Microbial Community Analysis Using Colony Phenotype Database

Student Handout

Objectives

- Manipulate large datasets to conduct community-level ecological analyses
- Use community-level data to address questions about insect microbiomes
- Use Excel or RStudio programs to calculate community ecology variables
- Compare microbial community using community ecology variables

Introduction

Microbiomes are the communities of microbes (bacteria, viruses, fungi and archaea) living symbiotically with all metazoans. In the past decade, both interest and research on microbiomes, including their implications for human health, have increased dramatically (Christian *et al.* 2015, Costello *et al.* 2012, McFall-Ngai *et al*. 2013, The Human Microbiome Consortium 2012, Young 2016). Insects have been used as model species to study the importance of microbiomes, because of their ease of use and the fact that microbial communities play diverse roles in insects (Engel and Moran 2013).

The data that are collected in any microbiome study consists of lists of the taxonomic units identified and their abundance. The same types of data are evaluated in an ecological community analysis, but now the communities are the collections of microbes that constitute different microbiomes. The community variables, "species" richness and relative abundance, are the same and the statistical methods used to compare communities, diversity and difference indices, also are the same. Perhaps the simplest measure of community structure used by ecologists is "species" or taxon richness, a count of the number of unique taxa in a sample. However, species richness does not consider the relative abundance of species in a community. Imagine two communities with five different species. In one community, all of the species have the same relative abundance. In the other community, one species dominates comprising 95% of individuals in the community. The other four species are very rare. Based on species richness as a measure of community structure, these two communities are the same, although they are clearly very different. As a result, ecologists use other species diversity indices that consider both the number of species and the relative abundance of species in a community. Two common indices are the Simpson Index and the Shannon-Weaver Index. Communities with greater numbers of species and higher evenness (i.e., similar relative abundance of species within a community) are considered more diverse. Finally, measures of species richness and species diversity do not consider the identity of species in a community. So, communities could have the same level of species diversity, but have completely different species. Measure of community similarity, such as the Bray-Curtis Index, compare the similarity (or dissimilarity) between two communities based on the identity of species in the communities, as well as their relative abundances. For more information on indices of species diversity and measures of community similarity, see Krebs (1999).

In this study, bean beetle gut microbiome data were collected by undergraduate students using the protocols developed by Cole *et al*. (2018). Three types of data were collected: colony phenotypes from cultured bacteria, 16s rRNA gene sequencing of specific bacterial colonies, and whole community 16s rRNA gene sequencing, but we will limit our analyses to the colony phenotype and colony-based 16s data.

Microbial Community Analysis Using Colony Phenotype Database

Questions

Using data from the colony phenotype database and the analyses described below, answer the following questions.

1. Based on the diversity indices that you calculated, which treatment had the highest (lowest) diversity?
2. Does the answer depend on the measure of species (taxon) diversity that you use?
3. Is there a relationship between number of samples and taxonomic diversity? If so, what might explain this?
4. Which communities are most similar (different)?

Database description

This database contains data for the microbial community of bean beetles based on colony phenotypes of individual bacterial colonies cultured from bean beetle homogenates plated on different media. The bacteria cultured from each beetle are represented by multiple rows of data with each row representing a different combination of phenotypic characters. A unique combination of phenotypic characters is taken to represent a unique bacterial taxon.

Access the database at https://www.beanbeetles.org/microbiome/phenotype-database-search/.

The database allows you to limit your search by bean host species, sex, life cycle stage, media on which bacteria were grown, and colony phenotype. EMB and PEA plates select for gram negative and gram positive bacteria, respectively. So, a colony with a particular combination of phenotypic characters on an EMB plate is a different bacterial taxon than a colony with the same combination of characters on a PEA plate. The same is not true if we include blood agar or nutrient agar plates in our sample. Those plates could be considered independently.

We want to limit our search to PEA and EMB plates (or Nutrient Agar only). After limiting by media type, click "Submit."

Downloading Data

While we can view the data on the website, we want to download the data to manipulate. Click the download link to download a csv file with the data. Then, re-save the file as an Excel file and rename the tab "raw data."

Screenshot 1 (Browser)

Phenotype Database Search Re... ×

https://www.beanbeetles.org/microbiome/phenotype-database-search-results/

Search

Most Visited   Getting Started

Bean Beetles
A Model Organism for Inquiry-based Undergraduate Laboratories

MOREHOUSE COLLEGE

EMORY UNIVERSITY

Search ...

Home   Laboratory Methods   Research   Genome   Microbiome   Laboratory Activities   Publications

Phenotype Database Search Results

Download search results

Show 10 entries

experiment_id	sample_id	host	sex	stage	media	color	gloss	form	elevation	CFU
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	EMB	clear		circular	flat	1000
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	EMB	purple	shiny	irregular	flat	30
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	EMB	yellow	shiny	irregular	flat	150
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	PEA	yellow	shiny	circular	flat	1500
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	EMB	clear		circular	flat	1000
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	PEA	white	matte	irregular	umbonate	50
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	EMB	blue	shiny	circular	raised	9
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	PEA	yellow	shiny	circular	flat	1500
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	PEA	white	matte	irregular	umbonate	50
EMORY_SP17	EMORY_SP17_106_1	mung	female	adult	EMB	blue	shiny	circular	raised	9

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Screenshot 2 (Excel)

phenotype_H0dgAwfObk [Read-Only] - Excel

Beck, Christopher

File   Home   Insert   Page Layout   Formulas   Data   Review   View

Q31

	A	B	C	D	E	F	G	H	I	J	K
1	experime	sample_i	host	sex	stage	media	color	gloss	form	elevation	CFU
2	EMORY_SI	EMORY_SI	adzuki	female	adult	PEA	offwhite	matte	circular	convex	4390
3	EMORY_SI	EMORY_SI	mung	female	adult	PEA	brown	matte	circular	raised	160000
4	EMORY_SI	EMORY_SI	adzuki	male	adult	PEA	white	shiny	circular	convex	420
5	EMORY_SI	EMORY_SI	mung	male	adult	PEA	white	shiny	circular	convex	250
6	EMORY_SI	EMORY_SI	mung	female	adult	PEA	white	matte	circular	raised	133
7	EMORY_SI	EMORY_SI	mung	female	adult	PEA	white	shiny	circular	raised	100000
8	EMORY_SI	EMORY_SI	pigeon	male	adult	PEA	white	shiny	circular	umbonate	190
9	EMORY_SI	EMORY_SI	adzuki	male	adult	EMB	red	shiny	circular	raised	100000
10	EMORY_SI	EMORY_SI	BEP	male	adult	PEA	white	shiny	circular	raised	60
11	EMORY_SI	EMORY_SI	adzuki	female	adult	PEA	offwhite	shiny	circular	flat	140
12	EMORY_SI	EMORY_SI	mung	male	adult	PEA	yellow	shiny	circular	raised	4350
13	EMORY_SI	EMORY_SI	adzuki	male	adult	EMB	brown	shiny	circular	raised	20
14	EMORY_SI	EMORY_SI	pigeon	male	adult	PEA	offwhite	shiny	circular	flat	10
15	EMORY_SI	EMORY_SI	mung	female	adult	EMB	clear	shiny	circular	raised	120
16	EMORY_SI	EMORY_SI	pigeon	male	adult	PEA	yellow	matte	circular	raised	200
17	EMORY_SI	EMORY_SI	adzuki	female	adult	EMB	red	shiny	circular	raised	6
18	EMORY_SI	EMORY_SI	pigeon	male	adult	PEA	white	shiny	circular	raised	3920
19	EMORY_SI	EMORY_SI	pigeon	male	adult	PEA	white	shiny	circular	raised	2
20	EMORY_SI	EMORY_SI	adzuki	female	adult	PEA	white	shiny	circular	convex	100
21	EMORY_SI	EMORY_SI	pigeon	male	adult	EMB	red	shiny	circular	raised	1220
22	EMORY_SI	EMORY_SI	mung	male	adult	PEA	orange	shiny	irregular	umbonate	2720
23	EMORY_SI	EMORY_SI	adzuki	female	adult	EMB	yellow	matte	circular	raised	1500
24	EMORY_SI	EMORY_SI	mung	female	adult	PEA	white	matte	circular	umbonate	11
25	EMORY_SI	EMORY_SI	adzuki	male	adult	PEA	yellow	matte	circular	raised	830
26	EMORY_SI	EMORY_SI	pigeon	female	adult	EMB	orange	shiny	circular	raised	80
27	EMORY_SI	EMORY_SI	adzuki	female	adult	PEA	white	matte	circular	raised	7
28	EMORY_SI	EMORY_SI	adzuki	male	adult	EMB	red	shiny	circular	flat	2800
29	EMORY_SI	EMORY_SI	BEP	female	adult	EMB	red	shiny	circular	raised	8
30	EMORY_SI	EMORY_SI	mung	female	adult	PEA	offwhite	shiny	circular	convex	112100
31	EMORY_SI	EMORY_SI	mung	female	adult	EMB	brown	shiny	circular	raised	400
32	EMORY_SI	EMORY_SI	BEP	female	adult	PEA	offwhite	shiny	circular	flat	1840
33	EMORY_SI	EMORY_SI	mung	male	adult	PEA	white	shiny	circular	convex	250

raw data

Ready

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Double click and relabel tab as "raw data"

4

Data manipulation

1. The raw data have some missing values for phenotypic characters or values of zero for CFU (colony forming units) (which represents missing data for CFU). These rows need to be deleted. The easiest way to do this is with the Filter function in Excel. In the "raw data" worksheet, turn on filtering by selecting Filter under Sort and Filter.
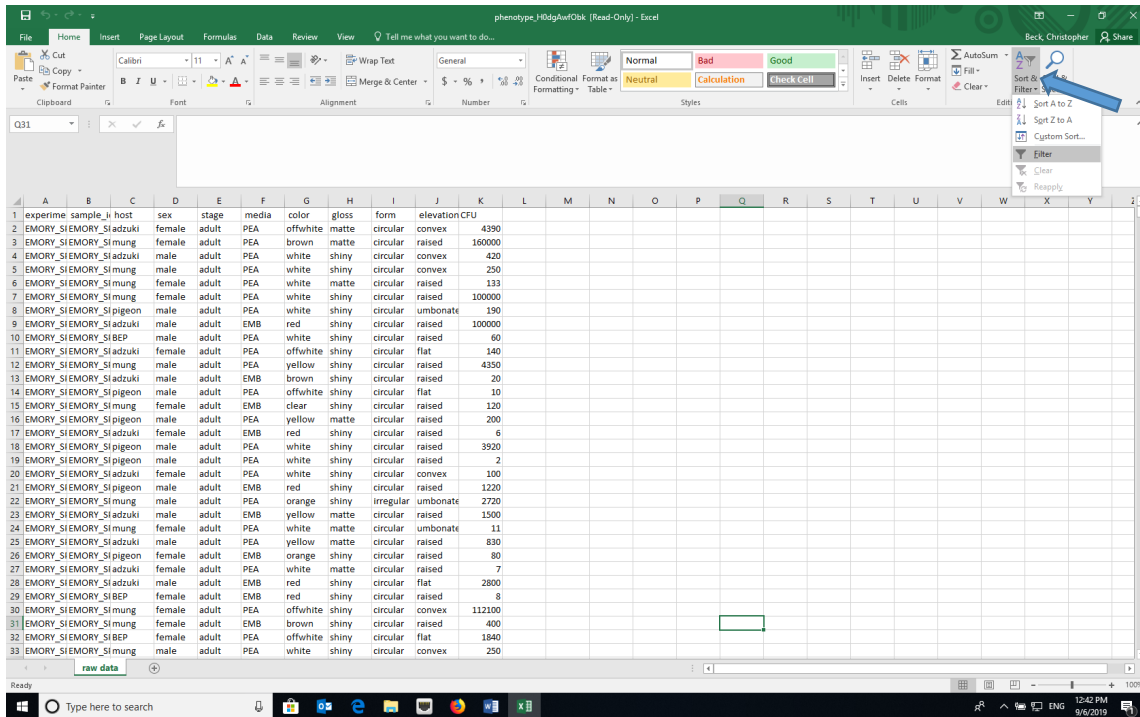

