Microbiome data science with R/Bioconductor

Welcome to Radboud Summer School, July 2022

2022-07-06

Contents

Chapter 1

Overview

1.1 Contents and learning goals

This course will focus on **microbiome data analysis with R/Bioconductor**, a popular open source environment for scientific data analysis. You will get an overview of the reproducible data analysis workflows in microbiome research, with a focus on gut-brain axis studies.

After the course you will know how to approach new tasks in the analysis of taxonomic profiling data by taking advantage of available documentation and R tools.

The teaching follows the open online documentation created by the course teachers, extending the online book Orchestrating Microbiome Analysis (https://microbiome.github.io/OMA). The openly licensed teaching material will be available online during and after the course, following Finnish national recommendations on open education.

The training material walks you through the standard steps of biomedical data analysis covering data access, exploration, analysis, visualization, reproducible reporting, and best practices in open science. We will teach generic data analytical skills that are applicable to common data analysis tasks encountered in modern omics research. The teaching format allows adaptations according to the student's learning speed.

1.2 Schedule and organizers

Format In-person course. For detailed schedule, see the course website.

Venue University of Radboud. July 11-15, 2022.

Expected background

Target audience The course is primarily designed for advanced MSc and PhD students, Postdocs, and biomedical researchers who wish to learn new skills in scientific programming and biomedical data analysis. Academic students and researchers encouraged to apply. Priority will be given for local students. Some earlier experience with R or another programming language is recommended.

Preparation Advance preparation is expected. Online support is available. See section 3 for instructions.

1.3 Acknowledgments

Citation We thank all developers and contributors who have contributed open resources that supported the development of the training material. Kindly cite the course material as Borman et al. (2022)

Contact See https://microbiome.github.io

Chapter 2

Code of Conduct

The Bioconductor community values an open approach to science that promotes the

- sharing of ideas, code, software and expertise
- collaboration
- diversity and inclusivity
- a kind and welcoming environment
- community contributions

We value your attendance and participation at Bioconductor events and in our community.

For the full version, enforcement, and reporting instructions, see the Bioconductor code of conduct.

Chapter 3

Getting started

3.1 Checklist (before the course)

3.1.1 Computer setup and installations

Setting up the system on your own computer is not required for the course but it can be useful for later use. The required software:

- R (version >4.2.0)
- RStudio; choose "Rstudio Desktop" to download the latest version. Optional but preferred. For further details, check the Rstudio home page.
- Install and load the required R packages (see Section 3.3)
- After a successful installation you can start with the case study examples in this training material

3.2 Support and resources

- We recommend to have a look at the additional reading tips and try out online material listed in Section 6.
- **You can run the workflows by simply copy-pasting the examples.** For further, advanced material, you can test and modify further examples from the online book, and apply these techniques to your own data.
- Online support on installation and other matters, join us at Gitter

3.3 Installing and loading the required R packages

You may need the examples from this subsection if you are installing the environment on your own computer. If you need to add new packages, you can modify the examples below.

This section shows how to install and load all required packages into the R session, if needed. Only uninstalled packages are installed.

Download the file pkgs.csv. This contains the list of packages that we recommend to preinstall. This can be done with the following code.

```
# List of packages that we need
pkg <- read.csv("pkgs.csv")[,1]

# List packages that are already installed
pkg_already_installed <- pkg[ pkg %in% installed.packages() ]

# List remaining packages that need to be installed
packages_to_install <- setdiff(pkg, pkg_already_installed)
```

```
# If there are packages that need to be installed, install them
if( length(packages_to_install) ) {
   BiocManager::install(packages_to_install)
}
```

Now all required packages are installed, so let's load them into the session. Some function names occur in multiple packages. That is why miaverse's packages mia and miaViz are prioritized. Packages that are loaded first have higher priority.

```
# Loading all packages into session. Returns true if package was successfully loaded.
loaded <- sapply(packages, require, character.only = TRUE)
as.data.frame(loaded)
```

Chapter 4

Importing microbiome data

This section demonstrates how to import microbiome profiling data in R.

4.1 Data access

Option 1

ADHD-associated changes in gut microbiota and brain in a mouse model

Tengeler AC *et al.* (2020) **Gut microbiota from persons with attention-deficit/hyperactivity disorder affects the brain in mice**. Microbiome 8:44.

In this study, mice are colonized with microbiota from participants with ADHD (attention deficit hyperactivity disorder) and healthy participants. The aim of the study was to assess whether the mice display ADHD behaviors after being inoculated with ADHD microbiota, suggesting a role of the microbiome in ADHD pathology.

Download the data from data subfolder.

Option 2

Open data set of your own choice, different options are listed in OMA.

4.2 Importing microbiome data in R

Import example data by modifying the examples in the online book section on data exploration and manipulation. The data files in our example are in *biom* format, which is a standard file format for microbiome data. Other file formats exist as well, and import details vary by platform.

Here, we import *biom* data files into a specific data container (structure) in R, *TreeSummarizedExperiment* (TSE) Huang et al. (2020). This provides the basis for downstream data analysis in the *miaverse* data science framework.

In this course, we focus on downstream analysis of taxonomic profiling data, and assume that the data has already been appropriately preprocessed and available in the TSE format. In addition to our example data, further demonstration data sets are readily available in the TSE format through microbiomeDataSets.

Figure sources:

Original article - Huang R *et al.* (2021) TreeSummarizedExperiment: a S4 class for data with hierarchical structure. F1000Research 9:1246.

Reference Sequence slot extension - Lahti L *et al.* (2020) Upgrading the R/Bioconductor ecosystem for microbiome research F1000Research 9:1464 (slides).

4.3 Example solutions

- Example code for data import: import.Rmd

Chapter 5

Reproducible reporting with Rmarkdown

Reproducible reporting is the starting point for robust interactive data science. Perform the following tasks:

- If you are entirely new to Markdown, take this 10 minute tutorial to get introduced to the most important functions within Markdown. Then experiment with different options with Rmarkdown
- Create a Rmarkdown template in RStudio, and render it into a document (markdown, PDF, docx or other format). In case you are new to Rmarkdown Rstudio provides resources to learn about the use cases and the basics of Rmarkdown.
- Further examples are tips for Rmarkdown are available in the online tutorial to reproducible reporting by Dr. C Titus Brown.

Chapter 6

Study material

6.1 Online tutorial

The course will utilize material from the online book (beta version) Orchestrating Microbiome Analysis with R/Bioconductor (OMA). We encourage to familiarize with this material and test examples already before the course.

6.2 Lecture slides

To be added.

