6. 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, you should **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 Posit.cloud workspace: `/cloud/project/QB-2025/Week4-Beta/`
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 (**6.BetaDiversity_2_Worksheet.Rmd**) with all code blocks filled out and questions answered) and the PDF output of `Knitr` (**6.BetaDiversity_2_Worksheet.pdf**).

The completed exercise is due on **Wednesday, February 12th, 2025 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 `Week4-Beta/` folder.
4. load the `vegan` R package (be sure to install if needed).

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

```
## [1] "/cloud/project/QB2025_Spencer/Week4-Beta"
```

```
require(vegan)
```

```
## Loading required package: vegan
```

```
## Loading required package: permute
```

```
## Loading required package: lattice
```

```
## This is vegan 2.6-8
```

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
# install.packages("indicspecies")
require(indicspecies)
```

```
## Loading required package: indicspecies
```

```
require(ade4)
```

```
## Loading required package: ade4
```

```
data(doubs)
```

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.

```
require(vegan)

fish <- doubs$fish
fish <- fish[-8, ]
str(fish)
```

```
## 'data.frame':    29 obs. of  27 variables:
##  $ Cogo: num  0 0 0 0 0 0 0 0 0 1 ...
##  $ Satr: num  3 5 5 4 2 3 5 0 1 3 ...
##  $ Phph: num  0 4 5 5 3 4 4 1 4 4 ...
##  $ Neba: num  0 3 5 5 2 5 5 3 4 1 ...
##  $ Thth: num  0 0 0 0 0 0 0 0 0 1 ...
##  $ Teso: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Chna: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Chto: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Lele: num  0 0 0 0 5 1 1 0 2 0 ...
##  $ Lece: num  0 0 0 1 2 2 1 5 2 1 ...
##  $ Baba: num  0 0 0 0 0 0 0 0 0 0 ...
```

```
##  $ Spbi: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Gogo: num  0 0 0 1 2 1 0 0 1 0 ...
##  $ Eslu: num  0 0 1 2 4 1 0 0 0 0 ...
##  $ Pefl: num  0 0 0 2 4 1 0 0 0 0 ...
##  $ Rham: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Legi: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Scer: num  0 0 0 0 2 0 0 0 0 0 ...
##  $ Cyca: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Titi: num  0 0 0 1 3 2 0 1 0 0 ...
##  $ Abbr: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Icme: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Acce: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Ruru: num  0 0 0 0 5 1 0 4 0 0 ...
##  $ Blbj: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Alal: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Anan: num  0 0 0 0 0 0 0 0 0 0 ...
```

```
env <- doubs$env
env <- env[-8, ]

xy <- doubs$xy
xy <- xy[-8, ]

quality <- c(rep("HQ", 13), rep("MQ", 5), rep("LQ", 6), rep("MQ", 5))


fish.ds <- vegdist(fish, method = "bray", diag = TRUE, binary = TRUE)
fish.db <- vegdist(fish, method = "bray", diag = TRUE)

adonis2(fish ~ quality, method = "bray", permutations = 999)
```

```
## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = fish ~ quality, permutations = 999, method = "bray")
##          Df SumOfSqs      R2     F Pr(>F)
## Model     2   3.0947 0.45765 10.97  0.001 ***
## Residual 26   3.6674 0.54235
## Total    28   6.7621 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
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   0.029 *
##
##  Group HQ+MQ  #sps.  2
##       stat p.value
## Satr 0.860   0.002 **
## Phph 0.859   0.021 *
##
##  Group LQ+MQ  #sps.  20
##       stat p.value
## Alal 0.935   0.001 ***
## Gogo 0.933   0.002 **
## Ruru 0.916   0.001 ***
## Legi 0.901   0.001 ***
## Baba 0.895   0.001 ***
## Chna 0.866   0.001 ***
## Spbi 0.866   0.001 ***
## Cyca 0.866   0.001 ***
## Acce 0.866   0.001 ***
## Lele 0.863   0.008 **
## Titi 0.853   0.003 **
## Chto 0.829   0.001 ***
## Rham 0.829   0.001 ***
## Anan 0.829   0.001 ***
## Eslu 0.827   0.031 *
## Pefl 0.806   0.021 *
## Blbj 0.791   0.002 **
## Scer 0.766   0.007 **
## Abbr 0.750   0.009 **
## Icme 0.661   0.026 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
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   0.037 *
##
##  Group MQ  #sps.  4
##       stat p.value
## Anan 0.571   0.013 *
## Spbi 0.557   0.015 *
## Chto 0.542   0.011 *
## Icme 0.475   0.029 *
##
##  Group LQ+MQ  #sps.  9
##       stat p.value
## Legi 0.658   0.002 **
## Baba 0.645   0.002 **
## Rham 0.600   0.010 **
## Acce 0.594   0.008 **
## Cyca 0.586   0.011 *
## Chna 0.571   0.005 **
## Blbj 0.571   0.006 **
## Gogo 0.523   0.018 *
## Abbr 0.499   0.031 *
## ---
## 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***: It appears as though water quality does significantly alter the diversity within the stream, as indicated by the PERMANOVA p-value being less than 0.001. The phi tests suggest that species *Salmo trutta fario* (Satr), *Phoxinus phoxinus* (Phph), *Nemacheilus barbatulus* (Neba) prefer high quality river sites, which aligns with our previous PCoA analyses that suggested these fish influence clustering of the "HQ" sites 1-14. The same can be said for the LQ species. In the case of the MQ species, there aren't very clear trends in these seen on other visualizations we have done prior. These analyses also align with the bray-curtis visualization that suggests farther apart sites are more dissimilar.
>
> The indval readout is a bit more difficult to interpret as most of the species are indicators of multiple river qualities, though the sites indicated line up well with the phi readout. In general, the different analyses seem to corroborate one another.

