

Social status effects on zebrafish gut microbiomes

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Project Description: Fill out

Initial Setup

#Import Files ## Environmental Data

Bacterial Data

Diversity Metrics - Hypothesis Testing

```
#install.packages("Rarefy")
#install.packages("rrarefy")
```

```
# Rarefy Abundances (min abundance is 10181. We are sampling to 10181)
min(rowSums(otu_final))
```

```
## [1] 10181
```

```
otus.r <- rrarefy(otu_final, 10181)

# Fisher's Alpha
fisher <- fisher.alpha(otus.r)

# Species Richness
#richness <- rowSums((PWESdata.r >= 1))
richness <- rowSums((otu_final >= 1))

# Shannon Diversity
shannon <- diversity(otus.r, "shannon")

# Simpson's Evenness
simp.even <- apply(otus.r, 1, simp_even)

#Pielou's evenness
J <- shannon/log(specnumber(otus.r[, -c(1:1)]))
```

```
#combined richness, diversity, evenness
diversity <- cbind(design_final,richness,shannon,simp.even,J)
#write.csv(diversity,"../Desktop/ES20_ZebrafishMicrobiomes/data/zf_diversity.csv")
```

Diversity Metrics - Hypothesis Testing - by species

```
#summary table for bacterial diversity
summary <- diversity %>% group_by(Social.Status, Day) %>% summarise(mean.richness=mean(richness), se.r
```

```
## 'summarise()' has grouped output by 'Social.Status'. You can override using the
## '.groups' argument.
```

```
print(summary)
```

```
## # A tibble: 16 x 6
## # Groups:   Social.Status [4]
##   Social.Status Day    mean.richness se.richness mean.shannon se.shannon
##   <fct>         <fct>         <dbl>         <dbl>         <dbl>         <dbl>
## 1 Communal     Day_0             278.           30.3           1.70          0.0428
## 2 Communal     Day_14            147.           22.3           1.75          0.0803
## 3 Communal     Day_7             305.           45.1           1.03          0.211
## 4 Communal     Day_IP            169.           25.8           1.44          0.101
## 5 Dominant     Day_0             368.          187.           1.12          0.0697
## 6 Dominant     Day_14            200.          107.           1.26          0.259
## 7 Dominant     Day_7             298.           75.8           1.82          0.478
## 8 Dominant     Day_IP            375.           75.7           1.52          0.106
## 9 Isolate      Day_0             251.           32.9           1.63          0.184
## 10 Isolate     Day_14            172.           15.7           1.24          0.230
## 11 Isolate     Day_7             265.           26.0           1.57          0.233
## 12 Isolate     Day_IP            172.           34.9           1.34          0.175
## 13 Subordinate Day_0             242.           48.3           1.62          0.127
## 14 Subordinate Day_14            108.           17.2           1.70          0.0907
## 15 Subordinate Day_7             177.           54.7           1.02          0.266
## 16 Subordinate Day_IP            506.          136.           1.81          0.226
```

```
#write.csv(summary,"../Desktop/ES20_ZebrafishMicrobiomes/data/diversity.bact.summary.csv")
```

```
library(lmerTest)
```

```
## Loading required package: lme4
## Loading required package: Matrix
##
## Attaching package: 'Matrix'
##
## The following objects are masked from 'package:tidyr':
##
##   expand, pack, unpack
##
## The following object is masked from 'package:reshape':
```

```
