RM-ANOVA for coevolution with a seed bank

phenotypic evolution

Load organized data

Adjusting variable types for lme

In the lme models used below the fixed effects need to be specified as factors. In this experiment these are: *seed.bank and time*. However for the auto correlation specification time needs to be specified as an integer.

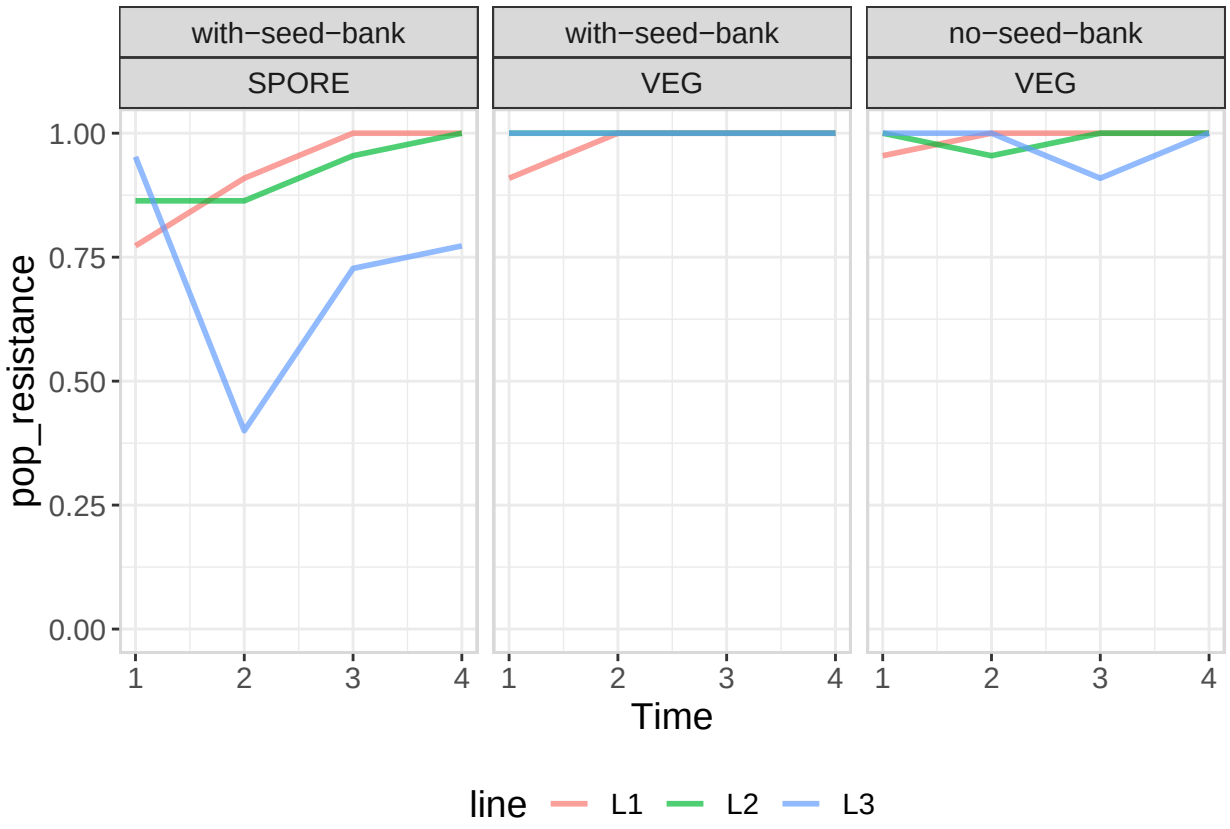
```
d <-
  d%>%
  rename(Time = t.host) %>%
  # for the lme model all fixed effects need to be factors
  mutate(time.fct=as.factor(Time))%>%
  mutate(pop=as.factor(pop))%>%
  mutate(seed.bank=as.factor(seed.bank))%>%
  #ajust factor order for seed bank
  mutate(seed.bank = fct_rev(seed.bank))
```

