9.Phylogenetic Diversity - Communities

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11 March, 2025

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 5th, 2025 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 `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.

```
getwd()
```

```
## [1] "/cloud/project/QB2025_Guevara/Week7-PhyloCom"
```

```
setwd("/cloud/project/QB2025_Guevara/Week7-PhyloCom")

package.list <- c('picante', 'ape', '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)
  }
}
```

```
##
## 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 1.9-1. For overview type 'help("mgcv-package")'.

## This is labdsv 2.1-0
## convert existing ordinations with as.dsvord()

##
## Attaching package: 'labdsv'

## The following objects are masked from 'package:vegan':
##
##     calibrate, pca, pco, scores

## The following objects are masked from 'package:stats':
##
##     density, loadings

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

##
## Attaching package: 'pROC'

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

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

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.

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

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

#Select DNA data using 'grep()'
comm <- comm[grep("*-DNA", rownames(comm)), ]

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

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

#Remove zero- abundance OTUS from data set
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 the alignment file ('seqinr')
library(seqinr)
```

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

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

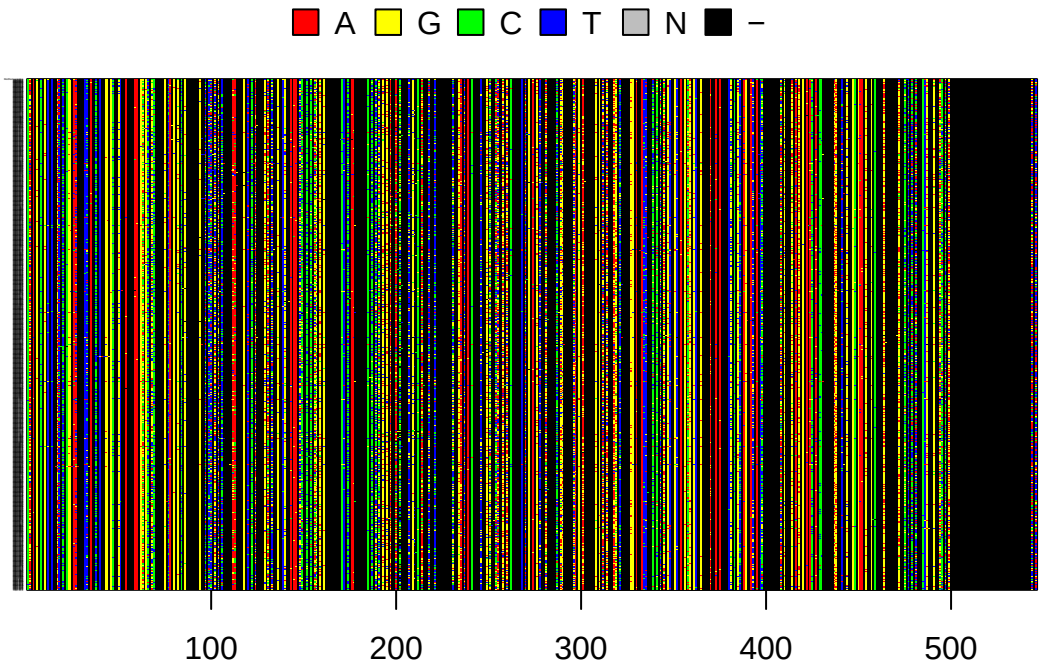
```
## The following object is masked from 'package:matrixStats':
##
##     count
```

```
## 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
```

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

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

##Import outgroup sequence
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 file (`ape`)
phy.all <- bionj(seq.dist.jc)

