Resource Heterogeneity Structures Microbial Communities

Mario E. Muscarella

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Introduction

Much is already know about how spatial gradients in resource availability contribution to the structure and fucntion of microbial communities. However, we are begining to appreciate the molecular diversity within the resource pool. In this study, we explore how both the concentration and diversity of resources contribute to the structure and function of aquatic microbial communities.

Initial Setup

```
rm(list=ls())
getwd()
setwd("~/GitHub/ResourceHeterogeneity/analyses")

# Import Tools and Standard Functions
source("../bin/MothurTools.R")
se <- function(x, ...){sd(x, na.rm = TRUE)/sqrt(length(na.omit(x)))}

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

# Load Required Packages
require("png")
require("grid")
require("vegan")
```

Supplemental Figure 1: Study System Map

We sampled 10 lakes in the Huron Mountains of Michigan. The Huron Mountains are located in the Superior Bedrock Uplands region of the Michigan Upper Peninsula (Schaetzl et al 2013). The region is classified as .... The forests around the lakes are .... The wetershed is ....

Lake Nutrient Concentrations

```
# DOC
DOC2011 <- read.delim("../data/2011DOC_data.txt", header=T)
DOC2012 <- read.delim("../data/2012DOC_data.txt", header=T)
DOC <- rbind(DOC2011, DOC2012)
```

```
DOC <- DOC[grep("MEM*", DOC$Sample), ]
colnames(DOC) <- c("sample", "conc", "LCL", "UCL", "se")
DOCkey <- read.delim("../data/DOC_Key_epi.txt", header=T)
DOC$code <- DOC$sample
DOC <- DOC[which(DOC$code %in% DOCkey$Sample.Name), ]
DOC$sample <- DOCkey$Site[match(DOCkey$Sample.Name, DOC$code)]
DOC$year <- substr(DOC$code, 4, 7)
DOC$conc <- pmax(DOC$conc, 0)
DOC2 <- data.frame("sample" = DOC$sample, "year" = DOC$year,
                   "conc" =  DOC$conc)[order(DOC$sample, DOC$year), ]
DOC$sample[grep("Pony", DOC$sample)] <- "Pony"
DOC <- droplevels(DOC)

# Total Nitrogen
TN2011 <- read.delim("../data/2011TN_data.txt", header=T)
TN2012 <- read.delim("../data/2012TN_data.txt", header=T)
TN <- rbind(TN2011, TN2012)
TN <- TN[grep("MEM*", TN$Sample), ]
colnames(TN) <- c("sample", "conc", "LCL", "UCL", "se")
TNkey <- read.delim("../data/DOC_Key_epi.txt", header=T)
TN$code <- TN$sample
TN <- TN[which(TN$code %in% TNkey$Sample.Name), ]
TN$sample <- TNkey$Site[match(TNkey$Sample.Name, TN$code)]
TN$year <- substr(TN$code, 4, 7)
TN$conc <- pmax(TN$conc, 0)
TN2 <- data.frame("sample" = TN$sample, "year" = TN$year,
                  "conc" = TN$conc)[order(TN$sample, TN$year), ]
TN$sample[grep("Pony", TN$sample)] <- "Pony"
TN <- droplevels(TN)

# Total Phosphorus
TP2011 <- read.delim("../data/2011TP_data.txt")
TP2012 <- read.delim("../data/2012TP_data.txt")
TP2011$year <- rep("2011", dim(TP2011)[1])
TP2012$year <- rep("2012", dim(TP2012)[1])
TP <- rbind(TP2011, TP2012)
TP <- TP[grep("*iltered", TP$Sample), ]
colnames(TP) <- c("sample", "conc", "LCL", "UCL", "se", "year")
TP$code <- TP$sample
TDP <- TP[grep("*Filtered", TP$sample), ]
TP <- TP[grep("*Unfiltered", TP$sample), ]
TP$sample <- gsub(" Unfiltered", "", TP$sample)
TDP$sample <- gsub(" Filtered", "", TDP$sample)
TP[6, ] <- TDP[6, ]
TP <- TP[-c(which(TP$sample == "CanyonHypo" | TP$sample == "CanyonChemo")), ]
TP$sample <- gsub("CanyonEpi", "Canyon", TP$sample)
TP$sample <- as.factor(TP$sample)
TP$conc <- pmax(TP$conc, 0)
TP2 <- data.frame("sample" = TP$sample, "year" = TP$year,
                  "conc" = TP$conc)[order(TP$sample, TP$year), ]
TP$sample[grep("Pony", TP$sample)] <- "Pony"
TP <- droplevels(TP)
```

Save Data Table

```
DOC2 <- aggregate(conc ~ sample + year, DOC2, mean)
TN2 <- aggregate(conc ~ sample + year, TN2, mean)
TP2 <- aggregate(conc ~ sample + year, TP2, mean)

nuts <- data.frame("sample" = DOC2$sample, "year" = DOC2$year,
              "DOC" = DOC2$conc, "TP" = TP2$conc, "TN" = TN2$conc)

nuts$sample[grep("Pony", nuts$sample)] <- "Pony"
nuts <- droplevels(nuts)
```

Figure 1: Lake Nutrients

```
png(filename="../figures/Figure1.png",
    width = 1600, height =1200, res = 96*2)
par(opar)
par(mfrow = c(1,1), mar = c(0, 6, 0, 0) + 0.5, oma = c(4, 0, 1, 1) + 0.5)
layout(rbind(1, 2, 3), height = c(3, 3, 3))

labs <- c("Ann", "Canyon", "Howe", "Ives", "Lily", "Mountain", "Pony", "Rush",
         "Second\nPine","Upper\nPine")

# DOC Plot
plot(DOC$conc ~ DOC$sample, ylim = c(0, 35), las = 1,
     xaxt="n", xlab = "", yaxt="n", ylab = "")
axis(side=1, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=1, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
axis(side=2, lwd.ticks = 2, labels = T, cex.axis = 1.2, las = 1,
     at = c(0, 10, 20, 30))
axis(side=2, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(0, 10, 20, 30))
axis(side=3, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=3, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
axis(side=4, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(0, 10, 20, 30))
axis(side=4, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(0, 10, 20, 30))
mtext(side = 2, expression(paste("DOC (mg C L" ^-1, ")", sep="")), line = 3.5, cex = 1)
legend("topleft", "A", bty = "n", x.intersp = 0, cex = 1.25)
box(lwd = 2)

# Total Nitrogen Plot
plot(TN$conc ~ TN$sample, ylim = c(0,0.65), las = 1,
     xaxt="n", xlab = "", yaxt="n", ylab = "")
axis(side=1, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=1, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
axis(side=2, lwd.ticks = 2, labels = T, cex.axis = 1.2, las = 1,
     at = c(0.0, 0.2, 0.4, 0.6))
axis(side=2, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(0.0, 0.2, 0.4, 0.6))
axis(side=3, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=3, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
axis(side=4, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(0.0, 0.2, 0.4, 0.6))
axis(side=4, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(0.0, 0.2, 0.4, 0.6))
```

```
mtext(side = 2, expression(paste("TN (mg N L" ^-1, ")")), line = 3.5, cex = 1)
legend("topleft", "B", bty = "n", x.intersp = 0, cex = 1.25)
box(lwd = 2)

# Total Phosphorus Plot
plot(TP$sample, TP$conc, ylim = c(0,50), las = 1,
     xaxt="n", xlab = "", yaxt="n", ylab = "")
axis(side=1, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=1, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
axis(side=2, lwd.ticks = 2, labels = T, cex.axis = 1.2, las = 1,
     at = c(0, 20, 40))
axis(side=2, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(0, 20, 40))
axis(side=3, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=3, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
axis(side=4, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(0, 20, 40))
axis(side=4, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(0, 20, 40))
mtext(side = 2, expression(paste("TP (",mu, "g P L" ^-1, ")")), line = 3.5, cex = 1)
mtext(side = 1, text = labs, line = 1, at = seq(1:10), padj = 0.5, cex = 0.8)
legend("topleft", "C", bty = "n", x.intersp = 0, cex = 1.25)
box(lwd = 2)


dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
par(opar)
```

Chlorophyll A and Bacterial Respiration

Figure 2: Chlorophyll A and BR

Patterns of Bacterial Diversity

Import Raw Data

```
# Define Inputs
# Design = general design file for experiment
# shared = OTU table from mothur with sequence similarity clustering
# Taxonomy = Taxonomic information for each OTU
design.in <- "../data/design.txt"
shared <- "../data/HMWF.bac.final.shared"
taxon  <- "../data/HMWF.bac.final.0.03.taxonomy"

# Import Design
design.raw <- read.delim(design.in, header=T, row.names=1)

# Import Shared Files
OTUs.in <- read.otu(shared = shared, cutoff = "0.03")    # 97% Similarity
```

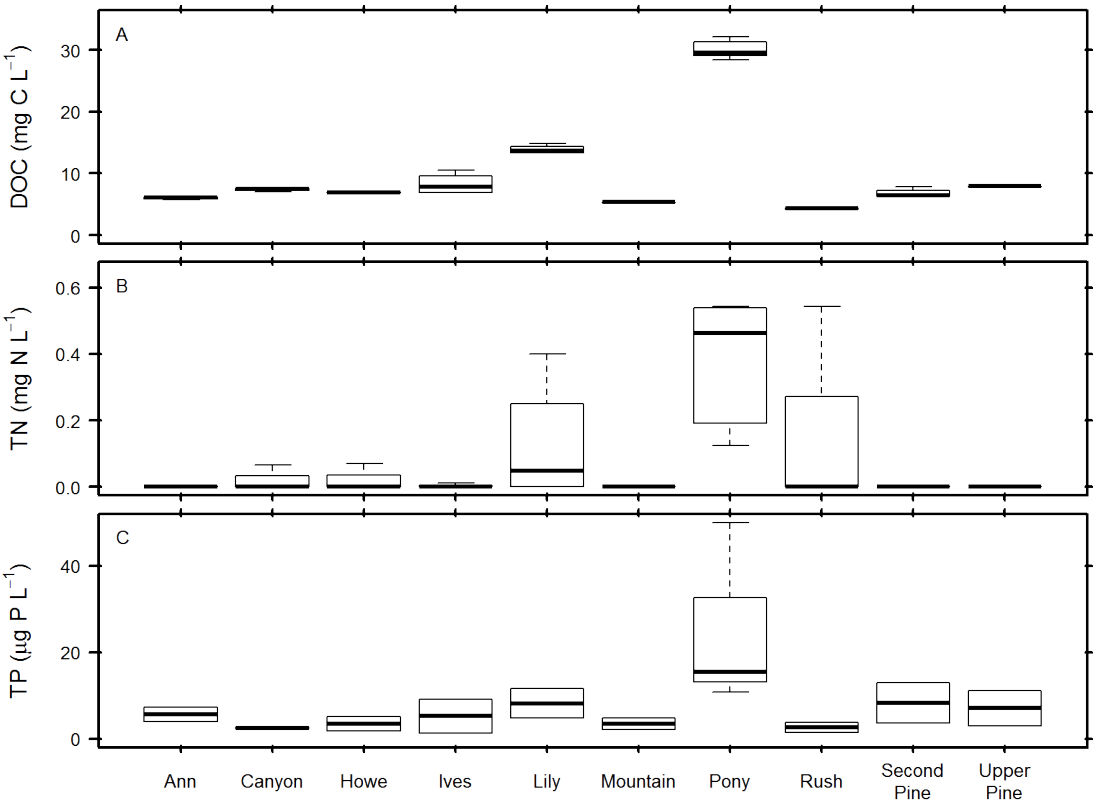

Figure 1: Lake Nutrients

```
# Import Taxonomy
OTU.tax <- read.tax(taxonomy = taxon, format = "rdp")
```

