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Author(s): Juuso Joonas, Eero Liski, Helena Kahiluoto

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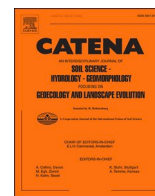
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Manure increases soil organic carbon most when allocated to annual cropping

Juuso Jooa^{a,*}, Eero Liski^b, Helena Kahiluoto^c

^a University of Helsinki, Department of Agricultural Sciences, University of Helsinki, P.O.B. 28, 00014, Finland

^b Natural Resources Institute Finland, Department of Natural Resources, Latokartanonkaari 9, 00790 Helsinki, Finland

^c LUT University, Sustainability Science, University of Helsinki, Mikkulankatu 19, 15210 Lahti, Finland

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ABSTRACT

Soil carbon sequestration has a great potential in climate change mitigation. To maximise carbon stocks in northern agricultural soils, the carbon sequestration determinants of manure application are crucial. We quantified the effect of manure on soil organic carbon (SOC) depending on the application rate, proportion of leys relative to annuals in crop rotation, and soil texture. We compared the steady-state SOC concentration under farm-yard manure application to a control treatment with no manure application based on 56 individual long-term experiments at 27 locations north of 50 degrees north latitude. At a low application rate of manure such as 0.7 Mg C/ha/y, SOC gradually increased with increasing ley proportions. At a moderate manure rate, such as 1.3 Mg C/ha/y, an increase in the ley proportion led to a decreasing growth of, or even a decrease in, SOC. At a low fixed ley proportion in crop rotation such as 20 %, SOC linearly increased with an increasing manure application rate. As the proportion of leys increased, saturation occurred at a certain ley proportion depending on soil texture. When leys occurred on sandy soils more than once out of seven years and the proportion of leys in crop rotation on clay soils was higher than 45 %, increasing the manure application rate led to decreasing SOC accrual. Compared to the absence of manure application, high manure rates even decreased SOC. We conclude that manure application in crop rotation with a low ley proportion maximises SOC accrual in the topsoil. The most effective manure application rate and ley proportion leys in regard to SOC in the northern agricultural landscape can be determined using a statistical model that we developed, depending on the management system and soil texture.

1. Introduction

Soil serves as the largest terrestrial pool of carbon (C) (Batjes, 1996; Smith, 2004a), which is also key to soil productivity (Oldfield et al., 2019). In agriculture, large quantities of harvested organic C end up in residues, such as manure, which are returned to agricultural soils. However, how to apply the organic C occurring in agricultural residues to cropping systems to maximise C accrual remains unclear.

The global technical C sequestration potential in agriculture has been estimated to range from 32 Gt (Sommer & Bossio, 2014) to 116 Gt (Sanderman et al., 2018) and 370 Gt (Lal et al., 2018) by the end of the twenty-first century, with the potential to account for 156 ppm of the atmospheric carbon dioxide (CO₂) level (Lal et al., 2018). Carbon occurs in soil organic matter (OM), which improves soil productivity through nutrient and water retention and by preventing the undesirable

mobilisation of harmful substances (Janzen, 2006; Sparling et al., 2006). Soil C sequestration; i.e., net removal of atmospheric C to soil (Don et al., 2023), is perceived as an increasingly important option for mitigating climate change while enhancing soil health (Paustian et al., 2016; Chenu et al., Bradford et al., 2019) but uncertainties remain. There are open questions regarding the soil organic C (SOC) formation process, SOC persistence, and achievable climate change mitigation potential (Bradford et al., 2019). Understanding SOC dynamics is crucial for climate change mitigation and for determining payments to farmers and land managers in terms of C sequestration.

In a system characterised by a particular climate, soil texture and crop management conditions, SOC requires decades to a century to achieve a management-system-specific steady state (Smith, 2004a; West & Six, 2007; Chenu et al., 2019). In a particular management system, SOC is controlled by the law of diminishing returns, i.e., the more C is

* Corresponding author.

E-mail addresses: juuso.jooa@helsinki.fi (J. Jooa), Eero.liski@luke.fi (E. Liski), Helena.kahiluoto@helsinki.fi (H. Kahiluoto).

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cumulatively amended, the less is accrued (Six et al., 2002; West & Six, 2007). At the steady state, C is no longer (net) accrued nor discharged. Rather, under particular management and other conditions, continued management sustains the dynamic C balance where C input equals C output (Six et al., 2002; West & Six, 2007).

SOC responds either linearly or asymptotically to C input increase among management systems. In the latter case, when comparing various management systems, the SOC concentration approaches the system-specific saturation under diminishing returns. Several SOC studies have demonstrated a linear SOC response (Paustian et al., 1997; Begill et al., 2023, Salonen et al., 2023). However other studies have indicated a declining soil C accrual capacity with increasing input (Six et al., 2002; Stewart et al., 2007). These latter studies, corroborating the steady-state saturation, have claimed that the linear responses result from low SOC and C inputs (Six et al., 2002; Stewart et al., 2007). Stewart et al. (2007) proposed, based on a comparison of two tillage methods on the same site, that soil contains an absolute, theoretical SOC saturation level and that tilled agroecosystems cannot reach that level but attain an effective stabilisation level. However recently, Begill et al. (2023) and Salonen et al. (2023) found that no absolute saturation even of mineral-associated SOC appeared, using data from the German Agricultural Soil Inventory and a Finnish long-term experiment, respectively. Consequently, the absolute, overall SOC saturation concentration is always specific to a particular system and depends on the system characteristics regardless of whether anthropogenic or natural systems are considered. The system-specific SOC saturation may be reached by increasing the C input and reducing decomposition, for example, by decreasing tillage or raising the water table. In northern agricultural soils, the characteristics of C saturation are insufficiently known, even though understanding these characteristics is crucial for maximising the impact of C inputs on SOC. Long-term experiments are unique sources of empirical data based on direct measurements. They are a valuable source for statistical analyses and unavoidable in the development and validation of models under certain conditions.

