Statistical analysis

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Data table

```
setDT(isumm)
```

Trials 1-4 (digestate treatment effects)

Subset.

```
isumm1 <- isumm[new.ID %in% as.character(1:4)]
```

Separate digestate type from treatment

```
isumm1[, dig.ID := substr(treat1, 1, 1)]
isumm1[, dig.treat := gsub('^[ABC]', '', treat1)]
isumm1[dig.treat == '', dig.treat := 'none']
isumm1[, dig.treat := relevel(factor(dig.treat), ref = 'none')]
```

Fit mixed-effects model and fixed-effects for comparison.

```
m0 <- lm(e.rel.150 ~ dig.treat * dig.ID + new.ID, data = isumm1)
m1 <- lmer(e.rel.150 ~ dig.treat + (1|dig.ID) + (1|new.ID), data = isumm1)
m1notreat <- lmer(e.rel.150 ~ (1|dig.ID) + (1|new.ID), data = isumm1)
m1nodig <- lmer(e.rel.150 ~ dig.treat + (1|new.ID), data = isumm1)
m2 <- lmer(e.rel.150 ~ dig.treat + (1|dig.ID/dig.treat) + (1|new.ID), data = isumm1)
m2b <- lmer(e.rel.150 ~ (1|dig.ID/dig.treat) + (1|new.ID), data = isumm1)
m3 <- lmer(e.rel.150 ~ treat1 + (1|new.ID), data = isumm1)
```

```
summary(m0)
```

```
##
## Call:
## lm(formula = e.rel.150 ~ dig.treat * dig.ID + new.ID, data = isumm1)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.08833 -0.01957  0.00625  0.02000  0.06763
```

```
## 
## Coefficients: (15 not defined because of singularities)
##                             Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 0.357632   0.020424  17.510  < 2e-16 ***
## dig.treat Acid             -0.126583   0.031198  -4.057 0.000205 ***
## dig.treat Dis               0.004368   0.031198   0.140 0.889299
## dig.treat Dis + acid       -0.068298   0.031198  -2.189 0.034060 *
## dig.treat MF liquid        -0.329802   0.031198 -10.571 1.56e-13 ***
## dig.treat MF slurry         0.059632   0.031198   1.911 0.062635 .
## dig.treat Sep-D            -0.325770   0.023584 -13.813  < 2e-16 ***
## dig.treat Sep-D + acid     -0.316265   0.031198 -10.137 5.70e-13 ***
## dig.treat Sep-S            -0.128618   0.028884  -4.453 5.93e-05 ***
## dig.treat Sep-S + acid     -0.119417   0.031198  -3.828 0.000414 ***
## dig.IDB                     0.122167   0.023584   5.180 5.60e-06 ***
## dig.IDC                    -0.160417   0.031198  -5.142 6.35e-06 ***
## new.ID2                    -0.016048   0.028884  -0.556 0.581353
## new.ID3                     0.126118   0.028884   4.366 7.81e-05 ***
## new.ID4                     0.035737   0.023584   1.515 0.137008
## dig.treat Acid:dig.IDB      0.185500   0.040848   4.541 4.48e-05 ***
## dig.treat Dis:dig.IDB             NA         NA      NA       NA
## dig.treat Dis + acid:dig.IDB      NA         NA      NA       NA
## dig.treat MF liquid:dig.IDB       NA         NA      NA       NA
## dig.treat MF slurry:dig.IDB       NA         NA      NA       NA
## dig.treat Sep-D:dig.IDB           NA         NA      NA       NA
## dig.treat Sep-D + acid:dig.IDB    NA         NA      NA       NA
## dig.treat Sep-S:dig.IDB    -0.072965   0.042516  -1.716 0.093326 .
## dig.treat Sep-S + acid:dig.IDB    NA         NA      NA       NA
## dig.treat Acid:dig.IDC            NA         NA      NA       NA
## dig.treat Dis:dig.IDC             NA         NA      NA       NA
## dig.treat Dis + acid:dig.IDC      NA         NA      NA       NA
## dig.treat MF liquid:dig.IDC       NA         NA      NA       NA
## dig.treat MF slurry:dig.IDC       NA         NA      NA       NA
## dig.treat Sep-D:dig.IDC     0.119370   0.040848   2.922 0.005519 **
## dig.treat Sep-D + acid:dig.IDC    NA         NA      NA       NA
## dig.treat Sep-S:dig.IDC           NA         NA      NA       NA
## dig.treat Sep-S + acid:dig.IDC    NA         NA      NA       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 0.04085 on 43 degrees of freedom
## Multiple R-squared:  0.9582, Adjusted R-squared:  0.9417
## F-statistic: 58.01 on 17 and 43 DF,  p-value: < 2.2e-16
```

```
anova(m0)
```

```
## Analysis of Variance Table
## 
## Response: e.rel.150
##                 Df  Sum Sq  Mean Sq F value    Pr(>F)
## dig.treat        9 1.27757 0.141952  85.075 < 2.2e-16 ***
## dig.ID           2 0.24833 0.124167  74.416 1.088e-14 ***
## new.ID           3 0.05598 0.018659  11.182 1.493e-05 ***
## dig.treat:dig.ID 3 0.06357 0.021189  12.699 4.453e-06 ***
## Residuals       43 0.07175 0.001669
```

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

```r
summary(m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ dig.treat + (1 | dig.ID) + (1 | new.ID)
##    Data: isumm1
##
## REML criterion at convergence: -123.9
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.2902 -0.7355  0.1204  0.5567  1.9408
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  new.ID   (Intercept) 0.002487 0.04987
##  dig.ID   (Intercept) 0.014034 0.11846
##  Residual             0.002945 0.05427
## Number of obs: 61, groups:  new.ID, 4; dig.ID, 3
##
## Fixed effects:
##                      Estimate Std. Error t value
## (Intercept)           0.41336    0.07456   5.544
## dig.treat Acid       -0.04325    0.03038  -1.424
## dig.treat Dis         0.01184    0.03921   0.302
## dig.treat Dis + acid -0.06082    0.03921  -1.551
## dig.treat MF liquid  -0.33145    0.03793  -8.740
## dig.treat MF slurry   0.05799    0.03793   1.529
## dig.treat Sep-D      -0.30086    0.02434 -12.360
## dig.treat Sep-D + acid -0.30879  0.03921  -7.875
## dig.treat Sep-S      -0.15722    0.02682  -5.862
## dig.treat Sep-S + acid -0.15216  0.03972  -3.831
##
## Correlation of Fixed Effects:
##            (Intr) dg.trA dg.trD dg.D+a dg.MFl dg.MFs dg.S-D d.S-D+a dg.S-S
## dig.tretAcd -0.058
## dig.treatDs -0.073  0.026
## dg.trtDs+ac -0.073  0.026  0.362
## dg.trtMFlqd -0.067  0.027  0.085  0.085
## dg.trtMFslr -0.067  0.027  0.085  0.085  0.318
## dig.trtSp-D -0.124  0.034  0.285  0.285  0.252  0.252
## dg.trSp-D+a -0.073  0.026  0.362  0.362  0.085  0.085  0.285
## dig.trtSp-S -0.075  0.039  0.085  0.085  0.310  0.310  0.250  0.085
## dg.trSp-S+a -0.070  0.368  0.013  0.013  0.031  0.031  0.028  0.013   0.089
```

```r
summary(m2)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ dig.treat + (1 | dig.ID/dig.treat) + (1 | new.ID)
##    Data: isumm1
##
```

```
## REML criterion at convergence: -141
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.2447 -0.5187  0.1327  0.4729  1.5858
##
## Random effects:
##  Groups           Name        Variance Std.Dev.
##  dig.treat:dig.ID (Intercept) 0.005960 0.07720
##  new.ID           (Intercept) 0.003555 0.05962
##  dig.ID           (Intercept) 0.013257 0.11514
##  Residual                     0.001676 0.04094
## Number of obs: 61, groups:  dig.treat:dig.ID, 15; new.ID, 4; dig.ID, 3
##
## Fixed effects:
##                       Estimate Std. Error t value
## (Intercept)           0.38753    0.08642   4.485
## dig.treat Acid       -0.01161    0.07746  -0.150
## dig.treat Dis         0.03652    0.10002   0.365
## dig.treat Dis + acid -0.03615    0.10002  -0.361
## dig.treat MF liquid  -0.29670    0.09969  -2.976
## dig.treat MF slurry   0.09273    0.09969   0.930
## dig.treat Sep-D      -0.27869    0.07561  -3.686
## dig.treat Sep-D + acid -0.28412  0.10002  -2.841
## dig.treat Sep-S      -0.13617    0.07648  -1.780
## dig.treat Sep-S + acid -0.10922  0.10172  -1.074
##
## Correlation of Fixed Effects:
##            (Intr) dg.trA dg.trD dg.D+a dg.MFl dg.MFs dg.S-D d.S-D+a dg.S-S
## dig.tretAcd -0.310
## dig.treatDs -0.248  0.306
## dg.trtDs+ac -0.248  0.306  0.348
## dg.trtMFlqd -0.249  0.307  0.320  0.320
## dg.trtMFslr -0.249  0.307  0.320  0.320  0.344
## dig.trtSp-D -0.327  0.299  0.313  0.313  0.312  0.312
## dg.trSp-D+a -0.248  0.306  0.348  0.348  0.320  0.320  0.313
## dig.trtSp-S -0.317  0.416  0.318  0.318  0.336  0.336  0.318  0.318
## dg.trSp-S+a -0.242  0.359  0.160  0.160  0.165  0.165  0.167  0.160   0.332
```

```r
summary(m2b)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ (1 | dig.ID/dig.treat) + (1 | new.ID)
##    Data: isumm1
##
## REML criterion at convergence: -145.6
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.2192 -0.5986  0.1588  0.4905  1.6799
##
## Random effects:
##  Groups           Name        Variance Std.Dev.
##  dig.treat:dig.ID (Intercept) 0.020448 0.14300
```

```
##  new.ID          (Intercept) 0.003628 0.06024
##  dig.ID          (Intercept) 0.016501 0.12846
##  Residual                    0.001670 0.04086
## Number of obs: 61, groups:  dig.treat:dig.ID, 15; new.ID, 4; dig.ID, 3
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)  0.28971    0.09093   3.186
```

summary(m3)

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ treat1 + (1 | new.ID)
##    Data: isumm1
##
## REML criterion at convergence: -136.7
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.1636 -0.4793  0.1600  0.4899  1.6282
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  new.ID   (Intercept) 0.003794 0.06159
##  Residual             0.001667 0.04083
## Number of obs: 61, groups:  new.ID, 4
##
## Fixed effects:
##                        Estimate Std. Error t value
## (Intercept)           0.3940833  0.0329751  11.951
## treat1A Acid         -0.1290595  0.0307427  -4.198
## treat1A Dis           0.0007752  0.0307427   0.025
## treat1A Dis + acid   -0.0718915  0.0307427  -2.338
## treat1A MF liquid    -0.3309564  0.0307427 -10.765
## treat1A MF slurry     0.0584769  0.0307427   1.902
## treat1A Sep-D        -0.3281439  0.0231922 -14.149
## treat1A Sep-D + acid -0.3198581  0.0307427 -10.404
## treat1A Sep-S        -0.1297731  0.0283938  -4.570
## treat1B               0.1245406  0.0231922   5.370
## treat1B Acid          0.1786072  0.0307427   5.810
## treat1B Sep-S        -0.0721926  0.0307427  -2.348
## treat1B Sep-S + acid  0.0002738  0.0307427   0.009
## treat1C              -0.1531926  0.0307427  -4.983
## treat1C Sep-D        -0.3595926  0.0307427 -11.697


##
## Correlation matrix not shown by default, as p = 15 > 12.
## Use print(value, correlation=TRUE)  or
##     vcov(value)        if you need it
```

AIC(m1, m2, m2b, m3)

```
##     df       AIC
```

```
## m1  13  -97.86348
## m2  14 -112.98432
## m2b  5 -135.58904
## m3  17 -102.74407
```

Check for treatment effect and interaction with likelihood ratio test.

```r
anova(m1, m1notreat, test = 'Chisq')
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: isumm1
## Models:
## m1notreat: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID)
## m1: e.rel.150 ~ dig.treat + (1 | dig.ID) + (1 | new.ID)
##           npar     AIC      BIC logLik deviance  Chisq Df Pr(>Chisq)
## m1notreat    4  -50.419  -41.975 29.209   -58.419
## m1          13 -147.357 -119.915 86.678 -173.357 114.94  9  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(m1, m1nodig, test = 'Chisq')
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: isumm1
## Models:
## m1nodig: e.rel.150 ~ dig.treat + (1 | new.ID)
## m1: e.rel.150 ~ dig.treat + (1 | dig.ID) + (1 | new.ID)
##         npar     AIC      BIC logLik deviance  Chisq Df Pr(>Chisq)
## m1nodig   12 -106.00  -80.668 64.999  -130.00
## m1        13 -147.36 -119.915 86.678  -173.36 43.359  1  4.557e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(m2, m1, test = 'Chisq')
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: isumm1
## Models:
## m1: e.rel.150 ~ dig.treat + (1 | dig.ID) + (1 | new.ID)
## m2: e.rel.150 ~ dig.treat + (1 | dig.ID/dig.treat) + (1 | new.ID)
##     npar    AIC      BIC logLik deviance  Chisq Df Pr(>Chisq)
## m1   13 -147.36 -119.92 86.678  -173.36
## m2   14 -152.17 -122.62 90.085  -180.17 6.8136  1   0.009047 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(m2b, m1, test = 'Chisq')
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: isumm1
## Models:
## m2b: e.rel.150 ~ (1 | dig.ID/dig.treat) + (1 | new.ID)
## m1: e.rel.150 ~ dig.treat + (1 | dig.ID) + (1 | new.ID)
##      npar    AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## m2b    5 -138.76 -128.20 74.379  -148.76
## m1    13 -147.36 -119.92 86.678  -173.36 24.599  8   0.001817 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(m2b, m2, test = 'Chisq')
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: isumm1
## Models:
## m2b: e.rel.150 ~ (1 | dig.ID/dig.treat) + (1 | new.ID)
## m2: e.rel.150 ~ dig.treat + (1 | dig.ID/dig.treat) + (1 | new.ID)
##      npar    AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## m2b    5 -138.76 -128.20 74.379  -148.76
## m2    14 -152.17 -122.62 90.085  -180.17 31.413  9  0.0002514 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Plot to check treatment effect and interaction.

```
ggplot(isumm1, aes(dig.treat, e.rel.150, colour = dig.ID)) +
  geom_jitter(height = 0) +
  theme_bw()
```
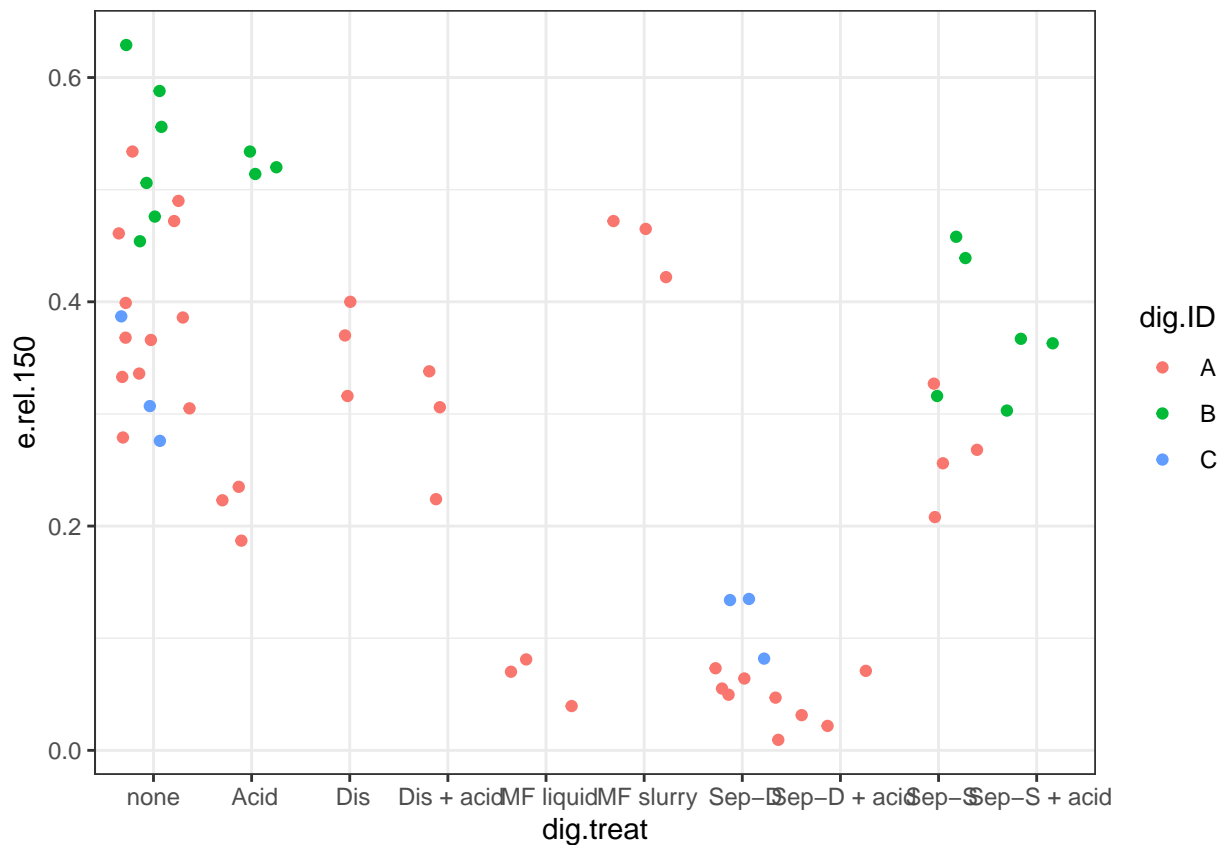
```
table(isumm1[, .(dig.ID, dig.treat)])
```

```
##       dig.treat
## dig.ID none  Acid  Dis  Dis + acid  MF liquid  MF slurry  Sep-D  Sep-D + acid
##      A   12     3    3           3          3          3      6             3
##      B    6     3    0           0          0          0      0             0
##      C    3     0    0           0          0          0      3             0
##       dig.treat
## dig.ID  Sep-S  Sep-S + acid
##      A      4             0
##      B      3             3
##      C      0             0
```

