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)

# Chao 1 species estimator
bc_alpha_div <- estimateR(otus.r)

bc_alpha_div_df <- as.data.frame(bc_alpha_div)

bc_alpha_div_df_t <- t(bc_alpha_div_df)

bc_alpha_div_df_t <- as.data.frame(bc_alpha_div_df_t)

write.csv(bc_alpha_div_df_t,"../data/zf_bact_diversity.csv")

# Species Richness
richness <- rowSums((otu_final >= 1))
```

```r
# 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,bc_alpha_div_df_t)
write.csv(diversity,"../data/zf_diversity.csv")
```

Diversity Metrics - Hypothesis Testing - by species

```r
#summary table for bacterial diversity
#diversity <- read.csv("../data/zf_diversity.csv", row.names=1)

diversity$Social.Status <- as.factor(diversity$Social.Status)
diversity$Day <- as.factor(diversity$Day)
str(diversity)
```

```
## 'data.frame':    93 obs. of  12 variables:
##  $ Social.Status: Factor w/ 7 levels "1_Dominant","2_Subordinate",..: 3 3 3 3 3 3 3 3 3 3 ...
##  $ Pair         : chr  "Non_Pair" "Non_Pair" "Non_Pair" "Non_Pair" ...
##  $ Day          : Factor w/ 7 levels "Day_0","Day_1",..: 1 6 4 7 1 6 4 7 1 6 ...
##  $ richness     : num  165 298 170 136 344 188 165 196 307 166 ...
##  $ shannon      : num  1.81 1.48 1.86 1.58 1.69 ...
##  $ simp.even    : num  0.0779 0.047 0.0451 0.0764 0.0243 ...
##  $ J            : num  0.443 0.354 0.407 0.397 0.347 ...
##  $ S.obs        : num  61 66 97 54 130 66 68 62 103 51 ...
##  $ S.chao1      : num  140 148 132 116 219 ...
##  $ se.chao1     : num  38.3 36.9 15.1 32.7 30 ...
##  $ S.ACE        : num  168 227 151 168 251 ...
##  $ se.ACE       : num  6.97 9.86 7.09 9.64 10.27 ...
```

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

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

```r
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 1_Dominant    Day_0            368        187.         1.11     0.0719
```

```
##  2 1_Dominant    Day_14          200.       107.          1.26     0.261
##  3 1_Dominant    Day_7           298.        75.8         1.82     0.480
##  4 1_Dominant    Day_IP          375.        75.7         1.52     0.107
##  5 2_Subordinate Day_0           242.        48.3         1.63     0.121
##  6 2_Subordinate Day_14          108.        17.2         1.70     0.0875
##  7 2_Subordinate Day_7           177.        54.7         1.03     0.268
##  8 2_Subordinate Day_IP          506.       136.          1.82     0.222
##  9 3_Communal    Day_0           278.        30.3         1.70     0.0404
## 10 3_Communal    Day_14          147.        22.3         1.74     0.0834
## 11 3_Communal    Day_7           305.        45.1         1.02     0.212
## 12 3_Communal    Day_IP          169.        25.8         1.45     0.0980
## 13 4_Isolate     Day_0           251.        32.9         1.63     0.181
## 14 4_Isolate     Day_14          172.        15.7         1.25     0.227
## 15 4_Isolate     Day_7           265.        26.0         1.58     0.234
## 16 4_Isolate     Day_IP          172         34.9         1.34     0.172
```

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

library(emmeans)
```

```
##
## Attaching package: 'emmeans'
##
## The following object is masked from 'package:devtools':
##
##     test
```

```
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.Status2_Subordinate
##                                  368.000                              -125.500
##                 Social.Status3_Communal                 Social.Status4_Isolate
##                                  -89.500                              -117.200
##                               DayDay_14                              DayDay_7
##                                 -168.167                               -69.667
##                               DayDay_IP  Social.Status2_Subordinate:DayDay_14
##                                   6.833                                33.267
##    Social.Status3_Communal:DayDay_14      Social.Status4_Isolate:DayDay_14
##                                  36.833                                89.567
##  Social.Status2_Subordinate:DayDay_7      Social.Status3_Communal:DayDay_7
##                                   4.000                                96.333
##      Social.Status4_Isolate:DayDay_7  Social.Status2_Subordinate:DayDay_IP
##                                  83.700                               256.167
##    Social.Status3_Communal:DayDay_IP      Social.Status4_Isolate:DayDay_IP
##                                -116.000                               -85.633
```

```
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)                         368.000     75.952   4.845 6.41e-06 ***
## Social.Status2_Subordinate         -125.500    107.412  -1.168   0.2463
## Social.Status3_Communal             -89.500    107.412  -0.833   0.4073
## Social.Status4_Isolate             -117.200    112.655  -1.040   0.3014
## DayDay_14                          -168.167    107.412  -1.566   0.1215
```

```
## DayDay_7                                -69.667    107.412  -0.649   0.5185
## DayDay_IP                                 6.833    107.412   0.064   0.9494
## Social.Status2_Subordinate:DayDay_14     33.267    155.655   0.214   0.8313
## Social.Status3_Communal:DayDay_14        36.833    151.904   0.242   0.8091
## Social.Status4_Isolate:DayDay_14         89.567    159.318   0.562   0.5756
## Social.Status2_Subordinate:DayDay_7       4.000    151.904   0.026   0.9791
## Social.Status3_Communal:DayDay_7         96.333    151.904   0.634   0.5278
## Social.Status4_Isolate:DayDay_7          83.700    155.655   0.538   0.5923
## Social.Status2_Subordinate:DayDay_IP    256.167    151.904   1.686   0.0958 .
## Social.Status3_Communal:DayDay_IP      -116.000    151.904  -0.764   0.4474
## Social.Status4_Isolate:DayDay_IP        -85.633    155.655  -0.550   0.5838
## ---
## 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
```

```
chao1.lm <- lm(S.chao1 ~ Social.Status*Day, data = diversity)
chao1.lm
```

```
##
## Call:
## lm(formula = S.chao1 ~ Social.Status * Day, data = diversity)
##
## Coefficients:
##                             (Intercept)            Social.Status2_Subordinate
##                                 316.191                               -98.978
##                Social.Status3_Communal                Social.Status4_Isolate
##                                -100.604                              -113.133
##                              DayDay_14                              DayDay_7
##                                -131.129                               -22.865
##                              DayDay_IP  Social.Status2_Subordinate:DayDay_14
##                                  23.673                                -7.768
##    Social.Status3_Communal:DayDay_14      Social.Status4_Isolate:DayDay_14
##                                   3.489                                98.635
##  Social.Status2_Subordinate:DayDay_7      Social.Status3_Communal:DayDay_7
##                                 -54.637                                68.452
##      Social.Status4_Isolate:DayDay_7  Social.Status2_Subordinate:DayDay_IP
##                                  36.480                               207.580
##    Social.Status3_Communal:DayDay_IP      Social.Status4_Isolate:DayDay_IP
##                                -130.012                               -59.739
```

```
summary(chao1.lm)
```

```
##
## Call:
## lm(formula = S.chao1 ~ Social.Status * Day, data = diversity)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -277.94  -91.99  -25.07   60.14  745.49
##
```

```
## Coefficients:
##                                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)                         316.191     71.061   4.450 2.86e-05 ***
## Social.Status2_Subordinate          -98.978    100.496  -0.985    0.328
## Social.Status3_Communal            -100.604    100.496  -1.001    0.320
## Social.Status4_Isolate             -113.133    105.401  -1.073    0.286
## DayDay_14                          -131.129    100.496  -1.305    0.196
## DayDay_7                            -22.865    100.496  -0.228    0.821
## DayDay_IP                            23.673    100.496   0.236    0.814
## Social.Status2_Subordinate:DayDay_14  -7.768    145.632  -0.053    0.958
## Social.Status3_Communal:DayDay_14      3.489    142.122   0.025    0.980
## Social.Status4_Isolate:DayDay_14      98.635    149.059   0.662    0.510
## Social.Status2_Subordinate:DayDay_7  -54.637    142.122  -0.384    0.702
## Social.Status3_Communal:DayDay_7      68.452    142.122   0.482    0.631
## Social.Status4_Isolate:DayDay_7       36.480    145.632   0.250    0.803
## Social.Status2_Subordinate:DayDay_IP 207.580    142.122   1.461    0.148
## Social.Status3_Communal:DayDay_IP   -130.012    142.122  -0.915    0.363
## Social.Status4_Isolate:DayDay_IP     -59.739    145.632  -0.410    0.683
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 174.1 on 77 degrees of freedom
## Multiple R-squared:  0.2675, Adjusted R-squared:  0.1249
## F-statistic: 1.875 on 15 and 77 DF,  p-value: 0.03896
```

```
anova(chao1.lm)
```

```
## Analysis of Variance Table
##
## Response: S.chao1
##                   Df  Sum Sq Mean Sq F value  Pr(>F)
## Social.Status      3  181859   60620  2.0008 0.12086
## Day                3  237131   79044  2.6089 0.05752 .
## Social.Status:Day  9  433182   48131  1.5886 0.13359
## Residuals         77 2332959   30298
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