##
## expand
##
##
## Attaching package: 'lme4'
##
## The following object is masked from 'package:labdsv':
##
## factorize
##
## The following object is masked from 'package:nlme':
##
## lmList
##
##
## Attaching package: 'lmerTest'
##
## The following object is masked from 'package:lme4':
##
## lmer
##
## The following object is masked from 'package:stats':
##
## step
```

```
richness.lm <- lm(richness ~ Social.Status*Day, data = diversity)
richness.lm
```

```
##
## Call:
## lm(formula = richness ~ Social.Status * Day, data = diversity)
##
## Coefficients:
##              (Intercept)              Social.StatusDominant
##                278.500                89.500
##      Social.StatusIsolate      Social.StatusSubordinate
##               -27.700               -36.000
##      DayDay_14      DayDay_7
##       -131.333        26.667
##      DayDay_IP      Social.StatusDominant:DayDay_14
##       -109.167        -36.833
##      Social.StatusIsolate:DayDay_14 Social.StatusSubordinate:DayDay_14
##                52.733          -3.567
##      Social.StatusDominant:DayDay_7      Social.StatusIsolate:DayDay_7
##               -96.333               -12.633
##      Social.StatusSubordinate:DayDay_7      Social.StatusDominant:DayDay_IP
##               -92.333               116.000
##      Social.StatusIsolate:DayDay_IP      Social.StatusSubordinate:DayDay_IP
##                30.367               372.167
```

```
summary(richness.lm)
```

```
##
```

```
## Call:
## lm(formula = richness ~ Social.Status * Day, data = diversity)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -298.00  -84.83  -29.33   62.17  912.00
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      278.500      75.952   3.667  0.00045 ***
## Social.StatusDominant      89.500     107.412   0.833  0.40729
## Social.StatusIsolate     -27.700     112.655  -0.246  0.80643
## Social.StatusSubordinate   -36.000     107.412  -0.335  0.73842
## DayDay_14     -131.333     107.412  -1.223  0.22517
## DayDay_7       26.667     107.412   0.248  0.80459
## DayDay_IP    -109.167     107.412  -1.016  0.31265
## Social.StatusDominant:DayDay_14   -36.833     151.904  -0.242  0.80905
## Social.StatusIsolate:DayDay_14    52.733     159.318   0.331  0.74155
## Social.StatusSubordinate:DayDay_14 -3.567     155.655  -0.023  0.98178
## Social.StatusDominant:DayDay_7   -96.333     151.904  -0.634  0.52785
## Social.StatusIsolate:DayDay_7   -12.633     155.655  -0.081  0.93552
## Social.StatusSubordinate:DayDay_7 -92.333     151.904  -0.608  0.54508
## Social.StatusDominant:DayDay_IP   116.000     151.904   0.764  0.44741
## Social.StatusIsolate:DayDay_IP    30.367     155.655   0.195  0.84584
## Social.StatusSubordinate:DayDay_IP 372.167     151.904   2.450  0.01656 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 186 on 77 degrees of freedom
## Multiple R-squared:  0.2573, Adjusted R-squared:  0.1127
## F-statistic: 1.779 on 15 and 77 DF, p-value: 0.05323
```

```
evenness.lm <- lm(simp.even ~ Social.Status*Day, data = diversity)
evenness.lm
```

```