Select response variable to be analyzed

```
var.response <- "pop_resistance"

d <- d %>%
  mutate(response=pop_resistance ) %>%
  mutate(flask = paste0(trt,"-", line))
```

Here we analyze **pop_resistance** .

```
d%>%
ggplot(aes(x=Time, y=response))+
      geom_line(aes(group=flask,color=line), size=1, alpha=0.7)+
        facet_wrap(seed.bank~pop)+
      theme_bw()+
      panel_border()+
      theme(legend.position = "bottom",
            text=element_text(size=14))+
      ylab(var.response)+
  ylim(0,NA)
```



Test data for homogeneity of variances

This is an assumption of ANOVA tests.

Based on : https://www.datanovia.com/en/lessons/homogeneity-of-variance-test-in-r/ Using "Levene's test" that according to website is the most commonly used test for this purpose. This test has a null hypothesis of equal variance. So getting P>0.05 suggests homogenic variance.

```
# Levene's test with multiple independent variables
car::leveneTest(response ~ pop*seed.bank*line, data = d)
```

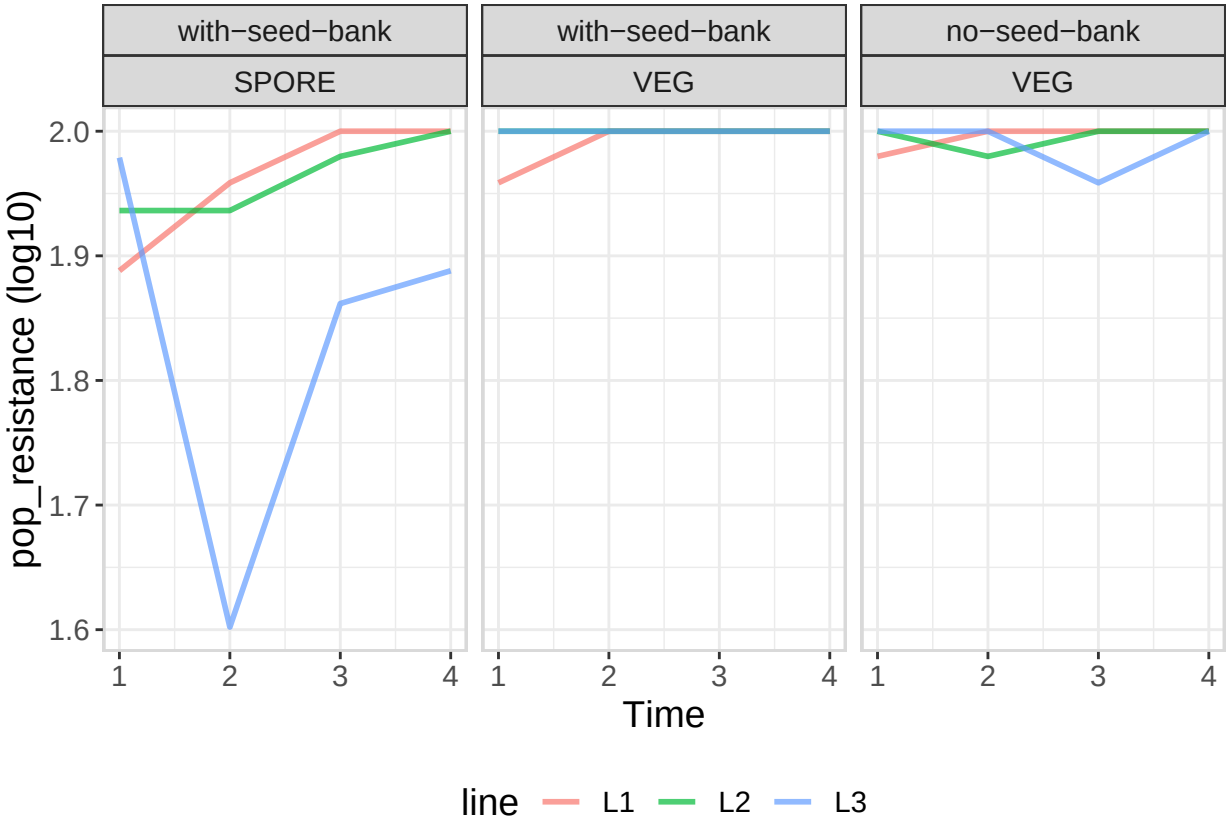
```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  8    2.45 0.0389 *
##       27
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The data does not fulfill the assumption of equal variance across test groups.

Log transform the data

Making it percent so that values remain positive

