

## 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  $\beta$ -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, 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 12<sup>th</sup>, 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_Yoo/Week4-Beta"
```

```
setwd("/cloud/project/QB2025_Yoo/Week4-Beta")

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 2.6-8
##
## 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

```
# note, please do not print the dataset when submitting

data(doubs)

fish <- doubs$fish
fish <- fish[-8, ] # Remove site 8 from data
```

## 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))

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

```
## Warning in adonis(fish ~ quality, method = "bray", permutations = 999): 'adonis' is deprecated.
## Use 'adonis2' instead.
## See help("Deprecated") and help("vegan-deprecated").

## $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 -1.699514e-17 -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,] 1.4761588
##      [2,] 2.4675141
##      [3,] 0.6451623
##      [4,] 0.7650236
##      [5,] 1.3284091
##      [6,] 0.3076341
##      [7,] 1.3806324
##      [8,] 0.8247590
##      [9,] 0.7641237
##     [10,] 1.8844037
##     [11,] 1.5807052
##     [12,] 0.6970637
##     [13,] 0.6273040
##     [14,] 0.8434048
##     [15,] 0.5804831
##     [16,] 0.9741364
##     [17,] 0.9738177
##     [18,] 0.4985102
##     [19,] 0.5499955
##     [20,] 1.8835808
##     [21,] 0.2287278
##     [22,] 1.6894975
##     [23,] 0.4099389
##     [24,] 0.5953520
##     [25,] 0.6868607
##     [26,] 0.3343245
##     [27,] 1.5089635
##     [28,] 1.1813171
##     [29,] 0.5358747
##     [30,] 1.5735401
##     [31,] 0.5461611
##     [32,] 0.6333158
##     [33,] 1.8483291
##     [34,] 0.4241359
##     [35,] 0.4415152
##     [36,] 0.5901819
##     [37,] 1.9005749
##     [38,] 0.7233381
##     [39,] 1.0101912
##     [40,] 1.1684187
##     [41,] 2.1977197
##     [42,] 0.4909446
##     [43,] 0.3891923
##     [44,] 0.5195919
##     [45,] 1.1048715
##     [46,] 1.1968744
##     [47,] 1.1943797
##     [48,] 0.7461452
##     [49,] 0.5783596

```

```
## [50,] 1.6449056
## [51,] 0.7697395
## [52,] 0.5463022
## [53,] 0.8732485
## [54,] 1.0186639
## [55,] 0.2759220
## [56,] 0.9668639
## [57,] 0.7023212
## [58,] 0.6000380
## [59,] 1.0608281
## [60,] 0.9639787
## [61,] 0.3074323
## [62,] 2.1911707
## [63,] 0.9626915
## [64,] 0.4799573
## [65,] 0.9262604
## [66,] 0.9722664
## [67,] 1.0113759
## [68,] 0.6469764
## [69,] 3.0186529
## [70,] 0.8280114
## [71,] 0.9371029
## [72,] 1.5932240
## [73,] 0.5429493
## [74,] 0.6179566
## [75,] 1.5671204
## [76,] 1.2144450
## [77,] 1.1491227
## [78,] 0.3573355
## [79,] 1.4927830
## [80,] 0.7536381
## [81,] 0.8466844
## [82,] 0.6220739
## [83,] 0.4424634
## [84,] 2.5605129
## [85,] 0.7055076
## [86,] 1.2494658
## [87,] 0.4233083
## [88,] 0.4696615
## [89,] 1.4834714
## [90,] 1.7182931
## [91,] 1.0669801
## [92,] 4.8617862
## [93,] 1.0175892
## [94,] 0.7776235
## [95,] 1.2993630
## [96,] 1.8576285
## [97,] 0.6595078
## [98,] 2.9164649
## [99,] 0.4371143
## [100,] 1.8661001
## [101,] 0.5947483
## [102,] 1.5790089
## [103,] 0.5812240
```

```
## [104,] 0.4634014
## [105,] 0.9056269
## [106,] 0.9438596
## [107,] 0.4925227
## [108,] 0.6332441
## [109,] 0.6668820
## [110,] 1.2378573
## [111,] 0.3833486
## [112,] 0.6307152
## [113,] 0.4806779
## [114,] 1.0552566
## [115,] 0.5130050
## [116,] 0.6526982
## [117,] 0.4614503
## [118,] 1.4923670
## [119,] 0.5507976
## [120,] 0.9024041
## [121,] 0.5410386
## [122,] 1.1339895
## [123,] 0.9664461
## [124,] 0.5551740
## [125,] 1.6169158
## [126,] 0.5819064
## [127,] 1.4256925
## [128,] 0.7715938
## [129,] 0.9212729
## [130,] 0.8902792
## [131,] 1.2133452
## [132,] 0.7608408
## [133,] 0.7746680
## [134,] 1.4492502
## [135,] 0.9314539
## [136,] 0.6224306
## [137,] 1.0174605
## [138,] 0.7298520
## [139,] 1.6952016
## [140,] 1.1030757
## [141,] 0.9211224
## [142,] 0.7579402
## [143,] 0.7498441
## [144,] 0.7521455
## [145,] 4.0064760
## [146,] 0.6410697
## [147,] 0.7563523
## [148,] 0.9210869
## [149,] 1.9660214
## [150,] 0.4037994
## [151,] 0.7368142
## [152,] 0.4298378
## [153,] 1.6821991
## [154,] 1.0479392
## [155,] 0.4149192
## [156,] 0.3838417
## [157,] 0.4734757
```

