

R version 4.5.0 (2025-04-11) -- "How About a Twenty-Six"

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Platform: aarch64-apple-darwin20

#####Permanova Analysis#####

#load packages

> library(vegan)

This is vegan 2.7-2

> library(dplyr)

> library(permute)

#import distance matrix into R from Qiime as .tsv

> bray <- read.table("bray-distance-matrix.tsv",

+ header = TRUE,

+ row.names = 1,

+ sep = "\t",

+ check.names = FALSE)

> jaccard <- read.table("jaccard-distance-matrix.tsv",

+ header = TRUE,

+ row.names = 1,

+ sep = "\t",

+ check.names = FALSE)

> unweighted<- read.table("unweighted-distance-matrix.tsv",

+ header = TRUE,

```
+      row.names = 1,
+
+      sep = "\t",
+
+      check.names = FALSE)

#import metadata

> meta <- read.table("InvasMeta.tsv",
+
+      header = TRUE,
+
+      row.names = 1,
+
+      sep = "\t",
+
+      check.names = FALSE)

#make sure all rows are in both files

> all(rownames(meta) %in% rownames(bray))

[1] TRUE

> all(rownames(bray) %in% rownames(meta))

[1] TRUE

# restrict permutations to site to remove effect of site

> ctrl <- how(nperm = 9999)

> setBlocks(ctrl) <- meta$site

# run permanova

> set.seed(42)

> permanova_fixed <- adonis2(
+
+   bray ~ Inoculant,
+
+   data     = meta,
+
+   permutations = ctrl,
```

```
+ by      = "margin"  
+ )  
> permanova_fixed
```

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

```
Marginal effects of terms
```

```
Blocks: meta$site
```

```
Permutation: free
```

```
Number of permutations: 9999
```

```
adonis2(formula = bray ~ Inoculant, data = meta, permutations = ctrl, by = "margin")
```

Df	SumOfSqs	R2	F	Pr(>F)
Inoculant	1	0.018528	0.12497	1.4282
Residual	10	0.129731	0.87503	
Total	11	0.148259	1.00000	

---

```
#####Partial Mantel Test#####
```

```
#load packages  
library(vegan)  
library(dplyr)  
library(permute)
```

```
#read metadata, with site as numeric
```

```
> meta <- read.table("InvasMetaMantel.txt",  
+ header = TRUE,  
+ row.names = 1,
```

```
+           sep = "\t",
+
+           check.names = FALSE)

# Convert the column to numeric (in case it's a character or factor)

> meta$CopiesPerGSoil <- as.numeric(meta$CopiesPerGSoil)

# Create Euclidean distance from that numeric variable

> copies_dist <- dist(meta$CopiesPerGSoil, method = "euclidean")

# Verify its a distance matrix

> inherits(copies_dist, "dist")

[1] TRUE

#import bc distance matrix

> bray <- read.table("bray-distance-matrix.tsv",
+
+           header = TRUE,
+
+           row.names = 1,
+
+           sep = "\t",
+
+           check.names = FALSE)

#control for site with partial mantel,
#make sure "site" is numeric

> meta$site <- as.numeric(meta$site)

#make a "site" distance matrix

> site_dist <- dist(meta$site, method = "euclidean")

#make sure it's a distance matrix

> inherits(site_dist, "dist")

[1] TRUE
```

```
#run partial mantel  
set.seed(123)  
> mantel.partial(bray, copies_dist, site_dist, permutations = 9999)
```

Partial Mantel statistic based on Pearson's product-moment correlation

Call:

```
mantel.partial(xdis = bray, ydis = copies_dist, zdis = site_dist, permutations = 9999)
```

Mantel statistic r: -0.1775

Significance: 0.8196

Upper quantiles of permutations (null model):

90% 95% 97.5% 99%

0.378 0.459 0.578 0.805

Permutation: free

```
#####Indicator analysis#####
```

```
> library(indicspecies)  
Loading required package: permute  
> library(permute)  
> library(tidyverse)
```

— Attaching core tidyverse packages

tidyverse 2.0.0 —

```
✓ dplyr  1.1.4  ✓ readr  2.1.5  
✓forcats 1.0.1  ✓ stringr 1.5.2  
✓ ggplot2 4.0.0  ✓ tibble  3.3.0  
✓ lubridate 1.9.4  ✓ tidyr  1.3.1  
✓ purrr  1.1.0
```

— Conflicts —

```
tidyverse_conflicts() —
```

```
✗ dplyr::filter() masks stats::filter()  
✗ dplyr::lag()  masks stats::lag()
```

 Use the conflicted package to force all conflicts to become errors

```
> library(dplyr)
```

```
#read metadata
```

```
> meta <- read.table("InvasMeta.txt",  
+ header = TRUE,  
+ row.names = 1,  
+ sep = "\t",  
+ check.names = FALSE,  
+ comment.char = "",
```

```

+           stringsAsFactors = FALSE)

#read feature table

> ft_raw <- read.table("exported-collapsed-table-L7-m2.txt",
+                       header = TRUE,
+                       row.names = 1,
+                       sep = "\t",
+                       check.names = FALSE,
+                       comment.char = "")

#ensure samples x taxa

> clean_ids <- function(x) {
+   x <- trimws(x)
+   x <- gsub("\u00A0", " ", x) # non-breaking space
+   x <- gsub("\s+", " ", x)
+   x
+ }

> rownames(meta) <- clean_ids(rownames(meta))
> rownames(ft_raw) <- clean_ids(rownames(ft_raw))
> colnames(ft_raw) <- clean_ids(colnames(ft_raw))

#If samples are columns (QIIME export), transpose to samples x taxa

> match_rows <- sum(rownames(ft_raw) %in% rownames(meta))
> match_cols <- sum(colnames(ft_raw) %in% rownames(meta))
> FT <- if (match_cols > match_rows) t(as.matrix(ft_raw)) else as.matrix(ft_raw)
> mode(FT) <- "numeric"