The climate and soil texture considerably affect SOC dynamics. When the mean annual temperature decreases, SOC tends to increase due to the lower decomposition rate (Post et al., 1982, Kirschbaum, 1995). Soil texture, especially clay concentration reportedly affects SOC accrual (Kätterer & Andren, 1999), but factors such as aluminium- and iron-oxhydroxides and exchangeable calcium, depending on climate and soil properties, are found to influence the accrual (Rasmussen et al., 2008). Clay functions as a physical protection and reactive surface, which stabilises organic compounds into aggregates and organo-mineral forms recalcitrant to microbial decomposition (Merckx et al., 1985; Schimel et al., 1994; Hassink, 1997; Six et al., 2004). Agricultural SOC accrual is determined by C input and decomposition, which are managed through crop rotation, crop cover, fertiliser application, soil amendment and tillage (Smith, 2004b; Lal et al., 2007; Aguilera et al., 2013; Chenu et al., 2019).

Manure application and forage leys, including grasses and legumes in crop rotation, are established practices in northern crop–livestock-integrated agriculture and their effectiveness at increasing SOC has been shown (Kirchmann et al., 1994; Blair et al., 2006). The potential of manure and other agricultural biomasses in SOC sequestration in agroecosystems has often been questioned with the argument that these biomasses are already utilised (Powlson, 2011). Although manure is mainly applied in agricultural soils, SOC accrual may be partially low. Manure is typically applied in ley-dominated rotations on animal farms where manure is available. There, leys increase the SOC concentration closer to potential saturation of the system and may thus reduce SOC accrual by added manure. On the other hand, in annual cropping with no perennial leys, mechanical tillage is more intensive, thus enhancing the decomposition of SOC added in manure (Six et al., 2000). However, to our knowledge neither evidence nor quantification has previously been published of whether manure application more effectively increases SOC when applied in rotations dominated by annual crops or leys.

Our objective was to investigate what type of crop rotation the C input of manure and other organic amendments should be applied to, to maximise soil organic C. We considered the following research question: How does SOC in northern agriculture depend on the manure application rate, the proportion of leys relative to annuals in crop rotation and the soil texture? The comprehensive, unique data from the geographically representative long-term experiments located north of 50 degrees north latitude at the steady-state regarding SOC were utilised. We used a machine-learning based statistical analysis to identify the model best fitting the data.

2. Materials and methods

2.1. Data

To analyse the SOC response to the manure application rate and ley proportion relative to annuals in crop rotation, data were collected from published literature reporting the results of northern long-term experiments. Data were derived from 27 long-term sites representing a total of 56 experiments (Table 1 and Fig. 1). Each observation was assumed to be independent. Some data were complementary primary, unpublished experimental data retrieved from known long-term experiments. The data coverage was cross-checked against published reviews.

Agricultural long-term experiments constitute a series of agricultural experiments established in national research centres worldwide. The longest experiment extends over 120 years and thus requires high societal perseverance. SOC changes under various constant management practices have been assessed in experiments, such as fertilisation, crop rotation, and tillage (Powlson et al., 1998). Management changes have occurred according to changes in common agricultural practices. Long-term experiments are unique direct, empirical sources of knowledge and understanding, invaluable sources of data for statistical modelling and examinations and unavoidable in the validation of process models under representative conditions.

In practical farming, forage and grazing leys are typically renewed after three to four harvest years, after which one to two annual crops are grown. Manure is typically applied to annual crops. In organic crop production, one- to three-year green manure leys are employed to improve the soil structure and nutrient stock for the following two- to four annual crops.

The long-term experiments considered here were fertilisation trials where manure application represented one treatment. The experiments comprised crop rotations varying from annual monocultures to a high proportion of harvested forage leys. The annual crops were mainly cereals and were annually ploughed. Ley crop mixtures and other practices reflected common (conventional) crop–livestock-integrated farming practices in northern regions.

The potentially confounding effect of the initial SOC concentration and variation in the experiment duration was eliminated by including only experiments at the steady state regarding SOC, as reported or observed in the data, or experiments based on their duration (>20 years). The analysed data included SOC concentration values retrieved from the latest publications of the selected long-term experiments, which represent the final or current conditions of these experiments. Variation was rarely given. Selected experiments had reached a management system-specific steady state, as reported (10 experiments) or interpreted based on the temporal development of the results (four experiments) or length of the experiments (41 experiments). One of the included experiments was reportedly not at steady state but was interpreted to be near the steady state based on its duration of 121 years (Jenkinson and Johnston, 1977). Experiment duration ranged from 12 to 121 years, while only two out of the 56 experiments had a shorter than 20-years duration (Table 1). The Intergovernmental Panel on Climate Change (IPCC) greenhouse gas inventory guidelines considered 20 years to be the time required for an agricultural management system to reach steady state (West & Six, 2007; IPCC, 2019).

Table 1
Long-term experiments providing data and their characteristics.

