# Chemo.05.GenomicTraits

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## Contents

0.1 Load packages . . . . . 1

**1 Setting up workspace** **2**

1.1 Load metadata . . . . . 2

1.2 Load predicted traits . . . . . 3

**2 Community indexes** **5**

2.1 Calculation of the Community weigthed mean (CWM) . . . . . 5

2.2 Estimate alpha diversity (Shannon diversity index) . . . . . 8

2.3 Community weigthed means (CWMs) . . . . . 9

**3 Community trait distribution during the experiment** **10**

3.1 Dataframe and format trait CWM values . . . . . 10

3.2 Summaring data replicate mean values . . . . . 11

3.3 Test for normality and homogeneity of variances . . . . . 11

3.4 Repeated measurement ANOVA . . . . . 13

**4 Paired-test per Genomic trait** **14**

4.1 Manuscript Figure 3 . . . . . 15

### 0.1 Load packages

```
rm(list = ls())
library(phyloseq)
library(reshape2)
library(ggplot2)
library(vegan)
library(rstatix)  #Homogeneity of variance test
library(olsrr)  # test_normality
library(egg)  # Tag figures
```

```r
library(rstatix)  #Tibble function
library(microViz)  #TO use re_order function
library(kableExtra)  #Table format

#######################################
cbbPalette <- c("#000000", "#E69F00", "#56B4E9", "#009E73", "#F0E442", "#0072B2",
    "#D55E00", "#CC79A7")
```

# 1 Setting up workspace

## 1.1 Load metadata

```r
# Create metadata for experimental setup
schema <- data.frame(sample.ID = paste0("C10-", rep(1:9, each = 12), "-", sprintf("%02d",
    1:12)), T = rep(1:9, each = 12), Chem.ID = sprintf("%02d", 1:12), DOM = rep(c("L",
    "H"), each = 6), Sal = c("C", "D"))

schema$T = as.factor(schema$T)
schema$DOM = as.factor(schema$DOM)
schema$Sal = as.factor(schema$Sal)

tibble(schema)
```

```
## # A tibble: 108 x 5
##    sample.ID T     Chem.ID DOM   Sal
##    <chr>     <fct> <chr>   <fct> <fct>
##  1 C10-1-01  1     01      L     C
##  2 C10-1-02  1     02      L     D
##  3 C10-1-03  1     03      L     C
##  4 C10-1-04  1     04      L     D
##  5 C10-1-05  1     05      L     C
##  6 C10-1-06  1     06      L     D
##  7 C10-1-07  1     07      H     C
##  8 C10-1-08  1     08      H     D
##  9 C10-1-09  1     09      H     C
## 10 C10-1-10  1     10      H     D
## # ... with 98 more rows
```

#Loading phyloseq object with ASV count table from #dada2

```r
# Loading phyloseq object from #dada2
ps <- readRDS("../data/dada2.output/chem.ps.rds")
# Phyloseq object contain abundance table, sample information, taxanomic
# information and the phylogenetic tree

# Loadgin phylogenetic tree
chem.tree = read_tree("../data/dada2.output/dada-chem.GTR2")
phy_tree(ps) <- chem.tree  #Adding phylo-tree to the phyloseq object

# Phyloseq object contain abundance table, sample information, taxonomic
```

```r
# information and the phylogenetic tree
ps
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 1447 taxa and 110 samples ]
## sample_data() Sample Data:       [ 110 samples by 3 sample variables ]
## tax_table()   Taxonomy Table:    [ 1447 taxa by 7 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 1447 tips and 1445 internal nodes ]
```

```r
# Removing initial inoculum samples for downstream analysis
ps = subset_samples(ps, sample.ID != "C10-0-LL" & sample.ID != "C10-0-HH")
ps
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 1447 taxa and 108 samples ]
## sample_data() Sample Data:       [ 108 samples by 3 sample variables ]
## tax_table()   Taxonomy Table:    [ 1447 taxa by 7 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 1447 tips and 1445 internal nodes ]
```

