Lab_10_Metagenomics_MBI3100A_2022_Tutorial

2022-11-12

R Markdown

Acknowledgement: The commands and datasets used in is tutorial are adapted from the phyloseq website and Yan Hui Microbiome tutorial.

Install required libraries

Installing these libraries may take some time. Try to update all other dependencies when prompted (type "a" and enter).

```
if (!require("BiocManager")) install.packages("BiocManager")

if (!require("phyloseq")) BiocManager::install("phyloseq")

if (!require("microbiomeMarker")) BiocManager::install("microbiomeMarkera")

if (!require("tidyverse")) install.packages("tidyverse")

if (!require("dendextend")) install.packages("dendextend")
```

Load Libraries

```
library(phyloseq)
library(ggplot2)
library(dplyr)
library(dendextend)
library(microbiomeMarker)
```

List of packages available in phyloseq

Phyloseq comes with preloaded datasets. The datasets can be explored using the following commands. The column named "Item" contains the list of all the datasets available in phyloseq package

```
phyloseq_datasets = data(package = "phyloseq")
phyloseq_datasets$results
```

```
##      Package    LibPath                                          Item
## [1,] "phyloseq" "C:/Users/HP/AppData/Local/R/win-library/4.2" "GlobalPatterns"
## [2,] "phyloseq" "C:/Users/HP/AppData/Local/R/win-library/4.2" "enterotype"
## [3,] "phyloseq" "C:/Users/HP/AppData/Local/R/win-library/4.2" "esophagus"
## [4,] "phyloseq" "C:/Users/HP/AppData/Local/R/win-library/4.2" "soilrep"
##      Title
## [1,] "(Data) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample (20
## [2,] "(Data) Enterotypes of the human gut microbiome (2011)"
## [3,] "(Data) Small example dataset from a human esophageal community (2004)"
## [4,] "(Data) Reproducibility of soil microbiome data (2011)"
```

```
# To load the GlobalPatterns dataset
data(GlobalPatterns)
```

```
GlobalPatterns
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 19216 taxa and 26 samples ]
## sample_data() Sample Data:       [ 26 samples by 7 sample variables ]
## tax_table()   Taxonomy Table:    [ 19216 taxa by 7 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 19216 tips and 19215 internal nodes ]
```

1. Data exploration

GlobalPatterns is a phyloseq object which contains 1 out_table, 1 sample_data table, 1 taxonomy table and 1 phylogenetic tree.

We can access the different data type of data and table using following commands

```
# otu_table(GlobalPatterns) %>% head()
# sample_data(GlobalPatterns) %>% head()
# tax_table(GlobalPatterns) %>% head()
# phy_tree(GlobalPatterns) %>% head()
```

For example: How many samples are there in the 'GlobalPatterns' data set?

```
sample_data(GlobalPatterns)
```

```
##          X.SampleID  Primer Final_Barcode Barcode_truncated_plus_T
## CL3             CL3 ILBC_01        AACGCA                   TGCGTT
## CC1             CC1 ILBC_02        AACTCG                   CGAGTT
## SV1             SV1 ILBC_03        AACTGT                   ACAGTT
## M31Fcsw     M31Fcsw ILBC_04        AAGAGA                   TCTCTT
## M11Fcsw     M11Fcsw ILBC_05        AAGCTG                   CAGCTT
## M31Plmr     M31Plmr ILBC_07        AATCGT                   ACGATT
## M11Plmr     M11Plmr ILBC_08        ACACAC                   GTGTGT
## F21Plmr     F21Plmr ILBC_09        ACACAT                   ATGTGT
## M31Tong     M31Tong ILBC_10        ACACGA                   TCGTGT
## M11Tong     M11Tong ILBC_11        ACACGG                   CCGTGT
```

```
## LMEpi24M   LMEpi24M ILBC_13        ACACTG                  CAGTGT
## SLEpi20M   SLEpi20M ILBC_15        ACAGAG                  CTCTGT
## AQC1cm       AQC1cm ILBC_16        ACAGCA                  TGCTGT
## AQC4cm       AQC4cm ILBC_17        ACAGCT                  AGCTGT
## AQC7cm       AQC7cm ILBC_18        ACAGTG                  CACTGT
## NP2             NP2 ILBC_19        ACAGTT                  AACTGT
## NP3             NP3 ILBC_20        ACATCA                  TGATGT
## NP5             NP5 ILBC_21        ACATGA                  TCATGT
## TRRsed1     TRRsed1 ILBC_22        ACATGT                  ACATGT
## TRRsed2     TRRsed2 ILBC_23        ACATTC                  GAATGT
## TRRsed3     TRRsed3 ILBC_24        ACCACA                  TGTGGT
## TS28           TS28 ILBC_25        ACCAGA                  TCTGGT
## TS29           TS29 ILBC_26        ACCAGC                  GCTGGT
## Even1         Even1 ILBC_27        ACCGCA                  TGCGGT
## Even2         Even2 ILBC_28        ACCTCG                  CGAGGT
## Even3         Even3 ILBC_29        ACCTGT                  ACAGGT
##          Barcode_full_length         SampleType
## CL3              CTAGCGTGCGT               Soil
## CC1              CATCGACGAGT               Soil
## SV1              GTACGCACAGT               Soil
## M31Fcsw          TCGACATCTCT              Feces
## M11Fcsw          CGACTGCAGCT              Feces
## M31Plmr          CGAGTCACGAT               Skin
## M11Plmr          GCCATAGTGTG               Skin
## F21Plmr          GTAGACATGTG               Skin
## M31Tong          TGTGGCTCGTG             Tongue
## M11Tong          TAGACACCGTG             Tongue
## LMEpi24M         CATGAACAGTG         Freshwater
## SLEpi20M         AGCCGACTCTG         Freshwater
## AQC1cm           GACCACTGCTG Freshwater (creek)
## AQC4cm           CAAGCTAGCTG Freshwater (creek)
## AQC7cm           ATGAAGCACTG Freshwater (creek)
## NP2              TCGCGCAACTG              Ocean
## NP3              GCTAAGTGATG              Ocean
## NP5              GAACGATCATG              Ocean
## TRRsed1          CACGTGACATG Sediment (estuary)
## TRRsed2          TGCGCTGAATG Sediment (estuary)
## TRRsed3          GATGTATGTGG Sediment (estuary)
## TS28             GCATCGTCTGG              Feces
## TS29             CTAGTCGCTGG              Feces
## Even1            TGACTCTGCGG               Mock
## Even2            TCTGATCGAGG               Mock
## Even3            AGAGAGACAGG               Mock
##                                        Description
## CL3          Calhoun South Carolina Pine soil, pH 4.9
## CC1          Cedar Creek Minnesota, grassland, pH 6.1
## SV1        Sevilleta new Mexico, desert scrub, pH 8.3
## M31Fcsw       M3, Day 1, fecal swab, whole body study
## M11Fcsw      M1, Day 1, fecal swab, whole body study
## M31Plmr       M3, Day 1, right palm, whole body study
## M11Plmr      M1, Day 1, right palm, whole body study
## F21Plmr     F1, Day 1,  right palm, whole body study
## M31Tong          M3, Day 1, tongue, whole body study
## M11Tong          M1, Day 1, tongue, whole body study
```

```
## LMEpi24M Lake Mendota Minnesota, 24 meter epilimnion
## SLEpi20M Sparkling Lake Wisconsin, 20 meter eplimnion
## AQC1cm                   Allequash Creek, 0-1cm depth
## AQC4cm                  Allequash Creek, 3-4 cm depth
## AQC7cm                  Allequash Creek, 6-7 cm depth
## NP2            Newport Pier, CA surface water, Time 1
## NP3            Newport Pier, CA surface water, Time 2
## NP5            Newport Pier, CA surface water, Time 3
## TRRsed1                Tijuana River Reserve, depth 1
## TRRsed2                Tijuana River Reserve, depth 2
## TRRsed3                Tijuana River Reserve, depth 2
## TS28                                         Twin #1
## TS29                                         Twin #2
## Even1                                          Even1
## Even2                                          Even2
## Even3                                          Even3
```

Answer: 26

Access the OTU table from a dataset

```
otu_table(GlobalPatterns) %>%  head()
```

