

# Oxalate-extractable aluminum alongside carbon inputs may be a major determinant for organic carbon content in agricultural topsoils in humid continental climate

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## ABSTRACT

The relative importance of various soil mineral constituents (e.g. clay-sized particles, aluminum- and iron-bearing mineral reactive phases) in protecting soil organic carbon (SOC) from decomposition is not yet fully understood in arable soils formed from quaternary deposits in humid continental climates. In this study, we investigated the relationships between soil physico-chemical properties (i.e. contents of oxalate-extractable aluminum (Alox) and iron (Feox) and clay size particle  $< 2 \mu\text{m}$ ), grain yield (as a proxy for carbon input) and total SOC as well as SOC in different soil fractions for samples taken from the topsoil of an arable field at Bjertorp in south-west Sweden. We found a positive correlation between Alox and total SOC content, where Alox explained ca. 48% of the spatial variation in SOC. We also found that ca. 80% of SOC was stored in silt- and clay-sized (SC) fractions, where Al-bearing reactive mineral phases (estimated by Alox) may be important for organic-mineral associations and clay aggregation. Our results were supported by data collated from the literature for arable topsoil in similar climates, which also showed positive correlations between SOC and Alox contents ( $R^2 = 23.1 - 74.5\%$ ). Multiple linear regression showed that including spatially-variable crop yields as a proxy for carbon inputs improved the prediction of SOC variation across the Bjertorp field. Other unquantified soil properties such as exchangeable calcium may account for the remaining unexplained variation in topsoil SOC. We conclude that Al-bearing reactive mineral phases are more important than clay content and Fe-bearing reactive mineral phases for SOC stabilization in arable topsoil in humid continental climates.

## 1. Introduction

Enhancing carbon (C) sequestration in arable soils has received attention for its potential to mitigate global warming and climate change as well as improving soil fertility (Lal, 2004; Chenu et al., 2019). To understand C cycling in agricultural soils, it is important to know how soil properties regulate the stabilization of soil organic carbon (SOC) against microbial decomposition. Today, it is widely acknowledged that the physico-chemical properties of soil rather than intrinsic biochemical recalcitrance of organic matter inputs determine the stability of SOC

(Schmidt et al., 2011; Dungait et al., 2012; Lehmann and Kleber, 2015). Clay content (particle size fraction  $< 2 \mu\text{m}$ ) has often been considered a key soil property for SOC stabilization because clay is a major component in the formation of soil aggregates in which SOC can be occluded and thereby physically protected from microbial decomposition (Horn et al., 1994; Totsche et al., 2018). Clay particles also have a large specific surface area and therefore a large capacity to protect SOC chemically by adsorption (Sollins et al., 1996; Lützwow et al., 2006). In addition, clay content has been reported to be positively correlated with the prevalence of anoxic sites, which limits aerobic microbial decomposition of

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SOC (Keiluweit et al., 2018). As a result, clay content (and sometimes fine silt content) has been used to estimate SOC turnover in C cycling models and to calculate the degree of carbon saturation to estimate the carbon sequestration potential of soils (Hassink, 1997; Feng et al., 2013; Wiesmeier et al., 2015; Rasmussen et al., 2018).

Indeed, many studies have reported positive correlations between clay and SOC contents at various scales (e.g. Arrouays et al., 2006; Homann et al., 2007; Li et al., 2020; Poepflau et al., 2020; Prout et al., 2020). However, in many other arable and grassland soils, clay or silt and clay content was not correlated with SOC content (e.g. Thomsen et al., 2009; Beare et al., 2014; Augustin and Cihacek, 2016; Van De Vreken et al., 2016; McNally et al., 2017; Mayer et al., 2019), which indicates either that the clay fraction was not saturated with SOC and/or that clay content itself was not a major determinant of physico-chemical stabilization of soil organic matter (SOM) for these soils. Rasmussen et al. (2018) showed that SOC was more strongly correlated with short range order (SRO) phases and organo-metal complexes (i.e. the reactive mineral fraction estimated from chemically extractable aluminum (Al) and iron (Fe)) for acidic to neutral soils and with the content of exchangeable calcium ions for alkaline soils, than with clay content. Their study was based on an analysis of the U.S. Department of Agriculture's National Cooperative Soil Survey. Positive correlations between SOC and chemically extractable Al and Fe have also previously been documented for acidic forest soils (e.g. Mikutta et al., 2006) and volcanic soils (i.e. Andisols, Percival et al., 2000; Matus et al., 2006; Asano and Wagai, 2014; Beare et al., 2014). In addition, recent studies have highlighted the importance of the reactive mineral fraction in the stabilization of SOC for soils of sub-Saharan Africa (Ouedraogo et al., 2020; Traoré et al., 2020; von Fromm et al., 2020). Typical pH values for arable soils in Sweden are below 7 (ca. 90% of arable soils according to the national soil inventory of Sweden; Eriksson et al., 2010), which suggests that the Al- and Fe-bearing reactive mineral fraction should also be important for SOC stabilization in Swedish agricultural soils. However, to the best of our knowledge, the relationship between SOC content and the physico-chemical properties of soil has not been fully examined in Swedish and other regions' arable soils formed from quaternary deposits with similar climatic conditions (i.e. humid continental climate; Beck et al., 2018).

Al- and Fe-bearing reactive mineral phases are considered to associate with SOC through organo-metal complexation and/or co-precipitation and thereby regulate microbial and enzymatic accessibility to SOC (Kleber et al., 2015). Wagai and Mayer (2007) argued that there should be other mechanisms that influence the overall protection of SOC because the proportion of SOC that can be directly associated with Fe- and Al-bearing reactive mineral phases is often small compared to bulk SOC contents. Asano et al. (2018) suggested that organo-metal association can facilitate the formation of clay-sized micro-aggregates and thereby enhance the physical stabilization of SOC. Since SOC is mainly associated with the silt and clay fraction in arable topsoil (ca. 90%; Gregorich et al., 2006; Matus, 2021), aggregation in silt- and / or clay-sizes may be more important than macro-aggregation for SOC stabilization (Wiesmeier et al., 2014). The studies cited above indicate that Al- and Fe-bearing reactive mineral phases can play an important role in the protection of SOC by both physical and chemical mechanisms.

SOC fractionation should be a useful method to investigate the relationship between the reactive mineral fraction, clay content and SOC contents, as it isolates SOM fractions with different stability against microbial decomposition. In particular, it is important to separate particulate organic matter (POM) from mineral-associated organic matter as these two fractions have been shown to differ in SOC stability and, hence, respond differently to environmental changes such as changes in land use (Lavalley et al., 2020). SOC in the mineral-associated organic matter fraction is considered to be more stable than POM and therefore important for long-term SOC stabilization, whereas the POM fraction is a useful indicator of short-term changes in SOC (Lavalley et al., 2020). Poeplau et al., (2018) studied SOC stability in different SOM fractions,

applying 20 different fractionation methods to soils from fields where C3 plants had been replaced by C4 plants. They reported that SOC in the mineral-associated organic matter fraction was more stable (i.e. had a higher enrichment of C3-derived SOC) than POM and SOM associated with sand-sized fractions. They also found that SOC that was resistant to oxidation (e.g. by NaOCl) was the most stable fraction (i.e. had the lowest enrichment in C4-derived SOC). The latter finding is consistent with results presented by Mikutta et al. (2006) based on  $^{14}\text{C}$  analysis, who found that oxidation-resistant SOC was older than the bulk SOC.

Apart from stabilization mechanisms, the SOC content is also strongly driven by carbon input from crop production (e.g. Bolinder et al., 2007; Börjesson et al., 2018; Ogle et al., 2012). Moni et al. (2010) found no significant correlations between SOC and reactive mineral phases in the topsoil of a mixed area of grassland and arable fields, leading them to conclude that variations in C input may have masked such relationships.

The objective of this study was to examine the relationships between physico-chemical properties (i.e. clay content and oxalate-extractable Al and Fe as a proxy for the reactive mineral phases), total SOC and SOC in different soil fractions in a Swedish arable topsoil. For this purpose, we chose a conventionally tilled field with large variations in soil texture and SOC content. By sampling soils at the field scale, we minimized the effects of variations in other factors such as management and climate that also influence SOC dynamics. Our field-scale relationships between physico-chemical properties and SOC were set in a wider context by comparison with available literature data from agricultural fields with similar climate and types of soils formed from quaternary deposit.

