8. Worksheet: Among Site (Beta) Diversity – Part 1

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16 April, 2021

OVERVIEW

In this worksheet, we move beyond the investigation of within-site α -diversity. We will explore β -diversity, which is defined as the diversity that occurs among sites. This requires that we examine the compositional similarity of assemblages that vary in space or time.

After completing this exercise you will know how to:

- 1. formally quantify β -diversity
- 2. visualize β -diversity with heatmaps, cluster analysis, and ordination
- 3. test hypotheses about β -diversity using multivariate statistics

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. The 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 (**8.BetaDiversity_1_Worksheet.Rmd**) with all code blocks filled out and questions answered) and the PDF output of Knitr (**8.BetaDiversity_1_Worksheet.pdf**).

The completed exercise is due on Friday, April 16th, 2021 before 09:00 AM.

1) R 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 "/8.BetaDiversity" folder, and
- 4. load the vegan R package (be sure to install if needed).

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

[1] "C:/Users/Danny/Desktop/GitHub/QB2021_Peltier-Thompson/2.Worksheets/8.BetaDiversity"

```
{\tt setwd("C:/Users/Danny/Desktop/GitHub/QB2021\_Peltier-Thompson/2.Worksheets/8.BetaDiversity")} \\ {\tt getwd()}
```

[1] "C:/Users/Danny/Desktop/GitHub/QB2021_Peltier-Thompson/2.Worksheets/8.BetaDiversity"

2) LOADING DATA

Load dataset

- 1. load the doubs dataset from the ade4 package, and
- 2. explore the structure of the dataset.

```
# note, please do not print the dataset when submitting
package.list <- c('vegan', 'ade4', 'viridis', 'gplots', 'BiodiversityR', 'indicspecies')
for (package in package.list){
   if(!require(package, character.only = TRUE, quietly = TRUE)) {
     install.packages(package)
     library(package, character.only = TRUE)
   }
}</pre>
```

```
## Warning: package 'vegan' was built under R version 4.0.5

## This is vegan 2.5-7

## Warning: package 'ade4' was built under R version 4.0.5

## Warning: package 'viridis' was built under R version 4.0.5

## Warning: package 'gplots' was built under R version 4.0.5

## ## Attaching package: 'gplots'

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

## lowess
```

```
## Warning: package 'BiodiversityR' was built under R version 4.0.5
## Registered S3 methods overwritten by 'lme4':
##
     method
##
     cooks.distance.influence.merMod car
##
     influence.merMod
##
     dfbeta.influence.merMod
                                      car
##
     dfbetas.influence.merMod
                                      car
## BiodiversityR 2.12-3: Use command BiodiversityRGUI() to launch the Graphical User Interface;
## to see changes use BiodiversityRGUI(changeLog=TRUE, backward.compatibility.messages=TRUE)
## Warning: package 'indicspecies' was built under R version 4.0.5
data(doubs)
str(doubs, max.level = 1)
## List of 4
    $ env
             :'data.frame': 30 obs. of
                                         11 variables:
    $ fish
             :'data.frame': 30 obs. of
                                         27 variables:
    $ xy
             :'data.frame': 30 obs. of
                                         2 variables:
   $ species:'data.frame': 27 obs. of
                                         4 variables:
head(doubs$env)
##
               slo flo pH har pho nit amm oxy bdo
## 1
       3 934 6.176 84 79
                           45
                                 1
                                    20
                                         0 122
                                                27
## 2 22 932 3.434 100 80
                                 2
                                    20
                                        10 103
                                                19
                           40
## 3 102 914 3.638 180 83
                           52
                                 5
                                    22
                                         5 105
                                                35
## 4 185 854 3.497 253 80
                           72
                                10
                                    21
                                         0 110
                                                13
## 5 215 849 3.178 264 81
                           84
                                38
                                    52
                                        20
                                            80
                                                62
## 6 324 846 3.497 286 79
                           60
                                20
                                    15
                                         0 102
                                                53
```

Question 1: Describe some of the attributes of the doubs dataset.

- a. How many objects are in doubs?
- b. How many fish species are there in the doubs dataset?
- c. How many sites are in the doubs dataset?

Answer 1a: 4 **Answer 1b**: 27 **Answer 1c**: 30

Visualizing the Doubs River Dataset

Question 2: Answer the following questions based on the spatial patterns of richness (i.e., α -diversity) and Brown Trout (Salmo trutta) abundance in the Doubs River.

