ENTMLGY 6702 Entomological Techniques and Data Analysis

Supplemental activity: Split Plots

Due: Never :)

1 Introduction

Stratified designs are used to increase precision of estimates by experimentally controlling or reducing variation. This tutorial covers how to analyze split-plot and split-split-plot designs. The examples include multiple ways of analyzing the same exact data.

You might recall that F-values in ANOVAs are calculated from F-ratios (e.g., mean squares for treatment divided by means squares for errors). Historically, for more complicated designs like split-plots, folks had to compute the F-ratios "by-hand": these designs have whole plot and sub plot factors and errors, and so F-ratios for whole plot factors use the the whole plot error as the denominator whereas sub plot factors use the sub plot error. When examining the R output below for split plots and split-split plots, note which mean square values are used in the F-ratios.

Nowadays, some folks use mixed-effects models to analyze stratified designs. Be aware that a fixed-effect only approach might be preferred, given that we assume random effects are normally distriubuted and it is hard to test that assumption when there are only a few levels of a random effect (e.g., 3 blocks or 4 whole plots).

Either way, you still have to be careful about specifying the random effects correctly. And you will notice that if you do, the sums of squares and F-statistics for each treatment are typically equivalent. There are sometimes differences, but rarely do they influence the overall conclusions.

The following packages are necessary to complete this tutorial.

```r
library(car)
library(lme4)
library(lmerTest)
library(tidyverse)
library(agricolae)
library(emmeans)
```

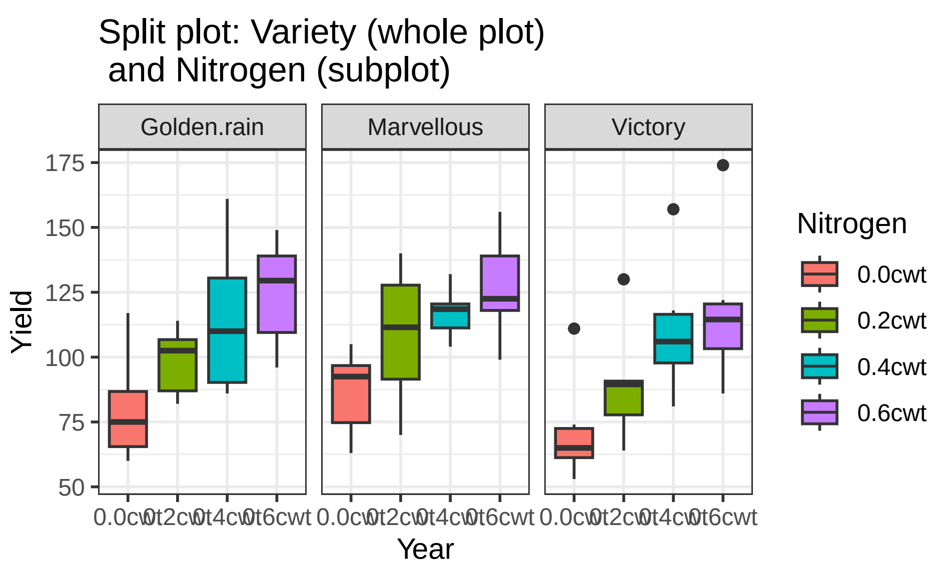
2 Split plot

These data report the yield of oats from a split-plot field trial. The treatment structure used in the experiment was a 3 x 4 full factorial, with three varieties of oats and four concentrations of nitrogen. The experimental units were arranged into six blocks, each with three whole-plots subdivided into four subplots. The varieties of oats were assigned randomly to the whole-plots and the concentrations of nitrogen to the subplots. All four concentrations of nitrogen were used on each whole-plot.

```
oats_df <- read.table("oats.txt", header=T, sep="\t",
                     colClasses = c("factor",   "factor","factor",
                                    "factor", "numeric"))
summary(oats_df)
```

```
##  Block          Variety     Nitrogen      Yield
##  I  :12   Golden.rain:24   0.0cwt:18   Min.   : 53.0
##  II :12   Marvellous :24   0.2cwt:18   1st Qu.: 86.0
##  III:12   Victory    :24   0.4cwt:18   Median :102.5
##  IV :12                    0.6cwt:18   Mean   :104.0
##  V  :12                                3rd Qu.:121.2
##  VI :12                                Max.   :174.0
```

```
ggplot(oats_df, aes(x=Nitrogen, y=Yield, fill=Nitrogen)) +
  geom_boxplot()+ theme_bw()+
  xlab("Year")+
  ylab("Yield")+
  facet_wrap(~Variety)+
  ggtitle("Split plot: Variety (whole plot) \n and Nitrogen (subplot)")
```