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.

```
fish.dist <- vegdist(fish, method = "bray")
env.dist <- vegdist(env, method = "euclid")

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: 0.4677
##       Significance: 0.001
##
## Upper quantiles of permutations (null model):
##   90%   95% 97.5%   99%
## 0.090 0.125 0.147 0.174
## 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?

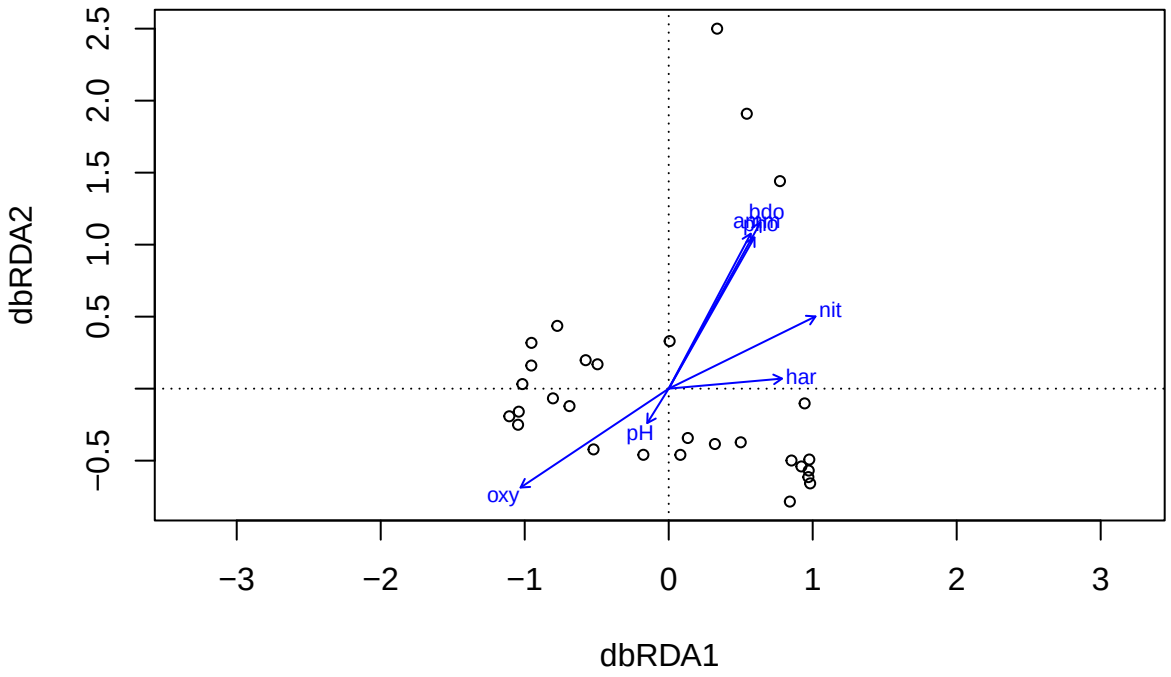
> ***Answer 2***: The mantel test produced a p-value of 0.001, suggesting there is a relationship between fish diversity and stream environmental conditions. This supports our hypothesis that that fish assemblages are correlated with stream environmental variables, but it does not provide more specific information like what environmental conditions, etc. This is functionally the same as the PERMANOVA test, but performed on data matrices that contain continuous variables rather than cateogorical, e.g. pH values rather than "acidic" vs. "basic".

ii. Constrained Ordination

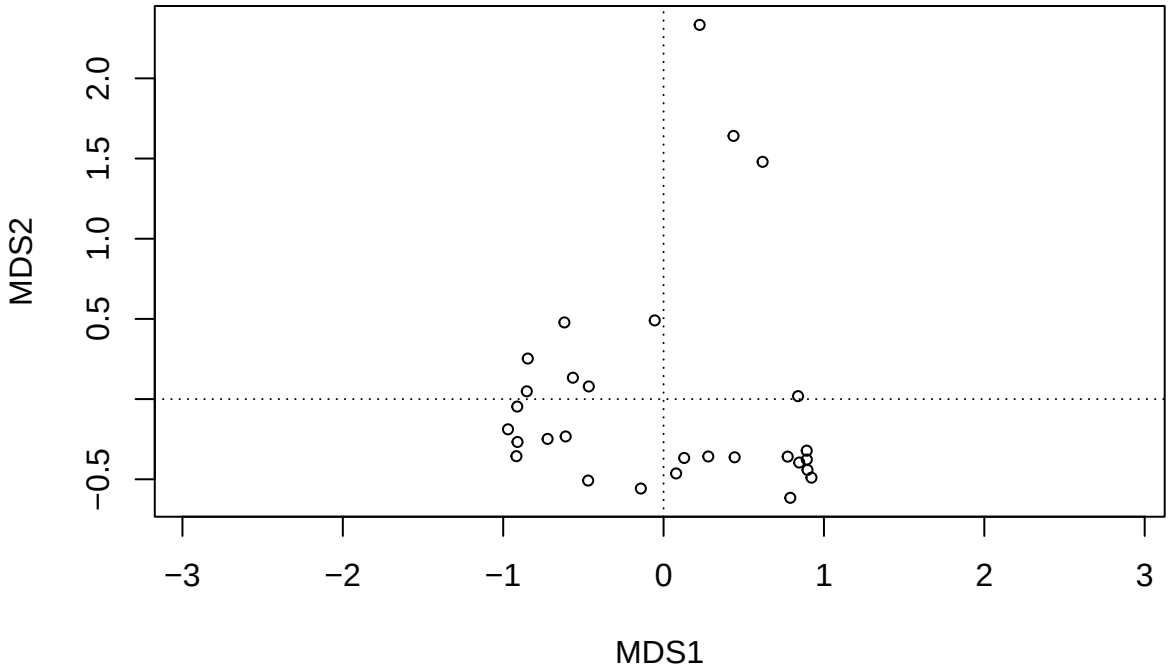
In the R code chunk below, do the following:

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.

