FuncionesNormalizado

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```r
library("phyloseq")
library("ggplot2")
library("vegan")
```

```
## Loading required package: permute
```

```
## Loading required package: lattice
```

```
## This is vegan 2.6-4
```

```r
library("RColorBrewer")
library("stringi")
```

Cargado de datos originales

```r
setwd("/home/camila/GIT/Tesis_Maestria/Data/fresa_solena/Data1")
outpath = "/home/camila/GIT/Tesis_Maestria/Analisis_Comparativo/Fresa_Solena/Results_img"

fresa_kraken <- import_biom("fresa_kraken.biom")
colnames(fresa_kraken@tax_table@.Data) <- c("Kingdom", "Phylum", "Class", "Order", "Family", "Genus", "S
fresa_kraken@tax_table@.Data <- substr(fresa_kraken@tax_table@.Data,4,100)
colnames(fresa_kraken@otu_table@.Data) <- substr(colnames(fresa_kraken@otu_table@.Data),1,6)
metadata_fresa <- read.csv2("/home/camila/GIT/Tesis_Maestria/Data/fresa_solena/Data1/metadata.csv",head
fresa_kraken@sam_data <- sample_data(metadata_fresa)
fresa_kraken@sam_data$Sample<-row.names(fresa_kraken@sam_data)
colnames(fresa_kraken@sam_data)<-c('Treatment','Samples')
samples_to_remove <- c("MP2079","MP2080","MP2088","MP2109","MP2137")
fresa_kraken_fil <- prune_samples(!(sample_names(fresa_kraken) %in% samples_to_remove), fresa_kraken)
percentages_fil <- transform_sample_counts(fresa_kraken_fil, function(x) x*100 / sum(x) )
percentages_df <- psmelt(percentages_fil)
```

Normalización de los datos

Cada una de las funciones de normalización toma un objeto phloseq y devuelve un objeto physeq cuya tabla otu se transforma.

edgeRnorm

Esta función escala datos NGS normalizados utilizando la función de normalización provista en edgeR.

```r
edgeRnorm = function(phy, ...){
  require("edgeR")
  require("phyloseq")
  if (!taxa_are_rows(phy)) {
    phy <- t(phy) #transpone el objeto phyoseq
  }
  x = as(otu_table(phy), "matrix")
  x = x + 1
  y = edgeR::DGEList(counts = x, remove.zeros = TRUE)
  z = edgeR::calcNormFactors(y, ...)
  if (!all(is.finite(z$samples$norm.factors))) {
    stop("Something wrong with edgeR::calcNormFactors on this data, non-finite $norm.factors")
  }
  return(z)
}
z <- edgeRnorm(fresa_kraken_fil, method = "TMM")
```

```
## Loading required package: edgeR
```

```
## Loading required package: limma
```

unimos z con el resto del objeto phyloseq

```r
nor_fresa_kraken_fil <- merge_phyloseq(otu_table(z@.Data[[1]], taxa_are_rows = TRUE),
                        tax_table(fresa_kraken_fil@tax_table@.Data),
                        fresa_kraken_fil@sam_data)
```

Subconjunto de "Eukaryota"

```r
merge_Eukaryota<-subset_taxa(nor_fresa_kraken_fil,Kingdom=="Eukaryota")
```

Subconjunto de "Bacteria"

```r
merge_Bacteria<-subset_taxa(nor_fresa_kraken_fil,Kingdom=="Bacteria")
```

Funciones

Crea los subconjuntos de datos input phy phyliseq total tax rango al que queremos recortar output

```r
glomToGraph<-function(phy,tax){
  ## creamos el subconjunto dependiendo del linaje taxonomico deseado
  glom <- tax_glom(phy, taxrank = tax)
  ## sacamos los porcentajes
  percentages <- transform_sample_counts(glom, function(x) x*100 / sum(x) )
  percentages_df <- psmelt(percentages)
  return(list(glom,percentages,percentages_df))
}
```

Graficar abundancias stackbar

input entra el percentages_df

```r
Abundance_barras <- function(phy,tax,attribute,abundance_percentage){
  ##llamar funcion de datos
  Data <- glomToGraph(phy,tax)
  glom <- Data[[1]] #phyloseq
  percentages <- Data[[2]] #phyloseq
  percentages_df <- Data[[3]] # dataframe
  ## Graficamos para cada subconjunto las barras de abundancia
  plot_barras <- ggplot(data=percentages_df, aes_string(x='Sample', y='Abundance', fill=tax ,color=attri
    scale_colour_manual(values=c('white','black')) +
    geom_bar(aes(), stat="identity", position="stack") +
    labs(title = "Abundance", x='Sample', y='Abundance', color = tax) +
    theme(legend.key.size = unit(0.2, "cm"),
          legend.key.width = unit(0.25,"cm"),
          legend.position = "bottom",
          legend.direction = "horizontal",
          legend.title=element_text(size=8, face = "bold"),
          legend.text=element_text(size=6),
          text = element_text(size=12),
          axis.text.x = element_text(angle=90, size=5, hjust=1, vjust=0.5))
  percentages_df$tax<-percentages_df[,ncol(percentages_df)]
  percentages_df$tax[percentages_df$Abundance < abundance_percentage] <- "Others"
  percentages_df$tax <- as.factor(percentages_df$tax)
  plot_percentages <- ggplot(data=percentages_df, aes_string(x='Sample', y='Abundance', fill='tax' ,col
    scale_colour_manual(values=c('white','black')) +
    geom_bar(aes(), stat="identity", position="stack") +
    labs(title = "Abundance", x='Sample', y='Abundance', color = tax) +
    theme(legend.key.size = unit(0.3, "cm"),
          legend.key.width = unit(0.5,"cm"),
          legend.position = "bottom",
          legend.direction = "horizontal",
          legend.title=element_text(size=10, face = "bold"),
          legend.text=element_text(size=8),
          text = element_text(size=12),
          axis.text.x = element_text(angle=90, size=5, hjust=1, vjust=0.5))
  return(list(plot_barras,plot_percentages))
}
```

Graficar betadiversity

```
Beta_diversity <- function(phy,tax,attribute,distance){
  Data <- glomToGraph(phy,tax)
  glom <- Data[[1]]
  #CREAR UN GLOM AL 10%
  percentages <- Data[[2]]
  percentages_df <- Data[[3]]
  ## Beta diversidad
  meta_ord <- ordinate(physeq = percentages, method = "NMDS", distance = distance)
  plot_beta <- plot_ordination(physeq = percentages, ordination = meta_ord, color = attribute) +
    geom_text(mapping = aes(label = colnames(phy@otu_table@.Data)), size = 3, vjust = 1.5)
  return(plot_beta)
}
```

Graficar alphadiversity

```
Alpha_diversity <- function(phy,tax,attribute){
  ## llamamos la funcion que crea los dataset
  Data <- glomToGraph(phy,tax)
  glom <- Data[[1]]

  percentages <- Data[[2]]
  percentages_df <- Data[[3]]
  ## Alfa diversidad
  plot_alpha <- plot_richness(physeq = glom, measures = c("Observed","Chao1","Shannon","simpson"),x = a
  return(plot_alpha)
}
```
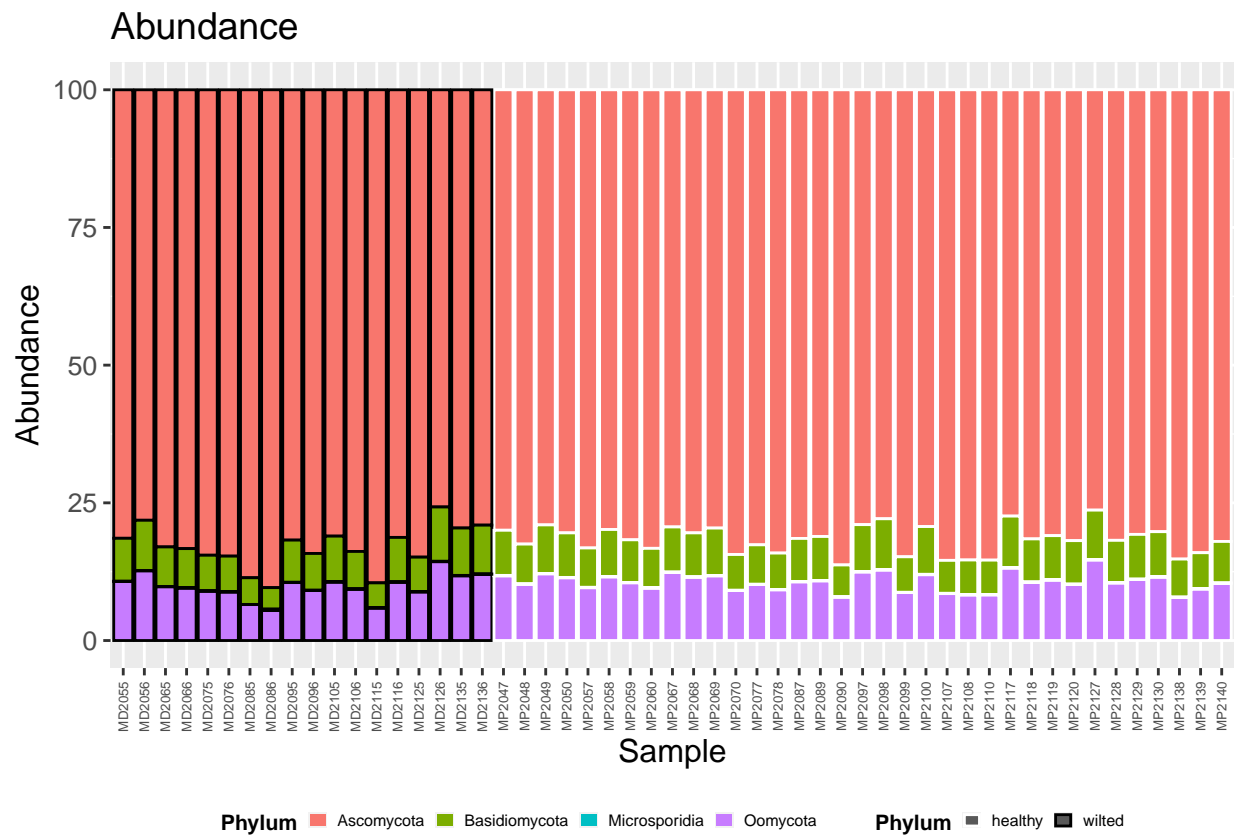
#————Eukarya by Phylum

```
Barras_Phylum <- Abundance_barras(merge_Eukaryota,'Phylum' , 'Treatment', 10.0)
```

```
## Warning: 'aes_string()' was deprecated in ggplot2 3.0.0.
## i Please use tidy evaluation ideoms with 'aes()'
```

