Data analysis for digestate experiments

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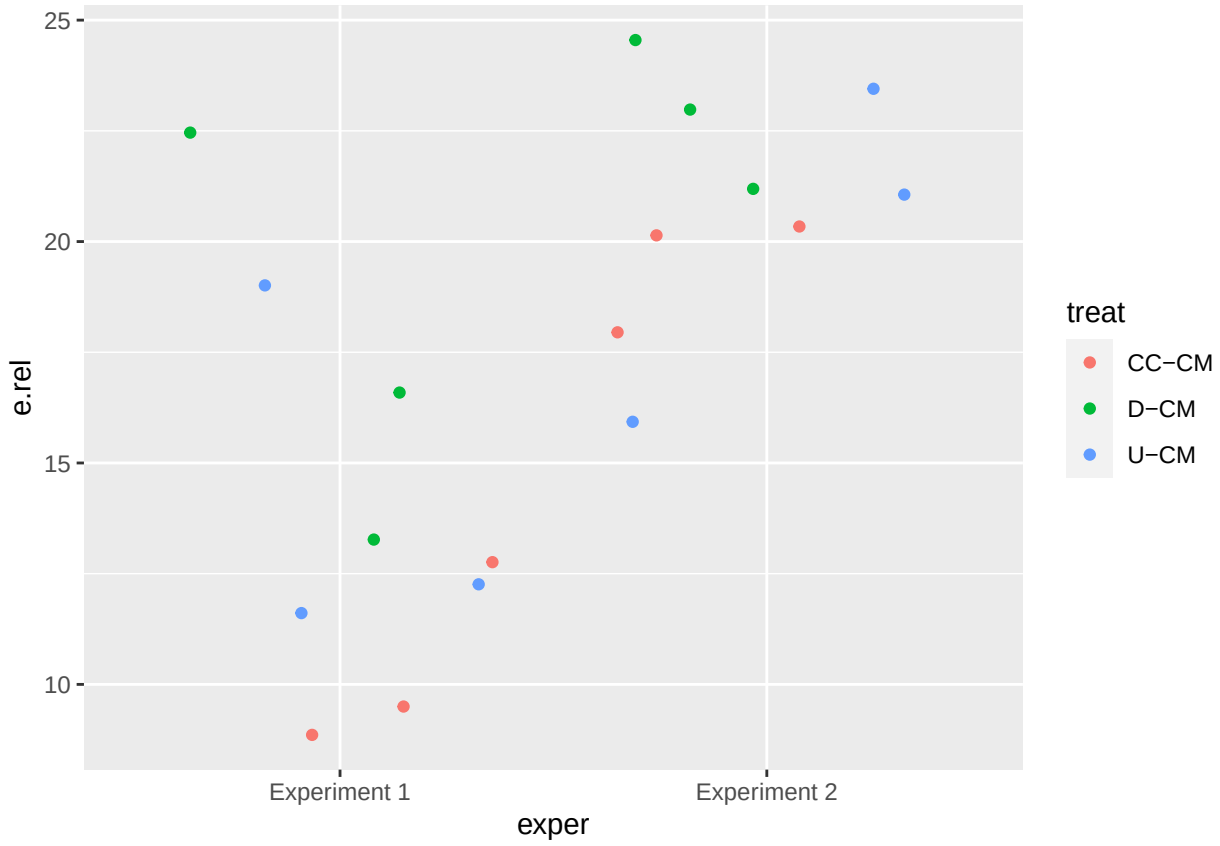
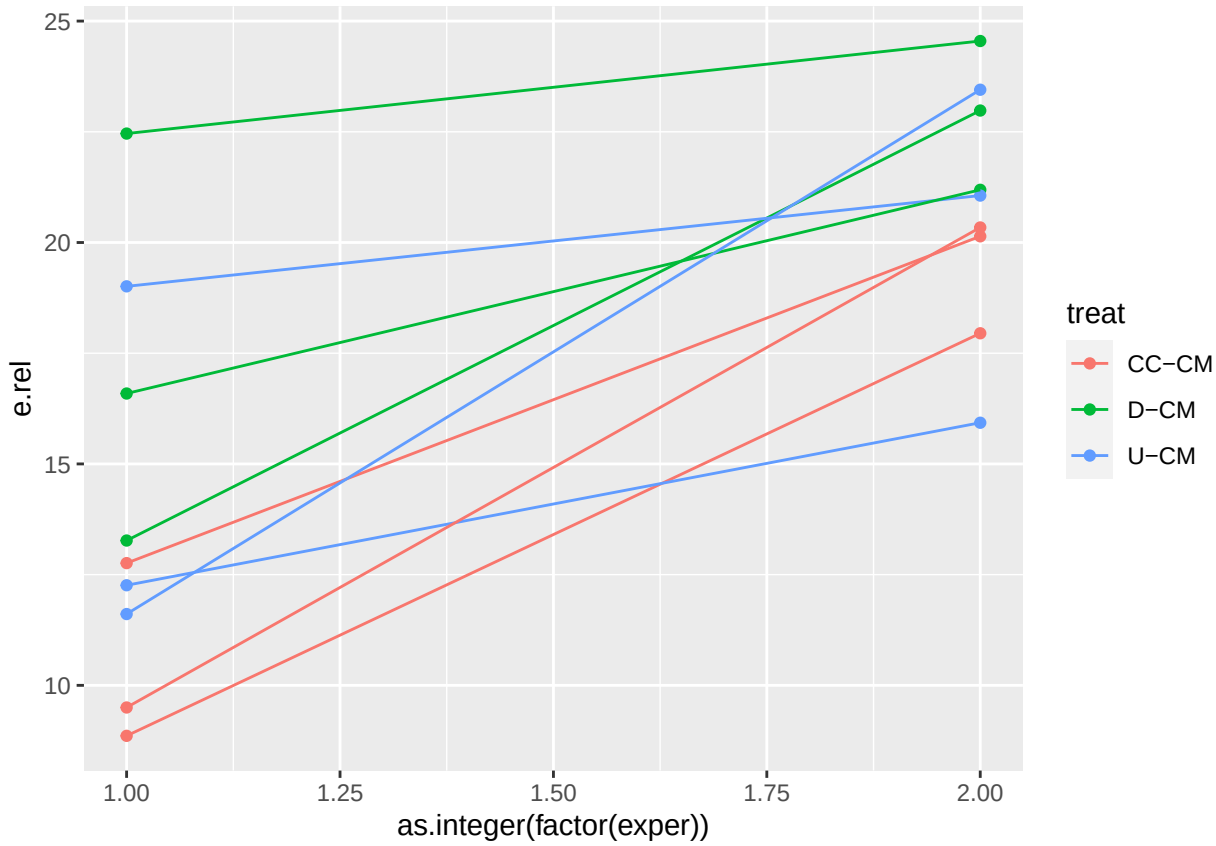
NH3 plots

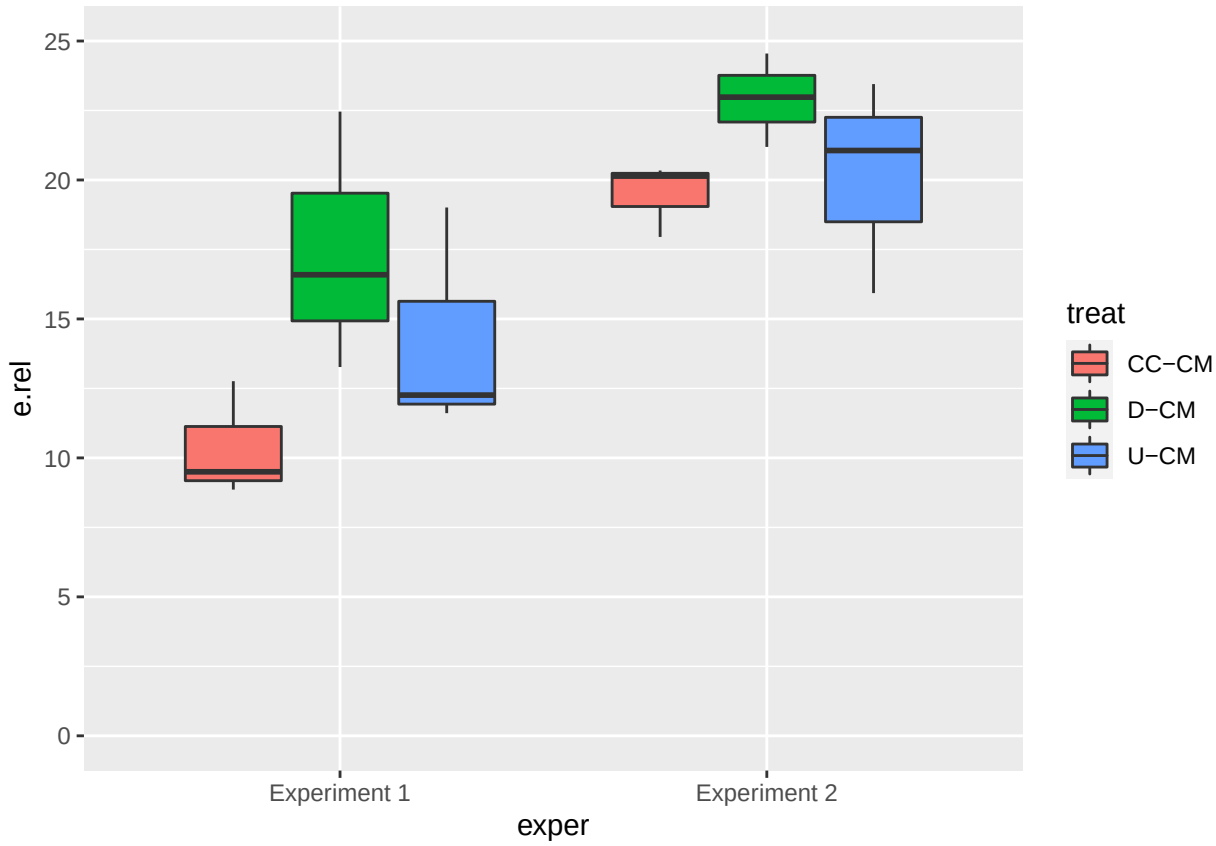
```
ggplot(dat, aes(exper, e.rel, colour = treat)) +
  geom_jitter(height = 0)
```




```
ggplot(dat, aes(as.integer(factor(exper)), e.rel, colour = treat, group = interaction(treat, rep))) +
  geom_point() +
  geom_line()
```




```
ggplot(dat, aes(exper, e.rel, fill = treat)) +
  geom_boxplot() +
  ylim(0, 25)
```