```
env.chem <- as.matrix(env[, 5:11])

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




```
# Model intercept
doubs.dbrda.mod0 <- dbrda(fish.dist ~ 1, as.data.frame(env.chem))
ordiplot(doubs.dbrda.mod0)
```




```
#Full model
doubs.dbrda.mod1 <- dbrda(fish.dist ~ ., as.data.frame(env.chem))

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  0.006 **
## ---
## 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
```

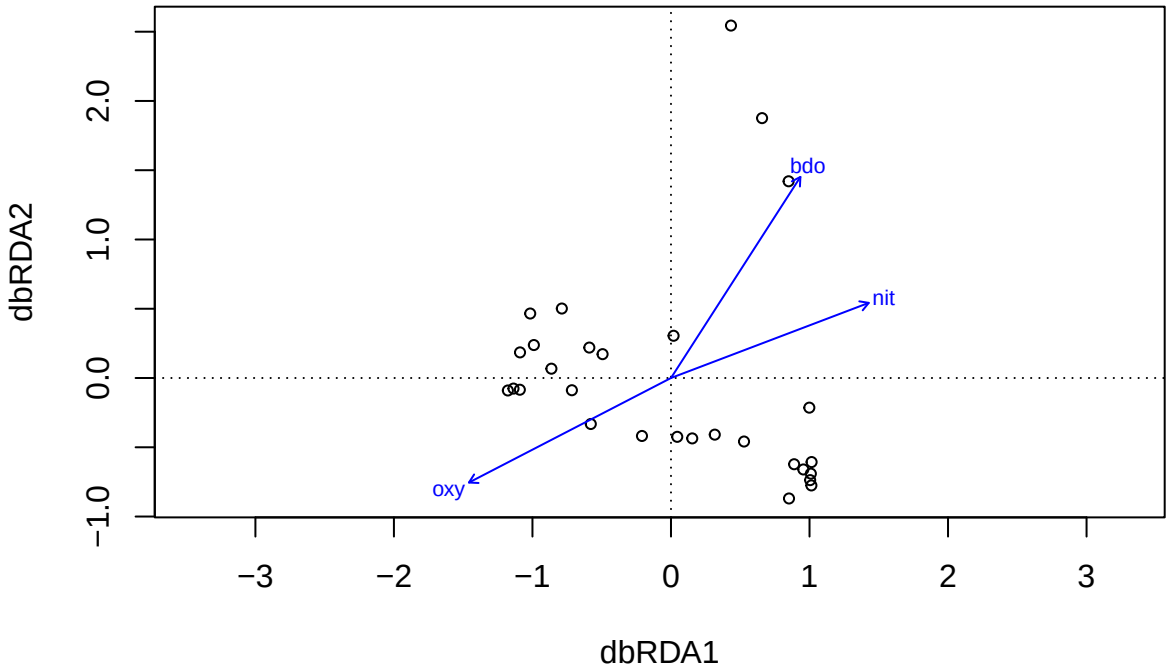
```
doubs.dbrda$call
```

```
## 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  0.006 **
## <All variables> 0.53033
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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




