# CART microbiome analysis

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# Contents

# Chapter 1

# Prerequisites

```r
install.packages("bookdown")
```

# Chapter 2

# Introduction

You can label chapter and section titles using `{#label}` after them, e.g., we can reference Chapter 2. If you do not manually label them, there will be automatic labels anyway, e.g., Chapter 4.

Figures and tables with captions will be placed in `figure` and `table` environments, respectively.

```
par(mar = c(4, 4, .1, .1))
plot(pressure, type = 'b', pch = 19)
```

Reference a figure by its code chunk label with the `fig:` prefix, e.g., see Figure 2.1. Similarly, you can reference tables generated from `knitr::kable()`, e.g., see Table 2.1.

```
knitr::kable(
  head(iris, 20), caption = 'Here is a nice table!',
  booktabs = TRUE
)
```

You can write citations, too. For example, we are using the **bookdown** package (Xie 2021) in this sample book, which was built on top of R Markdown and **knitr** (Xie 2015).

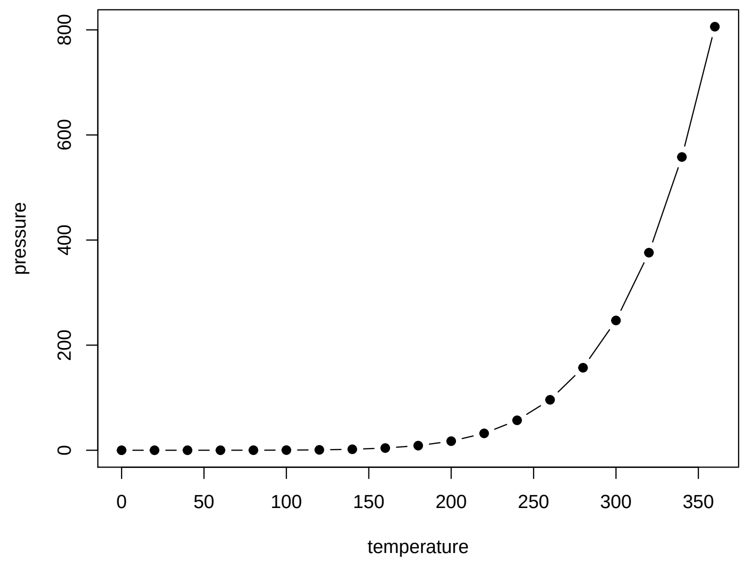



Figure 2.1: Here is a nice figure!

Table 2.1: Here is a nice table!

| Sepal.Length | Sepal.Width | Petal.Length | Petal.Width | Species |
|---:|---:|---:|---:|---|
| 5.1 | 3.5 | 1.4 | 0.2 | setosa |
| 4.9 | 3.0 | 1.4 | 0.2 | setosa |
| 4.7 | 3.2 | 1.3 | 0.2 | setosa |
| 4.6 | 3.1 | 1.5 | 0.2 | setosa |
| 5.0 | 3.6 | 1.4 | 0.2 | setosa |
| 5.4 | 3.9 | 1.7 | 0.4 | setosa |
| 4.6 | 3.4 | 1.4 | 0.3 | setosa |
| 5.0 | 3.4 | 1.5 | 0.2 | setosa |
| 4.4 | 2.9 | 1.4 | 0.2 | setosa |
| 4.9 | 3.1 | 1.5 | 0.1 | setosa |
| 5.4 | 3.7 | 1.5 | 0.2 | setosa |
| 4.8 | 3.4 | 1.6 | 0.2 | setosa |
| 4.8 | 3.0 | 1.4 | 0.1 | setosa |
| 4.3 | 3.0 | 1.1 | 0.1 | setosa |
| 5.8 | 4.0 | 1.2 | 0.2 | setosa |
| 5.7 | 4.4 | 1.5 | 0.4 | setosa |
| 5.4 | 3.9 | 1.3 | 0.4 | setosa |
| 5.1 | 3.5 | 1.4 | 0.3 | setosa |
| 5.7 | 3.8 | 1.7 | 0.3 | setosa |
| 5.1 | 3.8 | 1.5 | 0.3 | setosa |

