7. Worksheet: Diversity Synthesis

Atalanta Ritter; Z620: Quantitative Biodiversity, Indiana University

15 February, 2023

OVERVIEW

In this worksheet, you will conduct exercises that reinforce fundamental concepts of biodiversity. Specifically, you will construct a a site-by-species matrix by sampling confectionery taxa. With this primary data structure, you will then answer questions and generate figures using tools from previous weeks, along with wrangling techniques that we learned about in class.

Directions:

1. In the Markdown version of this document in your cloned repo, change "Student Name" on line 3 (above) to your name.
2. Complete as much of the worksheet as possible during class.
3. Refer to previous handouts to help with developing of questions and writing of code.
4. Answer questions in the worksheet. Space for your answer is provided in this document and indicated by the ">" character. If you need a second paragraph be sure to start the first line with ">". You should notice that the answer is highlighted in green by RStudio (color may vary if you changed the editor theme).
5. Before you leave the classroom, **push** this file to your GitHub repo.
6. For the assignment portion of the worksheet, follow the directions at the bottom of this file.
7. When you are done, **Knit** the text and code into a PDF file.
8. After Knitting, submit the completed exercise by creating a **pull request** via GitHub. Your pull request should include this file `7.DiversitySynthesis_Worskheet.Rmd` and the PDF output of `Knitr` (`DiversitySynthesis_Worskheet.pdf`).

CONFECTIONARY EXERCISE GOALS

We will construct a site-by-species matrix using confectionery taxa (i.e, gummies). The instructors have created distinct **sources communities** that vary in the composition of gummy taxa with even and uneven communities. It might be fun to consider them as distinct geographical regions experiencing different environmental regimes, or different experimental units under different treatments. Each student will sample a source community and then use a taxonomic key to identify gummies and their abundances.

In the end, students will use the site-by-species matrix to:

1) explore their sampling efforts and their effects on species richness using **coverage** and **rarefaction** concept,

2) measure **alpha diversity** for each sub-sample collated from data with their peers from the same source community,

3) examine **beta diversity** between each source community using the data generated across each source community, and

4) use **data wrangling** tools they have learned during the class to accomplish the above goals.

SAMPLING PROTOCOL TO CONSTRUCT A SITE-BY-SPECIES MATRIX

1. Instructors will assign you to sample confectionery taxa from one of the two designated source community bucket (A and B).
2. After randomly sampling one unit (imagine as an equal biomass) from the source community, each student will count the total number of individuals (N), identify the taxa using the species key and quantify the abundance of each taxon.
3. Work with other students in your group to assemble data into a site-by-species matrix on the white board. One person needs to create a .csv or .txt file and share your group's site-by-species matrix with the class using GitHub. Make sure that you include a sample identifier (student name) and what community you sampled from.

GROUP BRAINSTORM

In smaller groups, take 15 minutes to brainstorm questions, code, statistical tests, and "fantasy figures" using the site-by-species matrix the class generated.

1. Using this data, explore how well your sampling effort was. You can use rarefaction and coverage tools you have learned earlier.
2. Investigate alpha diversity based on the methods you have learned in the rest of the handout and accompanying worksheet. For example, you can measure richness, Shannon diversity and Simpson index. You can also convert them to effective number of species using the Hill numbers concept.
3. Measure beta diversity using ordination and multivariate statistical methods. For example, you can create a PCoA plot, based on Bray-Curtis dissimilarity, of sites and communities using different shape and color codes. Use Permanova to test if there are differences between communities.

