Data analysis for digestate experiments

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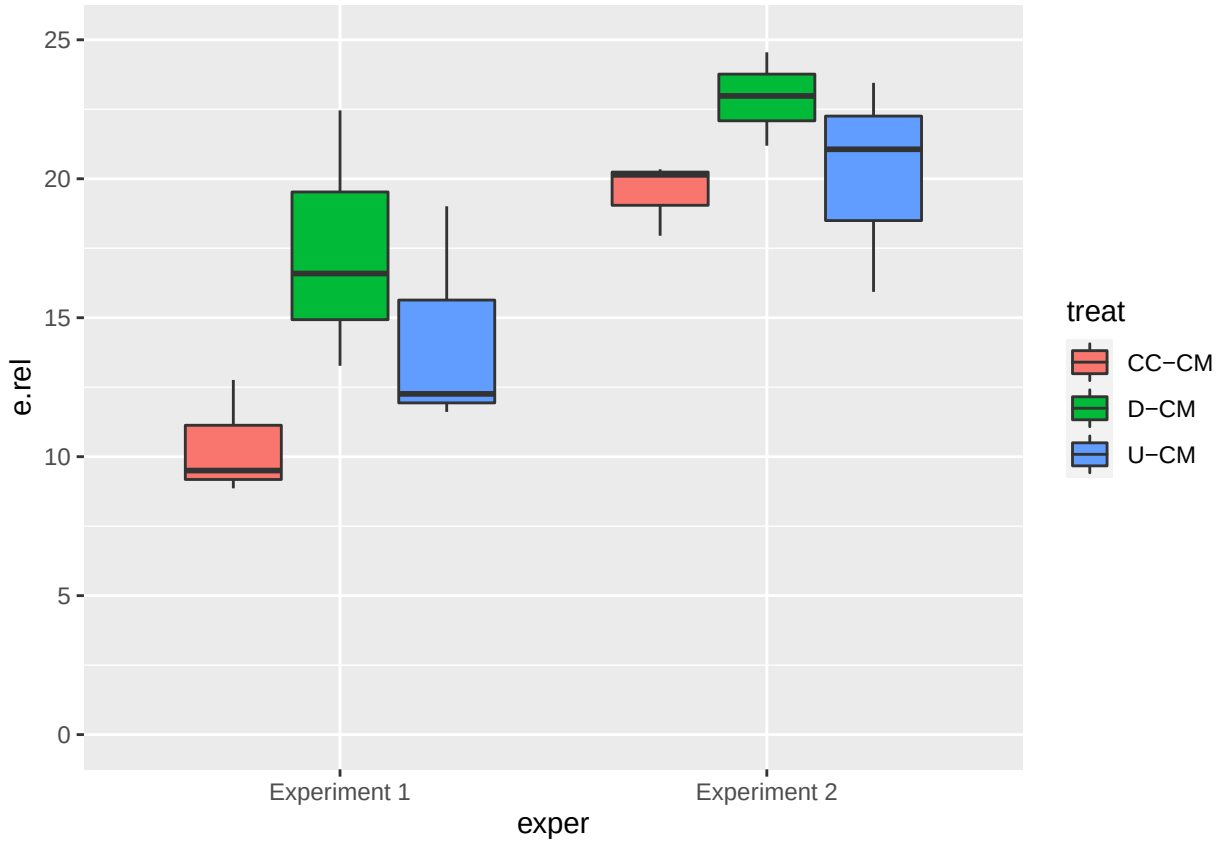
NH3 plots

```
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)
```

First model, with interaction, no transformation.

```r
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
```

```r
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
```

Without interaction.

```r
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
```

```r
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
```

This model is the one we should use in the paper. Diagnostic plots look better, and the boxplot above shows smaller differences for experiment 2 (not larger as expected if there were a fixed relative effect).

```
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)
```

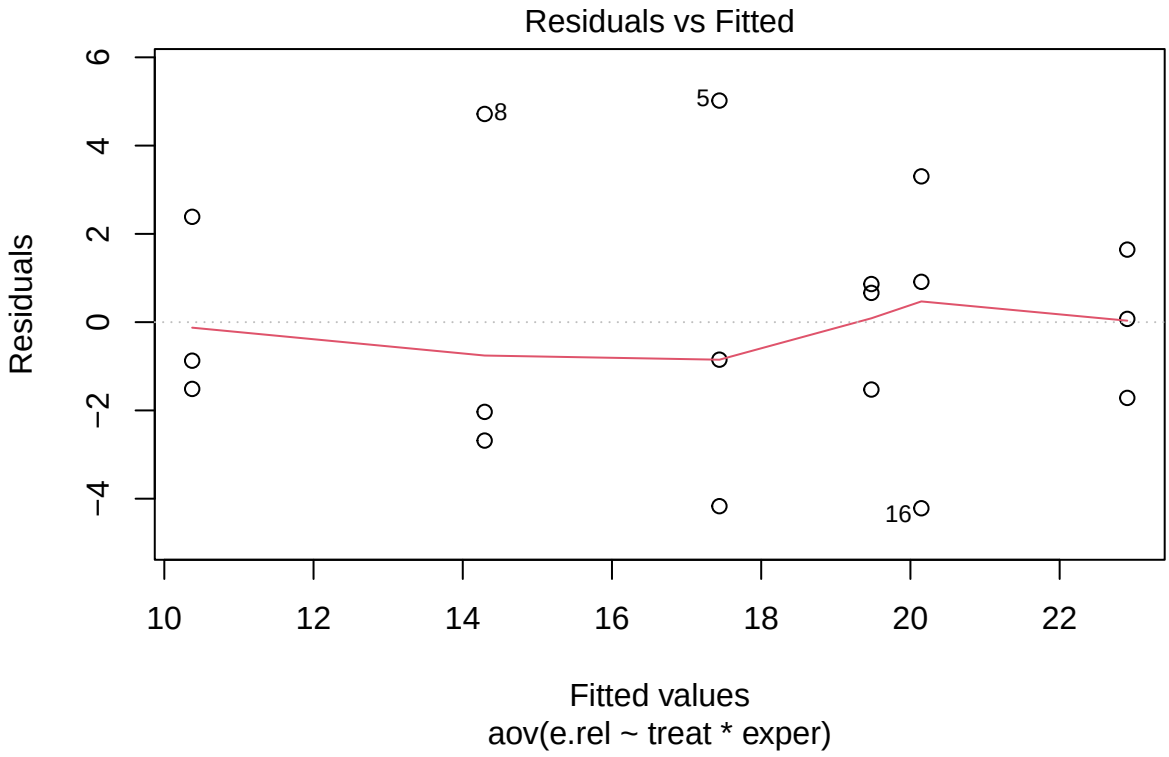
```
##
## Call:
## aov(formula = log10(e.rel) ~ treat + exper, data = dat)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.112604 -0.062945  0.004974  0.053927  0.151094
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        1.12789    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
```