```
# Permutation tests
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))
```

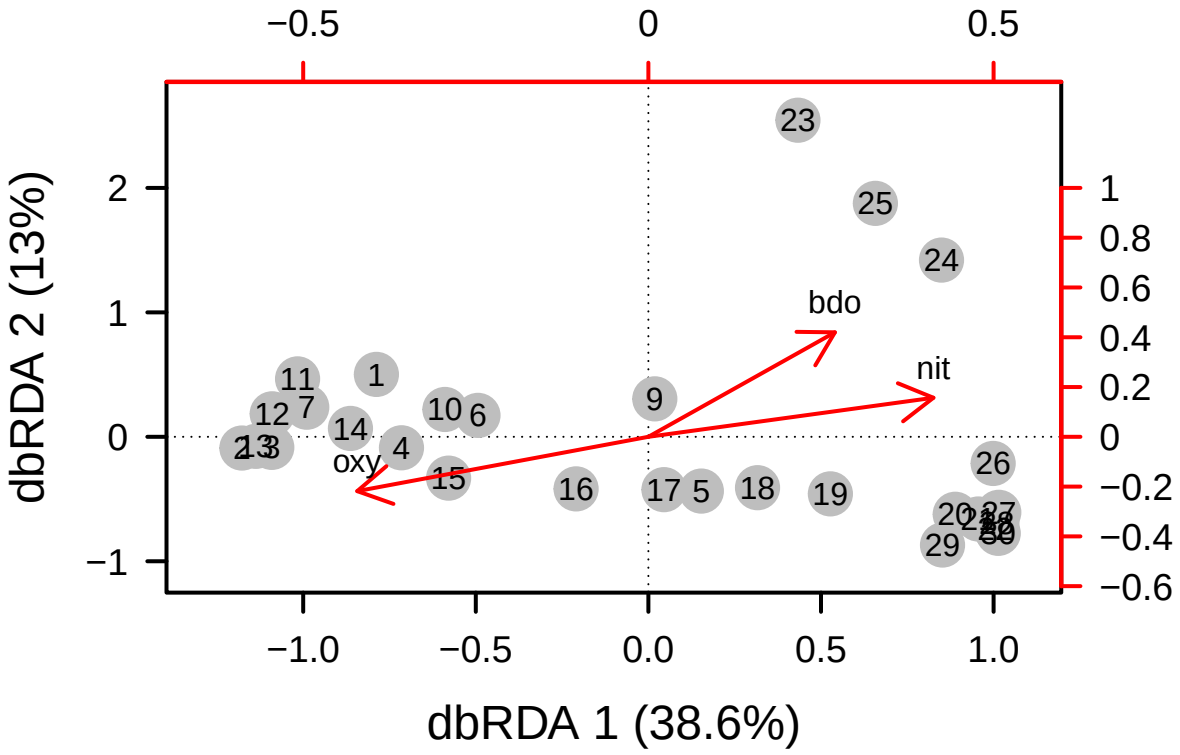
```
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     3  3.7317 10.262  0.001 ***
## Residual 25  3.0304
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
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
```

```
# 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

# Plotting ordination
par(mar = c(5, 5, 4, 4) + 0.1)

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)

# Axes
axis(side = 1, labels = TRUE, lwd.ticks = 2, cex.axis = 1.2, las = 1)
axis(side = 2, labels = TRUE, lwd.ticks = 2, cex.axis = 1.2, las = 1)
abline(h = 0, v = 0, lty = 3)
box(lwd = 2)

# Points and text
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")))

# Environmental vectors
vectors <- scores(doubs.dbrda, display = "bp")
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 environmental variables dissolved oxygen, biological demand for oxygen, and nitrate concentration contribute to variation in fish community strcture based on their vectors.