```
##
## Call:
## lm(formula = shannon ~ Social.Status * Day, data = diversity)
##
## Coefficients:
##                           (Intercept)            Social.Status2_Subordinate
##                                1.1111                                0.5170
##              Social.Status3_Communal                Social.Status4_Isolate
##                                0.5936                                0.5237
##                            DayDay_14                               DayDay_7
##                                0.1536                                0.7091
##                            DayDay_IP  Social.Status2_Subordinate:DayDay_14
```

```
##                                0.4095                                -0.0813
##    Social.Status3_Communal:DayDay_14     Social.Status4_Isolate:DayDay_14
##                               -0.1140                                -0.5395
##  Social.Status2_Subordinate:DayDay_7     Social.Status3_Communal:DayDay_7
##                               -1.3107                                -1.3913
##      Social.Status4_Isolate:DayDay_7  Social.Status2_Subordinate:DayDay_IP
##                               -0.7633                                -0.2127
##    Social.Status3_Communal:DayDay_IP     Social.Status4_Isolate:DayDay_IP
##                               -0.6690                                -0.7005
```

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

```
##
## Call:
## lm(formula = shannon ~ Social.Status * Day, data = diversity)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.56191 -0.26373  0.06193  0.25097  1.26019
##
## Coefficients:
##                                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)                           1.1111     0.2071   5.366  8.2e-07 ***
## Social.Status2_Subordinate            0.5170     0.2928   1.766  0.08141 .
## Social.Status3_Communal               0.5936     0.2928   2.027  0.04611 *
## Social.Status4_Isolate                0.5238     0.3071   1.705  0.09216 .
## DayDay_14                             0.1536     0.2928   0.525  0.60142
## DayDay_7                              0.7091     0.2928   2.422  0.01780 *
## DayDay_IP                             0.4095     0.2928   1.398  0.16604
## Social.Status2_Subordinate:DayDay_14 -0.0813     0.4243  -0.192  0.84856
## Social.Status3_Communal:DayDay_14    -0.1140     0.4141  -0.275  0.78392
## Social.Status4_Isolate:DayDay_14     -0.5395     0.4343  -1.242  0.21798
## Social.Status2_Subordinate:DayDay_7  -1.3107     0.4141  -3.165  0.00222 **
## Social.Status3_Communal:DayDay_7     -1.3913     0.4141  -3.360  0.00122 **
## Social.Status4_Isolate:DayDay_7      -0.7633     0.4243  -1.799  0.07597 .
## Social.Status2_Subordinate:DayDay_IP -0.2127     0.4141  -0.514  0.60905
## Social.Status3_Communal:DayDay_IP    -0.6690     0.4141  -1.615  0.11031
## Social.Status4_Isolate:DayDay_IP     -0.7005     0.4243  -1.651  0.10284
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.5072 on 77 degrees of freedom
## Multiple R-squared:  0.2517, Adjusted R-squared:  0.1059
## F-statistic: 1.727 on 15 and 77 DF,  p-value: 0.06287
```

```
anova(shannon.lm)
```

```
## Analysis of Variance Table
##
## Response: shannon
##                  Df  Sum Sq Mean Sq F value  Pr(>F)
## Social.Status     3  0.1536 0.05120  0.1990 0.89674
## Day               3  0.4277 0.14256  0.5542 0.64686
```

```
## Social.Status:Day  9  6.0809 0.67566  2.6266 0.01053 *
## Residuals         77 19.8073 0.25724
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans(shannon.lm, pairwise ~ Social.Status*Day)
```

