5. Worksheet: Alpha Diversity

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24 January, 2023

OVERVIEW

In this exercise, we will explore aspects of local or site-specific diversity, also known as alpha (α) diversity. First we will quantify two of the fundamental components of (α) diversity: **richness** and **evenness**. From there, we will then discuss ways to integrate richness and evenness, which will include univariate metrics of diversity along with an investigation of the **species abundance distribution (SAD)**.

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. Use the handout as a guide; it contains a more complete description of data sets along with the proper scripting needed to carry out the exercise.
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 `AlphaDiversity_Worskheet.Rmd` and the PDF output of `Knitr` (`AlphaDiversity_Worskheet.pdf`).

1) R SETUP

In the R code chunk below, please provide the code to: 1) Clear your R environment, 2) Print your current working directory, 3) Set your working directory to your `5.AlphaDiversity` folder, and 4) Load the `vegan` R package (be sure to install first if you haven't already).

```
r = getOption("repos")
r["CRAN"] = "http://cran.us.r-project.org"
options(repos = r)
rm(list = ls())
getwd()
```

```
## [1] "/Users/annawerkowski/Documents/Github/QB2023_Werkowski/2.Worksheets/5.AlphaDiversity"
```

```
setwd("~/Documents/Github/QB2023_Werkowski/2.Worksheets/5.AlphaDiversity")
install.packages("vegan")
```

```
##
## The downloaded binary packages are in
##  /var/folders/_6/rhn_z_2n7q15t3x1fmslmz1h0000gn/T//RtmpsuRXX6/downloaded_packages
```

```
require("vegan")
```

```
## Loading required package: vegan

## Loading required package: permute

## Loading required package: lattice

## This is vegan 2.6-4
```

2) LOADING DATA

In the R code chunk below, do the following: 1) Load the BCI dataset, and 2) Display the structure of the dataset (if the structure is long, use the `max.level = 0` argument to show the basic information).

```
data("BCI")
BCIdata <- BCI
str(BCIdata, max.level = 0)
```

```
## 'data.frame':    50 obs. of  225 variables:
##  - attr(*, "original.names")= chr [1:225] "Abarema.macradenium" "Acacia.melanoceras" "Acalypha.divers
```

3) SPECIES RICHNESS

Species richness (S) refers to the number of species in a system or the number of species observed in a sample.

Observed richness

In the R code chunk below, do the following:

1. Write a function called `S.obs` to calculate observed richness
2. Use your function to determine the number of species in `site1` of the BCI data set, and
3. Compare the output of your function to the output of the `specnumber()` function in `vegan`.

```
S.obs <- function(x = ""){
  rowSums(x > 0) * 1
}
site1 <- BCIdata[1, ]
S.obs(site1) #93 species in Site 1
```

```
##  1
## 93
```

```
specnumber(BCIdata) #also showed 93 species in Site 1
```

```
##   1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19  20
##  93  84  90  94 101  85  82  88  90  94  87  84  93  98  93  93  93  89 109 100
##  21  22  23  24  25  26  27  28  29  30  31  32  33  34  35  36  37  38  39  40
##  99  91  99  95 105  91  99  85  86  97  77  88  86  92  83  92  88  82  84  80
##  41  42  43  44  45  46  47  48  49  50
## 102  87  86  81  81  86 102  91  91  93
```

```
site2 <- BCIdata[2, ]
site3 <- BCIdata[3, ]
site4 <- BCIdata[4, ]
S.obs(site2)
```

```
##  2
## 84
```

```
S.obs(site3)
```

```
##  3
## 90
```

```
S.obs(site4)
```

```
##  4
## 94
```

Question 1: Does `specnumber()` from `vegan` return the same value for observed richness in `site1` as our function `S.obs`? What is the species richness of the first four sites (i.e., rows) of the BCI matrix?

> ***Answer 1***: Yes, both functions return the same value. The species richness of the first four sites is as follows: Site 1 = 93, Site 2 = 84, Site 3 = 90, Site 4 = 94.

Coverage: How well did you sample your site?

