Phyloseq tutorial

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Contents

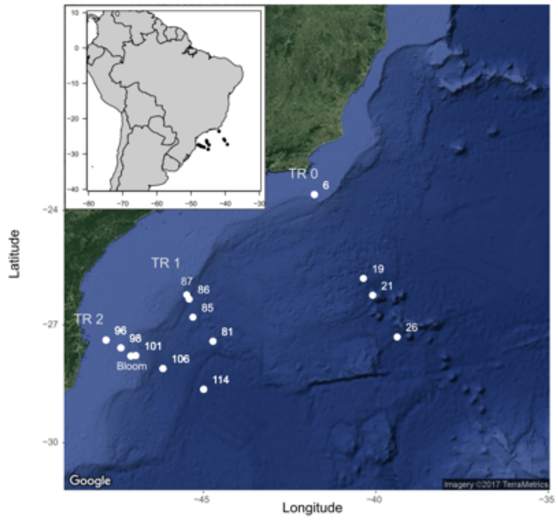
1 Aim

This document explains the use of the phyloseq R library to analyze metabarcoding data.

1.1 Phyloseq R library

- Phyloseq web site : https://joey711.github.io/phyloseq/index.html
- See in particular tutorials for
  - importing data: https://joey711.github.io/phyloseq/import-data.html
  - heat maps: https://joey711.github.io/phyloseq/plot_heatmap-examples.html

1.2 Data



This tutorial uses a reduced metabarcoding dataset obtained by C. Ribeiro and A. Lopes dos Santos. This dataset originates from the CARBOM cruise in 2013 off Brazil and corresponds to the 18S V4 region amplified on flow cytometry sorted samples (see pptx file for details) and sequenced on an Illumina run 2*250 bp analyzed with mothur.

1.3 References for data

- Gérikas Ribeiro, C., Lopes dos Santos, A., Marie, D., Helena Pellizari, V., Pereira Brandini, F., and Vaulot, D. (2016). Pico and nanoplankton abundance and carbon stocks along the Brazilian Bight. PeerJ 4, e2587. doi:10.7717/peerj.2587.
- Gérikas Ribeiro, C., Marie, D., Lopes dos Santos, A., Pereira Brandini, F., and Vaulot, D. (2016). Estimating microbial populations by flow cytometry: Comparison between instruments. Limnol. Oceanogr. Methods 14, 750–758. doi:10.1002/lom3.10135.
- Gérikas Ribeiro C, Lopes dos Santos A, Marie D, Brandini P, Vaulot D. (2018). Relationships between photosynthetic eukaryotes and nitrogen-fixing cyanobacteria off Brazil. ISME J in press.

1.4 To be added

- Exercices

2 Prerequisites to be installed

- R : https://pbil.univ-lyon1.fr/CRAN/
- R studio : https://www.rstudio.com/products/rstudio/download/#download
- Download and install the following libraries by running under R studio the following lines

```
install.packages("dplyr")      # To manipulate dataframes
install.packages("readxl")     # To read Excel files into R

install.packages("ggplot2")    # for high quality graphics
```

```r
source("https://bioconductor.org/biocLite.R")
biocLite("phyloseq")
```

3 Script description

- Load the script file : **Phyloseq_tutorial.R**

3.1 Load necessary libraries

```r
library("phyloseq")
library("ggplot2")      # graphics
library("readxl")       # necessary to import the data from Excel file
library("dplyr")        # filter and reformat data frames
```

```
##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

3.2 Read the data and create phyloseq objects

Three tables are needed

- OTU
- Taxonomy
- Samples

They are read from a single Excel file where each sheet contains one of the tables

OTU table

otu	X10n	X10p	X11n	X11p	X120n	X120p	X121n	X121p	X122n	X122p	X125n	X125p	X126n	X126p	X127n	X13n	X13p	X140n	X140p	X141n
Otu001	13679	6292	42	2500	18850	5	43	7138	9432	10541	9	9772	1388	7	31538	38	2338	23	9	1358
Otu002	18	7134	38	9830	45	61420	182	23751	36	11	4535	3502	11018	5473	26	14411	38	19018	12	3080
Otu003	9939	8983	31	13	24620	19	19	16	12502	3831	4621	2240	9924	4052	9292	18	0	37	7	3680
Otu004	3675	4234	24	22	11	16	32967	35	6	18	6908	5	16	8702	24	11	37717	0	25	4196
Otu005	0	5	0	7	0	8	0	16	20166	0	0	2	5	8	2	16	0	13	0	0
Otu006	0	8	0	0	0	8	0	0	5	3	3	0	0	9	0	5	4	0	0	3
Otu007	4587	518	4	386	8775	5	6	1102	14336	0	0	3626	51	0	6	12	0	10	0	395
Otu008	1	8	2	4408	3	29	6	12355	0	0	0	0	0	9	3	1588	0	6	3	3
Otu009	115	914	3	325	0	629	1	834	5	0	1354	2108	1117	67	0	2010	1897	11227	1	3
Otu010	780	8	23810	12	3279	0	12	7	3027	0	2	4156	0	0	18	0	0	0	0	0
Otu011	0	3	2	2	0	13	5	5	4	7	3081	11	4	6804	0	3	11	0	5	0
Otu012	0	0	0	6	0	0	0	16	3	0	0	0	0	0	0	17	0	6	0	0
Otu013	6321	2471	2	0	12	3	0	0	4	20272	0	15	9	0	5	0	11	0	14	0
Otu014	0	82	4	3304	1	1667	4	9233	13	3	0	2707	0	0	3	4806	9	3	5	0
Otu015	0	12	0	3	7	25	1	6	10	0	4	2772	1	3	0	2	0	10	13	8052
Otu016	1	0	0	9	5	0	0	14	0	0	0	0	2654	0	0	6	1	1	0	0
Otu017	0	0	0	0	0	0	0	0	17	8	0	0	0	0	0	17	24	48	35210	4
Otu018	1	0	9	911	0	0	15	2702	6	4	342	2217	606	0	13	3846	4	6	8513	1
Otu019	0	0	13	0	0	0	29	0	0	0	0	0	0	0	11	0	0	5	4	0
Otu020	425	0	1	0	1706	0	8447	1	0	0	0	0	0	26	0	0	3490	0	2620	0
Otu021	0	4	0	0	0	10	0	0	0	0	2	0	0	4	0	0	0	0	0	4
Otu022	0	0	0	4987	0	0	0	6	90	1	1	524	0	467	0	4	8	6198	0	1
Otu023	4	0	1	0	0	3	0	0	0	0	0	0	3351	3	0	3910	1	2	3	0
Otu024	0	0	0	0	0	0	0	0	0	0	0	17	0	0	0	0	0	2	1	0
Otu025	69	0	0	0	290	0	0	0	21	0	118	2	9	513	2	0	0	2	0	0
Otu026	0	2	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Otu027	6	2304	0	0	5	0	0	0	57	4	0	14529	9597	2	6	0	0	0	0	0

Taxon table

otu	Domain	Supergroup	Division	Class	Order	Family	Genus
Otu001	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Bathycoccaceae	Ostreococcus
Otu002	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Prymnesiophyceae_X	Braarudosphaeraceae	UCYN_A1_host
Otu003	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Bathycoccaceae	Bathycoccus
Otu004	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Prorocentrum
Otu005	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Mediophyceae	Mediophyceae_X	Thalassiosira
Otu006	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Bacillariophyceae	Bacillariophyceae_X	Pseudo_nitzschia
Otu007	Eukaryota	Stramenopiles	Ochrophyta	Pelagophyceae	Pelagophyceae_X	Pelagophyceae_X	Pelagomonas
Otu008	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Dinophyceae_X
Otu009	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Prymnesiales	Chrysochromulinaceae	Chrysochromulina
Otu010	Eukaryota	Opisthokonta	Metazoa	Craniata	Craniata_X	Craniata_XX	Craniata_XX_unclassified
Otu011	Eukaryota	Stramenopiles	Ochrophyta	Chrysophyceae	Chrysophyceae_X	Chrysophyceae_Clade_C	Chrysophyceae_Clade_C_X
Otu012	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Gonyaulax
Otu013	Eukaryota	Alveolata	Dinophyta	Syndiniales	Syndiniales_Group_III	Syndiniales_Group_III_X	Syndiniales_Group_III_X
Otu014	Eukaryota	Stramenopiles	Ochrophyta	Chrysophyceae	Chrysophyceae_X	Chrysophyceae_Clade_G	Chrysophyceae_Clade_G_X
Otu015	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Dinophyceae_X
Otu016	Eukaryota	Hacrobia	Centroheliozoa	Centroheliozoa_X	Pterocystida	Pterocystida_X	Pterocystida_X
Otu017	Eukaryota	Opisthokonta	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Hyphodontia
Otu018	Eukaryota	Stramenopiles	Ochrophyta	Dictyochophyceae	Dictyochophyceae_X	Pedinellales	Pedinellales_X
Otu019	Eukaryota	Opisthokonta	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Itersonilia
Otu020	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Prymnesiophyceae_X	Braarudosphaeraceae	Braarudosphaera
Otu021	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Dinophyceae_X
Otu022	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Prymnesiophyceae_X	Prymnesiophyceae_X	Syracosphaera
Otu023	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Bacillariophyceae	Bacillariophyceae_X	Bacillariophyceae_X
Otu024	Eukaryota	Archaeplastida	Streptophyta	Klebsormidiophyceae	Klebsormidiophyceae_X	Klebsormidiophyceae_XX	Klebsormidium
Otu025	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu026	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Bacillariophyceae	Bacillariophyceae_X	Cylindrotheca
Otu027	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Suessiales	Suessiales_X	Karlodinium
Otu028	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Isochrysidales	Noelaerhabdaceae	Emiliania
Otu029	Eukaryota	Opisthokonta	Fungi	Ascomycota	Saccharomycotina	Saccharomycetales	Debaryomyces
Otu030	Eukaryota	Hacrobia	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Teleaulax
Otu031	Eukaryota	Alveolata	Dinophyta	Syndiniales	Syndiniales_Group_I	Syndiniales_Group_I_Clade_1	Syndiniales_Group_I_Clade_1_X
Otu032	Eukaryota	Archaeplastida	Chlorophyta	Prasino_Clade_VII	Prasino_Clade_VII_X	Prasino_Clade_VII_A	Prasino_Clade_VII_A_4_X

Sample table

sample	fraction	Select_18S_nifH	total_18S	total_16S	total_nifH	sample_number	transect	station	depth	latitude	longitude
X10n	Nano	Yes	53230	8772	36	10	1	81	140	-27.42	-44.72
X10p	Pico	Yes	47390	4448	6241	10	1	81	140	-27.42	-44.72
X11n	Nano	No	24007	6193	3772	11	1	85	110	-26.8	-45.3
X11p	Pico	Yes	31899	14	10201	11	1	85	110	-26.8	-45.3
X120n	Nano	Yes	70455	5292	93	120	2	96	5	-27.39	-47.82
X120p	Pico	Yes	76182	53272	23147	120	2	96	5	-27.39	-47.82
X121n	Nano	Yes	52401	5958	26838	121	2	96	30	-27.39	-47.82
X121p	Pico	Yes	71785	10993	23706	121	2	96	30	-27.39	-47.82
X122n	Nano	Yes	78740	11730	15543	122	2	96	50	-27.39	-47.82
X122p	Pico	Yes	37364	11817	11045	122	2	96	50	-27.39	-47.82
X125n	Nano	Yes	27381	9	14331	125	2	98	5	-27.59	-47.39
X125p	Pico	Yes	55179	10419	21461	125	2	98	5	-27.59	-47.39
X126n	Nano	Yes	65714	15	16929	126	2	98	50	-27.59	-47.39
X126p	Pico	Yes	30406	3	10140	126	2	98	50	-27.59	-47.39
X127n	Nano	Yes	60610	9	11493	127	2	98	85	-27.59	-47.39
X13n	Nano	Yes	46001	33	21316	13	1	86	105	-26.33	-45.41
X13p	Pico	Yes	59626	7217	11954	13	1	86	105	-26.33	-45.41
X140n	Nano	Yes	48126	10428	25286	140	2	101	5	-27.79	-46.96
X140p	Pico	Yes	46569	10448	12301	140	2	101	5	-27.79	-46.96
X141n	Nano	Yes	30081	6394	21302	141	2	101	60	-27.79	-46.96
X141p	Pico	Yes	64221	11318	10428	141	2	101	60	-27.79	-46.96
X142n	Nano	Yes	85219	23243	11753	142	2	101	110	-27.79	-46.96
X142p	Pico	Yes	89797	9553	17156	142	2	101	110	-27.79	-46.96
X155n	Nano	Yes	54162	8237	20674	155	2	106	5	-28.12	-46.17
X155p	Pico	Yes	50782	7384	66172	155	2	106	5	-28.12	-46.17
X156n	Nano	Yes	55065	11371	14447	156	2	106	60	-28.12	-46.17
X156p	Pico	Yes	43917	9665	16093	156	2	106	60	-28.12	-46.17
X157n	Nano	Yes	29078	4978	15532	157	2	106	100	-28.12	-46.17
X157p	Pico	Yes	51848	9139	15204	157	2	106	100	-28.12	-46.17
X15n	Nano	Yes	22468	2887	2678	15	1	87	105	-26.22	-45.48
X15p	Pico	Yes	78390	13813	1033	15	1	87	105	-26.22	-45.48
X165n	Nano	Yes	50732	15337	14706	165	2	114	5	-28.65	-44.99
X165p	Pico	Yes	48514	10902	39918	165	2	114	5	-28.65	-44.99
X166n	Nano	Yes	53412	3411	24442	166	2	114	60	-28.65	-44.99