Then, for each of the phenotypic characters, click the down arrow in the column heading and unselect "Blank" at the bottom of the list and unselect "0" in the CFU column.
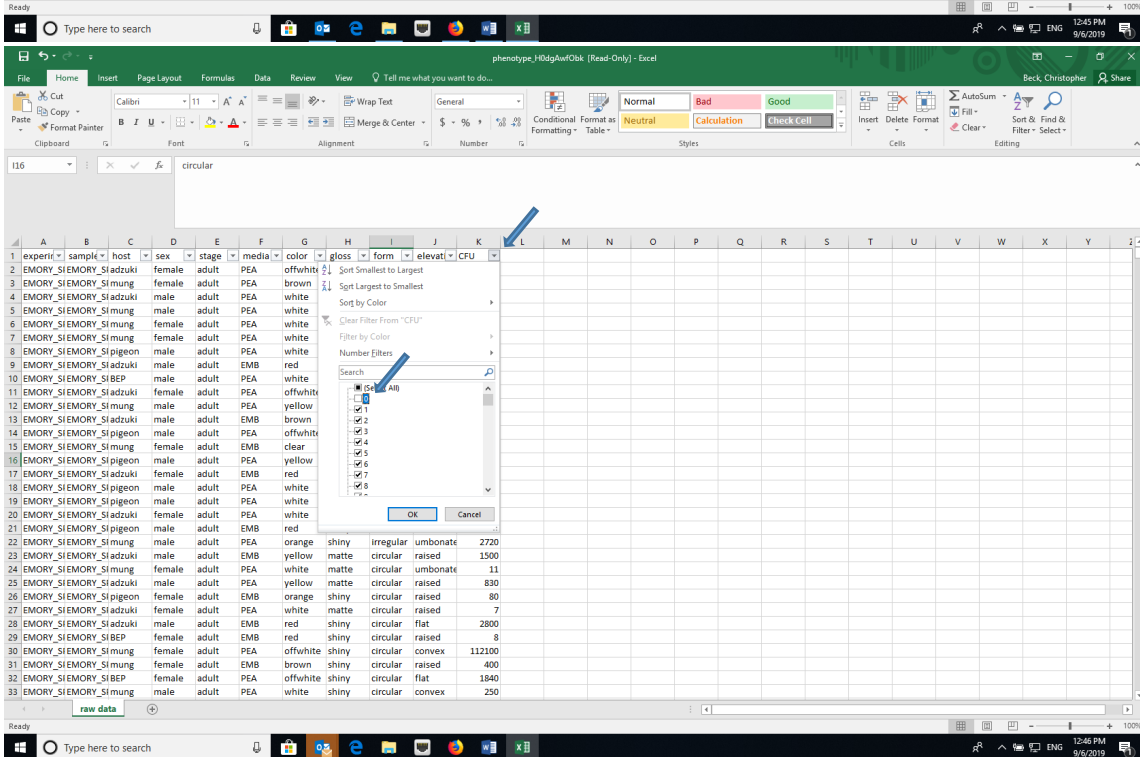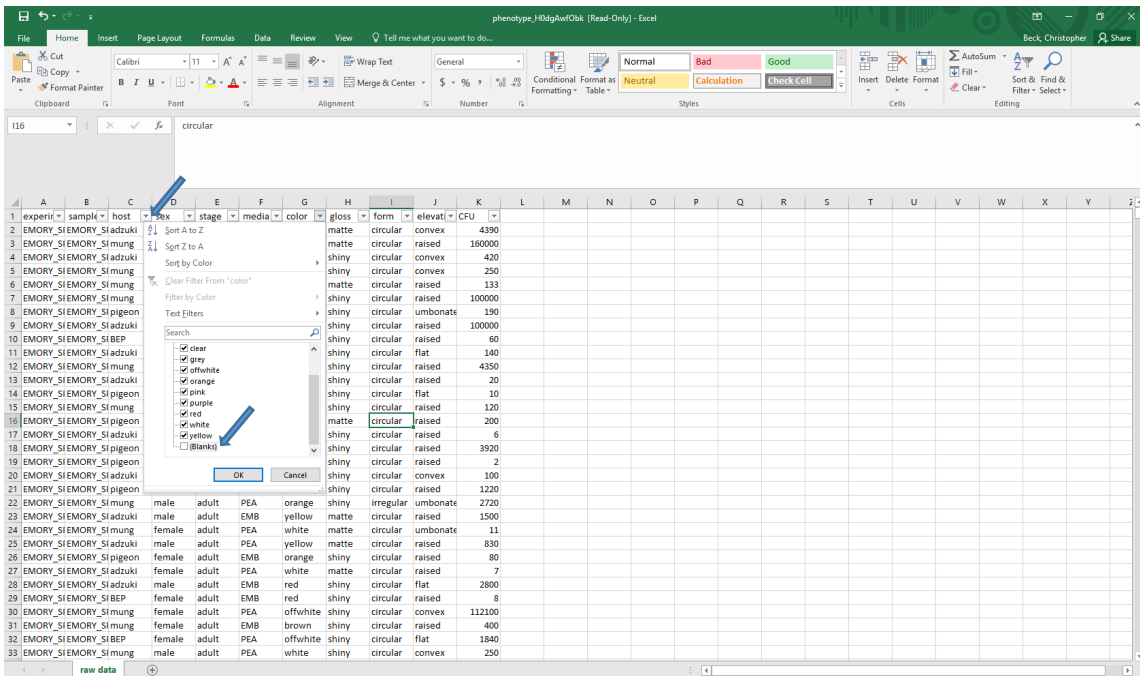
Next, select all (CRTL-A or CMD-A) and copy and paste into a new worksheet and name that sheet "phenotype". The "phenotype" worksheet represents the cleaned raw data. If you will be analyzing the data in R later, copy and paste a second time into another blank sheet and name that sheet "community".

2. With our new "phenotype" dataset, we need to define a bacterial "taxon" based on the combination of media and the four phenotypic characters (color, gloss, form and elevation). One way to do this is to create a "taxon" name by concatenating the media and the four different phenotypic traits. You can do this using the CONCATENATE function in Excel (=CONCATENATE(F2,G2,H2,I2,J2). After you create the "taxa" names, you might want to select the column and then re-paste it in the same column by pasting values (using paste special) to remove the formula.
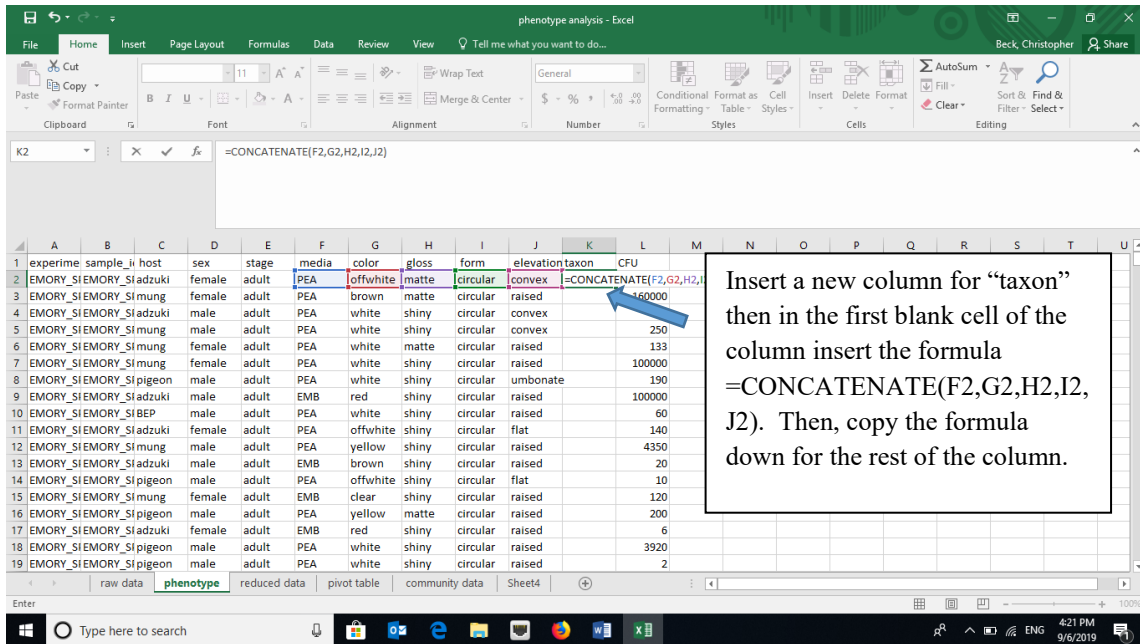
3. Now, we need to consolidate the data for each host species, each sex, or the combination of the two by the bacterial taxa. The easiest way to do this is with the Pivot Table function in Excel.

4. When clicked on a cell within the data, create a Pivot Table (Insert -> Pivot Table OR Data -> Summarize with Pivot Table, depending on your version of Excel). Make sure that the data source includes the top row, which has the column headings.
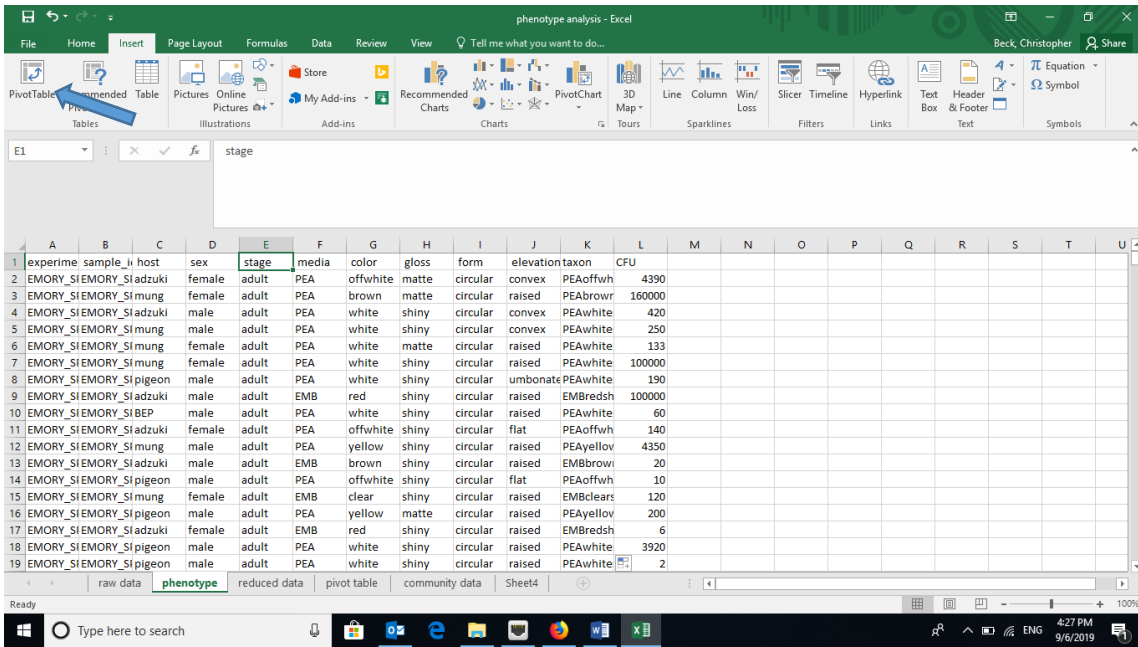
Set the host and sample_id as the rows as these represent the treatment and individual communities, respectively. The new "taxon" column should be the columns in the pivot table. The Values should be a SUM of the CFU (colony-forming units, a measure of density), which will be shown as "SUM of CFU" using the Options menu.
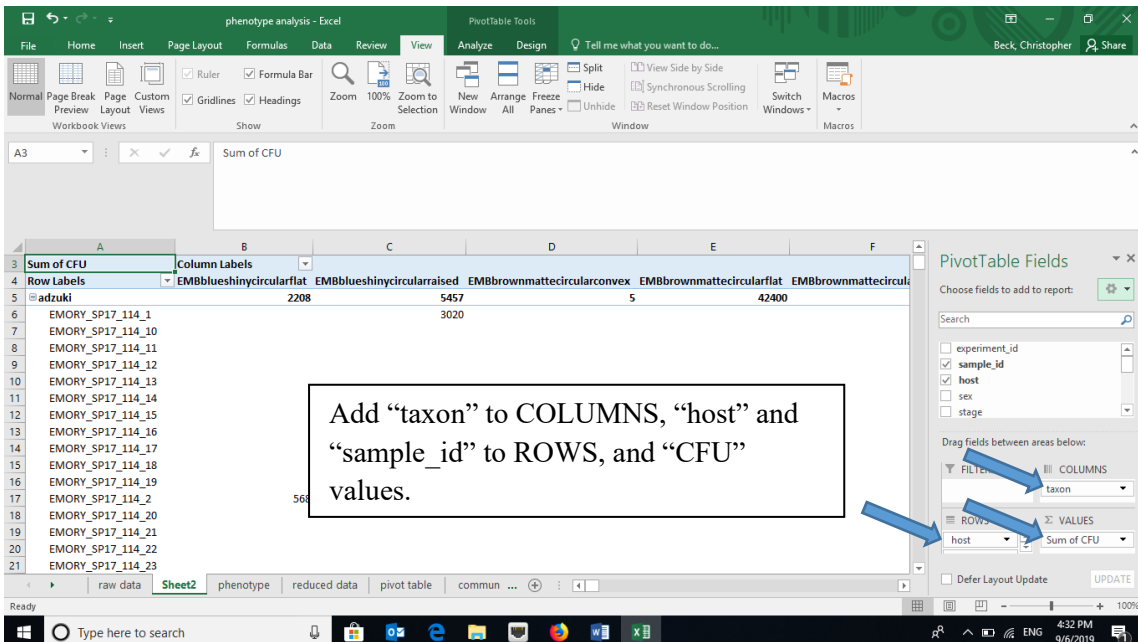

Add "taxon" to COLUMNS, "host" and "sample_id" to ROWS, and "CFU" values.