Since the OTU tables are very big we use the command head to read the top 6 rows of a matrix/dataframe

```
## OTU Table:          [6 taxa and 26 samples]
##                      taxa are rows
##        CL3 CC1 SV1 M31Fcsw M11Fcsw M31Plmr M11Plmr F21Plmr M31Tong M11Tong
## 549322   0   0   0       0       0       0       0       0       0       0
## 522457   0   0   0       0       0       0       0       0       0       0
## 951      0   0   0       0       0       0       1       0       0       0
## 244423   0   0   0       0       0       0       0       0       0       0
## 586076   0   0   0       0       0       0       0       0       0       0
## 246140   0   0   0       0       0       0       0       0       0       0
##        LMEpi24M SLEpi20M AQC1cm AQC4cm AQC7cm NP2 NP3 NP5 TRRsed1 TRRsed2
## 549322        0        1     27    100    130   1   0   0       0       0
## 522457        0        0      0      2      6   0   0   0       0       0
## 951           0        0      0      0      0   0   0   0       0       0
## 244423        0        0      0     22     29   0   0   0       0       0
## 586076        0        0      0      2      1   0   0   0       0       0
## 246140        0        0      0      1      3   0   0   0       0       0
##        TRRsed3 TS28 TS29 Even1 Even2 Even3
## 549322       0    0    0     0     0     0
## 522457       0    0    0     0     0     0
## 951          0    0    0     0     0     0
## 244423       0    0    0     0     0     0
## 586076       0    0    0     0     0     0
## 246140       0    0    0     0     0     0
```

```
otu_table(GlobalPatterns)[1:5, 1:5]
```

Another way to see the content of a big table

```
## OTU Table:          [5 taxa and 5 samples]
##                      taxa are rows
##        CL3 CC1 SV1 M31Fcsw M11Fcsw
## 549322   0   0   0       0       0
## 522457   0   0   0       0       0
## 951      0   0   0       0       0
## 244423   0   0   0       0       0
## 586076   0   0   0       0       0
```

```
sample_data(GlobalPatterns) %>% head()
```

```
##         X.SampleID  Primer Final_Barcode Barcode_truncated_plus_T
## CL3            CL3 ILBC_01        AACGCA                   TGCGTT
## CC1            CC1 ILBC_02        AACTCG                   CGAGTT
## SV1            SV1 ILBC_03        AACTGT                   ACAGTT
## M31Fcsw    M31Fcsw ILBC_04        AAGAGA                   TCTCTT
## M11Fcsw    M11Fcsw ILBC_05        AAGCTG                   CAGCTT
## M31Plmr    M31Plmr ILBC_07        AATCGT                   ACGATT
##         Barcode_full_length SampleType
## CL3              CTAGCGTGCGT       Soil
## CC1              CATCGACGAGT       Soil
## SV1              GTACGCACAGT       Soil
## M31Fcsw          TCGACATCTCT      Feces
## M11Fcsw          CGACTGCAGCT      Feces
## M31Plmr          CGAGTCACGAT       Skin
##                                      Description
## CL3       Calhoun South Carolina Pine soil, pH 4.9
## CC1       Cedar Creek Minnesota, grassland, pH 6.1
## SV1     Sevilleta new Mexico, desert scrub, pH 8.3
## M31Fcsw    M3, Day 1, fecal swab, whole body study
## M11Fcsw   M1, Day 1, fecal swab, whole body study
## M31Plmr    M3, Day 1, right palm, whole body study
```

Access the sample data table and the column content from a dataset

```
# To access the variables in the column 'SampleType'
# The column 'SampleType' is of class factor so get the levels using the command
sample_data(GlobalPatterns)$SampleType %>% levels()
```

```
## [1] "Feces"              "Freshwater"         "Freshwater (creek)"
## [4] "Mock"               "Ocean"              "Sediment (estuary)"
## [7] "Skin"               "Soil"               "Tongue"
```

```
sample_data(GlobalPatterns)$SampleType %>% levels() %>% as.data.frame()
```

Read with as.data.frame to see the results in a tabular format

```
##                     .
## 1               Feces
## 2          Freshwater
## 3 Freshwater (creek)
## 4                Mock
## 5               Ocean
## 6 Sediment (estuary)
## 7                Skin
## 8                Soil
## 9              Tongue
```

How many sample types are available under the SampleType column?

```
Answer: 9, "Feces", "Freshwater", "Freshwater (creek)", "Mock", "Ocean"     "Sediment (estuary)", "Skin
```

Explore the taxonomy table

```
tax_table(GlobalPatterns) %>% head() %>% DT::datatable()
```

Kingdom Phylum Class Order Family Genus Species

```
tax_table(GlobalPatterns) %>% head()
```

```
## Taxonomy Table:     [6 taxa by 7 taxonomic ranks]:
##        Kingdom   Phylum          Class          Order          Family
## 549322 "Archaea" "Crenarchaeota" "Thermoprotei" NA             NA
## 522457 "Archaea" "Crenarchaeota" "Thermoprotei" NA             NA
## 951    "Archaea" "Crenarchaeota" "Thermoprotei" "Sulfolobales" "Sulfolobaceae"
## 244423 "Archaea" "Crenarchaeota" "Sd-NA"        NA             NA
## 586076 "Archaea" "Crenarchaeota" "Sd-NA"        NA             NA
## 246140 "Archaea" "Crenarchaeota" "Sd-NA"        NA             NA
##        Genus        Species
## 549322 NA           NA
## 522457 NA           NA
## 951    "Sulfolobus" "Sulfolobusacidocaldarius"
## 244423 NA           NA
## 586076 NA           NA
## 246140 NA           NA
```

2. Importing data in R as phyloseq object

We will combine a phyloseq object using otu_table, sample_data and taxonomy file. Will will read these three file and then combine them to make a phyloseq object to work with them,

```
#data_dir =
```

OTU table

```
# To import otus column as rownames, as required by phyloseq
GP_sp_tutorial_otu_table = read.table("./tutorial_files/GP_sp_tutorial_otu_table_df.csv",
           sep = "\t",
           header =  T,
           row.names = "otus")
GP_sp_tutorial_otu_table[1:5, 1:5]
```

```
##        M31Plmr M11Plmr F21Plmr M31Tong M11Tong
## 951          0       1       0       0       0
## 155495       0       0       0       0       0
## 1029         0       0       0       0       0
## 341551       0       0       0       0       0
## 108964       0       2       6       1       0
```

Sample data

```
# Import with sampleid column as rownames, as required by phyloseq
GP_sp_tutorial_sample_data = read.table("./tutorial_files/GP_sp_tutorial_sample_data_df.csv",
           sep = "\t", header =  T,
           row.names = "sampleid")
GP_sp_tutorial_sample_data %>% head()
```

```
##          SampleOrigin X.SampleID  Primer Final_Barcode Barcode_truncated_plus_T
## M31Plmr         Human    M31Plmr ILBC_07        AATCGT                   ACGATT
## M11Plmr         Human    M11Plmr ILBC_08        ACACAC                   GTGTGT
## F21Plmr         Human    F21Plmr ILBC_09        ACACAT                   ATGTGT
## M31Tong         Human    M31Tong ILBC_10        ACACGA                   TCGTGT
## M11Tong         Human    M11Tong ILBC_11        ACACGG                   CCGTGT
## LMEpi24M   Freshwater   LMEpi24M ILBC_13        ACACTG                   CAGTGT
##          Barcode_full_length SampleType
## M31Plmr          CGAGTCACGAT       Skin
## M11Plmr          GCCATAGTGTG       Skin
## F21Plmr          GTAGACATGTG       Skin
## M31Tong          TGTGGCTCGTG     Tongue
## M11Tong          TAGACACCGTG     Tongue
## LMEpi24M         CATGAACAGTG Freshwater
##                                        Description
## M31Plmr       M3, Day 1, right palm, whole body study
## M11Plmr      M1, Day 1, right palm, whole body study
## F21Plmr     F1, Day 1,  right palm, whole body study
## M31Tong          M3, Day 1, tongue, whole body study
## M11Tong          M1, Day 1, tongue, whole body study
## LMEpi24M Lake Mendota Minnesota, 24 meter epilimnion
```