```
## $emmeans
##  Social.Status Day    emmean    SE df lower.CL upper.CL
##  1_Dominant    Day_0    1.11 0.207 77    0.699     1.52
##  2_Subordinate Day_0    1.63 0.207 77    1.216     2.04
##  3_Communal    Day_0    1.70 0.207 77    1.292     2.12
##  4_Isolate     Day_0    1.63 0.227 77    1.183     2.09
##  1_Dominant    Day_14   1.26 0.207 77    0.852     1.68
##  2_Subordinate Day_14   1.70 0.227 77    1.249     2.15
##  3_Communal    Day_14   1.74 0.207 77    1.332     2.16
##  4_Isolate     Day_14   1.25 0.227 77    0.797     1.70
##  1_Dominant    Day_7    1.82 0.207 77    1.408     2.23
##  2_Subordinate Day_7    1.03 0.207 77    0.614     1.44
##  3_Communal    Day_7    1.02 0.207 77    0.610     1.43
##  4_Isolate     Day_7    1.58 0.207 77    1.168     1.99
##  1_Dominant    Day_IP   1.52 0.207 77    1.108     1.93
##  2_Subordinate Day_IP   1.82 0.207 77    1.413     2.24
##  3_Communal    Day_IP   1.45 0.207 77    1.033     1.86
##  4_Isolate     Day_IP   1.34 0.207 77    0.931     1.76
##
## Confidence level used: 0.95
##
## $contrasts
##  contrast                                      estimate    SE df t.ratio p.value
##  1_Dominant Day_0 - 2_Subordinate Day_0      -0.51704 0.293 77  -1.766  0.9207
##  1_Dominant Day_0 - 3_Communal Day_0         -0.59360 0.293 77  -2.027  0.8025
##  1_Dominant Day_0 - 4_Isolate Day_0          -0.52375 0.307 77  -1.705  0.9391
##  1_Dominant Day_0 - 1_Dominant Day_14        -0.15359 0.293 77  -0.525  1.0000
##  1_Dominant Day_0 - 2_Subordinate Day_14     -0.58932 0.307 77  -1.919  0.8590
##  1_Dominant Day_0 - 3_Communal Day_14        -0.63323 0.293 77  -2.163  0.7192
##  1_Dominant Day_0 - 4_Isolate Day_14         -0.13787 0.307 77  -0.449  1.0000
##  1_Dominant Day_0 - 1_Dominant Day_7         -0.70914 0.293 77  -2.422  0.5379
##  1_Dominant Day_0 - 2_Subordinate Day_7       0.08449 0.293 77   0.289  1.0000
##  1_Dominant Day_0 - 3_Communal Day_7          0.08855 0.293 77   0.302  1.0000
##  1_Dominant Day_0 - 4_Isolate Day_7          -0.46959 0.293 77  -1.604  0.9629
##  1_Dominant Day_0 - 1_Dominant Day_IP        -0.40946 0.293 77  -1.398  0.9892
##  1_Dominant Day_0 - 2_Subordinate Day_IP     -0.71383 0.293 77  -2.438  0.5265
##  1_Dominant Day_0 - 3_Communal Day_IP        -0.33408 0.293 77  -1.141  0.9987
##  1_Dominant Day_0 - 4_Isolate Day_IP         -0.23268 0.293 77  -0.795  1.0000
##  2_Subordinate Day_0 - 3_Communal Day_0      -0.07656 0.293 77  -0.261  1.0000
##  2_Subordinate Day_0 - 4_Isolate Day_0       -0.00671 0.307 77  -0.022  1.0000
##  2_Subordinate Day_0 - 1_Dominant Day_14      0.36345 0.293 77   1.241  0.9968
##  2_Subordinate Day_0 - 2_Subordinate Day_14  -0.07229 0.307 77  -0.235  1.0000
##  2_Subordinate Day_0 - 3_Communal Day_14     -0.11620 0.293 77  -0.397  1.0000
##  2_Subordinate Day_0 - 4_Isolate Day_14       0.37917 0.307 77   1.235  0.9970
##  2_Subordinate Day_0 - 1_Dominant Day_7      -0.19210 0.293 77  -0.656  1.0000
##  2_Subordinate Day_0 - 2_Subordinate Day_7    0.60152 0.293 77   2.054  0.7869
##  2_Subordinate Day_0 - 3_Communal Day_7       0.60559 0.293 77   2.068  0.7786
```

```
##  2_Subordinate Day_0 - 4_Isolate Day_7        0.04745 0.293 77   0.162  1.0000
##  2_Subordinate Day_0 - 1_Dominant Day_IP      0.10758 0.293 77   0.367  1.0000
##  2_Subordinate Day_0 - 2_Subordinate Day_IP  -0.19679 0.293 77  -0.672  1.0000
##  2_Subordinate Day_0 - 3_Communal Day_IP      0.18296 0.293 77   0.625  1.0000
##  2_Subordinate Day_0 - 4_Isolate Day_IP       0.28436 0.293 77   0.971  0.9998
##  3_Communal Day_0 - 4_Isolate Day_0           0.06985 0.307 77   0.227  1.0000
##  3_Communal Day_0 - 1_Dominant Day_14         0.44001 0.293 77   1.503  0.9789
##  3_Communal Day_0 - 2_Subordinate Day_14      0.00427 0.307 77   0.014  1.0000
##  3_Communal Day_0 - 3_Communal Day_14        -0.03964 0.293 77  -0.135  1.0000
##  3_Communal Day_0 - 4_Isolate Day_14          0.45573 0.307 77   1.484  0.9812
##  3_Communal Day_0 - 1_Dominant Day_7         -0.11554 0.293 77  -0.395  1.0000
##  3_Communal Day_0 - 2_Subordinate Day_7       0.67808 0.293 77   2.316  0.6137
##  3_Communal Day_0 - 3_Communal Day_7          0.68215 0.293 77   2.330  0.6038
##  3_Communal Day_0 - 4_Isolate Day_7           0.12401 0.293 77   0.424  1.0000
##  3_Communal Day_0 - 1_Dominant Day_IP         0.18414 0.293 77   0.629  1.0000
##  3_Communal Day_0 - 2_Subordinate Day_IP     -0.12023 0.293 77  -0.411  1.0000
##  3_Communal Day_0 - 3_Communal Day_IP         0.25952 0.293 77   0.886  0.9999
##  3_Communal Day_0 - 4_Isolate Day_IP          0.36092 0.293 77   1.233  0.9970
##  4_Isolate Day_0 - 1_Dominant Day_14          0.37015 0.307 77   1.205  0.9977
##  4_Isolate Day_0 - 2_Subordinate Day_14      -0.06558 0.321 77  -0.204  1.0000
##  4_Isolate Day_0 - 3_Communal Day_14         -0.10949 0.307 77  -0.357  1.0000
##  4_Isolate Day_0 - 4_Isolate Day_14           0.38588 0.321 77   1.203  0.9977
##  4_Isolate Day_0 - 1_Dominant Day_7          -0.18539 0.307 77  -0.604  1.0000
##  4_Isolate Day_0 - 2_Subordinate Day_7        0.60823 0.307 77   1.980  0.8281
##  4_Isolate Day_0 - 3_Communal Day_7           0.61230 0.307 77   1.994  0.8210
##  4_Isolate Day_0 - 4_Isolate Day_7            0.05416 0.307 77   0.176  1.0000
##  4_Isolate Day_0 - 1_Dominant Day_IP          0.11429 0.307 77   0.372  1.0000
##  4_Isolate Day_0 - 2_Subordinate Day_IP      -0.19008 0.307 77  -0.619  1.0000
##  4_Isolate Day_0 - 3_Communal Day_IP          0.18967 0.307 77   0.618  1.0000
##  4_Isolate Day_0 - 4_Isolate Day_IP           0.29107 0.307 77   0.948  0.9999
##  1_Dominant Day_14 - 2_Subordinate Day_14    -0.43573 0.307 77  -1.419  0.9876
##  1_Dominant Day_14 - 3_Communal Day_14       -0.47964 0.293 77  -1.638  0.9558
##  1_Dominant Day_14 - 4_Isolate Day_14         0.01572 0.307 77   0.051  1.0000
##  1_Dominant Day_14 - 1_Dominant Day_7        -0.55554 0.293 77  -1.897  0.8691
##  1_Dominant Day_14 - 2_Subordinate Day_7      0.23808 0.293 77   0.813  1.0000
##  1_Dominant Day_14 - 3_Communal Day_7         0.24214 0.293 77   0.827  1.0000
##  1_Dominant Day_14 - 4_Isolate Day_7         -0.31599 0.293 77  -1.079  0.9993
##  1_Dominant Day_14 - 1_Dominant Day_IP       -0.25586 0.293 77  -0.874  0.9999
##  1_Dominant Day_14 - 2_Subordinate Day_IP    -0.56024 0.293 77  -1.913  0.8617
##  1_Dominant Day_14 - 3_Communal Day_IP       -0.18049 0.293 77  -0.616  1.0000
##  1_Dominant Day_14 - 4_Isolate Day_IP        -0.07909 0.293 77  -0.270  1.0000
##  2_Subordinate Day_14 - 3_Communal Day_14    -0.04391 0.307 77  -0.143  1.0000
##  2_Subordinate Day_14 - 4_Isolate Day_14      0.45146 0.321 77   1.407  0.9886
##  2_Subordinate Day_14 - 1_Dominant Day_7     -0.11981 0.307 77  -0.390  1.0000
##  2_Subordinate Day_14 - 2_Subordinate Day_7   0.67381 0.307 77   2.194  0.6983
##  2_Subordinate Day_14 - 3_Communal Day_7      0.67788 0.307 77   2.207  0.6893
##  2_Subordinate Day_14 - 4_Isolate Day_7       0.11974 0.307 77   0.390  1.0000
##  2_Subordinate Day_14 - 1_Dominant Day_IP     0.17987 0.307 77   0.586  1.0000
##  2_Subordinate Day_14 - 2_Subordinate Day_IP -0.12450 0.307 77  -0.405  1.0000
##  2_Subordinate Day_14 - 3_Communal Day_IP     0.25525 0.307 77   0.831  1.0000
##  2_Subordinate Day_14 - 4_Isolate Day_IP      0.35665 0.307 77   1.161  0.9984
##  3_Communal Day_14 - 4_Isolate Day_14         0.49536 0.307 77   1.613  0.9611
##  3_Communal Day_14 - 1_Dominant Day_7        -0.07590 0.293 77  -0.259  1.0000
##  3_Communal Day_14 - 2_Subordinate Day_7      0.71772 0.293 77   2.451  0.5170
```

