Week 3 Exercise

Z620: Quantitative Biodiversity, Indiana University

January 23, 2015

In this exercise, we will explore between site biodiversity. We will start by using the methods we learned for measuring biodiversity within a single site. We will then expand this to learn how to compare communities between communities. We will also learn various statitical tools for testing hypotheses.

A. Initial Setup

Retrieve and Set Your Working Directory

```
rm(list=ls())
getwd()
```

```
## [1] "C:/Users/Mario Muscarella/GitHub/QuantitativeBiodiversity/Assignments/Week3"
```

```
setwd("~/GitHub/QuantitativeBiodiversity/Assignments/Week3")
```

Install Packages

People develop different packages for certain tasks that can be carried out in the R enviornment. This week we will be using a few different R packages. You can use the 'help' funciton to learn more about each package. Let's go ahead and load a few of the packages that we will be using.

```
require("vegan")||install.packages("vegan");require("vegan")
require("ade4")||install.packages("ade4");require("ade4")
require("BiodiversityR")||install.packages("BiodiversityR");require("BiodiversityR")
```

At this point you should be familiar with `vegan`. The other packages (`ade4` and `BiodiversityR`) contain data sets that we will use this week.

User Defined Functions

It help to start scripts with any user defined functions that you know that you will be using. For example, throughout this assignment we will be using the `sem()` function we wrote in week 1. Let's go ahead and define this function.

```
sem <- function(x){
  sd(x, na.rm=TRUE)/sqrt(length(na.omit(x)))
  }
```

Import Data

We will again be using the BCI data set (in `vegan`) for part of this weeks exercises. In addition, we will be using environmental data for the BCI plots. The `BiodiversityR` package has some BCI environmental data (BCI.env). There is additional data avaiable for BCI and your Weeek3 folder has soil data for each plot (bci.soil.txt). We will go ahead and import all of this data now.

```
data(BCI)                                    # BCI Tree Abundance (vegan)
data(BCI.env)                                # BCI Environmental Data (BiodiversityR)
BCI.soil <- read.delim ('./bci.soil.txt')    # BCI Soil Data
```

B. Alpha Diversity (review)

Last week we learned about alpha diversity. We calculated things like species richness, evenness, and diversity. However, as you will soon see, these tools are not always adequate when comparing communities

Let's go back to the BCI dataset and calculate the tree richness across all sites.

```
bci.S <- specnumber(BCI)
mean(bci.S)
```

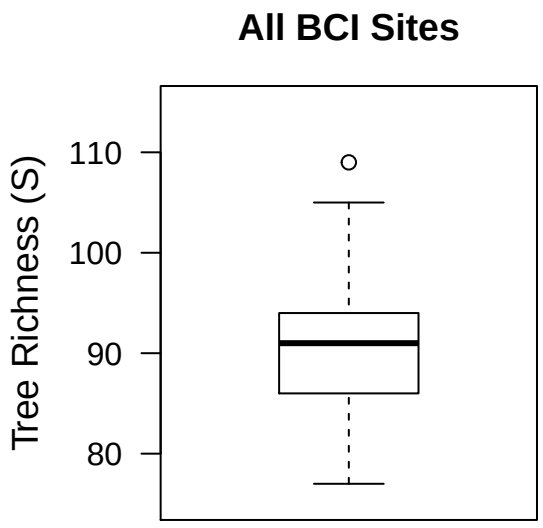
```
## [1] 90.78
```

```
sem(bci.S)
```

```
## [1] 0.9906872
```

We can visualize these data using a box and whisker plot. We are going to start our plot by changing the plotting environment using the `par()` function. Adjust the values in `mar` to see how it changes the plot. You can learn more about the diverse plotting options with the help files (`help(par)`)

```
par(mar=c(0.5, 4.5, 3, 0.5) + 0.1)
boxplot(bci.S, ylim=c(75, 115), las = 1, cex.lab = 1.2,
        main="All BCI Sites", ylab = "Tree Richness (S)")
```



However, this doesn't allow us to compare between site variation. One way we can viualize this is by seperating sites. Since there aren't any factors that seperate BCI sites into groups, we can just plot them based on location (based on XY-coordinates). To make it easy to visualize differences we can color code sites based on tree richness using a heatmap.

In R there are a few tools that allow you to make color pallets. Here we are going to use `terrain.colors` but you can learn about others by looking at the `Pallettes` help file `{r} help(Pallettes)`.

Let's start by making a color pallette for both Richness (S) and Abundance (N)

```
BCI.S.color <- rev(terrain.colors(151))
BCI.N.color <- rev(terrain.colors(41))
```

Notice that we reversed the sequence of colors in the pallette, this was done to make the colors more intuitive. You will have to make these decisions yourself so that your figure is intuitive to readers. Also, notice that we hade to define the number of colors in each pallette. Here we used 151 for S and 41 for N. You can adjust these values if needed. You will see soon why we used odd numbers.

BCI Tree Richness

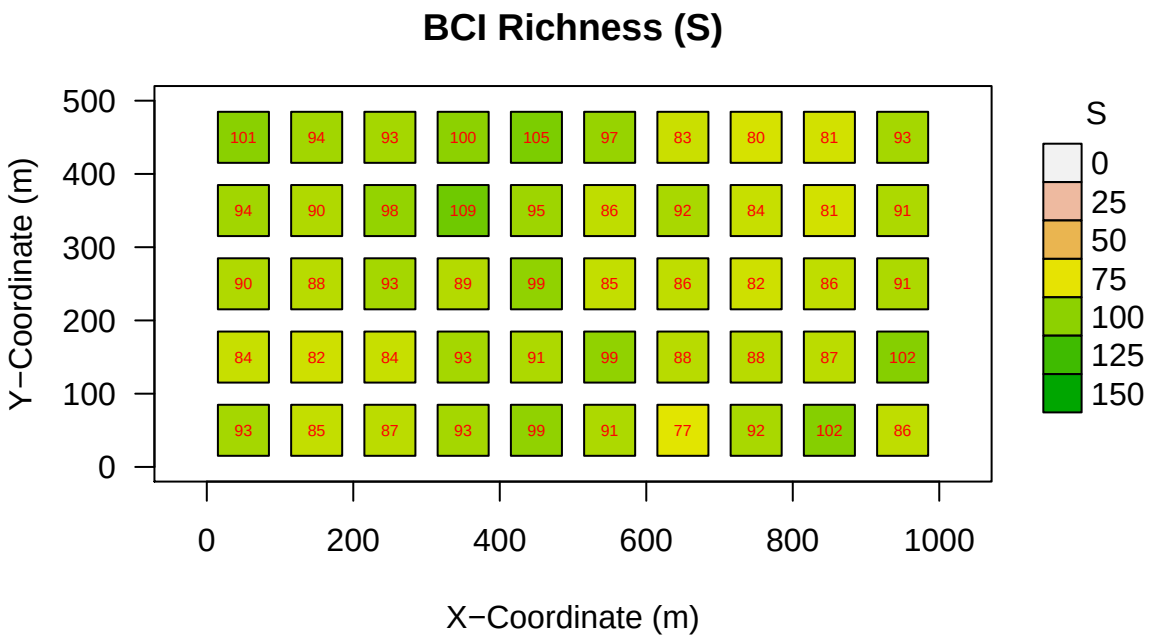
Let's start by plotting tree richness for each plot at BCI. But how do we know how these sites are arranged? Well lucky for us, the BCI soil dataset includes standardized XY-coordinates for each plot

```
head(BCI.soil)
```

```
##     x   y        Al       B       Ca      Cu       Fe         K       Mg
## 1  50  50  901.0908 0.79448 1680.021 6.20312 135.2870 141.88128 279.1291
## 2  50 150  954.2488 0.66968 1503.365 6.03148 141.8080 137.23932 280.4524
## 3  50 250 1114.1122 0.59516 1182.311 6.79768 157.0878  98.69056 230.3973
## 4  50 350 1023.5793 0.56780 1558.020 6.63400 153.1746  98.36412 228.9468
## 5  50 450 1001.8848 0.39876 1242.251 6.44428 149.2509  94.07208 202.6820
## 6 150  50 1091.4672 0.73120 1441.977 6.49552 173.8682 131.89280 276.5010
##         Mn       P      Zn        N   N.min.      pH
## 1 266.9997 1.95248 2.96948 18.46500 -3.88544 4.32432
## 2 320.4786 2.24740 2.53208 21.59896  5.64388 4.37548
## 3 445.0708 1.95484 2.24672 20.24516 -4.06408 4.34700
## 4 407.7580 2.63444 2.44284 20.84232  7.89012 4.46112
## 5 250.5403 1.86356 2.13748 16.94500  8.53716 4.40128
## 6 477.3249 1.61612 2.63148 20.29812  4.38948 4.57252
```