Taxonomy table

```
# To import otus column as rownames, as required by phyloseq
GP_sp_tutorial_tax_table = read.table("./tutorial_files/GP_sp_tutorial_tax_table_df.csv",
           sep = "\t",
           header =  T,
           row.names = "otus")
GP_sp_tutorial_tax_table %>% head()
```

```
##        Kingdom        Phylum          Class         Order         Family
## 951    Archaea Crenarchaeota   Thermoprotei  Sulfolobales  Sulfolobaceae
## 155495 Archaea Crenarchaeota Thaumarchaeota Cenarchaeales Cenarchaeaceae
## 1029   Archaea Crenarchaeota Thaumarchaeota Cenarchaeales Cenarchaeaceae
## 341551 Archaea Crenarchaeota Thaumarchaeota Cenarchaeales Cenarchaeaceae
## 108964 Archaea Crenarchaeota Thaumarchaeota Cenarchaeales Cenarchaeaceae
## 330416 Archaea Crenarchaeota Thaumarchaeota Cenarchaeales Cenarchaeaceae
##                 Genus                  Species
## 951        Sulfolobus Sulfolobusacidocaldarius
## 155495    Cenarchaeum     Cenarchaeumsymbiosum
## 1029      Cenarchaeum     Cenarchaeumsymbiosum
## 341551 Nitrosopumilus                   pIVWA5
## 108964 Nitrosopumilus                   pIVWA5
## 330416 Nitrosopumilus                   pIVWA5
```

```
my_OTU_table = otu_table(GP_sp_tutorial_otu_table, taxa_are_rows = TRUE)
my_OTU_table %>% head()
```

In order to read the OTU table as phyloseq object we need to use the following command

```
## OTU Table:          [6 taxa and 10 samples]
##                      taxa are rows
##        M31Plmr M11Plmr F21Plmr M31Tong M11Tong LMEpi24M SLEpi20M AQC1cm AQC4cm
## 951          0       1       0       0       0        0        0      0      0
## 155495       0       0       0       0       0        0        0      0      0
## 1029         0       0       0       0       0        0        0      0      0
## 341551       0       0       0       0       0        0        0      0      0
## 108964       0       2       6       1       0        1        0      1      0
## 330416       0       0       0       0       0        0        0      0      0
##        AQC7cm
## 951         0
## 155495      0
## 1029        0
## 341551      0
## 108964      1
## 330416      0
```

```
my_Sample_data = sample_data(GP_sp_tutorial_sample_data)
my_Sample_data %>% head()
```

Similarly to read sample data and taxonomy table as phyloseq objects

```
##          SampleOrigin X.SampleID  Primer Final_Barcode Barcode_truncated_plus_T
## M31Plmr         Human    M31Plmr ILBC_07        AATCGT                    ACGATT
## M11Plmr         Human    M11Plmr ILBC_08        ACACAC                    GTGTGT
## F21Plmr         Human    F21Plmr ILBC_09        ACACAT                    ATGTGT
## M31Tong         Human    M31Tong ILBC_10        ACACGA                    TCGTGT
## M11Tong         Human    M11Tong ILBC_11        ACACGG                    CCGTGT
## LMEpi24M   Freshwater   LMEpi24M ILBC_13        ACACTG                    CAGTGT
##          Barcode_full_length SampleType
## M31Plmr          CGAGTCACGAT       Skin
## M11Plmr          GCCATAGTGTG       Skin
## F21Plmr          GTAGACATGTG       Skin
## M31Tong          TGTGGCTCGTG     Tongue
## M11Tong          TAGACACCGTG     Tongue
## LMEpi24M         CATGAACAGTG Freshwater
##                                        Description
## M31Plmr       M3, Day 1, right palm, whole body study
## M11Plmr      M1, Day 1, right palm, whole body study
## F21Plmr     F1, Day 1,  right palm, whole body study
## M31Tong          M3, Day 1, tongue, whole body study
## M11Tong          M1, Day 1, tongue, whole body study
## LMEpi24M Lake Mendota Minnesota, 24 meter epilimnion
```

```
# the taxonomy table is required in matrix format
my_tax_table = tax_table(as.matrix(GP_sp_tutorial_tax_table))
my_tax_table %>% head()
```

Reading taxonomy file as phyloseq object

```
## Taxonomy Table:     [6 taxa by 7 taxonomic ranks]:
##        Kingdom   Phylum          Class            Order
## 951    "Archaea" "Crenarchaeota" "Thermoprotei"   "Sulfolobales"
## 155495 "Archaea" "Crenarchaeota" "Thaumarchaeota" "Cenarchaeales"
## 1029   "Archaea" "Crenarchaeota" "Thaumarchaeota" "Cenarchaeales"
## 341551 "Archaea" "Crenarchaeota" "Thaumarchaeota" "Cenarchaeales"
## 108964 "Archaea" "Crenarchaeota" "Thaumarchaeota" "Cenarchaeales"
## 330416 "Archaea" "Crenarchaeota" "Thaumarchaeota" "Cenarchaeales"
##        Family           Genus            Species
## 951    "Sulfolobaceae"  "Sulfolobus"     "Sulfolobusacidocaldarius"
## 155495 "Cenarchaeaceae" "Cenarchaeum"    "Cenarchaeumsymbiosum"
## 1029   "Cenarchaeaceae" "Cenarchaeum"    "Cenarchaeumsymbiosum"
## 341551 "Cenarchaeaceae" "Nitrosopumilus" "pIVWA5"
## 108964 "Cenarchaeaceae" "Nitrosopumilus" "pIVWA5"
## 330416 "Cenarchaeaceae" "Nitrosopumilus" "pIVWA5"
```

Combine to make a phyloseq object.

```
my_physeq = phyloseq(my_OTU_table, my_Sample_data, my_tax_table)
my_physeq
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 1413 taxa and 10 samples ]
## sample_data() Sample Data:       [ 10 samples by 8 sample variables ]
## tax_table()   Taxonomy Table:    [ 1413 taxa by 7 taxonomic ranks ]
```

'my_physeq' is now a new phyloseq object which contains the data (OTU table, sample data, and taxonomy table) that we just imported.

```
sample_data(my_physeq)$SampleType %>% as.factor %>% levels()
```

Explore the categories in a sample variable eg SampleType

```
## [1] "Freshwater"         "Freshwater (creek)" "Skin"
## [4] "Tongue"
```

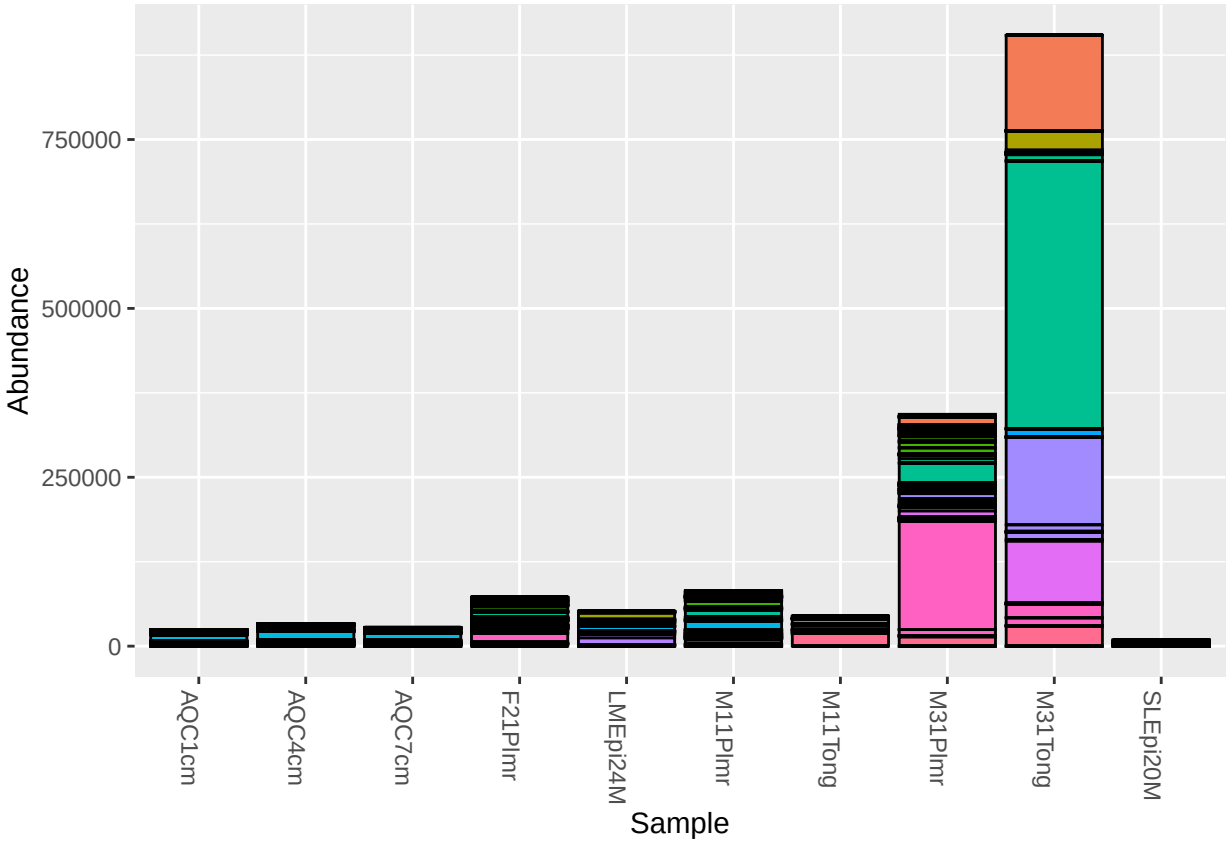
```
sample_data(my_physeq)$SampleOrigin %>% as.factor %>% levels()
```

```
## [1] "Freshwater" "Human"
```

