alpha/beta diversity for bruno ATTEMPT 2

Tom Fu

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```
library(betapart)
library(vegan)
```

```
## Loading required package: permute
```

```
## Loading required package: lattice
```

```
## This is vegan 2.6-2
```

```
library(ggplot2)
library(readxl)
library(ggnetwork)
```

1. all

1.1 data loading and cleaning

```
otu_mat<- read_excel("/Users/chenlianfu/Documents/GitHub/IBS_FructanSensitivity/diversityAnalysis/source/speciesBrunoOTUTable110422.xlsx") # CORRECT
row.names(otu_mat) <- otu_mat$otu
otu_mat <- as.matrix(otu_mat)
otu_mat_t = t(otu_mat)
otu_mat_t <- otu_mat_t[-1,]
class(otu_mat_t)<-"numeric"
```

```
samples_df <- read_excel("/Users/chenlianfu/Documents/GitHub/IBS_FructanSensitivity/diversityAnalysis/source/brunoMetadata110422_1.xlsx") # CORRECT
groups<-as.list(samples_df['FsenText'])$FsenText
groups
```

```
##  [1] "tolerant"  "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"
##  [7] "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"  "sensitive"
## [13] "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"  "tolerant"
## [19] "sensitive" "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"
## [25] "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"  "sensitive"
## [31] "sensitive" "tolerant"  "tolerant"  "tolerant"  "tolerant"  "sensitive"
## [37] "sensitive" "tolerant"  "tolerant"  "sensitive" "sensitive" "sensitive"
## [43] "sensitive" "tolerant"  "tolerant"  "sensitive" "sensitive" "tolerant"
## [49] "tolerant"  "sensitive" "sensitive" "sensitive" "sensitive" "tolerant"
## [55] "tolerant"  "sensitive" "sensitive" "sensitive" "sensitive" "tolerant"
## [61] "tolerant"  "sensitive" "sensitive" "sensitive" "sensitive" "tolerant"
## [67] "tolerant"  "tolerant"  "tolerant"  "sensitive" "sensitive" "tolerant"
## [73] "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"
## [79] "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"
## [85] "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"
## [91] "tolerant"  "sensitive" "sensitive" "sensitive" "tolerant"  "tolerant"
## [97] "tolerant"  "tolerant"
```

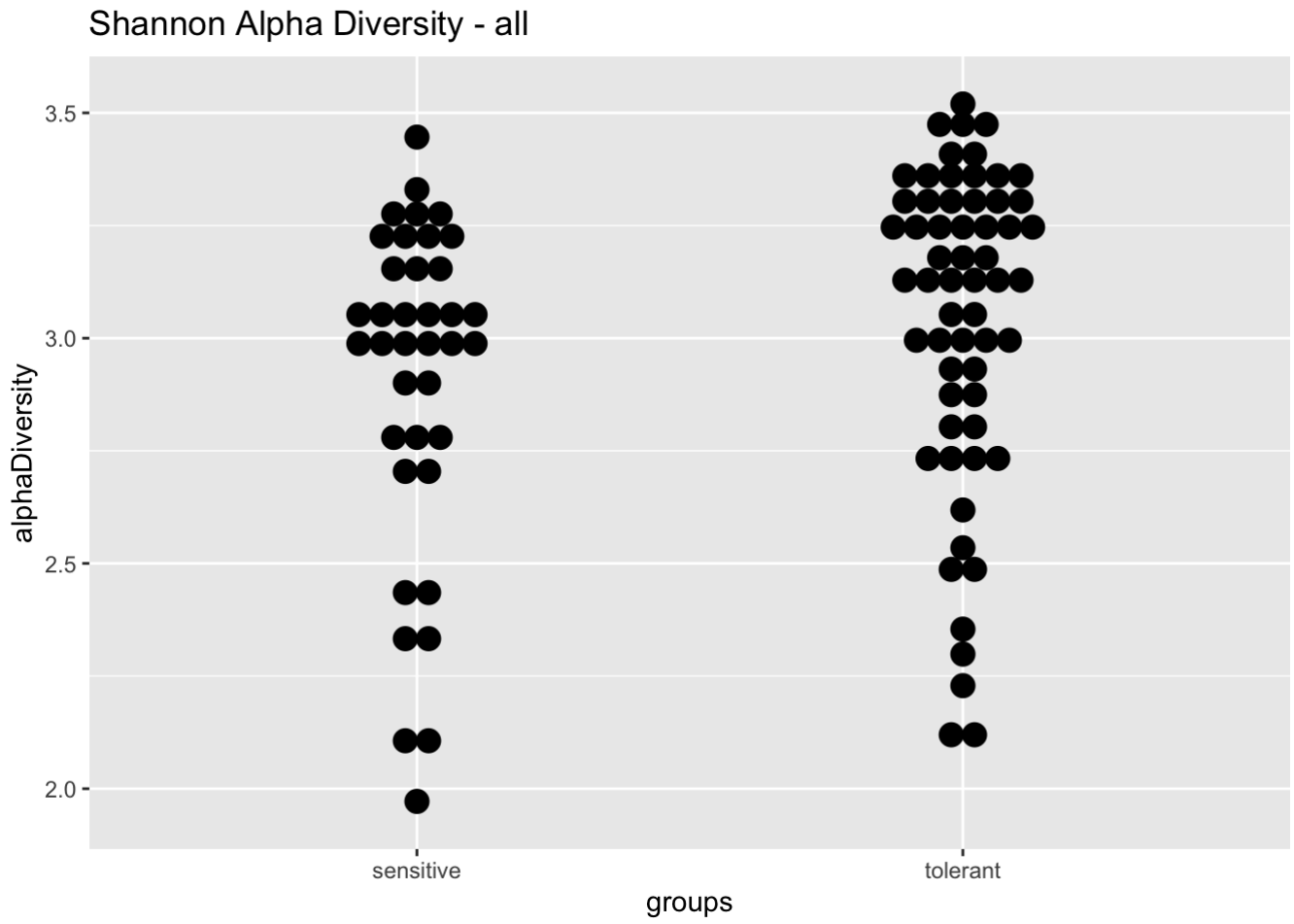
1.2 alpha diversity

```
alphaDiversity <- diversity(otu_mat_t, index="shannon")
summary(aov(alphaDiversity ~ groups))
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## groups       1  0.395  0.3953   3.016 0.0857 .
## Residuals   96 12.583  0.1311
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
alphaDataframe <- data.frame(alphaDiversity, groups)
write.csv(alphaDataframe, "alph_div_df_all.csv")
p<-ggplot(alphaDataframe, aes(x=groups, y=alphaDiversity)) +
  geom_dotplot(binaxis='y', stackdir='center')+
  ggtitle("Shannon Alpha Diversity - all")
p
```

```
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth
`.
```

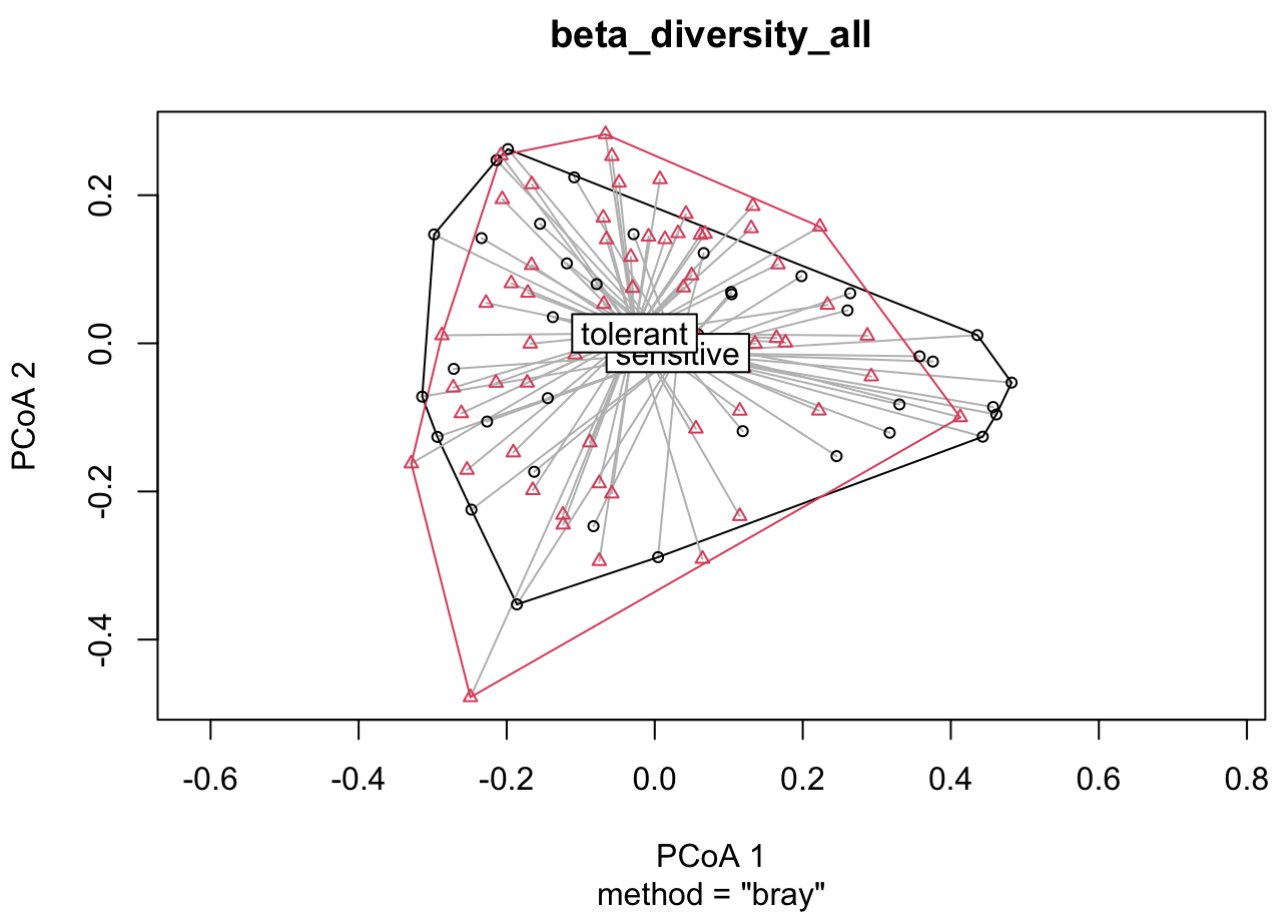



```
ggsave(file="alpha_div_all.pdf", width=10, height=8, dpi=300)
```

```
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth
`.
```

1.3 beta diversity

```
dist <- vegdist(otu_mat_t, method = "bray")
beta_diversity_all<-betadisper(dist,groups)
g <- plot(beta_diversity_all)
```




```
# ggsave(file="beta_div_all.pdf", width=10, height=8, dpi=300)
anova(beta_diversity_all)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df  Sum Sq  Mean Sq F value Pr(>F)
## Groups     1 0.01076 0.010756   2.089 0.1516
## Residuals 96 0.49430 0.005149
```

