

1. Biodiversity effects over time

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package loading

```
library(here)
library(tidyverse)
library(nlme)
library(emmeans)
library(ggpubr)
library(grid)
library(car)
library(rstatix)
library(formatR)
```

data importation

```
#Data on Net effect, CE, SE
NE_data<-read.csv(here("data", "NE_data.csv"), sep=";")

#transform Composition, Block, Days as factor for further analyses
NE_data<-NE_data[NE_data$Days!="0", -1]
NE_data<-NE_data %>%
  mutate(across(c("Composition", "Block", "Days"),
                 as.factor))
str(NE_data)

## 'data.frame':   99 obs. of  7 variables:
## $ Block      : Factor w/ 3 levels "1","2","3": 1 1 1 1 1 1 1 1 1 ...
## $ Composition: Factor w/ 11 levels "LmLt","LmSp",...: 2 6 1 9 8 11 4 3 7 10 ...
## $ Days       : Factor w/ 3 levels "20","40","60": 1 1 1 1 1 1 1 1 1 ...
## $ RYT        : num  0.962 0.952 1.373 1.012 1.547 ...
## $ NE         : num  -40.6 -53.7 318 11.4 355.9 ...
## $ CE         : num  -40.7 -50.5 227.7 11.3 281.9 ...
## $ SE         : num   0.00933 -3.20672 90.26404 0.05771 73.94945 ...
```

test homoscedasticity of variance

```
# Plot Raw data - NE against Days
PlotNE <- ggplot(NE_data, aes(x = Days, y = NE)) +
  geom_jitter(position=position_jitter(0.2)) +
  stat_summary(fun.data="mean_sdl", fun.args = list(mult=1),
              geom="pointrange", color = "red")+
  theme_classic() +
  labs(x="Days", y="NE (mg)") +
```

```

scale_y_continuous(limits=c(-2000, 2000), breaks=seq(-2000, 2000, by=500))

leveneTest(NE ~ Days, data=NE_data) #the variance among the factor Days is NOT equal

## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value    Pr(>F)
## group  2  23.415 5.22e-09 ***
##      96
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

PlotCE <- ggplot(NE_data, aes(x = Days, y = CE)) +
  geom_jitter(position=position_jitter(0.2)) +
  stat_summary(fun.data="mean_sdl", fun.args = list(mult=1),
    geom="pointrange", color = "red")+
  theme_classic() +
  labs(x="Days", y="CE (mg)") +
  scale_y_continuous(limits=c(-2000, 2000), breaks=seq(-2000, 2000, by=500))

leveneTest(CE ~ Days, data=NE_data) #variance among the factor Days is NOT equal

## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value    Pr(>F)
## group  2  33.672 8.319e-12 ***
##      96
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

PlotSE <- ggplot(NE_data, aes(x = Days, y = SE)) +
  geom_jitter(position=position_jitter(0.2)) +
  stat_summary(fun.data="mean_sdl", fun.args = list(mult=1),
    geom="pointrange", color = "red")+
  theme_classic() +
  labs(x="Days", y="SE (mg)") +
  scale_y_continuous(limits=c(-2000, 2000), breaks=seq(-2000, 2000, by=500))

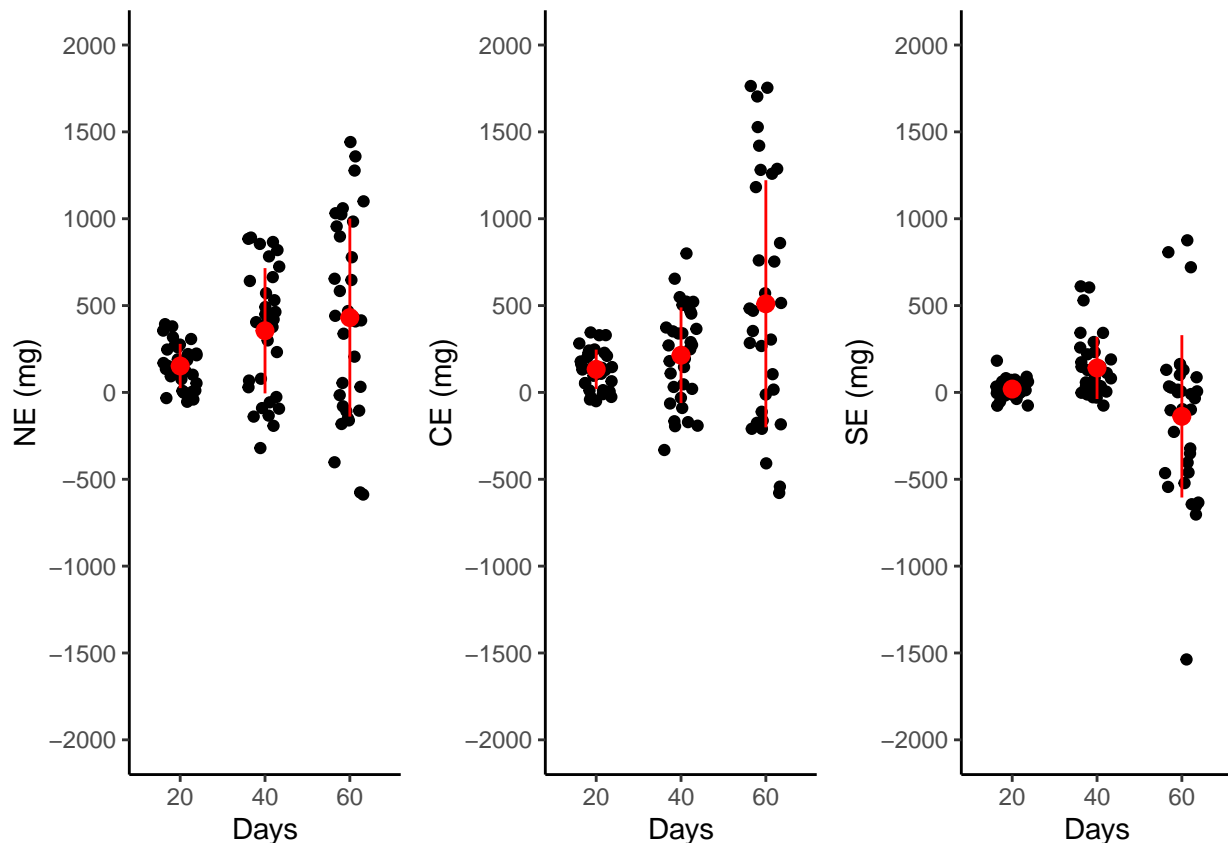
leveneTest(SE ~ Days, data=NE_data) #variance among the factor Days is NOT equal

## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value    Pr(>F)
## group  2  16.923 5.065e-07 ***
##      96
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#mean_sdl computes the mean plus or minus a constant times the standard deviation
#(here the constant (mult) =1), so we see mean + standard deviation in Red
#We can clearly see that the variance of NE, CE and SE increases over time

ggarrange(PlotNE, PlotCE, PlotSE, nrow=1, ncol=3)

