eDNA

Jay T. Lennon, Mario E. Muscarella, . . .

12 February, 2017

Analysis of community sequence data to test whether the structure and composition of bacterial communities is affected by extracellular DNA

Setup Work Environment

```
rm(list=ls())
getwd()
```

```
## [1] "/Users/mmuscarella/GitHub/eDNA/code"
```

```
setwd("~/GitHub/eDNA/code")

# Load dependencies
require("vegan")
```

```
## Loading required package: vegan
## Loading required package: permute
## Loading required package: lattice
## This is vegan 2.4-1
```

```
require("plyr")
```

```
## Loading required package: plyr
```

```
require("car")
```

```
## Loading required package: car
```

```
require("grid")
```

```
## Loading required package: grid
```

```
require("png")
```

```
## Loading required package: png
```

```
require("ape")
```

```
## Loading required package: ape
```

```
require("picante")
```

```
## Loading required package: picante
## Loading required package: nlme
```

```
require("ade4")
```

```
## Loading required package: ade4
```

```
##
## Attaching package: 'ade4'

## The following object is masked from 'package:vegan':
##
##     cca
```

```
#require("phytools")
require("phangorn")
```

```
## Loading required package: phangorn

##
## Attaching package: 'phangorn'

## The following objects are masked from 'package:vegan':
##
##     diversity, treedist
```

```
# Source code functions
source("../bin/DiversityFunctions.R")
source("../bin/MothurTools.R")
```

```
## Loading required package: reshape

##
## Attaching package: 'reshape'

## The following objects are masked from 'package:plyr':
##
##     rename, round_any
```

```
source("../bin/phylodiversity2.R")

# Small custom functions
sem <- function(x, ...){sd(x, na.rm = TRUE)/sqrt(length(na.omit(x)))}

# Save Default Plot Settings
opar <- par(no.readonly = TRUE)  # Saves plot defauxlts

# Run All: Select if all section are to be re-run
run.all <- FALSE
```

Phylogenetic Diversity Notes

A multi-fasta file was generated with representative sequences for each OTU. Representatives were picked based on the most abundant unique sequence in each OTU. FastTree was used to construct a phylogenetic tree. Note: This must be done before performing the following analyses. The following parameters were used:

```
Note: eDNA.bac.final.0.03.rep.fasta is larger than the github limits. Please obtain the file from SDA o

Note: The names must be renamed so that they match the OTU names:

> python ./bin/name_change.py "./eDNA.bac.final.0.03.rep.fasta" "./eDNA.bac.final.0.03.rep.rename.fasta

> FastTree -gtr -nt -gamma -fastest eDNA.bac.final.0.03.rep.rename.fasta > eDNA.bac.rename.tree

Output:
```

```
ML-NNI round 14: LogLk = -5469747.038 NNIs 18759 max delta 6.95 Time 4888.39 (final)ax delta 6.954)
Optimize all lengths: LogLk = -5469663.971 Time 5006.96
Gamma(20) LogLk = -5470984.148 alpha = 1.037 rescaling lengths by 1.659
Total time: 5529.61 seconds Unique: 174292/174292 Bad splits: 903/174289 Worst delta-LogLk 10.311
```

Data Input Section

Define Inputs

```
  # Design       = general design file for experiment
  # shared       = OTU table from mothur with sequence similarity clustering
  # tax          = Taxonomy for 97% similarity OTUs
design       <- "../data/eDNA_Design.txt"
shared       <- "../mothur/output/eDNA.bac.final.shared"
tax          <- "../mothur/output/eDNA.bac.final.0.03.taxonomy"
```

Import Design

```
design <- read.delim(design, header=T, row.names=1)
```

Import Shared, Taxonomy, and Phylogeny Files

```
OTU <- read.otu(shared = shared, cutoff = "0.03")
OTU.tax <- read.tax(taxonomy = tax, format = "rdp")
OTU.tre <- read.tree("../mothur/output/eDNA.bac.rename.tree")
```

Remove OTUs with less than two occurences across all sites

```
OTU <- OTU[, which(colSums(OTU) >= 2)]
```

Calculate Coverage Stats

```
cov.seqs <- count.groups(OTU)
cov.mean <- mean(cov.seqs) # 222701
cov.sem <- sem(cov.seqs) # 9560
cov.min <- min(cov.seqs) # 31,475
total.seqs <- sum(cov.seqs) # 12,916,632

# Good's coverage
goods.c <- function(x = ""){
              1 - (apply(OTU, 1, function(x){sum(x == 1)}) / rowSums(x))
}

goods.c.eDNA <- goods.c(OTU)
```

```
mean.good.c <- mean(goods.c.eDNA) # 0.99
min.good.c <- min(goods.c.eDNA) # 0.98
```

Alpha Diversity

Calculate Alpha diversity using Resampling

```
# Mario's resampling code to estimate alpha diversity (used if run.all = T)

if (run.all == TRUE){
  rich <- round(richness.iter(input = OTU, size = 30000,
                              iters = 100, shared = "FALSE"), 3)
  even <- round(evenness.iter(input = OTU, size = 30000,
                              iters = 100, shared = "FALSE",
                              method = "simp_even"), 3)

  rare <- rarefy(OTU, 30000, se = FALSE, MARGIN = 1)

  # Write output to files
  write.table(rich, "../data/rich.txt", sep = "\t",
              col.names = T, row.names = T)
  write.table(even, "../data/even.txt", sep = "\t",
              col.names = T, row.names = T)
}

# Read in alpha diversity files from above
rich2 <- read.table("../data/rich.txt", sep = "\t")
even2 <- read.table("../data/even.txt", sep = "\t")

# Merge data to design and calculate mean and sem per sample
rich.data <- merge(design, rich2, by = "row.names")
row.names(rich.data) <- rich.data$Row.names
rich.data <- rich.data[sort(row.names(rich.data)), ]
rich.mean <- round(apply(rich.data[8:(7 + dim(rich2)[2])], 1, mean, na.rm = TRUE),3)
rich.sem <- round(apply(rich.data[8:(7 + dim(rich2)[2])], 1, sem, na.rm = TRUE), 3)

even.data <- merge(design, even2, by = "row.names")
row.names(even.data) <- even.data$Row.names
even.data <- even.data[sort(row.names(even.data)), ]
even.mean <- round(apply(even.data[8:(7 + dim(even2)[2])], 1, mean, na.rm = TRUE),3)
even.sem <- round(apply(even.data[8:(7 + dim(even2)[2])], 1, sem, na.rm = TRUE),4)

# Make new dataframe merging design file and mean diversity
eDNA.div <- data.frame(design[sort(row.names(design)), ], rich.mean, even.mean)

# Take averages of technial reps
rich.rep.ave <- ddply(eDNA.div, .(env, treat, qPCR.match), summarize, rich = mean(rich.mean))
even.rep.ave <- ddply(eDNA.div, .(env, treat, qPCR.match), summarize, even = mean(even.mean))

# Reshape data
rich.2 <- reshape(rich.rep.ave[,1:4], timevar = "treat",
```

```
                  idvar = c("qPCR.match", "env"), direction = "wide")

even.2 <- reshape(even.rep.ave[,1:4], timevar = "treat",
                  idvar = c("qPCR.match", "env"), direction = "wide")
```

Richness: differences among sites?

```
rich.anova <- aov(rich.2$rich.C ~rich.2$env)
summary(rich.anova)
```

```
##             Df   Sum Sq  Mean Sq F value   Pr(>F)
## rich.2$env   3 34630542 11543514   14.95 5.08e-05 ***
## Residuals   17 13122506   771912
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(rich.anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = rich.2$rich.C ~ rich.2$env)
##
## $`rich.2$env`
##                    diff        lwr        upr     p adj
## sed-feces    2700.5429  1088.4587  4312.6271 0.0009436
## soil-feces   1379.7157  -295.6113  3055.0428 0.1276911
## water-feces  -447.6879 -2059.7721  1164.3963 0.8582474
## soil-sed    -1320.8272 -2833.0962   191.4418 0.0989233
## water-sed   -3148.2308 -4590.1228 -1706.3389 0.0000519
## water-soil  -1827.4037 -3339.6727  -315.1347 0.0151328
```

Richness: does eDNA "inflate" diversity?

```
# Take averages of technial reps
rich.rep.ave <- ddply(eDNA.div, .(env, treat, qPCR.match),
                      summarize, rich = mean(rich.mean))

# Reshape data
rich.2 <- reshape(rich.rep.ave[,1:4], timevar = "treat",
                  idvar = c("qPCR.match", "env"), direction = "wide")

# Calcualte means +/- SEM of control and treated richness
rich.2.ag.mean.control <- aggregate(rich.C ~ env, rich.2, mean)
rich.2.ag.sem.control <- aggregate(rich.C ~ env, rich.2, sem)
rich.2.ag.mean.treatment <- aggregate(rich.E ~ env, rich.2, mean)
rich.2.ag.sem.treatment <- aggregate(rich.E ~ env, rich.2, sem)

# Calculate ratios
rich.2$rich.ratio <- rich.2$rich.E / rich.2$rich.C
```

```r
# Richness table
rich.2.ag.mean <- aggregate(rich.ratio ~ env, rich.2, mean)
rich.2.ag.sem <- aggregate(rich.ratio ~ env, rich.2, sem)
rich.2.ag.95 <- aggregate(rich.ratio ~ env, rich.2,
                  FUN = function(x) t.test(x)$conf.int[1:2])
rich.table <- data.frame(rich.2.ag.mean$env, rich.2.ag.mean$rich.ratio,
                         rich.2.ag.sem$rich.ratio, rich.2.ag.95$rich.ratio)
colnames(rich.table) <- c("env", "mean", "sem", "LCI", "UCI")

# Regression: richness ratio vs. proportion eDNA
eDNA.prop <- read.table("../data/eDNA.prop.txt", sep = "\t", header = T)

eDNA.prop.sub <- eDNA.prop[eDNA.prop$sample.number %in% rich.2$qPCR.match, ]
eDNA.prop.sub2 <- eDNA.prop.sub[order(eDNA.prop.sub$sample.number), ]

rich.reg <- lm(rich.2[order(rich.2$qPCR.match), ]$rich.ratio ~ eDNA.prop.sub2$env + eDNA.prop.sub2$prop

summary(rich.reg)
```

```
##
## Call:
## lm(formula = rich.2[order(rich.2$qPCR.match), ]$rich.ratio ~
##     eDNA.prop.sub2$env + eDNA.prop.sub2$prop)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.24381 -0.09627 -0.00581  0.08514  0.32615
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)               1.07902    0.12570   8.584  2.2e-07 ***
## eDNA.prop.sub2$envsed    -0.18874    0.10774  -1.752   0.0989 .
## eDNA.prop.sub2$envsoil   -0.10213    0.12196  -0.837   0.4147
## eDNA.prop.sub2$envwater -0.09194    0.10728  -0.857   0.4040
## eDNA.prop.sub2$prop      -0.06588    0.19859  -0.332   0.7444
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1659 on 16 degrees of freedom
## Multiple R-squared:  0.1663, Adjusted R-squared:  -0.04211
## F-statistic: 0.798 on 4 and 16 DF,  p-value: 0.5438
```

```r
Anova(rich.reg)
```

```
## Anova Table (Type II tests)
##
## Response: rich.2[order(rich.2$qPCR.match), ]$rich.ratio
##                       Sum Sq Df F value Pr(>F)
## eDNA.prop.sub2$env  0.08657  3   1.048 0.3984
## eDNA.prop.sub2$prop 0.00303  1   0.110 0.7444
## Residuals           0.44055 16
```

```r
# MEM: what is the Anova() function? = prints the anova like table for the model
#                                      Anova uses type II sums of squares
# answer => no correlation between proportion eDNA and richness ratio.
```

```
# maybe a little in sediment
```

Evennes: differences among sites?

```
even.anova <- aov(even.2$even.C ~even.2$env)
summary(even.anova)
```

```
##             Df Sum Sq Mean Sq F value  Pr(>F)
## even.2$env   3 0.1247 0.04158   6.497 0.00397 **
## Residuals   17 0.1088 0.00640
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(even.anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = even.2$even.C ~ even.2$env)
##
## $`even.2$env`
##                     diff         lwr         upr     p adj
## sed-feces   -0.17916667 -0.32595852 -0.03237482 0.0140884
## soil-feces  -0.12695000 -0.27950056  0.02560056 0.1222474
## water-feces -0.21808333 -0.36487518 -0.07129148 0.0029099
## soil-sed     0.05221667 -0.08548629  0.18991963 0.7071690
## water-sed   -0.03891667 -0.17021129  0.09237795 0.8335247
## water-soil  -0.09113333 -0.22883629  0.04656963 0.2723574
```

Evenness: does eDNA "inflate" diversity?

```
# Take averages of technial reps
even.rep.ave <- ddply(eDNA.div, .(env, treat, qPCR.match),
                      summarize, even = mean(even.mean))

# Reshape data
even.2 <- reshape(even.rep.ave[,1:4], timevar = "treat",
                  idvar = c("qPCR.match", "env"), direction = "wide")

# Calcualte means +/- SEM of control and treated richness
even.2.ag.mean.control <- aggregate(even.C ~ env, even.2, mean)
even.2.ag.sem.control <- aggregate(even.C ~ env, even.2, sem)
even.2.ag.mean.treatment <- aggregate(even.E ~ env, even.2, mean)
even.2.ag.sem.treatment <- aggregate(even.E ~ env, even.2, sem)

# Calculate ratios
even.2$even.ratio <- even.2$even.E / even.2$even.C

# Evennes table
even.2.ag.mean <- aggregate(even.ratio ~ env, even.2, mean)
even.2.ag.sem <- aggregate(even.ratio ~ env, even.2, sem)
```

```
even.2.ag.95 <- aggregate(even.ratio ~ env, even.2,
                  FUN = function(x) t.test(x)$conf.int[1:2])
even.table <- data.frame(even.2.ag.mean$env, even.2.ag.mean$even.ratio,
                         even.2.ag.sem$even.ratio, even.2.ag.95$even.ratio)
colnames(even.table) <- c("env", "mean", "sem", "LCI", "UCI")


# Regression: evennes ratio vs. proportion eDNA
even.reg <- lm(even.2[order(even.2$qPCR.match), ]$even.ratio ~ eDNA.prop.sub2$prop + eDNA.prop.sub2$env
summary(even.reg)
```

```
##
## Call:
## lm(formula = even.2[order(even.2$qPCR.match), ]$even.ratio ~
##     eDNA.prop.sub2$prop + eDNA.prop.sub2$env)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.18494 -0.04681 -0.02213  0.05598  0.33792
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)               0.9491753  0.0896313  10.590 1.23e-08 ***
## eDNA.prop.sub2$prop       0.1413973  0.1416141   0.998    0.333
## eDNA.prop.sub2$envsed     0.0618303  0.0768245   0.805    0.433
## eDNA.prop.sub2$envsoil    0.0411972  0.0869705   0.474    0.642
## eDNA.prop.sub2$envwater 0.0006756  0.0764959   0.009    0.993
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1183 on 16 degrees of freedom
## Multiple R-squared:  0.105,  Adjusted R-squared:  -0.1188
## F-statistic: 0.4692 on 4 and 16 DF,  p-value: 0.7576
```

```
# answer => no correlation between proportion eDNA and evenness ratio.
```

Calculate Phylogenetic Alpha Diversity

```
# Test if all OTUs are in tree
sum(colnames(OTU) %in% OTU.tre$tip.label) == length(colnames(OTU) %in% OTU.tre$tip.label)
```

```
## [1] TRUE
```

```
# Root Tree if Needed
is.rooted(OTU.tre)
```

```
## [1] FALSE
```

```
OTU.tre.rooted <- midpoint(OTU.tre)

if (run.all == TRUE){
  OTU.2 <- rrarefy(OTU, 30000)
  OTU.2 <- OTU.2[,which(colSums(OTU.2) > 0)]
  OTU.tre.2 <- prune.sample(OTU.2, OTU.tre.rooted)
```

```
  # Calculate Faith's D
  eDNA.pd <- pd(OTU.2, OTU.tre.2, include.root = F)

  # Write output to files
  write.table(eDNA.pd, "../data/phylo.txt", sep = "\t",
              col.names = T, row.names = T)
}

# Read in alpha diversity files from above
eDNA.pd2 <- read.table("../data/phylo.txt", sep = "\t")

# Make new dataframe merging design file and phylo diversity
eDNA.phylo <- data.frame(design[sort(row.names(design)), ],
                         pd = eDNA.pd2$PD)

# Take averages of technial reps
pd.rep.ave <- ddply(eDNA.phylo, .(env, treat, qPCR.match),
                    summarize, phylo = mean(pd))

# Reshape data
phylo.2 <- reshape(pd.rep.ave[,1:4], timevar = "treat",
                   idvar = c("qPCR.match", "env"), direction = "wide")

# Calcualte means +/- SEM of control and treated richness
phylo.2.ag.mean.control <- aggregate(phylo.C ~ env, phylo.2, mean)
phylo.2.ag.sem.control <- aggregate(phylo.C ~ env, phylo.2, sem)
phylo.2.ag.mean.treatment <- aggregate(phylo.E ~ env, phylo.2, mean)
phylo.2.ag.sem.treatment <- aggregate(phylo.E ~ env, phylo.2, sem)
```

Phylogenetic: differences among sites?

```
faith.anova <- aov(phylo.2$phylo.C ~phylo.2$env)
summary(faith.anova)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## phylo.2$env  3 693939  231313   21.92 4.46e-06 ***
## Residuals   17 179413   10554
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(faith.anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = phylo.2$phylo.C ~ phylo.2$env)
##
## $`phylo.2$env`
##                   diff        lwr        upr     p adj
## sed-feces    492.7041  304.20628  681.20192 0.0000054
## soil-feces   262.8600   66.96729  458.75265 0.0068654
## water-feces  126.1452  -62.35265  314.64299 0.2638046
```

```
## soil-sed    -229.8441 -406.67076  -53.01750 0.0088154
## water-sed   -366.5589 -535.15651 -197.96136 0.0000546
## water-soil  -136.7148 -313.54143   40.11183 0.1637189
```

Phylogenetic Diversity: does eDNA "inflate" diversity?

```
# Calculate ratios
phylo.2$phylo.ratio <- phylo.2$phylo.E / phylo.2$phylo.C

# Richness table
phylo.2.ag.mean <- aggregate(phylo.ratio ~ env, phylo.2, mean)
phylo.2.ag.sem <- aggregate(phylo.ratio ~ env, phylo.2, sem)
phylo.2.ag.95 <- aggregate(phylo.ratio ~ env, phylo.2,
                  FUN = function(x) t.test(x)$conf.int[1:2])
phylo.table <- data.frame(phylo.2.ag.mean$env, phylo.2.ag.mean$phylo.ratio,
                          phylo.2.ag.sem$phylo.ratio, phylo.2.ag.95$phylo.ratio)
colnames(phylo.table) <- c("env", "mean", "sem", "LCI", "UCI")


# Regression: PD ratio vs. proportion eDNA
phylo.reg <- lm(phylo.2[order(phylo.2$qPCR.match), ]$phylo.ratio ~ eDNA.prop.sub2$prop * eDNA.prop.sub2$
summary(phylo.reg)
```

```
##
## Call:
## lm(formula = phylo.2[order(phylo.2$qPCR.match), ]$phylo.ratio ~
##     eDNA.prop.sub2$prop * eDNA.prop.sub2$env)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.13873 -0.05357 -0.01196  0.05997  0.22201
##
## Coefficients:
##                                             Estimate Std. Error t value
## (Intercept)                                  1.12141    0.15416   7.275
## eDNA.prop.sub2$prop                         -0.15341    0.30092  -0.510
## eDNA.prop.sub2$envsed                       -0.09207    0.18954  -0.486
## eDNA.prop.sub2$envsoil                      -0.15157    0.19583  -0.774
## eDNA.prop.sub2$envwater                     -0.24347    0.18909  -1.288
## eDNA.prop.sub2$prop:eDNA.prop.sub2$envsed   -0.20327    0.38449  -0.529
## eDNA.prop.sub2$prop:eDNA.prop.sub2$envsoil   0.04565    0.57247   0.080
## eDNA.prop.sub2$prop:eDNA.prop.sub2$envwater  0.32164    0.37402   0.860
##                                             Pr(>|t|)
## (Intercept)                                 6.23e-06 ***
## eDNA.prop.sub2$prop                            0.619
## eDNA.prop.sub2$envsed                          0.635
## eDNA.prop.sub2$envsoil                         0.453
## eDNA.prop.sub2$envwater                        0.220
## eDNA.prop.sub2$prop:eDNA.prop.sub2$envsed      0.606
## eDNA.prop.sub2$prop:eDNA.prop.sub2$envsoil     0.938
## eDNA.prop.sub2$prop:eDNA.prop.sub2$envwater    0.405
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Residual standard error: 0.1148 on 13 degrees of freedom
## Multiple R-squared:  0.3883, Adjusted R-squared:  0.05886
## F-statistic: 1.179 on 7 and 13 DF,  p-value: 0.378
```

```
# answer => no correlation between proportion eDNA and evenness ratio.
```

Alpha Diversity Plots

Richness plot

```
png(filename="../figures/ratio.richness.png",
    width = 800, height = 600, res = 96*2)

par(mar=c(3, 4, 1, 1) + 0.1)

rich.plot <- plot(rich.table$mean, ylim = c(0.5, 1.5),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 1, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = rich.table$mean, y1 = rich.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = rich.table$mean, y1 = rich.table$UCI, angle = 90,
       length=0.1, lwd = 2)
points(c(1:4), rich.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Richness Ratio'), side = 2,
      outer = F, cex = 1.5, line = 2.5, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = T, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
    labels = c("Gut", "Sediment", "Soil", "Water"), at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/ratio.richness.png")
grid.raster(img)
```

Evenness plot

```
png(filename="../figures/ratio.evenness.png",
    width = 800, height = 600, res = 96*2)

par(mar=c(3, 4, 1, 1) + 0.1)

even.plot <- plot(even.table$mean, ylim = c(0.5, 1.5),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 1, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = even.table$mean, y1 = even.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = even.table$mean, y1 = even.table$UCI, angle = 90,
       length=0.1, lwd = 2)
points(c(1:4), even.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Evenness Ratio'), side = 2,
        outer = F, cex = 1.5, line = 2.5, adj = 0.5)
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = T, at = c(0.5, 1, 1.5))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.5, 1, 1.5))

axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
    labels = c("Gut", "Sediment", "Soil", "Water"), at = c(1, 2, 3, 4))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/ratio.evenness.png")
grid.raster(img)
```

Faith's D plot

```
png(filename="../figures/ratio.phylo.png",
    width = 800, height = 600, res = 96*2)

par(mar=c(3, 4, 1, 1) + 0.1)

phylo.plot <- plot(phylo.table$mean, ylim = c(0.5, 1.5),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 1, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = phylo.table$mean, y1 = phylo.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = phylo.table$mean, y1 = phylo.table$UCI, angle = 90,
       length=0.1, lwd = 2)
points(c(1:4), phylo.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Faiths D Ratio'), side = 2,
        outer = F, cex = 1.5, line = 2.5, adj = 0.5)
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = T, at = c(0.5, 1, 1.5))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.5, 1, 1.5))

axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
    labels = c("Gut", "Sediment", "Soil", "Water"), at = c(1, 2, 3, 4))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```r
graphics.off()

# Show Plot
img <- readPNG("../figures/ratio.phylo.png")
grid.raster(img)
```