The BCI environmental dataset actually has the UTM coordinates for each plot, but the standarized version is easier to use for our purposes. <– Add info about plotting parameters

```
# BCI Tree Richness
par(mar=c(4,4,3,4) + 0.1, xpd=TRUE)
plot(BCI.soil$x, BCI.soil$y, asp = 1, pch = 22, cex = 4, las = 1,
     bg = BCI.S.color[bci.S + 1],
     xlim = c(0, 1000), ylim = c(0, 500),
     main = "BCI Richness (S)",
     xlab = "X-Coordinate (m)",
     ylab = "Y-Coordinate (m)")
text(BCI.soil$x, BCI.soil$y, bci.S, cex = 0.5, col = "red")
legend("topright", inset = c(-0.2, 0), pch = 22, pt.cex = 3, title = "S",
       bty='n', legend = seq(0, 150, 25),
       pt.bg = BCI.S.color[seq(1, 151, 25)])
```

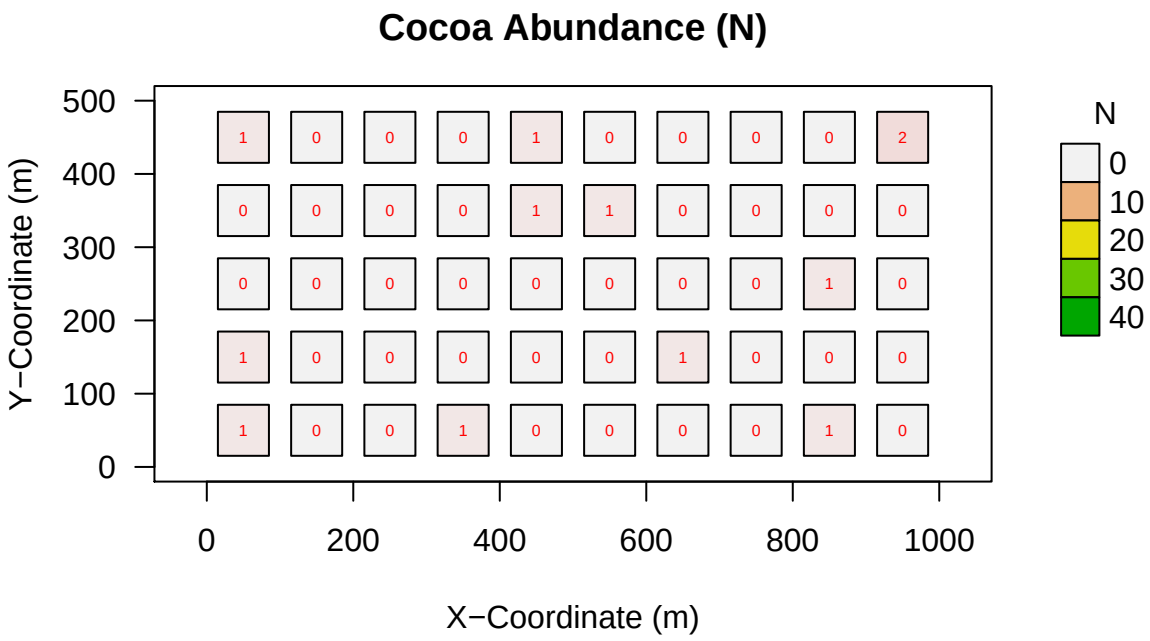


What do you notice when you compare all 50 sites? Is this a good way to compare the sites?