##
## Call:
## lm(formula = simp.even ~ Social.Status * Day, data = diversity)
##
## Coefficients:
##              (Intercept)              Social.StatusDominant
##              0.044361                -0.003681
##              Social.StatusIsolate              Social.StatusSubordinate
##              -0.003000                -0.001957
##              DayDay_14              DayDay_7
##              0.032202                -0.019648
##              DayDay_IP              Social.StatusDominant:DayDay_14
##              0.017335                -0.026437
##              Social.StatusIsolate:DayDay_14 Social.StatusSubordinate:DayDay_14
##              -0.044706                0.021697
##              Social.StatusDominant:DayDay_7              Social.StatusIsolate:DayDay_7
##              0.003857                0.009322
##              Social.StatusSubordinate:DayDay_7              Social.StatusDominant:DayDay_IP
##              0.024884                -0.030436
```

```
##      Social.StatusIsolate:DayDay_IP  Social.StatusSubordinate:DayDay_IP
##                                -0.011441                                -0.040437
```

```
summary(evenness.lm)
```

```
##
## Call:
## lm(formula = simp.even ~ Social.Status * Day, data = diversity)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.045893 -0.014853 -0.006465  0.008651  0.102099
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      0.044361   0.011947   3.713 0.000385 ***
## Social.StatusDominant -0.003681   0.016896  -0.218 0.828100
## Social.StatusIsolate -0.003000   0.017721  -0.169 0.865997
## Social.StatusSubordinate -0.001957   0.016896  -0.116 0.908096
## DayDay_14         0.032202   0.016896   1.906 0.060398 .
## DayDay_7        -0.019648   0.016896  -1.163 0.248477
## DayDay_IP         0.017335   0.016896   1.026 0.308109
## Social.StatusDominant:DayDay_14 -0.026437   0.023894  -1.106 0.271992
## Social.StatusIsolate:DayDay_14 -0.044706   0.025061  -1.784 0.078375 .
## Social.StatusSubordinate:DayDay_14 0.021697   0.024485   0.886 0.378288
## Social.StatusDominant:DayDay_7  0.003857   0.023894   0.161 0.872180
## Social.StatusIsolate:DayDay_7  0.009322   0.024485   0.381 0.704449
## Social.StatusSubordinate:DayDay_7 0.024884   0.023894   1.041 0.300941
## Social.StatusDominant:DayDay_IP -0.030436   0.023894  -1.274 0.206573
## Social.StatusIsolate:DayDay_IP -0.011441   0.024485  -0.467 0.641623
## Social.StatusSubordinate:DayDay_IP -0.040437   0.023894  -1.692 0.094627 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02926 on 77 degrees of freedom
## Multiple R-squared:  0.3405, Adjusted R-squared:  0.212
## F-statistic:  2.65 on 15 and 77 DF, p-value: 0.002789
```

```
shannon.lm <- lm(shannon ~ Social.Status*Day, data = diversity)
shannon.lm
```

```
##
## Call:
## lm(formula = shannon ~ Social.Status * Day, data = diversity)
##
## Coefficients:
##              (Intercept)              Social.StatusDominant
##              1.70363                  -0.58023
##      Social.StatusIsolate      Social.StatusSubordinate
##      -0.06944                  -0.08501
##      DayDay_14              DayDay_7
##      0.04176                 -0.67044
##      DayDay_IP      Social.StatusDominant:DayDay_14
```

```
##           -0.26168           0.09894
## Social.StatusIsolate:DayDay_14 Social.StatusSubordinate:DayDay_14
##           -0.43228           0.04259
## Social.StatusDominant:DayDay_7 Social.StatusIsolate:DayDay_7
##           1.36698           0.60643
## Social.StatusSubordinate:DayDay_7 Social.StatusDominant:DayDay_IP
##           0.07439           0.65391
## Social.StatusIsolate:DayDay_IP Social.StatusSubordinate:DayDay_IP
##           -0.03331           0.45777
```

