linear_models on alpha hill numbers

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4/21/2022

setting up the environment

Loading the data

```
####For some reason the dataframe has duplicates - remove these
alpha_diversity_tax <- read.csv('../hill_numbers/outputsR/diversity_table.csv', header=T, row.names=1) '
    unique(.) %>%
    as.tibble

## Warning: 'as.tibble()' was deprecated in tibble 2.0.0.
## i Please use 'as_tibble()' instead.
## i The signature and semantics have changed, see '?as_tibble'.

#####Combined dataframe containing functional diversities
alpha_diversity_fd <- read.csv('../hill_numbers/outputsR/diversity_table_fd.csv', header=T, row.names =
####Phylogenetic diversities
alpha_diversity_pd <- read.csv('../hill_numbers/outputsR/diversity_table_pd.csv', header=T, row.names =
####Metadata
metadata <- read.csv('../data/metadata.csv')</pre>
```

Combining the metadata with the dataframes

```
alph_div_tax_as <- alpha_diversity_tax %>%
  filter(species == 'AS') %>%
  dplyr::select(c(diversity, sample_id)) %>%
  left_join(metadata, by='sample_id') %>%
  mutate(days=case_when(treatment == 'Day1' ~ 0,
                        treatment == 'Acc' ~ 7,
                        treatment == 'Heat' ~ 19,
                        treatment == 'Cold' ~ 33,
                        treatment == 'Diet' ~ 45)) %>%
  mutate(treatment = factor(treatment, levels = c('Day1', 'Acc', 'Heat', 'Cold', 'Diet')))
alph_div_tax_cr <- alpha_diversity_tax %>%
  filter(species == 'CR') %>%
  dplyr::select(c(diversity, sample_id)) %>%
  left_join(metadata, by='sample_id') %>%
  mutate(days=case_when(treatment == 'Day1' ~ 0,
                        treatment == 'Acc' ~ 7,
                        treatment == 'Heat' ~ 19,
                        treatment == 'Cold' ~ 33,
```

```
treatment == 'Diet' ~ 45),
         cage=case_when(cage == 1 ~ 6,
                        cage == 2 \sim 7,
                        cage == 3 \sim 8,
                        cage == 4 \sim 9,
                        cage == 5 \sim 10,
                        cage == 6 \sim 11)) %>%
  mutate at(2:8, as.factor) %>%
  mutate(treatment = factor(treatment, levels = c('Day1', 'Acc', 'Heat', 'Cold', 'Diet')))
alph_div_fd_as <- alpha_diversity_fd %>%
  filter(species == 'AS') %>%
  dplyr::select(c(Q, sample_id)) %>%
  left_join(metadata, by='sample_id') %>%
  filter(treatment != 'FAST') %>%
  mutate(days=case_when(treatment == 'Day1' ~ 0,
                        treatment == 'Acc' ~ 7,
                        treatment == 'Heat' ~ 19,
                        treatment == 'Cold' ~ 33,
                        treatment == 'Diet' ~ 45)) %>%
  mutate_at(2:8, as.factor) %>%
  mutate(treatment = factor(treatment, levels = c('Day1', 'Acc', 'Heat', 'Cold', 'Diet')))
alph_div_fd_cr <- alpha_diversity_fd %>%
  filter(species == 'CR') %>%
  dplyr::select(c(Q, sample_id)) %>%
  left_join(metadata, by='sample_id') %>%
  mutate(days=case_when(treatment == 'Day1' ~ 0,
                        treatment == 'Acc' ~ 7,
                        treatment == 'Heat' ~ 19,
                        treatment == 'Cold' ~ 33,
                        treatment == 'Diet' ~ 45),
         cage=case_when(cage == 1 ~ 6,
                        cage == 2 \sim 7,
                        cage == 3 \sim 8,
                        cage == 4 \sim 9,
                        cage == 5 \sim 10,
                        cage == 6 \sim 11)) %>%
  mutate_at(2:8, as.factor) %>%
  mutate(treatment = factor(treatment, levels = c('Day1', 'Acc', 'Heat', 'Cold', 'Diet')))
alph_div_pd_as <- alpha_diversity_pd %>%
  filter(species == 'AS') %>%
  dplyr::select(c(diversity, sample_id)) %>%
  left_join(metadata, by='sample_id') %>%
 filter(treatment != 'FAST') %>%
  mutate(days=case_when(treatment == 'Day1' ~ 0,
                        treatment == 'Acc' ~ 7,
                        treatment == 'Heat' ~ 19,
                        treatment == 'Cold' ~ 33,
                        treatment == 'Diet' ~ 45)) %>%
  mutate_at(2:8, as.factor) %>%
  mutate(treatment = factor(treatment, levels = c('Day1', 'Acc', 'Heat', 'Cold', 'Diet')))
```

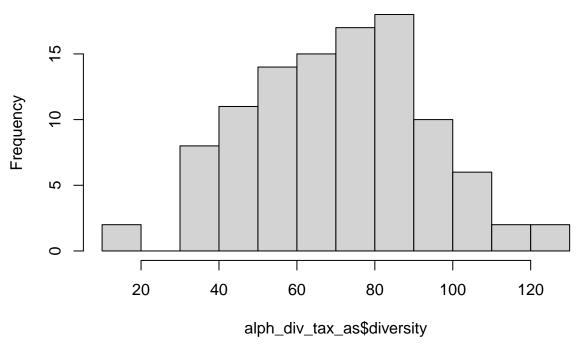
```
alph_div_pd_cr <- alpha_diversity_pd %>%
  filter(species == 'CR') %>%
  dplyr::select(c(diversity, sample_id)) %>%
  left join(metadata, by='sample id') %>%
 mutate(days=case_when(treatment == 'Day1' ~ 0,
                         treatment == 'Acc' ~ 7,
                         treatment == 'Heat' ~ 19,
                         treatment == 'Cold' ~ 33,
                         treatment == 'Diet' ~ 45),
         cage=case_when(cage == 1 ~ 6,
                         cage == 2 \sim 7,
                         cage == 3 \sim 8,
                         cage == 4 \sim 9,
                         cage == 5 \sim 10,
                         cage == 6 \sim 11)) %>%
  mutate_at(2:8, as.factor) %>%
  mutate(treatment = factor(treatment, levels = c('Day1', 'Acc', 'Heat', 'Cold', 'Diet')))
```

Sanity check of each factor

```
sapply(alph_div_tax_as, mode)
##
       diversity
                      sample_id
                                             X individual_id
                                                                  treatment
##
       "numeric"
                    "character"
                                     "numeric"
                                                 "character"
                                                                  "numeric"
            cage
                            sex
                                       species
                                                        days
                                                   "numeric"
##
       "numeric"
                    "character"
                                  "character"
contr <- rbind("Day1-Acc" = c(1, -1, 0, 0, 0),
               "Acc-Heat" = c(0, 1, -1, 0, 0),
               "Heat-Cold" = c(0, 0, 1, -1, 0),
               "Cold-Diet" = c(0, 0, 0, 1,-1))
contr_cr \leftarrow rbind("Day1-Acc" = c(1, -1, 0, 0, 0),
               "Acc-Heat" = c(0, 1, -1, 0, 0),
                "Heat-Cold" = c(0, 0, 1, -1, 0),
               "Cold-Diet" = c(0, 0, 0, 1,-1))
```

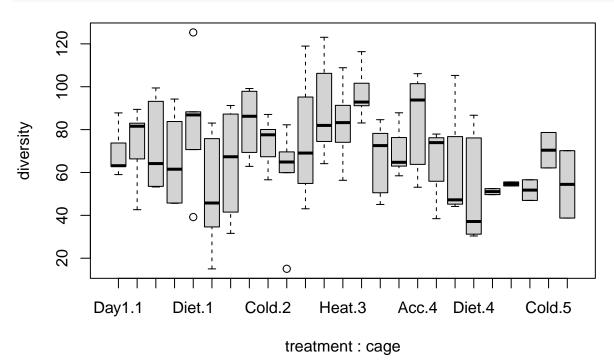
hist(alph_div_tax_as\$diversity)

Histogram of alph_div_tax_as\$diversity



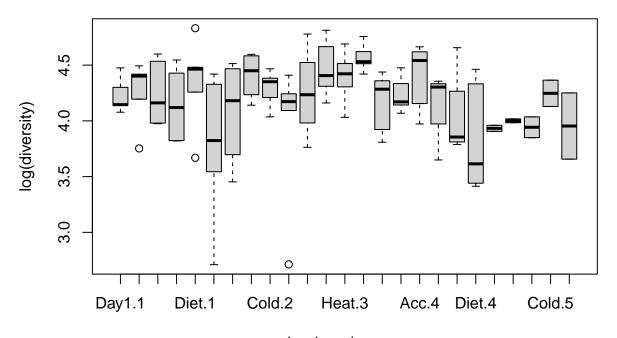
 $treatment_wise_boxplots \ from \ the \ taxonomic \ diversity \ Hill \ numbers$

boxplot(diversity~treatment*cage,data = alph_div_tax_as)



sessment of log transformation it looks tighter, however the non-transformed data still looks ok to me so i will continue with this

As-



treatment : cage

Create

the models for comparison - including the random effects results in the lowest AIC

```
###Try to include the other factors
model_as.a <- lme(diversity~treatment,</pre>
                   random = ~1|cage/individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_tax_as)
model_as.b <- lme(diversity~treatment,</pre>
                   random = ~1 | cage,
                   data=alph_div_tax_as)
model_as.c <- lme(diversity~treatment,</pre>
                   random = ~1|individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_tax_as)
#model_as.d <- lme(diversity~treatment,</pre>
                   \#random = ~1/cage,
                   #correlation = corCAR1(form = ~ days),
                   #data=alph_div_tax_as)
model_as.e <- lm(diversity~treatment,</pre>
                   data=alph_div_tax_as)
anova(model_as.a,model_as.b,model_as.c,model_as.e)
```

```
##
             Model df
                                           logLik
                                                    Test L.Ratio p-value
                           AIC
                                    BIC
                 1 9 934.9830 958.4295 -458.4915
## model_as.a
                 2 7 933.4924 951.7286 -459.7462 1 vs 2 2.509424
## model_as.b
                                                                   0.2852
                 3 8 933.6263 954.4677 -458.8132 2 vs 3 1.866080
## model_as.c
                                                                   0.1719
                 4 6 935.0961 950.7271 -461.5480 3 vs 4 5.469725 0.0649
## model_as.e
```

```
AIC(model_as.a) #931.8822

## [1] 934.983

AIC(model_as.b) #933.4933

## [1] 933.4924

AIC(model_as.c) #930.3255

## [1] 933.6263

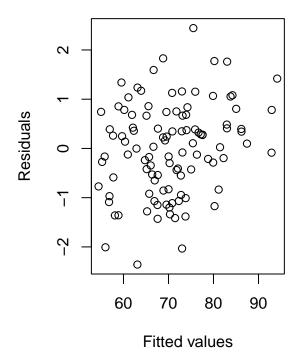
AIC(model_as.e) #960.1475
```