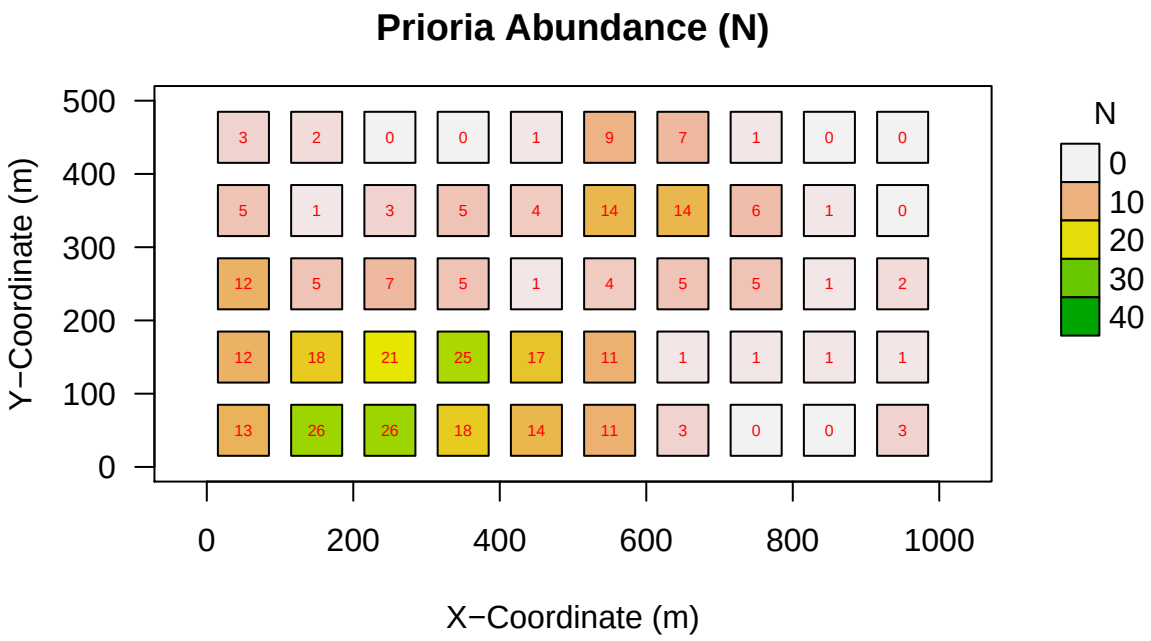
BCI Individual Tree Abunance

What would happen if we looked at only specific tree species? Let's use a similar plotting method and just focus on the abundance of two specific trees: the Cocoa tree and the Prioria tree. We can use the abundance heatmap pallette we created earlier.

```
# BCI Cocoa Abundance
par(mar=c(4,4,3,4) + 0.1, xpd=TRUE)
plot(BCI.soil$x, BCI.soil$y, asp = 1, pch = 22, cex = 4, las = 1,
     bg = BCI.N.color[BCI$Theobroma.cacao + 1],
     xlim = c(0, 1000), ylim = c(0, 500),
     main = "Cocoa Abundance (N)",
     xlab = "X-Coordinate (m)",
     ylab = "Y-Coordinate (m)")
text(BCI.soil$x, BCI.soil$y, BCI$Theobroma.cacao, cex = 0.5, col = "red")
legend("topright", inset=c(-0.2, 0), pch=22, pt.cex=3, bty='n',
       title="N", legend=seq(0, 40, 10),
       pt.bg = BCI.N.color[seq(1, 41, 10)])
```




```
# BCI Prioria Abundance
par(mar=c(4,4,3,4) + 0.1, xpd=TRUE)
plot(BCI.soil$x, BCI.soil$y, asp = 1, pch = 22, cex = 4, las = 1,
     bg = BCI.N.color[BCI$Prioria.copaifera + 1],
     xlim = c(0,1000), ylim = c(0, 500),
     main = "Prioria Abundance (N)",
     xlab = "X-Coordinate (m)",
     ylab = "Y-Coordinate (m)")
text(BCI.soil$x, BCI.soil$y, BCI$Prioria.copaifera, cex = 0.5, col="red")
legend("topright", inset=c(-0.2, 0), pch=22, pt.cex=3, bty='n',
       title="N", legend=seq(0, 40, 10),
       pt.bg = BCI.N.color[seq(1, 41, 10)])
```



What do you observe across BCI?

*** Possible Homework: Create similar plots but for Evenness and Diversity (you pick the measure, but justify)

Doubs River Fish Abundance

Similarly, some datasets might show variation in alpha diversity across samples or sites. The doubs data set in the `ade4` package has fish abundances, environmental variables, and spatial cooridinates for 30 sites in the Doubs River (runs near the France-Switzerland boarder in the Jura Mountains). This data set has been used to show that fish communities can be a good indicator of ecological zones in rivers and streams. We will use this data for similar purposes throughout today's exercise.

First we need to import the data from the `ade4`package. We can also look at the dataset to see what it contains. We will use the `{r} str()` function to see what information is in the dataset.

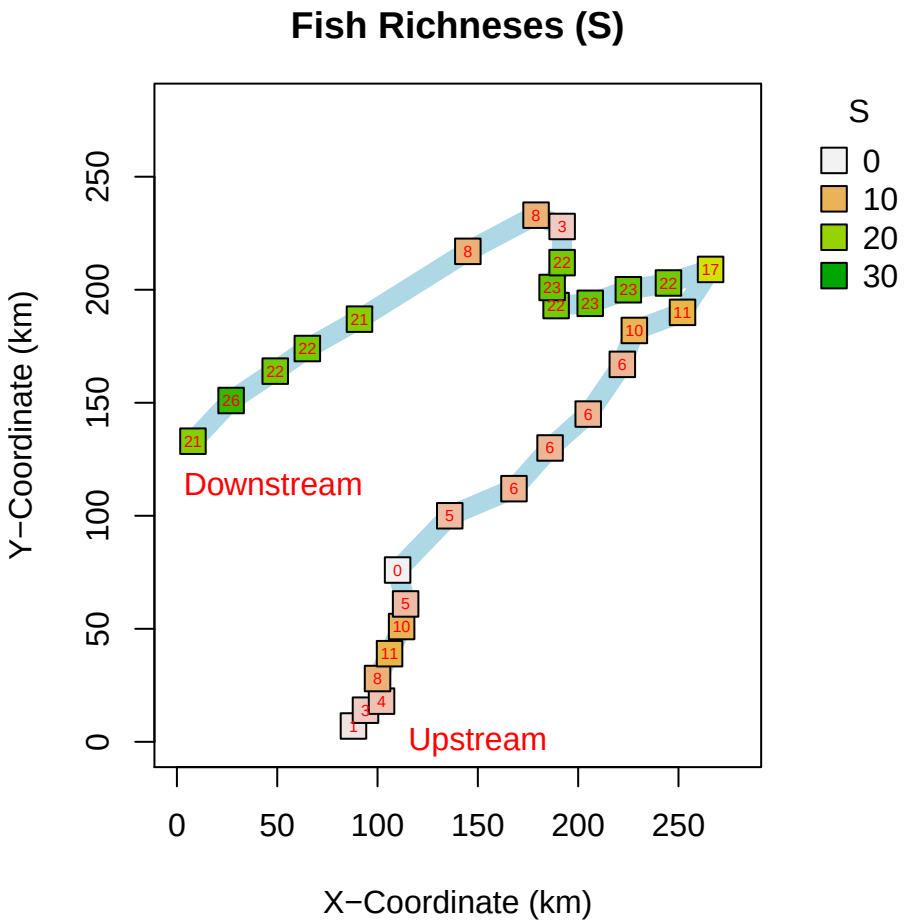
```
data(doubs)
str(doubs, max.level = 1)                # What does max.level do?
```

```
## List of 4
##  $ env    :'data.frame': 30 obs. of  11 variables:
##  $ fish   :'data.frame': 30 obs. of  27 variables:
##  $ xy     :'data.frame': 30 obs. of  2 variables:
##  $ species:'data.frame': 27 obs. of  4 variables:
```

Notice that the doubs data set is actully a list with 4 components (have we worked with this data structure yet?). The first component is the environmental data for each of the 30 sites (doubs$env). There are 11 environmental variables in this dataset. See the help file for more information on each (including units). The second component are the abundances at each site for 27 fish species. The third component has the xy spatial coordinates for each site. The last componet contains the names of each fish species.

