12.Phylogenetic Diversity - Communities

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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 worksheet, 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. In the Markdown version of this document in your cloned repo, change "Student Name" on line 3 (above) with 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 examples of proper scripting needed to carry out the exercises.
4. Answer questions in the worksheet. Space for your answers is provided in this document and is 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 today, it is *imperative* that you **push** this file to your GitHub repo, at whatever stage you are. This will enable you to pull your work onto your own computer.
6. When you have completed the worksheet, **Knit** the text and code into a single PDF file by pressing the `Knit` button in the RStudio scripting panel. This will save the PDF output in your '8.BetaDiversity' folder.
7. After Knitting, please submit the worksheet by making a **push** to your GitHub repo and then create a **pull request** via GitHub. Your pull request should include this file *12.PhyloCom_Worksheet.Rmd* and the PDF output of `Knitr` (*12.PhyloCom_Worksheet.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.

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

```
## [1] "/Users/lana/GitHub/QB2019_Bolin/2.Worksheets/12.PhyloCom"
```

```r
setwd("/Users/lana/GitHub/QB2019_Bolin/2.Worksheets/12.PhyloCom")

package.list <- c('ape', 'seqinr', 'picante', 'vegan', 'fossil', 'reshape', 'simba')
for (package in package.list) {
  if (!require(package, character.only=TRUE, quietly=TRUE)) {
    install.packages(package)
    library(package, character.only=TRUE)
  }
}
```

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

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

##
## Attaching package: 'permute'

## The following object is masked from 'package:seqinr':
##
##     getType

## This is vegan 2.5-3

##
## Attaching package: 'nlme'

## The following object is masked from 'package:seqinr':
##
##     gls

##
## 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")
```

2) DESCRIPTION OF DATA

need to discuss data set from spatial ecology!

In 2013 we sampled > 50 forested ponds in Brown County State Park, Yellowwood State Park, and Hoosier National Forest in southern Indiana. In addition to measuring a suite of geographic and environmental variables, we characterized the diversity of bacteria in the ponds using molecular-based approaches. Specifically, we amplified the 16S rRNA gene (i.e., the DNA sequence) and 16S rRNA transcripts (i.e., the RNA transcript of the gene) of bacteria. We used a program called `mothur` to quality-trim our data set and assign sequences to operational taxonomic units (OTUs), which resulted in a site-by-OTU matrix.
In this module we will focus on taxa that were present (i.e., DNA), but there will be a few steps where we need to parse out the transcript (i.e., RNA) samples. 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.

```
library(dplyr)
```

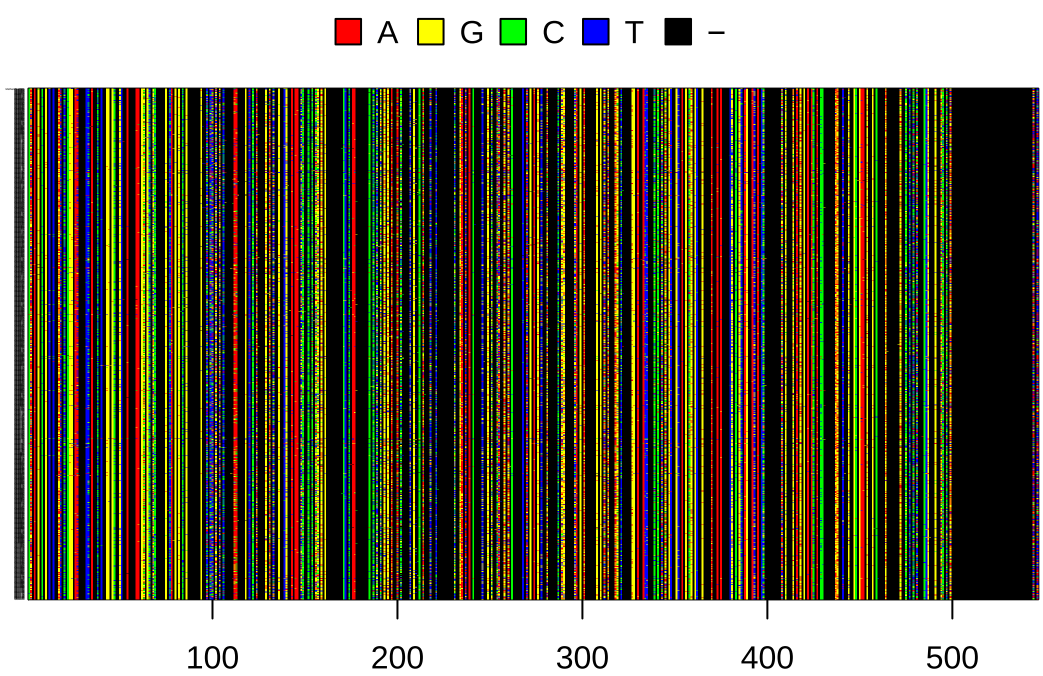
```
##
## Attaching package: 'dplyr'

