

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

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08 February, 2023

### OVERVIEW

In this worksheet, we continue to explore concepts, statistics, and visualizations related to  $\beta$ -diversity. Now that you know how to formally quantify  $\beta$ -diversity, we will learn how to test hypotheses about  $\beta$ -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 8<sup>th</sup>, 2023 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 “/6.BetaDiversity” folder, and

4. load the `vegan` R package (be sure to install if needed).

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

## [1] "/Users/ericanadolski/GitHub/QB2023_Nadolski/2.Worksheets/6.BetaDiversity"

package.list <- c("vegan", "ade4", "viridis", "gplots", "indicspecies")
for (package in package.list) {
  if (!require(package, character.only = TRUE, quietly=TRUE)) {
    install.packages(package)
    library(package, character.only = TRUE)
  }
}

## This is vegan 2.6-4

##
## Attaching package: 'gplots'

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

## 2) LOADING DATA

### Load dataset

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

```
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.

```
quality <- c(rep("HQ", 13), rep("MQ", 5), rep("LQ", 6), rep("MQ", 5))
fish <- doubs$fish
fish <- fish[-8,]

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

```
## 'adonis' will be deprecated: use 'adonis2' instead

## $aov.tab
## 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
##
## $call
## adonis(formula = fish ~ quality, permutations = 999, method = "bray")
##
## $coefficients
##           Cogo          Satr          Phph          Neba          Thth          Teso
## (Intercept) 0.4384615  1.546154  1.941880  2.191453  0.4230769  0.5948718
## quality1    0.1769231  1.992308  1.750427  1.116239  0.3461538 -0.2102564
## quality2   -0.4384615 -1.546154 -1.608547 -1.524786 -0.4230769 -0.5948718
##           Chna          Chto          Lele          Lece          Baba
## (Intercept) 0.7777778  1.000000e+00  1.5418803  2.0324786  1.7145299
## quality1   -0.7777778 -1.000000e+00 -0.8495726 -0.8017094 -1.6376068
## quality2    0.5555556 -1.444014e-16 -0.2085470  0.1341880  0.4521368
##           Spbi          Gogo          Eslu          Pefl Rham          Legi
## (Intercept) 1.03333333  2.1649573  1.46410256  1.3461538  1.3  1.1444444
## quality1   -1.03333333 -1.7034188 -0.77179487 -0.8076923 -1.3 -1.1444444
## quality2   -0.03333333  0.6683761  0.03589744  0.1538462  0.2  0.1888889
##           Scer          Cyca          Titi          Abbr          Icme          Acce
## (Intercept) 0.8401709  0.96666667  1.7128205  1.0444444  0.6666667  1.5333333
## quality1   -0.6863248 -0.96666667 -1.1743590 -1.0444444 -0.6666667 -1.5333333
## quality2    0.3264957  0.03333333  0.2871795  0.2888889 -0.1666667  0.4666667
##           Ruru          Blbj          Alal          Anan
## (Intercept) 2.4452991  1.2555556  2.455556  1.03333333
## quality1   -1.6760684 -1.2555556 -2.455556 -1.03333333
## quality2    0.7213675  0.4111111  1.711111 -0.03333333
##
## $coef.sites
##           1           2           3           4           5           6
## (Intercept) 0.8837625  0.7158590  0.6938684  0.6230418  0.593432800  0.5790159
## quality1   -0.1866775 -0.3635334 -0.3456333 -0.2543114  0.001651813 -0.2025678
## quality2    0.1162375  0.2564578  0.2548711  0.2057479  0.056023013  0.1732300
##           7           9          10          11          12          13
## (Intercept) 0.6581737  0.71175827  0.6442621  0.7094992  0.6760632  0.7319489
## quality1   -0.3307310 -0.05986164 -0.2179352 -0.2948057 -0.3340042 -0.3052481
## quality2    0.2381097  0.04794668  0.1706838  0.2036504  0.2492646  0.2428243
##          14          15          16          17          18          19
## (Intercept) 0.6625054  0.6126096  0.58629885  0.53831620  0.5265411  0.5207733
## quality1   -0.2452690 -0.1465108 -0.01793157  0.06448519  0.1353961  0.1853921
## quality2    0.2192664  0.2018642  0.14094735  0.08434935  0.0300669 -0.0183066
##          20          21          22          23          24          25
```

```

## (Intercept) 0.5199318 0.5323960 0.5576307 0.8035439 0.6877772 0.6964587
## quality1    0.2948405 0.3155710 0.3206040 0.1145069 0.2110503 0.1691525
## quality2    -0.1191038 -0.1333993 -0.1281596 -0.1892430 -0.2071918 -0.1908893
##              26          27          28          29          30
## (Intercept) 0.5546865 0.5573809 0.5683021 0.56565435 0.61089065
## quality1    0.2955016 0.3181323 0.3117246 0.28417790 0.30341313
## quality2    -0.1264475 -0.1150663 -0.1044695 -0.07583091 -0.09189382
##
## $f.perms
##           [,1]
##      [1,] 0.4764580
##      [2,] 0.8074977
##      [3,] 0.4885326
##      [4,] 1.7302479
##      [5,] 2.2991940
##      [6,] 1.7492334
##      [7,] 0.5693504
##      [8,] 1.2930181
##      [9,] 1.8716965
##     [10,] 0.9260482
##     [11,] 1.5318016
##     [12,] 1.1792445
##     [13,] 0.9273279
##     [14,] 0.7083475
##     [15,] 0.6551385
##     [16,] 1.6005829
##     [17,] 1.2254249
##     [18,] 1.0045719
##     [19,] 0.4857888
##     [20,] 0.6467672
##     [21,] 0.7238783
##     [22,] 0.9128139
##     [23,] 1.1803439
##     [24,] 0.9332384
##     [25,] 1.2541510
##     [26,] 0.8474058
##     [27,] 1.8733652
##     [28,] 1.0396851
##     [29,] 0.3570951
##     [30,] 0.7659412
##     [31,] 0.5089215
##     [32,] 0.6517048
##     [33,] 0.7044918
##     [34,] 0.4228663
##     [35,] 3.8703217
##     [36,] 0.5761153
##     [37,] 0.9026878
##     [38,] 0.7361772
##     [39,] 0.6764858
##     [40,] 0.6987975
##     [41,] 1.3902571
##     [42,] 0.8663034
##     [43,] 0.3529206
##     [44,] 0.6892482

```

```
## [45,] 0.6962706
## [46,] 1.1141528
## [47,] 1.4789257
## [48,] 0.3551746
## [49,] 0.7241373
## [50,] 0.6116049
## [51,] 0.7612271
## [52,] 3.5712703
## [53,] 0.4038222
## [54,] 0.8800852
## [55,] 1.3497964
## [56,] 0.4357954
## [57,] 1.0884890
## [58,] 2.0734072
## [59,] 0.6303964
## [60,] 1.2932408
## [61,] 1.6672089
## [62,] 1.0215526
## [63,] 0.9418131
## [64,] 0.8239514
## [65,] 0.9154520
## [66,] 0.6136820
## [67,] 1.0074773
## [68,] 0.8324387
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## [70,] 0.3974663
## [71,] 1.5691103
## [72,] 1.4297740
## [73,] 0.9729030
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## [75,] 1.7318970
## [76,] 1.5116129
## [77,] 0.9487972
## [78,] 0.6399474
## [79,] 0.6392029
## [80,] 0.7249586
## [81,] 0.6814756
## [82,] 1.4881634
## [83,] 0.2473441
## [84,] 0.6277440
## [85,] 0.9199629
## [86,] 2.3277901
## [87,] 1.4226783
## [88,] 0.6257980
## [89,] 0.4182401
## [90,] 0.5438472
## [91,] 0.3424419
## [92,] 0.6259133
## [93,] 0.9210348
## [94,] 1.2284818
## [95,] 0.4148106
## [96,] 2.6363754
## [97,] 0.4750244
## [98,] 1.5330042
```

```
## [99,] 0.7874703
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## [101,] 0.6343015
## [102,] 1.2751081
## [103,] 0.5610526
## [104,] 0.7151152
## [105,] 0.8896890
## [106,] 0.8352640
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## [109,] 1.1799947
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## [112,] 0.5516075
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## [134,] 0.7558229
## [135,] 0.6777827
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## [137,] 0.9506805
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## [139,] 1.1236885
## [140,] 1.6563802
## [141,] 0.8210050
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## [147,] 0.8342359
## [148,] 1.7598262
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## [150,] 0.5177281
## [151,] 0.3263226
## [152,] 3.3553393
```

