



Bioinformatic analysis of metabarcoding data using DADA2

Introduction to R

Clarisse Lemonnier

Program

DAY 1 (31st May) 09:00– 12:20

online

09:00-09:10	<i>Welcome speech</i> Dr Miloš Ćirić, UB-ICTM
09:10 – 10:40	<i>R Introduction, installation</i> Dr Clarisse Lemonnier, INRAE
10:40 – 11:00	Coffee break
11:00 – 12:00	<i>DADA2 - theoretical part</i> Dr Clarisse Lemonnier, INRAE
12:00 – 12:20	Q&A

- See if everything is ready for the practical part
- Theory on metabarcoding pipeline

Program

DAY 2 (3 rd Jun) 09:00– 15:00 <i>Onsite (UB-ICTM) and online (INRAE)</i>	
09:00-09:15	Registration of participants
09:15 – 10:30	<i>DADA2 – practical part, 1st part</i> Dr Clarisse Lemonnier, INRAE
10:30 – 10:50	Coffee break
10:50 – 12:15	<i>DADA2 – practical part, 2nd part</i> Dr Clarisse Lemonnier, INRAE
12:15 – 13:15	Lunch break (self-provided)
13:15 – 14:15	<i>DADA2 – practical part, 3rd part</i> Dr Clarisse Lemonnier, INRAE
14:15 – 15:00	Q&A

We will go through all the pipeline step by step
Listen carefully, because you will do it on real data on Tuesday !

Program

DAY 3 (4 th Jun) 09:00– 15:00 <i>Onsite (UB-ICTM) and online (INRAE)</i>	
09:00 – 09:15	Registration of participants
09:15 – 10:30	<i>DADA2 – practical part, 1st part</i> (participants will be divided into groups and work with different data sets)
10:30 – 10:50	Coffee break
10:50 – 12:15	<i>DADA2 – practical part, 2nd part</i> (work in group)
12:15 – 13:15	Lunch break (self-provided)
13:15 – 14:15	<i>DADA2 – practical part, 3rd part</i> (work in group)
14:15 – 15:00	Q&A

You will be divided into 3 groups - one with a specific dataset
Diatom (rbcl) - Phytoplankton 16S - Phytoplankton - 23S

Program

DAY 4 (5th Jun) 09:00– 11:00

Onsite (UB-ICTM) and online (INRAE)

09:00 – 09:15	Registration of participants
09:15 – 09:45	<i>Presentation of results (Group 1)</i>
09:45 – 10:05	Coffee break
10:05 – 10:35	<i>Presentation of results (Group 2)</i>
10:35 – 11:00	Q&A

DAY 5 (6th Jun) 09:00– 11:00

Onsite (UB-ICTM) and online (INRAE)

09:00 – 09:15	Registration of participants
09:15 – 09:45	<i>Presentation of results (Group 3)</i>
09:45 – 10:05	Coffee break
10:05 – 10:35	<i>Summarizing</i> Dr Clarisse Lemonnier, INRAE
10:35 – 11:00	Q&A

Each group will present the results they got at the end, the difficulties they had to run the dada2 pipeline

So don't forget to save figures, or take notes to make a little presentation

At the end there will be a little questionnaire :)

R use - basics

```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
(Intercept)  1.3831  0.24566  0.2330  2.3330 ***
x1           5.29776  0.12483  0.0000  0.0000 ***
x2           1.61055  0.0518  0.0000  0.0000 ***
x3           0.44947  0.016  0.0000  0.0000 ***
groupB       1.87978  0.16  0.0000  0.0000 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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
```

What is R ?

R is an **open source** programming language

It was designed for **statistical computing, data analysis and graph display**

It works in all operating system



It is widely used in academic research

```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.3831     0.24566   5.634  <0.0001 **
x1           5.2977     0.12483  42.440  <0.0001 **
x2           1.6105     0.0518    31.090  <0.0001 **
x3           0.44947    0.016    28.086  <0.0001 **
groupB       1.87978    0.16    11.746  <0.0001 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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
```

What can we do with R?

Analytics

Basic mathematics

Statistical tests

Big data analysis

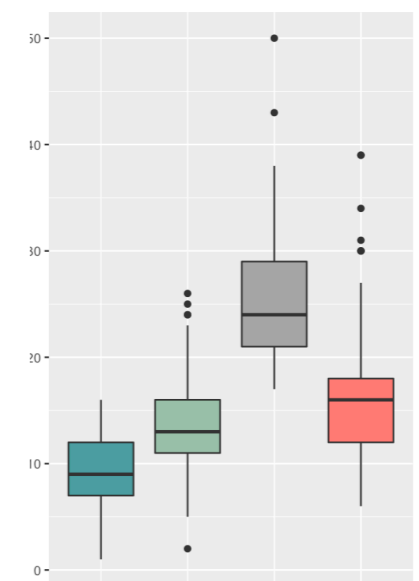
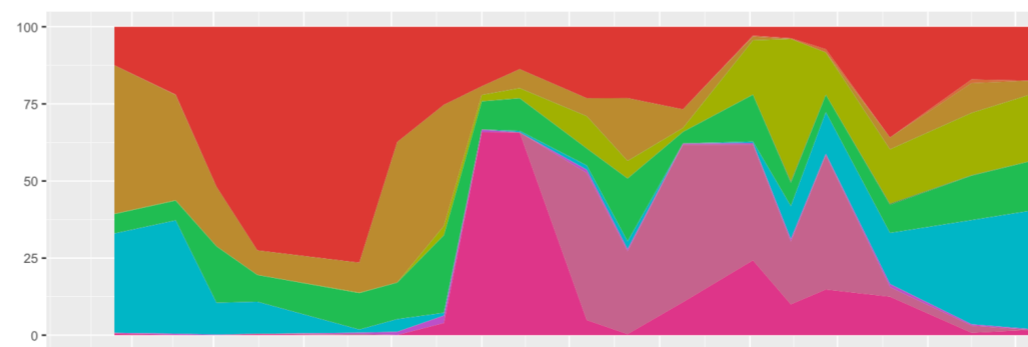
Statistical modeling

Machine learning

Graphics and visualisation

Static graphics

Cartography




```
Call:
lm(formula = y ~ ., data = data)

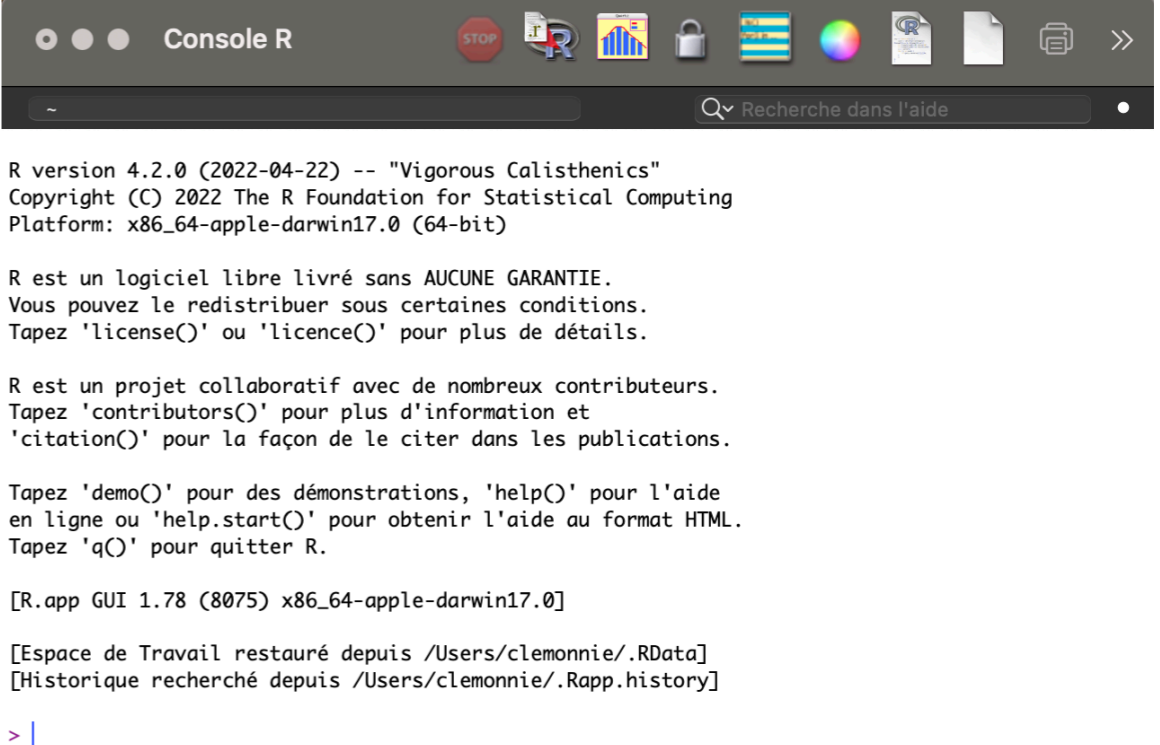
Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
            1          2          3          4          5          6
(Intercept) 1.3831  5.29776  1.61055  0.44947  1.87978  0.16000
            7          8          9         10         11         12
            13         14         15         16         17         18
            19         20         21         22         23         24
            25         26         27         28         29         30
            31         32         33         34         35         36
            37         38         39         40         41         42
            43         44         45         46         47         48
            49         50         51         52         53         54
            55         56         57         58         59         60
            61         62         63         64         65         66
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            541        542        543        544        545        546
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            553        554        555        556        557        558
            559        560        561        562        563        564
            565        566        567        568        569        570
            571        572        573        574        575        576
            577        578        579        580        581        582
            583        584        585        586        587        588
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            595        596        597        598        599        600
            601        602        603        604        605        606
            607        608        609        610        611        612
            613        614        615        616        617        618
            619        620        621        622        623        624
            625        626        627        628        629        630
            631        632        633        634        635        636
            637        638        639        640        641        642
            643        644        645        646        647        648
            649        650        651        652        653        654
            655        656        657        658        659        660
            661        662        663        664        665        666
            667        668        669        670        671        672
            673        674        675        676        677        678
            679        680        681        682        683        684
            685        686        687        688        689        690
            691        692        693        694        695        696
            697        698        699        700        701        702
            703        704        705        706        707        708
            709        710        711        712        713        714
            715        716        717        718        719        720
            721        722        723        724        725        726
            727        728        729        730        731        732
            733        734        735        736        737        738
            739        740        741        742        743        744
            745        746        747        748        749        750
            751        752        753        754        755        756
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            763        764        765        766        767        768
            769        770        771        772        773        774
            775        776        777        778        779        780
            781        782        783        784        785        786
            787        788        789        790        791        792
            793        794        795        796        797        798
            799        800        801        802        803        804
            805        806        807        808        809        810
            811        812        813        814        815        816
            817        818        819        820        821        822
            823        824        825        826        827        828
            829        830        831        832        833        834
            835        836        837        838        839        840
            841        842        843        844        845        846
            847        848        849        850        851        852
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            859        860        861        862        863        864
            865        866        867        868        869        870
            871        872        873        874        875        876
            877        878        879        880        881        882
            883        884        885        886        887        888
            889        890        891        892        893        894
            895        896        897        898        899        900
            901        902        903        904        905        906
            907        908        909        910        911        912
            913        914        915        916        917        918
            919        920        921        922        923        924
            925        926        927        928        929        930
            931        932        933        934        935        936
            937        938        939        940        941        942
            943        944        945        946        947        948
            949        950        951        952        953        954
            955        956        957        958        959        960
            961        962        963        964        965        966
            967        968        969        970        971        972
            973        974        975        976        977        978
            979        980        981        982        983        984
            985        986        987        988        989        990
            991        992        993        994        995

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```

R is used through a command line console

R console



```
Console R
~
Recherche dans l'aide

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)

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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]

> |
```

```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
(Intercept) 1.3831 24566 0.2... **
x1          5.29776 12483 ... **
x2          1.61055 518 ... ***
x3          0.44947 ... **
groupB     1.87978 0.16... **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2.817 on 995 degrees of freedom
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```



R console

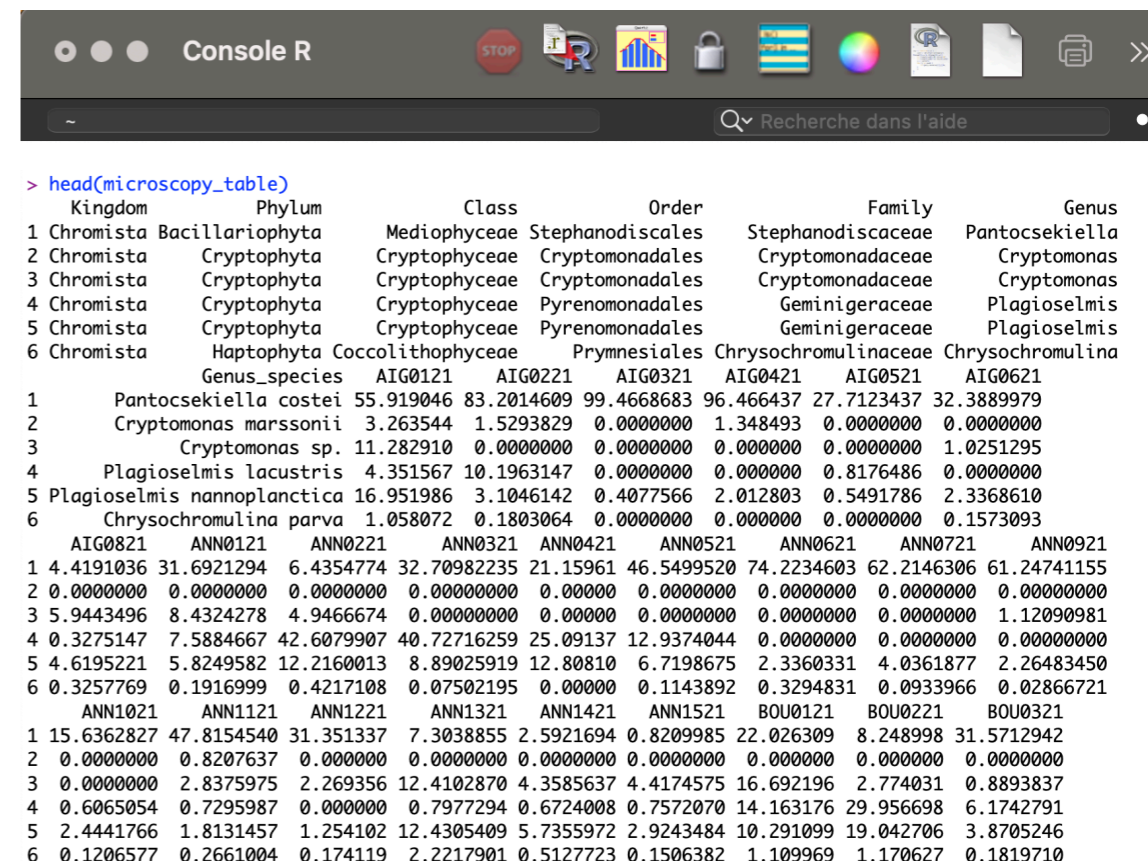
R is used through a command line console

