

MB590 Microbiome Analysis

Christine V. Hawkes

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Contents

EXPLORATORY ANALYSIS PRACTICUM	1
SETUP	1
LOAD AND PREPARE YOUR DATA	2
ALPHA DIVERSITY	2
BETA DIVERSITY	2
TRY SOMETHING NEW	3

EXPLORATORY ANALYSIS PRACTICUM

- Today we will practice characterizing alpha and beta diversity of microbial communities.
- Go through all of the following steps
- Annotate with comments that document your choices and results
- A knitted html or pdf file is due on 3/16 uploaded to your class GitHub repo

SETUP

The following packages are likely to be used, but this is not an exhaustive list.
Load any other libraries you need depending on what analyses you choose.

```
library(phyloseq)
library(tidyverse)
library(DESeq2)
library(microbiome)
library(vegan)
library(ggplot2)
```

LOAD AND PREPARE YOUR DATA

1. Select and load your data
 - Load your own data (must have at least ASV matrix and SampleData matrix)
 - Alternative: use Erlandson et al. data available on Dryad
 - Orig paper: <https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14576>
 - DRYAD entry: <https://datadryad.org/stash/dataset/doi:10.5061/dryad.5f24ks4>
 - Study of how abiotic and biotic environment affect Salix-assoc microbial comms
 - Load `wk9_data_dryad.RData` file
 - Contains many data subsets;
 - * use `wk9_README_dryad.txt` to choose your target files
 - * can delete the remaining files
 - * consider whether you need the `ps` object or separate files for your analyses
2. Examine, check quality, and subset the data if needed (e.g., remove singletons)
3. Transform the data (clr or vst) if needed
4. Take a minute to think through the data and what analyses would make the most sense before you begin the next steps

ALPHA DIVERSITY

Choose 2 of the following, plot results, and interpret results:

1. Calculate richness and diversity
2. Plot rank-abundance and plot species-accumulation or rarefaction curves
3. Run either of the above at a different taxonomic level and compare

BETA DIVERSITY

Choose 2 of the following, explain your selections of how to handle the data (including dissimilarity metric, ordination type, etc.), plot results, and interpret results:

1. Unconstrained ordination
 2. Constrained ordination
 3. Core microbiome analysis
-

TRY SOMETHING NEW

If you found the above exercises easy, select one of the following to learn a new skill:

1. Partition variance in beta-diversity due to env, spatial, or other vars with **vegan::varpart**

- partitions variance in community matrix due to 2-4 explanatory matrices
- <https://esajournals.onlinelibrary.wiley.com/doi/full/10.1890/05-0549>
- <https://cran.r-project.org/web/packages/vegan/vignettes/partitioning.pdf>
- <https://www.rdocumentation.org/packages/vegan/versions/2.4-2/topics/varpart>

2. Partition beta-diversity components with **betapart**

- calculates turnover and nestedness components of beta-diversity
- <https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/j.2041-210X.2012.00224.x>
- <https://cran.r-project.org/web/packages/betapart/index.html>

3. Compare different ordinations with one of the following

- **vegan::procrustes**
 - rotates one matrix to achieve maximum similarity with a target matrix
 - <https://link.springer.com/article/10.1007/s004420100720>
 - <https://www.rdocumentation.org/packages/vegan/versions/2.4-2/topics/procrustes>
- **dispRity**
 - calculates disparity in multidimensional space occupancy
 - <https://besjournals.onlinelibrary.wiley.com/doi/10.1111/2041-210X.13022>
 - <https://cran.r-project.org/web/packages/dispRity/dispRity.pdf>