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

Joy O'Brien; Z620: Quantitative Biodiversity, Indiana University

08 February, 2023

OVERVIEW

In this worksheet, we continue to explore concepts, statistics, and visualizations related to β-diversity. Now that you know how to formally quantify β-diversity, we will learn how to test hypotheses about β-diversity using multivariate statistics.

Directions:

1. In the Markdown version of this document in your cloned repo, change "Student Name" on line 3 (above) with your name.
2. Complete as much of the worksheet as possible during class.
3. Use the handout as a guide; it contains a more complete description of data sets along with examples of proper scripting needed to carry out the exercises.
4. Answer questions in the worksheet. Space for your answers is provided in this document and is indicated by the ">" character. If you need a second paragraph be sure to start the first line with ">". You should notice that the answer is highlighted in green by RStudio (color may vary if you changed the editor theme).
5. Before you leave the classroom today, it is *imperative* that you **push** this file to your GitHub repo, at whatever stage you are. This will enable you to pull your work onto your own computer.
6. When you have completed the worksheet, **Knit** the text and code into a single PDF file by pressing the `Knit` button in the RStudio scripting panel. This will save the PDF output in your '8.BetaDiversity' folder.
7. After Knitting, please submit the worksheet by making a **push** to your GitHub repo and then create a **pull request** via GitHub. Your pull request should include this file (**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 8th, 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/joyobrien/GitHub/QB2023_OBrien/2.Worksheets/6.BetaDiversity"
```

```
setwd("~/GitHub/QB2023_OBrien/2.Worksheets/6.BetaDiversity")
library(vegan)
```

```
## Loading required package: permute

## Loading required package: lattice

## This is vegan 2.6-4
```

2) LOADING DATA

Load dataset

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

```
# note, pleae do not print the dataset when submitting
library(ade4)
# Loading dataset
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.

```
# create factors vector
data(doubs)

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

fish <- doubs$fish

fish <- fish[-8, ] # Remove site 8 from data because it has no observations

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

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = fish ~ quality, permutations = 999, method = "bray")
##          Df SumOfSqs      R2     F Pr(>F)
## quality   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
```

```r
# Load package for indicator species analysis
library(indicspecies)

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

```
##
##  Multilevel pattern analysis
##  ---------------------------
##
##  Association function: IndVal.g
##  Significance level (alpha): 0.05
##
##  Total number of species: 27
##  Selected number of species: 23
##  Number of species associated to 1 group: 1
##  Number of species associated to 2 groups: 22
##
##  List of species associated to each combination:
##
##  Group MQ  #sps.  1
##       stat p.value
## Teso 0.686   0.029 *
##
##  Group HQ+MQ  #sps.  2
##       stat p.value
## Satr 0.860   0.007 **
## Phph 0.859   0.012 *
##
##  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   0.002 **
## Cyca 0.866   0.001 ***
```

```
## Acce 0.866   0.001 ***
## Lele 0.863   0.003 **
## Titi 0.853   0.001 ***
## Chto 0.829   0.001 ***
## Rham 0.829   0.001 ***
## Anan 0.829   0.001 ***
## Eslu 0.827   0.021 *
## Pefl 0.806   0.011 *
## Blbj 0.791   0.001 ***
## Scer 0.766   0.007 **
## Abbr 0.750   0.002 **
## Icme 0.661   0.017 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# Habitat preferences of species
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   0.002 **
## Ruru 0.473   0.027 *
##
##  Group MQ  #sps.  4
##       stat p.value
## Anan 0.571   0.005 **
## Spbi 0.557   0.008 **
## Chto 0.542   0.012 *
## Icme 0.475   0.030 *
##
##  Group LQ+MQ  #sps.  9
```

```
##       stat p.value
## Legi 0.658   0.003 **
## Baba 0.645   0.002 **
## Rham 0.600   0.003 **
## Acce 0.594   0.007 **
## Cyca 0.586   0.004 **
## Chna 0.571   0.007 **
## Blbj 0.571   0.008 **
## Gogo 0.523   0.012 *
## Abbr 0.499   0.026 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

> ***Answer 1***: The PERMANOVA results indicate that there is a significant amount of variation explained by the quality of water when determining fish species. In other words, habitat quality determines fish species composition in the Doubs river. The IndVal analysis indicates that there are a number of fish species that are significant indicators to determining river quality ("Alal", "Gogo", "Ruru", "Legi", "Baba", "Chna"–just to name a few). The phi coefficient analysis describes which species are associated with which quality of water. We see that "Phph", "Neba", and "Satr" are significantly associated with high quality water, while "Alal" and "Ruru" are assosicated with low quality water. These analyses are consistent with eachother and do agree with the visualizations from the previous worksheet, these outcome of analyses allow us to make these interpretations from the heatmaps, clusters, and ordinations with confidence.

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.

```r
# 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%
## 0.102 0.141 0.184 0.227
## Permutation: free
## Number of permutations: 999
```

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

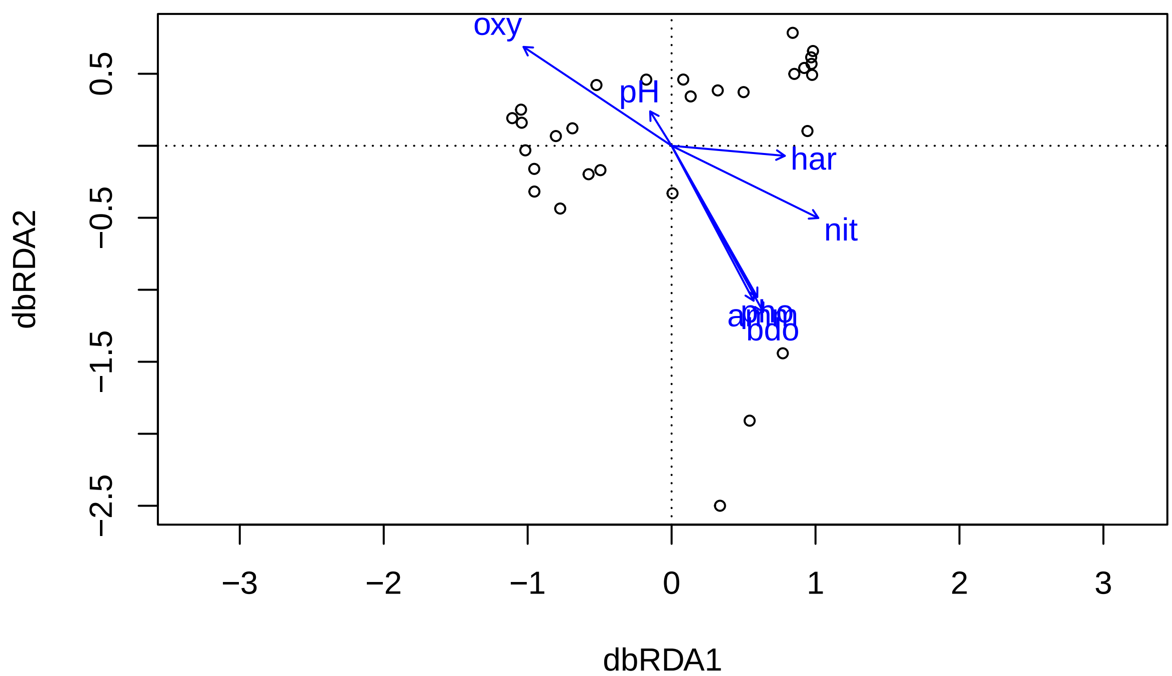
> ***Answer 2***: The mantel test results give an r-statistic of 0.604 and a p-value of 0.001. These results suggest that there is a positive correlation between fish diversity and stream environmental conditions in the Doubs river. Therefore, our hypothesis that fish assemblages are correlated with environmental variables is supported.