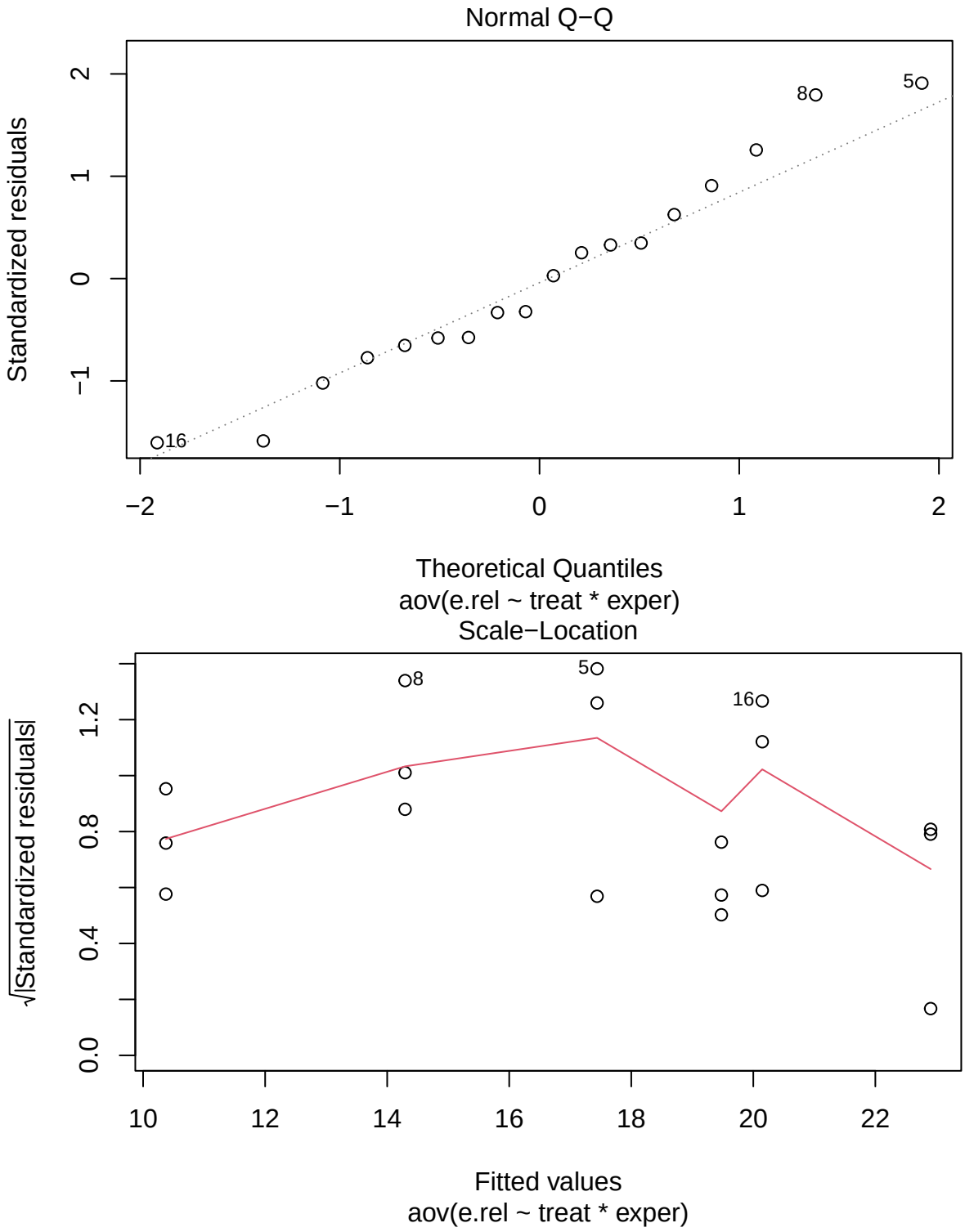
```
##
## Residual standard error: 0.08715 on 14 degrees of freedom
## Multiple R-squared:  0.6751, Adjusted R-squared:  0.6055
## F-statistic: 9.697 on 3 and 14 DF,  p-value: 0.001015
```

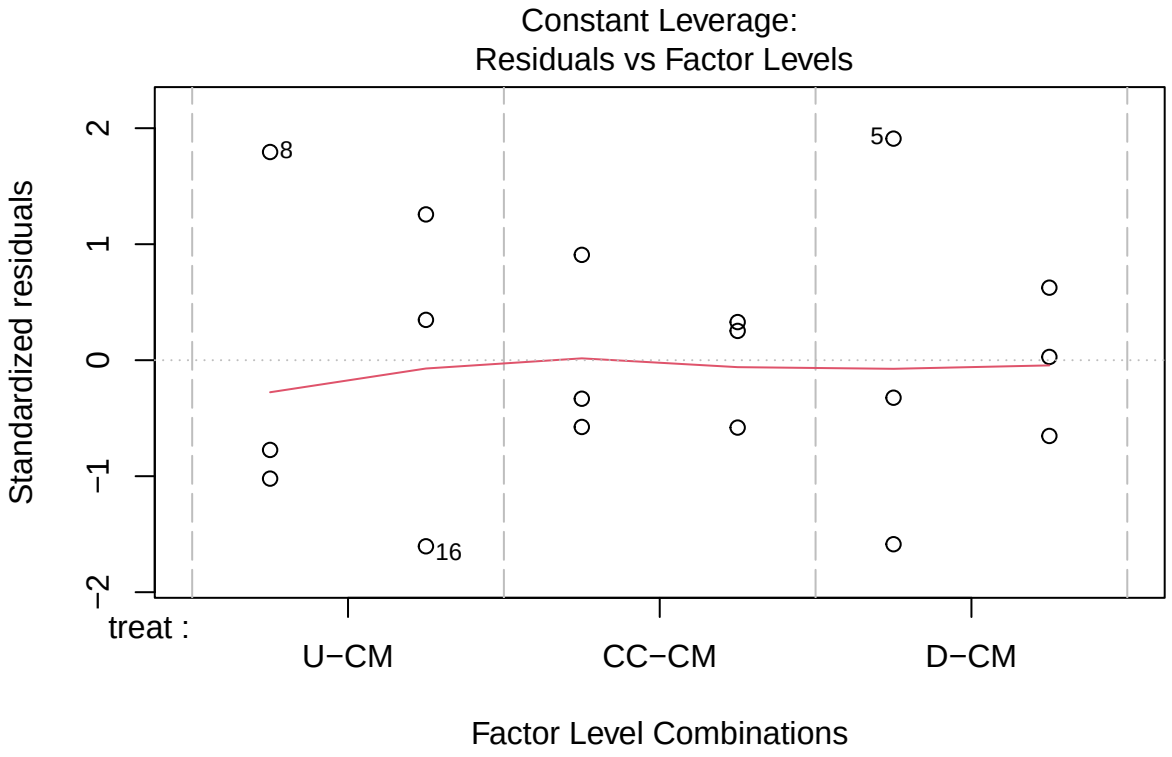
CC-CM and D-CM are clearly different. But neither is clearly different from the reference. Makes interpretation just a bit tricky but not terrible. Some evidence of a difference but presumably digestion pH effect is moderated by low DM, and variability was high, so power is low.