## 1.2 Load predicted traits

```r
# Resilience related genes load RRN predicted from rrnDB tree and trait data
pic.16s.custom <- read.table("../data/picrust2/trait.predicted/pic.chemo10.16S_predicted_custom_tree.tx
    header = T)
tibble(pic.16s.custom)
```

```
## # A tibble: 1,447 x 3
##    sequence                  X16S_rRNA_Count metadata_NSTI
##    <chr>                               <int>         <dbl>
##  1 SV_1000_Sphingomonadales                1        0.0377
##  2 SV_1001_Rhodospirillales                4        0.0525
##  3 SV_1002_Enterobacterales               12        0.283
##  4 SV_1003_NA                              2        0.686
##  5 SV_1004_Enterobacterales                5        0.0286
##  6 SV_1005_Flavobacteriales                3        0.263
##  7 SV_1006_Rhodospirillales                4        0.630
##  8 SV_1007_Flavobacteriales                3        0.0555
##  9 SV_1008_Enterobacterales                5        0.129
## 10 SV_1009_Rhodobacterales                 1        0.0286
## # ... with 1,437 more rows
```

```r
# load generation time predicted from PICRUST2 default tree and database
pic.d.gRodon.default <- read.table("../data/picrust2/trait.predicted/pic.d.gRodon.retransformed.txt",
    header = T)
tibble(pic.d.gRodon.default)
```

```
## # A tibble: 4,298 x 3
##    sequence           d.gRodon metadata_NSTI
##    <chr>                 <dbl>         <dbl>
```

```
##  1 2228664026            13.3       0.0395
##  2 2236661015            10.9       0.00632
##  3 2264265199            17.8       0.533
##  4 2264813001-cluster    11.7       1.26
##  5 2264867162            14.0       0.504
##  6 2265123003             5.37      0.120
##  7 2500069000             1.19      0.0820
##  8 2501846311             1.43      0.000002
##  9 2504557005            10.2       0.386
## 10 2504756036             3.29      0.00523
## # ... with 4,288 more rows
```

```
# Resistance-related genes load %TF predicted from PICRUSt2 default tree and
# database
pic.TFr.default <- read.table("../data/picrust2/trait.predicted/pic.TF_perc.retransformed.txt",
    header = T)
tibble(pic.TFr.default)
```

```
## # A tibble: 4,298 x 3
##    sequence           TF_perc metadata_NSTI
##    <chr>                <dbl>         <dbl>
##  1 2228664026           1.56       0.0395
##  2 2236661015           1.18       0.00632
##  3 2264265199           1.79       0.533
##  4 2264813001-cluster   1.79       1.26
##  5 2264867162           1.22       0.504
##  6 2265123003           1.23       0.120
##  7 2500069000           2.95       0.0820
##  8 2501846311           0.798      0.000002
##  9 2504557005           1.45       0.386
## 10 2504756036           1.43       0.00523
## # ... with 4,288 more rows
```

```
# load genome size predicted from PICRUSt2 default tree and database
pic.gs.default <- read.table("../data/picrust2/trait.predicted/pic.genome.size.retransformed.txt",
    header = T)
tibble(pic.gs.default)
```

```
## # A tibble: 3,687 x 3
##    sequence           genome.size metadata_NSTI
##    <chr>                    <dbl>         <dbl>
##  1 2228664026                2.45      0.0395
##  2 2236661015                1.41      0.00632
##  3 2264265199                1.68      0.533
##  4 2264813001-cluster        2.14      1.26
##  5 2264867162                2.88      0.504
##  6 2265123003                2.43      0.120
##  7 2500069000                2.05      0.0820
##  8 2501846311                2.39      0.000002
##  9 2504557005                4.87      0.386
## 10 2504756036                2.14      0.00523
## # ... with 3,677 more rows
```

# 2 Community indexes

## 2.1 Calculation of the Community weigthed mean (CWM)

CWMs were obtained by summing predicted and abundance-weighted trait-values for all ASVs in each community

### 2.1.1 Relative abundance data

```
# Rarefy by minimum read numbers and transform to relative data
ps = rarefy_even_depth(ps, min(rowSums(otu_table(ps))), rngseed = 1, replace = F,
    trimOTUs = F)
```

```
## `set.seed(1)` was used to initialize repeatable random subsampling.

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

## Try `set.seed(1); .Random.seed` for the full vector

## ...
```

```
# Estimating relative abundance
rOTUdf.rar <- prop.table(otu_table(ps), 1)

# New phyloseq-project with rarefied ASV table
otu_table(ps) <- otu_table(rOTUdf.rar, taxa_are_rows = FALSE)
ps
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 1447 taxa and 108 samples ]
## sample_data() Sample Data:       [ 108 samples by 3 sample variables ]
## tax_table()   Taxonomy Table:    [ 1447 taxa by 7 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 1447 tips and 1445 internal nodes ]
```

```
# Keep ASVs with prevalence equivalent to more 0 reads
ps <- prune_taxa(taxa_sums(ps) > 0, ps)
ps
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 973 taxa and 108 samples ]
## sample_data() Sample Data:       [ 108 samples by 3 sample variables ]
## tax_table()   Taxonomy Table:    [ 973 taxa by 7 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 973 tips and 971 internal nodes ]
```

```
# Setting up metadata

# Samples in phyloseq object did not correspond to the metadata (schema), so we
# proceed to reorder ps-data base in the schema$sample.ID ##SARA: ???; samples
# from chem3?
```

```r
new_order <- schema$sample.ID
ps = ps %>%
    ps_reorder(new_order)  #MicroViz package

# Extract ASV count table
counts = t(otu_table(ps))
```