In the R code chunk below, do the following:

1. Write a function to calculate Good's Coverage, and
2. Use that function to calculate coverage for all sites in the BCI matrix.

```
coverage <- function(x = ""){
  1 - (rowSums(x == 1) / rowSums(x))
}
coverage(BCIdata[1:50,])
```

```
##         1         2         3         4         5         6         7         8
## 0.9308036 0.9287356 0.9200864 0.9468504 0.9287129 0.9174757 0.9326923 0.9443155
##         9        10        11        12        13        14        15        16
## 0.9095355 0.9275362 0.9152120 0.9071038 0.9242054 0.9132420 0.9350649 0.9267735
##        17        18        19        20        21        22        23        24
## 0.8950131 0.9193084 0.8891455 0.9114219 0.8946078 0.9066986 0.8705882 0.9030612
##        25        26        27        28        29        30        31        32
## 0.9095023 0.9115479 0.9088729 0.9198966 0.8983516 0.9221053 0.9382423 0.9411765
##        33        34        35        36        37        38        39        40
## 0.9220183 0.9239374 0.9267887 0.9186047 0.9379310 0.9306488 0.9268868 0.9386503
##        41        42        43        44        45        46        47        48
## 0.8880597 0.9299517 0.9140049 0.9168704 0.9234234 0.9348837 0.8847059 0.9228916
##        49        50
## 0.9086651 0.9143519
```

Question 2: Answer the following questions about coverage:

a. What is the range of values that can be generated by Good's Coverage?
b. What would we conclude from Good's Coverage if n_i equaled N?
c. What portion of taxa in `site1` was represented by singletons?
d. Make some observations about coverage at the BCI plots.

Answer 2a: Values between 0.0 and 1.0 can be generated.

Answer 2b: If the two values match each other, then we can conclude that the number of singleton species is equal to the total number of individuals in the sample. When put into the equation C = 1 - n1/N, C would be 0 meaning that our sample is composed 100% of singleton species.

Answer 2c: 7% of the taxa in Site 1 were represented by singletons.

Answer 2d: The coverage at the BCI plots was composed of mostly doubleton species with the percentage of singleton species ranging between 6% to 13% among the sites.

Estimated richness

In the R code chunk below, do the following:

1. Load the microbial dataset (located in the `5.AlphaDiversity/data` folder),
2. Transform and transpose the data as needed (see handout),
3. Create a new vector (`soilbac1`) by indexing the bacterial OTU abundances of any site in the dataset,
4. Calculate the observed richness at that particular site, and
5. Calculate coverage of that site

```
soilbac <- read.table("data/soilbac.txt", sep = "\t", header = TRUE, row.names = 1)
soilbac.t <- as.data.frame(t(soilbac))
soilbac1 <- soilbac.t[1, ]
S.obs(soilbac1) #observed richness is 1074
```

```
## T1_1
## 1074
```

```
coverage(soilbac1) #coverage showed 65% doubletons and 35% singletons
```

```
##      T1_1
## 0.6479471
```

Question 3: Answer the following questions about the soil bacterial dataset.

a. How many sequences did we recover from the sample `soilbac1`, i.e. N?
b. What is the observed richness of `soilbac1`?
c. How does coverage compare between the BCI sample (`site1`) and the KBS sample (`soilbac1`)?

Answer 3a: There were 13,310 variables included within the Soilbac1 dataset.

Answer 3b: The observed richness of Soilbac1 was 1074.

Answer 3c: The coverage of the Soilbac1 site was 35% singletons and 65% doubletons. The BCI site 1 coverage had 7% singletons and 93% doubletons. It is fair to say that there is a more even distribution of singletons and doubletons in the Soilbac1 dataset.

Richness estimators

In the R code chunk below, do the following:

1. Write a function to calculate **Chao1**,
2. Write a function to calculate **Chao2**,
3. Write a function to calculate **ACE**, and
4. Use these functions to estimate richness at `site1` and `soilbac1`.

```
S.chao1 <- function(x = ""){
  S.obs(x) + (sum(x == 1)^2) / (2 * sum(x == 2))
}
S.chao2 <- function(site = "", SbyS = ""){
  SbyS = as.data.frame(SbyS)
  x = SbyS[site, ]
  SbyS.pa <- (SbyS < 0) * 1
  Q1 = sum(colSums(SbyS.pa) == 1)
  Q2 = sum(colSums(SbyS.pa) == 2)
  S.chao2 = S.obs(x) + (Q1^2) / (2 * Q2)
  return(S.chao2)
}
S.ace <- function(x = "", thresh = 10){
  x <- x[x>0]
  S.abund <- length(which(x > thresh))
  S.rare <- length(which(x <= thresh))
  singlt <- length(which(x == 1))
  N.rare <- sum(x[which(x <= thresh)])
```

```
  C.ace <- 1 - (singlt / N.rare)
  i <- c(1:thresh)
  count <- function(i, y){
    length(y[y == i])
  }
  a.1 <- sapply(i, count, x)
  f.1 <- (i * (i - 1)) * a.1
  G.ace <- (S.rare/C.ace) * (sum(f.1)/(N.rare*(N.rare-1)))
  S.ace <- S.abund + (S.rare/C.ace) + (singlt/C.ace) * max(G.ace, 0)
}

S.chao1(site1) #estimated richness is 119.69
```

```
##        1
## 119.6944
```

```
S.chao1(soilbac1) #estimated richness is 2628.51
```

```
##     T1_1
## 2628.514
```

```
S.ace(site1) #estimated richness is 159.3
S.ace(soilbac1)
S.chao2("1",BCI) #this produced NaN
```

```
##   1
## NaN
```

```
S.chao2("T1_1", soilbac1) #tis produced NaN
```

```
## T1_1
##  NaN
```

Question 4: What is the difference between ACE and the Chao estimators? Do the estimators give consistent results? Which one would you choose to use and why?

> ***Answer 4***: #Chao1, Chao2, and ACE all are slightly different. Chao1 is an abundance-based estimator used for examining richness of a single site. Chao2 is an incidence-based estimator that uses presence-absence data to examine richness across multiple sites. ACE is an abundance-based coverage estimator which has a threshold included to look at the abundance of other rare species. The estimators seem to give pretty consistent results. For what we are currently working on, using Chao1 seems like the best option because it examines observed richness using the singletons/doubletons we learned about previously. I don't believe we are relativizing any data right now, so that wouldn't impact the use of Chao1 at this point in time.

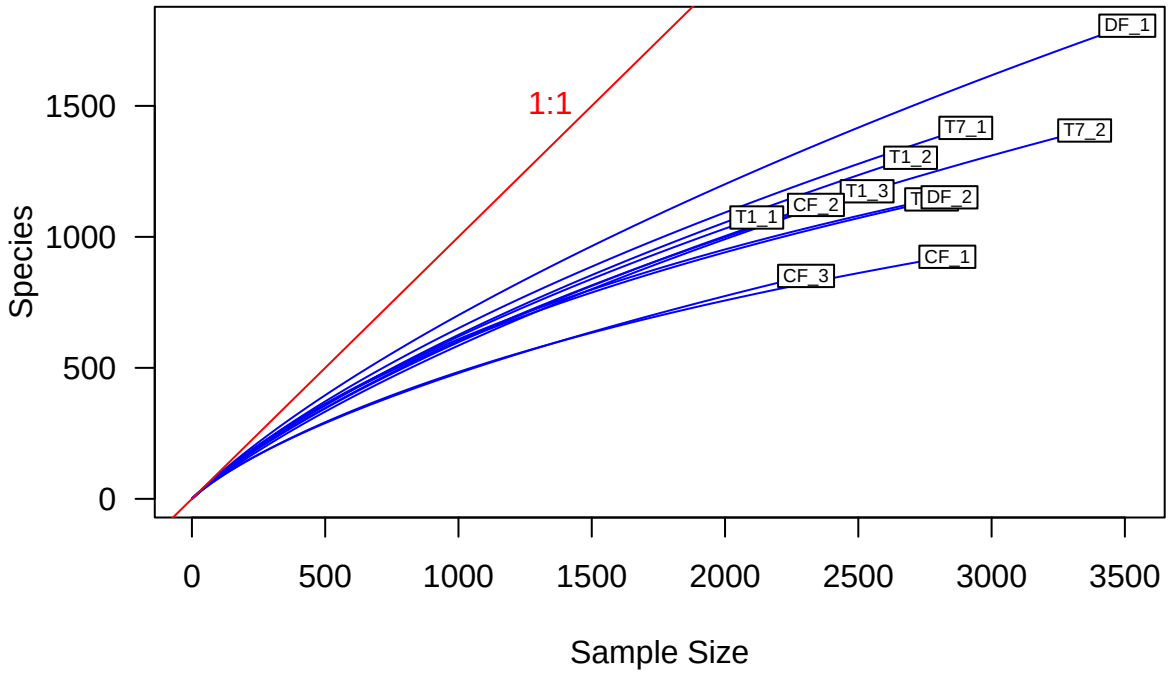
Rarefaction

In the R code chunk below, please do the following:

1. Calculate observed richness for all samples in `soilbac`,

2. Determine the size of the smallest sample,
3. Use the `rarefy()` function to rarefy each sample to this level,
4. Plot the rarefaction results, and
5. Add the 1:1 line and label.

```
soilbac.S <- S.obs(soilbac.t)
min.N <- min(rowSums(soilbac.t))
S.rarefy <- rarefy(x = soilbac.t, sample = min.N, se = TRUE)
rarecurve(x = soilbac.t, step = 20, col = "blue", cex = 0.6, las = 1)
abline(0, 1, col = 'red')
text(1500, 1500, "1:1", pos = 2, col = 'red')
```



4) SPECIES EVNENNESS

Here, we consider how abundance varies among species, that is, **species evenness**.

Visualizing evenness: the rank abundance curve (RAC)

One of the most common ways to visualize evenness is in a **rank-abundance curve** (sometime referred to as a rank-abundance distribution or Whittaker plot). An RAC can be constructed by ranking species from the most abundant to the least abundant without respect to species labels (and hence no worries about 'ties' in abundance).

In the R code chunk below, do the following:

1. Write a function to construct a RAC,
2. Be sure your function removes species that have zero abundances,

3. Order the vector (RAC) from greatest (most abundant) to least (least abundant), and
4. Return the ranked vector

```
RAC <- function(x = ""){
  x.ab = x[x >0]
  x.ab.ranked = x.ab[order(x.ab, decreasing = TRUE)]
  as.data.frame(lapply(x.ab.ranked, unlist))
  return(x.ab.ranked)
}
```

Now, let us examine the RAC for `site1` of the BCI data set.

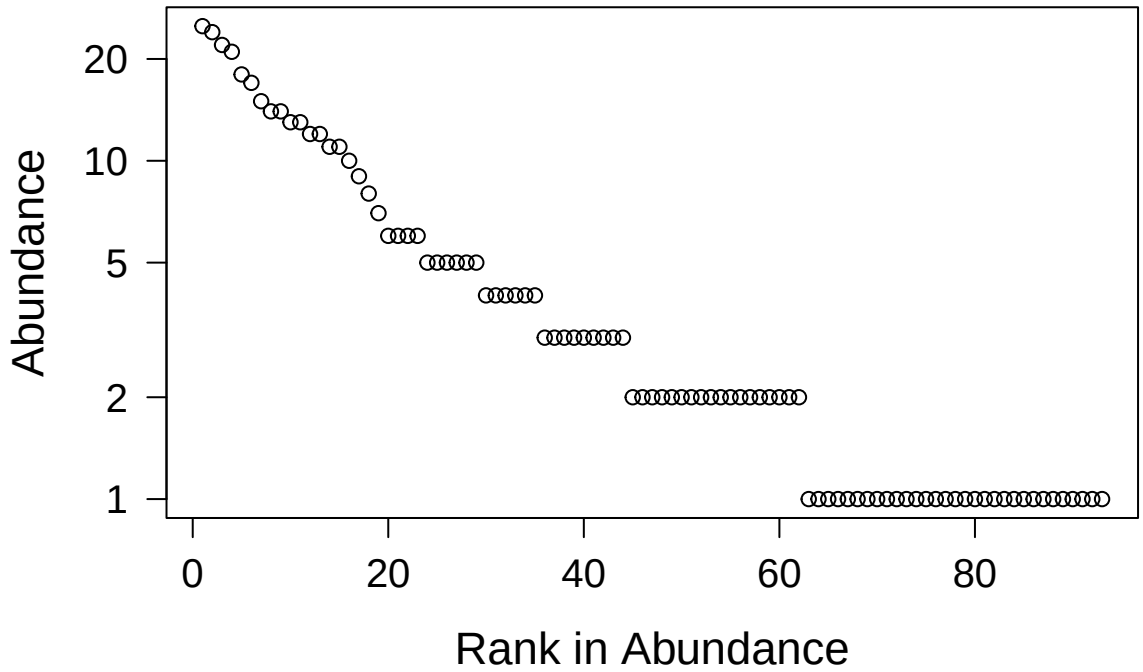
In the R code chunk below, do the following:

1. Create a sequence of ranks and plot the RAC with natural-log-transformed abundances,
2. Label the x-axis "Rank in abundance" and the y-axis "log(abundance)"

```
plot.new()
site1 <- BCI[1,]

rac <- RAC(x = site1)
ranks <- as.vector(seq(1, length(rac)))
opar <- par(no.readonly = TRUE)
par(mar = c(5.1, 5.1, 4.1, 2.1))
plot(ranks, log(rac), type = 'p', axes = F, xlab = "Rank in Abundance", ylab = "Abundance", las = 1, ce

box()
axis(side = 1, labels = T, cex.axis = 1.25)
axis(side = 2, las = 1, cex.axis = 1.25, labels = c(1, 2, 5, 10, 20), at = log(c(1, 2, 5, 10, 20)))
```



Question 5: What effect does visualizing species abundance data on a log-scaled axis have on how we interpret evenness in the RAC?

Answer 5: By visualizing the species abundance on a log-scaled axis we are able to see that there is an uneven distribution in the abundance.

Now that we have visualized unevenness, it is time to quantify it using Simpson's evenness ($E_{1/D}$) and Smith and Wilson's evenness index (E_{var}).

Simpson's evenness ($E_{1/D}$)

In the R code chunk below, do the following:

1. Write the function to calculate $E_{1/D}$, and
2. Calculate $E_{1/D}$ for `site1`.

```
SimpE <- function(x = ""){
  S <- S.obs(x)
  x = as.data.frame(x)
  D <- diversity(x, "inv")
  E <- (D)/S
  return(E)
}
SimpE(site1) #evenness value is 0.4238
```

```
##         1
## 0.4238232
```

Smith and Wilson's evenness index (E_{var})

In the R code chunk below, please do the following:

1. Write the function to calculate E_{var},
2. Calculate E_{var} for `site1`, and
3. Compare $E_{1/D}$ and E_{var}.

```
Evar <- function(x){
  x <- as.vector(x[x > 0])
  1 - (2/pi) * atan(var(log(x)))
}
Evar(site1) #evenness value is 0.5067
```

```
## [1] 0.5067211
```

Question 6: Compare estimates of evenness for `site1` of BCI using $E_{1/D}$ and E_{var}. Do they agree? If so, why? If not, why? What can you infer from the results.

Answer 6: The estimates of evenness do not agree, though they are close. This may be because the Simpson's evenness can be biased by the more abundant species. The Smith and Wilson function was less biased by adundant species. From these results, I am infering that the abundance of species for both BCI and Soilbac are pretty even.

5) INTEGRATING RICHNESS AND EVENNESS: DIVERSITY METRICS

So far, we have introduced two primary aspects of diversity, i.e., richness and evenness. Here, we will use popular indices to estimate diversity, which explicitly incorporate richness and evenness We will write our own diversity functions and compare them against the functions in `vegan`.

Shannon's diversity (a.k.a., Shannon's entropy)

In the R code chunk below, please do the following:

1. Provide the code for calculating H' (Shannon's diversity),
2. Compare this estimate with the output of `vegan`'s diversity function using method = "shannon".

```
ShanH <- function(x = ""){
  H = 0
  for (n_i in x){
    if(n_i > 0) {
      p = n_i / sum(x)
      H = H - p*log(p)
    }
  }
  return(H)
}
diversity(site1, index = "shannon") #Shannon index is 4.018
```

```
## [1] 4.018412
```

```
ShanH(site1) #Shannon index is 4.018
```

```
## [1] 4.018412
```

Simpson's diversity (or dominance)

In the R code chunk below, please do the following:

1. Provide the code for calculating D (Simpson's diversity),
2. Calculate both the inverse (1/D) and 1 - D,
3. Compare this estimate with the output of `vegan's` diversity function using method = "simp".

```
SimpD <- function(x = ""){
  D = 0
  N = sum(x)
  for (n_i in x) {
    D = D + (n_i^2)/(N^2)
  }
  return(D)
}
D.inv <- 1/SimpD(site1)
D.sub <- 1-SimpD(site1)
diversity(site1, "inv") #value equals 39.415
```

```
## [1] 39.41555
```

```
diversity(site1, "simp") #value equals 0.9746
```

```
## [1] 0.9746293
```

