

03 Community analysis: who are the winners?

Angel Rain

01/03/2024

Contents

1	Setting up the workspace	1
1.1	Loading Packages	1
1.2	Loading colorblind palette	2
1.3	Load OTU table and metadata	2
1.4	Subsetting dataset	3
1.5	Use Genus level for downstream analysis	4
2	Elo-rating	4
2.1	Setting up dataset	4
2.2	Screen distribution of the ranking during the cycles (C0 to C6)	4
2.3	Ranking plot figure S1a	5
3	Load Elo-rating from iterative calculations	6
3.1	Getting mean value from the 1000 iterations	6
3.2	Explore relationship from Elo-rating and Abundance and occurrence	7
4	Occurrence vs Elo-rating (Figure S1b)	7
4.1	Abundance vs Elo-rating (Figure S1c)	7
4.2	Apply linear regression to each dataset	9
5	Figure Elo-rating changes (Fig. 5 ms)	10

1 Setting up the workspace

1.1 Loading Packages

```
rm(list = ls())
library(cowplot)
library(egg)
library(readxl)
library(FactoMineR)
library(factoextra)
library(RColorBrewer) #Expando color palette
library(dplyr)
library(stringr) # FOr editing string
library(reshape2) #For 'melt' function
library(correlation)
```

1.2 Loading colorblind palette

```
# Setting the colorblind palette
cbp1 <- c("#999999", "#FFDB6D", "#E69F00", "#56B4E9", "#009E73", "#F0E442",
          "#0072B2", "#D55E00", "#CC79A7", "#293352")
# Expanding the standard pallete Pastel1 for downstream analysis
mycolors <- colorRampPalette(brewer.pal(8, "Pastel1"))(31)
```

1.3 Load OTU table and metadata

```
df1 = data.frame(read.csv("../data/OTU_table_merged200_SILVA_megablast.csv",
  row.names = 1))
tibble(df1)
```

```
## # A tibble: 1,047 x 107
##   Kingdom Phylum      Class Order Family Genus Specie      C01      C010      C011
##   <chr>    <chr>      <chr> <chr> <chr> <chr> <chr>    <dbl>    <dbl>    <dbl>
## 1 Bacteria Proteobacte~ Gamm~ Ente~ Alter~ Rhei~ marin~ 0      0      0
## 2 Bacteria Bacteroidota Bact~ Flav~ Flav~ Flav~ marin~ 0.00893 1.24e-2 0.00206
## 3 Bacteria Chloroflexi SL56~ mari~ <NA> o_ma~ <NA> 0      3.44e-4 0
## 4 Bacteria Actinobacte~ Acid~ Micr~ Iluma~ f_Il~ marin~ 0      0      0
## 5 Bacteria Proteobacte~ Gamm~ Burk~ Comam~ f_Co~ marin~ 0      0      0
## 6 Bacteria Proteobacte~ Gamm~ Burk~ Comam~ Pelo~ Pelom~ 0      0      0
## 7 Bacteria Proteobacte~ Gamm~ Burk~ Comam~ f_Co~ <NA> 0      0      0
## 8 Bacteria Proteobacte~ Gamm~ Pseu~ Pseud~ Pseu~ bacte~ 0      0      0
## 9 Bacteria Bacteroidota Bact~ Flav~ Flav~ Flav~ Flav~ 0      1.75e-2 0
## 10 Bacteria Proteobacte~ Gamm~ Pseu~ Pseud~ Pseu~ Pseud~ 0      3.44e-4 0
## # i 1,037 more rows
## # i 97 more variables: C012 <dbl>, C013 <dbl>, C014 <dbl>, C015 <dbl>,
## #   C016 <dbl>, C017 <dbl>, C018 <dbl>, C019 <dbl>, C02 <dbl>, C020 <dbl>,
## #   C03 <dbl>, C04 <dbl>, C05 <dbl>, C06 <dbl>, C07 <dbl>, C08 <dbl>,
## #   C09 <dbl>, C11 <dbl>, C110 <dbl>, C111 <dbl>, C112 <dbl>, C113 <dbl>,
## #   C114 <dbl>, C115 <dbl>, C116 <dbl>, C117 <dbl>, C118 <dbl>, C119 <dbl>,
## #   C12 <dbl>, C120 <dbl>, C13 <dbl>, C14 <dbl>, C15 <dbl>, C16 <dbl>, ...
```

```
meta = data.frame(read.csv("../data/metadata_merged200_SILVA_megablast.csv",
  row.names = 1))
tibble(meta)
```

```
## # A tibble: 100 x 3
##   Cycle Replicate M.ID
##   <chr>      <int> <chr>
## 1 C0         1 C01
## 2 C0        10 C010
## 3 C0        11 C011
## 4 C0        12 C012
## 5 C0        13 C013
## 6 C0        14 C014
## 7 C0        15 C015
## 8 C0        16 C016
## 9 C0        17 C017
## 10 C0       18 C018
## # i 90 more rows
```