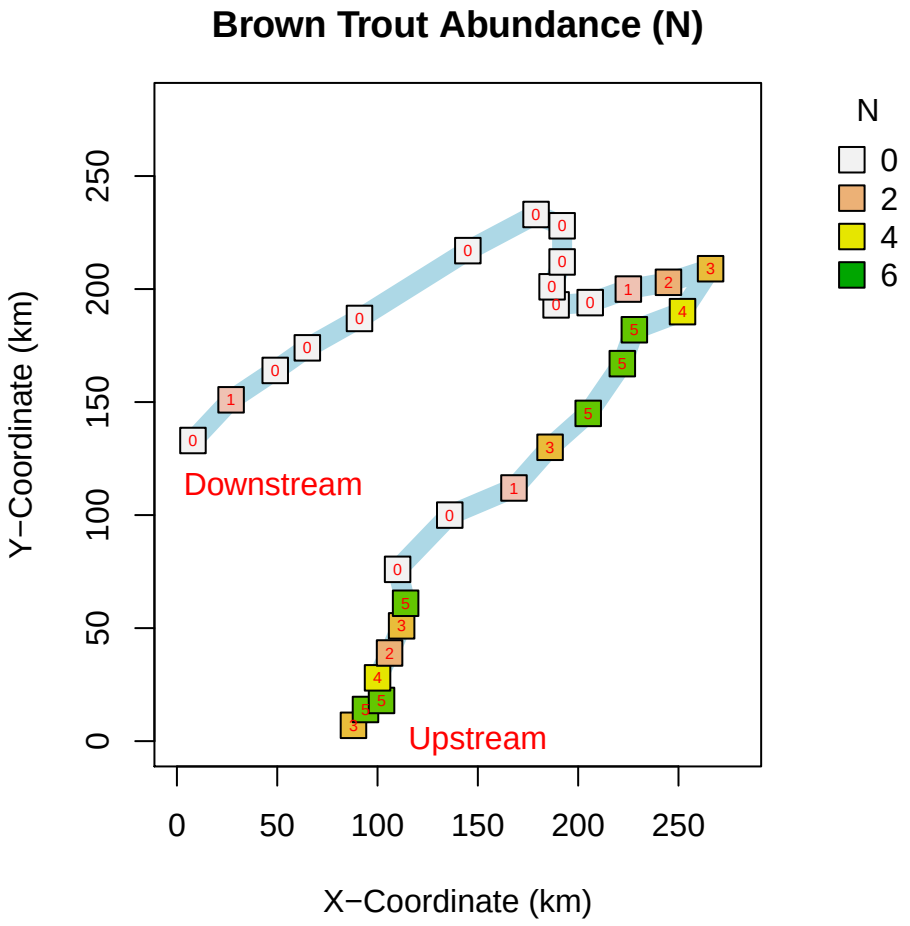
Let's plot fish richness in these stream communities. We will first need to calculate richness and define our color pallettes. Once we have these items we can plot stream fish richness in a similar fashion as we did with BCI tree communities.

```r
# Stream Fish
par(mar = c(4, 4, 3, 5) + 0.1, xpd = TRUE)       # Define Plot Parameters
spa.S <- specnumber(doubs$fish)            # Calculate Richness
spa.S.color <- rev(terrain.colors(31))     # Define Richness Color Pallette
spa.N.color <- rev(terrain.colors(7))      # Define Abundance Color Pallette

# Stream Fish Richness
plot(doubs$xy, type = 'l', col = "light blue", lwd = 10,
     xlim = c(0,280), ylim = c(0,280),
     main = "Fish Richneses (S)",
     xlab = "X-Coordinate (km)",
     ylab = "Y-Coordinate (km)")
points(doubs$xy, pch = 22, cex=2, bg = spa.S.color[spa.S + 1])
text(doubs$xy, as.character(spa.S), cex = 0.5, col="red")
text(150, 0, "Upstream", cex = 1, col = "red")
text(48, 114, "Downstream", cex = 1, col = "red")
legend("topright", inset=c(-0.25, 0), pch = 22, pt.cex = 2, bty = 'n',
       title = "S", legend = seq(0, 30, 10),
       pt.bg = spa.S.color[seq(1, 31, 10)])
```



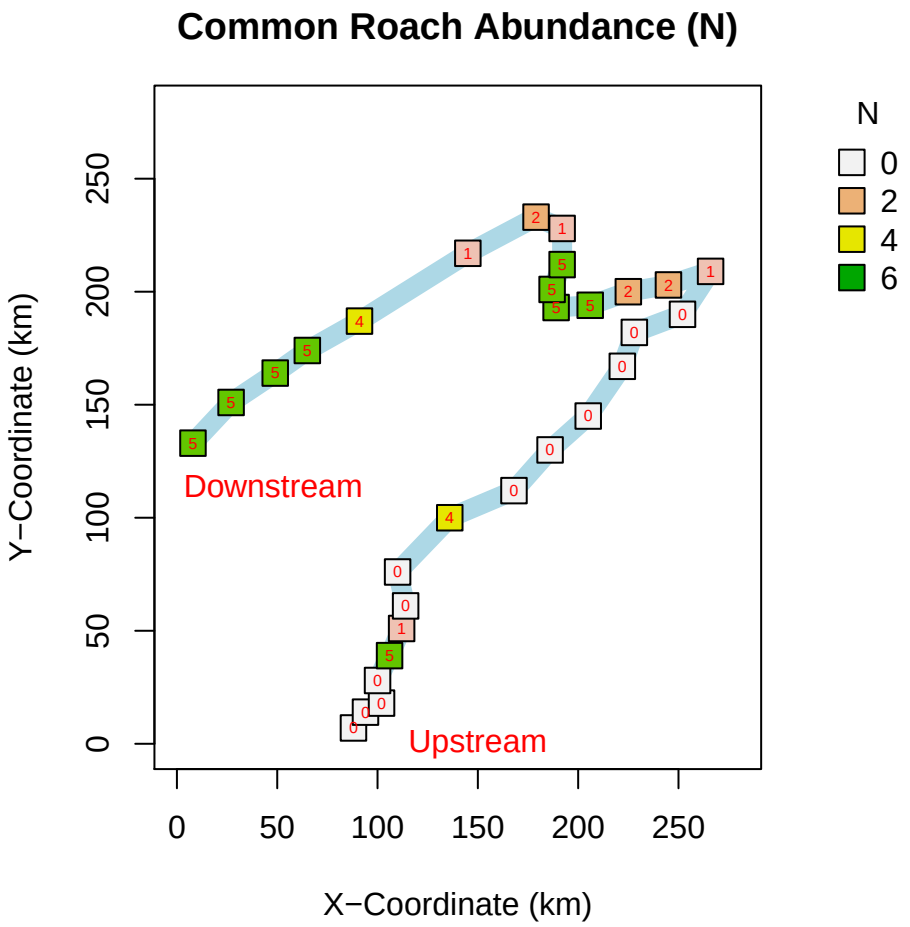

```r
# Brown Trout Abundance
plot(doubs$xy, type = 'l', col = "light blue", lwd = 10,
```

```
     xlim = c(0, 280), ylim = c(0, 280),
     main = "Brown Trout Abundance (N)",
     xlab = "X-Coordinate (km)",
     ylab = "Y-Coordinate (km)")
points(doubs$xy, pch = 22, cex=2, bg = spa.N.color[doubs$fish$Satr + 1])
text(doubs$xy, as.character(doubs$fish$Satr), cex = 0.5, col="red")
text(150, 0, "Upstream", cex=1, col="red")
text(48, 114, "Downstream", cex=1, col="red")
legend("topright", inset=c(-0.25, 0), pch = 22, pt.cex = 2, bty = 'n',
       title = "N", legend = seq(0, 6, 2),
       pt.bg = spa.N.color[seq(1, 7, 2)])
```




```
# Common Roach Abundance
plot(doubs$xy, type = 'l', col = "light blue", lwd = 10,
     xlim = c(0, 280), ylim = c(0, 280),
     main = "Common Roach Abundance (N)",
     xlab = "X-Coordinate (km)",
     ylab = "Y-Coordinate (km)")
points(doubs$xy, pch = 22, cex=2, bg = spa.N.color[doubs$fish$Ruru + 1])
text(doubs$xy, as.character(doubs$fish$Ruru), cex = 0.5, col="red")
text(150, 0, "Upstream", cex = 1, col = "red")
text(48, 114, "Downstream", cex = 1, col = "red")
```

```
legend("topright", inset=c(-0.25, 0), pch = 22, pt.cex = 2, bty = 'n',
       title = "N", legend = seq(0, 6, 2),
       pt.bg = spa.N.color[seq(1, 7, 2)])
```



How does this dataset differ from the BCI data? Is richness the most appropriate tool to compare communities? What does it miss?

