

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)
minotreat <- lmer(e.rel.150 ~ (1|dig.ID) + (1|new.ID), data = isumm1)
minodig <- 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

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.MF1 dg.MFs dg.S-D d.S-D+a dg.S-S
## dig.treatAcid -0.058
## dig.treatDs   -0.073  0.026
## dg.trtDs+ac   -0.073  0.026  0.362
## dg.trtMF1qd   -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
```

```
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.MF1 dg.MFs dg.S-D d.S-D+a dg.S-S
## dig.tretAcid -0.310
## dig.treatDs -0.248  0.306
## dg.trtDs+ac -0.248  0.306  0.348
## dg.trtMF1qd -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

```

```
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.

```
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
```

```
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
```

```
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
```

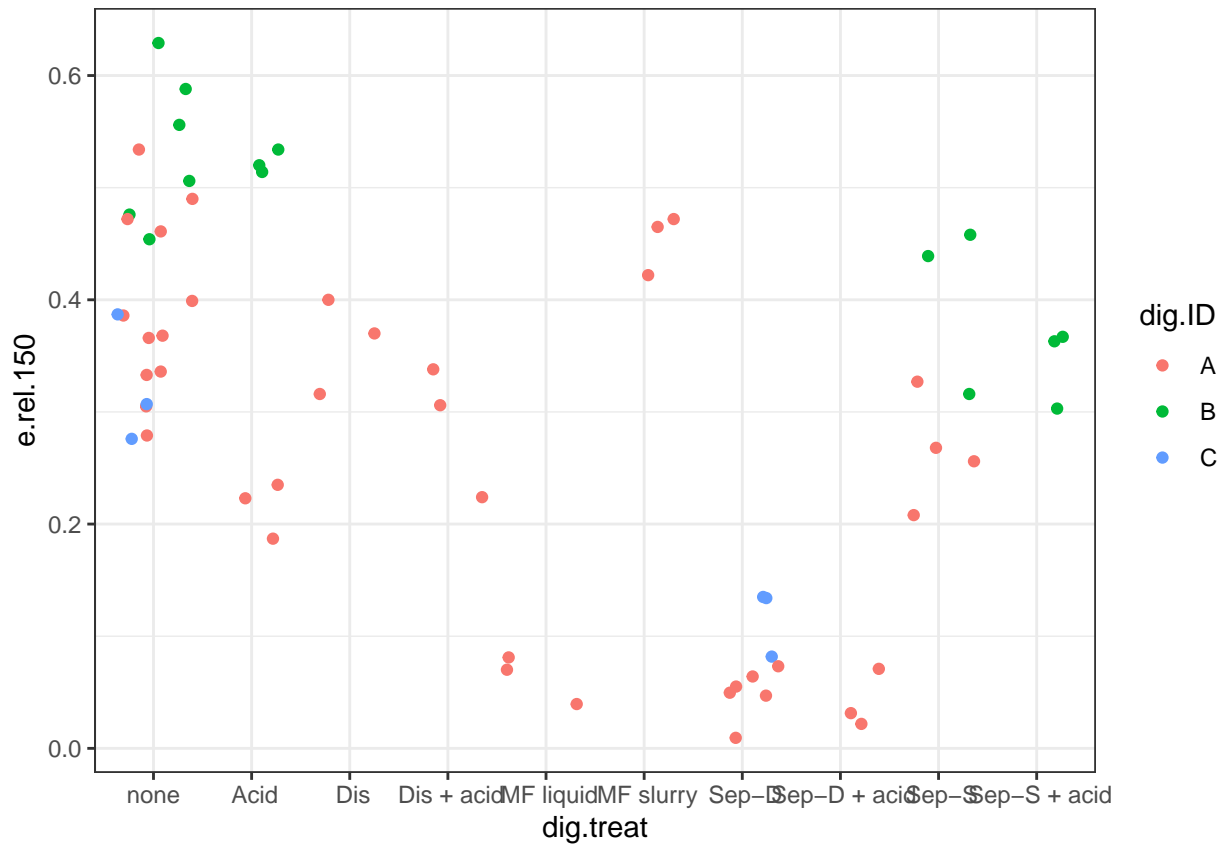
```
anova(m2b, m2, test = 'Chisq')
```

```
## 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.

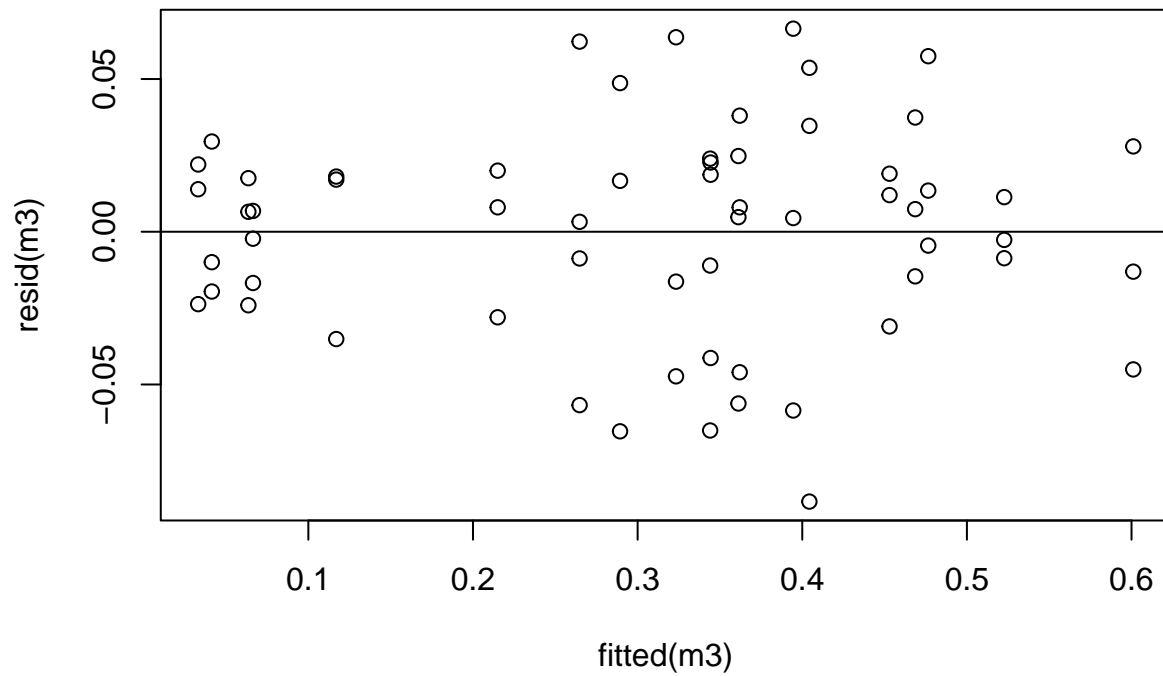
```
mmeans1_4 <- letters1_4[order(letters1_4$treat1), ]
```

And get a column for copy/paste into paper.

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

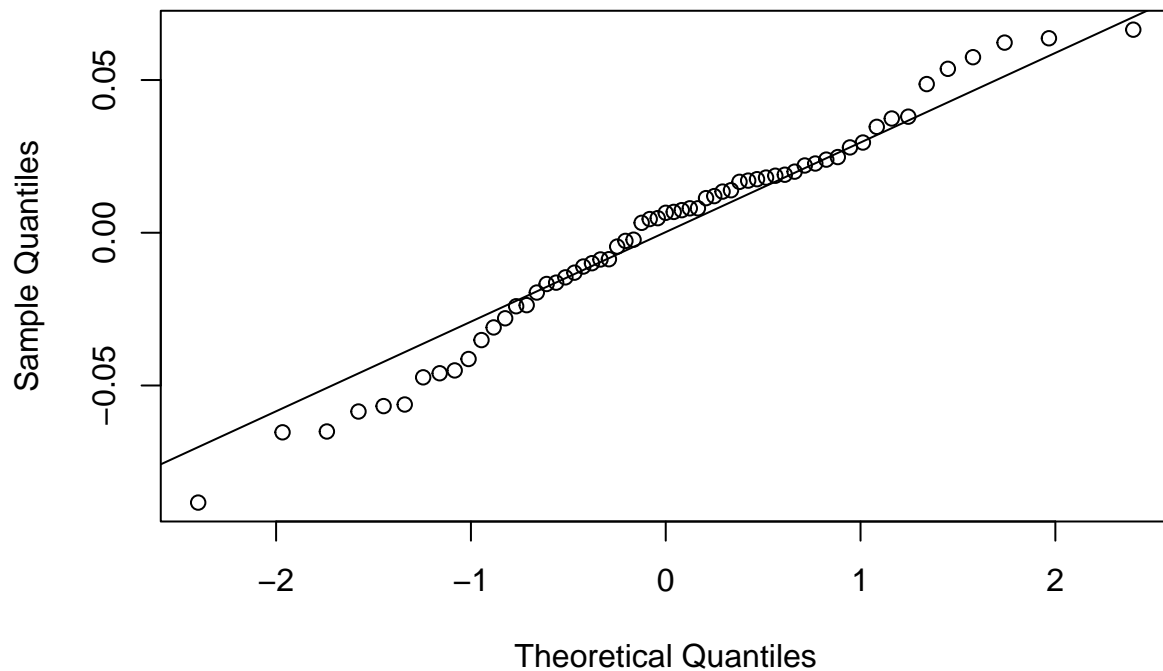
Residuals.