## [153,] 0.6020303  
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```
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```

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## [410,] 0.6248769  
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## [412,] 1.3154691  
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## [414,] 1.5220478  
## [415,] 0.8954512  
## [416,] 0.4658185  
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## [686,] 2.3644262  
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## [692,] 0.6026249



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## [695,] 1.0346093  
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## [766,] 0.4225762  
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## [930,] 1.6672526  
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## [938,] 1.1852420  
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## [945,] 1.0883950  
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## [947,] 0.7139862  
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## [949,] 0.3759979  
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## [958,] 2.3241672  
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## [961,] 0.4131232  
## [962,] 0.5241231

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## [963,] 0.9229976
## [964,] 0.6456638
## [965,] 1.4405091
## [966,] 2.8807934
## [967,] 1.8109521
## [968,] 0.8459932
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## [976,] 1.1360996
## [977,] 0.6808167
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## [979,] 0.6369361
## [980,] 1.0049180
## [981,] 0.9010021
## [982,] 0.8878818
## [983,] 1.0299756
## [984,] 0.4205549
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## [986,] 1.0598446
## [987,] 0.7004753
## [988,] 0.4610544
## [989,] 1.7365374
## [990,] 3.7640493
## [991,] 1.2540292
## [992,] 1.6094449
## [993,] 0.9611394
## [994,] 0.6794601
## [995,] 1.4137775
## [996,] 0.3513397
## [997,] 0.8459246
## [998,] 1.9327924
## [999,] 0.6057243
##
## $model.matrix
##      (Intercept) quality1 quality2
## 1             1         1         0
## 2             1         1         0
## 3             1         1         0
## 4             1         1         0
## 5             1         1         0
## 6             1         1         0
## 7             1         1         0
## 8             1         1         0
## 9             1         1         0
## 10            1         1         0
## 11            1         1         0
## 12            1         1         0
## 13            1         1         0
## 14            1        -1        -1

```

```
## 15      1      -1      -1
## 16      1      -1      -1
## 17      1      -1      -1
## 18      1      -1      -1
## 19      1       0       1
## 20      1       0       1
## 21      1       0       1
## 22      1       0       1
## 23      1       0       1
## 24      1       0       1
## 25      1      -1      -1
## 26      1      -1      -1
## 27      1      -1      -1
## 28      1      -1      -1
## 29      1      -1      -1
```

```
##
## $terms
## fish ~ quality
## attr("variables")
## list(fish, quality)
## attr("factors")
##      quality
## fish      0
## quality   1
## attr("term.labels")
## [1] "quality"
## attr("order")
## [1] 1
## attr("intercept")
## [1] 1
## attr("response")
## [1] 1
## attr(".Environment")
## <environment: R_GlobalEnv>
##
## attr("class")
## [1] "adonis"
```

```
# Indicator values
```

```
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    0.024 *
##
## Group HQ+MQ #sps. 2
##      stat p.value
## Satr 0.860    0.007 **
## Phph 0.859    0.008 **
##
## 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    0.001 ***
## Spbi 0.866    0.003 **
## Cyca 0.866    0.001 ***
## Acce 0.866    0.001 ***
## Lele 0.863    0.002 **
## Titi 0.853    0.003 **
## Chto 0.829    0.005 **
## Rham 0.829    0.002 **
## Anan 0.829    0.002 **
## Eslu 0.827    0.022 *
## Pefl 0.806    0.014 *
## Blbj 0.791    0.005 **
## Scer 0.766    0.008 **
## Abbr 0.750    0.006 **
## Icme 0.661    0.027 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

**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:** From the visualizations, we saw that Satr, Phph, and Neba were more abundant at sites near the river source, while, Alal, Lece, and Ruru were abundant downstream the river. The PERMANOVA told us that site quality explains ~45% of the variation in fish diversity across the sites, the IndVal told us that Satr and Phph were significantly associated with HQ+MQ while Alal and Ruru were associated with LQ+MQ sites, and the phi coefficient told us that Satr, Phph, and Neba were found to be significantly associated with HQ sites (near the source) while Alal and Ruru were significantly associated with LQ sites. Overall, the results of all the methods are generally in agreement, but different methods pick up difference nuances of the associations, especially for the fish-quality associations of smaller magnitudes.



## 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$fish[-8,], method="bray")
env.dist <- vegdist(doubs$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: 0.4677
##      Significance: 0.001
##
## Upper quantiles of permutations (null model):
##      90%      95%    97.5%     99%
## 0.0884 0.1294 0.1583 0.1809
## 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  $r$  value of the test is 0.4677 and is highly significant, meaning we can be confident that site quality explains about half of the variation in fish communities that we see in the Doubs data set; our hypothesis is supported by this analysis.

### ii. Constrained Ordination

In the R code chunk below, do the following:

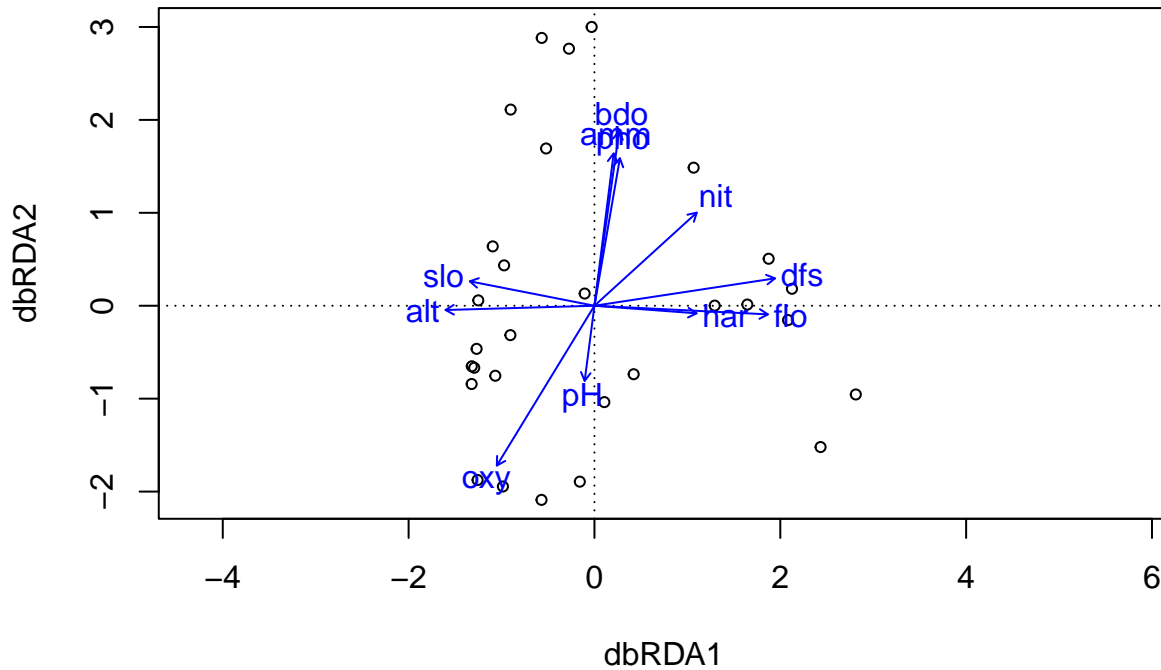
1. create an environmental matrix of the water chemistry data included in the `doubs` dataset using forward and reverse selection of variables,
2. conduct a redundancy analysis on the fish assemblages of the Doubs River,
3. use a permutation test to determine the significance of the constrained analysis,
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.

```
fish.db <- as.data.frame(fish)
```

