



# Ecological analysis of metabarcoding data

Introduction to R

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### Theory on all the steps of the metabarcoding pipeline



Raw data

BIOLAWEB



+ taxonomy



### Now : Ecological analysis of metabarcoding data













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## What is R?







# What can we do with R?

## **Analytics**

**Basic mathematics** 

Statistical tests

Big data analysis

Statistical modeling

Machine learning

### **Graphics and visualisation**

Static graphics

### Cartography







Residual standard error: 2.817 on 995 degrees of freedom Multiple R-squared: 0.7882, Adjusted R-squared: 0.7873 F-statistic: 925.6 on 4 and 995 DF, p-value: < 2.2e-16

# R console

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## R is used through a command line console



R version 4.2.0 (2022-04-22) -- "Vigorous Calisthenics" Copyright (C) 2022 The R Foundation for Statistical Computing Platform: x86\_64-apple-darwin17.0 (64-bit)

R est un logiciel libre livré sans AUCUNE GARANTIE. Vous pouvez le redistribuer sous certaines conditions. Tapez 'license()' ou 'licence()' pour plus de détails.

R est un projet collaboratif avec de nombreux contributeurs. Tapez 'contributors()' pour plus d'information et 'citation()' pour la façon de le citer dans les publications.

Tapez 'demo()' pour des démonstrations, 'help()' pour l'aide en ligne ou 'help.start()' pour obtenir l'aide au format HTML. Tapez 'q()' pour quitter R.

[R.app GUI 1.78 (8075) x86\_64-apple-darwin17.0]

[Espace de Travail restauré depuis /Users/clemonnie/.RData] [Historique recherché depuis /Users/clemonnie/.Rapp.history]

>



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## R console

## R is used through a command line console

### Set to the working directory

> setwd(dir="~/Desktop/R\_Introduction/")

### Load a table

> Microscopy\_table <- read.table("Microscopy\_table.csv", header=T, sep=";")</pre>

### Visualize the table

> head(microscopy\_table)

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hand(minutestation hash) a				
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Cryptomonas manssonij	3 263544 1 52038	33.4008083.50	348493 0 000000 0	0000000
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2 4441766 1 8131457 1 25	102 12 4305400 5 7	25972 2 9743494	10 201000 10 042706	3 8705246
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# **R** Studio<sup>®</sup> Is an integrated development environment (IDE)





**R** Studio<sup>®</sup> Is an integrated development environment (IDE)

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9 Eubacteria				Merismopediaceae	Aphanocapsa	Aphanocapsa delicatissima			
10 Eubacteria	Cyanobacteria			Merismopediaceae					
11 Plantae			Chlorellales						
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Residual standard error: 2.817 on 995 degrees of freedom Multiple R-squared: 0.7882, Adjusted R-squared: 0.7873 F-statistic: 925.6 on 4 and 995 DF, p-value: < 2.2e-16

## R Language

### Command line example







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## R Language

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### Command line example





## R Language

**RIOLAWEB** 

### Command line example



A variable in R is the memory allocated to the stockage of a specific **object** 

It is assigned to an object using either e or <-

A function() is made to perform a specific task.

It might works with arguments



## **R** Language

### Different type of objects in R



Vector

[1]	"Monday"	"Tuesday"
[3]	"Wednesday"	"Thursday"
[5]	"Friday"	"Saturday"
[7]	"Sunday"	



Matrix

	ANN0121 🗘	ANN0221 🗘
ASV1	3317	3583
ASV2	1040	359
ASV3	0	9
ASV4	673	509
ASV5	2698	3342



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Data frame

<b>^</b>	Month 🗘	Date 🗘	day 🗘
ANN0121	January	19/01/21	19
ANN0221	February	09/02/21	40
ANN0321	March	09/03/21	68
ANN0421	April	01/04/21	91
ANN0521	April	15/04/21	105



# R Language

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### Different type of objects in R



Source: R in Action, p.23



## R Language

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### Different classes of objects in R





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# R Language

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### Create objects





## R Language

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Manipulate objects using « [] »





## **R** Language

### Basic operations and functions

### **Different operators**

R o	perators
+	Addition
*	Multiplication
/	Division
-	Soustraction
Logica	l operators
&	And
I	Or
!	Not

Relational operators					
>	More than				
<	Less than				
>=	More or equal				
!=	Not equal to				

Miscellaneous oper.			
%in%	in		

### **Different functions**

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subset()
sort()
order()
factor()
str()
print()
plot()
merge()
na.omit()

To do a specific mathematical or logical operation

To do a specific task

. . .