```
d <- d%>%
  mutate(log.response=log10(100*response))

d%>%
ggplot(aes(x=Time, y=log.response,group=flask))+
      geom_line(aes(group=flask,color=line), size=1, alpha=0.7)+
        facet_wrap(seed.bank~pop)+
      theme_bw()+
      panel_border()+
      theme(legend.position = "bottom",
            text=element_text(size=14))+
      ylab(paste(var.response,"(log10)"))
```



Test transformed data for homogeneity of variances

```
# Levene's test with multiple independent variables
car::leveneTest(log.response ~ pop * seed.bank * line, data = d)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value  Pr(>F)
## group  8  2.1496 0.06561 .
##       27
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The data still does not fulfill the assumption of equal variance across test groups.

Box-Cox transformation

> `powerTransform` uses the maximum likelihood-like approach of Box and Cox (1964) to select a transformation of a univariate or multivariate response for normality, linearity and/or constant variance.
> (help page for `car::powerTransform`)

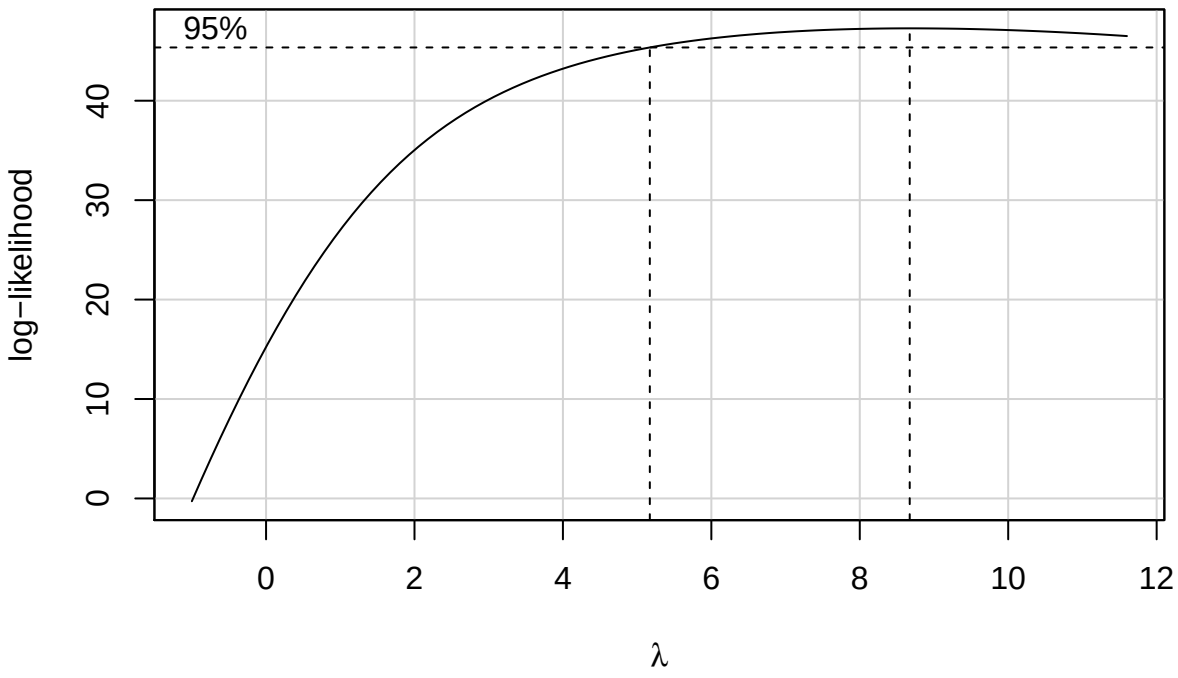
```
# Multivariate transformation to normality within levels of treatments
bx.cx <- powerTransform(response ~ pop*seed.bank*line, d)
summary(bx.cx)
```

```
## bcPower Transformation to Normality
##    Est Power Rounded Pwr Wald Lwr Bnd Wald Upr Bnd
## Y1    8.6886        8.69        4.509      12.8681
##
## Likelihood ratio test that transformation parameter is equal to 0
##  (log transformation)
##                            LRT df       pval
## LR test, lambda = (0) 64.09321  1 1.2212e-15
##
## Likelihood ratio test that no transformation is needed
##                            LRT df       pval
## LR test, lambda = (1) 40.55038  1 1.9161e-10
```

