Host Response

2025-08-04

```
#Load in all packages
library(readxl)
## Warning: package 'readxl' was built under R version 4.3.3
library(nlme)
## Warning: package 'nlme' was built under R version 4.3.3
library(lme4)
## Warning: package 'lme4' was built under R version 4.3.3
## Loading required package: Matrix
## Warning: package 'Matrix' was built under R version 4.3.3
## Attaching package: 'lme4'
## The following object is masked from 'package:nlme':
##
       lmList
library(DAAG)
## Warning: package 'DAAG' was built under R version 4.3.3
library(ggplot2)
## Warning: package 'ggplot2' was built under R version 4.3.3
library(dplyr)
## Warning: package 'dplyr' was built under R version 4.3.3
## Attaching package: 'dplyr'
```

```
## The following object is masked from 'package:nlme':
##
       collapse
##
## The following objects are masked from 'package:stats':
##
       filter, lag
## The following objects are masked from 'package:base':
##
##
       intersect, setdiff, setequal, union
library(MuMIn)
## Warning: package 'MuMIn' was built under R version 4.3.3
library(car)
## Warning: package 'car' was built under R version 4.3.3
## Loading required package: carData
## Warning: package 'carData' was built under R version 4.3.3
##
## Attaching package: 'car'
## The following object is masked from 'package:dplyr':
##
##
       recode
## The following object is masked from 'package:DAAG':
##
##
       vif
library(viridis)
## Warning: package 'viridis' was built under R version 4.3.3
## Loading required package: viridisLite
library(DHARMa)
## Warning: package 'DHARMa' was built under R version 4.3.3
## This is DHARMa 0.4.7. For overview type '?DHARMa'. For recent changes, type news(package = 'DHARMa')
```

```
library(FSA)
## Warning: package 'FSA' was built under R version 4.3.3
## Registered S3 methods overwritten by 'FSA':
     method
##
     confint.boot car
     hist.boot
## ## FSA v0.9.6. See citation('FSA') if used in publication.
## ## Run fishR() for related website and fishR('IFAR') for related book.
##
## Attaching package: 'FSA'
## The following object is masked from 'package:car':
##
##
       bootCase
library(emmeans)
## Warning: package 'emmeans' was built under R version 4.3.3
## Welcome to emmeans.
## Caution: You lose important information if you filter this package's results.
## See '? untidy'
library(ggpubr)
## Warning: package 'ggpubr' was built under R version 4.3.3
library(rstatix)
## Warning: package 'rstatix' was built under R version 4.3.3
## Attaching package: 'rstatix'
## The following object is masked from 'package:stats':
##
##
       filter
library(glmmTMB)
## Warning: package 'glmmTMB' was built under R version 4.3.3
```

library(boot)

```
## Warning: package 'boot' was built under R version 4.3.3
##
## Attaching package: 'boot'
## The following object is masked from 'package:car':
##
## logit
```

Import the dataset

```
#Import the dataset
data <- read.csv(file.path(</pre>
 "C:/Users/Joyalea/Documents/UBCO/Thesis",
 "Final_Files",
 "RQ2 HostResponse",
 "host_response_2024.csv"
))
#Add treatment group column
data <- data %>%
 mutate(treatment = ifelse(mesocosm_id %in% c(1:7), "Control",
                            ifelse(mesocosm_id %in% c(8:14), "197198",
                                   ifelse(mesocosm_id %in% c(15:21), "240448",
                            ifelse(mesocosm_id %in% c(22:28), "240720", NA)))),
         block = ifelse(mesocosm_id %in% c(15, 20, 22, 12, 11, 14, 24, 27, 19,
                                            21, 25, 3, 2, 7), "A",
                      ifelse(mesocosm_id %in% c(4, 17, 9, 28, 6, 1, 5, 18, 8,
                                                 26, 16, 23, 13, 10), "B", NA)))
#Review data import
head(data)
```

```
##
    mesocosm id
                   species biomass copies uL copies 20uL copies rxn
## 1
                    Bromus
                             14.39
                                                      0
                                                                 0 0.428
             1
                                          0
## 2
              1
                   Festuca
                            31.19
                                           0
                                                      0
                                                                 0 0.189
## 3
              1 Gaillardia
                             6.69
                                          0
                                                      0
                                                                 0 0.232
## 4
              1 Taraxacum 24.34
                                          0
                                                      0
                                                                 0 0.316
## 5
                             26.19
                                                                 0 0.412
              2
                    Bromus
                                          0
                                                      0
## 6
              2
                   Festuca
                            40.62
                                          0
                                                      0
                                                                 0 0.197
    seed_weight date_bio_harvested date_roots_havested number_plants treatment
##
## 1
             NA
                            27-Aug
                                                27-Aug
                                                                     Control
## 2
             NA
                            27-Aug
                                                27-Aug
                                                                      Control
                                                                  3
## 3
             NA
                                                                      Control
                            27-Aug
                                                27-Aug
## 4
             NA
                                                                  3 Control
                            27-Aug
                                                27-Aug
## 5
                                                                  3 Control
             NA
                            27-Aug
                                                27-Aug
## 6
             NA
                            27-Aug
                                                27-Aug
                                                                  3 Control
## block
## 1
```

```
## 3
         В
## 4
         В
## 5
         Α
## 6
         Α
str(data)
                    112 obs. of 13 variables:
## 'data.frame':
                         : int 1 1 1 1 2 2 2 2 3 3 ...
   $ mesocosm_id
## $ species
                                "Bromus" "Festuca" "Gaillardia" "Taraxacum" ...
                         : chr
                                14.39 31.19 6.69 24.34 26.19 ...
## $ biomass
                         : num
##
   $ copies_uL
                         : num
                                0 0 0 0 0 0 0 0 0 0 ...
   $ copies_20uL
                         : num
                                0 0 0 0 0 0 0 0 0 0 ...
##
                                0 0 0 0 0 0 0 0 0 0 ...
## $ copies_rxn
                         : num
## $ P
                                0.428 0.189 0.232 0.316 0.412 0.197 0.244 0.299 NA NA ...
                         : num
##
   $ seed_weight
                                NA NA NA NA NA NA NA NA NA ...
                         : num
                                "27-Aug" "27-Aug" "27-Aug" "27-Aug" ...
## $ date_bio_harvested : chr
## $ date roots havested: chr
                                "27-Aug" "27-Aug" "27-Aug" "27-Aug" ...
## $ number_plants
                                2 3 3 3 3 3 3 3 2 3 ...
                         : int
   $ treatment
                                "Control" "Control" "Control" ...
                         : chr
                                "B" "B" "B" "B" ...
##
   $ block
                         : chr
tail(data)
       mesocosm id
                      species biomass copies uL copies 20uL copies rxn
                                                                            Ρ
##
## 107
                27 Gaillardia
                                13.09
                                           0.56
                                                        11.2
                                                                  12.32
                                                                           NA
                                24.22
                                           0.22
                                                         4.4
                                                                   4.84
## 108
                27 Taraxacum
                                                                           NA
## 109
                                           0.00
                28
                       Bromus
                                23.48
                                                         0.0
                                                                   0.00 0.366
                                                         0.0
## 110
                28
                      Festuca
                               72.10
                                           0.00
                                                                   0.00 0.149
## 111
                28 Gaillardia
                               12.99
                                           0.50
                                                        10.0
                                                                  11.00 0.210
                28 Taraxacum
                               18.06
                                           0.00
                                                         0.0
                                                                   0.00 0.243
## 112
##
       seed_weight date_bio_harvested date_roots_havested number_plants treatment
## 107
          0.002060
                               28-Aug
                                                    04-Sep
                                                                       3
                                                                            240720
## 108
                NA
                               28-Aug
                                                    04-Sep
                                                                       3
                                                                            240720
## 109
                               28-Aug
                                                                       2
                                                                            240720
                NA
                                                    05-Sep
## 110
                NA
                               28-Aug
                                                    05-Sep
                                                                       3
                                                                            240720
## 111
          0.001730
                                                                       3
                                                                            240720
                               28-Aug
                                                    05-Sep
## 112
          0.000505
                               28-Aug
                                                    05-Sep
                                                                       3
                                                                            240720
##
       block
## 107
           Α
## 108
## 109
           В
           В
## 110
## 111
           В
## 112
           В
#Convert variables to appropriate form
#mesocosm id and species id as factors
data$mesocosm_id<-as.factor(data$mesocosm_id)</pre>
data$species<-as.factor(data$species)</pre>
data$treatment<-as.factor(data$treatment)</pre>
```

2

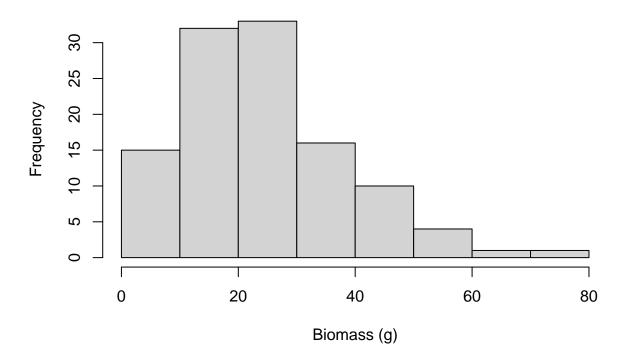
В

```
#biomass and seed weight as numeric
data$biomass<-as.numeric(data$biomass)</pre>
data$P<-as.numeric(data$P)</pre>
#summary of dataset
summary(data$mesocosm_id) #all mesocosms have four observations
## 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
## 27 28
## 4 4
#Create data frames to view species independently
#view species independently
data_gaillardia<-data%>%
 filter(species=="Gaillardia")
data_taraxacum<-data%>%
 filter(species=="Taraxacum")
data_bromus<-data%>%
 filter(species=="Bromus")
data_festuca<-data%>%
 filter(species=="Festuca")
#By treament
#197198
data_197198<-data%>%
 filter(treatment == "197198")
#240448
data 240448<-data%>%
 filter(treatment == "240448")
data 240720<-data%>%
 filter(treatment == "240720")
#control
data_control<-data%>%
filter(treatment == "control")
```

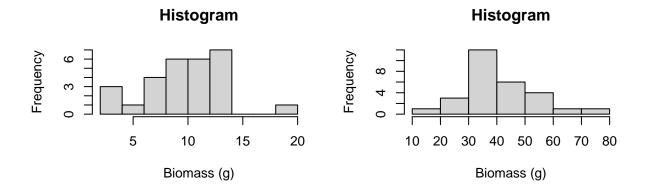
Visualisation and data exploration

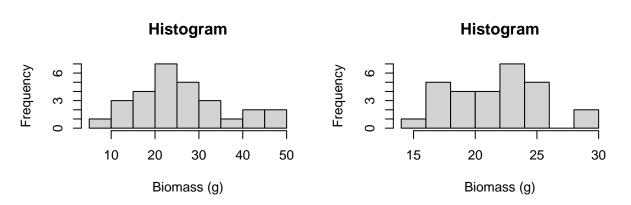
```
#Histogram of all biomass values
hist(data$biomass,
    main = "Histogram of Biomass",
    xlab = "Biomass (g)")
```

Histogram of Biomass



```
#near normal, slightly right skewed
par(mfrow = c(2, 2)) #2 rows, 2 columns
#View histogram by host species
#generate histogram of data distribution: GAILLARDIA
hist(data_gaillardia$biomass,
     main="Histogram",
     xlab="Biomass (g)")
#generate histogram of data distribution: FESTUCA
hist(data_festuca$biomass,
     main="Histogram",
     xlab="Biomass (g)")
#generate histogram of data distribution: BROMUS
hist(data_bromus$biomass,
     main="Histogram",
     xlab="Biomass (g)")
#generate histogram of data distribution: TARAXACUM
hist(data_taraxacum$biomass,
     main="Histogram",
     xlab="Biomass (g)")
```

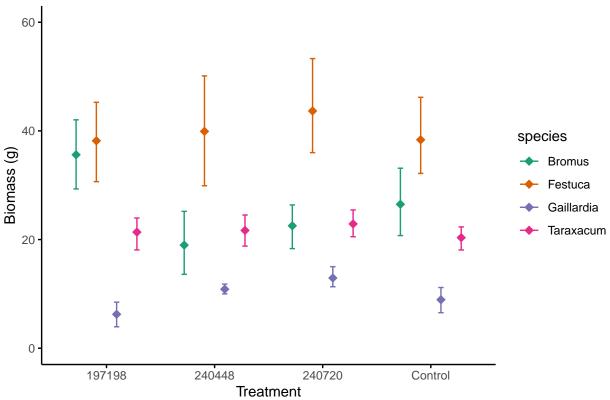




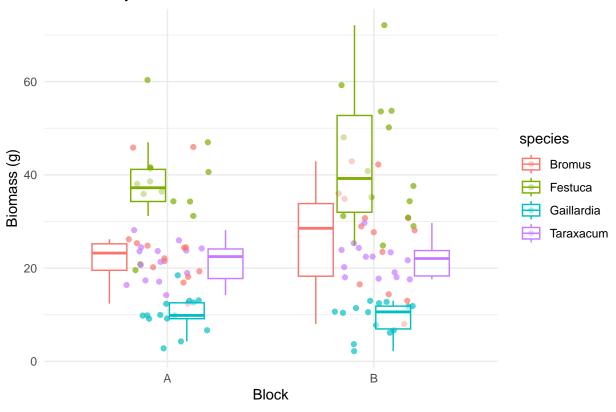
```
#Skewed histogram driven by slight differences in species group density centres
par(mfrow = c(1,1)) #return plot view to default
#Create custom function to bootsrap 95% confidence intervals
mean_cl_boot \leftarrow function(x, conf = 0.95, R = 999) {
 boot_mean <- function(x, i) mean(x[i])</pre>
  boot_out <- boot(x, boot_mean, R = R)</pre>
 ci <- boot.ci(boot_out, type = "perc", conf = conf)$percent[4:5]</pre>
  data.frame(y = mean(x), ymin = ci[1], ymax = ci[2])
}
#Create species labels for plots
species labels <- c(</pre>
  "Bromus"
               = expression(italic("B. tectorum")),
  "Taraxacum" = expression(italic("T. officinale")),
               = expression(italic("F. idahoensis")),
  "Gaillardia" = expression(italic("G. aristata"))
)
#Plot biomass by treatment and species (boxplot and bootstrapped mean)
print(plot_mean_biomass <- ggplot(data, aes(x = treatment, y = biomass, color = species)) +</pre>
  stat_summary(fun.data = mean_cl_boot, geom = "errorbar",
               position = position_dodge(0.75), width = 0.2) +
  stat summary(fun = mean, geom = "point", shape = 18, size = 3,
               position = position_dodge(0.75)) +
```

```
labs(title = "Biomass by Treatment and Species",
    x = "Treatment",
    y = "Biomass (g)") +
coord_cartesian(ylim = c(0, 60)) +
scale_colour_brewer(palette = "Dark2") +
theme_minimal() +
theme(panel.grid = element_blank(),
    axis.line = element_line(color = "black"),
    axis.ticks = element_line(color = "black")))
```

