230306_Reporte1Exploracion

Camila Silva

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R Markdown

reporte

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

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

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

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

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

Cargado de datos originales

```
setwd("/home/camila/GIT/Tesis_Maestria/Data/fresa_solena")
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", "
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/metadata.csv",header =
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)
```

Subconjunto de "Eukaryota"

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

Subconjunto de "Bacteria"

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

Funciones

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

```
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

```
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=attr
    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.position = "bottom",
          legend.title = element_text(face = "bold"),
          text = element_text(size=12),
          axis.text.x = element_text(angle=90, size=12, hjust=1, vjust=0.5))

  percentages_df$tax<-percentages_df[,ncol(percentages_df)]
  percentages_df$tax[percentages_df$Abundance < abundance_percentage] <- "abundance_percentage"
  percentages_df$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.position = "bottom",
          legend.title = element_text(face = "bold"),
```

```
            text = element_text(size=12),
            axis.text.x = element_text(angle=90, size=12, 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)
}
```
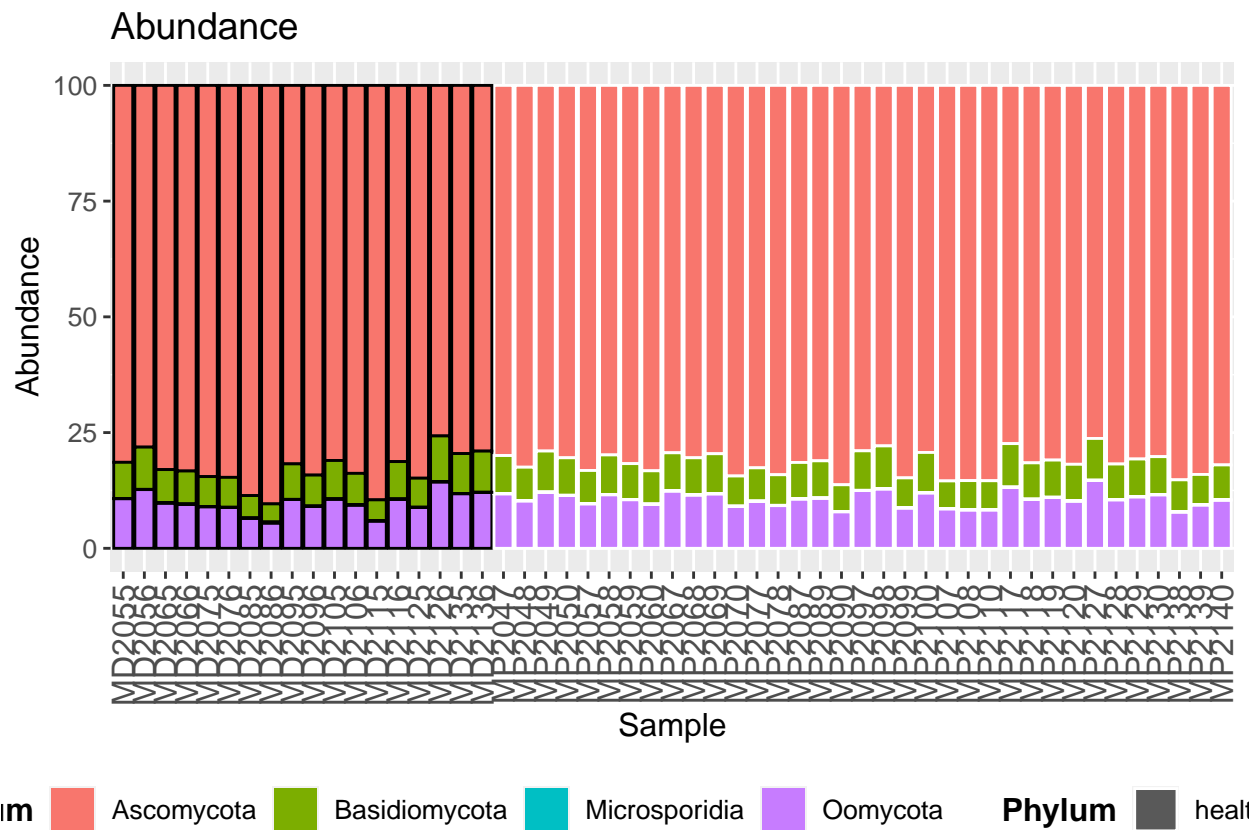
#————Eukarya by Phylum

```
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()'
```
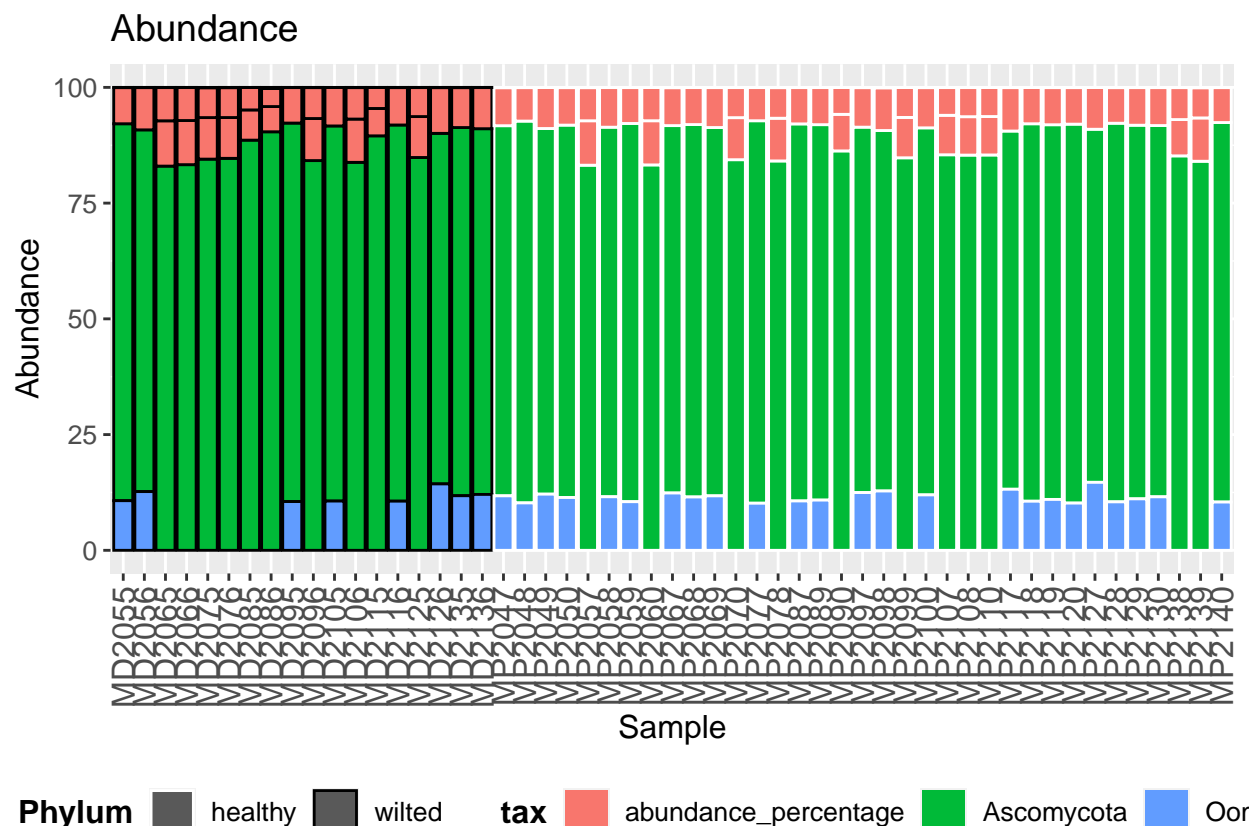
```
Barras_Phylum[1] # normal
```

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

Barras_Phylum[2]

[[1]]

Abundance

Phylum ▨ healthy ◼ wilted **tax** 🟥 abundance_percentage 🟩 Ascomycota 🟦 Oor

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

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.01148681
## Run 1 stress 8.581372e-05
## ... New best solution
## ... Procrustes: rmse 0.03464451  max resid 0.09086026
## Run 2 stress 0.3921933
## Run 3 stress 9.235661e-05
## ... Procrustes: rmse 8.175744e-05  max resid 0.0002288446
## ... Similar to previous best
## Run 4 stress 9.617614e-05
## ... Procrustes: rmse 0.0001008522  max resid 0.000269171
## ... Similar to previous best
## Run 5 stress 9.644717e-05
## ... Procrustes: rmse 7.91108e-05  max resid 0.0002130743
## ... Similar to previous best
## Run 6 stress 9.553648e-05
## ... Procrustes: rmse 5.57069e-05  max resid 0.0001792227
## ... Similar to previous best
## Run 7 stress 9.878097e-05
## ... Procrustes: rmse 8.529878e-05  max resid 0.0002173021
## ... Similar to previous best
## Run 8 stress 9.713914e-05
```

```
## ... Procrustes: rmse 0.0001313505  max resid 0.0003899858
## ... Similar to previous best
## Run 9 stress 9.742258e-05
## ... Procrustes: rmse 8.139225e-05  max resid 0.000230473
## ... Similar to previous best
## Run 10 stress 9.485504e-05
## ... Procrustes: rmse 7.85512e-05  max resid 0.0002270051
## ... Similar to previous best
## Run 11 stress 0.004528109
## Run 12 stress 9.76768e-05
## ... Procrustes: rmse 7.773256e-05  max resid 0.0002308454
## ... Similar to previous best
## Run 13 stress 3.426821e-05
## ... New best solution
## ... Procrustes: rmse 3.502835e-05  max resid 0.0001157903
## ... Similar to previous best
## Run 14 stress 0.4042142
## Run 15 stress 5.930543e-05
## ... Procrustes: rmse 2.516612e-05  max resid 5.308667e-05
## ... Similar to previous best
## Run 16 stress 9.713476e-05
## ... Procrustes: rmse 9.139138e-05  max resid 0.0002546255
## ... Similar to previous best
## Run 17 stress 0.004365444
## Run 18 stress 8.993984e-05
## ... Procrustes: rmse 6.829854e-05  max resid 0.0001603421
## ... Similar to previous best
## Run 19 stress 9.909522e-05
## ... Procrustes: rmse 0.0001362133  max resid 0.0003464382
## ... Similar to previous best
## Run 20 stress 8.874459e-05
## ... Procrustes: rmse 8.475813e-05  max resid 0.0002386906
## ... Similar to previous best
## *** Best solution repeated 6 times


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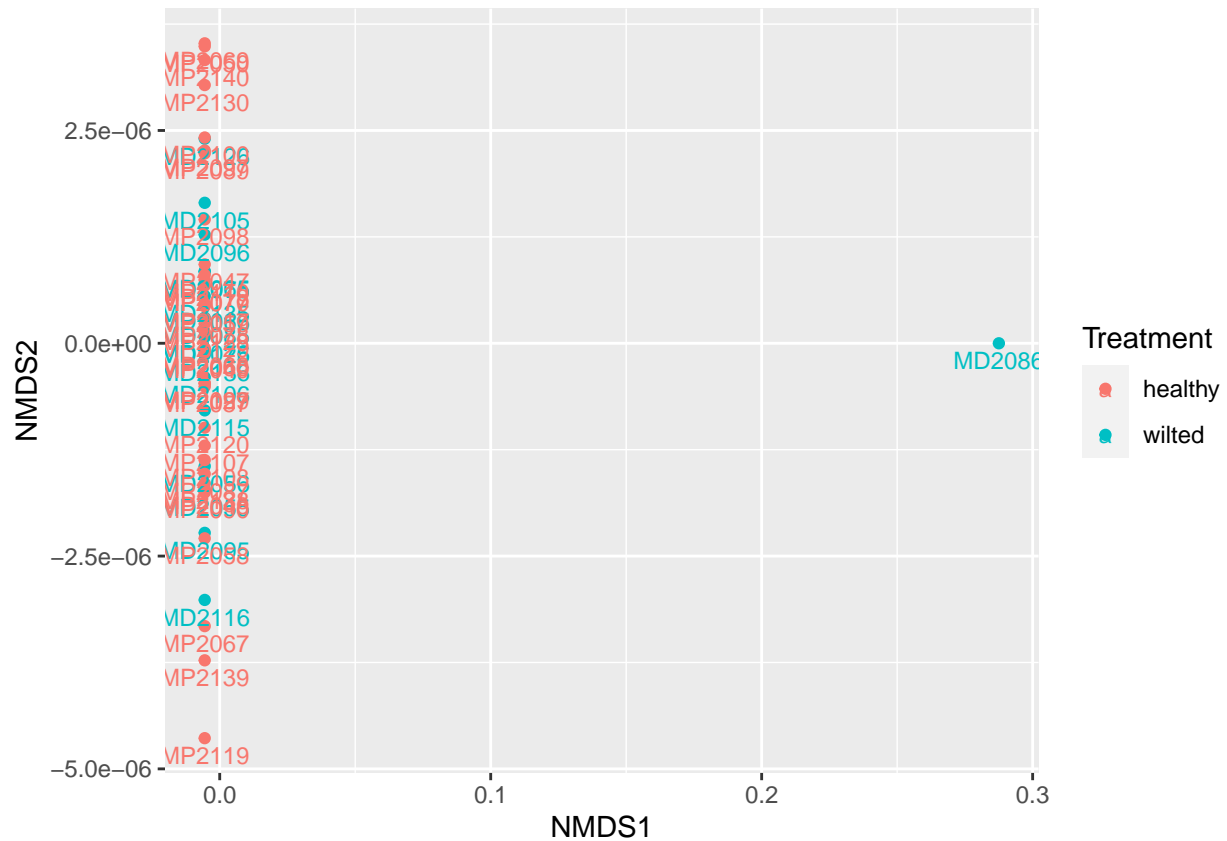
```r
Alpha_diversity(merge_Eukaryota , 'Phylum' , 'Treatment')
```