ii. Constrained Ordination

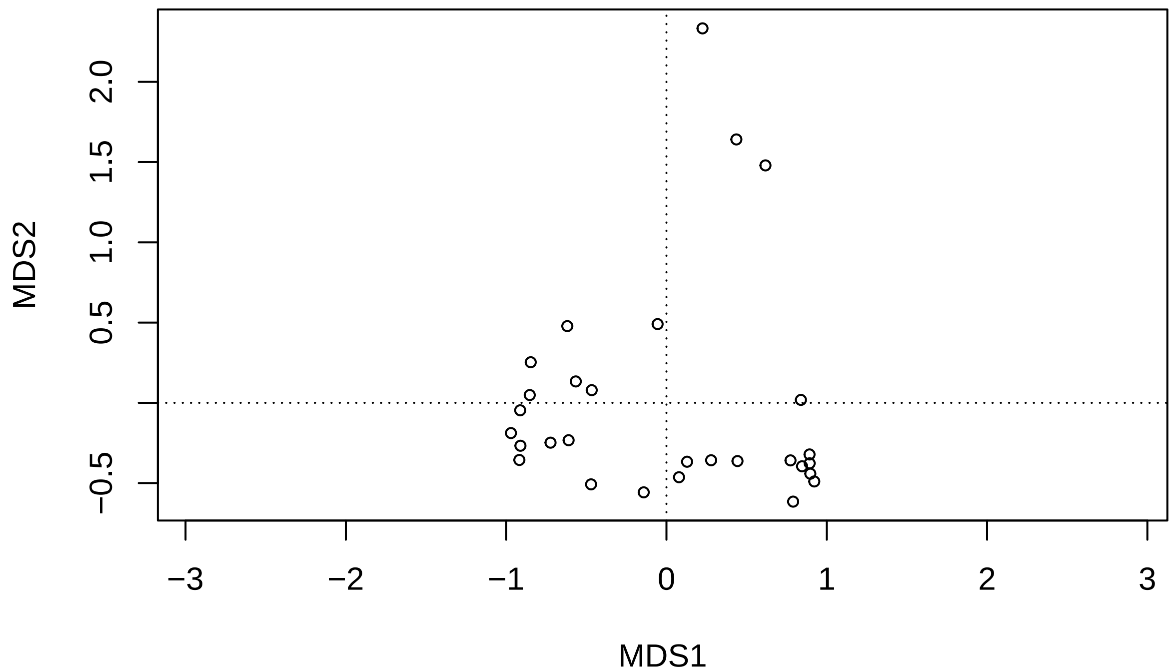
In the R code chunk below, do the following:

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.

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

# Creating fish.db again
fish.db <- vegdist(fish, method = "bray")

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




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

# No vectors here, axes suggest MDS
ordiplot(doubs.dbrda.mod0)
```




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

# Function returns the model with the lowest AIC
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
```

```
# Look at the selected model
doubs.dbrda$call
```

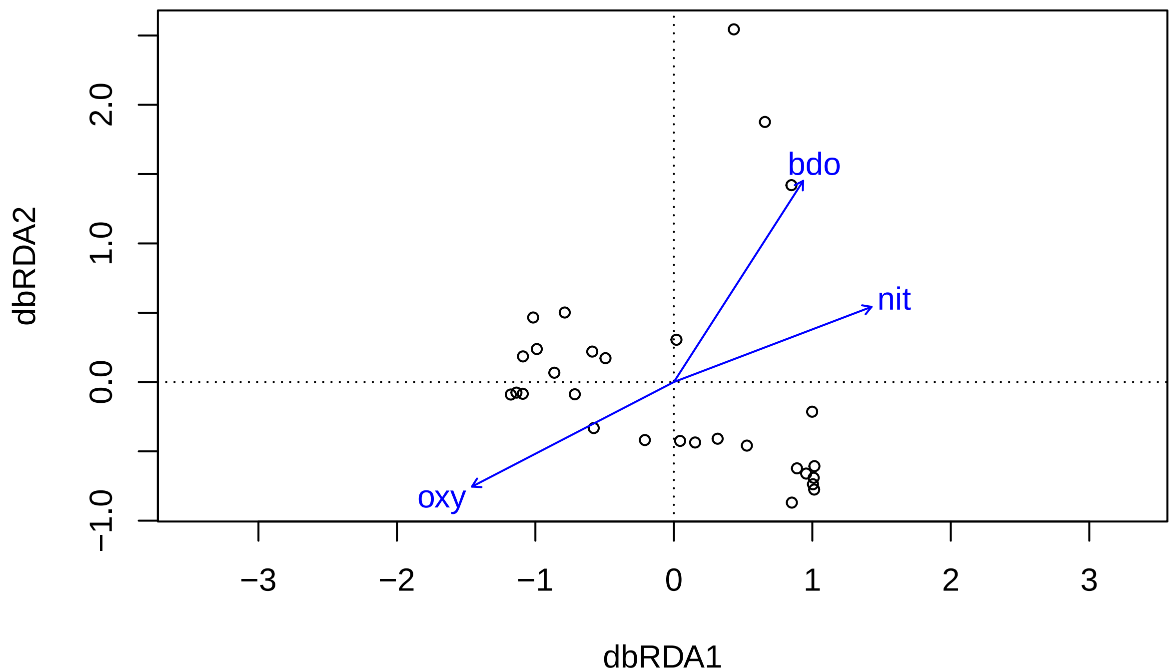
```
## dbrda(formula = fish.db ~ oxy + bdo + nit, data = as.data.frame(env.chem))
```

```
doubs.dbrda$anova
```

```
##                   R2.adj Df    AIC       F Pr(>F)
```

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

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



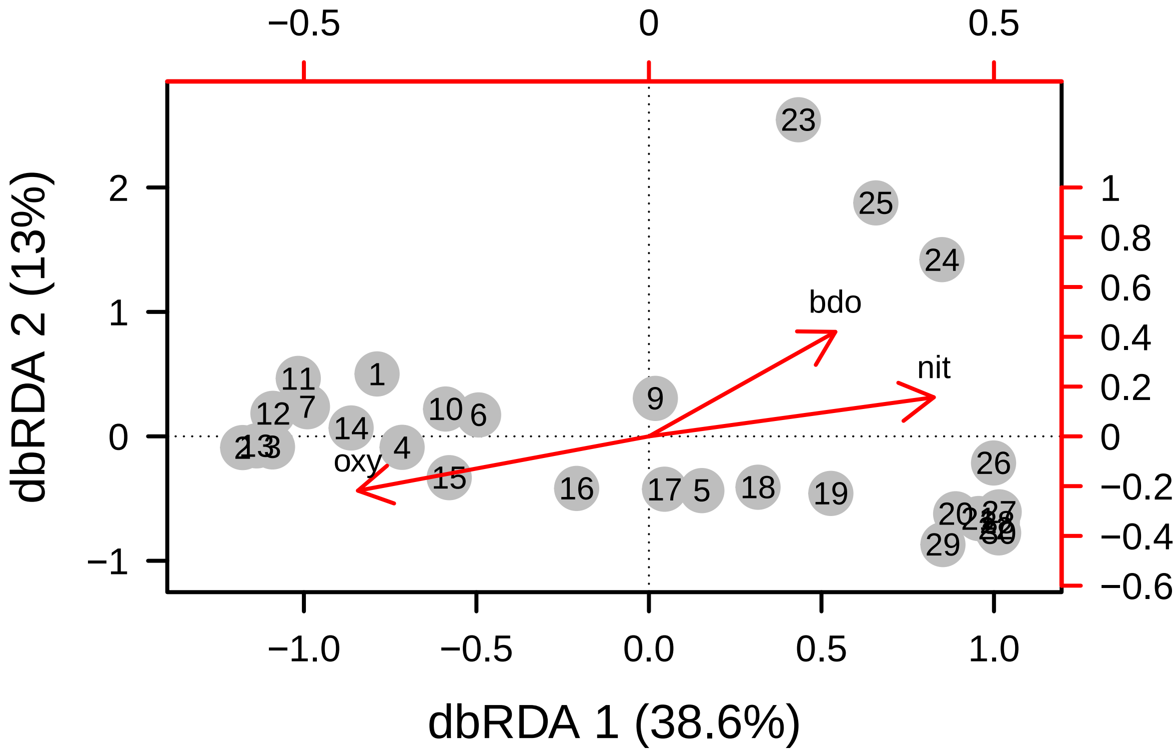

```
# Permutation tests to evaluate significance
permutest(doubs.dbrda, permutations = 999)
```