```



why we use the nlme::lme function to compute the ANOVAs

Since the variance of NE, CE, SE increases over time (heteroscedasticity in the residuals), we decided to use the function `nlme::lme` to make our ANOVAs, because the function allows to weight the variance by the factor Days. Observations with higher variability will have lower weights, allowing the model to give them less influence. This method is particularly relevant with repeated measures data as we have here.

The class `pdMat`, which represent positive-definite matrices, is used to represent variance-covariance matrices of random effects. We have have two blocks in the random effects variance-covariance matrix: one for Composition, and one for Block.

Sources: <https://stackoverflow.com/questions/36643713/how-to-specify-different-random-effects-in-nlme-vs-lme4> <https://biostatmatt.com/archives/2718>

The `varIdent` function allows different variances according to the levels of a classification factor.

ANOVA 1 : net effect through time

```
# Model testing the effect of Days on NE with Composition and Block
# as random factors and weighted by Days.

# Here we add a dummy factor just to include the whole dataset in a single block,
# to act as a grouping factor within which Composition and Block are both nested.
NE_data$dummy<-factor(1)

#1.1-ASSESSING WHETHER THERE IS ANY STATISTICALLY SIGNIFICANT DIFFERENCE AMONG DAYS
# this first p-value aims to understand whether there is any statistically significant
# difference among a set of treatments (Days). In the model, pdBlocked combines the
```

```

# covariance structures of pdIdent Composition and pdIdent Block (identity matrices)
# in a 2-block matrix. -1 means that there is no default overall intercept being
# estimated for the factors Composition and Block independently. pdIdent indicates that
# the random effects are assumed to have equal variances and no covariances. This whole
# syntax is actually a "trick" to specify non-nested crossed random effects in the nlme
# syntax. An equivalent way to specify the random effects could be :
# random=list(dummy=pdBlocked(list(pdIdent(~ 0 + Composition), pdIdent(~ 0 + Block)))),
modNE <- lme(NL ~ Days,
             random=list(dummy=pdBlocked(list(pdIdent(~Composition-1),
                                                pdIdent(~Block-1)))),
             data=NE_data,
             weights=varIdent(form=~ 1 | Days), #residuals are allowed to have different
             #variances for different levels of the "Days" variable
             method="REML",
             control=list(msMaxIter=1000,
                          msMaxEval=1000))

```

```

anova(modNE) #there is at least one statistically significant difference among certain

```

```

##          numDF denDF  F-value p-value
## (Intercept)      1    96 15.37169  2e-04
## Days              2    96 15.39105  <.0001

```

```

#levels of factor days

```

```

#1.2-ASSESSING OVERALL EFFECT OF DAYS : likelihood ratio test

```

```

# "when assessing the overall treatment effects using a likelihood ratio test, one should
# use maximum likelihood, rather than REML, when using lme or lmer"