## The following object is masked from 'package:reshape':
##
##     rename

## The following object is masked from 'package:nlme':
##
##     collapse

## The following object is masked from 'package:seqinr':
##
##     count

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
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))
```

```r
comm <- comm[rownames(comm) %in% env$Sample_ID, ]
comm <- comm[, colSums(comm) > 0]

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

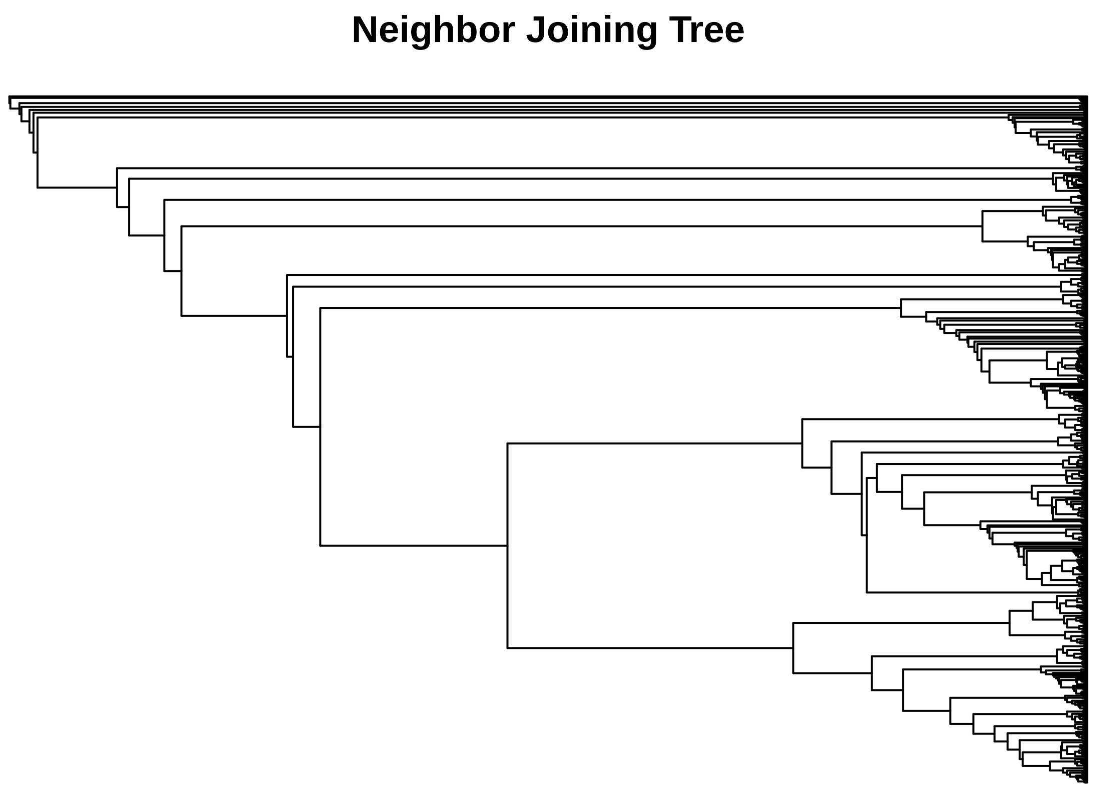
Next, in the R code chunk below, do the following:
1. load the FASTA alignment for the bacterial operational taxonomic units (OTUs),
2. rename the OTUs by removing everything before the tab (\t) and after the bar (|),
3. import the *Methanosarcina* outgroup FASTA file,
4. convert both FASTA files into 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.

```r
ponds.cons <- read.alignment(fil = "./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)
```




```r
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 = T)

par(mar = c(1, 1, 2, 1) + 0.1)
plot.phylo(phy, main = "Neighbor Joining Tree", "phylogram", show.tip.label = F, use.edge.length = F, di
```



