Analysis of community composition data using phyloseq

MAHENDRA MARIADASSOU, MARIA BERNARD, GERALDINE PASCAL, LAURENT CAUQUIL, STEPHANE CHAILLOU

Goals

- Learn about and become familiar with **phyloseq** R package for the analysis of microbial census data
- Exploratory Data Analysis
  - α-diversity: how diverse is my community?
  - β-diversity: how different are two communities?
  - Use a distance matrix to study structures:
    - Hierarchical clustering: how do the communities cluster?
    - Permutational ANOVA: are the communities structured by some known environmental factor (pH, height, etc)?
  - Visual assessment of the data
    - Bar plots: what is the composition of each community?
    - Multidimensional Scaling: how are communities related?
    - Heatmaps: are there interactions between species and (groups of) communities?

Training Data

Training data comes from a real analysis provided by Stéphane Chaillou et al.

The aim was to compare meat (4 types) and seafood (4 types) bacterial communities.

We use here, an extract of these public data :

- 64 samples of 16S V1-V3
- From 8 environment types :
  - Meat ➔ Ground Beef, Ground veal, Poultry sausage, Diced bacon
  - Seafood ➔ Cooked schrimps, Smoked salmon, Salmon filet, Cod filet
- 508 OTUs affiliated with the Greengenes database

Chaillou, S. et al (2015). Origin and ecological selection of core and food-specific bacterial communities associated with meat and seafood spoilage. ISME J, 9(5):1105-1118.

Training Data

➔ Take a look at the data

- data/chaillou/otu_table.tsv

	CDT0#LOT06	CDT0#LOT09	CDT0#LOT07	CDT0#LOT02
otu_00601	0	0	0	4
otu_01717	0	4	37	5

- data/chaillou/tax_table.tsv

	Kingdom	Phylum	Class	Order	…
otu_00001	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	…
otu_00004	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	…

Training Data

➔ Take a look at the data

- data/chaillou/sample_data.tsv

```
              SampleID     EnvType  Description  FoodType
BHT0.LOT01  BHT0#LOT01  BoeufHache         LOT1      Meat
BHT0.LOT03  BHT0#LOT03  BoeufHache         LOT3      Meat
```

- data/chaillou/tree.nwk

```
((((((((((((otu_00520:0.016,otu_00555:0.01122):0.01094,((otu_00568:0.003
 01,otu_00566:0.01354):0.00617,otu_00569:0.00821):0.00998):0.00828,otu_005
 45:0.03879):0.02824,((otu_00527:0.02225,otu_00521:0.00934): …
```

Phyloseq

Phyloseq

- About phyloseq
- phyloseq data structure
- Other accessors
- Manipulating a phyloseq object: Filtering
- Manipulating a phyloseq object: Smoothing
- Manipulating a phyloseq object: Abundance counts
- Importing a phyloseq object

About Phyloseq

- R package (McMurdie and Holmes, 2013) to analyze community composition data in a phylogenetic framework

It uses other R packages:

- Community ecology functions from vegan, ade4, picante
- Tree manipulation from ape
- Graphics from ggplot2

About Phyloseq

- Find help:

Phyloseq comes with two vignettes

```
vignette("phyloseq-basics")
```

```
vignette("phyloseq-analysis")
```

The first one gives insights about data structure and data manipulation (Section 2), the second one about data analysis (Section 3 to 5).

load-extra-functions.R

Phyloseq : Let's get started

➔ load the phyloseq package

```
library(phyloseq)
```

➔ and some additional custom functions

```
source("https://raw.githubusercontent.com/mahendra-
 mariadassou/phyloseq-extended/master/load-extra-functions.R" )
```

Phyloseq : Data import

The biom format natively supports

- OTU count tables (required)
- OTU description
- sample description

but each component can be stored in TSV files.

Other optional components must be stored in separate files

- phylogenetic tree in Newick format
- sequences in fasta format

Phyloseq : Data import

➔ Import biom file (from FROGS or anywhere else)

```
biomfile <- "data/chaillou/chaillou.biom"
food1 <-import_biom(biomfile)
food1
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 508 taxa and 64 samples ]
## tax_table()   Taxonomy Table:    [ 508 taxa by 7 taxonomic ranks ]
```

Phyloseq : Data import

➔ Add samples metadata (optional, these can be already stored in biom file)

```
sampledata <-read.csv("data/chaillou/sample_data.tsv", sep="\t", row.names = 1)
sample_data(food1) <- sampledata
food1
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 508 taxa and 64 samples ]
## sample_data() Sample Data:       [ 64 samples by 3 sample variables ]
## tax_table()   Taxonomy Table:    [ 508 taxa by 7 taxonomic ranks ]
```

Phyloseq : Data import

➔ Add phylogenetic tree (optional)

```
treefile <- read.tree("data/chaillou/tree.nwk")
phy_tree(food1) <- treefile
food1
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 508 taxa and 64 samples ]
## sample_data() Sample Data:       [ 64 samples by 3 sample variables ]
## tax_table()   Taxonomy Table:    [ 508 taxa by 7 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 508 tips and 507 internal nodes ]
```

Phyloseq : Data import

➔ More direct way, if sample metadata are in biom file

```
biomfile2 <- "data/chaillou/chaillou_with_sam_data.biom"
treefile <- read.tree("data/chaillou/tree.nwk")
food <-import_biom(biomfile2, treefile, parseFunction =
 parse_taxonomy_greengenes)
food
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 508 taxa and 64 samples ]
## sample_data() Sample Data:       [ 64 samples by 3 sample variables ]
## tax_table()   Taxonomy Table:    [ 508 taxa by 7 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 508 tips and 507 internal nodes ]
```

Phyloseq Data Structure

Our phyloseq object food is made up of four parts:

- OTU Table
- Sample Data
- Taxomony Table
- Phylogenetic Tree

Let's have a quick look at each using the hinted at functions:

- otu_table
- sample_data
- tax_table
- phy_tree

Phyloseq Data Structure

OTU_table object is a matrix-like object

```
head(otu_table(food))
##OTU Table:          [6 taxa and 64 samples]
##                     taxa are rows
##          DLT0.LOT08 DLT0.LOT05 DLT0.LOT03 DLT0.LOT07 DLT0.LOT06 DLT0.LOT01 DLT0.LOT04 DLT0.LOT10
##otu_00520          0          0          0          0          0          0          0          0
##otu_00555          4          0          8          3         15          4          2          0
##otu_00568          0          0          0          0          0          0          0          0
##otu_00566          0          3          0          0          0          0          0          0
##otu_00569         12          3         26         16         38          0          4          0
##otu_00545          0          0          0          0          0          0          0          0
##          MVT0.LOT05 MVT0.LOT01 MVT0.LOT06 MVT0.LOT07 MVT0.LOT03 MVT0.LOT09 MVT0.LOT08 MVT0.LOT10
##otu_00520          0          0          0          0          0          0          0          0
##otu_00555         12         17         25         31         11         40         12          4
##otu_00568          0          0          0          0          0          0          0          0
##otu_00566          0          0          0          0          0          0          0          0
##otu_00569         10         15         40         35         11        119          0          0
```

Phyloseq Data Structure

tax_table object is a matrix-like object

```
head(tax_table(food))
##Taxonomy Table:     [6 taxa by 7 taxonomic ranks]:
##           Kingdom    Phylum           Class                  Order                Family
##otu_00520 "Bacteria" "Proteobacteria" "Gammaproteobacteria" "Enterobacteriales" "Enterobacteriaceae"
##otu_00555 "Bacteria" "Proteobacteria" "Gammaproteobacteria" "Enterobacteriales" "Enterobacteriaceae"
##otu_00568 "Bacteria" "Proteobacteria" "Gammaproteobacteria" "Enterobacteriales" "Enterobacteriaceae"
##otu_00566 "Bacteria" "Proteobacteria" "Gammaproteobacteria" "Enterobacteriales" "Enterobacteriaceae"
##otu_00569 "Bacteria" "Proteobacteria" "Gammaproteobacteria" "Enterobacteriales" "Enterobacteriaceae"
##otu_00545 "Bacteria" "Proteobacteria" "Gammaproteobacteria" "Enterobacteriales" "Enterobacteriaceae"
##           Genus                     Species
##otu_00520 "Raoultella"              "Ornithinolytica"
##otu_00555 "Hafnia-Obesumbacterium" "Alveii"
##otu_00568 "Serratia"                "Fonticola"
##otu_00566 "Serratia"                "Liquefaciens"
##otu_00569 "Serratia"                "Proteamaculans"
```

➔ Try on food1

Phyloseq Data Structure

Sample_data object is a data.frame-like object

```
head(sample_data(food))
##Sample Data:        [6 samples by 3 sample variables]:
##              EnvType FoodType Description
##DLT0.LOT08 DesLardons     Meat        LOT8
##DLT0.LOT05 DesLardons     Meat        LOT5
##DLT0.LOT03 DesLardons     Meat        LOT3
##DLT0.LOT07 DesLardons     Meat        LOT7
##DLT0.LOT06 DesLardons     Meat        LOT6
##DLT0.LOT01 DesLardons     Meat        LOT1
```

Phyloseq Data Structure

Phy_tree object is a phylo_class(tree) object

```
phy_tree(food)
##Phylogenetic tree with 508 tips and 507 internal nodes.

Tip labels: otu_00520, otu_00555, otu_00568, otu_00566, otu_00569,
 otu_00545, ...

Rooted; includes branch lengths.
```

Phyloseq Data Structure

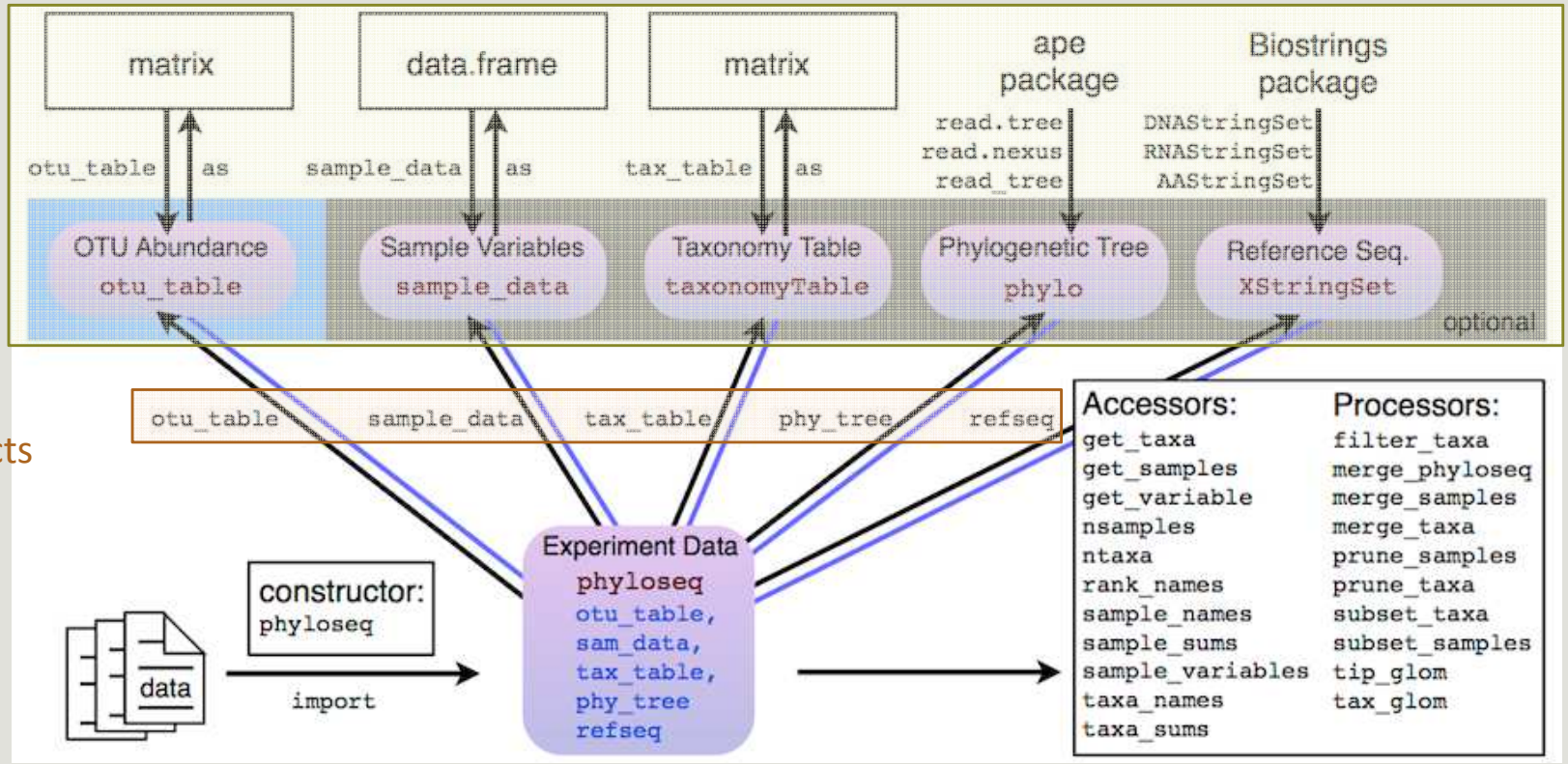
A phyloseq object is made of up to 5 **components** (or slots):

- otu_table: an OTU abundance table
- sample_data: a table of sample metadata, like sequencing technology, location of sampling, etc
- tax_table: a table of taxonomic descriptors for each OTU, typically the taxonomic assignation at different levels (Phylum, Order, Class, etc.)
- phy_tree: a phylogenetic tree of the OTUs
- refseq: a set of reference sequences (one per OTU), not present in food

Phyloseq Data Structure

Phyloseq objects

Functions to access/edit objects



Phyloseq : accessors

Phyloseq also offers the following accessors to extract parts of a phyloseq object:

- ntaxa / nsamples
- sample_names / taxa_names
- sample_sums / taxa_sums
- rank_names
- sample_variables
- get_taxa
- get_samples
- get_variable

➔ Try them on your own (on food) and guess what they do.

Phyloseq : accessors

```
ntaxa(food)
```

```
## [1] 508
```

ntaxa returns the number of taxa

```
nsamples(food)
```

```
## [1] 64
```

nsamples returns the number of samples

Phyloseq : accessors

```
head(sample_names(food))
```

```
## [1] "DLT0.LOT08" "DLT0.LOT05" "DLT0.LOT03" "DLT0.LOT07" "DLT0.LOT06"
## [6] "DLT0.LOT01"
```

```
head(taxa_names(food))
```

```
## [1] "otu_00520" "otu_00555" "otu_00568" "otu_00566" "otu_00569"
"otu_00545"
```

Names of the samples and taxa included in the phyloseq object

Phyloseq : accessors

```
head(sample_sums(food))
```

```
## DLT0.LOT08 DLT0.LOT05 DLT0.LOT03 DLT0.LOT07 DLT0.LOT06 DLT0.LOT01
##      11812      11787      11804      11806      11832      11857
```

```
head(taxa_sums(food))
```

```
## otu_00520 otu_00555 otu_00568 otu_00566 otu_00569 otu_00545
##        55       395        22        13      1998       210
```

Total count of each sample (i.e. samples library sizes) and total count of each taxa (i.e. overall abundances across all samples)

Phyloseq : accessors

```
rank_names(food)
```

```
## [1] "Kingdom" "Phylum"  "Class"   "Order"   "Family"  "Genus"
"Species"
```

Names of the taxonomic levels available in the tax_table slot

➔ Try on food1

```
sample_variables(food)
```

```
## [1] "EnvType" "FoodType" "Description"
```

Names of the contextual data recorded on the samples

Phyloseq : accessors

➔Find the

- library size of samples `MVT0.LOT01, MVT0.LOT07, MVT0.LOT09`
- overall abundance of OTUs `otu_00520, otu_00569, otu_00527`

Hint: What's the class of sample_sums(food) and taxa_sums(food)? How do you index them?

```
## sample library sizes
sample_sums(food)[c("MVT0.LOT01", "MVT0.LOT07", "MVT0.LOT09")]
## MVT0.LOT01 MVT0.LOT07 MVT0.LOT09
##      11743      11765      11739

## OTU overall abundances
taxa_sums(food)[c("otu_00520", "otu_00569", "otu_00527")]
## otu_00520 otu_00569 otu_00527
##        55      1998        58
```

Phyloseq : accessors

```
head(get_variable(food, varName = "EnvType"))
## [1] DesLardons DesLardons DesLardons DesLardons DesLardons DesLardons
## 8 Levels: BoeufHache VeauHache DesLardons MerguezVolaille ...
 Crevette
```

values for variable varName in sample data

```
head(get_sample(food, i = "otu_00520"))
## DLT0.LOT08 DLT0.LOT05 DLT0.LOT03 DLT0.LOT07 DLT0.LOT06 DLT0.LOT01
##          0          0          0          0          0          0
```

abundance values of OTU i in all samples (row of OTU table)

Phyloseq : accessors

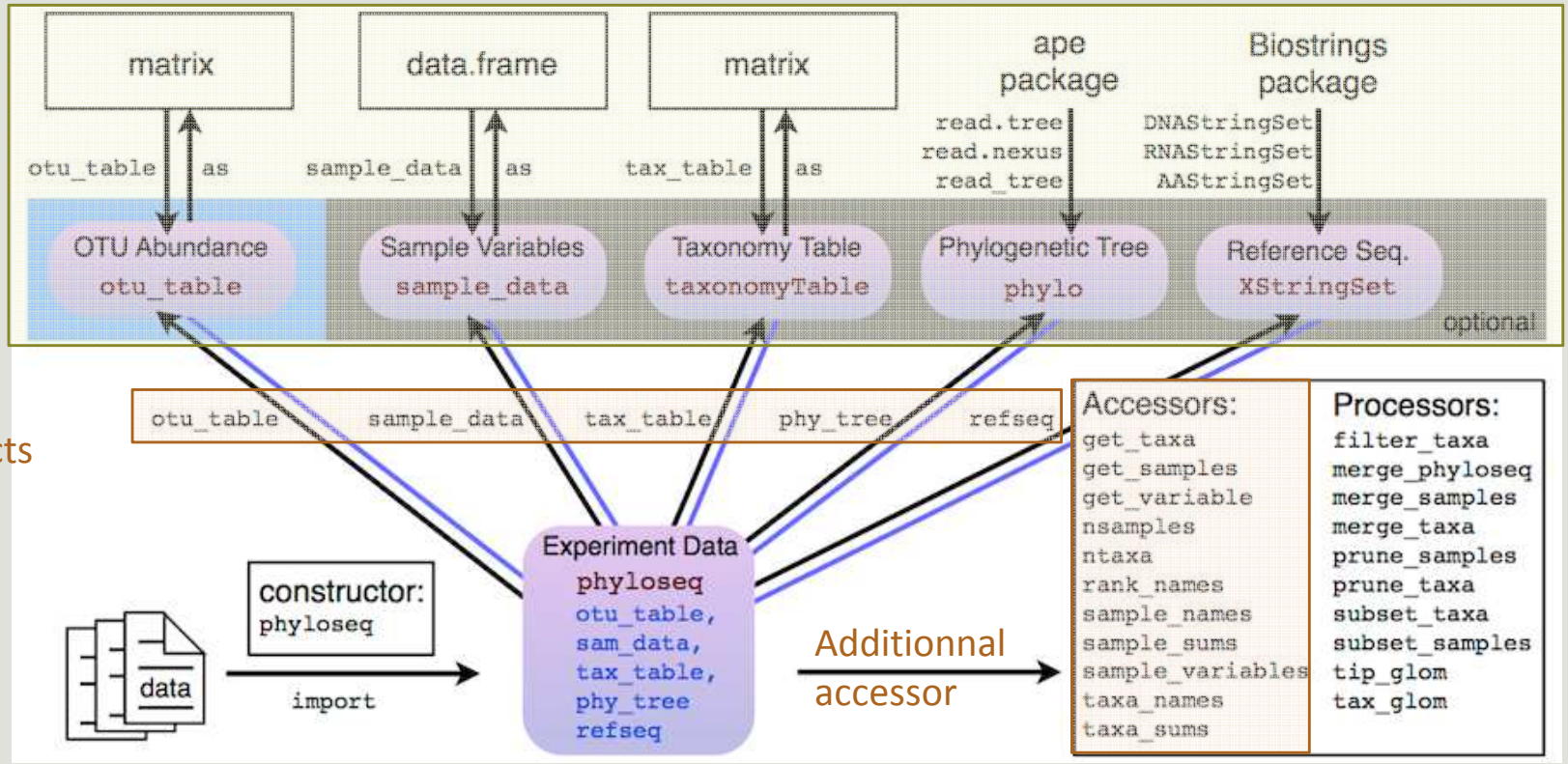
```
head(get_taxa(food, i = "MVT0.LOT07"))
## otu_00520 otu_00555 otu_00568 otu_00566 otu_00569 otu_00545
##         0        31         0         0        35         0
```

abundance values of all OTUs in sample i (column of OTU table)

Phyloseq Data Structure

Phyloseq objects

Functions to access/edit objects



Phyloseq : accessors/editors

➔ How to modify part of phyloseq objects?

- Change the taxonomic rank names in food1 object. Hint : use the (high level) accessors?

```
## desired rank names
new_rank <- c("Kingdom", "Phylum", "Class", "Order", "Family", "Genus", "Species")
## modify
colnames(tax_table(food1)) <- new_rank
## check
rank_names(food1) ## or head(tax_table(food1))
## [1] "Kingdom"    "Phylum"     "Class"      "Order"      "Family"     "Genus"
      "Species"
```

Phyloseq : accessors/editors

➔ How to modify part of phyloseq objects?