Alpha Diversity Multi-Panel Plot

```r
png(filename="../figures/alpha.ratios.png",
    width = 800, height = 1600, res = 96*2)

layout(matrix(c(1:3), byrow = T))
par(mar = c(0.5, 4, 0.5, 1), oma = c(3, 1, 1, 1))

# Richness Panel
rich.plot <- plot(rich.table$mean, ylim = c(0.5, 1.5),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 1, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = rich.table$mean, y1 = rich.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = rich.table$mean, y1 = rich.table$UCI, angle = 90,
       length=0.1, lwd = 2)
points(c(1:4), rich.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Richness Ratio'), side = 2,
      outer = F, cex = 1.5, line = 3, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, tck = -0.02, cex.axis = 1.5, las = 1,
    labels = T, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, tck = -0.02, cex.axis = 0.9, las = 1,
    labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))

# Evenness Panel
even.plot <- plot(even.table$mean, ylim = c(0.5, 1.5),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
```

```
abline(h = 1, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = even.table$mean, y1 = even.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = even.table$mean, y1 = even.table$UCI, angle = 90,
       length=0.1, lwd = 2)
points(c(1:4), even.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Evenness Ratio'), side = 2,
        outer = F, cex = 1.5, line = 3, adj = 0.5)
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    labels = T, at = c(0.5, 1, 1.5))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.5, 1, 1.5))

axis(side = 1, lwd.ticks = 2, tck = -0.02, cex.axis = 0.9, las = 1,
    labels = F, at = c(1, 2, 3, 4))

axis(side = 3, lwd.ticks = 2, tck = -0.02, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))

# Phylo Panel
phylo.plot <- plot(phylo.table$mean, ylim = c(0.5, 1.5),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 1, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = phylo.table$mean, y1 = phylo.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = phylo.table$mean, y1 = phylo.table$UCI, angle = 90,
       length=0.1, lwd = 2)
points(c(1:4), phylo.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Faiths D Ratio'), side = 2,
        outer = F, cex = 1.5, line = 3, adj = 0.5)
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    labels = T, at = c(0.5, 1, 1.5))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.5, 1, 1.5))

axis(side = 1, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    labels = c("Gut", "Sediment", "Soil", "Water"), at = c(1, 2, 3, 4))
```

```
axis(side = 3, lwd.ticks = 2, tck = -0.02, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/alpha.ratios.png")
grid.raster(img)
```

Beta diversity

Taxonomic Beta Diversity

```
# Make presence-absence matrix
OTU.PA <- (OTU > 0) * 1

# Make relative abundence matrix
OTU.REL <- OTU
for (i in 1:dim(OTU)[1]){
  OTU.REL[i,] <- OTU[i,]/sum(OTU[i,])
  }

# Log-transform relative abundances
OTU.REL.log <- decostand(OTU, method="log")

eDNA.bc.dis <- vegdist(OTU.REL.log, method = "bray", binary = "FALSE")
eDNA.dis.mean <- mean(eDNA.bc.dis)

# Principal Coordinates Analysis (PCoA)
eDNA.PCoA <- cmdscale(eDNA.bc.dis, eig = TRUE, k = 3)
explainvar1 <- round(eDNA.PCoA$eig[1] / sum(eDNA.PCoA$eig), 3) * 100
explainvar2 <- round(eDNA.PCoA$eig[2] / sum(eDNA.PCoA$eig), 3) * 100
explainvar3 <- round(eDNA.PCoA$eig[3] / sum(eDNA.PCoA$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

# OTU Scores
otu.scores <- t(cor(eDNA.PCoA$points, OTU.REL))
otu.scores <- as.matrix(otu.scores)[,1:2]
otu.scores <- otu.scores[abs(otu.scores[,1]) > 0.7|abs(otu.scores[,2]) > 0.7,]
```

Phylogenetic Beta Diversity

```
phylo.dist <- read.delim("../mothur/output/eDNA.bac.tree.weighted.phylip.dist",
                         skip = 1, row.names = 1, header = F, strip.white = T)

phylo.dist <- as.dist(phylo.dist)

# Principal Coordinates Analysis (PCoA)
eDNA.phylo.PCoA <- cmdscale(phylo.dist, eig = TRUE, k = 3)
explainvar1 <- round(eDNA.phylo.PCoA$eig[1] /
                       sum(eDNA.phylo.PCoA$eig), 3) * 100
explainvar2 <- round(eDNA.phylo.PCoA$eig[2] /
                       sum(eDNA.phylo.PCoA$eig), 3) * 100
explainvar3 <- round(eDNA.phylo.PCoA$eig[3] /
                       sum(eDNA.phylo.PCoA$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

# OTU Scores
otu.scores <- t(cor(eDNA.phylo.PCoA$points, OTU.REL))
otu.scores <- as.matrix(otu.scores)[,1:2]
otu.scores <- otu.scores[abs(otu.scores[,1]) > 0.7|
                           abs(otu.scores[,2]) > 0.7,]
```

PCoA Plots

Taxonomic PcoA Plot (Supplemental)

```
png(filename="../figures/ordination.png",
    width = 1200, height = 800, res = 96*2)
layout(matrix(1:2, 1, 2), widths = c(9, 3))
par(mar = c(4, 5, 1, 0) + 0.5)

plot(eDNA.PCoA$points[ ,1], eDNA.PCoA$points[ ,2],
     ylim = c(-0.4, 0.4), xlim = c(-0.5, 0.4),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""),
     #xlab = "", ylab = "", xaxt = "n", yaxt = "n",
     pch = 22, cex = 2.0, type = "n", cex.lab = 1.5, cex.axis = 1,
     axes = FALSE)

# Add Axes
axis(side = 1, labels = T, lwd.ticks = 2, cex.axis = 1.25, las = 1)
axis(side = 2, labels = T, lwd.ticks = 2, cex.axis = 1.25, las = 1)
axis(side = 3, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=-0.02)
axis(side = 4, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=-0.02)
axis(side = 1, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=0.01)
axis(side = 2, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=0.01)
axis(side = 3, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=0.01)
axis(side = 4, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=0.01)
abline(h = 0, v = 0, lty = 3)
box(lwd = 2)
```

```
# Subset data
eDNA.div.sort <- eDNA.div[order(eDNA.div[,1]) ,]
all.equal(row.names(eDNA.PCoA$points), rownames(eDNA.div.sort))
eDNA.points <- data.frame(eDNA.PCoA$points, eDNA.div.sort)

# Gut
eDNA.gut.C <- eDNA.points[ which(eDNA.points$env == "feces" &
                                   eDNA.points$treat == "C"), ]
eDNA.gut.E <- eDNA.points[ which(eDNA.points$env == "feces" &
                                   eDNA.points$treat == "E"), ]
# Sed
eDNA.sed.C <- eDNA.points[ which(eDNA.points$env == "sed" &
                                   eDNA.points$treat == "C"), ]
eDNA.sed.E <- eDNA.points[ which(eDNA.points$env == "sed" &
                                   eDNA.points$treat == "E"), ]
# Soil
eDNA.soil.C <- eDNA.points[ which(eDNA.points$env == "soil" &
                                    eDNA.points$treat == "C"), ]
eDNA.soil.E <- eDNA.points[ which(eDNA.points$env == "soil" &
                                    eDNA.points$treat == "E"), ]
# Water
eDNA.water.C <- eDNA.points[ which(eDNA.points$env == "water" &
                                     eDNA.points$treat == "C"), ]
eDNA.water.E <- eDNA.points[ which(eDNA.points$env == "water" &
                                     eDNA.points$treat == "E"), ]

# Add points
# Gut C
points(eDNA.gut.C[ ,1], eDNA.gut.C[ ,2], pch = 21,
       cex = 2, col = "red", bg = "white", lwd = 2)
# Gut E
points(eDNA.gut.E[ ,1], eDNA.gut.E[ ,2], pch = 21,
       cex = 2, col = "red", bg = "red", lwd = 2)
# Sed C
points(eDNA.sed.C[ ,1], eDNA.sed.C[ ,2], pch = 22,
       cex = 2, col = "darkgreen", bg = "white", lwd = 2)
# Sed E
points(eDNA.sed.E[ ,1], eDNA.sed.E[ ,2], pch = 22,
       cex = 2, col = "darkgreen", bg = "darkgreen", lwd = 2)
# Soil C
points(eDNA.soil.C[ ,1], eDNA.soil.C[ ,2], pch = 24,
       cex = 2, col = "brown", bg = "white", lwd = 2)
# Soil E
points(eDNA.soil.E[ ,1], eDNA.soil.E[ ,2], pch = 24,
       cex = 2, col = "brown", bg = "brown", lwd = 2)
# Water C
points(eDNA.water.C[ ,1], eDNA.water.C[ ,2], pch = 23,
       cex = 2, col = "blue", bg = "white", lwd = 2)
# Soil E
points(eDNA.water.E[ ,1], eDNA.water.E[ ,2], pch = 23,
       cex = 2, col = "blue", bg = "blue", lwd = 2)

# Add Legend Outside
```

```
par(mar = c(4, 0, 1, 1) + 0.5)
plot.new()
legend(0, 1, c("Gut", "Sediment", "Soil", "Water"),
       pch = c(21, 22, 24, 23),
       pt.bg = c("red", "darkgreen", "brown", "blue"),
       bty = "n", y.intersp = 1.5)
legend(0, 0.25, c("Control", "DNase"), pch = 22,
       pt.bg = c("white", "black"),
       bty = "n", y.intersp = 1.5)

# Close Plot Device
dev.off()
graphics.off()

# Show Plot
img <- readPNG("../figures/ordination.png")
grid.raster(img)
```

Phylogenetic PcoA Plot (Supplemental)

```
png(filename="../figures/phylo.ordination.png",
    width = 1200, height = 800, res = 96*2)
layout(matrix(1:2, 1, 2), widths = c(9, 3))
par(mar = c(4, 5, 1, 0) + 0.5)

plot(eDNA.phylo.PCoA$points[ ,1], eDNA.phylo.PCoA$points[ ,2],
     ylim = c(-0.3, 0.5), xlim = c(-0.3, 0.6),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""),
     #xlab = "", ylab = "", xaxt = "n", yaxt = "n",
     pch = 22, cex = 2.0, type = "n", cex.lab = 1.5, cex.axis = 1,
     axes = FALSE)

# Add Axes
axis(side = 1, labels = T, lwd.ticks = 2, cex.axis = 1.25, las = 1)
axis(side = 2, labels = T, lwd.ticks = 2, cex.axis = 1.25, las = 1)
axis(side = 3, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=-0.02)
axis(side = 4, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=-0.02)
axis(side = 1, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=0.01)
axis(side = 2, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=0.01)
axis(side = 3, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=0.01)
axis(side = 4, labels = F, lwd.ticks = 2, cex.axis = 1, las = 1, tck=0.01)
abline(h = 0, v = 0, lty = 3)
box(lwd = 2)

# Subset data
eDNA.div.sort <- eDNA.div[order(eDNA.div[,1]) ,]
all.equal(row.names(eDNA.phylo.PCoA$points), rownames(eDNA.div.sort))
eDNA.points <- data.frame(eDNA.phylo.PCoA$points, eDNA.div.sort)

# Gut
eDNA.gut.C <- eDNA.points[ which(eDNA.points$env == "feces" &
```

```
                                    eDNA.points$treat == "C"), ]
eDNA.gut.E <- eDNA.points[ which(eDNA.points$env == "feces" &
                                    eDNA.points$treat == "E"), ]
# Sed
eDNA.sed.C <- eDNA.points[ which(eDNA.points$env == "sed" &
                                    eDNA.points$treat == "C"), ]
eDNA.sed.E <- eDNA.points[ which(eDNA.points$env == "sed" &
                                    eDNA.points$treat == "E"), ]
# Soil
eDNA.soil.C <- eDNA.points[ which(eDNA.points$env == "soil" &
                                     eDNA.points$treat == "C"), ]
eDNA.soil.E <- eDNA.points[ which(eDNA.points$env == "soil" &
                                     eDNA.points$treat == "E"), ]
# Water
eDNA.water.C <- eDNA.points[ which(eDNA.points$env == "water" &
                                      eDNA.points$treat == "C"), ]
eDNA.water.E <- eDNA.points[ which(eDNA.points$env == "water" &
                                     eDNA.points$treat == "E"), ]

# Add points
# Gut C
points(eDNA.gut.C[ ,1], eDNA.gut.C[ ,2], pch = 21,
       cex = 2, col = "red", bg = "white", lwd = 2)
# Gut E
points(eDNA.gut.E[ ,1], eDNA.gut.E[ ,2], pch = 21,
       cex = 2, col = "red", bg = "red", lwd = 2)
# Sed C
points(eDNA.sed.C[ ,1], eDNA.sed.C[ ,2], pch = 22,
       cex = 2, col = "darkgreen", bg = "white", lwd = 2)
# Sed E
points(eDNA.sed.E[ ,1], eDNA.sed.E[ ,2], pch = 22,
       cex = 2, col = "darkgreen", bg = "darkgreen", lwd = 2)
# Soil C
points(eDNA.soil.C[ ,1], eDNA.soil.C[ ,2], pch = 24,
       cex = 2, col = "brown", bg = "white", lwd = 2)
# Soil E
points(eDNA.soil.E[ ,1], eDNA.soil.E[ ,2], pch = 24,
       cex = 2, col = "brown", bg = "brown", lwd = 2)
# Water C
points(eDNA.water.C[ ,1], eDNA.water.C[ ,2], pch = 23,
       cex = 2, col = "blue", bg = "white", lwd = 2)
# Soil E
points(eDNA.water.E[ ,1], eDNA.water.E[ ,2], pch = 23,
       cex = 2, col = "blue", bg = "blue", lwd = 2)

# Add Labels to Test Outgroups
# text(eDNA.gut.C[ ,1], eDNA.gut.C[ ,2], labels = eDNA.gut.C$label)
# text(eDNA.gut.E[ ,1], eDNA.gut.E[ ,2], labels = eDNA.gut.E$label)

# Add Legend Outside
par(mar = c(4, 0, 1, 1) + 0.5)
plot.new()
legend(0, 1, c("Gut", "Sediment", "Soil", "Water"),
```

```
       pch = c(21, 22, 24, 23),
       pt.bg = c("red", "darkgreen", "brown", "blue"),
       bty = "n", y.intersp = 1.5)
legend(0, 0.25, c("Control", "DNase"), pch = 22,
       pt.bg = c("white", "black"),
       bty = "n", y.intersp = 1.5)

# Close Plot Device
dev.off()
graphics.off()

# Show Plot
img <- readPNG("../figures/phylo.ordination.png")
grid.raster(img)
```

Beta Diversity Statistics

PERMANOVA: Taxonomic

```
# Check Order of Dataframes
all.equal(row.names(eDNA.div), row.names(OTU.REL.log))
```

```
## [1] TRUE
```

```
eDNA.permanova <- adonis(OTU.REL.log ~ eDNA.div$env * eDNA.div$treat,
                         method = "bray", binary = FALSE)
eDNA.permanova
```

```
##
## Call:
## adonis(formula = OTU.REL.log ~ eDNA.div$env * eDNA.div$treat,      method = "bray", binary = FALSE)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                              Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## eDNA.div$env                  3    7.6829 2.56095  9.3326 0.35195  0.001
## eDNA.div$treat                1    0.1142 0.11422  0.4163 0.00523  1.000
## eDNA.div$env:eDNA.div$treat   3    0.3119 0.10398  0.3789 0.01429  1.000
## Residuals                    50   13.7205 0.27441         0.62853
## Total                        57   21.8295                 1.00000
##
## eDNA.div$env                 ***
## eDNA.div$treat
## eDNA.div$env:eDNA.div$treat
## Residuals
## Total
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
eDNA.bc.dis <- vegdist(OTU.REL.log, method = "bray", binary = "FALSE")
beta.disp <- betadisper(d = eDNA.bc.dis, group = eDNA.div$env)
permutest(beta.disp, 99)
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df Sum Sq  Mean Sq      F N.Perm Pr(>F)
## Groups     3 0.2137 0.071233 13.787    999  0.001 ***
## Residuals 54 0.2790 0.005167
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##            feces        sed       soil water
## feces            2.0000e-03 1.0000e-03 0.004
## sed   5.7913e-04            4.4000e-02 0.221
## soil  2.0056e-05 4.2912e-02            0.002
## water 2.2807e-03 2.2940e-01 1.9272e-03
```

```
TukeyHSD(beta.disp)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = distances ~ group, data = df)
##
## $group
##                     diff         lwr         upr     p adj
## sed-feces   -0.11233229 -0.18206456 -0.04260002 0.0004509
## soil-feces  -0.17047994 -0.24324545 -0.09771443 0.0000005
## water-feces -0.08725668 -0.15462445 -0.01988891 0.0061528
## soil-sed    -0.05814765 -0.13310759  0.01681228 0.1806561
## water-sed    0.02507561 -0.04465666  0.09480788 0.7762587
## water-soil   0.08322326  0.01045775  0.15598877 0.0189601
```

PERMANOVA: Phylogenetic

```
# Check Order of Dataframes
all.equal(row.names(eDNA.div), row.names(as.matrix(phylo.dist)))
```

```
## [1] TRUE
```

```
eDNA.permanova <- adonis(phylo.dist ~ eDNA.div$env * eDNA.div$treat,
                         binary = FALSE)
eDNA.permanova
```

```
##
## Call:
## adonis(formula = phylo.dist ~ eDNA.div$env * eDNA.div$treat,      binary = FALSE)
```

```
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                             Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## eDNA.div$env                 3    4.1920 1.39733  31.821 0.65359  0.001
## eDNA.div$treat               1    0.0091 0.00907   0.207 0.00141  0.981
## eDNA.div$env:eDNA.div$treat  3    0.0171 0.00571   0.130 0.00267  1.000
## Residuals                   50    2.1956 0.04391         0.34233
## Total                       57    6.4138                 1.00000
##
## eDNA.div$env                ***
## eDNA.div$treat
## eDNA.div$env:eDNA.div$treat
## Residuals
## Total
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
beta.disp <- betadisper(d = phylo.dist, group = eDNA.div$env)
permutest(beta.disp, 99)
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
## Groups     3 0.26109 0.087030 13.403    999  0.001 ***
## Residuals 54 0.35064 0.006493
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##            feces        sed       soil water
## feces            0.00100000 0.00200000 0.001
## sed   0.00025791            0.46900000 0.999
## soil  0.00165032 0.43147784            0.525
## water 0.00016369 0.99827983 0.49095914
```

```
TukeyHSD(beta.disp)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = distances ~ group, data = df)
##
## $group
##                      diff         lwr         upr     p adj
## sed-feces   -1.535453e-01 -0.23171919 -0.07537138 0.0000179
## soil-feces  -1.398666e-01 -0.22144095 -0.05829226 0.0001797
```

```
## water-feces -1.535844e-01 -0.22910759 -0.07806125 0.0000093
## soil-sed     1.367868e-02 -0.07035574  0.09771310 0.9728051
## water-sed   -3.913702e-05 -0.07821304  0.07813477 1.0000000
## water-soil  -1.371782e-02 -0.09529216  0.06785653 0.9701593
```

Mantel Test: Taxonomic

```
# Check order of design
all.equal(row.names(eDNA.div), row.names(OTU.REL.log))
```

```
## [1] TRUE
```

```
# Subset OTU Matrix for Each Molecule
OTU.REL.c <- OTU.REL.log[which(eDNA.div$treat == "C"), ]
OTU.REL.e <- OTU.REL.log[which(eDNA.div$treat == "E"), ]

# Subset the Design Matrix
eDNA.div.c <- eDNA.div[which(eDNA.div$treat == "C"), ]
eDNA.div.e <- eDNA.div[which(eDNA.div$treat == "E"), ]

# Check Order of Subsets
all.equal(sort(eDNA.div.c$samp.code), sort(eDNA.div.e$samp.code))
```

```
## [1] TRUE
```

```
# Make Sure Subset OTU Matrices are Aligned by Sample Code
OTU.REL.c2 <- OTU.REL.c[order(eDNA.div.c$samp.code), ]
OTU.REL.e2 <- OTU.REL.e[order(eDNA.div.e$samp.code), ]

# Calculate Bray-Curtis Distances
dist.c <- vegdist(OTU.REL.c2, "bray")
dist.e <- vegdist(OTU.REL.e2, "bray")

# Run Mantel Test of Distance Matrix
mantel.rtest(dist.c, dist.e, nrepet = 999)
```

```
## Monte-Carlo test
## Observation: 0.9589581
## Call: mantel.rtest(m1 = dist.c, m2 = dist.e, nrepet = 999)
## Based on 999 replicates
## Simulated p-value: 0.001
```

Mantel Test: Phylogenetic

```
# Turn into square matrix
phylo.dist.m <- as.matrix(phylo.dist)

# Check order of design
all.equal(row.names(eDNA.div), row.names(phylo.dist.m))
```

```
## [1] TRUE
```

```
# Subset Phylo Distance Matrix Based on Design
dist.c <- phylo.dist.m[which(eDNA.div$treat == "C"),
```

```
                        which(eDNA.div$treat == "C")]
dist.e <- phylo.dist.m[which(eDNA.div$treat == "E"),
                        which(eDNA.div$treat == "E")]

# Define Order Based on Pairs
ord.c <- order(as.numeric(eDNA.div$pair[which(eDNA.div$treat =="C")]))
ord.e <- order(as.numeric(eDNA.div$pair[which(eDNA.div$treat =="E")]))

# Reorder Distance Matices
dist.c <- dist.c[ord.c, ord.c]
dist.e <- dist.e[ord.e, ord.e]

# Turn Square into Lower Triangle Matrix
dist.c <- as.dist(dist.c)
dist.e <- as.dist(dist.e)

# Run Mantel Test of Distance Matrix
mantel.rtest(dist.c, dist.e, nrepet = 999)
```

```
## Monte-Carlo test
## Observation: 0.9955652
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 999 replicates
## Simulated p-value: 0.001
```

