

# Beta diversity: constrained ordination (dbRDA)

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## Introduction

Genus level, relative abundances, weighted UniFrac distance. Use distance-based RDA, derived from R code of Shankar *et al.* 2017 (but using **dbrda()** instead of **capscale()** function).

## Load packages

```
library(phyloseq)
library(microbiome)
library(vegan)
library(ggplot2)
```

## Input files

```
pstot <- readRDS("./phyobjects/ps1.work.rds")
pstot.g <- microbiome::aggregate_taxa(pstot, "Genus")
pstot.g.r <- microbiome::transform(pstot.g, "compositional")
pstot.g.r
```

```
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 220 taxa and 93 samples ]
## sample_data() Sample Data: [ 93 samples by 16 sample variables ]
## tax_table() Taxonomy Table: [ 220 taxa by 6 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 220 tips and 219 internal nodes ]
```

## 1. Prepare data

Input files for dbRDA, balanced subsets required for permutation tests with stratified permutations.

```
# chicken feed, day 15
CF15 <- subset_samples(pstot.g.r, Diet == "CF" & Timepoint == 15)
CF15 <- prune_taxa(taxa_sums(otu_table(CF15)) > 0, CF15)
otu.cf15 <- as.data.frame(t(abundances(CF15)))
env.cf15 <- meta(CF15)
wuf.cf15 <- distance(CF15, "wunifrac")

# chicken manure, day 0
CM0 <- subset_samples(pstot.g.r, Diet == "CM" & Timepoint == 0)
CM0 <- prune_taxa(taxa_sums(otu_table(CM0)) > 0, CM0)
otu.cm0 <- as.data.frame(t(abundances(CM0)))
env.cm0 <- meta(CM0)
wuf.cm0 <- distance(CM0, "wunifrac")

# chicken manure, day 15
CM15 <- subset_samples(pstot.g.r, Diet == "CM" & Timepoint == 15 & Description != "39.N")
CM15 <- prune_taxa(taxa_sums(otu_table(CM15)) > 0, CM15)
otu.cm15 <- as.data.frame(t(abundances(CM15)))
env.cm15 <- meta(CM15)
wuf.cm15 <- distance(CM15, "wunifrac")

table(env.cm15[,c("Treatment", "Type")])
```

```
##           Type
## Treatment substrate larvae
##      S/E           5           5
##     Si/E           6           6
##    Si/Es           6           6
##     Ss/E           6           6
```

```
# balanced design, removed 39.N (because 39.M was excluded as it was likely
## mixed up with 26.K, see R markdown file 3_subsetting.Rmd)
```

