230306_Reporte1Exploracion

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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", "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)</pre>
```

Subconjunto de "Eukaryota"

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

Subconjunto de "Bacteria"

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

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))
}</pre>
```

Graficar abundancias stackbar

input entra el percentages_df

```
Abundance_barras <- function(phy,tax,attribute,abundance_percentage){
  ##llamar funcion de datos
  Data <- glomToGraph(phy,tax)</pre>
  glom <- Data[[1]] #phyloseq</pre>
  percentages <- Data[[2]] #phyloseq</pre>
  percentages_df <- Data[[3]] # dataframe</pre>
  ## 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.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$tax<-percentages_df[,ncol(percentages_df)]</pre>
  percentages_df$tax[percentages_df$Abundance < abundance_percentage] <- "abundance_percentage"
  percentages_df$tax <- as.factor(percentages_df$tax)</pre>
  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",
```

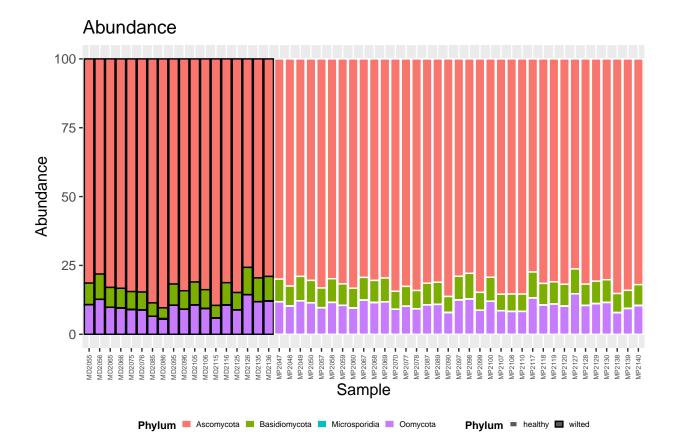
```
legend.direction = "horizontal",
legend.title=element_text(size=10, face = "bold"),
legend.text=element_text(size=8),
text = element_text(size=12),
axis.text.x = element_text(angle=90, size=5, hjust=1, vjust=0.5))
return(list(plot_barras,plot_percentages))
}
```

Graficar betadiversity

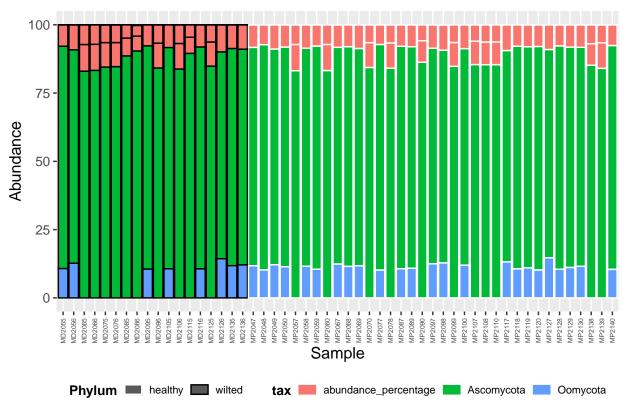
```
Beta_diversity <- function(phy,tax,attribute,distance){
  Data <- glomToGraph(phy,tax)
  glom <- Data[[1]]
#CREAR UN GLOM AL 10%
percentages <- Data[[2]]
percentages_df <- Data[[3]]
## Beta diversidad
meta_ord <- ordinate(physeq = percentages, method = "NMDS", distance = distance)
plot_beta <- plot_ordination(physeq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

Graficar alphadiversity

```
Alpha_diversity <- function(phy,tax,attribute){</pre>
  ## llamamos la funcion que crea los dataset
  Data <- glomToGraph(phy,tax)</pre>
  glom <- Data[[1]]</pre>
  percentages <- Data[[2]]</pre>
  percentages_df <- Data[[3]]</pre>
  ## Alfa diversidad
  plot_alpha <- plot_richness(physeq = glom, measures = c("Observed", "Chao1", "Shannon", "simpson"), x = a</pre>
  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]]
```



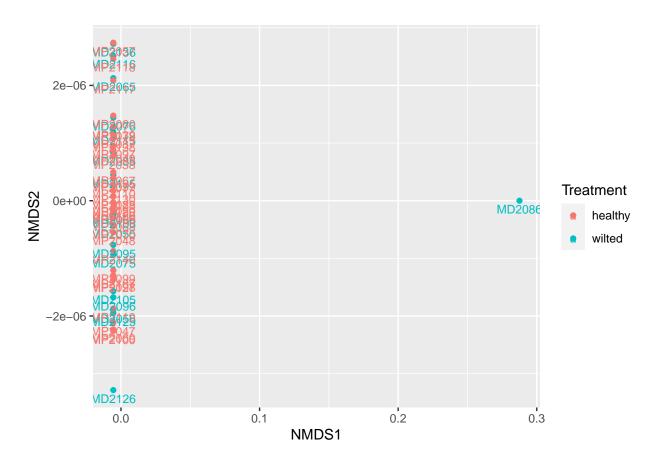
Barras_Phylum[2]



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

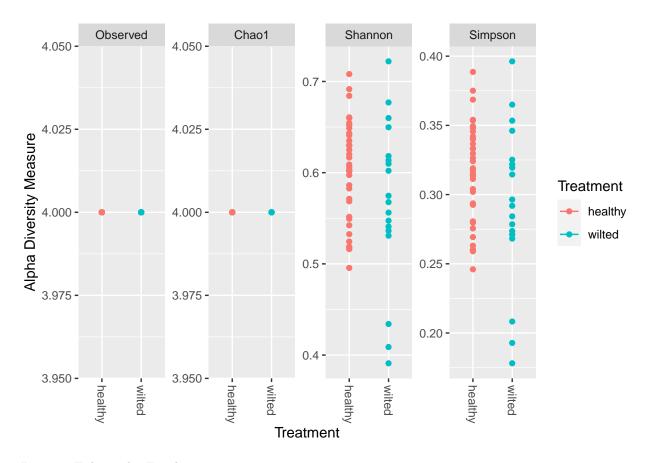
```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.01148681
## Run 1 stress 9.641699e-05
## ... New best solution
## ... Procrustes: rmse 0.03460127 max resid 0.09064286
## Run 2 stress 9.692958e-05
## ... Procrustes: rmse 0.0001280624 max resid 0.0004094851
## ... Similar to previous best
## Run 3 stress 0.0009993135
## Run 4 stress 2.875625e-05
## ... New best solution
## ... Procrustes: rmse 0.0001075607 max resid 0.0002893054
## ... Similar to previous best
## Run 5 stress 7.419065e-05
## ... Procrustes: rmse 4.598373e-05 max resid 0.0001193756
## ... Similar to previous best
## Run 6 stress 7.803609e-05
## ... Procrustes: rmse 2.872485e-05 max resid 6.902742e-05
## ... Similar to previous best
## Run 7 stress 9.725895e-05
## ... Procrustes: rmse 0.0002168401 max resid 0.0005770855
## ... Similar to previous best
```