DATA ANALYSIS

```
# set up
rm(list = ls())
getwd()
```

```
## [1] "/Users/Atalanta/GitHub/QB2023_Ritter/2.Worksheets/7.DiversitySynthesis"
```

```
setwd("~/GitHub/QB2023_Ritter/2.Worksheets/7.DiversitySynthesis")
# load packages
package.list <- c('vegan', 'tidyverse', 'ggplot2', 'dplyr', 'broom')
for (package in package.list) {
  if (!require(package, character.only = TRUE, quietly = TRUE)) {
    install.packages(package)
  }
  library(c(package), character.only = TRUE)
}
```

```
## This is vegan 2.6-4
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.2 --
## v ggplot2 3.3.6      v purrr   0.3.5
## v tibble  3.1.8      v dplyr   1.0.10
## v tidyr   1.2.1      v stringr 1.4.1
## v readr   2.1.3      v forcats 0.5.2
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

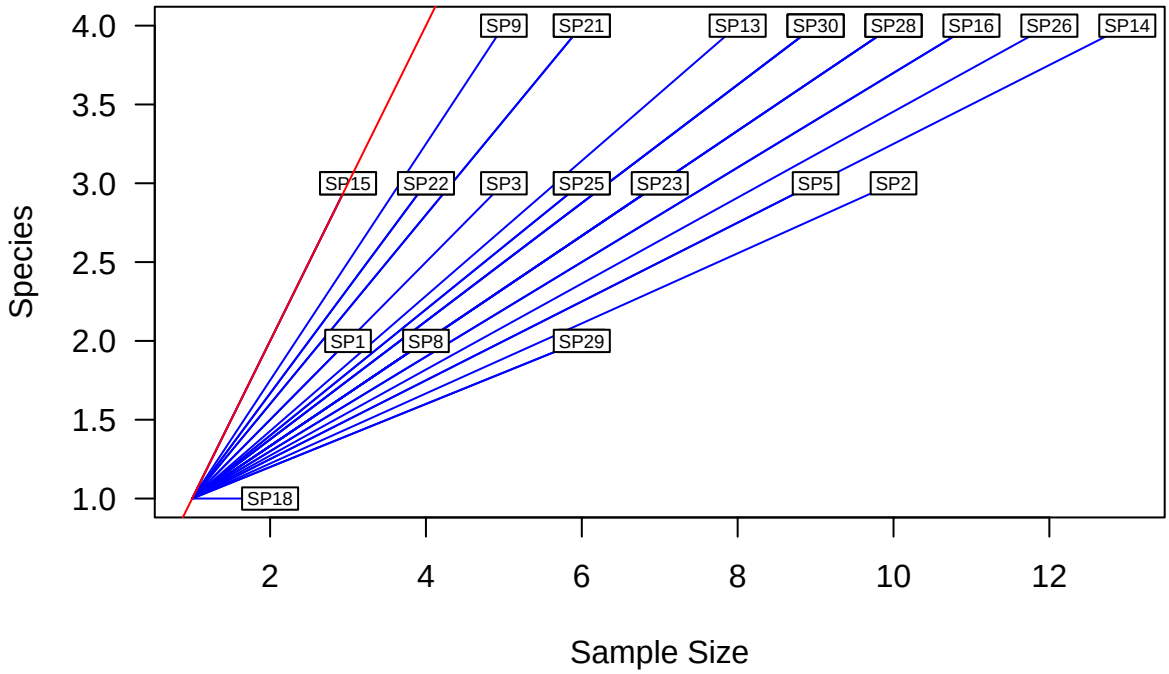