Data Transformations

```
# Remove Unwanted Sites
OTUs.hmwf <- OTUs.in[-c(7, 8 , 11, 12), ]

design <- design.raw[-c(7, 8 , 11, 12) , ]

# Reorder Site
OTUs.hmwf <- OTUs.hmwf[rownames(design), ]


# Remove OTUs with less than two occurences across all sites
OTUs <- OTUs.hmwf[, colSums((OTUs.hmwf > 0) * 1)  >= 2 | colSums(OTUs.hmwf >= 10)]

# Sequencing Coverage
coverage <- rowSums(OTUs)

# Good's Coverage
goods <- function(x = ""){
  1 - (sum(x == 1) / rowSums(x))
}
goods.c <- goods(OTUs)

# Make Presence Absence Matrix
OTUsPA <- (OTUs > 0) * 1

# Make Relative Abundence Matrices
OTUsREL <- OTUs
for(i in 1:dim(OTUs)[1]){
  OTUsREL[i,] <- OTUs[i,]/sum(OTUs[i,])
}
require(vegan)

# Log Transform Relative Abundances
OTUsREL.log <- decostand(OTUs, method="log")
```

Calculate Alpha Diversity

```
# Observed Richness
S.obs <- rowSums((OTUs > 0) * 1)

# Simpson's Evenness
SimpE <- function(x = ""){
  x <- as.data.frame(x)
  D <- diversity(x, "inv")
  S <- sum((x > 0) * 1)
```

```
  E <- (D)/S
  return(E)
}
simpsE <- round(apply(OTUs, 1, SimpE), 3)

# Shannon's Diversity
H <- function(x = ""){
  x <- x[x>0]
  H = 0
  for (n_i in x){
    p = n_i / sum(x)
    H = H - p*log(p)
  }
  return(H)
}

shan <- round(apply(OTUs, 1, H), 2)
shan2 <- diversity(OTUs, index = "shannon")

design <- droplevels(design)

alpha.div <- cbind(design, S.obs, simpsE, shan)
alpha.div <- alpha.div[order(alpha.div$Lake, alpha.div$Year, alpha.div$Molecule), ]
```

Figure 3: Lake Alpha Diversity

```
png(filename="../figures/Figure3.png",
    width = 1600, height = 1100, res = 96*2)
par(opar)
par(mfrow = c(1,1), mar = c(0, 6, 0, 0) + 0.5, oma = c(3, 0, 1, 1) + 0.5)
layout(rbind(1, 2), height = c(3, 3))

plot(alpha.div$S.obs ~ alpha.div$Lake,  ylim = c(0,7000), las = 1,
     xaxt="n", xlab = "", yaxt="n", ylab = "")
axis(side=1, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=1, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
axis(side=2, lwd.ticks = 2, labels = T, cex.axis = 1, las = 1,
     at = c(1000, 3000, 5000, 7000))
axis(side=2, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1000, 3000, 5000, 7000))
axis(side=3, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=3, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
axis(side=4, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1000, 3000, 5000, 7000))
axis(side=4, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1000, 3000, 5000, 7000))
mtext(side = 2, "Richness (S)", line = 4, cex = 1)
legend("topleft", "A", bty = "n", x.intersp = 0, cex = 1.25)
box(lwd = 2)

plot(alpha.div$simpsE ~ alpha.div$Lake, ylim = c(0,0.06), las = 1,
     xaxt="n", xlab = "", yaxt="n", ylab = "")
axis(side=1, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=1, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
```

```r
axis(side=2, lwd.ticks = 2, labels = T, cex.axis = 1, las = 1,
     at = c(0, 0.02, 0.04, 0.06))
axis(side=2, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(0, 0.02, 0.04, 0.06))
axis(side=3, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(1:10))
axis(side=3, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(1:10))
axis(side=4, lwd.ticks = 2, tck=-0.02, labels = F, cex.axis = 1, at = c(0, 0.02, 0.04, 0.06))
axis(side=4, lwd.ticks = 2, tck=0.01, labels = F, cex.axis = 1, at = c(0, 0.02, 0.04, 0.06))
mtext(side = 2, "Simpson's Eveness (E)", line = 4, cex = 1)
mtext(side = 1, text = labs, line = 0.5, at = seq(1:10), padj = 0.5, cex = 0.8)
legend("topleft", "B", bty = "n", x.intersp = 0, cex = 1.25)
box(lwd = 2)

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
par(opar)
```

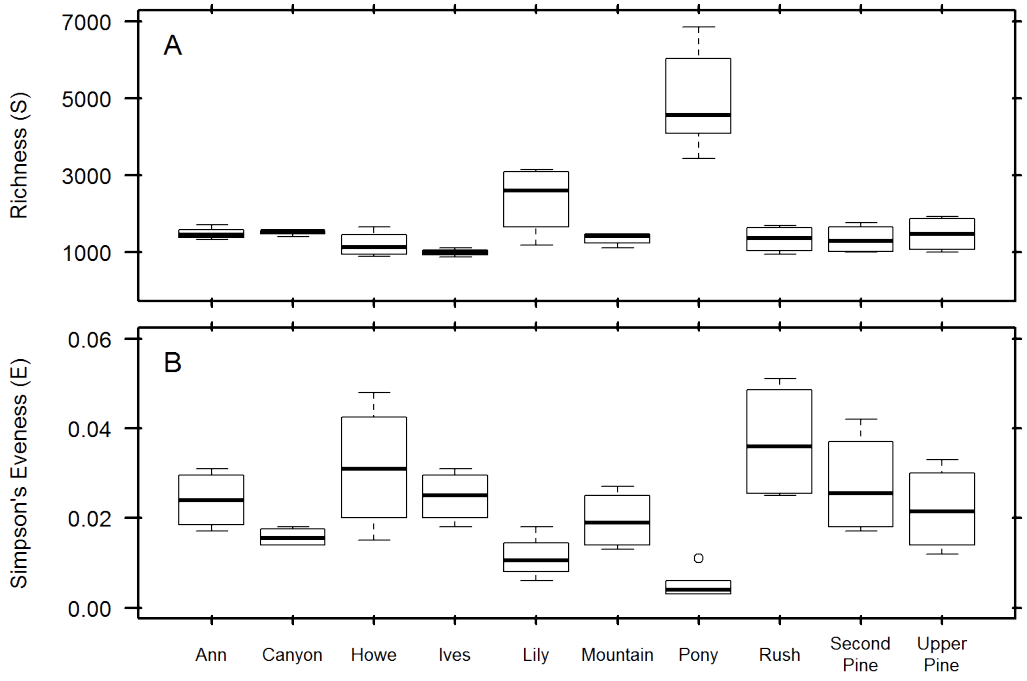

Figure 2: Lake Nutrients

Calculate and Visualize Beta Diversity

```r
beta.w <- function(site1 = "", site2 = ""){
  site1 = subset(site1, select = site1 > 0)             # Removes absences
  site2 = subset(site2, select = site2 > 0)             # Removes absences
  gamma = union(colnames(site1), colnames(site2))       # Gamma species pool
  s     = length(gamma)                                 # Gamma richness
```

```
  a.bar = mean(c(specnumber(site1), specnumber(site2)))   # Mean sample richness
  b.w   = round(s/a.bar - 1, 3)
  return(b.w)
}

# Calculate Bray-Curtis
hmwf.db <- vegdist(OTUsREL.log, method = "bray")
```

Principal Coordinates Analysis

```
par(opar)
hmwf.pcoa <- cmdscale(hmwf.db, eig = TRUE, k = 3)
explainvar1 <- round(hmwf.pcoa$eig[1] / sum(hmwf.pcoa$eig), 3) * 100
explainvar2 <- round(hmwf.pcoa$eig[2] / sum(hmwf.pcoa$eig), 3) * 100
explainvar3 <- round(hmwf.pcoa$eig[3] / sum(hmwf.pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

# Define Plot Parameters
par(mar = c(5, 5, 1, 2) + 0.1)

# Plot Eigenvalues
plot(hmwf.pcoa$eig, xlab = "PCoA Axis", ylab = "Eigenvalue",
     las = 1, cex.lab = 1.5, pch = 16)

# Add Expectation based on Kaiser-Guttman criterion and Broken Stick Model
abline(h = mean(hmwf.pcoa$eig), lty = 2, lwd = 2, col = "blue")
b.stick <- bstick(42, sum(hmwf.pcoa$eig))
lines(1:42, b.stick, type = "l", lty = 4, lwd = 2, col = "red")

# Add Legend
legend("topright", legend = c("Avg Eigenvalue", "Broken-Stick"),
       lty = c(2, 4), bty = "n", col = c("blue", "red"))
```

Figure 4: Bacterial Community Composition Ordination Figure

```
png(filename="../figures/Figure4.png",
    width = 1200, height = 1200, res = 96*2)
par(opar)
# Define Plot Parameters
par(mar = c(5, 5, 1, 1) + 0.1)

# Initiate Plot
plot(hmwf.pcoa$points[ ,1], hmwf.pcoa$points[ ,2], ylim = c(-0.3, 0.3),
     xlim = c(-0.3, 0.6),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""),
     pch = 16, cex = 2.0, type = "n", cex.lab = 1.5, cex.axis = 1.2, axes = FALSE)
```

