

# Funciones con datos normalizados

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```
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/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", "Species")
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",header=1)
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)
```

## Normalización de los datos

Cada una de las funciones de normalización toma un objeto phyloseq 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.

```
edgeRnorm = function(phy, ...){
  require("edgeR")
  require("phyloseq")
  if (!taxa_are_rows(phy)) {
    phy <- t(phy) #transpone el objeto phyloseq
  }
  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

```
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)
```

Haciendo el mismo análisis del reporte 3 tomando los datos normalizados para poder realizar una comparación en los resultados.

## Subconjunto de “Eukaryota”

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

## Subconjunto de “Bacteria”

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

## Funciones

Crea los subconjuntos de datos input phy phyloseq 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=attribute)) +
    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' ,color=attribute)) +
    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)
}
```

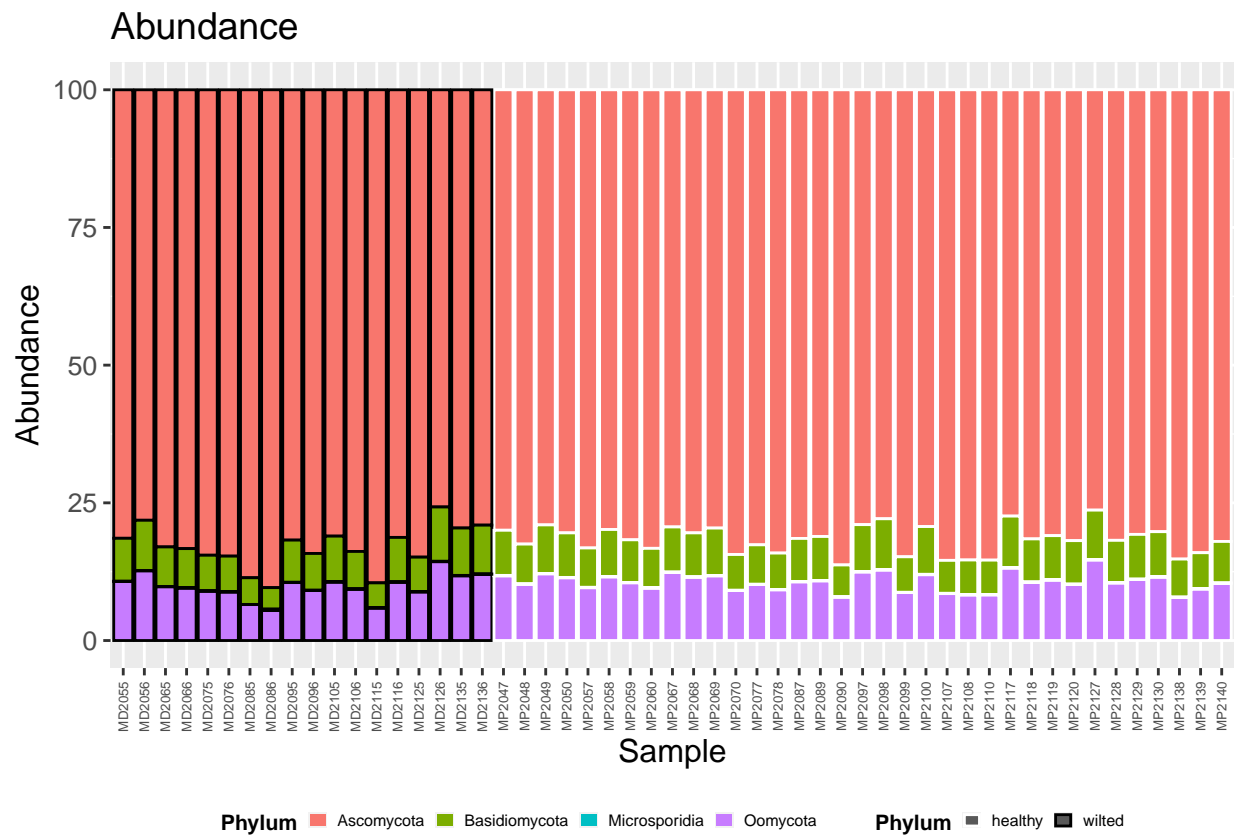
```
#———Eukarya by Phylum
```

```
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 idioms with 'aes()'.
## i See also 'vignette("ggplot2-in-packages")' for more information.
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was
## generated.
```

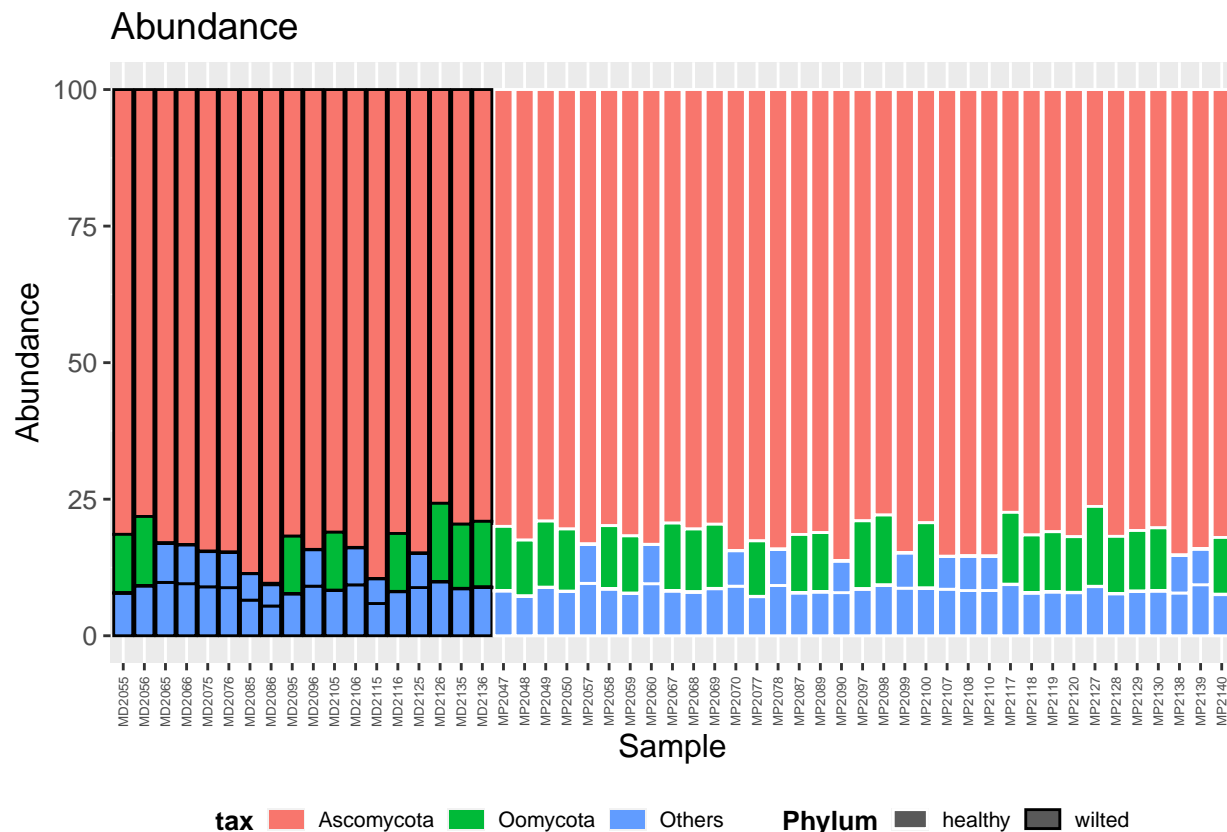
```
Barras_Phylum[1] # normal
```

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



```
Barras_Phylum[2]
```

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



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

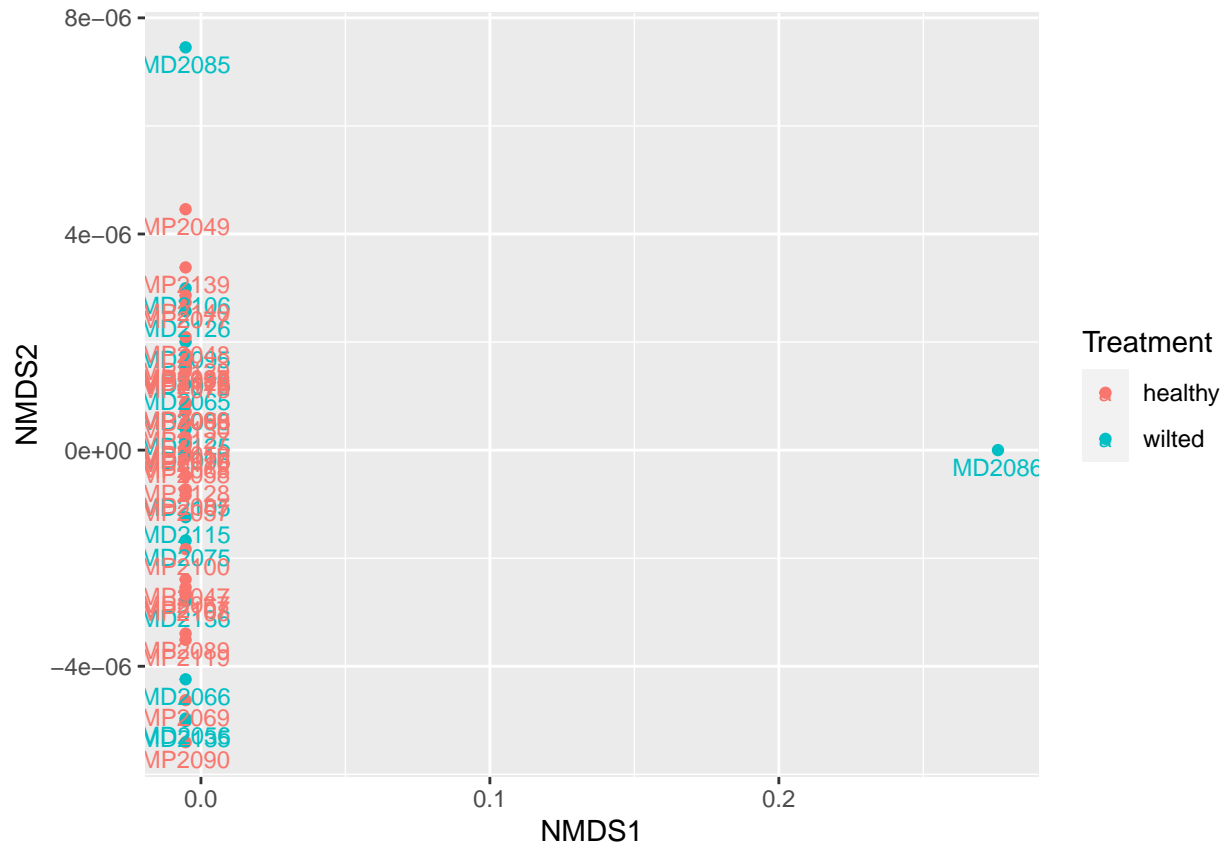
```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.01197068
## Run 1 stress 9.889248e-05
## ... New best solution
## ... Procrustes: rmse 0.03525563 max resid 0.09422638
## Run 2 stress 7.818682e-05
## ... New best solution
## ... Procrustes: rmse 0.0002159789 max resid 0.0005655097
## ... Similar to previous best
## Run 3 stress 0.0001011747
## ... Procrustes: rmse 0.0002355887 max resid 0.000621583
## ... Similar to previous best
## Run 4 stress 0.000309758
## ... Procrustes: rmse 0.0005170606 max resid 0.001251241
## ... Similar to previous best
## Run 5 stress 4.005523e-05
## ... New best solution
## ... Procrustes: rmse 8.15411e-05 max resid 0.0002405467
## ... Similar to previous best
## Run 6 stress 6.976465e-05
## ... Procrustes: rmse 2.73921e-05 max resid 6.102336e-05
## ... Similar to previous best
```

```

## Run 7 stress 0.0001000429
## ... Procrustes: rmse 0.0002890018  max resid 0.0007874319
## ... Similar to previous best
## Run 8 stress 9.895129e-05
## ... Procrustes: rmse 3.184906e-05  max resid 9.916759e-05
## ... Similar to previous best
## Run 9 stress 9.294297e-05
## ... Procrustes: rmse 9.371295e-05  max resid 0.0002302141
## ... Similar to previous best
## Run 10 stress 5.607435e-05
## ... Procrustes: rmse 2.143592e-05  max resid 4.889522e-05
## ... Similar to previous best
## Run 11 stress 0.0003939858
## ... Procrustes: rmse 0.001147254  max resid 0.003083277
## ... Similar to previous best
## Run 12 stress 5.443749e-05
## ... Procrustes: rmse 1.9537e-05  max resid 4.309105e-05
## ... Similar to previous best
## Run 13 stress 5.53932e-05
## ... Procrustes: rmse 2.099716e-05  max resid 4.579697e-05
## ... Similar to previous best
## Run 14 stress 0.0001078389
## ... Procrustes: rmse 0.0003120376  max resid 0.0008479884
## ... Similar to previous best
## Run 15 stress 8.570531e-05
## ... Procrustes: rmse 8.491113e-05  max resid 0.0002139273
## ... Similar to previous best
## Run 16 stress 0.001664373
## Run 17 stress 0.0009139555
## Run 18 stress 7.956569e-05
## ... Procrustes: rmse 2.811447e-05  max resid 5.75333e-05
## ... Similar to previous best
## Run 19 stress 9.211771e-05
## ... Procrustes: rmse 2.977756e-05  max resid 7.812541e-05
## ... Similar to previous best
## Run 20 stress 0.0009805078
## *** Best solution repeated 13 times

