# 4-by-4 factorial design

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  - o anv
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In an experiment with soybean, micronutrients were added to a fertilizer: copper (Cu) and/or manganeze (Mn) (values in % of fertilizer). Yield was then measured (kg/acre).

```
soy = read.table("soybean.txt", header=T)
```

### predictors as factors

Sometimes we will need to use cu as a numerical variable (to plot the data for instance), and sometimes as a factor with 4 categories (for the analysis), and similarly with mn. Below we define 2 new columns with the same data as in cu and mn, but considered as factors.

```
soy$cu_factor = factor(soy$cu)
soy$mn_factor = factor(soy$mn)
str(soy)
```

The experiments used 4 levels of cu addition, and 4 levels of mn addition, with 2 plots for each combination:

```
table(soy$cu, soy$mn)
```

```
20 50 80 110

1 2 2 2 2 2

3 2 2 2 2

5 2 2 2 2

7 2 2 2 2
```

Let's look at the data. We can also visualize and calculate the means at each combination:

```
library(dplyr)
soy %>% group_by(cu, mn) %>% summarize(mean = mean(yield), sd = sd(yield))
```

1

3

cu<sup>5</sup>

7

```
# A tibble: 16 \times 4
# Groups:
             cu [4]
             mn mean
                            sd
   <int> <int> <dbl>
                        <dbl>
 1
                        44.5
        1
             20 1526.
 2
        1
             50 2012
                        12.7
 3
             80 2448
                        59.4
        1
 4
        1
            110 2910 113.
 5
        3
             20 1606.
                        23.3
        3
 6
             50 1979
                        58.0
 7
        3
             80 2644.
                        34.6
        3
            110 2897
                        52.3
 8
 9
        5
             20 1579
                         41.0
10
        5
             50 1994.
                        21.9
11
        5
             80 2566. 107.
        5
            110 2770.
12
                        27.6
13
        7
             20 1297
                        43.8
14
        7
             50 1650. 156.
15
        7
             80 2336
                        79.2
        7
            110 2628
16
                          2.83
```

### analysis of variance

2

1

3

4

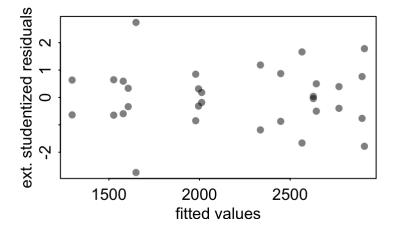
cu

5

6

7

Now to the formal analysis. It is extremely important to consider our two predictors as factors, to let each group have its own mean. Otherwise, and the mean for group cu=3 would be constrained to be half-way between the mean for group cu=1 and for group cu=5.



```
anova(fit)
```

```
Analysis of Variance Table

Response: yield

Df Sum Sq Mean Sq F value Pr(>F)

cu_factor 3 443636 147879 32.2943 5.084e-07

mn_factor 3 8161105 2720368 594.0845 < 2.2e-16

cu_factor:mn_factor 9 69190 7688 1.6789 0.1753

Residuals 16 73265 4579
```

In R, be really careful with this anova function: it returns the "type I" sums of squares, that is, when factors/predictors are added **sequentially**. Here, the design is balanced (n=2 in each treatment combination), so the type I and type III sums of squares are equal: the variance explained by each factor is the same whether it is added first or added last to the model. But in general, beware of the anova function: do not use it if you want to test a factor given that all others are in the model.

A safe way to get p-values based on type III sums of squares is using the <code>drop1</code> function, which drops only 1 term at a time:

```
drop1(fit, test="F")
```

Unfortunately (or fortunately), drop1 respects the hierarchy principle, and it won't drop the term for "cu" if an interaction involving "cu" is present in the model. So here we only get the test for the interaction term.

#### testing main effects

Why can't we test for main effects easily in R? We didn't get p-values for main effects with drop1, and we don't get them with anova unless the design is balanced (type I, or sequential SS). Here is why:

- if there is an interaction:
  - all terms in this interaction should be considered as having an effect: no need for further testing.
  - the "main" effects are not meaningful: the main effect of a factor does not represent any of its simple effects
- if there is no interaction, then we can drop the interaction term from the model, and testing main effects becomes easy.