```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided o
## 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.
```
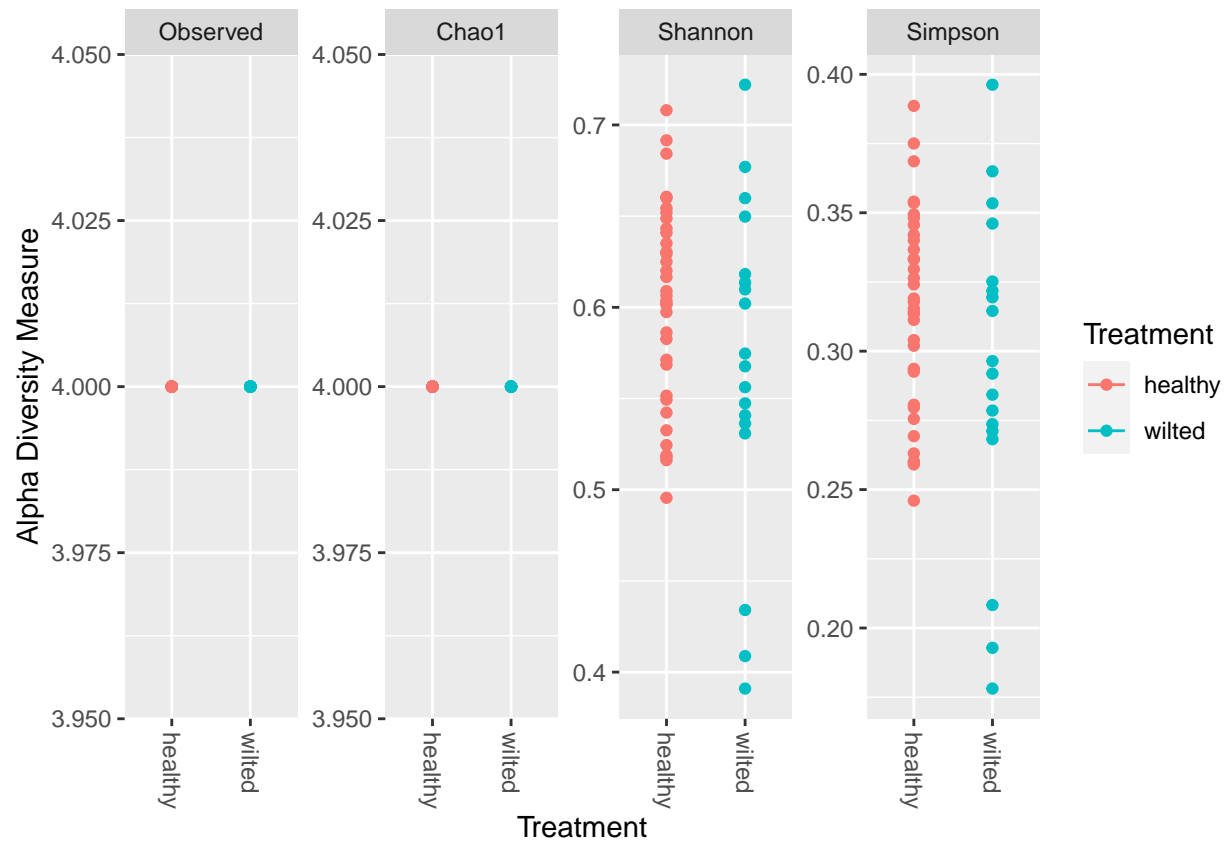
#———Eukarya by Family

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

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

Abundance

Family

Aspergillaceae	Debaryomycetaceae	Marasmiaceae	Pichiace
Bionectriaceae	Didymosphaeriaceae	Mycosphaerellaceae	Pyricula
Ceratobasidiaceae	Dipodascaceae	Nectriaceae	Pythiace
Chaetomiaceae	Glomerellaceae	Ophiocordycipitaceae	Sacchar
Clavicipitaceae	Hypocreaceae	Peronosporaceae	Sacchar
Cordycipitaceae	Leptosphaeriaceae	Phaffomycetaceae	Schizos:
Cryptococcaceae	Malasseziaceae	Phanerochaetaceae	Sclerotir

```
Barras_Species[2]
```

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

9

Abundance

Legend:
- abundance_percentage
- Aspergillaceae
- Chaetomiaceae
- Cordycipitaceae
- Debaryomycetaceae
- Hypocreaceae
- Lepto...
- Nectr...

tax (Wilted)

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

```
## Wisconsin double standardization
## Run 0 stress 0.1089081
## Run 1 stress 0.1088889
## ... New best solution
## ... Procrustes: rmse 0.001720324  max resid 0.009420874
## ... Similar to previous best
## Run 2 stress 0.1089102
## ... Procrustes: rmse 0.001709312  max resid 0.009429385
## ... Similar to previous best
## Run 3 stress 0.109113
## ... Procrustes: rmse 0.00999353  max resid 0.05406397
## Run 4 stress 0.135207
## Run 5 stress 0.1089297
## ... Procrustes: rmse 0.008437248  max resid 0.05478629
## Run 6 stress 0.1089296
## ... Procrustes: rmse 0.008439796  max resid 0.05478447
## Run 7 stress 0.1089299
## ... Procrustes: rmse 0.008608354  max resid 0.05452418
## Run 8 stress 0.1088885
## ... New best solution
## ... Procrustes: rmse 0.001575318  max resid 0.008989064
## ... Similar to previous best
## Run 9 stress 0.1312477
```