NH3 stats

Set reference to untreated cattle manure.

```
dat$treat <- factor(dat$treat, levels = c('U-CM', 'CC-CM', 'D-CM'))
dat$exper <- factor(dat$exper)
```

```
m1 <- aov(e.rel ~ treat * exper, data = dat)
summary(m1)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## treat        2  83.07   41.53   4.009 0.046418 *
## exper        1 208.56  208.56  20.128 0.000744 ***
## treat:exper  2  11.97    5.98   0.578 0.576130
## Residuals   12 124.34   10.36
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary.lm(m1)
```

```
##
## Call:
## aov(formula = e.rel ~ treat * exper, data = dat)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.2167 -1.6692 -0.3883  1.4608  5.0200
##
```

```
## Coefficients:
##                               Estimate Std. Error t value Pr(>|t|)
## (Intercept)                    14.2933     1.8584   7.691 5.61e-06 ***
## treatCC-CM                     -3.9200     2.6282  -1.491   0.1616
## treatD-CM                       3.1467     2.6282   1.197   0.2543
## experExperiment 2               5.8533     2.6282   2.227   0.0459 *
## treatCC-CM:experExperiment 2    3.2500     3.7169   0.874   0.3991
## treatD-CM:experExperiment 2    -0.3867     3.7169  -0.104   0.9189
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.219 on 12 degrees of freedom
## Multiple R-squared:  0.7094, Adjusted R-squared:  0.5884
## F-statistic:  5.86 on 5 and 12 DF,  p-value: 0.005746
```