2.1 Agricolae package

```
library(agricolae)
sp.plot(oats_df$Block, oats_df$Variety, oats_df$Nitrogen, oats_df$Yield)
```

```
##
## ANALYSIS SPLIT PLOT:  oats_df$Yield
## Class level information
```

```
##
## oats_df$Variety  :  Victory Golden.rain Marvellous
## oats_df$Nitrogen     :  0.0cwt 0.2cwt 0.4cwt 0.6cwt
## oats_df$Block    :  I II III IV V VI
##
## Number of observations:  72
##
## Analysis of Variance Table
##
## Response: oats_df$Yield
##                                  Df  Sum Sq Mean Sq F value    Pr(>F)
## oats_df$Block                     5 15875.3  3175.1 17.9297 9.525e-10 ***
## oats_df$Variety                   2  1786.4   893.2  1.4853    0.2724
## Ea                               10  6013.3   601.3
## oats_df$Nitrogen                  3 20020.5  6673.5 37.6856 2.458e-12 ***
## oats_df$Variety:oats_df$Nitrogen  6   321.8    53.6  0.3028    0.9322
## Eb                               45  7968.7   177.1
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## cv(a) = 23.6 %, cv(b) = 12.8 %, Mean = 103.9722
```

2.2 aov()

```
fit_aov_SP <- aov(Yield ~ Variety*Nitrogen + Error(Block/Variety), data=oats_df)
summary(fit_aov_SP)
```

```
##
## Error: Block
##           Df Sum Sq Mean Sq F value Pr(>F)
## Residuals  5  15875    3175
##
## Error: Block:Variety
##           Df Sum Sq Mean Sq F value Pr(>F)
## Variety    2   1786   893.2   1.485  0.272
## Residuals 10   6013   601.3
##
## Error: Within
##                  Df Sum Sq Mean Sq F value   Pr(>F)
## Nitrogen          3  20020    6673  37.686 2.46e-12 ***
## Variety:Nitrogen  6    322      54   0.303    0.932
## Residuals        45   7969     177
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.3 mixed-effects model

```
fit_lmer_SP <- lmer(Yield ~ Variety*Nitrogen + (1|Block) + (1|Block:Variety), data=oats_df)
anova(fit_lmer_SP, type=3)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                  Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Variety           526.1   263.0     2    10  1.4853    0.2724
## Nitrogen        20020.5  6673.5     3    45 37.6857 2.458e-12 ***
```

```
## Variety:Nitrogen   321.8    53.6     6    45  0.3028    0.9322
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.4 pairwise comparisons

```r
emmeans(fit_lmer_SP, pairwise~"Nitrogen")
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
## $emmeans
##  Nitrogen emmean   SE   df lower.CL upper.CL
##  0.0cwt     79.4 7.17 6.79     62.3     96.5
##  0.2cwt     98.9 7.17 6.79     81.8    116.0
##  0.4cwt    114.2 7.17 6.79     97.2    131.3
##  0.6cwt    123.4 7.17 6.79    106.3    140.5
##
## Results are averaged over the levels of: Variety
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast        estimate   SE df t.ratio p.value
##  0.0cwt - 0.2cwt   -19.50 4.44 45  -4.396  0.0004
##  0.0cwt - 0.4cwt   -34.83 4.44 45  -7.853  <.0001
##  0.0cwt - 0.6cwt   -44.00 4.44 45  -9.919  <.0001
##  0.2cwt - 0.4cwt   -15.33 4.44 45  -3.457  0.0064
##  0.2cwt - 0.6cwt   -24.50 4.44 45  -5.523  <.0001
##  0.4cwt - 0.6cwt    -9.17 4.44 45  -2.067  0.1797
##
## Results are averaged over the levels of: Variety
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
```