5. You can add zeros to all of the empty cells in the Pivot Table using the Options menu.

6. Remove the Grand totals for Columns using the Options menu or the Design tab depending of your version of Excel.

Unselect check boxes for grand totals for columns.
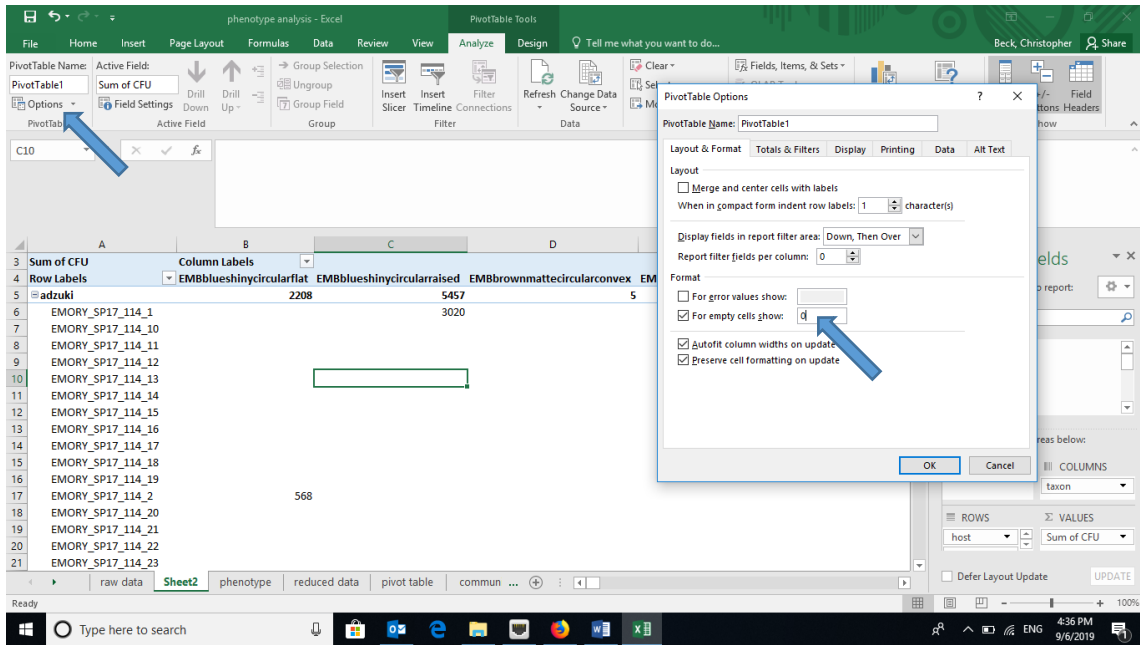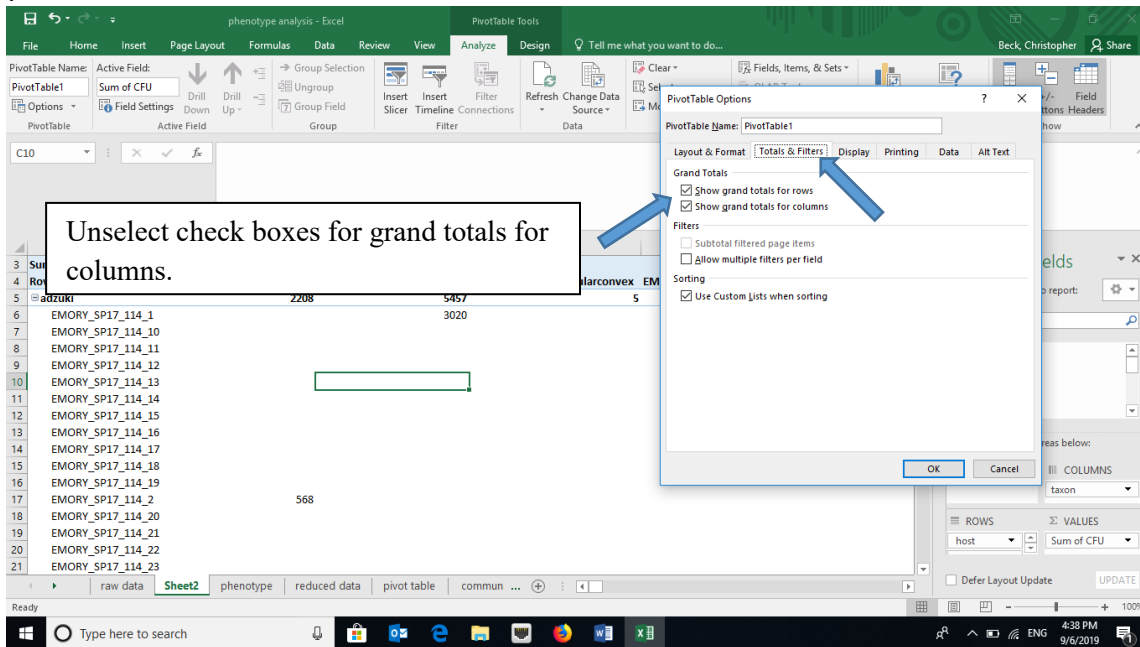
7. To get the treatment data to repeat for each sample, in the Design tab, select "Report Layout" and choose "Show in Tabular form" and "Repeat All Item Labels".
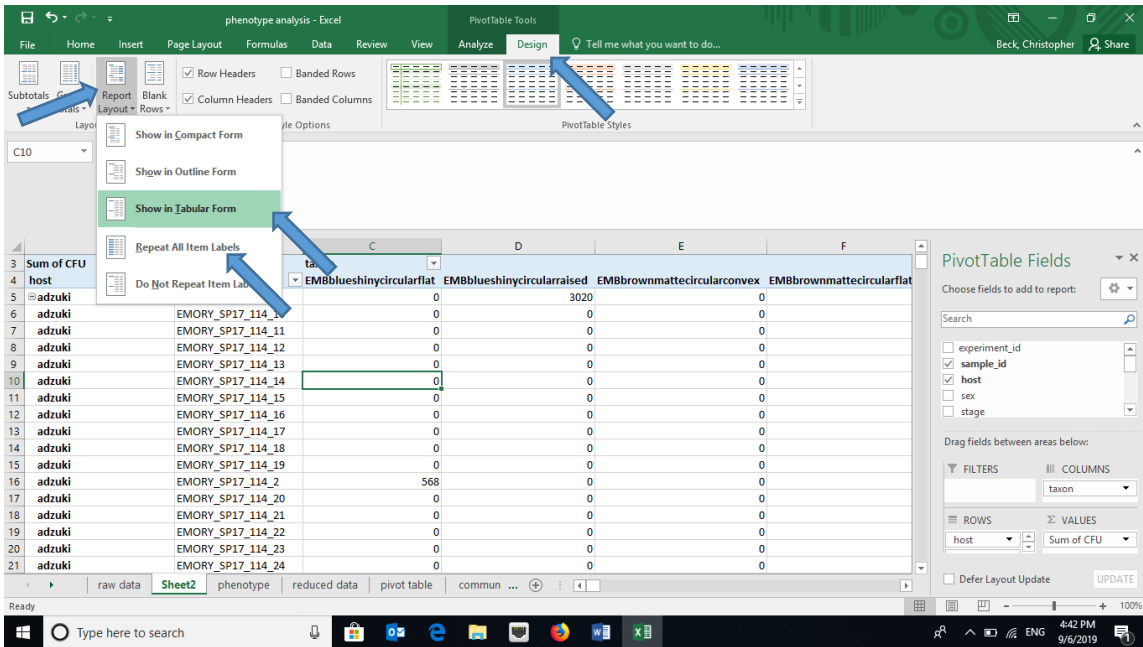
8. Copy and paste (as values) the pivot table to a new worksheet and remove any extra rows at the top. Each of the columns in this new worksheet represents a unique bacterial taxa. The exact phenotype doesn't matter, so we are going to rename them species_1, species_2, …. Title this worksheet tab "community data".
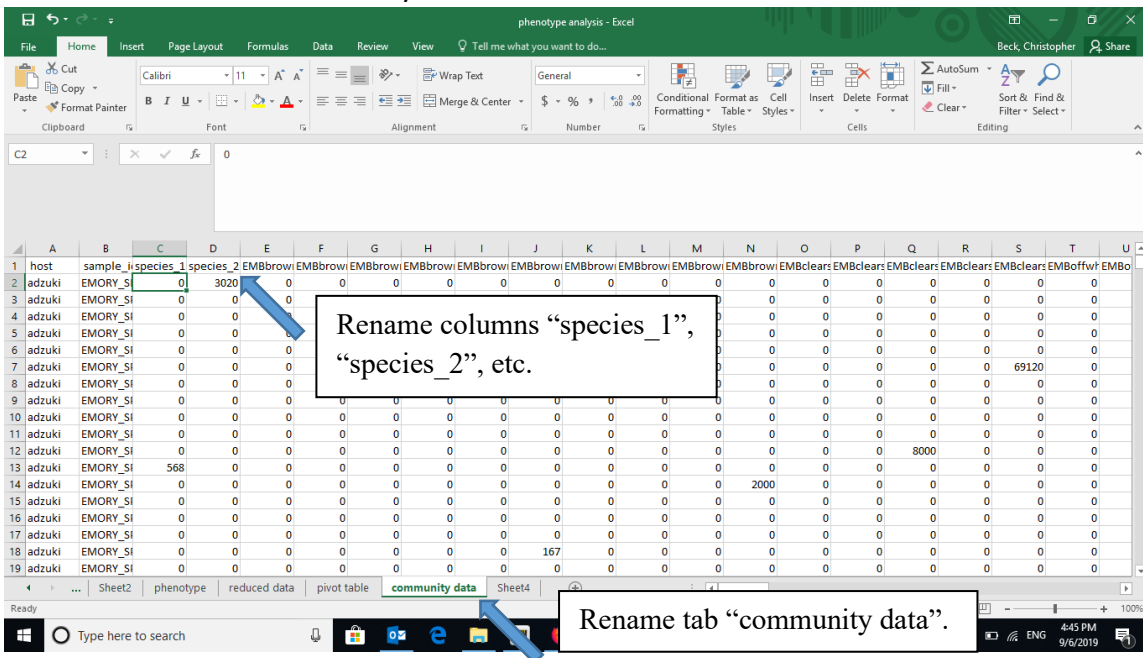
Rename columns "species_1", "species_2", etc.

Rename tab "community data".

Calculating diversity indices

1. Species (taxon) richness – the number of unique species (taxa) in a sample

a. Although you could manually count the number of cells with values greater than zero for each treatment, using the COUNTIF formula in Excel is easier (e.g., =COUNTIF(range,">0") where "range" the is range of cells in the spreadsheet containing the data, such as "C2:EW2").
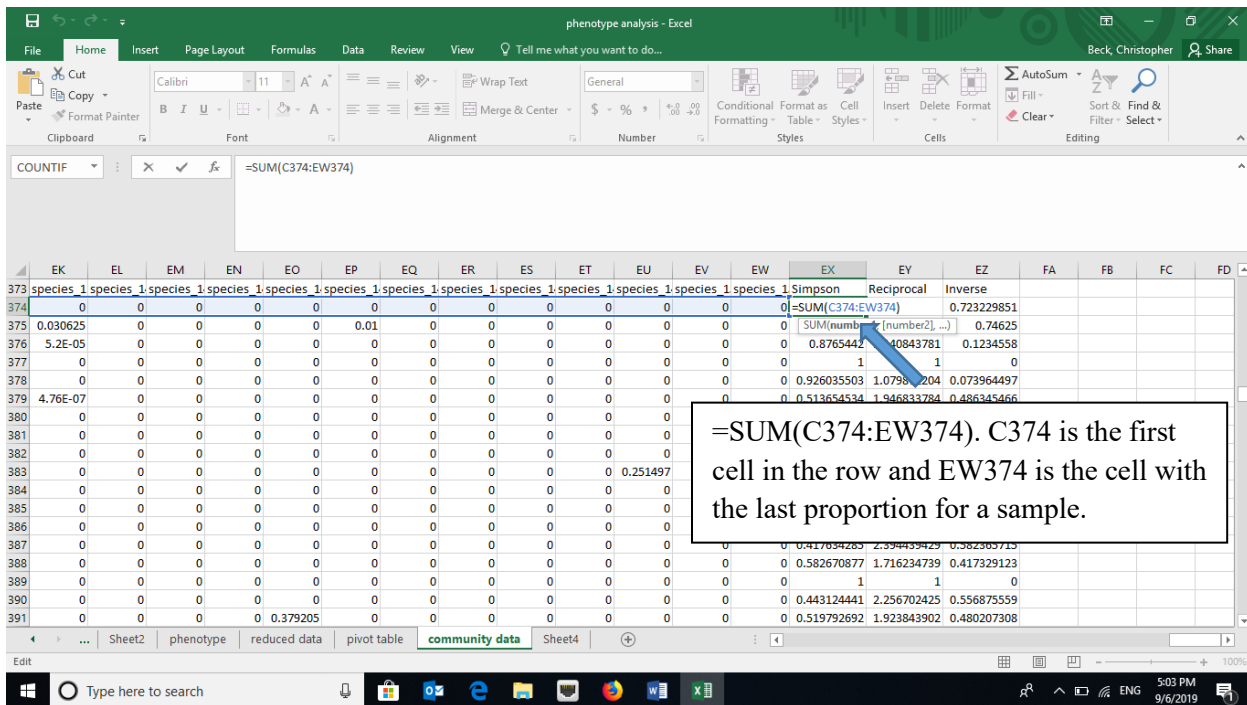