```
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)
m4b <- lmer(e.rel.150 ~ (1|dig.ID/new.ID), data = dref)
m5 <- lmer(e.rel.150 ~ (1|dig.ID) + (1|new.ID) + man.dm + man.ph, data = dref)
m6 <- lmer(e.rel.150 ~ (1|dig.ID/new.ID) + man.dm + man.ph, data = dref)
```

```
## boundary (singular) fit: see help('isSingular')
```

```
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(m4b)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ (1 | dig.ID/new.ID)
## Data: dref
##
## REML criterion at convergence: -48.9
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.40190 -0.56096  0.00607  0.63643  1.39169
##
## Random effects:
## Groups Name Variance Std.Dev.
## new.ID:dig.ID (Intercept) 0.005363 0.07323
## dig.ID (Intercept) 0.007081 0.08415
## Residual 0.002002 0.04475
## Number of obs: 21, groups: new.ID:dig.ID, 7; dig.ID, 3
##
## Fixed effects:
## Estimate Std. Error t value
## (Intercept) 0.42337 0.05855 7.231
```

```
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
```

```
summary(m6)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ (1 | dig.ID/new.ID) + man.dm + man.ph
## Data: dref
##
## REML criterion at convergence: -48.9
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.5803 -0.4847  0.0908  0.7587  1.3597
##
## Random effects:
## Groups      Name                Variance Std.Dev.
## new.ID:dig.ID (Intercept) 0.001500 0.03873
## dig.ID      (Intercept) 0.000000 0.00000
## Residual                    0.002002 0.04475
## Number of obs: 21, groups: new.ID:dig.ID, 7; dig.ID, 3
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)  2.364336    0.755467   3.130
```

```
## man.dm      0.037429  0.008942  4.186
## man.ph      -0.267593  0.093150 -2.873
##
## Correlation of Fixed Effects:
##      (Intr) man.dm
## man.dm -0.019
## man.ph -0.997 -0.054
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
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 <- update(m5, ~ . - man.ph)
m5nodm <- update(m5, ~ . - man.dm)
```

```
m6noph <- update(m6, ~ . - man.ph)
```

```
## boundary (singular) fit: see help('isSingular')
```

```
m6nodm <- update(m6, ~ . - man.dm)
```

```
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
```

```
anova(m6, m6noph, test = 'Chisq')
```

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

```
## Data: dref
## Models:
## m6noph: e.rel.150 ~ (1 | dig.ID/new.ID) + man.dm
## m6: e.rel.150 ~ (1 | dig.ID/new.ID) + man.dm + man.ph
##      npar      AIC      BIC logLik deviance  Chisq Df Pr(>Chisq)
## m6noph    5 -48.726 -43.503 29.363  -58.726
## m6        6 -54.562 -48.295 33.281  -66.562 7.836  1  0.005121 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

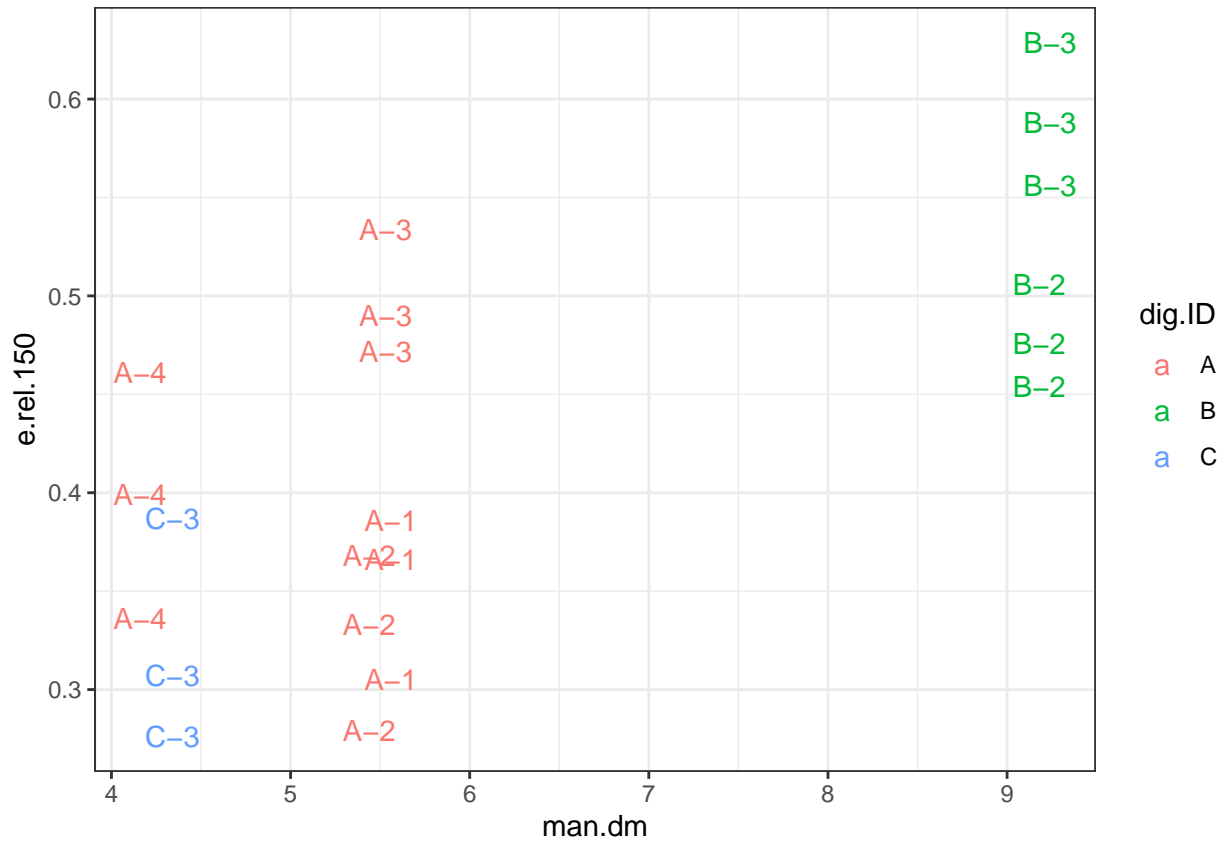
```
anova(m6, m6nodm, test = 'Chisq')
```