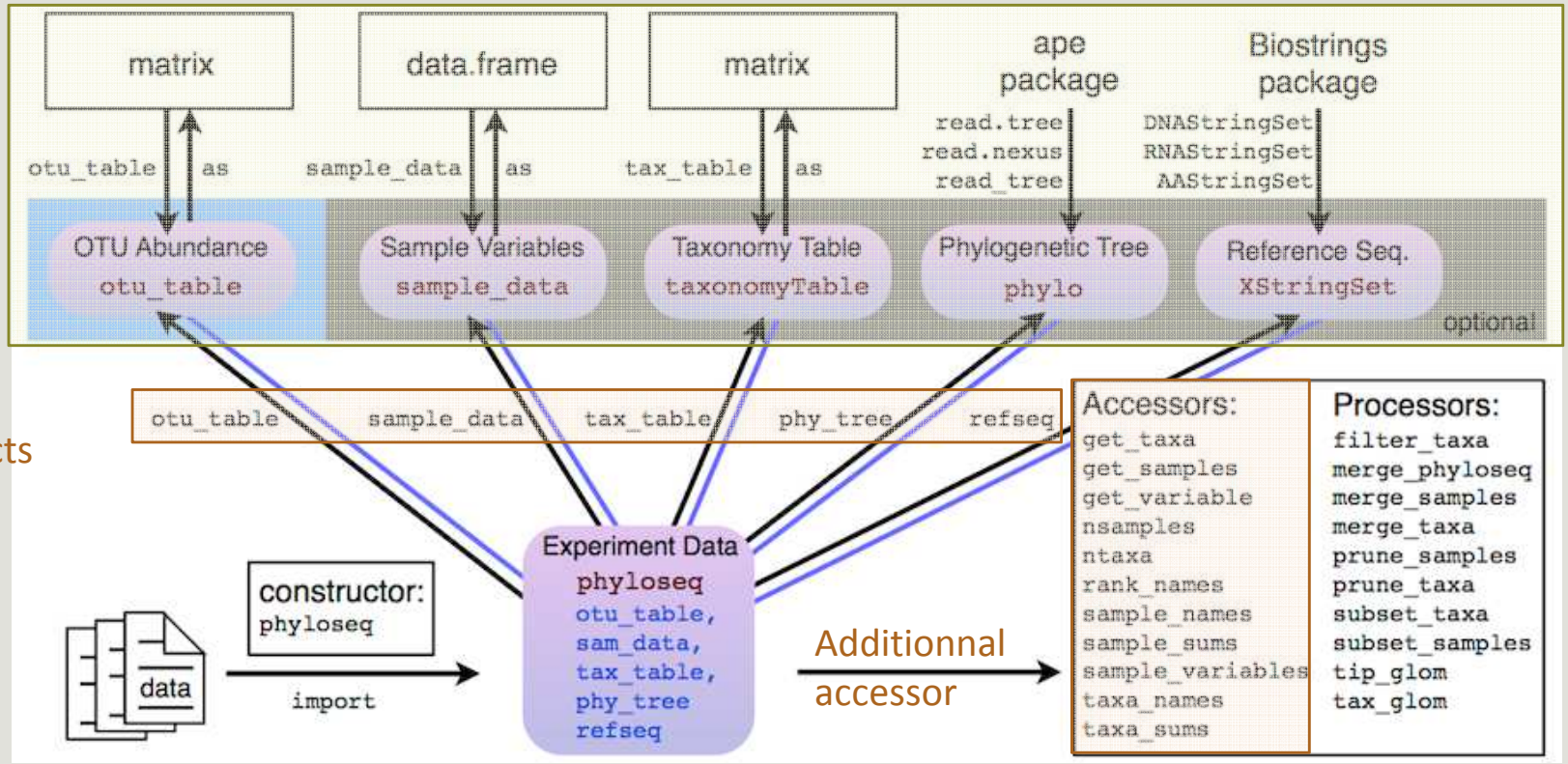
- Change the EnvType variable order to have meat products first and seafood second.

```
## desired order
correct.order <- c("BoeufHache", "VeauHache", "DesLardons"  , "MerguezVolaille",
"SaumonFume", "FiletSaumon"  , "FiletCabillaud", "Crevette")
## modify and convert in factor
sample_data(food)$EnvType <- factor(sample_data(food)$EnvType,levels = correct.order)
## check
levels(get_variable(food, "EnvType"))
## [1] "BoeufHache" "VeauHache" "DesLardons" "MerguezVolaille"
## [5] "SaumonFume" "FiletSaumon" "FiletCabillaud" "Crevette"
```

Phyloseq Data Structure

Phyloseq objects

Functions to access/edit objects



Phyloseq : Filtering Functions

- Prune
  - prune_taxa /prune_samples prunes unwanted taxa/samples from a phyloseq object based on a vector of taxa to keep
  - Taxa are passed as a vector, taxa, of character (“otu_1”, “otu_4”) or of logical (TRUE, FALSE, FALSE, TRUE)
  - Example : `prune_taxa(taxa, physeq)` would keep only OTUs otu_1, otu_4

- Subset
  - subset_taxa / subset_samples subsets unwanted taxa/samples from a phyloseq object based on conditions that must be met
  - The conditions (any number) can applied to any descriptor (e.g. taxonomy) of the OTUs included in the phyloseq object, physeq
  - `Subset_taxa(physeq, Phylum == "Firmicutes")` would keep only Firmicutes

Phyloseq : Filtering Functions

➔ Prune_samples: Keep only the first 10 samples of your phyloseq object food

```
## samplesToKeep
samplesToKeep <- sample_names(food)[1:10]
## prune_samples
prune_samples(samplesToKeep, food)
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 508 taxa and 10 samples ]
## sample_data() Sample Data: [ 10 samples by 3 sample variables ]
## tax_table() Taxonomy Table: [ 508 taxa by 7 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 508 tips and 507 internal nodes ]
```

Phyloseq : Filtering Functions

➔ Subset_samples :Keep only samples that corresponds to "DesLardons" and "MerguezVolaille"

```
subset_samples(food, EnvType %in% c("DesLardons", "MerguezVolaille"))
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 508 taxa and 16 samples ]
## sample_data() Sample Data: [ 16 samples by 3 sample variables ]
## tax_table() Taxonomy Table: [ 508 taxa by 7 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 508 tips and 507 internal nodes ]
```

Phyloseq : Filtering Functions

Advanced exercice : Subset and Multiple conditions combined

Keep only samples with Phylum affiliation equal to Firmicutes and Class affiliation to Bacilli.

Hint : AND is coded by « & » and OR is coded by « | »

```
small.food <- subset_taxa(food, Phylum == "Firmicutes" & Class == "Bacilli")
head(tax_table(small.food)[ , c("Phylum", "Class", "Order")])
## Taxonomy Table: [6 taxa by 3 taxonomic ranks]:
## Phylum Class Order
## otu_00583 "Firmicutes" "Bacilli" "Lactobacillales"
## otu_00574 "Firmicutes" "Bacilli" "Lactobacillales"
## otu_00581 "Firmicutes" "Bacilli" "Lactobacillales"
## otu_00591 "Firmicutes" "Bacilli" "Lactobacillales"
## otu_00582 "Firmicutes" "Bacilli" "Lactobacillales"
## otu_00586 "Firmicutes" "Bacilli" "Lactobacillales"

## Unique combinations (Phylum, Class)
unique(tax_table(small.food)[ , c("Phylum", "Class")])
## Taxonomy Table: [1 taxa by 2 taxonomic ranks]:
## Phylum Class
## otu_00583 "Firmicutes" "Bacilli"
```

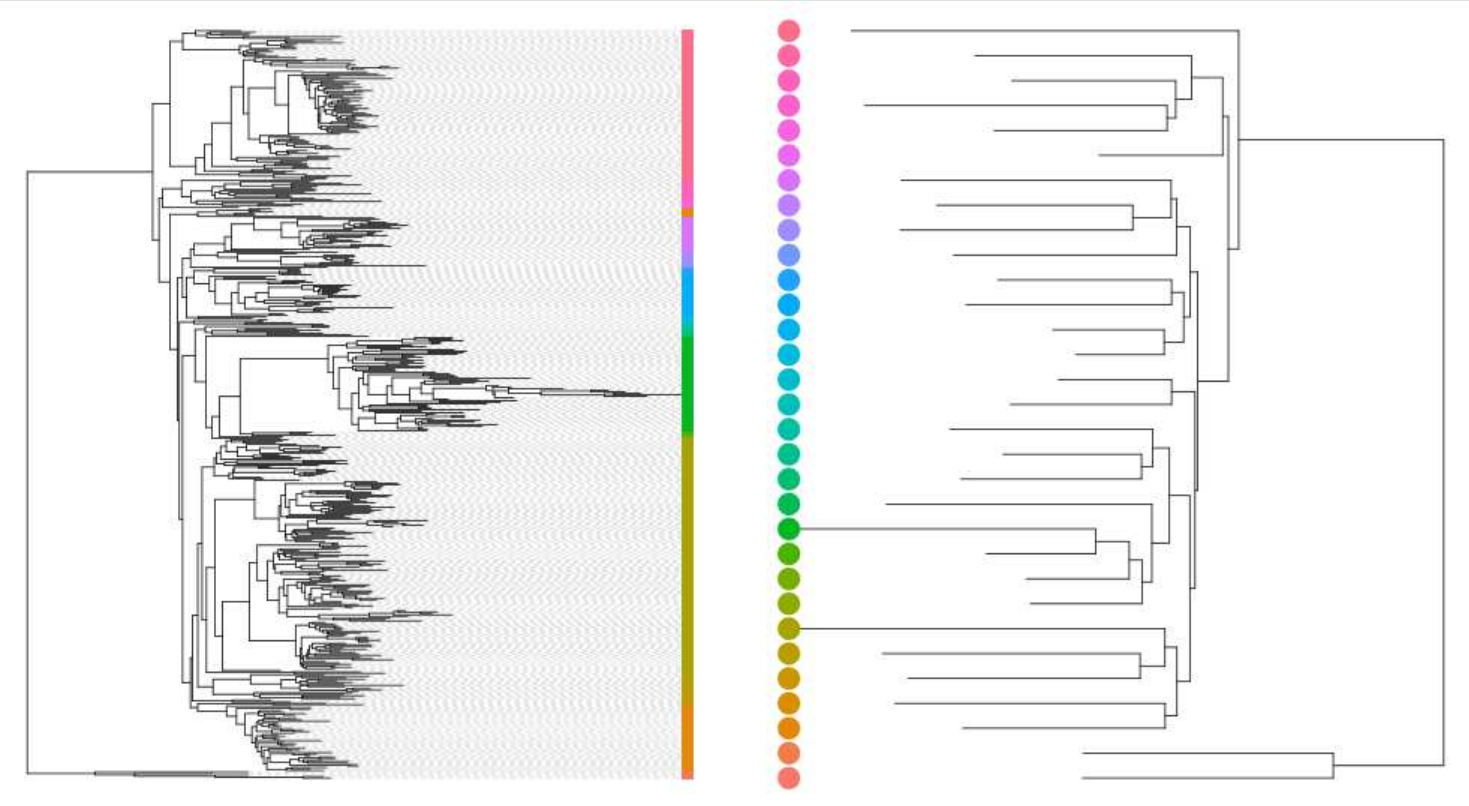
Phyloseq : Smoothing Functions

tax_glom agglomerates OTUs at a given taxonomic level. Finer taxonomic information is lost

```
mergedData <- tax_glom(food, "Phylum")
## check with ntaxa, tax_table
ntaxa(mergedData) ## number of different phyla
## [1] 11
tax_table(mergedData)[1:2, c("Phylum", "Order", "Class")]
## Taxonomy Table: [2 taxa by 3 taxonomic ranks]:
## Phylum Order Class
## otu_01101 "Proteobacteria" NA NA
## otu_01152 "Actinobacteria" NA NA
```

Phyloseq : Smoothing Functions

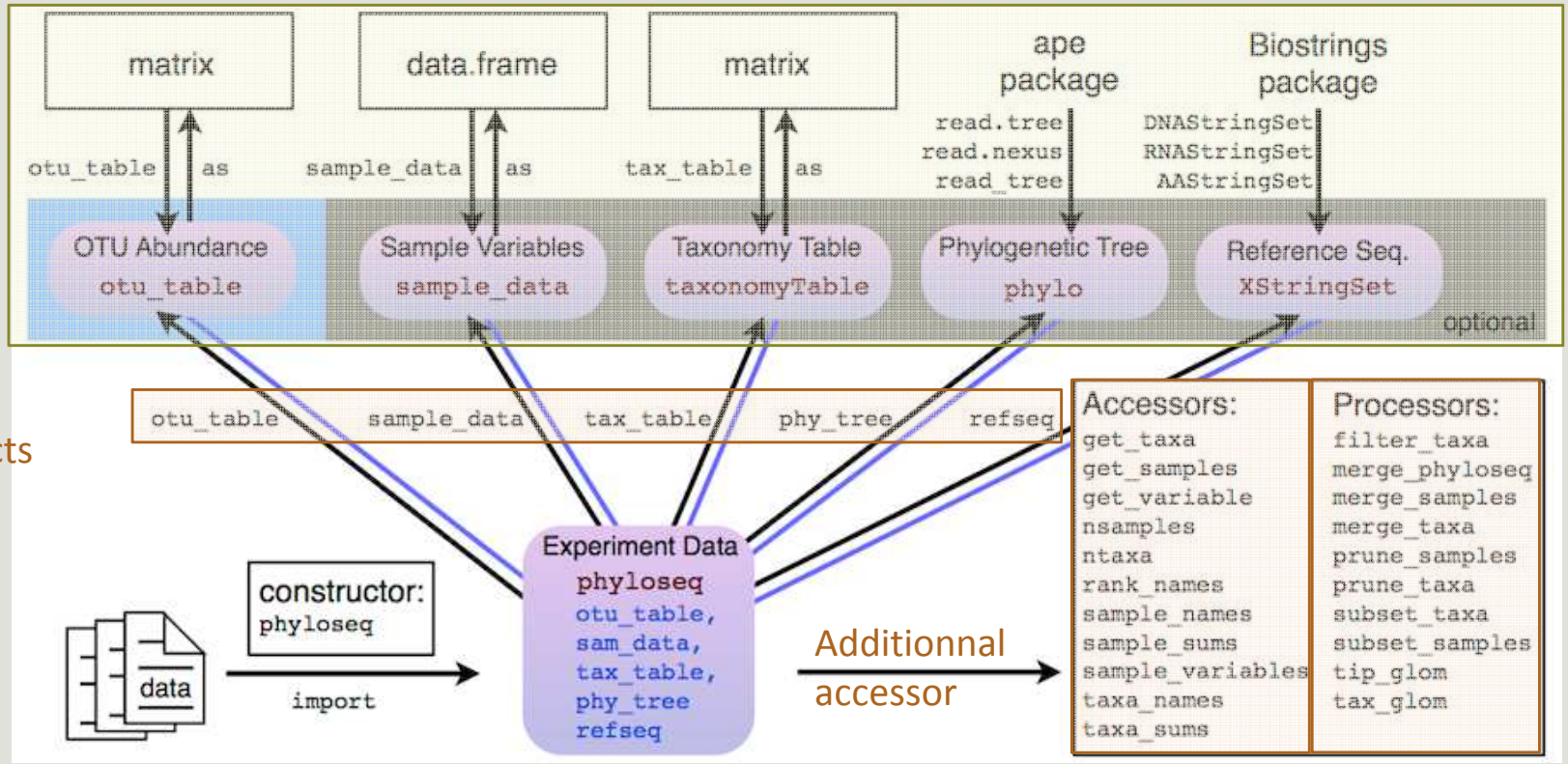
The effect of tax_glom is most obvious and best understood on the phylogenetic tree (OTUs are colored by phylum).



Phyloseq Data Structure

Phyloseq objects

Functions to access/edit objects



Additionnal editor

Phyloseq : Abundance manipulation

rarefy_even_depth downsamples/normalizes all samples to the same depth and prunes OTUs that disappear from all samples as a result

➔ Try on food

```
foodRare <- rarefy_even_depth(food, rngseed = 1121983)
## `set.seed(1121983)` was used to initialize repeatable random
subsampling.
## Please record this for your records so others can reproduce.
## Try `set.seed(1121983); .Random.seed` for the full vector
## ...
## Some OTUs were removed because they are no longer
## present in any sample after random subsampling
## check with sample_sums
sample_sums(foodRare)[1:5]
## DLT0.LOT08 DLT0.LOT05 DLT0.LOT03 DLT0.LOT07 DLT0.LOT06
##       11718       11718       11718       11718        11718
```

Phyloseq : Abundance manipulation

transform_sample_counts applies a function to the abundance vector of each sample. It can be useful for normalization

```
count_to_prop <- function(x) {return( x / sum(x) )}
```

➔transforms counts to proportions

```
foodTrans <- transform_sample_counts(food, count_to_prop)
sample_sums(foodTrans)[1:5] ## should be 1
## DLT0.LOT08 DLT0.LOT05 DLT0.LOT03 DLT0.LOT07 DLT0.LOT06
##          1          1          1          1          1
```

Phyloseq : in brief

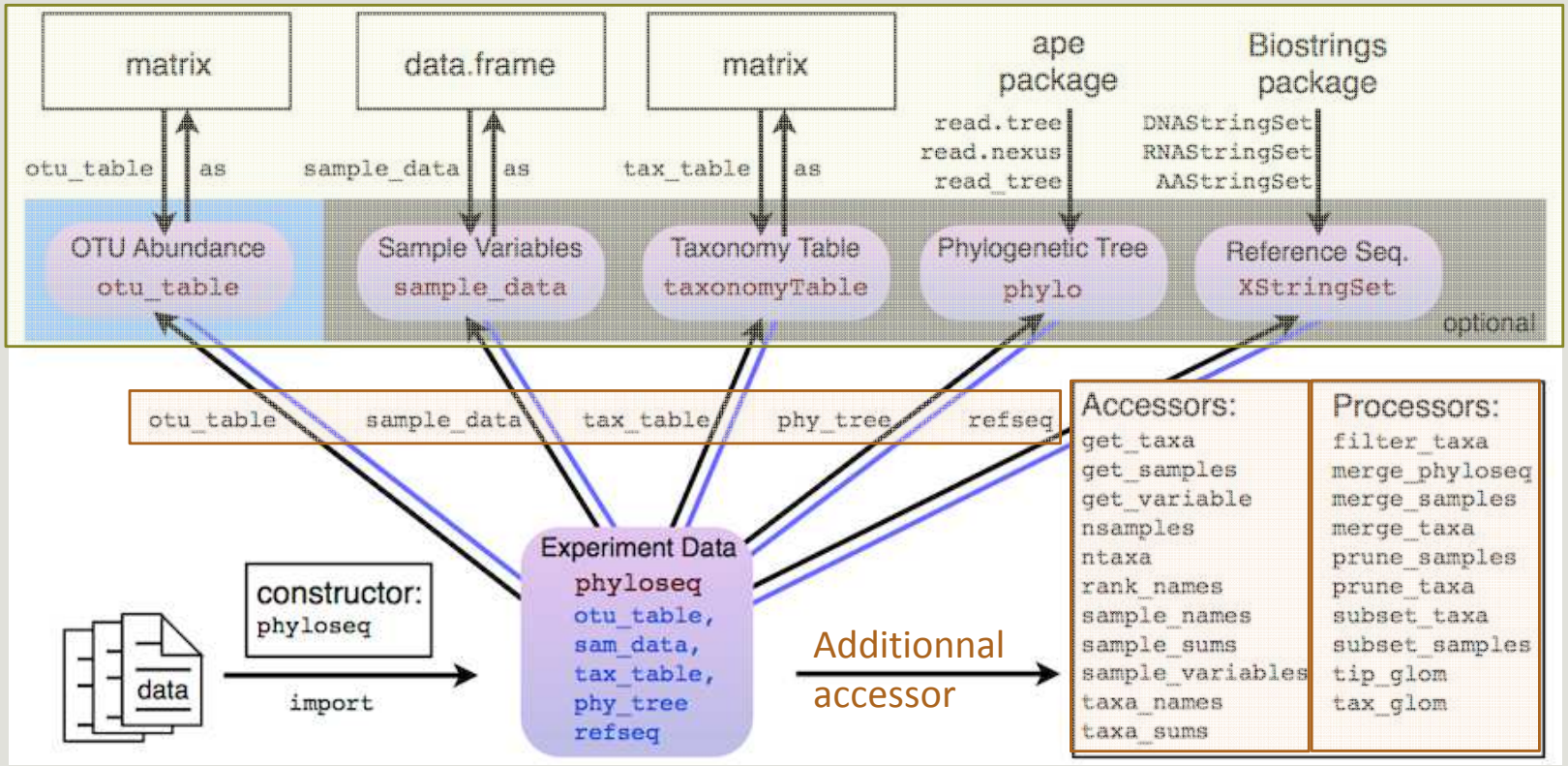
A nice data structure to store the count table, taxonomic information, contextual data and phylogenetic tree as different components of a single R object .

- Functions to import data from biom files, qiime output files or plain tabular files
- Accessors to access different component of your dataset
- Samples and taxa names are coherent between the different components
- Filters to keep only part of the dataset
- Smoothers to aggregate parts of the dataset
- Manipulators to rarefy and transform samples

Phyloseq in brief

Phyloseq objects

Functions to access objects



Additionnal editor

Biodiversity analysis

Biodiversity analysis

- Exploring the samples composition
- Notions of biodiversity
- α-diversity analysis
- β-diversity analysis

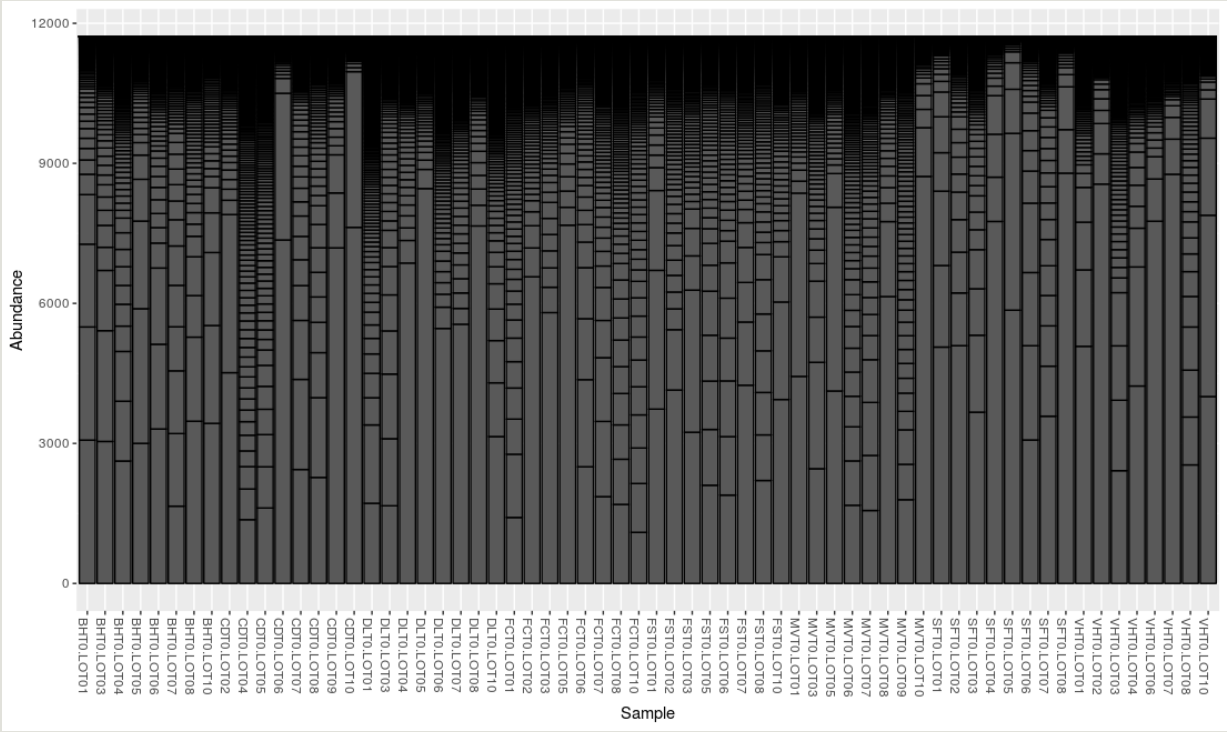
Biodiversity analysis

VISUALIZATION

Exploring biodiversity : visualization

Visualization of abundance per sample thanks to plot_bar().