### 2.1.2 Remove ASVs without close relatives in the default reference database (NSTI<1)

```r
counts.s.default <- counts[row.names(counts) %in% pic.gs.default[pic.gs.default$metadata_NSTI <
    1, 1], ]  #extract ASVs with NSTI<1 in default reference database
colSums(counts.s.default)  #check which proportion of sequences is left after removing ASVs with NSTI<1
```

```
##  C10-1-01  C10-1-02  C10-1-03  C10-1-04  C10-1-05  C10-1-06  C10-1-07  C10-1-08
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-1-09  C10-1-10  C10-1-11  C10-1-12  C10-2-01  C10-2-02  C10-2-03  C10-2-04
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-2-05  C10-2-06  C10-2-07  C10-2-08  C10-2-09  C10-2-10  C10-2-11  C10-2-12
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 0.9998938
##  C10-3-01  C10-3-02  C10-3-03  C10-3-04  C10-3-05  C10-3-06  C10-3-07  C10-3-08
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-3-09  C10-3-10  C10-3-11  C10-3-12  C10-4-01  C10-4-02  C10-4-03  C10-4-04
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-4-05  C10-4-06  C10-4-07  C10-4-08  C10-4-09  C10-4-10  C10-4-11  C10-4-12
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-5-01  C10-5-02  C10-5-03  C10-5-04  C10-5-05  C10-5-06  C10-5-07  C10-5-08
## 1.0000000 1.0000000 1.0000000 0.9998938 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-5-09  C10-5-10  C10-5-11  C10-5-12  C10-6-01  C10-6-02  C10-6-03  C10-6-04
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-6-05  C10-6-06  C10-6-07  C10-6-08  C10-6-09  C10-6-10  C10-6-11  C10-6-12
## 0.9998938 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-7-01  C10-7-02  C10-7-03  C10-7-04  C10-7-05  C10-7-06  C10-7-07  C10-7-08
## 0.9997875 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-7-09  C10-7-10  C10-7-11  C10-7-12  C10-8-01  C10-8-02  C10-8-03  C10-8-04
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-8-05  C10-8-06  C10-8-07  C10-8-08  C10-8-09  C10-8-10  C10-8-11  C10-8-12
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-9-01  C10-9-02  C10-9-03  C10-9-04  C10-9-05  C10-9-06  C10-9-07  C10-9-08
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-9-09  C10-9-10  C10-9-11  C10-9-12
## 1.0000000 1.0000000 1.0000000 1.0000000
```

```r
min(colSums(counts.s.default))
```

```
## [1] 0.9997875
```

```r
counts.s.rel.default <- as.data.frame.matrix(prop.table(t(t(counts.s.default)), 2))  #re-normalize rema
colSums(counts.s.rel.default)  #should sum up again to 1
```

```
## C10-1-01 C10-1-02 C10-1-03 C10-1-04 C10-1-05 C10-1-06 C10-1-07 C10-1-08
```

```
##        1        1        1        1        1        1        1        1
## C10-1-09 C10-1-10 C10-1-11 C10-1-12 C10-2-01 C10-2-02 C10-2-03 C10-2-04
##        1        1        1        1        1        1        1        1
## C10-2-05 C10-2-06 C10-2-07 C10-2-08 C10-2-09 C10-2-10 C10-2-11 C10-2-12
##        1        1        1        1        1        1        1        1
## C10-3-01 C10-3-02 C10-3-03 C10-3-04 C10-3-05 C10-3-06 C10-3-07 C10-3-08
##        1        1        1        1        1        1        1        1
## C10-3-09 C10-3-10 C10-3-11 C10-3-12 C10-4-01 C10-4-02 C10-4-03 C10-4-04
##        1        1        1        1        1        1        1        1
## C10-4-05 C10-4-06 C10-4-07 C10-4-08 C10-4-09 C10-4-10 C10-4-11 C10-4-12
##        1        1        1        1        1        1        1        1
## C10-5-01 C10-5-02 C10-5-03 C10-5-04 C10-5-05 C10-5-06 C10-5-07 C10-5-08
##        1        1        1        1        1        1        1        1
## C10-5-09 C10-5-10 C10-5-11 C10-5-12 C10-6-01 C10-6-02 C10-6-03 C10-6-04
##        1        1        1        1        1        1        1        1
## C10-6-05 C10-6-06 C10-6-07 C10-6-08 C10-6-09 C10-6-10 C10-6-11 C10-6-12
##        1        1        1        1        1        1        1        1
## C10-7-01 C10-7-02 C10-7-03 C10-7-04 C10-7-05 C10-7-06 C10-7-07 C10-7-08
##        1        1        1        1        1        1        1        1
## C10-7-09 C10-7-10 C10-7-11 C10-7-12 C10-8-01 C10-8-02 C10-8-03 C10-8-04
##        1        1        1        1        1        1        1        1
## C10-8-05 C10-8-06 C10-8-07 C10-8-08 C10-8-09 C10-8-10 C10-8-11 C10-8-12
##        1        1        1        1        1        1        1        1
## C10-9-01 C10-9-02 C10-9-03 C10-9-04 C10-9-05 C10-9-06 C10-9-07 C10-9-08
##        1        1        1        1        1        1        1        1
## C10-9-09 C10-9-10 C10-9-11 C10-9-12
##        1        1        1        1
```