Location	Latitude deg. N	Experiment period	Duration y	Management	No. of experiments	FYM C Mg/ha/y	Ley %	Clay %	Initial SOC %	Depth cm	Reference
GREAT BRITAIN											
Rothamstedt, Barnfield	51	1843–1964	121	FYM	1	4.7	0	32	–	15	Rose, 1991
Rothamstedt, Broadbalk	51	1882–1998	116	FYM	1	4.7	0	25	1.02	100	Blair et al., 2006
Rothamstedt, Broadbalk	51	1843–1964	121	FYM	1	4.7	0	21	–	15	Rose, 1991
Rothamstedt, Hoosfield	51	1852–1975	123	FYM	1	4.7	0	>20	1.17	23	Jenkinson and Johnstone, 1977
Rothamstedt, Hoosfield	51	1852–1964	112	FYM	1	4.7	0	24	–	15	Rose, 1991
Saxmundham	52	1899–1965	66	FYM	1	2	0	27	–	15	Rose, 1991
Wellesbourne	52	1954–1966	12	FYM	1	9.6	0	15	–	15	Rose, 1991
Woburn, Arable&ley	52	1938–2007	69	FYM, LEY	8	1	0–60	11–16	0.98	25	Johnston et al., 2017
Woburn, Lansome	52	1942–1965	23	FYM	1	10.4	0	14	–	15	Rose, 1991
Woburn, Market Garden	52	1942–1967	25	FYM	2	3.2–6.3	0	9.4	0.87	23	Johnston, 1975
SWEDEN											
Fors	60	1956–2010	54	FYM, LEY	1	0.7	33	<20	1.48	20	Kirchmann et al., 2013
Offer	63	1956–2008	30	FYM, LEY	3	0.5–0.7	33–83	20–25	2.8	25	Bolinder et al., Bolinder et al., 2012
Röbäcksdalen	64	1958–1987	29	FYM, LEY	3	0.5–0.7	33–83	10–15	4.84	25	Bolinder et al., Bolinder et al., 2012
Ultuna	60	1956–2009	53	FYM	1	4	0	37	3.39	30	Kirchmann et al., Gerzabek et al., Kätterer et al., 2011
Ås	62	1957–1987	30	FYM, LEY	3	0.5–0.7	33–83	20	3.85	25	Bolinder et al., 2010; Bolinder et al., 2012
Örja	56	1956–2010	54	FYM, LEY	1	0.7	25	>20	1.67	20	Kirchmann et al., 2013
NORWAY											
Møystad	60	1922–1970	49	FYM	2	2–2.3	0	15	2.9	20	Uhlen, 1976
Ås	59	1939–1968	29	FYM, LEY	1	1.3	50	25	3.2	25	Uhlen, 1976
GERMANY											
Bad Lauchstädt	51	1902–2010	108	FYM	2	1.3–2	0	21	3.39	100	Merbach and Schulz, 2013
Halle	51	1878–1987	109	FYM	3	1.6	0	8	1.24	20	Stumpe et al., 1990; Stumpe et al., 1995
RUSSIA											
Barybino	55	1940–1999	21–42	FYM, LEY	4	0.8–2.6	0–14	22	0.88–1.05	–	Shevtsova et al., 2003
Dolgoprudny	56	1931–1956	25	FYM	2	1.2	0	<20	–	–	Lyubarskaya, 1969
Solikamsk	60	1933–1948	15	FYM	1	0.9	0	8.33	–	18	Prokoshev, 1952
DENMARK											
Askov	55	1894–1985	91	FYM (+S), LEY	3	0.7–2	25	11	1.69–1.80	20	Kofoed, 1982; Christensen, 1996
Askov	55	1912–1976	64	FYM (+S), LEY	1	1.3	25	4	1.08	20	Kofoed, 1982; Christensen, 1996
POLAND											
Skiernewice	52	1923–1995	73	FYM	3	0.8–2.6	0	8	0.47	20	Mercik et al., 1997
LITHUANIA											
Vezaiciai	56	1949–2002	53	FYM + LEY	4	0.4–2.3	29	<20	–	–	Repsiene and Ciuberkis, 2005
				Total	56						

FYM denotes farm-yard manure, FYM C denotes farm-yard manure carbon, S denotes slurry and LEY denotes the ley proportion in crop rotation.

We adopted the difference in SOC concentration between the manure (without additional fertilisation) and control (no manure or additional fertilisation) treatments in each experiment with a certain ley proportion in the rotation (SOC_{DIFF} (%)) as the response variable during our analysis, to exclude the interference caused by SOC variation due to climatic and edaphic conditions (Rimhanen et al., 2016). SOC concentration was commonly expressed as a percentage of dry soil, whereas bulk density data were rarely available.

Derivating the changes in SOC stock based on the concentration via fixed-depth sampling often leads to incorrect estimations due to differences in bulk density between the sampling times or treatments. Examining the absolute quantitative differences in SOC stock among the various treatments therefore requires observing the C quantity related to the specific soil mass and entire depth affected. A certain layer below the

plough layer, at least up to the rooting depth, must also be included (Ellert & Bettany, 1995; Wendt & Hauser, 2013; Heikkinen et al., 2020). We focused on the relative changes in topsoil SOC, which makes observations of changes in the SOC concentration relevant, as Lee et al. (2009) described: “Due to measurement errors and spatial/temporal variation in BD (bulk density) (Amador et al., 2000; Kulmatiski and Beard, 2004; Logsdon and Cambardella, 2000), one could use soil C concentration data to reliably describe soil C changes rather than using soil C stock estimates based on the FD (fixed depth) method.”.

The depth of the soil surface layer sampled ranged from 15 to 100 cm, reaching a depth of 25 cm in most cases. The sampling depth was unknown in three experiments. Another three experiments exhibited a sampling depth deeper than 25 cm, which may result in lower SOC concentrations than those occurring at lower sampling depths due to



Fig. 1. Site locations of the long-term experiments used in the study.

dilution with SOC-poor subsoil. However, the significance of these cases for the final model was low. The variation in sampling depth was of low importance due to the repeated soil blending as a result of tillage.

The quantity of manure amendment was reported in many studies, while some studies reported C quantities based on C content analysis. The applied manure was mainly farm-yard manure (FYM) obtained from cows, which is a manure type where bedding material, such as cereal straw, is used to reduce and bind moisture. The reported manure quantities were converted into C quantities according to the Finnish average FYM dry matter content (22.7 %), which is based on a comprehensive analysis of farm results (Eurofins Viljavuusalvelu Ltd., 2016). Manure-OM was converted into organic carbon (OC) by the van Bemmelen factor 1.724. In some studies, slurry was also applied as a partial supplement or replacement of FYM. Slurry quantities were converted into C quantities similar to FYM (Eurofins Viljavuusalvelu Ltd., 2016).

The effect of the clay concentration on the steady-state SOC concentration was also investigated. A categorical variable was constructed based on the soil texture according to the international soil classification (IUSS Working Group WRB, 2015). To achieve a balanced distribution among classes and to include already classified data, soils containing ≤ 20 % clay were classified as sandy soils, soils containing > 20 % clay were classified as clay soils, and organic soils (>20 % OM) were not included. After the initial analysis, five observations were omitted because they were interpreted to not represent a steady-state system. Sandy and clay soils therefore comprised 21 and 35 observations, respectively. The data consisted of a total of 56 observations. Thus, the clay concentration was a two-level categorical variable (dummy; denoted as CLAY in the analysis), where the levels were sandy soils (clay = 0) and clay soils (clay = 1).