# Chapter 3

# Fig 2

## 3.1 2B: stacked bar chart.

```
library(tidyverse)
library(vdbR)
library(ggpubr)
connect_database('~/dbConfig.txt')
get_table_from_database("asv_annotation_blast_color_ag");
```

```
# my table of the CART stool cohort
stb <- read_csv('data/amplicon/stool/combined_2_meta.csv')
# get the counts from database and also the color for the asv
counts_data <- get_counts_subset(stb$sampleid)
```

```
dat <- counts_data %>%
  select(asv_key:count_total, count_relative) %>%
  left_join(asv_annotation_blast_color_ag %>%
              select(asv_key,color_label_group_distinct), by = "asv_key")
# there are some ASVs that don't have a color with it, but can use the color for the genus level
color_group <- dat %>%
  split(is.na(.$color_label_group_distinct))
# find the genus for these asv
get_table_from_database('asv_annotation_blast_ag')
no_color <- color_group %>%
  pluck('TRUE') %>%
  distinct(asv_key) %>%
  inner_join(asv_annotation_blast_ag %>%
               select(asv_key, genus))
```

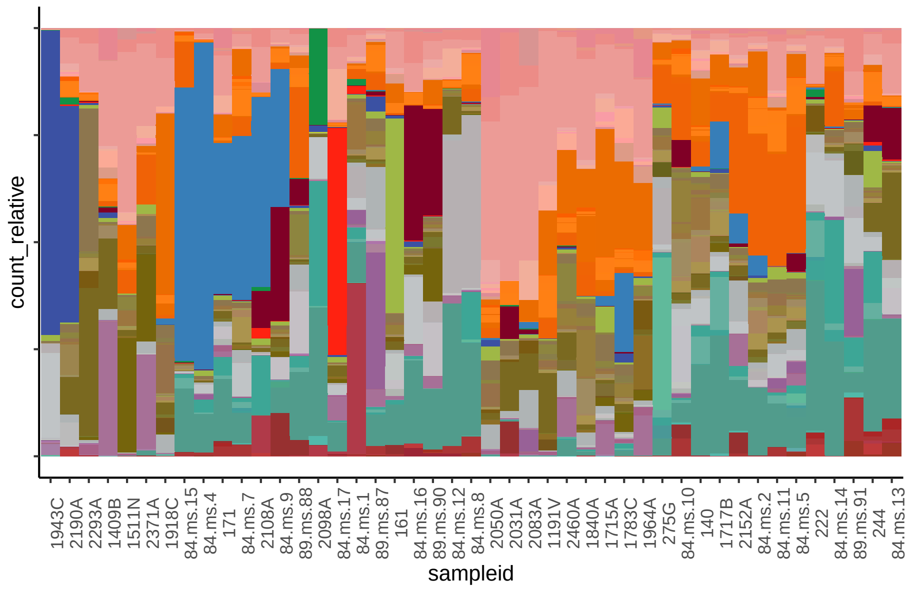
```
# find the colors for these genera
genera_colors <- no_color %>%
  distinct(genus) %>%
  inner_join(asv_annotation_blast_color_ag %>%
               distinct(genus, color_label_group_distinct))
# the full df for the no color genera
no_color_df <- no_color %>%
  left_join(genera_colors)
no_color_df_full <- color_group %>%
  pluck('TRUE') %>%
  select(-color_label_group_distinct) %>%
  left_join(no_color_df %>%
              select(- genus))

# so if the genus is unknown then it's gonna be assigned "other" gray color
# the question is do we go one taxa level higher or make a new color base and shades f
# after discussing with Tsoni, we decided that it's ok to assign gray to the unknown g
# merge the new no_color_df_full to the original df
dat <- bind_rows(
  no_color_df_full,
  color_group %>%
    pluck('FALSE')
)
dat %>%  write_csv('data/the_data_to_make_panel_B.csv')
```

```
# the color palette (inherited from Ying, used in lots of project in our lab, the pale
asv_color_set <- asv_annotation_blast_color_ag %>%
  distinct(color,color_label_group_distinct,color_label_group,color_base) %>%
  select(color_label_group_distinct, color) %>%
  deframe()
```

```
# calculate the beta diversity between the samples which deicide the order of the samp
cbd <- compute_beta_diversity_and_tsne(sampleid = dat$sampleid,
                                       taxonomy = dat$color_label_group_distinct,
                                       count = dat$count);
#compute beta diversity
cbd$compute_beta_diversity()
```

```
## Time:Composition_matrix:
## Time difference of 0.01151896 secs
## Time:Bray-Curtis matrix:
## Time difference of 0.002497911 secs
```

```
#get beta diversity
d_beta <- cbd$get_betadiversity()
#compute hierarchical cluster
hc <- hclust(as.dist(d_beta), method = 'complete')
dend <- as.dendrogram(hc)
sample_dendogram_order <- labels(dend)


#  dividing the samples to lower and higher diversity

div_order <- stb %>%
  arrange(simpson_reciprocal) %>%
  pull(sampleid)
###
# how about splitting the above dendrogram order into the low and higher diversity groups
div_med <- median(stb$simpson_reciprocal)
lower_samp <- stb %>%
  filter(simpson_reciprocal <= div_med) %>%
  pull(sampleid)
lower_samp_o <- sample_dendogram_order[sample_dendogram_order %in% lower_samp]
higher_samp_o <- sample_dendogram_order[!sample_dendogram_order %in% lower_samp]
dat$sampleid = factor(dat$sampleid,levels = c(lower_samp_o, higher_samp_o))
ggplot(dat,aes(sampleid, count_relative, fill = color_label_group_distinct) ) +
  geom_bar(stat = "identity", position="fill", width = 1) +
  theme_classic() +
  labs(title = '',
       ylab = 'Relative counts') +
  theme(axis.text.x = element_text(angle = 90),
        axis.text.y = element_blank(),
        legend.position = "none") +
  scale_fill_manual(values = asv_color_set) +
  ggsave('figs/amplicon/stacked_bar_sorted_with_hclust_lower_and_higher_diversity.pdf', width = 7
```