No need to look at interaction.

```
m2 <- aov(e.rel ~ treat + exper, data = dat)
summary(m2)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## treat        2  83.07   41.53   4.266 0.035753 *
## exper        1 208.56  208.56  21.421 0.000391 ***
## Residuals   14 136.31    9.74
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary.lm(m2)
```

```
##
## Call:
## aov(formula = e.rel ~ treat + exper, data = dat)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.6939 -2.1599 -0.2792  1.6681  5.6906
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)         13.816      1.471   9.393 2.02e-07 ***
## treatCC-CM          -2.295      1.801  -1.274 0.223428
## treatD-CM            2.953      1.801   1.639 0.123404
## experExperiment 2    6.808      1.471   4.628 0.000391 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.12 on 14 degrees of freedom
## Multiple R-squared:  0.6815, Adjusted R-squared:  0.6132
## F-statistic: 9.984 on 3 and 14 DF,  p-value: 0.0008875
```

```
m3 <- aov(log10(e.rel) ~ treat * exper, data = dat)
summary(m3)
```

```
##             Df  Sum Sq Mean Sq F value   Pr(>F)
## treat        2 0.06369 0.03184   4.396 0.036958 *
## exper        1 0.15725 0.15725  21.708 0.000552 ***
## treat:exper  2 0.01940 0.00970   1.339 0.298643
```

```
## Residuals   12 0.08692 0.00724
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
m4 <- aov(log10(e.rel) ~ treat + exper, data = dat)
summary(m4)
```

```
##             Df  Sum Sq Mean Sq F value   Pr(>F)
## treat        2 0.06369 0.03184   4.193 0.037415 *
## exper        1 0.15725 0.15725  20.706 0.000453 ***
## Residuals   14 0.10632 0.00759
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

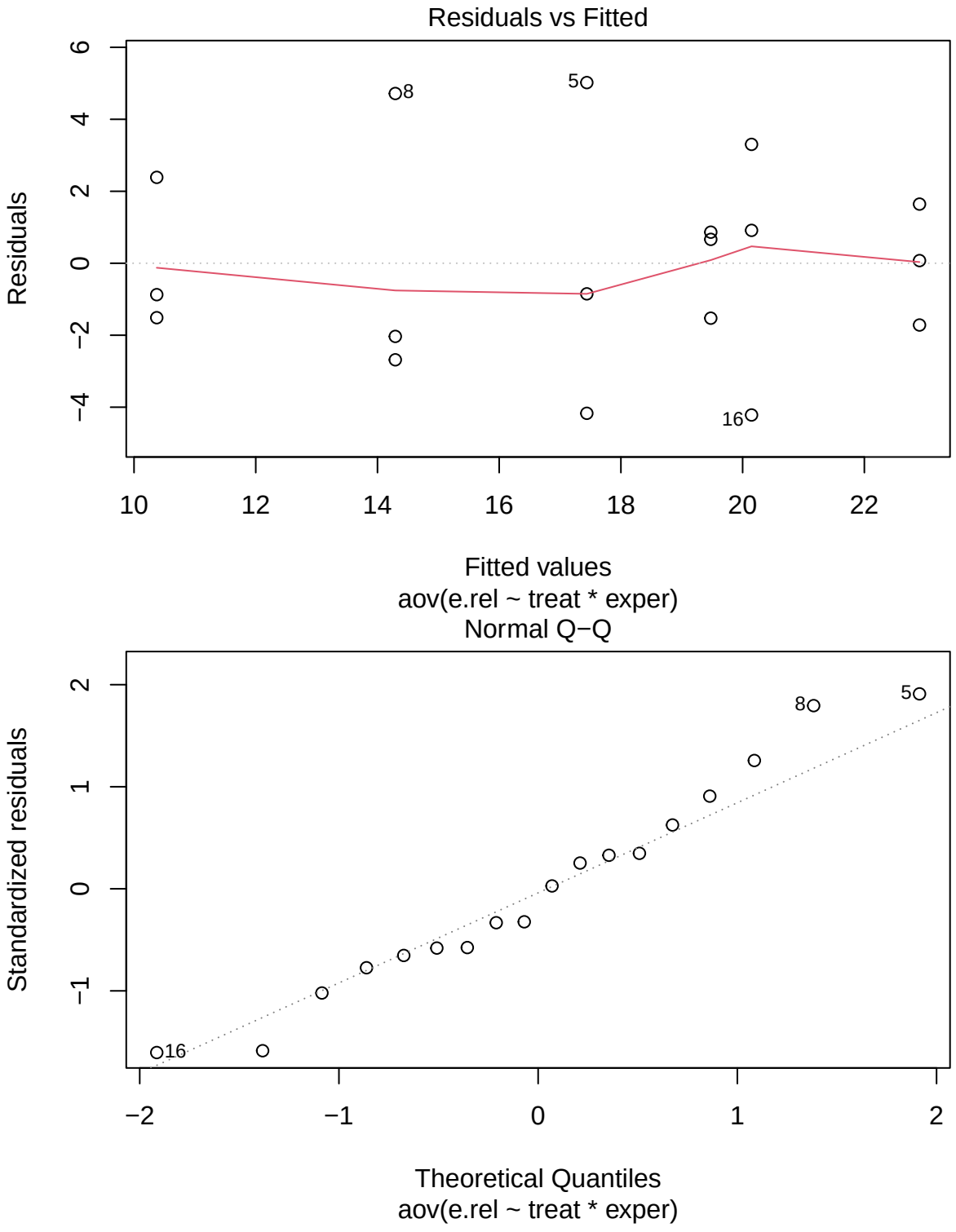
```
summary.lm(m4)
```

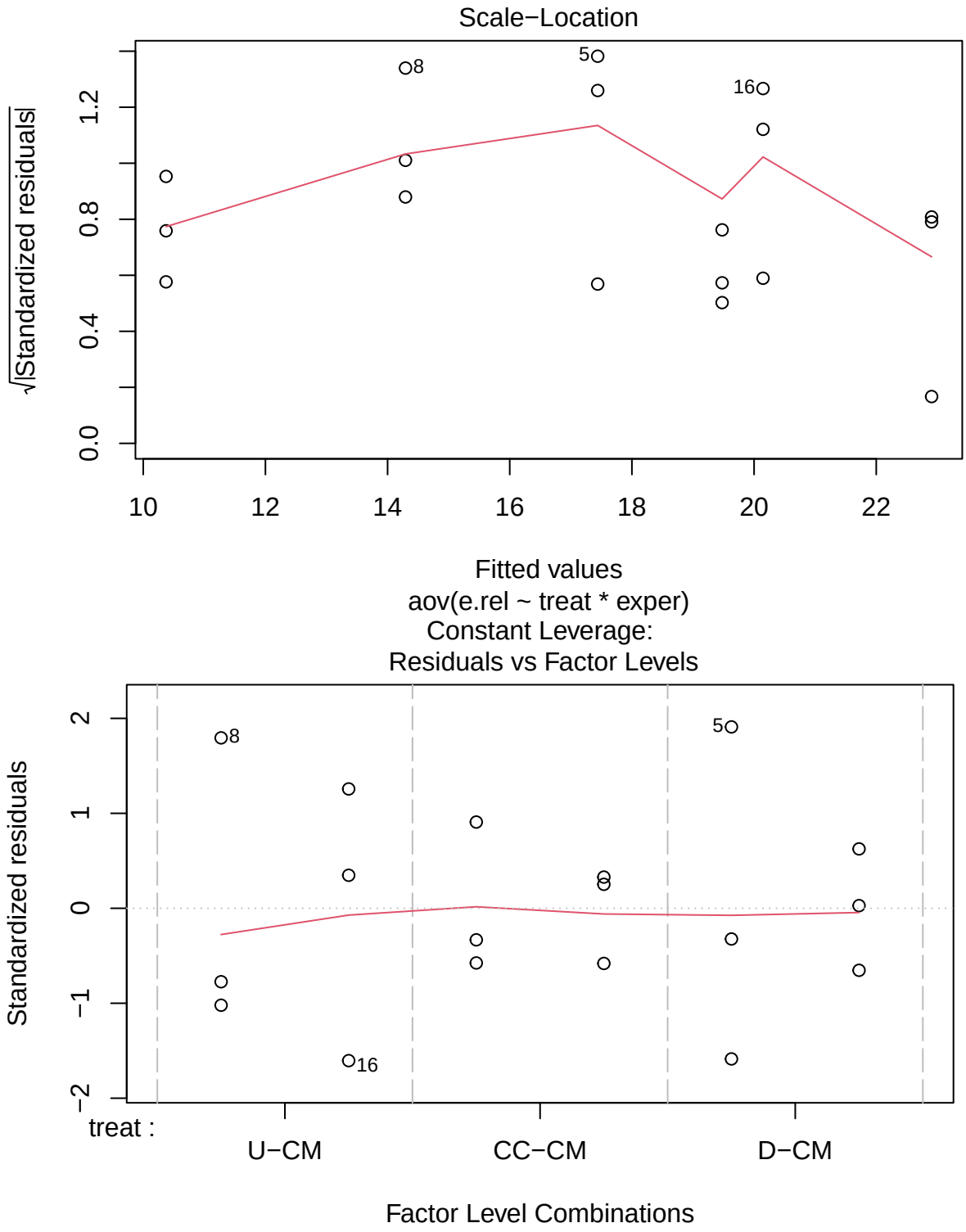
```
##
## Call:
## aov(formula = log10(e.rel) ~ treat + exper, data = dat)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.112604 -0.062945  0.004974  0.053927  0.151094
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        1.12789    0.04108  27.455 1.42e-13 ***
## treatCC-CM        -0.07177    0.05031  -1.427 0.175637
## treatD-CM          0.07392    0.05031   1.469 0.163876
## experExperiment 2  0.18693    0.04108   4.550 0.000453 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.08715 on 14 degrees of freedom
## Multiple R-squared:  0.6751, Adjusted R-squared:  0.6055
## F-statistic: 9.697 on 3 and 14 DF,  p-value: 0.001015
```

CC-CM and D-CM are clearly different. But neither is clearly different from the reference. Makes interpretation just a bit tricky but not terrible. Some evidence of a difference but presumably digestion pH effect is moderated by low DM, and variability was high, so power is low.

Check residuals.

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

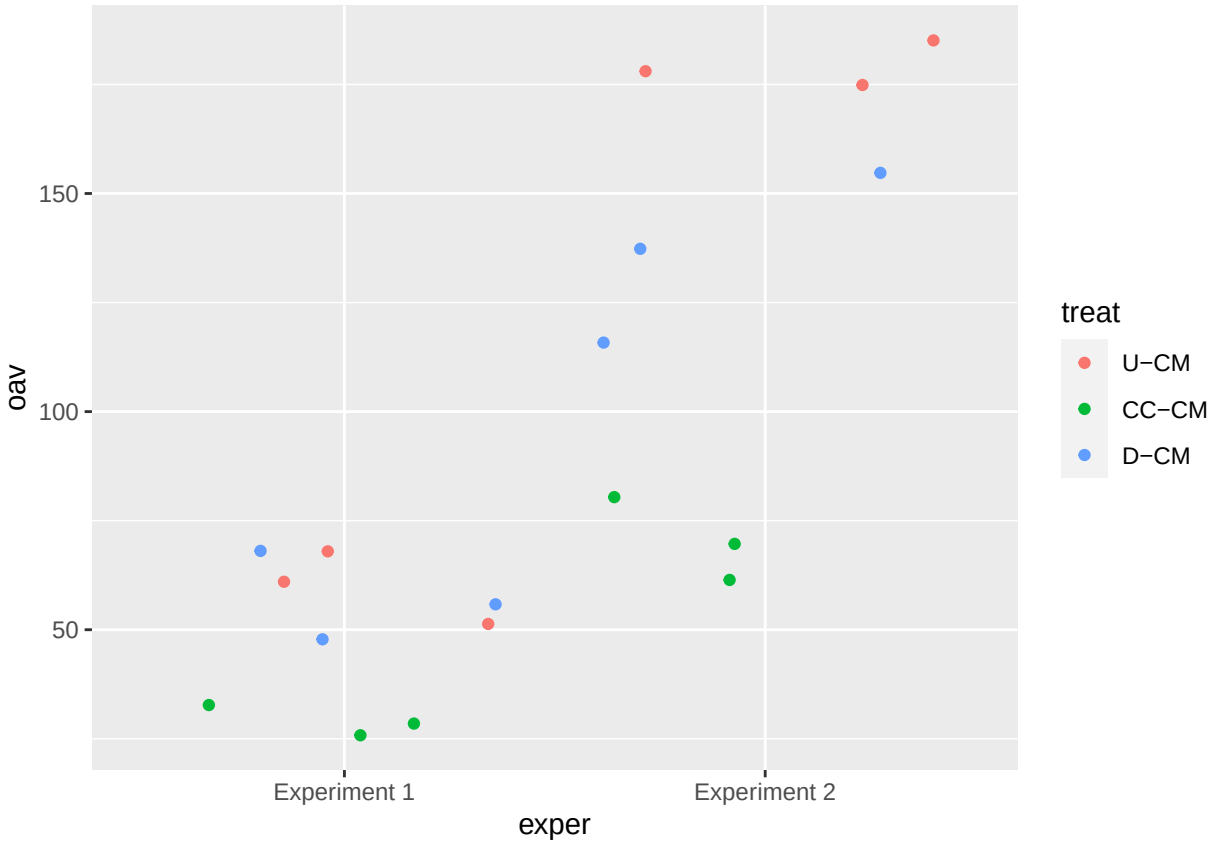

Residuals vs Fitted
Residuals
Fitted values
aov(e.rel ~ treat * exper)
Normal Q−Q
Standardized residuals
Theoretical Quantiles
aov(e.rel ~ treat * exper)



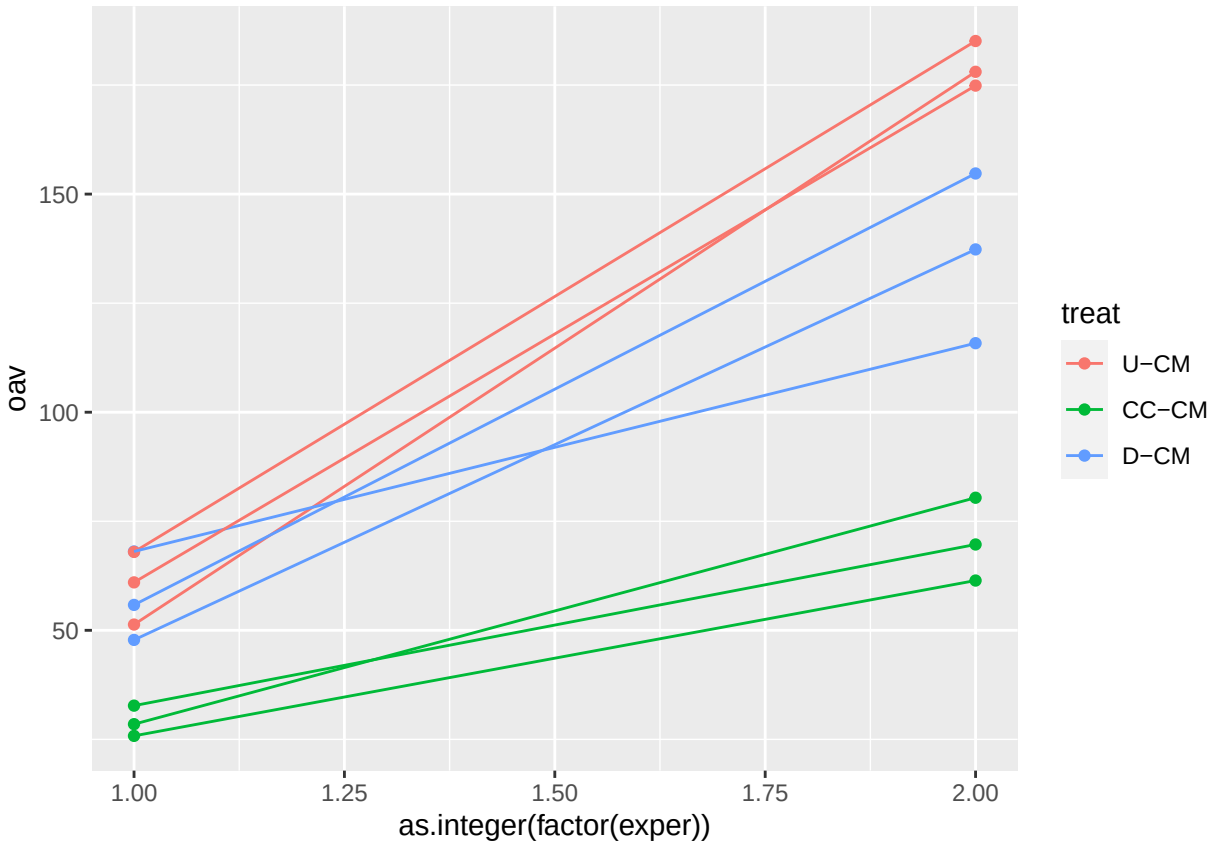
Looks fine, no need to transform.

OAV plots

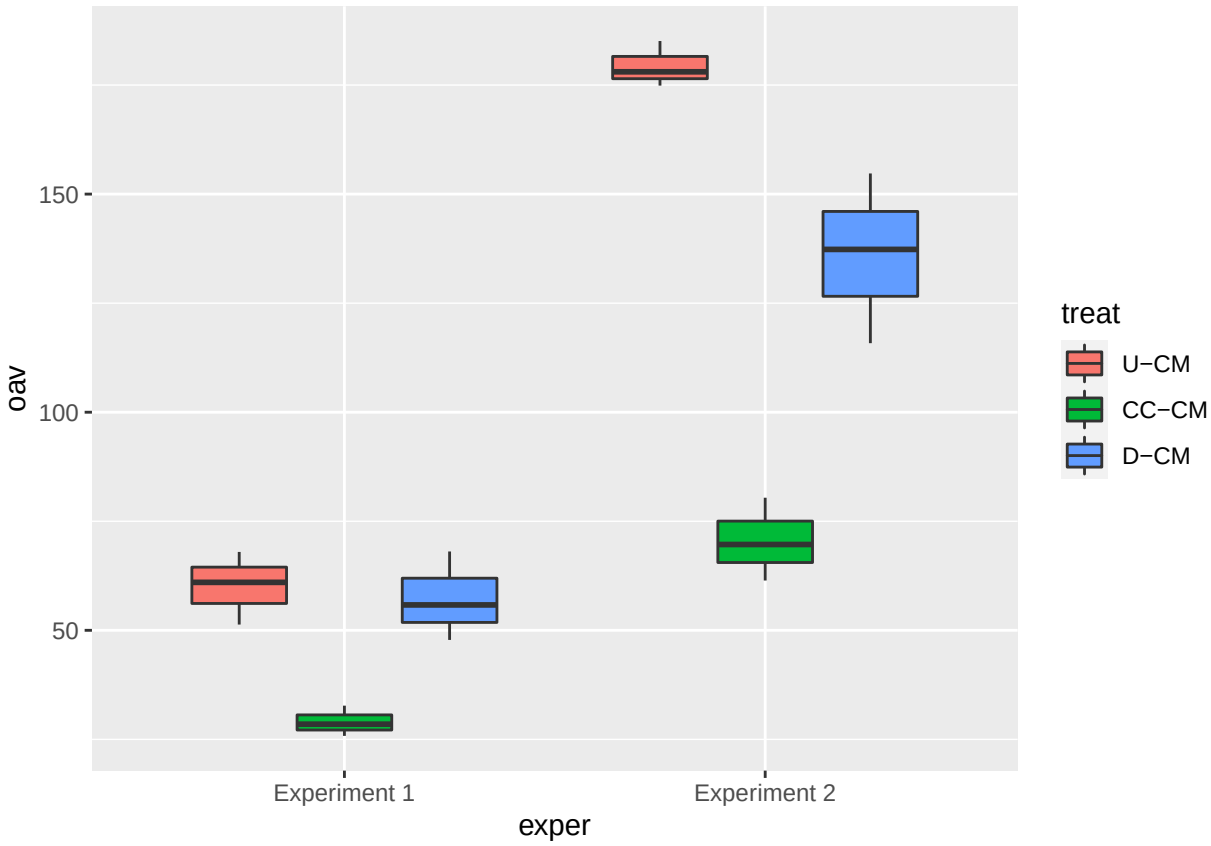
```
ggplot(dat, aes(exper, oav, colour = treat)) +
  geom_jitter(height = 0)
```