## 2. Materials and methods

### 2.1. Field description and soil sampling

The selected field (46.9 ha) is located at Bjertorp (58°14'00.0"N 13°08'00.0"E) in Västergötland in south-west Sweden (Lindahl et al., 2008). Mean annual precipitation and temperature are 624 mm and 7.3 °C, respectively (average for the period 2006 to 2019 taken from the nearby metrological station at Hällum, Swedish Meteorological and Hydrological Institute). The field has been used for conventional crop production for at least 60 years with winter wheat, barley, oats and oilseed rape as the dominant crops. Similar soil and crop management has been applied across the whole field with two exceptions: 1) different crops have occasionally been grown in different parts of the field, and 2) nitrogen, phosphorus and potassium fertilization has, since the late 1990s, varied across the field. Nitrogen fertilization has been optimized using a tractor mounted sensor (Yara N sensor, on average 167 kg ha<sup>-1</sup> year<sup>-1</sup>) while phosphorus and potassium fertilization rates were based on measurements of ammonium lactate extractable phosphorus and potassium contents (on average 25 kg ha<sup>-1</sup> year<sup>-1</sup> and 10 kg ha<sup>-1</sup> year<sup>-1</sup> respectively). The field has been mouldboard ploughed regularly down to 20 – 25 cm depth except during years when winter rape seed was grown, when the tillage depth was reduced. No farmyard manure has been applied since the 1960s. In September 2020, we carried out soil profile descriptions at three different locations following the FAO guidelines (FAO, 2006). According to the IUSS Working Group WRB (2015) classification system, these soil profiles were classified as Stagnic Eutric Cambisol, Eutric Stagnosol and Haplic Phaeozem respectively (Fig. 1). Further details of these soil profiles are available in Table S1. The parent material in this region is Quaternary silty clay, which was deposited during the Weichselian deglaciation (Stevens and Bayard, 1994; Simonsson et al., 2014). Eriksson (2016) reported soil mineralogy data from a field located approximately 500 m from our field; the proportion of quartz, plagioclase, K-feldspar, amphibole, iron oxides and clay minerals in bulk soil (<2 mm) were 33, 18, 16, 2.6, 2.7 and 15%, respectively. In the clay fraction (<2 µm), the proportions of kaolinite, mica and expandable minerals (e.g. smectite, vermiculite, hydroxy-interlayered minerals and mixed layer illite-smectite minerals) were

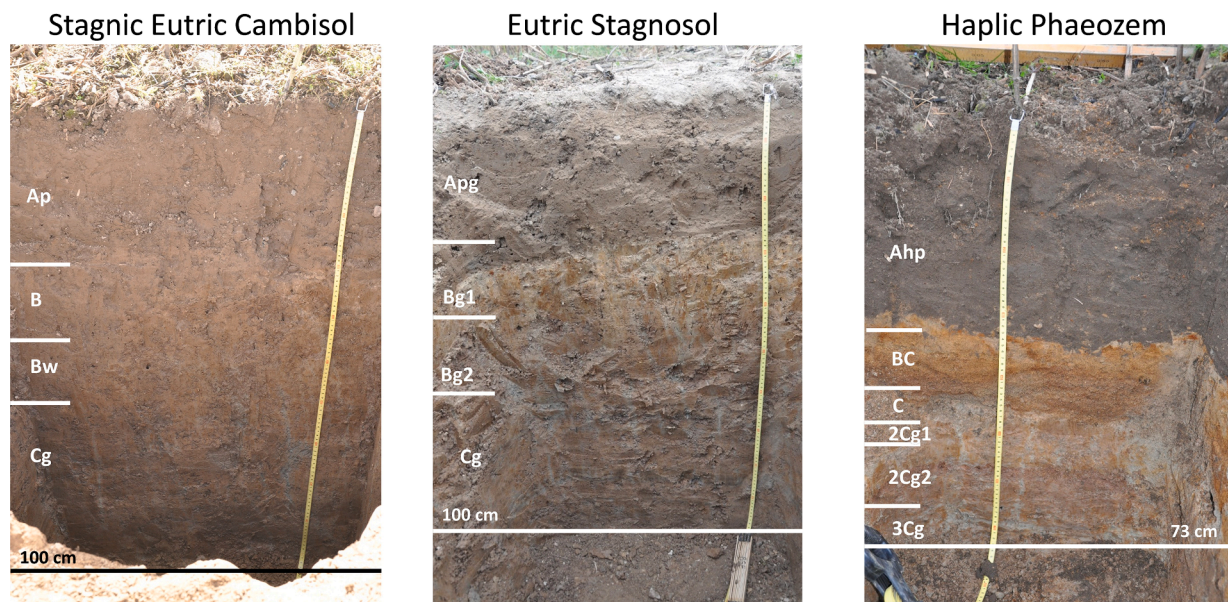


Fig. 1. Photos of soil profiles for three locations within the field. Elevation for Stagnic Eutric Cambisol, Eutric Stagnosol and Haplic Phaeozem are 90.6, 95.1 and 95.7 m respectively. These locations are shown in Fig. S1.

4, 27 and 69%, respectively. This field shows large variations in SOM and clay contents (Lindahl et al., 2008). SOM and clay contents were originally measured in topsoil samples (0 – 20 cm depth) in 2000. A weak negative correlation between clay content and bulk SOC (estimated from SOM) content was found (Fig. 2, original data from Lindahl et al., 2008). The elevation varies across the field from 87 to 95 m above sea level. Some significant statistical relationships were found between soil properties and elevation. These are reported in the supplementary material (Fig. S1). We do not know the origin of the spatial variation in soil texture at Bjertorp. However, the negative correlation between clay content and elevation (Fig. S1) suggests that it might be a result of sedimentation processes during the formation of the soil in the post-glacial period (Greve et al., 2012).

In late August 2017, after harvest of an oilseed rape crop, we sampled intact soil cores and loose soil at ca. 3 – 13 cm depth at 35 different locations in the field (Fig. S2). We used stratified sampling from the dataset collected in 2000 to get the best possible coverage of combinations of bulk SOC and clay contents (Fig. 2). The intact soil cores were first used for non-reactive solute transport experiments (Methodological detail in Larsbo et al., 2016) that are not reported here. After these experiments, the soil was dried at ca. 38 °C, crushed and sieved to < 2 mm (referred to as bulk soil). The dried bulk soil was used for soil texture analysis, SOM fractionation and analyses of oxalate-extractable Al (Alox) and Fe (Feox) contents. To assess potential effects of the irrigation experiment, we also measured bulk C and N contents and its ratio as well as Alox and Feox for a subset of the 35 sampling locations in soil samples that had not been irrigated. These properties were similar for irrigated and non-irrigated soil ( $R^2 > 0.87$ ). Based on these measurements of bulk soil chemical properties, we assume that the effects of the irrigation treatment on SOC fractions were also negligible. The loose soil was dried at ca. 38 °C, sieved to < 2 mm and used for soil pH measurement. A digital elevation model for the field was downloaded from Lantmateriet (resolution: 2 m, <https://www.lantmateriet.se/sv/>).

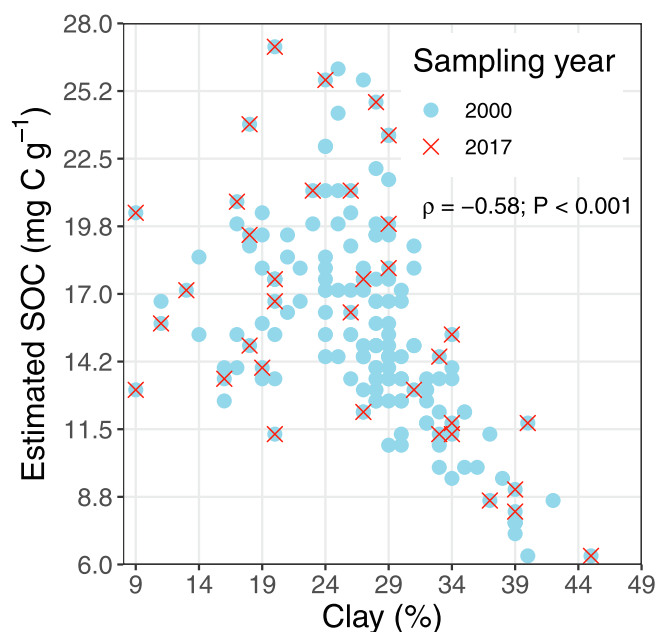


Fig. 2. A relationship between bulk soil organic carbon (SOC; estimated from soil organic matter) and clay content. Data from samples taken in 2000 (Lindahl et al., 2008).

