Social status effects on zebrafish gut microbiomes

Emily Scott, Fadi Issa, Michael Brewer, Ariane L. Peralta

Last updated on 10 March, 2023

Project Description: Fill out

Initial Setup

#Import Files ## Environmental Data

Bacterial Data

Diversity Metrics

```
# 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))

# 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

```
#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 1.49 1.85 1.59 1.67 ...
##  $ simp.even    : num  0.0746 0.0409 0.0425 0.0721 0.0223 ...
##  $ J            : num  0.439 0.345 0.402 0.394 0.338 ...
##  $ S.obs        : num  63 76 101 57 139 52 69 68 110 43 ...
##  $ S.chao1      : num  129 211 154 111 280 ...
##  $ se.chao1     : num  31.8 61 21.9 29 44.3 ...
##  $ S.ACE        : num  134 201 175 118 358 ...
##  $ se.ACE       : num  5.63 7.75 8.31 6.93 13.61 ...
```

```
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.
```

```
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     0.0781
##  2 1_Dominant    Day_14           200.       107.          1.27     0.261
##  3 1_Dominant    Day_7            298.        75.8         1.82     0.483
##  4 1_Dominant    Day_IP           375.        75.7         1.53     0.104
```

```
##  5 2_Subordinate Day_0          242.        48.3         1.62     0.125
##  6 2_Subordinate Day_14         108.        17.2         1.71     0.0870
##  7 2_Subordinate Day_7          177.        54.7         1.02     0.268
##  8 2_Subordinate Day_IP         506.       136.          1.82     0.222
##  9 3_Communal    Day_0          278.        30.3         1.70     0.0416
## 10 3_Communal    Day_14         147.        22.3         1.75     0.0823
## 11 3_Communal    Day_7          305.        45.1         1.02     0.211
## 12 3_Communal    Day_IP         169.        25.8         1.44     0.102
## 13 4_Isolate     Day_0          251.        32.9         1.64     0.184
## 14 4_Isolate     Day_14         172.        15.7         1.25     0.230
## 15 4_Isolate     Day_7          265.        26.0         1.59     0.233
## 16 4_Isolate     Day_IP         172         34.9         1.34     0.172
```

```
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
##                                316.530                               -99.358
##                Social.Status3_Communal                Social.Status4_Isolate
##                                -98.596                              -121.290
##                              DayDay_14                              DayDay_7
##                               -151.595                                -1.166
##                              DayDay_IP  Social.Status2_Subordinate:DayDay_14
##                                  7.387                                18.153
##    Social.Status3_Communal:DayDay_14     Social.Status4_Isolate:DayDay_14
##                                 36.150                               149.081
##  Social.Status2_Subordinate:DayDay_7     Social.Status3_Communal:DayDay_7
##                                -74.010                                21.098
##      Social.Status4_Isolate:DayDay_7  Social.Status2_Subordinate:DayDay_IP
##                                101.105                               218.902
##    Social.Status3_Communal:DayDay_IP     Social.Status4_Isolate:DayDay_IP
##                               -101.461                               -58.006
```

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

```
##
## Call:
## lm(formula = S.chao1 ~ Social.Status * Day, data = diversity)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -289.63  -80.91  -28.87   67.27  629.38
##
## Coefficients:
##                                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)                           316.530     66.999   4.724 1.02e-05 ***
## Social.Status2_Subordinate            -99.358     94.751  -1.049    0.298
## Social.Status3_Communal               -98.596     94.751  -1.041    0.301
```

```
## Social.Status4_Isolate               -121.290     99.375  -1.221    0.226
## DayDay_14                            -151.595     94.751  -1.600    0.114
## DayDay_7                               -1.166     94.751  -0.012    0.990
## DayDay_IP                               7.387     94.751   0.078    0.938
## Social.Status2_Subordinate:DayDay_14   18.153    137.307   0.132    0.895
## Social.Status3_Communal:DayDay_14      36.150    133.998   0.270    0.788
## Social.Status4_Isolate:DayDay_14      149.081    140.538   1.061    0.292
## Social.Status2_Subordinate:DayDay_7   -74.010    133.998  -0.552    0.582
## Social.Status3_Communal:DayDay_7       21.098    133.998   0.157    0.875
## Social.Status4_Isolate:DayDay_7       101.105    137.307   0.736    0.464
## Social.Status2_Subordinate:DayDay_IP  218.902    133.998   1.634    0.106
## Social.Status3_Communal:DayDay_IP    -101.461    133.998  -0.757    0.451
## Social.Status4_Isolate:DayDay_IP      -58.006    137.307  -0.422    0.674
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 164.1 on 77 degrees of freedom
## Multiple R-squared:  0.2876, Adjusted R-squared:  0.1488
## F-statistic: 2.072 on 15 and 77 DF,  p-value: 0.02026
```

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

```
## Analysis of Variance Table
##
## Response: S.chao1
##                   Df  Sum Sq Mean Sq F value  Pr(>F)
## Social.Status      3  149770   49923  1.8536 0.14451
## Day                3  219518   73173  2.7168 0.05040 .
## Social.Status:Day  9  467909   51990  1.9303 0.05978 .
## Residuals         77 2073843   26933
## ---
## 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
##                                 1.1060                               0.5144
##              Social.Status3_Communal               Social.Status4_Isolate
##                                 0.5965                               0.5293
##                             DayDay_14                             DayDay_7
##                                 0.1604                               0.7184
##                             DayDay_IP  Social.Status2_Subordinate:DayDay_14
##                                 0.4269                              -0.0750
##    Social.Status3_Communal:DayDay_14     Social.Status4_Isolate:DayDay_14
##                                -0.1149                              -0.5493
##  Social.Status2_Subordinate:DayDay_7     Social.Status3_Communal:DayDay_7
##                                -1.3161                              -1.3963
```

```
##      Social.Status4_Isolate:DayDay_7  Social.Status2_Subordinate:DayDay_IP
##                              -0.7638                               -0.2282
##    Social.Status3_Communal:DayDay_IP      Social.Status4_Isolate:DayDay_IP
##                              -0.6903                               -0.7247
```

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

```
##
## Call:
## lm(formula = shannon ~ Social.Status * Day, data = diversity)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.56272 -0.25447  0.05049  0.25083  1.26012
##
## Coefficients:
##                                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)                          1.1059     0.2079   5.320 9.87e-07 ***
## Social.Status2_Subordinate           0.5144     0.2940   1.750  0.08413 .
## Social.Status3_Communal              0.5965     0.2940   2.029  0.04590 *
## Social.Status4_Isolate               0.5293     0.3083   1.717  0.09005 .
## DayDay_14                            0.1604     0.2940   0.546  0.58696
## DayDay_7                             0.7184     0.2940   2.444  0.01683 *
## DayDay_IP                            0.4269     0.2940   1.452  0.15054
## Social.Status2_Subordinate:DayDay_14 -0.0750     0.4260  -0.176  0.86072
## Social.Status3_Communal:DayDay_14    -0.1149     0.4158  -0.276  0.78297
## Social.Status4_Isolate:DayDay_14     -0.5494     0.4360  -1.260  0.21154
## Social.Status2_Subordinate:DayDay_7  -1.3161     0.4158  -3.166  0.00222 **
## Social.Status3_Communal:DayDay_7     -1.3963     0.4158  -3.358  0.00122 **
## Social.Status4_Isolate:DayDay_7      -0.7639     0.4260  -1.793  0.07690 .
## Social.Status2_Subordinate:DayDay_IP -0.2282     0.4158  -0.549  0.58470
## Social.Status3_Communal:DayDay_IP    -0.6903     0.4158  -1.660  0.10089
## Social.Status4_Isolate:DayDay_IP     -0.7247     0.4260  -1.701  0.09297 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.5092 on 77 degrees of freedom
## Multiple R-squared:  0.252,  Adjusted R-squared:  0.1063
## F-statistic:  1.73 on 15 and 77 DF,  p-value: 0.06228
```

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