- a. How does fish richness vary along the sampled reach of the Doubs River?
- b. How does Brown Trout (Salmo trutta) abundance vary along the sampled reach of the Doubs River?
- c. What do these patterns say about the limitations of using richness when examining patterns of biodiversity?

Answer 2a: richness increases downstream $Answer \ 2b$: richness decreases downstream $Answer \ 2c$: the brown trout has oposite pattern than fish in general. Using richness only will show the most abundant pattern but not the variation

3) QUANTIFYING BETA-DIVERSITY

- 1. write a function (beta.w()) to calculate Whittaker's β -diversity (i.e., β_w) that accepts a site-by-species matrix with optional arguments to specify pairwise turnover between two sites, and
- 2. use this function to analyze various aspects of β -diversity in the Doubs River.

```
fish <- doubs$fish
beta.w <- function(site.by.species = ""){</pre>
  SbyS.pa <- decostand(site.by.species, method = "pa")</pre>
  S <- ncol(SbyS.pa[,which(colSums(SbyS.pa) > 0)])
  a.bar <- mean(specnumber(SbyS.pa))</pre>
  b.w \leftarrow round(S/a.bar, 3)
  return(b.w)
}
beta.wt <- function(site.by.species = "", sitenum1 = "", sitenum2 = "", pairwise = FALSE){
  if (pairwise == TRUE){
    if(sitenum1 == "" | sitenum2 == ""){
      print("Error: please specify sites to compare")
      return(NA)
    site1 = site.by.species[sitenum1,]
    site2 = site.by.species[sitenum2,]
    site1 = subset(site1, select = site1 > 0)
    site2 = subset(site2, select = site2 > 0)
    gamma = union(colnames(site1), colnames(site2))
    s = length(gamma)
    a.bar = mean(c(specnumber(site1), specnumber(site2)))
    b.w = round(s/a.bar - 1,3)
    return(b.w)
  }
  else{
    SbyS.pa <- decostand(site.by.species, method = "pa")</pre>
    S <- ncol(SbyS.pa[,which(colSums(SbyS.pa) > 0)])
    a.bar <- mean(specnumber(SbyS.pa))</pre>
    b.w <- round(S/a.bar, 3)
    return(b.w)
  }
}
bwt_doubs <- beta.wt(fish)</pre>
bw_doubs <- beta.w(fish)</pre>
bwt_1_2 \leftarrow beta.wt(fish[c(1,2),])
bwt_1_10 \leftarrow beta.wt(fish[c(1,10),])
```

Question 3: Using your beta.w() function above, answer the following questions:

- a. Describe how local richness (α) and turnover (β) contribute to regional (γ) fish diversity in the Doubs.
- b. Is the fish assemblage at site 1 more similar to the one at site 2 or site 10?
- c. Using your understanding of the equation $\beta_w = \gamma/\alpha$, how would your interpretation of β change if we instead defined beta additively (i.e., $\beta = \gamma \alpha$)?

Answer 3a: gamma is total number of species within the region. Alpha diversity how many of those species are found in one place/time. When beta diversity is large, the region is more diverse and gamma is a larger than alpha so there are a lot of species and each site has a different collection of species. When beta diversity is small, the alpha and gamma values are similar so there could be a lot of species but each site has the same species so diversity is low. Answer 3b: site 1 is more similar to site 2 Answer 3c: if alpha = gamma, than additive beta = 0 and multiplicative beta = 1. the greater gamma is compared to alpha, the larger beta will be in both the additive and multiplicative equations. the additive beta is how how many more species are in the region than the site but the multiplicative beta doesn't quantify the number of species but rather the ratio of species represented at site and total species

The Resemblance Matrix

In order to quantify β -diversity for more than two samples, we need to introduce a new primary ecological data structure: the **Resemblance Matrix**.

Question 4: How do incidence- and abundance-based metrics differ in their treatment of rare species?