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

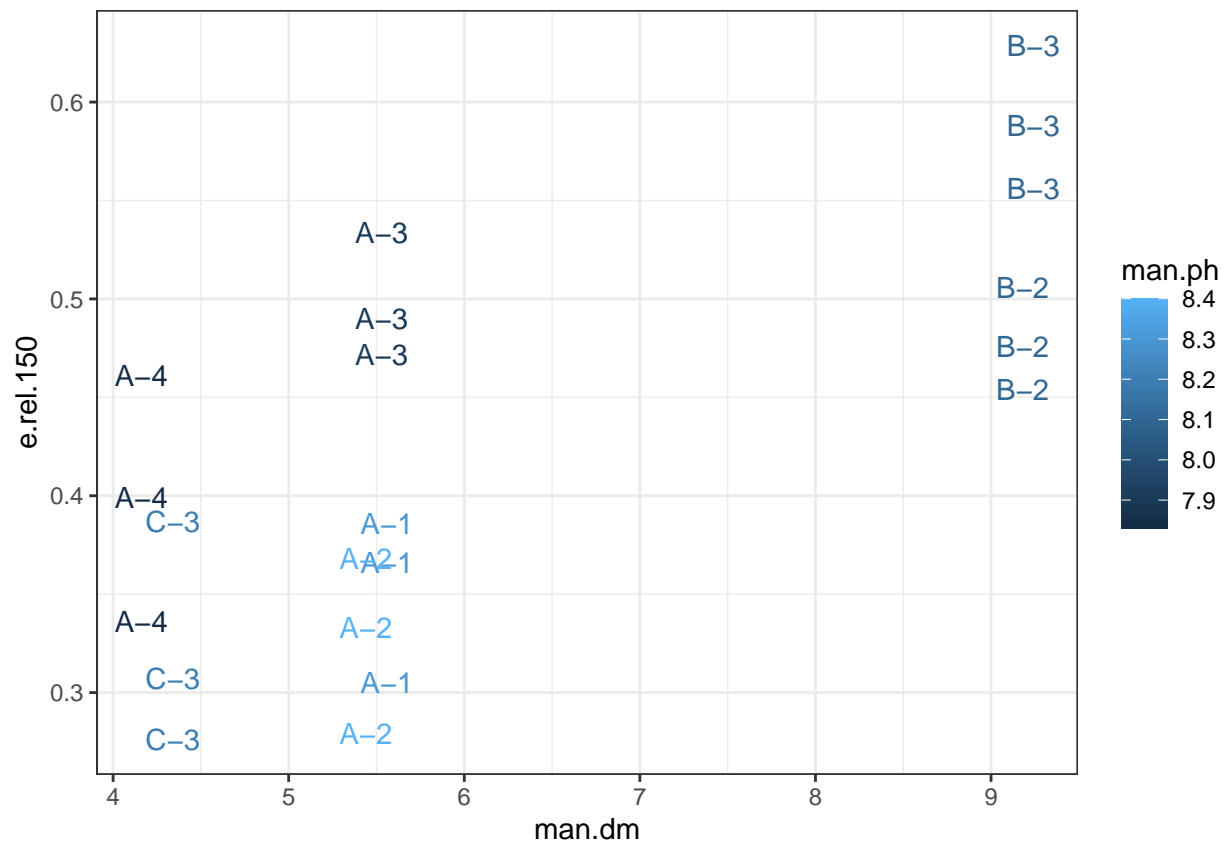
```
## Data: dref
## Models:
## m6nodm: e.rel.150 ~ (1 | dig.ID/new.ID) + man.ph
## m6: e.rel.150 ~ (1 | dig.ID/new.ID) + man.dm + man.ph
##      npar      AIC      BIC logLik deviance  Chisq Df Pr(>Chisq)
## m6nodm    5 -45.809 -40.586 27.904  -55.809
## m6        6 -54.562 -48.295 33.281  -66.562 10.753  1  0.001041 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Plots to look at apparent effects.

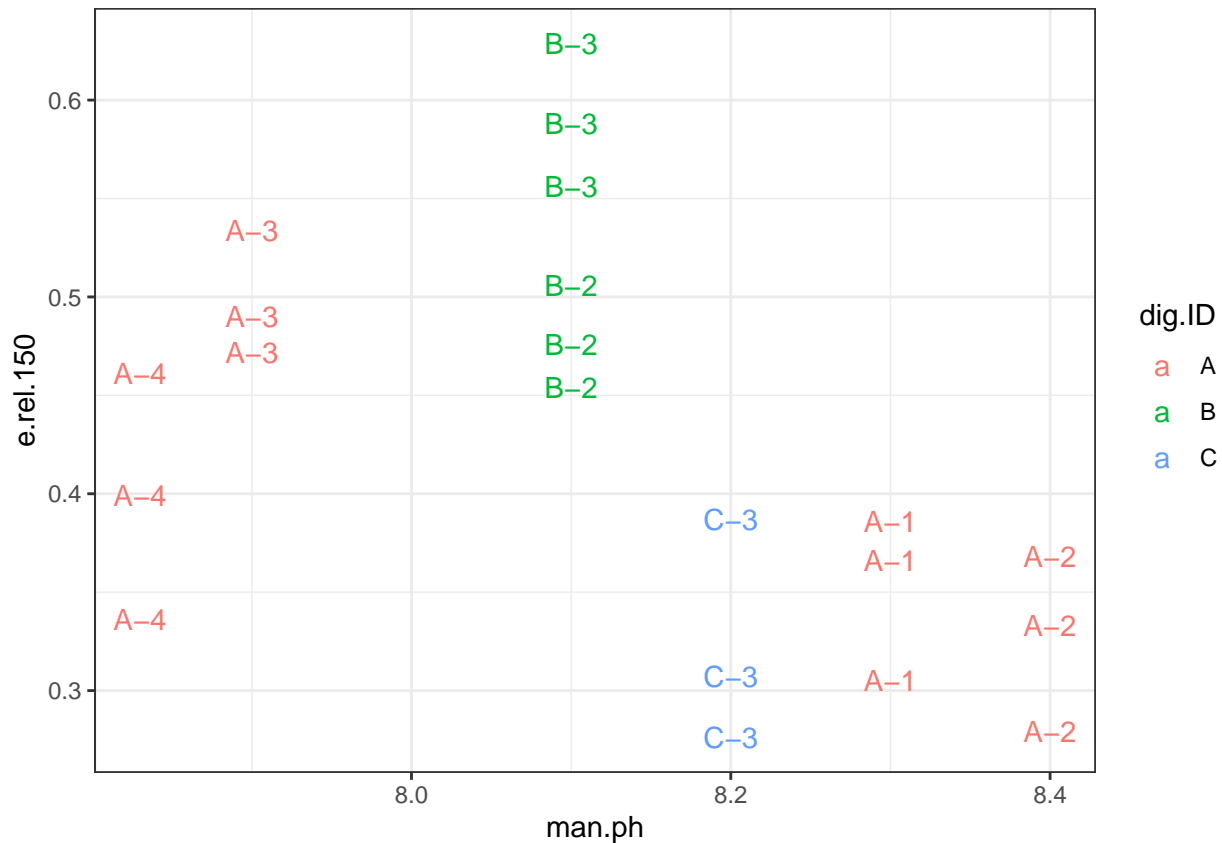
```
ggplot(dref, aes(man.dm, e.rel.150, colour = dig.ID)) +  
  geom_text(aes(label = paste0(dig.ID, '-', new.ID))) +  
  theme_bw()
```



```
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()
```



Take means—I do not believe pH result.

```
dmn <- dref[, .(e.rel.150 = mean(e.rel.150), man.dm = mean(man.dm), man.ph = mean(man.ph)),
  by = .(dig.ID, new.ID)]
```

```
m10 <- lmer(e.rel.150 ~ (1|dig.ID), data = dmn)
m10b <- lm(e.rel.150 ~ 1, data = dmn)
m11 <- lm(e.rel.150 ~ man.dm + man.ph, data = dmn)
```

```
summary(m10)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: e.rel.150 ~ (1 | dig.ID)
## Data: dmn
##
## REML criterion at convergence: -9.3
##
## Scaled residuals:
##    Min       1Q   Median       3Q      Max
## -0.9344 -0.5982 -0.2947  0.5724  1.2806
##
## Random effects:
## Groups Name Variance Std.Dev.
## dig.ID (Intercept) 0.007082 0.08415
## Residual 0.006030 0.07765
## Number of obs: 7, groups: dig.ID, 3
```

```
##
## Fixed effects:
##           Estimate Std. Error t value
## (Intercept)  0.42337    0.05855    7.231
```

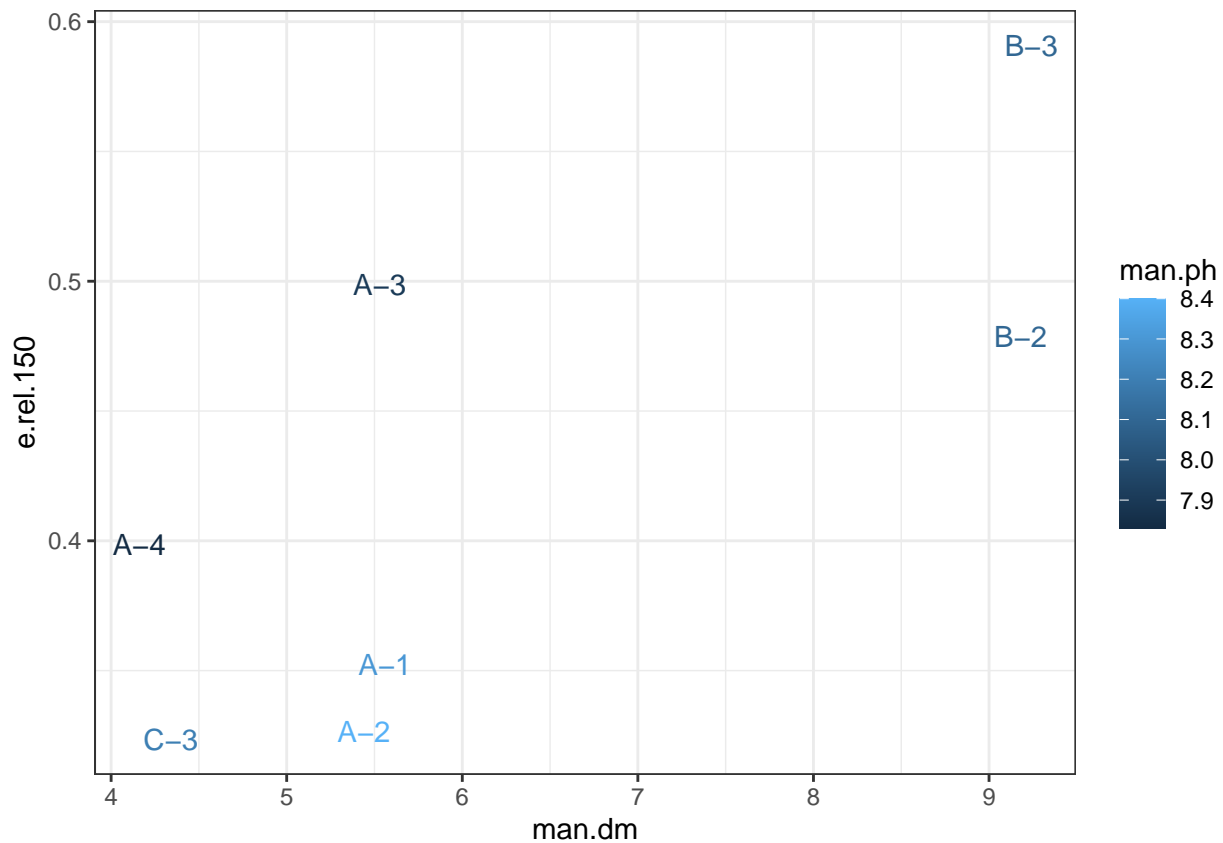
```
summary(m10b)
```

```
##
## Call:
## lm(formula = e.rel.150 ~ 1, data = dmn)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.10086 -0.08469 -0.02552  0.06448  0.16681
##
## Coefficients:
##           Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.42419    0.03837   11.05 3.26e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1015 on 6 degrees of freedom
```