```
## Analysis of Variance Table
##
## Response: shannon
##                   Df  Sum Sq Mean Sq F value   Pr(>F)
## Social.Status      3  0.1367 0.04556  0.1757 0.912525
## Day                3  0.4035 0.13449  0.5187 0.670667
## Social.Status:Day  9  6.1865 0.68739  2.6512 0.009889 **
## Residuals         77 19.9647 0.25928
## ---
## 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 0.208 77    0.692     1.52
##  2_Subordinate Day_0    1.62 0.208 77    1.206     2.03
##  3_Communal    Day_0    1.70 0.208 77    1.289     2.12
##  4_Isolate     Day_0    1.64 0.228 77    1.182     2.09
##  1_Dominant    Day_14   1.27 0.208 77    0.852     1.68
##  2_Subordinate Day_14   1.71 0.228 77    1.252     2.16
##  3_Communal    Day_14   1.75 0.208 77    1.334     2.16
##  4_Isolate     Day_14   1.25 0.228 77    0.793     1.70
##  1_Dominant    Day_7    1.82 0.208 77    1.410     2.24
##  2_Subordinate Day_7    1.02 0.208 77    0.609     1.44
##  3_Communal    Day_7    1.02 0.208 77    0.611     1.44
##  4_Isolate     Day_7    1.59 0.208 77    1.176     2.00
##  1_Dominant    Day_IP   1.53 0.208 77    1.119     1.95
##  2_Subordinate Day_IP   1.82 0.208 77    1.405     2.23
##  3_Communal    Day_IP   1.44 0.208 77    1.025     1.85
##  4_Isolate     Day_IP   1.34 0.208 77    0.924     1.75
##
## Confidence level used: 0.95
##
## $contrasts
##  contrast                                      estimate    SE df t.ratio p.value
##  1_Dominant Day_0 - 2_Subordinate Day_0      -0.51443 0.294 77  -1.750  0.9259
##  1_Dominant Day_0 - 3_Communal Day_0         -0.59653 0.294 77  -2.029  0.8014
##  1_Dominant Day_0 - 4_Isolate Day_0          -0.52932 0.308 77  -1.717  0.9359
##  1_Dominant Day_0 - 1_Dominant Day_14        -0.16038 0.294 77  -0.546  1.0000
##  1_Dominant Day_0 - 2_Subordinate Day_14     -0.59981 0.308 77  -1.945  0.8462
##  1_Dominant Day_0 - 3_Communal Day_14        -0.64199 0.294 77  -2.184  0.7051
##  1_Dominant Day_0 - 4_Isolate Day_14         -0.14035 0.308 77  -0.455  1.0000
##  1_Dominant Day_0 - 1_Dominant Day_7         -0.71840 0.294 77  -2.444  0.5223
##  1_Dominant Day_0 - 2_Subordinate Day_7       0.08328 0.294 77   0.283  1.0000
##  1_Dominant Day_0 - 3_Communal Day_7          0.08135 0.294 77   0.277  1.0000
##  1_Dominant Day_0 - 4_Isolate Day_7          -0.48387 0.294 77  -1.646  0.9540
##  1_Dominant Day_0 - 1_Dominant Day_IP        -0.42690 0.294 77  -1.452  0.9846
##  1_Dominant Day_0 - 2_Subordinate Day_IP     -0.71314 0.294 77  -2.426  0.5350
##  1_Dominant Day_0 - 3_Communal Day_IP        -0.33309 0.294 77  -1.133  0.9988
##  1_Dominant Day_0 - 4_Isolate Day_IP         -0.23153 0.294 77  -0.788  1.0000
##  2_Subordinate Day_0 - 3_Communal Day_0      -0.08210 0.294 77  -0.279  1.0000
##  2_Subordinate Day_0 - 4_Isolate Day_0       -0.01490 0.308 77  -0.048  1.0000
##  2_Subordinate Day_0 - 1_Dominant Day_14      0.35405 0.294 77   1.204  0.9977
##  2_Subordinate Day_0 - 2_Subordinate Day_14  -0.08538 0.308 77  -0.277  1.0000
##  2_Subordinate Day_0 - 3_Communal Day_14     -0.12756 0.294 77  -0.434  1.0000
##  2_Subordinate Day_0 - 4_Isolate Day_14       0.37407 0.308 77   1.213  0.9975
##  2_Subordinate Day_0 - 1_Dominant Day_7      -0.20397 0.294 77  -0.694  1.0000
##  2_Subordinate Day_0 - 2_Subordinate Day_7    0.59771 0.294 77   2.033  0.7991
##  2_Subordinate Day_0 - 3_Communal Day_7       0.59577 0.294 77   2.027  0.8029
##  2_Subordinate Day_0 - 4_Isolate Day_7        0.03056 0.294 77   0.104  1.0000
##  2_Subordinate Day_0 - 1_Dominant Day_IP      0.08753 0.294 77   0.298  1.0000
##  2_Subordinate Day_0 - 2_Subordinate Day_IP  -0.19871 0.294 77  -0.676  1.0000
##  2_Subordinate Day_0 - 3_Communal Day_IP      0.18134 0.294 77   0.617  1.0000
```