```
ggplot(dat, aes(as.integer(factor(exper)), oav, colour = treat, group = interaction(treat, rep))) +
  geom_point() +
  geom_line()
```




```
ggplot(dat, aes(exper, oav, fill = treat)) +
  geom_boxplot()
```



OAV stats

```
m1 <- aov(oav ~ treat * exper, data = dat)
summary(m1)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## treat        2  15249    7625   66.86 3.12e-07 ***
## exper        1  28672   28672  251.44 2.06e-09 ***
## treat:exper  2   4537    2268   19.89 0.000155 ***
## Residuals   12   1368     114
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary.lm(m1)
```

```
##
## Call:
## aov(formula = oav ~ treat * exper, data = dat)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -20.1353  -4.1476  -0.6712   5.2514  18.7727
##
## Coefficients:
##                             Estimate Std. Error t value Pr(>|t|)
## (Intercept)                   60.093      6.165   9.747 4.72e-07 ***
```

```
## treatCC-CM                    -31.090      8.719  -3.566  0.00388 **
## treatD-CM                      -2.863      8.719  -0.328  0.74827
## experExperiment 2             119.245      8.719  13.677 1.11e-08 ***
## treatCC-CM:experExperiment 2  -77.754     12.330  -6.306 3.91e-05 ***
## treatD-CM:experExperiment 2   -40.515     12.330  -3.286  0.00651 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 10.68 on 12 degrees of freedom
## Multiple R-squared:  0.9725, Adjusted R-squared:  0.9611
## F-statistic: 84.99 on 5 and 12 DF,  p-value: 6.069e-09
```

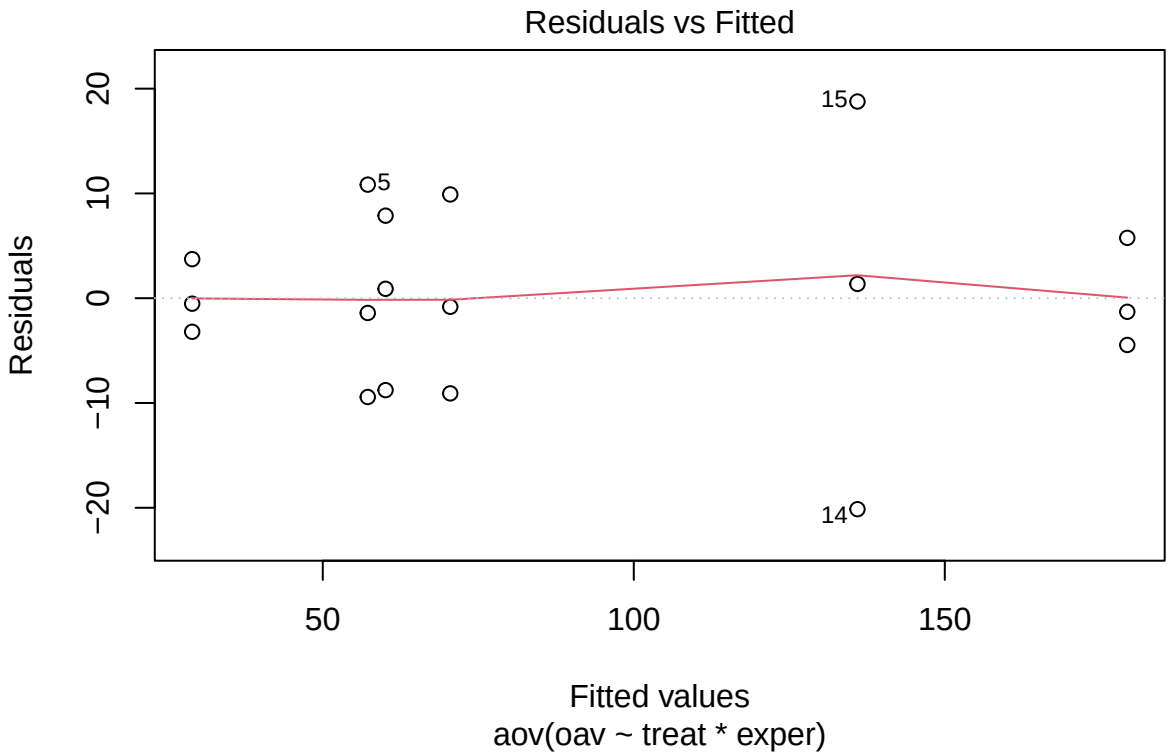
```
confint(m1)
```

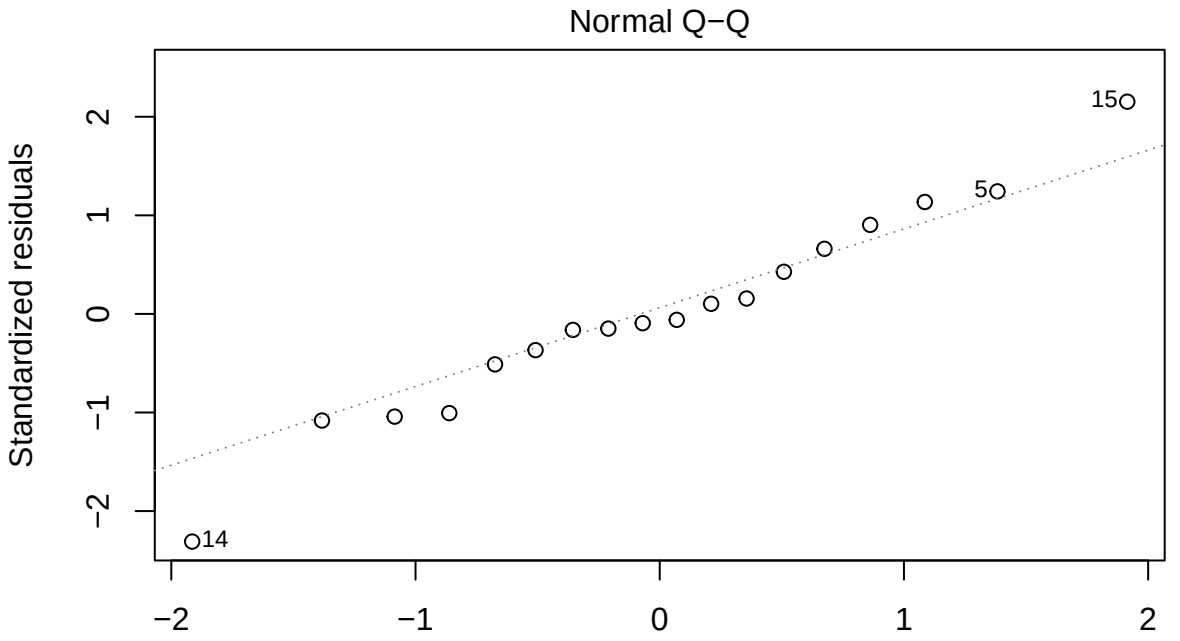
```
##                                 2.5 %    97.5 %
## (Intercept)                  46.66051  73.52616
## treatCC-CM                  -50.08689 -12.09311
## treatD-CM                   -21.86022  16.13355
## experExperiment 2           100.24811 138.24189
## treatCC-CM:experExperiment 2 -104.61999 -50.88868
## treatD-CM:experExperiment 2   -67.38032 -13.64901
```

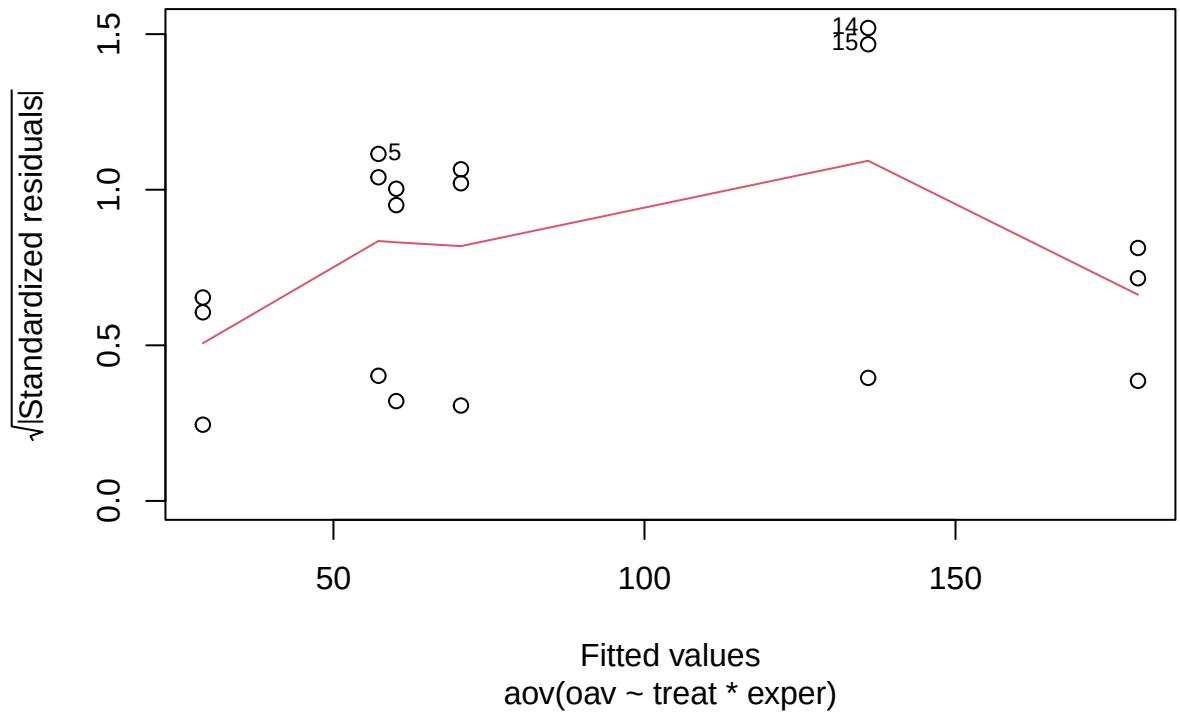
For OAV there is a clear interaction. D-CM is no different from the reference in the first experiment, but is in the second. The apparent CC-CM reduction is also larger in experiment 2.

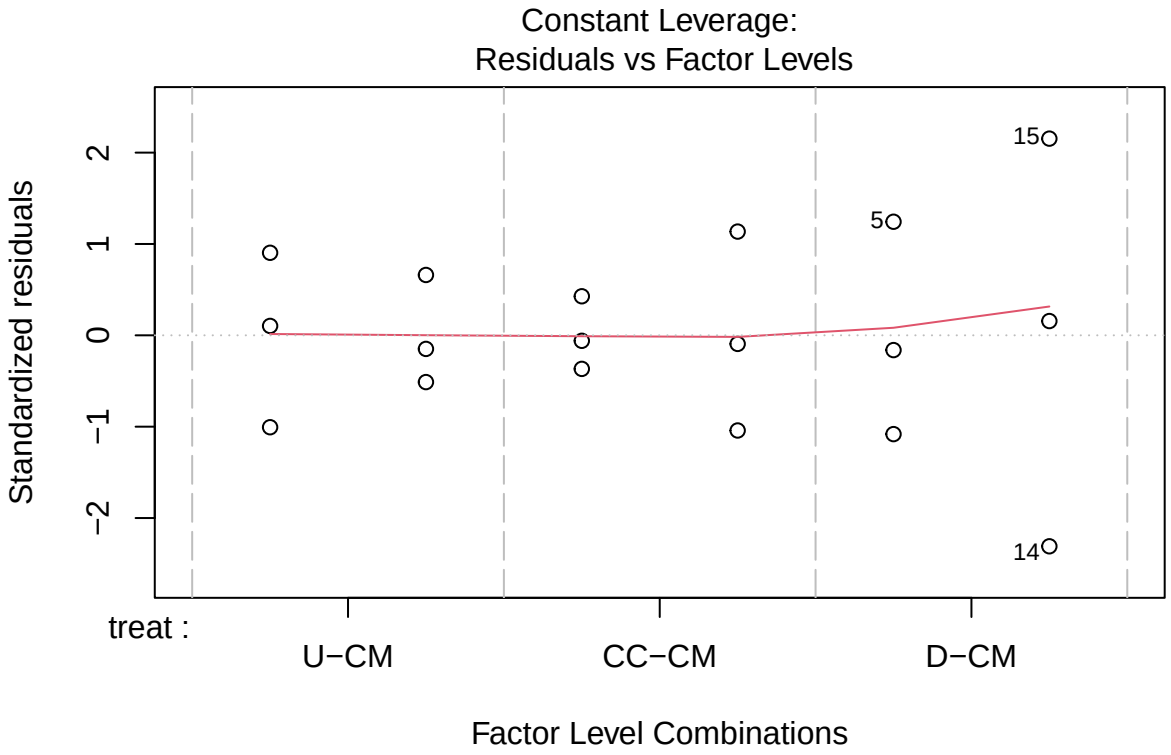
Check residuals.

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




Normal Q−Q
Standardized residuals
Theoretical Quantiles
aov(oav ~ treat * exper)
15
5
14
−2
−1
0
1
2


Scale−Location
√|Standardized residuals|
Fitted values
aov(oav ~ treat * exper)
14
15
5
0.0
0.5
1.0
1.5
50
100
150



No obviously improved with a transformation, but let's try log anyway to for fixed relative effects.