```
## Run 10 stress 0.1089289
## ... Procrustes: rmse 0.00838502  max resid 0.05449974
## Run 11 stress 0.1088904
## ... Procrustes: rmse 0.001868121  max resid 0.01070488
## Run 12 stress 0.1088886
## ... Procrustes: rmse 0.001486157  max resid 0.008465417
## ... Similar to previous best
## Run 13 stress 0.1089296
## ... Procrustes: rmse 0.008424576  max resid 0.05466056
## Run 14 stress 0.1089294
## ... Procrustes: rmse 0.008419728  max resid 0.05464795
## Run 15 stress 0.1089489
## ... Procrustes: rmse 0.00806154  max resid 0.05392253
## Run 16 stress 0.1088962
## ... Procrustes: rmse 0.001932239  max resid 0.009016564
## ... Similar to previous best
## Run 17 stress 0.108929
## ... Procrustes: rmse 0.008397748  max resid 0.0545526
## Run 18 stress 0.1089078
## ... Procrustes: rmse 0.002060412  max resid 0.009177838
## ... Similar to previous best
## Run 19 stress 0.1089523
## ... Procrustes: rmse 0.008114132  max resid 0.05378841
## Run 20 stress 0.1088882
## ... New best solution
## ... Procrustes: rmse 0.001363466  max resid 0.007742189
## ... Similar to previous best
## *** Best solution repeated 1 times
```
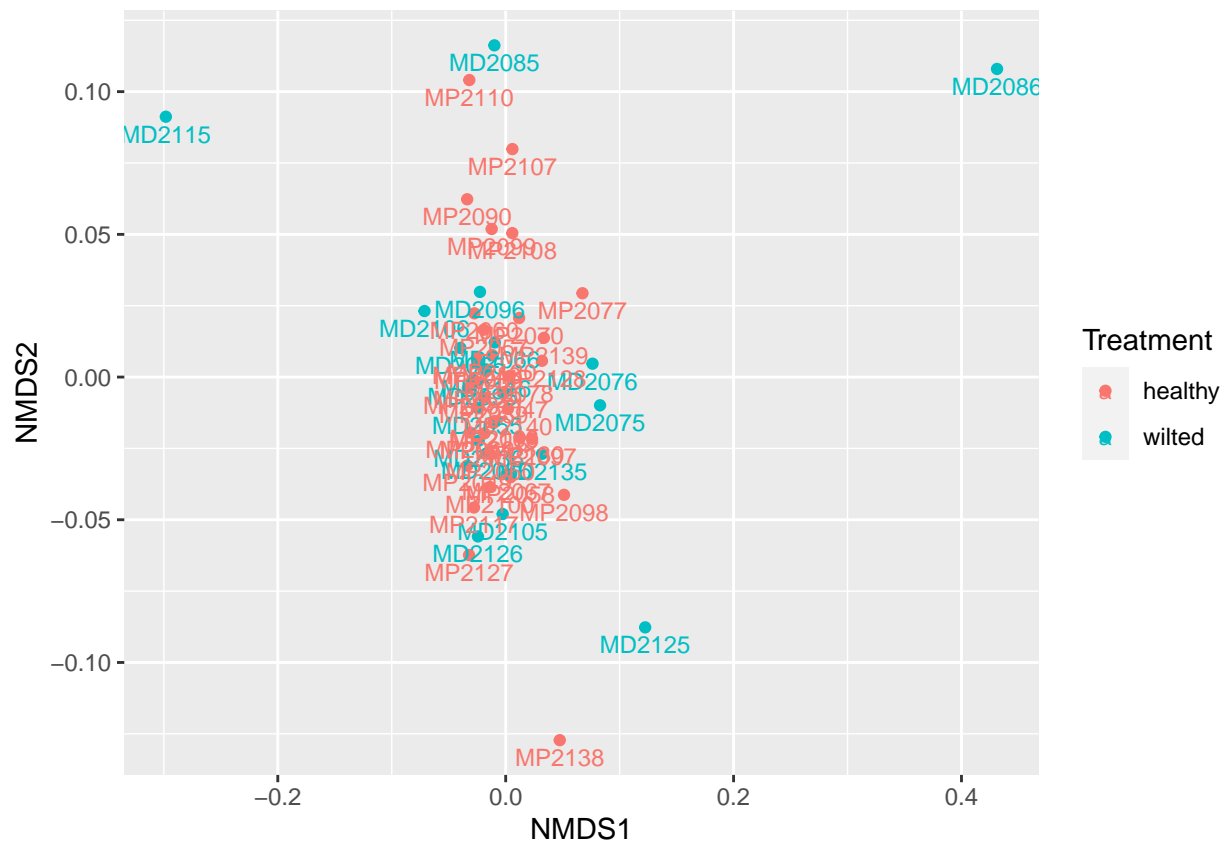
```
Alpha_diversity(merge_Eukaryota , 'Family' , 'Treatment')
```

```
## 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.
```
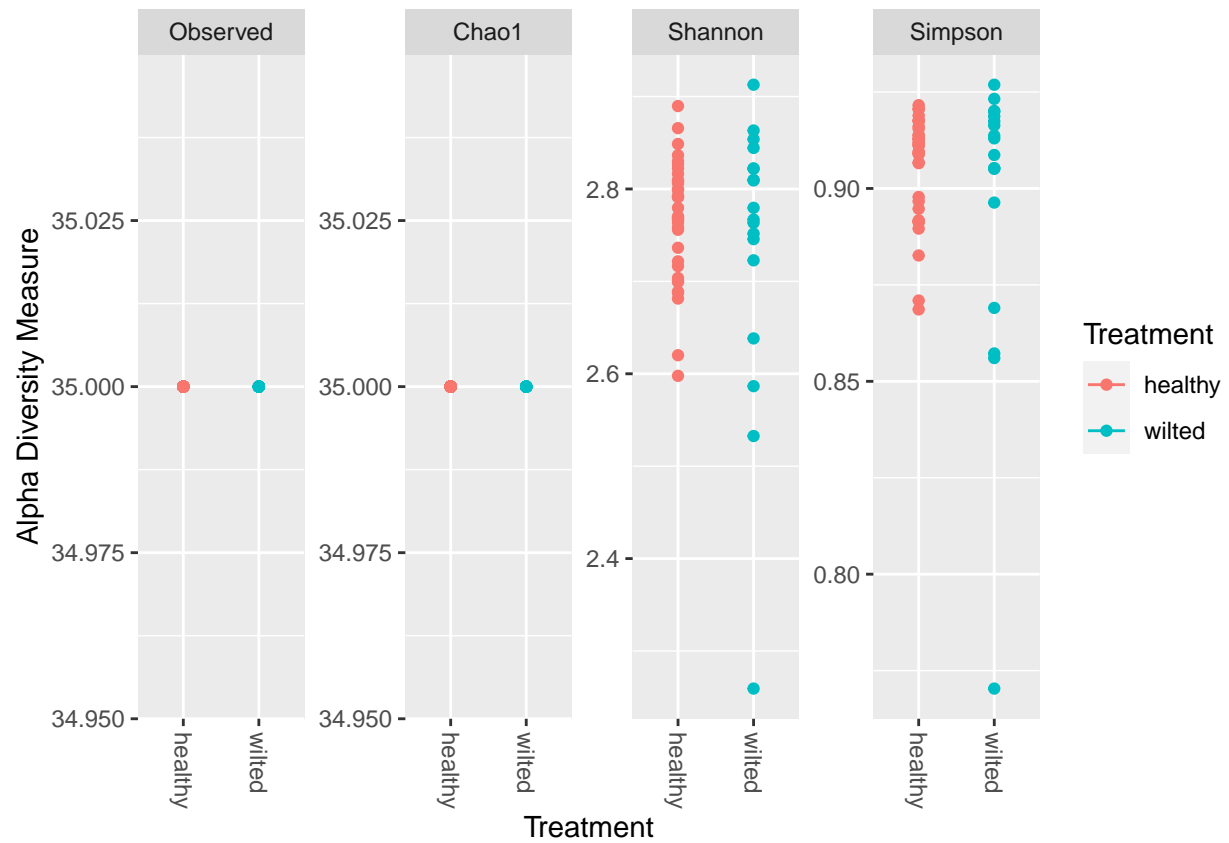
#———Eukarya by Genero

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

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

Abundance

Drechmeria
Encephalitozoon
Eremothecium
Fusarium
Kazachstania
Kluyveromyces
Komagataella
Lachancea
Malassezia
Marasmius
Metarhizium
Nakaseomyces

Naumovozyma
Neopestalotiopsis
Neurospora
Ogataea
Paecilomyces
Paraphaeosphaeria
Penicillium
Phlebiopsis
Phytophthora
Pichia
Pochonia
Pseudozyma

Purpureocillium
Pyricularia
Pythium
Rhizoctonia
Saccharomyces
Saccharomycodes
Scheffersomyces
Schizosaccharomyces
Sporisorium
Sugiyamaella
Talaromyces
Tetrapisispora

Thermothelomyces
Thermothielavioides
Torulaspora
Trichoderma
Ustilaginoidea
Ustilago
Yarrowia
Zygosaccharomyces
Zygotorulaspora
Zymoseptoria

```
Barras_Species[2]
```

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

Abundance

Legend:
- abundance_percentage
- Ampelomyces
- Aspergillus
- Candida
- Fusarium
- Neopestalotiopsis
- Phytophthora
- Trichoderma
- **Genus**

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

