

9. Phylogenetic Diversity - Communities

Jonathan Enriquez Madrid; Z620: Quantitative Biodiversity, Indiana University

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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 ‘9.PhyloCom’ 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 *9.PhyloCom_Worksheet.Rmd* and the PDF output of **Knitr** (*9.PhyloCom_Worksheet.pdf*).

The completed exercise is due on **Wednesday, March 1st, 2023 before 12:00 PM (noon)**.

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 /9.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] "C:/Users/jonat/GitHub/QB2023_Enriquez_Madrid/2.Worksheets/9.PhyloCom"
```

```
setwd("C:/Users/jonat/GitHub/QB2023_Enriquez_Madrid/2.Worksheets/9.PhyloCom")
```

```
package.list <- c('picante', 'ape', 'seqinr', 'vegan', 'fossil',
                  'reshape', 'devtools', 'BiocManager', 'ineq',
                  'labdsv', 'matrixStats', 'pROC')
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 2.6-4
```

```
##
```

```
## 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
```

```
##
```

```
## Attaching package: 'devtools'
```

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

```
##
```

```
## check
```

```
##
## Attaching package: 'BiocManager'

## The following object is masked from 'package:devtools':
##
##     install

## This is mgcv 1.8-41. For overview type 'help("mgcv-package")'.

## Registered S3 method overwritten by 'labdsv':
##   method      from
##   summary.dist ade4

## This is labdsv 2.0-1
## convert existing ordinations with as.dsvord()

##
## Attaching package: 'labdsv'

## The following object is masked from 'package:stats':
##
##     density

##
## Attaching package: 'matrixStats'

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

## Type 'citation("pROC")' for a citation.

##
## Attaching package: 'pROC'

## The following objects are masked from 'package:stats':
##
##     cov, smooth, var
```

2) DESCRIPTION OF DATA

need to discuss data set from spatial ecology!

We sampled >50 forested ponds in Brown County State Park, Yellowood 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.

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

#Site by species matrix
comm <- read.otu(shared = "../data/INPonds.final.rdp.shared", cutoff = "1")

#Select dna data
comm <- comm[grepl("*-DNA", rownames(comm)), ]

#perform replacement of all matches
rownames(comm) <- gsub("\\-DNA", "", rownames(comm))
rownames(comm) <- gsub("\\_", "", rownames(comm))

#Remove sites not in the environment data set
comm <- comm[rownames(comm) %in% env$sample_ID, ]

#Remove zero-abundance OTUs
comm <- comm[, colSums(comm) > 0]

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

```
## Warning in type.convert.default(as.character(x)): 'as.is' should be specified by
## the caller; using TRUE
```

```
## Warning in type.convert.default(as.character(x)): 'as.is' should be specified by
## the caller; using TRUE
```

```
## Warning in type.convert.default(as.character(x)): 'as.is' should be specified by
## the caller; using TRUE
```

```
## Warning in type.convert.default(as.character(x)): 'as.is' should be specified by
## the caller; using TRUE
```

```
## Warning in type.convert.default(as.character(x)): 'as.is' should be specified by
## the caller; using TRUE
```

```
## Warning in type.convert.default(as.character(x)): 'as.is' should be specified by
## the caller; using TRUE
```

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.

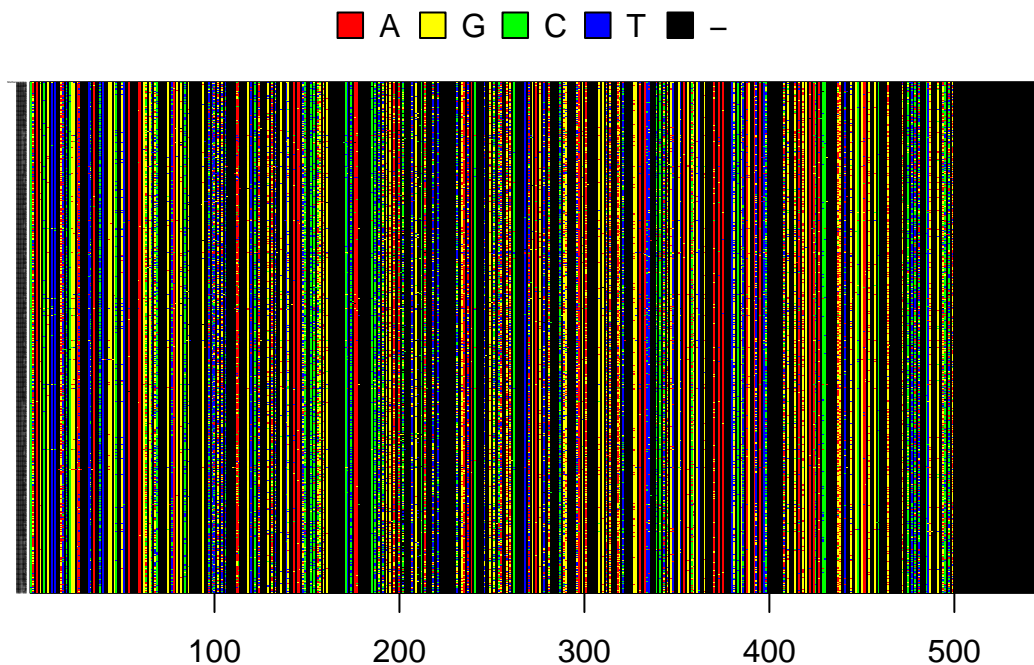
```
#Import alignment file
ponds.cons <- read.alignment(file = "./data/INPonds.final.rdp.1.rep.fasta",
                             format = "fasta")