```
m2 <- aov(log10(oav) ~ treat * exper, data = dat)
summary(m2)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## treat        2 0.4393  0.2196  65.937 3.37e-07 ***
## exper        1 0.7689  0.7689 230.831 3.36e-09 ***
## treat:exper  2 0.0093  0.0047   1.401    0.284
## Residuals   12 0.0400  0.0033
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary.lm(m2)
```

```
##
## Call:
## aov(formula = log10(oav) ~ treat * exper, data = dat)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.073630 -0.039267 -0.002736  0.044396  0.079898
##
## Coefficients:
##                                Estimate Std. Error t value Pr(>|t|)
## (Intercept)                     1.77595    0.03332  53.298 1.25e-15 ***
## treatCC-CM                     -0.31559    0.04712  -6.697 2.21e-05 ***
## treatD-CM                      -0.02290    0.04712  -0.486    0.636
## experExperiment 2               0.47760    0.04712  10.135 3.10e-07 ***
## treatCC-CM:experExperiment 2 -0.09245    0.06664  -1.387    0.191
## treatD-CM:experExperiment 2  -0.10028    0.06664  -1.505    0.158
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05771 on 12 degrees of freedom
## Multiple R-squared:  0.9682, Adjusted R-squared:  0.955
## F-statistic:  73.1 on 5 and 12 DF,  p-value: 1.451e-08
```

Quite interesting. Interaction drops out.

```
m3 <- aov(log10(oav) ~ treat + exper, data = dat)
summary(m3)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## treat        2 0.4393  0.2196   62.37 1.07e-07 ***
## exper        1 0.7689  0.7689  218.33 6.21e-10 ***
## Residuals   14 0.0493  0.0035
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary.lm(m3)
```

```
##
## Call:
## aov(formula = log10(oav) ~ treat + exper, data = dat)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.09779 -0.03169  0.01001  0.03816  0.09792
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        1.80807    0.02797  64.633  < 2e-16 ***
## treatCC-CM        -0.36181    0.03426 -10.560 4.74e-08 ***
## treatD-CM         -0.07304    0.03426  -2.132   0.0512 .
## experExperiment 2  0.41336    0.02797  14.776 6.21e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05934 on 14 degrees of freedom
## Multiple R-squared:  0.9608, Adjusted R-squared:  0.9524
## F-statistic: 114.4 on 3 and 14 DF,  p-value: 4.399e-10
```

```
confint(m3)
```

```
##                         2.5 %       97.5 %
## (Intercept)         1.7480751  1.8680745437
## treatCC-CM         -0.4352929 -0.2883242127
## treatD-CM          -0.1465197  0.0004489093
## experExperiment 2   0.3533555  0.4733548517
```

Look at back-transformed results. These are relative reductions in % of reference.

```
100 * (1 - 10^coef(m3))
```

```
##       (Intercept)        treatCC-CM         treatD-CM experExperiment 2
##       -6327.98483          56.52982          15.47901        -159.03303
```