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

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

# Root the tree (`ape`)
phy <- root(phy, outgroup, resolve.root = TRUE)

# Plot the rooted tree (`ape`)
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

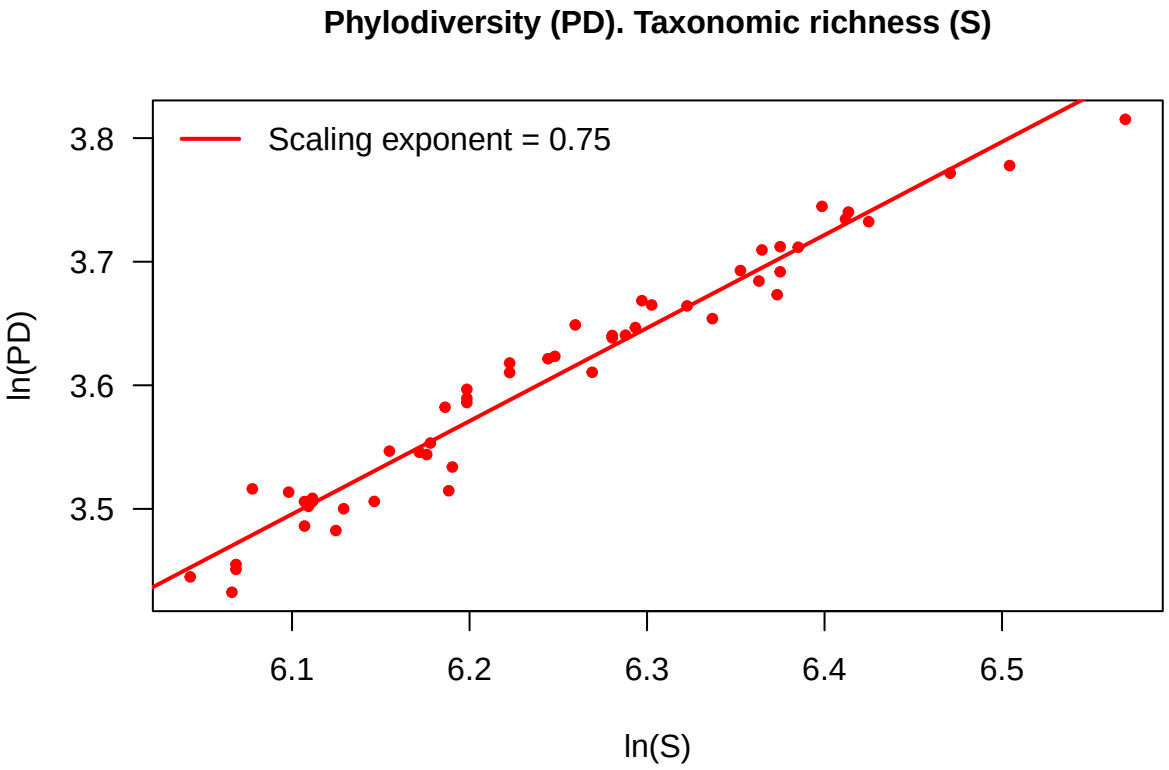
A. Faith's Phylogenetic Diversity (PD)

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.

```
#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 = "Phylodiversity (PD). 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 = "
```



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

> ***Answer 1a***: "Faith's PD sums the branch lengths for each species found in a sample from the root to the tip of the phylogenetic tree." This metric should be related to taxomic richness because it accounts for how much genetic diversity there is within the tree where a higher PD value would indicate more diverged taxa, thus greater taxonomic richness. Lower PD values would indicate less taxonomic richness in the generated tree. PD just gives a more complex understanding of the diversity as the PD values would indicate the nuances/differences in taxa at the genetic level as opposed to just the species or taxa level. ***Answer 1b***: I would expect these two to deviate in cases where there are many closely related species with few genetic differences among them. This would result in small branch lengths (smaller small, thus smaller faith's PD) with high levels of taxonomic richness. ***Answer 1c***: The scaling exponent is significant because it actively shows us at which PD increases compared to taxonomic richness. We see, with a exponent of 0.75, that PD increases at a slower rate compared to taxonomic richness in this case. This probably means that there is quite a bit of taxonomic clustering leading to shorter branch lengths (we do see this in the tree up above).

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 ('picante')
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:

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 null hypothesis is that faith's PD does not deviate from the expected PD which is calculated from randomized community data matrix abundances. The alt. hypothesis tests is that our PD does deviate from the random expected value.
Answer 2b: Since it maintains sample species richenss, thus not altering our overall richness. The only aspect of our ses.pd value that it would be the sample mean values in pd.rand.mean and used to calculate pd.rand.sd.

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.

```
#Create a phylogenetic distance matrix ('picante')
phydist <- cophenetic.phylo(phy)
#Estimate standardized effect size of NRIA via randomization ('picante')
ses.mpd <- ses.mpd(comm,phydist, null.model = "taxa.labels",
                   abundance.weighted = FALSE, runs = 25)
```

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.