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

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

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

```r
# Calculate explained variation
dbrda.explainvar1 <- round(doubs.dbrda$CCA$eig[1] /
                             sum(c(doubs.dbrda$CCA$eig, doubs.dbrda$CA$eig)), 3) * 100
dbrda.explainvar2 <- round(doubs.dbrda$CCA$eig[2] /
                             sum(c(doubs.dbrda$CCA$eig, doubs.dbrda$CA$eig)), 3) * 100

# Plot ordination
# Define plot parameters
par(mar = c(5, 5, 4, 4) + 0.1)

# Initiate plot
plot(scores(doubs.dbrda, display = "wa"), xlim = c(-1.3, 1.1),
     ylim = c(-1.1, 2.7), xlab = paste("dbRDA 1 (", dbrda.explainvar1, "%)",
     sep = ""), ylab = paste("dbRDA 2 (", dbrda.explainvar2, "%)", sep = ""),
     pch = 16, cex = 2.0, type = "n", cex.lab = 1.5,
     cex.axis = 1.2, axes = FALSE)
# Add axes
axis(side = 1, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
axis(side = 2, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
abline(h = 0, v = 0, lty = 3)
box(lwd = 2)

# Add points and labels
points(scores(doubs.dbrda, display = "wa"),
       pch = 19, cex = 3, bg = "gray", col = "gray")
text(scores(doubs.dbrda, display = "wa"),
     labels = row.names(scores(doubs.dbrda, display = "wa")))

# Add environmental vectors
vectors <- scores(doubs.dbrda, display = "bp")
#row.names(vectors) <- rownames(vectors)
arrows(0, 0, vectors[,1], vectors[,2],
       lwd = 2, lty = 1, length = 0.2, col = "red")
text(vectors[,1], vectors[, 2], pos = 3,
     labels = row.names(vectors))
axis(side = 3, lwd.ticks = 2, cex.axis = 1.2, las = 1, col = "red", lwd = 2.2,
     at = pretty(range(vectors[, 1])) * 2, labels = pretty(range(vectors[, 1])))
axis(side = 4, lwd.ticks = 2, cex.axis = 1.2, las = 1, col = "red", lwd = 2.2,
     at = pretty(range(vectors[, 2])) * 2, labels = pretty(range(vectors[, 2])))
```



Question 3: Based on the constrained ordination, what are the environmental variables (or groups of correlated variables) that seem to be contributing to variation in fish community structure?

> ***Answer 3***: The environmental variables that are contributing to the variation in fish community structure are "nit", "bdo", and "oxy" which are nitrogen, biological oxygen demand, and dissolved oxygen.

iii. Variation Partitioning

In the code chunk below,

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

```
doubs.dbrda$anova
```

```
##                   R2.adj Df    AIC       F Pr(>F)
## + oxy            0.27727  1 47.939 11.7421  0.002 **
```

```
## + bdo           0.40090  1 43.404  6.5716  0.002 **
## + nit           0.49808  1 39.134  6.0340  0.002 **
## <All variables> 0.53033
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

# Weight each site by rel. abundance
rs <- rowSums(fish)/sum(fish)
# PCNM
doubs.pcnmw <- pcnm(dist(doubs$xy[-8, ]), w = rs, dist.ret = T)
doubs.pcnmw$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
```

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

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

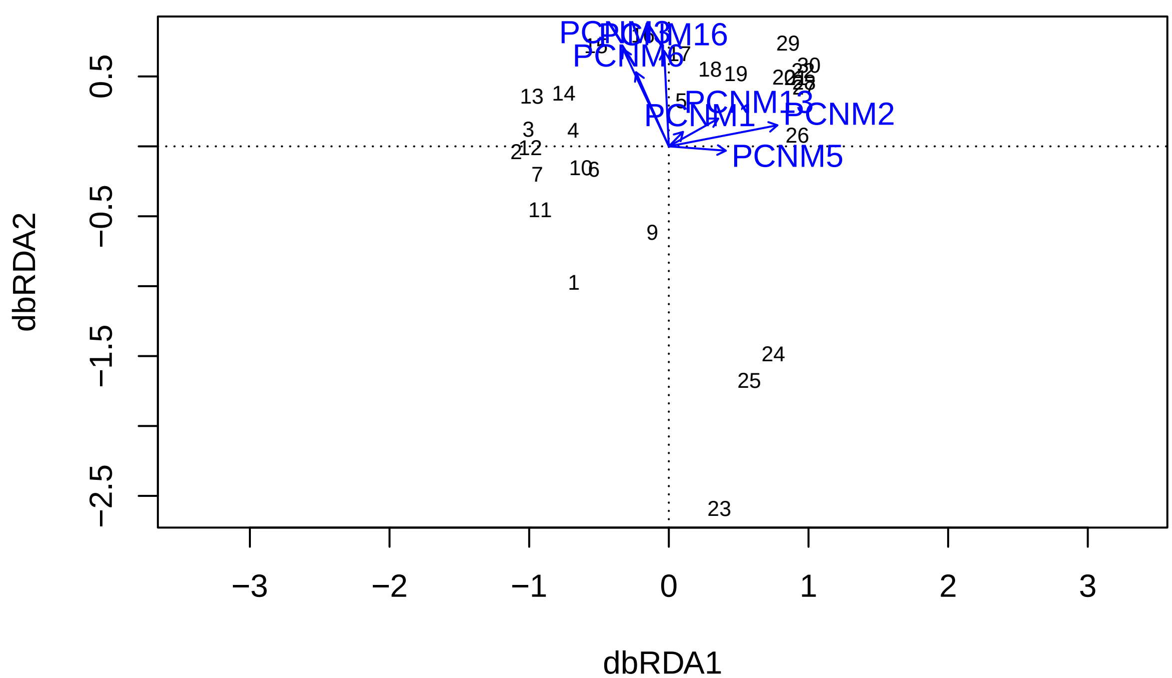
```
## Step: R2.adj= 0.2353704
## Call: fish.db ~ PCNM2
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM3           0.3429270
## + PCNM5           0.3057368
## + PCNM1           0.2885396
## + PCNM16          0.2786746
## + PCNM14          0.2744520
## + PCNM15          0.2692809
## + PCNM6           0.2659866
## + PCNM13          0.2636194
## + PCNM9           0.2517847
## + PCNM8           0.2496240
## + PCNM10          0.2434688
## + PCNM7           0.2431476
## + PCNM17          0.2404343
## + PCNM11          0.2366833
## <none>            0.2353704
## + PCNM12          0.2288789
## + PCNM4           0.2189522
##
##         Df    AIC      F Pr(>F)
## + PCNM3  1 46.083 5.4196  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.342927
## Call: fish.db ~ PCNM2 + PCNM3
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM5           0.4076020
## + PCNM1           0.3970300
## + PCNM16          0.3853210
## + PCNM15          0.3828748
## + PCNM14          0.3781827
## + PCNM13          0.3770376
## + PCNM6           0.3595644
## + PCNM8           0.3556885
## + PCNM7           0.3541631
## + PCNM10          0.3526775
## + PCNM17          0.3513683
## + PCNM9           0.3433672
## <none>            0.3429270
## + PCNM11          0.3416399
## + PCNM12          0.3396547
## + PCNM4           0.3311509
##
##         Df    AIC      F Pr(>F)
## + PCNM5  1 43.941 3.8385  0.012 *
## ---
## 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  0.012 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.4721469
## Call: fish.db ~ PCNM2 + PCNM3 + PCNM5 + PCNM1
##
##                 R2.adjusted
## <All variables>   0.6260113
## + PCNM13          0.5212427
## + PCNM16          0.5208668
## + PCNM15          0.5161770
## + PCNM14          0.5147355
## + PCNM6           0.4999020
## + PCNM7           0.4936559
## + PCNM8           0.4904113
## + PCNM17          0.4856884
## + PCNM10          0.4835952
## + PCNM11          0.4760087
## + PCNM9           0.4751424
## + PCNM12          0.4747221
## <none>            0.4721469
## + PCNM4           0.4651218
##
##          Df    AIC      F Pr(>F)
## + PCNM13  1 39.346 3.4612  0.024 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: R2.adj= 0.5212427
## Call: fish.db ~ PCNM2 + PCNM3 + PCNM5 + PCNM1 + PCNM13
```

```
##
##                  R2.adjusted
## <All variables>   0.6260113
## + PCNM16          0.5767968
## + PCNM15          0.5715331
## + PCNM14          0.5698343
## + PCNM6           0.5475140
## + PCNM7           0.5392074
## + PCNM8           0.5379134
## + PCNM11          0.5281106
## + PCNM9           0.5267003
## + PCNM10          0.5265029
## + PCNM12          0.5255581
## <none>            0.5212427
## + PCNM17          0.5171800
## + PCNM4           0.5152311
##
##          Df   AIC      F Pr(>F)
## + PCNM16  1 36.48 4.0192  0.012 *
## ---
## 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  0.026 *
## ---
## 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  0.066 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# Visualize biplot
plot(step.pcnm)
```




```r
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  0.012 *
## + PCNM13      0.52124  1 39.346 3.4612  0.024 *
## + PCNM16      0.57680  1 36.480 4.0192  0.012 *
```

```
## + PCNM6         0.60431  1 35.182 2.5296  0.026 *
## <All variables> 0.62601
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

# Conduct constrained ordinations
doubs.total.env <- dbrda(fish.db ~ env.mod)
doubs.total.space <- dbrda(fish.db ~ space.mod)

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

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

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

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

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

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

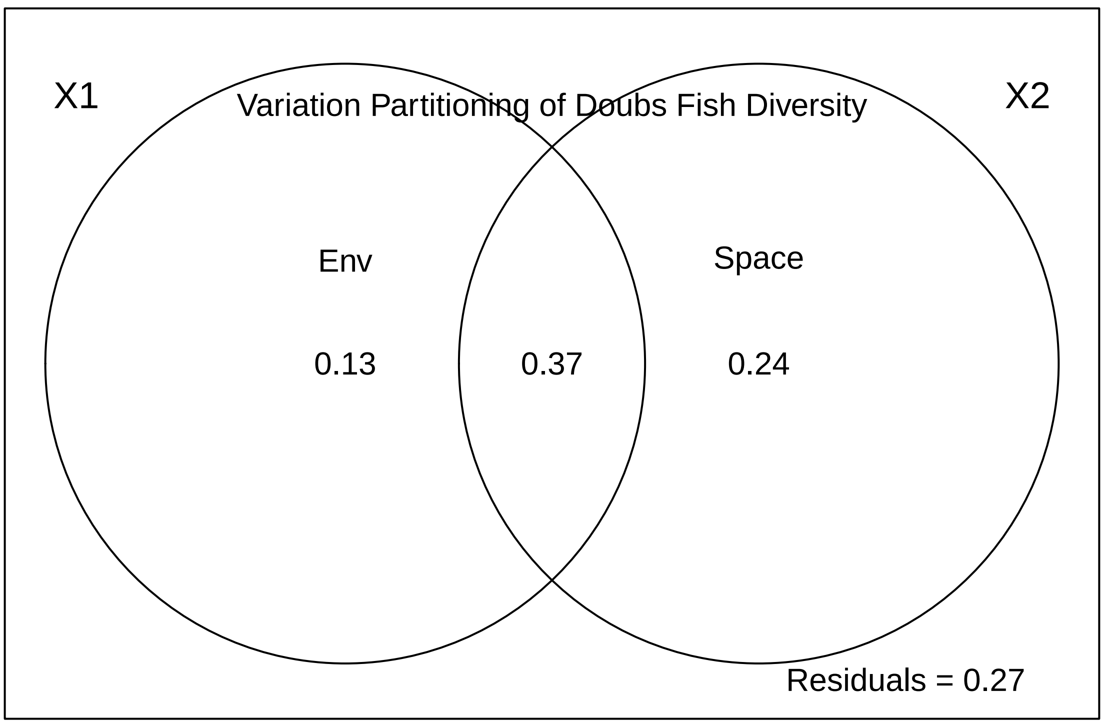
```
##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.db ~ space.mod)
## Permutation test for all constrained eigenvalues
##          Df Inertia      F Pr(>F)
## Model     7  4.7553 7.1089  0.001 ***
## Residual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

```
# 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 Doubs Fish Diversity", side = 3, line = -3)
```



