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RESEARCH ARTICLE

No saturation of soil carbon under long-term extreme manure additions

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Abstract
Background and aims According to the carbon (C) saturation concept, the capacity of soils to accumulate stabilized organic C is limited by the number of binding sites on mineral surfaces. The concept and its application are highly debated. Therefore, we aimed at testing this theory using field experimental data.

Methods Soils were sampled from four long-term field experiments with different amounts of organic fertilisation going up to extreme high C inputs (20 Mg C ha^{-1} yr^{-1}) five times higher than in common agricultural practice. Soils were fractionated by particle size to obtain sand-sized, coarse silt and fine silt plus clay fractions.

Results We found a linear relation between C input and soil organic carbon stocks (SOC) even with vast amounts of organic C inputs to the soil at three experimental sites. Across all experiments, C stocks in the sand-sized fraction increased on average by 146%, whereas C stocks in the fine silt plus clay fraction (< 20 µm) increased by just 17% without distinct saturation behaviour. The C sequestration efficiency (amount of C retained as SOC per amount of C input) tended to increase with initial SOC content which is not in line with the saturation theory.

Conclusion The experiments were subject to C inputs via organic fertilisation that would and should rarely be reached in agricultural practice due to negative side effects. Even under these artificial conditions experiments did not show a distinct saturation behaviour.

Initial SOC stocks or SOC in the mineral-associated fraction did not appear to limit the potential of soils to sequester additional SOC. It can be concluded that C sequestration is mainly limited by the availability of C inputs from biomass.

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Introduction

Soils are the largest pool of organic carbon (C) in the biosphere. Global soil organic carbon (SOC) stocks hold more than half of all terrestrial C (Jobbágy and Jackson 2000) and thus SOC plays a critical role in the global C cycle. The accumulation of C in soils can help mitigate climate change and sustaining agricultural productivity (Lal 2004; Minasny et al. 2017). In order to differentiate between the suitability of soils for C storage, several studies have applied what is known as the carbon saturation theory to identify soils with high C-sequestration potential (Angers et al. 2011; Carter et al. 2003; Chen et al. 2019; Wiesmeier et al. 2014). This approach assumes that the capacity of soils to store stabilised i.e., mineral associated, SOC is limited by the proportion of fine silt and clay particles (<20 µm). In general, two fractions are often separated: particulate organic carbon (POC) and mineral-associated organic carbon (MAOC). POC consists mainly of rather undecomposed, lightweight fragments that are not protected from decomposition. The simpler organic compounds of MAOC are associated with minerals and hence protected from decomposition (Schmidt et al. 2011; Lavallee et al. 2020). From a positive correlation between the amount of the fine fraction and the MAOC content in a soil, Hassink (1997) inferred that the capacity of soils to protect SOC from decomposition is limited by their clay and fine silt content. This assumption was supported by Six et al. (2002) and Chung et al. (2010).

Chung et al. (2010) found a finite capacity to store C in soil aggregate fractions, while only the particulate organic matter sequestered C infinitely. A recent study by Cotrufo et al. (2019) strengthened the concept of MAOC saturation and predicted an upper limit of 47 g C kg^{-1} soil for European soils. Further studies support the saturation concept with various experimental approaches (Brown et al. 2014; Chung et al. 2008; Du et al. 2014; Stewart et al. 2007) and it has also been suggested, that not only the fine fraction may saturate, but also the bulk SOC (Stewart et al. 2007). The saturation concept implies, that once a certain threshold is reached, additional carbon is only accumulating in a relatively labile form, with relatively faster turnover (Hassink 1997). Following this logic, soils with a relatively low content of SOC in the fine fraction are assumed to build up bulk SOC at a high rate per unit of C added to the soil and therefore having a high sequestration potential (Dexter et al. 2008), because the mineral phase provides space for this C input to become long-term stabilized. Thus, the C-sequestration efficiency, a ratio between C input to the soils and stored C, would be high in soils with a low SOC content, but low when soils are close to saturation (Georgiou et al. 2022). However, this coherence has been challenged by several studies that did not find an asymptotic relationship between C input and C accumulation in soils that are supposedly close to the C saturation level (Matus 2021; Orgill et al. 2017). Georgiou et al. (2022) have recently shown that the degree of MAOC saturation might negatively influence SOC accrual, which would be explicable by the fact that SOC tends to accumulate in fast cycling pools once a saturation of the slower-cycling MAOC pool is reached. Latest research has seriously challenged the concept of limited mineral-associated organic carbon accumulation (Begill et al. 2023) and has confirmed that clay-associated C storage can be driven by building a thicker loading of organic C, irrespective of limitations by clay content (Schweizer et al. 2021). Begill et al. (2023) used soil inventory data to explore a theoretical upper limit of MAOC in agricultural soils at large scale. However, this is only indirect evidence and lack direct field experimental evidence how increasing C input to the soil is transformed into different SOC pools and mineralised to CO_2. Due to contrasting results in the literature as well as methodological limitations to comprehensively test the saturation concept it remains unclear i) if MAOC saturation exists, ii) at which point MAOC saturates and how pedo-climatic conditions can influence this potential point, iii) if a potential MAOC saturation would be relevant for overall SOC dynamics i.e., the efficiency of C sequestration, or even bulk SOC saturation.

When testing for saturation behaviour of a certain SOC pool or bulk SOC, distinction between equilibrium SOC stock and SOC saturation need to be clear. SOC equilibrium or steady state can occur after a certain time under constant agronomic management and climatic conditions, when C input to the soil and output of C mainly via mineralisation balance each other (Stewart et al. 2007; West and Six 2007; Fig. 1a). If SOC is not changing it can be a result of SOC being in equilibrium, which is in independent of C saturation. With changes in agricultural management practice, C-input levels may change and SOC content will therefore increase or decrease until a new equilibrium SOC stock is reached (Fig. 1a). Soils with a SOC content that is close to the management-specific equilibrium SOC content will show slower changes in SOC than soils that are far from this steady state. Therefore, SOC contents will show asymptotic behaviour when expressed over time (Six et al. 2002; Stewart et al. 2007). This behaviour is often misinterpreted as soils reaching maximum storage capacity. However, C saturation in a SOC pool would only be reached when an increase in C input (e.g., via a change in management practice) does not result in rising SOC stocks in this pool. Thus, saturation must be assessed by monitoring SOC changes expressed over different C-input levels (Stewart et al. 2007). According to the saturation concept, there is a maximum level of stabilized SOC that will be reached under conditions with large amounts of C inputs to the soil (Fig. 1b). This is associated with a decreasing fraction of C inputs that will be retained as SOC, eventually resulting in no change in SOC due to additional C input. Without saturation, the SOC stock will continue to increase linearly with rising C inputs (Stewart et al. 2007; West and Six 2007).

According to the theory, saturation would occur in the MAOC fraction, while POM as larger size fractions may continue to increase with C input (Hassink 1997; Six et al. 2002). To detect SOC saturation behaviour in soils and soil fractions, C inputs have to be high enough and need to be applied over a long time (Stewart et al. 2007). Here we used long-term field experiments to make sure that saturation could be reached and to test the following research questions: (a) Does an asymptotic relationship occur between C inputs and SOC stocks of different sized fractions and how does this correspond with bulk SOC stocks for high C inputs? (b) Does the fine silt and clay fraction saturate while the larger sized fractions continue to accumulate SOC with increasing C inputs? An asymptotic relationship in the fine silt and clay fraction with larger fractions accumulating SOC would support the saturation hypothesis. The saturation theory would be contradicted by a linear relationship of bulk SOC or the fine silt and clay fraction with increasing C inputs. A non-declining C-sequestration efficiency across all fertilisation treatments would furthermore contradict the concept of saturation.

