OVERVIEW

Complementing taxonomic measures of α- and β-diversity with evolutionary information yields insight into a broad range of biodiversity issues including conservation, biogeography, and community assembly. In this assignment, you will be introduced to some commonly used methods in phylogenetic community ecology.

After completing this assignment you will know how to:

1. incorporate an evolutionary perspective into your understanding of community ecology
2. quantify and interpret phylogenetic α- and β-diversity
3. evaluate the contribution of phylogeny to spatial patterns of biodiversity

Directions:

1. Change "Student Name" on line 3 (above) with your name.
2. Complete as much of the assignment as possible during class; what you do not complete in class will need to be done outside of 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. Be sure to **answer the questions** in this exercise document; they also correspond to the handout. 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 ">".
5. Before you leave the classroom, **push** this file to your GitHub repo.
6. When you are done, **Knit** the text and code into a PDF file.
7. After Knitting, please submit the completed assignment by creating a **pull request** via GitHub. Your pull request should include this file *PhyloCom_assignment.Rmd* and the PDF output of `Knitr` (*PhyloCom_assignment.pdf*).

1) SETUP

Typically, the first thing you will do in either an R script or an RMarkdown file is setup your environment. This includes things such as setting the working directory and loading any packages that you will need.

In the R code chunk below, provide the code to:
1. clear your R environment,
2. print your current working directory,
3. set your working directory to your `/Week7-PhyloCom` folder,
4. load all of the required R packages (be sure to install if needed), and
5. load the required R source file.

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

```
## [1] "C:/Users/Michelle/GitHub/QB2017_Benavidez/Week7-PhyloCom"
```

```r
setwd("C:/Users/Michelle/GitHub/QB2017_Benavidez/Week7-PhyloCom/")

package.list <- c('picante', 'ape', 'seqinr', 'vegan', 'fossil', 'simba')
for (package in package.list) {
  if (!require(package, character.only = TRUE, quietly = TRUE)) {
    install.packages(package, repos='http://cran.us.r-project.org')
    library(package, character.only = TRUE)
  }
}
```

```
## This is vegan 2.4-2
```

```
##
## Attaching package: 'seqinr'
```

```
## The following object is masked from 'package:nlme':
##
##     gls
```

```
## The following object is masked from 'package:permute':
##
##     getType
```

```
## The following objects are masked from 'package:ape':
##
##     as.alignment, consensus
```

```
##
## Attaching package: 'shapefiles'
```

```
## The following objects are masked from 'package:foreign':
##
##     read.dbf, write.dbf
```

```
## This is simba 0.3-5
```

```
##
## Attaching package: 'simba'
```

```
## The following object is masked from 'package:picante':
##
##     mpd
```

```
## The following object is masked from 'package:stats':
##
##     mad
```

```r
source("./bin/MothurTools.R")
```

```
## Loading required package: reshape
```

2) DESCRIPTION OF DATA

We will revisit the data that was used in the Spatial Diversity module. As a reminder, in 2013 we sampled ~ 50 forested ponds located in Brown County State Park, Yellowwood State Park, and Hoosier National Forest in southern Indiana. See the handout for a further description of this week's dataset.

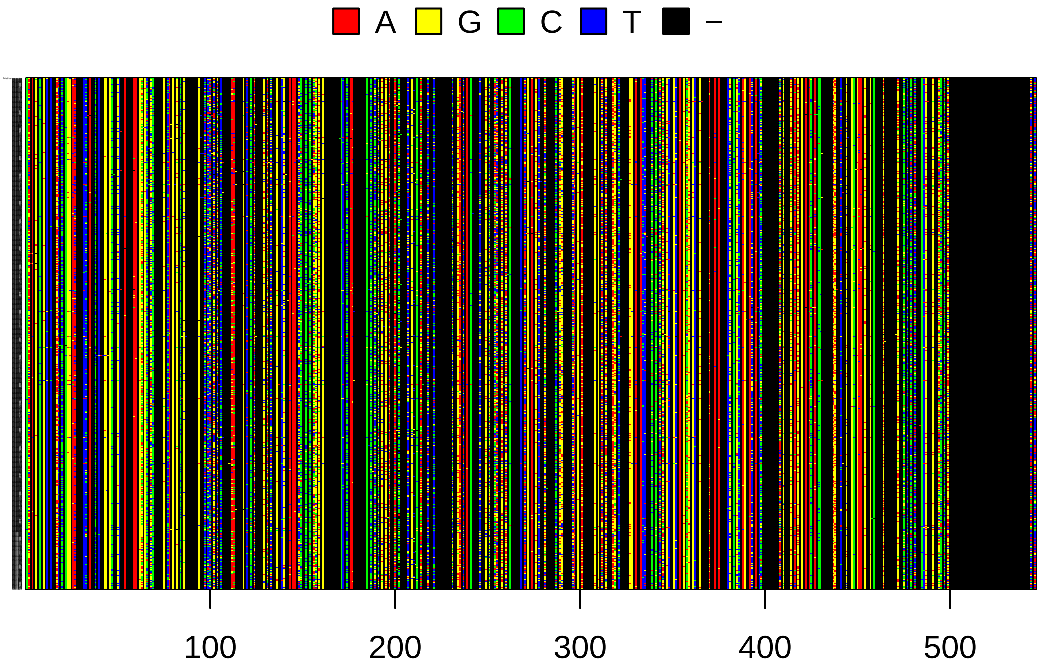
3) LOAD THE DATA

In the R code chunk below, do the following:
1. load the environmental data for the Brown County ponds (*20130801_PondDataMod.csv*),
2. load the site-by-species matrix using the `read.otu()` function,
3. subset the data to include only DNA-based identifications of bacteria,
4. rename the sites by removing extra characters,
5. remove unnecessary OTUs in the site-by-species, and
6. load the taxonomic data using the `read.tax()` function from the source-code file.

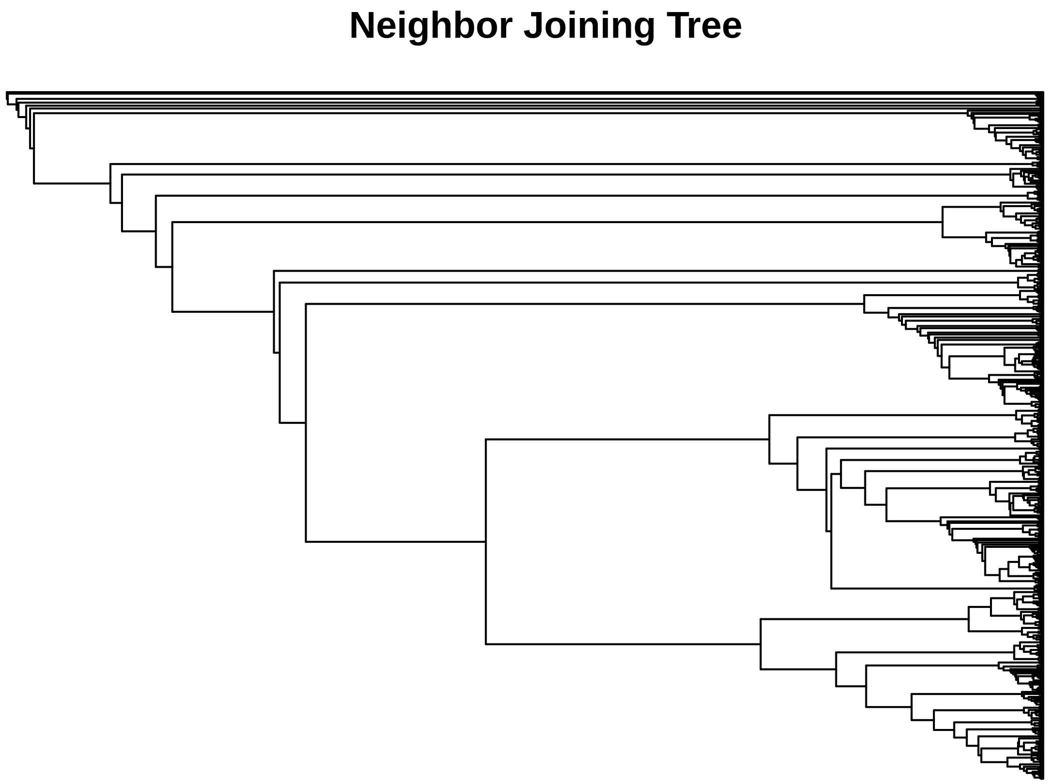
```
env <- read.table("data/20130801_PondDataMod.csv", sep = ",", header = TRUE)
env <- na.omit(env)

comm <- read.otu(shared = "./data/INPonds.final.rdp.shared", cutoff = "1")

comm <- comm[grep("*-DNA", rownames(comm)), ]

rownames(comm) <- gsub("\\-DNA", "", rownames(comm))
rownames(comm) <- gsub("\\_", "", rownames(comm))

comm <- comm[rownames(comm) %in% env$Sample_ID, ]

comm <- comm[ , colSums(comm) > 0]

tax <- read.tax(taxonomy = "./data/INPonds.final.rdp.1.cons.taxonomy")
```