3 Split-split splot

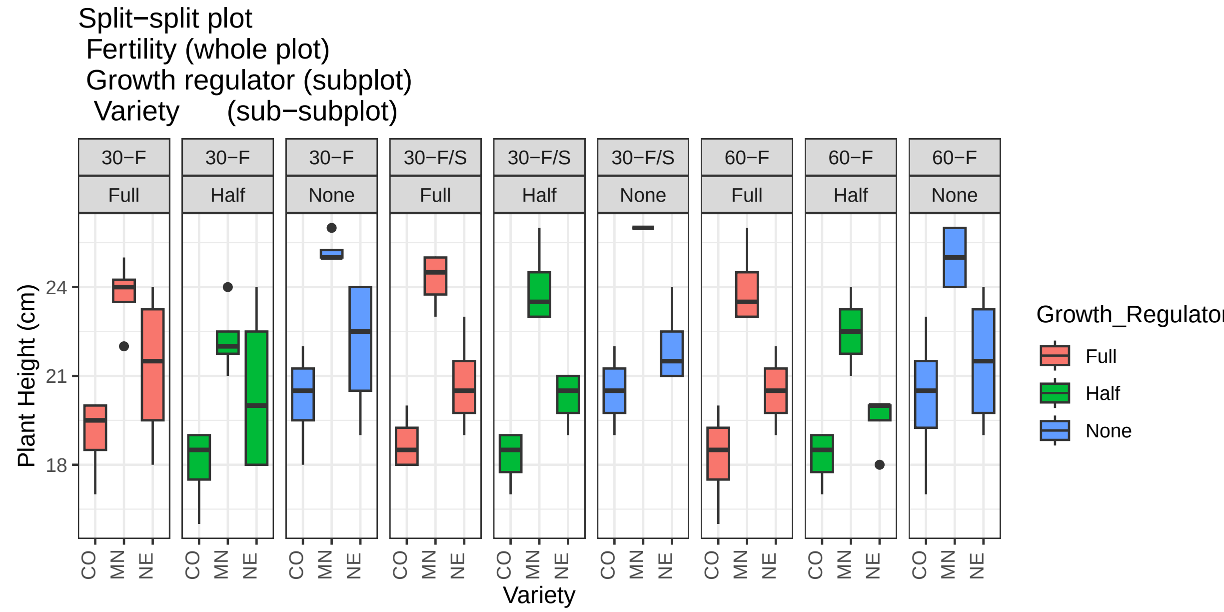
Prairie junegrass is a native species that has potential as a low input turf grass. Seed production strategies need to be investigated to determine best management practices. An experiment was conducted at the Sand Plain Research Center near Becker, MN to investigate varieties, fertility, and the use of growth regulators. Growth regulators in grasses tend to reduce plant height by influencing stem elongation. The experiment was a split split plot treatment design in a randomized complete block with 4 replications (blocks).

- Whole plots: Fertility
  - 30-F = 30 lbs N applied in Fall
  - 30-F/S = 30 lbs N applied in Fall + 30 lbs N applied in Spring
  - 60-F = 60 lbs N applied in Fall
- Subplots: Growth regulator applications
  - None = no Apogee growth regulator
  - Full = full rate (8 oz.)
  - Half = half rate (4 oz.)
- Sub-Subplots: 3 prairie junegrass populations
  - CO = Koeleria Colorada
  - NE = Koeleria Nebraska
  - MN = Koeleria Weaver Dunes, MN

```
june_df <- read.table('EPP_junegrass.txt', header=T, sep="\t",
                      colClasses = c("factor","factor","factor","factor",
                                     "numeric", "numeric", "numeric"))

summary(june_df)
```

```
##  Variety Growth_Regulator  Fertility  Rep     Seed_Yield_samples
##  CO:36   Full:36          30-F  :36   1:27   Min.   : 2.00
##  MN:36   Half:36          30-F/S:36   2:27   1st Qu.:11.00
##  NE:36   None:36          60-F  :36   3:27   Median :15.00
##                                       4:27   Mean   :15.81
##                                              3rd Qu.:20.25
##                                              Max.   :40.00
##  Seed_Yield_lbs_per_acre  Plant_Height
##  Min.   : 18.0           Min.   :16.00
##  1st Qu.: 98.0           1st Qu.:19.00
##  Median :134.0           Median :21.00
##  Mean   :140.7           Mean   :21.36
##  3rd Qu.:180.2           3rd Qu.:24.00
##  Max.   :356.0           Max.   :26.00
```