Centroid Distances Ratios: Taxonomic

```
# Data Check
all.equal(row.names(eDNA.div), row.names(OTU.REL.log))
```

```
## [1] TRUE
```

```
eDNA.dist <- vegdist(OTU.REL.log, method = "bray")
eDNA.env <- eDNA.div$env
eDNA.group <- paste(eDNA.div$env, eDNA.div$treat, sep = "")

disp.env <- betadisper(eDNA.dist, eDNA.env)
disp.group <- betadisper(eDNA.dist, eDNA.group)

# Soil
env.soi <- which(eDNA.env == "soil")
cent.soil <- as.data.frame(matrix(NA, nrow = length(env.soi), ncol = 3))
colnames(cent.soil) <- c("T", "C", "E")
rownames(cent.soil) <- rownames(eDNA.div)[env.soi]

for (i in 1:length(env.soi)){
  cent.soil[i, 1] <- dist(rbind(disp.env$vectors[env.soi[i]],
                      disp.env$centroids[3, ]), method = "euclidean")
  cent.soil[i, 2] <- dist(rbind(disp.group$vectors[env.soi[i]],
                      disp.group$centroids[5, ]), method = "euclidean")
  cent.soil[i, 3] <- dist(rbind(disp.group$vectors[env.soi[i]],
                      disp.group$centroids[6, ]),  method = "euclidean")
}
```

```
pairs.soil <- eDNA.div[env.soi, c(3,4,5,7)]

cent.soil.ratio <- as.data.frame(matrix(NA,
                   nrow = length(unique(pairs.soil$pair)), ncol = 3))
colnames(cent.soil.ratio) <- c("T", "C", "E")
rownames(cent.soil.ratio) <- paste(pairs.soil$env, pairs.soil$pair,
                             sep = "")[which(pairs.soil$treat == "E") ]

for (i in 1:dim(cent.soil.ratio)[1]){
  pair <- as.numeric(gsub("soil", "", rownames(cent.soil.ratio)[i]))
  ratio.temp.1 <- (cent.soil[, 1][which(pairs.soil$pair == pair &
                                        pairs.soil$treat == "C")]) /
                  (cent.soil[, 1][which(pairs.soil$pair == pair &
                                        pairs.soil$treat == "E")])
  cent.soil.ratio[i, 1] <- ratio.temp.1
  ratio.temp.2 <- (cent.soil[, 2][which(pairs.soil$pair == pair &
                                        pairs.soil$treat == "C")]) /
                  (cent.soil[, 2][which(pairs.soil$pair == pair &
                                        pairs.soil$treat == "E")])
  cent.soil.ratio[i, 2] <- ratio.temp.2
  ratio.temp.3 <- (cent.soil[, 3][which(pairs.soil$pair == pair &
                                        pairs.soil$treat == "C")]) /
                  (cent.soil[, 3][which(pairs.soil$pair == pair &
                                        pairs.soil$treat == "E")])
  cent.soil.ratio[i, 3] <- ratio.temp.3
}

# Water
env.wat <- which(eDNA.env == "water")
cent.water <- as.data.frame(matrix(NA, nrow = length(env.wat), ncol = 3))
colnames(cent.water) <- c("T", "C", "E")
rownames(cent.water) <- rownames(eDNA.div)[env.wat]

for (i in 1:length(env.wat)){
  cent.water[i, 1] <- dist(rbind(disp.env$vectors[env.wat[i]],
                      disp.env$centroids[4, ]), method = "euclidean")
  cent.water[i, 2] <- dist(rbind(disp.group$vectors[env.wat[i]],
                      disp.group$centroids[7, ]), method = "euclidean")
  cent.water[i, 3] <- dist(rbind(disp.group$vectors[env.wat[i]],
                      disp.group$centroids[8, ]), method = "euclidean")
}

pairs.water <- eDNA.div[env.wat, c(3,4,5,7)]

cent.water.ratio <- as.data.frame(matrix(NA,
                    nrow = length(unique(pairs.water$pair)), ncol = 3))
colnames(cent.water.ratio) <- c("T", "C", "E")
rownames(cent.water.ratio) <- paste(pairs.water$env, pairs.water$pair,
                              sep = "")[which(pairs.water$treat == "E") ]

for (i in 1:dim(cent.water.ratio)[1]){
  pair <- as.numeric(gsub("water", "", rownames(cent.water.ratio)[i]))
  ratio.temp.1 <- (cent.water[, 1][which(pairs.water$pair == pair &
```

```
                                        pairs.water$treat == "C")]) /
                  (cent.water[, 1][which(pairs.water$pair == pair &
                                        pairs.water$treat == "E")])
  cent.water.ratio[i, 1] <- ratio.temp.1
  ratio.temp.2 <- (cent.water[, 2][which(pairs.water$pair == pair &
                                        pairs.water$treat == "C")]) /
                  (cent.water[, 2][which(pairs.water$pair == pair &
                                        pairs.water$treat == "E")])
  cent.water.ratio[i, 2] <- ratio.temp.2
  ratio.temp.3 <- (cent.water[, 3][which(pairs.water$pair == pair &
                                        pairs.water$treat == "C")]) /
                  (cent.water[, 3][which(pairs.water$pair == pair &
                                        pairs.water$treat == "E")])
  cent.water.ratio[i, 3] <- ratio.temp.3
}

# Sediments
env.sed <- which(eDNA.env == "sed")
cent.sed <- as.data.frame(matrix(NA, nrow = length(env.sed), ncol = 3))
colnames(cent.sed) <- c("T", "C", "E")
rownames(cent.sed) <- rownames(eDNA.div)[env.sed]

for (i in 1:length(env.sed)){
  cent.sed[i, 1] <- dist(rbind(disp.env$vectors[env.sed[i]],
                         disp.env$centroids[2, ]), method = "euclidean")
  cent.sed[i, 2] <- dist(rbind(disp.group$vectors[env.sed[i]],
                         disp.group$centroids[3, ]), method = "euclidean")
  cent.sed[i, 3] <- dist(rbind(disp.group$vectors[env.sed[i]],
                         disp.group$centroids[4, ]), method = "euclidean")
}

pairs.sed <- eDNA.div[env.sed, c(3,4,5,7)]

cent.sed.ratio <- as.data.frame(matrix(NA,
                  nrow = length(unique(pairs.sed$pair)), ncol = 3))
colnames(cent.sed.ratio) <- c("T", "C", "E")
rownames(cent.sed.ratio) <- paste(pairs.sed$env, pairs.sed$pair,
                            sep = "")[which(pairs.sed$treat == "E") ]

for (i in 1:dim(cent.sed.ratio)[1]){
  pair <- as.numeric(gsub("sed", "", rownames(cent.sed.ratio)[i]))
  ratio.temp.1 <- (cent.sed[, 1][which(pairs.sed$pair == pair &
                                       pairs.sed$treat == "C")]) /
                  (cent.sed[, 1][which(pairs.sed$pair == pair &
                                       pairs.sed$treat == "E")])
  cent.sed.ratio[i, 1] <- ratio.temp.1
  ratio.temp.2 <- (cent.sed[, 2][which(pairs.sed$pair == pair &
                                       pairs.sed$treat == "C")]) /
                  (cent.sed[, 2][which(pairs.sed$pair == pair &
                                       pairs.sed$treat == "E")])
  cent.sed.ratio[i, 2] <- ratio.temp.2
  ratio.temp.3 <- (cent.sed[, 3][which(pairs.sed$pair == pair &
                                       pairs.sed$treat == "C")]) /
```

```
                  (cent.sed[, 3][which(pairs.sed$pair == pair &
                                       pairs.sed$treat == "E")])
  cent.sed.ratio[i, 3] <- ratio.temp.3
}

# Feces
env.fec <- which(eDNA.env == "feces")
cent.feces <- as.data.frame(matrix(NA, nrow = length(env.fec), ncol = 3))
colnames(cent.feces) <- c("T", "C", "E")
rownames(cent.feces) <- rownames(eDNA.div)[env.fec]

for (i in 1:length(env.fec)){
  cent.feces[i, 1] <- dist(rbind(disp.env$vectors[env.fec[i]],
                           disp.env$centroids[1, ]), method = "euclidean")
  cent.feces[i, 2] <- dist(rbind(disp.group$vectors[env.fec[i]],
                           disp.group$centroids[1, ]), method = "euclidean")
  cent.feces[i, 3] <- dist(rbind(disp.group$vectors[env.fec[i]],
                           disp.group$centroids[2, ]), method = "euclidean")
}

pairs.feces <- eDNA.div[env.fec, c(3,4,5,7)]

cent.feces.ratio <- as.data.frame(matrix(NA,
                    nrow = length(unique(pairs.feces$pair)), ncol = 3))
colnames(cent.feces.ratio) <- c("T", "C", "E")
rownames(cent.feces.ratio) <- paste(pairs.feces$env, pairs.feces$pair,
                              sep = "")[which(pairs.feces$treat == "E") ]

for (i in 1:dim(cent.feces.ratio)[1]){
  pair <- as.numeric(gsub("feces", "", rownames(cent.feces.ratio)[i]))
  ratio.temp.1 <- (cent.feces[, 1][which(pairs.feces$pair == pair &
                                         pairs.feces$treat == "C")]) /
                  (cent.feces[, 1][which(pairs.feces$pair == pair &
                                         pairs.feces$treat == "E")])
  cent.feces.ratio[i, 1] <- ratio.temp.1
  ratio.temp.2 <- (cent.feces[, 2][which(pairs.feces$pair == pair &
                                         pairs.feces$treat == "C")]) /
                  (cent.feces[, 2][which(pairs.feces$pair == pair &
                                         pairs.feces$treat == "E")])
  cent.feces.ratio[i, 2] <- ratio.temp.2
  ratio.temp.3 <- (cent.feces[, 3][which(pairs.feces$pair == pair &
                                        pairs.feces$treat == "C")]) /
                  (cent.feces[, 3][which(pairs.feces$pair == pair &
                                         pairs.feces$treat == "E")])
  cent.feces.ratio[i, 3] <- ratio.temp.3
}

# Ratio Data Table
ratio.list <- list(cent.feces.ratio[,1], cent.sed.ratio[,1],
                   cent.soil.ratio[,1], cent.water.ratio[,1])
cent.dist.table <- as.data.frame(matrix(NA, nrow = 4, ncol = 5))
colnames(cent.dist.table) <- c("env", "mean", "sem", "LCI", "UCI")
cent.dist.table$env <- c("Gut", "Sediment", "Soil", "Water")
```

```
cent.dist.table$mean <- unlist(lapply(ratio.list, mean))
cent.dist.table$sem <- unlist(lapply(ratio.list, sem))
cent.dist.table$LCI <- unlist(lapply(ratio.list, FUN = function(x) t.test(x)$conf.int[1]))
cent.dist.table$UCI <- unlist(lapply(ratio.list, FUN = function(x) t.test(x)$conf.int[2]))
t.cent.dist.table <- as.data.frame(cent.dist.table)

# Regression: centroid ratio vs. proportion eDNA
# Combine Centroid Distances
centroid.dists <- c(cent.feces.ratio$T, cent.sed.ratio$T, cent.soil.ratio$T,
                    cent.water.ratio$T)
names(centroid.dists) <- c(rownames(cent.feces.ratio), rownames(cent.sed.ratio),
                           rownames(cent.soil.ratio), rownames(cent.water.ratio))

# Reorganize
names(centroid.dists) %in% paste(eDNA.div.c$env, eDNA.div.c$pair, sep = "")
```

```
##  [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [15] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [29] TRUE
```

```
all.equal(names(centroid.dists), paste(eDNA.div.c$env, eDNA.div.c$pair, sep = ""))
```

```
## [1] "28 string mismatches"
```

```
cent.dist <- centroid.dists[match(names(centroid.dists),
                      paste(eDNA.div.c$env, eDNA.div.c$pair, sep = ""))]

# Combine Centroid Distance and Design Information
centroid.div <- cbind(eDNA.div.c[,c(1:3, 5:7)], cent.dist)

centroid.div$qPCR.match %in% eDNA.prop$sample.number
```

```
##  [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [15] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [29] TRUE
```

```
centroid.div$prop <- NA
for (i in 1:length(centroid.div$prop)){
  centroid.div$prop[i] <- eDNA.prop$prop[which(eDNA.prop$sample.number ==
                                                 centroid.div$qPCR.match[i])]
}

t.cent.reg <- lm(cent.dist ~ prop * env, data = centroid.div)
summary(t.cent.reg)
```

```
##
## Call:
## lm(formula = cent.dist ~ prop * env, data = centroid.div)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.45682 -0.18318 -0.03164  0.10082  0.60954
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)     1.3761     0.2560   5.375 2.48e-05 ***
```

```
## prop           -0.7145     0.4997  -1.430   0.1675
## envsed         -0.7833     0.3596  -2.179   0.0409 *
## envsoil        -0.3089     0.3821  -0.809   0.4278
## envwater       -0.5149     0.3470  -1.484   0.1527
## prop:envsed     1.5065     0.7163   2.103   0.0477 *
## prop:envsoil    1.7538     1.2275   1.429   0.1678
## prop:envwater   0.8900     0.7079   1.257   0.2224
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2696 on 21 degrees of freedom
## Multiple R-squared:  0.3892, Adjusted R-squared:  0.1855
## F-statistic: 1.911 on 7 and 21 DF,  p-value: 0.1185
```

```
# answer => no correlation between proportion eDNA and centroid dist ratio.
```