Load a table

```
> Microscopy_table <- read.table("Microscopy_table.csv", header=T, sep=";")
```

Visualize the table

```
> head(microscopy_table)
```

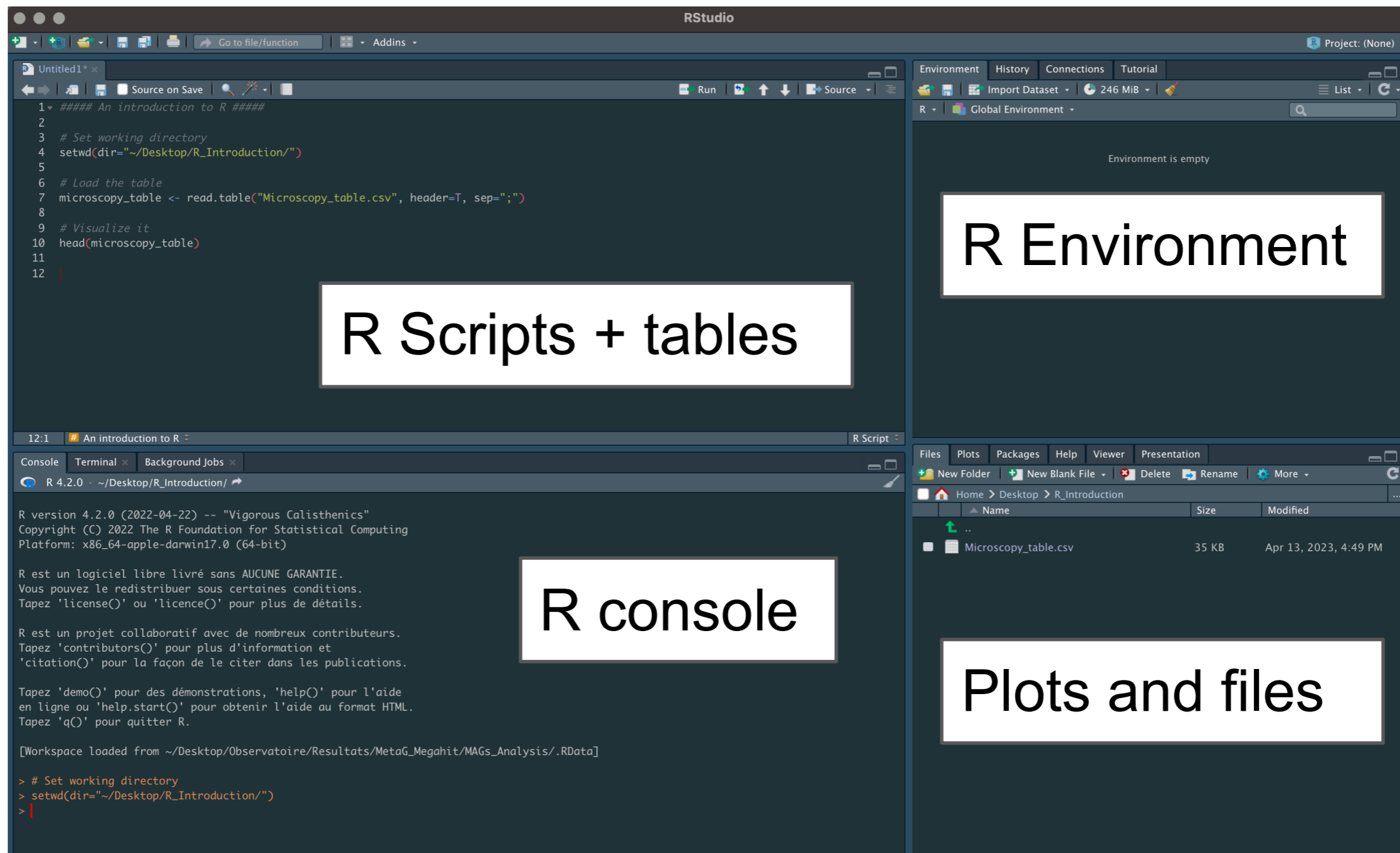


```
> head(microscopy_table)
  Kingdom      Phylum      Class      Order      Family      Genus
1 Chromista Bacillariophyta Mediophyceae Stephanodiscales Stephanodiscaceae Pantocsekiella
2 Chromista Cryptophyta Cryptophyceae Cryptomonadales Cryptomonadaceae Cryptomonas
3 Chromista Cryptophyta Cryptophyceae Cryptomonadales Cryptomonadaceae Cryptomonas
4 Chromista Cryptophyta Cryptophyceae Pyrenomonadales Geminigeraceae Plagioselmis
5 Chromista Cryptophyta Cryptophyceae Pyrenomonadales Geminigeraceae Plagioselmis
6 Chromista Haptophyta Coccolithophyceae Prymnesiales Chrysochromulinaceae Chrysochromulina
  Genus_species AIG0121 AIG0221 AIG0321 AIG0421 AIG0521 AIG0621
1 Pantocsekiella costei 55.919046 83.2014609 99.4668683 96.466437 27.7123437 32.3889979
2 Cryptomonas marssonii 3.263544 1.5293829 0.0000000 1.348493 0.0000000 0.0000000
3 Cryptomonas sp. 11.282910 0.0000000 0.0000000 0.0000000 0.0000000 1.0251295
4 Plagioselmis lacustris 4.351567 10.1963147 0.0000000 0.0000000 0.8176486 0.0000000
5 Plagioselmis nannoplanctica 16.951986 3.1046142 0.4077566 2.012803 0.5491786 2.3368610
6 Chrysochromulina parva 1.058072 0.1803064 0.0000000 0.0000000 0.0000000 0.1573093
  AIG0821 ANN0121 ANN0221 ANN0321 ANN0421 ANN0521 ANN0621 ANN0721 ANN0921
1 4.4191036 31.6921294 6.4354774 32.70982235 21.15961 46.5499520 74.2234603 62.2146306 61.24741155
2 0.0000000 0.0000000 0.0000000 0.0000000 0.00000 0.0000000 0.0000000 0.0000000 0.0000000
3 5.9443496 8.4324278 4.9466674 0.00000000 0.00000 0.0000000 0.0000000 0.0000000 1.12090981
4 0.3275147 7.5884667 42.6079907 40.72716259 25.09137 12.9374044 0.0000000 0.0000000 0.0000000
5 4.6195221 5.8249582 12.2160013 8.89025919 12.80810 6.7198675 2.3360331 4.0361877 2.26483450
6 0.3257769 0.1916999 0.4217108 0.07502195 0.00000 0.1143892 0.3294831 0.0933966 0.02866721
  ANN1021 ANN1121 ANN1221 ANN1321 ANN1421 ANN1521 BOU0121 BOU0221 BOU0321
1 15.6362827 47.8154540 31.351337 7.3038855 2.5921694 0.8209985 22.026309 8.248998 31.5712942
2 0.0000000 0.8207637 0.000000 0.0000000 0.0000000 0.0000000 0.000000 0.000000 0.0000000
3 0.0000000 2.8375975 2.269356 12.4102870 4.3585637 4.4174575 16.692196 2.774031 0.8893837
4 0.6065054 0.7295987 0.000000 0.7977294 0.6724008 0.7572070 14.163176 29.956698 6.1742791
5 2.4441766 1.8131457 1.254102 12.4305409 5.7355972 2.9243484 10.291099 19.042706 3.8705246
6 0.1206577 0.2661004 0.174119 2.2217901 0.5127723 0.1506382 1.109969 1.170627 0.1819710
```



Studio[®]

Is an integrated development environment (IDE)



The screenshot shows the RStudio IDE interface with four white boxes highlighting key components:

- R Scripts + tables**: Points to the source editor showing R code for reading a CSV file and displaying its head.
- R Environment**: Points to the Environment pane, which is currently empty.
- R console**: Points to the console window showing the R startup message and the execution of the code from the script.
- Plots and files**: Points to the Files pane showing the current directory structure and a file named 'Microscopy_table.csv'.

```
1 ##### An introduction to R #####
2
3 # Set working directory
4 setwd(dir=~ /Desktop/R_Introduction/")
5
6 # Load the table
7 microscopy_table <- read.table("Microscopy_table.csv", header=T, sep=";")
8
9 # Visualize it
10 head(microscopy_table)
11
12
```

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)

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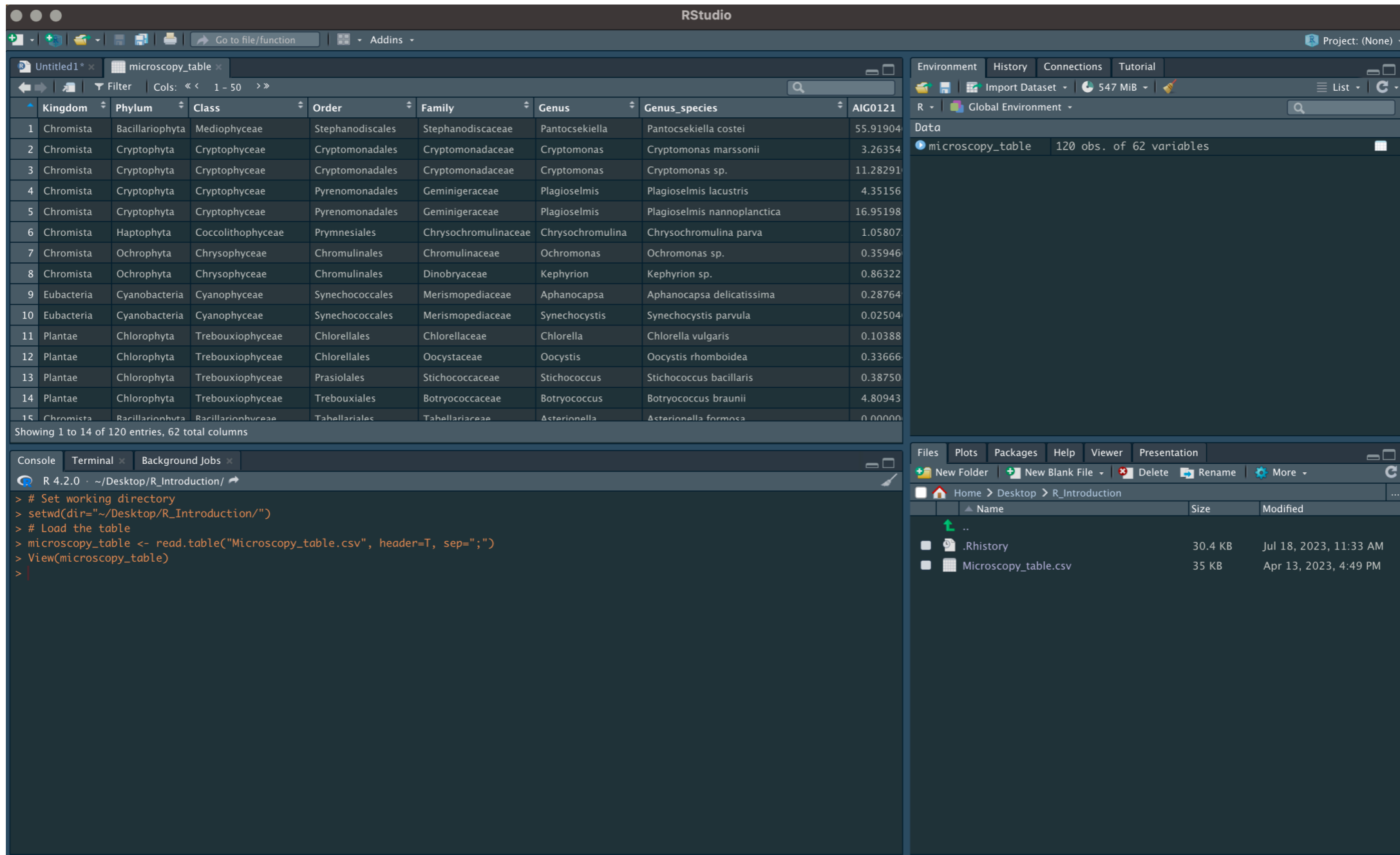
[Workspace loaded from ~/Desktop/Observatoire/Resultats/MetaG_Megahit/MAGs_Analysis/.RData]

```
> # Set working directory
> setwd(dir=~ /Desktop/R_Introduction/")
>
```

Name	Size	Modified
..		
Microscopy_table.csv	35 KB	Apr 13, 2023, 4:49 PM



Is an integrated development environment (IDE)



The screenshot displays the RStudio integrated development environment (IDE) interface. The main window shows a data table with 14 rows and 9 columns. The columns are: Kingdom, Phylum, Class, Order, Family, Genus, Genus_species, and AIG0121. The data represents various microorganisms, including Pantocsekiella, Cryptomonas, Plagioselmis, Chrysochromulina, and Aphanocapsa.