The values in both classes were of the same magnitude, but the steady-state SOC (SOC_{SS}) values for the clay soils were slightly higher than those for the sandy soils. The SOC maximum value was < 4 %, and

the mean and median were < 2 % for both soil classes (Table 2).

Every studied experiment represented manure application in crop rotation at various ley proportions (Fig. 2). The number of observations without leys in crop rotation was 26, and the number of observations with leys was 30.

2.2. Statistical analysis

Statistical analysis was performed by explaining the SOC difference factor (denoted as SOC_{DIFF} in the analysis) with explanatory variables of the manure application rate (denoted as ADDED C_{FYM} in the analysis), the proportion of leys in crop rotation (denoted as LEY in the analysis) and CLAY. The SOC difference factor was determined as the difference in steady-state SOC concentration between the treatment and control in a given trial. A single trial had a constant ley proportion but the control did not have manure application. Positive SOC_{DIFF} values indicated growth, and negative values indicated a decrease in SOC in a certain management system over the control. This procedure reduced the effect of other factors, such as climatic or edaphic conditions.

We adopted a machine learning approach, where the aim was to maximise prediction accuracy. The machine learning approach tackles the problem of underfitting and overfitting, which are well-known problems in statistical modelling (Hastie et al., 2013). We chose linear regression as the model type (for an example, please refer to Biggs et al., 2009). The interpretation process of linear regression is simpler than that of other approaches such as nonparametric methods (Biggs et al., 2009). The statistical analyses and generation of descriptive statistics were performed in the R software (R Core Team, 2014). By fitting several linear models with linear regression analysis, we examined the effects of ley proportion in the crop rotation and C addition quantity in manure (the explanatory variables) on the steady-state SOC concentration for the two soil classes (sandy and clay soils). As the modelling scheme consisted of both continuous variables (ley proportion and

Table 2
Range of the soil organic C concentration, manure addition level, and ley proportion in the experiments.

Variable	Sandy soils (n = 35)				Clay soils (n = 21)			
	Minimum	Maximum	Median	Mean	Minimum	Maximum	Median	Mean
SOC _{SS} (%)	0.44	3.73	1.33	1.63	0.85	3.75	2.70	2.43
SOC _{DIFF} (%)	0	1.76	0.24	0.37	0.05	2.50	0.77	0.92
LEY (%)	0	83.30	25.00	20.47	0	83.30	0	20.80
ADDED C _{FYM} (Mg)	0.38	10.37	1.19	1.96	0.50	4.68	1.58	2.08

SOC = soil organic carbon; SOC_{SS}: Steady-state SOC; SOC_{DIFF}: SOC difference; LEY: ley proportion in crop rotation; ADDED C_{FYM}: added farm-yard manure C.

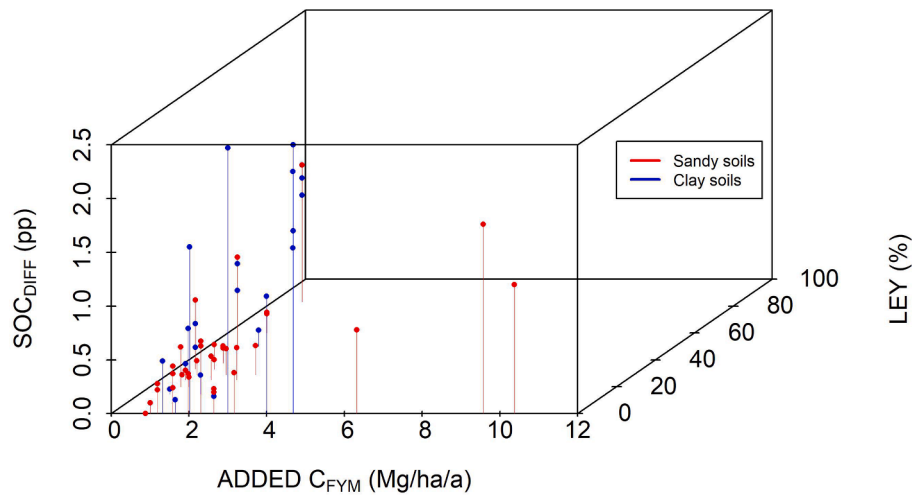


Fig. 2. Observations of the differences in soil organic carbon (SOC) relative to the control, depending on the quantity of added manure carbon and ley proportion in crop rotation. Red and blue correspond to sandy and clay soils, respectively. The data in the final analysis comprise 56 observations (35 sandy soils, and 21 clay soils). SOC_{DIFF}: SOC difference; LEY: proportion of leys in crop rotation; ADDED C_{FYM}: added farm-yard manure C.

manure application quantity) and a single categorical variable (soil class), the modelling approach could also be considered an analysis of covariance (ANCOVA) type of approach. Generally, the objective was to determine whether the SOC values differed among the various experiments considering varying values of the aforementioned explanatory variables. Furthermore, we specified different functional forms and investigated and obtained inferences from these models.

We fitted several models for which the second derivative could be negative. For example, a log-transformed predictor model was a valid option, but a log-transformed response model was not.

The full form of the fitted model is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1^2 + \beta_5 x_2^2 + \beta_6 x_1^2 x_3 + \beta_7 x_2^2 x_3 + \beta_8 x_1 x_3 + \beta_9 x_2 x_3 + \beta_{10} x_1 x_2 + \beta_{11} x_1 x_2 x_3 + \varepsilon$$

where y, x_1, x_2, x_3 and ε denote SOC_{DIFF}, ADDED C_{FYM}, LEY, CLAY and the error term, respectively. We assumed $\varepsilon \sim N(0, \sigma^2)$.

This general model form was applied as the full model and compared to several submodels (subsets of predictors). We also fitted models containing transformed predictors, obtained via transformation as $\log(x), \exp(-x)$ and $1/x$.

The model selection process used a grid search approach. For each submodel of the full model, including the full model itself, we performed leave-one-out cross-validation and calculated the coefficient of determination R^2 describing the prediction performance. We then chose the submodel for which the so-called cross-validation (CV) R^2 value was maximised. Model performance comparison must include the main effects of all terms included in the interactions. Based on the final model, we also calculated the Wald test value, variance inflation factor (VIF), and Cook's distance.