```
##  2_Subordinate Day_0 - 4_Isolate Day_IP       0.28290 0.294 77   0.962  0.9998
##  3_Communal Day_0 - 4_Isolate Day_0           0.06721 0.308 77   0.218  1.0000
##  3_Communal Day_0 - 1_Dominant Day_14         0.43615 0.294 77   1.484  0.9812
##  3_Communal Day_0 - 2_Subordinate Day_14     -0.00328 0.308 77  -0.011  1.0000
##  3_Communal Day_0 - 3_Communal Day_14        -0.04546 0.294 77  -0.155  1.0000
##  3_Communal Day_0 - 4_Isolate Day_14          0.45618 0.308 77   1.479  0.9817
##  3_Communal Day_0 - 1_Dominant Day_7         -0.12187 0.294 77  -0.415  1.0000
##  3_Communal Day_0 - 2_Subordinate Day_7       0.67981 0.294 77   2.312  0.6160
##  3_Communal Day_0 - 3_Communal Day_7          0.67788 0.294 77   2.306  0.6207
##  3_Communal Day_0 - 4_Isolate Day_7           0.11266 0.294 77   0.383  1.0000
##  3_Communal Day_0 - 1_Dominant Day_IP         0.16963 0.294 77   0.577  1.0000
##  3_Communal Day_0 - 2_Subordinate Day_IP     -0.11661 0.294 77  -0.397  1.0000
##  3_Communal Day_0 - 3_Communal Day_IP         0.26345 0.294 77   0.896  0.9999
##  3_Communal Day_0 - 4_Isolate Day_IP          0.36500 0.294 77   1.242  0.9968
##  4_Isolate Day_0 - 1_Dominant Day_14          0.36894 0.308 77   1.197  0.9978
##  4_Isolate Day_0 - 2_Subordinate Day_14      -0.07048 0.322 77  -0.219  1.0000
##  4_Isolate Day_0 - 3_Communal Day_14         -0.11267 0.308 77  -0.365  1.0000
##  4_Isolate Day_0 - 4_Isolate Day_14           0.38897 0.322 77   1.208  0.9976
##  4_Isolate Day_0 - 1_Dominant Day_7          -0.18908 0.308 77  -0.613  1.0000
##  4_Isolate Day_0 - 2_Subordinate Day_7        0.61260 0.308 77   1.987  0.8247
##  4_Isolate Day_0 - 3_Communal Day_7           0.61067 0.308 77   1.981  0.8281
##  4_Isolate Day_0 - 4_Isolate Day_7            0.04545 0.308 77   0.147  1.0000
##  4_Isolate Day_0 - 1_Dominant Day_IP          0.10243 0.308 77   0.332  1.0000
##  4_Isolate Day_0 - 2_Subordinate Day_IP      -0.18382 0.308 77  -0.596  1.0000
##  4_Isolate Day_0 - 3_Communal Day_IP          0.19624 0.308 77   0.636  1.0000
##  4_Isolate Day_0 - 4_Isolate Day_IP           0.29779 0.308 77   0.966  0.9998
##  1_Dominant Day_14 - 2_Subordinate Day_14    -0.43943 0.308 77  -1.425  0.9871
##  1_Dominant Day_14 - 3_Communal Day_14       -0.48161 0.294 77  -1.638  0.9557
##  1_Dominant Day_14 - 4_Isolate Day_14         0.02003 0.308 77   0.065  1.0000
##  1_Dominant Day_14 - 1_Dominant Day_7        -0.55802 0.294 77  -1.898  0.8687
##  1_Dominant Day_14 - 2_Subordinate Day_7      0.24366 0.294 77   0.829  1.0000
##  1_Dominant Day_14 - 3_Communal Day_7         0.24172 0.294 77   0.822  1.0000
##  1_Dominant Day_14 - 4_Isolate Day_7         -0.32349 0.294 77  -1.100  0.9992
##  1_Dominant Day_14 - 1_Dominant Day_IP       -0.26652 0.294 77  -0.907  0.9999
##  1_Dominant Day_14 - 2_Subordinate Day_IP    -0.55276 0.294 77  -1.880  0.8767
##  1_Dominant Day_14 - 3_Communal Day_IP       -0.17271 0.294 77  -0.587  1.0000
##  1_Dominant Day_14 - 4_Isolate Day_IP        -0.07115 0.294 77  -0.242  1.0000
##  2_Subordinate Day_14 - 3_Communal Day_14    -0.04218 0.308 77  -0.137  1.0000
##  2_Subordinate Day_14 - 4_Isolate Day_14      0.45945 0.322 77   1.427  0.9870
##  2_Subordinate Day_14 - 1_Dominant Day_7     -0.11859 0.308 77  -0.385  1.0000
##  2_Subordinate Day_14 - 2_Subordinate Day_7   0.68309 0.308 77   2.215  0.6838
##  2_Subordinate Day_14 - 3_Communal Day_7      0.68115 0.308 77   2.209  0.6880
##  2_Subordinate Day_14 - 4_Isolate Day_7       0.11593 0.308 77   0.376  1.0000
##  2_Subordinate Day_14 - 1_Dominant Day_IP     0.17291 0.308 77   0.561  1.0000
##  2_Subordinate Day_14 - 2_Subordinate Day_IP -0.11333 0.308 77  -0.368  1.0000
##  2_Subordinate Day_14 - 3_Communal Day_IP     0.26672 0.308 77   0.865  1.0000
##  2_Subordinate Day_14 - 4_Isolate Day_IP      0.36827 0.308 77   1.194  0.9979
##  3_Communal Day_14 - 4_Isolate Day_14         0.50164 0.308 77   1.627  0.9582
##  3_Communal Day_14 - 1_Dominant Day_7        -0.07641 0.294 77  -0.260  1.0000
##  3_Communal Day_14 - 2_Subordinate Day_7      0.72527 0.294 77   2.467  0.5057
##  3_Communal Day_14 - 3_Communal Day_7         0.72333 0.294 77   2.460  0.5103
##  3_Communal Day_14 - 4_Isolate Day_7          0.15812 0.294 77   0.538  1.0000
##  3_Communal Day_14 - 1_Dominant Day_IP        0.21509 0.294 77   0.732  1.0000
##  3_Communal Day_14 - 2_Subordinate Day_IP    -0.07115 0.294 77  -0.242  1.0000
```

```
##  3_Communal Day_14 - 3_Communal Day_IP        0.30890 0.294 77   1.051  0.9995
##  3_Communal Day_14 - 4_Isolate Day_IP         0.41046 0.294 77   1.396  0.9894
##  4_Isolate Day_14 - 1_Dominant Day_7         -0.57805 0.308 77  -1.875  0.8790
##  4_Isolate Day_14 - 2_Subordinate Day_7       0.22363 0.308 77   0.725  1.0000
##  4_Isolate Day_14 - 3_Communal Day_7          0.22170 0.308 77   0.719  1.0000
##  4_Isolate Day_14 - 4_Isolate Day_7          -0.34352 0.308 77  -1.114  0.9990
##  4_Isolate Day_14 - 1_Dominant Day_IP        -0.28654 0.308 77  -0.929  0.9999
##  4_Isolate Day_14 - 2_Subordinate Day_IP     -0.57279 0.308 77  -1.858  0.8863
##  4_Isolate Day_14 - 3_Communal Day_IP        -0.19273 0.308 77  -0.625  1.0000
##  4_Isolate Day_14 - 4_Isolate Day_IP         -0.09118 0.308 77  -0.296  1.0000
##  1_Dominant Day_7 - 2_Subordinate Day_7       0.80168 0.294 77   2.727  0.3335
##  1_Dominant Day_7 - 3_Communal Day_7          0.79974 0.294 77   2.720  0.3375
##  1_Dominant Day_7 - 4_Isolate Day_7           0.23453 0.294 77   0.798  1.0000
##  1_Dominant Day_7 - 1_Dominant Day_IP         0.29150 0.294 77   0.992  0.9997
##  1_Dominant Day_7 - 2_Subordinate Day_IP      0.00526 0.294 77   0.018  1.0000
##  1_Dominant Day_7 - 3_Communal Day_IP         0.38531 0.294 77   1.311  0.9943
##  1_Dominant Day_7 - 4_Isolate Day_IP          0.48687 0.294 77   1.656  0.9517
##  2_Subordinate Day_7 - 3_Communal Day_7      -0.00193 0.294 77  -0.007  1.0000
##  2_Subordinate Day_7 - 4_Isolate Day_7       -0.56715 0.294 77  -1.929  0.8541
##  2_Subordinate Day_7 - 1_Dominant Day_IP     -0.51018 0.294 77  -1.735  0.9304
##  2_Subordinate Day_7 - 2_Subordinate Day_IP  -0.79642 0.294 77  -2.709  0.3444
##  2_Subordinate Day_7 - 3_Communal Day_IP     -0.41636 0.294 77  -1.416  0.9878
##  2_Subordinate Day_7 - 4_Isolate Day_IP      -0.31481 0.294 77  -1.071  0.9994
##  3_Communal Day_7 - 4_Isolate Day_7          -0.56522 0.294 77  -1.923  0.8572
##  3_Communal Day_7 - 1_Dominant Day_IP        -0.50824 0.294 77  -1.729  0.9323
##  3_Communal Day_7 - 2_Subordinate Day_IP     -0.79448 0.294 77  -2.702  0.3484
##  3_Communal Day_7 - 3_Communal Day_IP        -0.41443 0.294 77  -1.410  0.9884
##  3_Communal Day_7 - 4_Isolate Day_IP         -0.31288 0.294 77  -1.064  0.9994
##  4_Isolate Day_7 - 1_Dominant Day_IP          0.05698 0.294 77   0.194  1.0000
##  4_Isolate Day_7 - 2_Subordinate Day_IP      -0.22927 0.294 77  -0.780  1.0000
##  4_Isolate Day_7 - 3_Communal Day_IP          0.15079 0.294 77   0.513  1.0000
##  4_Isolate Day_7 - 4_Isolate Day_IP           0.25234 0.294 77   0.858  1.0000
##  1_Dominant Day_IP - 2_Subordinate Day_IP    -0.28624 0.294 77  -0.974  0.9998
##  1_Dominant Day_IP - 3_Communal Day_IP        0.09381 0.294 77   0.319  1.0000
##  1_Dominant Day_IP - 4_Isolate Day_IP         0.19536 0.294 77   0.665  1.0000
##  2_Subordinate Day_IP - 3_Communal Day_IP     0.38005 0.294 77   1.293  0.9951
##  2_Subordinate Day_IP - 4_Isolate Day_IP      0.48161 0.294 77   1.638  0.9557
##  3_Communal Day_IP - 4_Isolate Day_IP         0.10155 0.294 77   0.345  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
##                             0.0412032                             0.0008122
##               Social.Status3_Communal                Social.Status4_Isolate
##                             0.0014363                            -0.0009784
##                             DayDay_14                              DayDay_7
```