```
g
```

```
## $sites
##              PCoA1         PCoA2
## 3003   0.164506517  0.0071442581
## 3004  -0.144515198 -0.0739727540
## 3005  -0.058005366 -0.2028920270
## 3007   0.198304768  0.0907124896
## 3008  -0.082797210 -0.2468649398
## 3009  -0.214429214 -0.0533822450
## 3012   0.330595212 -0.0825080261
## 3013   0.221795644 -0.0908629439
## 3014  -0.247625333 -0.2245389448
## 3016   0.264163034  0.0676033991
## 3020  -0.123732540 -0.2448121213
## 3021   0.317427117 -0.1206929229
## 3022  -0.198350990  0.2622066106
## 3023   0.130277754  0.1552851379
## 3025  -0.298074637  0.1470219884
## 3026  -0.314177266 -0.0719639093
## 3027  -0.171464730  0.0682151749
## 3028  -0.206016439  0.1945941794
## 3029   0.058601194  0.0120292127
## 3030   0.007078642  0.2215036261
## 3031   0.061781694  0.1463723504
## 3032   0.055631616 -0.1152121575
## 3033  -0.074903791 -0.2940232835
## 3036  -0.261303982 -0.0944831951
## 3037   0.176621805  0.0009342984
## 3039   0.114814733 -0.2333364584
## 3042   0.132578507  0.1853553769
## 3043   0.031479630  0.1486600770
## 3044  -0.166384295  0.1055721168
## 3046   0.457096490 -0.0859985776
## 3047   0.245694654 -0.1523022295
## 3048  -0.287470750  0.0107992654
## 3049  -0.008381539  0.1440716960
## 3003A  0.166655804  0.1064692513
## 3003B -0.065170271  0.1403686144
## 3004A -0.078511151  0.0799202229
## 3004B -0.154936352  0.1613891946
## 3005A -0.164720193 -0.1982785785
## 3005B  0.038784343  0.0753537637
## 3007A  0.435884191  0.0110960685
## 3007B  0.482103142 -0.0530206392
## 3008A  0.066158375  0.1218686225
## 3008B -0.234014003  0.1420354710
## 3009A -0.253649273 -0.1709028398
## 3009B -0.249017839 -0.4778230562
## 3012A  0.357880063 -0.0175426105
## 3012B  0.461532158 -0.0960799882
## 3013A -0.087738343 -0.1338242795
## 3013B -0.124078009 -0.2315286460
## 3014A -0.271511635 -0.0344814904
```

```
## 3014B -0.293628101 -0.1262911045
## 3016A  0.260446995  0.0444599828
## 3016B  0.375801799 -0.0246130579
## 3020A  0.287272666  0.0102207182
## 3020B  0.135524557 -0.0005918203
## 3021A  0.103171596  0.0693215110
## 3021B  0.103987615  0.0662089212
## 3022A -0.108765958  0.2241060128
## 3022B -0.028645639  0.1473676498
## 3023A  0.223141261  0.1574104232
## 3023B  0.412768082 -0.0999095263
## 3025A -0.118722345  0.1079421002
## 3025B -0.226484440 -0.1056204375
## 3026A -0.162911363 -0.1734252210
## 3026B  0.118898702 -0.1185863967
## 3027A -0.029940603  0.0747625257
## 3027B -0.227928456  0.0545104488
## 3028A -0.069581000  0.1695765038
## 3028B -0.272179020 -0.0598186655
## 3029A -0.137656009  0.0354103921
## 3029B -0.186207613 -0.3525011240
## 3030A -0.048184904  0.2169143388
## 3030B  0.233325022  0.0518841937
## 3031A  0.049943805  0.0917480625
## 3031B  0.042198804  0.1746623329
## 3032A -0.193854104  0.0809715237
## 3032B  0.055953006  0.0121253356
## 3033A  0.114925349 -0.0911050122
## 3033B  0.064484708 -0.2910569368
## 3036A -0.172263686 -0.0531622371
## 3036B -0.328856490 -0.1622649597
## 3037A  0.292600022 -0.0447472721
## 3037B  0.120593416 -0.0330658565
## 3039A -0.075067849 -0.1890866817
## 3039B -0.107641176 -0.0151425107
## 3042A -0.057867456  0.2527436993
## 3042B -0.066496555  0.2822484082
## 3043A  0.068085920  0.1477716749
## 3043B  0.013827992  0.1405386802
## 3044A -0.068801679  0.0531505815
## 3044B -0.168265518 -0.0005094445
## 3046A -0.213742841  0.2473035052
## 3046B  0.443121120 -0.1260523962
## 3047B  0.004506664 -0.2887330531
## 3048A -0.191079547 -0.1473707866
## 3048B -0.207825742  0.2537100326
## 3049A -0.166109984  0.2146473170
## 3049B -0.032337763  0.1166840223
##
## $centroids
##                 PCoA1       PCoA2
## sensitive  0.03115702 -0.01306529
```

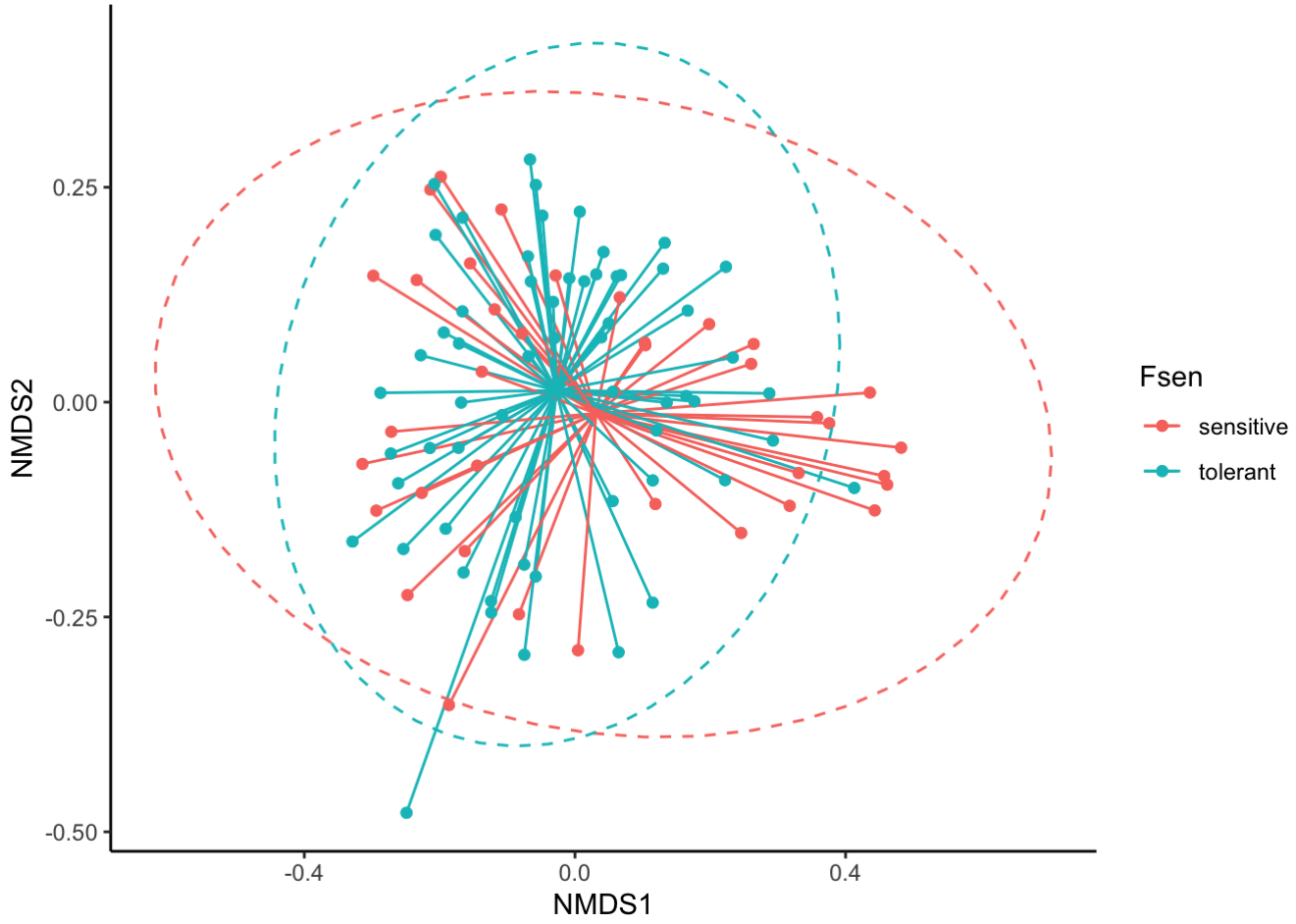
```
## tolerant  -0.02736797  0.01355755
##
## attr(,"class")
## [1] "ordiplot"
```

```
prepDataFrame <- as.data.frame(g$sites)
prepDataFrame$Fsen <- groups
prepDataFrame$PCoA1_centroids <- ifelse(prepDataFrame$Fsen == "sensitive", 0.03115702, -
0.02736797)
prepDataFrame$PCoA2_centroids <- ifelse(prepDataFrame$Fsen == "sensitive", -0.01306529,
0.01355755)
prepDataFrame
```