Fisher's α

In the R code chunk below, please do the following:

1. Provide the code for calculating Fisher's α,
2. Calculate Fisher's α for `site1` of BCI.

```
rac <- as.vector(site1[site1 > 0])
invD <- diversity(rac, "inv")
invD #value equals 39.415
```

```
## [1] 39.41555
```

```
Fisher <- fisher.alpha(rac)
Fisher #value equals 35.673
```

```
## [1] 35.67297
```

Question 7: How is Fisher's α different from $E_{H'}$ and E_{var}? What does Fisher's α take into account that $E_{H'}$ and E_{var} do not?

> ***Answer 7***: Fisher's Alpha Diversity is different from Smith and Wilson's Evenness Index and Shannon's Diversity because it generates an estimation while the other two generate metrics. Fisher's takes into account the aspect of sampling error because it is known that when sampling ecological communities we are not observing every single individual.

6) HILL NUMBERS

Remember that we have learned about the advantages of Hill Numbers to measure and compare diversity among samples. We also learned to explore the effects of rare species in a community by examining diversity for a series of exponents q.

Question 8: Using `site1` of BCI and `vegan` package, a) calculate Hill numbers for q exponent 0, 1 and 2 (richness, exponential Shannon's entropy, and inverse Simpson's diversity). b) Interpret the effect of rare species in your community based on the response of diversity to increasing exponent q.

> ***Answer 8a***: ***Answer 8b***:

```
Q0_Hill <- specnumber(site1)
Q0_Hill #value is 93
```

```
##  1
## 93
```

```
Q1_Hill <- exp(diversity(site1, index = "shannon"))
Q1_Hill #value is 55.613
```

```
## [1] 55.6127
```

```
Q2_Hill <- 1 / SimpD(site1)
Q2_Hill #value is 39.415
```

```
## [1] 39.41555
```

```
#The response of diversity to increasing the exponent was a negative correlation. The rare species are
```

##7) MOVING BEYOND UNIVARIATE METRICS OF α DIVERSITY

The diversity metrics that we just learned about attempt to integrate richness and evenness into a single, univariate metric. Although useful, information is invariably lost in this process. If we go back to the rank-abundance curve, we can retrieve additional information – and in some cases – make inferences about the processes influencing the structure of an ecological system.

Species abundance models

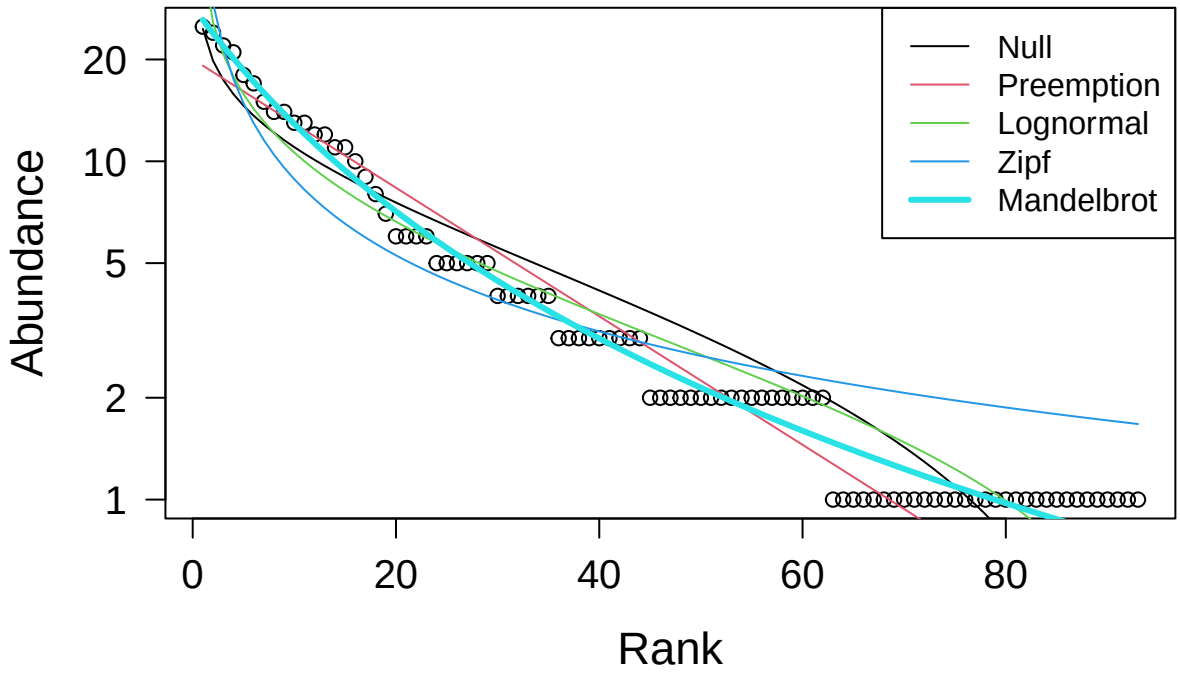
The RAC is a simple data structure that is both a vector of abundances. It is also a row in the site-by-species matrix (minus the zeros, i.e., absences).

