastq.gz: file that contains the forward sequence reads
2. reverse.fastq.gz: file that contains the reverse sequence reads
3. barcdes.fastq.gz: file that contains the barcode sequence reads

```
qiime tools import \
  --type EMPPairedEndSequences \
  --input-path extract_barcode_ste1 \
  --output-path ste1.qza
```

–type : type of file , in this case paired end sequences. Check other import types[1].

–input-path: directory with the files to import

–output-path: artifact name output

And then, we perform the demultiplexing:

```
qiime demux emp-paired --i-seqs ste1.qza \
--m-barcodes-file ../18S/STE1.txt \
--m-barcodes-column BarcodeSequence \
--output-dir demux_STE1\
--p-no-golay-error-correction
```

[1] https://docs.qiime2.org/2021.4/tutorials/importing/

–i-seqs : artifact with the import paired end sequences

–m-barcodes-file : mapping file containing information of the sequences

–m-barcodes-column: column name of the Barcode sequences

–output-dir : output directory with the demultiplexed samples and error correction details

–p-no-golay-error-correction: by default perform a correction with a barcode of 12 nt if not use this option (in our case is 16 nt)

Step 3: REMOVE PRIMERS AND VISUALIZATION

```
qiime cutadapt trim-paired \
--i-demultiplexed-sequences demux_STE1/per_sample_sequences.qza \
--p-cores 4 --p-front-f TTAGCATGGAATAATRRAATAGGA \
--p-front-r TCTGGACCTGGTGAGTTTCC  \
--o-trimmed-sequences demux_STE1_trimmed.qza
```

–i-demultiplexed-sequences : demultiplexed sequences (.qza artifact)

–p-cores : number of threads

–p-front-f : forward primer sequences (front if is in the beginning of the sequences)

–p-front-r : reverse primer sequences (front if is in the beginning of the sequences)

–o-trimmed-sequences : output

```
qiime demux summarize \
--i-data  demux_STE1_trimmed.qza
--o-visualization demux_STE1_trimmed.qzv
```

–i-data : demultiplexed and/or trimmed sequences

–o-visualization : output

In this case, due to de the low quality of reverse reads we will continue with the forward sequences and let's set the truncation length of 240 bp.

Step 4: RUN DADA2

In this step, we will perform as an example a loop that can be used in the previous steps and the next ones:

```
for i in demux_STE1_trimmed.qza demux_STE2_trimmed.qza demux_STE3.qza \
demux_STE4.qzA demux_STE5_trimmed.qza;
do qiime dada2 denoise-single \
--i-demultiplexed-seqs $i -\
-p-trunc-len 240 \
--output-dir dada_single_240_$i; done
```

–i-demultiplexed-seqs : demultiplexed and trimmed sequences

-p-trunc-len 240 : length to trunc sequences to obtain good quality (usually when sequencing drops)

–output-dir : output directory that will contain feature-table and representative sequences

Step 5: MERGING TABLES AND SEQUENCES

First, merge tables and seqs:

```
qiime feature-table merge \
--i-tables dada_single_240_demux_STE1_trimmed/table.qza \
--i-tables dada_single_240_demux_STE2_trimmed/table.qza \
--i-tables dada_single_240_demux_STE3_trimmed/table.qza \
--i-tables dada_single_240_demux_STE4_trimmed/table.qza \
--i-tables dada_single_240_demux_STE5_trimmed/table.qza \
--o-merged-table merge_table_240.qza
```

–i-tables : table to merge (put every time you want to add a different table)

–o-merged-table : output/merge table

```
qiime feature-table merge-seqs \
--i-data dada_single_240_demux_STE1_trimmed/representative_sequences.qza \
--i-data dada_single_240_demux_STE2_trimmed/representative_sequences.qza \
--i-data dada_single_240_demux_STE3_trimmed/representative_sequences.qza \
--i-data dada_single_240_demux_STE4_trimmed/representative_sequences.qza \
--i-data dada_single_240_demux_STE5_trimmed/representative_sequences.qza \
--o-merged-data merge_seqs_dada_240.qza
```

–i-data : sequences to merge (put every time you want to add a different sequence)

–o-merged-data : output/merge sequences

Then, let's visualize them:

```
qiime feature-table summarize \
--i-table merge_table_240.qza\
--m-sample-metadata-file MAPPINGS/FINALMAP18S
--o-visualization merge_table_240.qzv \
```

–i-table : merged table

–m-sample-metadata-file : mapping file containing all libraries

–o-visualization : output/ visualization artifact

```
qiime metadata tabulate  \
--m-input-file merge_seqs_dada_240.qza \
--o-visualization merge_seqs_dada_240.qzv\
```

–m-input-file : merged sequences

–o-visualization : output/ visualization artifact

Step 6: ASSIGN TAXONOMY

```
qiime feature-classifier classify-consensus-blast \
--i-query merge_seqs_dada_240.qza \
--i-reference-taxonomy /home/steph/Descargas/silva-138-99-tax.qza \
--i-reference-reads /home/steph/Descargas/silva-138-99-seqs.qza \
--o-classification taxonomy_blast_240_0.97.qza --p-perc-identity 0.97
```

classify-consensus-blast : using blast (other options are vsearch and sklearn)

–i-query : seqs merged

–i-reference-taxonomy : artifact imported of taxonomy silva reference database

–i-reference-reads : artifact imported of reads silva reference database

–o-classification output artifact with taxonomy

–p-perc-identity : percent of identity

Step 7: FILTERING AND GROUPING TABLE

- **Removing taxa of Plants**

  I checked the feature table and the division Phragmoplastophyta is all assigned to plants

```
 qiime taxa filter-table \
 --i-table merge_table_240.qza \
 --i-taxonomy taxonomy_blast_240_0.97.qza \
 --p-exclude Phragmoplastophyta \
 --o-filtered-table merge_table_240_noplant.qza
```

–i-table : merge table

–i-taxonomy : taxonomy (from assign taxonomy)

–p-exclude : taxa to exclude

–o-filtered-table : output/artifact

- **Filtering initial treatmens and min frequency**

```
qiime feature-table  filter-samples \
--i-table merge_table_240_noplant.qza \
--m-metadata-file ../../MAPPINGS/FINALMAP_GROUPED.txt \
--p-where "[Treatments]='T0'" --p-exclude-ids --p-min-frequency 520\
--o-filtered-table merge_table_240_noplant_filtered.qza
```

–i-table : input table

–m-metadata-file : mapping file

–p-where (“[Treatments]=‘T0’ ”) : sql code to indicate what column and condition to filter

–p-exclude-ids : to indicate that we will exclude base on the conditions (if not it will retain the data from the condition)

–p-min-frequency : min frequency to retain (sampling depth)

–o-filtered-table : output/table filtered

- **Filtering Uncultivated samples**

```
qiime feature-table  filter-samples \
--i-table merge_table_240_noplant_filtered.qza \
--m-metadata-file ../../MAPPINGS/FINALMAP_GROUPED.txt \
--p-where "[Type_of_soil]='Uncultivated'" --p-exclude-ids \
--o-filtered-table merge_table_240_noplant_filtered_nous.qza
```

–i-table : input table

–m-metadata-file : mapping file

–p-where (“[Type_of_soil]=‘Uncultivated’ ”) : sql code to indicate what column and condition to filter

–p-exclude-ids : to indicate that we will exclude base on the conditions (if not it will retain the data from the condition)

–o-filtered-table : output/table filtered

- **Grouping table (joining replicates and filtering)**

grouped_table_240.qza

```
qiime feature-table group \
--i-table merge_table_240.qza\
--m-metadata-file ../../../MAPPINGS/FINALMAP18s.tsv \
--m-metadata-column group \
--p-mode sum --p-axis sample\
--o-grouped-table grouped_table_240.qza
```

–i-table : input table

–m-metadata-file: metadata file

–m-metadata-column : column name from the metadata

–p-mode : mode of joining samples (in this case, sum the counts, other choices median, mean)

–p-axis : Along which axis to group (it can be features or sample)

–o-grouped-table : output/table

Now, let's filter this grouped table (as we did before, see parameters in the previous steps):

```
 qiime taxa filter-table \
 --i-table mgrouped_table_240.qza  \
 --i-taxonomy taxonomy_blast_240_0.97.qza \
 --p-exclude Phragmoplastophyta \
 --o-filtered-table grouped_table_240_noplant.qza
```

```
qiime feature-table  filter-samples \
--i-table grouped_table_240_noplant.qza \
--m-metadata-file ../../MAPPINGS/FINALMAP_GROUPED.txt \
--p-where "[Treatments]='T0'" --p-exclude-ids --p-min-frequency 1500 \
--o-filtered-table grouped_table_240_filt_noplant.qza
```

```
qiime  feature-table filter-samples \
--i-table grouped_240_fil_noplant.qza \
--p-where "[Type_of_soil]='Uncultivated'" \
--m-metadata-file ../../MAPPINGS/FINALMAP_GROUPED.txt \
--p-exclude-ids \
--o-filtered-table grouped_240_fil_noplant_nous.qza
```

Step 8: FILTERING SEQUENCES

For this step we will filter the representative sequences base on the table filtered.

```
qiime  feature-table filter-seqs \
--i-data seqs_and_taxonomy/merge_seqs_dada_240.qza \
--i-table merge_table_240_noplant_filtered_nous.qza  \
--o-filtered-data seqs_and_taxonomy/merge_seqs_dada_240_noplant_filtered_nous.qza

qiime  feature-table filter-seqs \
--i-data seqs_and_taxonomy/merge_seqs_dada_240.qza \
--i-table grouped_240_fil_noplant_nous.qza \
--o-filtered-data seqs_and_taxonomy/grouped_seqs_dada_240_noplant_filtered_nous.qza
```

–i-data : input sequences

–i-table : input table use to filter

–o-filtered-data : output/filtered sequences

Step 9: BUILDING THE TREE

For this step we will build the phylogenetic tree *denovo*.

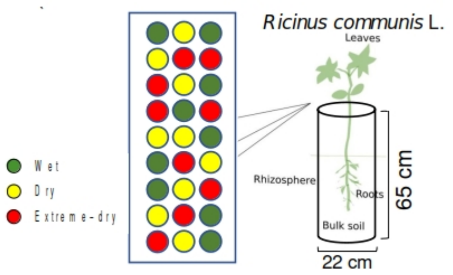
```
qiime phylogeny align-to-tree-mafft-fasttree  \
--i-sequences merge_seqs_dada_240_noplant_filtered_nous.qza  \
--output-dir tree_merge

qiime phylogeny align-to-tree-mafft-fasttree  \
--i-sequences grouped_seqs_dada_240_noplant_filtered_nous.qza  \
--output-dir tree_grouped
```

–i-sequences : sequences filtered

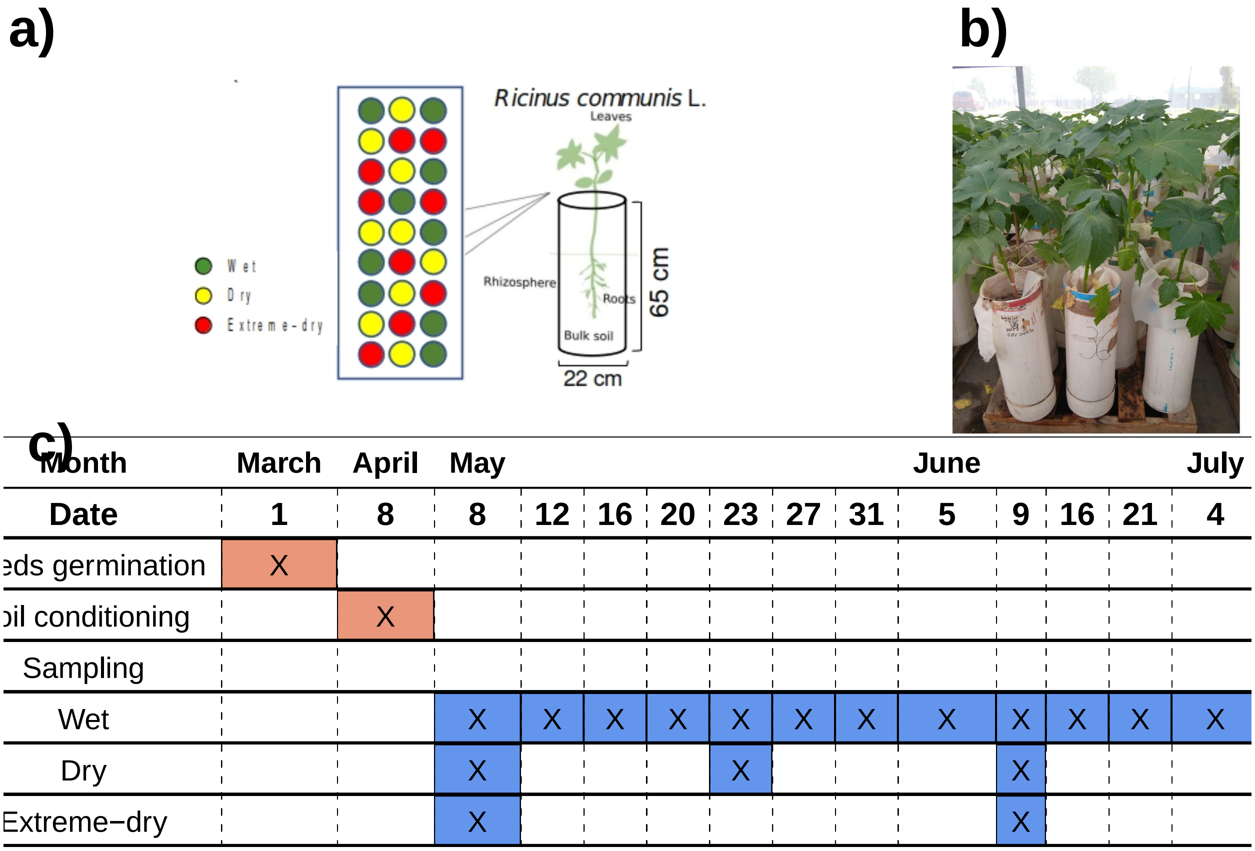
–output-dir : output director that will contain the alignment, masked alignment, the tree and the rooted treed.

Plot of the experimental design

```
#load libraries and files
library(imager)
library(cowplot)
library(ggpubr)
library(tidyverse)
library(magrittr)
library(ggpubr)
library(readxl)
library(viridis)

ricinus<-load.image(file = "../Figures/ricinus3.jpg")
exper<-load.image(file = "../Figures/exper.jpg")

photo_panel <- ggdraw() + draw_image(ricinus, scale = 1)
photo_panel2 <- ggdraw() + draw_image(exper)

plot_grid(photo_panel, photo_panel2)
```



```
table_exper<- read_excel("../Data/experiment.xls")

table_exper[is.na(table_exper)] <-""
names_cols<-c("Month", "March" ,"April" ,"May"  , "",  "" , "",  "",  ""  ,
              "" ,"June" , "", "", "", "July"  ,"")
colnames(table_exper)<-names_cols

table_experiment<-table_exper %>% ggtexttable(rows = NULL, theme = ttheme("blank"))%>%
tab_add_vline(at.column = 1:16, column.side = "left", from.row = 2, linetype = 2)%>%
tab_add_hline(at.row = 2:8, row.side = "bottom", linewidth = 3, linetype = 1) %>%
tbody_add_border() %>%
  thead_add_border()%>%
  table_cell_bg(row =6 , column = 4:15, fill="cornflowerblue")%>%
  table_cell_bg(row =7 , column = 4, fill="cornflowerblue")%>%
  table_cell_bg(row =8 , column = 4, fill="cornflowerblue")%>%
  table_cell_bg(row =7 , column = 8, fill="cornflowerblue")%>%
  table_cell_bg(row =7 , column = 12, fill="cornflowerblue")%>%
  table_cell_bg(row =8 , column = 12, fill="cornflowerblue")%>%
  table_cell_bg(row =3 , column = 2, fill="darksalmon")%>%
  table_cell_bg(row =4 , column = 3, fill="darksalmon")%>%
  table_cell_bg(row =5 , column = 16, fill="darksalmon")%>%
  table_cell_font(row = 2, column = 1:tab_ncol(.), face = "bold")%>%
  tab_add_footnote(
    text = "*cells in blue = Watering, cells in red = Experiment key points",
    size = 10, face = "italic")


p1<- plot_grid(photo_panel, photo_panel2, labels = c("a)", "b)"), label_size = 20, rel_widths = c(3,1),
p2<- plot_grid(p1, table_experiment, labels = c(NULL, "c)"), nrow =  2, label_size = 20, rel_heights =
p2
```

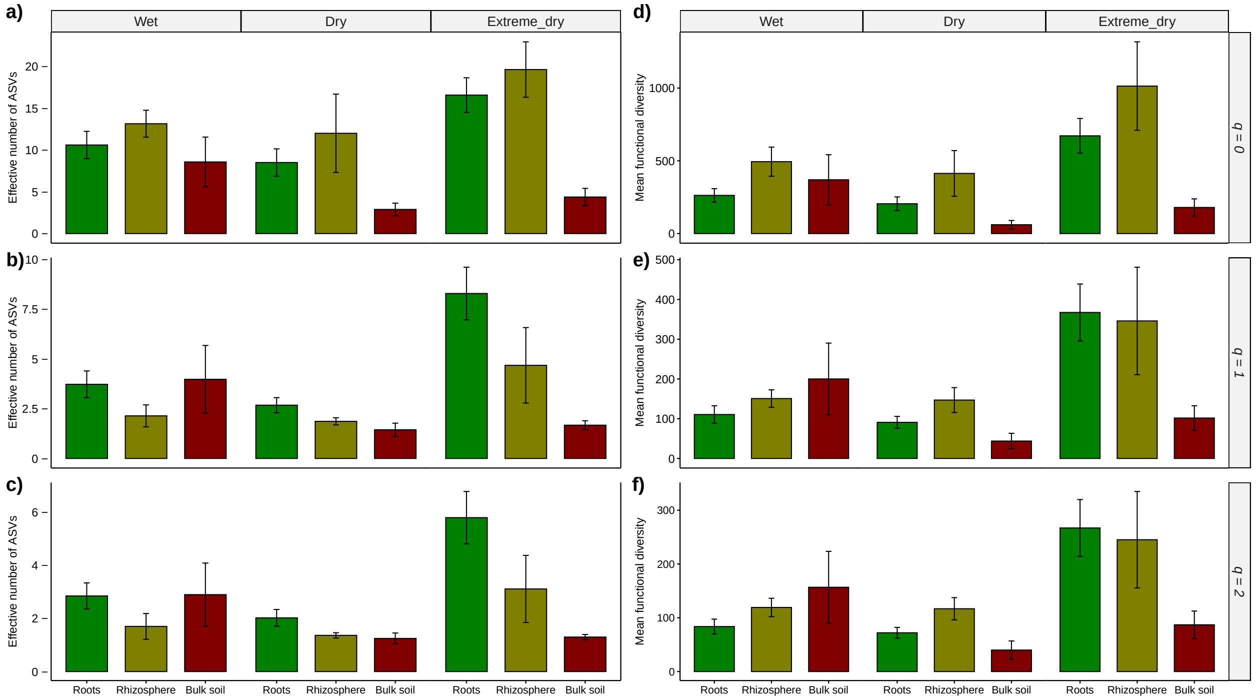


Month	March	April	May							June				July
Date	1	8	8	12	16	20	23	27	31	5	9	16	21	4
eds germination	X													
oil conditioning		X												
Sampling														
Wet			X	X	X	X	X	X	X	X	X	X	X	X
Dry			X				X				X			
Extreme−dry			X								X			

```
#ggsave('../../Figures_final/FigS1s.tiff', width = 7.5, height = 5, dpi = 300, plot = p2)
```

```
#loading libraries
library(hillR)
library(tidyverse)
library(qiime2R)
library(dplyr)
library(cowplot)
library(RColorBrewer)
library(gtable)
library(ggpubr)
```

```
#setwd("/home/steph/Documents/Documentos/fastas nuevos/18S/R_project")
MD_q<- read.delim("../Data/diversity.txt")%>%mutate(
                              qs= case_when(
                                str_detect(q, "q0") ~ "q = 0",
                                str_detect(q, "q1") ~ "q = 1",
                                str_detect(q, "q2") ~ "q = 2"))
MD_q0<- read.delim("../Data/MD_q0.txt")%>%mutate(
                              qs= case_when(
                                str_detect(q, "q0") ~ "q = 0",
                                str_detect(q, "q1") ~ "q = 1",
                                str_detect(q, "q2") ~ "q = 2"))
MD_q1<- read.delim("../Data/MD_q1.txt")%>%mutate(
                              qs= case_when(
                                str_detect(q, "q0") ~ "q = 0",
                                str_detect(q, "q1") ~ "q = 1",
                                str_detect(q, "q2") ~ "q = 2"))
MD_q2<- read.delim("../Data/MD_q2.txt")%>%mutate(
                              qs= case_when(
                                str_detect(q, "q0") ~ "q = 0",
                                str_detect(q, "q1") ~ "q = 1",
                                str_detect(q, "q2") ~ "q = 2"))



MD_q$Treatment <- factor(MD_q$Treatment,levels = c("Wet", "Dry", "Extreme_dry"))
MD_q0$Treatment <- factor(MD_q0$Treatment,levels = c("Wet", "Dry", "Extreme_dry"))
MD_q1$Treatment <- factor(MD_q1$Treatment,levels = c("Wet", "Dry", "Extreme_dry"))
MD_q2$Treatment <- factor(MD_q2$Treatment,levels = c("Wet", "Dry", "Extreme_dry"))


  a0<-MD_q  %>% filter(q=="q0" )%>% ggbarplot(., x = "Type", y = "value",
      facet.by = c( "Treatment"),fill = "Type_of_soil", add = "mean_se")+
  scale_y_continuous(labels = function(x){paste(x, "-")},
                     sec.axis = dup_axis(breaks = 0))+
    scale_fill_manual(
      values = c("#800000", "#808000", "#008000", "#D35400", "#2E4053"))+
    theme(legend.position = "none", axis.title.x = element_blank())+
    ylab("Effective number of ASVs")+
    theme( panel.border = element_blank(),
       panel.spacing.x = unit(0,"line"),
       axis.line= element_line(colour = "black"),
       strip.text.y = element_text(size=14, face="italic"),
       strip.text.x = element_text(size=14),
       axis.title = element_text(size = 12),
       axis.text.x = element_blank(),
       axis.ticks.x = element_blank(),
       panel.background=element_blank(),
       axis.title.y.right = element_blank(),
       axis.text.y.right = element_blank(),
       axis.ticks.y = element_blank(),
       axis.text.y = element_text(margin = margin(r = 0)),
       axis.text.y.left = element_text(colour = "black", size = 12))

  a1<-MD_q  %>% filter(q=="q1") %>% ggbarplot(., x = "Type", y = "value",
    facet.by = c("Treatment"),fill = "Type_of_soil", add = "mean_se")+
    scale_fill_manual(
      values = c("#800000", "#808000", "#008000", "#D35400", "#2E4053"))+
     scale_y_continuous(labels = function(x){paste(x, "-")},
                     sec.axis = dup_axis(breaks = 0))+
    theme(legend.position = "none",
          axis.title.x = element_blank())+
    ylab("Effective number of ASVs")+
    theme( panel.border = element_blank(),
       panel.spacing.x = unit(0,"line"),
       axis.line= element_line(colour = "black"),
        strip.text.y = element_text(size=14, face="italic"),
       strip.text.x = element_blank(),
       axis.title = element_text(size = 12),
        axis.text.x = element_blank(),
       axis.ticks.x = element_blank(),
       panel.background=element_blank(),
       axis.title.y.right = element_blank(),
       axis.text.y.right = element_blank(),
       axis.ticks.y = element_blank(),
       axis.text.y = element_text(margin = margin(r = 0)),
       axis.text.y.left = element_text(colour = "black", size = 12))

  a2<-MD_q  %>% filter(q=="q2" )%>% ggbarplot(., x = "Type", y = "value",
    facet.by = c("Treatment"),fill = "Type_of_soil", add = "mean_se")+
    scale_fill_manual(
      values = c("#800000", "#808000", "#008000", "#D35400", "#2E4053"))+
     scale_y_continuous(labels = function(x){paste(x, "-")},
                     sec.axis = dup_axis(breaks = 0))+
    theme(legend.position = "none",
          axis.title.x = element_blank())+
    ylab("Effective number of ASVs")+
    theme( panel.border = element_blank(),
       panel.spacing.x = unit(0,"line"),
       axis.line= element_line(colour = "black"),
        strip.text.y = element_text(size=14, face="italic"),
       strip.text.x = element_blank(),
       axis.title = element_text(size = 12),
       panel.background=element_blank(),
       axis.title.y.right = element_blank(),
       axis.text.y.right = element_blank(),
       axis.ticks.y = element_blank(),
        axis.text = element_text(size = 11),
       axis.text.y = element_text(margin = margin(r = 0)),
       axis.text.y.left = element_text(colour = "black", size = 12))


library(cowplot)
  tres<-plot_grid(a0, a1, a2, nrow = 3, labels = c("a)", "b)", "c)"), label_y = 1.03,
                   label_size = 20, hjust = -0.2, align = "v", rel_heights = c(1.1,1,1))
  MD_q$Treatment <- factor(MD_q$Treatment,levels = c("Wet", "Dry", "Extreme_dry"))

  b0<-MD_q0 %>% ggbarplot(., x = "Type", y = "MD_q", facet.by = c(
    "qs", "Treatment"),fill = "Type_of_soil", add = "mean_se")  +
    scale_fill_manual(
      values = c("#800000", "#808000", "#008000", "#D35400", "#2E4053"))+
    theme(
        legend.position = "none", axis.title.x = element_blank())+
    ylab("Mean functional diversity")+
    theme( panel.border = element_blank(),
       panel.spacing.x = unit(0,"line"),
       axis.line= element_line(colour = "black"),
        strip.text.y = element_text(size=14, face="italic"),
       strip.text.x = element_text(size=14),
       axis.title = element_text(size = 12),
       axis.ticks.x =element_blank(),
       axis.text.x = element_blank())

    b1<-MD_q1 %>% ggbarplot(., x = "Type", y = "MD_q", facet.by = c(
    "qs", "Treatment"),fill = "Type_of_soil", add = "mean_se")  +
      scale_fill_manual(
      values = c("#800000", "#808000", "#008000", "#D35400", "#2E4053"))+
      theme(
        legend.position = "none", axis.title.x = element_blank())+
      ylab("Mean functional diversity")+
      theme( panel.border = element_blank(),
     panel.spacing.x = unit(0,"line"),
     axis.line= element_line(colour = "black"),
     strip.text.y = element_text(size=14, face="italic"),
     axis.title = element_text(size = 12),
      axis.ticks.x =element_blank(),
      axis.text.x = element_blank(),
     strip.text.x = element_blank(),
     strip.background.x = element_blank(),
     panel.background = element_blank())

  b2<-MD_q2 %>% ggbarplot(., x = "Type", y = "MD_q", facet.by = c(
    "qs", "Treatment"),fill = "Type_of_soil", add = "mean_se")  +
    scale_fill_manual(
      values = c("#800000", "#808000", "#008000", "#D35400", "#2E4053"))+
    theme(
        legend.position = "none", axis.title.x = element_blank())+
    ylab("Mean functional diversity")+
    theme( panel.border = element_blank(),
 panel.spacing.x = unit(0,"line"),
 axis.line= element_line(colour = "black"),
        strip.text.y = element_text(size=14, face="italic"),
      axis.title = element_text(size = 12),
      strip.text.x = element_blank(),
     strip.background.x = element_blank(),
 axis.text = element_text(size = 11),
     panel.background =element_blank())

tres_b<- plot_grid(b0, b1, b2, nrow = 3, labels = c("d)", "e)", "f)"), label_y = 1.03,
                   label_size = 20, hjust = -0.2, align = "v", rel_heights = c(1.1,1,1))
all<-  plot_grid(tres, tres_b, ncol = 2)

all
```



```
#ggsave('/home/steph/Documents/Documentos/fastas nuevos/18S/R_project/Figures_final//Fig1.pdf',
#width = 16, height = 9, dpi = 300, plot = all)
```