## 2. dbRDA

### 2.1. Chicken feed, day 15

```
# Calculate dbRDA
dbRDA.cf <- dbrda(wuf.cf15 ~ env.cf15$Treatment * env.cf15$Type)
dbRDA.cf

## Call: dbrda(formula = wuf.cf15 ~ env.cf15$Treatment * env.cf15$Type)
##
##              Inertia Proportion Rank RealDims
## Total              1.2415      1.0000
## Constrained      0.6759      0.5444      5      5
## Unconstrained    0.5656      0.4556     18     12
## Inertia is squared Unknown distance
##
## Eigenvalues for constrained axes:
## dbRDA1 dbRDA2 dbRDA3 dbRDA4 dbRDA5
## 0.5738 0.0928 0.0064 0.0024 0.0005
##
## Eigenvalues for unconstrained axes:
##      MDS1      MDS2      MDS3      MDS4      MDS5      MDS6      MDS7      MDS8
## 0.27564 0.11622 0.08394 0.05224 0.02749 0.01345 0.00746 0.00627
## (Showing 8 of 18 unconstrained eigenvalues)

RsquareAdj(dbRDA.cf)

## $r.squared
## [1] 0.5444302
##
## $adj.r.squared
## [1] 0.417883

# permutation tests
dbRDA.cf0 <- dbrda(wuf.cf15 ~ 1)
anova(dbRDA.cf0, dbRDA.cf,
      permutations = how(within = Within(type = "none"),
                        plots = Plots(strata = env.cf15$ContainerID,
                                     type = "free"), nperm = 999))

## Permutation tests for dbrda under reduced model
## Plots: env.cf15$ContainerID, plot permutation: free
## Permutation: none
## Number of permutations: 999
##
## Model 1: wuf.cf15 ~ 1
## Model 2: wuf.cf15 ~ env.cf15$Treatment * env.cf15$Type
##      ResDf ResChiSquare Df ChiSquare      F Pr(>F)
## 1      23          1.2415
## 2      18          0.5656  5    0.67592 4.3022 0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

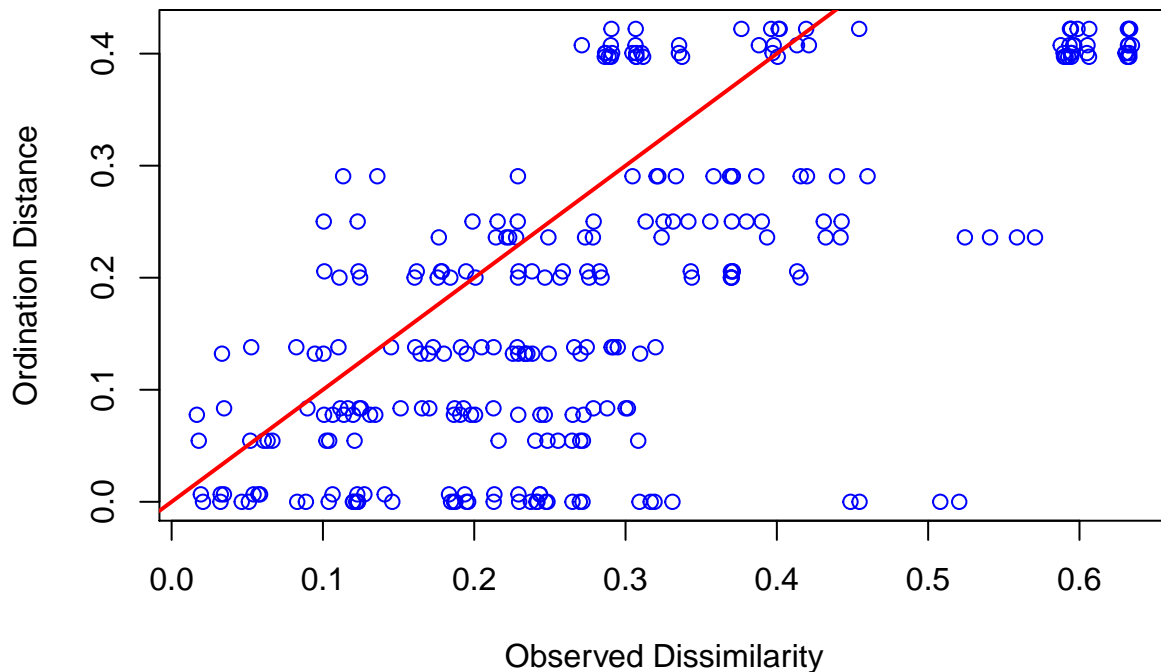
```

# by terms
aov.cf <- anova.cca(dbrDA.cf, model = "direct", by="terms",
  permutations = how(within = Within(type = "none"),
    plots = Plots(strata = env.cf15$ContainerID,
      type = "free"), nperm = 999))
aov.cf