Check diagnostic plots.

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

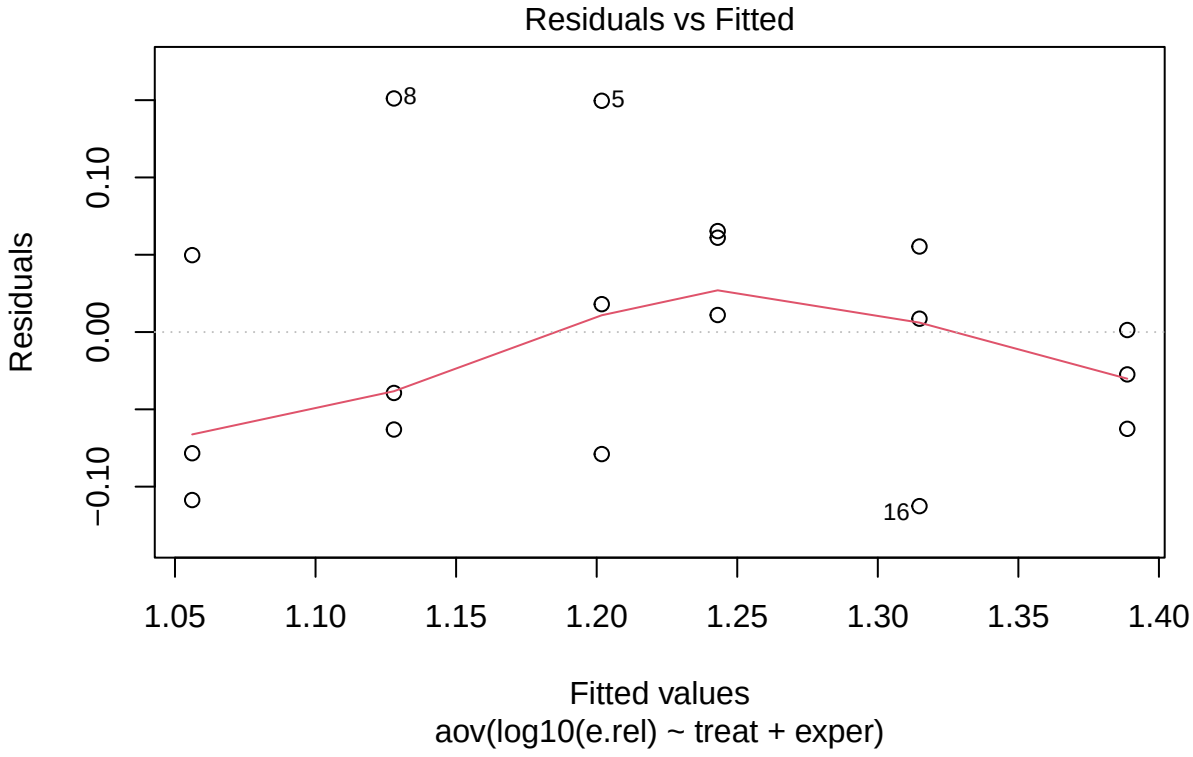


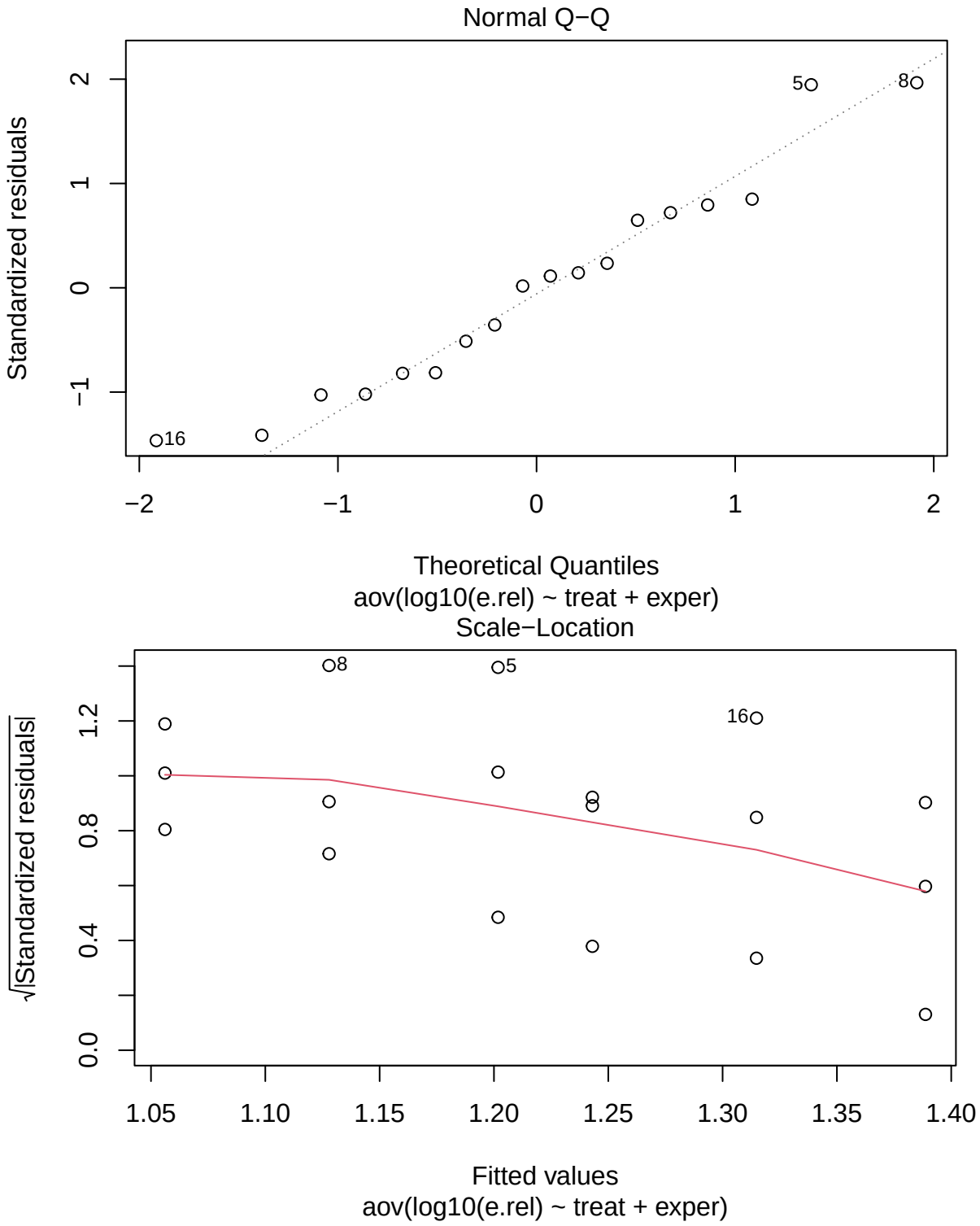

Normal Q−Q
Standardized residuals
Theoretical Quantiles
aov(e.rel ~ treat * exper)
Scale−Location
√|Standardized residuals|
Fitted values
aov(e.rel ~ treat * exper)