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.

```
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  0.006 **
## <All variables> 0.53033
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
env.mod <- model.matrix(~ oxy + bdo + nit, as.data.frame(env.chem))[, -1]

rs <- rowSums(fish) / sum(fish)

cat("Length of weights vector rs:", length(rs), "\n")
```

```
## Length of weights vector rs: 29
```

```r
cat("Number of rows in doubs$xy:", nrow(xy), "\n")
```

```
## Number of rows in doubs$xy: 29
```

```r
doubs.pcnmw <- pcnm(dist(xy), w = rs, dist.ret = TRUE)

positive_values <- doubs.pcnmw$values > 0

doubs.space <- as.data.frame(scores(doubs.pcnmw))
doubs.pcnm.mod0 <- dbrda(fish.db ~ 1, doubs.space)
doubs.pcnm.mod1 <- dbrda(fish.db ~ ., doubs.space)
step.pcnm <- ordiR2step(doubs.pcnm.mod0, doubs.pcnm.mod1, perm.max = 200)
```

```
## Step: R2.adj= 0
## Call: fish.db ~ 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.db ~ PCNM2
##
##                   R2.adjusted
## <All variables>   0.6260113
```

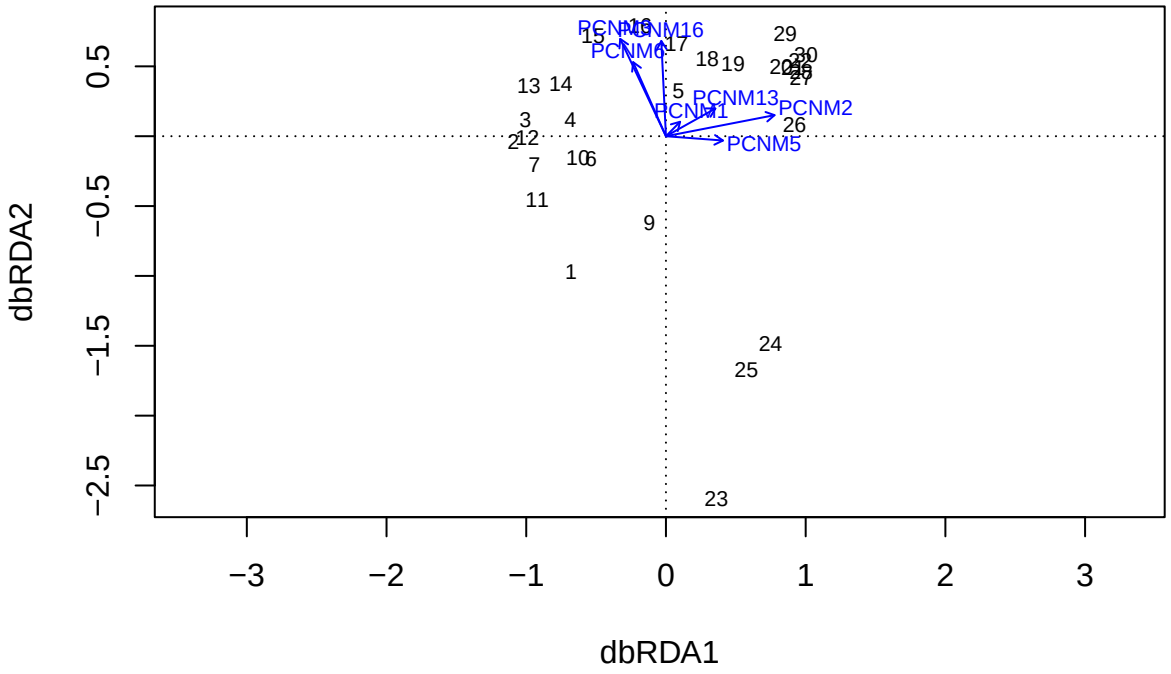
```
## + PCNM3           0.3429270
## + PCNM5           0.3057368
## + PCNM1           0.2885396
## + PCNM16          0.2786746
## + PCNM14          0.2744520
## + PCNM15          0.2692809
## + PCNM6           0.2659866
## + PCNM13          0.2636194
## + PCNM9           0.2517847
## + PCNM8           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  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.342927
## Call: fish.db ~ 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  0.008 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.407602
## Call: fish.db ~ 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  0.016 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.4721469
## Call: fish.db ~ 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  0.024 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.5212427
## Call: fish.db ~ 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  0.024 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.5767968
## Call: fish.db ~ 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  0.032 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.6043089
## Call: fish.db ~ 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  0.058 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
plot(step.pcnm)
```




```
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  0.002 **
## + PCNM5         0.40760  1 43.941 3.8385  0.008 **
## + PCNM1         0.47215  1 41.411 4.0570  0.016 *
## + PCNM13        0.52124  1 39.346 3.4612  0.024 *
## + PCNM16        0.57680  1 36.480 4.0192  0.024 *
## + PCNM6         0.60431  1 35.182 2.5296  0.032 *
## <All variables> 0.62601
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
space.mod <- model.matrix(~ PCNM2 + PCNM3 + PCNM5 + PCNM1 +
                            PCNM13 + PCNM16 + PCNM6, doubs.space)[, -1]

doubs.total.env <- dbrda(fish.db ~ env.mod)
doubs.total.space <- dbrda(fish.db ~ space.mod)

doubs.env.cond.space <- dbrda(fish.db ~ env.mod + Condition(space.mod))
doubs.env.cond.env <- dbrda(fish.db ~ space.mod + Condition(env.mod))

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

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.db ~ 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
```