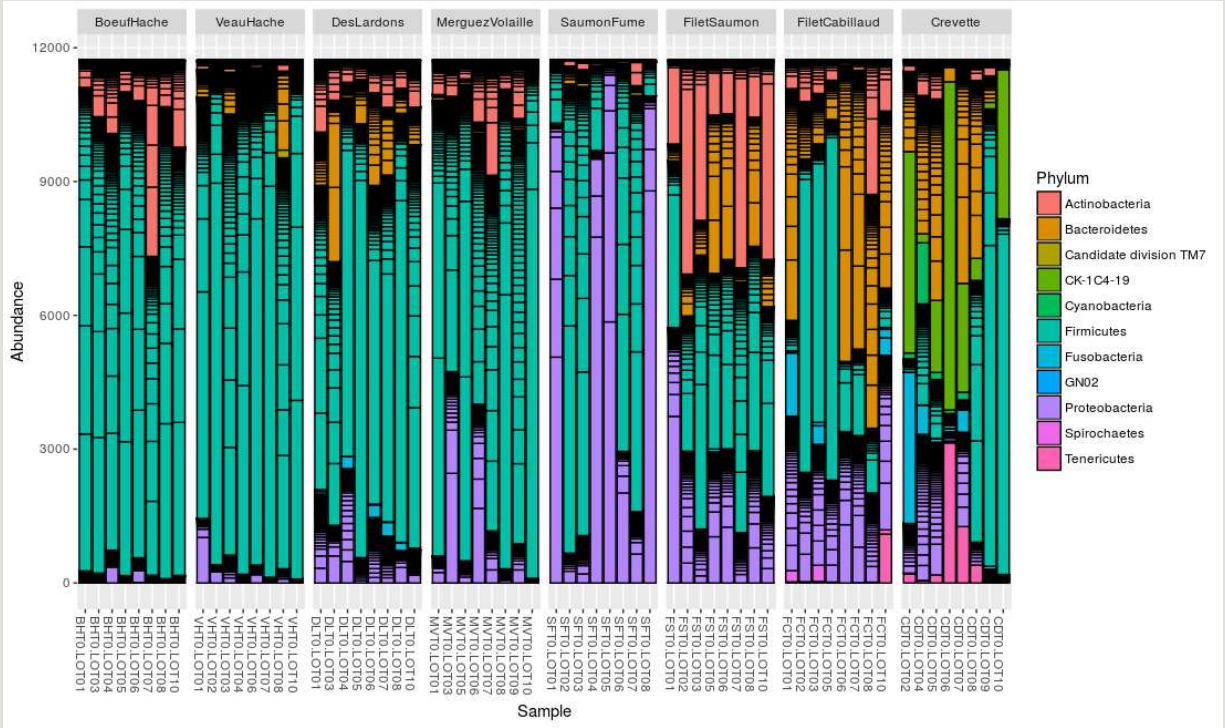
```
p <- plot_bar(foodRare)

plot(p) ## Base graphic, ugly
```



Exploring biodiversity : visualization

➔ Thanks to ggplot2, organize samples and color OTU by Phylum

```
## aes, fill bar according to phylum
p <- plot_bar(foodRare, fill = "Phylum")

## add facets
p <- p + facet_wrap(~EnvType, scales = "free_x", nrow = 1)

plot(p)
```



Exploring biodiversity : visualization

➔ Limitations:

- Plot bar works at the OTU-level...
- ...which may lead to graph cluttering and useless legends
- No easy way to look at a subset of the data
- Works with absolute counts (beware of unequal depths)

load-extra-functions.R

Exploring biodiversity : visualization

- Customization: plot_composition function
  - subsets OTUs at a given taxonomic level
  - aggregates OTUs at another taxonomic level
  - shows only a given number of OTUs (by default the 10 most abundant)
  - works with relative abundances

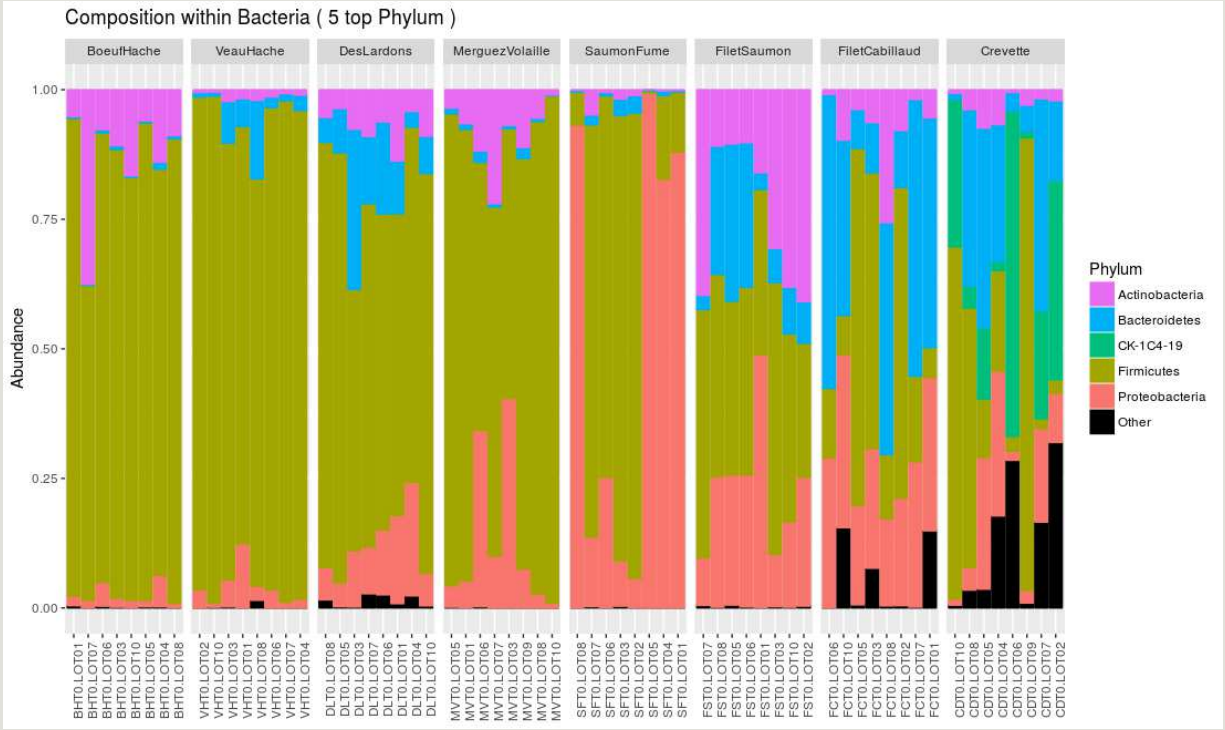
```
p <- plot_composition(physeq, "tax_level_selection",
 "tax_name_selection", "tax_level_aggregation",
 numberOfTaxa = NB_OTU, fill = "tax_color")
```

Exploring biodiversity : visualization

load-extra-functions.R

➔ Select Bacteria (at Kingdom level) and aggregate by Phylum, select the 5 most abundant

```
## plot_composition

p <- plot_composition(foodRare, "Kingdom", "Bacteria", "Phylum", numberOfTaxa = 5, fill = "Phylum")

## plot facetting by EnvType

p <- p + facet_wrap(~EnvType, scales = "free_x", nrow = 1)

plot(p)
```

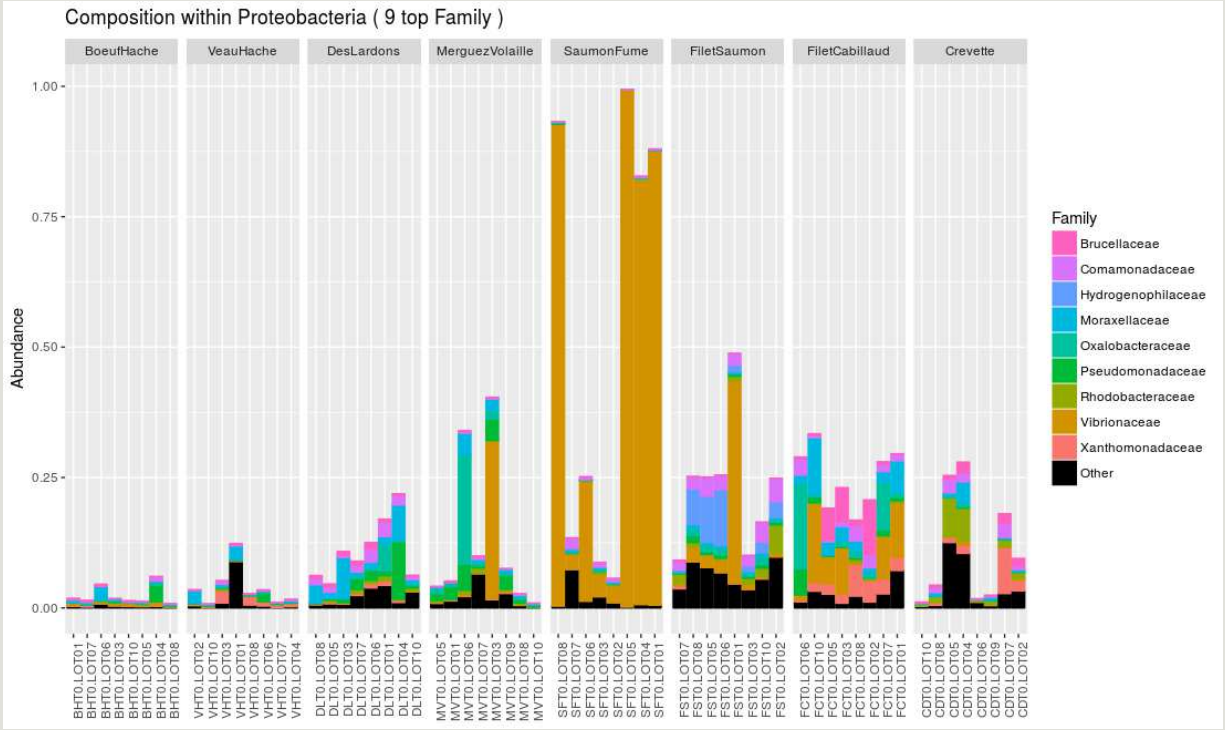


Exploring biodiversity : visualization

load-extra-functions.R

➔ Select Proteobacteria (at Phylum level) and aggregate by Family and select the 9 most abundant

```
##plot_composition

p <- plot_composition(foodRare, "Phylum", "Proteobacteria", "Family", numberOfTaxa = 9, fill = "Family")

## plot facetting

p <- p + facet_wrap(~EnvType, scales = "free_x", nrow = 1)

plot(p)
```

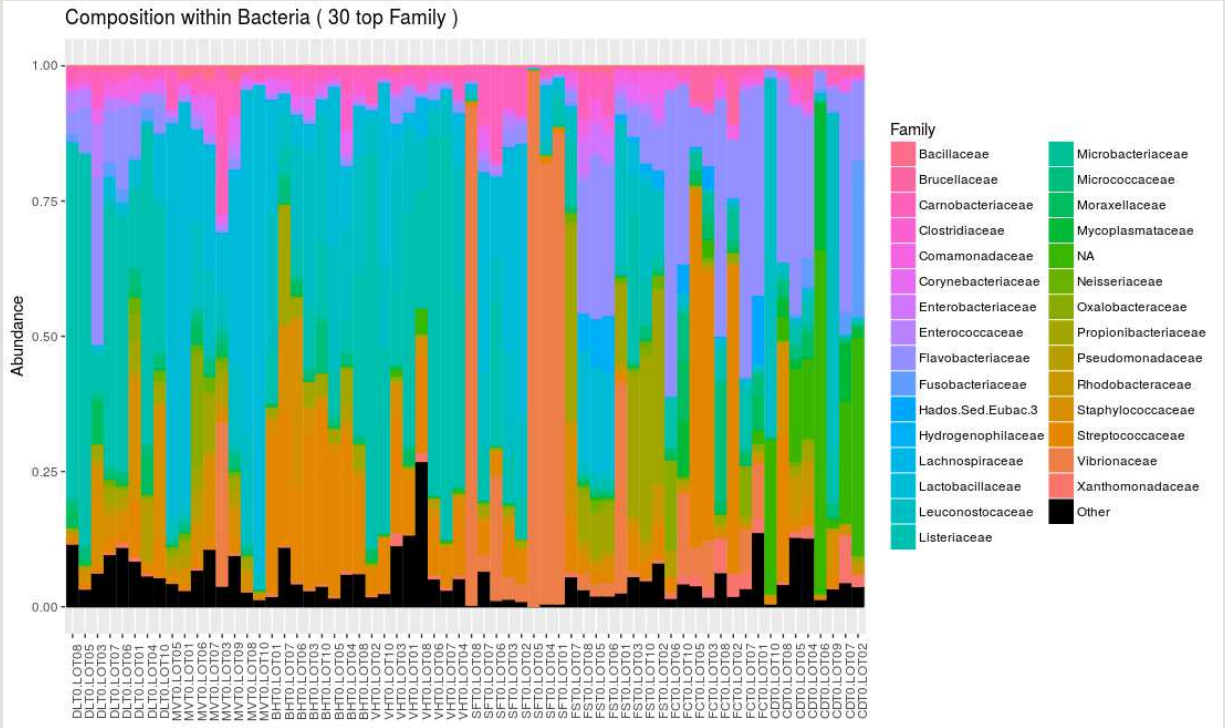


Exploring biodiversity : visualization

load-extra-functions.R

➔How to select the 30 most abundant Family ?

```
##plot_composition

p <- plot_composition(foodRare,
"Kingdom", "Bacteria", "Family",
numberOfTaxa = 30, fill =
"Family")

## plot facetting

p <- p + facet_wrap(~EnvType,
scales = "free_x", nrow = 1)

plot(p)
```



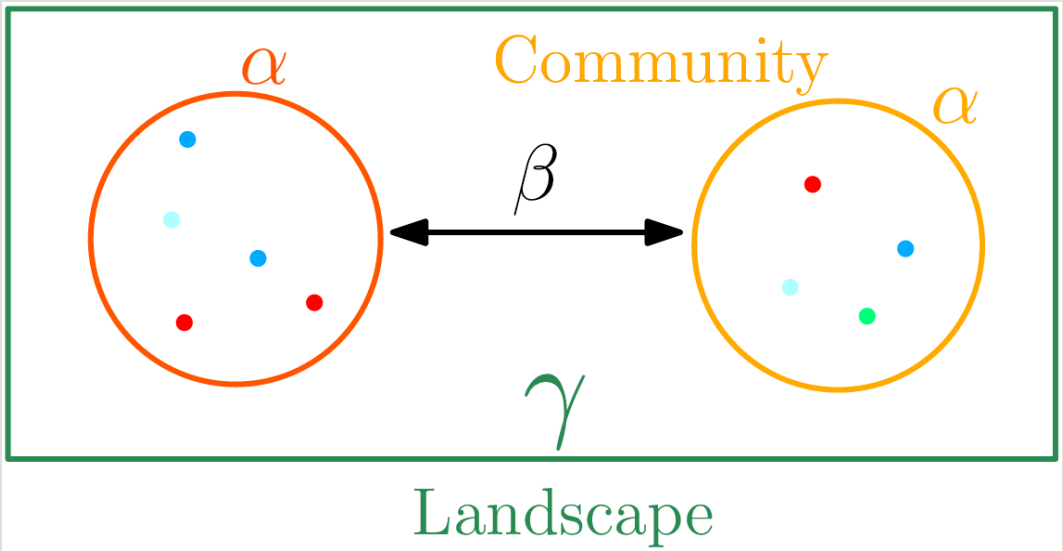
Biodiversity analysis

DIVERSITY INDICES

Exploring biodiversity : statistical indices

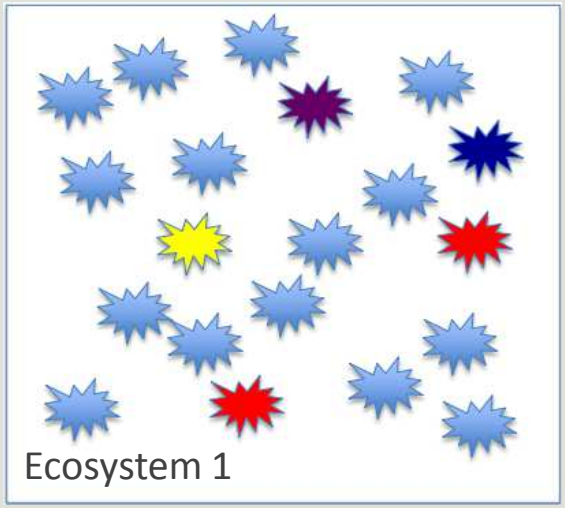
Second step, compute and compare diversity indices. Three flavors of diversity:

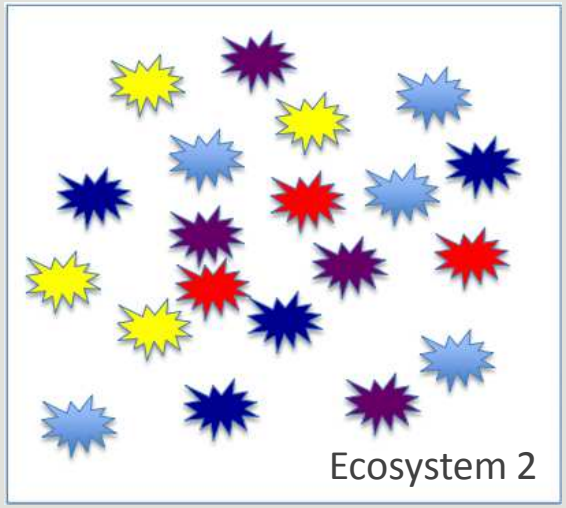
- **α-diversity**: diversity within a community;
- **β-diversity**: diversity between communities;
  - β-dissimilarities/distances
    - Dissimilarities between pairs of communities
    - Often used as a first step to compute-diversity
- **γ-diversity** : diversity at the landscape scale (blurry for bacterial communities)



Exploring biodiversity : descriptors

- The **richness** corresponds to number of OTUs or functional groups present in communities. It caracterizes the **composition**
- The **diversity** takes into account the relative abundancy of species, it caracterizes the **structure**





Richness : Eco1 = Eco2

Diversity: Eco2 > Eco1

Exploring biodiversity : statistical indices

Qualitative (Presence/Absence) vs. Quantitative (Abundance)

- Qualitative gives less weight to dominant species
- Qualitative is more sensitive to differences in sampling depths
- Qualitative emphasizes difference in taxa diversity rather than differences in composition

Compositional vs. Phylogenetic

- Compositional does not require a phylogenetic tree
- is more sensitive to erroneous OTU picking
- gives the same importance to all OTUs

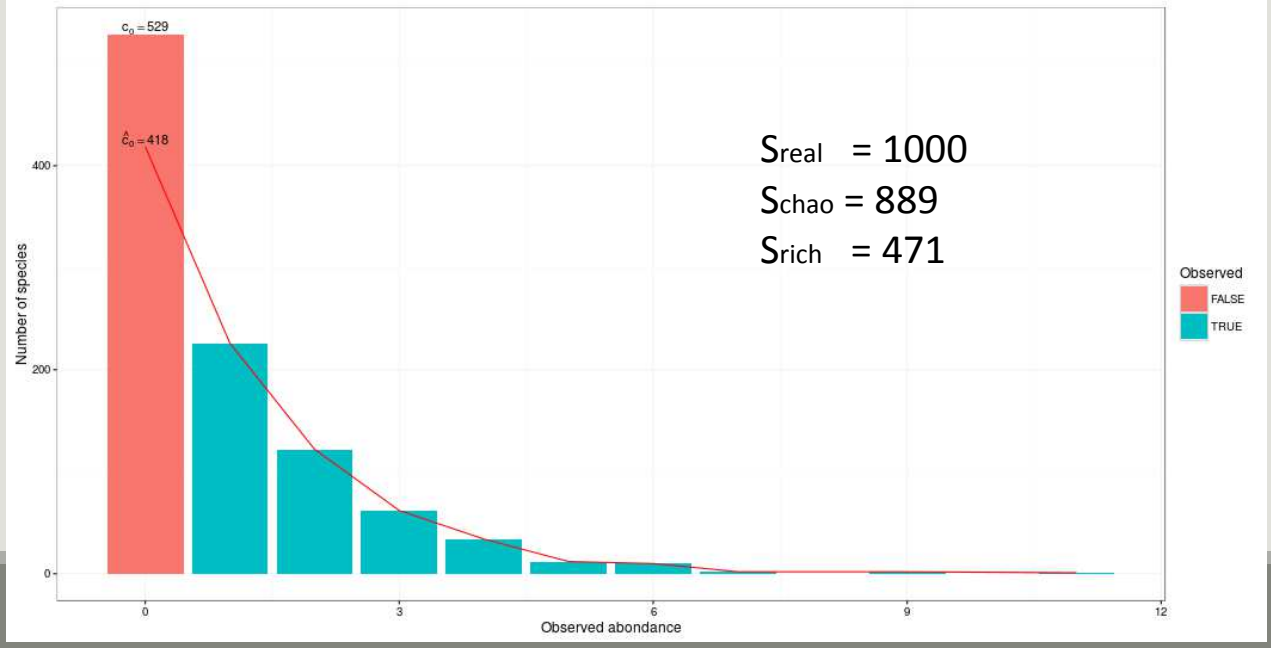
Biodiversity analysis

α-DIVERSITY INDICES

Exploring biodiversity : α-diversity

α-diversity is equivalent to the richness : number of species

Richness	Chao
Number of observed species	Richness + (estimated) number of unobserved species



S_{real} = 1000

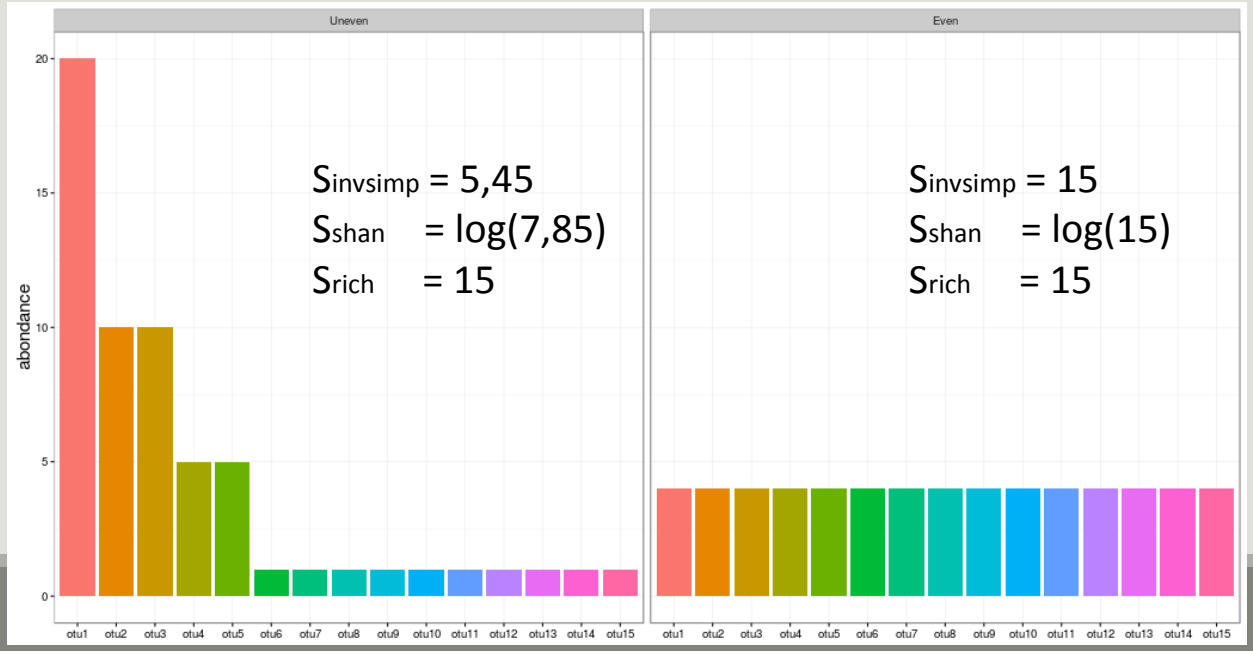
S_{chao} = 889

S_{rich} = 471

Exploring biodiversity : α-diversity

α-diversity is equivalent to the richness : number of species

Shannon	Inv-Simpson
Evenness of the species abundance distribution	Inverse probability that two sequences sampled at random come from the same species



Interpretation :
15 observed species, but according to Shannon, the left example acts like there is 7.85 equally abundant species (5.45 for invSimp)

Exploring biodiversity : α-diversity

α-diversity indices available in phyloseq :

- Species **richness** : number of observed OTUs
- **Chao1** : number of observed OTU + estimate of the number of unobserved OTUs
- **Shannon** entropy / **Jensen** : the width of the OTU relative abundance distribution. Roughly, it reflects our (in)ability to predict the OTU of a randomly picked bacteria
- **Simpson** : 1 - probability that two bacteria picked at random in the community belong to different OTU
- **Inverse Simpson** : inverse of the probability that two bacteria picked at random belong to the same OTU

Exploring biodiversity : α-diversity

Richness are plotted with plot_richness

➔ Try it on food

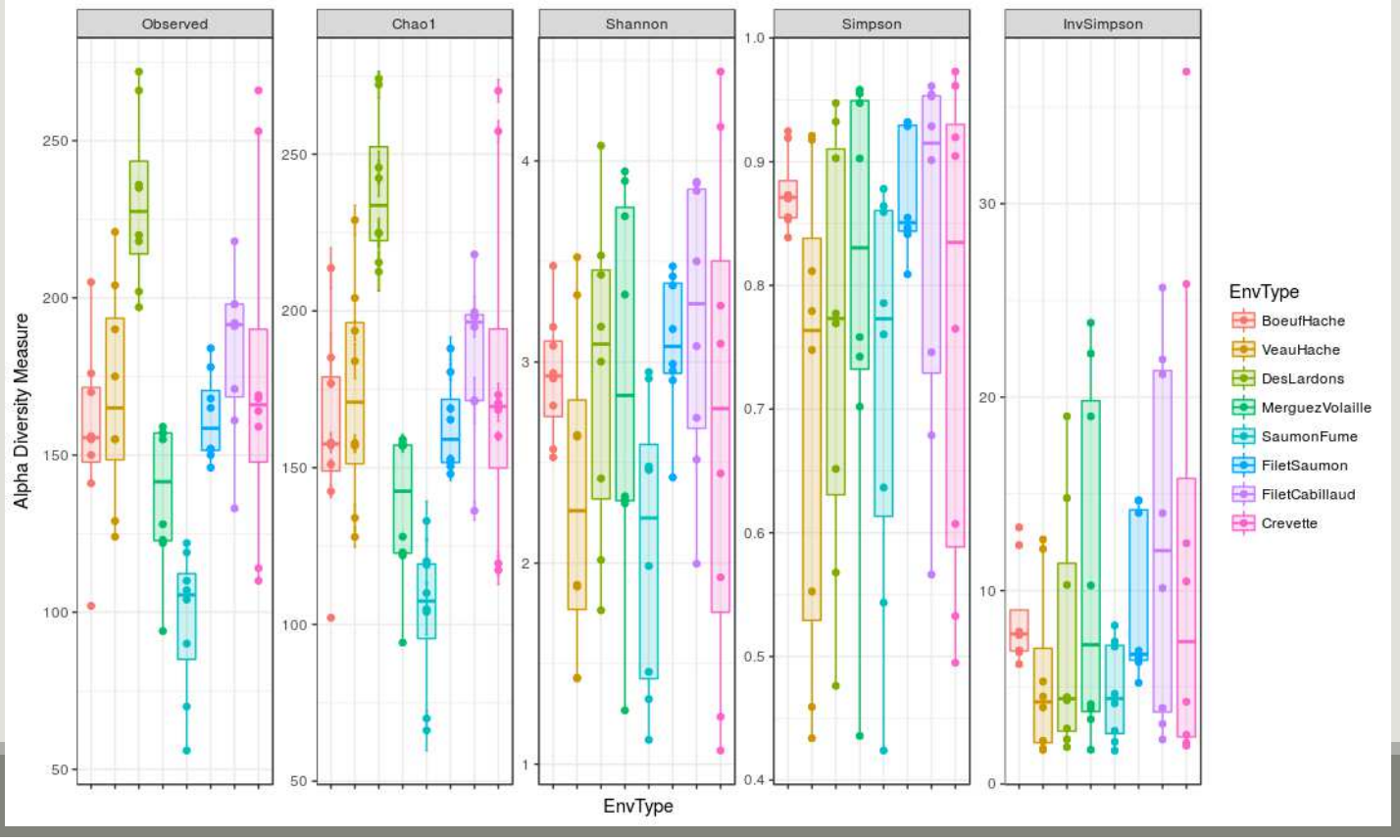
```
plot_richness(foodRare)
```

➔ Custom it on EnvType, color EnvType, select measures as "Observed", "Chao1", "Shannon", "Simpson", "InvSimpson", and plot as boxplot

Note the x = "EnvType " passed on to the aes mapping of a ggplot.