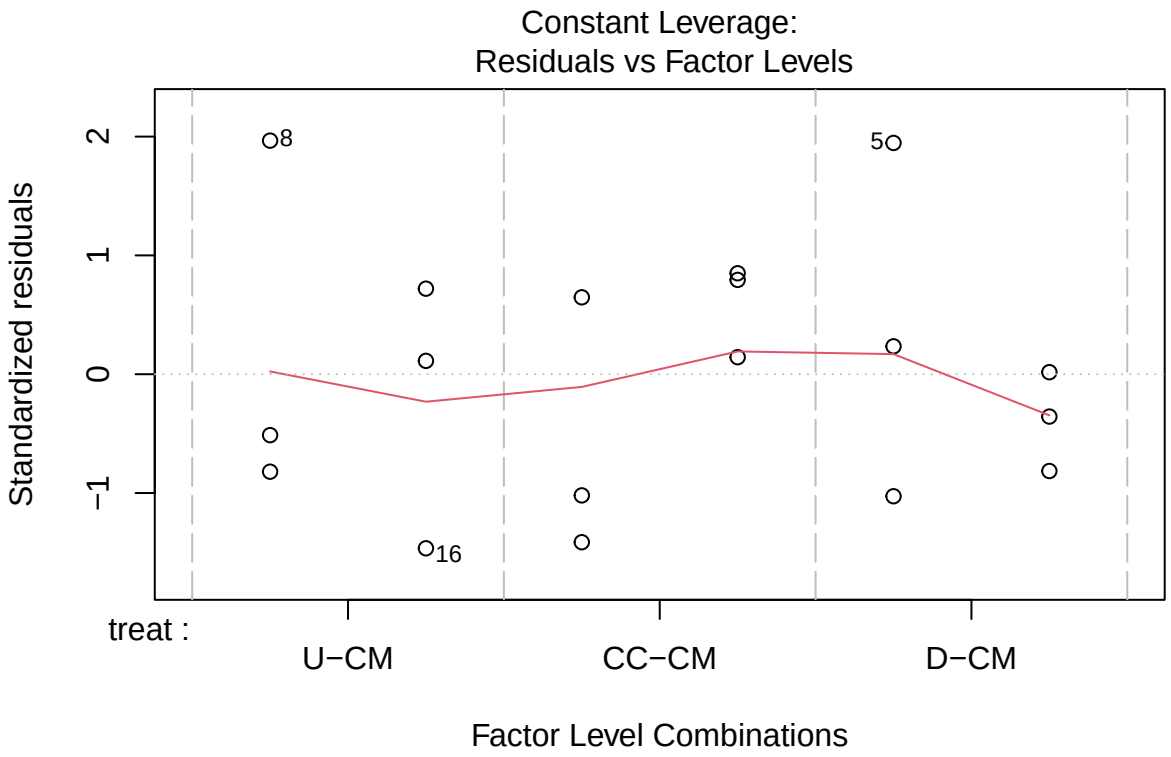


Diagnostic plots with transformation.

```
plot(m4, ask = FALSE)
```



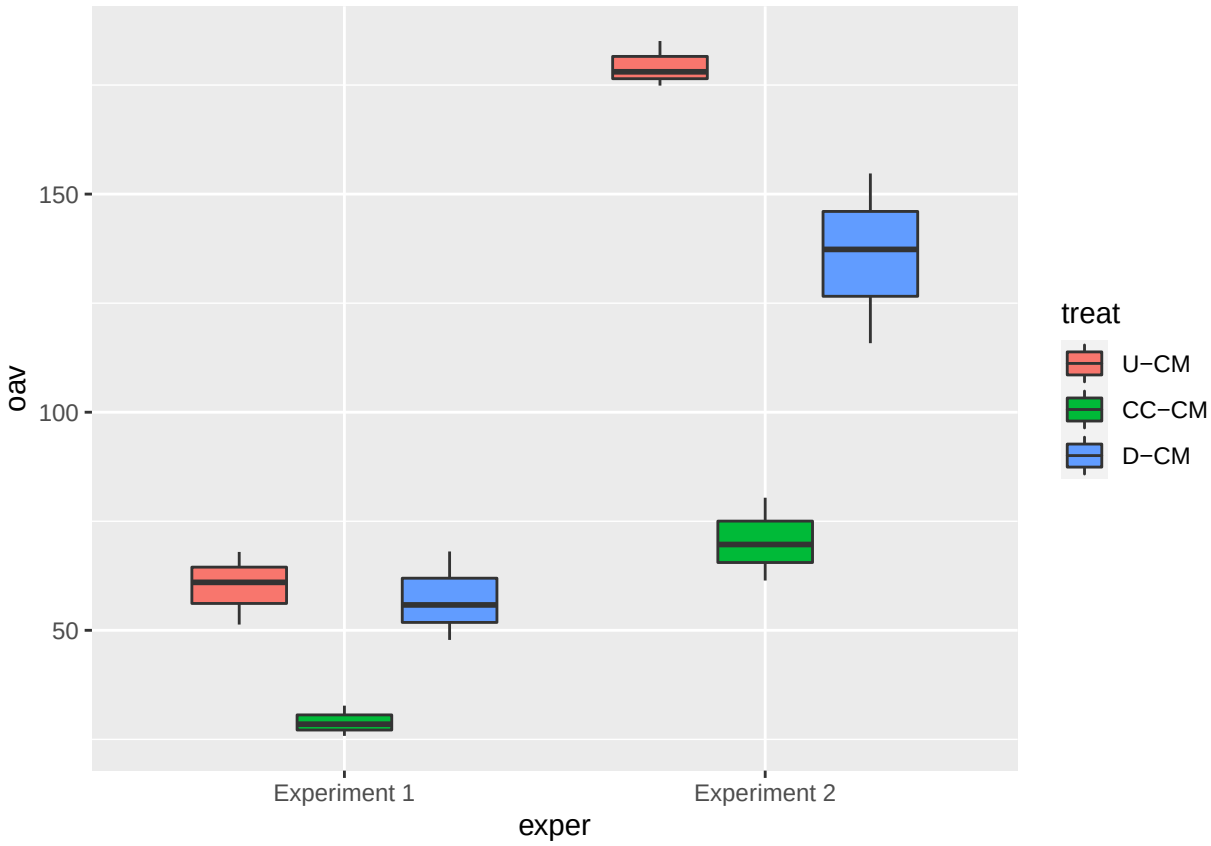

Normal Q−Q
Standardized residuals
Theoretical Quantiles
aov(log10(e.rel) ~ treat + exper)
Scale−Location
√|Standardized residuals|
Fitted values
aov(log10(e.rel) ~ treat + exper)



Actually looks better without the transformation.

OAV plots

```
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
```

```
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
```

This is the model we should use in the paper. Most importantly (I think) the boxplot shows that a relative effects concept fits better than absolute effects. And diagnostic plots are better with the transformation. Lastly, the interaction drops out, which is simpler and so better by some perspectives.

