**Using Mixed Effects Models for Field Experiments with Hierarchical Designs e.g. Split-plots**

***Some hints and tips for analysis and presentation of results from Nafferton field trials***

1. The field experiments at Nafferton are factorial in design – that means that they have at least two **treatment factors**, each with at least two **levels** and that all combinations of each factor are included in the experiment. First of all – make sure that you know: what are your treatment **factors** and what are the **levels** are for each factor?
2. The analysis that you do should be designed to test:
   1. whether there is a significant effect (difference) for each factor (sometimes called main effect) and,
   2. whether the effect of that particular factor is the same for other factors, regardless of the treatment level
      1. if the effect is the same at each level of another factor, we say that the interaction is not significant
      2. if the effect of one factor is not the same at each level of another factor, we say that there is a significant interaction between these two factors
3. The results of this analysis should be presented as shown in Table 2 below with main effect means for each factor and the results of the ANOVA for main effects and interactions. This is the **first step in your analysis and presentation of results**.

Table 2 Potentially mineralisable N concentrations and soil basal respiration rates (Main effect means (SE)

|  |  |  |
| --- | --- | --- |
|  | Potentially mineralisable N (mg N-NH4+ kg-1)b | Soil basal respiration (mg C-CO2 kg-1 h-1)b |
| Main effect means |  |  |
| CR |  |  |
| CON | 57.04 (5.7) | 1.10 (0.08) |
| ORG | 57.71 (4.7) | 1.12 (0.06) |
|  |  |  |
| CP |  |  |
| CON | 59.81 (6.2) | 1.04 (0.06) |
| ORG | 54.94 (4.0) | 1.18 (0.07) |
|  |  |  |
| FM |  |  |
| C | 63.71 (5.3) | 1.30 (0.11) a |
| CS | 60.46 (5.0) | 1.25 (0.10) a |
| CF | 58.49 (10.7) | 0.84 (0.07) b |
| F | 46.83 (6.7) | 1.06 (0.70) ab |
|  |  |  |
| ANOVA *P* values |  |  |
| CR | 0.598 | 0.828 |
| CP | 0.655 | 0.171 |
| FM | 0.066 | **0.003** |
| CR x CP | 0.877 | 0.557 |
| CR x FM | 0.918 | 0.826 |
| CP x FM | 0.684 | 0.861 |
| CR x CP x FM | 0.480 | 0.136 |

a CR, crop rotation; CP, crop protection; FM, fertility management; ORG, organic; CON,

conventional; C, compost; CS, compost-slurry; CF, compost-synthetic fertiliser; F,

synthetic fertiliser.

b Boldface is used for a significance (*P* < 0.05).

1. We will use linear mixed effects models to analyse the data in R. Mixed effects models have "fixed effects", which are the treatment factors we are interested in, and "random" effects, which are other factors that we aren't interested in, but that we have to take account of in the statistical analysis.
2. The Nafferton design is a bit complicated. The basic design is a split-split plot design, but in some cases you may have a more complicated split-split-split plot design. Because the treatments in these experiments are not completely randomly assigned to the plots, we need to account for this in our analysis. These designs are sometimes called nested or hierarchical and require you to specify an error term in the statistical model that indicates how the various treatments are "nested" within each other.

The data will be analysed using R software. This can be done directly in R using the R Console or you may prefer to do some of the data analysis using R Studio or R Commander. Below I will provide you with the simplified instructions for analysis using R Console. You should be able to adapt this for R Commander.

NB: all red type in this document can be copied and pasted directly into the R console. The names of data files and parameters will then have to be adjusted before running the analysis in R.

**The instructions below are for importing data and setting up analysis in R. You may already know how to do this in another, easier way! So no need to follow these steps if you already know how to import the data, define the factors and calculate means and standard errors. The red text below refers to a different dataset (whpot).**

**Step 1: Preparing data for analysis in R**

* data is prepared in Excel
* simplify all column headings so that the titles are short. Use no more than 5 characters, **do not use** a number at the beginning of a parameter, **do not use** capitals or spaces.
* arrange the columns of data containing the factors to reflect the nested structure of the design, this should be: year (if more than one year’s data), pc or rot (if more than one pre-crop or rotation), he, fe, fh, plot (it is useful to include plot numbers to keep track of the data), then columns containing the parameters to be analyzed
* save the file as a .txt tab delimited file
* open the data.txt file by right clicking and selecting open with Word
* view the codes in the document to check that tabs are in the correct places; also delete any extra rows at the bottom of the data(there might be columns to the right as well. This is best to be fixed in excel by deleting a couple of columns to the right of the data).
* close the file keeping it as a .txt file

**Step 2: Reading the data into R**

1. First change the directory to the folder where the data.txt file is located.

🖰 **File**

🖰 **Change dir…**

🖰 **Browse**

And select the directory where your data is located.