Lmer models with interaction effects -Taxonomic alpha diversity

```
library(lme4)
```

```
## Loading required package: Matrix

##
## Attaching package: 'Matrix'

## The following objects are masked from 'package:tidyr':
##
##     expand, pack, unpack
```

```
library(nlme)
```

```
##
## Attaching package: 'nlme'

## The following object is masked from 'package:lme4':
##
##     lmList

## The following object is masked from 'package:dplyr':
##
##     collapse
```

```
library(cowplot)
library(pgirmess)
```

```
## Registered S3 method overwritten by 'spdep':
##   method   from
##   plot.mst ape
```

```
library(emmeans)

alpha<-read.delim("../Data/alpha_all_filt.tsv") %>% dplyr::select(sampleid, q, value)
alpha<- alpha %>% spread(q, value)
metadata<- read.delim("../Data/FINALMAP18S_plant.csv", check.names = F)
alpha<- alpha %>% inner_join(metadata,by = c("sampleid"="#SampleID"))


alpha<- alpha %>% mutate(Treatments =case_when(
  Treatment =="1" ~ "Wet",
  Treatment =="2" ~ "Dry",
  Treatment =="3" ~ "Extreme-dry"))

#q0
q0_lme<-lme(q0~ Type_of_soil+Treatments+Type_of_soil*Treatments, random=~1 |Plant, data = alpha)
q0_lme_perm<-PermTest(q0_lme)
q0_lme_perm
```

```
##
## Monte-Carlo test
##
## Call:
## PermTest.lme(obj = q0_lme)
##
## Based on 1000 replicates
## Simulated p-value:
##                          p.value
## (Intercept)                0.999
## Type_of_soil               0.020
## Treatments                 0.178
## Type_of_soil:Treatments    0.084
```

```
#q1
q1_lme<-lme(q1~ Type_of_soil+Treatments+Type_of_soil*Treatments, random=~1 |Plant, data = alpha)
q1_lme_perm<-PermTest(q1_lme)
q1_lme_perm
```

```
##
## Monte-Carlo test
##
## Call:
## PermTest.lme(obj = q1_lme)
##
## Based on 1000 replicates
## Simulated p-value:
##                          p.value
## (Intercept)                0.979
## Type_of_soil               0.640
## Treatments                 0.163
## Type_of_soil:Treatments    0.013
```

```
#q2
q2_lme<-lme(q2~ Type_of_soil+Treatments+Type_of_soil*Treatments, random=~1 |Plant, data = alpha)
q2_lme_perm<-PermTest(q2_lme)
q2_lme_perm
```

```
##
## Monte-Carlo test
##
## Call:
## PermTest.lme(obj = q2_lme)
##
## Based on 1000 replicates
## Simulated p-value:
##                          p.value
## (Intercept)                0.947
## Type_of_soil               0.667
## Treatments                 0.188
## Type_of_soil:Treatments    0.014
```

Lmer models with interaction effects -Functional alpha diversity

```
#Functional
MD_q0<- read.delim("../Data/MD_q0.txt")  %>% mutate(cate="q0")
MD_q1<- read.delim("../Data/MD_q1.txt")%>% mutate(cate="q1")
MD_q2<- read.delim("../Data/MD_q2.txt")%>% mutate(cate="q2")

alpha_f<-rbind(MD_q0, MD_q1, MD_q2) %>% dplyr::select(sampleid, cate, MD_q)
alpha_f<- alpha_f %>% spread(cate, MD_q)
alpha_f<- alpha_f %>% inner_join(metadata,by = c("sampleid"="#SampleID")) %>% mutate(
  Treatments =case_when(
  Treatment =="1" ~ "Wet",
  Treatment =="2" ~ "Dry",
  Treatment =="3" ~ "Extreme-dry"))



#q0
q0_lme_f<-lme(q0~ Type_of_soil+Treatment+Type_of_soil*Treatment, random=~1 |Plant, data = alpha)
q0_lme_perm_f<-PermTest(q0_lme_f)
q0_lme_perm_f
```

```
##
## Monte-Carlo test
##
## Call:
## PermTest.lme(obj = q0_lme_f)
##
## Based on 1000 replicates
## Simulated p-value:
##                         p.value
## (Intercept)               0.336
## Type_of_soil              0.529
## Treatment                 0.185
## Type_of_soil:Treatment    0.014
```

```
#q1
q1_lme_f<-lme(q1~ Type_of_soil+Treatment+Type_of_soil*Treatment, random=~1 |Plant, data = alpha)
q1_lme_perm_f<-PermTest(q1_lme_f)
q1_lme_perm_f
```

```
##
```

```
## Monte-Carlo test
##
## Call:
## PermTest.lme(obj = q1_lme_f)
##
## Based on 1000 replicates
## Simulated p-value:
##                          p.value
## (Intercept)                0.150
## Type_of_soil               0.070
## Treatment                  0.159
## Type_of_soil:Treatment     0.003
```

```
#q2
q2_lme_f<-lme(q2~ Type_of_soil+Treatment+Type_of_soil*Treatment, random=~1 |Plant, data = alpha)
q2_lme_perm_f<-PermTest(q2_lme_f)
q2_lme_perm_f
```

```
##
## Monte-Carlo test
##
## Call:
## PermTest.lme(obj = q2_lme_f)
##
## Based on 1000 replicates
## Simulated p-value:
##                          p.value
## (Intercept)                0.157
## Type_of_soil               0.080
## Treatment                  0.134
## Type_of_soil:Treatment     0.005
```

Sorting by letters

```
#Letters were added manually resulting from the next code

alpha<- alpha %>% unite("interact", c("Type_of_soil", "Treatments"), remove = F)

q0_lme<-lme(q0~ interact, random=~1 |Plant, data = alpha)
q0_lme_means<-emmeans(q0_lme, pairwise ~ interact)
multcomp::cld(object = q0_lme_means$emmeans,
              Letters = letters)
```

```
##  interact                     emmean   SE df lower.CL upper.CL .group
##  Non-rizospheric_Dry            2.89 2.10 26   -1.436     7.21  a
##  Non-rizospheric_Extreme-dry    4.21 2.23 26   -0.373     8.78  a
##  Roots_Dry                      8.64 2.22 26    4.067    13.21  ab
##  Non-rizospheric_Wet            8.76 2.37 26    3.876    13.64  abc
##  Roots_Wet                      9.87 2.79 26    4.124    15.61  abc
##  Rizospheric_Dry               11.78 2.55 26    6.532    17.03  abc
##  Rizospheric_Wet               13.03 2.37 26    8.147    17.91  abc
##  Roots_Extreme-dry             16.47 2.37 26   11.594    21.35   bc
##  Rizospheric_Extreme-dry       19.46 2.22 26   14.885    24.03    c
##
## Degrees-of-freedom method: containment
## Confidence level used: 0.95
## P value adjustment: tukey method for comparing a family of 9 estimates
## significance level used: alpha = 0.05
## NOTE: Compact letter displays can be misleading
##       because they show NON-findings rather than findings.
##       Consider using ’pairs()’, ’pwpp()’, or ’pwpm()’ instead.
```

```
q1_lme<-lme(q1~ interact, random=~1 |Plant, data = alpha)
q1_lme_means<-emmeans(q1_lme, pairwise ~ interact)
multcomp::cld(object = q1_lme_means$emmeans,
              Letters = letters)
```

```
##  interact                     emmean    SE df lower.CL upper.CL .group
##  Non-rizospheric_Dry            1.44 0.932 26   -0.473     3.36  a
##  Non-rizospheric_Extreme-dry    1.64 0.988 26   -0.394     3.67  a
##  Rizospheric_Dry                1.83 1.138 26   -0.514     4.16  a
##  Rizospheric_Wet                2.11 1.055 26   -0.061     4.28  a
##  Roots_Dry                      2.67 0.987 26    0.644     4.70  a
##  Roots_Wet                      3.55 1.246 26    0.987     6.11  ab
##  Non-rizospheric_Wet            3.94 1.055 26    1.771     6.11  ab
##  Rizospheric_Extreme-dry        4.67 0.987 26    2.641     6.70  ab
##  Roots_Extreme-dry              8.26 1.055 26    6.093    10.43   b
##
## Degrees-of-freedom method: containment
## Confidence level used: 0.95
## P value adjustment: tukey method for comparing a family of 9 estimates
## significance level used: alpha = 0.05
## NOTE: Compact letter displays can be misleading
##       because they show NON-findings rather than findings.
##       Consider using ’pairs()’, ’pwpp()’, or ’pwpm()’ instead.
```

```
q2_lme<-lme(q2~ interact, random=~1 |Plant, data = alpha)
q2_lme_means<-emmeans(q2_lme, pairwise ~ interact)
multcomp::cld(object = q2_lme_means$emmeans,
              Letters = letters)
```

```
##  interact                     emmean    SE df lower.CL upper.CL .group
##  Non-rizospheric_Dry            1.25 0.650 26  -0.0889     2.58  a
##  Non-rizospheric_Extreme-dry    1.27 0.689 26  -0.1438     2.69  a
##  Rizospheric_Dry                1.33 0.794 26  -0.3004     2.96  a
##  Rizospheric_Wet                1.67 0.736 26   0.1600     3.19  a
##  Roots_Dry                      2.02 0.689 26   0.6022     3.43  a
##  Roots_Wet                      2.71 0.869 26   0.9231     4.50  ab
##  Non-rizospheric_Wet            2.86 0.736 26   1.3468     4.37  ab
##  Rizospheric_Extreme-dry        3.12 0.689 26   1.6993     4.53  ab
##  Roots_Extreme-dry              5.77 0.736 26   4.2603     7.29   b
##
## Degrees-of-freedom method: containment
## Confidence level used: 0.95
## P value adjustment: tukey method for comparing a family of 9 estimates
## significance level used: alpha = 0.05
## NOTE: Compact letter displays can be misleading
##       because they show NON-findings rather than findings.
##       Consider using ’pairs()’, ’pwpp()’, or ’pwpm()’ instead.
```

```
alpha_f<- alpha_f %>% unite("interact", c("Type_of_soil", "Treatments"), remove = F)
```

```
q0_lme_f<-lme(q0~ interact, random=~1 |Plant, data = alpha_f)
q0_lme_means_f<-emmeans(q0_lme_f, pairwise ~ interact)
multcomp::cld(object = q0_lme_means_f$emmeans,
              Letters = letters)
```

```
##  interact                    emmean  SE df lower.CL upper.CL .group
##  Non-rizospheric_Dry           60.9 130 26   -207.0      329  a    
##  Non-rizospheric_Extreme-dry  164.3 138 26   -119.4      448  a    
##  Roots_Dry                    209.0 138 26    -74.4      492  a    
##  Roots_Wet                    218.1 173 26   -137.8      574  a    
##  Non-rizospheric_Wet          374.0 147 26     71.6      676  ab   
##  Rizospheric_Dry              405.2 158 26     80.0      730  ab   
##  Rizospheric_Wet              487.2 147 26    184.8      790  ab   
##  Roots_Extreme-dry            657.7 147 26    355.3      960  ab   
##  Rizospheric_Extreme-dry     1009.1 138 26    725.8     1292   b   
## 
## Degrees-of-freedom method: containment 
## Confidence level used: 0.95 
## P value adjustment: tukey method for comparing a family of 9 estimates 
## significance level used: alpha = 0.05 
## NOTE: Compact letter displays can be misleading
##       because they show NON-findings rather than findings.
##       Consider using ’pairs()’, ’pwpp()’, or ’pwpm()’ instead.
```

```
q1_lme_f<-lme(q1~ interact, random=~1 |Plant, data = alpha_f)
q1_lme_means_f<-emmeans(q1_lme_f, pairwise ~ interact)
multcomp::cld(object = q1_lme_means_f$emmeans,
              Letters = letters)
```

```
##  interact                    emmean   SE df lower.CL upper.CL .group
##  Non-rizospheric_Dry           43.8 58.4 26    -76.3      164  a    
##  Roots_Dry                     91.3 61.8 26    -35.6      218  ab   
##  Roots_Wet                     93.4 77.6 26    -66.1      253  ab   
##  Non-rizospheric_Extreme-dry   96.8 61.9 26    -30.4      224  ab   
##  Rizospheric_Dry              142.8 70.9 26     -2.9      289  ab   
##  Rizospheric_Wet              148.0 65.9 26     12.5      284  ab   
##  Non-rizospheric_Wet          201.2 65.9 26     65.6      337  ab   
##  Rizospheric_Extreme-dry      345.8 61.8 26    218.8      473   b   
##  Roots_Extreme-dry            361.2 65.9 26    225.7      497   b   
## 
## Degrees-of-freedom method: containment 
## Confidence level used: 0.95 
## P value adjustment: tukey method for comparing a family of 9 estimates 
## significance level used: alpha = 0.05 
## NOTE: Compact letter displays can be misleading
##       because they show NON-findings rather than findings.
##       Consider using ’pairs()’, ’pwpp()’, or ’pwpm()’ instead.
```

```
q2_lme_f<-lme(q2~ interact, random=~1 |Plant, data = alpha_f)
q2_lme_means_f<-emmeans(q2_lme_f, pairwise ~ interact)
multcomp::cld(object = q2_lme_means_f$emmeans,
              Letters = letters)
```

```
##  interact                    emmean   SE df lower.CL upper.CL .group
##  Non-rizospheric_Dry           40.0 40.8 26   -43.88      124  a    
##  Roots_Wet                     70.5 54.0 26   -40.57      182  ab
##  Roots_Dry                     72.3 43.1 26   -16.28      161  ab
##  Non-rizospheric_Extreme-dry   83.4 43.2 26    -5.32      172  ab
##  Rizospheric_Dry              113.3 49.4 26    11.74      215  ab
##  Rizospheric_Wet              116.8 46.0 26    22.28      211  ab
##  Non-rizospheric_Wet          158.2 46.0 26    63.65      253  ab
##  Rizospheric_Extreme-dry      245.9 43.1 26   157.24      334   b
##  Roots_Extreme-dry            263.0 46.0 26   168.46      357   b
##
## Degrees-of-freedom method: containment
## Confidence level used: 0.95
## P value adjustment: tukey method for comparing a family of 9 estimates
## significance level used: alpha = 0.05
## NOTE: Compact letter displays can be misleading
##       because they show NON-findings rather than findings.
##       Consider using 'pairs()', 'pwpp()', or 'pwpm()' instead.
```

Loading files

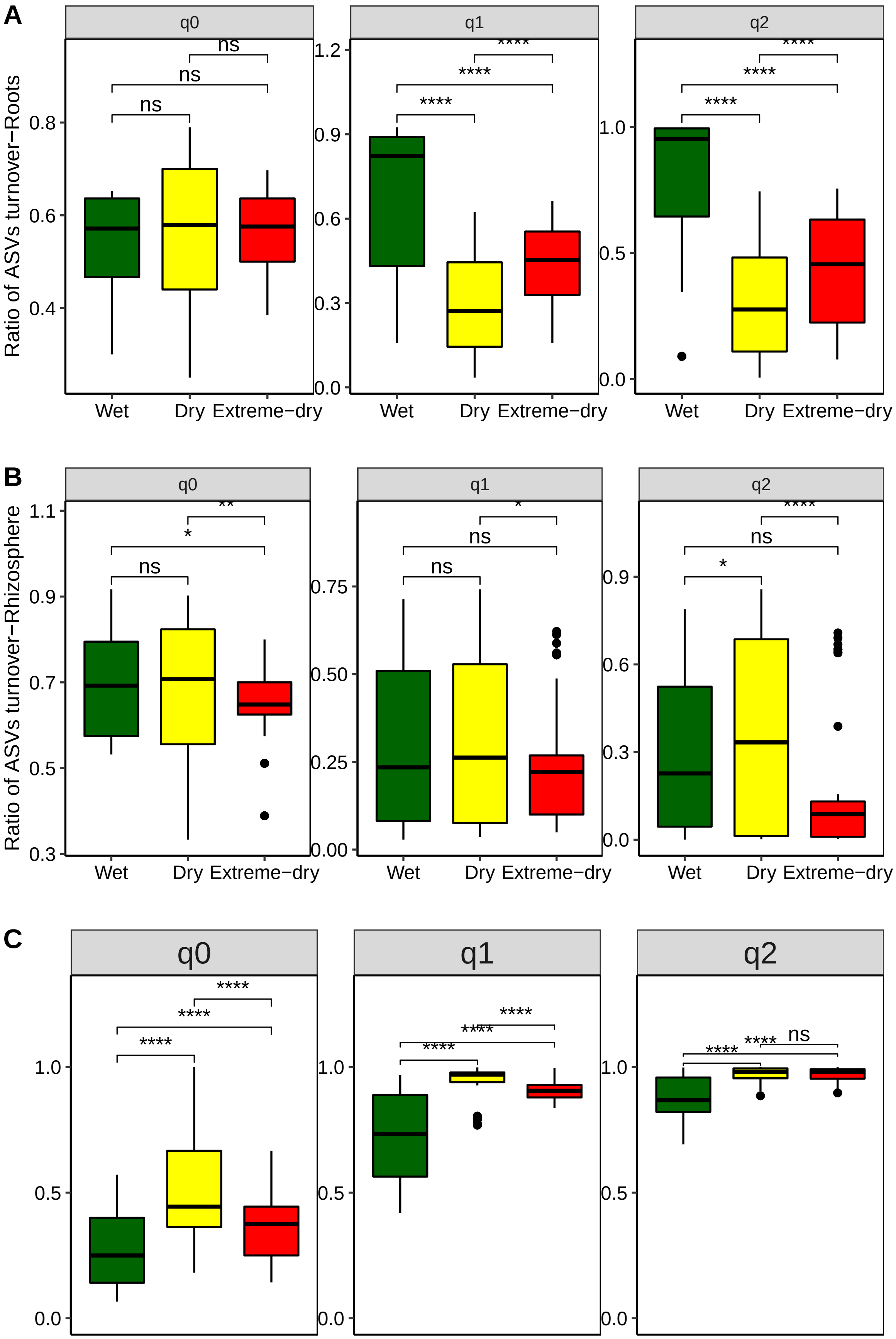
```
library(tidyverse)
intra_ro_wet_q0<- read.csv(
  "../Data/beta/Beta_diversityro/Intra_Beta_Similarity-q=0-table_wet_ro.txt.csv") %>% mutate(
    qs="q0") %>% mutate(type="Wet")
intra_ro_wet_q1<- read.csv(
  "../Data/beta/Beta_diversityro/Intra_Beta_Similarity-q=1-table_wet_ro.txt.csv") %>% mutate(
    qs="q1")%>% mutate(type="Wet")
intra_ro_wet_q2<- read.csv(
  "../Data/beta/Beta_diversityro/Intra_Beta_Similarity-q=2-table_wet_ro.txt.csv") %>% mutate(
    qs="q2")%>% mutate(type="Wet")

intra_ri_wet_q0<- read.csv(
  "../Data/beta/Beta_diversityri/Intra_Beta_Similarity-q=0-table_wet_ri.txt.csv") %>% mutate(
    qs="q0")%>% mutate(type="Wet")
intra_ri_wet_q1<- read.csv(
  "../Data/beta/Beta_diversityri/Intra_Beta_Similarity-q=1-table_wet_ri.txt.csv") %>% mutate(
    qs="q1")%>% mutate(type="Wet")
intra_ri_wet_q2<- read.csv(
  "../Data/beta/Beta_diversityri/Intra_Beta_Similarity-q=2-table_wet_ri.txt.csv") %>% mutate(
    qs="q2")%>% mutate(type="Wet")

intra_nr_wet_q0<- read.csv(
  "../Data/beta/Beta_diversitynr/Intra_Beta_Similarity-q=0-table_wet_nr.txt.csv") %>% mutate(
    qs="q0")%>% mutate(type="Wet")
intra_nr_wet_q1<- read.csv(
  "../Data/beta/Beta_diversitynr/Intra_Beta_Similarity-q=1-table_wet_nr.txt.csv") %>% mutate(
    qs="q1")%>% mutate(type="Wet")
intra_nr_wet_q2<- read.csv(
  "../Data/beta/Beta_diversitynr/Intra_Beta_Similarity-q=2-table_wet_nr.txt.csv") %>% mutate(
    qs="q2")%>% mutate(type="Wet")


intra_ro_dry_q0<- read.csv(
  "../Data/beta/Beta_diversityro/Intra_Beta_Similarity-q=0-table_dry_ro.txt.csv") %>% mutate(
    qs="q0")%>% mutate(type="Dry")
intra_ro_dry_q1<- read.csv(
  "../Data/beta/Beta_diversityro/Intra_Beta_Similarity-q=1-table_dry_ro.txt.csv") %>% mutate(
    qs="q1")%>% mutate(type="Dry")
intra_ro_dry_q2<- read.csv(
  "../Data/beta/Beta_diversityro/Intra_Beta_Similarity-q=2-table_dry_ro.txt.csv") %>% mutate(
    qs="q2")%>% mutate(type="Dry")

intra_ri_dry_q0<- read.csv(
  "../Data/beta/Beta_diversityri/Intra_Beta_Similarity-q=0-table_dry_ri.txt.csv") %>% mutate(
    qs="q0")%>% mutate(type="Dry")
intra_ri_dry_q1<- read.csv(
  "../Data/beta/Beta_diversityri/Intra_Beta_Similarity-q=1-table_dry_ri.txt.csv") %>% mutate(
    qs="q1")%>% mutate(type="Dry")
intra_ri_dry_q2<- read.csv(
  "../Data/beta/Beta_diversityri/Intra_Beta_Similarity-q=2-table_dry_ri.txt.csv") %>% mutate(
    qs="q2")%>% mutate(type="Dry")

intra_nr_dry_q0<- read.csv(
  "../Data/beta/Beta_diversitynr/Intra_Beta_Similarity-q=0-table_dry_nr.txt.csv") %>% mutate(
    qs="q0")%>% mutate(type="Dry")
intra_nr_dry_q1<- read.csv(
  "../Data/beta/Beta_diversitynr/Intra_Beta_Similarity-q=1-table_dry_nr.txt.csv") %>% mutate(
    qs="q1")%>% mutate(type="Dry")
intra_nr_dry_q2<- read.csv(
  "../Data/beta/Beta_diversitynr/Intra_Beta_Similarity-q=2-table_dry_nr.txt.csv") %>% mutate(
    qs="q2")%>% mutate(type="Dry")


intra_ro_exdry_q0<- read.csv(
  "../Data/beta/Beta_diversityro/Intra_Beta_Similarity-q=0-table_exdry_ro.txt.csv") %>% mutate(
    qs="q0")%>% mutate(type="Extreme-dry")
intra_ro_exdry_q1<- read.csv(
  "../Data/beta/Beta_diversityro/Intra_Beta_Similarity-q=1-table_exdry_ro.txt.csv") %>% mutate(
    qs="q1")%>% mutate(type="Extreme-dry")
intra_ro_exdry_q2<- read.csv(
  "../Data/beta/Beta_diversityro/Intra_Beta_Similarity-q=2-table_exdry_ro.txt.csv") %>% mutate(
    qs="q2")%>% mutate(type="Extreme-dry")

intra_ri_exdry_q0<- read.csv(
  "../Data/beta/Beta_diversityri/Intra_Beta_Similarity-q=0-table_exdry_ri.txt.csv") %>% mutate(
    qs="q0")%>% mutate(type="Extreme-dry")
intra_ri_exdry_q1<- read.csv(
  "../Data/beta/Beta_diversityri/Intra_Beta_Similarity-q=1-table_exdry_ri.txt.csv") %>% mutate(
    qs="q1")%>% mutate(type="Extreme-dry")
intra_ri_exdry_q2<- read.csv(
  "../Data/beta/Beta_diversityri/Intra_Beta_Similarity-q=2-table_exdry_ri.txt.csv") %>% mutate(
    qs="q2")%>% mutate(type="Extreme-dry")

intra_nr_exdry_q0<- read.csv(
  "../Data/beta/Beta_diversitynr/Intra_Beta_Similarity-q=0-table_exdry_nr.txt.csv") %>% mutate(
    qs="q0")%>% mutate(type="Extreme-dry")
intra_nr_exdry_q1<- read.csv(
  "../Data/beta/Beta_diversitynr/Intra_Beta_Similarity-q=1-table_exdry_nr.txt.csv") %>% mutate(
    qs="q1")%>% mutate(type="Extreme-dry")
```

```
intra_nr_exdry_q2<- read.csv(
  "../Data/beta/Beta_diversitynr/Intra_Beta_Similarity-q=2-table_exdry_nr.txt.csv") %>% mutate(
    qs="q2")%>% mutate(type="Extreme-dry")
```