## [158,] 0.8563159  
## [159,] 0.9242358  
## [160,] 0.5490713  
## [161,] 0.9536860  
## [162,] 0.4843905  
## [163,] 2.4958531  
## [164,] 0.6508472  
## [165,] 1.1775725  
## [166,] 0.3785332  
## [167,] 0.8988843  
## [168,] 0.3929436  
## [169,] 0.5161388  
## [170,] 0.7146199  
## [171,] 0.2568245  
## [172,] 1.0543903  
## [173,] 0.3138933  
## [174,] 0.9536706  
## [175,] 0.7205849  
## [176,] 0.4713999  
## [177,] 1.7858508  
## [178,] 0.6414664  
## [179,] 0.7964002  
## [180,] 0.7673623  
## [181,] 1.6626076  
## [182,] 0.2977715  
## [183,] 1.0304276  
## [184,] 1.3985214  
## [185,] 0.9803390  
## [186,] 1.2080078  
## [187,] 0.2926008  
## [188,] 0.4515020  
## [189,] 0.9302115  
## [190,] 0.3480419  
## [191,] 0.6977753  
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## [201,] 2.2015148  
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## [203,] 2.3026893  
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## [205,] 0.5239861  
## [206,] 1.6768188  
## [207,] 0.4805097  
## [208,] 0.3213491  
## [209,] 1.0098457  
## [210,] 0.7020403  
## [211,] 0.7163528

## [212,] 0.4051157  
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## [265,] 0.2876124



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## [292,] 1.6754891  
## [293,] 0.5293213  
## [294,] 1.3819647  
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## [319,] 1.0335561

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## [328,] 1.1329110  
## [329,] 0.7915504  
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## [331,] 0.2422918  
## [332,] 1.2623306  
## [333,] 0.3363792  
## [334,] 0.3596655  
## [335,] 0.7296588  
## [336,] 0.5106234  
## [337,] 1.6342067  
## [338,] 0.2818412  
## [339,] 1.9250257  
## [340,] 1.1676642  
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## [353,] 0.6197674  
## [354,] 0.5484151  
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## [359,] 1.3331345  
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## [365,] 1.6367975  
## [366,] 0.6830185  
## [367,] 0.2754131  
## [368,] 1.0623311  
## [369,] 1.1457324  
## [370,] 0.6926451  
## [371,] 1.2133724  
## [372,] 2.7993613  
## [373,] 1.6954634