#Rename OTUs in FASTA file
ponds.cons$nam <- gsub(".*\t", "", ponds.cons$nam)
ponds.cons$nam <- gsub("\\|.*", "", ponds.cons$nam)

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

#Convert alignment file to DNABin
DNABin <- rbind(as.DNABin(outgroup), as.DNABin(ponds.cons))

#Visualize alignment
image.DNABin(DNABin, show.labels = T, cex.lab = 0.05, las = 1)
```



```

#Make distance matrix ('ape')
seq.dist.jc <- dist.dna(DNABin, model = "JC", pairwise.deletion = FALSE)

#Make a neighbor-joining tree
phy.all <- bionj(seq.dist.jc)

#Drop sites of zero-occurrence
phy <- drop.tip(phy.all, phy.all$tip.label[!phy.all$tip.label %in%
                                                    c(colnames(comm), "Methanosarcina")])

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

#Root tree
phy <- root(phy, "Methanosarcina", resolve.root = TRUE)

#Plot rooted tree
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)

```

```

## Warning in plot.phylo(phy, main = "Neighbor Joining Tree", "phylogram", : found
## fewer than 2 tips in the tree

```

```
## NULL
```

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.

```

#Calculate PD and S
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.

```

#Biplot of S and PD
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 = "Phylogenetic diversity (PD) vs. Taxonomic richness (S)")

#test of power-law relationship

```

```
#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 taxonomic richness? b. Describe the relationship between taxonomic richness and phylogenetic diversity. 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 should be related to taxonomic richness as it measures how much evolutionary history taxa share. A high PD value indicates there is more evolutionary diverged taxa, which could mean a greater number of species and thus greater species richness. A lower PD value on the other hand shows less evolutionary diverged taxa, which could mean a smaller number of species and lower species richness. **Answer 1b:** As taxonomic richness increases, so does phylogenetic diversity. In other words, the more different species there are, the greater the chance these different species are greatly different from each other, and the greater the phylogenetic diversity will be. Species that are very different from each other have greater phylogenetic diversity than species who are less different from each other.

Answer 1c: Taxonomic diversity and phylogenetic diversity may deviate from each other when there is clustering in a phylogenetic tree. This is because species that cluster in a tree may be more related and have lower phylogenetic diversity. Even if species richness increases, clustering of species will mean that there will be low phylogenetic diversity between these sampled species.

Answer 1d: The significance of the PD-S scaling exponent is that it shows how PD scales with S. In this case, phylogenetic diversity increases as species richness increases.

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.

```
#estimate standardized effect size of PD via randomization
#ses.pd <- ses.pd(comm[1:2,], phy, null.model = "richness", 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:

- What are the null and alternative hypotheses you are testing via randomization when calculating `ses.pd`?
- 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: When testing the null hypothesis and alternative hypotheses, we are testing if our sample is more phylogenetically diverse than expected at random. The null is that our sample is not more phylogenetically diverse than expected at random and the alternative is that the sample is more phylogenetically diverse than expected at random.

Answer 2b: I could not get the code to work and cannot answer this question based on the values I did not receive. However, I would assume that if the sample truly is phylogenetically diverse, then it should make no difference on what null model one uses. The null model is there so that we can know for sure if our sample truly is phylogenetically diverse.