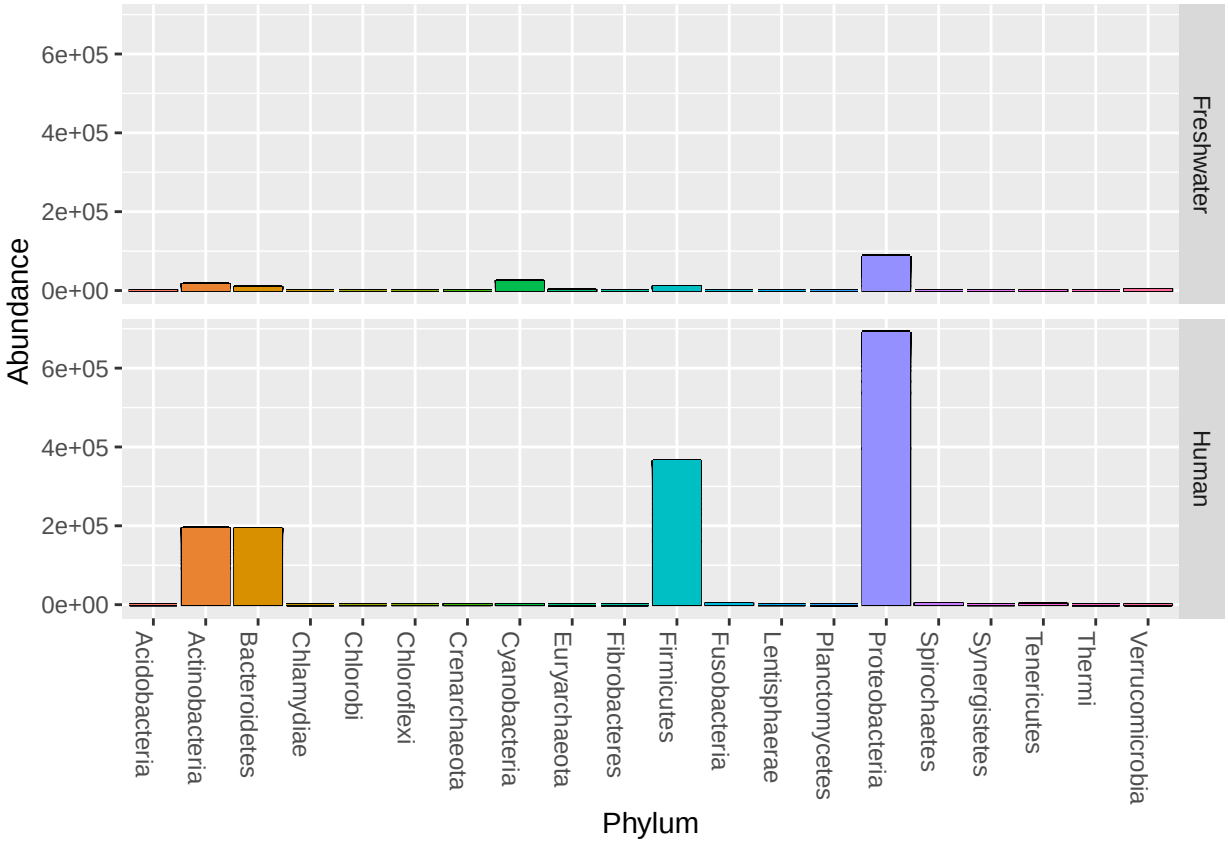
3. Data visualization and formatting

Basic plot

```
p = plot_bar(my_physeq, fill = "Species")
p + theme(legend.position="none")
```




```
p = plot_bar(my_physeq, x= "Phylum", fill = "Phylum", facet_grid=SampleOrigin~.)
p + theme(legend.position="none") + geom_bar(stat = "identity")
```



Relative abundance and filtering

Some of the analysis provide better results when we work with relative abundance data (frequency table for OTU numbers). To avoid the spurious results, we can also filter some taxa which have very low abundance.

```
# To convert to relative abundance
my_physeq_r  = transform_sample_counts(my_physeq, function(x) x / sum(x) )
# Keep the taxa which have a mean values at least 1e-5
my_physeq_rf = filter_taxa(my_physeq_r, function(x) mean(x) > 1e-5, TRUE)
my_physeq_rf
```

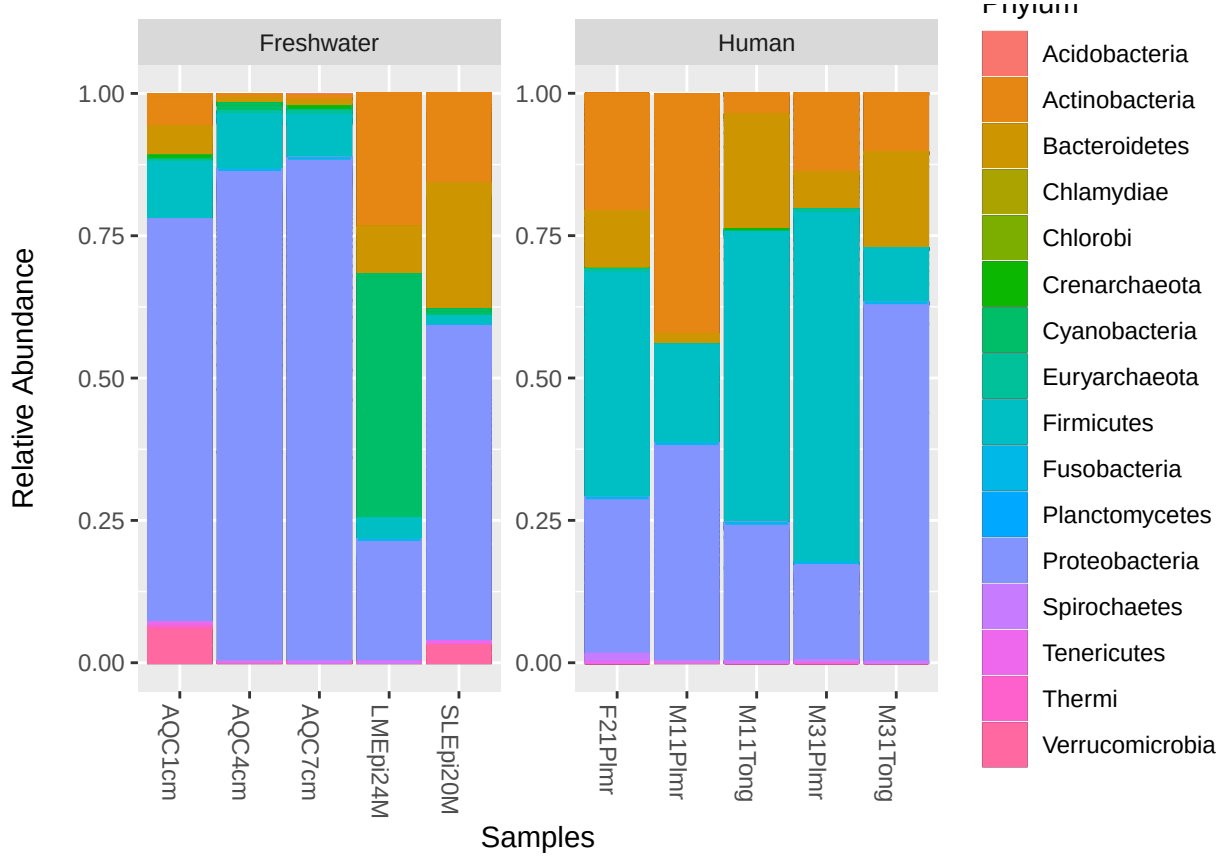
```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 619 taxa and 10 samples ]
## sample_data() Sample Data:       [ 10 samples by 8 sample variables ]
## tax_table()   Taxonomy Table:    [ 619 taxa by 7 taxonomic ranks ]
```

Now the number of remaining taxa after filtering low abundance taxa is 511 out of 1413 in the full dataset.