```
100 * (1 - 10^confint(m3))
```

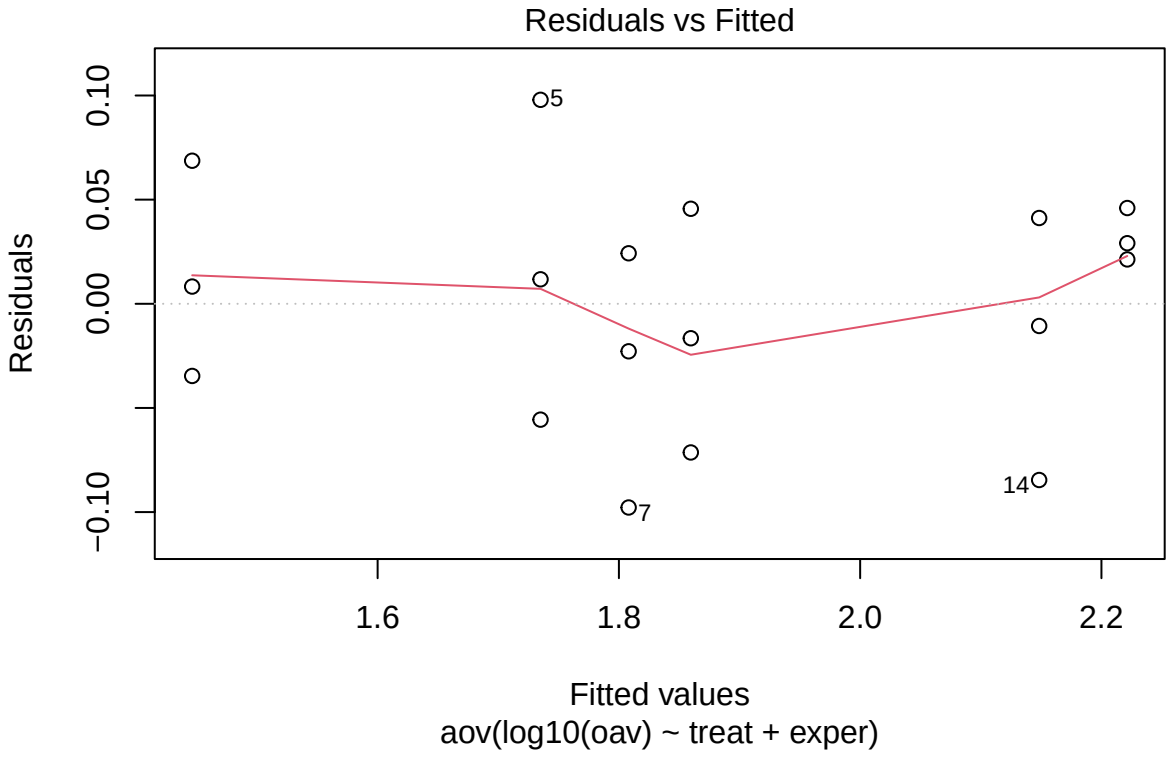
```
##                         2.5 %       97.5 %
```

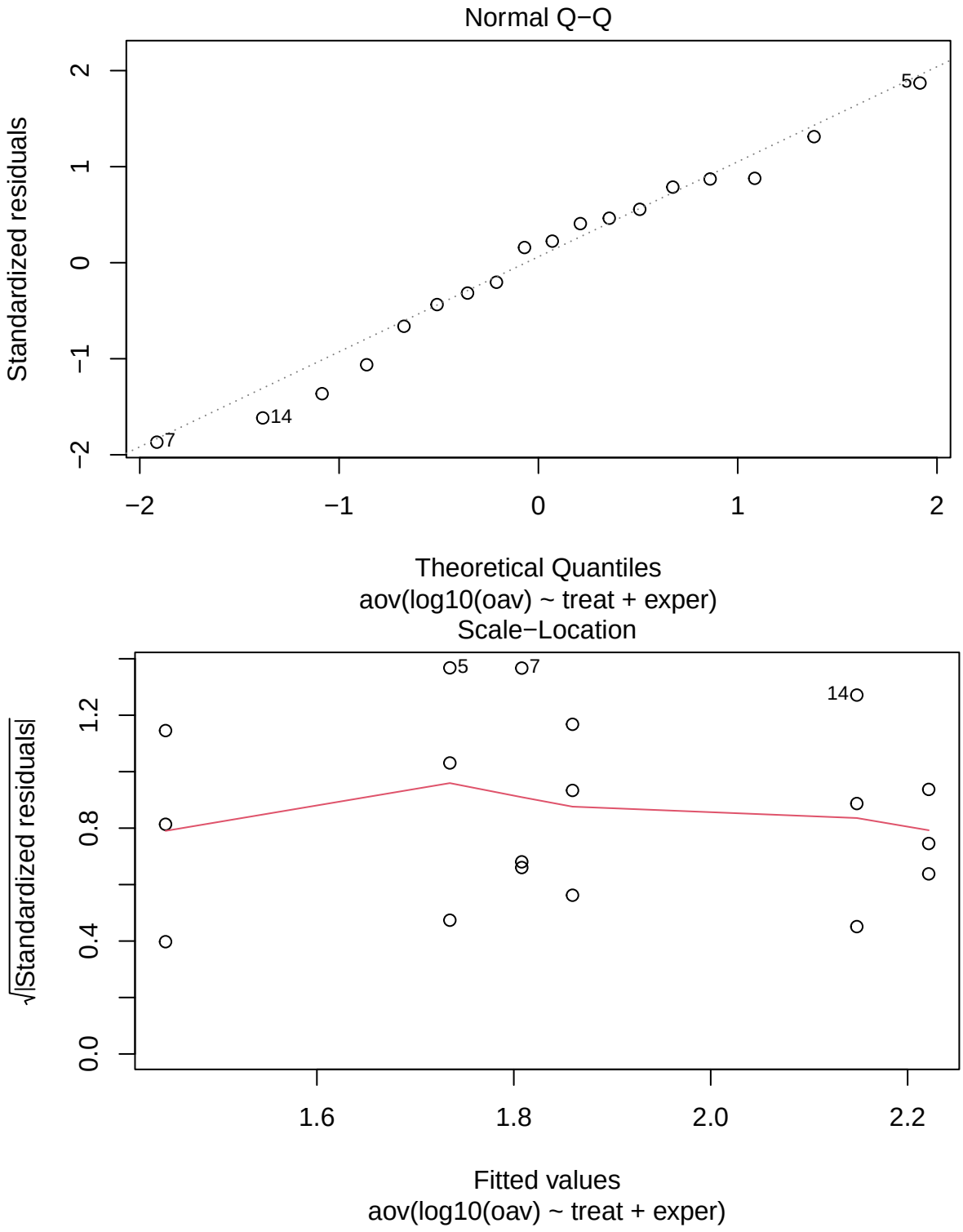
```
## (Intercept)       -5498.54461 -7280.3089721
## treatCC-CM           63.29653    48.5155844
## treatD-CM            28.63582    -0.1034186
## experExperiment 2  -125.60850  -197.4095101
```

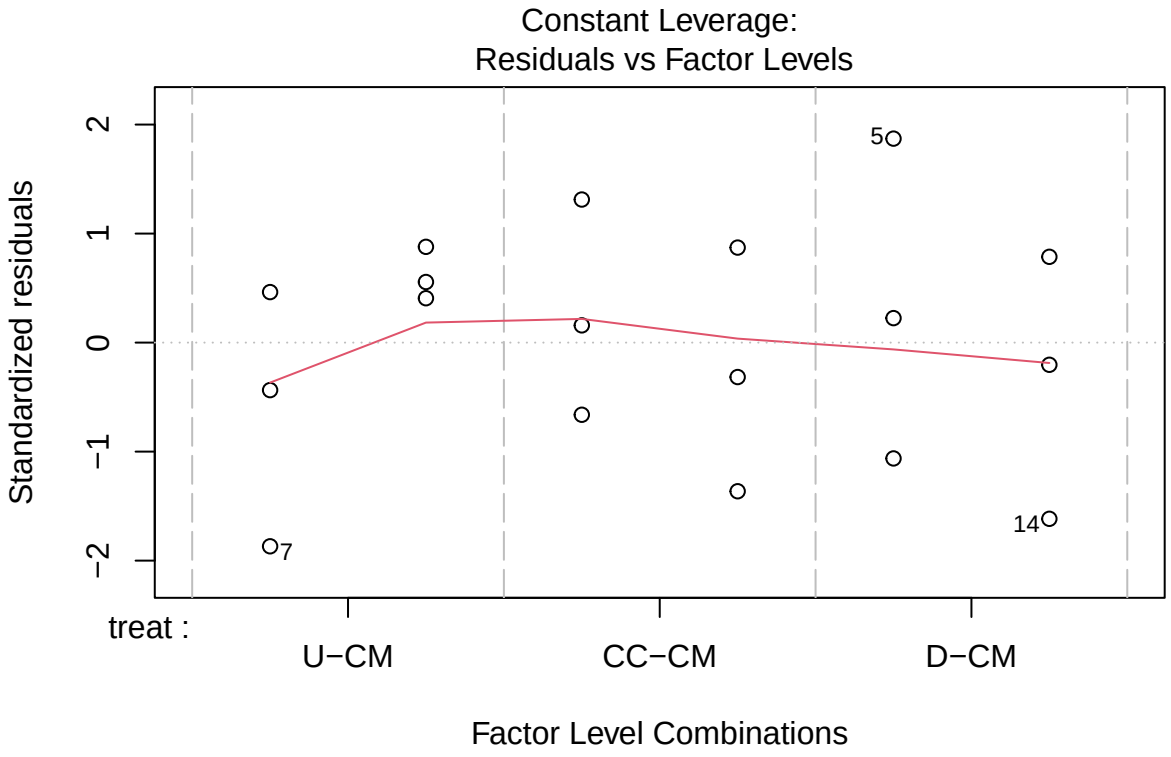
So we conclude CC-CM is lower, but D-CM difference isn't clear.

Check residuals.