```
##  3_Communal Day_14 - 3_Communal Day_7         0.72178 0.293 77   2.465  0.5072
##  3_Communal Day_14 - 4_Isolate Day_7          0.16365 0.293 77   0.559  1.0000
##  3_Communal Day_14 - 1_Dominant Day_IP        0.22378 0.293 77   0.764  1.0000
##  3_Communal Day_14 - 2_Subordinate Day_IP    -0.08060 0.293 77  -0.275  1.0000
##  3_Communal Day_14 - 3_Communal Day_IP        0.29915 0.293 77   1.022  0.9996
##  3_Communal Day_14 - 4_Isolate Day_IP         0.40055 0.293 77   1.368  0.9913
##  4_Isolate Day_14 - 1_Dominant Day_7         -0.57127 0.307 77  -1.860  0.8853
##  4_Isolate Day_14 - 2_Subordinate Day_7       0.22235 0.307 77   0.724  1.0000
##  4_Isolate Day_14 - 3_Communal Day_7          0.22642 0.307 77   0.737  1.0000
##  4_Isolate Day_14 - 4_Isolate Day_7          -0.33172 0.307 77  -1.080  0.9993
##  4_Isolate Day_14 - 1_Dominant Day_IP        -0.27159 0.307 77  -0.884  0.9999
##  4_Isolate Day_14 - 2_Subordinate Day_IP     -0.57596 0.307 77  -1.875  0.8788
##  4_Isolate Day_14 - 3_Communal Day_IP        -0.19621 0.307 77  -0.639  1.0000
##  4_Isolate Day_14 - 4_Isolate Day_IP         -0.09481 0.307 77  -0.309  1.0000
##  1_Dominant Day_7 - 2_Subordinate Day_7       0.79362 0.293 77   2.710  0.3436
##  1_Dominant Day_7 - 3_Communal Day_7          0.79769 0.293 77   2.724  0.3352
##  1_Dominant Day_7 - 4_Isolate Day_7           0.23955 0.293 77   0.818  1.0000
##  1_Dominant Day_7 - 1_Dominant Day_IP         0.29968 0.293 77   1.023  0.9996
##  1_Dominant Day_7 - 2_Subordinate Day_IP     -0.00469 0.293 77  -0.016  1.0000
##  1_Dominant Day_7 - 3_Communal Day_IP         0.37506 0.293 77   1.281  0.9955
##  1_Dominant Day_7 - 4_Isolate Day_IP          0.47646 0.293 77   1.627  0.9581
##  2_Subordinate Day_7 - 3_Communal Day_7       0.00407 0.293 77   0.014  1.0000
##  2_Subordinate Day_7 - 4_Isolate Day_7       -0.55407 0.293 77  -1.892  0.8714
##  2_Subordinate Day_7 - 1_Dominant Day_IP     -0.49394 0.293 77  -1.687  0.9441
##  2_Subordinate Day_7 - 2_Subordinate Day_IP  -0.79832 0.293 77  -2.726  0.3339
##  2_Subordinate Day_7 - 3_Communal Day_IP     -0.41856 0.293 77  -1.429  0.9867
##  2_Subordinate Day_7 - 4_Isolate Day_IP      -0.31716 0.293 77  -1.083  0.9993
##  3_Communal Day_7 - 4_Isolate Day_7          -0.55814 0.293 77  -1.906  0.8650
##  3_Communal Day_7 - 1_Dominant Day_IP        -0.49801 0.293 77  -1.701  0.9403
##  3_Communal Day_7 - 2_Subordinate Day_IP     -0.80238 0.293 77  -2.740  0.3257
##  3_Communal Day_7 - 3_Communal Day_IP        -0.42263 0.293 77  -1.443  0.9855
##  3_Communal Day_7 - 4_Isolate Day_IP         -0.32123 0.293 77  -1.097  0.9992
##  4_Isolate Day_7 - 1_Dominant Day_IP          0.06013 0.293 77   0.205  1.0000
##  4_Isolate Day_7 - 2_Subordinate Day_IP      -0.24424 0.293 77  -0.834  1.0000
##  4_Isolate Day_7 - 3_Communal Day_IP          0.13551 0.293 77   0.463  1.0000
##  4_Isolate Day_7 - 4_Isolate Day_IP           0.23691 0.293 77   0.809  1.0000
##  1_Dominant Day_IP - 2_Subordinate Day_IP    -0.30437 0.293 77  -1.039  0.9996
##  1_Dominant Day_IP - 3_Communal Day_IP        0.07538 0.293 77   0.257  1.0000
##  1_Dominant Day_IP - 4_Isolate Day_IP         0.17678 0.293 77   0.604  1.0000
##  2_Subordinate Day_IP - 3_Communal Day_IP     0.37975 0.293 77   1.297  0.9949
##  2_Subordinate Day_IP - 4_Isolate Day_IP      0.48115 0.293 77   1.643  0.9546
##  3_Communal Day_IP - 4_Isolate Day_IP         0.10140 0.293 77   0.346  1.0000
##
## P value adjustment: tukey method for comparing a family of 16 estimates
```

```
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.Status2_Subordinate
```

```
##                             0.0438940                             -0.0017794
##             Social.Status3_Communal                Social.Status4_Isolate
##                            -0.0001417                             -0.0048018
##                             DayDay_14                               DayDay_7
##                             0.0021991                             -0.0203039
##                             DayDay_IP  Social.Status2_Subordinate:DayDay_14
##                            -0.0141169                              0.0460850
##    Social.Status3_Communal:DayDay_14     Social.Status4_Isolate:DayDay_14
##                             0.0318522                             -0.0121161
##  Social.Status2_Subordinate:DayDay_7     Social.Status3_Communal:DayDay_7
##                             0.0234902                              0.0030726
##      Social.Status4_Isolate:DayDay_7  Social.Status2_Subordinate:DayDay_IP
##                             0.0136719                             -0.0090364
##    Social.Status3_Communal:DayDay_IP     Social.Status4_Isolate:DayDay_IP
##                             0.0318437                              0.0216599
```

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