Predicting the form of the RAC is the first test that any biodiversity theory must pass and there are no less than 20 models that have attempted to explain the uneven form of the RAC across ecological systems.

In the R code chunk below, please do the following:

1. Use the `radfit()` function in the `vegan` package to fit the predictions of various species abundance models to the RAC of `site1` in BCI,
2. Display the results of the `radfit()` function, and
3. Plot the results of the `radfit()` function using the code provided in the handout.

```
RACresults <- radfit(site1)
plot.new()
plot(RACresults, las = 1, cex.lab = 1.4, cex.axis = 1.25)
```



Question 9: Answer the following questions about the rank abundance curves: a) Based on the output of `radfit()` and plotting above, discuss which model best fits our rank-abundance curve for `site1`? b) Can we make any inferences about the forces, processes, and/or mechanisms influencing the structure of our system, e.g., an ecological community?

> ***Answer 9a***: I believe that the Mandelbrot model would best fit the rank-abundance curve seeing as it follows the line of best fit throughout the site1 data. ***Answer 9b***: The broken stick model asserts the idea that individuals are randomly distributed among species and there are no fitted parameters. There are realistic patterns at play that influence the structure of the system, but the majority is random chance.

Question 10: Answer the following questions about the preemption model: a. What does the preemption model assume about the relationship between total abundance (N) and total resources that can be preempted? b. Why does the niche preemption model look like a straight line in the RAD plot?

> ***Answer 10a***: I did not find anything in the literature about the preemption model assuming things about the total resources that can be preempted. From what I read, the preemption model has an estimated parameter that gives the decay rate of abundance per rank. ***Answer 10b***: The preemption model is a non-linear model and always looks like a straight line in a RAD plot.

Question 11: Why is it important to account for the number of parameters a model uses when judging how well it explains a given set of data?

> ***Answer 11***: Like the amount of replicates in an experiment, it is commonly believed that the more options there are to test if the hypothesis is true, the better. This allows for less error.

SYNTHESIS

1. As stated by Magurran (2004) the $D = \sum p_i^2$ derivation of Simpson's Diversity only applies to communities of infinite size. For anything but an infinitely large community, Simpson's Diversity index is calculated as $D = \sum \frac{n_i(n_i-1)}{N(N-1)}$. Assuming a finite community, calculate Simpson's D, 1 - D, and Simpson's inverse (i.e. 1/D) for `site 1` of the BCI site-by-species matrix.

```
SimpD <- function(x = ""){
  D = 0
  N = sum(x)
  for (n_i in x) {
    D = D + (n_i^2)/(N^2)
  }
  return(D)
}
D.inv <- 1/SimpD(site1)
D.sub <- 1-SimpD(site1)
diversity(site1, "inv") #value equals 39.415
```

```
## [1] 39.41555
```

```
diversity(site1, "simp") #value equals 0.9746
```

```
## [1] 0.9746293
```

```
SimpD(site1) #value equals 0.0253
```