```
## Wisconsin double standardization
## Run 0 stress 0.110709
## Run 1 stress 0.1119784
## Run 2 stress 0.1236297
## Run 3 stress 0.1222458
## Run 4 stress 0.1107325
## ... Procrustes: rmse 0.003155808  max resid 0.0178256
## Run 5 stress 0.1119814
## Run 6 stress 0.1107099
## ... Procrustes: rmse 0.001218476  max resid 0.006978283
## ... Similar to previous best
## Run 7 stress 0.1119817
## Run 8 stress 0.1107099
## ... Procrustes: rmse 0.0002610031  max resid 0.001454966
## ... Similar to previous best
## Run 9 stress 0.1107096
## ... Procrustes: rmse 0.001154233  max resid 0.006656173
## ... Similar to previous best
## Run 10 stress 0.1236305
## Run 11 stress 0.1223838
## Run 12 stress 0.1235029
## Run 13 stress 0.1107093
## ... Procrustes: rmse 8.867104e-05  max resid 0.0004837295
```
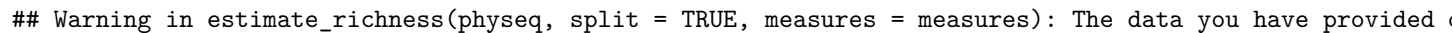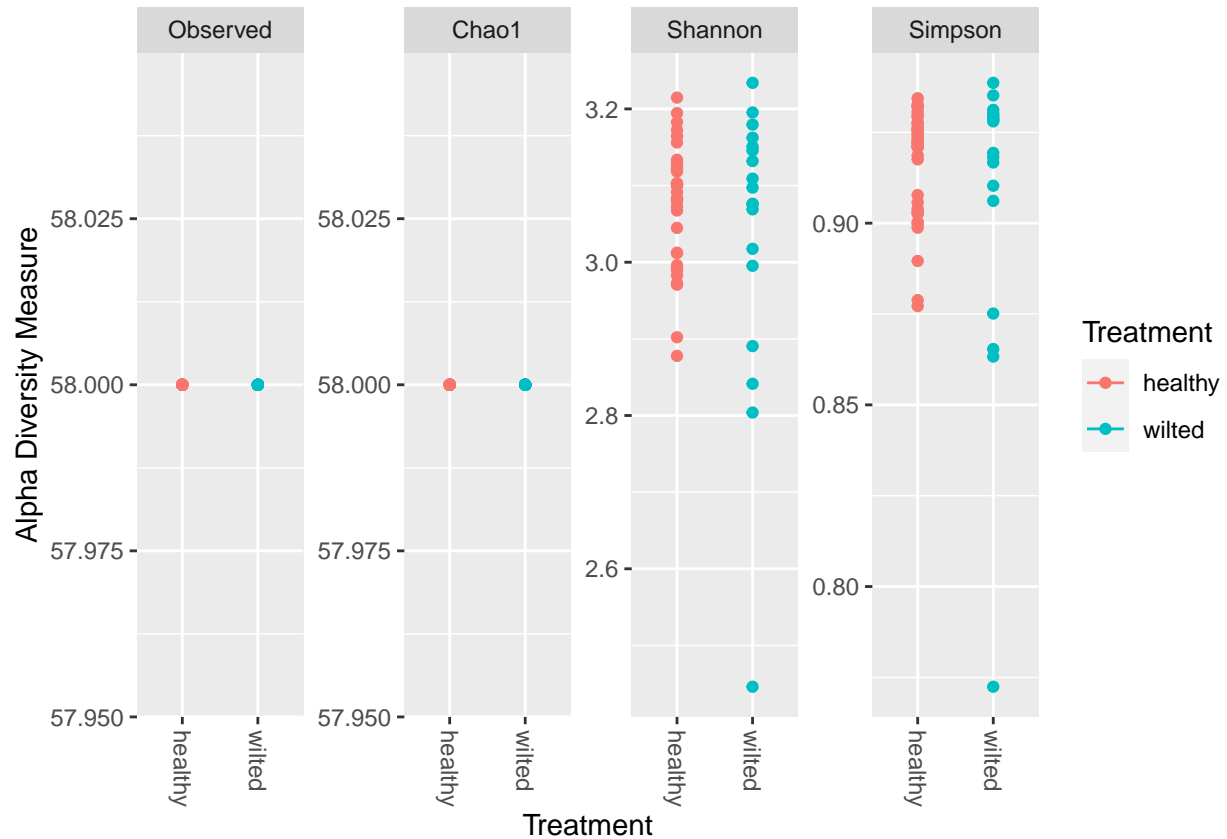
```
## ... Similar to previous best
## Run 14 stress 0.1223831
## Run 15 stress 0.1107092
## ... Procrustes: rmse 0.001046559  max resid 0.005988173
## ... Similar to previous best
## Run 16 stress 0.1107088
## ... New best solution
## ... Procrustes: rmse 9.242977e-05  max resid 0.0005407841
## ... Similar to previous best
## Run 17 stress 0.1107088
## ... New best solution
## ... Procrustes: rmse 0.0002525399  max resid 0.001356355
## ... Similar to previous best
## Run 18 stress 0.1107097
## ... Procrustes: rmse 0.0009608511  max resid 0.005945941
## ... Similar to previous best
## Run 19 stress 0.1119813
## Run 20 stress 0.1107099
## ... Procrustes: rmse 0.0005559224  max resid 0.0027921
## ... Similar to previous best
## *** Best solution repeated 3 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.
```
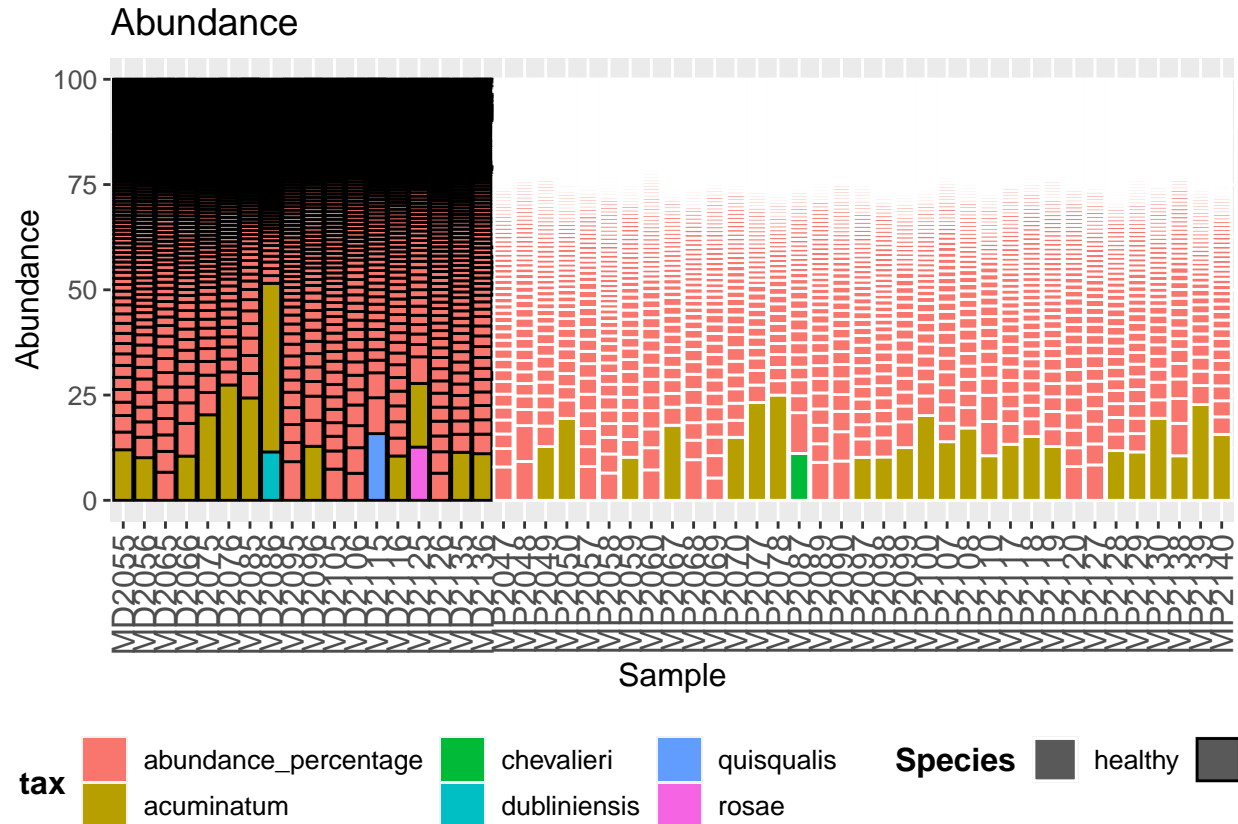

#———Eukarya by Species

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

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

	clavispora		graminearum		maydis		rileyi
	coniospora		graminicola		minitans		robertsii
	crassa		graminum		mrakii		romaleae
	cryptogea		grisea		musae		rosae
	culmorum		guizhouense		naganishii		rosea
	cuniculi		hamatum		nanus		rouxii
	cymbalariae		hansenii		nelsonii		rudraprayagi
	dairenensis		harzianum		neoformans		rugulosus
	decemcellulare		hellem		niveus		sambucinum
	delbrueckii		heterosporum		nygamai		scirpi
	digitatum		higginsianum		oreades		sinecaudum
	dimerum		humberi		orthopsilosis		solani
	dubliniensis		infestans		oryzae		sporotrichioides
	equiseti		intestinalis		oxysporum		stipitis
	erinaceum		italicum		paradoxus		terrestris
	eubayanus		koningii		parapolymorpha		thapsinum
	expansum		koningiopsis		parasitica		thermophilus
	felina		kudriavzevii		pennisetigena		thermotolerans
	flavus		lactis		phaffii		tritici

Barras_Species[2]

[[1]]

Abundance

Legend:
tax
- abundance_percentage
- acuminatum
- chevalieri
- dubliniensis
- quisqualis
- rosae

Species
- healthy

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

```
## Wisconsin double standardization
## Run 0 stress 0.1226512
## Run 1 stress 0.1272844
## Run 2 stress 0.1218539
## ... New best solution
## ... Procrustes: rmse 0.02022733  max resid 0.1284437
## Run 3 stress 0.121854
## ... Procrustes: rmse 0.0001884672  max resid 0.0008491261
## ... Similar to previous best
## Run 4 stress 0.121874
## ... Procrustes: rmse 0.002565178  max resid 0.01268243
## Run 5 stress 0.1218543
## ... Procrustes: rmse 0.0002458649  max resid 0.001223033
## ... Similar to previous best
## Run 6 stress 0.1218541
## ... Procrustes: rmse 0.0003502193  max resid 0.001741702
## ... Similar to previous best
## Run 7 stress 0.1218543
## ... Procrustes: rmse 0.0002170087  max resid 0.001060605
## ... Similar to previous best
## Run 8 stress 0.1218543
## ... Procrustes: rmse 0.0002443382  max resid 0.001196685
## ... Similar to previous best
```