Plot

```
library(ggpubr)
my_comparisons <- list( c("Wet", "Dry"), c("Wet", "Extreme-dry"), c("Dry", "Extreme-dry") )


intra_ro<- rbind(intra_ro_wet_q0, intra_ro_dry_q0, intra_ro_exdry_q0,
                 intra_ro_wet_q1, intra_ro_dry_q1, intra_ro_exdry_q1,
                 intra_ro_wet_q2, intra_ro_dry_q2, intra_ro_exdry_q2)

i1a<- intra_ro %>%mutate(TurnOver= 1-TurnoverComp, part="Roots") %>% filter(qs=="q0")%>%  ggpubr::ggbox
  x = "type", y="TurnOver", fill = "type")+
  facet_grid(.~part, scales = "free")+xlab("")+
  ylab("Relative turnover ratio of ASVs")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  #stat_compare_means(comparisons = my_comparisons, label = "p.signif")+
  theme_bw()+
  theme(legend.position = "none",
        axis.ticks.x = element_blank(),
        axis.text.x = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
       text = element_text(size = 14),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.5))+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))

i1d<- intra_ro %>%mutate(TurnOver= 1-TurnoverComp, part="Roots") %>% filter(qs=="q1")%>%  ggpubr::ggbox
  x = "type", y="TurnOver", fill = "type")+
  #facet_grid(.~part, scales = "free")+xlab("")+
  ylab("Relative turnover ratio of ASVs")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons, label = "p.signif")+
  theme_bw()+
  theme(legend.position = "none",
        axis.ticks.x = element_blank(),
        axis.text.x = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
       text = element_text(size = 14),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.3))+theme(
    axis.title.x = element_blank()  )+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))


i1g<- intra_ro %>%mutate(TurnOver= 1-TurnoverComp, part="Roots") %>% filter(qs=="q2")%>%  ggpubr::ggboxp
  x = "type", y="TurnOver", fill = "type")+
 # facet_grid(qs~part, scales = "free")+xlab("")+
  ylab("Relative turnover ratio of ASVs")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons, label = "p.signif")+
  theme_bw()+
  theme(legend.position = "none",
       # axis.ticks.x = element_blank(),
        axis.text.x = element_text(size = 14),
        axis.title.x = element_blank(),
        panel.grid.major = element_blank(),
       text = element_text(size = 14),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.3))+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))


i1<-intra_ro %>%mutate(TurnOver= 1-TurnoverComp) %>%  ggpubr::ggboxplot(
  x = "type", y="TurnOver", fill = "type")+
  facet_wrap( ~qs, scales = "free")+xlab("")+
  ylab("Ratio of ASVs turnover-Roots")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons, label = "p.signif")+
  theme_bw()+
  theme(panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
        axis.text = element_text(colour = "black", size = 10))

intra_ri<- rbind(intra_ri_wet_q0, intra_ri_dry_q0, intra_ri_exdry_q0,
                 intra_ri_wet_q1, intra_ri_dry_q1, intra_ri_exdry_q1,
                 intra_ri_wet_q2, intra_ri_dry_q2, intra_ri_exdry_q2)

i2<-intra_ri %>%mutate(TurnOver= 1-TurnoverComp)%>% ggpubr::ggboxplot(
  x = "type", y="TurnOver", fill = "type")+
  facet_wrap(~qs, scales = "free")+
  xlab("")+ylab("Ratio of ASVs turnover-Rhizosphere")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons, label = "p.signif")+
  theme_bw()+theme(panel.grid.major = element_blank(),
                   panel.grid.minor = element_blank(),
                   panel.background = element_blank(),
                   panel.border = element_rect(color = "black"),
                   panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
        axis.text = element_text(colour = "black", size = 10))

my_comparisons2 <- list(  c("Wet", "Extreme-dry"), c("Dry", "Extreme-dry") )

i2b<- intra_ri %>%mutate(TurnOver= 1-TurnoverComp, part="Rhizosphere") %>% filter(qs=="q0")%>%  ggpubr:
  x = "type", y="TurnOver", fill = "type")+
  facet_grid(.~part, scales = "free")+xlab("")+
  ylab("")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons2, label = "p.signif")+
  theme_bw()+
  theme(legend.position = "none",
        axis.ticks.x = element_blank(),
        axis.text.x = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
       text = element_text(size = 14),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.5))+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))

my_comparisons3 <- list( c("Dry", "Extreme-dry") )
```

```
i2e<- intra_ri %>%mutate(TurnOver= 1-TurnoverComp, part="Rhizosphere") %>% filter(qs=="q1")%>%  ggpubr:
  x = "type", y="TurnOver", fill = "type")+
  #facet_grid(.~part, scales = "free")+xlab("")+
  ylab("")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons3, label = "p.signif")+
  theme_bw()+
  theme(legend.position = "none",
        axis.ticks.x = element_blank(),
        axis.text.x = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
       text = element_text(size = 14),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.3))+theme(
    axis.title.x = element_blank()  )+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))

my_comparisons4 <- list(  c("Wet", "Dry"), c("Dry", "Extreme-dry") )

i2h<- intra_ri %>%mutate(TurnOver= 1-TurnoverComp, part="Rhizosphere") %>% filter(qs=="q2")%>%  ggpubr:
  x = "type", y="TurnOver", fill = "type")+
 # facet_grid(qs~part, scales = "free")+xlab("")+
  ylab("")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons4, label = "p.signif")+
  theme_bw()+
  theme(legend.position = "none",
       # axis.ticks.x = element_blank(),
        axis.text.x = element_text(size = 14),
        axis.title.x = element_blank(),
        panel.grid.major = element_blank(),
       text = element_text(size = 14),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.3))+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))



intra_nr<- rbind(intra_nr_wet_q0, intra_nr_dry_q0, intra_nr_exdry_q0,
                 intra_nr_wet_q1, intra_nr_dry_q1, intra_nr_exdry_q1,
                 intra_nr_wet_q2, intra_nr_dry_q2, intra_nr_exdry_q2)%>% mutate(
          qss=case_when(
            qs=="q0" ~ "q = 0",
            qs=="q1" ~ "q = 1",
            qs=="q2" ~ "q = 2"))

i3<-intra_nr%>%mutate(TurnOver= 1-TurnoverComp) %>% ggpubr::ggboxplot(
  x = "type", y="TurnoverComp", fill = "type")+
  facet_wrap(~qs, scales = "free")+xlab("")+
  ylab("")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons, label = "p.signif")+
  theme_bw()+theme( panel.grid.major = element_blank(),
                    panel.grid.minor = element_blank(),
                    panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
        axis.text = element_text(colour = "black", size = 10)) +
              theme(legend.position = "none",
        axis.ticks.x = element_blank(),
        axis.text.x = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
       text = element_text(size = 14),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.3))+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))

i3c<- intra_nr %>%mutate(TurnOver= 1-TurnoverComp, part="Bulk soil") %>% filter(qs=="q0")%>%  ggpubr::gg
  x = "type", y="TurnOver", fill = "type")+
  facet_grid(qss~part, scales = "free")+xlab("")+
  ylab("")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons, label = "p.signif")+
  theme_bw()+
  theme(legend.position = "none",
        axis.ticks.x = element_blank(),
        axis.text.x = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
       text = element_text(size = 14),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.5))+theme(
    axis.title.x = element_blank()  )+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))

i3f<- intra_nr %>%mutate(TurnOver= 1-TurnoverComp, part="Bulk soil") %>% filter(qs=="q1")%>%  ggpubr::gg
  x = "type", y="TurnOver", fill = "type")+
  facet_grid(qss~., scales = "free")+xlab("")+
  ylab("")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons, label = "p.signif")+
  theme_bw()+
  theme(legend.position = "none",
        axis.ticks.x = element_blank(),
        axis.text.x = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
       text = element_text(size = 14),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.3))+theme(
    axis.title.x = element_blank()  )+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))

my_comparisons5 <- list(  c("Wet", "Dry"), c("Wet", "Extreme-dry") )

i3i<- intra_nr %>%mutate(TurnOver= 1-TurnoverComp, part="Roots") %>% filter(qs=="q2")%>%  ggpubr::ggbox
  x = "type", y="TurnOver", fill = "type")+
  facet_grid(qss~., scales = "free")+xlab("")+
  ylab("")+
  scale_fill_manual(values = c("DarkGreen", "yellow", "red"))+
  stat_compare_means(comparisons = my_comparisons5, label = "p.signif")+
  theme_bw()+
  theme(legend.position = "none",
       # axis.ticks.x = element_blank(),
        axis.text.x = element_text(size = 14),
        axis.title.x = element_blank(),
        panel.grid.major = element_blank(),
       text = element_text(size = 14),
        panel.grid.minor = element_blank(),
        panel.background = element_blank(),
        panel.border = element_rect(color = "black"),
        panel.spacing.x = unit(0,"line"),
        axis.line= element_line(colour = "black"),
        axis.line.y.right = element_line(colour = "black" ),
        axis.text = element_text(colour = "black", size = 10))+
  scale_y_continuous(limits = c(0, 1.3))+theme(
     strip.text.y = element_text(
        size = 16, face = "italic"
        ),
      strip.text.x = element_text(
        size = 16 ))

library(cowplot)

p1<- plot_grid(i1a, i2b, i3c,
               i1d, i2e, i3f,
               i1g, i2h, i3i, rel_heights = c(1,1,1,
                                              1,1,1,
                                              1,1,1),
               labels = c("a)", "b)", "c)", "d)", "e)",
                          "f)", "g)", "h)", "i)"), align = "hv")

p<-plot_grid(
  i1+theme(legend.position = "none"),i2+theme(legend.position = "none"),
  i3+theme(legend.position = "none"), nrow = 3, labels = c("A", "B", "C"))
p
```


A
q0
q1
q2
Ratio of ASVs turnover−Roots
ns
ns
ns
****
****
****
****
****
****
0.4
0.6
0.8
0.0
0.3
0.6
0.9
1.2
0.0
0.5
1.0
Wet
Dry
Extreme−dry
B
Ratio of ASVs turnover−Rhizosphere
**
*
ns
*
ns
ns
****
ns
*
0.3
0.5
0.7
0.9
1.1
0.00
0.25
0.50
0.75
0.0
0.3
0.6
0.9
C
****
****
****
****
****
****
****
****
ns
0.0
0.5
1.0

```
#ggsave(plot=p1, "../../Figures_final/Fig2.intra-turnover-paired-mod.pdf", width = 10, height = 12)
#ggsave(plot=p1, "../../Figures_final/Fig2.tiff", width = 10, height = 12, dpi = 300)
```

```
#loading libraries
library(qiime2R)
library(tidyverse)
library(cowplot)
library(FactoMineR)
library(vegan)
library(ggpubr)
library(zCompositions)
library(compositions)
```

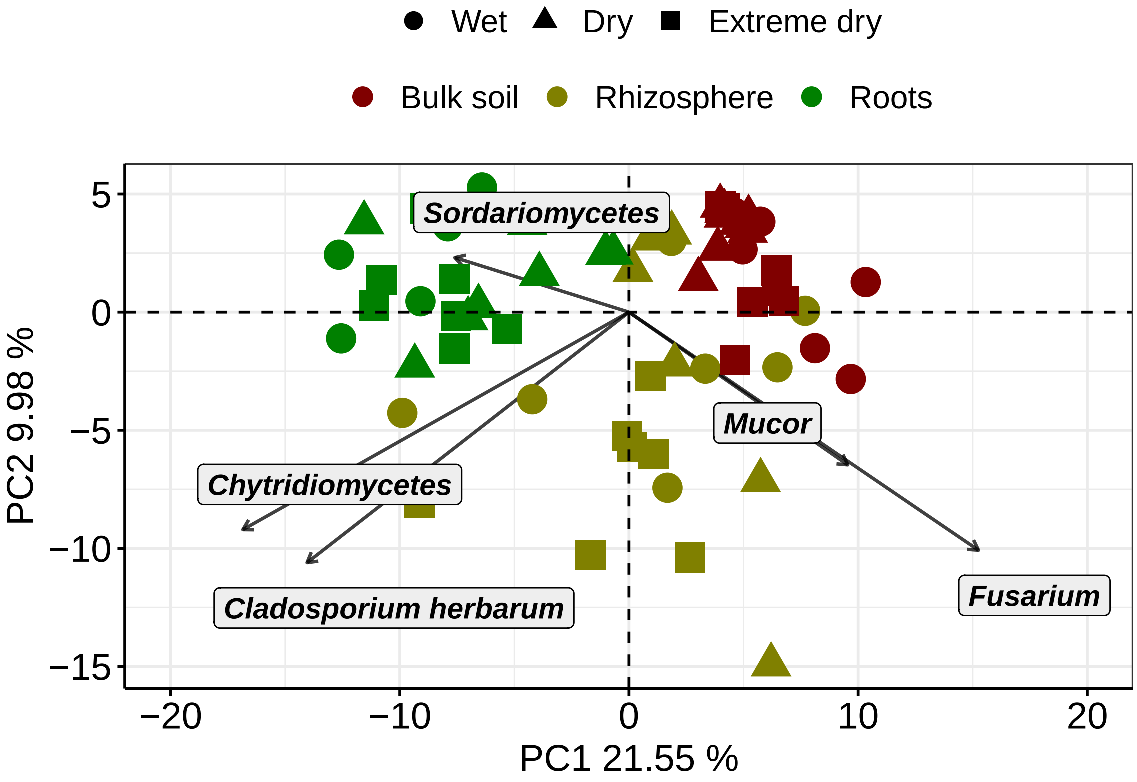
PCA plot all

```
#load file and correct zero values
table_240<- read_qza("../../Data/merge_table_240_noplant_filtered_nous.qza")$data %>%
  as.data.frame()

taxonomy_240<- read_qza("../Data/taxonomy_blast_240_0.97.qza")$data

#remove non-fungi taxa

taxonomy_filter<- taxonomy_240 %>% filter(
  !str_detect(Taxon, "ozoa"))%>% filter(
  !str_detect(Taxon, "helida")) %>% filter(
  !str_detect(Taxon, "ophyta")) %>% filter(
  !str_detect(Taxon, "Ciliophora")) %>% filter(
  !str_detect(Taxon, "Nucleariidae_and_Fonticula_group")) %>% filter(
  !str_detect(Taxon, "Arthrioida")) %>% filter(
  !str_detect(Taxon, "Labyrinthulomycetes"))  %>% filter(
  !str_detect(Taxon, "Apicomplexa")) %>% filter(
  !str_detect(Taxon, "Bicosoecida")) %>% filter(
  !str_detect(Taxon, "Breviatea")) %>% filter(
  !str_detect(Taxon, "Aphelidea"))  %>% filter(
  !str_detect(Taxon, "Arthropoda"))

table_filter<-  table_240[match(
  taxonomy_filter$Feature.ID, rownames(table_240)),] %>% drop_na(.)

d.pro.0<- table_filter %>% dplyr::select_at(vars(!contains("US")))
d.pro <- t(cmultRepl(t(d.pro.0), method="CZM", output="p-counts"))
```

```
## No. corrected values:  335
```

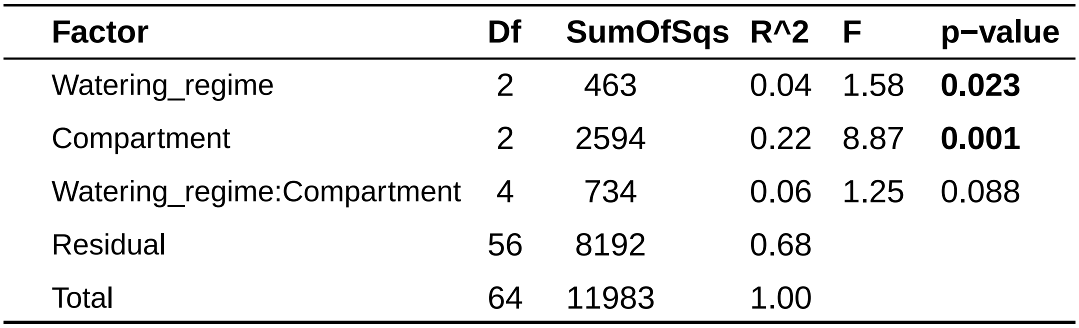
```
# make our compositional and run pca function
d.clr.abund <- t(apply(d.pro, 2, function(x){log(x) - mean(log(x))}))
pcx.abund <- prcomp(d.clr.abund)


#load metadata and taxonomy file
meta<-read_tsv(
  file = "/home/steph/Documents/Documentos/fastas nuevos/18S/MAPPINGS/FINALMAP18S") %>%
  rename(SampleID=`#SampleID`) %>%
  filter(SampleID!="#q2:types")
```

```r
meta$Compartment<- factor(meta$Type_of_soil,
                   levels = c( "Non-rizospheric", "Rizospheric",
                               "Roots", "Uncultivated"),
                   labels = c("Bulk soil", "Rhizosphere",
                              "Roots", "Uncultivated"))
meta$Watering_regime<- factor(meta$Treatment,
                        levels = c( "0", "1", "2", "3"),
                        labels = c("Initial","Wet", "Dry", "Extreme dry"))


tax<-read_qza("../Data/taxonomy_blast_240_0.97.qza")$data %>%
  rename(FeatureID=Feature.ID)

#Construsting PCA

#LABELS
PC1 <- paste("PC1", round(sum(pcx.abund$sdev[1] ^ 2) /
                            mvar(d.clr.abund) * 100, 2), "%")
PC2 <- paste("PC2", round(sum(pcx.abund$sdev[2] ^ 2) /
                            mvar(d.clr.abund) * 100, 2), "%")

#SHAPES AND COLOR OF ARROWS


vars_chosen<- c("588a0e16a32970569d10c88aaa93f050",
                "81979356618c524328e9a9fc41c30906",
                "ab657f069eebbddfa32f5fd2609e4b24",
                "0147e304b1ce1be9c4ff15b660605ef2",
                "1db96e3e66ec1535d586d1a3a954cb66")


vars_choosing<- data.frame(pcx.abund$rotation)%>%  rownames_to_column(
  var = "FeatureID")%>%
  filter(FeatureID %in% vars_chosen) %>%  mutate(a=sqrt(PC1^2+PC2^2)) %>%
  mutate(PC1=PC1*40, PC2=PC2*40) %>% left_join(tax)%>% dplyr::select(
    Taxon, PC1, PC2, FeatureID)%>%mutate(tax= str_extract(Taxon, "[^_]+$")) %>%
  mutate_at(c("tax"), funs(tax = case_when(
    tax=="herbarum" ~ "Cladosporium herbarum",
    TRUE~as.character(tax))))

color_type<- c("#800000", "#808000", "#008000", "#D35400", "#2E4053")

pca<- ggplot() +
  theme_bw()+
  xlab(PC1) +
  ylab(PC2) +
  geom_segment(data = vars_choosing, aes(x = 0, y = 0, xend = PC1, yend = PC2),
               arrow=arrow(length=unit(0.15,"cm")),
               alpha = 0.75, color = 'black', size= 0.6)+
  theme(axis.text = element_text(colour = "black", size = 14),
        axis.title = element_text(colour = "black", size = 14),
         legend.text = element_text(size = 9),
        axis.ticks = element_line(colour = "black"),
        axis.line = element_line(colour = "black"),
        axis.text.y.right = element_text(colour = "black"),
        axis.text.x.top = element_text(colour = "black"),
        legend.position = "top",
        legend.box = "vertical",
        legend.direction = "horizontal",
        legend.spacing.y = unit(0.01, 'cm'),
        legend.title = element_blank()) +
  geom_point(
    data=data.frame(pcx.abund$x) %>%   rownames_to_column(var = "SampleID")%>%
      left_join(meta, by = "SampleID") %>% filter(!Compartment =="NA"),
    aes(x=PC1, y=PC2, color=Compartment, shape=Watering_regime),
     size=5) + geom_vline(xintercept = 0, linetype = 2) +
  geom_hline(yintercept = 0, linetype = 2)+
  scale_color_manual(values = color_type) +
  scale_x_continuous(limits = c(-20,20))+
  ggrepel::geom_label_repel(data = vars_choosing, aes(x=PC1, y=PC2, label= tax),
  segment.colour = NA, col = 'black', fill= "#EEEEEE",
  fontface="bold.italic",  box.padding = 0.6, size=4)+ theme(
  plot.margin = unit(c(0.5, 1.5, 0.4, 0.1), "cm"))+
  guides(colour = guide_legend(override.aes = list(size=3)),
         shape = guide_legend(override.aes = list(size = 3)))+
  theme(legend.text = element_text(size = 12))

pca
```



PERMANOVAs

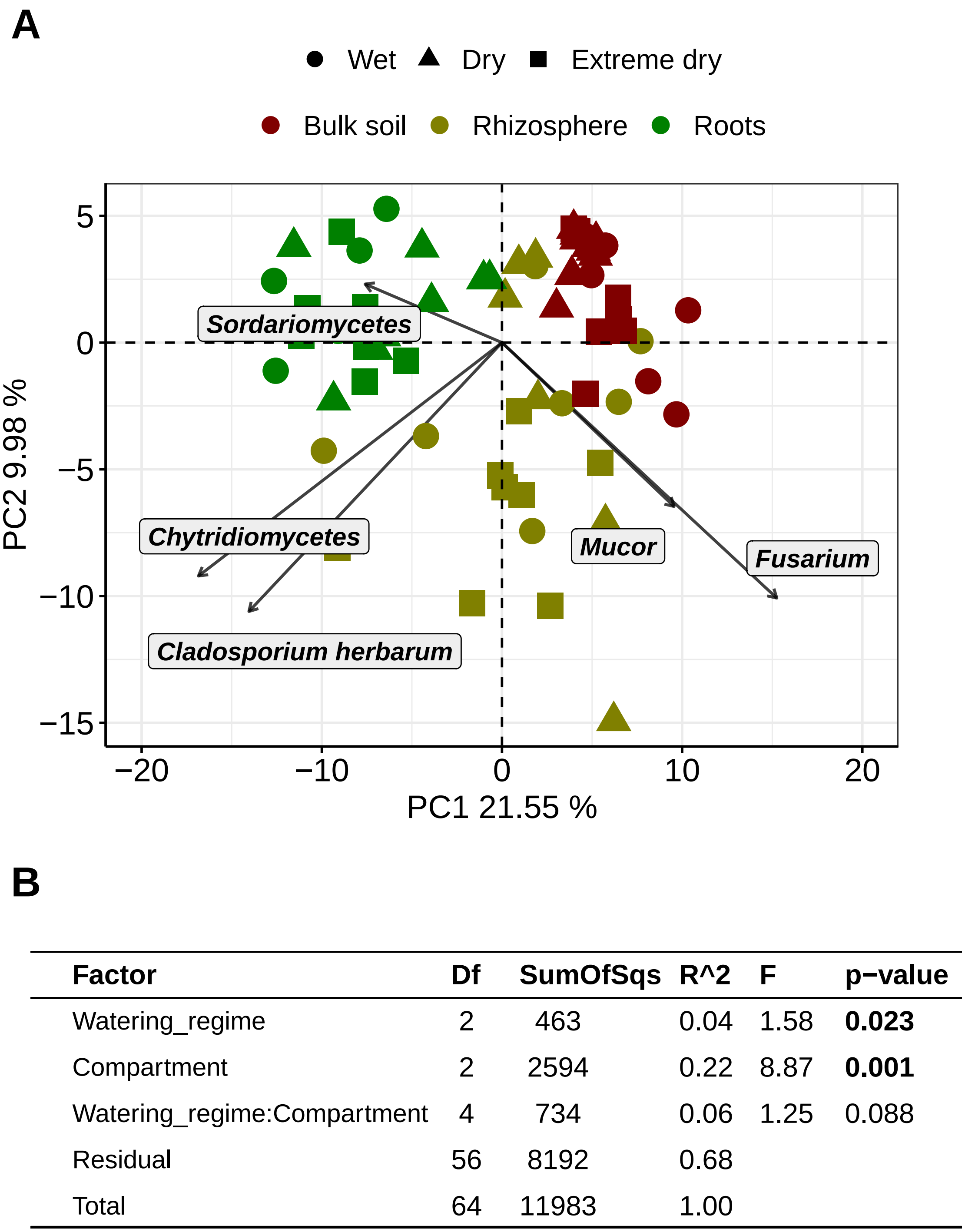
```r
d.clr.abund2<- data.frame(d.clr.abund, check.names = F) %>% rownames_to_column(
  var = "ids") %>% filter(!str_detect(ids, 'US')) %>% column_to_rownames(
    var = "ids")

meta_just<- data.frame(d.clr.abund2, check.names = F) %>% rownames_to_column(
  var = "SampleID") %>% inner_join(meta)


perm<- how(nperm = 999)
setBlocks(perm)<- with(meta_just, Plot)
ad_grouped<-adonis2(d.clr.abund2~Watering_regime*Compartment,
                    data = meta_just, method = "euclidian",
                    permutations =999) %>% round(
                      ., digits = 3) %>%replace(is.na(.), "-")

tbody.style = tbody_style(color = "black",
   fill = c("white"), hjust=0, x=0.1)

Permanova_grouped <-data.frame(ad_grouped, check.names = F) %>%
  rename("R^2" =  "R2", "p-value"="Pr(>F)") %>%
  rownames_to_column( var="Factor") %>%
  mutate_at(3, funs(round(., 0)))  %>%
    mutate_at(5:6, as.numeric) %>%
  mutate_at(4:5, funs(round(., 2))) %>%
  mutate_at(5:6, ~replace(., is.na(.), "")) %>%
    ggtexttable(., rows = NULL,  theme = ttheme(
             colnames.style = colnames_style(color = "black",
             tbody.style = tbody.style)) %>%
  tab_add_hline(at.row = 1:2, row.side = "top", linewidth = 2)%>%
  table_cell_font(., row = 3, column = 6, face = "bold") %>%
  table_cell_font(., row = 2, column = 6, face = "bold") %>%
  tab_add_hline(at.row = c(6), row.side = "bottom",
                linewidth = 3, linetype = 1) %>%
  table_cell_font(., row = 2:6, column = 1,
                     size = 11.3)

#%>%
  #tab_add_footnote(
   # text = "*p values in Bold are significant using \n an alpha value of 0.05",
    #size = 10, face = "italic")

Permanova_grouped
```