### 2.2. Basic soil properties

Soil texture (sand: 60 – 2000 μm, silt: 2 – 60 μm, clay: < 2 μm) was determined by the pipette method after SOM removal using 10 mL peroxide (35%) and dispersion with a few drops of hydrochloric acid (HCl) and 25 mL chemical dispersant (7 g L<sup>-1</sup> sodium carbonate, Na<sub>2</sub>CO<sub>3</sub> + 33 g L<sup>-1</sup> Sodium metaphosphate, (NaO<sub>3</sub>P)n). Soil pH was measured in ultrapure water at a soil:water mass ratio of 1:2.5. Alox and Feox contents were determined in 1 g of soil after extraction with 100 mL of oxalate solution (mixture of 4.8 L ammonium oxalate solution (0.2 mol L<sup>-1</sup>) and 3.6 L oxalic acid solution (0.2 mol L<sup>-1</sup>) adjusted to pH 3.0) carried out in the dark. We considered Alox and Feox to represent hydroxides complexed with SOC and SRO mineral phases (Panichini et al., 2012; Wagai et al., 2013; Rasmussen et al., 2018).

### 2.3. Bulk SOC, bulk N and fractionation

We first measured the inorganic carbon (C) content for five of the 35 samples with varying soil pH (5.9 – 6.3) and clay content (16.5 – 41.7%)



after removing organic carbon by oven-drying at 550 °C. We found that the inorganic C content was <0.024 mg g<sup>-1</sup> which corresponds to <0.2% of the total C. Therefore, we assumed that the inorganic C content was negligible, such that organic C equaled total C content for the bulk soil and for all soil fractions. SOM fractionation was conducted according to Zimmermann et al. (2007b) and Poeplau et al. (2013). Briefly, 30 g of soil was placed in a glass beaker and 150 mL of deionized water was added. To disperse soil aggregates, 22 J mL<sup>-1</sup> energy with a constant power of 20 W was then applied to the soil samples using an ultrasonic probe (Poeplau et al., 2013). The dispersed soils were then wet-sieved (63 µm mesh size) with ca. 2 L deionized water. The fraction >63 µm was first dried at ca. 36 °C and density fractionation was then conducted to separate POM from the SOM associated with the sand and aggregate fraction. For this purpose, a sodium polytungstate solution with 1.8 g cm<sup>-3</sup> density was used. This density fractionation step was carried out twice. After the density fractionation, the sand and aggregate fraction and the POM fraction were gently washed with deionized water. The weight of each fraction was measured after drying at ca. 36 °C. The fraction smaller than 63 µm (silt and clay (SC) fraction) was also dried at ca. 36 °C and ground using a mortar and pestle. This fraction was further used for chemical oxidation experiments to isolate chemically resistant SOM (rSOM) in the SC fraction. For this, 1 g of the SC fraction was placed in a 50 mL centrifuge tube and 50 mL of 7% NaOCl (adjusted to pH 8 by HCl addition) was added. The samples were left for 16 h and then washed with deionized water. This oxidation step was carried out three times and the samples were then dried at ca. 36 °C. The fractionation resulted in five different SOM fractions, namely POM, SOM associated with sand-sized aggregates (sand-OM), SOM associated with silt and clay (SC-OM), oxidation resistant SOM in the SC fraction (rSOM) and non-oxidation resistant SOM in the SC fraction (SC-OM-rSOM). Correspondingly, the SOC content in each fraction is expressed as POM-C, Sand-C, SC-C, rSOC and SC-C-rSOC. SOC and N contents in bulk soil and all soil fractions were measured by dry-combustion on a TruMac CN (LECO Corp.). In addition, we report SOC contents in each fraction on a bulk soil basis (mg C g<sup>-1</sup> bulk) and as the proportion of SOC in each fraction in relation to total SOC (SOC<sub>tot</sub>) content. The recovery of soil mass, C and N after the fractionation experiment was estimated by calculating ratios of total recovered mass, C and N to initial soil mass, SOC<sub>tot</sub> and total N contents. We do not show data on total N and N contents in soil fractions since N contents were strongly positively correlated with the SOC content in each fraction. We used the carbon to nitrogen ratio (C:N ratio) as an indicator for the degree of microbial decomposition of SOM (Gregorich et al., 2006).

#### 2.4. Silt-sized soil aggregates

We examined particle and aggregate size distributions in ca. 0.08 g of the SC fraction (particles size < 63 µm) using a laser particle size analyzer (Partica LA-950 V2, Horiba). The Mie Theory was used to obtain volume-based particle and aggregate size distributions. The dried SC fractions were either mechanically dispersed (MD) or dispersed by SOM removal (DSR). For the MD treatment, the samples were placed in ca. 40 mL deionized water and stirred overnight with a magnetic bar (350 rpm). For the DSR samples, 3 mL of hydrogen peroxide (35%) and one drop of HCl were added to the samples with ca. 15 mL of deionized water. The samples were then boiled for at least 6 h to enhance oxidation. Then 1 mL of chemical dispersant (sodium carbonate, Na<sub>2</sub>CO<sub>3</sub> 7 g L<sup>-1</sup> + Sodium metaphosphate, (NaO<sub>3</sub>P)<sub>n</sub> 33 g L<sup>-1</sup>) and distilled water were added, resulting in a final volume of ca. 40 mL. Finally, also the DSR samples were mechanically dispersed overnight (350 rpm). We assumed that the DSR treatment dispersed most of the soil aggregates formed via SOC and clay interactions and at least partly organo-mineral associations (Harada and Inoko, 1977; Poeplau et al., 2019), resulting in disaggregated primary particles (Mikutta et al., 2005; Jensen et al., 2017), while water stable aggregates remained in the MD treatment. We calculated the difference in volumes between the MD and DSR

treatments for different size classes (i.e. < 0.5 µm, 0.5 – 1 µm, 1 – 2 µm, 2 – 6 µm, 6 – 20 µm, 20 – 60 µm and 60 – 100 µm size ranges). When MD was smaller than DSR, we interpreted the difference as the volume of primary particles that had been dispersed from silt-sized aggregates upon SOM removal. When MD was larger than DSR, the difference was interpreted as the volume of silt-sized aggregates contained in the MD samples.

#### 2.5. Grain yield data

Grain yields across the Bjertorp field were measured using the yield monitoring system on a Claas combine harvester in 1997, 1999, 2000–2004, 2007, 2010–2013, 2015 and 2016. The grain yield data were filtered to remove extreme values likely caused by machine dynamics such as abrupt velocity changes (Keller et al., 2012). The grain yield data were then converted to relative yield (RY) within each year to account for different crop types (Blackmore et al., 2003; Keller et al., 2012) as:

$$RY_i = \frac{Y_i}{Y_{mean}}$$

where  $i$  is the individual location where grain yield was recorded,  $Y_{mean}$  is the average grain yield for the respective year. The RY data were then imported to ArcGIS 10.7.1 (ESRI, <http://www.esri.com>) where interpolation was conducted using the Inverse Distance Weighting (IDW) method implemented in the Geostatistical Analyst extension. We excluded the data from 2003, 2004 and 2011 because yield data were missing for large parts of the field (i.e. > 30% of the area). Details of the interpolation procedure are provided in the [supplementary material](#) (page S7). The interpolated relative yield was extracted for soil sampling locations where soil properties were known based on Lindahl et al. (2008) and the present study (Fig. S2). The mean of the relative yields (MRY) for each soil sampling location was then calculated as:

$$MRY_{SSL} = \frac{1}{n} \sum_{i=1}^n RY_{SSL}$$

where SSL is soil sampling location and  $n$  is the number of years. The  $n$  value differs between soil sampling locations because of missing RY data for some years. The MRY was used in the statistical analysis as described below. We used MRY instead of mean actual yield or mean C input estimated from actual yield (Bolinder et al., 2007), because the actual yields measured by the combine harvester might not be comparable between years due to uncertainties related to the measurement equipment. Yield data within a given year were recorded with the same equipment and settings and should, hence, be reliable.