Plot and compare the relative abundance

Notice the taxa abundant in only one group.

```
phyloseq::plot_bar(my_physeq_rf , fill = "Phylum") +
  geom_bar(aes(color = Phylum, fill = Phylum), stat = "identity", position = "stack") +
  labs(x = "Samples", y = "Relative Abundance\n") +
  facet_wrap(~ SampleOrigin, scales = "free")
```



An example of how to subset the data based on a condition

Here we will remove all the taxa which have "NA" in the Species column. i.e. Species is unknown for these OTUs

```
GP_subset_1 = subset_taxa(GlobalPatterns, Species!="NA")
```

```
## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'

## Also defined by 'tidytree'

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## Found more than one class "phylo" in cache; using the first, from namespace 'phyloseq'

## Also defined by 'tidytree'
```

```
GP_subset_1
```

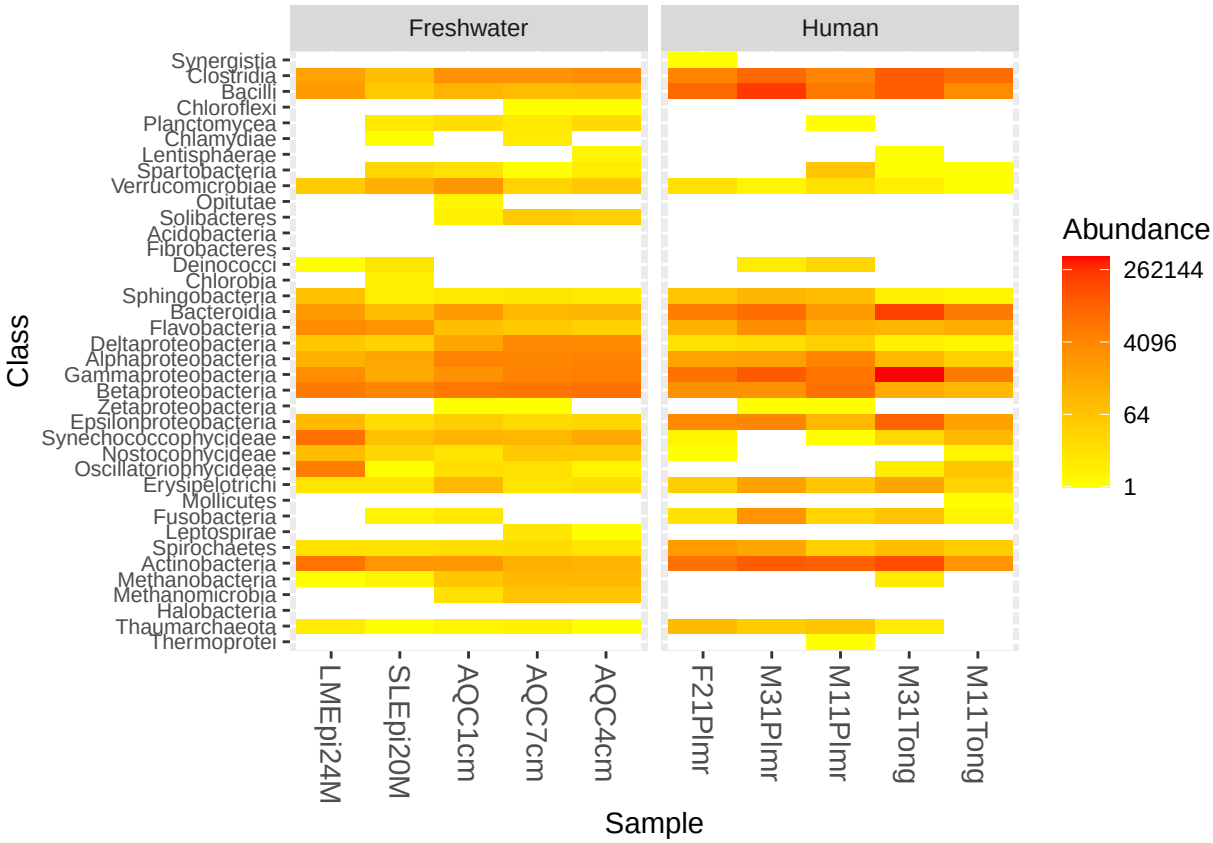
```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 1413 taxa and 26 samples ]
## sample_data() Sample Data:       [ 26 samples by 7 sample variables ]
## tax_table()   Taxonomy Table:    [ 1413 taxa by 7 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 1413 tips and 1412 internal nodes ]
```

Agglomerate taxa at Class level (required by plot_heatmap option)

```
my_physeq_glom = tax_glom(my_physeq, taxrank="Class")
```

```
phyloseq::plot_heatmap(my_physeq_glom, low = "yellow", high = "red", na.value = "white",taxa.label = "C
  facet_grid(~SampleOrigin, scales = "free_x")
```

```
## Warning: Transformation introduced infinite values in discrete y-axis
```



4. Hierarchical clustering

```
#Extract OTU table as data frame
my_physeq_otu_df = phyloseq::otu_table(my_physeq) %>%  data.frame()
my_physeq_otu_df[1:5, 1:5]
```

```
##        M31Plmr M11Plmr F21Plmr M31Tong M11Tong
## 951          0       1       0       0       0
## 155495       0       0       0       0       0
## 1029         0       0       0       0       0
## 341551       0       0       0       0       0
## 108964       0       2       6       1       0
```

```
# transpose the table (required by vegdist)
my_physeq_otu_df_t = t(my_physeq_otu_df)
my_physeq_otu_df_t[1:5, 1:5]
```

```
##         951 155495 1029 341551 108964
## M31Plmr   0      0    0      0      0
## M11Plmr   1      0    0      0      2
## F21Plmr   0      0    0      0      6
## M31Tong   0      0    0      0      1
## M11Tong   0      0    0      0      0
```

```
#compute Bray-Curtis dissimilarity
bc_dist = vegan::vegdist(my_physeq_otu_df_t, method = "bray")
bc_dist
```