2. Simpson Index – the Simpson Index incorporates both species (taxon) richness and species (taxon) evenness.
   a. $D = \Sigma(n/N)^2$, where n=number of individuals of a particular species (taxon) and N=total number of individuals in a sample. D increases as diversity decreases, which is counterintuitive. A reciprocal or inverse index would be more intuitive and are easily calculated.
   b. Reciprocal Simpson = 1/D and scales so the maximum value is the species richness of a community.
   c. Inverse Simpson = 1-D and scales to a maximum value of 1.0.
   d. Create a new data array below the original using the same row labels (treatment variables) and the same column labels (species).

e. To calculate the proportion squared for each taxa, use the grand totals for each treatment. Using the Excel trick that $ before a column or row prevents Excel from iterating when copying a formula makes this easy. For example, =(C2/$EX2)^2. Copy the formula across the row and then down.
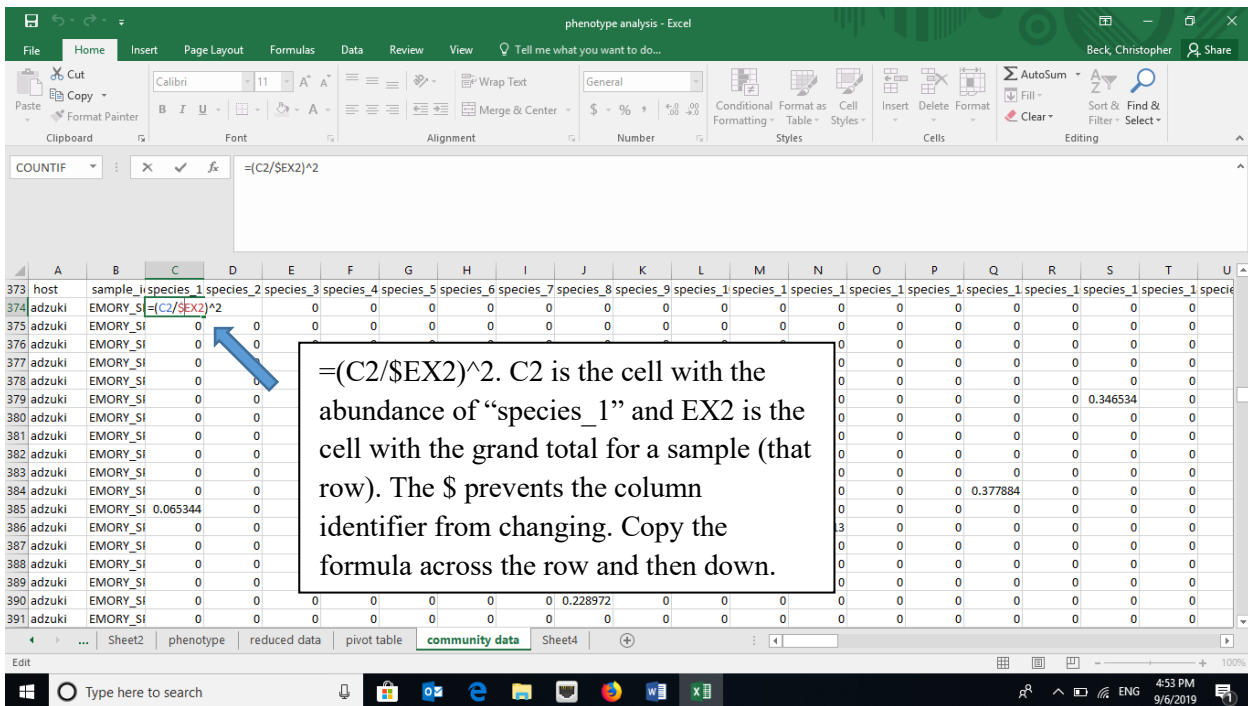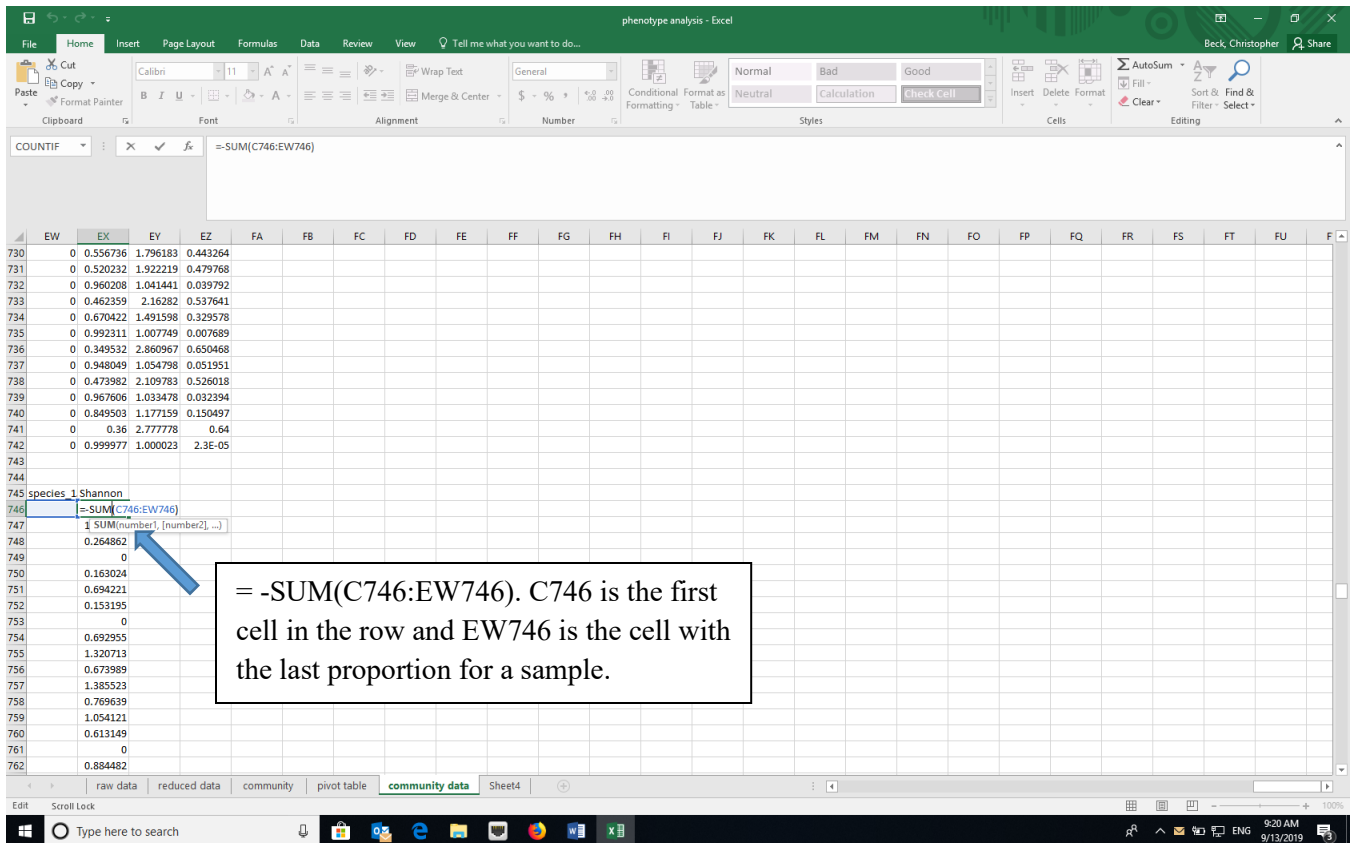

=(C2/$EX2)^2. C2 is the cell with the abundance of "species_1" and EX2 is the cell with the grand total for a sample (that row). The $ prevents the column identifier from changing. Copy the formula across the row and then down.

f. Calculate the sum of the proportions squared (=SUM in Excel for each row, a different microbial community) to calculate the Simpson Index.

12

=SUM(C374:EW374). C374 is the first cell in the row and EW374 is the cell with the last proportion for a sample.

g. Calculate the reciprocal (e.g., =1/EX374) and inverse Simpson (e.g., =1-EX374) using formulas in Excel.

3. Shannon-Weaver (Shannon-Weiner) Index – also incorporates species (taxon) richness and species (taxon) evenness
   a. H=-Σplnp, where p is the proportion of individuals of each species (taxon) in a community (i.e., n/N).
   b. Create a new data array below the original using the same row labels (treatment variables) and the same column labels (species).
   c. Using the grand totals for each community, calculate the proportions (plnp). Using the Excel trick that $ before a column or row prevents Excel from iterating when copying a formula makes this easy.
   d. Note that lnp is undefined if p=0, so you can use an "IF" statement in Excel. For example, =IF(C2>0,(C2/$EX2)*LN((C2/$EX2)),"")

COUNTIF =IF(C2>0,(C2/$EX2)*LN((C2/$EX2)),"")

=IF(C2>0,(C2/$EX2)*LN((C2/$EX2)),"").
C2 is the cell with the abundance of "species_1" and EX2 is the cell with the grand total for a sample. The $ prevents the column identifier from changing. Copy the formula across the row and then down.

e. Calculate the negative sum of the proportions (*p*ln*p*) (=-SUM in Excel for each row, a different microbial community) to calculate the Shannon-Weaver Index.

= -SUM(C746:EW746). C746 is the first cell in the row and EW746 is the cell with the last proportion for a sample.

Data Manipulation in R

1. Open RStudio and create a new project using the New Project option under File and select for the new project to be in an existing folder where your data are.
2. Install the following packages either using the Packages tab in RStudio or the command install.packages("name_of_package") in the console. Note that BiodiversityR requires QuartzX on a Mac. If you are using a MacOS and don't have QuartzX, install it first and restart your computer before install these packages.
   a. dplyr
   b. reshape2
   c. vegan
   d. BiodiversityR
   e. ggplot2

3. Load the packages listed above by clicking the checkboxes for the appropriate packages in the Packages tab or the command library("name_of_package") in the console.
4. Import the dataset ("community" that you created above in the Excel section) into RStudio.

Screenshot 1 (RStudio, Import Dataset dropdown):

R:/BeckLab/cbeck/MyFiles/bean beetle microbiome/ABLE 2019 mini - RStudio

File   Edit   Code   View   Plots   Session   Build   Debug   Profile   Tools   Help

Go to file/function   Addins

ABLE 2019 mini

Console   Terminal

R:/BeckLab/cbeck/MyFiles/bean beetle microbiome/ABLE 2019 mini/

R version 3.5.2 (2018-12-20) -- "Eggshell Igloo"
Copyright (C) 2018 The R Foundation for Statistical Computing
Platform: x86_64-w64-mingw32/x64 (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

Warning message:
File monitoring failed for project at "R:/BeckLab/cbeck/MyFiles/bean beetle microbiome/ABLE 2019 mini"
Error 2 (The system cannot find the file specified)
Features disabled: R source file indexing, Diagnostics
>

Environment   History   Connections

Import Dataset

From Text (base)...
From Text (readr)...
From Excel...
From SPSS...
From SAS...
From Stata...

Environment is empty

Files   Plots   Packages   Help   Viewer

Install   Update   Packrat

User Library

Name	Description	Version
abind	Combine Multidimensional Arrays	1.4-5
acepack	ACE and AVAS for Selecting Multiple Regression Transformations	1.4.1
applpack	Another Plot Package: 'Bagplots', 'Iconplots', 'Summaryplots', Slider Functions and Others	1.3.2
arm	Data Analysis Using Regression and Multilevel/Hierarchical Models	1.10-1
askpass	Safe Password Entry for R, Git, and SSH	1.1
assertthat	Easy Pre and Post Assertions	0.2.1
backports	Reimplementations of Functions Introduced Since R-3.0.0	1.1.3
base64enc	Tools for base64 encoding	0.1-3
BH	Boost C++ Header Files	1.69.0-1
Biobase	Biobase: Base functions for Bioconductor	2.42.0
BiocGenerics	S4 generic functions for Bioconductor	0.28.0
BiocManager	Access the Bioconductor Project Package Repository	1.30.4
BiocParallel	Bioconductor facilities for parallel evaluation	1.16.6
BiocVersion	Set the appropriate version of Bioconductor packages	3.8.0
BiodiversityR	Package for Community Ecology and Suitability Analysis	2.11-1
Biostrings	Efficient manipulation of biological strings	2.50.2
bitops	Bitwise Operations	1.0-6
broom	Convert Statistical Analysis Objects into Tidy Tibbles	0.5.2
callr	Call R from R	3.2.0
car	Companion to Applied Regression	3.0-2
carData	Companion to Applied Regression Data Sets	3.0-2
cellranger	Translate Spreadsheet Cell Ranges to Rows and Columns	1.1.0
checkmate	Fast and Versatile Argument Checks	1.9.1

1:03 PM   9/10/2019

Screenshot 2 (Import Excel Data dialog):

R:/BeckLab/cbeck/MyFiles/bean beetle microbiome/ABLE 2019 mini - RStudio

Import Excel Data

File/Url:
R:/BeckLab/cbeck/MyFiles/bean beetle microbiome/ABLE 2019 mini/phenotype analysis.xlsx   Browse...