## 3.2 2C: alpha and beta diversity between CART patients and healthy volunteers

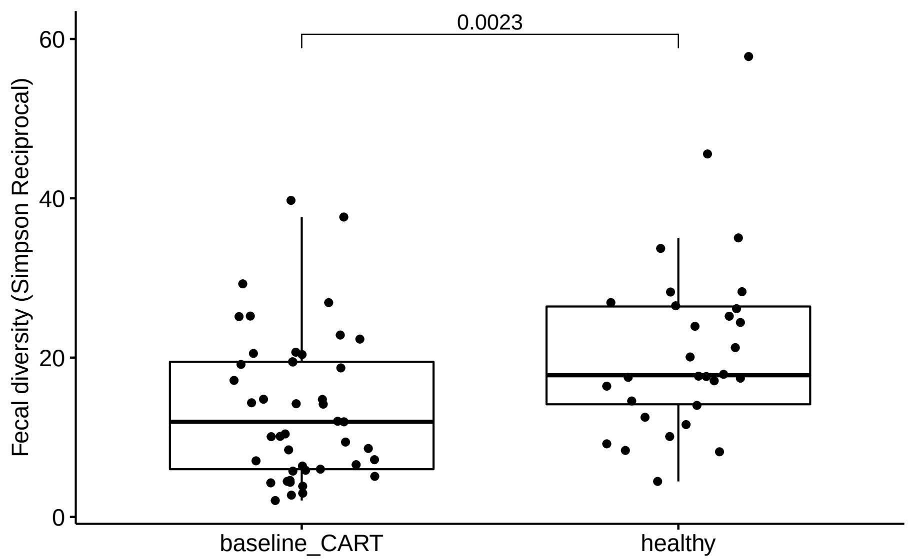
### 3.2.1 alpha diversity (Simpson's reciprocal)

```
library(vdbR)
connect_database('~/dbConfig.txt')
get_table_from_database("healthy_volunteers_ag")
get_table_from_database("asv_alpha_diversity_ag")
```

```
## [1] "table asv_alpha_diversity_ag is loaded and filtered for duplicates. Only the re
```

```
# a total of 75 samples
alpha <- bind_rows(
  stb %>% select(sampleid, simpson_reciprocal) %>% mutate(grp = 'baseline_CART'),
  asv_alpha_diversity_ag %>%
    select(sampleid, simpson_reciprocal) %>%
    inner_join(healthy_volunteers_ag %>% select(sampleid), by = "sampleid") %>%
    mutate(grp = 'healthy')
) %>%
```

```
  distinct(sampleid, .keep_all = T)

alpha %>%
  ggboxplot(x = 'grp', y = 'simpson_reciprocal', add = 'jitter',
            title = '', ylab = 'Fecal diversity (Simpson Reciprocal)', xlab = '',
            palette = c('#ED0000','#00468B')) +
            stat_compare_means(comparisons= list(c('healthy', 'baseline_CART')),
            label= "p.format",
            method= 'wilcox.test')
```