The console window at the bottom left shows the following R code being executed:

```
> # Set working directory
> setwd(dir="~/Desktop/R_Introduction/")
> # Load the table
> microscopy_table <- read.table("Microscopy_table.csv", header=T, sep=";")
> View(microscopy_table)
>
```

The file explorer on the right shows the current directory structure, including a file named "Microscopy_table.csv" with a size of 35 KB, last modified on April 13, 2023, at 4:49 PM.


```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
            1          2          3          4          5          6
(Intercept) 1.3831  5.2977  1.6105  0.4494  1.8797  0.1600
            7          8          9         10         11         12
            13         14         15         16         17         18
            19         20         21         22         23         24
            25         26         27         28         29         30
            31         32         33         34         35         36
            37         38         39         40         41         42
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            61         62         63         64         65         66
            67         68         69         70         71         72
            73         74         75         76         77         78
            79         80         81         82         83         84
            85         86         87         88         89         90
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            161        162        163        164        165        166
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            173        174        175        176        177        178
            179        180        181        182        183        184
            185        186        187        188        189        190
            191        192        193        194        195        196
            197        198        199        200

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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```

R Language

Command line example

```
> ASV_table <- read.table("ASV_table.txt", header=T, row.names=1, sep="\t")
```

```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
(Intercept)  1.3831  1.24566  1.0230  2.3000  *
x1           5.29776  1.2483  1.0000  0.0000  **
x2           1.61055  0.518  1.0000  0.0000  ***
x3           0.44947  0.16  1.0000  0.0000  ***
groupB       1.87978  0.16  1.0000  0.0000  ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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

```
> ASV_table <- read.table("ASV_table.txt", header=T, row.names=1, sep="\t")
```

function

arguments

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

It might works with arguments

```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
(Intercept)  1.3831  1.24566  0.24566 ***
x1           5.29776  1.2483  1.2483 ***
x2           1.61055  0.518  0.518 ***
x3           0.44947  0.16  0.16 ***
groupB       1.87978  0.16  0.16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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

```
> ASV_table <- read.table("ASV_table.txt", header=T, row.names=1, sep="\t")
```

variable

function

arguments

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

It is assigned to an object using either **=** or **<-**

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

It might works with arguments

```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

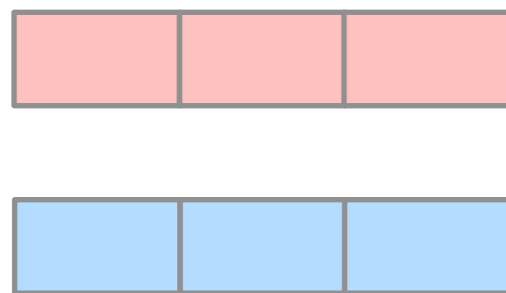
Coefficients:
(Intercept)  1.3831  0.24566  0.2023  0.2023  0.2023 ***
x1           5.29776  0.12483  0.0000  0.0000  0.0000 ***
x2           1.61055  0.0518  0.0000  0.0000  0.0000 ***
x3           0.44947  0.016  0.0000  0.0000  0.0000 ***
groupB       1.87978  0.16  0.0000  0.0000  0.0000 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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
```

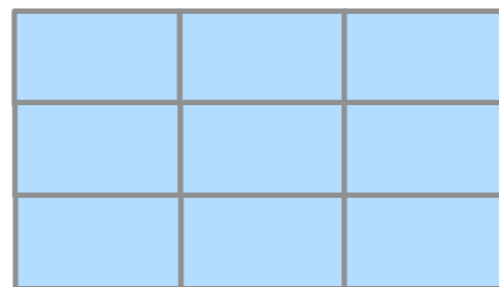


R Language

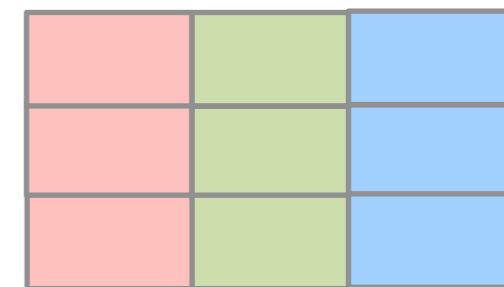
Different type of objects in R



Vector



Matrix



Data frame

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

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

	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


```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

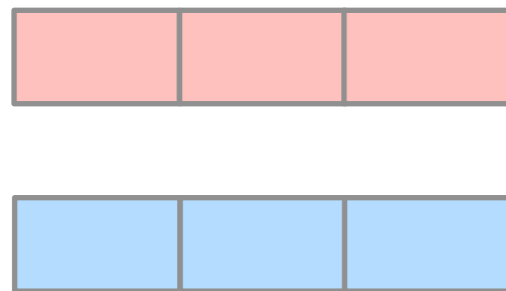
Coefficients:
(Intercept) 1.3831  2.24566  0.2300  0.0000  0.0000 *
x1          5.29776  0.12483  0.0000  0.0000  0.0000 ***
x2          1.61055  0.0518  0.0000  0.0000  0.0000 ***
x3          0.44947  0.0016  0.0000  0.0000  0.0000 ***
groupB      1.87978  0.1600  0.0000  0.0000  0.0000 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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
```

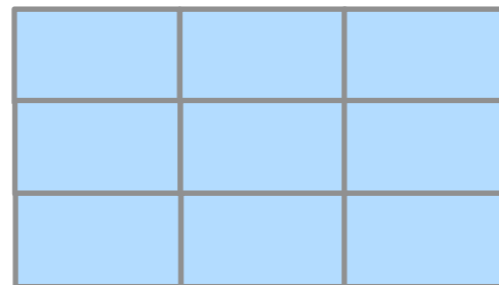


R Language

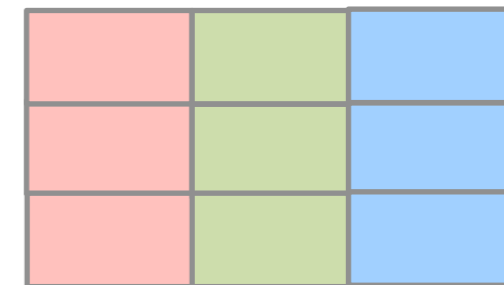
Different type of objects in R



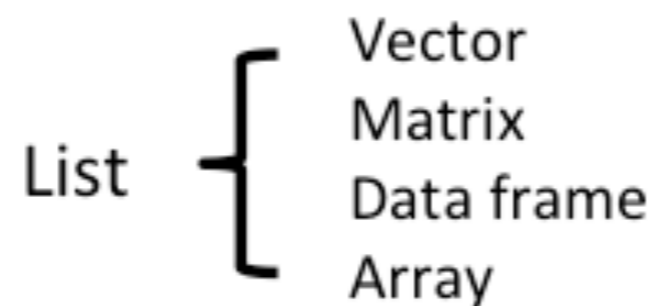
Vector



Matrix



Data frame



```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
(Intercept)  1.3831  1.24566  0.24566 ***
x1           5.29776  1.2483  1.2483 ***
x2           1.61055  0.518  0.518 ***
x3           0.44947  0.16  0.16 ***
groupB       1.87978  0.16  0.16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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

Different classes of objects in R

Character

« word »

Numeric

« 4.356 »

Integer

« 4 » or « 4L »

Date

« 01/10/2023 »

Logical

« TRUE » « FALSE »

Get the class of the object A

class(A)

Change A to a specific class

as.numeric(A)

```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
(Intercept)  1.3831  0.24566  0.2023  0.0000  0.0000 *
x1           5.29776  0.12483  0.0000  0.0000  0.0000 ***
x2           1.61055  0.0518  0.0000  0.0000  0.0000 ***
x3           0.44947  0.016  0.0000  0.0000  0.0000 ***
groupB       1.87978  0.16  0.0000  0.0000  0.0000 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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

Basic operations and functions

Different operators

R operators	
+	Addition
*	Multiplication
/	Division
-	Soustraction

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

Logical operators	
&	And
	Or
!	Not

Miscellaneous oper.	
%in%	in

Different functions

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

To do a specific mathematical or logical operation

To do a specific task

```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
            1          2          3          4          5          6
(Intercept) 1.3831    5.2977    1.6105    0.4494    1.8797    0.1600
x1          5.2977    1.6105    0.4494    1.8797    0.1600    0.1600
x2          1.6105    0.4494    1.8797    0.1600    0.1600    0.1600
x3          0.4494    1.8797    0.1600    0.1600    0.1600    0.1600
groupB      1.8797    0.1600    0.1600    0.1600    0.1600    0.1600
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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

Basic operations and functions

Load a file

```
> ASV_table <- read.table("ASV_table.txt", header=T, row.names=1, sep="\t")
```

```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
            1          2          3          4          5
(Intercept) 1.3831  5.2977  1.6105  0.4494  1.8797
            6          7          8          9         10
            11         12         13         14         15
            16         17         18         19         20
            21         22         23         24         25
            26         27         28         29         30
            31         32         33         34         35
            36         37         38         39         40
            41         42         43         44         45
            46         47         48         49         50
            51         52         53         54         55
            56         57         58         59         60
            61         62         63         64         65
            66         67         68         69         70
            71         72         73         74         75
            76         77         78         79         80
            81         82         83         84         85
            86         87         88         89         90
            91         92         93         94         95
            96         97         98         99        100
            101        102        103        104        105
            106        107        108        109        110
            111        112        113        114        115
            116        117        118        119        120
            121        122        123        124        125
            126        127        128        129        130
            131        132        133        134        135
            136        137        138        139        140
            141        142        143        144        145
            146        147        148        149        150
            151        152        153        154        155
            156        157        158        159        160
            161        162        163        164        165
            166        167        168        169        170
            171        172        173        174        175
            176        177        178        179        180
            181        182        183        184        185
            186        187        188        189        190
            191        192        193        194        195
            196        197        198        199        200
            201        202        203        204        205
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            211        212        213        214        215
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            231        232        233        234        235
            236        237        238        239        240
            241        242        243        244        245
            246        247        248        249        250
            251        252        253        254        255
            256        257        258        259        260
            261        262        263        264        265
            266        267        268        269        270
            271        272        273        274        275
            276        277        278        279        280
            281        282        283        284        285
            286        287        288        289        290
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            336        337        338        339        340
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            351        352        353        354        355
            356        357        358        359        360
            361        362        363        364        365
            366        367        368        369        370
            371        372        373        374        375
            376        377        378        379        380
            381        382        383        384        385
            386        387        388        389        390
            391        392        393        394        395
            396        397        398        399        400
            401        402        403        404        405
            406        407        408        409        410
            411        412        413        414        415
            416        417        418        419        420
            421        422        423        424        425
            426        427        428        429        430
            431        432        433        434        435
            436        437        438        439        440
            441        442        443        444        445
            446        447        448        449        450
            451        452        453        454        455
            456        457        458        459        460
            461        462        463        464        465
            466        467        468        469        470
            471        472        473        474        475
            476        477        478        479        480
            481        482        483        484        485
            486        487        488        489        490
            491        492        493        494        495
            496        497        498        499        500
            501        502        503        504        505
            506        507        508        509        510
            511        512        513        514        515
            516        517        518        519        520
            521        522        523        524        525
            526        527        528        529        530
            531        532        533        534        535
            536        537        538        539        540
            541        542        543        544        545
            546        547        548        549        550
            551        552        553        554        555
            556        557        558        559        560
            561        562        563        564        565
            566        567        568        569        570
            571        572        573        574        575
            576        577        578        579        580
            581        582        583        584        585
            586        587        588        589        590
            591        592        593        594        595
            596        597        598        599        600
            601        602        603        604        605
            606        607        608        609        610
            611        612        613        614        615
            616        617        618        619        620
            621        622        623        624        625
            626        627        628        629        630
            631        632        633        634        635
            636        637        638        639        640
            641        642        643        644        645
            646        647        648        649        650
            651        652        653        654        655
            656        657        658        659        660
            661        662        663        664        665
            666        667        668        669        670
            671        672        673        674        675
            676        677        678        679        680
            681        682        683        684        685
            686        687        688        689        690
            691        692        693        694        695
            696        697        698        699        700
            701        702        703        704        705
            706        707        708        709        710
            711        712        713        714        715
            716        717        718        719        720
            721        722        723        724        725
            726        727        728        729        730
            731        732        733        734        735
            736        737        738        739        740
            741        742        743        744        745
            746        747        748        749        750
            751        752        753        754        755
            756        757        758        759        760
            761        762        763        764        765
            766        767        768        769        770
            771        772        773        774        775
            776        777        778        779        780
            781        782        783        784        785
            786        787        788        789        790
            791        792        793        794        795
            796        797        798        799        800
            801        802        803        804        805
            806        807        808        809        810
            811        812        813        814        815
            816        817        818        819        820
            821        822        823        824        825
            826        827        828        829        830
            831        832        833        834        835
            836        837        838        839        840
            841        842        843        844        845
            846        847        848        849        850
            851        852        853        854        855
            856        857        858        859        860
            861        862        863        864        865
            866        867        868        869        870
            871        872        873        874        875
            876        877        878        879        880
            881        882        883        884        885
            886        887        888        889        890
            891        892        893        894        895
            896        897        898        899        900
            901        902        903        904        905
            906        907        908        909        910
            911        912        913        914        915
            916        917        918        919        920
            921        922        923        924        925
            926        927        928        929        930
            931        932        933        934        935
            936        937        938        939        940
            941        942        943        944        945
            946        947        948        949        950
            951        952        953        954        955
            956        957        958        959        960
            961        962        963        964        965
            966        967        968        969        970
            971        972        973        974        975
            976        977        978        979        980
            981        982        983        984        985
            986        987        988        989        990
            991        992        993        994        995

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 Packages

Combination of different functions for a specific purpose

Most of the time they are available in CRAN



But sometimes (particularly if they are recent) you can directly install them from Bioconductor or Github



```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
(Intercept)  1.3831  0.24566  0.2300  0.2300  0.2300 ***
x1           5.29776  0.12483  0.0000  0.0000  0.0000 ***
x2           1.61055  0.0518  0.0000  0.0000  0.0000 ***
x3           0.44947  0.016  0.0000  0.0000  0.0000 ***
groupB       1.87978  0.16  0.0000  0.0000  0.0000 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