6.3 Tasks

Seek guidance from the https://microbiome.github.io/OMA/

- Exercises
- Example solutions

6.4 Extra material on miaverse and R programming

Further information on the data science framework

Chapter 7

Alpha diversity demo

7.1 Alpha diversity estimation

First let's load the required packages and data set

```
library(mia)
```

```
## Loading required package: SummarizedExperiment
```

```
## Loading required package: MatrixGenerics
```

```
## Loading required package: matrixStats
```

```
##
## Attaching package: 'MatrixGenerics'
```

```
## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars
```

```
## Loading required package: GenomicRanges

## Loading required package: stats4

## Loading required package: BiocGenerics

##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##     union, unique, unsplit, which.max, which.min

## Loading required package: S4Vectors

##
## Attaching package: 'S4Vectors'

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname

## Loading required package: IRanges

## Loading required package: GenomeInfoDb

## Loading required package: Biobase

## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.

##
## Attaching package: 'Biobase'

## The following object is masked from 'package:MatrixGenerics':
##
##     rowMedians
```

```
## The following objects are masked from 'package:matrixStats':
##
##     anyMissing, rowMedians

## Loading required package: SingleCellExperiment

## Loading required package: TreeSummarizedExperiment

## Loading required package: Biostrings

## Loading required package: XVector

##
## Attaching package: 'Biostrings'

## The following object is masked from 'package:base':
##
##     strsplit

## Loading required package: MultiAssayExperiment
```

```
library(miaViz)
```

```
## Loading required package: ggplot2

## Loading required package: ggraph
```

```
library(tidyverse)
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --

## v tibble  3.1.7     v dplyr   1.0.9
## v tidyr   1.2.0     v stringr 1.4.0
## v readr   2.1.2     v forcats 0.5.1
## v purrr   0.3.4

## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::collapse()   masks Biostrings::collapse(), IRanges::collapse()
## x dplyr::combine()    masks Biobase::combine(), BiocGenerics::combine()
## x purrr::compact()    masks XVector::compact()
## x dplyr::count()      masks matrixStats::count()
## x dplyr::desc()       masks IRanges::desc()
## x tidyr::expand()     masks S4Vectors::expand()
## x dplyr::filter()     masks stats::filter()
## x dplyr::first()      masks S4Vectors::first()
## x dplyr::lag()        masks stats::lag()
## x ggplot2::Position() masks BiocGenerics::Position(), base::Position()
## x purrr::reduce()     masks GenomicRanges::reduce(), IRanges::reduce()
## x dplyr::rename()     masks S4Vectors::rename()
## x dplyr::slice()      masks XVector::slice(), IRanges::slice()
```

```
# library(vegan)

tse <- read_rds("data/Tengeler2020/tse.rds")

tse
```

```
## class: TreeSummarizedExperiment
## dim: 151 27
## metadata(0):
## assays(1): counts
## rownames(151): 1726470 1726471 ... 17264756 17264757
## rowData names(6): Kingdom Phylum ... Family Genus
## colnames(27): A110 A12 ... A35 A38
## colData names(4): patient_status cohort patient_status_vs_cohort
##   sample_name
## reducedDimNames(0):
## mainExpName: NULL
## altExpNames(0):
## rowLinks: a LinkDataFrame (151 rows)
## rowTree: 1 phylo tree(s) (151 leaves)
## colLinks: NULL
## colTree: NULL
```

Then let's estimate multiple diversity indices.

```
?estimateDiversity

tse <- estimateDiversity(tse,
                         index = c("shannon","gini_simpson","faith"),
                         name = c("shannon","gini_simpson","faith"))
head(colData(tse))
```

```
## DataFrame with 6 rows and 7 columns
##      patient_status      cohort patient_status_vs_cohort sample_name   shannon
##         <character> <character>             <character> <character> <numeric>
## A110           ADHD    Cohort_1           ADHD_Cohort_1        A110   1.76541
## A12            ADHD    Cohort_1           ADHD_Cohort_1         A12   2.71644
## A15            ADHD    Cohort_1           ADHD_Cohort_1         A15   3.17810
## A19            ADHD    Cohort_1           ADHD_Cohort_1         A19   2.89199
## A21            ADHD    Cohort_2           ADHD_Cohort_2         A21   2.84198
## A23            ADHD    Cohort_2           ADHD_Cohort_2         A23   2.79794
##      gini_simpson     faith
##         <numeric> <numeric>
## A110     0.669537   7.39224
## A12      0.871176   6.29378
## A15      0.930561   6.60608
## A19      0.899210   6.79708
## A21      0.885042   6.65110
## A23      0.859813   5.96246
```

We can see that the variables are included in the data. Similarly, let's calculate richness indices.

```
tse <- estimateRichness(tse,
                                index = c("chao1","observed"))
head(colData(tse))
```

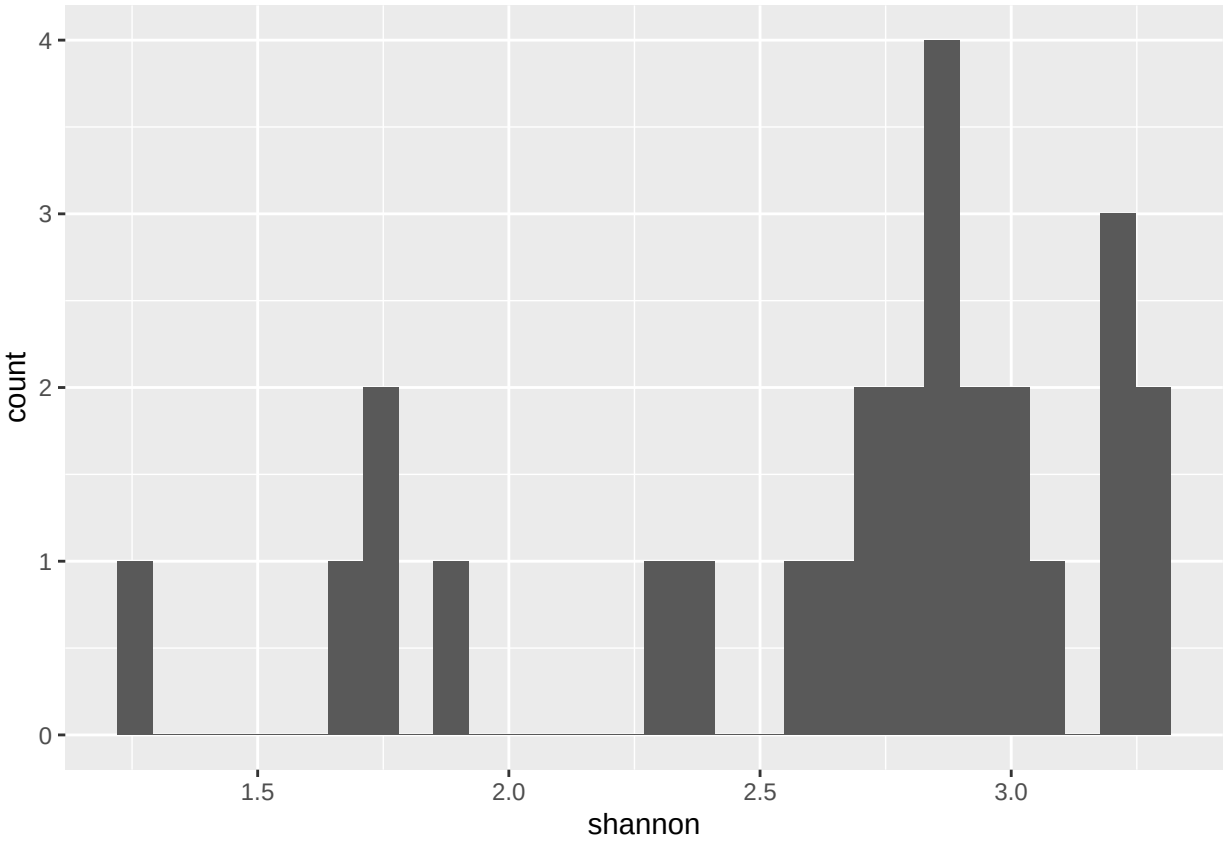
```
## DataFrame with 6 rows and 10 columns
##      patient_status      cohort patient_status_vs_cohort sample_name   shannon
##         <character> <character>              <character> <character> <numeric>
## A110           ADHD    Cohort_1            ADHD_Cohort_1        A110   1.76541
## A12            ADHD    Cohort_1            ADHD_Cohort_1         A12   2.71644
## A15            ADHD    Cohort_1            ADHD_Cohort_1         A15   3.17810
## A19            ADHD    Cohort_1            ADHD_Cohort_1         A19   2.89199
## A21            ADHD    Cohort_2            ADHD_Cohort_2         A21   2.84198
## A23            ADHD    Cohort_2            ADHD_Cohort_2         A23   2.79794
##      gini_simpson     faith     chao1  chao1_se  observed
##         <numeric> <numeric> <numeric> <numeric> <numeric>
## A110     0.669537   7.39224        68  0.000000        68
## A12      0.871176   6.29378        51  0.000000        51
## A15      0.930561   6.60608        68  0.000000        68
## A19      0.899210   6.79708        62  0.000000        62
## A21      0.885042   6.65110        58  0.000000        58
## A23      0.859813   5.96246        61  0.247942        61
```