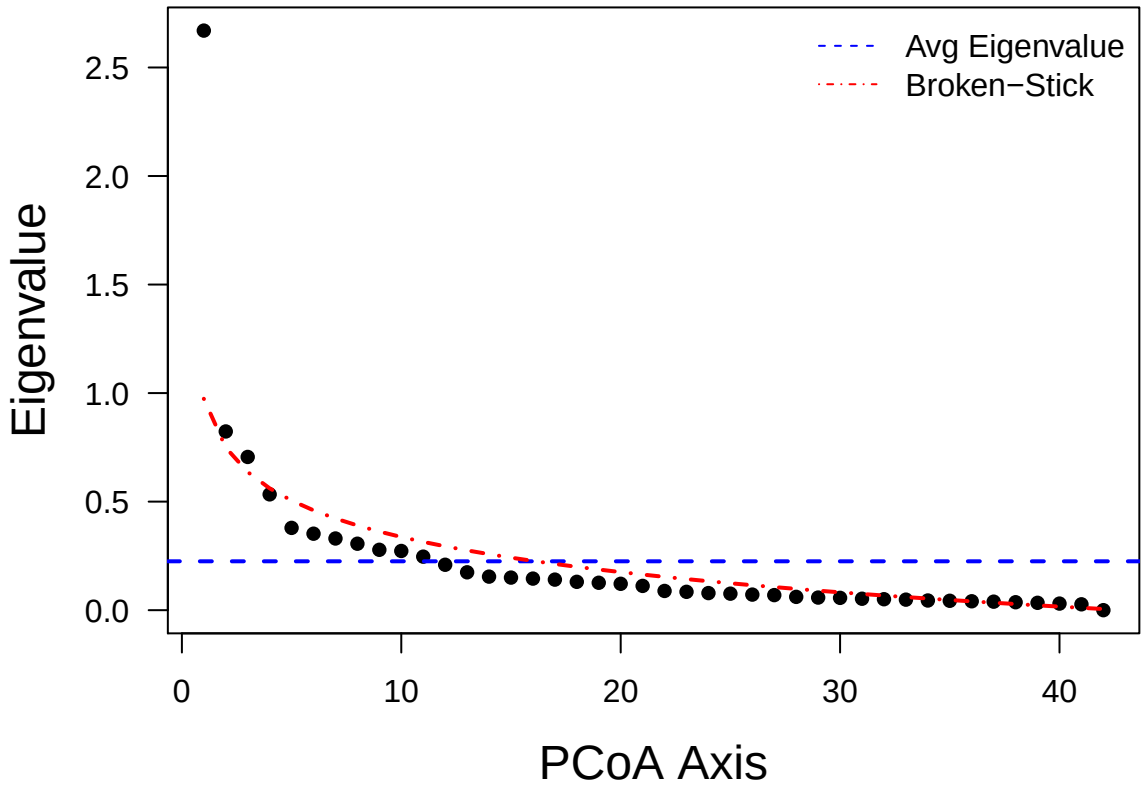
Avg Eigenvalue
Broken−Stick
Eigenvalue
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
PCoA Axis

Figure 3:

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

# Add Points & Labels
points(hmwf.pcoa$points[ ,1], hmwf.pcoa$points[ ,2],
       pch = 19, cex = 3, bg = "gray", col = "gray")

ordiellipse(cbind(hmwf.pcoa$points[ ,1], hmwf.pcoa$points[ ,2]),
    design$Lake, kind="se", conf=0.95,
    lwd=2, draw = "polygon", col="gray", border = "black", label=TRUE, cex=1, bty = 'n')

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
par(opar)
```

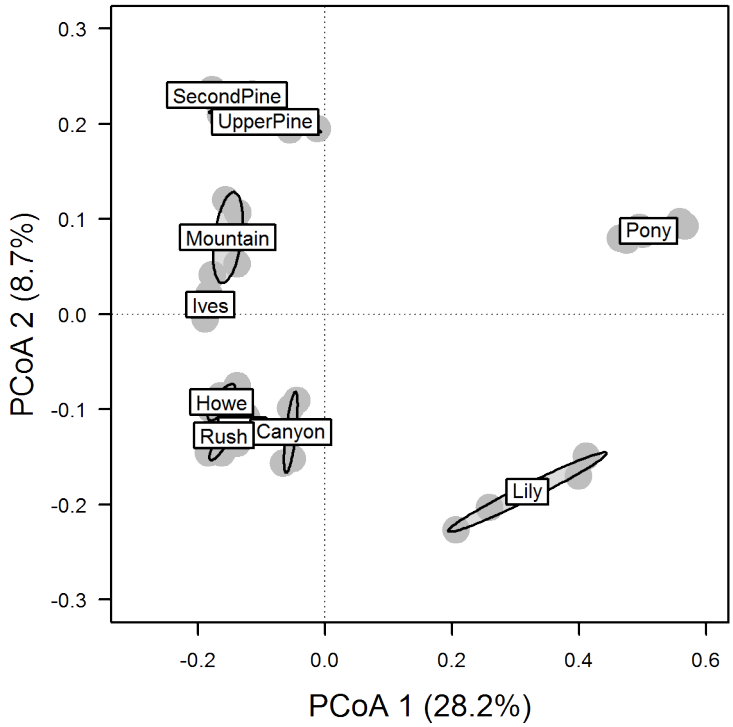

Figure 4: Lake Nutrients

Statistical Analyses

What are the differences between lakes and does resource concentration explain differences

```
nuts
```

```
##        sample year       DOC        TP          TN
```

```
## 1         Ann 2011  6.149285  3.977949 0.000000000
## 2      Canyon 2011  7.615331  2.452633 0.000000000
## 3        Howe 2011  6.875177  1.859455 0.035116957
## 4        Ives 2011  9.537734  1.351016 0.005848597
## 5        Lily 2011 13.358296  4.740607 0.249393025
## 6    Mountain 2011  5.411983  2.113674 0.000000000
## 7        Pony 2011 29.280871 15.417817 0.463248295
## 8        Pony 2011 31.648222  1.520496 0.541116330
## 9        Rush 2011  4.437947  3.554250 0.271601973
## 10 SecondPine 2011  7.197479 10.757130 0.000000000
## 11  UpperPine 2011  7.990399  2.961072 0.000000000
## 12        Ann 2012  5.973806  7.267117 0.000000000
## 13     Canyon 2012  7.233826  2.638631 0.032606559
## 14       Howe 2012  7.037040  5.210013 0.000000000
## 15       Ives 2012  6.913681  9.152797 0.000000000
## 16       Lily 2012 14.351915 11.552753 0.000000000
## 17   Mountain 2012  5.270368  4.867162 0.000000000
## 18       Pony 2012 28.986069 49.952043 0.158174426
## 19       Rush 2012  4.223288  3.838609 0.000000000
## 20 SecondPine 2012  6.261643 12.924156 0.000000000
## 21  UpperPine 2012  7.840340 11.209902 0.000000000
```

```
nuts2 <- data.frame(nuts[rep(seq_len(nrow(nuts)), each=2),])
nuts2$molecule <- rep(c("DNA", "RNA"), 21)
nuts2 <- nuts2[order(nuts2$sample, nuts2$year, nuts2$molecule), ]
as.character(nuts2$sample) == as.character(design$Lake)
```

```
##  [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 TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
```

```
beta.dis <- betadisper(vegdist(OTUsREL, "bray"), design$Lake)
permutest(beta.dis)
```

```
##
## 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     9 0.023336 0.0025929 1.0852    999  0.413
## Residuals 32 0.076459 0.0023894
```

```
adonis(OTUsREL ~ design$Lake + design$Molecule + design$Year, method = "bray", permutations = 999)
```

```
##
## Call:
## adonis(formula = OTUsREL ~ design$Lake + design$Molecule + design$Year,      permutations = 999, met
##
## Permutation: free
## Number of permutations: 999
```

```
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## design$Lake      9    5.7126 0.63473  9.6782 0.65353  0.001 ***
## design$Molecule  1    0.8004 0.80037 12.2038 0.09156  0.001 ***
## design$Year      1    0.2607 0.26069  3.9749 0.02982  0.001 ***
## Residuals       30    1.9675 0.06558         0.22509
## Total           41    8.7412                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
chem.dbrda <- capscale(OTUsREL ~ nuts2$DOC + nuts2$TN + nuts2$TP, add = T, distance = "bray")
anova(chem.dbrda)
```

```
## Permutation test for capscale under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: capscale(formula = OTUsREL ~ nuts2$DOC + nuts2$TN + nuts2$TP, distance = "bray", add = T)
##          Df Variance      F Pr(>F)
## Model     3   2.9476 4.4983  0.001 ***
## Residual 38   8.3002
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
RsquareAdj(chem.dbrda)
```

```
## $r.squared
## [1] 0.2620626
##
## $adj.r.squared
## [1] 0.2038044
```

```
coef(chem.dbrda)
```

```
##                   CAP1         CAP2        CAP3
## nuts2$DOC  0.020151356 -0.007345761  0.03829894
## nuts2$TN  -0.053186394  0.895450602 -1.74044847
## nuts2$TP  -0.001050423 -0.010015276 -0.01945302
```

```
anova.cca(chem.dbrda, step=1000)
```

```
## Permutation test for capscale under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: capscale(formula = OTUsREL ~ nuts2$DOC + nuts2$TN + nuts2$TP, distance = "bray", add = T)
##          Df Variance      F Pr(>F)
## Model     3   2.9476 4.4983  0.001 ***
## Residual 38   8.3002
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
plot(chem.dbrda)
```

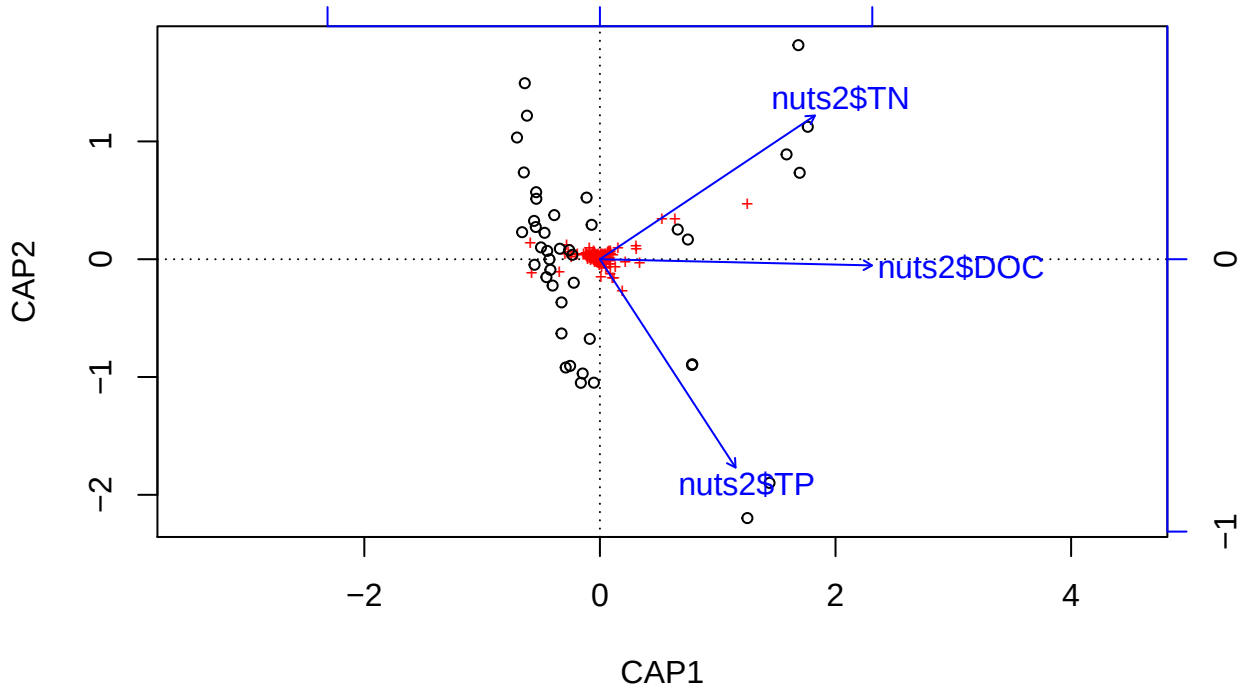

Figure 5:

```
lmod <- as.mlm(chem.dbrda)
lmod
```

```
##
## Call:
## lm(formula = x$CCA$wa ~ . - 1, data = as.data.frame(X))
##
## Coefficients:
##                CAP1       CAP2       CAP3
## `nuts2$DOC`   0.020151  -0.007346   0.038299
## `nuts2$TN`   -0.053186   0.895451  -1.740448
## `nuts2$TP`   -0.001050  -0.010015  -0.019453
```

```
summary(lmod)
```

```
## Response CAP1 :
##
## Call:
## lm(formula = CAP1 ~ (`nuts2$DOC` + `nuts2$TN` + `nuts2$TP`) -
```

```
##     1, data = as.data.frame(X))
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.062237 -0.026040 -0.006134  0.023474  0.111846
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## `nuts2$DOC`  0.0201514  0.0019405  10.385 8.69e-13 ***
## `nuts2$TN`  -0.0531864  0.0865557  -0.614    0.542
## `nuts2$TP`  -0.0010504  0.0009683  -1.085    0.285
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04421 on 39 degrees of freedom
## Multiple R-squared:  0.9292, Adjusted R-squared:  0.9237
## F-statistic: 170.6 on 3 and 39 DF,  p-value: < 2.2e-16
##
##
## Response CAP2 :
##
## Call:
## lm(formula = CAP2 ~ (`nuts2$DOC` + `nuts2$TN` + `nuts2$TP`) -
##     1, data = as.data.frame(X))
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.141550 -0.058758  0.006012  0.063185  0.248419
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## `nuts2$DOC` -0.007346   0.004068  -1.806   0.0787 .
## `nuts2$TN`   0.895451   0.181445   4.935 1.54e-05 ***
## `nuts2$TP`  -0.010015   0.002030  -4.934 1.54e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.09267 on 39 degrees of freedom
## Multiple R-squared:  0.7491, Adjusted R-squared:  0.7298
## F-statistic: 38.82 on 3 and 39 DF,  p-value: 8.634e-12
##
##
## Response CAP3 :
##
## Call:
## lm(formula = CAP3 ~ (`nuts2$DOC` + `nuts2$TN` + `nuts2$TP`) -
##     1, data = as.data.frame(X))
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.41902 -0.09057  0.01869  0.09790  0.26632
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
```

```
## `nuts2$DOC`  0.038299   0.006332   6.049 4.43e-07 ***
## `nuts2$TN`  -1.740448   0.282425  -6.163 3.08e-07 ***
## `nuts2$TP`  -0.019453   0.003160  -6.157 3.14e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1442 on 39 degrees of freedom
## Multiple R-squared:  0.5521, Adjusted R-squared:  0.5176
## F-statistic: 16.02 on 3 and 39 DF,  p-value: 6.08e-07
```

Generalists

```
active <- OTUs[design$Molecule == "RNA", ]
activePA <- (active > 0) * 1

total <- OTUs[design$Molecule == "DNA", ]
totalPA <- (total > 0) * 1

gens <- data.frame(matrix(NA, 21, 3))
colnames(gens) <- c("sites", "taxaA", "taxaT")
gens$sites <- c(1:21)

for (i in 1:21){
  gens$taxaA[i] <- sum(colSums(activePA) == i)
  gens$taxaT[i] <- sum(colSums(totalPA) == i)
}

# Define Plot Parameters
par(mar = c(5, 5, 1, 1) + 0.1)
plot(gens$taxaA ~ gens$sites, xlab = "Number of Sites", ylab = "Number of Taxa")
```



```
plot(gens$taxaT ~ gens$sites, xlab = "Number of Sites", ylab = "Number of Taxa")
```



```
# Total Taxa
total <- OTUs[design$Molecule == "DNA", ]
totalPA <- (total > 0) * 1

# Inactive Taxa
inactivePA <- totalPA - activePA
inactivePA <- pmax(inactivePA, 0)
sum(colSums(inactivePA)  > 10)
```

```
## [1] 13
```

```
rowSums(inactivePA)/rowSums(totalPA)
```

```
##        Ann2011_DNA        Ann2012_DNA     Canyon2011_DNA
##          0.5404130          0.4817619          0.5364583
##  CanyonEpi2012_DNA       Howe2011_DNA       Howe2012_DNA
```

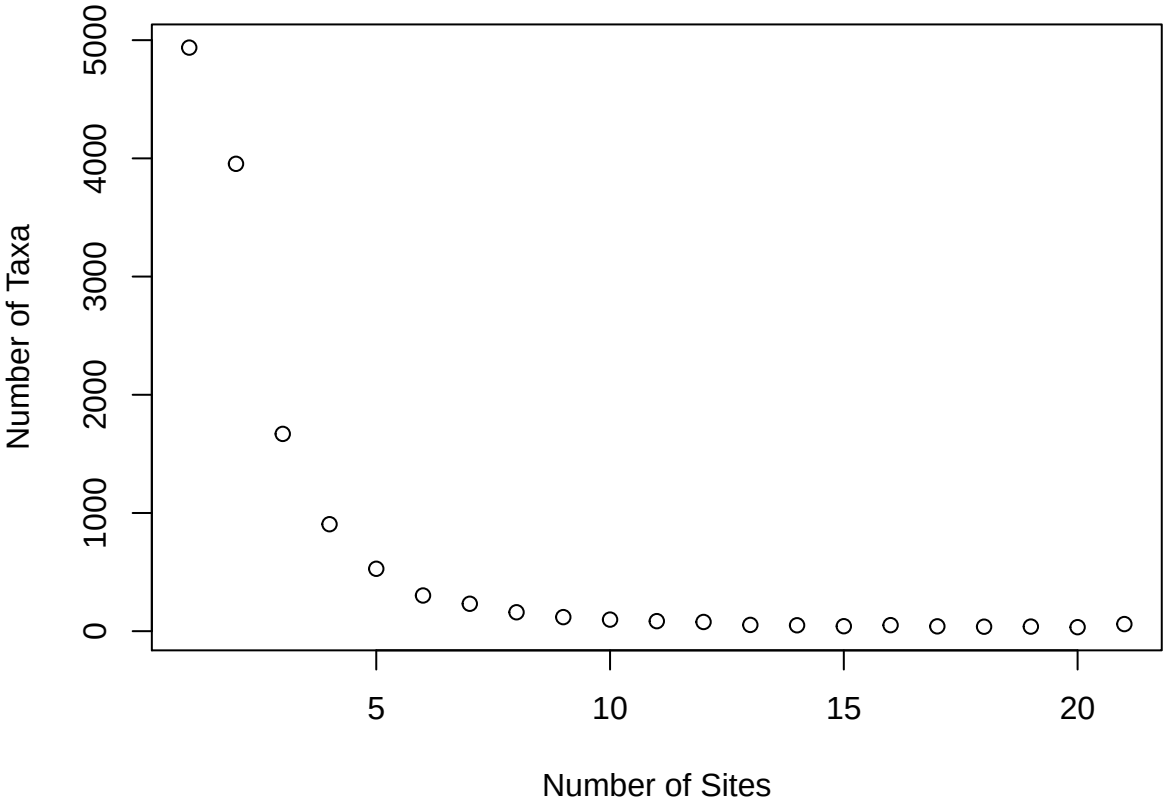
Number of Taxa
0
1000
2000
3000
4000
5000
5
10
15
20
Number of Sites

Figure 6:

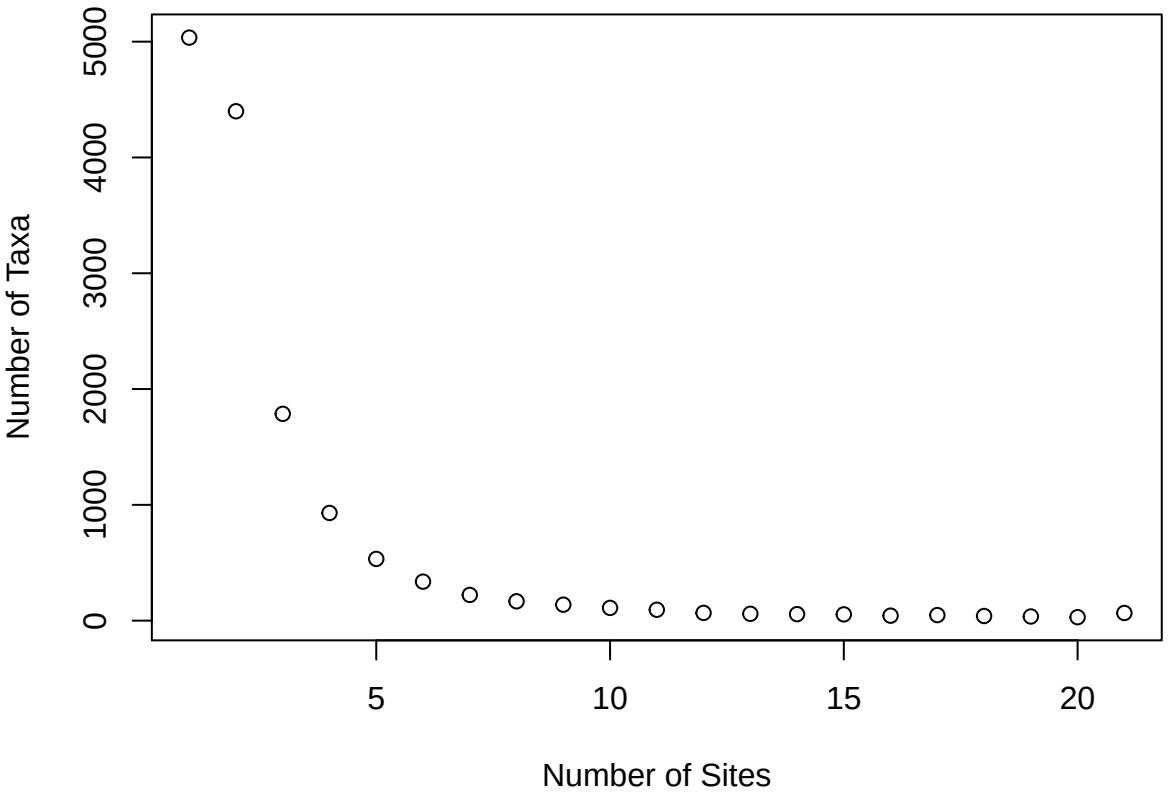
Number of Taxa
0
1000
2000
3000
4000
5000
5
10
15
20
Number of Sites

Figure 7:

```
##           0.5402447           0.6549424           0.5311005
##       Ives2011_DNA       Ives2012_DNA       Lily2011_DNA
##           0.5036430           0.4282869           0.6358517
##       Lily2012_DNA   Mountain2011_DNA   Mountain2012_DNA
##           0.3909898           0.4671683           0.5374150
##  NorthPony2011_DNA  SouthPony2011_DNA       Pony2012_DNA
##           0.3323388           0.5204007           0.3596533
##       Rush2011_DNA       Rush2012_DNA SecondPine2011_DNA
##           0.5092317           0.4916300           0.4213960
## SecondPine2012_DNA  UpperPine2011_DNA  UpperPine2012_DNA
##           0.3968566           0.4870666           0.4771300
```