```
##
## Call:
## lm(formula = simp.even ~ Social.Status * Day, data = diversity)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.045530 -0.016100 -0.003891  0.010166  0.098475
##
## Coefficients:
##                                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)                           0.0438940  0.0116098   3.781 0.000307 ***
## Social.Status2_Subordinate           -0.0017794  0.0164187  -0.108 0.913978
## Social.Status3_Communal              -0.0001417  0.0164187  -0.009 0.993135
## Social.Status4_Isolate               -0.0048018  0.0172201  -0.279 0.781110
## DayDay_14                             0.0021991  0.0164187   0.134 0.893799
## DayDay_7                             -0.0203039  0.0164187  -1.237 0.219983
## DayDay_IP                            -0.0141169  0.0164187  -0.860 0.392566
## Social.Status2_Subordinate:DayDay_14  0.0460850  0.0237930   1.937 0.056424 .
## Social.Status3_Communal:DayDay_14     0.0318522  0.0232195   1.372 0.174115
## Social.Status4_Isolate:DayDay_14     -0.0121161  0.0243529  -0.498 0.620239
## Social.Status2_Subordinate:DayDay_7   0.0234902  0.0232195   1.012 0.314871
## Social.Status3_Communal:DayDay_7      0.0030726  0.0232195   0.132 0.895071
## Social.Status4_Isolate:DayDay_7       0.0136719  0.0237930   0.575 0.567225
## Social.Status2_Subordinate:DayDay_IP -0.0090364  0.0232195  -0.389 0.698223
## Social.Status3_Communal:DayDay_IP     0.0318437  0.0232195   1.371 0.174229
## Social.Status4_Isolate:DayDay_IP      0.0216599  0.0237930   0.910 0.365478
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02844 on 77 degrees of freedom
## Multiple R-squared:  0.3311, Adjusted R-squared:  0.2008
## F-statistic: 2.541 on 15 and 77 DF,  p-value: 0.004065
```

```
anova(evenness.lm)
```

```
## Analysis of Variance Table
```

```
##
## Response: simp.even
##                   Df   Sum Sq   Mean Sq F value   Pr(>F)
## Social.Status      3 0.004555 0.0015183  1.8774 0.140397
## Day                3 0.010328 0.0034427  4.2570 0.007765 **
## Social.Status:Day  9 0.015946 0.0017718  2.1908 0.031543 *
## Residuals         77 0.062272 0.0008087
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
emmeans(evenness.lm, pairwise ~ Day)
```

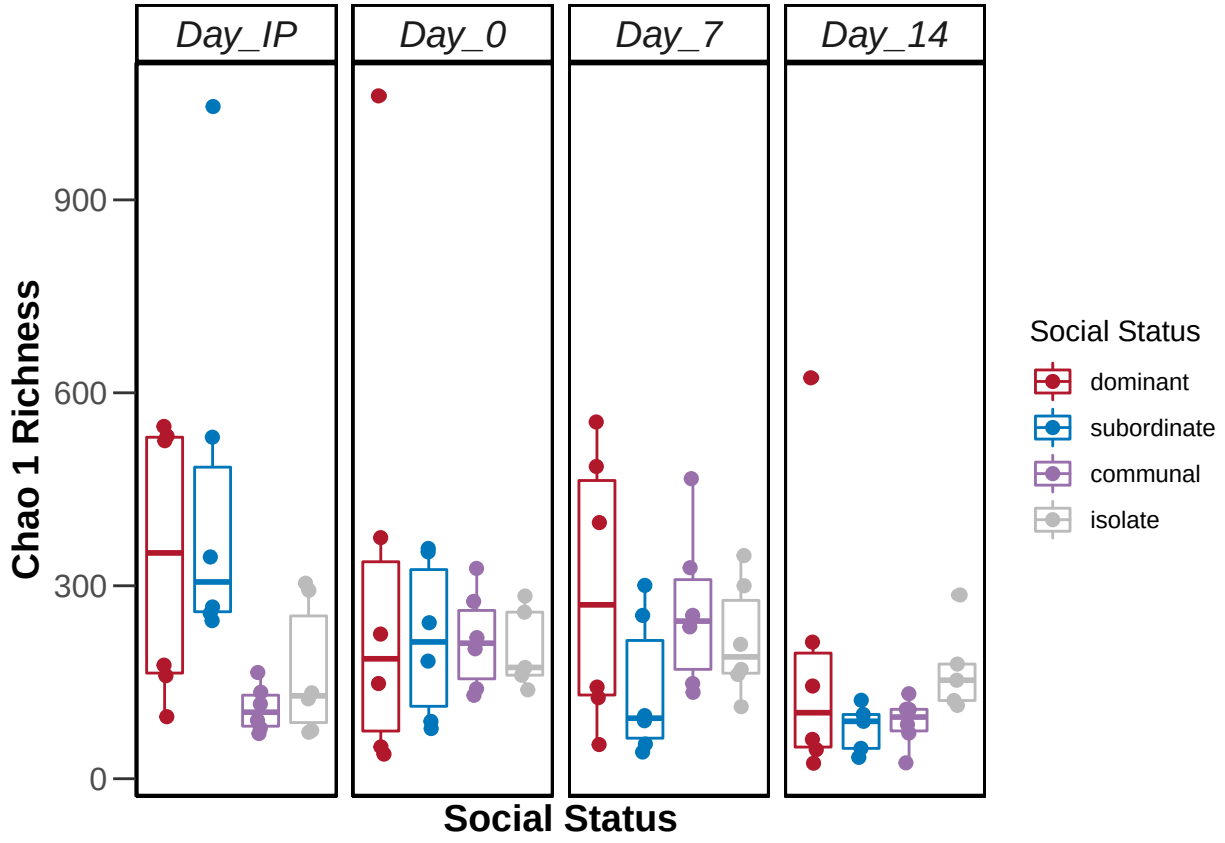
```
## NOTE: Results may be misleading due to involvement in interactions

## $emmeans
##  Day    emmean      SE df lower.CL upper.CL
##  Day_0  0.0422 0.00595 77   0.0304   0.0541
##  Day_14 0.0609 0.00609 77   0.0487   0.0730
##  Day_7  0.0320 0.00580 77   0.0204   0.0435
##  Day_IP 0.0392 0.00580 77   0.0277   0.0508
##
## Results are averaged over the levels of: Social.Status
## Confidence level used: 0.95
##
## $contrasts
##  contrast        estimate      SE df t.ratio p.value
##  Day_0 - Day_14  -0.01865 0.00851 77  -2.192  0.1347
##  Day_0 - Day_7    0.01025 0.00831 77   1.233  0.6082
##  Day_0 - Day_IP   0.00300 0.00831 77   0.361  0.9838
##  Day_14 - Day_7   0.02890 0.00841 77   3.435  0.0052
##  Day_14 - Day_IP  0.02165 0.00841 77   2.574  0.0568
##  Day_7 - Day_IP  -0.00725 0.00821 77  -0.883  0.8139
##
## Results are averaged over the levels of: Social.Status
## P value adjustment: tukey method for comparing a family of 4 estimates
```

```
# contrast        estimate      SE df t.ratio p.value
 # Day_7 - Day_14  -0.02593 0.00811 77  -3.195  0.0107
```

#Plot Richness

```
diversity$Day <- factor(diversity$Day,      # Reordering group factor levels
                        levels = c("Day_IP", "Day_0", "Day_7", "Day_14"))

# Graphing Chao1
p <- ggplot(diversity, aes(x=Social.Status, y=S.chao1, color=as.factor(Social.Status)))+
  geom_boxplot() + theme_bw() +
  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_grid(.~Day)
```