1.4 Subsetting dataset

```
# Overview by hierarchical level (Order, Family or Genus) aggregate
# counts by Genus
df2 <- aggregate(. ~ Genus, data = df1[, c(6, 8:107)], sum, na.rm = TRUE)
colSums(df2[, 2:101])
```

```
## C01 C010 C011 C012 C013 C014 C015 C016 C017 C018 C019 C02 C020 C03 C04 C05
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C06 C07 C08 C09 C11 C110 C111 C112 C113 C114 C115 C116 C117 C118 C119 C12
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C120 C13 C14 C15 C16 C17 C18 C19 C41 C410 C411 C412 C413 C414 C415 C416
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C417 C418 C419 C42 C420 C43 C44 C45 C46 C47 C48 C49 C61 C610 C611 C612
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C613 C614 C615 C616 C617 C618 C619 C62 C620 C63 C64 C65 C66 C67 C68 C69
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C71 C710 C711 C712 C713 C714 C715 C716 C717 C718 C719 C72 C720 C73 C74 C75
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C76 C77 C78 C79
## 1 1 1 1
```

```
order <- df2[, 1] #vector with orders
rownames(df2) <- df2[, 1] #rownames
df2 <- df2[, -1] #remove column with orders and keep only abundance data
colSums(df2) #test colSums to see if values close to 1 are reached
```

```
## C01 C010 C011 C012 C013 C014 C015 C016 C017 C018 C019 C02 C020 C03 C04 C05
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C06 C07 C08 C09 C11 C110 C111 C112 C113 C114 C115 C116 C117 C118 C119 C12
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
```

```
## C120 C13 C14 C15 C16 C17 C18 C19 C41 C410 C411 C412 C413 C414 C415 C416
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C417 C418 C419 C42 C420 C43 C44 C45 C46 C47 C48 C49 C61 C610 C611 C612
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C613 C614 C615 C616 C617 C618 C619 C62 C620 C63 C64 C65 C66 C67 C68 C69
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C71 C710 C711 C712 C713 C714 C715 C716 C717 C718 C719 C72 C720 C73 C74 C75
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
## C76 C77 C78 C79
## 1 1 1 1
```

1.5 Use Genus level for downstream analysis

```
### NEW STEP ## USE GENUS INSTEAD OF OTU LEVEL TAXONOMY ##
df2$Genus = row.names(df2)
tax.genus = unique(df1[, 1:6])
df1 = merge(tax.genus, df2, by = "Genus", all.x = TRUE)
row.names(df1) = df1$Genus
```

2 Elo-rating

2.1 Setting up dataset

```
meta$M.ID = as.factor(meta$M.ID)
meta$Replicate = as.factor(meta$Replicate)
```

```
# For microcosm
df.C0.C6 = df1[, c(7:106)]
dim(df.C0.C6)
```

```
## [1] 118 100
```

```
# Only those that represent 0.1% (comprising 41 Genera)
df.C0.C6 = df.C0.C6[which(rowMeans(df.C0.C6) > 0.001), ]
dim(df.C0.C6)
```

```
## [1] 41 100
```

```
# Get occurrence at C0 and C6
df.tmp.occurrence <- data.frame(C0 = rowSums(df.C0.C6[, c(1:20)] > 0),
  C6 = rowSums(df.C0.C6[, c(61:80)] > 0))
```