```
##                             0.0017846                             -0.0176223
##                             DayDay_IP  Social.Status2_Subordinate:DayDay_14
##                            -0.0131972                              0.0413659
##    Social.Status3_Communal:DayDay_14      Social.Status4_Isolate:DayDay_14
##                             0.0375360                             -0.0129372
##  Social.Status2_Subordinate:DayDay_7      Social.Status3_Communal:DayDay_7
##                             0.0186037                              0.0033917
##      Social.Status4_Isolate:DayDay_7  Social.Status2_Subordinate:DayDay_IP
##                             0.0061472                             -0.0091233
##    Social.Status3_Communal:DayDay_IP      Social.Status4_Isolate:DayDay_IP
##                             0.0316356                              0.0186529
```

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

```
##
## Call:
## lm(formula = simp.even ~ Social.Status * Day, data = diversity)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.051729 -0.014581 -0.003174  0.010015  0.105826
##
## Coefficients:
##                                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)                          0.0412032  0.0112315   3.669 0.000447 ***
## Social.Status2_Subordinate           0.0008122  0.0158838   0.051 0.959352
## Social.Status3_Communal              0.0014363  0.0158838   0.090 0.928183
## Social.Status4_Isolate              -0.0009784  0.0166590  -0.059 0.953319
## DayDay_14                            0.0017846  0.0158838   0.112 0.910836
## DayDay_7                            -0.0176223  0.0158838  -1.109 0.270687
## DayDay_IP                           -0.0131972  0.0158838  -0.831 0.408621
## Social.Status2_Subordinate:DayDay_14 0.0413659  0.0230177   1.797 0.076236 .
## Social.Status3_Communal:DayDay_14    0.0375360  0.0224630   1.671 0.098779 .
## Social.Status4_Isolate:DayDay_14    -0.0129372  0.0235594  -0.549 0.584506
## Social.Status2_Subordinate:DayDay_7  0.0186037  0.0224630   0.828 0.410122
## Social.Status3_Communal:DayDay_7     0.0033917  0.0224630   0.151 0.880380
## Social.Status4_Isolate:DayDay_7      0.0061472  0.0230177   0.267 0.790136
## Social.Status2_Subordinate:DayDay_IP -0.0091233  0.0224630  -0.406 0.685762
## Social.Status3_Communal:DayDay_IP    0.0316356  0.0224630   1.408 0.163054
## Social.Status4_Isolate:DayDay_IP     0.0186529  0.0230177   0.810 0.420224
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.02751 on 77 degrees of freedom
## Multiple R-squared:  0.3426, Adjusted R-squared:  0.2145
## F-statistic: 2.675 on 15 and 77 DF,  p-value: 0.002555
```

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

```
## Analysis of Variance Table
##
## Response: simp.even
##                   Df   Sum Sq   Mean Sq F value   Pr(>F)
```

```
## Social.Status      3 0.005842 0.0019475  2.5730 0.060097 .
## Day                3 0.010286 0.0034288  4.5301 0.005598 **
## Social.Status:Day  9 0.014243 0.0015826  2.0909 0.040392 *
## Residuals         77 0.058280 0.0007569
## ---
## 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  0.0415 0.00575 77   0.0301   0.0530
##  Day_14 0.0598 0.00589 77   0.0481   0.0715
##  Day_7  0.0309 0.00562 77   0.0198   0.0421
##  Day_IP 0.0386 0.00562 77   0.0274   0.0498
##
## 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  -0.01828 0.00823 77  -2.219  0.1271
##  Day_0 - Day_7    0.01059 0.00804 77   1.317  0.5553
##  Day_0 - Day_IP   0.00291 0.00804 77   0.361  0.9837
##  Day_14 - Day_7   0.02886 0.00814 77   3.547  0.0037
##  Day_14 - Day_IP  0.02118 0.00814 77   2.603  0.0529
##  Day_7 - Day_IP  -0.00768 0.00794 77  -0.967  0.7685
##
## Results are averaged over the levels of: Social.Status
## P value adjustment: tukey method for comparing a family of 4 estimates
```

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

```
## $emmeans
##  Social.Status Day    emmean     SE df lower.CL upper.CL
##  1_Dominant    Day_0  0.0412 0.0112 77  0.01884   0.0636
##  2_Subordinate Day_0  0.0420 0.0112 77  0.01965   0.0644
##  3_Communal    Day_0  0.0426 0.0112 77  0.02027   0.0650
##  4_Isolate     Day_0  0.0402 0.0123 77  0.01573   0.0647
##  1_Dominant    Day_14 0.0430 0.0112 77  0.02062   0.0654
##  2_Subordinate Day_14 0.0852 0.0123 77  0.06067   0.1097
##  3_Communal    Day_14 0.0820 0.0112 77  0.05960   0.1043
##  4_Isolate     Day_14 0.0291 0.0123 77  0.00457   0.0536
##  1_Dominant    Day_7  0.0236 0.0112 77  0.00122   0.0459
##  2_Subordinate Day_7  0.0430 0.0112 77  0.02063   0.0654
##  3_Communal    Day_7  0.0284 0.0112 77  0.00604   0.0508
##  4_Isolate     Day_7  0.0287 0.0112 77  0.00638   0.0511
##  1_Dominant    Day_IP 0.0280 0.0112 77  0.00564   0.0504
##  2_Subordinate Day_IP 0.0197 0.0112 77 -0.00267   0.0421
##  3_Communal    Day_IP 0.0611 0.0112 77  0.03871   0.0834
```