## [374,] 0.9279183  
## [375,] 0.3612498  
## [376,] 1.2753540  
## [377,] 0.6967792  
## [378,] 0.7256190  
## [379,] 0.9272262  
## [380,] 0.6024144  
## [381,] 1.3332424  
## [382,] 0.4284439  
## [383,] 0.9750207  
## [384,] 1.7315647  
## [385,] 1.0376889  
## [386,] 1.8300718  
## [387,] 0.9840959  
## [388,] 0.6524770  
## [389,] 1.1158993  
## [390,] 0.2811468  
## [391,] 0.9443019  
## [392,] 1.5953542  
## [393,] 0.5414628  
## [394,] 0.4358933  
## [395,] 0.9884177  
## [396,] 0.9687170  
## [397,] 0.9033346  
## [398,] 0.5108731  
## [399,] 0.9562963  
## [400,] 0.8436898  
## [401,] 0.9812497  
## [402,] 0.6683254  
## [403,] 0.4372448  
## [404,] 0.5240214  
## [405,] 1.2407631  
## [406,] 1.7677071  
## [407,] 1.0959245  
## [408,] 2.4029611  
## [409,] 0.6179147  
## [410,] 0.7958027  
## [411,] 2.0457860  
## [412,] 0.3435603  
## [413,] 0.7290479  
## [414,] 0.7455127  
## [415,] 1.6837450  
## [416,] 0.6313258  
## [417,] 1.5685231  
## [418,] 0.9723152  
## [419,] 1.9922983  
## [420,] 1.2654294  
## [421,] 2.1123104  
## [422,] 0.2791490  
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## [996,] 0.6545402
## [997,] 0.8427754
## [998,] 1.2080289
## [999,] 1.3174343
##
## $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"

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

# Indicator value
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  0.017 *
##
## Group HQ+MQ #sps. 2
##      stat p.value
## Satr 0.860  0.002 **
## Phph 0.859  0.007 **
##
## 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  0.001 ***
## Lele 0.863  0.002 **
## Titi 0.853  0.006 **
## Chto 0.829  0.001 ***
## Rham 0.829  0.002 **
## Anan 0.829  0.002 **
## Eslu 0.827  0.015 *
## Pefl 0.806  0.016 *
## Blbj 0.791  0.002 **
## Scer 0.766  0.006 **
## Abbr 0.750  0.005 **
## Icme 0.661  0.022 *
## ---
## 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))
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  0.031 *
##
## Group MQ #sps. 4
##      stat p.value
## Anan 0.571  0.010 **
## Spbi 0.557  0.008 **
## Chto 0.542  0.008 **
## Icme 0.475  0.036 *
##
## Group LQ+MQ #sps. 9
##      stat p.value
## Legi 0.658  0.003 **
## Baba 0.645  0.005 **
## Rham 0.600  0.009 **
## Acce 0.594  0.006 **
## Cyca 0.586  0.009 **
## Chna 0.571  0.008 **
## Blbj 0.571  0.009 **
## Gogo 0.523  0.015 *
## Abbr 0.499  0.038 *
## ---
## 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 dendrograms, ordinations) that you created?

**Answer 1:** P-value for testing the model that water quality affects fish diversity using PERMANOVA is 0.001 which means that we can reject the null hypothesis, and water quality affects fish diversity with strong statistical support. Indicator value explains fish species that are related to water quality habitat categories based on incidence data. Higher indicator indicates higher association with the categories. Based on IndVal, Teso is closely associated with MQ habitat, Satr and Phph are closely related with HQ and MQ habitat, and other 20 species are closely related with LQ and MQ habitat. Higher Phi coefficient of association with specific category means that species are associated with specific category based on species fidelity. Based on Phi, Phph, Neba

and Satr are related to HQ habitat, Alal and Ruru are relate to LQ habitat, Anan, Spbi, Chto and Icme are related to MQ habitat, and other 9 species are related to LQ and MQ habitat.

## 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%
## 0.100 0.136 0.171 0.202
## 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:** Mantel statistics is 0.604, and p-value is 0.001. Also, the upper quatiles of permuta-tions is 0.208 at 99th percentile. This suggest that the observed relationship between fish diversity and stream environmental conditions is greater than null hypothesis, so we can reject the null hypothesis that fish diversity and stream environmental conditions are not related.

