Microbial community analysis in R

Marko Suokas

Libraries

```
library(phyloseq);packageVersion("phyloseq")
```

```
[1] '1.48.0'
```

```
library(tidyverse);packageVersion("tidyverse")
```

```
[1] '2.0.0'
```

```
library(kableExtra);packageVersion("kableExtra")
```

```
[1] '1.4.0'
```

```
library(patchwork);packageVersion("patchwork")
```

```
[1] '1.2.0'
```

```
library(mia);packageVersion("mia")
```

```
[1] '1.12.0'
```

```
library(ggplot2);packageVersion("ggplot2")
```

```
[1] '3.5.1'
```

```
library(ggthemes);packageVersion("ggthemes")
```

```
[1] '5.1.0'
```

Reload results as a tse object named dada

```
# Path variables
asvfile <- "results_set1/asvs.tsv"
metafile <- "data/set1_meta.tsv"
taxafile <- "results_set1/taxonomy.tsv"
#Abundance data is imported from tabular txt file, rownames stored and emptied
counts <- read_tsv(asvfile, show_col_types = FALSE)
ASV_names <- counts$ASV_names
counts$ASV_names <- NULL
#Metadata is imported from tabular txt file, rownames stored and emptied
samples <- read_tsv(metafile, show_col_types = FALSE)
sampleid <- samples$sampleid
samples$sampleid <- NULL
#Taxonomy table is imported tabular txt file, rownames stored and emptied
taxonomy <-read_tsv(taxafile, show_col_types = FALSE)
taxanames <- taxonomy$ASV_names
taxonomy$ASV_names <- NULL
#Abundance values should be in numeric matrix format
counts <- as.matrix(counts)
#And should be added to a SimpleList
assays <-  SimpleList(counts = counts)
#colData and rowData should be in DataFrame format
colData <- DataFrame(colData)
rowData <- DataFrame(rowData)
#Create a TreeSummarized Experiment object
dada <- TreeSummarizedExperiment(assays = assays,
                                     colData = samples,
                                     rowData = taxonomy)
#Add amplicon variant names as rownames
rownames(dada) <- ASV_names
```

Load results from qiime pipelines

```
# vsearch97
asvfile <- "results_vsearch97/asvs_set1.tsv"
taxafile <- "results_vsearch97/taxonomy_set1.tsv"
#Abundance data is imported from tabular txt file, rownames stored and emptied
counts <- read_tsv(asvfile, show_col_types = FALSE)
ASV_names <- counts$ASV_names
counts$ASV_names <- NULL
#Metadata is imported from tabular txt file, rownames stored and emptied
samples <- read_tsv(metafile, show_col_types = FALSE)
sampleid <- samples$sampleid
samples$sampleid <- NULL
#Taxonomy table is imported tabular txt file, rownames stored and emptied
taxonomy <-read_tsv(taxafile, show_col_types = FALSE)
taxanames <- taxonomy$ASV_names
taxonomy$ASV_names <- NULL
#Abundance values should be in numeric matrix format
counts <- as.matrix(counts)
#And should be added to a SimpleList
assays <-  SimpleList(counts = counts)
#colData and rowData should be in DataFrame format
colData <- DataFrame(colData)
rowData <- DataFrame(rowData)
#Create a TreeSummarized Experiment object
vsearch97 <- TreeSummarizedExperiment(assays = assays,
                                      colData = samples,
                                      rowData = taxonomy)
#Add amplicon variant names as rownames
rownames(vsearch97) <- ASV_names
#vsearch99
asvfile <- "results_vsearch99/asvs_set1.tsv"
taxafile <- "results_vsearch99/taxonomy_set1.tsv"
#Abundance data is imported from tabular txt file, rownames stored and emptied
counts <- read_tsv(asvfile, show_col_types = FALSE)
ASV_names <- counts$ASV_names
counts$ASV_names <- NULL
#Metadata is imported from tabular txt file, rownames stored and emptied
samples <- read_tsv(metafile, show_col_types = FALSE)
sampleid <- samples$sampleid
samples$sampleid <- NULL
#Taxonomy table is imported tabular txt file, rownames stored and emptied
taxonomy <-read_tsv(taxafile, show_col_types = FALSE)
taxanames <- taxonomy$ASV_names
taxonomy$ASV_names <- NULL
#Abundance values should be in numeric matrix format
counts <- as.matrix(counts)
#And should be added to a SimpleList
assays <-  SimpleList(counts = counts)
#colData and rowData should be in DataFrame format
colData <- DataFrame(colData)
rowData <- DataFrame(rowData)
#Create a TreeSummarized Experiment object
vsearch99 <- TreeSummarizedExperiment(assays = assays,
                                      colData = samples,
                                      rowData = taxonomy)
#Add amplicon variant names as rownames
rownames(vsearch99) <- ASV_names
```