```
rowSums(activePA)/rowSums(totalPA)
```

```
##        Ann2011_RNA        Ann2012_RNA     Canyon2011_RNA
##          0.8572271          0.9029594          1.0162760
##  CanyonEpi2012_RNA       Howe2011_RNA       Howe2012_RNA
##          0.8969736          0.5300182          0.7886762
##       Ives2011_RNA       Ives2012_RNA       Lily2011_RNA
##          0.7877960          0.9820717          0.5527921
##       Lily2012_RNA   Mountain2011_RNA   Mountain2012_RNA
##          1.0302532          0.9377565          0.7503401
##  NorthPony2011_RNA  SouthPony2011_RNA       Pony2012_RNA
##          1.1359191          0.8394820          1.1918633
##       Rush2011_RNA       Rush2012_RNA SecondPine2011_RNA
##          0.9416319          0.8264317          1.1467710
## SecondPine2012_RNA  UpperPine2011_RNA  UpperPine2012_RNA
##          0.9813360          1.0528343          0.8968610
```

```
plot(rowSums(activePA)/rowSums(totalPA), rowSums(inactivePA)/rowSums(totalPA))
```

```
inactive <- total * inactivePA
active.N <- total * (1-inactivePA)
active.NPA <- (active.N > 0 ) * 1

plot(rowSums(active.NPA)/rowSums(totalPA), rowSums(inactivePA)/rowSums(totalPA))
```

```
plot(rowSums(active.NPA)/rowSums(totalPA) ~ nuts2$TP[nuts2$molecule == "DNA"],
     xlab = "Total Phosphorus", ylab = "Proportion of Active Taxa")
phos <- lm(rowSums(active.NPA)/rowSums(totalPA) ~ nuts2$TP[nuts2$molecule == "DNA"])
abline(phos)
```

```
dim(activePA)
```

```
## [1]    21 15998
```

```
dim(totalPA)
```

```
## [1]    21 15998
```

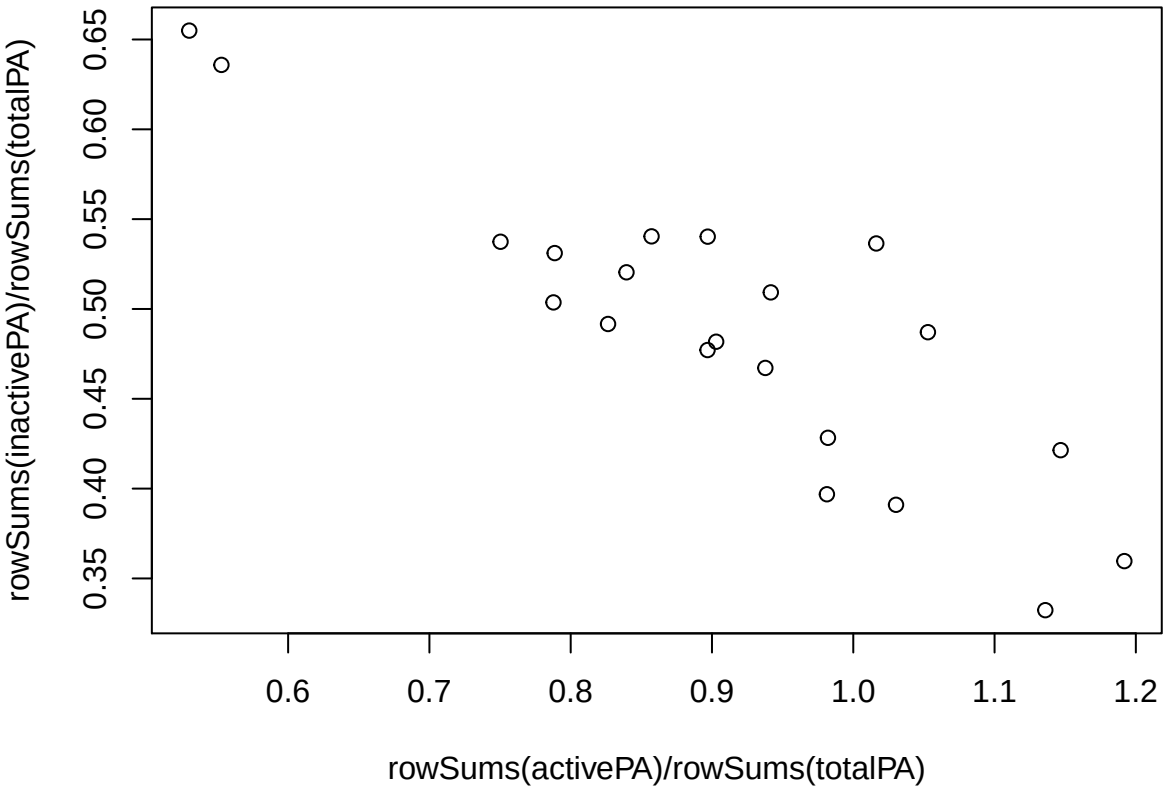
rowSums(inactivePA)/rowSums(totalPA)
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.6
0.7
0.8
0.9
1.0
1.1
1.2
rowSums(activePA)/rowSums(totalPA)

Figure 8:

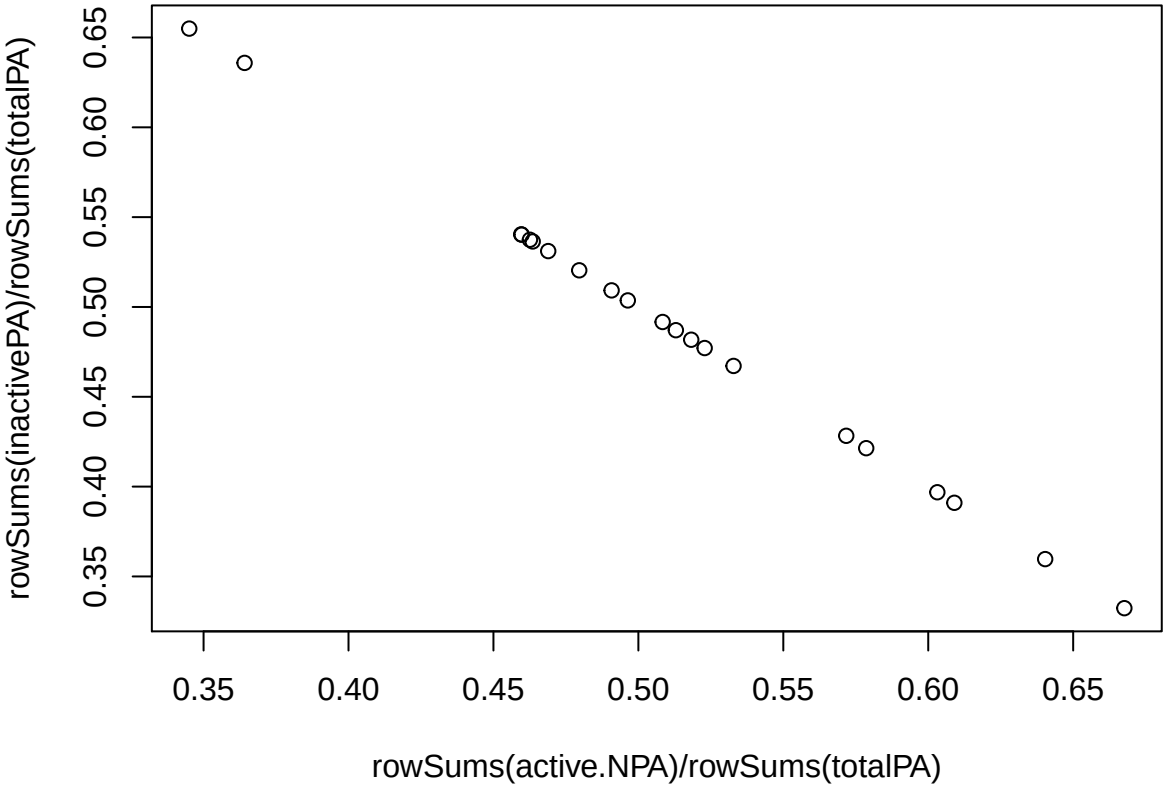
rowSums(inactivePA)/rowSums(totalPA)
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.35
0.40
0.45
0.50
0.55
0.60
0.65
rowSums(active.NPA)/rowSums(totalPA)

Figure 9:

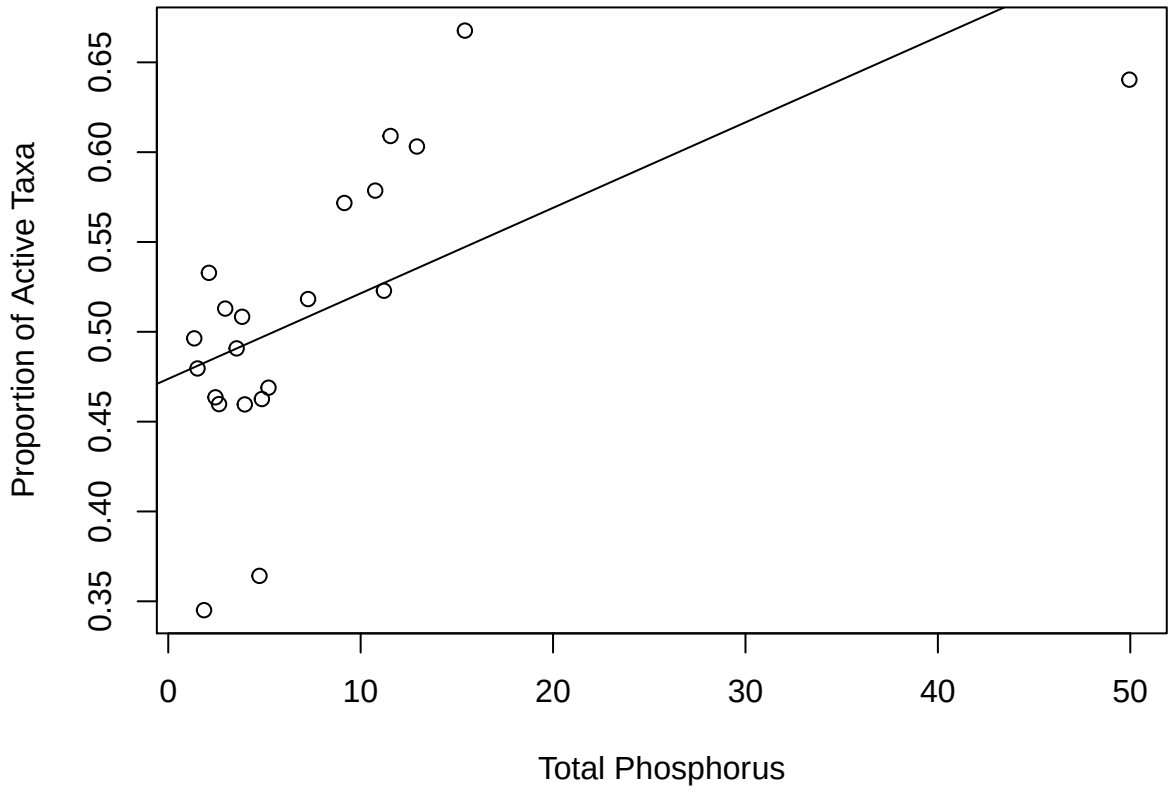
Proportion of Active Taxa
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0
10
20
30
40
50
Total Phosphorus

Figure 10:

Patterns of Resource Diversity