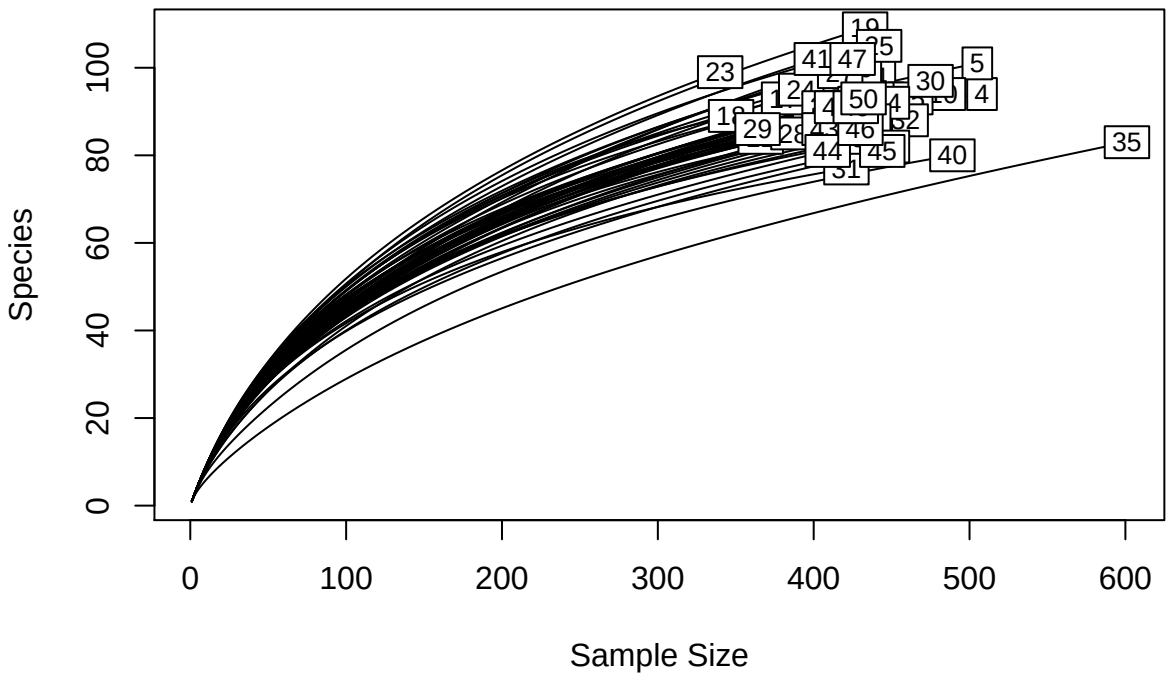
This week we are going to compare the diversity communities across sites. We will start by comparing diversity across sites. We will learn about the issues associated with making these comparisons and sampling.

*** We need a statement about sampling. See Chap 5 in Magurran

Comparing Communities with Rarefaction

First, we can plot the number of

```
rarecurve(BCI)
```



So sampling effor is not the same for all of the plot. Remember that in the BCI plots all individuals are surveyed. However, Cannon **et al.** 1998 points out that we can easily confused richness and individual density when we make such observations. So, we can standarize the number of individuals in each plot with a technique known as rarefaction

Often, it is common to rarefy all samples to the lowest abundance

```
min(apply(BCI, 1, sum))
```

```
## [1] 340
```

```
rarefy(BCI, 340, se = TRUE)[, 1:4]        # We can just look at a few to compare
```

```
##            1         2         3         4
## S  84.339919 76.531650 79.115036 82.465714
## se  2.470344  2.325785  2.708913  2.735269
```

C. Beta Diversity

Beta diversity is a measure of between-habitat diversity. <– We need more details here

Turnover

One way to think about beta diversity is the change in communities over time and space. This concept is generally refered to as *turnover*.

$t = \frac{b+c}{S_1+S_2}$ Is this the type of turnover we want to calculate?

Other measures of betadiversity

One of the classic measures of β diversity was developed by Whittaker (1960): $\beta_W = \frac{S}{\bar{\alpha}}$ where S = the total number of species recorded in a system and $\bar{\alpha}$ os the average sample richness

```
S <- specnumber(colSums(BCI[1:2,]))
a.avg <- mean(specnumber(BCI[1:2,]))
B <- S/a.avg
B - 1
```

```
## [1] 0.2768362
```

β_w ranges from 0 (minimum β diversity) to 1 (maximum β diverstity). However, this measure works best for comparing two sites.

Measures of compositional similarity

When comparing multiple communities, measures of resemblance (similarity). These measures can either be based on incidence (presence-absence; qualitative) data, or abundance (absolute or total; quantitative) data.

	Index	Equation	Properties	Reference
1. Incidence-Based	Jaccard	$S_7 = \frac{a}{a+b+c}$		Jaccard 1900
	Sørensen	$S_8 = \frac{2a}{(2a+b+c)}$		Sørensen 1948

where a = the number of shared speces, b = the number of unique species in the first assemblage, and c = the number of unique species in the second assemblage

	Index	Equation	Properties	Re
2. Abundance-Based	Bray-Curtis Dissimilarity	$D_{14} = \frac{\sum_{j=1}^{p} \lvert y_{1j} \cdot y_{2j} \rvert}{\sum_{j=1}^{p} (y_{1j} + y_{2j})}$		
	Chord Distance	$D_3 = \sqrt{2(1 - \frac{\sum_{j=1}^{p} y_{1j} \cdot y_{2j}}{\sum_{j=1}^{p} y_{1j}^2 \cdot \sum_{j=1}^{p} y_{2j}^2})}$	Range: $\sqrt{2}$ (no species in common) to 0 (two sites share the sampe speces in the sampe proportions)	Orl
	Chi-Squared Distance	$D_16 = \frac{1}{2}$		

3. Other Measures (may not be appropriate for species abundaces) |Euclidean

Calculate Bray Curtis Dissimilarity

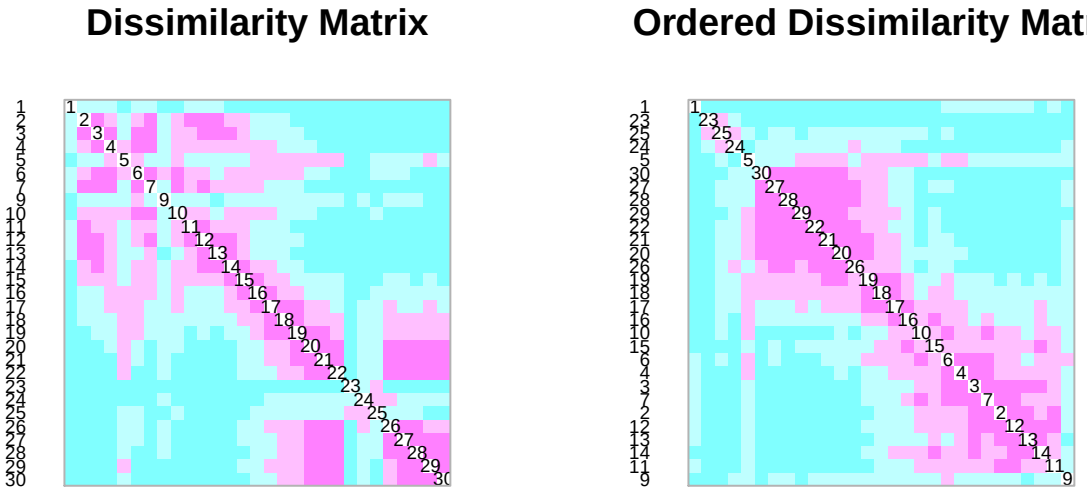
Many of these similarity metrixs are in the vegan `vegdist()` function

```
spe <- doubs$fish
env <- doubs$env

# It is always good to check your sites
rowSums(spe)    # Notice site 8 is empty
```