7.2 Visualizing alpha diversity

We can plot the distributions of individual indices:

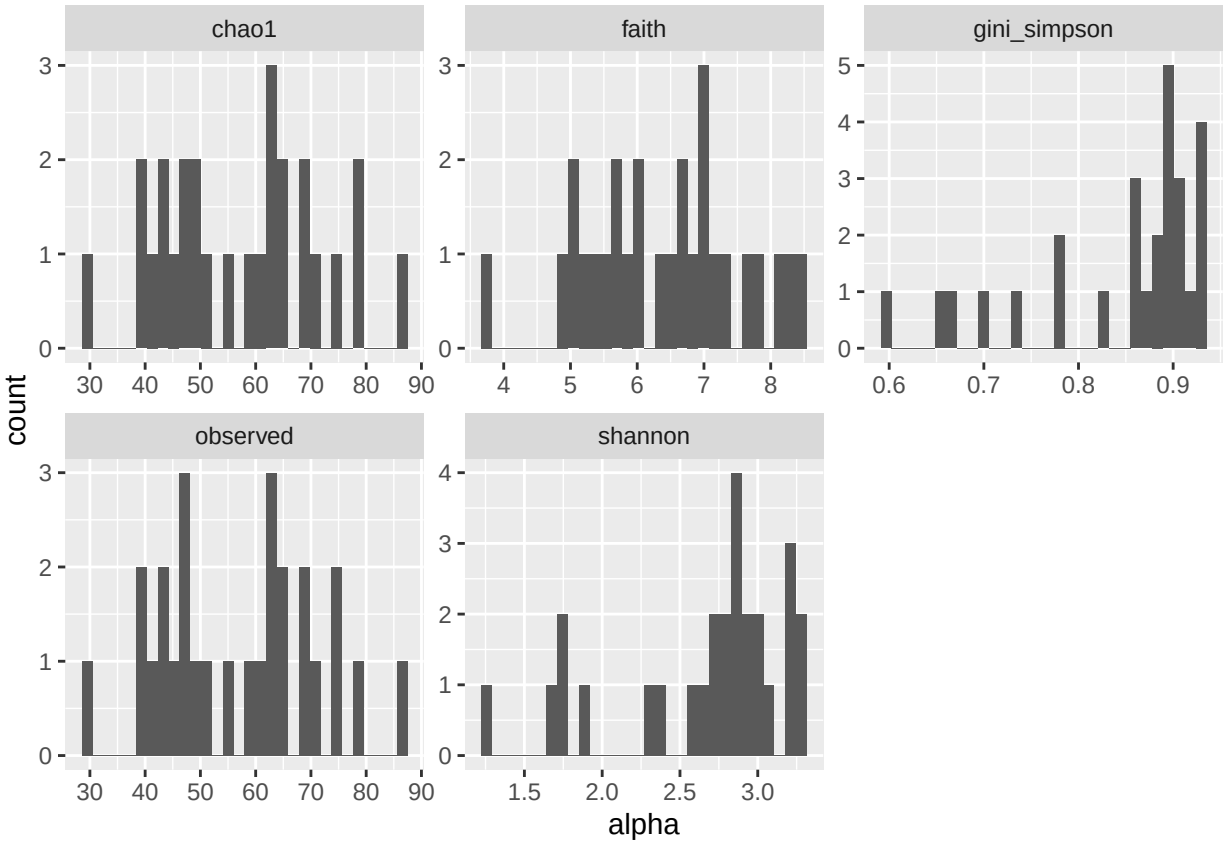
```
#individual plot
p <- as_tibble(colData(tse)) %>%
  ggplot(aes(shannon)) +
  geom_histogram()

print(p)
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```




```
#multiple plots

p <- as_tibble(colData(tse)) %>%
  pivot_longer(cols = c("shannon","gini_simpson","faith","chao1","observed"), names_to = "index", values_to =
  ggplot(aes(alpha)) +
  geom_histogram() +
  facet_wrap(vars(index), scales = "free")


