8. Worksheet: Among Site (Beta) Diversity – Part 2

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OVERVIEW

In this worksheet, we continue to explore concepts, statistics, and visualizations related to β-diversity. Now that you know how to formally quantify β-diversity, we will learn how to 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. 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 (**8.BetaDiversity_2_Worksheet.Rmd**) with all code blocks filled out and questions answered) and the PDF output of `Knitr` (**8.BetaDiversity_2_Worksheet.pdf**).

The completed exercise is due on **Wednesday, February 13th, 2019 before 12:00 PM (noon)**.

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] "/Users/mcaple/GitHub/QB2019_Caple/2.Worksheets/8.BetaDiversity"
```

```
setwd("~/GitHub/QB2019_Caple/2.Worksheets/8.BetaDiversity/")

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)
  }
}
```

```
## Warning: package 'vegan' was built under R version 3.5.2
```

```
## This is vegan 2.5-4
```

```
## Warning: package 'gplots' was built under R version 3.5.2
```

```
##
## Attaching package: 'gplots'
```

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

```
## Warning: package 'BiodiversityR' was built under R version 3.5.2
```

```
## BiodiversityR 2.11-1: Use command BiodiversityRGUI() to launch the Graphical User Interface;
## to see changes use BiodiversityRGUI(changeLog=TRUE, backward.compatibility.messages=TRUE)
```

2) LOADING DATA

Load dataset

In the R code chunk below, load the `doubs` dataset from the `ade4` package

```
# note, pleae do not print the dataset when submitting
data(doubs)

fish <- doubs$fish
fish <- fish[-8, ] # remove site 8 from the data (it has no observations)
```

3) HYPOTHESIS TESTING

A. Multivariate Procedures for Categorical Designs

Earlier work done in the Doubs River suggested that the river has four distinct regions of habitat quality: the first region (sites 1-14) of "high quality"; the second (sites 15 - 19) and fourth (sites 26 - 30) of "moderate quality"; and the third (sites 20 - 25) of "low quality".

In the code chunk below, test the hypothesis that fish community composition varies with river quality.

1. create a factor vector that categorizes habitat quality in the Doubs River,
2. use the multivariate analyses for categorical predictors to describe how fish community structure relates to habitat quality.

```
# create "factors" vector
quality <- c(rep("HQ", 13), rep("MQ", 5), rep("LQ", 6), rep("MQ", 5))
```

```r
# run PERMANOVA with adonis function
adonis(fish ~ quality, method = "bray", permutations = 999)
```

```
##
## Call:
## adonis(formula = fish ~ quality, permutations = 999, method = "bray")
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## quality    2    3.0947 1.54733   10.97 0.45765  0.001 ***
## Residuals 26    3.6674 0.14105         0.54235
## Total     28    6.7621                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# identify indicator species
indval <- multipatt(fish, cluster = quality, func = "IndVal.g", control = how(nperm = 999))
summary(indval)
```

```
##
##  Multilevel pattern analysis
##  ---------------------------
##
##  Association function: IndVal.g
##  Significance level (alpha): 0.05
##
##  Total number of species: 27
##  Selected number of species: 23
##  Number of species associated to 1 group: 1
##  Number of species associated to 2 groups: 22
##
##  List of species associated to each combination:
##
##  Group MQ  #sps.  1
##       stat p.value
## Teso 0.686    0.03 *
##
##  Group HQ+MQ  #sps.  2
##       stat p.value
## Satr 0.860   0.006 **
## Phph 0.859   0.013 *
##
##  Group LQ+MQ  #sps.  20
##       stat p.value
## Alal 0.935   0.001 ***
## Gogo 0.933   0.001 ***
## Ruru 0.916   0.001 ***
## Legi 0.901   0.001 ***
## Baba 0.895   0.001 ***
## Chna 0.866   0.002 **
## Spbi 0.866   0.001 ***
```

```
## Cyca 0.866   0.001 ***
## Acce 0.866   0.002 **
## Lele 0.863   0.006 **
## Titi 0.853   0.008 **
## Chto 0.829   0.001 ***
## Rham 0.829   0.003 **
## Anan 0.829   0.003 **
## Eslu 0.827   0.023 *
## Pefl 0.806   0.018 *
## Blbj 0.791   0.007 **
## Scer 0.766   0.012 *
## Abbr 0.750   0.013 *
## Icme 0.661   0.029 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# calculate phi coefficient of association to examine habitat preference
fish.rel <- decostand(fish, method = "total")
phi <- multipatt(fish.rel, cluster = quality, func = "r.g", control = how(nperm = 999))
summary(phi)
```

```
##
##  Multilevel pattern analysis
##  ---------------------------
##
##  Association function: r.g
##  Significance level (alpha): 0.05
##
##  Total number of species: 27
##  Selected number of species: 18
##  Number of species associated to 1 group: 9
##  Number of species associated to 2 groups: 9
##
##  List of species associated to each combination:
##
##  Group HQ  #sps.  3
##       stat p.value
## Phph 0.802   0.001 ***
## Neba 0.734   0.001 ***
## Satr 0.650   0.001 ***
##
##  Group LQ  #sps.  2
##       stat p.value
## Alal 0.693   0.001 ***
## Ruru 0.473   0.024 *
##
##  Group MQ  #sps.  4
##       stat p.value
## Anan 0.571   0.006 **
## Spbi 0.557   0.007 **
## Chto 0.542   0.015 *
## Icme 0.475   0.029 *
##
##  Group LQ+MQ  #sps.  9
##       stat p.value
```

```
## Legi 0.658   0.002 **
## Baba 0.645   0.004 **
## Rham 0.600   0.003 **
## Acce 0.594   0.005 **
## Cyca 0.586   0.004 **
## Chna 0.571   0.007 **
## Blbj 0.571   0.008 **
## Gogo 0.523   0.010 **
## Abbr 0.499   0.026 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Question 1: Based on the PERMANOVA, IndVal, and phi coefficient analyses, what did you learn about the relationship between habitat quality and the fish species composition? Are the different analyses consistent with one another and do they agree with the visualizations (heat maps, cluster dendograms, ordinations) that you created?

> ***Answer 1***: Fish species do seem to tend to group by water quality, which agrees with the visualizations from last week. There is some overlap between HQ and MQ, and some overlap between MQ and LQ, but very very little between HQ and LQ; since our quality groupings were essentially rough estimates, this makes some sense.