4) PHYLOGENETIC ALPHA DIVERSITY

A. Faith's Phylogenetic Diversity (PD)

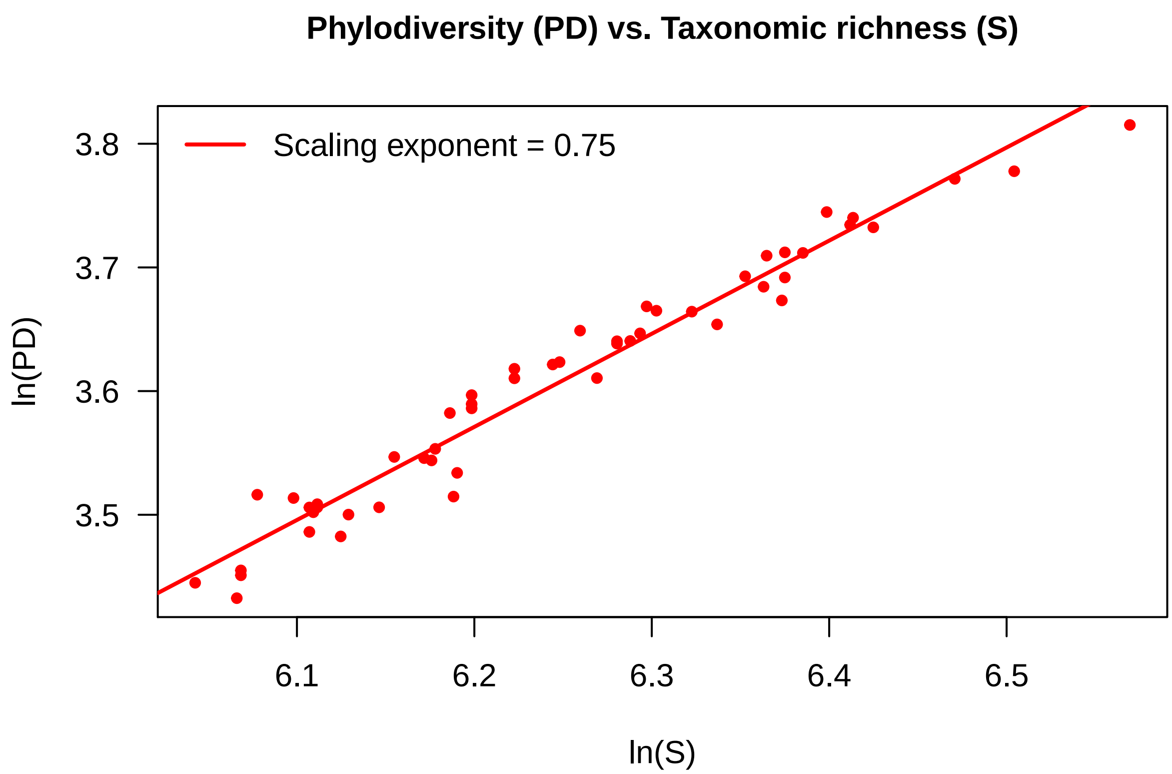
In the R code chunk below, do the following:
1. calculate Faith's D using the `pd()` function.

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

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$SR), log(pd$PD),
     pch = 20, col = "red", las = 1,
     xlab = "ln(S)", ylab = "ln(PD)", cex.main = 1,
```

```r
    main = "Phylodiversity (PD) vs. Taxonomic richness (S)")
fit <- lm("log(pd$PD) ~ log(pd$SR)")
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 = "
```



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***: Less-related species will have a longer summed branch length, meaning a higher Faith's PD.
> ***Answer 1b***: Species richness scales with (phylogenetic distance)^0.75, so as species richness gets really high we see diminishing returns in terms of phylogenetic diversity. ***Answer 1c***: When species in your sample are very closely related. Species richness may be high, but PD will stay quite low. ***Answer 1d***: It tells us how quickly we get diminishing PD returns as we add species.

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.

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

```
##       ntaxa   pd.obs pd.rand.mean pd.rand.sd pd.obs.rank   pd.obs.z
## BC001   668 43.71912     44.11530  0.9458113           9 -0.4188739
```

```
## BC002   587 40.94334     39.97948  0.7888796          22  1.2218117
##        pd.obs.p runs
## BC001 0.3461538   25
## BC002 0.8461538   25
```

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