```
chao1 <- p +
    theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line
          =element_line(colour = "black")) +
    theme(axis.ticks.length=unit(0.3,"cm")) +
    theme(axis.text.y = element_text(size=12)) +
  labs(x = "Social Status", y = "Chao 1 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")) +
      theme(axis.title=element_text(vjust=1,size=14,face="bold"),
          axis.text=element_text(size=12), panel.border = element_rect(colour = "black",size=1)) +
      theme(axis.text.x = element_text(angle = 45))

chao1
```

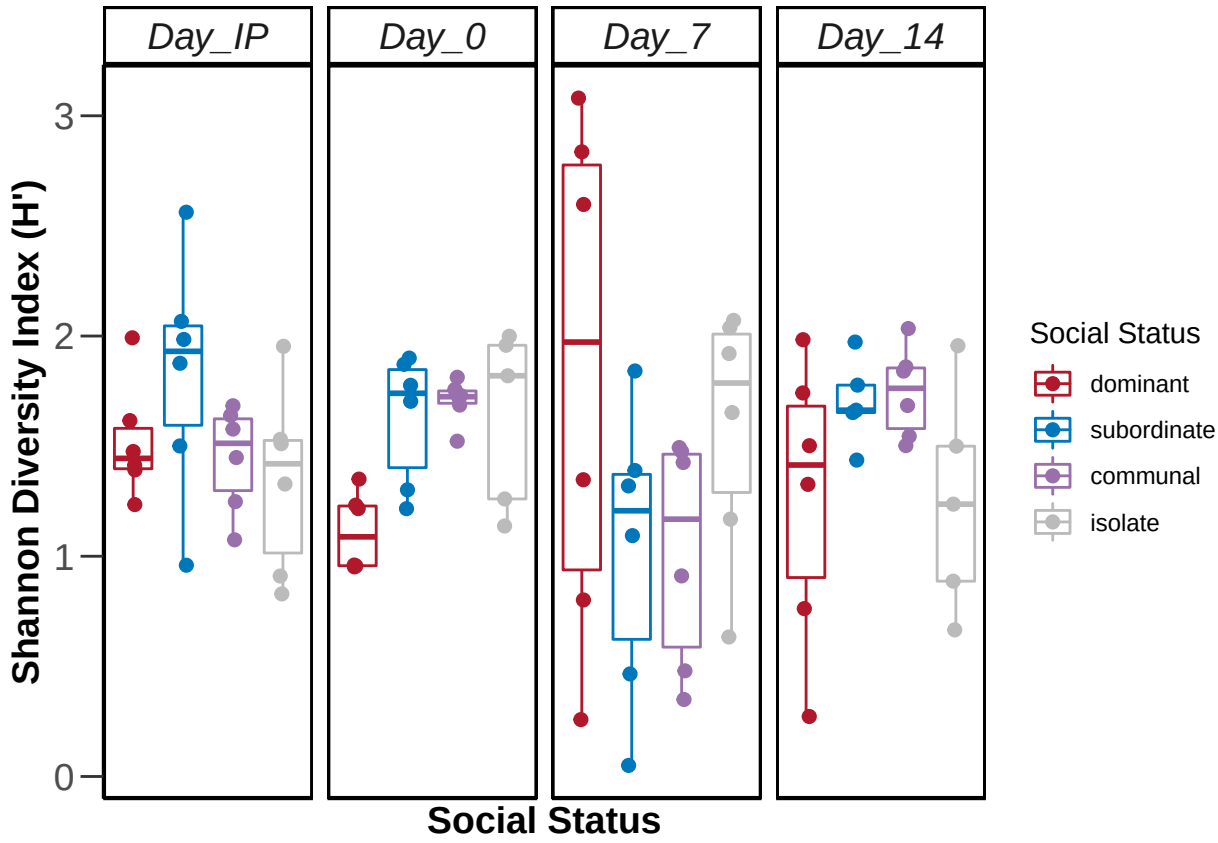



```
ggsave("../figures/chao1_updated.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=8, heig
```

#Plot shannon diversity

```
# Graphing Shannon Diversity
p <- ggplot(diversity, aes(x=Social.Status, y=shannon, color=as.factor(Social.Status)))+
  geom_boxplot() +
```

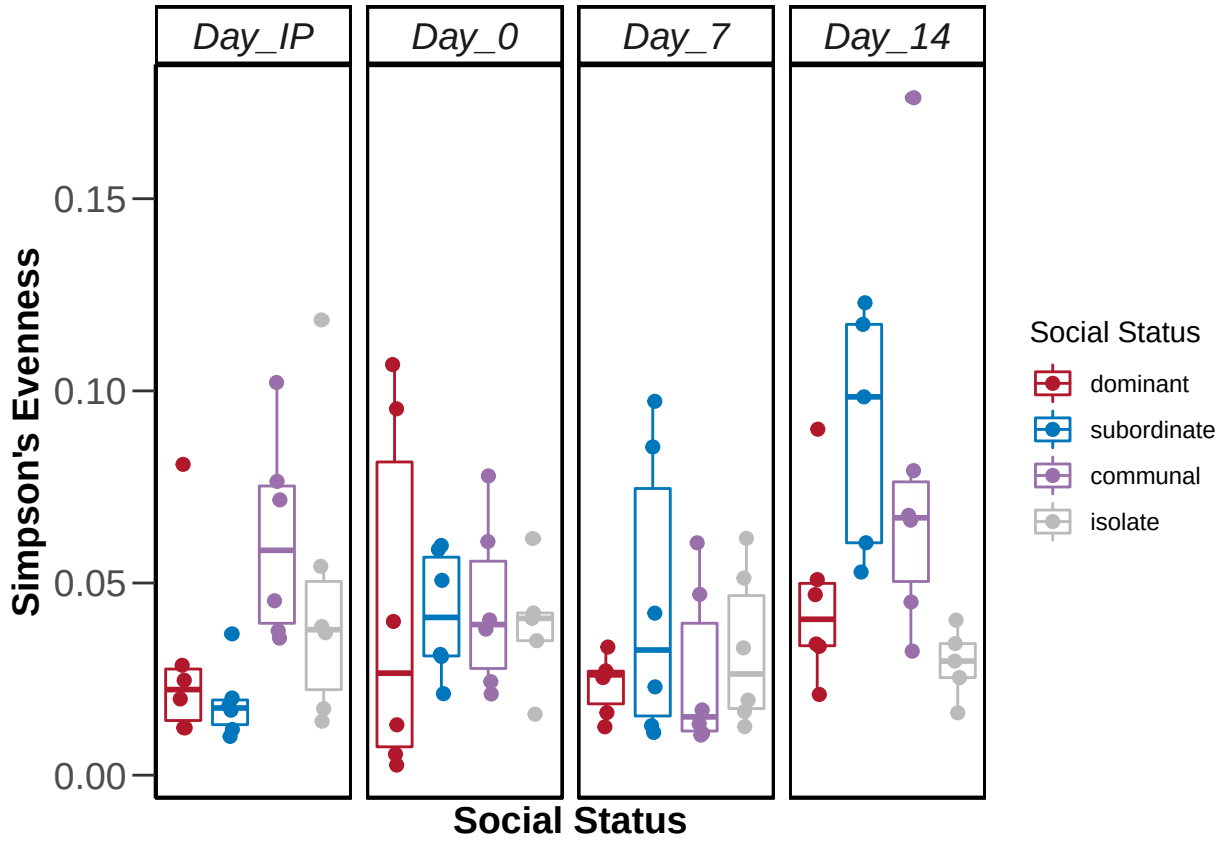
```
  geom_point(aes(color=factor(Social.Status)), size=2, position = position_jitterdodge()) +
  scale_color_manual(name="Social Status", values=c("#B2182B", "#0077BB", "#9970AB", "#BBBBBB"),
                     labels = c("dominant", "subordinate", "communal", "isolate")) +
  facet_grid(.~Day)