Show number of variants in each object

```
#create new dataframe
variants <- data.frame(Dada = nrow(dada), Vsearch97 = nrow(vsearch97),
                       Vsearch99 = nrow(vsearch99))
#table
kable(variants, caption = "Number of variants" )  %>%
  kable_styling(latex_options = c("HOLD_position", "striped")) %>%
  row_spec(0, background = "teal", color = "ivory")
```

Table 1: Number of variants

Dada	Vsearch97	Vsearch99
63	985	9195

As species level taxonomic information is fairly unreliable, we agglomerate objects to genus level

```
#agglomeration to genus level
DADA<- mergeFeaturesByRank(dada, rank = "Genus", onRankOnly = FALSE,
                           na.rm = TRUE)
VS97 <- agglomerateByRank(vsearch97, rank = "Genus", onRankOnly = FALSE,
                          na.rm = TRUE)
VS99 <- agglomerateByRank(vsearch99, rank = "Genus", onRankOnly = FALSE,
                          na.rm = TRUE)
#check number of variants
nrow(DADA)
```

```
[1] 18
```

```
nrow(VS97)
```

```
[1] 33
```

```
nrow(VS99)
```

```
[1] 32
```

Clear difference between methods remains, but in much smaller extent

Next, we convert counts to relative abundance values

```
#relabundance
DADA <- transformAssay(DADA, assay.type = "counts",
                                   method = "relabundance")
VS97 <- transformAssay(VS97, assay.type = "counts",
                                   method = "relabundance")
VS99 <- transformAssay(VS99, assay.type = "counts",
                                   method = "relabundance")
```

Then, we pick five most abundant features

```
#get top5 features
top5_DADA <- getTopFeatures(DADA, top = 5, method = "mean",
                              assay.type = "relabundance")
top5_VS97 <- getTopFeatures(VS97, top = 5, method = "mean",
                             assay.type = "relabundance")
top5_VS99 <- getTopFeatures(VS99, top = 5, method = "mean",
                             assay.type = "relabundance")
#subset top features based on top5 list
DADA <- subsetFeatures(DADA, rowData(DADA)$Genus %in% top5_DADA)
VS97 <- subsetFeatures(VS97, rowData(VS97)$Genus %in% top5_VS97)
VS99 <- subsetFeatures(VS99, rowData(VS99)$Genus %in% top5_VS99)
#create dataframes
df_DADA <- data.frame(assays(DADA)$relabundance)
df_DADA <- df_DADA %>% mutate(Genus = rownames(df_DADA)) %>%
  filter(Genus %in% top5_DADA) %>% arrange(Genus)
rownames(df_DADA) <- NULL
df_DADA <- df_DADA[,c(7,1,2,3,4,5,6)]
df_VS97 <- data.frame(assays(VS97)$relabundance)
df_VS97 <- df_VS97 %>% mutate(Genus = rownames(df_VS97)) %>%
  filter(Genus %in% top5_VS97) %>% arrange(Genus) %>%
  mutate_at("Genus", str_replace, "Genus:", "")
rownames(df_VS97) <- NULL
df_VS97 <- df_VS97[,c(7,1,2,3,4,5,6)]
df_VS99 <- data.frame(assays(VS99)$relabundance)
df_VS99 <- df_VS99 %>% mutate(Genus = rownames(df_VS99)) %>%
  filter(Genus %in% top5_VS99) %>% arrange(Genus) %>%
  mutate_at("Genus", str_replace, "Genus:", "")
rownames(df_VS99) <- NULL
df_VS99 <- df_VS99[,c(7,1,2,3,4,5,6)]
```

```
kable(df_DADA, digits=2) %>%
  kable_styling(latex_options = c("HOLD_position", "striped")) %>%
  row_spec(0, background = "teal", color = "ivory")
```

Genus	barcode01	barcode02	barcode03	barcode04	barcode05	barcode06
Aeromonas	0	1	0	0	0	0
Delftia	0	0	0	0	1	0
Providencia	0	0	0	1	0	0
Pseudomonas	1	0	0	0	0	0
Stenotrophomonas	0	0	1	0	0	1

```
kable(df_VS97, digits=2) %>%
  kable_styling(latex_options = c("HOLD_position", "striped")) %>%
  row_spec(0, background = "teal", color = "ivory")
```

Genus	barcode01	barcode02	barcode03	barcode04	barcode05	barcode06
Aeromonas	0	1	0	0	0	0
Delftia	0	0	0	0	1	0
Providencia	0	0	0	1	0	0
Pseudomonas	1	0	0	0	0	0
Stenotrophomonas	0	0	1	0	0	1

```
kable(df_VS99, digits=2) %>%
  kable_styling(latex_options = c("HOLD_position", "striped")) %>%
  row_spec(0, background = "teal", color = "ivory")
```

Genus	barcode01	barcode02	barcode03	barcode04	barcode05	barcode06
Aeromonas	0	1	0	0	0	0
Delftia	0	0	0	0	1	0
Providencia	0	0	0	1	0	0
Pseudomonas	1	0	0	0	0	0
Stenotrophomonas	0	0	1	0	0	1

