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# Long-Term Experiments with cropping systems: Case studies on data analysis

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ABSTRACT

Data analysis for Long-Term Experiments (LTEs) with cropping systems requires some careful thinking, especially for the most complex designs, characterised by rotations with different durations and/or a different number of test-crops per rotation cycle. This paper takes an example-based approach, built upon a number of datasets, covering the main types of LTEs, with increasing levels of complexity. A procedure is outlined to build statistical models for data analysis that is useful for all LTEs characterised by the simultaneous presence of all rotation phases in all years, together with within-year replication. This procedure is based on the assumption that correct analyses can be performed separately for each year. The use of mixed models and REML estimation is advocated for model fitting with all LTEs, due to the fact that most designs are non-orthogonal, as plots may not produce data for the test-crop under study in all years. Mixed models are also useful to account for the autocorrelation of residuals over time and hints are given for the selection of an appropriate variance-covariance structure. For all our examples, variances were not constant across years and compound symmetry correlation structures with variance heterogeneity of years proved to be the best compromise between parsimony and statistical accuracy. Methods are outlined to test for the need of other more complex correlation structures and examples are also given on how to test for fixed effects, model fertility trends and assess the long-term stability of cropping systems.



## 1. Introduction

Long-Term Experiments (LTEs) represent an invaluable tool to detect possible slow changes produced by the cropping systems in the long run and reveal possible threats to the environment and to the future fertility of agricultural land. From a methodological point of view, LTEs may be regarded as a particular class of multi-environment experiments, wherein observations are repeated on the same plots for a long period of time, usually from 20 years upwards (Frye and Thomas, 1991). The experimental treatments under comparison may be highly variable (rotations, fertilisations, tillage systems . . .), but in most cases they represent several cropping systems, to be investigated by repeated observations of yield or other relevant indicators of productivity, sustainability, efficiency and so on.

In general, data analysis for LTEs is not trivial, due to the peculiar characteristics of this class of experiments. In a recent review, Payne (2015) highlighted several special issues, such as: (i) non-uniform correlation structure of within-plot observations; (ii) heterogeneity of variances in different years; (iii) residual effects of agronomic practices, leading to fertility trends over time; (iv) changes in the experimental protocol, due to, e.g., the adoption of new varieties, harvesting methods or weed control methods. Unfortunately, the statistical solutions to these (and other) problems may not be unique and straightforward. Indeed, a survey of literature shows that LTEs employ a wide array of experimental designs with their own peculiarities. For example, rotation experiments may be designed so that all crops in the rotation are grown every year (Cochran, 1939). However, in order to avoid an excessive increase in the total number of plots, LTEs may also be designed without within-year replicates (Patterson, 1964), thus requiring a different approach to data analysis. Another example relates to the

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frequency of repeated measures: in long rotations the test crop may return on the same plot after a relatively long period of time, which may create a totally different correlation structure compared to short rotations.

We will not list all the possible peculiarities of LTEs in terms of experimental design, as this has already been accomplished in literature (see, e.g., Frye and Thomas, 1991). It should be clear that each LTE, according to its aims and experimental design, poses peculiar problems in terms of data analysis and may create difficulties in the selection of an appropriate statistical method.

In this regard, a survey of literature shows a number of early reviews, discussing ANOVA-like models for LTEs with a wide array of experimental designs (Patterson, 1964; Yates, 1954). However, these reviews use traditional methods of data analyses, while the current availability of personal computers and mixed model procedures gives scientists some new challenges and perspectives. On the other hand, more recent reviews about LTEs give examples relating to few experimental designs and do not consider the most complex situations, such as when LTEs include rotations of different lengths and/or with a different number of test crops (see for example Richter and Kroschewski, 2006; Singh and Jones, 2002). Another review about repeated measures includes only an example referring to a LTE comparing rotations of equal lengths (Piepho et al., 2004). A further recent review takes an example-based approach and presents a stepwise procedure for data analysis, though this is based on a single experiment, characterised by no within-year replication (Payne, 2015).

Considering the above background, we felt that a further example-based treatment of the statistical analysis of LTE data would be useful. Therefore, we used a number of datasets with contrasting characteristics and elaborated several case studies to give examples on how to: (i) build an appropriate model, (ii) inspect the validity of basic assumptions, (iii) test hypotheses of interest and (iv) obtain reliable measures of productivity, stability and sustainability for the cropping systems under comparison. The code to perform the analyses is made available as supplemental material, with the aim of giving practical directions when dealing with LTEs.