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

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

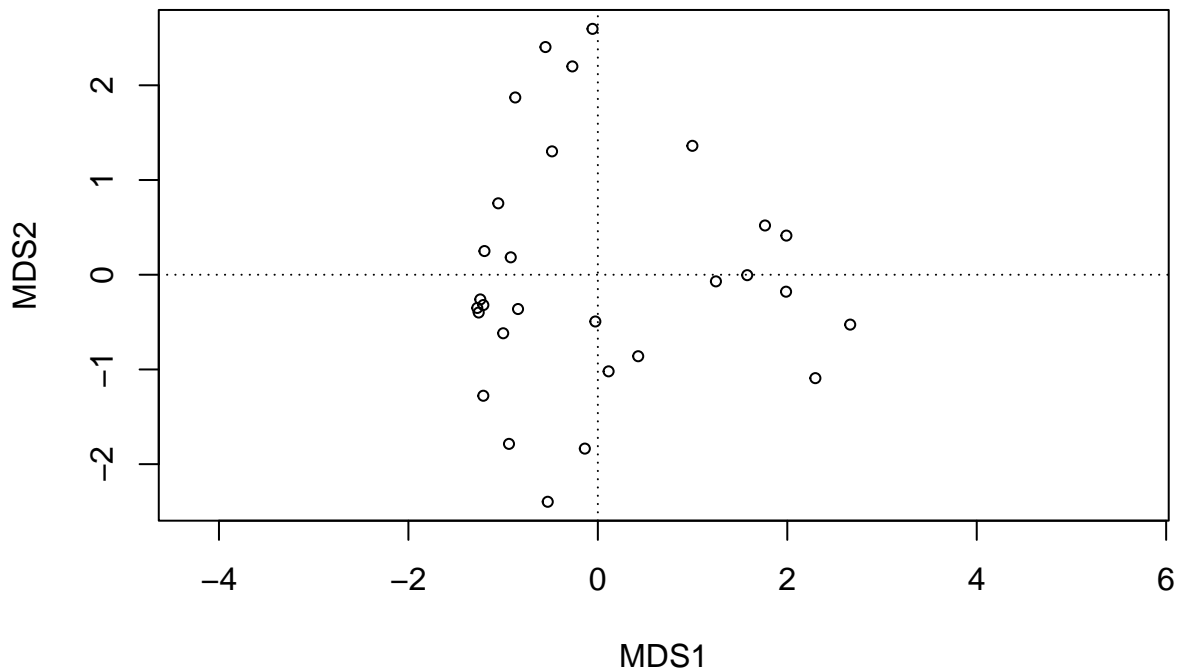
```
ordiplot(doubs.dbrda)
```



```
# first model only intercept
```

```
doubs.dbrda.mod0 <- dbrda(fish.db ~ 1, as.data.frame(env.chem))
```

```
ordiplot(doubs.dbrda.mod0) # no vectors constrained here, so its a simple MDS (PCoA)
```



```
# now model with all explanatory variables
doubts.dbrda.mod1 <- dbrda(fish.db ~ ., as.data.frame(env.chem))
# now iterate through all combos of explanatory variables, return model with lowest AIC
doubts.dbrda <- ordiR2step(doubts.dbrda.mod0,doubts.dbrda.mod1, perm.max=200)
```

```
## Step: R2.adj= 0
## Call: fish.db ~ 1
##
##               R2.adjusted
## <All variables> 0.61674085
## + dfs          0.42741273
## + flo          0.39139827
## + alt          0.28815032
## + slo          0.19091501
## + oxy          0.14851920
## + nit          0.13741705
## + har          0.11933638
## + bdo          0.03421527
## + pho          0.01943949
## + amm          0.01803524
## <none>         0.00000000
## + pH          -0.01846260
##
##      Df    AIC      F Pr(>F)
## + dfs  1 108.53 21.901 0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.4274127
## Call: fish.db ~ dfs
##
##               R2.adjusted
## <All variables> 0.6167408
## + bdo          0.5247385
## + pho          0.5223629
## + amm          0.5080947
## + alt          0.4945241
## + oxy          0.4734211
## + nit          0.4701579
## + flo          0.4471745
## + har          0.4454607
## + slo          0.4314037
## <none>         0.4274127
## + pH          0.4256566
##
##      Df    AIC      F Pr(>F)
## + bdo  1 104.03  6.5292 0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.5247385
## Call: fish.db ~ dfs + bdo
##
```

```
##                                R2.adjusted
## <All variables>      0.6167408
## + alt                0.6059729
## + oxy                0.6036302
## + har                0.5459153
## + flo                0.5356872
## + slo                0.5329456
## + pho                0.5266412
## + pH                0.5255286
## <none>                0.5247385
## + nit                0.5224083
## + amm                0.5147677
##
##      Df      AIC      F Pr(>F)
## + alt  1 99.458 6.3603 0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.6059729
## Call: fish.db ~ dfs + bdo + alt
##
##                                R2.adjusted
## + oxy                0.6448443
## + flo                0.6384164
## <All variables>      0.6167408
## + slo                0.6078485
## + har                0.6067391
## <none>                0.6059729
## + pH                0.6058566
## + pho                0.6042463
## + nit                0.5997052
## + amm                0.5945204
```