```
##  4_Isolate     Day_IP 0.0457 0.0112 77  0.02332   0.0680
##
## Confidence level used: 0.95
##
## $contrasts
##  contrast                                      estimate     SE df t.ratio
##  1_Dominant Day_0 - 2_Subordinate Day_0      -8.12e-04 0.0159 77  -0.051
##  1_Dominant Day_0 - 3_Communal Day_0         -1.44e-03 0.0159 77  -0.090
##  1_Dominant Day_0 - 4_Isolate Day_0           9.78e-04 0.0167 77   0.059
##  1_Dominant Day_0 - 1_Dominant Day_14        -1.78e-03 0.0159 77  -0.112
##  1_Dominant Day_0 - 2_Subordinate Day_14     -4.40e-02 0.0167 77  -2.639
##  1_Dominant Day_0 - 3_Communal Day_14        -4.08e-02 0.0159 77  -2.566
##  1_Dominant Day_0 - 4_Isolate Day_14          1.21e-02 0.0167 77   0.728
##  1_Dominant Day_0 - 1_Dominant Day_7          1.76e-02 0.0159 77   1.109
##  1_Dominant Day_0 - 2_Subordinate Day_7      -1.79e-03 0.0159 77  -0.113
##  1_Dominant Day_0 - 3_Communal Day_7          1.28e-02 0.0159 77   0.805
##  1_Dominant Day_0 - 4_Isolate Day_7           1.25e-02 0.0159 77   0.784
##  1_Dominant Day_0 - 1_Dominant Day_IP         1.32e-02 0.0159 77   0.831
##  1_Dominant Day_0 - 2_Subordinate Day_IP      2.15e-02 0.0159 77   1.354
##  1_Dominant Day_0 - 3_Communal Day_IP        -1.99e-02 0.0159 77  -1.251
##  1_Dominant Day_0 - 4_Isolate Day_IP         -4.48e-03 0.0159 77  -0.282
##  2_Subordinate Day_0 - 3_Communal Day_0      -6.24e-04 0.0159 77  -0.039
##  2_Subordinate Day_0 - 4_Isolate Day_0        1.79e-03 0.0167 77   0.107
##  2_Subordinate Day_0 - 1_Dominant Day_14     -9.72e-04 0.0159 77  -0.061
##  2_Subordinate Day_0 - 2_Subordinate Day_14  -4.32e-02 0.0167 77  -2.590
##  2_Subordinate Day_0 - 3_Communal Day_14     -3.99e-02 0.0159 77  -2.515
##  2_Subordinate Day_0 - 4_Isolate Day_14       1.29e-02 0.0167 77   0.777
##  2_Subordinate Day_0 - 1_Dominant Day_7       1.84e-02 0.0159 77   1.161
##  2_Subordinate Day_0 - 2_Subordinate Day_7   -9.81e-04 0.0159 77  -0.062
##  2_Subordinate Day_0 - 3_Communal Day_7       1.36e-02 0.0159 77   0.857
##  2_Subordinate Day_0 - 4_Isolate Day_7        1.33e-02 0.0159 77   0.835
##  2_Subordinate Day_0 - 1_Dominant Day_IP      1.40e-02 0.0159 77   0.882
##  2_Subordinate Day_0 - 2_Subordinate Day_IP   2.23e-02 0.0159 77   1.405
##  2_Subordinate Day_0 - 3_Communal Day_IP     -1.91e-02 0.0159 77  -1.200
##  2_Subordinate Day_0 - 4_Isolate Day_IP      -3.67e-03 0.0159 77  -0.231
##  3_Communal Day_0 - 4_Isolate Day_0           2.41e-03 0.0167 77   0.145
##  3_Communal Day_0 - 1_Dominant Day_14        -3.48e-04 0.0159 77  -0.022
##  3_Communal Day_0 - 2_Subordinate Day_14     -4.25e-02 0.0167 77  -2.553
##  3_Communal Day_0 - 3_Communal Day_14        -3.93e-02 0.0159 77  -2.476
##  3_Communal Day_0 - 4_Isolate Day_14          1.36e-02 0.0167 77   0.814
##  3_Communal Day_0 - 1_Dominant Day_7          1.91e-02 0.0159 77   1.200
##  3_Communal Day_0 - 2_Subordinate Day_7      -3.57e-04 0.0159 77  -0.022
##  3_Communal Day_0 - 3_Communal Day_7          1.42e-02 0.0159 77   0.896
##  3_Communal Day_0 - 4_Isolate Day_7           1.39e-02 0.0159 77   0.874
##  3_Communal Day_0 - 1_Dominant Day_IP         1.46e-02 0.0159 77   0.921
##  3_Communal Day_0 - 2_Subordinate Day_IP      2.29e-02 0.0159 77   1.445
##  3_Communal Day_0 - 3_Communal Day_IP        -1.84e-02 0.0159 77  -1.161
##  3_Communal Day_0 - 4_Isolate Day_IP         -3.04e-03 0.0159 77  -0.191
##  4_Isolate Day_0 - 1_Dominant Day_14         -2.76e-03 0.0167 77  -0.166
##  4_Isolate Day_0 - 2_Subordinate Day_14      -4.49e-02 0.0174 77  -2.583
##  4_Isolate Day_0 - 3_Communal Day_14         -4.17e-02 0.0167 77  -2.505
##  4_Isolate Day_0 - 4_Isolate Day_14           1.12e-02 0.0174 77   0.641
##  4_Isolate Day_0 - 1_Dominant Day_7           1.66e-02 0.0167 77   0.999
##  4_Isolate Day_0 - 2_Subordinate Day_7       -2.77e-03 0.0167 77  -0.166
```

```
##  4_Isolate Day_0 - 3_Communal Day_7           1.18e-02 0.0167 77   0.709
##  4_Isolate Day_0 - 4_Isolate Day_7            1.15e-02 0.0167 77   0.689
##  4_Isolate Day_0 - 1_Dominant Day_IP          1.22e-02 0.0167 77   0.733
##  4_Isolate Day_0 - 2_Subordinate Day_IP       2.05e-02 0.0167 77   1.232
##  4_Isolate Day_0 - 3_Communal Day_IP         -2.09e-02 0.0167 77  -1.252
##  4_Isolate Day_0 - 4_Isolate Day_IP          -5.46e-03 0.0167 77  -0.327
##  1_Dominant Day_14 - 2_Subordinate Day_14    -4.22e-02 0.0167 77  -2.532
##  1_Dominant Day_14 - 3_Communal Day_14       -3.90e-02 0.0159 77  -2.454
##  1_Dominant Day_14 - 4_Isolate Day_14         1.39e-02 0.0167 77   0.835
##  1_Dominant Day_14 - 1_Dominant Day_7         1.94e-02 0.0159 77   1.222
##  1_Dominant Day_14 - 2_Subordinate Day_7     -8.98e-06 0.0159 77  -0.001
##  1_Dominant Day_14 - 3_Communal Day_7         1.46e-02 0.0159 77   0.918
##  1_Dominant Day_14 - 4_Isolate Day_7          1.42e-02 0.0159 77   0.896
##  1_Dominant Day_14 - 1_Dominant Day_IP        1.50e-02 0.0159 77   0.943
##  1_Dominant Day_14 - 2_Subordinate Day_IP     2.33e-02 0.0159 77   1.466
##  1_Dominant Day_14 - 3_Communal Day_IP       -1.81e-02 0.0159 77  -1.139
##  1_Dominant Day_14 - 4_Isolate Day_IP        -2.69e-03 0.0159 77  -0.170
##  2_Subordinate Day_14 - 3_Communal Day_14     3.21e-03 0.0167 77   0.192
##  2_Subordinate Day_14 - 4_Isolate Day_14      5.61e-02 0.0174 77   3.224
##  2_Subordinate Day_14 - 1_Dominant Day_7      6.16e-02 0.0167 77   3.697
##  2_Subordinate Day_14 - 2_Subordinate Day_7   4.22e-02 0.0167 77   2.531
##  2_Subordinate Day_14 - 3_Communal Day_7      5.68e-02 0.0167 77   3.407
##  2_Subordinate Day_14 - 4_Isolate Day_7       5.64e-02 0.0167 77   3.387
##  2_Subordinate Day_14 - 1_Dominant Day_IP     5.72e-02 0.0167 77   3.431
##  2_Subordinate Day_14 - 2_Subordinate Day_IP  6.55e-02 0.0167 77   3.930
##  2_Subordinate Day_14 - 3_Communal Day_IP     2.41e-02 0.0167 77   1.446
##  2_Subordinate Day_14 - 4_Isolate Day_IP      3.95e-02 0.0167 77   2.370
##  3_Communal Day_14 - 4_Isolate Day_14         5.29e-02 0.0167 77   3.175
##  3_Communal Day_14 - 1_Dominant Day_7         5.84e-02 0.0159 77   3.675
##  3_Communal Day_14 - 2_Subordinate Day_7      3.90e-02 0.0159 77   2.453
##  3_Communal Day_14 - 3_Communal Day_7         5.36e-02 0.0159 77   3.371
##  3_Communal Day_14 - 4_Isolate Day_7          5.32e-02 0.0159 77   3.350
##  3_Communal Day_14 - 1_Dominant Day_IP        5.40e-02 0.0159 77   3.397
##  3_Communal Day_14 - 2_Subordinate Day_IP     6.23e-02 0.0159 77   3.920
##  3_Communal Day_14 - 3_Communal Day_IP        2.09e-02 0.0159 77   1.315
##  3_Communal Day_14 - 4_Isolate Day_IP         3.63e-02 0.0159 77   2.284
##  4_Isolate Day_14 - 1_Dominant Day_7          5.49e-03 0.0167 77   0.330
##  4_Isolate Day_14 - 2_Subordinate Day_7      -1.39e-02 0.0167 77  -0.836
##  4_Isolate Day_14 - 3_Communal Day_7          6.63e-04 0.0167 77   0.040
##  4_Isolate Day_14 - 4_Isolate Day_7           3.23e-04 0.0167 77   0.019
##  4_Isolate Day_14 - 1_Dominant Day_IP         1.07e-03 0.0167 77   0.064
##  4_Isolate Day_14 - 2_Subordinate Day_IP      9.38e-03 0.0167 77   0.563
##  4_Isolate Day_14 - 3_Communal Day_IP        -3.20e-02 0.0167 77  -1.921
##  4_Isolate Day_14 - 4_Isolate Day_IP         -1.66e-02 0.0167 77  -0.997
##  1_Dominant Day_7 - 2_Subordinate Day_7      -1.94e-02 0.0159 77  -1.222
##  1_Dominant Day_7 - 3_Communal Day_7         -4.83e-03 0.0159 77  -0.304
##  1_Dominant Day_7 - 4_Isolate Day_7          -5.17e-03 0.0159 77  -0.325
##  1_Dominant Day_7 - 1_Dominant Day_IP        -4.43e-03 0.0159 77  -0.279
##  1_Dominant Day_7 - 2_Subordinate Day_IP      3.89e-03 0.0159 77   0.245
##  1_Dominant Day_7 - 3_Communal Day_IP        -3.75e-02 0.0159 77  -2.361
##  1_Dominant Day_7 - 4_Isolate Day_IP         -2.21e-02 0.0159 77  -1.391
##  2_Subordinate Day_7 - 3_Communal Day_7       1.46e-02 0.0159 77   0.918
##  2_Subordinate Day_7 - 4_Isolate Day_7        1.42e-02 0.0159 77   0.897
##  2_Subordinate Day_7 - 1_Dominant Day_IP      1.50e-02 0.0159 77   0.944
```