```
Barras_Phylum[1] # normal
```

```
## [[1]]
```
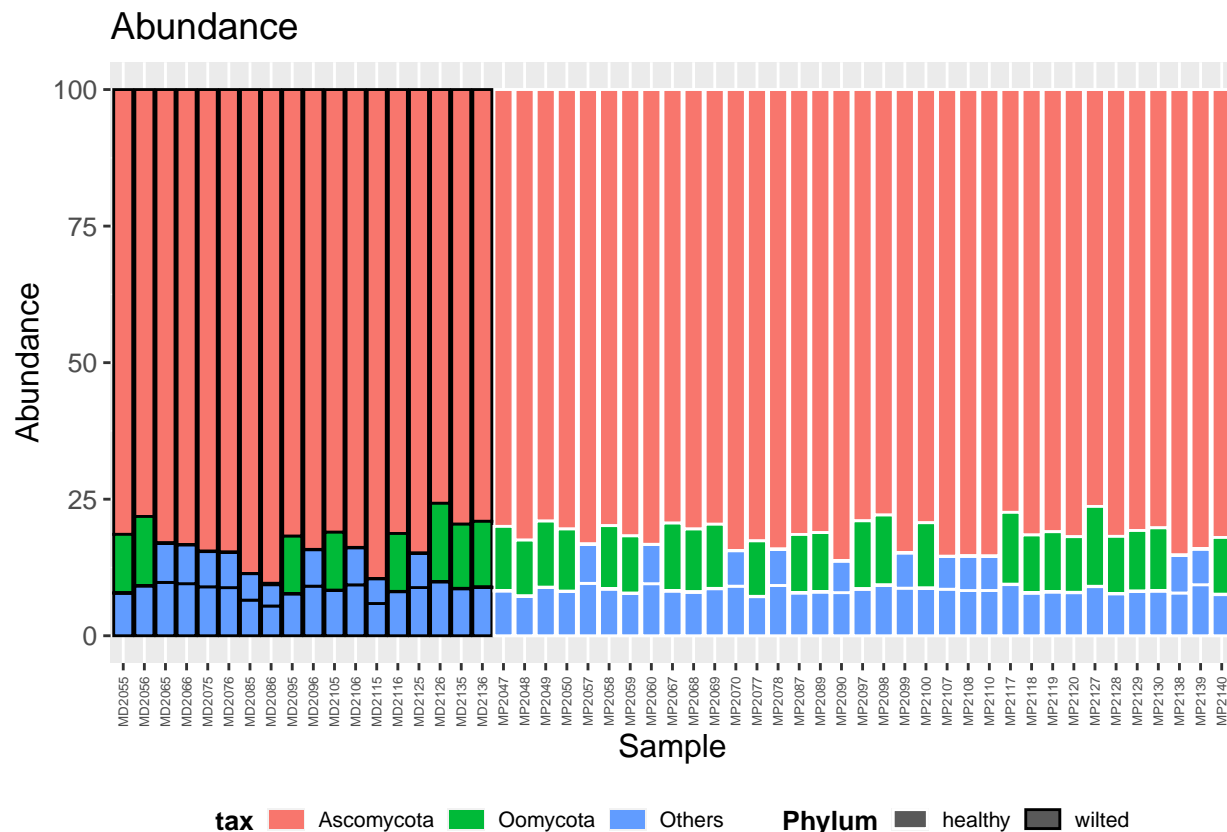
Abundance


```
Barras_Phylum[2]
```

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

```
Beta_diversity(merge_Eukaryota , 'Phylum' , 'Treatment', 'bray')
```

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.01197068
## Run 1 stress 0.0001004821
## ... New best solution
## ... Procrustes: rmse 0.03520944   max resid 0.09412419
## Run 2 stress 9.820099e-05
## ... New best solution
## ... Procrustes: rmse 0.0002390628   max resid 0.0005672624
## ... Similar to previous best
## Run 3 stress 9.453209e-05
## ... New best solution
## ... Procrustes: rmse 0.0001722163   max resid 0.0003906395
## ... Similar to previous best
## Run 4 stress 9.93216e-05
## ... Procrustes: rmse 3.47028e-05   max resid 0.0001782403
## ... Similar to previous best
## Run 5 stress 9.149589e-05
## ... New best solution
## ... Procrustes: rmse 0.0001072471   max resid 0.0003010075
## ... Similar to previous best
## Run 6 stress 9.384861e-05
## ... Procrustes: rmse 0.0001226776   max resid 0.000347392
```

```
## ... Similar to previous best
## Run 7 stress 9.985626e-05
## ... Procrustes: rmse 9.297206e-05  max resid 0.0003299359
## ... Similar to previous best
## Run 8 stress 0.0006522342
## Run 9 stress 9.985536e-05
## ... Procrustes: rmse 0.0001710838  max resid 0.0004062972
## ... Similar to previous best
## Run 10 stress 9.196438e-05
## ... Procrustes: rmse 0.0001009413  max resid 0.0002533249
## ... Similar to previous best
## Run 11 stress 9.574355e-05
## ... Procrustes: rmse 0.0001449805  max resid 0.0004382373
## ... Similar to previous best
## Run 12 stress 9.996973e-05
## ... Procrustes: rmse 0.0001166248  max resid 0.0002784036
## ... Similar to previous best
## Run 13 stress 9.40336e-05
## ... Procrustes: rmse 0.0001231315  max resid 0.0003346891
## ... Similar to previous best
## Run 14 stress 9.805572e-05
## ... Procrustes: rmse 0.0001365674  max resid 0.0004091787
## ... Similar to previous best
## Run 15 stress 9.926294e-05
## ... Procrustes: rmse 0.0001938839  max resid 0.0004565536
## ... Similar to previous best
## Run 16 stress 9.638448e-05
## ... Procrustes: rmse 6.160214e-05  max resid 0.0002680359
## ... Similar to previous best
## Run 17 stress 9.526674e-05
## ... Procrustes: rmse 6.685518e-05  max resid 0.0003079584
## ... Similar to previous best
## Run 18 stress 0.0002867444
## ... Procrustes: rmse 0.000726448  max resid 0.001882133
## ... Similar to previous best
## Run 19 stress 9.40921e-05
## ... Procrustes: rmse 0.0001404129  max resid 0.000460821
## ... Similar to previous best
## Run 20 stress 0.0002706644
## ... Procrustes: rmse 0.000677052  max resid 0.001751565
## ... Similar to previous best
## *** Best solution repeated 15 times


## Warning in metaMDS(veganifyOTU(physeq), distance, ...): stress is (nearly) zero:
## you may have insufficient data
```
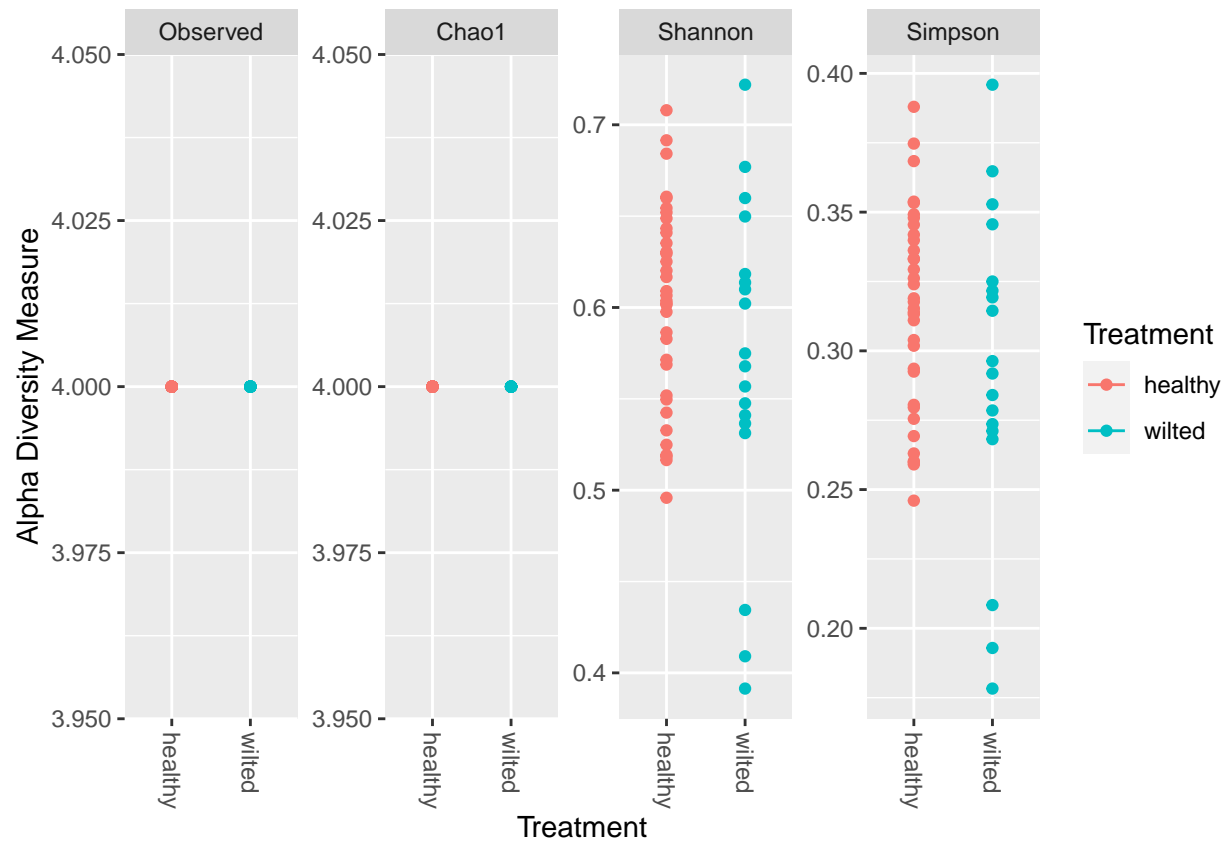
```
Alpha_diversity(merge_Eukaryota , 'Phylum' , 'Treatment')
```

```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided 
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##
## We recommended that you find the un-trimmed data and retry.
```

#————Eukarya by Family

```
Barras_Species <- Abundance_barras(merge_Eukaryota,'Family','Treatment',10.0)
Barras_Species[1]
```
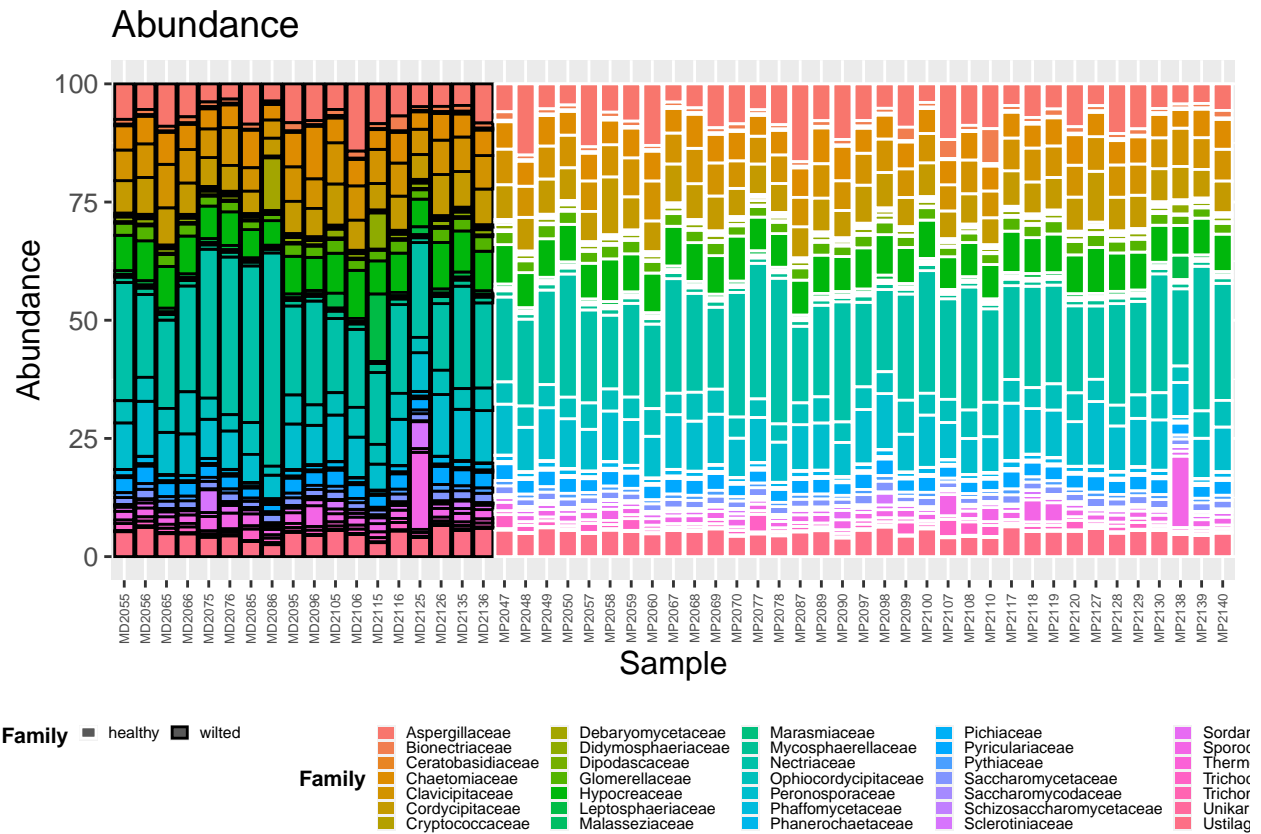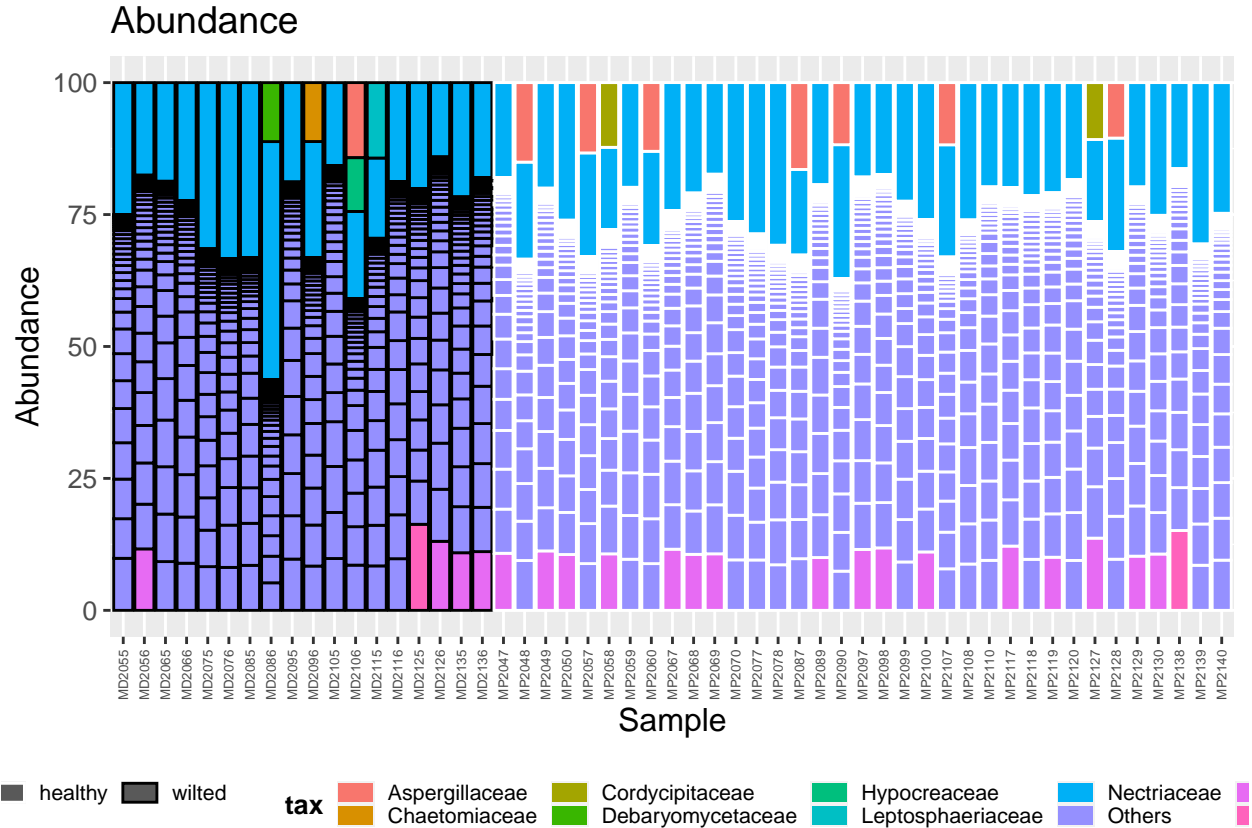
```
## [[1]]
```