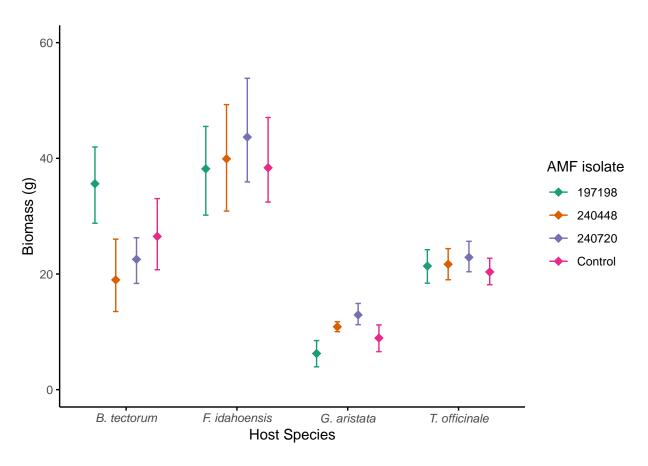
Biomass by Treatment and Species

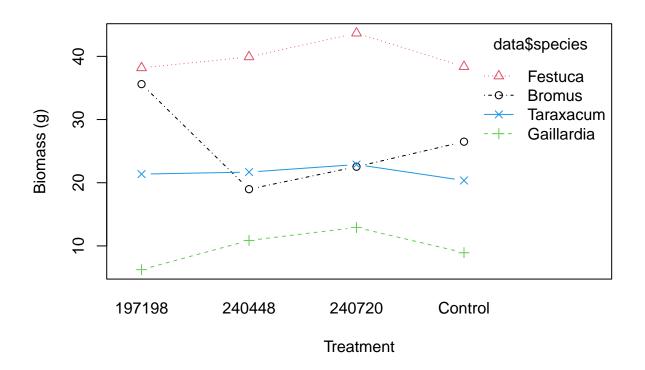


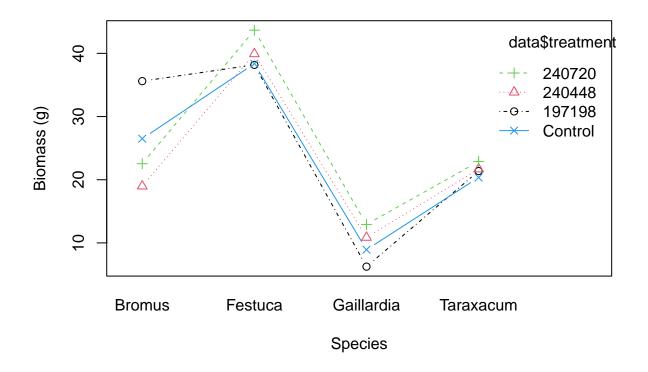
Biomass by Block



```
#Biomass by species
print(biomass_mean_species <- ggplot(data, aes(x = species, y = biomass, colour = treatment)) +</pre>
  stat_summary(fun.data = mean_cl_boot, geom = "errorbar",
               position = position_dodge(0.75), width = 0.2) +
 stat_summary(fun = mean, geom = "point", shape = 18, size = 3,
               position = position_dodge(0.75)) +
 labs(color = "AMF isolate",
       x = "Host Species",
       y = "Biomass (g)") +
  scale_colour_brewer(palette = "Dark2") +
  scale_x_discrete(labels = species_labels) +
  coord_cartesian(ylim = c(0, 60)) +
  theme minimal() +
  theme(panel.grid = element_blank(),
       axis.line = element_line(color = "black"),
        axis.ticks = element_line(color = "black")))
```





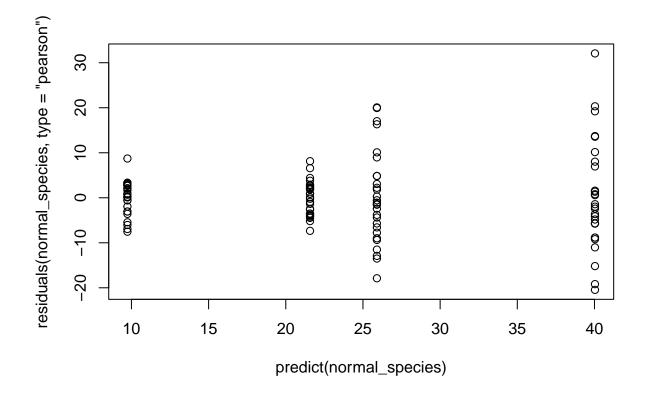


#Treatments follow roughly equivalent patterns within species (species identity #has the strongest effect on biomass), excepting bromus * 197198, #which remains equivalent to the biomass of festuca.

Model selection

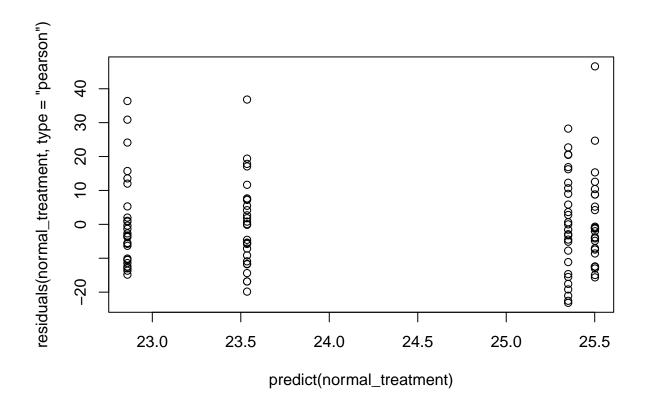
```
#Start with the simplest model
#normal distribution with species as the predictor
normal_species<-glm(biomass~species,</pre>
                    family = gaussian(link="identity"),
                    data = data)
summary(normal_species)
##
## Call:
  glm(formula = biomass ~ species, family = gaussian(link = "identity"),
##
       data = data)
##
## Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
##
## (Intercept)
                       25.904
                                   1.569 16.513 < 2e-16 ***
## speciesFestuca
                                   2.218
                                            6.369 4.76e-09 ***
                       14.129
## speciesGaillardia -16.164
                                   2.218 -7.286 5.50e-11 ***
```

```
## speciesTaraxacum -4.333     2.218 -1.953     0.0534 .
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 68.90103)
##
## Null deviance: 20589.0 on 111 degrees of freedom
## Residual deviance: 7441.3 on 108 degrees of freedom
## AIC: 797.83
##
## Number of Fisher Scoring iterations: 2
#*Check heteroskedasticity
plot(predict(normal_species), residuals(normal_species, type = "pearson"))
```



Call:

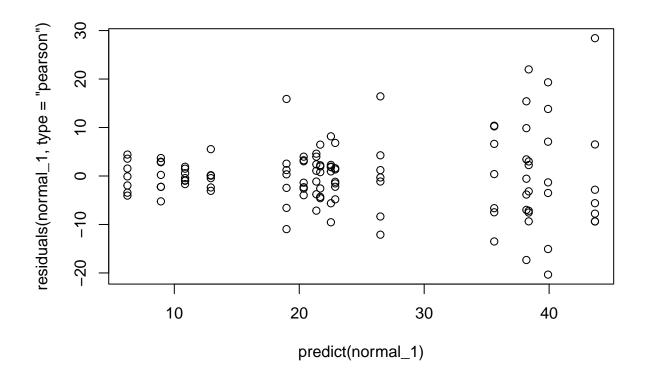
```
## glm(formula = biomass ~ treatment, family = gaussian(link = "identity"),
##
       data = data)
##
## Coefficients:
##
                    Estimate Std. Error t value Pr(>|t|)
                     25.3500
                                 2.6001
                                          9.750
                                                  <2e-16 ***
## (Intercept)
## treatment240448
                     -2.4907
                                 3.6770
                                         -0.677
                                                   0.500
## treatment240720
                                 3.6770
                                                   0.967
                      0.1514
                                          0.041
## treatmentControl
                    -1.8146
                                 3.6770 -0.494
                                                   0.623
##
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
##
## (Dispersion parameter for gaussian family taken to be 189.2889)
##
##
       Null deviance: 20589
                             on 111 degrees of freedom
## Residual deviance: 20443
                             on 108 degrees of freedom
## AIC: 911.02
##
## Number of Fisher Scoring iterations: 2
#Check heteroskedasticity
plot(predict(normal_treatment), residuals(normal_treatment, type = "pearson"))
```



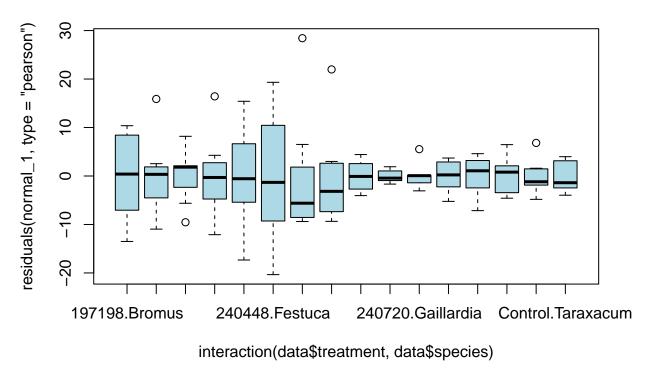
#Patterns in residual variance, large residual spread, less hetero. than species #only model

```
#Interaction model
normal_1<-glm(biomass~treatment*species,
             family = gaussian(link="identity"),
             data = data)
summary(normal_1)
##
## Call:
## glm(formula = biomass ~ treatment * species, family = gaussian(link = "identity"),
      data = data)
## Coefficients:
##
                                     Estimate Std. Error t value Pr(>|t|)
                                                   2.997 11.880 < 2e-16 ***
## (Intercept)
                                       35.606
                                                   4.239 -3.923 0.000165 ***
## treatment240448
                                      -16.626
## treatment240720
                                      -13.071
                                                   4.239 -3.084 0.002668 **
## treatmentControl
                                       -9.111
                                                   4.239 -2.150 0.034095 *
## speciesFestuca
                                                   4.239 0.607 0.545051
                                        2.574
## speciesGaillardia
                                      -29.359
                                                   4.239 -6.927 4.91e-10 ***
                                                   4.239 -3.359 0.001122 **
## speciesTaraxacum
                                      -14.239
## treatment240448:speciesFestuca
                                       18.360
                                                   5.994 3.063 0.002844 **
                                                   5.994 3.097 0.002567 **
## treatment240720:speciesFestuca
                                       18.561
## treatmentControl:speciesFestuca
                                       9.299
                                                   5.994 1.551 0.124130
## treatment240448:speciesGaillardia
                                       21.240
                                                   5.994 3.543 0.000612 ***
## treatment240720:speciesGaillardia
                                      19.747
                                                  5.994 3.294 0.001382 **
## treatmentControl:speciesGaillardia
                                      11.790
                                                   5.994 1.967 0.052082 .
## treatment240448:speciesTaraxacum
                                       16.940
                                                   5.994 2.826 0.005734 **
## treatment240720:speciesTaraxacum
                                      14.583
                                                   5.994 2.433 0.016833 *
## treatmentControl:speciesTaraxacum
                                      8.099
                                                   5.994 1.351 0.179849
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
## (Dispersion parameter for gaussian family taken to be 62.87825)
##
##
      Null deviance: 20589.0 on 111 degrees of freedom
## Residual deviance: 6036.3 on 96 degrees of freedom
## AIC: 798.39
## Number of Fisher Scoring iterations: 2
#Check heteroskedasticity
```

plot(predict(normal_1), residuals(normal_1, type = "pearson"))

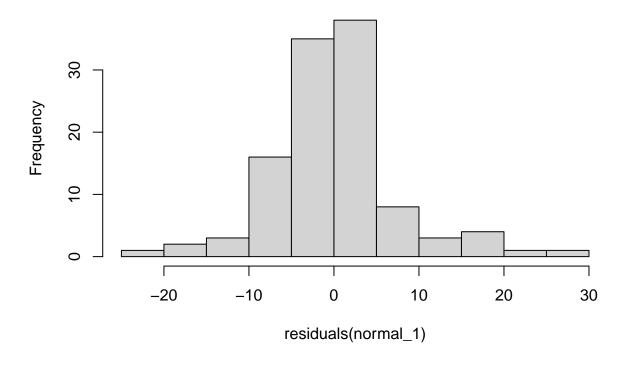


Boxplot of Residuals per Treatment

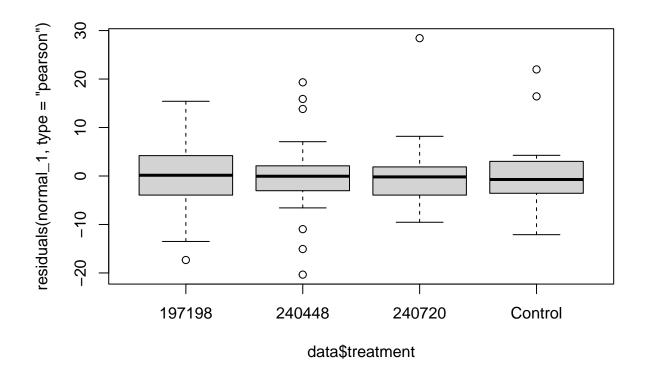


#Differences in residual variance between treatment*species interactions
hist(residuals(normal_1))

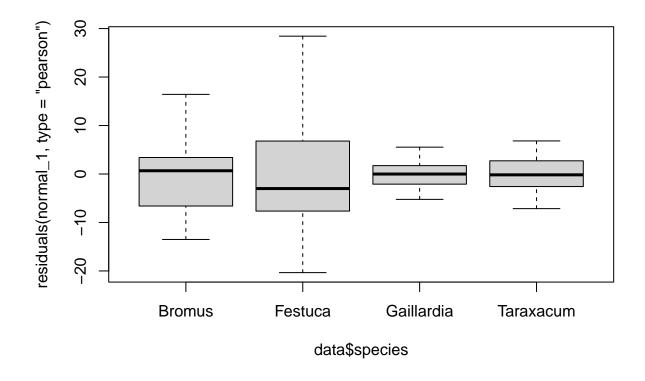
Histogram of residuals(normal_1)



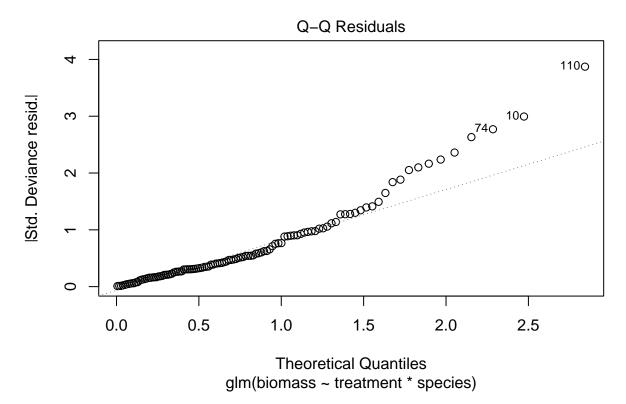
#Good histogram shape
boxplot(residuals(normal_1, type ="pearson")~data\$treatment) #Looks good



boxplot(residuals(normal_1, type = "pearson")~data\$species)

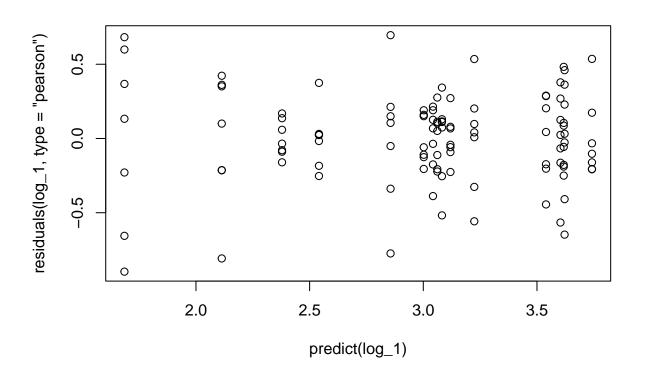


```
#Variance between species
#Check Q-Q residuals
plot(normal_1, which =2) #Struggles to predict higher values
```