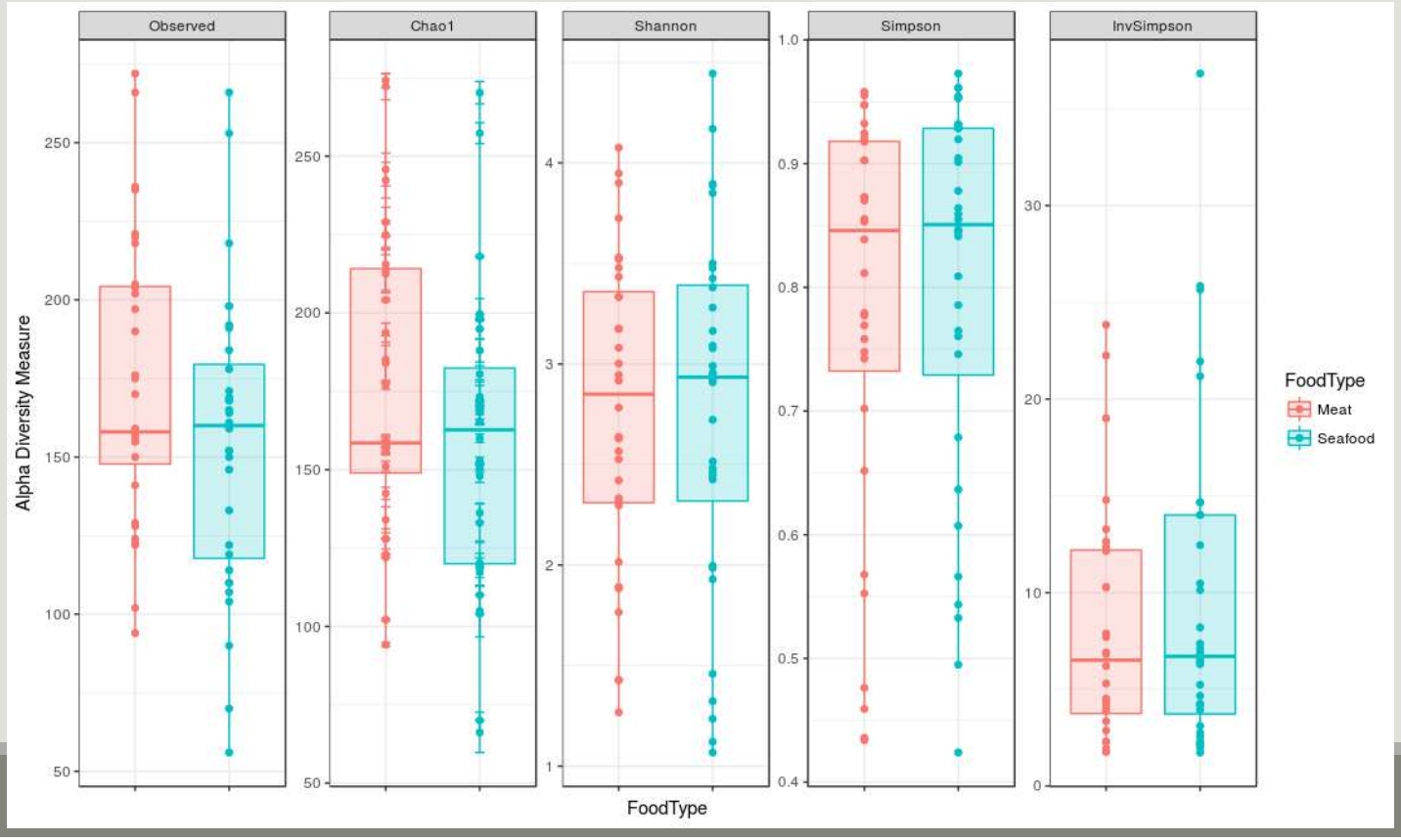
```
## plot_richness
p <- plot_richness(foodRare, color = "EnvType", x = "EnvType",
 measures = c("Observed", "Chao1", "Shannon", "Simpson",
 "InvSimpson"))
## plot as boxplot
p <- p + geom_boxplot(aes(fill = EnvType), alpha=0.2)
plot(p)
```

Exploring biodiversity : α-diversity



Exploring biodiversity : α-diversity

➔ Try with FoodType instead of EnvType



Exploring biodiversity : α-diversity

Numeric values of α-diversities are given by estimate_richness (used internally by plot_richness)

```
alpha.diversity <- estimate_richness(foodRare, measures = c("Observed", "Chao1", "Shannon"))
head(alpha.diversity)
```

```
##              Observed     Chao1   se.chao1   Shannon
## DLT0.LOT08        202  212.5000   6.024155  2.015369
## DLT0.LOT05        197  215.4545   9.049244  1.765450
## DLT0.LOT03        218  224.9545   4.521082  3.433389
## DLT0.LOT07        220  224.7143   3.779245  3.002275
## DLT0.LOT06        266  272.1818   4.171422  3.175710
## DLT0.LOT01        272  274.1176   2.148341  4.075913
```

```
write.table(alpha.diversity, "myfile.txt")
```

Exploring biodiversity : α-diversity

A quick ANOVA : tests if observed richness is significantly different in function of EnvType

```
data <- cbind(sample_data(foodRare), alpha.diversity)
foodRare.anova <- aov(Observed ~ EnvType, data)
summary(foodRare.anova)
##              Df  Sum Sq  Mean Sq  F value    Pr(>F)
## EnvType       7   81674    11668    11.51  5.96e-09 ***
## Residuals    56   56774     1014
## ---
## Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

There is a significant effect of environment type on richness

Exploring biodiversity : α-diversity

A quick ANOVA : tests if Shannon indices is significantly different in function of EnvType

```
foodRare.anova <- aov(Shannon ~ EnvType, data)
summary(foodRare.anova)
##              Df  Sum Sq  Mean Sq  F value  Pr(>F)
## EnvType       7    7.91   1.1300    1.771   0.111
## Residuals    56   35.72   0.6379
```

EnvType effect on Shannon diversity is not significant

Exploring biodiversity : α-diversity

Interpretation

- Many taxa observed in Deslardons (high Chao1, high Observed)…
- ...but low Shannon and Inverse-Simpson

➔ communities dominated by a few abundant taxa

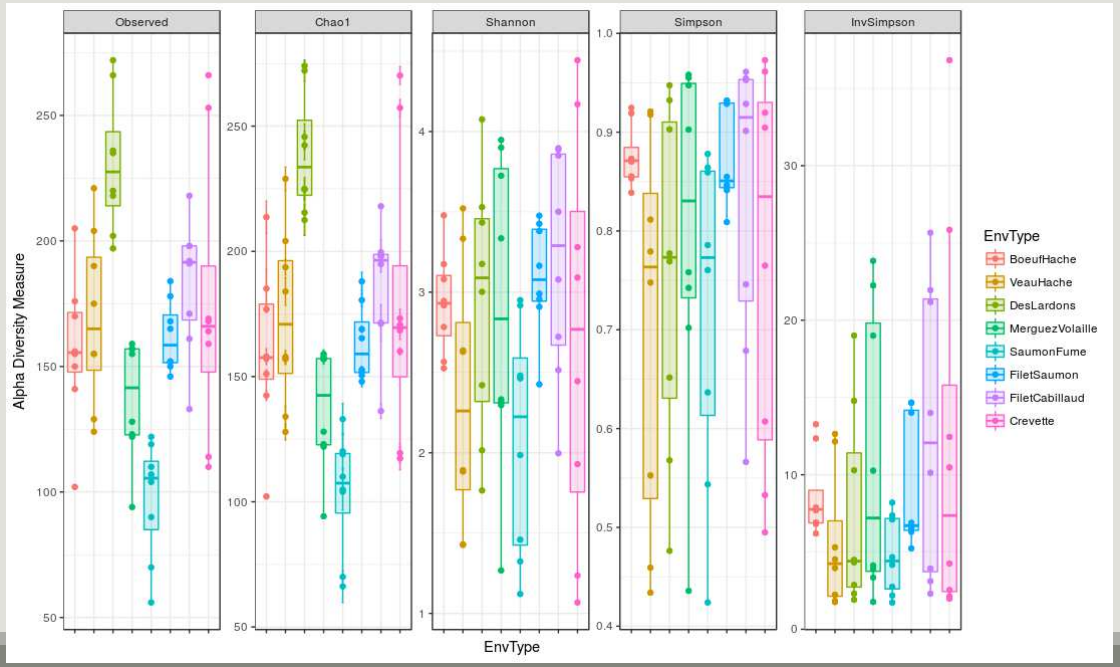
- Environments differ a lot in terms of richness...
- ...but not so much in terms of Shannon diversity

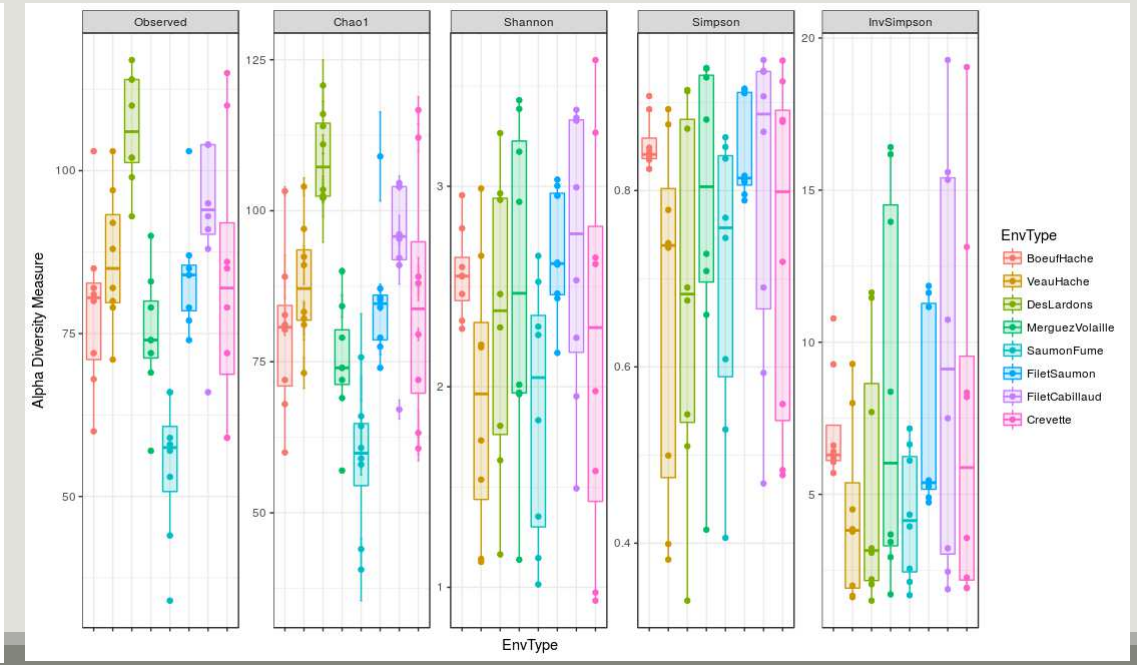
➔ Effective diversities are quite similar

Exploring biodiversity : α-diversity

WARNING : Many diversities (richness, Chao) depend a lot on rare OTUs. Do not trim rare OTUs before computing them as it can drastically alter the result.

α-diversity: without (left) and with (right) trimming on rare OTU (total abundance < 500)





Biodiversity analysis

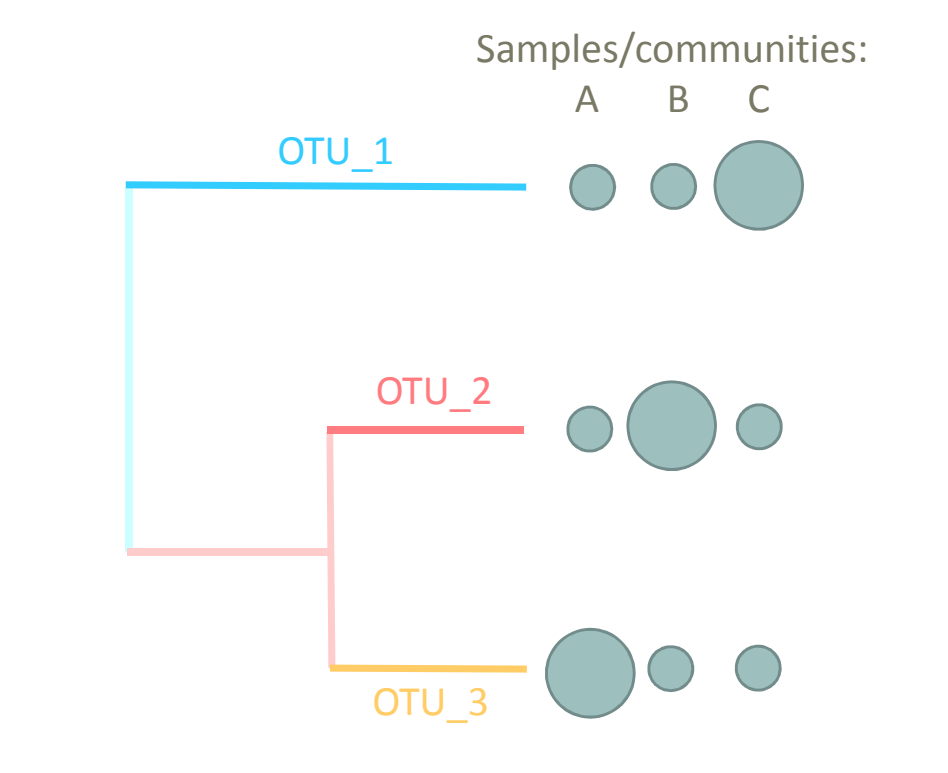
β-DIVERSITY INDICES

Exploring biodiversity : β-diversity

Many diversities (both compositional and phylogenetic) offered by Phyloseq through the generic distance function.

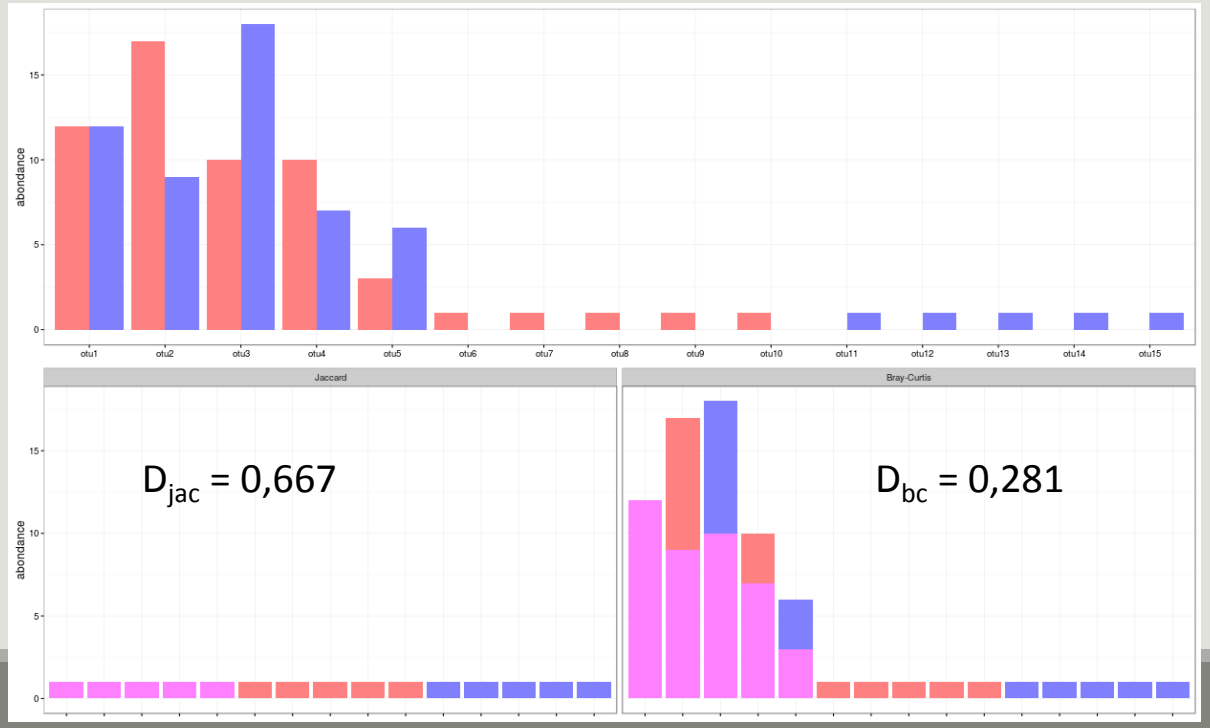
Different dissimilarities capture different features of the communities:

- qualitatively, communities are very similar
- quantitatively they are very different
- phylogeneticaly two communities seems to be closer than the third one.



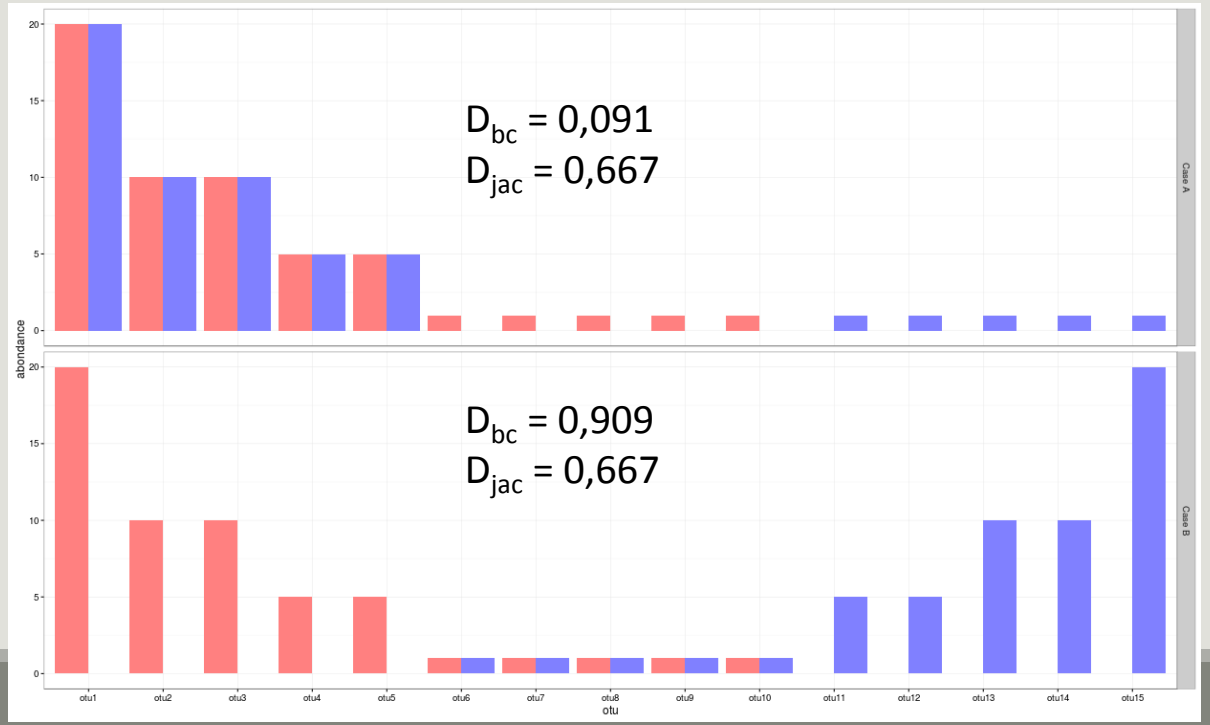
Exploring biodiversity : β-diversity

Jaccard	Bray-Curtis
Fraction of species specific to either 1 or 2	Fraction of the community specific to 1 or to 2



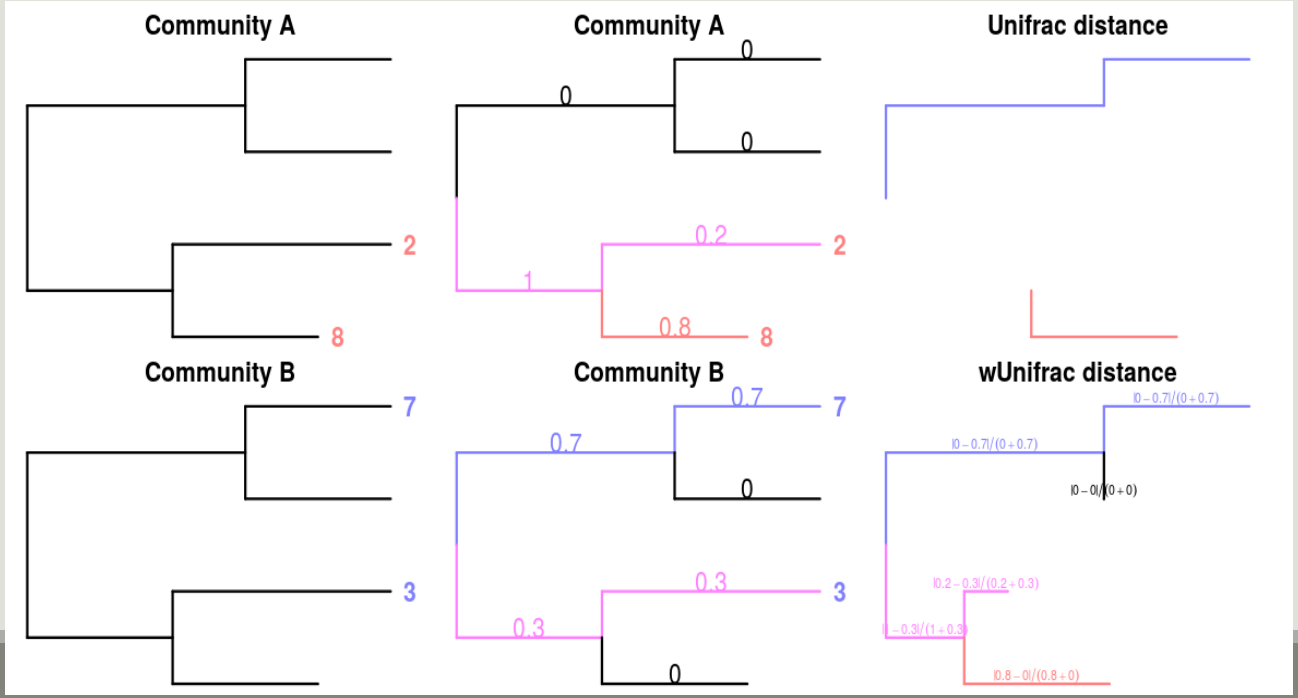
Exploring biodiversity : β-diversity

Jaccard	Bray-Curtis
Fraction of species specific to either 1 or 2	Fraction of the community specific to 1 or to 2



Exploring biodiversity : β-diversity

Unifrac	Weigthed-Unifrac
Fraction of the tree specific to either 1 or 2	Fraction of the diversity specific to 1 or to 2



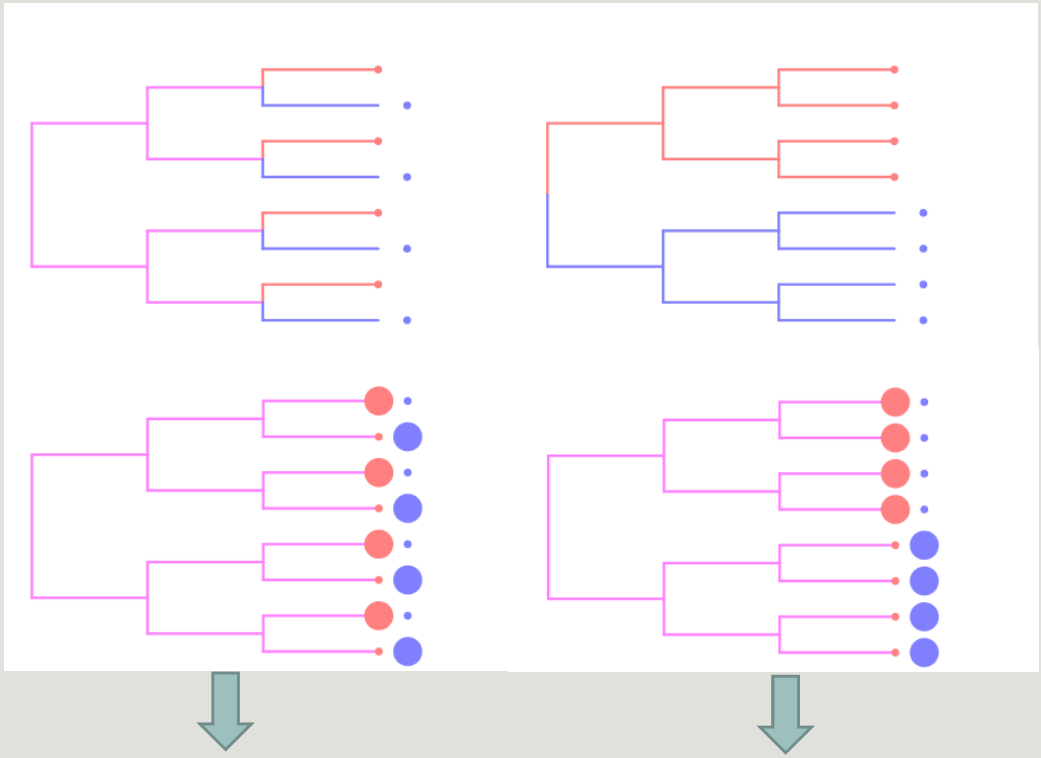
If all branch length are equal to 1, only branches present in at least one community are taken into account :

$$Unifrac - \frac{\sum specific\_branch\_length}{\sum all \quad branch \quad length} - 0.6$$

$$WUnifrac - \frac{\sum reduced\_branch\_length}{\sum non\_reduced\_branch\_length} - 0.74$$

Exploring biodiversity : β-diversity

➔ What do you conclude in terme of Jaccard, Bray Curtis, Unifrac and weigthed Unifrac values?



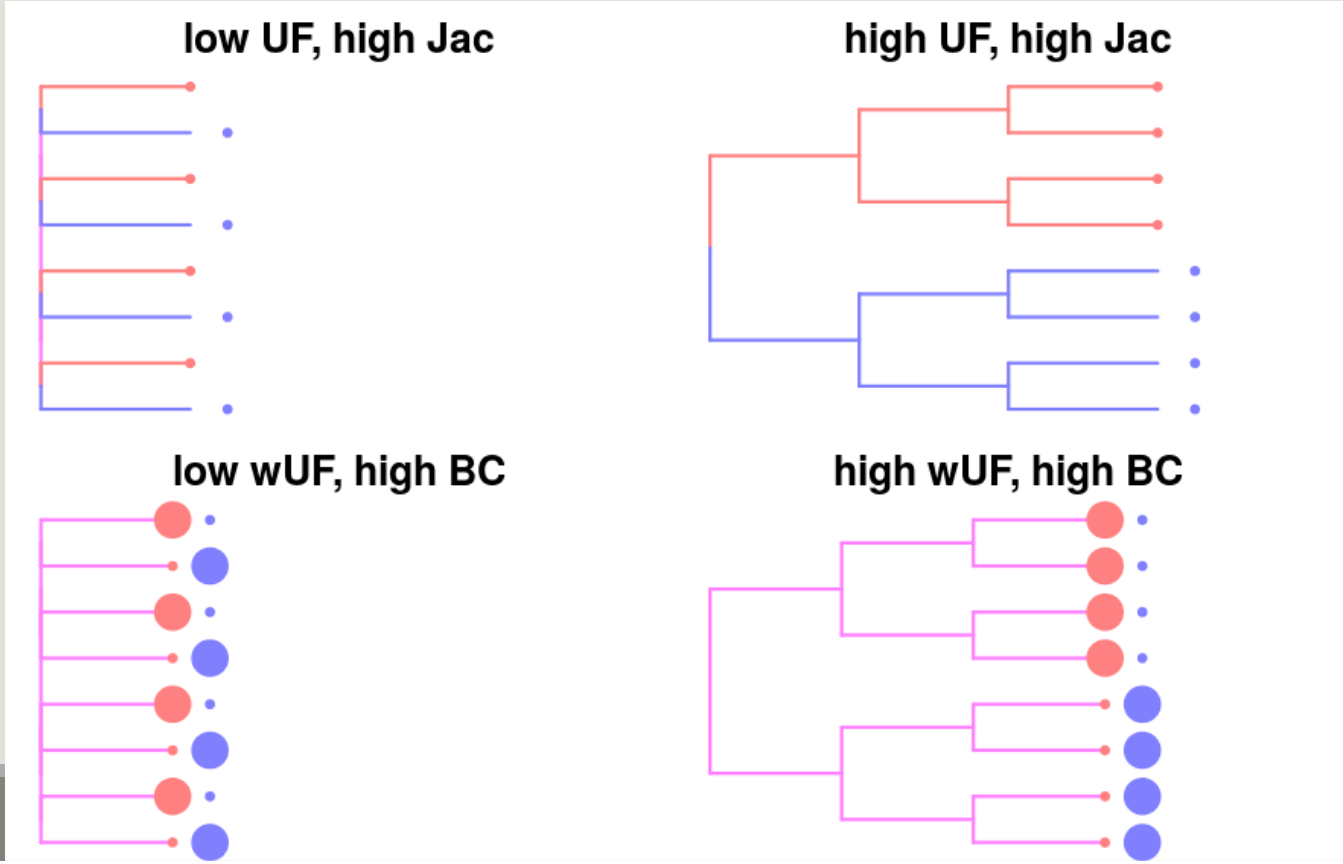
Qualitative impact

Quantitative impact

Low phylogenetic impact

Highphylogenetic impact

Exploring biodiversity : β-diversity



Exploring biodiversity : β-diversity

Dissimilarities are computed with distance