Centroid Distances Ratios: Phylogenetic

```
# Data Check
all.equal(row.names(eDNA.div), row.names(phylo.dist.m))
```

```
## [1] TRUE
```

```
eDNA.dist <- as.dist(phylo.dist.m)
eDNA.env <- eDNA.div$env
eDNA.group <- paste(eDNA.div$env, eDNA.div$treat, sep = "")

disp.env <- betadisper(eDNA.dist, eDNA.env)
disp.group <- betadisper(eDNA.dist, eDNA.group)

disp.group$vectors
```

```
##               PCoA1        PCoA2        PCoA3         PCoA4         PCoA5
## eDNA_01 -0.13887108 -0.113744265  0.148915828 -0.0187348393  3.229884e-04
## eDNA_02 -0.14432245  0.045760113 -0.092823923  0.0911463532  1.329472e-02
## eDNA_03 -0.13347184  0.019785511 -0.081307708  0.0964246861  2.062611e-02
## eDNA_04 -0.17372768  0.004956970 -0.067521221  0.1492361538  2.050991e-02
## eDNA_05 -0.14740165 -0.084168080  0.182728764 -0.0313473532 -1.818740e-02
## eDNA_06  0.45819121 -0.035639120 -0.015356712  0.0005375485  1.263160e-01
## eDNA_07 -0.08974623  0.018964339 -0.104419835  0.1466444586  1.304599e-02
## eDNA_08 -0.15040514 -0.087445637  0.180724842 -0.0264791122 -1.634051e-02
## eDNA_09 -0.15576427 -0.064524158  0.099536567  0.0002918775 -2.659837e-03
## eDNA_10  0.35786453 -0.126809628 -0.012969061 -0.0619094968  1.152163e-01
## eDNA_11  0.35186294  0.384284549  0.175650888  0.0566234249 -7.220159e-02
## eDNA_12 -0.14703301 -0.078771528  0.147484251 -0.0465827280 -9.900697e-03
## eDNA_13  0.39127600 -0.129312846 -0.024796196 -0.0223439585  1.221936e-01
## eDNA_14  0.44206721 -0.082891258 -0.032157599 -0.0007412091  1.104368e-01
## eDNA_15  0.45496212 -0.082913477 -0.020162441  0.0179377124  1.292853e-01
## eDNA_16  0.46853197 -0.054256805 -0.008367318  0.0156497209  1.437989e-01
## eDNA_17 -0.17608872  0.013122425 -0.062174286  0.0997215035  1.575171e-02
## eDNA_18 -0.14807533  0.086492529 -0.121935470 -0.0054977632  9.192956e-03
## eDNA_19 -0.14272123 -0.089141638  0.154927597 -0.0565422183 -8.919142e-03
## eDNA_20 -0.15465025  0.077572001 -0.115958902  0.0204696922  1.074766e-02
## eDNA_21 -0.16578271 -0.049511758  0.098578089 -0.0115604085 -4.909437e-03
```

```
## eDNA_22 -0.17219486  0.031807886 -0.098361623  0.1141303986  1.857999e-02
## eDNA_23 -0.11041976 -0.102187826  0.055647846  0.0324638239  1.609050e-02
## eDNA_24 -0.11612508 -0.093058323  0.042926097  0.0351115733  1.568372e-02
## eDNA_25  0.30785981 -0.171408873 -0.103526202  0.0032092297 -2.338961e-01
## eDNA_26  0.33403702  0.347440988  0.160835874  0.0541657651 -5.099250e-02
## eDNA_27 -0.06006449  0.135072027 -0.126146457 -0.1842597747  4.697203e-03
## eDNA_28 -0.16559047 -0.078716662  0.152356549 -0.0227536927 -4.244886e-03
## eDNA_29 -0.15756180  0.051118855 -0.038334090 -0.1083715400 -1.755319e-03
## eDNA_30 -0.15838179  0.051663319 -0.035416894 -0.1117616115 -2.455067e-03
## eDNA_31  0.30650883 -0.183486778 -0.100735851  0.0062625963 -2.427458e-01
## eDNA_32  0.34489255 -0.156251158 -0.096239424  0.0074124011 -2.159014e-01
## eDNA_33 -0.05836522  0.133277549 -0.118453616 -0.1949018342  2.492396e-03
## eDNA_34 -0.17145252  0.033055144 -0.099688981  0.1136270064  1.894753e-02
## eDNA_35 -0.16682467 -0.083335244  0.151747418 -0.0143969314 -2.445223e-03
## eDNA_36 -0.15623972  0.082634741 -0.081942896 -0.1012535080 -8.565257e-05
## eDNA_37 -0.14079384  0.088262193 -0.069853679 -0.1696581338 -9.686604e-03
## eDNA_38 -0.14860794 -0.080698010  0.174314413 -0.0321870685 -1.639609e-02
## eDNA_39 -0.15272153 -0.083483936  0.171573213 -0.0273405802 -1.455097e-02
## eDNA_40  0.34946122  0.365871669  0.177840357  0.0804402896 -5.966594e-02
## eDNA_41 -0.17339488  0.016381250 -0.063427103  0.0836172199  1.686201e-02
## eDNA_42 -0.15147358  0.086708077 -0.083081911 -0.1209415415 -2.849224e-03
## eDNA_43 -0.14791033  0.087767347 -0.074780218 -0.1562359456 -6.082842e-03
## eDNA_44 -0.16432929 -0.076944554  0.102897286 -0.0255434767  5.510990e-03
## eDNA_45  0.31485364 -0.185455321 -0.100591424  0.0074105109 -2.359080e-01
## eDNA_46 -0.14801951 -0.111011226  0.139661885 -0.0097495967  1.457497e-03
## eDNA_47 -0.04003714  0.137473461 -0.117780281 -0.2150447065 -1.300527e-03
## eDNA_48  0.33927146 -0.138505246 -0.036791434 -0.0490148993  1.000670e-01
## eDNA_49 -0.14586016  0.026727690 -0.081840798  0.1123185564  1.398562e-02
## eDNA_50 -0.16386689  0.009824799 -0.080469406  0.1590369626  2.147929e-02
## eDNA_51 -0.17180480  0.005516216 -0.055797040  0.1106095390  1.783409e-02
## eDNA_52 -0.07099080  0.050005250 -0.115286169  0.1086473579  9.079120e-03
## eDNA_53 -0.06926685  0.129146016 -0.110350150 -0.1983343776  3.201907e-03
## eDNA_54  0.33412287  0.321800217  0.157048370  0.0715676929 -4.239369e-02
## eDNA_55 -0.14776790 -0.111428197  0.143654063 -0.0194012192  1.303598e-03
## eDNA_56  0.37909964 -0.121710938 -0.021892683 -0.0380869543  1.179231e-01
## eDNA_57 -0.17028834  0.009121198 -0.073849899  0.1277328518  2.068884e-02
## eDNA_58 -0.16644730  0.005198155 -0.074461296  0.1585295727  1.985107e-02
##                 PCoA6        PCoA7         PCoA8         PCoA9
## eDNA_01  0.0016338620 -0.019609578  0.0096218904  2.688449e-02
## eDNA_02 -0.0359885813  0.045306409  0.0424549814 -1.993553e-04
## eDNA_03  0.0644458905 -0.014279183 -0.0578159263 -1.163173e-02
## eDNA_04  0.0109430733 -0.029852960 -0.0074591384 -1.504662e-02
## eDNA_05 -0.0327992221 -0.001837232  0.0228021420 -6.351836e-02
## eDNA_06 -0.0439166536 -0.112445989 -0.0514265136 -2.009659e-02
## eDNA_07 -0.1438317639  0.040989519  0.0552596389 -4.419240e-03
## eDNA_08 -0.0249328895 -0.012566976  0.0266094086 -5.353080e-02
## eDNA_09 -0.0423399252  0.031796370 -0.1175743027  1.009166e-01
## eDNA_10  0.0224962703  0.078631555  0.0413615196  2.255719e-02
## eDNA_11  0.0236773398  0.008632553  0.0101957501  1.982284e-02
## eDNA_12 -0.0475121701  0.047841935 -0.0678258915  7.906557e-03
## eDNA_13  0.0299005460  0.083011266  0.0274643851  5.858388e-03
## eDNA_14 -0.0181826766 -0.058355828 -0.0400860786 -2.410674e-02
## eDNA_15  0.0021803014 -0.028387709 -0.0409299865 -3.045176e-02
## eDNA_16 -0.0092941503 -0.056403098 -0.0452681407 -2.643809e-02
```

```
## eDNA_17  0.0793423243 -0.054255517 -0.0282188398 -2.716187e-02
## eDNA_18  0.0175033047  0.069429834 -0.0270078907  3.038935e-02
## eDNA_19 -0.0299253467  0.032763640 -0.0365545944 -1.864893e-02
## eDNA_20  0.0254975104  0.052554475 -0.0118883334  1.566946e-02
## eDNA_21 -0.0518326927  0.044313159 -0.1233029290  8.701100e-02
## eDNA_22 -0.0020715093  0.001392134 -0.0438221591  7.635016e-05
## eDNA_23  0.0876429650 -0.057646355  0.1184035282  8.234861e-02
## eDNA_24  0.0886070212 -0.075055880  0.1205692711  8.551374e-02
## eDNA_25 -0.0006266059 -0.002691174  0.0005298785  1.982630e-03
## eDNA_26  0.0121422473  0.007255251  0.0052440133  7.487237e-03
## eDNA_27 -0.0575081976 -0.074986634  0.0010362742  2.528342e-02
## eDNA_28 -0.0203506990  0.008541791  0.0187765106 -5.127172e-02
## eDNA_29  0.0612139171  0.036420313 -0.0052891164 -4.745949e-02
## eDNA_30  0.0616921536  0.036355673 -0.0053040353 -4.904813e-02
## eDNA_31  0.0087061259  0.001320261 -0.0088155731 -4.092760e-03
## eDNA_32 -0.0055816087 -0.017989604 -0.0076552118 -3.005899e-03
## eDNA_33 -0.0539545420 -0.076858623  0.0129230600  1.734522e-02
## eDNA_34 -0.0027078941  0.001119881 -0.0405281115 -1.883438e-04
## eDNA_35 -0.0116048566  0.003088284  0.0022183821 -3.488150e-02
## eDNA_36  0.0525239958  0.054776318 -0.0146238296 -2.535990e-04
## eDNA_37  0.0350047431  0.038013059  0.0094035294 -3.208103e-02
## eDNA_38 -0.0270245339 -0.009068394  0.0301552862 -6.292899e-02
## eDNA_39 -0.0141169866 -0.017366085  0.0339065498 -5.270927e-02
## eDNA_40  0.0157702262  0.011906395  0.0092340166  1.029985e-02
## eDNA_41  0.0832517121 -0.020872502 -0.0499166824 -1.007866e-02
## eDNA_42  0.0523634220  0.057787377 -0.0100838524 -1.028667e-02
## eDNA_43  0.0367028127  0.030760164  0.0065317993 -2.565771e-02
## eDNA_44 -0.0018409376 -0.042950918 -0.0008169888  3.966586e-02
## eDNA_45  0.0105136869  0.002111162 -0.0102841515 -5.005653e-03
## eDNA_46  0.0080643443 -0.029185149 -0.0017913136  4.889566e-02
## eDNA_47 -0.0606663523 -0.076225550  0.0188985866  2.371086e-02
## eDNA_48  0.0167548754  0.081713448  0.0418468290  1.957848e-02
## eDNA_49 -0.0332293256  0.026244261  0.0463037317 -5.603277e-03
## eDNA_50 -0.0324355950 -0.009390412  0.0137849007 -9.808737e-03
## eDNA_51  0.0830666471 -0.059998801 -0.0163599305 -2.347848e-02
## eDNA_52 -0.1492251202  0.051971011  0.0819823207  7.237150e-03
## eDNA_53 -0.0507990107 -0.060625882  0.0154873003  1.223096e-02
## eDNA_54  0.0058410983  0.013029014  0.0069882220 -3.747693e-03
## eDNA_55  0.0063753091 -0.017627928  0.0016573764  3.501957e-02
## eDNA_56  0.0208993765  0.075881969  0.0361576063  1.752844e-02
## eDNA_57  0.0087042854 -0.017419553 -0.0142579865 -1.195463e-02
## eDNA_58 -0.0291615407 -0.021004966  0.0170988189 -1.242761e-02
##              PCoA10        PCoA11        PCoA12        PCoA13
## eDNA_01  0.024021703 -5.133890e-03  0.0204391920  0.0238877249
## eDNA_02  0.047570762 -3.511342e-02  0.0122560834  0.0012001612
## eDNA_03 -0.028605292 -5.201966e-02  0.0388949314 -0.0496491757
## eDNA_04 -0.031173906  2.626122e-02 -0.0157379851  0.0379134624
## eDNA_05 -0.014928752 -3.969598e-02 -0.0501515521 -0.0097030325
## eDNA_06  0.061488825 -2.977847e-03  0.0096768923 -0.0115576810
## eDNA_07  0.022538566  1.904297e-02  0.0104337559 -0.0400070322
## eDNA_08 -0.014849757 -4.039063e-02 -0.0440502964 -0.0088334406
## eDNA_09 -0.000925671  6.555052e-03 -0.0497429321 -0.0242836078
## eDNA_10 -0.041229208  5.353806e-03  0.0044348366 -0.0041321481
## eDNA_11  0.007808336  2.996740e-03  0.0191019643  0.0182865631
```

```
## eDNA_12 -0.002418567  4.075164e-02  0.0657996127 -0.0326053527
## eDNA_13 -0.054537698 -5.427675e-03 -0.0037307145  0.0109396602
## eDNA_14  0.032199603 -1.886252e-03 -0.0066401903 -0.0030182660
## eDNA_15  0.052377897  6.331978e-03 -0.0155192305  0.0070618883
## eDNA_16  0.062933609  6.415434e-03 -0.0084675402  0.0067460314
## eDNA_17 -0.040562438 -8.537188e-03  0.0072450596 -0.0326600194
## eDNA_18  0.048578645 -6.238044e-02 -0.0010375193  0.0117862948
## eDNA_19  0.002827383  4.230367e-02  0.0575438216 -0.0277999782
## eDNA_20  0.040416997 -5.875014e-02  0.0009568382  0.0051811642
## eDNA_21 -0.005528948  3.539678e-03 -0.0518393381 -0.0098969066
## eDNA_22 -0.034632985  2.713478e-02 -0.0317086812  0.0052169946
## eDNA_23  0.035195091  2.520837e-02 -0.0164828310 -0.0305404659
## eDNA_24  0.034094661  1.993170e-02 -0.0161321524 -0.0260339489
## eDNA_25 -0.002359715 -3.892424e-03  0.0037291865 -0.0030874771
## eDNA_26  0.002855288 -1.910143e-03 -0.0012313651  0.0010058764
## eDNA_27 -0.048880696 -6.566685e-03  0.0094698828 -0.0008173060
## eDNA_28 -0.010930560  6.698701e-03  0.0489274553  0.0186775580
## eDNA_29  0.025796417  7.897704e-02 -0.0189223324 -0.0275191749
## eDNA_30  0.023503912  7.851801e-02 -0.0152869830 -0.0241148289
## eDNA_31 -0.003575084  6.303433e-04 -0.0025123240  0.0023996388
## eDNA_32  0.019308391  1.036123e-03  0.0065733879 -0.0004516448
## eDNA_33 -0.044476243 -4.475989e-03  0.0012826494 -0.0061104058
## eDNA_34 -0.034016659  2.456598e-02 -0.0284882973  0.0068106459
## eDNA_35 -0.008968516 -4.080839e-03  0.0459403010  0.0260533076
## eDNA_36  0.036963668 -1.509006e-02 -0.0038635281  0.0176985469
## eDNA_37  0.035698568  1.800599e-02 -0.0110930379  0.0164298236
## eDNA_38 -0.009687343 -3.440764e-02 -0.0468034972 -0.0085645510
## eDNA_39 -0.008761610 -3.334874e-02 -0.0393323410 -0.0119547055
## eDNA_40 -0.021209297  3.019325e-03 -0.0009720696  0.0046949794
## eDNA_41 -0.016778265 -4.673119e-02  0.0411543850 -0.0135517766
## eDNA_42  0.050583194 -2.527828e-02 -0.0025768266  0.0235775903
## eDNA_43  0.020856172  1.464289e-02 -0.0103891609  0.0131672443
## eDNA_44 -0.005429068 -5.949592e-03  0.0172307806  0.0352623418
## eDNA_45 -0.005050847  2.848692e-03 -0.0068455581  0.0074661650
## eDNA_46  0.018039550 -6.061671e-03  0.0164227574  0.0351859382
## eDNA_47 -0.036889539 -1.457880e-02  0.0041773152 -0.0031623639
## eDNA_48 -0.076918032 -1.052199e-02  0.0040022590  0.0001543353
## eDNA_49  0.034055620 -2.007944e-02  0.0024526508 -0.0198073878
## eDNA_50 -0.016197872  3.128045e-02 -0.0069956224  0.0519447672
## eDNA_51 -0.037418987 -6.627373e-03  0.0204329234 -0.0319678492
## eDNA_52  0.030819458  2.305175e-03  0.0183681417 -0.0254556754
## eDNA_53 -0.024836825 -9.155484e-04  0.0081023596  0.0045598276
## eDNA_54 -0.018624609  7.263762e-03 -0.0127472657 -0.0215079850
## eDNA_55  0.024043836 -7.662799e-05  0.0344316338  0.0332837763
## eDNA_56 -0.050779271 -4.591402e-03  0.0056484865  0.0033987346
## eDNA_57 -0.023513763  1.454861e-02 -0.0054345510  0.0341442765
## eDNA_58 -0.019880128  4.132942e-02 -0.0103938205  0.0446588687
##                PCoA14        PCoA15        PCoA16        PCoA17
## eDNA_01  0.0303783157  1.671978e-03 -1.365818e-02  0.0079074975
## eDNA_02 -0.0037273997  1.686180e-02 -3.566190e-03  0.0021587737
## eDNA_03  0.0320913404 -1.583357e-02  1.275687e-02  0.0216122082
## eDNA_04 -0.0137822141  2.073378e-02  9.745178e-03 -0.0084722679
## eDNA_05 -0.0070190395  3.669966e-03  6.141829e-03  0.0021387138
## eDNA_06 -0.0152223091 -7.075294e-03  4.085037e-02 -0.0387231939
```

```
## eDNA_07  0.0182663388  2.277032e-04  4.652688e-03  0.0028862241
## eDNA_08 -0.0028007672  4.917110e-03  4.172632e-03  0.0060222743
## eDNA_09  0.0120969579  1.246926e-02  1.761961e-02  0.0050863652
## eDNA_10  0.0045415537 -3.541527e-03  7.752090e-03  0.0016864592
## eDNA_11 -0.0025885802 -8.133641e-03  7.020893e-02  0.0169344185
## eDNA_12 -0.0573323749  2.711996e-02 -1.492742e-02  0.0193567099
## eDNA_13  0.0086359605  7.186226e-03 -8.545110e-03  0.0276488375
## eDNA_14 -0.0056447818  3.866801e-03 -1.916339e-02 -0.0229622695
## eDNA_15  0.0059632082  1.547086e-03 -2.388273e-02  0.0272413402
## eDNA_16 -0.0001654146 -3.249077e-03 -2.196808e-03  0.0290236866
## eDNA_17 -0.0102194529  3.382606e-02 -7.789082e-03 -0.0101093054
## eDNA_18 -0.0237280131 -2.949708e-02 -5.712462e-03 -0.0162720650
## eDNA_19 -0.0496065294 -4.468569e-03  2.471389e-03  0.0118236040
## eDNA_20 -0.0284776381 -1.269781e-02 -2.194183e-02 -0.0165757847
## eDNA_21  0.0169730027  1.318495e-02  1.299468e-02 -0.0159515412
## eDNA_22 -0.0274803080 -6.045582e-02 -9.523255e-03  0.0087545229
## eDNA_23 -0.0230329017 -1.228629e-02  8.485747e-03  0.0033510692
## eDNA_24 -0.0274048962 -1.096271e-02 -3.151843e-03 -0.0017659857
## eDNA_25 -0.0013155961 -1.531829e-04  9.503385e-03 -0.0093841995
## eDNA_26 -0.0011677689  9.168207e-05 -7.089620e-03 -0.0011159685
## eDNA_27 -0.0051513020 -2.484128e-03 -6.559343e-03 -0.0002200829
## eDNA_28 -0.0104231598 -3.012915e-02  3.379169e-03 -0.0228405261
## eDNA_29  0.0382586917 -1.442134e-03  1.087475e-04 -0.0226502736
## eDNA_30  0.0410670361 -7.858714e-03 -7.228448e-04 -0.0211135004
## eDNA_31 -0.0021352291 -1.934564e-04  1.556784e-03 -0.0016057074
## eDNA_32  0.0043792043 -8.239723e-04 -9.188319e-03  0.0133543996
## eDNA_33 -0.0054017869 -3.553079e-03 -7.640323e-03  0.0013066181
## eDNA_34 -0.0274551099 -6.121869e-02 -1.320612e-02  0.0091424739
## eDNA_35 -0.0022557695 -2.739000e-02  4.775526e-03 -0.0269493324
## eDNA_36 -0.0169733547  3.432246e-02 -5.462585e-03 -0.0205055915
## eDNA_37  0.0044485827 -2.762839e-03  1.785803e-02  0.0308950292
## eDNA_38 -0.0015991630 -4.880816e-03  4.522081e-03  0.0034348856
## eDNA_39 -0.0022347488  8.650674e-05  4.098895e-03  0.0042127638
## eDNA_40 -0.0025243646  5.529614e-03 -8.596153e-03 -0.0027437183
## eDNA_41  0.0441344288 -1.697817e-02  9.763337e-03  0.0075328157
## eDNA_42 -0.0140318596  1.536661e-02  1.929134e-03 -0.0096077053
## eDNA_43 -0.0005842972 -1.591869e-03  8.508352e-03  0.0348376893
## eDNA_44  0.0270019643  6.260450e-03 -1.972060e-02  0.0156871748
## eDNA_45 -0.0020623979  4.288653e-05 -4.380896e-03 -0.0008448824
## eDNA_46  0.0363098271 -1.369760e-03 -1.161504e-02 -0.0065786262
## eDNA_47 -0.0033095247  3.976071e-03  5.683443e-03 -0.0078727371
## eDNA_48 -0.0063407147  5.804487e-03  1.918775e-02 -0.0315169510
## eDNA_49 -0.0242241207  3.420331e-02  1.790055e-03  0.0138907333
## eDNA_50 -0.0029269475  2.170028e-02  4.704648e-03 -0.0054096505
## eDNA_51  0.0110321023  3.404355e-02  3.286056e-03 -0.0035609080
## eDNA_52  0.0523347076 -1.352883e-02  1.800880e-03 -0.0036673430
## eDNA_53  0.0051716911  5.908209e-03 -7.638026e-03  0.0129967591
## eDNA_54  0.0099813570  2.977533e-03 -6.551865e-02 -0.0152991949
## eDNA_55  0.0296972848 -3.591946e-03 -1.272628e-02 -0.0026596414
## eDNA_56  0.0025185117 -7.985169e-04 -1.248655e-06 -0.0010090626
## eDNA_57  0.0059798158  4.739107e-03  9.037542e-03  0.0086495390
## eDNA_58 -0.0109120467  2.661518e-02  4.778526e-03 -0.0015855700
##                PCoA18        PCoA19        PCoA20        PCoA21
## eDNA_01  2.343733e-02 -0.0016219990 -2.075460e-03 -0.0095080179
```

```
## eDNA_02 -2.462970e-04 -0.0412676689 -1.391355e-02 -0.0064423192
## eDNA_03  8.797513e-03 -0.0082747558  9.834250e-04  0.0184752332
## eDNA_04  1.141968e-02  0.0077990153  2.408075e-02  0.0164570858
## eDNA_05  8.378250e-03  0.0052841336  5.010824e-03 -0.0018388133
## eDNA_06  2.080348e-02 -0.0193867576  6.520018e-03 -0.0126131484
## eDNA_07 -3.148164e-03  0.0340838758  4.482734e-03 -0.0075566945
## eDNA_08  1.368196e-02  0.0038583258  5.681936e-03 -0.0010524834
## eDNA_09 -1.571690e-02 -0.0109321226 -2.012251e-04  0.0123058243
## eDNA_10 -6.782872e-03  0.0005915104  1.882494e-02  0.0057651483
## eDNA_11  7.812327e-03  0.0155099591 -1.470718e-02 -0.0198111137
## eDNA_12  3.201339e-02  0.0095841772  5.527588e-03 -0.0032502353
## eDNA_13  1.067939e-03  0.0087693774 -1.290157e-02 -0.0142860704
## eDNA_14 -1.074974e-04 -0.0124200299 -5.511094e-03  0.0210143382
## eDNA_15 -1.678517e-02  0.0166953649 -6.213998e-03  0.0082612778
## eDNA_16 -7.790701e-03  0.0199045211 -6.071998e-03 -0.0032820279
## eDNA_17 -2.456396e-02  0.0204652410 -6.499690e-03 -0.0315984797
## eDNA_18 -1.774435e-03  0.0027565179 -4.130075e-04 -0.0043049738
## eDNA_19 -6.249816e-04 -0.0100677313  4.578813e-04  0.0158107309
## eDNA_20  6.157044e-04  0.0091012727 -9.776719e-03 -0.0130631347
## eDNA_21 -2.843812e-02  0.0018583178 -9.874528e-04  0.0009594615
## eDNA_22  1.215311e-02  0.0017627274 -2.013019e-05 -0.0146689126
## eDNA_23 -1.061229e-02 -0.0015995387  6.958982e-03  0.0158799152
## eDNA_24 -3.103512e-06  0.0087753002 -1.028197e-03  0.0078198021
## eDNA_25  4.305669e-04 -0.0137174856  1.361474e-02 -0.0118344920
## eDNA_26 -1.608081e-03  0.0102547283 -1.014015e-02  0.0097423522
## eDNA_27 -5.043143e-03 -0.0008706450 -8.852743e-03 -0.0102472957
## eDNA_28 -3.919410e-02  0.0009981233 -4.012874e-03  0.0065082027
## eDNA_29  1.515574e-02 -0.0050400857 -6.498082e-03 -0.0149813554
## eDNA_30  1.315360e-02 -0.0075882279 -8.583462e-03 -0.0122142429
## eDNA_31  7.896579e-03  0.0117119378 -2.902257e-02  0.0153247818
## eDNA_32 -2.015502e-02 -0.0174652617  5.426229e-02 -0.0276304303
## eDNA_33  3.084752e-03  0.0015589355 -4.948643e-03 -0.0059293447
## eDNA_34  1.031213e-02  0.0001521340 -1.119237e-03 -0.0147784043
## eDNA_35 -4.131580e-02  0.0031410685 -1.434791e-03  0.0080443180
## eDNA_36  1.550176e-02  0.0311181305  1.475837e-02  0.0004550892
## eDNA_37 -2.626548e-02 -0.0171530510 -8.023057e-04  0.0143839173
## eDNA_38  7.183772e-03  0.0012827505  8.338928e-04 -0.0013237950
## eDNA_39  2.188750e-03 -0.0006209452  7.757656e-04 -0.0011050591
## eDNA_40 -2.995046e-04 -0.0068053159  2.160945e-02 -0.0042190072
## eDNA_41  3.576791e-03 -0.0071726546  3.571776e-03  0.0172007819
## eDNA_42  5.297893e-03  0.0190404654  2.159169e-02  0.0116105473
## eDNA_43 -1.693541e-02 -0.0151096950 -4.393710e-03  0.0039498525
## eDNA_44  1.299162e-02 -0.0071201413 -1.467228e-02 -0.0275488424
## eDNA_45  8.295406e-03  0.0175827884 -3.414523e-02  0.0199581784
## eDNA_46  7.733819e-03  0.0037327633 -2.569445e-03 -0.0101088848
## eDNA_47  4.980658e-03 -0.0051388617  1.595352e-03  0.0044575580
## eDNA_48  1.570027e-02 -0.0095094223 -2.238324e-02  0.0072410002
## eDNA_49  1.316711e-03 -0.0395622840 -2.369641e-02 -0.0130179155
## eDNA_50 -1.461524e-02 -0.0093265588 -6.555181e-03  0.0044621399
## eDNA_51 -2.061655e-02  0.0140813531  9.174259e-04 -0.0059894155
## eDNA_52  1.444564e-03  0.0186517721  9.242448e-03  0.0116193998
## eDNA_53 -3.913249e-03  0.0098355198  6.939224e-03  0.0166587146
## eDNA_54 -4.253365e-03 -0.0216810647  9.754456e-05  0.0187858999
## eDNA_55  1.353369e-02  0.0012075129 -2.700215e-04 -0.0054000951
```

```
## eDNA_56 -3.977470e-03 -0.0072263339  2.351892e-02 -0.0149068424
## eDNA_57  4.184143e-02 -0.0113969675  2.171421e-02  0.0322963850
## eDNA_58 -1.701426e-02 -0.0030740152 -9.150526e-03 -0.0009360887
##                PCoA22        PCoA23        PCoA24        PCoA25
## eDNA_01  0.0159572420 -0.0006136865 -0.0022640925 -1.108936e-02
## eDNA_02 -0.0108884602 -0.0257334708  0.0039570570 -9.645022e-03
## eDNA_03  0.0117892963 -0.0100458822  0.0056996241 -8.303572e-03
## eDNA_04 -0.0224668377  0.0136126811  0.0047160613 -1.363016e-02
## eDNA_05  0.0036403972  0.0039656147 -0.0073605405  7.062220e-03
## eDNA_06  0.0144142199 -0.0034558324  0.0134453574  1.124698e-02
## eDNA_07  0.0180530648  0.0144106986  0.0089424849 -9.012854e-03
## eDNA_08  0.0015778040 -0.0022669753 -0.0100861626  3.249333e-03
## eDNA_09 -0.0032327896  0.0039393392  0.0007745108  5.027365e-03
## eDNA_10 -0.0131952043 -0.0214086647 -0.0002889249  2.277982e-03
## eDNA_11 -0.0168848539 -0.0014849796 -0.0174613970 -5.597915e-03
## eDNA_12  0.0059814008  0.0010437719 -0.0146808927  7.539283e-04
## eDNA_13 -0.0026871714  0.0024920215  0.0053363259  5.578391e-03
## eDNA_14  0.0041152608  0.0061377912 -0.0255473788 -1.115286e-02
## eDNA_15 -0.0049449866  0.0043833712 -0.0013023370 -1.135673e-02
## eDNA_16 -0.0088221574 -0.0036194663  0.0133649126  2.799609e-03
## eDNA_17 -0.0059092399  0.0109210090  0.0125169239 -1.164282e-03
## eDNA_18 -0.0051992488  0.0295734553 -0.0092717040  9.417521e-03
## eDNA_19 -0.0014326827  0.0110767060  0.0055925567  1.264658e-02
## eDNA_20 -0.0092153376  0.0259176755  0.0026709495  1.096466e-02
## eDNA_21 -0.0065722866 -0.0093950299  0.0055080580 -6.681769e-03
## eDNA_22  0.0086422865 -0.0174612937 -0.0019515624 -7.980177e-03
## eDNA_23  0.0018310355  0.0048312149 -0.0038830618  1.858929e-07
## eDNA_24 -0.0007427253 -0.0049746148  0.0033376828  2.298615e-03
## eDNA_25  0.0131487705  0.0176525924 -0.0074473504 -3.300648e-02
## eDNA_26 -0.0151896699 -0.0018471470 -0.0218128838 -2.301560e-03
## eDNA_27 -0.0138687877  0.0090070789  0.0014237785 -1.213092e-03
## eDNA_28 -0.0122628005 -0.0122026921  0.0093308871 -1.166859e-02
## eDNA_29 -0.0111027155  0.0023288586 -0.0009316015  5.166156e-04
## eDNA_30 -0.0077457843  0.0015524306 -0.0039636690  7.934622e-03
## eDNA_31 -0.0038625825 -0.0082246081  0.0077451907  1.256227e-02
## eDNA_32 -0.0130531012 -0.0035318525 -0.0116836644  9.287602e-03
## eDNA_33 -0.0107381210  0.0027431919 -0.0008873684  2.676446e-03
## eDNA_34  0.0107232189 -0.0174132350 -0.0031122592 -7.203126e-03
## eDNA_35 -0.0143416278 -0.0037954968  0.0081077568 -1.991033e-03
## eDNA_36  0.0002169072 -0.0297903534  0.0009700752 -5.954018e-03
## eDNA_37  0.0195240004  0.0101952079  0.0065361217 -5.867785e-03
## eDNA_38  0.0010870242  0.0015363471 -0.0008319875  1.586098e-03
## eDNA_39 -0.0018227355  0.0002972303  0.0029423607  3.186199e-04
## eDNA_40  0.0344463874  0.0051070385  0.0389348850  6.947238e-03
## eDNA_41  0.0085045243 -0.0029208336 -0.0167699332  1.921915e-02
## eDNA_42  0.0178345968 -0.0196178526  0.0045779628 -4.682263e-03
## eDNA_43  0.0104440170  0.0198980383  0.0040399873 -1.882698e-03
## eDNA_44 -0.0063099733 -0.0017233928  0.0133564533 -4.222591e-03
## eDNA_45  0.0002728595 -0.0080285148  0.0131623253  1.251212e-02
## eDNA_46  0.0049269602  0.0083396517 -0.0024132691 -4.221016e-04
## eDNA_47  0.0040104788 -0.0118670444  0.0057808592  1.208790e-02
## eDNA_48  0.0195967129  0.0153915244 -0.0008172273 -1.856188e-02
## eDNA_49 -0.0167312154 -0.0133937030  0.0040347387 -4.798884e-03
## eDNA_50  0.0222871407 -0.0017672454 -0.0154598302  1.800763e-02
```

```