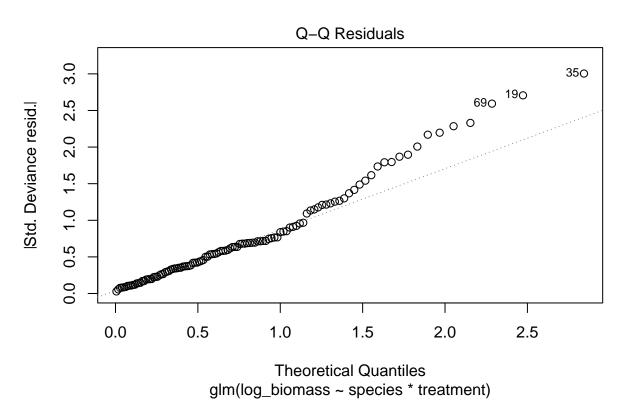


```
##
## Call:
  glm(formula = log_biomass ~ species * treatment, family = gaussian(link = "identity"),
##
       data = data)
##
## Coefficients:
##
                                       Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                                         3.5398
                                                    0.1219
                                                            29.045
                                                                    < 2e-16 ***
## speciesFestuca
                                         0.0636
                                                    0.1724
                                                              0.369
                                                                     0.71293
## speciesGaillardia
                                        -1.8545
                                                    0.1724 -10.760
                                                                     < 2e-16 ***
## speciesTaraxacum
                                        -0.4972
                                                    0.1724
                                                            -2.885
                                                                     0.00484 **
## treatment240448
                                        -0.6837
                                                    0.1724
                                                            -3.967
                                                                     0.00014 ***
                                                            -2.655
## treatment240720
                                                    0.1724
                                        -0.4577
                                                                     0.00928 **
## treatmentControl
                                        -0.3155
                                                    0.1724
                                                            -1.830
                                                                     0.07031 .
## speciesFestuca:treatment240448
                                         0.7019
                                                    0.2437
                                                              2.880
                                                                     0.00491 **
## speciesGaillardia:treatment240448
                                         1.3771
                                                    0.2437
                                                              5.650 1.64e-07 ***
                                         0.7028
                                                    0.2437
                                                              2.883 0.00485 **
## speciesTaraxacum:treatment240448
```

```
0.2437
## speciesFestuca:treatment240720
                                          0.5963
                                                              2.446 0.01625 *
## speciesGaillardia:treatment240720
                                          1.3135
                                                     0.2437
                                                              5.389 5.06e-07 ***
## speciesTaraxacum:treatment240720
                                          0.5344
                                                     0.2437
                                                              2.193
                                                                      0.03076 *
                                         0.3298
                                                     0.2437
                                                               1.353
                                                                      0.17915
## speciesFestuca:treatmentControl
   speciesGaillardia:treatmentControl
                                          0.7437
                                                     0.2437
                                                              3.051
                                                                      0.00295 **
   speciesTaraxacum:treatmentControl
                                         0.2749
                                                     0.2437
                                                               1.128
                                                                      0.26224
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
##
   (Dispersion parameter for gaussian family taken to be 0.1039667)
##
##
##
       Null deviance: 46.2342
                                on 111
                                        degrees of freedom
                                        degrees of freedom
## Residual deviance: 9.9808
                                on
                                   96
## AIC: 81.045
##
## Number of Fisher Scoring iterations: 2
#Much better AIC
#Review residual diagnostics
res_log<-summary(residuals(log_1, type = "pearson"))</pre>
exp_res<-exp(res_log)</pre>
pred_log<-predict(log_1)</pre>
exp_pred<-exp(pred_log)</pre>
#Low variance in residuals, visually assess
plot(predict(log_1), residuals(log_1, type = "pearson"))
```

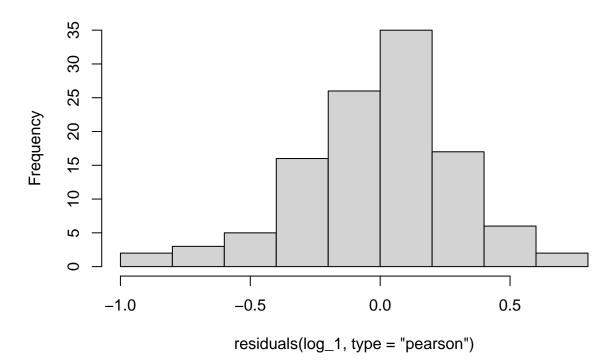


#Better residuals, but fan shaped, heteroskedastic residual variance persists
#Model is likely underdispersed. Residual spread expected between ~ -2 and 2
#wiht pearson residuals, log model has a spread of ~-0.5 to 0.5.
#Check Q-Q residuals
plot(log_1, which = 2)



#Model struggles to predict higher values
#Check histogram of residuals
hist(residuals(log_1, type = "pearson")) #Compressed residual variance

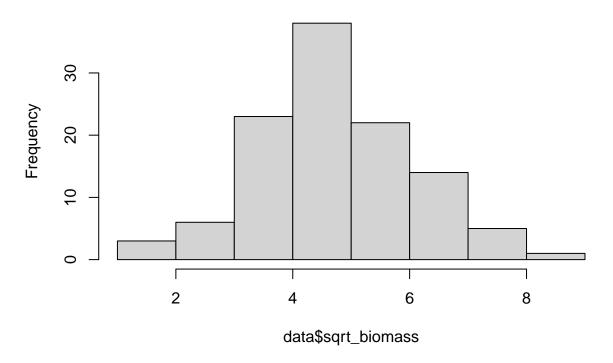
Histogram of residuals(log_1, type = "pearson")



```
#Square root transformation
data$sqrt_biomass<-sqrt(data$biomass)

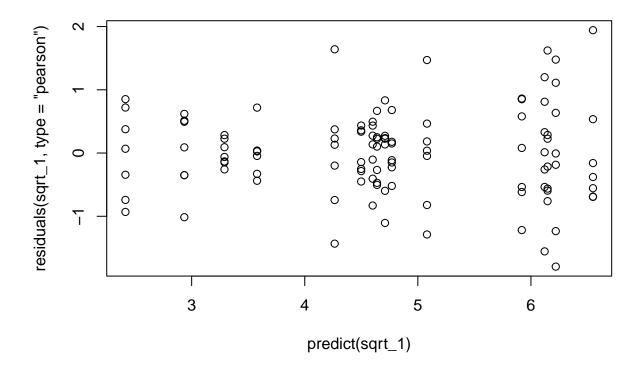
#Create the histogram
hist(data$sqrt_biomass) #Looks good</pre>
```

Histogram of data\$sqrt_biomass



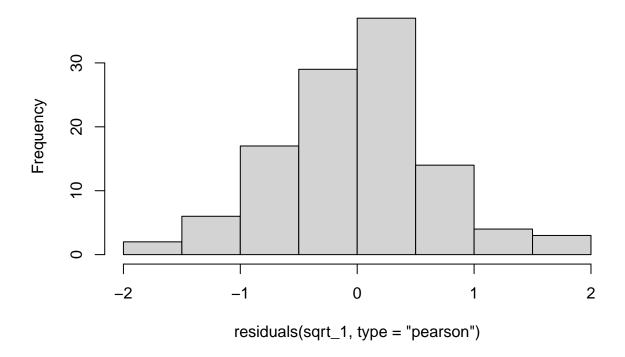
```
##
## Coefficients:
##
                                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                                        5.9194
                                                   0.2733 21.656 < 2e-16 ***
## speciesFestuca
                                        0.2017
                                                   0.3866
                                                            0.522 0.602947
## speciesGaillardia
                                       -3.5050
                                                   0.3866 -9.067 1.50e-14 ***
                                                   0.3866 -3.411 0.000948 ***
## speciesTaraxacum
                                       -1.3185
## treatment240448
                                       -1.6547
                                                   0.3866 -4.281 4.41e-05 ***
## treatment240720
                                       -1.2098
                                                   0.3866 -3.130 0.002319 **
## treatmentControl
                                                   0.3866 -2.169 0.032538 *
                                       -0.8385
## speciesFestuca:treatment240448
                                        1.7533
                                                   0.5467
                                                            3.207 0.001821 **
## speciesGaillardia:treatment240448
                                        2.5305
                                                   0.5467
                                                            4.629 1.15e-05 ***
## speciesTaraxacum:treatment240448
                                        1.6930
                                                   0.5467
                                                            3.097 0.002564 **
## speciesFestuca:treatment240720
                                        1.6372
                                                   0.5467
                                                            2.995 0.003492 **
## speciesGaillardia:treatment240720
                                        2.3738
                                                   0.5467
                                                            4.342 3.50e-05 ***
## speciesTaraxacum:treatment240720
                                        1.3790
                                                   0.5467
                                                            2.522 0.013298 *
```

```
0.5467 1.581 0.117080
## speciesFestuca:treatmentControl
                                  0.8645
                                                 0.5467
                                                          2.487 0.014624 *
## speciesGaillardia:treatmentControl 1.3593
## speciesTaraxacum:treatmentControl 0.7365
                                                 0.5467 1.347 0.181078
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
## (Dispersion parameter for gaussian family taken to be 0.5229848)
##
      Null deviance: 213.216 on 111 degrees of freedom
## Residual deviance: 50.207 on 96 degrees of freedom
## AIC: 261.98
## Number of Fisher Scoring iterations: 2
#Better AIC fit than no transformation, worse than log,
#but better dispersion. Underdispserion (dispersion parameter ~0.5) persists
#but may be acceptable
summary(residuals(sqrt_1, type = "pearson")) #Residual spread of pearson
      Min. 1st Qu.
                      Median
                                 Mean 3rd Qu.
                                                  Max.
## -1.79594 -0.41386 0.02776 0.00000 0.36289 1.94251
#residuals expected between -2 and 2 for a normally distributed model, this
#residual spread looks good
#Plot residuals
#predict vs. residuals
plot(predict(sqrt_1), residuals(sqrt_1, type = "pearson"))
```

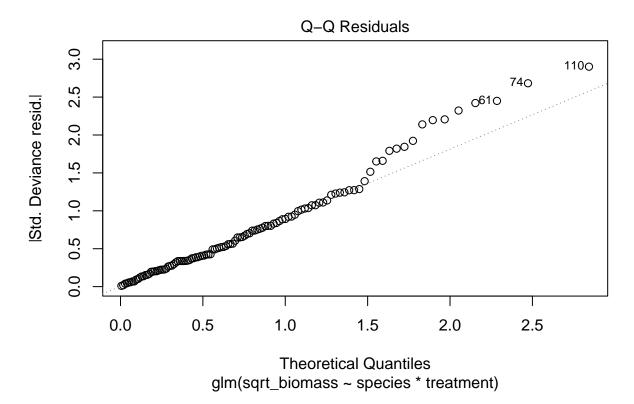


#Looks okay, slightly underdispersed. Persistence of heteroskedastic fan shape.
#Check histogram
hist(residuals(sqrt_1, type="pearson"))

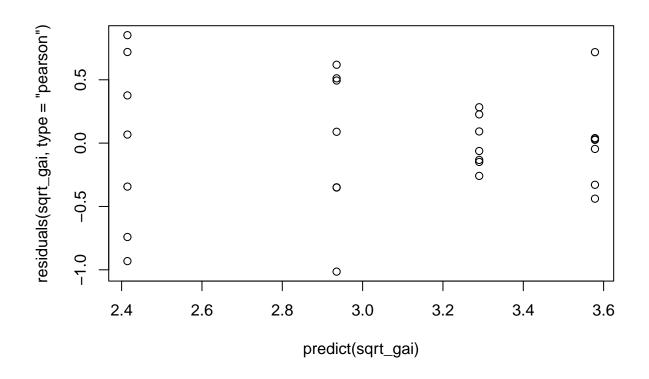
Histogram of residuals(sqrt_1, type = "pearson")

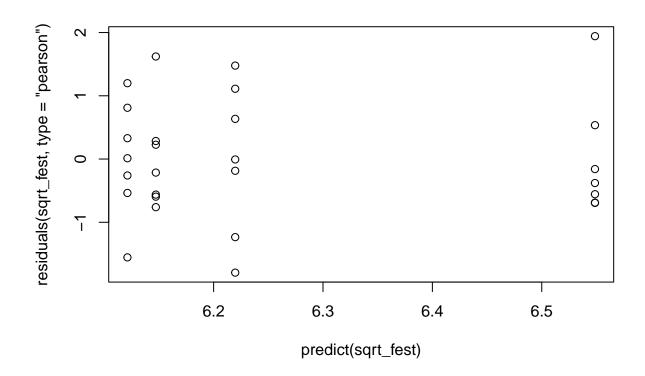


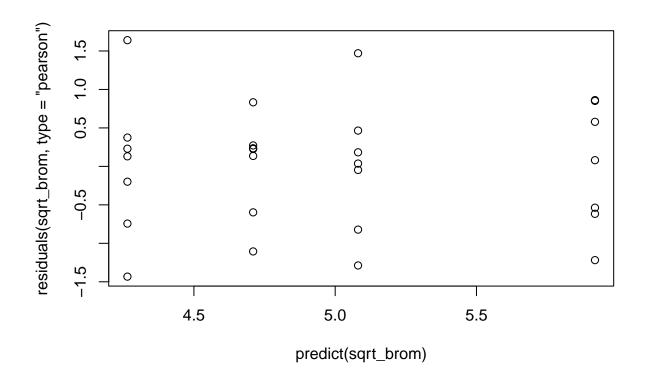
```
#looks good
#Check QQplot
plot(sqrt_1, which =2) #struggles to predict on higher biomass
```

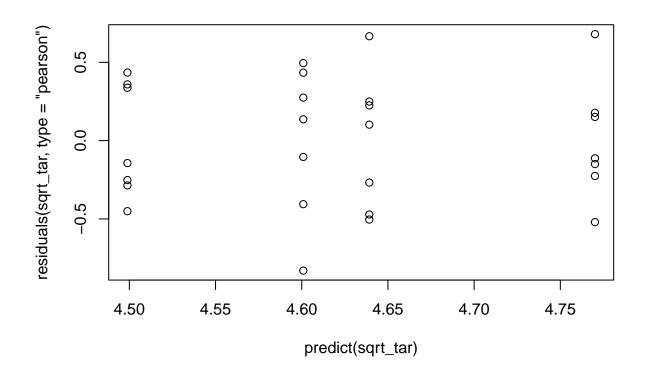


```
#Try analysing the data by species to account for heterogeneity of variance
#Add the sqrt transformation to species groups
data_gaillardia<-data%>%
  filter(species=="Gaillardia")
data_taraxacum<-data%>%
  filter(species=="Taraxacum")
data_bromus<-data%>%
  filter(species=="Bromus")
data_festuca<-data%>%
  filter(species=="Festuca")
#Model diagnostics for each species (sqrt transformed)
#GAILLARDIA
sqrt_gai<-glm(sqrt_biomass~treatment,</pre>
              family=gaussian(link="identity"),
              data=data_gaillardia)
#Check heteroskedasticity
plot(predict(sqrt_gai), residuals(sqrt_gai, type = "pearson"))
```