### 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.

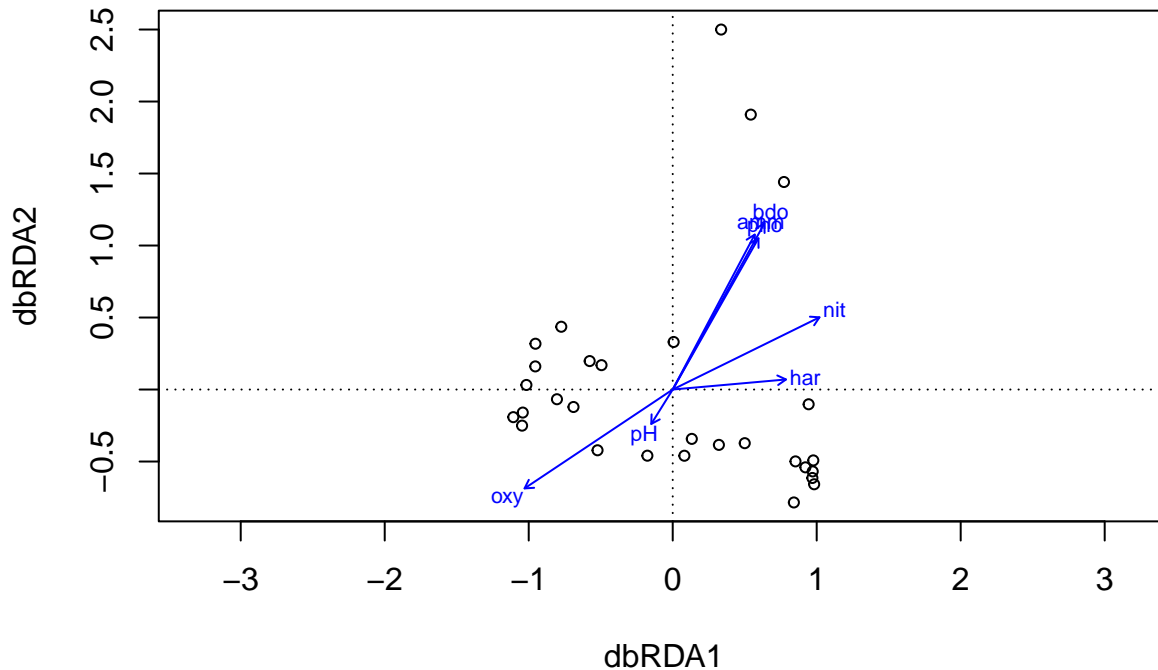
```

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

# Calculate Bray-Curtis
fish.db <- vegdist(fish, method = "bray", diag = TRUE)

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

```



```

# First, we will model only the intercept
doubs.dbrda.mod0 <- dbrda(fish.db ~ 1, as.data.frame(env.chem))

# Note 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)

# Next, we will model the full model, with all explanatory variables
doubs.dbrda.mod1 <- dbrda(fish.db ~ ., as.data.frame(env.chem))

# Now we step through all combinations of explanatory variables in our model
doubs.dbrda <- ordiR2step(doubs.dbrda.mod0, doubs.dbrda.mod1, perm.max = 200)

```

```

## Step: R2.adj= 0
## Call: fish.db ~ 1
##
##               R2.adjusted
## <All variables> 0.53032584
## + oxy          0.27727176
## + nit          0.25755208
## + bdo          0.17477787
## + pho          0.14568614
## + har          0.14174915