```
##              PCoA1         PCoA2      Fsen PCoA1_centroids PCoA2_centroids
## 3003   0.164506517  0.0071442581  tolerant     -0.02736797      0.01355755
## 3004  -0.144515198 -0.0739727540 sensitive      0.03115702     -0.01306529
## 3005  -0.058005366 -0.2028920270  tolerant     -0.02736797      0.01355755
## 3007   0.198304768  0.0907124896 sensitive      0.03115702     -0.01306529
## 3008  -0.082797210 -0.2468649398 sensitive      0.03115702     -0.01306529
## 3009  -0.214429214 -0.0533822450  tolerant     -0.02736797      0.01355755
## 3012   0.330595212 -0.0825080261 sensitive      0.03115702     -0.01306529
## 3013   0.221795644 -0.0908629439  tolerant     -0.02736797      0.01355755
## 3014  -0.247625333 -0.2245389448 sensitive      0.03115702     -0.01306529
## 3016   0.264163034  0.0676033991 sensitive      0.03115702     -0.01306529
## 3020  -0.123732540 -0.2448121213  tolerant     -0.02736797      0.01355755
## 3021   0.317427117 -0.1206929229 sensitive      0.03115702     -0.01306529
## 3022  -0.198350990  0.2622066106 sensitive      0.03115702     -0.01306529
## 3023   0.130277754  0.1552851379  tolerant     -0.02736797      0.01355755
## 3025  -0.298074637  0.1470219884 sensitive      0.03115702     -0.01306529
## 3026  -0.314177266 -0.0719639093 sensitive      0.03115702     -0.01306529
## 3027  -0.171464730  0.0682151749  tolerant     -0.02736797      0.01355755
## 3028  -0.206016439  0.1945941794  tolerant     -0.02736797      0.01355755
## 3029   0.058601194  0.0120292127 sensitive      0.03115702     -0.01306529
## 3030   0.007078642  0.2215036261  tolerant     -0.02736797      0.01355755
## 3031   0.061781694  0.1463723504  tolerant     -0.02736797      0.01355755
## 3032   0.055631616 -0.1152121575  tolerant     -0.02736797      0.01355755
## 3033  -0.074903791 -0.2940232835  tolerant     -0.02736797      0.01355755
## 3036  -0.261303982 -0.0944831951  tolerant     -0.02736797      0.01355755
## 3037   0.176621805  0.0009342984  tolerant     -0.02736797      0.01355755
## 3039   0.114814733 -0.2333364584  tolerant     -0.02736797      0.01355755
## 3042   0.132578507  0.1853553769  tolerant     -0.02736797      0.01355755
## 3043   0.031479630  0.1486600770  tolerant     -0.02736797      0.01355755
## 3044  -0.166384295  0.1055721168  tolerant     -0.02736797      0.01355755
## 3046   0.457096490 -0.0859985776 sensitive      0.03115702     -0.01306529
## 3047   0.245694654 -0.1523022295 sensitive      0.03115702     -0.01306529
## 3048  -0.287470750  0.0107992654  tolerant     -0.02736797      0.01355755
## 3049  -0.008381539  0.1440716960  tolerant     -0.02736797      0.01355755
## 3003A  0.166655804  0.1064692513  tolerant     -0.02736797      0.01355755
## 3003B -0.065170271  0.1403686144  tolerant     -0.02736797      0.01355755
## 3004A -0.078511151  0.0799202229 sensitive      0.03115702     -0.01306529
## 3004B -0.154936352  0.1613891946 sensitive      0.03115702     -0.01306529
## 3005A -0.164720193 -0.1982785785  tolerant     -0.02736797      0.01355755
## 3005B  0.038784343  0.0753537637  tolerant     -0.02736797      0.01355755
## 3007A  0.435884191  0.0110960685 sensitive      0.03115702     -0.01306529
## 3007B  0.482103142 -0.0530206392 sensitive      0.03115702     -0.01306529
## 3008A  0.066158375  0.1218686225 sensitive      0.03115702     -0.01306529
## 3008B -0.234014003  0.1420354710 sensitive      0.03115702     -0.01306529
## 3009A -0.253649273 -0.1709028398  tolerant     -0.02736797      0.01355755
## 3009B -0.249017839 -0.4778230562  tolerant     -0.02736797      0.01355755
## 3012A  0.357880063 -0.0175426105 sensitive      0.03115702     -0.01306529
## 3012B  0.461532158 -0.0960799882 sensitive      0.03115702     -0.01306529
## 3013A -0.087738343 -0.1338242795  tolerant     -0.02736797      0.01355755
## 3013B -0.124078009 -0.2315286460  tolerant     -0.02736797      0.01355755
## 3014A -0.271511635 -0.0344814904 sensitive      0.03115702     -0.01306529
## 3014B -0.293628101 -0.1262911045 sensitive      0.03115702     -0.01306529
```

```
## 3016A  0.260446995  0.0444599828 sensitive      0.03115702     -0.01306529
## 3016B  0.375801799 -0.0246130579 sensitive      0.03115702     -0.01306529
## 3020A  0.287272666  0.0102207182  tolerant     -0.02736797      0.01355755
## 3020B  0.135524557 -0.0005918203  tolerant     -0.02736797      0.01355755
## 3021A  0.103171596  0.0693215110 sensitive      0.03115702     -0.01306529
## 3021B  0.103987615  0.0662089212 sensitive      0.03115702     -0.01306529
## 3022A -0.108765958  0.2241060128 sensitive      0.03115702     -0.01306529
## 3022B -0.028645639  0.1473676498 sensitive      0.03115702     -0.01306529
## 3023A  0.223141261  0.1574104232  tolerant     -0.02736797      0.01355755
## 3023B  0.412768082 -0.0999095263  tolerant     -0.02736797      0.01355755
## 3025A -0.118722345  0.1079421002 sensitive      0.03115702     -0.01306529
## 3025B -0.226484440 -0.1056204375 sensitive      0.03115702     -0.01306529
## 3026A -0.162911363 -0.1734252210 sensitive      0.03115702     -0.01306529
## 3026B  0.118898702 -0.1185863967 sensitive      0.03115702     -0.01306529
## 3027A -0.029940603  0.0747625257  tolerant     -0.02736797      0.01355755
## 3027B -0.227928456  0.0545104488  tolerant     -0.02736797      0.01355755
## 3028A -0.069581000  0.1695765038  tolerant     -0.02736797      0.01355755
## 3028B -0.272179020 -0.0598186655  tolerant     -0.02736797      0.01355755
## 3029A -0.137656009  0.0354103921 sensitive      0.03115702     -0.01306529
## 3029B -0.186207613 -0.3525011240 sensitive      0.03115702     -0.01306529
## 3030A -0.048184904  0.2169143388  tolerant     -0.02736797      0.01355755
## 3030B  0.233325022  0.0518841937  tolerant     -0.02736797      0.01355755
## 3031A  0.049943805  0.0917480625  tolerant     -0.02736797      0.01355755
## 3031B  0.042198804  0.1746623329  tolerant     -0.02736797      0.01355755
## 3032A -0.193854104  0.0809715237  tolerant     -0.02736797      0.01355755
## 3032B  0.055953006  0.0121253356  tolerant     -0.02736797      0.01355755
## 3033A  0.114925349 -0.0911050122  tolerant     -0.02736797      0.01355755
## 3033B  0.064484708 -0.2910569368  tolerant     -0.02736797      0.01355755
## 3036A -0.172263686 -0.0531622371  tolerant     -0.02736797      0.01355755
## 3036B -0.328856490 -0.1622649597  tolerant     -0.02736797      0.01355755
## 3037A  0.292600022 -0.0447472721  tolerant     -0.02736797      0.01355755
## 3037B  0.120593416 -0.0330658565  tolerant     -0.02736797      0.01355755
## 3039A -0.075067849 -0.1890866817  tolerant     -0.02736797      0.01355755
## 3039B -0.107641176 -0.0151425107  tolerant     -0.02736797      0.01355755
## 3042A -0.057867456  0.2527436993  tolerant     -0.02736797      0.01355755
## 3042B -0.066496555  0.2822484082  tolerant     -0.02736797      0.01355755
## 3043A  0.068085920  0.1477716749  tolerant     -0.02736797      0.01355755
## 3043B  0.013827992  0.1405386802  tolerant     -0.02736797      0.01355755
## 3044A -0.068801679  0.0531505815  tolerant     -0.02736797      0.01355755
## 3044B -0.168265518 -0.0005094445  tolerant     -0.02736797      0.01355755
## 3046A -0.213742841  0.2473035052 sensitive      0.03115702     -0.01306529
## 3046B  0.443121120 -0.1260523962 sensitive      0.03115702     -0.01306529
## 3047B  0.004506664 -0.2887330531 sensitive      0.03115702     -0.01306529
## 3048A -0.191079547 -0.1473707866  tolerant     -0.02736797      0.01355755
## 3048B -0.207825742  0.2537100326  tolerant     -0.02736797      0.01355755
## 3049A -0.166109984  0.2146473170  tolerant     -0.02736797      0.01355755
## 3049B -0.032337763  0.1166840223  tolerant     -0.02736797      0.01355755
```

```
write.csv(prepDataFrame, "beta_div_df_all.csv")
library(readr)
# a dataset edited from NMDSNetworkData.csv
library(ggplot2)
ggplot(prepDataFrame, aes(x = PCoA1, y = PCoA2, xend = PCoA1_centroids, yend = PCoA2_cen
troids, color = Fsen), axes=TRUE) +
  geom_point(aes(color = Fsen)) +
  geom_edges(aes(color = Fsen)) +
  geom_nodes(aes(color = Fsen)) +
  xlab("NMDS1") +
  ylab("NMDS2")+
  stat_ellipse(type = "norm", linetype = 2) +
  theme_classic()
```




```
ggsave(file="beta_div_all.pdf", width=10, height=8, dpi=300)
```

2. BS

2.1 data loading and cleaning

```
otu_mat<- read_excel("/Users/chenlianfu/Documents/GitHub/IBS_FructanSensitivity/diversit
yAnalysis/source/dietSubsets/speciesOTUTable_BS_110422.xlsx") # CORRECT
row.names(otu_mat) <- otu_mat$otu
otu_mat <- as.matrix(otu_mat)
otu_mat_t = t(otu_mat)
otu_mat_t <- otu_mat_t[-1,]
class(otu_mat_t)<-"numeric"
```

```
samples_df <- read_excel("/Users/chenlianfu/Documents/GitHub/IBS_FructanSensitivity/dive
rsityAnalysis/source/dietSubsets/brunoMetadata_BS_110422.xlsx") # CORRECT
groups<-as.list(samples_df['FsenText'])$FsenText
groups
```

```
##  [1] "tolerant"  "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"
##  [7] "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"  "sensitive"
## [13] "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"  "tolerant"
## [19] "sensitive" "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"
## [25] "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"  "sensitive"
## [31] "sensitive" "tolerant"  "tolerant"
```

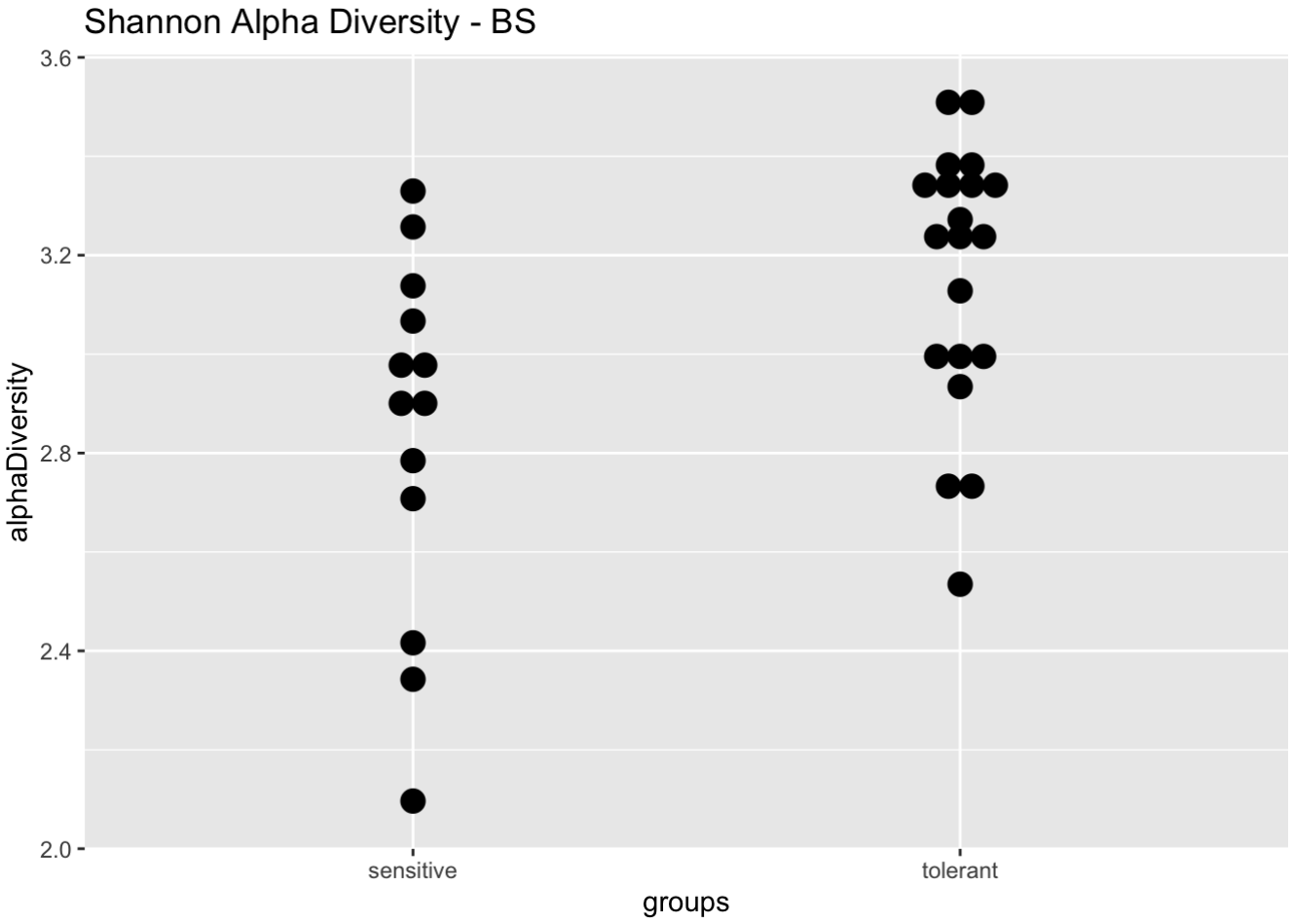
2.2 alpha diversity