```
dist.bc <- distance(foodRare, method = "bray") ## Bray-Curtis
```

All available distances are available with

```
distanceMethodList ## or distance("list") depending on phyloseq version
## $UniFrac
## [1] "unifrac" "wunifrac"
## $DPCoA
## [1] "dpcoa"
## $JSD
## [1] "jsd"
## $vegdist
## [1] "manhattan" "euclidean" "canberra" "bray" "kulczynski " "jaccard" "gower"
 "altGower" "morisita" "horn"
## [11] "mountford" "raup" "binomial" "chao" "cao"
## $betadiver
## [1] "w" "-1" "c" "wb" "r" "I" "e" "t" "me" "j" "sor" ...
```

Exploring biodiversity : β-diversity

- Bray-Curtis, Jaccard and Kulczynski are good at detecting underlying ecological gradients
- Morisita-Horn, Cao and Jensen-Shannon are good at handling different sample sizes
- All take value in [0; 1] except JSD and Cao.

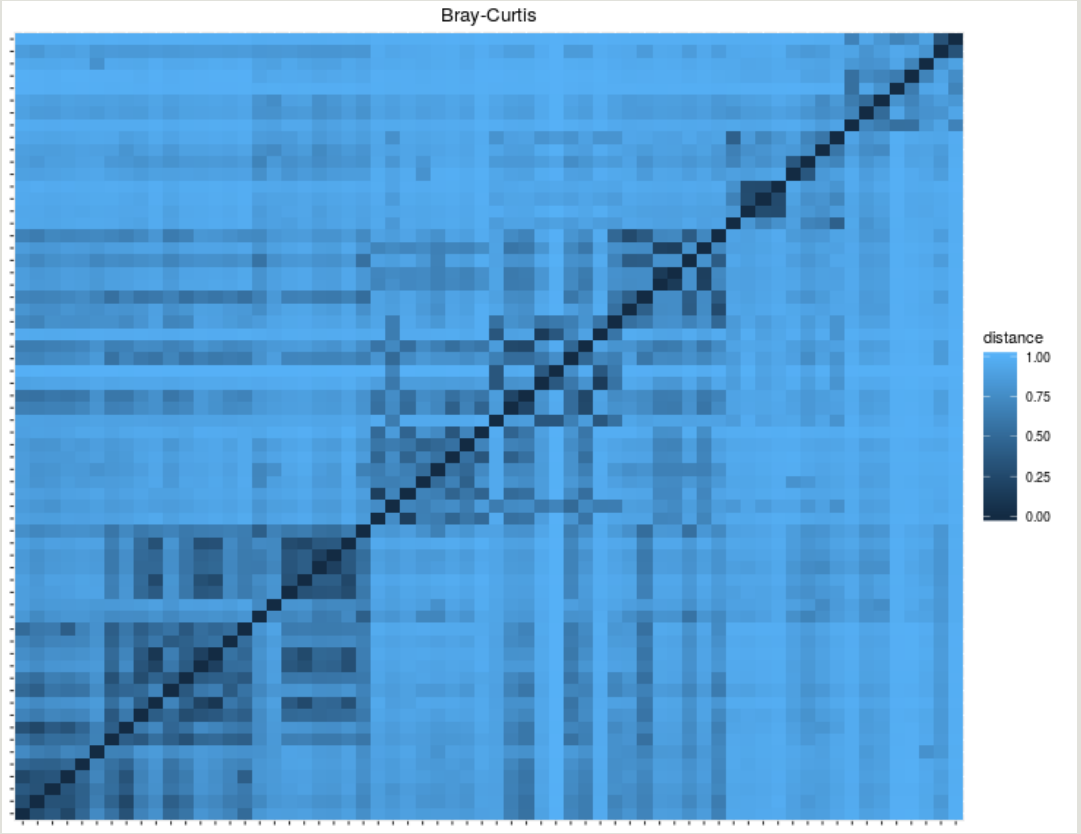
➔ Compute Jaccard, Bray Curtis, Unifrac and weighted Unifrac distance

Exploring biodiversity : β-diversity

β-diversity indices can be visualized thanks to a color matrix.

```
## custom function to implement

p <- plot_dist_as_heatmap(dist.bc, title = "Bray-Curtis")

plot(p)
```

➔ Try it on the other distance matrices



Exploring biodiversity : β-diversity

- Jaccard lower than Bray-Curtis

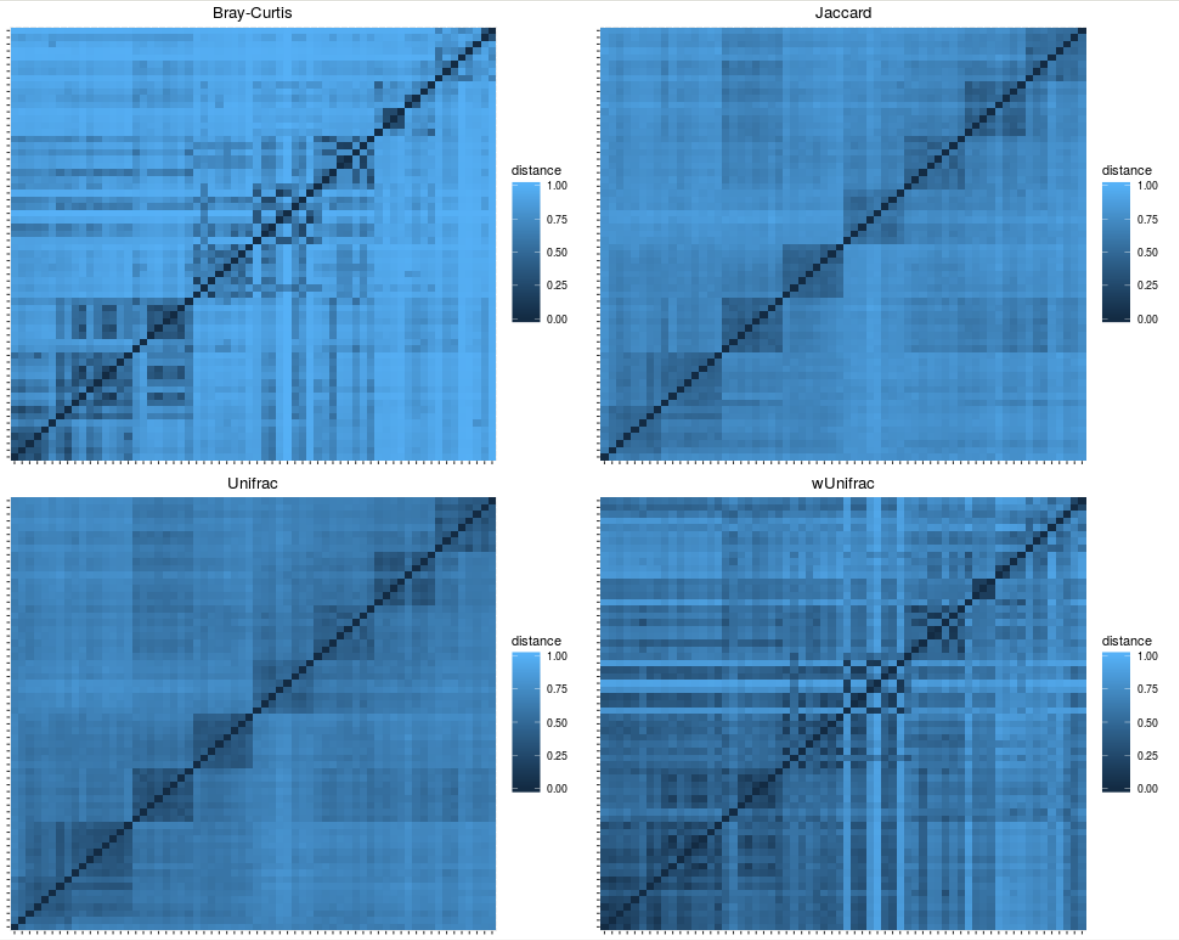
➔ abundant taxa are not shared

- Jaccard higher than Unifrac

➔ communities' taxa are distinct but phylogenetically related

- Unifrac higher than weighted Unifrac

➔ abondant taxa in both communities are phylogenetically closed



Exploring biodiversity : β-diversity

- In general, qualitative diversities are more sensitive to factors that affect presence/absence of organisms (such as pH, salinity, depth, etc) and therefore useful to study and define bioregions (regions with little of no flow between them)...
- ... whereas quantitative distances focus on factors that affect relative changes (seasonal changes, nutrient availability, concentration of oxygen, depth, etc) and therefore useful to monitor communities over time or along an environmental gradient

Different distances capture different features of the samples

There is no "one size fits all"

Exploring the structure

Exploring the structure

ORDINATION

Exploring the structure : ordination

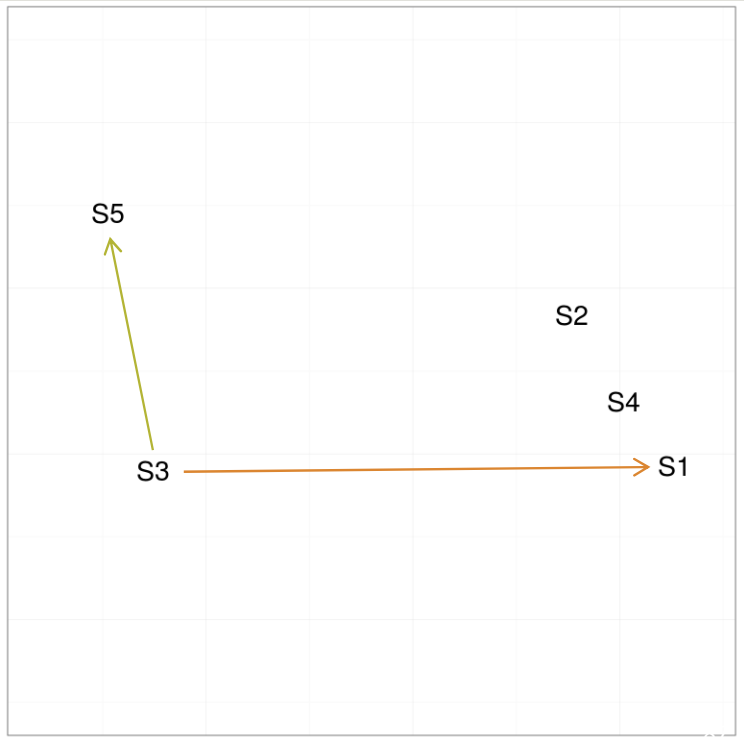
- Each community is described by OTUs abundances
- OTUs abundance may be correlated
- PCA finds linear combinations of OTUs that
  - are uncorrelated
  - capture well the variance of community composition

But variance is not a very good measure of β-diversity

Exploring the structure : ordination

The Multidimensionnal Scaling (MDS or PCoA) is equivalent to a principal component Analysis (PCA) but preserves the β-diversity instead of the variance.
The MDS try to represent samples in two dimensions
➔ The samples ordination

	Distance Matrix				
	S1	S2	S3	S4	S5
S1	0.00	2.21	6.31	0.99	7.50
S2	2.21	0.00	5.40	1.22	5.74
S3	6.31	5.40	0.00	5.75	3.16
S4	0.99	1.22	5.75	0.00	6.64
S5	7.50	5.74	3.16	6.64	0.00



Exploring the structure : ordination

Ordination is done through the ordinate function

You can pass the distance either by name (and phyloseq will call distance) :

```
ord <- ordinate(foodRare, method = "MDS", distance = "bray")
```

or by passing a distance matrix directly (useful if you already computed it)

```
dist.bc <- distance(foodRare, method = "bray")
ord <- ordinate(foodRare, method = "MDS", distance = dist.bc)
```

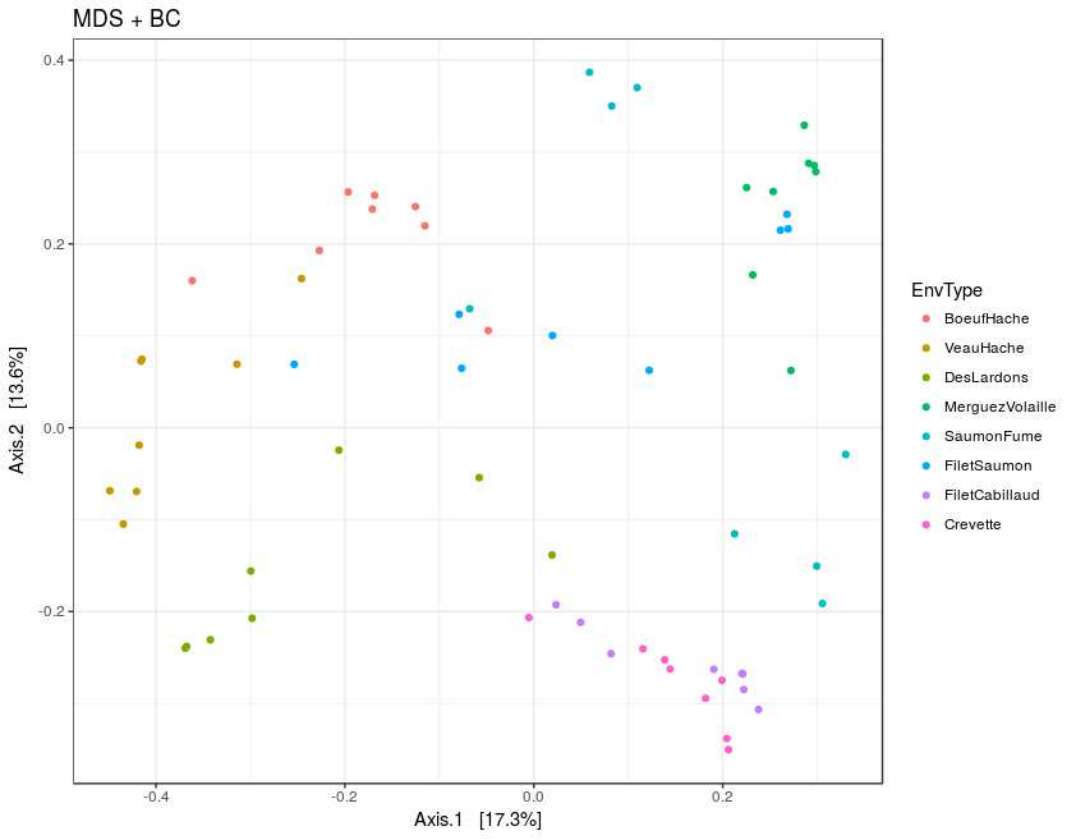
Exploring the structure : ordination

The graphic is then produced with plot_ordination

➔ Try it on foodRare with ord

➔ Custom color by EnvType

```
p <- plot_ordination(foodRare, ord, color = "EnvType")