#### 2.6. Literature search on the relationship between SOC, clay, Alox and Feox in agricultural soils

To set the results from our field-scale study in a wider context, we searched the literature for datasets containing clay, total SOC (or total C), Alox and Feox contents for arable topsoils (0 – 20 cm) under humid continental climates. We focused on these conditions, since positive correlations between SOC and oxalate-extractable Al and Fe are already well known for forest soils (e.g. Mikutta et al., 2006) and some other soil types such as Andisols and Oxisols (e.g. Kramer et al., 2012; Souza et al., 2017b). We found one study from Sweden (Blombäck et al., 2020), one from Norway (Grønsten and Børresen 2009), one from Canada (Beauchemin et al., 2003) and one from Denmark (Paradelo et al., 2016). The dataset from Blombäck et al. (2020) covered agricultural fields mostly located in central and southern Sweden ( $n = 61$ ) including different types of soils (i.e. texture) and under different management practices. Grønsten and Børresen (2009) sampled soils from different arable fields located in Akershus County in south-east Norway that covered various combinations of clay and SOM contents ( $n = 21$ ). For this dataset, we

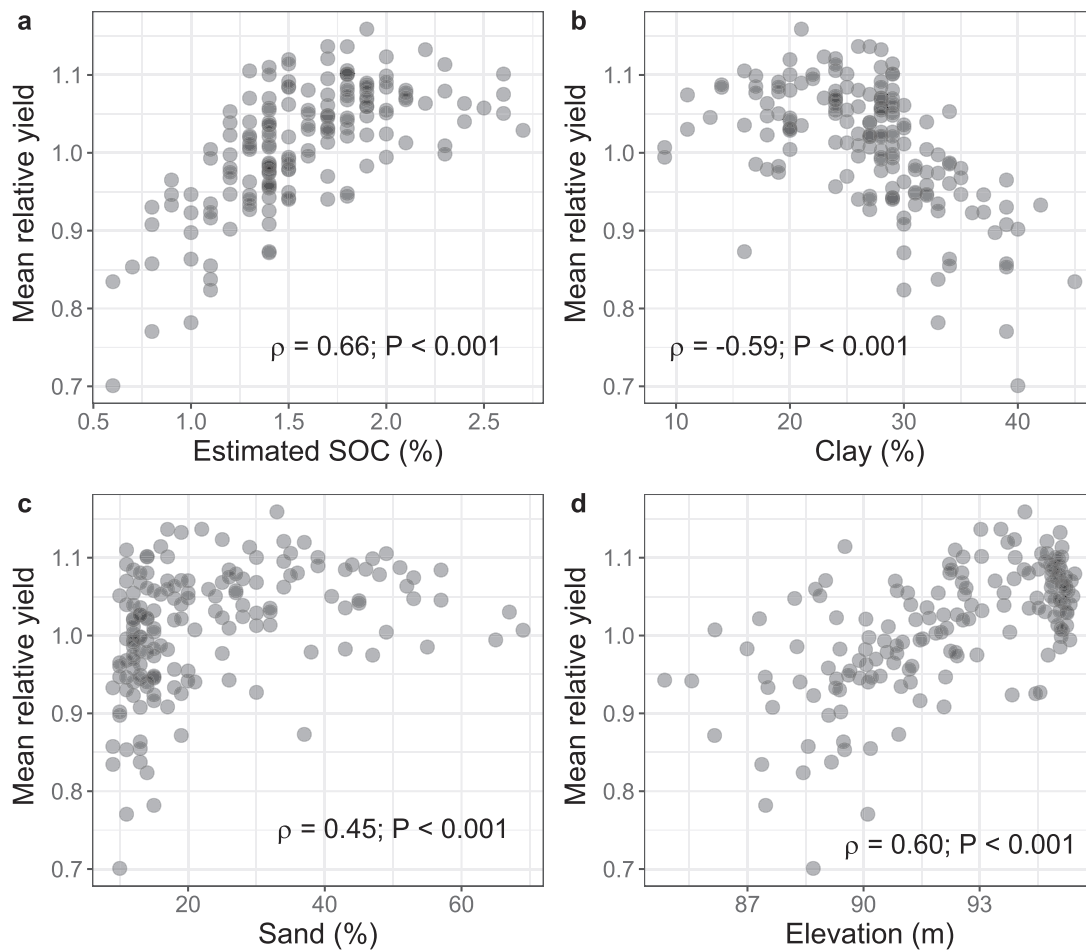


Fig. 3. Relationships between eleven-year mean relative yield and (a) estimated SOC, (b) clay, (c) sand and (d) elevation (n = 162).

Table 1

Mean, standard deviation (S.D.), maximum and minimum values of basic soil properties (n = 35) measured on samples taken in august 2017 and elevation and eleven-year mean relative yield (MRY) at respective soil sampling locations.

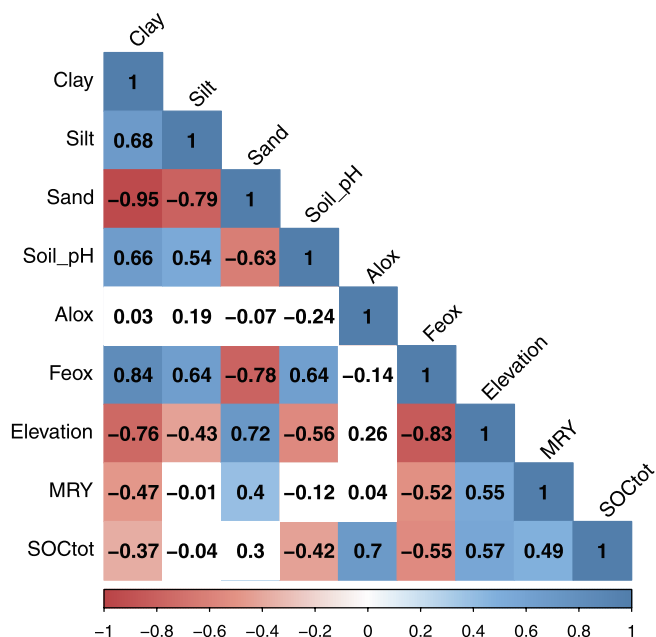
	Clay (%)	Silt (%)	Sand (%)	Soil pH	Alox (mg g <sup>-1</sup> )	Feox (mg g <sup>-1</sup> )	Elevation (m)	MRY	SOctot (mg C g <sup>-1</sup> )
Mean	27.2	47.0	25.8	6.02	1.61	5.64	92.6	1.00	17.0
S.D.	9.1	12.0	20.4	0.25	0.25	1.91	2.7	0.07	4.5
Max.	42.2	61.3	72.0	6.57	2.13	8.81	95.4	1.08	27.2
Min.	8.4	19.5	4.7	5.63	1.08	2.53	87.4	0.82	11.2

converted bulk SOM to bulk C content using a conversion factor of 0.58. [Beauchemin et al. \(2003\)](#) investigated soils sampled from tile-drained, cultivated areas in the Montreal Lowlands, Canada (n = 10). [Paradelo et al. \(2016\)](#) investigated soils taken from one agricultural field (1.26 ha) with a large spatial variation in SOC contents. A statistical summary of these studies is given in [Table S2](#). We combined the data from these studies into one dataset, except for the study by [Paradelo et al. \(2016\)](#), for which raw data were unavailable. From now on, we refer to this as the combined dataset.

### 2.7. Data analysis and statistics

Some of the soil properties were not normally distributed. Spearman rank correlation coefficients were therefore determined between basic

soil properties, SOctot, SOC in different soil fractions, elevation and MRY for the field scale study and also between SOC and soil properties for the individual and combined datasets from the literature. Spearman correlation coefficients were also calculated for the data obtained in the analysis of silt-sized aggregation. For the Bjertorp field, stepwise and multiple linear regression analyses were conducted to identify potentially important variables for predicting variations in topsoil SOC. Additionally, we used simple linear regression to investigate relationships between physico-chemical properties and SOC for the Bjertorp field as well as for the individual studies from the literature. For all regression analyses, assumptions such as normality and homoscedasticity of the residuals were visually checked. All the statistical analyses were performed in R ([R Core Team, 2019](#)). P = 0.05 was used as the significance level.



**Fig. 4.** Spearman rank correlation coefficients ( $\rho$ ) for relationships between soil physico-chemical properties, elevation and mean relative yield ( $n = 35$ ). Significant correlations ( $P < 0.05$ ) were highlighted either in red (negative) or in blue (positive). MRY: mean relative yield; SOctot: total SOC content.

**Table 2**

Simple and multiple regression analysis between bulk SOC, physico-chemical properties and mean relative yield (MRY). Model 1 is the result of stepwise regression analysis.

Regression model	Adj. R <sup>2</sup>
1 SOctot = 10.1 Alox - 1.4 Feox + 0.1 Silt + 12.8 MRY - 9.3	77.1%
2 SOctot = 11.8 Alox - 1.1 Feox + 5.0	69.6%
3 SOctot = 12.5 Alox + 30 MRY - 33.2	69.6%
4 SOctot = 12.8 Alox - 3.6	48.4%

### 3. Results

#### 3.1. Soil properties, elevation and mean relative yield in the entire Bjertorp field

For the sampling locations of Lindahl et al. (2008) which covered the entire field (Fig. S2), mean relative yield (MRY) was positively

**Table 3**

Mean, standard deviation (S.D.), maximum and minimum values of SOM fractions ( $n = 35$ ).