#### 2.1.3 Remove ASVs without close relatives in the custom reference database (NSTI<1)

```
counts.s.custom <- counts[row.names(counts) %in% pic.16s.custom[pic.16s.custom$metadata_NSTI <
    1, 1], ]  #extract ASVs with NSTI<1 (= ASVs with no close relative in the picrust2 reference databa
colSums(counts.s.custom)  #check which proportion of sequences is left after removing ASVs with NSTI<1
```

```
##  C10-1-01  C10-1-02  C10-1-03  C10-1-04  C10-1-05  C10-1-06  C10-1-07  C10-1-08
## 0.9989375 0.9993625 0.9993625 0.9993625 0.9998938 0.9997875 1.0000000 0.9998938
##  C10-1-09  C10-1-10  C10-1-11  C10-1-12  C10-2-01  C10-2-02  C10-2-03  C10-2-04
## 1.0000000 0.9997875 0.9996813 0.9997875 1.0000000 0.9996813 1.0000000 0.9998938
##  C10-2-05  C10-2-06  C10-2-07  C10-2-08  C10-2-09  C10-2-10  C10-2-11  C10-2-12
## 1.0000000 0.9998938 1.0000000 0.9998938 0.9997875 1.0000000 1.0000000 1.0000000
##  C10-3-01  C10-3-02  C10-3-03  C10-3-04  C10-3-05  C10-3-06  C10-3-07  C10-3-08
## 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 0.9992563 0.9997875
##  C10-3-09  C10-3-10  C10-3-11  C10-3-12  C10-4-01  C10-4-02  C10-4-03  C10-4-04
## 0.9998938 0.9998938 0.9998938 0.9996813 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-4-05  C10-4-06  C10-4-07  C10-4-08  C10-4-09  C10-4-10  C10-4-11  C10-4-12
## 1.0000000 1.0000000 0.9989375 0.9997875 0.9995750 0.9994688 0.9997875 0.9995750
##  C10-5-01  C10-5-02  C10-5-03  C10-5-04  C10-5-05  C10-5-06  C10-5-07  C10-5-08
## 1.0000000 1.0000000 0.9998938 1.0000000 1.0000000 1.0000000 0.9993625 0.9996813
##  C10-5-09  C10-5-10  C10-5-11  C10-5-12  C10-6-01  C10-6-02  C10-6-03  C10-6-04
## 0.9998938 0.9997875 0.9997875 0.9994688 1.0000000 1.0000000 1.0000000 1.0000000
##  C10-6-05  C10-6-06  C10-6-07  C10-6-08  C10-6-09  C10-6-10  C10-6-11  C10-6-12
## 0.9998938 1.0000000 0.9991500 0.9994688 0.9997875 1.0000000 0.9994688 0.9997875
```

```
##  C10-7-01  C10-7-02  C10-7-03  C10-7-04  C10-7-05  C10-7-06  C10-7-07  C10-7-08
## 1.0000000 1.0000000 1.0000000 1.0000000 0.9998938 1.0000000 1.0000000 0.9986188
##  C10-7-09  C10-7-10  C10-7-11  C10-7-12  C10-8-01  C10-8-02  C10-8-03  C10-8-04
## 1.0000000 0.9996813 1.0000000 1.0000000 0.9994688 0.9998938 1.0000000 1.0000000
##  C10-8-05  C10-8-06  C10-8-07  C10-8-08  C10-8-09  C10-8-10  C10-8-11  C10-8-12
## 1.0000000 1.0000000 0.9991500 0.9993625 0.9997875 0.9997875 1.0000000 1.0000000
##  C10-9-01  C10-9-02  C10-9-03  C10-9-04  C10-9-05  C10-9-06  C10-9-07  C10-9-08
## 0.9997875 1.0000000 1.0000000 1.0000000 1.0000000 1.0000000 0.9997875 0.9987250
##  C10-9-09  C10-9-10  C10-9-11  C10-9-12
## 1.0000000 0.9996813 1.0000000 1.0000000
```

```
min(colSums(counts.s.custom))
```

```
## [1] 0.9986188
```

```
counts.s.rel.custom <- as.data.frame.matrix(prop.table(t(t(counts.s.custom)), 2))  #re-normalize remain
colSums(counts.s.rel.custom)  #should sum up again to 1
```

```
## C10-1-01 C10-1-02 C10-1-03 C10-1-04 C10-1-05 C10-1-06 C10-1-07 C10-1-08
##        1        1        1        1        1        1        1        1
## C10-1-09 C10-1-10 C10-1-11 C10-1-12 C10-2-01 C10-2-02 C10-2-03 C10-2-04
##        1        1        1        1        1        1        1        1
## C10-2-05 C10-2-06 C10-2-07 C10-2-08 C10-2-09 C10-2-10 C10-2-11 C10-2-12
##        1        1        1        1        1        1        1        1
## C10-3-01 C10-3-02 C10-3-03 C10-3-04 C10-3-05 C10-3-06 C10-3-07 C10-3-08
##        1        1        1        1        1        1        1        1
## C10-3-09 C10-3-10 C10-3-11 C10-3-12 C10-4-01 C10-4-02 C10-4-03 C10-4-04
##        1        1        1        1        1        1        1        1
## C10-4-05 C10-4-06 C10-4-07 C10-4-08 C10-4-09 C10-4-10 C10-4-11 C10-4-12
##        1        1        1        1        1        1        1        1
## C10-5-01 C10-5-02 C10-5-03 C10-5-04 C10-5-05 C10-5-06 C10-5-07 C10-5-08
##        1        1        1        1        1        1        1        1
## C10-5-09 C10-5-10 C10-5-11 C10-5-12 C10-6-01 C10-6-02 C10-6-03 C10-6-04
##        1        1        1        1        1        1        1        1
## C10-6-05 C10-6-06 C10-6-07 C10-6-08 C10-6-09 C10-6-10 C10-6-11 C10-6-12
##        1        1        1        1        1        1        1        1
## C10-7-01 C10-7-02 C10-7-03 C10-7-04 C10-7-05 C10-7-06 C10-7-07 C10-7-08
##        1        1        1        1        1        1        1        1
## C10-7-09 C10-7-10 C10-7-11 C10-7-12 C10-8-01 C10-8-02 C10-8-03 C10-8-04
##        1        1        1        1        1        1        1        1
## C10-8-05 C10-8-06 C10-8-07 C10-8-08 C10-8-09 C10-8-10 C10-8-11 C10-8-12
##        1        1        1        1        1        1        1        1
## C10-9-01 C10-9-02 C10-9-03 C10-9-04 C10-9-05 C10-9-06 C10-9-07 C10-9-08
##        1        1        1        1        1        1        1        1
## C10-9-09 C10-9-10 C10-9-11 C10-9-12
##        1        1        1        1
```

## 2.2 Estimate alpha diversity (Shannon diversity index)

```
# Shannon diversity
H <- diversity(counts, index = "shannon", MARGIN = 2, base = exp(1))
tibble(H)
```

```
## # A tibble: 108 x 1
##        H
##    <dbl>
##  1  1.41
##  2  1.56
##  3  1.26
##  4  1.42
##  5  1.20
##  6  1.43
##  7  1.75
##  8  1.86
##  9  1.28
## 10  1.67
## # ... with 98 more rows
```