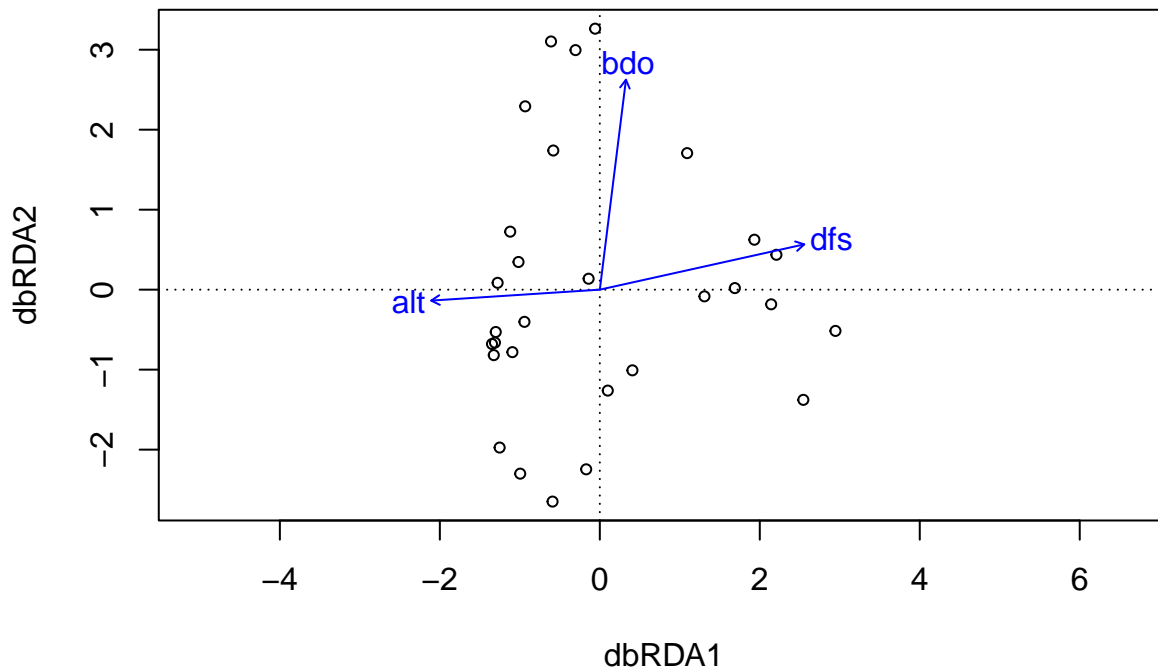
```
# look at model that was selected
doubts.dbrda$call
```

```
## dbrda(formula = fish.db ~ dfs + bdo + alt, data = as.data.frame(env.chem))
```

```
doubts.dbrda$anova
```

```
##                                R2.adj Df      AIC      F Pr(>F)
## + dfs                0.42741  1 108.528 21.9008 0.002 **
## + bdo                0.52474  1 104.031  6.5292 0.002 **
## + alt                0.60597  1  99.458  6.3603 0.002 **
## <All variables>      0.61674
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ordiplot(doubts.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.db ~ dfs + bdo + alt, data =
## as.data.frame(env.chem))
## Permutation test for all constrained eigenvalues
##      Df Inertia      F Pr(>F)
## Model    3  44.696 15.354 0.001 ***
## Residual 25  24.259
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

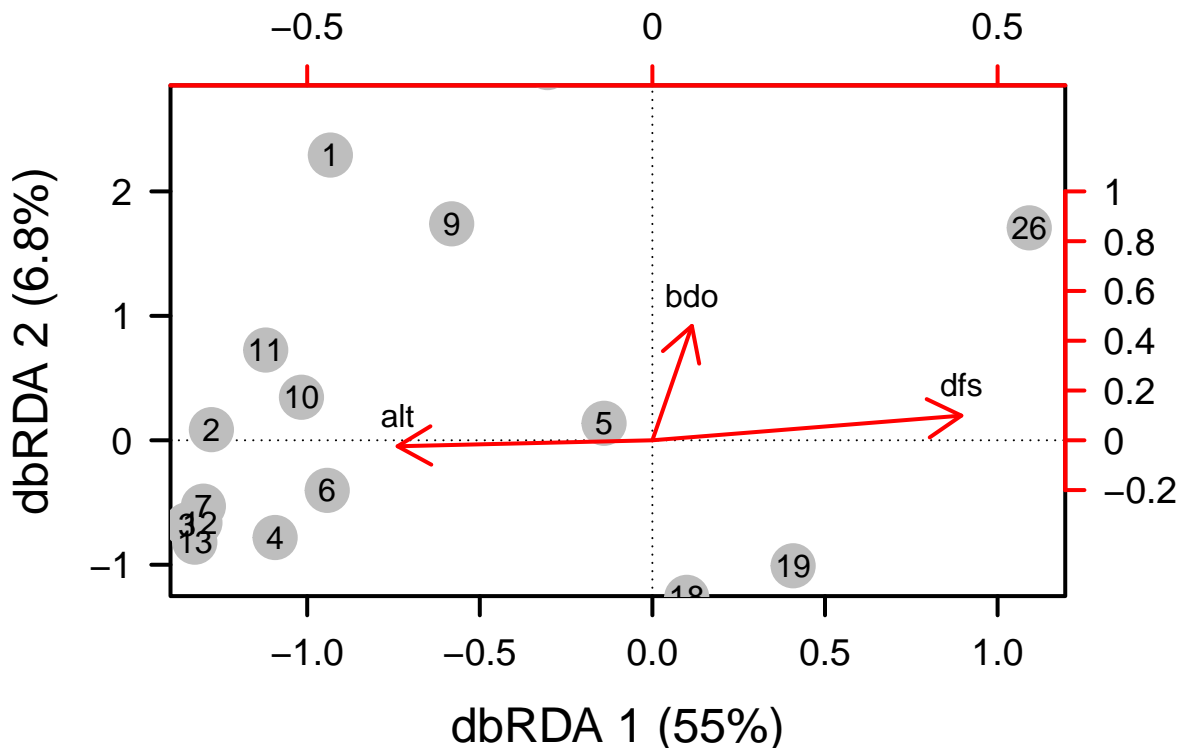
```
##
## ***VECTORS
##
##      dbRDA1  dbRDA2    r2 Pr(>r)
## flo  0.99999 -0.00368 0.6320 0.001 ***
## har  0.99943 -0.03376 0.2184 0.047 *
## pho  0.16809  0.98577 0.3854 0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

```

#calculate explained variation
dbrda.explainvar1 <- round(doubs.dbrda$CCA$eig[1] / sum(c(doubs.dbrda$CCA$eig, doubs.dbrda$CA$eig)), 3)
dbrda.explainvar2 <- round(doubs.dbrda$CCA$eig[2] / sum(c(doubs.dbrda$CCA$eig, doubs.dbrda$CA$eig)), 3)

# now plot selected model, define plot parameters
par(mar=c(5,5,4,4)+0.1)
vectors <- scores(doubs.dbrda,display="bp")
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);
axis(side=1, labels=T, lwd.ticks=2, cex.axis=1.2, las=1);
axis(side=2, labels=T, lwd.ticks=2, cex.axis=1.2, las=1);
abline(h=0,v=0,lty=3);
box(lwd=2);
points(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")));
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 variables dfs (discharge flow per second) and alt (altitude) contribute heavily to the variation in fish communities across the river; specifically, they map onto the first RDA

axis which explains 55% of the variation. The variable bdo (?) maps onto the second RDA axis, which explains an additional 7% of the variation.

### 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)
## + dfs          0.42741  1 108.528 21.9008 0.002 **
## + bdo          0.52474  1 104.031  6.5292 0.002 **
## + alt          0.60597  1  99.458  6.3603 0.002 **
## <All variables> 0.61674
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# create a matrix model for our environmental data
env.mod <- model.matrix( ~oxy + bdo + nit, as.data.frame(env.chem))[, -1]
# spatial model
rs <- rowSums(fish)/sum(fish) #weight each site by relative abundance
doubs.pcnmw <- pcnm(dist(doubs$xy[-8,]), w=rs, dist.ret=T) # perform PCNM
doubs.pcnmw$values > 0 # keep only the positive eigenvalues
```

```
## [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [13] TRUE TRUE TRUE TRUE TRUE TRUE FALSE FALSE FALSE FALSE FALSE FALSE
## [25] FALSE FALSE
```

```
# perform model selection
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> 0.572085682
## + PCNM2          0.231215878
## + PCNM3          0.059236621
## + PCNM1          0.048452431
```

```

## + PCNM5          0.011878086
## + PCNM13         0.011382477
## <none>           0.000000000
## + PCNM15        -0.004103784
## + PCNM6          -0.004305671
## + PCNM16        -0.010316626
## + PCNM14        -0.015089646
## + PCNM9          -0.016415162
## + PCNM8          -0.016589781
## + PCNM4          -0.017513855
## + PCNM7          -0.019315072
## + PCNM17        -0.023426151
## + PCNM10        -0.023962155
## + PCNM11        -0.026011191
## + PCNM12        -0.029124765
##
##           Df      AIC      F Pr(>F)
## + PCNM2   1 117.07 9.4211 0.004 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.2312159
## Call: fish.db ~ PCNM2
##
##           R2.adjusted
## <All variables> 0.5720857
## + PCNM1         0.3903810
## + PCNM3         0.3147907
## + PCNM5         0.2631795
## + PCNM15        0.2525901
## + PCNM16        0.2449515
## + PCNM6         0.2387263
## + PCNM14        0.2337845
## <none>          0.2312159
## + PCNM9         0.2271297
## + PCNM4         0.2259698
## + PCNM13        0.2246320
## + PCNM8         0.2217435
## + PCNM7         0.2201187
## + PCNM11        0.2180744
## + PCNM17        0.2146517
## + PCNM10        0.2140924
## + PCNM12        0.2136165
##
##           Df      AIC      F Pr(>F)
## + PCNM1   1 111.25 8.0494 0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.390381
## Call: fish.db ~ PCNM2 + PCNM1
##
##           R2.adjusted
## <All variables> 0.5720857