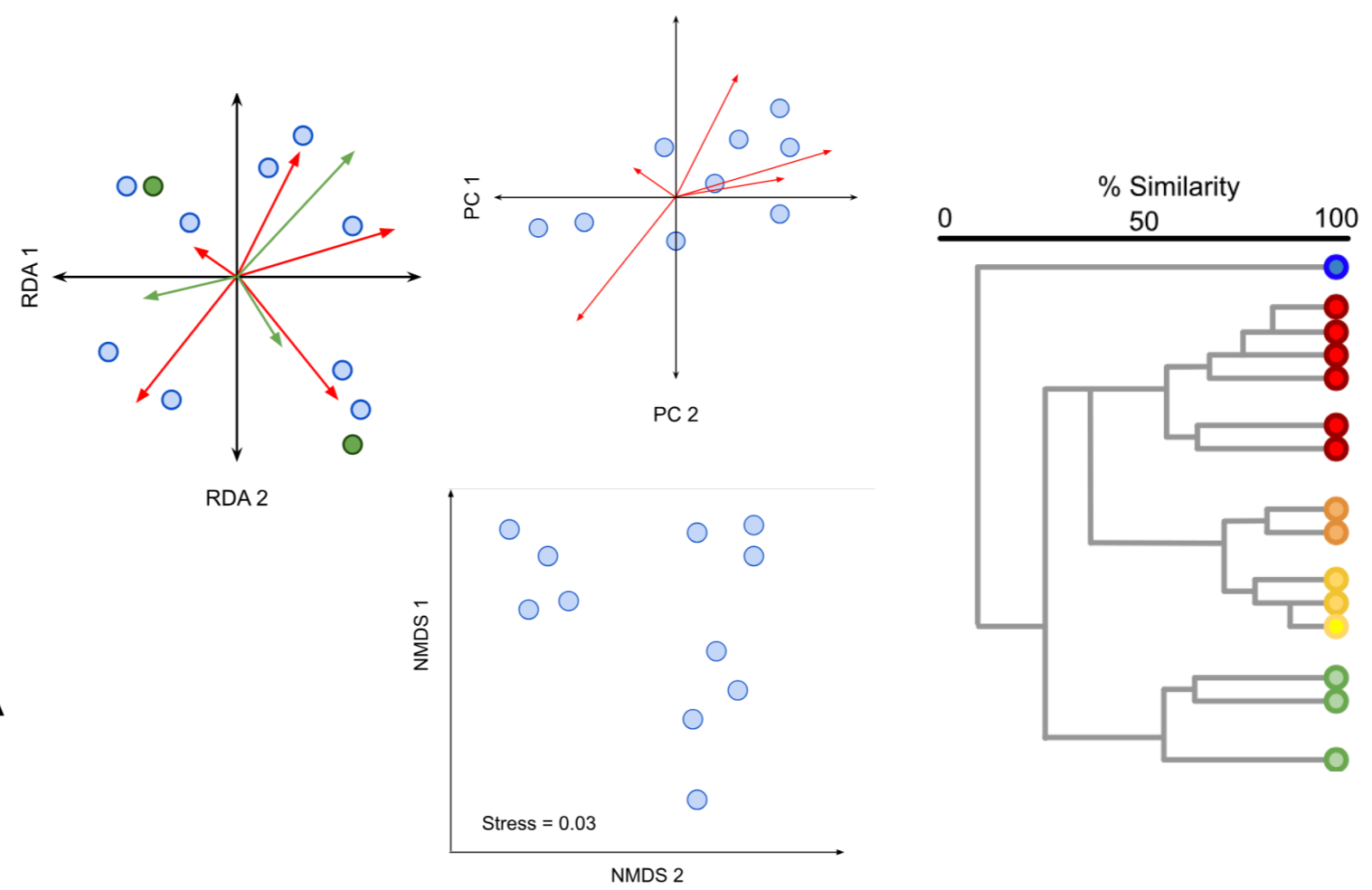
Residual standard error: 2.817 on 995 degrees of freedom
Multiple R-squared:  0.7882,    Adjusted R-squared:  0.7873
F-statistic: 925.6 on 4 and 995 DF,  p-value: < 2.2e-16
```



R Packages

Community ecology : vegan package / ade4 package

- Test hypothesis
- Multivariate analysis
- Constrained analysis
- Composant analysis, PCA




```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

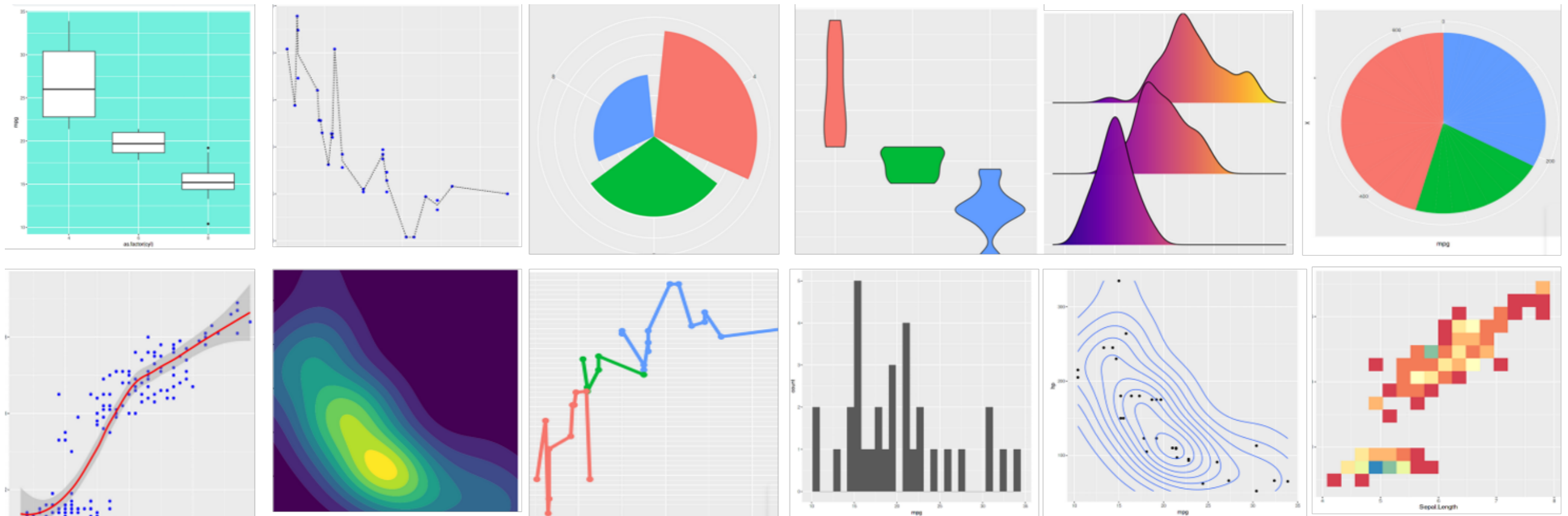
Coefficients:
(Intercept)  1.3831  2.24566  0.2330  2.3330 ***
x1           5.29776  1.2483  0.0000  0.0000 ***
x2           1.61055  0.518  0.0000  0.0000 ***
x3           0.44947  0.16  0.0000  0.0000 ***
groupB       1.87978  0.16  0.0000  0.0000 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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 Packages

Graphic display : ggplot2



```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

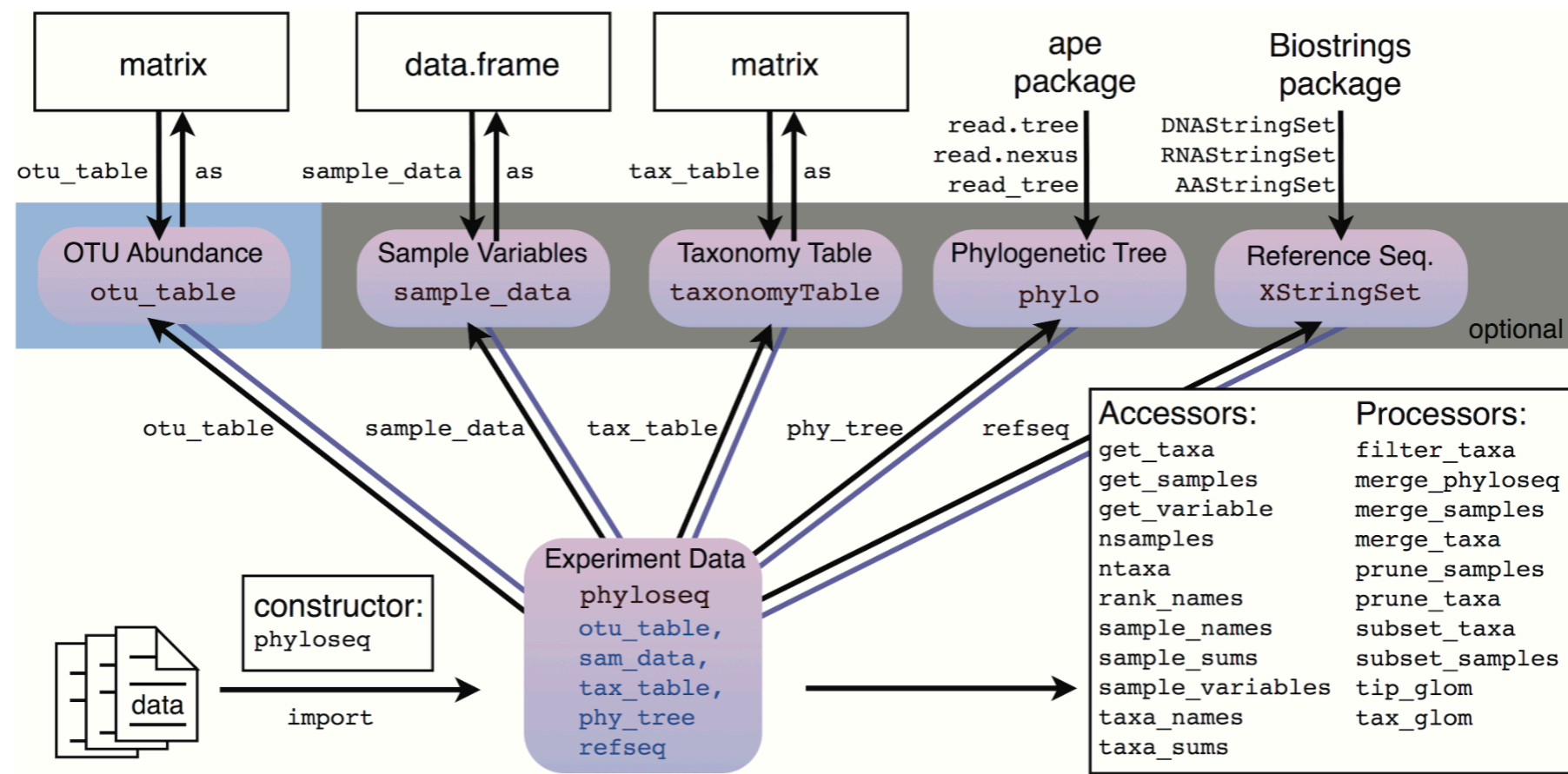
Coefficients:
(Intercept) 1.3831 0.24566 0.2023 0.0000 0.0000 ***
x1          5.29776 0.12483 0.0000 0.0000 0.0000 ***
x2          1.61055 0.0518 0.0000 0.0000 0.0000 ***
x3          0.44947 0.016 0.0000 0.0000 0.0000 ***
groupB      1.87978 0.16 0.0000 0.0000 0.0000 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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 Packages

A package dedicated to metabarcoding data analysis : phyloseq



```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
            1          2          3          4          5
(Intercept)  1.3831  1.24566  1.02023  0.24566  0.24566 *
x1           5.29776  1.2483  1.2483  1.2483  1.2483 **
x2           1.61055  1.518  1.518  1.518  1.518 ***
x3           0.44947  1.916  1.916  1.916  1.916 ***
groupB       1.87978  0.166  0.166  0.166  0.166 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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 Help

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

```
Call:
lm(formula = y ~ ., data = data)

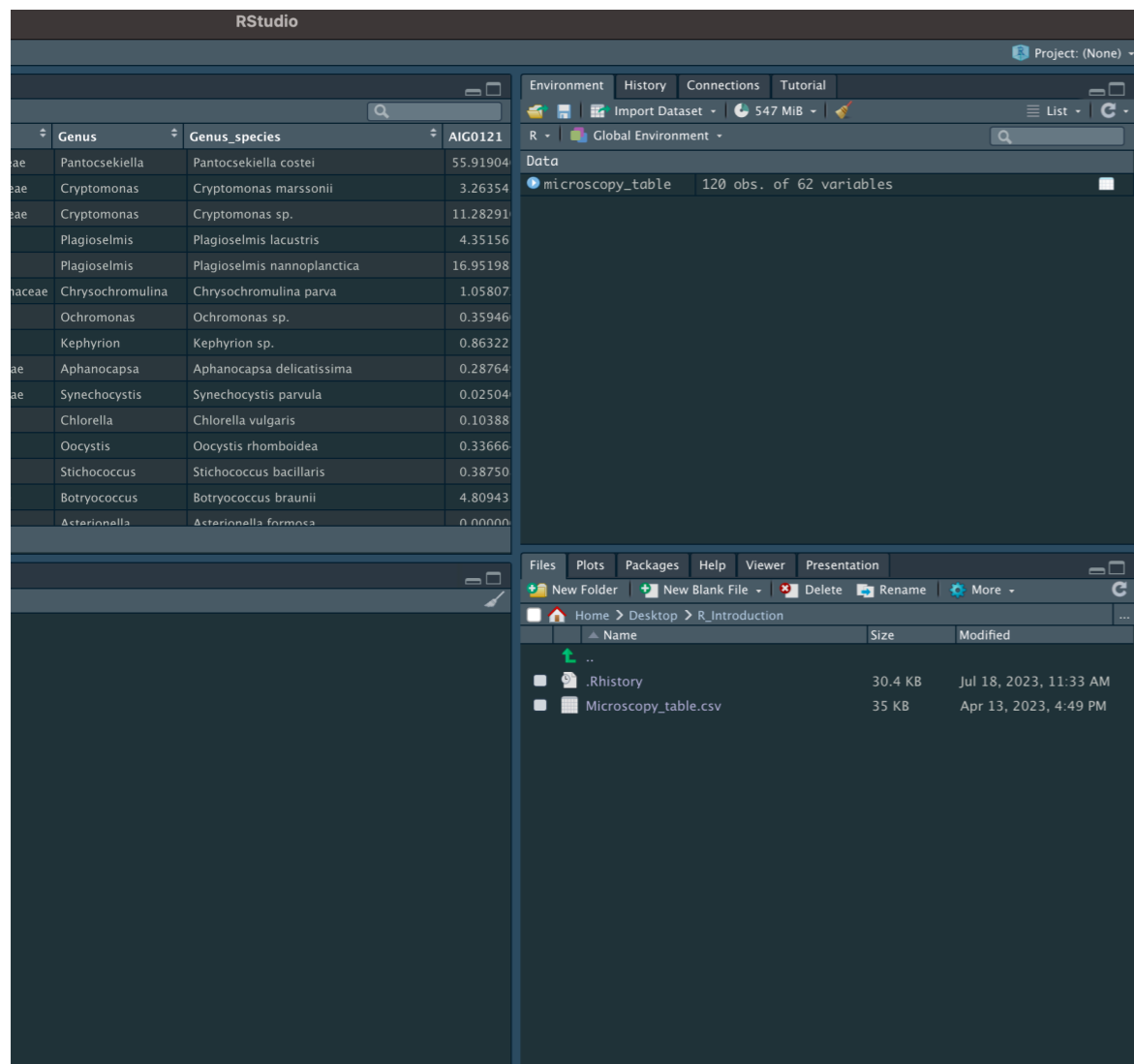
Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
(Intercept) 1.3831  0.24566  0.2023  0.2023 ***
x1          5.29776  0.12483  0.0000  0.0000 ***
x2          1.61055  0.0518  0.0000  0.0000 ***
x3          0.44947  0.019  0.0000  0.0000 ***
groupB      1.87978  0.16  0.0000  0.0000 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

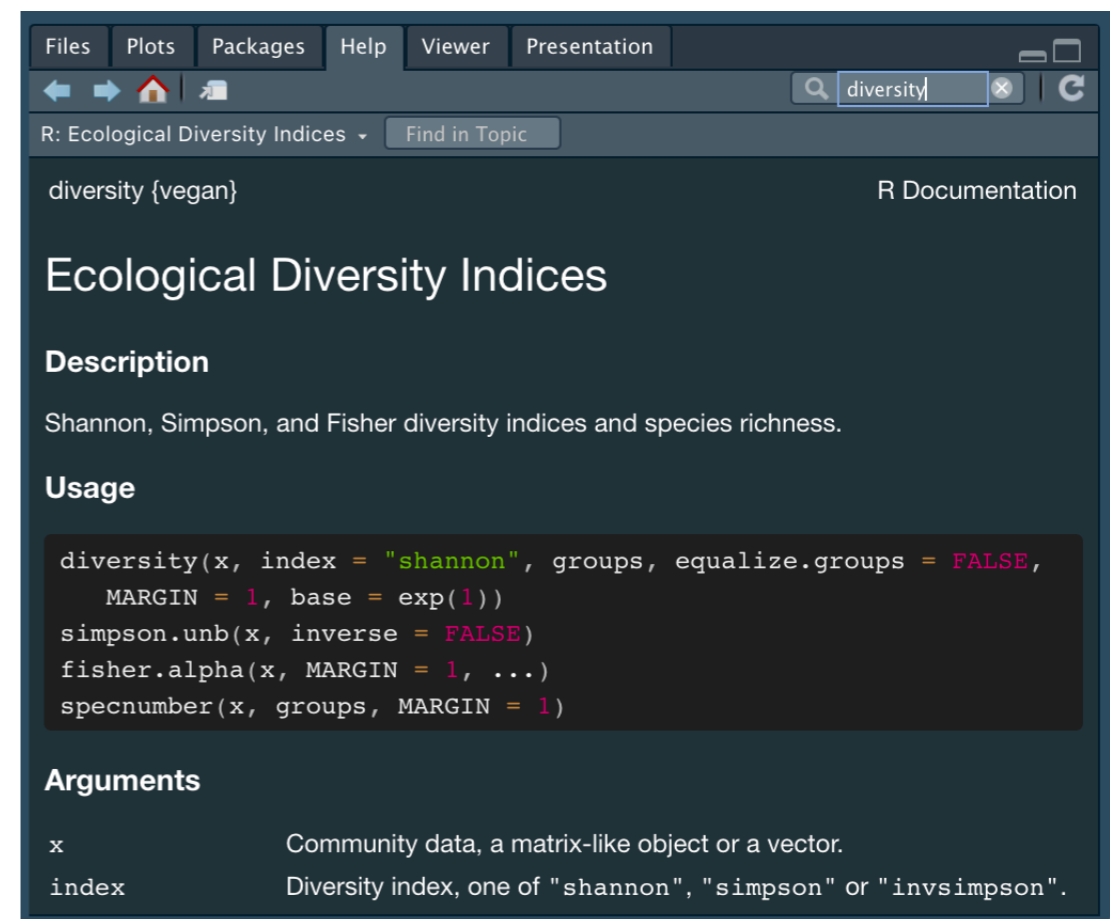
Residual standard error: 2.817 on 995 degrees of freedom
Multiple R-squared:  0.7882,    Adjusted R-squared:  0.7873
F-statistic: 925.6 on 4 and 995 DF,  p-value: < 2.2e-16
```