Tranformation is required, but not a simple log transformation

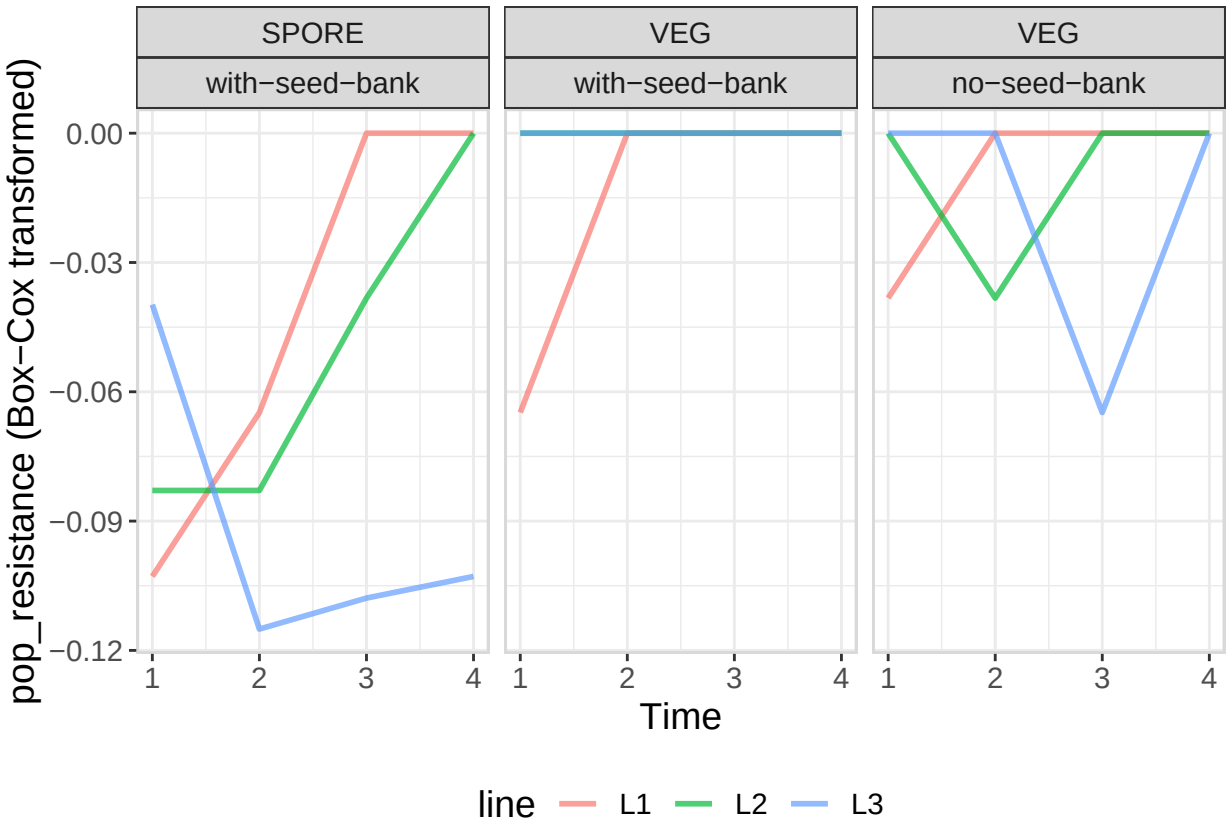
Plot the profile log-likelihood for Box-Cox transformations.

```
  with(d, boxCox(response ~ pop*seed.bank*line,
                 lambda = seq(-1, bx.cx$roundlam+3, by = 0.1),
                 family="bcPower"))
```