## add title and plain background
p <- p + theme_bw() + ggtitle("MDS + BC")

plot(p)
```



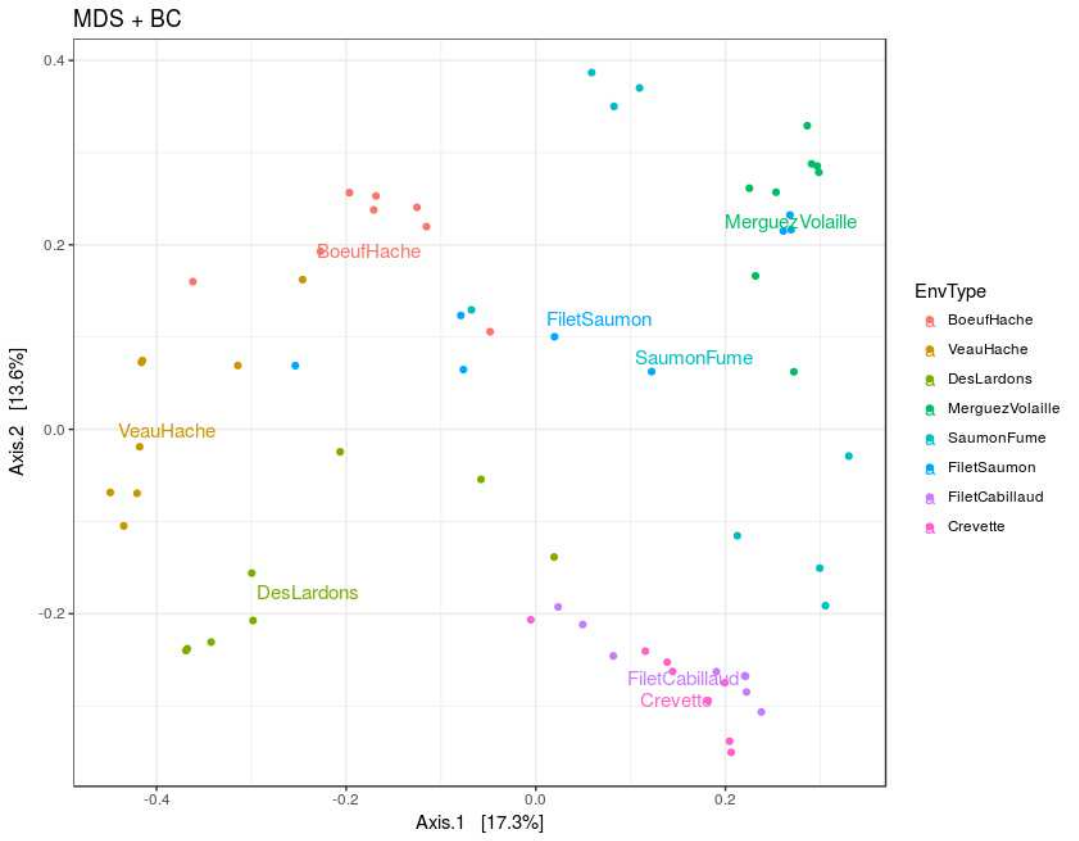
Exploring the structure : ordination

load-extra-functions.R

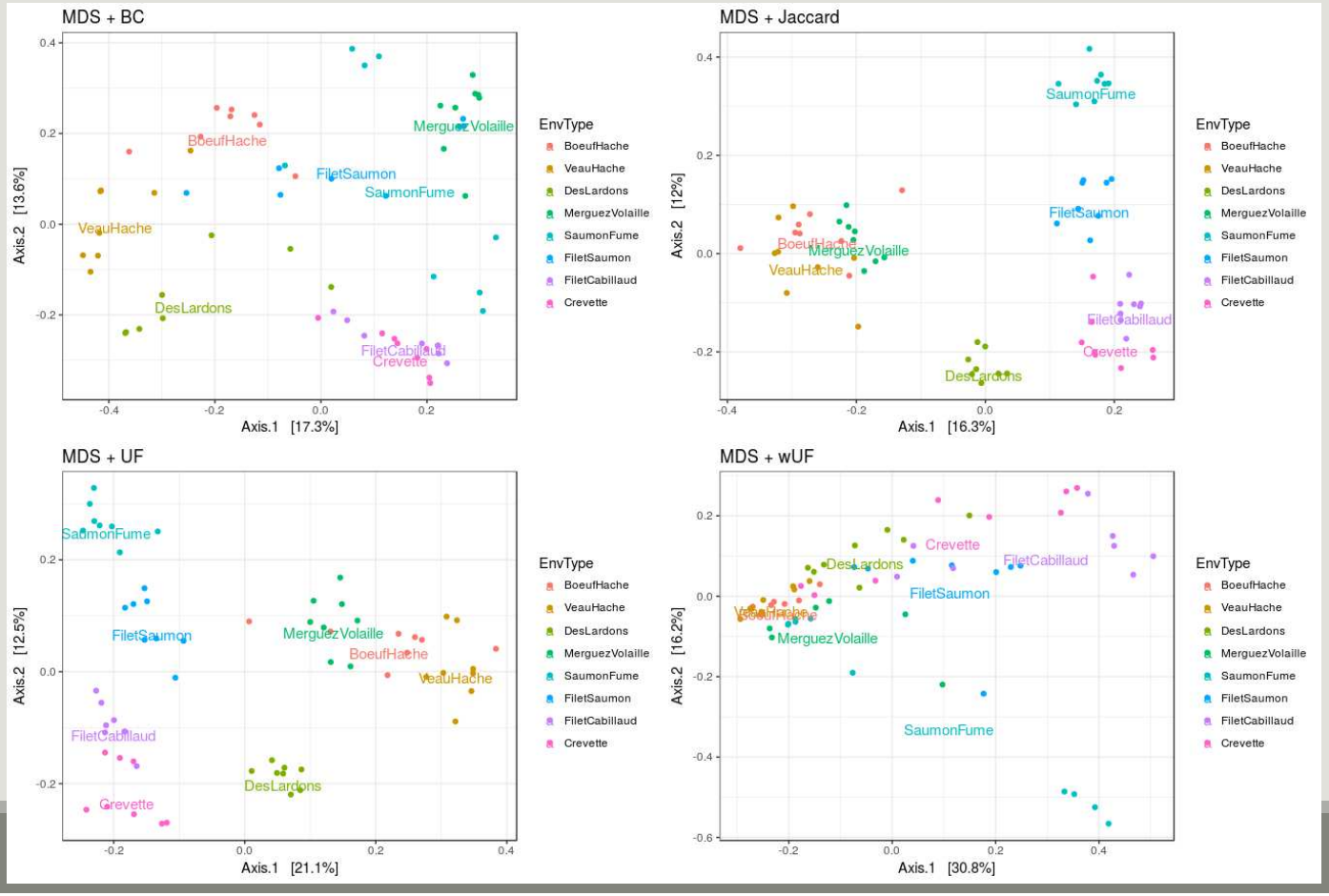
Custom plot_samples function is built around plot_ordination to represent groups (extra replicate parameter)

➔ Try it on food and ord, and choose EnvType for color and replicate

```
p <- plot_samples(foodRare, ord, color = "EnvType", replicate = "EnvType")

## add title

p <- p + theme_bw() + ggtitle("MDS + BC")

plot(p)
```



Exploring the structure : ordination



Exploring the structure : ordination

- Qualitative distances (Unifrac, Jaccard) separate meat products from seafood ones

➔ detected taxa segregate by origin

- DesLardons is somewhere in between

➔ contamination induced by sea salt

- Quantitative distances (wUnifrac) exhibit a gradient meat – seafood (on axis 1) with DesLardons in the middle and a gradient SaumonFume - everything else on axis 2
- Large overlap between groups in terms of relative composition but less so in term of species composition (a side effect of undersampling?)
- Note the difference between wUniFrac and Bray-Curtis for the distances between BoeufHache and VeauHache
- Warning The 2-D representation captures only part of the original distances

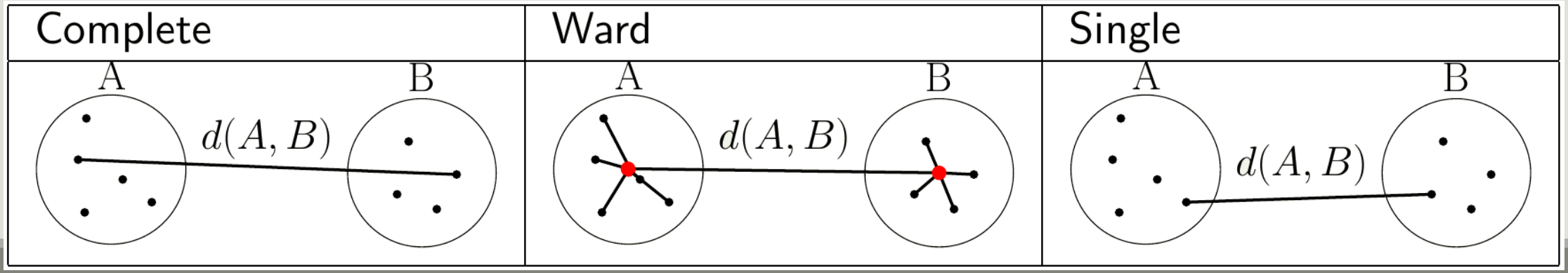
Exploring the structure

CLUSTERING

Exploring the structure : clustering

The clustering aims to represent samples in a tree based on a distance matrix and a linkage function:

- Complete linkage: tends to produce compact, spherical clusters and guarantees that all samples in a cluster are similar to each other
- Ward: tends to also produces spherical clusters but has better theoretical properties than complete linkage
- single: friend of friend approach, tends to produce banana-shaped or chains-like clusters



Exploring the structure : clustering

➔ Choose a distance (among Jaccard, Bray-Curtis, Unifrac, etc)

➔ Choose a linkage function

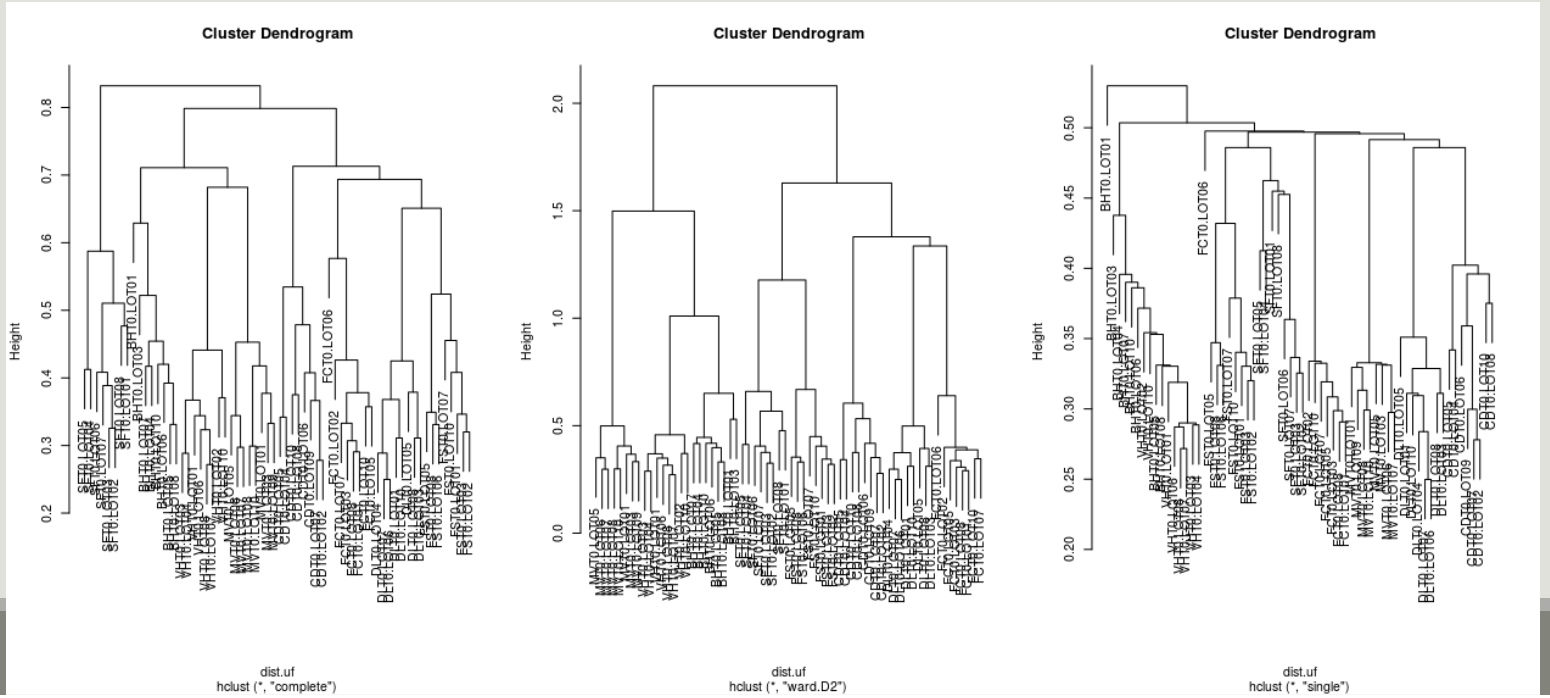
- complete (“complete”): tends to produce compact, spherical clusters and guarantees that all samples in a cluster are similar to each other
- ward (“ward.D2”): tends to also produces spherical clusters but has better theoretical properties than complete linkage
- single (“single”): friend of friend approach, tends to produce banana-shaped or chains-like clusters

➔ Feed to hclust and plot

```
clustering <- hclust(distance.matrix, method = "linkage.function")
plot(clustering)
```

Exploring the structure : clustering

```
par(mfcol = c(1, 3)) ## To plot the three clustering trees side-by-side
plot(hclust(dist.uf, method = "complete"))
plot(hclust(dist.uf, method = "ward.D2"))
plot(hclust(dist.uf, method = "single"))
```



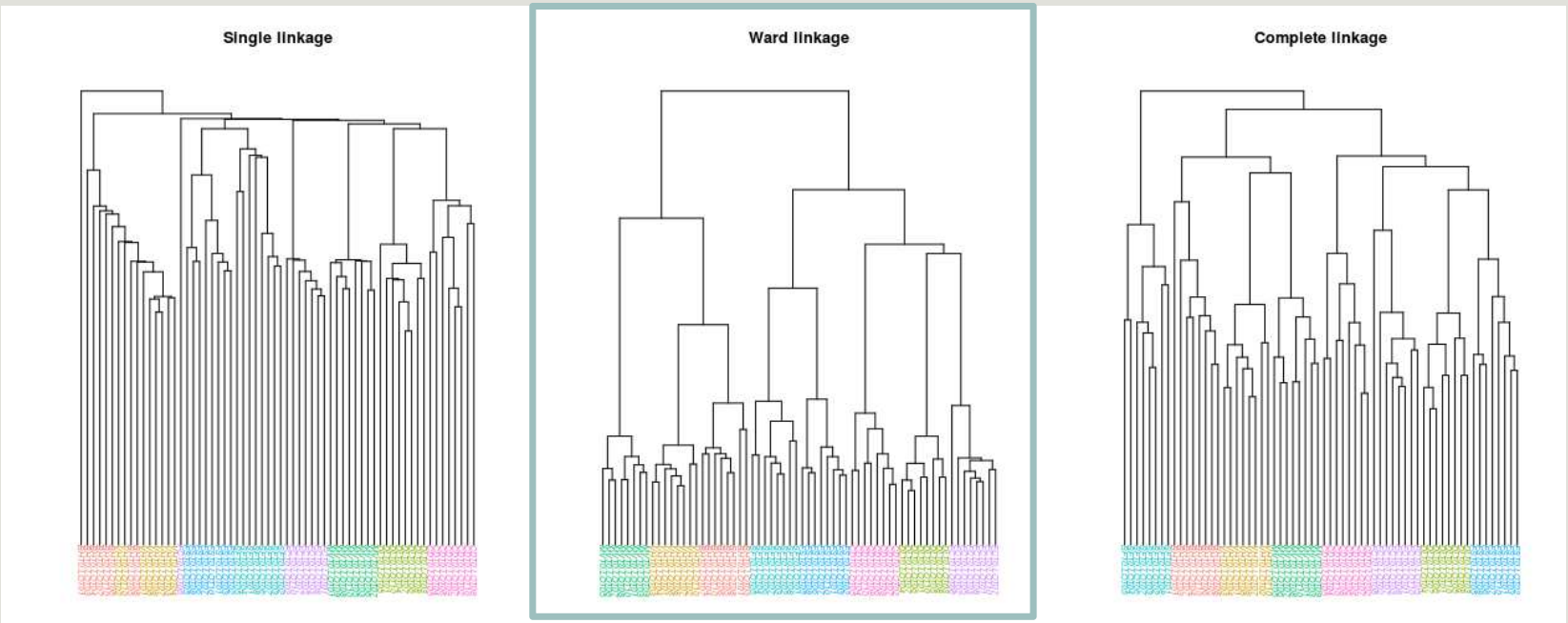
load-extra-functions.R

Exploring the structure : clustering

With some effort (see companion R script), we can produce better dendrograms and color sample by food type.

```
## Env types
envtype <- get_variable(foodRare, "EnvType")
## automatic color palette: one color per different sample type
palette <- hue_pal()(length(levels(envtype)))
## Map sample type to color
tipColor <- col_factor(palette, levels = levels(envtype))(envtype)
## Change hclust object to phylo object and plot
par(mar = c(0, 0, 2, 0))
dist.uf <- distance(foodRare, method = "unifrac")## if not already done
clust.uf <- as.phylo(hclust(dist.uf, method = "complete"))
plot(clust.uf, tip.color = tipColor, direction = "downwards",
main = "Ward linkage")
```

Exploring the structure : clustering



- Crevette
- FiletCabillaud
- FiletSaumon
- SaumonFume
- MerguezVolaille
- DesLardons
- BoeufHache
- VeauHache

Exploring the structure : clustering

Remarks

- Consistent with the ordination plots, clustering works quite well for the UniFrac distance for some linkage (Ward)
- Clustering is based on the whole distance whereas ordination represents parts of the distance (the most it can with 2 dimensions)

Exploring the structure

HEATMAP

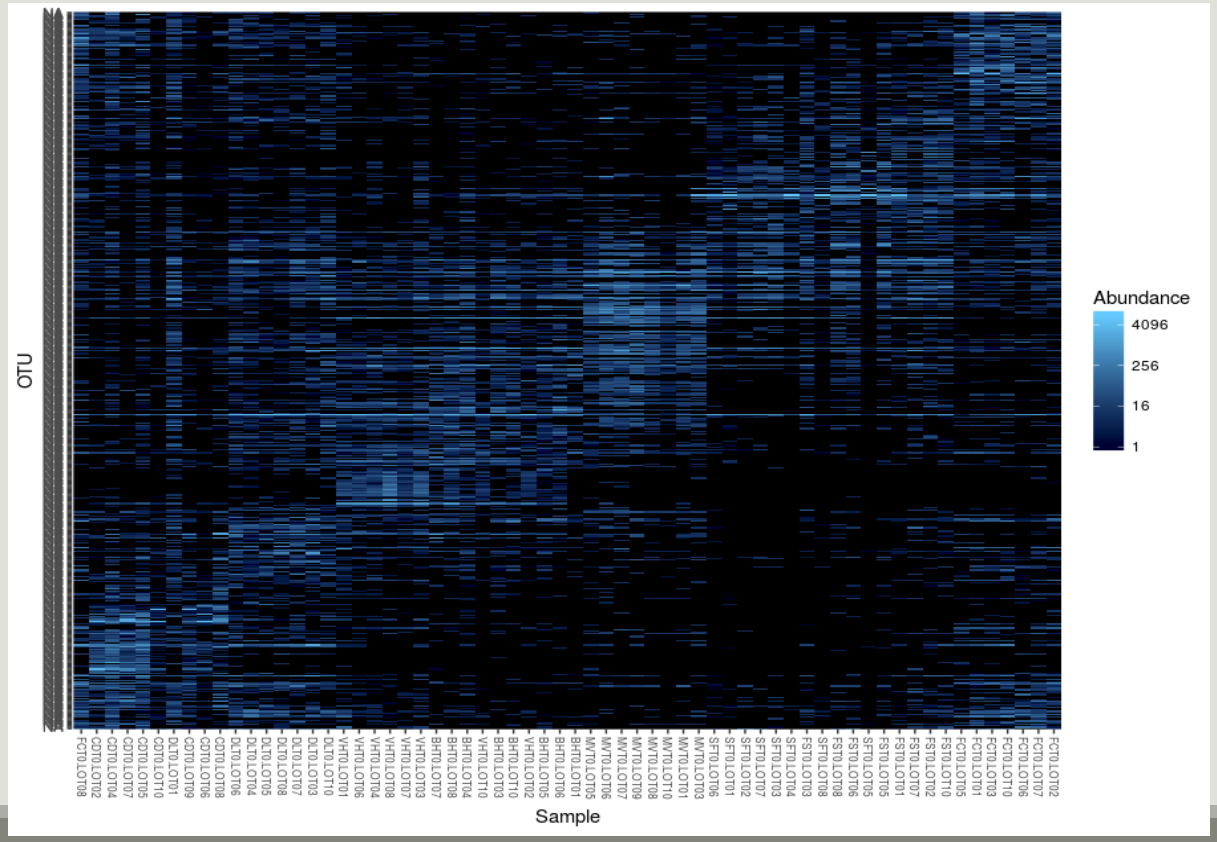
Exploring the structure : Heatmap

- The heatmap is an other representation of the distance matrix
- It tries to reveal if there is a structure between group of OTU and group of samples
- plot_heatmap is a versatile function to visualize the count table:
  - Finds a meaningful order of the samples and the OTUs
  - Allows the user to choose a custom order
  - Allows the user to change the color scale
  - Produces a gpplot2 object, easy to manipulate and customize

Exploring the structure : Heatmap

➔ Try it on food

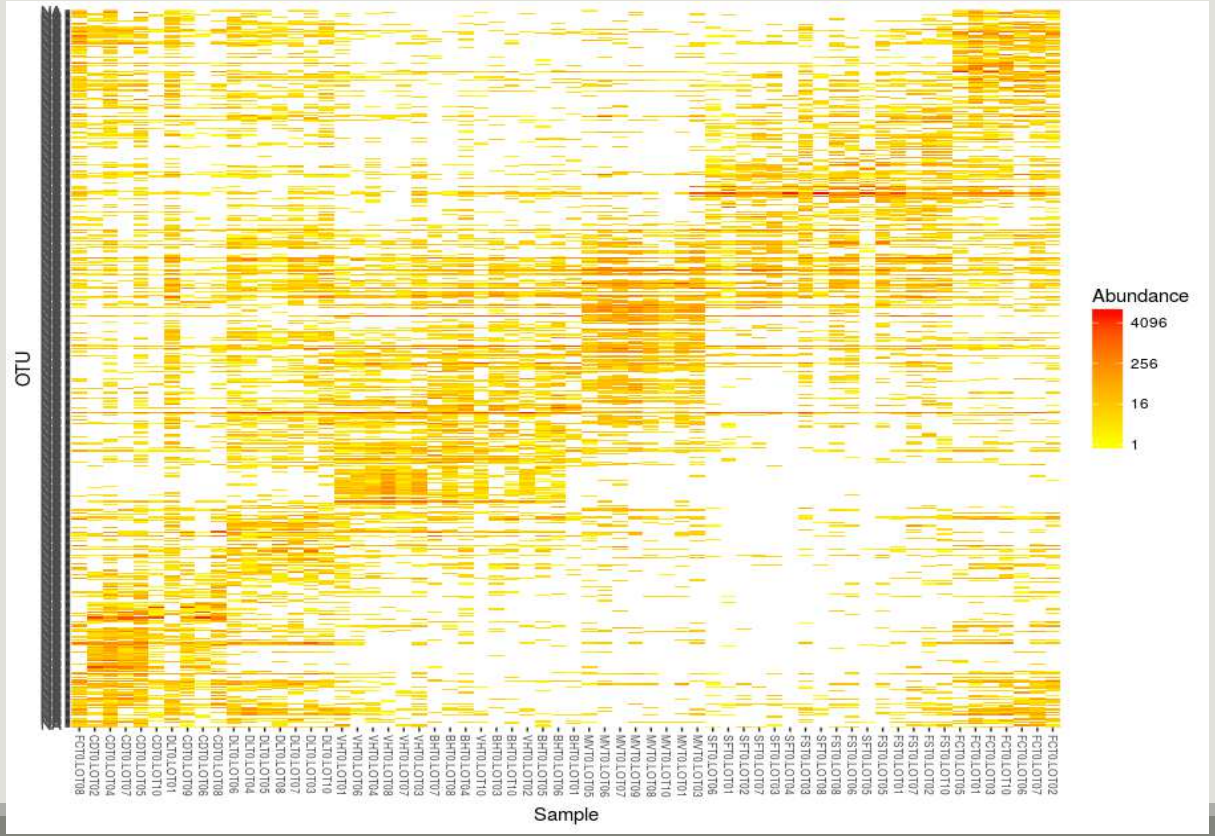
```
p <- plot_heatmap(foodRare)
plot(p)
```



Exploring the structure : Heatmap

➔ Change color scale in plot_heatmap