```
# load in dataset
raw.candy <- read.csv("/Users/Atalanta/Downloads/QB Data Wrangling Lab SP23 - Sheet1.csv")
# get rid of names column
candy <- raw.candy[,c(1,3:32)]
```

1) Sampling coverage and rarefaction curves

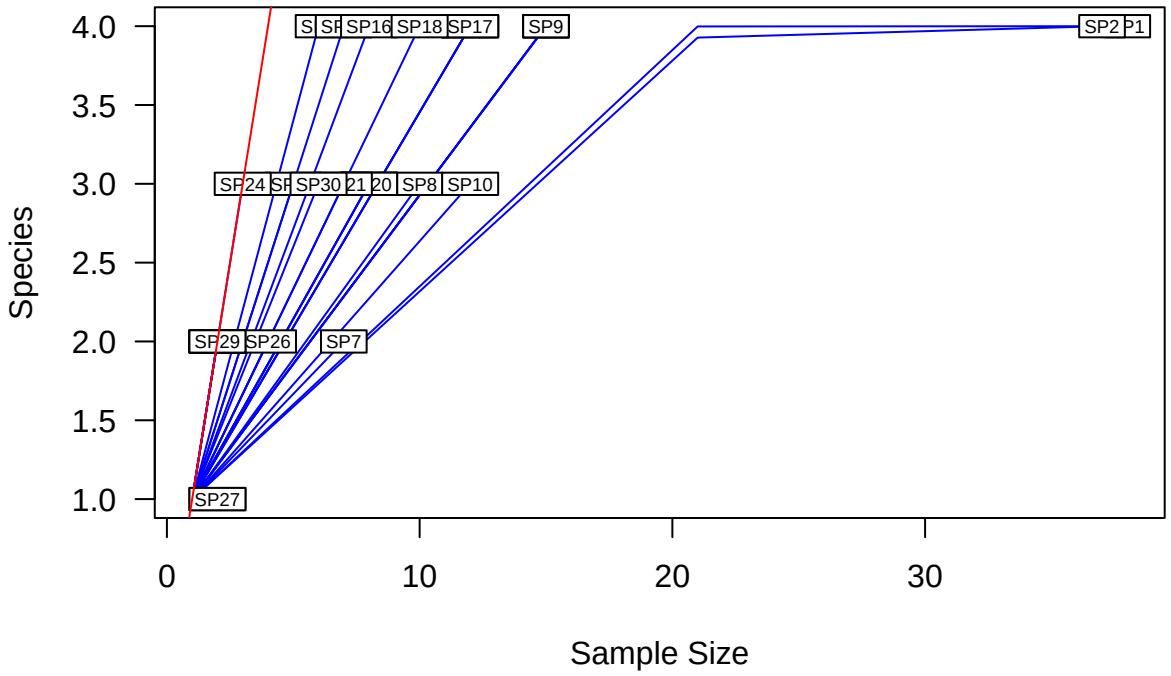
Question 1: Using this data, explore how well your sampling effort was. Compare your sampling efforts with other groups. Do you think that your samples cover the actual diversity found in each source community? You can use rarefaction and coverage tools you have learned earlier.

Answer 1: Use the space below to generate a rarefaction curve/sample coverage based on the data we collected in class for each community. Make sure to annotate your code using # symbols so others (including instructors) understand what you have done and why you have done it.