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

#Estimate standardized effect size for NRI via randomizaiton
ses.mntd <- ses.mntd(comm, phydist, null.model = "taxa.labels",
                     abundance.weighted = FALSE, runs = 25)
```

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.

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

```
## NULL
```

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, we are essentially performing a Tukey test where we perform pairwise comparisons between each branch length and average the difference across all

comparisons. We are also essentially following the same formula as for our standard effect size of phylogenetic diversity (PD) but instead of PD, it now becomes our mean phylogenetic distance. ***Answer 3b***: In calculating the NTI, we basically do the same formual again as for standard effect size of PD, but now using mean nearest phylogenetic neighbor distance values instead. This only considers the most closely related taxa which can highlight taxa clustering at the branch tips. ***Answer 3c***: From our NTI values, which vary quite a bit but are generally negative, we can see that the taxa our generally overdispersed. There are few ponds resulting in positive NTI values indicating clustering, but the clustering we see is minor as indicated y our NTI. From our NRI, we see a lot more variation between negative and positive, but more positives than negatives. This would lead us to the interpretation of clustering. ***Answer 3d***: When we changed whether we are weighting abundance data for NTI, we see that it actually reverses our interpretation, now making all of the values (except one) greater than zero. This would lead us to interpret what we are seeing as clustered or undispersed. For our NRI, the values become slightly positive with very few surpassing one or some become just very slightly negative values. It seems that generally, when we weight our abundances, our interpretations of clustering or overdispersion tend to flip.

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.

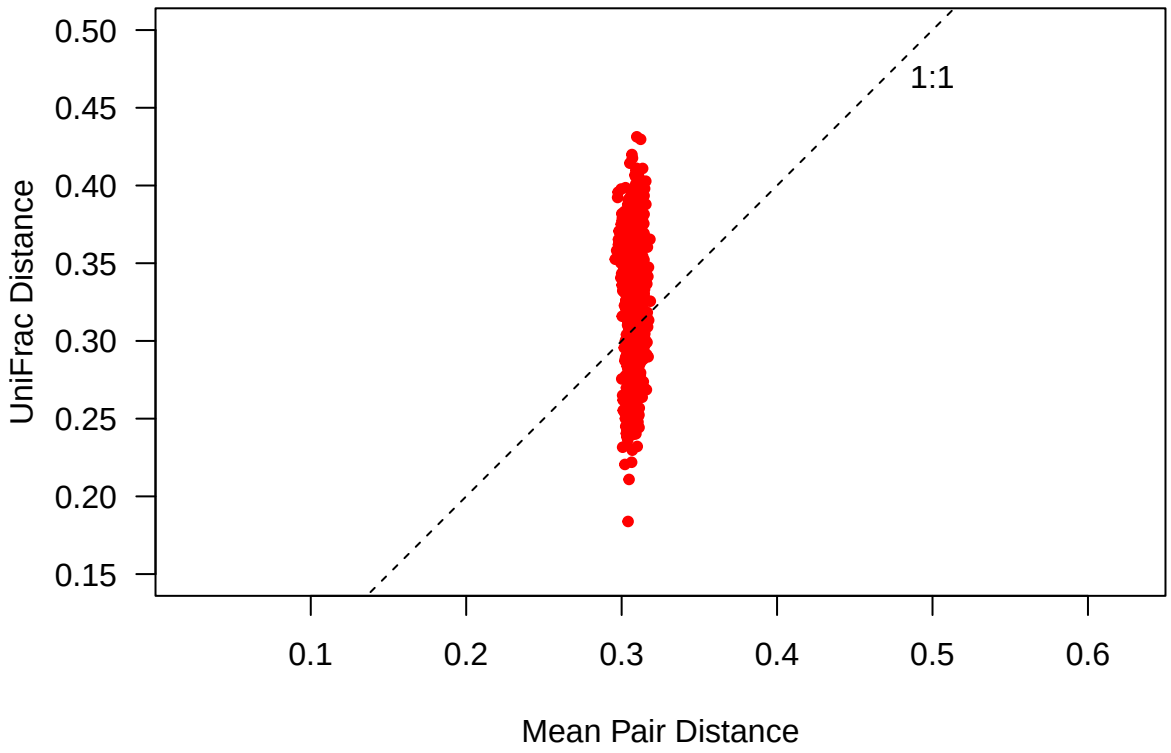
```
#Mean pairwise distance
dist.mp <- comdist(comm, phydist)
```

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