```
summary(m11)
```

```
##
## Call:
## lm(formula = e.rel.150 ~ man.dm + man.ph, data = dmn)
##
## Residuals:
##      1      2      3      4      5      6      7
## 0.0009136 -0.0617638  0.0064977  0.0413327  0.0483238 -0.0091826 -0.0261214
##
## Coefficients:
##           Estimate Std. Error t value Pr(>|t|)
## (Intercept)  2.364336    0.755467   3.130  0.0352 *
## man.dm       0.037429    0.008942   4.186  0.0139 *
## man.ph      -0.267593    0.093150  -2.873  0.0453 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.04655 on 4 degrees of freedom
## Multiple R-squared:  0.8598, Adjusted R-squared:  0.7897
## F-statistic: 12.27 on 2 and 4 DF,  p-value: 0.01965
```

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



Trials 5-10 (app tech on winter wheat)

Subset.

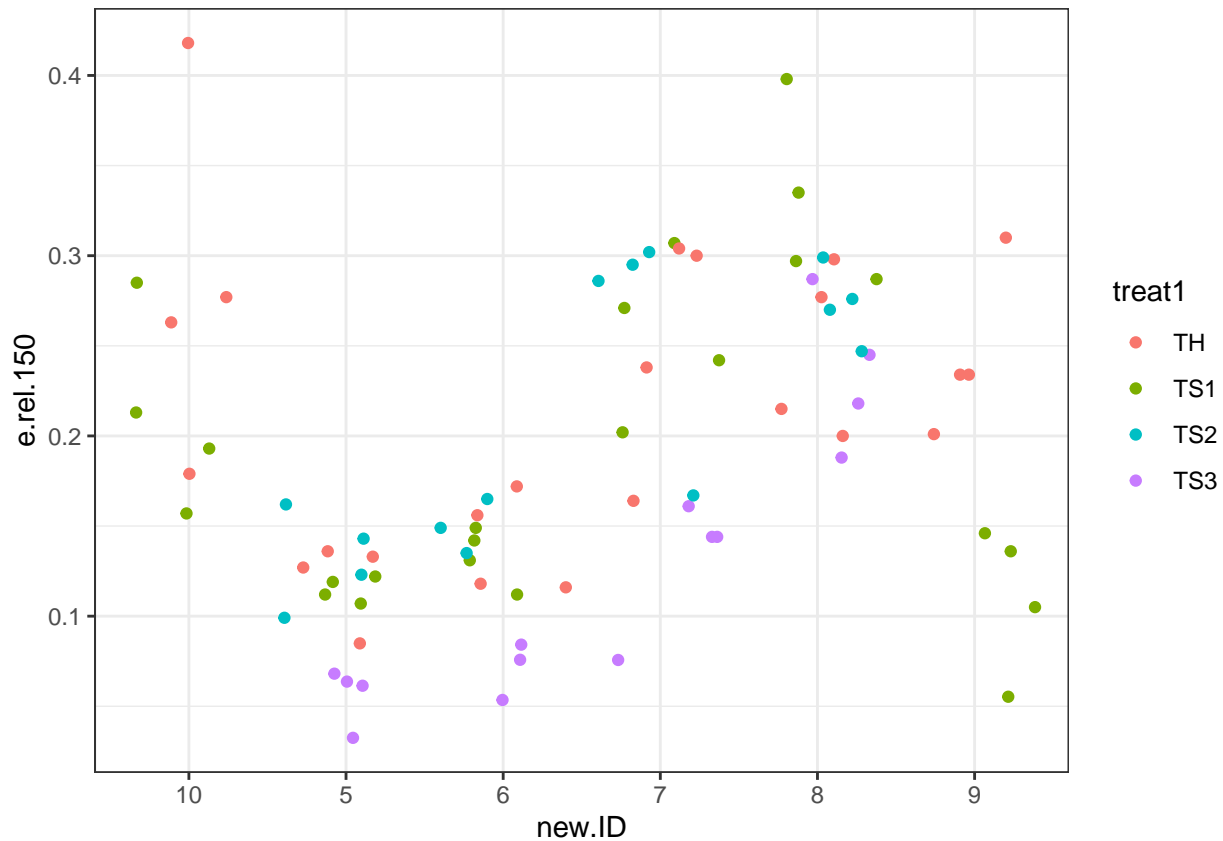
```
isumm1 <- droplevels(isumm[new.ID %in% c('5', '6', '7', '8', '9', '10') & !treat1 %in% c('TH-4', 'TS1-4')])

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.

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

```
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.
```

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

And get a column for copy/paste into paper.

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

Add 11-12

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

```
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.

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

```
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)])
```

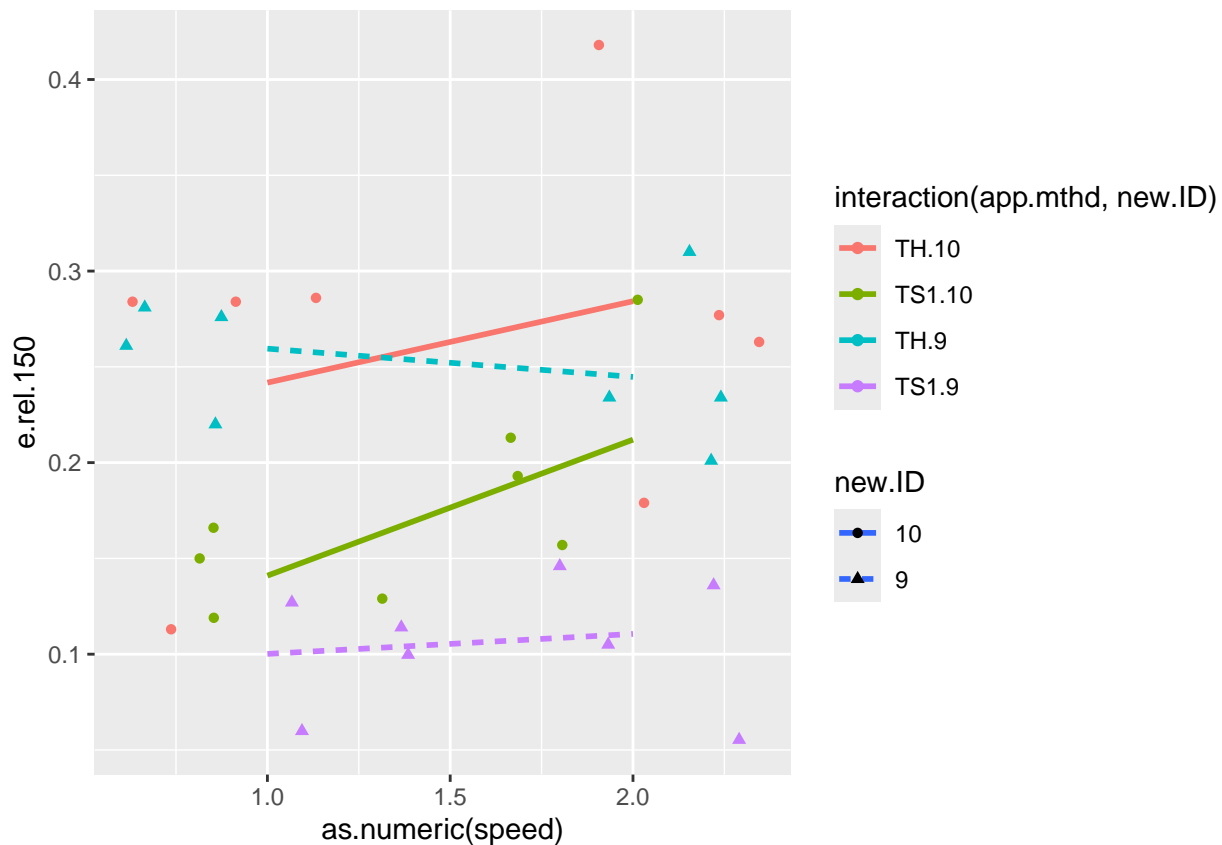
```
## , , speed = 4
##
##      app.mthd
## new.ID TH TS1
##     10  4  4
##      9  4  4
##
## , , speed = 12
##
##      app.mthd
## new.ID TH TS1
##     10  4  4
##      9  4  4
```

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')
```