```
##  2_Subordinate Day_7 - 2_Subordinate Day_IP   2.33e-02 0.0159 77   1.467
##  2_Subordinate Day_7 - 3_Communal Day_IP     -1.81e-02 0.0159 77  -1.138
##  2_Subordinate Day_7 - 4_Isolate Day_IP      -2.68e-03 0.0159 77  -0.169
##  3_Communal Day_7 - 4_Isolate Day_7          -3.41e-04 0.0159 77  -0.021
##  3_Communal Day_7 - 1_Dominant Day_IP         4.03e-04 0.0159 77   0.025
##  3_Communal Day_7 - 2_Subordinate Day_IP      8.71e-03 0.0159 77   0.549
##  3_Communal Day_7 - 3_Communal Day_IP        -3.27e-02 0.0159 77  -2.057
##  3_Communal Day_7 - 4_Isolate Day_IP         -1.73e-02 0.0159 77  -1.087
##  4_Isolate Day_7 - 1_Dominant Day_IP          7.44e-04 0.0159 77   0.047
##  4_Isolate Day_7 - 2_Subordinate Day_IP       9.05e-03 0.0159 77   0.570
##  4_Isolate Day_7 - 3_Communal Day_IP         -3.23e-02 0.0159 77  -2.035
##  4_Isolate Day_7 - 4_Isolate Day_IP          -1.69e-02 0.0159 77  -1.066
##  1_Dominant Day_IP - 2_Subordinate Day_IP     8.31e-03 0.0159 77   0.523
##  1_Dominant Day_IP - 3_Communal Day_IP       -3.31e-02 0.0159 77  -2.082
##  1_Dominant Day_IP - 4_Isolate Day_IP        -1.77e-02 0.0159 77  -1.113
##  2_Subordinate Day_IP - 3_Communal Day_IP    -4.14e-02 0.0159 77  -2.605
##  2_Subordinate Day_IP - 4_Isolate Day_IP     -2.60e-02 0.0159 77  -1.636
##  3_Communal Day_IP - 4_Isolate Day_IP         1.54e-02 0.0159 77   0.969
##  p.value
##   1.0000
##   1.0000
##   1.0000
##   1.0000
##   0.3884
##   0.4369
##   1.0000
##   0.9991
##   1.0000
##   1.0000
##   1.0000
##   1.0000
##   0.9921
##   0.9965
##   1.0000
##   1.0000
##   1.0000
##   1.0000
##   0.4206
##   0.4721
##   1.0000
##   0.9985
##   1.0000
##   1.0000
##   1.0000
##   0.9999
##   0.9887
##   0.9978
##   1.0000
##   1.0000
##   1.0000
##   0.4459
##   0.4997
##   1.0000
##   0.9978
```

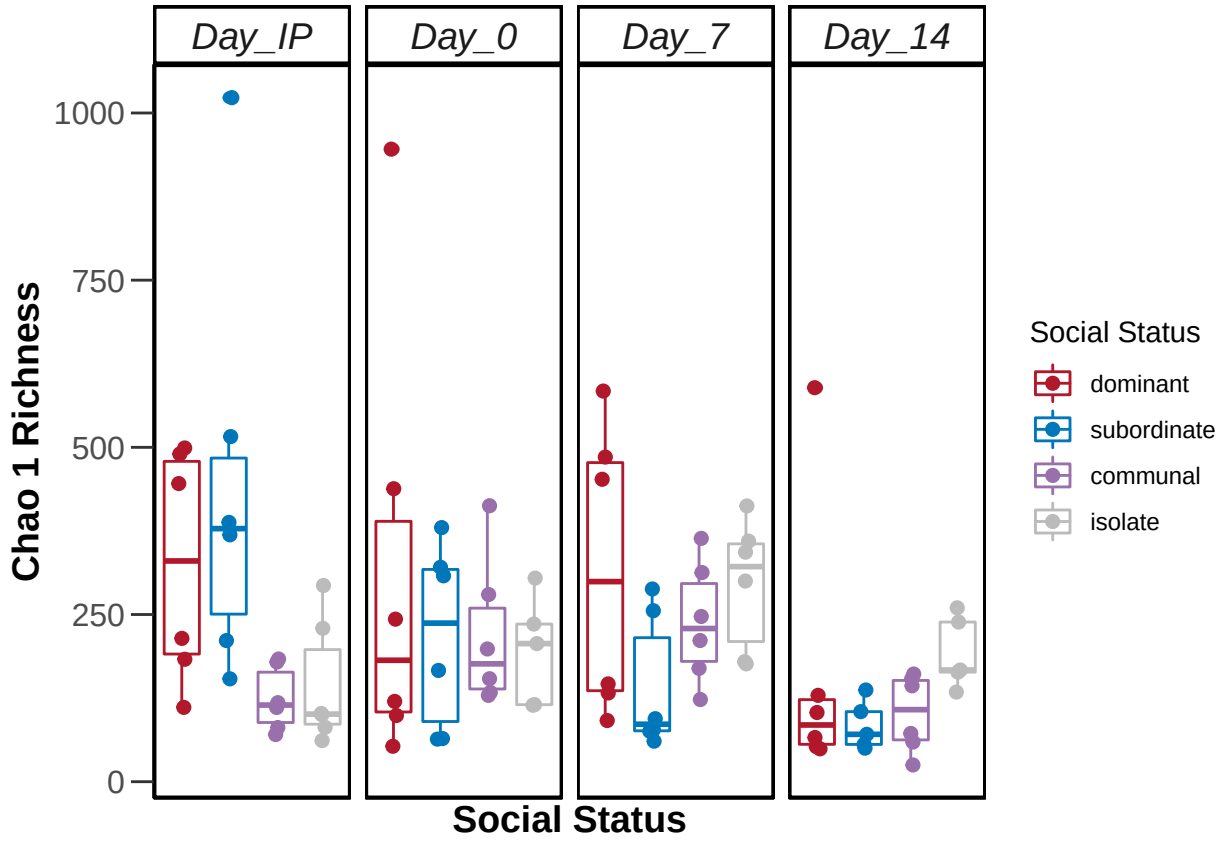
```
##   1.0000
##   0.9999
##   0.9999
##   0.9999
##   0.9853
##   0.9984
##   1.0000
##   1.0000
##   0.4255
##   0.4788
##   1.0000
##   0.9997
##   1.0000
##   1.0000
##   1.0000
##   1.0000
##   0.9970
##   0.9965
##   1.0000
##   0.4603
##   0.5152
##   1.0000
##   0.9973
##   1.0000
##   0.9999
##   0.9999
##   0.9999
##   0.9831
##   0.9987
##   1.0000
##   1.0000
##   0.1153
##   0.0320
##   0.4607
##   0.0722
##   0.0762
##   0.0677
##   0.0158
##   0.9852
##   0.5748
##   0.1299
##   0.0341
##   0.5156
##   0.0793
##   0.0839
##   0.0742
##   0.0163
##   0.9942
##   0.6361
##   1.0000
##   1.0000
##   1.0000
##   1.0000
##   1.0000
```