```
alphaDiversity <- diversity(otu_mat_t, index="shannon")
summary(aov(alphaDiversity ~ groups))
```

```
##             Df Sum Sq Mean Sq F value  Pr(>F)
## groups       1  0.807  0.8070   8.385 0.00688 **
## Residuals   31  2.983  0.0962
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
alphaDataframe <- data.frame(alphaDiversity, groups)
write.csv(alphaDataframe, "alph_div_df_BS.csv")
p<-ggplot(alphaDataframe, aes(x=groups, y=alphaDiversity)) +
  geom_dotplot(binaxis='y', stackdir='center')+
  ggtitle("Shannon Alpha Diversity - BS")
p
```

```
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth
`.
```

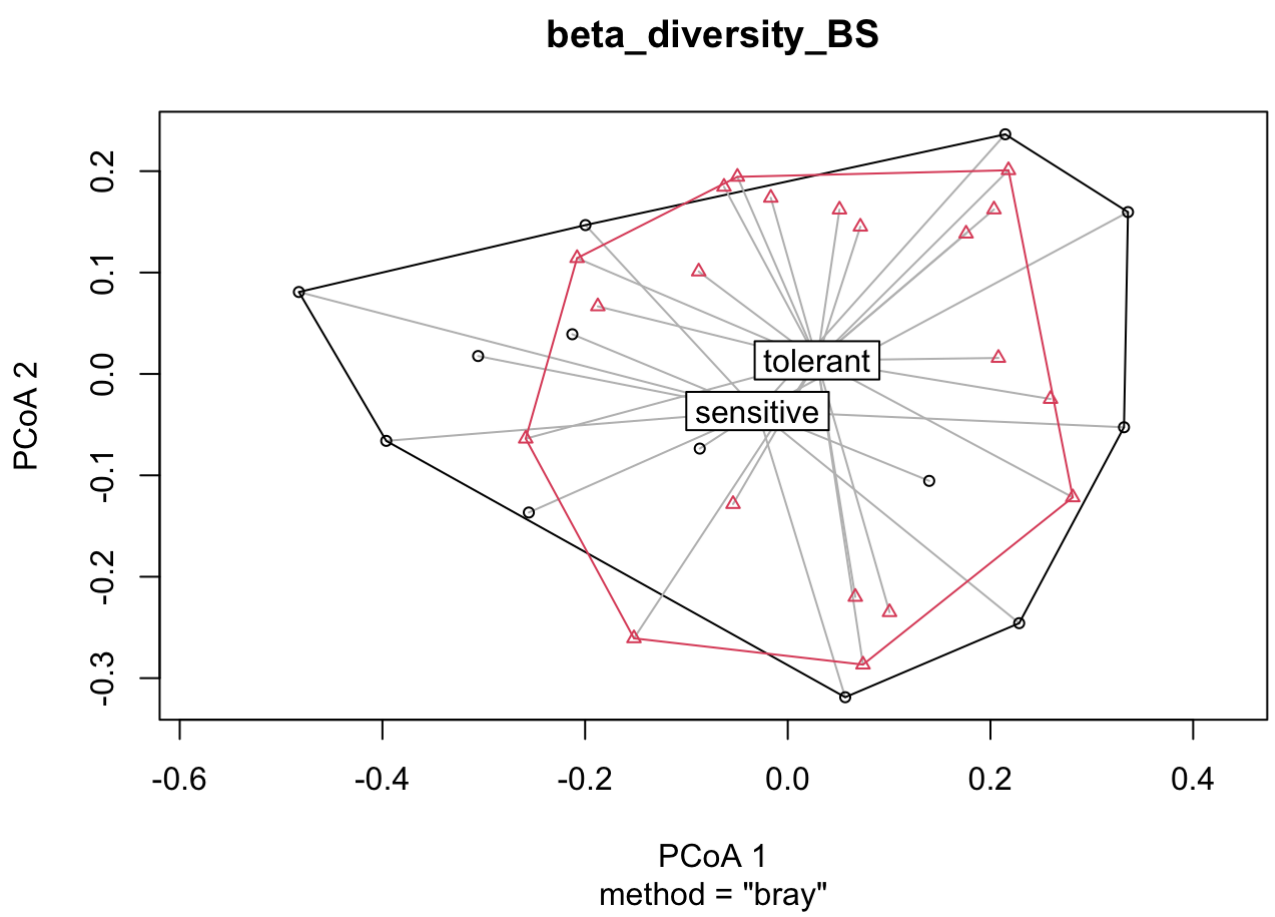



```
ggsave(file="alpha_div_BS.pdf", width=10, height=8, dpi=300)
```

```
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth
`.
```

2.3 beta diversity

```
dist <- vegdist(otu_mat_t, method = "bray")
beta_diversity_BS<-betadisper(dist,groups)
g <- plot(beta_diversity_BS)
```




```
# ggsave(file="beta_div_BS.pdf", width=10, height=8, dpi=300)
anova(beta_diversity_BS)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value  Pr(>F)
## Groups     1 0.021436 0.0214357  4.6206 0.03951 *
## Residuals 31 0.143815 0.0046392
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

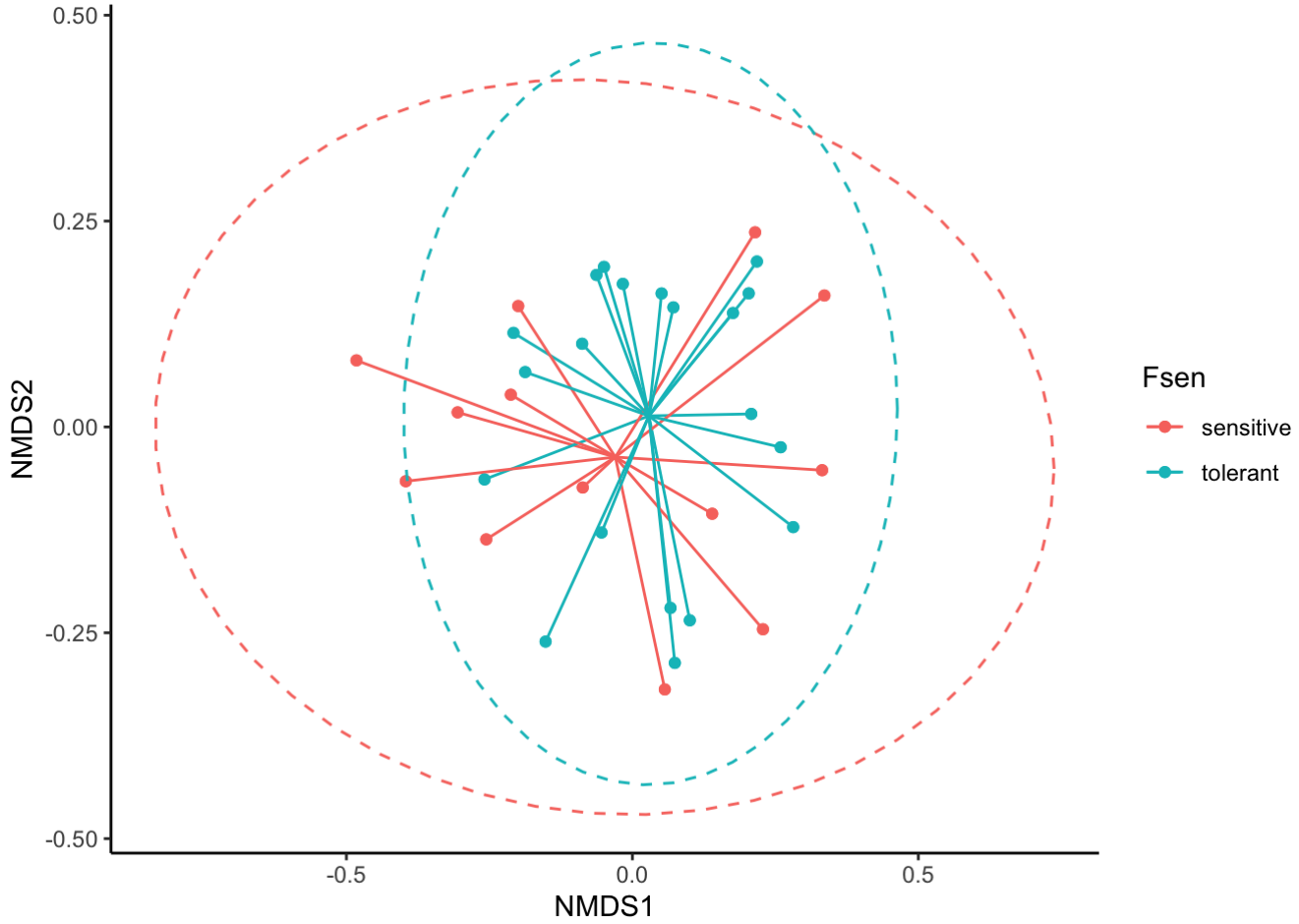
```
g
```

```
## $sites
##            PCoA1       PCoA2
## 3003 -0.20791478  0.11422094
## 3004  0.13956311 -0.10546715
## 3005  0.06664968 -0.21987513
## 3007 -0.19967150  0.14679642
## 3008  0.05668736 -0.31882804
## 3009  0.20785624  0.01578278
## 3012 -0.30560243  0.01768410
## 3013 -0.25863253 -0.06381954
## 3014  0.22833680 -0.24579008
## 3016 -0.21262209  0.03914801
## 3020  0.10041025 -0.23493157
## 3021 -0.39622781 -0.06599891
## 3022  0.21459022  0.23635072
## 3023 -0.08791699  0.10100606
## 3025  0.33572960  0.15962945
## 3026  0.33175450 -0.05256500
## 3027  0.21776886  0.20090082
## 3028  0.20346407  0.16221774
## 3029 -0.08685991 -0.07360836
## 3030  0.05101955  0.16208886
## 3031 -0.06293778  0.18456687
## 3032 -0.05400512 -0.12831342
## 3033  0.07422754 -0.28663245
## 3036  0.28121917 -0.12170410
## 3037 -0.18747194  0.06665638
## 3039 -0.15180356 -0.26072639
## 3042 -0.04970932  0.19439143
## 3043 -0.01674040  0.17371747
## 3044  0.17593483  0.13838541
## 3046 -0.48243493  0.08082018
## 3047 -0.25555445 -0.13663593
## 3048  0.25926168 -0.02468912
## 3049  0.07163208  0.14522155
##
## $centroids
##                 PCoA1       PCoA2
## sensitive -0.02985877 -0.03657319
## tolerant   0.02887691  0.01340628
##
## attr(,"class")
## [1] "ordiplot"
```

```
prepDataFrame <- as.data.frame(g$sites)
prepDataFrame$Fsen <- groups
prepDataFrame$PCoA1_centroids <- ifelse(prepDataFrame$Fsen == "sensitive", -0.02985877,
0.02887691)
prepDataFrame$PCoA2_centroids <- ifelse(prepDataFrame$Fsen == "sensitive", -0.03657319,
0.01340628)
prepDataFrame
```

```
##            PCoA1       PCoA2      Fsen PCoA1_centroids PCoA2_centroids
## 3003 -0.20791478  0.11422094  tolerant      0.02887691      0.01340628
## 3004  0.13956311 -0.10546715 sensitive     -0.02985877     -0.03657319
## 3005  0.06664968 -0.21987513  tolerant      0.02887691      0.01340628
## 3007 -0.19967150  0.14679642 sensitive     -0.02985877     -0.03657319
## 3008  0.05668736 -0.31882804 sensitive     -0.02985877     -0.03657319
## 3009  0.20785624  0.01578278  tolerant      0.02887691      0.01340628
## 3012 -0.30560243  0.01768410 sensitive     -0.02985877     -0.03657319
## 3013 -0.25863253 -0.06381954  tolerant      0.02887691      0.01340628
## 3014  0.22833680 -0.24579008 sensitive     -0.02985877     -0.03657319
## 3016 -0.21262209  0.03914801 sensitive     -0.02985877     -0.03657319
## 3020  0.10041025 -0.23493157  tolerant      0.02887691      0.01340628
## 3021 -0.39622781 -0.06599891 sensitive     -0.02985877     -0.03657319
## 3022  0.21459022  0.23635072 sensitive     -0.02985877     -0.03657319
## 3023 -0.08791699  0.10100606  tolerant      0.02887691      0.01340628
## 3025  0.33572960  0.15962945 sensitive     -0.02985877     -0.03657319
## 3026  0.33175450 -0.05256500 sensitive     -0.02985877     -0.03657319
## 3027  0.21776886  0.20090082  tolerant      0.02887691      0.01340628
## 3028  0.20346407  0.16221774  tolerant      0.02887691      0.01340628
## 3029 -0.08685991 -0.07360836 sensitive     -0.02985877     -0.03657319
## 3030  0.05101955  0.16208886  tolerant      0.02887691      0.01340628
## 3031 -0.06293778  0.18456687  tolerant      0.02887691      0.01340628
## 3032 -0.05400512 -0.12831342  tolerant      0.02887691      0.01340628
## 3033  0.07422754 -0.28663245  tolerant      0.02887691      0.01340628
## 3036  0.28121917 -0.12170410  tolerant      0.02887691      0.01340628
## 3037 -0.18747194  0.06665638  tolerant      0.02887691      0.01340628
## 3039 -0.15180356 -0.26072639  tolerant      0.02887691      0.01340628
## 3042 -0.04970932  0.19439143  tolerant      0.02887691      0.01340628
## 3043 -0.01674040  0.17371747  tolerant      0.02887691      0.01340628
## 3044  0.17593483  0.13838541  tolerant      0.02887691      0.01340628
## 3046 -0.48243493  0.08082018 sensitive     -0.02985877     -0.03657319
## 3047 -0.25555445 -0.13663593 sensitive     -0.02985877     -0.03657319
## 3048  0.25926168 -0.02468912  tolerant      0.02887691      0.01340628
## 3049  0.07163208  0.14522155  tolerant      0.02887691      0.01340628
```

```
write.csv(prepDataFrame, "beta_div_df_BS.csv")
library(readr)
# a dataset edited from NMDSNetworkData.csv
library(ggplot2)
ggplot(prepDataFrame, aes(x = PCoA1, y = PCoA2, xend = PCoA1_centroids, yend = PCoA2_cen
troids, color = Fsen), axes=TRUE) +
  geom_point(aes(color = Fsen)) +
  geom_edges(aes(color = Fsen)) +
  geom_nodes(aes(color = Fsen)) +
  xlab("NMDS1") +
  ylab("NMDS2")+
  stat_ellipse(type = "norm", linetype = 2) +
  theme_classic()
```




```
ggsave(file="beta_div_BS.pdf", width=10, height=8, dpi=300)
```