```
##       ntaxa   pd.obs pd.rand.mean pd.rand.sd pd.obs.rank  pd.obs.z
## BC001   668 43.71912     42.21117  0.6384794          26  2.361783
## BC002   587 40.94334     42.38532  0.6104976           1 -2.361979
##         pd.obs.p runs
## BC001 1.00000000   25
## BC002 0.03846154   25
```

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

```
##       ntaxa   pd.obs pd.rand.mean pd.rand.sd pd.obs.rank   pd.obs.z
## BC001   668 43.71912     44.07853  0.6195849           9 -0.5800721
## BC002   587 40.94334     39.99830  0.6819488          24  1.3857973
##        pd.obs.p runs
## BC001 0.3461538   25
## BC002 0.9230769   25
```

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: The phylogenetic diversity observed in our samples is different from a null expectation.
    Answer 2b: The choice of null model didn't change observed ses.pd values because the null model doesn't describe our observed data - it describes a null expectation to which we compare our data.

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 = F, runs = 25)
NRI <- as.matrix(-1 * ((ses.mpd[, 2] - ses.mpd[, 3]) / ses.mpd[, 4]))
rownames(NRI) <- row.names(ses.mpd)
colnames(NRI) <- "NRI"
```

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 = F, 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.mpd.ab <- ses.mpd(comm, phydist, null.model = "taxa.labels", abundance.weighted = T, runs = 25)
NRI.ab <- as.matrix(-1 * ((ses.mpd.ab[, 2] - ses.mpd.ab[, 3]) / ses.mpd.ab[, 4]))
rownames(NRI.ab) <- row.names(ses.mpd.ab)
colnames(NRI.ab) <- "NRI"

ses.mntd.ab <- ses.mntd(comm, phydist, null.model = "taxa.labels", abundance.weighted = T, runs = 25)
NTI.ab <- as.matrix(-1 * ((ses.mntd.ab[, 2] - ses.mntd.ab[, 3]) / ses.mntd.ab[, 4]))
rownames(NTI.ab) <- row.names(ses.mntd.ab)
colnames(NTI.ab) <- "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: You're comparing the mean phylogenetic distance between any two taxa in your sample to that of a null model. ***Answer 3b***: You're comparing the mean distance between each taxon and its closest neighbor in your sample to that of a null model. ***Answer 3c***: Some ponds are phylogenetically underdispersed (positive NRI and NTI values), while some are phylogenetically overdispersed (negative NRI and NTI values). These two Indices generally agree. ***Answer 3d***: Now the relative abundance of taxa will be included, so evenness will contribute to phylogenetic diversity.

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)
```

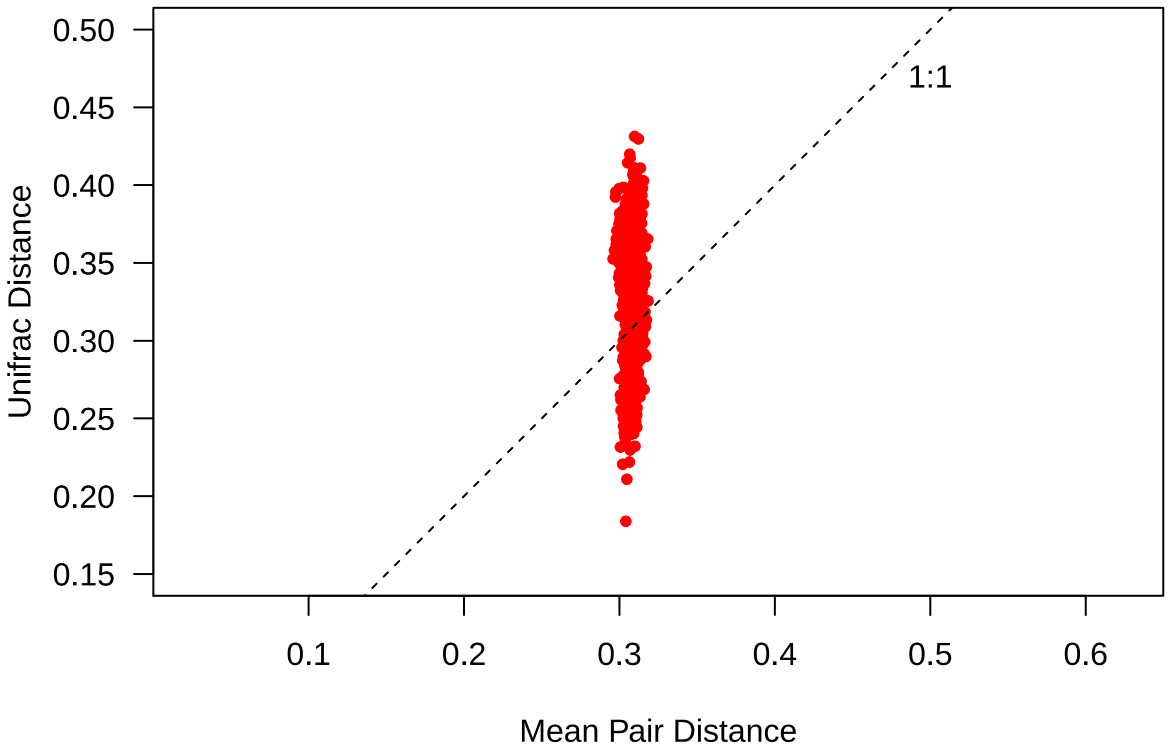
```
## [1] "Dropping taxa from the distance matrix because they are not present in the community data:"
## [1] "Methanosarcina"
```