		Bulk	POM	Sand-OM	SC-OM	rSOM in SC	SC-OM-rSOM
SOC content (mg C g <sup>-1</sup> bulk soil)	Mean	17.0	0.83	0.92	14.0	2.56	11.4
	S.D.	4.5	0.29	0.38	4.2	1.36	2.9
	Max.	27.2	1.77	2.18	23.2	5.78	17.9
	Min.	11.2	0.39	0.46	9.0	1.06	7.4
SOC content (mg C g <sup>-1</sup> fraction)	Mean	17.0	320	1.64	21.7	4.10	17.6
	S.D.	4.5	16.8	1.63	10.9	3.05	8.0
	Max.	11.2	364	7.76	57.7	15.1	42.6
	Min.	17.0	295	0.56	10.1	1.24	8.37
Proportion of SOC fractions to SOctot (%)	Mean	100	5.0	5.6	81.7	14.2	67.5
	S.D.	-	1.8	2.3	4.4	3.9	4.5
	Max.	-	10.8	11.8	90.6	23.8	75.2
	Min.	-	2.7	1.9	70.9	8.0	55.7
C:N ratio	Mean	11.6	19.8	20.7	11.3	49.2	9.7
	S.D.	1.2	1.8	5.0	1.2	18.6	0.60
	Max.	14.2	24.4	34.5	14.0	92.3	10.9
	Min.	10.1	15.4	14.9	10.0	21.8	8.3

POM: particulate organic matter, sand-OM: SOM in the sand fraction, SC-OM: SOM in the silt + clay (SC) fraction, rSOM: oxidation resistant SOM in the SC fraction, SC-OM-rSOM: non-oxidation resistant SOM in the SC fraction, SOctot: total SOC content.

correlated with SOC ( $\rho = 0.66, P < 0.001$ ; Fig. 3a), sand ( $\rho = 0.45, P < 0.001$ ; Fig. 3c) and elevation ( $\rho = 0.60, P < 0.001$ ; Fig. 3d), whereas it was negatively correlated with clay ( $\rho = -0.59, P < 0.001$ ; Fig. 3b).

#### 3.2. Physio-chemical properties, elevation, MRY and SOctot for soil sampling locations in 2017

Clay and total SOC (SOctot) contents of soils sampled in 2017 (Table 1) were very similar to the properties measured in 2000 (clay: 9–45%; total SOC: 0.6–2.7%). Alox contents (mean 1.61 g kg<sup>-1</sup>) were much smaller than Feox contents (mean 5.64 g kg<sup>-1</sup>). The MRY (based on eleven years of data) varied between 0.82 and 1.08 at the field locations sampled in 2017.

The SOctot content was positively correlated with Alox content ( $\rho = 0.70, P < 0.05$ ), elevation ( $\rho = 0.57, P < 0.05$ ) and MRY ( $\rho = 0.49, P < 0.05$ ), whereas it was negatively correlated with clay ( $\rho = -0.37, P < 0.05$ ), Feox ( $\rho = -0.65, P < 0.05$ ), and soil pH ( $\rho = -0.55, P < 0.05$ ) respectively (Fig. 4). Alox content was not correlated with any variables except SOctot, while clay, Feox, MRY and elevation were significantly correlated with one another (Fig. 4).

In the stepwise regression analysis (Table 2), Alox, Feox, Silt and MRY were selected as explanatory variables for SOctot, where the coefficients of Alox and Feox were significantly different from zero ( $P < 0.005$ ) while the coefficients of Silt and MRY were not ( $P = 0.08$  and  $P = 0.09$  respectively). This best-fit model explained 77% of the variation in SOctot. Simple linear regression with Alox as an explanatory variable explained 48% of the variation in SOctot ( $P < 0.001$ ). Also, multiple linear regression analysis with combinations of Alox and either MRY or Feox explained about 70% of the variation in SOctot and the slopes of these regressions were significantly different from zero ( $P < 0.001$ ; Table 2).

#### 3.3. SOC in different soil fractions

Recoveries of total mass, carbon and nitrogen after the SOM fractionation were on average 98.2% ( $\pm 0.4$  S.D.), 92.3% ( $\pm 2.2$  S.D.) and 90.3% ( $\pm 2.3$  S.D.) respectively. The mass proportion of the sand and aggregate fraction was similar to the sand content determined in the texture analysis for all samples (i.e. all data points fell on the 1:1 line, Fig. S3). This means that most aggregates  $> 63 \mu\text{m}$  were destroyed by the ultrasonic treatment and wet-sieving procedures. Thus, in the following, we refer to the SOC in the sand-sized aggregate fraction as SOC associated with sand only (Sand-C). Most SOC (ca. 81  $\pm$  4.4%) was contained in the SC fraction (Table 3). The oxidation-resistant SOC (rSOC) in the SC fraction constituted, on average, 14.2 ( $\pm 3.9$ ) % of SOctot. POM-C

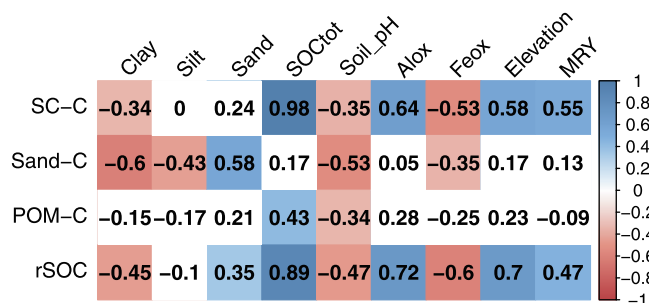


Fig. 5. Spearman rank correlation coefficients ( $\rho$ ) for relationships between soil physico-chemical properties, elevation, SOCtot and SOC content of each fraction in bulk soil ( $\text{mg C g}^{-1}$  bulk soil). Significant correlations ( $P < 0.05$ ,  $n = 35$ ) were highlighted either in red (negative) or in blue (positive). SC-C: SOC content in the silt and clay (SC) fraction; Sand-C: SOC content in the sand fraction; POM-C: SOC in the POM fraction; rSOC: oxidation resistant SOC content in the SC fraction.

and Sand-C were minor constituents, with average proportions of 5.0 ( $\pm 1.8$ ) % and 5.6 ( $\pm 2.3$ ) % respectively (Table 3). C:N ratios were larger for the more labile fractions (POM and Sand-OM fractions) compared to the SC-OM fraction (Table 3). C:N ratios for the oxidation-resistant SOM (rSOM) in the SC fraction were even larger (Table 3), but this result should be interpreted with care, since N contents in the rSOM of the SC fraction were close to the detection limit.

SC-C and rSOC contents in bulk soil were positively correlated with Alox, SOCtot, elevation and MRY (Fig. 5), whereas SC-C, Sand-C and rSOC contents were negatively correlated with clay, Feox and soil pH (Fig. 5). POM-C content was weakly but negatively correlated with soil pH and positively correlated with SOCtot (Fig. 5). Additionally, we found that the proportion of rSOC to SOCtot was positively correlated with SOCtot and Alox contents (Fig. 6). The C:N ratio for bulk SOM and SOM in the SC fraction was negatively correlated with clay, Feox and soil pH and positively correlated with Alox and SOCtot or SC-C (Table 4). For the labile fractions, the C:N ratio for POM fraction was not correlated with any soil variables, whereas the C:N ratio for the sand-OM fraction was negatively correlated with clay, Feox and soil pH (Table 4).

### 3.4. Clay aggregation

Fig. 7a shows that the dispersion by SOM removal (DSR) changed the particle and aggregate size distributions relative to mechanical dispersion (MD). There were some clear differences between the two dispersion treatments with a larger volume in the silt-sized (2 – 20  $\mu\text{m}$ ) range for the MD treatment relative to the DSR treatment, whereas the volume in the clay-sized (<2  $\mu\text{m}$ ) range was larger in the DSR treatment (Fig. 7b). The volume of the released clay-sized particles upon SOM removal (i.e. the volume of DSR-MD in the < 2  $\mu\text{m}$  size range) was positively correlated with the SC-C content (Fig. 7c).