```
## Run 9 stress 0.1218541
## ... Procrustes: rmse 0.000372195  max resid 0.001835571
## ... Similar to previous best
## Run 10 stress 0.1218545
## ... Procrustes: rmse 0.0003347888  max resid 0.001663274
## ... Similar to previous best
## Run 11 stress 0.1272837
## Run 12 stress 0.1272835
## Run 13 stress 0.1517477
## Run 14 stress 0.1547184
## Run 15 stress 0.121854
## ... Procrustes: rmse 4.026617e-05  max resid 0.0001777355
## ... Similar to previous best
## Run 16 stress 0.1218549
## ... Procrustes: rmse 0.0004050457  max resid 0.002010646
## ... Similar to previous best
## Run 17 stress 0.1351259
## Run 18 stress 0.1218542
## ... Procrustes: rmse 0.0004154338  max resid 0.002196092
## ... Similar to previous best
## Run 19 stress 0.1226506
## Run 20 stress 0.1227525
## *** Best solution repeated 10 times
```
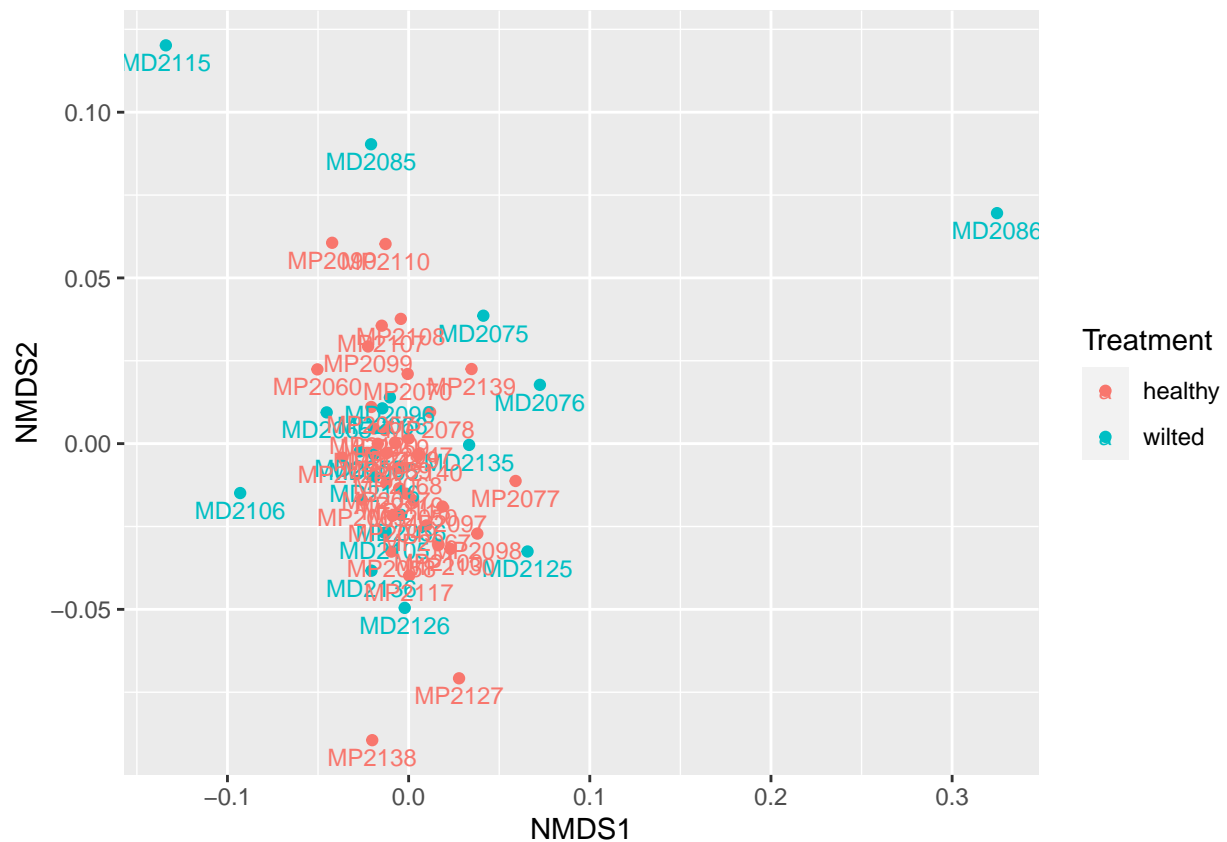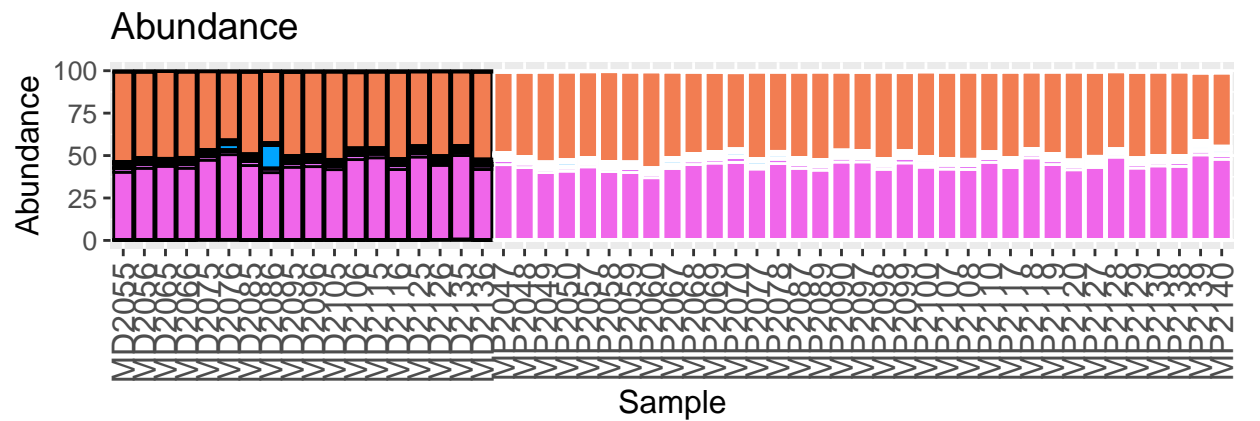
```
Alpha_diversity(merge_Eukaryota , 'Species' , 'Treatment')
```
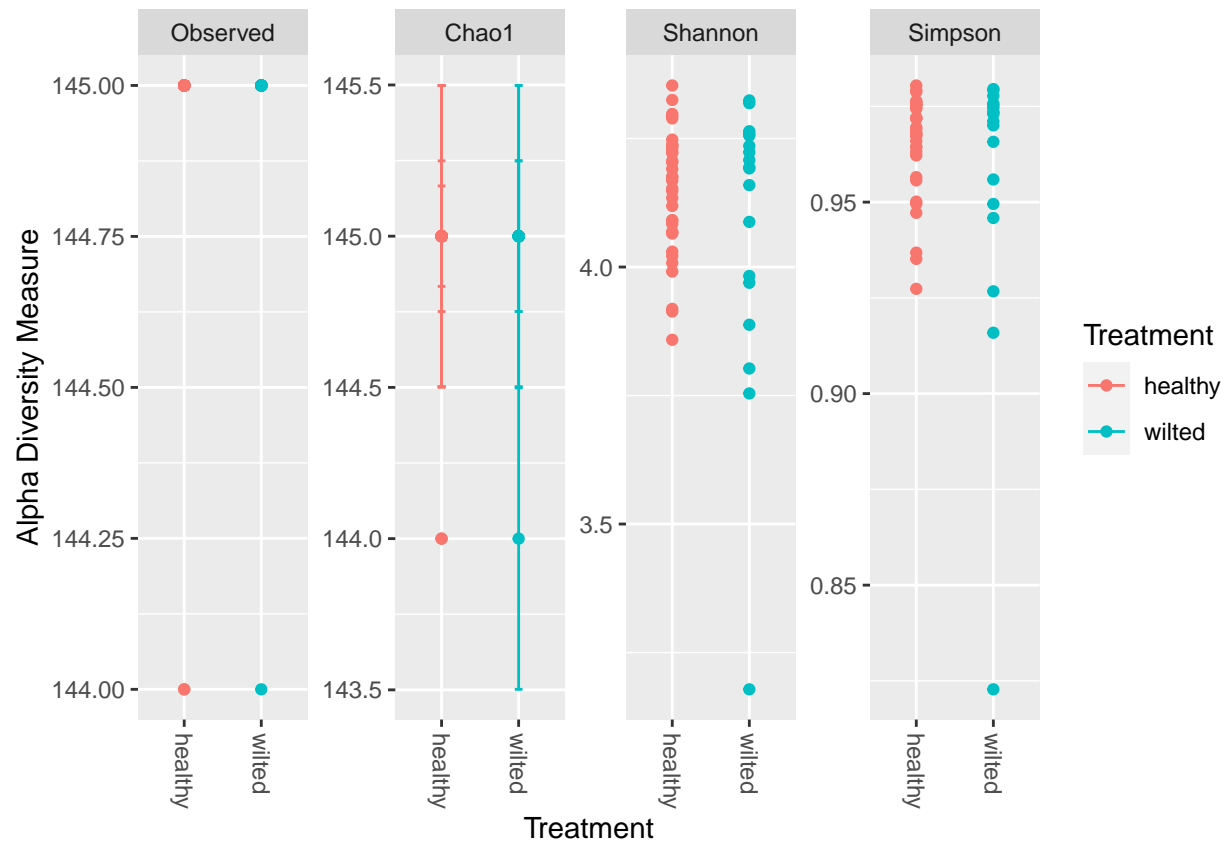

#————Bacteria by Phylum

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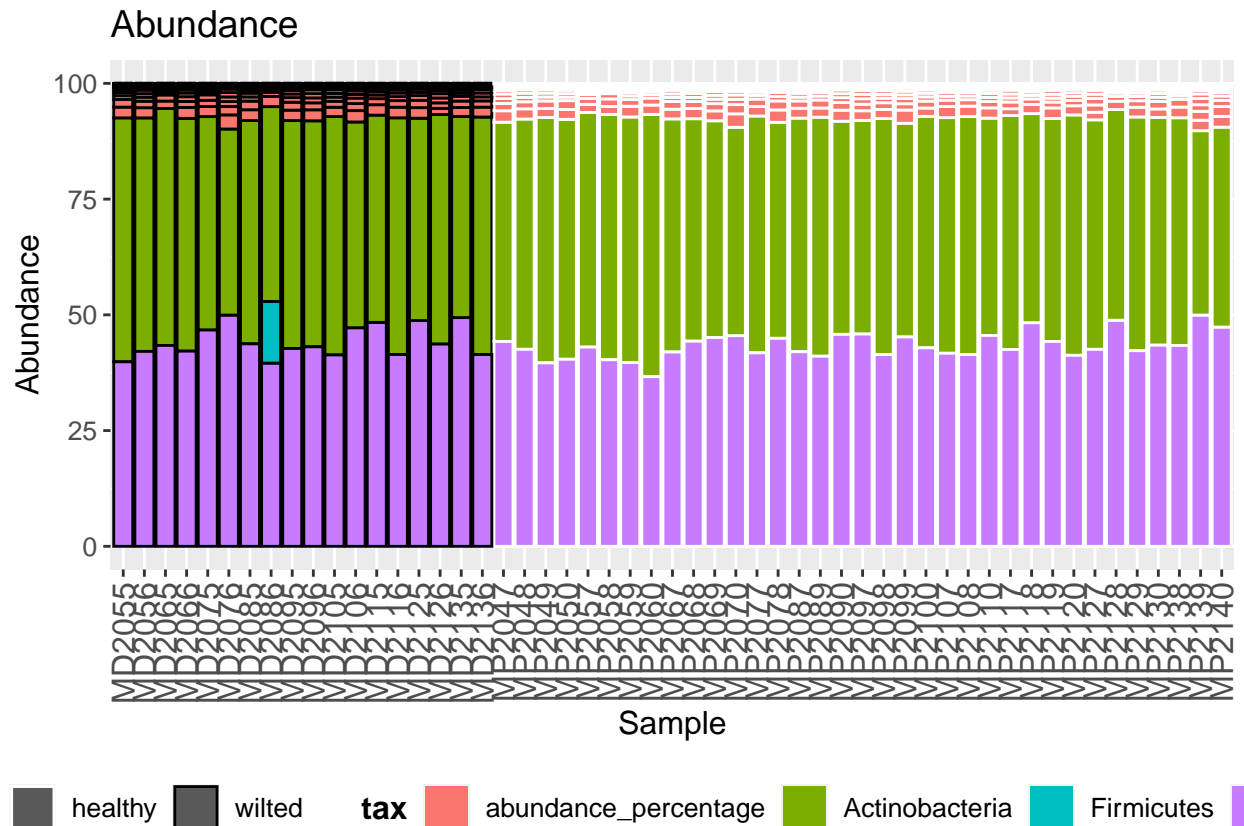
```
## [[1]]
```

Abundance

alditrichaeota	Chloroflexi	Fibrobacteres	Proteobacteri
andidatus Absconditabacteria	Chrysiogenetes	Firmicutes	Spirochaetes
andidatus Bipolaricaulota	Coprothermobacterota	Fusobacteria	Synergistetes
andidatus Cloacimonetes	Cyanobacteria	Gemmatimonadetes	Tenericutes