```



```

## + PCNM3          0.4754979
## + PCNM5          0.4374583
## + PCNM6          0.4081993
## + PCNM15         0.4051835
## + PCNM4          0.4041234
## + PCNM16         0.3976791
## + PCNM14         0.3916984
## <none>           0.3903810
## + PCNM13         0.3900540
## + PCNM9          0.3894225
## + PCNM8          0.3868595
## + PCNM7          0.3855378
## + PCNM10         0.3808845
## + PCNM11         0.3804068
## + PCNM17         0.3792106
## + PCNM12         0.3771900
##
##           Df      AIC      F Pr(>F)
## + PCNM3    1 107.75 5.2193 0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.4754979
## Call: fish.db ~ PCNM2 + PCNM1 + PCNM3
##
##           R2.adjusted
## <All variables> 0.5720857
## + PCNM5         0.5216867
## + PCNM15        0.4956369
## + PCNM6         0.4946094
## + PCNM4         0.4929907
## + PCNM16        0.4853069
## + PCNM13        0.4776429
## + PCNM14        0.4766851
## <none>          0.4754979
## + PCNM9         0.4753106
## + PCNM7         0.4736775
## + PCNM8         0.4726987
## + PCNM12        0.4698956
## + PCNM11        0.4694465
## + PCNM10        0.4685059
## + PCNM17        0.4662512
##
##           Df      AIC      F Pr(>F)
## + PCNM5    1 105.9 3.4141 0.024 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.5216867
## Call: fish.db ~ PCNM2 + PCNM1 + PCNM3 + PCNM5
##
##           R2.adjusted
## <All variables> 0.5720857
## + PCNM15        0.5512193

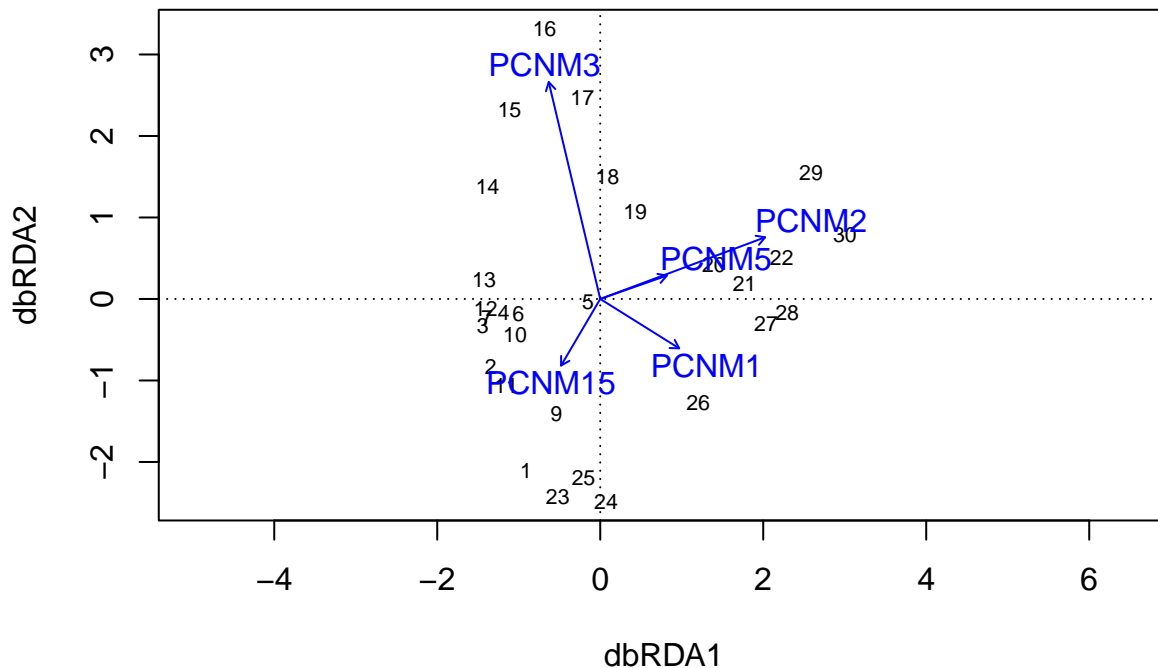
```

```

## + PCNM16      0.5440665
## + PCNM4       0.5431513
## + PCNM6       0.5385303
## + PCNM14      0.5326653
## + PCNM9       0.5301706
## + PCNM13      0.5296995
## + PCNM12      0.5240003
## + PCNM7       0.5225683
## <none>        0.5216867
## + PCNM11      0.5210653
## + PCNM8       0.5209816
## + PCNM17      0.5135661
## + PCNM10      0.5132748
##
##           Df      AIC      F Pr(>F)
## + PCNM15  1 104.81 2.5794 0.05 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.5512193
## Call: fish.db ~ PCNM2 + PCNM1 + PCNM3 + PCNM5 + PCNM15
##
##           R2.adjusted
## + PCNM4      0.5766918
## <All variables> 0.5720857
## + PCNM6      0.5697239
## + PCNM9      0.5651178
## + PCNM13     0.5623506
## + PCNM12     0.5609834
## + PCNM7      0.5556769
## + PCNM16     0.5520003
## + PCNM8      0.5518718
## <none>       0.5512193
## + PCNM10     0.5482104
## + PCNM14     0.5469739
## + PCNM11     0.5450627
## + PCNM17     0.5441366

```

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



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

```
##               R2.adj Df      AIC      F Pr(>F)
## + PCNM2       0.23122  1 117.07  9.4211  0.004 **
## + PCNM1       0.39038  1 111.25  8.0494  0.002 **
## + PCNM3       0.47550  1 107.75  5.2193  0.002 **
## + PCNM5       0.52169  1 105.89  3.4141  0.024 *
## + PCNM15      0.55122  1 104.81  2.5794  0.050 *
## <All variables> 0.57209
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

# Partial constrained ordination to determine independent and joint explanatory power of spatial and env
# constrained ordinations
doub.s.total.env <- dbrda(fish.db ~ env.mod)
doub.s.total.space <- dbrda(fish.db ~ space.mod)
# partial constrained ordinations
doub.s.env.cond.space <- dbrda(fish.db ~ env.mod + Condition(space.mod))
doub.s.space.cond.env <- dbrda(fish.db ~ space.mod + Condition(env.mod))
# test for significance of the dbrda fractions
permutest(doub.s.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  8.3688 3.7061 0.001 ***
## Residual 18 13.5486
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
permutest(doubs.space.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 23.946 4.5448 0.001 ***
## Residual 18 13.549
## ---
## 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 31.461 6.9924 0.001 ***
## Residual 25 37.495
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

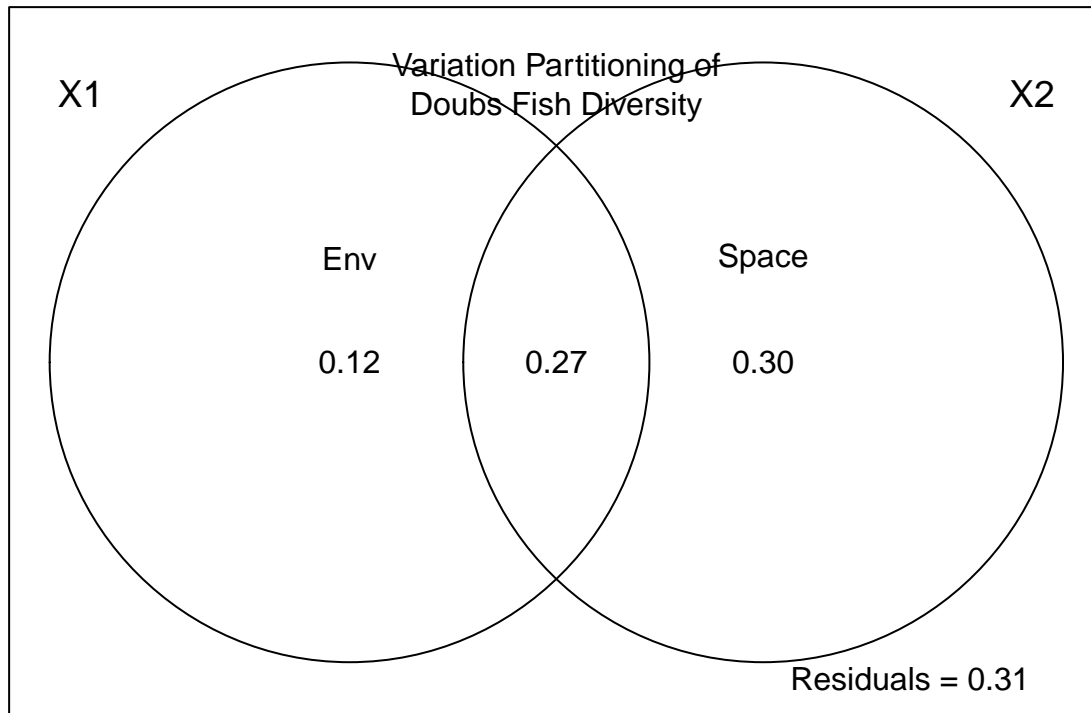
```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.db ~ space.mod)
## Permutation test for all constrained eigenvalues
##      Df Inertia      F Pr(>F)
```