Abundance

```
Barras_Species[2]
```

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

Abundance

Legend:
- **tax**
- Aspergillaceae
- Chaetomiaceae
- Cordycipitaceae
- Debaryomycetaceae
- Hypocreaceae
- Leptosphaeriaceae
- Nectriaceae
- Others

```
Beta_diversity(merge_Eukaryota , 'Family' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1060678
## Run 1 stress 0.1061084
## ... Procrustes: rmse 0.008635432  max resid 0.04964329
## Run 2 stress 0.1254714
## Run 3 stress 0.1061184
## ... Procrustes: rmse 0.008964249  max resid 0.04938607
## Run 4 stress 0.106098
## ... Procrustes: rmse 0.002871765  max resid 0.01667967
## Run 5 stress 0.1060652
## ... New best solution
## ... Procrustes: rmse 0.007432752  max resid 0.04979235
## Run 6 stress 0.1060676
## ... Procrustes: rmse 0.007453764  max resid 0.04976001
## Run 7 stress 0.1061121
## ... Procrustes: rmse 0.004311605  max resid 0.02302031
## Run 8 stress 0.1060649
## ... New best solution
## ... Procrustes: rmse 0.0001066042  max resid 0.0006098323
## ... Similar to previous best
## Run 9 stress 0.1254597
## Run 10 stress 0.1060662
## ... Procrustes: rmse 0.001292552  max resid 0.006677735
```

```
## ... Similar to previous best
## Run 11 stress 0.1060648
## ... New best solution
## ... Procrustes: rmse 0.0007977151  max resid 0.004088471
## ... Similar to previous best
## Run 12 stress 0.1060638
## ... New best solution
## ... Procrustes: rmse 0.002062903  max resid 0.01003506
## Run 13 stress 0.1060834
## ... Procrustes: rmse 0.008604535  max resid 0.04975318
## Run 14 stress 0.1060746
## ... Procrustes: rmse 0.008273184  max resid 0.04969505
## Run 15 stress 0.1060755
## ... Procrustes: rmse 0.008216248  max resid 0.04965764
## Run 16 stress 0.1061146
## ... Procrustes: rmse 0.004308367  max resid 0.02319213
## Run 17 stress 0.1061215
## ... Procrustes: rmse 0.009031352  max resid 0.05008506
## Run 18 stress 0.1107719
## Run 19 stress 0.1060712
## ... Procrustes: rmse 0.008121355  max resid 0.04966343
## Run 20 stress 0.1228161
## *** Best solution was not repeated -- monoMDS stopping criteria:
##      9: no. of iterations >= maxit
##      11: stress ratio > sratmax
```
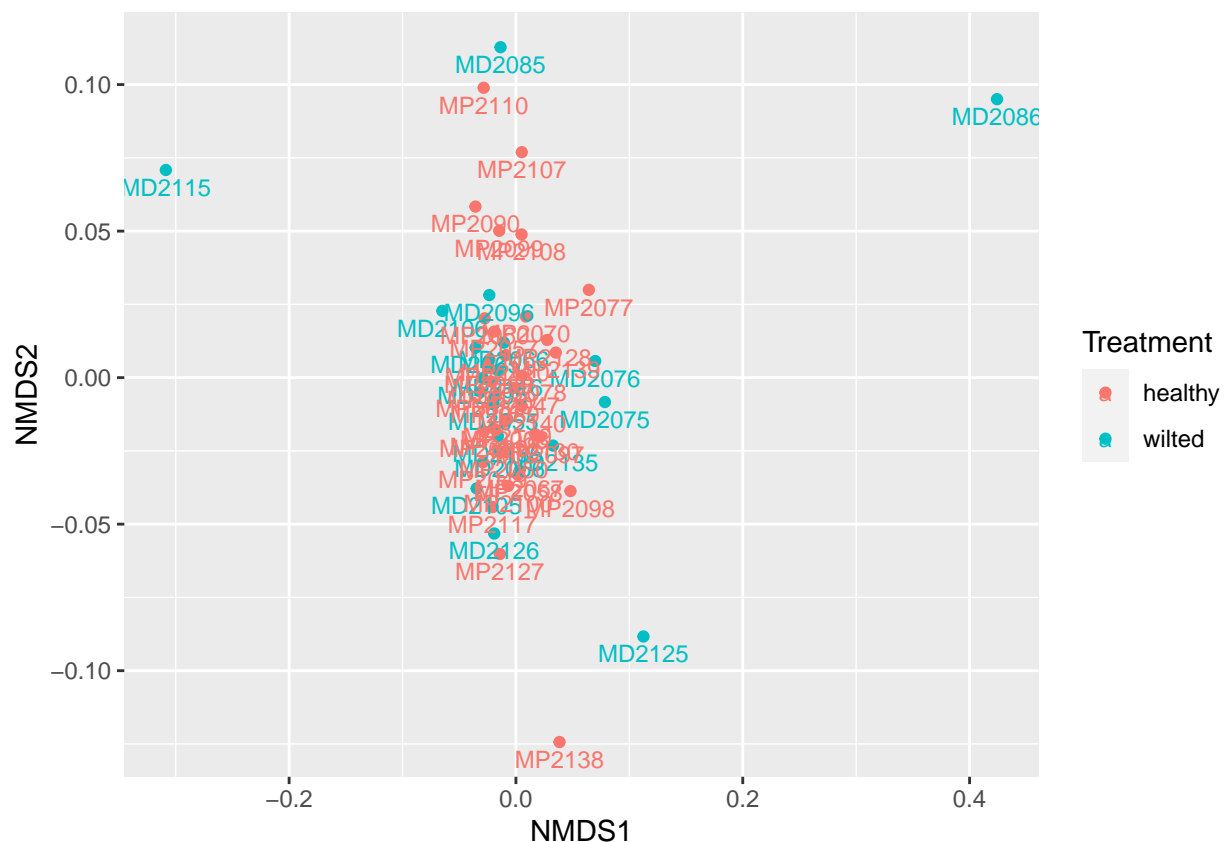
```
Alpha_diversity(merge_Eukaryota , 'Family' , 'Treatment')
```

```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##
## We recommended that you find the un-trimmed data and retry.
```
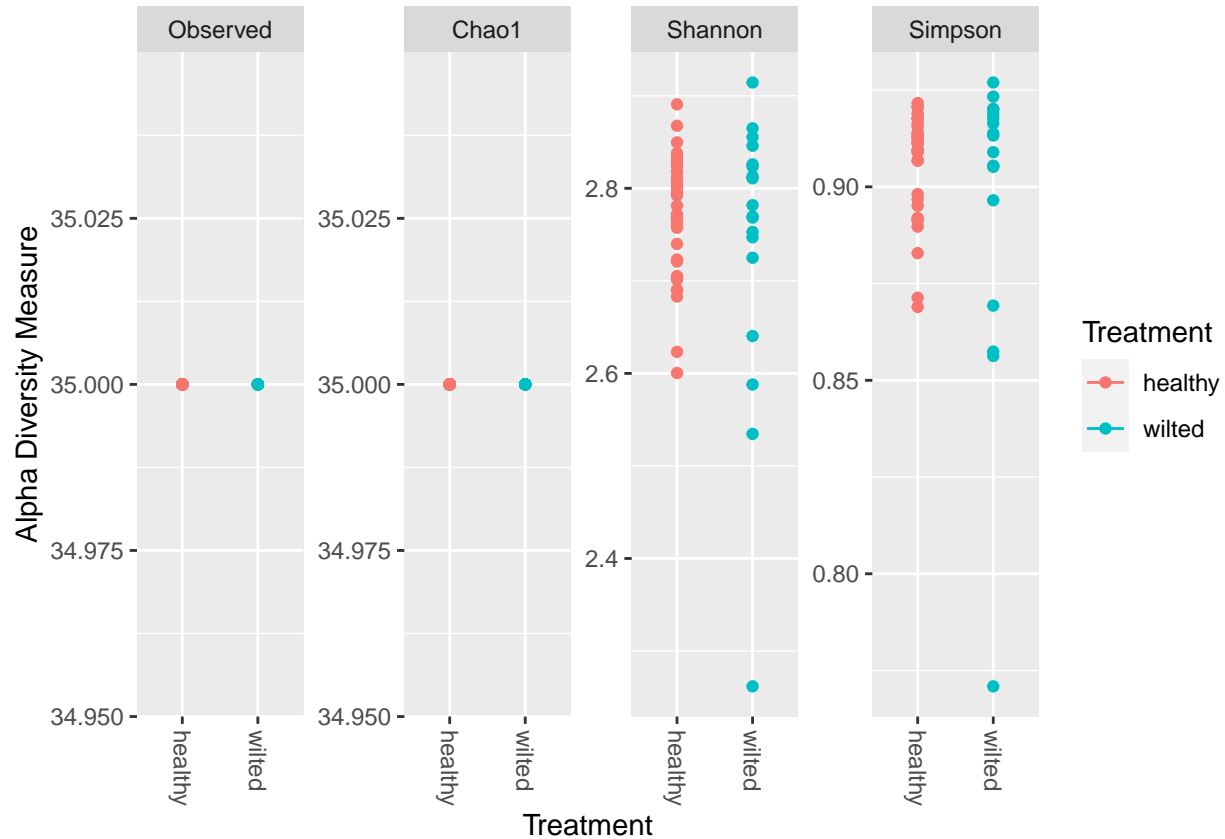

#———Eukarya by Genero

```
Barras_Species <- Abundance_barras(merge_Eukaryota,'Genus','Treatment',10.0)
Barras_Species[1]
```

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

Abundance

Genus

Ampelomyces	Drechmeria	Naumovozyma	Purpureocillium	Thermothelomyces
Aspergillus	Encephalitozoon	Neopestalotiopsis	Pyricularia	Thermothielavioides
Beauveria	Eremothecium	Neurospora	Pythium	Torulaspora
Botrytis	Fusarium	Ogataea	Rhizoctonia	Trichoderma
Brettanomyces	Kazachstania	Paecilomyces	Saccharomyces	Ustilaginoidea
Candida	Kluyveromyces	Paraphaeosphaeria	Saccharomycodes	Ustilago
Cercospora	Komagataella	Penicillium	Scheffersomyces	Yarrowia
Clonostachys	Lachancea	Phlebiopsis	Schizosaccharomyces	Zygosaccharomyces
Colletotrichum	Malassezia	Phytophthora	Sporisorium	Zygotorulaspora
Cordyceps	Marasmius	Pichia	Sugiyamaella	Zymoseptoria
Cryptococcus	Metarhizium	Pochonia	Talaromyces	
Debaryomyces	Nakaseomyces	Pseudozyma	Tetrapisispora	

Genus ▪ healthy ▪

```
Barras_Species[2]
```

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

Abundance

```
Beta_diversity(merge_Eukaryota , 'Genus' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1074764
## Run 1 stress 0.1196324
## Run 2 stress 0.1074756
## ... New best solution
## ... Procrustes: rmse 0.0002909911  max resid 0.001596314
## ... Similar to previous best
## Run 3 stress 0.1074759
## ... Procrustes: rmse 0.0001390156  max resid 0.0007188292
## ... Similar to previous best
## Run 4 stress 0.1558809
## Run 5 stress 0.1198394
## Run 6 stress 0.1074765
## ... Procrustes: rmse 0.001143739  max resid 0.006109848
## ... Similar to previous best
## Run 7 stress 0.107476
## ... Procrustes: rmse 0.001004827  max resid 0.005355567
## ... Similar to previous best
## Run 8 stress 0.108695
## Run 9 stress 0.1074761
## ... Procrustes: rmse 0.001028037  max resid 0.005489472
## ... Similar to previous best
## Run 10 stress 0.1196315
```

```
## Run 11 stress 0.1074769
## ... Procrustes: rmse 0.001230951  max resid 0.006571815
## ... Similar to previous best
## Run 12 stress 0.1218237
## Run 13 stress 0.1074757
## ... Procrustes: rmse 4.839244e-05  max resid 0.0002571386
## ... Similar to previous best
## Run 14 stress 0.1215604
## Run 15 stress 0.1086937
## Run 16 stress 0.1165622
## Run 17 stress 0.107483
## ... Procrustes: rmse 0.001349481  max resid 0.006991788
## ... Similar to previous best
## Run 18 stress 0.108831
## Run 19 stress 0.1074766
## ... Procrustes: rmse 0.0002990836  max resid 0.001556706
## ... Similar to previous best
## Run 20 stress 0.1074767
## ... Procrustes: rmse 0.0003706979  max resid 0.002170874
## ... Similar to previous best
## *** Best solution repeated 10 times
```



```
Alpha_diversity(merge_Eukaryota , 'Genus' , 'Treatment')
```
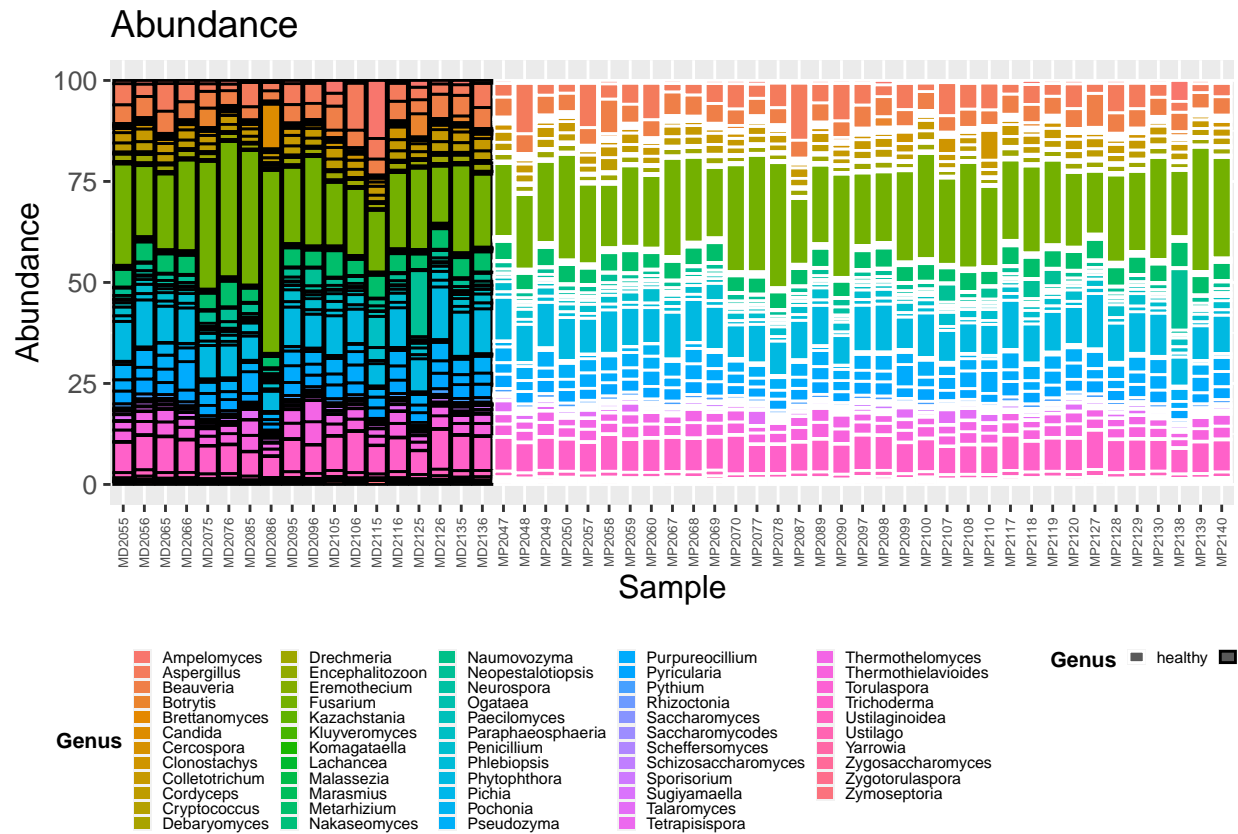
```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided
```

```
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##
## We recommended that you find the un-trimmed data and retry.
```