```
## Run 8 stress 8.900437e-05
## ... Procrustes: rmse 8.648394e-05 max resid 0.0002263783
## ... Similar to previous best
## Run 9 stress 9.712047e-05
## ... Procrustes: rmse 0.0001019901 max resid 0.0002717338
## ... Similar to previous best
## Run 10 stress 9.473721e-05
## ... Procrustes: rmse 0.0001079967 max resid 0.0002832011
## ... Similar to previous best
## Run 11 stress 9.169436e-05
## ... Procrustes: rmse 6.777917e-05 max resid 0.0001896028
## ... Similar to previous best
## Run 12 stress 0.0001125098
## ... Procrustes: rmse 0.000332578 max resid 0.0008723591
## ... Similar to previous best
## Run 13 stress 9.913374e-05
## ... Procrustes: rmse 0.000169905 max resid 0.0004530579
## ... Similar to previous best
## Run 14 stress 9.120442e-05
## ... Procrustes: rmse 2.554051e-05 max resid 0.0001256882
## ... Similar to previous best
## Run 15 stress 9.384635e-05
## ... Procrustes: rmse 7.33919e-05 max resid 0.0001795472
## ... Similar to previous best
## Run 16 stress 6.475988e-05
## ... Procrustes: rmse 3.209143e-05 max resid 7.189593e-05
## ... Similar to previous best
## Run 17 stress 0.0007519899
## Run 18 stress 0.0001067793
## ... Procrustes: rmse 0.0003143495 max resid 0.0008254772
## ... Similar to previous best
## Run 19 stress 9.906154e-05
## ... Procrustes: rmse 0.0001095915 max resid 0.0002853942
## ... Similar to previous best
## Run 20 stress 9.462542e-05
## ... Procrustes: rmse 0.0001393057 max resid 0.0003633612
## ... Similar to previous best
## *** Best solution repeated 16 times
## Warning in metaMDS(veganifyOTU(physeq), distance, ...): stress is (nearly) zero:
## you may have insufficient data
```



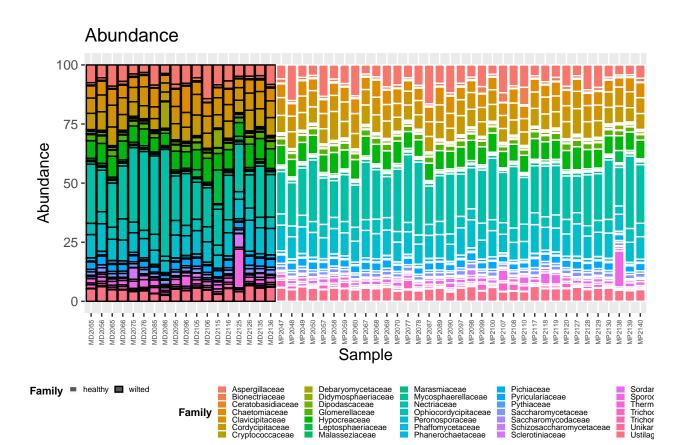
```
Alpha_diversity(merge_Eukaryota , 'Phylum' , 'Treatment')
```

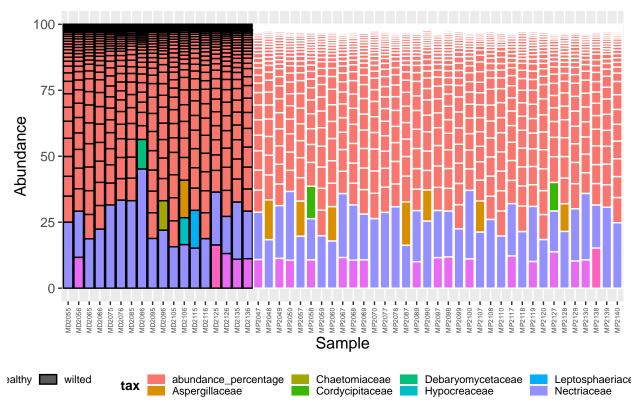
```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##
## 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]</pre>

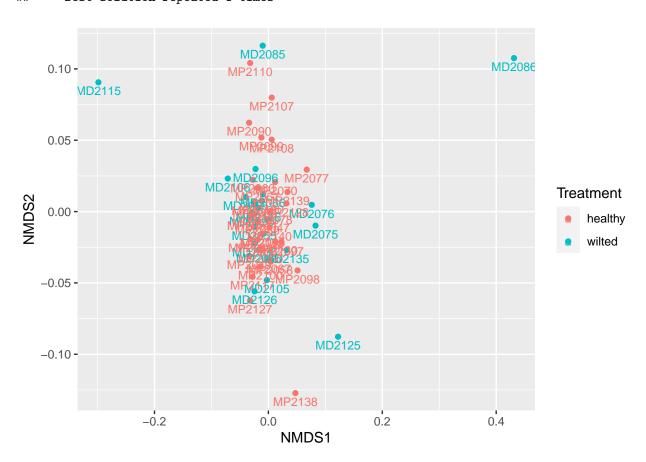




Beta_diversity(merge_Eukaryota , 'Family' , 'Treatment', 'bray')