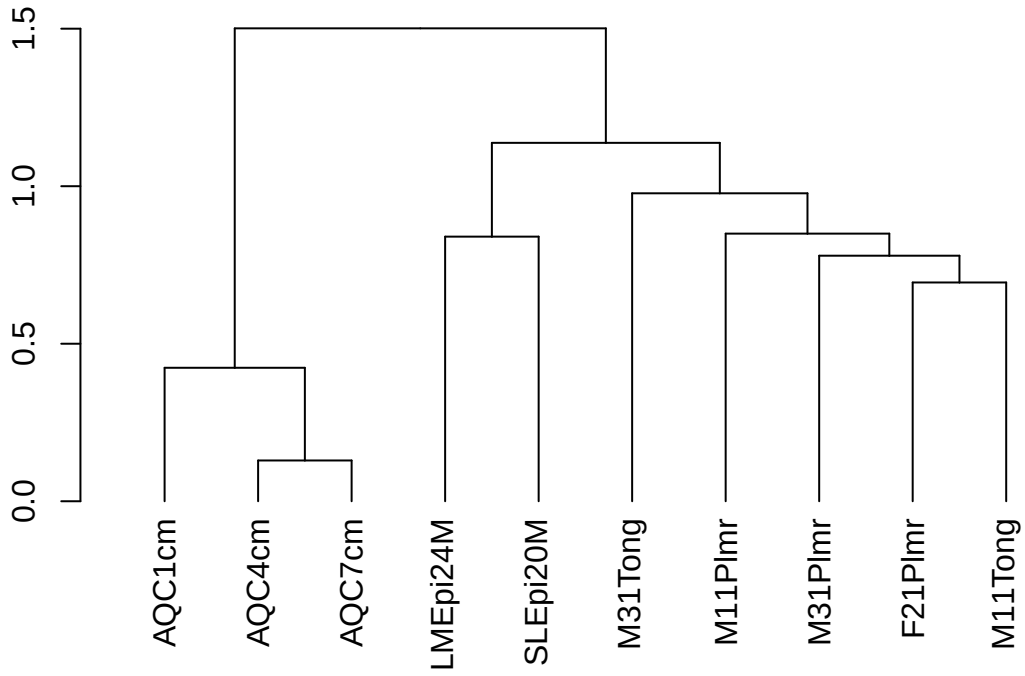
```
##            M31Plmr   M11Plmr   F21Plmr   M31Tong   M11Tong  LMEpi24M  SLEpi20M
## M11Plmr  0.8201904
## F21Plmr  0.7088825 0.7171987
## M31Tong  0.8100538 0.9747491 0.9275711
## M11Tong  0.8061918 0.8950000 0.6944554 0.9097781
## LMEpi24M 0.9651206 0.9228413 0.8950359 0.9863850 0.8614094
## SLEpi20M 0.9903308 0.9559947 0.9673560 0.9984609 0.9663373 0.8399396
## AQC1cm   0.9885630 0.9196260 0.9576297 0.9951015 0.9537315 0.9318942 0.8449668
## AQC4cm   0.9922834 0.9462591 0.9733061 0.9976856 0.9738446 0.9451736 0.8869454
## AQC7cm   0.9919732 0.9472929 0.9722075 0.9978891 0.9716969 0.9485904 0.8737116
##             AQC1cm    AQC4cm
## M11Plmr
## F21Plmr
## M31Tong
## M11Tong
## LMEpi24M
## SLEpi20M
## AQC1cm
## AQC4cm   0.3939152
## AQC7cm   0.3498352 0.1294176
```

```
# View the distance matrix as matrix
as.matrix(bc_dist)[1:5, 1:5]
```

```
##           M31Plmr   M11Plmr   F21Plmr   M31Tong   M11Tong
## M31Plmr 0.0000000 0.8201904 0.7088825 0.8100538 0.8061918
## M11Plmr 0.8201904 0.0000000 0.7171987 0.9747491 0.8950000
## F21Plmr 0.7088825 0.7171987 0.0000000 0.9275711 0.6944554
## M31Tong 0.8100538 0.9747491 0.9275711 0.0000000 0.9097781
## M11Tong 0.8061918 0.8950000 0.6944554 0.9097781 0.0000000
```

The distance table records the Bray_Curtis distance between all samples. For example, the distance between M31Plmr and M11Plmr is 0.8201904. Next this matrix will be used for plotting the dendrogram. 'hclust' is the command for hierarchical clustering on distance matrix

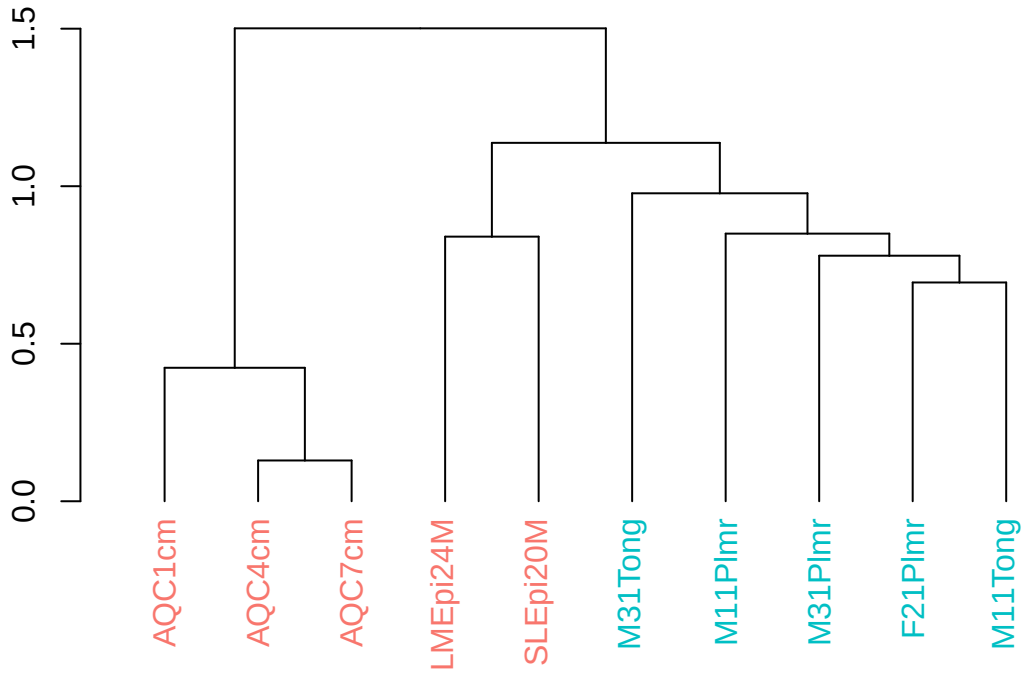
```
#Save as dendrogram
ward = as.dendrogram(hclust(bc_dist, method = "ward.D2"))

#Plot
plot(ward)
```



A nicer dendrogram plot with color coding

Below is the code to color the samples based on their category in SampleOrigin.

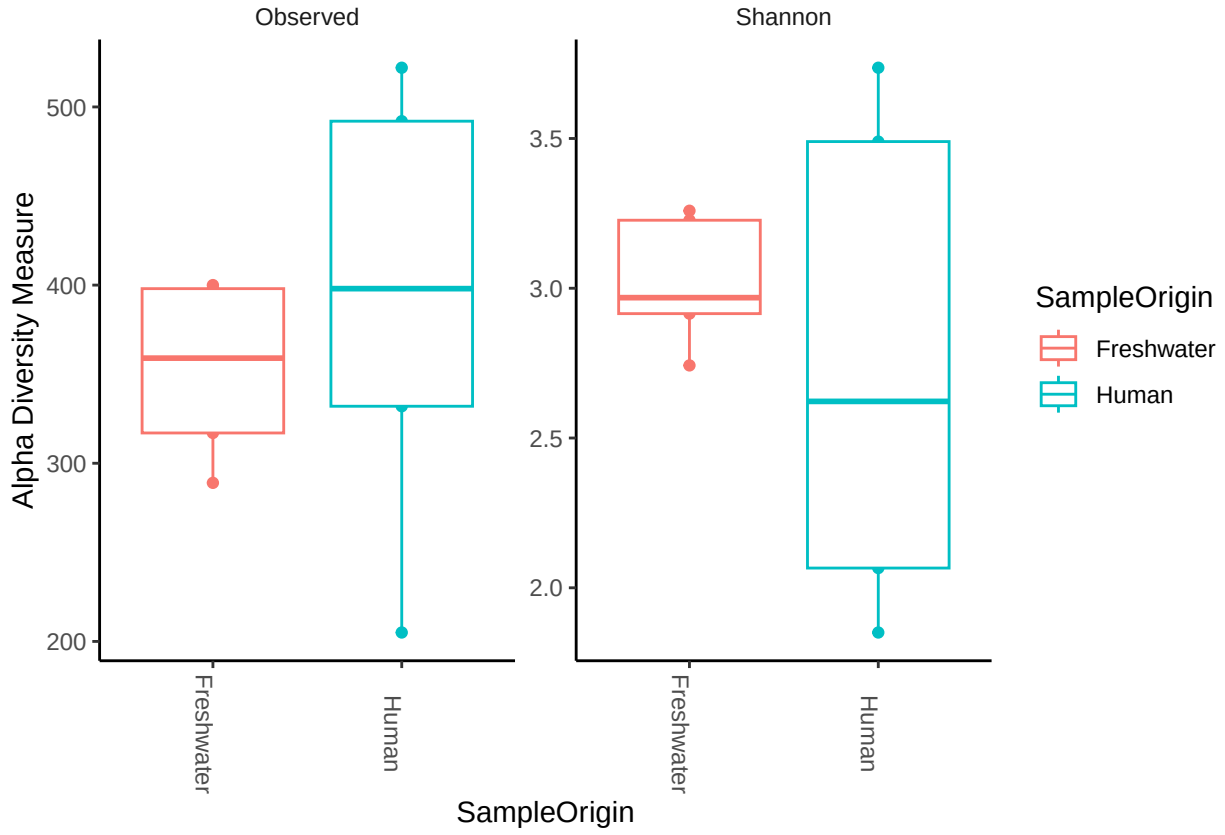
```
#Provide color codes
meta = data.frame(phyloseq::sample_data(my_physeq))
colorCode = c(`Freshwater` = "#F8766D", Human = "#00BFC4" )
labels_colors(ward) = colorCode[meta$SampleOrigin][order.dendrogram(ward)]
#Plot
plot(ward)
```