Factor	Df	SumOfSqs	R^2	F	p−value
Watering_regime	2	463	0.04	1.58	**0.023**
Compartment	2	2594	0.22	8.87	**0.001**
Watering_regime:Compartment	4	734	0.06	1.25	0.088
Residual	56	8192	0.68		
Total	64	11983	1.00		

```
#ggsave('../../Figures_final/paired_perma.pdf',
 #   width = 6, height = 2, dpi = 300, plot =Pairwsie_permanova_grouped)
#ggsave('../../Figures_final/pca_all.pdf',
 #    width = 6, height = 2, dpi = 300, plot =pca)
leg<- get_legend(pca)
part1<- plot_grid(leg, NULL, ncol = 2)
part2<- plot_grid(part1, Permanova_grouped, nrow = 2, labels = c("", "b)"), vjust = 0, rel_heights = c(
part3<- plot_grid(pca+theme(legend.position = "none"),
                  part2, ncol = 2, rel_widths =  c(1.2,1), labels = c("a)", ""))
```

```
b<-cowplot::plot_grid(pca,Permanova_grouped,
                      nrow=2, rel_heights = c(1.8,1),
                      labels = c("A", "B"), label_size = 18)

b
```



Factor	Df	SumOfSqs	R^2	F	p−value
Watering_regime	2	463	0.04	1.58	**0.023**
Compartment	2	2594	0.22	8.87	**0.001**
Watering_regime:Compartment	4	734	0.06	1.25	0.088
Residual	56	8192	0.68		
Total	64	11983	1.00		

```
#ggsave('../../Figures_final/beta.png',
#       width = 14, height = 9.5, dpi = 300, plot =c)
```

Water regime PCA AND PERMANOVA

```
#barplots

table_all<- data.frame(read_qza(
  "../Data/merge_table_240_noplant_filtered_nous.qza")$data ,
                      check.names = F)
```

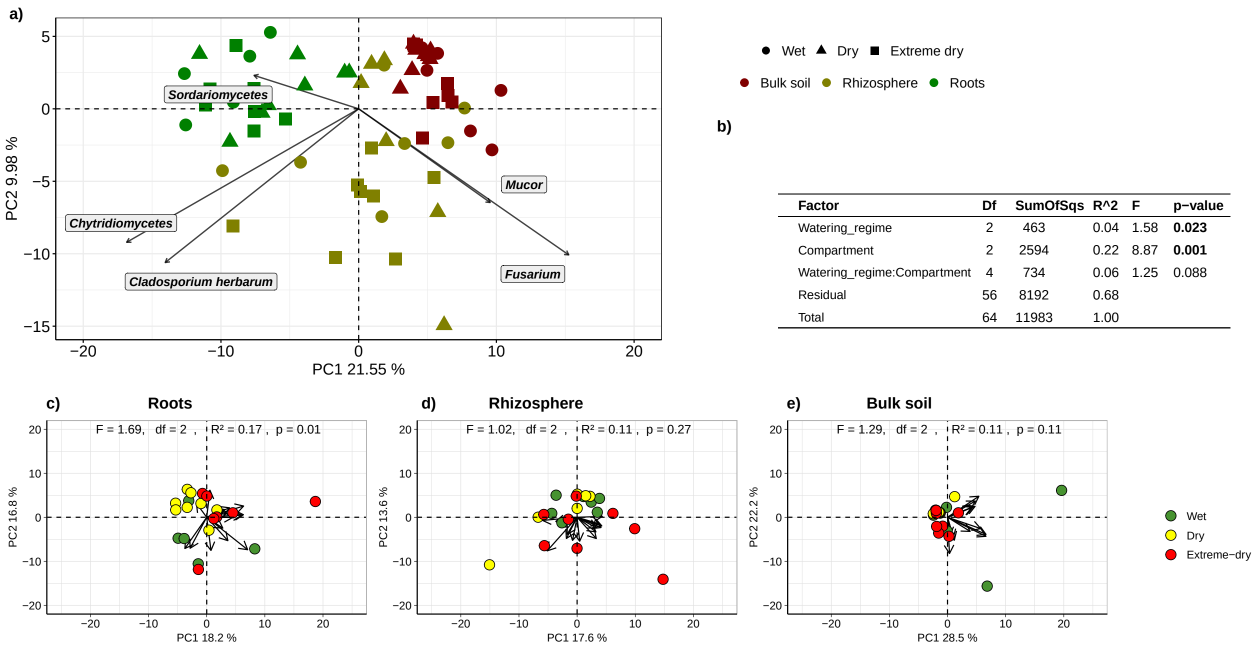
```r
taxonomy<- read_qza("../Data//taxonomy_blast_240_0.97.qza")$data
metadata<- read_tsv("../Data/FINALMAP18S") %>% dplyr::select(
  -Month)

metadata$Compartment<- factor(
  metadata$Type_of_soil,
  levels = c( "non-rizospheric", "Non-rizospheric", "Rizospheric",
              "Roots", "Seeds", "Uncultivated"),
  labels = c("Initials", "Non-rhizospheric" ,"Rhizoshpere",
             "Roots", "Initials", "Uncultivated"))

metadata$Watering_regime<- factor(metadata$Treatment,
                       levels = c( "0", "1", "2", "3"),
                       labels = c("Initials", "Wet" ,"Dry", "Extreme-dry"))

#ALL
table_all_ro<- table_filter %>% dplyr::select_at(vars(
  matches("RO"))) %>% t()%>% as.data.frame(  )%>% rownames_to_column(
  var = "#SampleID") %>% inner_join(metadata)  %>% group_by(
    Watering_regime) %>% summarise_at(c(2:152), mean) %>% column_to_rownames(
      var = "Watering_regime") %>% t()

table_all_ri<- table_filter %>% dplyr::select_at(vars(
  matches("RI"))) %>% t()%>% as.data.frame(
  )%>% rownames_to_column(
  var = "#SampleID") %>% inner_join(metadata)  %>% group_by(
    Watering_regime) %>% summarise_at(c(2:152), mean) %>% column_to_rownames(
      var = "Watering_regime") %>% t()

table_all_nr<- table_filter%>% dplyr::select_at(vars(
  matches("NR"))) %>% t()%>% as.data.frame(
  )%>% rownames_to_column(
  var = "#SampleID") %>% inner_join(metadata)  %>% group_by(
    Watering_regime) %>% summarise_at(c(2:152), mean) %>% column_to_rownames(
      var = "Watering_regime") %>% t()



#PCA'S
all_ro<- table_filter %>% dplyr::select_at(vars(
  matches("RO"))) %>% t()%>% as.data.frame(
)%>% rownames_to_column(
  var = "#SampleID") %>% inner_join(metadata) %>%arrange(
    Watering_regime) %>% filter(
    !Watering_regime=="T0") %>%  dplyr::select(
    -BarcodeSequence:-Watering_regime) %>% column_to_rownames(
      var = "#SampleID") %>% t()

all_ri<- table_filter %>% dplyr::select_at(vars(
  matches("RI"))) %>% t()%>% as.data.frame(
)%>% rownames_to_column(
  var = "#SampleID") %>% inner_join(metadata) %>%arrange(
    Watering_regime) %>% filter(
    !Watering_regime=="T0") %>%  dplyr::select(
    -BarcodeSequence:-Watering_regime) %>% column_to_rownames(
      var = "#SampleID") %>% t()

all_nr<- table_filter %>% dplyr::select_at(vars(
  matches("NR"))) %>% t()%>% as.data.frame(
)%>% rownames_to_column(
  var = "#SampleID") %>% inner_join(metadata) %>%arrange(
    Watering_regime) %>% filter(
    !Watering_regime=="T0") %>%  dplyr::select(
    -BarcodeSequence:-Watering_regime) %>% column_to_rownames(
      var = "#SampleID") %>% t()

list_pca<- list(all_ro, all_ri, all_nr)
zero_func <- function(x){ t(cmultRepl(t(x), method="CZM", output="p-counts"))}
clr_func<-function(x){t(CoDaSeq::codaSeq.clr(x ,samples.by.row = F))}

zero_list<- lapply(list_pca, zero_func)
```

```
## No. corrected values:  46
```

```
clr_list<- lapply(zero_list, clr_func)
pcx.abund_list <- lapply(clr_list, prcomp)

#create the base plot with only the arrows
PC1.f<- function(x,y){paste("PC1", round(sum(x$sdev[1] ^ 2) /
                                           mvar(y) * 100, 1), "%")}
PC2.f <- function(x,y){paste("PC2", round(sum(x$sdev[2] ^ 2) /
                                            mvar(y) * 100, 1), "%")}

PC1_all<- mapply(PC1.f, pcx.abund_list, clr_list)
PC2_all<- mapply(PC2.f, pcx.abund_list, clr_list)

list2<- list(pcx.abund_list[[1]],pcx.abund_list[[2]] , pcx.abund_list[[3]])

pca_tables2<- function(tab){ggplot() +
    geom_segment(data=data.frame(tab$rotation) %>%
                   rownames_to_column(var = "FeatureID")%>%
                   mutate(a=sqrt(PC1^2+PC2^2)) %>%
                   top_n(15, a) %>%
                   mutate(PC1=PC1*20, PC2=PC2*20),
                 aes(x=0, xend=PC1, y=0, yend=PC2),
                 arrow = arrow(length = unit(0.3,"cm")))+
    geom_point(data=data.frame(tab$x) %>%
                 rownames_to_column(var = "#SampleID")%>%
                 left_join(metadata, by = "#SampleID"),
               aes(x=PC1, y=PC2, fill=Watering_regime),shape=21, size=4) +
    geom_vline(xintercept = 0, linetype = 2) +
    geom_hline(yintercept = 0, linetype = 2) +theme_light()+
    scale_x_continuous(limits = c(-25,25))+
    scale_y_continuous(limits = c(-20,20))+
    scale_fill_manual(values = c("#479330","#FFFF00", "#FF0000"))+
  theme(axis.text = element_text(colour = "black", size = 10),
        axis.title = element_text(colour = "black", size = 10),
         legend.text = element_text(size = 10),
        axis.ticks = element_line(colour = "black"),
        axis.line = element_line(colour = "black"),
        axis.text.y.right = element_text(colour = "black"),
        axis.text.x.top = element_text(colour = "black"),
        legend.position = "top",
        legend.direction = "vertical",
        legend.box = "vertical",
        legend.title = element_blank())  }

figures_pca2<- lapply(list2, pca_tables2)
pca_ro_all<- figures_pca2[[1]]+ xlab(PC1_all[[1]]) +ylab(PC2_all[[1]])  + theme(
  legend.position="top", legend.title=element_blank())+
annotate("text", x=0, y=20,label = paste(" F = 1.69, ",
" df = 2  ,",
"   R² = 0.17 ,",
" p = 0.01"))
pca_ri_all<- figures_pca2[[2]]+ xlab(PC1_all[[2]]) +ylab(PC2_all[[2]])  + theme(
  legend.position="top", legend.title=element_blank())+
annotate("text", x=-0, y=20,label = paste(" F = 1.02, ",
" df = 2  ,",
"   R² = 0.11 ,",
" p = 0.27"))
pca_nr_all<- figures_pca2[[3]]+ xlab(PC1_all[[3]]) +ylab(PC2_all[[3]]) + theme(
  legend.position="top", legend.title=element_blank())+
annotate("text", x=0, y=20,label = paste(" F = 1.29, ",
" df = 2  ,",
"   R² = 0.11 ,",
" p = 0.11"))


pca_allthree<- plot_grid(pca_ro_all, pca_ri_all, pca_nr_all,ncol = 3,
labels = c("C                    Roots", "E            Rhizosphere",
           "G               Bulk soil"), rel_widths = c(1,1,1) )



#PERMANOVA'S
clr_function_perma<-function(x){data.frame(x, check.names = F) %>%
    rownames_to_column(
  var = "ids")%>% column_to_rownames(var = "ids")}

meta_just_func2<- function(x){data.frame(x, check.names = F) %>%
    rownames_to_column(
  var = "#SampleID") %>% inner_join(metadata)}
clr_perma<- lapply(clr_list, clr_function_perma)
meta_just_list2<- lapply(clr_perma, meta_just_func2)




library(vegan)
perm<- how(nperm = 999)
```

```
setBlocks(perm)<- with(meta_just_list2[[1]], Plot)
a5<-adonis2(clr_perma[[1]]~Watering_regime, data = meta_just_list2[[1]], method =
              "euclidian", permutations =perm) %>% round(., digits = 2) %>%replace(
                is.na(.), "-")


setBlocks(perm)<- with(meta_just_list2[[2]], Plot)
a6<-adonis2(clr_perma[[2]]~Watering_regime, data = meta_just_list2[[2]], method =
              "euclidian", permutations =perm) %>% round(., digits = 2) %>%replace(
                is.na(.), "-")
setBlocks(perm)<- with(meta_just_list2[[3]], Plot)
a7<-adonis2(clr_perma[[3]]~Watering_regime, data = meta_just_list2[[3]], method =
              "euclidian", permutations =perm) %>% round(., digits = 2) %>%replace(
                is.na(.), "-")


library(ggpubr)

Permanova_a5<-data.frame(a5, check.names = F) %>% rownames_to_column(
  var="Factor") %>% ggtexttable(., rows = NULL, theme = ttheme(
    "blank", base_size = 10)) %>%
  tab_add_hline(at.row = 1:2, row.side = "top", linewidth = 2)%>%
  tab_add_hline(at.row = c(4), row.side = "bottom", linewidth = 3, linetype = 1)

Permanova_a6<-data.frame(a6, check.names = F) %>% rownames_to_column(
  var="Factor") %>% ggtexttable(., rows = NULL, theme = ttheme(
    "blank", base_size = 10)) %>%
  tab_add_hline(at.row = 1:2, row.side = "top", linewidth = 2)%>%
  tab_add_hline(at.row = c(4), row.side = "bottom", linewidth = 3, linetype = 1)

Permanova_a7<-data.frame(a7, check.names = F) %>% rownames_to_column(
  var="Factor") %>% ggtexttable(., rows = NULL,theme = ttheme(
    "blank", base_size = 10)) %>%
  tab_add_hline(at.row = 1:2, row.side = "top", linewidth = 2)%>%
  tab_add_hline(at.row = c(4), row.side = "bottom", linewidth = 3, linetype = 1)

#all
perm_all_three<- plot_grid(Permanova_a5, Permanova_a6, Permanova_a7,
                           ncol = 3, labels = c("D", "F", "H"), label_y = 0.8)


pca_ro_all<- pca_ro_all+ theme(legend.position="top",
                               legend.direction="vertical")
legends<- get_legend(pca_ro_all)
pca_allthree<- plot_grid(
                 pca_ro_all+theme(legend.position = "none"),
                 pca_ri_all+theme(legend.position = "none"),
                 pca_nr_all+theme(legend.position = "none"),
                 legends,ncol =  4, rel_widths= c(1,1,1,0.5),
                 labels = c("c)                    Roots",
                            "d)            Rhizosphere",
                            "e)               Bulk soil",
                            ""),
                 hjust = -0.3, vjust = -0.5)
part4<- plot_grid(NULL,pca_allthree, nrow = 2, rel_heights = c(0.1,1))


part5<- plot_grid(part3, part4, nrow = 2, rel_heights = c(1.5,1))

#plot_all_three<- plot_grid( pca_allthree, perm_all_three,
 #                            nrow = 2, rel_heights = c(1.5,1))

#plot_all_three
```

```
#joinning plots
#per_pca<-plot_grid(b,plot_all_three, rel_widths =  c(1,2))
#per_pca
part5
```



Factor	Df	SumOfSqs	R^2	F	p−value
Watering_regime	2	463	0.04	1.58	**0.023**
Compartment	2	2594	0.22	8.87	**0.001**
Watering_regime:Compartment	4	734	0.06	1.25	0.088
Residual	56	8192	0.68		
Total	64	11983	1.00		

```
#ggsave('../../Figures_final/Fig3.tiff',
#width = 12, height = 8, dpi = 300, plot =part5)
#ggsave('../../Figures_final/Fig2.Permanova_PCA_NEW.png',
 # width = 16, height = 8, dpi = 300, plot =per_pca)
#ggsave('../../Figures_final/Permanova-pairwise_filt.pdf',
 #   width = 6, height = 4, dpi = 300, plot =Pairwsie_permanova)
```

Aldex analysis

```
#Loading libraries
library(dplyr)
library(stringr)
library(purrr)
library(tidyverse)
library(tibble)
library(tidyr)
library(qiime2R)
library(ALDEx2)
```

```r
library(tidyverse)
library(ComplexHeatmap)
library(viridis)
library(circlize)
library(RColorBrewer)
library(cowplot)
library(tidyverse)
library(qiime2R)
library(cowplot)
require(compositions)
require(zCompositions)
library(ggrepel)
library(vegan)
library(ggpubr)
library(RVAideMemoire)

relabunda<- function(x){(t(t(x)/colSums(x)))*100}
```

```r
#Importing data
table_240<- read_qza("../../Data/grouped_240_fil_noplant_nous.qza")$data
metadata<- read_tsv("../Data/FINALMAP_GROUPED.txt")
table_240<- table_240 %>% t() %>% data.frame(
  .,check.names = F) %>% rownames_to_column(
    var = "#SampleID") %>% inner_join(
  metadata) %>% arrange(Type_of_soil) %>% dplyr::select(
    -Type_of_soil:-Month) %>% column_to_rownames(
      var = "#SampleID") %>% t() %>% as.data.frame()
table_240_t<- table_240 %>% t()
taxonomy_240<- read_qza("../Data/taxonomy_blast_240_0.97.qza")$data

#remove non-fungi taxa

taxonomy_filter<- taxonomy_240 %>% filter(
  !str_detect(Taxon, "ozoa"))%>% filter(
  !str_detect(Taxon, "helida")) %>% filter(
  !str_detect(Taxon, "ophyta")) %>% filter(
  !str_detect(Taxon, "Ciliophora")) %>% filter(
  !str_detect(Taxon, "Nucleariidae_and_Fonticula_group")) %>% filter(
  !str_detect(Taxon, "Arthrioida")) %>% filter(
  !str_detect(Taxon, "Labyrinthulomycetes"))  %>% filter(
  !str_detect(Taxon, "Apicomplexa")) %>% filter(
  !str_detect(Taxon, "Bicosoecida")) %>% filter(
  !str_detect(Taxon, "Breviatea")) %>% filter(
  !str_detect(Taxon, "Aphelidea"))  %>% filter(
  !str_detect(Taxon, "Arthropoda"))

table_filter<-  table_240[match(taxonomy_filter$Feature.ID,
                                rownames(table_240)),] %>% drop_na(.)


#by taxonomic levels
parse_taxa_240<- parse_taxonomy(taxonomy_filter)

summarize_taxa_240<-summarize_taxa(table_filter,
                                   parse_taxa_240)
Phylum_240<- summarize_taxa_240$Phylum
Class_240<- summarize_taxa_240$Class
Genus_240<- summarize_taxa_240$Genus

#analysis kruskal wallis all levels
conds<- c(rep("NR", 9), rep("RI", 9), rep("RO", 9))

#avss
aldex_analysis<- aldex.clr(table_filter,
                           mc.samples = 1000, denom="all", verbose = TRUE, conds )

differentials <- aldex.kw(aldex_analysis,  useMC = F, verbose = F)
aldex_240<- differentials %>% rownames_to_column(
  var = "Feature.ID") %>% left_join(taxonomy_240)%>% filter(kw.eBH < 0.05)


#taxonomic levels
aldex_list<- list(Phylum_240, Class_240, Genus_240)


aldex_analysis_function<-function(x){ aldex.clr(x,
mc.samples = 1000, denom="all", verbose = TRUE, conds)}

aldex_analysis<- lapply(aldex_list, aldex_analysis_function)

differentials_function <- function(x){aldex.kw(x,  useMC = F, verbose = F)}

differentials_tables_class <- lapply(aldex_analysis, differentials_function)


aldex_phylum<- differentials_tables_class[[1]] %>% rownames_to_column(
  var = "Feature.ID")  %>% filter(kw.eBH<0.05)
aldex_class<- differentials_tables_class[[2]] %>% rownames_to_column(
  var = "Feature.ID")  %>% filter(kw.eBH<0.05)
aldex_genus<- differentials_tables_class[[3]] %>% rownames_to_column(
  var = "Feature.ID")  %>% filter(kw.eBH<0.05)


#Paired test just with asv's
table_240_t<- table_filter %>% t()
otutable1<- data.frame(t(table_240_t),
                       check.names = F, stringsAsFactors = F) %>% dplyr::select_at(
                         vars(contains("Roots")))
otutable2<- data.frame(t(table_240_t),
                       check.names = F, stringsAsFactors = F) %>% dplyr::select_at(
                         vars(starts_with("Ri")))
otutable3<- cbind(otutable1, otutable2)

otutable4<- data.frame(t(table_240_t),
                       check.names = F, stringsAsFactors = F) %>% dplyr::select_at(
                         vars(contains("Roots")))
otutable5<- data.frame(t(table_240_t),
                       check.names = F, stringsAsFactors = F) %>% dplyr::select_at(
                         vars(starts_with("N")))
otutable6<- cbind(otutable4, otutable5)

otutable7<- data.frame(t(table_240_t),
                       check.names = F, stringsAsFactors = F)%>%dplyr::select_at(
                         vars(contains("Non")))
otutable8<- data.frame(t(table_240_t),
                       check.names = F, stringsAsFactors = F) %>% dplyr::select_at(
                         vars(starts_with("Ri")))
otutable9<- cbind(otutable7, otutable8)

conds<- c(rep("A", 9), rep("B", 9))

aldex_list_paired<- list(otutable3, otutable6, otutable9)


aldex_analysis_function_paired<-function(x){ aldex(x,
     mc.samples = 1000, denom="all", verbose = TRUE, conds,  effect = T, test = "t")}

aldex_analysis_paired<- lapply(aldex_list_paired, aldex_analysis_function_paired)


aldex_ro_ri<-aldex_analysis_paired[[1]] %>% rownames_to_column(
  var = "Feature.ID")  %>% filter(wi.eBH < 0.05) %>% left_join(taxonomy_filter)
aldex_ro_nr<- aldex_analysis_paired[[2]] %>% rownames_to_column(
  var = "Feature.ID")  %>% filter(wi.eBH < 0.05) %>% left_join(taxonomy_filter)
aldex_nr_ri<- aldex_analysis_paired[[3]] %>% rownames_to_column(
  var = "Feature.ID")  %>% filter(wi.eBH < 0.05) %>% left_join(taxonomy_filter)


#write.table(aldex_nr_ri,"dif_aldex_nr_ri.tsv", sep = "\t", row.names = T)

#Aldex for functional predictions of Picrust2

EC_predicted <- read.delim("../Data/pathways_table_all.txt",
                           check.names = F) %>% dplyr::select(
                             -"#OTU ID") %>% column_to_rownames(
                               var = "taxonomy")

Alpha.t_asv_table<- read.delim("../Data/alpha_all.tsv")


table<- EC_predicted %>% t() %>% as.data.frame() %>% rownames_to_column(
  var = "sampleid")
table_meta<- table %>% inner_join(Alpha.t_asv_table) %>% group_by(
  Type, Treatment, Plot) %>% summarise_if(is.numeric, sum) %>% unite(
    "ids",Type:Plot,  sep = "_") %>% column_to_rownames(
      var = "ids") %>% dplyr::select(-Loc:-value) %>% t() %>%
  as.data.frame() %>% mutate_if(
      is.numeric, as.integer)

table_nr<- table_meta %>% dplyr::select_at(vars(contains("Bulk")))
```

```
table_ri<- table_meta %>% dplyr::select_at(vars(contains("Rhizosphere")))
table_ro<- table_meta %>% dplyr::select_at(vars(contains("Root")))

table_nr_ri<- cbind(table_nr, table_ri)
table_nr_ro<- cbind(table_nr, table_ro)
table_ro_ri<- cbind(table_ro, table_ri)

conds1<- c(rep("bs", 9), rep("ri", 9))
conds2<- c(rep("bs", 9), rep("ro", 9))
conds3<- c(rep("ro", 9), rep("ri", 9))

library(ALDEx2)
aldex_nr_ri<-aldex(table_nr_ri, conditions = conds1, mc.samples = 1000,
                   test = "t", effect = T, denom = "all")
aldex_nr_ro<-aldex(table_nr_ro, conditions = conds2, mc.samples = 1000,
                   test = "t", effect = T, denom = "all")
aldex_ro_ri<-aldex(table_nr_ri, conditions = conds3, mc.samples = 1000,
                   test = "t", effect = T, denom = "all")

#write.table(aldex_nr_ri, "aldex_nr_ri_funct.txt", sep = "\t")
#write.table(aldex_nr_ro, "aldex_nr_ro_funct.txt", sep = "\t")
#write.table(aldex_ro_ri, "aldex_ro_ri_funct.txt", sep = "\t")
```

Let's plot heatmap with aldex values!

```
#loading files
meta<-read_tsv("../Data/FINALMAP_GROUPED.txt")
table_240<- read_qza("../../Data/grouped_240_fil_noplant_nous.qza")$data %>% as.data.frame()
taxonomy_240<- read_qza("../Data/taxonomy_blast_240_0.97.qza")$data
#remove non-fungi taxa
taxonomy_filter<- taxonomy_240 %>% filter(
  !str_detect(Taxon, "ozoa"))%>% filter(
  !str_detect(Taxon, "helida")) %>% filter(
  !str_detect(Taxon, "ophyta")) %>% filter(
  !str_detect(Taxon, "Ciliophora")) %>% filter(
  !str_detect(Taxon, "Nucleariidae_and_Fonticula_group")) %>% filter(
  !str_detect(Taxon, "Arthrioida")) %>% filter(
  !str_detect(Taxon, "Labyrinthulomycetes"))  %>% filter(
  !str_detect(Taxon, "Apicomplexa")) %>% filter(
  !str_detect(Taxon, "Bicosoecida")) %>% filter(
  !str_detect(Taxon, "Breviatea")) %>% filter(
  !str_detect(Taxon, "Aphelidea"))  %>% filter(
  !str_detect(Taxon, "Arthropoda"))

table_filter<-  table_240[match(taxonomy_filter$Feature.ID, rownames(table_240)),] %>% drop_na(.)


#by taxonomic levels
parse_taxa_240<- parse_taxonomy(taxonomy_filter)

summarize_taxa_240<-summarize_taxa(table_filter, parse_taxa_240)
Phylum_240<- summarize_taxa_240$Phylum
Class_240<- summarize_taxa_240$Class
Genus_240<- summarize_taxa_240$Genus
```