```
#UniFrac Distance (note: takes a few mins)
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 is essentially taking the pairwise distance between every single pair of taxa and then taking the average value of each of those distances. UniFrac distance is another average, but this time specifically focusing only on the sum of unique branch lengths between each pair of taxa within a given sample to be divided to by the sum of all branch lengths. I would characterize the difference between these two is whether we want to focus on uniqueness or distances generally where UniFrac seems to focus on dissimilarity with the summation of unique branch lengths and MPD focuses on the general distance between each pair, disregarding unique branch lengths and focusing simply on distance between pairs of taxa. ***Answer 4b***: It seems that MPD is essentially constant (slight variations) while UniFrac varies much more so. There seems to be a direct correlation or a 1:1 relationship where the two distances would grow at an equal rate, and in this case, are equal to each other. ***Answer 4c***: MPD might show less variation because it focuses solely on average phylogenetic distance between every pair of species/taxa which shouldn't really change so long as the distances don't, but the slight variations are in each of the tree formations. UniFrac varies much more so because each community would have different number of unshared branch lengths between each taxa pair leading to much more variation but keeping the total branch lengths constant, leading to all the variation but keeping it within a certain range.

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.

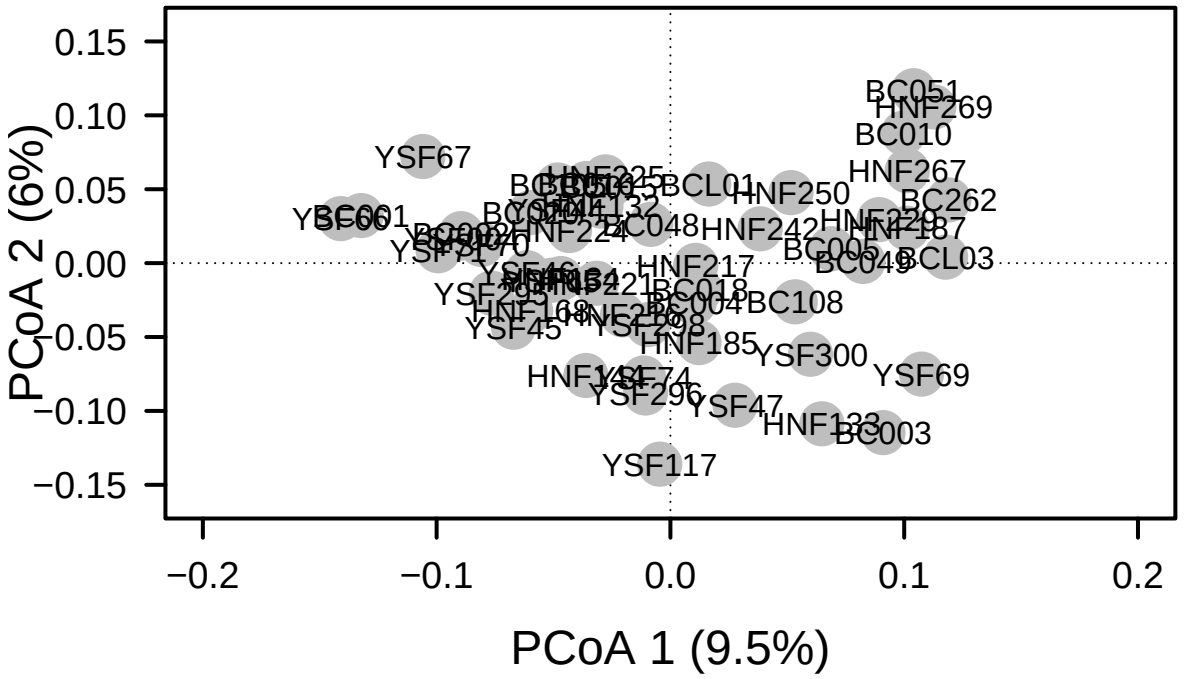
In the R code chunk below, do the following:
1. perform a PCoA based on the UniFrac distances, and
2. calculate the explained variation for the first three PCoA axes.

```
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)

#Define plot parameters
par(mar = c(5,5,1,2) +0.1)
```