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
```

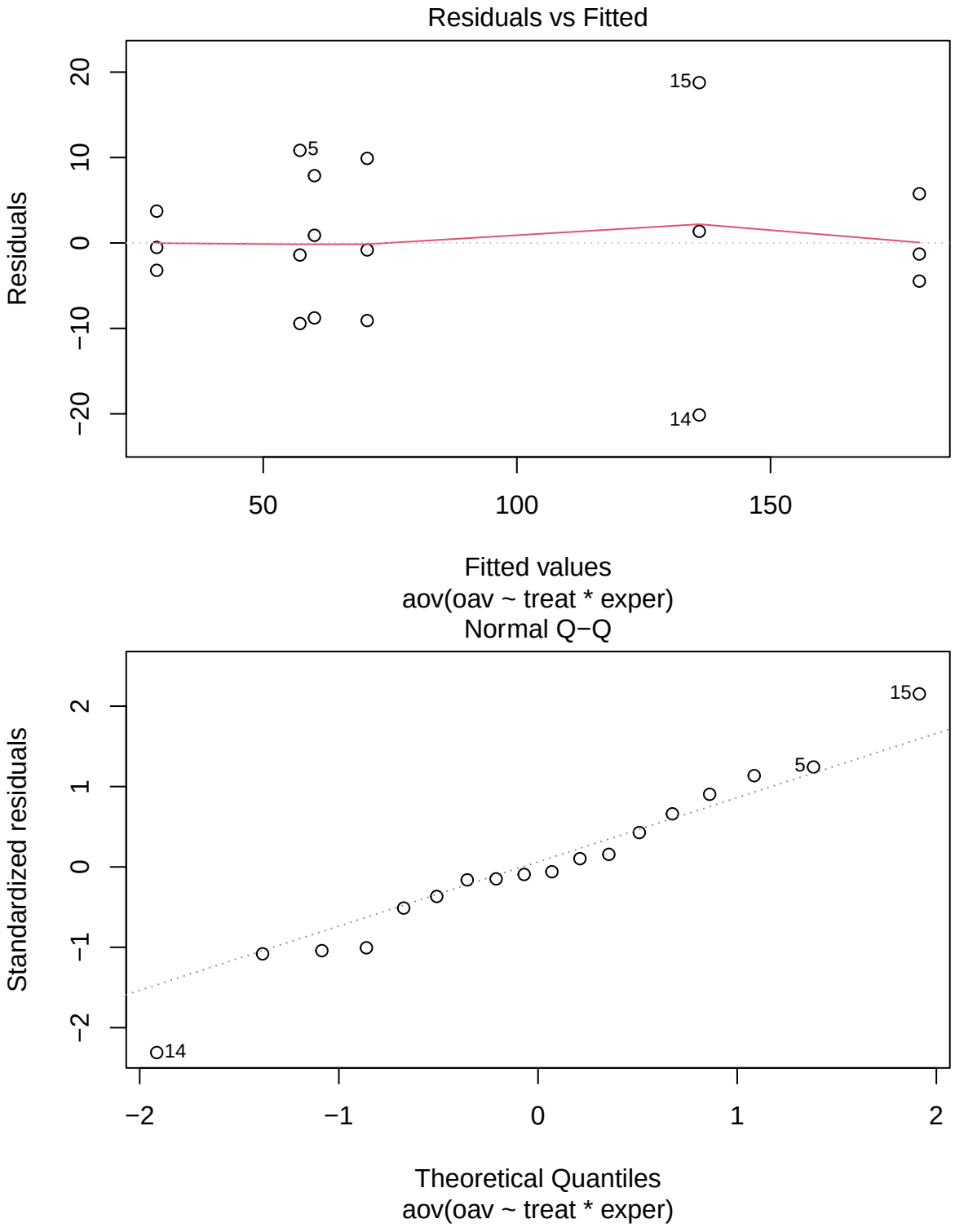
```
100 * (1 - 10^confint(m3))
```

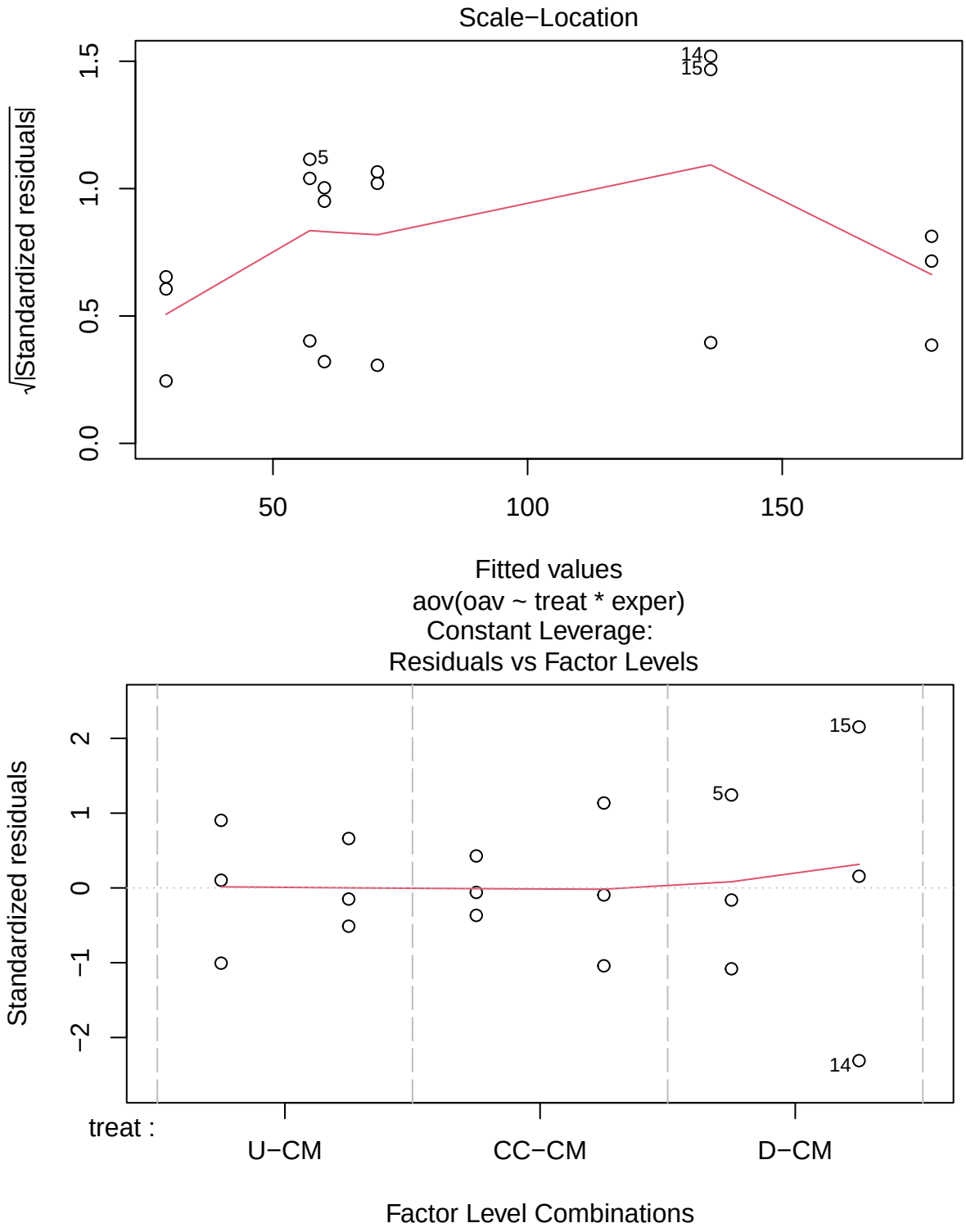
```
##                          2.5 %        97.5 %
## (Intercept)       -5498.54461 -7280.3089721
## treatCC-CM           63.29653    48.5155844
## treatD-CM            28.63582    -0.1034186
## experExperiment 2  -125.60850  -197.4095101
```