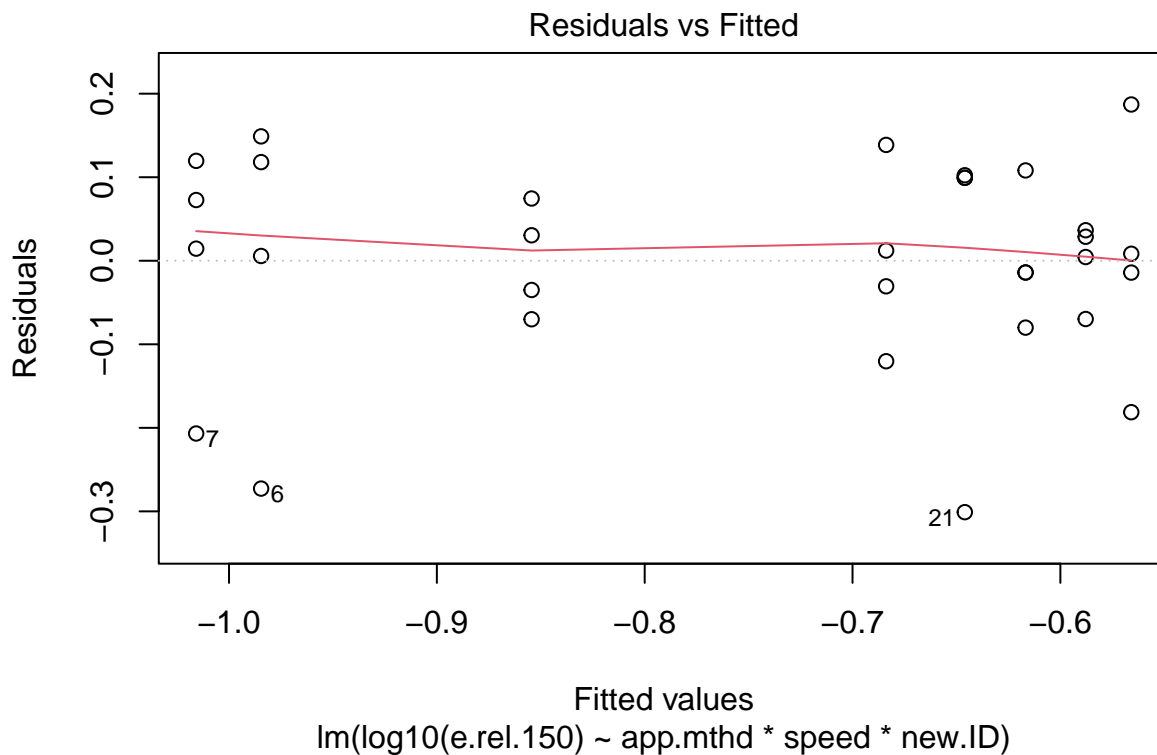
```
## Single term deletions
```

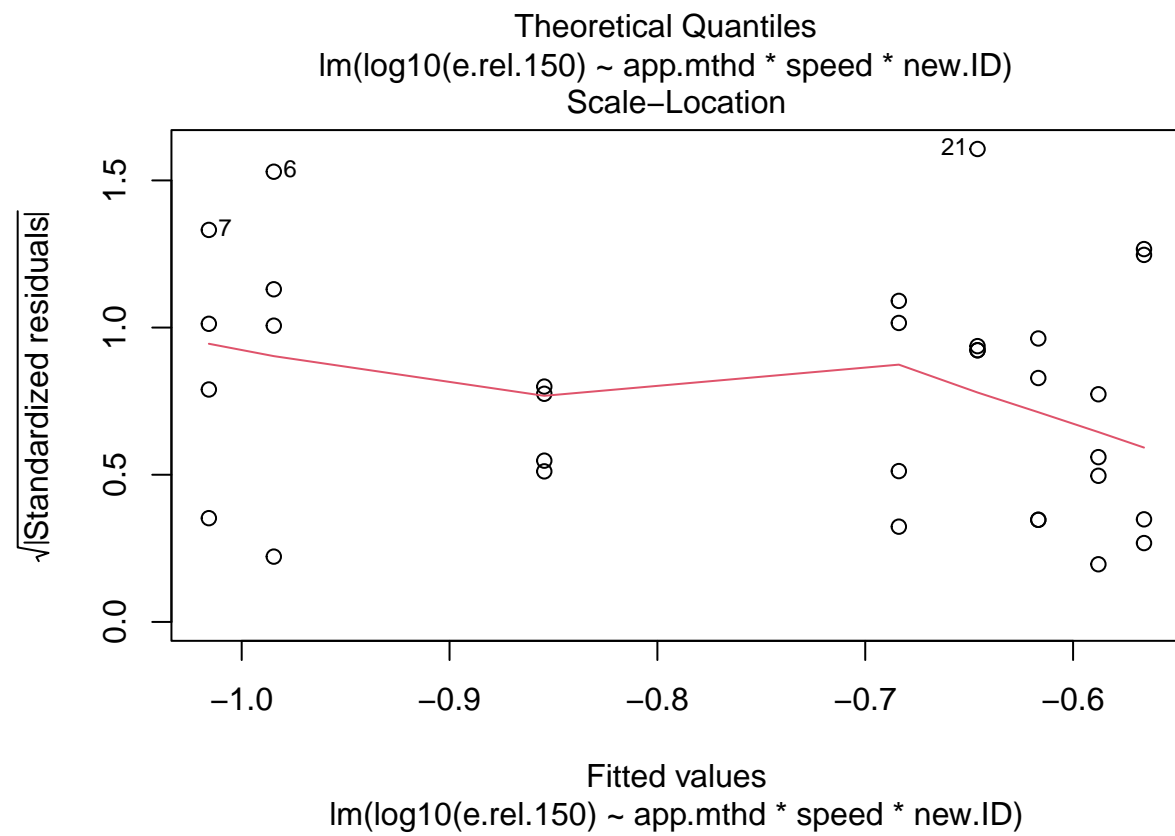
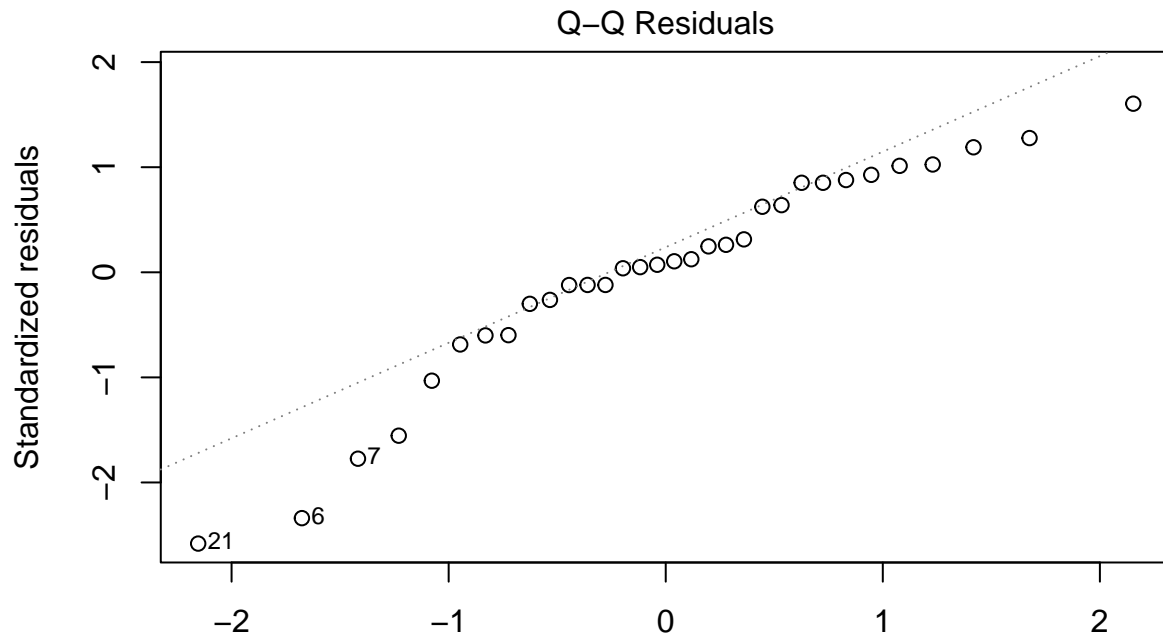
```
##
## Model:
## e.rel.150 ~ (app.mthd + speed + new.ID)^2
##           Df Sum of Sq      RSS       AIC F value Pr(>F)
## <none>                0.077852 -178.60
## app.mthd:speed    1 0.0014405 0.079292 -180.01   0.4626 0.5027
## app.mthd:new.ID   1 0.0072631 0.085115 -177.74   2.3324 0.1393
## speed:new.ID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

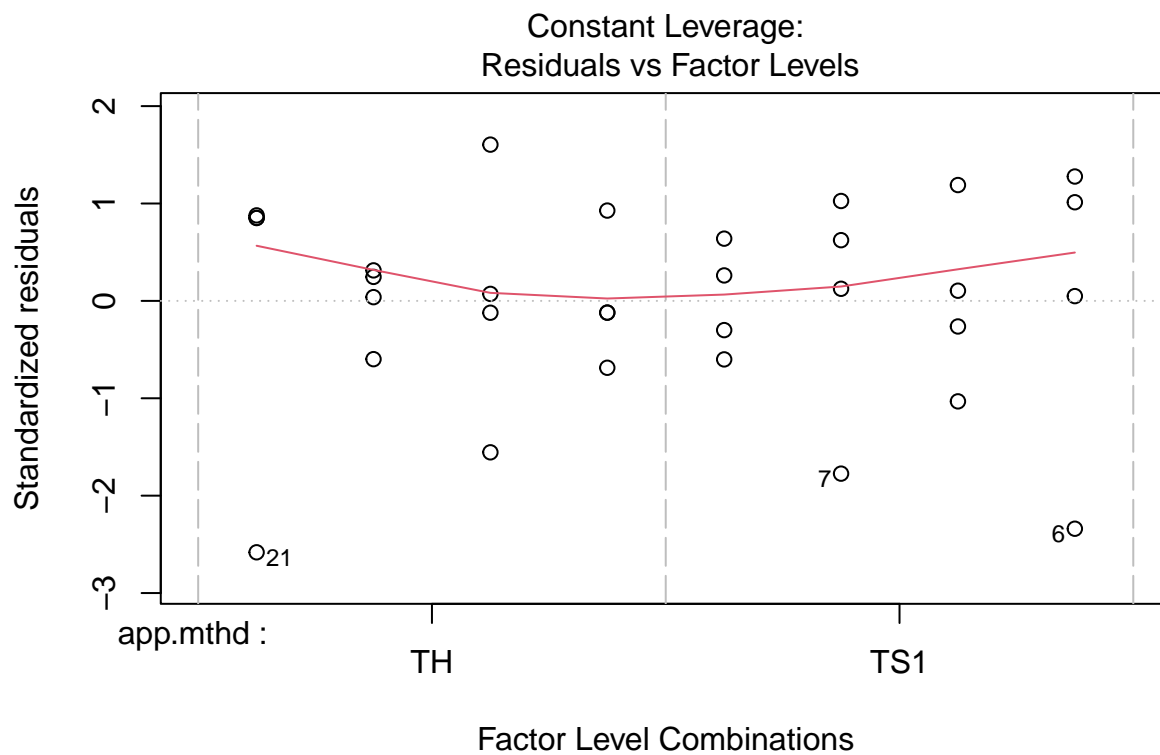
```
summary(m3, test = 'F')
```