print(p)
```

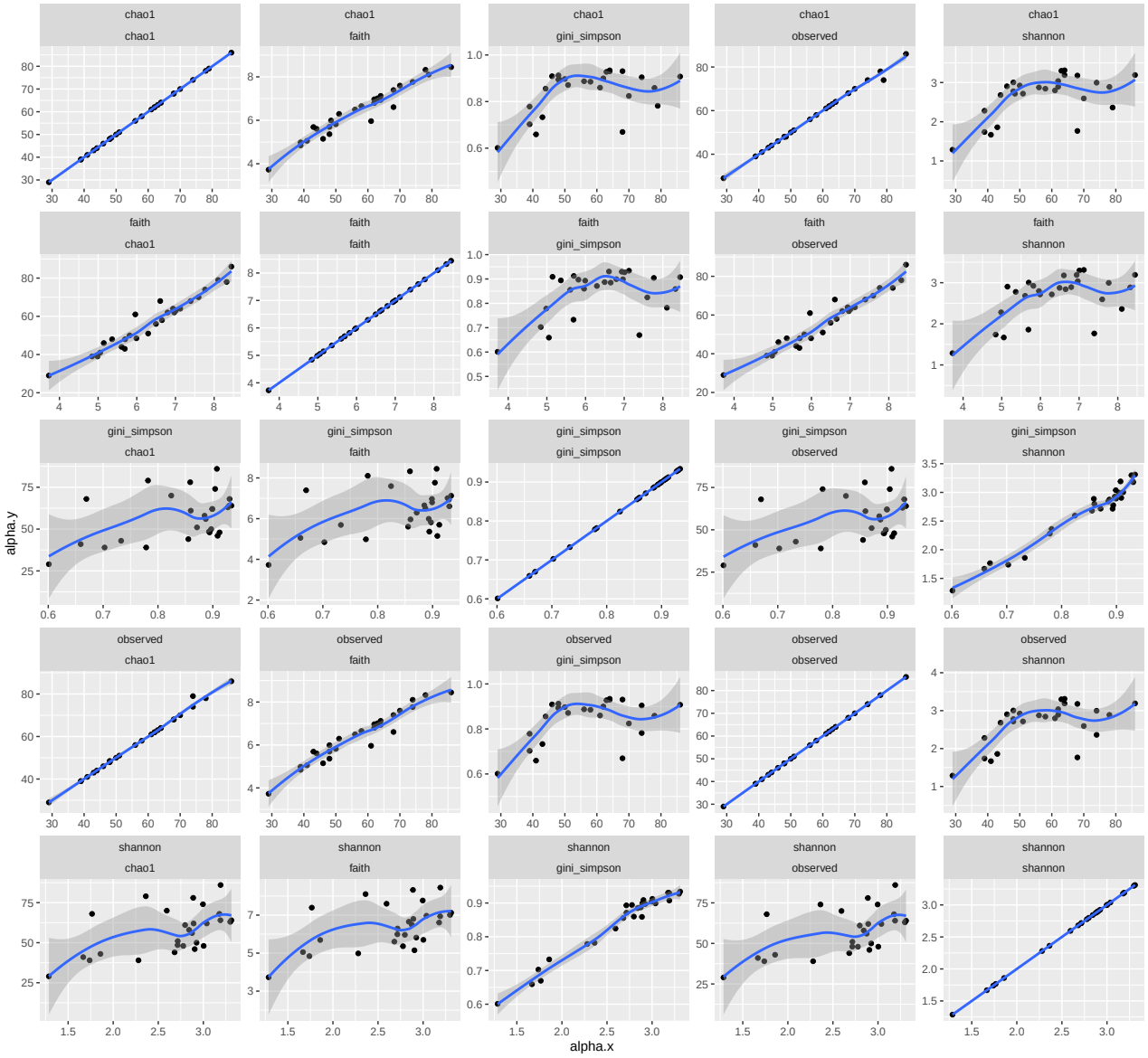
```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



and the correlation between indices:

```
p <- as_tibble(colData(tse)) %>%
  pivot_longer(cols = c("shannon","gini_simpson","faith","chao1","observed"), names_to = "index", values_to =
  full_join(.,., by = "sample_name") %>%
  ggplot( aes(x = alpha.x, y = alpha.y)) +
  geom_point() +
  geom_smooth() +
  facet_wrap(index.x ~ index.y, scales = "free")

print(p)
```

```
## `geom_smooth()` using method = 'loess' and formula 'y ~ x'
```

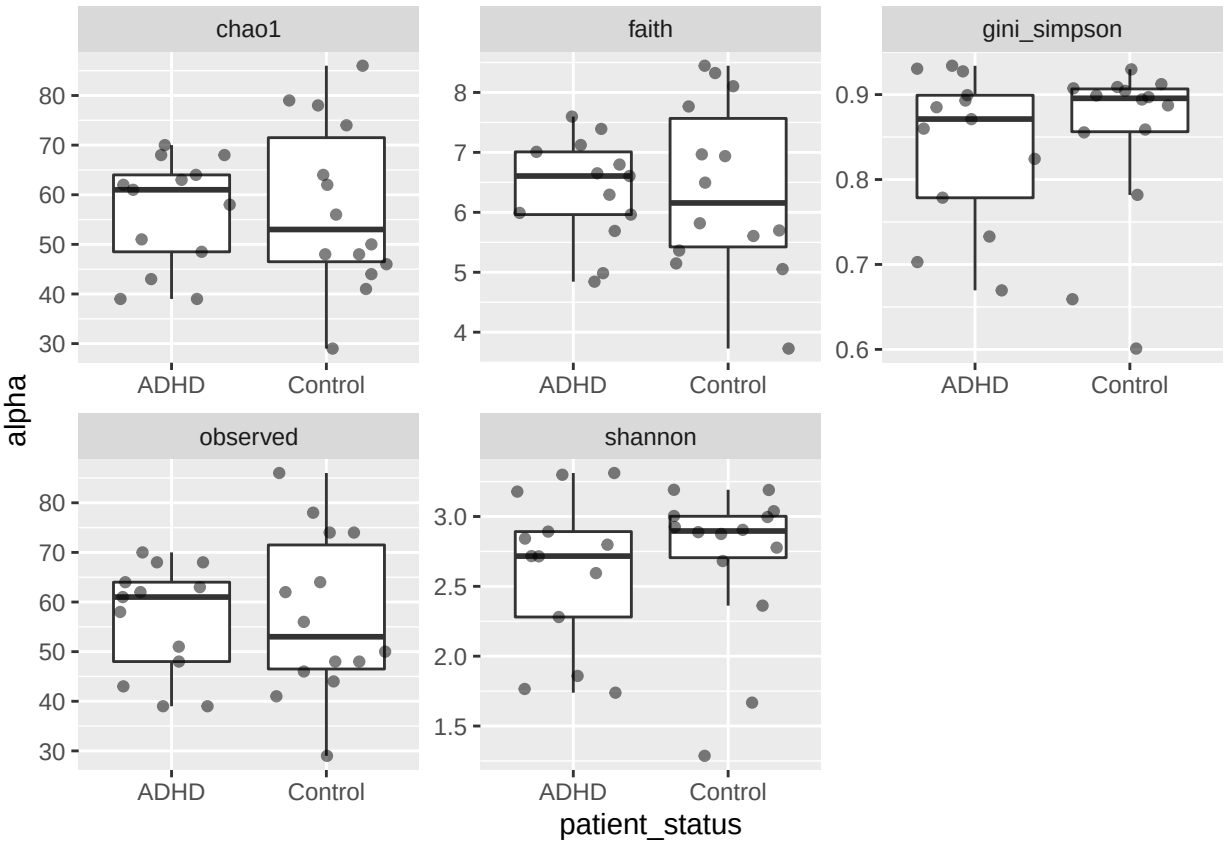


7.3 Comparing alpha diversity

It is often interesting to look for any group differences:

```
p <- as_tibble(colData(tse)) %>%
  pivot_longer(cols = c("shannon","gini_simpson","faith","chao1","observed"), names_to = "index", values_to =
  ggplot( aes(x = patient_status, y = alpha)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(alpha =0.5) +
  facet_wrap(vars(index), scales = "free")
```

```
print(p)
```



Moreover, we can test the group differences by parametric or non-parametric tests:

```
df1 <- as_tibble(colData(tse)) %>%
  pivot_longer(cols = c("faith","chao1","observed"), names_to = "index", values_to = "alpha") %>%
  group_by(index) %>%
  nest() %>%
  mutate(test_pval = map_dbl(data, ~ t.test(alpha ~ patient_status, data = .x)$p.value)) %>%
  mutate(test = "ttest" )

df2 <- as_tibble(colData(tse)) %>%
  pivot_longer(cols = c("shannon","gini_simpson"), names_to = "index", values_to = "alpha") %>%
  group_by(index) %>%
  nest() %>%
  mutate(test_pval = map_dbl(data, ~ wilcox.test(alpha ~ patient_status, data = .x)$p.value))%>%
  mutate(test = "wilcoxon" )

df <- rbind(df1,df2) %>% select(-data) %>% arrange(test_pval) %>% ungroup()

df
```

```
## # A tibble: 5 x 3
```

```
##   index        test_pval test
##   <chr>            <dbl> <chr>
## 1 shannon          0.488 wilcoxon
## 2 gini_simpson     0.685 wilcoxon
## 3 chao1            0.856 ttest
## 4 observed         0.900 ttest
## 5 faith            0.983 ttest
```

End of the demo.