Next, in the R code chunk below, do the following:
1. load the FASTA alignment for the bacterial operational taxonomic units (OTUs),
2. rename the OTUs by removing everything before the tab (\t) and after the bar (|),
3. import the *Methanosarcina* outgroup FASTA file,
4. convert both FASTA files into the DNAbin format and combine using `rbind()`,
5. visualize the sequence alignment,
6. using the alignment (with outgroup), pick a DNA substitution model, and create a phylogenetic distance matrix,
7. using the distance matrix above, make a neighbor joining tree,
8. remove any tips (OTUs) that are not in the community data set,
9. plot the rooted tree.

```
ponds.cons <- read.alignment(file = "./data/INPonds.final.rdp.1.rep.fasta",
                             format = "fasta")

ponds.cons$nam <- gsub("\\|.*$", "", gsub("^.*?\t", "", ponds.cons$nam))

outgroup <- read.alignment(file = "./data/methanosarcina.fasta", format = "fasta")

DNAbin <- rbind(as.DNAbin(outgroup), as.DNAbin(ponds.cons))

image.DNAbin(DNAbin, show.labels=T, cex.lab = 0.05, las = 1)
```



```
seq.dist.jc <- dist.dna(DNAbin, model = "JC", pairwise.deletion = F)

phy.all <- bionj(seq.dist.jc)

phy <- drop.tip(phy.all, phy.all$tip.label[!phy.all$tip.label %in%
                                             c(colnames(comm), "Methanosarcina")])

outgroup <- match("Methanosarcina", phy$tip.label)

phy <- root(phy, outgroup, resolve.root = TRUE)

par(mar = c(1, 1, 2, 1) + 0.1)
plot.phylo(phy, main = "Neighbor Joining Tree", "phylogram", show.tip.label = FALSE,
           use.edge.length = FALSE, direction = "right", cex = 0.6, label.offset = 1)
```



4) PHYLOGENETIC ALPHA DIVERSITY

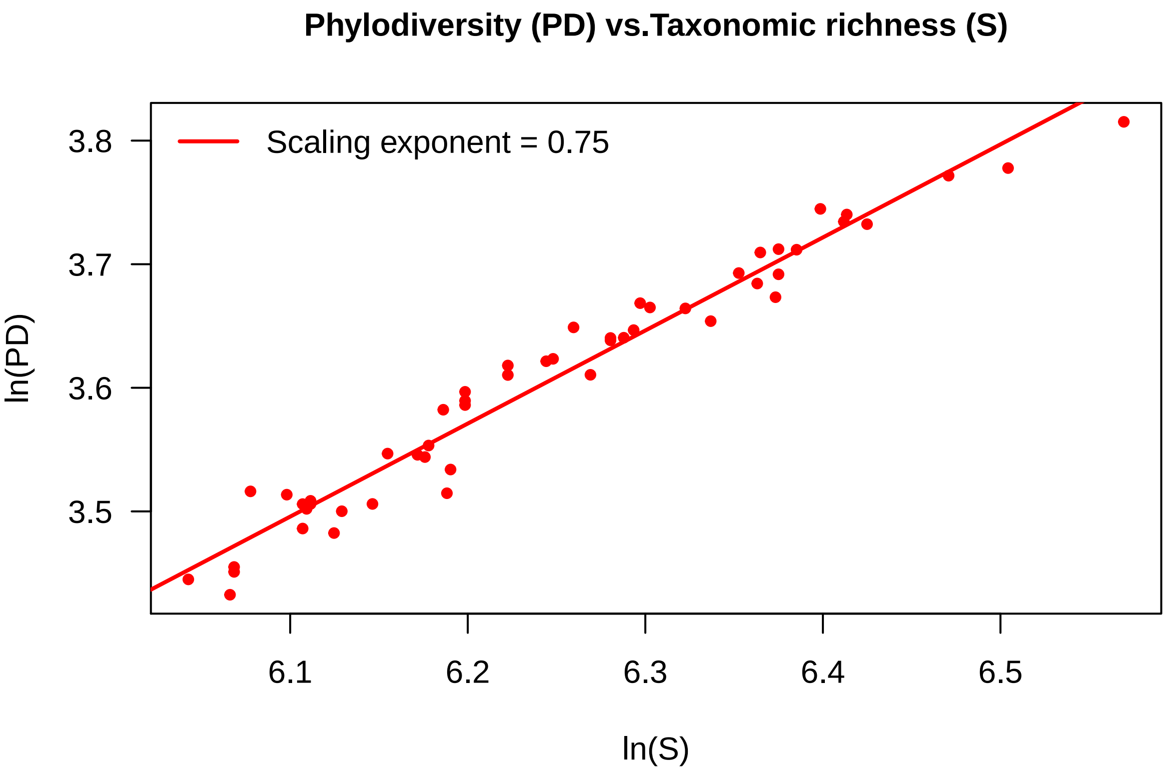
A. Faith's Phylogenetic Diversity (PD)

In the R code chunk below, do the following:
1. calculate Faith's D using the `pd()` function.

```
pd <- pd(comm, phy, include.root = FALSE)
```

In the R code chunk below, do the following:
1. plot species richness (S) versus phylogenetic diversity (PD),
2. add the trend line, and
3. calculate the scaling exponent.

```
par(mar = c(5, 5, 4, 1) + 0.1)

plot(log(pd$S), log(pd$PD),
     pch = 20, col = "red", las = 1,
     xlab = "ln(S)", ylab = "ln(PD)", cex.main = 1,
     main="Phylodiversity (PD) vs.Taxonomic richness (S)")

fit <- lm('log(pd$PD) ~ log(pd$S)')
abline(fit, col = "red", lw = 2)
exponent <- round(coefficients(fit)[2], 2)
legend("topleft", legend=paste("Scaling exponent = ", exponent, sep = ""),
       bty = "n", lw = 2, col = "red")
```



Question 1: Answer the following questions about the PD-S pattern.
a. Based on how PD is calculated, why should this metric be related to taxonmic richness? b. Describe the relationship between taxonomic richness and phylodiversity. c. When would you expect these two estimates of diversity to deviate from one another? d. Interpret the significance of the scaling PD-S scaling exponent.