For stacked barplots, we melt assay data

```
#transform data to ggplots
assay_dada <- meltAssay(DADA, assay.type="relabundance")
names(assay_dada) <- c("Genus","Sample","Abundance")
assay_vs97 <- meltAssay(VS97, assay.type="relabundance")
names(assay_vs97) <- c("Genus", "Sample", "Abundance")
assay_vs99 <- meltAssay(VS99, assay.type="relabundance")
names(assay_vs99) <- c("Genus", "Sample", "Abundance")
```

Plot objects

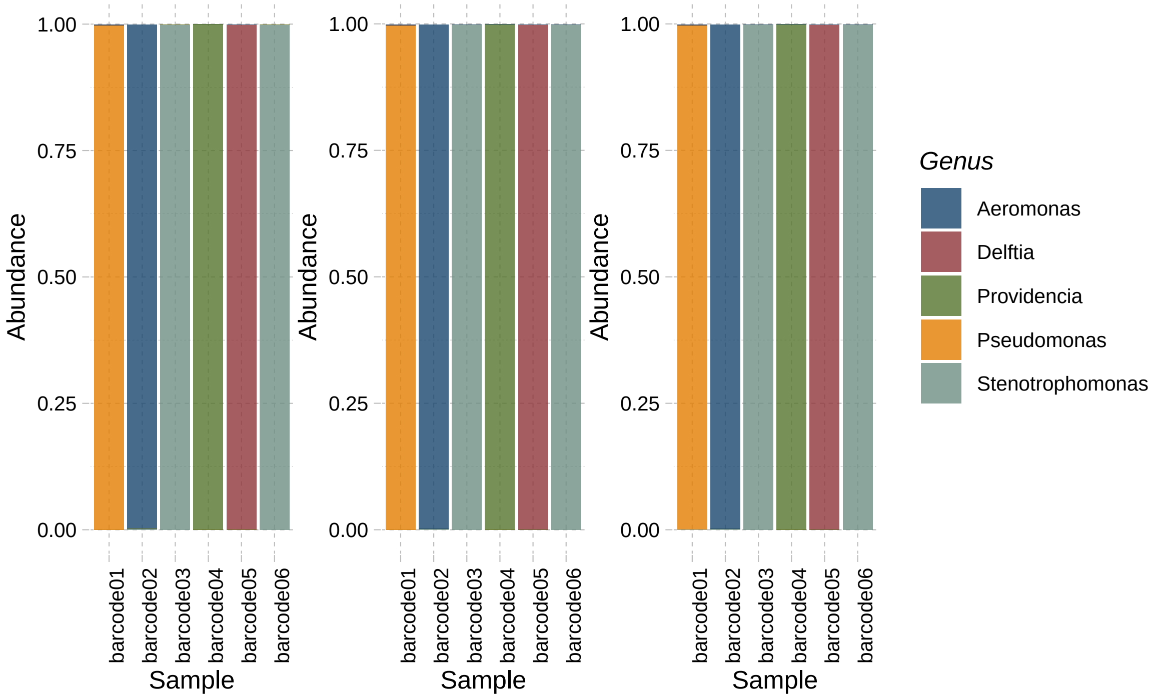
```
#Create stacked barplot
dada_plot <- ggplot(assay_dada, aes(x = Sample, y = Abundance, fill = Genus)) +
  geom_bar(stat = "identity", show.legend = FALSE, alpha=0.8) +
  theme_pander(base_size = 10) + scale_fill_stata() + theme(axis.text.x =
                                                              element_text(angle = 90))

vs97_plot <- ggplot(assay_vs97, aes(x = Sample, y = Abundance, fill = Genus)) +
  geom_bar(stat = "identity", show.legend=FALSE, alpha=0.8) +
  theme_pander(base_size = 10) + scale_fill_stata() + theme(axis.text.x =
                                                              element_text(angle = 90))

vs99_plot <- ggplot(assay_vs99, aes(x = Sample, y = Abundance, fill = Genus)) +
  geom_bar(stat = "identity", alpha=0.8) + theme_pander(base_size = 10) +
  scale_fill_stata() + theme(axis.text.x = element_text(angle = 90))
```

Results side by side

```
#show plots side by side
dada_plot+vs97_plot+vs99_plot
```



Observations

In this sample set, where we evidently have pure microbial cultures, each method gave exactly same result. Low diversity might explain why dada2 error profile looks clean. In more complex communities, it's possible that denoising doesn't function as well. We already have previous evidence that dada2 can function accurately in mock communities consisting few microbes.

On the other hand, clustering methods produce lot more low abundance noise, but it doesn't show in the end results (with two digits accuracy). There are two discrepancies in 10 most commond features between denoising and clustering. Dada picked up fonticella and clustering methods acidithiobacillus.