In our example the interaction is not significant, so we could consider dropping it to test each factor individually:

```
fit.noint = lm(yield ~ cu_factor + mn_factor, data=soy)
drop1(fit.noint, test="F")
```

However we changed the model: the interaction was removed, so the tests for the main effects have changed (notice the slightly different p-values): for instance, we tested "all  $a_i$ =0 |  $\mu$ ,  $\beta_j$ " instead of "all  $a_i$ =0 |  $\mu$ ,  $\beta_j$ ". Many researchers would argue **against** removing the interaction from the model, even if it is not significant, because the experiment was designed as a 2-factorial CRD.

### group means and treatment differences

The estimated coefficients tell us about the estimated group means, but getting all the group means is not straightforward.

```
summary(fit)$coefficients
```

```
Estimate Std. Error
                                               t value
                                                           Pr(>|t|)
                          1526.5
                                   47.84921 31.9023032 6.512077e-16
(Intercept)
cu_factor3
                            80.0
                                   67.66900 1.1822252 2.543885e-01
cu_factor5
                            52.5
                                   67.66900 0.7758353 4.491629e-01
                          -229.5
                                   67.66900 -3.3915086 3.726336e-03
cu_factor7
                           485.5
                                   67.66900 7.1746293 2.210244e-06
mn_factor50
mn factor80
                           921.5
                                   67.66900 13.6177567 3.226611e-10
                          1383.5
                                   67.66900 20.4451074 6.813319e-13
mn_factor110
cu_factor3:mn_factor50
                                   95.69842 -1.1807928 2.549412e-01
                          -113.0
cu factor5:mn factor50
                          -70.0
                                   95.69842 -0.7314645 4.750730e-01
cu_factor7:mn_factor50
                          -133.0
                                   95.69842 -1.3897826 1.836308e-01
cu_factor3:mn_factor80
                           116.5
                                   95.69842 1.2173660 2.411158e-01
cu_factor5:mn_factor80
                           65.0
                                   95.69842 0.6792171 5.067079e-01
cu factor7:mn factor80
                           117.5
                                   95.69842 1.2278155 2.372737e-01
cu_factor3:mn_factor110
                           -93.0
                                   95.69842 -0.9718029 3.456119e-01
cu_factor5:mn_factor110
                          -193.0
                                   95.69842 -2.0167522 6.082519e-02
cu factor7:mn factor110
                           -52.5
                                   95.69842 -0.5485984 5.908547e-01
```

- For example, the levels **cu=1** and **mn=20** do not appear in the coefficient names. They are the baseline levels, with values of 0 for all dummy variables that represent cu and mn. Do model.matrix(fit) to see these dummy variables. So the mean yield in the baseline group, cu=1 and mn=20 is the intercept: 1526.50. Its standard error is given as 47.85, which would allow us to get a confidence interval for instance (using a multiplier from the t-distribution with dfError = 16 df).
- For the group mean in group **cu=3 and mn=50**, it's more complicated: we need to add the intercept, the coefficient for cu=3, and coefficient for mn=50, and the interaction coefficient for "cu=3 and mn=50": 1526.50+80+485.5+(-113) = 1979. But the coefficient table does not give the SE for this estimate.

#### using emmeans

The package emmeans (https://cran.r-project.org/web/packages/emmeans/) is the best to do inference in treament means, their pairwise differences, and other contrasts. It has great documentation, is very flexible (e.g. to models with random effects), and easier syntax than other packages to specify contrats.

EMM stands for "estimated marginal means" (estimated from a fitted model), sometimes called adjusted means when the model has covariates. Let's look at treatment means first:

```
library(emmeans)
emmeans(fit, ~ mn_factor)
```

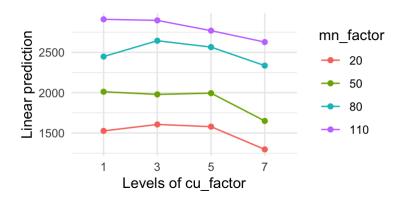
```
mn factor emmean
                    SE df lower.CL upper.CL
 20
            1502 23.9 16
                              1452
                                       1553
 50
             1909 23.9 16
                              1858
                                       1959
 80
            2498 23.9 16
                              2448
                                       2549
 110
            2801 23.9 16
                              2750
                                       2852
Results are averaged over the levels of: cu_factor
Confidence level used: 0.95
```

