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# Towards an unbiased method for quantifying treatment effects on soil carbon in long-term experiments considering initial within-field variation

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ABSTRACT

Agricultural management effects on soil organic carbon (SOC) can be significant, but are often relatively small compared with the total SOC pool and its field–scale variability. In field experiments, completely uniform initial SOC concentrations across all plots are unlikely. Therefore, measured treatment effects through time are biased by these initial differences. This study questioned how much this bias can represent and whether it can be neglected or must be accounted for. In 1116 investigated pairs of treatments in 10 meta-replicated Swedish long-term soil fertility experiments, the average absolute initial difference in SOC was 1.3 g C $kg^{-1}$, whereas the average absolute difference after about 50 years of contrasting management was 1.5 g C $kg^{-1}$. Initial differences in SOC were significantly correlated with final differences ($R^2 = 0.14$) and in 47% of all investigated pairs the absolute initial difference was higher than the absolute final difference. However, simple subtraction of initial differences between pairs from final differences to isolate the treatment effect neglect the fact that the soil with the higher initial SOC content will lose proportionally more C through mineralization during the experimental period higher SOC content caused higher SOC mineralisation during the experimental period. Therefore, a first-order kinetic model was used to predict the decrease in initial SOC differences over time as influenced by climate. We derived a generic empirical equation that can be used to account for this severe but inherent problem with plot experiments. According to the equation, 65 and 69% of the initial differences in SOC between treatment pairs persisted after 50 years under the climate conditions in southern and central Sweden, respectively. We conclude that initial differences can be severe and have to be accounted for. The proposed empirical equation can reduce the bias in treatment comparisons for different climatic conditions and contribute to improve the quality of data interpretation from long-term field experiments.



## 1. Introduction

Agricultural management can have significant effects on soil organic carbon (SOC). This is most often determined in long-term field experiments around the globe in order to obtain generalisable results, e.g. to parameterise models (Smith et al., 1997). Debreczeni and Körschens (2003) estimated the number of long-term (10 to >100 years) field experiments in the world to be 625, with 185 trials considered as classical (>50 years), most of them located in Europe. However, even in long-term experiments, detecting SOC changes as a function of varying management practices is challenging (Knebl et al., 2015), since field–scale spatial heterogeneity in SOC can be as high as the long-term effect of a management practice. Field–scale variability of SOC can have various causes, such as prior land-use, topographical or pedological variability, patchiness of organic amendments or spots of charcoal derived from burning (Conant et al., 2003). Locations for field experiments are therefore carefully selected and, if possible, installed in flat terrain without any detectable variability in soil type or texture (Cerri et al., 2004). However, SOC variability is still detected (Lück et al., 2011).

To account for this problem, the conventional approach is to lay out the experiments in a thoroughly replicated, randomised design, so that any existing gradients in SOC are minimised. Ideally, with regard to e.g. SOC, the difference between the average values of the plots designated for each treatment should be zero. However, this is often not the case, particularly for some of the classical long-term field experiments (Poeplau et al., 2015b). One of the reasons for this problem relates to the fact that they are often assigned to relatively large plots, or they may be subject to deficiencies in the experimental design, such as lack of adequate replication (Janzen, 1995). This deficiency in replication is not necessarily attributable to poor design, but in many cases such experiments were actually established before the statistical methods used today were developed (Machado and Petrie, 2006). Furthermore, the classical long-term experiments were not initially designed to study the temporal dynamics of SOC. As a consequence, detected differences in SOC between treatments may be biased to

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some degree by initial average differences between the plots in which the treatments were laid out. Treatment effects on SOC can thereby be overestimated or underestimated when the latest measured SOC differences are ascribed to the treatment alone. As a result, inaccurate conclusions may be drawn, results may be inexplicable and there may be high variability among different experiments (Poeplau and Don, 2015). The majority of available studies focusing on SOC dynamics after differing agricultural management practices only report final differences in SOC contents or stocks between treatments, assuming that the initial difference between those treatments is zero or negligible compared with the treatment effect (Dolan et al., 2006). In some studies only one initial value is given, which more likely indicates that spatial variability in SOC was ignored in the beginning, rather than non-existent (Zanatta et al., 2007; Zhu et al., 2007). Only scattered studies report treatment-wise initial values (Pampolino et al., 2008; Liu et al., 2014). When initial differences between two treatments are present, they are usually subtracted from final measured differences. This simple subtraction assumes that without any treatment contrast the SOC dynamic would have been the same in both soils. However, SOC decay is a function of available SOC (first order decay) (Andrén and Kätterer, 1997), therefore a correction needs to account for the fact that the plots with higher SOC would lose more SOC under equal management. Ignoring this, a simple subtraction of initial differences would overestimate their influence on final differences, which depends on i) length of the experiment, and ii) climatic conditions driving SOC decay and thus the time needed to approximate SOC levels.

The initial differences between treatments in agricultural long-term experiments in relation to final differences have never been investigated comprehensively. It is not clear whether plot-wise initial SOC contents should be corrected for, or if it is a reasonable assumption that they are negligible in the long term. The objective of this study was to shed light on this potentially underestimated problem in soil carbon research by using a large number of treatment combinations from 10 Swedish meta-replicated long-term field experiments. We further aimed to develop a simple method to correct for the potential bias made when final SOC differences between treatments are fully ascribed to the treatment alone, considering length of the experiment and climatic conditions driving SOC decay.