```r
otu_mat<- read_excel("CARBOM data.xlsx", sheet = "OTU matrix")
tax_mat<- read_excel("CARBOM data.xlsx", sheet = "Taxonomy table")
samples_df <- read_excel("CARBOM data.xlsx", sheet = "Samples")
```

Phyloseq objects need to have row.names

- define the row names from the otu column

```
row.names(otu_mat) <- otu_mat$otu
```

```
## Warning: Setting row names on a tibble is deprecated.
```

- remove the column otu since it is now used as a row name

```
otu_mat <- otu_mat %>% select (-otu)
```

- Idem for the two other matrixes

```
row.names(tax_mat) <- tax_mat$otu
```

```
## Warning: Setting row names on a tibble is deprecated.
```

```
tax_mat <- tax_mat %>% select (-otu)

row.names(samples_df) <- samples_df$sample
```

```
## Warning: Setting row names on a tibble is deprecated.
```

```
samples_df <- samples_df %>% select (-sample)
```

Transform into matrixes otu and tax tables (sample table can be left as data frame)

```
otu_mat <- as.matrix(otu_mat)
tax_mat <- as.matrix(tax_mat)
```

Transform to phyloseq objects

```
OTU = otu_table(otu_mat, taxa_are_rows = TRUE)
TAX = tax_table(tax_mat)
samples = sample_data(samples_df)

carbom <- phyloseq(OTU, TAX, samples)
carbom
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 287 taxa and 55 samples ]
## sample_data() Sample Data:       [ 55 samples by 27 sample variables ]
## tax_table()   Taxonomy Table:    [ 287 taxa by 7 taxonomic ranks ]
```

Visualize data

```
sample_names(carbom)
```

```
##  [1] "X10n"    "X10p"    "X11n"    "X11p"    "X120n"   "X120p"   "X121n"
##  [8] "X121p"   "X122n"   "X122p"   "X125n"   "X125p"   "X126n"   "X126p"
## [15] "X127n"   "X13n"    "X13p"    "X140n"   "X140p"   "X141n"   "X141p"
## [22] "X142n"   "X142p"   "X155n"   "X155p"   "X156n"   "X156p"   "X157n"
## [29] "X157p"   "X15n"    "X15p"    "X165n"   "X165p"   "X166n"   "X166p"
## [36] "X167n"   "X167p"   "X1n"     "X1p"     "X2n"     "X2p"     "X3n"
## [43] "X3p"     "X5n"     "X5p"     "X7n"     "X7p"     "X9n"     "X9p"
## [50] "tri01n" "tri01p" "tri02n" "tri02p" "tri03n" "tri03p"
```

```
rank_names(carbom)
```

```
## [1] "Domain"      "Supergroup" "Division"    "Class"       "Order"
## [6] "Family"      "Genus"
```

```r
sample_variables(carbom)
```

```
##  [1] "fraction"          "Select_18S_nifH"   "total_18S"
##  [4] "total_16S"         "total_nifH"        "sample_number"
##  [7] "transect"          "station"           "depth"
## [10] "latitude"          "longitude"         "picoeuks"
## [13] "nanoeuks"          "bottom_depth"      "level"
## [16] "transect_distance" "date"              "time"
## [19] "phosphates"        "silicates"         "ammonia"
## [22] "nitrates"          "nitrites"          "temperature"
## [25] "fluorescence"      "salinity"          "sample_label"
```

Keep only samples to be analyzed

```r
carbom <- subset_samples(carbom, Select_18S_nifH =="Yes")
carbom
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 287 taxa and 54 samples ]
## sample_data() Sample Data:       [ 54 samples by 27 sample variables ]
## tax_table()   Taxonomy Table:    [ 287 taxa by 7 taxonomic ranks ]
```

Keep only photosynthetic taxa