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

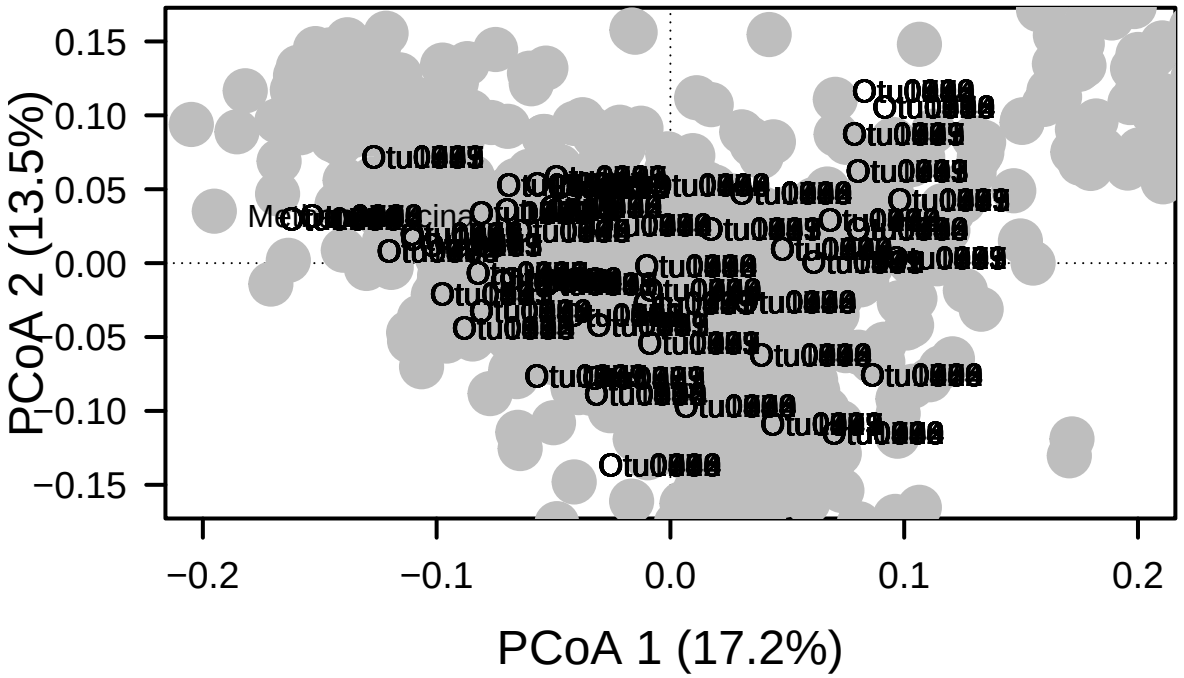
In the R code chunk below, do the following:
1. plot the PCoA results using either the R base package or the `ggplot` package,
2. include the appropriate axes,
3. add and label the points, and
4. customize the plot.

```
#Initiate Plto
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)

#Add axes
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)

#Add points and labels
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.

```
library(vegan)
library(ape)
#Taxonomic data
tax.pcoa <- cmdscale(seq.dist.jc, eig = T, k = 3)
explainvar1.tax <- round(tax.pcoa$eig[1] / sum(tax.pcoa$eig), 3) * 100
explainvar2.tax <- round(tax.pcoa$eig[2] / sum(tax.pcoa$eig), 3) * 100
explainvar3.tax <- round(tax.pcoa$eig[3] / sum(tax.pcoa$eig), 3) * 100
sum.eig.tax <- sum(explainvar1.tax, explainvar2.tax, explainvar3.tax)

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

#Add axes
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)

#Add points and labels
points(tax.pcoa$points[ ,1], tax.pcoa$points[ , 2],
       pch = 19, cex = 3, bg = "gray", col = "gray")
text(pond.pcoa$points[ , 1], pond.pcoa$points[ , 2],
     labels = row.names(tax.pcoa$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***: Compared to just our taxa, our phylogenetic data provides a much clearer insight on the actual beta diversity than we are seeing where as our taxonomic data, even though it uses a jaccard method of similarity that incorporates incidence values only. Taxonomy seems to lead to much greater variation as taxa composition is much greater than UniFrac calculations focusing on how different/how related taxa are evolutionarily. Phylogeneitc information is very important because there are so many taxa in this system, thus there will be too much variation for us to get a sense of any actual patterns from the plots we are capable of visualizing.

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.

```
##Defining environmental category
watershed <- env$Location

#Run PERMANOVA iwth adonis
phylos.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()`).