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

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.db ~ 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
```

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

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.db ~ 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)
```

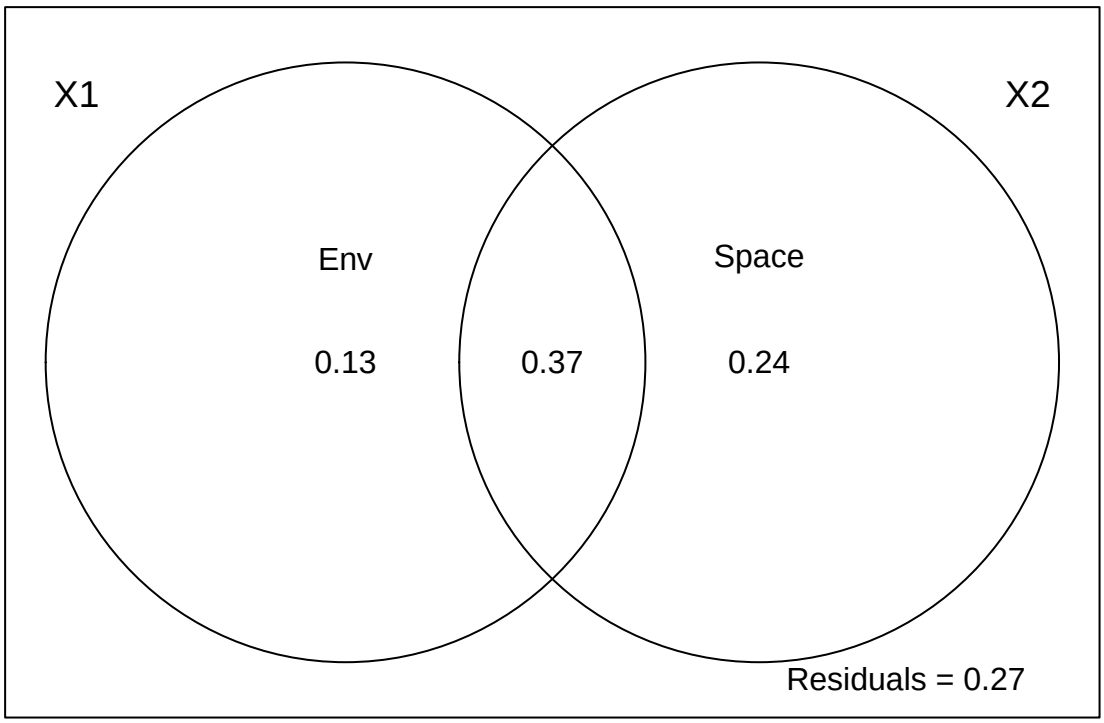
```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.db ~ space.mod)
## Permutation test for all constrained eigenvalues
```

```
##          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
```

```
doubs.varpart <- varpart(fish.db, env.mod, space.mod)
print(doubs.varpart)
```

```
##
## Partition of squared Bray distance in dbRDA
##
## Call: varpart(Y = fish.db, 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+c] = 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] = X2|X1            7                 0.23618     TRUE
## [c]                    0                 0.36813    FALSE
## [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")
```



Question 4: Interpret the variation partitioning results.

> ***Answer 4***: Our partitioning results determined that 0.13 of the variation in fish diversity is explained by environment alone, 0.24 by space alone, 0.37 by both space and environment, and 0.27 neither space nor environment.

SYNTHESIS

Load the dataset from that you and your partner are using for the team project. Use one of the hypothesis-testing tools introduced in the beta diversity module. Interpret the findings of your data with respect to principles of biodiversity.

```
#install.packages(dplyr)
#install.packages("tidyr")
require(dplyr)
```

```
## Loading required package: dplyr
```

```
##
## 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
```

```
require(tidyr)
```

```
## Loading required package: tidyr
```