B. Multivariate Procedures for Continuous Designs

i. Mantel Test

In the R code chunk below, do the following:

1. create distance matrices for both fish communities and environmental factors, and
2. use a Mantel test to test the hypothesis that fish assemblages are correlated with stream environmental variables.

```
# define matrices
fish.dist <- vegdist(doubs$fish[-8, ], method = "bray")
env.dist <- vegdist(scale(doubs$env[-8, ]), method = "euclid")

# Mantel test
mantel(fish.dist, env.dist)
```

```
##
## Mantel statistic based on Pearson's product-moment correlation
##
## Call:
## mantel(xdis = fish.dist, ydis = env.dist)
##
## Mantel statistic r: 0.604
##       Significance: 0.001
##
## Upper quantiles of permutations (null model):
##   90%   95% 97.5%   99%
## 0.104 0.140 0.166 0.207
## Permutation: free
## Number of permutations: 999
```

Question 2: What do the results from our Mantel test suggest about fish diversity and stream environmental conditions? How does this relate to your hypothesis about stream quality influencing fish communities?

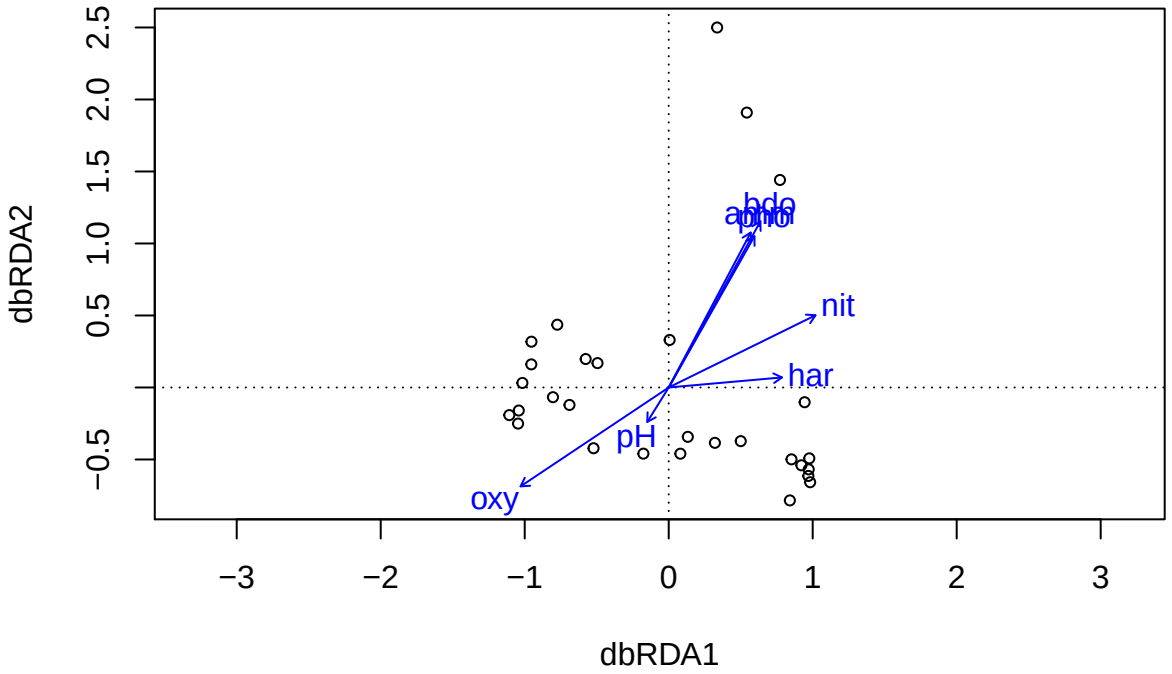
Answer 2: The two matrices are highly significantly correlated, which supports our hypothesis that water quality is a major driver of fish community composition.

ii. Constrained Ordination

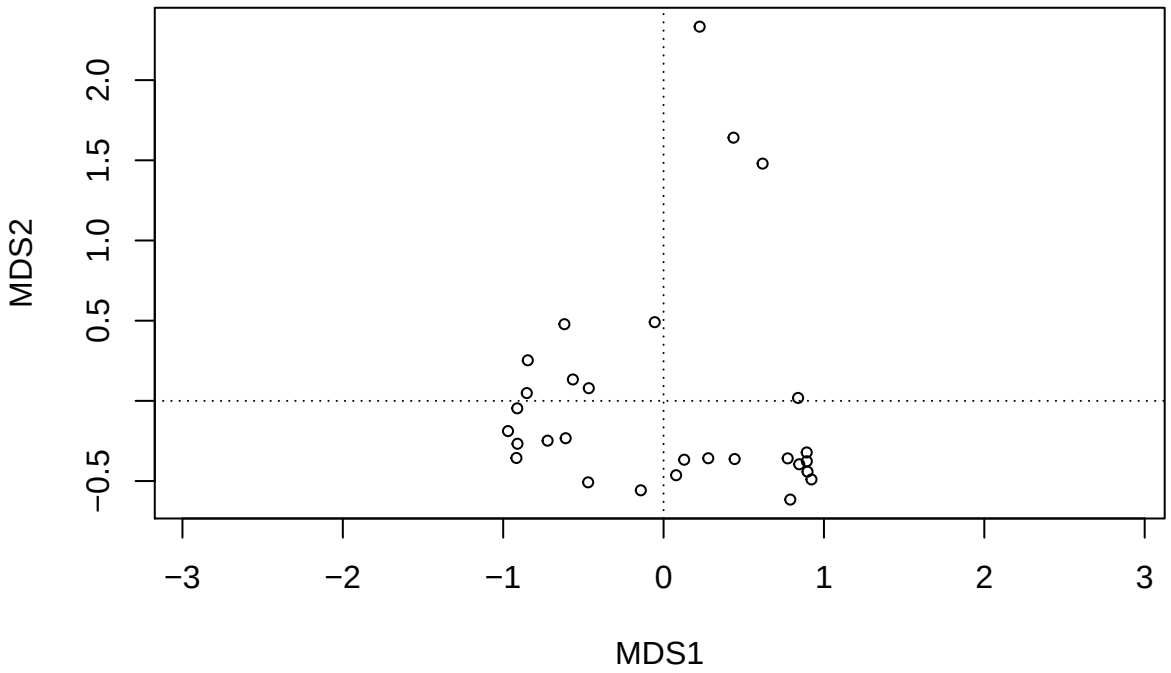
In the R code chunk below, do the following:

1. create an environmental matrix of the water chemistry data included in the `doubs` dataset using forward and reverse selection of variables,
2. conduct a redundancy analysis on the fish assemblages of the Doubs River,
3. use a permutation test to determine the significance of the constrained analysis,
4. use a permutation test to determine the correlation of each environmental factor on the constrained axes,
5. calculate the explained variation on the first and second constrained axes,
6. plot the constrained ordination results including labeled points for each site, and
7. add vectors that demonstrate the influence of each environmental factor the constrained ordination.

```
# define environmental matrix
env.chem <- as.matrix(doubs$env[-8, 5:11])

# perform dbRDA
doubs.dbrda <- dbrda(fish.dist ~ ., as.data.frame(env.chem))
ordiplot(doubs.dbrda)
```




```
# first, model only the intercept
doubs.dbrda.mod0 <- dbrda(fish.dist ~ 1, as.data.frame(env.chem))

# note that there are no vectors here (we didn't constrain anything)
# therefore, the axes suggest this is a simple MDS (i.e. PCoA)
ordiplot(doubs.dbrda.mod0)
```




```
# next, model the full model, with all the explanatory variables
doubs.dbrda.mod1 <- dbrda(fish.dist ~ ., as.data.frame(env.chem))

# now step through all combinations of explanatory variables in our model
# the function returns the model with the lowest AIC value
doubs.dbrda <- ordiR2step(doubs.dbrda.mod0, doubs.dbrda.mod1, perm.max = 200)
```

```
## Step: R2.adj= 0
## Call: fish.dist ~ 1
##
##                 R2.adjusted
## <All variables>  0.53032584
## + oxy            0.27727176
## + nit            0.25755208
## + bdo            0.17477787
## + pho            0.14568614
## + har            0.14174915
## + amm            0.14142804
## <none>           0.00000000
## + pH            -0.01827054
##
##       Df    AIC      F Pr(>F)
## + oxy  1 47.939 11.742  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.2772718
## Call: fish.dist ~ oxy
##
##                 R2.adjusted
## <All variables>   0.5303258
## + bdo             0.4009000
## + amm             0.3474192
```

```
## + pho             0.3452702
## + har             0.3331357
## + nit             0.3316120
## <none>            0.2772718
## + pH              0.2586983
##
##       Df    AIC      F Pr(>F)
## + bdo  1 43.404 6.5716  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.4009
## Call: fish.dist ~ oxy + bdo
##
##                 R2.adjusted
## <All variables>   0.5303258
## + nit             0.4980793
## + har             0.4695121
## <none>            0.4009000
## + pho             0.3938042
## + amm             0.3869134
## + pH              0.3865240
##
##       Df    AIC     F Pr(>F)
## + nit  1 39.134 6.034  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.4980793
## Call: fish.dist ~ oxy + bdo + nit
##
##                 R2.adjusted
## + amm             0.5415705
## <All variables>   0.5303258
## + pho             0.5277128
## + har             0.5218852
## <none>            0.4980793
## + pH              0.4843267
```

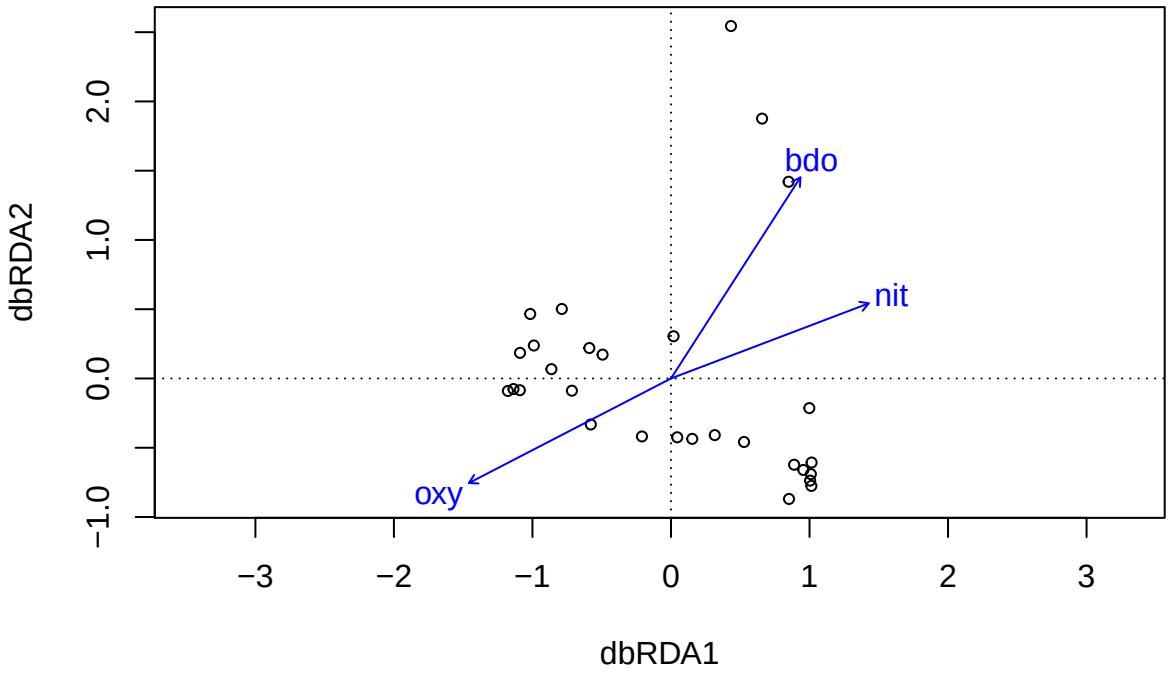
```
# look at the model that was selected
doubs.dbrda$call
```

```
## dbrda(formula = fish.dist ~ oxy + bdo + nit, data = as.data.frame(env.chem))
```

```
doubs.dbrda$anova
```

```
##                  R2.adj Df    AIC       F Pr(>F)
## + oxy           0.27727  1 47.939 11.7421  0.002 **
## + bdo           0.40090  1 43.404  6.5716  0.002 **
## + nit           0.49808  1 39.134  6.0340  0.002 **
## <All variables> 0.53033
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ordiplot(doubs.dbrda)
```