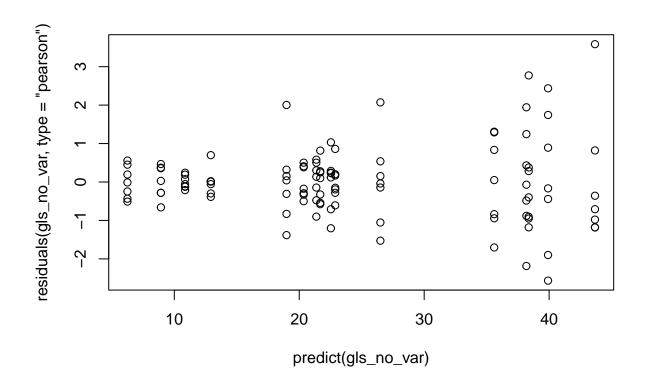




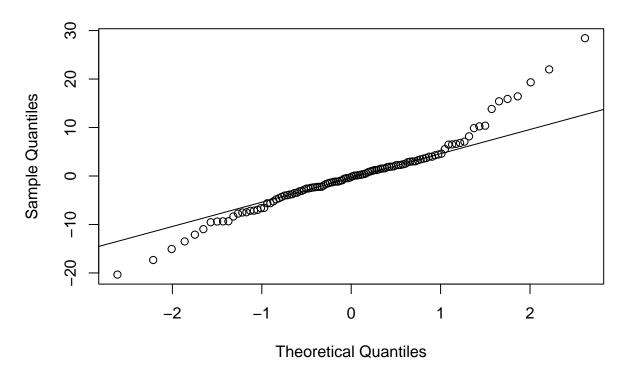
```
## Generalized least squares fit by REML
##
    Model: biomass ~ treatment * species
##
     Data: data
##
         AIC
                  BIC
                        logLik
     735.126 778.7199 -350.563
##
##
## Coefficients:
                                                            t-value p-value
##
                                          Value Std.Error
## (Intercept)
                                       35.60571 2.997100 11.880056 0.0000
## treatment240448
                                      -16.62571 4.238539 -3.922511 0.0002
## treatment240720
                                      -13.07143 4.238539 -3.083947 0.0027
```

```
## treatmentControl
                                        -9.11143 4.238539 -2.149662 0.0341
                                                                      0.5451
## speciesFestuca
                                         2.57429 4.238539 0.607352
                                                                      0.0000
## speciesGaillardia
                                       -29.35857 4.238539 -6.926578
## speciesTaraxacum
                                       -14.23857
                                                  4.238539 -3.359311
                                                                     0.0011
## treatment240448:speciesFestuca
                                        18.36000
                                                  5.994200
                                                            3.062961
                                                                      0.0028
## treatment240720:speciesFestuca
                                        18.56143
                                                 5.994200
                                                           3.096565
                                                                      0.0026
## treatmentControl:speciesFestuca
                                         9.29857
                                                  5.994200
                                                           1.551262
                                                                      0.1241
## treatment240448:speciesGaillardia
                                        21.24000
                                                  5.994200
                                                            3.543426
                                                                      0.0006
## treatment240720:speciesGaillardia
                                        19.74714
                                                  5.994200
                                                            3.294375
                                                                      0.0014
## treatmentControl:speciesGaillardia
                                        11.79000
                                                  5.994200
                                                            1.966901
                                                                      0.0521
## treatment240448:speciesTaraxacum
                                        16.94000
                                                  5.994200
                                                            2.826065
                                                                      0.0057
## treatment240720:speciesTaraxacum
                                        14.58286
                                                  5.994200
                                                            2.432828
                                                                      0.0168
## treatmentControl:speciesTaraxacum
                                         8.09857
                                                  5.994200 1.351068 0.1798
##
##
   Correlation:
##
                                       (Intr) tr240448 tr240720 trtmnC spcsFs
## treatment240448
                                       -0.707
## treatment240720
                                       -0.707
                                               0.500
## treatmentControl
                                       -0.707 0.500
                                                        0.500
## speciesFestuca
                                       -0.707 0.500
                                                        0.500
                                                                 0.500
## speciesGaillardia
                                       -0.707 0.500
                                                        0.500
                                                                 0.500 0.500
## speciesTaraxacum
                                                        0.500
                                                                 0.500 0.500
                                       -0.707 0.500
## treatment240448:speciesFestuca
                                        0.500 - 0.707
                                                       -0.354
                                                                -0.354 - 0.707
## treatment240720:speciesFestuca
                                        0.500 - 0.354
                                                       -0.707
                                                                -0.354 - 0.707
## treatmentControl:speciesFestuca
                                        0.500 - 0.354
                                                       -0.354
                                                                -0.707 - 0.707
## treatment240448:speciesGaillardia
                                        0.500 - 0.707
                                                       -0.354
                                                                -0.354 -0.354
## treatment240720:speciesGaillardia
                                        0.500 - 0.354
                                                       -0.707
                                                                -0.354 -0.354
## treatmentControl:speciesGaillardia
                                       0.500 - 0.354
                                                       -0.354
                                                                -0.707 -0.354
## treatment240448:speciesTaraxacum
                                        0.500 - 0.707
                                                       -0.354
                                                                -0.354 - 0.354
## treatment240720:speciesTaraxacum
                                        0.500 - 0.354
                                                       -0.707
                                                                -0.354 -0.354
## treatmentControl:speciesTaraxacum
                                        0.500 - 0.354
                                                       -0.354
                                                                -0.707 - 0.354
##
                                       spcsGl spcsTr t240448:F t240720:F trtC:F
## treatment240448
## treatment240720
## treatmentControl
## speciesFestuca
## speciesGaillardia
## speciesTaraxacum
                                       0.500
## treatment240448:speciesFestuca
                                       -0.354 -0.354
## treatment240720:speciesFestuca
                                       -0.354 -0.354 0.500
## treatmentControl:speciesFestuca
                                                                0.500
                                       -0.354 -0.354 0.500
## treatment240448:speciesGaillardia
                                      -0.707 -0.354
                                                     0.500
                                                                0.250
                                                                          0.250
## treatment240720:speciesGaillardia
                                      -0.707 -0.354
                                                     0.250
                                                                0.500
                                                                          0.250
## treatmentControl:speciesGaillardia -0.707 -0.354
                                                     0.250
                                                                0.250
                                                                          0.500
## treatment240448:speciesTaraxacum
                                       -0.354 -0.707
                                                      0.500
                                                                0.250
                                                                          0.250
## treatment240720:speciesTaraxacum
                                       -0.354 -0.707
                                                      0.250
                                                                0.500
                                                                          0.250
## treatmentControl:speciesTaraxacum
                                      -0.354 - 0.707
                                                     0.250
                                                                0.250
                                                                          0.500
##
                                       t240448:G t240720:G trtC:G t240448:T
## treatment240448
## treatment240720
## treatmentControl
## speciesFestuca
## speciesGaillardia
## speciesTaraxacum
```

```
## treatment240448:speciesFestuca
## treatment240720:speciesFestuca
## treatmentControl:speciesFestuca
## treatment240448:speciesGaillardia
## treatment240720:speciesGaillardia
                                       0.500
## treatmentControl:speciesGaillardia 0.500
                                                 0.500
## treatment240448:speciesTaraxacum
                                       0.500
                                                 0.250
                                                           0.250
## treatment240720:speciesTaraxacum
                                       0.250
                                                 0.500
                                                           0.250 0.500
## treatmentControl:speciesTaraxacum
                                       0.250
                                                 0.250
                                                           0.500 0.500
##
                                      t240720:T
## treatment240448
## treatment240720
## treatmentControl
## speciesFestuca
## speciesGaillardia
## speciesTaraxacum
## treatment240448:speciesFestuca
## treatment240720:speciesFestuca
## treatmentControl:speciesFestuca
## treatment240448:speciesGaillardia
## treatment240720:speciesGaillardia
## treatmentControl:speciesGaillardia
## treatment240448:speciesTaraxacum
## treatment240720:speciesTaraxacum
## treatmentControl:speciesTaraxacum
                                       0.500
## Standardized residuals:
           Min
                                   Med
                                                QЗ
                                                           Max
## -2.56561939 -0.47705640 -0.02468154 0.37409653 3.58530942
##
## Residual standard error: 7.929581
## Degrees of freedom: 112 total; 96 residual
#Check residuals
plot(predict(gls_no_var), residuals(gls_no_var, type = "pearson"))
```

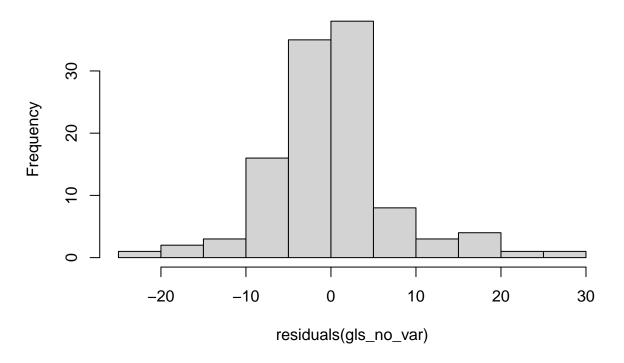


qqnorm(residuals(gls_no_var)); qqline(residuals(gls_no_var))



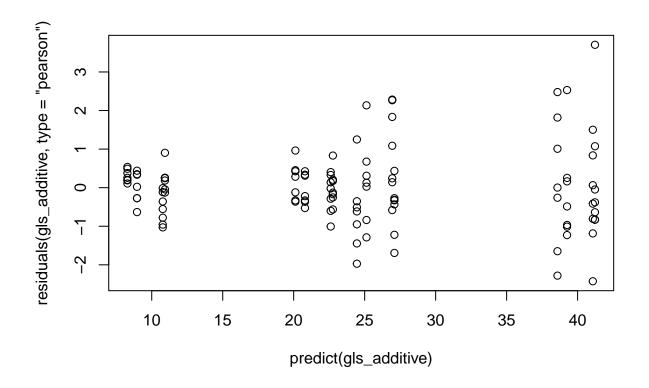
hist(residuals(gls_no_var))

Histogram of residuals(gls_no_var)



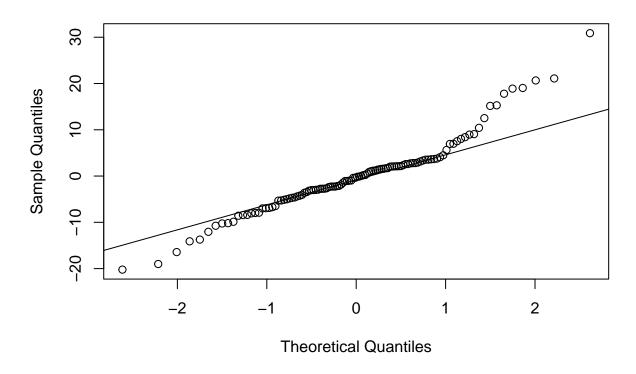
```
#Heteroskedastic
#additive model
gls_additive <- gls(biomass ~ treatment + species,</pre>
                    data = data)
summary(gls_additive)
## Generalized least squares fit by REML
##
     Model: biomass ~ treatment + species
##
     Data: data
##
          AIC
                   BIC
                          logLik
     781.2275 802.4592 -382.6137
##
##
## Coefficients:
##
                          Value Std.Error
                                             t-value p-value
## (Intercept)
                      26.942054 2.083888 12.928743 0.0000
## treatment240448
                      -2.490714
                                 2.227770 -1.118030
                                                      0.2661
## treatment240720
                       0.151429
                                 2.227770
                                            0.067973
                                                      0.9459
## treatmentControl
                      -1.814643
                                 2.227770 -0.814556
                                                      0.4172
## speciesFestuca
                                            6.342345
                                                     0.0000
                      14.129286
                                 2.227770
## speciesGaillardia -16.164286
                                 2.227770 -7.255814
                                                      0.0000
## speciesTaraxacum
                      -4.333214 2.227770 -1.945090 0.0544
##
##
   Correlation:
##
                     (Intr) t24044 t24072 trtmnC spcsFs spcsGl
## treatment240448
                     -0.535
```

```
## treatment240720
                     -0.535
                             0.500
## treatmentControl -0.535
                             0.500
                                    0.500
## speciesFestuca
                     -0.535
                             0.000
                                    0.000
                                           0.000
## speciesGaillardia -0.535
                             0.000
                                    0.000
                                           0.000 0.500
                                           0.000 0.500
##
   speciesTaraxacum -0.535
                             0.000
                                    0.000
##
## Standardized residuals:
##
                                                Q3
           Min
                                   Med
                                                           Max
## -2.42591473 -0.53476831 -0.03227355 0.33993805
                                                   3.70428147
##
## Residual standard error: 8.335552
## Degrees of freedom: 112 total; 105 residual
#Check residuals
plot(predict(gls_additive), residuals(gls_additive, type = "pearson"))
```



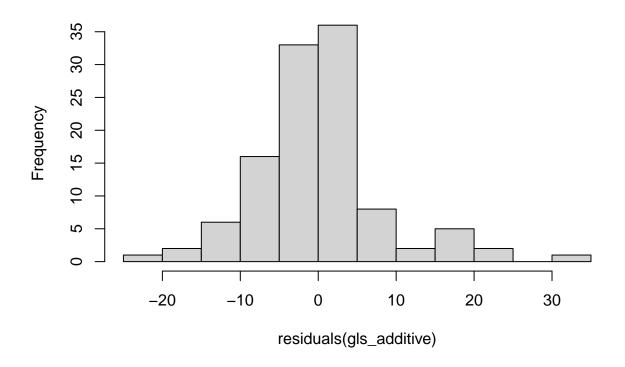
```
qqnorm(residuals(gls_additive)); qqline(residuals(gls_additive))
```

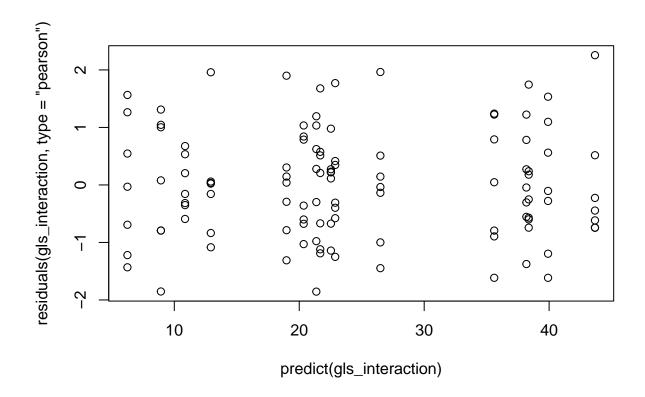
Normal Q-Q Plot



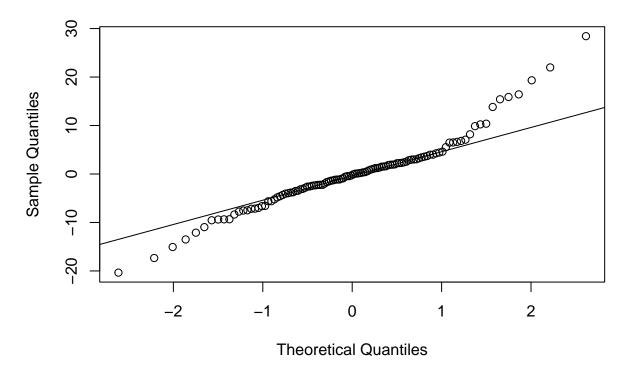
hist(residuals(gls_additive))

Histogram of residuals(gls_additive)



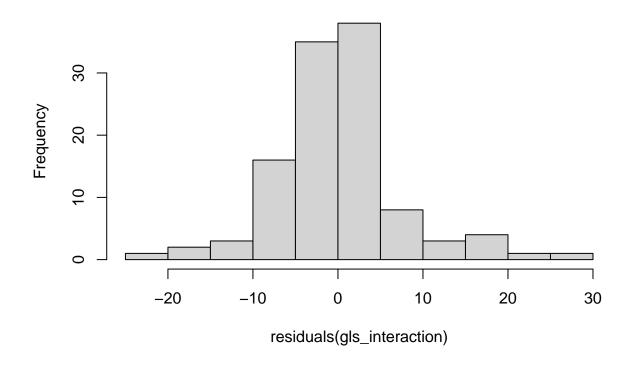


qqnorm(residuals(gls_interaction)); qqline(residuals(gls_interaction))



#Struggles to predicat at higher and lower values
hist(residuals(gls_interaction))

Histogram of residuals(gls_interaction)



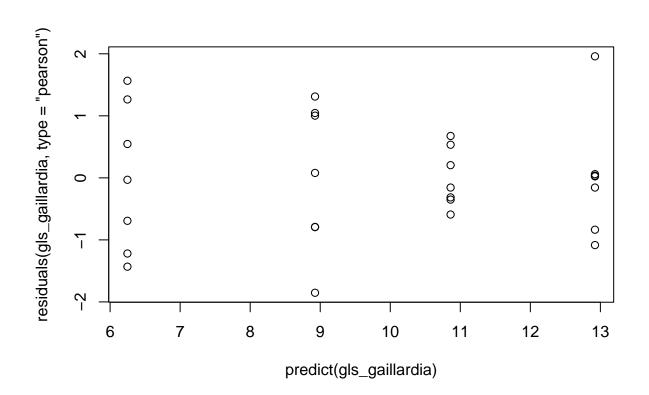
#Good summary(gls_interaction)