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

Check diagnostic plots.

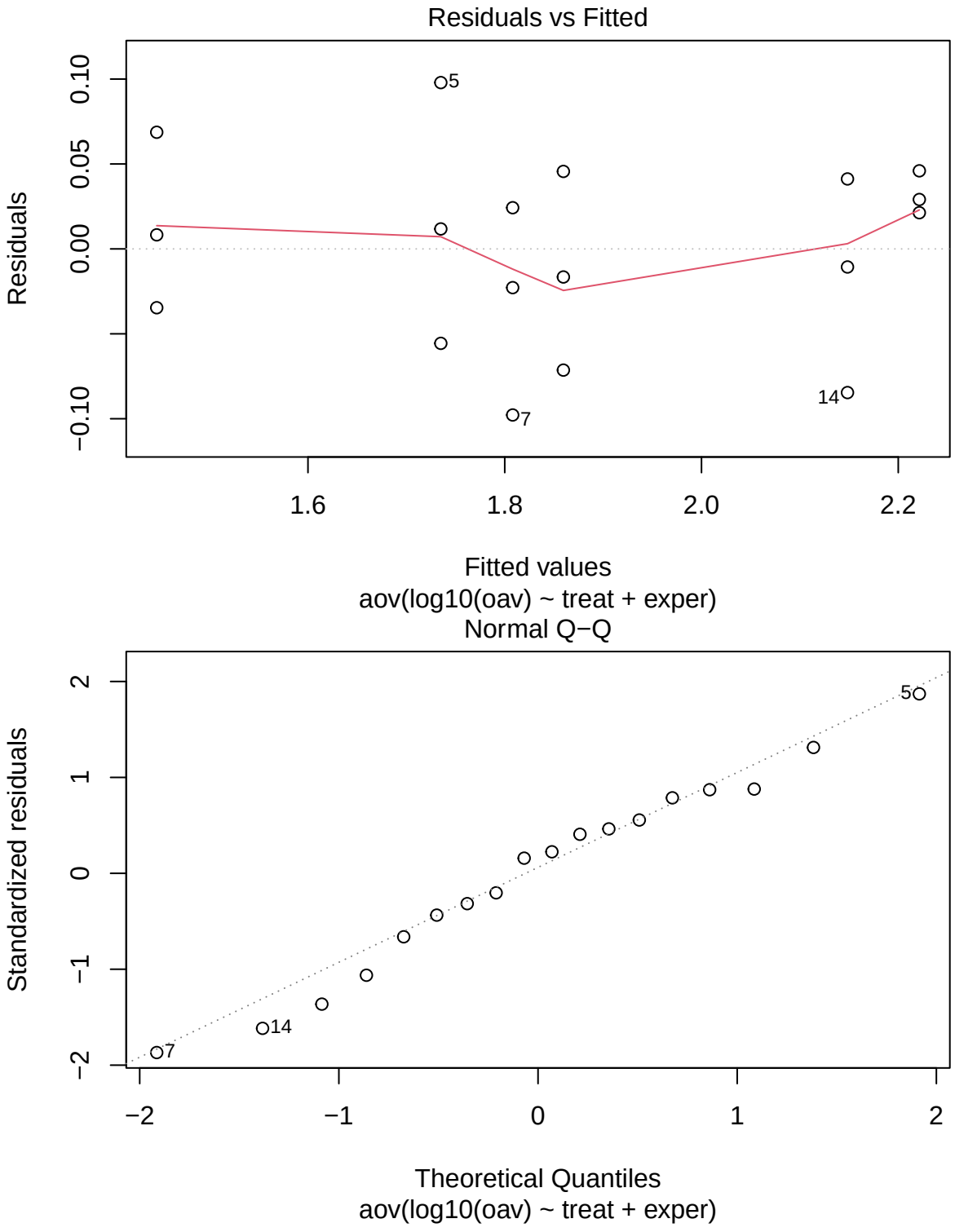
```
plot(m1, ask = FALSE)
```

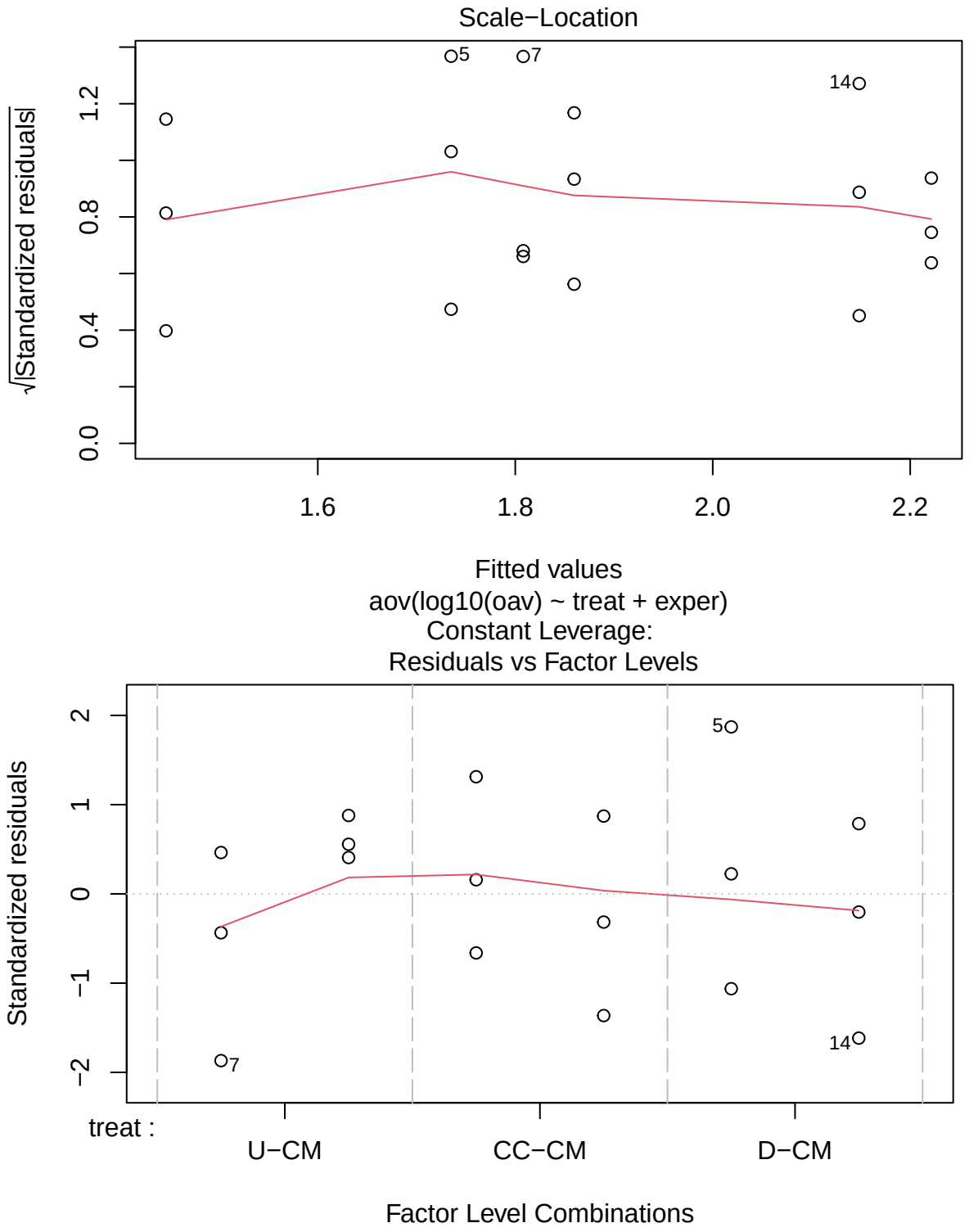

Residuals vs Fitted
Residuals
Fitted values
aov(oav ~ treat * exper)
Normal Q−Q
Standardized residuals
Theoretical Quantiles
aov(oav ~ treat * exper)