# emmeans(fit, ~ mn\_factor | cu\_factor) # same as below, presented differently
emmeans(fit, ~ mn\_factor:cu\_factor)

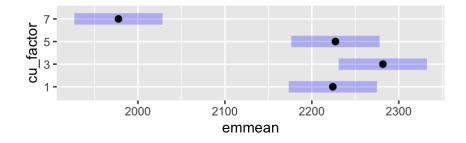
_	_				_	
mn_factor	cu_factor	emmean	SE	df	lower.CL	upper.CL
20	1	1526	47.8	16	1425	1628
50	1	2012	47.8	16	1911	2113
80	1	2448	47.8	16	2347	2549
110	1	2910	47.8	16	2809	3011
20	3	1606	47.8	16	1505	1708
50	3	1979	47.8	16	1878	2080
80	3	2644	47.8	16	2543	2746
110	3	2897	47.8	16	2796	2998
20	5	1579	47.8	16	1478	1680
50	5	1994	47.8	16	1893	2096
80	5	2566	47.8	16	2464	2667
110	5	2770	47.8	16	2668	2871
20	7	1297	47.8	16	1196	1398
50	7	1650	47.8	16	1548	1751
80	7	2336	47.8	16	2235	2437
110	7	2628	47.8	16	2527	2729

Confidence level used: 0.95

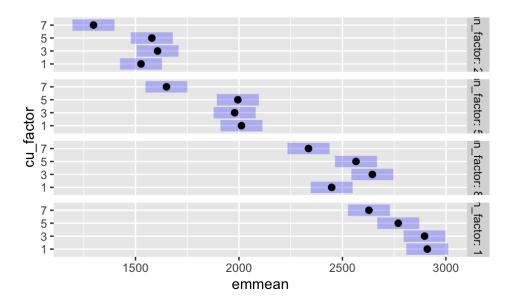
```
library(ggplot2)
emmip(fit, mn_factor ~ cu_factor) + theme_minimal() # ip = interaction plot
```



plot(emmeans(fit, ~ cu\_factor))



```
plot(emmeans(fit, ~ cu_factor | mn_factor), by = "mn_factor")
```



by the way: why are all intervals of the same length? Now, let's look at pairwise comparisons:

```
em_cu = emmeans(fit, ~ cu_factor)
pairs(em_cu) # default: Tukey
```

```
contrast estimate
                    SE df t.ratio p.value
            -57.6 33.8 16 -1.703 0.3542
1 - 5
            -3.0 33.8 16 -0.089 0.9997
 1 - 7
            246.5 33.8 16
                           7.285 <.0001
            54.6 33.8 16
                          1.614 0.3987
 3 - 7
            304.1 33.8 16
                            8.989 <.0001
            249.5 33.8 16
                          7.374 <.0001
Results are averaged over the levels of: mn factor
P value adjustment: tukey method for comparing a family of 4 estimates
```

```
pairs(em_cu, adjust = "none") # for LSD
```

```
contrast estimate
                    SE df t.ratio p.value
            -57.6 33.8 16 -1.703 0.1079
 1 - 5
             -3.0 33.8 16 -0.089 0.9304
 1 - 7
            246.5 33.8 16
                            7.285 <.0001
  - 5
             54.6 33.8 16
                           1.614 0.1260
  - 7
            304.1 33.8 16
                           8.989 <.0001
            249.5 33.8 16
                            7.374 <.0001
Results are averaged over the levels of: mn factor
```

```
pwpm(em_cu, adjust = "none") # pw = pairwise pm = p-value matrix
```

```
1 3 5 7

1 [2224] 0.1079 0.9304 <.0001

3 -57.6 [2282] 0.1260 <.0001

5 -3.0 54.6 [2227] <.0001

7 246.5 304.1 249.5 [1978]

Row and column labels: cu_factor
Upper triangle: P values
Diagonal: [Estimates] (emmean)
Lower triangle: Comparisons (estimate) earlier vs. later
```

```
em_cuxmn = emmeans(fit, ~ cu_factor:mn_factor)[1:10] # first 10 only: to fit page width
pwpm(em_cuxmn)
```

```
1 20
              3 20
                             7 20
                                    1 50
                                           3 50
                                                   5 50
                                                          7 50
                     5 20
                                                                 1 80
                                                                        3 80
1 20 [1526] 0.9651 0.9980 0.0789 <.0001 0.0002 0.0001 0.7170 <.0001 <.0001
     -80.0 [1606] 1.0000 0.0085 0.0006 0.0014 0.0009 0.9996
                                                               <.0001 <.0001
3 20
5 20
      -52.5 27.5 [1579] 0.0185 0.0003 0.0007 0.0004 0.9842 <.0001 <.0001
                   282.0 [1297] <.0001 <.0001 <.0001 0.0025 <.0001 <.0001
7 20
      229.5 309.5
1 50
    -485.5 -405.5 -433.0 -715.0 [2012] 1.0000 1.0000 0.0019 0.0003 < .0001
3 50
    -452.5 -372.5 -400.0 -682.0
                                  33.0 [1979] 1.0000 0.0048 0.0001 <.0001
                                    17.5 -15.5 [1994] 0.0031 0.0002 <.0001
5 50 -468.0 -388.0 -415.5 -697.5
7 50 -123.0 -43.0 -70.5 -352.5
                                                               <.0001 <.0001
                                   362.5
                                        329.5
                                                345.0 [1649]
1 80 -921.5 -841.5 -869.0 -1151.0 -436.0 -469.0 -453.5 -798.5
                                                               [2448] 0.1824
3 80 -1118.0 -1038.0 -1065.5 -1347.5 -632.5 -665.5 -650.0 -995.0 -196.5 [2644]
Row and column labels: cu factor:mn factor:.wgt.
Upper triangle: P values
                        adjust = "tukey"
Diagonal: [Estimates] (emmean)
Lower triangle: Comparisons (estimate)
                                     earlier vs. later
```