```

```

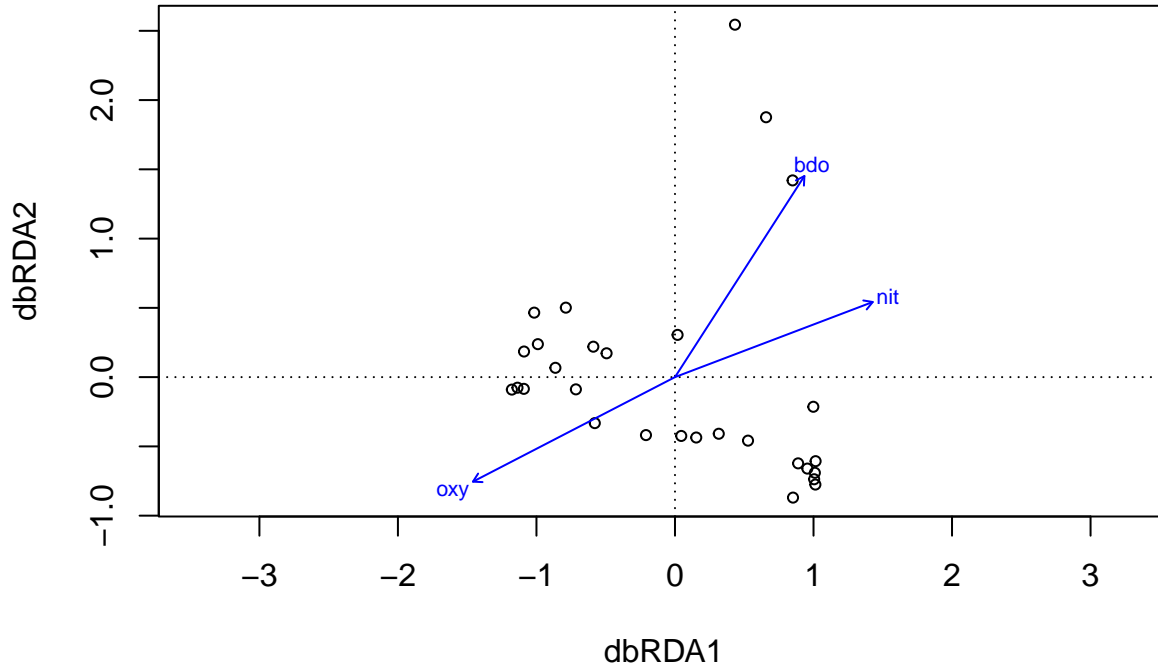
## + amm          0.14142804
## <none>          0.00000000
## + pH           -0.01827054
##
##      Df      AIC      F Pr(>F)
## + oxy  1 47.939 11.742 0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.2772718
## Call: fish.db ~ oxy
##
##      R2.adjusted
## <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.db ~ 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.db ~ oxy + bdo + nit
##
##      R2.adjusted
## + amm          0.5415705
## <All variables> 0.5303258
## + pho          0.5277128
## + har          0.5218852
## <none>         0.4980793
## + pH           0.4843267

```

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

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

```
ordiplot(doubts.dbrda)
```



```
# Permutation tests to evaluate significance
permutest(doubts.dbrda, permutations = 999)
```

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

# Calculate Explained Variation
dbrda.explainvar1 <- round(doubs.dbrda$CCA$eig[1] /
                          sum(doubs.dbrda$CCA$eig), 3) * 100
dbrda.explainvar2 <- round(doubs.dbrda$CCA$eig[2] /
                          sum(doubs.dbrda$CCA$eig), 3) * 100

# Plot
# 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)

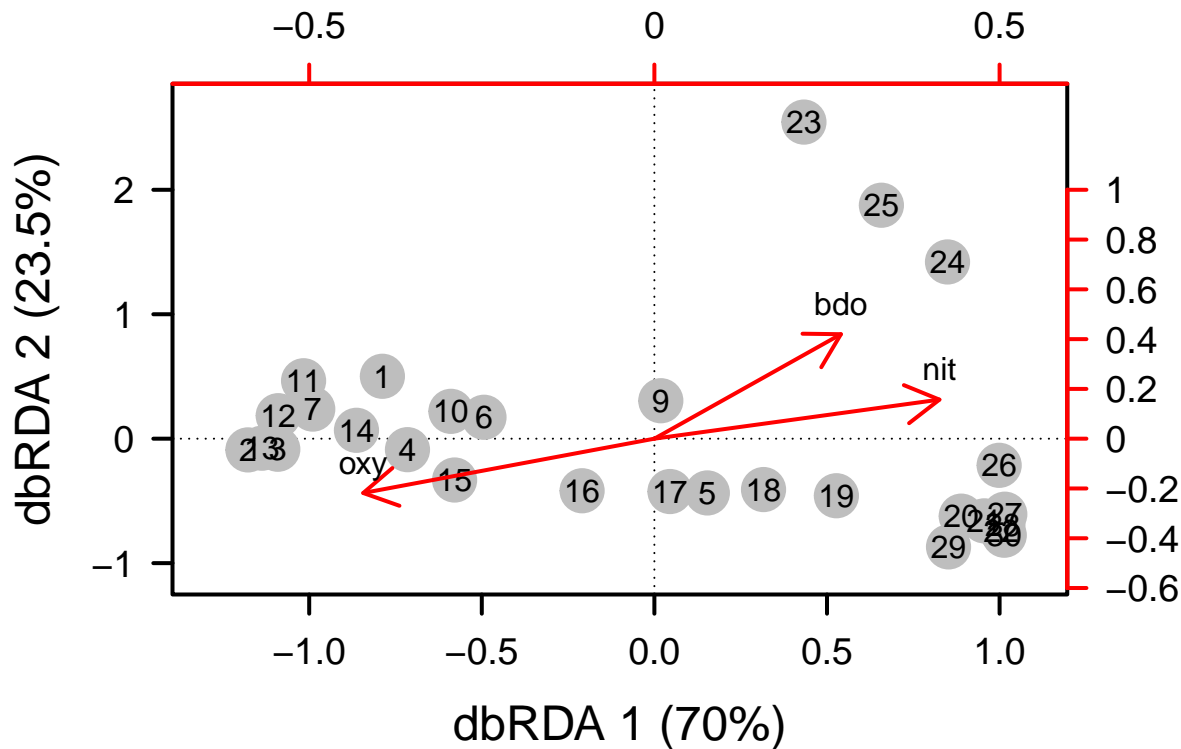
#Add axes
axis(side = 1, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
axis(side = 2, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
abline(h = 0, v = 0, lty = 3)
box(lwd = 2)