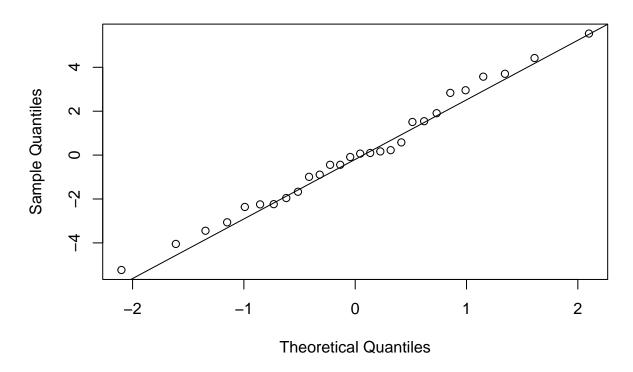
```
## Generalized least squares fit by REML
##
    Model: biomass ~ treatment * species
##
     Data: data
          AIC
##
                   BIC
                          logLik
     681.7433 733.0302 -320.8716
##
##
## Variance function:
   Structure: Different standard deviations per stratum
   Formula: ~1 | species
##
##
   Parameter estimates:
##
       Bromus
                 Festuca Gaillardia Taraxacum
##
   1.0000000 1.5063050 0.3378848 0.4606768
##
  Coefficients:
##
##
                                          Value Std.Error
                                                            t-value p-value
## (Intercept)
                                       35.60571 3.161265 11.263121
                                                                    0.0000
## treatment240448
                                                4.470704 -3.718813
                                                                    0.0003
                                      -16.62571
## treatment240720
                                      -13.07143
                                                 4.470704 -2.923796
## treatmentControl
                                                4.470704 -2.038030
                                       -9.11143
                                                                     0.0443
## speciesFestuca
                                        2.57429
                                                 5.715647
                                                          0.450393
                                                                     0.6534
## speciesGaillardia
                                      -29.35857
                                                 3.336844 -8.798304
                                                                     0.0000
## speciesTaraxacum
                                      -14.23857
                                                 3.480585 -4.090856 0.0001
## treatment240448:speciesFestuca
                                       18.36000 8.083146 2.271393 0.0254
```

```
## treatment240720:speciesFestuca
                                       18.56143 8.083146 2.296312 0.0238
## treatmentControl:speciesFestuca
                                       9.29857 8.083146 1.150365 0.2529
## treatment240448:speciesGaillardia
                                       21.24000 4.719010 4.500944 0.0000
## treatment240720:speciesGaillardia
                                       19.74714
                                                4.719010 4.184594 0.0001
## treatmentControl:speciesGaillardia
                                       11.79000
                                                4.719010 2.498405 0.0142
## treatment240448:speciesTaraxacum
                                                4.922291 3.441487
                                                                    0.0009
                                       16.94000
## treatment240720:speciesTaraxacum
                                       14.58286 4.922291 2.962616 0.0038
## treatmentControl:speciesTaraxacum
                                       8.09857 4.922291 1.645285 0.1032
##
##
   Correlation:
##
                                      (Intr) tr240448 tr240720 trtmnC spcsFs
## treatment240448
                                      -0.707
## treatment240720
                                      -0.707
                                             0.500
                                      -0.707 0.500
                                                       0.500
## treatmentControl
## speciesFestuca
                                      -0.553 0.391
                                                       0.391
                                                                0.391
## speciesGaillardia
                                      -0.947
                                              0.670
                                                       0.670
                                                                0.670 0.524
## speciesTaraxacum
                                      -0.908 0.642
                                                       0.642
                                                                0.642 0.502
## treatment240448:speciesFestuca
                                       0.391 - 0.553
                                                      -0.277
                                                               -0.277 - 0.707
## treatment240720:speciesFestuca
                                       0.391 -0.277
                                                              -0.277 - 0.707
                                                      -0.553
## treatmentControl:speciesFestuca
                                       0.391 - 0.277
                                                      -0.277
                                                               -0.553 - 0.707
## treatment240448:speciesGaillardia
                                       0.670 -0.947
                                                      -0.474
                                                              -0.474 -0.371
## treatment240720:speciesGaillardia
                                       0.670 - 0.474
                                                      -0.947
                                                               -0.474 -0.371
                                       0.670 -0.474
## treatmentControl:speciesGaillardia
                                                      -0.474
                                                              -0.947 -0.371
## treatment240448:speciesTaraxacum
                                       0.642 - 0.908
                                                      -0.454
                                                               -0.454 -0.355
## treatment240720:speciesTaraxacum
                                                      -0.908
                                       0.642 - 0.454
                                                              -0.454 - 0.355
## treatmentControl:speciesTaraxacum
                                       0.642 - 0.454
                                                      -0.454
                                                               -0.908 -0.355
                                      spcsGl spcsTr t240448:F t240720:F trtC:F
## treatment240448
## treatment240720
## treatmentControl
## speciesFestuca
## speciesGaillardia
## speciesTaraxacum
                                       0.860
## treatment240448:speciesFestuca
                                      -0.371 -0.355
## treatment240720:speciesFestuca
                                      -0.371 -0.355 0.500
## treatmentControl:speciesFestuca
                                      -0.371 -0.355 0.500
                                                               0.500
## treatment240448:speciesGaillardia
                                     -0.707 -0.608 0.524
                                                               0.262
                                                                         0.262
## treatment240720:speciesGaillardia -0.707 -0.608 0.262
                                                               0.524
                                                                         0.262
## treatmentControl:speciesGaillardia -0.707 -0.608 0.262
                                                               0.262
                                                                         0.524
## treatment240448:speciesTaraxacum
                                                               0.251
                                      -0.608 -0.707 0.502
                                                                         0.251
## treatment240720:speciesTaraxacum
                                      -0.608 -0.707 0.251
                                                               0.502
                                                                         0.251
## treatmentControl:speciesTaraxacum -0.608 -0.707 0.251
                                                               0.251
                                                                         0.502
                                      t240448:G t240720:G trtC:G t240448:T
## treatment240448
## treatment240720
## treatmentControl
## speciesFestuca
## speciesGaillardia
## speciesTaraxacum
## treatment240448:speciesFestuca
## treatment240720:speciesFestuca
## treatmentControl:speciesFestuca
## treatment240448:speciesGaillardia
## treatment240720:speciesGaillardia
```

```
## treatmentControl:speciesGaillardia 0.500
                                                 0.500
## treatment240448:speciesTaraxacum
                                                 0.430
                                                           0.430
                                       0.860
## treatment240720:speciesTaraxacum
                                       0.430
                                                 0.860
                                                           0.430 0.500
## treatmentControl:speciesTaraxacum    0.430
                                                           0.860 0.500
                                                 0.430
                                      t240720:T
## treatment240448
## treatment240720
## treatmentControl
## speciesFestuca
## speciesGaillardia
## speciesTaraxacum
## treatment240448:speciesFestuca
## treatment240720:speciesFestuca
## treatmentControl:speciesFestuca
## treatment240448:speciesGaillardia
## treatment240720:speciesGaillardia
## treatmentControl:speciesGaillardia
## treatment240448:speciesTaraxacum
## treatment240720:speciesTaraxacum
## treatmentControl:speciesTaraxacum
                                       0.500
##
## Standardized residuals:
                      Q1
##
         Min
                                Med
                                            QЗ
                                                      Max
## -1.8549239 -0.6800594 -0.0336082 0.5651681 2.2565967
##
## Residual standard error: 8.363922
## Degrees of freedom: 112 total; 96 residual
AIC(gls_no_var)
## [1] 735.126
AIC(gls_additive) #Worse fit
## [1] 781.2275
AIC(gls_interaction) #Best fit (delta AIC>50)
## [1] 681.7433
#Check GLS by species group (simpler models for n=7 sample size)
#Gaillardia
gls_gaillardia <- gls(biomass ~ treatment,</pre>
                      data = data_gaillardia)
summary(gls_gaillardia)
## Generalized least squares fit by REML
##
    Model: biomass ~ treatment
##
    Data: data_gaillardia
##
                  BIC
                         logLik
    135.7588 141.6491 -62.8794
##
```

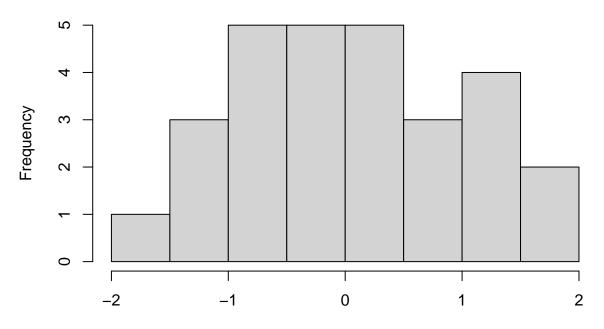
```
##
## Coefficients:
##
                       Value Std.Error t-value p-value
                    6.247143
                              1.068143 5.848599 0.0000
##
  (Intercept)
   treatment240448
                    4.614286
                              1.510583 3.054639
   treatment240720 6.675714
                             1.510583 4.419296
                                                  0.0002
   treatmentControl 2.678571 1.510583 1.773204
##
##
    Correlation:
##
                    (Intr) t24044 t24072
##
   treatment240448
                    -0.707
   treatment240720
                    -0.707
                            0.500
   treatmentControl -0.707
                           0.500
##
##
  Standardized residuals:
##
            Min
                          Q1
                                      Med
                                                     Q3
                                                                 Max
  -1.852666730 -0.717181698 -0.003538518
                                           0.578294881
                                                        1.959327761
##
##
## Residual standard error: 2.826042
## Degrees of freedom: 28 total; 24 residual
#Check residuals
plot(predict(gls_gaillardia), residuals(gls_gaillardia, type = "pearson"))
```





hist(residuals(gls_gaillardia, type = "pearson")) #Okay

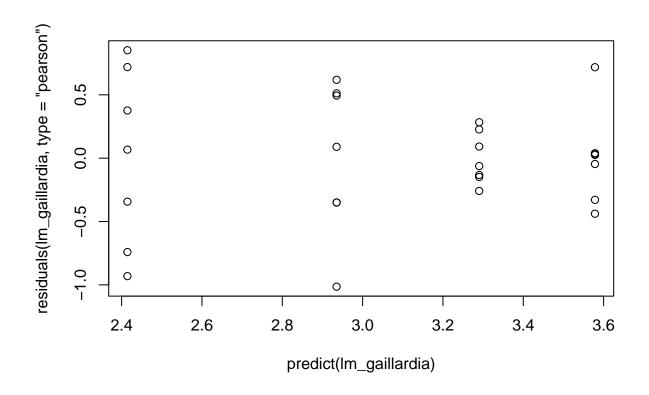
Histogram of residuals(gls_gaillardia, type = "pearson")



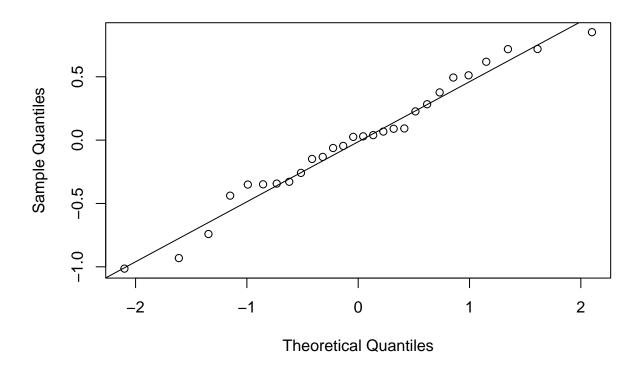
residuals(gls_gaillardia, type = "pearson")

```
##
## Call:
## lm(formula = sqrt_biomass ~ treatment, data = data_gaillardia)
##
## Residuals:
##
       Min
                 1Q
                      Median
                                   3Q
## -1.01432 -0.33245 0.02776 0.30662 0.85207
##
## Coefficients:
##
                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                     2.4144
                                0.1916 12.603 4.5e-12 ***
## treatment240448
                     0.8758
                                0.2709
                                         3.233 0.003548 **
## treatment240720
                     1.1641
                                0.2709
                                         4.297 0.000248 ***
                     0.5208
                                0.2709
                                         1.922 0.066498 .
## treatmentControl
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## Residual standard error: 0.5069 on 24 degrees of freedom
## Multiple R-squared: 0.4612, Adjusted R-squared: 0.3939
## F-statistic: 6.849 on 3 and 24 DF, p-value: 0.001708
```

```
#Check residuals
plot(predict(lm_gaillardia), residuals(lm_gaillardia, type = "pearson"))
```

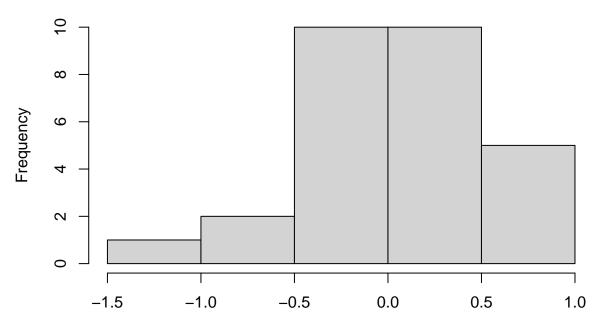


qqnorm(residuals(lm_gaillardia)); qqline(residuals(lm_gaillardia)) #God



hist(residuals(lm_gaillardia, type = "pearson"))

Histogram of residuals(Im_gaillardia, type = "pearson")

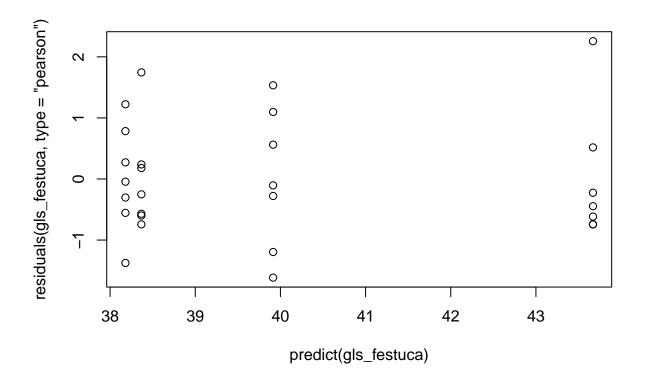


residuals(Im_gaillardia, type = "pearson")

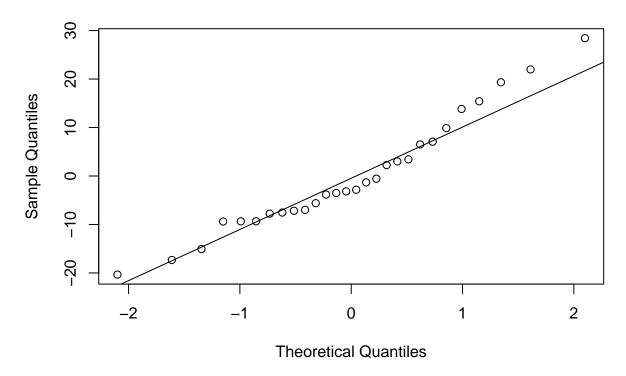
```
#FESTUCA
gls_festuca<- gls(biomass ~ treatment,</pre>
                  data = data_festuca)
summary(gls_festuca)
## Generalized least squares fit by REML
##
     Model: biomass ~ treatment
##
     Data: data festuca
##
          AIC
                   BIC
                          logLik
     207.5049 213.3951 -98.75244
##
##
## Coefficients:
##
                       Value Std.Error t-value p-value
## (Intercept)
                    38.18000 4.761830 8.017926 0.0000
  treatment240448
                     1.73429
                              6.734244 0.257532
                                                 0.7990
## treatment240720
                     5.49000
                              6.734244 0.815236
                                                 0.4230
   treatmentControl 0.18714
                              6.734244 0.027790 0.9781
##
##
   Correlation:
##
                    (Intr) t24044 t24072
## treatment240448
                    -0.707
## treatment240720 -0.707 0.500
## treatmentControl -0.707 0.500 0.500
##
```