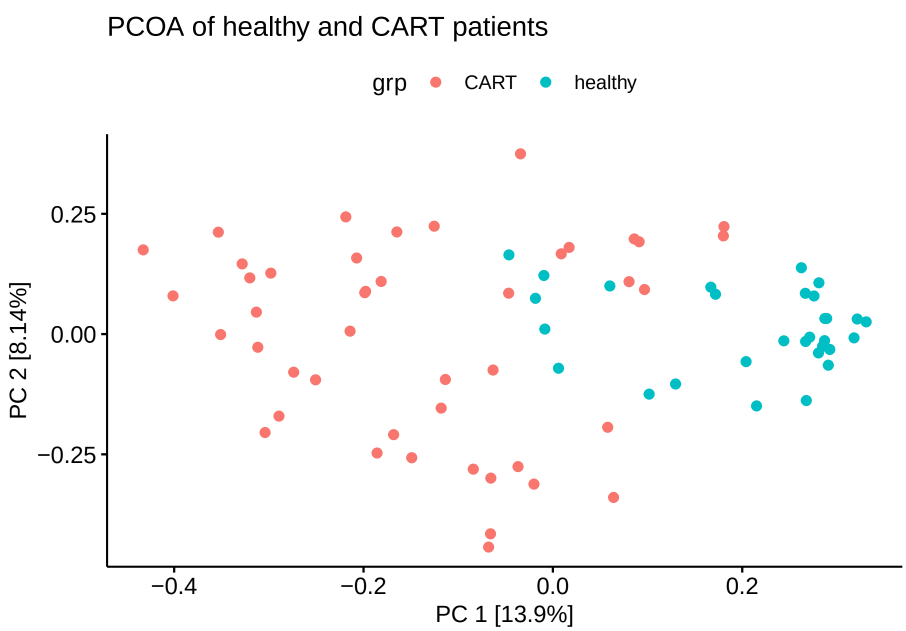



### 3.2.2 beta diversity (PCOA of Bray-Curtis)

```
library(vdbR)
connect_database('~/dbConfig.txt')

healthy <- healthy_volunteers_ag %>%
  inner_join(asv_alpha_diversity_ag, by = c("sampleid", "oligos_id"))
cts <- get_counts_subset(c(stb$sampleid, healthy %>% pull(sampleid)))

# a total of 75 samples counts. there are 3 healthy samples I don't have count .
nsamp <- cts %>%
  distinct(sampleid) %>%
```