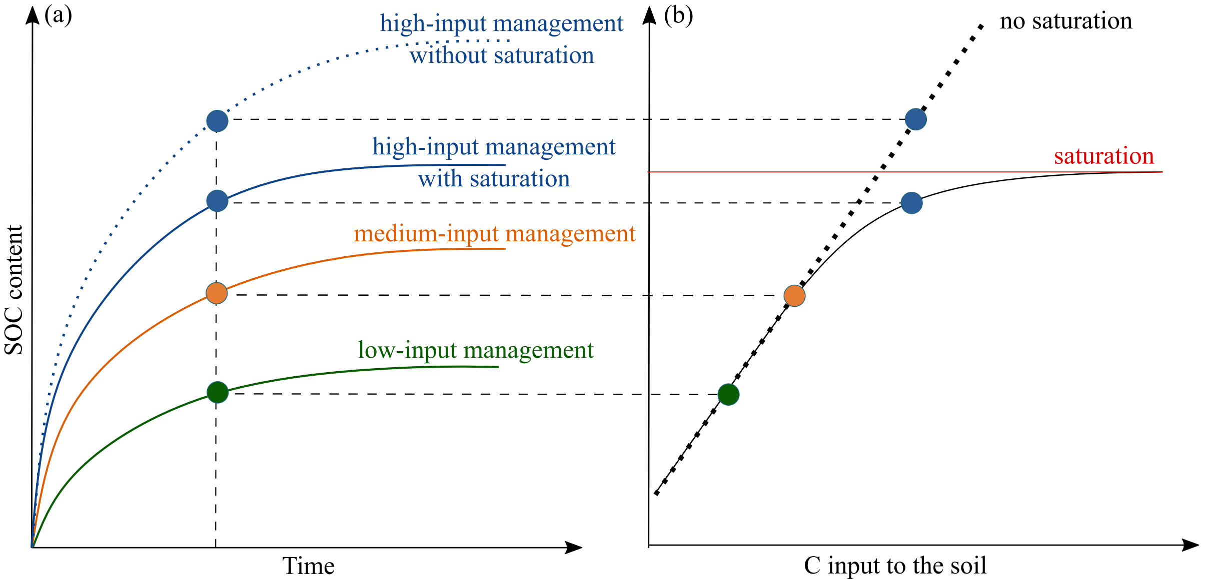

Fig. 1 Dynamics of SOC or C fractions expressed over time (**a**) to illustrate steady-state behaviour, and expressed over C input to the soil (**b**) to illustrate saturation behaviour

Materials & methods

Study sites & soil sampling

Three German field sites with four long-term experiments under different land uses were selected for this study. The sites differed in their soil type, texture and soil pH (Table 1). Each experiment comprised a large range of C inputs with organic fertilisation, as well as varying crop rotations (Table 2).

The Incremental Farmyard Manure Experiment in Bad Lauchstädt in eastern Germany was laid out in 1983 as a two-factorial block experiment in two replications. The first factor is organic fertilisation in four increments (0, 50, 100 and 200 Mg fresh matter ha^{-1} yr^{-1}) applied as farmyard manure (Table 2), giving a wide range of C inputs for fertilisation of 0, 4.7, 9.4 and 18.7 Mg C ha^{-1} yr^{-1} (Table 2). The second factor is crop rotation versus a mechanical bare fallow. The crop rotation has been continuous silage maize since 2015. The historical management prior to maize was a crop rotation of potato, sugar beet and silage maize, which is described in detail in Schulz (2016).

The experimental site in Kiel is part of a long-term grassland re-sowing experiment (Chen et al. 2016; Reinsch et al. 2018). Experimental plots were established in 2005 in a randomised design on grassland seeded in 1994 at the Lindhof experimental farm of Kiel University. The farm is located in northern Germany close to the Baltic Coast and is managed in accordance with the German organic farmers' association Bioland, which prohibits the use of mineral fertilisers or pesticides. Organic fertiliser is applied as cattle slurry in three different nitrogen (N) fertilisation doses (0, 240 and 480 kg N ha^{-1} yr^{-1}), which correspond to average fertilisation treatments of 0, 1.7 and 3.3 Mg C ha^{-1} yr^{-1}. Additional organic C inputs have occurred with grassland litter or via root residues (Table 2). In order to maintain the soil's nutrient

Table 1 Site characteristics with mean annual temperature (MAT) and mean annual precipitation (MAP)

Site	Location degrees minutes (WGS84)	MAT (°C)	MAP (mm)	Soil type (FAO 2014)	Soil pH ($CaCl_2$)	Soil texture		
						sand (%)	silt (%)	clay (%)
Bad Lauchstädt	51°24'N 11°53'E	8.9	481	Haplic Chernozem	6.9	6	73	21
Kiel	54°27'N 9°57'E	8.9	768	Eutric Cambisol	6.1	63	27	10
Meckenheim	50°32'N 6°59'E	9.3	594	Luvisol	5.9	8	78	14

Data derived from Reinsch et al. (2018) and Scherer et al. (2011) except for soil texture and pH value

Table 2 Land use, fertilisation treatments and corresponding organic C inputs, experimental duration, and soil disturbance at the sampling sites

Site	Current land use	Fertiliser	Fertilisation treatments (Mg dry matter ha^{-1} yr^{-1})	Organic C fertiliser inputs (Mg C ha^{-1} yr^{-1})	Duration (years)	Soil disturbance
Bad Lauchstädt	crop rotation and mechanical bare fallow	farmyard manure	0	0	37	ploughing
			12.6	4.7		
			25.2	9.4		
			50.4	18.7		
Kiel	grassland	cattle slurry	0	0	15	non
			5	1.7		
			10	3.3		
Meckenheim	crop rotation	farmyard manure	0	0	44	ploughing
			20	2.8		
			40	5.6		

status, all treatments are fertilised at irregular intervals with rock phosphate and potassium-magnesium sulphate (Loges et al. 2018).

The long-term field experiment in Meckenheim in western Germany was established in 1962 as a randomised complete block design with four replications. The selected treatments are mineral fertiliser and farmyard manure with three fertilisation treatments of 0, 20 and 40 Mg C ha^{-1} yr^{-1} (Table 2). Since 1997 sugar beet, wheat and barley have been grown in rotation (Scherer et al. 2011). The soil pH values of the experimental sites distinguished by fertilisation treatments are given in Supplementary Table 1.

Disturbed soil samples were collected from the topsoil using a soil corer. The sampling of the croplands was performed according to ploughing depth at 0–25 cm in Bad Lauchstädt (sampled in 2020, in the centre parts of the plots) and at 0–30 cm depth in Meckenheim (sampled in 2006). In Kiel, samples were taken at depths of 0 to 5 cm (sampled in 2018). Since it is a grassland, SOC inputs are mainly deposited in the upper horizon and SOC stocks decline exponentially with increasing depth (Don et al. 2007). Thus, the upper horizon is especially interesting when testing for C saturation.