```
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 Pa
```

```r
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: Mean Pairwise Distance compares the mean relatedness of pairs of taxa between samples, while UniFrac distance describes the fraction of the total branch length that is unshared between samples. ***Answer 4b***: Mean pairwise distance is nearly constant at 0.31 between all site pairs, while Unifrac Distance ranges from ~0.2 to ~0.45. ***Answer 4c***: Is it because the proportion of branch length that is unshared between samples can vary quite a lot without the mean distance between pairs of taxa changing? (I'm having a hard time thinking through this one…)

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.

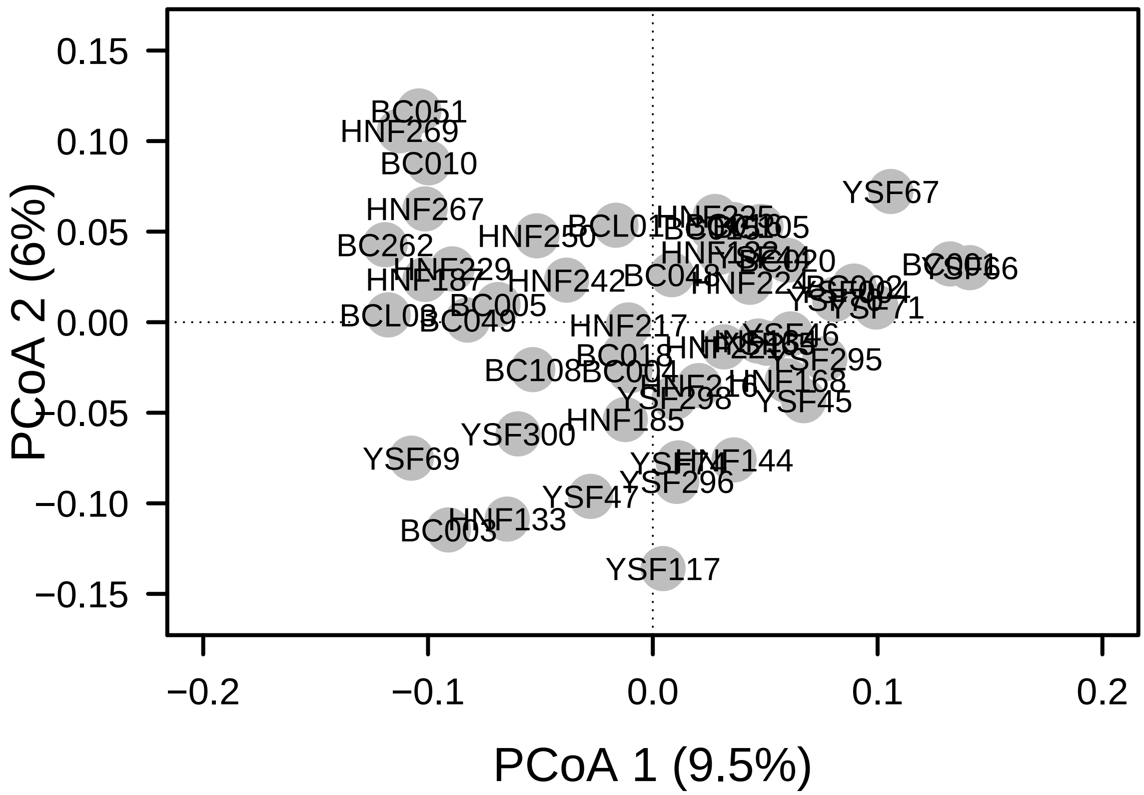
```r
pond.pcoa <- cmdscale(dist.uf, eig = T, k = 3)

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)
sum.eig
```

```
## [1] 20.9
```

