

# Group Project

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## 1. Create site-by-species matrix

```
site_species_matrix <- read.csv("site_species_matrix.csv")

# View the resulting matrix (row 1~8 = Trop_AM1~7, Trop_EcM1~7, Temp_AM1~4, Temp_EcM1~4)
#print(site_species_matrix)
LDW<-site_species_matrix[,-1]
rownames(LDW) <- site_species_matrix$site
```

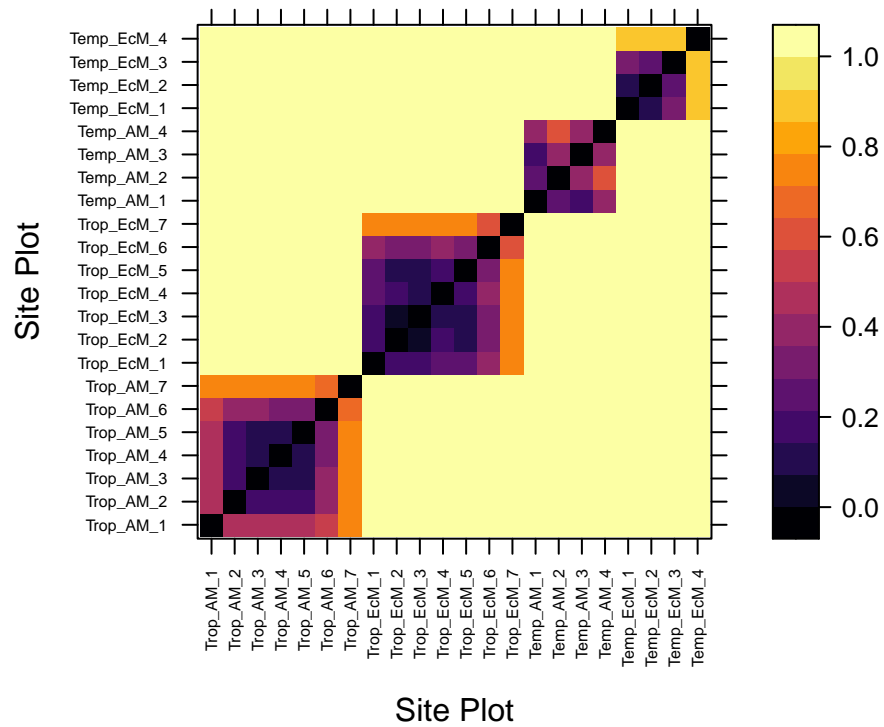
## 2. Visualise Beta-Diversity

```
# Calculate Jaccard
LDW.dj <- vegdist(LDW, method = "jaccard", binary = TRUE)

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

# Plot Heatmaps
levelplot(as.matrix(LDW.dj),
          aspect = "iso",
          col.regions = inferno,
          xlab = "Site Plot",
          ylab = "Site Plot",
          scales = list(x = list(rot=90, cex = 0.5), y = list(cex = 0.5)),
          main = "Jaccard Distance")
```

## Jaccard Distance

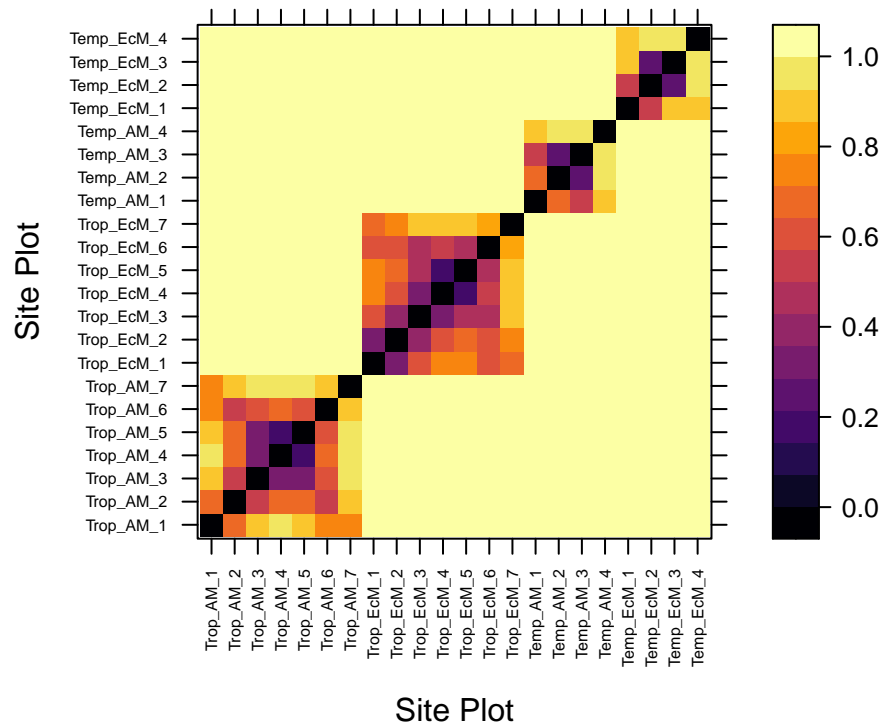


```

levelplot(as.matrix(LDW.db),
  aspect = "iso",
  col.regions = inferno,
  xlab = "Site Plot",
  ylab = "Site Plot",
  scales = list(x = list(rot=90, cex = 0.5), y = list(cex = 0.5)),
  main = "Bray-Curtis Distance")