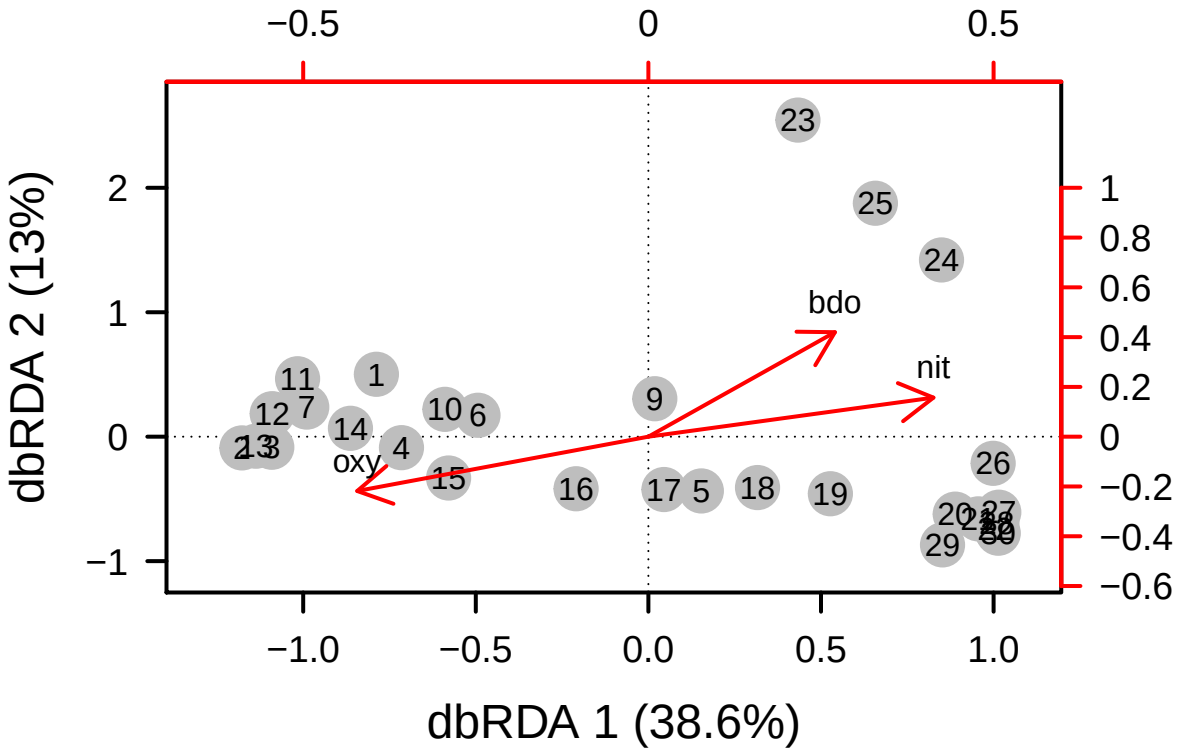

```
# permutation tests to evaluate significance
permutest(doubs.dbrda, permutations = 999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.dist ~ oxy + bdo + nit, data =
## as.data.frame(env.chem))
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     3  3.7317 10.262  0.001 ***
## Residual 25  3.0304
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
envfit(doubs.dbrda, env.chem[, c(4, 6, 7)], perm = 999)
```

```
##
## ***VECTORS
##
##       dbRDA1   dbRDA2     r2 Pr(>r)
## nit  0.87724  0.48005 0.6431  0.001 ***
## oxy -0.82864 -0.55979 0.7656  0.001 ***
## bdo  0.55603  0.83116 0.8939  0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

```r
# calculate explained variation
dbrda.explainvar1 <- round(doubs.dbrda$CCA$eig[1] /
                           sum(c(doubs.dbrda$CCA$eig, doubs.dbrda$CA$eig)), 3) * 100

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


# plot the ordination for the selected model

# define plot parameters
par(mar = c(5, 5, 4, 4) + 0.1)

# initiate plot
plot(scores(doubs.dbrda, display = "wa"), xlim = c(-1.3, 1.1), ylim = c(-1.1, 2.7),
     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)
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(doubs.dbrda, display = "wa"),
       pch = 19, cex = 3, bg = "gray", col = "gray")
text(scores(doubs.dbrda, display = "wa"),
     labels = row.names(scores(doubs.dbrda, display = "wa")))

# add environmental vectors
vectors <- scores(doubs.dbrda, display = "bp")
#row.names(vectors) <- rownames(vectors)
arrows(0, 0, vectors[ , 1], vectors[ , 2],
       lwd = 2, lty = 1, length = 0.2, col = "red")
text(vectors[ , 1], vectors[ , 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 3: Based on the constrained ordination, what are the environmental variables (or groups of correlated variables) that seem to be contributing to variation in fish community structure?

> ***Answer 3***: The three main environmental variables seem to be oxy (dissolved oxygen), bdo (biological demand for oxygen), and nit (nitrates)