## eDNA_51  0.0042094466 -0.0007272151 -0.0070024629 -5.101804e-03
## eDNA_52 -0.0063515272 -0.0004492586 -0.0014082963  6.396795e-03
## eDNA_53  0.0049305013 -0.0056562597 -0.0120577733 -1.078192e-02
## eDNA_54 -0.0012059557 -0.0007872146 -0.0015997457 -1.166162e-03
## eDNA_55  0.0078778358  0.0011157286 -0.0033127791  1.627215e-03
## eDNA_56 -0.0066523357  0.0003565591 -0.0049973532  1.750944e-02
## eDNA_57 -0.0370121234  0.0176851853  0.0196256073 -1.841584e-03
## eDNA_58  0.0204016482 -0.0013081982 -0.0158239858  1.576913e-02
##                PCoA26        PCoA27        PCoA28        PCoA29
## eDNA_01  1.752012e-02  2.328511e-03  0.0010068059  0.0024429797
## eDNA_02  5.938828e-06  9.967441e-03 -0.0033277364  0.0002462371
## eDNA_03  2.058964e-02 -8.008062e-03  0.0182083004 -0.0011379620
## eDNA_04  9.569906e-03  2.006063e-02 -0.0184327442  0.0077910817
## eDNA_05  5.224104e-03 -9.606583e-04  0.0080626657  0.0039031963
## eDNA_06  3.825808e-03  6.762535e-05 -0.0027216490 -0.0021177059
## eDNA_07 -5.265030e-03 -2.212223e-02 -0.0126656927 -0.0097128658
## eDNA_08  8.177578e-04  5.924965e-03 -0.0035796290  0.0011977772
## eDNA_09 -3.908768e-03  6.483779e-04  0.0011633468  0.0038277422
## eDNA_10  1.243135e-02 -7.771283e-03  0.0026644004  0.0013460765
## eDNA_11  4.961814e-03 -6.421282e-03 -0.0040614142  0.0006807783
## eDNA_12  3.380410e-03  8.106056e-03 -0.0067340278  0.0002831559
## eDNA_13 -1.471891e-02  8.941975e-03 -0.0044530143 -0.0155672631
## eDNA_14  1.054698e-02 -1.840085e-02 -0.0130361579 -0.0015717248
## eDNA_15 -6.503153e-03 -3.514896e-03  0.0016390638  0.0031096349
## eDNA_16 -8.079173e-03  2.163185e-02  0.0133586515  0.0034133975
## eDNA_17  1.669585e-03 -1.236705e-02  0.0060139431  0.0035577721
## eDNA_18 -1.676376e-04  1.170597e-03 -0.0007482842  0.0086238815
## eDNA_19 -8.468964e-03 -3.379651e-03  0.0105808572  0.0006129789
## eDNA_20  1.369447e-02  5.917145e-03  0.0065747392 -0.0100239082
## eDNA_21  1.106596e-03  9.064093e-04 -0.0011669006 -0.0026606441
## eDNA_22 -2.767711e-03 -1.300005e-03 -0.0036706874 -0.0007937139
## eDNA_23  1.268844e-02  1.267609e-02  0.0011660451 -0.0229636995
## eDNA_24 -1.602929e-02 -1.195389e-02  0.0013667615  0.0216525485
## eDNA_25 -2.022055e-02  7.698624e-03  0.0150515986 -0.0019986020
## eDNA_26 -6.126328e-03 -2.175581e-03  0.0086970023 -0.0057731325
## eDNA_27  5.471085e-03  1.865083e-03 -0.0016236299  0.0034951576
## eDNA_28  8.728334e-03  3.499331e-03  0.0009812832  0.0020388207
## eDNA_29  2.387338e-03  3.896724e-03  0.0016341606 -0.0011884945
## eDNA_30 -3.350184e-03  2.253776e-03  0.0031959945  0.0011329395
## eDNA_31  7.796744e-03  1.960280e-03 -0.0008120906  0.0036305769
## eDNA_32  1.903631e-03 -4.111450e-03 -0.0009602887 -0.0014552767
## eDNA_33 -3.372602e-03  1.662988e-03  0.0047861035  0.0018833314
## eDNA_34  8.450672e-04  1.918003e-03 -0.0029461940 -0.0003055332
## eDNA_35 -1.272572e-02 -3.432591e-03 -0.0030200214 -0.0044003859
## eDNA_36 -4.224703e-03 -8.295559e-03  0.0065215682 -0.0003328023
## eDNA_37  6.133158e-03 -4.374005e-03 -0.0039618002 -0.0040661467
## eDNA_38  1.024041e-03 -1.690954e-03  0.0038809539 -0.0049636871
## eDNA_39 -8.526226e-03 -1.594721e-03 -0.0063832387  0.0019176390
## eDNA_40  1.862992e-03  6.738803e-03 -0.0032853288  0.0060947441
## eDNA_41 -2.730118e-02  4.523993e-03 -0.0140885596  0.0023027462
## eDNA_42 -8.723947e-03 -9.230941e-04 -0.0015477368 -0.0033891283
## eDNA_43  5.783433e-03 -4.354852e-03 -0.0108667861  0.0084576552
## eDNA_44  3.080980e-04 -1.110979e-02  0.0028375591  0.0002445803
## eDNA_45  9.528110e-03 -3.348358e-03 -0.0116151768 -0.0002216989
```

```
## eDNA_46 -8.544899e-04 -2.585118e-03 -0.0049361480 -0.0057794316
## eDNA_47 -1.030426e-02  1.939705e-03 -0.0043550816 -0.0137172586
## eDNA_48 -7.132961e-03  5.217061e-03  0.0047631431  0.0060639147
## eDNA_49 -6.042068e-03 -3.549486e-03 -0.0057197287  0.0021401804
## eDNA_50  5.459540e-03 -5.265957e-04  0.0176101554  0.0010477156
## eDNA_51  5.193353e-03  1.431933e-02 -0.0097710462 -0.0020935335
## eDNA_52  3.350427e-03  1.494398e-02  0.0061416423  0.0096586716
## eDNA_53  4.554647e-03  2.527983e-03  0.0047894829  0.0061455124
## eDNA_54 -1.111135e-03 -6.150516e-04 -0.0015287240 -0.0017587767
## eDNA_55 -1.740288e-03  2.539289e-03 -0.0022491802  0.0022805791
## eDNA_56  1.045570e-02 -7.485193e-03 -0.0042162760  0.0072105716
## eDNA_57 -7.307375e-03 -1.571564e-02  0.0087081607 -0.0081397011
## eDNA_58 -3.845950e-03 -3.764722e-03  0.0070805854 -0.0023014976
##               PCoA30        PCoA31        PCoA32        PCoA33
## eDNA_01  9.164011e-03  0.0055410895  4.365473e-03 -0.0028366932
## eDNA_02  4.030028e-04 -0.0037923989  3.231901e-03  0.0046554555
## eDNA_03  9.742965e-04  0.0102378175  4.054576e-03  0.0022791850
## eDNA_04 -6.479899e-03  0.0107111184  1.554957e-02  0.0038172240
## eDNA_05  6.269572e-05 -0.0037541029  2.824906e-03  0.0023658219
## eDNA_06  3.708334e-04  0.0021281425 -7.094086e-04 -0.0051655740
## eDNA_07 -1.253515e-03  0.0102844641  6.877158e-04  0.0038820161
## eDNA_08 -1.143687e-03 -0.0007324861 -6.443455e-03 -0.0011309750
## eDNA_09  5.945540e-03  0.0009064582 -9.496556e-04  0.0018642604
## eDNA_10 -1.043215e-02 -0.0010902520 -6.453611e-03 -0.0062226497
## eDNA_11  3.328971e-03 -0.0072727725  2.158488e-03  0.0011898659
## eDNA_12  1.855979e-04 -0.0083654732 -6.747968e-03 -0.0057217745
## eDNA_13  1.448972e-02  0.0022320102  5.986507e-03  0.0057663896
## eDNA_14  5.333854e-03 -0.0058155714 -1.761713e-04  0.0106771075
## eDNA_15 -1.025650e-02 -0.0010674250  4.590584e-03 -0.0146859275
## eDNA_16  1.673597e-03  0.0014857583 -5.800329e-03  0.0083328718
## eDNA_17 -7.839851e-03 -0.0200870659  4.289930e-03  0.0047000682
## eDNA_18 -8.565085e-03  0.0083655107 -2.685042e-03  0.0041733317
## eDNA_19 -3.117564e-03  0.0085433950  1.109840e-02  0.0050215430
## eDNA_20  1.378171e-02 -0.0056803480  3.333029e-03 -0.0054622896
## eDNA_21 -1.400003e-03 -0.0032934524  2.452721e-03 -0.0013980905
## eDNA_22 -1.187293e-03  0.0007320226 -2.595828e-03  0.0034425440
## eDNA_23 -1.532173e-02 -0.0031592461 -6.292406e-03  0.0053714968
## eDNA_24  1.574340e-02  0.0018368588  6.520966e-03 -0.0046545708
## eDNA_25 -1.579193e-03 -0.0005281669 -7.126103e-04 -0.0035906414
## eDNA_26 -3.816155e-03  0.0105710861  3.774291e-03 -0.0028705308
## eDNA_27 -8.489779e-04  0.0053425207 -8.223348e-03 -0.0049251921
## eDNA_28 -4.873900e-04 -0.0062046328  6.186081e-03 -0.0031488570
## eDNA_29  4.043066e-03  0.0046814498  1.147408e-03  0.0034683931
## eDNA_30 -2.024172e-03 -0.0010074977 -8.319373e-04  0.0010094965
## eDNA_31 -1.204500e-03  0.0044030528 -5.251056e-03  0.0034708007
## eDNA_32  3.781500e-03  0.0006347257  2.575446e-03  0.0013239167
## eDNA_33 -7.185677e-03  0.0037649189  1.421026e-03  0.0054096631
## eDNA_34  2.525524e-03 -0.0047316404 -1.671494e-04 -0.0048638836
## eDNA_35  8.069926e-03  0.0069947714 -1.252485e-02  0.0030800507
## eDNA_36 -2.246951e-03  0.0085350472 -5.610598e-03 -0.0035796545
## eDNA_37  7.259065e-03 -0.0048971453  6.778469e-03 -0.0028521457
## eDNA_38 -1.466710e-03  0.0031625973  4.188345e-03 -0.0021673795
## eDNA_39  2.697266e-04  0.0008316986 -3.181576e-03  0.0005054393
## eDNA_40 -1.954772e-04 -0.0009447943 -6.537545e-03  0.0042339200
```

```
## eDNA_41 -8.472749e-03 -0.0047653440  7.912422e-04  0.0004059687
## eDNA_42 -2.871946e-03 -0.0049690298  4.413905e-03  0.0060210382
## eDNA_43 -7.035779e-03  0.0009447395 -9.229848e-03 -0.0071467147
## eDNA_44 -1.384799e-02  0.0049979764  3.334284e-03  0.0032671525
## eDNA_45 -9.524470e-04 -0.0037175238  2.794154e-03 -0.0012393502
## eDNA_46  4.669983e-04  0.0022897618 -5.985247e-03 -0.0033206624
## eDNA_47  6.004178e-04 -0.0024216599  1.282807e-02 -0.0103312466
## eDNA_48 -3.665524e-03 -0.0029264222 -7.780936e-04 -0.0008638449
## eDNA_49 -4.358468e-04  0.0035594338 -5.357395e-03  0.0004066505
## eDNA_50  1.606076e-03  0.0044065641  3.563754e-04 -0.0044839745
## eDNA_51  1.184586e-02  0.0090150543 -7.460911e-03 -0.0086049132
## eDNA_52 -1.224966e-03 -0.0115626595 -2.705532e-05 -0.0047980005
## eDNA_53  1.069031e-02 -0.0055160694 -5.457274e-03  0.0140815413
## eDNA_54 -1.752505e-04 -0.0015552619  1.180446e-03 -0.0028471442
## eDNA_55 -2.125401e-03 -0.0098135697  2.798609e-03  0.0034666091
## eDNA_56  1.920087e-03  0.0033231591  2.089423e-03  0.0019193488
## eDNA_57  7.444786e-03 -0.0097440022 -9.390749e-03 -0.0034480564
## eDNA_58 -3.120199e-03 -0.0010471894 -2.221232e-03  0.0027515664
##                PCoA34        PCoA35        PCoA36        PCoA37
## eDNA_01 -0.0036562981  5.978413e-03 -0.0027468674  0.0023293969
## eDNA_02  0.0069448159 -6.093947e-03  0.0054946491 -0.0014875670
## eDNA_03  0.0073731366 -4.779715e-03  0.0027567986  0.0005567719
## eDNA_04  0.0028937577  3.329089e-03  0.0011467113  0.0007354773
## eDNA_05  0.0026746908  1.815578e-03 -0.0013520111  0.0019982183
## eDNA_06  0.0023287411  2.430732e-03  0.0008316570 -0.0003078633
## eDNA_07  0.0045686912  2.799676e-03  0.0002764380  0.0008722415
## eDNA_08  0.0044104419 -2.131857e-03 -0.0027998549  0.0007001620
## eDNA_09 -0.0068633424  1.170766e-02  0.0090011278  0.0008067811
## eDNA_10 -0.0004490519  3.498420e-03  0.0039665123 -0.0007611225
## eDNA_11 -0.0014346878 -2.637564e-03  0.0025182593 -0.0035635499
## eDNA_12  0.0106253639 -2.809361e-03  0.0021179680  0.0022214636
## eDNA_13  0.0070359714  3.402156e-03 -0.0011044752  0.0025871816
## eDNA_14 -0.0031200224  1.380826e-03 -0.0016210981 -0.0006560309
## eDNA_15  0.0015092633 -3.391120e-03  0.0022963709 -0.0049520551
## eDNA_16 -0.0013410914 -9.536753e-04 -0.0016117396  0.0046248875
## eDNA_17  0.0023439918  4.111883e-03 -0.0005330517  0.0044335170
## eDNA_18  0.0059614531 -8.306801e-03 -0.0024344890  0.0062081270
## eDNA_19 -0.0124335228 -2.030245e-03 -0.0026547326 -0.0039112614
## eDNA_20 -0.0052346960  5.502312e-03  0.0041767953 -0.0073755870
## eDNA_21  0.0065972033 -9.788673e-03 -0.0087539217 -0.0014993286
## eDNA_22 -0.0043092666  8.142333e-04  0.0010865987  0.0004445628
## eDNA_23 -0.0008505508  1.310436e-03  0.0000707971  0.0014196552
## eDNA_24  0.0066328468 -2.189190e-03 -0.0002914611 -0.0001177460
## eDNA_25 -0.0008159112  1.006141e-03  0.0021964318  0.0011921333
## eDNA_26  0.0007057519  3.805869e-03 -0.0019562543  0.0050549306
## eDNA_27  0.0021893910  6.305796e-03 -0.0006857178  0.0052044946
## eDNA_28  0.0003133501  6.699175e-03 -0.0059309135  0.0032653208
## eDNA_29  0.0017772826  1.106780e-03  0.0073275833 -0.0008483468
## eDNA_30  0.0020315098 -3.271912e-03 -0.0047196612  0.0026131744
## eDNA_31  0.0115444183  9.803152e-03 -0.0088346186 -0.0133458969
## eDNA_32 -0.0003416086 -6.611020e-04 -0.0006523708  0.0005570330
## eDNA_33 -0.0028680567 -6.485453e-03 -0.0017513995 -0.0082258783
## eDNA_34 -0.0019575656  4.119241e-04 -0.0026011841 -0.0013044073
## eDNA_35  0.0045845560 -2.720136e-03  0.0077945138 -0.0003622894
```

```
## eDNA_36 -0.0071981855  5.498947e-03 -0.0045639067  0.0032143024
## eDNA_37 -0.0006118224 -1.637284e-03 -0.0089091844  0.0013727912
## eDNA_38 -0.0035742796 -5.246744e-04 -0.0018306226  0.0003754240
## eDNA_39 -0.0025508172 -1.586977e-03  0.0085929858 -0.0036619258
## eDNA_40 -0.0013685744 -5.337040e-04 -0.0025574119  0.0007659664
## eDNA_41 -0.0020027982  7.912318e-03 -0.0031962464  0.0016418155
## eDNA_42  0.0007178373 -1.257486e-03  0.0051712377 -0.0055652492
## eDNA_43  0.0022263293  4.359460e-03  0.0056651397 -0.0015742366
## eDNA_44 -0.0026318419 -2.239010e-04  0.0051469577 -0.0040590940
## eDNA_45 -0.0102485413 -1.017818e-02  0.0076179512  0.0122192811
## eDNA_46 -0.0025205585 -6.093389e-03 -0.0078913181  0.0014951979
## eDNA_47  0.0036729857 -1.072691e-03  0.0016673186  0.0023149581
## eDNA_48 -0.0033947881 -1.504296e-03 -0.0008960657 -0.0011662325
## eDNA_49 -0.0075064900  4.204668e-03 -0.0078419680  0.0040296285
## eDNA_50  0.0069226408  1.736927e-03  0.0057547714  0.0066591757
## eDNA_51 -0.0091603587 -7.803970e-03 -0.0005859245 -0.0058932052
## eDNA_52 -0.0068167955 -4.981268e-04 -0.0006804175 -0.0020853002
## eDNA_53 -0.0015202588 -1.531345e-04  0.0008227566  0.0022699081
## eDNA_54  0.0020282333 -6.564796e-05  0.0019901848 -0.0022866083
## eDNA_55  0.0015410386  1.536745e-03  0.0026295279 -0.0013193115
## eDNA_56 -0.0033332703 -4.478291e-03 -0.0019774677 -0.0005081166
## eDNA_57  0.0005610696 -2.829109e-03 -0.0027814937 -0.0000409788
## eDNA_58 -0.0026017102 -3.777713e-03 -0.0013701941 -0.0073047900
##                PCoA38        PCoA39        PCoA40        PCoA41
## eDNA_01  2.013847e-03 -4.620210e-03  2.810811e-03 -8.695169e-04
## eDNA_02  1.096651e-02 -1.556504e-03 -2.474158e-03 -2.718912e-03
## eDNA_03 -1.727536e-03  7.687365e-04  2.575613e-03 -1.185811e-04
## eDNA_04 -3.216135e-03 -2.700026e-05  1.500460e-04 -1.878347e-04
## eDNA_05 -3.788811e-03  1.133421e-03 -3.860451e-03 -3.334067e-03
## eDNA_06  2.055984e-04  2.213088e-04  9.827235e-04 -6.615952e-04
## eDNA_07  7.230831e-04  2.172235e-03  6.503017e-04 -3.274774e-04
## eDNA_08  3.784746e-03  1.047520e-02 -2.723305e-03  3.396623e-04
## eDNA_09  3.801796e-03  3.981352e-03 -1.167677e-04  2.735570e-03
## eDNA_10 -5.750307e-03  3.877003e-03  1.793625e-04 -1.302769e-03
## eDNA_11 -2.564666e-03  7.683694e-04 -3.162597e-03 -9.477079e-04
## eDNA_12  1.123283e-03 -1.654179e-03  1.940456e-03  1.415433e-03
## eDNA_13 -1.117452e-03 -9.703750e-04 -6.701635e-04 -2.160334e-03
## eDNA_14  1.575730e-04 -2.890211e-03 -1.230223e-03 -3.882404e-04
## eDNA_15  5.824693e-04  3.190056e-03 -3.269166e-03  7.765036e-04
## eDNA_16 -4.149064e-04 -8.560773e-04  2.291300e-03  2.099983e-04
## eDNA_17  1.827056e-03 -2.534897e-03 -1.765564e-03  2.005432e-03
## eDNA_18 -2.636069e-04  3.319666e-03  7.021824e-04  2.723798e-03
## eDNA_19 -8.667554e-04 -3.069297e-04 -4.238498e-03 -1.947828e-03
## eDNA_20 -2.213505e-03  4.600885e-04  1.167043e-03 -2.959491e-03
## eDNA_21 -3.087512e-03 -4.409353e-03  1.545484e-03 -3.528857e-03
## eDNA_22  4.162588e-03 -1.405243e-03 -7.088239e-03 -8.121432e-03
## eDNA_23  1.184136e-03 -1.388143e-03 -7.553020e-04  8.589141e-04
## eDNA_24 -4.722969e-05  5.855870e-04  9.483929e-04 -2.269803e-03
## eDNA_25 -1.203232e-03  6.039916e-04  9.311817e-04 -1.345085e-03
## eDNA_26  5.065961e-03 -3.242305e-03  5.700200e-03  2.253618e-03
## eDNA_27  4.597904e-03  1.675578e-03  1.978690e-03 -2.938653e-03
## eDNA_28  2.524672e-03  2.045602e-03  2.567265e-03 -3.956245e-04
## eDNA_29  1.043068e-03  4.785861e-03 -2.664251e-03  4.770656e-03
## eDNA_30 -2.870182e-03 -2.398798e-03  4.118989e-03 -4.498241e-03
```

```
## eDNA_31  4.493538e-04 -1.427906e-03 -2.054765e-03  2.195084e-03
## eDNA_32  2.823961e-04 -3.619592e-04 -5.990546e-05  1.144692e-04
## eDNA_33  1.447309e-03 -2.353738e-03 -3.043437e-03  2.781753e-03
## eDNA_34 -4.154216e-03  2.696381e-04  4.634712e-03  9.543735e-03
## eDNA_35 -2.361429e-03 -5.051905e-04 -8.314676e-04  7.093933e-04
## eDNA_36  5.890042e-03 -1.214261e-03 -3.334620e-03 -2.763156e-04
## eDNA_37  6.026302e-03  3.106514e-03 -2.464374e-03  5.879146e-03
## eDNA_38 -1.223207e-03 -1.807614e-03  3.306904e-03 -4.121587e-03
## eDNA_39  2.385052e-03 -9.924382e-03  3.508099e-03  7.671616e-03
## eDNA_40  5.208126e-04  5.991655e-04 -5.154409e-04  1.333937e-04
## eDNA_41  9.893364e-04 -1.035790e-03 -4.262998e-04 -1.157648e-03
## eDNA_42 -5.790053e-03 -5.482391e-04  2.767834e-03  3.209073e-04
## eDNA_43 -2.395636e-03 -6.207770e-03  1.615612e-03 -6.330407e-03
## eDNA_44 -5.706944e-04  2.027483e-03  9.509248e-04  7.555864e-04
## eDNA_45  3.445373e-04  1.356542e-03  1.644104e-03 -1.074256e-03
## eDNA_46 -3.384693e-03 -2.056497e-03 -6.035521e-03  1.730320e-03
## eDNA_47 -2.533770e-03  6.017675e-04 -1.917596e-04  5.606654e-04
## eDNA_48  1.535554e-03 -3.112648e-04 -5.326706e-04  1.329265e-03
## eDNA_49 -1.168230e-02  2.217586e-03  1.095900e-03  2.552032e-03
## eDNA_50 -4.079056e-03 -4.591634e-03 -8.101318e-03  3.144160e-03
## eDNA_51  1.708686e-04  1.734333e-03 -1.389704e-03 -6.898214e-04
## eDNA_52  3.060639e-04 -3.468487e-03 -1.405704e-03  6.048658e-04
## eDNA_53 -4.974888e-03  1.776437e-03  8.320424e-04 -4.880450e-04
## eDNA_54 -3.041172e-03  1.946410e-03 -1.840486e-03 -1.316711e-03
## eDNA_55 -6.253758e-04  5.680508e-03  1.229300e-03 -2.460756e-04
## eDNA_56  4.882255e-03 -2.395618e-03  2.090243e-03  2.293391e-03
## eDNA_57  2.756453e-03 -2.157066e-04  7.358944e-04  1.430059e-05
## eDNA_58  4.197702e-03  5.305843e-03  1.059455e-02 -3.700749e-03
##                PCoA42        PCoA43        PCoA44        PCoA45
## eDNA_01 -2.285074e-05  2.171878e-04  7.076807e-05  1.616844e-03
## eDNA_02 -2.070204e-03  1.310490e-03 -1.921290e-03  5.343610e-04
## eDNA_03 -2.918883e-04  3.545673e-04  7.916899e-04 -2.983852e-04
## eDNA_04  1.827817e-04  9.100381e-04  6.588452e-05 -2.887728e-04
## eDNA_05 -1.966697e-03  2.364683e-03  1.016682e-03  7.373498e-04
## eDNA_06 -5.975352e-04  3.503910e-04  4.288385e-04 -1.004814e-04
## eDNA_07 -1.063838e-03  6.056331e-04 -3.272400e-04  1.368637e-04
## eDNA_08 -2.530193e-03  1.233611e-03  4.298591e-04  1.764833e-03
## eDNA_09  2.221147e-03  9.465996e-04 -9.589025e-04  8.181020e-04
## eDNA_10 -1.089988e-03  3.684600e-04  2.825882e-04 -2.346675e-04
## eDNA_11 -1.849510e-04  1.072394e-03 -7.364877e-04 -3.939351e-04
## eDNA_12  1.542729e-03 -1.660134e-03  3.453902e-04 -2.805345e-04
## eDNA_13 -3.099704e-03  1.396509e-03  5.112866e-04 -5.074843e-04
## eDNA_14 -2.021548e-03  1.943234e-04 -4.996527e-07 -3.742033e-04
## eDNA_15  3.232343e-03  7.810606e-04 -2.261793e-04  2.830042e-04
## eDNA_16 -7.457108e-04 -1.130132e-03 -2.986873e-04  7.960387e-05
## eDNA_17  8.553368e-04 -4.764907e-04 -7.360958e-04  6.134383e-04
## eDNA_18  2.650589e-03  2.547259e-04 -4.757711e-04 -5.620235e-04
## eDNA_19 -1.920893e-03  1.903455e-03 -8.870530e-04  2.912273e-04
## eDNA_20 -8.301605e-04  3.467149e-04  8.277769e-04  7.374326e-06
## eDNA_21 -3.056246e-03 -1.089145e-03  1.045181e-03 -7.687768e-04
## eDNA_22  6.997829e-03 -2.246062e-03  1.921202e-03  1.022887e-04
## eDNA_23  1.539178e-04  6.450501e-04 -1.413661e-04 -1.346482e-04
## eDNA_24 -1.444391e-03 -7.214795e-04  1.795078e-04 -2.323557e-05
## eDNA_25 -1.334954e-03  2.247184e-04  3.049355e-04 -1.055351e-04
```

```
## eDNA_26  9.893725e-04 -1.764260e-03  1.103277e-03  1.124284e-03
## eDNA_27  5.988875e-05  8.872954e-04 -2.934573e-04 -1.113863e-04
## eDNA_28 -1.259158e-03 -9.625835e-04  7.473141e-04  6.825772e-04
## eDNA_29 -3.506553e-03 -5.639291e-03  5.085772e-04  8.673078e-05
## eDNA_30  4.702357e-03  5.957351e-03 -5.429950e-04  3.239349e-04
## eDNA_31  3.513960e-03 -3.560566e-04 -7.452571e-04 -4.592162e-05
## eDNA_32  7.384105e-05  9.920519e-05  2.862984e-05 -8.663514e-05
## eDNA_33 -6.555460e-03 -1.076448e-03  1.540099e-03  4.240232e-05
## eDNA_34 -5.738621e-03  2.312931e-03 -2.099916e-03  8.308822e-06
## eDNA_35  1.226197e-03  4.356128e-04 -3.432245e-04 -2.293663e-05
## eDNA_36 -3.420226e-03  2.304702e-03 -2.711155e-04 -2.126149e-03
## eDNA_37  3.058308e-03  1.641162e-04  9.013143e-04 -1.085378e-03
## eDNA_38  1.862240e-03 -5.670748e-03 -3.541469e-03 -2.277326e-03
## eDNA_39  3.023879e-03  2.509358e-03  1.927048e-03 -1.050548e-03
## eDNA_40 -3.326348e-04 -1.886481e-04  1.196406e-04 -1.889347e-04
## eDNA_41 -2.705997e-03 -9.629111e-04 -4.130043e-05 -8.460116e-07
## eDNA_42  2.228617e-03 -1.810703e-03  2.957370e-05  2.633610e-03
## eDNA_43 -5.500647e-03 -9.465485e-04 -2.551451e-04  3.853459e-04
## eDNA_44  2.111983e-03  9.253077e-04 -1.894385e-04 -7.926054e-05
## eDNA_45 -2.346566e-03 -1.188410e-05  4.483248e-04  2.428352e-04
## eDNA_46 -5.502266e-04 -1.085530e-03 -8.628704e-04  2.001786e-03
## eDNA_47  4.088048e-03 -1.832094e-05 -4.718857e-04  3.172139e-04
## eDNA_48  1.694526e-03 -4.191921e-04 -3.669318e-04  1.840583e-04
## eDNA_49  2.428275e-03 -1.663547e-03  1.466941e-03 -5.782592e-05
## eDNA_50 -1.961537e-04 -1.848789e-04 -1.436240e-04 -4.708494e-04
## eDNA_51  1.980117e-03  4.072919e-04 -3.494435e-04 -3.204051e-04
## eDNA_52  5.337974e-04 -3.509755e-04  3.258979e-04 -1.517268e-04
## eDNA_53  3.159144e-03  3.589645e-04 -1.022783e-03 -1.413758e-04
## eDNA_54 -3.765111e-04  8.100757e-04 -4.540987e-04 -4.772985e-04
## eDNA_55  2.915677e-04  7.271897e-05  1.154160e-03 -3.491083e-03
## eDNA_56  2.760757e-03 -1.581704e-03 -3.823625e-04  6.382720e-04
## eDNA_57 -1.558977e-05 -5.341013e-04 -5.459296e-05  3.081380e-04
## eDNA_58 -8.474531e-04 -1.737677e-04  6.190952e-04  2.937822e-04
##                PCoA46        PCoA47        PCoA48        PCoA49
## eDNA_01 -4.307939e-04 -3.757632e-03 -3.962273e-04  1.132929e-02
## eDNA_02 -1.154819e-03 -5.475520e-05  7.442641e-04 -3.340796e-03
## eDNA_03  5.160123e-04  1.349497e-03 -4.376440e-03 -1.107131e-03
## eDNA_04  5.145859e-04 -4.363245e-04 -3.944985e-03 -1.684098e-03
## eDNA_05 -6.088897e-03  1.680982e-03 -2.851872e-04 -4.930715e-03
## eDNA_06  3.940332e-05 -2.477605e-04 -1.075096e-03 -1.012437e-03
## eDNA_07  1.422394e-04 -9.085622e-04  2.693273e-03 -1.587581e-04
## eDNA_08  5.226608e-03  2.775946e-03 -4.122739e-04  5.246493e-03
## eDNA_09  4.114820e-04 -2.018829e-03 -2.870575e-03 -5.620106e-03
## eDNA_10 -4.020291e-04 -6.244517e-03  2.794069e-03 -6.103337e-03
## eDNA_11  3.728894e-05 -1.592672e-03  6.605398e-04  4.147875e-03
## eDNA_12  1.621111e-04 -3.396083e-03 -1.207222e-03 -2.807649e-03
## eDNA_13  6.319371e-05 -7.469645e-04 -3.372590e-04 -1.085338e-03
## eDNA_14  7.875221e-04  7.545893e-04  1.777008e-03 -3.397018e-03
## eDNA_15 -7.162337e-04  4.134580e-04 -2.421704e-03  6.180794e-03
## eDNA_16  3.376313e-05 -7.329994e-04  1.826285e-03 -9.229275e-04
## eDNA_17  3.347950e-04  8.077051e-04 -3.586156e-03  6.792516e-04
## eDNA_18 -1.698485e-03 -3.968698e-03  2.169202e-03  2.195191e-03
## eDNA_19 -3.230023e-04  3.697780e-03  2.734393e-03  3.240168e-03
## eDNA_20  1.704292e-03 -1.188463e-03 -5.228731e-04  4.361153e-05
```

```
## eDNA_21  1.936506e-04  1.948327e-03  3.448928e-03  6.806011e-03
## eDNA_22  9.522456e-05 -1.014047e-04 -9.947457e-04 -5.075632e-04
## eDNA_23 -3.403900e-04  1.081022e-03 -1.198828e-04  4.175704e-03
## eDNA_24  5.339953e-04 -9.974734e-04  6.750842e-04 -2.221133e-03
## eDNA_25  2.599634e-04 -8.782356e-04  4.101544e-04 -5.641408e-04
## eDNA_26  1.777511e-04  4.939237e-03 -8.788328e-04 -7.747982e-03
## eDNA_27 -9.043683e-04  5.243624e-03  6.883609e-03  7.334056e-05
## eDNA_28  1.259785e-04 -3.986600e-03  4.473192e-03 -9.937903e-04
## eDNA_29 -2.424473e-03  1.672459e-04  1.044511e-03  5.463239e-03
## eDNA_30  2.406977e-03 -1.422728e-03 -5.147164e-04 -4.029725e-03
## eDNA_31 -5.695410e-04  7.950223e-04  2.213823e-04  1.553005e-04
## eDNA_32  7.689146e-05  8.144269e-05 -3.361835e-04  5.259672e-04
## eDNA_33  2.234070e-03 -5.216813e-03 -2.966331e-03 -5.205272e-03
## eDNA_34 -6.623711e-04  3.254378e-03 -6.127326e-05 -9.737927e-04
## eDNA_35  3.784167e-04  1.787482e-03 -7.608546e-03  2.547742e-03
## eDNA_36 -3.090033e-04 -4.623695e-04 -4.323376e-03  3.309862e-03
## eDNA_37  3.437589e-04 -1.898398e-03 -1.960716e-04 -4.895011e-03
## eDNA_38  1.575181e-03 -2.329866e-03 -3.871897e-04 -3.429483e-03
## eDNA_39 -6.025677e-04 -2.490641e-03  3.058932e-03  2.654289e-03
## eDNA_40  5.544842e-05 -7.969495e-04  1.680687e-04 -6.805269e-04
## eDNA_41 -8.491112e-05 -3.185171e-03  2.489650e-03  4.093735e-03
## eDNA_42  8.557224e-04  3.695874e-03  5.689178e-03 -3.422718e-03
## eDNA_43  1.116308e-04  3.696882e-03 -3.317565e-03 -1.703419e-04
## eDNA_44  7.330588e-04 -1.215004e-03  5.024335e-03 -1.234324e-03
## eDNA_45  2.368198e-04 -3.026294e-04 -1.464302e-04 -6.134714e-05
## eDNA_46 -1.319126e-03 -9.729299e-04 -4.723813e-03 -9.354002e-03
## eDNA_47 -1.327261e-03  4.577488e-04 -3.209191e-03  3.386984e-03
## eDNA_48 -2.898493e-05  1.393174e-03 -7.361202e-04  1.496488e-03
## eDNA_49  3.787022e-04  1.531747e-03 -2.402511e-03  2.214543e-03
## eDNA_50  2.369575e-03  5.781821e-05  5.547661e-03  7.128729e-04
## eDNA_51 -1.076533e-03  1.229146e-03  6.132123e-03 -4.625175e-03
## eDNA_52  2.960430e-04  2.256179e-03 -2.287604e-03 -1.117150e-03
## eDNA_53 -5.835389e-04 -1.690085e-03 -7.905091e-04  5.439243e-03
## eDNA_54 -2.946654e-04 -2.277360e-03  9.680147e-05  3.915106e-03
## eDNA_55  1.075896e-04  8.207020e-03 -5.605225e-04 -4.112888e-03
## eDNA_56  3.184968e-04  5.178729e-03 -1.486811e-03  4.248754e-03
## eDNA_57 -5.166615e-04  1.704959e-04  3.064015e-03  1.218738e-03
## eDNA_58 -1.979587e-03 -3.133635e-03 -4.342433e-03  2.016078e-03
##                PCoA50        PCoA51        PCoA52        PCoA53
## eDNA_01 -0.0095727561 -7.450487e-04  0.0089449626 -0.0053786135
## eDNA_02 -0.0004453259 -1.863236e-03 -0.0025815996  0.0001761155
## eDNA_03  0.0055891449  1.316716e-03  0.0022653086  0.0054786454
## eDNA_04  0.0040586373  1.775648e-03 -0.0007419782 -0.0030793700
## eDNA_05 -0.0055132615  8.132572e-03 -0.0054106542 -0.0032556781
## eDNA_06 -0.0011371739 -1.136625e-05  0.0008630637 -0.0004519912
## eDNA_07 -0.0016656107  6.621378e-04 -0.0084025140  0.0026030108
## eDNA_08  0.0023494513 -6.004934e-03  0.0000102342 -0.0035152795
## eDNA_09 -0.0035004779  7.608658e-04  0.0012713514  0.0073304565
## eDNA_10  0.0011869429 -7.242073e-03  0.0036904921 -0.0103093507
## eDNA_11  0.0019284370  2.419292e-03  0.0016675928  0.0034402425
## eDNA_12  0.0005936027  5.028186e-03 -0.0021161873 -0.0017371382
## eDNA_13 -0.0008075542 -2.401383e-03  0.0111087302  0.0046907779
## eDNA_14  0.0011261213 -2.301619e-03  0.0044621827 -0.0045229045
## eDNA_15 -0.0024783053  4.500023e-03  0.0029909960  0.0098736406
```

```