```r
carbom <- subset_taxa(carbom, Division %in% c("Chlorophyta", "Dinophyta", "Cryptophyta",
                                               "Haptophyta", "Ochrophyta", "Cercozoa"))
carbom <- subset_taxa(carbom, !(Class %in% c("Syndiniales", "Sarcomonadea")))
carbom
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 205 taxa and 54 samples ]
## sample_data() Sample Data:       [ 54 samples by 27 sample variables ]
## tax_table()   Taxonomy Table:    [ 205 taxa by 7 taxonomic ranks ]
```

Normalize number of reads in each sample using median sequencing depth.

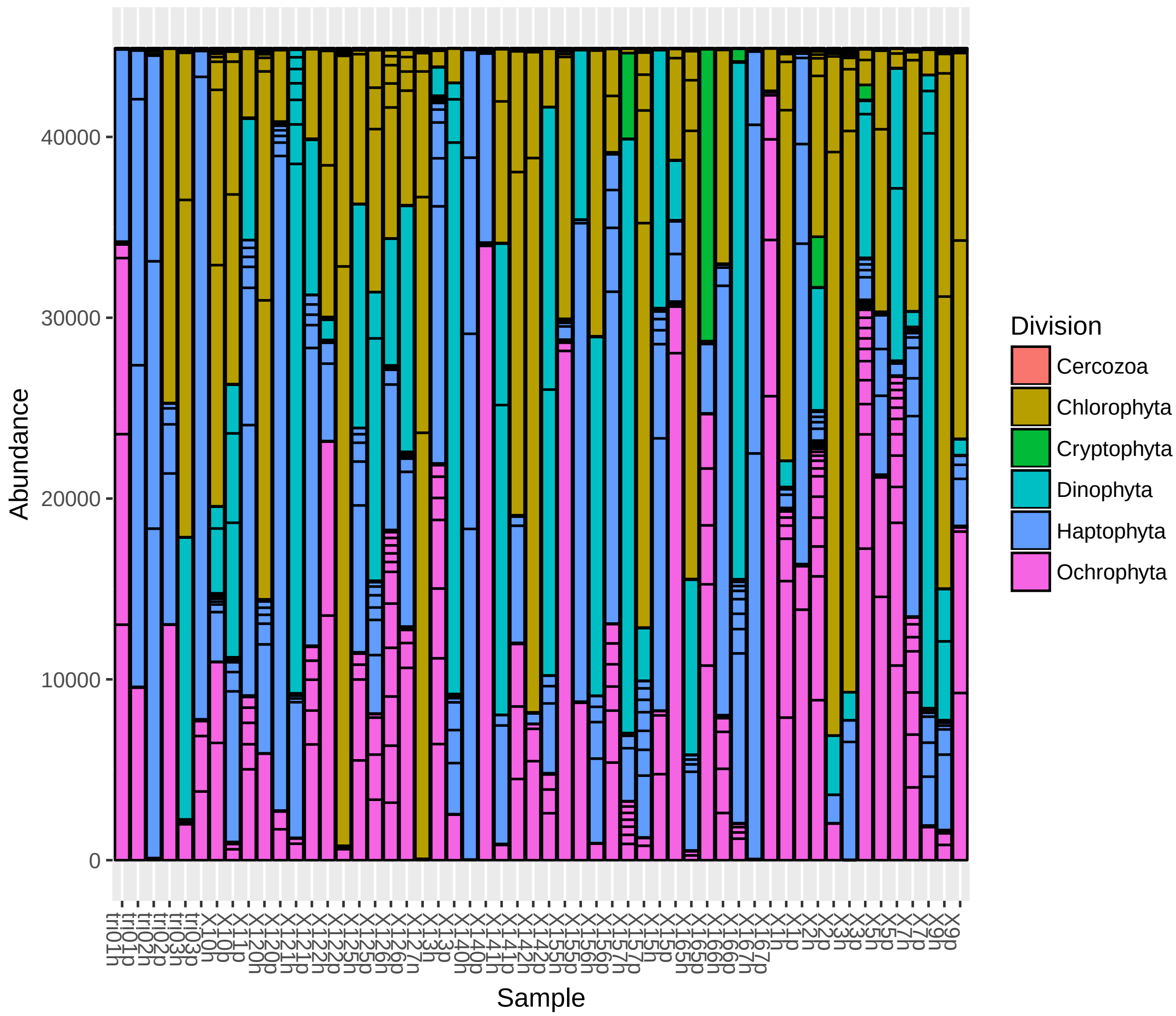
```r
total = median(sample_sums(carbom))
standf = function(x, t=total) round(t * (x / sum(x)))
carbom = transform_sample_counts(carbom, standf)
```

The number of reads used for normalization is **44903**.

3.3 Bar graphs

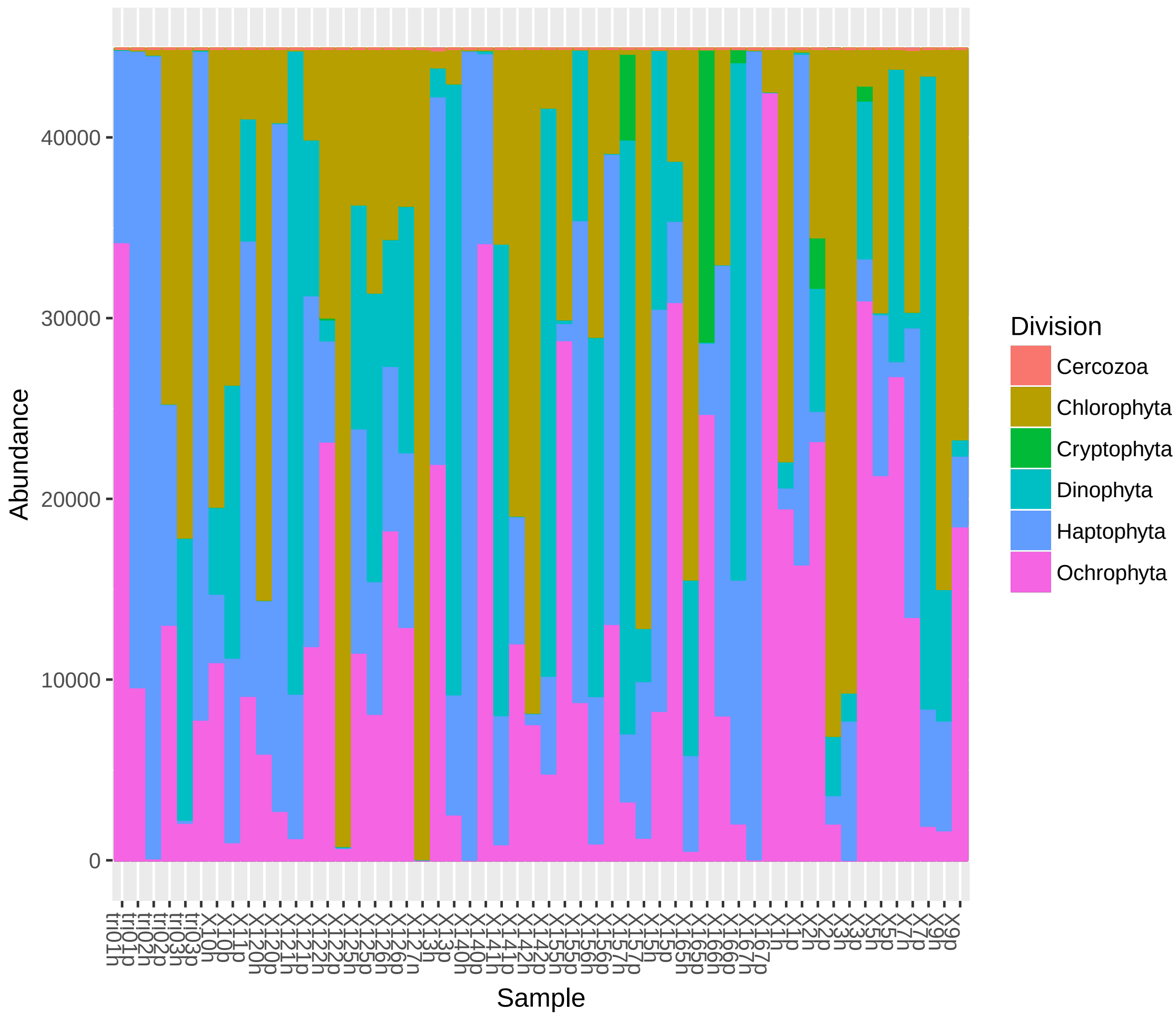
Basic bar graph based on Division

```r
plot_bar(carbom, fill = "Division")
```



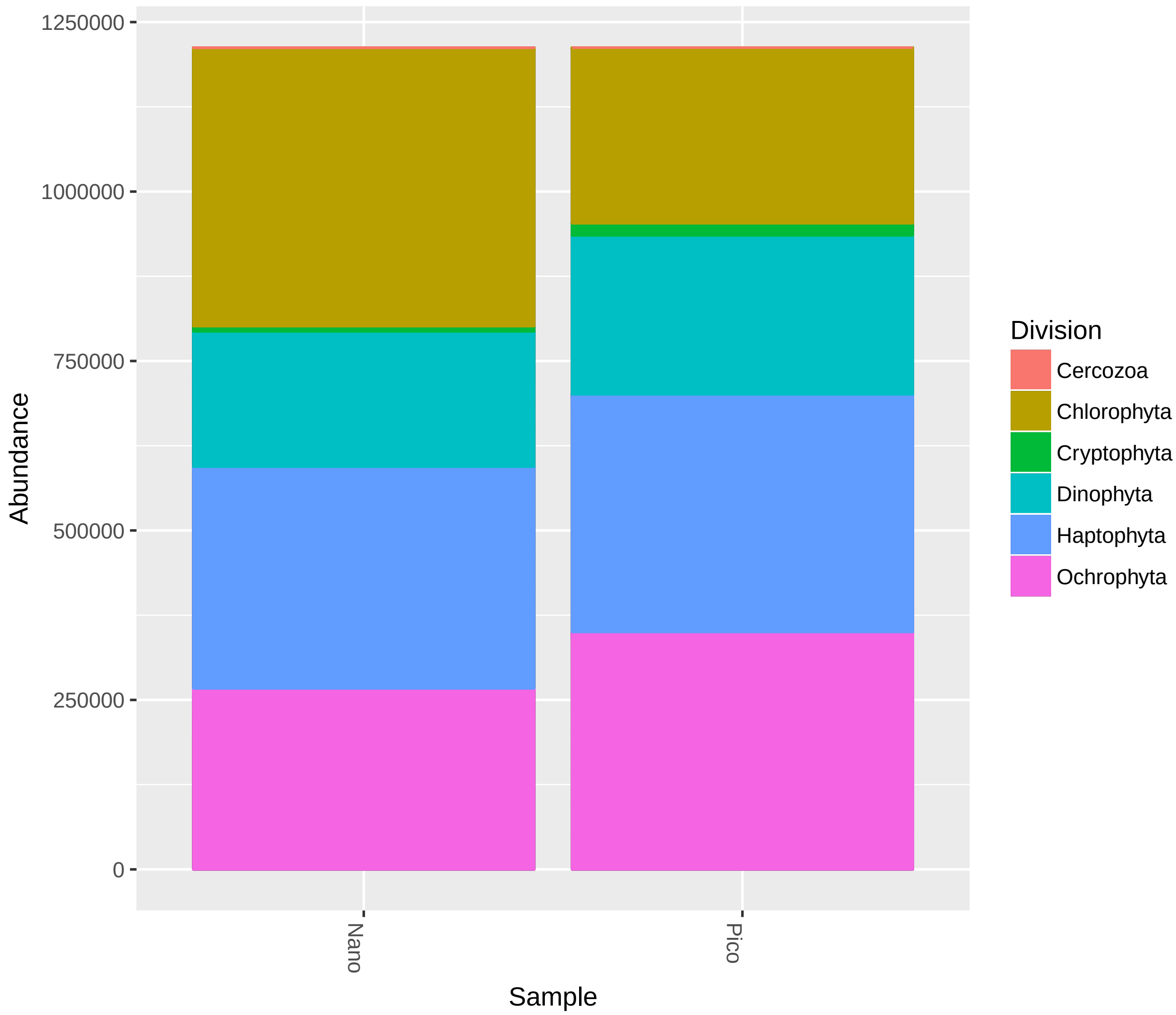
Make the bargraph nicer by removing OTUs boundaries. This is done by adding ggplot2 modifier.