> ***Answer 1a***: PD is based on incidence data, so when estimating shared branch length sums it is directly associated with evolutionary taxa richness.
> ***Answer 1b***: When you have a larger number of evolutionary divergent taxa in the communitiy, you will have high PD values. When you have fewer divergent taxa, you will have few shared branches; therefore you will have lower PD values.
> ***Answer 1c***: Taxonomic rishness and and phylodivserity should deviate when taxa within the dataset are less phylogenetically divergent. ***Answer 1d***: Pd-S tells us that the relationship between PD (evolutionary diversity metric) and S (ruchness metric) scales to a strength of 0.75.

i. Randomizations and Null Models

In the R code chunk below, do the following:
1. estimate the standardized effect size of PD using the `richness` randomization method.

```
ses.pd <- ses.pd(comm[1:2,], phy, null.model = "richness", runs = 25,
                 include.root = FALSE)

ses.pd.1 <- ses.pd(comm[1:2,], phy, null.model = "independentswap", runs = 25,
                 include.root = FALSE)

ses.pd.2 <- ses.pd(comm[1:2,], phy, null.model = "trialswap", runs = 25,
                 include.root = FALSE)
```

Question 2: Using `help()` and the table above, run the `ses.pd()` function using two other null models and answer the following questions:

a. What are the null and alternative hypotheses you are testing via randomization when calculating `ses.pd`?
b. How did your choice of null model influence your observed ses.pd values? Explain why this choice affected or did not affect the output.

   Answer 2a: Based on randomized null model, the null hypothesis is ses.pd $<$ 0 [i.e. less phylogenetically diverse than expected by chance] and the alternative hypothesis is that ses.pd $>$ 0 [i.e. more phylogenetically diverse than expected by chance]. ***Answer 2b***: The randomly generated mean was higher and the standard deviation was lower than when using the richness null. These changes in turn alter the values of rank, effect size, and p - values.

B. Phylogenetic Dispersion Within a Sample

Another way to assess phylogenetic α-diversity is to look at dispersion within a sample.

i. Phylogenetic Resemblance Matrix

In the R code chunk below, do the following:
1. calculate the phylogenetic resemblance matrix for taxa in the Indiana ponds data set.

```
phydist <- cophenetic.phylo(phy)
```

ii. Net Relatedness Index (NRI)

In the R code chunk below, do the following:
1. Calculate the NRI for each site in the Indiana ponds data set.

```
ses.mpd <- ses.mpd(comm, phydist, null.model = "taxa.labels",
                   abundance.weighted = FALSE, runs = 25)

NRI <- as.matrix(-1 * ((ses.mpd[,2] - ses.mpd[,3]) / ses.mpd[,4]))
rownames(NRI) <- row.names(ses.mpd)
colnames(NRI) <- "NRI"


ses.mpd.1 <- ses.mpd(comm, phydist, null.model = "taxa.labels",
                   abundance.weighted = T, runs = 25)

NRI.1 <- as.matrix(-1 * ((ses.mpd.1[,2] - ses.mpd.1[,3]) / ses.mpd.1[,4]))
rownames(NRI.1) <- row.names(ses.mpd.1)
colnames(NRI.1) <- "NRI.1"
```

iii. Nearest Taxon Index (NTI)

In the R code chunk below, do the following: 1. Calculate the NTI for each site in the Indiana ponds data set.

```
ses.mntd <- ses.mntd(comm, phydist, null.model = "taxa.labels",
                     abundance.weighted = FALSE, runs = 25)

NTI <- as.matrix(-1 * ((ses.mntd[,2] - ses.mntd[,3]) / ses.mntd[,4]))

rownames(NTI) <- row.names(ses.mntd)
colnames(NTI) <- "NTI"
```

```
ses.mntd.1 <- ses.mntd(comm, phydist, null.model = "taxa.labels",
                      abundance.weighted = T, runs = 25)

NTI.1 <- as.matrix(-1 * ((ses.mntd.1[,2] - ses.mntd.1[,3]) / ses.mntd.1[,4]))

rownames(NTI.1) <- row.names(ses.mntd.1)
colnames(NTI.1) <- "NTI"
```

Question 3:

a. In your own words describe what you are doing when you calculate the NRI.
b. In your own words describe what you are doing when you calculate the NTI.
c. Interpret the NRI and NTI values you observed for this dataset.
d. In the NRI and NTI examples above, the arguments "abundance.weighted = FALSE" means that the indices were calculated using presence-absence data. Modify and rerun the code so that NRI and NTI are calculated using abundance data. How does this affect the interpretation of NRI and NTI?

> ***Answer 3a***: Net Relatedness Index - This seems essentially to be a measure of phylogenetic diversity. It measures the liklihood that species in a dataset are more closely related than you would expect by random chance - so in other words NRI tests for overdispersion or clustering within a dataset.
> ***Answer 3b***: Nearest Taxon Index - This is basically the same thing as NRI except that it uses mean distances to the nearest phylogenetic neighbor instead of the mean phylogenetic distance that NRI uses.
> ***Answer 3c***: On the whole, all values are negative (only exception is BC001 for NTI estimate); therefore NRI and NTI values generally suggest that these taxa are less phylogenetically diverse than you would expect by chance [i.e. the samples are overdispersed]. ***Answer 3d***: In both cases (NRI and NTI), using abundance data generated more positive values than using incidence data; therefore, using abundance data gives an opposite intrepretation and clustering may be suspected.

5) PHYLOGENETIC BETA DIVERSITY

A. Phylogenetically Based Community Resemblance Matrix

In the R code chunk below, do the following:
1. calculate the phylogenetically based community resemblance matrix using Mean Pair Distance, and
2. calculate the phylogenetically based community resemblance matrix using UniFrac distance.

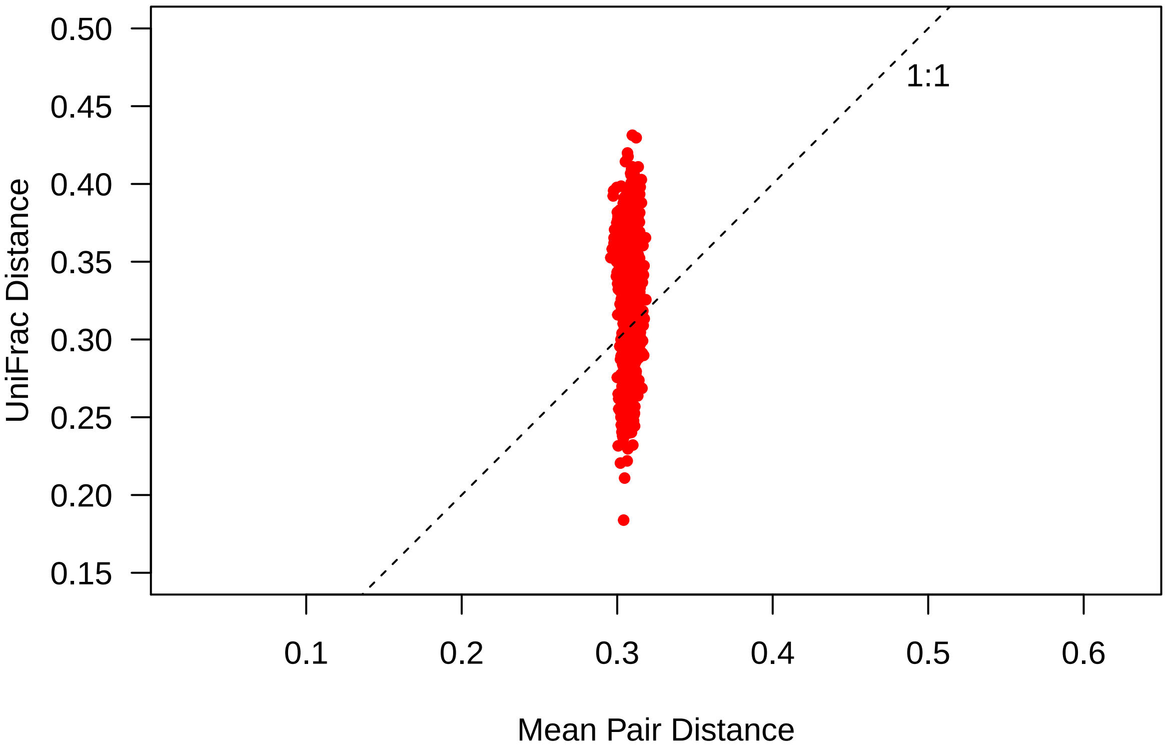
```
dist.mp <- comdist(comm, phydist)
```

```
## [1] "Dropping taxa from the distance matrix because they are not present in the community data:"
## [1] "Methanosarcina"
```

```
dist.uf <- unifrac(comm, phy)
```