```
p <- plot_heatmap(foodRare,
 low = "yellow", high = "red",
 na.value = "white")

plot(p)
```

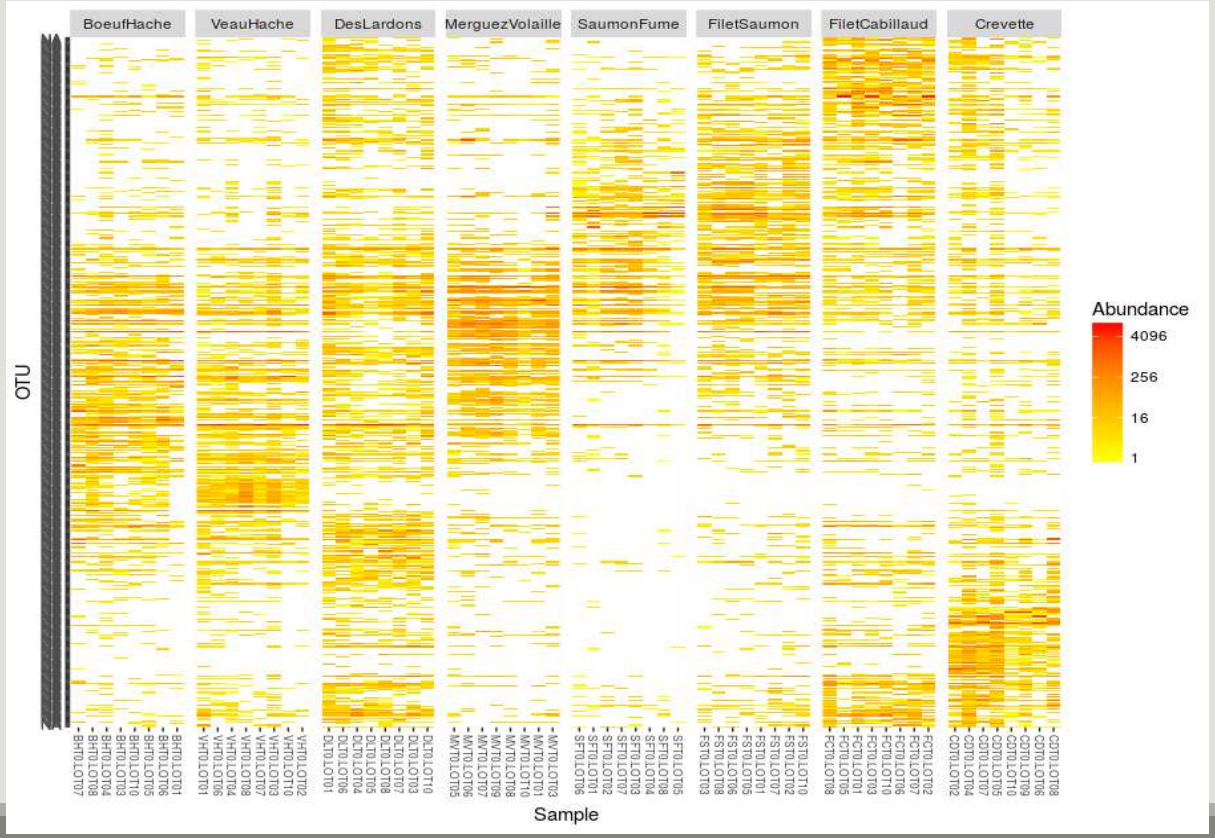


Exploring the structure : Heatmap

```
p <- plot_heatmap(foodRare,
 low = "yellow", high = "red",
 na.value = "white")
```

➔ Facet your plot by EnvType

```
p <- p + facet_grid(~EnvType,
 scales = "free_x")

plot(p)
```



Exploring the structure : Heatmap

➔ Use scale_fill_gradient layers of ggplot2 to change color scale

```
p <- plot_heatmap(foodRare)

p <- p +
 scale_fill_gradient2(low =
 "#1a9850", mid = "#ffffbf",
 high = "#d73027“, na.value =
 white", trans = log_trans(4),

  midpoint = log(100, base = 4))

p <- p + facet_grid(~EnvType,
 scales = "free_x")

plot(p)
```

Exploring the structure : Heatmap

- Block-like structure of the abundance table
- Interaction between (groups of) taxa and (groups of) samples
- Core and condition-specific microbiota

➔ Classification of taxa and use of custom taxa order to highlight structure

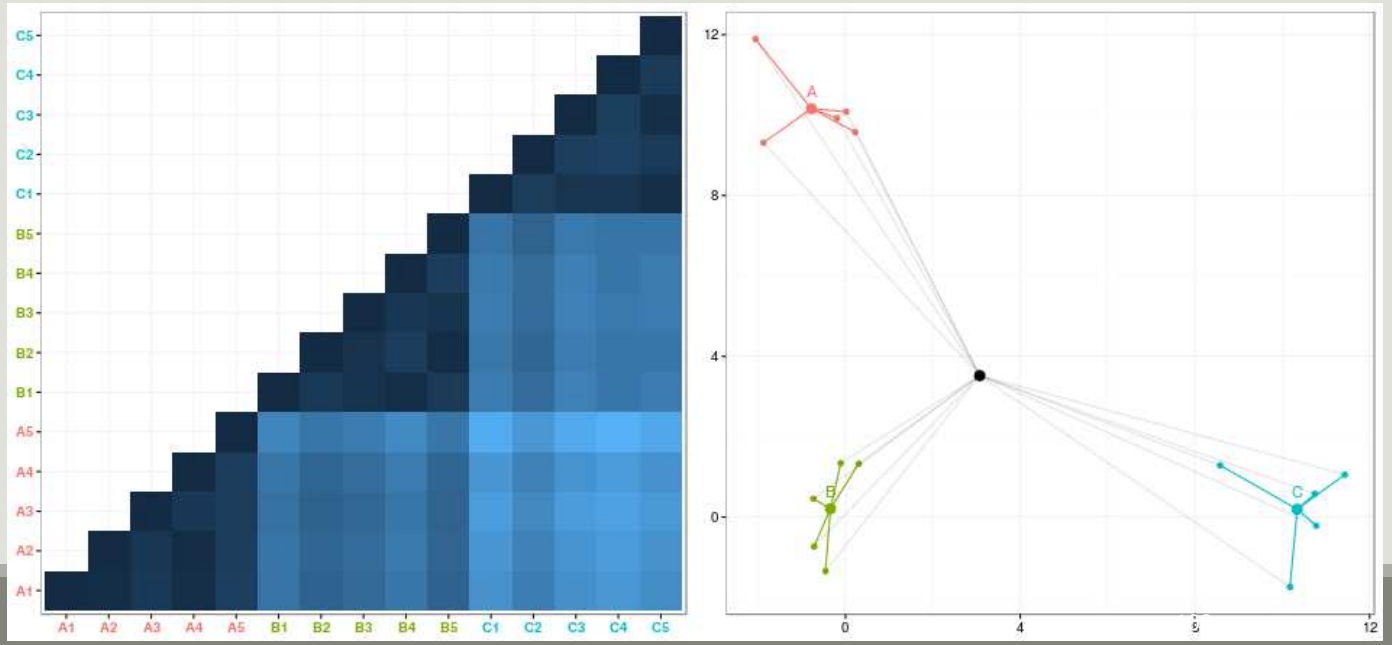
Diversity partitioning

Diversity partitioning

Are the structures seen linked to metadata ? Do the metadata have an effect on our communities composition ?

To answer these questions, **multivariate analyses**:

- tests composition differences of communities from different groups using a distance matrix
- compares within group to between group distances



Diversity partitioning : CAP

Constrained Analysis of Principal Coordinates (CAP) tries to :

- Find associations between community composition and environmental variables (pH, group)
- Quantify differences between groups of samples

How it works : Regress a distance matrix against some covariates using the standard R syntax for linear models.

- Project distance matrix on metadata variables ➔ communities are constrained to depend on metadata
- Look if constrained distance fit to non-constrained distance

```
## convert sample_data to data.frame
metadata <- as(sample_data(foodRare), "data.frame")
cap <- capscale(dist.uf ~ EnvType, data = metadata)
```

Diversity partitioning : CAP

```
cap
## Call:
## capscale(formula = dist.uf ~ EnvType, data = metadata)
##
##               Inertia Proportion Eigenvals Rank
## Total         12.39764    1.00000  12.42360
## Constrained    7.66026    0.61788   7.66073    7
## Unconstrained  4.73738    0.38212   4.76287   56
## Imaginary                           -0.02595    4
## Inertia is squared Unknown distance
##
## Eigenvalues for constrained axes:
## CAP1   CAP2   CAP3   CAP4   CAP5   CAP6   CAP7
## 2.4953 1.4544 1.1004 0.9350 0.7181 0.5074 0.4501

## Eigenvalues for unconstrained axes:
## MDS1   MDS2   MDS3   MDS4   MDS5   MDS6   MDS7   MDS8
## 0.4494 0.2990 0.2672 0.2378 0.2176 0.2000 0.1745 0.1626
## Showed only 8 of all 56 unconstrained eigenvalues)
```

Environment type explains roughly **62%** of the total variation between samples (as measured by Unifrac)

Diversity partitioning : CAP

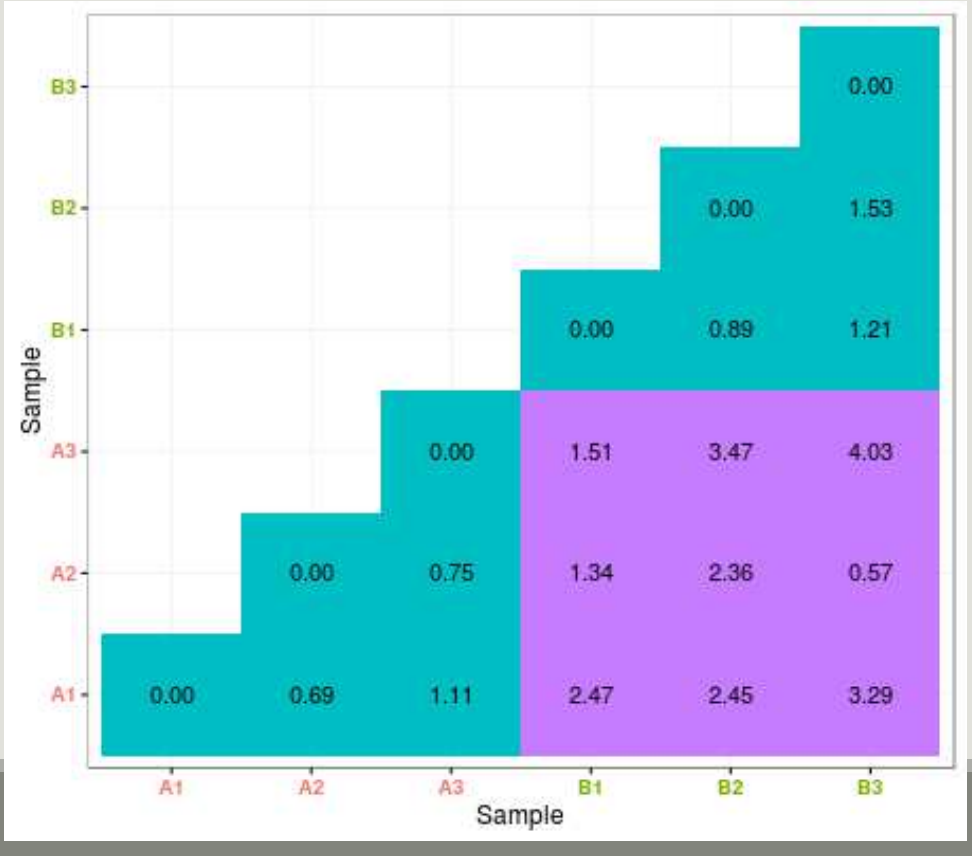
```
## test the confidence of CAP
anova <- anova(cap, permutations = 999)
## Permutation test for capscale under reduced model
## Permutation: freeNumber of permutations: 999
## Model: capscale(formula = dist.uf ~ EnvType, data = metadata)
##          Df   SumOfSqs       F  Pr(>F)
## Model     7     7.6603  12.936   0.001 ***
## Residual  56    4.7374
## ---
## Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Diversity partitioning : Multivariate ANOVA

Idea : test differences in the community composition from different groups using a distance matrix

How it works ?

- No projection of distance matrix, but compute sum of square distance
- Variance analysis



Diversity partitioning : Multivariate ANOVA

```
metadata <- as(sample_data(foodRare), "data.frame")
adonis(dist.uf ~ EnvType, data = metadata, perm = 9999)
##
## Call:
## adonis(formula = dist.uf ~ EnvType, data = metadata, permutations = 9999)

## Permutation: free
## Number of permutations: 9999

## Terms added sequentially (first to last)
##
## Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## EnvType    7    7.6603  1.0943  12.936 0.61788  1e-04 ***
## Residuals 56    4.7374  0.0846         0.38212
## Total     63   12.3976                 1.00000
## ---
## Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Environment type explains roughly **62%** of the total variation.

Annexes

References

- Chaillou, S., Chaulot-Talmon, A., Caekebeke, H., Cardinal, M., Christieans, S., Denis, C., Desmonts, M. H., Dousset, X., Feurer, C., Hamon, E., Joraud, J.-J., La Carbona, S., Leroi, F., Leroy, S., Lorre, S., Mace, S., Pilet, M.-F., Prevost, H., Rivollier, M., Roux, D., Talon, R., Zagorec, M., and Champomier-Verges, M.-C. (2015). Origin and ecological selection of core and food-specific bacterial communities associated with meat and seafood spoilage. ISME J, 9(5):1105{1118.
- McMurdie, P. J. and Holmes, S. (2013). phyloseq: An r package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE, 8(4):e61217.
- Shade, A., Jones, S. E., Caporaso, J. G., Handelsman, J., Knight, R., Fierer, N., and Gilbert, J. A. (2014). Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. MBio, 5(4):e01371{e01314.

Installing Phyloseq

- From bioconductor

```
## try http if https is not available
source("https://bioconductor.org/biocLite.R")
biocLite("phyloseq")
```

- From developer's website

```
install.packages("devtools") ## If not installed previously
library("devtools")
install_github("phyloseq", "joey711")
```

Go further : phyloseq import

- It is possible to build a phyloseq object from plain tabular files
- Since OTUs/sample names are not always consistent (unlike in a biom), some care must be taken
- Otherwise the phyloseq objects consists only of OTUs and samples with consistent names and may end up empty

- Import each component separately
- Convert to correct base R data type (matrix for otu_table and tax_table, data.frame for sample_data)
- Convert to phyloseq data type (otu_table, tax_table, sample_data)
- Check name consistency
- Build phyloseq object

Go further : phyloseq import

- Import each component

```
sampledata <- read.csv("data/manual/sampledata.tsv", sep = "nt",
row.names = 1)
taxtable <- read.csv("data/manual/taxtable.tsv", sep = "nt",
row.names = 1)
otutable <- read.csv("data/manual/otutable.tsv", sep = "nt",
row.names = 1)
tree <- read.tree("data/manual/tree.phy")
```

- Convert to base R type

```
taxtable <- as.matrix(taxtable)
otutable <- as.matrix(otutable)
```

- Convert to phyloseq base type

```
sampledata <- sample_data(sampledata)
taxtable <- tax_table(taxtable)
otutable <- otu_table(otutable, taxa_are_rows = TRUE)
```

Go further : phyloseq import

- Check name consistency
  - Abundance table and sample data (sample names)

```
all(colnames(otutable) %in% rownames(sampledata)) ## sample names
## [1] TRUE
```

  - Abundance table and taxonomy table (taxa names)

```
all(rownames(otutable) %in% rownames(taxtable)) ## taxa names
## [1] TRUE
```

  - Abundance table and tree leaves (taxa names)

```
all(rownames(otutable) %in% tree$tip.label) ## taxa names
## [1] TRUE
```

Go further : phyloseq import

- Build object

```
manualData <- phyloseq(sampledata, otutable, taxtable, tree)
manualData
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 500 taxa and 26 samples ]
## sample_data() Sample Data: [ 26 samples by 6 sample variables ]
## tax_table() Taxonomy Table: [ 500 taxa by 7 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 500 tips and 499 internal nodes ]
```

Go further : phyloseq filters

- Specify a sample wise, OTU wide condition (e.g. abundance greater than 2, in the top ten OTUs, etc)
- Specify a number of samples A
- Select only OTUs satisfying condition in at least A samples (usually to prune them)

Examples (what do they do?)

```
condition <- function(x) { x > 0 }
taxaToKeep <- genefilter_sample(food, condition, 5)
prune_taxa(taxaToKeep, food)

condition <- function(x) {order(x, decreasing = TRUE) <= 250 }
taxaToKeep <- genefilter_sample(food, condition, 3)
prune_taxa(taxaToKeep, food)
```

Go further : phyloseq filters

First example

- condition is TRUE if a taxa is present in a sample
- condition must be met 5 times

➔ Select taxa that appear in at least 5 samples

Second example

- condition is TRUE if a taxa is among the 250 most abundant ones in the sample
- condition must be met 3 times

➔ Select taxa that are very abundant (top 250) in at least 3 samples

Go further : phyloseq filters

- Specify an OTU wise, sample wide on the OTU abundance vector condition (e.g. overall abundance greater than 3, etc)
- Selects only OTUs satisfying condition (usually to prune them)
- Works on an OTU-by-OTU basis. Beware of bad normalizations

Examples (what do they do?)

```
condition <- function(x) { sum(x > 0) >= 5 }
taxaToKeep <- filter_taxa(food, condition)
prune_taxa(taxaToKeep, food)

condition <- function(x) { sum(x) >= 100 }
taxaToKeep <- filter_taxa(food, condition)
prune_taxa(taxaToKeep, food)
```

Go further : phyloseq filters

First example

- condition is TRUE if a taxa has at least 5 positive counts (across samples)

➔ Select taxa that appear in at least 5 samples

Probably better done with genefilter_sample

Second example

- condition is TRUE if a taxa has global abundance at least 100

➔ Select taxa with overall abundance at least 100 (beware of unequal sample sizes)

Probably done better with sample_sums

Go further : phyloseq smoothing

merge_samples merges samples according to a factor by summing their abundances (beware of different group sizes and library sizes)

```
mergedData <- merge_samples(food, "EnvType")
## Warning in asMethod(object): NAs introduits lors de la conversion automatique
## Warning in asMethod(object): NAs introduits lors de la conversion automatique
mergedData
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 508 taxa and 8 samples ]
## sample_data() Sample Data: [ 8 samples by 3 sample variables ]
## tax_table() Taxonomy Table: [ 508 taxa by 7 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 508 tips and 507 internal nodes ]
sample_names(mergedData)
## [1] "BoeufHache" "VeauHache" "DesLardons" "MerguezVolaille"
## [5] "SaumonFume" "FiletSaumon" "FiletCabillaud" "Crevette"
```

Go further : phyloseq smoothing

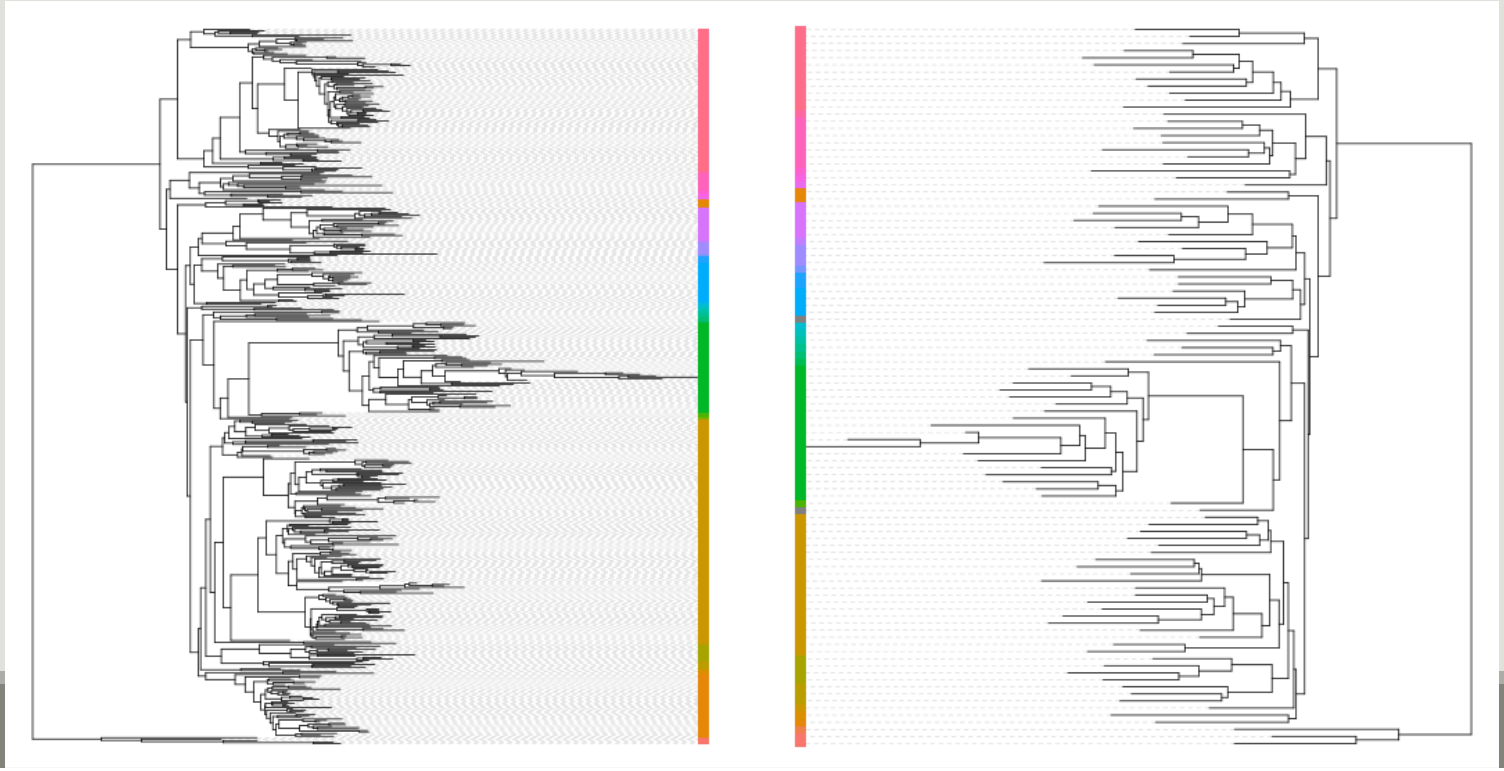
Unfortunately, merging the contextual data is hard to do automatically in a meaningful way and information is lost in the process...