Major imbalance. Get marginal means.

```
emmeans(m3, 'treat1')
```

```
##   treat1        emmean      SE   df lower.CL  upper.CL
##   A             0.3941  0.0330 3.28   0.2941     0.494
##   A Acid        0.2650  0.0423 7.90   0.1672     0.363
##   A Dis         0.3949  0.0423 7.90   0.2970     0.493
##   A Dis + acid  0.3222  0.0423 7.90   0.2244     0.420
##   A MF liquid   0.0631  0.0423 7.90  -0.0347     0.161
##   A MF slurry   0.4526  0.0423 7.90   0.3547     0.550
##   A Sep-D       0.0659  0.0370 4.85  -0.0301     0.162
```

```
##  A Sep-D + acid 0.0742 0.0423 7.90  -0.0236    0.172
##  A Sep-S        0.2643 0.0407 6.76   0.1675    0.361
##  B              0.5186 0.0370 4.85   0.4226    0.615
##  B Acid         0.5727 0.0423 7.90   0.4748    0.671
##  B Sep-S        0.3219 0.0423 7.90   0.2240    0.420
##  B Sep-S + acid 0.3944 0.0423 7.90   0.2965    0.492
##  C              0.2409 0.0423 7.90   0.1430    0.339
##  C Sep-D        0.0345 0.0423 7.90  -0.0634    0.132
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
```

```
mmeans1_4 <- emmeans(m3, 'treat1')
mmeans1_4
```

```
##  treat1         emmean     SE   df lower.CL upper.CL
##  A              0.3941 0.0330 3.28   0.2941    0.494
##  A Acid         0.2650 0.0423 7.90   0.1672    0.363
##  A Dis          0.3949 0.0423 7.90   0.2970    0.493
##  A Dis + acid   0.3222 0.0423 7.90   0.2244    0.420
##  A MF liquid    0.0631 0.0423 7.90  -0.0347    0.161
##  A MF slurry    0.4526 0.0423 7.90   0.3547    0.550
##  A Sep-D        0.0659 0.0370 4.85  -0.0301    0.162
##  A Sep-D + acid 0.0742 0.0423 7.90  -0.0236    0.172
##  A Sep-S        0.2643 0.0407 6.76   0.1675    0.361
##  B              0.5186 0.0370 4.85   0.4226    0.615
##  B Acid         0.5727 0.0423 7.90   0.4748    0.671
##  B Sep-S        0.3219 0.0423 7.90   0.2240    0.420
##  B Sep-S + acid 0.3944 0.0423 7.90   0.2965    0.492
##  C              0.2409 0.0423 7.90   0.1430    0.339
##  C Sep-D        0.0345 0.0423 7.90  -0.0634    0.132
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
```

```
pairs(mmeans1_4)
```

```
##  contrast                   estimate     SE   df t.ratio p.value
##  A - A Acid                 0.129059 0.0313 45.0   4.118  0.0117
##  A - A Dis                 -0.000775 0.0313 45.0  -0.025  1.0000
##  A - (A Dis + acid)         0.071891 0.0313 45.0   2.294  0.5999
##  A - A MF liquid            0.330956 0.0313 45.0  10.559  <.0001
##  A - A MF slurry           -0.058477 0.0313 45.0  -1.866  0.8571
##  A - (A Sep-D)              0.328144 0.0237 45.2  13.854  <.0001
##  A - (A Sep-D + acid)       0.319858 0.0313 45.0  10.205  <.0001
##  A - (A Sep-S)              0.129773 0.0290 45.2   4.468  0.0041
##  A - B                     -0.124541 0.0237 45.2  -5.258  0.0003
##  A - B Acid                -0.178607 0.0313 45.0  -5.699  0.0001
##  A - (B Sep-S)              0.072193 0.0313 45.0   2.303  0.5934
##  A - (B Sep-S + acid)      -0.000274 0.0313 45.0  -0.009  1.0000
##  A - C                      0.153193 0.0313 45.0   4.888  0.0011
##  A - (C Sep-D)              0.359593 0.0313 45.0  11.473  <.0001
##  A Acid - A Dis            -0.129835 0.0444 45.8  -2.921  0.2252
```

```
## A Acid - (A Dis + acid)         -0.057168 0.0444 45.8   -1.286   0.9922
## A Acid - A MF liquid             0.201897 0.0444 45.8    4.542   0.0032
## A Acid - A MF slurry            -0.187536 0.0444 45.8   -4.219   0.0086
## A Acid - (A Sep-D)               0.199084 0.0394 45.9    5.050   0.0006
## A Acid - (A Sep-D + acid)        0.190799 0.0444 45.8    4.293   0.0069
## A Acid - (A Sep-S)               0.000714 0.0429 45.9    0.017   1.0000
## A Acid - B                      -0.253600 0.0313 43.8   -8.114  <.0001
## A Acid - B Acid                 -0.307667 0.0333 43.0   -9.229  <.0001
## A Acid - (B Sep-S)              -0.056867 0.0411 44.7   -1.385   0.9846
## A Acid - (B Sep-S + acid)       -0.129333 0.0333 43.0   -3.880   0.0237
## A Acid - C                       0.024133 0.0411 44.7    0.588   1.0000
## A Acid - (C Sep-D)               0.230533 0.0411 44.7    5.615   0.0001
## A Dis - (A Dis + acid)           0.072667 0.0333 43.0    2.180   0.6764
## A Dis - A MF liquid              0.331732 0.0411 44.7    8.081  <.0001
## A Dis - A MF slurry             -0.057702 0.0411 44.7   -1.406   0.9825
## A Dis - (A Sep-D)                0.328919 0.0313 43.8   10.523  <.0001
## A Dis - (A Sep-D + acid)         0.320633 0.0333 43.0    9.618  <.0001
## A Dis - (A Sep-S)                0.130548 0.0393 44.9    3.320   0.0959
## A Dis - B                       -0.123765 0.0394 45.9   -3.139   0.1433
## A Dis - B Acid                  -0.177832 0.0444 45.8   -4.001   0.0161
## A Dis - (B Sep-S)                0.072968 0.0444 45.8    1.642   0.9395
## A Dis - (B Sep-S + acid)         0.000501 0.0444 45.8    0.011   1.0000
## A Dis - C                        0.153968 0.0444 45.8    3.464   0.0673
## A Dis - (C Sep-D)                0.360368 0.0444 45.8    8.108  <.0001
## (A Dis + acid) - A MF liquid     0.259065 0.0411 44.7    6.310  <.0001
## (A Dis + acid) - A MF slurry    -0.130368 0.0411 44.7   -3.176   0.1332
## (A Dis + acid) - (A Sep-D)       0.256252 0.0313 43.8    8.198  <.0001
## (A Dis + acid) - (A Sep-D + acid) 0.247967 0.0333 43.0   7.439  <.0001
## (A Dis + acid) - (A Sep-S)       0.057882 0.0393 44.9    1.472   0.9741
## (A Dis + acid) - B              -0.196432 0.0394 45.9   -4.983   0.0008
## (A Dis + acid) - B Acid         -0.250499 0.0444 45.8   -5.636   0.0001
## (A Dis + acid) - (B Sep-S)       0.000301 0.0444 45.8    0.007   1.0000
## (A Dis + acid) - (B Sep-S + acid) -0.072165 0.0444 45.8 -1.624   0.9442
## (A Dis + acid) - C               0.081301 0.0444 45.8    1.829   0.8737
## (A Dis + acid) - (C Sep-D)       0.287701 0.0444 45.8    6.473  <.0001
## A MF liquid - A MF slurry       -0.389433 0.0333 43.0  -11.682  <.0001
## A MF liquid - (A Sep-D)         -0.002812 0.0313 43.8   -0.090   1.0000
## A MF liquid - (A Sep-D + acid)  -0.011098 0.0411 44.7   -0.270   1.0000
## A MF liquid - (A Sep-S)         -0.201183 0.0312 43.0   -6.452  <.0001
## A MF liquid - B                 -0.455497 0.0394 45.9  -11.554  <.0001
## A MF liquid - B Acid            -0.509564 0.0444 45.8  -11.465  <.0001
## A MF liquid - (B Sep-S)         -0.258764 0.0444 45.8   -5.822   0.0001
## A MF liquid - (B Sep-S + acid)  -0.331230 0.0444 45.8   -7.452  <.0001
## A MF liquid - C                 -0.177764 0.0444 45.8   -4.000   0.0162
## A MF liquid - (C Sep-D)          0.028636 0.0444 45.8    0.644   1.0000
## A MF slurry - (A Sep-D)          0.386621 0.0313 43.8   12.369  <.0001
## A MF slurry - (A Sep-D + acid)   0.378335 0.0411 44.7    9.216  <.0001
## A MF slurry - (A Sep-S)          0.188250 0.0312 43.0    6.037  <.0001
## A MF slurry - B                 -0.066064 0.0394 45.9   -1.676   0.9298
## A MF slurry - B Acid            -0.120130 0.0444 45.8   -2.703   0.3354
## A MF slurry - (B Sep-S)          0.130669 0.0444 45.8    2.940   0.2170
## A MF slurry - (B Sep-S + acid)   0.058203 0.0444 45.8    1.310   0.9908
## A MF slurry - C                  0.211670 0.0444 45.8    4.762   0.0016
## A MF slurry - (C Sep-D)          0.418069 0.0444 45.8    9.406  <.0001
```

```
## (A Sep-D) - (A Sep-D + acid)         -0.008286 0.0313 43.8  -0.265  1.0000
## (A Sep-D) - (A Sep-S)                 -0.198371 0.0289 43.9  -6.852 <.0001
## (A Sep-D) - B                         -0.452685 0.0337 46.0 -13.450 <.0001
## (A Sep-D) - B Acid                    -0.506751 0.0394 45.9 -12.854 <.0001
## (A Sep-D) - (B Sep-S)                 -0.255951 0.0394 45.9  -6.493 <.0001
## (A Sep-D) - (B Sep-S + acid)          -0.328418 0.0394 45.9  -8.331 <.0001
## (A Sep-D) - C                         -0.174951 0.0394 45.9  -4.438  0.0045
## (A Sep-D) - (C Sep-D)                  0.031449 0.0394 45.9   0.798  1.0000
## (A Sep-D + acid) - (A Sep-S)          -0.190085 0.0393 44.9  -4.834  0.0013
## (A Sep-D + acid) - B                  -0.444399 0.0394 45.9 -11.273 <.0001
## (A Sep-D + acid) - B Acid             -0.498465 0.0444 45.8 -11.215 <.0001
## (A Sep-D + acid) - (B Sep-S)          -0.247666 0.0444 45.8  -5.572  0.0001
## (A Sep-D + acid) - (B Sep-S + acid)   -0.320132 0.0444 45.8  -7.203 <.0001
## (A Sep-D + acid) - C                  -0.166665 0.0444 45.8  -3.750  0.0322
## (A Sep-D + acid) - (C Sep-D)           0.039734 0.0444 45.8   0.894  0.9998
## (A Sep-S) - B                         -0.254314 0.0376 46.0  -6.760 <.0001
## (A Sep-S) - B Acid                    -0.308380 0.0429 45.9  -7.196 <.0001
## (A Sep-S) - (B Sep-S)                 -0.057580 0.0429 45.9  -1.344  0.9884
## (A Sep-S) - (B Sep-S + acid)          -0.130047 0.0429 45.9  -3.035  0.1791
## (A Sep-S) - C                          0.023419 0.0429 45.9   0.546  1.0000
## (A Sep-S) - (C Sep-D)                  0.229820 0.0429 45.9   5.363  0.0002
## B - B Acid                            -0.054067 0.0313 43.8  -1.730  0.9119
## B - (B Sep-S)                          0.196733 0.0313 43.8   6.294 <.0001
## B - (B Sep-S + acid)                   0.124267 0.0313 43.8   3.976  0.0179
## B - C                                  0.277733 0.0313 43.8   8.886 <.0001
## B - (C Sep-D)                          0.484133 0.0313 43.8  15.489 <.0001
## B Acid - (B Sep-S)                     0.250800 0.0411 44.7   6.109 <.0001
## B Acid - (B Sep-S + acid)              0.178333 0.0333 43.0   5.350  0.0003
## B Acid - C                             0.331800 0.0411 44.7   8.082 <.0001
## B Acid - (C Sep-D)                     0.538200 0.0411 44.7  13.110 <.0001
## (B Sep-S) - (B Sep-S + acid)          -0.072467 0.0411 44.7  -1.765  0.8994
## (B Sep-S) - C                          0.081000 0.0333 43.0   2.430  0.5079
## (B Sep-S) - (C Sep-D)                  0.287400 0.0333 43.0   8.621 <.0001
## (B Sep-S + acid) - C                   0.153467 0.0411 44.7   3.738  0.0337
## (B Sep-S + acid) - (C Sep-D)           0.359866 0.0411 44.7   8.766 <.0001
## C - (C Sep-D)                          0.206400 0.0333 43.0   6.192 <.0001
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 15 estimates
```