Question 4: Interpret the variation partitioning results.

> ***Answer 4***: Based on the Venn diagram, we see that 13% of the variation within the fish community is due to environmental factors, 24% of the variation is based on space, and 37% of the variation is explained by the environment and space at the same time.

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. > Answer: Based on a permanova between the total microbial community composition and location of the sample, we can say that location explains a significant amount of variation (p-value = 0.003) within the microbial community (both active and total). Additionally, I ran an IndVal analysis (which I am not sure if that was a good idea or not) but there are a number of OTUs that are strongly associated with site. For example, Otu00101 is significantly associated with BCSP, as is Otu00104 with HNF, and Otu00340 with YSF.

```
library(vegan)
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
```

```
library(tidyverse)
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.2 --

## v ggplot2 3.4.0     v purrr   0.3.5
## v tibble  3.1.8     v stringr 1.4.1
## v tidyr   1.2.1     v forcats 0.5.2
## v readr   2.1.3
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
library(ggplot2)


data <- load("/Users/joyobrien/GitHub/team2/INPond_Initial.RData")

DormDecay_env <- readRDS("/Users/joyobrien/GitHub/team2/DormDecay_env.rds")

# DNA
total_matrix <- Pond97[grep('-DNA', rownames(Pond97)),]

# Permanova
adonis <- adonis2(total_matrix ~ DormDecay_env$Location, method = "bray", permutations = 999)
print(adonis)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = total_matrix ~ DormDecay_env$Location, permutations = 999, method = "bray")
```

```
##                          Df SumOfSqs      R2      F Pr(>F)
## DormDecay_env$Location  2   0.9476 0.07876 2.3511  0.003 **
## Residual                55  11.0832 0.92124
## Total                   57  12.0307 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
library(indicspecies)
indval_pond <- multipatt(total_matrix, cluster = DormDecay_env$Location, func = "IndVal.g",
                         control = how(nperm = 999))
summary(indval_pond)
```

```
##
##  Multilevel pattern analysis
##  ---------------------------
##
##  Association function: IndVal.g
##  Significance level (alpha): 0.05
##
##  Total number of species: 34059
##  Selected number of species: 822
##  Number of species associated to 1 group: 659
##  Number of species associated to 2 groups: 163
##
##  List of species associated to each combination:
##
##  Group BCSP  #sps.  198
##          stat p.value
## Otu00101 0.883   0.003 **
## Otu03616 0.770   0.002 **
## Otu03415 0.756   0.002 **
## Otu00891 0.722   0.048 *
## Otu02732 0.701   0.001 ***
## Otu00313 0.701   0.018 *
## Otu02041 0.695   0.006 **
## Otu02463 0.695   0.007 **
## Otu01438 0.662   0.002 **
## Otu07834 0.649   0.003 **
## Otu04418 0.649   0.002 **
## Otu02974 0.646   0.001 ***
## Otu06844 0.645   0.001 ***
## Otu01451 0.625   0.003 **
## Otu02599 0.619   0.005 **
## Otu02911 0.618   0.013 *
## Otu04773 0.603   0.001 ***
## Otu04819 0.592   0.003 **
## Otu13892 0.580   0.040 *
## Otu03364 0.577   0.001 ***
## Otu04417 0.577   0.002 **
## Otu14824 0.577   0.001 ***
## Otu11035 0.574   0.013 *
## Otu03401 0.572   0.002 **
## Otu01893 0.571   0.004 **
## Otu02377 0.569   0.015 *
```

```
## Otu04523 0.561   0.039 *
## Otu00575 0.553   0.015 *
## Otu06097 0.550   0.004 **
## Otu10843 0.548   0.022 *
## Otu03378 0.547   0.007 **
## Otu01946 0.545   0.002 **
## Otu02688 0.543   0.014 *
## Otu02823 0.539   0.005 **
## Otu01026 0.537   0.039 *
## Otu04398 0.532   0.010 **
## Otu10232 0.527   0.003 **
## Otu11163 0.527   0.002 **
## Otu17808 0.527   0.003 **
## Otu54684 0.527   0.005 **
## Otu08593 0.524   0.008 **
## Otu02176 0.522   0.010 **
## Otu04488 0.522   0.006 **
## Otu06513 0.518   0.013 *
## Otu04189 0.517   0.026 *
## Otu02591 0.515   0.019 *
## Otu18369 0.511   0.011 *
## Otu12630 0.509   0.009 **
## Otu03074 0.508   0.048 *
## Otu06098 0.508   0.008 **
## Otu05084 0.506   0.005 **
## Otu50831 0.503   0.013 *
## Otu05682 0.502   0.033 *
## Otu13450 0.501   0.012 *
## Otu03333 0.501   0.015 *
## Otu02959 0.500   0.024 *
## Otu06220 0.500   0.016 *
## Otu05594 0.499   0.012 *
## Otu01108 0.498   0.027 *
## Otu06398 0.495   0.017 *
## Otu05158 0.493   0.017 *
## Otu05674 0.493   0.022 *
## Otu01349 0.486   0.008 **
## Otu03738 0.486   0.017 *
## Otu07705 0.483   0.010 **
## Otu08615 0.480   0.042 *
## Otu04526 0.480   0.030 *
## Otu03185 0.477   0.038 *
## Otu08170 0.476   0.025 *
## Otu02685 0.476   0.045 *
## Otu02513 0.475   0.035 *
## Otu01744 0.471   0.007 **
## Otu03398 0.471   0.016 *
## Otu04320 0.471   0.010 **
## Otu05774 0.471   0.008 **
## Otu05864 0.471   0.013 *
## Otu06957 0.471   0.016 *
## Otu07075 0.471   0.015 *
## Otu07395 0.471   0.011 *
## Otu07863 0.471   0.014 *
```

```