3. A

3.1 data loading and cleaning

```
otu_mat<- read_excel("/Users/chenlianfu/Documents/GitHub/IBS_FructanSensitivity/diversit
yAnalysis/source/dietSubsets/speciesOTUTable_A_110422.xlsx") # CORRECT
row.names(otu_mat) <- otu_mat$otu
otu_mat <- as.matrix(otu_mat)
otu_mat_t = t(otu_mat)
otu_mat_t <- otu_mat_t[-1,]
class(otu_mat_t)<-"numeric"
```

```
samples_df <- read_excel("/Users/chenlianfu/Documents/GitHub/IBS_FructanSensitivity/dive
rsityAnalysis/source/dietSubsets/brunoMetadata_A_110422.xlsx") # CORRECT
groups<-as.list(samples_df['FsenText'])$FsenText
groups
```

```
##  [1] "tolerant"  "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"
##  [7] "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"  "sensitive"
## [13] "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"  "tolerant"
## [19] "sensitive" "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"
## [25] "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"  "sensitive"
## [31] "tolerant"  "tolerant"
```

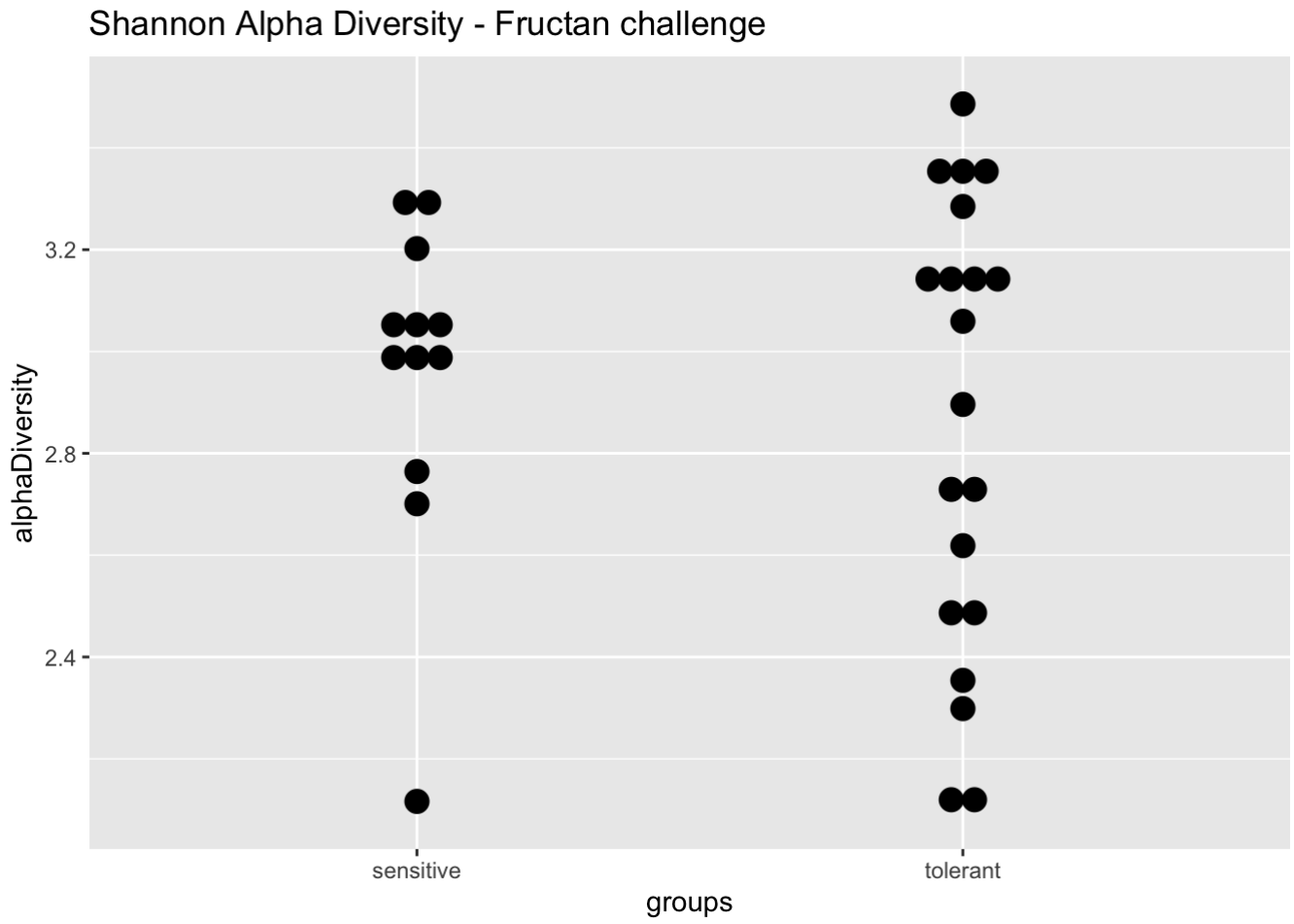
3.2 alpha diversity

```
alphaDiversity <- diversity(otu_mat_t, index="shannon")
summary(aov(alphaDiversity ~ groups))
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## groups       1  0.067 0.06657   0.412  0.526
## Residuals   30  4.841 0.16138
```

```
alphaDataframe <- data.frame(alphaDiversity, groups)
write.csv(alphaDataframe, "alph_div_df_A.csv")
p<-ggplot(alphaDataframe, aes(x=groups, y=alphaDiversity)) +
  geom_dotplot(binaxis='y', stackdir='center')+
  ggtitle("Shannon Alpha Diversity - Fructan challenge")
p
```

```
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth
`.
```