## Permutation test for dbrda under direct model
## Terms added sequentially (first to last)
## Plots: env.cf15$ContainerID, plot permutation: free
## Permutation: none
## Number of permutations: 999
##
## Model: dbrda(formula = wuf.cf15 ~ env.cf15$Treatment * env.cf15$Type)
##
##              Df SumOfSqs      F Pr(>F)
## env.cf15$Treatment      2  0.50925 8.1034  0.002 **
## env.cf15$Type            1  0.04813 1.5317  0.011 *
## env.cf15$Treatment:env.cf15$Type  2  0.11854 1.8862  0.001 ***
## Residual                18  0.56560
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# goodness of fit
stressplot(dbrDA.cf)

```



```

# dispersion test
anova(betadisper(wuf.cf15, group = interaction(env.cf15$Treatment,
env.cf15$Type, drop = T)),
      permutations = how(within = Within(type = "none"),
        plots = Plots(strata = env.cf15$ContainerID,
          type = "free"), nperm = 999))

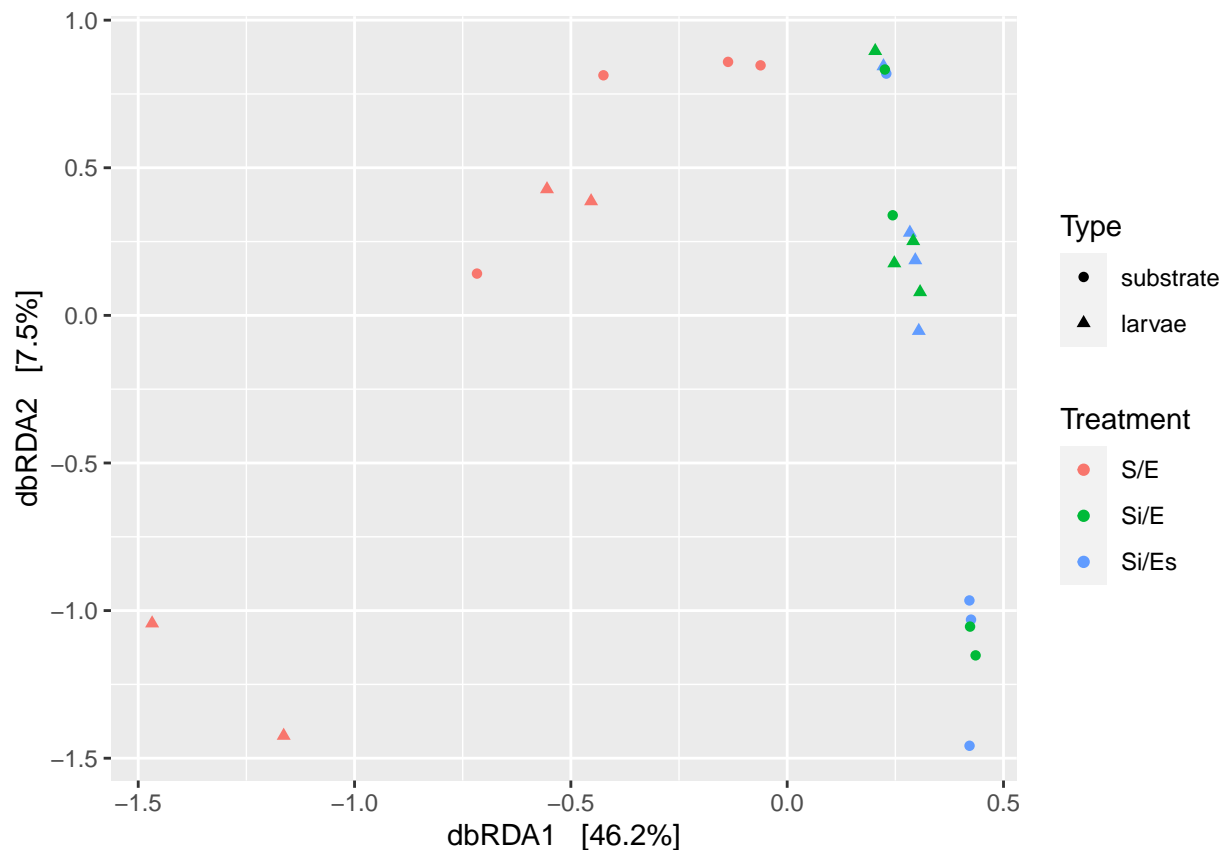
## Warning in betadisper(wuf.cf15, group = interaction(env.cf15$Treatment, : some
## squared distances are negative and changed to zero