## Warning in metaMDS(veganifyOTU(physeq), distance, ...): stress is (nearly)
## zero: you may have insufficient data

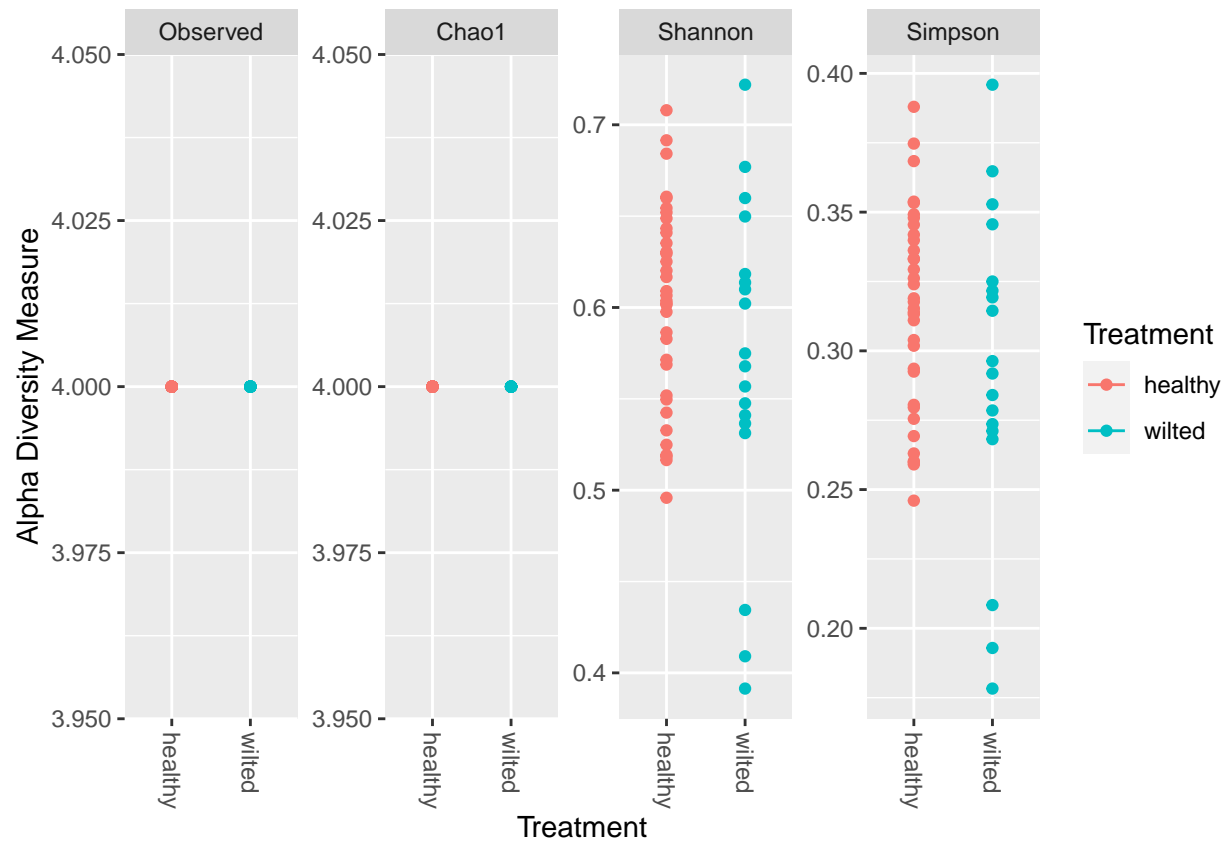
```



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

```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided contains
## 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.
```

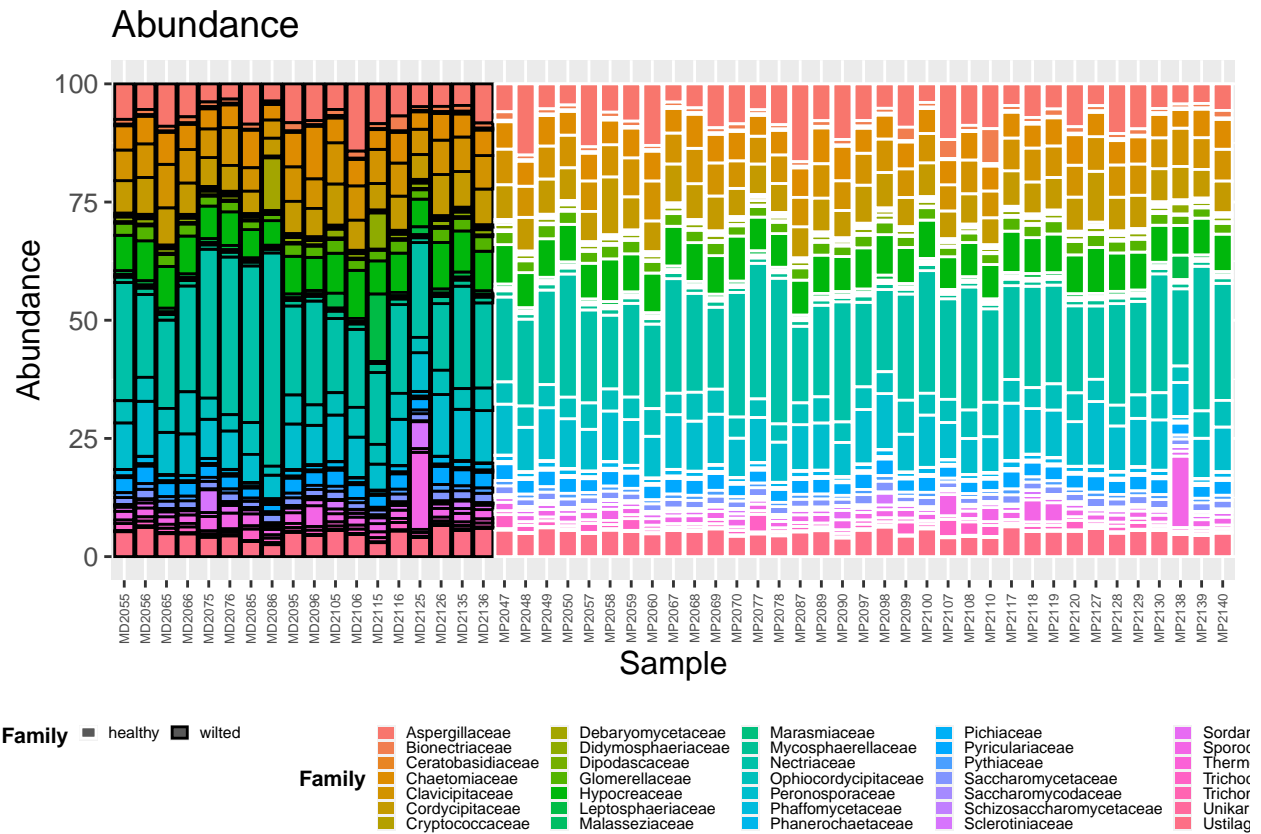




#—— Eukarya by Family

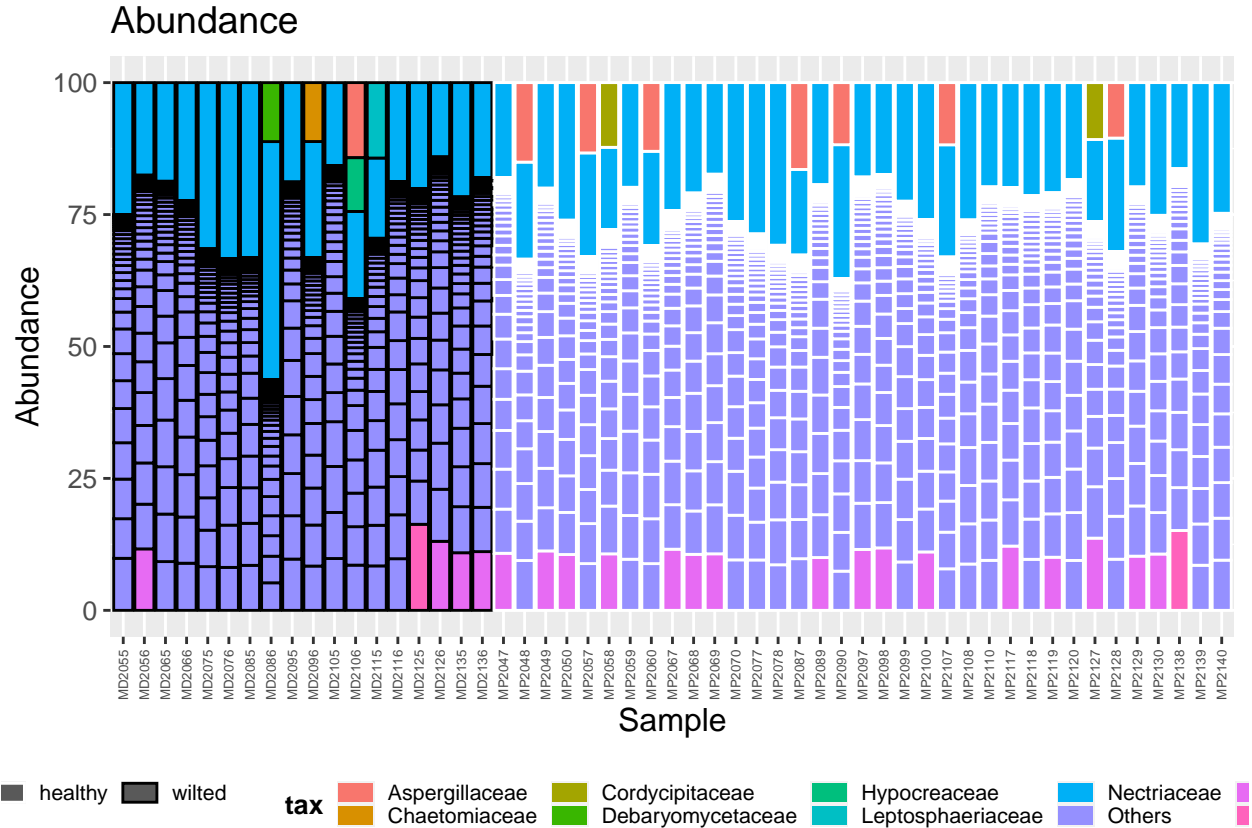
```
Barras_Species <- Abundance_barras(merge_Eukaryota, 'Family', 'Treatment', 10.0)
Barras_Species[1]
```

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



Barras\_Species [2]

## [[1]]

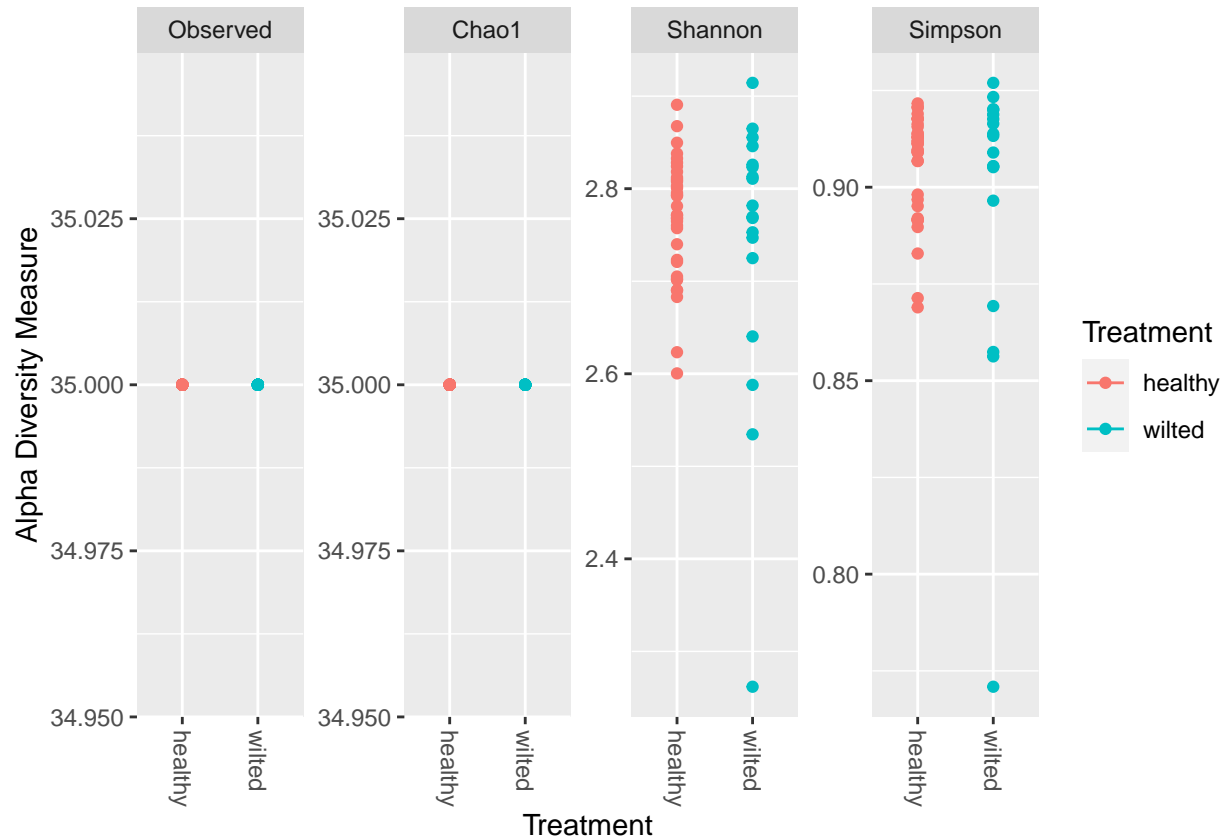