```
# dividing site x sp matrix into the 2 communities
community.A <- as.data.frame(t(candy[1:4,-c(1)]))
community.B <- as.data.frame(t(candy[5:8,-c(1)]))
# observed species in community A
candy.SA <- specnumber(community.A)
# observed species in community B
candy.SB <- specnumber(community.B)
# minimum number of species observed in community A
min.N.A <- min(rowSums(community.A))
# minimum number of species observed in community B
min.N.B <- min(rowSums(community.B))
# community A rarefaction curve
A.rarefy <- rarefy(x = community.A, sample = min.N.A, se = TRUE)
rarecurve(x = community.A, step = 20, col = "blue", cex = 0.6, las = 1)
abline(0, 1, col = "red")
text(1500, 1500, "1:1", pos = 2, col = "red")
```



```r
# community B rarefaction curve
B.rarefy <- rarefy(x = community.B, sample = min.N.A, se = TRUE)
rarecurve(x = community.B, step = 20, col = "blue", cex = 0.6, las = 1)
abline(0, 1, col = "red")
text(1500, 1500, "1:1", pos = 2, col = "red")
```



2) Alpha diversity

Question 2: Compare alpha diversity measures within sites and among communities. You can calculate and plot richness, Shannon diversity, and Simpson index. You can also convert these indices to effective number of species using the Hill numbers concept by generating a diversity profile, which will make comparisons easier across sites.

What is the variation among the samples in your group and between the communities of other groups for the alpha diversity indices? Generate a hypothesis around the diversity metrics you chose and test your hypothesis. Interpret your findings.

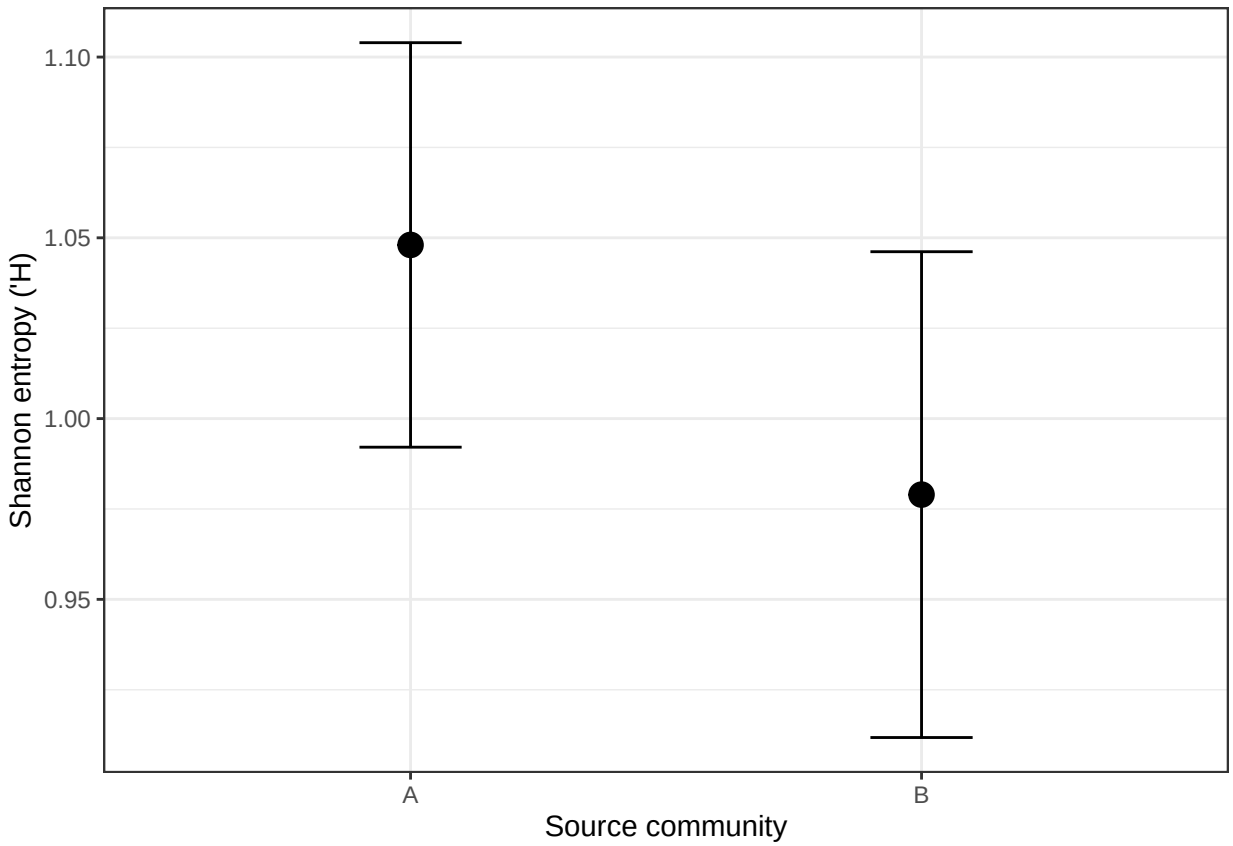
Answer 2a - Analysis: Use the space below for code that is being used to analyze your data and test your hypotheses on your chosen alpha diversity tool. Make sure to annotate your code using # symbols so others (including instructors) understand what you have done and why you have done it.