Soil fractionation

The soil samples were separated into three size fractions (> 63 µm, 63–20 µm and < 20 µm) based on a revised method of van Wesemael et al. (2019). All the samples were dried and sieved at ≤ 2 mm. A subsample of 10 g of fine earth (≤ 2 mm) was mixed with 100 ml of deionised water and shaken horizontally for 20 min at 20 rpm. The suspension was then washed through sieves of different sizes, and carefully rinsed with a rubber spatula and a spray bottle filled with deionised water. First the suspension was poured through a 63-µm sieve into a beaker until the liquid that passed through the sieve contained no visible particles. The material that remained on the sieve formed the sand-sized fraction, according to the German Soil Classification (Ad-hoc-Arbeitsgruppe Boden der Staatlichen Geologischen Dienste und der Bundesanstalt für Geowissenschaften und Rohstoffe 2005). The material that passed the 63-µm sieve was then poured through a 20-µm sieve until the water was clear. The material that stayed on the 20-µm sieve consisted of coarse silt. The fractions remaining on the sieves were collected separately and dried at 60 °C. The liquid that passed the 20-µm sieve was then centrifuged for 30 min at 3515 rpm and the clear liquid was decanted. The remaining material (fine silt and clay) was transferred into a beaker and dried at 60 °C.

After drying, all the fractions were weighed, ground and analysed for their concentration of organic carbon by combustion using an elemental analyser (LECO, TruMac, St. Joseph, MI, USA). After particle size fractionation, the mean mass recovery was 96.7%, while the minimal mass recovery was 94.9%. The mean and minimal C recoveries were 91.5% and 79.8% respectively.

Calculations & statistics

Data analysis was performed using R version 4.3.0. To test for saturation behaviour versus non-saturation behaviour, two different models were fitted for the individual experiments and size fractions. Before fitting a model, the data was inspected for heteroscedasticity visually. Heteroscedasticity was detected for Kiel, bulk soil. To account for this, the variance structure was allowed to increase with higher C inputs for both models.

A linear model (Eq. 1) represented ‘no saturation behaviour’. The SOC content was a linear function of organic C input per year and hectare:

$$SOC = a \times C_{input} + b \quad (1)$$

where: a, b= coefficients of the model and

$$C_{input} = C_{fertiliser} + C_{plant} \quad (2)$$

where: $C_{fertiliser}$= C inputs from fertiliser application (Mg C ha^{-1} yr^{-1}).

C_{plant} = C inputs from aboveground and belowground plant material (non- harvested plant residues).

$C_{fertiliser}$ was calculated from the fertiliser application (Mg dry matter ha^{-1} yr^{-1}) and its C content. C_{plant} was calculated from the yield data of the main crops with regional specific allocation functions from Jacobs et al. (2020). Yield data from the last 10 years before sampling were obtained for the Kiel site, while yield data from the Bad Lauchstädt site covered the last 20 years. Yield data for Meckenheim were not available and were therefore obtained from a long-term experiment on the Dikopshof estate located 20 km from Meckenheim to estimate the yield level under different fertilisation treatments. Mean average yield per treatment per experiment was used to estimate plant residual C inputs (Supplementary Table 2).

'Saturation behaviour' was defined as described in Eq. 3, where the SOC content approached a certain asymptotic value with increasing C inputs:

$$SOC = a \times \left(1 - e^{C_{input} \times b}\right) + c \tag{3}$$

where: $a, b, c =$ coefficients of the model.

The asymptotic model was fitted using the function *nlsLM* for sites with four different fertilisation treatments (e.g. Bad Lauchstädt) and a generalised additive model (*gam*) for sites with three different treatments. This was done because the *nlsLM* function cannot be applied to data sets with only three treatments, but it would easier to interpret. The *gam* was fitted using the *gam* function in the 'mgcv' R package (Wood 2011).

To compare the models, the Akaike Information Criterion was used with a correction for small sample sizes (AICc). The AICc values for the linear model were compared with the asymptotic models (*gam* or *nlsLM*). The site and fraction were matched to the category 'no saturation behaviour' when the linear model had a lower AICc than the asymptotic model. Burnham and Anderson (2004) outlined the following rules for correctly interpreting differences in AICc values: a model scoring less well has a) still substantial support if the difference in AICc scores is smaller than two, b) it has 'considerably less support' if the score difference ranges between four to seven and c) has 'essentially no support' if the score is higher than 10. If the linear and asymptotic model showed the same AICc, the simplest (e.g. linear) model was chosen and the site was assigned to 'no saturation behaviour'. To exclude the possibility of the C content already having reached the saturation plateau at the asymptote, the procedure included testing for a slope > 0 in the linear model at the significance level $p < 0.05$.

C stocks (Mg ha^{-1}) were calculated from the thickness and bulk density of the horizons according to Ellert and Bettany (1995):

$$Cstocks = conc \times \rho_b \times T \times mp_{fraction} \tag{4}$$

where: $conc =$ C concentration (%).

$\rho_b =$ bulk density (g cm^{-3}).

T= thickness of soil layer (cm)

$mp_{fraction} =$ mass portion of individual fractions.

The bulk density in Kiel (1.13 g cm^{-3}) was measured, the bulk density in Bad Lauchstädt (1.40 g cm^{-3}) was measured from Körschens and Waldschmidt (1995). No bulk density data were available for Meckenheim. To calculate the bulk density for the 0–30 cm depth (1.24 g cm^{-3}), data from 21 cropland sites from the same region with the same soil type, land use and texture were taken from the First German Agricultural Soil Inventory – Core dataset (Poeplau et al. 2020). The C stocks were mass corrected for changing proportions of mineral and organic compounds of each fraction with increasing C input. Thus, we accounted for the effect of different bulk density due to changes in C content with manure addition.

The C-sequestration efficiency of bulk soil per fertilisation treatment was calculated as follows:

$$C - sequestration\ efficiency = \frac{SOC_{high} - SOC_{ref}}{Cinput_{high} - Cinput_{ref}} \tag{5}$$

where: $SOC =$ mean SOC stocks of bulk soil (Mg ha^{-1}).

$Cinput =$ C inputs from fertiliser and aboveground and belowground plant material (Mg ha^{-1} yr^{-1}).

$ref =$ foregone fertilisation treatment as reference.

The ratio between actual and expected SOC (SOC:SOC$_{\text{exp}}$) was calculated according to (Poeplau and Don 2023) where:

$$SOC_{exp} = clay \times 0.0288 + 13.674 \tag{6}$$

where: clay = clay content (%).

In addition, the maximum amount of C (g kg^{-1}) associated with particles < 20 µm was calculated according to Hassink (1997) as follows:

$$C\ in\ fraction < 20\mu m = 4.09 + 0.37 * \%particles < 20\mu m \tag{7}$$

where $particles < 20\mu m =$ fine silt and clay content (%).