andidatus Omnitrophica	Deferribacteres	Ignavibacteriae	Thermodesul
andidatus Saccharibacteria	Deinococcus−Thermus	Kiritimatiellaeota	Thermotogae
hlamydiae	Dictyoglomi	Nitrospirae	Verrucomicro
hlorobi	Elusimicrobia	Planctomycetes	

Barras_Species[2]

[[1]]

Abundance

Legend: healthy, wilted, **tax**, abundance_percentage, Actinobacteria, Firmicutes

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

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.1520004
## Run 1 stress 0.1742641
## Run 2 stress 0.1593175
## Run 3 stress 0.1573463
## Run 4 stress 0.161734
## Run 5 stress 0.1537339
## Run 6 stress 0.1573473
## Run 7 stress 0.1667699
## Run 8 stress 0.1744041
## Run 9 stress 0.1697749
## Run 10 stress 0.1744627
## Run 11 stress 0.1711649
## Run 12 stress 0.1707681
## Run 13 stress 0.1656732
## Run 14 stress 0.1692037
## Run 15 stress 0.1668935
## Run 16 stress 0.1693494
## Run 17 stress 0.162697
## Run 18 stress 0.157849
## Run 19 stress 0.1680263
## Run 20 stress 0.1573461
```

```
## *** Best solution was not repeated -- monoMDS stopping criteria:
##      2: no. of iterations >= maxit
##      18: stress ratio > sratmax
```
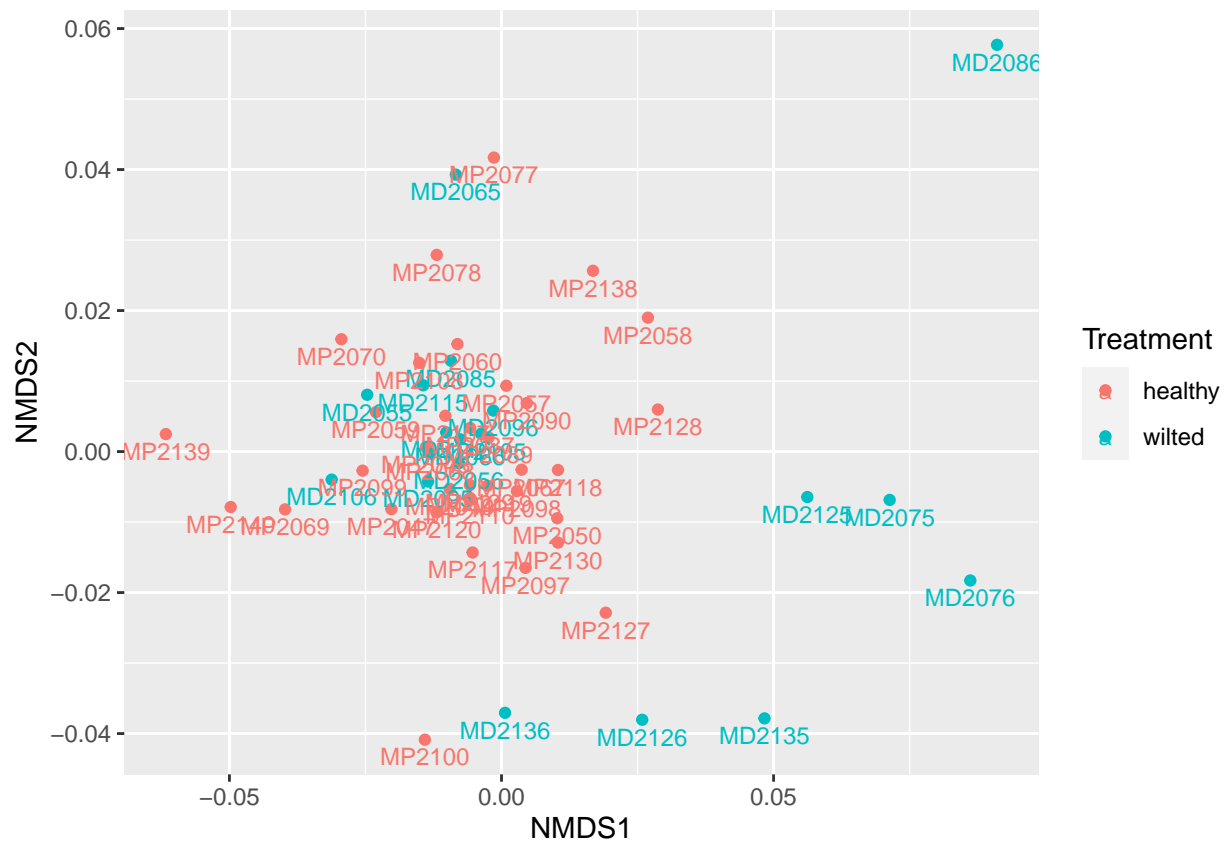


```
Alpha_diversity(merge_Bacteria , 'Phylum' , 'Treatment')
```

```
## 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.
```
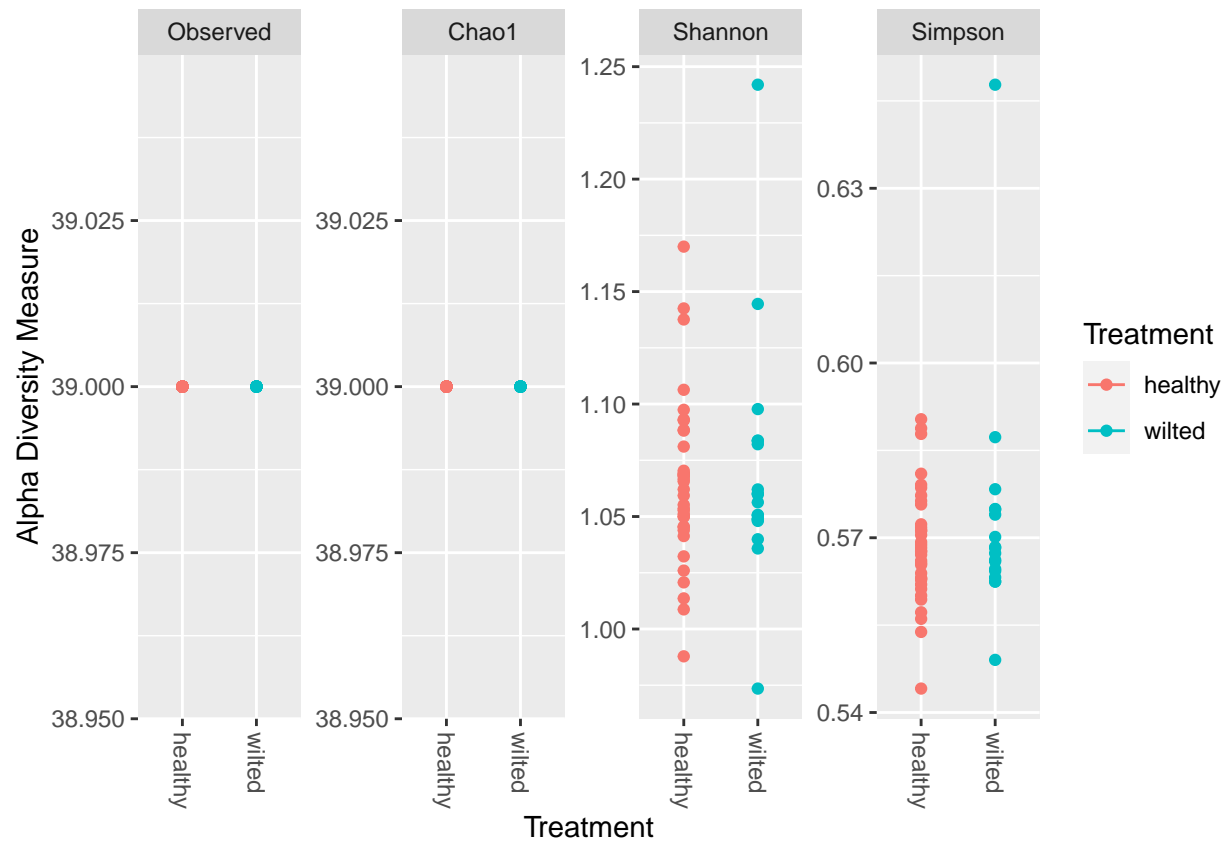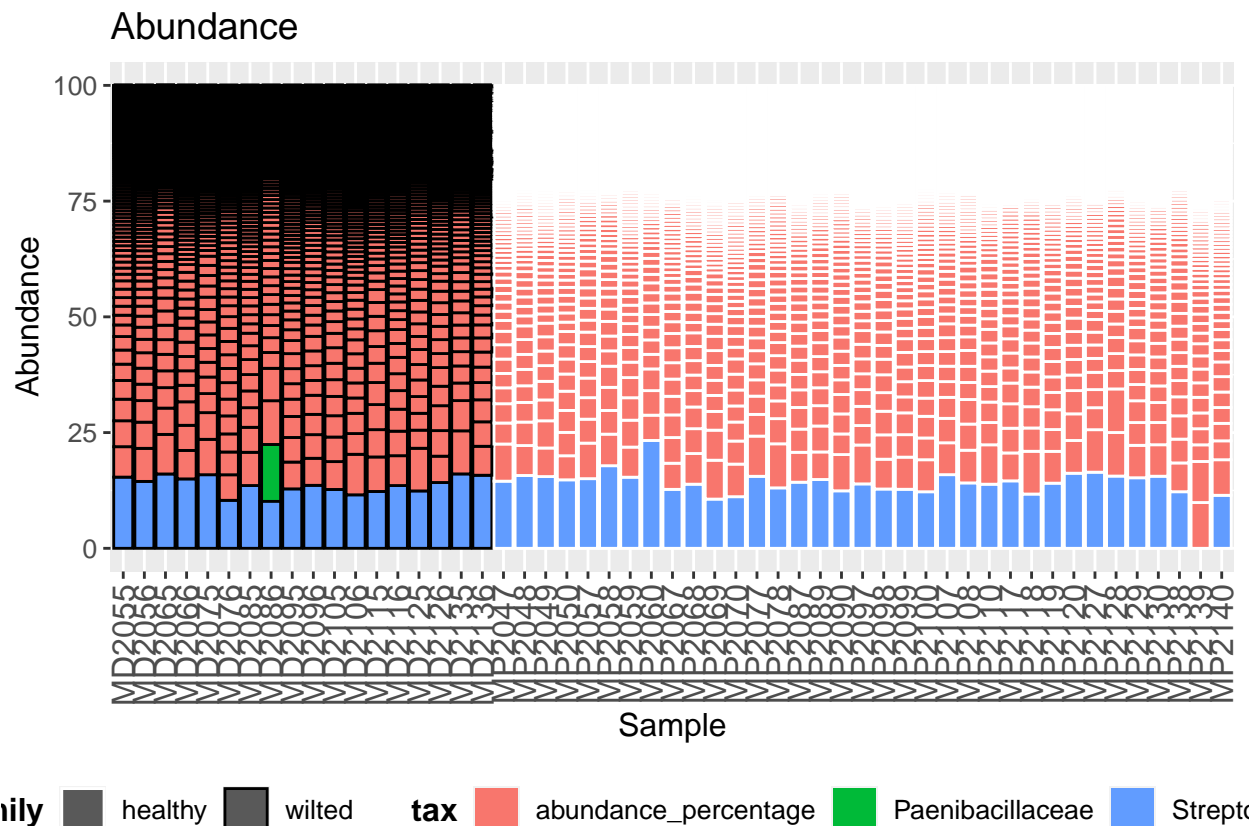
#———Bacteria by Familia