R Help



The screenshot shows the RStudio environment. On the left, a data table is displayed with columns for Genus, Genus_species, and AIG0121. The table lists various species such as Pantocsekiella, Cryptomonas, and Plagioselmis. The bottom right pane shows a file explorer for the 'R_Introduction' directory, listing files like '.Rhistory' and 'Microscopy_table.csv'.



The screenshot shows the R documentation page for the 'diversity' function from the 'vegan' package. The page title is 'Ecological Diversity Indices'. It includes a description: 'Shannon, Simpson, and Fisher diversity indices and species richness.' and a usage section with the following code examples:

```
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)
```

The 'Arguments' section defines the parameters:

- x**: Community data, a matrix-like object or a vector.
- index**: Diversity index, one of "shannon", "simpson" or "invsimpson".



```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
            1          2          3          4          5          6
(Intercept) 1.3831  0.24566  0.2023  0.2023  0.2023  0.2023 ***
x1          5.29776  0.12483  0.12483  0.12483  0.12483  0.12483 ***
x2          1.61055  0.0518  0.0518  0.0518  0.0518  0.0518 ***
x3          0.44947  0.016  0.016  0.016  0.016  0.016 ***
groupB      1.87978  0.16  0.16  0.16  0.16  0.16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

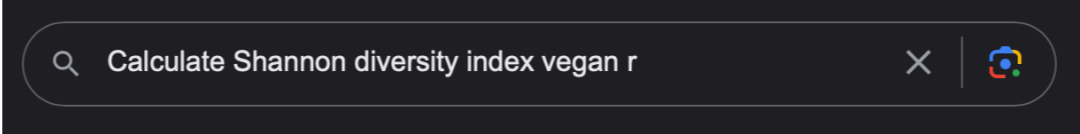
Residual standard error: 2.817 on 995 degrees of freedom
Multiple R-squared:  0.7882,    Adjusted R-squared:  0.7873
F-statistic: 925.6 on 4 and 995 DF,  p-value: < 2.2e-16
```



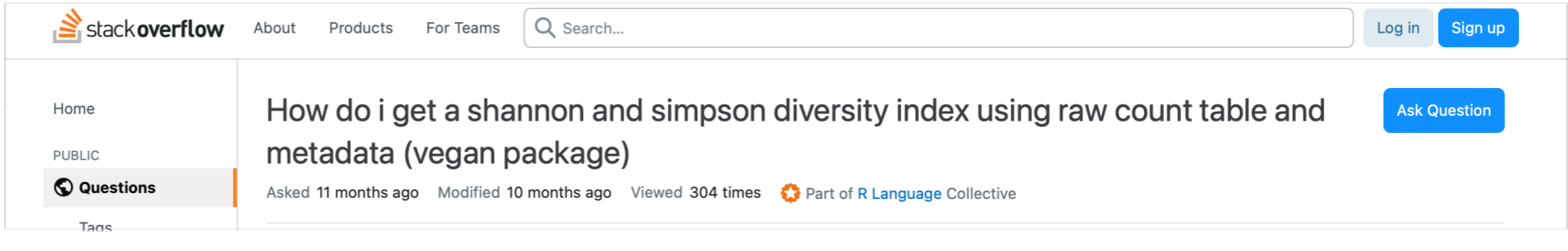
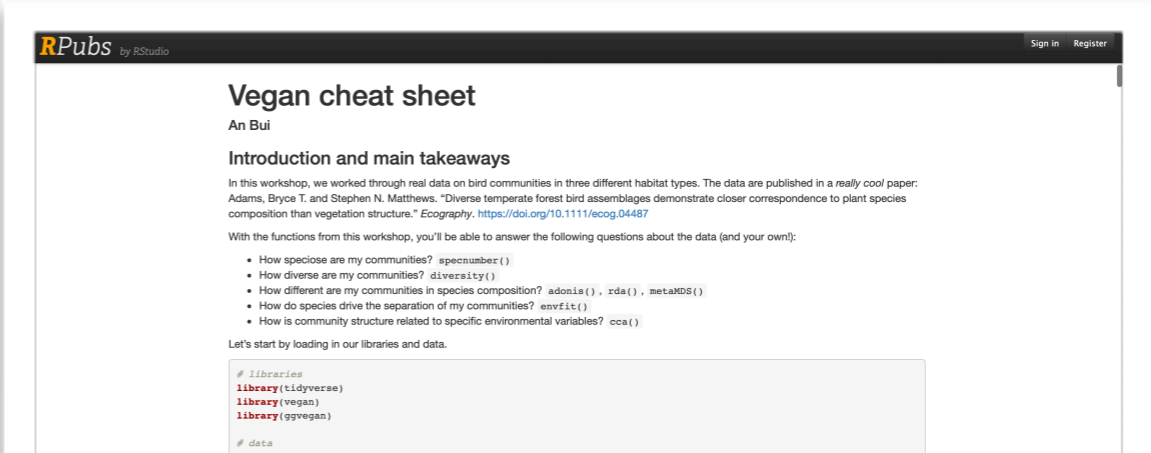
R Help

Any question has an answer on internet !!

Official documentation of Vegan package



Tutorial made by other scientists



Forums

```
Call:
lm(formula = y ~ ., data = data)

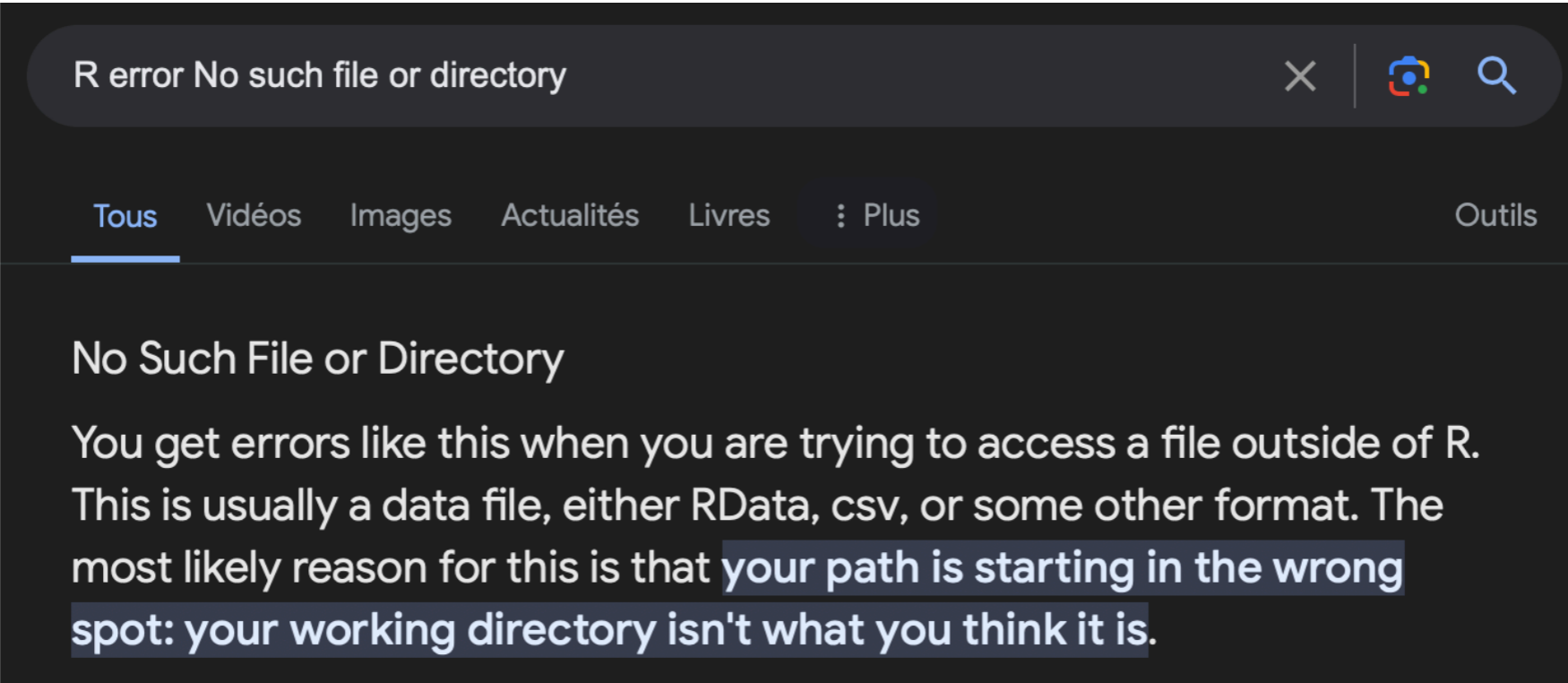
Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9711  9.7121

Coefficients:
(Intercept)  1.3831  1.24566  10.2300  2.3000  *
x1           5.29776  1.2483  1.0000  0.0000  **
x2           1.61055  0.518  1.0000  0.0000  ***
x3           0.44947  0.16  1.0000  0.0000  ***
groupB       1.87978  0.16  1.0000  0.0000  ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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 Help

Any question has an answer on internet !!



R error No such file or directory

Tous Vidéos Images Actualités Livres : Plus Outils

No Such File or Directory

You get errors like this when you are trying to access a file outside of R. This is usually a data file, either RData, csv, or some other format. The most likely reason for this is that **your path is starting in the wrong spot: your working directory isn't what you think it is.**


```
Call:
lm(formula = y ~ ., data = data)

Residuals:
    Min       1Q   Median       3Q      Max
-8.7859 -2.0085  0.0000  2.9712  9.7121

Coefficients:
            1          2          3          4          5          6
(Intercept) 1.3831  5.29776  1.61055  0.44947  1.87978  0.16000
            Error      t-value      Pr(>|t|)
            2.4566      2.1483      0.0330 ***
            1.2483      1.518      0.0666 ***
            0.518      0.16      0.8766
            0.16      0.016      0.9866 ***
            0.016      0.0016      0.9996 ***
            0.0016      0.00016      0.99996 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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
```

TYPICAL ANALYSIS WITH R

1. OPEN OR START AN R SCRIPT
2. SET WORKING DIRECTORY - FILE PATHS
3. LOAD R PACKAGES
4. BE CAREFUL OF ERRORS
5. ONCE A SCRIPT WORKED, SAVE IT

TYPICAL ANALYSIS WITH R

1. OPEN OR START AN R SCRIPT

```
# input data
# store the raw fastq files path in 2 vectors (one for R1, and one for R2)
# of course the pattern must correspond exactly to the end of your file
# e.g. it will end with "fastq.gz" if the files are zipped
raw_F_reads <- sort(list.files(path, pattern="_R1.fastq", full.names = TRUE))
raw_R_reads <- sort(list.files(path, pattern="_R2.fastq", full.names = TRUE))