Diagnostic plots with transformation.

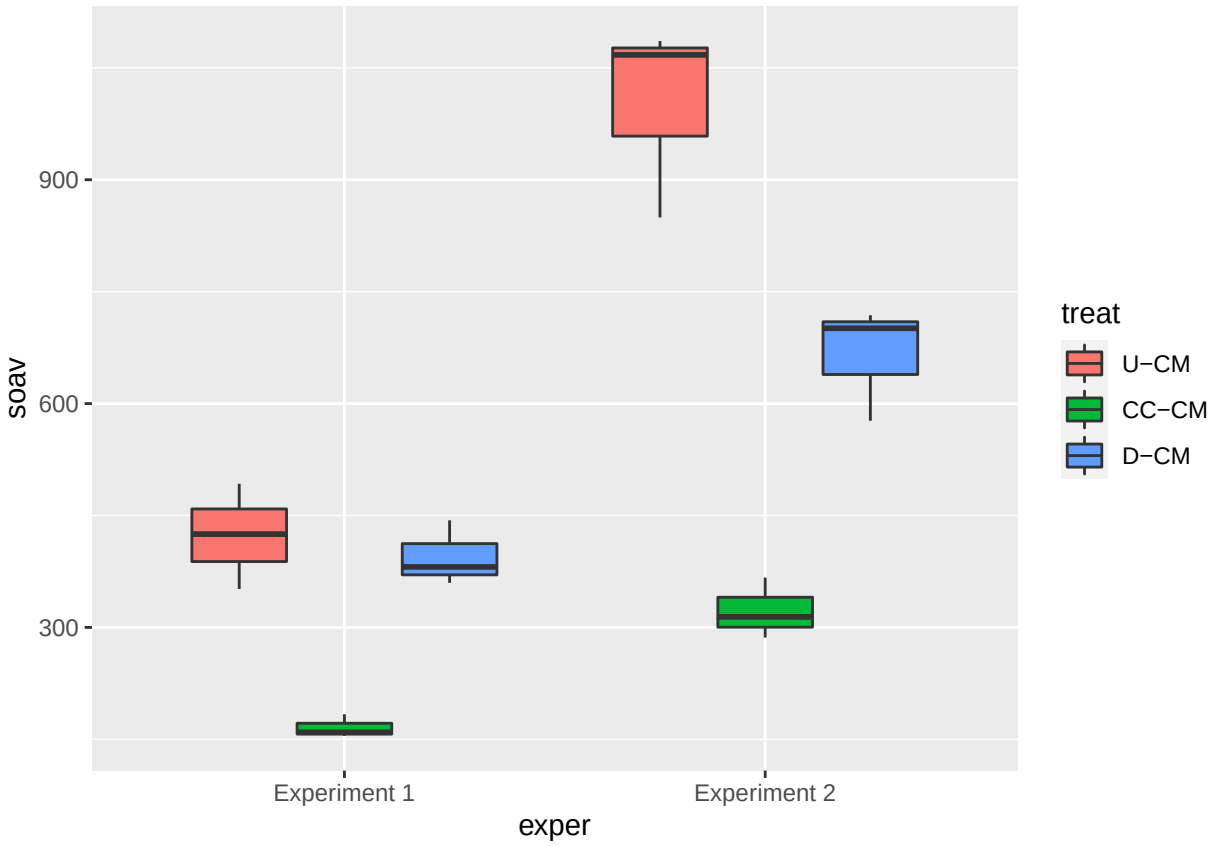
```
plot(m3, ask = FALSE)
```


Residuals vs Fitted
Residuals
0.10
0.05
0.00
−0.10
5
7
14
1.6
1.8
2.0
2.2
Fitted values
aov(log10(oav) ~ treat + exper)
Normal Q−Q
Standardized residuals
2
1
0
−1
−2
5
14
7
−2
−1
0
1
2
Theoretical Quantiles
aov(log10(oav) ~ treat + exper)



SOAV plots