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.

```r
par(mar = c(5, 5, 1, 2) + 0.1)
plot(pond.pcoa$points[, 1], pond.pcoa$points[, 2],
     xlim = c(-.2, .2), ylim = c(-.16, .16),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""),
     pch = 16, cex = 2, type = "n", cex.lab = 1.5, cex.axis = 1.2, axes = F)
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 = 19, cex = 3, bg = "gray", col = "gray")
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.

```
require(vegan)
pond.db <- vegdist(comm, method = "bray")
pond.tax.pcoa <- cmdscale(pond.db, eig = T, k = 3)

explainvar1.tax <- round(pond.tax.pcoa$eig[1] / sum(pond.tax.pcoa$eig), 3) * 100
explainvar2.tax <- round(pond.tax.pcoa$eig[2] / sum(pond.tax.pcoa$eig), 3) * 100
explainvar3.tax <- round(pond.tax.pcoa$eig[3] / sum(pond.tax.pcoa$eig), 3) * 100
sum.eig.tax <- sum(explainvar1.tax, explainvar2.tax, explainvar3.tax)
sum.eig.tax
```

```
## [1] 49
```

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***: The phylogenetically based ordiation explains 20.9% of variation while the taxonomic ordination explains 49%, which tells us phylogenetic information is important for explaining diversity in this system.

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  0.026 *
## 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
```

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()`).

```
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.

```
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: 0.066
##
## Upper quantiles of permutations (null model):
##   90%   95% 97.5%   99%
## 0.128 0.173 0.207 0.239
## 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")
```

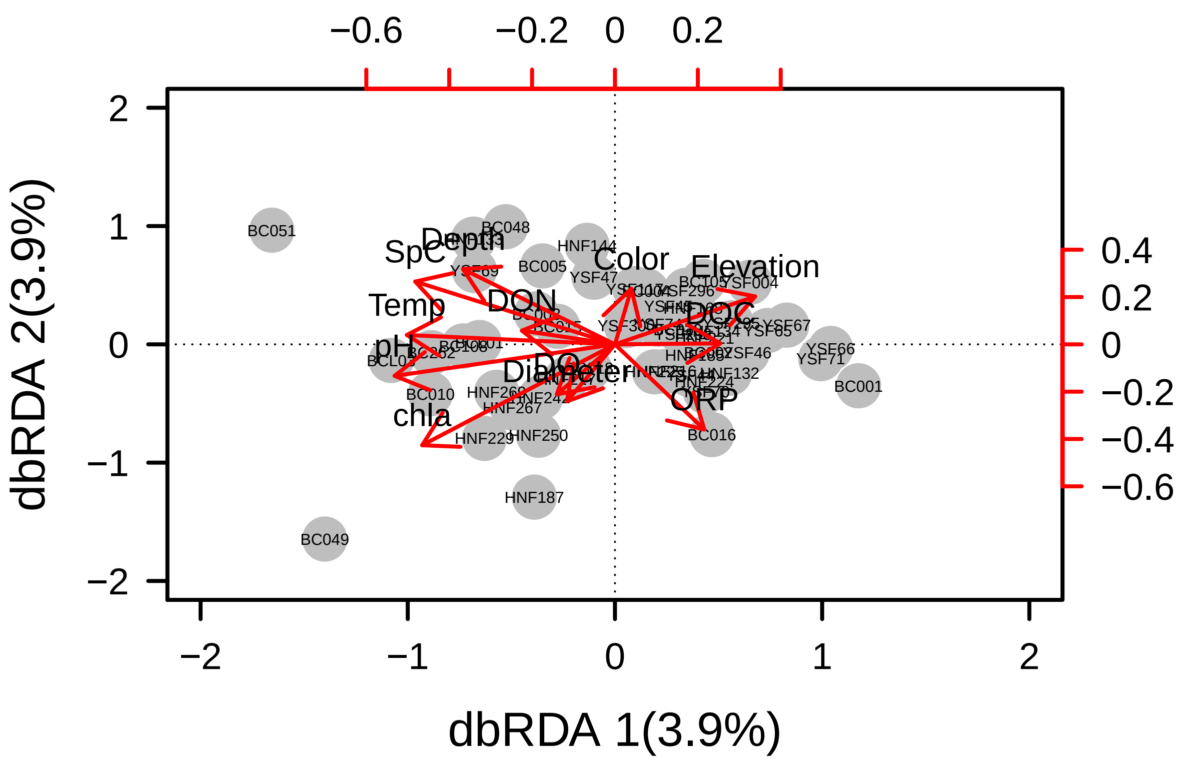
```
## Permutation test for dbrda under reduced model
## Forward tests for axes
## Permutation: free
## Number of permutations: 999
##
## Model: vegan::dbrda(formula = dist.uf ~ Elevation + Diameter + Depth + ORP + Temp + SpC + DO + pH + C
##          Df SumOfSqs      F Pr(>F)
## dbRDA1    1  0.10566 2.0152  0.435
## dbRDA2    1  0.09258 1.7658  0.640
## dbRDA3    1  0.07555 1.4409  0.967
## dbRDA4    1  0.06677 1.2735  0.997
## dbRDA5    1  0.05666 1.0807  1.000
## dbRDA6    1  0.05293 1.0095  1.000
## dbRDA7    1  0.04750 0.9059  1.000
## dbRDA8    1  0.03941 0.7517  1.000
## dbRDA9    1  0.03775 0.7201  1.000
## dbRDA10   1  0.03280 0.6256  1.000
## dbRDA11   1  0.02876 0.5485  1.000
## dbRDA12   1  0.02501 0.4770  1.000
## Residual 39  2.04482
```

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