```r
# Define environmental variables
envs <- env[, 5:19]

# Remove redundant variables
envs <- envs[, -which(names(envs) %in% c("TDS", "Salinity", "Cal_Volume"))]

# Create distance matrix for environmental variables
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
# Conduct Mantel Test (`vegan`)
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: 0.048
##
## Upper quantiles of permutations (null model):
##   90%   95% 97.5%   99%
## 0.122 0.157 0.193 0.231
## Permutation: free
## Number of permutations: 999
```

```r
# Conduct dbRDA (`vegan`)
ponds.dbrda <- dbrda(dist.uf ~ ., data = as.data.frame(scale(envs)))

# Permutation tests: axes and environmental variables
anova(ponds.dbrda, by = "axis")
```

```
## Permutation test for dbrda under reduced model
## Forward tests for axes
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = dist.uf ~ Elevation + Diameter + Depth + ORP + Temp + SpC + DO + pH + Color +
##          Df SumOfSqs      F Pr(>F)
## dbRDA1    1  0.10566 2.0152  0.448
## dbRDA2    1  0.09258 1.7658  0.633
## dbRDA3    1  0.07555 1.4409  0.981
## dbRDA4    1  0.06677 1.2735  0.995
## dbRDA5    1  0.05666 1.0807  1.000
## dbRDA6    1  0.05293 1.0095
## dbRDA7    1  0.04750 0.9059
## dbRDA8    1  0.03941 0.7517
## dbRDA9    1  0.03775 0.7201
## dbRDA10   1  0.03280 0.6256
## dbRDA11   1  0.02876 0.5485
```

```
## dbRDA12   1  0.02501 0.4770
## 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  0.094 .
## Diameter   0.27972 -0.96008 0.0541  0.251
## Depth      0.63137  0.77548 0.1756  0.011 *
## ORP       -0.41879 -0.90808 0.1437  0.026 *
## Temp       0.98250  0.18628 0.1523  0.014 *
## SpC        0.77101  0.63682 0.2087  0.008 **
## DO         0.39318 -0.91946 0.0464  0.285
## pH         0.96210 -0.27270 0.1756  0.012 *
## Color     -0.06353  0.99798 0.0464  0.317
## chla       0.60392 -0.79704 0.2626  0.012 *
## DOC       -0.99847 -0.05526 0.0382  0.388
## DON        0.91633  0.40042 0.0339  0.423
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