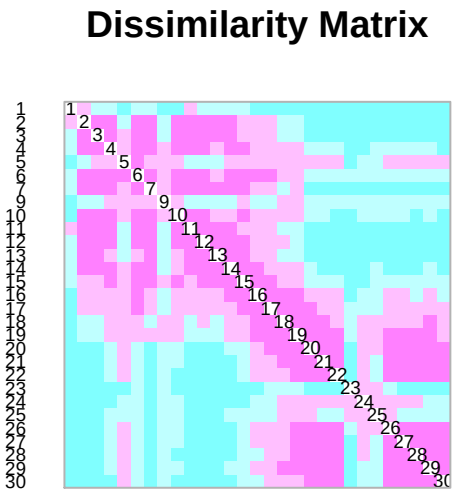
```
##  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
##  3 12 16 21 34 21 16  0 14 14 11 18 19 28 33 40 44 42 46 56 62 72  4 15 11
## 26 27 28 29 30
## 43 63 70 87 89
```

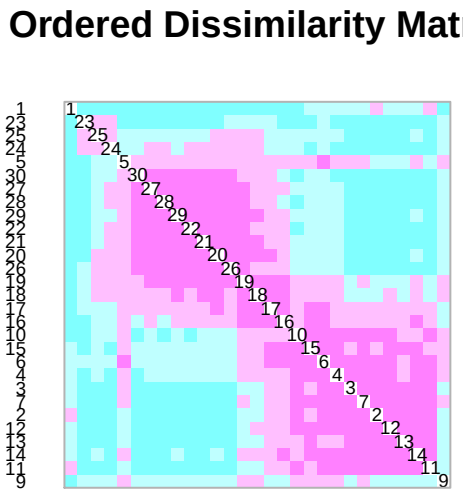
```
spe <- spe[-8, ]
env <- env[-8, ]

# Calculate Bray-Curtis Dissimilarty between doubs river sites
spe.db <- vegdist(spe, method="bray")

# Calculate Jaccard Dissimilarty
spe.dj <- vegdist(spe, method="jaccard", binary=TRUE)

# Visualize association matrices
source("coldiss.R")
coldiss(spe.db, byrank=FALSE, diag=TRUE)
```

```
## Loading required package: gclus
## Loading required package: cluster
```




```
coldiss(spe.db, byrank=TRUE, diag=TRUE)
```