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"),
                           labels=c("dominant", "subordinate", "communal", "isolate"))
shannon
```




```
ggsave("../figures/shannon_updated.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=8, he
```

#Plot Evenness

```r
# 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_grid(.~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"),
                           labels=c("dominant", "subordinate", "communal", "isolate"))
simpeven
```




```r
ggsave("../figures/simpeven_updated.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=8, h
```

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 0.003996 **
## 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
```

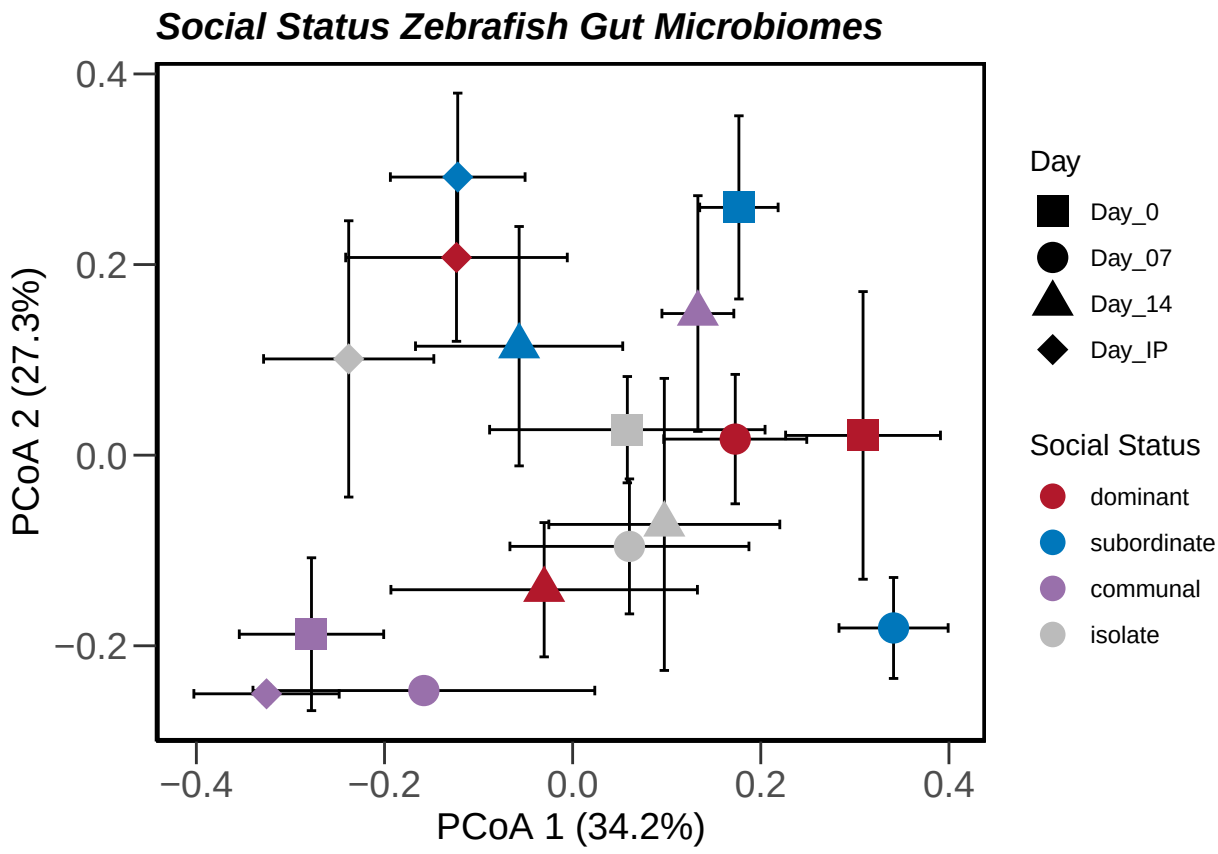
```
## [1] 34.2
```

```
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
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
pcoa.cent.treats <- rownames(pcoa.cent.dataframe)

Social.Status <- c("communal","communal","communal","communal","dominant","dominant","dominant","dominan
Day <- c("Day_0","Day_14","Day_07","Day_IP","Day_0","Day_14","Day_07","Day_IP","Day_0","Day_14","Day_07
pcoa.cent.dataframe.trts <- as.data.frame(pcoa.cent.dataframe)
pcoa.cent.dataframe.trts$Social.Status <- as.factor(Social.Status)
pcoa.cent.dataframe.trts$Day <- as.factor(Day)

#Plot
plot1a <- ggplot(pcoa.cent.dataframe.trts, aes(x=V1, y=V2, colour=Social.Status, shape=Day)) + theme_bw
p <-plot1a + theme(panel.grid.major = element_blank(),
                   panel.grid.minor = element_blank(),
                   axis.line = element_line(colour = "black")) +
  theme(panel.background = element_blank()) +
  geom_errorbarh(aes(xmax=V1+V1e, xmin=V1-V1e, height=0.01), colour="black") +
  geom_errorbar(aes(ymax=V2+V2e, ymin=V2-V2e, width=0.01), colour="black") +
  geom_point(aes(colour=Social.Status), size=5, stroke = 0.75, show.legend = TRUE) +
  scale_colour_manual(labels = c("dominant","subordinate","communal","isolate"),
                      values = c("#B2182B", "#0077BB", "#9970AB", "#BBBBBB")) +
  scale_shape_manual(values = c(15,16,17,18)) +
  theme(axis.title = element_text(size=14), axis.text=element_text(size=14),
          axis.text.x = element_text(size=14),
          panel.border = element_rect(colour = "black", size=1.25)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  xlab("PCoA 1 (34.2%)") + ylab("PCoA 2 (27.3%)") +
  labs(colour = "Social Status") +
  guides(colour = guide_legend(override.aes = list(pch=16,size = 4)))+
  ggtitle(label="Social Status Zebrafish Gut Microbiomes") +
  theme(plot.title = element_text(color="black", size=14, face="bold.italic"))
p
```




```
ggsave("../figures/zf_PCoAordination_updated.png", plot=last_plot(), device=NULL, path=NULL, scale=1, w
```