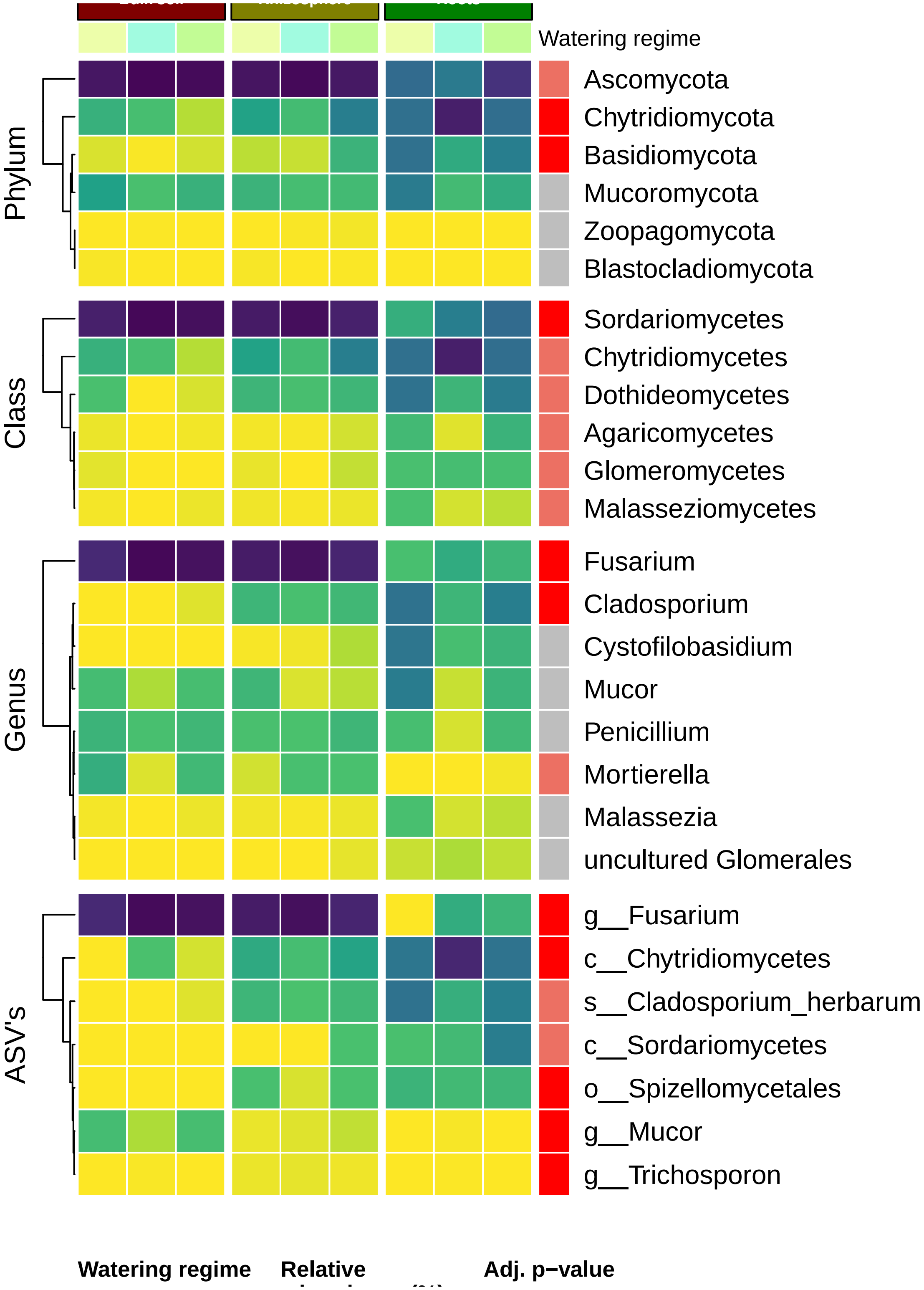
```r
#PHYLUM
phylum_240<- summarize_taxa_240$Phylum %>%  mutate(means = rowMeans(.)) %>%mutate_at(
  "means", as.numeric) %>%  arrange(-means)
phylum_240_abund<-data.frame( relabunda(phylum_240[1:28]) , check.names = F)

phylum_240_relab<-phylum_240_abund %>%  dplyr::select_at(
  vars(!contains("0"))) %>% dplyr::slice(
  c(1:7)) %>% rownames_to_column(var = "#OTU ID") %>% filter(
    !`#OTU ID` =="d__Eukaryota; Phragmoplastophyta") %>% dplyr::select(-means)

phylum_aldex_240<- read_tsv("../Data/aldex_results/dif_240_phylum.tsv") %>%
  rename("#OTU ID"=Feature.ID) %>%
  right_join( phylum_240_relab)%>%dplyr::mutate_at(
    c(1),~str_extract(., "[^;]+$")) %>% column_to_rownames(var = "#OTU ID") %>%
  mutate_at(c("kw.eBH"), funs(p.value = case_when(
    . <= 0.001 ~ "<0.001",
    . >  0.001 & .  <= 0.01 ~ "<0.01",
    . >  0.01 & .  < 0.05 ~ "<0.05",
    . >=  0.05 ~ ">0.05")))%>% rownames_to_column(
      var = "ids")  %>% filter(!ids == " NA") %>%  arrange(
        ids) %>% column_to_rownames(var = "ids")

heatmap<- phylum_240_relab %>% column_to_rownames(
  var = "#OTU ID")  %>% t() %>% as.data.frame() %>%  rownames_to_column(
  var = "#SampleID") %>% inner_join(meta) %>% group_by(
    Type_of_soil, Treatments) %>% summarise_if(
    is.numeric, mean) %>%  unite(
  "ids", Type_of_soil:Treatments) %>% column_to_rownames(
  var = "ids") %>%dplyr::select_at(vars(
    contains("d__")),~str_extract(
      ., "[^;]+$")) %>% t() %>% as.data.frame( ) %>% rownames_to_column(
      var = "ids")  %>% arrange(ids) %>% column_to_rownames(var = "ids")


#CLASS
class_240<- summarize_taxa_240$Class %>%  mutate(
  means = rowMeans(.)) %>%mutate_at(
  "means", as.numeric) %>%  arrange(-means)
class_240_abund<-data.frame(relabunda(class_240[1:28]) , check.names = F)
class_240_relab<-class_240_abund %>%  dplyr::select_at(
  vars(!contains("0"))) %>% dplyr::slice(
  c(1:20)) %>% rownames_to_column(
    var = "#OTU ID") %>% filter(
      !`#OTU ID` =="d__Eukaryota; Phragmoplastophyta") %>% dplyr::select(-means)

remove<- c(" Pezizomycetes", " Aconoidasida" , " Chromadorea" ,
           " Aphelidea",  " Discosea" , " Imbricatea" )

class_aldex_240<- read_tsv(
  "../Data/aldex_results/dif_240_class.tsv") %>% rename(
    "#OTU ID"=Feature.ID) %>%  right_join(
  class_240_relab)%>%dplyr::mutate_at(c(1),~str_extract(., "[^;]+$"))%>% filter(
    !`#OTU ID` == " Incertae_Sedis" & !`#OTU ID` == " NA"& !
      `#OTU ID` == " Eurotiomycetes" ) %>% arrange(
      `#OTU ID`)%>% column_to_rownames(var = "#OTU ID") %>%
  mutate_at(c("kw.eBH"), funs(p.value = case_when(
    . <= 0.001 ~ "<0.001",
    . >  0.001 & .  <= 0.01 ~ "<0.01",
    . >  0.01 & .  < 0.05 ~ "<0.05",
    . >=  0.05 ~ ">0.05"))) %>% rownames_to_column(
      var = "ids")  %>% filter(!ids %in% remove) %>%  arrange(
        ids) %>% column_to_rownames(var = "ids")


heatmap_class<- class_240_relab %>% column_to_rownames(
  var = "#OTU ID")  %>% t() %>% as.data.frame(
  ) %>%  rownames_to_column(
  var = "#SampleID") %>% inner_join(meta) %>% group_by(
    Type_of_soil, Treatments) %>% summarise_if(
    is.numeric, mean) %>%  unite(
      "ids", Type_of_soil:Treatments) %>% column_to_rownames(
        var = "ids") %>%dplyr::select(
          -"d__Eukaryota; Zoopagomycota; Incertae_Sedis" ,
          -"d__Eukaryota; Mucoromycota; Incertae_Sedis") %>% dplyr::select_at(vars(
          contains("d__")),~str_extract(
            ., "[^;]+$")) %>% t() %>% as.data.frame( ) %>% rownames_to_column(
            var = "ids")  %>% filter(!ids %in% remove) %>%  arrange(
              ids) %>% filter(!ids==" Eurotiomycetes") %>% column_to_rownames(var = "ids")


#GENUS
genus_240<- summarize_taxa_240$Genus %>%  mutate(
  means = rowMeans(.)) %>%mutate_at(
  "means", as.numeric) %>%  arrange(-means)

genus_240_abund<-data.frame( relabunda(genus_240[1:28]) , check.names = F)
genus_240_relab<-genus_240_abund %>%  dplyr::select_at(vars(!contains("0"))) %>% dplyr::slice(
  c(1:27)) %>% rownames_to_column(var = "#OTU ID") %>% filter(
    !`#OTU ID` =="d__Eukaryota; Phragmoplastophyta")
remove2<- c(" Ophiostoma", " Naganishia" , " Aspergillus" , " Magnoliophyta" )

genus_aldex_240<- read_tsv("../Data/aldex_results/dif_240_genus.tsv")%>%
  rename("#OTU ID"=Feature.ID) %>%  right_join(
 genus_240_relab)%>%dplyr::mutate_at(c(1),~str_extract(., "[^;]+$"))%>% filter(
    !`#OTU ID` == " Incertae_Sedis" & !`#OTU ID` == " NA" ) %>% arrange(
      `#OTU ID`)%>% column_to_rownames(var = "#OTU ID") %>%
  mutate_at(c("kw.eBH"), funs(p.value = case_when(
    . <= 0.001 ~ "<0.001",
    . >  0.001 & .  <= 0.01 ~ "<0.01",
    . >  0.01 & .  < 0.05 ~ "<0.05",
    . >=  0.05 ~ ">0.05"))) %>% rownames_to_column(
      var = "ids")  %>% filter(
        !ids %in% remove2) %>%  arrange(ids) %>% mutate(ids=case_when(
          ids ==" uncultured"  ~ " uncultured Glomerales",
          TRUE~ as.character(ids))) %>%
  column_to_rownames(var = "ids")


heatmap_genus<- genus_240_relab %>% column_to_rownames(
  var = "#OTU ID")  %>% t() %>% as.data.frame(
) %>%  rownames_to_column(
  var = "#SampleID") %>% inner_join(meta) %>% group_by(
    Type_of_soil, Treatments) %>% summarise_if(
    is.numeric, mean) %>%  unite(
      "ids", Type_of_soil:Treatments) %>% column_to_rownames(
        var = "ids") %>%dplyr::select_at(vars(
          -ends_with("NA"))) %>% dplyr::select_at(vars(
            contains("d__")),~str_extract(
              ., "[^;]+$")) %>% t() %>% as.data.frame( ) %>% rownames_to_column(
              var = "ids")  %>% filter(
                !ids %in% remove2) %>%  arrange(ids) %>% mutate(ids=case_when(
          ids ==" uncultured"  ~ " uncultured Glomerales",
          TRUE~ as.character(ids))) %>%
  column_to_rownames(var = "ids")

#ASV's

asv_240<- data.frame(table_filter, check.names = F)  %>%  mutate(
  means = rowMeans(.)) %>%mutate_at(
    "means", as.numeric) %>%  arrange(-means)%>% rownames_to_column(
      var = "Feature.ID")%>%  inner_join(
        taxonomy_240)%>% dplyr::select(-Consensus)

asv_240_abund<-data.frame(asv_240[c(1,30)],
                          relabunda(asv_240[2:28]) , check.names = F)

names<- asv_240_abund[2]



asv_aldex_240<- read.delim("../Data/aldex_results/dif_240_asv.tsv") %>%
  rename("#OTU ID"=Feature.ID) %>% filter(
  kw.eBH < 0.05) %>%dplyr::mutate_at(c("Taxon"),~str_extract(., "[^;]+$"))%>% filter(
    !`#OTU ID` == " Incertae_Sedis" & !`#OTU ID` == " NA" ) %>% arrange(
      `#OTU ID`)%>% remove_rownames() %>% column_to_rownames(var = "#OTU ID") %>%
  mutate_at(c("kw.eBH"), funs(p.value = case_when(
    . <= 0.001 ~ "<0.001",
    . >  0.001 & .  <= 0.01 ~ "<0.01",
    . >  0.01 & .  < 0.05 ~ "<0.05",
    . >=  0.05 ~ ">0.05"))) %>% rownames_to_column(
      var = "Feature.ID") %>% arrange(Taxon)  %>%drop_na(.) %>%  remove_rownames(
        )%>% column_to_rownames(var = "Taxon")


asv_240_relab<-asv_240_abund %>%  dplyr::select_at(
  vars(!contains("0"))) %>% right_join(
    asv_aldex_240, by = c("Feature.ID")) %>% dplyr::select(
      Taxon, `Non-rizospheric22`:Roots11) %>%drop_na(.) %>%  column_to_rownames(var = "Taxon")
```

```
heatmap_asv<- asv_240_relab %>% t() %>% as.data.frame(
) %>%  rownames_to_column(
  var = "#SampleID") %>% inner_join(meta) %>% group_by(Type_of_soil, Treatments) %>% summarise_if(
    is.numeric, mean) %>%  unite(
      "ids", Type_of_soil:Treatments) %>% column_to_rownames(
        var = "ids") %>% dplyr::select_at(vars(
 contains("__")),~str_extract(., "[^;]+$")) %>% t() %>% as.data.frame(
   ) %>% rownames_to_column(var = "ids")  %>%  arrange(
     ids) %>% column_to_rownames(var = "ids")
```

Heatmap!

```
col_fun2 = colorRamp2(c(0,  1, 1+1e-5,10,10+1e-5,50,50+1e-5, 100),
                      viridis(8, option = "D", direction = -1))

#pvalue annotation
mypalette<-brewer.pal(10,"BrBG")
cols_pvalue <- list('Adj. p-value' = c("<0.001" = '#AB0000',
                                  "<0.01"  =  '#FF0000',
                                "<0.05"="#EC7063",
                               ">0.05"="#F9EBEA"))
split = rep(1:3, each = 3)
treats<- c("1.Wet", "2.Dry", "3.Extreme-dry")
cols_ho<- list("Water regime" = c("1.Wet" = '#479330',
                                  "2.Dry"  =  '#FFFF00',
                                  "3.Extreme-dry"="#FF0000"))
```

```
annP = HeatmapAnnotation("Adj. p-value" = phylum_aldex_240$p.value,
                          which = "row", col = cols_pvalue,
                         show_legend = F, gp = gpar(col = "white"),
                         show_annotation_name = F)


ha = HeatmapAnnotation(foo = anno_block(gp = gpar(
  fill = c("#800000" ,"#808000" ,"#008000", "#D35400")),
labels = c("Bulk soil", "Rhizosphere", "Roots"),
labels_gp = gpar(col = "white", fontsize = 7, fontface= "bold")))



ho = HeatmapAnnotation("Watering regime" = c(rep(treats, 3)),
                       which = "col", col = cols_ho,
                       annotation_name_gp = gpar(fontsize=10),
                       show_legend = T, gp = gpar(col = "white", fontize=12),
                       show_annotation_name = T)


ht1<-Heatmap(heatmap,col = col_fun2,
             heatmap_legend_param = list(direction = "horizontal",
                                         title = "Relative \n abundance (%)",
                                         at = c(0,1, 10,  50, 100), break_dist = 1),
             rect_gp = gpar(col = "white", lwd = 1),
             cluster_columns = F, cluster_rows = T,
             show_heatmap_legend = TRUE, top_annotation = c(ha, ho),
                           height = nrow(heatmap)*unit(6, "mm"),
             row_dend_width = unit(0.5, "cm"),right_annotation =  c(annP),
             column_order = sort(colnames(heatmap)),width = ncol(heatmap)*unit(3, "mm"),
             column_split = split, column_title = NULL,
             row_names_gp = gpar(fontsize=12),row_title = "Phylum",
             column_title_gp = gpar(
               fill = c("#800000" ,"#808000" ,"#008000", "#D35400", "#2E4053" )))



class_aldex<- class_aldex_240 %>% drop_na(.)
annP2 = HeatmapAnnotation("Adj. p-value" = class_aldex$p.value,
                          which = "row", col = cols_pvalue,name = ".",
                          show_annotation_name = F,
                         show_legend = T, gp = gpar(col = "white"))

heat_class<- heatmap_class %>% rownames_to_column(var = "ids") %>% inner_join(
  class_aldex %>%  mutate(ids = rownames(class_aldex)),
  by = 'ids') %>% column_to_rownames(var = "ids") %>%  dplyr::select(
    `Non-rizospheric_TC`:`Roots_TSD`)
ht2<-Heatmap(heat_class,col = col_fun2,
             heatmap_legend_param = list(direction = "horizontal",
                                         col_fun = col_fun2,
                                         title = "Relative \n abundance (%)",
                                         at = c(0,1, 10,  50, 100),
                                         break_dist = 1),
             rect_gp = gpar(col = "white", lwd = 1),
                           height = nrow(ht1)*unit(6, "mm"),
             row_title = "Class",width = ncol(heatmap)*unit(3, "mm"),
             row_dend_width = unit(0.5, "cm"),cluster_columns = F,
             cluster_rows = T,show_heatmap_legend = FALSE,
             row_names_gp = gpar(fontsize=12), right_annotation =  c(annP2))




annP3 = HeatmapAnnotation("Adj. p-value" = genus_aldex_240$p.value,
                          which = "row", col = cols_pvalue,name = ".",
                          show_annotation_name = F,
                          show_legend = T, gp = gpar(col = "white"))



ht3<-Heatmap(heatmap_genus,col = col_fun2,
             heatmap_legend_param = list(direction = "horizontal",
                                         col_fun = col_fun2,
                                         title = "Relative \n abundance (%)",
                                         at = c(0,1, 10,  50, 100),
                                         break_dist = 1),
             rect_gp = gpar(col = "white", lwd = 1), cluster_columns = F,
             cluster_rows = T,show_heatmap_legend = FALSE,row_title = "Genus",
                           height = nrow(ht1)*unit(9, "mm"),
             width = ncol(heatmap)*unit(3, "mm"),
             row_dend_width = unit(0.5, "cm"),  right_annotation =  c(annP3),
             column_order = sort(colnames(heatmap_genus)),
             row_names_gp = gpar(fontsize=12),
             column_split = split, column_title = NULL)




annP4 = HeatmapAnnotation('Adj. p-value' = asv_aldex_240$p.value,
                          which = "row", col = cols_pvalue,name = ".",
                          annotation_name_gp = gpar(fontsize=10),
                          show_legend = F, gp = gpar(col = "white"),
                          show_annotation_name = F)


ht4<-Heatmap(heatmap_asv,col = col_fun2,
             heatmap_legend_param = list(direction = "horizontal",
                                         col_fun = col_fun2,
                                         title = "Relative \n abundance (%)",
                                         at = c(0,1, 10,  50, 100),
                                         break_dist = 1),
             rect_gp = gpar(col = "white", lwd = 1), row_title = "ASV's",
             cluster_columns = F, cluster_rows = T,
             show_heatmap_legend = FALSE,row_names_gp = gpar(fontsize=12),
             width = ncol(heatmap)*unit(3, "mm"),
              height = nrow(ht1)*unit(8, "mm"),
             row_dend_width = unit(0.5, "cm"),  right_annotation =  c(annP4),
             column_order = sort(colnames(heatmap_asv)), show_column_names = F)


#ht1%v%ht2%v%ht3%v%ht4
ht_list = ht1 %v% ht2 %v% ht3 %v% ht4

heatm<-grid.grabExpr(draw(ht_list, heatmap_legend_side = "bottom",
                          annotation_legend_side = "bottom",
                          merge_legend=T, width = ncol(ht1)*unit(8, "mm")))

plot_grid(heatm)
```



Aldex paired plots

```
#load and format files
aldex_ro_ri<- read.delim("../Data/aldex_results/dif_aldex_ro_ri.tsv",
                         ) %>%
  mutate_at(c("diff.btw"), funs(Type = case_when(
    . < 0 ~ "Roots",
    . >  0 ~ "Rhizosphere"))) %>% arrange(diff.btw)%>%mutate(
      taxa= str_extract(Taxon, "[^_]+$"))%>%
 mutate(taxa=case_when(
   taxa=="Sordariomycetes" ~ "c__Sordariomycetes",
   taxa=="Trichosporon" ~ "g__Trichosporon",
   taxa=="Fusarium" ~ "g__Fusarium",
   taxa=="Mucor" ~ "g__Mucor" )) %>%
  mutate_at(c("wi.eBH"), funs(p.value = case_when(
    . <= 0.001 ~ "<0.001",
    . >  0.001 & .  <= 0.01 ~ "<0.01",
    . >  0.01 & .  < 0.05 ~ "<0.05",
    . >=  0.05 ~ ">0.05")))



aldex_nr_ri<- read.delim("../Data/aldex_results/dif_aldex_nr_ri.tsv" ) %>%
  mutate_at(c("diff.btw"), funs(Type = case_when(
    . < 0 ~ "Non-rhizospheric",
    . >  0 ~ "Rhizosphere"))) %>% arrange(diff.btw)%>%mutate(
      taxa= str_extract(Taxon, "[^_]+$"))%>%
 mutate(taxa=case_when(
   taxa=="Spizellomycetales" ~ "o__Spizellomycetales",
   taxa=="Trichosporon" ~ "g__Trichosporon",
   taxa=="Chytridiomycetes" ~ "c__Chytridiomycetes")) %>%
  mutate_at(c("wi.eBH"), funs(p.value = case_when(
    . <= 0.001 ~ "<0.001",
    . >  0.001 & .  <= 0.01 ~ "<0.01",
    . >  0.01 & .  < 0.05 ~ "<0.05",
    . >=  0.05 ~ ">0.05")))


aldex_ro_nr<- read.delim("../Data/aldex_results/dif_aldex_ro_nr.tsv") %>%
  mutate_at(c("diff.btw"), funs(Type = case_when(
    . < 0 ~ "Roots",
    . >  0 ~ "Non-rhizospheric"))) %>% arrange(diff.btw)%>%mutate(
      taxa= str_extract(Taxon, "[^_]+$"))%>%
  mutate_at(c("wi.eBH"), funs(p.value = case_when(
    . <= 0.001 ~ "<0.001",
    . >  0.001 & .  <= 0.01 ~ "<0.01",
    . >  0.01 & .  < 0.05 ~ "<0.05",
    . >=  0.05 ~ ">0.05")))%>%
  mutate_at(c("taxa"), funs(taxa = case_when(
    taxa=="herbarum" ~ "s__Cladosporium herbarum",
       taxa=="Chytridiomycetes" ~ "c__Chytridiomycetes",
       taxa=="Fusarium" ~ "g__Fusarium",
       taxa=="Spizellomycetales" ~ "o__Spizellomycetales",
       taxa=="Sordariomycetes" ~ "c__Sordariomycetes",
       taxa=="Mucor" ~ "g__Mucor" )))


cols_pvalue <- list('Adj. p-value' = c(
  "<0.001" = '#C70039',
  "<0.01"='#FF5733',
  "<0.05"="#FFC300",
  ">0.05"="#DAF7A6"))
effect_col_fun =colorRamp2(
  c(-3.5, 0, 3.5),
  c("lightsalmon4", "white", "lightseagreen"))

aldex_nr_ro_funct<- read.delim("../Data/aldex_nr_ro_funct.txt" ) %>% rownames_to_column(
  var = "Taxon") %>% filter(
  wi.eBH<0.05) %>%   mutate_at(c("diff.btw"), funs(Type = case_when(
    . < 0 ~ "Non-rhizospheric",
    . >  0 ~ "Roots"))) %>% arrange(diff.btw)%>%mutate(
      taxa= str_extract(Taxon, "[^_]+$"))%>%
  mutate_at(c("wi.eBH"), funs(p.value = case_when(
    . <= 0.001 ~ "<0.001",
    . >  0.001 & .  <= 0.01 ~ "<0.01",
    . >  0.01 & .  < 0.05 ~ "<0.05",
    . >=  0.05 ~ ">0.05")))



heat<- aldex_ro_nr %>% dplyr::select(
  taxa, "RO"= rab.win.A, "BS"= rab.win.B)
heat1<- heat[c(-5),] %>% remove_rownames() %>% column_to_rownames(var = "taxa")
heat2<- aldex_nr_ri %>% dplyr::select(
  taxa, "BS"= rab.win.A, "R"= rab.win.B)  %>% remove_rownames() %>% column_to_rownames(var = "taxa")
heat3<- aldex_ro_ri %>% dplyr::select(
  taxa, "RO"= rab.win.A, "R"= rab.win.B)  %>% remove_rownames() %>% column_to_rownames(var = "taxa")
heat4<- aldex_nr_ro_funct %>% dplyr::select(
  taxa, "BS"= rab.win.bs, "RO"= rab.win.ro)  %>% remove_rownames() %>% column_to_rownames(var = "taxa")
```

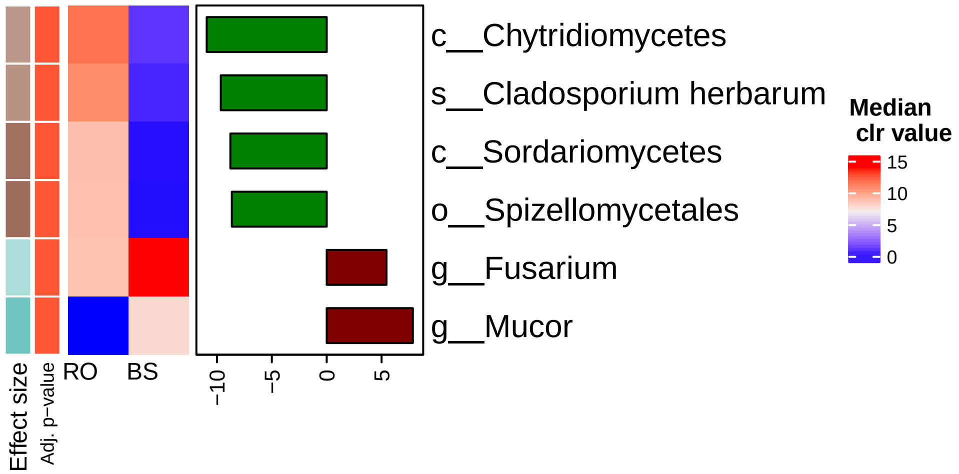
Let's plot!

```
treatment_col = structure(c("#008000", "#800000"),
                          names = c("Roots", "Non-rhizospheric"))

barpl = rowAnnotation("difference \nbetween groups" = anno_barplot(
  aldex_ro_nr[c(-5),]$diff.btw, which = "row",
  gp = gpar(fill = treatment_col[aldex_ro_nr[c(-5),]$Type]),
  width = unit(3, "cm")),  show_annotation_name = F,
  annotation_name_gp =gpar(fontsize = 0),
  annotation_name_rot = 0)

annP = HeatmapAnnotation("Adj. p-value" =aldex_ro_nr[c(-5),]$p.value,
                         simple_anno_size = unit(0.35, "cm"),
                         annotation_name_gp =gpar(fontsize = 7),
                         which = 'row',
                         annotation_legend_param = list(
                           title_gp = gpar(fontsize = 9, fontface="bold"),
                           labels_gp = gpar(fontsize = 9),
                           direction ="vertical"),
                         col = cols_pvalue,
                        show_legend = F, gp = gpar(col = "white"),
                        show_annotation_name = T)
```