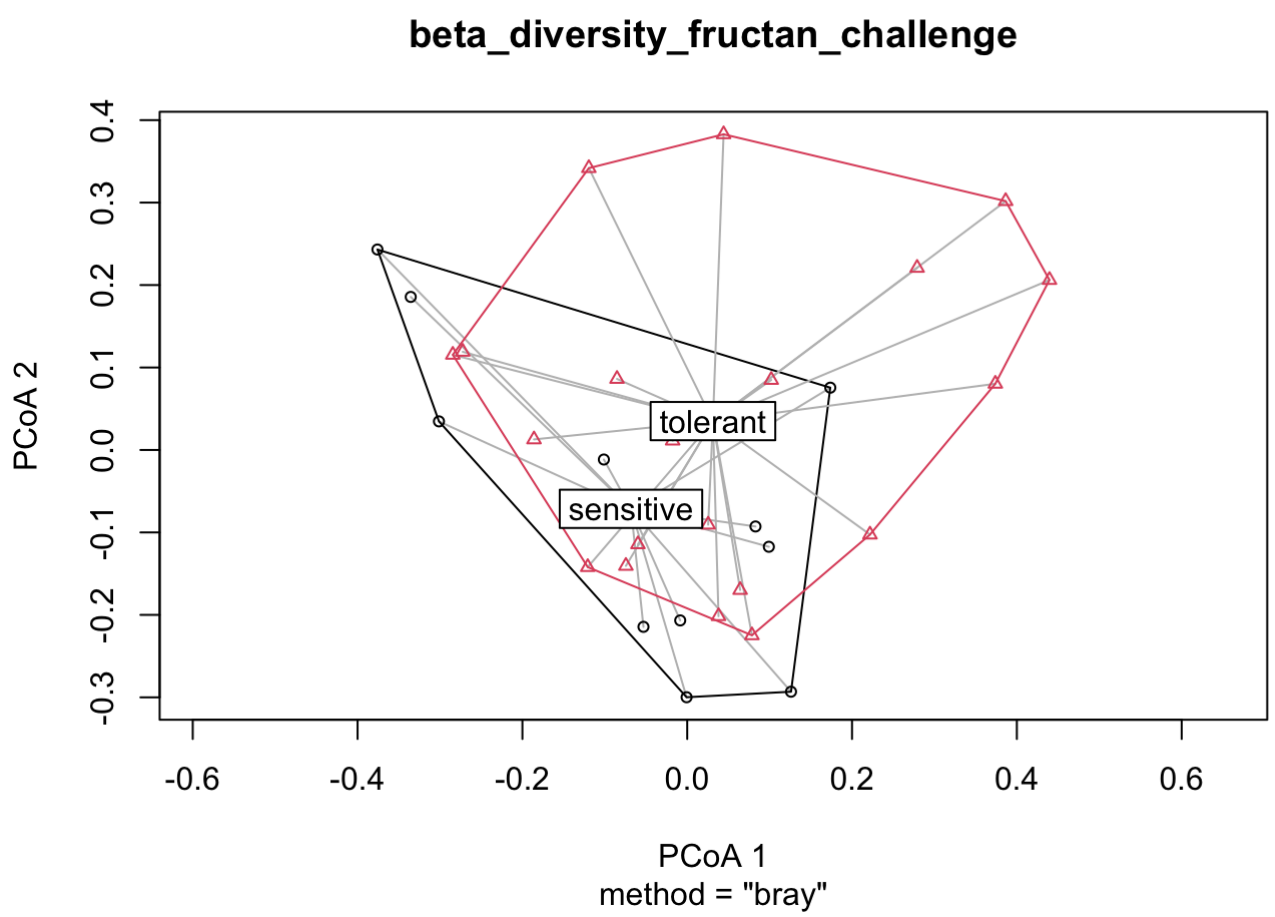



```
ggsave(file="alpha_div_A.pdf", width=10, height=8, dpi=300)
```

```
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth
`.
```

3.3 beta diversity

```
dist <- vegdist(otu_mat_t, method = "bray")
beta_diversity_fructan_challenge<-betadisper(dist,groups)
g <- plot(beta_diversity_fructan_challenge)
```




```
dev.off
```

```
## function (which = dev.cur())
## {
##     if (which == 1)
##         stop("cannot shut down device 1 (the null device)")
##     .External(C_devoff, as.integer(which))
##     dev.cur()
## }
## <bytecode: 0x7f8b99cafbe8>
## <environment: namespace:grDevices>
```

```
# ggsave(file="beta_div_A.pdf", width=10, height=8, dpi=300)
anova(beta_diversity_fructan_challenge)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value Pr(>F)
## Groups     1 0.000273 0.0002735  0.0582  0.811
## Residuals 30 0.141004 0.0047001
```

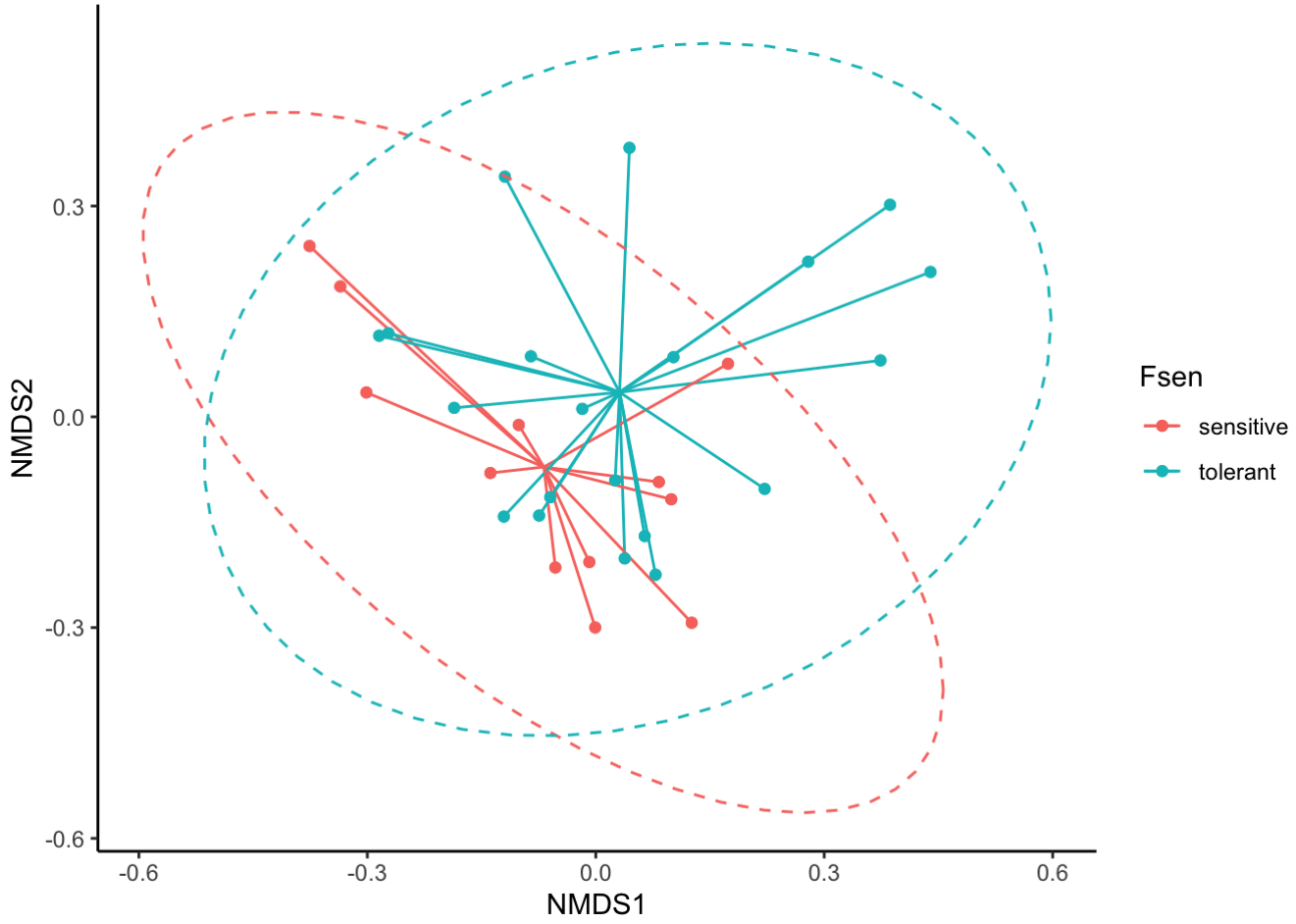
```
g
```

```
## $sites
##               PCoA1       PCoA2
## 3003A  0.0441677429  0.38283619
## 3004A  0.0828625107 -0.09278056
## 3005A  0.0379890702 -0.20157629
## 3007A -0.3759037336  0.24310633
## 3008A -0.1383976477 -0.07998188
## 3009A  0.3738847502  0.08029102
## 3012A -0.3354912089  0.18561258
## 3013A  0.2790018697  0.22083642
## 3014A  0.1259877419 -0.29315664
## 3016A -0.3012640720  0.03467458
## 3020A -0.1193846188  0.34179879
## 3021A -0.1011613674 -0.01159111
## 3022A  0.1736487599  0.07555590
## 3023A -0.2844635558  0.11542452
## 3025A -0.0530972414 -0.21449097
## 3026A  0.0990924366 -0.11741128
## 3027A  0.1019807428  0.08492518
## 3028A -0.0744293570 -0.14057275
## 3029A -0.0085005473 -0.20671649
## 3030A -0.1207205523 -0.14203642
## 3031A -0.0177008476  0.01135208
## 3032A  0.0785315462 -0.22475149
## 3033A -0.0851998964  0.08615089
## 3036A  0.4395745192  0.20615216
## 3037A -0.2723518847  0.11905957
## 3039A  0.0254193862 -0.09057678
## 3042A -0.0598141462 -0.11441495
## 3043A -0.1859450415  0.01279322
## 3044A  0.3864154615  0.30177975
## 3046A -0.0007573109 -0.29986991
## 3048A  0.2217916349 -0.10262622
## 3049A  0.0642348566 -0.16979544
##
## $centroids
##                PCoA1       PCoA2
## sensitive -0.06823887 -0.07139907
## tolerant   0.03122648  0.03500215
##
## attr(,"class")
## [1] "ordiplot"
```

```
prepDataFrame <- as.data.frame(g$sites)
prepDataFrame$Fsen <- groups
prepDataFrame$PCoA1_centroids <- ifelse(prepDataFrame$Fsen == "sensitive", -0.06823887,
0.03122648)
prepDataFrame$PCoA2_centroids <- ifelse(prepDataFrame$Fsen == "sensitive", -0.07139907,
0.03500215)
prepDataFrame
```

```
##               PCoA1       PCoA2      Fsen PCoA1_centroids PCoA2_centroids
## 3003A  0.0441677429  0.38283619  tolerant      0.03122648      0.03500215
## 3004A  0.0828625107 -0.09278056 sensitive     -0.06823887     -0.07139907
## 3005A  0.0379890702 -0.20157629  tolerant      0.03122648      0.03500215
## 3007A -0.3759037336  0.24310633 sensitive     -0.06823887     -0.07139907
## 3008A -0.1383976477 -0.07998188 sensitive     -0.06823887     -0.07139907
## 3009A  0.3738847502  0.08029102  tolerant      0.03122648      0.03500215
## 3012A -0.3354912089  0.18561258 sensitive     -0.06823887     -0.07139907
## 3013A  0.2790018697  0.22083642  tolerant      0.03122648      0.03500215
## 3014A  0.1259877419 -0.29315664 sensitive     -0.06823887     -0.07139907
## 3016A -0.3012640720  0.03467458 sensitive     -0.06823887     -0.07139907
## 3020A -0.1193846188  0.34179879  tolerant      0.03122648      0.03500215
## 3021A -0.1011613674 -0.01159111 sensitive     -0.06823887     -0.07139907
## 3022A  0.1736487599  0.07555590 sensitive     -0.06823887     -0.07139907
## 3023A -0.2844635558  0.11542452  tolerant      0.03122648      0.03500215
## 3025A -0.0530972414 -0.21449097 sensitive     -0.06823887     -0.07139907
## 3026A  0.0990924366 -0.11741128 sensitive     -0.06823887     -0.07139907
## 3027A  0.1019807428  0.08492518  tolerant      0.03122648      0.03500215
## 3028A -0.0744293570 -0.14057275  tolerant      0.03122648      0.03500215
## 3029A -0.0085005473 -0.20671649 sensitive     -0.06823887     -0.07139907
## 3030A -0.1207205523 -0.14203642  tolerant      0.03122648      0.03500215
## 3031A -0.0177008476  0.01135208  tolerant      0.03122648      0.03500215
## 3032A  0.0785315462 -0.22475149  tolerant      0.03122648      0.03500215
## 3033A -0.0851998964  0.08615089  tolerant      0.03122648      0.03500215
## 3036A  0.4395745192  0.20615216  tolerant      0.03122648      0.03500215
## 3037A -0.2723518847  0.11905957  tolerant      0.03122648      0.03500215
## 3039A  0.0254193862 -0.09057678  tolerant      0.03122648      0.03500215
## 3042A -0.0598141462 -0.11441495  tolerant      0.03122648      0.03500215
## 3043A -0.1859450415  0.01279322  tolerant      0.03122648      0.03500215
## 3044A  0.3864154615  0.30177975  tolerant      0.03122648      0.03500215
## 3046A -0.0007573109 -0.29986991 sensitive     -0.06823887     -0.07139907
## 3048A  0.2217916349 -0.10262622  tolerant      0.03122648      0.03500215
## 3049A  0.0642348566 -0.16979544  tolerant      0.03122648      0.03500215
```

```
write.csv(prepDataFrame, "beta_div_df_A.csv")
library(readr)
# a dataset edited from NMDSNetworkData.csv
library(ggplot2)
ggplot(prepDataFrame, aes(x = PCoA1, y = PCoA2, xend = PCoA1_centroids, yend = PCoA2_cen
troids, color = Fsen), axes=TRUE) +
  geom_point(aes(color = Fsen)) +
  geom_edges(aes(color = Fsen)) +
  geom_nodes(aes(color = Fsen)) +
  xlab("NMDS1") +
  ylab("NMDS2")+
  stat_ellipse(type = "norm", linetype = 2) +
  theme_classic()
```




```
ggsave(file="beta_div_A.pdf", width=10, height=8, dpi=300)
```