# Extract sample names in a new variable
# /!\ be careful, this you might change the field separator "_R" depending on the file names /!\
sample.names <- sapply(strsplit(basename(raw_F_reads), "_R"), function(x) x[1])

# We check if the command worked well :
sample.names

# DO NOT CONTINUE IF YOU DON'T HAVE THE GOOD SAMPLE NAMES
```

An R script is a succession of command lines (functions) to perform an analysis

It needs to be adapted to your data / computer

It is important to carefully do it in order and regularly check if everything worked

If one step was not successful, the rest of the script will not work

TYPICAL ANALYSIS WITH R

1. OPEN OR START AN R SCRIPT

```
# input data
# store the raw fastq files path in 2 vectors (one for R1, and one for R2)
# of course the pattern must correspond exactly to the end of your file
# e.g. it will end with "fastq.gz" if the files are zipped
raw_F_reads <- sort(list.files(path, pattern="_R1.fastq", full.names = TRUE))
raw_R_reads <- sort(list.files(path, pattern="_R2.fastq", full.names = TRUE))

# Extract sample names in a new variable
# /!\ be careful, this you might change the field separator "_R" depending on the file names /!\
sample.names <- sapply(strsplit(basename(raw_F_reads), "_R"), function(x) x[1])

# We check if the command worked well :
sample.names

# DO NOT CONTINUE IF YOU DON'T HAVE THE GOOD SAMPLE NAMES
```

When you want to run a command line in a script :

Click somewhere in the line

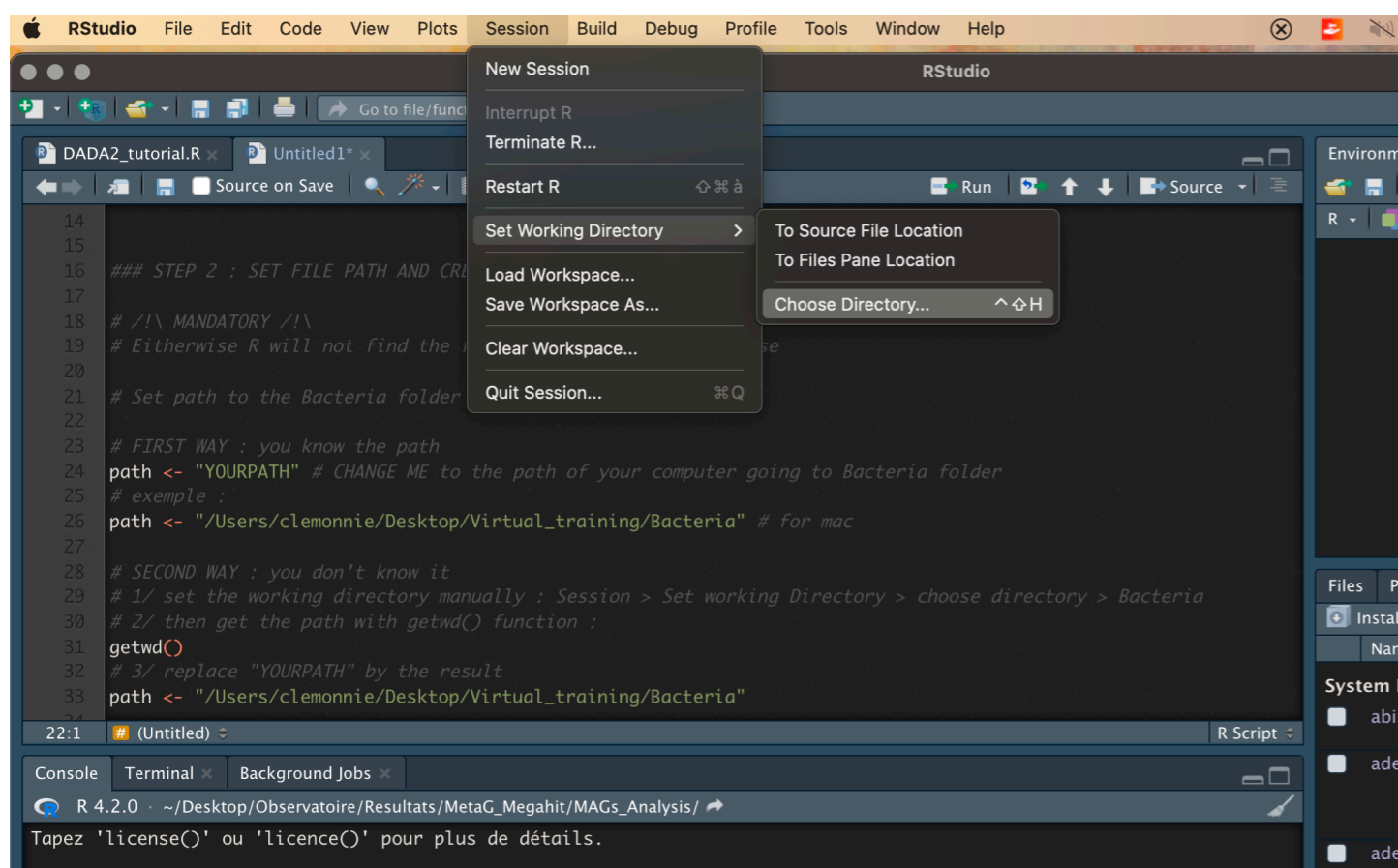
And then do : ctrl + enter (for Mac it is command + enter)



TYPICAL ANALYSIS WITH R

1. OPEN OR START AN R SCRIPT
2. SET WORKING DIRECTORY - FILE PATHS

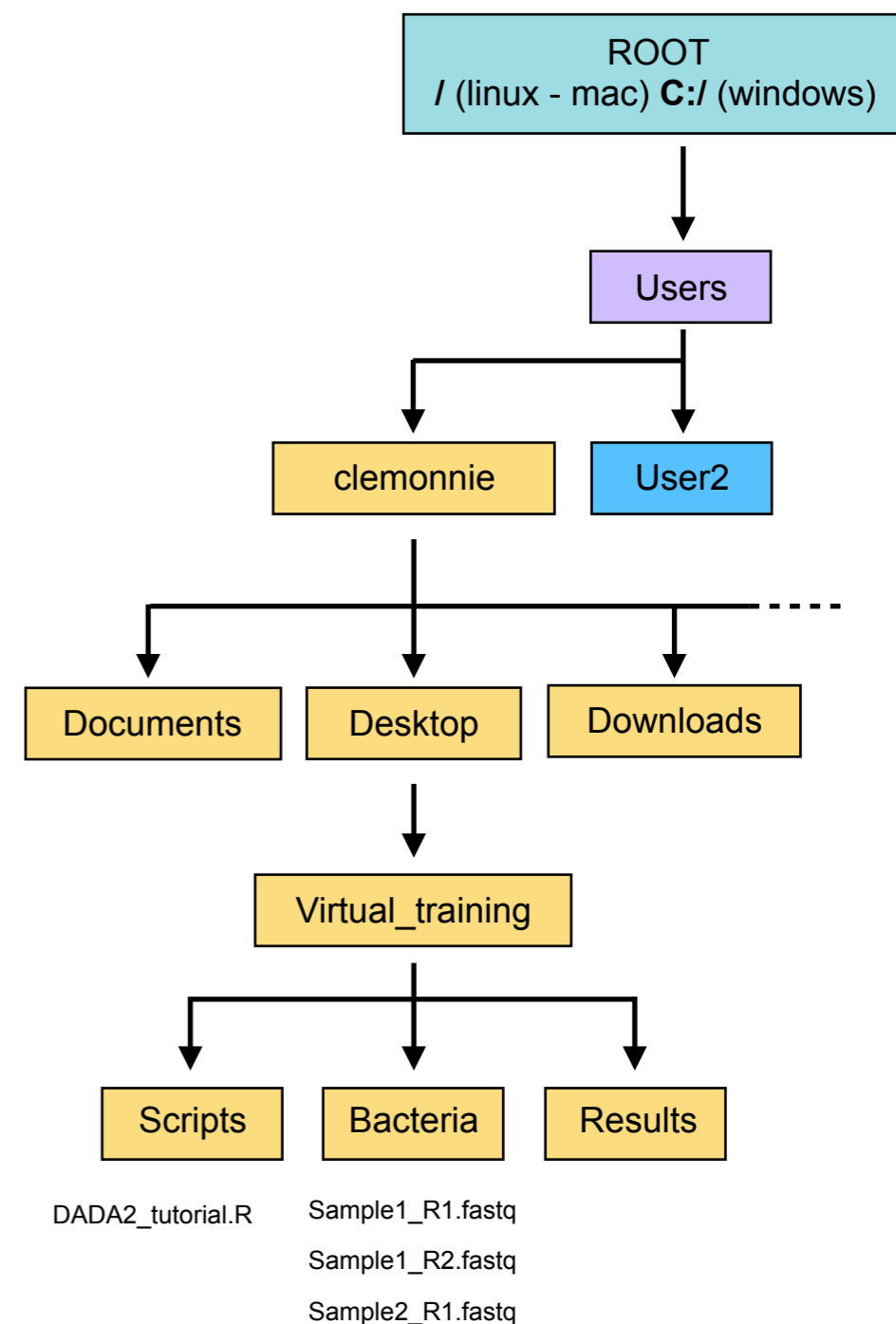
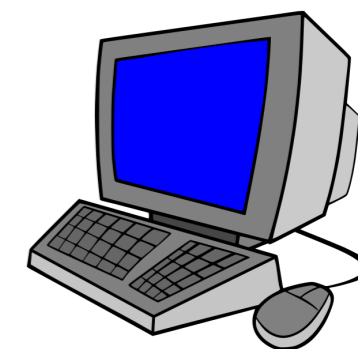
R does not know where are the files you want to analyse. You need to specify it.



```

RStudio File Edit Code View Plots Session Build Debug Profile Tools Window Help
DADA2_tutorial.R x Untitled1* x
Source on Save Run Source
14
15
16 ### STEP 2 : SET FILE PATH AND CR
17
18 # /!\ MANDATORY /!\
19 # Eitherwise R will not find the
20
21 # Set path to the Bacteria folder
22
23 # FIRST WAY : you know the path
24 path <- "YOURPATH" # CHANGE ME to the path of your computer going to Bacteria folder
25 # exemple :
26 path <- "/Users/clemonnie/Desktop/Virtual_training/Bacteria" # for mac
27
28 # SECOND WAY : you don't know it
29 # 1/ set the working directory manually : Session > Set working Directory > choose directory > Bacteria
30 # 2/ then get the path with getwd() function :
31 getwd()
32 # 3/ replace "YOURPATH" by the result
33 path <- "/Users/clemonnie/Desktop/Virtual_training/Bacteria"
22:1 # (Untitled) R Script
Console Terminal Background Jobs
R 4.2.0 ~ /Desktop/Observatoire/Resultats/MetaG_Megahit/MAGs_Analysis/
Tapez 'license()' ou 'licence()' pour plus de détails.
  
```

```
> setwd(dir="/Users/clemonnie/Desktop/Virtual_training/Bacteria")
```



TYPICAL ANALYSIS WITH R

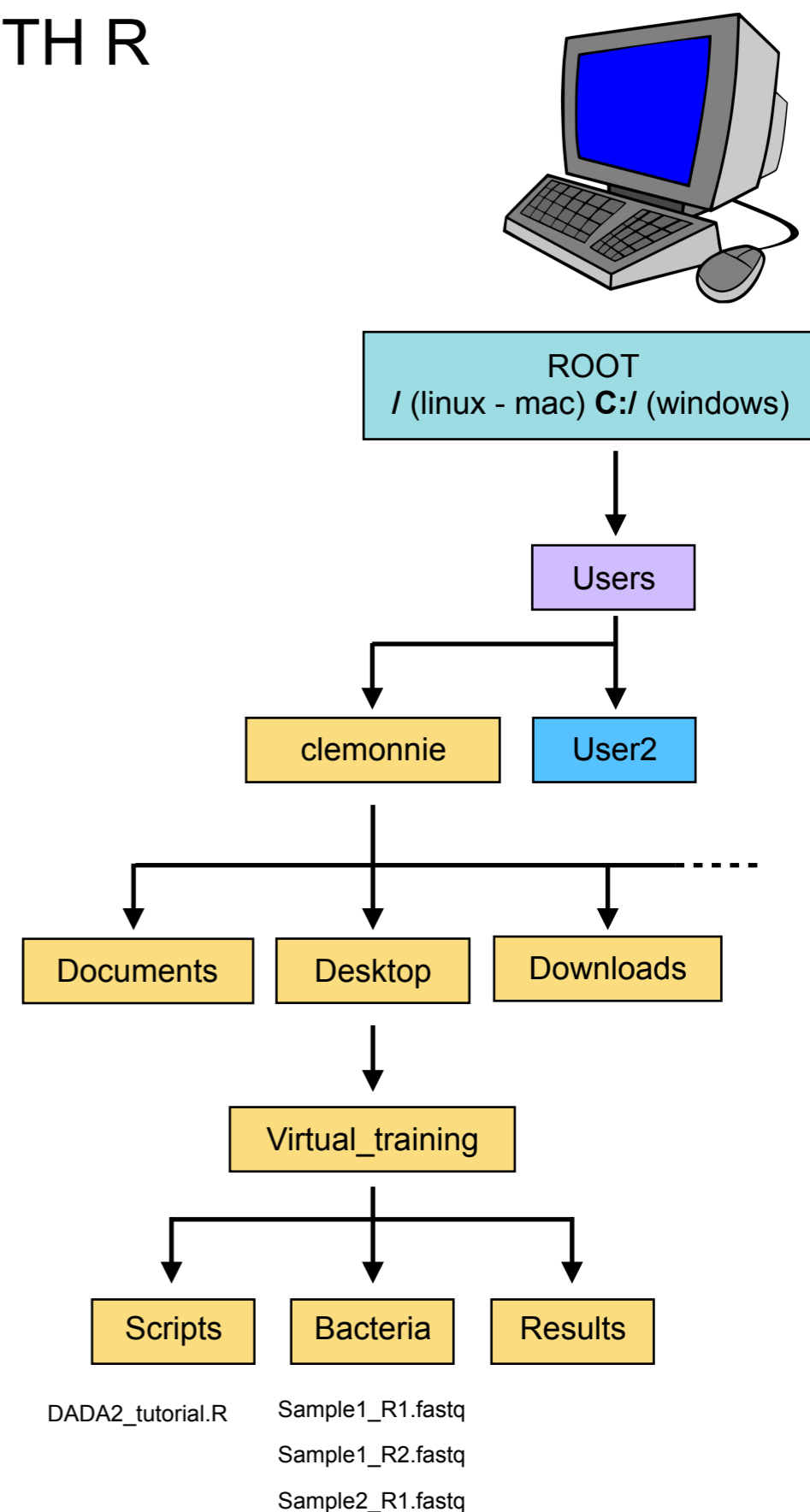
1. OPEN OR START AN R SCRIPT
2. SET WORKING DIRECTORY - FILE PATHS

In the DADA2 pipeline, we will tell the path