# Add Points & 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])))

```



*##doubts*

**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:** Nitrate concentration (nit), dissolved oxygen (oxy), and bdo (biological demand for oxygen) seems contributing to variation in fish community structure. nit and oxy have longer vector than bdo, which means they have stronger effect on fish community structure than bdo. Also, bdo and nit aligns closely with dbRDA1, which means they contribute to variations explained with dbRDA1 strongly. Also, Sites related to higher oxy is related to lower nit and bdo.

### 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*  
doubts.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

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

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

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

# PCNM can return negative eigenvalues, but only the eigenvectors associated with the positive eigenval
doubts.pcnm$values > 0

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

doubts.space <- as.data.frame(scores(doubts.pcnm))
doubts.pcnm.mod0 <- dbrda(fish.db ~ 1, doubts.space)
doubts.pcnm.mod1 <- dbrda(fish.db ~ ., doubts.space)
step.pcnm <- ordiR2step(doubts.pcnm.mod0, doubts.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  0.012 *
## ---
## 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 0.002 **
## ---
## 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 0.016 *
## ---
## 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

```

```

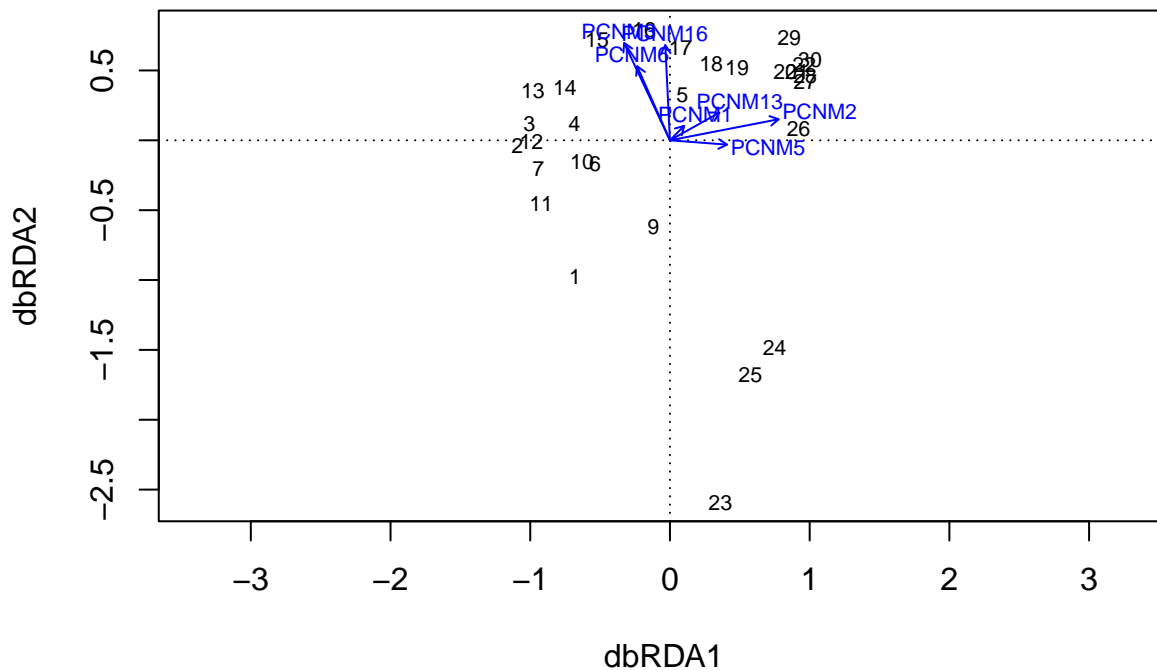
## + PCNM16      0.5767968
## + PCNM15      0.5715331
## + PCNM14      0.5698343
## + PCNM6       0.5475140
## + PCNM7       0.5392074
## + PCNM8       0.5379134
## + PCNM11      0.5281106
## + PCNM9       0.5267003
## + PCNM10      0.5265029
## + PCNM12      0.5255581
## <none>        0.5212427
## + PCNM17      0.5171800
## + PCNM4       0.5152311
##
##           Df    AIC      F Pr(>F)
## + PCNM16  1 36.48 