## 2. Five datasets for five types of Long-Term Experiments

Focusing on LTEs characterised by the presence of within-year replicates, five main groups can be found in literature, with increasing levels of complexity in terms of data analysis:

1 LTEs with monocultures or perennial crops;
2 LTEs with different rotations of same length and one test crop per rotation cycle;
3 LTEs with a fixed rotation (one test crop per rotation cycle) and different treatments;
4 LTEs with more than one phase per crop and rotation cycle;
5 LTEs with several rotations of different lengths and/or different numbers of phases per crop and rotation cycle.

Relating to the terms rotation, phase, cycle, sequence, we will refer to the terminology used in Yates (1954), which is summarised in Table 1.

We cannot cover all these situations with datasets from real and independent experiments and, therefore, instead of using simulated data, we decided to take a 'hybrid' approach: we started from two real field experiments and formed different subsets of the data to fit the above list. In more detail, we used the datasets from two real long-term field experiments: the first was established around 1974 near Perugia (central Italy), at the Experimental Farm of the Department of Agricultural, Food and Environmental Sciences and the second was established in 1994 at the Pasquale Rosati experimental farm in Agugliano (central Italy). Both experiments are still running at present (2016) and a detailed description is given as supplemental material, while other information can be found in Perucci et al. (1997) and elsewhere in this same special issue (Bonciarelli et al., 2016; Seddaiu et al., 2016).

These two real LTEs were used to extract five exemplary datasets, representing the five types of LTE listed above. It is important to point out that, for the sake of our exercise, these five datasets need not be considered in connection with the two original LTEs and may be taken to represent five 'ideal' and independent LTEs. Readers interested in full-fledged analyses of the original datasets are referred to the aforementioned references.

Table 2 summarises the contrasting characteristics of these datasets that will be described in detail afterwards. For each dataset, some records are given as supplemental materials, in order to show the exact structure of data tables.

### 2.1. LTEs with monocultures or perennial crops (Dataset 1)

This type of LTE does not involve rotations and the treatments under comparison consist of different cropping practices (e.g., fertilisations). In terms of data analysis, these LTEs are similar to experiments with perennial crops, as observations are taken all years on the same plot, for each treatment level and replicate (Eckl and Piepho, 2015; Piepho and Eckl, 2014; Smith et al., 2007).

#### 2.1.1. Dataset 1: continuous wheat cropping

Wheat is grown in continuous cropping from 1983 to 2012, with three fertilisation levels (150, 200 and 250 Kg N $ha^{-1}$), randomly assigned to three plots in each of three blocks. In all, there are nine plots with yearly sampling, with a total of 270 wheat yield observations in 30 years.

### 2.2. LTEs with different rotations of the same length and one test crop per rotation cycle (Dataset 2)

If we have, e.g., two crops in a rotation (maize and wheat), both crops will be grown in different plots in the same year and we will have two possible sequences in time (maize–wheat and wheat–maize). If we consider only one of the two crops, the main difference with respect to the previous situation is that the data obtained in two consecutive years for the same treatment and block are independent, in the sense that they are obtained in different plots. Otherwise, data obtained in a two-year interval (on different rotation cycles) on the same block are correlated, as they originate from the same plot.

#### 2.2.1. Dataset 2: comparing wheat yield for several biennial rotations

Wheat is grown in five types of two-year rotations, with either pea (*Pisum sativum* L.), grain sorghum (*Sorghum bicolor* (L.) Moench), sugar beet (*Beta vulgaris* L. subsp. *saccharifera*), sunflower (*Helianthus annuus* L.) and faba bean (*Vicia faba* L. subsp. *minor*). For each rotation, there are two possible sequences (wheat in odd years and wheat in even years) and the ten combinations (five rotations by two sequences) are completely randomised to ten plots per each of three blocks (Table 3). Therefore, five wheat plots out of the available ten plots are used from each block and year, for a total of 450 observations, from 1983 to 2012.

### 2.3. LTEs with a fixed rotation (one test crop per cycle) and different treatments (Dataset 3)

This type of LTE is very similar to the previous one, though we will introduce a dataset with a different experimental layout.