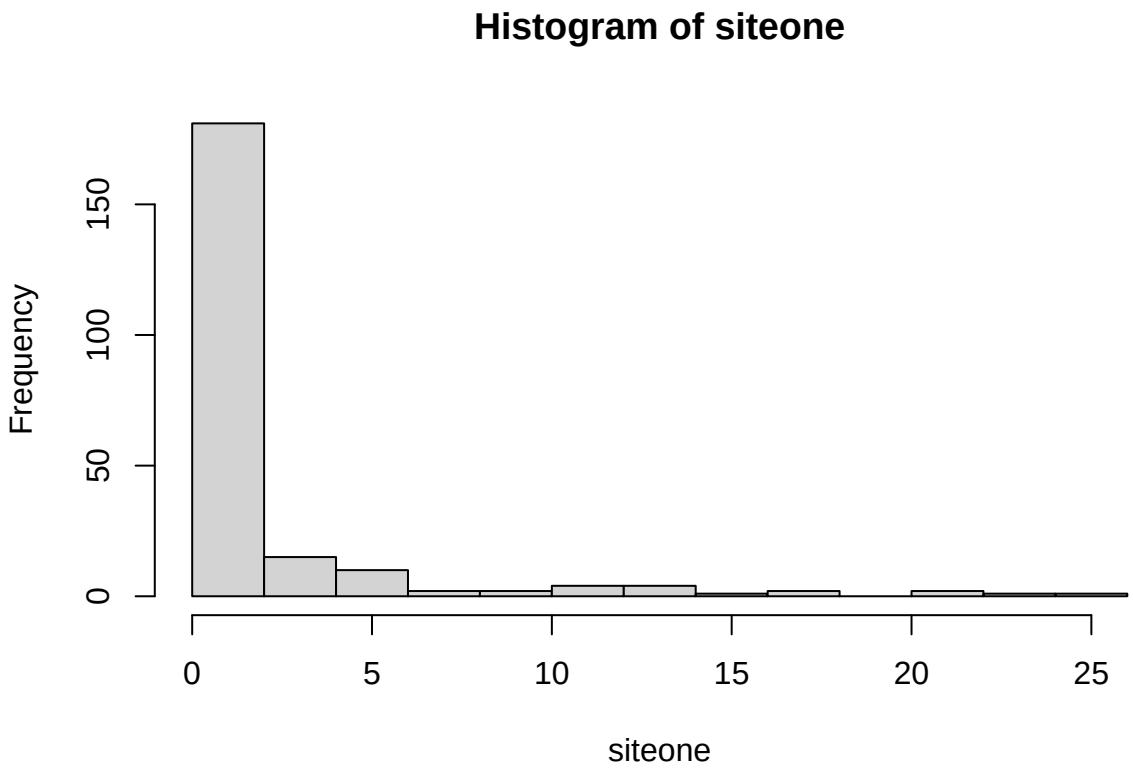
```
## [1] 0.0253707
```

2. Along with the rank-abundance curve (RAC), another way to visualize the distribution of abundance among species is with a histogram (a.k.a., frequency distribution) that shows the frequency of different abundance classes. For example, in a given sample, there may be 10 species represented by a single individual, 8 species with two individuals, 4 species with three individuals, and so on. In fact, the rank-abundance curve and the frequency distribution are the two most common ways to visualize the species-abundance distribution (SAD) and to test species abundance models and biodiversity theories. To address this homework question, use the R function **hist()** to plot the frequency distribution for `site 1` of the BCI site-by-species matrix, and describe the general pattern you see.

```
class(site1) #determining why R won't let me make a histogram
```

```
## [1] "data.frame"
```

```
siteone <- as.numeric(site1) #changing the site one data to numeric
hist(siteone)
```




```
#the general pattern that you can see when you plot the frequency of distribution for site 1 is that th
```

3. We asked you to find a biodiversity dataset with your partner. This data could be one of your own or it could be something that you obtained from the literature. Load that dataset. How many sites are there? How many species are there in the entire site-by-species matrix? Any other interesting observations based on what you learned this week?

```
#loaded dataset is Soil+Inorganic+Nitrogen+.+Early+Sucesional+Microplots
#the amount of sites and species is currently unknown to us and we are working on it
```

SUBMITTING YOUR ASSIGNMENT

Use Knitr to create a PDF of your completed 5.AlphaDiversity_Worksheet.Rmd document, push it to GitHub, and create a pull request. Please make sure your updated repo include both the pdf and RMarkdown files.

Unless otherwise noted, this assignment is due on **Wednesday, January 25th, 2023 at 12:00 PM (noon)**.