In the R code chunk below, do the following:
1. plot Mean Pair Distance versus UniFrac distance and compare.

```
par(mar = c(5, 5, 2, 1) + 0.1)
plot(dist.mp, dist.uf,
     pch = 20, col = "red", las = 1, asp = 1, xlim = c(0.15, 0.5), ylim = c(0.15, 0.5),
     xlab = "Mean Pair Distance", ylab = "UniFrac Distance")
abline(b = 1, a = 0, lty = 2)
text(0.5, 0.47, "1:1")
```



Question 4:

a. In your own words describe Mean Pair Distance, UniFrac distance, and the difference between them.
b. Using the plot above, describe the relationship between Mean Pair Distance and UniFrac distance. Note: we are calculating unweighted phylogenetic distances (similar to incidence based measures). That means that we are not taking into account the abundance of each taxon in each site.
c. Why might MPD show less variation than UniFrac?

> ***Answer 4a***: Both mean pair distance and unifrqac distance are methods to calculate the phylogenetic distance between two samples, however they are mathamatically different. Mean pairwise difference uses the average phylogenetic difference between samples while unifrac uses the ratio of unshared branch lengths to the total branch lengths in a rooted tree. ***Answer 4b***: There is a narrow range of variation in mean pair distance and wide variation in unifrac distances. ***Answer 4c***: Since UniFrac is taking the ratio of unshared to total branch length, there should be more variation if a larger proportion of branches are shared. This pattern would be less noticable with mean pair ditance since it calculates distance based on mean phylogenetic difference between samples.

B. Visualizing Phylogenetic Beta-Diversity

Now that we have our phylogenetically based community resemblance matrix, we can visualize phylogenetic diversity among samples using the same techniques that we used in the β-diversity module from earlier in the course.

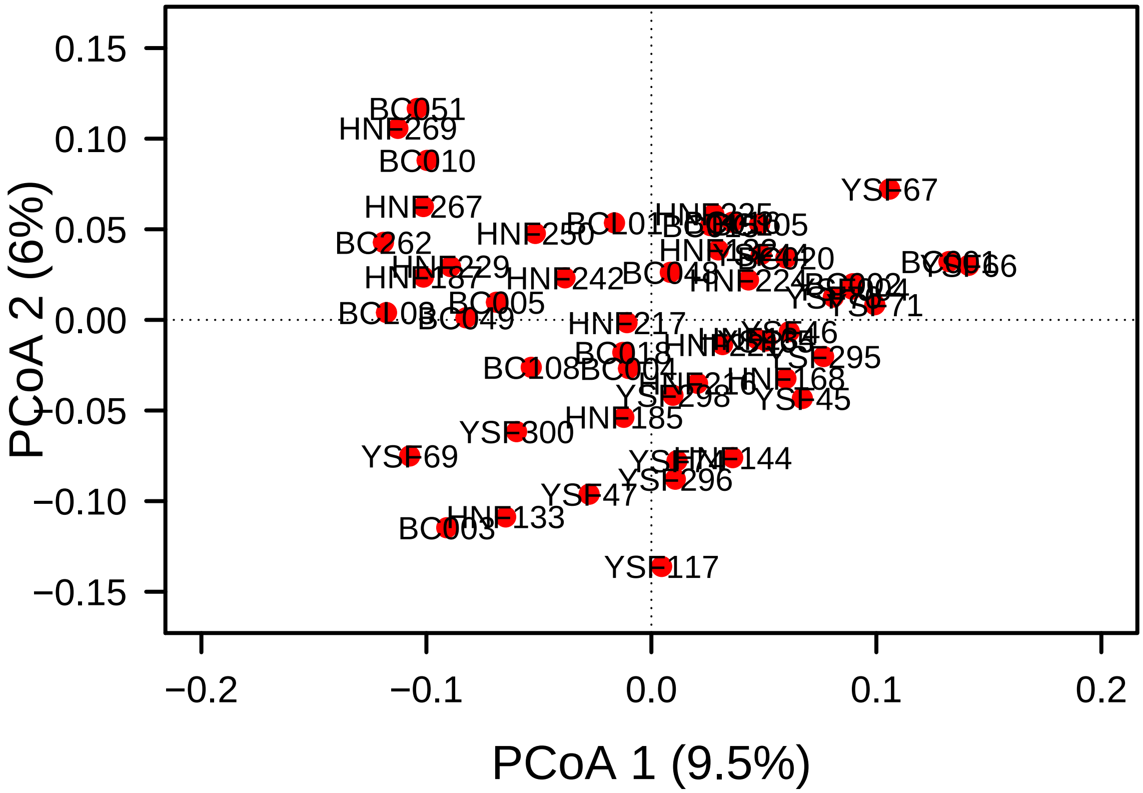
In the R code chunk below, do the following:
1. perform a PCoA based on the UniFrac distances, and
2. calculate the explained variation for the first three PCoA axes.

```
pond.pcoa <- cmdscale(dist.uf, eig = T, k = 3)
explainvar1 <- round(pond.pcoa$eig[1] / sum(pond.pcoa$eig), 3) * 100
explainvar2 <- round(pond.pcoa$eig[2] / sum(pond.pcoa$eig), 3) * 100
explainvar3 <- round(pond.pcoa$eig[3] / sum(pond.pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)
```

Now that we have calculated our PCoA, we can plot the results.

In the R code chunk below, do the following:
1. plot the PCoA results using either the R base package or the `ggplot` package,
2. include the appropriate axes,
3. add and label the points, and
4. customize the plot.