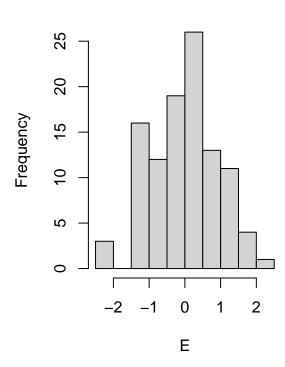
[1] 960.1465

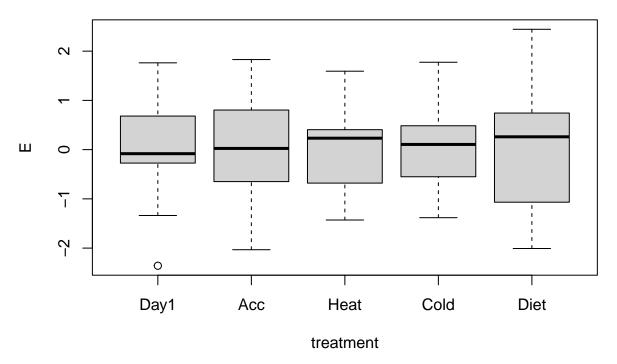
Residuals looks like they are randomly distributed around 0

Residuals versus fitted values

Histogram of E







summary(model_as.a)

```
## Linear mixed-effects model fit by REML
##
     Data: alph_div_tax_as
##
         AIC
                  BIC
                         logLik
     934.983 958.4295 -458.4915
##
##
## Random effects:
##
    Formula: ~1 | cage
##
           (Intercept)
## StdDev:
               5.81433
##
   Formula: ~1 | individual_id %in% cage
##
##
           (Intercept) Residual
## StdDev:
              8.42224 20.38083
##
## Correlation Structure: Continuous AR(1)
    Formula: ~as.numeric(days) | cage/individual_id
##
    Parameter estimate(s):
##
         Phi
## 0.2000667
## Fixed effects: diversity ~ treatment
                    Value Std.Error DF
##
                                         t-value p-value
## (Intercept)
                 64.02810 5.494296 79 11.653560 0.0000
## treatmentAcc 11.12264 6.306979 79
                                        1.763545 0.0817
## treatmentHeat 9.90226 6.394579 79
                                        1.548540 0.1255
## treatmentCold 9.86497 6.307017 79
                                       1.564126 0.1218
```

```
## treatmentDiet -0.79601 6.225929 79 -0.127854 0.8986
##
    Correlation:
##
                 (Intr) trtmnA trtmnH trtmnC
                 -0.574
##
  treatmentAcc
##
   treatmentHeat -0.567
                         0.493
   treatmentCold -0.574
                         0.500
                                0.493
   treatmentDiet -0.581 0.507
                                0.500
##
##
   Standardized Within-Group Residuals:
##
           Min
                        Q1
                                    Med
                                                            Max
   -2.35827091 -0.64938041
                            0.09777876
                                        0.68155759
                                                     2.44381609
##
  Number of Observations: 105
##
  Number of Groups:
##
                      cage individual_id %in% cage
                         5
##
```

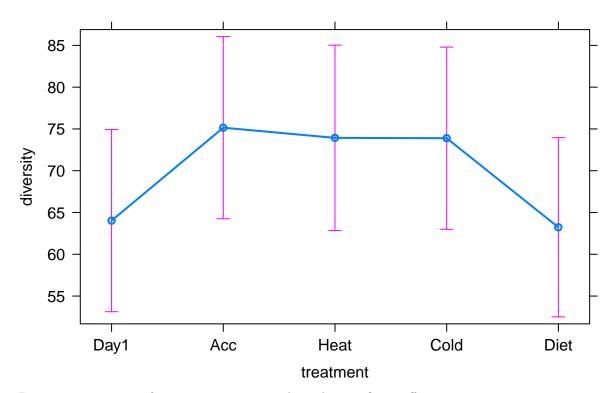
We have a slightly significant treatment effect

```
anova(model_as.a)
```

```
## numDF denDF F-value p-value
## (Intercept) 1 79 342.5953 <.0001
## treatment 4 79 1.7623 0.1448
```

plot(allEffects(model_as.a))

treatment effect plot



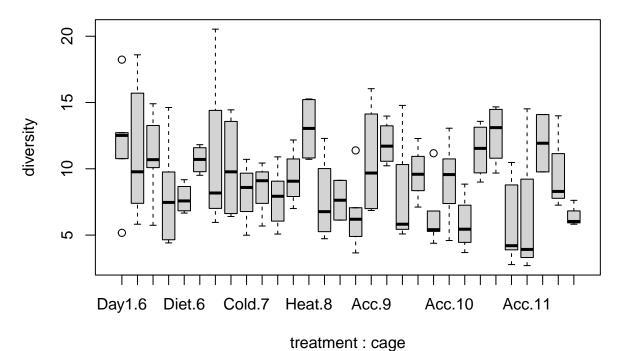
Pairwise comparison of treatments to assess where the significant effects are

```
summary(glht(model_as.a, linfct = mcp(treatment=contr),
                   test = adjusted("holm")))
##
     Simultaneous Tests for General Linear Hypotheses
##
##
## Multiple Comparisons of Means: User-defined Contrasts
##
##
## Fit: lme.formula(fixed = diversity ~ treatment, data = alph_div_tax_as,
       random = ~1 | cage/individual_id, correlation = corCAR1(form = ~as.numeric(days)))
##
##
## Linear Hypotheses:
                   Estimate Std. Error z value Pr(>|z|)
##
## Day1-Acc == 0 -11.12264
                               6.30698 -1.764
                                                   0.248
                                         0.191
                                                   0.999
## Acc-Heat == 0
                    1.22038
                               6.39240
## Heat-Cold == 0
                    0.03729
                               6.39240
                                         0.006
                                                   1.000
## Cold-Diet == 0 10.66098
                               6.22593
                                          1.712
                                                   0.273
```

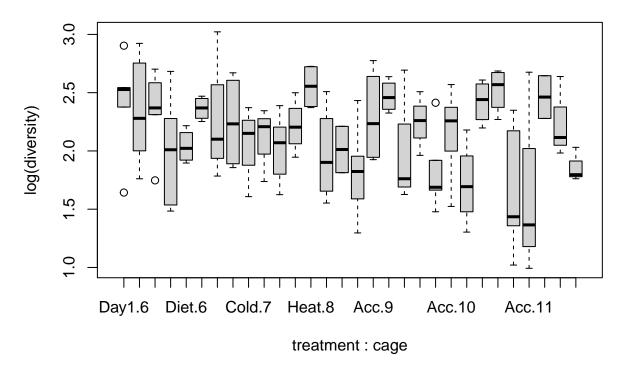
```
#plot_model(model_as1, type='Day1d', terms = c('treatment'))
```

boxplot(diversity~treatment*cage,data = alph_div_tax_cr)

(Adjusted p values reported -- single-step method)

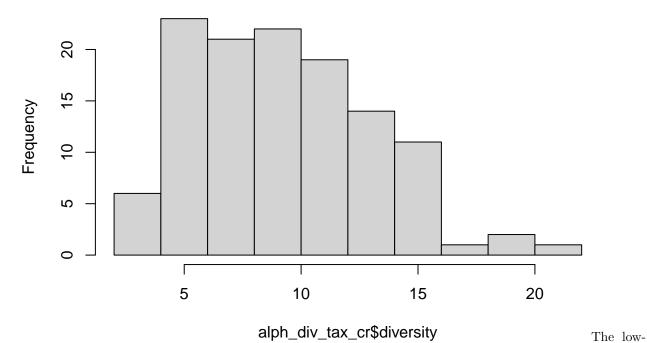


boxplot(log(diversity)~treatment*cage,data = alph_div_tax_cr)



hist(alph_div_tax_cr\$diversity)

Histogram of alph_div_tax_cr\$diversity

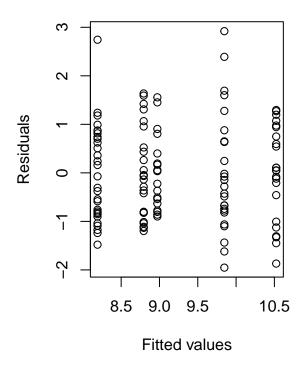


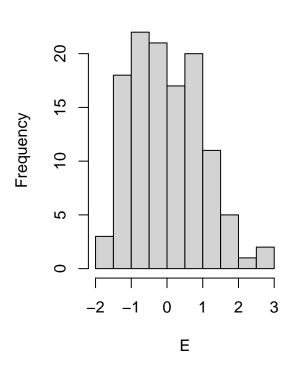
est AIC is the gls without random effects - however i consider the random effects to be an essential study design parameter - i'll continue with the random effects included