Results

Total annual organic C input

Total annual organic C inputs differed greatly between fertilisation treatments and the four experiments. The range of maximum total annual organic C input to the soil was between 6.5 Mg ha^{-1} yr^{-1} in grassland at Kiel and 20.2 Mg ha^{-1} yr^{-1} in cropland at Bad Lauchstädt (Table 3). In Bad Lauchstädt two different experiments were sampled, one with a crop rotation and one with continuous bare fallow. Thus, there was no organic C input at all in the no fertilisation treatment of the bare fallow at Bad Lauchstädt, but still 18.7 Mg organic C input ha^{-1} yr^{-1} from organic fertilisation with the highest treatment. Especially under low organic C fertiliser input, crop residues contributed up to 49% of total organic C input to the soil, with maximum fertilisation at the grassland site in Kiel (Table 3). As fertiliser organic C inputs were high at Bad Lauchstädt, residues only contributed between 7.3% and 25% of total annual organic C input to the soil.

Changes in SOC content of bulk soil

The long-term fertilisation treatments in each of the four experiments resulted in a wide range of SOC contents. The SOC content under no organic fertilisation ranged from 11 g kg^{-1} (Meckenheim) to 30 g kg^{-1} (Bad Lauchstädt – crop rotation). The highest fertiliser application in each experiment led to SOC contents of between 15 g kg^{-1} at Meckenheim and 57 g kg^{-1} at Bad Lauchstädt (crop rotation) (Fig. 2).

At three of four sites, a linear model was the best to describe the relationship between C input to soil and SOC content (Fig. 2, Table 4), based on the fitting of the linear and asymptotic models. Only at Bad Lauchstädt, the crop rotation showed a slightly lower AICc score for the asymptotic model and thus possibly indicates a non-linear fit (Table 4). As the AICc value is almost 5 units lower, the asymptotic model has considerable support (Burnham and Anderson 2004). All models showed a significant positive trend in SOC with increasing C input (Table 4). At Meckenheim, the asymptotic model even showed an exponential trend, which was in direct contrast to the assumed asymptotic behaviour with saturation (Fig. 2d). As SOC stocks were calculated from SOC contents with equivalent soil mass (Ellert and Bettany 1995), the models for SOC stocks were equal to the models fitted through SOC content data (Supplementary Fig. 1).

The C-sequestration efficiency of the bulk soil (Eq. 5) did not generally increase with an increasing fine silt and clay fraction (Fig. 3a). Bad Lauchstädt had the highest content of the fine silt and clay and a storage efficiency ranging from 0.05 to 0.24, while the C-sequestration efficiency at Kiel, the site with the lowest fine silt and clay content, ranged from 0.09 to 0.12. Bad Lauchstädt showed the widest range of C-sequestration efficiency, but the bare fallow was generally less efficient than the crop rotation.

Table 3 Amounts of derived organic C from different sources and total annual organic C input per treatment

Site	Land use/ management	Organic C fertiliser input levels (Mg ha^{-1} yr^{-1})	Mean annual organic C input levels from residues (Mg ha^{-1} yr^{-1})	Total annual organic C input levels (Mg ha^{-1} yr^{-1})
Bad Lauchstädt	crop rotation	0	1.4	1.4
		4.7	1.6	6.3
		9.4	1.5	10.9
		18.7	1.5	20.2
	bare fallow	0	0	0
		4.7		4.7
		9.4		9.4
		18.7		18.7
Kiel	grassland	0	2.9	2.9
		1.7	3.1	4.8
		3.3	3.2	6.5
Meckenheim	crop rotation	0	1.1	1.1
		2.8	1.8	4.6
		5.6	2.0	7.6

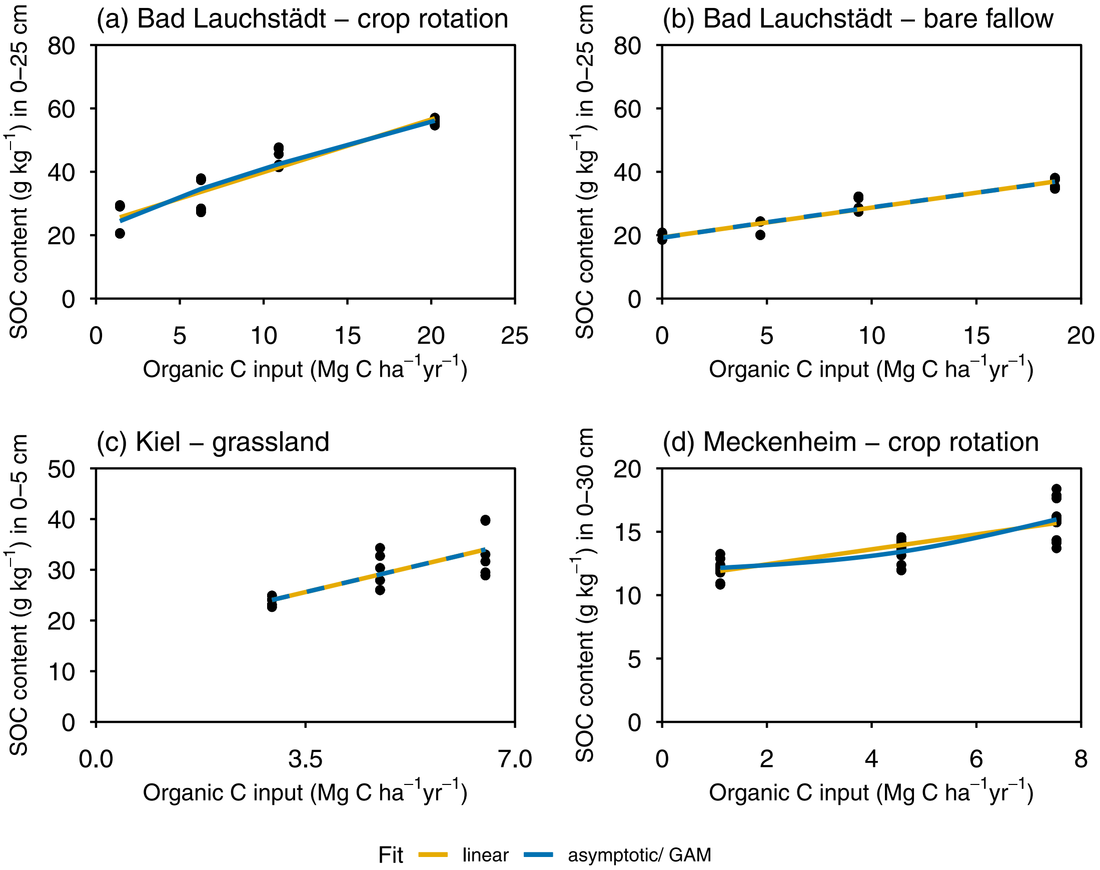

Fig. 2 SOC content (g kg^{-1}) of bulk soil at each sampling site with fitted linear, asymptotic and generalised additive models (GAM)

Table 4 AICc of the linear (lin), asymptotic (asymp) and generalised additive model (GAM) fitted to the SOC stocks at each sampling site

Site	Management	lin AICc	GAM AICc	asymp AICc	Indicates saturation	Linear fit slope
Bad Lauchstädt	crop rotation	782.10	-	777.31	yes	5.82 *
	bare fallow	661.70	-	663.65	no	3.29 *
Kiel	grassland	71.74	69.11	-	no	1.58 *
Meckenheim	crop rotation	819.81	802.42	-	no	2.19 *

Slope of the linear model with * showing significant differences from zero at $p < 0.05$

Meckenheim showed the lowest C-sequestration efficiency while at the same time the initial SOC content in the fine silt and clay fraction was the lowest of all experiments (Fig. 3b). Kiel showed the highest initial SOC:SOC$_{exp}$ (Eq. 6) of all experiments (Fig. 3c) and highest initial SOC content in the fine silt and clay fraction (Fig. 3b). At the same time the C sequestration efficiency in Kiel was with around 10% similar as in Bad Lauchstädt and higher as in Meckenheim.