```r
# shannon's entropy 'H for community A
community.A.H <- diversity(community.A, index = "shannon")
# shannon's entropy 'H for community A
community.B.H <- diversity(community.B, index = "shannon")
# write function for SEM
SEM <- function(x) {
  return(sd(x)/sqrt(length(x)))
}
# bind vectors as columns in a single dataframe
communities <- t(cbind.data.frame(community.A.H, community.B.H))
# rename row names
row.names(communities) <- c("Community A", "Community B")
# create an empty data grame to fill with summary statistics
```

```r
com_div_sum <- as.data.frame(matrix(ncol = 2, nrow = 2))
colnames(com_div_sum) <- c("mean", "sem")
# loop that iteratively calculates the mean and SE for each row
for(i in 1:2) {
  x = as.numeric(communities[i,])
  com_div_sum[i,1] <- mean(x) # calculate mean, save to diversity indices data
  com_div_sum[i,2] <- SEM(x) # calculate SEM, save to diversity indices data
}
# add grouping column to data
com_div_sum$community <- c("A", "B")
```

Answer 2b - Plot: With your analysis, create one (and only one, although it can have multiple panels) *publication-quality* figure.

```r
# plot mean and SE of Shannon entropy for communities A and B
ggplot(data = com_div_sum, aes(x = community, y= mean)) +
  geom_point(size = 4) +
  geom_errorbar(aes(ymin = mean - sem, ymax = mean + sem), width = 0.2) +
  ylab("Shannon entropy ('H)") +
  xlab("Source community") +
  theme_bw()
```



Answer 2c - Interpret results: Write an informative yet succinct (~5 sentences) caption that creates a "stand-alone" figure. Take a peek at figures and figure captions in a paper published in your favorite journal for inspiration.

> The figure above plots Shannon's entropy ('H) across sites within two different communities. Community A's average 'H value was 1.048 +/- 0.056 and Community B's average 'H was 0.979

+/- 0.067. Overall, these values are quite similar to each other, suggesting that alpha diversity in terms of species richness and evenness is similar across communities (although this does not mean that the species composition is similar). However, there is considerable overlap in their standard error, probably due to inconsistent sampling efforts.

3) Beta diversity

Question 3: Measure beta diversity using ordination and multivariate statistics methods. You can create a PCoA plot, based on Bray-Curtis dissimilarity, of sites and communities using different shape and color codes. Then, you can use a Permanova to test if there are differences between communities. Generate a hypothesis around your chosen analysis and test your hypothesis. Interpret your findings.

Can you detect compositional differences between each source community sampled?

Answer 3a - Analysis: Use the space below for code that is being used to analyze your data and test your hypotheses on your chosen beta diversity tool. Make sure to annotate your code using # symbols so others (including instructors) understand what you have done and why you have done it.

```
# PERMANOVA
# test hypothesis that community composition varies between A and B
library(indicspecies)
# create factors vector, where factor is the community
community <- c(rep("A", 4), rep("B", 4))
# run PERMANOVA with adonis function
adonis(candy[,2:31] ~ community, method = "bray", permutations = 999)
```

```
## 'adonis' will be deprecated: use 'adonis2' instead
```

```
## $aov.tab
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## community  1   0.31895 0.31895  3.8605 0.39151  0.032 *
## Residuals  6   0.49571 0.08262         0.60849
## Total      7   0.81466                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## $call
## adonis(formula = candy[, 2:31] ~ community, permutations = 999,
##     method = "bray")
##
## $coefficients
##                SP1    SP2   SP3    SP4   SP5   SP6           SP7   SP8   SP9
## (Intercept)  5.125  5.875  2.50  2.625  3.00 2.125  1.750000e+00  1.75  2.50
## community1  -4.375 -3.375 -1.25 -1.125 -0.75 0.625 -7.850462e-17 -0.75 -1.25
##               SP10  SP11  SP12          SP13  SP14   SP15  SP16 SP17 SP18 SP19
## (Intercept)  1.875 2.125  2.75  2.000000e+00 3.125  1.375 2.375    2  1.5  1.0
## community1  -1.125 0.375 -0.25 -1.570092e-16 0.125 -0.625 0.375   -1 -1.0  0.5
##              SP20   SP21   SP22  SP23 SP24 SP25 SP26  SP27 SP28 SP29  SP30
## (Intercept)  1.75  1.625  1.125 1.125 1.50  1.0    2 1.375  1.5  1.0 1.875
## community1  -0.25 -0.125 -0.125 0.625 0.75  0.5    1 0.875  1.0  0.5 0.375
##
## $coef.sites
```