Get letters.

```
letters1_4 <- cld(object = mmeans1_4,
                  adjust = "Tukey",
                  Letters = letters,
                  alpha = 0.05)
```

```
## Note: adjust = "tukey" was changed to "sidak"
## because "tukey" is only appropriate for one set of pairwise comparisons
```

```
letters1_4
```

```
##  treat1          emmean    SE   df lower.CL upper.CL .group
```

```
##  C Sep-D        0.0345 0.0423 7.90  -0.1402    0.209  a
##  A MF liquid    0.0631 0.0423 7.90  -0.1115    0.238  a
##  A Sep-D        0.0659 0.0370 4.85  -0.1313    0.263  a
##  A Sep-D + acid 0.0742 0.0423 7.90  -0.1004    0.249  a
##  C              0.2409 0.0423 7.90   0.0662    0.416   b
##  A Sep-S        0.2643 0.0407 6.76   0.0853    0.443   bc
##  A Acid         0.2650 0.0423 7.90   0.0904    0.440   b
##  B Sep-S        0.3219 0.0423 7.90   0.1472    0.497   bcd
##  A Dis + acid   0.3222 0.0423 7.90   0.1475    0.497   bcd
##  A              0.3941 0.0330 3.28   0.1430    0.645    d
##  B Sep-S + acid 0.3944 0.0423 7.90   0.2197    0.569    cd
##  A Dis          0.3949 0.0423 7.90   0.2202    0.570   bcde
##  A MF slurry    0.4526 0.0423 7.90   0.2779    0.627    def
##  B              0.5186 0.0370 4.85   0.3214    0.716     ef
##  B Acid         0.5727 0.0423 7.90   0.3980    0.747      f
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Conf-level adjustment: sidak method for 15 estimates
## P value adjustment: tukey method for comparing a family of 15 estimates
## significance level used: alpha = 0.05
## NOTE: If two or more means share the same grouping symbol,
##       then we cannot show them to be different.
##       But we also did not show them to be the same.
```

Sort letters by treat1 and replace mmeans.

```
mmeans1_4 <- letters1_4[order(letters1_4$treat1), ]
```

And get a column for copy/paste into paper.

```
mmeans1_4$tabval <- paste(round(100 * mmeans1_4$emmean, 1), gsub(' ', '', mmeans1_4$.group))
```
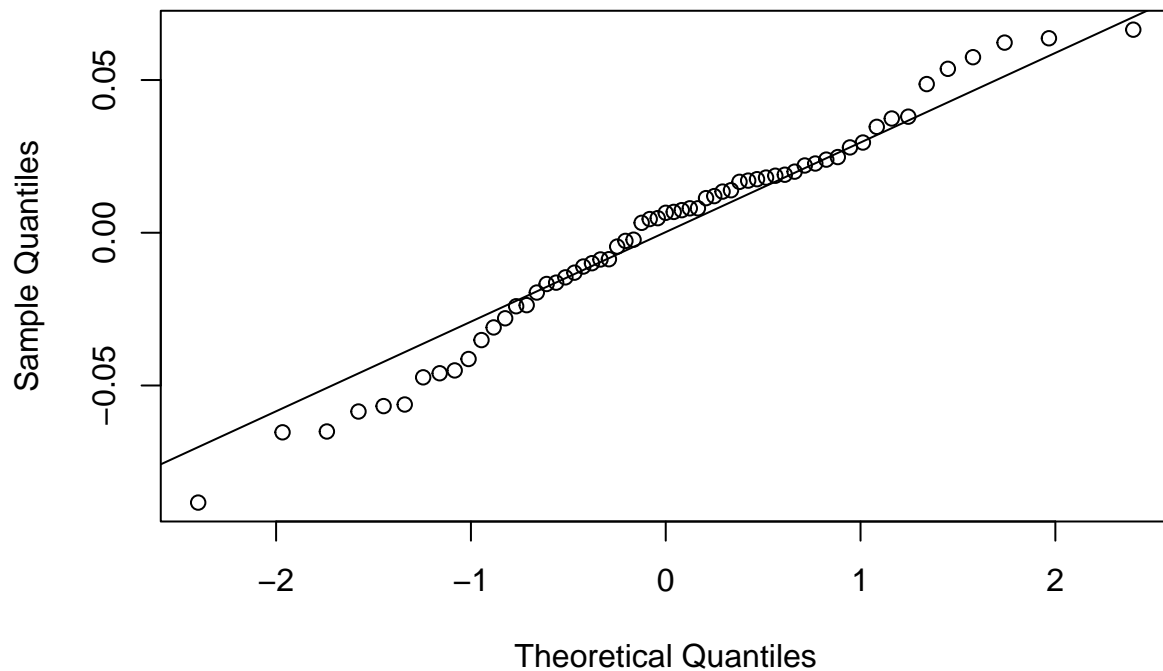
Residuals.

```
plot(fitted(m3), resid(m3))
abline(0,0)
```
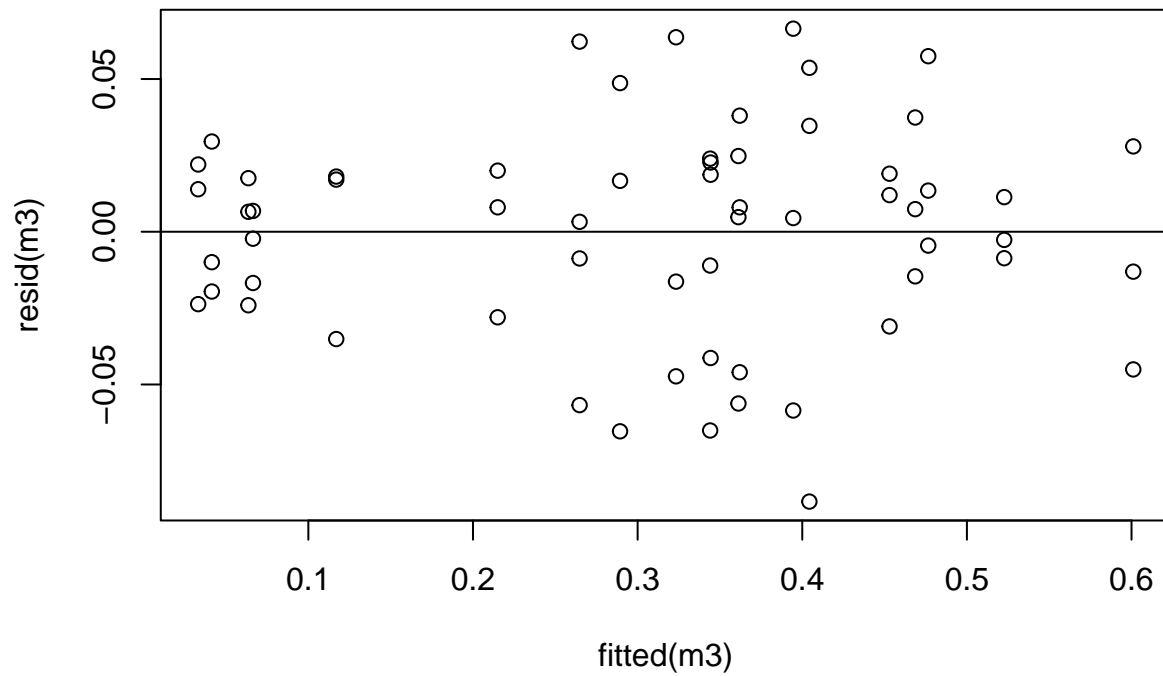
```
qqnorm(resid(m3))
qqline(resid(m3))
```

Normal Q–Q Plot

More on trials 1-4, DM and pH effects

Look for DM and pH explanation of digestate variability.

```
dref <- isumm1[dig.treat == 'none', ]
m4 <- lmer(e.rel.150 ~ (1|dig.ID) + (1|new.ID), data = dref)
m5 <- lmer(e.rel.150 ~ (1|dig.ID) + (1|new.ID) + man.dm + man.ph, data = dref)
```

summary(m4)

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID)
##    Data: dref
##
## REML criterion at convergence: -49.9
##
## Scaled residuals:
##      Min      1Q   Median       3Q      Max
## -1.47340 -0.47349  0.02298  0.60460  1.39387
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  new.ID   (Intercept) 0.003812 0.06174
##  dig.ID   (Intercept) 0.018496 0.13600
##  Residual             0.002045 0.04523
## Number of obs: 21, groups:  new.ID, 4; dig.ID, 3
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)  0.38646    0.08562   4.514
```

summary(m5)

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID) + man.dm + man.ph
##    Data: dref
##
## REML criterion at convergence: -48.4
##
## Scaled residuals:
##      Min      1Q   Median       3Q      Max
## -1.42953 -0.58352  0.01923  0.62873  1.32172
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  new.ID   (Intercept) 0.001349 0.03673
##  dig.ID   (Intercept) 0.001390 0.03729
##  Residual             0.002064 0.04543
## Number of obs: 21, groups:  new.ID, 4; dig.ID, 3
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)  1.82322    0.61703   2.955
## man.dm       0.04232    0.01132   3.740
## man.ph      -0.20688    0.07496  -2.760
##
```

```
## Correlation of Fixed Effects:
##        (Intr) man.dm
## man.dm -0.145
## man.ph -0.992  0.032
```

```
AIC(m4, m5)
```

```
##    df       AIC
## m4  4 -41.88321
## m5  6 -36.39417
```

```
anova(m4, m5, test = 'Chisq')
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: dref
## Models:
## m4: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID)
## m5: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID) + man.dm + man.ph
##    npar     AIC     BIC logLik deviance Chisq Df Pr(>Chisq)
## m4    4 -45.128 -40.950 26.564  -53.128
## m5    6 -54.238 -47.971 33.119  -66.238 13.11  2   0.001423 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

I do not like how AIC difference flips with ML and REML! But see reduction in dig.ID standard deviation when DM and pH are included. And see likelihood ratio tests below.

```
m5noph <- lmer(e.rel.150 ~ (1|dig.ID) + (1|new.ID) + man.dm, data = dref)
m5nodm <- lmer(e.rel.150 ~ (1|dig.ID) + (1|new.ID) + man.ph, data = dref)
```

```
summary(m5noph)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID) + man.dm
##    Data: dref
##
## REML criterion at convergence: -46.9
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.4247 -0.5232  0.1227  0.5865  1.5221
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  new.ID   (Intercept) 0.004851 0.06965
##  dig.ID   (Intercept) 0.004059 0.06371
##  Residual             0.002048 0.04526
## Number of obs: 21, groups:  new.ID, 4; dig.ID, 3
##
## Fixed effects:
```

```
##              Estimate Std. Error t value
## (Intercept)  0.10914    0.12057   0.905
## man.dm       0.04611    0.01783   2.586
##
## Correlation of Fixed Effects:
##        (Intr)
## man.dm -0.900
```

summary(m5nodm)

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID) + man.ph
##    Data: dref
##
## REML criterion at convergence: -49.8
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.57949 -0.48978 -0.01658  0.70174  1.31183
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  new.ID   (Intercept) 0.002640 0.05138
##  dig.ID   (Intercept) 0.014155 0.11898
##  Residual             0.001972 0.04440
## Number of obs: 21, groups:  new.ID, 4; dig.ID, 3
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)  1.62305    0.68550   2.368
## man.ph      -0.15157    0.08363  -1.812
##
## Correlation of Fixed Effects:
##        (Intr)
## man.ph -0.994
```

anova(m5, m5noph, test = 'Chisq')

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: dref
## Models:
## m5noph: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID) + man.dm
## m5: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID) + man.dm + man.ph
##        npar     AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## m5noph    5 -47.936 -42.713 28.968  -57.936
## m5        6 -54.238 -47.971 33.119  -66.238 8.3024  1   0.003959 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(m5, m5nodm, test = 'Chisq')
```

