

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

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## OVERVIEW

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

## Directions:

1. In the Markdown version of this document in your cloned repo, change “Student Name” on line 3 (above) with your name.
2. Complete as much of the worksheet as possible during class.
3. Use the handout as a guide; it contains a more complete description of data sets along with examples of proper scripting needed to carry out the exercises.
4. Answer questions in the worksheet. Space for your answers is provided in this document and is indicated by the “>” character. If you need a second paragraph be sure to start the first line with “>”. You should notice that the answer is highlighted in green by RStudio (color may vary if you changed the editor theme).
5. Before you leave the classroom today, it is *imperative* that you **push** this file to your GitHub repo, at whatever stage you are. This will enable you to pull your work onto your own computer.
6. When you have completed the worksheet, **Knit** the text and code into a single PDF file by pressing the **Knit** button in the RStudio scripting panel. This will save the PDF output in your ‘8.BetaDiversity’ folder.
7. After Knitting, please submit the worksheet by making a **push** to your GitHub repo and then create a **pull request** via GitHub. Your pull request should include this file (**8.BetaDiversity\_\_2\_\_Worksheet.Rmd**) with all code blocks filled out and questions answered) and the PDF output of Knitr (**8.BetaDiversity\_\_2\_\_Worksheet.pdf**).

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

## 1) R SETUP

Typically, the first thing you will do in either an R script or an RMarkdown file is setup your environment. This includes things such as setting the working directory and loading any packages that you will need.

In the R code chunk below, provide the code to:

1. clear your R environment,
2. print your current working directory,
3. set your working directory to your “/8.BetaDiversity” folder, and
4. load the **vegan** R package (be sure to install if needed).

```
remove(list=ls())  
getwd()
```

```
## [1] "/Users/mark/Box Sync/Courses/Quantitative Biodiversity/QB2019_Hibbins/2.Worksheets/8.BetaDiversity"  
setwd('/Users/mark/Box Sync/Courses/Quantitative Biodiversity/QB2019_Hibbins/2.Worksheets/8.BetaDiversity')  
library(vegan)
```

```
## Loading required package: permute
## Loading required package: lattice
## This is vegan 2.5-3
```

## 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
library(ade4)
data('doubs')
```

## 3) HYPOTHESIS TESTING

### A. Multivariate Procedures for Categorical Designs

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

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

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

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

```
adonis(fish ~ quality, method = 'bray', permutations = 999)
```

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

```
library(indicspecies)
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 0.021 *
##
## Group HQ+MQ #sps. 2
##      stat p.value
## Satr 0.860 0.001 ***
## Phph 0.859 0.009 **
##
## Group LQ+MQ #sps. 20
##      stat p.value
## Alal 0.935 0.001 ***
## Gogo 0.933 0.001 ***
## Ruru 0.916 0.001 ***
## Legi 0.901 0.001 ***
## Baba 0.895 0.001 ***
## Chna 0.866 0.001 ***
## Spbi 0.866 0.003 **
## Cyca 0.866 0.002 **
## Acce 0.866 0.001 ***
## Lele 0.863 0.005 **
## Titi 0.853 0.008 **
## Chto 0.829 0.001 ***
## Rham 0.829 0.002 **
## Anan 0.829 0.002 **
## Eslu 0.827 0.021 *
## Pefl 0.806 0.012 *
## Blbj 0.791 0.005 **
## Scer 0.766 0.013 *
## Abbr 0.750 0.014 *
## Icme 0.661 0.040 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

fish.rel <- decostand(fish, method = 'total')
phi <- multipatt(fish.rel, cluster = quality, func = 'r.g', control = how(nperm=999))
summary(phi)