```
##                       1           2          3           4         5         6
## (Intercept)  0.4220687  0.39065129  0.4587502  0.42922560 0.4120827 0.4275198
## community1  -0.1239824 -0.08059001 -0.1215706 -0.09283702 0.1202197 0.1363959
##                       7         8
## (Intercept) 0.4069227 0.3793954
## community1  0.1059075 0.1312320
##
## $f.perms
##              [,1]
##    [1,] 1.8393788
##    [2,] 1.2095051
##    [3,] 0.3276923
##    [4,] 0.4244291
##    [5,] 0.3276923
##    [6,] 0.5980672
##    [7,] 3.8605106
##    [8,] 0.7315165
##    [9,] 0.3802881
##   [10,] 1.3349363
##   [11,] 0.6687867
##   [12,] 0.4244291
##   [13,] 1.1229696
##   [14,] 1.2095051
##   [15,] 0.6723741
##   [16,] 1.2304356
##   [17,] 0.3276923
##   [18,] 0.4244291
##   [19,] 1.8251915
##   [20,] 1.2095051
##   [21,] 1.1411912
##   [22,] 0.8636075
##   [23,] 1.8393788
##   [24,] 1.3564416
##   [25,] 0.6687867
##   [26,] 0.7827233
##   [27,] 1.5386435
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##   [30,] 1.5386435
##   [31,] 0.3802881
##   [32,] 1.1229696
##   [33,] 1.2304356
##   [34,] 0.7385108
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##   [37,] 0.8636075
##   [38,] 0.4244291
##   [39,] 0.8636075
##   [40,] 1.1229696
##   [41,] 0.8805548
##   [42,] 0.4244291
##   [43,] 0.8636075
##   [44,] 0.4774510
##   [45,] 0.8636075
```

```
##   [46,] 0.4244291
##   [47,] 0.8805548
##   [48,] 1.8393788
##   [49,] 0.5325287
##   [50,] 0.5980672
##   [51,] 0.5325287
##   [52,] 1.8393788
##   [53,] 0.6747114
##   [54,] 0.8320900
##   [55,] 0.7315165
##   [56,] 0.7448797
##   [57,] 0.3276923
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##   [60,] 0.7315165
##   [61,] 1.1411912
##   [62,] 0.3276923
##   [63,] 1.5268480
##   [64,] 1.1411912
##   [65,] 0.7385108
##   [66,] 1.2450232
##   [67,] 0.4244291
##   [68,] 0.7315165
##   [69,] 3.8605106
##   [70,] 1.3573663
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```

```
##  [100,] 0.8592754
##  [101,] 0.6723741
##  [102,] 0.7448797
##  [103,] 1.8393788
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##  [154,] 0.7827233
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##  [910,] 1.3564416
##  [911,] 0.6687867
##  [912,] 0.3276923
##  [913,] 0.7827233
##  [914,] 0.6069838
##  [915,] 0.3276923
##  [916,] 1.1411912
##  [917,] 1.3573663
##  [918,] 1.2095051
##  [919,] 0.7827233
##  [920,] 1.2643171
##  [921,] 0.8592754
##  [922,] 0.7827233
##  [923,] 1.3349363
##  [924,] 1.5386435
##  [925,] 1.5386435
##  [926,] 1.2457273
##  [927,] 0.4244291
##  [928,] 0.6723741
##  [929,] 0.6747114
##  [930,] 1.5386435
##  [931,] 0.5325287
##  [932,] 0.8636075
##  [933,] 1.2450232
##  [934,] 0.8636075
##  [935,] 1.1411912
##  [936,] 1.5268480
##  [937,] 0.8805548
##  [938,] 0.8592754
##  [939,] 0.5325287
##  [940,] 1.1495976
##  [941,] 0.5325287
##  [942,] 0.8636075
##  [943,] 0.5980672
##  [944,] 1.2457273
##  [945,] 1.8251915
##  [946,] 0.8592754
##  [947,] 0.6687867
##  [948,] 0.6687867
##  [949,] 0.4774510
##  [950,] 0.7385108
##  [951,] 0.6747114
##  [952,] 1.1411912
##  [953,] 0.5325287
##  [954,] 0.6747114
##  [955,] 0.8805548
##  [956,] 0.5325287
##  [957,] 0.3276923
##  [958,] 1.3573663
##  [959,] 0.3802881
##  [960,] 0.6687867
##  [961,] 0.7827233
##  [962,] 0.5679079
##  [963,] 0.4244291
```