```
## Model      7  47.038 6.4385  0.001 ***
## Residual 21  21.917
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# variation partitioning
doubs.varpart <- varpart(fish.db, env.mod, space.mod)
doubs.varpart
```

```
##
## Partition of variance in RDA
##
## 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): 1930.8
##           Variance: 68.956
## No. of observations: 29
##
## Partition table:
##
##      Df R.squared Adj.R.squared Testable
## [a+c] = X1      3  0.45625      0.39100    TRUE
## [b+c] = X2      7  0.68215      0.57620    TRUE
## [a+b+c] = X1+X2 10  0.80352      0.69436    TRUE
## Individual fractions
## [a] = X1|X2      3              0.11816    TRUE
## [b] = X2|X1      7              0.30336    TRUE
## [c]              0              0.27284    FALSE
## [d] = Residuals              0.30564    FALSE
## ---
## Use function 'rda' 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:** The variance partitioning indicates that there are significant associations of environment and space independently and together; spatial distribution explains 30% of the variation, environmental conditions explain 12%, and the joint interaction/correlation between both explains another 27% of variation, with residuals of 0.31.

## 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.

```
##
## 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

## Warning in FUN(newX[, i], ...): NAs introduced by coercion
```

## 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
OTU.dist <- vegdist(total, method="bray")
pond.env.dist <- vegdist(env, method="euclid")

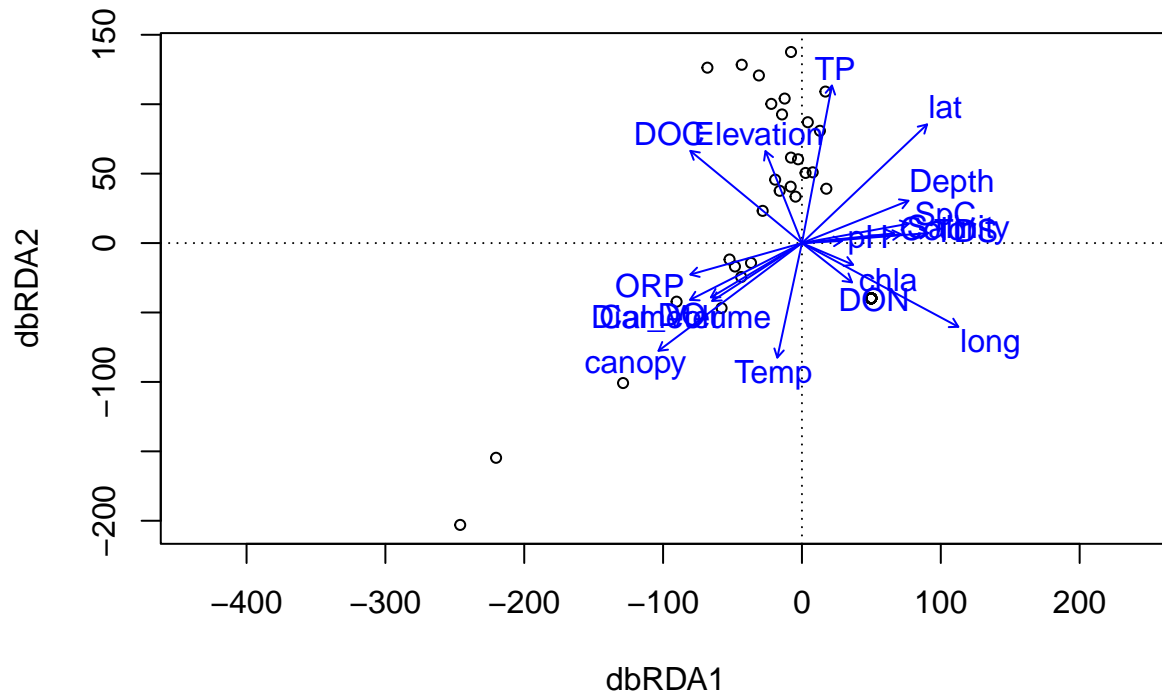
# mantel test
mantel(OTU.dist,pond.env.dist)

##
## Mantel statistic based on Pearson's product-moment correlation
##
## Call:
## mantel(xdis = OTU.dist, ydis = pond.env.dist)
##
## Mantel statistic r: 0.07691
##      Significance: 0.009
##
## Upper quantiles of permutations (null model):
##      90%      95%     97.5%      99%
## 0.0318 0.0462 0.0580 0.0742
## Permutation: free
## Number of permutations: 999
```

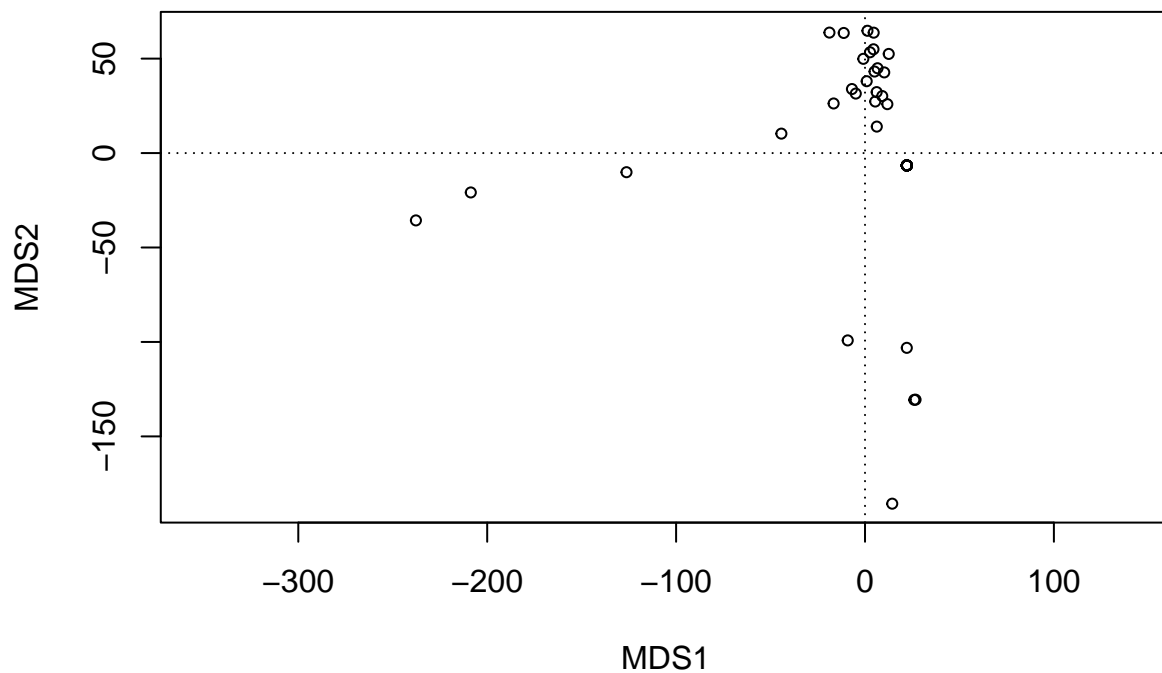
## Constrained Ordination of Indiana Ponds data

1. create an environmental matrix of the pond data using forward and reverse selection of variables
2. conduct a redundancy analysis on the bacterial assemblages
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.