iii. Variation Partitioning

In the code chunk below,

1. Create a matrix model of the selected environmental variables,
2. Create a matrix model of the selected PCNM axes,
3. Perform constrained and partial constrained ordinations using the spatial and environmental models you just created,
4. Test the significance of each of your constrained ordinations using permutation tests,
5. Partition the variation among sites into the relative importance of space, environment, spatially structured environment, and residuals,
6. Plot the variation partitioning output to visualize it.

```
# remember, our environmental model uses oxy, bdo, and nit, and has R2 of 0.53
doubs.dbrda$anova
```

```
##                  R2.adj Df    AIC       F Pr(>F)
## + oxy           0.27727  1 47.939 11.7421  0.002 **
## + bdo           0.40090  1 43.404  6.5716  0.002 **
## + nit           0.49808  1 39.134  6.0340  0.002 **
## <All variables> 0.53033
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# let's create a matrix model for our environmental data
env.mod <- model.matrix(~ oxy + bdo + nit, as.data.frame(env.chem))[ , -1]


# create the spatial model

# first, weight each site by its relative abundance
rs <- rowSums(fish)/sum(fish)

# next, we will perform PCNM
doubs.pcnmw <- pcnm(dist(doubs$xy[-8, ]), w = rs, dist.ret = T)

#PCNM can return negative eigenvalues, but only the
# eigenvectors associated with the positive eigenvalues are meaningful
doubs.pcnmw$values > 0
```

```
##  [1]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
## [12]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE FALSE FALSE FALSE FALSE FALSE
## [23] FALSE FALSE FALSE FALSE
```

```r
# model selection to determine which eigenvalues create the best model
doubs.space <- as.data.frame(scores(doubs.pcnmw))
doubs.pcnm.mod0 <- dbrda(fish.dist ~ 1, doubs.space)
doubs.pcnm.mod1 <- dbrda(fish.dist ~ ., doubs.space)
step.pcnm <- ordiR2step(doubs.pcnm.mod0, doubs.pcnm.mod1, perm.max = 200)
```

```
## Step: R2.adj= 0
## Call: fish.dist ~ 1
##
##                   R2.adjusted
## <All variables>  0.626011301
## + PCNM2          0.235370423
## + PCNM3          0.078394885
## + PCNM13         0.065305668
## + PCNM5          0.046185074
## + PCNM6          0.032809156
## + PCNM16         0.030486700
## + PCNM14         0.029680999
## + PCNM9          0.020357410
## + PCNM15         0.013632610
## + PCNM8          0.009411968
## + PCNM1          0.003986221
## + PCNM17         0.002415012
## + PCNM10         0.001326442
## <none>           0.000000000
## + PCNM7         -0.001861430
## + PCNM11        -0.006841522
## + PCNM4         -0.007089863
## + PCNM12        -0.014396973
##
##         Df    AIC     F Pr(>F)
## + PCNM2  1 49.574 9.619  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

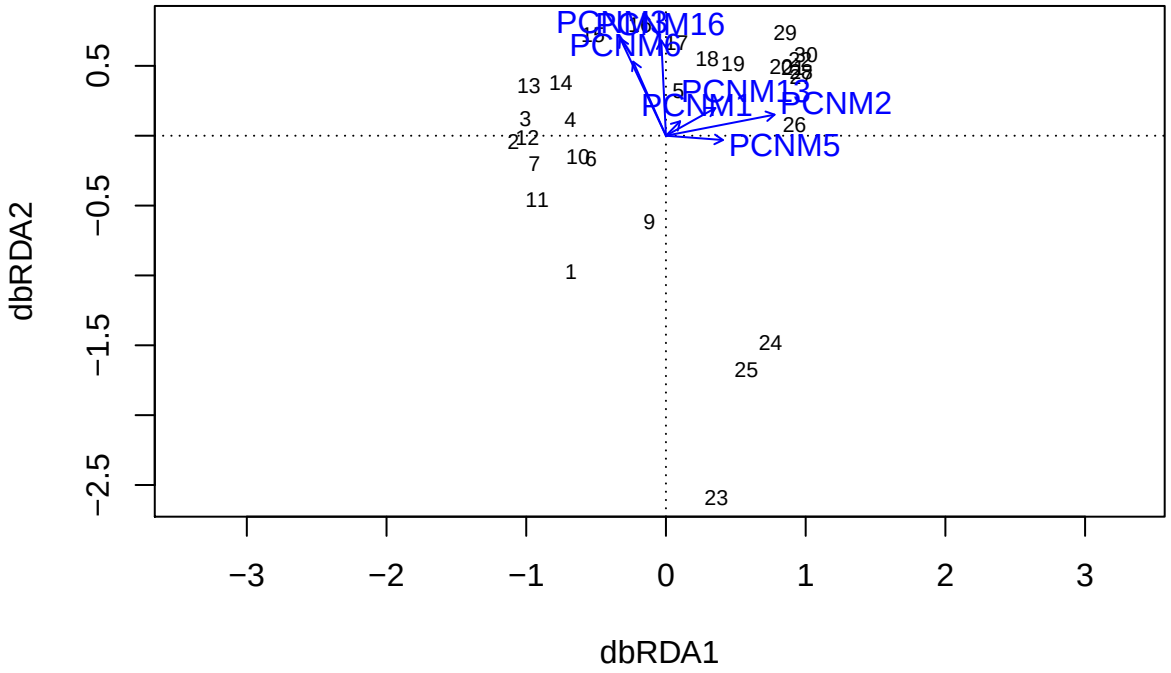
```
##
## Step: R2.adj= 0.2353704
## Call: fish.dist ~ PCNM2
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM3           0.3429270
## + PCNM5           0.3057368
## + PCNM1           0.2885396
## + PCNM16          0.2786746
## + PCNM14          0.2744520
## + PCNM15          0.2692809
## + PCNM6           0.2659866
## + PCNM13          0.2636194
## + PCNM9           0.2517847
## + PCNM8           0.2496240
## + PCNM10          0.2434688
## + PCNM7           0.2431476
## + PCNM17          0.2404343
## + PCNM11          0.2366833
## <none>            0.2353704
## + PCNM12          0.2288789
## + PCNM4           0.2189522
##
##         Df    AIC      F Pr(>F)
## + PCNM3  1 46.083 5.4196  0.004 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.342927
## Call: fish.dist ~ PCNM2 + PCNM3
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM5           0.4076020
## + PCNM1           0.3970300
## + PCNM16          0.3853210
## + PCNM15          0.3828748
## + PCNM14          0.3781827
## + PCNM13          0.3770376
## + PCNM6           0.3595644
## + PCNM8           0.3556885
## + PCNM7           0.3541631
## + PCNM10          0.3526775
## + PCNM17          0.3513683
## + PCNM9           0.3433672
## <none>            0.3429270
## + PCNM11          0.3416399
## + PCNM12          0.3396547
## + PCNM4           0.3311509
##
##         Df    AIC      F Pr(>F)
## + PCNM5  1 43.941 3.8385  0.014 *
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.407602
## Call: fish.dist ~ PCNM2 + PCNM3 + PCNM5
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM1           0.4721469
## + PCNM16          0.4631976
## + PCNM15          0.4589111
## + PCNM14          0.4535248
## + PCNM13          0.4511582
## + PCNM6           0.4305640
## + PCNM7           0.4261965
## + PCNM8           0.4224505
## + PCNM17          0.4181666
## + PCNM10          0.4154485
## + PCNM11          0.4112178
## + PCNM9           0.4111995
## + PCNM12          0.4087602
## <none>            0.4076020
## + PCNM4           0.3976526
##
##         Df    AIC     F Pr(>F)
## + PCNM1  1 41.411 4.057  0.004 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.4721469
## Call: fish.dist ~ PCNM2 + PCNM3 + PCNM5 + PCNM1
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM13          0.5212427
## + PCNM16          0.5208668
## + PCNM15          0.5161770
## + PCNM14          0.5147355
## + PCNM6           0.4999020
## + PCNM7           0.4936559
## + PCNM8           0.4904113
## + PCNM17          0.4856884
## + PCNM10          0.4835952
## + PCNM11          0.4760087
## + PCNM9           0.4751424
## + PCNM12          0.4747221
## <none>            0.4721469
## + PCNM4           0.4651218
##
##          Df    AIC      F Pr(>F)
## + PCNM13  1 39.346 3.4612  0.018 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.5212427
```