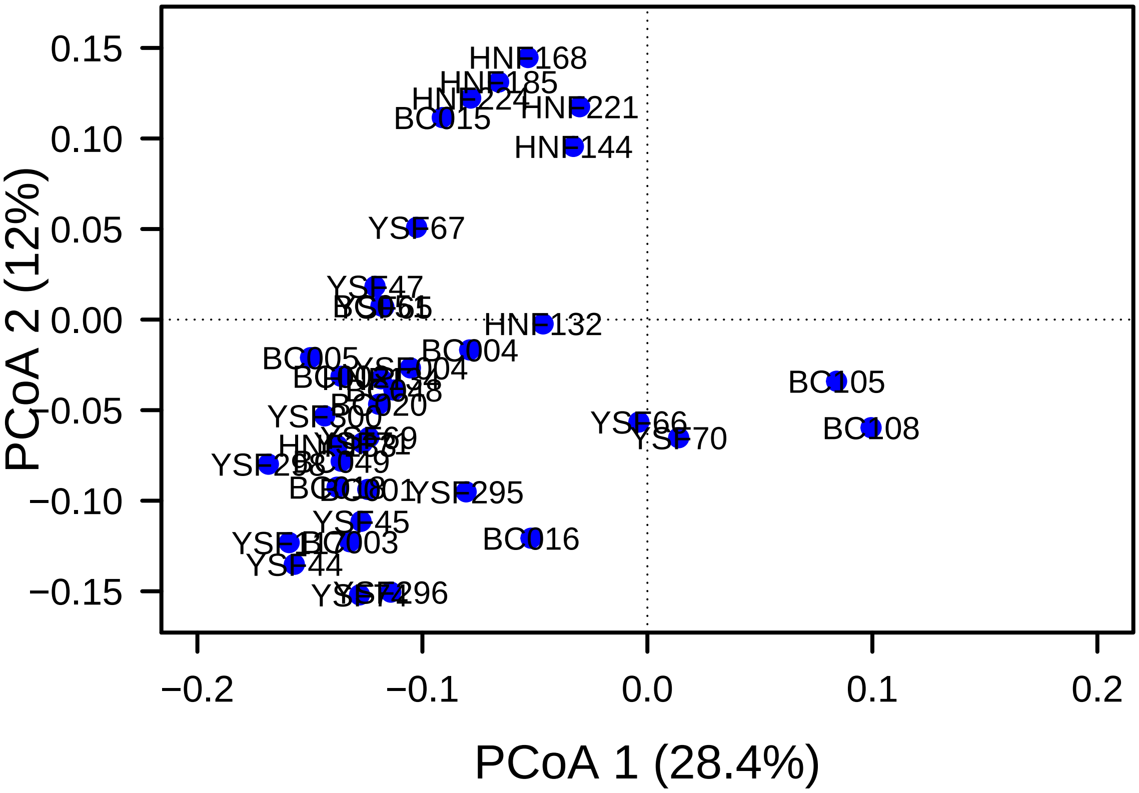
```
par(mar = c(5, 5, 1, 2) + 0.1)

plot(pond.pcoa$points[ ,1], pond.pcoa$points[ ,2],
     xlim = c(-0.2, 0.2), ylim = c(-.16, 0.16),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""),
     pch = 16, cex = 2.0, type = "n", cex.lab = 1.5, cex.axis = 1.2, axes = FALSE)

axis(side = 1, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
axis(side = 2, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
abline(h = 0, v = 0, lty = 3)
box(lwd = 2)

points(pond.pcoa$points[ ,1], pond.pcoa$points[ ,2],
       pch = 20, cex = 2, bg = "grey", col = "red")
text(pond.pcoa$points[ ,1], pond.pcoa$points[ ,2],
     labels = row.names(pond.pcoa$points))
```



In the following R code chunk: 1. perform another PCoA on taxonomic data using an appropriate measure of dissimilarity, and 2. calculate the explained variation on the first three PCoA axes.

```
comm.db=vegdist(comm,method="bray",upper=T,diag=T)


pond.pcoa.1 <- cmdscale(comm.db, eig = T, k = 3)
explainvar1.1 <- round(pond.pcoa.1$eig[1] / sum(pond.pcoa.1$eig), 3) * 100
explainvar2.1 <- round(pond.pcoa.1$eig[2] / sum(pond.pcoa.1$eig), 3) * 100
explainvar3.1 <- round(pond.pcoa.1$eig[3] / sum(pond.pcoa.1$eig), 3) * 100
sum.eig <- sum(explainvar1.1, explainvar2.1, explainvar3.1)

par(mar = c(5, 5, 1, 2) + 0.1)

plot(pond.pcoa.1$points[ ,1], pond.pcoa.1$points[ ,2],
     xlim = c(-0.2, 0.2), ylim = c(-.16, 0.16),
     xlab = paste("PCoA 1 (", explainvar1.1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2.1, "%)", sep = ""),
     pch = 16, cex = 2.0, type = "n", cex.lab = 1.5, cex.axis = 1.2, axes = FALSE)

axis(side = 1, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
axis(side = 2, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
abline(h = 0, v = 0, lty = 3)
box(lwd = 2)

points(pond.pcoa.1$points[ ,1], pond.pcoa.1$points[ ,2],
       pch = 20, cex = 2, bg = "grey", col = "blue")
```

```
text(pond.pcoa.1$points[ ,1], pond.pcoa.1$points[ ,2],
     labels = row.names(pond.pcoa.1$points))
```



Question 5: Using a combination of visualization tools and percent variation explained, how does the phylogenetically based ordination compare or contrast with the taxonomic ordination? What does this tell you about the importance of phylogenetic information in this system?

> ***Answer 5***: Taxonomic data shows more clustering than the phylogenetic PCoA, and in the taxonomic PCoA more variation is explained by PCoA 1 and 2. Accounting for phylogenetics in this case was imporant because it reducing the chances of Type 1 error. The taxonomic data alone was cryptically influenced by phylogony within the dataset and was unable to estimate true variation between samples.

C. Hypothesis Testing

i. Categorical Approach

In the R code chunk below, do the following:
1. test the hypothesis that watershed has an effect on the phylogenetic diversity of bacterial communities.

```
watershed <- env$Location
adonis(dist.uf ~ watershed, permutations = 999)
```

```
##
## Call:
## adonis(formula = dist.uf ~ watershed, permutations = 999)
##
```

```
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model     R2 Pr(>F)
## watershed  2   0.13316 0.066579  1.2679 0.0492  0.024 *
## Residuals 49   2.57305 0.052511         0.9508
## Total     51   2.70621                  1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
adonis(
  vegdist(
    decostand(comm, method = "log"),
    method = "bray") ~ watershed,
  permutations = 999)
```

```
##
## Call:
## adonis(formula = vegdist(decostand(comm, method = "log"), method = "bray") ~      watershed, permutat
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## watershed  2   0.16601 0.083003  1.5689 0.06018  0.009 **
## Residuals 49   2.59229 0.052904         0.93982
## Total     51   2.75829                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

ii. Continuous Approach

In the R code chunk below, do the following: 1. from the environmental data matrix, subset the variables related to physical and chemical properties of the ponds, and
2. calculate environmental distance between ponds based on the Euclidean distance between sites in the environmental data matrix (after transforming and centering using `scale()`).

```r
envs <- env[, 5:19]
envs <- envs[, -which(names(envs) %in% c("TDS", "Salinity", "Cal_Volume"))]
env.dist <- vegdist(scale(envs), method = "euclid")
```

In the R code chunk below, do the following:
1. conduct a Mantel test to evaluate whether or not UniFrac distance is correlated with environmental variation.