#———Eukarya by Species

```
Barras_Species <- Abundance_barras(merge_Eukaryota,'Species','Treatment',10.0)
Barras_Species[1]
```
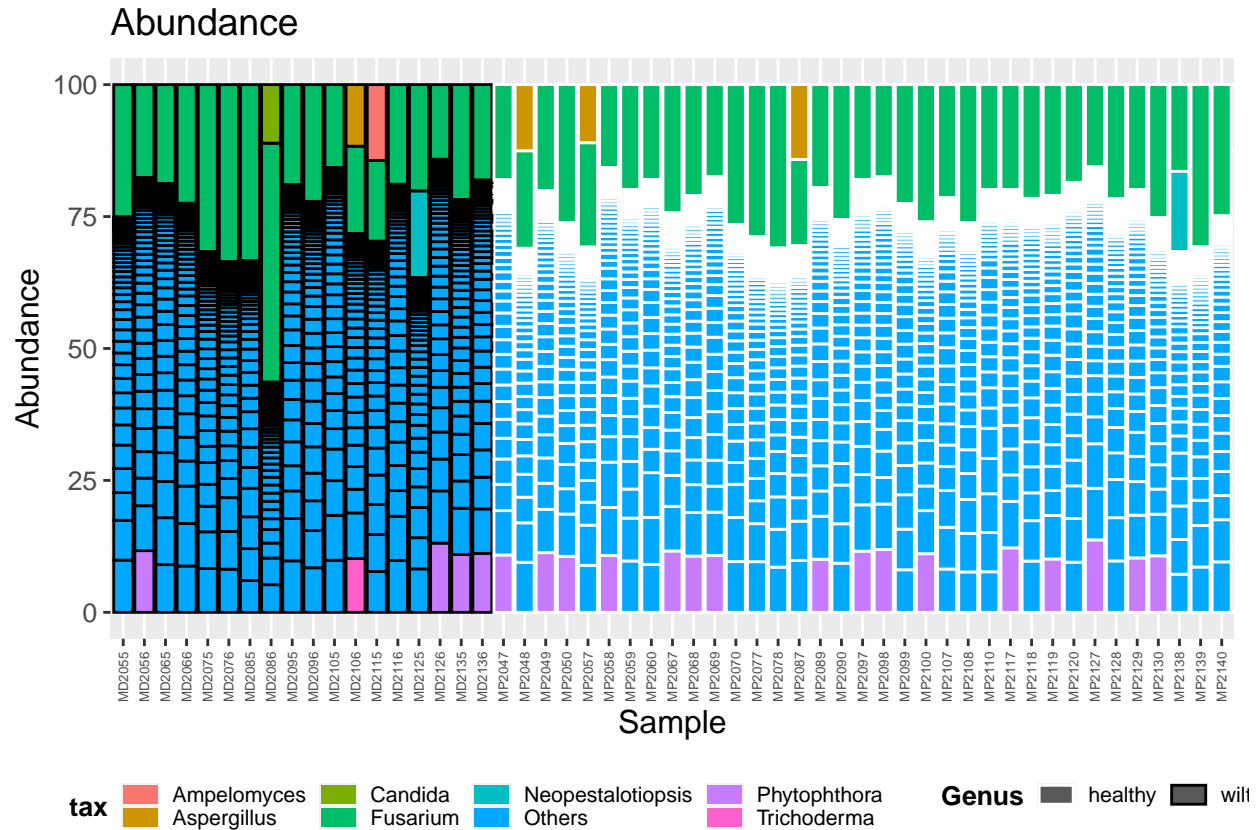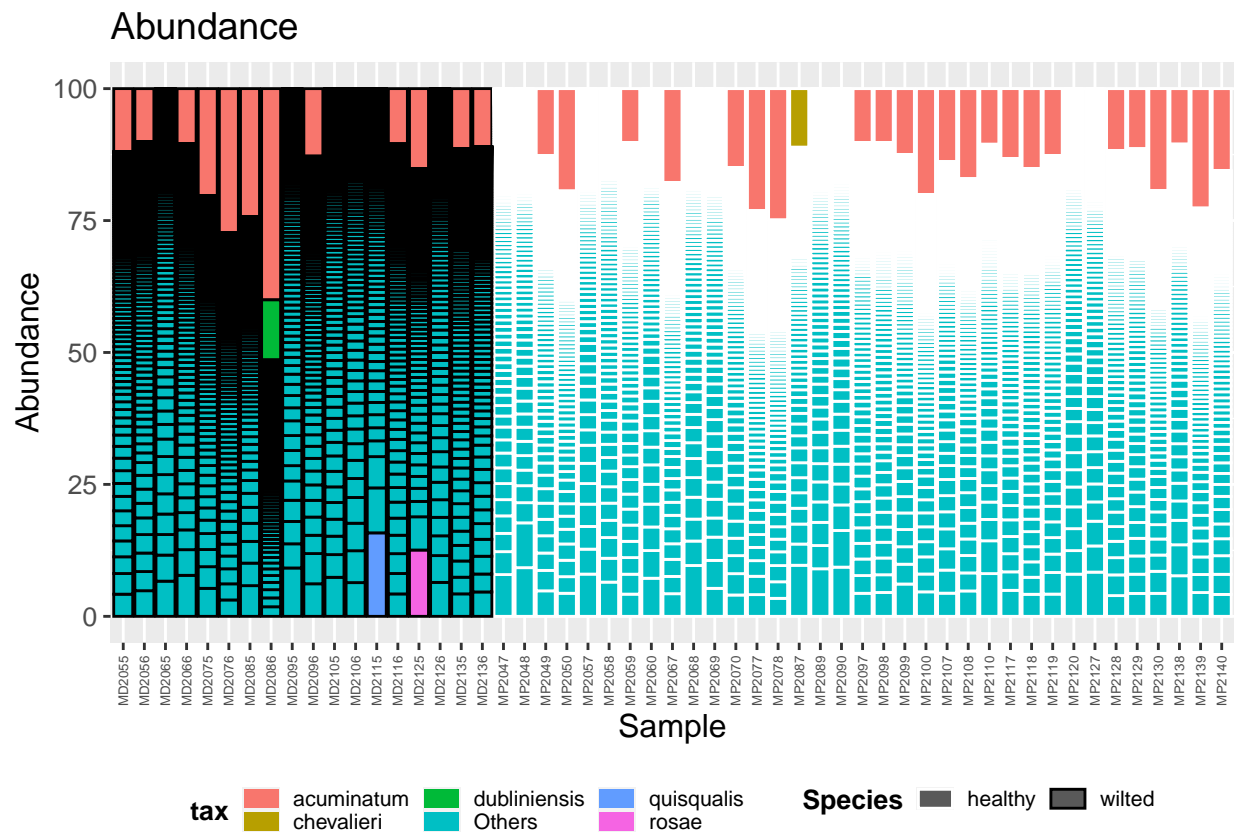
```
## [[1]]
```
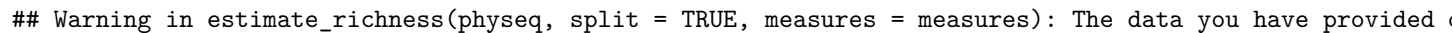
Abundance


```
Barras_Species[2]
```

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

```
Beta_diversity(merge_Eukaryota , 'Species' , 'Treatment', 'bray')
```
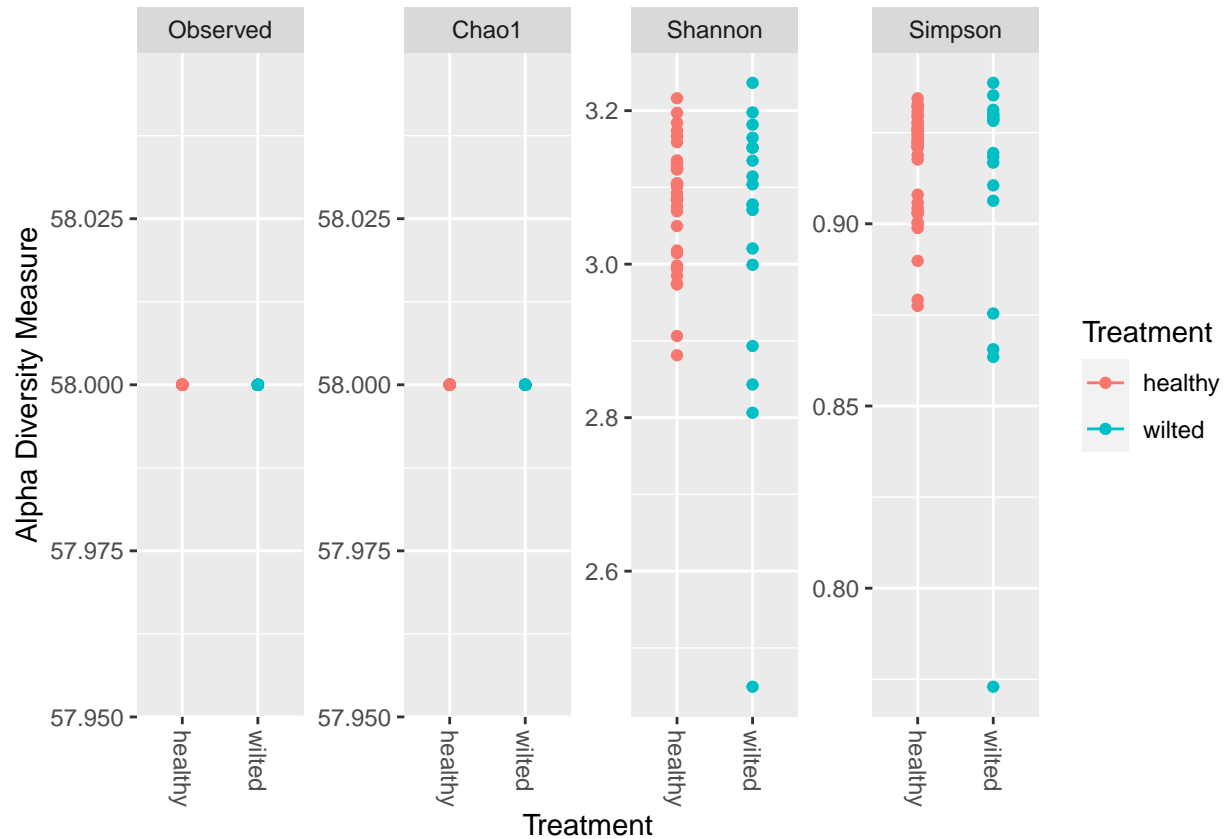
```
## Wisconsin double standardization
## Run 0 stress 0.1207974
## Run 1 stress 0.1248608
## Run 2 stress 0.1200528
## ... New best solution
## ... Procrustes: rmse 0.02422446  max resid 0.1311347
## Run 3 stress 0.1200533
## ... Procrustes: rmse 0.0001736559  max resid 0.000816172
## ... Similar to previous best
## Run 4 stress 0.1200159
## ... New best solution
## ... Procrustes: rmse 0.003386851  max resid 0.0173403
## Run 5 stress 0.120014
## ... New best solution
## ... Procrustes: rmse 0.001187857  max resid 0.006252773
## ... Similar to previous best
## Run 6 stress 0.1485627
## Run 7 stress 0.1200142
## ... Procrustes: rmse 7.579589e-05  max resid 0.00041313
## ... Similar to previous best
## Run 8 stress 0.1249142
## Run 9 stress 0.1206969
## Run 10 stress 0.1249155
```

```
## Run 11 stress 0.1200526
## ... Procrustes: rmse 0.003417562  max resid 0.01757972
## Run 12 stress 0.1207972
## Run 13 stress 0.124862
## Run 14 stress 0.1248483
## Run 15 stress 0.1200146
## ... Procrustes: rmse 0.001271214  max resid 0.006227727
## ... Similar to previous best
## Run 16 stress 0.1248546
## Run 17 stress 0.1202944
## ... Procrustes: rmse 0.01110346  max resid 0.07714005
## Run 18 stress 0.1207359
## Run 19 stress 0.1513819
## Run 20 stress 0.1356645
## *** Best solution repeated 3 times
```



```
Alpha_diversity(merge_Eukaryota , 'Species' , 'Treatment')
```

#———Bacteria by Phylum

```
Barras_Species <- Abundance_barras(merge_Bacteria,'Phylum','Treatment',10.0)
Barras_Species[1]
```

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

Abundance

Phylum
- Acidobacteria
- Actinobacteria
- Aquificae
- Armatimonadetes
- Atribacterota
- Bacteroidetes
- Balneolaeota
- Caldiserica
- Calditrichaeota
- Candidatus Absconditabacteria
- Candidatus Bipolaricaulota
- Candidatus Cloacimonetes
- Candidatus Omnitrophica
- Candidatus Saccharibacteria
- Chlamydiae
- Chlorobi
- Chloroflexi
- Chrysiogenetes
- Coprothermobacterota
- Cyanobacteria
- Deferribacteres
- Deinococcus–Thermus
- Dictyoglomi
- Elusimicrobia
- Fibrobacteres
- Firmicutes
- Fusobacteria
- Gemmatimonadetes
- Ignavibacteriae
- Kiritimatiellaeota
- Nitrospirae
- Planctomycetes
- Proteobacteria
- Spirochaetes
- Synergistetes
- Tenericutes
- Thermodesulfobacteria
- Thermotogae
- Verrucomicrobia

Phylum = h

```
Barras_Species[2]
```

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

Abundance

Phylum: ☐ healthy ☐ wilted   tax: ☐ Actinobacteria ☐ Firmicutes ☐ Others ☐ Proteobacteria

```
Beta_diversity(merge_Bacteria , 'Phylum' , 'Treatment', 'bray')
```

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.1513513
## Run 1 stress 0.1719374
## Run 2 stress 0.1588152
## Run 3 stress 0.1504234
## ... New best solution
## ... Procrustes: rmse 0.00934479  max resid 0.04542046
## Run 4 stress 0.1581249
## Run 5 stress 0.1709639
## Run 6 stress 0.1721019
## Run 7 stress 0.1606131
## Run 8 stress 0.1592882
## Run 9 stress 0.1591807
## Run 10 stress 0.1581926
## Run 11 stress 0.1690259
## Run 12 stress 0.1683791
## Run 13 stress 0.169534
## Run 14 stress 0.1695877
## Run 15 stress 0.1574731
## Run 16 stress 0.1506768
## ... Procrustes: rmse 0.04446673  max resid 0.2965404
## Run 17 stress 0.1660446
```

```
## Run 18 stress 0.1593645
## Run 19 stress 0.1641685
## Run 20 stress 0.1672763
## *** Best solution was not repeated -- monoMDS stopping criteria:
##      1: no. of iterations >= maxit
##      19: stress ratio > sratmax
```