### 2.3 Community weigthed means (CWMs)

For each sample and genomic trait (16S rRNA gene copy number, generation time, %transcription factors ,and generation time), the community weighted mean (CWM) was used for downstream statistical analyses.

```
## 16s rRNA gene copy number
counts.16s <- merge(pic.16s.custom, counts.s.rel.custom, by.x = "sequence", by.y = 0)
row.names(counts.16s) <- counts.16s[, 1]
counts.16s <- counts.16s[, c(2, 4:dim(counts.16s)[2])]
# CWM 16S rRNA gene copy per sample
av.16s <- colSums(counts.16s[, 1] * counts.16s[, 2:dim(counts.16s)[2]])
av.16s[1:10]
```

```
## C10-1-01 C10-1-02 C10-1-03 C10-1-04 C10-1-05 C10-1-06 C10-1-07 C10-1-08
## 3.571474 3.757602 3.548480 3.706464 3.472957 3.653666 3.274543 3.507916
## C10-1-09 C10-1-10
## 3.206120 3.527205
```

```
## Generation time gRodon (from codon usage bias using the gRodon R package)
counts.generationstime.gR <- merge(pic.d.gRodon.default, counts.s.rel.default, by.x = "sequence",
    by.y = 0)
row.names(counts.generationstime.gR) <- counts.generationstime.gR[, 1]
# Select sample columns
counts.generationstime.gR <- counts.generationstime.gR[, c(2, 4:dim(counts.generationstime.gR)[2])]
# CWM generation time
av.dgR <- colSums(counts.generationstime.gR[, 1] * counts.generationstime.gR[, 2:dim(counts.generations
av.dgR[1:10]
```

```
## C10-1-01 C10-1-02 C10-1-03 C10-1-04 C10-1-05 C10-1-06 C10-1-07 C10-1-08
## 2.920043 2.719025 2.880545 2.737744 2.948559 2.804541 3.311073 3.206684
## C10-1-09 C10-1-10
## 3.334865 3.161886
```

```
# Percent transcription factors (%TF)
counts.TFr <- merge(pic.TFr.default, counts.s.rel.default, by.x = "sequence", by.y = 0)  #create a colu
row.names(counts.TFr) <- counts.TFr[, 1]
# Select sample columns
```

```r
counts.TFr <- counts.TFr[, c(2, 4:dim(counts.TFr)[2])]
# CWM generation time
av.TFr <- colSums(counts.TFr[, 1] * counts.TFr[, 2:dim(counts.TFr)[2]])
av.TFr[1:10]
```

```
## C10-1-01 C10-1-02 C10-1-03 C10-1-04 C10-1-05 C10-1-06 C10-1-07 C10-1-08
## 2.576288 2.643172 2.562466 2.607937 2.501393 2.602768 2.604296 2.641633
## C10-1-09 C10-1-10
## 2.465664 2.571326
```

```r
## Genome size (in Mbp)
counts.gs <- merge(pic.gs.default, counts.s.rel.default, by.x = "sequence", by.y = 0)
row.names(counts.gs) <- counts.gs[, 1]
# Select sample columns
counts.gs <- counts.gs[, c(2, 4:dim(counts.gs)[2])]
# CWM Genome size
av.gs <- colSums(counts.gs[, 1] * counts.gs[, 2:dim(counts.gs)[2]])
av.gs[1:10]
```

```
## C10-1-01 C10-1-02 C10-1-03 C10-1-04 C10-1-05 C10-1-06 C10-1-07 C10-1-08
## 4.045784 4.108859 4.045568 4.085424 3.996843 4.069437 3.989177 4.035152
## C10-1-09 C10-1-10
## 3.924678 4.010054
```

```r
# NSTI custom
counts.NSTIs <- merge(pic.16s.custom, counts.s.rel.custom, by.x = "sequence", by.y = 0)  #create a colu
row.names(counts.NSTIs) <- counts.NSTIs[, 1]
counts.NSTIs <- counts.NSTIs[, c(3, 4:dim(counts.NSTIs)[2])]  #select releavnt samples
av.NSTI <- colSums(counts.NSTIs[, 1] * counts.NSTIs[, 2:dim(counts.NSTIs)[2]])  #average number Of 16s
summary(av.NSTI)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
## 0.02578 0.06757 0.10371 0.09982 0.12602 0.17881
```

```r
# NSTI default
counts.NSTIs <- merge(pic.gs.default, counts.s.rel.default, by.x = "sequence", by.y = 0)  #create a col
row.names(counts.NSTIs) <- counts.NSTIs[, 1]
counts.NSTIs <- counts.NSTIs[, c(3, 4:dim(counts.NSTIs)[2])]  #select releavnt samples
av.NSTI <- colSums(counts.NSTIs[, 1] * counts.NSTIs[, 2:dim(counts.NSTIs)[2]])  #average number Of
summary(av.NSTI)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
## 0.01122 0.02949 0.05102 0.05674 0.08241 0.12767
```

# 3 Community trait distribution during the experiment

## 3.1 Dataframe and format trait CWM values

```r
# Data frame with CWM trait data and sample schema
traits <- cbind(schema, av.16s, av.gs, av.dgR, av.TFr, H)

# Formatting data set from wide to long format
traits.w <- melt(traits[, 2:10], id.vars = c("Sal", "DOM", "T"), measure.vars = c("av.16s",
    "av.dgR", "av.TFr", "av.gs", "H"))

# Add column with Replicate ID
traits.w$Rep = rep(c("1", "2", "3"), each = 2)
traits.w$Rep = as.factor(traits.w$Rep)

# Add Column with sample time (day)
traits.w$Time = as.numeric(rep(c(4, 8, 15, 18, 22, 29, 36, 39, 41), each = 12))
```