2.2 Screen distribution of the ranking during the cycles (C0 to C6)

```

ave.genus.cycles <- data.frame(Mean.abundance = rowMeans(df.C0.C6)) # Estimate average relative abundance

# Create dataframe for model fitting
M <- data.frame(Mean.abundance = ave.genus.cycles$Mean.abundance[order(-ave.genus.cycles$Mean.abundance)],
  PA = 1:dim(ave.genus.cycles)[1])

# Fitting model estimation
results.nls <- nls(Mean.abundance ~ ((alpha^(dim(ave.genus.cycles)[1] -
  PA)) - 1)/sum((alpha^(dim(ave.genus.cycles)[1] - PA)) - 1), data = M,
  start = list(alpha = 0.1))
summary(results.nls)

##
## Formula: Mean.abundance ~ ((alpha^(dim(ave.genus.cycles)[1] - PA)) - 1)/sum((alpha^(dim(ave.genus.cycles)[1] -
## PA)) - 1)
##
## Parameters:
##      Estimate Std. Error t value Pr(>|t|)
## alpha  1.22303    0.01409   86.81  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.01252 on 40 degrees of freedom
##
## Number of iterations to convergence: 10
## Achieved convergence tolerance: 3.057e-06

# Save results from nls model
M$Expected <- predict(results.nls)

# Get Pseudo-R2-square
print(1 - (deviance(results.nls)/sum((M$Mean.abundance - mean(M$Mean.abundance))^2)))

## [1] 0.9062178

```

2.3 Ranking plot figure S1a

```

plot.rank <- M %>%
  ggplot(aes(PA, Mean.abundance)) + geom_col(alpha = 0.7) + theme_bw() +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank()) +
  # geom_point(aes(PA,Expected),size=0.5)+
  geom_line(aes(PA, Expected)) + labs(y = "Average relative abundance", x = "") +
  geom_label(x = 20, y = 0.15, label = expression(frac("1.223"^{
    (italic(N) - italic(Pa))
  } * -1, sum(, italic(i) == 1, italic(N))("1.223"^{
    (italic(N) - italic(i))
  } * -1))), size = 3, label.size = NA)

```

3 Load Elo-rating from iterative calculations

Elo-rating calculated values from 1000 iterations we calculated in Python3 according <https://github.com/djcunningham0/multielo> were retrieve for downstream analysis in R.

```
Elo.0 = read.csv("../data/EloRating_C0_results1000iter.csv", header = T)[,
-1]
Elo.1 = read.csv("../data/EloRating_C1_results1000iter.csv", header = T)[,
-1]
Elo.4 = read.csv("../data/EloRating_C4_results1000iter.csv", header = T)[,
-1]
Elo.6 = read.csv("../data/EloRating_C6_results1000iter.csv", header = T)[,
-1]
```

3.1 Getting mean value from the 1000 iterations

```
sum.elo.0 = aggregate(. ~ player_id + n_games, data = Elo.0[, -1], mean)
sum.elo.0$Cycle = "C00"
head(sum.elo.0)
```

##	player_id	n_games	rating	Cycle
## 1	Bradyrhizobium	1	1000.1723	C00
## 2	Emticicia	1	990.3320	C00
## 3	Brevundimonas	2	995.2917	C00
## 4	f_Caulobacteraceae	2	1064.3713	C00
## 5	Caulobacter	3	1065.1167	C00
## 6	Chitinibacter	3	990.1293	C00

```
sum.elo.1 = aggregate(. ~ player_id + n_games, data = Elo.1[, -1], mean)
sum.elo.1$Cycle = "C01"
head(sum.elo.1)
```

##	player_id	n_games	rating	Cycle
## 1	Bradyrhizobium	1	1024.644	C01
## 2	Emticicia	1	1015.626	C01
## 3	Brevundimonas	2	1040.649	C01
## 4	f_Caulobacteraceae	2	1062.287	C01
## 5	Caulobacter	3	1102.510	C01
## 6	Chitinibacter	3	1023.327	C01

```
sum.elo.4 = aggregate(. ~ player_id + n_games, data = Elo.4[, -1], mean)
sum.elo.4$Cycle = "C04"
head(sum.elo.4)
```

##	player_id	n_games	rating	Cycle
## 1	Bradyrhizobium	1	1050.645	C04
## 2	Emticicia	1	1053.112	C04
## 3	Brevundimonas	2	1037.692	C04
## 4	f_Caulobacteraceae	2	1070.247	C04
## 5	Caulobacter	3	1063.884	C04
## 6	Chitinibacter	3	1006.275	C04

```
sum.elo.6 = aggregate(rating ~ player_id + n_games, data = Elo.6[, -1],
  mean)
sum.elo.6$Cycle = "C06"
head(sum.elo.6)
```

```
##           player_id n_games    rating Cycle
## 1   Bradyrhizobium      1 1049.6802   C06
## 2      Emticicia       1 1070.9531   C06
## 3   Brevundimonas      2 1040.2766   C06
## 4 f_Caulobacteraceae    2 1066.6892   C06
## 5      Caulobacter      3 1070.2905   C06
## 6    Chitinibacter      3  984.0774   C06
```

```
sum.elo = rbind(sum.elo.0, sum.elo.1, sum.elo.4, sum.elo.6)
sum.elo$Cycle.n = as.numeric(str_remove(sum.elo$Cycle, "C"))
sum.elo$Cycle.label = sum.elo$Cycle.n + 2
```