Answer 4:

- 1. make a new object, fish, containing the fish abundance data for the Doubs River,
- 2. remove any sites where no fish were observed (i.e., rows with sum of zero),
- 3. construct a resemblance matrix based on Sørensen's Similarity ("fish.ds"), and
- 4. construct a resemblance matrix based on Bray-Curtis Distance ("fish.db").

```
fish <- doubs$fish
fish \leftarrow fish[-8,]
fish.dj <- vegdist(fish, method = "jaccard", binary = TRUE)</pre>
fish.db <- vegdist(fish, method = "bray")</pre>
fish.ds <- vegdist(fish, method = "bray", binary = TRUE)
fish.db
##
               1
                          2
                                      3
                                                            5
                                                                        6
                                                                                   7
## 2
      0.60000000
      0.68421053 0.14285714
## 4
      0.75000000 0.33333333 0.18918919
     0.89189189 0.69565217 0.68000000 0.49090909
      0.75000000 0.39393939 0.29729730 0.19047619 0.41818182
      0.68421053 0.14285714 0.12500000 0.24324324 0.64000000 0.24324324
      1.00000000 0.69230769 0.73333333 0.65714286 0.58333333 0.54285714 0.66666667
## 10 0.88235294 0.38461538 0.40000000 0.37142857 0.54166667 0.25714286 0.26666667
## 11 0.57142857 0.30434783 0.40740741 0.43750000 0.68888889 0.43750000 0.33333333
## 12 0.71428571 0.20000000 0.23529412 0.33333333 0.69230769 0.38461538 0.17647059
## 13 0.72727273 0.29032258 0.31428571 0.45000000 0.73584906 0.55000000 0.37142857
## 14 0.80645161 0.40000000 0.31818182 0.34693878 0.67741935 0.42857143 0.36363636
## 15 0.83333333 0.51111111 0.46938776 0.40740741 0.55223881 0.37037037 0.38775510
## 16 0.86046512 0.65384615 0.57142857 0.47540984 0.45945946 0.37704918 0.53571429
## 17 0.91489362 0.67857143 0.63333333 0.50769231 0.51282051 0.44615385 0.60000000
## 18 0.95555556 0.74074074 0.72413793 0.58730159 0.50000000 0.52380952 0.68965517
## 19 1.00000000 0.79310345 0.70967742 0.61194030 0.50000000 0.52238806 0.67741935
```

```
## 20 1.00000000 0.91176471 0.88888889 0.74025974 0.48888889 0.68831169 0.86111111
## 21 1.00000000 0.94594595 0.92307692 0.78313253 0.50000000 0.73493976 0.89743590
## 22 1.00000000 0.97619048 0.95454545 0.82795699 0.52830189 0.78494624 0.93181818
## 23 1.00000000 1.00000000 1.00000000 0.92000000 0.89473684 0.84000000 0.90000000
## 24 1.00000000 1.00000000 1.00000000 0.88888889 0.79591837 0.77777778 0.93548387
## 25 1.00000000 1.00000000 0.92592593 0.81250000 0.68888889 0.68750000 0.85185185
## 26 1.00000000 0.96363636 0.93220339 0.78125000 0.55844156 0.68750000 0.89830508
## 27 1.00000000 0.97333333 0.94936709 0.83333333 0.56701031 0.76190476 0.92405063
## 28 1.00000000 0.97560976 0.95348837 0.82417582 0.57692308 0.78021978 0.93023256
## 29 0.97777778 0.93939394 0.92233010 0.81481481 0.53719008 0.77777778 0.90291262
## 30 1.00000000 1.00000000 0.98095238 0.87272727 0.59349593 0.83636364 0.96190476
##
               9
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                                    11
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## 2