```r
mantel(dist.uf, env.dist)
```

```
##
## Mantel statistic based on Pearson's product-moment correlation
##
## Call:
## mantel(xdis = dist.uf, ydis = env.dist)
##
```

```
## Mantel statistic r: 0.1604
##       Significance: 0.069
##
## Upper quantiles of permutations (null model):
##   90%   95% 97.5%   99%
## 0.136 0.173 0.208 0.261
## Permutation: free
## Number of permutations: 999
```

Last, conduct a distance-based Redundancy Analysis (dbRDA).

In the R code chunk below, do the following:
1. conduct a dbRDA to test the hypothesis that environmental variation effects the phylogenetic diversity of bacterial communities,
2. use a permutation test to determine significance, and 3. plot the dbRDA results

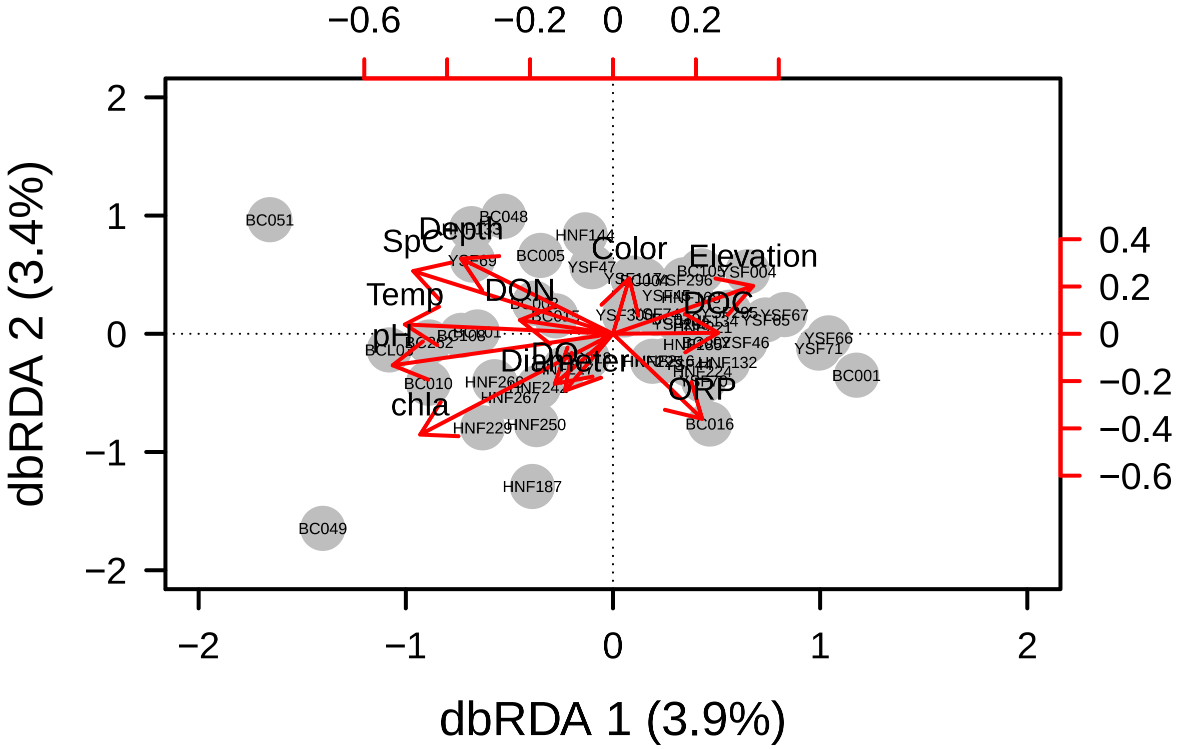
```
ponds.dbrda <- vegan::dbrda(dist.uf ~ ., data = as.data.frame(scale(envs)))

anova(ponds.dbrda, by = "axis")
```

```
## Permutation test for dbrda under reduced model
## Marginal tests for axes
## Permutation: free
## Number of permutations: 999
##
## Model: vegan::dbrda(formula = dist.uf ~ Elevation + Diameter + Depth + ORP + Temp + SpC + DO + pH + C
##          Df SumOfSqs      F Pr(>F)
## dbRDA1    1  0.10566 2.0152  0.001 ***
## dbRDA2    1  0.09258 1.7658  0.004 **
## dbRDA3    1  0.07555 1.4409  0.036 *
## dbRDA4    1  0.06677 1.2735  0.092 .
## dbRDA5    1  0.05666 1.0807  0.302
## dbRDA6    1  0.05293 1.0095  0.437
## dbRDA7    1  0.04750 0.9059  0.638
## dbRDA8    1  0.03941 0.7517  0.893
## dbRDA9    1  0.03775 0.7201  0.936
## dbRDA10   1  0.03280 0.6256  0.985
## dbRDA11   1  0.02876 0.5485  0.996
## dbRDA12   1  0.02501 0.4770  1.000
## Residual 39  2.04482
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ponds.fit <- envfit(ponds.dbrda, envs, perm = 999)
ponds.fit
```

```
##
## ***VECTORS
##
##              dbRDA1   dbRDA2     r2 Pr(>r)
## Elevation  0.77670  0.62986 0.0959  0.097 .
## Diameter  -0.27972 -0.96008 0.0541  0.273
## Depth     -0.63137  0.77548 0.1756  0.007 **
## ORP        0.41879 -0.90808 0.1437  0.024 *
## Temp      -0.98250  0.18628 0.1523  0.026 *
## SpC       -0.77101  0.63682 0.2087  0.003 **
## DO        -0.39318 -0.91946 0.0464  0.308
## pH        -0.96210 -0.27270 0.1756  0.005 **
## Color      0.06353  0.99798 0.0464  0.320
## chla      -0.60392 -0.79704 0.2626  0.004 **
## DOC        0.99847 -0.05526 0.0382  0.350
## DON       -0.91633  0.40042 0.0339  0.426
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

```
dbrda.explainvar1 <- round(ponds.dbrda$CCA$eig[1] /
                             sum(c(ponds.dbrda$CCA$eig, ponds.dbrda$CA$eig)), 3) * 100
dbrda.explainvar2 <- round(ponds.dbrda$CCA$eig[2] /
                             sum(c(ponds.dbrda$CCA$eig, ponds.dbrda$CA$eig)), 3) * 100

par(mar = c(5, 5, 4, 4) + 0.1)

plot(scores(ponds.dbrda, display = "wa"), xlim = c(-2, 2), ylim = c(-2, 2),
     xlab = paste("dbRDA 1 (", dbrda.explainvar1, "%)", sep = ""),
     ylab = paste("dbRDA 2 (", dbrda.explainvar2, "%)", sep = ""),
     pch = 16, cex = 2.0, type = "n", cex.lab = 1.5, cex.axis = 1.2, axes = FALSE)

axis(side = 1, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
axis(side = 2, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
abline(h = 0, v = 0, lty = 3)
box(lwd = 2)

points(scores(ponds.dbrda, display = "wa"),
       pch = 19, cex = 3, bg = "gray", col = "gray")
text(scores(ponds.dbrda, display = "wa"),
     labels = row.names(scores(ponds.dbrda, display = "wa")), cex = 0.5)

vectors <- scores(ponds.dbrda, display = "bp")

arrows(0, 0, vectors[,1] * 2, vectors[, 2] * 2,
       lwd = 2, lty = 1, length = 0.2, col = "red")
text(vectors[,1] * 2, vectors[, 2] * 2, pos = 3,
     labels = row.names(vectors))
axis(side = 3, lwd.ticks = 2, cex.axis = 1.2, las = 1, col = "red", lwd = 2.2,
     at = pretty(range(vectors[, 1])) * 2, labels = pretty(range(vectors[, 1])))
axis(side = 4, lwd.ticks = 2, cex.axis = 1.2, las = 1, col = "red", lwd = 2.2,at = pretty(range(vectors
       pretty(range(vectors[, 2])))
```



Question 6: Based on the multivariate procedures conducted above, describe the phylogenetic patterns of β-diversity for bacterial communities in the Indiana ponds.

> ***Answer 6***: Watershed does influence baterical communities and this is particularly determined by water depth, ORP, water teperature, SpC, water pH, and Chla.

6) SPATIAL PHYLOGENETIC COMMUNITY ECOLOGY

A. Phylogenetic Distance-Decay (PDD)

First, calculate distances for geographic data, taxonomic data, and phylogenetic data among all unique pair-wise combinations of ponds.