```
Alpha_diversity(merge_Bacteria , 'Phylum' , 'Treatment')
```

```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##
## We recommended that you find the un-trimmed data and retry.
```
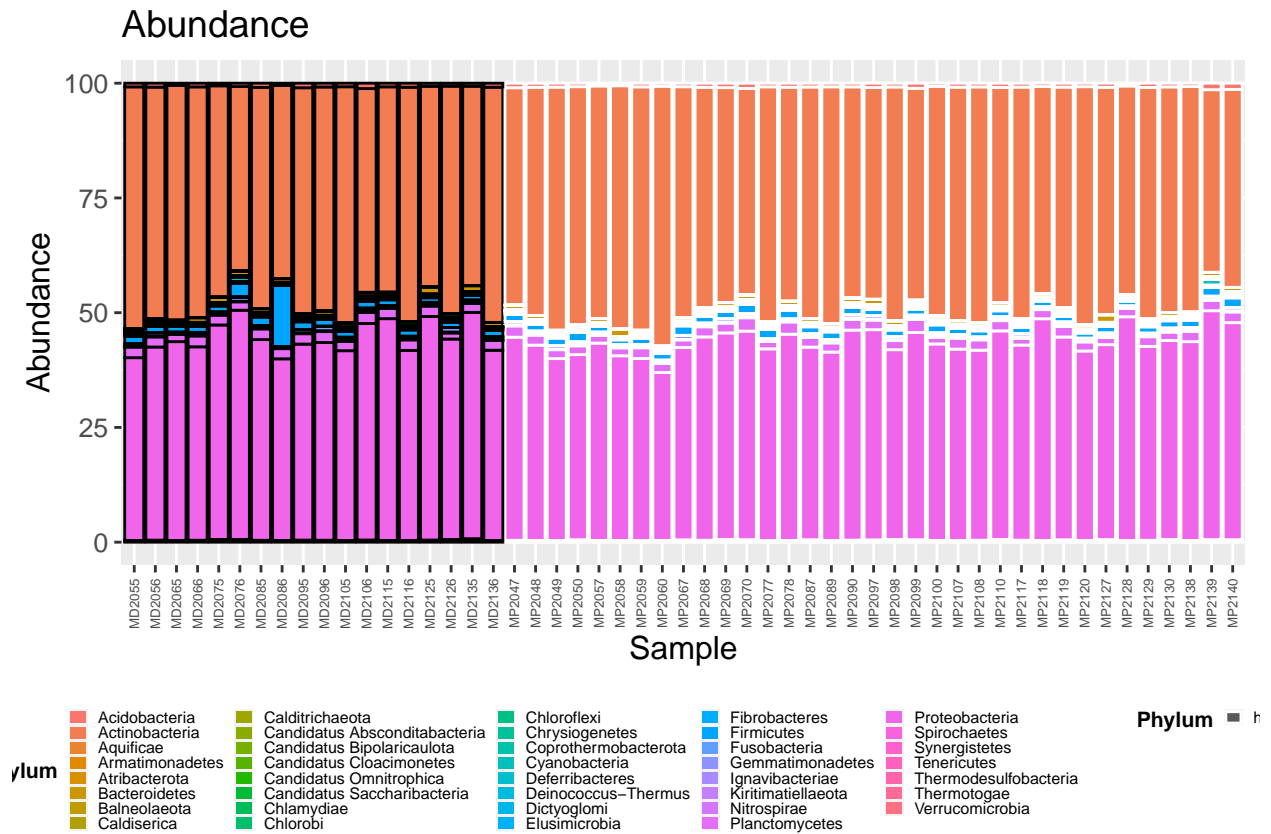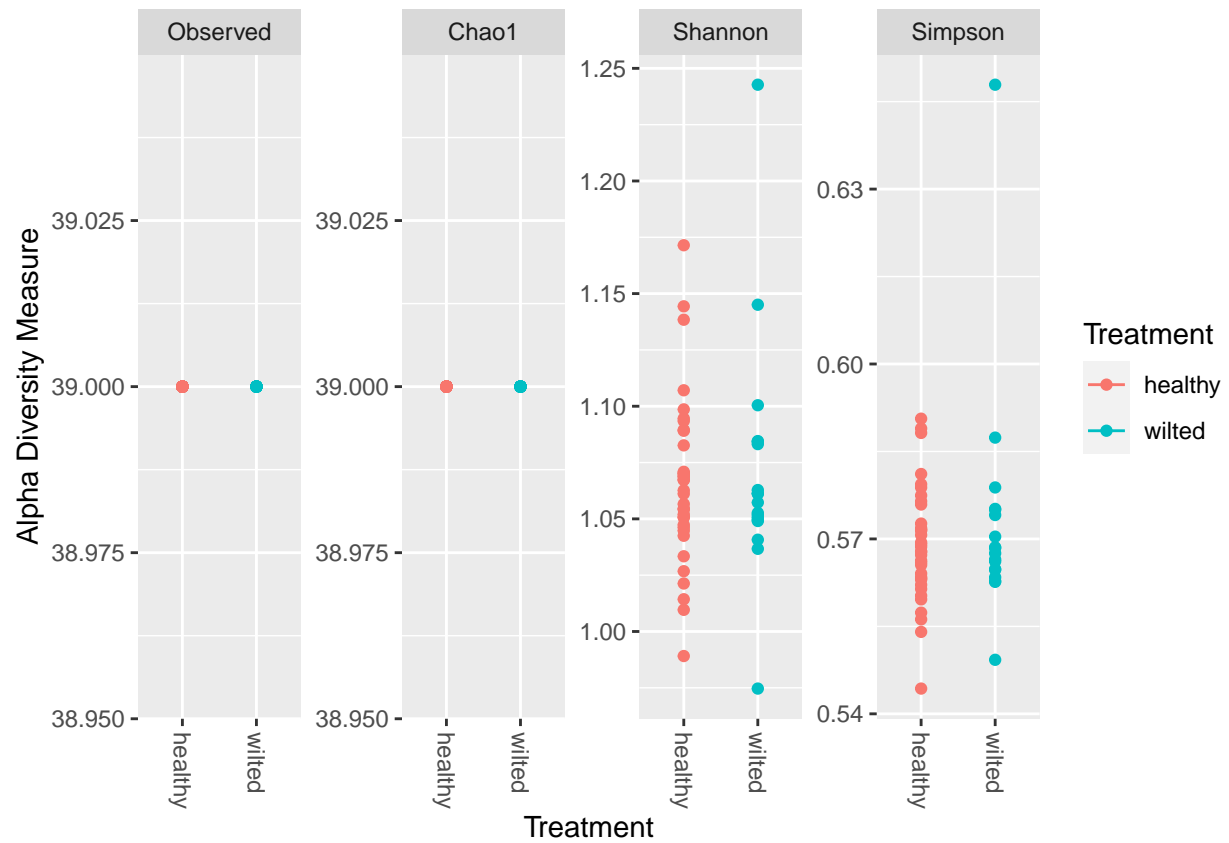
#———Bacteria by Familia

```
Barras_Species <- Abundance_barras(merge_Bacteria,'Family','Treatment',10.0)
Barras_Species[1]
```

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

Family

Column 1:
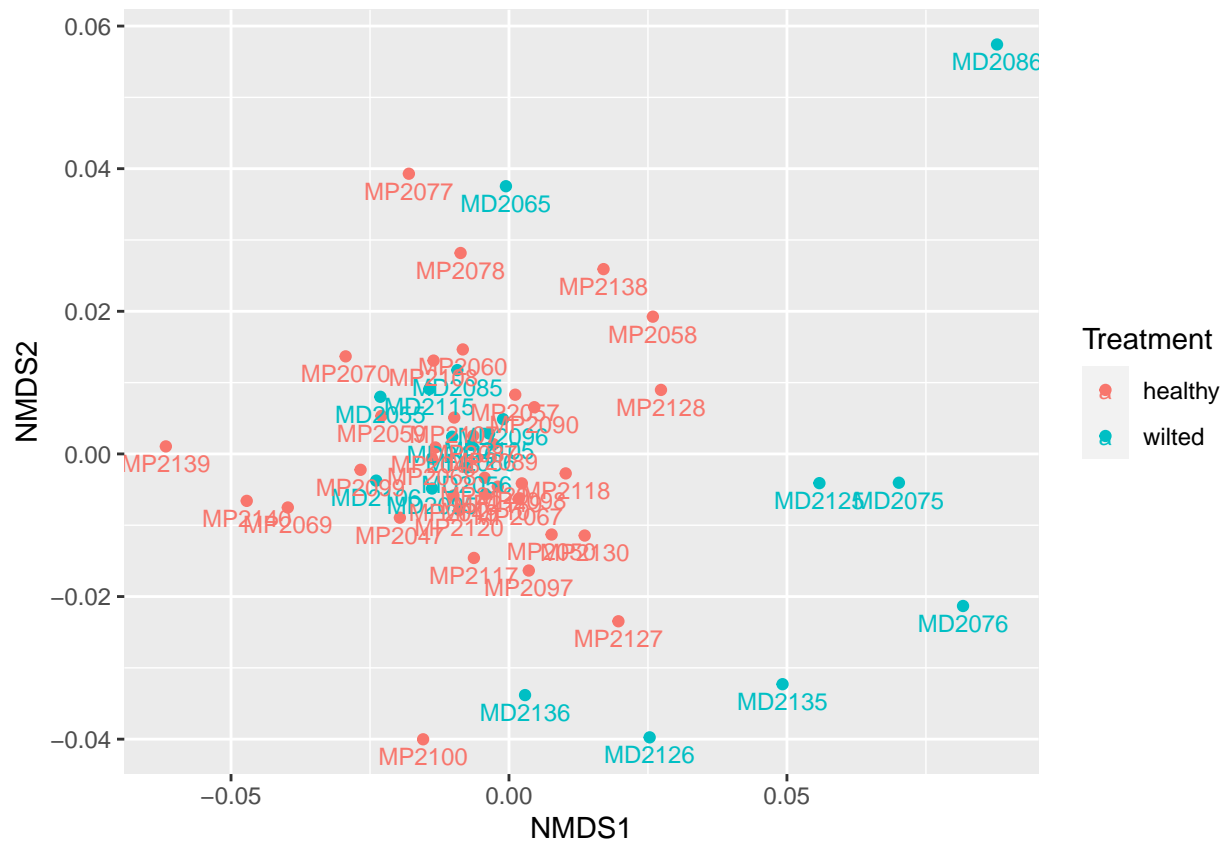- Actinomycetaceae
- Actinopolymorphaceae
- Actinopolysporaceae
- Aerococcaceae
- Aeromonadaceae
- Akkermansiaceae
- Alcaligenaceae
- Alcanivoracaceae
- Alicyclobacillaceae
- Alteromonadaceae
- Amoebophilaceae
- Amorphaceae
- Anaerohalosphaeraceae
- Anaerolineaceae
- Anaeromyxobacteraceae
- Anaplasmataceae
- Aphanizomenonaceae
- Aphanothecaceae
- Aquificaceae
- Archangiaceae
- Arcobacteraceae
- Ardenticatenaceae
- Atopobiaceae
- Atribacteraceae
- Aurantimonadaceae
- Azonexaceae
- Azospirillaceae
- Bacillaceae
- Bacteriovoracaceae
- Bacteroidaceae
- Baekduiaceae
- Barnesiellaceae
- Bartonellaceae
- Bdellovibrionaceae
- Beijerinckiaceae
- Bernardetiaceae
- Beutenbergiaceae
- Bifidobacteriaceae
- Blastochloridaceae
- Blattabacteriaceae
- Bogoriellaceae
- Borreliaceae
- Boseaceae
- Brachyspiraceae
- Bradymonadaceae
- Bradyrhizobiaceae
- Breoghaniaceae
- Brevibacteriaceae
- Brucellaceae
- Bruguierivoracaceae
- Bryobacteraceae
- Budviciaceae
- Burkholderiaceae
- Caedimonadaceae
- Caldilineaceae
- Caldisericaceae
- Calditrichaceae
- Calotrichaceae

Column 2:
- Chthonomonadaceae
- Clostridiaceae
- Clostridiales Family XVI. Incertae Sedis
- Clostridiales Family XVII. Incertae Sedis
- Cohaesibacteraceae
- Coleofasciculaceae
- Colwelliaceae
- Comamonadaceae
- Conexibacteraceae
- Coprothermobacteraceae
- Coriobacteriaceae
- Corynebacteriaceae
- Coxiellaceae
- Crocinitomicaceae
- Cyclobacteriaceae
- Cytophagaceae
- Deferribacteraceae
- Dehalococcoidaceae
- Deinococcaceae
- Demequinaceae
- Dermabacteraceae
- Dermacoccaceae
- Dermatophilaceae
- Dermocarpellaceae
- Desulfallaceae
- Desulfarculaceae
- Desulfitobacteriaceae
- Desulfobacteraceae
- Desulfobulbaceae
- Desulfocapsaceae
- Desulfohalobiaceae
- Desulfomicrobiaceae
- Desulfosudaceae
- Desulfotomaculaceae
- Desulfovibrionaceae
- Desulfurellaceae
- Desulfurobacteriaceae
- Desulfuromonadaceae
- Devosiaceae
- Dictyoglomaceae
- Dietziaceae
- Dissulfurispiraceae
- Dysgonomonadaceae
- Ectothiorhodospiraceae
- Eggerthellaceae
- Egibacteraceae
- Egicoccaceae
- Elioraeaceae
- Elusimicrobiaceae
- Emcibacteraceae
- Endomicrobiaceae
- Endozoicomonadaceae
- Enterobacteriaceae
- Enterococcaceae
- Entomoplasmataceae
- Erwiniaceae
- Erysipelotrichaceae
- Erythrobacteraceae

Column 3:
- Halobacteriovoraceae
- Halobacteroidaceae
- Halomonadaceae
- Halothiobacillaceae
- Hapalosiphonaceae
- Helicobacteraceae
- Heliobacteriaceae
- Holosporaceae
- Hydrogenimonaceae
- Hydrogenophilaceae
- Hydrogenothermaceae
- Hyellaceae
- Hymenobacteraceae
- Hyphomicrobiaceae
- Hyphomonadaceae
- Iamiaceae
- Ichthyobacteriaceae
- Idiomarinaceae
- Ignavibacteriaceae
- Ilumatobacteraceae
- Immundisolibacteraceae
- Intrasporangiaceae
- Isosphaeraceae
- Jatrophihabitantaceae
- Jiangellaceae
- Jonesiaceae
- Kaistiaceae
- Kangiellaceae
- Kineosporiaceae
- Kiritimatiellaceae
- Kofleriaceae
- Koleobacteraceae
- Kordiimonadaceae
- Kosmotogaceae
- Kribbellaceae
- Ktedonosporobacteraceae
- Kytococcaceae
- Labilitrichaceae
- Lachnospiraceae
- Lacipirellulaceae
- Lactobacillaceae
- Lawsonellaceae
- Legionellaceae
- Leptolyngbyaceae
- Leptospiraceae
- Leptotrichiaceae
- Lichenihabitantaceae
- Limnochordaceae
- Listeriaceae
- Litoricolaceae
- Magnetococcaceae
- Mangrovivirgaceae
- Maricaulaceae
- Marinifilaceae
- Marinilabiliaceae
- Marinobacteraceae
- Mariprofundaceae
- Marivirgaceae

Column 4:
- Nocardiaceae
- Nocardioidaceae
- Nocardiopsaceae
- Nostocaceae
- Oceanospirillaceae
- Oculatellaceae
- Odoribacteraceae
- Oleiphilaceae
- Opitutaceae
- Orbaceae
- Ornithinimicrobiaceae
- Oscillatoriaceae
- Oscillospiraceae
- Oxalobacteraceae
- Paenibacillaceae
- Paludibacteraceae
- Parachlamydiaceae
- Parvibaculaceae
- Parvularculaceae
- Pasteurellaceae
- Pectobacteriaceae
- Pelagibacteraceae
- Peptococcaceae
- Peptoniphilaceae
- Peptostreptococcaceae
- Petrotogaceae
- Phototrophicaceae
- Phreatobacteraceae
- Phycisphaeraceae
- Phyllobacteriaceae
- Pirellulaceae
- Piscirickettsiaceae
- Planctomycetaceae
- Planococcaceae
- Pleomorphomonadaceae
- Polyangiaceae
- Porphyromonadaceae
- Prevotellaceae
- Prochlorococcaceae
- Prochlorotrichaceae
- Prolixibacteraceae
- Promicromonosporaceae
- Propionibacteriaceae
- Proteinivoraceae
- Pseudanabaenaceae
- Pseudoalteromonadaceae
- Pseudomonadaceae
- Pseudonocardiaceae
- Psychromonadaceae
- Puniceicoccaceae
- Rhabdochlamydiaceae
- Rhizobiaceae
- Rhodanobacteraceae
- Rhodobacteraceae
- Rhodocyclaceae
- Rhodospirillaceae
- Rhodothermaceae
- Rickettsiaceae

Column 5:
- Sp...
- Sp...
- St...
- St...
- St...
- St...
- St...
- St...
- St...
- Su...
- Su...
- Su...
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- Sy...
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- Tr...
- Tr...