```
path <- "YOURPATH" # CHANGE ME
```

```
list.files(path)
```

```
> list.files(path)
[1] "cut_fastq"          "filtered"          "results"
[5] "S3_16fev15_R2.fastq" "S3_1sept14_R1.fastq" "S3_1sept14_R2.fastq"
[9] "S3_24jui14_R2.fastq" "S3_8aou14_R1.fastq" "S3_8aou14_R2.fastq"
[13] "S3_9janv15_R2.fastq"
```



TYPICAL ANALYSIS WITH R

1. OPEN OR START AN R SCRIPT
2. SET WORKING DIRECTORY - FILE PATHS

In the DADA2 pipeline, we will tell the path

BE CAREFUL :

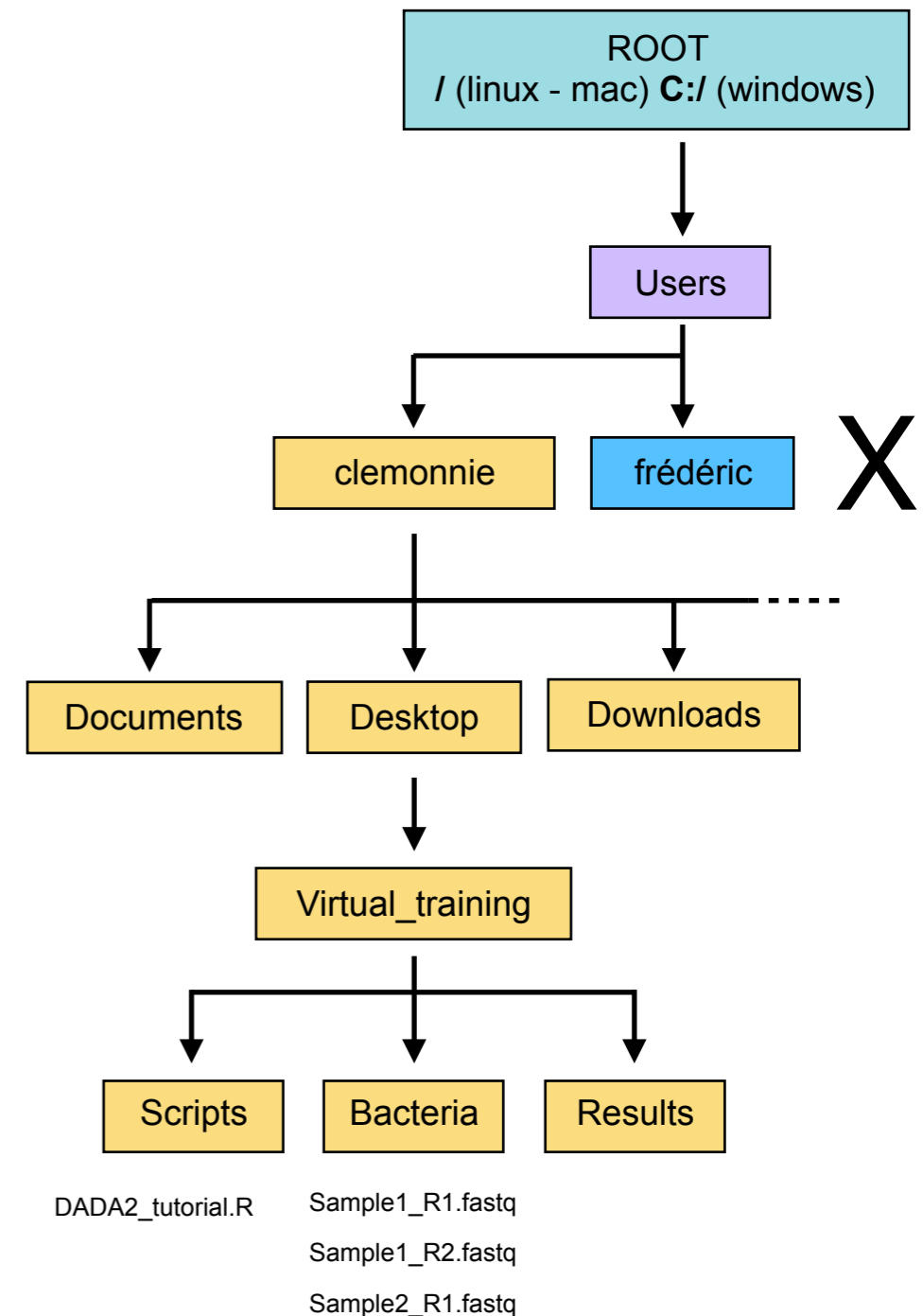
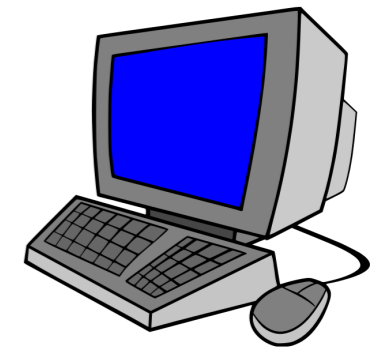
To space in the folder names

Virtual_training

Virtual training

X

To special characters (e.g. accent)



TYPICAL ANALYSIS WITH R

1. OPEN OR START AN R SCRIPT
2. SET WORKING DIRECTORY - FILE PATHS
3. LOAD R PACKAGES

```
### STEP 1 : LOAD PACKAGES ###
```

```
library(dada2)  
library(ggplot2)  
library(reshape2)
```

```
> plotQualityProfile(filt_F_reads[1:2])  
Erreur dans plotQualityProfile(filt_F_reads[1:2]) :  
  impossible de trouver la fonction "plotQualityProfile"
```

TYPICAL ANALYSIS WITH R

1. OPEN OR START AN R SCRIPT
2. SET WORKING DIRECTORY - FILE PATHS
3. LOAD R PACKAGES
4. BE CAREFUL OF ERRORS

Sometimes R write down the error directly

```
> plotQualityProfile(filt_F_reads[1:2])  
Erreur dans plotQualityProfile(filt_F_reads[1:2]) :  
  impossible de trouver la fonction "plotQualityProfile"
```

But sometime it does not know that you made a mistake :

```
path <- "/Users/clemonnie/Desktop/Virtual_training/Bacteri"
```

```
> # Check if the path is correct by listing files it is containing  
> list.files(path)  
character(0)
```

TYPICAL ANALYSIS WITH R

1. OPEN OR START AN R SCRIPT
2. SET WORKING DIRECTORY - FILE PATHS
3. LOAD R PACKAGES
4. BE CAREFUL OF ERRORS

All things written in red does not mean an error

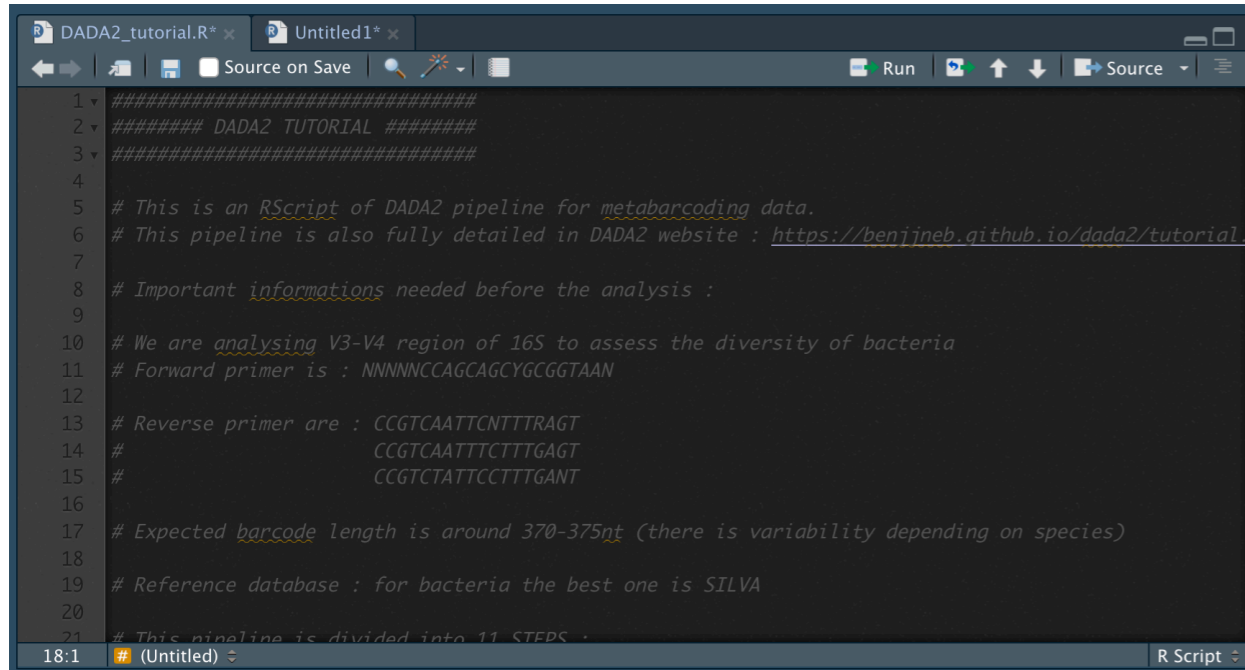
Sometimes it is just that R wants to speak to you

```
> library(dada2)  
Le chargement a nécessité le package : Rcpp
```

```
> plotQualityProfile(cut_F_reads[1:5])  
Message d'avis :  
The `<scale>` argument of `guides()` cannot be `FALSE`. Use "none" instead as of ggplot2 3.3.4.  
i The deprecated feature was likely used in the dada2 package.  
Please report the issue at https://github.com/benjaneb/dada2/issues.  
This warning is displayed once every 8 hours.  
Call `lifecycle::last_lifecycle_warnings()` to see where this warning was generated.
```

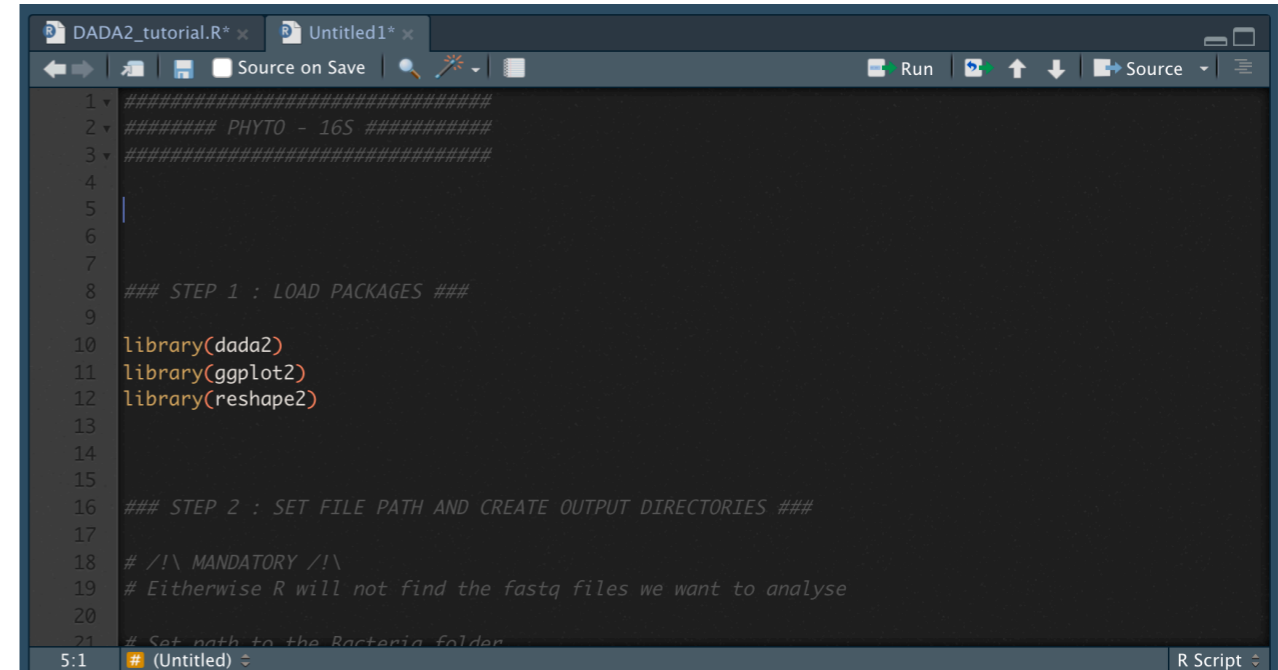
TYPICAL ANALYSIS WITH R

1. OPEN OR START AN R SCRIPT
2. SET WORKING DIRECTORY - FILE PATHS
3. LOAD R PACKAGES
4. BE CAREFUL OF ERRORS
5. ONCE A SCRIPT WORKED, SAVE IT - so you can re-use it later



```
1 #####
2 ##### DADA2 TUTORIAL #####
3 #####
4
5 # This is an RScript of DADA2 pipeline for metabarcoding data.
6 # This pipeline is also fully detailed in DADA2 website : https://benjjneb.github.io/dada2/tutorial.
7
8 # Important informations needed before the analysis :
9
10 # We are analysing V3-V4 region of 16S to assess the diversity of bacteria
11 # Forward primer is : NNNNNCCAGCAGCYGCGGTAAN
12
13 # Reverse primer are : CCGTCAATTCNTTTRAGT
14 #                       CCGTCAATTCTTTGAGT
15 #                       CCGTCTATTCCTTTGANT
16
17 # Expected barcode length is around 370-375nt (there is variability depending on species)
18
19 # Reference database : for bacteria the best one is SILVA
20
21 # This pipeline is divided into 11 STEPS :
18:1 # (Untitled) R Script
```

DADA2_tutorial.R



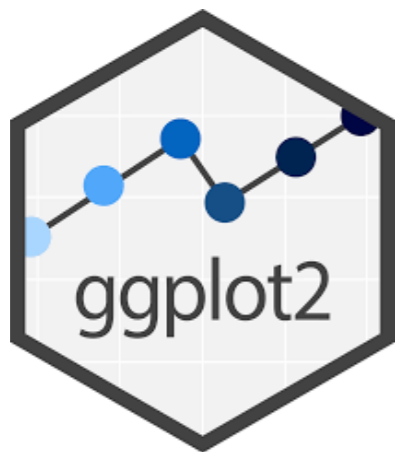
```
1 #####
2 ##### PHYTO - 16S #####
3 #####
4
5
6
7
8 ### STEP 1 : LOAD PACKAGES ###
9
10 library(dada2)
11 library(ggplot2)
12 library(reshape2)
13
14
15
16 ### STEP 2 : SET FILE PATH AND CREATE OUTPUT DIRECTORIES ###
17
18 # !\ MANDATORY !\
19 # Otherwise R will not find the fastq files we want to analyse
20
21 # Set path to the Bacteria folder
5:1 # (Untitled) R Script
```

Phyto_16S.R

ANY QUESTIONS ?

PREPARATION OF THE PRACTICAL PART

Does everyone has RStudio and all the following packages ?