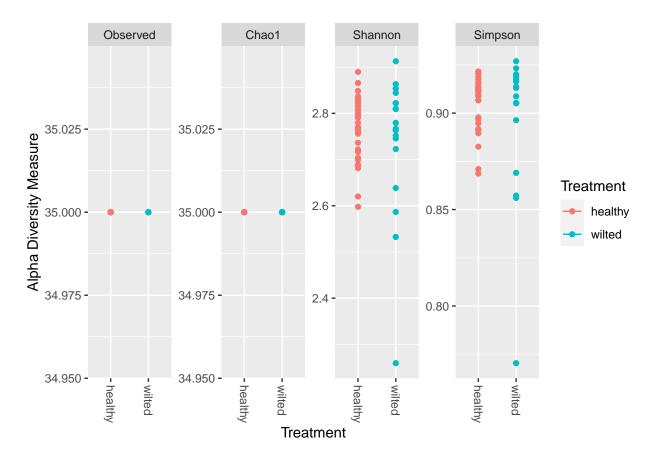
```
## Wisconsin double standardization
## Run 0 stress 0.1089081
## Run 1 stress 0.1088964
## ... New best solution
## ... Procrustes: rmse 0.002397465 max resid 0.009337499
## ... Similar to previous best
## Run 2 stress 0.1089479
## ... Procrustes: rmse 0.008318336 max resid 0.0543661
## Run 3 stress 0.1089298
## ... Procrustes: rmse 0.008610539 max resid 0.05495783
## Run 4 stress 0.1088985
## ... Procrustes: rmse 0.000636777
                                    max resid 0.00368301
## ... Similar to previous best
## Run 5 stress 0.1352082
## Run 6 stress 0.109113
## ... Procrustes: rmse 0.01003345 max resid 0.05422836
## Run 7 stress 0.1088882
## ... New best solution
## ... Procrustes: rmse 0.00173353
                                    max resid 0.009003435
## ... Similar to previous best
## Run 8 stress 0.1089152
## ... Procrustes: rmse 0.001735834 max resid 0.009378948
## ... Similar to previous best
```

```
## Run 9 stress 0.1185617
## Run 10 stress 0.1088982
## ... Procrustes: rmse 0.00155193 max resid 0.007565839
## ... Similar to previous best
## Run 11 stress 0.1089574
## ... Procrustes: rmse 0.00861713 max resid 0.05374258
## Run 12 stress 0.1089481
## ... Procrustes: rmse 0.008203471 max resid 0.05409664
## Run 13 stress 0.4042251
## Run 14 stress 0.1089316
## ... Procrustes: rmse 0.008488576 max resid 0.05456272
## Run 15 stress 0.1089084
## ... Procrustes: rmse 0.001691858 max resid 0.009408485
## ... Similar to previous best
## Run 16 stress 0.1113091
## Run 17 stress 0.1091144
## ... Procrustes: rmse 0.0100354 max resid 0.05392358
## Run 18 stress 0.1091131
## ... Procrustes: rmse 0.009953453 max resid 0.05403308
## Run 19 stress 0.1088877
## ... New best solution
## ... Procrustes: rmse 0.0001671813 max resid 0.0009656288
## ... Similar to previous best
## Run 20 stress 0.109126
## ... Procrustes: rmse 0.01001698 max resid 0.05420395
## *** Best solution repeated 1 times
```



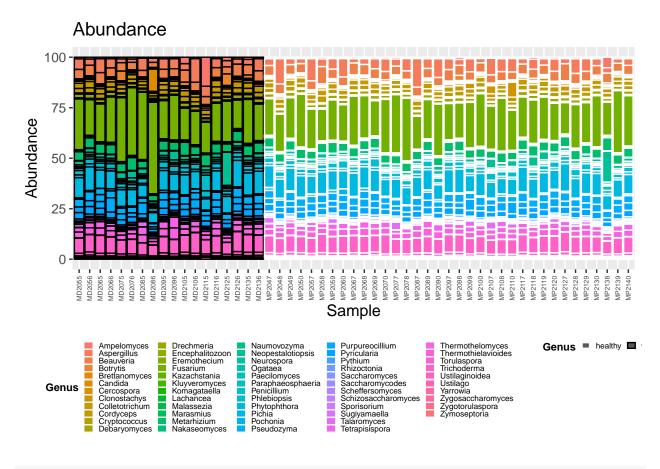
```
Alpha_diversity(merge_Eukaryota , 'Family' , 'Treatment')
```

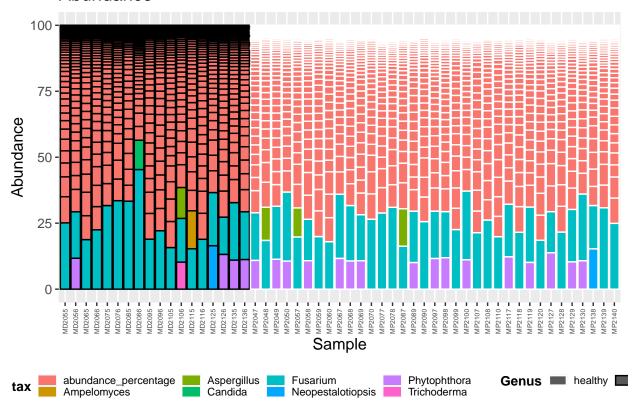
```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##
## 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]</pre>

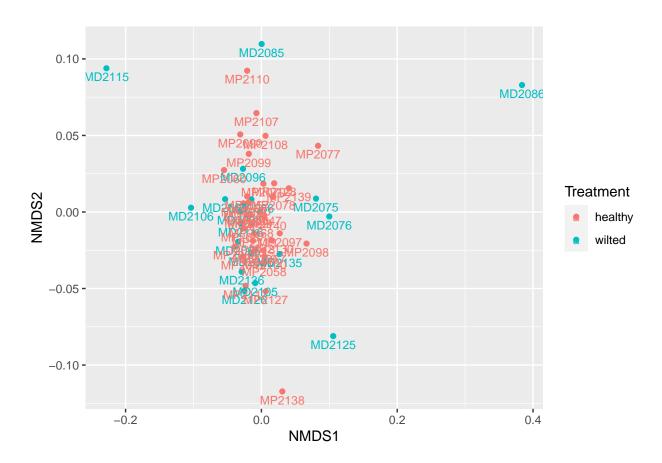