7.4 Exercises

Do “Alpha diversity basics” from the exercises.

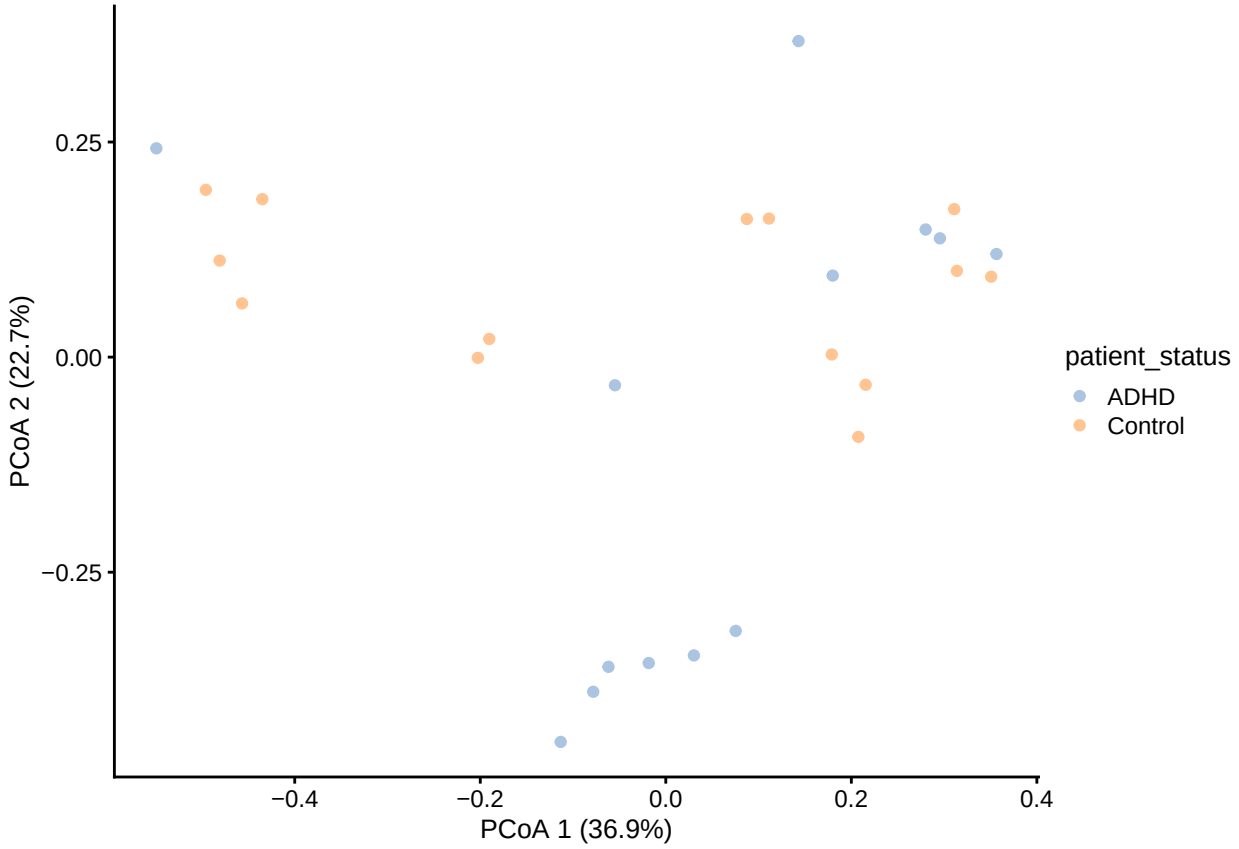
Chapter 8

Beta diversity demo

8.1 Visualizations

Lets generate ordination plots with different methods and transformations.

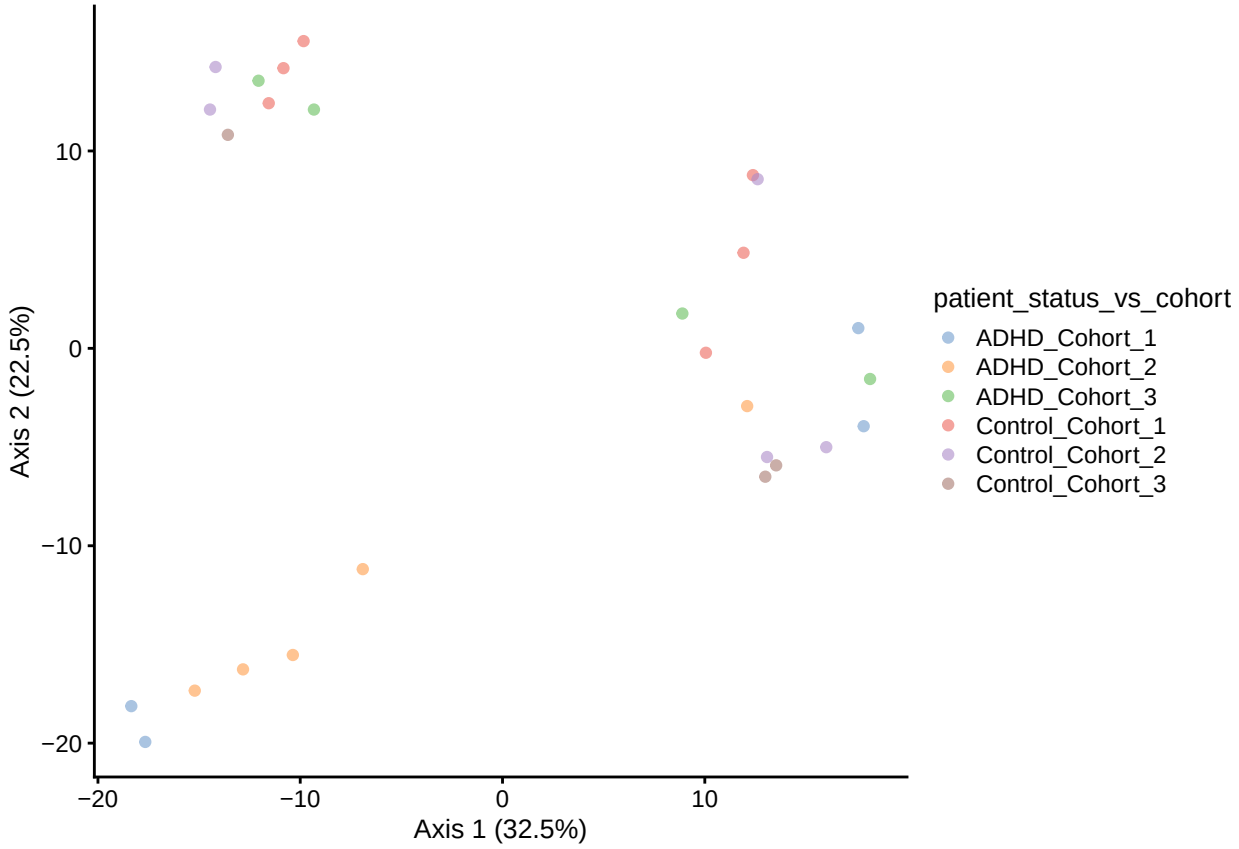
```
#### calculating Bray Curtis dissimilarity and PCoA

tse <- transformSamples(tse, method = "relabundance")
tse <- runMDS(tse, FUN = vegan::vegdist, method = "bray", name = "PCoA_BC", exprs_values = "relabundance")


p <- plotReducedDim(tse, "PCoA_BC", colour_by = "patient_status")

# Add explained variance for each axis
e <- attr(reducedDim(tse, "PCoA_BC"), "eig");
rel_eig <- e/sum(e[e>0])
p <- p + labs(x = paste("PCoA 1 (", round(100 * rel_eig[[1]],1), "%", ")", sep = ""),
              y = paste("PCoA 2 (", round(100 * rel_eig[[2]],1), "%", ")", sep = ""))

print(p)
```




```
#### Aitchinson distances and PCA

tse <- transformSamples(tse, method = "clr", pseudocount = 1)
```