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

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

e	

Dermocarpellaceae

Desulfallaceae

Desulfarculaceae

Desulfitobacteriaceae

Desulfobacteraceae

e

Desulfobulbaceae

Desulfocapsaceae

Desulfohalobiaceae

Desulfomicrobiaceae

Desulfosudaceae

Desulfotomaculaceae

Desulfovibrionaceae

Desulfurellaceae

Desulfurobacteriaceae

Desulfuromonadaceae

Devosiaceae

Dictyoglomaceae

Dietziaceae

Dissulfurispiraceae

Jatrophihabitantaceae

Jiangellaceae

Jonesiaceae

Kaistiaceae

Kangiellaceae

Kineosporiaceae

Kiritimatiellaceae

Kofleriaceae

Koleobacteraceae

Kordiimonadaceae

Kosmotogaceae

Kribbellaceae

Ktedonosporobacteraceae

Kytococcaceae

Labilitrichaceae

Lachnospiraceae

Lacipirellulaceae

Lactobacillaceae

Lawsonellaceae

Pe

Pe

Pe

Pl

Pl

Pl

Pl

Pi

Pi

Pl

Pl

Pl

Po

Po

Pr

Pr

Pr

Pr

Pr

```
Barras_Species[2]
```

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

Abundance

Legend: **Family** healthy | wilted **tax** abundance_percentage | Paenibacillaceae | Strepto...
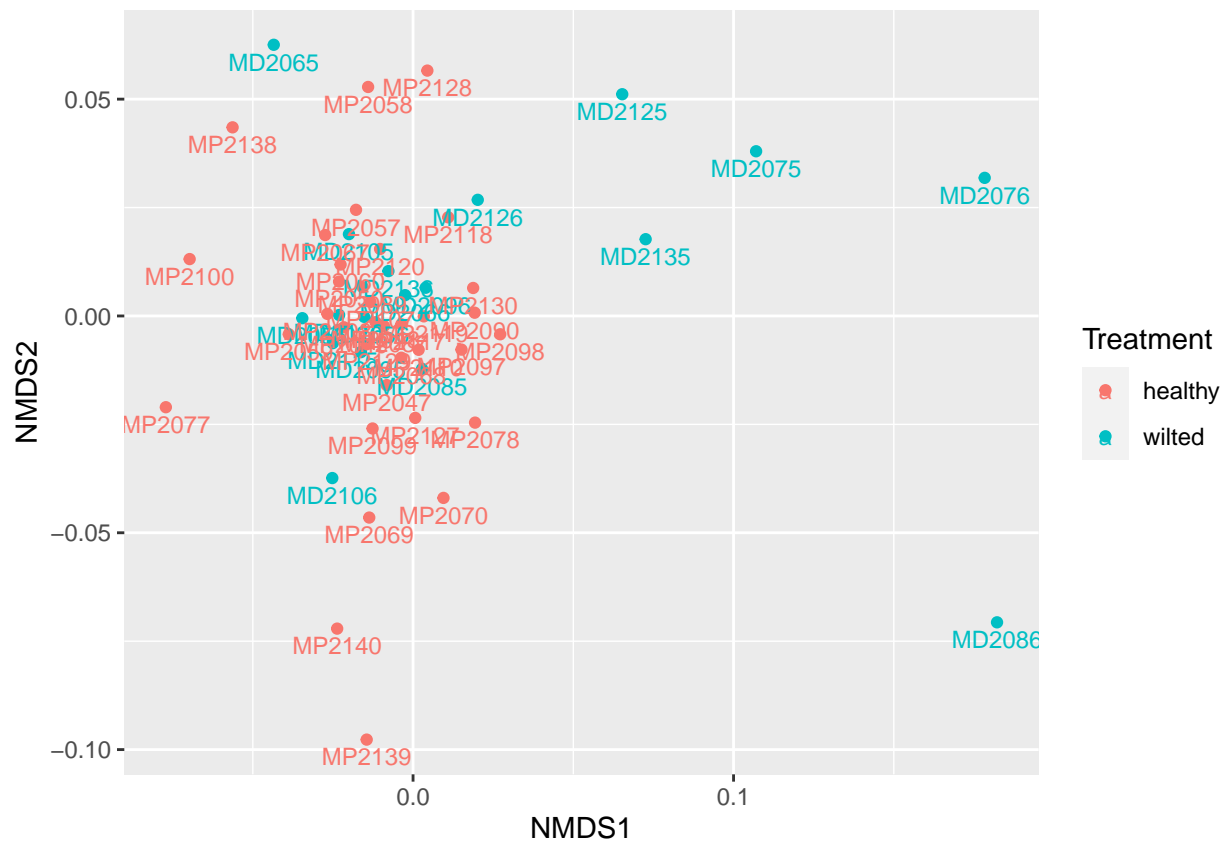
```
Beta_diversity(merge_Bacteria , 'Family' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1412073
## Run 1 stress 0.1567789
## Run 2 stress 0.1400264
## ... New best solution
## ... Procrustes: rmse 0.02602484  max resid 0.1381912
## Run 3 stress 0.1631861
## Run 4 stress 0.1455823
## Run 5 stress 0.1412069
## Run 6 stress 0.137377
## ... New best solution
## ... Procrustes: rmse 0.05943273  max resid 0.3596099
## Run 7 stress 0.1412066
## Run 8 stress 0.1396568
## Run 9 stress 0.1401651
## Run 10 stress 0.1369869
## ... New best solution
## ... Procrustes: rmse 0.01588383  max resid 0.05975398
## Run 11 stress 0.1571927
## Run 12 stress 0.1418143
## Run 13 stress 0.1418148
## Run 14 stress 0.1373772
## ... Procrustes: rmse 0.01593279  max resid 0.05952602
```

```
## Run 15 stress 0.1373768
## ... Procrustes: rmse 0.01582816   max resid 0.05945794
## Run 16 stress 0.1401068
## Run 17 stress 0.1647408
## Run 18 stress 0.1517988
## Run 19 stress 0.140104
## Run 20 stress 0.1369864
## ... New best solution
## ... Procrustes: rmse 0.0001686947   max resid 0.0005752435
## ... Similar to previous best
## *** Best solution repeated 1 times
```



```
Alpha_diversity(merge_Bacteria , 'Family' , 'Treatment')
```
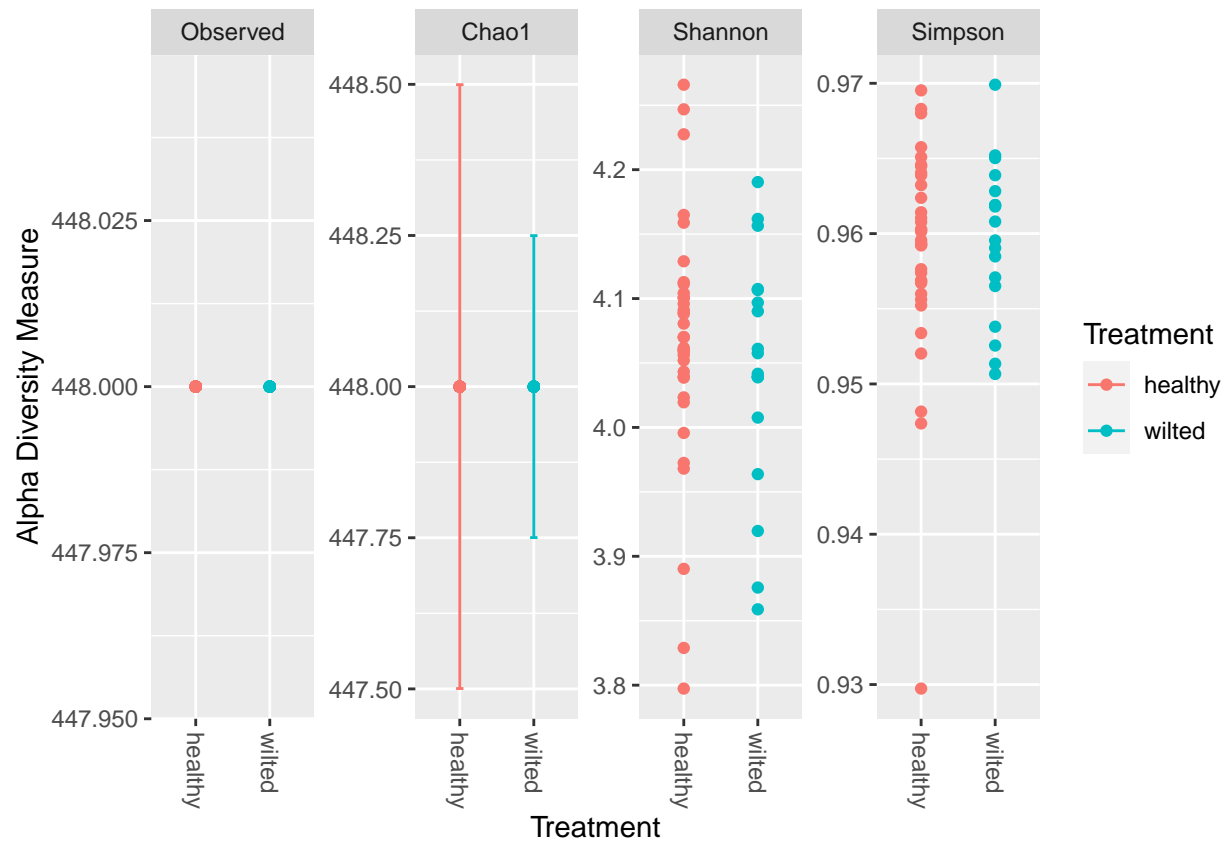
#———Bacteria by Genero
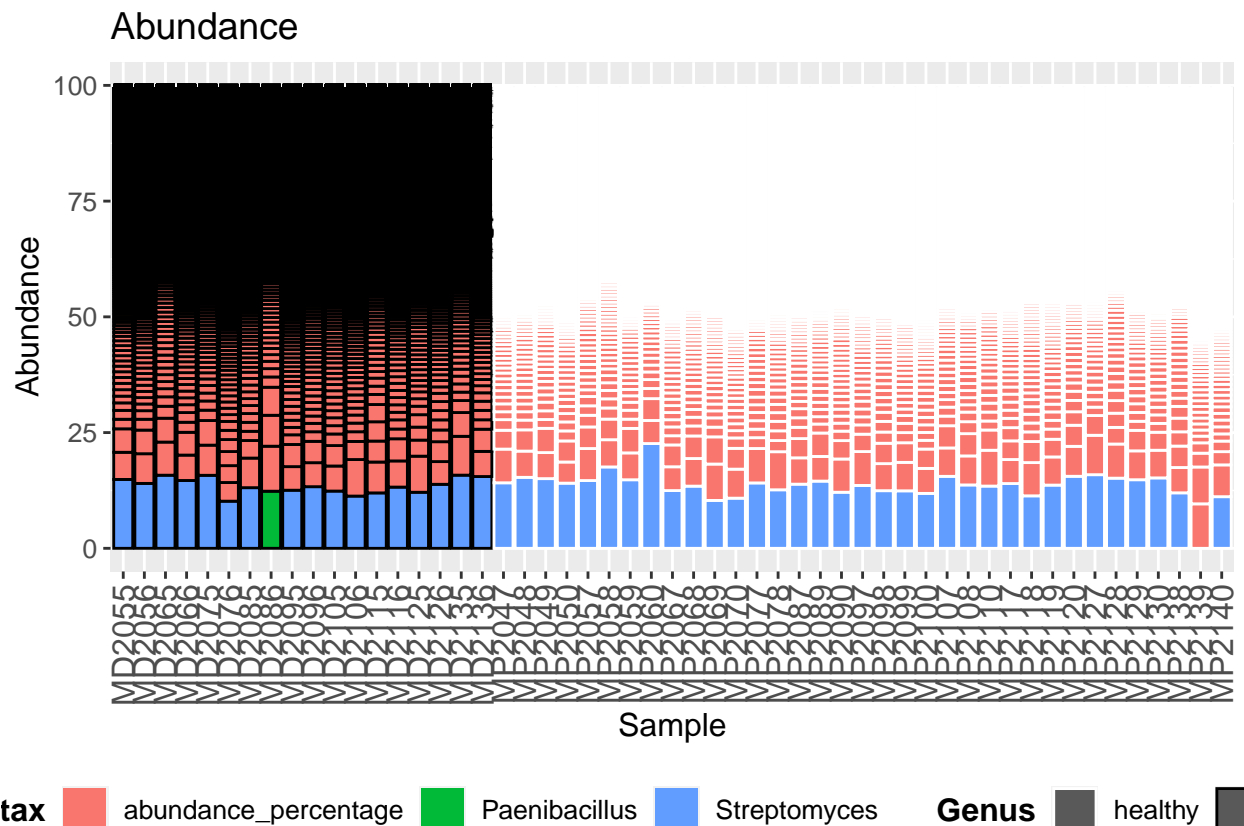
```
Barras_Species <- Abundance_barras(merge_Bacteria,'Genus','Treatment',10.0)
Barras_Species[1]
```