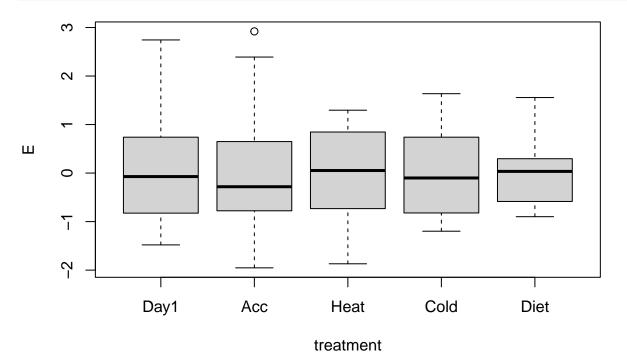
```
\#data = alph\_div\_tax\_cr)
model_cr.b <- lme(diversity~treatment,</pre>
                   random = ~1 | cage,
                   data=alph_div_tax_cr)
model_cr.c <- lme(diversity~treatment,</pre>
                   random = ~1|individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_tax_cr)
model_cr.e <- lm(diversity~treatment,</pre>
                   data=alph_div_tax_cr)
#AIC(model_cr.a) #657.3954
AIC(model_cr.b) #654.6046
## [1] 654.6036
AIC(model_cr.c) #655.3954
## [1] 656.4563
\#AIC(model\_cr.d) \#NA
AIC(model_cr.e) #658.816
## [1] 658.8157
model_cr1=model_cr.b
E <- resid(model_cr1, type = "normalized")</pre>
Fit <- fitted(model_cr1)</pre>
op \leftarrow par(mfrow = c(1, 2))
plot(x = Fit, y = E,
     xlab = "Fitted values", ylab = "Residuals",
     main = "Residuals versus fitted values")
hist(E, nclass = 15)
```

Residuals versus fitted values

Histogram of E







Again we have a slightly significance treatment effect on taxonomy

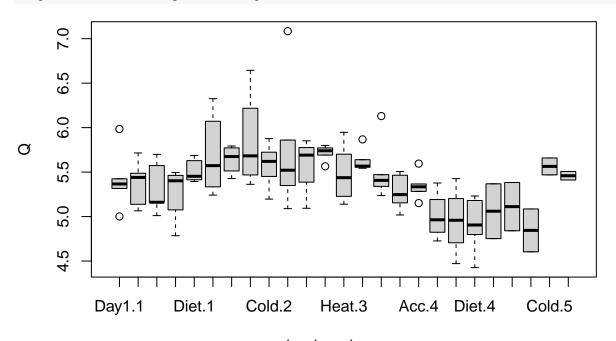
```
anova(model_cr1)
```

```
## numDF denDF F-value p-value
## (Intercept) 1 110 762.8574 <.0001
## treatment 4 110 1.5882 0.1825
```

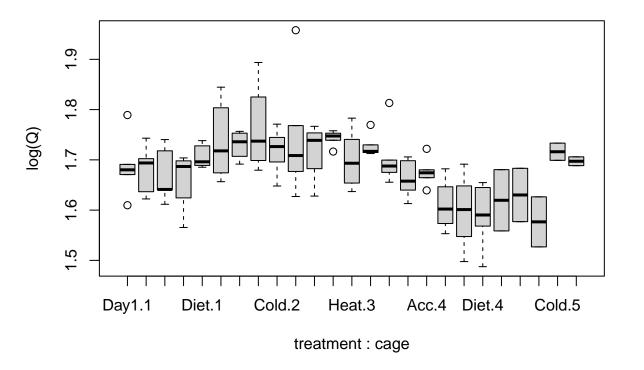
Pairwise comparison of treatments

```
##
##
     Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: User-defined Contrasts
##
##
## Fit: lme.formula(fixed = diversity ~ treatment, data = alph_div_tax_cr,
##
       random = ~1 | cage)
##
## Linear Hypotheses:
##
                  Estimate Std. Error z value Pr(>|z|)
## Day1-Acc == 0
                  -1.6551
                               0.9988 -1.657
                                                  0.302
## Acc-Heat == 0
                   -0.6770
                               1.0574 -0.640
                                                  0.924
## Heat-Cold == 0
                  1.7296
                               1.0679
                                        1.620
                                                  0.322
## Cold-Diet == 0 -0.1784
                               1.1239 -0.159
                                                  1.000
## (Adjusted p values reported -- single-step method)
```

```
boxplot(Q~treatment*cage,data = alph_div_fd_as)
```

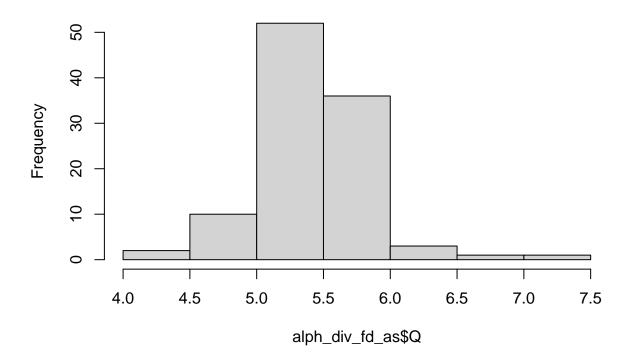


treatment : cage



hist(alph_div_fd_as\$Q)

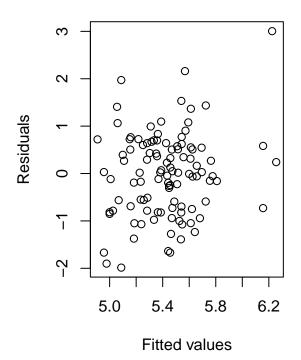
Histogram of alph_div_fd_as\$Q

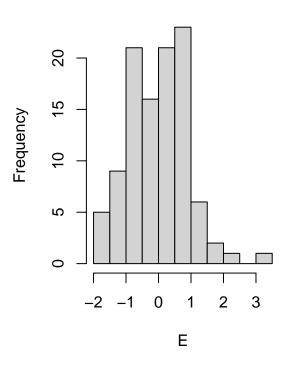


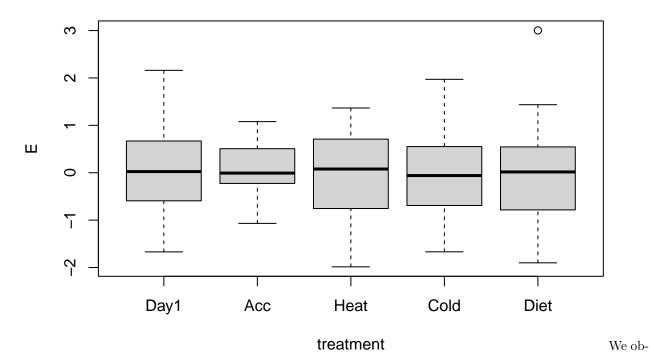
```
model_as.a_fd <- lme(Q~treatment,</pre>
                   random = ~1|cage/individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_fd_as)
model_as.b_fd <- lme(Q~treatment,</pre>
                   random = ~1|individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_fd_as)
model_as.c_fd <- lme(Q~treatment,</pre>
                   random = ~1 | cage,
                   data=alph_div_fd_as)
model_as.d_fd <- lme(Q~treatment,</pre>
                   random = ~1|cage/individual_id,
                   data=alph_div_fd_as)
model_as.e_fd <- lm(Q~treatment,</pre>
                   data=alph_div_fd_as)
AIC(model_as.a_fd) #292.0188
## [1] 92.69364
AIC(model_as.b_fd) #290.6965
## [1] 95.5458
AIC(model_as.c_fd) #292.2867
## [1] 108.2607
AIC(model_as.d_fd) #292.3475
## [1] 93.27356
AIC(model_as.e_fd) #286.632
## [1] 116.5169
model_as1_fd=model_as.a_fd
E <- resid(model_as1_fd, type = "normalized")</pre>
Fit <- fitted(model_as.a_fd)</pre>
op \leftarrow par(mfrow = c(1, 2))
plot(x = Fit, y = E,
     xlab = "Fitted values", ylab = "Residuals",
     main = "Residuals versus fitted values")
hist(E, nclass = 15)
```

Residuals versus fitted values

Histogram of E







serve no significant difference in the effect of treatment on functional alpha diversity

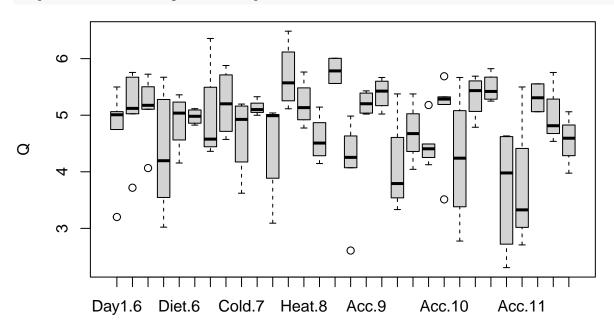
anova(model_as1_fd)

```
## numDF denDF F-value p-value
## (Intercept) 1 79 2026.1679 <.0001
## treatment 4 79 1.0907 0.367
```

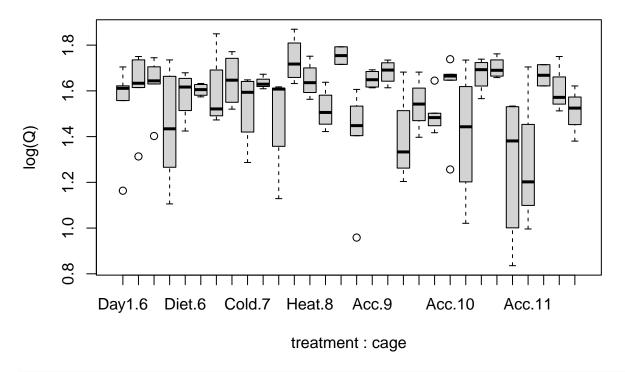
This is useless - ignore it for now but just wanted to sanity check

```
##
##
     Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: User-defined Contrasts
##
##
##
  Fit: lme.formula(fixed = Q ~ treatment, data = alph_div_fd_as, random = ~1 |
##
       cage/individual_id, correlation = corCAR1(form = ~as.numeric(days)))
##
## Linear Hypotheses:
##
                    Estimate Std. Error z value Pr(>|z|)
## Day1-Acc == 0 -5.243e-02 7.089e-02 -0.740
                                                   0.894
## Acc-Heat == 0
                  1.514e-01
                             8.189e-02
                                          1.849
                                                   0.218
## Heat-Cold == 0 5.619e-05 8.422e-02
                                          0.001
                                                   1.000
## Cold-Diet == 0 -6.825e-02 7.958e-02 -0.858
                                                   0.834
## (Adjusted p values reported -- single-step method)
```

boxplot(Q~treatment*cage,data = alph_div_fd_cr)