## Otu08012 0.471   0.009 **
## Otu09186 0.471   0.017 *
## Otu09246 0.471   0.009 **
## Otu11026 0.471   0.014 *
## Otu11772 0.471   0.021 *
## Otu14142 0.471   0.012 *
## Otu14612 0.471   0.016 *
## Otu15384 0.471   0.012 *
## Otu18083 0.471   0.011 *
## Otu18292 0.471   0.016 *
## Otu19304 0.471   0.008 **
## Otu20384 0.471   0.015 *
## Otu39134 0.471   0.015 *
## Otu48049 0.471   0.014 *
## Otu53632 0.471   0.010 **
## Otu54467 0.471   0.009 **
## Otu11739 0.469   0.007 **
## Otu41841 0.467   0.018 *
## Otu07319 0.467   0.017 *
## Otu08224 0.464   0.038 *
## Otu03167 0.464   0.030 *
## Otu08532 0.463   0.036 *
## Otu04518 0.461   0.038 *
## Otu05740 0.461   0.038 *
## Otu14781 0.459   0.029 *
## Otu03399 0.458   0.036 *
## Otu03577 0.457   0.024 *
## Otu03213 0.456   0.033 *
## Otu04180 0.456   0.039 *
## Otu07253 0.456   0.032 *
## Otu09178 0.456   0.031 *
## Otu03950 0.452   0.028 *
## Otu14827 0.452   0.027 *
## Otu06890 0.451   0.031 *
## Otu03269 0.451   0.041 *
## Otu06279 0.450   0.030 *
## Otu11698 0.450   0.026 *
## Otu10018 0.448   0.033 *
## Otu22373 0.447   0.034 *
## Otu13019 0.443   0.038 *
## Otu02814 0.441   0.042 *
## Otu08497 0.439   0.048 *
## Otu09501 0.439   0.047 *
## Otu10306 0.439   0.036 *
## Otu13413 0.439   0.045 *
## Otu06021 0.438   0.034 *
## Otu03547 0.437   0.047 *
## Otu10938 0.435   0.048 *
## Otu06051 0.431   0.039 *
## Otu06662 0.431   0.049 *
## Otu07542 0.431   0.043 *
## Otu09171 0.429   0.046 *
## Otu06448 0.428   0.047 *
## Otu10949 0.428   0.050 *
```

```
## Otu15428 0.420   0.049 *
## Otu07990 0.419   0.037 *
## Otu06252 0.414   0.044 *
## Otu05638 0.414   0.046 *
## Otu01736 0.408   0.039 *
## Otu02014 0.408   0.045 *
## Otu03842 0.408   0.042 *
## Otu05211 0.408   0.046 *
## Otu05739 0.408   0.039 *
## Otu05917 0.408   0.044 *
## Otu06205 0.408   0.045 *
## Otu06576 0.408   0.039 *
## Otu07524 0.408   0.048 *
## Otu07742 0.408   0.048 *
## Otu07828 0.408   0.047 *
## Otu08429 0.408   0.049 *
## Otu08645 0.408   0.048 *
## Otu08850 0.408   0.049 *
## Otu09722 0.408   0.043 *
## Otu09854 0.408   0.040 *
## Otu10021 0.408   0.047 *
## Otu10626 0.408   0.048 *
## Otu10630 0.408   0.043 *
## Otu10682 0.408   0.050 *
## Otu10918 0.408   0.035 *
## Otu10931 0.408   0.050 *
## Otu11111 0.408   0.036 *
## Otu11203 0.408   0.043 *
## Otu11571 0.408   0.039 *
## Otu11587 0.408   0.039 *
## Otu12479 0.408   0.050 *
## Otu13134 0.408   0.037 *
## Otu13499 0.408   0.048 *
## Otu13600 0.408   0.044 *
## Otu13610 0.408   0.042 *
## Otu13909 0.408   0.043 *
## Otu14569 0.408   0.044 *
## Otu14743 0.408   0.039 *
## Otu16790 0.408   0.035 *
## Otu17171 0.408   0.042 *
## Otu17390 0.408   0.043 *
## Otu17752 0.408   0.046 *
## Otu18543 0.408   0.044 *
## Otu19178 0.408   0.047 *
## Otu19336 0.408   0.050 *
## Otu20058 0.408   0.048 *
## Otu20116 0.408   0.044 *
## Otu20379 0.408   0.049 *
## Otu20527 0.408   0.042 *
## Otu22263 0.408   0.045 *
## Otu22361 0.408   0.050 *
## Otu23055 0.408   0.044 *
## Otu41100 0.408   0.042 *
## Otu41854 0.408   0.047 *
```

```
## Otu48260 0.408   0.050 *
## Otu48502 0.408   0.049 *
## Otu49051 0.408   0.047 *
## Otu50432 0.408   0.047 *
## Otu50598 0.408   0.038 *
## Otu53671 0.408   0.044 *
## Otu55080 0.408   0.050 *
## Otu55464 0.408   0.050 *
## Otu04439 0.405   0.047 *
## Otu11484 0.393   0.048 *
##
##  Group HNF  #sps.  123
##           stat p.value
## Otu00104 0.915   0.023 *
## Otu00458 0.851   0.048 *
## Otu00318 0.844   0.002 **
## Otu01259 0.763   0.002 **
## Otu00978 0.760   0.002 **
## Otu00302 0.747   0.018 *
## Otu00348 0.744   0.008 **
## Otu00418 0.738   0.021 *
## Otu01130 0.735   0.005 **
## Otu00956 0.724   0.001 ***
## Otu01832 0.709   0.025 *
## Otu00400 0.709   0.029 *
## Otu00413 0.705   0.038 *
## Otu01312 0.691   0.021 *
## Otu00599 0.688   0.001 ***
## Otu00602 0.661   0.047 *
## Otu03788 0.645   0.004 **
## Otu01865 0.636   0.019 *
## Otu01792 0.615   0.025 *
## Otu01906 0.588   0.028 *
## Otu05351 0.587   0.002 **
## Otu03008 0.579   0.015 *
## Otu02372 0.567   0.036 *
## Otu02725 0.557   0.027 *
## Otu03558 0.555   0.014 *
## Otu03344 0.553   0.024 *
## Otu10326 0.552   0.001 ***
## Otu04139 0.550   0.009 **
## Otu00991 0.549   0.008 **
## Otu04548 0.543   0.025 *
## Otu03551 0.541   0.045 *
## Otu06506 0.533   0.008 **
## Otu02932 0.530   0.007 **
## Otu05448 0.521   0.008 **
## Otu03552 0.519   0.036 *
## Otu00963 0.511   0.010 **
## Otu03354 0.511   0.011 *
## Otu03775 0.511   0.013 *
## Otu09275 0.511   0.012 *
## Otu10440 0.511   0.014 *
## Otu13608 0.511   0.008 **
```

```
## Otu13873 0.511   0.018 *
## Otu15226 0.511   0.016 *
## Otu16495 0.511   0.009 **
## Otu07211 0.510   0.043 *
## Otu07196 0.507   0.022 *
## Otu11377 0.497   0.024 *
## Otu04276 0.495   0.025 *
## Otu09989 0.489   0.025 *
## Otu08865 0.488   0.036 *
## Otu10450 0.483   0.029 *
## Otu02069 0.475   0.044 *
## Otu05200 0.472   0.032 *
## Otu02803 0.466   0.029 *
## Otu03266 0.466   0.021 *
## Otu04436 0.466   0.031 *
## Otu05498 0.466   0.022 *
## Otu07122 0.466   0.010 **
## Otu07356 0.466   0.033 *
## Otu07971 0.466   0.011 *
## Otu10174 0.466   0.006 **
## Otu12409 0.466   0.027 *
## Otu13914 0.466   0.019 *
## Otu17230 0.466   0.017 *
## Otu50318 0.466   0.023 *
## Otu02575 0.465   0.042 *
## Otu21162 0.464   0.039 *
## Otu05780 0.461   0.046 *
## Otu09136 0.454   0.050 *
## Otu06459 0.454   0.020 *
## Otu10936 0.453   0.046 *
## Otu05451 0.450   0.043 *
## Otu23312 0.447   0.031 *
## Otu03275 0.444   0.037 *
## Otu07920 0.444   0.036 *
## Otu04877 0.443   0.042 *
## Otu21500 0.441   0.045 *
## Otu04259 0.435   0.046 *
## Otu02310 0.417   0.033 *
## Otu03003 0.417   0.039 *
## Otu03355 0.417   0.039 *
## Otu04328 0.417   0.032 *
## Otu04632 0.417   0.041 *
## Otu05090 0.417   0.043 *
## Otu05550 0.417   0.036 *
## Otu07293 0.417   0.041 *
## Otu07661 0.417   0.028 *
## Otu07956 0.417   0.026 *
## Otu08075 0.417   0.038 *
## Otu08157 0.417   0.033 *
## Otu08525 0.417   0.033 *
## Otu08620 0.417   0.024 *
## Otu08630 0.417   0.030 *
## Otu08972 0.417   0.026 *
## Otu09383 0.417   0.029 *
```