```

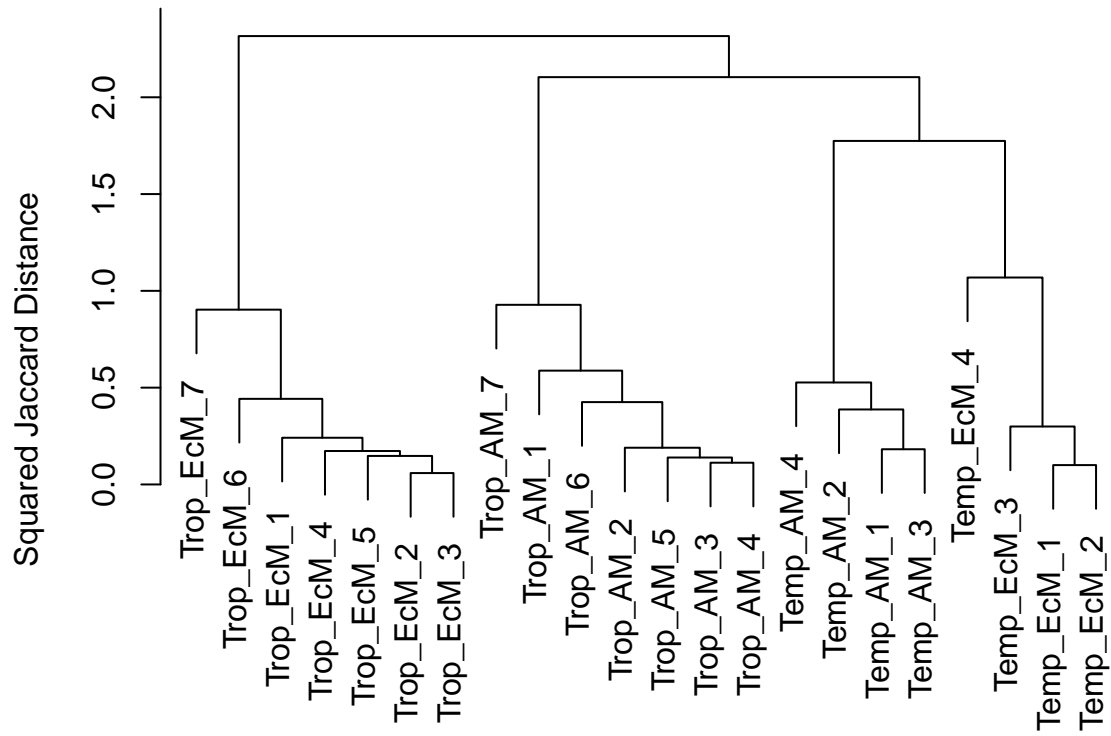
## Bray-Curtis Distance



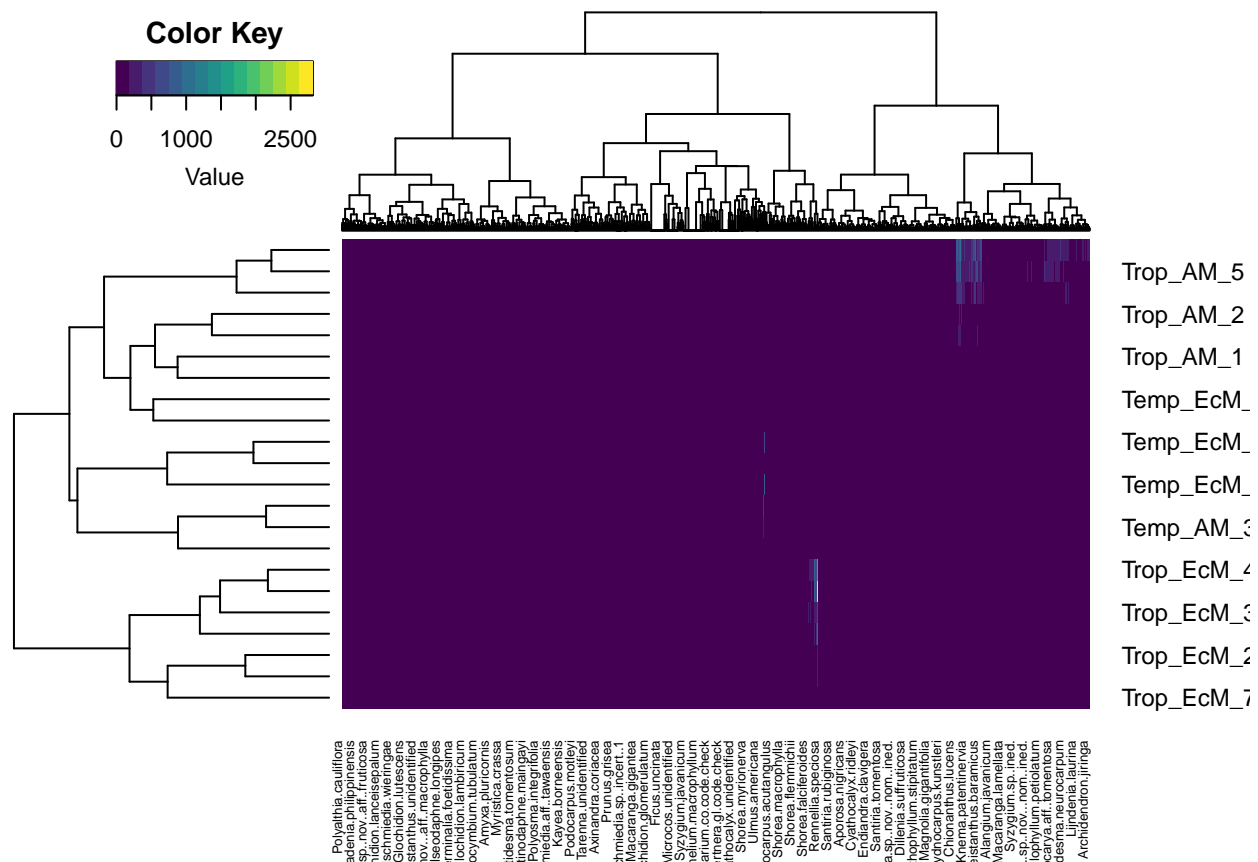
```
## Perform cluster analysis for Jaccard distance
LDW.ward.dj <- hclust(LDW.dj, method = "ward.D2")

# Plot cluster
par(mar = c(1, 5, 2, 2) + 0.1)
plot(LDW.ward.dj, main = "LDW: Ward's Clustering",