```
Beta_diversity(merge_Eukaryota , 'Genus' , 'Treatment', 'bray')
```

```
## Wisconsin double standardization
## Run 0 stress 0.110709
## Run 1 stress 0.1111514
## ... Procrustes: rmse 0.01374689 max resid 0.06906551
## Run 2 stress 0.1235012
## Run 3 stress 0.1119814
## Run 4 stress 0.1107094
## ... Procrustes: rmse 0.001118204 max resid 0.006393413
## ... Similar to previous best
## Run 5 stress 0.123502
## Run 6 stress 0.1235014
## Run 7 stress 0.1223845
## Run 8 stress 0.1107098
## ... Procrustes: rmse 0.0002377637 max resid 0.001329702
## ... Similar to previous best
## Run 9 stress 0.1236307
## Run 10 stress 0.1236309
## Run 11 stress 0.110709
## ... New best solution
## ... Procrustes: rmse 1.991006e-05 max resid 0.0001234856
## ... Similar to previous best
## Run 12 stress 0.1235025
## Run 13 stress 0.1236304
```

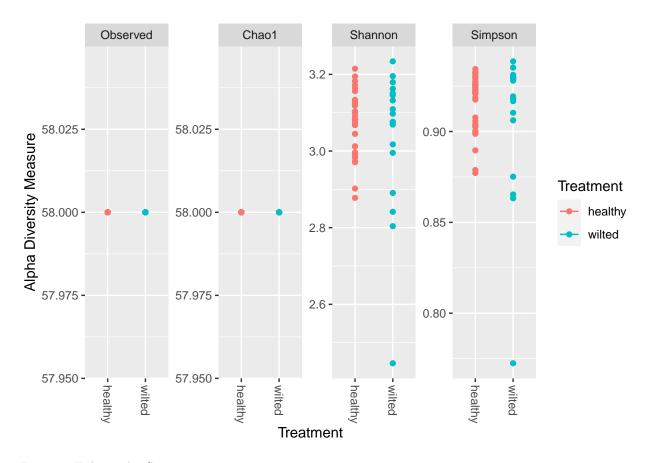
```
## Run 14 stress 0.1107086
## ... New best solution
## ... Procrustes: rmse 0.000252225 max resid 0.001539016
## ... Similar to previous best
## Run 15 stress 0.1107097
## ... Procrustes: rmse 0.0009435275 max resid 0.005267492
## ... Similar to previous best
## Run 16 stress 0.1223877
## Run 17 stress 0.1107172
## ... Procrustes: rmse 0.002093797 max resid 0.01195467
## Run 18 stress 0.1235015
## Run 19 stress 0.1223843
## Run 20 stress 0.1107093
## ... Procrustes: rmse 0.000821115 max resid 0.00457383
## ... Similar to previous best
## *** Best solution repeated 3 times
```



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

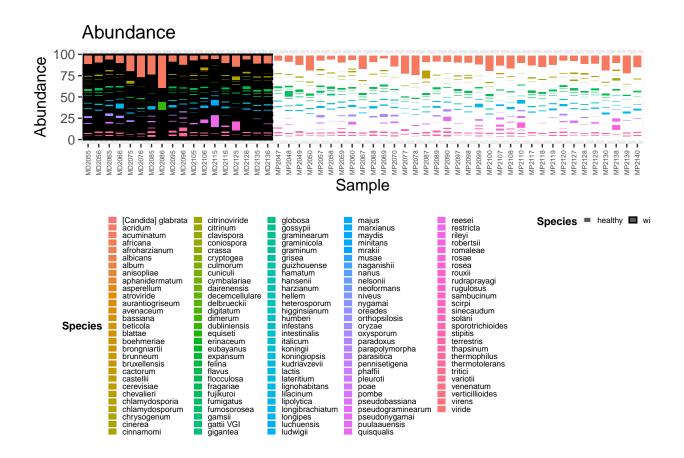
```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##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

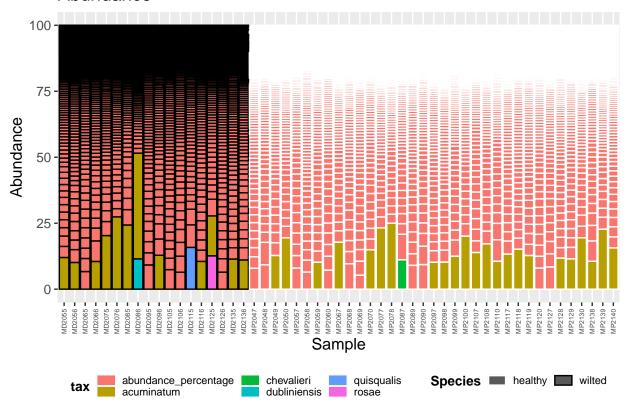
We recommended that you find the un-trimmed data and retry.