4. B

4.1 data loading and cleaning

```
otu_mat<- read_excel("/Users/chenlianfu/Documents/GitHub/IBS_FructanSensitivity/diversit
yAnalysis/source/dietSubsets/speciesOTUTable_B_110422.xlsx") # CORRECT
row.names(otu_mat) <- otu_mat$otu
otu_mat <- as.matrix(otu_mat)
otu_mat_t = t(otu_mat)
otu_mat_t <- otu_mat_t[-1,]
class(otu_mat_t)<-"numeric"
```

```
samples_df <- read_excel("/Users/chenlianfu/Documents/GitHub/IBS_FructanSensitivity/dive
rsityAnalysis/source/dietSubsets/brunoMetadata_B_110422.xlsx") # CORRECT
groups<-as.list(samples_df['FsenText'])$FsenText
groups
```

```
##  [1] "tolerant"  "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"
##  [7] "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"  "sensitive"
## [13] "sensitive" "tolerant"  "sensitive" "sensitive" "tolerant"  "tolerant"
## [19] "sensitive" "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"
## [25] "tolerant"  "tolerant"  "tolerant"  "tolerant"  "tolerant"  "sensitive"
## [31] "sensitive" "tolerant"  "tolerant"
```

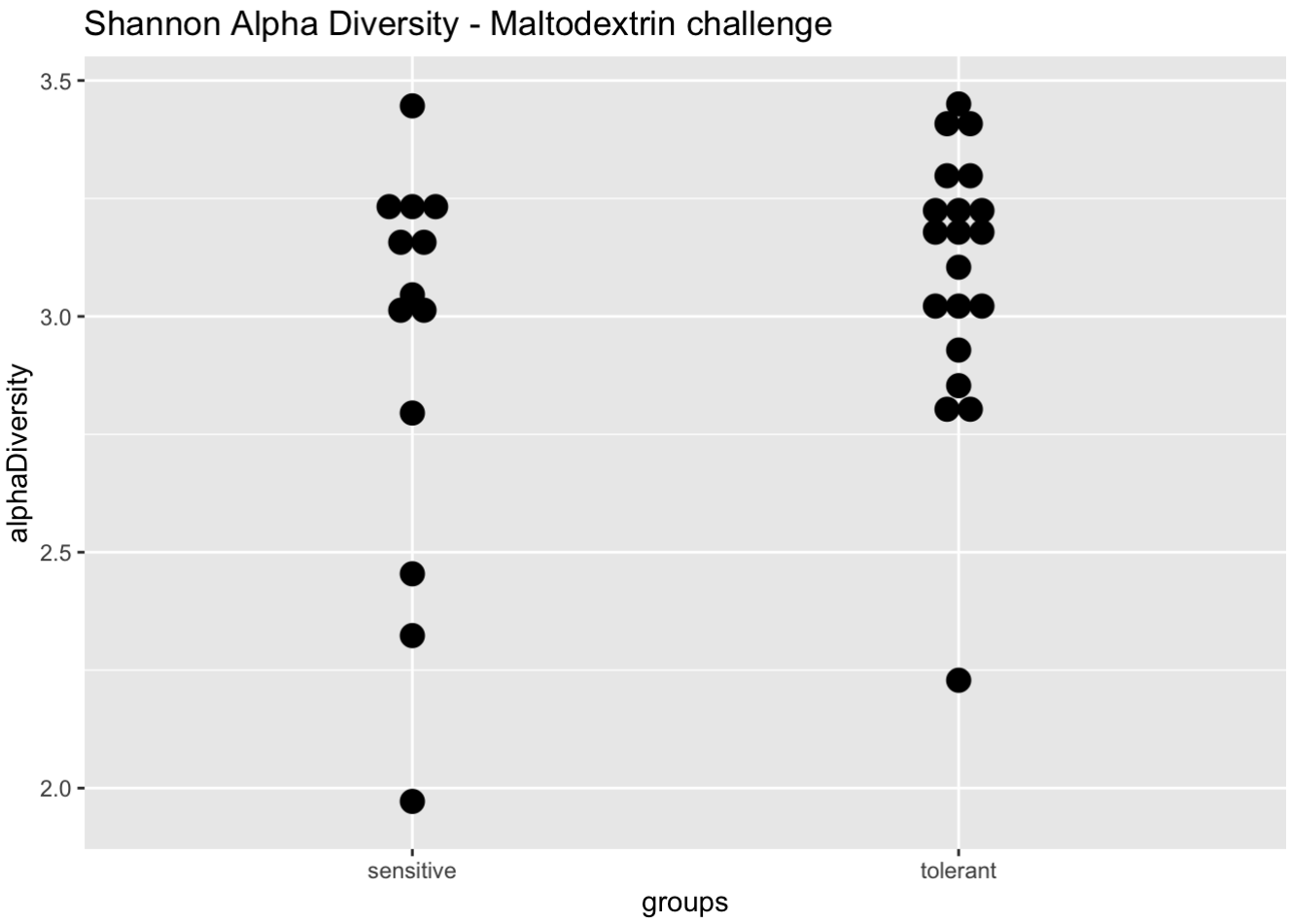
4.2 alpha diversity

```
alphaDiversity <- diversity(otu_mat_t, index="shannon")
summary(aov(alphaDiversity ~ groups))
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## groups       1  0.205  0.2048   1.709  0.201
## Residuals   31  3.716  0.1199
```

```
alphaDataframe <- data.frame(alphaDiversity, groups)
write.csv(alphaDataframe, "alph_div_df_B.csv")
p<-ggplot(alphaDataframe, aes(x=groups, y=alphaDiversity)) +
  geom_dotplot(binaxis='y', stackdir='center')+
  ggtitle("Shannon Alpha Diversity - Maltodextrin challenge")
p
```

```
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth
`.
```

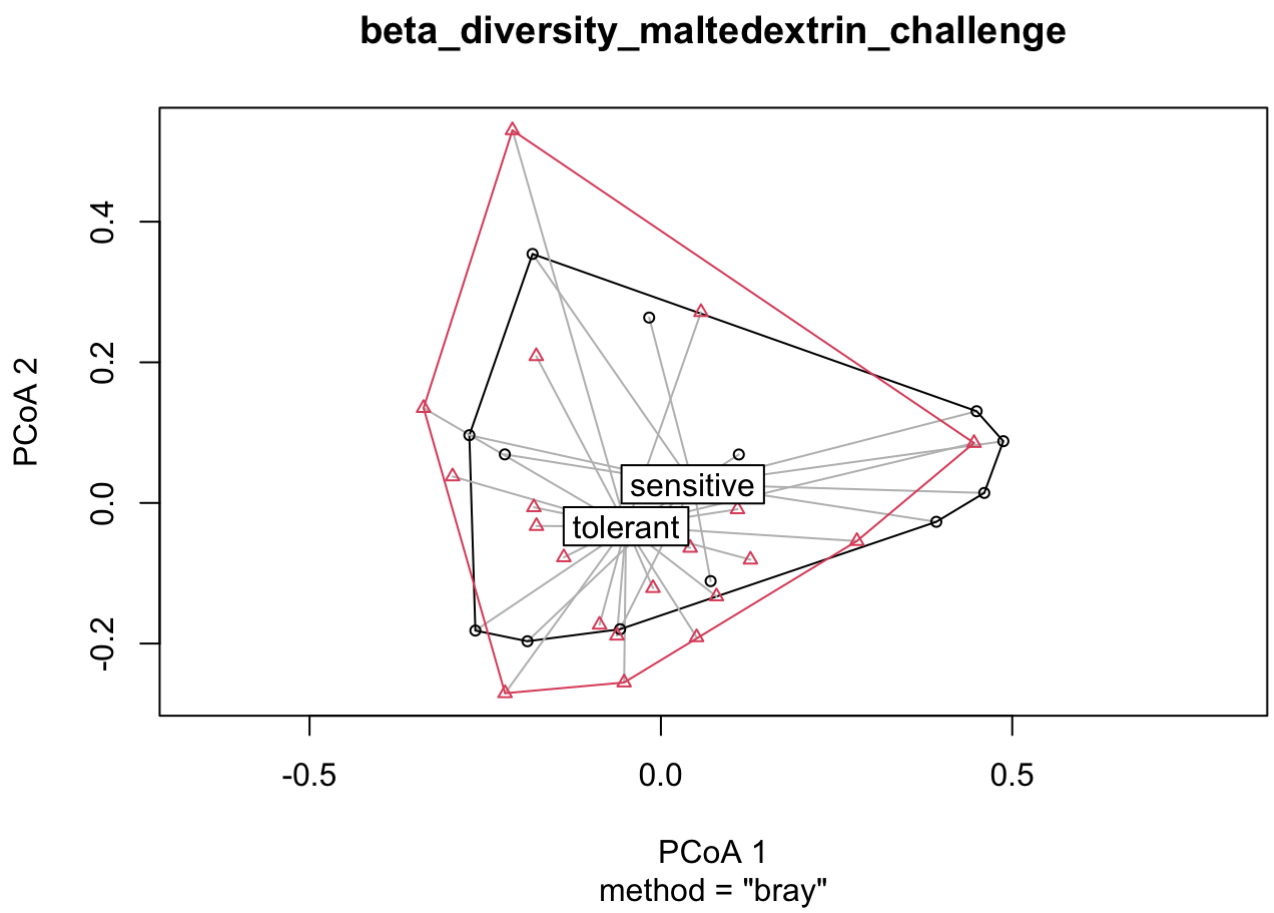



```
ggsave(file="alpha_div_B.pdf", width=10, height=8, dpi=300)
```

```
## Bin width defaults to 1/30 of the range of the data. Pick better value with `binwidth
`.
```