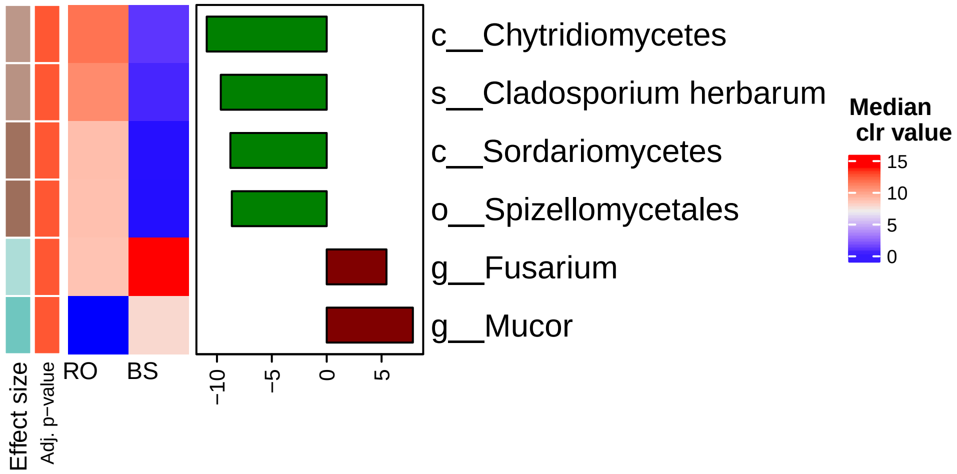
```
annE = rowAnnotation("Effect size" = aldex_ro_nr[c(-5),]$effect,
                     annotation_name_gp =gpar(fontsize = 9),
                     col = list("Effect size" = effect_col_fun),
                     simple_anno_size = unit(0.35, "cm"),
                     annotation_legend_param = list(title_gp = gpar(fontsize = 9,
                                                                    fontface="bold"),
                                                    labels_gp = gpar(fontsize = 9),
                                                    direction ="vertical"),
                     show_legend = F, gp = gpar(col = "white"), show_annotation_name = T)




H1<-Heatmap(heat1, cluster_rows = F,
            cluster_columns = F,  width = ncol(heat1)*unit(8, "mm"),
            height = nrow(heat1)*unit(7.7, "mm"),column_names_rot = 0,
            left_annotation = c(annE, annP),
            right_annotation=c(barpl),
            name = "Median \n clr value",
            heatmap_legend_param = list(direction = "vertical" ,
                                        labels_gp = gpar(fontsize = 7),
                                        title_gp = gpar(fontsize = 9,  fontface="bold"),
                                        legend_height = unit(1.4, "cm")),
            column_names_gp = gpar(fontsize = 9),
            row_names_gp = gpar(fontsize = 12), show_heatmap_legend = T)
```

```
## Warning: The input is a data frame, convert it to the matrix.
```

```
H1.1<- draw(H1,  heatmap_legend_side = "right")
```



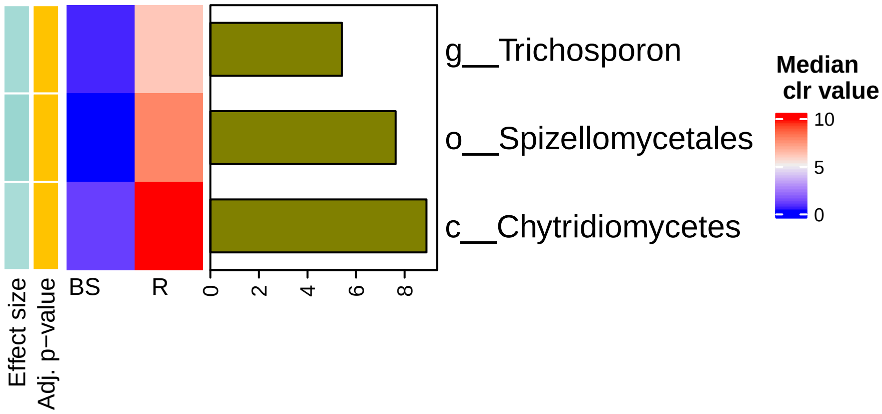
```
H1.1
```



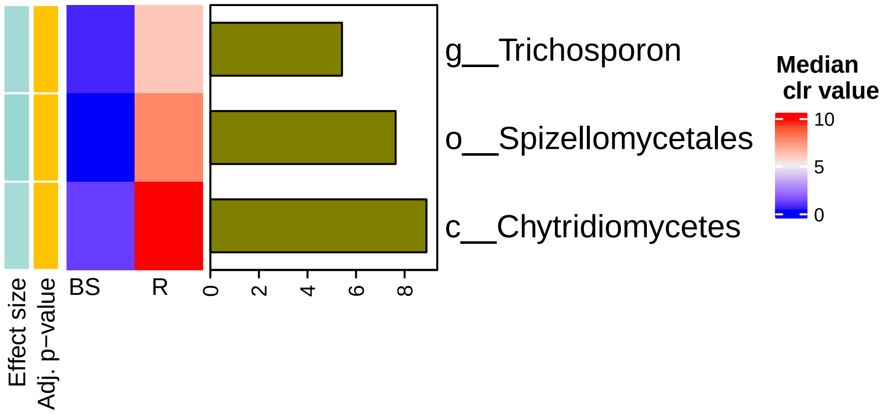
```
#heat2

treatment_col = structure(c("#808000", "#800000"),
                          names = c("Rhizosphere", "Non-rhizospheric"))
barpl = rowAnnotation("difference \nbetween groups" = anno_barplot(
  aldex_nr_ri$diff.btw,
  gp = gpar(fill = treatment_col[aldex_nr_ri$Type]),
  width = unit(3, "cm"),   height = unit(2, "cm")),
  show_annotation_name = F,annotation_name_gp =gpar(fontsize = 9),
  annotation_name_rot = 0)

annP = rowAnnotation("Adj. p-value" = aldex_nr_ri$p.value,
                     simple_anno_size = unit(0.35, "cm"),
                     col = cols_pvalue,
                     annotation_name_gp =gpar(fontsize = 9),
                     show_legend = F, gp = gpar(col = "white"),
                     show_annotation_name = T)

annE = rowAnnotation("Effect size" = aldex_nr_ri$effect,
                     simple_anno_size = unit(0.35, "cm"),
                     col = list("Effect size" = effect_col_fun),
                     annotation_name_gp =gpar(fontsize = 9),
                     show_legend = F, gp = gpar(col = "white"),
                     show_annotation_name = T)




H2<-Heatmap(heat2, cluster_rows = F, cluster_columns = F,
            width = ncol(heat)*unit(6, "mm"),
            height = nrow(heat)*unit(5, "mm"),column_names_rot = 0,
            left_annotation = c(annE, annP),
            right_annotation = barpl,
            name = "Median \n clr value",
            heatmap_legend_param = list(direction = "vertical" ,
                                        labels_gp = gpar(fontsize = 7),
                                        title_gp = gpar(fontsize = 9,
                                                        fontface="bold"),
                                        legend_height = unit(1.4, "cm")),
            column_names_gp = gpar(fontsize = 9),
            row_names_gp = gpar(fontsize = 12))
```

```
## Warning: The input is a data frame, convert it to the matrix.
```

```
H2.1<- draw(H2, heatmap_legend_side = "right")
```



```
H2.1
```

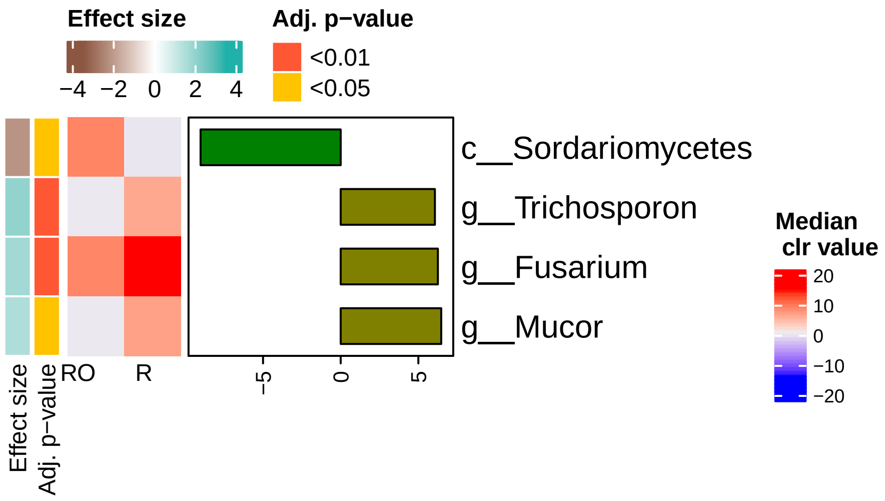


```
#heat3

treatment_col = structure(c("#008000", "#808000"),
                          names = c("Roots", "Rhizosphere"))
barpl = rowAnnotation("difference \nbetween groups" = anno_barplot(
  aldex_ro_ri$diff.btw,
  gp = gpar(fill = treatment_col[aldex_ro_ri$Type]),
  width = unit(3.5, "cm"),   height = unit(2, "cm")),
  show_annotation_name = F,
  annotation_name_gp =gpar(fontsize = 9),
  annotation_name_rot = 0)

annP = rowAnnotation("Adj. p-value" = aldex_ro_ri$p.value,
                     annotation_name_gp =gpar(fontsize = 9),
                     simple_anno_size = unit(0.35, "cm"),
                     annotation_legend_param = list(title_gp = gpar(fontsize = 9,
                                                    fontface="bold"),
                                                    labels_gp = gpar(fontsize = 9),
                                                    direction ="horizontal"),
                     col = cols_pvalue,show_legend = T,
                     gp = gpar(col = "white"), show_annotation_name = T)

annE = rowAnnotation("Effect size" = aldex_ro_ri$effect,
                     annotation_name_gp =gpar(fontsize = 9),
                     col = list("Effect size" = effect_col_fun),
                     simple_anno_size = unit(0.35, "cm"),
                     annotation_legend_param = list(title_gp = gpar(fontsize = 9,
                                                                    fontface="bold"),
                                                    labels_gp = gpar(fontsize = 9),
                                                    direction ="horizontal"),
                     show_legend = T, gp = gpar(col = "white"), show_annotation_name = T)




H3<-Heatmap(heat3, cluster_rows = F, cluster_columns = F,
            width = ncol(heat)*unit(5, "mm"),
            height = nrow(heat)*unit(4.5, "mm"),column_names_rot = 0,
            left_annotation = c(annE, annP), name = "Median \n clr value",
            right_annotation = barpl,
            heatmap_legend_param = list(direction = "vertical" , labels_gp = gpar(fontsize = 7),
                                        title_gp = gpar(fontsize = 9,  fontface="bold"),
                                        legend_height = unit(1.4, "cm")),
            column_names_gp = gpar(fontsize = 9),
            row_names_gp = gpar(fontsize = 12)) %v% NULL
```

```
## Warning: The input is a data frame, convert it to the matrix.
```

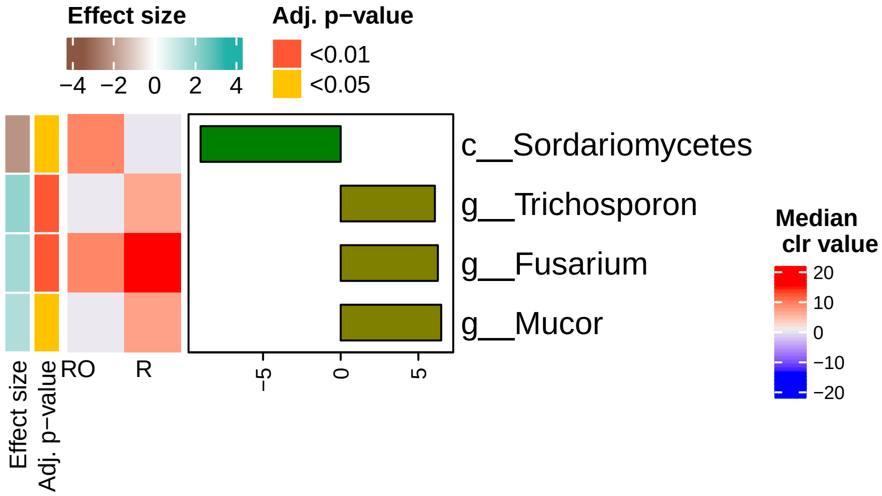
```
H3.1<- draw(H3, annotation_legend_side = "top", heatmap_legend_side="right")
```

```
## Warning: ‘legend_height‘ you specified is too small, use the default minimal
## height.

## Warning: ‘legend_height‘ you specified is too small, use the default minimal
## height.

## Warning: ‘legend_height‘ you specified is too small, use the default minimal
## height.
```



```
H3.1
```

```
## Warning: ‘legend_height‘ you specified is too small, use the default minimal
## height.

## Warning: ‘legend_height‘ you specified is too small, use the default minimal
## height.
```

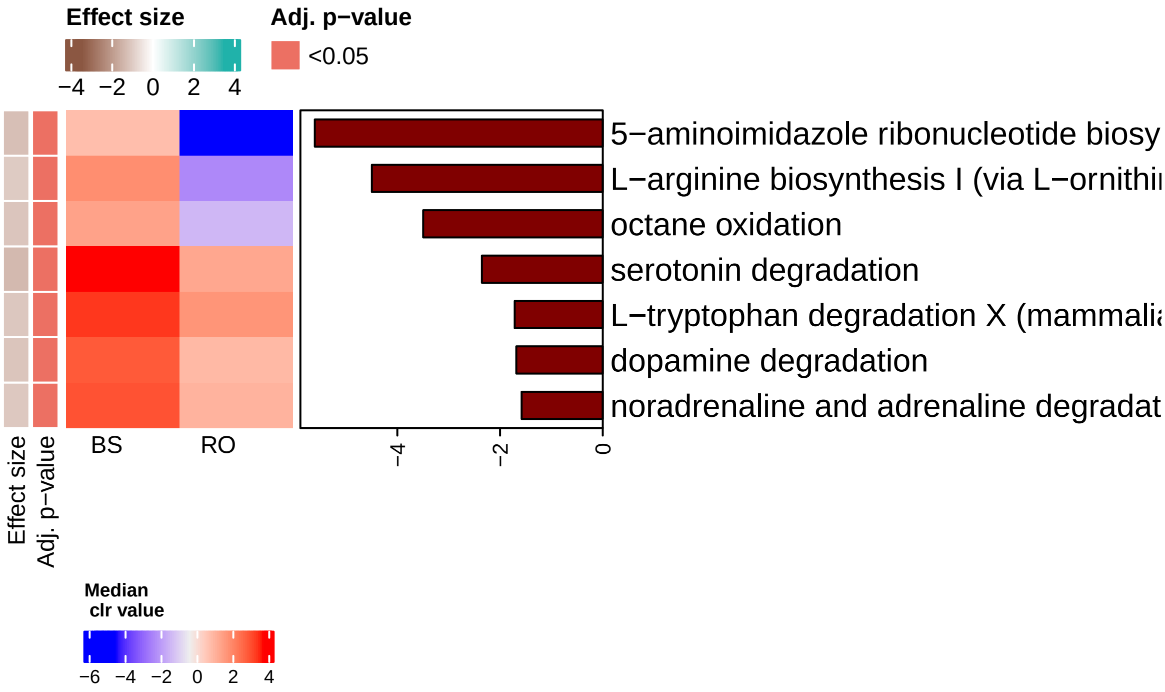


```
#heat4
#heatmap funciones
cols_pvalue <- list('Adj. p-value' = c("<0.001" = '#AB0000',
                                   "<0.01"  =  '#FF0000',
                                 "<0.05"="#EC7063",
                                ">0.05"="#F9EBEA"))

treatment_col = structure(c("#008000", "#800000"),
                          names = c("Roots", "Non-rhizospheric"))
barpl = rowAnnotation("difference \nbetween groups" = anno_barplot(
  aldex_nr_ro_funct$diff.btw,
  gp = gpar(fill = treatment_col[aldex_nr_ro_funct$Type]),
  width = unit(4, "cm"),   height = unit(2, "cm")),
show_annotation_name = F,
  annotation_name_gp =gpar(fontsize = 8),
  annotation_name_rot = 0)

annP = rowAnnotation('Adj. p-value' = aldex_nr_ro_funct$p.value,
                     annotation_name_gp =gpar(fontsize = 9),
                     simple_anno_size = unit(0.35, "cm"),
                     annotation_legend_param = list(title_gp = gpar(fontsize = 9,
                                                    fontface="bold"),
                                                    labels_gp = gpar(fontsize = 9),
                                                    direction ="vertical"),
                     col = cols_pvalue,show_legend = T,
                     gp = gpar(col = "white"), show_annotation_name = T)

annE = rowAnnotation("Effect size" = aldex_nr_ro_funct$effect,
                     annotation_name_gp =gpar(fontsize = 9),
                     col = list("Effect size" = effect_col_fun),
```

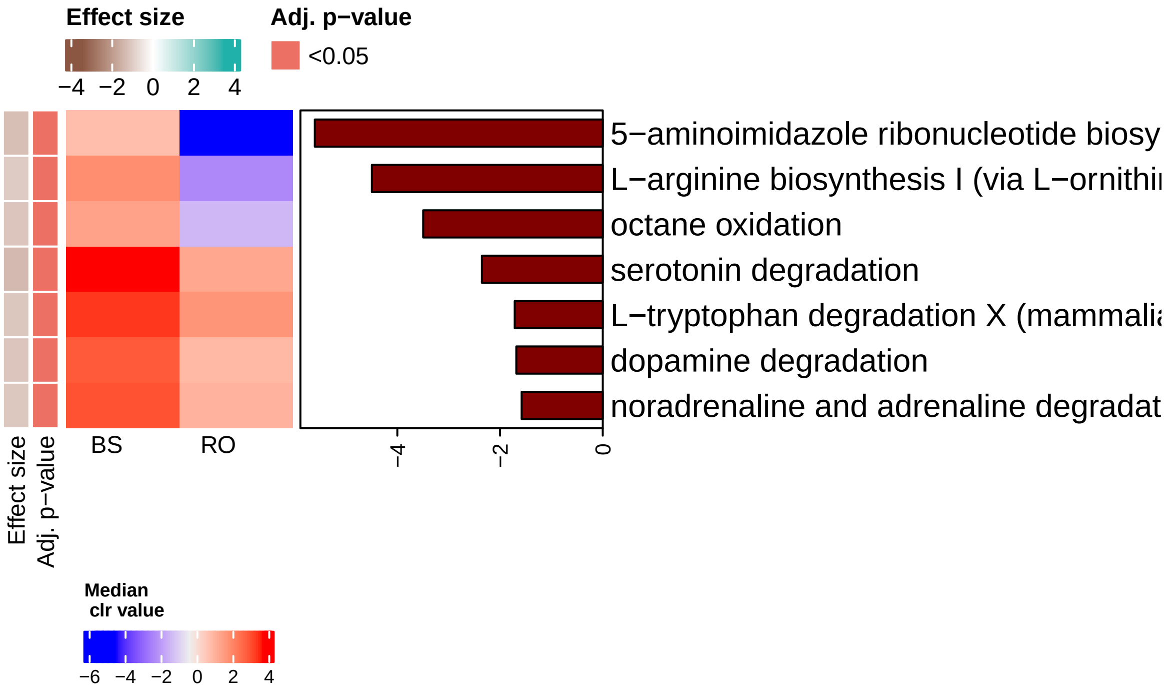
```r
                      simple_anno_size = unit(0.35, "cm"),
                      annotation_legend_param = list(title_gp = gpar(fontsize = 9,
                                                   fontface="bold"),
                                                labels_gp = gpar(fontsize = 9),
                                                direction ="horizontal"),
                      show_legend = T, gp = gpar(col = "white"), show_annotation_name = T)




H4<-Heatmap(heat4, cluster_rows = F, cluster_columns = F,  width = ncol(heat)*unit(10, "mm"),
            height = nrow(heat)*unit(6, "mm"),column_names_rot = 0,
            left_annotation = c(annE, annP), name = "Median \n clr value",
            right_annotation = barpl,
            heatmap_legend_param = list(direction = "horizontal" , labels_gp = gpar(fontsize = 7),
                                        title_gp = gpar(fontsize = 7,  fontface="bold"),
                                        legend_height = unit(1.4, "cm")),
            column_names_gp = gpar(fontsize = 9),
            row_names_gp = gpar(fontsize = 12)) %v% NULL
```

```
## Warning: The input is a data frame, convert it to the matrix.
```

```r
H4.1<- draw(H4, annotation_legend_side = "top", heatmap_legend_side="bottom")
```



```r
H4.1
```

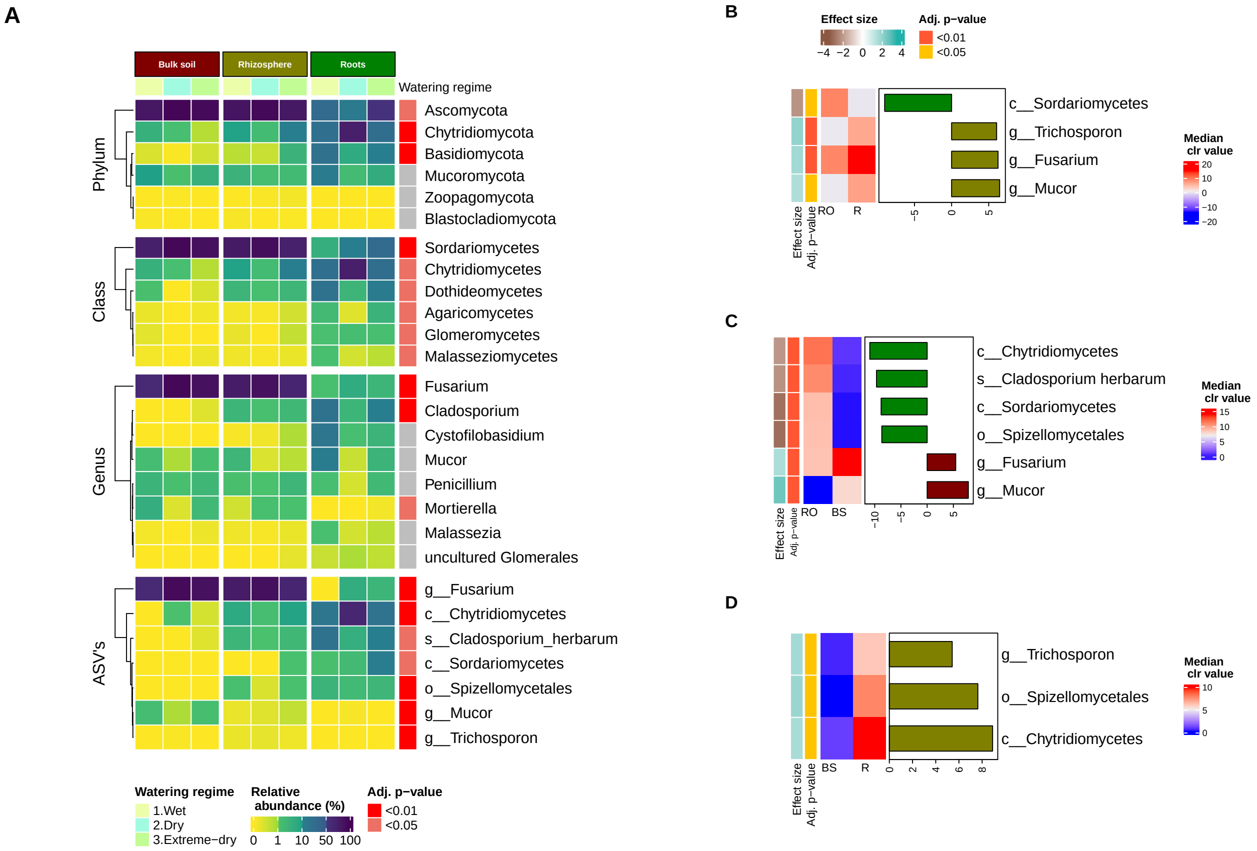


```
aldex_H1<- grid.grabExpr(draw(H1.1))
aldex_H2<- grid.grabExpr(draw(H2.1))
aldex_H3<- grid.grabExpr(draw(H3.1))
```

```
## Warning: ‘legend_height‘ you specified is too small, use the default minimal
## height.

## Warning: ‘legend_height‘ you specified is too small, use the default minimal
## height.
```

```
aldex_H4<- grid.grabExpr(draw(H4.1))


paired_grouped<-plot_grid(aldex_H3, aldex_H1, aldex_H2, nrow =  3,
                          rel_heights = c(1.1, 1, 1),
                          labels = c("B", "C", "D"))+ theme(
                            plot.margin = unit(c(0, 0, 0, 0), "cm"))
```