In the R code chunk below, do the following:
1. calculate the geographic distances among ponds,
2. calculate the taxonomic similarity among ponds,
3. calculate the phylogenetic similarity among ponds, and
4. create a dataframe that includes all of the above information.

```
long.lat <- as.matrix(cbind(env$long, env$lat))
coord.dist <- earth.dist(long.lat, dist = TRUE)

bray.curtis.dist <- 1 - vegdist(comm)

unifrac.dist <- 1 - dist.uf

unifrac.dist.ls <- liste(unifrac.dist, entry = "unifrac")
```

```
bray.curtis.dist.ls <- liste(bray.curtis.dist, entry = "bray.curtis")
coord.dist.ls <- liste(coord.dist, entry = "geo.dist")
env.dist.ls <- liste(env.dist, entry = "env.dist")

df <- data.frame(coord.dist.ls, bray.curtis.dist.ls[, 3], unifrac.dist.ls[, 3],
                 env.dist.ls[, 3])
names(df)[4:6] <- c("bray.curtis", "unifrac", "env.dist")
```

Now, let's plot the DD relationships:
In the R code chunk below, do the following:
1. plot the taxonomic distance decay relationship,
2. plot the phylogenetic distance decay relationship, and
3. add trend lines to each.

```
par(mfrow=c(2, 1), mar = c(1, 5, 2, 1) + 0.1, oma = c(2, 0, 0, 0))

plot(df$geo.dist, df$bray.curtis, xlab = "", xaxt = "n", las = 1, ylim = c(0.1, 0.9),
     ylab="Bray-Curtis Similarity",
     main = "Distance Decay", col = "SteelBlue")

DD.reg.bc <- lm(df$bray.curtis ~ df$geo.dist)
summary(DD.reg.bc)
```

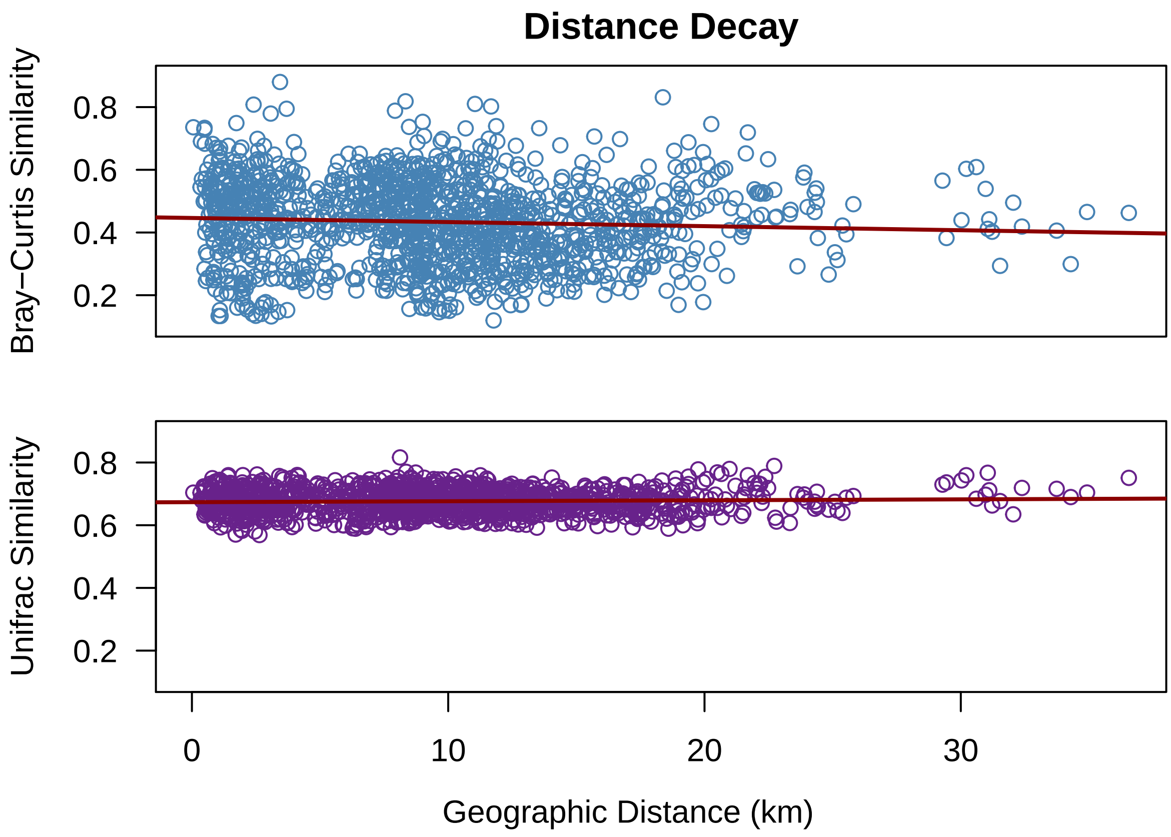
```
##
## Call:
## lm(formula = df$bray.curtis ~ df$geo.dist)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.31151 -0.08843  0.00315  0.09121  0.43817
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.4463453  0.0066883  66.735   <2e-16 ***
## df$geo.dist -0.0013051  0.0005864  -2.226   0.0262 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1303 on 1324 degrees of freedom
## Multiple R-squared:  0.003728,   Adjusted R-squared:  0.002975
## F-statistic: 4.954 on 1 and 1324 DF,  p-value: 0.0262
```

```
abline(DD.reg.bc , col = "red4", lwd = 2)


par(mar = c(2, 5, 1, 1) + 0.1)

plot(df$geo.dist, df$unifrac, xlab = "", las = 1, ylim = c(0.1, 0.9),
     ylab = "Unifrac Similarity", col = "darkorchid4")

DD.reg.uni <- lm(df$unifrac ~ df$geo.dist)
summary(DD.reg.uni)
```

```
##
## Call:
```

```
## lm(formula = df$unifrac ~ df$geo.dist)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.105629 -0.027107 -0.000077  0.026761  0.140215
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.6735186  0.0019206 350.677   <2e-16 ***
## df$geo.dist 0.0002976  0.0001684   1.767   0.0774 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.03741 on 1324 degrees of freedom
## Multiple R-squared:  0.002354,   Adjusted R-squared:  0.0016
## F-statistic: 3.124 on 1 and 1324 DF,  p-value: 0.07738
```

```
abline(DD.reg.uni, col = "red4", lwd = 2)

mtext("Geographic Distance (km)", side = 1, adj = 0.55,
      line = 0.5, outer = TRUE)
```



In the R code chunk below, test if the trend lines in the above distance decay relationships are different from one another.

```
diffslope(df$geo.dist, df$unifrac, df$geo.dist, df$bray.curtis)
```

```
##
```

```
## Is difference in slope significant?
## Significance is based on 1000 permutations
##
## Call:
## diffslope(x1 = df$geo.dist, y1 = df$unifrac, x2 = df$geo.dist,      y2 = df$bray.curtis)
##
## Difference in Slope: 0.001603
## Significance: 0.004
##
## Empirical upper confidence limits of r:
##      90%      95%    97.5%      99%
## 0.000727 0.000991 0.001170 0.001361
```

Question 7: Interpret the slopes from the taxonomic and phylogenetic DD relationships. If there are differences, hypothesize why this might be.

> ***Answer 7***: The slopes are statistically different. Since Bray-Curtis incorporates taxa abundance and UniFrac accounts for phylogenetic relatedness, then genetic relatedness may play a key role in explaining the distribution of these taxa.