```
## Call: fish.dist ~ PCNM2 + PCNM3 + PCNM5 + PCNM1 + PCNM13
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM16          0.5767968
## + PCNM15          0.5715331
## + PCNM14          0.5698343
## + PCNM6           0.5475140
## + PCNM7           0.5392074
## + PCNM8           0.5379134
## + PCNM11          0.5281106
## + PCNM9           0.5267003
## + PCNM10          0.5265029
## + PCNM12          0.5255581
## <none>            0.5212427
## + PCNM17          0.5171800
## + PCNM4           0.5152311
##
##          Df   AIC      F Pr(>F)
## + PCNM16  1 36.48 4.0192  0.006 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.5767968
## Call: fish.dist ~ PCNM2 + PCNM3 + PCNM5 + PCNM1 + PCNM13 + PCNM16
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM6           0.6043089
## + PCNM8           0.5970286
## + PCNM12          0.5946888
## + PCNM7           0.5946475
## + PCNM9           0.5883735
## + PCNM10          0.5851333
## + PCNM15          0.5846468
## <none>            0.5767968
## + PCNM17          0.5748533
## + PCNM4           0.5733749
## + PCNM11          0.5711176
## + PCNM14          0.5652509
##
##         Df    AIC      F Pr(>F)
## + PCNM6  1 35.182 2.5296  0.044 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.6043089
## Call: fish.dist ~ PCNM2 + PCNM3 + PCNM5 + PCNM1 + PCNM13 + PCNM16 +      PCNM6
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM8           0.6248697
## + PCNM12          0.6208788
## + PCNM10          0.6170988
```

```
## + PCNM7           0.6142419
## + PCNM15          0.6140369
## + PCNM9           0.6107110
## <none>            0.6043089
## + PCNM17          0.6037430
## + PCNM11          0.5978305
## + PCNM4           0.5963667
## + PCNM14          0.5932113
##
##         Df    AIC     F Pr(>F)
## + PCNM8  1 34.219 2.151  0.088 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# because this is another dbRBA, we could visualize the biplot
# showing how each vector explains variation across the sites
plot(step.pcnm)
```




```r
# the object 'step.pcnm' now contains the selected model
step.pcnm$anova
```

```
##                  R2.adj Df    AIC      F Pr(>F)
## + PCNM2         0.23537  1 49.574 9.6190  0.002 **
## + PCNM3         0.34293  1 46.083 5.4196  0.004 **
## + PCNM5         0.40760  1 43.941 3.8385  0.014 *
## + PCNM1         0.47215  1 41.411 4.0570  0.004 **
## + PCNM13        0.52124  1 39.346 3.4612  0.018 *
## + PCNM16        0.57680  1 36.480 4.0192  0.006 **
## + PCNM6         0.60431  1 35.182 2.5296  0.044 *
## <All variables> 0.62601
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# we can construct a spatial model using only the selected PCNM axes
space.mod <- model.matrix(~ PCNM2 + PCNM3 + PCNM5 + PCNM1 +
                            PCNM13 + PCNM16 + PCNM6, doubs.space)[ , -1]


# perform partial constrained ordination

# first conduct constrained ordinations
doubs.total.env <- dbrda(fish.dist ~ env.mod)
doubs.total.space <- dbrda(fish.dist ~ space.mod)

# next construct partial constrained ordinations
doubs.env.cond.space <- dbrda(fish.dist ~ env.mod + Condition(space.mod))
doubs.space.cond.env <- dbrda(fish.dist ~ space.mod + Condition(env.mod))

# next test for significance of dbRDA fractions
permutest(doubs.env.cond.space, permutations = 999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.dist ~ env.mod + Condition(space.mod))
## Permutation test for all constrained eigenvalues
##          Df Inertia     F Pr(>F)
## Model     3 0.85158 4.423  0.001 ***
## Residual 18 1.15519
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
permutest(doubs.space.cond.env, permutations = 999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.dist ~ space.mod + Condition(env.mod))
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     7  1.8752 4.1741  0.001 ***
## Residual 18  1.1552
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
permutest(doubs.total.env, permutations = 999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
```

```
##
## Model: dbrda(formula = fish.dist ~ env.mod)
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     3  3.7317 10.262  0.001 ***
## Residual 25  3.0304
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

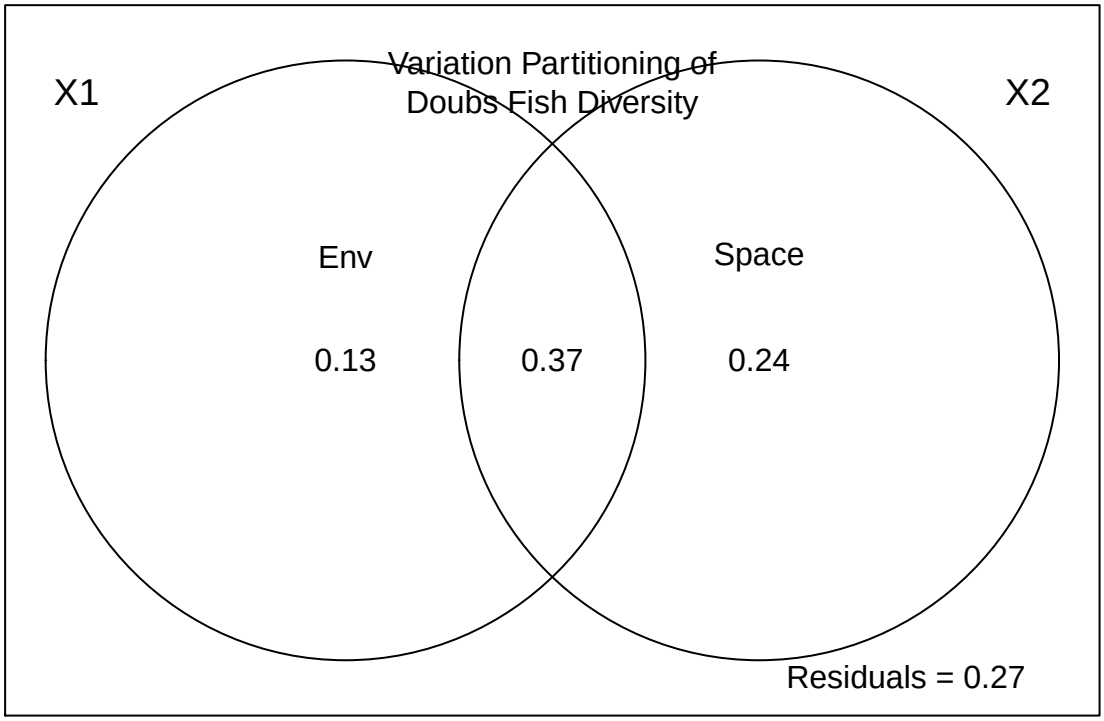
```
permutest(doubs.total.space, permutations = 999)
```