##
## Multilevel pattern analysis
## -----

```

```

##
## Association function: r.g
## Significance level (alpha): 0.05
##
## Total number of species: 27
## Selected number of species: 18
## Number of species associated to 1 group: 9
## Number of species associated to 2 groups: 9
##
## List of species associated to each combination:
##
## Group HQ #sps. 3
##      stat p.value
## Phph 0.802 0.001 ***
## Neba 0.734 0.001 ***
## Satr 0.650 0.001 ***
##
## Group LQ #sps. 2
##      stat p.value
## Alal 0.693 0.001 ***
## Ruru 0.473 0.031 *
##
## Group MQ #sps. 4
##      stat p.value
## Anan 0.571 0.008 **
## Spbi 0.557 0.009 **
## Chto 0.542 0.018 *
## Icme 0.475 0.033 *
##
## Group LQ+MQ #sps. 9
##      stat p.value
## Legi 0.658 0.002 **
## Baba 0.645 0.004 **
## Rham 0.600 0.005 **
## Acce 0.594 0.004 **
## Cyca 0.586 0.009 **
## Chna 0.571 0.007 **
## Blbj 0.571 0.008 **
## Gogo 0.523 0.007 **
## Abbr 0.499 0.030 *
## ---
## 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:** All the analyses agree that river quality is a significant predictor of species composition. The IndVal and phi analyses disagree somewhat about what species are important indicators for each quality level. The ‘Satr’ and ‘Phph’ are indicators of high river quality in both analyses, but the IndVal analysis lumps almost all the species together into the low and medium quality category, while the phi analysis finds more indicator species for low and medium quality specifically.

## B. Multivariate Procedures for Continuous Designs

### i. Mantel Test

In the R code chunk below, do the following:

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

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

mantel(fish.dist, env.dist)
```

```
##
## Mantel statistic based on Pearson's product-moment correlation
##
## Call:
## mantel(xdis = fish.dist, ydis = env.dist)
##
## Mantel statistic r: 0.604
##      Significance: 0.001
##
## Upper quantiles of permutations (null model):
##   90%   95% 97.5%   99%
## 0.106 0.139 0.172 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:** The similarity matrix and environment matrix are significantly correlated ( $p = 0.001$ ), with a correlation coefficient of 60%. Since many of the environmental variables will contribute to stream quality, this is consistent with the hypothesis that quality affects fish community structure.

### ii. Constrained Ordination

In the R code chunk below, do the following:

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

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

#Ordination analyses
fish.db <- vegdist(fish, method = 'bray')
doubs.dbrda <- dbrda(fish.db ~ ., as.data.frame(env.chem))
```

```

#Permutation tests
permutest(doubs.dbrda, permutations = 999)

##
## Permutation test for dbrda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = fish.db ~ pH + har + pho + nit + amm + oxy
## + bdo, data = as.data.frame(env.chem))
## Permutation test for all constrained eigenvalues
##           Df Inertia      F Pr(>F)
## Model      7  4.3801 5.5165  0.001 ***
## Residual  21  2.3820
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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

##
## ***VECTORS
##
##           dbRDA1   dbRDA2      r2 Pr(>r)
## nit   0.86441   0.50279 0.6386  0.001 ***
## oxy  -0.79947  -0.60071 0.7699  0.001 ***
## bdo   0.49508   0.86885 0.8927  0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999

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

#plot

par(mar = c(5, 5, 4, 4) + 0.1)

plot(scores(doubs.dbrda, display = 'wa'),
  xlim = c(-1.3, 1.1),
  ylim = c(-1.1, 2.7),
  xlab = paste('dbRDA 1 (', dbrda.explainvar1, '%)', sep = ''),
  ylab = paste('dbRDA 2 (', dbrda.explainvar2, '%)', sep = ''),
  pch = 16,
  cex = 2.0,
  type = 'n',
  cex.lab = 1.5,
  cex.axis = 1.2, axes = FALSE)

```

```

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)

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'))))

vectors <- scores(doubs.dbrda,
                 display = 'bp')
#Add 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])))