Transform using Box-Cox λ (rounded).

```
d <- d%>%
  mutate(bxcx.response=bcPower(response, bx.cx$roundlam))

d%>%
ggplot(aes(x=Time, y=bxcx.response))+
      geom_line(aes(group=flask,color=line), size=1, alpha=0.7)+
        facet_wrap(pop~ seed.bank)+
      theme_bw()+
      panel_border()+
      theme(legend.position = "bottom",
            text=element_text(size=14))+
      ylab(paste(var.response,"(Box-Cox transformed)"))
```



Test transformed data for homogeneity of variances

```
# Levene's test with multiple independent variables
car::leveneTest(bxcx.response ~ pop*seed.bank*line, data = d)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  8  1.5376 0.1908
##       27
```

The data now fulfills the assumption of equal variance across test groups.

#RM-ANOVA

vs. Spores in "with-seed-bank" populations

```
cur.model <- d%>%
      # compare veg and spore in with-seed-bank
      filter(seed.bank == "with-seed-bank") %>%
      lme(bxcx.response ~ pop * time.fct ,
          random = ~1|flask ,
          correlation=corARMA(form = ~ 1 | flask,  p = 1, q = 1),
```

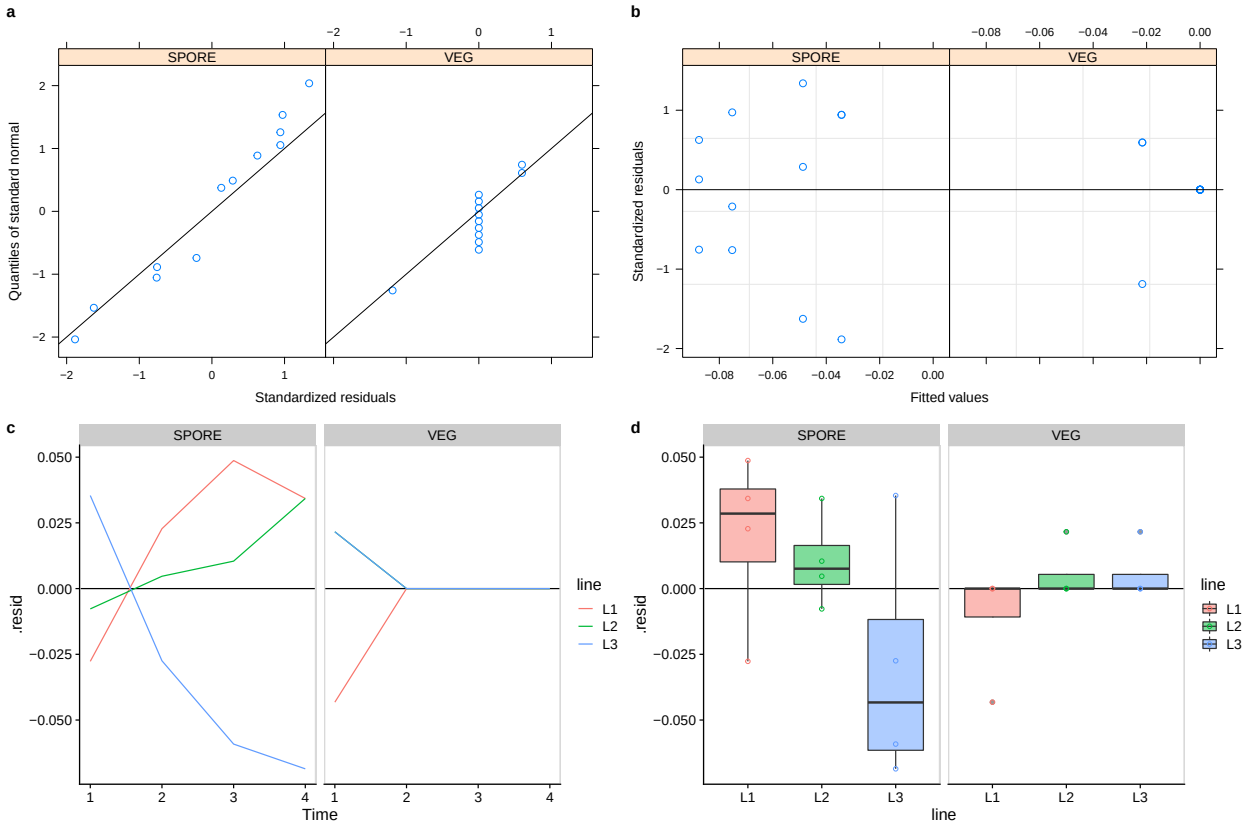
```
            data = .)


anova(cur.model)
```

```
##              numDF denDF   F-value p-value
## (Intercept)      1    14  7.785109  0.0145
## pop              1    14 12.499046  0.0033
## time.fct         3    14  1.089454  0.3859
## pop:time.fct     3    14  1.214008  0.3411
```