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.dist ~ space.mod)
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     7  4.7553 7.1089  0.001 ***
## Residual 21  2.0068
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# use built-in varpart() function to calculate fractions of variation explained by each
doubs.varpart <- varpart(fish.dist, env.mod, space.mod)
doubs.varpart
```

```
##
## Partition of squared Bray distance in dbRDA
##
## Call: varpart(Y = fish.dist, X = env.mod, space.mod)
##
## Explanatory tables:
## X1:  env.mod
## X2:  space.mod
##
## No. of explanatory tables: 2
## Total variation (SS): 6.7621
## No. of observations: 29
##
## Partition table:
##                       Df R.squared Adj.R.squared Testable
## [a+b] = X1             3   0.55186       0.49808     TRUE
## [b+c] = X2             7   0.70323       0.60431     TRUE
## [a+b+c] = X1+X2       10   0.82917       0.73426     TRUE
## Individual fractions
## [a] = X1|X2            3                 0.12995     TRUE
## [b]                    0                 0.36813    FALSE
## [c] = X2|X1            7                 0.23618     TRUE
## [d] = Residuals                          0.26574    FALSE
## ---
## Use function 'dbrda' to test significance of fractions of interest
```

```
par(mar = c(2, 2, 2, 2))
plot(doubs.varpart)
text(1, 0.25, "Space")
text(0, 0.25, "Env")
mtext("Variation Partitioning of\nDoubs Fish Diversity", side = 3, line = -3)
```



Question 4: Interpret the variation partitioning results.

> ***Answer 4***: Space and environment together predict ~72% of variation in Doubs fish diversity, with 13% explained by the environment alone, 24% explained by space alone, and 37% explained by the interaction of space and environment.

SYNTHESIS

1) Using the jelly bean data from class (i.e., JellyBeans.txt), perform a PERMANOVA to test whether or not the vicariance event (random splitting of source community) had an affect on jelly bean composition. Based on your previous analyses with this data set, what are your thoughts about the importance of stochastic vs. deterministic factors on estimates of biodiversity?

```
setwd("~/GitHub/QB2019_Caple/2.Worksheets/6.DiversitySampling")

jellysample <- read.table("JellyBeans.std.txt", sep = "\t", header = TRUE)
jellysample.sort <- jellysample[order(jellysample$Group),]
jellysample.numeric = jellysample.sort[ , 3:28]

subcommunities <- c(rep("A", 5), rep("B", 4))

adonis(jellysample.numeric ~ subcommunities, method = "bray", permutations = 999)
```

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

```
## adonis(formula = jellysample.numeric ~ subcommunities, permutations = 999,      method = "bray")
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                Df SumsOfSqs  MeanSqs F.Model     R2 Pr(>F)
## subcommunities  1   0.08741 0.087413  1.9732 0.2199  0.041 *
## Residuals       7   0.31011 0.044301         0.7801
## Total           8   0.39752                  1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# I thought this may be a more elegant way to get the same results-- but not being
# familiar with these methods and using a stochastic test it's hard to be sure.
# I tried running both with an increased number of permutations and it does seem to give the same resul
subcoms <- jellysample[ , 1]
jellysample.num <- jellysample[ , 3:28]
adonis(jellysample.num ~ subcoms, method = "bray", permutations = 999)
```

```
##
## Call:
## adonis(formula = jellysample.num ~ subcoms, permutations = 999,      method = "bray")
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs  MeanSqs F.Model     R2 Pr(>F)
## subcoms    1   0.08741 0.087413  1.9732 0.2199  0.059 .
## Residuals  7   0.31011 0.044301         0.7801
## Total      8   0.39752                  1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The PERMANOVA results say that the jelly bean community composition varies significantly with subcommunity– however, although the p-value is below the commonly accepted significance threshold, it is not below it by much; some runs of the PERMANOVA function at a fairly low number of permutations (999) gave a p-value slightly above 0.05. However, if the PERMANOVA is run with a greater number of permutations (99999), the p-values seem to converge around 0.047 (I removed this edit as it takes a little while to run and I didn't want to bog you down if you decided to run the code), so I feel reasonable confident in accepting this p-value and saying that it is likely that the vicariance event had a significant effect on the community composition. Running the tests multiple times, in multiple ways, and with multiple numbers of permutations has made me appreciate the stochastic nature of this test; although I think it's sometimes very important to use tests that are non-parametric, this exercise has shown me how important it is to use a large number of permutations if I'm doing more than exploratory statistics, since the differences in p-values between runs was large enough to shift the p-value back and forth over the commonly-accepted threshold of significance (though how seriously some people take that threshold is a debate for another time...)