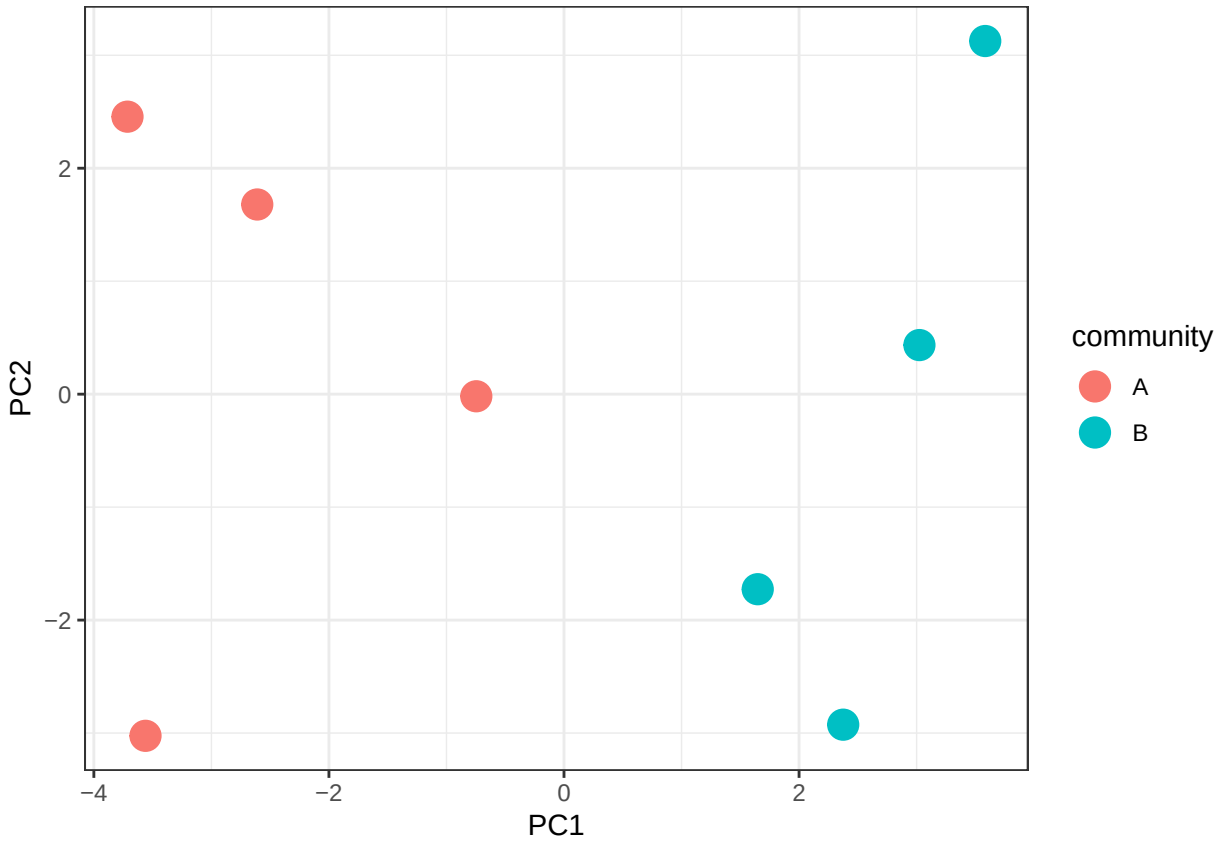
```
##  [964,] 0.4244291
##  [965,] 0.8636075
##  [966,] 3.8605106
##  [967,] 0.6069838
##  [968,] 1.2457273
##  [969,] 0.7315165
##  [970,] 1.8251915
##  [971,] 1.8251915
##  [972,] 1.8393788
##  [973,] 1.1229696
##  [974,] 1.3349363
##  [975,] 0.8636075
##  [976,] 1.1495976
##  [977,] 1.2450232
##  [978,] 1.1411912
##  [979,] 0.6747114
##  [980,] 1.3573663
##  [981,] 0.6687867
##  [982,] 1.5386435
##  [983,] 0.8592754
##  [984,] 0.8320900
##  [985,] 1.5268480
##  [986,] 1.8393788
##  [987,] 1.2095051
##  [988,] 0.8636075
##  [989,] 1.2450232
##  [990,] 0.8636075
##  [991,] 0.7385108
##  [992,] 1.8393788
##  [993,] 0.8320900
##  [994,] 0.5679079
##  [995,] 1.3349363
##  [996,] 3.8605106
##  [997,] 0.6723741
##  [998,] 3.8605106
##  [999,] 1.2304356
##
## $model.matrix
##   (Intercept) community1
## 1           1          1
## 2           1          1
## 3           1          1
## 4           1          1
## 5           1         -1
## 6           1         -1
## 7           1         -1
## 8           1         -1
##
## $terms
## candy[, 2:31] ~ community
## attr(,"variables")
## list(candy[, 2:31], community)
## attr(,"factors")
##               community
```

```
## candy[, 2:31]         0
## community             1
## attr(,"term.labels")
## [1] "community"
## attr(,"order")
## [1] 1
## attr(,"intercept")
## [1] 1
## attr(,"response")
## [1] 1
## attr(,".Environment")
## <environment: R_GlobalEnv>
##
## attr(,"class")
## [1] "adonis"
```

```
# I got a p value of 0.031, so community composition does significantly vary
# based on community
```