Distribution of model residuals

Is the model any good?

```
p1 <-
  #qqplot by seed bank
  qqnorm(cur.model,~ resid(., type = "p")|pop, abline = c(0, 1))

p2 <-
  # standardized residuals versus fitted values by seed.bank
plot(cur.model, resid(., type = "p") ~ fitted(.) | pop, abline = 0)

p3 <-
broom.mixed::augment(cur.model)%>%
  ggplot(aes(Time,.resid)) +
  geom_hline(yintercept = 0)+
  geom_line(aes(color=line))+
  facet_wrap(~pop)+
  theme_cowplot()+panel_border()

p4 <-
broom.mixed::augment(cur.model)%>%
  ggplot(aes(line,.resid)) +
  geom_hline(yintercept = 0)+
  geom_boxplot(aes(fill=line),alpha=.5, position = position_dodge(width = .9))+
  geom_point(aes(color=line), position = position_dodge(width = .9), shape=21)+
  facet_wrap(~pop)+
  theme_cowplot()+panel_border()



plot_grid(p1,p2,p3,p4, nrow = 2, labels = 'auto')
```



ok (L3 not great...)

Seed-bank effect on veg populations

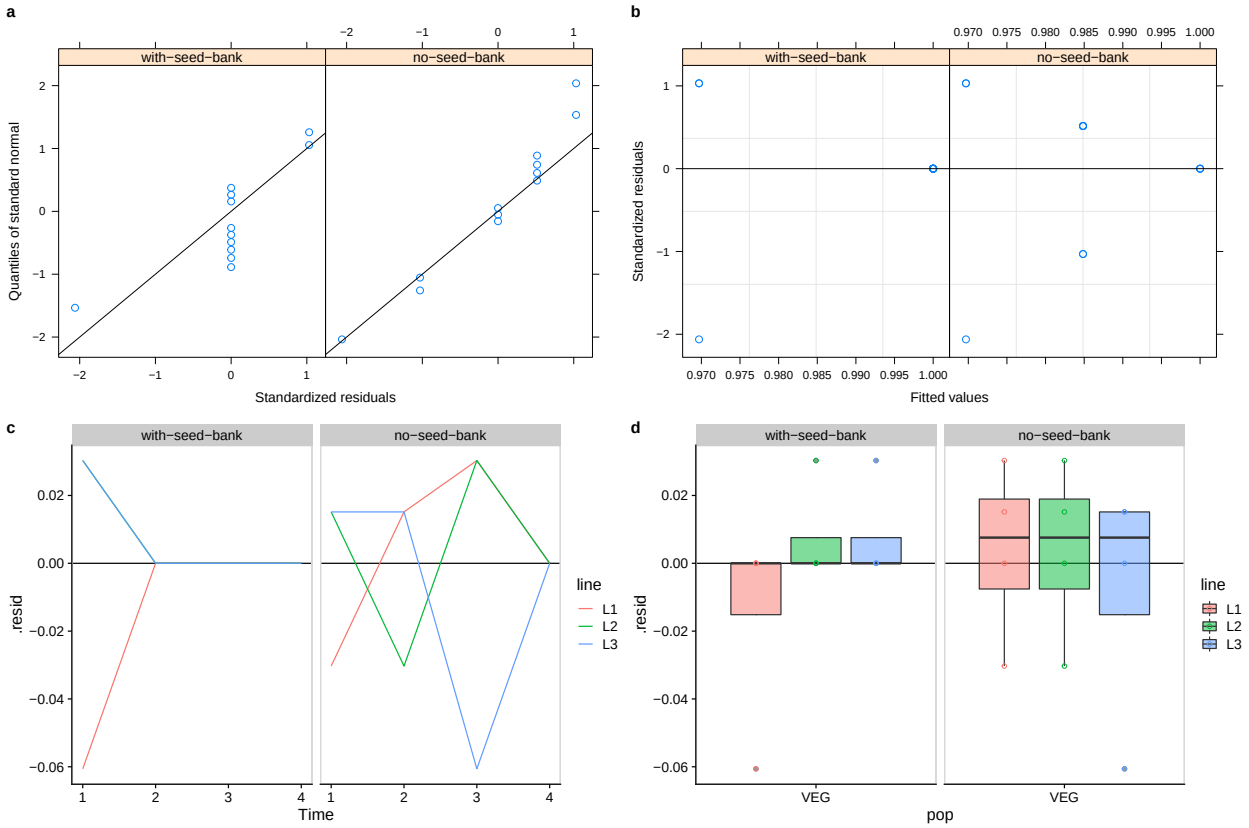
```
cur.model <- d%>%
      # compare veg and spore in with-seed-bank
      filter(pop == "VEG") %>%
      lme(response ~ seed.bank * time.fct ,
          random = ~1|flask ,
          correlation=corARMA(form = ~ 1 | flask, p=1, q=1),
            data = .)

anova(cur.model)
```

```