```
# Define Inputs
# Resource = raw site-by-resource matrix
resource.pos <- "../data/SpecAbundAvePos.csv"
resource.neg <- "../data/SpecAbundAveNeg.csv"

# Import Resources
res.in <- read.csv(resource.pos, header=T, row.names=1)

rownames(res.in)
```

```
##  [1] "Ann_Lake-5.1906"      "blank-5.7312"         "Canyon_Chemo-NA"
##  [4] "Canyon_Epi-8.0847"    "Canyon_Hypo-5.2494"   "Canyon_I-8.72"
##  [7] "Canyon_II-5.4808"     "Canyon_III-7.392"     "Canyon_IV-5.41395"
## [10] "Cowe_Lake-5.39"       "Ives_Lake-7.512"      "Jordan_River-0"
## [13] "Lily_Pond-7.6638"     "Mountain_lake-12.915" "Pony_Lake-8.9376"
## [16] "Rush-16.299"          "Second_Pine-9.0368"   "Upper_Pine-13.9104"
```

```
rownames(res.in) <- c("Ann", "blank", "CanyonChemo", "Canyon", "CanyonHypo",
                      "CanyonI", "CanyonII", "CanyonIII", "CanyonIV", "Howe",
                      "Ives", "Jordan", "Lily", "Mountain", "Pony", "Rush",
                      "SecondPine", "UpperPine")

blank <- unlist(res.in["blank", ])
res.hmwf <- res.in[-c(which(rownames(res.in) %in% c("blank", "CanyonChemo",
                           "CanyonHypo", "CanyonI", "CanyonII",
                           "CanyonIII", "CanyonIV", "Jordan"))), ]
```

Remove Major Peaks from Blanks

```
summary(blank)
```

```
##      Min.   1st Qu.    Median      Mean   3rd Qu.      Max.
##     44.82   1316.00   2538.00   3257.00   4153.00 301900.00
```

```
blank[which(blank > 2 * sd(blank))]
```

```
##       C18       C30       C51       C71       C74       C79      C100
##  14959.37  22063.16 106190.60  26631.05  20680.04  17271.76  22571.21
##      C101      C138      C188      C196      C359      C370      C442
##  30197.26 301905.92  33679.78  41662.87  14751.05 143840.32  63741.27
##      C485      C530      C694      C789     C1100     C1184     C1938
##  15882.99  17158.63  15492.82  16451.88  13853.99  56389.92  14744.39
##     C1939     C1941     C1942     C1943     C1944     C1945     C1950
##  16596.80  15668.18  23674.26  14348.07  14463.70  16458.16  18022.09
##     C1955     C2613     C3285     C3659     C3662     C3664     C3666
##  18956.26  25926.43  14089.19  28085.24  16266.40  15196.54  14060.33
##     C3667     C3668
##  13874.05  16777.74
```

```
# res.hmwf <- res.hmwf[, -c(which(blank > sd(blank)))]

# What other peaks should be removed
for (i in 1:dim(res.hmwf)[1]){
  res.hmwf[i, ] <- res.hmwf[i, ] - blank * 1.1
}

res.hmwf[res.hmwf < 0] <- 0
res.hmwf <- res.hmwf[,colSums(res.hmwf) > 0]
```

Data Transformations

```
# Remove OTUs with less than two occurences across all sites
res <- res.hmwf

# Sequencing Coverage
coverage <- rowSums(res)

# # Remove Low Coverage Samples (This code removes two sites: Site 5DNA, Site 6cDNA)
# lows <- which(coverage < 10000)
# OTUs <- OTUs[-which(coverage < 10000), ]
# design <- design[-which(coverage < 10000), ]

# Make Relative Abundence Matrices
resREL <- res
for(i in 1:dim(res)[1]){
  resREL[i,] <- res[i,]/sum(res[i,])
}
```

Calculate Alpha Diversity

```
# Observed Richness
S.res <- rowSums((res > 0) * 1)

# Simpson's Evenness
res.simpsE <- round(apply(res, 1, SimpE), 3)

# Shannon's Diversity
res.shan <- round(apply(res, 1, H), 2)
res.shan2 <- round(diversity(res, index = "shannon"), 2)

res.div <- as.data.frame(cbind(S.res, res.simpsE, res.shan2))
```

Figure 5: Resource Diversity

```
png(filename="../figures/Figure5.png",
    width = 1600, height = 1200, res = 96*2)
```

```
par(opar)
par(mfrow = c(1,1), mar = c(0, 9, 0, 0) + 0.5, oma = c(5, 0, 1, 1) + 0.5)
layout(rbind(1, 2, 3), height = c(3, 3, 3))
labs <- c("Ann", "Canyon", "Howe", "Ives", "Lily", "Mountain", "Pony", "Rush",
          "Second\nPine","Upper\nPine")
rich <- barplot(res.div$S.res, names.arg = NULL, las =1, ylim=c(0, 2000),
     xlab = "", ylab = "")
mtext(side = 2, text = "Resource\nRichness", cex.lab = 1.2, line = 4.5)
even <- barplot(res.div$res.simpsE, names.arg = NULL, las =1, ylim=c(0, 0.27),
     xlab = "", ylab = "")
mtext(side = 2, text = "Simpson's\nEvenness", cex.lab = 1.2, line = 4.5)
shan <- barplot(res.div$res.shan, names.arg = NULL, las = 1, ylim = c(0, 9),
     xlab = "", ylab = "")
mtext(side = 2, text = "Shannon\nDiversity", cex.lab = 1.2, line = 4.5)
mtext(side = 1, text = labs, line = 2, at = shan, padj = 0.5, cex = 0.8)

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
par(opar)
```

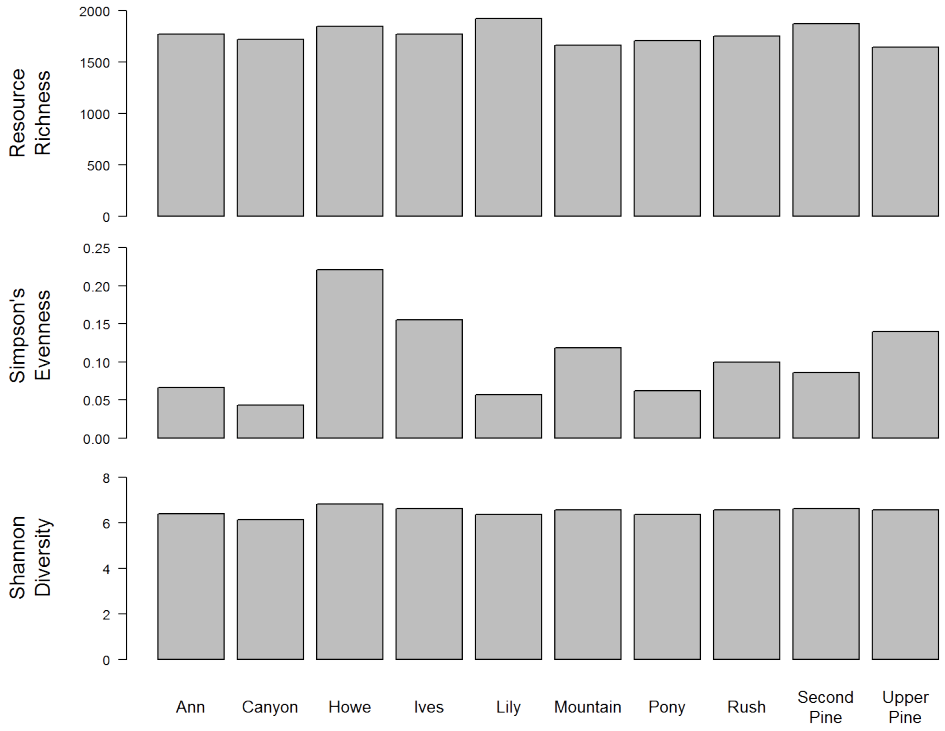

Figure 11: Resource Alpha Diversity

Hypothesis that resource diversity is related to nutrient concentration

```
nuts2012 <- nuts2[nuts2$year == "2012" & nuts2$molecule == "DNA", ]
evenmod <- lm(res.div$res.simpsE ~ nuts2012$DOC*nuts2012$TN*nuts2012$TP)
richmod <- lm(res.div$S.res ~ nuts2012$DOC*nuts2012$TN*nuts2012$TP)
summary(evenmod)
```

```
##
## Call:
## lm(formula = res.div$res.simpsE ~ nuts2012$DOC * nuts2012$TN *
##     nuts2012$TP)
##
## Residuals:
##          1          2          3          4          5          6
## -7.338e-02  2.082e-17  2.423e-02  1.873e-02 -9.299e-03 -9.183e-03
##          7          8          9         10
## -3.469e-18  2.028e-02 -6.428e-03  3.505e-02
##
## Coefficients: (2 not defined because of singularities)
##                                       Estimate Std. Error t value
## (Intercept)                           -0.257513   0.201644  -1.277
## nuts2012$DOC                           0.075643   0.036349   2.081
## nuts2012$TN                          -22.014401   8.533039  -2.580
## nuts2012$TP                            0.034057   0.018697   1.822
## nuts2012$DOC:nuts2012$TN               2.180300   0.927042   2.352
## nuts2012$DOC:nuts2012$TP              -0.006968   0.003213  -2.168
## nuts2012$TN:nuts2012$TP                      NA         NA      NA
## nuts2012$DOC:nuts2012$TN:nuts2012$TP         NA         NA      NA
##                                     Pr(>|t|)
## (Intercept)                           0.2707
## nuts2012$DOC                          0.1059
## nuts2012$TN                           0.0613 .
## nuts2012$TP                           0.1426
## nuts2012$DOC:nuts2012$TN              0.0784 .
## nuts2012$DOC:nuts2012$TP              0.0960 .
## nuts2012$TN:nuts2012$TP                   NA
## nuts2012$DOC:nuts2012$TN:nuts2012$TP      NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04521 on 4 degrees of freedom
## Multiple R-squared:  0.7004, Adjusted R-squared:  0.3258
## F-statistic:  1.87 on 5 and 4 DF,  p-value: 0.282
```

```
summary(richmod)
```

```
##
## Call:
## lm(formula = res.div$S.res ~ nuts2012$DOC * nuts2012$TN * nuts2012$TP)
##
## Residuals:
##          1          2          3          4          5          6
##  1.202e+01  2.087e-14  5.657e+01 -1.038e+01  2.630e+01 -8.083e+01
##          7          8          9         10
```