Answer 3b - Plot: With your analysis, create one (and only one, although it can have multiple panels) *publication-quality* figure.

```
# Wrangle data and obtain principal components scores
com_candy_tidy <- candy[,2:31] %>%
# identify data frame of interest
mutate_if(is.integer, as.numeric) %>%
# basic statements and operations.here we convert integers to numeric values
prcomp(center = T, scale = T) %>%
# run a pca with correlation matrix
tidy('scores') %>%
# this is a useful broom function to tidy model outputs as data frames
filter(PC<3) %>%
# filter out principle components > 2
pivot_wider(names_from = PC, values_from = value) %>%
# converts the data to wide format, opposite function is pivot_longer
mutate(site = rep(c('1','2','3','4'), times = 2)) %>%
# add a site column for plotting
mutate(community = rep(c('A', 'B'), each = 4)) %>%
# add a community column for plotting
dplyr::rename('PC1' = '1', 'PC2' = '2') %>%
# you can rename variable names
select(community, PC1, PC2)
# let us plot ordination results
ggplot(data = com_candy_tidy, aes(x = PC1, y = PC2, color = community))+
geom_point(size = 5)+
theme_bw()
```



Answer 3c - Interpret results: Write an informative yet succinct (~5 sentences) caption that creates a "stand-alone" figure. Take a peek at figures and figure captions in a paper published in your favorite journal for inspiration.

> This is an ordination plot of two ecological communities A and B, which were each independently sampled 4 times. Along the PC1 axis, there is a clear distinction between the communities, with A having predominantly lower values and B having higher values. This is most likely indicative of differences in community composition, which did vary significantly between A and B (F-model = 3.8605, P = 0.031). There is less of a distinction along the PC2 axis; values within both communities range from low to high. This could be a result of differences in sampling effort or accuracy.

SUBMITTING YOUR ASSIGNMENT

Use Knitr to create a PDF of your completed 7.DiversitySynthesis_Worksheet.Rmd document, push it to GitHub, and create a pull request. Please make sure your updated repo includes both the pdf and RMarkdown files.

Unless otherwise noted, this assignment is due on **Wednesday, February 15th, 2023 at 12:00 PM (noon)**.