```
## refitting model(s) with ML (instead of REML)
```

```
## Data: dref
## Models:
## m5nodm: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID) + man.ph
## m5: e.rel.150 ~ (1 | dig.ID) + (1 | new.ID) + man.dm + man.ph
##        npar     AIC     BIC logLik deviance  Chisq Df Pr(>Chisq)
## m5nodm    5 -46.496 -41.274 28.248  -56.496
## m5        6 -54.238 -47.971 33.119  -66.238 9.7418  1   0.001801 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```
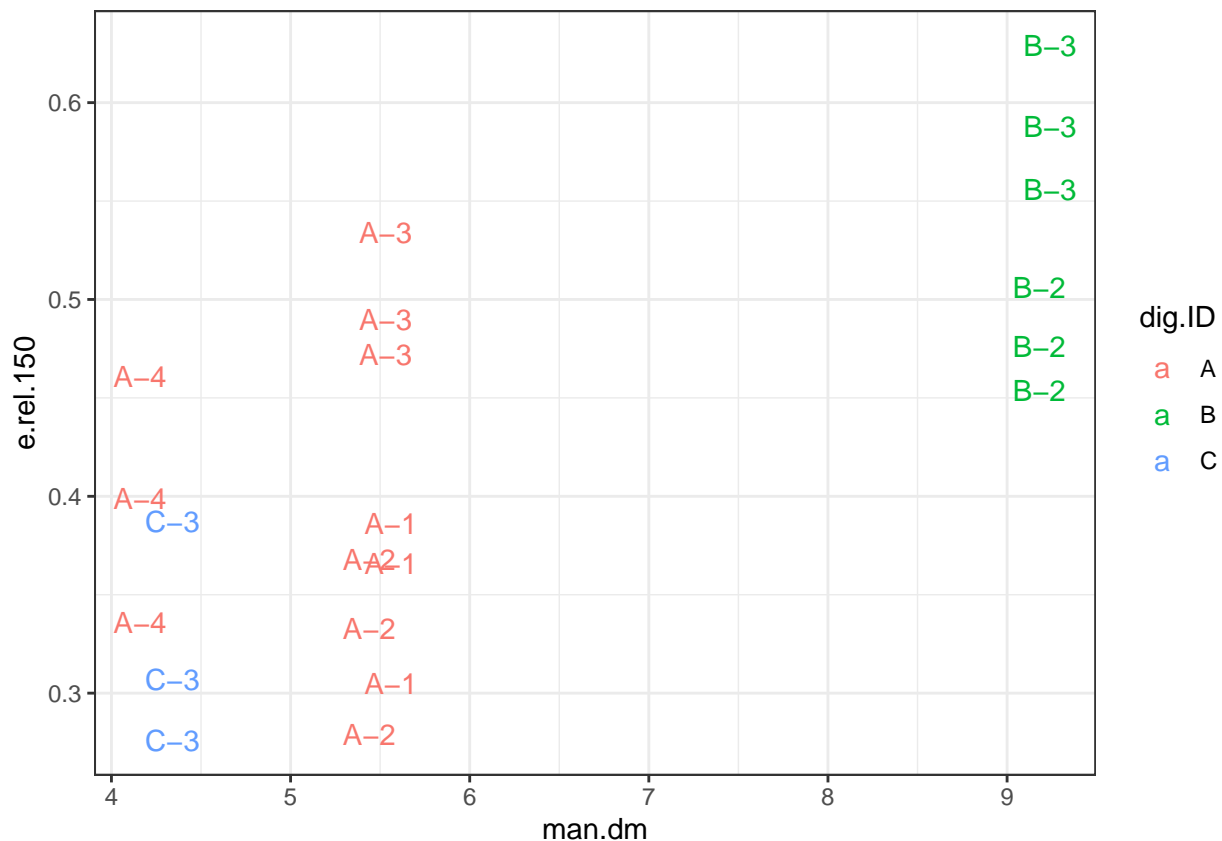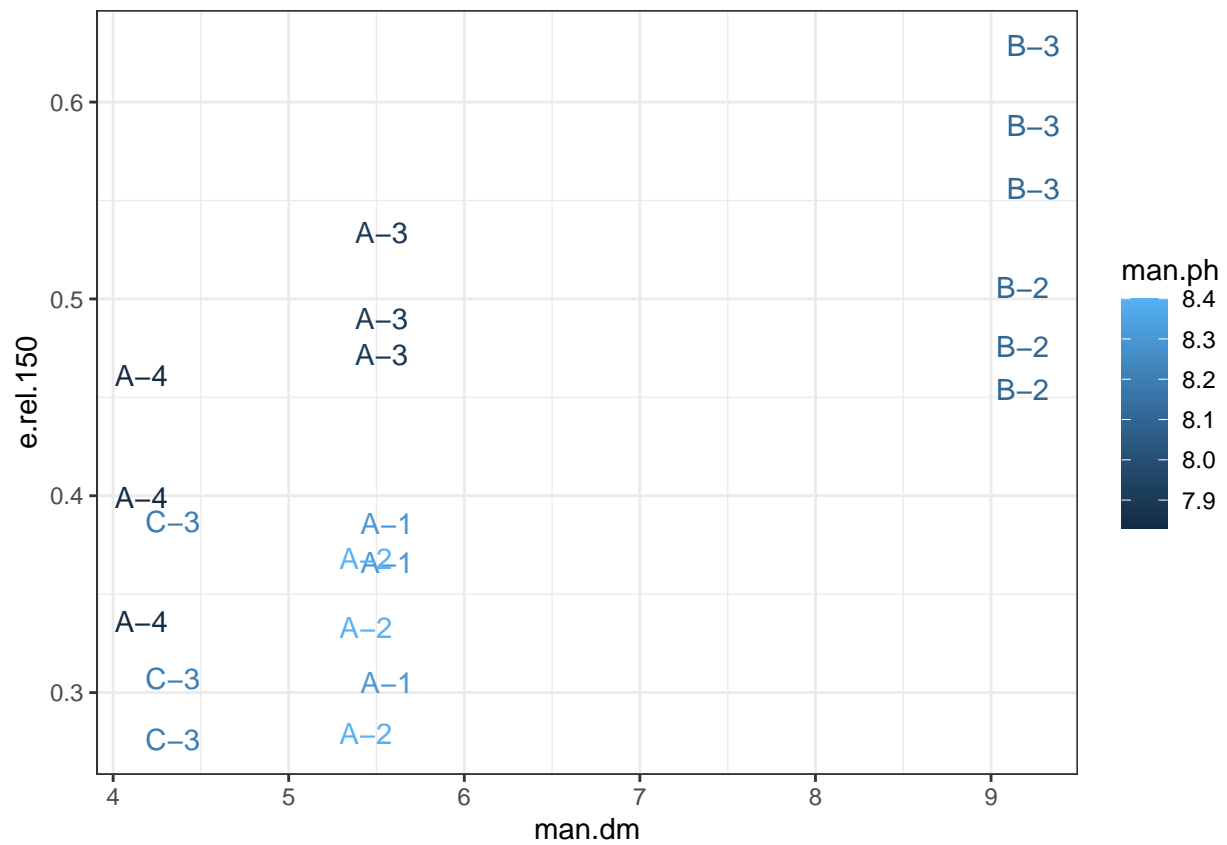
Plots to look at apparent effects.

```
ggplot(dref, aes(man.dm, e.rel.150, colour = dig.ID)) +
  geom_text(aes(label = paste0(dig.ID, '-', new.ID))) +
  theme_bw()
```



```
ggplot(dref, aes(man.dm, e.rel.150, colour = man.ph)) +
  geom_text(aes(label = paste0(dig.ID, '-', new.ID))) +
  theme_bw()
```

```
ggplot(dref, aes(man.ph, e.rel.150, colour = dig.ID)) +
  geom_text(aes(label = paste0(dig.ID, '-', new.ID))) +
  theme_bw()
```
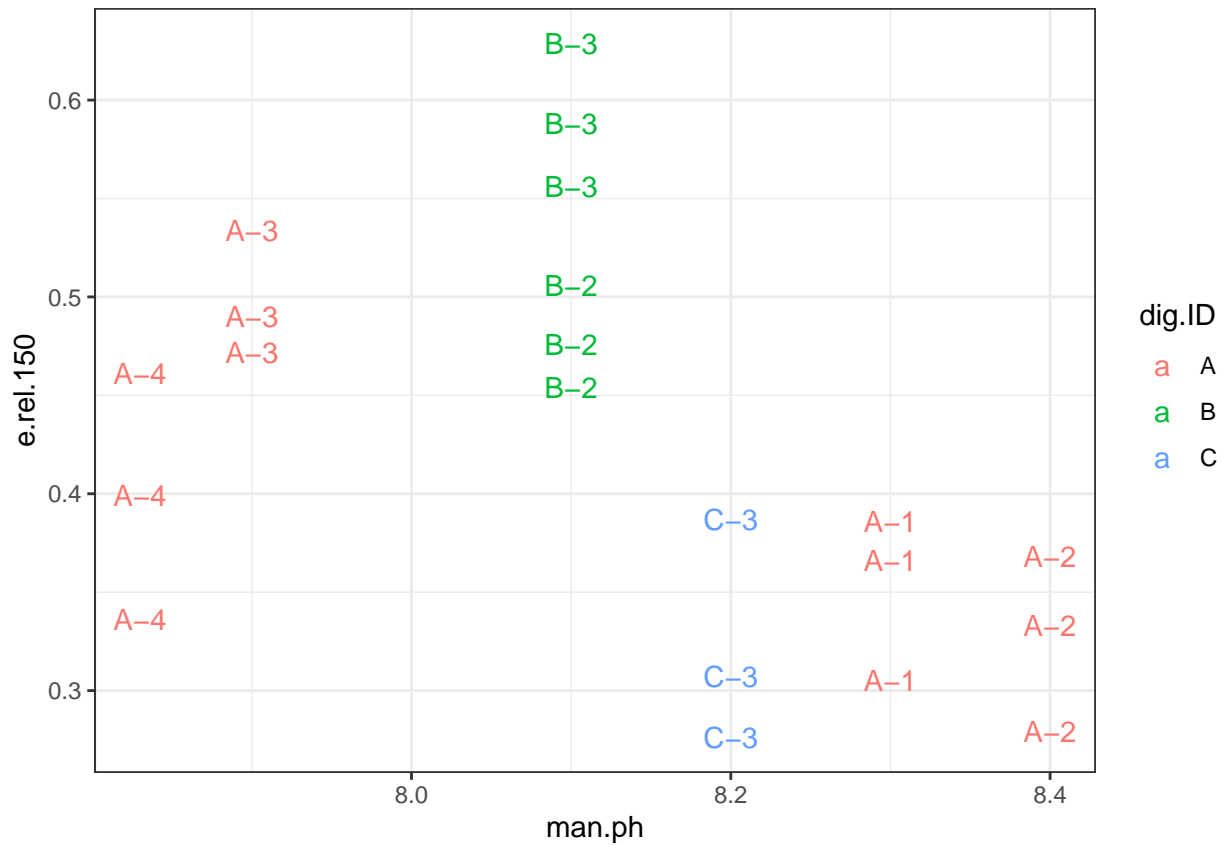
Trials 5-10 (app tech on winter wheat)

Subset.

```
isumm1 <- droplevels(isumm[new.ID %in% c('5', '6', '7', '8', '9', '10') & !treat1 %in% c('TH-4', 'TS1-4
```
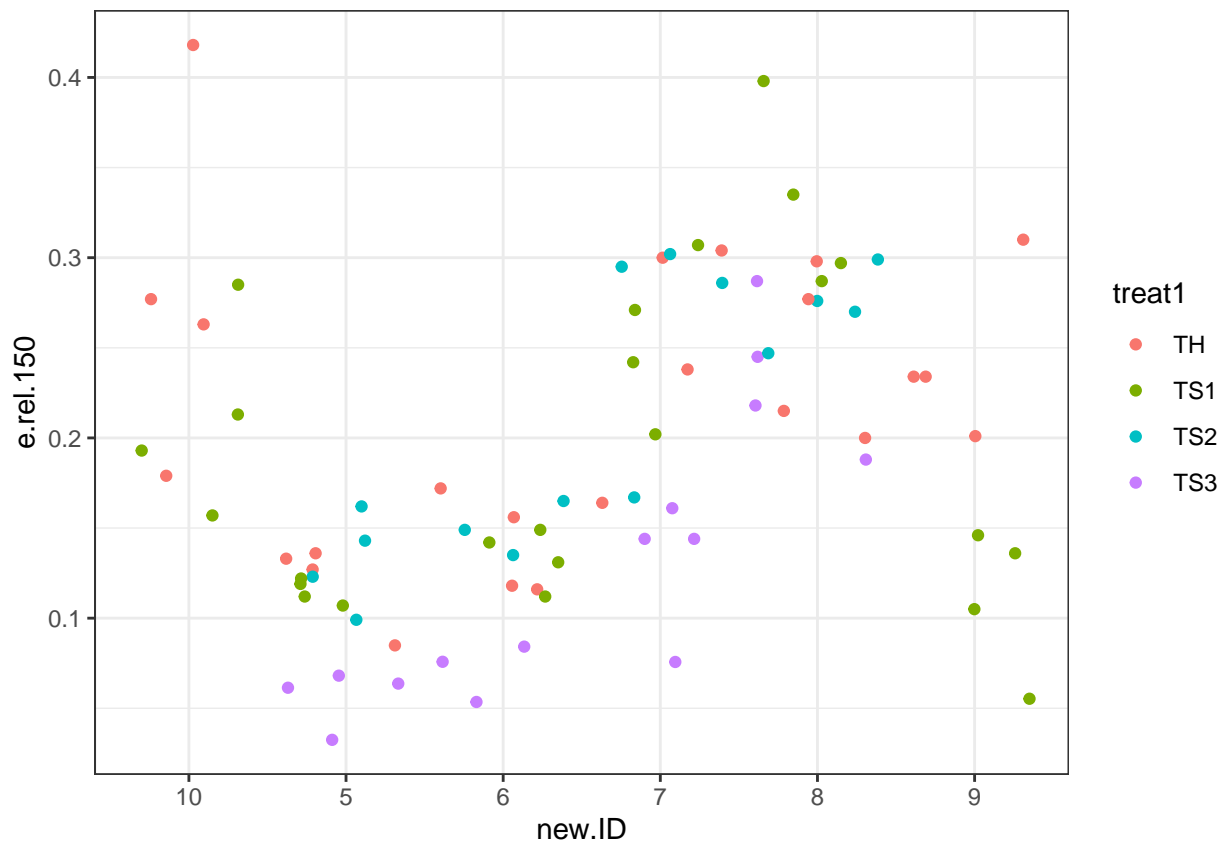
```
table(isumm1[, .(new.ID, treat1)])
```

```
##       treat1
## new.ID TH TS1 TS2 TS3
##     10  4   4   0   0
##      5  4   4   4   4
##      6  4   4   3   3
##      7  4   4   4   4
##      8  4   4   4   4
##      9  4   4   0   0
```

Close to balanced.

```
ggplot(isumm1, aes(new.ID, e.rel.150, colour = treat1)) +
  geom_jitter(height = 0) +
  theme_bw()
```

Fit mixed-effects model.

```r
m1 <- lmer(e.rel.150 ~ treat1 + (1|new.ID), data = isumm1)
```

```r
summary(m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ treat1 + (1 | new.ID)
##    Data: isumm1
##
## REML criterion at convergence: -203.3
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.2137 -0.4155  0.0176  0.3447  3.1690
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  new.ID   (Intercept) 0.004217 0.06494
##  Residual             0.002613 0.05112
## Number of obs: 78, groups:  new.ID, 6
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)  0.214788   0.028492   7.539
## treat1TS1   -0.022150   0.014756  -1.501
## treat1TS2   -0.006375   0.017576  -0.363
```

```
## treat1TS3   -0.087455    0.017576   -4.976
##
## Correlation of Fixed Effects:
##           (Intr) tr1TS1 tr1TS2
## treat1TS1 -0.259
## treat1TS2 -0.217  0.420
## treat1TS3 -0.217  0.420  0.436
```

Marginal means.

```
mmeans <- emmeans(m1, 'treat1')
mmeans
```

```
##  treat1 emmean     SE   df lower.CL upper.CL
##  TH      0.215 0.0285 5.99   0.1450    0.285
##  TS1     0.193 0.0285 5.99   0.1229    0.262
##  TS2     0.208 0.0301 7.36   0.1380    0.279
##  TS3     0.127 0.0301 7.36   0.0569    0.198
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
```

Need name for export.