3.1 Agricolae package

```
ssp.plot(june_df$Rep, june_df$Fertility, june_df$Growth_Regulator,
         june_df$Variety, june_df$Plant_Height)
```

```
##
## ANALYSIS SPLIT-SPLIT PLOT:  june_df$Plant_Height
## Class level information
##
## june_df$Fertility    :  30-F 30-F/S 60-F
## june_df$Growth_Regulator     :  None Full Half
## june_df$Variety  :  CO NE MN
## june_df$Rep  :  1 2 3 4
##
## Number of observations:  108
##
## Analysis of Variance Table
##
## Response: june_df$Plant_Height
##                                                              Df Sum Sq Mean Sq
## june_df$Rep                                                   3 109.88  36.627
## june_df$Fertility                                             2   5.56   2.778
## Ea                                                            6  13.26   2.210
## june_df$Growth_Regulator                                      2  84.39  42.194
## june_df$Fertility:june_df$Growth_Regulator                    4   1.39   0.347
## Eb                                                           18  18.44   1.025
## june_df$Variety                                               2 470.72 235.361
## june_df$Variety:june_df$Fertility                             4   5.72   1.431
## june_df$Variety:june_df$Growth_Regulator                      4   2.72   0.681
## june_df$Variety:june_df$Fertility:june_df$Growth_Regulator    8   2.67   0.333
## Ec                                                           54  64.17   1.188
##                                                               F value    Pr(>F)
## june_df$Rep                                                   30.8234 9.508e-12
```

```
## june_df$Fertility                                           1.2570    0.3500
## Ea
## june_df$Growth_Regulator                                   41.1777 1.921e-07
## june_df$Fertility:june_df$Growth_Regulator                  0.3389    0.8482
## Eb
## june_df$Variety                                           198.0701 < 2.2e-16
## june_df$Variety:june_df$Fertility                           1.2039    0.3198
## june_df$Variety:june_df$Growth_Regulator                    0.5727    0.6836
## june_df$Variety:june_df$Fertility:june_df$Growth_Regulator   0.2805    0.9696
## Ec
##
## june_df$Rep                                               ***
## june_df$Fertility
## Ea
## june_df$Growth_Regulator                                  ***
## june_df$Fertility:june_df$Growth_Regulator
## Eb
## june_df$Variety                                           ***
## june_df$Variety:june_df$Fertility
## june_df$Variety:june_df$Growth_Regulator
## june_df$Variety:june_df$Fertility:june_df$Growth_Regulator
## Ec
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## cv(a) = 7 %, cv(b) = 4.7 %, cv(c) = 5.1 %, Mean = 21.36111
```

3.2 aov()

```
fit_aov_SSP <-aov(Plant_Height   ~  Rep + Fertility*Growth_Regulator*Variety +
                   Error(Rep/Fertility/Growth_Regulator),data=june_df)
summary(fit_aov_SSP)
```

```
##
## Error: Rep
##     Df Sum Sq Mean Sq
## Rep  3  109.9   36.63
##
## Error: Rep:Fertility
##           Df Sum Sq Mean Sq F value Pr(>F)
## Fertility  2  5.556   2.778   1.257   0.35
## Residuals  6 13.259   2.210
##
## Error: Rep:Fertility:Growth_Regulator
##                            Df Sum Sq Mean Sq F value   Pr(>F)
## Growth_Regulator            2  84.39   42.19  41.178 1.92e-07 ***
## Fertility:Growth_Regulator  4   1.39    0.35   0.339    0.848
## Residuals                  18  18.44    1.02
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##                                      Df Sum Sq Mean Sq F value Pr(>F)
## Variety                               2  470.7  235.36 198.070 <2e-16 ***
```

```
## Fertility:Variety                     4    5.7    1.43   1.204  0.320
## Growth_Regulator:Variety              4    2.7    0.68   0.573  0.684
## Fertility:Growth_Regulator:Variety    8    2.7    0.33   0.281  0.970
## Residuals                            54   64.2    1.19
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

3.3 mixed-effects model