3.2 Explore relationship from Elo-rating and Abundance and occurrence

4 Occurrence vs Elo-rating (Figure S1b)

```
plot.Elo.occurrence<-sum.elo%>%
ggplot(aes(x=n_games,y=(rating),fill=Cycle,colour=Cycle))+
  geom_point(alpha=0.5,shape=21,size=1,color="black")+
  theme_bw()+labs(y="Elo-rating",x="occurrence (n)")+
  scale_color_brewer(palette = "Dark2")+scale_fill_brewer(palette = "Dark2")+ylim(0,11)+
  theme(legend.position="none", panel.grid.minor = element_blank(),panel.grid.major = element_blank())+
  geom_smooth(method='lm', formula = y~poly(x,3),se=F,size=0.5)
```

```
## Warning: Using 'size' aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use 'linewidth' instead.
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_lifecycle_warnings()' to see where this warning was
## generated.
```

4.1 Abundance vs Elo-rating (Figure S1c)

```
df.comparison <- data.frame(Cycle = rep(c("C00", "C01", "C04", "C06"),
  each = 41), Genus = row.names(df.C0.C6), m.rel.abundance = c(rowMeans(df.C0.C6[,
  c(1:20)]), rowMeans(df.C0.C6[, c(21:40)]), rowMeans(df.C0.C6[, c(41:60)]),
  rowMeans(df.C0.C6[, c(61:80)])))

elo.temporal <- sum.elo
elo.temporal$index <- paste0(elo.temporal$Cycle, ".", elo.temporal$player_id)
df.comparison$index <- paste0(df.comparison$Cycle, ".", df.comparison$Genus)
df.comparison <- merge(df.comparison, elo.temporal, by = "index")
```

```
plot.elo.abundance <- df.comparison %>%
  ggplot(aes(x = log10(m.rel.abundance + 0.001), y = (rating), fill = Cycle.x,
    colour = Cycle.x)) + geom_point(alpha = 0.5, shape = 21, size = 1,
    color = "black") + theme_bw() + labs(y = "Elo-rating", x = "log10-trans rel. abundance") +
  scale_color_brewer(name = "Cycle", palette = "Dark2") + scale_fill_brewer(name = "Cycle",
  palette = "Dark2") + theme(legend.position = "right", panel.grid.minor = element_blank(),
  panel.grid.major = element_blank()) + geom_smooth(method = "lm", se = F,
  size = 0.5)
```

```
jpeg("../Figures/Elo_panel_supplementary.jpg", width = 21, height = 6.8,
  units = "cm", res = 300)
plot_grid(plot.rank, plot.Elo.occurrence, plot.elo.abundance, rel_widths = c(0.8,
  0.78, 1), nrow = 1, labels = c("a)", "b)", "c)"))
```

```
## Warning in is.na(x): is.na() applied to non-(list or vector) of type
## 'expression'
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```

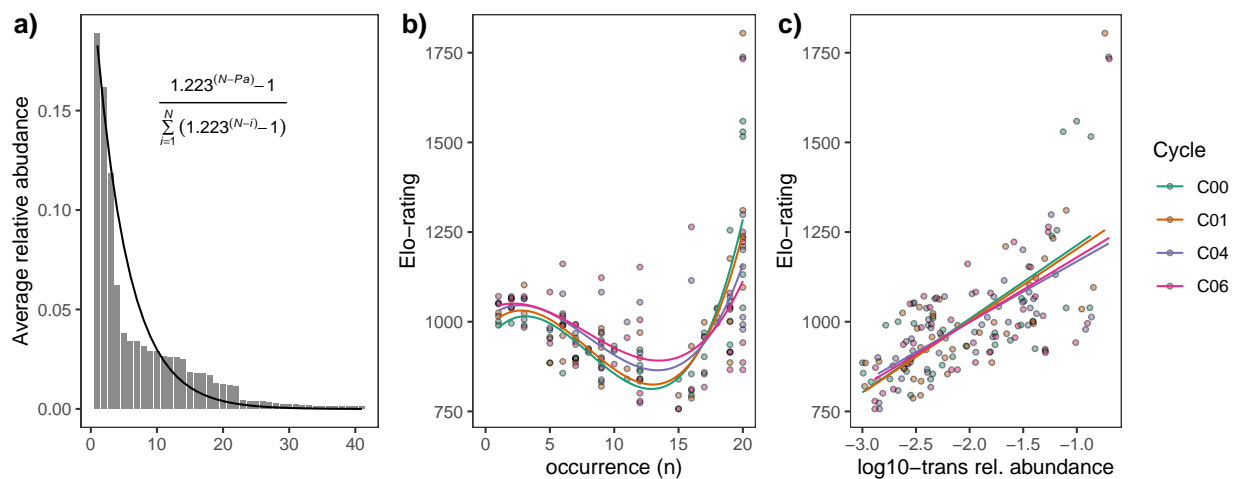
```
dev.off()
```