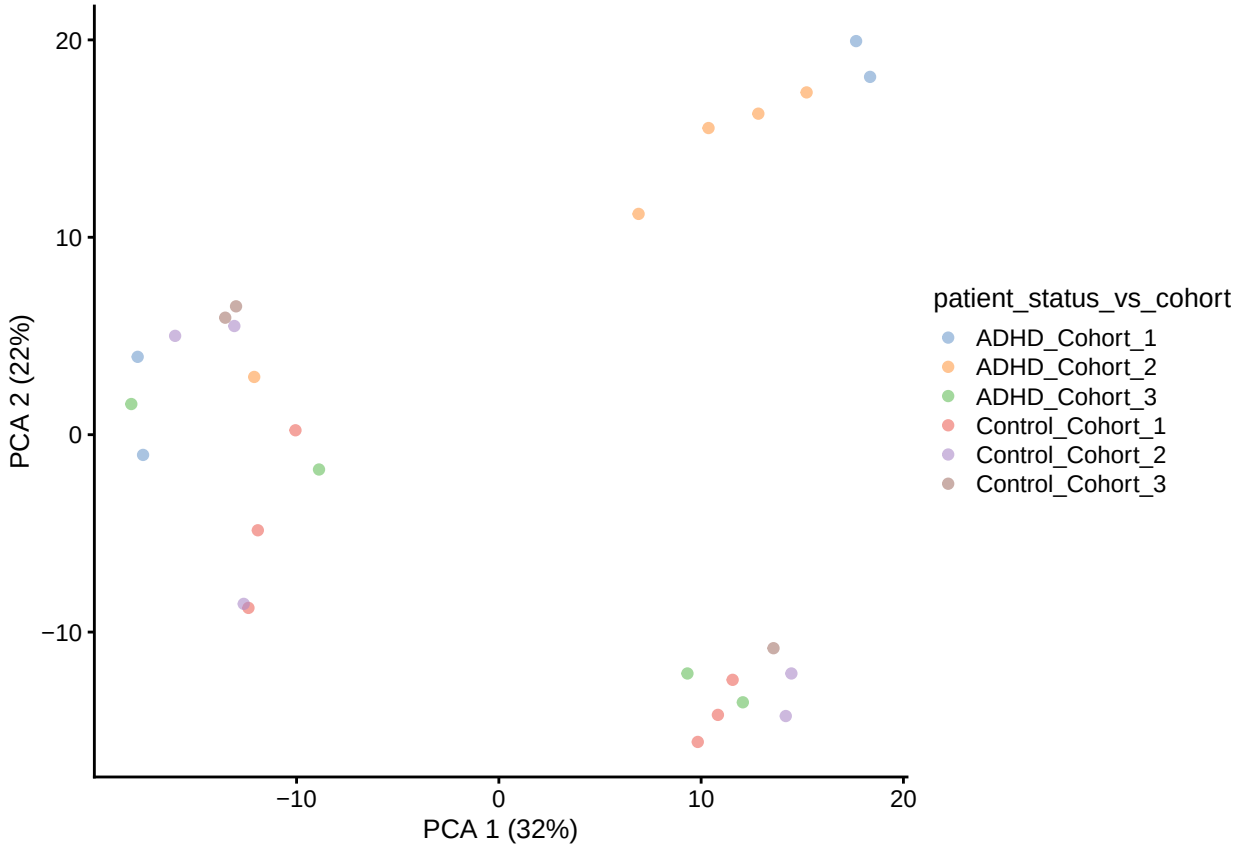
```
## Warning: All the total abundances of samples do not sum-up to a fixed constant.
## Please consider to apply, e.g., relative transformation in prior to CLR
## transformation.
```

```
tse <- runMDS(tse, FUN = vegan::vegdist, name = "MDS_euclidean",
              method = "euclidean", exprs_values = "clr")

p <- plotReducedDim(tse, "MDS_euclidean", colour_by = "patient_status_vs_cohort")

# Add explained variance for each axis
e <- attr(reducedDim(tse, "MDS_euclidean"), "eig");
rel_eig <- e/sum(e[e>0])
p <- p + labs(x = paste("Axis 1 (", round(100 * rel_eig[[1]],1), "%", ")", sep = ""),
              y = paste("Axis 2 (", round(100 * rel_eig[[2]],1), "%", ")", sep = ""))

print(p)
```