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

esulfosediminicola	■	Lawsonia	■	Pengzhenrongella	■	■
esulfosporosinus		Leadbetterella		Peptacetobacter		
esulfosudis		Leclercia		Peptoclostridium		
esulfotalea		Lederbergia		Peptoniphilus		
esulfotomaculum		Leeuwenhoekiella		Peribacillus		
esulfovibrio		Legionella		Periweissella		
esulfurispirillum		Leifsonia		Permianibacter		
esulfurivibrio		Leisingera		Persephonella		
esulfurobacterium		Lelliottia		Persicimonas		
esulfuromonas		Leminorella		Peteryoungia		
evosia		Lentibacillus		Petrimonas		
evriesea		Lentilactobacillus		Petrocella		
ialister		Lentilitoribacter		Petrotoga		
iaminobutyricimonas		Lentzea		Phaeobacter		
iaphorobacter		Leptodesmis		Phascolarctobacterium		
ichelobacter		Leptolyngbya		Phenylobacterium		
ickeya		Leptospira		Phnomibacter		
ictyoglomus		Leptospirillum		Phocaeicola		
ietzia		Leptothermofonsia		Phoenicibacter		

Barras_Species[2]

[[1]]

Abundance

tax ▇ abundance_percentage ▇ Paenibacillus ▇ Streptomyces   **Genus** ▇ healthy ▇

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

```
## Wisconsin double standardization
## Run 0 stress 0.1467945
## Run 1 stress 0.1432832
## ... New best solution
## ... Procrustes: rmse 0.08897178  max resid 0.3295048
## Run 2 stress 0.1496771
## Run 3 stress 0.1475446
## Run 4 stress 0.1454655
## Run 5 stress 0.1468279
## Run 6 stress 0.143337
## ... Procrustes: rmse 0.004699465  max resid 0.02896213
## Run 7 stress 0.1693988
## Run 8 stress 0.1439567
## Run 9 stress 0.1538104
## Run 10 stress 0.145199
## Run 11 stress 0.1490812
## Run 12 stress 0.1474936
## Run 13 stress 0.1496766
## Run 14 stress 0.1669881
## Run 15 stress 0.1432834
## ... Procrustes: rmse 0.0001297085  max resid 0.0005894101
## ... Similar to previous best
## Run 16 stress 0.148677
```

```
## Run 17 stress 0.1437853
## Run 18 stress 0.1475447
## Run 19 stress 0.1433508
## ... Procrustes: rmse 0.006150377   max resid 0.03676621
## Run 20 stress 0.1505683
## *** Best solution repeated 1 times
```



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

#————Bacteria by Species

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

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

orei	▪ sp. 1(2017)	▪ sp. L
	▪ sp. 103DPR2	▪ sp. L
	▪ sp. 1063	▪ sp. L
	▪ sp. 107–1	▪ sp. L
	▪ sp. 1070	▪ sp. L
	▪ sp. 10FS3–1	▪ sp. L
orus	▪ sp. 10M–3C3	▪ sp. L
dii	▪ sp. 10RB9215	▪ sp. L
	▪ sp. 11–1–2	▪ sp. L
tivorans	▪ sp. 11–B–312	▪ sp. L
lae	▪ sp. 113–1–2	▪ sp. L
	▪ sp. 113–3–3	▪ sp. L
eri	▪ sp. 113–3–9	▪ sp. L
hoekii	▪ sp. 1137	▪ sp. L
osarum	▪ sp. 113P3	▪ sp. L
;	▪ sp. 114	▪ sp. L
rous	▪ sp. 11515TR	▪ sp. L
cum	▪ sp. 116–D4	▪ sp. L
)	▪ sp. 119287	▪ sp. L

Barras_Species[2]

[[1]]
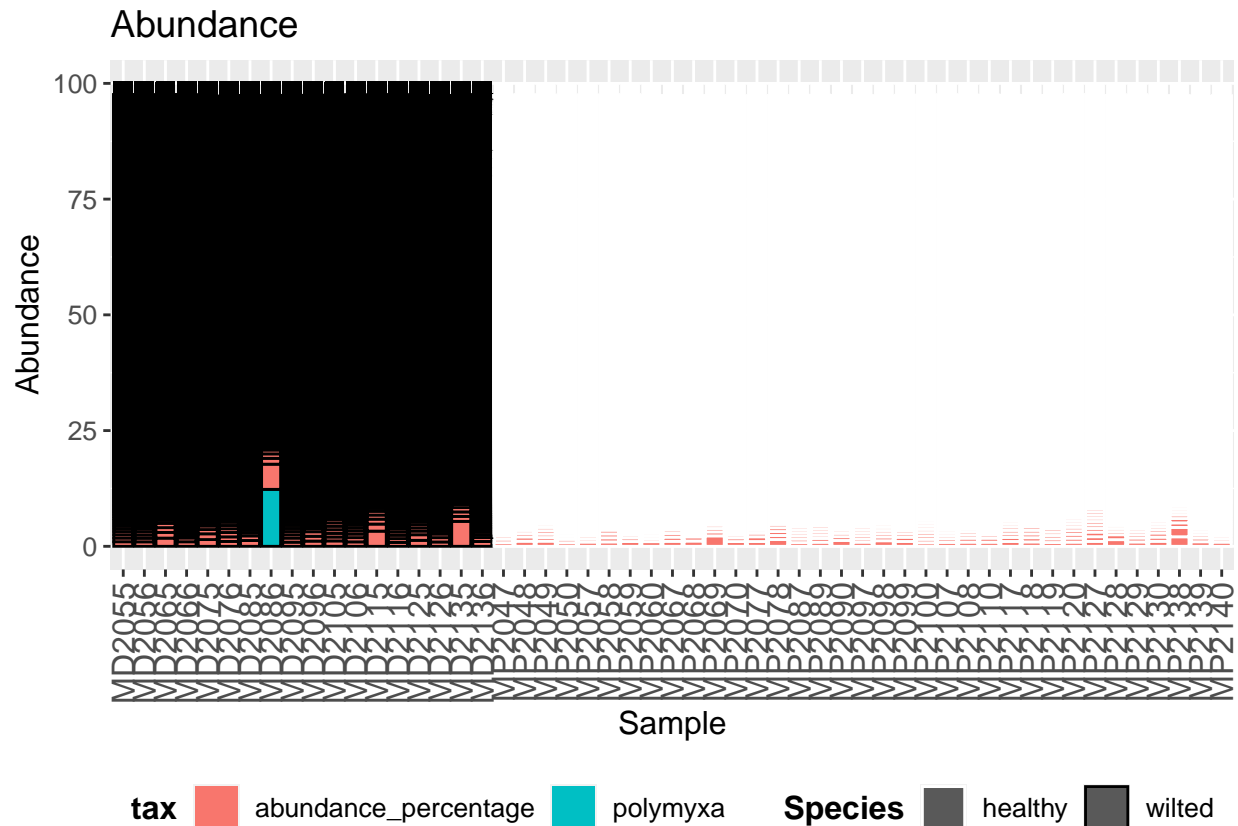
Abundance


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

```
## Wisconsin double standardization
## Run 0 stress 0.1597841
## Run 1 stress 0.1600856
## ... Procrustes: rmse 0.05681376  max resid 0.275897
## Run 2 stress 0.1576765
## ... New best solution
## ... Procrustes: rmse 0.05506173  max resid 0.2871601
## Run 3 stress 0.1598505
## Run 4 stress 0.1738726
## Run 5 stress 0.16716
## Run 6 stress 0.1639717
## Run 7 stress 0.208091
## Run 8 stress 0.1597922
## Run 9 stress 0.175953
## Run 10 stress 0.179444
## Run 11 stress 0.1618986
## Run 12 stress 0.1783748
## Run 13 stress 0.1577445
## ... Procrustes: rmse 0.03464099  max resid 0.1600732
## Run 14 stress 0.1640697
## Run 15 stress 0.1604227
## Run 16 stress 0.160562
## Run 17 stress 0.1692767
```

```
## Run 18 stress 0.1632417
## Run 19 stress 0.1705406
## Run 20 stress 0.1586886
## *** Best solution was not repeated -- monoMDS stopping criteria:
##      4: no. of iterations >= maxit
##      16: stress ratio > sratmax
```



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