### 3.5. Literature data

We found positive correlations between Alox and total SOC (or total C) contents for all individual studies, whereas SOCtot was not correlated with clay, pH and Feox (Table 5). In addition, the adjusted coefficients of determination for linear regressions showed that Alox could explain 40.5% of the variation in SOC in Grønsten and Børresen (2009), 74.5% in Beauchemin et al. (2003) and 23.1% in Blombäck et al. (2020). The coefficient of determination for samples in Blombäck et al. (2020) increased to 41.3%, when only samples with clay contents >15% were included. Total SOC was positively correlated with Alox content ( $\rho = 0.51$ ,  $P < 0.001$ ; Fig. 8a) for the combined dataset, whereas it was not correlated with clay, Feox and soil pH (Fig. 8b,c).

## 4. Discussion

About 80% of SOCtot was contained in the SC fraction and SC-C was

Table 4

Spearman rank correlation coefficients between soil properties and C:N ratios for SOM of bulk soil and each SOM fraction. Significant correlations ( $P < 0.05$ ) were indicated in bold.

	Clay	Silt	Sand	Soil pH	Alox	Feox	SOC*
Bulk SOM	-0.81	-0.52	<b>0.78</b>	-0.74	0.41	-0.81	<b>0.66</b> <sup>1</sup>
POM	-0.28	-0.07	0.27	-0.25	-0.28	-0.01	-0.14 <sup>2</sup>
sand-OM	-0.58	-0.35	<b>0.63</b>	-0.41	-0.01	-0.47	-0.06 <sup>3</sup>
SC-OM	-0.75	-0.44	<b>0.70</b>	-0.69	<b>0.47</b>	-0.82	<b>0.69</b> <sup>4</sup>
rSOM in SC	-0.80	-0.43	<b>0.74</b>	-0.57	0.21	-0.70	<b>0.65</b> <sup>5</sup>

\*SOC content in bulk soil (SOCtot)<sup>1</sup> or each SOM fraction (POM-C<sup>2</sup>, sand-C<sup>3</sup>, SC-C<sup>4</sup> or rSOC<sup>5</sup>). The unit is  $\text{mg C g}^{-1}$  bulk soil.

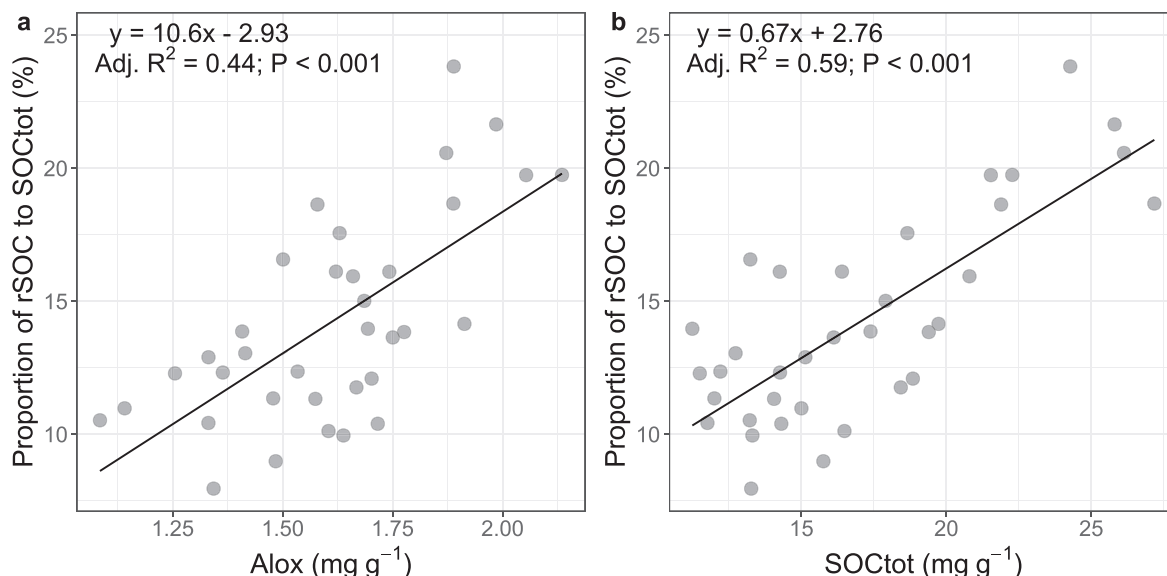
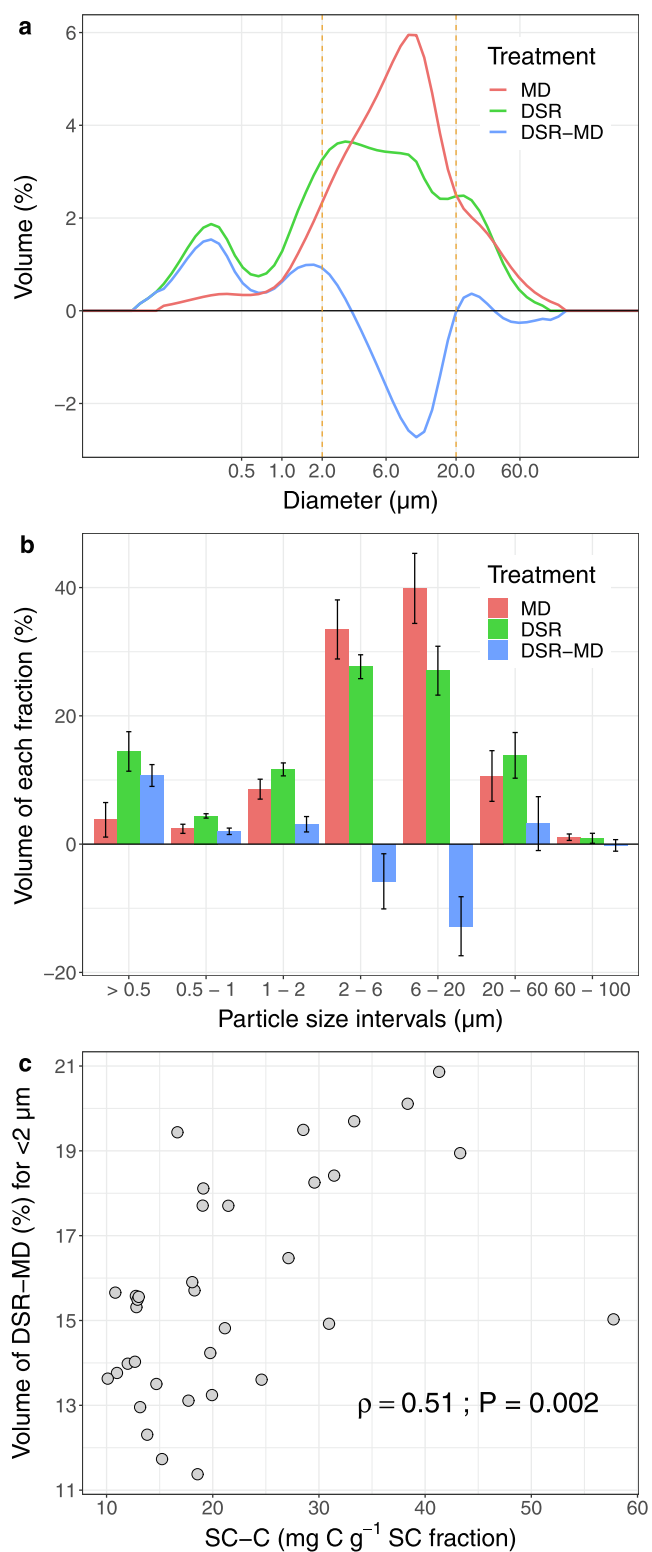


Fig. 6. Relationships between the proportion of rSOC content to SOCtot content and (a) Alox and (b) SOCtot.





**Fig. 7.** (a) An example of particle and aggregate size distributions for the silt- and clay-sized (SC) fraction after mechanical dispersion (MD), after dispersion with SOM removal (DSR) and their difference (DSR-MD); (b) Mean of volumes for particle size intervals ( $> 0.5 \mu\text{m}$ ,  $0.5 - 1 \mu\text{m}$ ,  $1 - 2 \mu\text{m}$ ,  $2 - 6 \mu\text{m}$ ,  $6 - 20 \mu\text{m}$ ,  $20 - 60 \mu\text{m}$  and  $60 - 100 \mu\text{m}$ ) with MD and DSR treatments and their difference (DSR-MD). Error bars indicate standard deviation ( $n = 35$ ); (c) The relationship between SOC in the SC fraction and the volume of DSR-MD for size classes  $< 2 \mu\text{m}$  (i.e. particles  $< 2 \mu\text{m}$  released upon SOM removal,  $n = 35$ ).