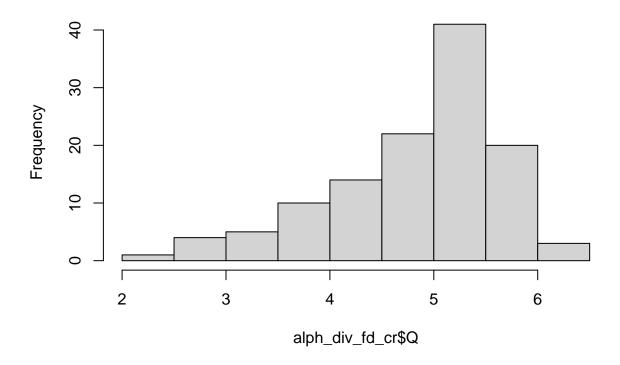


treatment: cage



hist(alph_div_fd_cr\$Q)

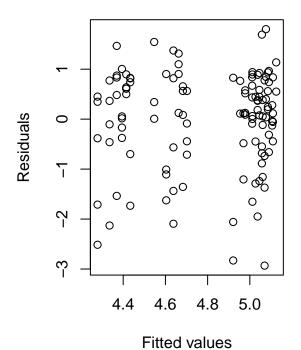
Histogram of alph_div_fd_cr\$Q

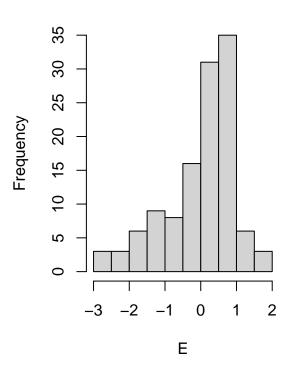


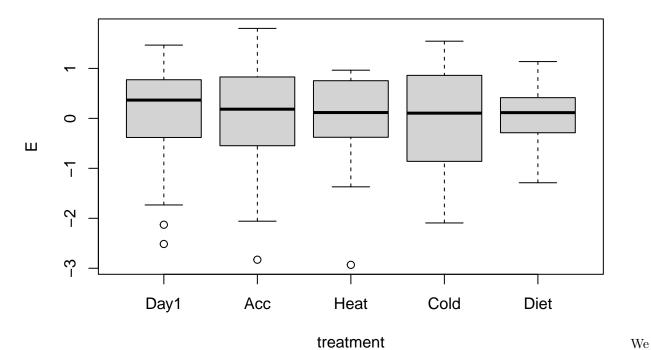
```
model_cr.a_fd <- lme(Q~treatment,</pre>
                   random = ~1|cage/individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_fd_cr)
model_cr.b_fd <- lme(Q~treatment,</pre>
                   random = ~1|individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_fd_cr)
model_cr.c_fd <- lme(Q~treatment,</pre>
                   random = ~1 | cage,
                   data=alph_div_fd_cr)
model_cr.d_fd <- lme(Q~treatment,</pre>
                   random = ~1|individual id,
                   data=alph_div_fd_cr)
model_cr.e_fd <- lm(Q~treatment,</pre>
                   data=alph_div_fd_cr)
AIC(model_cr.a_fd) #377.5765
## [1] 304.8715
AIC(model_cr.b_fd) #376.0824
## [1] 303.0507
AIC(model_cr.c_fd) #379.5765
## [1] 301.5524
AIC(model_cr.d_fd) #374.0913
## [1] 301.7523
AIC(model_cr.e_fd) #370.5743
## [1] 290.9681
model_cr1_fd=model_cr.a_fd
E <- resid(model_cr1_fd, type = "normalized")</pre>
Fit <- fitted(model_cr1_fd)</pre>
op \leftarrow par(mfrow = c(1, 2))
plot(x = Fit, y = E,
     xlab = "Fitted values", ylab = "Residuals",
     main = "Residuals versus fitted values")
hist(E, nclass = 15)
```

Residuals versus fitted values

Histogram of E





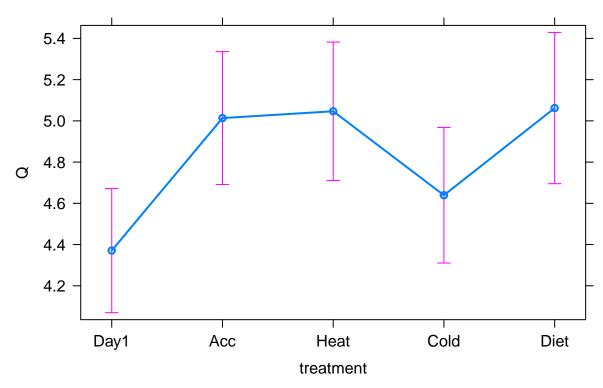


have a highly significant effect of treatment on functional alpha diversity

```
anova(model_cr1_fd)
##
              numDF denDF F-value p-value
## (Intercept)
               1
                       87 3038.5944 <.0001
                             4.2982 0.0032
## treatment
                  4
                       87
summary(glht(model_cr1_fd, linfct = mcp(treatment=contr_cr),
                  test = adjusted("holm")))
##
##
    Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: User-defined Contrasts
##
##
## Fit: lme.formula(fixed = Q ~ treatment, data = alph div fd cr, random = ~1 |
      cage/individual_id, correlation = corCAR1(form = ~as.numeric(days)))
##
##
## Linear Hypotheses:
                 Estimate Std. Error z value Pr(>|z|)
## Day1-Acc == 0 -0.64267
                            0.19986 -3.216 0.00494 **
                             0.22256 -0.148 0.99968
## Acc-Heat == 0 -0.03303
## Heat-Cold == 0 0.40729
                             0.22636 1.799 0.23403
## Cold-Diet == 0 -0.42289
                             0.23702 -1.784 0.24094
## ---
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

plot(allEffects(model_cr1_fd))

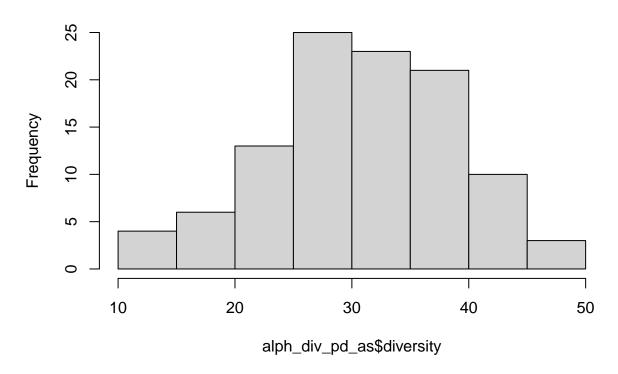
treatment effect plot



Phylogenetic linear models

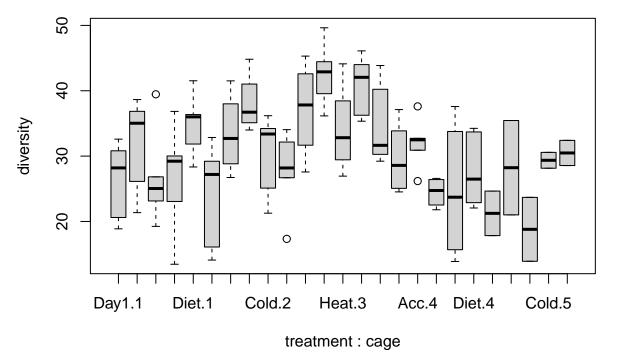
hist(alph_div_pd_as\$diversity)

Histogram of alph_div_pd_as\$diversity



 $treatment_wise_boxplots \ from \ the \ taxonomic \ diversity \ Hill \ numbers$

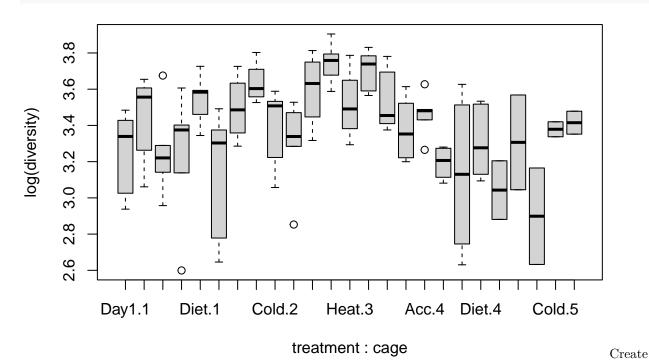
boxplot(diversity~treatment*cage,data = alph_div_pd_as)



sessment of log transformation it looks tighter, however the non-transformed data still looks ok to me so i will continue with this

As-

boxplot(log(diversity)~treatment*cage,data = alph_div_pd_as)



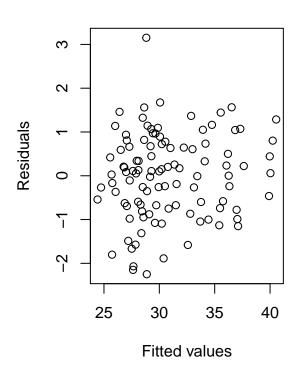
the models for comparison - including the random effects results in the lowest AIC

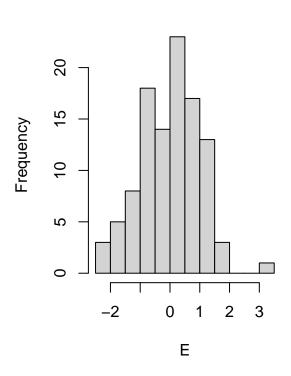
```
###Try to include the other factors
model_as.a.pd <- lme(diversity~treatment,</pre>
                  random = ~1|cage/individual id,
                  correlation = corCAR1(form = ~ as.numeric(days)),
                  data=alph_div_pd_as)
model_as.b.pd <- lme(diversity~treatment,</pre>
                  random = ~1|individual_id,
                  data=alph_div_pd_as)
model_as.c.pd <- lme(diversity~treatment,</pre>
                  random = ~1|individual_id,
                  correlation = corCAR1(form = ~ as.numeric(days)),
                  data=alph_div_pd_as)
model_as.d.pd <- lme(diversity~treatment,</pre>
                  random = ~1 | cage,
                  data=alph_div_pd_as)
model_as.e.pd <- lm(diversity~treatment,</pre>
                  data=alph_div_pd_as)
anova(model_as.a.pd,model_as.b.pd,model_as.c.pd,model_as.e.pd)
                 Model df
                                                        Test L.Ratio p-value
                               AIC
                                        BIC
                                               logLik
## model_as.a.pd 1 9 707.3642 730.8108 -344.6821
## model_as.b.pd
                    2 7 712.2172 730.4534 -349.1086 1 vs 2 8.852929 0.0120
## model_as.c.pd 3 8 714.2171 735.0584 -349.1085 2 vs 3 0.000091 0.9924
## model_as.e.pd 4 6 724.2139 739.8449 -356.1069 3 vs 4 13.996777 0.0009
AIC(model_as.a.pd ) #525.004
## [1] 707.3642
AIC(model_as.b.pd ) #529.4421
## [1] 712.2172
AIC(model_as.c.pd ) #529.9477
## [1] 714.2171
AIC(model_as.d.pd ) #523.7998
## [1] 706.5748
AIC(model_as.e.pd ) #546.8065
## [1] 738.7202
```

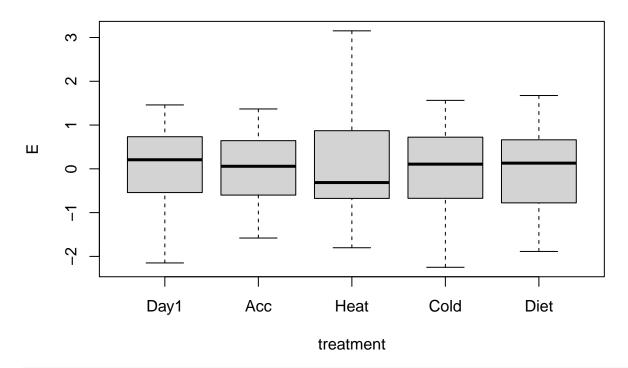
Residuals looks like they are randomly distributed around 0

Residuals versus fitted values

Histogram of E







summary(model_as1_pd)