#GLS is a better it for gaillardia based on residual diagnostics

```
## Standardized residuals:
## Min Q1 Med Q3 Max
## -1.6148030 -0.6026738 -0.2384048 0.5279491 2.2565967
##
## Residual standard error: 12.59862
## Degrees of freedom: 28 total; 24 residual
##Check residuals
plot(predict(gls_festuca), residuals(gls_festuca, type = "pearson"))
```

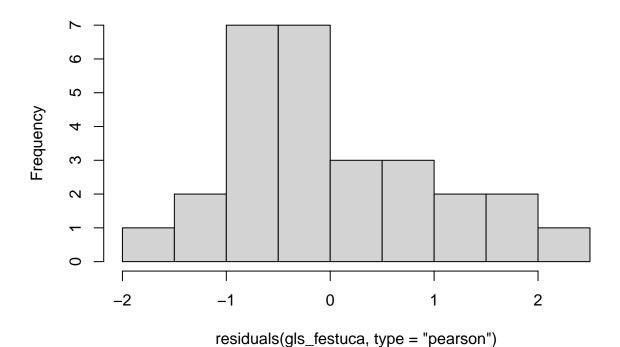


qqnorm(residuals(gls_festuca)); qqline(residuals(gls_festuca)) #okay



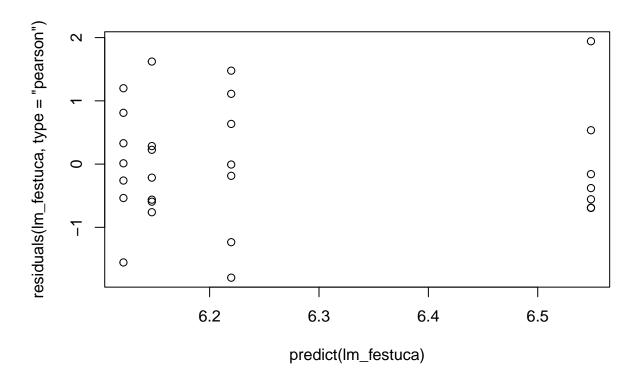
hist(residuals(gls_festuca, type = "pearson")) #Okay, slight left tail

Histogram of residuals(gls_festuca, type = "pearson")

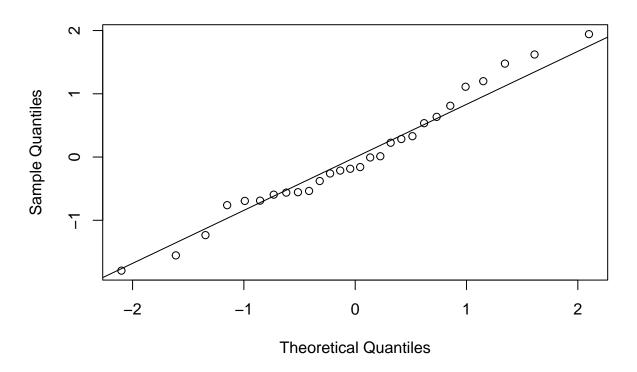


```
##
## Call:
## lm(formula = sqrt_biomass ~ treatment, data = data_festuca)
## Residuals:
##
      Min
               1Q Median
                               3Q
## -1.7959 -0.5705 -0.1722 0.5601
                                  1.9425
##
## Coefficients:
##
                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                    6.12118
                               0.37107 16.496 1.35e-14 ***
## treatment240448
                    0.09855
                                         0.188
                                                  0.853
                               0.52477
## treatment240720
                    0.42748
                               0.52477
                                         0.815
                                                  0.423
## treatmentControl 0.02599
                                         0.050
                                                  0.961
                               0.52477
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9818 on 24 degrees of freedom
## Multiple R-squared: 0.03418, Adjusted R-squared: -0.08655
## F-statistic: 0.2831 on 3 and 24 DF, p-value: 0.8371
```

```
#Check residuals
plot(predict(lm_festuca), residuals(lm_festuca, type = "pearson"))
```

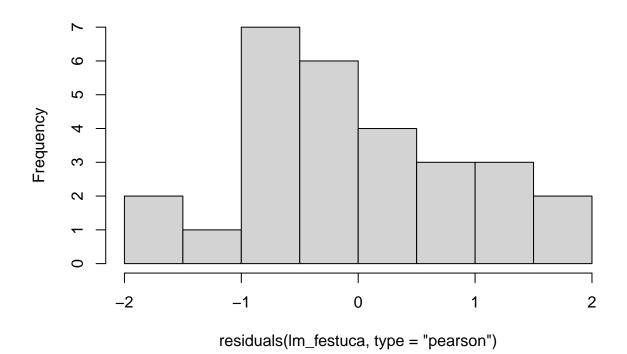


qqnorm(residuals(lm_festuca)); qqline(residuals(lm_festuca)) #okay



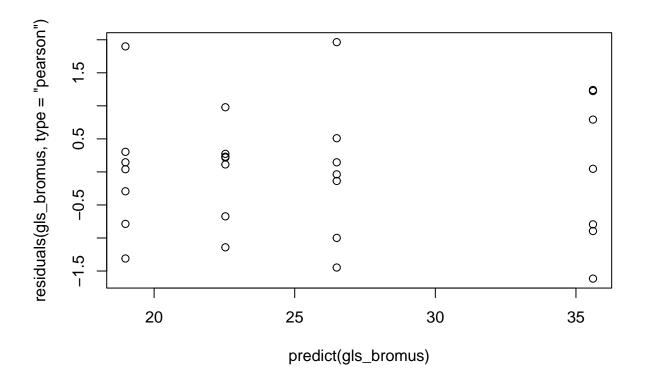
hist(residuals(lm_festuca, type = "pearson"))

Histogram of residuals(Im_festuca, type = "pearson")

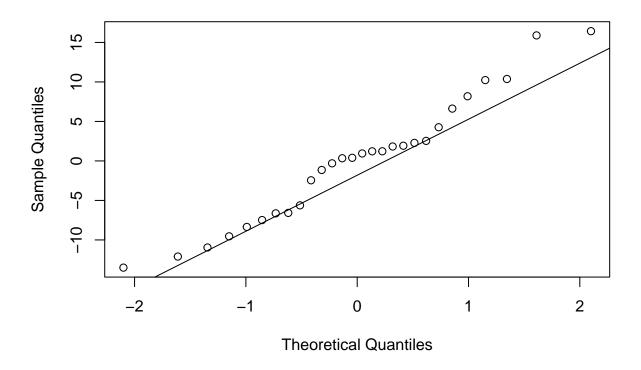


```
## Generalized least squares fit by REML
##
    Model: biomass ~ treatment
##
     Data: data_bromus
##
         AIC
                  BIC
                        logLik
     187.8412 193.7315 -88.9206
##
##
## Coefficients:
##
                       Value Std.Error t-value p-value
## (Intercept)
                    35.60571 3.161265 11.263121 0.0000
## treatment240448 -16.62571 4.470705 -3.718813 0.0011
## treatment240720 -13.07143 4.470705 -2.923796 0.0074
  treatmentControl -9.11143 4.470705 -2.038030 0.0527
##
##
   Correlation:
##
                    (Intr) t24044 t24072
## treatment240448
                   -0.707
## treatment240720 -0.707 0.500
## treatmentControl -0.707 0.500 0.500
##
```

```
## Standardized residuals:
## Min Q1 Med Q3 Max
## -1.61475846 -0.78837755 0.08010596 0.35526736 1.96387696
##
## Residual standard error: 8.363922
## Degrees of freedom: 28 total; 24 residual
#check residuals
plot(predict(gls_bromus), residuals(gls_bromus, type = "pearson")) #Okay residual
```

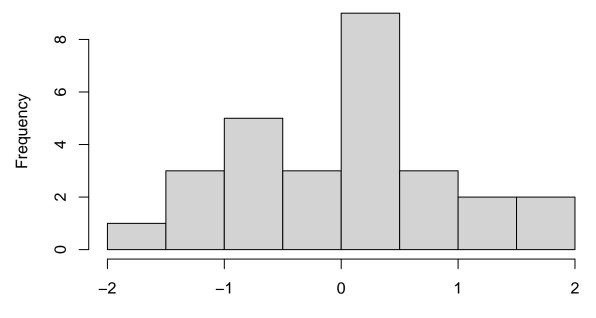


```
#spread
qqnorm(residuals(gls_bromus)); qqline(residuals(gls_bromus)) #okay
```



hist(residuals(gls_bromus, type="pearson")) #Okay

Histogram of residuals(gls_bromus, type = "pearson")

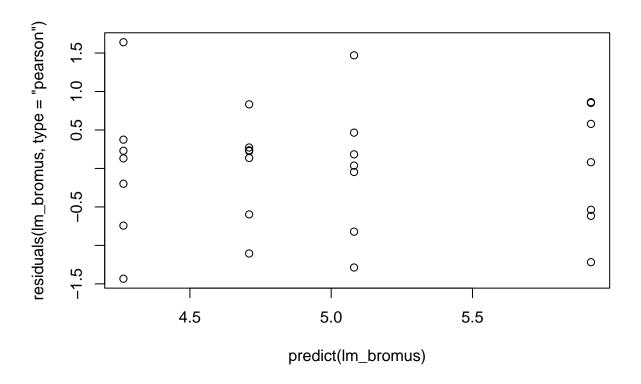


residuals(gls_bromus, type = "pearson")

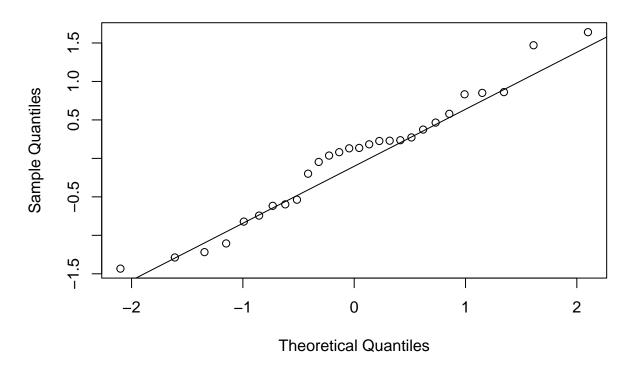
```
lm_bromus <- lm(sqrt_biomass ~ treatment,</pre>
                data = data_bromus)
summary(lm_bromus)
##
## Call:
## lm(formula = sqrt_biomass ~ treatment, data = data_bromus)
## Residuals:
      Min
                1Q Median
                                3Q
                                       Max
## -1.4327 -0.6023 0.1333 0.3970 1.6404
##
## Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
##
## (Intercept)
                     5.9194
                                 0.3155 18.759 7.62e-16 ***
## treatment240448
                     -1.6547
                                 0.4463
                                        -3.708
                                                  0.0011 **
## treatment240720
                     -1.2098
                                 0.4463
                                         -2.711
                                                  0.0122 *
                                 0.4463 -1.879
                                                  0.0724 .
## treatmentControl -0.8385
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
## Residual standard error: 0.8349 on 24 degrees of freedom
## Multiple R-squared: 0.3819, Adjusted R-squared: 0.3047
## F-statistic: 4.944 on 3 and 24 DF, p-value: 0.008196
```

#residuals still centered around O

```
#Check residuals
plot(predict(lm_bromus), residuals(lm_bromus, type = "pearson"))
```

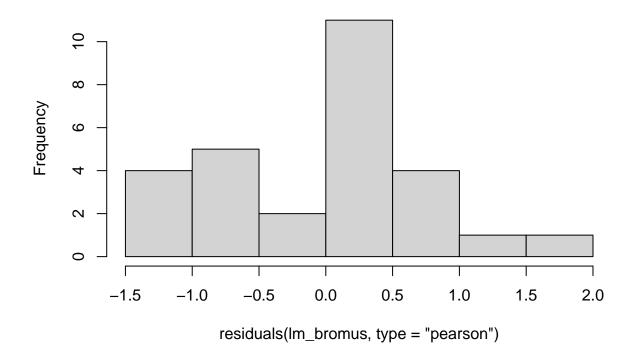


qqnorm(residuals(lm_bromus)); qqline(residuals(lm_bromus)) #meh



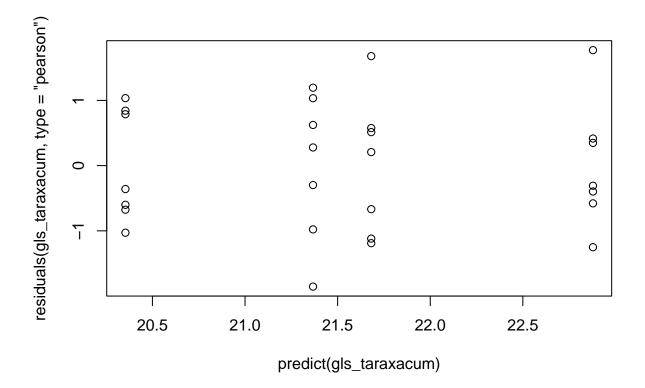
hist(residuals(lm_bromus, type = "pearson")) #Does not fit expected distribution, bimodal

Histogram of residuals(Im_bromus, type = "pearson")

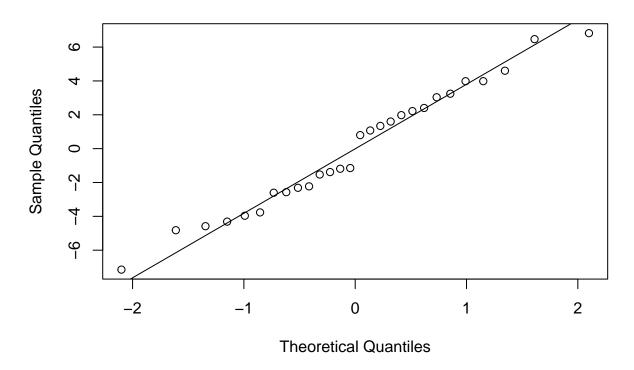


```
## Generalized least squares fit by REML
##
    Model: biomass ~ treatment
##
    Data: data_taraxacum
##
         AIC
                 BIC
                       logLik
    150.6384 156.5287 -70.3192
##
##
## Coefficients:
##
                      Value Std.Error
                                       t-value p-value
## (Intercept)
                  treatment240448
                   0.314286
                            2.059550
                                     0.152599
                                               0.8800
  treatment240720
                   1.511429
                            2.059550 0.733863 0.4701
  treatmentControl -1.012857 2.059550 -0.491786 0.6273
##
##
   Correlation:
##
                   (Intr) t24044 t24072
## treatment240448
                  -0.707
## treatment240720 -0.707 0.500
## treatmentControl -0.707 0.500 0.500
##
```

```
## Standardized residuals:
## Min Q1 Med Q3 Max
## -1.8549239 -0.6695041 -0.0452330 0.6646842 1.7703901
##
## Residual standard error: 3.853065
## Degrees of freedom: 28 total; 24 residual
#Check residuals
plot(predict(gls_taraxacum), residuals(gls_taraxacum, type = "pearson"))
```