```

```

modNE.ml <- lme(NL ~ Days,
               random=list(dummy=pdBlocked(list(pdIdent(~Composition-1),
                                                  pdIdent(~Block-1)))),
               data=NE_data,
               weights=varIdent(form=~ 1 | Days),
               method="ML",
               control=list(msMaxIter=1000,
                           msMaxEval=1000))

```

```

#null model

```

```

modNE0.ml <- lme(NL ~ 1,
                random=list(dummy=pdBlocked(list(pdIdent(~Composition-1),
                                                    pdIdent(~Block-1)))),
                data=NE_data,
                weights=varIdent(form=~ 1 | Days),
                method="ML",
                control=list(msMaxIter=1000,
                            msMaxEval=1000))

```

```

#overall p-value modNE

```

```

anova(modNE0.ml, modNE.ml) #overall, the factor days has a statistically

```

```

##          Model df      AIC      BIC    logLik    Test  L.Ratio p-value
## modNE0.ml      1   6 1395.617 1411.187 -691.8082
## modNE.ml       2   8 1374.020 1394.781 -679.0100 1 vs 2 25.59642  <.0001

```

#significant effect on net effect

#1.3-COMPARISON BETWEEN EACH DAY AND DAY 20 (CONTROL LEVEL)

summary(modNE)

```
## Linear mixed-effects model fit by REML
##   Data: NE_data
##       AIC      BIC    logLik
## 1344.593 1365.108 -664.2964
##
## Random effects:
## Composite Structure: Blocked
##
## Block 1: CompositionLmLt, CompositionLmSp, CompositionLmSpLt, CompositionLmSpWc, CompositionLmSpWcLt
## Formula: ~Composition - 1 | dummy
## Structure: Multiple of an Identity
##           CompositionLmLt CompositionLmSp CompositionLmSpLt CompositionLmSpWc
## StdDev:      135.6742      135.6742      135.6742      135.6742
##           CompositionLmSpWcLt CompositionLmWc CompositionLmWcLt CompositionSpLt
## StdDev:      135.6742      135.6742      135.6742      135.6742
##           CompositionSpWc CompositionSpWcLt CompositionWcLt
## StdDev:      135.6742      135.6742      135.6742
##
## Block 2: Block1, Block2, Block3
## Formula: ~Block - 1 | dummy
## Structure: Multiple of an Identity
##           Block1  Block2  Block3 Residual
## StdDev: 20.45318 20.45318 20.45318 71.08742
##
## Variance function:
## Structure: Different standard deviations per stratum
## Formula: ~1 | Days
## Parameter estimates:
##      20      40      60
## 1.000000 3.499917 6.492142
## Fixed effects: NE ~ Days
##           Value Std.Error DF  t-value p-value
## (Intercept) 152.2370  44.33945 96  3.433443  9e-04
## Days40      202.7507  45.04372 96  4.501199  0e+00
## Days60      278.7358  81.28599 96  3.429075  9e-04
## Correlation:
##      (Intr) Days40
## Days40 -0.077
## Days60 -0.042  0.042
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -2.0964613 -0.6720728  0.0518790  0.7502074  1.9399138
##
## Number of Observations: 99
## Number of Groups: 1
```

#here we can see that NE is significantly greater at day 40 and at day 60 than at day 20

#WHICH TREATMENTS ARE DIFFERENT FROM EACH OTHER?

```
contrast(emmeans(modNE, specs="Days"), "pairwise") #the effect of days on NE isn't
```

```
## contrast      estimate    SE df t.ratio p.value
## Days20 - Days40    -203 45.0 96  -4.501  0.0001
## Days20 - Days60    -279 81.3 96  -3.429  0.0026
## Days40 - Days60     -76 91.3 96  -0.833  0.6838
##
```