```
fit_lmer_SSP <- lmer(Plant_Height ~ Fertility*Growth_Regulator*Variety +
                   (1|Rep) + (1:Rep:Fertility) + (1:Rep:Fertility:Growth_Regulator),
                   data=june_df)
anova(fit_lmer_SSP, type=3)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##                                    Sum Sq Mean Sq NumDF DenDF  F value
## Fertility                            5.56   2.778     2    78   2.2600
## Growth_Regulator                    84.39  42.194     2    78  34.3293
## Variety                            470.72 235.361     2    78 191.4895
## Fertility:Growth_Regulator           1.39   0.347     4    78   0.2825
## Fertility:Variety                    5.72   1.431     4    78   1.1639
## Growth_Regulator:Variety             2.72   0.681     4    78   0.5537
## Fertility:Growth_Regulator:Variety   2.67   0.333     8    78   0.2712
##                                       Pr(>F)
## Fertility                             0.1111
## Growth_Regulator                   2.022e-11 ***
## Variety                            < 2.2e-16 ***
## Fertility:Growth_Regulator            0.8885
## Fertility:Variety                     0.3333
## Growth_Regulator:Variety              0.6969
## Fertility:Growth_Regulator:Variety    0.9735
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

3.4 pairwise comparisons

```
emmeans(fit_lmer_SSP, pairwise~Variety)
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
## $emmeans
##  Variety emmean    SE   df lower.CL upper.CL
##  CO        19.1 0.602 3.42     17.3     20.8
##  MN        24.1 0.602 3.42     22.3     25.9
##  NE        20.9 0.602 3.42     19.1     22.7
##
## Results are averaged over the levels of: Fertility, Growth_Regulator, Rep
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast estimate    SE df t.ratio p.value
##  CO - MN     -5.06 0.261 78 -19.347  <.0001
##  CO - NE     -1.86 0.261 78  -7.122  <.0001
```

```
##  MN - NE      3.19 0.261 78  12.225  <.0001
##
## Results are averaged over the levels of: Fertility, Growth_Regulator, Rep
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
```

4 R Activity

You will need the following packages to complete this problem set:

```
library(tidyverse)
library(car)
library(lme4)
library(lmerTest)
library(emmeans)
```

An experiment was designed to assess the effect of nitrogen fertilizer on yield (grams) from three varieties of wheat. The experimental design was a split plot with replicates (=blocks). Five rates of nitrogen fertilizer were applied to whole plots at rates of 0, 40, 80, 120 and 160 kg/ha, and the three varieties were planted in sub plots. The data are in the "`EPP_yield.txt`" data file. Even though split plots are often designed to evaluate interactions, please ignore interactions for this activity.

1. Load in the data. Note that column names starting with `Rep` represent a block.

```
yield_df <- read.table('EPP_yield.txt', header=T, sep="\t",
                       colClasses = c("factor","factor",NA,NA,NA))
summary(yield_df)
```

```
##  Nitrogen Variety      Rep1           Rep2           Rep3      
##  0  :3    1:5     Min.   :3.60   Min.   :4.30   Min.   :4.200  
##  120:3    2:5     1st Qu.:5.35   1st Qu.:5.30   1st Qu.:5.300  
##  160:3    3:5     Median :6.40   Median :6.50   Median :5.900  
##  40 :3            Mean   :6.58   Mean   :6.58   Mean   :6.313  
##  80 :3            3rd Qu.:8.00   3rd Qu.:7.75   3rd Qu.:7.300  
##                   Max.   :9.20   Max.   :9.70   Max.   :9.300  
```