     ylab = "Squared Jaccard Distance",
     )
```

## LDW: Ward's Clustering



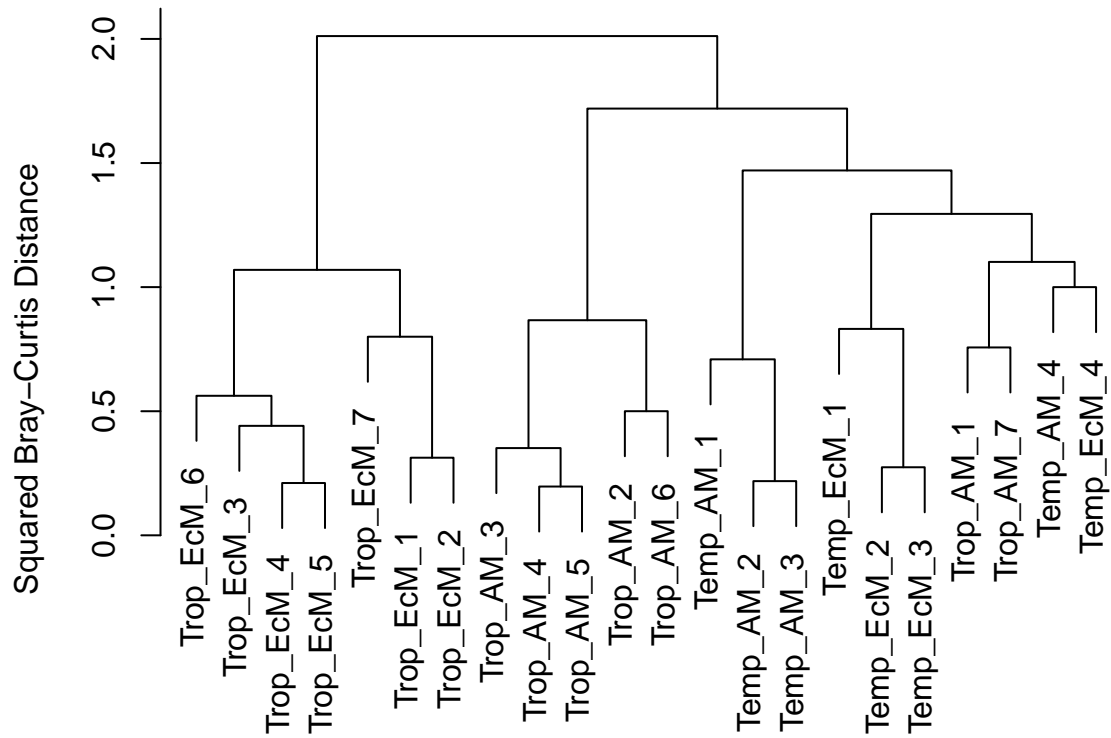
```
gplots::heatmap.2(as.matrix(LDW),
  distfun = function(x) vegdist(x, method = "jaccard"),
  hclustfun = function(x) hclust(x, method = "ward.D2"),
  col = viridis, trace = "none", density.info = "none")
```