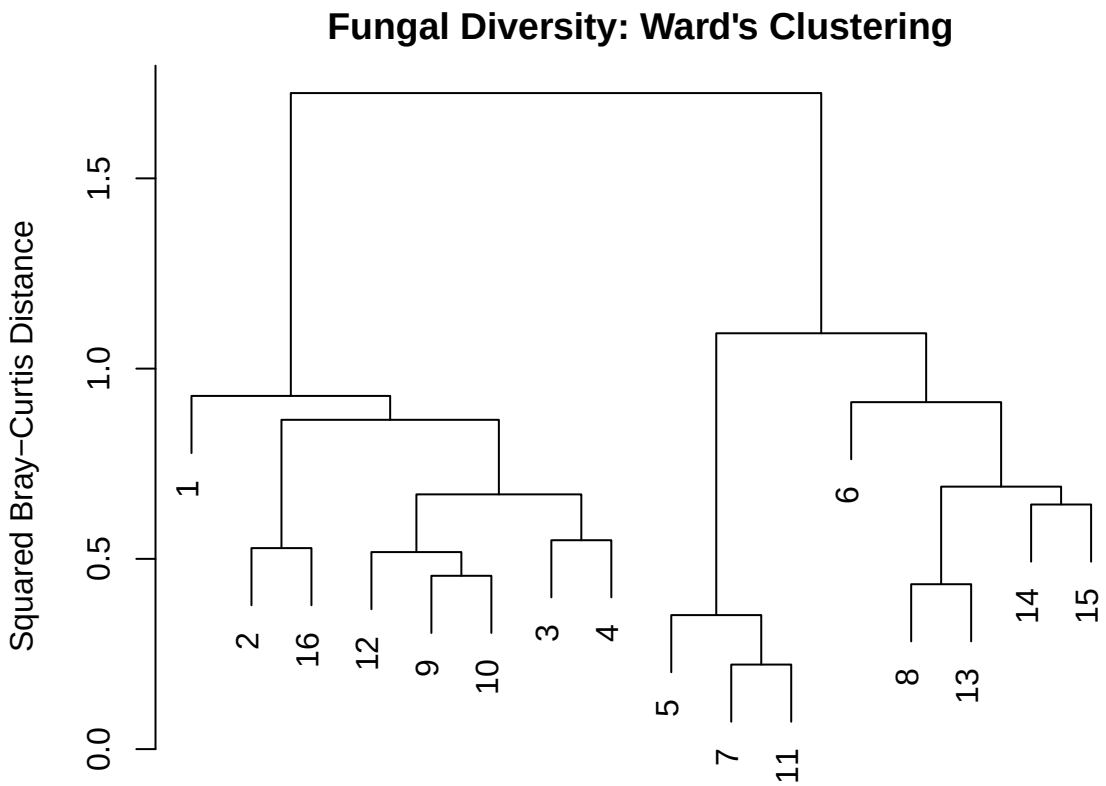
```
require(gplots)
```

```
## Loading required package: gplots

##
## Attaching package: 'gplots'

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

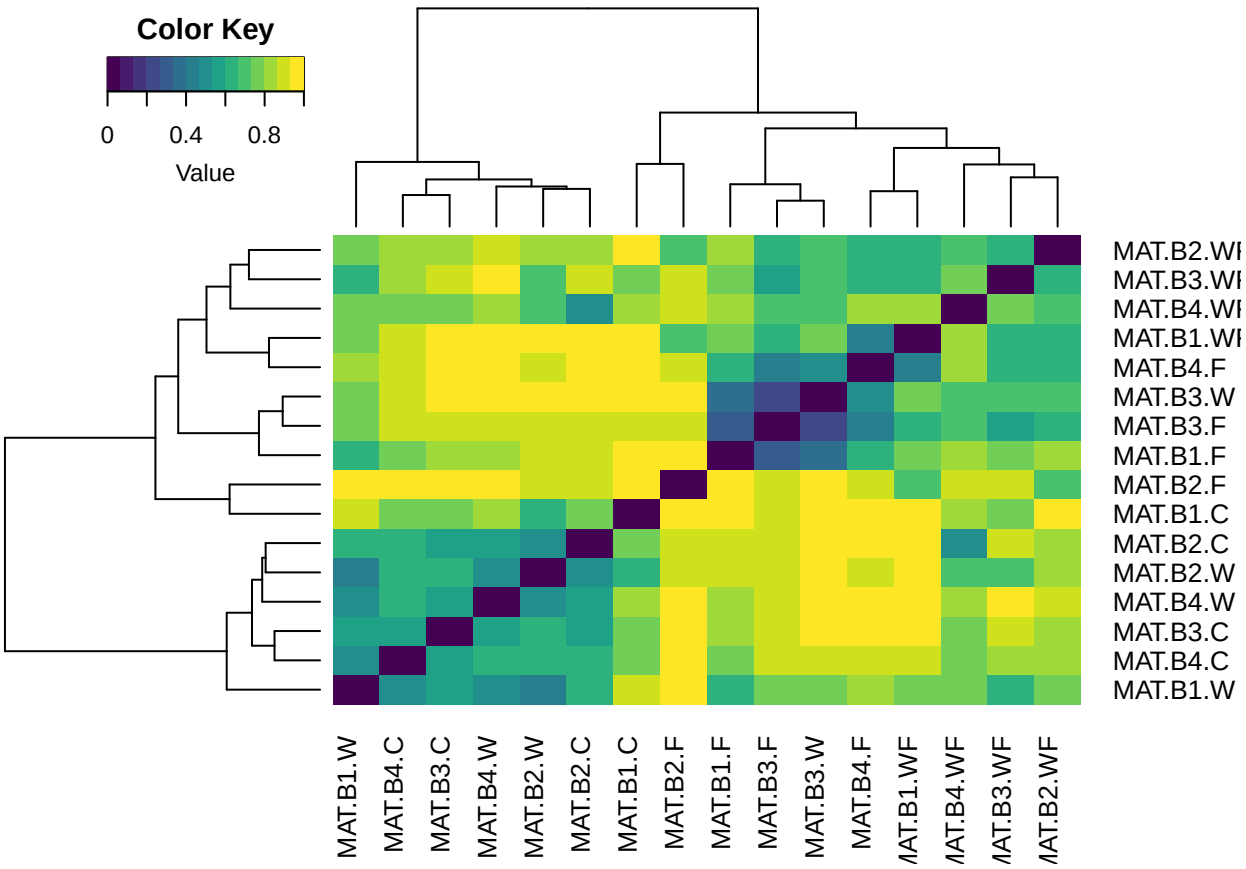
```
require(viridis)
```