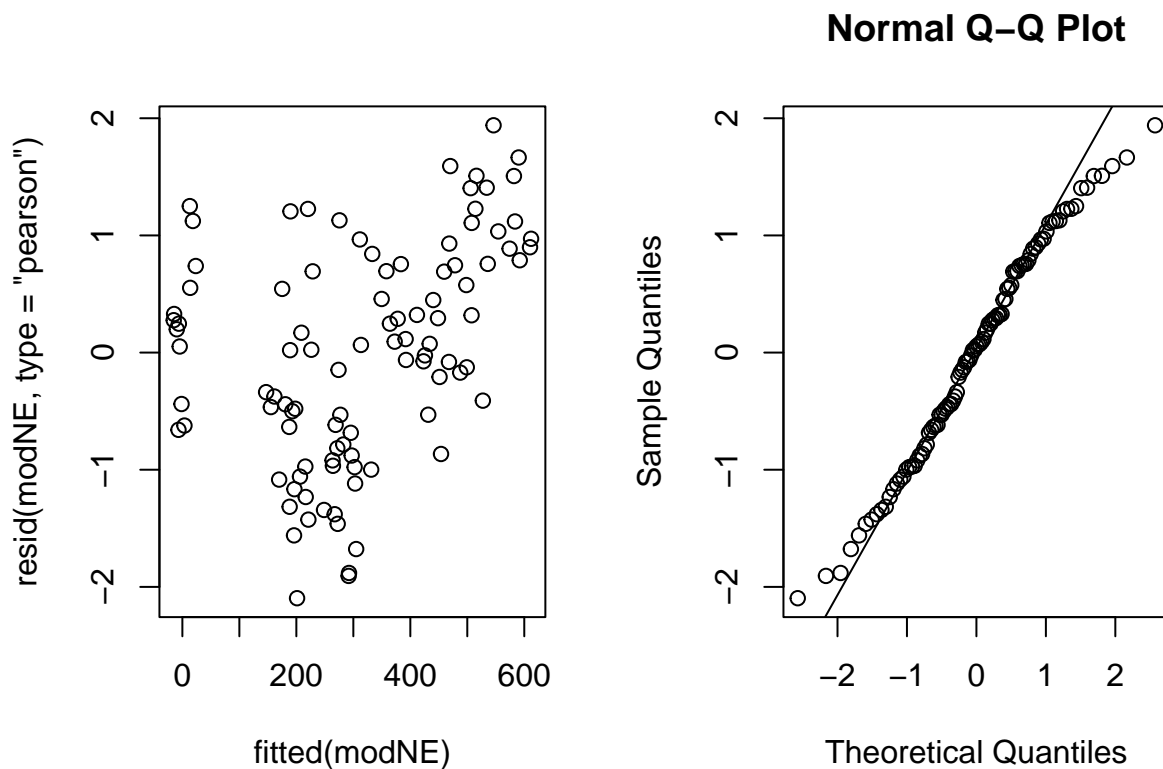
```
## Degrees-of-freedom method: containment
```

```
## P value adjustment: tukey method for comparing a family of 3 estimates
```

statistically different between Day 40 and Day 60 only. All other comparisons are significant.

#MODEL VALIDATION

```
par(mfrow=c(1,2))
plot(fitted(modNE), resid(modNE,type="pearson"))
qqnorm(resid(modNE,type="pearson"))
qqline(resid(modNE,type="pearson"))
```



##PLOT

```
hcl.colors(4, palette="Blue-Yellow")
```

```
## [1] "#2D3184" "#32AAB5" "#B3E7C5" "#F3F1E4"
```

#computing mean and standard deviation per day from raw data

```
mean<-as.vector(c(mean(NE_data[NE_data$Days==20, ]$NE), mean(NE_data[NE_data$Days==40, ]$NE), mean(NE_data[NE_data$Days==60, ]$NE)))
```

```
se<-as.vector(c(sd(NE_data[NE_data$Days==20, ]$NE)/sqrt(length(NE_data[NE_data$Days==20, ]$NE)), sd(NE_data[NE_data$Days==40, ]$NE)/sqrt(length(NE_data[NE_data$Days==40, ]$NE)), sd(NE_data[NE_data$Days==60, ]$NE)/sqrt(length(NE_data[NE_data$Days==60, ]$NE))))
```

```
NE_meanse<-data.frame(
  days=c("20", "40", "60"),
  mean=mean,
  se=se)
```

```

mean=mean,
se=se,
label=c("a", "b", "b"))
NE_meanse$ci<-NE_meanse$se*1.96
NE_meanse$stars<-NE_meanse$mean + NE_meanse$ci + 125

NEp<-ggplot(NE_meanse) +
  geom_bar(aes(x=days, y=mean), colour="black", fill="#2D3184", stat="identity", alpha=0.7) +
  #geom_errorbar(aes(x=days, ymin=mean-se, ymax=mean+se), width=0.4, colour="red", alpha=0.9, size=1.3)
  geom_errorbar(aes(x=days, ymin=mean-ci, ymax=mean+ci), width=0.4, colour="black", alpha=0.9, size=1.3)
  geom_text(aes(x=days, y=mean+ci+100, label=label,), position=position_dodge(0.9), vjust=1, size=7) +
  theme_classic() +
  theme(text=element_text(size=15), plot.title=element_text(vjust=2), panel.grid.major.y = element_line
  labs(x="Days of growth", y="NE", title="Net biodiversity effect", pch=8) +
  scale_y_continuous(limits=c(0, 1000), breaks=seq(0, 1000, by=200)) + # Add stars based
  #on significance levels
  annotate(
    "text",
    x = c(1, 2, 3), # x-coordinates where stars should be placed
    y = c(320.6969, 603.0179, 750.5188), # y-coordinate (adjust based on your plot)
    label = c("****", "****", "***"), # significance labels
    size = 5, # adjust the size of the stars
    color = "black" # color of the stars
  )

```

ANOVA 2 : complementarity effect through time

```

modCE <- lme(CE ~ Days,
  random=list(dummy=pdBlocked(list(pdIdent(~Composition-1),
                                     pdIdent(~Block-1)))),
  data=NE_data,
  weights=varIdent(form=~ 1 | Days),
  method="REML",
  control=list(msMaxIter=1000,
               msMaxEval=1000))
anova(modCE) #there is at least one statistically significant difference among certain