```
Barras_Species[2]
```

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

Abundance

Family ▬ healthy ▬ wilted   **tax** ▮ Others ▮ Paenibacillaceae ▮ Streptomycetaceae

```
Beta_diversity(merge_Bacteria , 'Family' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1371579
## Run 1 stress 0.1341091
## ... New best solution
## ... Procrustes: rmse 0.04803585  max resid 0.2920653
## Run 2 stress 0.1339662
## ... New best solution
## ... Procrustes: rmse 0.009204567  max resid 0.05534446
## Run 3 stress 0.139773
## Run 4 stress 0.1373333
## Run 5 stress 0.1371518
## Run 6 stress 0.1339661
## ... New best solution
## ... Procrustes: rmse 2.003629e-05  max resid 7.346251e-05
## ... Similar to previous best
## Run 7 stress 0.1373217
## Run 8 stress 0.1373152
## Run 9 stress 0.1397729
## Run 10 stress 0.1342677
## ... Procrustes: rmse 0.01075626  max resid 0.05759144
## Run 11 stress 0.1733268
## Run 12 stress 0.1810067
## Run 13 stress 0.1371796
```
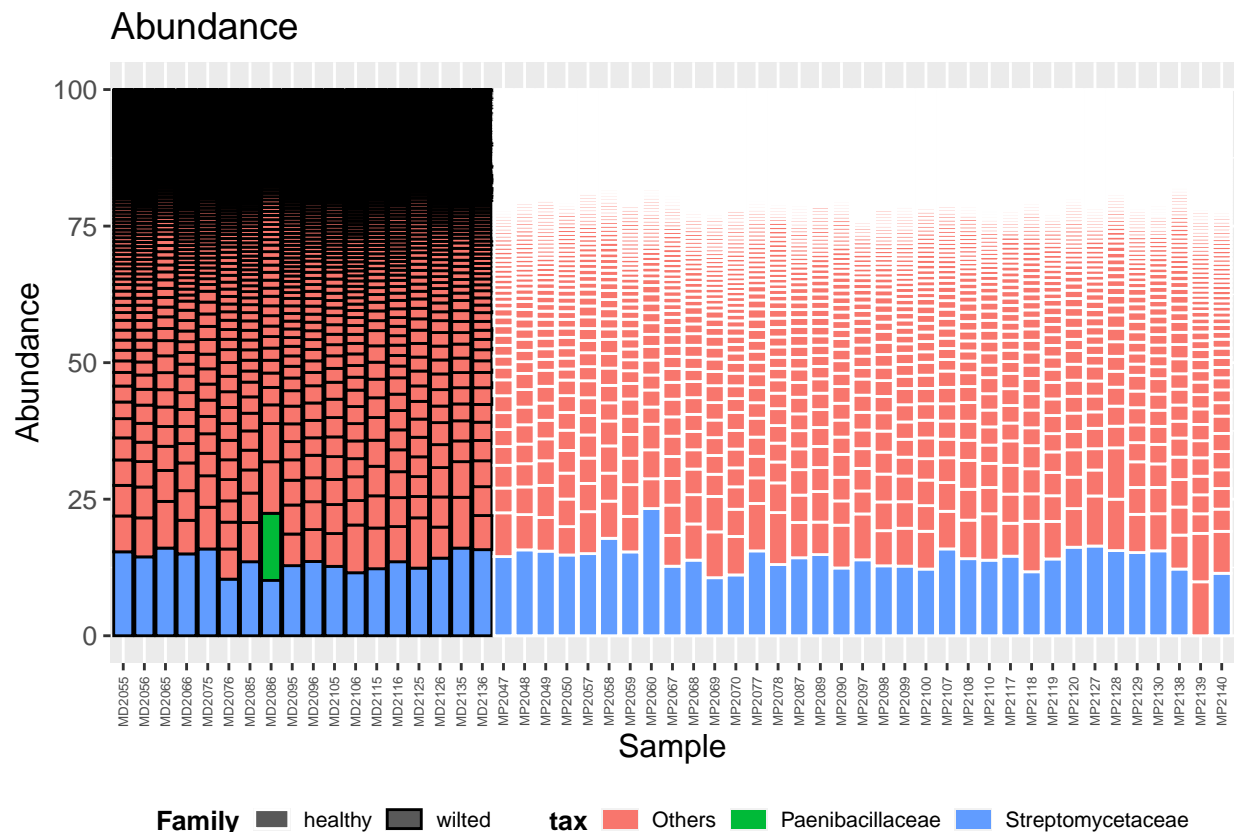
```
## Run 14 stress 0.1339659
## ... New best solution
## ... Procrustes: rmse 0.0002044604  max resid 0.0007869028
## ... Similar to previous best
## Run 15 stress 0.1771062
## Run 16 stress 0.1373154
## Run 17 stress 0.1734309
## Run 18 stress 0.1372212
## Run 19 stress 0.1341087
## ... Procrustes: rmse 0.009067051  max resid 0.05479245
## Run 20 stress 0.1372461
## *** Best solution repeated 1 times
```



```
Alpha_diversity(merge_Bacteria , 'Family' , 'Treatment')
```

```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##
## We recommended that you find the un-trimmed data and retry.
```

#———Bacteria by Genero