```
## Loading required package: viridis

## Loading required package: viridisLite
```

```
Fungi_path <- "/cloud/project/QB2025_Spencer/MAT_fungal_abundances.txt"
Fungi_data <- read.table(Fungi_path, header = TRUE, sep = "\t", stringsAsFactors = TRUE)
Fungi_data$Relative.Abundance <- as.numeric(Fungi_data$Relative.Abundance)

# Slim down the data to only columns needed
slim <- Fungi_data %>%
  select(Plot, Treatment, Species, Relative.Abundance)

# Change data so that each species is a column, values are the relative.abundance, and rows are each pl
ExpandFungi_data <- slim %>%
  pivot_wider(names_from = Species, values_from = Relative.Abundance, values_fill = list(Relative.Abunda

# Sort the expanded data
Sorted_ExpandFungi_data <- ExpandFungi_data %>%
  arrange(Treatment)

# Calculate Bray-Curtis distance
ExpandFun_data.db <- vegdist(Sorted_ExpandFungi_data[, -c(1,2)], method = "bray", upper = TRUE, diag = T

# Create a new variable and classify it as a matrix
heatmap_matrix <- as.matrix(ExpandFun_data.db)

# Assign row and column names using the "Plot" column from Sorted_ExpandFungi_data
rownames(heatmap_matrix) <- Sorted_ExpandFungi_data$Plot
colnames(heatmap_matrix) <- Sorted_ExpandFungi_data$Plot

#Ward's Clustering with heat map
fun.ward <- hclust(ExpandFun_data.db, method = "ward.D2")

par(mar = c(1, 5, 2, 2) + 0.1)
plot(fun.ward, main = "Fungal Diversity: Ward's Clustering",
     ylab = "Squared Bray-Curtis Distance")
```




```
gplots::heatmap.2(heatmap_matrix,
                  distfun = function(x) vegdist(x, method = "bray"),
                  hclustfun = function(x) hclust(x, method = "ward.D2"),
                  col = "viridis", trace = "none", density.info = "none")
```




```
# Check and handle missing values
Fungi_data <- na.omit(Fungi_data)

# Ensure correct data types
Fungi_data$Treatment <- as.factor(Fungi_data$Treatment)
Fungi_data$Relative.Abundance <- as.numeric(Fungi_data$Relative.Abundance)

# Verify no negative values
if (any(Fungi_data$Relative.Abundance < 0)) {
  stop("Abundance data contains negative values.")
}

# Replace zero values
Fungi_data$Relative.Abundance[Fungi_data$Relative.Abundance == 0] <- 0.0001

# Run adonis2
adonis2_result <- adonis2(Fungi_data$Relative.Abundance ~ Fungi_data$Treatment, method = "bray", permuta
print(adonis2_result)
```

```
## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Fungi_data$Relative.Abundance ~ Fungi_data$Treatment, permutations = 999, method =
##            Df SumOfSqs      R2      F Pr(>F)
## Model       3    0.661 0.00603 1.4166  0.223
## Residual 700  108.829 0.99397
```

```
## Total    703  109.490 1.00000
```

The clustering of the treatments is surprising - there isn't an apparent trend to clustering based on treatment that I had initially anticipated. It seems the warmed and fertilized treatments tend to cluster together, but the warmed, fertilized, and control groups cluster more randomly than by treatment. This suggests that there may be no individual impact of either warming or fertilizer, but an effect when in tandem. The PERMANOVA results, however, yield an insignificant p-value. This means that there is no interaction between treatment and fungal diversity. Diversity amongst treatments may be due to more random ecological impacts like dispersal and speciation.