The C-sequestration efficiency of bulk soil increased at Bad Lauchstädt under both types of management in relation to the none fertilisation treatment, for the first fertilisation level to 0.24 under crop

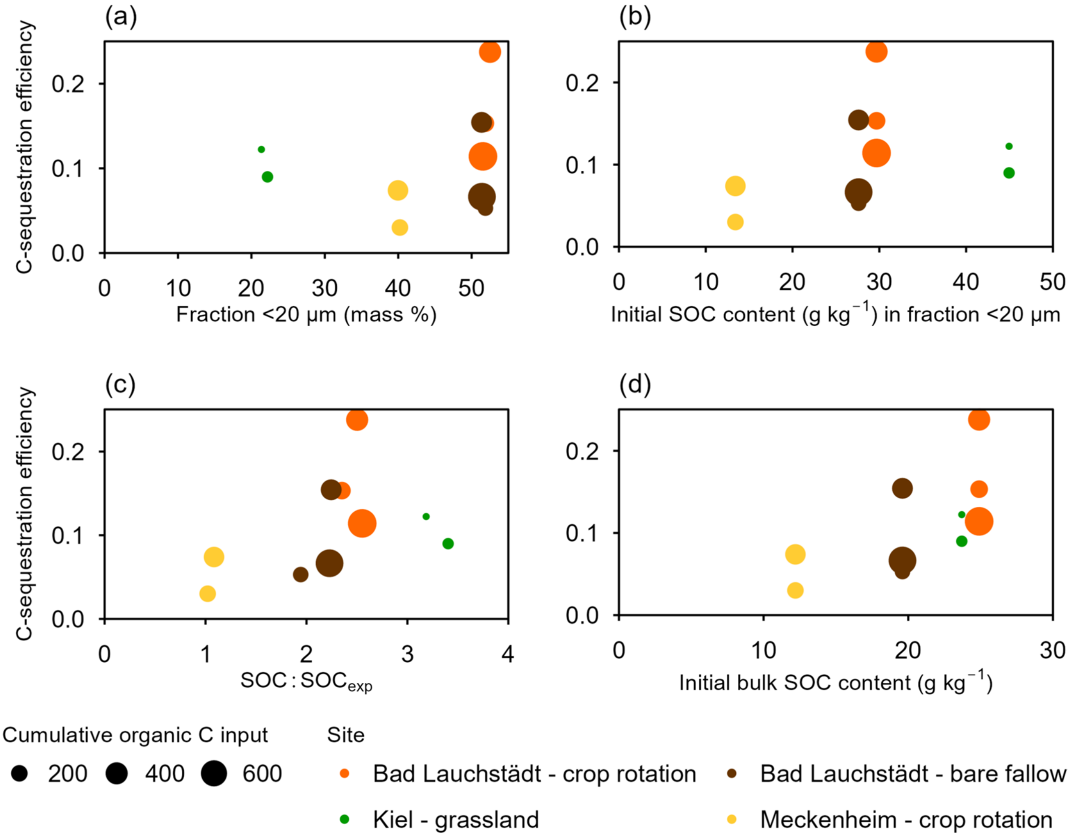

Fig. 3 C-sequestration efficiency of bulk soil defined as the ration between cumulative C input to the soil over the experimental duration and the SOC stock change. The SOC:SOCexp is an indicator for the SOC status of the soils taking into account the effect of clay (Poeplau and Don 2023)

rotation and 0.15 under bare fallow (Fig. 3). Under the highest fertilisation level, the C-sequestration efficiency of the crop rotation declined to 0.11 and the bare fallow declined to 0.06. Thus, the crop rotation stored C more efficiently than the bare fallow although the initial bulk SOC content was higher in the crop rotation (Fig. 3d). At Kiel, the C-sequestration efficiency declined in relation to the foregone fertilisation level from 0.12 to 0.09 with increasing bulk SOC contents. In contrast, Meckenheim showed an increase in C-sequestration efficiency from 0.03 to 0.07 with increasing bulk SOC contents.

Changes in size fractions with increasing C inputs

At all three sites, C stocks in the sand-sized fraction increased the most with increasing C input. At Kiel, the sand-sized fraction stored three times more C than the smaller fractions, irrespective of the fertilisation level (Supplementary Fig. 2). Based on the AICc values, the linear regression was a better fit than the asymptotic regression for the fine silt and clay fraction in all four experiments (Table 5). The sand-sized fraction was the best described with a linear model at one experimental site as the AICc value was lower. For the sand-sized and coarse silt fraction at Meckenheim a nonlinear model had a lower AICc, but instead of asymptotic behaviour it showed exponential growth (Fig. 4d). At the cropland site in Bad Lauchstädt the sand size fraction showed a non-linear behaviour with an asymptotic model being the best. All other fractions at all sites were best described by linear models without indication of saturation.

To test whether C fractions were increasing with C input, we tested if the slopes of linear regressions differed significantly from zero. C stocks of

Table 5 AICc of the linear (lin), asymptotic (asymp) and generalised additive model (GAM) fitted to C stocks of different size fractions for each sampling site

Site	Land use	Fraction	lin AICc	GAM AICc	asymp AICc	Indicates saturation	Linear fit Slope
Bad Lauchstädt	crop rotation	Fine silt & clay	95.10		95.10	no	0.47 *
		Coarse silt	105.93		106.05	no	1.32 *
		Sand	100.45		97.71	yes	1.94 *
	bare fallow	Fine silt & clay	57.49		63.42	no	0.40*
		Coarse silt	57.47		62.23	no	0.98*
		Sand	56.04		60.25	no	1.28 *
Kiel	grassland	Fine silt & clay	3.63	3.85		no	0.07
		Coarse silt	16.75	16.75		no	0.28 *
		Sand	60.12	60.72		no	1.06
Meckenheim	crop rotation	Fine silt & clay	80.55	80.55		no	0.43 *
		Coarse silt	88.09	87.85		no	0.79 *
		Sand	91.84	90.73		no	0.77 *

Slopes of the linear model with * indicate a significant difference from zero ($p < 0.05$)

the sand-sized fraction increased significantly with increasing fertiliser C input in all experiments, except at Kiel. The coarse silt fraction showed a significant slope of C content in all four experiments. C stocks in the fine silt and clay fraction increased significantly in Meckenheim and in Bad Lauchstädt (bare fallow and crop rotation) but not in Kiel (Table 5).

C stored in the fine silt and clay fraction

Soils in the four experiments with organic fertilisation were potentially closer to a hypothetic saturation than agricultural soils under common practices due to excessive C inputs via fertilisation. Figure 5 shows a comparison of the actual stored C in the fine silt plus clay fraction with storage capacity calculated as described in Eq. 7 in accordance with Hassink (1997). At Bad Lauchstädt, the highest hypothetical storage capacity was calculated according to Hassink (1997) due to the highest fine silt and clay content. Both crop rotation and bare fallow exceeded the calculated storage capacity even without organic C inputs (Fig. 5a, b). At Kiel, the C storage capacity was calculated to be 12 g C kg^{-1} soil and was the lowest of all the sites. This storage capacity was exceeded by almost five times (Fig. 5c). The storage capacity of the fine silt and clay fraction was not reached in Meckenheim only.