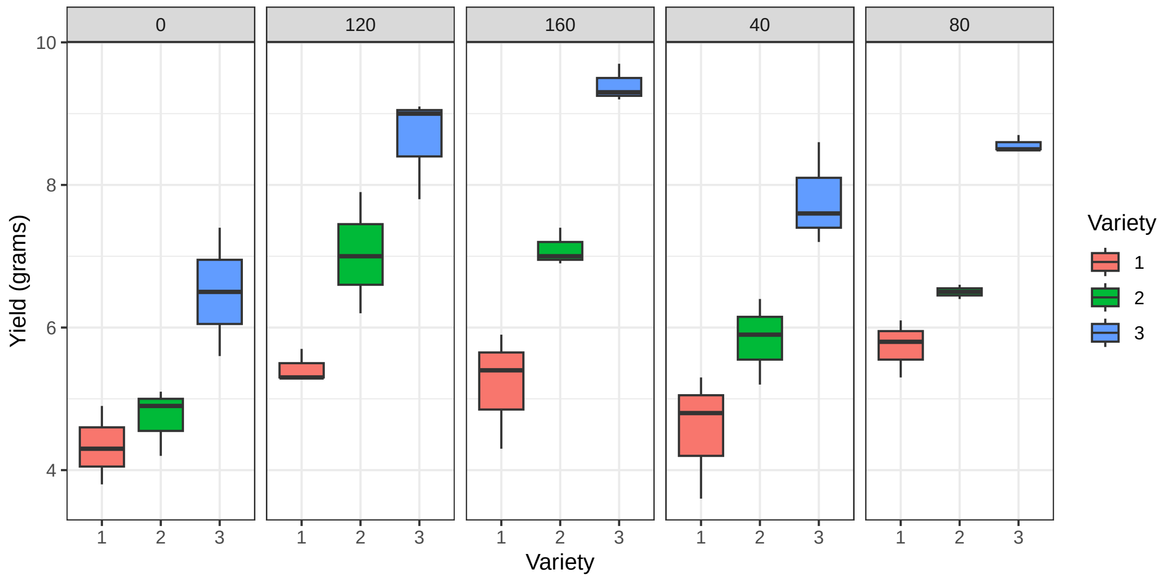
2. You might notice the data do not have one observation per row (which R expects when fitting linear models). Convert the data from "wide" to "long" format using R. Check out last week's R activity for some example code.

```
yield_df_long <- yield_df %>%
   pivot_longer(
   cols = starts_with("Rep"),
   names_to = "Rep",
   values_to = "Yield")
summary(yield_df_long)
```

```
##  Nitrogen Variety     Rep                Yield      
##  0  :9    1:15    Length:45          Min.   :3.600  
##  120:9    2:15    Class :character   1st Qu.:5.300  
##  160:9    3:15    Mode  :character   Median :6.400  
##  40 :9                               Mean   :6.491  
##  80 :9                               3rd Qu.:7.600  
##                                      Max.   :9.700  
```

3. Graph the data using a boxplot. In the plot, group the data by the whole plot factor (one panel for each level of `Nitrogen`) and display the sub plot factor on the x-axis.

```
ggplot(yield_df_long, aes(x=Variety, y=Yield, fill=Variety)) +
  geom_boxplot()+ theme_bw()+
  xlab("Variety")+
  ylab("Yield (grams)")+
  facet_wrap(~Nitrogen, ncol=5)
```



4. Conduct an analysis of variance (ANOVA) using the `aov()` command (i.e., assess if `Nitrogen` and `Variety` explain variation in `yield`). Treat `Nitrogen` as a factor for all analyses.

```
aov_yield_1 <- aov(Yield ~ Rep + Nitrogen + Variety + Error(Rep/Nitrogen),
data=yield_df_long)
summary(aov_yield_1)
```

```
##
## Error: Rep
##     Df Sum Sq Mean Sq
## Rep  2 0.7111  0.3556
##
## Error: Rep:Nitrogen
##           Df Sum Sq Mean Sq F value   Pr(>F)
## Nitrogen   4 26.248   6.562   23.25 0.000184 ***
## Residuals  8  2.258   0.282
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##           Df Sum Sq Mean Sq F value   Pr(>F)
## Variety    2  74.74   37.37   90.66 5.87e-13 ***
## Residuals 28  11.54    0.41
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

5. Conduct the same ANOVA as you did in the previous step but use the `lmer()` command from the `lme4` package and the `anova()` command from the `lmerTest` package. Note: if you get an error that says `boundary (singular) fit: see ?isSingular`, ignore it.