Tukey's test

```
mmeans5_10 <- emmeans(m1, 'treat1')
pairs(mmeans5_10)
```

```
##  contrast   estimate     SE   df t.ratio p.value
##  TH - TS1    0.02215 0.0148 69.0   1.501  0.4424
##  TH - TS2    0.00638 0.0176 69.8   0.362  0.9837
##  TH - TS3    0.08746 0.0176 69.8   4.963  <.0001
##  TS1 - TS2  -0.01577 0.0176 69.8  -0.895  0.8074
##  TS1 - TS3   0.06531 0.0176 69.8   3.706  0.0023
##  TS2 - TS3   0.08108 0.0187 69.0   4.344  0.0003
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
```

```
letters5_10 <- cld(object = mmeans5_10,
                   adjust = "Tukey",
                   Letters = letters,
                   alpha = 0.05)
```

```
## Note: adjust = "tukey" was changed to "sidak"
## because "tukey" is only appropriate for one set of pairwise comparisons
```

```
letters5_10
```

```
##  treat1 emmean     SE   df lower.CL upper.CL .group
```

```
## TS3      0.127 0.0301 7.36    0.0290     0.226  a
## TS1      0.193 0.0285 5.99    0.0927     0.293   b
## TS2      0.208 0.0301 7.36    0.1100     0.307   b
## TH       0.215 0.0285 5.99    0.1149     0.315   b
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Conf-level adjustment: sidak method for 4 estimates
## P value adjustment: tukey method for comparing a family of 4 estimates
## significance level used: alpha = 0.05
## NOTE: If two or more means share the same grouping symbol,
##       then we cannot show them to be different.
##       But we also did not show them to be the same.
```

```r
mmeans5_10 <- letters5_10[order(letters5_10$treat1), ]
```

And get a column for copy/paste into paper.

```r
mmeans5_10$tabval <- paste(round(100 * mmeans5_10$emmean, 1), gsub(' ', '', mmeans5_10$.group))
```

Add 11-12

```r
isumm1 <- droplevels(isumm[new.ID %in% c('11', '12') & treat1 != 'TS1 + acid', ])
```

```r
table(isumm1[, .(new.ID, treat1)])
```

```
##       treat1
## new.ID OSI TH TS1
##     11   4  4   4
##     12   4  4   4
```

Completely balanced and only two experiments, so no need for mixed-effects model.

```r
m1 <- lm(e.rel.150 ~ treat1 + new.ID, data = isumm1)
```

```r
summary(m1)
```

```
##
## Call:
## lm(formula = e.rel.150 ~ treat1 + new.ID, data = isumm1)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.08313 -0.04784 -0.01006  0.03072  0.16487
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.29213    0.02727  10.713 9.81e-10 ***
```

```
## treat1TH       0.12425     0.03340    3.720   0.00135 **
## treat1TS1      0.07063     0.03340    2.115   0.04720 *
## new.ID12      -0.04500     0.02727   -1.650   0.11450
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.06679 on 20 degrees of freedom
## Multiple R-squared:  0.4543, Adjusted R-squared:  0.3725
## F-statistic: 5.551 on 3 and 20 DF,  p-value: 0.006143
```

Marginal means.

```
mmeans <- emmeans(m1, 'treat1')
mmeans
```

```
##  treat1 emmean      SE df lower.CL upper.CL
##  OSI     0.270 0.0236 20    0.220    0.319
##  TH      0.394 0.0236 20    0.345    0.443
##  TS1     0.340 0.0236 20    0.291    0.390
##
## Results are averaged over the levels of: new.ID
## Confidence level used: 0.95
```

Need name for export.

Tukey's test

```
mmeans11_12 <- emmeans(m1, 'treat1')
pairs(mmeans11_12)
```

```
##  contrast  estimate     SE df t.ratio p.value
##  OSI - TH   -0.1242 0.0334 20  -3.720  0.0037
##  OSI - TS1  -0.0706 0.0334 20  -2.115  0.1121
##  TH - TS1    0.0536 0.0334 20   1.606  0.2664
##
## Results are averaged over the levels of: new.ID
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
letters11_12 <- cld(object = mmeans11_12,
                    adjust = "Tukey",
                    Letters = letters,
                    alpha = 0.05)
```

```
## Note: adjust = "tukey" was changed to "sidak"
## because "tukey" is only appropriate for one set of pairwise comparisons
```

Order.

```
mmeans11_12 <- letters11_12[c(3, 2, 1), ]
```

And get a column for copy/paste into paper.

```
mmeans11_12$tabval <- paste(round(100 * mmeans11_12$emmean, 1), gsub(' ', '', mmeans11_12$.group))
```

Trials 9 and 10 for driving speed

Subset.

```
isumm1 <- isumm[new.ID %in% as.character(c(9, 10))]
```

Extract driving speed and application method.

```
table(isumm1$treat)
```

```
##
##    TH  TH-4   TS1 TS1-4
##     8     8     8     8
```

```
isumm1[, speed := factor('12', levels = c(4, 12))]
isumm1[grepl('-4', treat1), speed := '4']
```

```
isumm1[, app.mthd := factor(sub('-4', '', treat1), levels = c('TH', 'TS1'))]
```

```
table(isumm1[, .(new.ID, app.mthd, speed)])
```
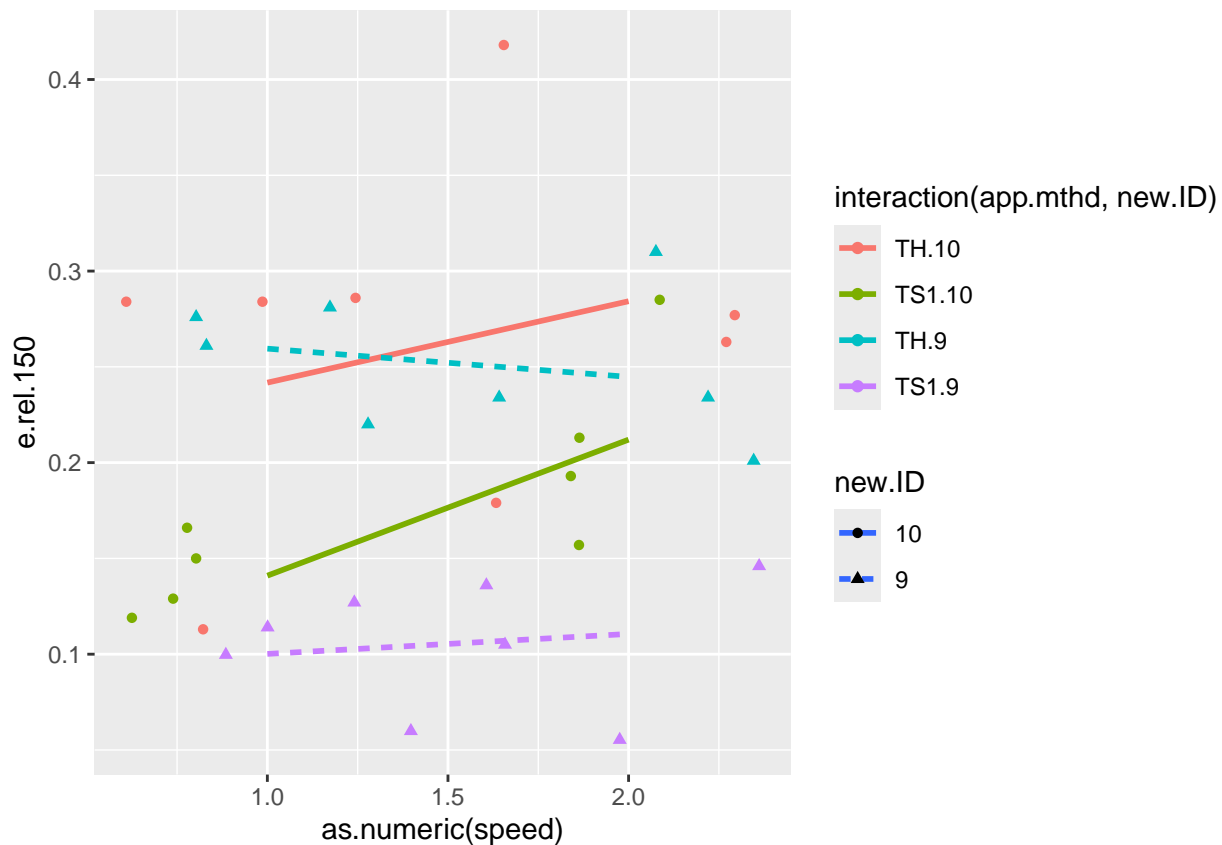
```
## , , speed = 4
##
##        app.mthd
## new.ID TH TS1
##     10  4   4
##      9  4   4
##
## , , speed = 12
##
##        app.mthd
## new.ID TH TS1
##     10  4   4
##      9  4   4
```

Completely crossed, perfectly balanced.

Plot.

```
ggplot(isumm1, aes(as.numeric(speed), e.rel.150, colour = interaction(app.mthd, new.ID), shape = new.ID
    geom_smooth(method = lm, se = FALSE) +
    geom_jitter(height = 0)
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

Fit model.

```
m1 <- lm(log10(e.rel.150) ~ app.mthd * speed * new.ID, data = isumm1)
m2 <- aov(log10(e.rel.150) ~ (app.mthd + speed + new.ID)^2, data = isumm1)
m3 <- aov(e.rel.150 ~ (app.mthd + speed + new.ID)^2, data = isumm1)
```

```
summary(m1)
```

```
##
## Call:
## lm(formula = log10(e.rel.150) ~ app.mthd * speed * new.ID, data = isumm1)
##
## Residuals:
##      Min      1Q  Median      3Q     Max
## -0.30094 -0.04368  0.01029  0.09930  0.18706
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)              -0.64598    0.06731  -9.598 1.09e-09 ***
## app.mthdTS1              -0.20844    0.09519  -2.190   0.0385 *
## speed12                   0.08010    0.09519   0.841   0.4084
## new.ID9                   0.05815    0.09519   0.611   0.5470
## app.mthdTS1:speed12       0.09049    0.13461   0.672   0.5079
## app.mthdTS1:new.ID9      -0.21953    0.13461  -1.631   0.1160
## speed12:new.ID9          -0.10902    0.13461  -0.810   0.4260
## app.mthdTS1:speed12:new.ID9 -0.03032  0.19037  -0.159   0.8748
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1346 on 24 degrees of freedom
## Multiple R-squared:  0.6785, Adjusted R-squared:  0.5847
## F-statistic: 7.236 on 7 and 24 DF,  p-value: 0.0001066
```

```
anova(m1)
```

```
## Analysis of Variance Table
##
## Response: log10(e.rel.150)
##                        Df  Sum Sq Mean Sq F value    Pr(>F)
## app.mthd                1 0.62960 0.62960 34.7448 4.419e-06 ***
## speed                   1 0.03201 0.03201  1.7662   0.19635
## new.ID                  1 0.10343 0.10343  5.7077   0.02510 *
## app.mthd:speed          1 0.01135 0.01135  0.6263   0.43647
## app.mthd:new.ID         1 0.11016 0.11016  6.0791   0.02122 *
## speed:new.ID            1 0.03084 0.03084  1.7020   0.20440
## app.mthd:speed:new.ID   1 0.00046 0.00046  0.0254   0.87477
## Residuals              24 0.43490 0.01812
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
drop1(m1, test = 'F')
```

```
## Single term deletions
##
## Model:
## log10(e.rel.150) ~ app.mthd * speed * new.ID
##                       Df  Sum of Sq     RSS     AIC F value Pr(>F)
## <none>                               0.43490 -121.55
## app.mthd:speed:new.ID  1 0.00045978 0.43536 -123.51  0.0254 0.8748
```

```
drop1(m2, test = 'F')
```

```
## Single term deletions
##
## Model:
## log10(e.rel.150) ~ (app.mthd + speed + new.ID)^2
##                 Df Sum of Sq     RSS     AIC F value Pr(>F)
## <none>                       0.43536 -123.51
## app.mthd:speed   1  0.011349 0.44671 -124.69  0.6517 0.4271
## app.mthd:new.ID  1  0.110157 0.54551 -118.30  6.3257 0.0187 *
## speed:new.ID     1  0.030842 0.46620 -123.32  1.7710 0.1953
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
drop1(m3, test = 'F')
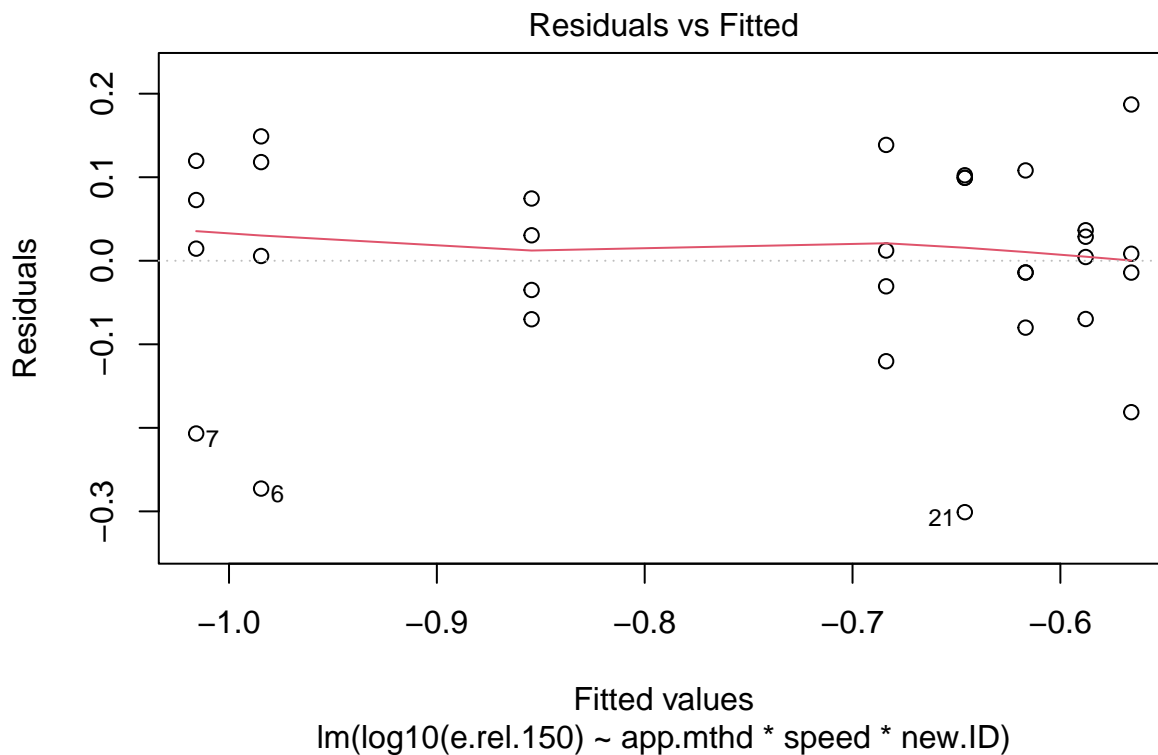```

```
Single term deletions
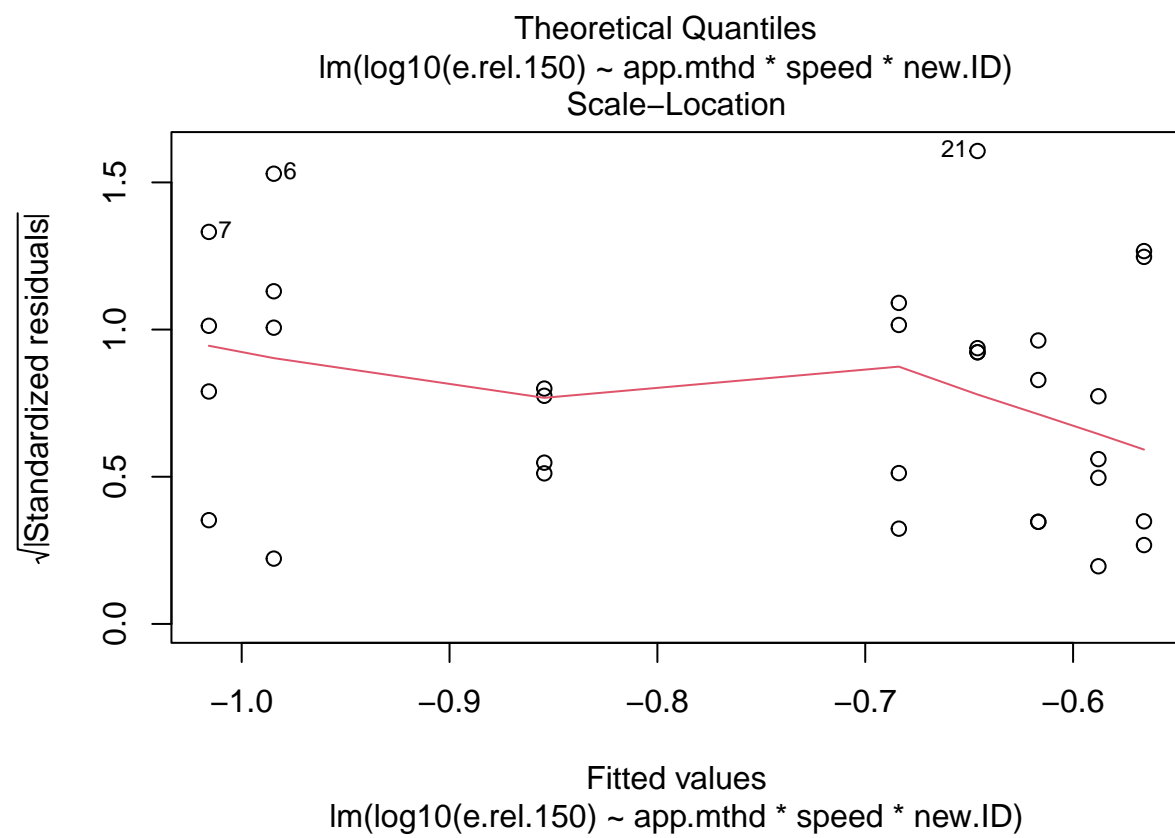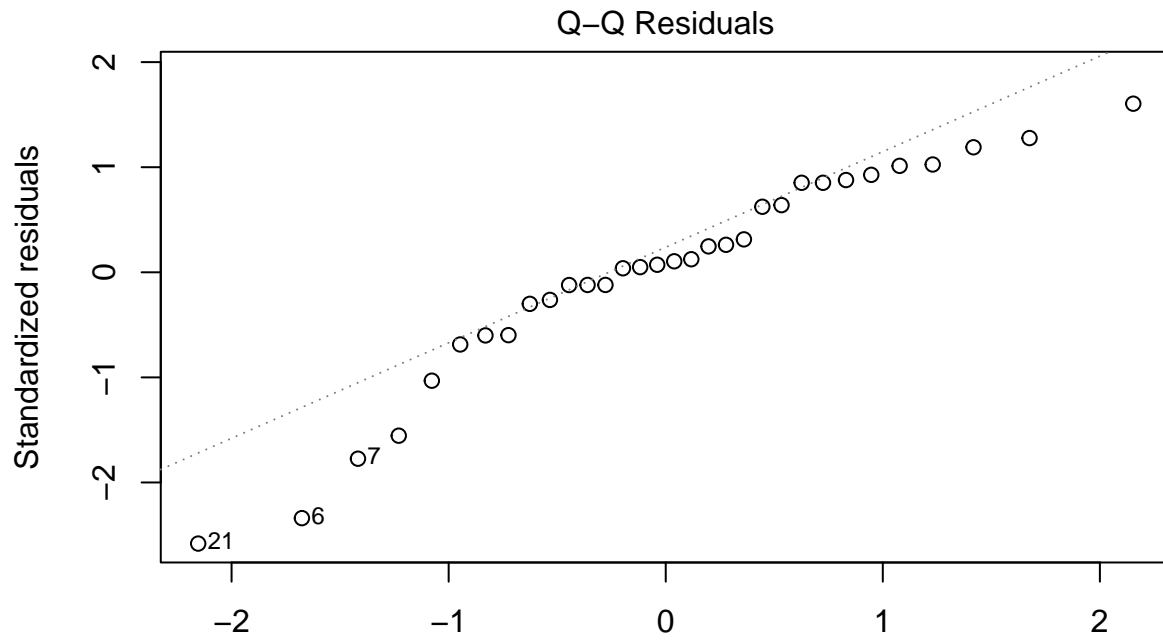```