```
## -5.329e-15  4.465e+01  1.009e+02 -1.492e+02
##
## Coefficients: (2 not defined because of singularities)
##                                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)                         1552.050    473.645   3.277   0.0306
## nuts2012$DOC                          34.749     85.382   0.407   0.7049
## nuts2012$TN                        -5272.547  20043.447  -0.263   0.8055
## nuts2012$TP                           10.596     43.917   0.241   0.8212
## nuts2012$DOC:nuts2012$TN             409.064   2177.549   0.188   0.8601
## nuts2012$DOC:nuts2012$TP              -1.673      7.548  -0.222   0.8354
## nuts2012$TN:nuts2012$TP                   NA         NA      NA       NA
## nuts2012$DOC:nuts2012$TN:nuts2012$TP      NA         NA      NA       NA
##
## (Intercept)                         *
## nuts2012$DOC
## nuts2012$TN
## nuts2012$TP
## nuts2012$DOC:nuts2012$TN
## nuts2012$DOC:nuts2012$TP
## nuts2012$TN:nuts2012$TP
## nuts2012$DOC:nuts2012$TN:nuts2012$TP
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 106.2 on 4 degrees of freedom
## Multiple R-squared:  0.3762, Adjusted R-squared:  -0.4035
## F-statistic: 0.4825 on 5 and 4 DF,  p-value: 0.7778
```

Hypothesis that resource diversity influences consumer diversity

```
alpha.div2012 <- alpha.div[alpha.div$Year == "2012" & alpha.div$Molecule == "DNA", c(1, 4:6)]
rownames(alpha.div2012) <- alpha.div2012[, 1]
alpha.div2012 <- alpha.div2012[, -1]
rownames(res.div) == rownames(alpha.div2012)
```

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

```
cor(res.div, alpha.div2012)
```

```
##                  S.obs      simpsE        shan
## S.res       0.06268514 -0.02491091 -0.09933677
## res.simpsE -0.47586109  0.18756375 -0.20676202
## res.shan2  -0.47661995  0.34065546 -0.12295892
```

```
rich.mod1 <- lm(alpha.div2012$S.obs ~ res.div$S.res)
rich.mod2 <- lm(alpha.div2012$S.obs ~ res.div$res.simpsE)
summary(rich.mod1)
```

```
##
## Call:
```

```
## lm(formula = alpha.div2012$S.obs ~ res.div$S.res)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -776.9 -562.7 -394.0 -143.7 2479.3
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)    431.3215  7260.7275   0.059    0.954
## res.div$S.res    0.7284     4.1002   0.178    0.863
##
## Residual standard error: 1102 on 8 degrees of freedom
## Multiple R-squared:  0.003929,   Adjusted R-squared:  -0.1206
## F-statistic: 0.03156 on 1 and 8 DF,  p-value: 0.8634
```

```
summary(rich.mod2)
```

```
##
## Call:
## lm(formula = alpha.div2012$S.obs ~ res.div$res.simpsE)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -871.8 -625.8 -276.7  403.8 2048.1
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)          2664.0      689.3   3.865  0.00478 **
## res.div$res.simpsE  -9001.6     5882.2  -1.530  0.16447
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 971.6 on 8 degrees of freedom
## Multiple R-squared:  0.2264, Adjusted R-squared:  0.1297
## F-statistic: 2.342 on 1 and 8 DF,  p-value: 0.1645
```

Between site comparisions of resources

```
# Calculate Bray-Curtis
res.db <- vegdist(resREL, method = "bray")

res.pcoa <- cmdscale(res.db, eig = TRUE, k = 3)
explainvar1 <- round(res.pcoa$eig[1] / sum(res.pcoa$eig), 3) * 100
explainvar2 <- round(res.pcoa$eig[2] / sum(res.pcoa$eig), 3) * 100
explainvar3 <- round(res.pcoa$eig[3] / sum(res.pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

# Define Plot Parameters
par(mar = c(5, 5, 1, 2) + 0.1)

# Plot Eigenvalues
```

```r
plot(res.pcoa$eig, xlab = "PCoA Axis", ylab = "Eigenvalue",
     las = 1, cex.lab = 1.5, pch = 16)

# Add Expectation based on Kaiser-Guttman criterion and Broken Stick Model
abline(h = mean(res.pcoa$eig), lty = 2, lwd = 2, col = "blue")
b.stick <- bstick(10, sum(res.pcoa$eig))
lines(1:10, b.stick, type = "l", lty = 4, lwd = 2, col = "red")

# Add Legend
legend("topright", legend = c("Avg Eigenvalue", "Broken-Stick"),
       lty = c(2, 4), bty = "n", col = c("blue", "red"))
```

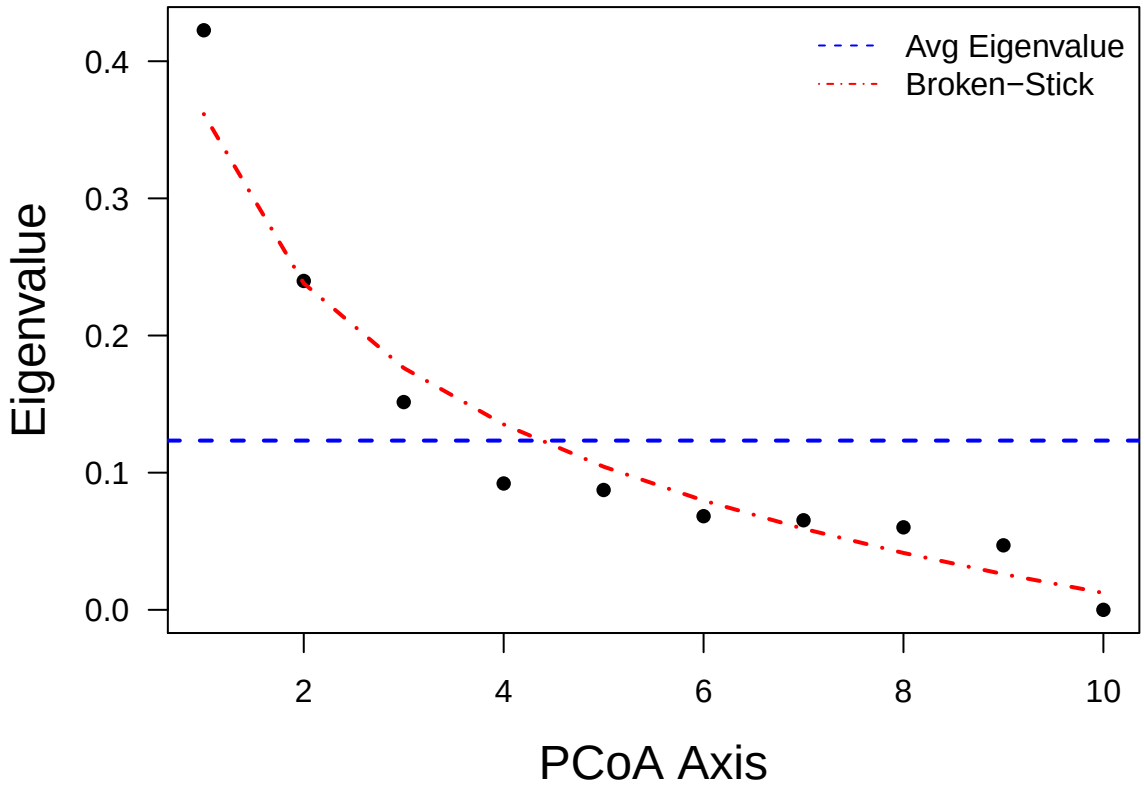

Figure 12:

Figre 6: Resource Differences Across Sites

```r
png(filename="../figures/Figure6.png",
    width = 1200, height = 1200, res = 96*2)

# Define Plot Parameters
par(mar = c(5, 5, 1, 1) + 0.1)
```

```r
# Initiate Plot
plot(res.pcoa$points[ ,1], res.pcoa$points[ ,2], ylim = c(-0.2, 0.4),
     xlim = c(-0.5, 0.5),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""),
     pch = 16, cex = 2.0, type = "n", cex.lab = 1.5, cex.axis = 1.2, axes = FALSE)

# Add Axes
axis(side = 1, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
axis(side = 2, labels = T, lwd.ticks = 2, cex.axis = 1.2, las = 1)
abline(h = 0, v = 0, lty = 3)
box(lwd = 2)

# Add Points & Labels
points(res.pcoa$points[ ,1], res.pcoa$points[ ,2],
       pch = 19, cex = 3, bg = "gray", col = "gray")
text(res.pcoa$points[ ,1], res.pcoa$points[ ,2],
     labels = row.names(res.pcoa$points))
dev.off() # this writes plot to folder
graphics.off() # shuts doum open devices
par(opar)
```

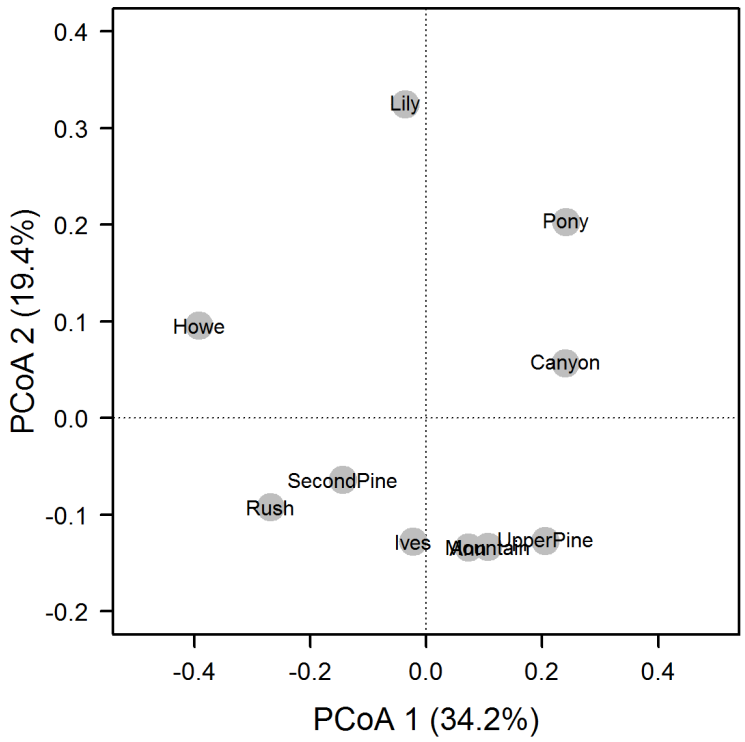

Figure 13: Resource Ordination

Resource Explainations of Differences