```
plot_bar(carbom, fill = "Division") +
geom_bar(aes(color=Division, fill=Division), stat="identity", position="stack")
```



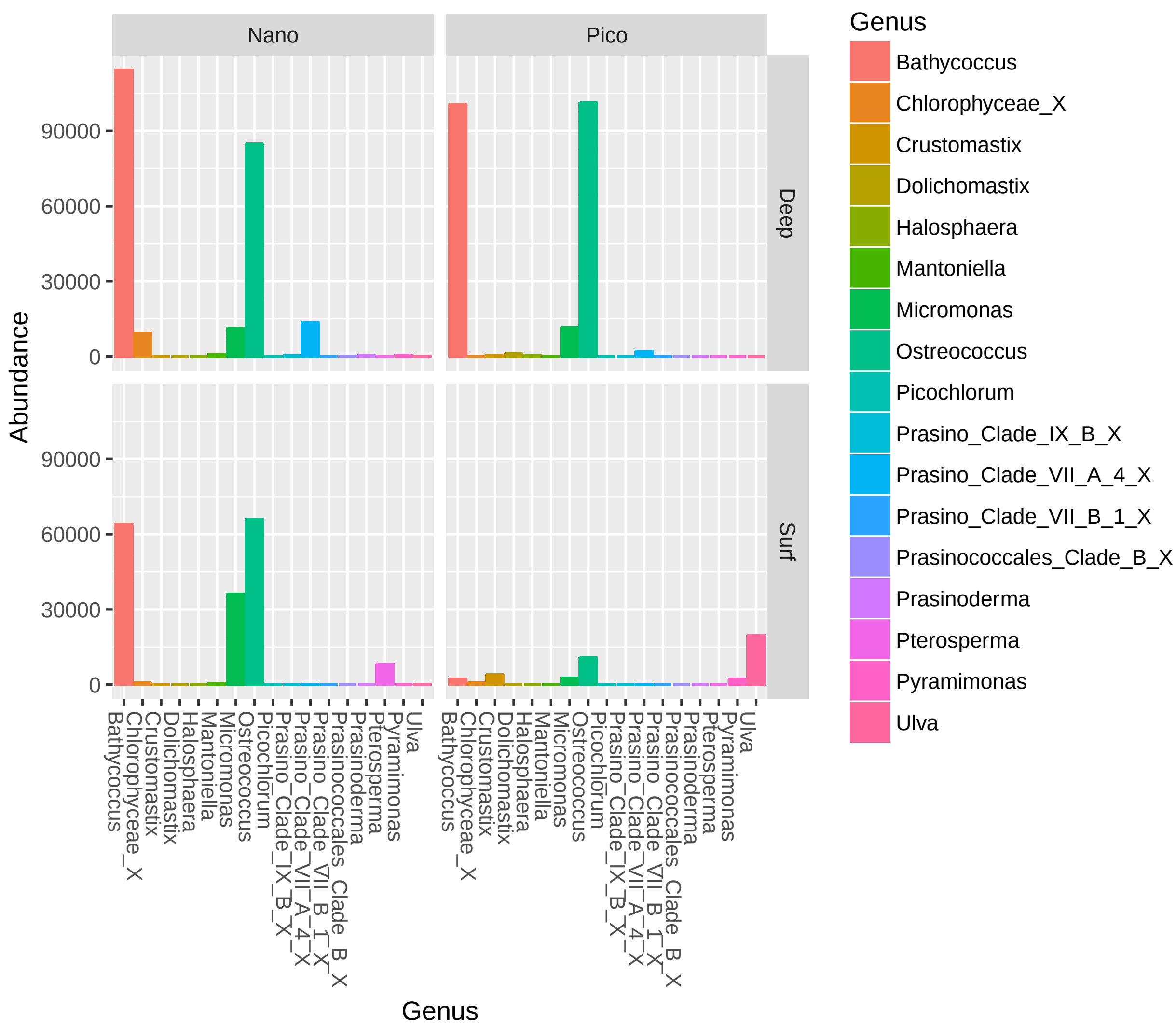
Regroup together Pico vs Nano samples

```
carbom_fraction <- merge_samples(carbom, "fraction")
plot_bar(carbom_fraction, fill = "Division") +
geom_bar(aes(color=Division, fill=Division), stat="identity", position="stack")
```



Keep only Chlorophyta and use color according to genus. Do separate panels Pico vs Nano and Surface vs Deep samples.

```
carbom_chloro <- subset_taxa(carbom, Division %in% c("Chlorophyta"))
plot_bar(carbom_chloro, x="Genus", fill = "Genus", facet_grid = level~fraction) +
geom_bar(aes(color=Genus, fill=Genus), stat="identity", position="stack")
```

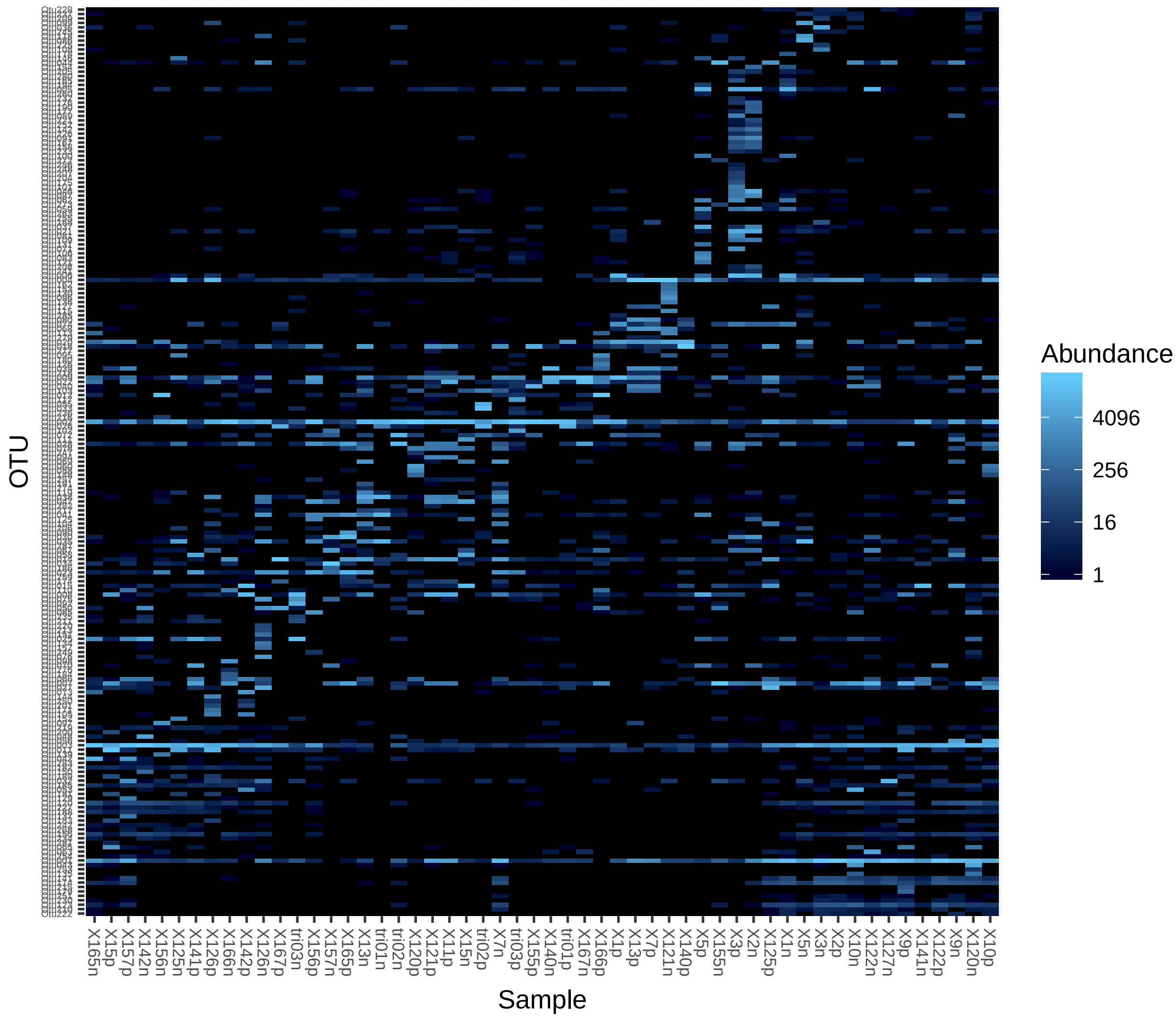


3.4 Heatmaps

A basic heatmap using the default parameters.

```
plot_heatmap(carbom, method = "NMDS", distance = "bray")
```

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



It is very very cluttered. It is better to only consider the most abundant OTUs for heatmaps. For example one can only take OTUs that represent at least 20% of reads in at least one sample. Remember we normalized all the sampples to median number of reads (total). We are left with only 33 OTUS which makes the reading much more easy.