```
Beta_diversity(merge_Eukaryota , 'Family' , 'Treatment' , 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1060678
## Run 1 stress 0.1060682
## ... Procrustes: rmse 0.0001028798 max resid 0.0005915666
## ... Similar to previous best
## Run 2 stress 0.1061217
## ... Procrustes: rmse 0.0041568 max resid 0.02267181
## Run 3 stress 0.1060657
## ... New best solution
## ... Procrustes: rmse 0.007448014 max resid 0.04961758
## Run 4 stress 0.1060685
## ... Procrustes: rmse 0.007469715 max resid 0.04966758
## Run 5 stress 0.1060659
## ... Procrustes: rmse 9.97509e-05 max resid 0.0005361477
## ... Similar to previous best
## Run 6 stress 0.1060673
## ... Procrustes: rmse 0.007408574 max resid 0.04955511
## Run 7 stress 0.1226876
## Run 8 stress 0.1231561
## Run 9 stress 0.1061211
## ... Procrustes: rmse 0.008498236 max resid 0.05011349
## Run 10 stress 0.1061092
## ... Procrustes: rmse 0.004302652 max resid 0.02332515
```



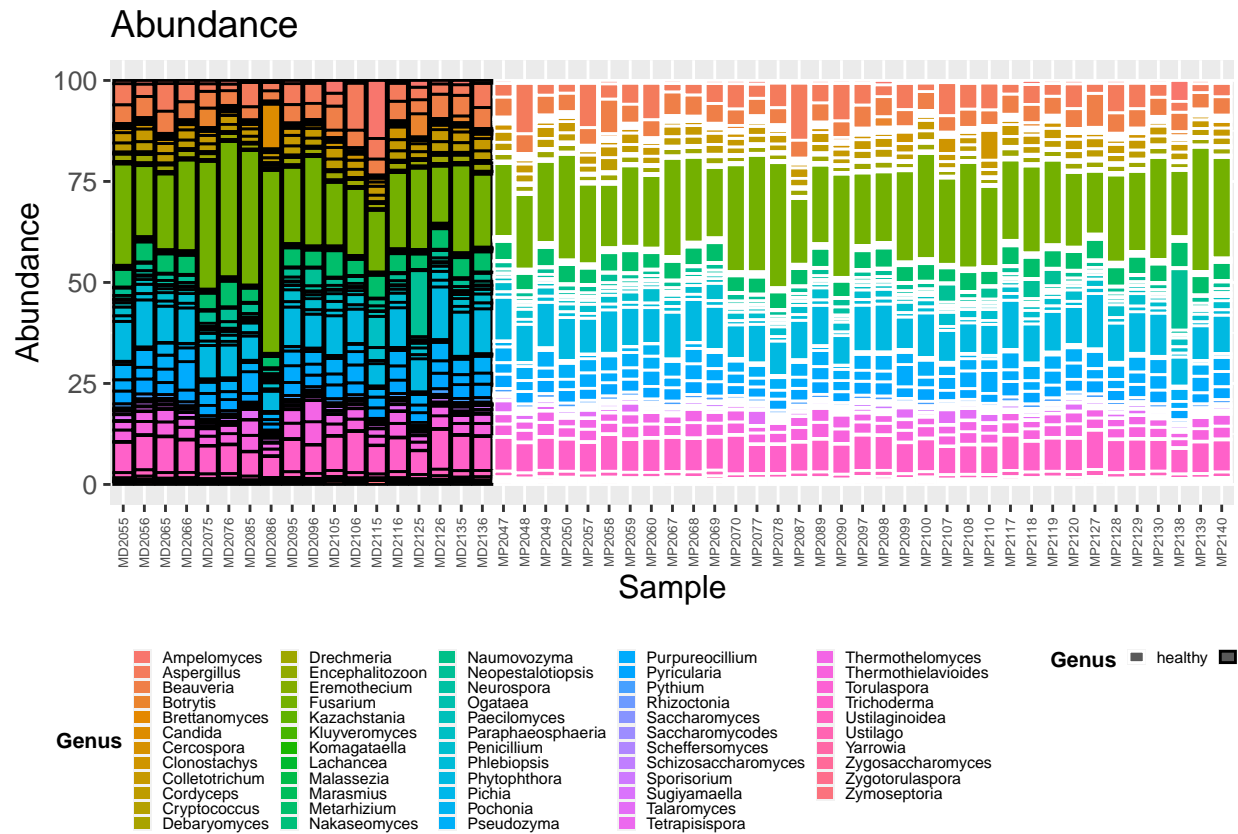
```
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##
## We recommended that you find the un-trimmed data and retry.
```



```
#———Eukarya by Genero
```

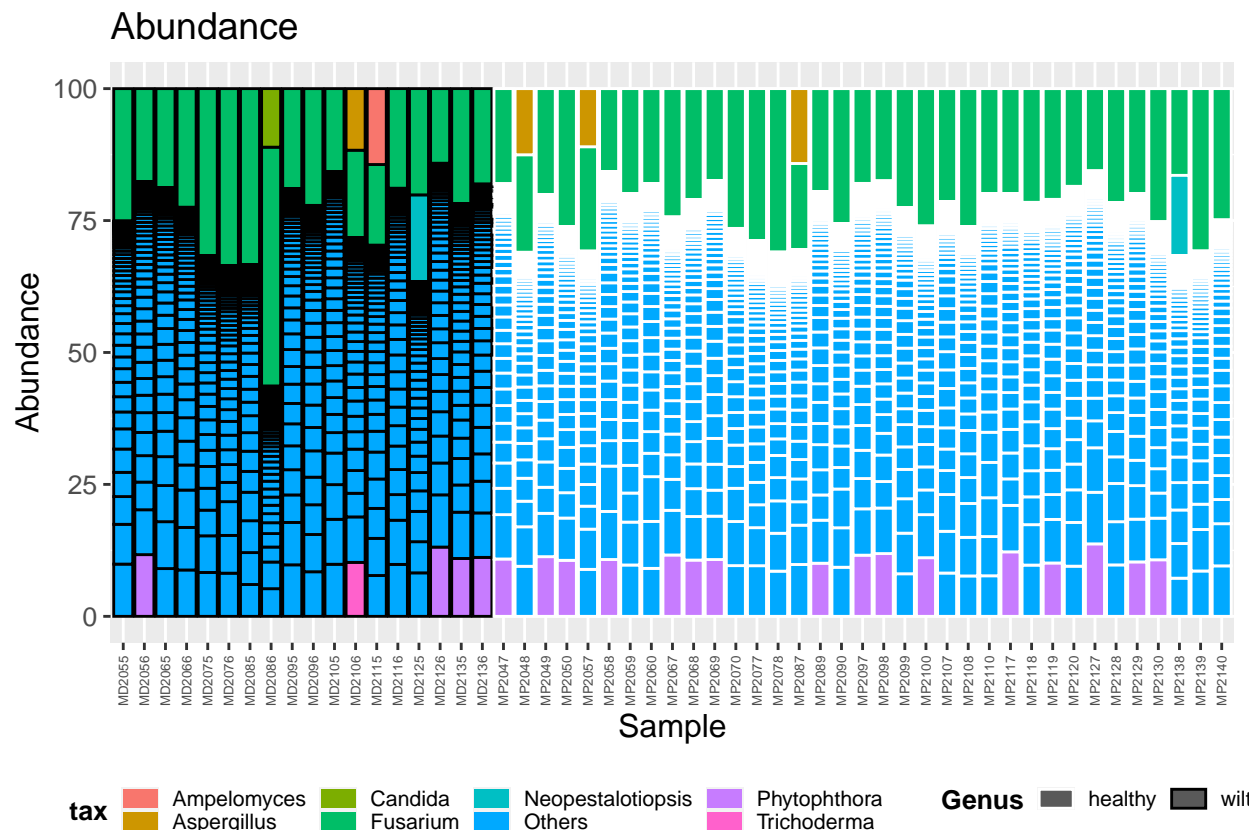
```
Barras_Species <- Abundance_barras(merge_Eukaryota, 'Genus', 'Treatment', 10.0)
Barras_Species[1]
```

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



Barras\_Species [2]

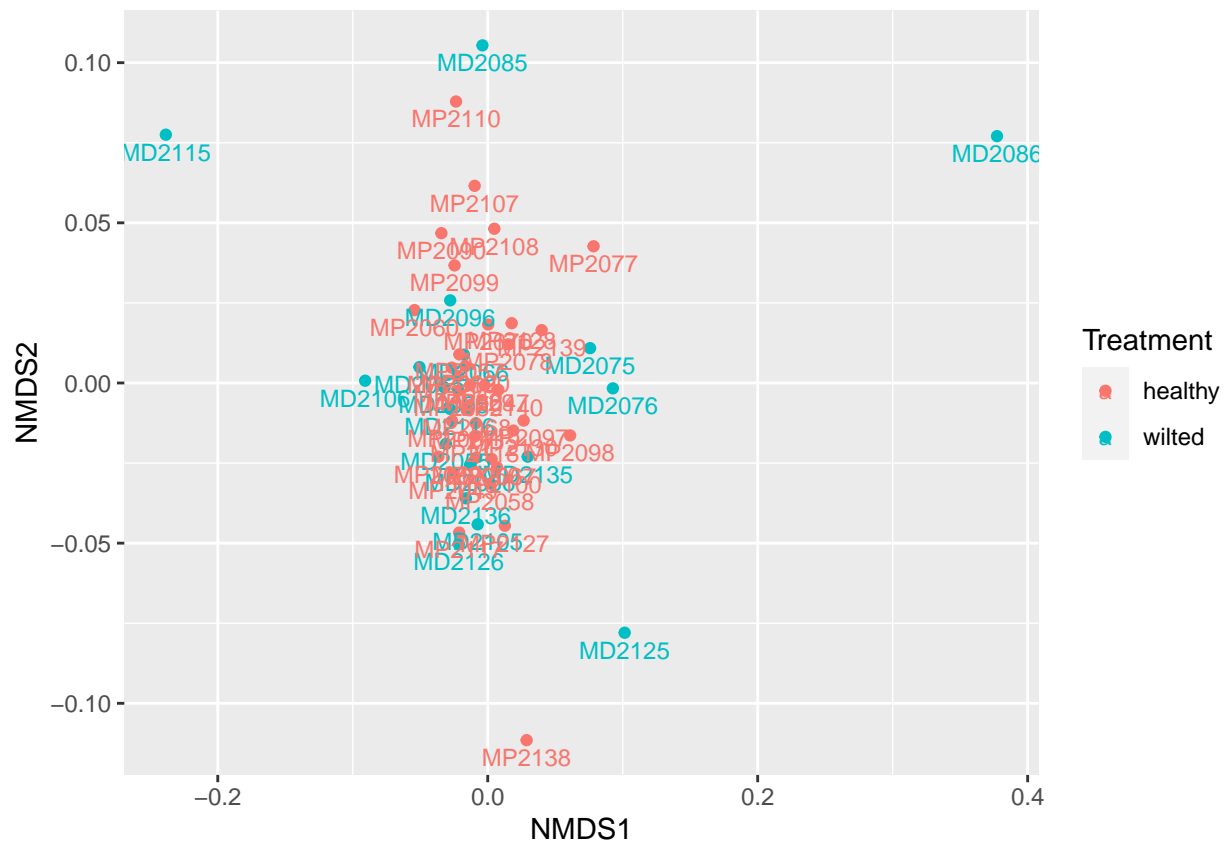
## [[1]]



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

