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Letter to the editor

Analysis of variance on gross nitrogen mineralization data

Gross N mineralization rates in soil, can be measured by the use of NH_4^+-pool dilution technique, where the NH_4^+ pool is labelled with ^{15}N by adding small amounts of $^{15}NH_4^+$ to soil (Kirkham and Bartholomew, 1954). The main principle of this technique is that the ^{15}N-enrichment ($^{15}N/(^{14}N + {}^{15}N)$-ratio) in the NH_4^+-pool decreases due to mineralization of ^{14}N. Any consumption of NH_4^+ will only change the size of the NH_4^+ pool, not the ^{15}N enrichment; so according to Eq. (1) gross N mineralization rate is calculated from change in size of the NH_4^+ pool and its ^{15}N dilution

$$m = \frac{\ln(x_4/x_2)}{\Delta t}\,\frac{x_1 - x_3}{\ln(x_3/x_1)} \tag{1}$$

where m is the gross N mineralization rate, Δt is the time step, x_1 and x_2 are the values of pool size and ^{15}N-enrichment of the ammonium pool at $t = 0$ and x_3 and x_4 are the values pool size and ^{15}N-enrichment of the ammonium pool at $t = \Delta t$. Each variable ($i = 1,\ldots,4$) in Eq. (1), will usually be determined by several replicates ($j = 1,\ldots,n$). In field trials, there will usually be field replicates of each treatment; hence x_{1j}, x_{2j}, x_{3j} and x_{4j} will be determined from the same field replicate. Then, it would be possible to calculate n replicates of m according to Eq. (2)

$$m_j = f(x_{1j}, x_{2j}, x_{3j}, x_{4j}) \tag{2}$$

where the formula for the function f, is given by Eq. (1). n replicates of each treatment gives the foundation for a standard analysis of variance in the SAS GLM procedure (SAS software, 1999–2001). In many laboratory studies, the replication is performed in a slightly alternative way, as shown in Fig. 1. $^{15}NH_4^+$ is applied to, e.g. eight (in theory) identical soil samples. Four of the soil samples are analysed at $t = 0$ for x_{1j} and x_{2j}, while the remaining four are analysed at $t = \Delta t$ for x_{3j} and x_{4j}. In practice, the outcome of this alternative replication procedure is that x_{1j} and x_{2j} are independent of x_{3j} and x_{4j}, so there is no unique way of combining these replications into four sets of replicates. Hence, it is not possible to calculate four replicates of m, and it is not possible to run a standard analysis of variance in the SAS GLM procedure.

After a survey in literature of gross N mineralization, it has come to our attention, that data sets from many laboratory investigations of gross N mineralization were analysed without an analysis of variance, probably due to the problem described above. Hence, we find it adequate to present a method to overcome this problem. It is possible to estimate the standard error (s) for m, using the procedure for *propagation of error* from knowledge of the variability of $\widehat{x}'$s (Cramér, 1946). The procedure is based on the Tayler series, and is given by Eq. (3)

$$s^2 = \sum_i \left(\frac{\partial f}{\partial \widehat{x}_i}\right)^2 \mathrm{var}(\widehat{x}_i) + 2\sum_{i,j}\left(\frac{\partial f}{\partial \widehat{x}_i}\right)\left(\frac{\partial f}{\partial \widehat{x}_j}\right)\mathrm{cov}(\widehat{x}_i, \widehat{x}_j) \tag{3}$$

The partial derivatives of Eq. (1) are

$$\widehat{x}_1: \quad \frac{\partial m}{\partial \widehat{x}_1} = \frac{\ln(\widehat{x}_4/\widehat{x}_2)}{\Delta t}\,\frac{1 - (\widehat{x}_3/\widehat{x}_1) + \ln(\widehat{x}_3/\widehat{x}_1)}{[\ln(\widehat{x}_3/\widehat{x}_1)]^2} \tag{4a}$$

$$\widehat{x}_2: \quad \frac{\partial m}{\partial \widehat{x}_2} = -\frac{1}{\Delta t}\,\frac{\widehat{x}_1 - \widehat{x}_3}{x_4 \ln(\widehat{x}_3/\widehat{x}_1)} \tag{4b}$$

$$\widehat{x}_3: \quad \frac{\partial m}{\partial \widehat{x}_3} = \frac{\ln(\widehat{x}_4/\widehat{x}_2)}{\Delta t}\,\frac{1 - (\widehat{x}_1/\widehat{x}_3) - \ln(\widehat{x}_3/\widehat{x}_1)}{[\ln(\widehat{x}_3/\widehat{x}_1)]^2} \tag{4c}$$

$$\widehat{x}_4: \quad \frac{\partial m}{\partial \widehat{x}_4} = \frac{1}{\Delta t}\,\frac{\widehat{x}_1 - \widehat{x}_3}{\widehat{x}_4 \ln(\widehat{x}_3/\widehat{x}_1)} \tag{4d}$$

Co-variation between $\widehat{x}_1$ and $\widehat{x}_2$ should be taking into account in Eq. (3), since these are obtained from the same soil sample. Likewise, co-variation between $\widehat{x}_3$ and $\widehat{x}_4$ should also be taking into account. After calculation of

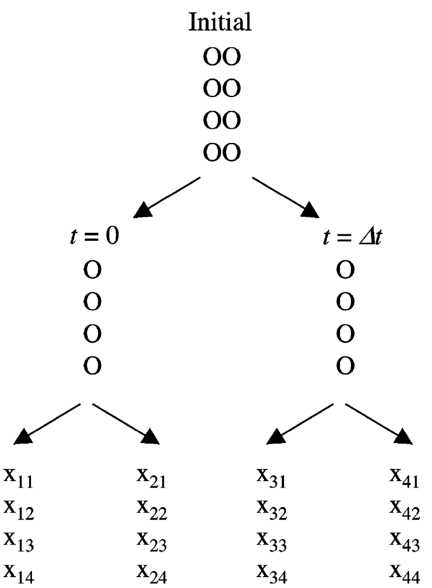

Fig. 1. Replication procedure in laboratory experiments.

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mean rates and standard errors for the gross N mineralization, the data set can be analysed for variance in the GLM procedure for Least Squares of Means in SAS (SAS software, 1999–2001). Below is given an example of how to set-up the SAS program for a Least Squares of Means analysis:

```
data example;
input factor1 $ factor2 $ factor3 m s2;
cards;
Input file;

data example;
set example;
weight = 1/s2;
run;

proc glm data = example;
class factor1 factor2 factor3;
model m = factor1 factor2 factor3/solution;
weight weight;
lsmeans factor1 factor2 factor3/pdiff cl;
run;
quit;
```

References

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Kirkham, D., Bartholomew, W.V., 1954. Equations for following nutrients transformantions in soil, utilizing tracer data. Soil Science Society of America Proceeding 18, 33–34.

SAS software, 1999–2001. SAS Institute Inc, Cary, NC, USA.

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