```
lmer_yield_1 <- lmer(Yield ~ Nitrogen + Variety +  (1|Rep) + (1|Rep:Nitrogen),
  data=yield_df_long)
```

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

```
anova(lmer_yield_1, type=3)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##          Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## Nitrogen 26.248   6.562     4    38  17.185 3.976e-08 ***
## Variety  74.739  37.370     2    38  97.868 1.024e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

6. Pick one of the variables to conduct pairwise comparisons of treatment levels. Explain the reasoning for your choice and conduct the comparisons using the `lmer()` model.

```r
emmeans(lmer_yield_1, pairwise~Nitrogen)
```

```
## $emmeans
##  Nitrogen emmean    SE df lower.CL upper.CL
##  0          5.19 0.206 10     4.73     5.65
##  120        7.03 0.206 10     6.57     7.49
##  160        7.23 0.206 10     6.77     7.69
##  40         6.07 0.206 10     5.61     6.53
##  80         6.93 0.206 10     6.47     7.39
##
## Results are averaged over the levels of: Variety
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast                  estimate    SE df t.ratio p.value
##  Nitrogen0 - Nitrogen120     -1.844 0.291  8  -6.332  0.0015
##  Nitrogen0 - Nitrogen160     -2.044 0.291  8  -7.018  0.0008
##  Nitrogen0 - Nitrogen40      -0.878 0.291  8  -3.013  0.0914
##  Nitrogen0 - Nitrogen80      -1.744 0.291  8  -5.989  0.0022
##  Nitrogen120 - Nitrogen160   -0.200 0.291  8  -0.687  0.9539
##  Nitrogen120 - Nitrogen40     0.967 0.291  8   3.319  0.0602
##  Nitrogen120 - Nitrogen80     0.100 0.291  8   0.343  0.9964
##  Nitrogen160 - Nitrogen40     1.167 0.291  8   4.005  0.0239
##  Nitrogen160 - Nitrogen80     0.300 0.291  8   1.030  0.8352
##  Nitrogen40 - Nitrogen80     -0.867 0.291  8  -2.975  0.0963
##
## Results are averaged over the levels of: Variety
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```r
emmeans(lmer_yield_1, pairwise~Variety)
```

```
## $emmeans
##  Variety emmean   SE df lower.CL upper.CL
##  1         5.05 0.16 14     4.71     5.40
##  2         6.24 0.16 14     5.90     6.58
##  3         8.18 0.16 14     7.84     8.52
##
## Results are averaged over the levels of: Nitrogen
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $contrasts
##  contrast            estimate    SE df t.ratio p.value
##  Variety1 - Variety2    -1.19 0.226 28  -5.259  <.0001
```

```
##  Variety1 - Variety3    -3.13 0.226 28 -13.857  <.0001
##  Variety2 - Variety3    -1.94 0.226 28  -8.598  <.0001
##
## Results are averaged over the levels of: Nitrogen
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
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7. Write 4-5 sentences comparing the conclusions from the two approaches (i.e., using `aov()` vs. `lmer()`) including conclusions drawn from any pairwise comparisons you conducted. At least 1-2 of your sentences should include a conclusion written in "biologically meaningful" terms.

   Answer: The analyses using `aov()` and `lmer()` were equivalent in terms of sums of squares and mean squares, but the F-values differed (FYI: it's due to the differences in residual degrees of freedom). Yield varied across nitrogen levels ($F_{4,38} = 17.19, p < 0.0001$) and between varieties ($F_{2,38} = 97.87, p < 0.0001$). Pairwise comparisons indicated that variety 3 produced a higher yield than variety 1 (Tukey's range test (TRT): $t_{28} = 13.86, p < 0.0001$) or variety 2 (TRT: $t_{28} = 8.60, p < 0.0001$). Even though nitrogen applications of 160 kg/ha were associated with the highest yield, yield at that rate did not differ from yield at applications 80 kg/ha (TRT: $t_8 = 1.03, p = 0.84$); thus, to maximize yield, plant variety 3 should be planted and returns on yield from nitrogen applications beyond 80-100 kg/ha might be considered negligible.