## Analysis of Variance Table
##
## Response: Distances
##          Df Sum Sq Mean Sq F value Pr(>F)
## Groups    5 0.076827 0.0153653  1.7281 0.1792
## Residuals 18 0.160048 0.0088916

# no differences in dispersion.

# plot
plot_ordination(CF15, dbRDA.cf, type = "samples", color = "Treatment", shape = "Type")

```



## 2.2. Chicken manure, day 0

```
# Calculate dbRDA
dbRDA.cmb <- dbrda(wuf.cm0 ~ env.cm0$Treatment)
dbRDA.cmb

## Call: dbrda(formula = wuf.cm0 ~ env.cm0$Treatment)
##
##              Inertia Proportion Rank RealDims
## Total          0.6150      1.0000
## Constrained    0.3126      0.5083      2      2
## Unconstrained  0.3024      0.4917     14     10
## Inertia is squared Unknown distance
##
## Eigenvalues for constrained axes:
## dbRDA1 dbRDA2
## 0.29839 0.01423
##
## Eigenvalues for unconstrained axes:
##      MDS1      MDS2      MDS3      MDS4      MDS5      MDS6      MDS7      MDS8
## 0.16030 0.07484 0.04031 0.01832 0.01270 0.00513 0.00340 0.00291
##      MDS9      MDS10     iMDS1     iMDS2     iMDS3     iMDS4
## 0.00205 0.00044 -0.00118 -0.00258 -0.00337 -0.01092

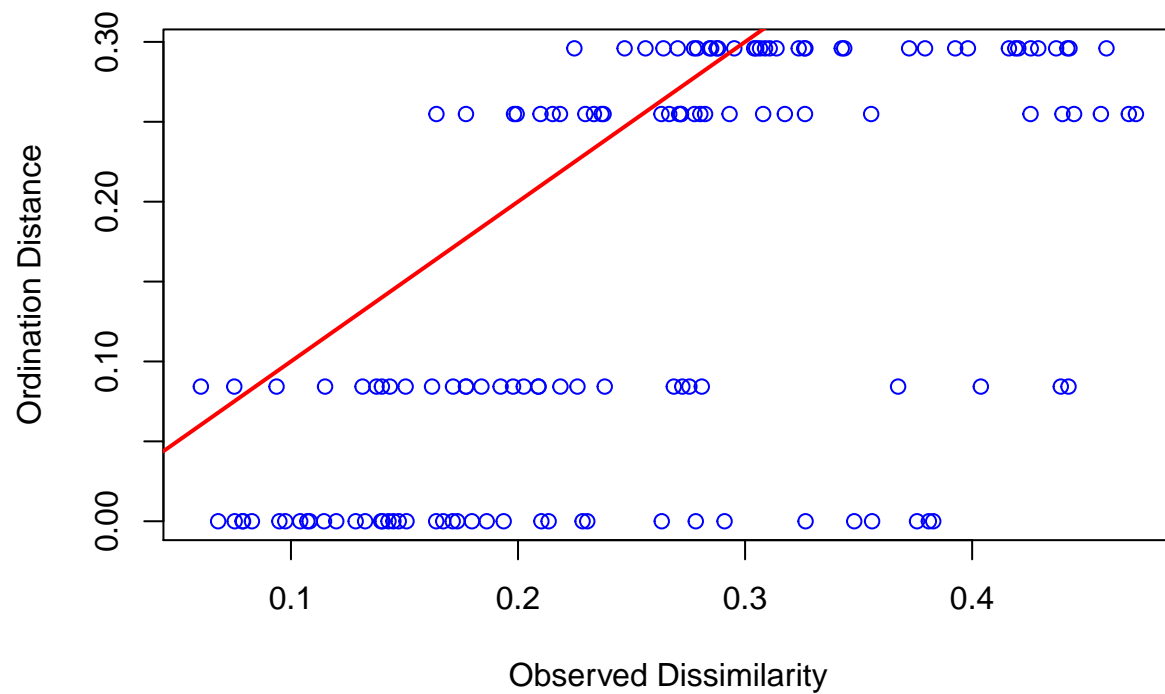
RsquareAdj(dbRDA.cmb)