##                     numDF denDF  F-value p-value
## (Intercept)             1    12 56972.94  <.0001
## seed.bank               1     4     1.10  0.3544
## time.fct                3    12     0.59  0.6356
## seed.bank:time.fct      3    12     0.57  0.6471
```

Distribution of model residuals

Is the model any good?

```r
p1 <-
  #qqplot by seed bank
  qqnorm(cur.model,~ resid(., type = "p")|seed.bank, abline = c(0, 1))

p2 <-
  # standardized residuals versus fitted values by seed.bank
plot(cur.model, resid(., type = "p") ~ fitted(.) | seed.bank, abline = 0)

p3 <-
broom.mixed::augment(cur.model)%>%
  ggplot(aes(Time,.resid)) +
  geom_hline(yintercept = 0)+
  geom_line(aes(color=line))+
  facet_wrap(~seed.bank)+
  theme_cowplot()+panel_border()

p4 <-
broom.mixed::augment(cur.model)%>%
  ggplot(aes(pop,.resid)) +
  geom_hline(yintercept = 0)+
  geom_boxplot(aes(fill=line),alpha=.5, position = position_dodge(width = .9))+
  geom_point(aes(color=line), position = position_dodge(width = .9), shape=21)+
  facet_wrap(~seed.bank)+
  theme_cowplot()+panel_border()



plot_grid(p1,p2,p3,p4, nrow = 2, labels = 'auto')
```


a
with−seed−bank
no−seed−bank
Quantiles of standard normal
Standardized residuals
b
with−seed−bank
no−seed−bank
Standardized residuals
Fitted values
c
with−seed−bank
no−seed−bank
.resid
Time
line
L1
L2
L3
d
with−seed−bank
no−seed−bank
.resid
VEG
VEG
pop
line
L1
L2
L3

ok