1. Now type in the code that reads the data file into R.

In this example the data is located in the file: whpot.txt

whpot<-read.delim(“whpot.txt”, header=T)

**Note: when you paste this into R, you will need to delete and retype the " " symbols as for some reason they don't paste correctly…**

*Note: The <- symbol is used to define what R calls “objects” i.e. sets of data, models, factors, variables. The name before the <- symbol can be anything you like. At any point if you want to find out what objects you have defined so far, you can type the command ls(), and a list of objects will be printed on screen.*

Hint: at any time the data can be checked by typing:

summary(whpot)

or the data can be viewed in a spreadsheet by typing

fix(whpot)

or by

🖰 **Edit**

🖰 **Data editor**

and typing in the filename, e.g. whpot

**Step 3: Defining the factors**

The next step is to tell R which columns in your data

are factors. The rest will be assigned as variables by default. The following format is used, with whpot in this example as the name of the data file.

whpot$bl<-factor(whpot$bl)

whpot$fe<-factor(whpot$fe)

whpot$he<-factor(whpot$he)

whpot$fh<-factor(whpot$fh)

You can use the up arrow to recall the last command typed into R. Then arrow across to change the factor names as required. For this example the factors have been defined for a set of data that does not include a pre-crop. When pre-crop is included, this factor must be defined as well.

**Step 4: Calculation of means, standard deviations and standard errors**

For the 2-way analysis with fe and he as the factors, the means and standard deviations are calculated as follows:

tapply(whpot$prot, whpot$fe, mean)

1 2

9.5125 13.2250

tapply(whpot$prot, whpot$fe,sd)

1 2

0.6057758 0.5092011

tapply(whpot$prot, whpot$he, mean)

1 2

11.7375 11.0000

tapply(whpot$prot, whpot$he,sd)

1 2

1.969001 2.077086

tapply(whpot$prot, whpot$fh, mean)

1 2 3 4

9.950 9.075 13.525 12.925

tapply(whpot$prot, whpot$fh,sd)

1 2 3 4

0.4203173 0.4112988 0.5909033 0.1258306

If we want to report standard errors of means, R does not have a built in function for calculating this figure. Crawley (2007) p. 54, suggests that we define the function and then use it as follows.

NB: the standard deviation is the square root of the variance; this formula could also have been written as sd(x)/sqrt(length(x))

First, define the function:

se<-function(x) sqrt(var(x)/length(x))

Then call the function for the different parameters where we need standard errors, just as we do for means and standard deviations.

tapply(whpot$prot,whpot$fe,se)

1 2

0.2141741 0.1800298

**This coding allows the calculation of interaction means.**

tapply(cab$egg3,list(cab$ne,cab$fe), mean)

1 2

1 0.0625 0.25

2 0.7500 3.50

Note for the tapply statement, the first factor named will be in the rows and the second factor is in the columns

## Case 1: Vizura trial

**ANOVA for main effects:**

library(nlme)

*Note: The library command calls up the R “library” of functions that will be used in this analysis. There are dozens of libraries that can be downloaded from the R site depending on the analysis to be done. This command only needs to be included once at the beginning of the R session. In this case the nlme library that is used for mixed-effects models (both linear and non-linear), is called.*

The Vizura trial has two experimental factors and two levels for each, there are also four replicate blocks (bl):

|  |  |
| --- | --- |
| **Factor** | **Levels** |
| Inhibitor (I) | +Vizura, -Vizura |
| Historical fertility management (FM) | Compost, NPK |

Sample coding and output:

Model.lme<-lme(nitrate~I\*FM, data=nitrate, random=~1|bl/FM)

anova(model.lme)

**Checking the model**

In order for the output of the anova to be valid, the residuals must be normally distributed. These can be checked using the qqnorm command:

Insert model name here.

qqnorm(whpot2.lme, ~resid(.)|bl)

gives quantiles of standard normal versus residuals. See **Figure 1** for a sample of the output.

The points in the plot should run diagonally across each quadrat in a straight line (e.g. the residuals in this example would be considered to be normally distributed).