We observed that the model with the maximum CV R^2 value exhibited the following expression, which was applied thereafter:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1 x_3 + \beta_5 x_1 x_2 + \beta_6 x_2^2 + \varepsilon$$

3. Results

The effects of the manure application rate and the ley proportion in the crop rotation on the SOC concentration were interdependent. Manure application was most effective at low ley proportions in the crop rotation. The effect on the SOC concentration was more notable in the clay soils, and the difference over the sandy soils increased with increasing manure application rate.

The model selection approach considered prediction performance maximisation based on unseen data. Thus, each coefficient (Table 3) in the final model was statistically significant in this respect. The CV R^2 value of this model was 0.65. The residual diagnostics indicated that the model assumptions were met. The largest Cook's distance value reached

Table 3
Interdependence of the effects of the manure carbon application rate (ADDED C_{FYM}) and proportion of leys in crop rotation (LEY) on the difference in SOC between the manure and control (no manure) treatments (SOC_{DIFF}). The intercept is the value of SOC_{DIFF} for sandy soils when both LEY and ADDED C_{FYM} are zero.

	Coefficient	SE
Intercept	0.0581	0.1173
ADDED C _{FYM}	0.1378	0.0288
LEY	0.0057	0.0086
CLAY (clay soils)	-0.2232	0.1562
LEY ²	0.0002	0.0001
LEY:ADDED C _{FYM}	-0.0101	0.0053
ADDED C _{FYM} :CLAY	0.3190	0.0586

LEY: Proportion of leys in crop rotation; ADDED C_{FYM}: added farm-yard manure C; SE: Standard error.

0.2, which is small. Hence, no overly influential observations were present. High VIF values were observed, which occurred due to the interaction and polynomial terms. The VIF value was calculated for a model including only quantitative main effects (x_1 and x_2), which yielded a VIF value of 1.3. Multicollinearity was thus not a problem. The best-fitted model included the interaction term.

The interaction term between ley proportion and manure application rate indicates that 1) while the ley proportion remains fixed at a low value (such as 20 %), increasing the manure application rate increases SOC_{DIFF} , and 2) when the ley proportion remains fixed at a high value (such as 80 %), increasing the manure application rate decreases SOC_{DIFF} depending on the soil texture. Thus, the slope of ADDED C_{FYM} varies depending on the LEY value.

Regarding LEY, at a low manure application rate, such as 0.7 Mg FYM C/ha/y as in Fig. 3a, increasing the proportion of leys in crop rotation leads to a higher SOC concentration per increase in ley proportion (SOC_{DIFF} grows increasingly; i.e., grows with a positive second derivative), with a minimal or no difference between the soil texture (Fig. 3a). This is revealed by the impact of the interaction term on the parabolic curve when the ADDED C_{FYM} value is varied (Fig. 3). In addition, the LEY^2 term indicates that the shape of the fitted curve is parabolic with respect to the LEY axis and the curve is convex. This means SOC_{DIFF} varies nonlinearly when LEY varies. At moderate manure application rates, such as 1.3 Mg FYM C/ha/y as shown in Fig. 3b, SOC_{DIFF} first decreases decreasingly (with a positive second derivative) until turning into increasing growth with an increasing ley proportion (Fig. 3b). Increasing the manure application rate causes decreasing SOC growth with increasing ley proportions, thereby even yielding negative values and a decrease in SOC at high ley proportions in the sandy soils (Fig. 3c). Results based on high manure applications and high ley

proportion in crop rotation are uncertain, which is seen from the confidence intervals shown in Fig. 3c. The sign of the second derivative determines the rate of change. For an upward sloping curve, a negative second derivative indicates that the slope of the curve is decreasing whereas a positive second derivative indicates that the slope is increasing.

The shape of the fitted model is linear with respect to the ADDED C_{FYM} axis (Fig. 4). The lower the proportion of leys in crop rotation, the greater the positive effect of manure application on the SOC concentration is. According to the model, when LEY remains fixed below 14 % in sandy soils and below 45 % in clay soils, SOC_{DIFF} linearly increases with ADDED C_{FYM} , and the increase occurs faster (higher slope) in the clay soils than in the sandy soils (Fig. 4a). The slope is zero at a certain proportion of leys (14 % in sandy soils and 45 % in clay soils), indicating that these systems are saturated irrespective of the manure application rate. At this ley proportion, manure addition imposes a constant effect on the SOC_{DIFF} value regardless of the application rate within the examined range. Here C input equals output by decomposition. The slope is negative (decreasing SOC_{DIFF}) at higher ley proportions, which indicates, first, a decrease in SOC growth (positive values) at low manure application rates and, second, a decrease in SOC concentration compared to the control (negative values) at high manure application rates. For example at a 60 % ley proportion in crop rotation (Fig. 4d), negative values were observed at ADDED C_{FYM} values of > 2.04 (ca. 15 Mg FYM/ha/y) and > 4.92 (ca. 38 Mg FYM/ha/y) in the sandy and clay soils, respectively. For example, in a 5-year rotation, this would occur at an application rate of 37.5 Mg FYM/ha/y on sandy soils and at an application rate of 95 Mg FYM/ha/y on clay soils applied over two-years of annual crops in the rotation system. The application rate for the clay soils is untypically high.