## eDNA_16  0.0028301925 -7.421772e-04 -0.0084225858 -0.0048119531
## eDNA_17 -0.0007185688 -4.648190e-03  0.0053577594 -0.0044275296
## eDNA_18 -0.0048227078 -7.348027e-03  0.0069686020  0.0015558823
## eDNA_19  0.0033021237 -6.119498e-03  0.0044120522 -0.0022958374
## eDNA_20  0.0049535915  2.389095e-03 -0.0095120165 -0.0029397272
## eDNA_21  0.0012945202  3.896907e-05 -0.0023697833 -0.0050201766
## eDNA_22  0.0002242544 -2.566634e-03  0.0007839037 -0.0005480711
## eDNA_23 -0.0016250305  3.649793e-03 -0.0002200010  0.0025530880
## eDNA_24  0.0020274056  8.651351e-04 -0.0001718806 -0.0021557677
## eDNA_25  0.0014399104  3.096248e-04 -0.0025399585 -0.0011703928
## eDNA_26 -0.0037312414 -5.582648e-03 -0.0030866177 -0.0066884221
## eDNA_27  0.0089526116  9.984763e-03  0.0126035554  0.0020272563
## eDNA_28 -0.0006440640 -2.697559e-03 -0.0058273598  0.0160643089
## eDNA_29  0.0033154676 -1.759392e-03  0.0007159108 -0.0005203163
## eDNA_30 -0.0025022024  2.373625e-03 -0.0015733264  0.0038934758
## eDNA_31 -0.0007141637 -2.570824e-03 -0.0006338108 -0.0004896720
## eDNA_32  0.0001304445 -3.916117e-04  0.0027183402  0.0010758651
## eDNA_33 -0.0102025149  5.286017e-03  0.0017688200  0.0064620733
## eDNA_34 -0.0014705255  3.478616e-04 -0.0004147925 -0.0022074341
## eDNA_35 -0.0038332282  6.996281e-03  0.0034833008 -0.0113848821
## eDNA_36  0.0022169025  6.498411e-04 -0.0024143192  0.0017935604
## eDNA_37  0.0018571143  5.507039e-03  0.0005242782 -0.0124904482
## eDNA_38 -0.0008743895  3.190427e-03  0.0045537189  0.0015956848
## eDNA_39  0.0084321947 -5.383755e-03  0.0015301745  0.0059203927
## eDNA_40 -0.0008551371 -3.685072e-04  0.0020346316  0.0014380052
## eDNA_41  0.0013354563  5.943014e-03 -0.0051832142 -0.0034801306
## eDNA_42 -0.0009887662  5.421829e-03  0.0087227855  0.0039240817
## eDNA_43 -0.0021442995 -8.151291e-03 -0.0046339728  0.0017335749
## eDNA_44  0.0009385614  2.894936e-03 -0.0074009219 -0.0132307261
## eDNA_45 -0.0008434932  2.680406e-03 -0.0003807837  0.0005499788
## eDNA_46  0.0124994358 -3.012059e-03 -0.0005578730  0.0078367676
## eDNA_47 -0.0007222173 -9.489152e-03 -0.0046159437 -0.0030765733
## eDNA_48  0.0001116027  1.292733e-03 -0.0027783494 -0.0012202267
## eDNA_49 -0.0015860859  2.361318e-03  0.0013156703  0.0037262252
## eDNA_50 -0.0013740923  1.398432e-03 -0.0019702925  0.0031072125
## eDNA_51 -0.0076260215 -4.468924e-03 -0.0013849182 -0.0008959914
## eDNA_52  0.0016341830 -4.738608e-04  0.0089828927 -0.0065619506
## eDNA_53  0.0012512258 -4.582980e-03 -0.0091894991  0.0024087574
## eDNA_54  0.0024596902  3.190311e-03 -0.0008691261  0.0010977561
## eDNA_55 -0.0053011891 -4.838000e-03 -0.0001610223  0.0072515174
## eDNA_56 -0.0007885689  6.481633e-03 -0.0123553955  0.0054264278
## eDNA_57 -0.0046915142 -1.518844e-03 -0.0006614507  0.0022433421
## eDNA_58  0.0051412611 -5.649299e-04  0.0048308382  0.0005884309
##                PCoA54        PCoA55        PCoA56        PCoA57
## eDNA_01  1.512371e-03 -1.360979e-02 -2.014687e-05 -5.295121e-04
## eDNA_02 -7.918960e-03 -7.721453e-03 -3.790284e-04  4.075854e-03
## eDNA_03  4.677167e-03  3.083571e-03  5.017406e-03  2.790796e-03
## eDNA_04 -1.335585e-03 -2.482543e-03 -1.202101e-03 -2.594097e-04
## eDNA_05  1.102212e-03  2.343216e-03  2.095526e-03  2.472274e-03
## eDNA_06 -8.106486e-03  7.297289e-03 -1.660469e-02 -2.339848e-02
## eDNA_07  7.435055e-05 -1.048918e-02  3.935530e-03 -4.967435e-03
## eDNA_08  1.582824e-03  1.383527e-03 -4.015532e-04  1.710495e-04
## eDNA_09  5.385577e-03  2.448844e-03  5.259000e-04  6.899163e-04
## eDNA_10 -6.834679e-03 -1.617866e-02 -4.408381e-03  4.724248e-03
```

```
## eDNA_11  7.513391e-03 -1.887961e-03  1.434819e-03  1.087891e-03
## eDNA_12  1.302353e-03  5.205668e-03  1.953338e-03  2.668925e-03
## eDNA_13 -2.038685e-03  1.545075e-02 -8.990755e-03 -3.851257e-03
## eDNA_14  8.118780e-03  7.379740e-03  7.240759e-03  2.513190e-02
## eDNA_15 -1.504300e-02  2.059259e-03  3.682420e-03  1.094562e-02
## eDNA_16  1.715372e-02 -1.263868e-02  9.827565e-03 -2.611439e-03
## eDNA_17 -2.937003e-04 -5.560279e-03 -6.862663e-03 -6.490486e-03
## eDNA_18  5.165921e-03  5.189055e-03 -3.959304e-03 -6.156675e-04
## eDNA_19 -4.292250e-03 -4.646117e-03 -3.780319e-03 -7.853080e-04
## eDNA_20 -8.760865e-03 -4.821641e-03  5.785477e-03  6.152811e-03
## eDNA_21 -5.642288e-03 -5.356727e-04 -2.856563e-04 -4.343798e-04
## eDNA_22  1.709181e-03 -1.416769e-03 -1.542795e-03 -3.344646e-03
## eDNA_23 -3.026788e-05  4.267105e-03  2.518687e-04  5.698371e-04
## eDNA_24  2.254073e-04  2.554568e-03  2.123783e-03  1.215330e-03
## eDNA_25  4.831309e-03 -2.326985e-03 -2.375489e-02  1.663213e-02
## eDNA_26 -1.329692e-02  1.553456e-03 -2.684531e-04 -3.826022e-03
## eDNA_27 -8.692176e-04 -7.316282e-03  1.699345e-03  2.678821e-03
## eDNA_28  5.304917e-03  1.132324e-02  2.234546e-03  7.626995e-04
## eDNA_29 -3.361885e-03  2.675076e-03  2.899424e-03  6.978484e-03
## eDNA_30 -1.991384e-03 -2.428473e-03  4.140817e-03  7.084529e-03
## eDNA_31  1.305983e-03 -1.322465e-03 -3.126568e-03  1.430105e-03
## eDNA_32 -3.118456e-03  4.183345e-03  2.910412e-02 -1.620845e-02
## eDNA_33  4.162974e-04 -6.801637e-03  3.113471e-04 -6.529535e-04
## eDNA_34 -1.676558e-03  7.965323e-04 -7.043179e-05  1.337083e-07
## eDNA_35 -4.939338e-04 -1.922040e-03  1.604384e-03  3.516228e-03
## eDNA_36  5.069141e-03  7.182917e-03  1.256317e-03 -4.573286e-04
## eDNA_37  8.234949e-04 -3.931699e-03 -6.441375e-03 -9.359422e-03
## eDNA_38 -3.314381e-03  5.168981e-05 -4.330258e-04  9.771533e-04
## eDNA_39 -1.888447e-03 -5.228499e-03 -8.069973e-04 -1.417064e-03
## eDNA_40 -9.594044e-03  4.030468e-03  8.898614e-03  2.680385e-02
## eDNA_41 -6.871730e-03 -2.617252e-03  9.213241e-04  2.778662e-03
## eDNA_42  4.657845e-03 -1.456199e-03 -2.191859e-03 -6.926127e-03
## eDNA_43  6.474822e-03  6.607272e-03  8.170154e-05 -7.735134e-03
## eDNA_44  3.177217e-03  2.519518e-02 -5.816462e-05 -1.063936e-03
## eDNA_45 -3.319313e-03 -7.891190e-04 -2.641190e-03 -3.711322e-03
## eDNA_46 -2.291651e-03 -4.770643e-03 -2.742741e-03 -3.658502e-03
## eDNA_47  1.389341e-02 -1.450609e-03  9.771293e-03  1.744311e-02
## eDNA_48 -1.269955e-03  4.110763e-04  3.078369e-02 -1.917215e-02
## eDNA_49 -3.942249e-04 -9.758823e-04  1.001237e-03  3.978799e-03
## eDNA_50 -1.121844e-03 -1.131721e-03  1.107762e-03  2.037717e-03
## eDNA_51  3.308745e-03  3.837433e-03 -2.252194e-03 -2.464624e-03
## eDNA_52  4.785175e-03  1.523459e-02 -4.812032e-03  3.928125e-05
## eDNA_53 -2.091906e-02  6.330411e-03 -9.824647e-03 -9.978128e-03
## eDNA_54  1.555507e-02 -4.488680e-03 -1.076388e-02 -2.752978e-02
## eDNA_55  2.282552e-06 -1.492797e-02  9.738000e-05 -2.784300e-03
## eDNA_56  7.033640e-03 -3.064485e-03 -2.199480e-02  9.529459e-03
## eDNA_57  9.588355e-04  6.394657e-04  7.266899e-04 -1.851723e-03
## eDNA_58  2.968322e-03  2.246524e-04  1.062543e-04  7.173720e-04
```

```
disp.group$centroids
```

```
##             PCoA1       PCoA2       PCoA3        PCoA4         PCoA5
## fecesC  0.3878748 -0.05983260 -0.00827372 -0.002572693  4.520720e-02
## fecesE  0.3846140 -0.05281179 -0.01768682 -0.003620721  2.563516e-02
## sedC   -0.1621571  0.01383030 -0.07574180  0.129742326  1.774716e-02
```

```
## sedE   -0.1536365  0.02393042 -0.08484757  0.114095079  1.808900e-02
## soilC  -0.1310322  0.09076200 -0.08660862 -0.124608286  8.663795e-05
## soilE  -0.1272992  0.09265998 -0.08386905 -0.139828718 -2.432636e-03
## waterC -0.1520673 -0.08814663  0.13910634 -0.019183111 -3.623969e-03
## waterE -0.1477446 -0.09197941  0.14612311 -0.021572728 -4.926486e-03
##                PCoA6        PCoA7         PCoA8        PCoA9        PCoA10
## fecesC  0.0098589442  0.016397777 -9.495006e-04 -0.002803429 -0.0005967342
## fecesE -0.0009809179 -0.002832237 -4.382681e-03 -0.002298915 -0.0032227201
## sedC    0.0019230882 -0.018076518 -7.021646e-05 -0.012981242 -0.0190403798
## sedE   -0.0016667448  0.001182872 -8.991959e-03 -0.006189935 -0.0097486094
## soilC   0.0207659427  0.014086669 -1.810293e-03 -0.007799465  0.0113697682
## soilE   0.0203669867  0.021771870  3.555331e-04 -0.011077239  0.0238205868
## waterC -0.0067040486 -0.013388065  3.212156e-03  0.005526618  0.0001780629
## waterE -0.0113894267 -0.004726205  3.909079e-03  0.001114462  0.0064050182
##               PCoA11       PCoA12       PCoA13       PCoA14        PCoA15
## fecesC  0.003183113 -0.003963531 0.0020243773  0.002531363 -0.0005298405
## fecesE -0.003608571  0.002141896 0.0003440503 -0.002857064  0.0013013718
## sedC    0.014102263 -0.005045631 0.0031010033 -0.010395640  0.0137423428
## sedE   -0.005464704  0.006464936 0.0060758761  0.009384678 -0.0088932577
## soilC   0.005083942 -0.005300623 0.0029716511 -0.002363583  0.0044321589
## soilE   0.001742570 -0.004551787 0.0082913536  0.001480640 -0.0003408614
## waterC -0.006252494  0.002339251 0.0061153259  0.003829896 -0.0030460972
## waterE -0.004545598  0.006421805 0.0064820664  0.004093985 -0.0018753856
##               PCoA16        PCoA17        PCoA18        PCoA19
## fecesC -0.008224039  0.0084617606 -0.0053131147  0.0056286235
## fecesE  0.007316486 -0.0093475111  0.0040293886 -0.0069442978
## sedC    0.001547744 -0.0005069016 -0.0050878748  0.0032158632
## sedE    0.002813347  0.0061489242  0.0091063204 -0.0099944064
## soilC  -0.002956428  0.0001524823  0.0007159359  0.0027013102
## soilE   0.004296706  0.0023177233 -0.0043380514 -0.0002855787
## waterC -0.001176543  0.0004675680 -0.0026140779 -0.0015345967
## waterE -0.004077742 -0.0007148156  0.0050376444  0.0023202964
##                PCoA20       PCoA21        PCoA22        PCoA23
## fecesC  1.871564e-03  0.003013167 -0.0044011219 -0.0046322545
## fecesE -5.434625e-04 -0.003286834  0.0035344766  0.0047069736
## sedC   -2.851750e-04 -0.005990463 -0.0003391233  0.0006712215
## sedE    2.273680e-03  0.009031973 -0.0010661459 -0.0051716875
## soilC  -1.869136e-03 -0.004531683 -0.0027033546  0.0040171382
## soilE   4.693306e-03  0.007112144  0.0090301300 -0.0002756054
## waterC -1.642061e-03 -0.001620279 -0.0026177886  0.0021203775
## waterE  6.578565e-05 -0.002305885  0.0037932998 -0.0016959900
##               PCoA24        PCoA25        PCoA26        PCoA27
## fecesC  0.0046528856  0.0044767226  0.0032243942  0.0001289074
## fecesE -0.0068589271 -0.0052939926 -0.0026994272 -0.0016560634
## sedC   -0.0004938348 -0.0029892349  0.0005104500  0.0012973139
## sedE   -0.0001994614  0.0018385272 -0.0008720932 -0.0006985110
## soilC   0.0015694339 -0.0002737461  0.0029289752 -0.0012816005
## soilE   0.0003684004 -0.0007059869 -0.0013453850 -0.0004637273
## waterC  0.0024004004  0.0011297401 -0.0035325443 -0.0016815309
## waterE -0.0015916923 -0.0027233255  0.0046684005  0.0011911673
##               PCoA28        PCoA29       PCoA30        PCoA31
## fecesC  0.0007728879  0.0032624291 -0.003361671  0.0003789649
## fecesE -0.0014147490 -0.0029653558  0.003188719 -0.0006940785
## sedC   -0.0051082562  0.0007276507 -0.001683197  0.0019425900
```

```
## sedE    0.0046446095 -0.0005163410  0.001132926 -0.0026507819
## soilC  -0.0002875452  0.0016014629 -0.001186218  0.0032208943
## soilE  -0.0009291542 -0.0014376238  0.001516174 -0.0027138314
## waterC -0.0010066063 -0.0022936813 -0.001829522  0.0030269221
## waterE  0.0007992513  0.0021226193  0.002051291 -0.0024826671
##              PCoA32       PCoA33        PCoA34        PCoA35
## fecesC -0.0013059273 -0.001943739 -0.0005138131 -0.0010967522
## fecesE  0.0013108361  0.001941544  0.0001957928  0.0010952512
## sedC    0.0012836986  0.001619802 -0.0020419565  0.0003208542
## sedE   -0.0006910528 -0.001585903  0.0020532735 -0.0007074935
## soilC  -0.0040256369 -0.002804686 -0.0013101646  0.0031776381
## soilE   0.0031338563  0.001988470  0.0011337385 -0.0022734283
## waterC -0.0030216159  0.001318552 -0.0018196958 -0.0012894450
## waterE  0.0031054014 -0.001198342  0.0012177101  0.0015232918
##              PCoA36        PCoA37        PCoA38        PCoA39
## fecesC  0.0004016044 -0.0006053044 -1.470032e-04  8.496251e-04
## fecesE -0.0003741919  0.0003595004  2.107090e-04 -9.186449e-04
## sedC   -0.0010018429 -0.0008153850 -4.312031e-04  1.167712e-03
## sedE    0.0006294952  0.0007610820  6.198903e-04 -1.258176e-03
## soilC   0.0019571170 -0.0011021402  1.194394e-03 -1.289248e-03
## soilE  -0.0019165877  0.0006419656 -1.037696e-03  1.043581e-03
## waterC  0.0018718789 -0.0011305951  6.683771e-05 -1.935014e-05
## waterE -0.0016902142  0.0010388946 -1.089476e-04 -1.002142e-04
##              PCoA40        PCoA41        PCoA42        PCoA43
## fecesC  1.080751e-05  0.0004003521  0.0008716828 -0.0002607005
## fecesE -3.741882e-05 -0.0003551539 -0.0008217638  0.0002395858
## sedC    8.338249e-04 -0.0014510852  0.0014757244 -0.0003460347
## sedE   -5.563145e-04  0.0016365865 -0.0016387248  0.0003404978
## soilC  -6.576134e-04 -0.0014593249 -0.0036800377 -0.0006346475
## soilE   7.302543e-04  0.0013620383  0.0031691596  0.0005411815
## waterC -1.379796e-03  0.0017412925  0.0005010704  0.0008559772
## waterE  1.453107e-03 -0.0014752124 -0.0002709734 -0.0007859971
##              PCoA44        PCoA45        PCoA46        PCoA47
## fecesC -1.571741e-04  3.525010e-05 -2.939244e-04  0.0000696631
## fecesE  1.512704e-04 -3.646330e-05  1.531149e-04  0.0004055686
## sedC    4.323787e-04  7.442799e-05 -7.709173e-05 -0.0001688912
## sedE   -5.348709e-04 -1.301651e-05  1.090552e-04  0.0008936589
## soilC   1.924022e-04 -4.424521e-04 -2.555528e-04  0.0001607612
## soilE  -5.122270e-05  3.135128e-04  1.944968e-04 -0.0009056166
## waterC -6.862448e-05  4.640367e-04  7.697569e-04  0.0007055191
## waterE  1.107679e-04 -5.111751e-04 -1.345287e-04 -0.0008686371
##              PCoA48        PCoA49        PCoA50        PCoA51
## fecesC  0.0001470747  3.473135e-04 -0.0004111759 -0.0005314460
## fecesE -0.0000429035 -7.783467e-04 -0.0001489254 -0.0008428236
## sedC   -0.0013375844 -3.095198e-04  0.0003027371 -0.0014969960
## sedE    0.0014127665 -6.589522e-06 -0.0008708656  0.0005784394
## soilC  -0.0009371561  1.544345e-03  0.0014810810  0.0006483433
## soilE   0.0010455542 -9.707283e-04 -0.0015220046 -0.0006286481
## waterC -0.0004774724  1.274815e-03  0.0019980741 -0.0013172151
## waterE  0.0004496811 -1.219755e-03 -0.0006554063  0.0019986355
##              PCoA52        PCoA53        PCoA54        PCoA55
## fecesC -0.0010244745 -6.765172e-05  1.985580e-04 -0.0025363545
## fecesE  0.0019230797 -1.467412e-04 -7.821703e-04  0.0039068044
## sedC    0.0007216908 -5.083225e-04  1.109365e-03 -0.0018822708
```

```
## sedE   -0.0006868360  1.677050e-04 -1.305983e-03  0.0002159500
## soilC  -0.0008457973  1.114935e-03 -6.922759e-05  0.0010052332
## soilE   0.0016351417  1.514065e-05  1.152270e-03  0.0001913697
## waterC  0.0008151452  8.764571e-05  1.050853e-06  0.0008271082
## waterE -0.0003077652 -4.924643e-04 -3.484497e-04  0.0009596914
##               PCoA56        PCoA57
## fecesC -0.0027385306  0.0013860052
## fecesE  0.0010564733 -0.0026147247
## sedC   -0.0012657501 -0.0022066403
## sedE    0.0005512717  0.0009778711
## soilC   0.0020974559  0.0017207758
## soilE  -0.0013019983 -0.0004730241
## waterC -0.0007002882 -0.0001164310
## waterE  0.0012092374  0.0010468986
```

```
# Soil
env.soi <- which(eDNA.env == "soil")
cent.soil <- as.data.frame(matrix(NA, nrow = length(env.soi), ncol = 3))
colnames(cent.soil) <- c("T", "C", "E")
rownames(cent.soil) <- rownames(eDNA.div)[env.soi]

# Centriod Ratio Calculaed for 1: vs Env; 2: vs C; 3 vs E
for (i in 1:length(env.soi)){
  cent.soil[i, 1] <- dist(rbind(disp.env$vectors[env.soi[i]],
                                disp.env$centroids[3, ]), method = "euclidean")
  cent.soil[i, 2] <- dist(rbind(disp.group$vectors[env.soi[i]],
                                disp.group$centroids[5, ]), method = "euclidean")
  cent.soil[i, 3] <- dist(rbind(disp.group$vectors[env.soi[i]],
                                disp.group$centroids[6, ]), method = "euclidean")
}

pairs.soil <- eDNA.div[env.soi, c(3,4,5,7)]

cent.soil.ratio <- as.data.frame(matrix(NA, nrow = length(unique(pairs.soil$pair)),
                                        ncol = 3))
colnames(cent.soil.ratio) <- c("T", "C", "E")
rownames(cent.soil.ratio) <- paste(pairs.soil$env, pairs.soil$pair,
                                   sep = "")[which(pairs.soil$treat == "E") ]

for (i in 1:dim(cent.soil.ratio)[1]){
  pair <- as.numeric(gsub("soil", "", rownames(cent.soil.ratio)[i]))
  cent.soil.ratio[i, 1] <- (cent.soil[, 1][which(pairs.soil$pair == pair &
                                                   pairs.soil$treat == "C")]) /
                           (cent.soil[, 1][which(pairs.soil$pair == pair &
                                                   pairs.soil$treat == "E")])
  cent.soil.ratio[i, 2] <- (cent.soil[, 2][which(pairs.soil$pair == pair &
                                                  pairs.soil$treat == "C")]) /
                           (cent.soil[, 2][which(pairs.soil$pair == pair &
                                                  pairs.soil$treat == "E")])
  cent.soil.ratio[i, 3] <- (cent.soil[, 3][which(pairs.soil$pair == pair &
                                                  pairs.soil$treat == "C")]) /
                           (cent.soil[, 3][which(pairs.soil$pair == pair &
                                                  pairs.soil$treat == "E")])
}
```

```r
# Water
env.wat <- which(eDNA.env == "water")
cent.water <- as.data.frame(matrix(NA, nrow = length(env.wat), ncol = 3))
colnames(cent.water) <- c("T", "C", "E")
rownames(cent.water) <- rownames(eDNA.div)[env.wat]

for (i in 1:length(env.wat)){
  cent.water[i, 1] <- dist(rbind(disp.env$vectors[env.wat[i]],
                                 disp.env$centroids[4, ]), method = "euclidean")
  cent.water[i, 2] <- dist(rbind(disp.group$vectors[env.wat[i]],
                                 disp.group$centroids[7, ]), method = "euclidean")
  cent.water[i, 3] <- dist(rbind(disp.group$vectors[env.wat[i]],
                                 disp.group$centroids[8, ]), method = "euclidean")
}

pairs.water <- eDNA.div[env.wat, c(3,4,5,7)]

cent.water.ratio <- as.data.frame(matrix(NA, nrow = length(unique(pairs.water$pair)),
                                         ncol = 3))
colnames(cent.water.ratio) <- c("T", "C", "E")
rownames(cent.water.ratio) <- paste(pairs.water$env, pairs.water$pair,
                                    sep = "")[which(pairs.water$treat == "E") ]

for (i in 1:dim(cent.water.ratio)[1]){
  pair <- as.numeric(gsub("water", "", rownames(cent.water.ratio)[i]))
  cent.water.ratio[i, 1] <- (cent.water[, 1][which(pairs.water$pair == pair &
                                                   pairs.water$treat == "C")]) /
                            (cent.water[, 1][which(pairs.water$pair == pair &
                                                   pairs.water$treat == "E")])
  cent.water.ratio[i, 2] <- (cent.water[, 2][which(pairs.water$pair == pair &
                                                  pairs.water$treat == "C")]) /
                            (cent.water[, 2][which(pairs.water$pair == pair &
                                                  pairs.water$treat == "E")])
  cent.water.ratio[i, 3] <- (cent.water[, 3][which(pairs.water$pair == pair &
                                                  pairs.water$treat == "C")]) /
                            (cent.water[, 3][which(pairs.water$pair == pair &
                                                  pairs.water$treat == "E")])
}

# Sediments
env.sed <- which(eDNA.env == "sed")
cent.sed <- as.data.frame(matrix(NA, nrow = length(env.sed), ncol = 3))
colnames(cent.sed) <- c("T", "C", "E")
rownames(cent.sed) <- rownames(eDNA.div)[env.sed]

for (i in 1:length(env.sed)){
  cent.sed[i, 1] <- dist(rbind(disp.env$vectors[env.sed[i]],
                               disp.env$centroids[2, ]), method = "euclidean")
  cent.sed[i, 2] <- dist(rbind(disp.group$vectors[env.sed[i]],
                               disp.group$centroids[3, ]), method = "euclidean")
  cent.sed[i, 3] <- dist(rbind(disp.group$vectors[env.sed[i]],
                               disp.group$centroids[4, ]), method = "euclidean")
}
```

```
pairs.sed <- eDNA.div[env.sed, c(3,4,5,7)]

cent.sed.ratio <- as.data.frame(matrix(NA, nrow = length(unique(pairs.sed$pair)),
                                       ncol = 3))
colnames(cent.sed.ratio) <- c("T", "C", "E")
rownames(cent.sed.ratio) <- paste(pairs.sed$env, pairs.sed$pair,
                                  sep = "")[which(pairs.sed$treat == "E") ]

for (i in 1:dim(cent.sed.ratio)[1]){
  pair <- as.numeric(gsub("sed", "", rownames(cent.sed.ratio)[i]))
  cent.sed.ratio[i, 1] <- (cent.sed[, 1][which(pairs.sed$pair == pair &
                                                 pairs.sed$treat == "C")]) /
                          (cent.sed[, 1][which(pairs.sed$pair == pair &
                                                 pairs.sed$treat == "E")])
  cent.sed.ratio[i, 2] <- (cent.sed[, 2][which(pairs.sed$pair == pair &
                                                pairs.sed$treat == "C")]) /
                          (cent.sed[, 2][which(pairs.sed$pair == pair &
                                                pairs.sed$treat == "E")])
  cent.sed.ratio[i, 3] <- (cent.sed[, 3][which(pairs.sed$pair == pair &
                                                pairs.sed$treat == "C")]) /
                          (cent.sed[, 3][which(pairs.sed$pair == pair &
                                                pairs.sed$treat == "E")])
}

# Feces
env.fec <- which(eDNA.env == "feces")
cent.feces <- as.data.frame(matrix(NA, nrow = length(env.fec), ncol = 3))
colnames(cent.feces) <- c("T", "C", "E")
rownames(cent.feces) <- rownames(eDNA.div)[env.fec]

for (i in 1:length(env.fec)){
  cent.feces[i, 1] <- dist(rbind(disp.env$vectors[env.fec[i]],
                                 disp.env$centroids[1, ]), method = "euclidean")
  cent.feces[i, 2] <- dist(rbind(disp.group$vectors[env.fec[i]],
                                 disp.group$centroids[1, ]), method = "euclidean")
  cent.feces[i, 3] <- dist(rbind(disp.group$vectors[env.fec[i]],
                                 disp.group$centroids[2, ]), method = "euclidean")
}

pairs.feces <- eDNA.div[env.fec, c(3,4,5,7)]

cent.feces.ratio <- as.data.frame(matrix(NA, nrow = length(unique(pairs.feces$pair)),
                                         ncol = 3))
colnames(cent.feces.ratio) <- c("T", "C", "E")
rownames(cent.feces.ratio) <- paste(pairs.feces$env, pairs.feces$pair,
                                    sep = "")[which(pairs.feces$treat == "E") ]

for (i in 1:dim(cent.feces.ratio)[1]){
  pair <- as.numeric(gsub("feces", "", rownames(cent.feces.ratio)[i]))
  cent.feces.ratio[i, 1] <- (cent.feces[, 1][which(pairs.feces$pair == pair &
                                                   pairs.feces$treat == "C")]) /
                            (cent.feces[, 1][which(pairs.feces$pair == pair &
                                                   pairs.feces$treat == "E")])
```

```
  cent.feces.ratio[i, 2] <- (cent.feces[, 2][which(pairs.feces$pair == pair &
                                                   pairs.feces$treat == "C")]) /
                            (cent.feces[, 2][which(pairs.feces$pair == pair &
                                                   pairs.feces$treat == "E")])
  cent.feces.ratio[i, 3] <- (cent.feces[, 3][which(pairs.feces$pair == pair &
                                                   pairs.feces$treat == "C")]) /
                            (cent.feces[, 3][which(pairs.feces$pair == pair &
                                                   pairs.feces$treat == "E")])
}

# Ratio Data Table
ratio.list <- list(cent.feces.ratio[,1], cent.sed.ratio[,1],
                   cent.soil.ratio[,1], cent.water.ratio[,1])
cent.dist.table <- as.data.frame(matrix(NA, nrow = 4, ncol = 5))
colnames(cent.dist.table) <- c("env", "mean", "sem", "LCI", "UCI")
cent.dist.table$env <- c("Gut", "Sediment", "Soil", "Water")
cent.dist.table$mean <- unlist(lapply(ratio.list, mean))
cent.dist.table$sem <- unlist(lapply(ratio.list, sem))
cent.dist.table$LCI <- unlist(lapply(ratio.list, FUN = function(x) t.test(x)$conf.int[1]))
cent.dist.table$UCI <- unlist(lapply(ratio.list, FUN = function(x) t.test(x)$conf.int[2]))
p.cent.dist.table <- as.data.frame(cent.dist.table)

# Regression: centroid ratio vs. proportion eDNA
# Combine Centroid Distances
centroid.dists <- c(cent.feces.ratio$T, cent.sed.ratio$T, cent.soil.ratio$T,
                    cent.water.ratio$T)
names(centroid.dists) <- c(rownames(cent.feces.ratio), rownames(cent.sed.ratio),
                           rownames(cent.soil.ratio), rownames(cent.water.ratio))

# Reorganize
names(centroid.dists) %in% paste(eDNA.div.c$env, eDNA.div.c$pair, sep = "")
```

```
##  [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [15] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [29] TRUE
```

```
all.equal(names(centroid.dists), paste(eDNA.div.c$env, eDNA.div.c$pair, sep = ""))
```

```
## [1] "28 string mismatches"
```

```
cent.dist <- centroid.dists[match(names(centroid.dists),
                      paste(eDNA.div.c$env, eDNA.div.c$pair, sep = ""))]

# Combine Centroid Distance and Design Information
centroid.div <- cbind(eDNA.div.c[,c(1:3, 5:7)], cent.dist)

centroid.div$qPCR.match %in% eDNA.prop$sample.number
```

```
##  [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [15] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
## [29] TRUE
```

```
centroid.div$prop <- NA
for (i in 1:length(centroid.div$prop)){
  centroid.div$prop[i] <- eDNA.prop$prop[which(eDNA.prop$sample.number ==
                                               centroid.div$qPCR.match[i])]
```

```
}

t.cent.reg <- lm(cent.dist ~ prop * env, data = centroid.div)
summary(t.cent.reg)
```

```
##
## Call:
## lm(formula = cent.dist ~ prop * env, data = centroid.div)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.16662 -0.05189 -0.01227  0.01581  0.26899
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.98695    0.10256   9.623 3.78e-09 ***
## prop           0.05988    0.20021   0.299    0.768
## envsed         0.14548    0.14406   1.010    0.324
## envsoil       -0.03073    0.15307  -0.201    0.843
## envwater       0.07209    0.13904   0.518    0.610
## prop:envsed   -0.25185    0.28697  -0.878    0.390
## prop:envsoil   0.39154    0.49179   0.796    0.435
## prop:envwater -0.14768    0.28361  -0.521    0.608
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.108 on 21 degrees of freedom
## Multiple R-squared:  0.1243, Adjusted R-squared:  -0.1677
## F-statistic: 0.4257 on 7 and 21 DF,  p-value: 0.8752
```

```
# answer => no correlation between proportion eDNA and centroid dist ratio.
```

