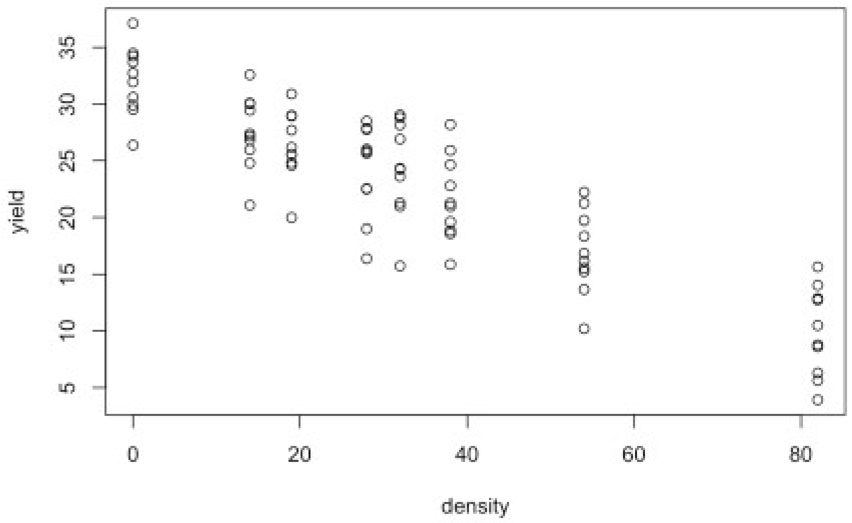
An example with linear regression

Let’s take a look at the ‘yieldDensity.csv’ dataset, that is available on gitHub. It represents an experiment where sunflower was tested with increasing weed densities (0, 14, 19, 28, 32, 38, 54, 82 plants per \(m^2\)), on a randomised complete block design, with 10 blocks. a swift plot shows that yield is linearly related to weed density, which calls for linear regression analysis.

```
rm(list=ls())
library(nlme)
library(lattice)
dataset <- read.csv("https://raw.githubusercontent.com/
OnofriAndreaPG/agroBioData/master/yieldDensityB.csv",
  header=T)
dataset$block <- factor(dataset$block)
head(dataset)
##   block density yield
## 1     1       0 29.90
## 2     2       0 34.23
## 3     3       0 37.12
## 4     4       0 26.37
## 5     5       0 34.48
## 6     6       0 33.70
plot(yield ~ density, data = dataset)
```



We might be tempted to neglect the block effect and run a linear regression analysis of yield against density. This is clearly wrong (I am violating the independence assumption) and inefficient, as any block-to-block variability goes into the residual error term, which is, therefore, inflated.

Some of my collegues would take the means for densities and use those to fit a linear regression model (two-steps analysis). By doing so, block-to-block variability is cancelled out and the analysis becomes more efficient. However, such a solution is not general, as it is not feasible, e.g., when we have unbalanced designs and heteroscedastic data. With the appropriate approach, sound analyses can also be made in two-steps (Damesa et al., 2017). From my point of view, it is reasonable to search for more general solutions to deal with one-step analyses.

Based on our experience with traditional ANOVA models, we might think of taking the block effect as fixed and fit it as and additive term. See the code below.

```
mod.reg <- lm(yield ~ block + density, data=dataset)
summary(mod.reg)
##
## Call:
## lm(formula = yield ~ block + density, data = dataset)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.6062 -0.8242 -0.3315  0.7505  4.6244
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 29.10462    0.57750  50.397  < 2e-16 ***
## block2       4.57750    0.74668   6.130 4.81e-08 ***
## block3       7.05875    0.74668   9.453 4.49e-14 ***
## block4      -3.98000    0.74668  -5.330 1.17e-06 ***
## block5       6.17625    0.74668   8.272 6.37e-12 ***
## block6       5.92750    0.74668   7.938 2.59e-11 ***
## block7       1.23750    0.74668   1.657  0.10199
## block8       1.25500    0.74668   1.681  0.09733 .
## block9       2.34875    0.74668   3.146  0.00245 **
## block10      2.25125    0.74668   3.015  0.00359 **
## density     -0.26744    0.00701 -38.149  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.493 on 69 degrees of freedom
## Multiple R-squared:  0.9635, Adjusted R-squared:  0.9582
## F-statistic: 181.9 on 10 and 69 DF,  p-value: < 2.2e-16
```

With regression, this solution is not convincing. Indeed, the above model assumes that the blocks produce an effect only on the intercept of the regression line, while the slope is unaffected. Is this a reasonable assumption? I vote no.