## 3
## 4
## 5
## 6
## 7
## 9
## 10 0.57142857
## 11 0.76000000 0.44000000
## 12 0.68750000 0.37500000 0.24137931
## 13 0.81818182 0.57575758 0.33333333 0.18918919
## 14 0.76190476 0.47619048 0.43589744 0.21739130 0.19148936
## 15 0.65957447 0.40425532 0.50000000 0.33333333 0.38461538 0.24590164
## 16 0.70370370 0.51851852 0.64705882 0.55172414 0.59322034 0.44117647 0.26027397
## 17 0.68965517 0.51724138 0.63636364 0.58064516 0.61904762 0.50000000 0.40259740
## 18 0.64285714 0.57142857 0.69811321 0.66666667 0.70491803 0.60000000 0.46666667
## 19 0.66666667 0.63333333 0.82456140 0.75000000 0.81538462 0.67567568 0.56962025
## 20 0.68571429 0.77142857 0.91044776 0.89189189 0.92000000 0.83333333 0.70786517
## 21 0.76315789 0.81578947 0.91780822 0.92500000 0.95061728 0.86666667 0.76842105
## 22 0.76744186 0.86046512 0.95180723 0.95555556 0.97802198 0.90000000 0.77142857
## 23 0.77777778 0.88888889 0.86666667 0.90909091 1.00000000 0.93750000 0.94594595
## 24 0.72413793 0.79310345 0.92307692 0.93939394 1.00000000 0.90697674 0.87500000
## 25 0.84000000 0.76000000 0.90909091 0.93103448 1.00000000 0.84615385 0.81818182
## 26 0.71929825 0.82456140 0.92592593 0.93442623 0.96774194 0.85915493 0.76315789
## 27 0.76623377 0.84415584 0.94594595 0.95061728 0.97560976 0.89010989 0.77083333
## 28 0.76190476 0.85714286 0.95061728 0.95454545 0.97752809 0.89795918 0.78640777
## 29 0.78217822 0.84158416 0.89795918 0.90476190 0.90566038 0.84347826 0.73333333
## 30 0.84466019 0.90291262 0.98000000 0.98130841 1.00000000 0.93162393 0.81967213
                         17
                                    18
                                              19
                                                          20
## 2
## 3
## 4
## 5
## 6
## 7
## 9
## 10
## 11
## 12
## 13
## 14
## 15
```

```
## 16
## 17 0.26190476
## 18 0.34146341 0.13953488
## 19 0.39534884 0.31111111 0.25000000
## 20 0.58333333 0.42000000 0.32653061 0.23529412
## 21 0.62745098 0.49056604 0.40384615 0.29629630 0.10169492
## 22 0.66071429 0.55172414 0.47368421 0.38983051 0.18750000 0.10447761
## 23 0.90909091 0.83333333 0.82608696 0.84000000 0.86666667 0.87878788 0.89473684
## 24 0.81818182 0.69491525 0.64912281 0.63934426 0.57746479 0.61038961 0.65517241
## 25 0.76470588 0.74545455 0.66037736 0.61403509 0.67164179 0.69863014 0.73493976
## 26 0.63855422 0.54022989 0.45882353 0.32584270 0.21212121 0.20000000 0.25217391
## 27 0.66990291 0.57009346 0.48571429 0.37614679 0.19327731 0.13600000 0.12592593
  28 0.69090909 0.57894737 0.50000000 0.41379310 0.22222222 0.16666667 0.12676056
## 29 0.65354331 0.51145038 0.44186047 0.41353383 0.24475524 0.18120805 0.11949686
## 30 0.72093023 0.57894737 0.52671756 0.48148148 0.29655172 0.23178808 0.18012422
##
              23
                         24
                                     25
                                                26
                                                           27
                                                                      28
                                                                                 29
## 2
## 3
## 4
## 5
## 6
## 7
## 9
## 10
## 11
## 12
## 13
## 14
## 15
## 16
## 17
## 18
## 19
## 20
## 21
## 22
## 23
## 24 0.57894737
## 25 0.46666667 0.46153846
## 26 0.82978723 0.48275862 0.59259259
## 27 0.88059701 0.61538462 0.70270270 0.18867925
## 28 0.89189189 0.64705882 0.72839506 0.23893805 0.09774436
## 29 0.91208791 0.70588235 0.77551020 0.33846154 0.18666667 0.14649682
## 30 0.91397849 0.71153846 0.78000000 0.36363636 0.19736842 0.15723270 0.14772727
fish.db <- vegdist(fish, method = "bray", upper = TRUE, diag = TRUE)
```