Data Preview:

experiment_id	sample_id	host	sex	stage	media	color	gloss	form	elevation	CFU
EMORY_SP18	EMORY_SP18_116_5_2	adzuki	female	adult	PEA	offwhite	matte	circular	convex	4390
EMORY_SP17	EMORY_SP17_106_27	mung	female	adult	PEA	brown	matte	circular	raised	160000
EMORY_SP18	EMORY_SP18_116_3	adzuki	male	adult	PEA	white	shiny	circular	convex	420
EMORY_SP17	EMORY_SP17_110_41	mung	male	adult	PEA	white	shiny	circular	convex	
EMORY_SP17	EMORY_SP17_106_33	mung	female	adult	PEA	white	matte	circular	raised	
EMORY_SP17	EMORY_SP17_106_6	mung	female	adult	PEA	white	shiny	circular	raised	1000
EMORY_SP18	EMORY_SP18_110_6_10	pigeon	male	adult	PEA	white	shiny	circular	umbonate	
EMORY_SP17	EMORY_SP17_110_9	adzuki	male	adult	EMB	red	shiny	circular	raised	1000
EMORY_SP18	EMORY_SP18_106_2_10	BEP	male	adult	PEA	white	shiny	circular	raised	60
EMORY_SP18	EMORY_SP18_110_1_5	adzuki	female	adult	PEA	offwhite	shiny	circular	flat	140
EMORY_SP17	EMORY_SP17_110_23	mung	male	adult	PEA	yellow	shiny	circular	raised	4350
EMORY_SP18	EMORY_SP18_114_3_7	adzuki	male	adult	EMB	brown	shiny	circular	raised	20
EMORY_SP18	EMORY_SP18_110_7_4	pigeon	male	adult	PEA	offwhite	shiny	circular	flat	10
EMORY_SP17	EMORY_SP17_106_20	mung	female	adult	EMB	clear	shiny	circular	raised	120
EMORY_SP18	EMORY_SP18_110_6_5	pigeon	male	adult	PEA	yellow	matte	circular	raised	200
EMORY_SP18	EMORY_SP18_106_7_5	adzuki	female	adult	EMB	red	shiny	circular	raised	6
EMORY_SP18	EMORY_SP18_110_7_2	pigeon	male	adult	PEA	white	shiny	circular	raised	3920
EMORY_SP18	EMORY_SP18_116_6_7	pigeon	male	adult	PEA	white	shiny	circular	raised	2
EMORY_SP18	EMORY_SP18_110_1_11	adzuki	female	adult	PEA	white	shiny	circular	convex	100
EMORY_SP18	EMORY_SP18_114_3_8	pigeon	male	adult	EMB	red	shiny	circular	raised	1220
EMORY_SP17	EMORY_SP17_110_24	mung					irregular		umbonate	2720

Previewing first 50 entries.

Import Options:

Name: phenotype   Max Rows:
Sheet: community
Range: A1:D10   NA:

is <- read_excel("phenotype analysis.xlsx",
                 munity")
nalysis)

Reading Excel files using readxl

Import   Cancel

Callout box (right): Browse to select file from your computer

Callout box (center): Set Name to "phenotype" and use Sheet dropdown to select appropriate worksheet from Excel file.

1:12 PM   9/10/2019

17

5. Attach the imported dataset to the dataframe using the attach command in the console (attach(phenotype))
6. Create a community matrix for each sample. Since each "taxon" is defined by the combination of media type and the four phenotypic characters, we can use the dcast function in the reshape2 library, using the following command.

```
> comm_pheno=dcast(phenotype,sample_id~media+color+gloss+form+elevation,value.var = "CFU",fun.aggregate = sum)
```

7. The first column in the resulting data table is the sample_id. The sample_id needs to become the row name and then deleted, using the following commands.

```
> row.names(comm_pheno)<-(comm_pheno$sample_id)
> comm_pheno<-comm_pheno[,-1]
```

8. Now, we need to create an environment matrix with the sample metadata. First, we create a data table with the sample names and sample metadata, which are in the second through fourth columns of the "phenotype" dataframe. Second, we remove all of the duplicate values for the sample_id using the distinct function in the dplyr package. Third, we need the sample_id to become the row name. (Note that this causes an error message and for some reason if you delete the column with the row name, the row names disappear. In this case, we do not need to delete the sample_id column because we can specify the factor of interest.) Last, we need to set host and sex as factors.

```
> pheno_meta<-phenotype[,2:4]
> pheno_meta<-pheno_meta %>% distinct(sample_id, .keep_all = TRUE)
> row.names(pheno_meta)<-(pheno_meta$sample_id)
> pheno_meta$host<-as.factor(pheno_meta$host)
> pheno_meta$sex<-as.factor(pheno_meta$sex)
```

You can ignore the error message about setting row names on a tibble being deprecated.

Species accumulation curves

Species accumulation curves are often used to visualize whether all of the taxa in a community have been sampled. As the number of samples increases, the total number of unique species sampled should increase. However, the relationship between the number of samples and the number of unique species should asymptote. If so, we can say that we have sampled the majority of species in the community. However, if the slope of the relationship is steep, this suggests that the community is incompletely sampled.

For each treatment separately, create the data for the species accumulation curve using:

```
> Accum.label1<-specaccum(comm_pheno,method='exact',permutations = 100, conditioned=TRUE,gamma='jack1',w=NULL, subset=pheno_meta$factor_variable=="factor")
```

factor_variable=="factor" refers to the factor being evaluated such as host in our dataset, and the "factor" is one state of that variable. For example, host=="mung" would do a species accumulation curve for microbiome communities of beetles that emerged from mung beans. Note that two equals signs are necessary

You need to run the command above for each treatment group separately. Change the label and factor terms appropriately for additional analyses.

      a. Plot the first species accumulation curve using:

> plot(Accum.label1,col="red")

      b. Plot each additional species accumulation curve using:

> plot(Accum.label2, add=TRUE, col="blue")

If the second curve extends beyond the y-axis, replot the curves in the opposite order (i.e., plot curve 2 first and then curve 1).

Calculating diversity indices

You can calculate the diversity indices described above in the Excel exercise using functions in the BiodiversityR package.

1. Species Richness
    > diversityresult(comm_pheno,index="richness",method="each site")

2. Simpson

    > diversityresult(comm_pheno,index="Simpson",method="each site")

    This calculates the inverse Simpson described above.

    > diversityresult(comm_pheno,index="inverseSimpson",method="each site")

    This calculates the reciprocal Simpson described above (confusing that it is called in the inverseSimpson).

3. Shannon
    > diversityresult(comm_pheno,index="Shannon",method="each site")

4. To calculate all of the biodiversity indices and merge them with the metadata for future analysis. You can then use this dataset for analysis of differences between treatments with t-tests, ANOVAs, or their non-parametric equivalents.

> pheno_diversity<-diversityvariables(comm_pheno,pheno_meta)

Because the diversityvariables function calculates a range of diversity indices, sometimes an error occurs because a particular index cannot be calculated with the dataset (e.g., requires taking log of zero). If this is the case, you can use the diversityresult function above and place the results in a dataframe. Then, you can combine the dataframes with the metadata using the cbind function.

> Simpson<-diversityresult(comm_pheno,index="Simpson",method="each site")
> Shannon<-diversityresult(comm_pheno,index="Shannon",method="each site")
> pheno_diversity<-cbind(pheno_meta,Simpson,Shannon)

Calculating community similarity (distance)

Sometimes we are interested in how similar (or different) two communities are based on what species (taxa) are present and the relative abundance of those species (taxa) in the two communities. One of the most common measures of distance is the Bray Curtis Dissimilarity. Similarity can be measured as 1-BC.

$$BC_{ij} = 1 - \frac{2C_{ij}}{S_i \div S_j}$$

Where:

- i & j are the two samples,
- S_i is the total number of specimens counted in sample i,
- S_j is the total number of specimens counted in sample j,
- C_{ij} is the sum of only the lesser counts for each taxa found in both sites.

Although Bray-Curtis Dissimilarity is often used in community ecology, it is not robust to incomplete sampling of the community (all taxa are not sampled) or unbalanced sampling (all treatments are not equally sampled). An alternative is the Morista-Horn Index of Dissimilarity (1-C_H). Morista-Horn Index of Similarity is

$$C_H = \frac{2 \sum_{i=1}^{D_{12}} \frac{X_i}{n} \frac{Y_i}{m}}{\sum_{i=1}^{D_1} \left(\frac{X_i}{n}\right)^2 + \sum_{i=1}^{D_2} \left(\frac{Y_i}{m}\right)^2}$$

Where

- D_1=number of taxa in sample 1
- D_2=number of taxa in sample 2
- D_{12}=number of taxa in shared in both communities
- X_i=number of individuals of taxon i in sample 1
- Y_i=number of individuals of taxon i in sample 2
- n=total number of individuals in sample 1
- m=total number of individuals in sample 2

So that X_i/n and Y_i/m are proportion of individuals of taxon i in each of the samples (communities).

To produce a matrix of all of the pair-wise distances between samples using the Bray Curtis index of dis tance, use the   following command.

```
> vegdist(comm_pheno, method="bray", binary=FALSE, diag=FALSE, upper=FALSE)
```

To produce a matrix of all of the pair-wise distances between samples using the Morista-Horn index of distance.

```
> vegdist(comm_pheno, method="horn", binary=FALSE, diag=FALSE, upper=FALSE)
```

How different (similar) are the communities?

Adonis is an approach to testing whether communities differ based on a treatment. It is the equivalent of an analysis of variance, but comparing distance matrices. "community_adonis" stores the results of the analysis, "comm_pheno" is the community matrix, "factor_variable" is the treatment (e.g., host), and "pheno_meta" is the name of the dataset that has the treatment data for each community. In the code below, we use Morista-Horn to estimate distance between communities.

```
> community_adonis<-adonis2(comm_pheno ~ factor_variable, data = pheno_meta, method="horn")
> community_adonis
```

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This study is based on Blumer LS, Beck CW 2020. **Introducing community ecology and data skills with the bean beetle microbiome project**. Advances in Biology Laboratory Education 41.

Microbial Community Analysis Using Colony-based Sequencing Database

Student Handout

Objectives

- Manipulate large datasets to conduct community-level ecological analyses
- Use community-level data to address questions about insect microbiomes
- Use Excel or RStudio programs to calculate community ecology variables
- Compare microbial community using community ecology variables

Introduction

Microbiomes are the communities of microbes (bacteria, viruses, fungi and archaea) living symbiotically with all metazoans. In the past decade, both interest and research on microbiomes, including their implications for human health, have increased dramatically (Christian *et al.* 2015, Costello *et al.* 2012, McFall-Ngai *et al*. 2013, The Human Microbiome Consortium 2012, Young 2016). Insects have been used as model species to study the importance of microbiomes, because of their ease of use and the fact that microbial communities play diverse roles in insects (Engel and Moran 2013).

The data that are collected in any microbiome study consists of lists of the taxonomic units identified and their abundance. The same types of data are evaluated in an ecological community analysis, but now the communities are the collections of microbes that constitute different microbiomes. The community variables, "species" richness and relative abundance, are the same and the statistical methods used to compare communities, diversity and difference indices, also are the same. Perhaps the simplest measure of community structure used by ecologists is "species" or taxon richness, a count of the number of unique taxa in a sample. However, species richness does not consider the relative abundance of species in a community. Imagine two communities with five different species. In one community, all of the species have the same relative abundance. In the other community, one species dominates comprising 95% of individuals in the community. The other four species are very rare. Based on species richness as a measure of community structure, these two communities are the same, although they are clearly very different. As a result, ecologists use other species diversity indices that consider both the number of species and the relative abundance of species in a community. Two common indices are the Simpson Index and the Shannon-Weaver Index. Communities with greater numbers of species and higher evenness (i.e., similar relative abundance of species within a community) are considered more diverse. Finally, measures of species richness and species diversity do not consider the identity of species in a community. So, communities could have the same level of species diversity, but have completely different species. Measure of community similarity, such as the Bray-Curtis Index, compare the similarity (or dissimilarity) between two communities based on the identity of species in the communities, as well as their relative abundances. For more information on indices of species diversity and measures of community similarity, see Krebs (1999).

In this study, bean beetle gut microbiome data were collected by undergraduate students using the protocols developed by Cole *et al*. (2018). Three types of data were collected: colony phenotypes from cultured bacteria, 16s rRNA gene sequencing of specific bacterial colonies, and whole community 16s rRNA gene sequencing, but we will limit our analyses to the colony phenotype and colony-based 16s data.

Questions

Using data from the colony-based sequencing database and the analyses described below, answer the following questions.

1. Which taxa are most prevalent in the bacterial communities in bean beetles?
2. Do the most prevalent taxa vary based on host bean type?
3. Based on the diversity indices that you calculated, which treatment had the highest (lowest) diversity?
4. Does the answer depend on the measure of species (taxon) diversity that you use?
5. Is there a relationship between number of samples and taxonomic diversity? If so, what might explain this?
6. Which communities are most similar (different)?
7. Do your answers to the questions above depend on the taxonomic level of analysis?

Database description

This database contains data for the microbial community of bean beetles based on 16s rRNA sequencing of individual bacterial colonies cultured from bean beetle homogenates plated on different media. Since only a small number of colonies are sequenced from each plate, the data do not represent the entire microbial community for a particular sample. However, qualitative comparisons can be made based on bean host species, sex of beetle, and other variables.

Access the database at https://www.beanbeetles.org/microbiome/colony-database-search/.

The database allows you to limit your search by bean host type, sex, life cycle stage, media on which bacteria were grown, colony phenotype, and bacterial taxonomy. Since we are interested in making comparisons between bacterial communities based on host species and sex, we want to download the entire database. Clicking "Submit" without limiting the search will allow you to view all of the data.