```

```

#align samples

> keep_ids <- intersect(rownames(FT), rownames(meta))

> if (length(keep_ids) < 2L) stop("No overlapping sample IDs between FT and metadata.")

> FT <- FT[keep_ids, , drop = FALSE]

> meta <- meta[keep_ids, , drop = FALSE]

#build groups from CopiesPerGSoil (Low/High)

# make numeric safely (strip commas if present)

> meta$CopiesPerGSoil <- as.numeric(gsub(", ", "", as.character(meta$CopiesPerGSoil)))

> if (all(is.na(meta$CopiesPerGSoil))) stop("CopiesPerGSoil is all NA after numeric conversion.")

> group <- cut(meta$CopiesPerGSoil,
+               breaks = c(-Inf, 25000, Inf),
+               labels = c("Low", "High"),
+               include.lowest = TRUE)

#drop samples with NA group (if any)

> has_group <- !is.na(group)

> FT <- FT[has_group, , drop = FALSE]

> meta <- meta[has_group, , drop = FALSE]

> group <- droplevels(group[has_group])

> if (nlevels(group) < 2L || any(table(group) < 2L)) {
+
+   print(table(group))
+
+   stop("Indicator analysis needs at least 2 groups with ≥2 samples each.")
+
+ }

> nlevels(group)

```

```
[1] 2  
> table(group)  
group  
Low High  
6 6
```

```
# run IndVal.g  
> set.seed(123)  
> ctrl <- how(nperm = 9999)  
> indval <- multipatt(FT, cluster = group, func = "IndVal.g", control = ctrl)  
> summary(indval, indvalcomp = TRUE, alpha = 1)
```

#### Multilevel pattern analysis

---

Association function: IndVal.g

Significance level (alpha): 1

Total number of species: 21

Selected number of species: 5

Number of species associated to 1 group: 5

List of species associated to each combination:

Group Low #sps. 1

A

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomeromycetes;f\_Glomeromycete  
s;g\_Glomeromycetes;s\_Paraglomus\_brasiliandum 0.8571

B

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomeromycetes;f\_Glomeromycete  
s;g\_Glomeromycetes;s\_Paraglomus\_brasiliandum 0.5000

stat

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomeromycetes;f\_Glomeromycete  
s;g\_Glomeromycetes;s\_Paraglomus\_brasiliandum 0.655

p.value

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomeromycetes;f\_Glomeromycete  
s;g\_Glomeromycetes;s\_Paraglomus\_brasiliandum 0.302

Group High #sps. 4

A

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_uncultured;s\_ uncultured\_fungus 1.0000

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Diversisporales;f\_Gigasporaceae;g\_Dentiscutata;s\_Dentiscutata\_reticulata 0.6263

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Claroideoglomeracea  
e;g\_Claroideogloimus;s\_Claroideogloimus\_etunicatum 1.0000

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_Glo  
mus;s\_ uncultured\_Glomeromycotina 1.0000

B

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_uncultured;s\_ uncultured\_fungus 0.6667

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Diversisporales;f\_Gigasporaceae;g\_Dentiscutata;s\_Dentiscutata\_reticulata 0.8333

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Claroideoglomeracea  
e;g\_Claroideogloimus;s\_Claroideogloimus\_etunicatum 0.3333

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_Glo  
mus;s\_uncultured\_Glomeromycotina 0.3333

stat

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_unc  
ultured;s\_uncultured\_fungus 0.816

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Diversisporales;f\_Gigasporaceae;g\_  
\_Dentiscutata;s\_Dentiscutata\_reticulata 0.722

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Claroideoglomeracea  
e;g\_Claroideoglmus;s\_Claroideoglmus\_etunicatum 0.577

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_Glo  
mus;s\_uncultured\_Glomeromycotina 0.577

p.value

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_unc  
ultured;s\_uncultured\_fungus 0.0633

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Diversisporales;f\_Gigasporaceae;g\_  
\_Dentiscutata;s\_Dentiscutata\_reticulata 0.1766

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Claroideoglomeracea  
e;g\_Claroideoglmus;s\_Claroideoglmus\_etunicatum 0.4599

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_Glo  
mus;s\_uncultured\_Glomeromycotina 0.4494

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_unc  
ultured;s\_uncultured\_fungus .

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Diversisporales;f\_Gigasporaceae;g\_  
\_Dentiscutata;s\_Dentiscutata\_reticulata

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Claroideoglomeracea  
e;g\_Claroideoglmus;s\_Claroideoglmus\_etunicatum

d\_Eukaryota;p\_Mucoromycota;c\_Glomeromycetes;o\_Glomerales;f\_Glomeraceae;g\_Glo  
mus;s\_uncultured\_Glomeromycotina

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1