## 2. Materials and methods

We used data from 10 sites of the Swedish long-term soil fertility experiments initiated in 1957–1966, with the latest sampling in 2007–2011 (Carlgren and Mattsson, 2001). Five sites are situated in southern Sweden (55°N) and five in central Sweden (58–60°N). The trials in southern Sweden were started in 1957 and the current experimental design exists since 1961. These sites were sampled plot-wise since 1962, which is therefore the reference year for those sites in this study. The other sites were sampled plot-wise since the establishment of the experiments. The period between first and last plot-wise sampling exceeded 45 years at all sites (Table 1). The experiments were laid out with a total of 16 different combinations of NPK fertilisation (four N levels each associated with four PK levels) within two different crop rotations in a replicated ($n = 2$) randomised split-split-block design for a total of 64 plots. In this study, we only considered the rotation without manure application ($n = 32$). Such a meta-replication, with several sites with identical experimental design across a wide range of pedological conditions, is quite unique (Johnson, 2006). Only the crop types and rotation length varied slightly between the sites in central and southern Sweden, according to regional agricultural practices. The experiments were laid out on old farmland, cultivated for at least one century. Only the experiment at Kungsängen (central Sweden) was converted from permanent grassland to cropland in the beginning of the last century (1907) (Kirchmann and Eriksson, 1993; Kirchmann et al., 1996; Kirchmann et al., 1999; Kirchmann et al., 2005; Kirchmann et al., 2013). Plot-wise initial SOC concentration data were available for all experiments, which we averaged treatment-wise ($n = 16$).

### 2.1. Calculation of treatment-wise differences in soil carbon

In this study, we calculated initial SOC differences ($\Delta SOC_{Initial}$) and final SOC differences ($\Delta SOC_{Final}$) for all possible treatment combinations. For each experiment with all 16 combinations, a total of 120 pairs were available for investigation. In two out of the 10 experiments (Kungsängen and Fors), only 13 combinations were present, which enabled the formation of 78 pairs. In total, we investigated 1116 pairs. In total, we investigated 1116 treatment combinations. The treatment combination effect on SOC ($\Delta SOC$) (in this case treatments x and y) was calculated as:

$$\Delta SOC(x, y) = \Delta SOC(x, y)_{Final} - \Delta SOC(x, y)_{Initial}. \quad (1)$$

A treatment effect of zero thus implies that initial differences were equivalent to those present at the final sampling. We calculated different measures to compare all three terms in Eq. (1) both across experiments and for each experiment separately. These were:

i) Mean absolute SOC difference ($|\overline{\Delta soc}|$) between treatments for initial and final sampling and for the calculated treatment combination effect ($\Delta SOC(x,y)$) (Eq. (1));
ii) Number of combinations for which $\Delta SOC(x,y)_{Initial} > \Delta SOC(x,y)_{Final}$; and
iii) Number of combinations for which $\Delta SOC(x,y)_{Initial} > \Delta SOC(x,y)$.

### 2.2. Statistics and modelling

We used linear mixed effect models to explore the correlation of all three terms in Eq. (1). Experimental site was used as a random effect to

**Table 1**
List of sites with coordinates [°N/°E] period between initial and final sampling [years], clay and sand content [%], average initial soil organic carbon content [%], soil pH, bulk density [g $cm^{-3}$], mean annual temperature (MAT) [°C], mean annual precipitation (MAP) [mm] and the condensed decomposition rate modifier $r_e$.

| Site | Coordinates | Duration | Clay | Sand | SOC | pH | BD | MAT | MAP | re |
|---|---|---|---|---|---|---|---|---|---|---|
| Fjärdingslöv | 55 40/13 23 | 49 | 14 | 62 | 1.4 | 7.9 | 1.66 | 7.7 | 550 | 1.26 |
| Orup | 55 82/13 50 | 49 | 12 | 59 | 2.4 | 6.6 | 1.51 | 8.3 | 769 | 1.31 |
| Örja | 55 88/12 87 | 49 | 23 | 52 | 1.1 | 7.8 | 1.72 | 8.3 | 593 | 1.24 |
| S. Ugglarp | 55 63/13 43 | 45 | 12 | 63 | 1.5 | 6.7 | 1.5 | 7.7 | 686 | 1.28 |
| Ekebo | 55 98/12 87 | 49 | 18 | 47 | 3.1 | 6.9 | 1.44 | 8.2 | 622 | 1.32 |
| Klostergården | 58 50/15 50 | 45 | 48 | 8 | 2.1 | 6.2 | 1.43 | 6.4 | 527 | 1.19 |
| Högåsa | 58 50/15 45 | 45 | 7 | 78 | 2.4 | 6.7 | 1.38 | 6.4 | 527 | 1.23 |
| Bjertorp | 58 23/13 13 | 45 | 30 | 16 | 2.2 | 6 | 1.37 | 6.5 | 593 | 1.18 |
| Kungsängen | 59 83/17 67 | 49 | 56 | 4 | 2.1 | 7.1 | 1.31 | 6 | 543 | 1.04 |
| Fors | 60 33/17 48 | 49 | 18 | 24 | 2.2 | 7.7 | 1.49 | 5.5 | 613 | 1.15 |