4.0192  0.01 **
## ---
## 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  0.04 *
## ---
## 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  0.078 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

*# Because this is another dbRDA, we could visualize the biplot showing how each vector explains variability*

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



*# 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  0.002 **
## + PCNM5      0.40760 1 43.941 3.8385  0.012 *
## + PCNM1      0.47215 1 41.411 4.0570  0.002 **
## + PCNM13     0.52124 1 39.346 3.4612  0.016 *
## + PCNM16     0.57680 1 36.480 4.0192  0.010 **
## + PCNM6      0.60431 1 35.182 2.5296  0.040 *
## <All variables> 0.62601
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

*# We can now construct a spatial model using only the selected PCNM axes.*

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

*# First conduct constrained ordinations*

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

```

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

# Next construct partial constrained ordinations
doubts.env.cond.space <- dbrda(fish.db ~ env.mod + Condition(space.mod))
doubts.space.cond.env <- dbrda(fish.db ~ space.mod + Condition(env.mod))

# Next test for significance of the dbRDA fractions
permutest(doubts.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  0.002 **
## Residual 18 1.15519
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
permutest(doubts.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 1.8752 4.1741 0.001 ***
## Residual 18 1.1552
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
permutest(doubts.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)

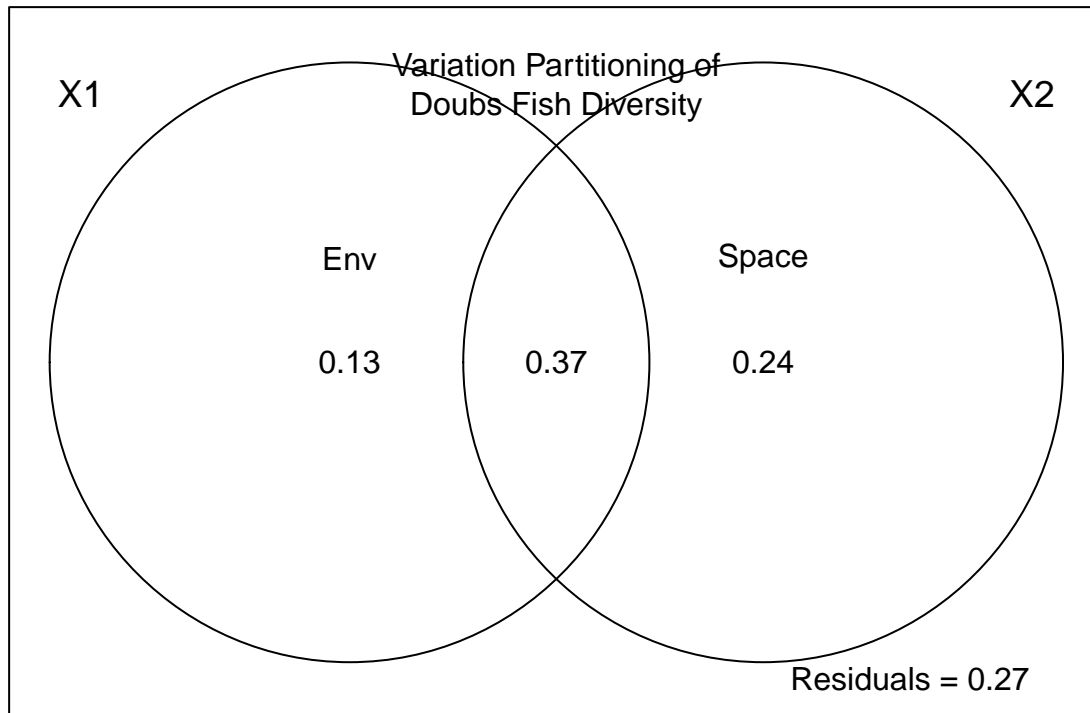
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.db ~ space.mod)
## Permutation test for all constrained eigenvalues
##           Df Inertia      F Pr(>F)
## Model      7  4.7553 7.1089  0.001 ***
## Residual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