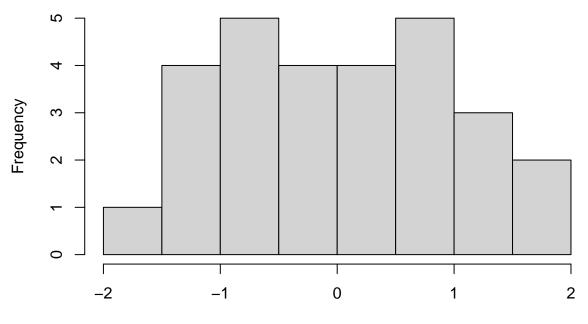


```
#Okay, possible heteroskedasticity
qqnorm(residuals(gls_taraxacum)); qqline(residuals(gls_taraxacum)) #okay
```



hist(residuals(gls_taraxacum, type = "pearson")) #Resdiusal centered around 0

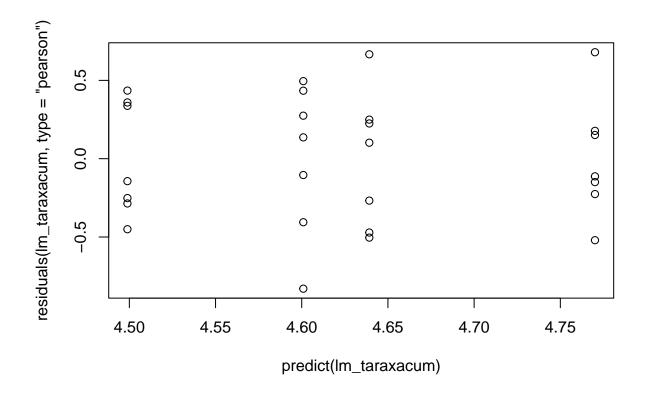
Histogram of residuals(gls_taraxacum, type = "pearson")



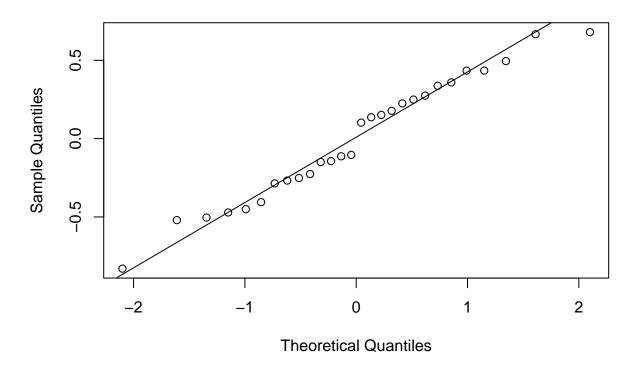
residuals(gls_taraxacum, type = "pearson")

```
##
## Call:
## lm(formula = sqrt_biomass ~ treatment, data = data_taraxacum)
## Residuals:
##
        Min
                  1Q
                      Median
                                    3Q
## -0.82996 -0.27222 -0.00106 0.29027 0.67967
##
## Coefficients:
##
                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                     4.60090
                               0.15775 29.166
                                                  <2e-16 ***
## treatment240448
                    0.03828
                               0.22309
                                                  0.865
                                         0.172
## treatment240720
                     0.16919
                               0.22309
                                         0.758
                                                  0.456
## treatmentControl -0.10202
                               0.22309 -0.457
                                                  0.652
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4174 on 24 degrees of freedom
## Multiple R-squared: 0.0594, Adjusted R-squared: -0.05817
## F-statistic: 0.5052 on 3 and 24 DF, p-value: 0.6823
```

```
#Check residuals
plot(predict(lm_taraxacum), residuals(lm_taraxacum, type = "pearson"))
```

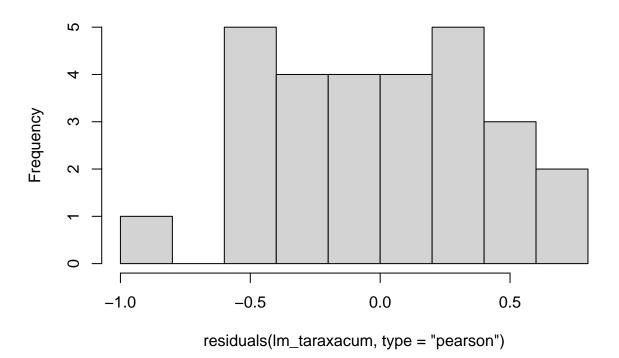


qqnorm(residuals(lm_taraxacum)); qqline(residuals(lm_taraxacum)) #okay



hist(residuals(lm_taraxacum, type = "pearson"))

Histogram of residuals(Im_taraxacum, type = "pearson")



#GLS is a better fit than lm models based on residual diagnostics

Extract estimated marginal means

```
#Compute estimated marginal means
#infer = (c(TRUE, FALSE)) includes confidence intervals, excludes
#p vlaues testing significant difference form 0
#Gaillardia
emm_gaillardia <- emmeans(gls_gaillardia, ~ treatment, mode = "df.error")</pre>
summary(emm_gaillardia)
                       SE df lower.CL upper.CL
  treatment emmean
## 197198
                6.25 1.07 24
                                 4.04
                                          8.45
               10.86 1.07 24
                                         13.07
   240448
                                 8.66
## 240720
               12.92 1.07 24
                                10.72
                                         15.13
  Control
               8.93 1.07 24
                                         11.13
##
## Degrees-of-freedom method: df.error
## Confidence level used: 0.95
pairs(emm_gaillardia, adjust = "tukey")
                                SE df t.ratio p.value
## contrast
                     estimate
```

```
## 197198 - Control
                      -2.68 1.51 24 -1.773 0.3102
## 240448 - 240720
                    -2.06 1.51 24 -1.365 0.5327
## 240448 - Control
                      1.94 1.51 24
                                     1.281 0.5831
## 240720 - Control
                       4.00 1.51 24
                                     2.646 0.0634
## Degrees-of-freedom method: df.error
## P value adjustment: tukey method for comparing a family of 4 estimates
#Festuca
emm_festuca <- emmeans(gls_festuca, ~ treatment, mode = "df.error")</pre>
summary(emm_festuca)
## treatment emmean
                     SE df lower.CL upper.CL
## 197198
             38.2 4.76 24
                              28.4
## 240448
              39.9 4.76 24
                              30.1
                                       49.7
## 240720
             43.7 4.76 24
                              33.8
                                       53.5
             38.4 4.76 24
                              28.5
                                       48.2
## Control
##
## Degrees-of-freedom method: df.error
## Confidence level used: 0.95
pairs(emm_festuca, adjust = "tukey")
                              SE df t.ratio p.value
## contrast
               estimate
## 197198 - 240448 -1.734 6.73 24 -0.258 0.9938
## 197198 - 240720
                     -5.490 6.73 24 -0.815 0.8467
## 197198 - Control -0.187 6.73 24 -0.028 1.0000
## 240448 - 240720
                     -3.756 6.73 24 -0.558 0.9435
## 240448 - Control
                     1.547 6.73 24
                                     0.230 0.9956
## 240720 - Control
                      5.303 6.73 24
                                     0.787 0.8594
##
## Degrees-of-freedom method: df.error
## P value adjustment: tukey method for comparing a family of 4 estimates
#Bromus
emm_bromus <- emmeans(gls_bromus, ~ treatment, mode = "df.error")</pre>
summary(emm_bromus)
## treatment emmean
                     SE df lower.CL upper.CL
## 197198
             35.6 3.16 24
                                       42.1
                              29.1
## 240448
               19.0 3.16 24
                              12.5
                                       25.5
## 240720
              22.5 3.16 24
                              16.0
                                       29.1
## Control
              26.5 3.16 24
                              20.0
                                       33.0
## Degrees-of-freedom method: df.error
## Confidence level used: 0.95
pairs(emm_bromus, adjust = "tukey")
```

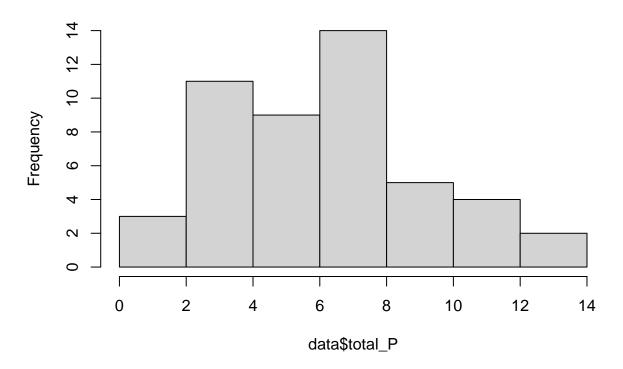
```
estimate SE df t.ratio p.value
   contrast
3.719 0.0055
## 197198 - 240720
                    13.07 4.47 24
                                     2.924 0.0349
                      9.11 4.47 24
                                     2.038 0.2024
## 197198 - Control
   240448 - 240720
                      -3.55 4.47 24 -0.795 0.8560
                      -7.51 4.47 24 -1.681 0.3553
## 240448 - Control
## 240720 - Control
                      -3.96 4.47 24 -0.886 0.8122
##
## Degrees-of-freedom method: df.error
## P value adjustment: tukey method for comparing a family of 4 estimates
# Taraxacum
emm_taraxacum <- emmeans(gls_taraxacum, ~ treatment, mode = "df.error")</pre>
summary(emm taraxacum)
## treatment emmean
                     SE df lower.CL upper.CL
## 197198
             21.4 1.46 24
                              18.4
                                       24.4
## 240448
                                       24.7
              21.7 1.46 24
                              18.7
                              19.9
                                       25.9
## 240720
             22.9 1.46 24
## Control
             20.4 1.46 24
                              17.3
                                       23.4
##
## Degrees-of-freedom method: df.error
## Confidence level used: 0.95
pairs(emm_taraxacum, adjust = "tukey")
## contrast
              estimate
                             SE df t.ratio p.value
## 197198 - 240448 -0.314 2.06 24 -0.153 0.9987
## 197198 - 240720
                     -1.511 2.06 24 -0.734 0.8825
## 197198 - Control
                     1.013 2.06 24
                                    0.492 0.9602
## 240448 - 240720
                     -1.197 2.06 24 -0.581 0.9368
## 240448 - Control 1.327 2.06 24
                                    0.644 0.9165
## 240720 - Control
                      2.524 2.06 24
                                     1.226 0.6171
##
## Degrees-of-freedom method: df.error
## P value adjustment: tukey method for comparing a family of 4 estimates
Total phosphorous analysis (exploration and model selection)
```

```
#Convert % P to toal P per species
data$total P<-data$P*data$biomass
str(data$biomass)
## num [1:112] 14.39 31.19 6.69 24.34 26.19 ...
#Add total phosphorous to the species datasets
data_gaillardia<-data%>%
  filter(species=="Gaillardia")
data taraxacum<-data%>%
 filter(species=="Taraxacum")
```

```
data_bromus<-data%>%
  filter(species=="Bromus")
data_festuca<-data%>%
  filter(species=="Festuca")

#Histogram of P distribution
hist(data$total_P) #near approximation of a normal distribution. May be bimodal
```

Histogram of data\$total_P

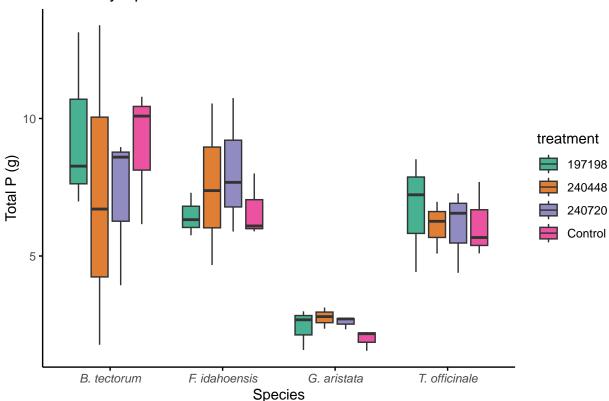


```
#Visualise total P within species groups/treatment
print(total_P_median_species <- ggplot(data,</pre>
                                        aes(x = species,
                                            y = total_P,
                                            fill = treatment)) +
        # Boxplot per species × treatment
        geom_boxplot(position = position_dodge(width = 0.75),
                     width = 0.6, alpha = 0.8,
                     outlier.shape = NA) + # Hide default outlier points
        labs(title = "Total P by Species and Treatment",
             x = "Species",
             y = "Total P (g)") +
        scale_fill_brewer(palette = "Dark2") +
        scale_colour_brewer(palette = "Dark2") +
        scale_x_discrete(labels = species_labels) +
        theme_minimal() +
        theme(
```

```
panel.grid = element_blank(),
  axis.line = element_line(color = "black"),
  axis.ticks = element_line(color = "black")
))
```

Warning: Removed 64 rows containing non-finite outside the scale range
('stat_boxplot()').

Total P by Species and Treatment



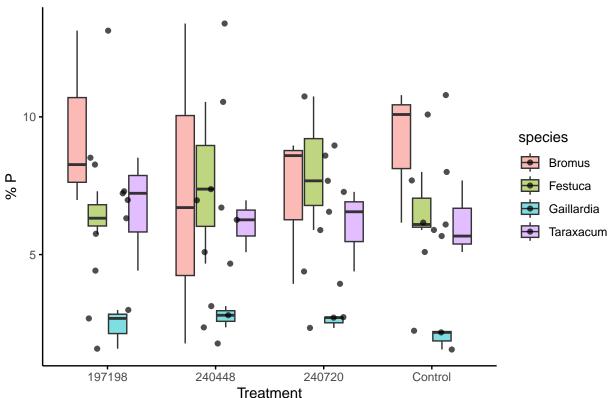
```
#Median
print(total_P_median<-ggplot(data, aes(x = treatment, y = total_P, fill = species)) +</pre>
        geom_boxplot(outlier.shape = NA, alpha = 0.5) +
        geom_jitter(width = 0.2, alpha = 0.7, color = "black") +
        labs(title = "Gaillardia Phosphorus by Treatment",
             x = "Treatment",
             y ="Phosphorus (% total foliar biomass)") +
        labs(title = "P by Species and Treatment",
            x = "Treatment",
             y = "% P") +
        scale_colour_brewer(palette = "Dark2") +
        scale_x_discrete(labels = species_labels) +
        theme_minimal() +
        theme(
          panel.grid = element_blank(),
                                                     #Removes all grid lines
          axis.line = element_line(color = "black"), #Adds black x and y axis lines
```

```
axis.ticks = element_line(color = "black") #show tick marks
))
```

Warning: Removed 64 rows containing non-finite outside the scale range
('stat_boxplot()').

Warning: Removed 64 rows containing missing values or values outside the scale range
('geom_point()').

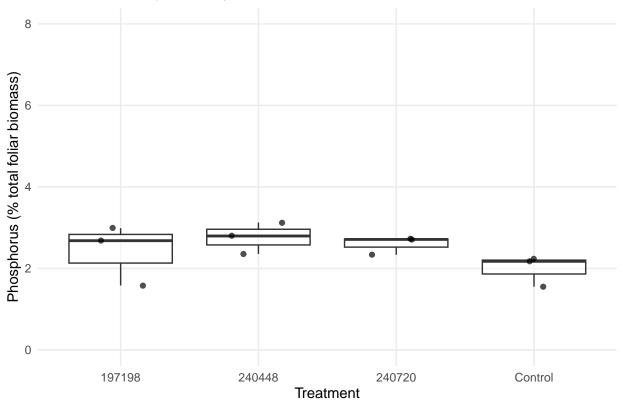
P by Species and Treatment



Warning: Removed 16 rows containing non-finite outside the scale range
('stat_boxplot()').

Warning: Removed 16 rows containing missing values or values outside the scale range
('geom_point()').

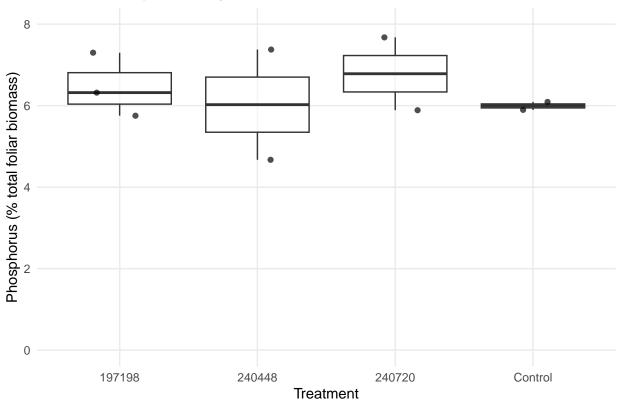
Gaillardia Phosphorus by Treatment



```
## Warning: Removed 19 rows containing non-finite outside the scale range
## ('stat_boxplot()').
```

Warning: Removed 19 rows containing missing values or values outside the scale range
('geom_point()').