```
## Otu09911 0.417   0.038 *
## Otu10526 0.417   0.037 *
## Otu10565 0.417   0.032 *
## Otu11098 0.417   0.031 *
## Otu11422 0.417   0.026 *
## Otu12025 0.417   0.039 *
## Otu12487 0.417   0.034 *
## Otu12794 0.417   0.032 *
## Otu13210 0.417   0.023 *
## Otu13248 0.417   0.038 *
## Otu13673 0.417   0.029 *
## Otu15058 0.417   0.039 *
## Otu17339 0.417   0.031 *
## Otu18056 0.417   0.037 *
## Otu18129 0.417   0.035 *
## Otu18608 0.417   0.037 *
## Otu19285 0.417   0.026 *
## Otu19905 0.417   0.030 *
## Otu20678 0.417   0.041 *
## Otu23624 0.417   0.032 *
## Otu39800 0.417   0.025 *
## Otu46796 0.417   0.046 *
## Otu48429 0.417   0.037 *
## Otu49086 0.417   0.029 *
## Otu49335 0.417   0.036 *
## Otu49509 0.417   0.041 *
## Otu50905 0.417   0.036 *
## Otu07801 0.416   0.038 *
##
##  Group YSF  #sps.  338
##          stat p.value
## Otu00340 0.935   0.001 ***
## Otu00289 0.858   0.001 ***
## Otu00233 0.800   0.010 **
## Otu00495 0.728   0.001 ***
## Otu00662 0.717   0.002 **
## Otu00538 0.693   0.002 **
## Otu02759 0.686   0.001 ***
## Otu00929 0.676   0.007 **
## Otu03609 0.673   0.015 *
## Otu05275 0.667   0.001 ***
## Otu01232 0.666   0.008 **
## Otu03128 0.647   0.017 *
## Otu01887 0.640   0.002 **
## Otu02367 0.634   0.008 **
## Otu02501 0.628   0.002 **
## Otu03654 0.627   0.002 **
## Otu17380 0.626   0.002 **
## Otu02216 0.625   0.002 **
## Otu12157 0.622   0.008 **
## Otu01335 0.620   0.002 **
## Otu01947 0.615   0.004 **
## Otu01417 0.610   0.005 **
## Otu01236 0.604   0.013 *
```

```
## Otu01129 0.600   0.007 **
## Otu08857 0.594   0.001 ***
## Otu10164 0.594   0.001 ***
## Otu01809 0.593   0.010 **
## Otu00834 0.589   0.001 ***
## Otu00678 0.586   0.002 **
## Otu01142 0.581   0.016 *
## Otu02461 0.580   0.006 **
## Otu01193 0.576   0.001 ***
## Otu00864 0.575   0.032 *
## Otu00854 0.571   0.001 ***
## Otu04291 0.570   0.018 *
## Otu17861 0.568   0.002 **
## Otu01822 0.567   0.024 *
## Otu05926 0.563   0.003 **
## Otu03040 0.561   0.003 **
## Otu03403 0.560   0.007 **
## Otu04596 0.558   0.011 *
## Otu06619 0.552   0.013 *
## Otu03514 0.552   0.005 **
## Otu07224 0.551   0.010 **
## Otu08136 0.551   0.007 **
## Otu02597 0.550   0.049 *
## Otu01322 0.548   0.014 *
## Otu08584 0.547   0.012 *
## Otu04099 0.542   0.002 **
## Otu06921 0.542   0.002 **
## Otu08313 0.542   0.001 ***
## Otu19761 0.542   0.002 **
## Otu02332 0.537   0.019 *
## Otu03024 0.537   0.010 **
## Otu03736 0.534   0.017 *
## Otu05057 0.533   0.013 *
## Otu06795 0.529   0.006 **
## Otu12817 0.529   0.007 **
## Otu07768 0.527   0.018 *
## Otu01350 0.525   0.004 **
## Otu02729 0.517   0.037 *
## Otu03242 0.516   0.005 **
## Otu18652 0.516   0.006 **
## Otu01656 0.514   0.034 *
## Otu01515 0.514   0.023 *
## Otu04973 0.514   0.021 *
## Otu12609 0.512   0.024 *
## Otu02428 0.506   0.038 *
## Otu02696 0.497   0.009 **
## Otu05425 0.495   0.024 *
## Otu05077 0.495   0.015 *
## Otu02578 0.493   0.010 **
## Otu03713 0.492   0.038 *
## Otu01143 0.489   0.038 *
## Otu05329 0.487   0.025 *
## Otu10891 0.486   0.009 **
## Otu03009 0.485   0.006 **
```

```
## Otu05316 0.485   0.006 **
## Otu06598 0.485   0.006 **
## Otu07019 0.485   0.004 **
## Otu07517 0.485   0.007 **
## Otu07867 0.485   0.006 **
## Otu08008 0.485   0.005 **
## Otu08031 0.485   0.007 **
## Otu08351 0.485   0.004 **
## Otu08790 0.485   0.007 **
## Otu08957 0.485   0.011 *
## Otu09053 0.485   0.011 *
## Otu12057 0.485   0.003 **
## Otu15976 0.485   0.007 **
## Otu19008 0.485   0.007 **
## Otu21438 0.485   0.006 **
## Otu43192 0.485   0.003 **
## Otu04297 0.484   0.014 *
## Otu03662 0.483   0.036 *
## Otu04950 0.483   0.032 *
## Otu04715 0.482   0.019 *
## Otu06331 0.481   0.016 *
## Otu26779 0.481   0.012 *
## Otu26730 0.480   0.010 **
## Otu03281 0.479   0.046 *
## Otu09793 0.479   0.022 *
## Otu02951 0.479   0.023 *
## Otu05000 0.479   0.034 *
## Otu08665 0.478   0.033 *
## Otu16793 0.476   0.007 **
## Otu03340 0.476   0.048 *
## Otu21399 0.475   0.009 **
## Otu03927 0.475   0.044 *
## Otu12635 0.474   0.009 **
## Otu19174 0.473   0.025 *
## Otu03690 0.471   0.021 *
## Otu03114 0.470   0.015 *
## Otu05305 0.470   0.013 *
## Otu04049 0.469   0.007 **
## Otu02768 0.466   0.012 *
## Otu09130 0.466   0.003 **
## Otu03425 0.466   0.025 *
## Otu05454 0.465   0.017 *
## Otu06557 0.465   0.026 *
## Otu07897 0.464   0.018 *
## Otu06038 0.462   0.033 *
## Otu03541 0.461   0.020 *
## Otu02900 0.461   0.023 *
## Otu05187 0.461   0.023 *
## Otu07568 0.460   0.034 *
## Otu12283 0.459   0.028 *
## Otu05132 0.458   0.031 *
## Otu15774 0.458   0.024 *
## Otu05907 0.458   0.012 *
## Otu02755 0.456   0.029 *
```

```