## $r.squared
## [1] 0.5083456
##
## $adj.r.squared
## [1] 0.4381092

# permutation tests
dbRDA.cmb0 <- dbrda(wuf.cm0 ~ 1)
anova(dbRDA.cmb0, dbRDA.cmb, permutations = 999)

## Permutation tests for dbrda under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model 1: wuf.cm0 ~ 1
## Model 2: wuf.cm0 ~ env.cm0$Treatment
##   ResDf ResChiSquare Df ChiSquare      F Pr(>F)
## 1    16      0.61498
## 2    14      0.30236  2   0.31262 7.2376 0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# goodness of fit
stressplot(dbRDA.cmb)
```

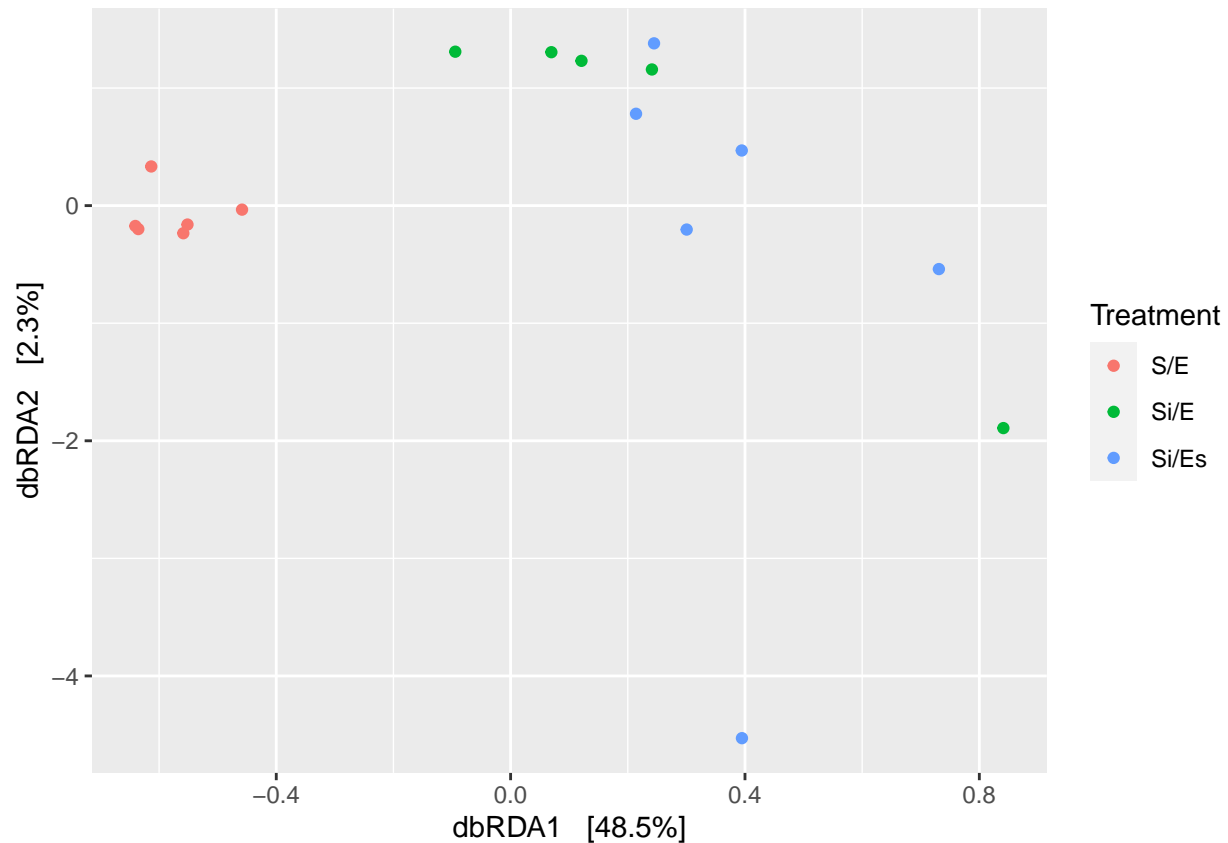