```
## 
## Model:
## e.rel.150 ~ (app.mthd + speed + new.ID)^2
##                Df Sum of Sq      RSS      AIC F value Pr(>F)
## <none>                      0.077852 -178.60
## app.mthd:speed  1 0.0014405 0.079292 -180.01  0.4626 0.5027
## app.mthd:new.ID 1 0.0072631 0.085115 -177.74  2.3324 0.1393
## speed:new.ID    1 0.0069414 0.084793 -177.87  2.2290 0.1480
```
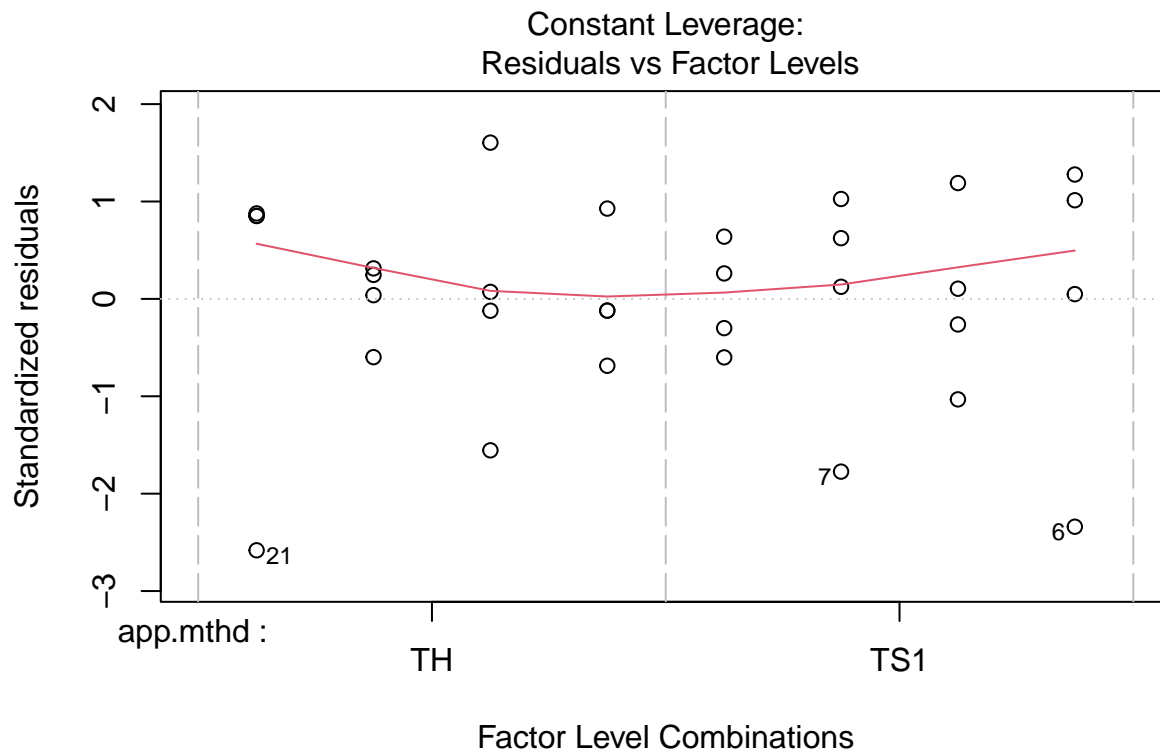
```r
summary(m3, test = 'F')
```

```
##                 Df  Sum Sq Mean Sq F value    Pr(>F)
## app.mthd         1 0.10882 0.10882  34.946 3.61e-06 ***
## speed            1 0.00596 0.00596   1.914   0.1788
## new.ID           1 0.01345 0.01345   4.320   0.0481 *
## app.mthd:speed   1 0.00144 0.00144   0.463   0.5027
## app.mthd:new.ID  1 0.00726 0.00726   2.332   0.1393
## speed:new.ID     1 0.00694 0.00694   2.229   0.1480
## Residuals       25 0.07785 0.00311
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
plot(m1, ask = FALSE)
```


Residuals vs Fitted

Fitted values
lm(log10(e.rel.150) ~ app.mthd * speed * new.ID)
```

Q–Q Residuals

Standardized residuals

Theoretical Quantiles
lm(log10(e.rel.150) ~ app.mthd * speed * new.ID)

Scale–Location

√|Standardized residuals|

Fitted values
lm(log10(e.rel.150) ~ app.mthd * speed * new.ID)

Constant Leverage:
Residuals vs Factor Levels

```r
model.tables(m2, 'means')
```

```
## Tables of means
## Grand mean
##
## -0.7443791
##
##   app.mthd
## app.mthd
##       TH      TS1
## -0.6041 -0.8846
##
##   speed
## speed
##        4       12
## -0.7760 -0.7128
##
##   new.ID
## new.ID
##       10        9
## -0.6875 -0.8012
##
##   app.mthd:speed
##          speed
## app.mthd 4       12
##       TH  -0.6169 -0.5913
##       TS1 -0.9351 -0.8342
##
##   app.mthd:new.ID
```

```
##         new.ID
## app.mthd 10       9
##      TH  -0.6059 -0.6023
##      TS1 -0.7691 -1.0002
##
##  speed:new.ID
##       new.ID
## speed 10       9
##     4  -0.7502 -0.8018
##    12 -0.6249 -0.8007
```

More useful means.

```
isumm1[, .(mn = mean(e.rel.150), sd = sd(e.rel.150)), by = .(new.ID, app.mthd, speed)]
```

```
##     new.ID app.mthd  speed        mn         sd
##     <char>   <fctr> <fctr>     <num>      <num>
## 1:       9       TH     12 0.244750 0.04619794
## 2:       9      TS1     12 0.110575 0.04077486
## 3:       9      TS1      4 0.100150 0.02905747
## 4:       9       TH      4 0.259500 0.02767068
## 5:      10      TS1      4 0.141000 0.02108712
## 6:      10       TH      4 0.241750 0.08583851
## 7:      10       TH     12 0.284250 0.09911399
## 8:      10      TS1     12 0.212000 0.05390114
```

Trials 13 and 14 for hose distance effect

Subset.

```
isumm1 <- isumm[new.ID %in% as.character(c(13, 14))]
```

Extract separation status and hose distance.

```
table(isumm1$treat)
```

```
##
## Sep25 Sep30  Un12  UN25  Un30
##     6     6     6     6     6
```

```
class(isumm1)
```

```
## [1] "data.table" "data.frame"
```

```
isumm1[, treat1 := tolower(treat1)]
isumm1[, distancef := factor(sub('sep|un', '', treat1))]
isumm1[, distancen := as.numeric(sub('sep|un', '', treat1))]
isumm1[, sep := factor(sub('[0-9]+$', '', treat1))]
```

```
table(isumm1[, .(new.ID, sep, distancef)])
```

```
## , , distancef = 12
##
##       sep
## new.ID sep un
##     13   0 3
##     14   0 3
##
## , , distancef = 25
##
##       sep
## new.ID sep un
##     13   3 3
##     14   3 3
##
## , , distancef = 30
##
##       sep
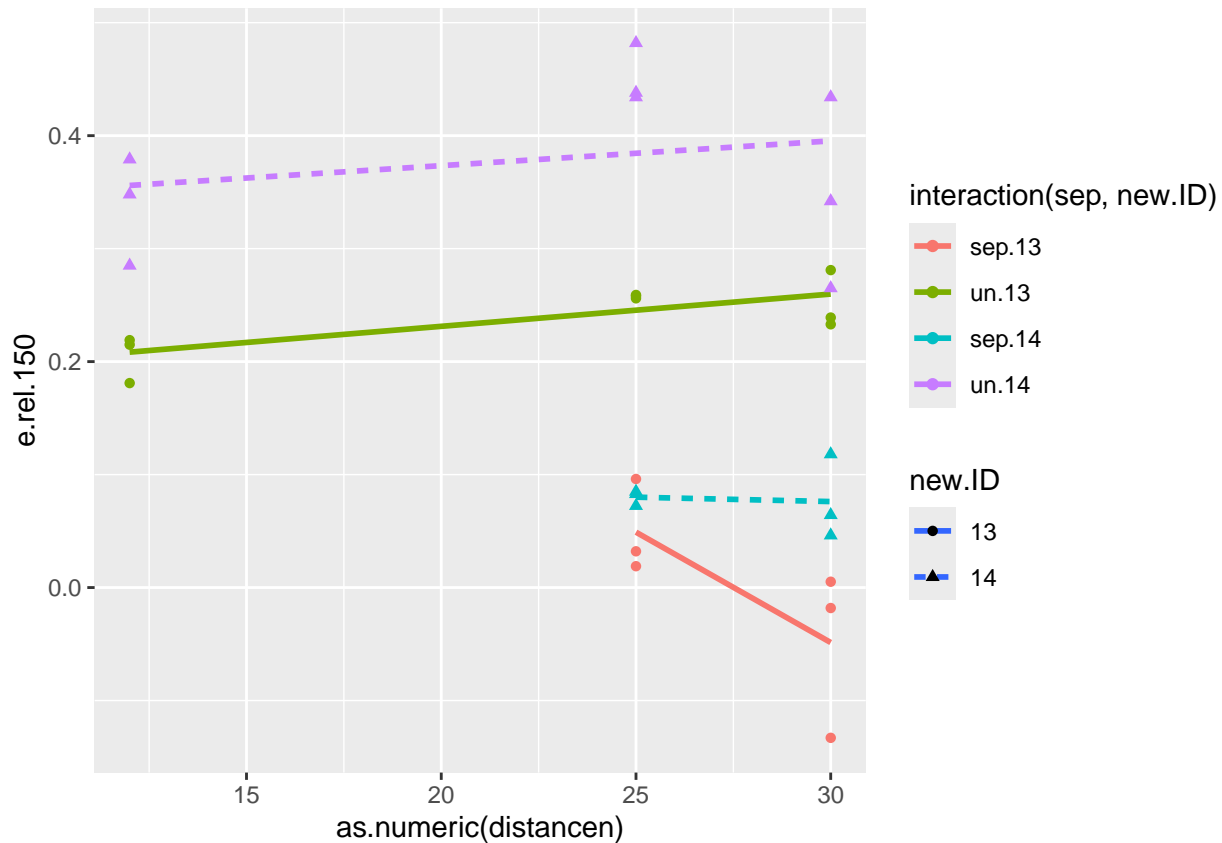## new.ID sep un
##     13   3 3
##     14   3 3
```

Subset is completely crossed, perfectly balanced. But un has an additional distance of 12 cm.

Plot.

```
ggplot(isumm1, aes(as.numeric(distancen), e.rel.150, colour = interaction(sep, new.ID), shape = new.ID,
    geom_smooth(method = lm, se = FALSE) +
    geom_point(height = 0)
```

```
## Warning in geom_point(height = 0): Ignoring unknown parameters: 'height'
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```

Hmm, might be some interactions. Fit model.