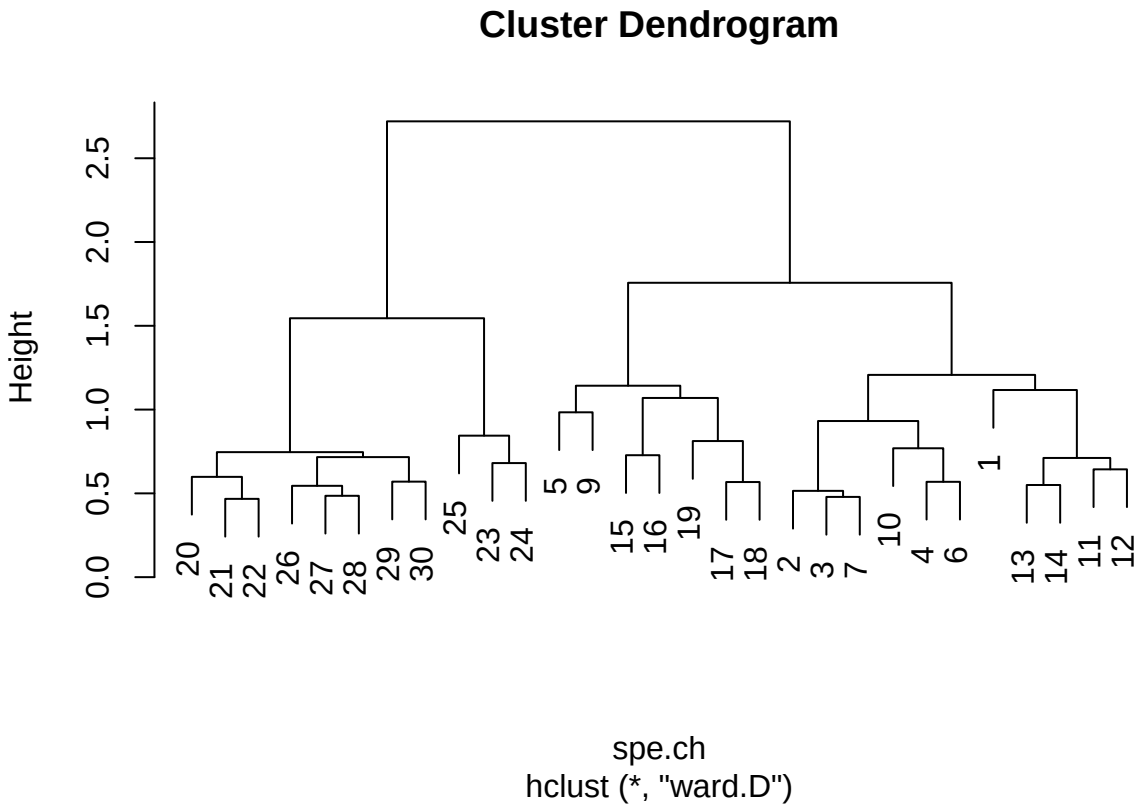


D. Visualizaton

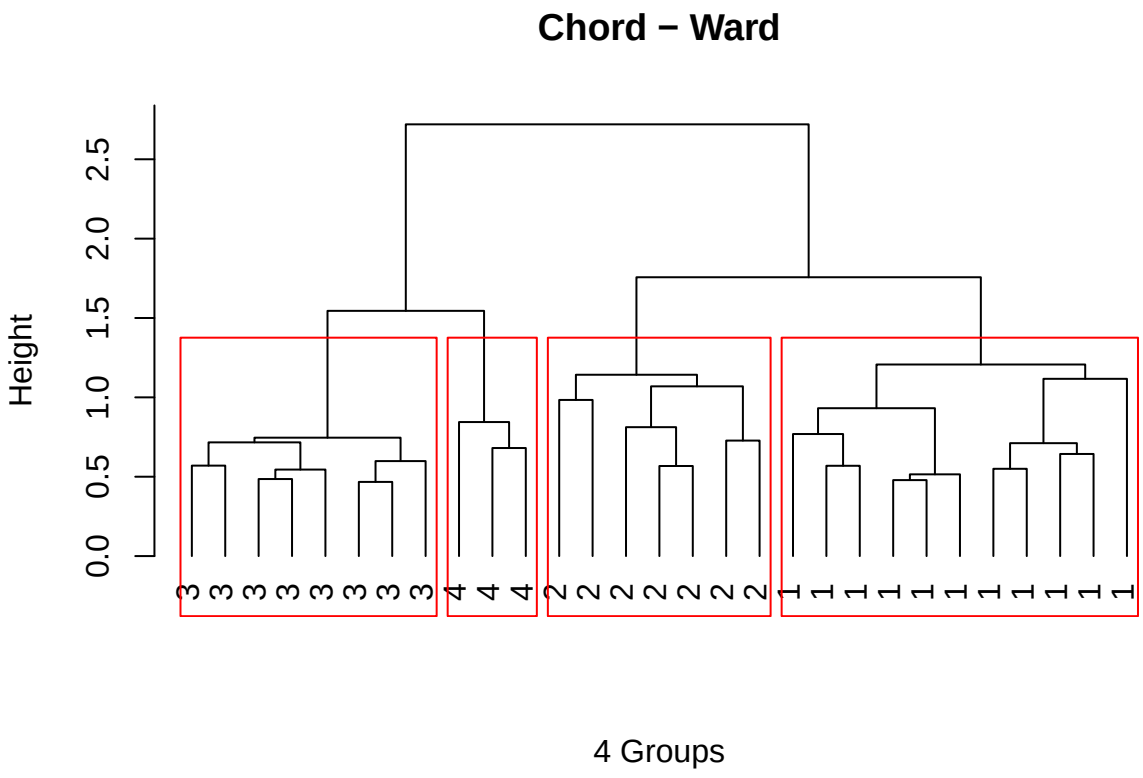
Cluster Analysis

Hierarchial Clustering (Ward Clustering)

```
spe.norm <- decostand(spe, "normalize")
spe.ch <- vegdist(spe.norm, "euc")
spe.ch.ward <- hclust(spe.ch, method="ward.D")
spe.ch.ward$height <- sqrt(spe.ch.ward$height)
plot(spe.ch.ward)
```




```
# Groups
spe.chwo <- reorder.hclust(spe.ch.ward, spe.ch)
plot(spe.chwo, hang=-1, xlab="4 Groups", sub="", ylab="Height", main="Chord - Ward", labels=cutree(spe.
rect.hclust(spe.chwo, k=4)
```



Visualization using Ordination

Various techniques | Method | Distance Preserved | Variables | | Principapl component analysis (PCA) | Euclidean distance | Quantiative data, linear relationships | | Correspondence analysis (CA) | χ^2 distance | Non-negative, dimensionally homogeneous quantita6tive or binary data; species frequencies or presence/absence data | | Principal coordinate analysis (PCoA), metric (multidimensional) scalling, classical scaling | Any distance measure | Quantitative, semiquantitative, qualitative, or mixed | |Nonmetric multidimensional scalling (nMDS) | Any distance measure | Quantitative, semiquantitative, qualitative, or mixed |

(REF: L&L , Table 9.1)

How does ordination work?

NMDS

```
spe.nmds <- metaMDS(spe, distance="bray")
```

```
## Run 0 stress 0.07477822
## Run 1 stress 0.08841675
## Run 2 stress 0.1196716
## Run 3 stress 0.1176196
## Run 4 stress 0.0898819
## Run 5 stress 0.08695583
## Run 6 stress 0.1204806
```

```
## Run 7 stress 0.1133756
## Run 8 stress 0.1209556
## Run 9 stress 0.08843915
## Run 10 stress 0.07376475
## ... New best solution
## ... procrustes: rmse 0.01947969  max resid 0.09468015
## Run 11 stress 0.1243961
## Run 12 stress 0.118898
## Run 13 stress 0.07477799
## Run 14 stress 0.1219309
## Run 15 stress 0.1234437
## Run 16 stress 0.08695648
## Run 17 stress 0.1226497
## Run 18 stress 0.1203766
## Run 19 stress 0.111104
## Run 20 stress 0.07477896
```

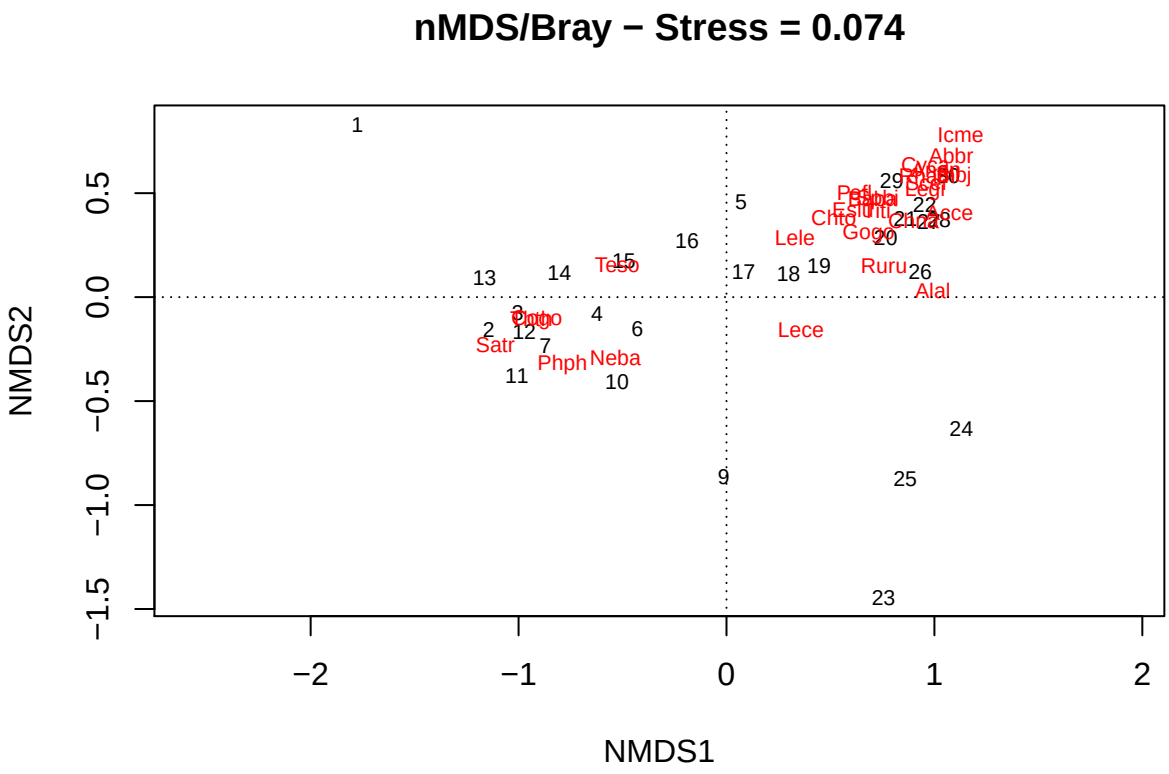
```
spe.nmds
```

```
##
## Call:
## metaMDS(comm = spe, distance = "bray")
##
## global Multidimensional Scaling using monoMDS
##
## Data:     spe
## Distance: bray
##
## Dimensions: 2
## Stress:     0.07376475
## Stress type 1, weak ties
## No convergent solutions - best solution after 20 tries
## Scaling: centring, PC rotation, halfchange scaling
## Species: expanded scores based on 'spe'
```

```
spe.nmds$stress
```

```
## [1] 0.07376475
```

```
plot(spe.nmds, type="t", main=paste("nMDS/Bray - Stress =", round(spe.nmds$stress, 3)))
abline(h=0, lty=3)
abline(v=0, lty=3)
```