```
carbom_abund <- filter_taxa(carbom, function(x) sum(x > total*0.20) > 0, TRUE)
carbom_abund
```

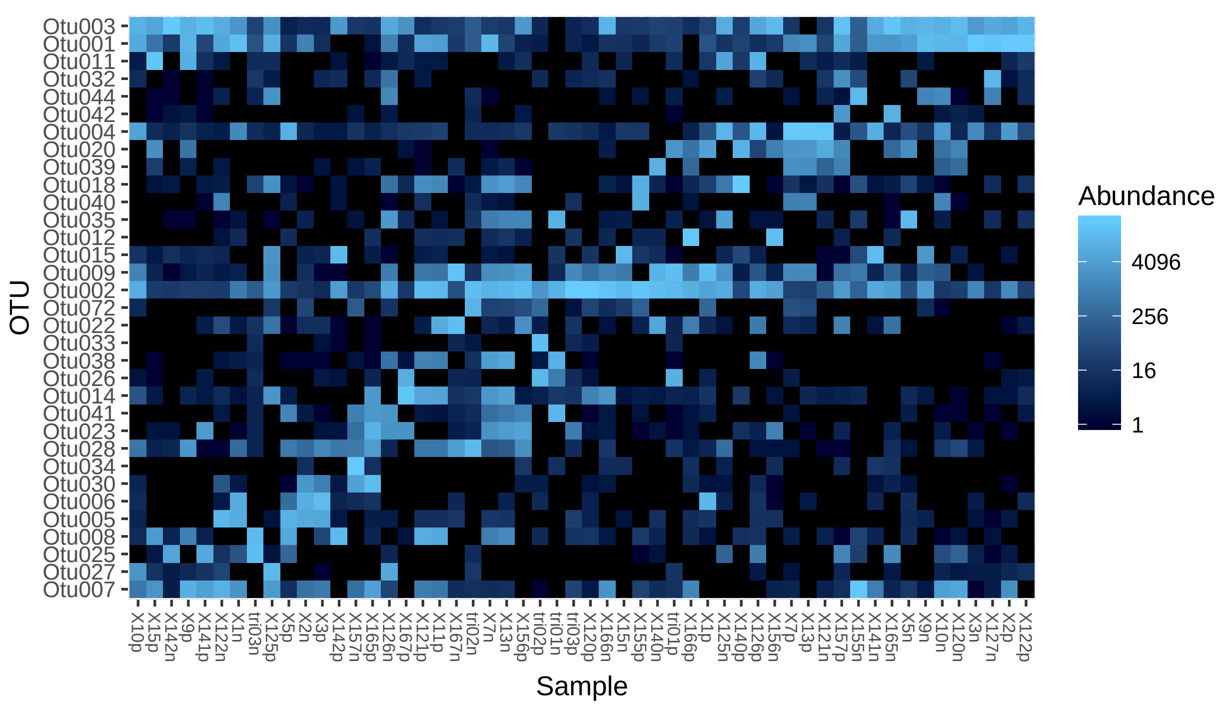
```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 33 taxa and 54 samples ]
## sample_data() Sample Data:       [ 54 samples by 27 sample variables ]
## tax_table()   Taxonomy Table:    [ 33 taxa by 7 taxonomic ranks ]
```

```
otu_table(carbom_abund)[1:8, 1:5]
```

```
## OTU Table:          [8 taxa and 5 samples]
##                      taxa are rows
##         X10n  X10p  X11p X120n X120p
## Otu001 13339  7346  3804 12662     3
## Otu002    18  8329 14958    30 36206
## Otu003  9692 10488    20 16537    11
## Otu004  3584  4943    33     7     9
## Otu005     0     6    11     0     5
## Otu006     0     9     0     0     5
## Otu007  4473   605   587  5894     3
## Otu008     1     9  6707     2    17
```

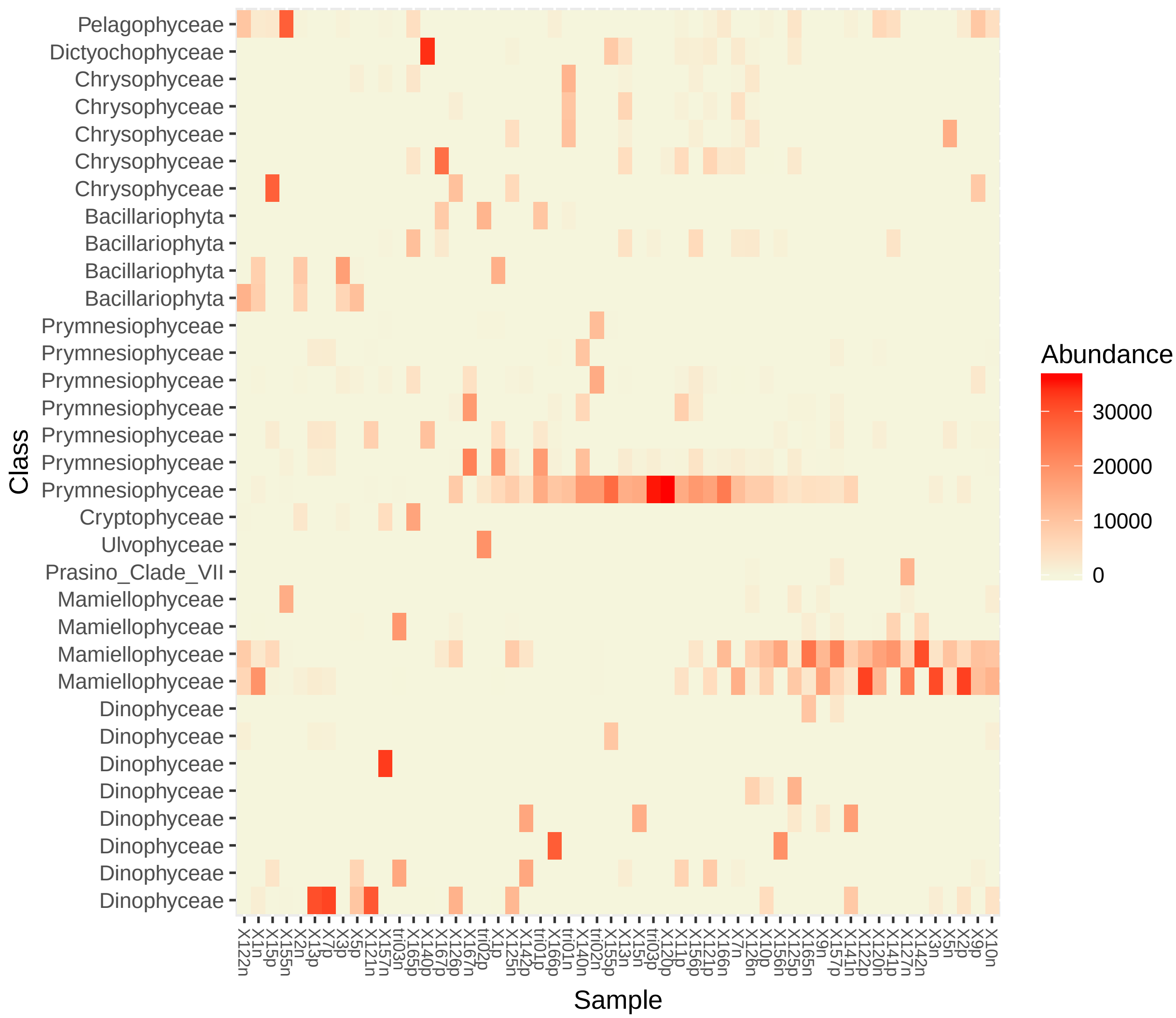
```
plot_heatmap(carbom_abund, method = "NMDS", distance = "bray")
```

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



It is possible to use different distances and different multivaraite methods. For example Jaccard distance and MDS and label OTUs with Class, order by Class. We can also change the Palette (the default palette is a bit ugly…).

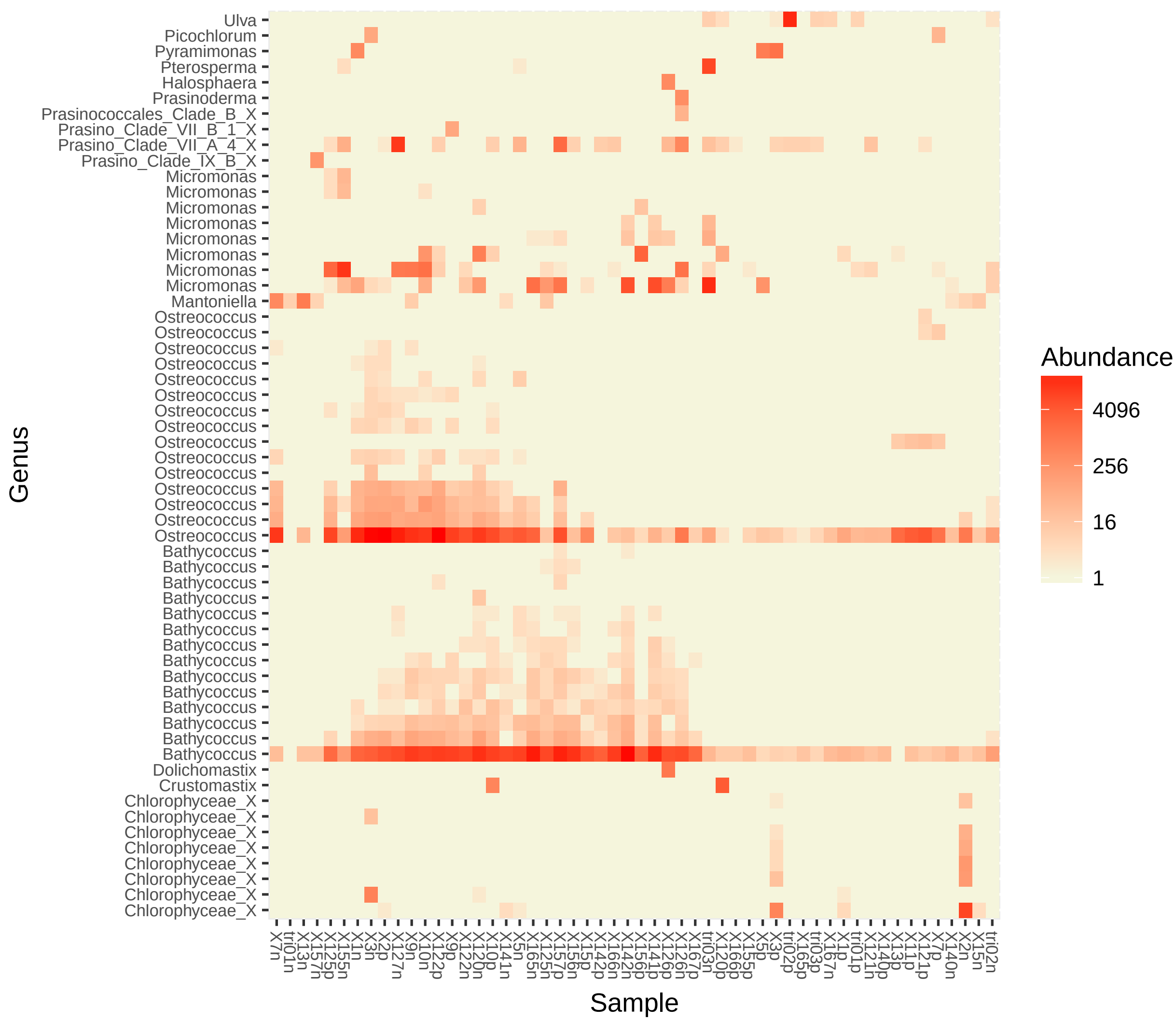
```
plot_heatmap(carbom_abund, method = "MDS", distance = "jaccard",
             taxa.label = "Class", taxa.order = "Class",
             trans=NULL, low="beige", high="red", na.value="beige")
```



Another strategy is to do a heatmap for a specific taxonomy group. For example we can taget the Chlorophyta and then label the OTUs using the Genus.