```
##
## ***VECTORS
##
##             dbRDA1   dbRDA2     r2 Pr(>r)
## Elevation  0.77670  0.62986 0.0959  0.086 .
## Diameter  -0.27972 -0.96008 0.0541  0.267
## Depth     -0.63137  0.77548 0.1756  0.008 **
## ORP        0.41879 -0.90808 0.1437  0.022 *
## Temp      -0.98250  0.18628 0.1523  0.016 *
## SpC       -0.77101  0.63682 0.2087  0.003 **
## DO        -0.39318 -0.91946 0.0464  0.276
## pH        -0.96210 -0.27270 0.1756  0.009 **
## Color      0.06353  0.99798 0.0464  0.288
## chla      -0.60392 -0.79704 0.2626  0.008 **
## DOC        0.99847 -0.05526 0.0382  0.387
## DON       -0.91633  0.40042 0.0339  0.424
## ---
## 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)
dbrda.explainvar2 <- round(ponds.dbrda$CCA$eig[2] / sum(c(ponds.dbrda$CCA$eig, ponds.dbrda$CA$eig)), 3)

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

```r
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.explainvar1, "%)", sep = ""),
     pch = 16, cex = 2, type = "n", cex.lab = 1.5, cex.axis = 1.2, axes = F)
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(vector
axis(side = 4, lwd.ticks = 2, cex.axis = 1.2, las = 1, col = "red", lwd = 2.2, at = pretty(range(vector
```



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***: UniFrac distance is correlated with environmental variation (Mantel test p = 0.5). The environmental variables that significantly affect the phylogenetic diversity of pond communities are: depth, redox potential, temperature, specific conductivity of water, pH, and chlorophyll a.

6) SPATIAL PHYLOGENETIC COMMUNITY ECOLOGY

A. Phylogenetic Distance-Decay (PDD)

A distance decay (DD) relationship reflects the spatial autocorrelation of community similarity. That is, communities located near one another should be more similar to one another in taxonomic composition than distant communities. (This is analagous to the isolation by distance (IBD) pattern that is commonly found when examining genetic similarity of a populations as a function of space.) Historically, the two most common explanations for the taxonomic DD are that it reflects spatially autocorrelated environmental variables and the influence of dispersal limitation. However, if phylogenetic diversity is also spatially autocorrelated, then evolutionary history may also explain some of the taxonomic DD pattern. Here, we will construct the phylogenetic distance-decay (PDD) relationship