```
## pdf
## 2
```

```
plot_grid(plot.rank, plot.Elo.occurrence, plot.elo.abundance, rel_widths = c(0.8,
  0.78, 1), nrow = 1, labels = c("a)", "b)", "c)"))
```

```
## Warning in is.na(x): is.na() applied to non-(list or vector) of type
## 'expression'
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
#Include quantile
```



```

q = quantile(sum.elo$rating[sum.elo$Cycle == "C00"])
res.C00 = aggregate(rating ~ player_id, sum.elo[sum.elo$Cycle == "C00",
], mean)
res.C00$quantileC0 = "q1"
res.C00$quantileC0[which(res.C00$rating > q[2])] = "q2"
res.C00$quantileC0[which(res.C00$rating > q[3])] = "q3"
res.C00$quantileC0[which(res.C00$rating > q[4])] = "q4"

# Merging quantile and dataset
sum.elo = merge(sum.elo, res.C00, by = "player_id")

```

4.2 Apply linear regression to each dataset

```

df_lm <- sum.elo[, c(1, 3, 5)] %>%
  group_by(player_id) %>%
  correlation(method = "spearman", p_adjust = "BH")

## Merging regression results with dataset
sum.elo = merge(sum.elo, df_lm, by.x = "player_id", by.y = "Group")
tibble(sum.elo)

## # A tibble: 164 x 18
##   player_id      n_games rating.x Cycle Cycle.n Cycle.label rating.y quantileC0
##   <chr>          <int>    <dbl> <chr>   <dbl>      <dbl>    <dbl> <chr>
## 1 Acidovorax      20    1733. C06      6         8    1516. q4
## 2 Acidovorax      20    1738. C04      4         6    1516. q4
## 3 Acidovorax      20    1805. C01      1         3    1516. q4
## 4 Acidovorax      20    1516. C00      0         2    1516. q4
## 5 Aeromonas       20    1039. C00      0         2    1039. q4
## 6 Aeromonas       20     942. C06      6         8    1039. q4
## 7 Aeromonas       20     979. C04      4         6    1039. q4
## 8 Aeromonas       20    1096. C01      1         3    1039. q4
## 9 Allorhizobium~   5     886. C00      0         2     886. q2
##10 Allorhizobium~   5    1083. C06      6         8     886. q2
## # i 154 more rows
## # i 10 more variables: Parameter1 <chr>, Parameter2 <chr>, rho <dbl>, CI <dbl>,
## #   CI_low <dbl>, CI_high <dbl>, S <dbl>, p <dbl>, Method <chr>, n_Obs <int>

## include values from correlation
sum.elo$sig = NA
sum.elo$sig = ifelse(sum.elo$rho > 0, "Increased", "Decreased")

tibble(sum.elo)

## # A tibble: 164 x 19
##   player_id      n_games rating.x Cycle Cycle.n Cycle.label rating.y quantileC0
##   <chr>          <int>    <dbl> <chr>   <dbl>      <dbl>    <dbl> <chr>
## 1 Acidovorax      20    1733. C06      6         8    1516. q4
## 2 Acidovorax      20    1738. C04      4         6    1516. q4
## 3 Acidovorax      20    1805. C01      1         3    1516. q4

```