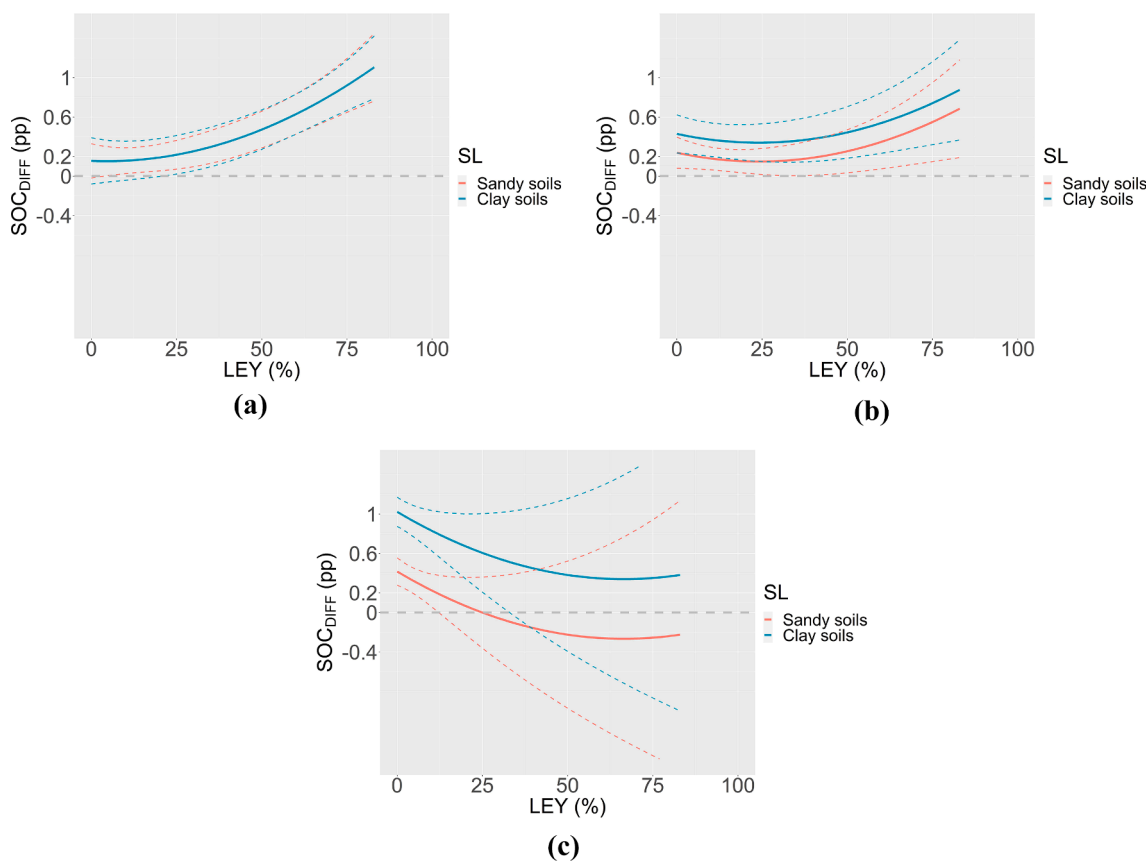


Fig. 3. Dependence of the ley proportion at fixed manure application rates on SOC response. Final model SOC_{DIFF} predictions (solid curves) along the LEY (%) axis with ADDED C_{FYM} fixed at values a) 0.7 Mg FYM C/ha/y (5 Mg FYM/ha/y), b) 1.3 Mg FYM C/ha/y (10 Mg FYM/ha/y), and c) 2.6 Mg FYM C/ha/y (20 Mg FYM/ha/y) in two soil texture categories (SL) sandy soils and clay soils. The dotted curves represent 90 % confidence intervals for sandy soils and clay soils, respectively.

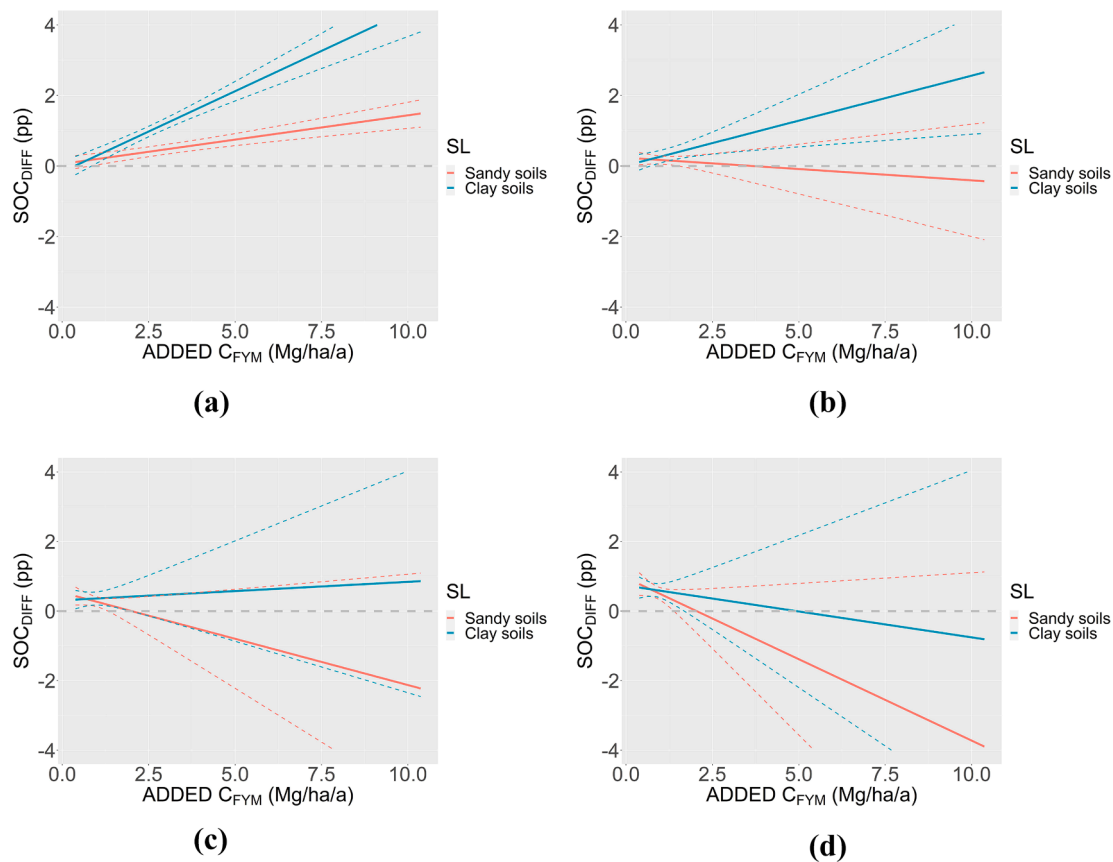


Fig. 4. Dependence of the manure application rate at fixed ley proportion on SOC response. Final model SOC_{DIFF} predictions (solid curves) along the ADDED C_{FYM} (t/ha/y) axis with LEY fixed at values of a) 0%, b) 20%, c) 40%, and d) 60% in two soil texture categories (SL) sandy soils and clay soils. The dotted curves represent 90% confidence intervals for sandy soils and clay soils, respectively.