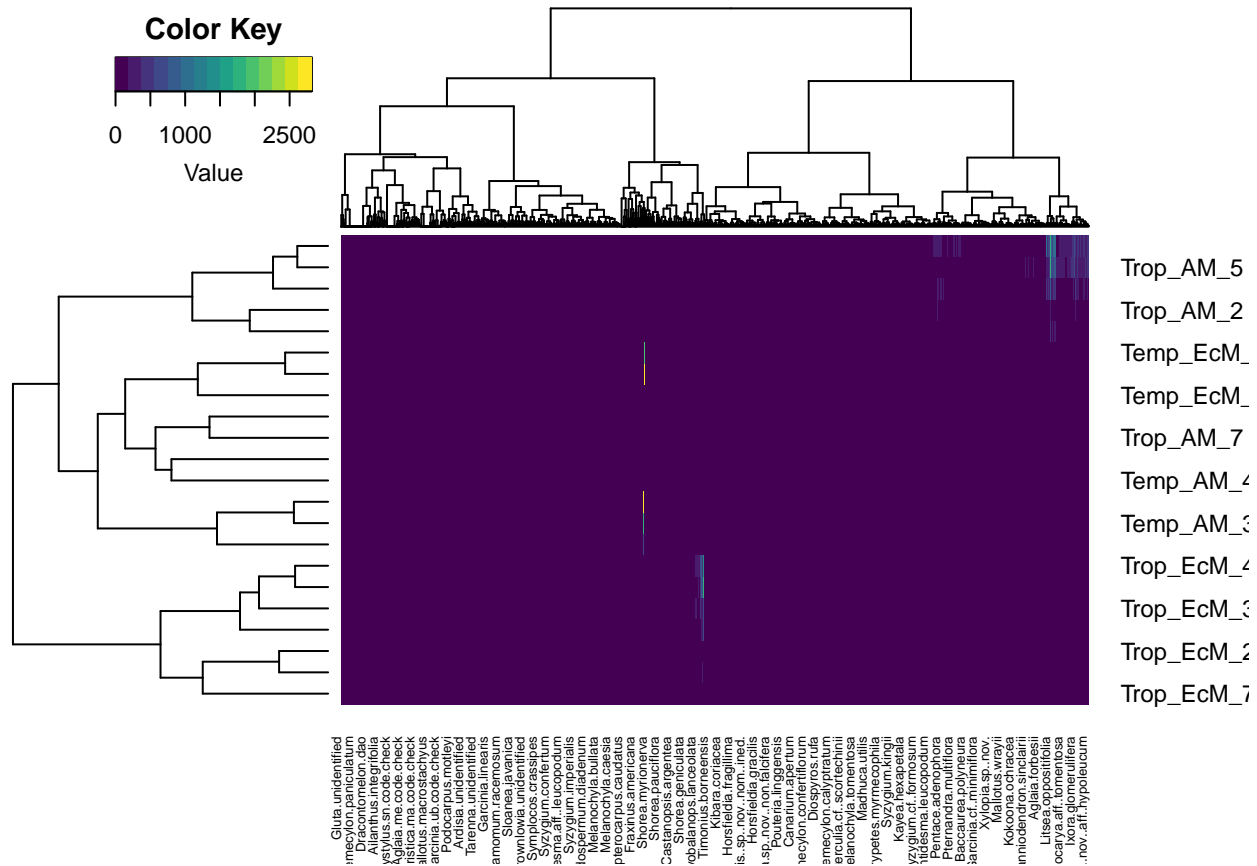
```
## Perform cluster analysis for Bray-Curtis distance
LDW.ward.db <- hclust(LDW.db, method = "ward.D2")
```

```
# Plot cluster
par(mar = c(1, 5, 2, 2) + 0.1)
plot(LDW.ward.db, main = "LDW: Ward's Clustering",
      ylab = "Squared Bray-Curtis Distance")
```