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# R Language

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### Basic operations and functions

### Multiply one matrix by one column of a data frame



new\_matrix <- matrix \* df\$col3



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# R Language

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Basic operations and functions

Keep only rows that are in category « a »







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Combination of different functions for a specific purpose

Most of the time they are available in CRAN









# R Packages

Community ecology : vegan package / ade4 package

Test hypothesis

Multivariate analysis

Constrained analysis

Composant analysis, PCA





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## R Packages

### Graphic display : ggplot2





## **R** Packages

### Data manipulation : Dplyr

## Data Transformation with dplyr : : **CHEAT SHEET**

dplyr functions work with pipes and expect tidy data. In tidy data:



pipes

### Summarise Cases

These apply summary functions to columns to create a new table of summary statistics. Summary functions take vectors as input and return one value (see back).





Compute table of summaries. summarise(mtcars, avg = mean(mpg))



count(x, ..., wt = NULL, sort = FALSE) Count number of rows in each group defined by the variables in ... Also tally(). count(iris, Species)

#### Manipulate Cases

#### EXTRACT CASES

-

Row functions return a subset of rows as a new table.



distinct(.data, ..., .keep\_all = FALSE) Remove rows with duplicate values. distinct(iris, Species)

sample\_frac(tbl, size = 1, replace = FALSE, → weight = NULL, env = parent, frame()) Randomly select fraction of rows. sample\_frac(iris, 0.5, replace = TRUE)

> sample\_n(tbl, size, replace = FALSE, weight = NULL, .env = parent.frame()) Randomly select size rows. sample\_n(iris, 10, replace = TRUE)

slice(.data, ...) Select rows by position. slice(iris, 10:15)

top\_n(x, n, wt) Select and order top n entries (by group if grouped data). top\_n(iris, 5, Sepal.Width)

### Manipulate Variables

#### EXTRACT VARIABLES

Column functions return a set of columns as a new vector or table.



pull(.data, var = -1) Extract column values as a vector. Choose by name or index. pull(iris, Sepal.Length)

dply

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select(.data, ...) Extract columns as a table. Also select\_if(). select(iris, Sepal.Length, Species)

#### Use these helpers with select (), e.g. select(iris, starts\_with("Sepal"))

contains(match) num\_range(prefix, range) :, e.g. mpg:cyl ends\_with(match) one\_of(...) -, e.g, -Species matches(match) starts\_with(match)

#### MAKE NEW VARIABLES

These apply vectorized functions to columns. Vectorized funs take vectors as input and return vectors of the same length as output (see back).

vectorized function





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### Learning how to code in R language could be the subject of an entire workshop

It takes time to understand how the R function works, how to have the good code that will do what we want...

An important reflex : search for help



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#### Files Plots Packages Help Viewer Presentation Q diversity 8 C 🔶 📫 🏠 🚛 diversity {vegan} R Documentation **Ecological Diversity Indices** Description Shannon, Simpson, and Fisher diversity indices and species richness. Usage diversity(x, index = "shannon", groups, equalize.groups = FALSE, MARGIN = 1, base = exp(1)) simpson.unb(x, inverse = FALSE) fisher.alpha(x, MARGIN = 1, ...) specnumber(x, groups, MARGIN = 1)

#### Arguments

Comn	nunit	y data,	, a matrix-like	object or	a vector.	

index

Diversity index, one of "shannon", "simpson" or "invsimpson".





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Any question has an answer on internet !!

l documentation of package		culate Shannon diversity ir	ndex vegan r	×   C	Tutorial made by	/ other sc
Vegan: ecolog Jari Ok processed with vegan 2.6-4 in It version Abstract This document explains diversity related methods in vegan. The methods are briefly described, and	ical diversity sanen L2.1 (2022-06-23) on October 11, 2022 The vegan package has two major com methods for diversity analysis of ecological methods for diversity analysis of ecological	€ © + © liversity 246-23) on October 11, 2022 gan package has two major components: iate analysis (recological commu-		Clean and the second seco	Sign in Register rest habitist spas. The data are published in a mady coor paper: robliges demonstrate dozer correspondence to plant spacies plantar rota() , metal00(1): coe()	
The equations used them are given orten in detail than in their help pages. The methods cussed include common diversity indices and effaction, families of diversity indices, species a dance models, species accumulation models beta diversity, extrapolated richness and prol ity of being a member of the species pool, document is still incomplete and does not ow	stack <b>overflow</b>	About Products For Teams	Q Search			Log in Sign up
	Home	How do i get a sha metadata (veqan r	nnon and simp backage)	son diversity index using	g raw count table and	Ask Question
Forums	<b>Questions</b>	Asked 11 months ago Modified 1	0 months ago Viewed 304	times 🔅 Part of R Language Collective		



Now let's practice !