```

```

##           numDF denDF  F-value p-value
## (Intercept)      1    96 16.55500 1e-04
## Days             2    96  8.50478 4e-04

```

#levels of factor days

```

modCE.ml <- lme(CE ~ Days,
  random=list(dummy=pdBlocked(list(pdIdent(~Composition-1),
                                     pdIdent(~Block-1)))),
  data=NE_data,
  weights=varIdent(form=~ 1 | Days),
  method="ML",
  control=list(msMaxIter=1000,
               msMaxEval=1000))

```

#null model

```

modCE0.ml <- lme(CE ~ 1,
  random=list(dummy=pdBlocked(list(pdIdent(~Composition-1),

```

```

pdIdent(~Block-1))),
data=NE_data,
weights=varIdent(form=~ 1 | Days),
method="ML",
control=list(msMaxIter=1000,
             msMaxEval=1000))

anova(modCEO.ml, modCE.ml) #overall, the factor days has a statistically significant

```

```

##           Model df      AIC      BIC    logLik    Test  L.Ratio p-value
## modCEO.ml      1   6 1392.966 1408.537 -690.4833
## modCE.ml       2   8 1381.734 1402.495 -682.8671 1 vs 2 15.23235   5e-04

```

```

#effect on complementarity effect

```

```

summary(modCE)

```

```

## Linear mixed-effects model fit by REML
##   Data: NE_data
##       AIC      BIC    logLik
##   1352.301 1372.815 -668.1503
##
## Random effects:
##   Composite Structure: Blocked
##
##   Block 1: CompositionLmLt, CompositionLmSp, CompositionLmSpLt, CompositionLmSpWc, CompositionLmSpWcLt
##   Formula: ~Composition - 1 | dummy
##   Structure: Multiple of an Identity
##           CompositionLmLt CompositionLmSp CompositionLmSpLt CompositionLmSpWc
## StdDev:      112.2394      112.2394      112.2394      112.2394
##           CompositionLmSpWcLt CompositionLmWc CompositionLmWcLt CompositionSpLt
## StdDev:      112.2394      112.2394      112.2394      112.2394
##           CompositionSpWc CompositionSpWcLt CompositionWcLt
## StdDev:      112.2394      112.2394      112.2394
##
##   Block 2: Block1, Block2, Block3
##   Formula: ~Block - 1 | dummy
##   Structure: Multiple of an Identity
##           Block1      Block2      Block3 Residual
## StdDev: 0.006820958 0.006820958 0.006820958 69.93059
##
## Variance function:
##   Structure: Different standard deviations per stratum
##   Formula: ~1 | Days
##   Parameter estimates:
##           20      40      60
## 1.000000 2.885068 9.987727
## Fixed effects: CE ~ Days
##           Value Std.Error DF  t-value p-value
## (Intercept) 132.8559  35.96436 96  3.694097  0.0004
## Days40      81.2908  37.17084 96  2.186951  0.0312
## Days60     435.7584 122.19203 96  3.566177  0.0006
## Correlation:
##   (Intr) Days40

```



```
## Days40 -0.111
## Days60 -0.034 0.033
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -2.0306808 -0.7444088 -0.1976629 0.6529373 2.5658965
##
## Number of Observations: 99
## Number of Groups: 1
```

#here we can see that CE is significantly greater at day 40 and at day 60 than at day 20

```
contrast(emmeans(modCE, specs="Days"), "pairwise") #the effect of days on CE isn't
```

```
## contrast      estimate      SE df t.ratio p.value
## Days20 - Days40    -81.3   37.2 96  -2.187 0.0786
## Days20 - Days60   -435.8 122.2 96  -3.566 0.0016
## Days40 - Days60   -354.5 126.6 96  -2.801 0.0168
##
```