Discussion

No saturation of bulk SOC

No saturation behaviour of bulk SOC was detected at all three sites in this study. For one experiment (Bad Lauchstädt – crop rotation) it was less clear. In this case, the AIC was only slightly lower for the asymptotic model than for the linear model. According to Burnham and Anderson (2004) the difference of AICc scores is too small to completely omit the ‘weaker’ model but it has ‘considerably less support’ than the better fitting one. In the case of Bad Lauchstädt – crop rotation this means, that there could be a tendency to saturation but it is not distinctive. Still, even with using extreme agricultural measures such as the heavy organic fertilisation we could not detect a systematically decreasing proportion of C input that was retained in the soil. In order to be able to detect C saturation effects, long-term high C inputs are required. Was there sufficiently high C input in the selected long-term field experiments for C-saturation behaviour to be determined? Mean inputs of organic C from crop residues and organic fertilisers to agricultural soils (arable land and grassland) in Germany are 3.7 Mg C ha^{-1} yr^{-1} (Jacobs et al. 2020). In the long-term field experiments chosen for the present study, C inputs exceeded this average C input rate up to 5.5-fold. Such high C inputs would rarely be applied

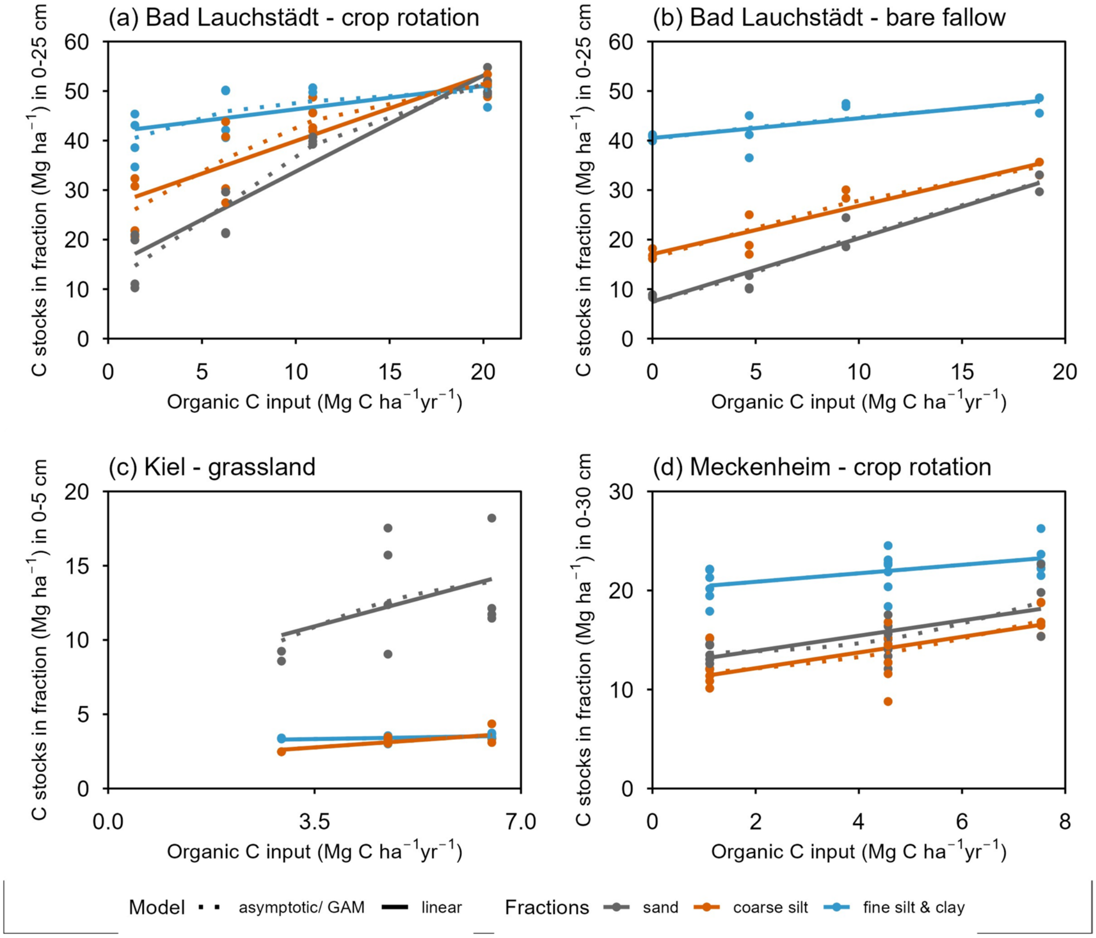

Fig. 4 Soil C stocks of different size fractions with fitted linear, asymptotic or generalised additive models (GAM)

in agricultural reality. This leads to the conclusion that bulk SOC saturation is unlikely to occur under common agricultural land use. Furthermore, our findings are in line with other studies such as Orgill et al. (2017), who found no limited C-storage capacity in SOC with increasing C inputs in a laboratory incubation experiment. Even though their C input was equal to 40.6 Mg C ha^{-1} and their mineral soils had moderate to high mean initial SOC contents of 32 to 63 g kg^{-1}, C input and SOC were linearly correlated. Thus, under controlled lab conditions with an extreme C input treatment soil saturation behaviour was not detectable. Kong et al. (2005) found no saturation behaviour of bulk SOC in a Californian long-term (10 years) experiment featuring 10 different cropping systems with C inputs ranging from 0.8 to 9.0 Mg C ha^{-1} yr^{-1} either.

The findings at Bad Lauchstädt are in contrast to those reported by Franko and Schulz (2020) who found C saturation behaviour at the bare fallow of Bad Lauchstädt. The different outcomes might be driven by the sampling design that could explain the different findings: Due to the absence of buffer strips between each plot, there might be a carryover of organic fertilisers or soil. Whereas we sampled only the centre of the plots, Franko and Schulz (2020) sampled the whole plots potentially including edge effects. Thus, the carry over might have a higher influence when sampling the whole plots rather than sampling in the middle of the plots.