```
## Wisconsin double standardization
## Run 0 stress 0.1074764
## Run 1 stress 0.107476
## ... New best solution
## ... Procrustes: rmse 0.001314157 max resid 0.00705542
## ... Similar to previous best
## Run 2 stress 0.1074767
## ... Procrustes: rmse 0.001313808 max resid 0.006996822
## ... Similar to previous best
## Run 3 stress 0.1074765
## ... Procrustes: rmse 0.001319793 max resid 0.007012956
## ... Similar to previous best
## Run 4 stress 0.1074767
## ... Procrustes: rmse 0.0001846096 max resid 0.001024319
## ... Similar to previous best
## Run 5 stress 0.1086937
## Run 6 stress 0.1199206
## Run 7 stress 0.1074768
## ... Procrustes: rmse 0.001390626 max resid 0.007388438
## ... Similar to previous best
## Run 8 stress 0.1074893
## ... Procrustes: rmse 0.002917775 max resid 0.01522508
## Run 9 stress 0.1086943
```

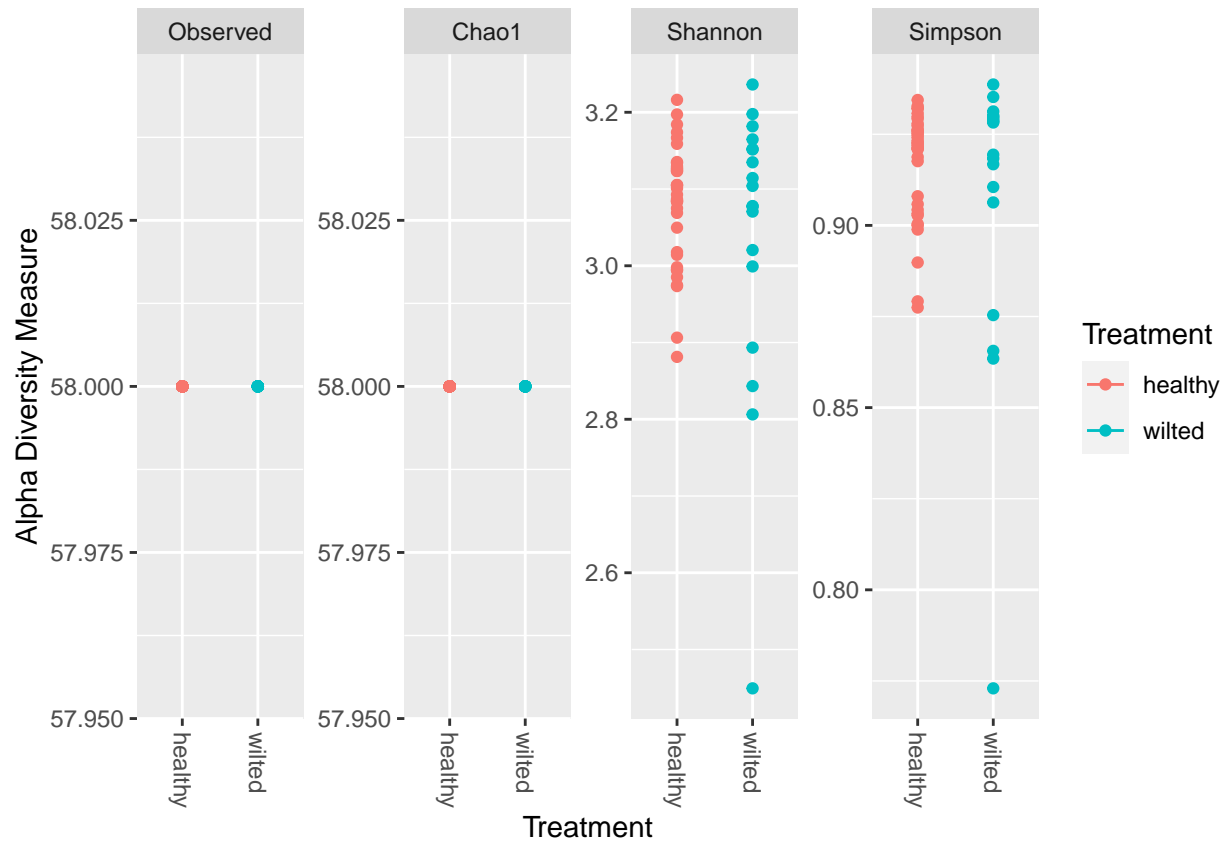
```
## Run 10 stress 0.11992
## Run 11 stress 0.1215632
## Run 12 stress 0.1169324
## Run 13 stress 0.1074765
## ... Procrustes: rmse 0.001329751  max resid 0.007069924
## ... Similar to previous best
## Run 14 stress 0.1198
## Run 15 stress 0.1196314
## Run 16 stress 0.127567
## Run 17 stress 0.1074767
## ... Procrustes: rmse 0.0001797517  max resid 0.0009988099
## ... Similar to previous best
## Run 18 stress 0.1074766
## ... Procrustes: rmse 0.00135151  max resid 0.007172921
## ... Similar to previous best
## Run 19 stress 0.1074756
## ... New best solution
## ... Procrustes: rmse 0.0001882877  max resid 0.001036694
## ... Similar to previous best
## Run 20 stress 0.1074764
## ... Procrustes: rmse 0.001121884  max resid 0.005921689
## ... Similar to previous best
## *** Best solution repeated 2 times
```





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

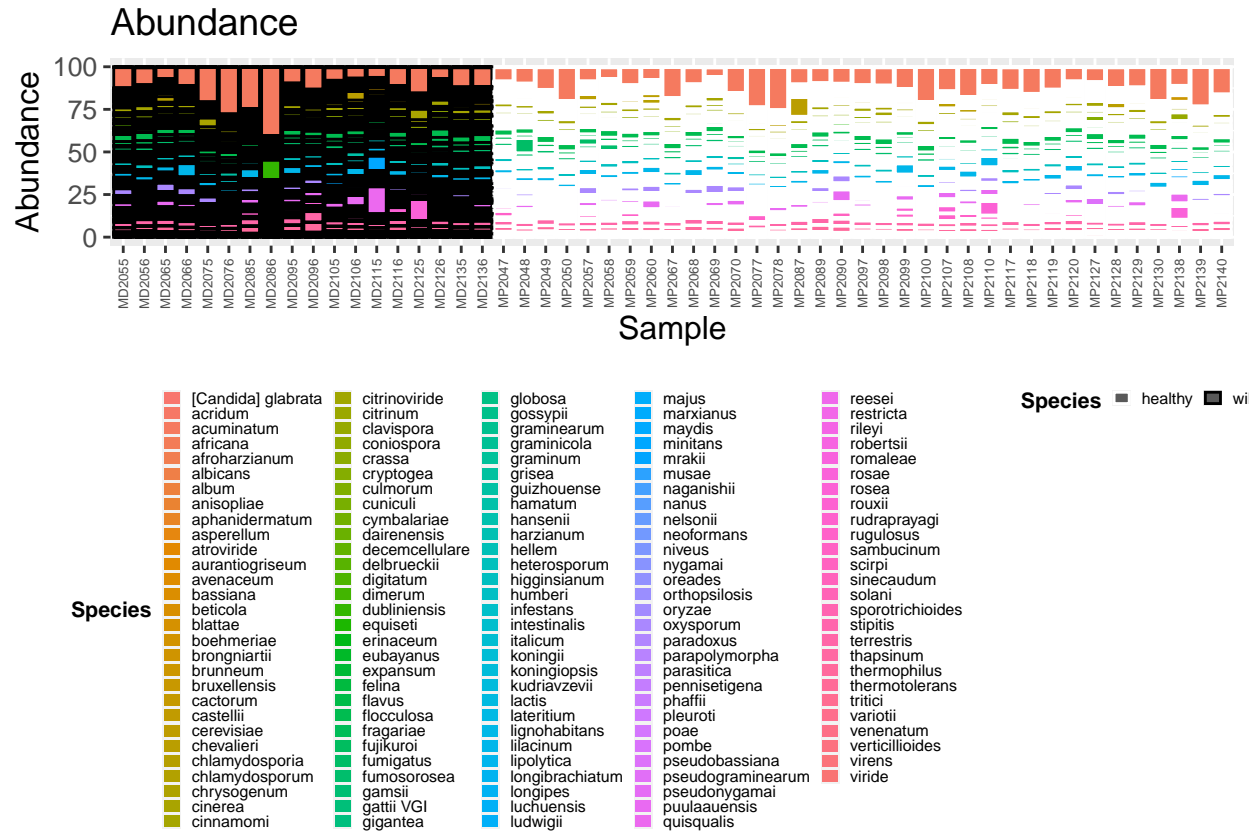
```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##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



```
#———Eukarya by Species
```

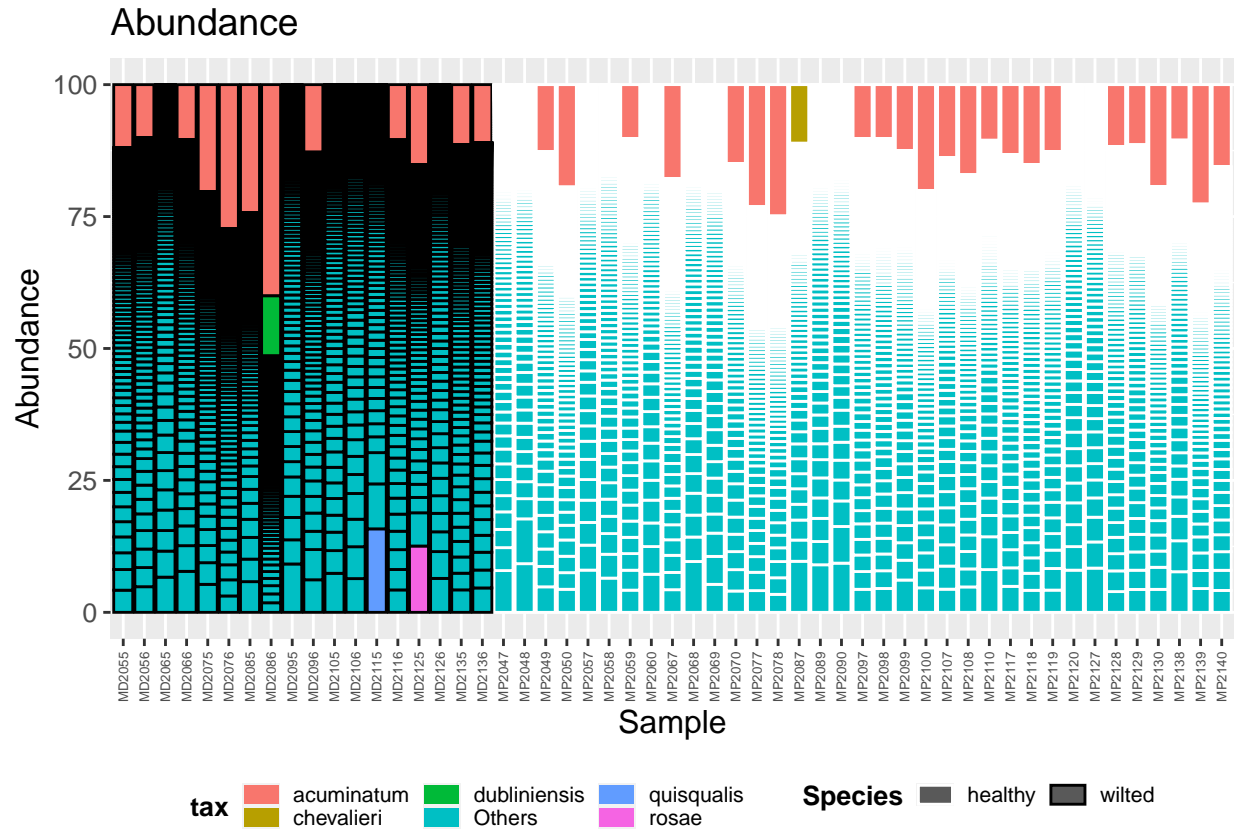
```
Barras_Species <- Abundance_barras(merge_Eukaryota, 'Species', 'Treatment', 10.0)
Barras_Species[1]
```

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



Barras\_Species [2]

## [[1]]



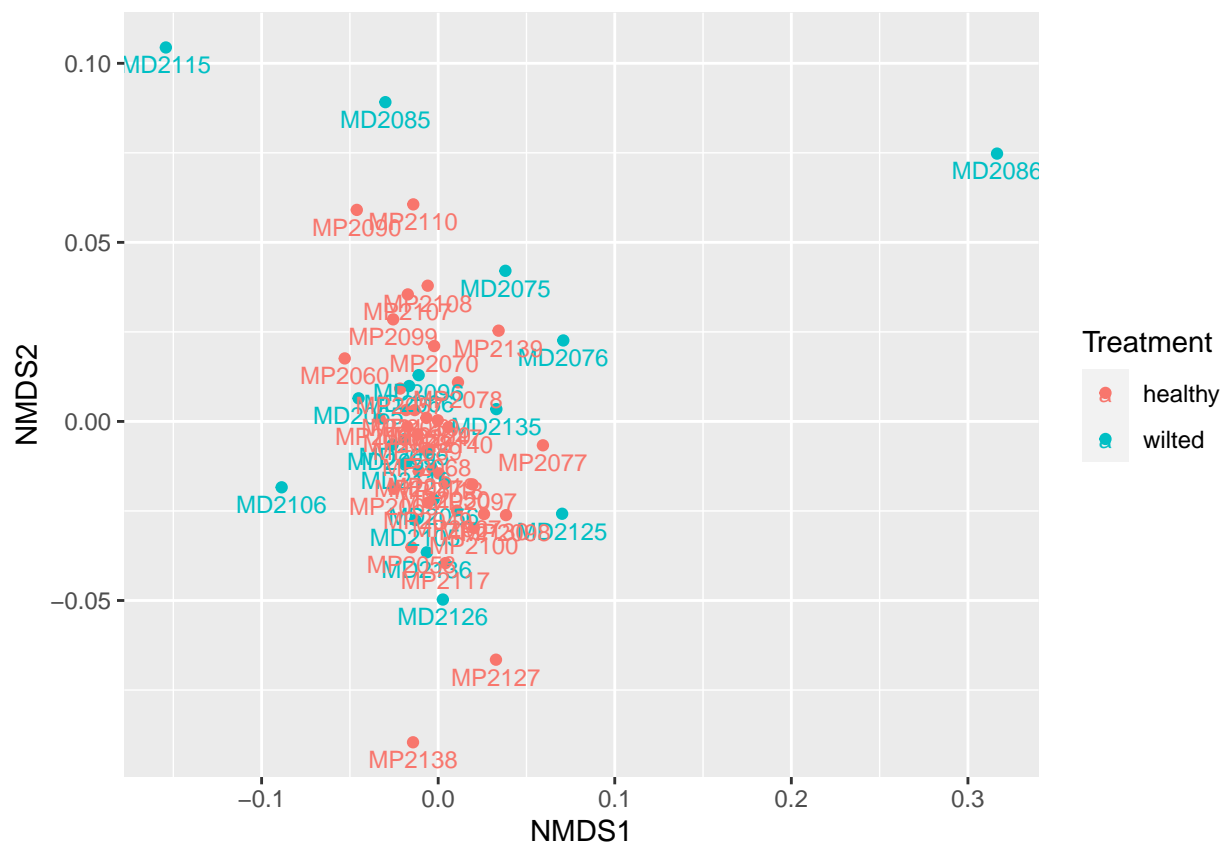
```
Beta_diversity(merge_Eukaryota , 'Species' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1207974
## Run 1 stress 0.1203176
## ... New best solution
## ... Procrustes: rmse 0.02159519 max resid 0.1291859
## Run 2 stress 0.1200151
## ... New best solution
## ... Procrustes: rmse 0.01150802 max resid 0.0769741
## Run 3 stress 0.120014
## ... New best solution
## ... Procrustes: rmse 0.0002715031 max resid 0.001355024
## ... Similar to previous best
## Run 4 stress 0.1202943
## ... Procrustes: rmse 0.01095976 max resid 0.07705545
## Run 5 stress 0.1249151
## Run 6 stress 0.1388923
## Run 7 stress 0.120658
## Run 8 stress 0.124854
## Run 9 stress 0.1249818
## Run 10 stress 0.1200138
## ... New best solution
## ... Procrustes: rmse 0.001034876 max resid 0.005056942
## ... Similar to previous best
```