```r
plot_heatmap(carbom_chloro, method = "NMDS", distance = "bray",
             taxa.label = "Genus", taxa.order = "Genus",
             low="beige", high="red", na.value="beige")
```

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

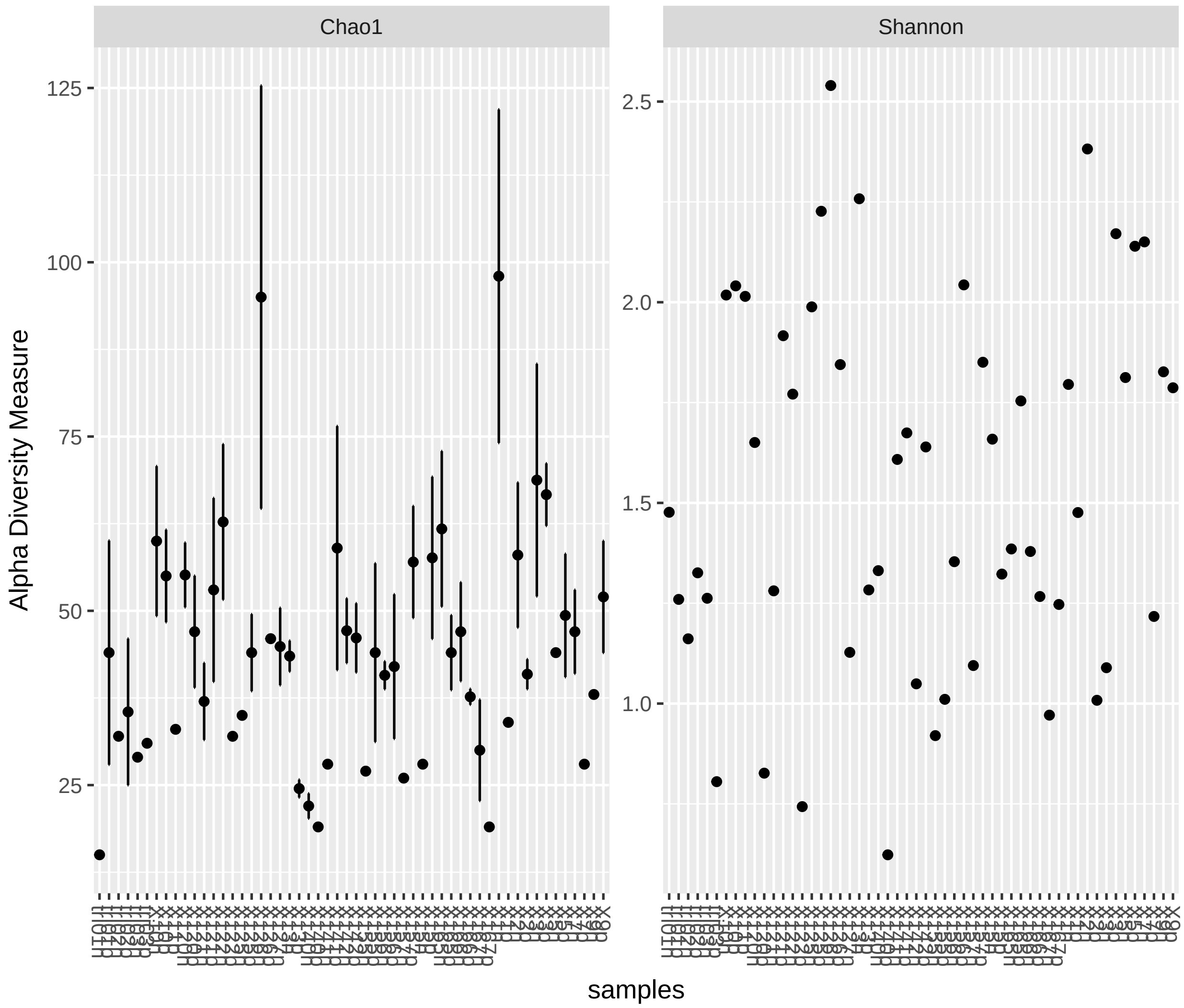


3.5 Alpha diversity

Plot Chao1 richness estimator and Shannon diversity estimator.

```r
plot_richness(carbom, measures=c("Chao1", "Shannon"))
```

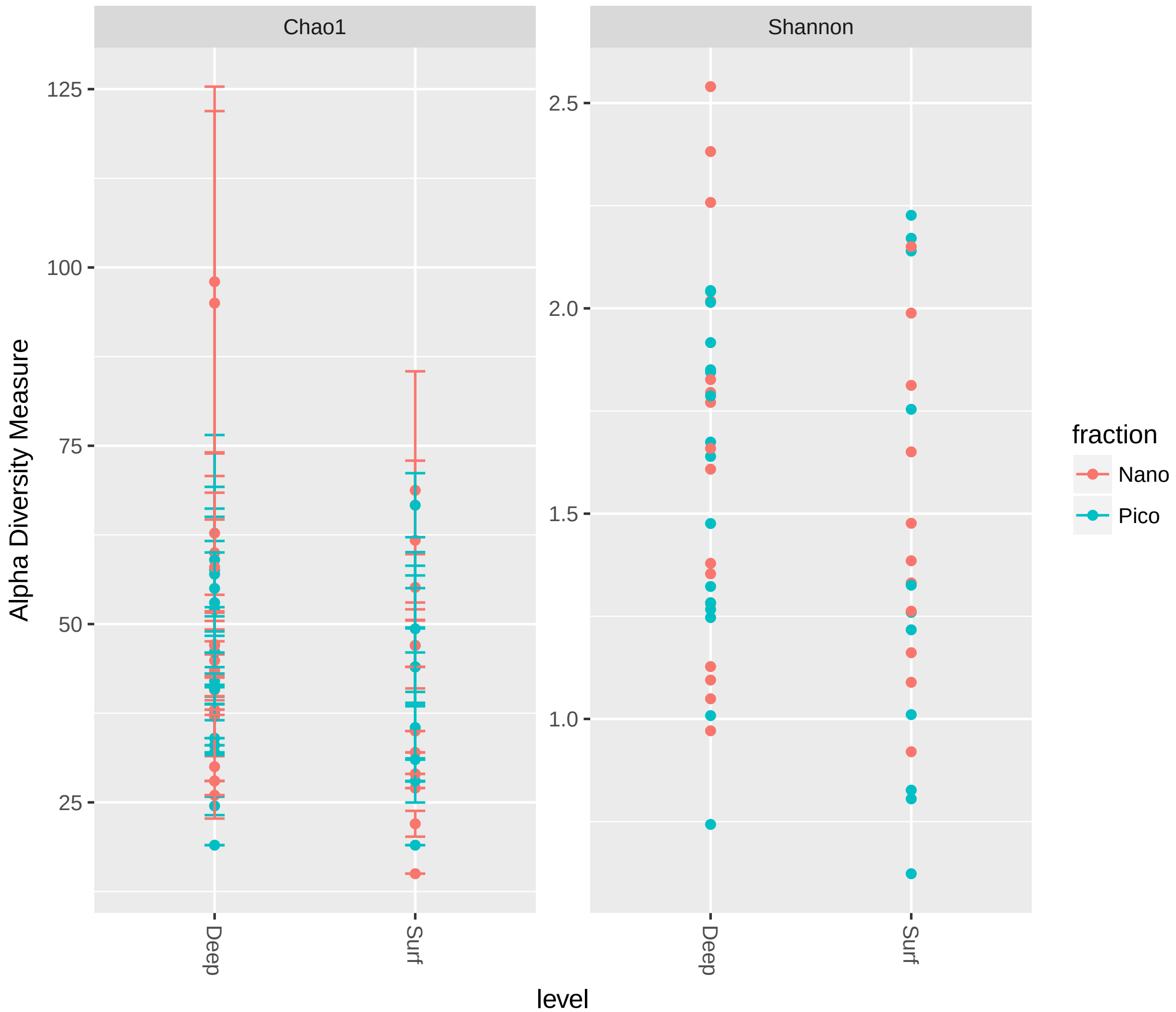
```
## Warning: Removed 54 rows containing missing values (geom_errorbar).
```



Regroup together samples from the same fraction.