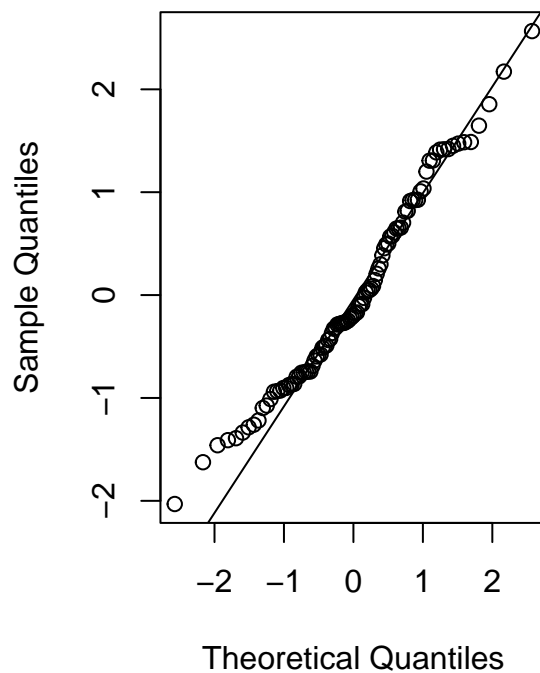
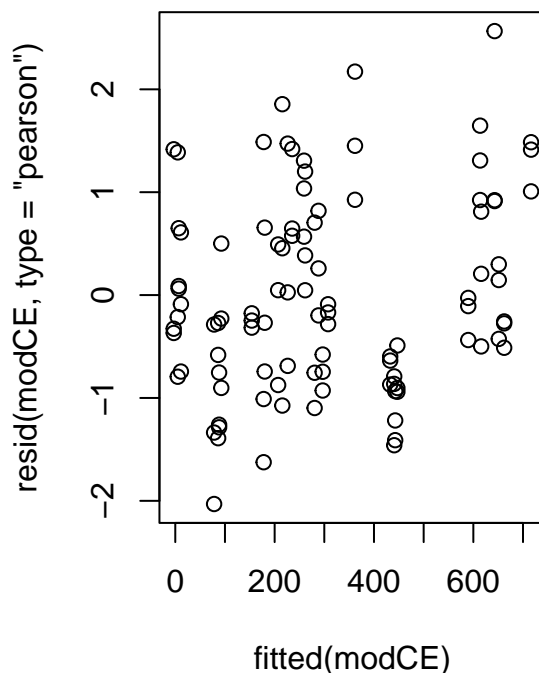
```
## Degrees-of-freedom method: containment
```

```
## P value adjustment: tukey method for comparing a family of 3 estimates
```

*#statistically different between Day 20 and Day 40, and between Day 40 and Day 60.
#All other comparisons are significant.*

```
par(mfrow=c(1,2))
plot(fitted(modCE), resid(modCE,type="pearson"))
qqnorm(resid(modCE,type="pearson"))
qqline(resid(modCE,type="pearson"))
```

Normal Q-Q Plot



```

meanCE<-as.vector(c(mean(NE_data[NE_data$Days==20, ]$CE), mean(NE_data[NE_data$Days==40, ]$CE), mean(NE_data[NE_data$Days==60, ]$CE)))
seCE<-as.vector(c(sd(NE_data[NE_data$Days==20, ]$CE)/sqrt(length(NE_data[NE_data$Days==20, ]$CE)), sd(NE_data[NE_data$Days==40, ]$CE), sd(NE_data[NE_data$Days==60, ]$CE)))

CE_meanse<-data.frame(
  days=c("20", "40", "60"),
  mean=meanCE,
  se=seCE,
  label=c("a", "ab", "b"))
CE_meanse$ci<-CE_meanse$se*1.96
CE_meanse$stars<-CE_meanse$mean + CE_meanse$ci + 125

CEp<-ggplot(CE_meanse) +
  geom_bar(aes(x=days, y=mean), colour="black", fill="#2D3184", stat="identity", alpha=0.7) +
  #geom_errorbar(aes(x=days, ymin=mean-se, ymax=mean+se), width=0.4, colour="red", alpha=0.9, size=1.3)
  geom_errorbar(aes(x=days, ymin=mean-ci, ymax=mean+ci), width=0.4, colour="black", alpha=0.9, size=1.3)
  geom_text(aes(x=days, y=mean+ci+100, label=label), position=position_dodge(0.9), vjust=1, size=7) +
  theme_classic() +
  theme(text=element_text(size=15), plot.title=element_text(vjust=2), panel.grid.major.y = element_line(linetype="dotted"),
  labs(x="Days of growth", y="CE", title="Complementarity effect", pch=8) +
  scale_y_continuous(limits=c(0, 1000), breaks=seq(0, 1000, by=200)) +
  annotate(
    "text",
    x = c(1, 2, 3),
    y = c(296.5644, 433.0912, 958.4401),
    label = c("****", "****", "***"),
    size = 5,
    color = "black"
  )

```

ANOVA 3 : Selection effect through time

```

modSE <- lme(SE ~ Days,
  random=list(dummy=pdBlocked(list(pdIdent(~Composition-1),
                                     pdIdent(~Block-1)))),
  data=NE_data,
  weights=varIdent(form=~ 1 | Days),
  method="REML",
  control=list(msMaxIter=1000,
               msMaxEval=1000))
anova(modSE) #there is at least one statistically significant difference among certain

```

```

##          numDF denDF    F-value p-value
## (Intercept)      1    96   1.677636  0.1983
## Days           2    96  10.284224  0.0001

```

#levels of factor days

```

modSE.ml <- lme(SE ~ Days,
  random=list(dummy=pdBlocked(list(pdIdent(~Composition-1),
                                     pdIdent(~Block-1)))),
  data=NE_data,
  weights=varIdent(form=~ 1 | Days),
  method="ML",

```

```

        control=list(msMaxIter=1000,
                     msMaxEval=1000))
#null model
modSEO.ml <- lme(SE ~ 1,
               random=list(dummy=pdBlocked(list(pdIdent(~Composition-1),
                                                  pdIdent(~Block-1)))),
               data=NE_data,
               weights=varIdent(form=~ 1 | Days),
               method="ML",
               control=list(msMaxIter=1000,
                           msMaxEval=1000))

anova(modSEO.ml, modSE.ml) #overall, the factor days has a statistically significant