Bacterial community indicator species analysis

```
zebrafish_16s <- import_mothur(mothur_shared_file = "../data/EM20_ZF_16S.opti_mcc.shared", mothur_consta
zebrafish_16s
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 10765 taxa and 101 samples ]
## tax_table()   Taxonomy Table:    [ 10765 taxa by 6 taxonomic ranks ]
```

```
sample <- sample_data(design)
sample_data(zebrafish_16s)<- sample

zebrafish_16s
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 10765 taxa and 101 samples ]
## sample_data() Sample Data:       [ 101 samples by 3 sample variables ]
## tax_table()   Taxonomy Table:    [ 10765 taxa by 6 taxonomic ranks ]
```

```
colnames(tax_table(zebrafish_16s))
```

```
## [1] "Rank1" "Rank2" "Rank3" "Rank4" "Rank5" "Rank6"
```

```
colnames(tax_table(zebrafish_16s)) <- c("Kingdom", "Phylum", "Class",
  "Order", "Family", "Genus")

after_remove_low_depth <- prune_samples(sample_sums(zebrafish_16s) >= 6000, zebrafish_16s)
head(sample_sums(after_remove_low_depth))
```

```
##  F_Com1_D0 F_Com1_D14  F_Com1_D7  F_Com1_IP  F_Com2_D0 F_Com2_D14
##      66860      44916     125876      93268      58884      71393
```

```
set.seed(1)
rare <- rarefy_even_depth(after_remove_low_depth, sample.size = 6000,rngseed=TRUE)
```

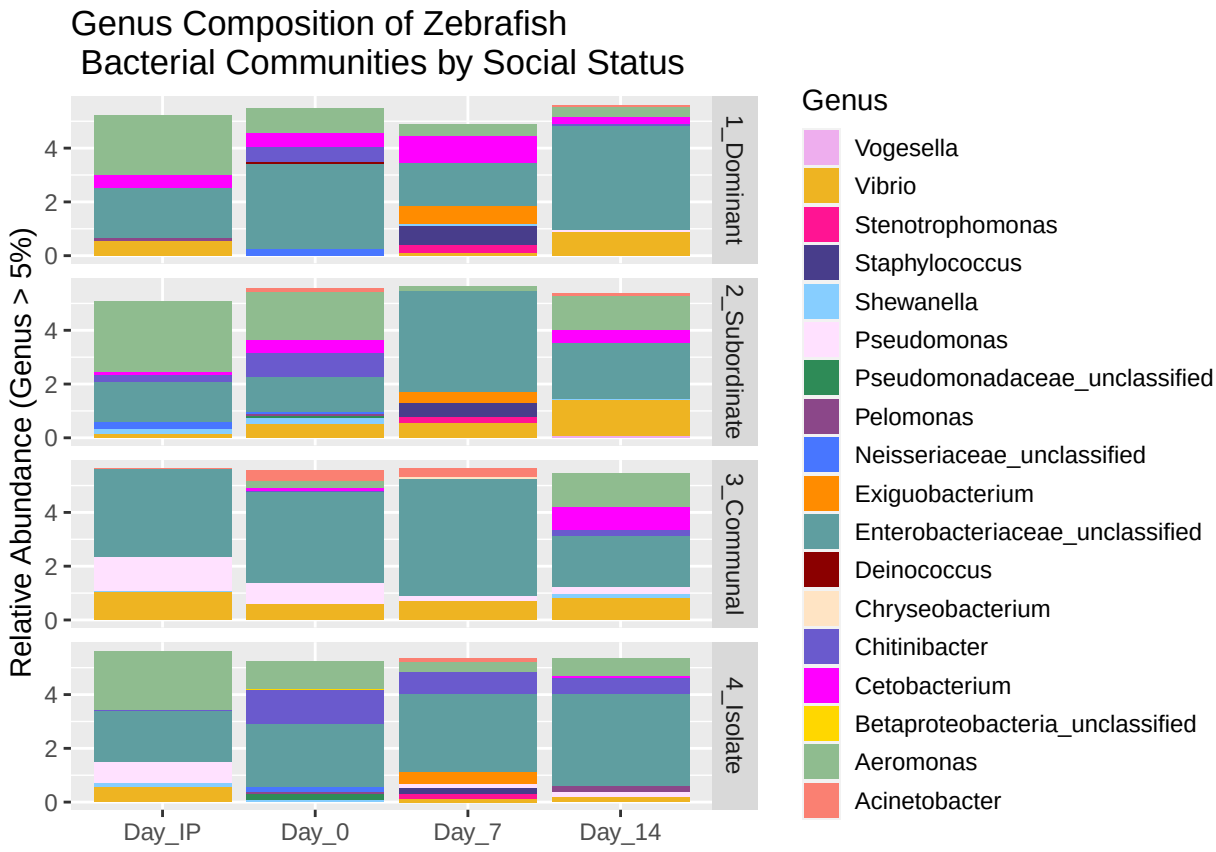
```
## `set.seed(TRUE)` was used to initialize repeatable random subsampling.

## Please record this for your records so others can reproduce.

## Try `set.seed(TRUE); .Random.seed` for the full vector

## ...

## 68320TUs were removed because they are no longer
## present in any sample after random subsampling

## ...
```

```
head(sample_sums(rare))
```

```
##  F_Com1_D0 F_Com1_D14  F_Com1_D7  F_Com1_IP  F_Com2_D0 F_Com2_D14
##       6000       6000       6000       6000       6000       6000
```

```
#remove the NTC sample. Check to make sure it doesn't have too many sequences before you through it awa
to_remove <- c("NTC")
pruned <- prune_samples(!(rownames(sample_data(rare)) %in% to_remove), rare)

#filter out OTUs less than 10
#darte_ed_16s_filter <- filter_taxa(pruned, function(x) sum(x) > 10, TRUE)

 #relative abundance
zebrafish_16s_filter_re <- transform_sample_counts(pruned, function(x) x /sum(x))

#Get rid of small taxa
zebrafish_16s_filter2 <- filter_taxa(zebrafish_16s_filter_re, function(x) sum(x) > .001, TRUE)

#Combine OTUs with common taxa
zebrafish_16s_filter_re_g = tax_glom(zebrafish_16s_filter2, "Phylum")
```

```r
zebrafish_16s_filter_re_g2 = tax_glom(zebrafish_16s_filter2, "Genus")


zebrafish_genus <- zebrafish_16s %>%
  tax_glom(taxrank = "Genus") %>%                      # agglomerate at phylum level
  transform_sample_counts(function(x) {x/sum(x)} ) %>% # Transform to rel. abundance
  psmelt() %>%                                          # Melt to long format
  filter(Abundance > 0.05) %>%                          # Filter out low abundance taxa
  arrange(Genus)                                       # Sort data frame alphabetically by phylum
# Set colors for plotting
genus_colors <- c(
  "salmon", "darkseagreen", "gold", "magenta", "slateblue", "bisque", "darkred", "cadetblue", "darkoran
)


# Plot
a <- list(
  font = list(size = 14),
  xref = "paper",
  yref = "paper",
  yanchor = "bottom",
  xanchor = "center",
  align = "center",
  x = 0.5,
  y = 1,
  showarrow = FALSE)

zebrafish_genus_v2 <- subset(zebrafish_genus, Social.Status == "1_Dominant" | Social.Status == "2_Subor

ggplot(transform(zebrafish_genus_v2, Day=factor(Day, levels=c("Day_IP","Day_0","Day_7","Day_14"))), aes
  facet_grid(Social.Status~.) +
  geom_bar(stat = "identity") +
  scale_fill_manual(values = genus_colors) +
  scale_x_discrete(labels = c("Day_IP", "Day_0", "Day_7", "Day_14"), drop = TRUE) +
  # Remove x axis title
  theme(axis.title.x = element_blank()) +
  #
  guides(fill = guide_legend(reverse = TRUE, keywidth = 1, keyheight = 1)) +
  ylab("Relative Abundance (Genus > 5%)") +
  ggtitle("Genus Composition of Zebrafish \n Bacterial Communities by Social Status")
```




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