5. Alpha diversity

Now we will plot for alpha diversity using two measures, c("Observed", "Shannon"),

```
plot_richness(my_physeq, x="SampleOrigin", measures=c("Observed", "Shannon"), color = "SampleOrigin") +
  geom_boxplot() +
  theme_classic() +
  theme(strip.background = element_blank(), axis.text.x.bottom = element_text(angle = -90))
```



Identifying the level of significance for the diversity between Feces and Freshwater

```
# Make a dataframe to combine the ouputs of Observed, Shannon and SampleOrigin
my_alph_div = data.frame(
  "Observed" = phyloseq::estimate_richness(my_physeq, measures = "Observed"),
  "Shannon" = phyloseq::estimate_richness(my_physeq, measures = "Shannon"),
  "SampleOrigin" = phyloseq::sample_data(my_physeq)$SampleOrigin)
head(my_alph_div)
```

```
##          Observed  Shannon SampleOrigin
## M31Plmr       492 2.622235        Human
## M11Plmr       522 3.736234        Human
## F21Plmr       398 3.489691        Human
## M31Tong       332 1.850676        Human
## M11Tong       205 2.065849        Human
## LMEpi24M      317 2.742431   Freshwater
```

Check the level of significance

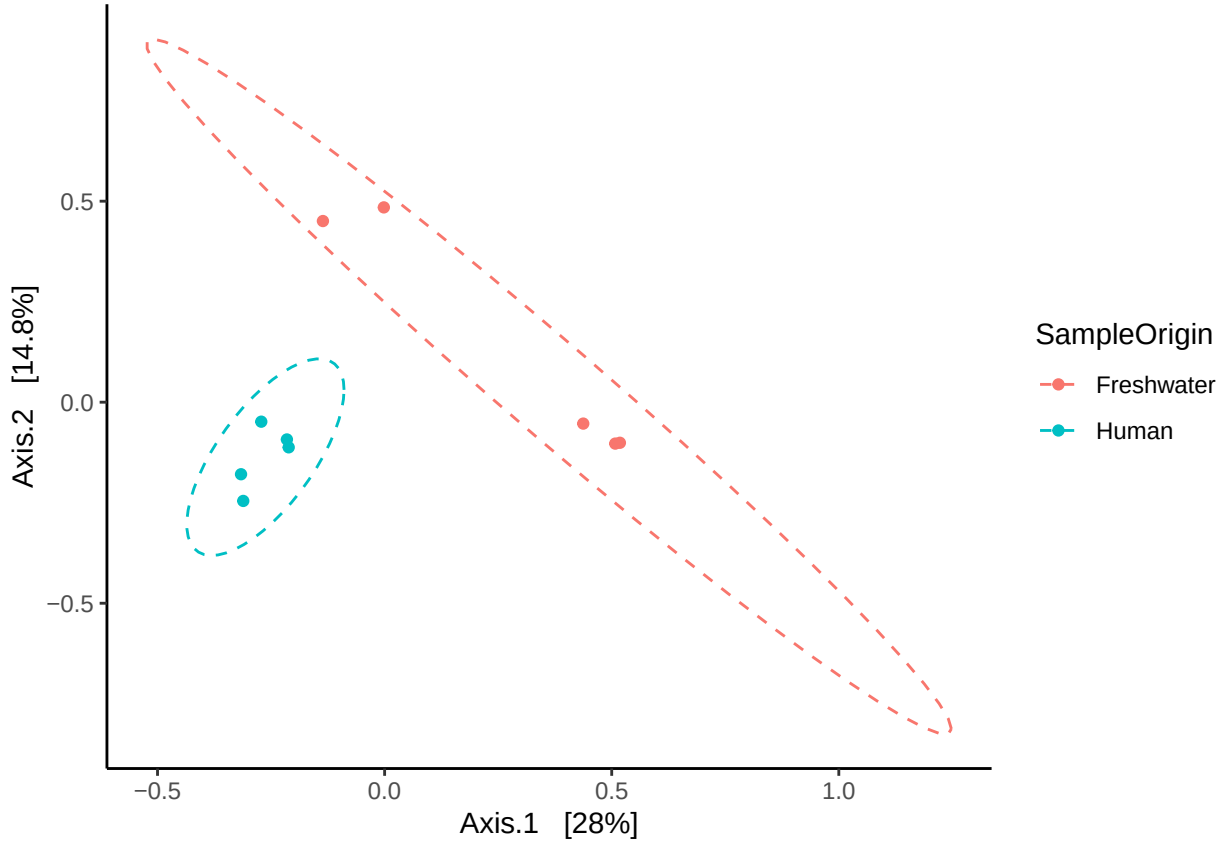
```
#Wilcoxon test for Shannon diversity for categories in SampleOrigin
my_alph_div_wt = wilcox.test(Shannon ~ SampleOrigin, data = my_alph_div, exact = FALSE, conf.int = TRUE)
print(my_alph_div_wt$p.value)
```

```
## [1] 0.6761033
```

p-value = 0.6761. Here the p-value is more than 0.05 If the p-value in the output is less than 0.05, it means the difference is significant. In this case we can **NOT** accept the alternative hypothesis which means that the diversity is **NOT** significantly different between Freshwater and Human samples.

6. Beta diversity

```
ordination = ordinate(my_physeq, method="PCoA", distance="jaccard")
plot_ordination(my_physeq, ordination, color="SampleOrigin") +
  theme_classic() +
  theme(strip.background = element_blank()) +
  stat_ellipse(linetype = 2)
```



It shows that the between sample diversity is very high between Freshwater and very low between Feces samples

7. Differential abundance (DA) analysis using deseq2

```
set.seed(2345)
# run_deseq2 command run the program deseq2 to identify DA taxa
# Running this command takes a few seconds
my_physeq_deseq2 = run_deseq2(my_physeq,
                              group = "SampleOrigin",
                              transform = "log10p", # log transformation
                              norm = "rarefy",  # common method for normalization
                              p_adjust = "BH", # adjusted p-value methods
                              )
```

```
## You set `rngseed` to FALSE. Make sure you've set & recorded
##  the random seed of your session for reproducibility.
## See `?set.seed`
```

```
## ...
```

```
## 461OTUs were removed because they are no longer
## present in any sample after random subsampling
```

```
## ...
```

```
## converting counts to integer mode
```

```
## -- note: fitType='parametric', but the dispersion trend was not well captured by the
##    function: y = a/x + b, and a local regression fit was automatically substituted.
##    specify fitType='local' or 'mean' to avoid this message next time.
```

```
my_physeq_deseq2
```

```
## microbiomeMarker-class inherited from phyloseq-class
## normalization method:              [ RLE ]
## microbiome marker identity method: [ DESeq2: Wald ]
## marker_table() Marker Table:       [ 16 microbiome markers with 5 variables ]
## otu_table()    OTU Table:          [ 959 taxa and  10 samples ]
## sample_data()  Sample Data:        [ 10 samples by  8 sample variables ]
## tax_table()    Taxonomy Table:     [ 959 taxa by 1 taxonomic ranks ]
```