Question 5: Using the distance matrices from above, answer the following questions:

- a. Does the resemblance matrix (fish.db) represent similarity or dissimilarity? What information in the resemblance matrix led you to arrive at your answer?
- b. Compare the resemblance matrices (fish.db or fish.ds) you just created. How does the choice of the Sørensen or Bray-Curtis distance influence your interpretation of site (dis)similarity?

Answer 5a: dissimilarity because it is the difference between 2 sites/ total **Answer 5b**: sorensen is based on presence absence data but bray-curtis is based on abundance data so in the sorensen distance would give an equal weight to rare and abundant species so to more qualitative similarity while bray-curtis is more quantitative because it measures similarity in abundance as well as presence

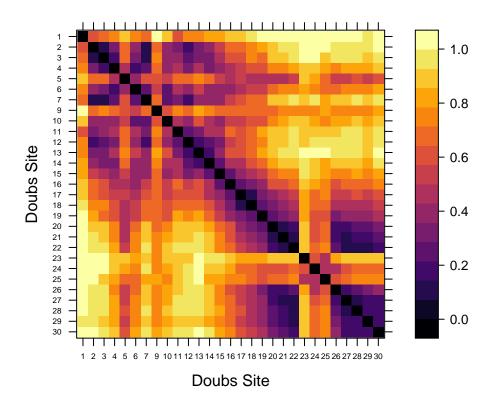
4) VISUALIZING BETA-DIVERSITY

A. Heatmaps

In the R code chunk below, do the following:

- 1. define a color palette,
- 2. define the order of sites in the Doubs River, and
- 3. use the levelplot() function to create a heatmap of fish abundances in the Doubs River.

Bray-Curtis Distance



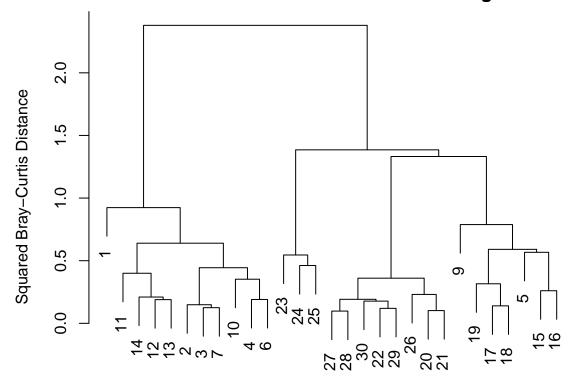
B. Cluster Analysis

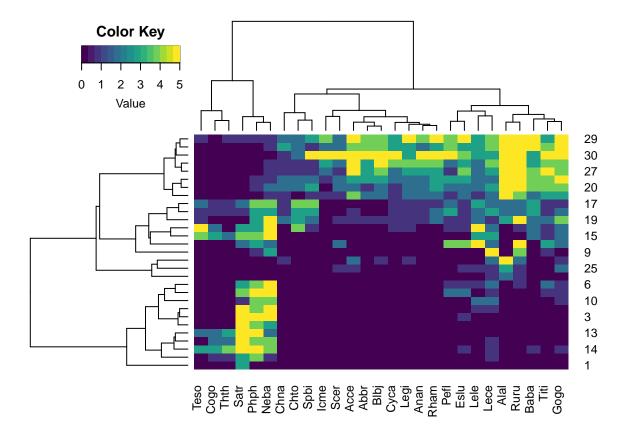
- 1. perform a cluster analysis using Ward's Clustering, and
- 2. plot your cluster analysis (use either hclust or heatmap.2).

```
fish.ward <- hclust(fish.db, method = "ward.D2")

par(mar = c(1, 5, 2, 2) + 0.1)
plot(fish.ward, main = "Doubs River Fish: Ward's Clustering",
    ylab = "Squared Bray-Curtis Distance")</pre>
```

Doubs River Fish: Ward's Clustering





Question 6: Based on cluster analyses and the introductory plots that we generated after loading the data, develop an ecological hypothesis for fish diversity the doubs data set?

Answer 6: My hypothesis for why there is a high amount of fish diversity in the river is because there is variation in conditions throughout the river which leads to clustering of fish presense/abundance at different locations.

C. Ordination

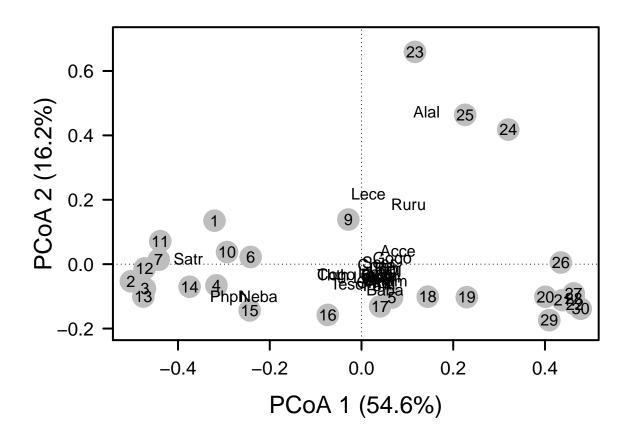
Principal Coordinates Analysis (PCoA)