#——Eukarya by Species

Barras_Species <- Abundance_barras(merge_Eukaryota, 'Species', 'Treatment', 10.0)
Barras_Species[1]</pre>

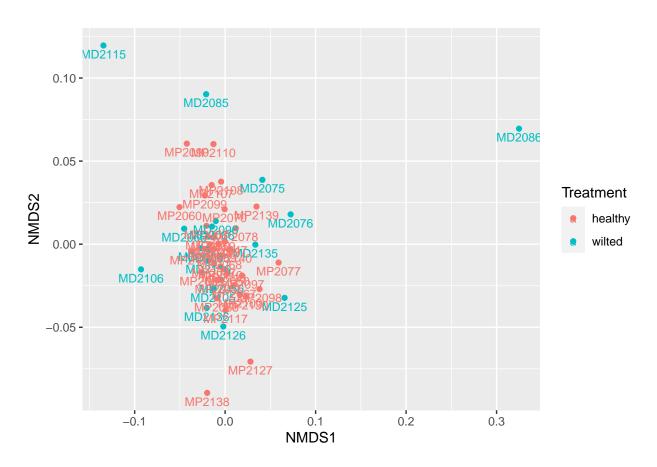




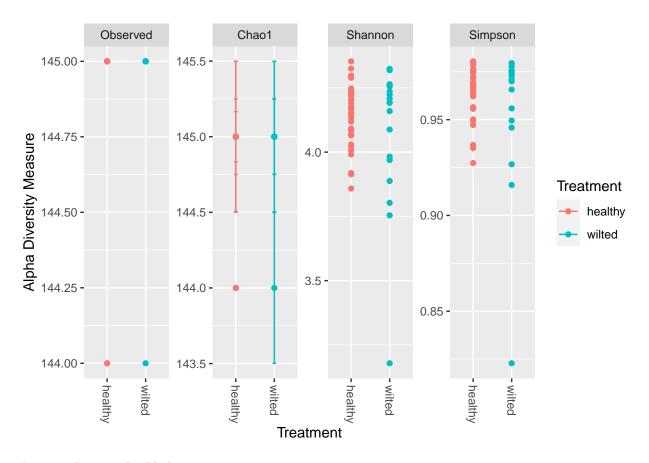
Beta_diversity(merge_Eukaryota , 'Species' , 'Treatment', 'bray')

```
## Wisconsin double standardization
## Run 0 stress 0.1226512
## Run 1 stress 0.1226515
## ... Procrustes: rmse 0.0009565164 max resid 0.004818758
## ... Similar to previous best
## Run 2 stress 0.1233302
## Run 3 stress 0.1218539
## ... New best solution
## ... Procrustes: rmse 0.02026332 max resid 0.1282693
## Run 4 stress 0.1278289
## Run 5 stress 0.1218549
## ... Procrustes: rmse 0.0006613939 max resid 0.003265407
## ... Similar to previous best
## Run 6 stress 0.1227526
## Run 7 stress 0.1226511
## Run 8 stress 0.1218544
## ... Procrustes: rmse 0.00050139 max resid 0.002464929
## ... Similar to previous best
## Run 9 stress 0.1218543
## ... Procrustes: rmse 0.0002429245 max resid 0.001192044
## ... Similar to previous best
## Run 10 stress 0.1233307
## Run 11 stress 0.1272847
```

```
## Run 12 stress 0.1218539
## ... Procrustes: rmse 0.0001695548 max resid 0.0008627428
## ... Similar to previous best
## Run 13 stress 0.121854
## ... Procrustes: rmse 0.0003043927 max resid 0.001496288
## ... Similar to previous best
## Run 14 stress 0.1226509
## Run 15 stress 0.1218543
## ... Procrustes: rmse 0.0004881011 max resid 0.002431532
## ... Similar to previous best
## Run 16 stress 0.1226506
## Run 17 stress 0.1539498
## Run 18 stress 0.1535018
## Run 19 stress 0.1218548
## ... Procrustes: rmse 0.0003979232 max resid 0.001941218
## ... Similar to previous best
## Run 20 stress 0.122651
## *** Best solution repeated 7 times
```

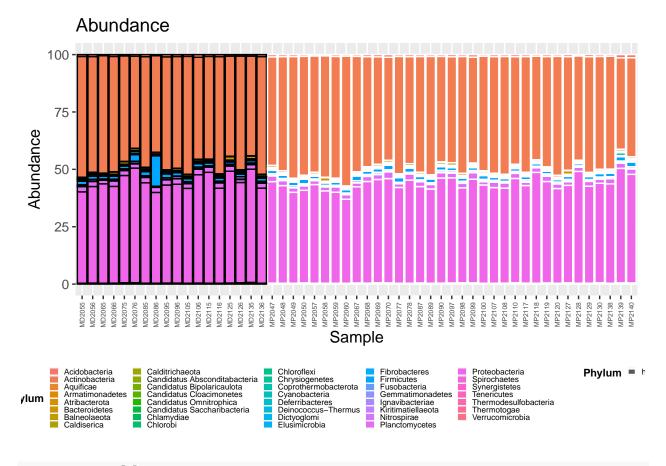


Alpha_diversity(merge_Eukaryota , 'Species' , 'Treatment')

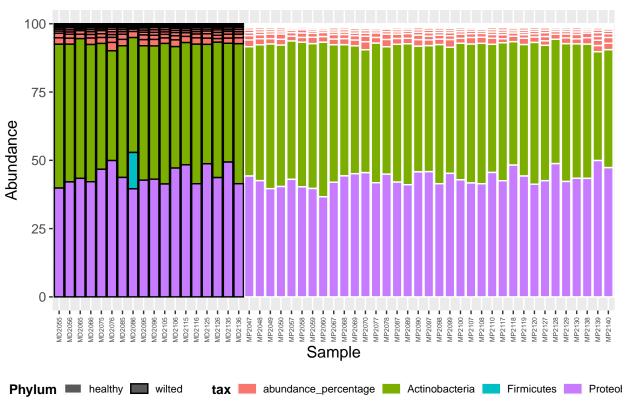


#——Bacteria by Phylum

Barras_Species <- Abundance_barras(merge_Bacteria, 'Phylum', 'Treatment', 10.0)
Barras_Species[1]</pre>