### 3.2 Summaring data replicate mean values

```r
tibble(aggregate(value ~ Sal + DOM + variable, traits.w, mean))
```

```
## # A tibble: 20 x 4
##    Sal   DOM   variable value
##    <fct> <fct> <fct>    <dbl>
##  1 C     H     av.16s    2.64
##  2 D     H     av.16s    2.84
##  3 C     L     av.16s    2.86
##  4 D     L     av.16s    2.93
##  5 C     H     av.dgR    4.28
##  6 D     H     av.dgR    4.03
##  7 C     L     av.dgR    4.72
##  8 D     L     av.dgR    4.39
##  9 C     H     av.TFr    2.72
## 10 D     H     av.TFr    2.79
## 11 C     L     av.TFr    2.76
## 12 D     L     av.TFr    2.78
## 13 C     H     av.gs     3.93
## 14 D     H     av.gs     3.98
## 15 C     L     av.gs     4.02
## 16 D     L     av.gs     3.99
## 17 C     H     H         2.39
## 18 D     H     H         2.67
## 19 C     L     H         1.81
## 20 D     L     H         1.90
```

### 3.3 Test for normality and homogeneity of variances

```r
# Normality Kolmogovor smirnov test
l = length(levels(traits.w$variable))
traits.w$T = factor(traits.w$T)
sum.normality = data.frame(variable = rep(NA, l), L_C = rep(NA, l), L_D = rep(NA,
```

```r
    1), H_C = rep(NA, 1), H_D = rep(NA, 1))

for (i in 1:length(levels(traits.w$variable))) {
    tmp = traits.w[traits.w$variable == levels(traits.w$variable)[i], ]
    sum.normality$variable[i] = levels(traits.w$variable)[i]
    sum.normality$L_C[i] = ols_test_normality((tmp$value[tmp$DOM == "L" & tmp$Sal ==
        "C"]))[[1]][[2]]
    sum.normality$L_D[i] = ols_test_normality((tmp$value[tmp$DOM == "L" & tmp$Sal ==
        "D"]))[[1]][[2]]
    sum.normality$H_C[i] = ols_test_normality((tmp$value[tmp$DOM == "H" & tmp$Sal ==
        "C"]))[[1]][[2]]
    sum.normality$H_D[i] = ols_test_normality((tmp$value[tmp$DOM == "H" & tmp$Sal ==
        "D"]))[[1]][[2]]
}
sum.normality[, 2:5] = round(sum.normality[, 2:5], 3)
tibble(sum.normality)
```

```
## # A tibble: 5 x 5
##   variable   L_C   L_D   H_C   H_D
##   <chr>    <dbl> <dbl> <dbl> <dbl>
## 1 av.16s   0.682 0.757 0.127 0.753
## 2 av.dgR   0.503 0.385 0.871 0.897
## 3 av.TFr   0.79  0.724 0.512 0.987
## 4 av.gs    0.565 0.536 0.415 0.762
## 5 H        0.504 0.342 0.252 0.115
```

```r
# Homogeneity of variances
HV = traits.w %>%
    group_by(variable, DOM, Sal) %>%
    levene_test(value ~ T)
tibble(HV)
```

```
## # A tibble: 20 x 7
##    Sal   DOM   variable   df1   df2 statistic     p
##    <fct> <fct> <fct>    <int> <int>     <dbl> <dbl>
##  1 C     H     av.16s       8    18     0.695 0.691
##  2 D     H     av.16s       8    18     0.606 0.761
##  3 C     L     av.16s       8    18     0.433 0.886
##  4 D     L     av.16s       8    18     0.994 0.473
##  5 C     H     av.dgR       8    18     0.493 0.846
##  6 D     H     av.dgR       8    18     1.00  0.467
##  7 C     L     av.dgR       8    18     0.541 0.811
##  8 D     L     av.dgR       8    18     0.594 0.770
##  9 C     H     av.TFr       8    18     0.871 0.558
## 10 D     H     av.TFr       8    18     0.741 0.656
## 11 C     L     av.TFr       8    18     0.944 0.507
## 12 D     L     av.TFr       8    18     1.31  0.299
## 13 C     H     av.gs        8    18     1.49  0.229
## 14 D     H     av.gs        8    18     0.727 0.667
## 15 C     L     av.gs        8    18     0.515 0.830
## 16 D     L     av.gs        8    18     1.05  0.440
## 17 C     H     H            8    18     1.10  0.409
## 18 D     H     H            8    18     0.373 0.921
```

```
## 19 C     L     H            8     18      0.842 0.579
## 20 D     L     H            8     18      0.500 0.840
```