```
plot(m3, ask = FALSE)
```

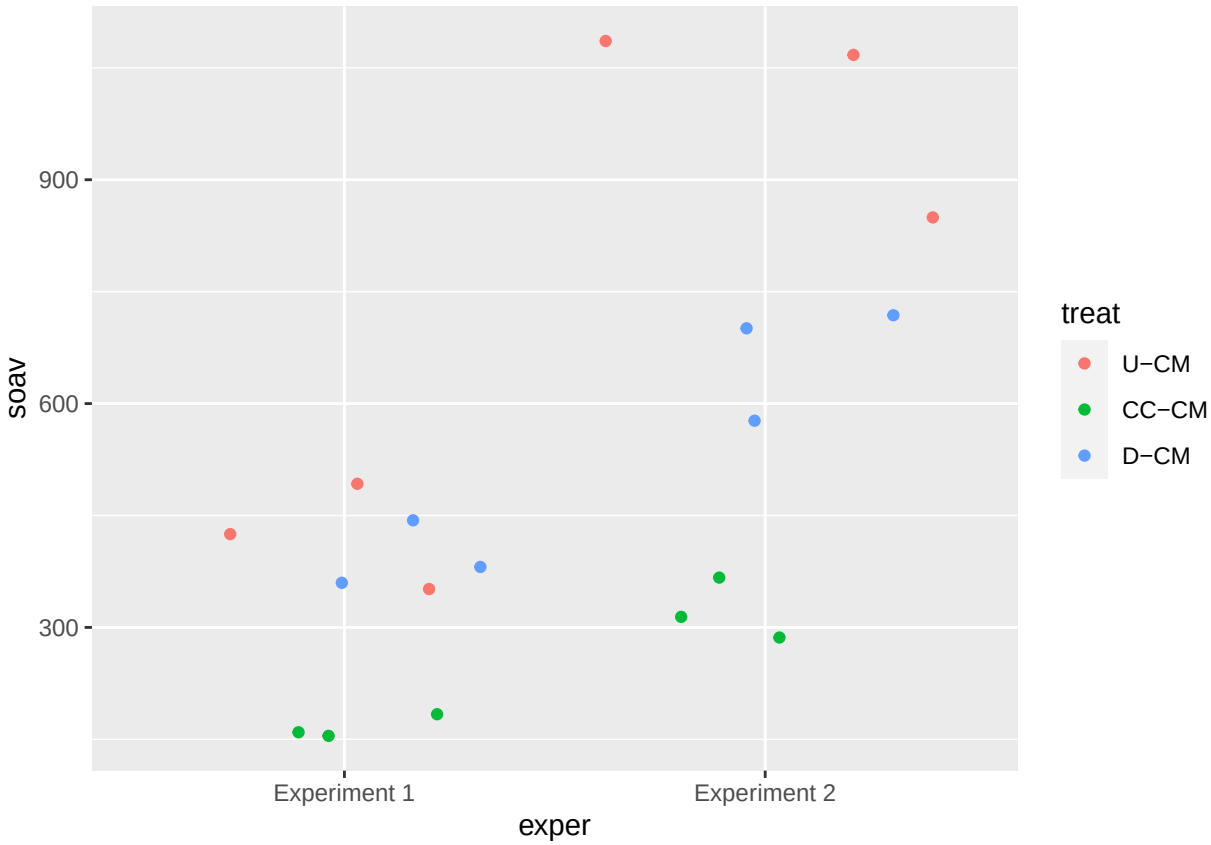



Normal Q−Q
Standardized residuals
Theoretical Quantiles
aov(log10(oav) ~ treat + exper)
Scale−Location
√|Standardized residuals|
Fitted values
aov(log10(oav) ~ treat + exper)

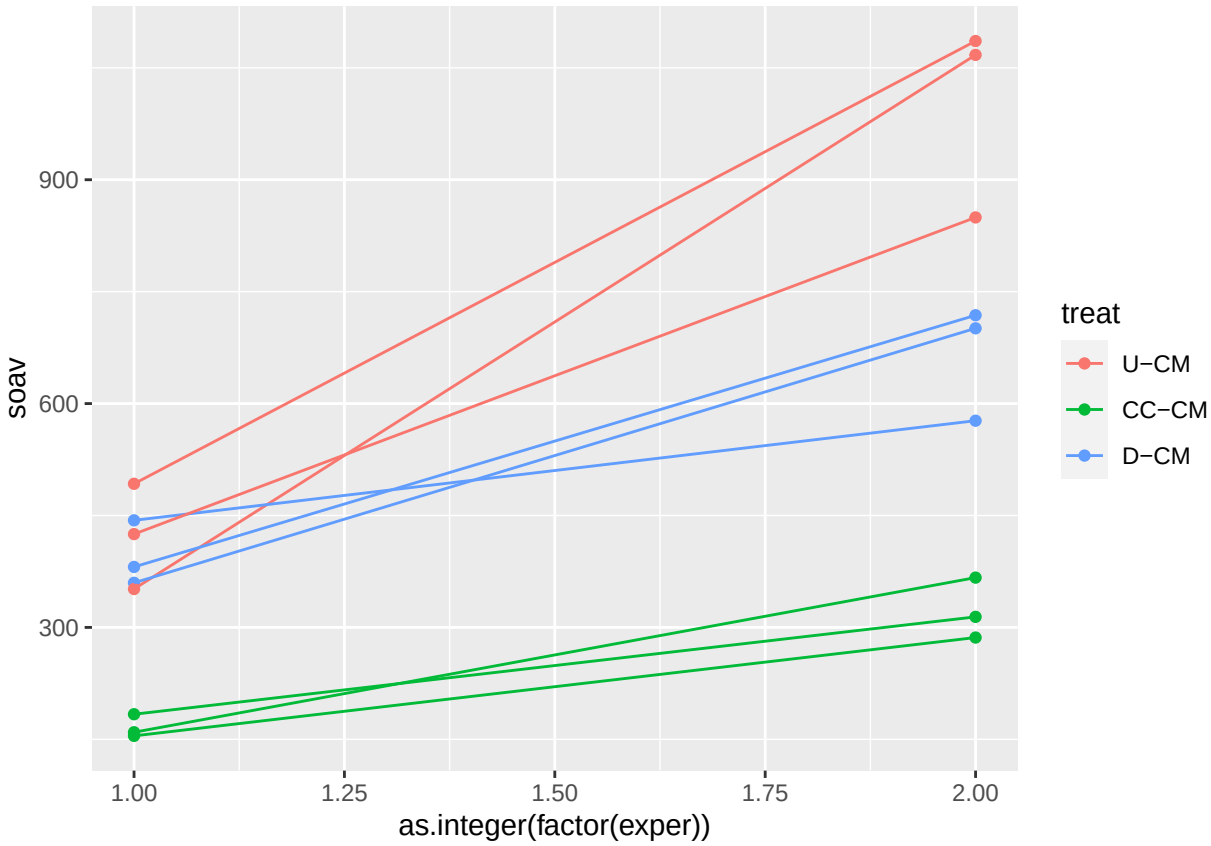


SOAV plots

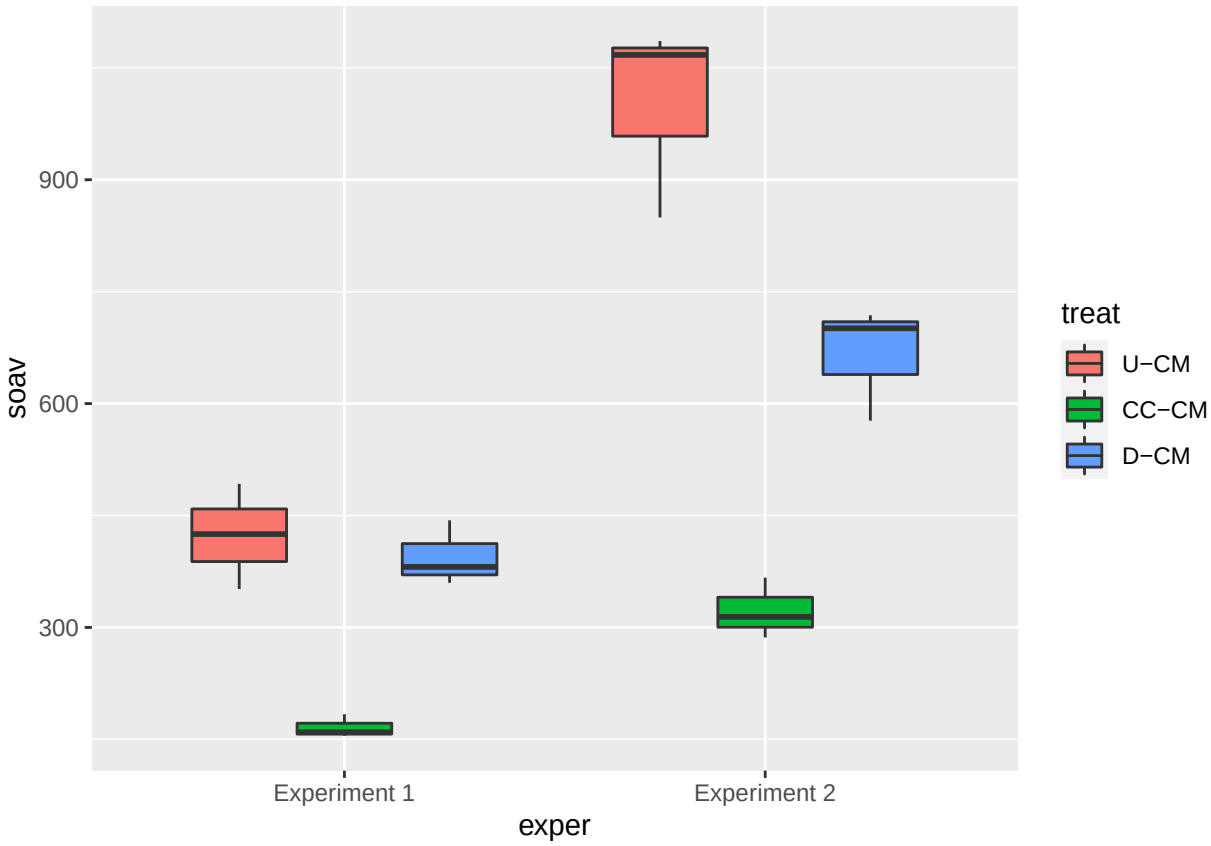
```
ggplot(dat, aes(exper, soav, colour = treat)) +
  geom_jitter(height = 0)
```




```
ggplot(dat, aes(as.integer(factor(exper)), soav, colour = treat, group = interaction(treat, rep))) +
  geom_point() +
  geom_line()
```




```
ggplot(dat, aes(exper, soav, fill = treat)) +
  geom_boxplot()
```



SOAV stats

```
m1 <- aov(soav ~ treat * exper, data = dat)
summary(m1)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## treat        2 667283  333641   62.57 4.49e-07 ***
## exper        1 505073  505073   94.72 4.80e-07 ***
## treat:exper  2 142495   71247   13.36 0.000886 ***
## Residuals   12  63990    5333
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary.lm(m1)
```

```
##
## Call:
## aov(formula = soav ~ treat * exper, data = dat)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -151.424  -29.693   -2.195   47.632   85.004
##
## Coefficients:
##                             Estimate Std. Error t value Pr(>|t|)
## (Intercept)                   423.02      42.16  10.033 3.45e-07 ***
```

```
## treatCC-CM                    -257.09      59.62  -4.312 0.001011 **
## treatD-CM                      -28.23      59.62  -0.473 0.644415
## experExperiment 2              577.90      59.62   9.692 5.01e-07 ***
## treatCC-CM:experExperiment 2  -421.38      84.32  -4.997 0.000311 ***
## treatD-CM:experExperiment 2   -307.25      84.32  -3.644 0.003365 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 73.02 on 12 degrees of freedom
## Multiple R-squared:  0.9536, Adjusted R-squared:  0.9343
## F-statistic: 49.31 on 5 and 12 DF,  p-value: 1.378e-07
```

Transform.

```
m2 <- aov(log10(soav) ~ treat * exper, data = dat)
summary(m2)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## treat        2 0.6602  0.3301 107.720 2.16e-08 ***
## exper        1 0.3955  0.3955 129.079 8.87e-08 ***
## treat:exper  2 0.0168  0.0084   2.749    0.104
## Residuals   12 0.0368  0.0031
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary.lm(m2)
```

```
##
## Call:
## aov(formula = log10(soav) ~ treat * exper, data = dat)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.076356 -0.036302 -0.001423  0.037355  0.070152
##
## Coefficients:
##                              Estimate Std. Error t value Pr(>|t|)
## (Intercept)                   2.62226    0.03196  82.050  < 2e-16 ***
## treatCC-CM                   -0.40357    0.04520  -8.929 1.20e-06 ***
## treatD-CM                    -0.02760    0.04520  -0.611   0.5528
## experExperiment 2             0.37550    0.04520   8.308 2.55e-06 ***
## treatCC-CM:experExperiment 2 -0.08801    0.06392  -1.377   0.1937
## treatD-CM:experExperiment 2  -0.14908    0.06392  -2.332   0.0379 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05536 on 12 degrees of freedom
## Multiple R-squared:  0.9669, Adjusted R-squared:  0.953
## F-statistic:    70 on 5 and 12 DF,  p-value: 1.863e-08
```

We lose the interaction again.