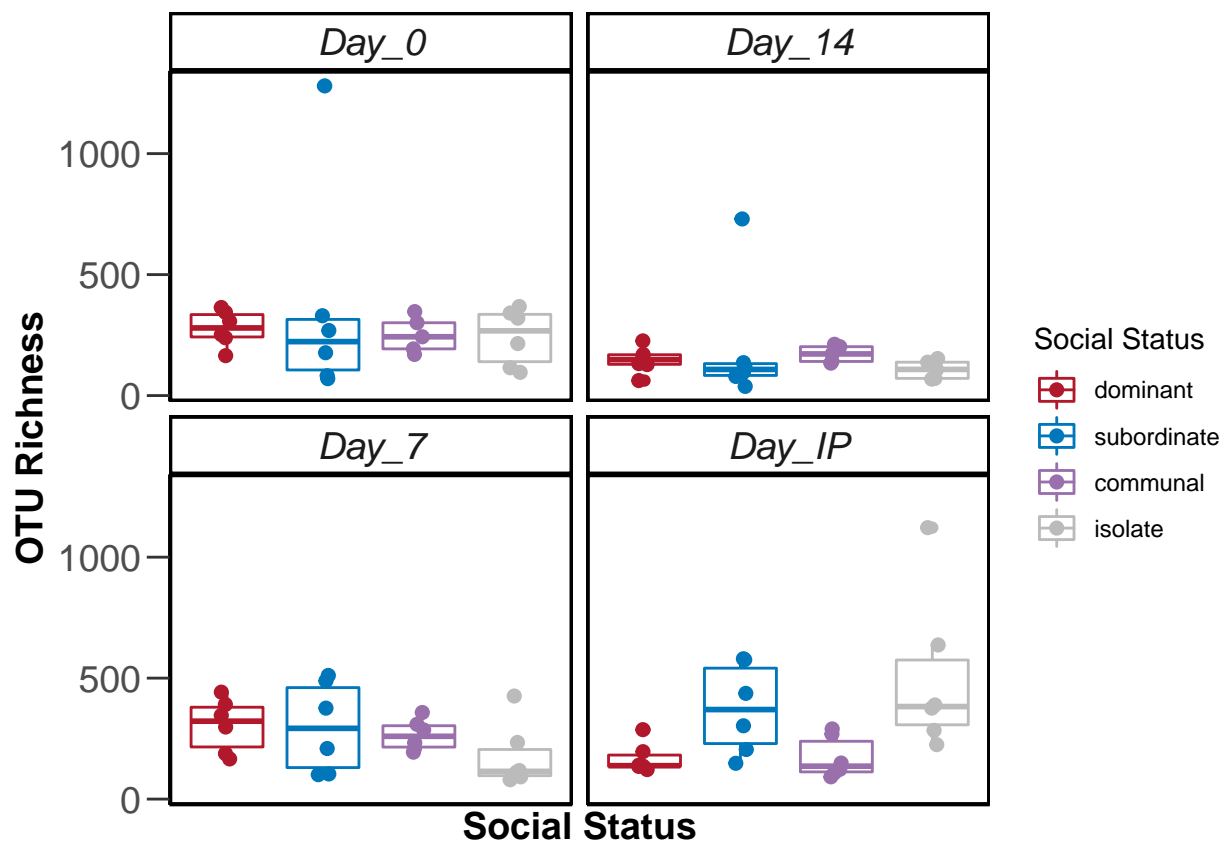
```
summary(shannon.lm)
```

```
##
## Call:
## lm(formula = shannon ~ Social.Status * Day, data = diversity)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.55581 -0.27010  0.05623  0.25156  1.25331
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      1.70363    0.20730   8.218 3.87e-12 ***
## Social.StatusDominant      -0.58023    0.29317  -1.979  0.05138 .
## Social.StatusIsolate      -0.06944    0.30748  -0.226  0.82192
## Social.StatusSubordinate    -0.08501    0.29317  -0.290  0.77261
## DayDay_14           0.04176    0.29317   0.142  0.88711
## DayDay_7           -0.67044    0.29317  -2.287  0.02495 *
## DayDay_IP          -0.26168    0.29317  -0.893  0.37485
## Social.StatusDominant:DayDay_14  0.09894    0.41461   0.239  0.81203
## Social.StatusIsolate:DayDay_14 -0.43228    0.43484  -0.994  0.32328
## Social.StatusSubordinate:DayDay_14 0.04259    0.42484   0.100  0.92040
## Social.StatusDominant:DayDay_7  1.36698    0.41461   3.297  0.00148 **
## Social.StatusIsolate:DayDay_7  0.60643    0.42484   1.427  0.15750
## Social.StatusSubordinate:DayDay_7 0.07439    0.41461   0.179  0.85809
## Social.StatusDominant:DayDay_IP  0.65391    0.41461   1.577  0.11885
## Social.StatusIsolate:DayDay_IP -0.03331    0.42484  -0.078  0.93770
## Social.StatusSubordinate:DayDay_IP 0.45777    0.41461   1.104  0.27299
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.5078 on 77 degrees of freedom