## Otu03803 0.456   0.031 *
## Otu02701 0.455   0.046 *
## Otu02588 0.455   0.022 *
## Otu03246 0.455   0.028 *
## Otu14924 0.455   0.019 *
## Otu02733 0.454   0.027 *
## Otu03168 0.453   0.045 *
## Otu02544 0.453   0.023 *
## Otu04522 0.453   0.023 *
## Otu02658 0.452   0.038 *
## Otu02965 0.451   0.027 *
## Otu09424 0.451   0.027 *
## Otu02817 0.450   0.027 *
## Otu05719 0.450   0.028 *
## Otu04865 0.449   0.036 *
## Otu07244 0.448   0.050 *
## Otu03872 0.447   0.014 *
## Otu04290 0.446   0.022 *
## Otu04316 0.446   0.031 *
## Otu05194 0.446   0.022 *
## Otu05480 0.446   0.022 *
## Otu06718 0.446   0.020 *
## Otu09287 0.446   0.024 *
## Otu12629 0.446   0.016 *
## Otu05627 0.445   0.027 *
## Otu12102 0.445   0.028 *
## Otu11394 0.445   0.050 *
## Otu06847 0.445   0.037 *
## Otu11205 0.445   0.017 *
## Otu01243 0.443   0.029 *
## Otu07102 0.441   0.024 *
## Otu02405 0.441   0.022 *
## Otu03353 0.441   0.028 *
## Otu02358 0.437   0.047 *
## Otu02704 0.436   0.025 *
## Otu03613 0.436   0.029 *
## Otu04476 0.436   0.037 *
## Otu04713 0.436   0.033 *
## Otu04399 0.436   0.033 *
## Otu06284 0.436   0.031 *
## Otu03405 0.435   0.034 *
## Otu05974 0.434   0.042 *
## Otu06181 0.434   0.025 *
## Otu09295 0.434   0.042 *
## Otu10381 0.434   0.049 *
## Otu18952 0.434   0.042 *
## Otu05716 0.430   0.037 *
## Otu08780 0.430   0.041 *
## Otu06188 0.429   0.043 *
## Otu13624 0.429   0.034 *
## Otu10511 0.429   0.042 *
## Otu03117 0.423   0.045 *
## Otu06589 0.423   0.040 *
## Otu12112 0.423   0.048 *
```

```
## Otu00441 0.420   0.029 *
## Otu01379 0.420   0.023 *
## Otu02762 0.420   0.025 *
## Otu03301 0.420   0.021 *
## Otu03331 0.420   0.026 *
## Otu03476 0.420   0.023 *
## Otu04172 0.420   0.027 *
## Otu04321 0.420   0.018 *
## Otu04355 0.420   0.023 *
## Otu04622 0.420   0.023 *
## Otu04991 0.420   0.016 *
## Otu05009 0.420   0.024 *
## Otu05065 0.420   0.031 *
## Otu05079 0.420   0.028 *
## Otu05326 0.420   0.024 *
## Otu05685 0.420   0.025 *
## Otu06006 0.420   0.021 *
## Otu06043 0.420   0.026 *
## Otu06072 0.420   0.027 *
## Otu06227 0.420   0.015 *
## Otu06394 0.420   0.021 *
## Otu06465 0.420   0.024 *
## Otu06882 0.420   0.023 *
## Otu06972 0.420   0.024 *
## Otu07130 0.420   0.023 *
## Otu07206 0.420   0.022 *
## Otu07247 0.420   0.024 *
## Otu07375 0.420   0.031 *
## Otu07426 0.420   0.022 *
## Otu07533 0.420   0.028 *
## Otu07576 0.420   0.022 *
## Otu07812 0.420   0.022 *
## Otu07928 0.420   0.021 *
## Otu08119 0.420   0.024 *
## Otu08226 0.420   0.023 *
## Otu08243 0.420   0.015 *
## Otu08765 0.420   0.016 *
## Otu09057 0.420   0.031 *
## Otu09338 0.420   0.027 *
## Otu09556 0.420   0.023 *
## Otu09561 0.420   0.020 *
## Otu09619 0.420   0.023 *
## Otu09683 0.420   0.020 *
## Otu09707 0.420   0.022 *
## Otu09723 0.420   0.023 *
## Otu09775 0.420   0.022 *
## Otu09851 0.420   0.031 *
## Otu09951 0.420   0.023 *
## Otu10162 0.420   0.029 *
## Otu10180 0.420   0.024 *
## Otu10187 0.420   0.022 *
## Otu10193 0.420   0.021 *
## Otu10323 0.420   0.031 *
## Otu10508 0.420   0.016 *
```

```
## Otu10561 0.420   0.023 *
## Otu10669 0.420   0.033 *
## Otu10704 0.420   0.023 *
## Otu10778 0.420   0.019 *
## Otu10786 0.420   0.032 *
## Otu10958 0.420   0.024 *
## Otu10962 0.420   0.021 *
## Otu10983 0.420   0.020 *
## Otu10992 0.420   0.023 *
## Otu11003 0.420   0.011 *
## Otu11225 0.420   0.015 *
## Otu11229 0.420   0.023 *
## Otu11382 0.420   0.022 *
## Otu11448 0.420   0.027 *
## Otu11574 0.420   0.024 *
## Otu11702 0.420   0.024 *
## Otu11784 0.420   0.025 *
## Otu12163 0.420   0.028 *
## Otu12277 0.420   0.022 *
## Otu12307 0.420   0.019 *
## Otu12445 0.420   0.024 *
## Otu12543 0.420   0.024 *
## Otu12647 0.420   0.017 *
## Otu12721 0.420   0.024 *
## Otu12770 0.420   0.024 *
## Otu12825 0.420   0.025 *
## Otu12962 0.420   0.024 *
## Otu13126 0.420   0.019 *
## Otu13273 0.420   0.022 *
## Otu13462 0.420   0.033 *
## Otu13481 0.420   0.023 *
## Otu13552 0.420   0.019 *
## Otu13778 0.420   0.023 *
## Otu13937 0.420   0.025 *
## Otu14080 0.420   0.029 *
## Otu14199 0.420   0.025 *
## Otu14287 0.420   0.023 *
## Otu14291 0.420   0.023 *
## Otu14328 0.420   0.025 *
## Otu14412 0.420   0.024 *
## Otu14436 0.420   0.023 *
## Otu14498 0.420   0.018 *
## Otu14656 0.420   0.016 *
## Otu14952 0.420   0.018 *
## Otu15129 0.420   0.024 *
## Otu17848 0.420   0.027 *
## Otu17888 0.420   0.024 *
## Otu17977 0.420   0.025 *
## Otu18097 0.420   0.021 *
## Otu18229 0.420   0.031 *
## Otu18237 0.420   0.021 *
## Otu18355 0.420   0.026 *
## Otu18465 0.420   0.020 *
## Otu18510 0.420   0.020 *
```

```
## Otu18595 0.420   0.029 *
## Otu18772 0.420   0.023 *
## Otu19357 0.420   0.020 *
## Otu19414 0.420   0.031 *
## Otu19437 0.420   0.023 *
## Otu19491 0.420   0.023 *
## Otu19512 0.420   0.020 *
## Otu19725 0.420   0.013 *
## Otu20242 0.420   0.026 *
## Otu20320 0.420   0.021 *
## Otu22057 0.420   0.027 *
## Otu22101 0.420   0.021 *
## Otu22285 0.420   0.016 *
## Otu23304 0.420   0.022 *
## Otu23539 0.420   0.019 *
## Otu24748 0.420   0.031 *
## Otu25114 0.420   0.026 *
## Otu25928 0.420   0.026 *
## Otu26892 0.420   0.022 *
## Otu28192 0.420   0.023 *
## Otu28287 0.420   0.016 *
## Otu28691 0.420   0.025 *
## Otu30260 0.420   0.024 *
## Otu32889 0.420   0.022 *
## Otu36856 0.420   0.023 *
## Otu50033 0.420   0.021 *
## Otu51019 0.420   0.016 *
## Otu06989 0.419   0.021 *
## Otu07420 0.419   0.026 *
## Otu05442 0.419   0.035 *
## Otu02821 0.414   0.044 *
## Otu18329 0.413   0.048 *
## Otu02445 0.409   0.034 *
## Otu04698 0.409   0.043 *
## Otu12472 0.405   0.044 *
## Otu03423 0.404   0.045 *
## Otu05271 0.400   0.032 *
## Otu06191 0.397   0.048 *
## Otu05133 0.396   0.044 *
## Otu07254 0.396   0.041 *
## Otu21803 0.396   0.044 *
## Otu20247 0.394   0.041 *
## Otu03813 0.392   0.045 *
## Otu12442 0.392   0.049 *
## Otu11046 0.385   0.050 *
##
##  Group BCSP+HNF  #sps.  20
##           stat p.value
## Otu00586 0.802   0.010 **
## Otu01009 0.773   0.007 **
## Otu00704 0.746   0.010 **
## Otu00974 0.721   0.004 **
## Otu01365 0.706   0.031 *
## Otu00846 0.692   0.015 *
```