It is assumed that saturation only occurs in the fine silt and clay fraction, while particulate, non-protected matter may increase linearly with increasing C input (Castellano et al. 2015; Cotrufo et al. 2019; Stewart

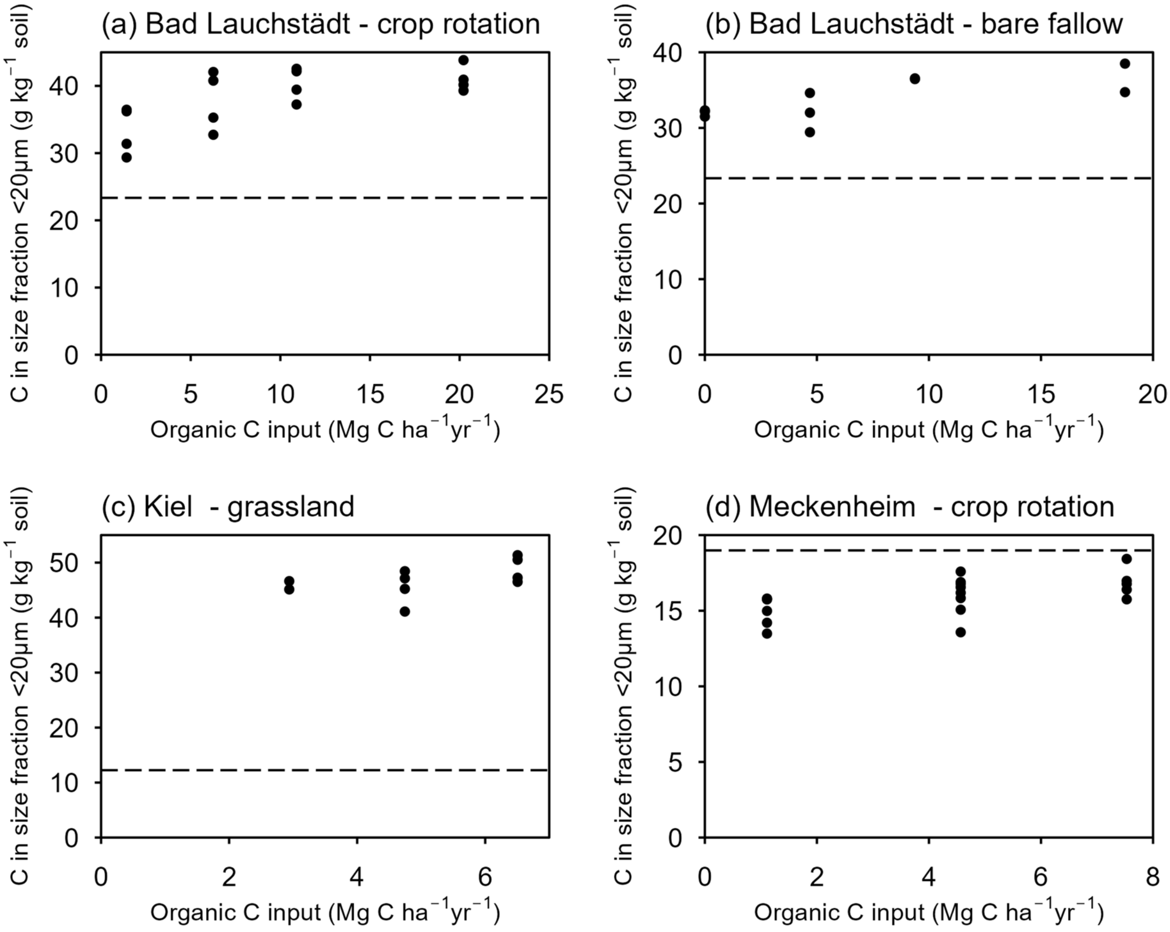

Fig. 5 C concentrations in fine silt + clay fraction (< 20 µm) of the fertilisation treatment. Dashed line indicates the C-storage capacity calculated according to Hassink (1997)

et al. 2008, 2012). However, if the fine silt and clay fraction would saturate at high C inputs, no linear relation between C input and bulk SOC could be obtained given the current kinetic conceptualisation of the fractions is approximately correct. Only if the fine fraction would decrease its turnover, a linear relationship might be maintained between total SOC and C input when the fine fraction would saturate. However, this hypothetical decrease in the fine fraction C turnover would need to be exactly to such an extent that it compensates the missing build-up of mineral associated C, which is unlikely the case.

In the present study, the fraction of C input that is retained as SOC (C-sequestration efficiency) was largely independent of the amount of manure that was added and also independent of the status of the soil in terms of SOC stocks and mineral associated C (Fig. 3). The SOC:SOC$_{exp}$ is a new indicator for the SOC level in relation to a clay determined average SOC content (SOC$_{exp}$) (Poeplau and Don 2023). It ranged from 0.95 (Meckenheim) to 3.22 (Kiel), with Bad Lauchstädt in between (1.93 bare fallow and 2.08 crop rotation) for the unfertilised plots respectively. This indicates that even SOC contents in unfertilised plots without organic amendments would be classified as good or very good according to Poeplau and Don (2023) indicating high SOC levels as compared to average German agricultural soils. With increasing C input, SOC:SOC$_{exp}$ ratios rose up to 3.40 (Kiel), 2.55 (Bad Lauchstädt—crop rotation), 2.23 (Bad Lauchstädt – bare fallow) and 1.02 (Meckenheim). Furthermore, we found the lowest C-sequestration efficiency in the soil with the lowest SOC:SOC$_{exp}$ ratio (Fig. 3c), which contradicts the notion that soils with a low SOC might sequester SOC more efficiently (Georgiou et al. 2022).

The crop rotation at Bad Lauchstädt was generally more efficient than the bare fallow in converting C input into bulk SOC (Fig. 3). Thus, the additional C input from plant residues did not reduce the efficiency as it would be expected under C saturation conditions. Root C inputs are known to be a main source of stabilized SOC (Poeplau et al. 2021). Thus, the additional C inputs from belowground plant material could be sequestered more efficiently than the manure amendments, leading to greater total C-sequestration efficiency in the crop rotation. This becomes apparent by taking a closer look at the C-sequestration efficiency (Fig. 3): The grassland site Kiel showed a higher C-sequestration efficiency than the cropland site Meckenheim even though the clay content was lower. But this behaviour could of course occur due to a time effect (Fig. 1) as the experiment in Kiel is younger than the experiment in Meckenheim. This indicates that the grassland might not be in a steady state and could therefore show a higher C-sequestration efficiency.

Generally, permanent grasslands are known to have higher SOC contents than arable land (Poeplau and Don 2013) because of high root C inputs and low soil disturbance, and thus are expected to be more easily C saturated (Hassink 1997; Guillaume et al. 2022). The additional SOC stored in grasslands is thought to be non-complexed, unprotected organic matter and thus to be sensitive to soil disturbance (Carter et al. 2003; Dexter et al. 2008) because particulate, non-protected organic matter has a higher turnover than mineral-associated organic matter (Buyanovsky et al. 1994; Poeplau et al. 2018). Thus, the high storage efficiency and high SOC stocks at Kiel could be unprotected C accumulating not in the fine silt and clay fraction. However, this was not the case, as we discuss in the next chapter.

Differing responses of the size fractions

Hassink (1997) proposed a saturation line in dependence on the clay content of soils and further studies supported or developed this approach (Cotrufo et al. 2019; Dexter et al. 2008; Guillaume et al. 2022). Dexter et al. (2008) calculated a new saturation line relating SOC stocks to clay content. They concluded that the bulk SOC content of pasture mostly exceeds the saturation line, while most arable soils fall below the saturation line. The additional stored SOC in grasslands is thought to be non-complexed, unprotected organic matter and thus sensitive to soil disturbance. Our findings are not in line with this because the silt and clay fraction exceeded the calculated C-storage capacity of 1 g OC per 10 g of clay at all experiments except at Meckenheim. Therefore, three experiments stored more C in the fine silt and clay fraction than the mineral surfaces should have been able to bind. Guillaume et al. (2022) suggested an even lower maximal value of C stored in the mineral-associated OC (43 mg C g^{-1} silt and clay) for permanent grasslands. This threshold is exceeded in the fine silt and clay fraction of the grassland at Kiel under all fertilisation treatments (Fig. 5c). In contrast to the perception by Guillaume et al. (2022) that grasslands are C saturated in the fine fraction, the present study and Fornara et al. (2016) found linear increases in SOC stocks with increasing C input to the soil in intensively fertilised grasslands. This suggests that long-term C sequestration in permanent grasslands is not limited by the fine silt and clay content and highlights the potential for carbon sequestration also in land use types with relatively high SOC stocks.