## Multiple R-squared:  0.2474, Adjusted R-squared:  0.1008
## F-statistic: 1.687 on 15 and 77 DF, p-value: 0.07121
```

```
#Plot Richness
```

```
# Graphing Richness
p <- ggplot(diversity, aes(x=Social.Status, y=richness, color=as.factor(Social.Status)))+
  geom_boxplot() +
  geom_point(aes(color=factor(Social.Status)), size=2, position = position_jitterdodge()) +
  scale_color_manual(name="Social Status", values=c("#B2182B", "#0077BB", "#9970AB", "#BBBBBB"),
    labels = c("dominant", "subordinate", "communal", "isolate")) + facet_wrap(~Day)
```

```
richness <- p + theme_bw() +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line
    =element_line(colour = "black")) +
  theme(axis.title=element_text(vjust=1,size=14,face="bold"),
    axis.text=element_text(size=14), axis.text.x = element_text(vjust=0.65, hjust=0.5,
    size=14), panel.border = element_rect(colour = "black",size=1)) +
  theme(axis.ticks.length=unit(0.3,"cm")) + labs(x = "Social Status", y = "OTU Richness") +
  theme(strip.text.x = element_text(size=14, face="italic"), strip.text.y =
    element_text(size=14, face="bold"), strip.background = element_rect(colour="black",
    fill="white", size=1)) +
  scale_x_discrete(breaks=c("dominant", "subordinate", "communal", "isolate"),
    labels=c("dominant", "subordinate", "communal", "isolate"))
richness
```



```
ggsave("../figures/richness.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5,
```