```
## Linear mixed-effects model fit by REML
##
     Data: alph_div_pd_as
##
          AIC
                   BIC
                          logLik
     707.3642 730.8108 -344.6821
##
##
  Random effects:
##
##
   Formula: ~1 | cage
##
           (Intercept)
  StdDev:
              4.095198
##
##
   Formula: ~1 | individual_id %in% cage
##
##
           (Intercept) Residual
## StdDev:
             0.9957605 6.854969
## Correlation Structure: Continuous AR(1)
   Formula: ~as.numeric(days) | cage/individual_id
##
   Parameter estimate(s):
##
         Phi
## 0.8551492
## Fixed effects: diversity ~ treatment
##
                     Value Std.Error DF
                                          t-value p-value
## (Intercept)
                 27.950951 2.385399 79 11.717514 0.0000
## treatmentAcc
                  5.803850
                           1.740702 79
                                         3.334201
                                                   0.0013
## treatmentHeat 1.312992 2.088097 79
                                         0.628798
                                                   0.5313
## treatmentCold 2.096134 2.107776 79
                                         0.994476
## treatmentDiet 2.876805 2.088303 79
                                        1.377580 0.1722
##
   Correlation:
##
                 (Intr) trtmnA trtmnH trtmnC
## treatmentAcc -0.365
## treatmentHeat -0.426 0.477
```

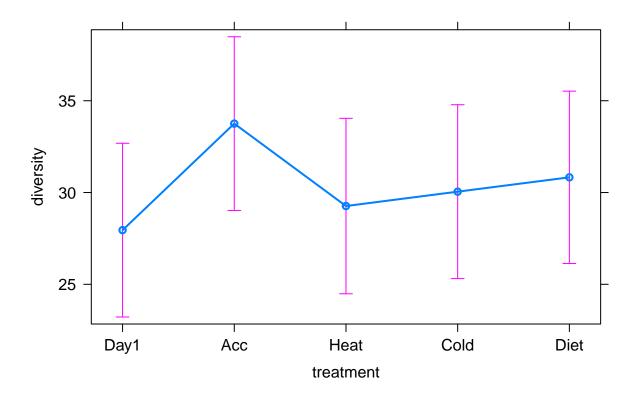
```
## treatmentCold -0.441 0.420 0.534
## treatmentDiet -0.447 0.418 0.494 0.577
##
## Standardized Within-Group Residuals:
##
          Min
                      Q1
                                Med
                                            QЗ
   -2.2488677 -0.7535860
                         0.1316047 0.7156377 2.3338739
##
##
## Number of Observations: 105
## Number of Groups:
##
                      cage individual_id %in% cage
##
```

####There is an interesting sex effect on Phylogenetic diversity sexMALE -5.406496 1.414101

We have a slightly significant treatment effect

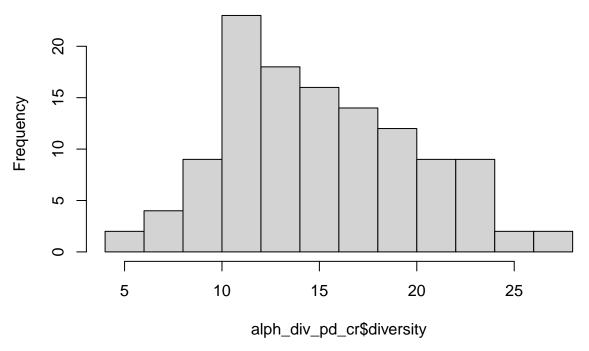
plot(allEffects(model_as1_pd))

treatment effect plot



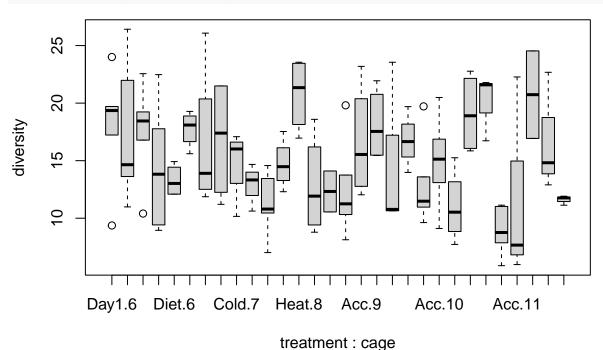
hist(alph_div_pd_cr\$diversity)

Histogram of alph_div_pd_cr\$diversity



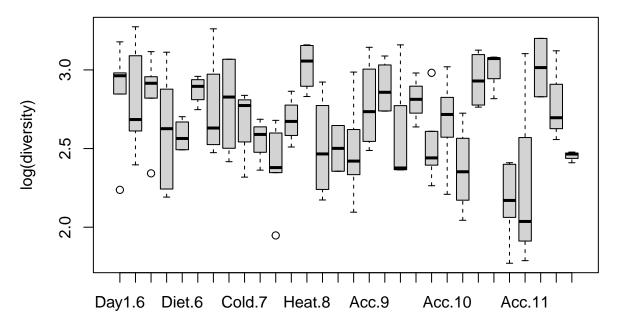
 $treatment_wise_boxplots \ from \ the \ taxonomic \ diversity \ Hill \ numbers$

boxplot(diversity~treatment*cage,data = alph_div_pd_cr)



sessment of log transformation it looks tighter, however the non-transformed data still looks ok to me so i

boxplot(log(diversity)~treatment*cage,data = alph_div_pd_cr)



treatment: cage

Create

the models for comparison - including the random effects results in the lowest AIC

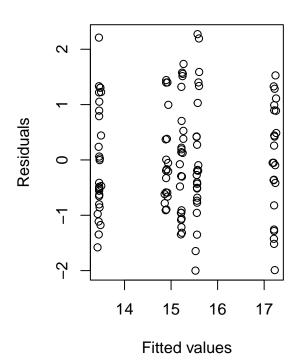
```
###Try to include the other factors
model_cr.a.pd <- lme(diversity~treatment,</pre>
                   random = ~1|cage/individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_pd_cr)
model_cr.b.pd <- lme(diversity~treatment,</pre>
                   random = ~1|individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_pd_cr)
model_cr.c.pd <- lme(diversity~treatment,</pre>
                   random = ~1 | cage,
                   data=alph_div_pd_cr)
model_cr.d.pd <- lme(diversity~treatment,</pre>
                   random = ~1|individual_id,
                   data=alph_div_pd_cr)
model_cr.e.pd <- lm(diversity~treatment,</pre>
                      data=alph_div_pd_cr)
anova(model_cr.a.pd,model_cr.b.pd,model_cr.c.pd,model_cr.e.pd)
```

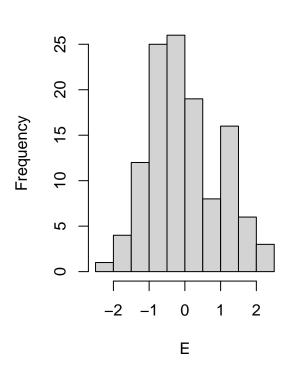
```
## model_cr.a.pd 1 9 719.9922 744.6966 -350.9961  
## model_cr.b.pd 2 8 717.9922 739.9517 -350.9961 1 vs 2 0.0000243801 0.9961  
## model_cr.c.pd 3 7 715.9930 735.2076 -350.9965 2 vs 3 0.0007876453 0.9776  
## model_cr.e.pd 4 6 713.9930 730.4626 -350.9965 3 vs 4 0.0000001073 0.9997
```