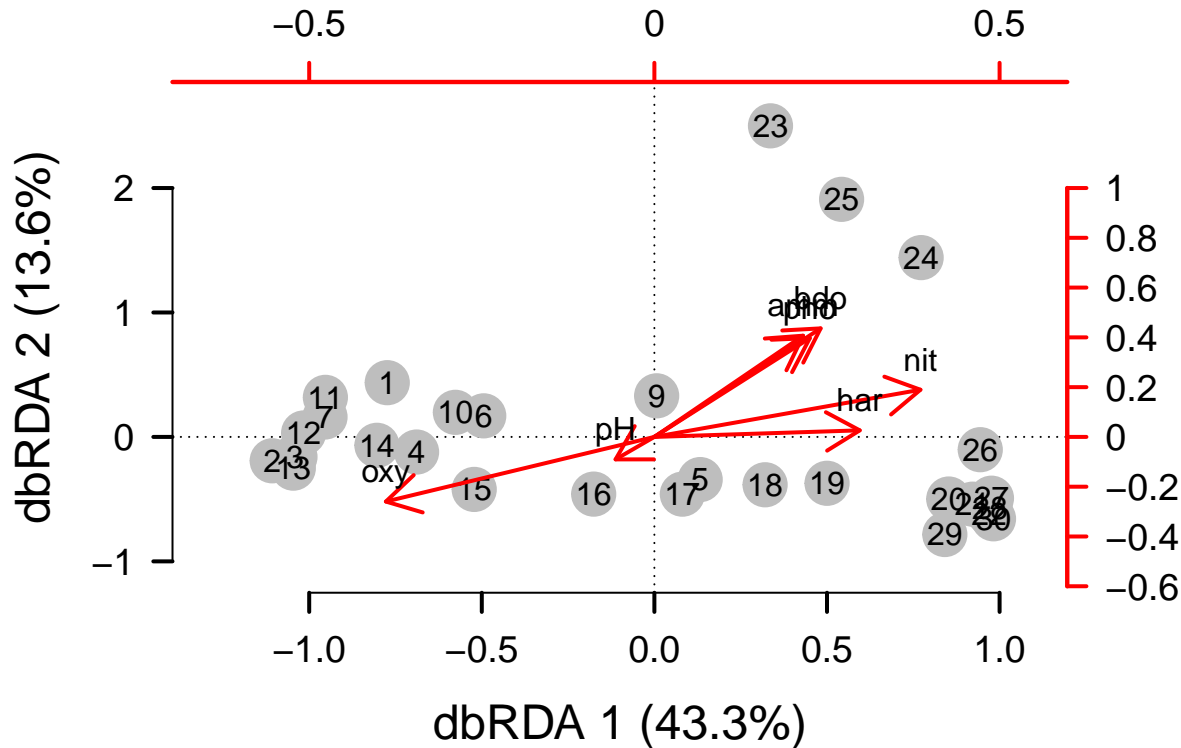
axis(side = 4,
     lwd.ticks = 2,

```

```

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:** Based on the size and direction of the vectors on the ordination plot, and the significant results of the permutation test, the three major explanatory variables are nit (nitrate concentration), oxy (dissolved oxygen), and bdo (biological demand for oxygen). Oxy and nit are near exact opposites of one another. This makes sense, since higher nitrate levels often lead to eutrophication and the depletion of oxygen levels. This will also increase the demand for oxygen, which is similar in direction.

### 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.



```

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

#PCNM matrix model

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

doubts.pcnmw <- pcnm(dist(doubts$xy[-8,]),
                     w = rs,
                     dist.ret = T)

doubts.space <- as.data.frame(scores(doubts.pcnmw))
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,
                       trace = FALSE)

#step.pcnm$anova #info used to make model

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

#Constrained ordinations
doubts.total.env <- dbrda(fish.db ~ env.mod)
doubts.total.space <- dbrda(fish.db ~ space.mod)

#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))

#Permutation tests for signifiance
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 0.001 ***
## 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
```