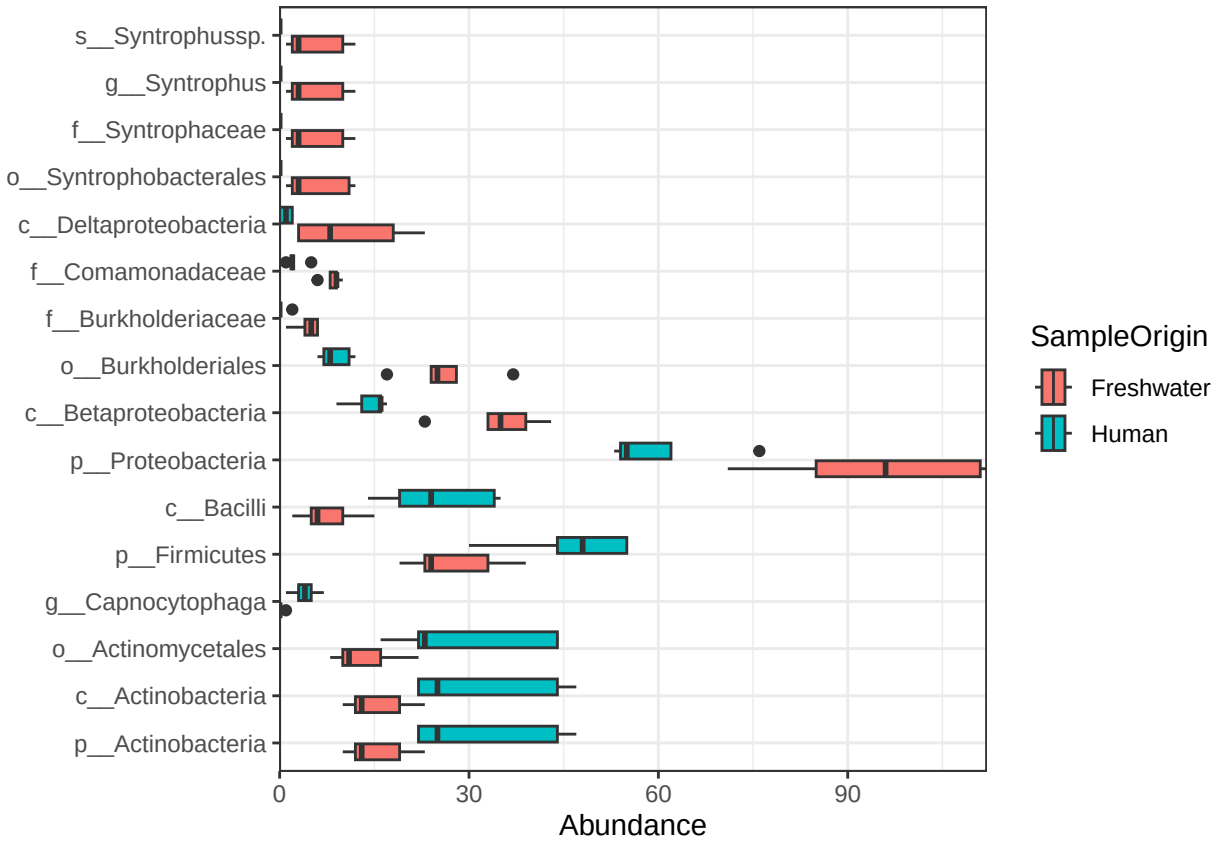
Plot the differentially abundant taxa identified by deseq2 method

```
marker_table(my_physeq_deseq2) %>% head()
```

```
##
## marker1                                                                    k__Bacteria|p__
## marker2                                                  k__Bacteria|p__Proteobacteria|c__Beta
## marker3                                      k__Bacteria|p__Proteobacteria|c__Betaproteobacteria|o__Bu
## marker4                                  k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Syntr
## marker5               k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Syntrophobacterales|f_
## marker6 k__Bacteria|p__Proteobacteria|c__Deltaproteobacteria|o__Syntrophobacterales|f__Syntrophaceae
```

```
##         enrich_group   ef_logFC       pvalue        padj
## marker1   Freshwater -0.6629649 4.009616e-05 0.001116965
## marker2   Freshwater -1.2848808 3.611545e-05 0.001116965
## marker3   Freshwater -1.5739909 3.313312e-05 0.001116965
## marker4   Freshwater -4.9787402 2.470348e-05 0.001116965
## marker5   Freshwater -4.9281142 3.137184e-05 0.001116965
## marker6   Freshwater -4.9281142 3.137184e-05 0.001116965
```

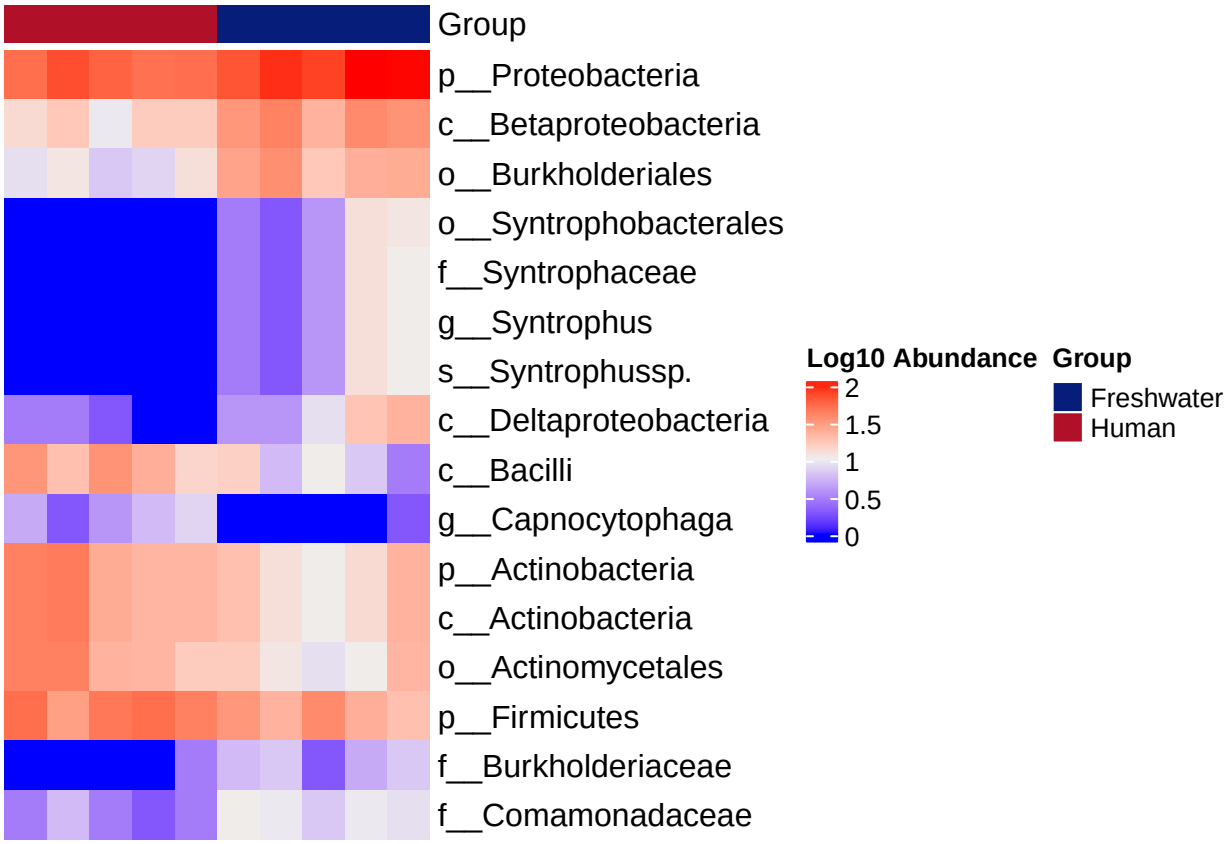
```
plot_DA =  microbiomeMarker::plot_abundance(my_physeq_deseq2, group = "SampleOrigin")
plot_DA
```




```
plot_DA_hmap = microbiomeMarker::plot_heatmap(my_physeq_deseq2, group = "SampleOrigin")
```

```
## Warning in transform_log10(otu): OTU table contains zeroes. Using log10(1 + x)
## instead.
```

```
plot_DA_hmap
```




```
#otu_table(my_physeq_deseq2) %>% head()
```