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.

```
#Phylogenetic distance matrix
#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)

#Calculate NRI
#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 = FALSE, runs = 25)

#Calculate NTI
#NTI <- as.matrix(-1 * ((ses.mntd[,2] - ses.mntd[,3]) / ses.mntd[,4]))
#rownames(NTI) <- row.names(ses.mntd)
#colnames(NTI) <- "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: When calculating the NRI one is measuring the relatedness between taxa by measuring the branch distances between taxa. Taxa that have a smaller branch distance between them are less evolutionary divergent, while taxa that have a greater branch distance between them are more evolutionary divergent.

Answer 3b: When calculating the NTI one is also measuring the relatedness between taxa, but by measuring the distance from the tip of the tree to the closest node. One is comparing taxa based on their nearest common ancestor. Taxa that have a lower distance to their nearest common

ancestor are more closely related than taxa that have a greater distance to their nearest common ancestor.

Answer 3c: I could not get the code to work so I cannot interpret the NRI and NTI values I did not receive. However, A positive NRI and NTI would indicate that the species are more closely related than expected (clustered), while a negative NRI and NTI would indicate the species are less related than expected (overdispersion).

Answer 3d: I do not know how “abundance.weighted = TRUE” affects the NRI and NTI values as I could not produce NTI and NRI values. However, if abundance data leads to more branches of the same species, I am not sure if this will mean that clustering (due to more branches of the same species) will be over expressed.

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)

#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: Mean Pair distance and UniFrac distance are both ways of measuring beta diversity between different sites. Mean Pair distance measures the distance between two sites based on the phylogenetic (branch) distance between taxa in each sample. UniFrac distance measures the beta diversity between sites by calculating the value of unshared branch lengths between sites.

Answer 4b: Unifrac distance values seem to cluster around a very narrow range of mean pair distance values. In the plot above, all the Unifrac distance values (ranging from 0.20 to 0.45) cluster around a mean Pair distance value of 0.3. **Answer 4c:** Mean Pair distance values may be showing less variation than Unifrac values because there might be a larger range of values that are reported when measuring Unifrac distance as it does not measure the mean like the Mean Pair distance does.

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 = 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.

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: I would assume that the phylogenetically based ordination explains more of the variation than the taxonomic ordination as the phylogenetic ordination is better at defining (with more certainty) which organisms are different species, and how different they are in terms of evolutionary history.

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
#phylo.adonis <- adonis2(dist.uf ~ watershed, permutations = 999)
#tax.adonis <- adonis2(vegdist(
  #decostand(comm, method = "log"),
  #method = "bray") ~ watershed,
  #permutations = 999)
```

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)
```

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

#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(slide = 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)

#add points and labels
#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)

#environmental vector
#vectors <- scores(ponds.dbrda, display = "bp")
#row.names(vectors) <- c("Temp", "DO", "chla", "DON")
#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[,2])) * 2, labels = 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: The phylogenetic patterns of beta-diversity seem to be driven by temperature, depth, pH, diameter, and elevation, chla, ORP, DOC, SPC, and DON of the different ponds. It is the differences found in these factors at different ponds that influence the phylogenetic differences between species at different sites, causing some taxa to be more similar based on their shared preference for temp., pH, or depth.

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 analogous 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 = TRUE)

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

#unifrac.dist <- 1 - dist.uf

#unifrac.dist.mlt <- melt(as.matrix(unifrac.dist))[melt(upper.tri(as.matrix(unifrac.dist)))$value,]

#bray.curtis.dist.mlt <- melt(as.matrix(bray.curtis.dist))[melt(upper.tri(as.matrix(bray.curtis.dist)))$value,]

# coord.dist.mlt <- melt(as.matrix(coord.dist))[melt(upper.tri(as.matrix(coord.dist)))$value,]

#env.dist.mlt <- melt(as.matrix(env.dist))[melt(upper.tri(as.matrix(env.dist)))$value,]

#df <- data.frame(coord.dist.mlt, bray.curtis.dist.mlt[,3], unifrac.dist.mlt[,3],
# env.dist.mlt[, 3])
#names(df) [3:6] <- c("geo.dist", "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)
#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)

#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.

```
#source("./bin/diffslope.R")  
#diffslope(df$geo.dist, df$unifrac, df$geo.dist, df$bray.curtis)
```

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

Answer 7: I could not get the code to work so I cannot test if there are differences between the taxonomic and phylogenetic DD relationships. However, by simply looking at the two plots one can see that there are differences. For the taxonomic DD relationship there seems to be both high and low similarity between species at short geographical distances, but intermediate similarity between species at longer distances. As for the Unifrac DD relationship, there seems to be less variation in differences in branch length between species as geographic distance increases. In other words, one plot is showing great variation in species across geographical distance and the other plot is showing low variation in species across geographical distance. These differences may arise because one plot is running off taxonomic data, and the other off phylogenetic data. The phylogenetic data may provide more information as it takes into account shared evolutionary history, while the taxonomic data does not. The phylogenetic data may also be more reliable in showing more accurate species differences along geographical 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.

*** Answer *** Phylogenetic information could be useful in my own research as it would clarify if the different species of nematodes I am working with truly are separate species. Understanding if the nematodes I am working with really are different species is important as I am asking questions about reproductive barriers between sympatric nematode species via host preference. If I am exploring reproductive barriers between sympatric nematode species, but all my nematodes are actually the same species, then there is no point in asking these questions. I would need to acquire phylogenetic data on the genus of nematode that I am working on (*Steinernema*) to see if the three sympatric species are actually different species. In doing so I could then ask my questions pertaining to reproductive barriers between sympatric populations and how this could contribute to local species diversity.