Centroid Distance Plots

Taxonomic Centoid Distance Ratio Plot

```
png(filename="../figures/ratio.centroid.png",
    width = 800, height = 600, res = 96*2)

par(mar=c(3, 4, 1, 1) + 0.1)

cent.dist.table <- t.cent.dist.table

centroid.plot <- plot(cent.dist.table$mean, ylim = c(0.5, 1.7),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 1, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = cent.dist.table$mean, y1 = cent.dist.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = cent.dist.table$mean, y1 = cent.dist.table$UCI, angle = 90,
```

```
       length=0.1, lwd = 2)
points(c(1:4), cent.dist.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Centroid Distance Ratio'), side = 2,
        outer = F, cex = 1.25, line = 2.5, adj = 0.5)
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = T, at = c(0.5, 1, 1.5))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.5, 1, 1.5))

axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
    labels = c("Gut", "Sediment", "Soil", "Water"), at = c(1, 2, 3, 4))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/ratio.centroid.png")
grid.raster(img)
```

Phylogenetic Centoid Distance Ratio Plot

```
png(filename="../figures/ratio.phylo.centroid.png",
    width = 800, height = 600, res = 96*2)

par(mar=c(3, 4, 1, 1) + 0.1)

cent.dist.table <- p.cent.dist.table

centroid.plot <- plot(cent.dist.table$mean, ylim = c(0.5, 1.5),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 1, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = cent.dist.table$mean, y1 = cent.dist.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
```

```
arrows(x0 = c(1,2,3,4), y0 = cent.dist.table$mean, y1 = cent.dist.table$UCI, angle = 90,
       length=0.1, lwd = 2)
points(c(1:4), cent.dist.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Centroid Distance Ratio'), side = 2,
        outer = F, cex = 1.25, line = 2.5, adj = 0.5)
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = T, at = c(0.5, 1, 1.5))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.5, 1, 1.5))

axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
    labels = c("Gut", "Sediment", "Soil", "Water"), at = c(1, 2, 3, 4))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/ratio.phylo.centroid.png")
grid.raster(img)
```

Multipanel Centoid Distance Ratio Plot

```
png(filename="../figures/panel.ratio.centroid.png",
    width = 800, height = 1200, res = 96*2)

layout(matrix(1:2, 2, 1))
par(mar = c(0.5, 5, 0.5, 0) + 0.1, oma = c(4, 1, 1, 1))

# Bray Curtis Plot
cent.dist.table <- t.cent.dist.table
centroid.plot <- plot(cent.dist.table$mean, ylim = c(0.5, 1.6),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 1, lty = 3, lwd = 2)
```

```
arrows(x0 = c(1,2,3,4), y0 = cent.dist.table$mean, y1 = cent.dist.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = cent.dist.table$mean, y1 = cent.dist.table$UCI, angle = 90,
       length=0.1, lwd = 2)
points(c(1:4), cent.dist.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext('Centroid Distance Ratio\n(Bray Curtis Distance)', side = 2,
        outer = F, cex = 1.25, line = 2.5, adj = 0.5)
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = T, at = c(0.7, 1, 1.3, 1.6))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.7, 1, 1.3, 1.6), tck = -0.02)

axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
    labels = F, at = c(1, 2, 3, 4), tck = -0.02)

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F, tck = -0.02)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F,
     at = c(0.7, 1, 1.3, 1.6))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F,
     at = c(0.7, 1, 1.3, 1.6))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))


# Phylogenetic Plot
cent.dist.table <- p.cent.dist.table
centroid.plot <- plot(cent.dist.table$mean, ylim = c(0.6, 1.4),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 1, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = cent.dist.table$mean, y1 = cent.dist.table$LCI, angle = 90,
       length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = cent.dist.table$mean, y1 = cent.dist.table$UCI, angle = 90,
       length=0.1, lwd = 2)
points(c(1:4), cent.dist.table$mean,pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext('Centroid Distance Ratio\n(UniFrac Distance)', side = 2,
        outer = F, cex = 1.25, line = 2.5, adj = 0.5)
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = T, at = c(0.7, 1, 1.3))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(0.7, 1, 1.3), tck = -0.02)
```

```r
axis(side = 1, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = c("Gut", "Sediment", "Soil", "Water"), at = c(1, 2, 3, 4))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F, tck = -0.02)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.7, 1, 1.3))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.7, 1, 1.3))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```r
graphics.off()

# Show Plot
img <- readPNG("../figures/panel.ratio.centroid.png")
grid.raster(img)
```