```
##           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
```

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







```
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
```

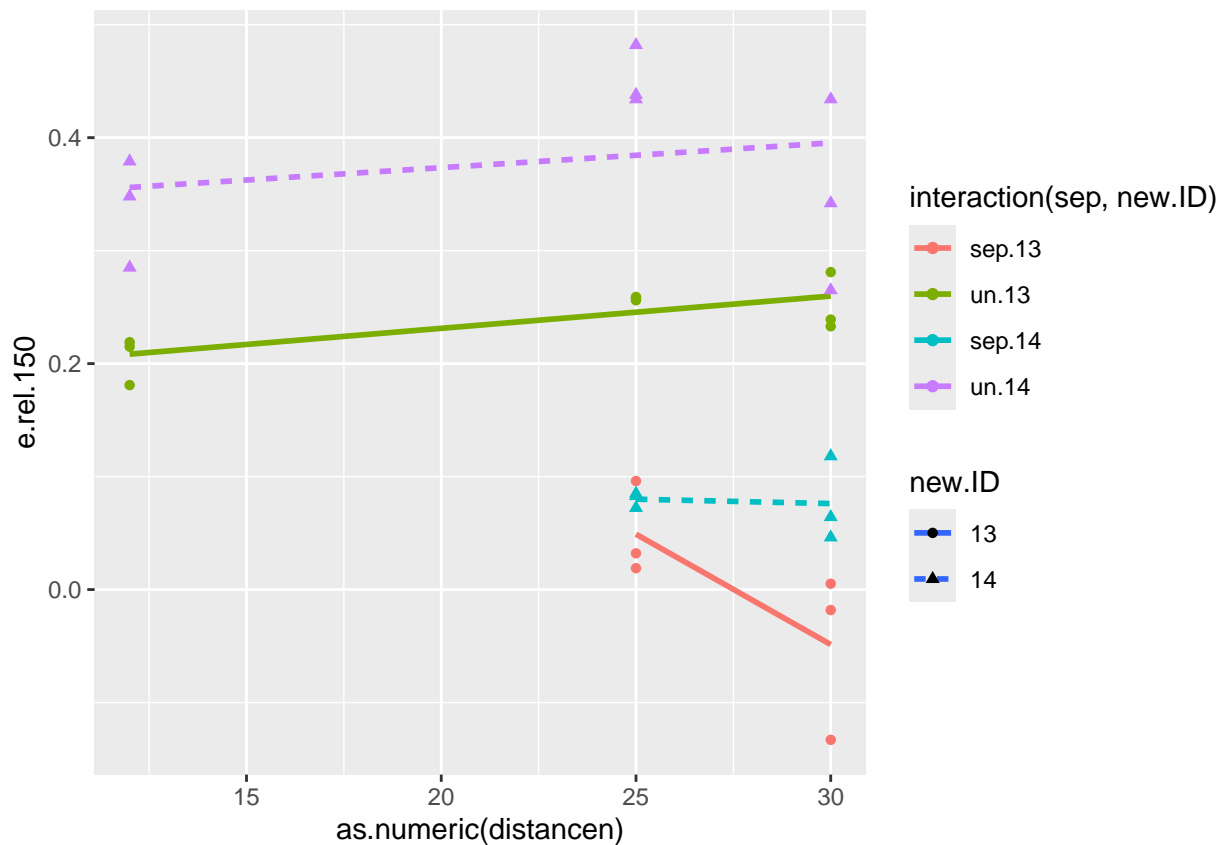
Subset is completely crossed, perfectly balanced. But un has an additional distance of 12 cm.

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
```