Finally, let's consider a contrast other than a pairwise difference. Say, we want to see if the response at cu=3 (averaged over the mn values) is the average between the mean at cu=1 and at cu=5 (again, averaged over the mn values). We find weak evidence against:

```
contrast(em_cu, list(cu3_vsland5 = c(-1/2, 1,-1/2,0)))
```

```
contrast estimate SE df t.ratio p.value
cu3_vsland5 56.1 29.3 16 1.915 0.0735

Results are averaged over the levels of: mn_factor
```

## other functions and packages for comparisons

Here are other tools that you may encounter, good to know about. But emmeans is the best so far, I think.

#### aov

The function and gives us another way to fit the same model (and get the same ANOVA table), and has many tools for post-hoc group comparisons, like Tukey's honest significant differences. But it is limited to simple designs, and balanced designs.

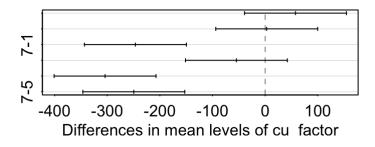
```
fit.aov = aov(yield ~ cu_factor * mn_factor, data=soy)
anova(fit.aov)
```

```
Analysis of Variance Table
Response: yield
                   Df Sum Sq Mean Sq F value
                                                 Pr(>F)
cu factor
                    3 443636 147879 32.2943 5.084e-07
mn_factor
                    3 8161105 2720368 594.0845 < 2.2e-16
cu_factor:mn_factor 9
                                      1.6789
                        69190
                                 7688
                                                 0.1753
Residuals
                   16
                        73265
                                 4579
```

```
TukeyHSD(fit.aov, "cu_factor")
```

```
plot(TukeyHSD(fit.aov, "cu_factor"))
```

#### 95% family-wise confidence level



Estimated group means are also easier to obtain after fitting the model with aov:

```
model.tables(fit.aov, type="means", se=TRUE)
```

```
Tables of means
Grand mean
2177,656
cu factor
cu_factor
           3
                  5
    1
2224.1 2281.7 2227.1 1977.6
mn factor
mn_factor
   20
        50
                 80
                       110
1502.2 1908.7 2498.5 2801.1
cu_factor:mn_factor
        mn factor
cu_factor 20
                50
                     80
                             110
       1 1526.5 2012.0 2448.0 2910.0
       3 1606.5 1979.0 2644.5 2897.0
       5 1579.0 1994.5 2565.5 2769.5
       7 1297.0 1649.5 2336.0 2628.0
Standard errors for differences of means
       cu_factor mn_factor cu_factor:mn_factor
           33.83 33.83
                                        67.67
replic.
               8
                         8
                                            2
```

The last table gives SEs to calculate various things:

- the LSD (least significant difference) values: to compare the overall means among cu groups at the 5% level, for instance, the LSD is 33.83 \* 2.12 = 71.72. Group means that differ by more than 71.72 would be said statistically different (but recall that LSD is liberal). The 2.12 is the t multiplier for 95% confidence, from 16 df (dfError): qt(.975, df=16) = 2.12. So for instance, the means for cu=1 and cu=3 are not statistically different according to LSD: 2281.7 2224.1=57.6, which is less than 71.72.
- test for differences between means: to compare the mean at cu=3 versus cu=1, we would divide the observed difference in means, 57.6, by the standard error of this difference: 33.83 (last table in the output above). Note that this SE can be derived manually using the MSError (4579) and the formula for the variance of a contrast: sqrt(4579\*(1/8+1/8)) = 33.83. So our t-value is 57.6/33.83 = 1.702, to be compared with a t-distribution with 16 df (error df): pt(1.702, df=16, lower.tail=FALSE)\*2 =0.13. Again, the difference is not significant.
- confidence interval for a **single mean**: we would follow a similar strategy. For the overal mean 2224.1 in groups cu=1, for example, the standard error of this mean is sqrt(4579\*(1/8)) = 23.92. The t multiplier for 95% confidence is still 2.12 (16 df), so our confidence interval has limits 2224.1 ± 2.12 \* 23.92.

- confidence interval for the group cu=1 and mn=20. Here we would use the estimated mean in that group: 1526.5. Its standard error is calculated on the basis of only 2 observation for that group: sqrt(4579\*(1/2)) = 47.85.
- difference between the means in group "cu=1 and mn=20" and group "cu=3 and mn=20": same strategy as before, but the standard error for the difference of interest is sqrt(4579\*(1/2 + 1/2)), because each group only has 2 observations. In the output above, the last row of the last table is useful to determine the appropriate SE, based on which group(s) we are interested in and the sample size for that (those) groups.

We can also get the "effects" that we need to add to the overall mean (intercept in the model). Using the course notations, these effects are the the  $a_i$ ,  $\beta_j$  and  $(a\beta)_{ij}$  terms. Note that they do sum to zero, in each row and each column:

```
model.tables(fit.aov) # default is type="effects"
```

```
Tables of effects
cu_factor
cu factor
       3 5 7
    1
 46.47 104.09 49.47 -200.03
mn_factor
mn factor
   2.0
      50
               80
                    110
-675.4 -268.9 320.8 623.5
cu factor:mn factor
       mn factor
cu_factor 20 50 80 110
      1 -22.22 56.78 -96.97 62.41
      3 0.16 -33.84 41.91 -8.22
      5 27.28 36.28 17.53 -81.09
      7 -5.22 -59.22 37.53 26.91
```

#### general contrasts using package multcomp

```
library(multcomp)
```

First, let's look at a very specific contrast: at mn=20, is the response to cu=3 in between those at cu=1 and cu=5? Warning: the coefficient named  $cu\_factor3$  is the difference between cu=3 and cu=1 (etc.), so the contrast coefficient is 0 for the intercept, below.

```
# coef(fit) # look at coefficients and what they mean, to define the constrast next K = matrix(c(0,-2,1, rep(0,13)), 1) t = glht(fit, linfct = K) summary(t)
```

```
Simultaneous Tests for General Linear Hypotheses

Fit: lm(formula = yield ~ cu_factor * mn_factor, data = soy)

Linear Hypotheses:

Estimate Std. Error t value Pr(>|t|)

1 == 0 -107.5 117.2 -0.917 0.373

(Adjusted p values reported -- single-step method)
```

testing main effect of cu using Tukey's HSD or Fisher's LSD:

```
lh_cu = glht(fit, linfct = mcp(cu_factor="Tukey", interaction_average=T))
summary(lh_cu) # p-values: for Tukey's HSD
```

```
Simultaneous Tests for General Linear Hypotheses
Multiple Comparisons of Means: Tukey Contrasts
Fit: lm(formula = yield ~ cu_factor * mn_factor, data = soy)
Linear Hypotheses:
          Estimate Std. Error t value Pr(>|t|)
3 - 1 == 0 57.62 33.84 1.703
                                       0.354
            3.00 33.84 0.005
246.50 33.84 -7.285 <0.001
5 - 1 == 0
7 - 1 == 0 -246.50
5 - 3 == 0 -54.62
7 - 3 == 0 -304.12
                        33.84 -8.989 <0.001
7 - 5 == 0 -249.50
                        33.84 -7.374 <0.001
(Adjusted p values reported -- single-step method)
```

summary(lh cu, test=univariate()) # no correction for multiple comparisons: Fisher LSD

applying Tukey's HSD to compare all 16 treatment means (try for yourself):

```
soy$cuxmn = with(soy, interaction(cu, mn, sep = "x"))
head(soy$cuxmn)
fit2 = lm(yield ~ cuxmn, data=soy)
lh2 = glht(fit2, linfct = mcp(cuxmn="Tukey"))
summary(lh2)
```

