**­­Microbial Diversity and Ecology Lab, Fall 2020**

**Week 9 Lab 15/16: Phyloseq Part 1**

**Due**: Friday October 23, 11:59 pm

**Task**: upload this document in the assignment on WyoCourse; Week 9 Lab 15/16 Phyloseq Part1, with your answers embedded and rename the file by adding your last name at the end of the file name, e.g. Week9\_lab15/16\_Phyloseq\_Assignment\_vanDiepen. Please save your file as a Word document.

**Points**: 2.0

**Objective**: In this tutorial you will be become familiar with phyloseq and the options it offers. You will make pretty graphs and calculate p-values for various alpha and beta diversity metrics using the tables that you created in the previous lab DADA2. What fun!!

**Instructions:** Download the Rmd file “Phyloseq\_final” and the "Metadata.csv" from WyoCourse Week 9 Module “Lab Materials”. Copy these files to your Desktop in your course folder, in which you could create a separate folder named “Phyloseq” to keep everything together.

Open the “Phyloseq\_final.Rmd” file. This will open the program R studio and the tutorial.

You can begin reading in the R tutorial and refer back to this document when you are ready to answer a question or complete one of the exercises.

Exercise 1 (~Line 23): What is the code you used to load the metadata.csv?

Answer:

Exercise 2 (~Line 41): Load Phyloseq package and record how you did so in the handout.

Answer:

Exercise 3 (~Line 61): Look at your phyloseq object. What does it contain. What type of summary info is available from your phyloseq object? Record a summary of your ps object in your handout.

Answer:

Exercise 4 (~Line 67): Record how many taxa did not assign to the Kingdom Bacteria. You can do this by looking at the total number of taxa in the original ps object and ps\_bac object after you run the previous section.

Answer:

Exercise 5 (~Line 69): Make another Phyloseq object named ps\_firm which only includes taxa assigned to the phylum Firmicutes. Make a third Phyloseq object (named ps\_not\_firm) that includes every member that did not assign to Firmicutes. Hint: "!" is a symbol for "not". If "==" means all that equal, might "!=" mean not equal? Record your code in the handout, and how many taxa belong to the Firmicutes.

Answer: ­­

Exercise 6 (~Line 84): Choose whether or not you want to remove sample CLEX1\_4 and provide a brief justification. Include this in your handout.

Answer:

Exercise 7 (~Line 107): Examine the rarefied ps object and the transformed ps object. What do you notice about the otu tables?

Answer:

Exercise 8 (~Line 111): What is alpha diversity? How does it compare to beta diversity and gamma diversity?

Answer:

Exercise 9 (~Line 115): Finish this lines of code by using the help page. Remember to use the rarefied data! Run the code and record the code here.

Answer:

Exercise 10 (~Line 122): Now let’s use the same methods we used in the "Intro to R" tutorial to extract information from the sample names and include it as metadata as an additional column in the richness\_full data frame.

Answer:

Exercise 11 (~Line 132): Decide between an ANOVA and Tukey's HSD test or its non-parametric equivalent (Kruskal-Walis) to compare the 'observed' alpha diversity metric among treatments. You can use the assumptions of ANOVA to guide your decision process. In your lab handout, detail which test you decided upon and whether or not you found any significant differences.

Exercise 12 (Lines ~138): Run the same analysis for Shannon diversity and then choose one more diversity metric and do the same. What are the strengths and weaknesses of the three alpha diversity metrics you examined (observed, Shannon and your choice)? When would someone want to use Shannon diversity as opposed to richness? Record this in your handout. Were there significant differences (P<0.05)?

Answer:

Exercise 13 (Lines 150): Install and load the package "psych" which contains the describeBy() function and use this function to summarize the richness stats by treatment. You will want to run this function on the richness statistics you calculated for the full dataset (Richness\_full). Copy the code you used to install and load the package and calculate the summary statistics into your lab handout.

Exercise 14 (Line ~167): By using the help page examine the functions (plot\_richness and geom\_boxplot) below and alter it to create a plot which examines the richness measures you chose above and compares the different treatments. Edit the code below and create a graph the same three metrics you examined above. Copy your codes to the handout.

Exercise 15 (~Lines 190): Save one of your alpha diversity plots and insert in here.

Answer:

#STOPPING POINT FOR THIS WEEK 9 assignment

NOTE: we'll continue next week with the remainder of this tutorial to look at beta-diversity

1) Make sure you save your environment for future work (Go to Environment tab in upper right pane) and click on the "save" symbol, and save it as LastName\_phyloseq\_Env2.

2) Then save your .Rmd file with all the updated information and code by clicking the "save" symbol in this pane.