```
# Constrained Ordination
pond.dbrda <- dbrda(total ~ ., as.data.frame(env)) #perform dbRDA
ordiplot(pond.dbrda)
```



```
# first model only intercept
pond.dbrda.mod0 <- dbrda(total ~ 1, as.data.frame(env))
ordiplot(pond.dbrda.mod0) # no vectors constrained here, so its a simple MDS (PCoA)
```



```
# now model with all explanatory variables
pond.dbrda.mod1 <- dbrda(total ~ ., as.data.frame(env))
# now iterate through all combos of explanatory variables, return model with lowest AIC
# pond.dbrda <- ordiR2step(pond.dbrda.mod0,pond.dbrda.mod1, permutations = how(nperm = 200))
# couldn't get ordiR2step to work, error that upper scope cannot be reached
pond.dbrda <- ordistep(pond.dbrda.mod0,pond.dbrda.mod1, permutations = how(nperm = 200))
```



```

##
## Start: total ~ 1
##
##           Df      AIC      F    Pr(>F)
## + canopy    1 1021.2 4.2672 0.004975 **
## + lat        1 1021.7 3.7835 0.004975 **
## + long       1 1021.1 4.3887 0.009950 **
## + TDS        1 1021.8 3.6640 0.009950 **
## + TP         1 1022.9 2.5795 0.014925 *
## + DOC        1 1022.4 3.0719 0.019900 *
## + SpC        1 1022.6 2.8611 0.019900 *
## + Diameter   1 1023.1 2.3307 0.029851 *
## + Depth      1 1023.3 2.1260 0.039801 *
## + Salinity   1 1023.0 2.5027 0.064677 .
## + pH         1 1023.5 1.9807 0.084577 .
## + Cal_Volume 1 1023.7 1.7619 0.089552 .
## + ORP        1 1023.3 2.1264 0.099502 .
## + DO         1 1023.8 1.6693 0.164179
## + Color      1 1024.1 1.3931 0.194030
## + DON        1 1024.2 1.2450 0.233831
## + Temp       1 1024.1 1.3351 0.253731
## + Elevation  1 1024.3 1.1388 0.323383
## + chla       1 1025.0 0.4653 0.597015
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: total ~ canopy
##
##           Df      AIC      F    Pr(>F)
## - canopy    1 1023.5 4.2672 0.004975 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           Df      AIC      F    Pr(>F)
## + long       1 1019.4 3.7620 0.004975 **
## + DOC        1 1020.0 3.0966 0.014925 *
## + TDS        1 1020.8 2.3815 0.034826 *
## + DON        1 1021.6 1.6000 0.074627 .
## + pH         1 1021.3 1.8648 0.089552 .
## + TP         1 1021.3 1.8174 0.119403
## + SpC        1 1021.5 1.6515 0.124378
## + Salinity   1 1021.5 1.6156 0.134328
## + Elevation  1 1021.9 1.2487 0.238806
## + lat        1 1021.9 1.2945 0.298507
## + Temp       1 1022.2 0.9425 0.427861
## + Depth      1 1022.4 0.7845 0.601990
## + Color      1 1022.6 0.6335 0.691542
## + ORP        1 1022.6 0.6057 0.726368
## + chla       1 1022.9 0.2938 0.771144
## + Diameter   1 1022.7 0.4640 0.865672
## + Cal_Volume 1 1022.9 0.3356 0.945274
## + DO         1 1022.9 0.3246 0.975124

```

```

## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: total ~ canopy + long
##
##           Df      AIC      F Pr(>F)
## - canopy  1 1021.1 3.6441 0.004975 **
## - long    1 1021.2 3.7620 0.004975 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           Df      AIC      F Pr(>F)
## + DOC      1 1019.2 2.1016 0.04975 *
## + pH       1 1019.3 1.9632 0.07463 .
## + TDS      1 1019.3 1.9829 0.08955 .
## + TP       1 1019.7 1.6041 0.10945
## + DON      1 1019.7 1.5657 0.12438
## + Cal_Volume 1 1019.6 1.6530 0.12935
## + Depth    1 1019.8 1.5358 0.13433
## + lat      1 1019.4 1.8414 0.14428
## + SpC      1 1019.8 1.5077 0.19403
## + Salinity  1 1019.8 1.5274 0.20398
## + Diameter  1 1020.1 1.2288 0.28358
## + Temp     1 1020.3 1.0336 0.42786
## + Elevation 1 1020.7 0.6565 0.66667
## + chla     1 1021.1 0.2718 0.70647
## + ORP      1 1020.9 0.4604 0.86070
## + Color    1 1021.0 0.3249 0.96020
## + DO       1 1021.0 0.3299 0.97015
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: total ~ canopy + long + DOC
##
##           Df      AIC      F Pr(>F)
## - DOC      1 1019.4 2.1016 0.05970 .
## - long     1 1020.0 2.7415 0.01990 *
## - canopy   1 1021.1 3.7346 0.00995 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           Df      AIC      F Pr(>F)
## + TDS      1 1019.0 2.0094 0.04478 *
## + pH       1 1019.1 1.8864 0.11443
## + lat      1 1019.3 1.7500 0.11940
## + DON      1 1019.5 1.5308 0.13433
## + SpC      1 1019.6 1.4728 0.14428
## + Salinity  1 1019.7 1.3779 0.19900
## + Temp     1 1019.8 1.2555 0.24876
## + TP       1 1019.8 1.2653 0.25871
## + Cal_Volume 1 1020.0 1.1041 0.31841
## + Depth    1 1019.9 1.1875 0.35821
## + Diameter  1 1020.2 0.8481 0.48259
## + Elevation 1 1020.4 0.6609 0.71642

```

```
## + chla      1 1020.9 0.2807 0.72139
## + ORP       1 1020.7 0.4389 0.81592
## + Color     1 1020.7 0.3872 0.91045
## + DO        1 1020.8 0.3373 0.94527
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: total ~ canopy + long + DOC + TDS
##
##           Df      AIC      F Pr(>F)
## - TDS      1 1019.2 2.0094 0.08458 .
## - long     1 1019.1 1.9968 0.07960 .
## - DOC      1 1019.3 2.1258 0.05473 .
## - canopy   1 1020.4 3.1397 0.01990 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           Df      AIC      F Pr(>F)
## + Salinity  1 1019.2 1.5600 0.1542
## + lat       1 1019.1 1.7031 0.1592
## + TP        1 1019.4 1.3968 0.2139
## + Depth     1 1019.5 1.3134 0.2289
## + Temp      1 1019.6 1.2792 0.2488
## + pH        1 1019.8 1.0257 0.3184
## + Cal_Volume 1 1019.6 1.1919 0.3284
## + Diameter  1 1019.9 0.9397 0.4876
## + DON       1 1020.0 0.9199 0.4925
## + ORP       1 1020.4 0.5024 0.8308
## + chla      1 1020.8 0.1993 0.8408
## + DO        1 1020.5 0.4271 0.9104
## + Elevation 1 1020.5 0.3883 0.9104
## + Color     1 1020.5 0.3976 0.9154
## + SpC       1 1021.0 0.0143 1.0000
```

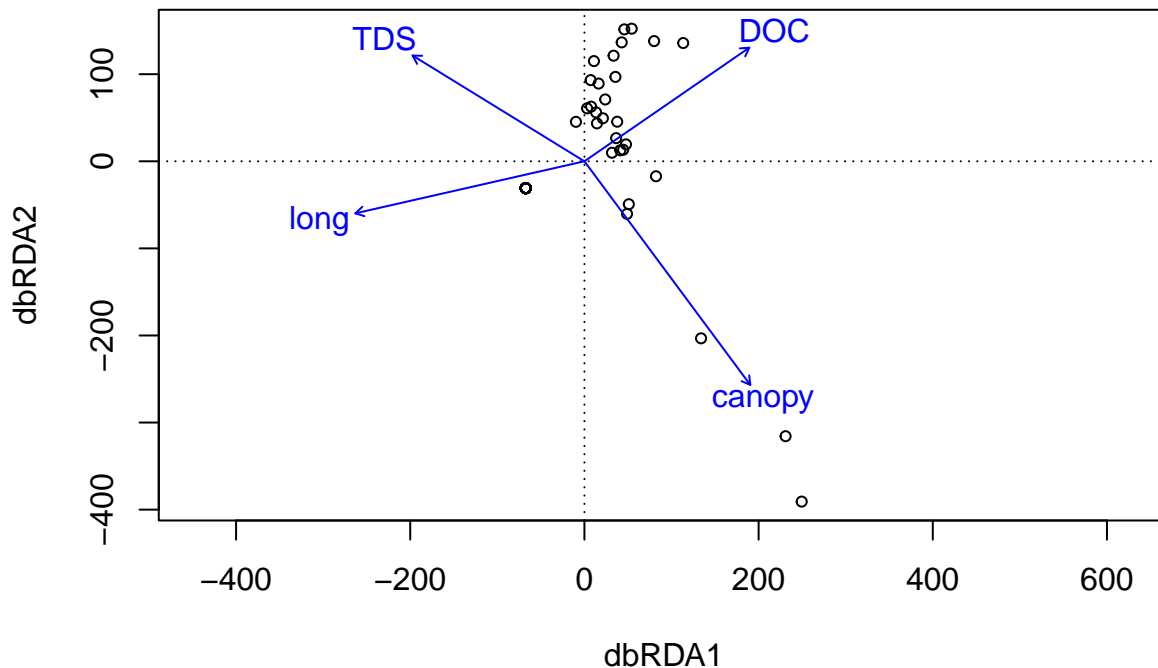
```
# look at model that was selected
pond.dbrda$call
```