```
## Otu00583 0.660   0.036 *
## Otu00890 0.654   0.017 *
## Otu00918 0.653   0.036 *
## Otu02497 0.641   0.040 *
## Otu04473 0.638   0.020 *
## Otu02826 0.636   0.021 *
## Otu00810 0.634   0.048 *
## Otu01403 0.614   0.041 *
## Otu01102 0.599   0.049 *
## Otu01086 0.597   0.045 *
## Otu01245 0.596   0.023 *
## Otu01014 0.593   0.039 *
## Otu02541 0.558   0.042 *
## Otu01320 0.544   0.047 *
##
##  Group BCSP+YSF  #sps.  114
##           stat p.value
## Otu00241 0.871   0.001 ***
## Otu00148 0.858   0.005 **
## Otu01661 0.853   0.003 **
## Otu00488 0.848   0.003 **
## Otu00300 0.840   0.006 **
## Otu00015 0.830   0.002 **
## Otu00064 0.828   0.007 **
## Otu00483 0.825   0.018 *
## Otu00337 0.808   0.011 *
## Otu00336 0.798   0.009 **
## Otu00730 0.788   0.024 *
## Otu00589 0.776   0.009 **
## Otu00862 0.748   0.013 *
## Otu01033 0.746   0.027 *
## Otu00902 0.738   0.001 ***
## Otu00038 0.730   0.041 *
## Otu21429 0.725   0.012 *
## Otu05925 0.721   0.014 *
## Otu00887 0.719   0.005 **
## Otu00776 0.718   0.007 **
## Otu00274 0.717   0.029 *
## Otu00517 0.716   0.015 *
## Otu00735 0.716   0.009 **
## Otu00108 0.715   0.012 *
## Otu00642 0.715   0.036 *
## Otu00266 0.705   0.042 *
## Otu02958 0.705   0.003 **
## Otu01424 0.695   0.002 **
## Otu03821 0.694   0.029 *
## Otu00819 0.683   0.013 *
## Otu00562 0.678   0.011 *
## Otu02717 0.676   0.011 *
## Otu00138 0.675   0.015 *
## Otu01542 0.674   0.040 *
## Otu01251 0.674   0.019 *
## Otu02430 0.674   0.029 *
## Otu00215 0.674   0.033 *
```

```
## Otu01047 0.667   0.023 *
## Otu01127 0.667   0.036 *
## Otu00652 0.659   0.012 *
## Otu00012 0.657   0.019 *
## Otu03030 0.654   0.012 *
## Otu03630 0.648   0.006 **
## Otu00796 0.638   0.041 *
## Otu00941 0.635   0.005 **
## Otu00685 0.631   0.015 *
## Otu00782 0.626   0.016 *
## Otu01031 0.622   0.038 *
## Otu00975 0.620   0.024 *
## Otu03446 0.617   0.004 **
## Otu01021 0.616   0.049 *
## Otu01833 0.614   0.025 *
## Otu02529 0.612   0.029 *
## Otu01770 0.602   0.003 **
## Otu00833 0.602   0.041 *
## Otu01355 0.597   0.016 *
## Otu01343 0.594   0.023 *
## Otu01869 0.593   0.037 *
## Otu04250 0.586   0.008 **
## Otu01477 0.581   0.041 *
## Otu01658 0.580   0.050 *
## Otu01352 0.579   0.009 **
## Otu04196 0.579   0.042 *
## Otu13919 0.578   0.025 *
## Otu02874 0.577   0.018 *
## Otu01170 0.576   0.020 *
## Otu01829 0.576   0.026 *
## Otu03113 0.574   0.045 *
## Otu01550 0.573   0.037 *
## Otu01552 0.569   0.036 *
## Otu01942 0.560   0.027 *
## Otu01620 0.558   0.019 *
## Otu02187 0.557   0.049 *
## Otu01530 0.555   0.023 *
## Otu00969 0.554   0.020 *
## Otu02472 0.554   0.013 *
## Otu00363 0.553   0.041 *
## Otu04351 0.552   0.015 *
## Otu01646 0.552   0.025 *
## Otu00749 0.550   0.050 *
## Otu02508 0.550   0.007 **
## Otu01239 0.550   0.023 *
## Otu01167 0.549   0.035 *
## Otu01664 0.535   0.033 *
## Otu03329 0.535   0.016 *
## Otu04156 0.535   0.017 *
## Otu09691 0.535   0.009 **
## Otu13152 0.535   0.023 *
## Otu02278 0.531   0.035 *
## Otu03659 0.526   0.024 *
## Otu03111 0.525   0.040 *
```

```
## Otu00802 0.524   0.026 *
## Otu02013 0.523   0.035 *
## Otu02317 0.514   0.047 *
## Otu01881 0.507   0.029 *
## Otu02512 0.507   0.032 *
## Otu03964 0.507   0.017 *
## Otu09055 0.507   0.032 *
## Otu09961 0.507   0.018 *
## Otu00746 0.502   0.049 *
## Otu03222 0.501   0.045 *
## Otu01864 0.499   0.035 *
## Otu08386 0.498   0.050 *
## Otu01184 0.498   0.047 *
## Otu01871 0.498   0.050 *
## Otu03430 0.478   0.034 *
## Otu03473 0.478   0.038 *
## Otu03889 0.478   0.033 *
## Otu04412 0.478   0.030 *
## Otu04585 0.478   0.027 *
## Otu04782 0.478   0.029 *
## Otu07639 0.478   0.035 *
## Otu09663 0.478   0.022 *
## Otu01525 0.447   0.046 *
##
##  Group HNF+YSF  #sps.  29
##           stat p.value
## Otu00231 0.880   0.024 *
## Otu00109 0.878   0.030 *
## Otu00155 0.829   0.048 *
## Otu00304 0.809   0.033 *
## Otu00325 0.804   0.006 **
## Otu00803 0.769   0.021 *
## Otu00526 0.746   0.025 *
## Otu02031 0.730   0.006 **
## Otu01518 0.714   0.008 **
## Otu00130 0.703   0.038 *
## Otu00163 0.668   0.039 *
## Otu04367 0.653   0.049 *
## Otu02227 0.639   0.036 *
## Otu00799 0.637   0.024 *
## Otu01563 0.631   0.039 *
## Otu02130 0.629   0.017 *
## Otu01591 0.595   0.043 *
## Otu02186 0.592   0.028 *
## Otu02444 0.592   0.007 **
## Otu01788 0.591   0.028 *
## Otu02919 0.570   0.045 *
## Otu05410 0.570   0.015 *
## Otu01397 0.558   0.044 *
## Otu01918 0.534   0.049 *
## Otu04945 0.524   0.036 *
## Otu08141 0.524   0.027 *
## Otu08841 0.524   0.028 *
## Otu04241 0.500   0.038 *
```

```
## Otu02741 0.474   0.040 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```