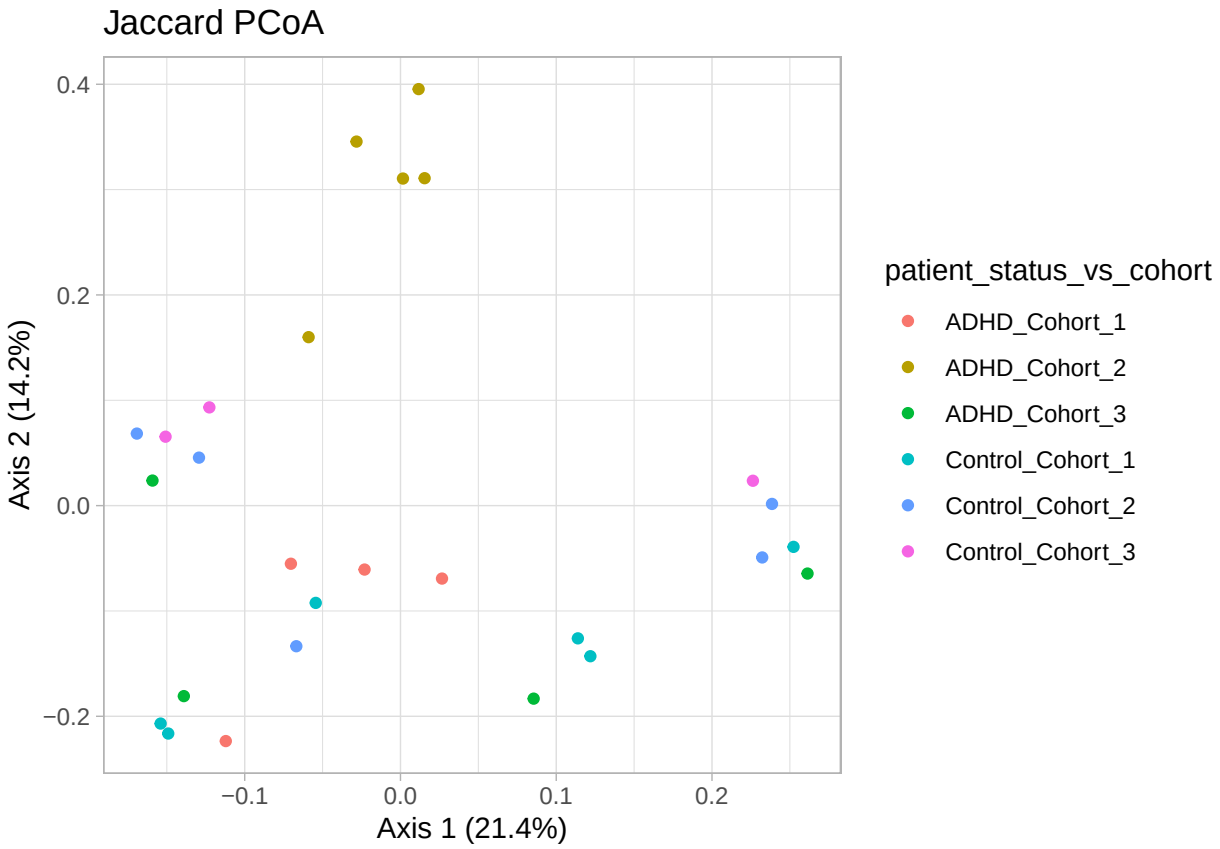
PCA is a subtype of MDS with Euclidean distances, below is a different alternative for running the same analysis.

```
# alternative method

tse <- runPCA(tse, name = "PCA", exprs_values = "clr", ncomponents = 10)
plotReducedDim(tse, "PCA", colour_by = "patient_status_vs_cohort")
```



One can use also ggplot for ordination plots for the flexible adaptablity.

```
dis <- vegan::vegdist(t(assays(tse)$counts), method = "jaccard")

# principal coordinate analysis
jaccard_pcoa <- ecodist::pco(dis)

# a data frame from principal coordinates and groupng variable
jaccard_pcoa_df <- data.frame(pcoa1 = jaccard_pcoa$vectors[,1],
                              pcoa2 = jaccard_pcoa$vectors[,2],
                            patient_status_vs_cohort =  colData(tse)$patient_status_vs_cohort)


# plot
jaccard_plot <- ggplot(data = jaccard_pcoa_df, aes(x=pcoa1, y=pcoa2, color = patient_status_vs_cohort)) +
  geom_point() +
  labs(x = paste("Axis 1 (", round(100 * jaccard_pcoa$values[[1]] / sum(jaccard_pcoa$values), 1), "%", ")", s
       y = paste("Axis 2 (", round(100 * jaccard_pcoa$values[[2]] / sum(jaccard_pcoa$values), 1), "%", ")", s
       title = "Jaccard PCoA") +
  theme(title = element_text(size = 12)) +
  theme_light()

jaccard_plot
```



8.2 Hypothesis testing

PERMANOVA with the function adonis is most commonly used to detect differences in multivariate data. adonis function was recently updated with slightly different functionality. Now the adonis2 allows independent analysis of terms.

```
variable_names <- c("patient_status", "cohort")

tse_genus <- agglomerateByRank(tse, "Genus")
```

```
## Warning: 'clr' includes negative values.
## Agglomeration of it might lead to meaningless values.
## Check the assay, and consider doing transformation again manually with agglomerated data.
```

```
# Apply relative transform
tse_genus <- transformSamples(tse_genus, method = "relabundance")


set.seed(12346)
# We choose 99 random permutations for speed. Consider applying more (999 or 9999)
```

```
assay <- t(assay(tse_genus,"relabundance"))

mod <- paste("assay ~", paste(variable_names, collapse="+")) %>% as.formula()

permanova2 <- vegan::adonis2(mod,
                     by = "margin", # each term analyzed individually
                     data = colData(tse),
                     method = "bray",
                     permutations = 99)

print(permanova2)
```

```
## Permutation test for adonis under reduced model
## Marginal effects of terms
## Permutation: free
## Number of permutations: 99
##
## vegan::adonis2(formula = mod, data = colData(tse), permutations = 99, method = "bray", by = "margin")
##                Df SumOfSqs      R2     F Pr(>F)
## patient_status  1   0.1885 0.05817 1.490   0.23
## cohort          2   0.1450 0.04474 0.573   0.75
## Residual       23   2.9104 0.89787
## Total          26   3.2414 1.00000
```

```
# older adonis for reference
permanova <- vegan::adonis(mod,
                           #by = "margin", # each term analyzed sequentially
                           data = colData(tse),
                           method = "bray",
                           permutations = 99)
```