```
plot_richness(carbom, measures=c("Chao1", "Shannon"), x="level", color="fraction")
```

```
## Warning: Removed 54 rows containing missing values (geom_errorbar).
```



3.6 Ordination

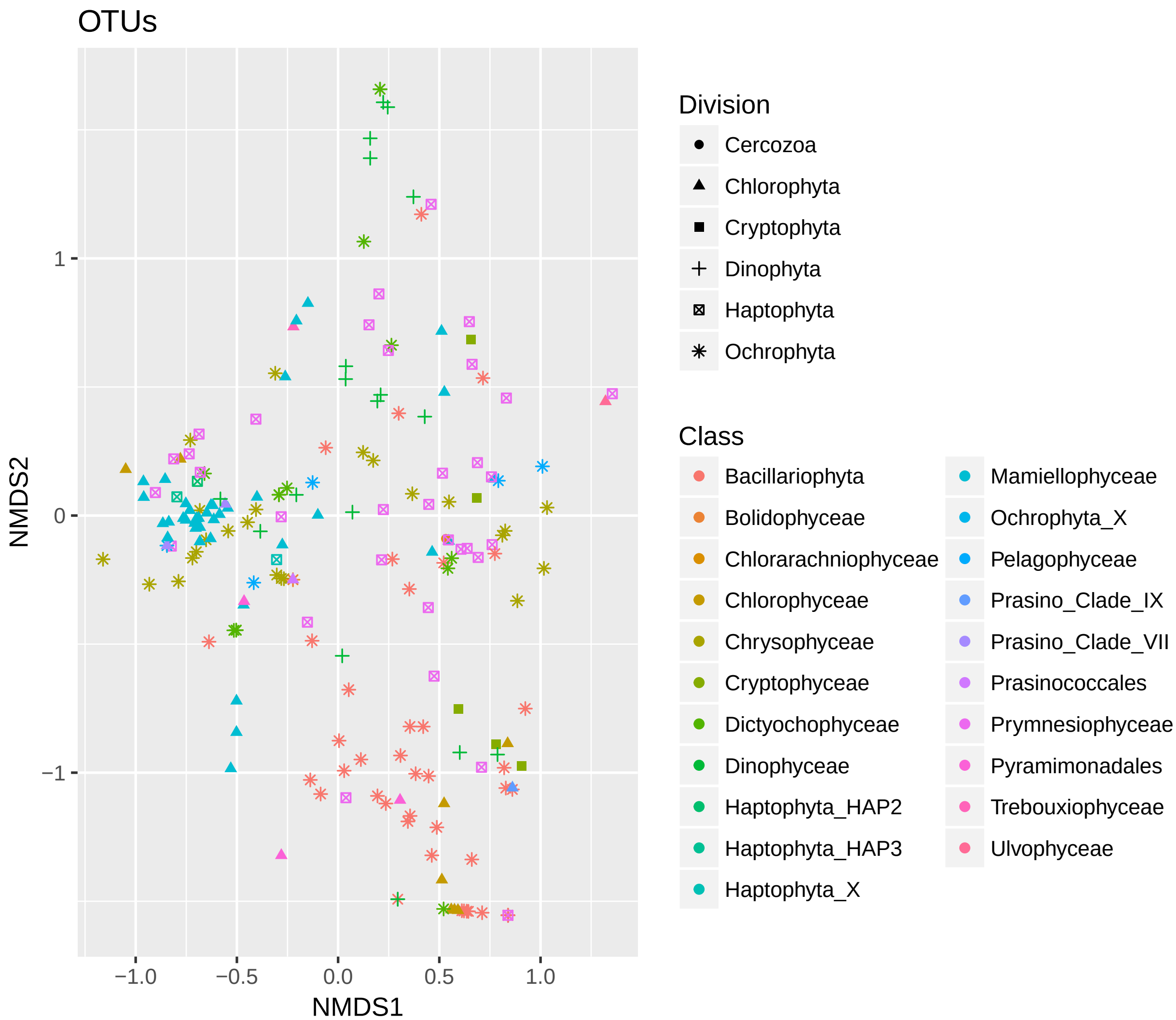
Do multivariate analysis based on Bray-Curtis distance and NMDS ordination.

```r
carbom.ord <- ordinate(carbom, "NMDS", "bray")
```

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.2317058
## Run 1 stress 0.2488414
## Run 2 stress 0.2534458
## Run 3 stress 0.2359226
## Run 4 stress 0.2576921
## Run 5 stress 0.2576468
## Run 6 stress 0.2472519
## Run 7 stress 0.2510679
## Run 8 stress 0.2519793
## Run 9 stress 0.2302728
## ... New best solution
## ... Procrustes: rmse 0.1066564  max resid 0.3796017
## Run 10 stress 0.2450473
## Run 11 stress 0.2444403
## Run 12 stress 0.2348005
## Run 13 stress 0.2422467
## Run 14 stress 0.2566082
## Run 15 stress 0.2407624
## Run 16 stress 0.2542353
## Run 17 stress 0.2632189
## Run 18 stress 0.2585642
## Run 19 stress 0.2532353
## Run 20 stress 0.2449511
## *** No convergence -- monoMDS stopping criteria:
##     20: stress ratio > sratmax
```

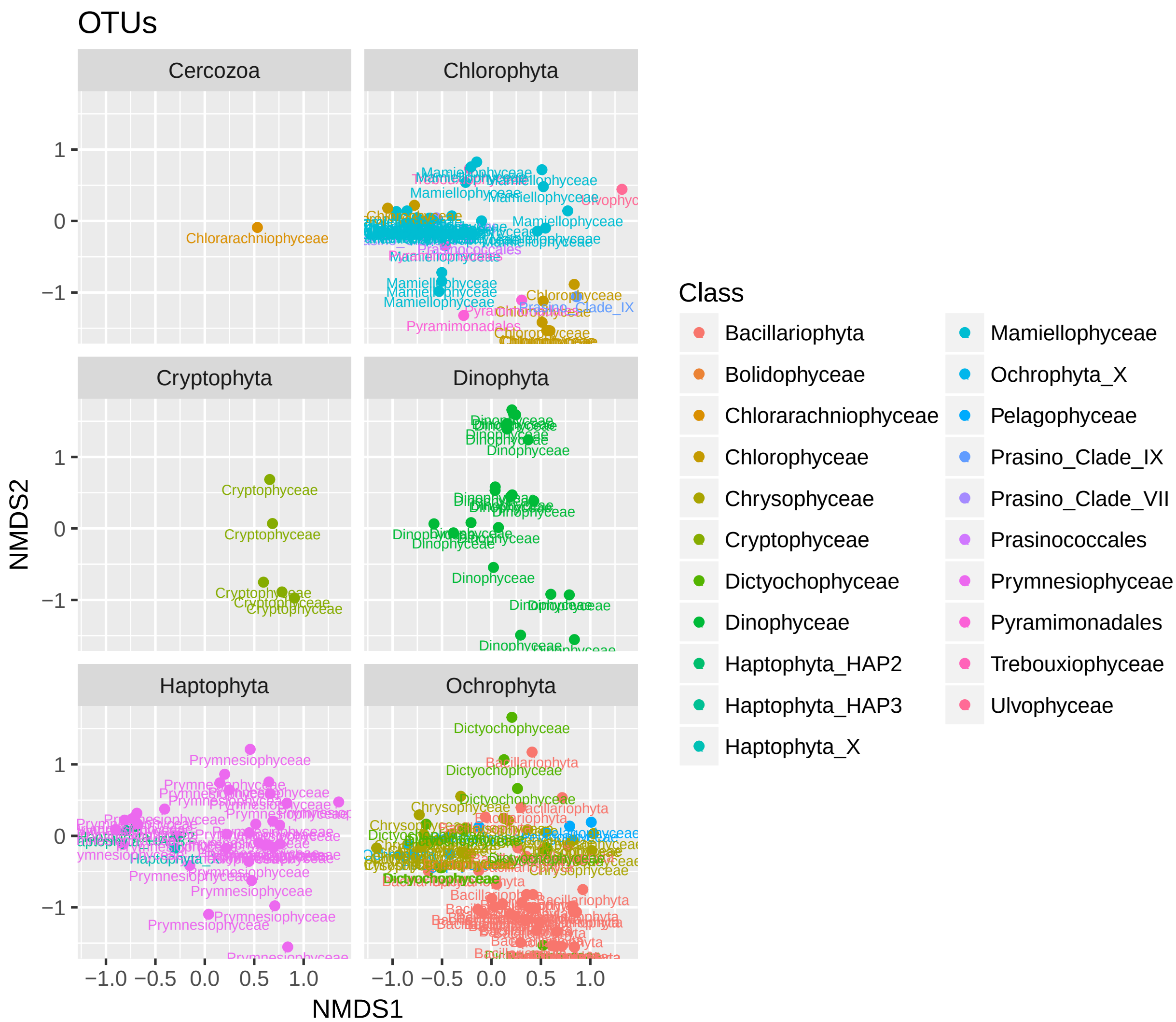
Plot **OTUs**

```
plot_ordination(carbom, carbom.ord, type="taxa", color="Class", shape= "Division",
                title="OTUs")
```



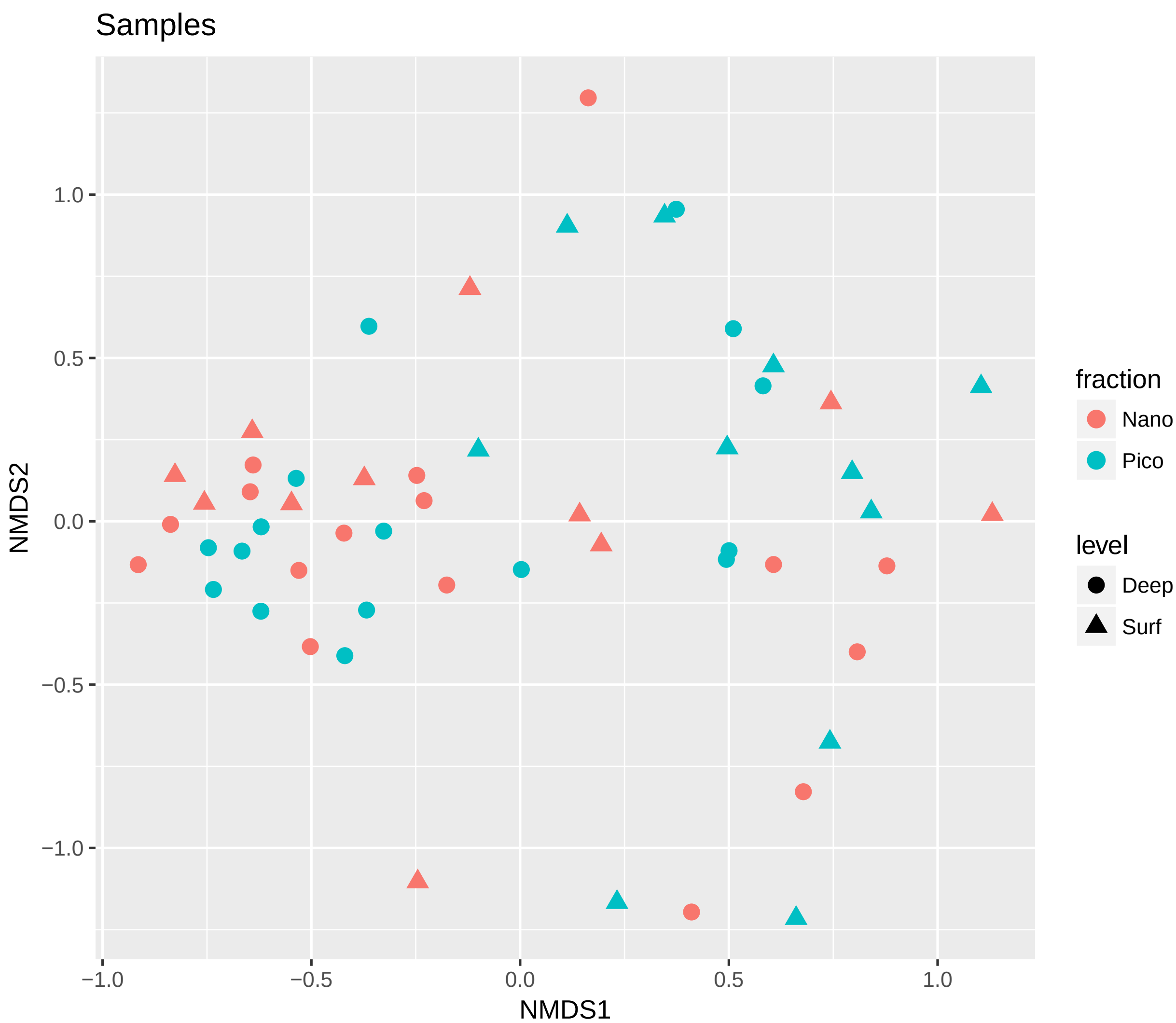
A bit confusing, so make it more easy to visualize by breaking according to taxonomic division.

```
plot_ordination(carbom, carbom.ord, type="taxa", color="Class",
                title="OTUs", label="Class") +
facet_wrap(~Division, 3)
```