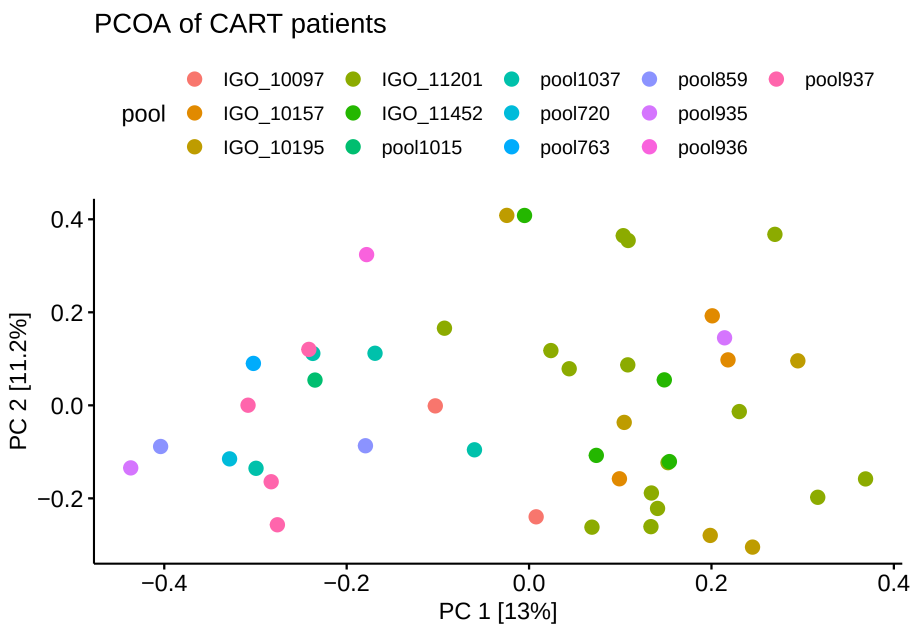
```
  nrow

all_pheno <- bind_rows(healthy %>%
  select(sampleid) %>%
  mutate(grp = 'healthy', center = 'healthy'),
  stb %>% select(sampleid, center)  %>%
    mutate(grp = 'CART') %>%
    select(sampleid, grp, center)
  ) %>%
  ungroup %>%
  distinct(sampleid, .keep_all = T) %>%
  inner_join(asv_alpha_diversity_ag %>%
               distinct(sampleid, .keep_all = T) %>%
               distinct(path_pool, sampleid))


# filter >0.01% in more than 25% samples
keepa <- cts %>%
  filter(count_relative > 0.0001) %>%
  count(asv_key) %>%
  filter(n > floor(nsamp * 0.25)) %>%
  pull(asv_key)

cts_fil <- cts %>%
  filter(asv_key %in% keepa) %>%
  select(sampleid, asv_key,count_relative ) %>%
  spread(key = 'asv_key', value = 'count_relative', fill = 0) %>%
  column_to_rownames('sampleid')

library(vegan)
dist_ <- vegdist(cts_fil, method = 'bray')
eigen <- pcoa(dist_)$values$Eigenvalues
percent_var <- signif(eigen/sum(eigen), 3)*100

bc <- cmdscale(dist_, k = 2)

bc %>%
  as.data.frame() %>%
  rownames_to_column('sampleid') %>%
  ungroup() %>%
  inner_join(all_pheno) %>%
  distinct() %>%
  ggscatter(x = 'V1', y = 'V2', color =  'grp') +
  labs(title = 'PCOA of healthy and CART patients') +
  xlab(paste0("PC 1 [",percent_var[1],"%]")) +
```

```
  ylab(paste0("PC 2 [",percent_var[2],"%]")) +
  #theme_void() +
  ggsave('figs/PCOA(bray-curtis) of healthy and CART patients.pdf')
```




```
# a pcoa at asv level to show they are from different pools and well mixed
cts <- get_counts_subset(c(stb$sampleid))

# filter >0.01% in more than 25% samples
keepa <- cts %>%
  filter(count_relative > 0.0001) %>%
  count(asv_key) %>%
  filter(n > floor(nsamp * 0.25)) %>%
  pull(asv_key)

cts_fil <- cts %>%
  filter(asv_key %in% keepa) %>%
  select(sampleid, asv_key,count_relative ) %>%
  spread(key = 'asv_key', value = 'count_relative', fill = 0) %>%
  column_to_rownames('sampleid')

dist_ <- vegdist(cts_fil, method = 'bray')
eigen <- pcoa(dist_)$values$Eigenvalues
percent_var <- signif(eigen/sum(eigen), 3)*100
```

```
bc <- cmdscale(dist_, k = 2)

mp <- bc %>%
  as.data.frame() %>%
  rownames_to_column('sampleid') %>%
  ungroup() %>%
  inner_join(all_pheno) %>%
  distinct(sampleid, .keep_all = T)  %>%
  mutate(pool = str_extract(path_pool, 'Sample.+/')) %>%
  mutate(pool = str_replace(pool, 'Sample_','')) %>%
  mutate(pool = if_else(str_detect(pool, 'IGO'), str_extract(pool, 'IGO.+$'), pool)) %
  mutate(pool = str_replace(pool, '_1/|_comple.+$',''))

mp %>%
  ggscatter(x = 'V1', y = 'V2', color =  'pool', size = 3) +
  labs(title = 'PCOA of CART patients') +
  xlab(paste0("PC 1 [",percent_var[1],"%]")) +
  ylab(paste0("PC 2 [",percent_var[2],"%]")) +
  #theme_void() +
  ggsave('figs/PCOA(bray-curtis) (ASV level)of CART patients_pool.pdf', width = 9, hei
```



# Chapter 4

# Methods

We describe our methods in this chapter.

# Chapter 5

# Applications

Some *significant* applications are demonstrated in this chapter.

## 5.1 Example one

## 5.2 Example two

# Chapter 6

# Final Words

We have finished a nice book.

Xie, Yihui. 2015. *Dynamic Documents with R and Knitr*. 2nd ed. Boca Raton, Florida: Chapman; Hall/CRC. http://yihui.org/knitr/.

———. 2021. *Bookdown: Authoring Books and Technical Documents with r Markdown*. https://CRAN.R-project.org/package=bookdown.