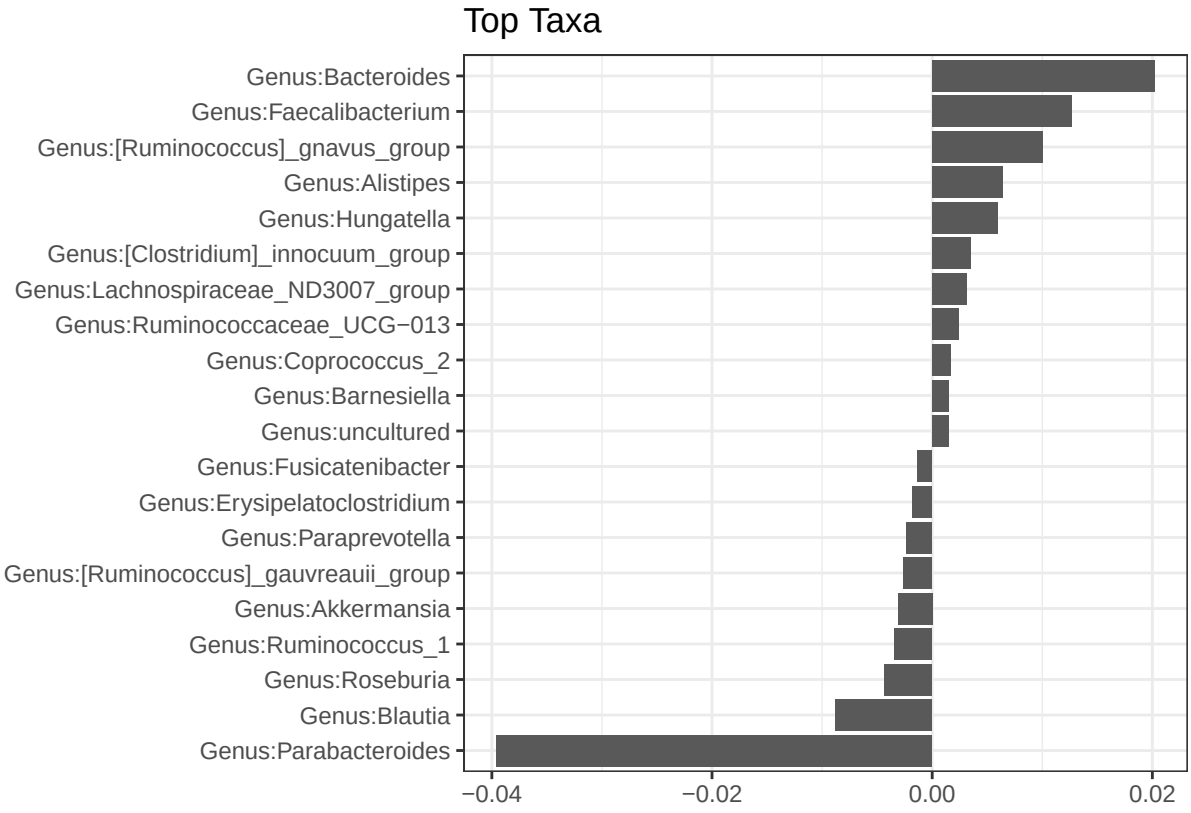
```
## 'adonis' will be deprecated: use 'adonis2' instead
```

```
permanova$aov.tab
```

```
## Permutation: free
## Number of permutations: 99
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## patient_status  1    0.1860 0.186024 1.47011 0.05739   0.22
## cohort          2    0.1450 0.072503 0.57298 0.04474   0.79
## Residuals      23    2.9104 0.126537         0.89787
## Total          26    3.2414                  1.00000
```

With older adonis version one cam calculate top coefficients driving the differences between groups.

```
# older adonis supplies the coefficients
coef <- coefficients(permanova)["cohort1",]
top.coef <- sort(head(coef[rev(order(abs(coef)))],20))

# plot
top_taxa_coeffient_plot <- ggplot(data.frame(x = top.coef,
                                             y = factor(names(top.coef),
                                                        unique(names(top.coef)))),
                                  aes(x = x, y = y)) +
  geom_bar(stat="identity") +
  labs(x="", y="", title="Top Taxa") +
  theme_bw()

top_taxa_coeffient_plot
```



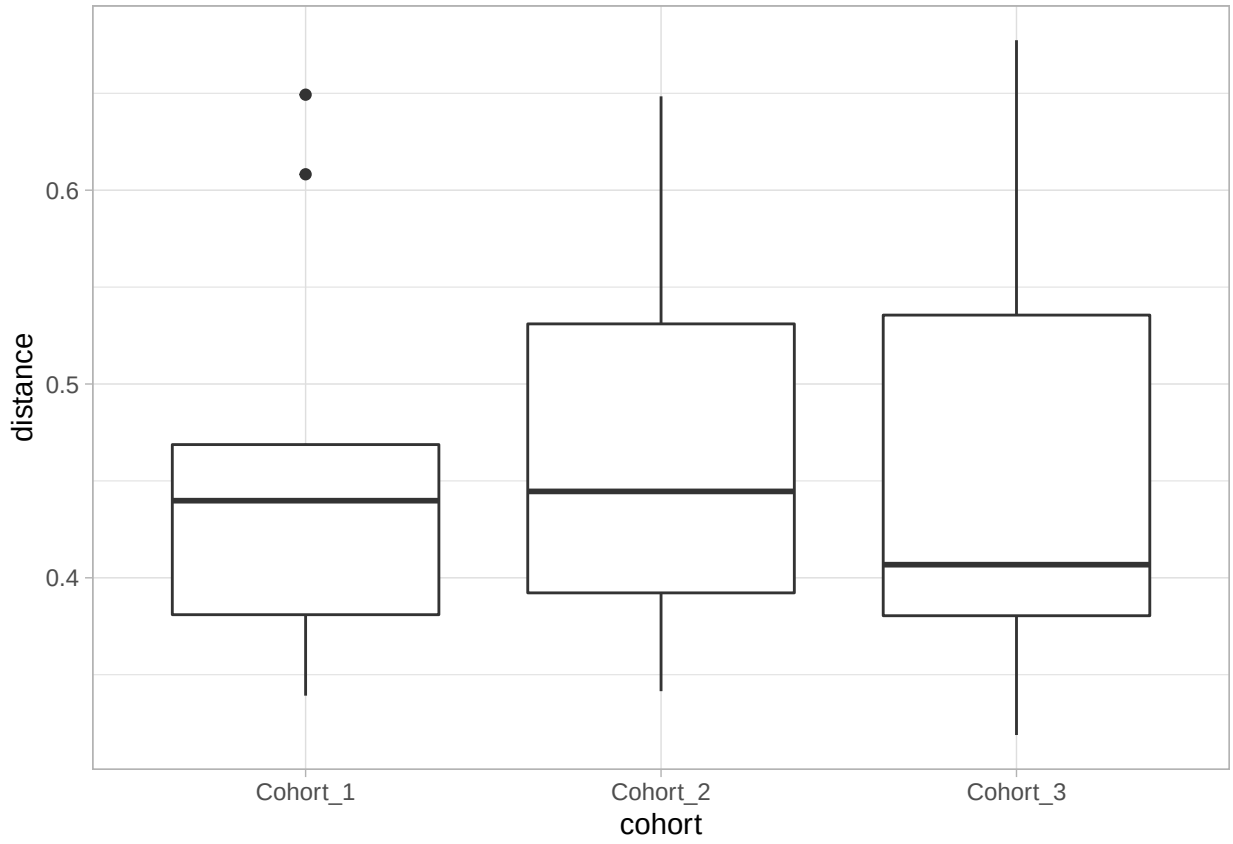
8.2.1 Testing the differences in dispersion

PEMRANOVA doesn't differentiate between different within-group variation, i.e. dispersion, or the mean differences between groups, i.e. the location of the centroid. Follow-up testing can be done with PERMDISP2 implemented in the vegan package.

```
dis <- vegan::vegdist(t(assays(tse)$counts), method = "bray")
b <- vegan::betadisper(dis, colData(tse)$cohort)
print(anova(b))
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value Pr(>F)
## Groups     2 0.000375 0.0001875  0.0166 0.9835
## Residuals 24 0.270795 0.0112831
```

```
# boxplor for distances to centroid
p <- cbind(distance = as.numeric(b$distances),
           cohort = colData(tse)$cohort) %>%
  as_tibble() %>%
  mutate(distance = as.numeric(distance)) %>%
  ggplot(aes(cohort, distance)) +
  geom_boxplot() +
  theme_light()

print(p)
```



End of the demo.

8.3 Exercises

Do “Beta diversity” from the exercises.

Bibliography

Borman, T., Eckerman, H., Aatsinki, A., and Lahti, L. (2022). *Microbiome data science with R/Bioconductor. Radboud Summer School.*