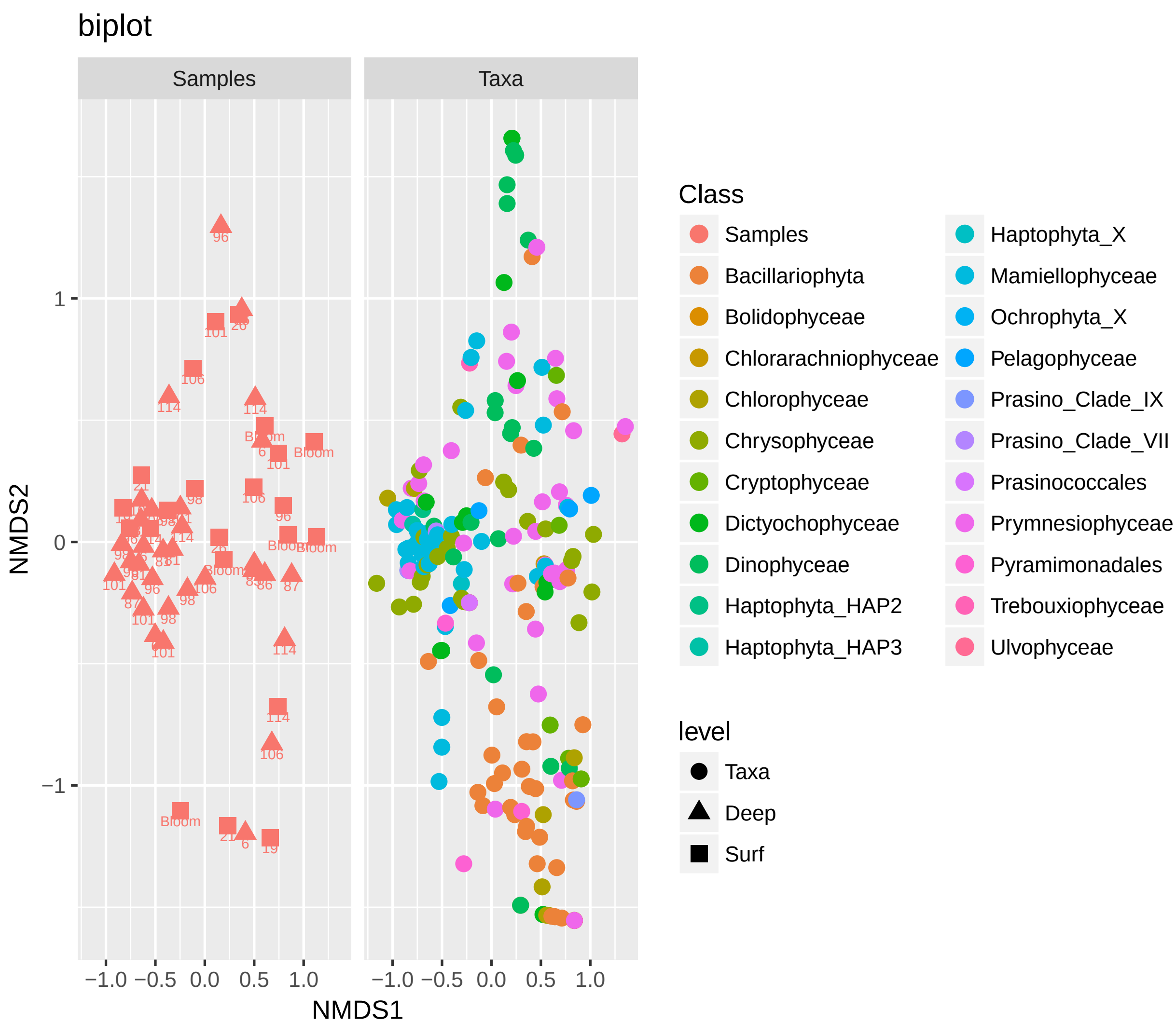
Now display **samples** and enlarge the points to make it more easy to read.

```
plot_ordination(carbom, carbom.ord, type="samples", color="fraction",
                shape="level", title="Samples") + geom_point(size=3)
```



Display both samples and OTUs but in 2 different panels.

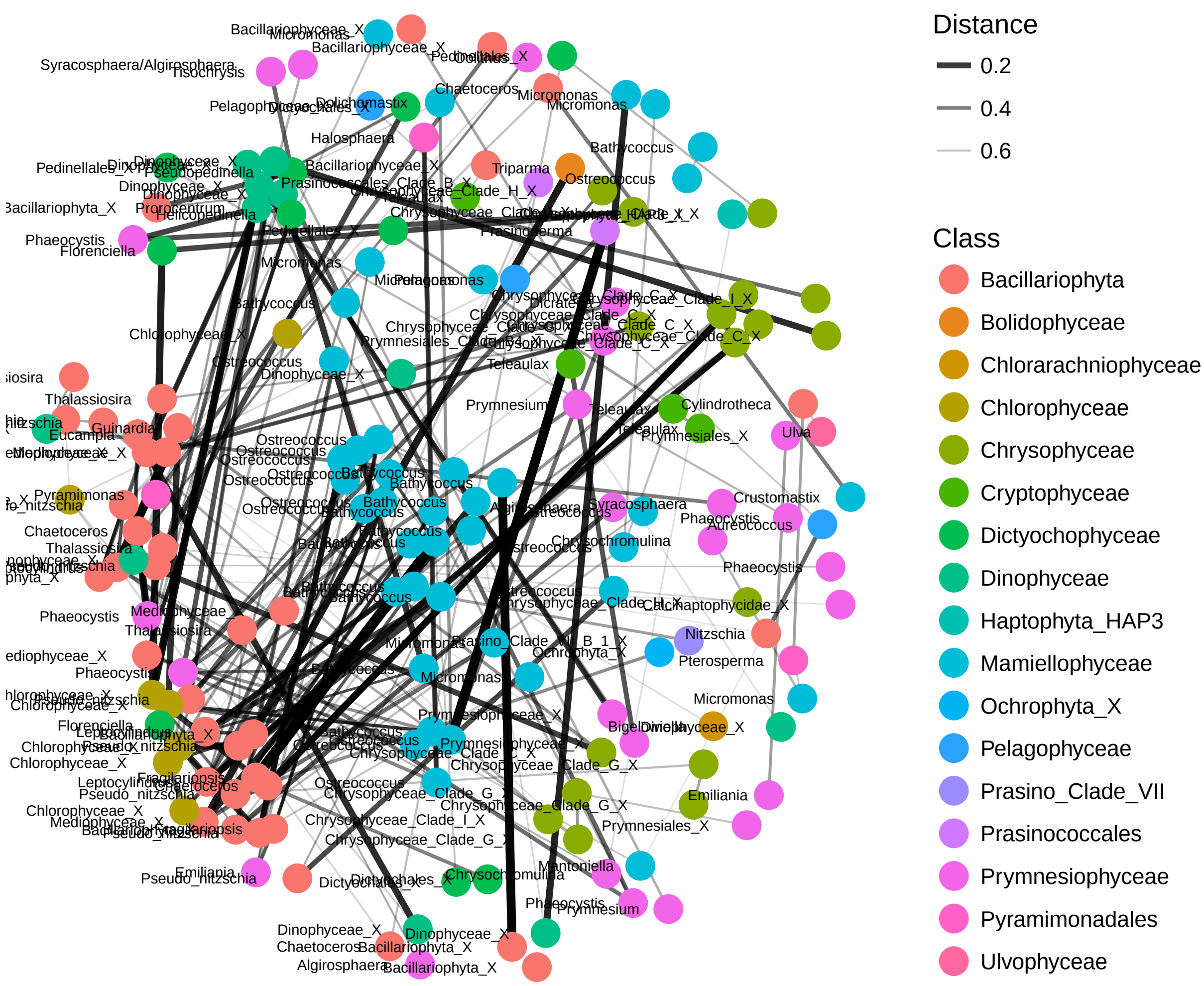
```
plot_ordination(carbom, carbom.ord, type="split", color="Class",
                shape="level", title="biplot", label = "station") +
geom_point(size=3)
```



3.7 Network analysis

Simple network analysis

```
plot_net(carbom, distance = "bray", type = "taxa",
         maxdist = 0.7, color="Class", point_label="Genus")
```



This is quite confusing. Let us make it more simple by using only major OTUs

```
plot_net(carbom_abund, distance = "bray", type = "taxa",
         maxdist = 0.8, color="Class", point_label="Genus")
```