```

```

##           Model df      AIC      BIC    logLik    Test  L.Ratio p-value
## modSEO.ml      1  6 1306.742 1322.313 -647.3710
## modSE.ml       2  8 1293.125 1313.886 -638.5627 1 vs 2 17.61664 1e-04

```

```

#effect on complementarity effect

```

```

summary(modSE)

```

```

## Linear mixed-effects model fit by REML
##   Data: NE_data
##       AIC      BIC    logLik
## 1266.343 1286.858 -625.1715
##
## Random effects:
##   Composite Structure: Blocked
##
##   Block 1: CompositionLmLt, CompositionLmSp, CompositionLmSpLt, CompositionLmSpWc, CompositionLmSpWcLt
##   Formula: ~Composition - 1 | dummy
##   Structure: Multiple of an Identity
##           CompositionLmLt CompositionLmSp CompositionLmSpLt CompositionLmSpWc
## StdDev:      33.16856      33.16856      33.16856      33.16856
##           CompositionLmSpWcLt CompositionLmWc CompositionLmWcLt CompositionSpLt
## StdDev:      33.16856      33.16856      33.16856      33.16856
##           CompositionSpWc CompositionSpWcLt CompositionWcLt
## StdDev:      33.16856      33.16856      33.16856
##
##   Block 2: Block1, Block2, Block3
##   Formula: ~Block - 1 | dummy
##   Structure: Multiple of an Identity
##           Block1  Block2  Block3 Residual
## StdDev: 24.64374 24.64374 24.64374 36.34131
##
## Variance function:
##   Structure: Different standard deviations per stratum
##   Formula: ~1 | Days
##   Parameter estimates:
##           20      40      60
## 1.000000 4.609739 12.803021
## Fixed effects: SE ~ Days
##           Value Std.Error DF   t-value p-value

```

```
## (Intercept) 19.38113 18.50602 96 1.047288 0.2976
## Days40      121.45993 29.84047 96 4.070308 0.0001
## Days60      -157.02261 81.24129 96 -1.932793 0.0562
## Correlation:
##      (Intr) Days40
## Days40 -0.072
## Days60 -0.027 0.017
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -2.9642952 -0.6574072 -0.1313255 0.4989232 2.7041302
##
## Number of Observations: 99
## Number of Groups: 1
```

#here we can see that CE is significantly greater at day 40 than at day 20, but CE isn't significantly greater at day 60 than at day 20.

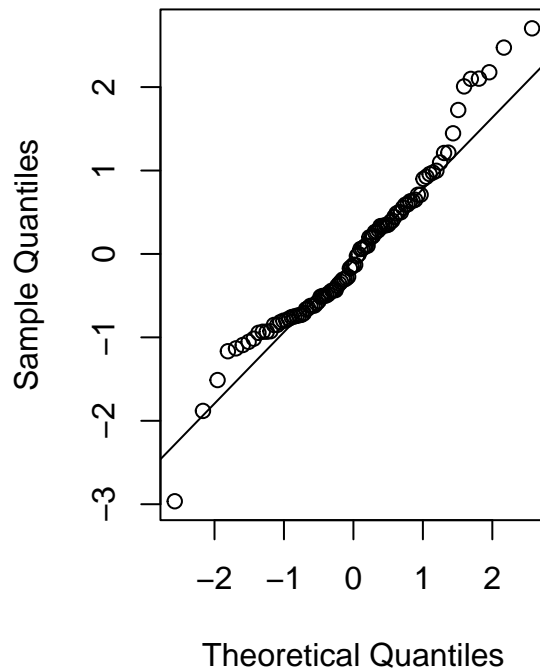
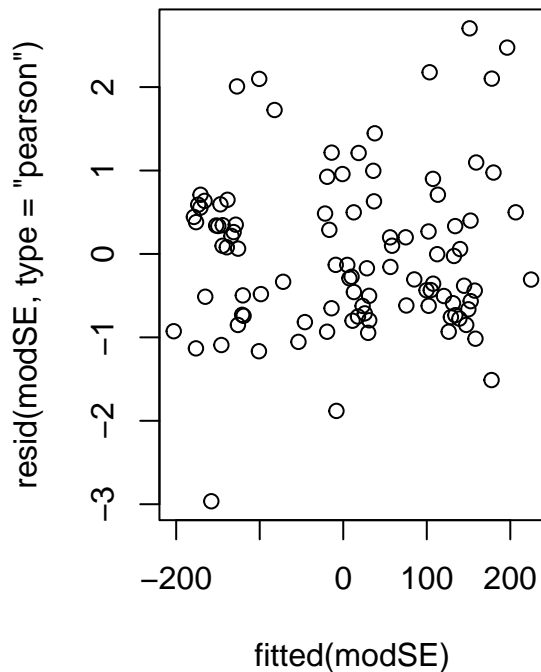
```
contrast(emmeans(modSE, specs="Days"), "pairwise") #the effect of days on SE is only
```

```
## contrast      estimate    SE df t.ratio p.value
## Days20 - Days40    -121 29.8 96  -4.070 0.0003
## Days20 - Days60     157 81.2 96   1.933 0.1351
## Days40 - Days60     278 86.1 96   3.235 0.0047
##
## Degrees-of-freedom method: containment
## P value adjustment: tukey method for comparing a family of 3 estimates
```