## LDW: Ward's Clustering



```
gplots::heatmap.2(as.matrix(LDW),
  distfun = function(x) vegdist(x, method = "bray"),
  hclustfun = function(x) hclust(x, method = "ward.D2"),
  col = viridis, trace = "none", density.info = "none")
```



```
## PCoA for Jaccard distance
LDW.pcoa <- cmdscale(LDW.dj, eig = TRUE, k = 3)

explainvar1 <- round(LDW.pcoa$eig[1] / sum(LDW.pcoa$eig), 3) * 100
explainvar2 <- round(LDW.pcoa$eig[2] / sum(LDW.pcoa$eig), 3) * 100
explainvar3 <- round(LDW.pcoa$eig[3] / sum(LDW.pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

# Define plot parameters
colors <- c("#CCFFCC", "#99FF99", "#66FF66", "#33FF33", "#33CC33", "#009900", "#006600",
            "#FFFF99", "#FFFF66", "#FFFF33", "#FFFF00", "#CCCC00", "#999900", "#666600",
            "#CCFFCC", "#66FF66", "#33CC33", "#006600",
            "#FFFF99", "#FFFF33", "#CCCC00", "#666600")

shapes <- c(16, 16, 16, 16, 16, 16, 16,
            16, 16, 16, 16, 16, 16, 16,
            17, 17, 17, 17,
            17, 17, 17, 17)

par(mar = c(5, 5, 1, 2) + 0.1)

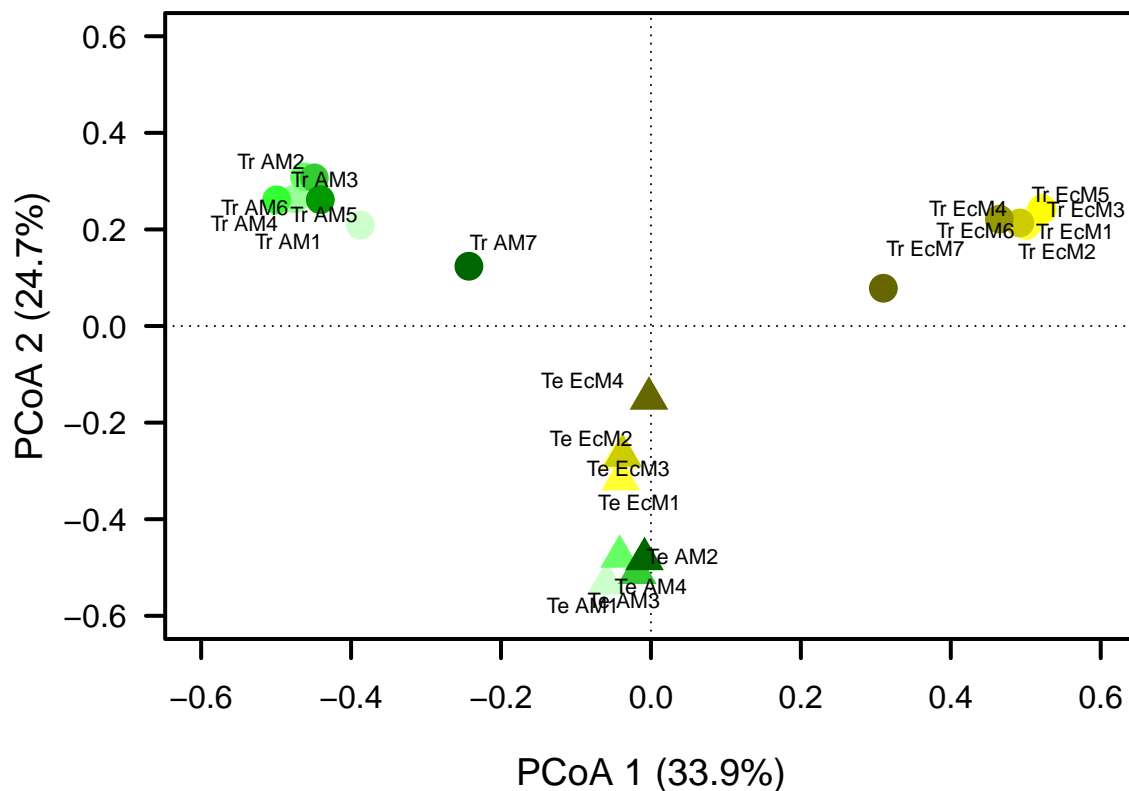
# Initiate plot
plot(LDW.pcoa$points[, 1], LDW.pcoa$points[, 2],
     xlim = c(-0.6, 0.6),
     ylim = c(-0.6, 0.6),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""),
     pch = 16, cex = 1.0, type = "n", cex.lab = 1.2, cex.axis = 0.8, axes = FALSE)
```

```

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

# Add points & labels
points(jitter(LDW.pcoa$points[, 1], amount = 0.03),
       jitter(LDW.pcoa$points[, 2], amount = 0.03),
       pch = shapes, cex = 2, bg = colors, col = colors)
text(jitter(LDW.pcoa$points[, 1], amount = 0.08),
     jitter(LDW.pcoa$points[, 2], amount = 0.08),
     cex = 0.7,
     labels = c("Tr AM1", "Tr AM2", "Tr AM3", "Tr AM4", "Tr AM5", "Tr AM6", "Tr AM7",
                "Tr EcM1", "Tr EcM2", "Tr EcM3", "Tr EcM4", "Tr EcM5", "Tr EcM6", "Tr EcM7",
                "Te AM1", "Te AM2", "Te AM3", "Te AM4",
                "Te EcM1", "Te EcM2", "Te EcM3", "Te EcM4"))