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 = T)

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

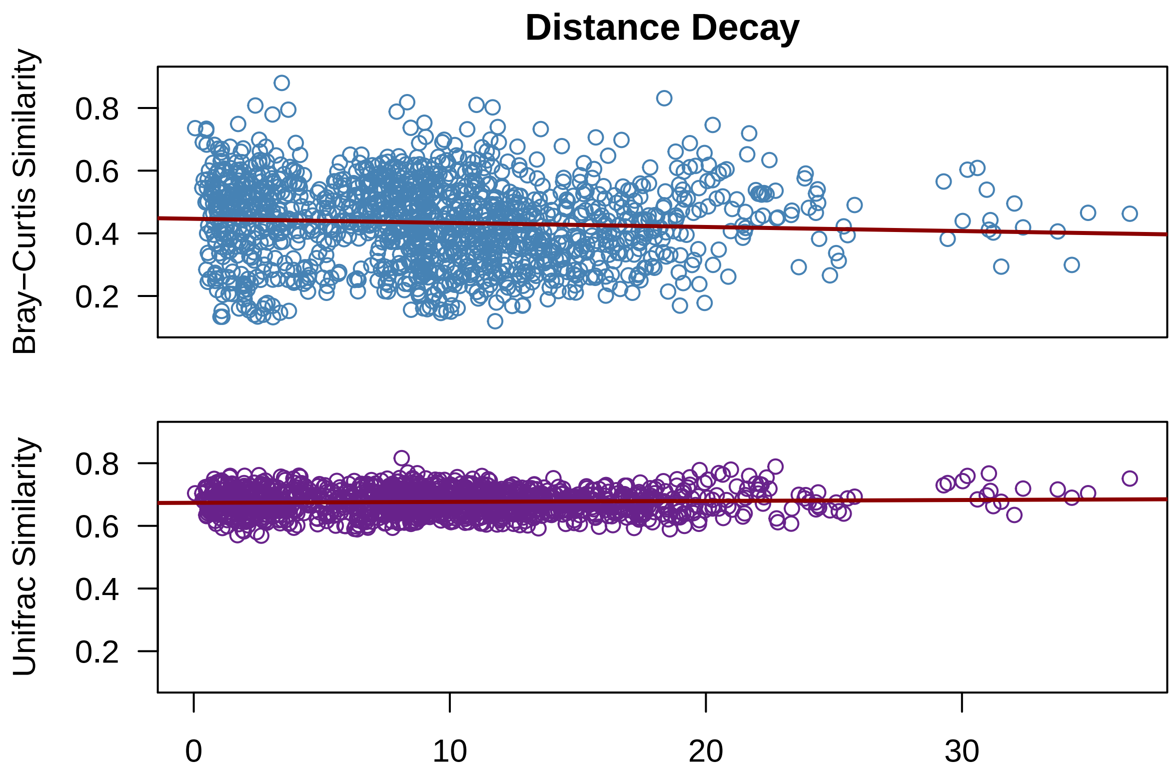
```
##
## Call:
## lm(formula = df$bray.curtis ~ df$geo.dist)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.31151 -0.08843  0.00315  0.09121  0.43817
##
## Coefficients:
```

```
##                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 =
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)
```



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: 0.003
##
## Empirical upper confidence limits of r:
##      90%      95%    97.5%      99%
## 0.000813 0.001006 0.001143 0.001316
```

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

> ***Answer 7***: The slope is negative when using Bray-Curtis similarity, indicating that ponds become less similar taxonomically with geographic distance, but the slope is positive when looking at Unifrac similarity, indicating that ponds become more phylogenetically similar with distance. These slopes were significantly different from one another. We might see this pattern if the species in the regional species pool share evolutionary history, but similar species may competitively exclude one another in local ponds. In other words, limited dispersal may drive decreasing Bray-

Curtis similarity with distance, while functionally similar and closely related pond assemblages may drive increasing Unifrac similarity with distance.

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.

> I could see myself wanting to account for phylogenetic relatedness in models looking at communities. For example, one project I'm working on is looking at whether variation in restored prairie community composition is best explained by the plant community that was established prior to restoration (indicating a soil legacy effect) or by the weed community that established immediately after seeding in the prairie (in which case competition is the likely mechanism). When looking at prairie community diversity I should account for phylogeny, both in terms of looking at alpha diversity as well as beta diversity between replicate prairies.