```
# dispersion test
anova(betadisper(wuf.cm0, group = env.cm0$Treatment), permutations = 999)
```

```
## Analysis of Variance Table
##
## Response: Distances
##          Df  Sum Sq  Mean Sq F value Pr(>F)
## Groups    2  0.012860  0.0064298   1.0212  0.3855
## Residuals 14  0.088148  0.0062963
```

```
# no differences in dispersion.
```

```
# plot
plot_ordination(CM0, dbRDA.cmb, type = "samples", color = "Treatment")
```



### 2.3. Chicken manure, day 15

```
# Calculate dbRDA
dbRDA.cm <- dbrda(wuf.cm15 ~ env.cm15$Treatment * env.cm15$Type)
dbRDA.cm

## Call: dbrda(formula = wuf.cm15 ~ env.cm15$Treatment * env.cm15$Type)
##
##              Inertia Proportion Rank RealDims
## Total           4.2120      1.0000
## Constrained     3.1685      0.7523    7        7
## Unconstrained   1.0435      0.2477   38       25
## Inertia is squared Unknown distance
##
## Eigenvalues for constrained axes:
## dbRDA1 dbRDA2 dbRDA3 dbRDA4 dbRDA5 dbRDA6 dbRDA7
## 2.5506 0.3680 0.1868 0.0353 0.0184 0.0078 0.0015
##
## Eigenvalues for unconstrained axes:
## MDS1 MDS2 MDS3 MDS4 MDS5 MDS6 MDS7 MDS8
## 0.31152 0.16592 0.14126 0.08829 0.07358 0.06074 0.04642 0.03882
## (Showing 8 of 38 unconstrained eigenvalues)
```