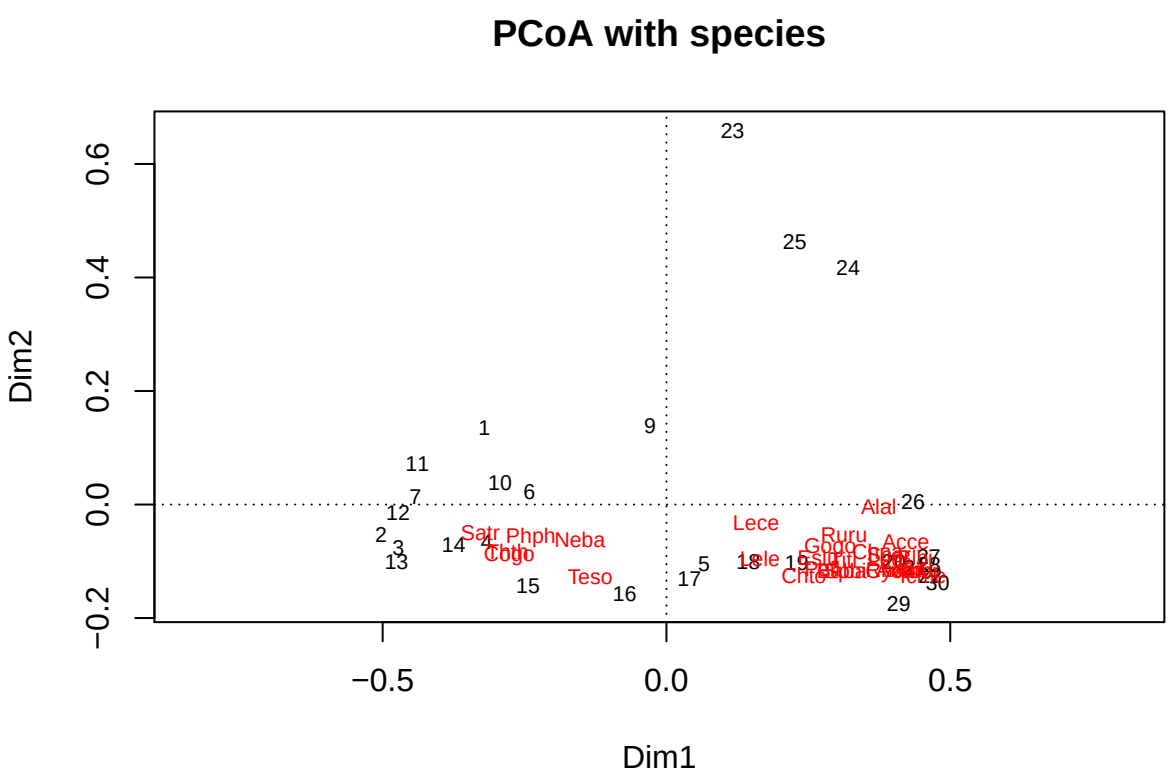
What is the nMDS stress? How is this used to judge the quality of the ordination

Classic Multidemensional Scalling (PCoA)

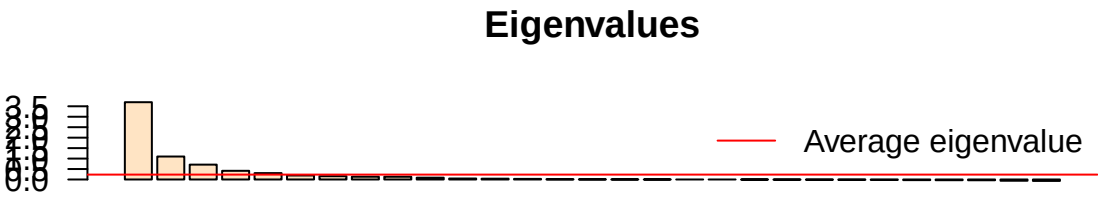
```
spe.bray <- vegdist(spe, method="bray")
spe.b.pcoa <- cmdscale(spe.bray, eig=TRUE)

ordiplot(scores(spe.b.pcoa)[,c(1,2)], type='t', main="PCoA with species")
```

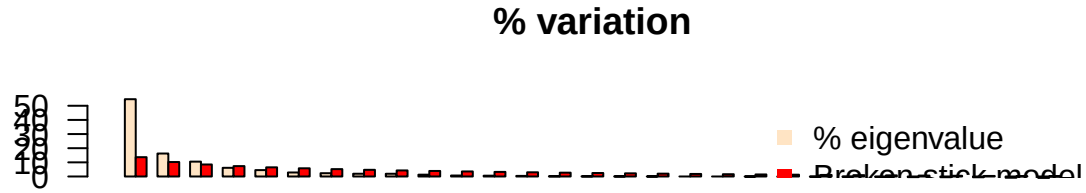
```
## Warning in ordiplot(scores(spe.b.pcoa)[, c(1, 2)], type = "t", main =
## "PCoA with species"): Species scores not available
```

```
abline(h=0, lty=3)
abline(v=0, lty=3)

# Add Species
spe.wa <- wascores(spe.b.pcoa$points[,1:2], spe)
text(spe.wa, rownames(spe.wa), cex=0.7, col="red")
```




```
evplot <- function(ev)
{
  # Broken stick model (MacArthur 1957)
    n <- length(ev)
    bsm <- data.frame(j=seq(1:n), p=0)
    bsm$p[1] <- 1/n
    for (i in 2:n) bsm$p[i] <- bsm$p[i-1] + (1/(n + 1 - i))
    bsm$p <- 100*bsm$p/n
    # Plot eigenvalues and % of variation for each axis
    op <- par(mfrow=c(2,1))
    barplot(ev, main="Eigenvalues", col="bisque", las=2)
    abline(h=mean(ev), col="red")
    legend("topright", "Average eigenvalue", lwd=1, col=2, bty="n")
    barplot(t(cbind(100*ev/sum(ev), bsm$p[n:1])), beside=TRUE,
        main="% variation", col=c("bisque",2), las=2)
    legend("topright", c("% eigenvalue", "Broken stick model"),
        pch=15, col=c("bisque",2), bty="n")
    par(op)
}

evplot(spe.b.pcoa$eig)



spe.pcoa.env <- envfit(spe.b.pcoa, env)
evplot(spe.b.pcoa$eig)
```





Why not PCA?

Constrained Ordination

```
# subset explanatory varaibles
envdas <- env[,1]
envtopo <- env[,c(2:4)]
envchem <- env[,c(5:11)]
spe.hel <- decostand(spe, method="hellinger")

spe.rda <- rda(spe.hel ~ ., envchem)
coef(spe.rda)
```

```
##              RDA1          RDA2          RDA3         RDA4         RDA5
## pH   0.0020384098 -0.0031031275 -0.0190886957 -0.016823415  0.004127536
## har -0.0011912221  0.0007238813 -0.0066409901 -0.010576449  0.002295200
## pho  0.0008030375  0.0006560255  0.0002170545  0.002050325  0.007613774
## nit -0.0011933640  0.0011698470 -0.0005397298  0.001754538 -0.001273360
## amm  0.0024457337 -0.0049289180  0.0006951098 -0.004139305 -0.004732773
## oxy  0.0066262263 -0.0008245053 -0.0127449854  0.006086134 -0.005810343
## bdo -0.0002256933 -0.0042878090 -0.0058318080 -0.000140698 -0.011305550
##              RDA6          RDA7
```

```
## pH  -0.0455461425  0.0996685848
## har  0.0074031347 -0.0009897574
## pho -0.0045440954 -0.0038977199
## nit -0.0008977745 -0.0002185856
## amm  0.0193330610  0.0115315915
## oxy -0.0015043312 -0.0046358191
## bdo -0.0080555706 -0.0033015665
```

```
spechem.physio <- rda(spe.hel, envchem, envtopo)

# Permutatoin Test
anova.cca(spe.rda, step=1000)
```

```
## Permutation test for rda under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: rda(formula = spe.hel ~ pH + har + pho + nit + amm + oxy + bdo, data = envchem)
##          Df Variance      F Pr(>F)
## Model     7  0.30442 4.6102  0.001 ***
## Residual 21  0.19809
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Variance Partitioning

```
spe.part.all <- varpart(spe.hel, envchem, envtopo)
```

Hypothesis Testing

Homework