```
AIC(model_cr.a.pd ) #383.197
## [1] 719.9922
AIC(model_cr.b.pd ) #381.197
## [1] 717.9922
AIC(model_cr.c.pd ) #379.439
## [1] 715.993
AIC(model_cr.d.pd ) #379.439
## [1] 715.9922
AIC(model_cr.e.pd ) #377.9547
## [1] 722.8743
Residuals looks like they are randomly distributed around 0
model_cr1_pd=model_cr.a.pd
E <- resid(model_cr1_pd, type = "normalized")</pre>
Fit <- fitted(model_cr1_pd)</pre>
op \leftarrow par(mfrow = c(1, 2))
plot(x = Fit, y = E,
     xlab = "Fitted values", ylab = "Residuals",
     main = "Residuals versus fitted values")
hist(E, nclass = 15)
```

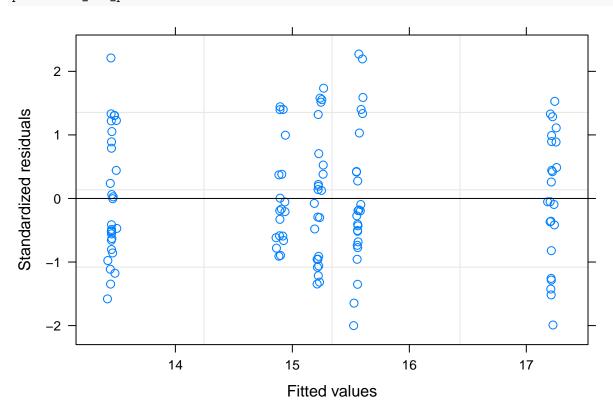
Residuals versus fitted values

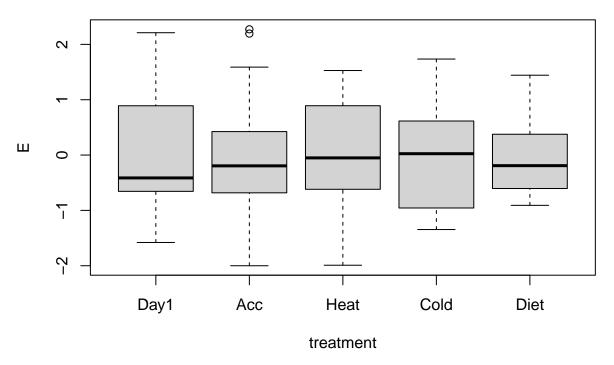
Histogram of E





plot(model_cr1_pd)





summary(model_cr1_pd)

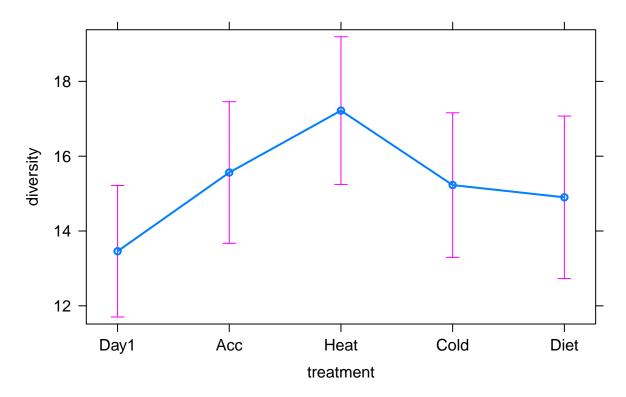
```
## Linear mixed-effects model fit by REML
##
     Data: alph_div_pd_cr
##
          AIC
                   BIC
                          logLik
     719.9922 744.6966 -350.9961
##
##
## Random effects:
##
    Formula: ~1 | cage
##
            (Intercept)
##
  StdDev: 0.0009642443
##
    Formula: ~1 | individual_id %in% cage
##
##
           (Intercept) Residual
## StdDev:
             0.2099112 4.774799
##
## Correlation Structure: Continuous AR(1)
    Formula: ~as.numeric(days) | cage/individual_id
##
##
    Parameter estimate(s):
##
         Phi
## 0.1363406
## Fixed effects: diversity ~ treatment
##
                     Value Std.Error DF
                                          t-value p-value
## (Intercept)
                 13.459064 0.8875144 87 15.164897 0.0000
                  2.104398 1.3032082 87
## treatmentAcc
                                        1.614783 0.1100
## treatmentHeat 3.759593 1.3333436 87
                                         2.819673 0.0060
## treatmentCold 1.768294 1.3177344 87 1.341920 0.1831
```

```
## treatmentDiet 1.441405 1.4095706 87 1.022584 0.3093
##
    Correlation:
##
                 (Intr) trtmnA trtmnH trtmnC
## treatmentAcc -0.680
## treatmentHeat -0.664 0.453
  treatmentCold -0.672 0.458
                               0.448
  treatmentDiet -0.628  0.428  0.418  0.423
##
## Standardized Within-Group Residuals:
##
          Min
                      Q1
                                Med
                                                2.2725295
   -2.0001993 -0.6960988 -0.1741151 0.8149149
##
## Number of Observations: 120
## Number of Groups:
##
                      cage individual_id %in% cage
##
                         6
```

####There is an interesting sex effect on Phylogenetic diversity sexMALE -5.406496 1.414101

We have a slightly significant treatment effect

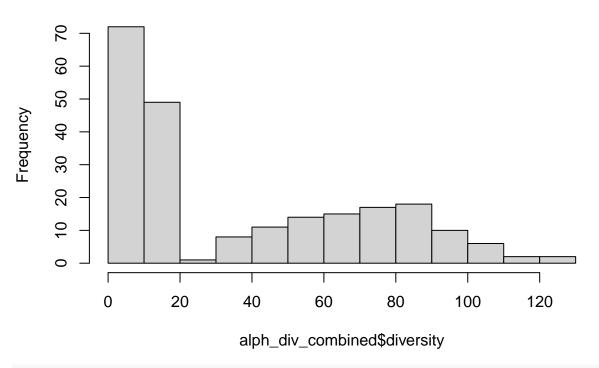
treatment effect plot



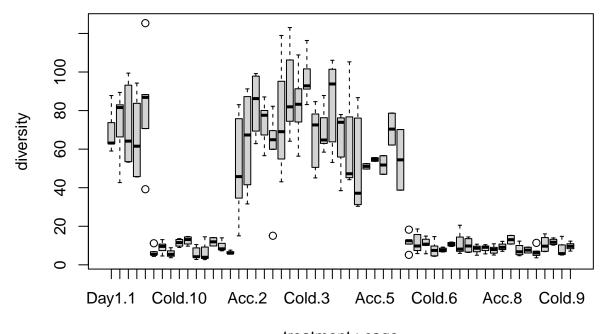
Species-species alpha diversity linear model

alph_div_combined <- rbind(alph_div_tax_as, alph_div_tax_cr)
hist(alph_div_combined\$diversity)</pre>

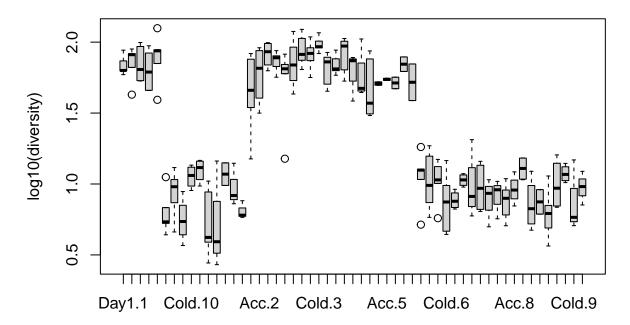
Histogram of alph_div_combined\$diversity



boxplot(diversity~treatment*cage,data = alph_div_combined)



treatment : cage



treatment : cage

```
###Try to include the other factors
model_div_combined.a <- lme(log(diversity)~species,</pre>
                   random = ~1|cage/individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_combined)
model_div_combined.b <- lme(diversity~species,</pre>
                   random = ~1|individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_combined)
model_div_combined.c <- lme(diversity~species,</pre>
                   random = ~1 | cage,
                   data=alph_div_combined)
model_div_combined.d <- lme(diversity~species,</pre>
                   random = ~1|individual_id,
                   data=alph_div_combined)
model_div_combined.e <- lm(diversity~species,</pre>
                      data=alph_div_combined)
AIC(model_div_combined.a) #1868.937
```

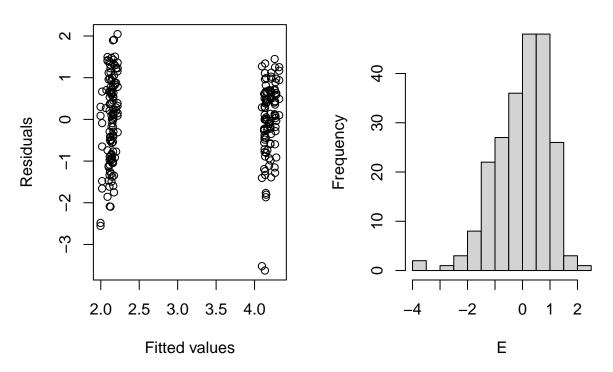
[1] 249.1726

```
AIC(model_div_combined.b) #1868.075
```

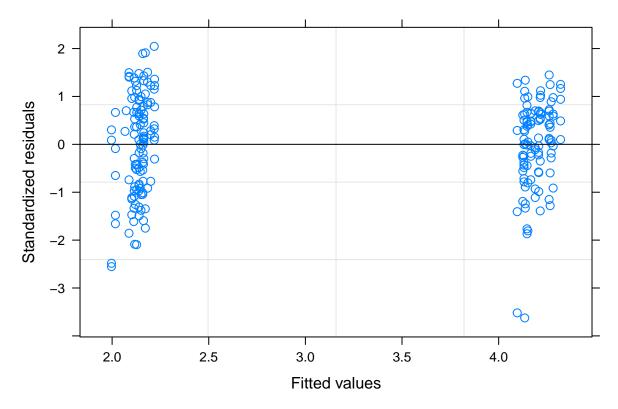
[1] 1874.692

Residuals versus fitted values

Histogram of E



plot(model_alph_combined)



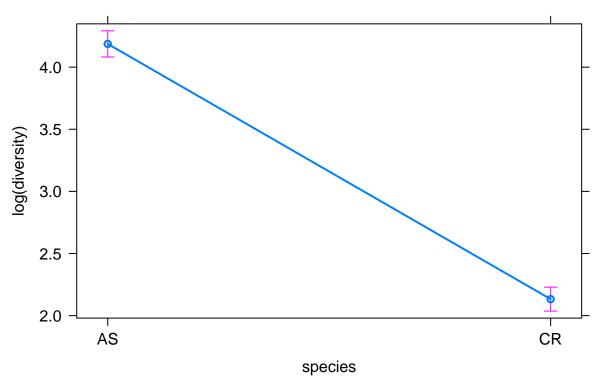
summary(model_alph_combined)

```
## Linear mixed-effects model fit by REML
     Data: alph_div_combined
##
##
          AIC
                  BIC
##
     249.1726 269.6157 -118.5863
##
## Random effects:
    Formula: ~1 | cage
##
           (Intercept)
## StdDev: 0.07296413
##
##
   Formula: ~1 | individual_id %in% cage
           (Intercept) Residual
##
## StdDev: 0.07484818 0.3930167
##
## Correlation Structure: Continuous AR(1)
  Formula: ~as.numeric(days) | cage/individual_id
   Parameter estimate(s):
##
         Phi
##
## 0.2000606
## Fixed effects: log(diversity) ~ species
##
                   Value Std.Error DF
                                          t-value p-value
## (Intercept) 4.187049 0.05336890 174 78.45484
                                    9 -28.36199
## speciesCR
              -2.054082 0.07242374
```