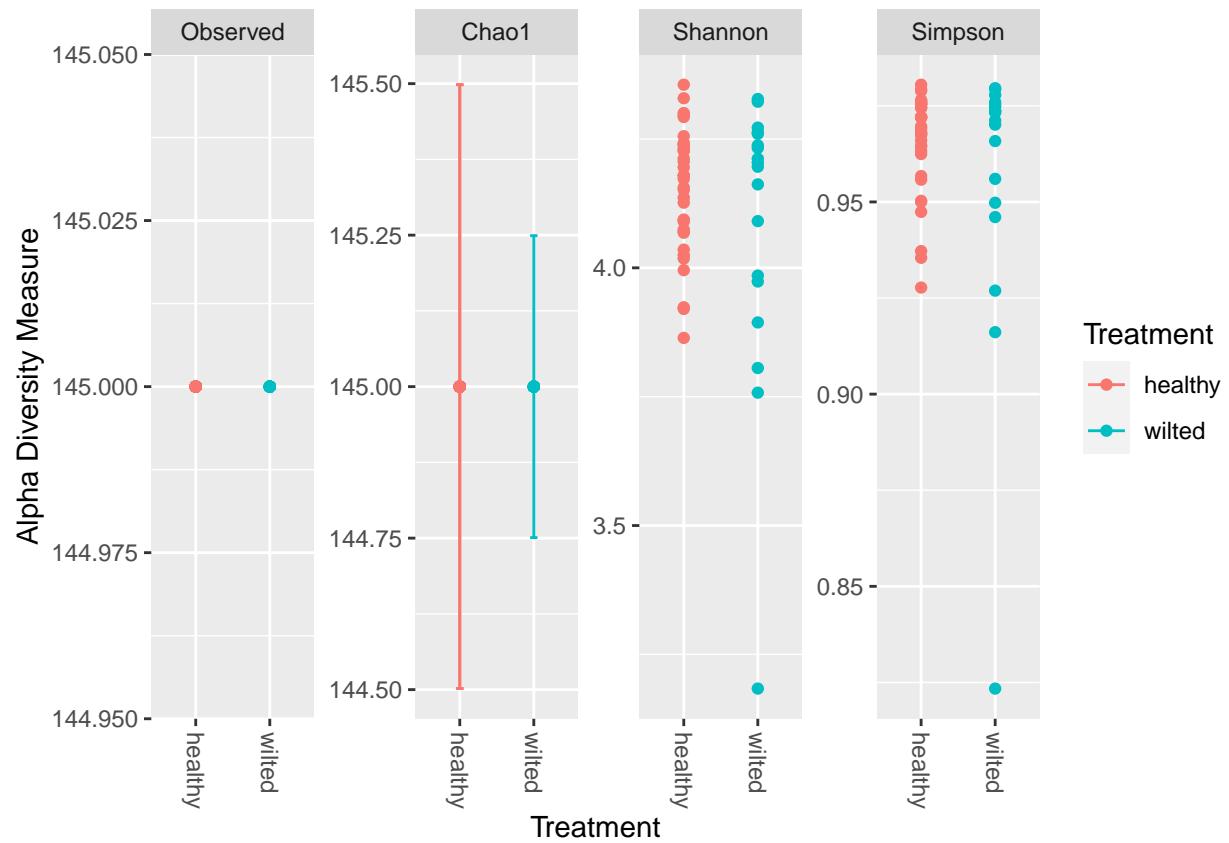
```

## Run 11 stress 0.1200136
## ... New best solution
## ... Procrustes: rmse 0.0002684173  max resid 0.001116539
## ... Similar to previous best
## Run 12 stress 0.133594
## Run 13 stress 0.1248472
## Run 14 stress 0.1200142
## ... Procrustes: rmse 0.0009451305  max resid 0.004285167
## ... Similar to previous best
## Run 15 stress 0.120053
## ... Procrustes: rmse 0.003444614  max resid 0.0173501
## Run 16 stress 0.1249152
## Run 17 stress 0.1248538
## Run 18 stress 0.1200146
## ... Procrustes: rmse 0.001058271  max resid 0.004855841
## ... Similar to previous best
## Run 19 stress 0.1200134
## ... New best solution
## ... Procrustes: rmse 0.0001936794  max resid 0.001210324
## ... Similar to previous best
## Run 20 stress 0.1248475
## *** Best solution repeated 1 times

```



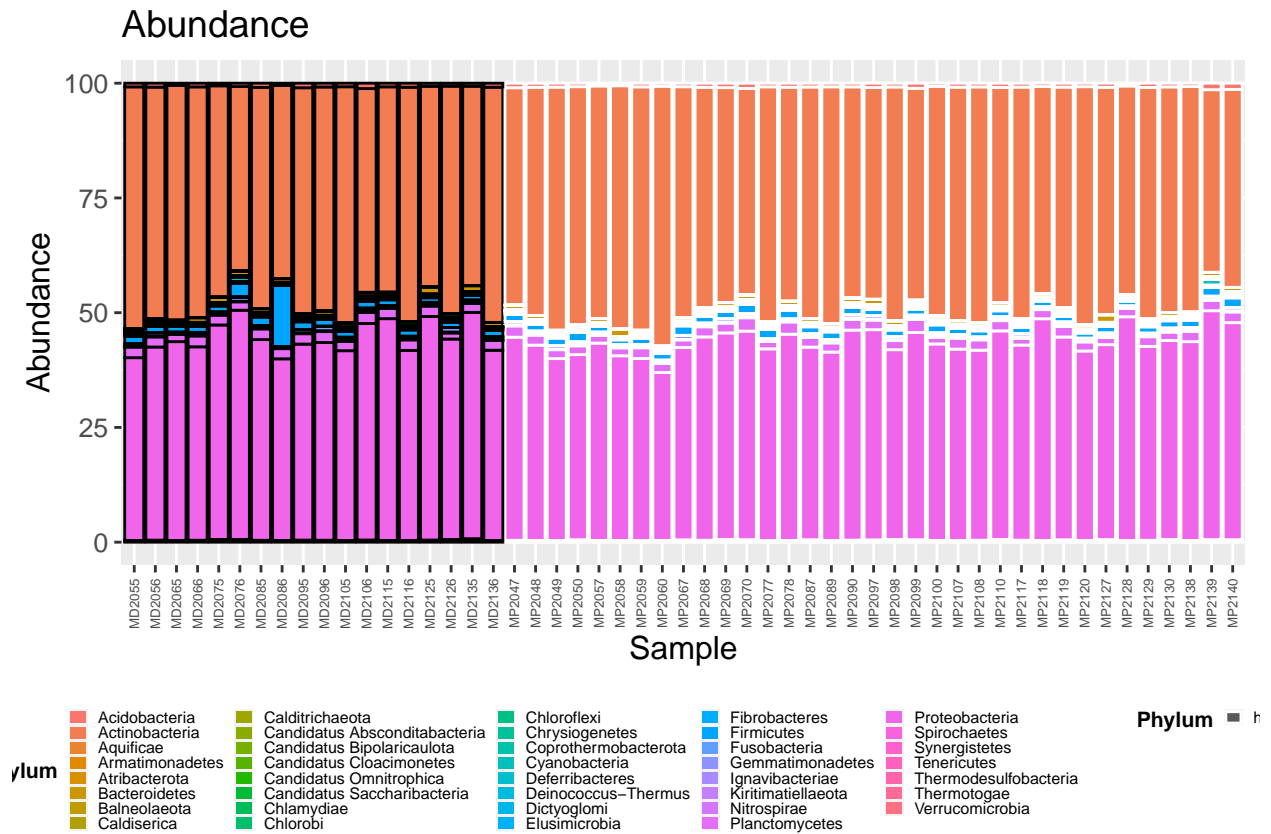
```
Alpha_diversity(merge_Eukaryota , 'Species' , 'Treatment')
```



#——Bacteria by Phylum

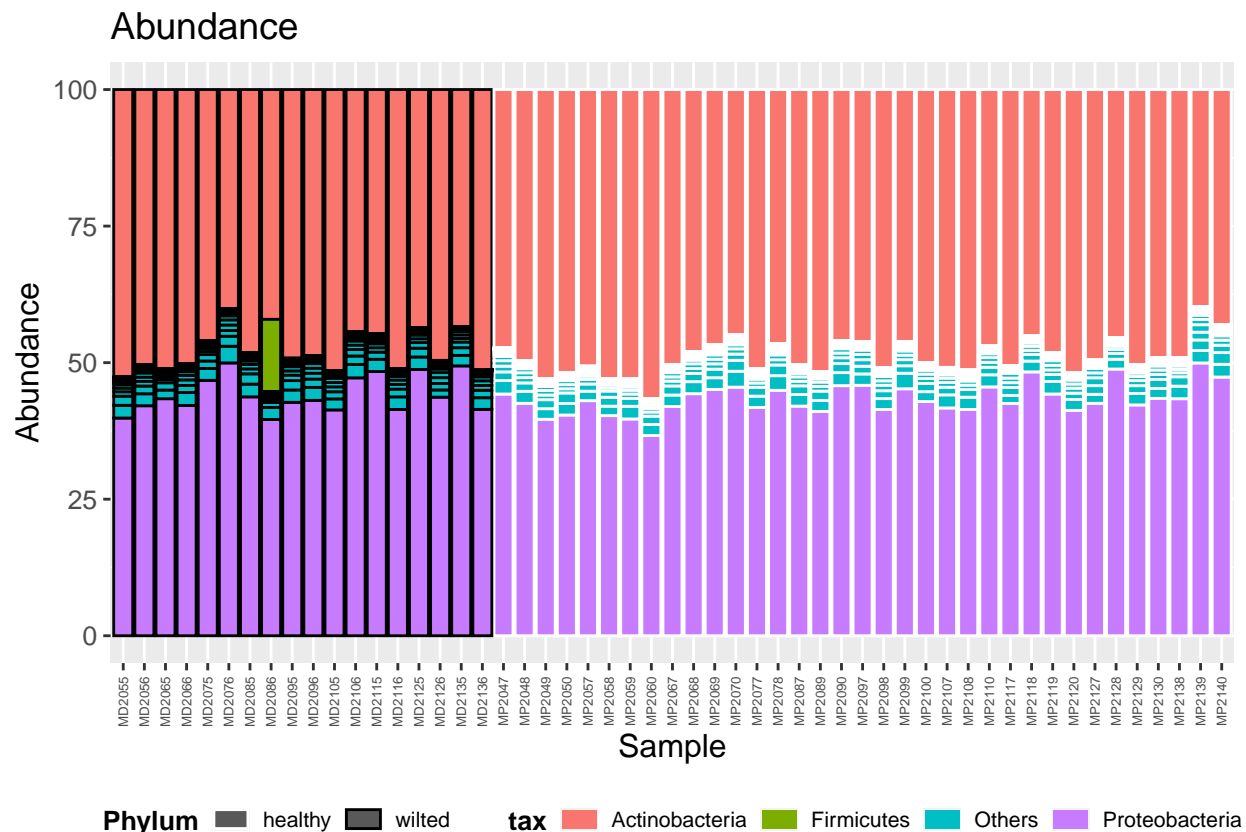
```
Barras_Species <- Abundance_barras(merge_Bacteria, 'Phylum', 'Treatment', 10.0)
Barras_Species[1]
```

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



Barras\_Species[2]

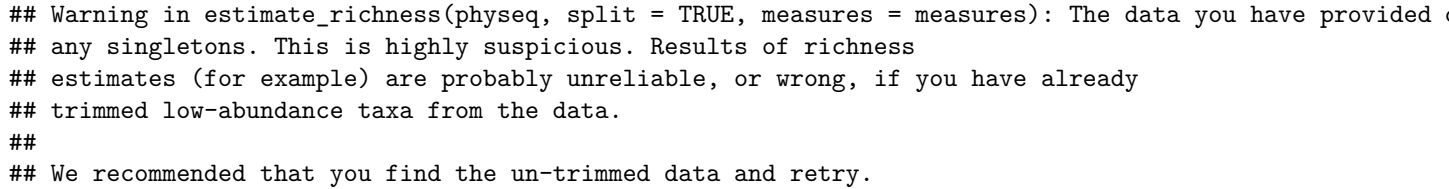
## [[1]]



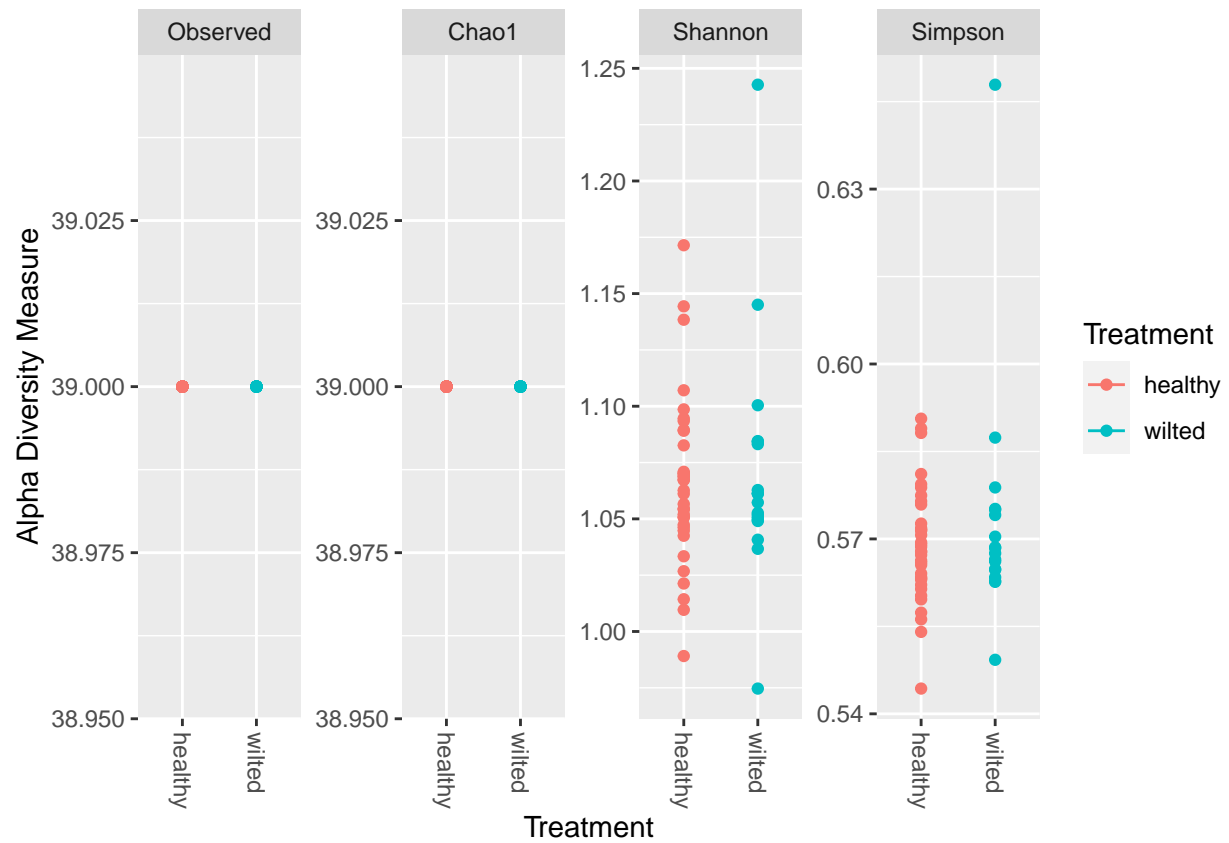
```
Beta_diversity(merge_Bacteria , 'Phylum' , 'Treatment', 'bray')
```