Whilst Cotrufo et al. (2019) also found an upper limit of mineral-associated OC storage of approximately 47 g kg^{-1} bulk soil, this was recently questioned by Begill et al. (2023) based on a comprehensive data set of the German Agricultural Soil Inventory. The authors found a linear correlation between SOC and MAOC up to 12% SOC. Also, Matus (2021) and Georgiou et al. (2022) found MAOC contents of more than 80 g kg^{-1}. This argues against the notion of C saturation due to limited mineral surfaces. It also shows that the experiments in this study are not near the upper limits that were recently proposed (Supplementary Fig. 1). This indicates that even under high C inputs that are far beyond common agricultural practice, saturation of MAOC is not relevant.

A linear relationship between the SOC content of the fine silt and clay fraction and C input to the soil is often explained by a large saturation deficit at which no non-linear saturation behaviour is expected (Feng et al. 2014; Kong et al. 2005). For example, Feng et al. (2014) did not detect saturation behaviour of fine silt and clay at four Chinese sites and one Canadian agricultural site with different crop rotations where initial C contents ranged from 2 to 35 g C kg^{-1}. The authors argued that the intensively cultivated soils in China and Canada exhibited a large C-saturation deficit due to management with low C inputs and a large storage potential due to high clay contents (Feng et al. 2014). C concentrations in the fine silt and clay fraction in our study ranged from 13 to 45 g C kg^{-1} which is at least partly above proposed saturation lines.

The C-sequestration efficiency of the crop rotation was higher than that of the bare fallow, which suggests that the sequestration efficiency might also be driven by the quality of C input. Differences due to the tillage regime can be excluded because soil management was the same in both treatments. The quality of C input differs between sources, e.g., root C input and straw. It is known that root C has a residence time in soils two to three times longer than that of other crop residues or manure-derived C inputs (Kätterer et al. 2012; Menichetti et al. 2015) and is therefore more effective at maintaining and building up SOC. This interpretation is underlined by the fact that the C-storage efficiency in Kiel was similar high even though this site stored more initial C in the fine silt and clay fraction (Fig. 4).

The storage of C in soils is influenced not only by the quality of C inputs, but also by the decomposition processes that depend on the quality and dynamics of SOC) as well as the role of decomposers. The size of the microbial biomass pool, on the other hand, may have a negative effect on SOC size due to its positive impact on carbon decomposition (Wieder et al. 2013). Modelling results suggest that microbially derived necromass C could contribute anywhere from 10 to 80% to the formation of SOC (Fan et al. 2021; Liang et al. 2019). Additionally, the composition of OC compounds plays a role in the long-term storage of SOC. This is because smaller, hydrophobic, and nitrogenous OC compounds, derived from microbes, exhibit preferential sorption (Lehmann and Kleber 2015; Barré et al. 2018). Therefore, decomposers can impact the storage of SOC by altering the composition of OC compounds (Kiem and Kögel-Knabner 2002; Wiesmeier et al. 2019). This study explores the results of biological processes, but was not able to measure them. Further research is necessary to capture the seasonal dynamics while considering the long-term trend in the experiments.

Several findings and concepts are proposing other C-storage mechanisms than sorption to mineral surfaces (Kleber et al. 2007; Schweizer et al. 2018, 2021; Vogel et al. 2014). The concept of organo-mineral interactions was promoted by Kleber et al. (2007). According to this model, C sorbs onto mineral surfaces but also onto organic matter in a zonal sequence. Thus, under increasing C input, the mineral surface coverage with OC will not necessarily increase, but the OC layer will grow thicker. The protection of C is greatest near to mineral surfaces in the 'contact zone', where strong organo-mineral associations form (Kleber et al. 2007). Taking this model into account, the C-storage capacity of soils is at least partly dependent on the binding sites of mineral surfaces and there should be saturation behaviour with decreasing C stabilisation with increasing C inputs (Kögel-Knabner et al. 2008). In contrast, findings from Vogel et al. (2014) showed that additional C input binds mainly to already existing OC clusters, while mineral surfaces are not completely occupied. In a study along a clay gradient of cropland sites it was shown that clay-associated C storage is driven by thicker accumulation of organic C (Schweizer et al. 2021). Thus, according to these findings the capacity of soil to accumulate C seems to be largely independent of the mineral surface area (Schweizer et al. 2018, 2021; Vogel et al. 2014). These nano-scale studies support our finding that silt and clay mineral surfaces are not imposing limitations for C stabilisation in agricultural soils.

Implications for C sequestration

The sequestration of SOC in soils can be an important mean to mitigate climate change (Chenu et al. 2019). Sequestration is the retention of atmospheric C in the soil as part of soil organic matter (Don et al. 2023). To mitigate climate change, long-term C sequestration is preferable. Thus, C should be stored in stabile soil fractions such as the fine silt and clay fraction (Six et al. 2002). However, in contrast to this our experiments sequestered C with a high efficiency even though the additional C input was not stored in the fine silt and clay fraction (Fig. 4). This study does not claim to be representative for Germany and should be treated as a case study. Therefore, caution should be used when trying to extrapolate these results. Furthermore, the experiments were subject to C inputs via organic fertilisation that would rarely be reached in agricultural reality (Jacobs et al. 2020). Organic fertilisation is used for agricultural nutrient management, and SOC effects may thus be a by-product. Nutrient applications need to be restricted and efficient in order to avoid negative side effects. Increasing manure application may increase environmental costs due to nitrate leaching and may also result in increased N_2O emissions (Wang et al. 2019). Thus, C sequestration via organic fertilisation is restricted in practice by these potential environmental costs rather than the soil´s capacity to stabilise SOC.

With increasing C input, more C accumulated in the coarse silt and sand-sized fraction. Coarse silt can contribute to the stabilisation of organic matter by entrapping organic C within silt-sized aggregates, where it is physically protected (Virto et al. 2008). The sand-sized fraction consists of partially decomposed plant residues. This particulate organic matter is not physically stabilised (Gregorich et al. 2006). Thus, C in the sand-sized fraction may easily be affected by soil management and climate changes (Carter et al. 2003). However, the field experiments that we investigated were between 15 and 44 years old and showed a stable C retention also in the coarse fraction. At least as transitional stage until land use and management related steady state conditions are reached also for the C pool distribution.

Overall, this data set suggests that long-term C sequestration in soils is not limited by the ability of the fine silt and clay content to stabilise SOC and highlights the potential for C sequestration in different land use types even when SOC stocks are already high. C sequestration in soils seems thus possible in all soils, regardless of their actual SOC content or their SOC in the fine silt and clay fraction. Limited is the availability and quality of biomass C which depends on ecosystem properties, management and productivity. However, this study does not aim to evaluate organic fertilisers. Instead, it focuses on the principles of carbon saturation.

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