2) Load the dataset you are using for your Team Project. Perform an ordination to visualize your dataset. Using this ordination, develop some hypotheses relevant to β-diversity. Use a statistic to test one of these hypotheses. Succinctly explain the finding and its relevance to your system.

```r
# unconstrained ordination


rm(list = ls())
setwd("~/GitHub/QB19_IndependentProject/")
package.list <- c('vegan', 'ade4', 'viridis', 'gplots', 'BiodiversityR', 'indicspecies', 'dplyr')
for(package in package.list){
  if(!require(package, character.only = TRUE, quietly = TRUE)){
    install.packages(package)
    library(package, character.only = TRUE)
  }
}
```

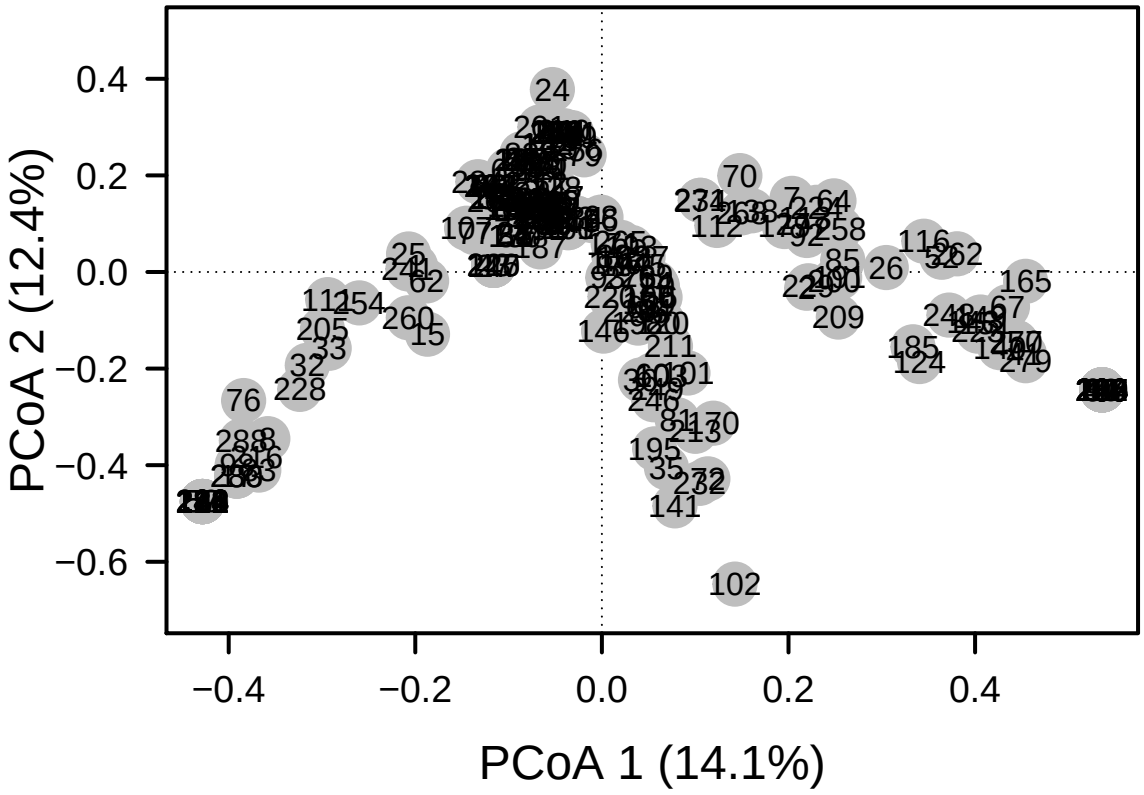
```
##
## Attaching package: 'dplyr'
```

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

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

```r
crawley.full <- read.csv("Crawley.csv")

# filter data
spec.all <- crawley.full[1:292, 4:23]

# remove rows with no observations
spec <- spec.all[-c(9, 23, 28, 29, 49, 50, 53, 71, 87, 90, 91, 139, 154, 161,
                    166, 168, 174, 188, 202, 215, 218, 219, 227, 234, 259, 263, 266, 290), ]

# calculate Sørensen
spec.ds <- vegdist(spec, method = "bray", binary = TRUE, upper = TRUE, diag = TRUE)

# conduct PCoA
spec.pcoa <- cmdscale(spec.ds, eig = TRUE, k = 3)

# examine eigenvalues
explainvar1 <- round(spec.pcoa$eig[1] / sum(spec.pcoa$eig), 3) *100
explainvar2 <- round(spec.pcoa$eig[2] / sum(spec.pcoa$eig), 3) *100
explainvar3 <- round(spec.pcoa$eig[3] / sum(spec.pcoa$eig), 3) *100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)


# create PCoA ordination plot

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

# initiate plot
plot(spec.pcoa$points[ , 1], spec.pcoa$points[ , 2], ylim = c(-0.7, 0.5),
     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(spec.pcoa$points[ , 1], spec.pcoa$points[ , 2],
       pch = 19, cex = 3, bg = "gray", col = "gray")
text(spec.pcoa$points[ , 1], spec.pcoa$points[ , 2],
     labels = row.names(spec.pcoa$points))
```




```
# hypothesis testing

# filter out nativity status into a vector
nat.full <- crawley.full[1:292, 2]

# remove rows with no observations
nat <- nat.full[-c(9, 23, 28, 29, 49, 50, 53, 71, 87, 90, 91, 139, 154, 161,
                   166, 168, 174, 188, 202, 215, 218, 219, 227, 234, 259, 263, 266, 290)]

# run PERMANOVA
adonis(spec ~ nat, method = "bray", permutations = 999)
```

```
##
## Call:
## adonis(formula = spec ~ nat, permutations = 999, method = "bray")
```

```
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##            Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## nat         4     2.292  0.5729  1.4754 0.02228  0.019 *
## Residuals 259   100.569  0.3883         0.97772
## Total     263   102.861                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# I couldn't figure out how to get this to work... I'll try to work on it more this week

# test hypothesis: there are relatively few species driving community differences

# create "factors" vector
# richness <- colSums(spec)

# run PERMANOVA with adonis function
# adonis(spec ~ richness, method = "bray", permutations = 999)

# identify indicator species
# indval <- multipatt(spec, cluster = richness, func = "IndVal.g", control = how(nperm = 999))
# summary(indval)

# calculate phi coefficient of association to examine habitat preference
# spec.rel <- decostand(spec, method = "total")
# phi <- multipatt(spec.rel, cluster = quality, func = "r.g", control = how(nperm = 999))
# summary(phi)
```

It's interesting that there are three main groupings, that all converge near the origin. This tells me that although most species tend to be in most communities, there are some species that tend to associate with others, forming three distinct community types that overlap with the 'base' community but not much with each other. I wanted to be able to see which points were from invasive vs native species, but I couldn't figure out how– I'll work on that for next week.

I decided to test the hypothesis of whether the nativity status of a species had a significant effect on the communities in which it is found. I ran a PERMANOVA comparing the nativity status vector to the site by species matrix, and found that this relationship is indeed significant. This is relevent to our system because it tells us that the nativity status of plants have a significant impact on community composition.