#### multiple comparisons using package DescTools

and aov fit (try for yourself):

```
library(DescTools)
PostHocTest(fit.aov, method = "lsd")
PostHocTest(fit.aov, method = "scheffe")
PostHocTest(fit.aov, method = "hsd")
```

## using specific contrasts as coefficients

In our model fit, all levels except the first have their own coefficients. For each factor, the first level is the "base" level. The intercept corresponds to the mean yield when all the factors are at their "base" level. This parametrization of the design matrix and coefficient is said to use what's called, in R, the "treatment" contrasts. It facilitates pairwise comparisons between treatments.

```
options()$contrasts # default is "contr.treatment"
```

```
unordered ordered
"contr.treatment" "contr.poly"
```

A different parametrization is with the "sum" contrasts, where the cu coefficients are  $a_i$  with  $\Sigma a_i = 0$  for example. Because of the constraint that a's (and a's, a's etc) sum to 0, R does not estimate the last a, here the coefficient associated with level a0. It takes it as the negative sum of the others: a0 to index the a1 coefficients.

```
lm(formula = yield ~ cu_factor * mn_factor, data = soy, contrasts = list(cu_factor = con
tr.sum,
    mn_factor = contr.sum))
Residuals:
    Min 1Q Median
                               3Q
                                         Max
-110.50 -31.12 0.00 31.12 110.50
Coefficients:
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)
                         2177.6562 11.9623 182.043 < 2e-16
cu factor1
                          46.4687
                                      20.7193 2.243 0.039426
cu_factor2
cu_factor3
                        104.093820.71935.0240.00012549.468820.71932.3880.029643
                       -675.4063 20.7193 -32.598 4.63e-16
mn factor1
mn factor2
                        -268.9062 20.7193 -12.979 6.56e-10
mn_factor3
                         320.8438 20.7193 15.485 4.74e-11
cu_factor1:mn_factor1 -22.2188 35.8869 -0.619 0.544545
cu_factor2:mn_factor1     0.1563     35.8869     0.004     0.996580
cu_factor3:mn_factor1 27.2813 35.8869 0.760 0.458191
cu_factor1:mn_factor2 56.7813 35.8869 1.582 0.133162

      cu_factor2:mn_factor2
      -33.8438
      35.8869
      -0.943
      0.359670

      cu_factor3:mn_factor2
      36.2812
      35.8869
      1.011
      0.327067

      cu_factor1:mn_factor3
      -96.9687
      35.8869
      -2.702
      0.015705

cu factor2:mn factor3 41.9062 35.8869 1.168 0.260023
cu factor3:mn factor3 17.5312
                                        35.8869 0.489 0.631814
Residual standard error: 67.67 on 16 degrees of freedom
Multiple R-squared: 0.9916, Adjusted R-squared:
F-statistic: 126.3 on 15 and 16 DF, p-value: 1.024e-13
```

With these "sum" contrasts, the intercept represents the mean of all group means, which is very nice. But getting individual group means is much more tedious. For example, to get the mean at cu=3 (second level) and mn=80 (third level):

```
2177.6562 + 104.0938 + 320.8438 + 41.9062 # 2644.5
```

Group means involving the last level for one or more factors are more complicated. For example, the mean yield at cu=7 (last level) and mn=20 (first level) is:

```
2177.6562 -(46.4687+104.0938+49.4688) + -675.4063 -(-22.2188+0.1563+27.2813) # 1297
```

An alternative way to fit the model with the "sum" contrasts is to change the global default contrast settings, and the "sum" contrasts will be use for all factors, now and later:

```
options(contrasts = c("contr.sum", "contr.poly"))
fit.sum = lm(yield ~ cu_factor * mn_factor, data=soy)
summary(fit.sum) # same as above
```