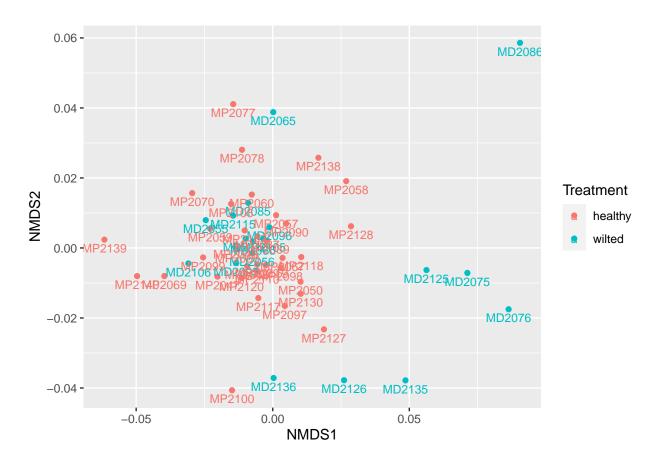




```
Beta_diversity(merge_Bacteria , 'Phylum' , 'Treatment', 'bray')
```

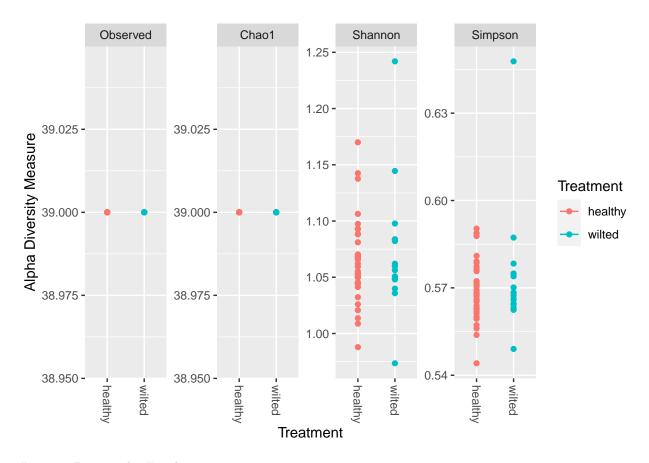
```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.1520004
## Run 1 stress 0.1636958
## Run 2 stress 0.1700011
## Run 3 stress 0.1519724
## ... New best solution
## ... Procrustes: rmse 0.008285382 max resid 0.04681388
## Run 4 stress 0.1552698
## Run 5 stress 0.1519014
## ... New best solution
## ... Procrustes: rmse 0.04885038 max resid 0.3152661
## Run 6 stress 0.1614796
## Run 7 stress 0.1513921
## ... New best solution
## ... Procrustes: rmse 0.04582075 max resid 0.3024696
## Run 8 stress 0.1779282
## Run 9 stress 0.1548791
## Run 10 stress 0.1629442
## Run 11 stress 0.16964
## Run 12 stress 0.1524009
## Run 13 stress 0.1662298
## Run 14 stress 0.1613925
```

```
## Run 15 stress 0.1652755
## Run 16 stress 0.1633312
## Run 17 stress 0.1607191
## Run 18 stress 0.1672632
## Run 19 stress 0.1513905
## ... New best solution
## ... Procrustes: rmse 0.0004428603 max resid 0.002388818
## ... Similar to previous best
## Run 20 stress 0.1513906
## ... Procrustes: rmse 3.760188e-05 max resid 0.0002150336
## ... Similar to previous best
## *** Best solution repeated 2 times
```



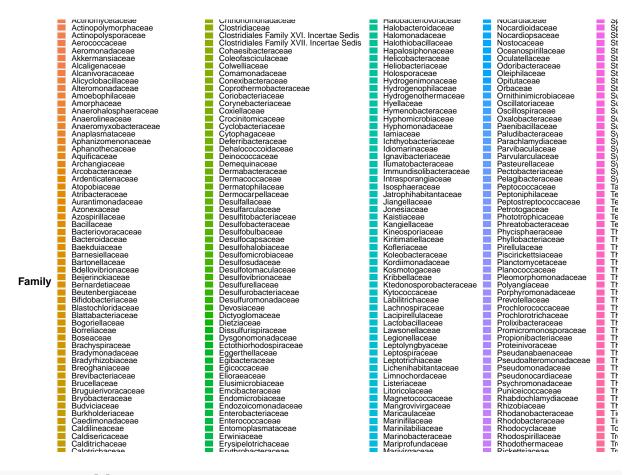
Alpha_diversity(merge_Bacteria , 'Phylum' , 'Treatment')

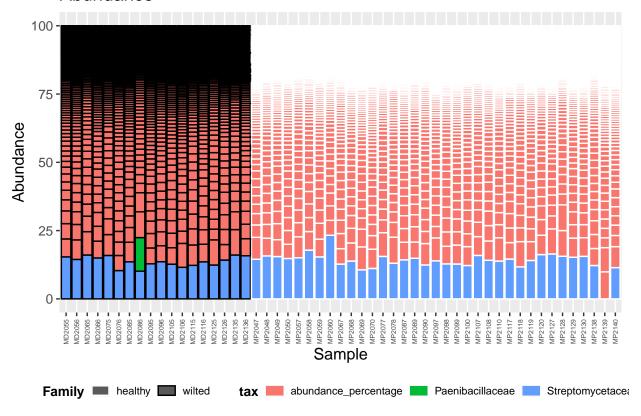
```
## Warning in estimate_richness(physeq, split = TRUE, measures = measures): The data you have provided of
## any singletons. This is highly suspicious. Results of richness
## estimates (for example) are probably unreliable, or wrong, if you have already
## trimmed low-abundance taxa from the data.
##
## 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]</pre>

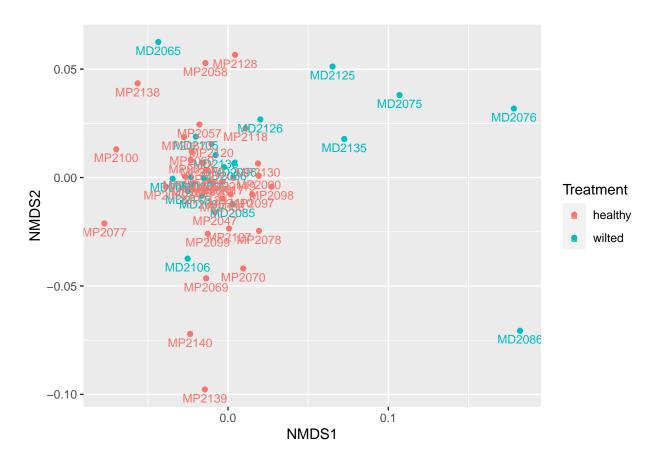




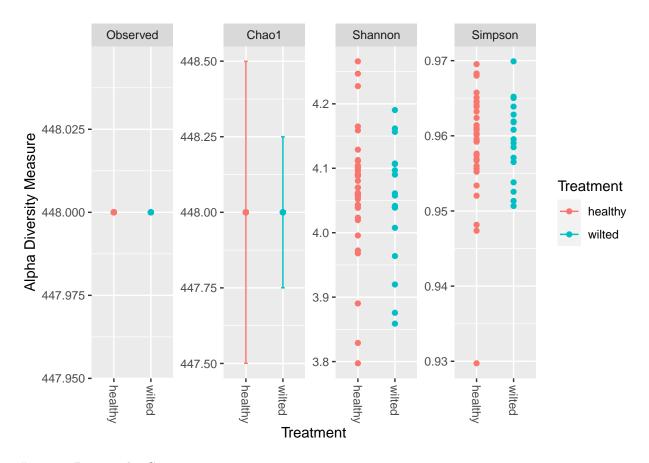
Beta_diversity(merge_Bacteria , 'Family' , 'Treatment', 'bray')