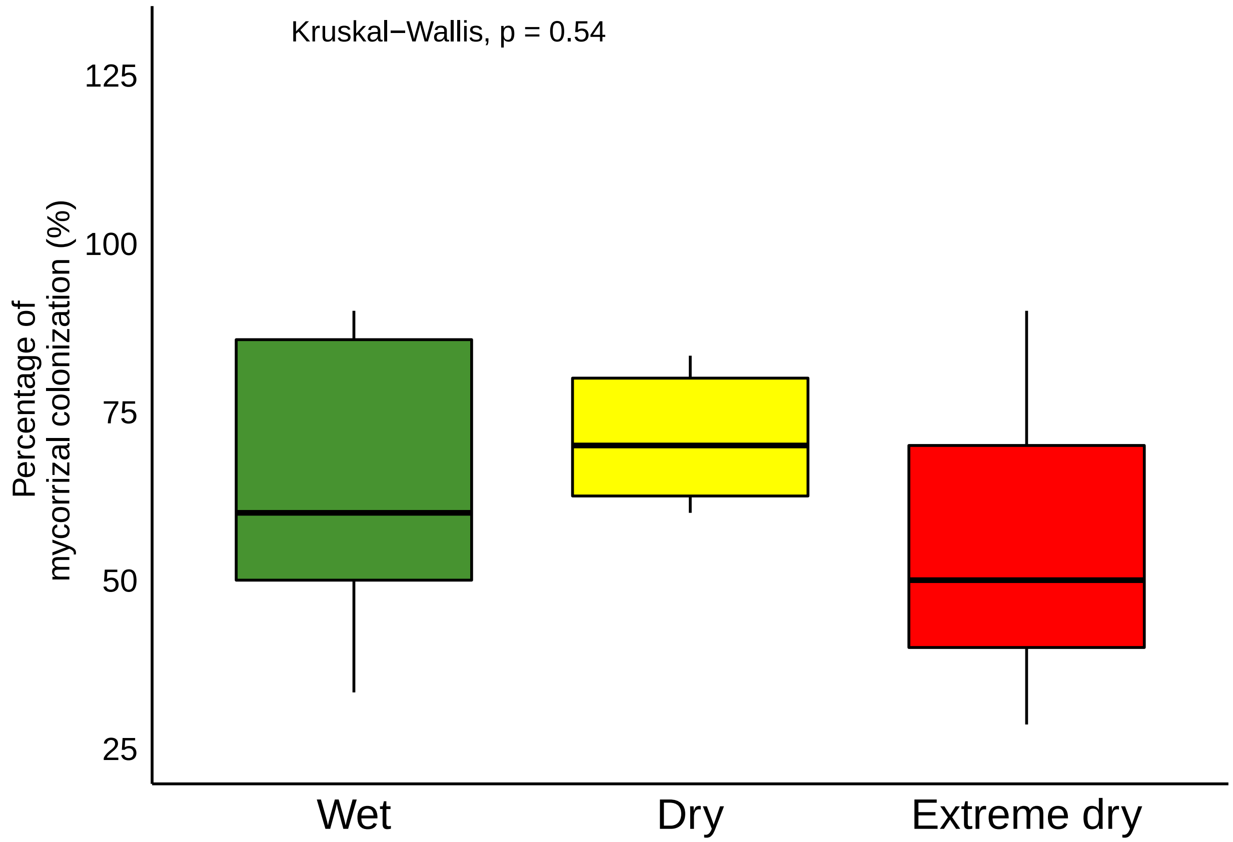
```
c<-cowplot::plot_grid(heatm ,paired_grouped,  ncol = 2,  rel_widths = c(1.8,1.4), labels = c("A", NULL)
c
```



```
#ggsave('../../Figures_final/Fig4.tiff',
 #  width = 12.5, height = 9, dpi = 300, plot =c)
#ggsave('../../Figures_final/FigS2.tiff',
 # width = 10, height = 6, dpi = 300, plot =aldex_H4)
```

```
#load libraries and files
library(imager)
library(cowplot)
library(ggpubr)
library(tidyverse)
library(magrittr)
library(ggpubr)
library(readxl)
library(viridis)
arbus2<-load.image(file = "../Figures/arbus_raw.png")
photo_panel <- ggdraw() + draw_image(arbus2, scale = 1)

#grafica arbusculos


dosmeses<- readxl::read_excel("../Data/MICOR ARBUS.xlsx", sheet = 2, range = "A1:O28")
cuatromeses<- readxl::read_excel("../Data/MICOR ARBUS.xlsx", sheet = 3, range = "A1:O29")
seismeses<- readxl::read_excel("../Data/MICOR ARBUS.xlsx", sheet = 4, range = "A1:O28")

arbus<- bind_rows(list(m2 = dosmeses, m4 = cuatromeses, m6=seismeses), .id = "Tiempos")
arbusc<- arbus %>% dplyr::select(L, P, "T", C, Tiempos, Porcentaje="%")
```

```r
#ggbloxplot de todas

arbusc$Time <- factor(arbusc$Tiempos,
                        levels = c("0", "m2", "m4", "m6"),
                        labels = c("day0", "2 months", "4 months", "6 months"))
arbusc$Treatment <- factor(arbusc$T,
                             levels = c("1", "2", "3"),
                             labels = c("Wet", "Dry", "Extreme dry"))


pallete = c("#479330", "#FFFF00", "#FF0000")

p<-arbusc %>% filter(Time == "2 months") %>%  ggboxplot(
              ., x = "Treatment", y = "Porcentaje", #add = "mean_se",
              palette = pallete, color= "black", fill = "Treatment",outlier.shape = NA,
             # position = position_dodge(0.8) ,
             xlab = "Time",
              ylab = "Percentage of \n mycorrizal colonization (%)")

p1<-p + #stat_compare_means(aes(group= Treatment))+
                               # ,label= paste0(..method..,"\n", "p=",..p.format..)))+
  stat_compare_means(label.y = 130, show.legend = F )+ font("legend.title", size = 12)+
  font("legend.text", size = 12)+theme(axis.title.x = element_blank(),
                                       axis.text.x = element_text(size = 16),
                                       legend.position = "none",
                                       axis.ticks = element_blank())+theme(legend.position = "none")+ gu



p1
```



```
#Guilds files

#Guild level
filo<- read_tsv("../Data/merge_table_240_noplant_filtered.guilds.txt") %>%
  rename( OTUID = "#OTU ID")
metadata<- read.csv("../Data/FINALMAP18S_2.csv") %>% dplyr::select(
  key=sampleid, groups, Treatment, Type_of_soil)

relabunda<- function(x){(t(t(x)/colSums(x)))*100}

filo2<- data.frame(filo[1], relabunda(filo[2:66]),
                   filo[67:76] , check.names = F)


guilds_gather<-filo2%>%dplyr::select(`1.2RO`:`2.10NR`, `Guild` )%>% gather(
  -`Guild`,key = "key", value = "value")

guilds_gather_sum<- guilds_gather %>% group_by(
  key,`Guild`)%>% summarise(prop =sum(value)) %>%
    mutate(Type = case_when(
      str_detect(key, "RI") ~ "Rhizosphere",
      str_detect(key,"US") ~ "Uncultivated",
      str_detect(key,"RO") ~ "Roots",
      str_detect(key,"SE") ~ "Initials",
      str_detect(key,"P") ~ "Initials",
      str_detect(key,"NR") ~ "Non-Rhizosphere")) %>% inner_join(metadata)
```

```
#Trophic level

trophic_gather<-filo2%>%dplyr::select(`1.2RO`:`7.23NR`, `Trophic Mode` )%>% gather(
  -`Trophic Mode`,key = "key", value = "value")

trophic_gather_sum<-trophic_gather %>% group_by(
  key,`Trophic Mode`)%>% summarise(prop =sum(value)) %>%
  mutate(Type = case_when(
    str_detect(key, "RI") ~ "Rhizosphere",
    str_detect(key,"US") ~ "Uncultivated",
    str_detect(key,"RO") ~ "Roots",
    str_detect(key,"SE") ~ "Initials",
    str_detect(key,"P") ~ "Initials",
    str_detect(key,"NR") ~ "Non-Rhizosphere"))%>% inner_join(metadata)

#Growth level

growth_gather<-filo2%>%dplyr::select(`1.2RO`:`7.23NR`, `Growth Morphology` )%>% gather(
  -`Growth Morphology`,key = "key", value = "value")

growth_gather_sum<-growth_gather %>% group_by(
  key,`Growth Morphology`)%>% summarise(prop =sum(value)) %>%
  mutate(Type = case_when(
    str_detect(key, "RI") ~ "Rhizosphere",
    str_detect(key,"US") ~ "Uncultivated",
    str_detect(key,"RO") ~ "Roots",
    str_detect(key,"SE") ~ "Initials",
    str_detect(key,"P") ~ "Initials",
    str_detect(key,"NR") ~ "Non-Rhizosphere"))%>% inner_join(metadata)
```

Let's plot!

```
#colors plot
color_type<- c("#800000", "#808000", "#008000", "#D35400", "#2E4053")


guilds_gather_sum_join<- guilds_gather_sum  %>%
  rename(Level = Guild) %>% mutate(
  type = "Guild")%>%filter(
Level %in% c("Leaf Saprotroph" , "Arbuscular Mycorrhizal"))
trophic_gather_sum_join<-trophic_gather_sum %>%  rename(
  Level =`Trophic Mode` ) %>% mutate(
  type = "Trophic")%>%filter(
 Level  %in% c("Saprotroph" , "Symbiotroph"))

gather_sum<- rbind(guilds_gather_sum_join, trophic_gather_sum_join) %>%
  mutate(Treatments = case_when(
    str_detect(Treatment, "1") ~ "Wet",
    str_detect(Treatment, "2") ~ "Dry",
    str_detect(Treatment, "3") ~ "Extreme-dry"))
gather_sum$Treatments<- factor(gather_sum$Treatments, levels = c(
  "Wet", "Dry", "Extreme-dry"))
```

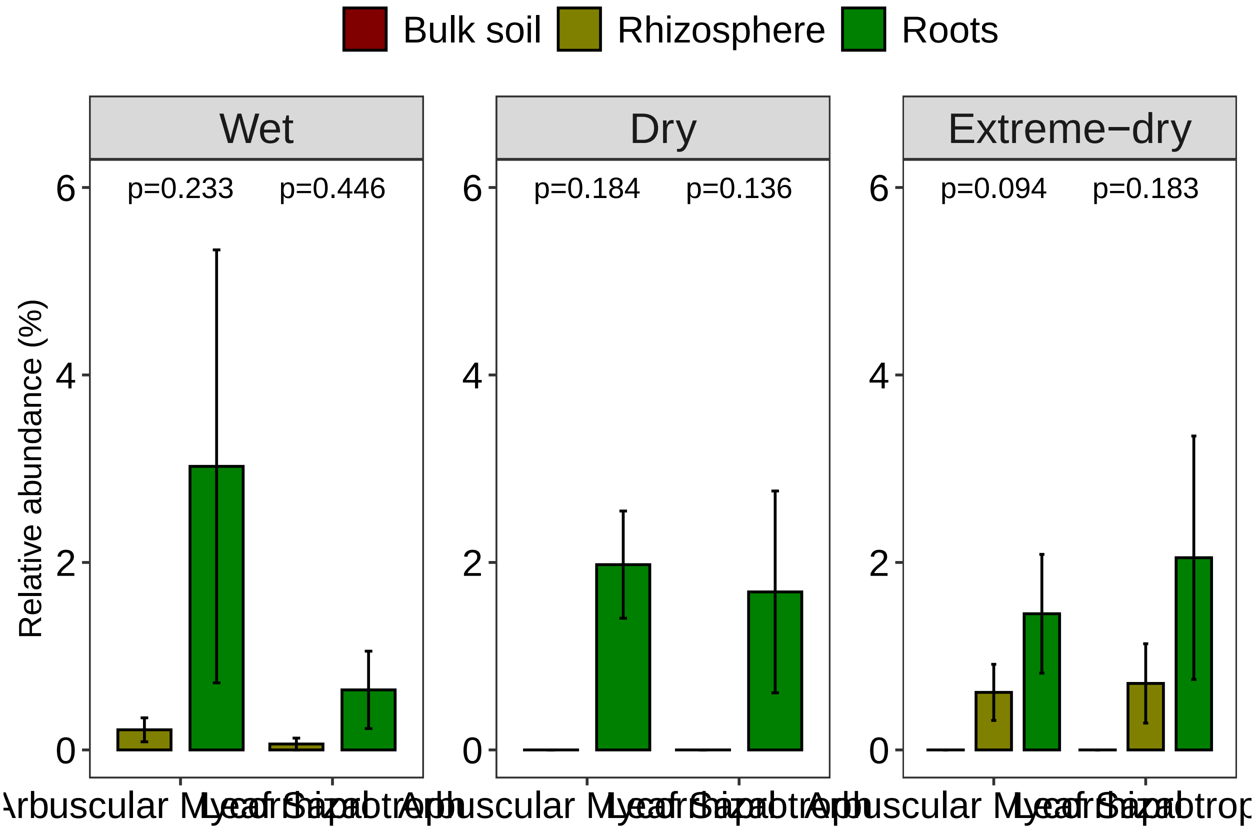
```
#compartment effect
library(lme4)
library(nlme)
library(pgirmess)
meta<- read.delim("../Data/FINALMAP18S_plant.csv",
                  check.names = F) %>% dplyr::select("#SampleID", "Plant")

gather_sum_plant<- gather_sum %>% inner_join(meta, by = c("key"="#SampleID"))

guild_wet_mico<- gather_sum_plant %>%  filter(type=="Guild")  %>% filter(
  Treatments =="Wet") %>% filter(Level=="Arbuscular Mycorrhizal")
guild_wet_leafsap<- gather_sum_plant %>%  filter(type=="Guild")  %>% filter(
  Treatments =="Wet") %>% filter(Level=="Leaf Saprotroph")

guild_dry_mico<- gather_sum_plant %>%  filter(type=="Guild")  %>% filter(
  Treatments =="Dry") %>% filter(Level=="Arbuscular Mycorrhizal")
guild_dry_leafsap<- gather_sum_plant %>%  filter(type=="Guild")  %>% filter(
  Treatments =="Dry") %>% filter(Level=="Leaf Saprotroph")


guild_exdry_mico<- gather_sum_plant %>%  filter(type=="Guild")  %>% filter(
  Treatments =="Extreme-dry") %>% filter(Level=="Arbuscular Mycorrhizal")
guild_exdry_leafsap<- gather_sum_plant %>%  filter(type=="Guild")  %>% filter(
  Treatments =="Extreme-dry") %>% filter(Level=="Leaf Saprotroph")


guild_wet_mico1<-lme(prop~ Type_of_soil, random=~1 |Plant, data = guild_wet_mico)
guild_wet_mico2<-PermTest(guild_wet_mico1)
guild_wet_mico2
```

```
##
## Monte-Carlo test
##
## Call:
## PermTest.lme(obj = guild_wet_mico1)
##
## Based on 1000 replicates
## Simulated p-value:
##              p.value
## (Intercept)    0.853
## Type_of_soil   0.233
```

```
guild_wet_leafsap1<-lme(prop~ Type_of_soil, random=~1 |Plant, data = guild_wet_leafsap)
guild_wet_leafsap2<-PermTest(guild_wet_leafsap1)

guild_dry_mico1<-lm(prop~ Type_of_soil,  data = guild_dry_mico)
guild_dry_mico2<-PermTest(guild_dry_mico1)
guild_dry_leafsap1<-lm(prop~ Type_of_soil, data = guild_dry_leafsap)
guild_dry_leafsap2<-PermTest(guild_dry_leafsap1)

guild_exdry_mico1<-lme(prop~ Type_of_soil, random=~1 |Plant, data = guild_exdry_mico)
guild_exdry_mico2<-PermTest(guild_exdry_mico1)
```

```
guild_exdry_leafsap1<-lme(prop~ Type_of_soil, random=~1 |Plant, data = guild_exdry_leafsap)
guild_exdry_leafsap2<-PermTest(guild_exdry_leafsap1)


annot_df_guild<- data.frame(
  Level= c("Arbuscular Mycorrhizal", "Leaf Saprotroph",
           "Arbuscular Mycorrhizal", "Leaf Saprotroph",
           "Arbuscular Mycorrhizal", "Leaf Saprotroph"),
  prop=c(6,6,6, 6, 6,6),
  Treatments=c("Wet", "Dry", "Extreme-dry"),
  label=c(
paste0("p=",guild_wet_mico2$resultats$p.value[2]),
paste0("p=",guild_wet_leafsap2$resultats$p.value[2]),
paste0("p=",guild_dry_mico2$resultats$p.value[1]),
paste0("p=",guild_dry_leafsap2$resultats$p.value[1]),
paste0("p=",guild_exdry_mico2$resultats$p.value[2]),
paste0("p=",guild_exdry_leafsap2$resultats$p.value[2])),  check.names = F)

annot_df_guild$Treatments<- factor(annot_df_guild$Treatments,
                                   levels =c("Wet", "Dry", "Extreme-dry") )
```

```
phyloplot4 <- gather_sum %>%  mutate(Type=case_when(
  Type =="Non-Rhizosphere"~"Bulk soil",
  TRUE ~ as.character(Type)
))%>%  filter(!key=="6.13RO"&!key=="5.14RO")  %>%   filter(
  type=="Guild") %>%
  ggbarplot(x = "Level", y="prop", color = "black", fill = "Type",
            position = position_dodge(), add = "mean_se",
            facet.by = "Treatments")+
  facet_wrap(~Treatments, scales = "free", ncol=3, strip.position = "top")+
  theme_bw()+
  theme(panel.spacing=unit(1,"lines"),
        strip.text.x = element_text(size = 16),
        axis.text =  element_text(colour = "black", size = 14),
        axis.title.x = element_blank(),
        axis.title.y = element_text(size = 12),
        legend.title = element_blank(),
        legend.text = element_text(size=14),
        #axis.text.x = element_blank(),
        panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        legend.position = "top")+scale_fill_manual(values =color_type)+
  ylab("Relative abundance (%)")+geom_text(data = annot_df_guild,label=annot_df_guild$label)
phyloplot4
```

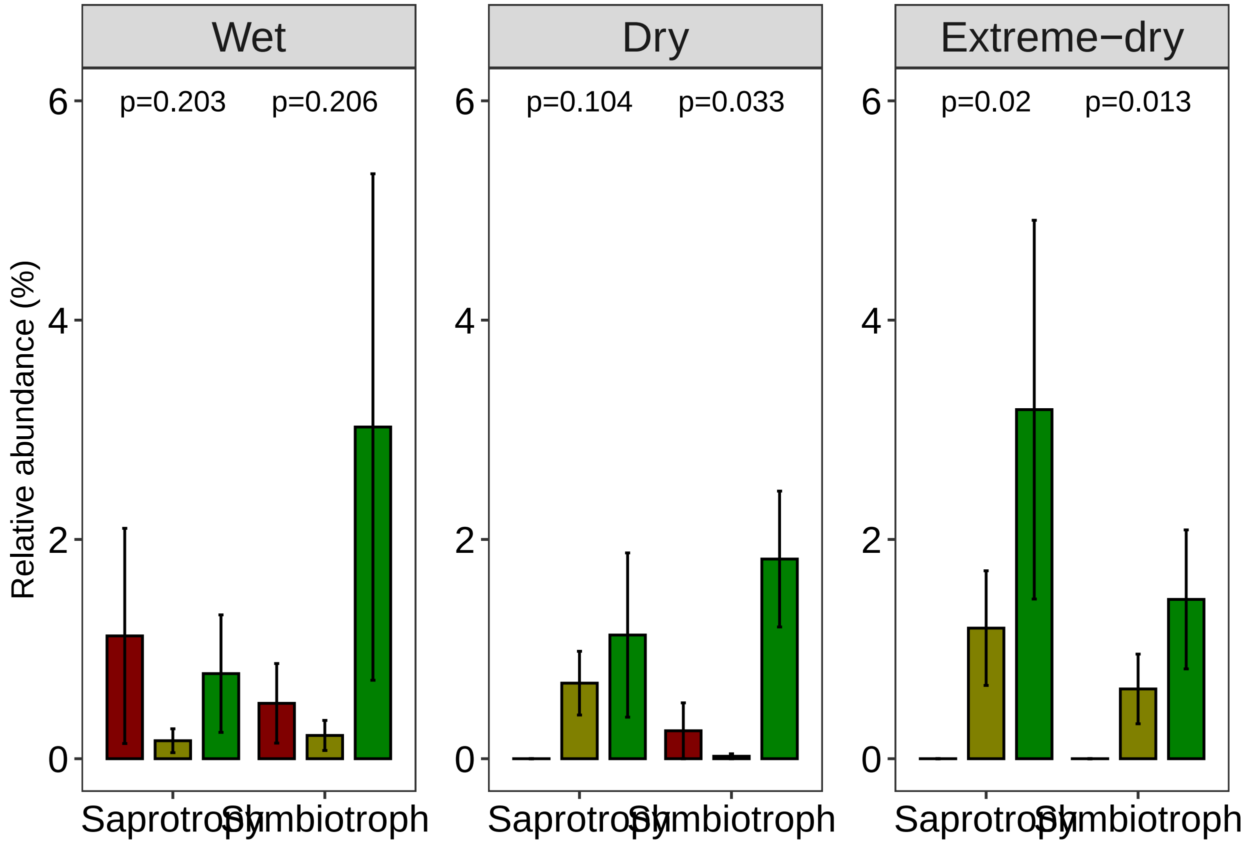


```
#compartment effect
library(lme4)
library(nlme)
library(pgirmess)
meta<- read.delim("../Data/FINALMAP18S_plant.csv",
                  check.names = F) %>% dplyr::select("#SampleID", "Plant")
gather_sum_plant<- gather_sum %>% inner_join(meta, by = c("key"="#SampleID"))

guild_wet_mico<- gather_sum_plant %>%  filter(type=="Trophic")  %>% filter(
  Treatments =="Wet") %>% filter(Level=="Symbiotroph")
guild_wet_leafsap<- gather_sum_plant %>%  filter(type=="Trophic")  %>% filter(
  Treatments =="Wet") %>% filter(Level=="Saprotroph")

guild_dry_mico<- gather_sum_plant %>%  filter(type=="Trophic")  %>% filter(
  Treatments =="Dry") %>% filter(Level=="Symbiotroph")
guild_dry_leafsap<- gather_sum_plant %>%  filter(type=="Trophic")  %>% filter(
  Treatments =="Dry") %>% filter(Level=="Saprotroph")


guild_exdry_mico<- gather_sum_plant %>%  filter(type=="Trophic")  %>% filter(
  Treatments =="Extreme-dry") %>% filter(Level=="Symbiotroph")
guild_exdry_leafsap<- gather_sum_plant %>%  filter(type=="Trophic")  %>% filter(
  Treatments =="Extreme-dry") %>% filter(Level=="Saprotroph")

guild_wet_mico1<-lme(prop~ Type_of_soil, random=~1 |Plant, data = guild_wet_mico)
guild_wet_mico2<-PermTest(guild_wet_mico1)
guild_wet_mico2
```

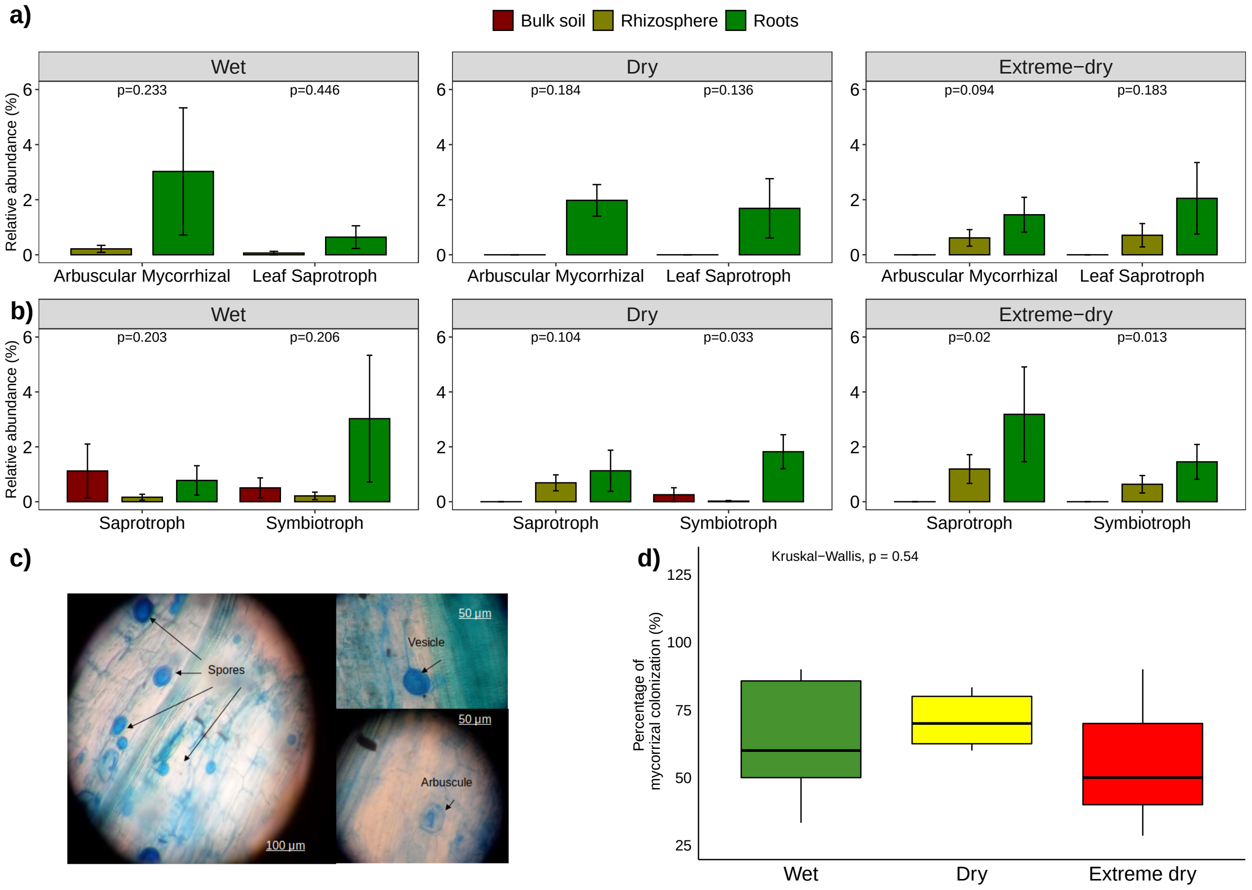
```
##
## Monte-Carlo test
##
## Call:
## PermTest.lme(obj = guild_wet_mico1)
##
## Based on 1000 replicates
## Simulated p-value:
##               p.value
## (Intercept)    0.583
## Type_of_soil   0.206
```

```
guild_wet_leafsap1<-lme(prop~ Type_of_soil, random=~1 |Plant, data = guild_wet_leafsap)
guild_wet_leafsap2<-PermTest(guild_wet_leafsap1)

guild_dry_mico1<-lm(prop~ Type_of_soil,  data = guild_dry_mico)
guild_dry_mico2<-PermTest(guild_dry_mico1)

guild_dry_leafsap1<-lm(prop~ Type_of_soil, data = guild_dry_leafsap)
guild_dry_leafsap2<-PermTest(guild_dry_leafsap1)

guild_exdry_mico1<-lme(prop~ Type_of_soil, random=~1 |Plant, data = guild_exdry_mico)


guild_exdry_mico2<-PermTest(guild_exdry_mico1)
guild_exdry_leafsap1<-lme(prop~ Type_of_soil, random=~1 |Plant, data = guild_exdry_leafsap)
guild_exdry_leafsap2<-PermTest(guild_exdry_leafsap1)


annot_df_guild<- data.frame(
  Level= c("Symbiotroph", "Saprotroph", "Symbiotroph",
           "Saprotroph","Symbiotroph", "Saprotroph"),
  prop=c(6,6,6, 6, 6,6),
  Treatments=c("Wet", "Dry", "Extreme-dry"),
  label=c(
paste0("p=",guild_wet_mico2$resultats$p.value[2]),
paste0("p=",guild_wet_leafsap2$resultats$p.value[2]),
paste0("p=",guild_dry_mico2$resultats$p.value[1]),
paste0("p=",guild_dry_leafsap2$resultats$p.value[1]),
paste0("p=",guild_exdry_mico2$resultats$p.value[2]),
paste0("p=",guild_exdry_leafsap2$resultats$p.value[2])),  check.names = F)

annot_df_guild$Treatments<- factor(annot_df_guild$Treatments,
                                   levels =c("Wet", "Dry", "Extreme-dry") )
```

```
phyloplot5 <- gather_sum %>%  mutate(Type=case_when(
  Type =="Non-Rhizosphere"~"Bulk soil",
  TRUE ~ as.character(Type)
))%>%  filter(!key=="6.13RO"&!key=="5.14RO")  %>%   filter(type=="Trophic") %>%
  ggbarplot(x = "Level", y="prop", color = "black", fill = "Type",
            position = position_dodge(), add = "mean_se",
            facet.by = "Treatments")+
  facet_wrap(~Treatments, scales = "free",
             ncol=3, strip.position = "top")+
  theme_bw()+
  theme(panel.spacing=unit(1,"lines"),
        strip.text.x = element_text(size = 16),
        axis.text =  element_text(colour = "black", size = 14),
        axis.title.x = element_blank(),
        axis.title.y = element_text(size = 12),
        legend.title = element_blank(),
        legend.text = element_text(size=14),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        legend.position = "top")+
  scale_fill_manual(values =color_type)+
  ylab("Relative abundance (%)")+
  geom_text(data = annot_df_guild,label=annot_df_guild$label)+
  theme(legend.position = "none")

phyloplot5
```