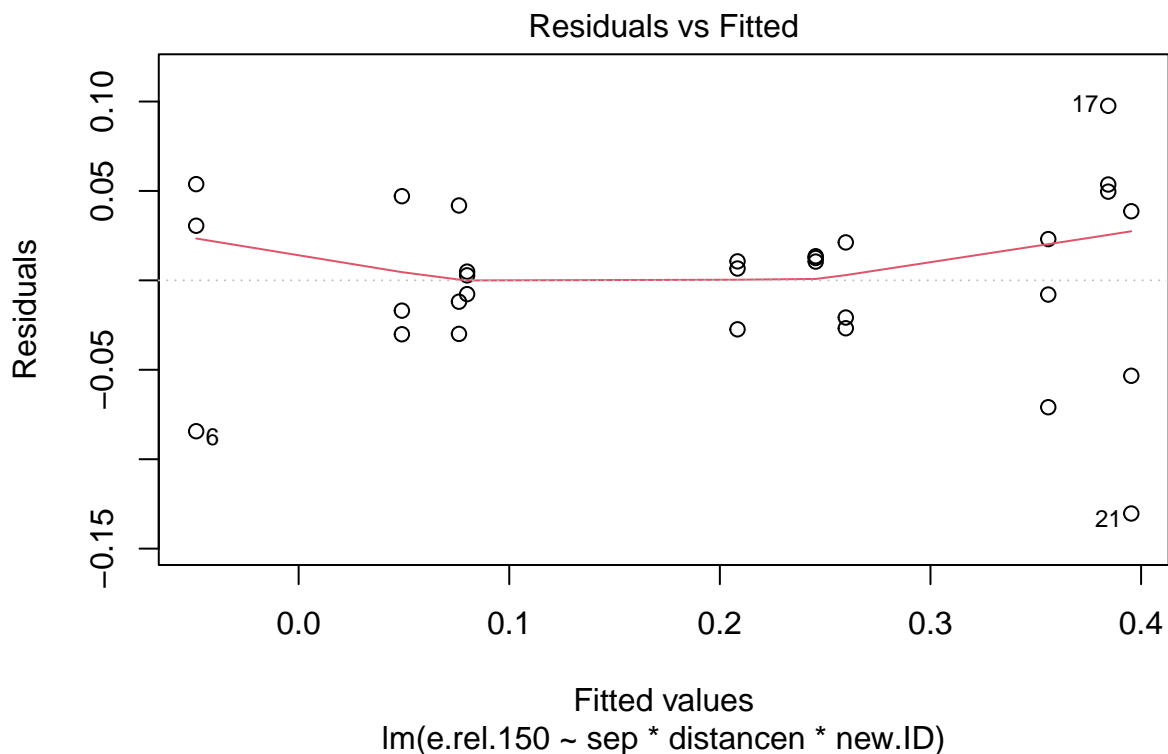
```
summary(m2b)
```

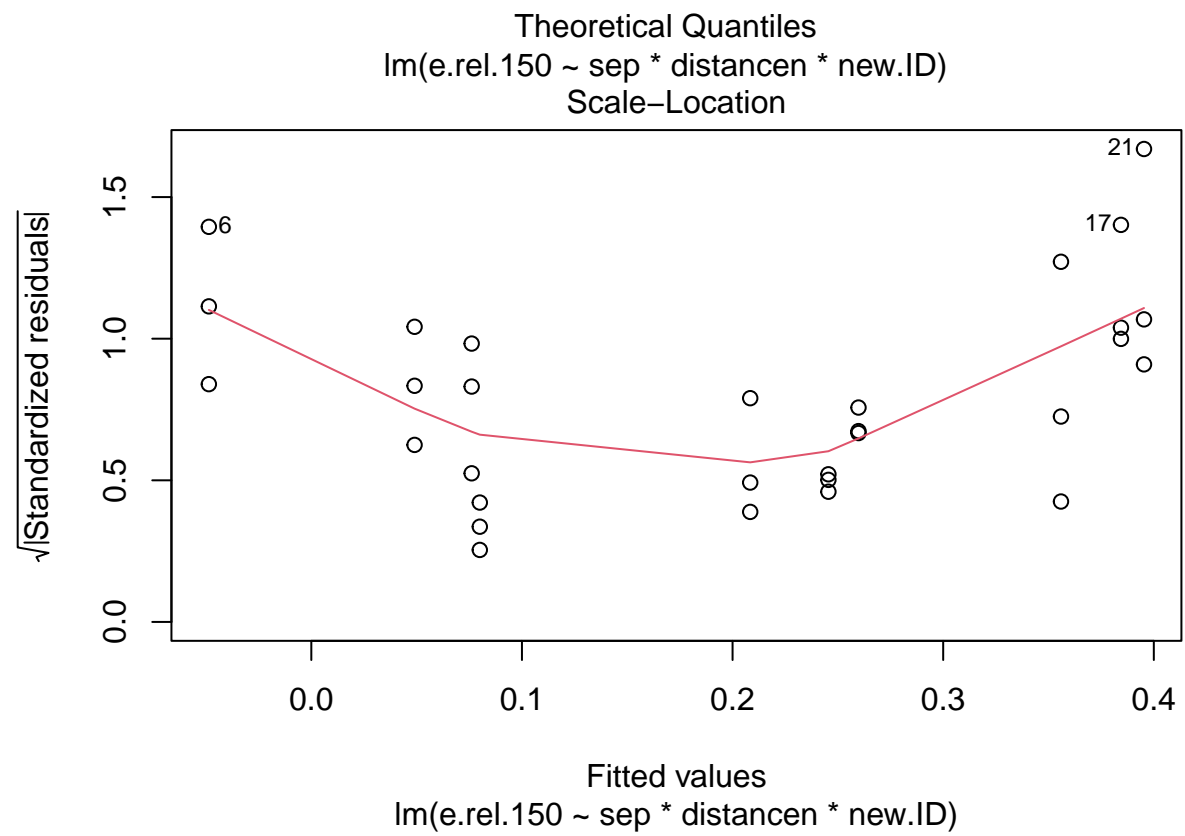
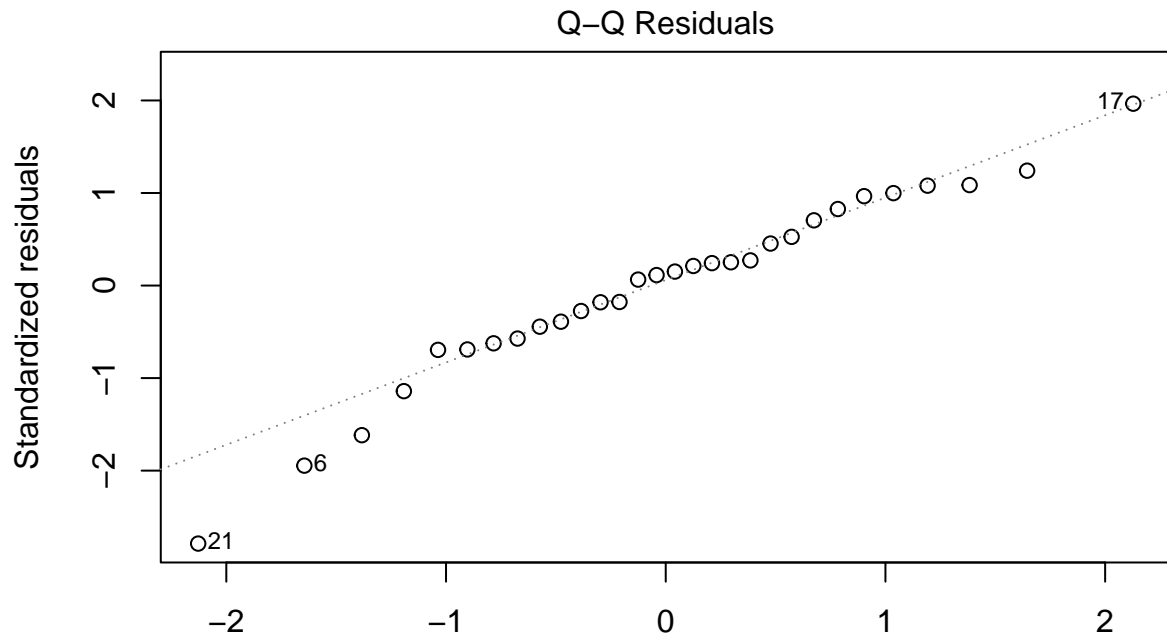
```
##
```

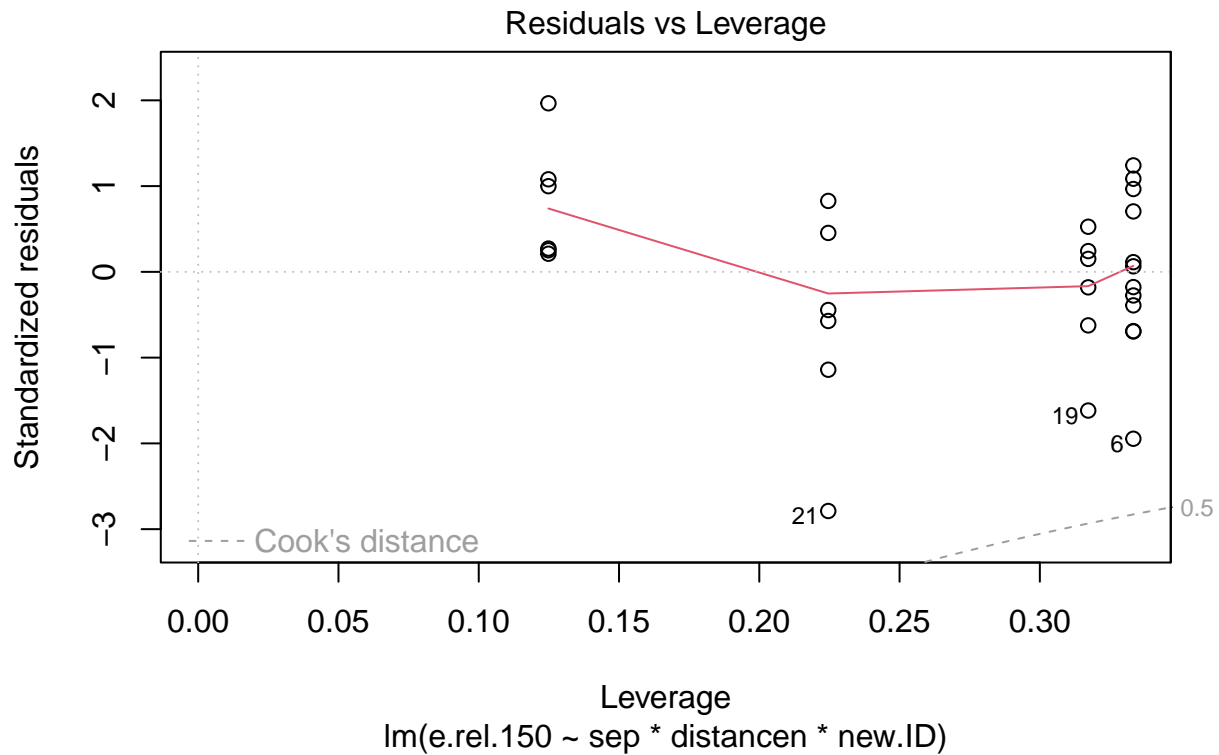
```
## Call:
## lm(formula = e.rel.150 ~ (sep + distancen + new.ID)^2, data = isumm1,
##     subset = sep != "sep" | new.ID != 13)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.130344 -0.022284  0.005761  0.021680  0.097605
##
## Coefficients: (1 not defined because of singularities)
##              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)
```







Means.

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

##	new.ID	sep	distancen	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.

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

Set reference.

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

Fit model.


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

Tukey's test.

```
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
```

```
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
```