4.2 beta diversity

```
dist <- vegdist(otu_mat_t, method = "bray")
beta_diversity_maltedextrin_challenge<-betadisper(dist,groups)
g <- plot(beta_diversity_maltedextrin_challenge)
```




```
dev.off
```

```
## function (which = dev.cur())
## {
##     if (which == 1)
##         stop("cannot shut down device 1 (the null device)")
##     .External(C_devoff, as.integer(which))
##     dev.cur()
## }
## <bytecode: 0x7f8b99cafbe8>
## <environment: namespace:grDevices>
```

```
# ggsave(file="beta_div_B.pdf", width=10, height=8, dpi=300)
anova(beta_diversity_maltedextrin_challenge)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value Pr(>F)
## Groups     1 0.001532 0.0015322  0.2712 0.6062
## Residuals 31 0.175136 0.0056495
```

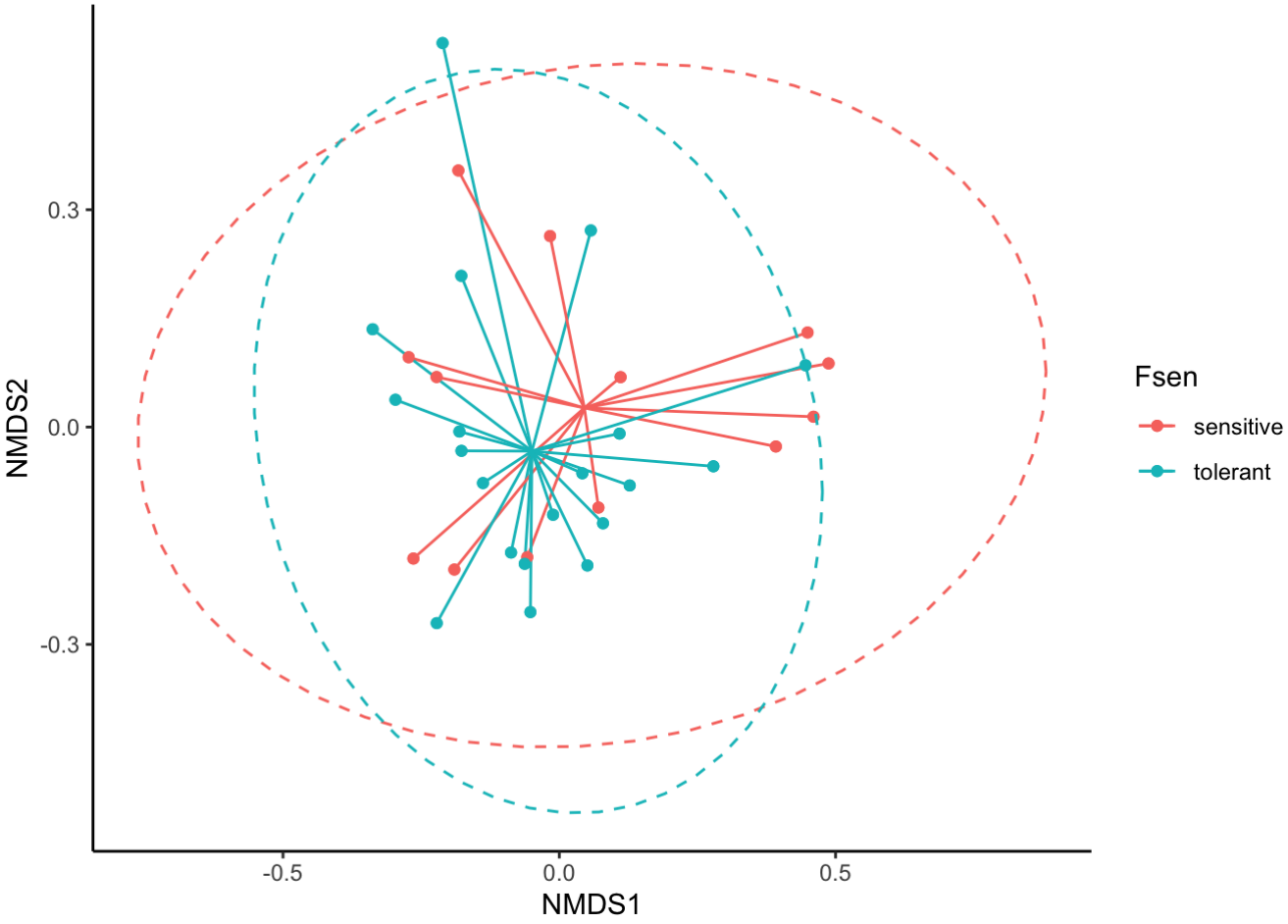
```
g
```

```
## $sites
##             PCoA1        PCoA2
## 3003B -0.08733821 -0.173222333
## 3004B -0.18993246 -0.196702847
## 3005B  0.07910034 -0.132872041
## 3007B  0.46019004  0.014244311
## 3008B -0.26403615 -0.181499463
## 3009B -0.21126392  0.530018157
## 3012B  0.48733934  0.087823518
## 3013B -0.17735839  0.208717224
## 3014B -0.27260492  0.096391525
## 3016B  0.39195588 -0.026829965
## 3020B  0.12745157 -0.080654528
## 3021B  0.07081405 -0.111158123
## 3022B -0.05802723 -0.179591617
## 3023B  0.44563164  0.085265541
## 3025B -0.22210600  0.069081959
## 3026B  0.11092528  0.068925851
## 3027B -0.17704762 -0.032816227
## 3028B -0.29646655  0.037732560
## 3029B -0.18266949  0.354129866
## 3030B  0.27892574 -0.054185874
## 3031B  0.05084523 -0.191064095
## 3032B  0.04167573 -0.063838746
## 3033B  0.05710156  0.271372567
## 3036B -0.33775822  0.135001634
## 3037B  0.10904056 -0.009033567
## 3039B -0.13816327 -0.077334095
## 3042B -0.05233209 -0.255374026
## 3043B -0.06259435 -0.188746235
## 3044B -0.18081621 -0.006173986
## 3046B  0.44932490  0.130474310
## 3047B -0.01670551  0.263649343
## 3048B -0.22181849 -0.270641239
## 3049B -0.01128278 -0.121089358
##
## $centroids
##                PCoA1       PCoA2
## sensitive  0.04535485  0.02646003
## tolerant  -0.04962727 -0.03320186
##
## attr(,"class")
## [1] "ordiplot"
```

```
prepDataFrame <- as.data.frame(g$sites)
prepDataFrame$Fsen <- groups
prepDataFrame$PCoA1_centroids <- ifelse(prepDataFrame$Fsen == "sensitive", 0.04535485, -
0.04962727)
prepDataFrame$PCoA2_centroids <- ifelse(prepDataFrame$Fsen == "sensitive", 0.02646003, -
0.03320186)
prepDataFrame
```

```
##             PCoA1        PCoA2      Fsen PCoA1_centroids PCoA2_centroids
## 3003B -0.08733821 -0.173222333  tolerant     -0.04962727     -0.03320186
## 3004B -0.18993246 -0.196702847 sensitive      0.04535485      0.02646003
## 3005B  0.07910034 -0.132872041  tolerant     -0.04962727     -0.03320186
## 3007B  0.46019004  0.014244311 sensitive      0.04535485      0.02646003
## 3008B -0.26403615 -0.181499463 sensitive      0.04535485      0.02646003
## 3009B -0.21126392  0.530018157  tolerant     -0.04962727     -0.03320186
## 3012B  0.48733934  0.087823518 sensitive      0.04535485      0.02646003
## 3013B -0.17735839  0.208717224  tolerant     -0.04962727     -0.03320186
## 3014B -0.27260492  0.096391525 sensitive      0.04535485      0.02646003
## 3016B  0.39195588 -0.026829965 sensitive      0.04535485      0.02646003
## 3020B  0.12745157 -0.080654528  tolerant     -0.04962727     -0.03320186
## 3021B  0.07081405 -0.111158123 sensitive      0.04535485      0.02646003
## 3022B -0.05802723 -0.179591617 sensitive      0.04535485      0.02646003
## 3023B  0.44563164  0.085265541  tolerant     -0.04962727     -0.03320186
## 3025B -0.22210600  0.069081959 sensitive      0.04535485      0.02646003
## 3026B  0.11092528  0.068925851 sensitive      0.04535485      0.02646003
## 3027B -0.17704762 -0.032816227  tolerant     -0.04962727     -0.03320186
## 3028B -0.29646655  0.037732560  tolerant     -0.04962727     -0.03320186
## 3029B -0.18266949  0.354129866 sensitive      0.04535485      0.02646003
## 3030B  0.27892574 -0.054185874  tolerant     -0.04962727     -0.03320186
## 3031B  0.05084523 -0.191064095  tolerant     -0.04962727     -0.03320186
## 3032B  0.04167573 -0.063838746  tolerant     -0.04962727     -0.03320186
## 3033B  0.05710156  0.271372567  tolerant     -0.04962727     -0.03320186
## 3036B -0.33775822  0.135001634  tolerant     -0.04962727     -0.03320186
## 3037B  0.10904056 -0.009033567  tolerant     -0.04962727     -0.03320186
## 3039B -0.13816327 -0.077334095  tolerant     -0.04962727     -0.03320186
## 3042B -0.05233209 -0.255374026  tolerant     -0.04962727     -0.03320186
## 3043B -0.06259435 -0.188746235  tolerant     -0.04962727     -0.03320186
## 3044B -0.18081621 -0.006173986  tolerant     -0.04962727     -0.03320186
## 3046B  0.44932490  0.130474310 sensitive      0.04535485      0.02646003
## 3047B -0.01670551  0.263649343 sensitive      0.04535485      0.02646003
## 3048B -0.22181849 -0.270641239  tolerant     -0.04962727     -0.03320186
## 3049B -0.01128278 -0.121089358  tolerant     -0.04962727     -0.03320186
```

```
write.csv(prepDataFrame, "beta_div_df_B.csv")
library(readr)
# a dataset edited from NMDSNetworkData.csv
library(ggplot2)
ggplot(prepDataFrame, aes(x = PCoA1, y = PCoA2, xend = PCoA1_centroids, yend = PCoA2_cen
troids, color = Fsen), axes=TRUE) +
  geom_point(aes(color = Fsen)) +
  geom_edges(aes(color = Fsen)) +
  geom_nodes(aes(color = Fsen)) +
  xlab("NMDS1") +
  ylab("NMDS2")+
  stat_ellipse(type = "norm", linetype = 2) +
  theme_classic()
```




```
ggsave(file="beta_div_B.pdf", width=10, height=8, dpi=300)
```