```
sample_data(mergedData)[1:2, ]
## Sample Data: [2 samples by 3 sample variables]:
## EnvType FoodType Description
## BoeufHache 1 NA NA
## VeauHache 2 NA NA
sample_data(food)[1:2, ]
## Sample Data: [2 samples by 3 sample variables]:
## EnvType FoodType Description
## DLT0.LOT08 DesLardons Meat LOT8
## DLT0.LOT05 DesLardons Meat LOT5
```

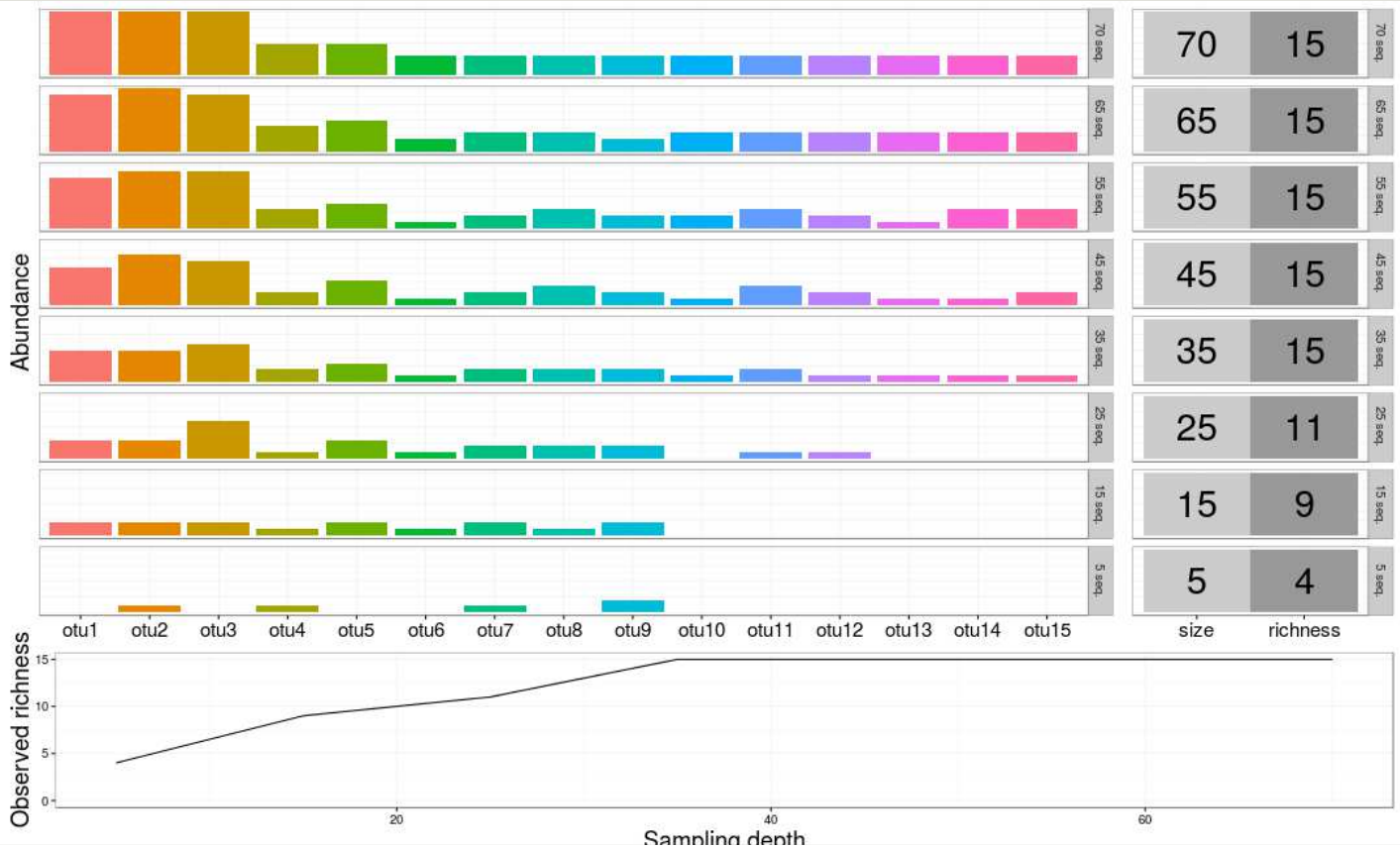
Go further : phyloseq smoothing

tip_glom agglomerates OTUs at a given height in the tree

```
mergedData <- tip_glom(food, h = 0.3)
```

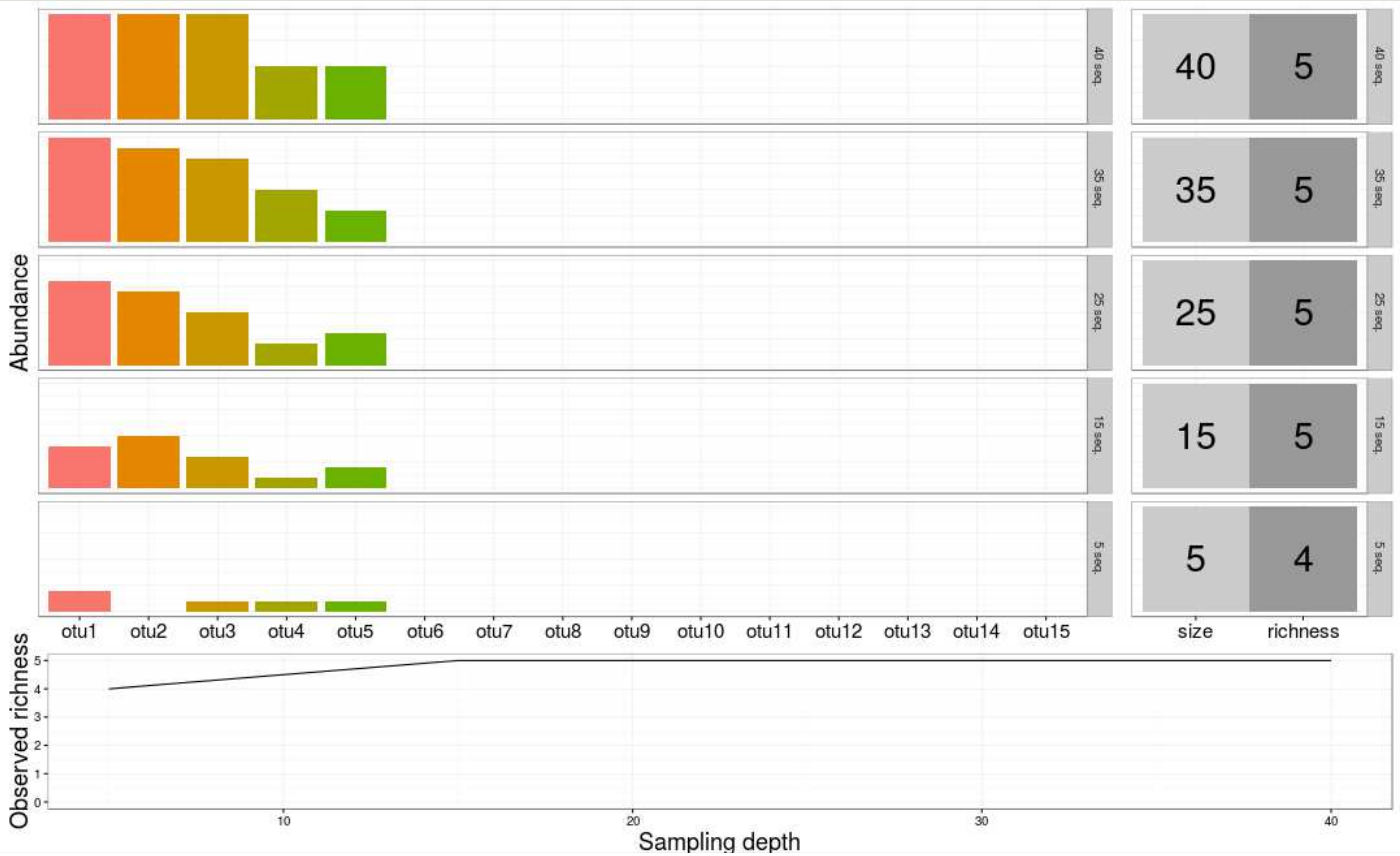


Go further : rarefaction curves



Go further : rarefaction curves

.. After filtering on rare OTUs



load-extra-functions.R

Go further : rarefaction curves

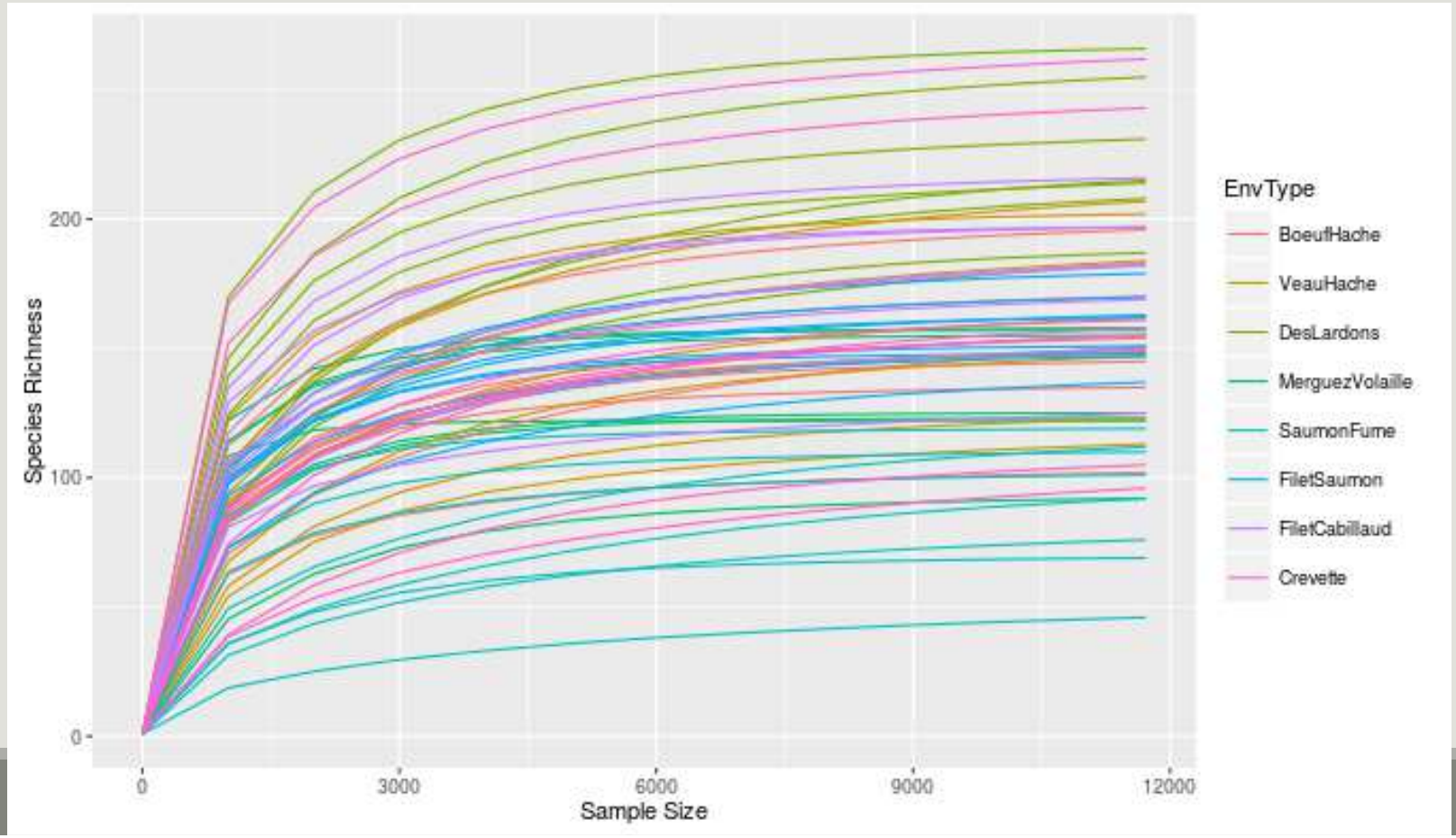
- Why?
  - diversity indices are heavily influenced by sampling depths
  - rarefaction curves assess if sampling has exhausted the diversity
- How?
  - Rarefy all samples to the same depth (optional, for speed here)
  - Use custom function ggrare and specify a step size

```
food <- rarefy_even_depth(food, rngseed = 1121983)
p <- ggrare(food, step = 1000, color = "SampleType", se = FALSE)
```

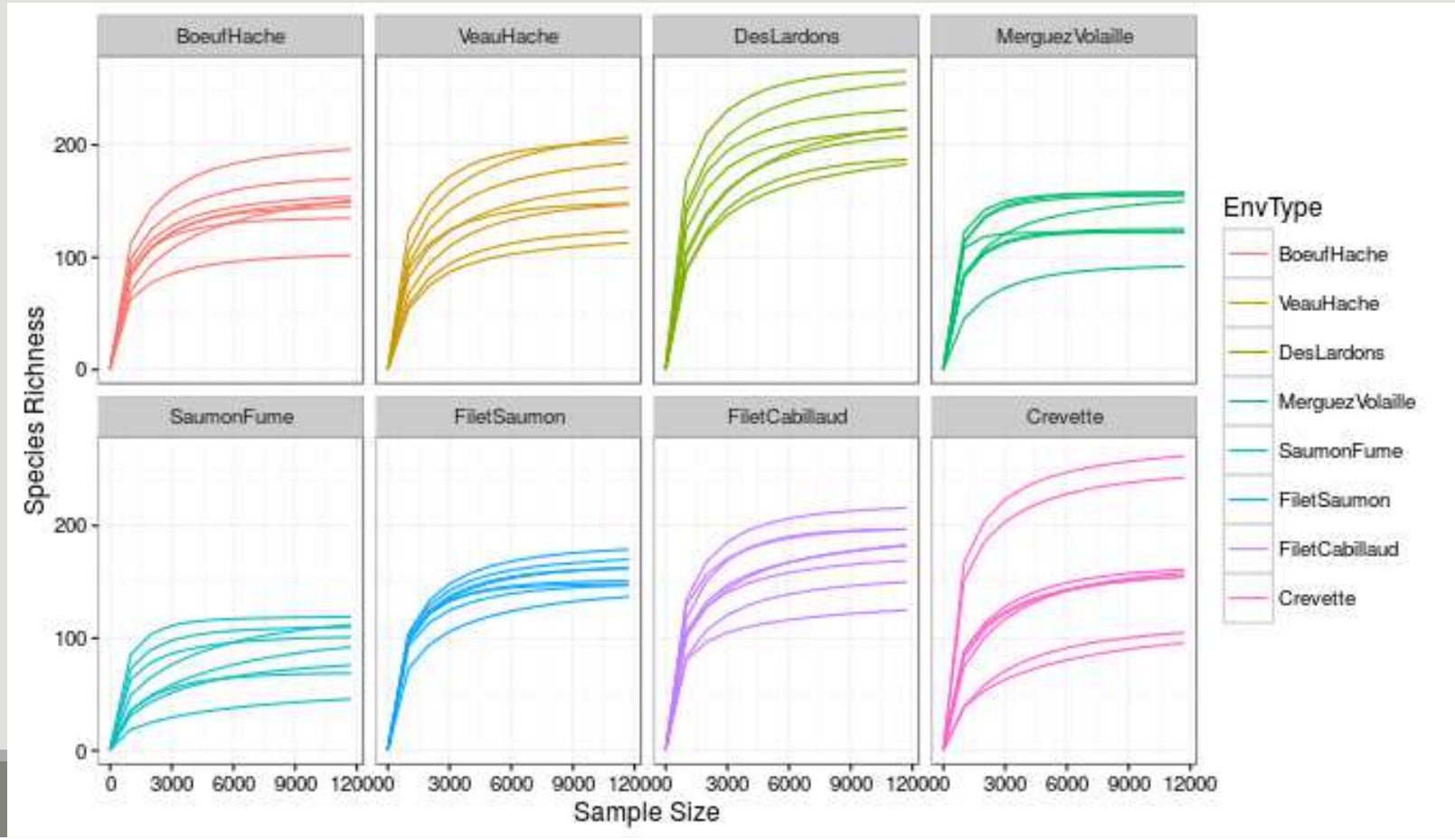
  - To distinguish different environments easily, use facetting (and plain background)

```
plot(p + facet_wrap(~EnvType) + theme_bw())
```

Go further : rarefaction curves



Go further : rarefaction curves



Go further : use your own color palette

We already order the food types to make graphs easy to read:

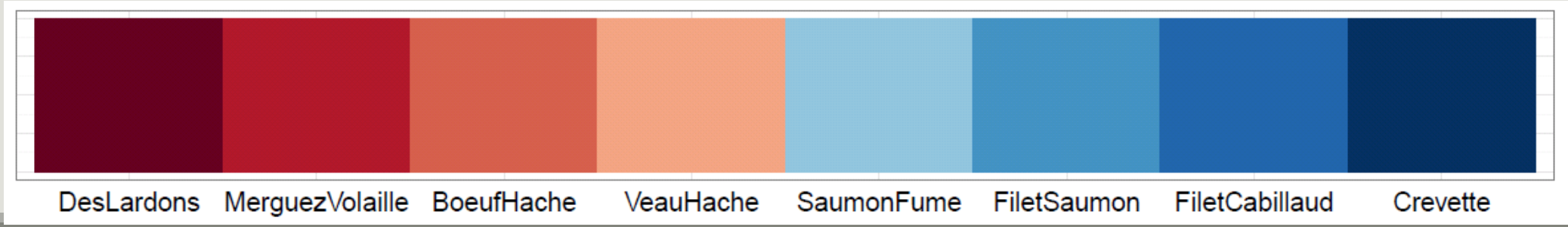
```
levels(sample_data(food)$EnvType)
```

```
## [1] "BoeufHache" "VeauHache" "DesLardons" "MerguezVolaille"
```

```
## [5] "SaumonFume" "FiletSaumon" "FiletCabillaud" "Crevette"
```

Likewise, we're going to use a custom color palette

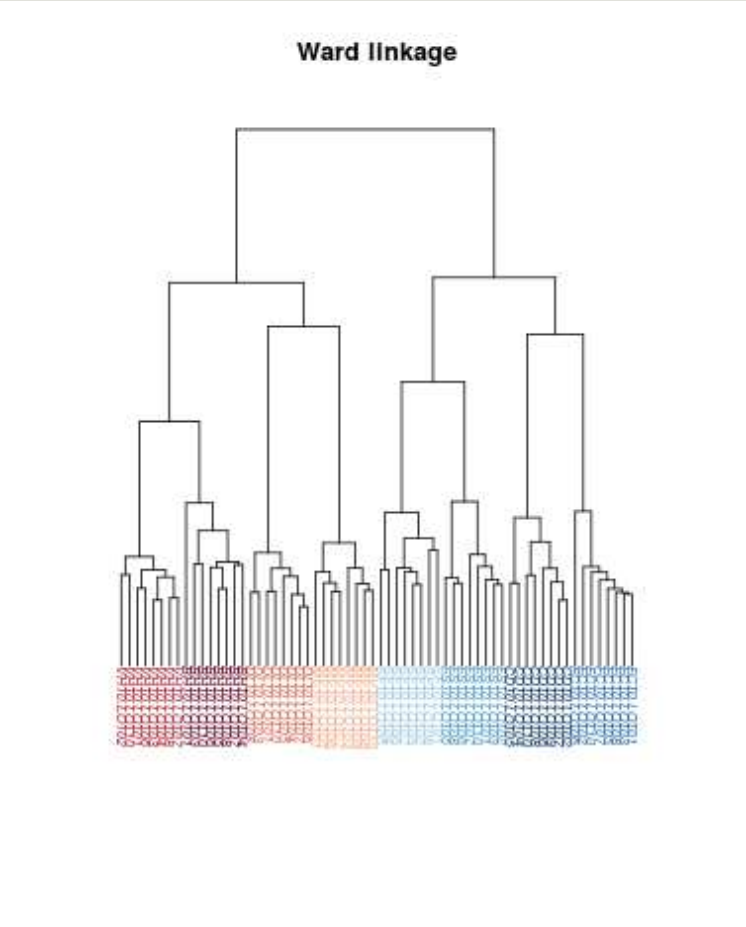
```
foodPalette <- c("#67001f", "#b2182b", "#d6604d", "#f4a582",
"#92c5de", "#4393c3", "#2166ac", "#053061")
```



Go further : use your own color palette

Try this palette on clustering plot

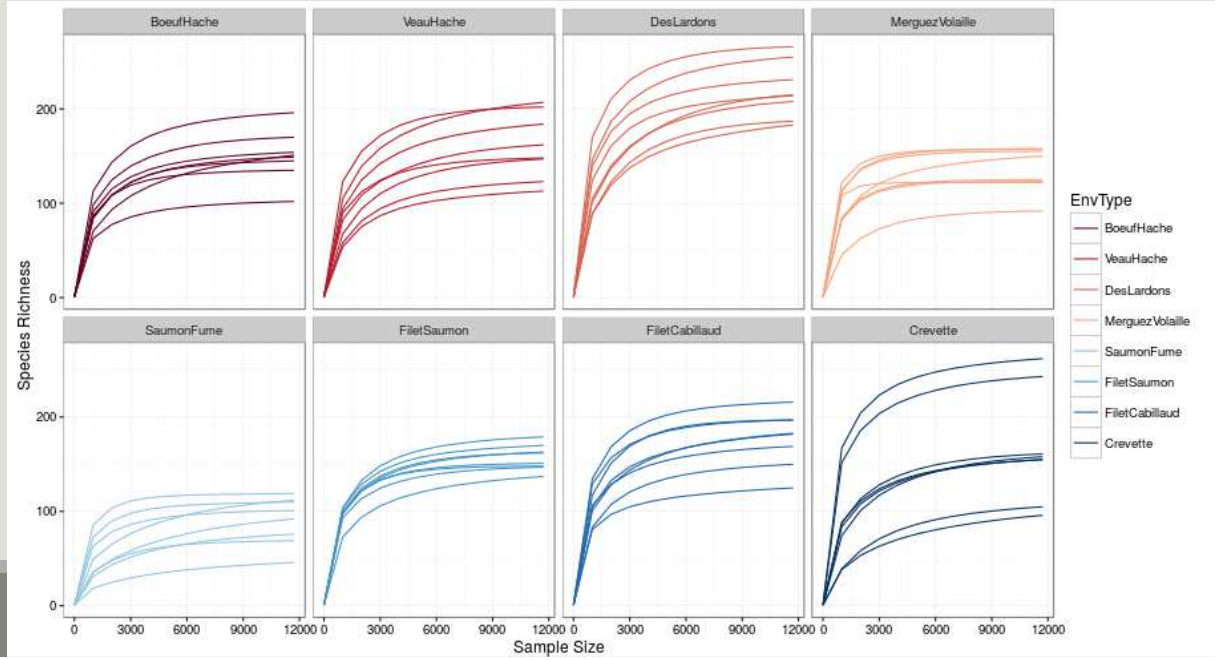
```
## Map sample type to color
tipColor <- col_factor(foodPalette, levels =
 levels(envtype))(envtype)

## Change hclust object to phylo object and
 plot
clust.uf <- as.phylo(hclust(dist.uf, method =
 "ward.D2"))

plot(clust.uf, tip.color = tipColor, direction
 = "downwards", main = "Ward linkage")
```



Go further : use your own color palette

Try this on any ggplot

```
food <- rarefy_even_depth(food, rngseed = 1121983)
p <- ggrare(food, step = 1000, color = "EnvType", se = FALSE)
p <- p + facet_wrap(~EnvType, ncol = 4) + theme_bw()
p <- p + scale_color_manual(values = foodPalette)
plot(p)
```



Go further : heatmap order

Custom OTU order in function of prevalence

```
prevalence <- estimate_prevalence(food,"EnvType")
```

get in a different format to estimate correlations

```
correlationData <- estimate_prevalence(food, "EnvType", format = "wide")
correlationData <- t(correlationData)
correlation <- cor(correlationData, method="pearson")
```

clustering sample and order OTU according to the tree

```
otu.clust <- hclust(as.dist(1-correlation), method = "complete")
otuOrder <- otu.clust$labels[otu.clust$order]
```

plot

```
p <- plot_heatmap(food, taxa.order = otuOrder)
p <- p + facet_grid(~EnvType, scales = "free", space = "free")
p <- p + scale_fill_gradient2(low="#1a9850", mid = "#ffffbf", high="#d73027",
 na.value = "white", trans = log_trans(4), midpoint = log(100,base= 4 ))
plot(p)
```

Go further : heatmap order