Let’s check this by fitting a different regression model per block (ten different slopes + ten different intercepts):

```
mod.reg2 <- lm(yield ~ block/density + block, data=dataset)
anova(mod.reg, mod.reg2)
## Analysis of Variance Table
##
## Model 1: yield ~ block + density
## Model 2: yield ~ block/density + block
##   Res.Df    RSS Df Sum of Sq      F  Pr(>F)
## 1     69 153.88
## 2     60 115.75  9    38.135 2.1965 0.03465 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The p-level confirms that the block had a significant effect both on the intercept and on the slope. To describe such an effect we need 20 parameters in the model, which is not very parsimonious. And above all: which regression line do we use for predictions? Taking the block effect as fixed is clearly sub-optimal with regression models.

The question is: can we fit a simpler and clearer model? The answer is: yes. Why don't we take the block effect as random? This is perfectly reasonable. Let's do it.

```
modMix.1 <- lme(yield ~ density, random = ~ density|block,
data=dataset)
summary(modMix.1)
## Linear mixed-effects model fit by REML
##  Data: dataset
##        AIC      BIC    logLik
##   340.9166 355.0569 -164.4583
##
## Random effects:
##  Formula: ~density | block
##  Structure: General positive-definite, Log-Cholesky parametrization
##             StdDev     Corr
## (Intercept) 3.16871858 (Intr)
## density     0.02255249 0.09
## Residual    1.38891957
##
## Fixed effects: yield ~ density
##                Value Std.Error DF   t-value p-value
## (Intercept) 31.78987 1.0370844 69  30.65311       0
## density     -0.26744 0.0096629 69 -27.67704       0
##  Correlation:
##         (Intr)
## density -0.078
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -1.9923722 -0.5657555 -0.1997103  0.4961675  2.6699060
##
## Number of Observations: 80
## Number of Groups: 10
```

The above fit shows that the random effects (slope and intercept) are sligthly correlated (r = 0.091). We might like to try a simpler model, where random effects are independent. To do so, we need to consider that the above model is equivalent to the following model:

```
modMix.1 <- lme(yield ~ density, random = list(block =
pdSymm(~density)), data=dataset)
```

It's just two different ways to code the very same model. However, this latter coding, based on a 'pdMat' structure, can be easily modified to remove the correlation. Indeed, 'pdSymm' specifies a totally unstructured variance-covariance matrix for random effects and it can be replaced by 'pdDiag', which specifies a diagonal matrix, where covariances (off-diagonal terms) are constrained to 0. The coding is as follows:

```
modMix.2 <- lme(yield ~ density, random = list(block =
pdDiag(~density)), data=dataset)
summary(modMix.2)
## Linear mixed-effects model fit by REML
##  Data: dataset
##       AIC      BIC   logLik
##   338.952 350.7355 -164.476
##
## Random effects:
##  Formula: ~density | block
##  Structure: Diagonal
##         (Intercept)    density Residual
## StdDev:    3.198267 0.02293222 1.387148
##
## Fixed effects: yield ~ density
##                 Value Std.Error DF   t-value p-value
## (Intercept) 31.78987 1.0460282 69  30.39102       0
## density     -0.26744 0.0097463 69 -27.44020       0
##  Correlation:
##         (Intr)
## density -0.139
##
## Standardized Within-Group Residuals:
##        Min          Q1         Med          Q3         Max
## -1.9991174 -0.5451478 -0.1970267  0.4925092  2.6700388
##
## Number of Observations: 80
## Number of Groups: 10
anova(modMix.1, modMix.2)
##          Model df      AIC      BIC    logLik   Test    L.Ratio
p-value
## modMix.1     1  6 340.9166 355.0569 -164.4583
## modMix.2     2  5 338.9520 350.7355 -164.4760 1 vs 2 0.03535079
0.8509
```

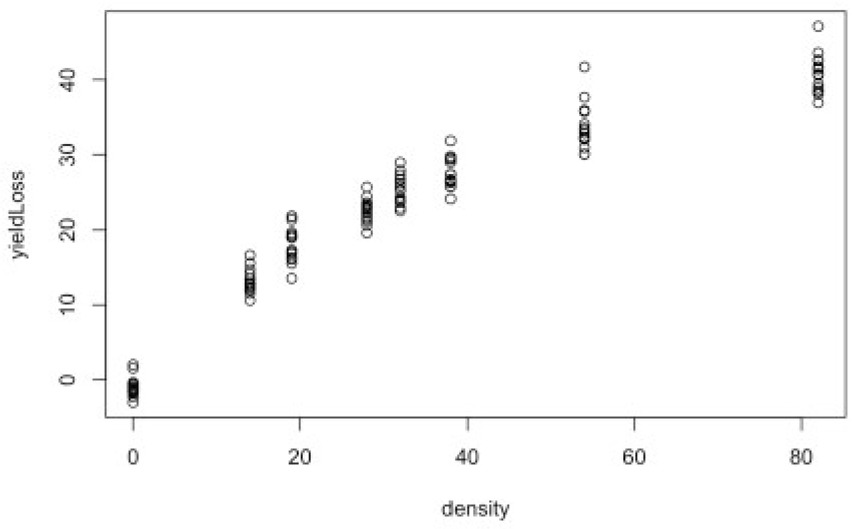
The model could be further simplified. For example, the code below shows how we could fit models with either random intercept or random slope.

```
#Model with only random intercept
modMix.3 <- lme(yield ~ density, random = list(block = ~1),
data=dataset)

#Alternative
#random = ~ 1|block

#Model with only random slope
modMix.4 <- lme(yield ~ density, random = list(block = ~ density - 1),
data=dataset)

#Alternative
#random = ~density - 1 | block
```

An example with nonlinear regression

The problem may become trickier if we have a nonlinear relationship. Let’s have a look at another similar dataset (‘YieldLossB.csv’), that is also available on gitHub. It represents another experiment where sunflower was grown with the same increasing densities of another weed (0, 14, 19, 28, 32, 38, 54, 82 plants per m^2), on a randomised complete block design, with 8 blocks. In this case, the yield loss was recorded and analysed.

```
rm(list=ls())
dataset <- read.csv("https://raw.githubusercontent.com/
OnofriAndreaPG/agroBioData/master/YieldLossB.csv",
  header=T)
dataset$block <- factor(dataset$block)
head(dataset)
##   block density yieldLoss
## 1     1       0     1.532
## 2     2       0    -0.661
## 3     3       0    -0.986
## 4     4       0    -0.697
## 5     5       0    -2.264
## 6     6       0    -1.623
plot(yieldLoss ~ density, data = dataset)
```



A swift plot shows that the relationship between density and yield loss is not linear. Literature references (Cousens, 1985) show that this could be modelled by using a rectangular hyperbola:

$$YL = \frac{i \, D}{1 + \frac{i \, D}{a}}$$

where YL is the yield loss, D is weed density, i is the slope at the origin of axes and a is the maximum asymptotic yield loss. This function, together with self-starters, is available in the ‘NLS.YL()’ function in the ‘aomisc’ package, which is the accompanying package for this blog. If you do not have this package, please refer to this link to download it.

The problem is the very same as above: the block effect may produce random fluctuations for both model parameters. The only difference is that we need to use the ‘nlme()’ function instead of ‘lme()’. With nonlinear mixed models, I strongly suggest you use a ‘groupedData’ object, which permits to avoid several problems. The second line below shows how to turn a data frame into a ‘groupedData’ object.