### 3.4 Repeated measurement ANOVA

A repeated measurement anova was applied seperately for the two DOM regimes to test the effect of the disturbance regime on the distribution of the resilience- and resistance-related genomic traits.

```
# Repeated measurements ANOVA for LDOM

list.rm_anova = list()
m.rm_anova = data.frame(variable = rep(NA, 1), F_Time = rep(NA, 1), P_Time = rep(NA,
    1), F_Sal = rep(NA, 1), P_Sal = rep(NA, 1))
for (i in 1:length(levels(traits.w$variable))) {
    list.rm_anova[[i]] <- with(traits.w[traits.w$DOM == "L" & traits.w$variable ==
        levels(traits.w$variable)[i], ], aov(value ~ T * Sal + Error(Rep)))
    m.rm_anova$variable[i] = levels(traits.w$variable)[i]
    m.rm_anova$F_Time[i] = unlist(summary(list.rm_anova[[i]]))["Error: Within.F value1"]
    m.rm_anova$P_Time[i] = unlist(summary(list.rm_anova[[i]]))["Error: Within.Pr(>F)1"]
    m.rm_anova$F_Sal[i] = unlist(summary(list.rm_anova[[i]]))["Error: Within.F value2"]
    m.rm_anova$P_Sal[i] = unlist(summary(list.rm_anova[[i]]))["Error: Within.Pr(>F)2"]
}

m.rm_anova[, 2:5] = round(m.rm_anova[, 2:5], 3)
a.rm_anova <- m.rm_anova
tibble(a.rm_anova)
```

```
## # A tibble: 5 x 5
##   variable F_Time P_Time F_Sal P_Sal
##   <chr>     <dbl>  <dbl> <dbl> <dbl>
## 1 av.16s    3.57   0.004 0.268 0.608
## 2 av.dgR   24.5    0     6.19  0.018
## 3 av.TFr    5.25   0     0.115 0.737
## 4 av.gs     0.342  0.943 0.167 0.685
## 5 H         8.45   0     0.699 0.409
```

```
# Repeated measurements ANOVA for HDOM
list.rm_anova = list()
m.rm_anova = data.frame(variable = rep(NA, 1), F_Time = rep(NA, 1), P_Time = rep(NA,
    1), F_Sal = rep(NA, 1), P_Sal = rep(NA, 1))
for (i in 1:length(levels(traits.w$variable))) {
    list.rm_anova[[i]] <- with(traits.w[traits.w$DOM == "H" & traits.w$variable ==
        levels(traits.w$variable)[i], ], aov(value ~ T * Sal + Error(Rep)))
    m.rm_anova$variable[i] = levels(traits.w$variable)[i]
    m.rm_anova$F_Time[i] = unlist(summary(list.rm_anova[[i]]))["Error: Within.F value1"]
    m.rm_anova$P_Time[i] = unlist(summary(list.rm_anova[[i]]))["Error: Within.Pr(>F)1"]
    m.rm_anova$F_Sal[i] = unlist(summary(list.rm_anova[[i]]))["Error: Within.F value2"]
    m.rm_anova$P_Sal[i] = unlist(summary(list.rm_anova[[i]]))["Error: Within.Pr(>F)2"]
}

m.rm_anova[, 2:5] = round(m.rm_anova[, 2:5], 3)
a.rm_anova <- m.rm_anova
tibble(a.rm_anova)
```

```
## # A tibble: 5 x 5
##   variable F_Time P_Time F_Sal P_Sal
##   <chr>     <dbl>  <dbl> <dbl> <dbl>
## 1 av.16s     7.26  0      5.98 0.02
## 2 av.dgR     8.99  0      5.19 0.029
## 3 av.TFr     4.73  0.001  4.02 0.053
## 4 av.gs      3.37  0.006  3.80 0.06
## 5 H         16.1   0     14.6  0.001
```

# 4 Paired-test per Genomic trait

```
traits.w.mean = aggregate(value ~ Sal + DOM + T + variable, data = traits.w, mean)
res.ttest = list()
res.ttest.df = data.frame(variable = levels(traits.w.mean$variable), direction = c("greater",
    "less", "greater", "greater", "greater"), LDOM.pvalue = NA, HDOM.pvalue = NA)

for (i in 1:length(levels(traits.w.mean$variable))) {
    tmp = traits.w.mean[traits.w.mean$variable == levels(traits.w$variable)[i], ]
    value.control = tmp[tmp$DOM == "L" & tmp$Sal == "C", ]
    value.disturbance = tmp[tmp$DOM == "L" & tmp$Sal == "D", ]
    res.ttest[[i]] = t.test(value.disturbance$value, value.control$value, alternative = res.ttest.df$di
        var.equal = T, paired = T)
    res.ttest.df$LDOM.pvalue[i] = res.ttest[[i]]$p.value
}


res.ttest = list()
for (i in 1:length(levels(traits.w.mean$variable))) {
    tmp = traits.w.mean[traits.w.mean$variable == levels(traits.w$variable)[i], ]
    value.control = tmp[tmp$DOM == "H" & tmp$Sal == "C", ]
    value.disturbance = tmp[tmp$DOM == "H" & tmp$Sal == "D", ]
    res.ttest[[i]] = t.test(value.disturbance$value, value.control$value, alternative = res.ttest.df$di
        var.equal = T, paired = T)
    res.ttest.df$HDOM.pvalue[i] = res.ttest[[i]]$p.value
}

tibble(res.ttest.df)
```

```
## # A tibble: 5 x 4
##   variable direction LDOM.pvalue HDOM.pvalue
##   <chr>    <chr>           <dbl>       <dbl>
## 1 av.16s   greater        0.138     0.00739
## 2 av.dgR   less           0.0154    0.00334
## 3 av.TFr   greater        0.334     0.00112
## 4 av.gs    greater        0.753     0.00781
## 5 H        greater        0.160     0.000358
```