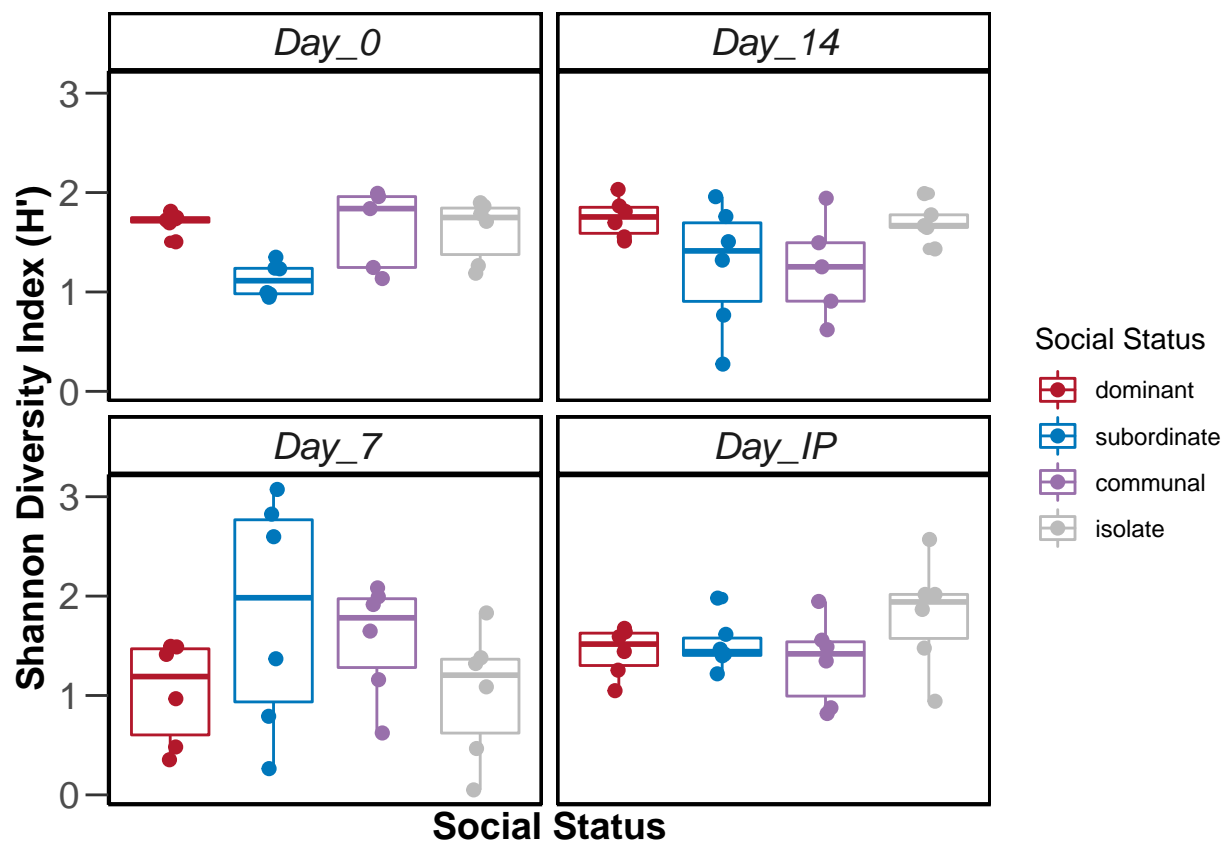
```
#Plot shannon diversity
```

```
# Graphing Shannon Diversity
p <- ggplot(diversity, aes(x=Social.Status, y=shannon, color=as.factor(Social.Status)))+
  geom_boxplot() +
  geom_point(aes(color=factor(Social.Status)), size=2, position = position_jitterdodge()) +
  scale_color_manual(name="Social Status", values=c("#B2182B", "#0077BB", "#9970AB", "#BBBBBB"),
    labels = c("dominant", "subordinate", "communal", "isolate")) + facet_wrap(~Day)
```

```

shannon <- p + theme_bw() +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line
        =element_line(colour = "black")) +
  theme(axis.title=element_text(vjust=1,size=14,face="bold"),
        axis.text=element_text(size=14), axis.text.x = element_text(vjust=0.65, hjust=0.5,
        size=14), panel.border = element_rect(colour = "black",size=1)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  labs(x = "Social Status", y = "Shannon Diversity Index (H')") +
  theme(strip.text.x = element_text(size=14, face="italic"), strip.text.y =
        element_text(size=14, face="bold"), strip.background = element_rect(colour="black",
        fill="white", size=1)) +
  scale_x_discrete(breaks=c("dominant", "subordinate", "communal", "isolate", "water", "pos con
shannon