# Using the built-in varpart() function
doubs.varpart <- varpart(fish.db, env.mod, space.mod)
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")
mtext("Variation Partitioning of\nDoubs Fish Diversity", side = 3, line = -3)

```



**Question 4:** Interpret the variation partitioning results.

**Answer 4:** There is 37% shared variation between environmental factors and space, which means that 37% of fish community variation is explained by environmental factors aligned with space distribution. Environmental factors alone can explain 13% of the variation, and spatial difference alone can explain 24% of the total variation. Residual is 27%, which means 27% of fish community variation is not explained by environmental factors and space included in the dataset.

## 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.

```
LDW_canopy <- read.csv("/cloud/project/QB2025_Yoo/GroupProject/LDW_canopy.csv")

library(tidyr)
library(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

# Combine 'myc_type' and 'canopy_layer' into a single 'site' column
LDW_canopy$site <- paste(LDW_canopy$myc_type, LDW_canopy$canopy_layer, sep = "_")

# Create a site-by-species matrix
```

```

site_species_matrix <- LDW_canopy %>%
  group_by(site, Latin) %>% # Group by 'site' and 'Latin' (species)
  summarise(count = n(), .groups = 'drop') %>% # Count occurrences of each species in each site
  pivot_wider(names_from = Latin, # Spread species into columns
              values_from = count,
              values_fill = 0) # Replace NA with 0

# View the resulting matrix (row 1-8 = AM1,AM2,AM3,AM4,ECM1,ECM2,ECM3,ECM4)
LDW<-site_species_matrix[,-1]
LDW_env <- LDW
LDW_env$mycor <- c("AM", "AM", "AM", "AM", "ECM", "ECM", "ECM", "ECM")
LDW_env$canopy <- c("a", "b", "c", "d", "a", "b", "c", "d")
env.chem <- as.matrix(LDW_env[,26:27])

# Run PERMANOVA with adonis function (Canopy layer)
adonis2(LDW ~ LDW_env$canopy, method = "bray", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ LDW_env$canopy, permutations = 999, method = "bray")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      3  0.89219 0.30848 0.5948      1
## Residual   4  2.00000 0.69152
## Total      7  2.89219 1.00000

# Indicator value
indval <- multipatt(LDW, cluster = LDW_env$canopy, 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: 25
## Selected number of species: 0
## Number of species associated to 1 group: 0
## Number of species associated to 2 groups: 0
## Number of species associated to 3 groups: 0
##
## List of species associated to each combination:
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Phi coefficient of association
LDW.rel <- decostand(LDW, method = "total")
phi <- multipatt(LDW.rel, cluster = LDW_env$canopy, 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: 25
## Selected number of species: 0
## Number of species associated to 1 group: 0
## Number of species associated to 2 groups: 0
## Number of species associated to 3 groups: 0
##
## List of species associated to each combination:
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

The canopy layer is a categorical value from layer 1-4 (here, a-d), so we had to use multivariate analyses for categorical predictors. P-value for testing the model that canopy layer affects tree diversity using the analysis is 1 probably because the number of rows are too small. We have to decide a way to increase the number of samples by divide 25ha site into small sections and conduct analyses.