**Table 5**

Correlation coefficients for relationships between total C or SOC and physico-chemical properties in different studies on arable topsoils ( $0 - 20 \text{ cm}$ ) from four different studies. Significant correlations ( $P < 0.05$ ) are indicated in bold.

Reference (n: sample size)		clay	Alox	Feox	Soil pH
Grønsten and Børresen (2009) (n = 21) <sup>a</sup>	Total C	0.11	<b>0.54</b>	0.06	0.16
Beauchemin et al., (2003) (n = 10) <sup>a</sup>	SOC	0.31	<b>0.90</b>	0.61	-0.36
Blombäck et al., (2021) (n = 61) <sup>ac</sup>	Total C	0.02	<b>0.48</b>	0.03	-0.19
Blombäck et al., (2021) (n = 45, clay $> 15\%$ ) <sup>ac</sup>	Total C	<b>0.43</b>	<b>0.69</b>	0.24	-0.15
Paradelo et al (2016) (n = 45) <sup>b</sup>	SOC	-0.18	<b>0.97</b>	-0.15	0.25

<sup>a</sup> Spearman rank correlation coefficients were determined from reported raw data.

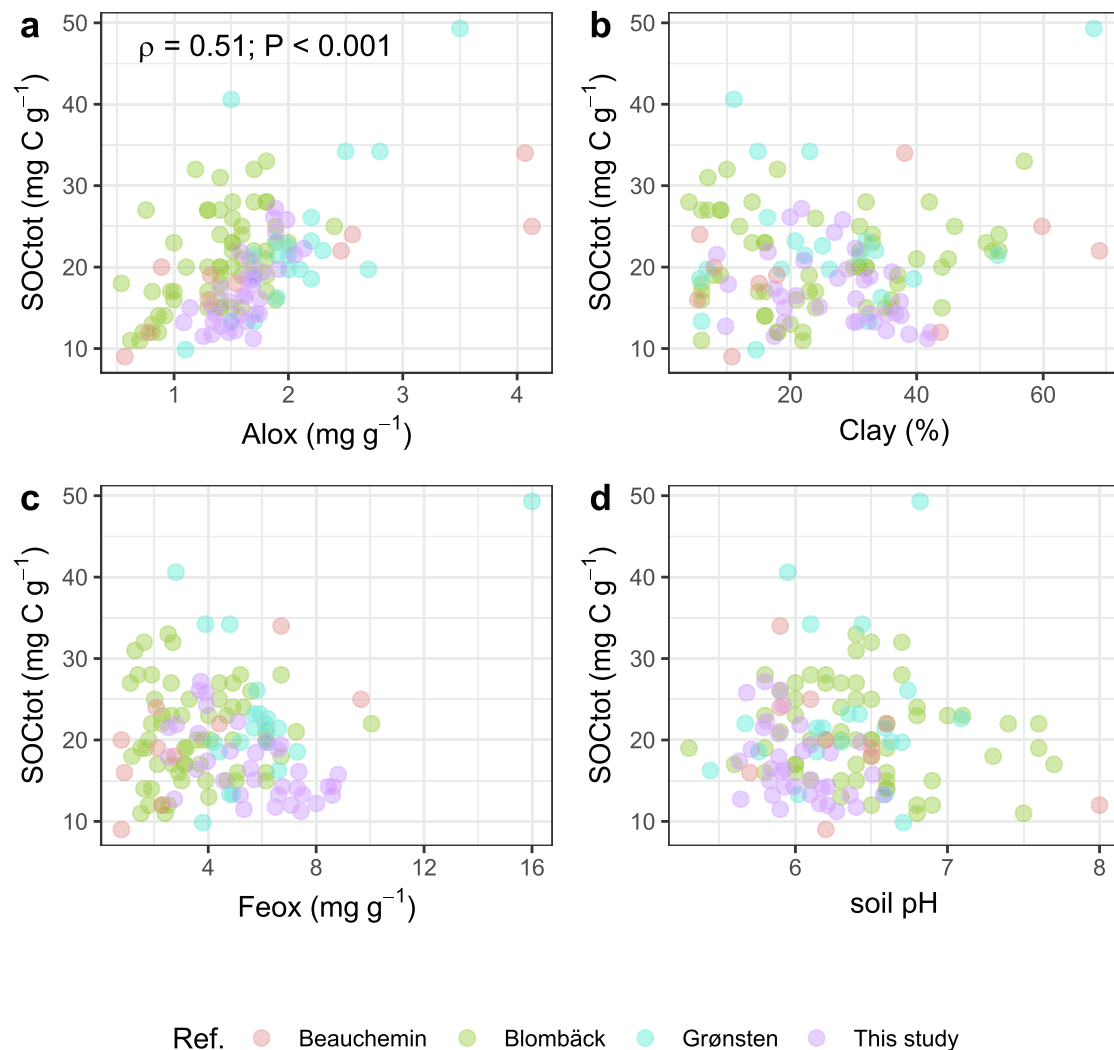
<sup>b</sup> Reported Pearson correlation coefficients were extracted from the paper since raw data were not available.

<sup>c</sup> Dataset excluding one sample with total C content  $> 9\%$ .

strongly positively correlated with SOC<sub>tot</sub>. These results are consistent with previous studies in Swedish arable soils (Simonsson et al., 2014) and internationally (e.g. Gregorich et al., 2006; Zimmermann et al., 2007a; McNally et al., 2017; Matus, 2021). Even for the samples with sand contents  $> 68\%$ , about 70% of SOC<sub>tot</sub> was contained in the SC fraction, suggesting that carbon stabilization is mainly restricted to SC sized aggregates and particles. Sand-C was positively correlated with sand content, which is in line with results presented in Li et al. (2013). However, the C:N ratio of sand-OM was similar to that of POM ( $\sim 20$ ), indicating that sand-OM was also dominated by relatively fresh, untransformed plant-derived OM. The POM-C content was only weakly correlated with soil pH and SOC<sub>tot</sub>. This may be because the spatial variation in POM-C may have been large compared to the other fractions (Simonsson et al., 2014).

The positive correlation between SOC<sub>tot</sub> and Alox suggests that Alox may be important for the protection of SOC against microbial decomposition in the Bjertorp field. Here, we discuss possible mechanisms behind this correlation. We also assume that Alox measured in bulk soil was mainly present in the silt + clay fraction (Curtin et al., 2016). Firstly, the oxidation-resistant SOC (rSOC) content was positively correlated with Alox content (Fig. 5). Additionally, the proportion of rSOC to SOC<sub>tot</sub> increased with Alox contents (Fig. 6a). These results indicate that Alox may have mediated the formation of stable SOC. Indeed, several studies have reported similar relationships between Alox/Feox and rSOC content (Mikutta et al., 2006; Wiaux et al., 2014). Based on <sup>14</sup>C analysis, Mikutta et al. (2006) also reported that oxidation-resistant SOC was older than SOC<sub>tot</sub>. Furthermore, Siregar et al. (2004) and Mikutta and Kaiser (2011) reported that NaOCl-resistant SOC seemed to be protected by associations with Al- and Fe-bearing reactive mineral phases since (1) the NaOCl treatment did not affect Alox and Feox contents and (2) larger amounts of these reactive phases resulted in larger rSOC contents. Secondly, Alox may also be important for physical protection of SOC. In this respect, we acknowledge that rSOC and non-oxidation resistant SOC (SC-C-rSOC) contents do not provide a clear division between physically and chemically protected SOC. For example, some chemically adsorbed SOC may be desorbed and oxidized at the pH value ( $= 8$ ) used in our oxidation experiment (Mikutta et al., 2005). Nevertheless, since about 67% of the SOC was contained in the SC-C-rSOC fraction, our data indicate that a large proportion of SOC may have been physically protected in the SC fraction. Accordingly, we found that when SOM was removed by peroxide, silt-sized aggregates were destroyed and clay-sized particles were released (Fig. 7b). Additionally, the volume of released clay particles upon SOM removal was positively correlated with the SC-C content (Fig. 7c). These results are consistent with the results presented by Jensen et al. (2017), who also reported an increase in the clay-sized fraction upon SOM removal





**Fig. 8.** Relationships between SOC<sub>tot</sub> (or total C) and (a) Alox, (b) Clay, (c) Feox and (d) soil pH. One sample with total C content > 9% in Blombäck et al. (2021) was not included. Data from Paradelo et al. (2016) were not openly available and hence not included here.

compared to samples without SOM removal. We hypothesize that the observed aggregation may also be important for the physical protection of SOC. In such aggregates, fine POM may be occluded and thereby physically protected from microbial decomposition (Shang and Tiessen, 1998; Virto et al., 2008; Steffens et al., 2017). The C:N ratios of SC-OM were also positively correlated with SC-C content (Table 4), which indicates that clay aggregation may have contributed to the protection of fresh, less decomposed SOM. Additionally, Alox was positively correlated with the C:N ratio of SC-OM (Table 4). Since reactive mineral phases have been suggested as key binding agents for clay aggregation (Shang and Tiessen, 1998; Asano et al., 2018; Totsche et al., 2018), we suggest that Alox content may also have been important for the physical stabilization of SOC in the Bjertorp field. This interpretation is of course only based on correlation analysis; further studies of the mechanisms behind the effects of Al-bearing reactive mineral phases on SOC turnover are needed.