```
m1 <- lm(e.rel.150 ~ sep * distancen * new.ID, data = isumm1)
m2 <- lm(e.rel.150 ~ (sep + distancen + new.ID)^2, data = isumm1)
m2b <- lm(e.rel.150 ~ (sep + distancen + new.ID)^2, data = isumm1, subset = sep != 'sep' | new.ID != 13)
```

```
summary(m1)
```

```
##
## Call:
## lm(formula = e.rel.150 ~ sep * distancen * new.ID, data = isumm1)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.130344 -0.025284  0.005761  0.028678  0.097605
##
## Coefficients:
##                         Estimate Std. Error t value Pr(>|t|)
## (Intercept)             0.537433   0.239393   2.245   0.0352 *
## sepun                  -0.363325   0.245634  -1.479   0.1533
## distancen              -0.019536   0.008669  -2.253   0.0345 *
## new.ID14               -0.438100   0.338553  -1.294   0.2091
## sepun:distancen         0.022392   0.008978   2.494   0.0206 *
## sepun:new.ID14          0.593641   0.347379   1.709   0.1015
## distancen:new.ID14      0.018763   0.012260   1.530   0.1402
## sepun:distancen:new.ID14 -0.019429  0.012696  -1.530   0.1402
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05309 on 22 degrees of freedom
## Multiple R-squared:  0.9129, Adjusted R-squared:  0.8851
## F-statistic: 32.93 on 7 and 22 DF,  p-value: 3.107e-10
```

anova(m1)

```
## Analysis of Variance Table
##
## Response: e.rel.150
##                     Df  Sum Sq Mean Sq  F value    Pr(>F)
## sep                  1 0.52136 0.52136 184.9781 3.464e-12 ***
## distancen            1 0.00309 0.00309   1.0954   0.30663
## new.ID               1 0.10014 0.10014  35.5289 5.342e-06 ***
## sep:distancen        1 0.01124 0.01124   3.9880   0.05834 .
## sep:new.ID           1 0.00710 0.00710   2.5182   0.12681
## distancen:new.ID     1 0.00012 0.00012   0.0411   0.84127
## sep:distancen:new.ID 1 0.00660 0.00660   2.3416   0.14021
## Residuals           22 0.06201 0.00282
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

drop1(m1, test = **'F'**)

```
## Single term deletions
##
## Model:
## e.rel.150 ~ sep * distancen * new.ID
##                      Df  Sum of Sq      RSS     AIC F value Pr(>F)
## <none>                             0.062006 -169.45
## sep:distancen:new.ID  1 0.0065998 0.068606 -168.42  2.3416 0.1402
```

drop1(m2, test = **'F'**)

```
## Single term deletions
##
## Model:
## e.rel.150 ~ (sep + distancen + new.ID)^2
##                  Df  Sum of Sq      RSS     AIC F value  Pr(>F)
## <none>                          0.068606 -168.42
## sep:distancen     1 0.0112402 0.079847 -165.87  3.7682 0.06459 .
## sep:new.ID        1 0.0067105 0.075317 -167.62  2.2497 0.14725
## distancen:new.ID  1 0.0001157 0.068722 -170.37  0.0388 0.84557
## ---
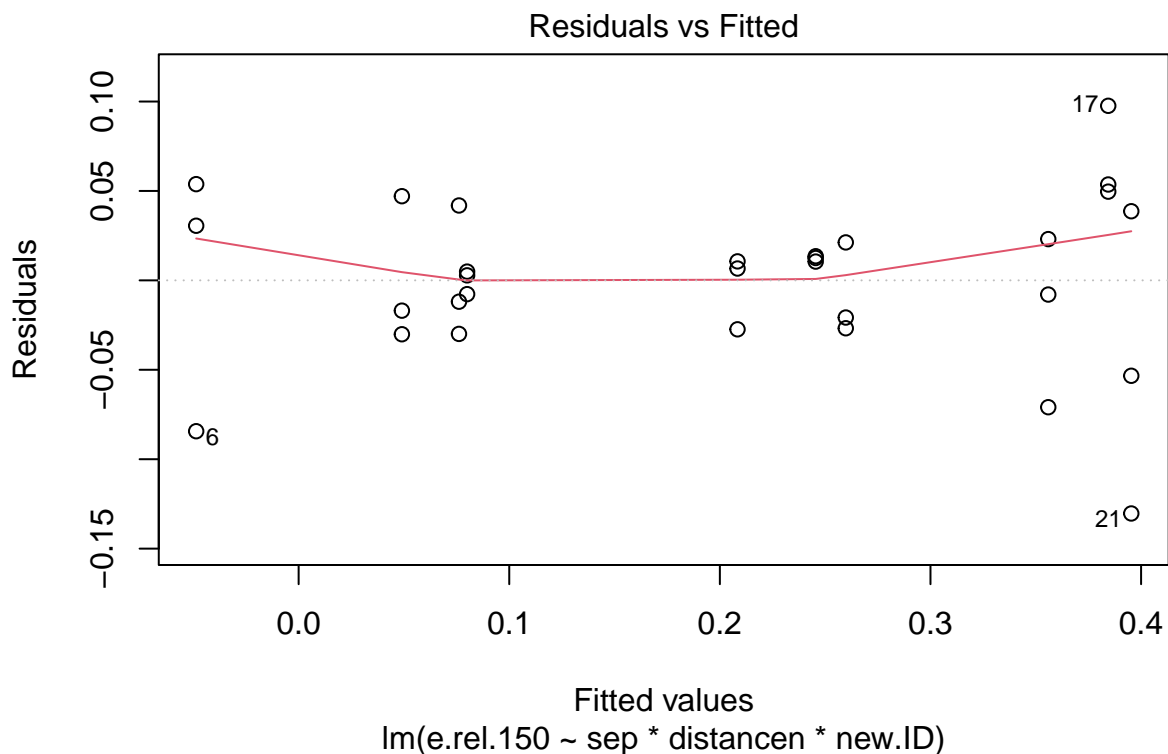## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
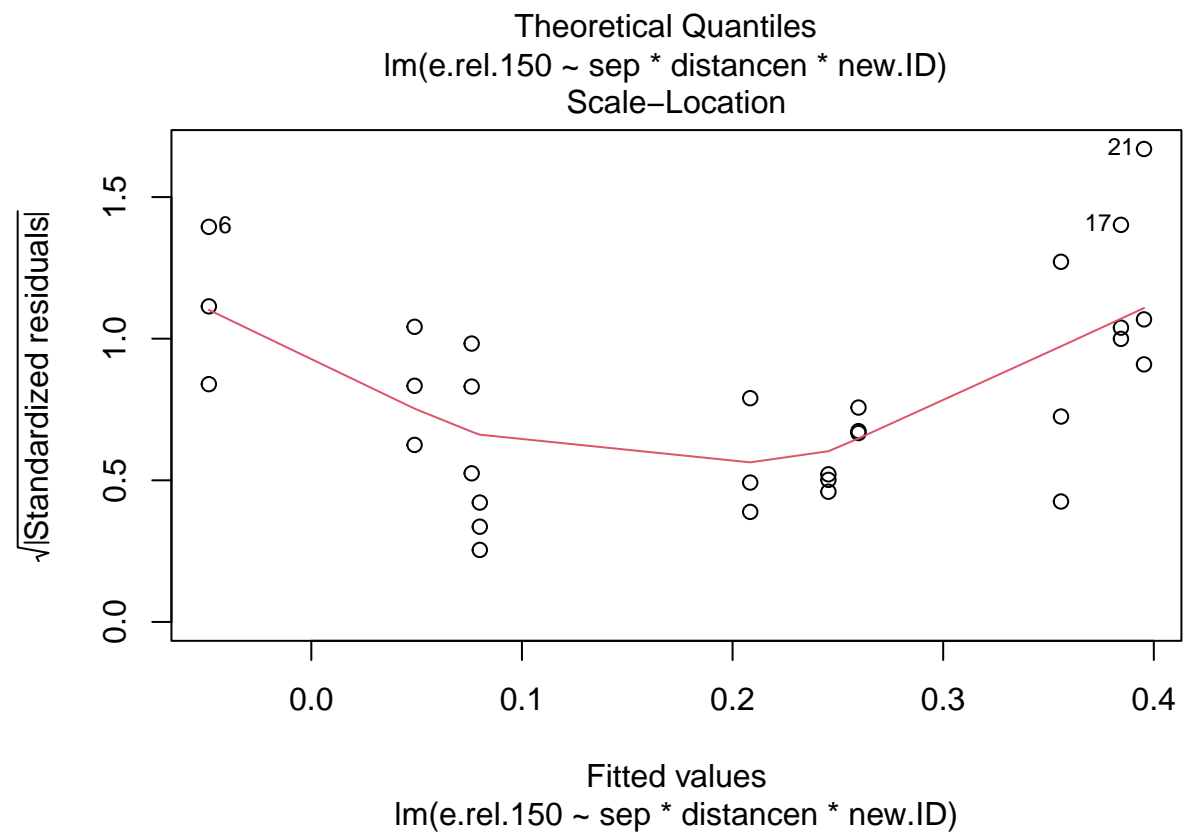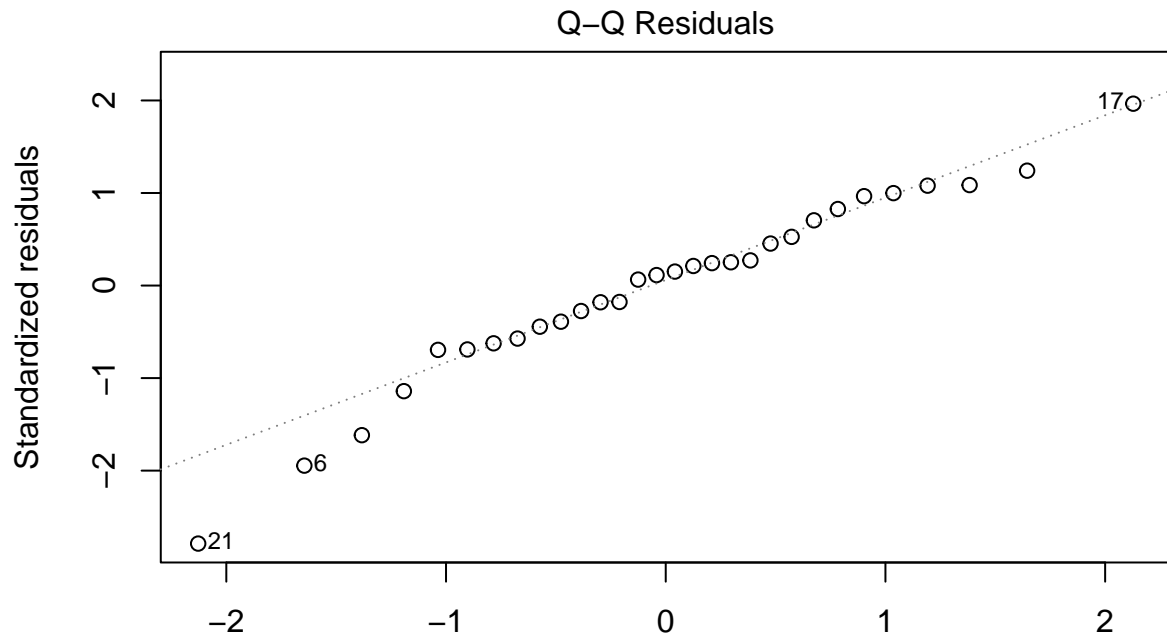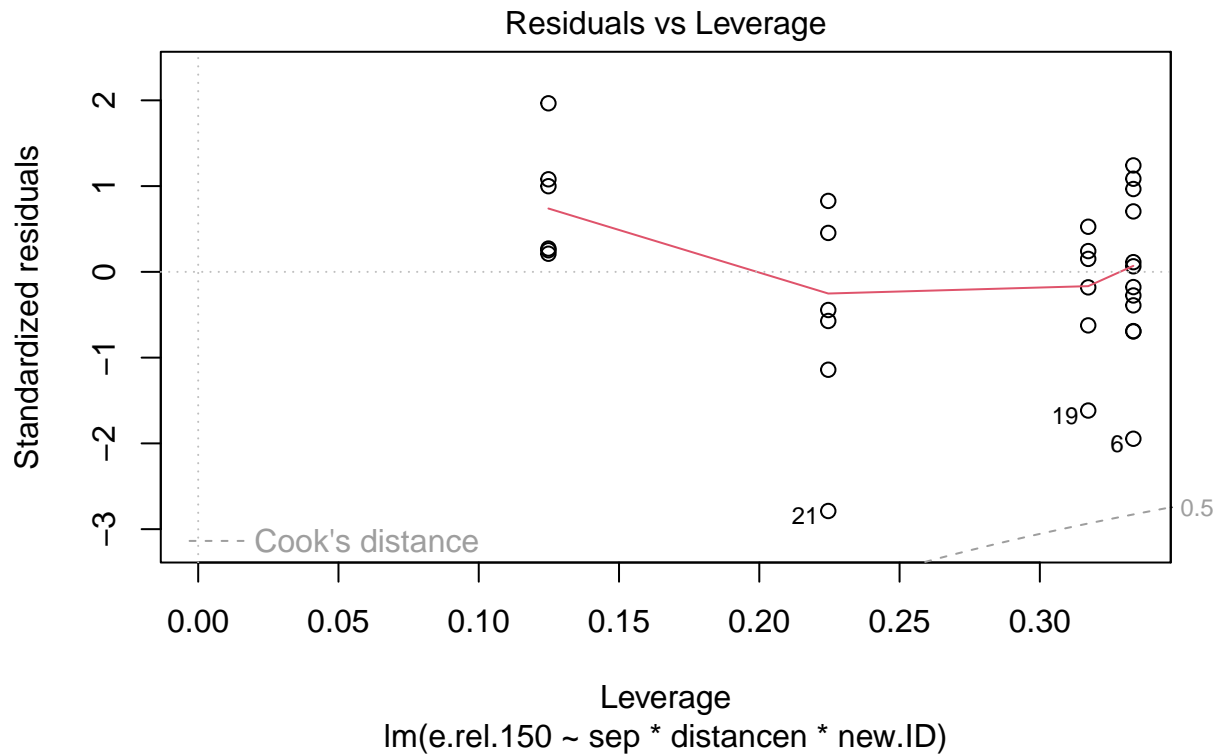```

**summary**(m2b)

```
##
```

```
## Call:
## lm(formula = e.rel.150 ~ (sep + distancen + new.ID)^2, data = isumm1,
##     subset = sep != "sep" | new.ID != 13)
##
## Residuals:
##       Min       1Q    Median        3Q       Max
## -0.130344 -0.022284  0.005761  0.021680  0.097605
##
## Coefficients: (1 not defined because of singularities)
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)        -0.0562079  0.2439600  -0.230   0.8204
## sepun               0.2303160  0.2380613   0.967   0.3461
## distancen          -0.0001073  0.0089899  -0.012   0.9906
## new.ID14            0.1555412  0.0754094   2.063   0.0539 .
## sepun:distancen     0.0029632  0.0087010   0.341   0.7374
## sepun:new.ID14            NA         NA      NA       NA
## distancen:new.ID14 -0.0006660  0.0031971  -0.208   0.8373
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05145 on 18 degrees of freedom
## Multiple R-squared:  0.8752, Adjusted R-squared:  0.8405
## F-statistic: 25.24 on 5 and 18 DF,  p-value: 1.516e-07
```

No clear distance effects, marginal interaction evidence, but all related to those negative values.

```
plot(m1, ask = FALSE)
```


Residuals vs Fitted

Fitted values
lm(e.rel.150 ~ sep * distancen * new.ID)

Q–Q Residuals

lm(e.rel.150 ~ sep * distancen * new.ID)

Scale–Location

lm(e.rel.150 ~ sep * distancen * new.ID)

Residuals vs Leverage

Residuals vs Leverage plot: Standardized residuals vs Leverage, lm(e.rel.150 ~ sep * distancen * new.ID). Points labeled 19, 6, 21. Cook's distance line at 0.5.

Means.