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.1513513
## Run 1 stress 0.1591989
## Run 2 stress 0.1646307
## Run 3 stress 0.1569451
## Run 4 stress 0.1689358
## Run 5 stress 0.1611884
## Run 6 stress 0.155079
## Run 7 stress 0.1566522
## Run 8 stress 0.1501999
## ... New best solution
## ... Procrustes: rmse 0.04681636  max resid 0.3138002
## Run 9 stress 0.1595085
## Run 10 stress 0.1564686
## Run 11 stress 0.1664798
## Run 12 stress 0.1607572
## Run 13 stress 0.16398
## Run 14 stress 0.1504408
## ... Procrustes: rmse 0.04432778  max resid 0.2971084
## Run 15 stress 0.170348
## Run 16 stress 0.1702325
## Run 17 stress 0.1552923
```

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







#——Bacteria by Familia

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

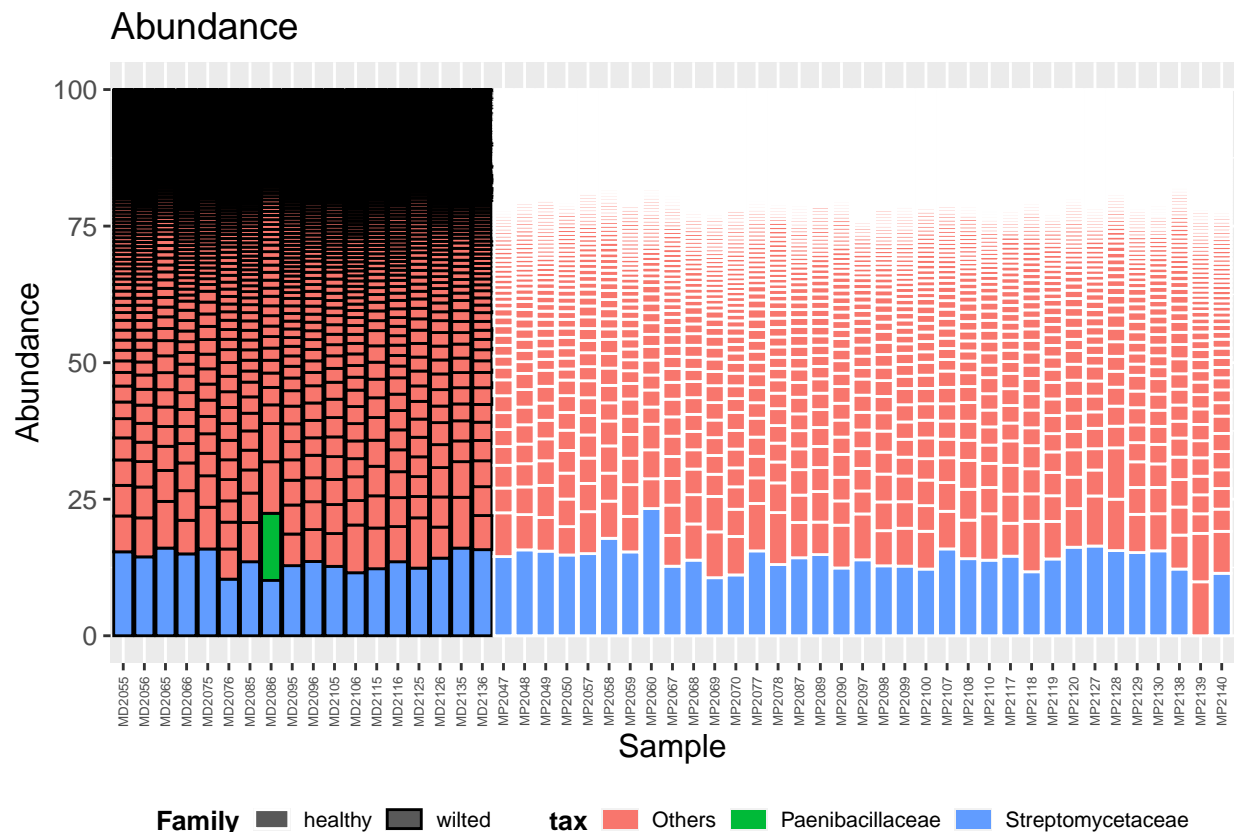
```
## [[1]]
```

# Family

Actinomycesaceae	Chloromonadaceae	Halobacterioviraceae	Nocardiaceae	St
Actinopolymorphaceae	Clostridiaceae	Halobacteroidaceae	Nocardioidaceae	St
Actinopolysporaceae	Clostridiales Family XVI. Incertae Sedis	Halomonadaceae	Nocardiopsaceae	St
Aerococcaceae	Clostridiales Family XVII. Incertae Sedis	Halothiobacillaceae	Nostocaceae	St
Aeromonadaceae	Cohaesibacteraceae	Hapalosiphonaceae	Oceanospirillaceae	St
Akkermansiaceae	Coleofasciculaceae	Helicobacteraceae	Oculatellaceae	St
Alcaligenaceae	Colwelliaceae	Hellobacteriaceae	Odoribacteraceae	St
Alcanivoracaceae	Comamonadaceae	Holospiraceae	Oleiphilaceae	St
Alicyclobacillaceae	Conexibacteraceae	Hydrogenimonaceae	Opitutaceae	St
Alteromonadaceae	Coprothermobacteraceae	Hydrogenophilaceae	Orbaceae	St
Amoebophilaceae	Coriobacteriaceae	Hydrogenothermaceae	Ornithinimicrobiaceae	Su
Amorphaceae	Corynebacteriaceae	Hyellaceae	Oscillatoriaceae	Su
Anaerohalosphaeraceae	Coxiellaceae	Hymenobacteraceae	Oscillospiraceae	Su
Anaerolineaceae	Crocinitomicaceae	Hyphomicrobiaceae	Oxalobacteraceae	Su
Anaeromyxobacteraceae	Cyclobacteriaceae	Hyphomonadaceae	Paenibacillaceae	Su
Anaplasmataceae	Cytophagaceae	Iamiaceae	Paludibacteraceae	Sy
Aphanizomenonaceae	Deferribacteraceae	Ichthyobacteriaceae	Parachiamydiaceae	Sy
Aphanothecaceae	Dehalococcoidaceae	Idiomarinaceae	Parvibaculaceae	Sy
Aquificaceae	Deinococcaceae	Ignavibacteriaceae	Parvularculaceae	Sy
Archangiaceae	Demeguinaceae	Ilumatobacteraceae	Pasteurellaceae	Sy
Arcobacteraceae	Dermabacteraceae	Immundisolibacteraceae	Pectobacteriaceae	Sy
Ardenticatenaceae	Dermacoccaceae	Intrasporangiaceae	Pelagibacteraceae	Sy
Atopobiaceae	Dermatophilaceae	Isosphaeraceae	Peptococcaceae	Ta
Atribacteraceae	Dermocarpellaceae	Jatrophihabitantaceae	Peptoniphilaceae	Te
Aurantimonadaceae	Desulfallaceae	Jiangellaceae	Peptostreptococcaceae	Te
Azonexaceae	Desulfarculaceae	Jonesiaceae	Petrotoaceae	Te
Azospirillaceae	Desulfatobacteriaceae	Kaistiaceae	Phototrophicaceae	Te
Bacillaceae	Desulfobacteriaceae	Kangiellaceae	Phreatobacteraceae	Te
Bacterioviraceae	Desulfobulbaceae	Kineosporiaceae	Phycisphaeraceae	Tr
Bacteroidaceae	Desulfocapsaceae	Kiritimatiellaceae	Phyllobacteriaceae	Tr
Baekduiaceae	Desulfohalobiaceae	Koferiaceae	Pirellulaceae	Tr
Barnesiellaceae	Desulfomicrobiaceae	Koleobacteraceae	Piscirickettsiaceae	Tr
Bartonellaceae	Desulfosudaceae	Kordiimonadaceae	Planctomycetaceae	Tr
Bdellovibrionaceae	Desulfotomaculaceae	Kosmotogaceae	Planococcaceae	Tr
Beijerinckiaceae	Desulfotomaculaceae	Kribbellaceae	Pleomorphomonadaceae	Tr
Bernardetiaceae	Desulfurellaceae	Ktedonosporobacteraceae	Polyangiaceae	Tr
Beutenbergiaceae	Desulfurobacteriaceae	Kytococcaceae	Porphyromonadaceae	Tr
Bifidobacteriaceae	Desulfuromonadaceae	Labilitrachaceae	Prevotellaceae	Tr
Blastochloridaceae	Devosiaceae	Lachnospiraceae	Prochlorococcaceae	Tr
Blattabacteriaceae	Dictyoglomaceae	Lacipirellulaceae	Prochlorotrichaceae	Tr
Bogoriellaceae	Dietziaceae	Lactobacillaceae	Prolixibacteraceae	Tr
Borrelliaceae	Dissulfurispiraceae	Lawsonellaceae	Promicromonosporaceae	Tr
Boseaceae	Dysgonomonadaceae	Legionellaceae	Propionibacteriaceae	Tr
Brachyspiraceae	Ectothiorhodospiraceae	Leptolyngbyaceae	Proteinivoraceae	Tr
Bradymonadaceae	Eggerthellaceae	Leptospiaceae	Pseudanabaenaceae	Tr
Bradyrhizobiaceae	Egibacteraceae	Leptotrichiaceae	Pseudoalteromonadaceae	Tr
Breoghanaceae	Egicoccaceae	Lichenihabitantaceae	Pseudomonadaceae	Tr
Brevibacteriaceae	Elioraeaceae	Limnochordaceae	Pseudonocardaceae	Tr
Brucellaceae	Elusimicrobiaceae	Listeriaceae	Psychromonadaceae	Tr
Bruguierivoracaceae	Emcibacteraceae	Litoricolaceae	Puniceococcaceae	Tr
Bryobacteraceae	Endomicrobiaceae	Magnetococcaceae	Rhabdochlamydiaceae	Tr
Budviciaceae	Endozoicomonadaceae	Mangrovivirgaceae	Rhizobiaceae	Tr
Burkholderiaceae	Enterobacteriaceae	Maricaulaceae	Rhodanobacteraceae	Ti
Caedimonadaceae	Enterococcaceae	Marinifiliaceae	Rhodobacteraceae	Ti
Caldilineaceae	Entomoplasmataceae	Marinifiliaceae	Rhodocyclaceae	Tc
Caldiseriaceae	Erwinaceae	Marinobacteraceae	Rhodospirillaceae	Tr
Calditrichaceae	Erysipelotrichaceae	Mariprofundaceae	Rhodothermaceae	Tr
Calothrixaceae	Erythrobacteraceae	Marinimaceae	Rickettsiaceae	Tr

Barras\_Species [2]

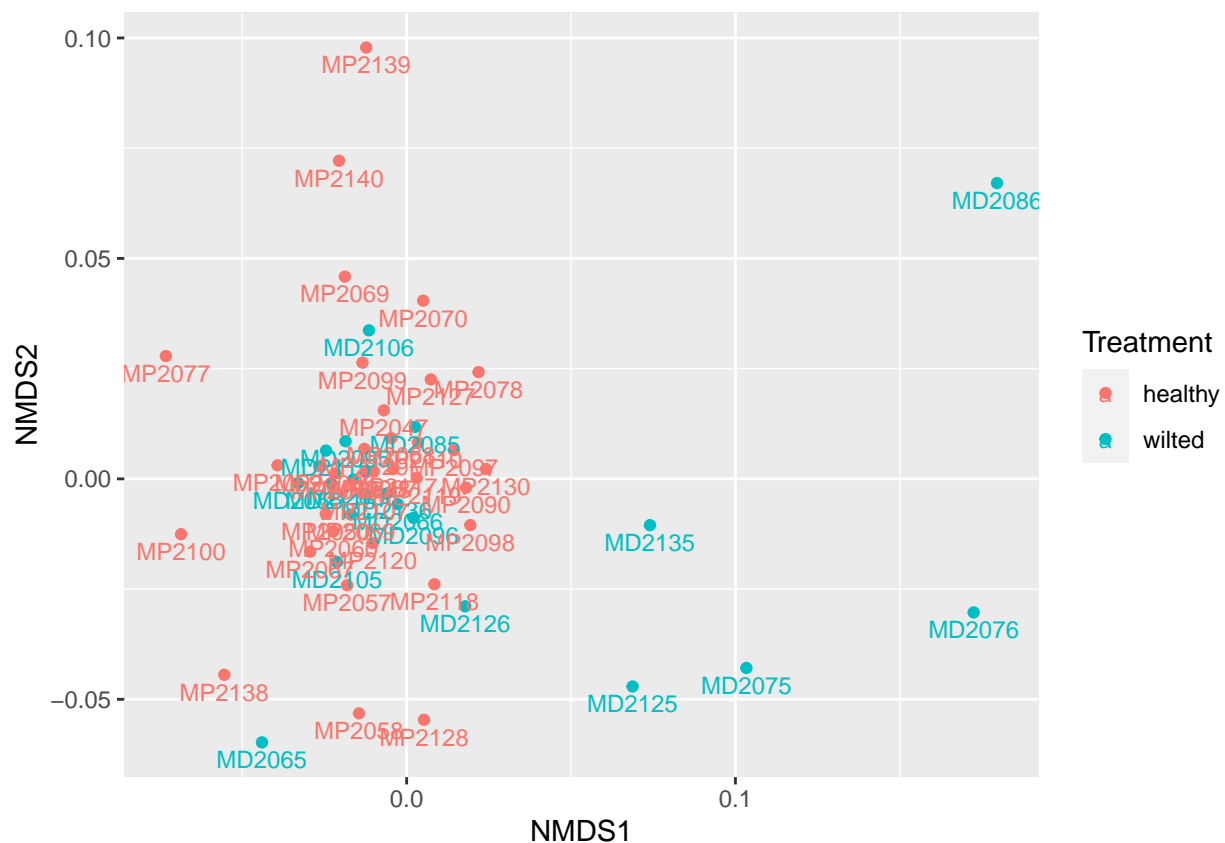
## [[1]]



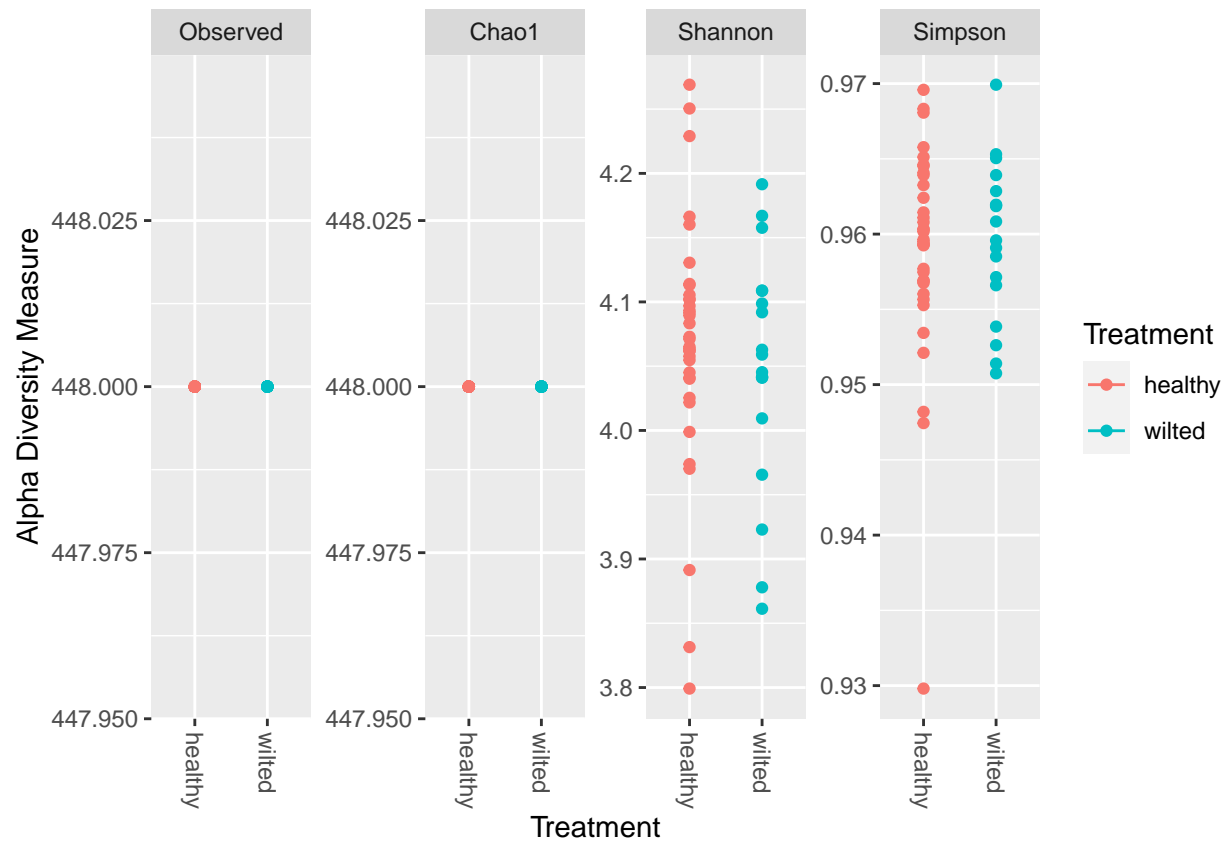
```
Beta_diversity(merge_Bacteria , 'Family' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1371579
## Run 1 stress 0.133966
## ... New best solution
## ... Procrustes: rmse 0.05000407 max resid 0.3009845
## Run 2 stress 0.1340831
## ... Procrustes: rmse 0.002883314 max resid 0.0144938
## Run 3 stress 0.1342678
## ... Procrustes: rmse 0.01080321 max resid 0.05772273
## Run 4 stress 0.1341087
## ... Procrustes: rmse 0.008984848 max resid 0.05444024
## Run 5 stress 0.1341086
## ... Procrustes: rmse 0.009118656 max resid 0.05504089
## Run 6 stress 0.1749506
## Run 7 stress 0.1480008
## Run 8 stress 0.1339659
## ... New best solution
## ... Procrustes: rmse 8.251938e-05 max resid 0.000305396
## ... Similar to previous best
## Run 9 stress 0.1397728
## Run 10 stress 0.1342675
## ... Procrustes: rmse 0.01062292 max resid 0.05713492
## Run 11 stress 0.1373272
```

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



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#——Bacteria by Genero

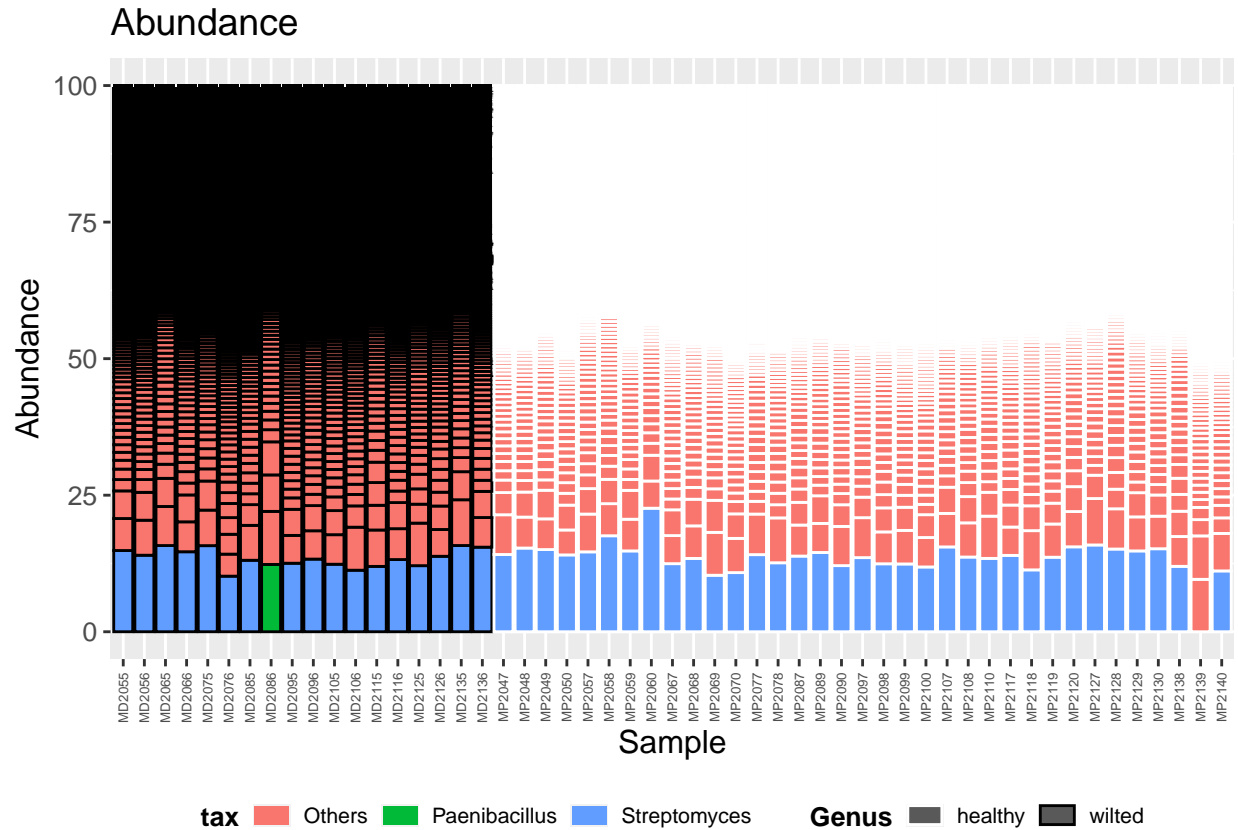
```
Barras_Species <- Abundance_barras(merge_Bacteria, 'Genus', 'Treatment', 10.0)
Barras_Species[1]
```

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

lex	Desulfuricoccus	Labyrinthula	Paramecium	Sulfolobus
flexum	Desulfatibacillum	Labrys	Paroceanicella	Succinivibrio