```
m3 <- aov(log10(soav) ~ treat + exper, data = dat)
summary(m3)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## treat        2 0.6602  0.3301   86.18 1.35e-08 ***
```

```
## exper        1 0.3955  0.3955  103.27 7.65e-08 ***
## Residuals   14 0.0536  0.0038
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary.lm(m3)
```

```
##
## Call:
## aov(formula = log10(soav) ~ treat + exper, data = dat)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.115870 -0.028025 -0.007021  0.045010  0.087298
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         2.66177    0.02917  91.239  < 2e-16 ***
## treatCC-CM         -0.44757    0.03573 -12.526 5.38e-09 ***
## treatD-CM          -0.10214    0.03573  -2.859   0.0126 *
## experExperiment 2   0.29647    0.02917  10.162 7.65e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.06189 on 14 degrees of freedom
## Multiple R-squared:  0.9517, Adjusted R-squared:  0.9413
## F-statistic: 91.88 on 3 and 14 DF,  p-value: 1.896e-09
```

```
confint(m3)
```

```
##                         2.5 %      97.5 %
## (Intercept)         2.5991979  2.72434015
## treatCC-CM         -0.5242056 -0.37093831
## treatD-CM          -0.1787759 -0.02550862
## experExperiment 2   0.2338977  0.35903992
```

Look at back-transformed results. These are relative reductions in % of reference.

```
100 * (1 - 10^coef(m3))
```

```
##       (Intercept)         treatCC-CM          treatD-CM experExperiment 2
##      -45795.38788           64.31974           20.95803          -97.91049
```

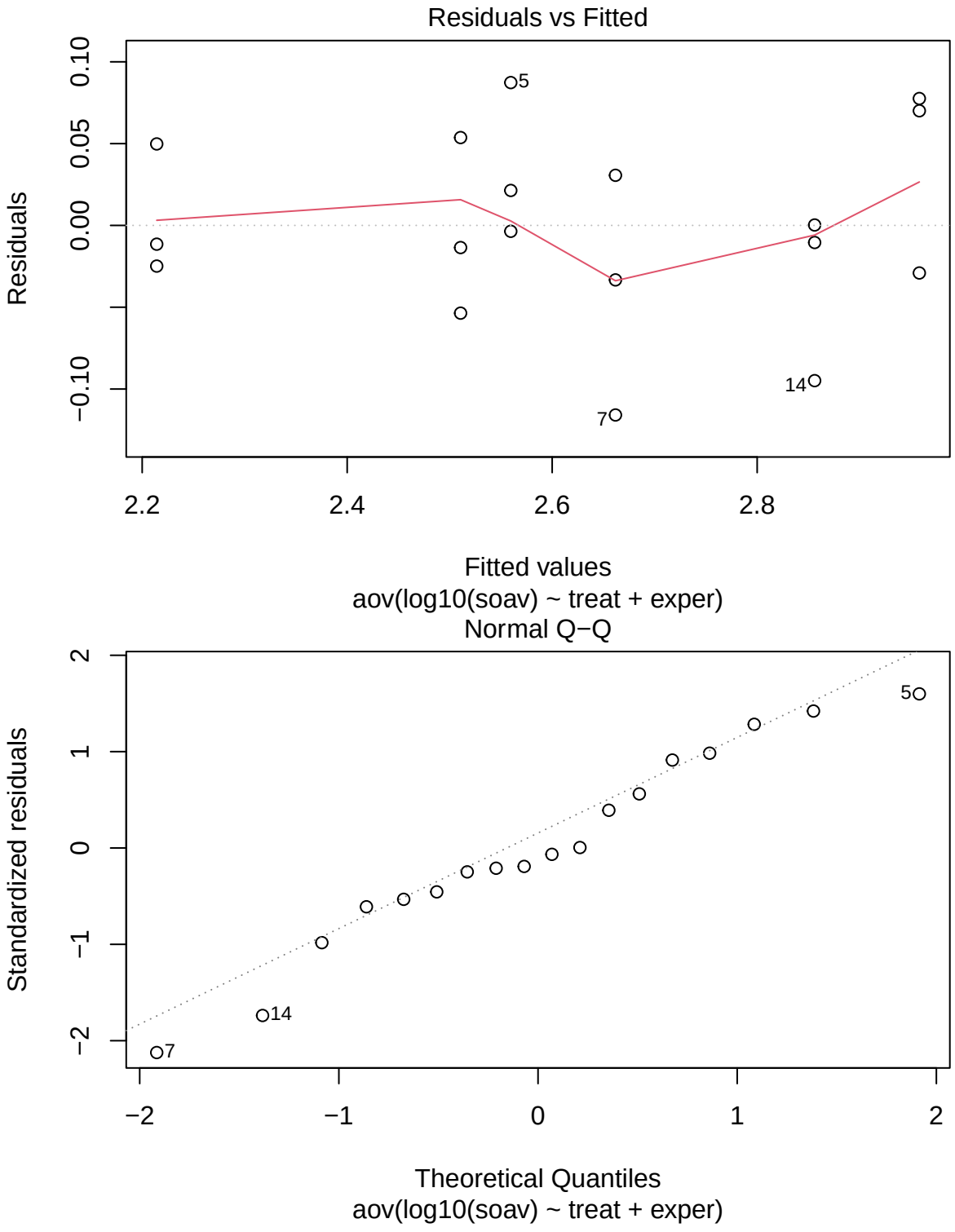
```
100 * (1 - 10^confint(m3))
```

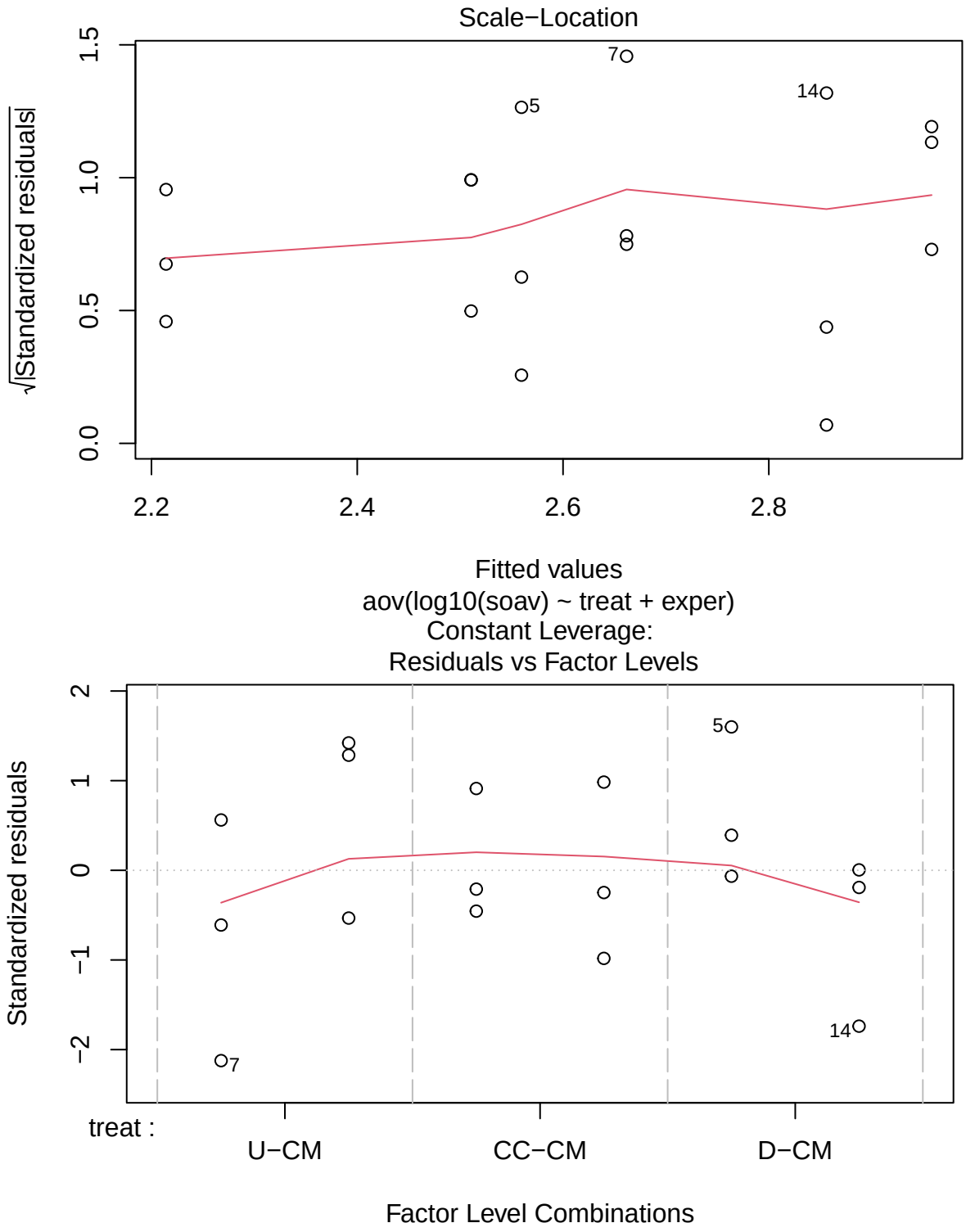
```
##                          2.5 %       97.5 %
## (Intercept)       -39637.26227 -52907.84474
## treatCC-CM            70.09151     57.43411
## treatD-CM             33.74417      5.70441
## experExperiment 2    -71.35537   -128.58089
```

So we conclude both CC-CM and D-CM are lower.

Check residuals.

```
plot(m3, ask = FALSE)
```


Residuals vs Fitted
Residuals
0.10
0.05
0.00
−0.10
5
14
7
2.2
2.4
2.6
2.8
Fitted values
aov(log10(soav) ~ treat + exper)
Normal Q−Q
Standardized residuals
2
1
0
−1
−2
5
14
7
−2
−1
0
1
2
Theoretical Quantiles
aov(log10(soav) ~ treat + exper)


Scale−Location
√|Standardized residuals|
0.0
0.5
1.0
1.5
2.2
2.4
2.6
2.8
7
5
14
Fitted values
aov(log10(soav) ~ treat + exper)
Constant Leverage:
Residuals vs Factor Levels
Standardized residuals
−2
−1
0
1
2
5
14
7
treat :
U−CM
CC−CM
D−CM
Factor Level Combinations