```
library(ggplot2)
```



```
library(dada2)
```

RESHAPE2

```
library(reshape2)
```


PREPARATION OF THE PRACTICAL PART

Does everyone has cutadapt and python ?

Installation on Windows

For some releases of Cutadapt, a single-file executable (`cutadapt.exe`) is made available on the [GitHub releases page](#). Try that first, and if it does not work for you, please report the issue.

To install Cutadapt manually, keep reading.

There is no Bioconda package for Windows because Bioconda does not produce Windows packages. To install Cutadapt, you can use `pip`, but because Cutadapt contains components that need to be compiled, you also need to install a compiler.

1. Download a recent version (at least 3.7) of Python for Windows from <https://www.python.org/> and install it.
2. Download and install “Build Tools for Visual Studio 2019” from <https://visualstudio.microsoft.com/downloads/#build-tools-for-visual-studio-2019>. (There are many similarly named downloads on that page, ensure you get the right one.)

During installation, when the dialog about which components to install pops up, ensure that “C++ Build tools” is ticked. The download is quite big and can take a long time.

3. Open the command line (`cmd.exe`) and run `py -m pip install cutadapt`.
4. Test whether it worked by running `py -m cutadapt --version`. You should see the version number of Cutadapt.

When running Cutadapt this way, you will need to remember to write `py -m cutadapt` instead of just `cutadapt`.

PREPARATION OF THE PRACTICAL PART

Does everyone has discord?



PREPARATION OF THE PRACTICAL PART

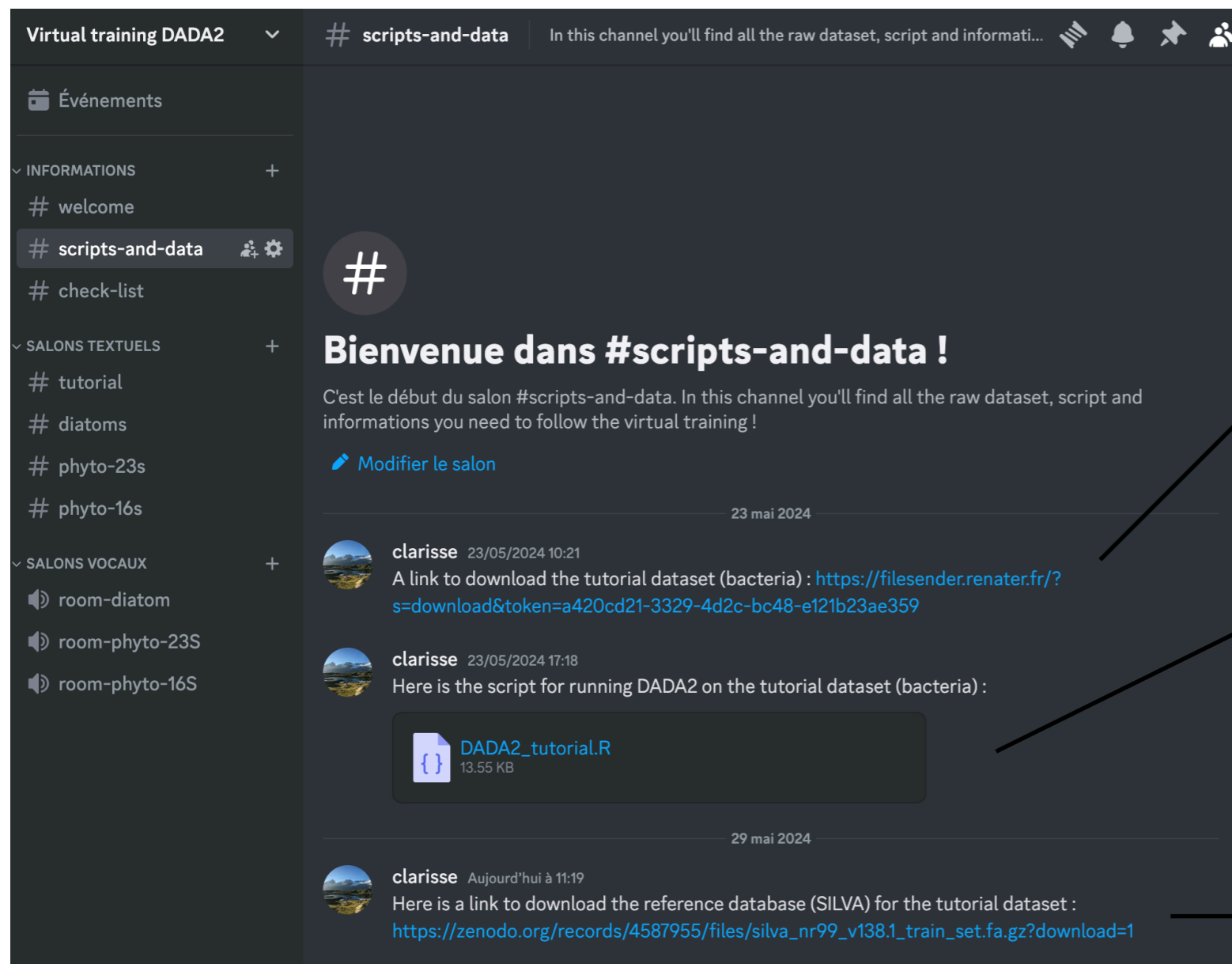
FOR MONDAY : download tutorial dataset

On your desktop, on a folder « **virtual_training** »

Put fastq files in a subfolder named « **bacteria** »

Put the script DADA2_tutorial.R in a subfolder named « **scripts** »

Put the script DADA2_tutorial.R in a subfolder named « **database** »



Virtual training DADA2 # scripts-and-data In this channel you'll find all the raw dataset, script and informati...

Événements

INFORMATIONS +

- # welcome
- # scripts-and-data
- # check-list

SALONS TEXTUELS +

- # tutorial
- # diatoms
- # phyto-23s
- # phyto-16s

SALONS VOCAUX +

- room-diatom
- room-phyto-23S
- room-phyto-16S

Bienvenue dans #scripts-and-data !

C'est le début du salon #scripts-and-data. In this channel you'll find all the raw dataset, script and informations you need to follow the virtual training !

[Modifier le salon](#)

23 mai 2024

clarisse 23/05/2024 10:21
A link to download the tutorial dataset (bacteria) : <https://filesender.renater.fr/?s=download&token=a420cd21-3329-4d2c-bc48-e121b23ae359>

clarisse 23/05/2024 17:18
Here is the script for running DADA2 on the tutorial dataset (bacteria) :

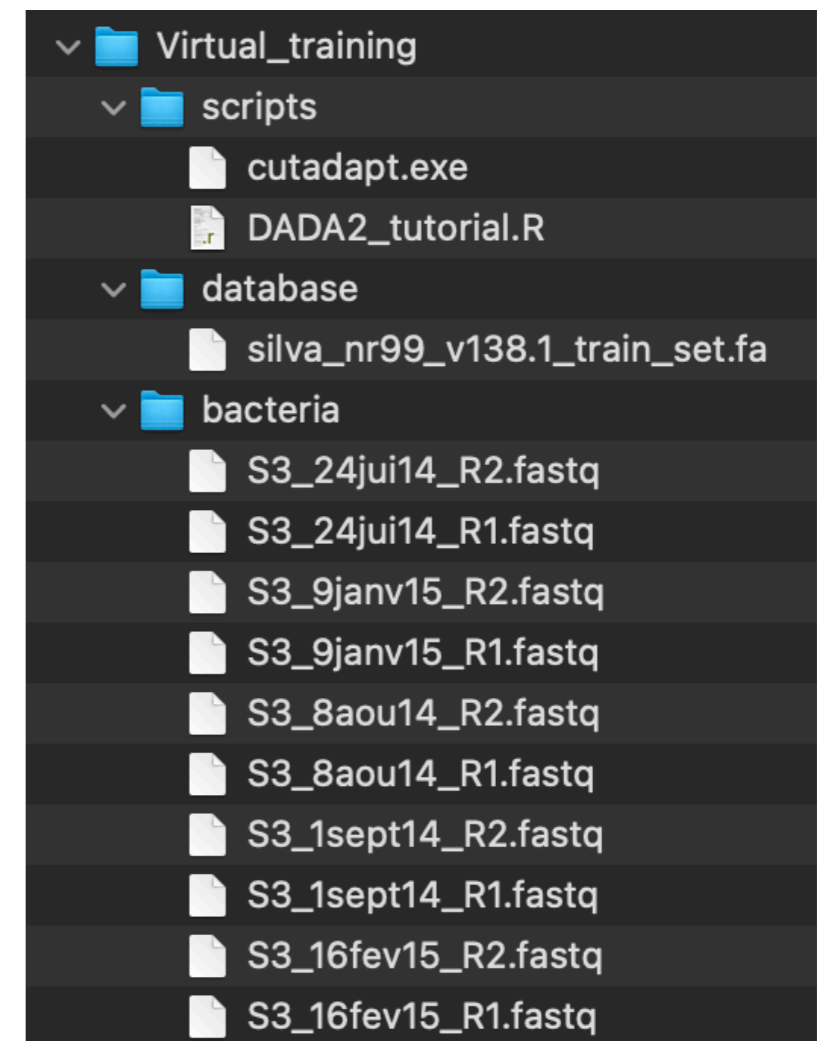
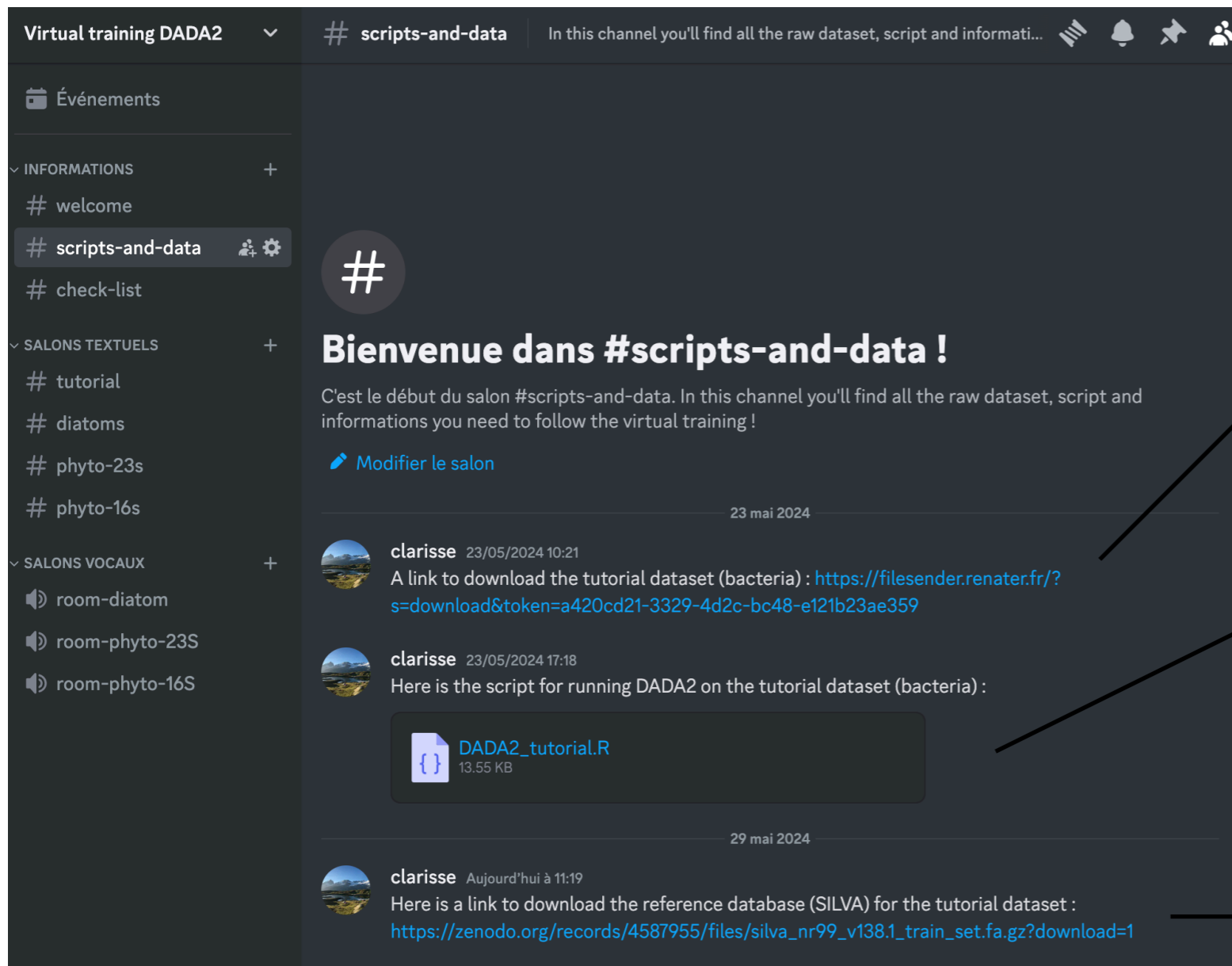
DADA2_tutorial.R
13.55 KB

29 mai 2024

clarisse Aujourd'hui à 11:19
Here is a link to download the reference database (SILVA) for the tutorial dataset : https://zenodo.org/records/4587955/files/silva_nr99_v138.1_train_set.fa.gz?download=1

PREPARATION OF THE PRACTICAL PART

FOR MONDAY : download tutorial dataset



ANY QUESTIONS ?