```
## Wisconsin double standardization
## Run 0 stress 0.1412073
## Run 1 stress 0.1418145
## Run 2 stress 0.1390466
## ... New best solution
## ... Procrustes: rmse 0.02128683 max resid 0.1331159
## Run 3 stress 0.1405626
## Run 4 stress 0.145559
## Run 5 stress 0.1863488
## Run 6 stress 0.1412066
## Run 7 stress 0.1369866
## ... New best solution
## ... Procrustes: rmse 0.06315741 max resid 0.3787318
## Run 8 stress 0.1551948
## Run 9 stress 0.142348
## Run 10 stress 0.1405103
## Run 11 stress 0.1372656
## ... Procrustes: rmse 0.01219911 max resid 0.0572946
## Run 12 stress 0.1369866
## ... Procrustes: rmse 5.037779e-05 max resid 0.0001538958
## ... Similar to previous best
## Run 13 stress 0.1412071
## Run 14 stress 0.1402344
```

```
## Run 15 stress 0.1373769
## ... Procrustes: rmse 0.01547261 max resid 0.05919644
## Run 16 stress 0.1412072
## Run 17 stress 0.1373766
## ... Procrustes: rmse 0.01563171 max resid 0.05932202
## Run 18 stress 0.1441744
## Run 19 stress 0.1552141
## Run 20 stress 0.1625797
## *** Best solution repeated 1 times
```



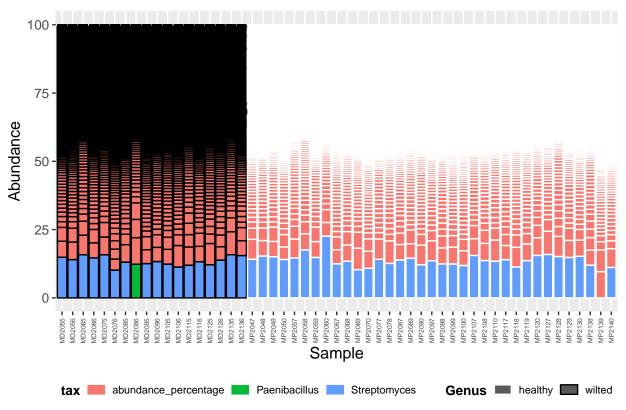
Alpha_diversity(merge_Bacteria , 'Family' , 'Treatment')



#——Bacteria by Genero

Barras_Species <- Abundance_barras(merge_Bacteria, 'Genus', 'Treatment', 10.0)
Barras_Species[1]</pre>

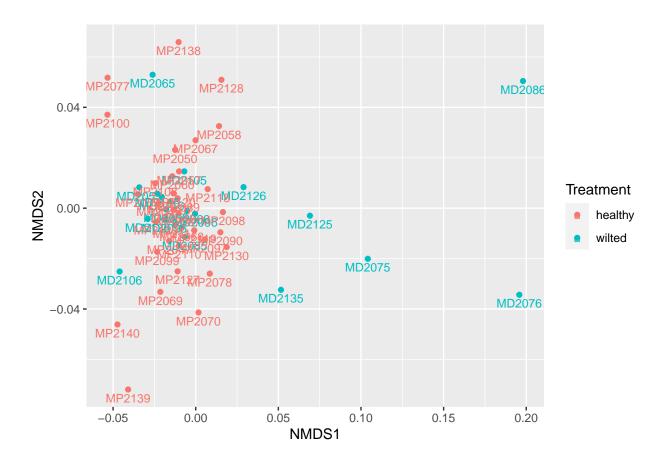
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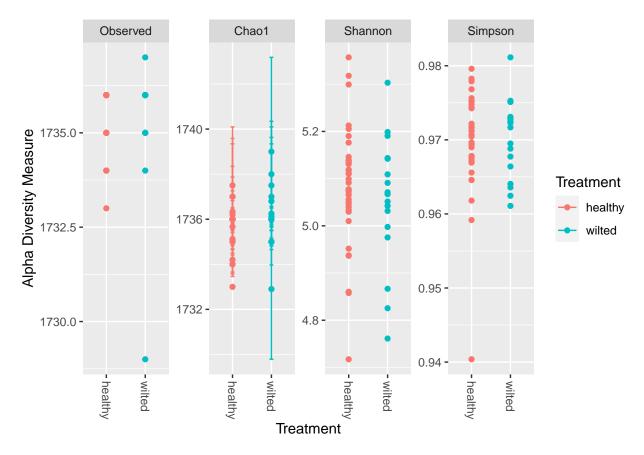
Beta_diversity(merge_Bacteria , 'Genus' , 'Treatment', 'bray')