habitans	Desulfatobacterium	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	Desulfoglobium	Lactiplantibacillus	Pediococcus	Sulfurimicrobium
ibacter	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	Pelolistega	Sulfurospirillum
cibacterium	Desulforamulus	Larkinella	Pelobacter	Sulfurovum
ibacter	Desulforapulum	Latlactobacillus	Pelodictyon	Sutcliffeella
imonas	Desulfosarcina	Lautropia	Pelolinea	Sutterella
ratoleum	Desulfoscipio	Lawsonella	Pelosinus	Suttonella
nicrococcus	Desulfosediminicola	Lawsonia	Pengzhengrongella	Swingsia
nophonus	Desulfosporosinus	Leadbetterella	Peptacetobacter	Symbiobacterium
obacter	Desulfosudis	Leclercia	Peptoclostridium	Symmachella
a	Desulfotalea	Lederbergia	Peptoniphilus	Synechococcus
acaulis	Desulfotomaculum	Leeuwenhoekella	Peribacillus	Synechocystis
lassotoga	Desulfovibrio	Legionella	Periweissella	Syntrophobacter
tibacter	Desulfurispirillum	Leifsonia	Permianibacter	Syntrophobotulus
obium	Desulfurivibrio	Leisingera	Persephonella	Syntrophomonas
acter	Desulfurobacterium	Lelliottia	Persicimonas	Syntrophotalea
ntiibacter	Desulfuromonas	Leminorella	Peteryoungia	Syntrophothermus
ntimicrobium	Devosia	Lentibacillus	Petrimonas	Syntrophus
ntimonas	Devriesea	Lentilactobacillus	Petrocella	Tabrizicola
ticoccus	Dialister	Lentilitoribacter	Petrotoga	Tamiana
imonas	Diaminobutyricimonas	Lentzea	Phaeobacter	Tannerella
liella	Diaphorobacter	Leptodesmis	Phascolarctobacterium	Tardibacter
idibacter	Dichelobacter	Leptolyngbya	Phenyllobacterium	Tardiphaga
wickia	Dickeya	Leptosira	Phnomibacter	Tateyamaria
acterium	Dictyoglomus	Leptospirillum	Phocaeicola	Tatlockia
rcus	Dietzia	Leptothermofonsia	Phoenicibacter	Tatumella
hizobium	Dinoroseobacter	Leptothrix	Photobacterium	Tautonia
pira	Dissulfurimicrobium	Leptotrichia	Photorhabdus	Taylorella
pirillum	Dissulfurispira	Leucobacter	Phototrophicus	Telmatocola
obacter	Dokdonella	Leuconostoc	Phreatobacter	Tenacibaculum
llus	Dokdonia	Levilactobacillus	Phycococcus	Tenuifilum
eriplanes	Dolichospermum	Liberibacter	Phycisphaera	Tepidanaerobacter
eriovorax	Dolosigranulum	Lichenicola	Phyllobacterium	Tepidibacter
eroides	Dongshaeta	Lichenihabitans	Phytobacter	Tepidiforma
duia	Dorea	Ligilactobacillus	Phytohabitans	Tepidimonas
esiella	Draconibacterium	Lignipirellula	Pigmentiphaga	Tepiditoga
ia	Duganella	Limihaloglobus	Pikeienueella	Terasakiella
lea	Duncaniella	Limnobacter	Pimelobacter	Teredinibacter
lovibrio	Dyadobacter	Limnobaculum	Pirellula	Terribacillus
giatota	Dyella	Limnochorda	Pirellulimonas	Terricaulis
irickia	Dysgonomonas	Limnoglobus	Piscirickettsia	Terriglobus
	Dysnomobacter	Limnolobus	Piscirickettsia	Terrirhabdus

Barras\_Species [2]

## [[1]]



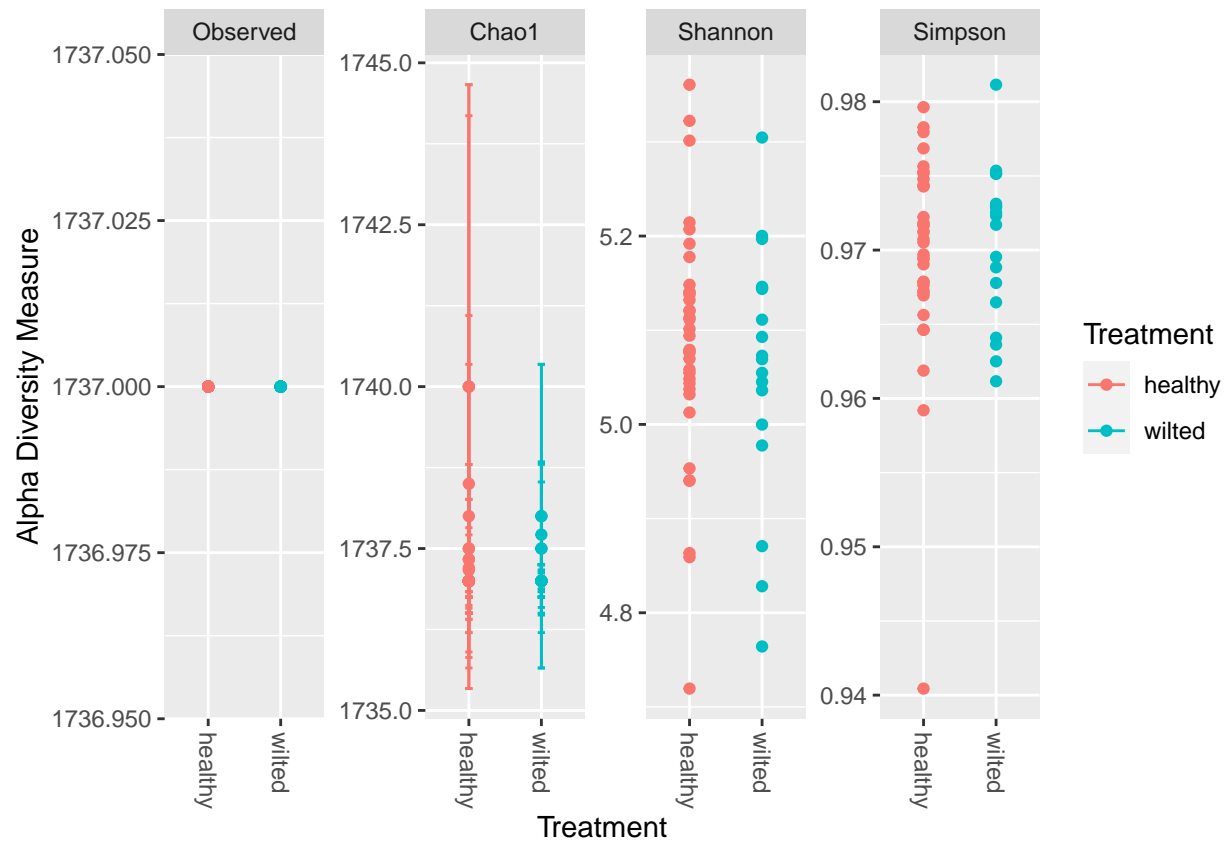
```
Beta_diversity(merge_Bacteria , 'Genus' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.1449554
## Run 1 stress 0.1568268
## Run 2 stress 0.4042205
## Run 3 stress 0.1381229
## ... New best solution
## ... Procrustes: rmse 0.05965033  max resid 0.3019309
## Run 4 stress 0.1616195
## Run 5 stress 0.1396337
## Run 6 stress 0.1394425
## Run 7 stress 0.1449217
## Run 8 stress 0.1391332
## Run 9 stress 0.1478272
## Run 10 stress 0.1381222
## ... New best solution
## ... Procrustes: rmse 0.0002157188  max resid 0.0007001074
## ... Similar to previous best
## Run 11 stress 0.1446967
## Run 12 stress 0.1394436
## Run 13 stress 0.1394896
## Run 14 stress 0.1538027
## Run 15 stress 0.1394435
## Run 16 stress 0.1446968
```

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







#——Bacteria by Species

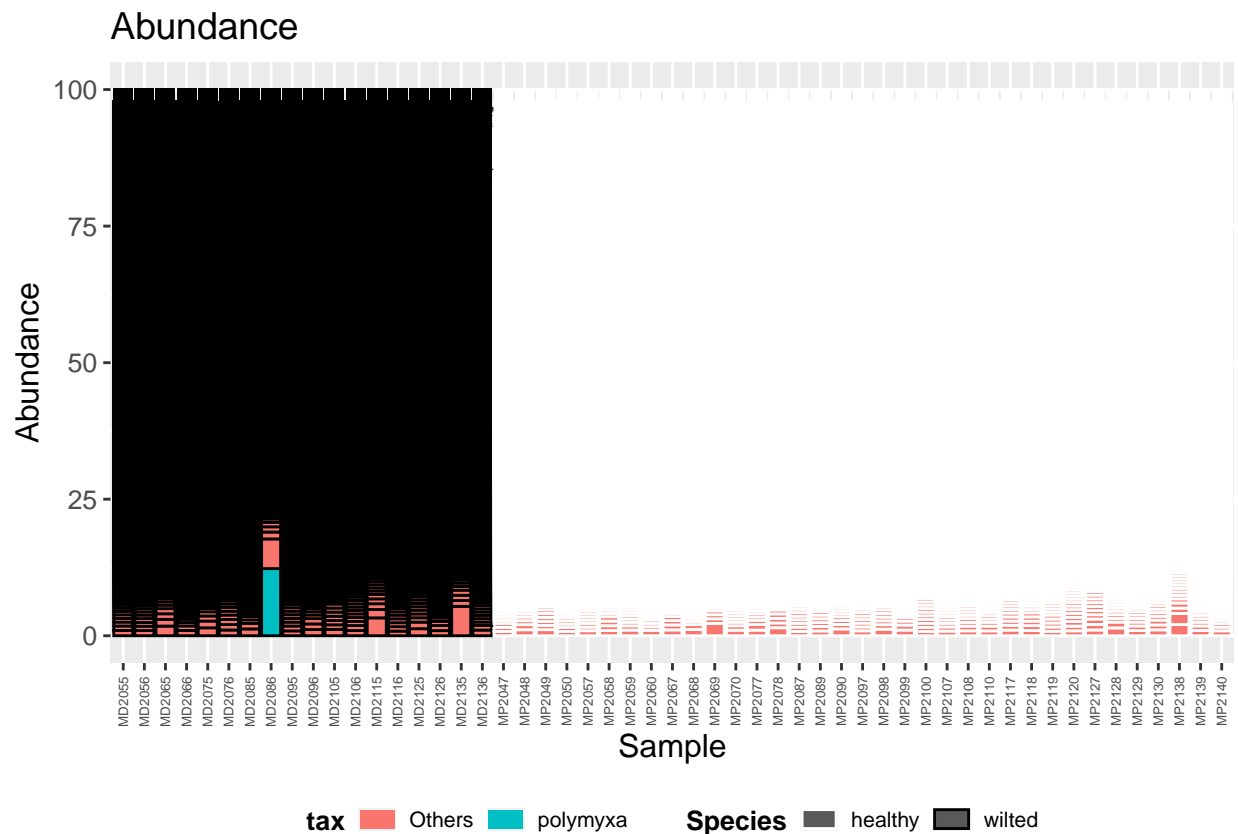
```
Barras_Species <- Abundance_barras(merge_Bacteria, 'Species', 'Treatment', 10.0)
Barras_Species[1]
```

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

e		lactamica	sp. 'Soap Lake #7'	sp. KSB-15	sp. X
		lactarius	sp. (Blaberus giganteus)	sp. KSM-R2A25	sp. X
		lactatifermentans	sp. (Blatta orientalis)	sp. KSM-R2A30	sp. X
		lactatiformans	sp. (Blattella germanica)	sp. KT 15	sp. X
		lactis	sp. (Cryptocercus kyebangensis)	sp. KT25b	sp. X
		lactucaae	sp. (Cryptocercus punctulatus) str. Cpu	sp. KTR9	sp. X
		lacunae	sp. (ex Adelges kitamiensis)	sp. KUDC0405	sp. X
		lacus	sp. (ex Biomphalaria glabrata)	sp. KUDC0406	sp. X
		lacuslunae	sp. (Mastotermes darwiniensis)	sp. KUDC1026	sp. X
		lacustris	sp. (Nauphoeta cinerea)	sp. KUDC1714	sp. X
		laguerreae	sp. (Periplaneta americana)	sp. KUIN-1	sp. X
		laidlawii	sp. 001	sp. Kuro-4	sp. X
		laixinhei	sp. 007	sp. KW1	sp. X
		laikuanensis	sp. 008	sp. KX20019	sp. X
		laminatus	sp. 02C 26	sp. KY-GH-1	sp. X
		lanienae	sp. 09C 129	sp. KY-YJ-3	sp. X
		lanii	sp. 09RB8471	sp. KY3	sp. Y
		lansingensis	sp. 09RB8910	sp. KY5	sp. Y
		lanthieri	sp. 1_2014MBL_MicDiv	sp. KY70	sp. Y
		lapagei	sp. 1_2015MBL_MicDiv	sp. KY75	sp. Y
		lapidicaptus	sp. 1-1C	sp. L-07	sp. Y
		lapsinanis	sp. 1.5R	sp. L-2-11	sp. Y
		lari	sp. 1(2017)	sp. L-8-10	sp. Y
		lariciata	sp. 103DPR2	sp. L-8-3	sp. Y
		larrymorei	sp. 1063	sp. L-A4	sp. Y
		larvae	sp. 107-1	sp. L12M9	sp. Y
		lata	sp. 1070	sp. L1A13	sp. Y
		latens	sp. 10FS3-1	sp. L1A34	sp. Y
		laterosporus	sp. 10M-3C3	sp. L1A9	sp. Y
		laumondii	sp. 10RB9215	sp. L1139	sp. Y
		lautus	sp. 11-1-2	sp. L1SW	sp. Y
		lavamentivorans	sp. 11-B-312	sp. L2A1	sp. Y
		lavendulae	sp. 113-1-2	sp. L2A11	sp. Y
		leachii	sp. 113-3-3	sp. L3-i22	sp. Y
		leadbetteri	sp. 113-3-9	sp. L3-i23	sp. Y
		leeuwenhoekii	sp. 1137	sp. L3A3	sp. Y
		leguminosarum	sp. 113P3	sp. L3A6	sp. Y
		lehensis	sp. 114	sp. L3A8	sp. Y
		lekithochrous	sp. 11515TR	sp. L5	sp. Y
		lemovicicum	sp. 116-D4	sp. L51/94	sp. Y
		lemurum	sp. 119287	sp. L5B5	sp. Y
		lenghuensis	sp. 11B	sp. L6-1	sp. Y
		lenta	sp. 11kri321	sp. L9-4	sp. Y
		lentiflavum	sp. 12200R-103	sp. LA-2-3-30-S1-D2	sp. Y
		lentis	sp. 13-15	sp. LA112445	sp. Y
		lentocellum	sp. 131	sp. LA31	sp. Y
		lentus	sp. 131-2-1	sp. LAB-08	sp. Y
		leopoldii	sp. 131-2-5	sp. LADL05-105	sp. Y
		leprae	sp. 131-3-5	sp. LAS2	sp. Y
		lepromatosis	sp. 13159349	sp. LB1	sp. Y
		leptomitiformis	sp. 135	sp. LBG001	sp. Y
		lettingae	sp. 140616W15	sp. LBUM 1475	sp. Y
andiella		liangguodongii	sp. 14171R-50	sp. LBUM 1480	sp. Y
ibbosus		liangshanensis	sp. 14181154	sp. LBUM 1482	sp. Y
ifera		liaowanqingii	sp. 143-21	sp. LBUM920	sp. Y
		libanensis	sp. 144S4	sp. LC2018020214	sp. Y
		licheniformis	sp. 15-184	sp. LC6	sp. Y

Barras\_Species[2]

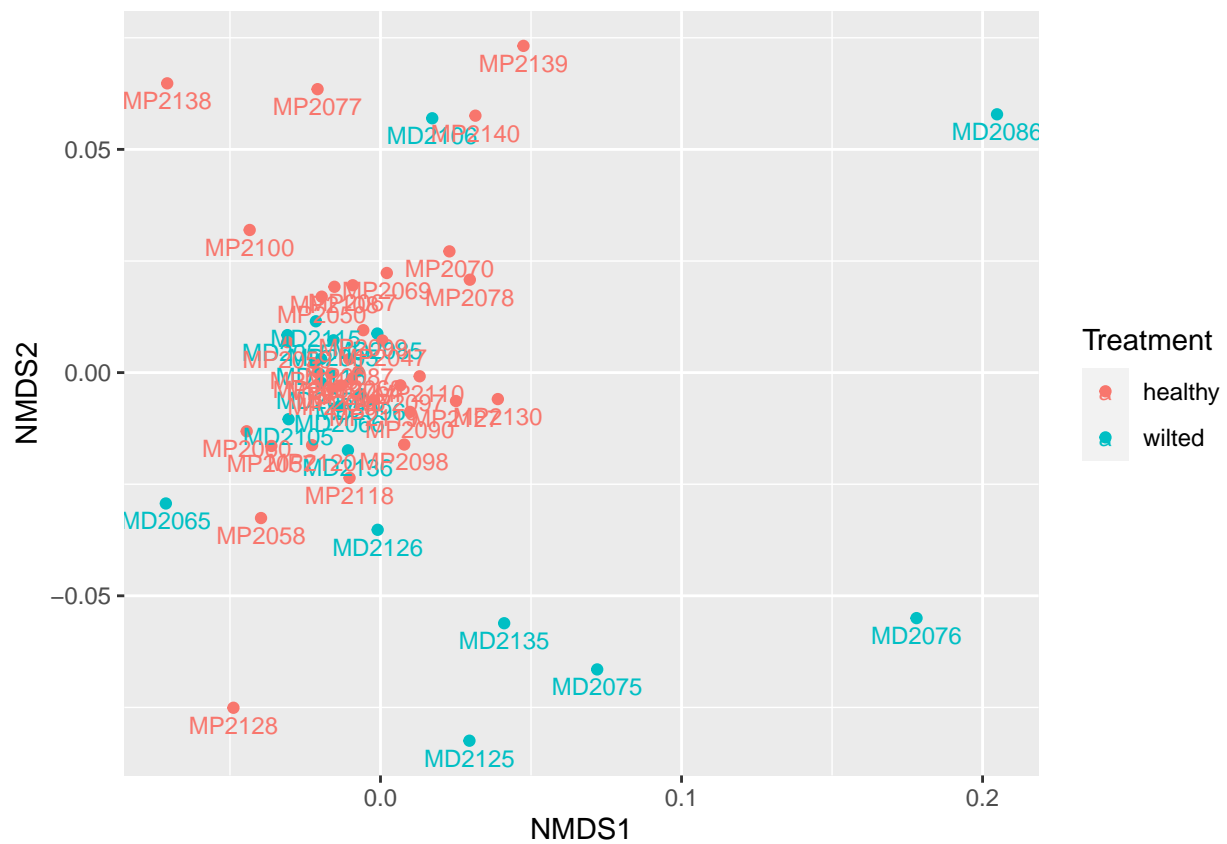
## [[1]]



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