If the residuals are not normally distributed, then the data must be transformed. Some simple transformations that can work are a square root which is done directly in the model statement:

whpot2.lme<-lme(prot^0.5~fe\*he, data=whpot, random=~1|bl/he)

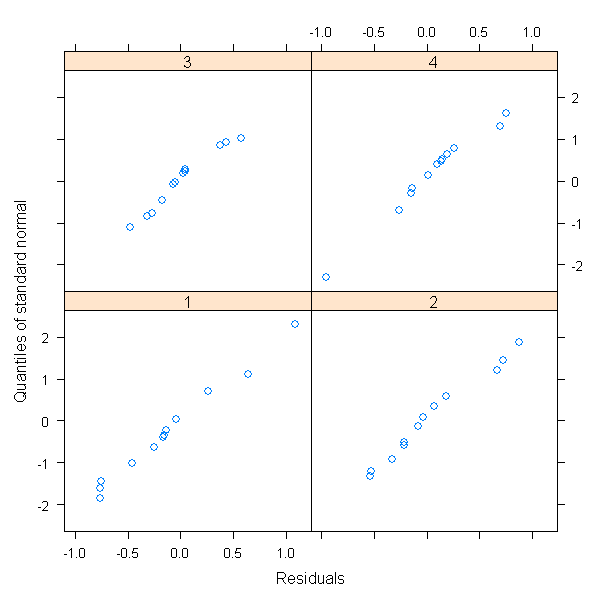
or cube root transformation…

whpot2.lme<-lme(prot^0.3333~fe\*he, data=whpot, random=~1|bl/he)

or a log transformation….

whpot2.lme<-lme(log10(prot)~fe\*he, data=whpot, random=~1|bl/he)

If the data has satisfied the requirement for normally distributed residuals, then the output of the anova is valid and the p values are reported in the results tables.



**Figure 1. Example of output from the qqnorm command in R for testing the normality of residuals of fitted models**

**Next steps – dealing with interactions**

If you have no significant interactions then you are finished with the analysis. You simply present your main effect means and the significance of these effects in your results table.

If you find that you have a significant interaction between two or three of the factors in your experiment, then you need to investigate what is going on. There are different ways to do this. For a simple two-way interaction with just four interaction means, it may be best to produce the four interaction means, and then do a Tukey test to compare those four means and determine if they are significantly different or not. You will need to include another factor in your data set (you may want to do this when you set up the data set) which represents the interaction means, and will have four levels.

For the Vizura trial, these would be something like this:

|  |  |  |
| --- | --- | --- |
| **Inhibitor** | **Fertility management** | **Interaction code (IFM)** |
| +I | compost | +IC |
| +I | NPK | +INPK |
| -I | compost | -IC |
| -I | NPK | -INPK |

Because the experiment is a split-split plot, you still need to use the lme model with the correct random term specified to do these means comparisons. The coding for this is shown below.

## Tukey contrasts using the lme model

First load the library "multcomp"

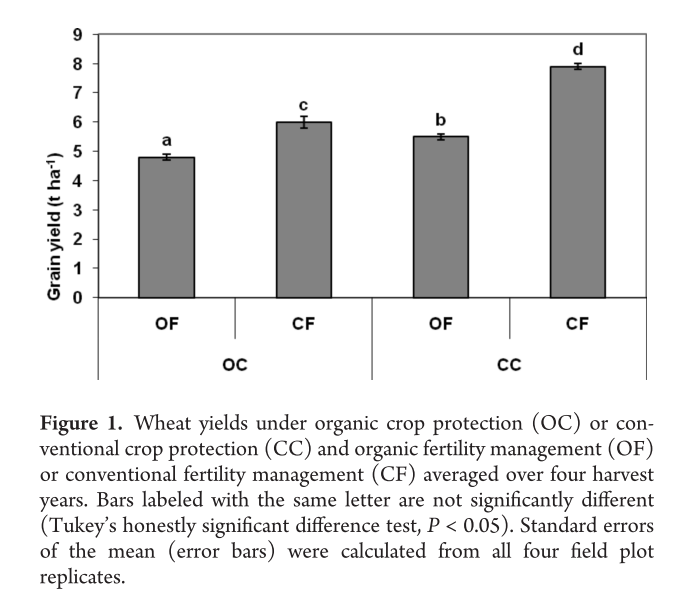
library(multcomp)

model.lme<-lme(nitrate~IFM,data=nitrate,random=~1|bl/FM)

anova(model.lme)

summary(glht(model.lme,linfct=mcp(IFM="Tukey")),test=univariate())

The chart below shows how you might want to present the interaction means if you get a significant IxFM interaction.



## Nutrisphere trial

The Nutrisphere trial has only one experimental factor and three levels, there are also four replicate blocks (bl):

|  |  |
| --- | --- |
| **Factor** | **Levels** |
| Fertiliser management (FM) | NS, NS15, urea |

Sample coding and output:

Model.lme<-lme(nitrate~FM, data=nitrate, random=~1|bl)

anova(model.lme)

Check the normality of the residuals as shown above.

If the P value for FM is significant, then you will need to use Tukey comparisons to determine which means are different.

Follow the instructions above for Tukey Contrasts and adapt the code so that you compare the three means for FM.