```
RsquareAdj(dbrDA.cm)
```

```
## $r.squared
## [1] 0.7522626
##
## $adj.r.squared
## [1] 0.7066267
```

```
# permutation tests
```

```
dbRDA.cm0 <- dbrda(wuf.cm15 ~ 1)
anova(dbRDA.cm0, dbRDA.cm,
       permutations = how(within = Within(type = "none"),
                           plots = Plots(strata = env.cm15$ContainerID,
                                           type = "free"), nperm = 999))
```

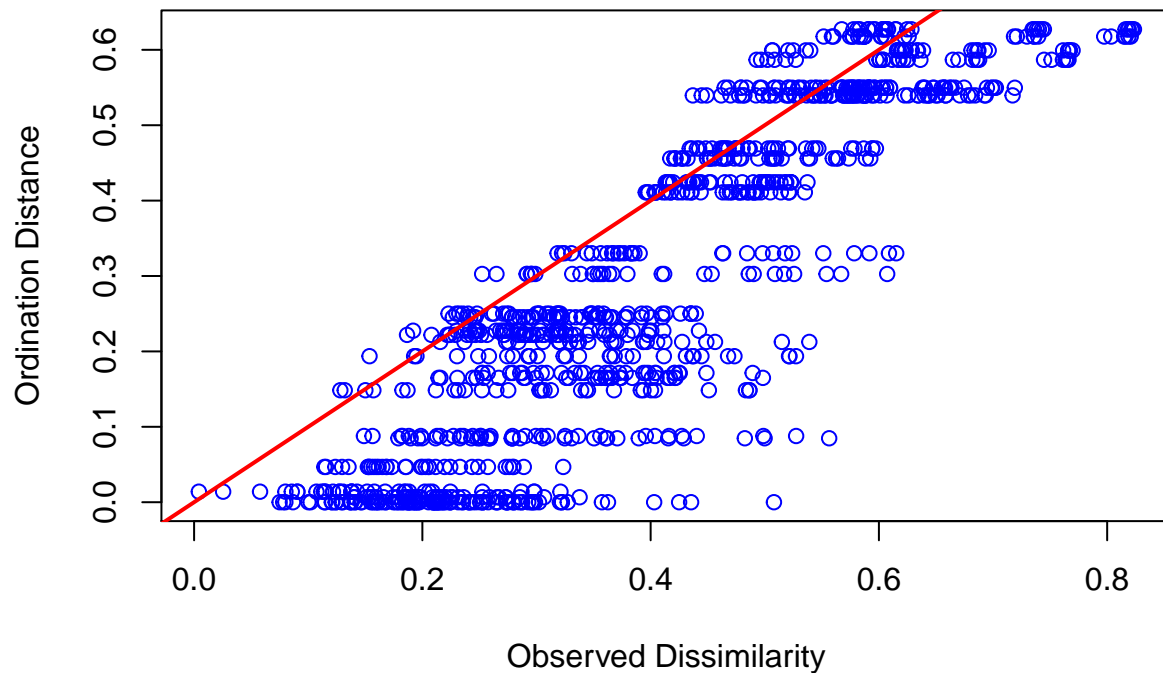
```
## Permutation tests for dbrda under reduced model
## Plots: env.cm15$ContainerID, plot permutation: free
## Permutation: none
## Number of permutations: 999
##
## Model 1: wuf.cm15 ~ 1
## Model 2: wuf.cm15 ~ env.cm15$Treatment * env.cm15$Type
##   ResDf ResChiSquare Df ChiSquare      F Pr(>F)
## 1     45          4.2120      3.1685 16.484 0.001 ***
## 2     38          1.0435      7      3.1685 16.484 0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# by terms
```

```
aov.cm <- anova.cca(dbRDA.cm, model = "direct", by="terms",
                   permutations = how(within = Within(type = "none"),
                                       plots = Plots(strata = env.cm15$ContainerID,
                                                       type = "free"), nperm = 999))
aov.cm
```