#different at Day 40

```
par(mfrow=c(1,2))
plot(fitted(modSE), resid(modSE,type="pearson"))
qqnorm(resid(modSE,type="pearson"))
qqline(resid(modSE,type="pearson"))
```

Normal Q-Q Plot



```
meanSE<-as.vector(c(mean(NE_data[NE_data$Days==20, ]$SE), mean(NE_data[NE_data$Days==40, ]$SE), mean(NE_data[NE_data$Days==60, ]$SE)))
seSE<-as.vector(c(sd(NE_data[NE_data$Days==20, ]$SE)/sqrt(length(NE_data[NE_data$Days==20, ]$SE)), sd(NE_data[NE_data$Days==40, ]$SE)/sqrt(length(NE_data[NE_data$Days==40, ]$SE)), sd(NE_data[NE_data$Days==60, ]$SE)/sqrt(length(NE_data[NE_data$Days==60, ]$SE))))

SE_meanse<-data.frame(
  days=c("20", "40", "60"),
  mean=meanSE,
  se=seSE,
  label=c("a", "b", "a"))
SE_meanse$ci<-SE_meanse$se*1.96
SE_meanse$stars<-SE_meanse$mean + SE_meanse$ci + 125

SEp<-ggplot(SE_meanse) +
  geom_bar(aes(x=days, y=mean), colour="black", fill="#2D3184", stat="identity", alpha=0.7) +
  #geom_errorbar(aes(x=days, ymin=mean-se, ymax=mean+se), width=0.4, colour="red", alpha=0.9, size=1.3)
  geom_errorbar(aes(x=days, ymin=mean-ci, ymax=mean+ci), width=0.4, colour="black", alpha=0.9, size=1.3)
  geom_text(aes(x=days, y=mean+ci+100, label=label,), position=position_dodge(0.9), vjust=1, size=7) +
  theme_classic() +
  theme(text=element_text(size=15), plot.title=element_text(vjust=2), panel.grid.major.y = element_line(linetype="none"))
  labs(x="Days of growth", y="SE", title="Selection effect", pch=8) +
  scale_y_continuous(limits=c(-400, 600), breaks=seq(-400, 600, by=200)) +
  annotate(
    "text",
    x = c(1, 2, 3),
    y = c(161.9431, 326.8847, 146.8014),
    label = c("a", "b", "n.s."),
    size = 5,
    color = "black"
  )
```

Two-tailed t-tests.

Here we want to see if the global NE, CE and SE is different from 0 for each level of the factor Days.

```
NE_ttests<-NE_data %>%
  group_by(Days) %>%
  t_test(NE ~ 0) %>%
  add_significance()
NE_ttests
```

```
## # A tibble: 3 x 9
##   Days .y. group1 group2      n statistic    df          p p.signif
##   <fct> <chr> <chr> <chr>    <int>    <dbl> <dbl>    <dbl> <chr>
## 1 20    NE     1     null model    33      6.87    32 0.0000000909 ****
## 2 40    NE     1     null model    33      5.66    32 0.00000295 ****
## 3 60    NE     1     null model    33      4.34    32 0.000133 ***
```

```
CE_ttests<-NE_data %>%
  group_by(Days) %>%
  t_test(CE ~ 0) %>%
  add_significance()
CE_ttests
```

```
## # A tibble: 3 x 9
##   Days .y. group1 group2      n statistic    df          p p.signif
##   <fct> <chr> <chr> <chr>    <int>    <dbl> <dbl>    <dbl> <chr>
## 1 20    CE     1     null model    33      6.73    32 0.000000135 ****
## 2 40    CE     1     null model    33      4.47    32 0.0000926 ****
## 3 60    CE     1     null model    33      4.21    32 0.000194 ***
```

```
SE_ttests<-NE_data %>%
  group_by(Days) %>%
  t_test(SE ~ 0) %>%
  add_significance()
SE_ttests
```

```
## # A tibble: 3 x 9
##   Days .y. group1 group2      n statistic    df          p p.signif
##   <fct> <chr> <chr> <chr>    <int>    <dbl> <dbl>    <dbl> <chr>
## 1 20    SE     1     null model    33      2.16    32 0.0381 *
## 2 40    SE     1     null model    33      4.52    32 0.0000792 ****
## 3 60    SE     1     null model    33     -1.69    32 0.1 ns
```

###Final plots BEF through time

```
BEFplots<-ggarrange(NEp, CEp, SEp, nrow=1, ncol=3, common.legend = TRUE)
BEFplots
```