Downloading Data

While we can view the data on the website, we want to download the data to manipulate. Click the download link to download a csv file with the data. Then, save the file as an Excel file (name the file "colony-based sequence data") and rename the tab "raw data."

Double click tab and rename as "raw data"

Data Reduction

1. Make a copy of the raw data in a new sheet using the sheet copy function in Excel (right click on the tab and select "Move or copy" and rename the tab ("reduced raw data").

Select "Move to end" and check "Create a copy"

2. In the "reduced raw data" sheet, delete any columns that we don't need, such as the colony phenotype (color, gloss, form, elevation) and sequence data columns. The "reduced raw data" sheet is the data source if you choose to analyze these data in RStudio. Additional data manipulation and formatting (below) is required if you choose to analyze these data in Excel.

Data manipulation

1. We need to consolidate the data for each host species, each sex, or the combination of the two by the bacterial taxa. The easiest way to do this is with the Pivot Table function in Excel.
2. When clicked on a cell within the data, create a Pivot Table (Insert -> Pivot Table OR Data -> Summarize with Pivot Table). Make sure that the data source includes the top row (the cell range should include "A1"), which has the column headings. Click OK to create the pivot table in a new worksheet and label the tab "pivot table".

Make sure that the cell range includes A1

3. Set the treatment(s) that you are interested (for example, host species) in as the rows and the bacterial taxonomic level you are interested in as the columns. The Values should be a COUNT of the sample_id, as each row in the dataset represents a single sample.

Drag treatment of interest (e.g., host) to ROWS, taxonomic level (e.g., genus) to COLUMNS, and sample_id to VALUES

4. You can add zeros to all of the empty cells in the Pivot Table using the Options menu and remove the Grand totals for Columns using the Options menu or the Design tab depending of your version of Excel. (You want to keep the Grand totals for rows to calculate diversity indices.)

Unselect checkbox for "Show grand totals for columns"

5. You can remove the "blanks" column using the Column labels dropdown (located at upper left of the sheet) and unselecting "blank".

Unselect checkbox for "blanks"
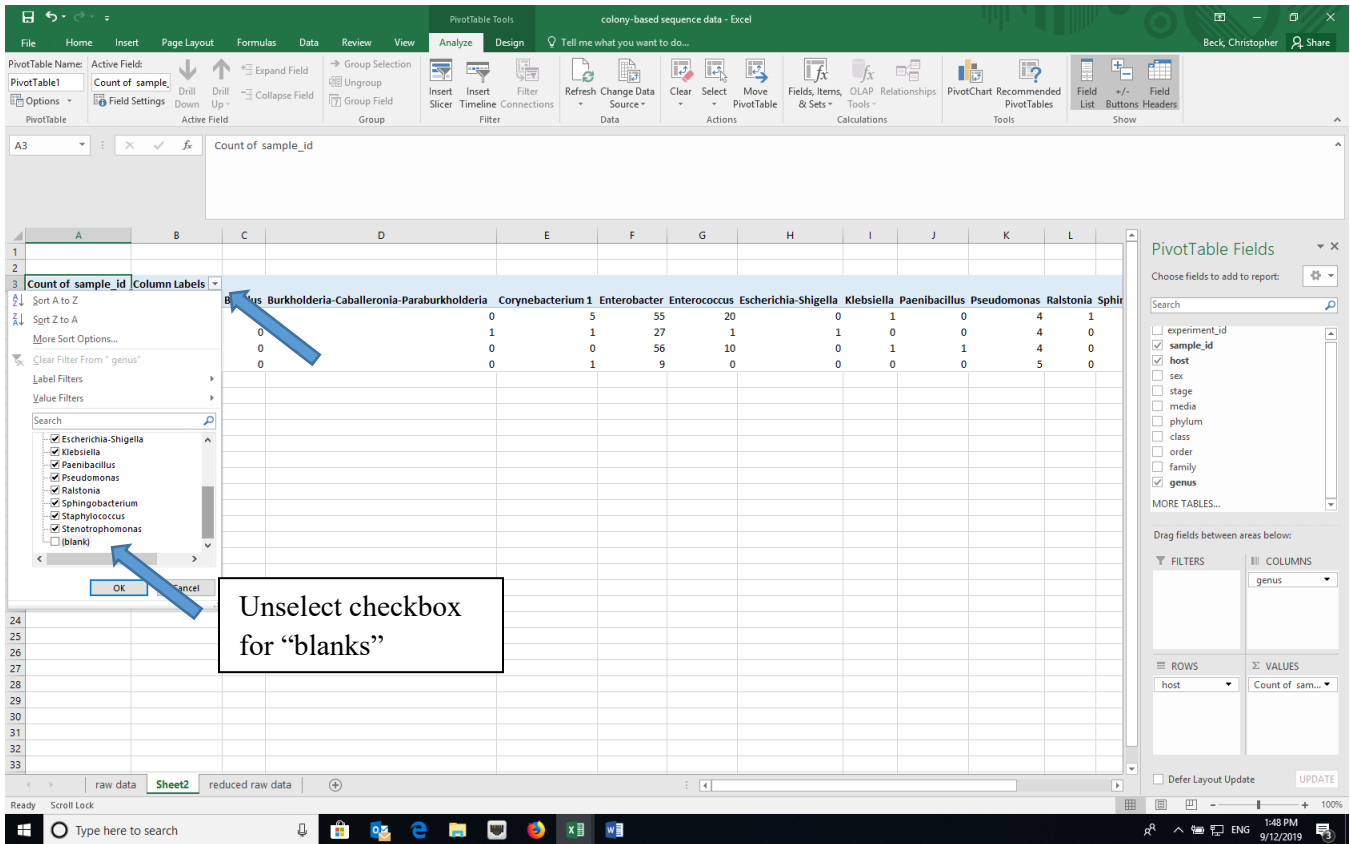
6. If you selected more than one treatment for the rows, you can get the treatment data to repeat for each sample. In the Design tab, select "Report Layout" and choose "Show in Tabular form" and "Repeat All Item Labels".
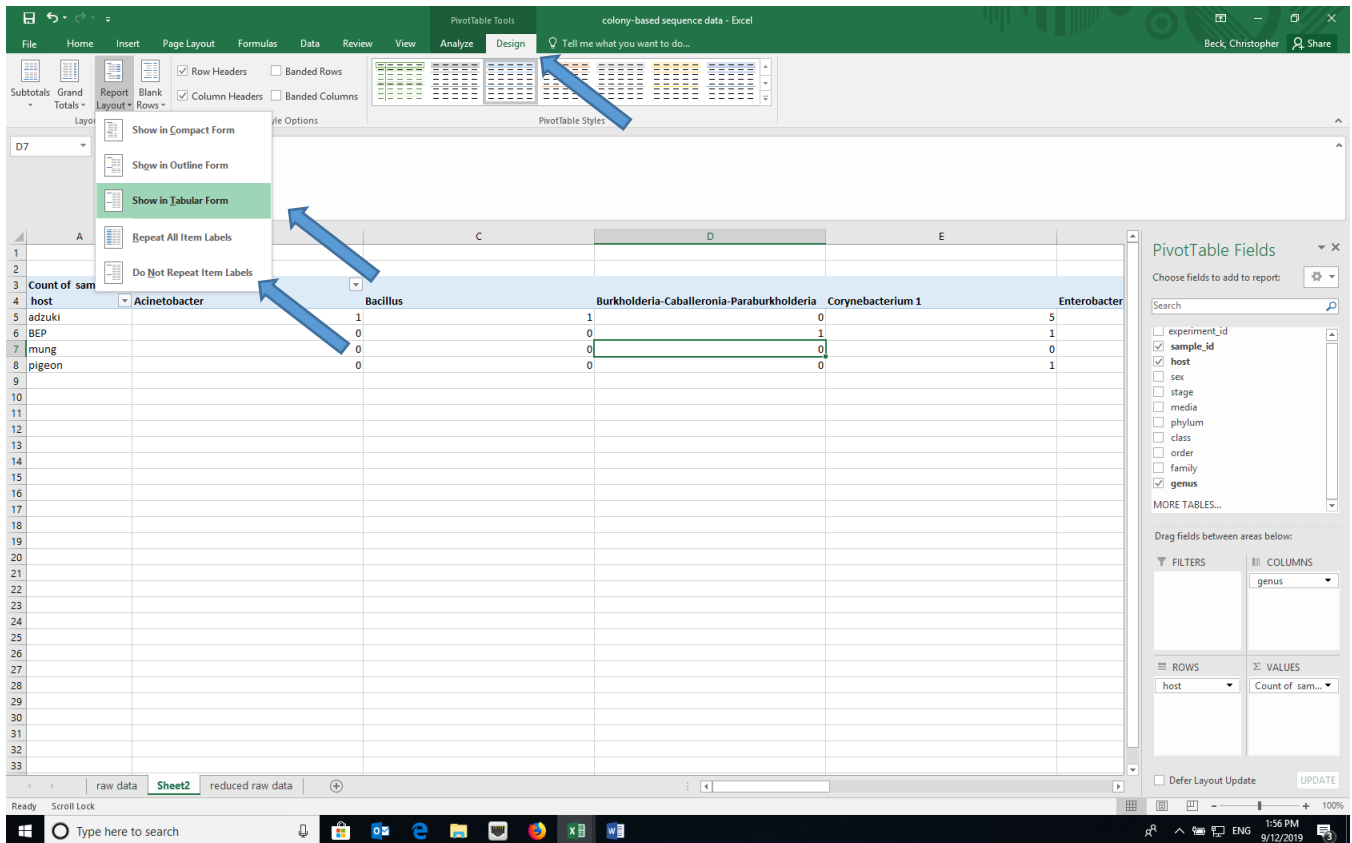
7. Copy and paste (as values) the pivot table to a new worksheet and remove any extra rows at the top. The top row should now have the taxa names. Name this tab "analysis". Conduct the community ecology analyses that follow in Excel on the "analysis" sheet.

host	Acinetoba	Bacillus	Burkholde	Corynebac	Enterobac	Enterococ	Escherichi	Klebsiella	Paenibaci	Pseudom	Ralstonia	Sphingoba	Staphyloc	Stenotrop	Grand Total
adzuki	1	1	0	5	55	20	0	1	0	4	1	1	64	1	154
BEP	0	0	1	1	27	1	1	0	0	4	0	0	50	2	87
mung	0	0	0	0	56	10	0	1	1	4	0	0	22	0	94
pigeon	0	0	0	1	9	0	0	0	0	5	0	0	7	0	22

Calculating diversity indices

1. Species (taxon) richness – the number of unique species (taxa) in a sample
   a. Although you could manually count the number of cells with values greater than zero for each treatment, using the COUNTIF formula in Excel is easier (e.g., =COUNTIF(range,">0")). Where "range" is the cell range in the datasheet, for example "C2:M2", a single row or treatment.
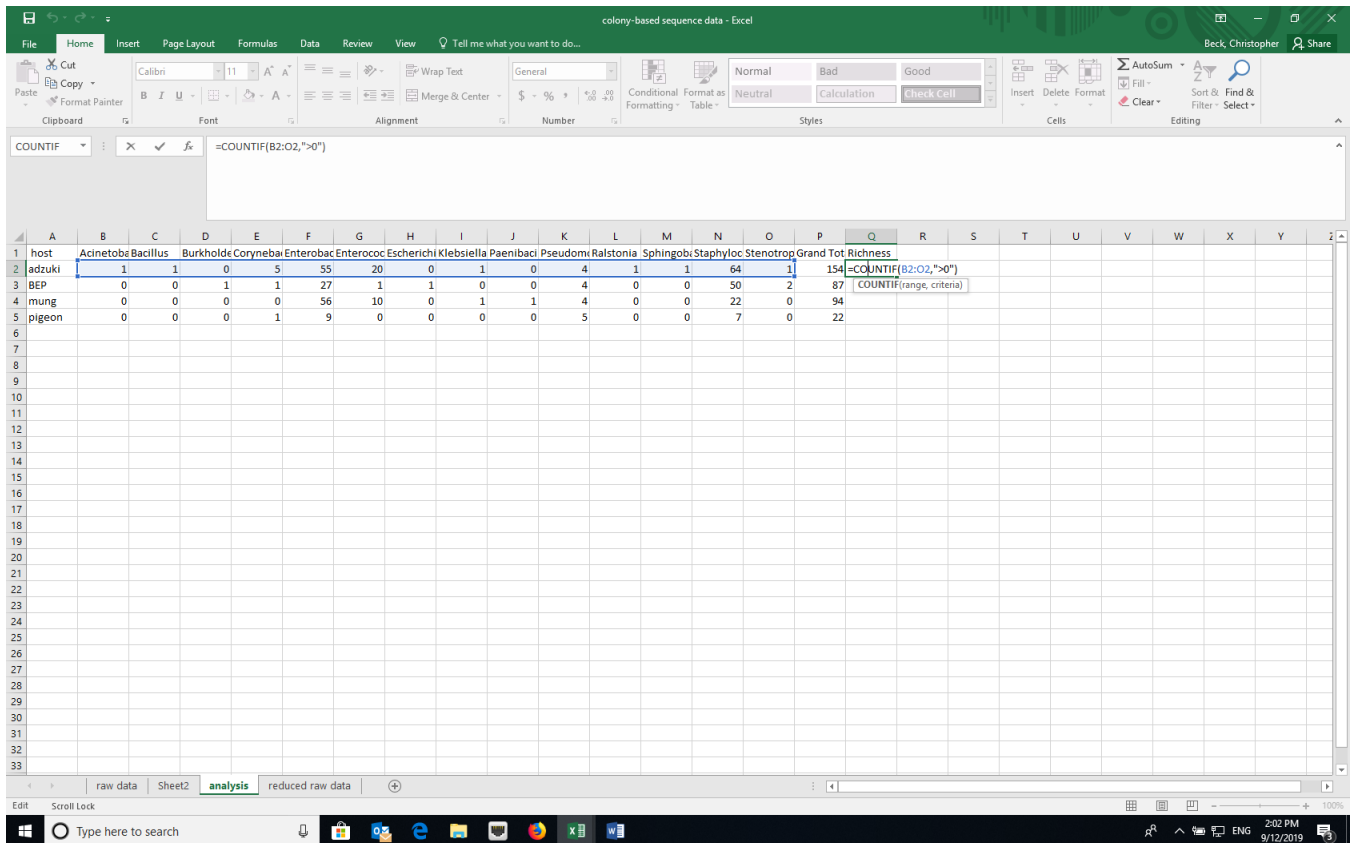
2. Simpson Index – the Simpson Index incorporates both species (taxon) richness and species (taxon) evenness.