```



```

ggsave("../figures/shannon.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5, c

```

#Plot Evenness

```

# Graphing Simpson's Evenness
p <- ggplot(diversity, aes(x=Social.Status, y=simp.even, color=as.factor(Social.Status)))+
  geom_boxplot() +
  geom_point(aes(color=factor(Social.Status)), size=2, position = position_jitterdodge()) +
  scale_color_manual(name="Social Status", values=c("#B2182B", "#0077BB", "#9970AB", "#BBBBBB"),
        labels = c("dominant", "subordinate", "communal", "isolate")) + facet_wrap(~Day)

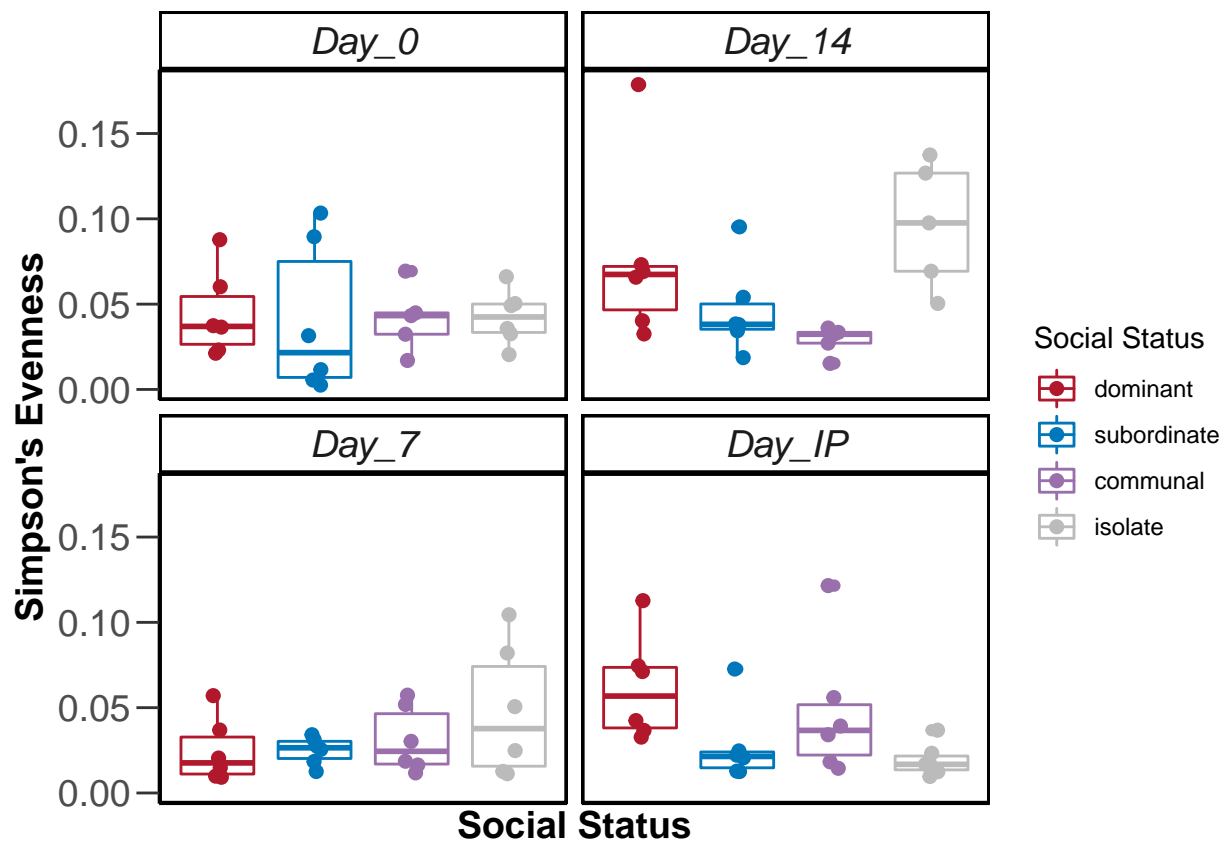
```



```

simpeven <- p + theme_bw() +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line
        =element_line(colour = "black")) +
  theme(axis.title=element_text(vjust=1,size=14,face="bold"),
        axis.text=element_text(size=14), axis.text.x = element_text(vjust=0.65, hjust=0.5,
        size=14), panel.border = element_rect(colour = "black",size=1)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  labs(x = "Social Status", y = "Simpson's Evenness") +
  theme(strip.text.x = element_text(size=14, face="italic"), strip.text.y =
        element_text(size=14, face="bold"), strip.background = element_rect(colour="black",
        fill="white", size=1)) +
  scale_x_discrete(breaks=c("dominant", "subordinate", "communal", "isolate", "water", "pos con
simpeven

```



```

ggsave("../figures/simpeven.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=5,

```

Community Composition Analyses

```

# Make Relative Abundance Matrices
dataREL <- otu_final
for(i in 1:dim(otu_final)[1]){
  dataREL[i,] <- otu_final[i,]/sum(otu_final[i,])

