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myOtus <- read.delim("otu_table.txt", header = T, row.names = 1, skip = 1) #load in OTU table

head(myOtus)
library(vegan)

taxa <- data.frame(otuID = rownames(myOtus), taxonomy = myOtus$taxonomy) # make an object for taxa information
myOtus <- myOtus[, -ncol(myOtus)] # remove taxa from OTU table
myOtus <- as.data.frame(t(myOtus)) #transpose OTU table

#normalise seq depth to min
otuNorm <- rrarefy(myOtus, min(rowSums(myOtus)))

# OTU richness per sample
otuRichness <- specnumber(otuNorm)

print(otuRichness)
write.table(otuRichness, "otuRichness.txt", sep="\t")

# shannon div

otuShannon <- diversity(otuNorm, "shannon")
write.table(otuShannon, "otuShannon.txt", sep="\t")

# simpsons

otuSimpsons <- diversity(otuNorm, "simpson")
write.table(otuSimpsons, "otuSimpsons.txt", sep="\t")

# alpha div test statistics

envData <- read.csv("env.csv", header = T)

modRichness <- lm(otuRichness ~ envData$stage)
modSimpson <- lm(otuSimpsons ~ envData$stage)
modShannon <- lm(otuShannon ~ envData$stage)

summary(modRichness)

#Coefficients:
# Estimate Std. Error t value Pr(>|t|)
#(Intercept)      1201.99      48.56   24.75 < 2e-16 ***
# envData$stage   -153.91      12.47  -12.34 4.11e-14 ***
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Residual standard error: 127.8 on 34 degrees of freedom
#Multiple R-squared:  0.8176, Adjusted R-squared:  0.8122
#F-statistic: 152.4 on 1 and 34 DF, p-value: 4.106e-14

summary(modSimpson)

#Coefficients:
# Estimate Std. Error t value Pr(>|t|)
#(Intercept)      1.004277    0.005185 193.674 < 2e-16 ***
# envData$stage   -0.010282    0.001331  -7.722 5.57e-09 ***
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Residual standard error: 0.01364 on 34 degrees of freedom
#Multiple R-squared:  0.6369, Adjusted R-squared:  0.6262
#F-statistic: 59.63 on 1 and 34 DF, p-value: 5.566e-09

summary(modShannon)

#Coefficients:
# Estimate Std. Error t value Pr(>|t|)
#(Intercept)      6.18168    0.13493   45.81 < 2e-16 ***
# envData$stage   -0.42111    0.03465  -12.15 6.32e-14 ***
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Residual standard error: 0.355 on 34 degrees of freedom
#Multiple R-squared:  0.8129, Adjusted R-squared:  0.8074
#F-statistic: 147.7 on 1 and 34 DF, p-value: 6.316e-14

# beta div

# make dist matrix

commSim <- vegdist(otuNorm, "jaccard")

par(mfrow = c(1, 2)) # make heat map and cluster
heatmap(as.matrix(commSim))
plot(hclust(commSim))

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# permanover to test cluters

permResult <- adonis(commSim ~ envData$set + envData$stage, permutations = 1000)
permResult

#Permutation: free
#Number of permutations: 1000

#Terms added sequentially (first to last)

#Df SumsOfSqs MeanSqs F.Model      R2    Pr(>F)
#envData$set      2      0.9281 0.46404  2.6949 0.10714 0.002997 **
#  envData$stage  1      2.2244 2.22437 12.9179 0.25678 0.000999 ***
#  Residuals     32      5.5102 0.17219          0.63609
#Total           35      8.6626          1.00000
#---
#  Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#NMds of data

nmdsResult <- metaMDS(otuNorm, "jaccard")

plot(nmdsResult, type = "n")
points(nmdsResult$points, display = "sites")

nmdsResult

# make better nmDsPlot with sets as shapes and colour as stage

with(envData, levels(stage))
scl <- 6
colvec <- c("grey1", "grey20", "grey40", "grey60", "grey70", "grey80")
treatvec <- c(15, 16, 17)

plot(nmdsResult, type = "n", scaling = scl)

with(envData, points(nmdsResult, display = "sites", col = colvec[stage], scaling = scl, cex = 1, pch = treatvec[set],
bg = colvec[stage]))

#cant get legend to work?
legend('topright', col=colvec[stage], legend=levels(envData$site), pch = 16, cex = 0.7)

envData$set

```