```
Barras_Species <- Abundance_barras(merge_Bacteria,'Genus','Treatment',10.0)
Barras_Species[1]
```

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

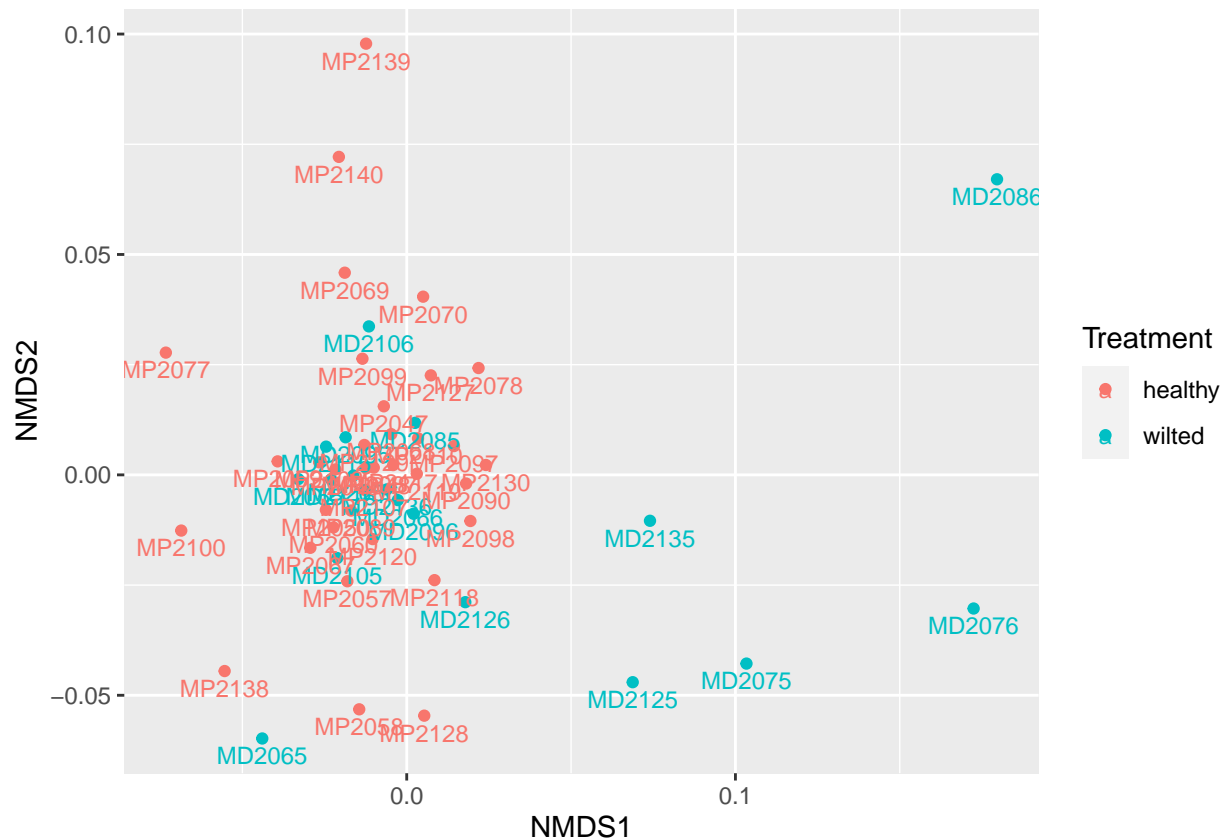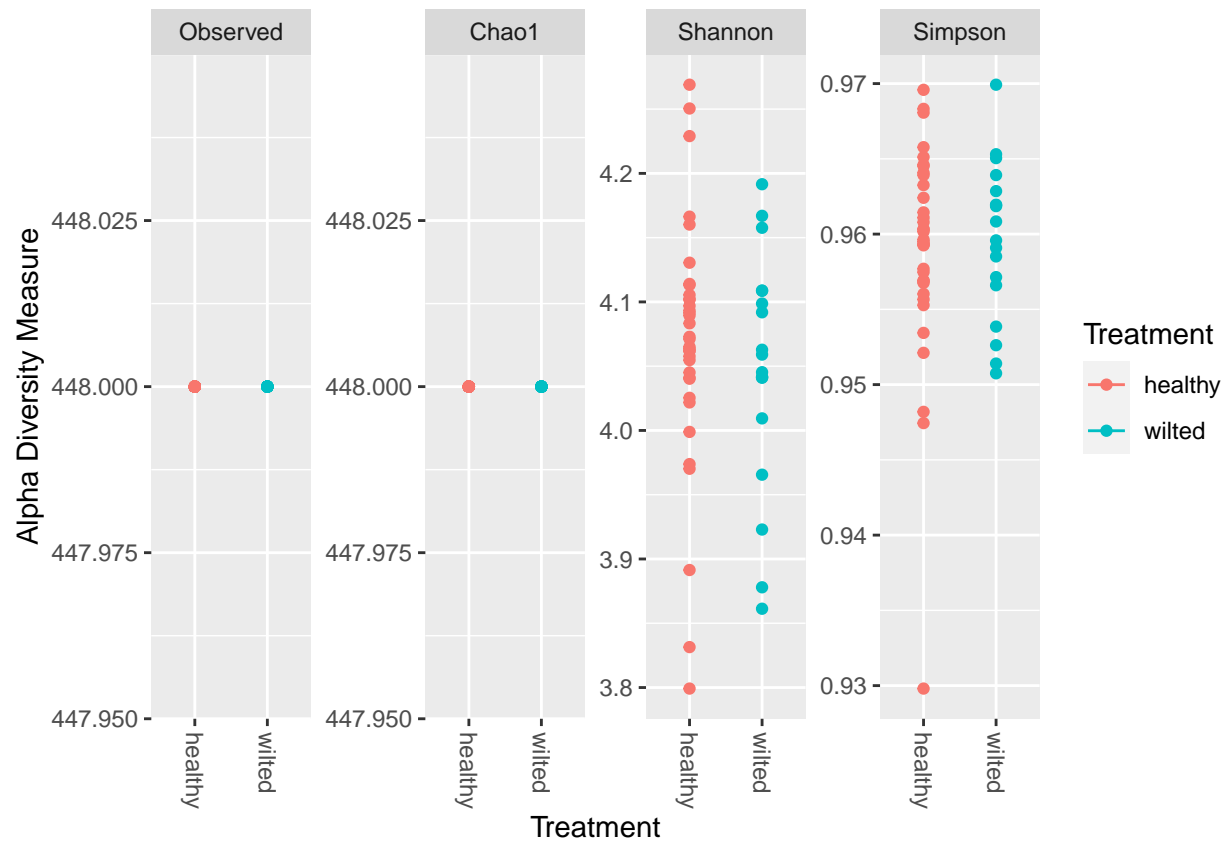|				
lex	Desulfarculus	Labrenzia	Paremcibacter	Subtercola
flexum	Desulfatibacillum	Labrys	Paroceanicella	Succinivibrio
habitans	Desulfitobacterium	Laceyella	Parolsenella	Suicoccus
luna	Desulfobacca	Lachnoanaerobaculum	Parvibaculum	Sulfidibacter
marina	Desulfobacter	Lachnoclostridium	Parvimonas	Sulfitobacter
ncola	Desulfobacula	Lachnospira	Parvularcula	Sulfuricaulis
rhabdus	Desulfobulbus	Lacibacter	Pasteurella	Sulfuricella
rufa	Desulfocapsa	Lacimicrobium	Paucibacter	Sulfuricurvum
salimonas	Desulfococcus	Lacinutrix	Paucilactobacillus	Sulfuriferula
salinus	Desulfocurvibacter	Lacipirellula	Pauljensenia	Sulfuriflexus
sediminimonas	Desulfofarcimen	Lacrimispora	Pectinatus	Sulfurifustis
sphaera	Desulfoglaeba	Lacticaseibacillus	Pectobacterium	Sulfurihydrogenibium
talea	Desulfohalobium	Lactiplantibacillus	Pediococcus	Sulfurimicrobium
iibacter	Desulfoluna	Lactobacillus	Pedobacter	Sulfurimonas
hidicoccus	Desulfolutivibrio	Lactococcus	Pedococcus	Sulfuriroseicoccus
hnia	Desulfomarina	Lacunisphaera	Pelagerythrobacter	Sulfuritalea
nobacterium	Desulfomicrobium	Lancefieldella	Pelagibacterium	Sulfuritortus
angium	Desulfomonile	Lapidilactobacillus	Pelagovum	Sulfurivermis
bacter	Desulfonema	Laribacter	Pelistega	Sulfurospirillum
cibacterium	Desulforamulus	Larkinella	Pelobacter	Sulfurovum
ibacter	Desulforapulum	Latilactobacillus	Pelodictyon	Sutcliffiella
imonas	Desulfosarcina	Lautropia	Pelolinea	Sutterella
natoleum	Desulfoscipio	Lawsonella	Pelosinus	Suttonella
nicicoccus	Desulfosediminicola	Lawsonia	Pengzhenrongella	Swingsia
nophonus	Desulfosporosinus	Leadbetterella	Peptacetobacter	Symbiobacterium
robacter	Desulfosudis	Leclercia	Peptoclostridium	Symmachiella
a	Desulfotalea	Lederbergia	Peptoniphilus	Synechococcus
ccacaulis	Desulfotomaculum	Leeuwenhoekiella	Peribacillus	Synechocystis
lassotoga	Desulfovibrio	Legionella	Periweissella	Syntrophobacter
ntibacter	Desulfurispirillum	Leifsonia	Permianibacter	Syntrophobotulus
obium	Desulfurivibrio	Leisingera	Persephonella	Syntrophomonas
acter	Desulfurobacterium	Lelliottia	Persicimonas	Syntrophotalea
ntiacibacter	Desulfuromonas	Leminorella	Peteryoungia	Syntrophothermus
ntimicrobium	Devosia	Lentibacillus	Petrimonas	Syntrophus
ntimonas	Devriesea	Lentilactobacillus	Petrocella	Tabrizicola
ticoccus	Dialister	Lentilitoribacter	Petrotoga	Tamlana
imonas	Diaminobutyricimonas	Lentzea	Phaeobacter	Tannerella
liella	Diaphorobacter	Leptodesmis	Phascolarctobacterium	Tardibacter
idibacter	Dichelobacter	Leptolyngbya	Phenylobacterium	Tardiphaga
wickia	Dickeya	Leptospira	Phnomibacter	Tateyamaria
acterium	Dictyoglomus	Leptospirillum	Phocaeicola	Tatlockia
rcus	Dietzia	Leptothermofonsia	Phoenicibacter	Tatumella
hizobium	Dinoroseobacter	Leptothrix	Photobacterium	Tautonia
spira	Dissulfurimicrobium	Leptotrichia	Photorhabdus	Taylorella
spirillum	Dissulfurispira	Leucobacter	Phototrophicus	Telmatocola
obacter	Dokdonella	Leuconostoc	Phreatobacter	Tenacibaculum
llus	Dokdonia	Levilactobacillus	Phycicoccus	Tenuifilum
erioplanes	Dolichospermum	Liberibacter	Phycisphaera	Tepidanaerobacter
eriovorax	Dolosigranulum	Lichenicola	Phyllobacterium	Tepidibacter
eroides	Dongshaea	Lichenihabitans	Phytobacter	Tepidiforma
duia	Dorea	Ligilactobacillus	Phytohabitans	Tepidimonas
esiella	Draconibacterium	Lignipirellula	Pigmentiphaga	Tepiditoga
onella	Duganella	Limihaloglobus	Pikeienuella	Terasakiella
ia	Duncaniella	Limnobacter	Pimelobacter	Teredinibacter
lea	Dyadobacter	Limnobaculum	Pirellula	Terribacillus
lovibrio	Dyella	Limnochorda	Pirellulimonas	Terricaulis
giatoa	Dysgonomonas	Limnoglobus	Piscirickettsia	Terriglobus
rinckia	Dysosmobacter	Limnohabitans	Pistricoccus	Terrihabitans

Barras_Species[2]

[[1]]
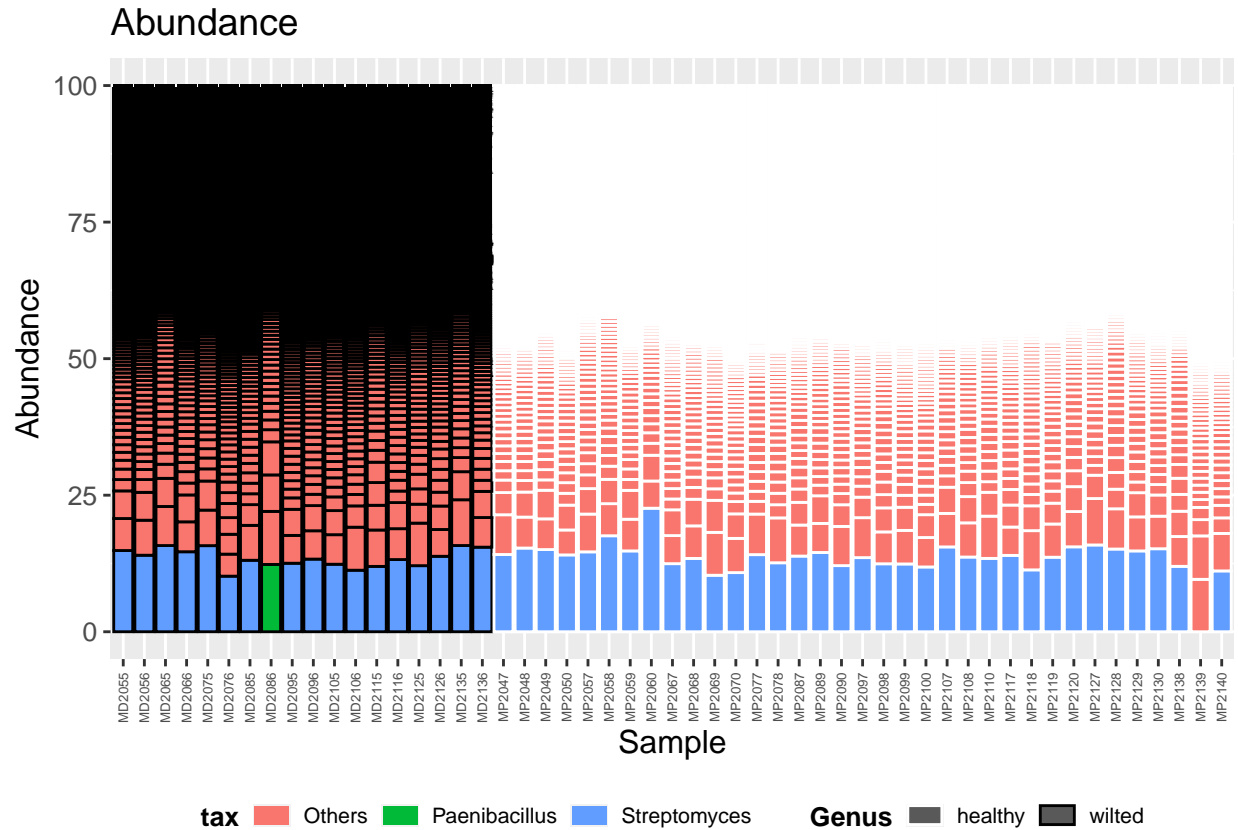
Abundance

```
Beta_diversity(merge_Bacteria , 'Genus' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1449554
## Run 1 stress 0.1394427
## ... New best solution
## ... Procrustes: rmse 0.08796529  max resid 0.3619209
## Run 2 stress 0.1479265
## Run 3 stress 0.1491912
## Run 4 stress 0.1394925
## ... Procrustes: rmse 0.004473006  max resid 0.02666874
## Run 5 stress 0.1650072
## Run 6 stress 0.1382025
## ... New best solution
## ... Procrustes: rmse 0.06369859  max resid 0.3720801
## Run 7 stress 0.1394869
## Run 8 stress 0.1647696
## Run 9 stress 0.1528891
## Run 10 stress 0.1640323
## Run 11 stress 0.1394429
## Run 12 stress 0.1392722
## Run 13 stress 0.1478283
## Run 14 stress 0.1381219
## ... New best solution
## ... Procrustes: rmse 0.005056097  max resid 0.02717121
```

```
## Run 15 stress 0.1396336
## Run 16 stress 0.1396658
## Run 17 stress 0.138123
## ... Procrustes: rmse 0.0003474481   max resid 0.001143416
## ... Similar to previous best
## Run 18 stress 0.1381221
## ... Procrustes: rmse 7.638288e-05   max resid 0.0002501497
## ... Similar to previous best
## Run 19 stress 0.1396338
## Run 20 stress 0.1425795
## *** Best solution repeated 2 times
```



```
Alpha_diversity(merge_Bacteria , 'Genus' , 'Treatment')
```

#———Bacteria by Species