```

```

}

#PERMANOVA
new.data <- cbind(design_final, dataREL)
adonis = adonis2(new.data[, -c(1:3)] ~ Social.Status * Day, method = "bray", data = new.data, perm=1000, set
adonis

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 1000
##
## adonis2(formula = new.data[, -c(1:3)] ~ Social.Status * Day, data = new.data, permutations = 1000, m
##
##          Df SumOfSqs      R2      F    Pr(>F)
## Social.Status      3    2.3013 0.09145 3.6706 0.000999 ***
## Day                3    3.1563 0.12544 5.0345 0.000999 ***
## Social.Status:Day   9    3.6138 0.14361 1.9214 0.002997 **
## Residual           77   16.0916 0.63949
## Total              92   25.1630 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Principal Coordinates Analysis
dataREL.dist <- vegdist(dataREL, method="bray")

pcoa <- cmdscale(dataREL.dist, k=3, eig=TRUE, add=FALSE)
# Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
# eig=TRUE returns eigenvalues; k = # of dimensions to calculate

explainvar1b <- round(pcoa$eig[1] / sum(pcoa$eig), 3) * 100
explainvar2b <- round(pcoa$eig[2] / sum(pcoa$eig), 3) * 100
sum.eigb <- sum(explainvar1b, explainvar2b)

explainvar1b #34.2

## [1] 34.2

explainvar2b #27.3

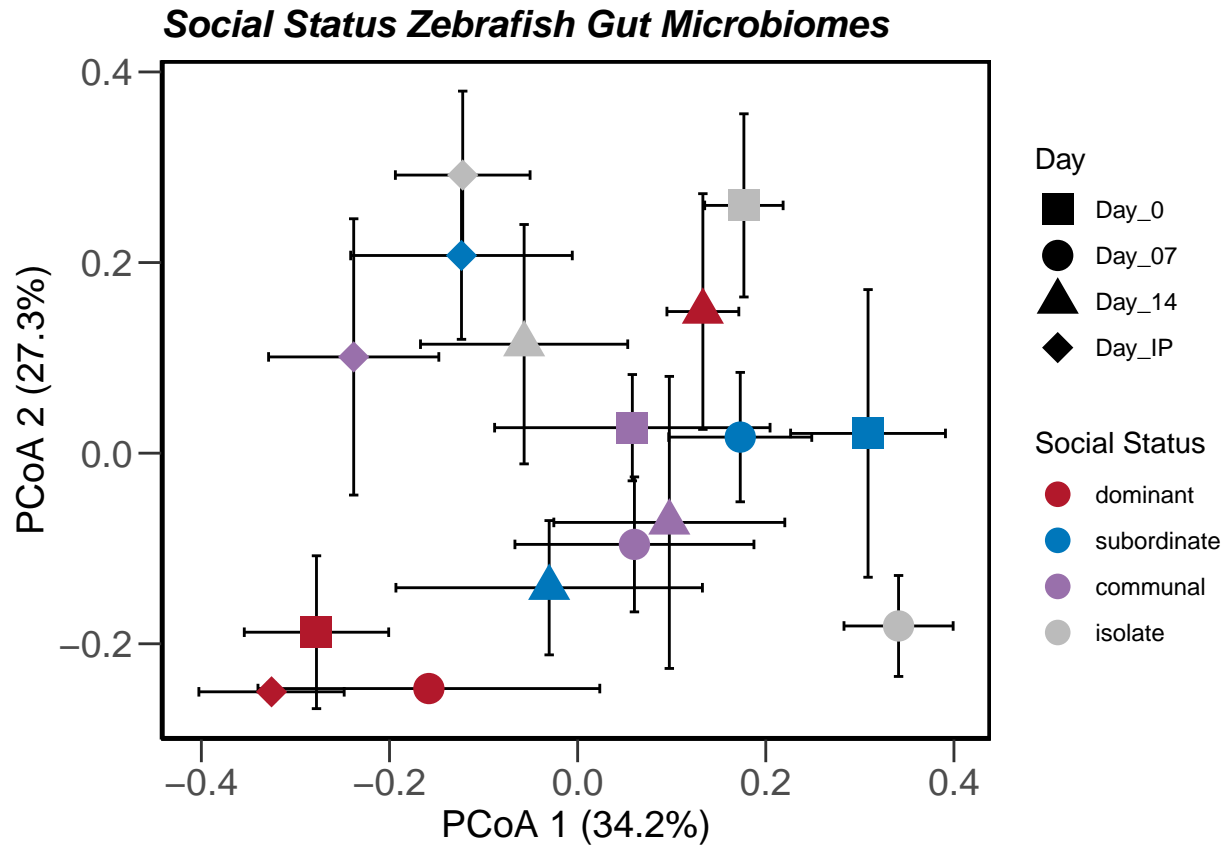
## [1] 27.3

pcoa.groups <- paste(new.data$Social.Status, new.data$Day, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)

# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable ~ group, sd)

# Combine

```

```
ggsave("../figures/zf_PCoAordination.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=7, height=7)
```

Bacterial community indicator species analysis - need to update