```
Correlation:
##
             (Intr)
   speciesCR -0.737
##
## Standardized Within-Group Residuals:
##
                      Q1
                                Med
                                            QЗ
                                                       Max
   -3.6249532 -0.5974494 0.1201001 0.6679768 2.0459622
##
## Number of Observations: 225
  Number of Groups:
##
                      cage individual_id %in% cage
                        11
##
anova(model_alph_combined)
               numDF denDF F-value p-value
## (Intercept)
                       174 7248.809 <.0001
                   1
## species
                            804.403 <.0001
```

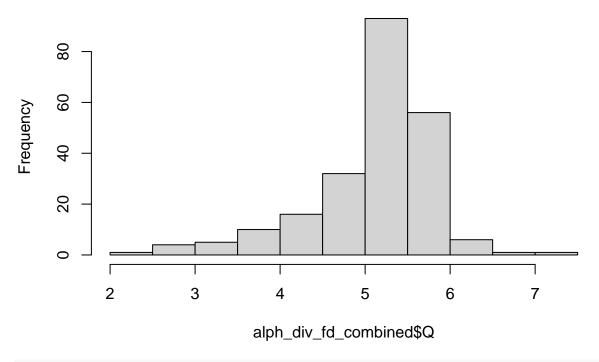
plot(allEffects(model_alph_combined))

species effect plot

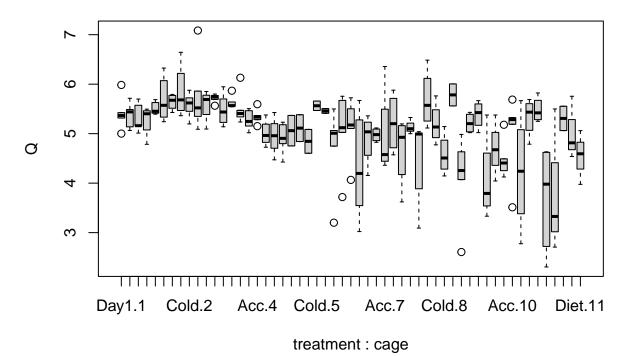


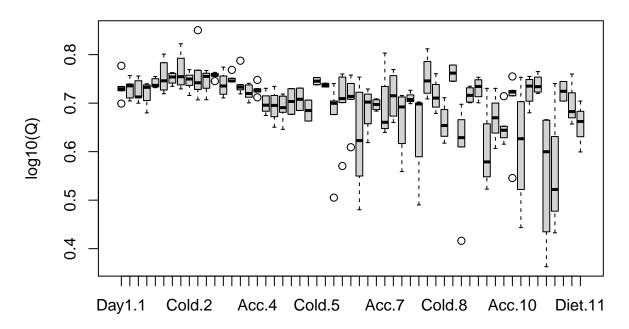
```
###Try to include the other factors
alph_div_fd_combined <- rbind(alph_div_fd_as, alph_div_fd_cr)
hist(alph_div_fd_combined$Q)</pre>
```

Histogram of alph_div_fd_combined\$Q



boxplot(Q~treatment*cage,data = alph_div_fd_combined)





treatment : cage

```
model_div_fd_combined.a <- lme(Q~species,</pre>
                   random = ~1|cage/individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_fd_combined)
model_div_fd_combined.b <- lme(Q~species,</pre>
                   random = ~1|individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_fd_combined)
model_div_fd_combined.c <- lme(Q~species,</pre>
                   random = ~1 | cage,
                   data=alph_div_fd_combined)
model_div_fd_combined.d <- lme(Q~species,</pre>
                   random = ~1|individual_id,
                   data=alph_div_fd_combined)
model_div_fd_combined.e <- lm(Q~species,</pre>
                      data=alph_div_fd_combined)
AIC(model_div_fd_combined.a) #940.832
```

[1] 464.4085

```
AIC(model_div_fd_combined.b) #939.4217
```

[1] 466.2917

```
AIC(model_div_fd_combined.c) #941.6157

## [1] 460.9967

AIC(model_div_fd_combined.d) #937.4217

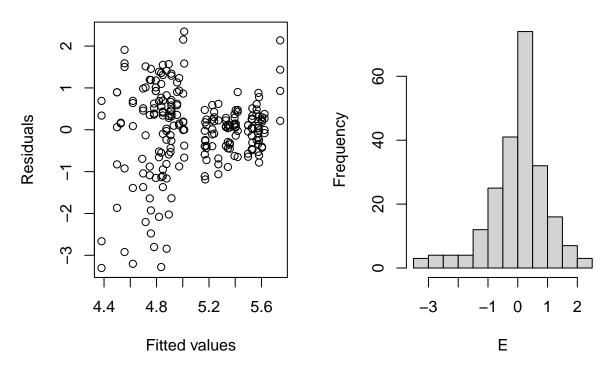
## [1] 464.2916

AIC(model_div_fd_combined.e) #939.4188
```

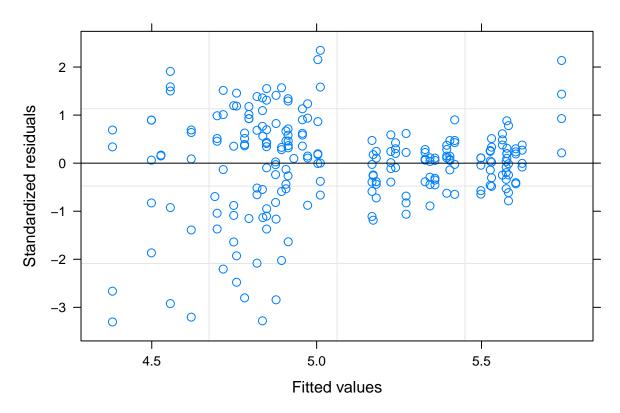
[1] 458.8496

Residuals versus fitted values

Histogram of E



plot(model_alph_fd_combined)



summary(model_alph_fd_combined)

```
## Linear mixed-effects model fit by REML
     Data: alph_div_fd_combined
##
##
          AIC
                   BIC
                          logLik
##
     464.4085 484.8515 -226.2042
##
## Random effects:
    Formula: ~1 | cage
##
##
           (Intercept)
  StdDev:
             0.1965538
##
##
##
    Formula: ~1 | individual_id %in% cage
           (Intercept) Residual
##
## StdDev:
             0.1345171 0.6280142
##
## Correlation Structure: Continuous AR(1)
    Formula: ~as.numeric(days) | cage/individual_id
    Parameter estimate(s):
##
##
         Phi
## 0.1998651
## Fixed effects: Q ~ species
##
                   Value Std.Error DF t-value p-value
## (Intercept) 5.411605 0.1122283 174 48.21961 0.0000
## speciesCR
              -0.623273 0.1518137
                                     9 -4.10551 0.0027
```

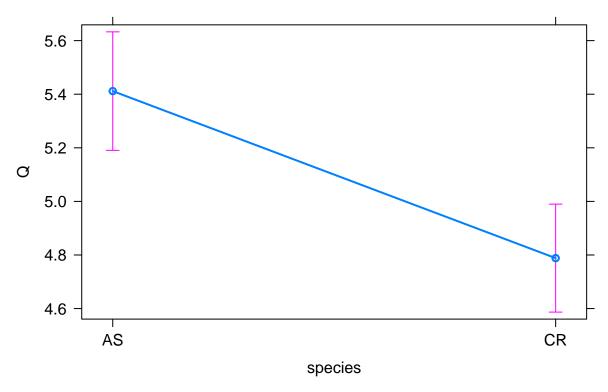
```
Correlation:
##
             (Intr)
   speciesCR -0.739
##
## Standardized Within-Group Residuals:
##
                      Q1
                                Med
                                             QЗ
                                                       Max
   -3.3036141 -0.4388384 0.1065470 0.5129885 2.3472157
##
## Number of Observations: 225
  Number of Groups:
##
                      cage individual_id %in% cage
##
                        11
```

anova(model_alph_fd_combined)

```
## numDF denDF F-value p-value
## (Intercept) 1 174 4501.890 <.0001
## species 1 9 16.855 0.0027
```

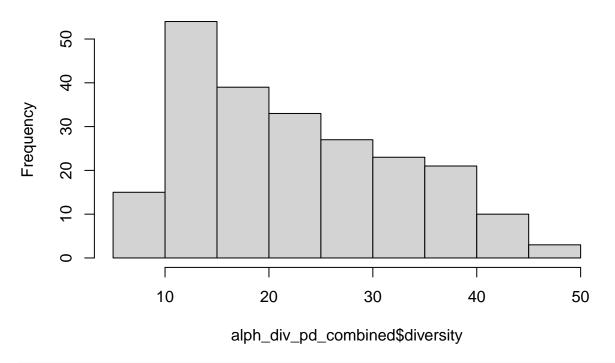
plot(allEffects(model_alph_fd_combined))

species effect plot

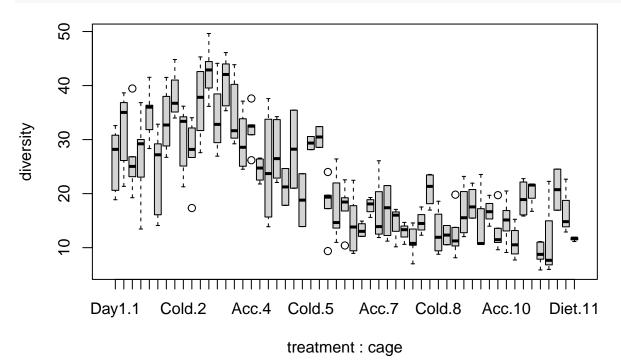


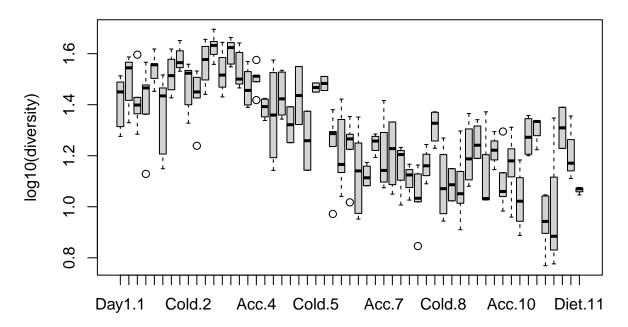
```
###Try to include the other factors
alph_div_pd_combined <- rbind(alph_div_pd_as, alph_div_pd_cr)
hist(alph_div_pd_combined$diversity)</pre>
```

Histogram of alph_div_pd_combined\$diversity



boxplot(diversity~treatment*cage,data = alph_div_pd_combined)





treatment : cage