```
##   1.0000
##   0.8579
##   0.9997
##   0.9973
##   1.0000
##   1.0000
##   1.0000
##   1.0000
##   0.5816
##   0.9898
##   0.9999
##   0.9999
##   0.9999
##   0.9831
##   0.9988
##   1.0000
##   1.0000
##   1.0000
##   1.0000
##   0.7854
##   0.9993
##   1.0000
##   1.0000
##   0.7979
##   0.9994
##   1.0000
##   0.7702
##   0.9990
##   0.4105
##   0.9562
##   0.9998
##
## P value adjustment: tukey method for comparing a family of 16 estimates
```

#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
```

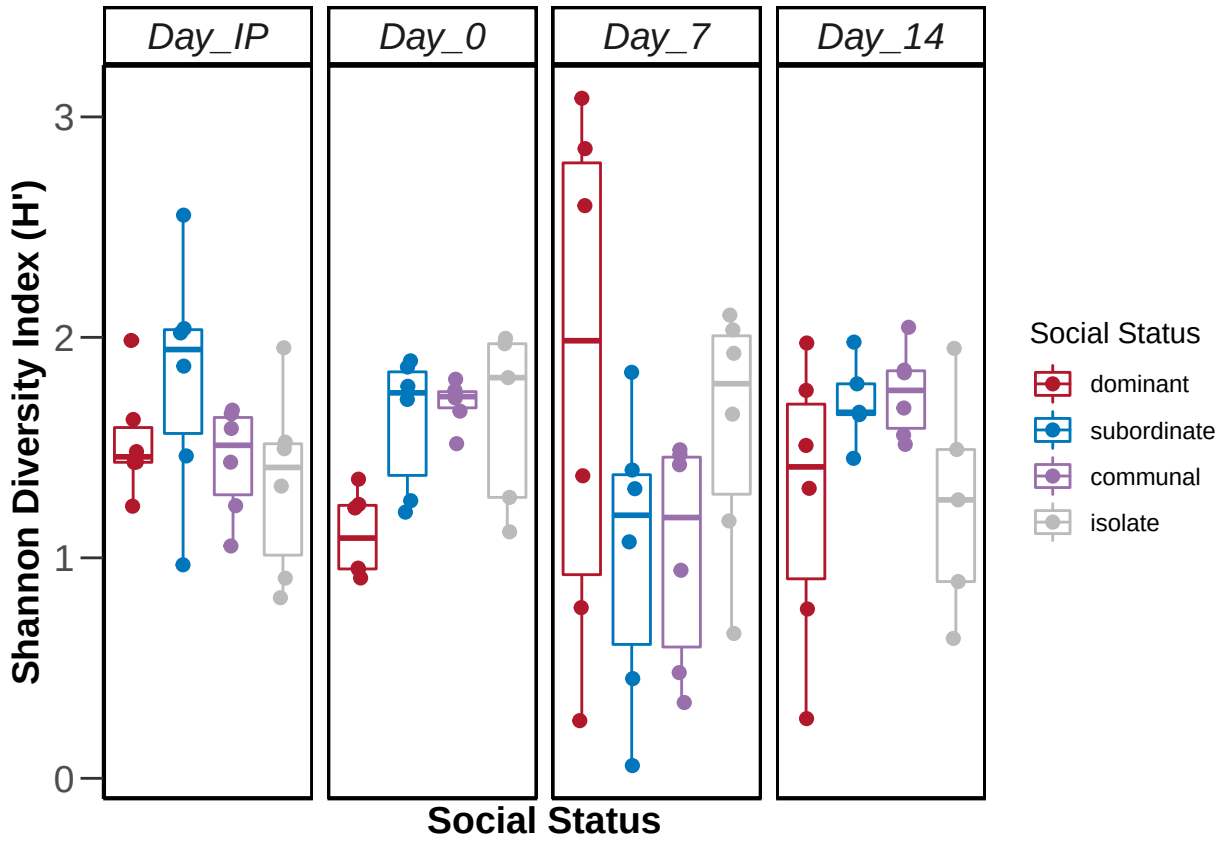



```
ggsave("../figures/chao1_updated.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=8, heigh
```

#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
```

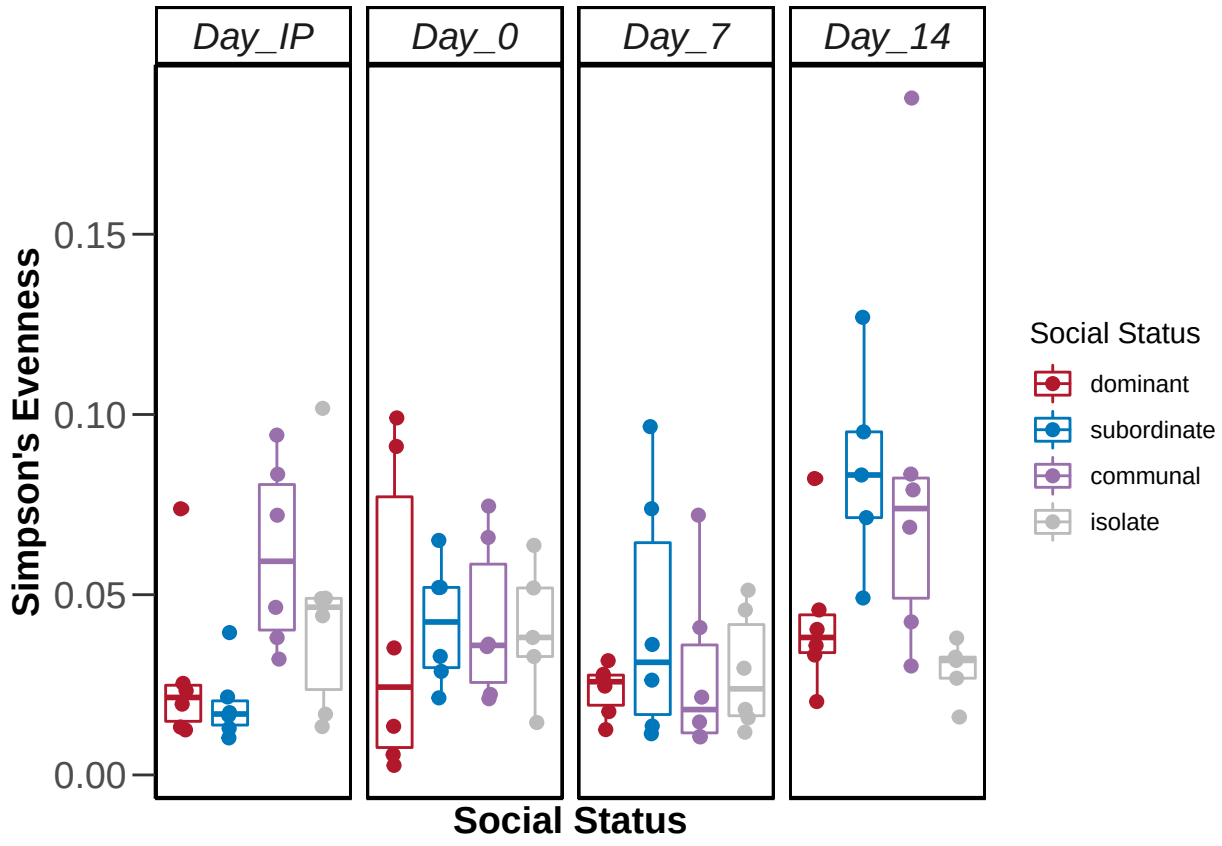