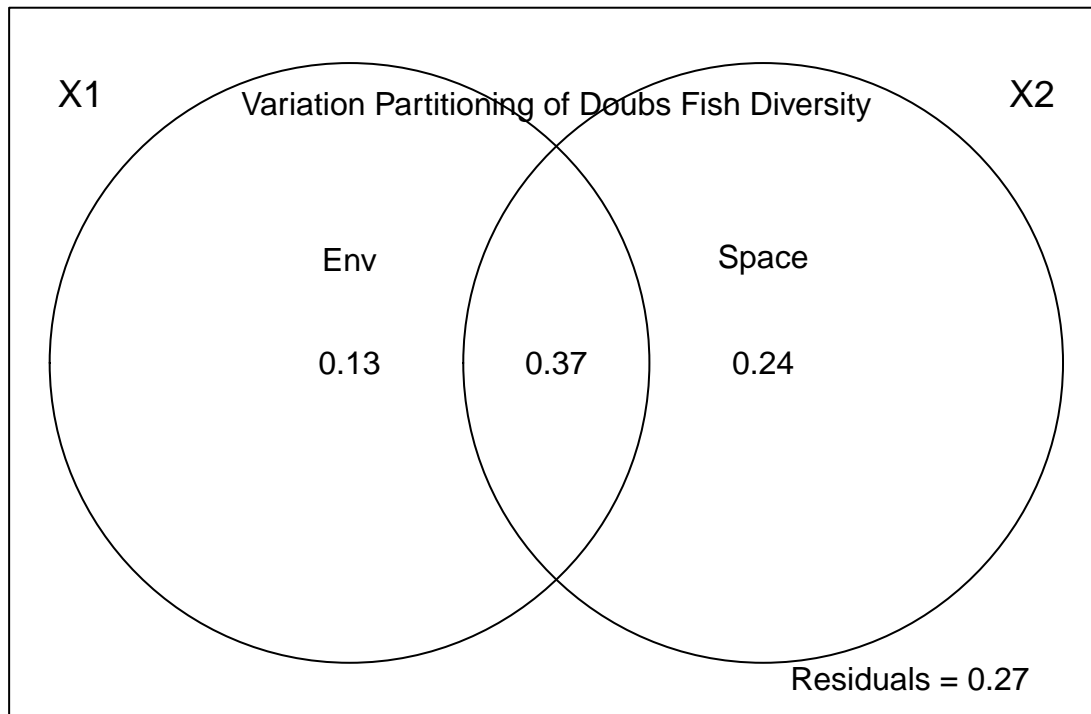
```
#Partitioning of variance
```

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

```
#Plot
```

```
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:** Out of the total variance in fish diversity in the Doubs river, 13% is explained by environmental variation, 24% is explained by geographical space, 37% is explained by environmental variation over space, and the remaining 27% is unexplained.

## SYNTHESIS

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

```
JellyBeans <- read.delim("~/Box Sync/Courses/Quantitative Biodiversity/QB2019_Hibbins/2.Worksheets/6.Di
```

```
group <- c(rep('A', 3), rep('B', 2), 'A', 'B', 'A', 'B')
```

```
Jellybeans <- JellyBeans[3:30]
```

```
adonis(Jellybeans ~ group, method = 'bray', permutations = 999)
```

```
##
```

```
## Call:
```

```
## adonis(formula = Jellybeans ~ group, permutations = 999, method = "bray")
```

```
##
```

```
## Permutation: free
```

```
## Number of permutations: 999
```

```
##
## Terms added sequentially (first to last)
##
##           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## group      1  0.09247 0.092468  2.0401 0.22568  0.06 .
## Residuals   7  0.31727 0.045324          0.77432
## Total       8  0.40974          1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

This analysis returned a similar p-value to the t-test performed in beta diversity 1. There appears to be a marginally significant effect of the vicariance event on the jelly bean community. This analysis shows that if the sampling effort is small enough, stochastic forces can have a significant effect on measures of biodiversity, and potentially bias results. While only marginally significant in this case, if it was paired with some other deterministic effect it could generate a misleadingly low p-value. It is therefore important to control for stochastic factors (ie. random effects) when doing these tests.

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

```
#Load dataset
epiphytes <- read.csv("~/Box Sync/Courses/Quantitative Biodiversity/QB2019_Hibbins/2.Worksheets/5.Alpha
epiphytes_SbyS <- epiphytes[,c(7:42)]

#Ordination
```