```r
isumm1[, .(mn = mean(e.rel.150), sd = sd(e.rel.150)), by = .(new.ID, sep, distancef)]
```

```
##      new.ID    sep distancef          mn           sd
##      <char> <fctr>    <fctr>       <num>        <num>
##  1:      13     un        30  0.25100000  0.026153394
##  2:      13     un        25  0.25766667  0.001527525
##  3:      13    sep        25  0.04903333  0.041291807
##  4:      13    sep        30 -0.04864667  0.073972093
##  5:      13     un        12  0.20500000  0.020880613
##  6:      14     un        12  0.33733333  0.047899200
##  7:      14     un        25  0.45133333  0.026633312
##  8:      14    sep        30  0.07613333  0.037357909
##  9:      14    sep        25  0.08000000  0.006750556
## 10:      14     un        30  0.34700000  0.084610874
```

Trial 15, chamber movement

Subset.

```r
isumm1 <- isumm[new.ID == 15, ]
```

Set reference.

```r
isumm1[, treat1 := factor(treat1, levels = c('No', '2-Pos', '7-Pos'))]
```

Fit model.

```r
m1 <- aov(e.rel.150 ~ treat1, data = isumm1)
```

Tukey's test.

```r
summary(m1)
```

```
##              Df  Sum Sq  Mean Sq F value Pr(>F)
## treat1        2 0.01633 0.008163   5.433 0.0209 *
## Residuals    12 0.01803 0.001502
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
TukeyHSD(m1)
```

```
##    Tukey multiple comparisons of means
##      95% family-wise confidence level
##
## Fit: aov(formula = e.rel.150 ~ treat1, data = isumm1)
##
## $treat1
##                diff          lwr        upr      p adj
## 2-Pos-No     0.0148 -0.050599854 0.08019985 0.8207809
## 7-Pos-No     0.0762  0.010800146 0.14159985 0.0228266
## 7-Pos-2-Pos  0.0614 -0.003999854 0.12679985 0.0664770
```

Dunnett test.

```r
mmeans15 <- emmeans(m1, 'treat1')
mmeans15
```

```
##  treat1 emmean     SE df lower.CL upper.CL
##  No      0.309 0.0173 12    0.271    0.347
##  2-Pos   0.324 0.0173 12    0.286    0.361
##  7-Pos   0.385 0.0173 12    0.347    0.423
##
## Confidence level used: 0.95
```

```r
contrast(mmeans15, 'trt.vs.ctrl')
```

```
##  contrast      estimate     SE df t.ratio p.value
##  (2-Pos) - No    0.0148 0.0245 12   0.604  0.7681
##  (7-Pos) - No    0.0762 0.0245 12   3.108  0.0172
##
## P value adjustment: dunnettx method for 2 tests
```

```r
contrast(mmeans15, 'tukey')
```

```
##  contrast           estimate     SE df t.ratio p.value
##  No - (2-Pos)        -0.0148 0.0245 12  -0.604  0.8208
##  No - (7-Pos)        -0.0762 0.0245 12  -3.108  0.0228
##  (2-Pos) - (7-Pos)   -0.0614 0.0245 12  -2.505  0.0665
##
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
pairs(mmeans15)
```

```
##  contrast            estimate     SE df t.ratio p.value
##  No - (2-Pos)         -0.0148 0.0245 12  -0.604  0.8208
##  No - (7-Pos)         -0.0762 0.0245 12  -3.108  0.0228
##  (2-Pos) - (7-Pos)    -0.0614 0.0245 12  -2.505  0.0665
##
## P value adjustment: tukey method for comparing a family of 3 estimates
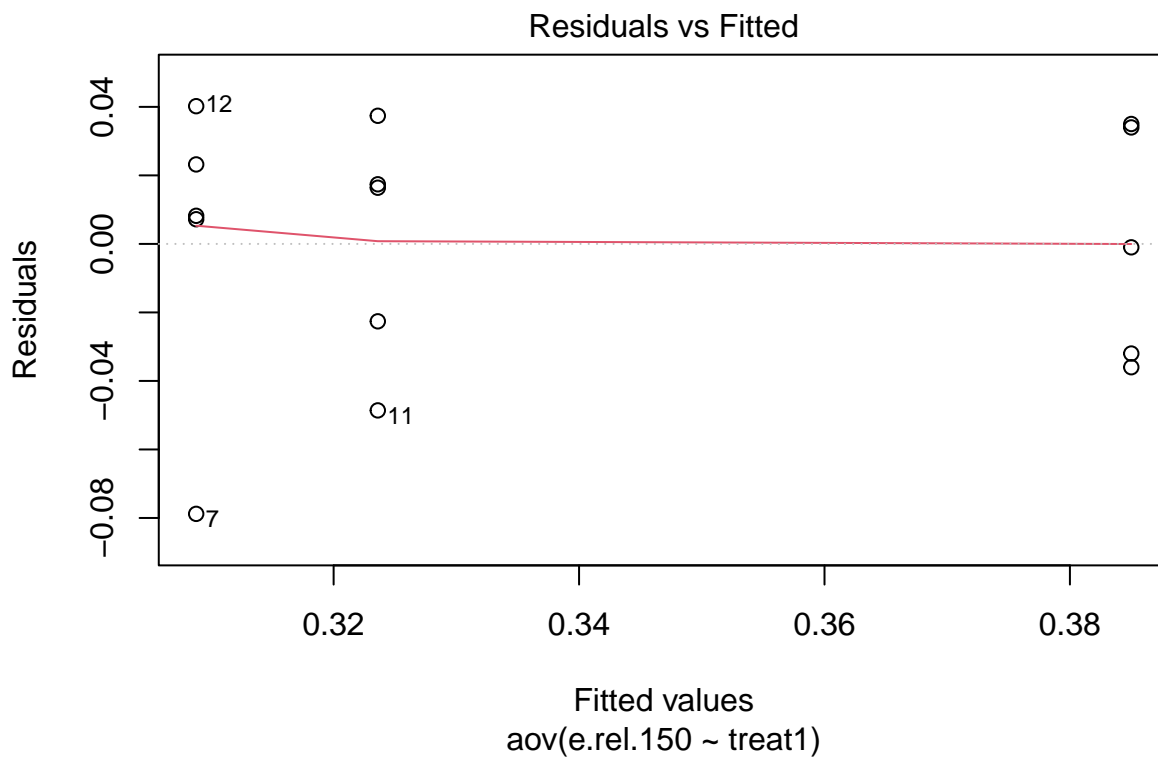```

Relative effects.

```
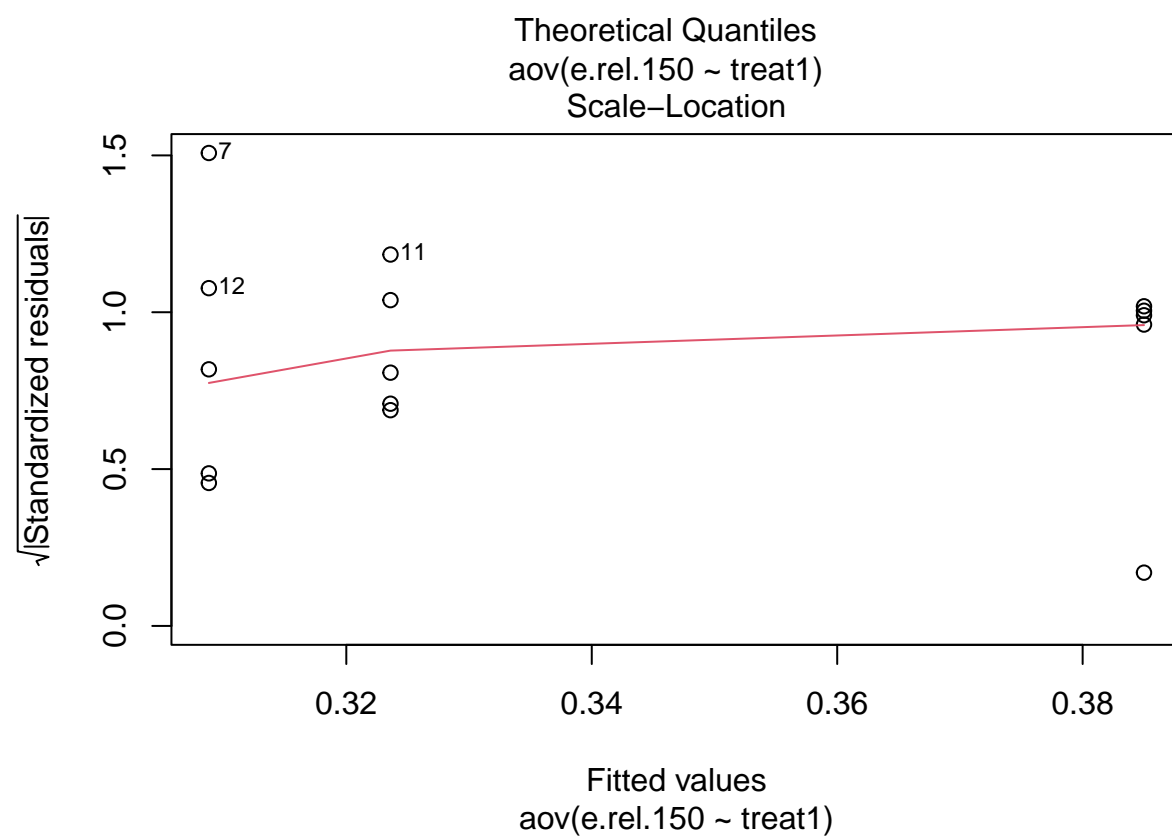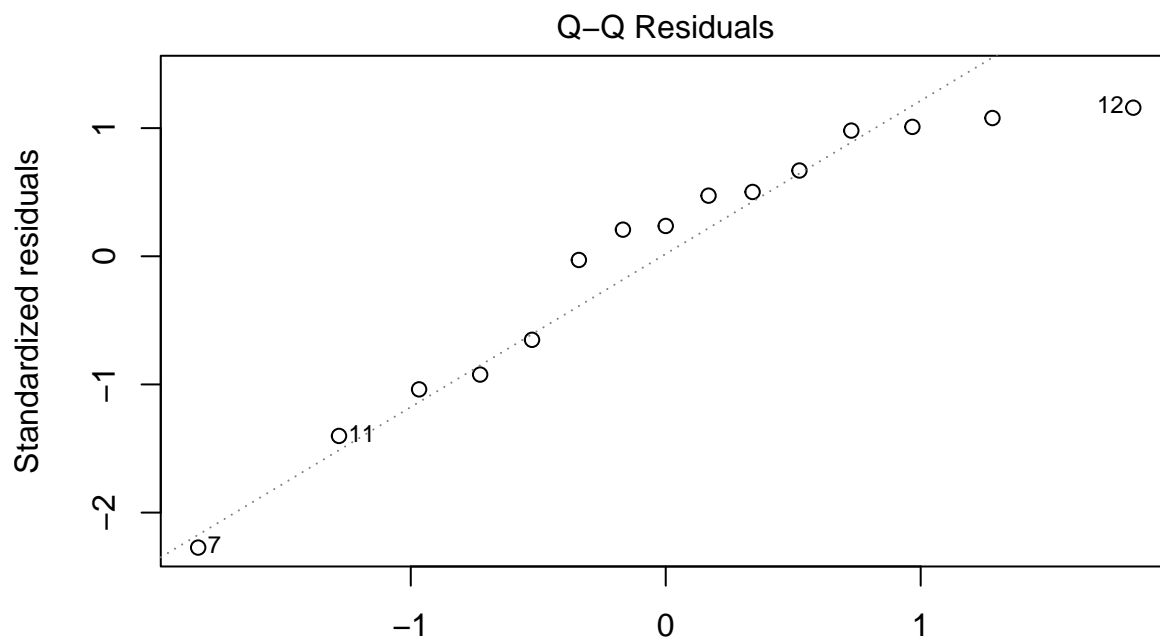coef(m1)
```

```
## (Intercept) treat12-Pos treat17-Pos
##      0.3088      0.0148      0.0762
```

```
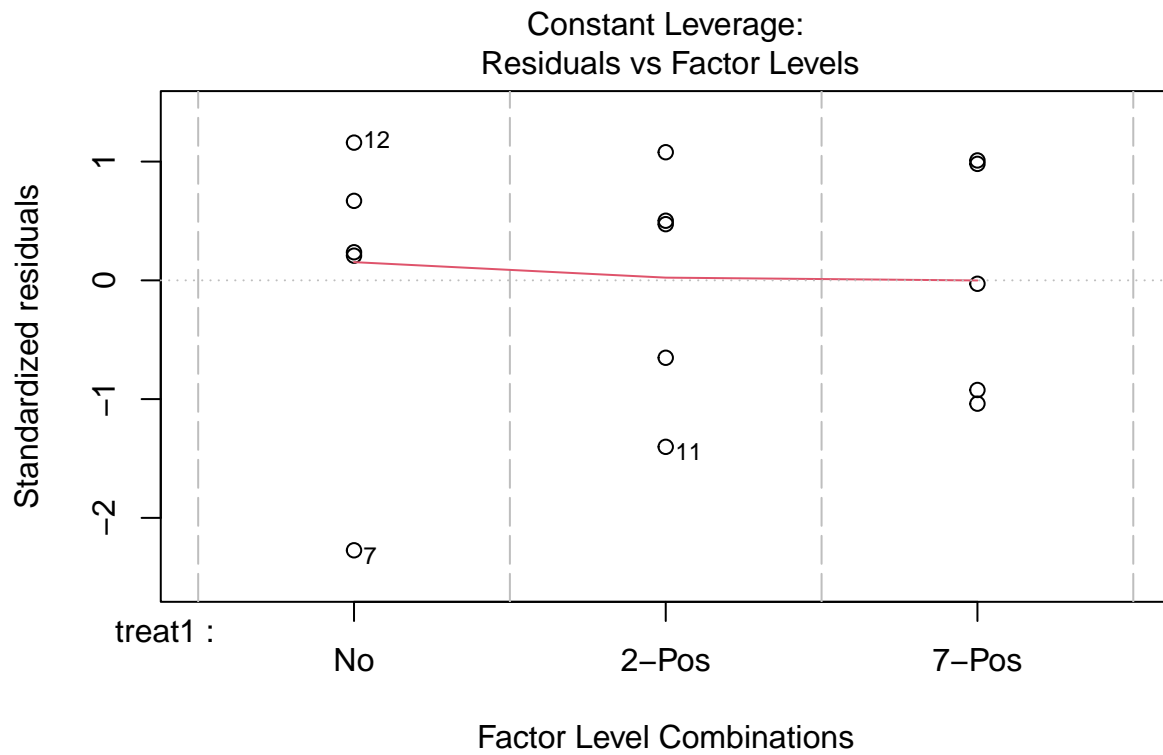coef(m1) / coef(m1)[1]
```

```
## (Intercept) treat12-Pos treat17-Pos
##  1.00000000  0.04792746  0.24676166
```

```
plot(m1)
```


Fitted values
aov(e.rel.150 ~ treat1)

Q–Q Residuals

Theoretical Quantiles
aov(e.rel.150 ~ treat1)

Scale–Location

Fitted values
aov(e.rel.150 ~ treat1)

Constant Leverage:
Residuals vs Factor Levels

Other stuff

I still need to sort this stuff out!

Then temperature stuff Add to supporting material