```
## 4 Acidovorax          20    1516. C00          0          2    1516. q4
## 5 Aeromonas           20    1039. C00          0          2    1039. q4
## 6 Aeromonas           20     942. C06          6          8    1039. q4
## 7 Aeromonas           20     979. C04          4          6    1039. q4
## 8 Aeromonas           20    1096. C01          1          3    1039. q4
## 9 Allorhizobium~       5     886. C00          0          2     886. q2
## 10 Allorhizobium~      5    1083. C06          6          8     886. q2
## # i 154 more rows
## # i 11 more variables: Parameter1 <chr>, Parameter2 <chr>, rho <dbl>, CI <dbl>,
## #   CI_low <dbl>, CI_high <dbl>, S <dbl>, p <dbl>, Method <chr>, n_Obs <int>,
## #   sig <chr>
```

5 Figure Elo-rating changes (Fig. 5 ms)

```
## Preparing figure
sum.elo$player_id[sum.elo$player_id == "Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium"] <- "ANPR"
sum.elo$player_id <- str_replace(sum.elo$player_id, "f_", "uncl_")

plot.elo.T0 <- sum.elo[sum.elo$Cycle == "C00", ] %>%
  ggplot(aes(x = reorder(player_id, rating.y), y = (rating.y), colour = sig)) +
  geom_hline(yintercept = 1000, color = "grey") + geom_point(aes(colour = ifelse(p <
0.05, sig, "No change")), size = 1.5) + scale_color_manual(values = c("red3",
"steelblue1", "black"), name = NULL) + theme(legend.position = "none") +
  labs(x = NULL, y = bquote(Elo - rating[C0]), title = "") + geom_segment(aes(y = 1000,
colour = ifelse(p < 0.05, sig, "No change"), x = player_id, yend = (rating.y),
xend = player_id)) + geom_vline(xintercept = 11, linetype = "dashed",
linewidth = 0.3) + geom_label(label = "25th Percentile", x = 10, y = 1600,
color = "black", size = 2, fontface = "italic", label.size = NA) +
  geom_vline(xintercept = 31.5, linetype = "dashed", linewidth = 0.3) +
  geom_label(label = "75th Percentile", x = 33, y = 1600, color = "black",
size = 2, fontface = "italic", label.size = NA) + geom_vline(xintercept = 21,
linetype = "dashed", linewidth = 0.3) + geom_label(label = "Median",
x = 23, y = 1600, color = "black", size = 2, fontface = "italic", label.size = NA) +
  theme_bw() + coord_flip() + theme(text = element_text(size = 8), legend.position = c(0.65,
0.1), legend.key.height = unit(0, "cm"), legend.margin = margin(0,
0, 0, 0, "cm")) + ylim(750, 1850) + theme(panel.grid.minor = element_blank(),
panel.grid.major = element_blank())

## Fig Elo rating at C6
plot.elo.T6 <- sum.elo[sum.elo$Cycle == "C06", ] %>%
  ggplot(aes(x = reorder(player_id, rating.y), y = (rating.x), colour = sig)) +
  geom_hline(yintercept = 1000, color = "grey") + geom_point(aes(colour = ifelse(p <
0.05, sig, "No change")), size = 1.5) + scale_color_manual(values = c("red3",
"steelblue1", "black"), name = NULL) + theme(legend.position = "none") +
  labs(x = NULL, y = bquote(Elo - rating[C6]), title = "") + geom_segment(aes(y = 1000,
colour = ifelse(p < 0.05, sig, "No change"), x = player_id, yend = (rating.x),
xend = player_id)) + geom_vline(xintercept = 11.5, linetype = "dashed",
linewidth = 0.3) + geom_vline(xintercept = 31.5, linetype = "dashed",
linewidth = 0.3) + geom_vline(xintercept = 21, linetype = "dashed",
linewidth = 0.3) + theme_bw() + coord_flip() + theme(text = element_text(size = 8),
```

```

legend.position = "none", axis.text.y = element_blank()) + ylim(750,
1850) + theme(panel.grid.minor = element_blank(), panel.grid.major = element_blank())

```

```

# Export only relative abundance plots

```

```

pdf("../Figures/Fig5.pdf", width = 4.33071, height = 4)

```

```

plot_grid(plot.elo.T0, NULL, plot.elo.T6, rel_widths = c(0.23, -0.005,
0.147), nrow = 1, labels = c("a)", "", "b)", vjust = 1.3, label_size = 14,
align = "h")

```

```

dev.off()

```

```

## pdf

```

```

## 2

```