Dunnnett test.

```
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
```

```
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: dunnett method for 2 tests
```

```
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.

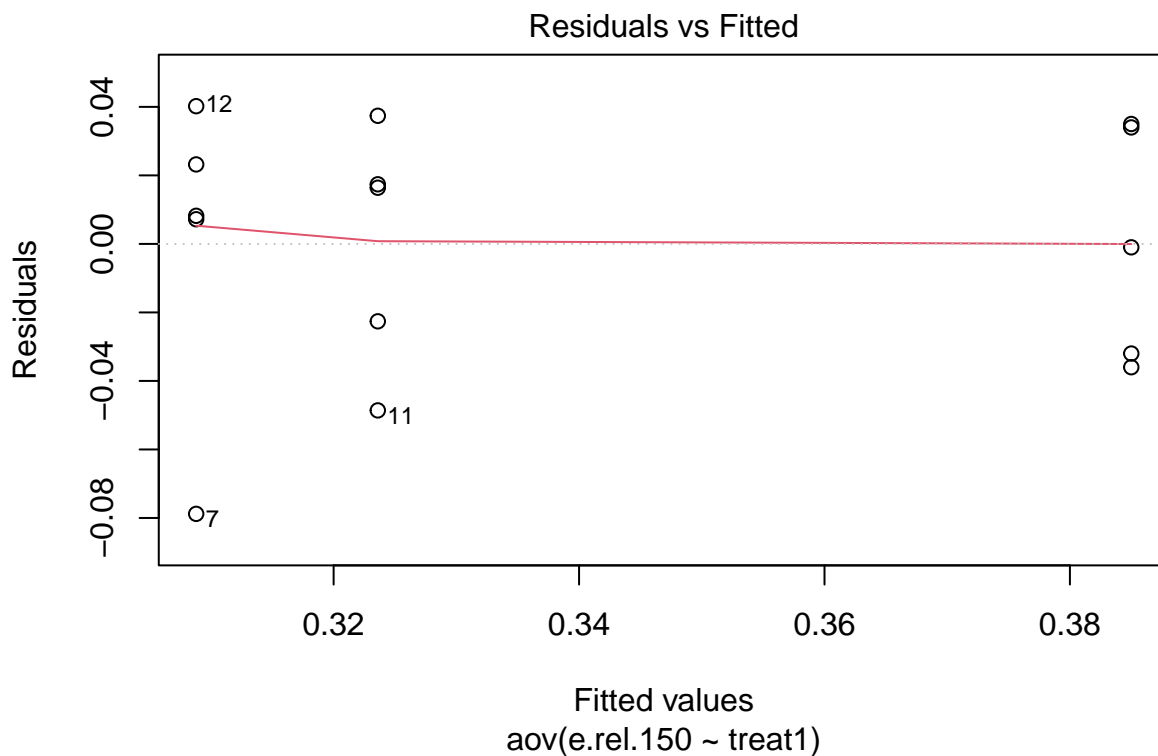
```
coef(m1)
```

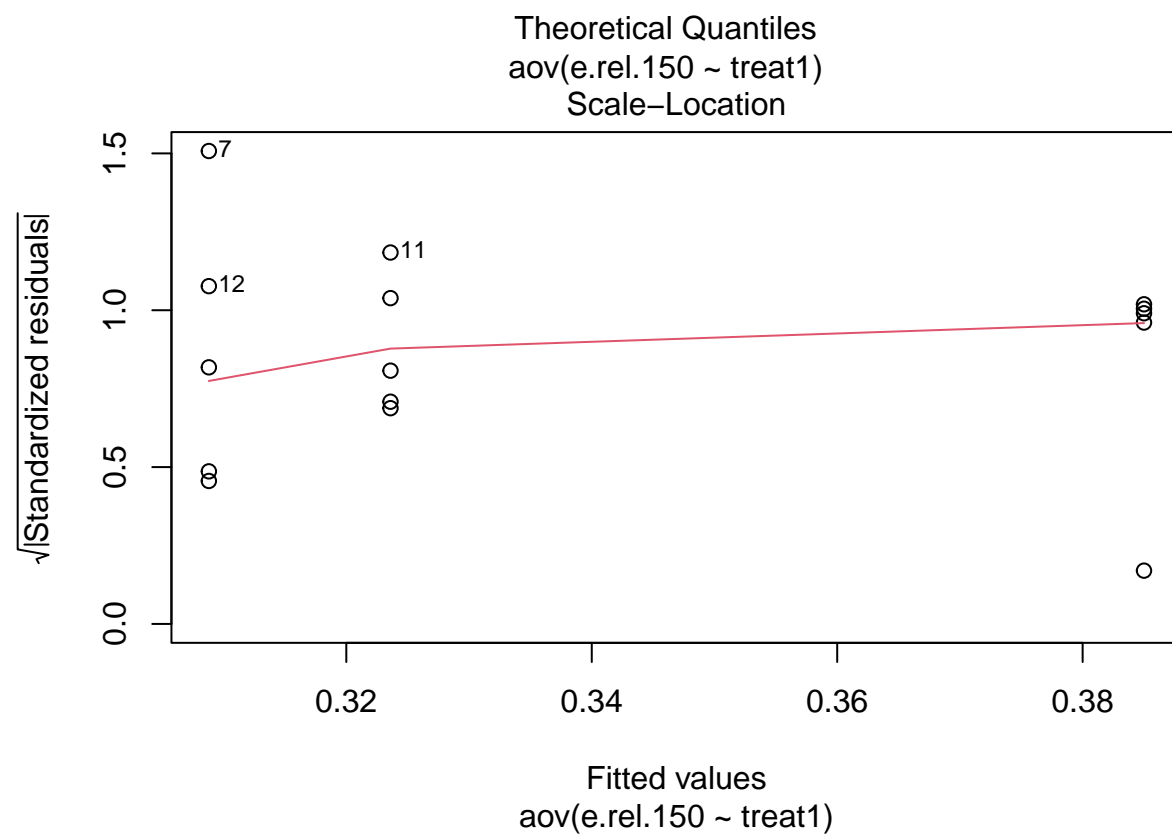
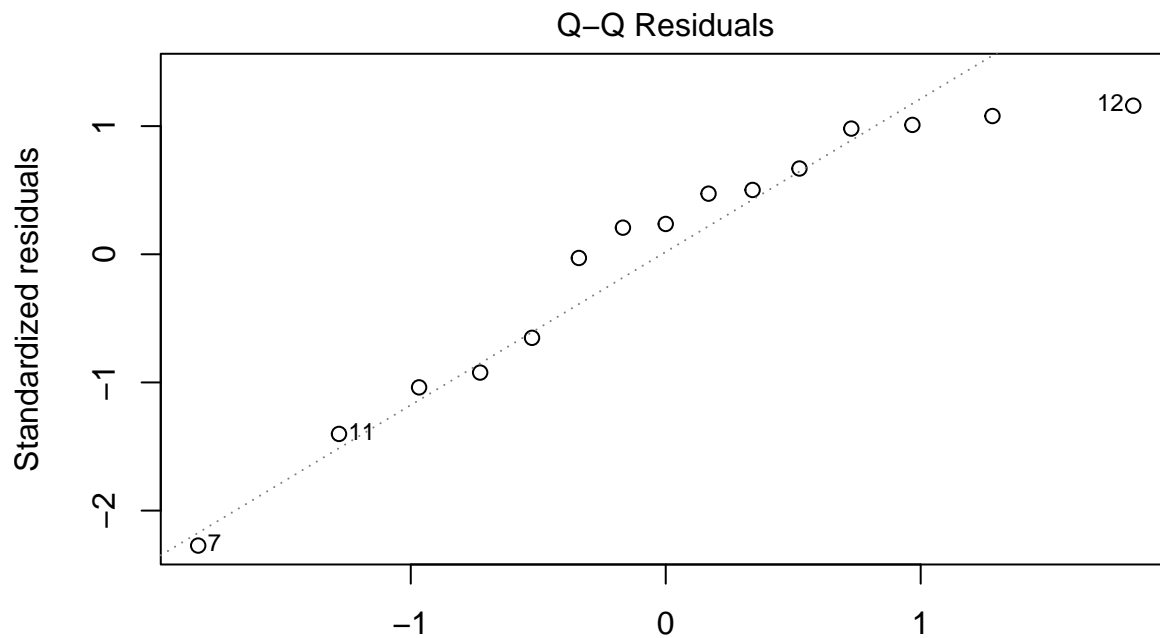
```
## (Intercept) treat12-Pos treat17-Pos
##      0.3088      0.0148      0.0762
```

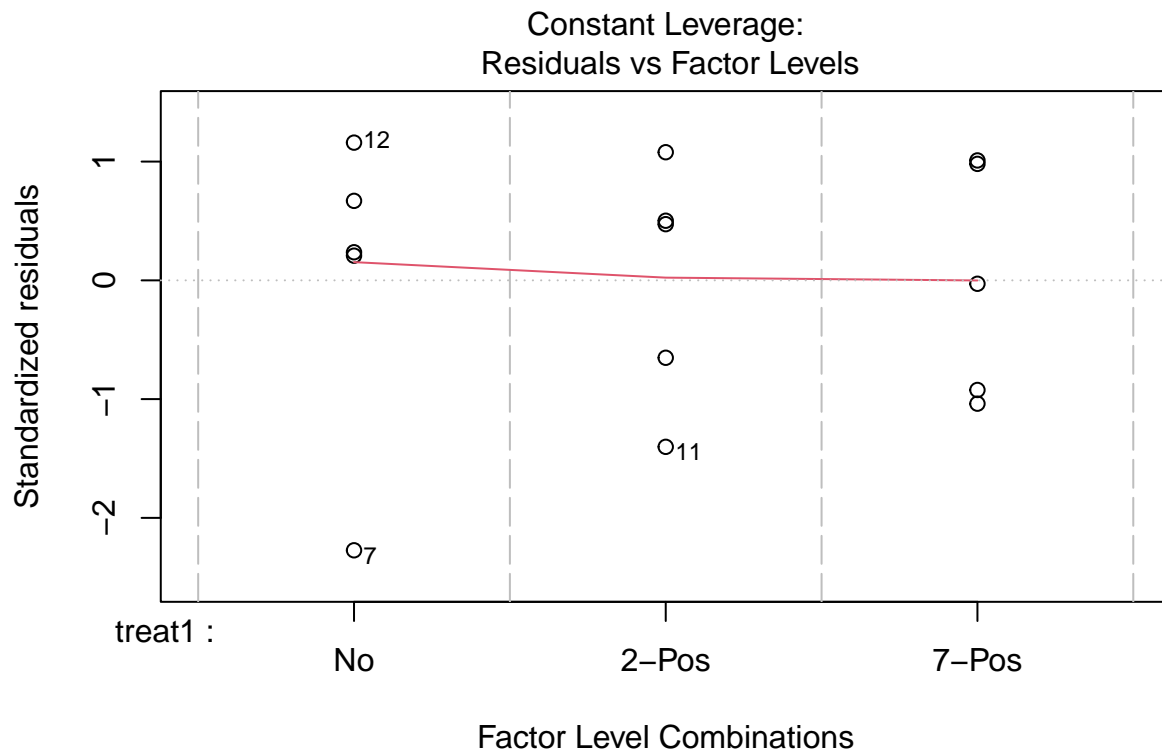
```
coef(m1) / coef(m1)[1]
```

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

```
plot(m1)
```







Other stuff

I still need to sort this stuff out!

Then temperature stuff Add to supporting material