   a. $D = \Sigma(n/N)^2$, where n=number of individuals of a particular species (taxon) and N=total number of individuals in a sample. D increases as diversity decreases, which is counterintuitive. A reciprocal or inverse index would be more intuitive and are easily calculated.

   b. Reciprocal Simpson = 1/D and scales so the maximum value is the species richness of a community.

   c. Inverse Simpson = 1-D and scales to a maximum value of 1.0.

   d. Create a new data array below the original using the same row labels (treatment variables) and the same column labels (bacterial taxa).

host	Acinetoba	Bacillus	Burkholde	Corynebac	Enterobac	Enterococc	Escherichi	Klebsiella	Paenibaci	Pseudom	Ralstonia	Sphingoba	Staphyloc	Stenotrop	Grand Tot	Richness
adzuki	1	1	0	5	55	20	0	1	0	4	1	1	64	1	154	11
BEP	0	0	1	1	27	1	1	0	0	4	0	0	50	2	87	
mung	0	0	0	0	56	10	0	1	1	4	0	0	22	0	94	
pigeon	0	0	0	1	9	0	0	0	0	5	0	0	7	0	22	

host	Acinetoba	Bacillus	Burkholde	Corynebac	Enterobac	Enterococc	Escherichi	Klebsiella	Paenibaci	Pseudom	Ralstonia	Sphingoba	Staphyloc	Stenotrophomonas
adzuki														
BEP														
mung														
pigeon														

e. To calculate the proportion squared for each taxa, use the grand totals for each treatment. Using the Excel trick that $ before a column or row prevents Excel from iterating when copying a formula makes this easy. For example, =(C2/$P2)^2. Copy the formula across the row and then down.

The image shows a screenshot of Excel ("colony-based sequence data - Excel") displaying an "analysis" worksheet with abundance data and proportion-squared calculations.

The data table (rows 1–5):

host	Acinetobacter	Bacillus	Burkholderia	Corynebacterium	Enterobacter	Enterococcus	Escherichia	Klebsiella	Paenibacillus	Pseudomonas	Ralstonia	Sphingobacterium	Staphylococcus	Stenotrophomonas	Grand Total	Richness
adzuki	1	1	0	5	55	20	0	1	0	4	1	1	64	1	154	11
BEP	0	0	1	1	27	1	1	0	0	4	0	0	50	2	87	8
mung	0	0	0	0	56	10	0	1	1	4	0	0	22	0	94	6
pigeon	0	0	0	1	9	0	0	0	0	5	0	0	7	0	22	4

The second table (rows 8–9):

host	Acinetobacter	Bacillus	Burkholderia	Corynebacterium	Enterobacter	Enterococcus	Escherichia	Klebsiella	Paenibacillus	Pseudomonas	Ralstonia	Sphingobacterium	Staphylococcus	Stenotrophomonas
adzuki	=(B2/$P2)^2	4.22E-05	0	0.001054	0.127551	0.016866	0	4.22E-05	0	0.000675	4.22E-05	4.22E-05	0.17271	4.22E-05
BEP														
mung														
pigeon														

Formula bar: =(B2/$P2)^2

Callout box text:

=(B2/$P2)^2. B2 is the cell with the abundance of the first taxon and P2 is the cell with the grand total for a treatment. The $ prevents the column identifier from changing. Copy the formula across the row and then down.

f. Calculate the sum of the proportions squared (=SUM in Excel) to calculate the Simpson Index.

host	Acinetobacte	Bacillus	Burkholde	Coryneba	Enterobac	Enterococ	Escherichi	Klebsiella	Paenibaci	Pseudome	Ralstonia	Sphingobi	Staphyloc	Stenotrop	Grand Tot	Richness
adzuki	1	1	0	5	55	20	0	1	0	4	1	1	64	1	154	11
BEP	0	0	1	1	27	1	1	0	0	4	0	0	50	2	87	8
mung	0	0	0	0	56	10	0	1	1	4	0	0	22	0	94	6
pigeon	0	0	0	1	9	0	0	0	0	5	0	0	7	0	22	4

host	Acinetobacte	Bacillus	Burkholde	Coryneba	Enterobac	Enterococ	Escherichi	Klebsiella	Paenibaci	Pseudome	Ralstonia	Sphingobi	Staphyloc	Stenotrop	Simpson	Reciprocal	Inverse
adzuki	4.21656E-05	4.22E-05	0	0.001054	0.127551	0.016866	0	4.22E-05	0	0.000675	4.22E-05	4.22E-05	0.17271	4.22E-05	=SUM(B9:O9)		0.680891
BEP	0	0	0.000132	0.000132	0.096314	0.000132	0.000132	0	0	0.002114	0	0	0.330295	0.000528	SUM(number1, [number2], …)	21
mung	0	0	0	0.354912	0.011317	0	0.000113	0.000113	0.001811	0	0	0.054776	0	0.423042	…8309	0.576958
pigeon	0	0	0	0.002066	0.167355	0	0	0	0.051653	0	0	0.10124	0	0.322314	3.10…	0.677686

=SUM(C9:O9). C9 is the first cell in the row and O9 is the cell with the last proportion for a treatment.

g. Calculate the reciprocal (e.g., =1/P9) and inverse Simpson (e.g., =1-P9) using formulas in Excel.

3. Shannon-Weaver (Shannon-Weiner) Index – also incorporates species (taxon) richness and species (taxon) evenness
   a. $H = -\Sigma p \ln p$, where p is the proportion of individuals of each bacterial taxon in a community (i.e., n/N).
   b. Create a new data array below the original using the same row labels (treatment variables) and the same column labels (species).
   c. Using the grand totals for each treatment, calculate the proportions ($p \ln p$). Using the Excel trick that $ before a column or row prevents Excel from iterating when copying a formula makes this easy.
   d. Note that $\ln p$ is undefined if $p=0$, so you can use an "IF" statement in Excel. For example, =IF(B2>0,(B2/$P2)*LN((B2/$P2)),"")

COUNTIF   =IF(B2>0,(B2/$P2)*LN((B2/$P2)),"")

	host	Acinetobacte	Bacillus	Burkholde	Corynebac	Enterobac	Enterococ	Escherichi	Klebsiella	Paenibaci	Pseudom	Ralstonia	Sphingoba	Staphyloc	Stenotrop	Grand Tot	Richness
1	host															Grand Tot	Richness
2	adzuki	1	1	0	5	55	20	0	1	0	4	1	1	64	1	154	11
3	BEP	0	0	1	1	27	1	1	0	0	4	0	0	50	2	87	8
4	mung	0	0	0	0	56	10	0	1	1	4	0	0	22	0	94	6
5	pigeon	0	0	0	1	9	0	0	0	0	5	0	0	7	0	22	4

	host	Acinetobacte	Bacillus	Burkholde	Corynebac	Enterobac	Enterococ	Escherichi	Klebsiella	Paenibaci	Pseudom	Ralstonia	Sphingoba	Staphyloc	Stenotrop	Simpson	Reciprocal	Inverse
8	host															Simpson	Reciprocal	Inverse
9	adzuki	4.21656E-05	4.22E-05	0	0.001054	0.127551	0.016866	0	4.22E-05	0	0.000675	4.22E-05	4.22E-05	0.17271	4.22E-05	0.319109	3.1337209	0.680891
10	BEP	0	0	0.000132	0.000132	0.096314	0.000132	0.000132	0	0	0.002114	0	0	0.330295	0.000528	0.429779	2.3267753	0.570221
11	mung	0	0	0	0	0.354912	0.011317	0	0.000113	0.000113	0.001811	0	0	0.054776	0	0.423042	2.3638309	0.576958
12	pigeon	0	0	0	0.002066	0.167355	0	0	0	0	0.051653	0	0	0.10124	0	0.322314	3.1025641	0.677686

	host	Acinetobacte	Bacillus	Burkholde	Corynebac	Enterobac	Enterococ	Escherichi	Klebsiella	Paenibaci	Pseudom	Ralstonia	Sphingoba	Staphyloc	Stenotrop	Shannon
14	host															Shannon
15	adzuki	=IF(B2>0,(B2/$P2)*LN((B2/$P2)),"")				-0.36772	-0.26509		-0.03271		-0.09482	-0.03271	-0.03271	-0.36491	-0.03271	1.400077
16	BEP	IF(logical_test, [value_if_true], [value_if_false])				-0.36313	-0.05133	-0.05133			-0.14159			-0.31832	-0.08673	1.115101
17	mung					-0.30856	-0.23837		-0.04833	-0.04833	-0.13434			-0.33989		1.11783
18	pigeon				-0.1405	-0.36565					-0.33673			-0.36436		1.207243

=IF(B2>0,(B2/$P2)*LN((B2/$P2)),""). B2 is the cell with the abundance of the first taxon and P2 is the cell with the grand total for a treatment. The $ prevents the column identifier from changing. Copy the formula across the row and then down.
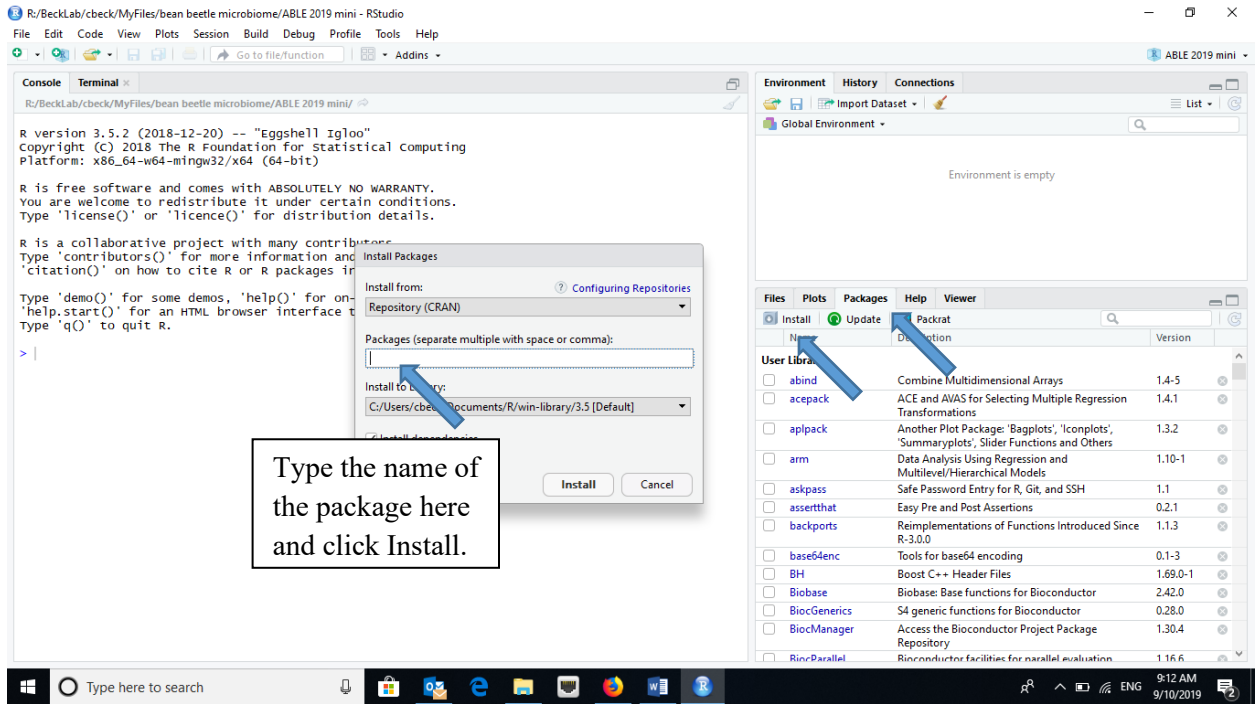
raw data | Sheet2 | **analysis**

e. Calculate the negative sum of the proportions ($p\ln p$) (=-SUM in Excel for each row, a different microbial community) to calculate the Shannon-Weaver Index.