```
OTUsREL2012.DNA <- OTUsREL[which(design$Year == "2012" & design$Molecule == "DNA"), ]
OTUsREL2012.DNA <- OTUsREL2012.DNA[ , colSums(OTUsREL2012.DNA > 0)]
OTUsREL2012.RNA <- OTUsREL[which(design$Year == "2012" & design$Molecule == "DNA"), ]
OTUsREL2012.RNA <- OTUsREL2012.RNA[ , colSums(OTUsREL2012.RNA > 0)]
active.N2012 <- active.N[grep("2012", rownames(active.N)), ]
rownames(OTUsREL2012.DNA) <- design$Lake[which(design$Year == "2012" & design$Molecule == "DNA")]
rownames(OTUsREL2012.RNA) <- design$Lake[which(design$Year == "2012" & design$Molecule == "DNA")]
rownames(active.N2012) <- design$Lake[which(design$Year == "2012" & design$Molecule == "DNA")]

# DNA
dbrda2012 <- capscale(OTUsREL2012.DNA ~ nuts2012$DOC + nuts2012$TN + nuts2012$TP, add =T, distance = "bi
anova(dbrda2012)
```

```
## Permutation test for capscale under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: capscale(formula = OTUsREL2012.DNA ~ nuts2012$DOC + nuts2012$TN + nuts2012$TP, distance = "bra
##          Df Variance      F Pr(>F)
## Model     3  0.16451 1.8665  0.151
## Residual  6  0.17628
```

```
RsquareAdj(dbrda2012)
```

```
## $r.squared
## [1] 0.4827334
##
## $adj.r.squared
## [1] 0.2241002
```

```
coef(dbrda2012)
```

```
##                      CAP1        CAP2        CAP3
## nuts2012$DOC   0.13283664  0.02907314  0.05137881
## nuts2012$TN  -11.22365318 -1.67797999 13.95264996
## nuts2012$TP   -0.03461051  0.01447238 -0.07330024
```

```
# RNA
dbrda2012 <- capscale(OTUsREL2012.RNA ~ nuts2012$DOC + nuts2012$TN + nuts2012$TP, add =T, distance = "bi
anova(dbrda2012)
```

```
## Permutation test for capscale under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: capscale(formula = OTUsREL2012.RNA ~ nuts2012$DOC + nuts2012$TN + nuts2012$TP, distance = "bra
##          Df Variance      F Pr(>F)
## Model     3  0.16451 1.8665  0.172
## Residual  6  0.17628
```

```
RsquareAdj(dbrda2012)
```

```
## $r.squared
## [1] 0.4827334
##
## $adj.r.squared
## [1] 0.2241002
```

```
coef(dbrda2012)
```

```
##                       CAP1        CAP2        CAP3
## nuts2012$DOC   0.13283664  0.02907314  0.05137881
## nuts2012$TN  -11.22365318 -1.67797999 13.95264996
## nuts2012$TP   -0.03461051  0.01447238 -0.07330024
```

```
# Active
dbrda2012 <- capscale(active.N2012 ~ nuts2012$DOC + nuts2012$TN + nuts2012$TP, add =T, distance = "bray
anova(dbrda2012)
```

```
## Permutation test for capscale under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: capscale(formula = active.N2012 ~ nuts2012$DOC + nuts2012$TN + nuts2012$TP, distance = "bray"
##          Df Variance      F Pr(>F)
## Model     3  0.88006 2.4136  0.004 **
## Residual  6  0.72925
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
RsquareAdj(dbrda2012)
```

```
## $r.squared
## [1] 0.5468577
##
## $adj.r.squared
## [1] 0.3202866
```

```
coef(dbrda2012)
```

```
##                      CAP1        CAP2        CAP3
## nuts2012$DOC  0.10137061 -0.09407207  0.04477777
## nuts2012$TN  -6.38022991  8.71627698 14.37990370
## nuts2012$TP  -0.01310565  0.03550141 -0.07313108
```

```
# Resoruces
chem.dbrda <- capscale(resREL ~ nuts2012$DOC + nuts2012$TN + nuts2012$TP, add =T, distance = "bray")
anova(chem.dbrda)
```

```
## Permutation test for capscale under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: capscale(formula = resREL ~ nuts2012$DOC + nuts2012$TN + nuts2012$TP, distance = "bray", add =
##          Df Variance      F Pr(>F)
## Model     3  0.45421 1.1649  0.257
## Residual  6  0.77985
```

```
RsquareAdj(chem.dbrda)
```

```
## $r.squared
## [1] 0.3680597
##
## $adj.r.squared
## [1] 0.05208953
```

```
coef(chem.dbrda)
```

```
##                      CAP1         CAP2         CAP3
## nuts2012$DOC  0.10045342   0.09751333  -0.03912624
## nuts2012$TN   1.61289193 -14.38332312 -10.67619053
## nuts2012$TP  -0.04053988  -0.01129384   0.07077591
```

```
anova.cca(chem.dbrda, step=1000)
```

```
## Permutation test for capscale under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: capscale(formula = resREL ~ nuts2012$DOC + nuts2012$TN + nuts2012$TP, distance = "bray", add =
##          Df Variance      F Pr(>F)
## Model     3  0.45421 1.1649  0.242
## Residual  6  0.77985
```

```
plot(chem.dbrda)
```

```
lmod <- as.mlm(chem.dbrda)
lmod
```

```
##
## Call:
## lm(formula = x$CCA$wa ~ . - 1, data = as.data.frame(X))
##
## Coefficients:
##                  CAP1       CAP2       CAP3
## `nuts2012$DOC`    0.10045    0.09751   -0.03913
## `nuts2012$TN`     1.61289  -14.38332  -10.67619
## `nuts2012$TP`    -0.04054   -0.01129    0.07078
```

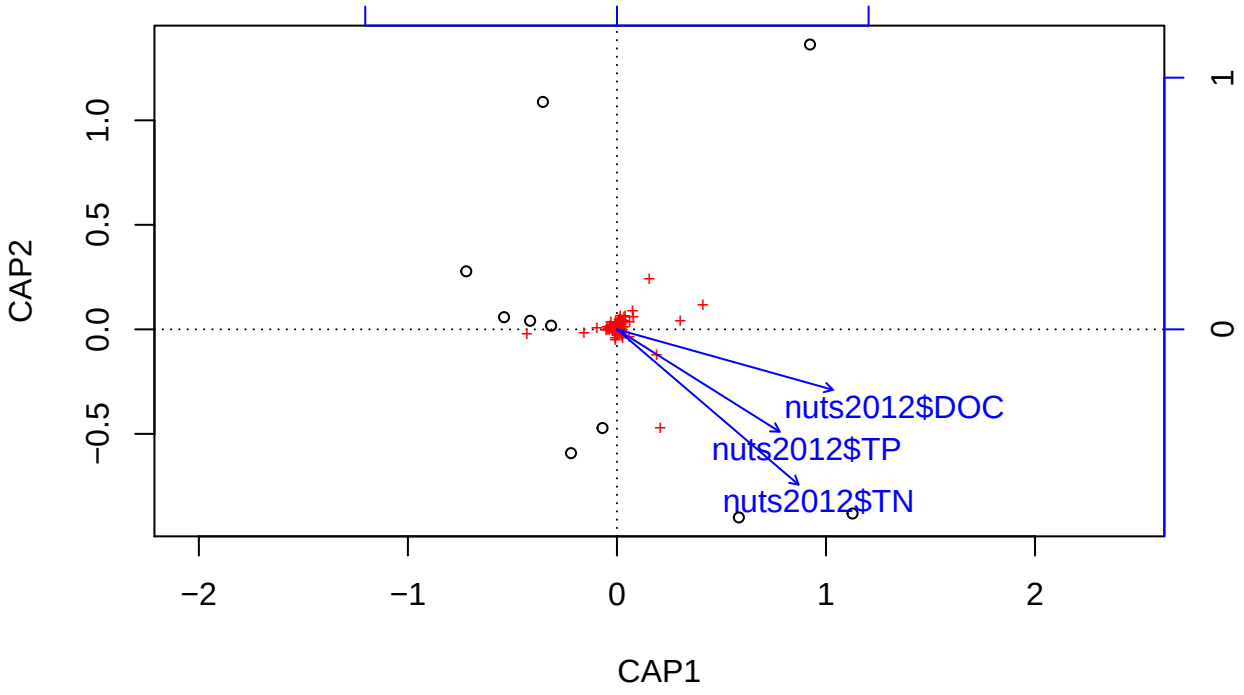
CAP2
1.0
0.5
0.0
−0.5
CAP1
−2
−1
0
1
2
1
0
nuts2012$DOC
nuts2012$TP
nuts2012$TN

Figure 14:

```r
summary(lmod)
```

```
## Response CAP1 :
##
## Call:
## lm(formula = CAP1 ~ (`nuts2012$DOC` + `nuts2012$TN` + `nuts2012$TP`) -
##     1, data = as.data.frame(X))
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.19464 -0.04927  0.02239  0.08138  0.14234
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## `nuts2012$DOC`  0.10045    0.01885   5.330  0.00109 **
## `nuts2012$TN`   1.61289    2.33193   0.692  0.51143
## `nuts2012$TP`  -0.04054    0.01068  -3.797  0.00674 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1297 on 7 degrees of freedom
## Multiple R-squared:  0.8947, Adjusted R-squared:  0.8496
## F-statistic: 19.83 on 3 and 7 DF,  p-value: 0.0008449
##
##
## Response CAP2 :
##
## Call:
## lm(formula = CAP2 ~ (`nuts2012$DOC` + `nuts2012$TN` + `nuts2012$TP`) -
##     1, data = as.data.frame(X))
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.38734 -0.15254  0.00246  0.06711  0.47775
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## `nuts2012$DOC`   0.09751    0.04202   2.321   0.0533 .
## `nuts2012$TN`  -14.38332    5.19862  -2.767   0.0278 *
## `nuts2012$TP`   -0.01129    0.02380  -0.475   0.6496
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2891 on 7 degrees of freedom
## Multiple R-squared:  0.631,  Adjusted R-squared:  0.4728
## F-statistic:  3.99 on 3 and 7 DF,  p-value: 0.05996
##
##
## Response CAP3 :
##
## Call:
## lm(formula = CAP3 ~ (`nuts2012$DOC` + `nuts2012$TN` + `nuts2012$TP`) -
##     1, data = as.data.frame(X))
```

```
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.214008 -0.070633  0.004382  0.049866  0.226041
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## `nuts2012$DOC`  -0.03913    0.02064  -1.896 0.099825 .
## `nuts2012$TN`  -10.67619    2.55352  -4.181 0.004132 **
## `nuts2012$TP`    0.07078    0.01169   6.054 0.000514 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.142 on 7 degrees of freedom
## Multiple R-squared:  0.8763, Adjusted R-squared:  0.8233
## F-statistic: 16.54 on 3 and 7 DF,  p-value: 0.001472
```

```
require(cocorresp)
```

```
## Loading required package: cocorresp
```

```
#test1 <- coca(OTUsREL2012.RNA, resREL, n.axes = 4)
```

Phylogenetic Approach

Resource distirubiton is not able to explain the distribution of all organisms combined. But why should we expect this assumption?

Remove Cyanobacteria

Microbial Functional Groups

Define RDP microbial groups Test each along with resource differences Who are the generalist taxa (which are active everywhere) Are generalists more abundant when resource concentration is higher?

Can we group resources

What are the similar groups of resources: cluster resources based on abundance Can we cluster based on chemical data?