```
## Permutation test for dbrda under direct model
## Terms added sequentially (first to last)
## Plots: env.cm15$ContainerID, plot permutation: free
## Permutation: none
## Number of permutations: 999
##
## Model: dbrda(formula = wuf.cm15 ~ env.cm15$Treatment * env.cm15$Type)
##               Df SumOfSqs      F Pr(>F)
## env.cm15$Treatment      3  2.66493 32.3498 0.001 ***
## env.cm15$Type           1  0.32503 11.8366 0.001 ***
## env.cm15$Treatment:env.cm15$Type 3  0.17855  2.1674 0.001 ***
## Residual               38  1.04346
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# goodness of fit
stressplot(dbrDA.cm)
```

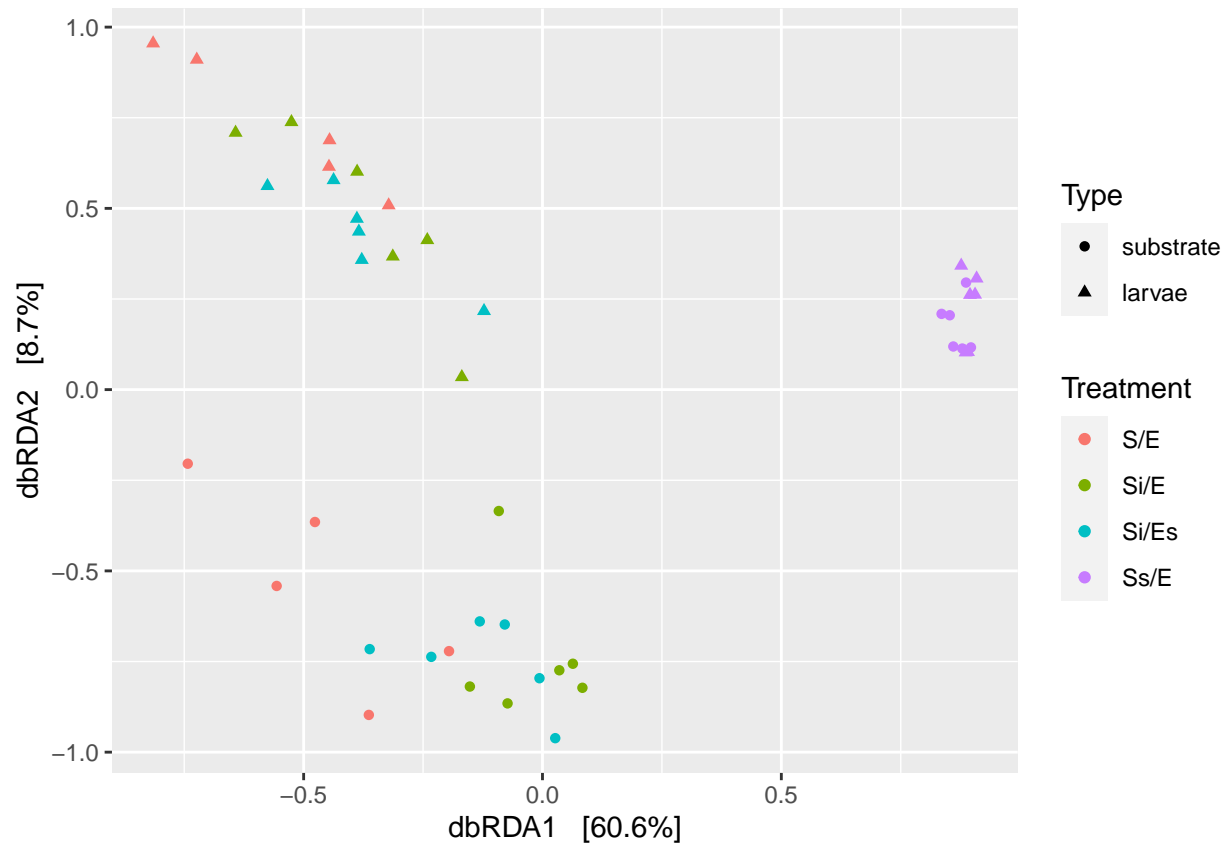


```
# dispersion test
anova(betadisper(wuf.cm15, group = interaction(env.cm15$Treatment,
  env.cm15$Type, drop = T)),
  permutations = how(within = Within(type = "none"),
    plots = Plots(strata = env.cm15$ContainerID,
      type = "free"), nperm = 999))
```

```
## Analysis of Variance Table
##
## Response: Distances
##      Df Sum Sq Mean Sq F value Pr(>F)
## Groups    7  0.04524  0.0064629    2.368  0.04123 *
## Residuals 38  0.10371  0.0027292
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# significant differences in dispersion, interpret anova.cca with caution.
```

```
# plot
plot_ordination(CM15, dbrDA.cm, type = "samples", color = "Treatment", shape = "Type")
```



### 3. Export tables

Supplementary Tables S6-7.

```
write.csv(aov.cf, "./tables/Supplementary_Table_S6.csv")
write.csv(aov.cm, "./tables/Supplementary_Table_S7.csv")
```