B. Phylogenetic diversity-area relationship (PDAR)

i. Constructing the PDAR

In the R code chunk below, write a function to generate the PDAR.

```
PDAR <- function(comm, tree){
  areas <- c()
  diversity <- c()
  num.plots <- c(2, 4, 8, 16, 32, 51)
  for (i in num.plots){
    areas.iter <- c()
    diversity.iter <- c()
    for (j in 1:10){
      pond.sample <- sample(51, replace = FALSE, size = i)
      area <- 0
      sites <- c()
      for (k in pond.sample) {
        area <- area + pond.areas[k]
        sites <- rbind(sites, comm[k, ])
      }
      areas.iter <- c(areas.iter, area)
      psv.vals <- psv(sites, tree, compute.var = FALSE)
      psv <- psv.vals$PSVs[1]
      diversity.iter <- c(diversity.iter, as.numeric(psv))
    }
    diversity <- c(diversity, mean(diversity.iter))
    areas <- c(areas, mean(areas.iter))
    print(c(i, mean(diversity.iter), mean(areas.iter)))
  }
  return(cbind(areas, diversity))
  }
```

ii. Evaluating the PDAR

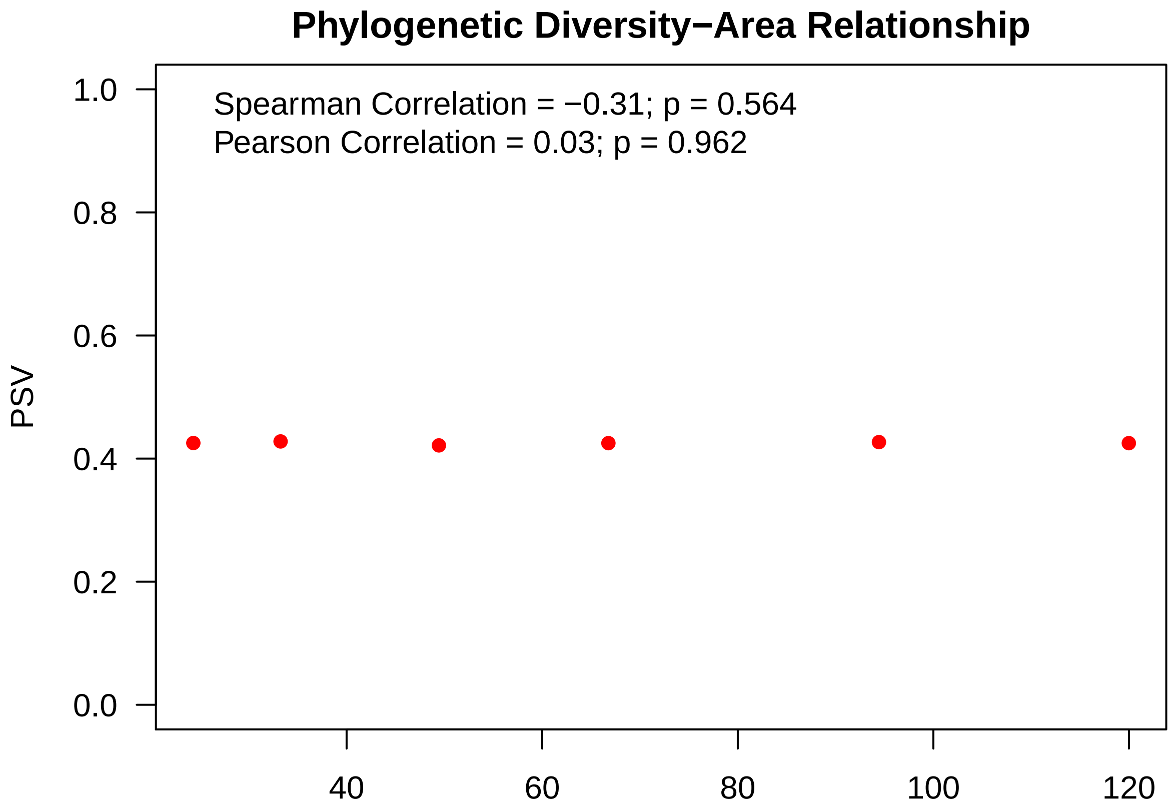
In the R code chunk below, do the following:

1. calculate the area for each pond,
2. use the PDAR() function you just created to calculate the PDAR for each pond,
3. calculate the Pearson's and Spearman's correlation coefficients,
4. plot the PDAR and include the correlation coefficients in the legend, and
5. customize the PDAR plot.

```r
pond.areas <- as.vector(pi * (env$Diameter/2)^2)

pdar <- PDAR(comm, phy)
```

```
## [1]   2.0000000   0.4252762 591.7291865
## [1]    4.0000000    0.4278672 1105.3450278
## [1]    8.0000000    0.4214464 2444.2085646
## [1]   16.0000000    0.4250919 4458.3385927
## [1]   32.0000000    0.4267584 8918.5668533
## [1] 5.100000e+01 4.250856e-01 1.439763e+04
```

```r
pdar <- as.data.frame(pdar)
pdar$areas <- sqrt(pdar$areas)

Pearson <- cor.test(pdar$areas, pdar$diversity, method = "pearson")
P <- round(Pearson$estimate, 2)
P.pval <- round(Pearson$p.value, 3)

Spearman <- cor.test(pdar$areas, pdar$diversity, method = "spearman")
rho <- round(Spearman$estimate, 2)
rho.pval <- round(Spearman$p.value, 3)

plot.new()
par(mfrow=c(1, 1), mar = c(1, 5, 2, 1) + 0.1, oma = c(2, 0, 0, 0))
plot(pdar[, 1], pdar[, 2], xlab = "Area", ylab = "PSV", ylim = c(0, 1),
     main = "Phylogenetic Diversity-Area Relationship",
     col = "red", pch = 16, las = 1)

legend("topleft", legend= c(paste("Spearman Correlation = ", rho, "; p = ", rho.pval, sep = ""),
                                  paste("Pearson Correlation = ", P, "; p = ", P.pval, sep = "")),
                                  bty = "n", col = "red")
```



Question 8: Compare your observations of the microbial PDAR and SAR in the Indiana ponds? How might you explain the differences between the taxonomic (SAR) and phylogenetic (PDAR)?

> ***Answer 8***: The slope of the SAR from the spaptial assignment was steeper than the PDAR for this assignment. Since the taxa are phylogentically similar, as the area gets larger there is not necessarily higher richness when accounting for phylogenetic relatedness.

SYNTHESIS

Ignoring technical or methodological constraints, discuss how phylogenetic information could be useful in your own research. Specifically, what kinds of phylogenetic data would you need? How could you use it to answer important questions in your field? In your response, feel free to consider not only phylogenetic approaches related to phylogenetic community ecology, but also those we discussed last week in the PhyloTraits module, or any other concepts that we have not covered in this course.

> A major component of my research is intepreting gut microbial diversity of howler monkeys in relation to measures of health (IgA levels, parasitic infections, hormones). Estimating the phylogenetic relationship of members of the bacterial community may provide more insightful results concerning any possible associations between microbial diversity of the gut and other measures in the study. For this, I would need to generate sequences from the 16s rRNA region of my samples along with an outgroup (which I could likely get from GenBank). Accounting for these potential relationships will be especially useful when I begin to compare diversity among contrasting habitat types through Panama since a major question in the field is if/why gut microbial diversity is varies in relation to environmental conditions. If more is understood about this relationship then it could potentially improve noninvasive monitoring methods for rare and endangered species, especially if they are sensitive to habitat encroachment and stress.