```
model_div_pd_combined.a <- lme(diversity~species,</pre>
                   random = ~1|cage/individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_pd_combined)
model_div_pd_combined.b <- lme(diversity~species,</pre>
                   random = ~1|individual_id,
                   correlation = corCAR1(form = ~ as.numeric(days)),
                   data=alph_div_pd_combined)
model_div_pd_combined.c <- lme(diversity~species,</pre>
                   random = ~1 | cage,
                   data=alph_div_pd_combined)
model_div_pd_combined.d <- lme(diversity~species,</pre>
                   random = ~1|individual_id,
                   data=alph_div_pd_combined)
model_div_pd_combined.e <- lm(diversity~species,</pre>
                      data=alph_div_pd_combined)
AIC(model_div_pd_combined.a) #940.832
```

[1] 1464.487

```
AIC(model_div_pd_combined.b) #939.4217
```

[1] 1473.215

```
AIC(model_div_pd_combined.c) #941.6157

## [1] 1462.028

AIC(model_div_pd_combined.d) #937.4217

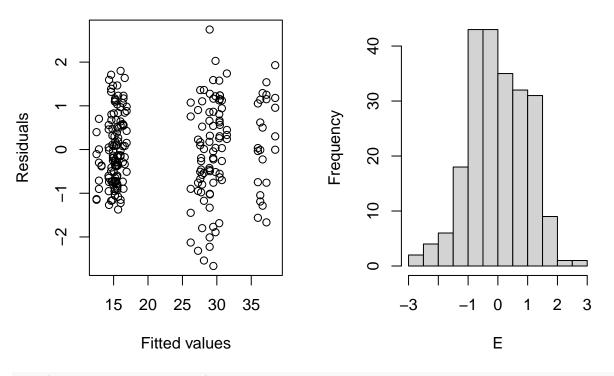
## [1] 1471.215

AIC(model_div_pd_combined.e) #939.4188
```

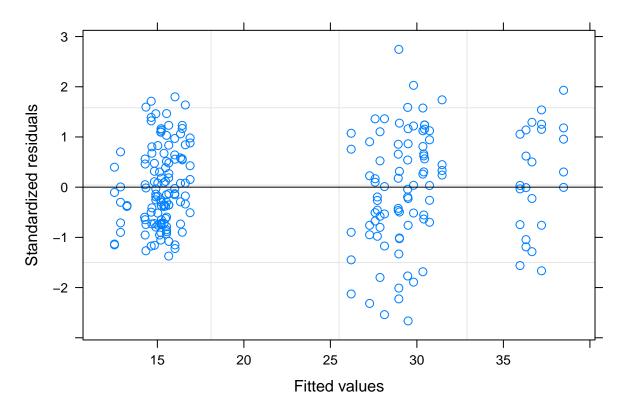
[1] 1486.344

Residuals versus fitted values

Histogram of E



plot(model_alph_pd_combined)



summary(model_alph_pd_combined)

```
## Linear mixed-effects model fit by REML
     Data: alph_div_pd_combined
##
##
          AIC
                  BIC
                         logLik
##
     1464.487 1484.93 -726.2435
##
## Random effects:
    Formula: ~1 | cage
##
##
           (Intercept)
## StdDev:
              2.822594
##
##
    Formula: ~1 | individual_id %in% cage
           (Intercept) Residual
##
## StdDev:
              1.609097 5.77962
##
## Correlation Structure: Continuous AR(1)
   Formula: ~as.numeric(days) | cage/individual_id
   Parameter estimate(s):
##
##
         Phi
## 0.1998991
## Fixed effects: diversity ~ species
##
                   Value Std.Error DF
                                         t-value p-value
## (Intercept) 30.42152 1.438039 174 21.154860
## speciesCR
              -15.36202 1.943356
                                     9 -7.904892
                                                        0
```

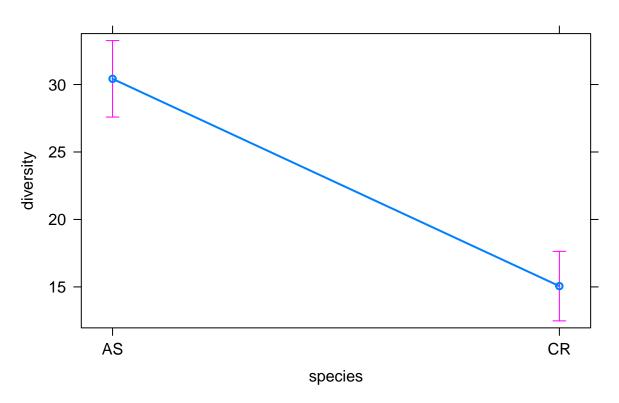
```
Correlation:
##
             (Intr)
   speciesCR -0.74
##
## Standardized Within-Group Residuals:
##
                        Q1
                                                 QЗ
                                                            Max
   -2.66450014 -0.71754267 -0.02619648 0.70257927 2.74515129
##
## Number of Observations: 225
  Number of Groups:
##
                      cage individual_id %in% cage
                        11
##
```

anova(model_alph_pd_combined)

```
## numDF denDF F-value p-value
## (Intercept) 1 174 517.7681 <.0001
## species 1 9 62.4873 <.0001
```

plot(allEffects(model_alph_pd_combined))

species effect plot



sessionInfo()

```
## R version 4.2.2 (2022-10-31)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Linux Mint 20.1
```

```
##
## Matrix products: default
           /usr/lib/x86 64-linux-gnu/blas/libblas.so.3.9.0
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.9.0
## locale:
                                   LC NUMERIC=C
  [1] LC CTYPE=en US.UTF-8
## [3] LC TIME=en US.UTF-8
                                   LC COLLATE=en US.UTF-8
   [5] LC_MONETARY=en_US.UTF-8
                                   LC MESSAGES=en US.UTF-8
  [7] LC_PAPER=en_US.UTF-8
                                   LC_NAME=C
   [9] LC_ADDRESS=C
                                   LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
## attached base packages:
## [1] stats
                 graphics grDevices utils
                                                datasets methods
                                                                    base
##
## other attached packages:
  [1] effects 4.2-2
                        carData 3.0-5
                                         sjPlot_2.8.11
                                                         itools 2.2.0
## [5] lme4_1.1-31
                        Matrix_1.5-1
                                         tinytex_0.42
                                                         multcomp_1.4-20
   [9] TH.data 1.1-1
                        MASS 7.3-58.1
                                         survival_3.4-0
                                                         mvtnorm 1.1-3
## [13] ggplotify_0.1.0 broom_1.0.1
                                         nlme_3.1-160
                                                         forcats_0.5.2
                                                         readr 2.1.3
## [17] stringr_1.4.1
                        dplyr_1.0.10
                                         purrr_0.3.5
                        tibble_3.1.8
## [21] tidyr_1.2.1
                                         ggplot2_3.3.6
                                                         tidyverse 1.3.2
## loaded via a namespace (and not attached):
## [1] fs_1.5.2
                            lubridate_1.8.0
                                                 insight_0.18.5
## [4] httr_1.4.4
                            tools_4.2.2
                                                 backports_1.4.1
## [7] utf8_1.2.2
                            R6_2.5.1
                                                 sjlabelled_1.2.0
                            colorspace_2.0-3
## [10] DBI_1.1.3
                                                 nnet_7.3-18
## [13] withr_2.5.0
                            tidyselect_1.2.0
                                                 emmeans_1.8.1-1
## [16] compiler_4.2.2
                            performance_0.10.0
                                                 cli_3.4.1
## [19] rvest_1.0.3
                            xm12_1.3.3
                                                 sandwich_3.0-2
## [22] bayestestR_0.13.0
                            scales_1.2.1
                                                 digest_0.6.30
## [25] yulab.utils_0.0.5
                            minqa_1.2.5
                                                 rmarkdown_2.17
## [28] pkgconfig_2.0.3
                            htmltools_0.5.3
                                                 highr_0.9
## [31] dbplyr_2.2.1
                            fastmap_1.1.0
                                                 rlang_1.0.6
## [34] readxl 1.4.1
                            rstudioapi 0.14
                                                 gridGraphics_0.5-1
## [37] generics_0.1.3
                            zoo_1.8-11
                                                 jsonlite_1.8.3
## [40] googlesheets4_1.0.1 magrittr_2.0.3
                                                 parameters_0.19.0
## [43] Rcpp_1.0.9
                            munsell_0.5.0
                                                 fansi_1.0.3
## [46] lifecycle 1.0.3
                            stringi 1.7.8
                                                 yaml 2.3.6
## [49] grid 4.2.2
                            sjmisc_2.8.9
                                                 crayon 1.5.2
## [52] lattice_0.20-45
                            ggeffects_1.1.3
                                                 haven 2.5.1
## [55] splines_4.2.2
                            pander_0.6.5
                                                 sjstats_0.18.1
## [58] hms_1.1.2
                            knitr_1.40
                                                 pillar_1.8.1
## [61] boot_1.3-28
                            estimability_1.4.1
                                                 effectsize_0.8.1
## [64] codetools_0.2-18
                            reprex_2.0.2
                                                 glue_1.6.2
## [67] evaluate_0.17
                            mitools_2.4
                                                 modelr_0.1.9
## [70] vctrs_0.5.0
                            nloptr_2.0.3
                                                 tzdb_0.3.0
## [73] cellranger_1.1.0
                            gtable_0.3.1
                                                 assertthat_0.2.1
## [76] datawizard_0.6.2
                            xfun_0.34
                                                 xtable_1.8-4
## [79] survey_4.1-1
                            coda 0.19-4
                                                 googledrive 2.0.0
## [82] gargle_1.2.1
                            ellipsis_0.3.2
```