```
ggsave("../figures/shannon_updated.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=8, hei
```

#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_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
```




```
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 0.000999 ***
## 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
```

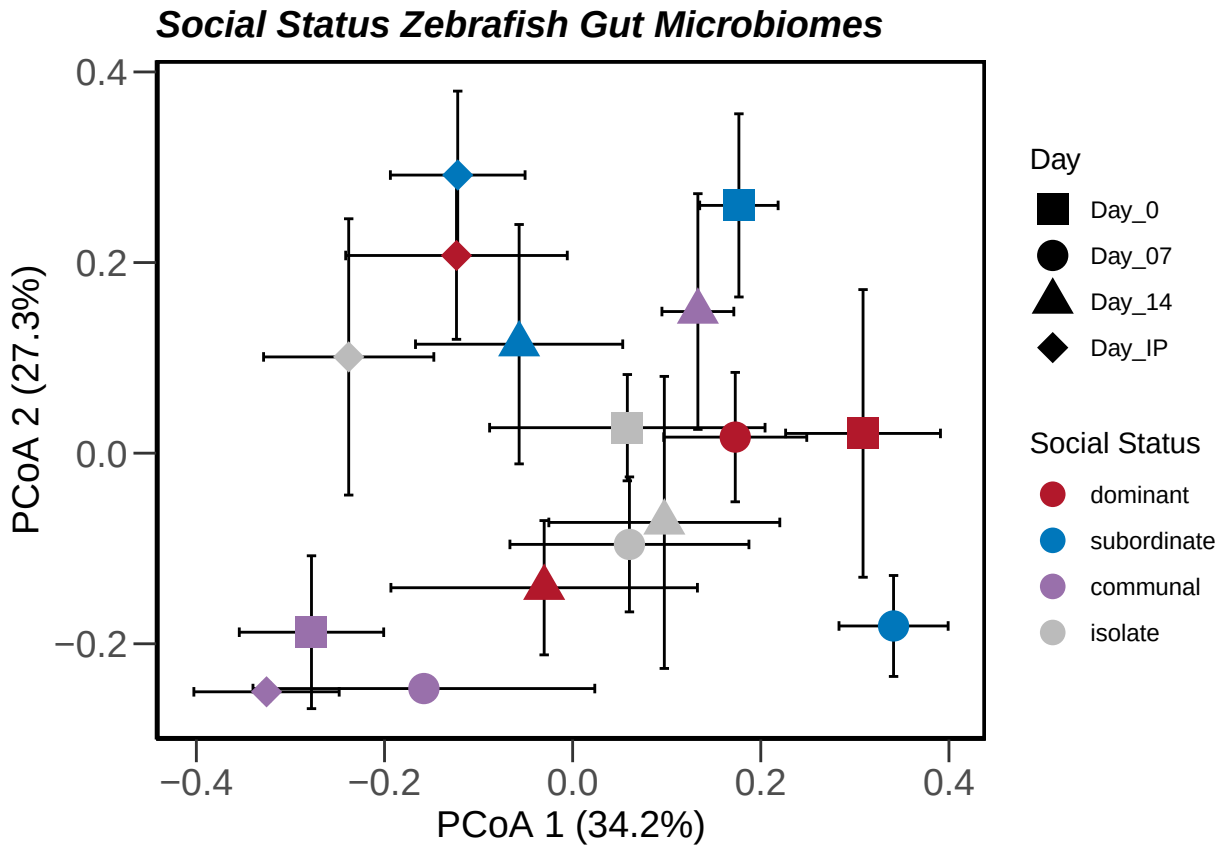
```
## [1] 34.2
```

```
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)
```

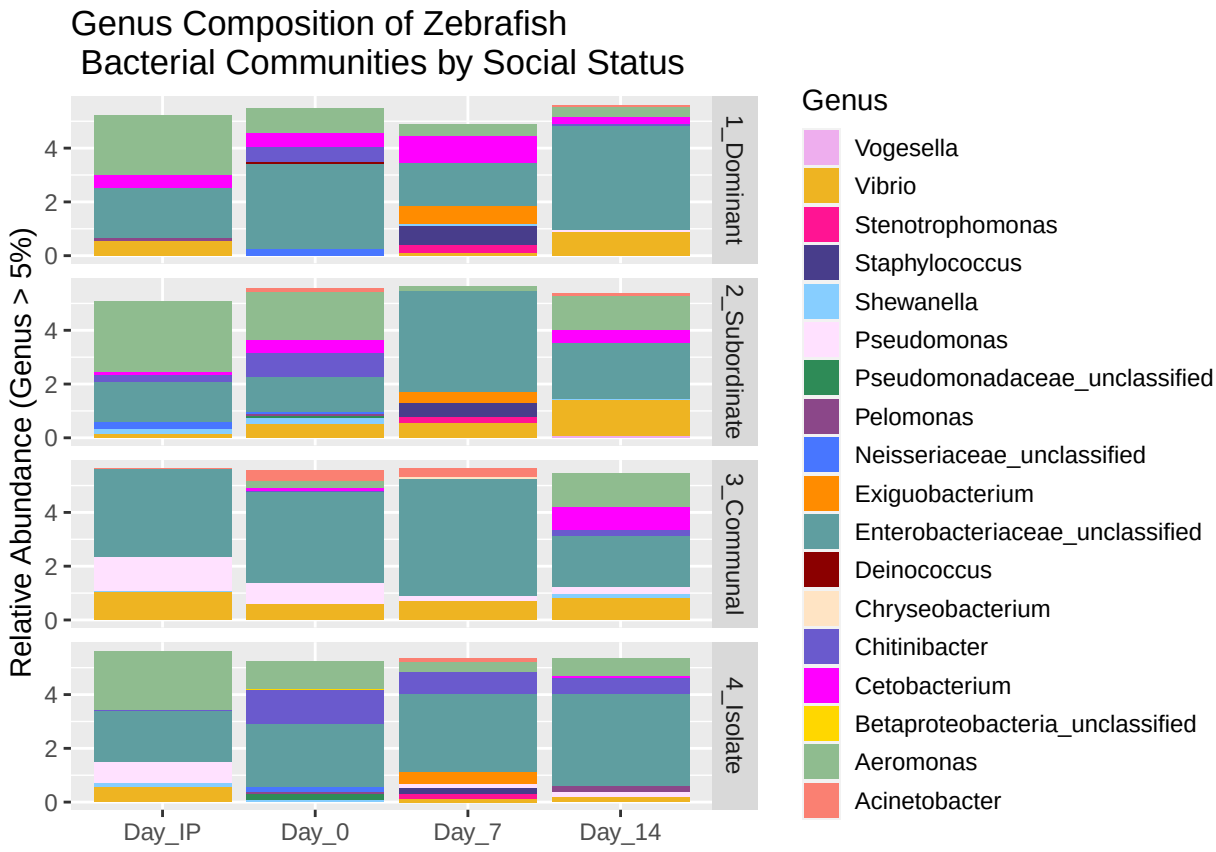
```
## `set.seed(TRUE)` was used to initialize repeatable random subsampling.

## 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
```