- 1. perform a Principal Coordinates Analysis to visualize beta-diversity
- 2. calculate the variation explained by the first three axes in your ordination
- 3. plot the PCoA ordination,
- 4. label the sites as points using the Doubs River site number, and
- 5. identify influential species and add species coordinates to PCoA plot.

```
fish.pcoa <- cmdscale(fish.db, eig = TRUE, k = 3)

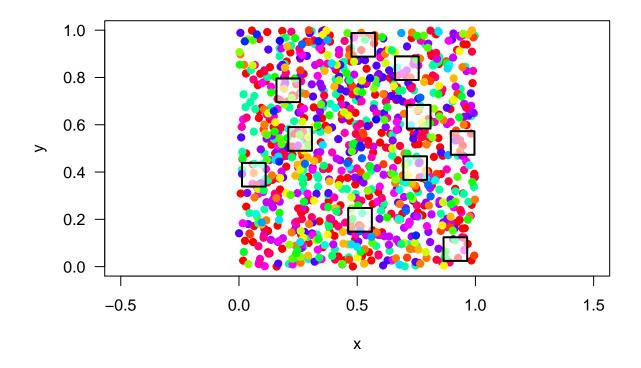
explainvar1 <- round(fish.pcoa$eig[1] / sum(fish.pcoa$eig), 3) * 100
explainvar2 <- round(fish.pcoa$eig[2] / sum(fish.pcoa$eig), 3) * 100
explainvar3 <- round(fish.pcoa$eig[3] / sum(fish.pcoa$eig), 3) * 100</pre>
```

```
sum.eig <- sum(explainvar1, explainvar2, explainvar3)</pre>
par(mar = c(5, 5, 1, 2) + 0.1)
plot(fish.pcoa$points[,1], fish.pcoa$points[,2], ylim = c(-0.2, 0.7),
     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(1wd = 2)
points(fish.pcoa$points[ ,1], fish.pcoa$points[ ,2],
       pch = 19, cex = 3, bg = "gray", col = "gray")
text(fish.pcoa$points[ ,1], fish.pcoa$points[ ,2],
     labels = row.names(fish.pcoa$points))
fishREL <- fish
for(i in 1:nrow(fish)){
  fishREL[i, ] = fish[i, ] / sum(fish[i, ])
}
fish.pcoa <- add.spec.scores(fish.pcoa, fishREL, method = "pcoa.scores")
text(fish.pcoa$cproj[ ,1], fish.pcoa$cproj[ ,2],
     labels = row.names(fish.pcoa$cproj), col = "black")
```

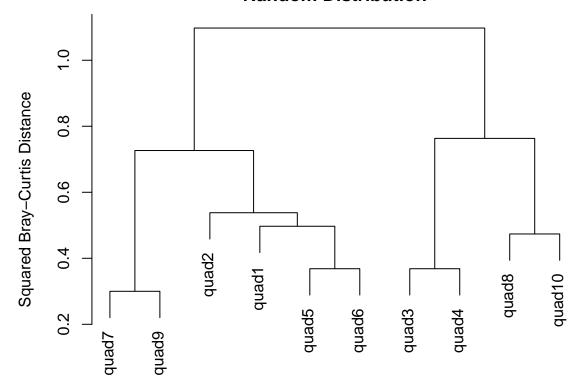


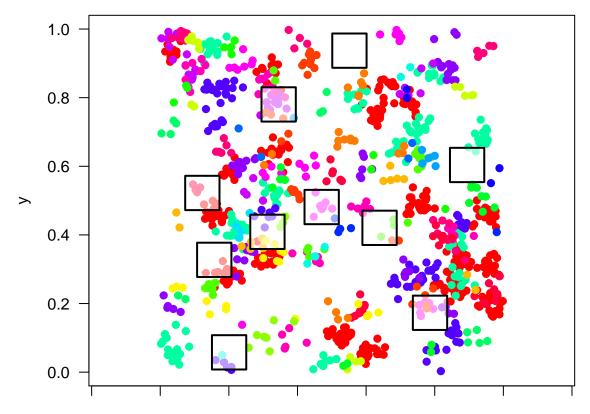
- 1. identify influential species based on correlations along each PCoA axis (use a cutoff of 0.70), and
- 2. use a permutation test (999 permutations) to test the correlations of each species along each axis.