```



```

# First we calculate the relative abundances of each species at each site
LDW[] <- lapply(LDW, as.numeric)

LDWREL <- LDW
for (i in 1:nrow(LDW)) {
  LDWREL[i, ] = LDW[i, ] / sum(LDW[i, ])
}

# Now, we use this information to calculate and add species scores
LDWREL <- as.data.frame(LDWREL)

```



```

LDWREL[] <- lapply(LDWREL, as.numeric)

LDW.pcoa <- add.spec.scores(LDW.pcoa, LDWREL, method = "pcoa.scores")
#text(LDW.pcoa$cproj[, 1], LDW.pcoa$cproj[, 2],
#      labels = row.names(LDW.pcoa$cproj), col = "black")

spe.corr <- add.spec.scores(LDW.pcoa, LDWREL, method = "cor.scores")$cproj
corrcut <- 0.7 # User-defined cutoff
imp.spp <- spe.corr[abs(spe.corr[, 1]) >= corrcut | abs(spe.corr[, 2]) >= corrcut, ]

# Permutation test for species abundances across axes
fit <- envfit(LDW.pcoa, LDWREL, perm = 999)

## PCoA for Bray-Curtis distance
LDW.pcoa <- cmdscale(LDW.db, eig = TRUE, k = 3)

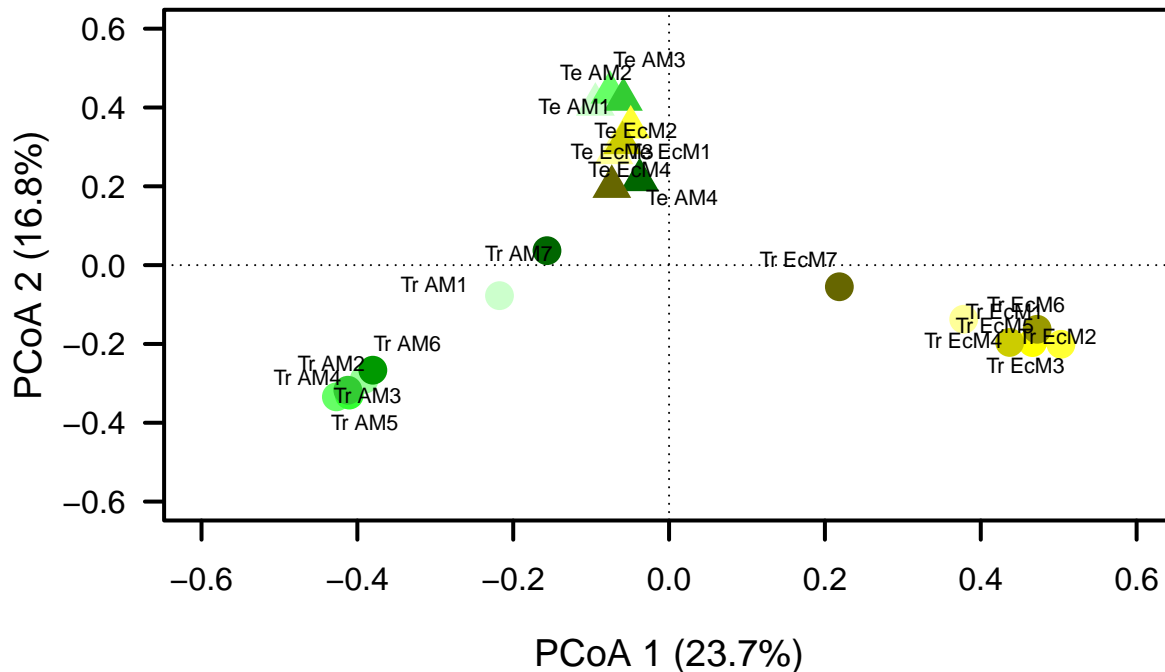
explainvar1 <- round(LDW.pcoa$eig[1] / sum(LDW.pcoa$eig), 3) * 100
explainvar2 <- round(LDW.pcoa$eig[2] / sum(LDW.pcoa$eig), 3) * 100
explainvar3 <- round(LDW.pcoa$eig[3] / sum(LDW.pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

# Initiate plot
plot(LDW.pcoa$points[, 1], LDW.pcoa$points[, 2],
      xlim = c(-0.6, 0.6),
      ylim = c(-0.6, 0.6),
      xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
      ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""),
      pch = 16, cex = 1.0, type = "n", cex.lab = 1.2, cex.axis = 0.8, axes = FALSE)

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

# Add points & labels
points(jitter(LDW.pcoa$points[, 1], amount = 0.03),
       jitter(LDW.pcoa$points[, 2], amount = 0.03),
       pch = shapes, cex = 2, bg = colors, col = colors)
text(jitter(LDW.pcoa$points[, 1], amount = 0.08),
     jitter(LDW.pcoa$points[, 2], amount = 0.08),
     cex = 0.7,
     labels = c("Tr AM1", "Tr AM2", "Tr AM3", "Tr AM4", "Tr AM5", "Tr AM6", "Tr AM7",
                "Tr EcM1", "Tr EcM2", "Tr EcM3", "Tr EcM4", "Tr EcM5", "Tr EcM6", "Tr EcM7",
                "Te AM1", "Te AM2", "Te AM3", "Te AM4",
                "Te EcM1", "Te EcM2", "Te EcM3", "Te EcM4"))