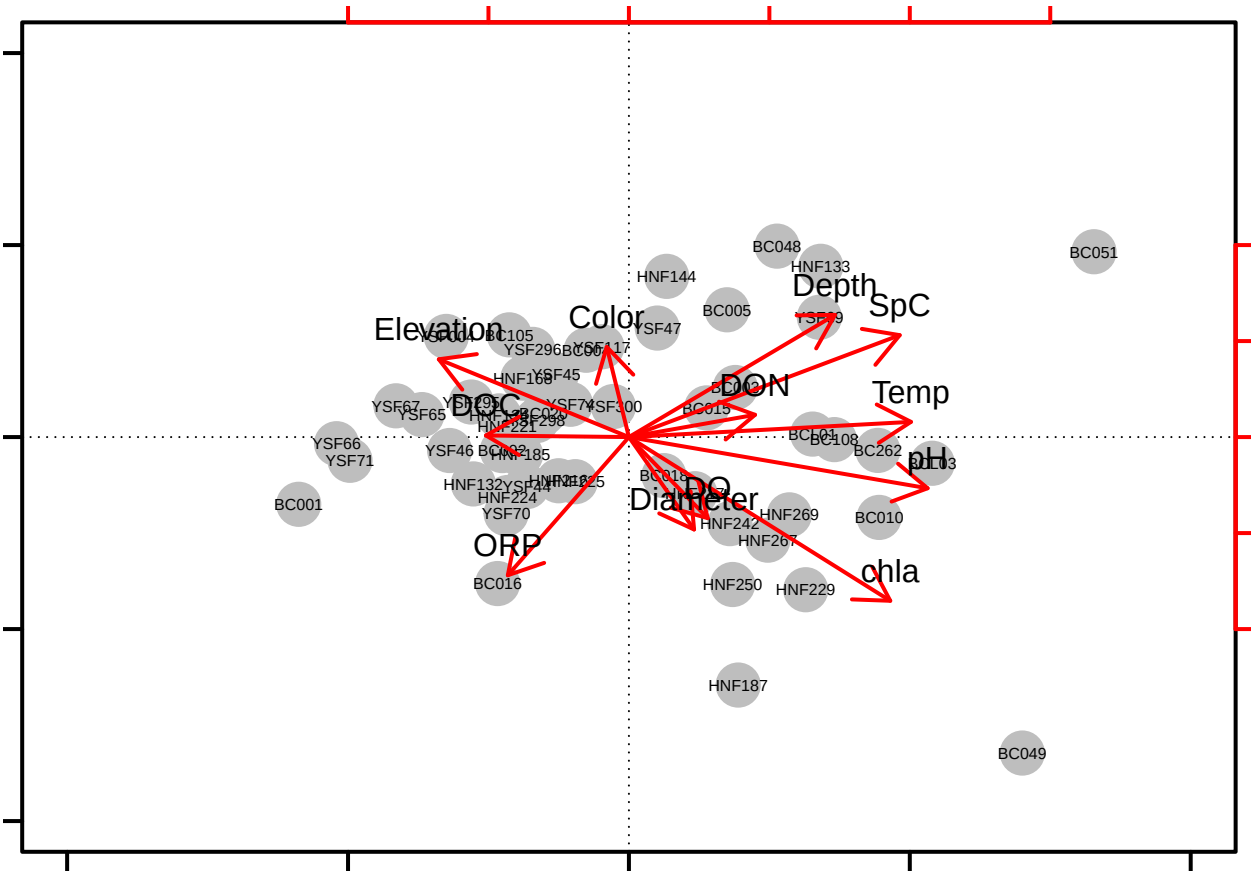
```
# Calculate explained variation
dbrda.explainvar1 <- round(ponds.dbrda$CCA$eig[1] /
    sum(c(ponds.dbrda$CCA$eig, ponds.dbrda$CA$eig)), 3) * 100
dbrda.explainvar2 <- round(ponds.dbrda$CCA$eig[2] /
    sum(c(ponds.dbrda$CCA$eig, ponds.dbrda$CA$eig)), 3) * 100
```

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

```
# Make dbRDA plot

# Extract scores from the dbRDA object
ponds_scores <- vegan::scores(ponds.dbrda, display = "sites")

# Define plot parameters
par(mar = c(5, 5, 4, 4) * 0.1)

# Initiate plot
plot(ponds_scores, 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)

# Add axes
axis(side = 1, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
```

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

# Extract site scores
wa_scores <- vegan::scores(ponds.dbrda, display = "sites")

# Add points and labels
points(wa_scores, pch = 19, cex = 3, col = "gray")

# Add labels
text(wa_scores, labels = rownames(wa_scores), cex = 0.5)

# Extract environmental vectors (biplot scores)
vectors <- vegan::scores(ponds.dbrda, display = "bp")

# Add environmental vectors to the plot
arrows(0, 0, vectors[,1] * 2, vectors[,2] * 2,
    lwd = 2, lty = 1, length = 0.2, col = "red")

# Add labels for the environmental vectors
text(vectors[,1] * 2, vectors[,2] * 2, pos = 3,
    labels = rownames(vectors))

# Add axes for the 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]) * 2))

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]) * 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***: We see that Temp, SpC, chla, pH, ORP, Elevation, and Depth seem to strongly influence bacterial community composition the most in the Indiana Ponds. This basically means that overall, phylogenetic patterns of beta diversity can be explained largely due to these environmental variables, where the more similar two environments are, more simlar the bacterial communities will likely be.

SYNTHESIS

Question 7: 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 7***: For my research, phylogenetic data would be useful for determining phylogenetic ordiance of closely related prairie species, their invasive counterparts, and locally adapted counterparts to get a good idea of what genetic differences are arising. I would primarily focus on the two populations of the same species where one shows evidence of local adaptation to determine which genetic changes are responsible for the local adaptation. I would need fasta files for each of my populations per species. Then, if other species show local adaptations to these same conditions after a certain amount of time, I would compare the phylogenetic distances of these species to see how similar or dissimilar they are from one another to see if that correlates with ability to adapt rapidly. I could use this to help me answer if local adaptation is possible at a rapid scale in plants, are specific taxa more prone to the changes compared to others? Then, using further

fasta data, I could determine whether these taxa are more prone because of specific gene or DNA region they possess and others lack.