As the data do not contain observations with high values of both the manure application rate (ADDED C_{FYM} > ~2) and ley proportion in crop rotation (LEY > ~50) but, rather, observations containing high values of either variable, combined high values are extrapolated and should therefore be interpreted with caution. The confidence intervals in Figs. 3 and 4 represent the uncertainty in the area of extrapolation.

4. Discussion

Our findings indicated that the effects of the manure application rate and ley proportion on SOC were interdependent and the effect was greater in the clay soils than in the sandy soils. System-specific soil C saturation occurred with manure application in crop rotation at a ley proportion of 14 % in the sandy soils and at a ley proportion of 45 % in the clay soils.

4.1. Reliability and generalisability of the results

The data were derived from long-term studies in the steady-state north of 50 degrees north latitude, mostly from Europe, were comprised of 56 trials, and are thus highly representative empirical data in regard to SOC in northern agricultural land. As we used a categorical soil classification and the SOC difference factor ((SOC_{DIFF} (%), see 2.1), our results are also applicable to other northern farming regions. The data cover practically relevant ranges of the manure application rate and ley proportion in crop rotation. However, the data do not include high values of both the manure application rate and ley proportion combined. We adopted prediction performance maximisation as our model selection approach. The results were affected by the sample size, as is the case in traditional model selection approaches, such as stepwise regression. A

larger sample size may have yielded lower variance estimates and thus more accurate results.

The considered manure was mostly FYM, and generalising the obtained results to other organic amendments requires caution. Properties such as the C/nitrogen (N) ratio, C and N quality, nutrient composition, and physical structure of the organic amendment affect SOC decomposition and accrual in soil (Six et al., 2002). Nevertheless, Mikutta et al. (2019) showed that the accumulation of mineral-associated OM did not depend on litter quality. FYM often contains not only a higher dry matter content than that of slurries but also organic bedding material. The C/N in slurries is much lower than that in FYM, which could affect the SOC concentration. Currently in the EU, FYM occupies approximately half of the manure management, while slurry covers the other half (European Commission, 2011). Considering earlier studies regarding manure and SOC, aligned with our results, Angers et al. (2010) observed that liquid hog manure application decreased the SOC stock on grassland soil in a long-term experiment compared to the mineral fertilised control. However, a global meta-analysis (Maillard and Angers, 2014) reported that the SOC response to added C in poultry and pig FYM was linear. Further, the expanding practices of manure separation and anaerobic digestion produce materials with a dry matter content and physical properties more comparable to those of FYM than to slurry (European Commission, 2011). The ley plant composition has rarely been described and has probably developed according to general practices over time.

We considered the difference in SOC concentration between each treatment and its corresponding control treatment in all the experiments in our analysis to increase the comparability of the SOC responses between the treatments in the various experiments and locations. In this way, potentially confounding differences among the experiments and locations were minimised, except for the factors interacting with the

studied variables. For example, we assumed that the confounding effect of the climate was mitigated via determination of the difference. Using difference decreased the variation and increased the reliability of the results and coefficient of determination.

In a particular experiment, the initial SOC concentration affects the final (or the most recently reported) SOC concentration if the system has not yet reached the steady state. As we only included experiments in which the steady state had been reached, we did not consider the effect of the initial SOC concentration, which was often not reported. The steady-state SOC concentration does not depend on the initial SOC concentration, which reflects not only the soil texture and climate but also previous land use or management and duration. In long-term experiments, changes in practices such as tillage depth or quality of the organic amendment may affect the SOC concentration, and climate change may also be a source of error (Powelson et al., 1998; Ellmer & Baumecker, 2005) over the initial SOC concentration.

Many other studies on saturation (i.e., Six et al., 2002; Stewart et al., 2008a&b) have only sampled the topsoil, which does not indisputably demonstrate that the entire soil column is saturated. As our results suggest, the topsoil may be saturated with C obtained from manure and ley application. Leys may exhibit roots below the topsoil and consequently a potentially high C input (Liebig et al., 2008; Taghizadeh-Toosi et al., 2014). Even though concentration sampling without considering the bulk density avoids this confounding effect, the conclusions are only valid regarding the sampled soil layer.

In SOC-saturated systems, changes in management (e.g., tillage) may again turn the soil into a C sink or source. The characteristics of a given system define the SOC concentration where saturation occurs. Our data describe management systems yielding relatively low SOC concentrations (average values of 1.63 % and 2.43 % in the sandy and clay soils, respectively). However, since the establishment of these long-term experiments, several cropping system elements have been developed that enable higher SOC concentrations and stocks. Approaches such as minimum tillage, trace nutrient/balanced fertilisation, mixed and cover crops, deep-rooted perennials, and organic amendments (such as biochar and wood-based materials) may contribute to higher SOC stocks (Phillips et al., 1997; Six et al., 2002; Lehmann, 2007; Liebig et al., 2008; Kell, 2011; Aguilera et al., 2013).

4.2. Joint effects of the manure application rate and ley proportion on soil organic carbon

The observed main differences between the effects of manure and ley application on SOC involve the stability of the added C and the soil horizon where deposition occurs. Manure-derived C decomposes faster than root-derived C, which under Swedish conditions resulted in a 1.2-fold slower humification of ley-derived C than that of manure-derived C (Kätterer et al., 2011). Perennial leys also strongly contribute to SOC below the topsoil. In Denmark, a notable effect on SOC at 25 to 50 cm was reported but not at depths below 50 cm (Taghizadeh-Toosi et al., 2014). In contrast, an increase in SOC occurred even at a depth of 1.2 m in the US (Liebig et al., 2008).