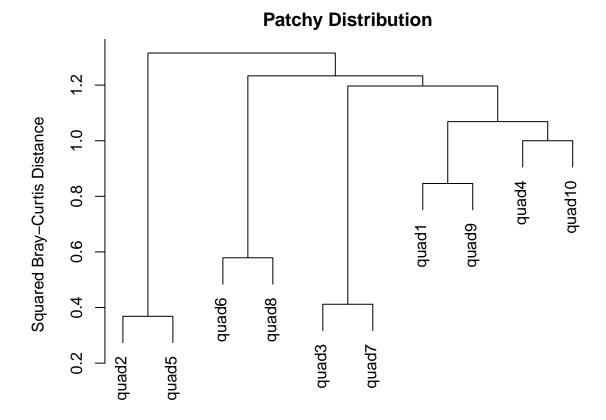
```
spe.corr <- add.spec.scores(fish.pcoa, fishREL, method = "cor.scores")$cproj</pre>
corrcut <- 0.7
imp.spp <- spe.corr[abs(spe.corr[,1]) >= corrcut | abs(spe.corr[, 2]) >= corrcut, ]
fit <- envfit(fish.pcoa, fishREL, perm = 999)
package.list <- c('mobsim', 'knitr', 'vegan', 'tidyr', 'dplyr', 'ggplot2', 'formatR')</pre>
for (package in package.list) {
  if (!require(package, character.only = TRUE, quietly = TRUE)) {
    install.packages(package)
    library(package, character.only = TRUE)
}
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##
       filter, lag
## The following objects are masked from 'package:base':
##
##
       intersect, setdiff, setequal, union
# set page dimensions for printing
opts_chunk$set(tidy.opts = list(width.cutoff = 70),
        tidy = TRUE, fig.width = 5, fig.height = 5)
#synthesis random
com_r <- sim_poisson_community(s_pool = 25, n_sim = 1000, sad_type = "lnorm", sad_coef = list("meanlog"</pre>
# visualize site
plot(com_r)
com_rs <- sample_quadrats(com_r, n_quadrats = 10, quadrat_area = 0.01,</pre>
             method = "random", avoid_overlap = T)
```



Random Distribution







Question 7: Address the following questions about the ordination results of the doubs data set:

- a. Describe the grouping of sites in the Doubs River based on fish community composition.
- b. Generate a hypothesis about which fish species are potential indicators of river quality.

Answer 7a: most of the fish are found in many of the sites and cluster around the center. I think PCoA2 is similarity because large PCoA2 values correspont to sites that are very dissimilar to any other sites while sites with negative values have similarities. The PCoA1 is roughly upstream vs. downstream. Sites 19-30 have PCoA1 values >0.2 and other than site 5 (which is ~0.07) sites 1-16 have negative PCoA1 values. Most fish species are near zero in the lower right quadrant of PC space so they are common in many parts of the upstream river except the far downstream. Answer 7b: I think it depends on the question your asking. For overall river health my hypothesis is a fish that is common throughout the river, like SCER would be a potential indicator of river quality because if its abundance or presence decreases you would know the overall river quality is changing. On the other hand, looking at a fish that is only found in certain areas like Alal which is mainly found upstream or Php which is mainly found downstream could be a useful indicator of change because if their abundance/presence becomes more widespread the river is changing

SYNTHESIS

Using the mobsim package from the DataWrangling module last week, simulate two local communities each containing 1000 individuals (N) and 25 species (S), but with one having a random spatial distribution and the other having a patchy spatial distribution. Take ten (10) subsamples from each site using the quadrat function and answer the following questions:

- 1) Compare the average pairwise similarity among subsamples in site 1 (random spatial distribution) to the average pairswise similarity among subsamples in site 2 (patchy spatial distribution). Use a t-test to determine whether compositional similarity was affected by the spatial distribution. Finally, compare the compositional similarity of site 1 and site 2 to the source community?
- 2) Create a cluster diagram or ordination using your simulated data. Are there any visual trends that would suggest a difference in composition between site 1 and site 2? Describe.