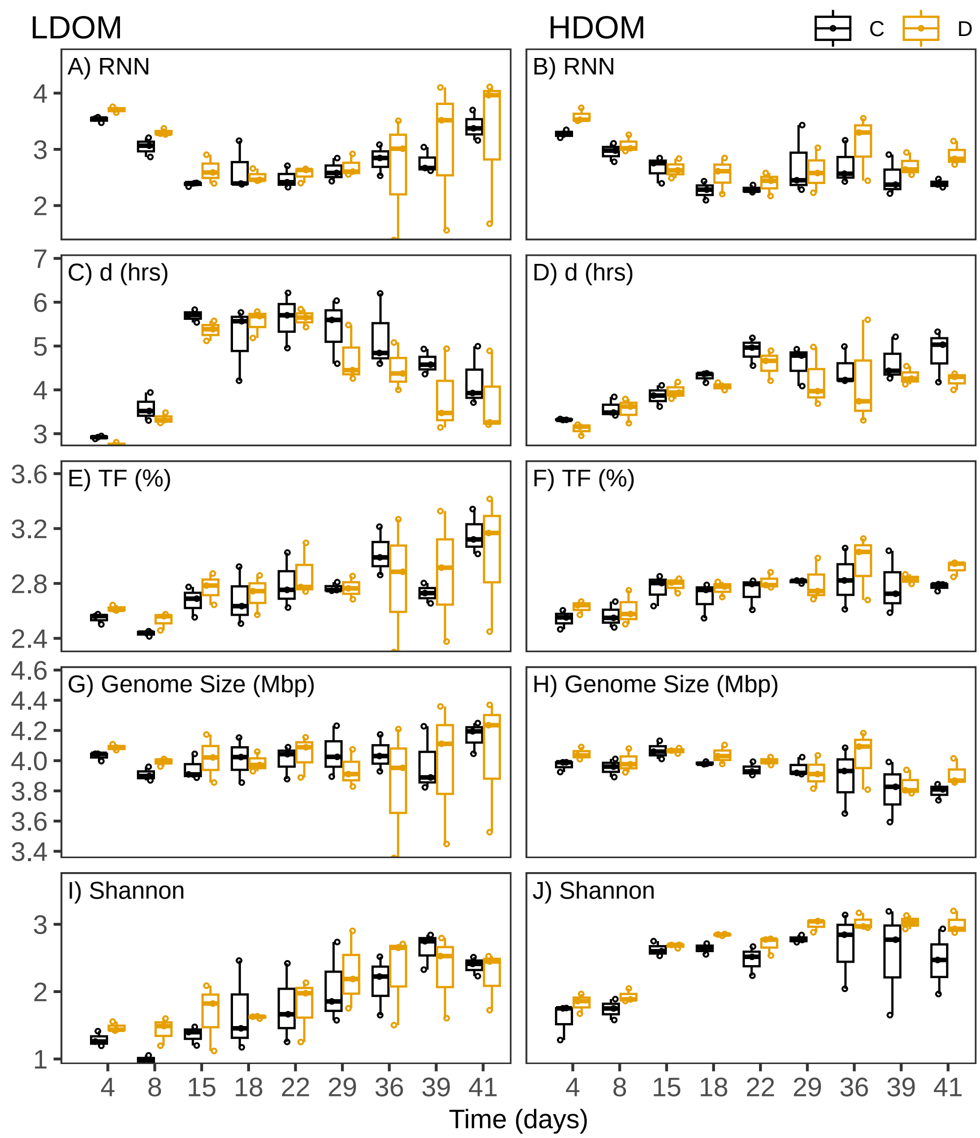
## 4.1 Manuscript Figure 3

```
# New facet label names for dose variable
bxp_labs <- c("", "", "", " ", "")
names(bxp_labs) <- levels(traits.w$variable)
traits.w$DOM = factor(traits.w$DOM, levels = c("L", "H"))

levels(traits.w$T) = c("4", "8", "15", "18", "22", "29", "36", "39", "41")

bxp = traits.w %>%
    ggplot(aes(x = T, y = value, colour = Sal)) + geom_boxplot(aes(colour = (Sal)),
    outlier.shape = NA, alpha = 0.3, size = 0.4) + geom_jitter(aes(colour = Sal),
    shape = 21, size = 0.5, position = position_jitterdodge()) + scale_colour_manual(values = cbbPalett
    name = "") + theme_bw() + ylab("") + scale_y_continuous(expand = expansion(mult = c(0,
    0.25))) + theme(panel.grid.minor = element_blank(), panel.grid.major = element_blank(),
    axis.text.x = element_text(size = 10), axis.text.y = element_text(size = 10)) +
    theme(legend.position = c(0.9, 1.02), legend.direction = "horizontal", legend.key = element_blank()
        legend.background = element_blank()) + theme(text = element_text(size = 10,
    family = "ArialMT")) + facet_grid(variable ~ DOM, scale = "free_y", switch = "y",
    labeller = labeller(variable = bxp_labs)) + xlab("Time (days)") + theme(strip.placement.y = "outsid
    strip.text.y = element_text(angle = 270), strip.background = element_blank()) +
    labs(tag = "LDOM                                               HDOM") + theme(plot.tag.position = 
    1.02))

# Labels using facet_tag
bxp = tag_facet(bxp, open = "", close = "", tag_pool = c(" A) RNN", " B) RNN ", " C) d (hrs)",
    " D) d (hrs)", " E) TF (%)", " F) TF (%)", " G) Genome Size (Mbp)", " H) Genome Size (Mbp)",
    " I) Shannon", " J) Shannon"), x = 0, fontface = 1, size = 3, hjust = 0)
bxp = bxp + theme(plot.margin = margin(t = 20, r = 5, b = 5, l = 5, unit = "pt"))
bxp
```




Boxplots displaying CWMs of genomic traits. LDOM and HDOM in the left and right panels restively for A, B), RRN, C, D) minimal generation time (d), E, F), %TF G, H) genome size and I, J) Shannon diversity index.