```
## Wisconsin double standardization
## Run 0 stress 0.1467945
## Run 1 stress 0.145778
## ... New best solution
## ... Procrustes: rmse 0.04626627 max resid 0.2220378
## Run 2 stress 0.1433318
## ... New best solution
## ... Procrustes: rmse 0.08271459 max resid 0.3946675
## Run 3 stress 0.1467201
## Run 4 stress 0.1531013
## Run 5 stress 0.1467425
## Run 6 stress 0.146185
## Run 7 stress 0.1505679
## Run 8 stress 0.1500532
## Run 9 stress 0.145519
## Run 10 stress 0.151215
## Run 11 stress 0.1512369
## Run 12 stress 0.1490812
## Run 13 stress 0.148721
## Run 14 stress 0.1432834
## ... New best solution
## ... Procrustes: rmse 0.004246344 max resid 0.02586992
## Run 15 stress 0.1454653
```

```
## Run 16 stress 0.1432838
## ... Procrustes: rmse 0.0007481338 max resid 0.003205415
## ... Similar to previous best
## Run 17 stress 0.1519958
## Run 18 stress 0.1499637
## Run 19 stress 0.1438174
## Run 20 stress 0.1484465
## *** Best solution repeated 1 times
```

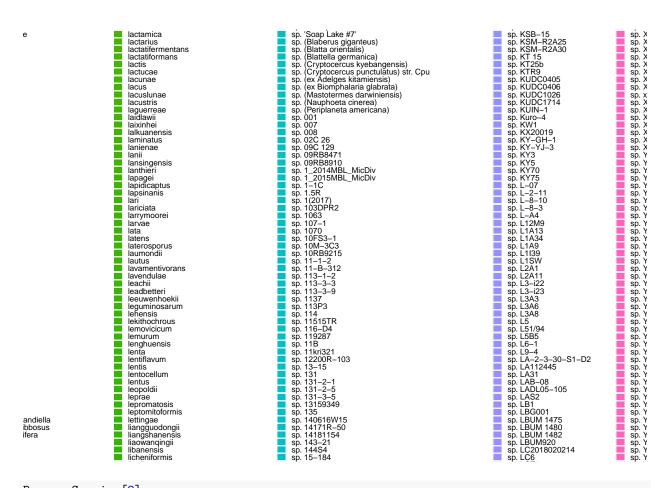


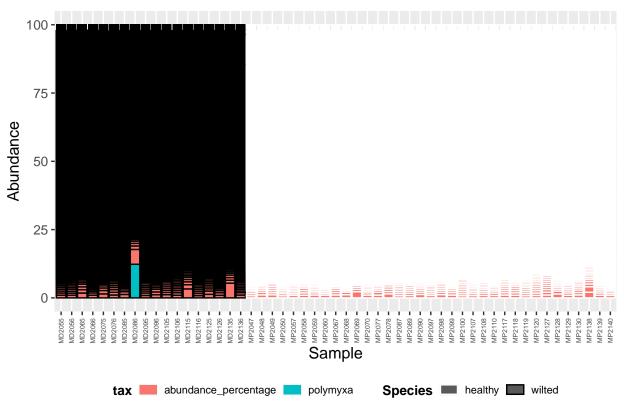
Alpha_diversity(merge_Bacteria , 'Genus' , 'Treatment')



#——Bacteria by Species

Barras_Species <- Abundance_barras(merge_Bacteria, 'Species', 'Treatment', 10.0)
Barras_Species[1]</pre>

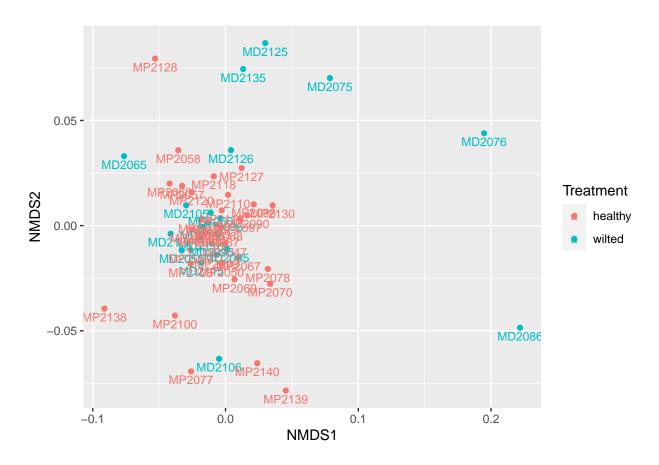




Beta_diversity(merge_Bacteria , 'Species' , 'Treatment', 'bray')

```
## Wisconsin double standardization
## Run 0 stress 0.1597841
## Run 1 stress 0.1616467
## Run 2 stress 0.1596992
## ... New best solution
## ... Procrustes: rmse 0.05927183 max resid 0.3719294
## Run 3 stress 0.1703226
## Run 4 stress 0.1633768
## Run 5 stress 0.1622795
## Run 6 stress 0.166837
## Run 7 stress 0.1577449
## ... New best solution
## ... Procrustes: rmse 0.02327626 max resid 0.1144114
## Run 8 stress 0.1676656
## Run 9 stress 0.1603708
## Run 10 stress 0.1596212
## Run 11 stress 0.1635446
## Run 12 stress 0.1605549
## Run 13 stress 0.1637696
## Run 14 stress 0.1641623
## Run 15 stress 0.1602436
## Run 16 stress 0.1603294
## Run 17 stress 0.1587399
```

```
## Run 18 stress 0.1789824
## Run 19 stress 0.1571292
## ... New best solution
## ... Procrustes: rmse 0.03053161 max resid 0.1408118
## Run 20 stress 0.1576528
## *** Best solution was not repeated -- monoMDS stopping criteria:
## 4: no. of iterations >= maxit
## 16: stress ratio > sratmax
```



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