COUNTIF =-SUM(B15:O15)

host	Acinetobacter	Bacillus	Burkholderia	Corynebacterium	Enterobacter	Enterococcus	Escherichia	Klebsiella	Paenibacillus	Pseudomonas	Ralstonia	Sphingobacterium	Staphylococcus	Stenotrophomonas	Grand Total	Richness
adzuki	1	1	0	5	55	20	0	1	0	4	1	1	64	1	154	11
BEP	0	0	1	1	27	1	1	0	0	4	0	0	50	2	87	8
mung	0	0	0	0	56	10	0	1	1	4	0	0	22	0	94	6
pigeon	0	0	0	1	9	0	0	0	0	5	0	0	7	0	22	4

host	Acinetobacter	Bacillus	Burkholderia	Corynebacterium	Enterobacter	Enterococcus	Escherichia	Klebsiella	Paenibacillus	Pseudomonas	Ralstonia	Sphingobacterium	Staphylococcus	Stenotrophomonas	Simpson	Reciprocal	Inverse
adzuki	4.21656E-05	4.22E-05	0	0.001054	0.127551	0.016866	0	4.22E-05	0	0.000675	4.22E-05	4.22E-05	0.17271	4.22E-05	0.319109	3.1337209	0.680891
BEP	0	0	0.000132	0.000132	0.096314	0.000132	0.000132	0	0	0.002114	0	0	0.330295	0.000528	0.429779	2.3267753	0.570221
mung	0	0	0	0	0.354912	0.011317	0	0.000113	0.000113	0.001811	0	0	0.054776	0	0.423042	2.3638309	0.576958
pigeon	0	0	0	0.002066	0.167355	0	0	0	0	0.051653	0	0	0.10124	0	0.322314	3.1025641	0.677686

host	Acinetobacter	Bacillus	Burkholderia	Corynebacterium	Enterobacter	Enterococcus	Escherichia	Klebsiella	Paenibacillus	Pseudomonas	Ralstonia	Sphingobacterium	Staphylococcus	Stenotrophomonas	Shannon
adzuki	-0.03270748	-0.03271		-0.11128	-0.36772	-0.26509		-0.03271		-0.09482	-0.03271	-0.03271	-0.36491	-0.03271	=-SUM(B15:O15)
BEP			-0.05133	-0.05133	-0.36313	-0.05133	-0.05133			-0.14159			-0.31832	-0.08673	1 SUM(number1, [number2], ...)
mung					-0.30856	-0.23837		-0.04833	-0.04833	-0.13434			-0.33989		1.11783
pigeon				-0.1405	-0.36565					-0.33673			-0.36436		1.207243

= -SUM(B15:O15). B15 is the first cell in the row and O15 is the cell with the last proportion for a treatment.

Data Manipulation in R

1. Open RStudio and create a new project using the New Project option under File and select for the new project to be in an existing folder where your data are.
2. Install the following packages either using the Packages tab in RStudio or the command install.packages("name_of_package") in the console. Note that BiodiversityR requires QuartzX on a Mac. If you are using a MacOS and don't have QuartzX, install it first and restart your computer before install these packages.
   a. dplyr
   b. reshape2
   c. vegan
   d. BiodiversityR
   e. ggplot2

3. Load the packages listed above by clicking the checkboxes for the appropriate packages in the Packages tab or the command library("name_of_package") in the console.

4. Import the dataset "reduced raw data" (dataset without the extra metadata that you created in the Excel section) into RStudio.

Browse to select file
from your computer

Set Name to "colony"
and use Sheet dropdown
to select appropriate
worksheet from Excel
file.
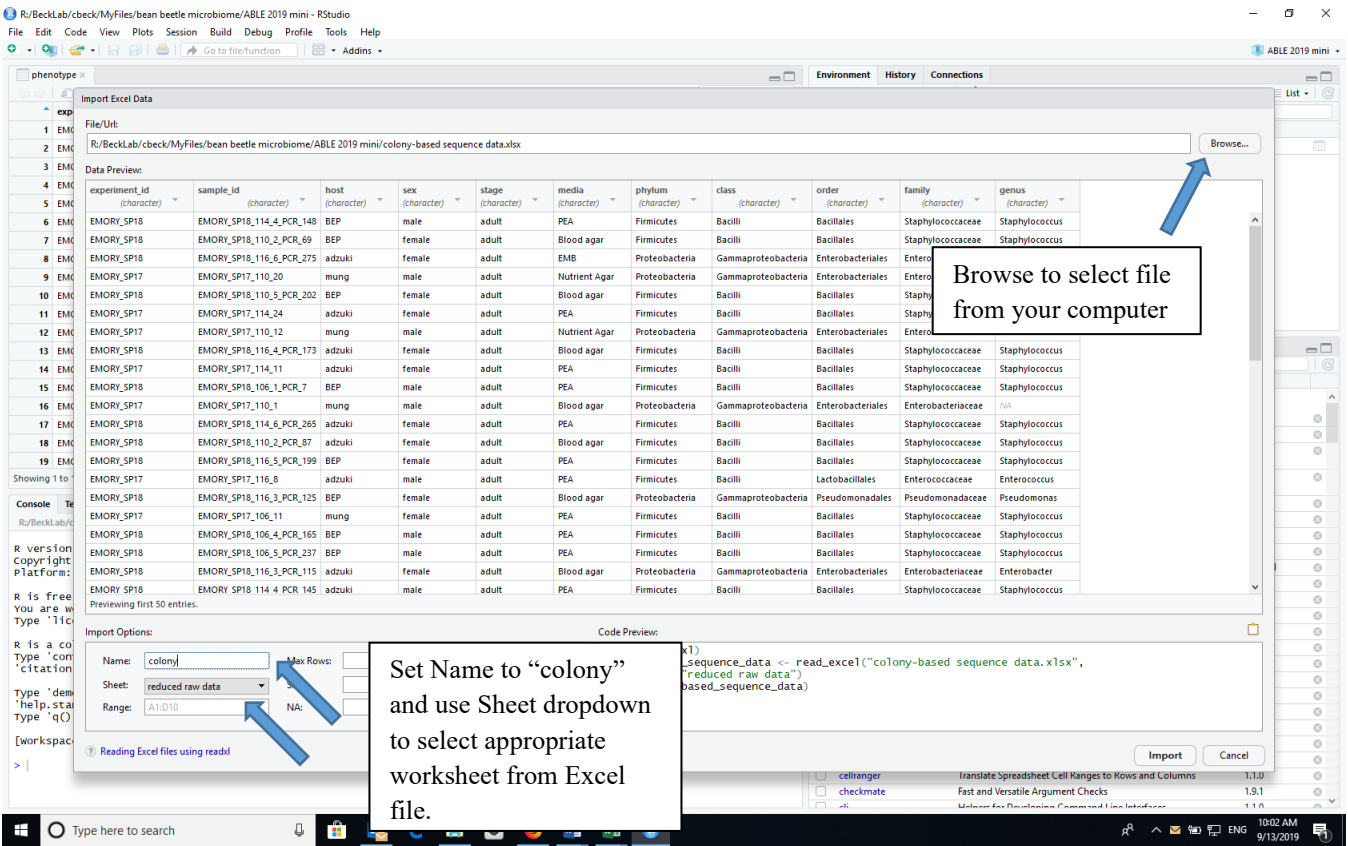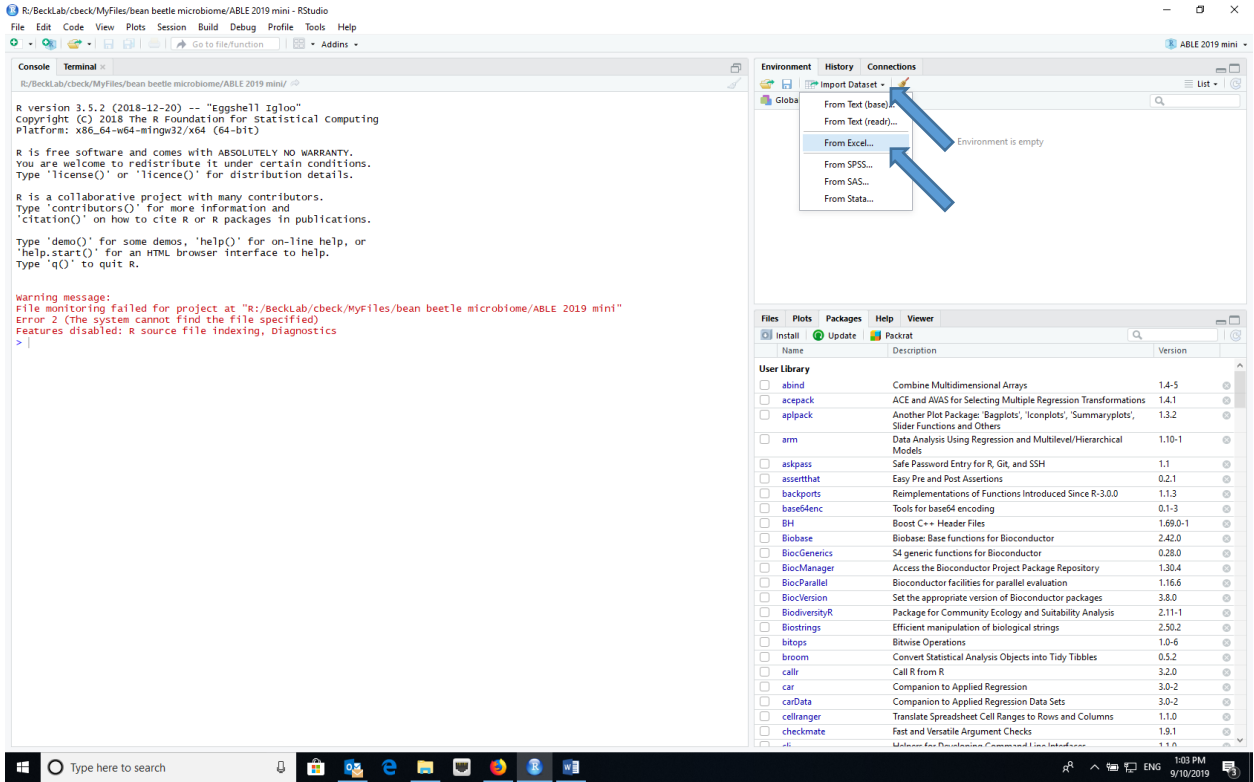
5. Attach the imported dataset ("colony") to the dataframe using the attach command in the console (attach(colony))

6. Create a community matrix (named "community" in this example) for a particular treatment. This example assumes that you are doing the analysis at the genus level. This can be changed to other taxonomic levels using the appropriate variable name

    >community<-table(host,genus)

7. If you want to look at two factors at the same time, creating the community matrix is a little more complicated. The first command calculates the count of each genus by each sex and host combination and drops any missing values. The second command creates a community matrix.

> community_2 <- colony %>% count(sex,host,genus) %>% drop_na()

> comm2<-dcast(community_2, sex+host~genus, value.var = "n", fun.aggregate = sum)

"genus" in both command lines may be whatever taxon level you wish to evaluate in the dataset. For example, it could be changed to "family" or "order".

Calculating diversity indices

Note: "community" is the name of the community matrix

1. Species Richness

    > diversityresult(community,index="richness",method="each site")

2. Simpson

    > diversityresult(community,index="Simpson",method="each site")

    This calculates the inverse Simpson described above

    > diversityresult(community,index="inverseSimpson",method="each site")

    This calculates the reciprocal Simpson described above. (confusing that it is called in the inverseSimpson)

3. Shannon

    > diversityresult(community,index="Shannon",method="each site")

Calculating community similarity (distance)

Sometimes we are interested in how similar (or different) two communities are based on what species (taxa) are present and the relative abundance of those species (taxa) in the two communities. One of the most common measures of distance is the Bray Curtis Dissimilarity. Similarity can be measured as 1-BC.

$$BC_{ij} = 1 - \frac{2C_{ij}}{S_i \div S_j}$$

Where:

- i & j are the two samples,
- S_i is the total number of specimens counted in sample i,
- S_j is the total number of specimens counted in sample j,
- C_{ij} is the sum of only the lesser counts for each taxa found in both sites.

Although Bray-Curtis Dissimilarity is often used in community ecology, it is not robust to incomplete sampling of the community (all taxa are not sampled) or unbalanced sampling (all treatments are not equally sampled). An alternative is the Morista-Horn Index of Dissimilarity (1-C_H). Morista-Horn Index of Similarity is

$$C_H = \frac{2 \sum_{i=1}^{D_{12}} \frac{X_i}{n} \frac{Y_i}{m}}{\sum_{i=1}^{D_1} \left(\frac{X_i}{n}\right)^2 + \sum_{i=1}^{D_2} \left(\frac{Y_i}{m}\right)^2}$$

Where

- D_1=number of taxa in sample 1
- D_2=number of taxa in sample 2
- D_{12}=number of taxa in shared in both communities
- X_i=number of individuals of taxon *i* in sample 1
- Y_i=number of individuals of taxon *i* in sample 2
- n=total number of individuals in sample 1
- m=total number of individuals in sample 2

So that X_i/n and Y_i/m are proportion of individuals of taxon *i* in each of the samples (communities).

To produce a matrix of all of the pair-wise distances between samples using the Bray Curtis index of dis tance, use the   following command.

> vegdist(community, method="bray", binary=FALSE, diag=FALSE, upper=FALSE)

To produce a matrix of all of the pair-wise distances between samples using the Morista-Horn index of distance.

> vegdist(community, method="horn", binary=FALSE, diag=FALSE, upper=FALSE)

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