```
Barras_Species <- Abundance_barras(merge_Bacteria,'Species','Treatment',10.0)
Barras_Species[1]
```

[[1]]

e

<table>
<tr><td>lactamica</td><td>sp. 'Soap Lake #7'</td><td>sp. KSB–15</td><td>sp. X</td></tr>
<tr><td>lactarius</td><td>sp. (Blaberus giganteus)</td><td>sp. KSM–R2A25</td><td>sp. X</td></tr>
<tr><td>lactatifermentans</td><td>sp. (Blatta orientalis)</td><td>sp. KSM–R2A30</td><td>sp. X</td></tr>
<tr><td>lactatiformans</td><td>sp. (Blattella germanica)</td><td>sp. KT 15</td><td>sp. X</td></tr>
<tr><td>lactis</td><td>sp. (Cryptocercus kyebangensis)</td><td>sp. KT25b</td><td>sp. X</td></tr>
<tr><td>lactucae</td><td>sp. (Cryptocercus punctulatus) str. Cpu</td><td>sp. KTR9</td><td>sp. X</td></tr>
<tr><td>lacunae</td><td>sp. (ex Adelges kitamiensis)</td><td>sp. KUDC0405</td><td>sp. X</td></tr>
<tr><td>lacus</td><td>sp. (ex Biomphalaria glabrata)</td><td>sp. KUDC0406</td><td>sp. X</td></tr>
<tr><td>lacuslunae</td><td>sp. (Mastotermes darwiniensis)</td><td>sp. KUDC1026</td><td>sp. x</td></tr>
<tr><td>lacustris</td><td>sp. (Nauphoeta cinerea)</td><td>sp. KUDC1714</td><td>sp. X</td></tr>
<tr><td>laguerreae</td><td>sp. (Periplaneta americana)</td><td>sp. KUIN–1</td><td>sp. X</td></tr>
<tr><td>laidlawii</td><td>sp. 001</td><td>sp. Kuro–4</td><td>sp. X</td></tr>
<tr><td>laixinhei</td><td>sp. 007</td><td>sp. KW1</td><td>sp. X</td></tr>
<tr><td>lalkuanensis</td><td>sp. 008</td><td>sp. KX20019</td><td>sp. X</td></tr>
<tr><td>laminatus</td><td>sp. 02C 26</td><td>sp. KY–GH–1</td><td>sp. X</td></tr>
<tr><td>lanienae</td><td>sp. 09C 129</td><td>sp. KY–YJ–3</td><td>sp. X</td></tr>
<tr><td>lanii</td><td>sp. 09RB8471</td><td>sp. KY3</td><td>sp. Y</td></tr>
<tr><td>lansingensis</td><td>sp. 09RB8910</td><td>sp. KY5</td><td>sp. Y</td></tr>
<tr><td>lanthieri</td><td>sp. 1_2014MBL_MicDiv</td><td>sp. KY70</td><td>sp. Y</td></tr>
<tr><td>lapagei</td><td>sp. 1_2015MBL_MicDiv</td><td>sp. KY75</td><td>sp. Y</td></tr>
<tr><td>lapidicaptus</td><td>sp. 1–1C</td><td>sp. L–07</td><td>sp. Y</td></tr>
<tr><td>lapsinanis</td><td>sp. 1.5R</td><td>sp. L–2–11</td><td>sp. Y</td></tr>
<tr><td>lari</td><td>sp. 1(2017)</td><td>sp. L–8–10</td><td>sp. Y</td></tr>
<tr><td>lariciata</td><td>sp. 103DPR2</td><td>sp. L–8–3</td><td>sp. Y</td></tr>
<tr><td>larrymoorei</td><td>sp. 1063</td><td>sp. L–A4</td><td>sp. Y</td></tr>
<tr><td>larvae</td><td>sp. 107–1</td><td>sp. L12M9</td><td>sp. Y</td></tr>
<tr><td>lata</td><td>sp. 1070</td><td>sp. L1A13</td><td>sp. Y</td></tr>
<tr><td>latens</td><td>sp. 10FS3–1</td><td>sp. L1A34</td><td>sp. Y</td></tr>
<tr><td>laterosporus</td><td>sp. 10M–3C3</td><td>sp. L1A9</td><td>sp. Y</td></tr>
<tr><td>laumondii</td><td>sp. 10RB9215</td><td>sp. L1I39</td><td>sp. Y</td></tr>
<tr><td>lautus</td><td>sp. 11–1–2</td><td>sp. L1SW</td><td>sp. Y</td></tr>
<tr><td>lavamentivorans</td><td>sp. 11–B–312</td><td>sp. L2A1</td><td>sp. Y</td></tr>
<tr><td>lavendulae</td><td>sp. 113–1–2</td><td>sp. L2A11</td><td>sp. Y</td></tr>
<tr><td>leachii</td><td>sp. 113–3–3</td><td>sp. L3–i22</td><td>sp. Y</td></tr>
<tr><td>leadbetteri</td><td>sp. 113–3–9</td><td>sp. L3–i23</td><td>sp. Y</td></tr>
<tr><td>leeuwenhoekii</td><td>sp. 1137</td><td>sp. L3A3</td><td>sp. Y</td></tr>
<tr><td>leguminosarum</td><td>sp. 113P3</td><td>sp. L3A6</td><td>sp. Y</td></tr>
<tr><td>lehensis</td><td>sp. 114</td><td>sp. L3A8</td><td>sp. Y</td></tr>
<tr><td>lekithochrous</td><td>sp. 11515TR</td><td>sp. L5</td><td>sp. Y</td></tr>
<tr><td>lemovicicum</td><td>sp. 116–D4</td><td>sp. L51/94</td><td>sp. Y</td></tr>
<tr><td>lemurum</td><td>sp. 119287</td><td>sp. L5B5</td><td>sp. Y</td></tr>
<tr><td>lenghuensis</td><td>sp. 11B</td><td>sp. L6–1</td><td>sp. Y</td></tr>
<tr><td>lenta</td><td>sp. 11kri321</td><td>sp. L9–4</td><td>sp. Y</td></tr>
<tr><td>lentiflavum</td><td>sp. 12200R–103</td><td>sp. LA–2–3–30–S1–D2</td><td>sp. Y</td></tr>
<tr><td>lentis</td><td>sp. 13–15</td><td>sp. LA112445</td><td>sp. Y</td></tr>
<tr><td>lentocellum</td><td>sp. 131</td><td>sp. LA31</td><td>sp. Y</td></tr>
<tr><td>lentus</td><td>sp. 131–2–1</td><td>sp. LAB–08</td><td>sp. Y</td></tr>
<tr><td>leopoldii</td><td>sp. 131–2–5</td><td>sp. LADL05–105</td><td>sp. Y</td></tr>
<tr><td>leprae</td><td>sp. 131–3–5</td><td>sp. LAS2</td><td>sp. Y</td></tr>
<tr><td>lepromatosis</td><td>sp. 13159349</td><td>sp. LB1</td><td>sp. Y</td></tr>
<tr><td>leptomitoformis</td><td>sp. 135</td><td>sp. LBG001</td><td>sp. Y</td></tr>
<tr><td>lettingae</td><td>sp. 140616W15</td><td>sp. LBUM 1475</td><td>sp. Y</td></tr>
<tr><td>liangguodongii</td><td>sp. 14171R–50</td><td>sp. LBUM 1480</td><td>sp. Y</td></tr>
<tr><td>liangshanensis</td><td>sp. 14181154</td><td>sp. LBUM 1482</td><td>sp. Y</td></tr>
<tr><td>liaowanqingii</td><td>sp. 143–21</td><td>sp. LBUM920</td><td>sp. Y</td></tr>
<tr><td>libanensis</td><td>sp. 144S4</td><td>sp. LC2018020214</td><td>sp. Y</td></tr>
<tr><td>licheniformis</td><td>sp. 15–184</td><td>sp. LC6</td><td>sp. Y</td></tr>
</table>
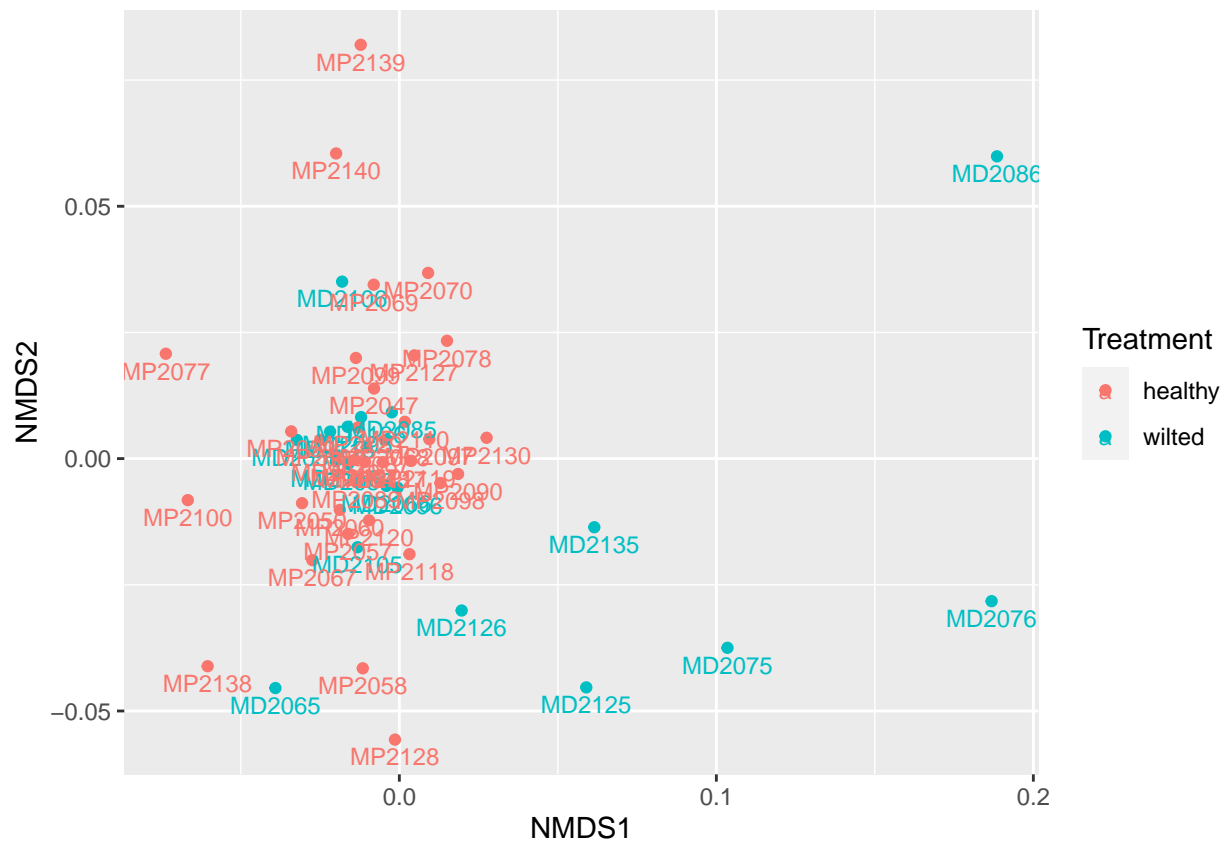
andiella
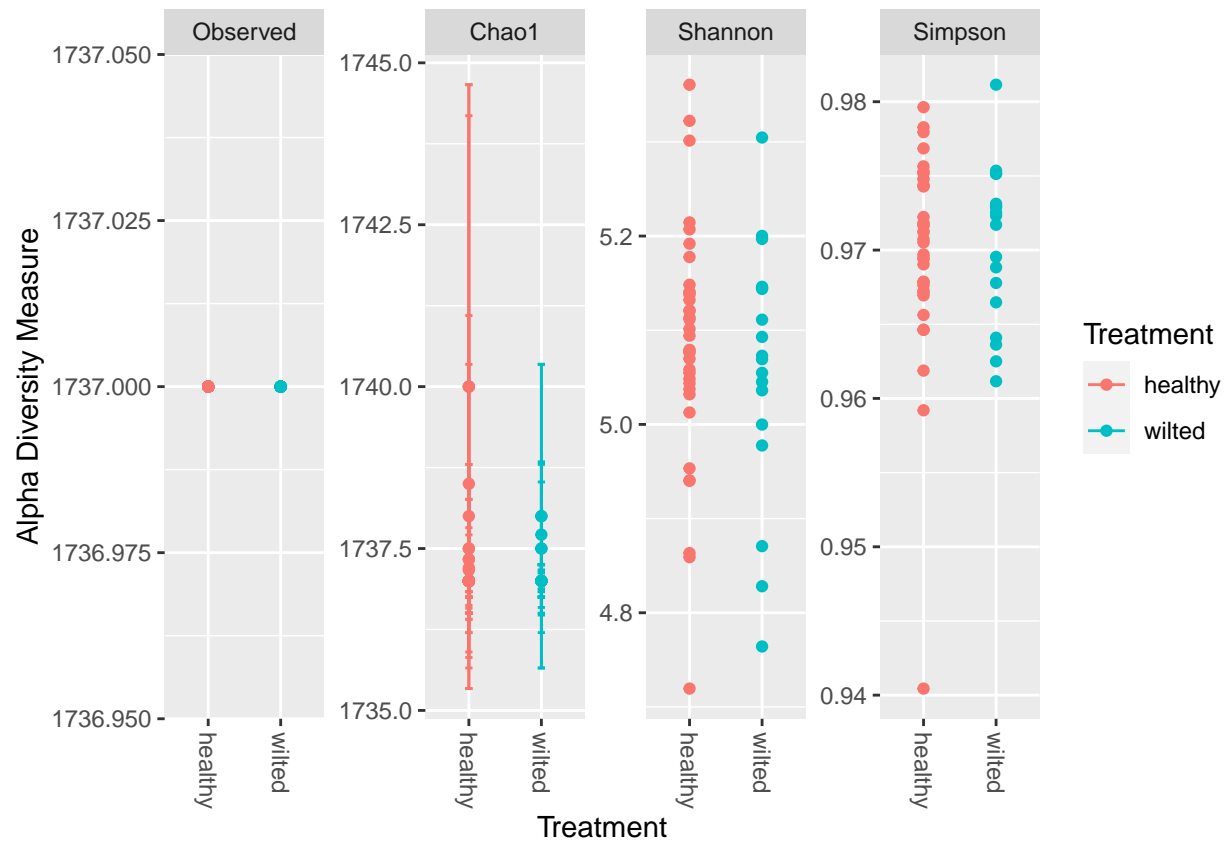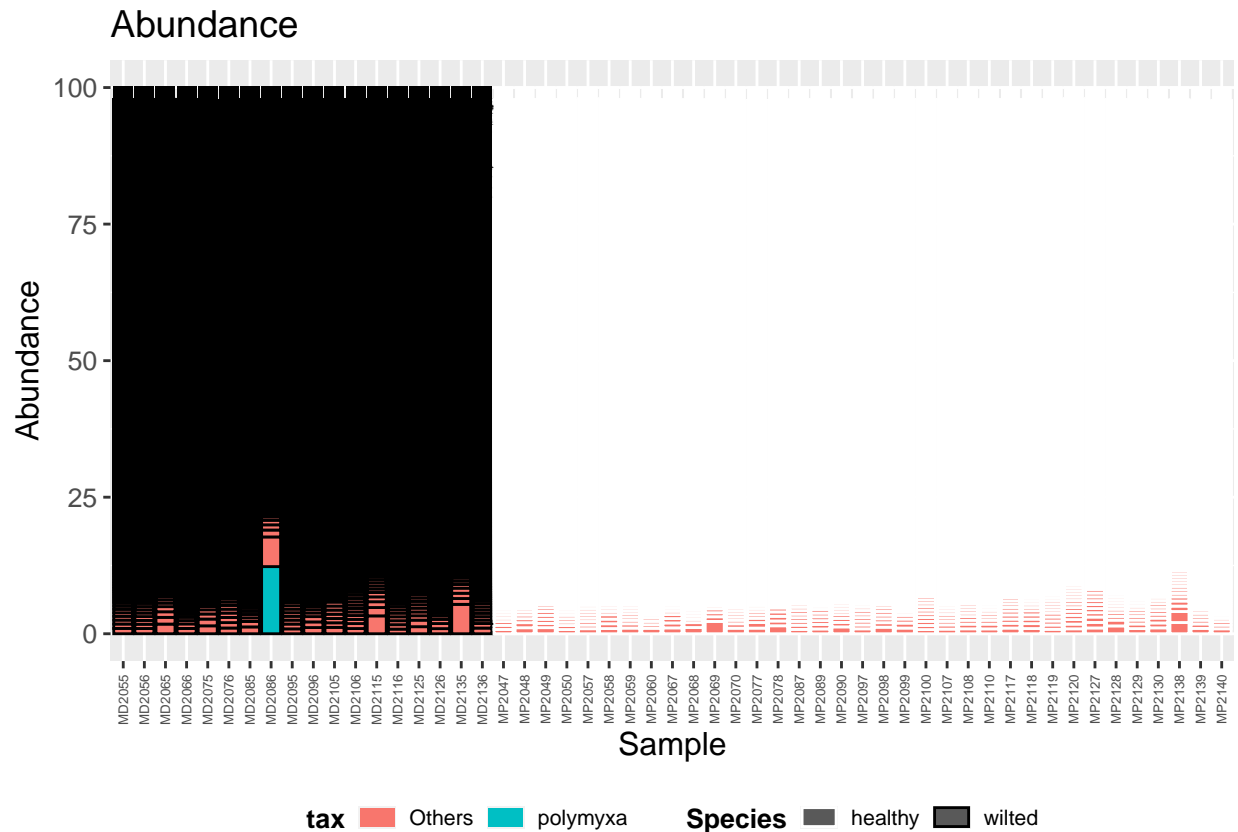ibbosus
ifera

Barras_Species[2]

[[1]]

Abundance

```
Beta_diversity(merge_Bacteria , 'Species' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1576092
## Run 1 stress 0.1866397
## Run 2 stress 0.1692535
## Run 3 stress 0.4024183
## Run 4 stress 0.1519158
## ... New best solution
## ... Procrustes: rmse 0.06346224  max resid 0.3602169
## Run 5 stress 0.1521401
## ... Procrustes: rmse 0.01671034  max resid 0.08898904
## Run 6 stress 0.1521391
## ... Procrustes: rmse 0.01626585  max resid 0.08838474
## Run 7 stress 0.174973
## Run 8 stress 0.1591658
## Run 9 stress 0.1696163
## Run 10 stress 0.1563336
## Run 11 stress 0.1521405
## ... Procrustes: rmse 0.01603465  max resid 0.08803435
## Run 12 stress 0.1515455
## ... New best solution
## ... Procrustes: rmse 0.006646715  max resid 0.03451472
## Run 13 stress 0.1693012
## Run 14 stress 0.1676826
```

```
## Run 15 stress 0.1563338
## Run 16 stress 0.1821167
## Run 17 stress 0.1578266
## Run 18 stress 0.1595337
## Run 19 stress 0.1586321
## Run 20 stress 0.1622876
## *** Best solution was not repeated -- monoMDS stopping criteria:
##       1: no. of iterations >= maxit
##      19: stress ratio > sratmax
```



```
Alpha_diversity(merge_Bacteria , 'Species' , 'Treatment')
```