```



```
# First we calculate the relative abundances of each species at each site
LDW[] <- lapply(LDW, as.numeric)

LDWREL <- LDW
for (i in 1:nrow(LDW)) {
  LDWREL[i, ] = LDW[i, ] / sum(LDW[i, ])
}

# Now, we use this information to calculate and add species scores
LDWREL <- as.data.frame(LDWREL)
LDWREL[] <- lapply(LDWREL, as.numeric)

LDW.pcoa <- add.spec.scores(LDW.pcoa, LDWREL, method = "pcoa.scores")
??add.spec.scores
#text(LDW.pcoa$cproj[, 1], LDW.pcoa$cproj[, 2],
#      labels = row.names(LDW.pcoa$cproj), col = "black")

spe.corr <- add.spec.scores(LDW.pcoa, LDWREL, method = "cor.scores")$cproj
corrcut <- 0.7 # User-defined cutoff
imp.spp <- spe.corr[abs(spe.corr[, 1]) >= corrcut | abs(spe.corr[, 2]) >= corrcut, ]

# Permutation test for species abundances across axes
fit <- envfit(LDW.pcoa, LDWREL, perm = 999)
```

### 3. Beta-Diversity - Hypothesis testing

```
# Create "Factors" vector
Mycorr <- c(rep("AM", 7), rep("ECM", 7), rep("AM", 4), rep("ECM", 4))
Forest <- c(rep("Tropical", 14), rep("Temperate", 8))
Canopy <- c(1,2,3,4,5,6,7,
            1,2,3,4,5,6,7,
            1,2,3,4,
```

1,2,3,4)

*# Run PERMANOVA with adonis function*

```
adonis2(LDW ~ Canopy, method = "jaccard", permutations = 999)
```

```
## Permutation test for adonis under reduced model
```

```
## Permutation: free
```

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

```
##
```

```
## adonis2(formula = LDW ~ Canopy, permutations = 999, method = "jaccard")
```

```
##           Df SumOfSqs      R2      F Pr(>F)
```

```
## Model      1   0.4781 0.04975 1.0471   0.36
```

```
## Residual  20   9.1323 0.95025
```

```
## Total     21   9.6104 1.00000
```

```
adonis2(LDW ~ Mycorr + Canopy, method = "jaccard", permutations = 999)
```

```
## Permutation test for adonis under reduced model
```

```
## Permutation: free
```

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

```
##
```

```
## adonis2(formula = LDW ~ Mycorr + Canopy, permutations = 999, method = "jaccard")
```

```
##           Df SumOfSqs      R2      F Pr(>F)
```

```
## Model      2   1.8677 0.19434 2.2916 0.001 ***
```

```
## Residual  19   7.7428 0.80566
```

```
## Total     21   9.6104 1.00000
```