Old Code

```r
# Delta Distance as Funciton of Proportion eDNA
eDNA.pairs
eDNA.prop

eDNA.pairs$prop <- eDNA.prop.sub2$prop[match(eDNA.prop.sub2$sample.number, eDNA.pairs$qPCR)]

# Reshape data
eDNA.pairs.2 <- reshape(eDNA.pairs[,1:4], timevar = "treat",
                  idvar = c("qPCR.match", "env"), direction = "wide")

# Regression: PD ratio vs. proportion eDNA
phylo.reg <- lm(phylo.2[order(phylo.2$qPCR.match), ]$phylo.ratio ~ eDNA.prop.sub2$prop * eDNA.prop.sub2$
summary(phylo.reg)

# Separate by Treatments
eDNA.div.c <- eDNA.div[which(eDNA.div$treat == "C"), ]
eDNA.div.e <- eDNA.div[which(eDNA.div$treat == "E"), ]
OTU.REL.log.c <- OTU.REL.log[which(eDNA.div$treat == "C"), ]
OTU.REL.log.e <- OTU.REL.log[which(eDNA.div$treat == "E"), ]

# Order Each by Pairings
eDNA.div.c <- eDNA.div.c[order(eDNA.div.c$pair), ]
eDNA.div.e <- eDNA.div.e[order(eDNA.div.e$pair), ]
OTU.REL.log.c <- OTU.REL.log.c[order(eDNA.div.c$pair), ]
OTU.REL.log.e <- OTU.REL.log.e[order(eDNA.div.e$pair), ]
all.equal(eDNA.div.e$env, eDNA.div.c$env)
```

```r
# Create Distance Matrix for Treatments
dist.c <- vegdist(OTU.REL.log.c, method = "bray", upper = T, diag = T)
dist.e <- vegdist(OTU.REL.log.e, method = "bray", upper = T, diag = T)

pcoa.c <- cmdscale(dist.c, k = 3)
pcoa.e <- cmdscale(dist.e, k = 3)

# Procrustes Analysis
eDNA.ProC <- protest(pcoa.c, pcoa.e, scores = "sites", permutations = how(nperm = 999))
eDNA.ProC
summary(eDNA.ProC)

# Delta Distances
## Taxonomic Distances Between Pairs
# Data Check
all.equal(row.names(eDNA.div), row.names(OTU.REL.log))

# Create Raw Distance Matrix
dist.raw <- as.matrix(vegdist(OTU.REL.log, method = "bray", upper = T, diag = T))

# Calculate Average Distance For Each Environment
mean.dist.env <- matrix(NA, length(unique(eDNA.div$env)), 3)
rownames(mean.dist.env) <- unique(eDNA.div$env)
colnames(mean.dist.env) <- c("Total", "C", "E")
for (i in 1:dim(mean.dist.env)[1]){
  mean.dist.env[i, 1] <- mean(dist.raw[eDNA.div$env == rownames(mean.dist.env)[i],
                                       eDNA.div$env == rownames(mean.dist.env)[i]])
  mean.dist.env[i, 2] <- mean(dist.raw[eDNA.div$env == rownames(mean.dist.env)[i] &
                                           eDNA.div$treat == "C",
                                       eDNA.div$env == rownames(mean.dist.env)[i] &
                                           eDNA.div$treat == "C"])
  mean.dist.env[i, 3] <- mean(dist.raw[eDNA.div$env == rownames(mean.dist.env)[i] &
                                           eDNA.div$treat == "E",
                                       eDNA.div$env == rownames(mean.dist.env)[i] &
                                           eDNA.div$treat == "E"])
}

# Create Pairing Matrix
pair.names <- levels(as.factor(eDNA.div$pair))
eDNA.pairs <- as.data.frame(matrix(NA, length(pair.names), 4))
colnames(eDNA.pairs) <- c("pair", "env", "dis", "qPCR")
eDNA.pairs$pair <- pair.names

# Calculate Distance for Pairs
for (i in 1:length(pair.names)){
  pair.temp <- eDNA.pairs$pair[i]
  samp.temp <- rownames(eDNA.div)[which(eDNA.div$pair == pair.temp)]
  if (length(samp.temp) != 2){
    stop("more than two samples in pair")
  }
  dist.temp <- dist.raw[which(colnames(dist.raw) == samp.temp[1]),
                        which(colnames(dist.raw) == samp.temp[2])]
  eDNA.pairs$env[i] <- eDNA.div$env[which(row.names(eDNA.div) == samp.temp[1])]
```

```
  eDNA.pairs$dis[i] <- dist.temp
  eDNA.pairs$qPCR[i] <- eDNA.div$qPCR.match[which(row.names(eDNA.div) == samp.temp[1])]
}

# Organize Data
pair.dis.mean <- aggregate(dis ~ env, eDNA.pairs, mean)
pair.dis.sem <- aggregate(dis ~ env, eDNA.pairs, sem)
pair.dis.95 <- aggregate(dis ~ env, eDNA.pairs,
                  FUN = function(x) t.test(x)$conf.int[1:2])
t.pair.dis.table <- data.frame(env = pair.dis.mean$env, mean = pair.dis.mean$dis,
                         sem = pair.dis.sem$dis, ci = pair.dis.95$dis)
colnames(t.pair.dis.table) <- c("env", "mean", "sem", "LCI", "UCI")

# Organize Data as Ratio
env.means <-


## Phylogenetic Distances between Pairs

# Data Check
all.equal(row.names(eDNA.div), row.names(as.matrix(phylo.dist.m)))

# Create Raw Distance Matrix
dist.raw <- phylo.dist.m
colnames(dist.raw) <- rownames(dist.raw)

# Create Pairing Matrix
pair.names <- levels(as.factor(eDNA.div$pair))
eDNA.pairs <- as.data.frame(matrix(NA, length(pair.names), 4))
colnames(eDNA.pairs) <- c("pair", "env", "dis", "qPCR")
eDNA.pairs$pair <- pair.names

# Calculate Distance for Pairs
for (i in 1:length(pair.names)){
  pair.temp <- eDNA.pairs$pair[i]
  samp.temp <- rownames(eDNA.div)[which(eDNA.div$pair == pair.temp)]
  if (length(samp.temp) != 2){
    stop("more than two samples in pair")
  }
  dist.temp <- dist.raw[which(colnames(dist.raw) == samp.temp[1]),
                        which(colnames(dist.raw) == samp.temp[2])]
  eDNA.pairs$env[i] <- eDNA.div$env[which(row.names(eDNA.div) == samp.temp[1])]
  eDNA.pairs$dis[i] <- dist.temp
  eDNA.pairs$qPCR[i] <- eDNA.div$qPCR.match[which(row.names(eDNA.div) == samp.temp[1])]
}

pair.dis.mean <- aggregate(dis ~ env, eDNA.pairs, mean)
pair.dis.sem <- aggregate(dis ~ env, eDNA.pairs, sem)
pair.dis.95 <- aggregate(dis ~ env, eDNA.pairs,
                  FUN = function(x) t.test(x)$conf.int[1:2])
p.pair.dis.table <- data.frame(env = pair.dis.mean$env, mean = pair.dis.mean$dis,
                         sem = pair.dis.sem$dis, ci = pair.dis.95$dis)
colnames(p.pair.dis.table) <- c("env", "mean", "sem", "LCI", "UCI")
```

```r
# Delta Distance Plots
## Taxonomic Plot

png(filename="../figures/delta.dist.png",
    width = 800, height = 600, res = 96*2)

par(mar=c(3, 4, 1, 1) + 0.1)

distance.plot <- plot(t.pair.dis.table$mean, ylim = c(-0.1, 0.6),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 0, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = delta.dis.table$mean, y1 = delta.dis.table$LCI,
       angle = 90, length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = delta.dis.table$mean, y1 = delta.dis.table$UCI,
       angle = 90, length=0.1, lwd = 2)
points(c(1:4), delta.dis.table$mean, pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Distance'), side = 2,
      outer = F, cex = 1.5, line = 3, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = T, at = c(seq(-0.1, 0.3, 0.1)))
axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(-0.1, 0.3, 0.1))
axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
    labels = c("Gut", "Sediment", "Soil", "Water"), at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(-0.1, 0.3, 0.1))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(-0.1, 0.3, 0.1))

# Close Plot Device
dev.off()
graphics.off()

# Show Plot
img <- readPNG("../figures/delta.dist.png")
grid.raster(img)


## Phylogenetic Plot

png(filename="../figures/phylo.delta.dist.png",
    width = 800, height = 600, res = 96*2)
```

```
par(mar=c(3, 4, 1, 1) + 0.1)

distance.plot <- plot(delta.dis.table$mean, ylim = c(-0.1, 0.3),
      xlim = c(0.5, 4.5), pch = 22, bg = "white", lwd = 2,
      cex = 3, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 1.5,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
abline(h = 0, lty = 3, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = delta.dis.table$mean, y1 = delta.dis.table$LCI,
       angle = 90, length = 0.1, lwd = 2)
arrows(x0 = c(1,2,3,4), y0 = delta.dis.table$mean, y1 = delta.dis.table$UCI,
       angle = 90, length=0.1, lwd = 2)
points(c(1:4), delta.dis.table$mean, pch = 22, cex = 3, bg = "gray90", lwd = 2)

mtext(expression('Distance'), side = 2,
      outer = F, cex = 1.5, line = 3, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
    labels = T, at = c(seq(-0.1, 0.3, 0.1)))
axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
   labels = F, at = c(-0.1, 0.3, 0.1))
axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
    labels = c("Gut", "Sediment", "Soil", "Water"), at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
    at = c(1, 2, 3, 4), labels = F)

# Minor ticks
axis(side = 2, lwd.ticks = 2, tck = 0.02, labels = F, at = c(0.5, 1, 1.5))
axis(side = 4, lwd.ticks = 2, tck = 0.02, labels = F, at = c(-0.1, 0.3, 0.1))
axis(side = 1, lwd.ticks = 2, tck = 0.02, labels = F, at = c(1, 2, 3, 4))
axis(side = 3, lwd.ticks = 2, tck = 0.02, labels = F, at = c(-0.1, 0.3, 0.1))

# Close Plot Device
dev.off()
graphics.off()

# Show Plot
img <- readPNG("../figures/phylo.delta.dist.png")
grid.raster(img)


## Delta-Abundances

# Check order of design
all.equal(row.names(eDNA.div), row.names(OTU.REL.log))

OTU.REL.c <- OTU.REL.log[which(eDNA.div$treat == "C"), ]
OTU.REL.e <- OTU.REL.log[which(eDNA.div$treat == "E"), ]

eDNA.div.c <- eDNA.div[which(eDNA.div$treat == "C"), ]
eDNA.div.e <- eDNA.div[which(eDNA.div$treat == "E"), ]
```

```
all.equal(sort(eDNA.div.c$samp.code), sort(eDNA.div.e$samp.code))

eDNA.div.c <- eDNA.div.c[order(eDNA.div.c$samp.code), ]
eDNA.div.e <- eDNA.div.e[order(eDNA.div.e$samp.code), ]

OTU.REL.c2 <- OTU.REL.c[order(eDNA.div.c$samp.code), ]
OTU.REL.e2 <- OTU.REL.e[order(eDNA.div.e$samp.code), ]

dim(OTU.REL.c2); dim(OTU.REL.e2)
all.equal(eDNA.div.e$samp.code, eDNA.div.c$samp.code)

delta.abund <- OTU.REL.c2 - OTU.REL.e2

eDNA.permanova <- adonis(delta.abund ~ eDNA.div.c$env,
                         method = "euclidean", binary = FALSE)
eDNA.permanova




# What is this stuff for????? Check all calculations
# Variation Parititioning

# Turn Distance Matrices into Matrices
dist.raw <- as.matrix(dist.raw)
dist.e <- as.matrix(dist.e)
dist.c <- as.matrix(dist.c)

# Seperate by Habitat
eDNA.div.gut <- eDNA.div[which(eDNA.div$env == "feces"), ]
eDNA.div.sed <- eDNA.div[which(eDNA.div$env == "sed"), ]
eDNA.div.sol <- eDNA.div[which(eDNA.div$env == "soil"), ]
eDNA.div.wat <- eDNA.div[which(eDNA.div$env == "water"), ]

OTU.REL.log.gut <- OTU.REL.log[which(eDNA.div$env == "feces"), ]
OTU.REL.log.sed <- OTU.REL.log[which(eDNA.div$env == "sed"), ]
OTU.REL.log.sol <- OTU.REL.log[which(eDNA.div$env == "soil"), ]
OTU.REL.log.wat <- OTU.REL.log[which(eDNA.div$env == "water"), ]

# Order Each by Pairings
eDNA.div.gut <- eDNA.div.gut[order(eDNA.div.gut$pair), ]
eDNA.div.sed <- eDNA.div.sed[order(eDNA.div.sed$pair), ]
eDNA.div.sol <- eDNA.div.sol[order(eDNA.div.sol$pair), ]
eDNA.div.wat <- eDNA.div.wat[order(eDNA.div.wat$pair), ]
OTU.REL.log.gut <- OTU.REL.log.gut[order(eDNA.div.gut$pair), ]
OTU.REL.log.sed <- OTU.REL.log.sed[order(eDNA.div.sed$pair), ]
OTU.REL.log.sol <- OTU.REL.log.sol[order(eDNA.div.sol$pair), ]
OTU.REL.log.wat <- OTU.REL.log.wat[order(eDNA.div.wat$pair), ]

# Create Distance Matrix for Treatments
dist.gut <- vegdist(OTU.REL.log.gut, method = "bray", upper = F, diag = F)
```

```
dist.sed <- vegdist(OTU.REL.log.sed, method = "bray", upper = F, diag = F)
dist.sol <- vegdist(OTU.REL.log.sol, method = "bray", upper = F, diag = F)
dist.wat <- vegdist(OTU.REL.log.wat, method = "bray", upper = F, diag = F)

# Turn Distance Matrices into Matrices
dist.gut2 <- as.matrix(dist.gut)
dist.sed2 <- as.matrix(dist.sed)
dist.sol2 <- as.matrix(dist.sol)
dist.wat2 <- as.matrix(dist.wat)

# what are the factors I have to work with
head(eDNA.div.gut)
levels(eDNA.div.gut$treat)
levels(as.factor(eDNA.div.gut$qPCR.match))

# Variance Partitioning
var.gut <- varpart(Y = dist.gut, as.numeric(eDNA.div.gut$treat), (eDNA.div.gut$qPCR.match))
plot(var.gut)

gut.RDA <- capscale(dist.gut ~ eDNA.div.gut$treat + as.factor(eDNA.div.gut$qPCR.match))
anova(gut.RDA, by = "term")
RsquareAdj(gut.RDA)

# Adjusted RSquare by Terms
rda.aov <- (anova(gut.RDA, by = "term"))
r2 <- (rda.aov$SumOfSqs[1:2] / sum(rda.aov$SumOfSqs[1:3]))
m <- c(length(levels(eDNA.div.gut$treat)) - 1,
       length(levels(as.factor(eDNA.div.gut$qPCR.match))) - 1)
n <- nrow(gut.RDA$CCA$u)
adjr2 <- 1 - (1 - r2) * (n - 1)/(n - m - 1)
r2; adjr2; sum(r2); sum(adjr2)


var.r2 <- as.data.frame(matrix(NA, 4, 3))
colnames(var.r2) <- c("eDNA", "replicates", "unexplained")
row.names(var.r2) <- c("gut", "sed", "sol", "wat")
for (i  in 1:dim(var.r2)[1]){
  env.temp <- rownames(var.r2)[i]
  dis.temp <- get(paste("dist", env.temp, sep = "."))
  dat.temp <- get(paste("eDNA.div", env.temp, sep = "."))
  rda.temp <- capscale(dis.temp ~ dat.temp$treat +
                         as.factor(dat.temp$qPCR.match))
  aov.temp <- anova(rda.temp, by = "term")
  r2 <- (aov.temp$SumOfSqs[1:2] / sum(aov.temp$SumOfSqs[1:3]))
  var.r2[i, 1] <- r2[1]
  var.r2[i, 2] <- r2[2]
  var.r2[i, 3] <- (1 - sum(r2))
}

png(filename="../figures/variation.png",
    width = 1200, height = 800, res = 96*2)
layout(matrix(1:2, 2, 1) , heights = c(8, 2))
par(mar = c(2, 5, 1, 0) + 0.5, oma = c(0.5, 0.5, 0.5, 0.5))
```

```r
barplot(t(as.matrix(var.r2)),
        names.arg = c("Gut", "Sediment", "Soil", "Water"),
        las = 1, ylab = "")

mtext("Proportion of Variation", side = 2, cex = 1.25, line = 3)

# Add Legend Outside
par(mar = c(1, 5, 0, 1) + 0.5)
plot.new()
legend("top", c("DNase", "Replicates", "Unexplained"),
       pch = 22, pt.bg = c("gray40", "gray60", "gray90"),
      bty = "n", y.intersp = 1.5, horiz = T)


# Close Plot Device
dev.off()
graphics.off()

# Show Plot
img <- readPNG("../figures/variation.png")
grid.raster(img)


# Variation based on Bray Curtis Distance

bc.var <- as.data.frame(matrix(NA, 4, 3))
colnames(bc.var) <- c("reps", "treat", "env")
row.names(bc.var) <- c("gut", "sed", "sol", "wat")
for (i  in 1:dim(bc.var)[1]){
  env.temp <- rownames(bc.var)[i]
  dis.temp <- get(paste("dist", env.temp, sep = "."))
  dat.temp <- get(paste("eDNA.div", env.temp, sep = "."))
  dat.temp <- dat.temp[order(dat.temp$samp.num), ]
  if (all(attributes(dis.temp)$Labels != rownames(dat.temp))){
    stop("distance matix and data are not arranged properly")
  }
  dis.temp.C <- as.matrix(dis.temp)[which(dat.temp$treat == "C"),
                                    which(dat.temp$treat == "C")]
  dis.temp.C <- as.dist(dis.temp.C)
  bc.var[i,3] <- mean(dis.temp.C)
  dis.pair <- as.data.frame(matrix(NA, length(unique(dat.temp$pair)), 3))
  colnames(dis.pair) <- c("C", "E", "dis")
  dis.pair[,1] <- rownames(dat.temp)[dat.temp$treat == "C"]
  for (j in 1:length(dis.pair$C)){
    pair <- dat.temp$pair[which(rownames(dat.temp) == dis.pair$C[j])]
    dis.pair[j, 2] <- rownames(dat.temp)[which(dat.temp$treat == "E" &
                                          dat.temp$pair == pair)]
    dis.pair[j, 3] <- as.matrix(dis.temp)[which(rownames(dat.temp) == dis.pair[j, 1]),
                                    which(rownames(dat.temp) == dis.pair[j, 2])]
  }
  bc.var[i,2] <- mean(dis.pair[,3])
  reps <- names(which(table(dat.temp$qPCR.match) > 2))
  dis.rep <- as.data.frame(matrix(NA, length(reps), 3))
```

```
  colnames(dis.rep) <- c("rep1", "rep2", "dis")
  for (k in 1:length(reps)){
    dis.rep[k, 1:2] <- rownames(dat.temp)[which(dat.temp$treat == "C" &
                                                 dat.temp$qPCR.match == reps[k])]
    dis.rep[k, 3] <- as.matrix(dis.temp)[which(rownames(dat.temp) == dis.rep[k, 1]),
                                         which(rownames(dat.temp) == dis.rep[k, 2])]
  }
  bc.var[i, 1] <- mean(dis.rep[, 3])
}

bc.var

# bc.var <- bc.var/rowSums(bc.var)

# Standardized BC distances
bc.var.std <- bc.var/rowSums(bc.var)


## Distance Partitioning Plot

png(filename="../figures/BC_dist.png",
    width = 1200, height = 800, res = 96*2)
layout(matrix(1:2, 2, 1) , heights = c(8, 2))
par(mar = c(2, 5, 1, 0) + 0.5, oma = c(0.5, 0.5, 0.5, 0.5))

barplot(t(as.matrix(bc.var)),
        names.arg = c("Gut", "Sediment", "Soil", "Water"),
        las = 1, ylab = "", ylim = c(0, 1))

mtext("Bray Curtis Distance", side = 2, cex = 1.25, line = 3)

# Add Legend Outside
par(mar = c(1, 5, 0, 1) + 0.5)
plot.new()
legend("top", c("Replicates", "Treatments", "Environments"),
       pch = 22, pt.bg = c("gray40", "gray60", "gray90"),
      bty = "n", y.intersp = 1.5, horiz = T)


# Close Plot Device
dev.off()
graphics.off()

# Show Plot
img <- readPNG("../figures/BC_dist.png")
grid.raster(img)

# PERMANOVA on Delta-Distances

# Check order of design
all.equal(row.names(eDNA.div), row.names(as.matrix(phylo.dist)))

OTU.REL.c <- OTU.REL.log[which(eDNA.div$treat == "C"), ]
```

```r
OTU.REL.e <- OTU.REL.log[which(eDNA.div$treat == "E"), ]

eDNA.div.c <- eDNA.div[which(eDNA.div$treat == "C"), ]
eDNA.div.e <- eDNA.div[which(eDNA.div$treat == "E"), ]

all.equal(sort(eDNA.div.c$samp.code), sort(eDNA.div.e$samp.code))

eDNA.div.c <- eDNA.div.c[order(eDNA.div.c$samp.code), ]
eDNA.div.e <- eDNA.div.e[order(eDNA.div.e$samp.code), ]

OTU.REL.c2 <- OTU.REL.c[order(eDNA.div.c$samp.code), ]
OTU.REL.e2 <- OTU.REL.e[order(eDNA.div.e$samp.code), ]

dim(OTU.REL.c2); dim(OTU.REL.e2)
all.equal(eDNA.div.e$samp.code, eDNA.div.c$samp.code)

delta.abund <- OTU.REL.c2 - OTU.REL.e2

eDNA.permanova <- adonis(delta.abund ~ eDNA.div.c$env,
                         method = "euclidean", binary = FALSE)
eDNA.permanova

rowSums(delta.abund)

# Effect Size from lda
require(MASS)
eDNA.lda <- lda(delta.abund ~ eDNA.div.c$env, method = "euclidean")
anova(eDNA.dbRDA)
anova(eDNA.dbRDA, by = "terms")

# Distance Moved in PCoA Space
eDNA.points.c <- eDNA.points[which(eDNA.points$treat == "C"), ]
eDNA.points.e <- eDNA.points[which(eDNA.points$treat == "E"), ]

eDNA.points.env <- eDNA.points.c$env

eDNA.points.c <- eDNA.points.c[order(eDNA.points.c$samp.code), c(1:3)]
eDNA.points.e <- eDNA.points.e[order(eDNA.points.e$samp.code), c(1:3)]

delta.dis <- matrix(NA, dim(eDNA.points.c)[1], 2)
rownames(delta.dis) <- eDNA.points.c$samp.code
colnames(delta.dis) <- c("Abs", "Non_Abs")

for (i in 1:dim(eDNA.points.c)[1]){
  dis.temp <- matrix(NA, 3, 2)
  for (j in 1:3){
    dis.temp[j, 1] <- abs(eDNA.points.c[i,j] - eDNA.points.e[i,j])
    dis.temp[j, 2] <- (eDNA.points.c[i,j] - eDNA.points.e[i,j])
  }
  delta.dis[i, 1] <- sum(abs(dis.temp[,1]))
  delta.dis[i, 2] <- sum(abs(dis.temp[,2]))
}
```

```
delta.dis <- as.data.frame(delta.dis)

delta.dis$env <- eDNA.points.env

delta.dis.mean <- aggregate(Non_Abs ~ env, delta.dis, mean)
delta.dis.sem <- aggregate(Non_Abs ~ env, delta.dis, sem)
delta.dis.95 <- aggregate(Non_Abs ~ env, delta.dis,
                  FUN = function(x) t.test(x)$conf.int[1:2])
delta.dis.table <- data.frame(env = delta.dis.mean$env, mean = delta.dis.mean$Non_Abs,
                        sem = delta.dis.sem$Non_Abs, ci = delta.dis.95$Non_Abs)
colnames(delta.dis.table) <- c("env", "mean", "sem", "LCI", "UCI")
```