Our field-scale results were supported by the analysis of individual studies in the literature (Table 5) and the combined dataset (Fig. 8a). The coefficients of determination for linear regressions showed that Alox could explain 23.1 – 74.5% of the variation in SOC in these studies. Other factors that may contribute to the unexplained variation in SOC, include (1) unquantified physico-chemical properties such as

exchangeable calcium (Rasmussen et al., 2018) and crystallized iron (Hall et al., 2018) and (2) variations in carbon input (Moni et al., 2010; Ogle et al., 2012). The relatively small coefficient of determination (23.1%) for the data from Blombäck et al. (2020) may be due to the fact that this study covered a wide range of soil types and land management across Sweden. The coefficient of determination increased to 41.3% when samples with clay content smaller than 15% were excluded. This may be because clay particles are important as building blocks for silt-sized aggregates (Souza et al., 2017a), as discussed earlier. It should be noted that extractable aluminum concentrations are dependent on the extraction method. For example, Panichini et al. (2012, 2017) reported a strong positive correlation between SOC and pyrophosphate-extractable Al in Andisols. In this study we used Alox as an explanatory variable for SOC since the pH in our soil samples was in the range of 5.5 – 6.5, which indicated that not only organically-complexed SOC but also SRO phases would be important for SOC stabilization (Rasmussen et al., 2018).

It is not clear why Feox content, which was much larger than Alox (Table 1), was not positively correlated with SOC<sub>tot</sub> (Fig. 4), SC-C and rSOC (Fig. 5) at Bjertorp and for the other arable topsoils listed in Table 5. However, our results are consistent with those of some earlier studies in different climates and soil types, such as Andisols (e.g. Beare

et al. 2014) and Oxisols and Ultisols (Barthès et al., 2008; Souza et al., 2017b). In contrast, other studies have suggested that Feox (or pedogenic Fe) may be more important than Alox in SOC stabilization, for example for an agroforestry field in North Carolina (Deiss et al., 2017), Swiss cropland soils (Zimmermann et al., 2007a) and European forest soils (Kaiser and Guggenberger 2000). There are two plausible reasons why Feox may not be effective in SOC protection in the Bjertorp field. First, soil pH in our field was relatively high (5.6 – 6.5) compared to studies on soils under other land uses, such as acidic forest soils. Additionally, there was a positive correlation between Feox content and soil pH in the Bjertorp field (Fig. 4). Lower soil pH may lead to stronger chemical association (e.g. complexation and precipitation) between Fe and SOM (Wagai and Mayer, 2007). Second, the SOC adsorption capacity of Fe-bearing reactive mineral phases is smaller under anoxic conditions, where SRO Fe may be reduced and adsorbed SOC may be released (Hall et al., 2018; Bailey et al., 2019; Huang et al., 2020). We do not know the redox conditions in the topsoil of the Bjertorp field. However, the Feox content was positively correlated with clay content and negatively with elevation, and hence sampling positions in low-lying areas with larger clay contents may have been more prone to anoxic conditions due to higher water contents (Manns et al., 2014; Keiluweit et al., 2018; Inagaki et al., 2020). We did observe signs of temporary anoxic conditions below the plough pan (28 – 43 cm; Fig. 1; Table S1) for one of the soil profiles (i.e. Eutric Stagnosol, Fig. 2). Thus, redox processes may also partly explain the lack of positive correlation between SOC and Feox at Bjertorp.

A weak negative correlation between clay and SOC<sub>tot</sub> contents suggests that clay content itself did not play a major role in the protection of SOC in the topsoil of the Bjertorp field. However, a number of authors have reported positive correlations between SOC and clay contents in other regions and climates (e.g. Homann et al., 2007; Wiesmeier et al., 2019; Poeplau et al., 2020). One possible explanation for the often observed positive correlations between SOC and clay contents is that clay content may be positively correlated with the contents of Al- and/or Fe-bearing reactive minerals. Several studies show that clay, Alox, Feox and SOC are indeed often positively correlated with each other (e.g. Zinn et al., 2007; Deiss et al., 2017). We suggest that, in addition to clay content, measurements of Alox and Feox may be useful for predicting the spatial variation of SOC in arable topsoils in continental humid climates and hence for modelling C dynamics and estimating the potential for SOC sequestration (Rasmussen et al., 2018; Basile-Doelsch et al., 2020; Wagai et al., 2020).

C:N ratios of bulk SOM and SC-OM were negatively correlated with clay and Feox contents, suggesting that there may be a greater accumulation of microbially-derived SOM with higher clay and Feox contents. This may be because N-rich SOM (such as proteins) is preferentially adsorbed on clay surfaces (Kleber et al., 2007; Lutfalla et al., 2019). Keiluweit et al., (2012) also reported that microbial N was preferentially associated with Fe-oxyhydroxides. However, the negative correlation between clay and the C:N ratio of bulk SOM and SC-OM can also be due to increased adsorption/fixation of inorganic nitrogen to clay particles (Jensen et al., 1989; Röing et al., 2006).

We used the eleven-year mean relative yield (MRY) as a proxy for carbon inputs from roots and above-ground residues. The multiple linear regression analyses indicate that C input was also a significant predictor of the spatial variation of SOC in the Bjertorp field. Positive correlations between SOC content and crop (or grain) yield have also been reported globally (Oldfield et al., 2019) and at the field scale (e.g. Kravchenko and Bullock, 2000). It should be noted that the positive correlations between SOC and MRY can be due to the beneficial effects of SOC on crop production (Henryson et al., 2018; Oldfield et al., 2020). Furthermore, the ratio of below-ground biomass to above-ground biomass or grain yield may not be constant. For example, Poeplau and Kätterer (2017) reported lower root biomass and root-to-shoot ratio in soils with larger clay content. The spatial variation of clay content in the field may also result in different soil mechanical properties and availability of

water and oxygen (Horn et al., 1994; Gregorich et al., 2011), which would affect root growth and crop production (e.g. Delin and Berglund, 2005; Gregorich et al., 2011; Wood et al., 2016; Börjesson et al., 2018). Correspondingly, we found that the MRY was negatively correlated with clay content and positively with elevation (Fig. 3), which indicates that the low-lying areas of the field with larger clay content may be less suitable for crop production and thereby lead to lower C inputs. This may also explain why SOC was negatively correlated not only with clay but also with Feox, since clay and Feox are highly correlated with each other (Fig. 4).

## 5. Conclusions

We examined relationships between physico-chemical properties, eleven-year mean relative grain yield (as a proxy for C input) and total SOC and SOC in different soil fractions for a Swedish arable topsoil. We found that total SOC content was positively correlated with oxalate-extractable aluminum (Alox) and that Alox explained ca. 48% of the variability in SOC. The results of SOM fractionation and analysis of clay aggregation further indicated that Alox may be important for physico-chemical stabilization of SOC in silt + clay sized fraction in the Bjertorp field. Our field-scale findings were supported by other studies that showed positive correlations between SOC and Alox contents in agricultural topsoils under climates compatible with our field. Simple linear regression analyses showed that Alox explained 23.1 – 74.5% of the SOC variations for these studies, highlighting the importance of other soil properties and variability in C input for better predictions of SOC variability. On the other hand, both clay (particle size < 2 µm) and oxalate-extractable Fe (Feox) contents seemed to be less important for SOC stabilization in the Bjertorp field. Additionally, a positive relationship between the mean relative yield and total SOC content indicated that C input from crop production could improve the predictions of SOC variability in the Bjertorp field. In future research, larger-scale studies (e.g. national soil inventories) exploring relationships between SOC and Alox and Feox contents may show if these variables are useful for estimating the potential for SOC sequestration and for modelling SOC dynamics in arable topsoils under humid continental climates.

## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2021.115345>.

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