```
## dbrda(formula = total ~ canopy + long + DOC + TDS, data = as.data.frame(env))
```

```
pond.dbrda$anova
```

```
##           Df      AIC      F Pr(>F)
## + canopy   1 1021.2 4.2672 0.004975 **
## + long     1 1019.4 3.7620 0.004975 **
## + DOC      1 1019.2 2.1016 0.049751 *
## + TDS      1 1019.0 2.0094 0.044776 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ordiplot(pond.dbrda)
```



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

```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = total ~ canopy + long + DOC + TDS, data =
## as.data.frame(env))
## Permutation test for all constrained eigenvalues
##      Df  Inertia    F Pr(>F)
## Model   4 74101522 3.1959 0.001 ***
## Residual 47 272441001
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
envfit(pond.dbrda, env[,c()], perm=999)
```

```
## Warning in max(cumDim[cumDim <= lstats]): no non-missing arguments to max;
## returning -Inf

##
## ***VECTORS

## Warning in cbind(x$arrows, r2 = x$r, 'Pr(>r)' = x$pvals): number of rows of
## result is not a multiple of vector length (arg 3)

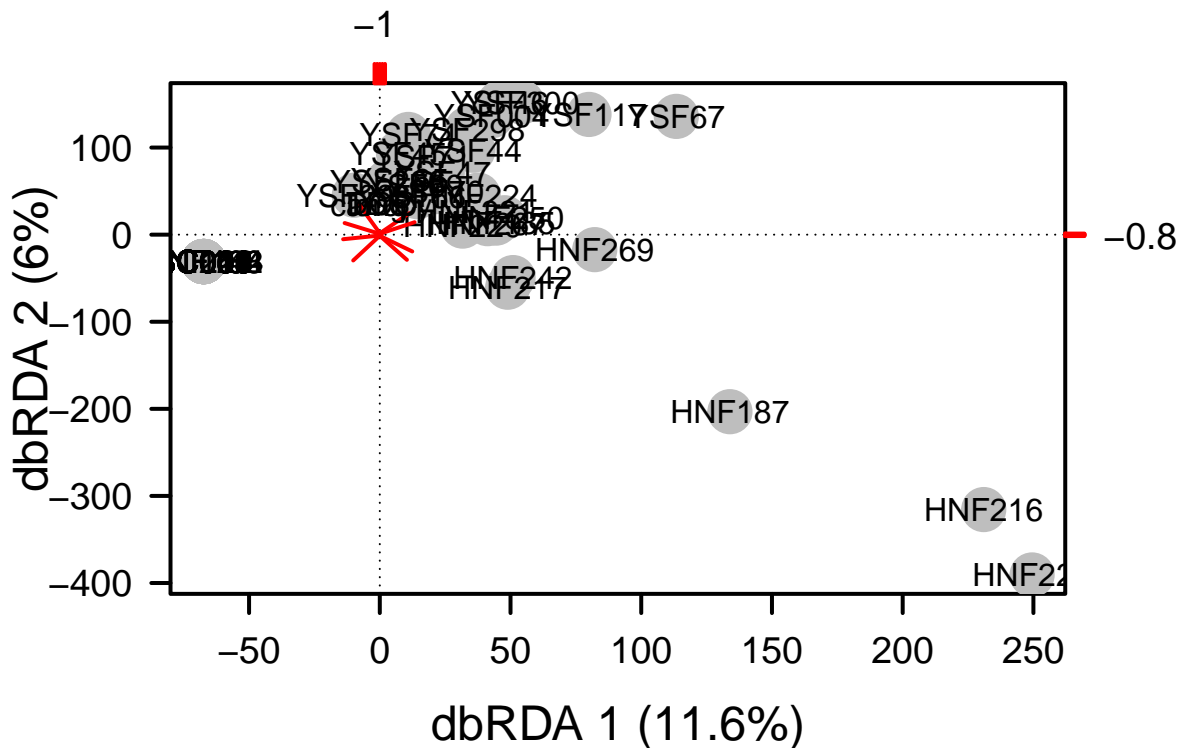
##      dbRDA1 dbRDA2 Pr(>r)
## Permutation: free
## Number of permutations: 999
```

```

#calculate explained variation
pond.dbrda.explainvar1 <- round(pond.dbrda$CCA$eig[1] / sum(c(pond.dbrda$CCA$eig, pond.dbrda$CA$eig)), 2)
pond.dbrda.explainvar2 <- round(pond.dbrda$CCA$eig[2] / sum(c(pond.dbrda$CCA$eig, pond.dbrda$CA$eig)), 2)

# now plot selected model, define plot parameters
par(mar=c(5,5,4,4)+0.1)
pond.vectors <- scores(pond.dbrda,display="bp")
plot(scores(pond.dbrda, display="wa"), xlab=paste("dbRDA 1 (",pond.dbrda.explainvar1,"%)", sep=""),
      ylab=paste("dbRDA 2 (",pond.dbrda.explainvar2,"%)", sep=""),
      pch=16, cex=2.0, type="n", cex.lab=1.5, cex.axis=1.2, axes=FALSE);
axis(side=1, labels=T, lwd.ticks=2, cex.axis=1.2,las=1);
axis(side=2, labels=T, lwd.ticks=2, cex.axis=1.2,las=1);
abline(h=0,v=0,lty=3);
box(lwd=2);
points(scores(pond.dbrda,display="wa"), pch=19,cex=3,bg="gray",col="gray");
text(scores(pond.dbrda,display="wa"),labels=row.names(scores(pond.dbrda,display="wa")));
arrows(0,0,pond.vectors[,1], pond.vectors[,2], lwd=2, lty=1, length=0.2, col="red");
text(pond.vectors[,1], pond.vectors[,2], pos=3,labels=row.names(pond.vectors));
axis(side=3, lwd.ticks=2, cex.axis=1.2,las=1, col="red", lwd=2.2,
      at=pretty(range(pond.vectors[,1]))*2, labels=pretty(range(pond.vectors[,1])));
axis(side=4, lwd.ticks=2, cex.axis=1.2,las=1, col="red", lwd=2.2,
      at=pretty(range(pond.vectors[,2]))*2, labels=pretty(range(pond.vectors[,2])))

```



```

## something isn't quite right about the plot output --- is it just the sizing or other?

## note for Erica to think about -- way to add spec scores like from last week, with a cut off only shown

```

**Synthesis Answer:** According to the Mantel test, 7% of the variation in pond bacterial communities can be explained by association with the ponds' corresponding environmental variables.

This is much lower than what was found with the Doubs river data, but the bacterial OTUs are much more diverse than the fish species in the Doubs. The constrained ordination via dbRDA indicated that the variable “chl<sub>a</sub>” was associated with much of the variation in pond data.