```
library(aomisc)
datasetG <- groupedData(yieldLoss ~ 1|block, dataset)
nlin.mix <- nlme(yieldLoss ~ NLS.YL(density, i, A), data=datasetG,
                        fixed = list(i ~ 1, A ~ 1),
            random = i + A ~ 1|block)
summary(nlin.mix)
## Nonlinear mixed-effects model fit by maximum likelihood
##   Model: yieldLoss ~ NLS.YL(density, i, A)
##  Data: datasetG
##        AIC      BIC    logLik
##   474.8228 491.5478 -231.4114
##
## Random effects:
##  Formula: list(i ~ 1, A ~ 1)
##  Level: block
##  Structure: General positive-definite, Log-Cholesky parametrization
##          StdDev    Corr
## i        0.1112839 i
## A        4.0444538 0.195
## Residual 1.4142272
##
## Fixed effects: list(i ~ 1, A ~ 1)
##      Value Std.Error  DF  t-value p-value
## i  1.23238 0.0382246 104 32.24038       0
## A 68.52305 1.9449745 104 35.23082       0
##  Correlation:
##   i
## A -0.408
##
## Standardized Within-Group Residuals:
##        Min          Q1         Med          Q3         Max
## -2.4416770 -0.7049388 -0.1805690  0.3385458  2.8788981
##
## Number of Observations: 120
## Number of Groups: 15
```

Similarly to linear mixed models, the above coding implies correlated random effects (r = 0.194). Alternatively, the above model can be coded by using a ’pdMat construct, as follows:

```
nlin.mix2 <- nlme(yieldLoss ~ NLS.YL(density, i, A), data=datasetG,
                              fixed = list(i ~ 1, A ~ 1),
                  random = pdSymm(list(i ~ 1, A ~ 1)))
summary(nlin.mix2)
## Nonlinear mixed-effects model fit by maximum likelihood
##   Model: yieldLoss ~ NLS.YL(density, i, A)
##  Data: datasetG
##        AIC      BIC    logLik
##   474.8225 491.5475 -231.4113
##
## Random effects:
```

```
##  Formula: list(i ~ 1, A ~ 1)
##  Level: block
##  Structure: General positive-definite
##          StdDev    Corr
## i        0.1112839 i
## A        4.0466971 0.194
## Residual 1.4142009
##
## Fixed effects: list(i ~ 1, A ~ 1)
##      Value Std.Error  DF  t-value p-value
## i  1.23242  0.038225 104 32.24107       0
## A 68.52068  1.945173 104 35.22600       0
##  Correlation:
##   i
## A -0.409
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -2.4414051 -0.7049356 -0.1805322  0.3385275  2.8787362
##
## Number of Observations: 120
## Number of Groups: 15
```

Now we can try to simplify the model, for example by excluding the correlation between random effects.

```
nlin.mix3 <- nlme(yieldLoss ~ NLS.YL(density, i, A), data=datasetG,
                              fixed = list(i ~ 1, A ~ 1),
                  random = pdDiag(list(i ~ 1, A ~ 1)))
summary(nlin.mix3)
## Nonlinear mixed-effects model fit by maximum likelihood
##   Model: yieldLoss ~ NLS.YL(density, i, A)
##  Data: datasetG
##        AIC      BIC    logLik
##   472.9076 486.8451 -231.4538
##
## Random effects:
##  Formula: list(i ~ 1, A ~ 1)
##  Level: block
##  Structure: Diagonal
##                 i        A Residual
## StdDev: 0.1172791 4.389173 1.408963
##
## Fixed effects: list(i ~ 1, A ~ 1)
##      Value Std.Error  DF  t-value p-value
## i  1.23243 0.0393514 104 31.31852       0
## A 68.57655 1.9905549 104 34.45097       0
##  Correlation:
##   i
## A -0.459
##
## Standardized Within-Group Residuals:
```

```
##        Min         Q1        Med         Q3        Max 
## -2.3577291 -0.6849962 -0.1785860  0.3255925  2.8592764 
## 
## Number of Observations: 120
## Number of Groups: 15
```

With a little fantasy, we can easily code several alternative models to represent alternative hypotheses about the observed data. Obviously, the very same method can be used (and SHOULD be used) to account for other grouping factors, such as main-plots in split-plot designs or plots in repeated measure designs.