```
## Wisconsin double standardization
## Run 0 stress 0.1576092
## Run 1 stress 0.1716447
## Run 2 stress 0.1884511
## Run 3 stress 0.1586321
## Run 4 stress 0.1576103
## ... Procrustes: rmse 0.0005398961 max resid 0.002832279
## ... Similar to previous best
## Run 5 stress 0.1520053
## ... New best solution
## ... Procrustes: rmse 0.06042515 max resid 0.3534131
## Run 6 stress 0.1657862
## Run 7 stress 0.1625793
## Run 8 stress 0.1599885
## Run 9 stress 0.1861631
## Run 10 stress 0.1586322
## Run 11 stress 0.1786974
## Run 12 stress 0.1519651
## ... New best solution
## ... Procrustes: rmse 0.006402111 max resid 0.04373792
## Run 13 stress 0.1586322
## Run 14 stress 0.1569664
## Run 15 stress 0.1752804
```

```
## Run 16 stress 0.1549728
## Run 17 stress 0.1566349
## Run 18 stress 0.1561432
## Run 19 stress 0.1515452
## ... New best solution
## ... Procrustes: rmse 0.01317253  max resid 0.08874532
## Run 20 stress 0.1519647
## ... Procrustes: rmse 0.01321349  max resid 0.08868381
## *** Best solution was not repeated -- monoMDS stopping criteria:
##      6: no. of iterations >= maxit
##     14: stress ratio > sratmax
```



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