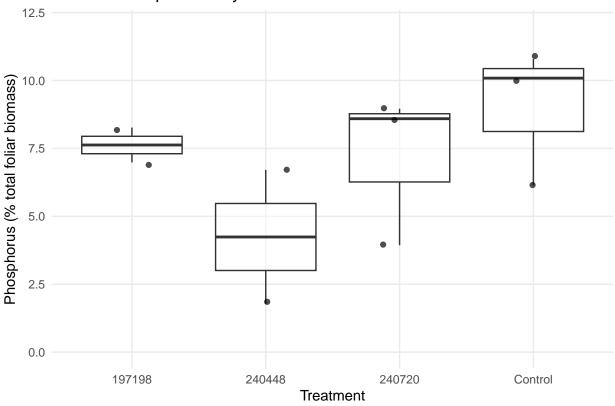
Festuca Phosphorous by Treatment



```
## Warning: Removed 18 rows containing non-finite outside the scale range
## ('stat_boxplot()').
```

Warning: Removed 18 rows containing missing values or values outside the scale range ## ('geom_point()').

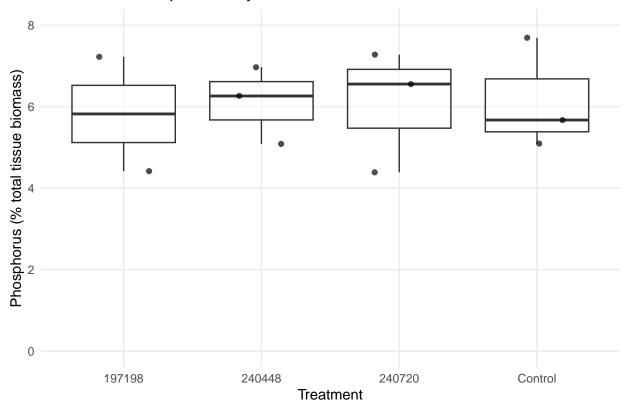
Bromus Phosphorous by Treatment



```
## Warning: Removed 17 rows containing non-finite outside the scale range
## ('stat_boxplot()').

## Warning: Removed 17 rows containing missing values or values outside the scale range
## ('geom_point()').
```

Taraxacum Phosphorous by Treatment



```
## Warning: Removed 18 rows containing non-finite outside the scale range
## ('stat_boxplot()').

## Warning: Removed 18 rows containing missing values or values outside the scale range
## ('geom_point()').

## Warning: Removed 19 rows containing non-finite outside the scale range
## ('stat_boxplot()').

## Warning: Removed 19 rows containing missing values or values outside the scale range
## ('geom_point()').

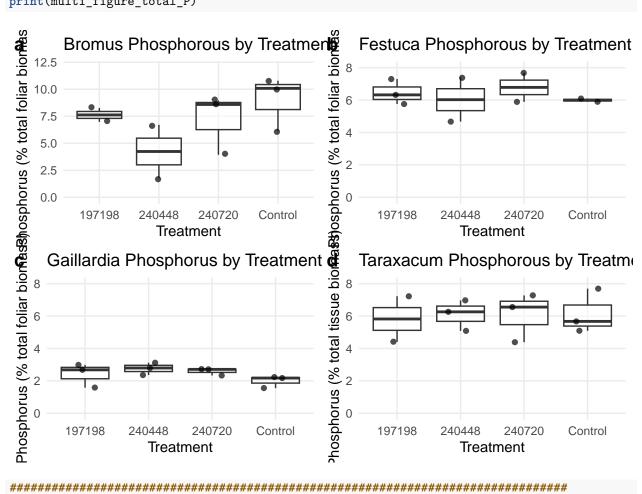
## Warning: Removed 16 rows containing non-finite outside the scale range
## ('stat_boxplot()').

## Warning: Removed 16 rows containing missing values or values outside the scale range
## ('geom_point()').
```

```
## Warning: Removed 17 rows containing non-finite outside the scale range
## ('stat_boxplot()').
```

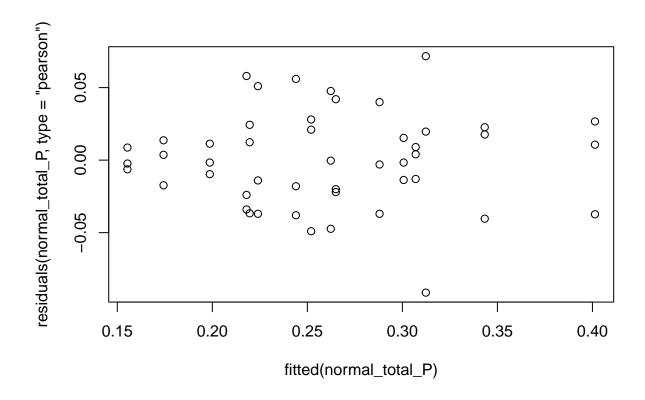
Warning: Removed 17 rows containing missing values or values outside the scale range
('geom_point()').

```
#Display the combined figure
print(multi_figure_total_P)
```

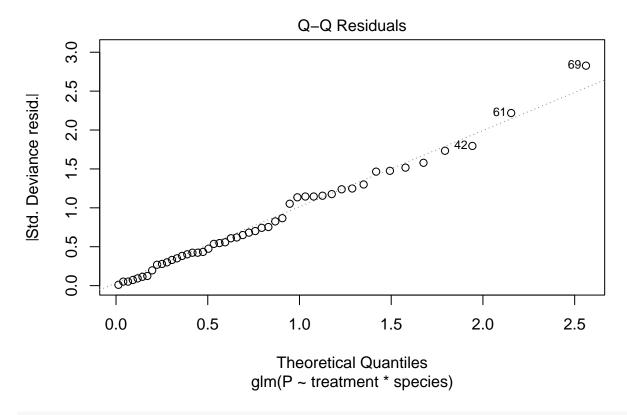


```
##
## Call:
## glm(formula = P ~ treatment * species, family = gaussian(link = "identity"),
## data = data)
##
```

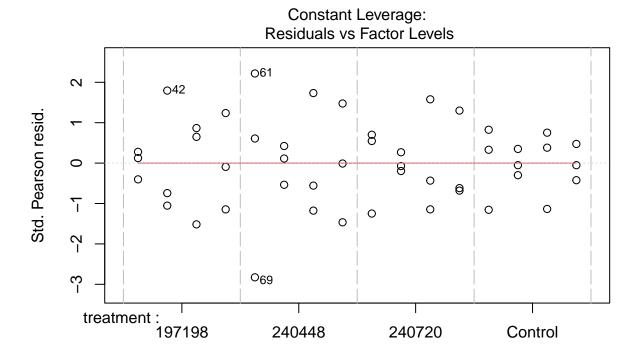
```
## Coefficients:
##
                                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)
                                      0.307000
                                                0.022843 13.440 1.05e-14 ***
## treatment240448
                                      0.005333
                                                0.032305
                                                           0.165 0.86991
## treatment240720
                                      0.036333
                                                0.032305
                                                           1.125
                                                                  0.26908
## treatmentControl
                                                           2.920 0.00636 **
                                      0.094333 0.032305
## speciesFestuca
                                                0.032305 -2.755 0.00960 **
                                     -0.089000
## speciesGaillardia
                                     -0.055000
                                                0.032305 -1.703 0.09835 .
                                                0.032305 -0.588 0.56056
## speciesTaraxacum
                                     -0.019000
## treatment240448:speciesFestuca
                                     -0.049000
                                                0.045686 -1.073 0.29150
## treatment240720:speciesFestuca
                                     -0.099000
                                                0.045686 -2.167 0.03779 *
## treatmentControl:speciesFestuca
                                                0.045686 -2.488 0.01824 *
                                     -0.113667
## treatment240448:speciesGaillardia -0.013333
                                                0.045686 -0.292 0.77228
## treatment240720:speciesGaillardia -0.064333
                                                0.045686 -1.408 0.16872
## treatmentControl:speciesGaillardia -0.126667
                                                0.045686 -2.773 0.00920 **
## treatment240448:speciesTaraxacum
                                     -0.031000
                                                0.045686 -0.679
                                                                  0.50230
## treatment240720:speciesTaraxacum
                                                                  0.20332
                                     -0.059333
                                                0.045686 -1.299
## treatmentControl:speciesTaraxacum -0.081667
                                                0.045686 -1.788 0.08332 .
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.001565375)
##
      Null deviance: 0.234279 on 47 degrees of freedom
##
## Residual deviance: 0.050092 on 32 degrees of freedom
     (64 observations deleted due to missingness)
## AIC: -159.31
## Number of Fisher Scoring iterations: 2
#Residual diagnostics
#Fitted vs. residuals
plot(fitted(normal_total_P), residuals(normal_total_P, type = "pearson"))
```



```
#some heteroskedasticity
#qqplot
plot(normal_total_P, which =2) #Looks good
```



#Residuals by factor level
plot(normal_total_P, which =5) #Looks good, decrease in residual variance



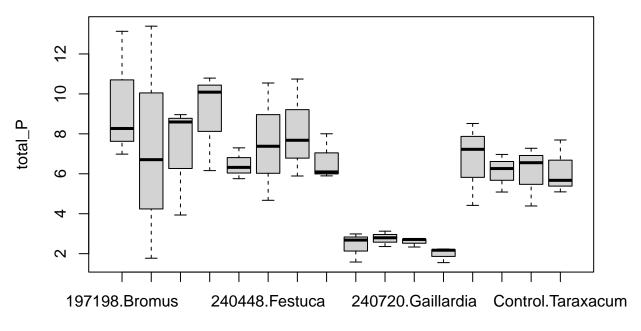
240448

Factor Level Combinations

240720

Control

```
#within control
#Check variance between groups
boxplot(total_P~interaction(treatment, species),
        data=data)
```



interaction(treatment, species)

```
#There are difference in variation between species and within treatment groups,
#try accounting for variance structure. There are only 3 observations/group,
#modelling a variance structure is too complex for the number
#of observations. Use Welch's ANOVA (rstatix package) to account for group
#variance. Welch's ANOVA must be run on each species group independently as it
#does not account for interactions. Games-Howell (rstatix package) is the posthoc test for Welch's ANOV.
#https://search.r-project.org/CRAN/refmans/rstatix/html/games_howell_test.html
#GAILLARDIA
print(gaillardia_welch_P<-oneway.test(total_P ~ treatment, data = data_gaillardia,</pre>
                                       var.equal = FALSE) )
##
##
   One-way analysis of means (not assuming equal variances)
## data: total P and treatment
## F = 1.8844, num df = 3.0000, denom df = 4.1762, p-value = 0.2685
#Not significant
#FESTUCA
#Run Welch's one way comparisons (to account for variance between groups)
print(festuca_welch_P<-oneway.test(total_P ~ treatment, data = data_festuca,</pre>
                                   var.equal = FALSE) )
```

```
## One-way analysis of means (not assuming equal variances)
##
## data: total P and treatment
## F = 0.37683, num df = 3.0000, denom df = 4.0602, p-value = 0.7755
#Not significant
#TARAXACUM
#Run Welch's one way comparisons (to account for variance between groups)
print(taraxacum_welch_P<-oneway.test(total_P ~ treatment, data = data_taraxacum,</pre>
                                     var.equal = FALSE) )
##
##
   One-way analysis of means (not assuming equal variances)
## data: total_P and treatment
## F = 0.059325, num df = 3.0000, denom df = 4.2941, p-value = 0.9787
#Not significant
#BROMUS
#Run Welch's one way comparisons (to account for variance between groups)
print(bromus_welch_P<-oneway.test(total_P ~ treatment, data = data_bromus,</pre>
                                  var.equal = FALSE) )
##
   One-way analysis of means (not assuming equal variances)
## data: total_P and treatment
## F = 0.3046, num df = 3.000, denom df = 4.338, p-value = 0.8218
#Not significant
```

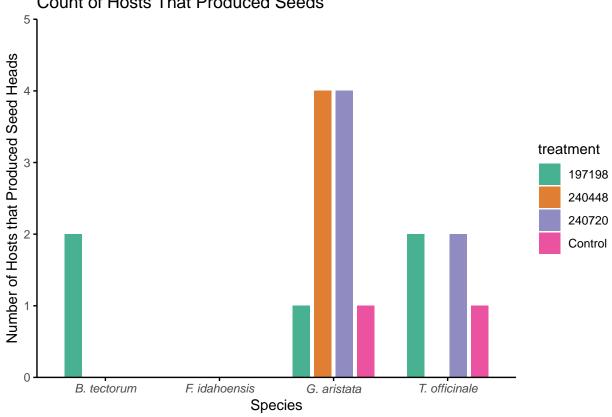
Seed production data

```
#Can not statistically compare seed weight or seed head count, visualise.
#Create a new dataframe that subset the treatments
#Add seed coutn data
data$seed_count<-data$seed_weight>0
data$seed_count<-as.numeric(data$seed_count)

#Do descriptive statistics and show plots of seed weight
#Summarize seed count
seed_summary <- data %>%
    group_by(species, treatment) %>%
    summarize(total_seed_count = sum(seed_count, na.rm = TRUE), .groups = "drop")

#Plot
seed_count<-ggplot(seed_summary, aes(x = species, y = total_seed_count, fill = treatment)) +
    geom_col(position = position_dodge(width = 0.75), width = 0.6, alpha = 0.8) +</pre>
```

Count of Hosts That Produced Seeds

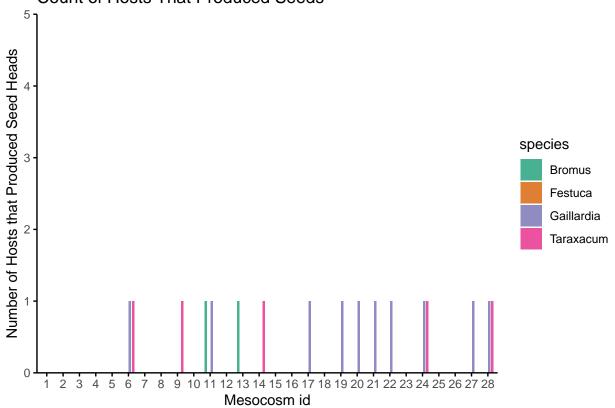


```
#View seed ocunt by mesocosm
#Summarize seed count
seed_summary <- data %>%
   group_by(species, treatment, mesocosm_id) %>%
   summarize(total_seed_count = sum(seed_count, na.rm = TRUE), .groups = "drop")

#Plot
seed_count_meso<-ggplot(seed_summary, aes(x = mesocosm_id, y = total_seed_count, fill = species)) +
   geom_col(position = position_dodge(width = 0.75), width = 0.6, alpha = 0.8) +
   labs(title = "Count of Hosts That Produced Seeds",</pre>
```

```
x = "Mesocosm id",
y = "Number of Hosts that Produced Seed Heads") +
scale_y_continuous(limits = c(0, 5), expand = c(0, 0)) +  # Removes space on x-axis
scale_fill_brewer(palette = "Dark2") +
scale_x_discrete(labels = species_labels) +
theme_minimal() +
theme(
   panel.grid = element_blank(),
   axis.line = element_line(color = "black"),
   axis.ticks = element_line(color = "black")
)
print(seed_count_meso)
```

Count of Hosts That Produced Seeds



#Alternative reasons variation in seed production could be observed:

#Not enough growing time

#Nested within mesocosms (mesocosm specific)