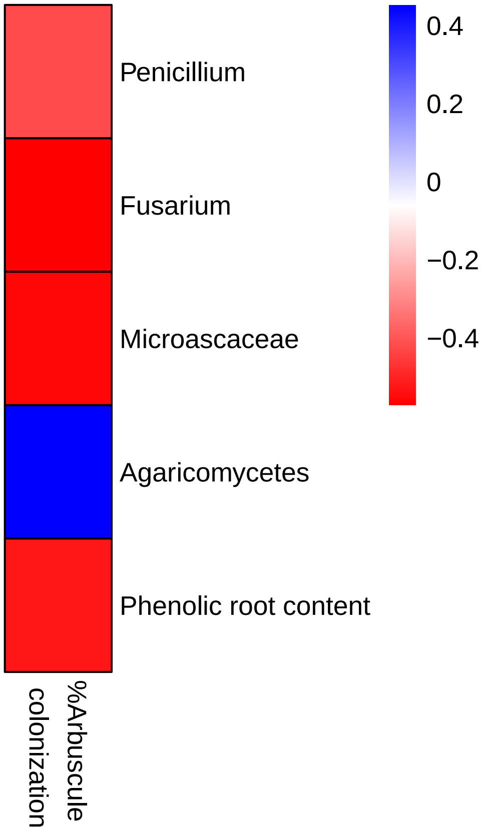
```
library(cowplot)
a1<-plot_grid(photo_panel, p1, labels = c("c)", "d)"), label_size = 20)
a2<- plot_grid(phyloplot4,phyloplot5,nrow = 2, rel_heights = c(1.2,1), labels = c("a)", "b)"), label_si
b1<- plot_grid( a2,a1 ,nrow = 2, rel_heights = c(2,1.3))
b1
```



```
#ggsave('../../Figures_final//Fig5.tiff',
#width = 14, height = 10, dpi = 300, plot = b1)
```

```
#ARBUSCULES IMAGE
library(imager)
library(cowplot)
library(viridis)
library(tidyverse)
library(ggpubr)
library(tidyverse)
library(qiime2R)
library(circlize)
library(viridis)
library(ggpubr)
library(ComplexHeatmap)
```

```
metadata2<- read_tsv("../Data/meta_dos.txt")
otutable<- read_qza("../../Data/merge_table_240_noplant_filtered_nous.qza")$data
taxonomy<- read_qza("../Data/taxonomy_blast_240_0.97.qza")$data

parse<- qiime2R::parse_taxonomy(taxonomy)
phyl<- qiime2R::summarize_taxa(features = otutable, taxonomy = parse)$Genus
phy.ra <- function(x){(t(x)/colSums(x))}

phyl_ro<- phyl %>% dplyr::select_at(vars(contains("RO"))) %>% filter_all(
  ., any_vars(. != 0)) %>%   phy.ra(
  .) %>% as.data.frame() %>% rownames_to_column(var = "SampleID")

phy_met<- phyl_ro %>% inner_join(metadata2) %>% column_to_rownames(var = "SampleID") %>% dplyr::select(
  contains("d__"), hojas, raices, Arbus_per)

library(Hmisc)
cors<- phy_met
corr <- rcorr(as.matrix(cors), type=c("spearman"))
#print(corr)
cor.out <-corr$r
#write.table (cor.out, "yenCorrgen", sep="\t")

cor.plot<- cor.out %>% as.data.frame() %>% dplyr::select(Arbus_per) %>% filter(abs(Arbus_per)>=abs(0.4)

cor_heat<-cor.plot%>% rownames_to_column(var="id") %>%  mutate_at(
  "id", str_replace, "; NA", "")%>%  mutate_at(
  "id", str_replace, "; NA", "")%>%  mutate_at(
  "id", str_replace, "; NA", "")%>% mutate( tax= str_extract(id, "[^; ]+$")) %>% mutate(
    tax= case_when(
      tax=="raices"~ "Phenolic root content",
      TRUE~as.character(tax))) %>% filter(!id=="Arbus_per") %>%
  dplyr::select(tax, "%Arbuscule \n colonization"=Arbus_per) %>%
  column_to_rownames(var = "tax")



test_labels<- cor_heat[1]

my_palette <- colorRampPalette(c("red", "white","blue"))(n=599)
library(pheatmap)
library(gridExtra)
heats<-pheatmap(cor_heat, color = my_palette,legend = TRUE,cellheight = 50,
        cellwidth = 40,border_color = "black",
         cluster_cols = F, cluster_rows = F, fontsize = 10)
```



```
#heat<- grid.grabExpr(draw(heats))
library(ggplotify)
heat<- as.ggplot(heats)
```

```
otu_grouped<- read.delim("/home/steph/Documents/Documentos/fastas nuevos/18S/demultiplexed/demux/table_g
skip = 1, check.names = F) %>% column_to_rownames(var = "#OTU ID") %>% dplyr::select(-taxonomy)

taxo<- read_qza("/home/steph/Documents/Documentos/fastas nuevos/18S//demultiplexed/demux/seqs_and_taxono
phyra<-t(otu_grouped)/colSums(otu_grouped) *100

metadata<- read_tsv("/home/steph/Documents/Documentos/fastas nuevos/18S//MAPPINGS/FINALMAP_GROUPED.txt")
```

```
## Rows: 41 Columns: 27
```

```
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr  (3): #SampleID, Type_of_soil, Treatments
## dbl (23): T, Loc, FW, Root_L, Stem_L, Root_DW, Treatment, TOC, Root_FW, Leav...
## lgl  (1): Month
```

```
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
otu_grouped_relab<- phyra %>% t() %>% as.data.frame() %>% rownames_to_column(
  var = "Feature.ID") %>% inner_join(taxonomy) %>% dplyr::select(-Feature.ID, -Consensus)
```

```
## Joining, by = "Feature.ID"
```

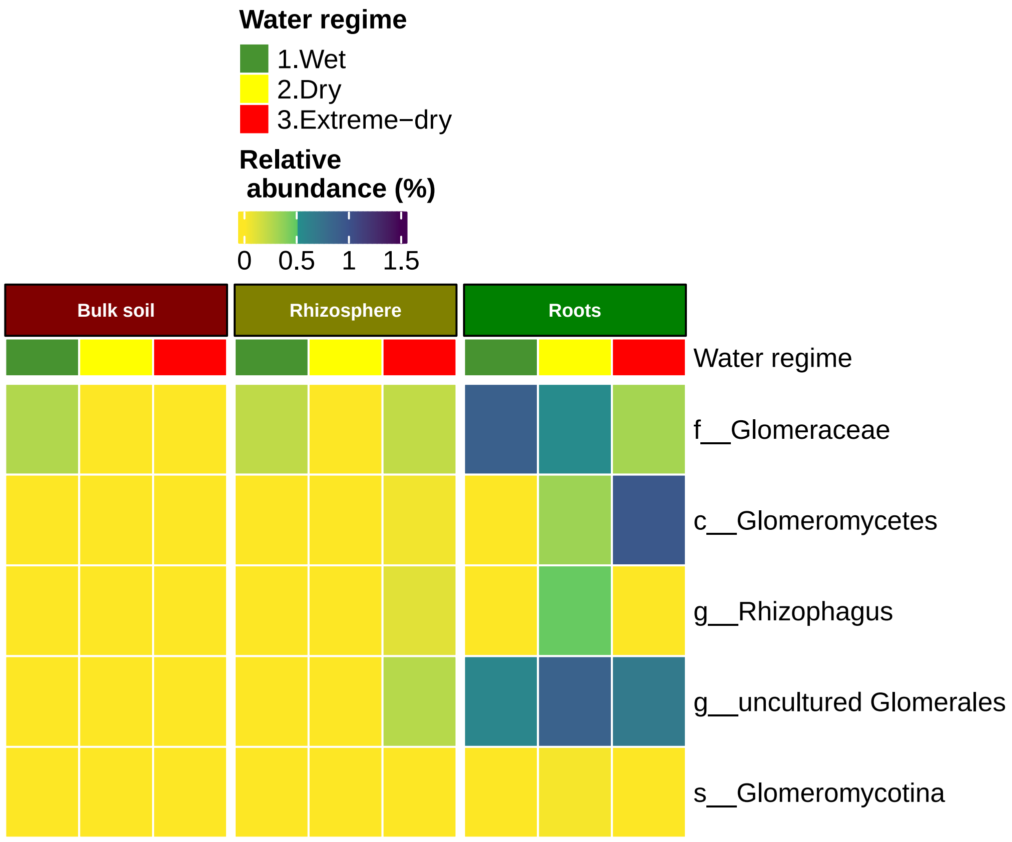
```
otu_glom<-otu_grouped_relab %>%
  filter(str_detect(Taxon, 'Glom')) %>% rownames_to_column(var = "rows") %>% unite(
    names, c(rows, Taxon), sep = "_"  ) %>% column_to_rownames(
      var = "names") %>% t() %>% as.data.frame(
        ) %>% rownames_to_column(var = "#SampleID") %>% inner_join(metadata)
```

```
## Joining, by = "#SampleID"
```

```
rel<-otu_glom %>% group_by(Type_of_soil, Treatments) %>% summarise_if(
  is.numeric, mean) %>% dplyr::select(
    Type_of_soil,Treatments,contains("d__")) %>% unite(
    "ids", Type_of_soil:Treatments) %>% column_to_rownames(var = "ids") %>%
  t() %>% as.data.frame() %>% rownames_to_column(var = "taxa") %>% mutate(
    tax= str_extract(taxa, "[^__]+$")) %>% mutate(
   taxon = case_when(
 tax=="sp."~"s__Glomeromycotina",
tax=="uncultured"~"g__uncultured Glomerales",
tax=="Glomeraceae"~"f__Glomeraceae",
tax=="Rhizophagus"~"g__Rhizophagus",
tax=="Glomeromycetes"~"c__Glomeromycetes",
      TRUE ~ as.character(tax))) %>% filter(!taxon=="Plectosphaerellaceae")

relab<-otu_glom %>% dplyr::select("#SampleID",contains("d__")) %>% column_to_rownames(
  var = "#SampleID") %>%
  t() %>% as.data.frame() %>% rownames_to_column(var = "taxa") %>% mutate(
    tax= str_extract(
    taxa, "[^__]+$")) %>% mutate(
    taxon = case_when(
 tax=="sp."~"s__Glomeromycotina",
tax=="uncultured"~"g__uncultured Glomerales",
tax=="Glomeraceae"~"f__Glomeraceae",
tax=="Rhizophagus"~"g__Rhizophagus",
tax=="Glomeromycetes"~"c__Glomeromycetes",
      TRUE ~ as.character(tax))) %>% filter(
        !taxon=="Plectosphaerellaceae")
rel_sum<- rel %>% group_by(taxon) %>% summarise_if(
  is.numeric, sum) %>% column_to_rownames(
  var = "taxon")


target <- c("f__Glomeraceae","c__Glomeromycetes",
            "g__Rhizophagus" ,"g__uncultured Glomerales","s__Glomeromycotina")
rel_sum2<-rel_sum %>%rownames_to_column(var = "name") %>% arrange(
  factor(name, levels = target)) %>% column_to_rownames(var = "name")

#other heatmap
col_fun2 = colorRamp2(c(0, 0.5, 0.5+1e-5, 1,1.5), viridis(5, option = "D", direction = -1))
split = rep(1:3, each = 3)
treats<- c("1.Wet", "2.Dry", "3.Extreme-dry")
cols_ho<- list("Water regime" = c("1.Wet" = '#479330',
                               "2.Dry"  =  '#FFFF00',
                               "3.Extreme-dry"="#FF0000"))
ha = HeatmapAnnotation(foo = anno_block(gp = gpar(
  fill = c("#800000" ,"#808000" ,"#008000", "#D35400")),
  labels = c("Bulk soil", "Rhizosphere", "Roots"),
  labels_gp = gpar(col = "white", fontsize = 7, fontface= "bold")))

ho = HeatmapAnnotation("Water regime" = c(rep(treats, 3)),
                       which = "col", col = cols_ho,
                       annotation_name_gp = gpar(fontsize=10),
                       show_legend = T, gp = gpar(col = "white",
                                                  fontize=12),
                       show_annotation_name = T)

ht<-Heatmap(rel_sum2, col = col_fun2,
            heatmap_legend_param = list(direction = "horizontal",
                 col_fun = col_fun2, title = "Relative \n abundance (%)",
                 at = c(0,0.5, 1, 1.5), break_dist = 1), rect_gp = gpar(col = "white", lwd = 1),
            top_annotation = c(ha, ho),column_split = split,
           cluster_columns = F, cluster_rows = F,show_heatmap_legend = TRUE,
         show_column_names = FALSE,
           width = ncol(rel_sum2)*unit(10, "mm"),
           height = nrow(rel_sum2)*unit(12, "mm"),
           column_title = NULL,row_names_gp = gpar(fontsize=10))+
  guides(fill=guide_legend(title="Water regime"))
```

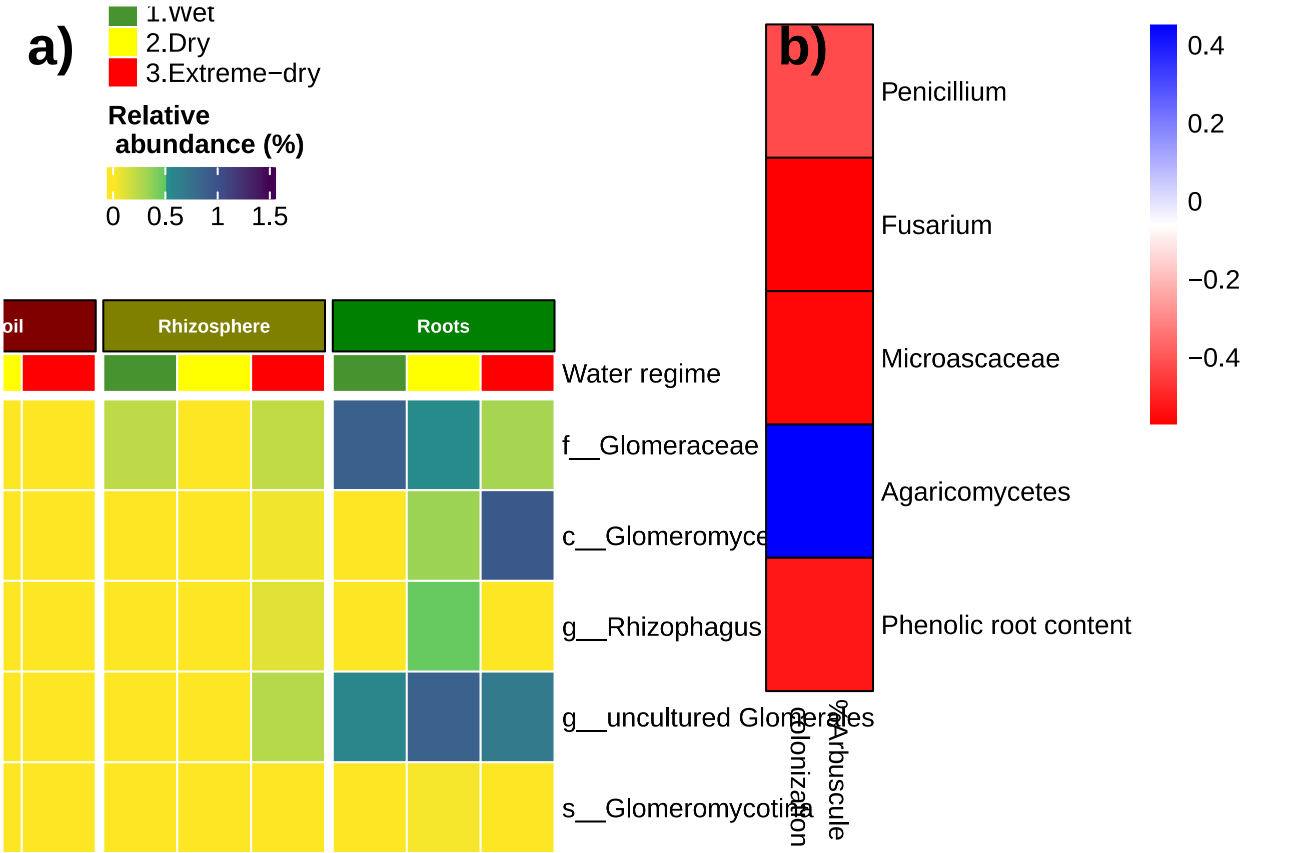
```
## Warning: The input is a data frame, convert it to the matrix.
```

```
abund<-draw(ht, heatmap_legend_side = "top", annotation_legend_side = "top")
```



```
abunds<- grid.grabExpr(draw(abund))
```

```
one<-plot_grid(abunds, heat, rel_widths = c(1.5,1), labels = c("a)", "b)"), label_size = 20)
one
```



```
#ggsave('../../Figures_final/FigS3.tiff',width = 10, height = 6, dpi = 300, plot =one)
```

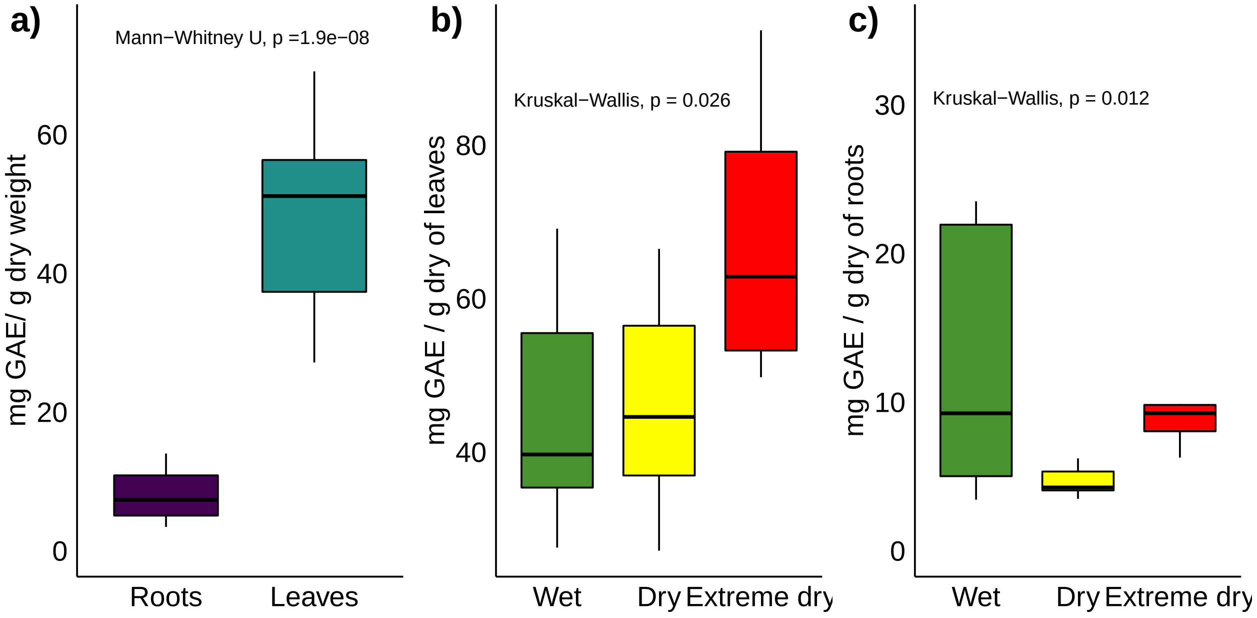
```
#PHENOLIC CONTENT
library(tidyverse)
library(ggpubr)
library(viridis)
library(cowplot)
pallete = c("#479330", "#FFFF00", "#FF0000")


fenoles<- readxl::read_excel("../Data/FENOLES_FINAL.xlsx", sheet = 1, range = "A1:G82")
fenoles$Time <- factor(fenoles$tiempo,
                       levels = c("0", "2", "4", "6"),
                       labels = c("day0", "2 months", "4 months", "6 months"))
fenoles$Treatment <- factor(fenoles$T,
                            levels = c("1", "2", "3"),
                            labels = c("Wet", "Dry", "Extreme dry"))



q<- fenoles %>% filter(Time == "2 months") %>% ggboxplot(
  ., x = "Treatment", y = "hojas",outlier.shape = NA,
  palette = pallete, color= "black", fill = "Treatment",
  xlab = "Time" ,
  ylab = "mg GAE / g dry of leaves")

q1<-q + stat_compare_means(aes(group= Treatment), label.y = 85)+
  font("legend.title", size = 8)+
  font("legend.text", size = 8)+theme(
    axis.title.x = element_blank(),
    legend.position = "none",
    axis.ticks = element_blank(),
    axis.text = element_text(size = 16),
         axis.title.y = element_text(size = 16))



r<-  fenoles %>% filter(Time == "2 months") %>%
  ggboxplot(
  ., x = "Treatment", y = "raices",
  palette = pallete, color= "black", fill = "Treatment",
  legend="none",outlier.shape = NA,
  xlab = "Time", ylab = "mg GAE / g dry of roots")
r1<-r +  stat_compare_means(aes(group= Treatment), label.y = 30)+
  theme(
      axis.title.x = element_blank(),
  axis.ticks = element_blank(),
  axis.text = element_text(size = 16),
         axis.title.y = element_text(size = 16))+scale_y_continuous(limits = c(0,35))


pallete2<- viridis_pal()(3)

fenoles2<- fenoles %>% gather(raices:hojas ,key = "parte", value = "fenoles")
colnames(fenoles2)
```

```
## [1] "L"         "P"         "T"         "C"         "tiempo"    "Time"
## [7] "Treatment" "parte"     "fenoles"
```

```
head(fenoles2)
```

```
## # A tibble: 6 x 9
##       L     P     T     C tiempo Time     Treatment   parte  fenoles
##   <dbl> <dbl> <dbl> <dbl>  <dbl> <fct>    <fct>       <chr>    <dbl>
## 1     7     1     1     1      2 2 months Wet         raices    9.24
## 2     4     3     1     1      2 2 months Wet         raices   21.8
## 3     6     1     2     1      6 6 months Dry         raices   31.6
## 4     1     2     2     1      4 4 months Dry         raices   NA
## 5     2     2     2     1      6 6 months Dry         raices   30.3
## 6     1     2     3     2      2 2 months Extreme dry raices    9.80
```

```
fenoles2$Part <- factor(fenoles2$parte,
                        levels = c("raices", "hojas"),
                        labels = c("Roots", "Leaves"))

s<-fenoles2 %>% filter(Time == "2 months") %>% ggboxplot(
  ., x = "Part", y = "fenoles", outlier.shape = NA,
  palette = pallete2, color= "black", fill = "Part",
  xlab = "Part of the plant",
  ylab = "mg GAE/ g dry weight")

s1<-s +   stat_compare_means(label.y = 73, show.legend = F, aes(
  label = paste0("Mann-Whitney U, p =", ..p.format..)))+
  font( "legend.title", size = 16)+
  font("legend.text", size = 16) +
  theme(legend.key.height= unit(4, 'mm'),legend.title = element_blank(),
        legend.key.width= unit(4, 'mm'),legend.position = "none")+
  theme( axis.title.x = element_blank(),
         axis.ticks = element_blank(),
         axis.text = element_text(size = 16),
         axis.title.y = element_text(size = 16))+
  scale_y_continuous(limits = c(0,75))




#joinning plots


third<- plot_grid(s1, q1,
                  r1, ncol = 3, align = "vh",axis = "l",
                  labels = c( "a)","b)", "c)"), label_size = 20)

third
```



```
#ggsave('../../Figures_final/Fig6.tiff', width = 12, height = 5, dpi = 300, plot = third)
```