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(LDW ~ Forest + Canopy, method = "jaccard", permutations = 999)
```

```
## Permutation test for adonis under reduced model
```

```
## Permutation: free
```

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

```
##
```

```
## adonis2(formula = LDW ~ Forest + Canopy, permutations = 999, method = "jaccard")
```

```
##           Df SumOfSqs      R2      F Pr(>F)
```

```
## Model      2   1.5816 0.16457 1.8714 0.005 **
```

```
## Residual  19   8.0288 0.83543
```

```
## Total     21   9.6104 1.00000
```

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(LDW ~ Mycorr + Forest + Canopy, method = "jaccard", permutations = 999)
```

```
## Permutation test for adonis under reduced model
```

```
## Permutation: free
```

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

```
##
```

```
## adonis2(formula = LDW ~ Mycorr + Forest + Canopy, permutations = 999, method = "jaccard")
```

```
##           Df SumOfSqs      R2      F Pr(>F)
```

```
## Model      3   2.9712 0.30916 2.6851 0.001 ***
```

```
## Residual  18   6.6393 0.69084
```

```
## Total     21   9.6104 1.00000
```

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```

adonis2(LDW ~ Mycorr + Forest * Canopy, method = "jaccard", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ Mycorr + Forest * Canopy, permutations = 999, method = "jaccard")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      4   3.3861 0.35233 2.312 0.001 ***
## Residual  17   6.2244 0.64767
## Total     21   9.6104 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(LDW ~ Forest + Mycorr * Canopy, method = "jaccard", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ Forest + Mycorr * Canopy, permutations = 999, method = "jaccard")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      4   3.4493 0.35891 2.3793 0.001 ***
## Residual  17   6.1611 0.64109
## Total     21   9.6104 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(LDW ~ Mycorr * Forest * Canopy, method = "jaccard", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ Mycorr * Forest * Canopy, permutations = 999, method = "jaccard")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      7   5.3826 0.56007 2.5462 0.001 ***
## Residual  14   4.2279 0.43993
## Total     21   9.6104 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(LDW ~ Canopy, method = "bray", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ Canopy, permutations = 999, method = "bray")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      1   0.4373 0.04716 0.9899 0.441
## Residual  20   8.8351 0.95284
## Total     21   9.2724 1.00000

adonis2(LDW ~ Mycorr + Canopy, method = "bray", permutations = 999)

## Permutation test for adonis under reduced model

```

```

## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ Mycorr + Canopy, permutations = 999, method = "bray")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      2   2.1649 0.23348 2.8937 0.001 ***
## Residual  19   7.1075 0.76652
## Total     21   9.2724 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(LDW ~ Forest + Canopy, method = "bray", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ Forest + Canopy, permutations = 999, method = "bray")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      2   1.7534 0.1891 2.2154 0.005 **
## Residual  19   7.5190 0.8109
## Total     21   9.2724 1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(LDW ~ Mycorr + Forest + Canopy, method = "bray", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ Mycorr + Forest + Canopy, permutations = 999, method = "bray")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      3   3.4810 0.37542 3.6065 0.001 ***
## Residual  18   5.7913 0.62458
## Total     21   9.2724 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(LDW ~ Mycorr + Forest * Canopy, method = "bray", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ Mycorr + Forest * Canopy, permutations = 999, method = "bray")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      4   3.8446 0.41462 3.0103 0.001 ***
## Residual  17   5.4278 0.58538
## Total     21   9.2724 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(LDW ~ Forest + Mycorr * Canopy, method = "bray", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free

```

```

## Number of permutations: 999
##
## adonis2(formula = LDW ~ Forest + Mycorr * Canopy, permutations = 999, method = "bray")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      4   3.9183 0.42258 3.1103 0.001 ***
## Residual  17   5.3540 0.57742
## Total     21   9.2724 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(LDW ~ Mycorr * Forest * Canopy, method = "bray", permutations = 999)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = LDW ~ Mycorr * Forest * Canopy, permutations = 999, method = "bray")
##           Df SumOfSqs      R2      F Pr(>F)
## Model      7   5.9615 0.64293 3.6011 0.001 ***
## Residual  14   3.3109 0.35707
## Total     21   9.2724 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Indicator value
indval <- multipatt(LDW, cluster = 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: 1285
## 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
## Number of species associated to 4 groups: 0
## Number of species associated to 5 groups: 0
## Number of species associated to 6 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 = 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: 1285
## 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
## Number of species associated to 4 groups: 0
## Number of species associated to 5 groups: 0
## Number of species associated to 6 groups: 0
##
## List of species associated to each combination:
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```