The decreasing positive effect of manure application on SOC concentration when the ley proportion in crop rotation is increased is supposedly the result of the priming effect caused by the low C/N ratio of the fresh OM and soluble N in manure, which may have resulted in the decomposition of OM with a high C/N ratio in the leys. According to Kuzakov et al. (2000), “priming effects are strong short-term changes in the turnover of soil OM caused by comparatively moderate treatments of the soil”. Both C and N may be drivers of the priming effect and may interact as energy sources and nutrients for soil microbes. Although the understanding of the role of labile OM digested by microbes to stabilise SOC has recently grown (Kallenbach et al., 2015; Mikutta et al., 2019; Woolf & Lehmann, 2019), manure applications may be especially important, as high rates contain more soluble nutrients than can be utilised by crops in the short term. This could lead to increased microbial activity and

decomposition of the C stock (Fangueiro et al., 2007; Angers et al., 2010).

4.3. Overall soil carbon saturation depends on the management system

We interpret that our results indicate an overall system-specific SOC saturation where both mineral-associated and particulate OM pools are saturated in the specific management system. SOC is stabilised in soil via the following three main mechanisms: chemical stabilisation, physical protection, and biochemical stabilisation (for a review, please refer to Six et al., 2002). Silt and especially clay in soil plays a major role in chemical and physicochemical binding and physical aggregation in the SOC stabilisation process. The long-held view of separate SOC pools has subsequently been challenged with the soil-continuum model, where SOC is continuously processed and controlled by ecological conditions (Lehmann & Kleber, 2015). However, soil OM may also be separated into particulate and mineral-associated OM forms, which are fundamentally different in their formation, persistence and function (Lavalley et al., 2020).

Mineral-associated OM is considered a stable fraction where saturation occurs, and particulate OM is considered a labile fast-turnover fraction. Recently, the saturation theory of mineral-associated OM has been challenged by Begill et al. (2023) and Salonen et al. (2023) and the role of particulate OM in SOC accrual has been emphasised by Angst et al. (2023). Nevertheless, the particulate fraction may also reach saturation (Six et al., 2002; Stewart et al., 2008a), even if contrasting results have also been reported (e.g., Stewart, 2007; Gulde et al., 2008; Cotrufo et al., 2019). In agroecosystems where the mineral-protected SOC fraction is saturated, the increase in the particulate fraction defines the overall saturation. Consequently, the labile particulate OM fraction is sensitive to changes in farming practices, such as tillage, and to the quality and quantity of the C input (Six et al., 2002). According to our results regarding topsoil SOC concentration, the total SOC may reach a system-specific saturation and even decrease with increasing C input under certain combinations of the ley proportion and manure application rate.

Our results show that sandy soils exhibit system-specific saturation at a considerably lower proportion of leys than those attained in clay soils. Sandy soils contain less C-protecting clay and silt minerals, and their SOC consists more of particulate OM, with an overall lower C accrual potential under a specific management system and climatic conditions than that contained in clay soils (Six et al., 2002; Begill et al., 2023). Therefore, SOC in sandy soils is more susceptible to changes in farming practices. Although sandy soils contain lower C concentrations and stocks than do clay soils with similar management, soil OM (SOM) and especially particulate OM may exhibit a greater importance in regard to soil productivity (i.e., through cation exchange and water-holding capacity) than those in clay soils.

4.4. Managing the manure application rate and ley proportion to maximise the carbon sink

Based on our results, considering the topsoil SOC concentration, manure is most effectively utilised when applied at agronomically relevant rates in crop rotation systems where the ley proportion is low (below 14 % or 45 %), depending on the soil texture. However, the SOC accrual efficiency of leys increases with an increasing proportion of leys in crop rotation, or, in other words, with the increasing perennial nature of leys. Even though the topsoil achieved system-specific saturation at the determined C input levels with the manure application rate and ley proportion, SOC accrual may have continued in the subsoil. Our results also demonstrated the susceptibility of the sandy soils OC to a high manure application rate, indicated by a possible decrease in SOC concentration due to unusually high manure application rates. Our results are supported by the recent meta-analysis on manure C accrual, which nevertheless did not consider crop rotation but emphasised the need for

studying SOC saturation (Gross and Glaser, 2021).

According to a simplification of Loveland and Webb (2003) and Oldfield et al. (2019), the critical SOC concentration required for soil productivity in temperate climates is approximately 2 %. From this perspective, it may be reasonable to apply manure in crop rotation systems at a low proportion of leys (relative to annual crops) and introduce perennial leys into annual cropping practices to reach the critical SOC concentration.

The joint effects of various farming practices on the SOC and system-dependent SOC saturation concentrations should be examined while considering the input quality and practices controlling the output. This consideration should preferably rely on data obtained from long-term experiments, which, however, are not immediately available regarding recently developed practices. Therefore, farmer-led research approaches should be considered (Mattila et al., 2022). Data on replicated adjacent management systems should be gathered from farms where relevant practices have already been applied for sufficiently long to reach the steady state, as suggested by Kahiluoto et al. (2014). The approach applied in our study based on the difference between a given treatment and its corresponding control treatment in each experiment allows for trial setups and their comparison at the farm scale. These results may help scientists develop soil C models and farmers target their inputs most effectively for SOC accrual and productivity purposes.

5. Conclusions

Applying manure in crop rotation systems at a low ley proportion relative to annual crops maximises the SOC gain in the topsoil. System-specific saturation appeared to be reached at a lower share of ley in crop rotation in sandy soils than in clay soils. In clay soils, manure increased SOC accrual more than in sandy soils, and after system-specific saturation, manure decreased SOC accrual more in sandy soils than in clay soils. The difference between the soil classes increased with increasing manure application rates. This highlights the system dependency of SOC accrual and the importance of understanding the management practices and local biophysical conditions when targeting SOC sequestration. Further, to enhance SOC sequestration, leys and organic amendments should be integrated into crop rotations on arable farms especially on clay soils low in SOC. The measures should be explored to feasibly utilise leys, manure and other organic amendments on arable farms.

The most effective manure application rate and ley proportion in regard to SOC in northern agricultural landscapes can be determined with the developed statistical model, depending on the management system and soil texture.

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CRedit authorship contribution statement

Juuso Joon: Conceptualization, Data curation, Investigation, Visualisation, Writing – Original draft, Review & Editing. **Eero Liski:** Formal analysis, Methodology, Software, Writing – review and editing. **Helena Kahiluoto:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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