```
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
```

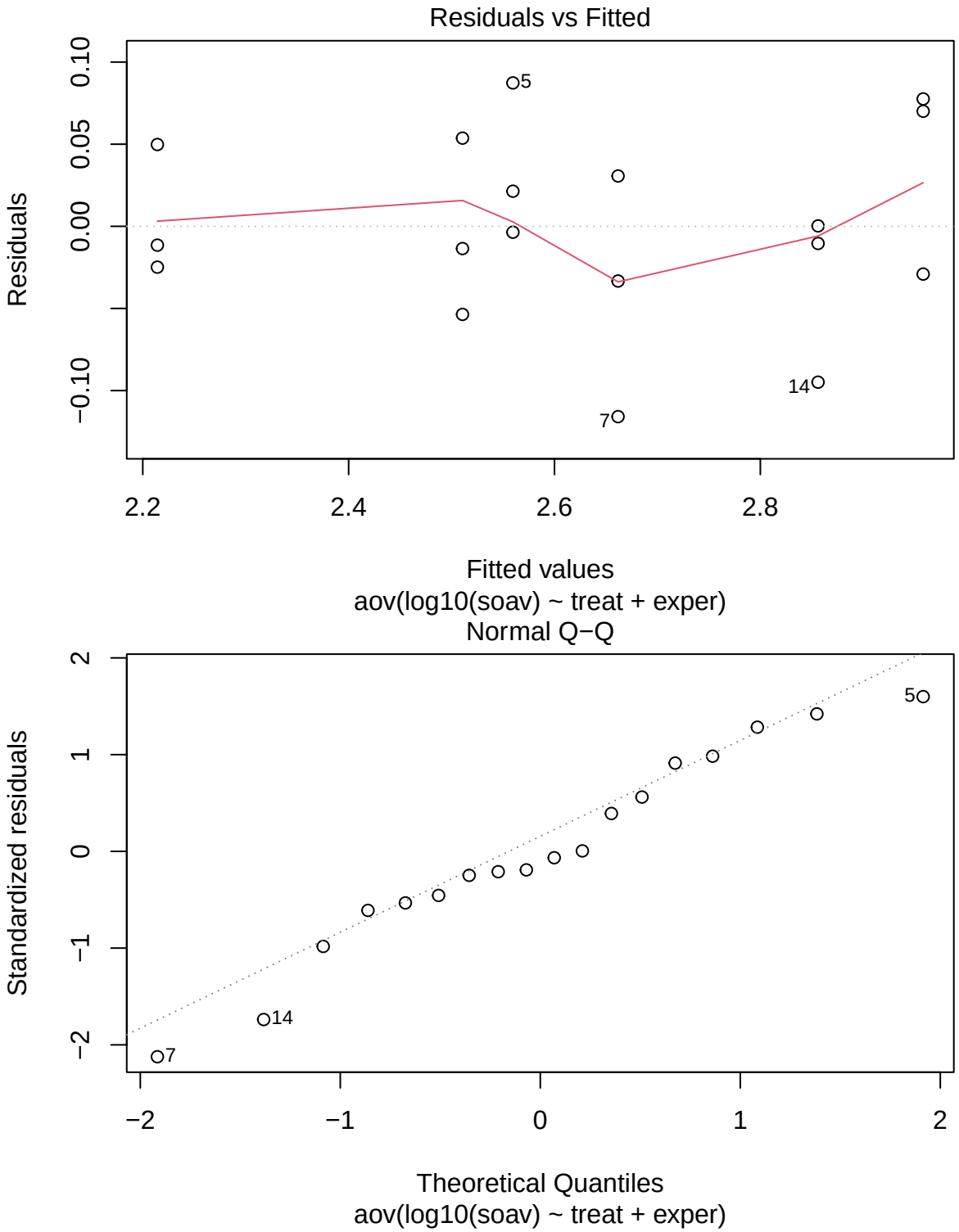
```
100 * (1 - 10^confint(m3))
```

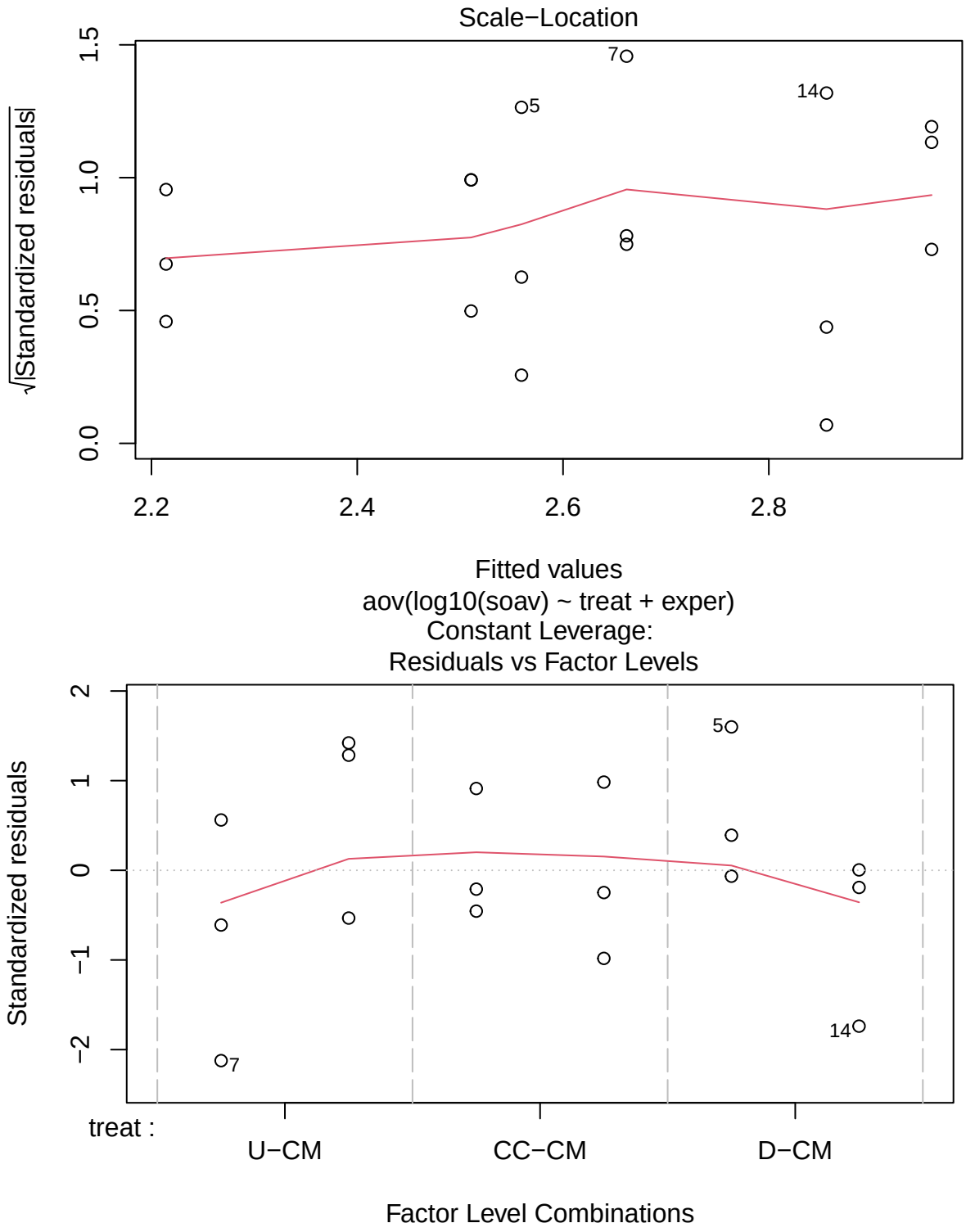
```
##                          2.5 %       97.5 %
## (Intercept)       -39637.26227 -52907.84474
## treatCC-CM            70.09151     57.43411
## treatD-CM             33.74417      5.70441
## experExperiment 2    -71.35537   -128.58089
```

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|
1.5
1.0
0.5
0.0
7
5
14
8
2.2
2.4
2.6
2.8
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